INVITED LECTURES – SYMPOSIA AREA

S1 – The genome in the 3rd millennium

S1.1 Coding and noncoding information in genome function

S1.1.1
Epigenetic control by histone methylation

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Epigenetic mechanisms, such as histone modifications, control eukaryotic development beyond DNA-stored information. Intriguingly, there is an under-representation of repressive marks in quiescent (resting) cells, stem cells and regenerating cells, but a selective accumulation of aberrant histone lysine methylation profiles in aging, ''stressed'' and tumor cells, particularly for the H3K9, H3K27 and H4K20 methyl marks. To examine this notion in functional detail, we have generated mutant mice that lack crucial HMTases, such as e.g. the Suv39h and Suv4-20h enzymes. In addition, we have been characterizing jumonjiC-containing proteins that represent histone lysine demethylases with the potential to remove H3K9me3 marks. We have also screened chemical libraries (in collaboration with Boehringer Ingelheim, Ridgefield, USA) and identified a small molecule inhibitor for the G9a HMTase. We have done extensive profiling by ChIP-chip micro-arrays for many histone modifications in chromatin from ES cells and from a variety of differentiated cells. Our data indicate that distinct histone lysine methylation profiles contribute to the epigenetic ''make-up'' of stem cells versus more committed cells. Surprisingly, epigenetic variation appears to reside in repeat-associated heterochromatic islands and much less at annotated genes. Together, these functional approaches promise to yield new insights into the plasticity of cell fate decisions and will provide novel strategies to modulate epigenetic control in normal and aberrant development.

S1.1.2
Three-dimensional architecture of the human genome

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The spatial organization of the genome plays a critical role in its regulation, including the control of gene expression. Enhancers, insulators, and repressors can act over large genomic distances. This often involves direct looping interactions between regulatory elements and their target genes, giving rise to complex spatial organization of chromosomes.

To probe the spatial arrangement of genomes we developed Hi-C, a method that combines 3C and high-throughput sequencing to map chromatin interactions in an unbiased, genome-wide fashion. Application of Hi-C to the human genome revealed a novel layer of genome organization in which open and closed chromatin are spatially segregated, forming two genome-wide compartments. The contents of the compartments are dynamic: changes in chromatin state and/or expression correlate with movement from one compartment to the other.

To explore the properties of three-dimensional chromatin interaction networks at higher resolution, we employed 5C technology. We generated a comprehensive long-range interaction map between 166 gene promoters and 1193 loci distributed evenly along human chromosome 21 and identified approximately 3000 specific long-range looping interactions. Analysis of this set of interactions provides new insights into the architecture of long-range control in the human genome. First, promoters are found to interact with a surprisingly large number of distant elements. Second, many distant elements also loop to multiple promoters. Third, the interacting elements frequently contain DNAse I hypersensitive sites, predicted enhancer elements, and/or CTCF-bound elements. This suggests that our analysis identified bona fide regulatory elements interacting with promoters. Fourth, only a small fraction of the observed interactions are very frequent and span a relatively small genomic distance, whereas the large majority of interactions are infrequent and long-range (>2 Mb). Finally, promoters preferentially interact with elements that belong to the same compartment (as determined by Hi-C), though elements belonging to the other compartment may be closer in the linear genome.

Combined, our Hi-C and 5C data provide a first view of the architecture and specificity of gene-element associations and of the potential role of higher order folding of chromosomes in facilitating gene regulation.

S1.1.3
Important lessons from a complex genome

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The three billion base pairs of the human genome represent a storage devise encoding information for hundreds of thousands of processes that can go on within and outside of a human cell. This information is revealed in the RNAs that are transcribed and processed and in interaction of DNA with the protein and RNA products encoded within it. Part of the results stemming from the efforts to catalogue and analyze the RNA products made by human cells in the ENCODE project has shed light on both the functional content and how this information is stored. A total of ˜142,000 transcripts present within ˜50,000 genic regions represent our current best manually-curated annotation (Gencode) of the transcriptome. However, data obtained from the use of deep sequencing of polyadenyated and non-polyadenylated long, as well as short (<200nt) RNAs isolated from subcellular compartments indicate that these estimates will continue to grow substantially as the exploration of transcripts present at low copy numbers improves. Such low copy number RNAs are being found as part of the transcriptional outputs of specialized cells or specifically enriched in sub-cellular compartments. The ENCODE project on the transcript analyses have resulted in important and often under appreciated lessons such as (i) low levels of expression does not equate to non-functionality, (ii) the fate of most long transcripts is likely to be processed into stable, sometimes capped short RNAs, (iii) a large and specific fraction

of human transcripts are selectively enriched in sub-cellular compartments in a cell thus increasing their relative copy number, (iv) non-polyadenylated transcripts abound in cells and possess unique characteristics distinct from polyadenylated transcripts and (v) RNAs are transported outside of the cell of origin in protective vessicles. These and other lessons drawn from the landscape of both coding and non-coding RNAs present in human can be used to assist in understanding and organizing what is often seen as dauntingly complex genome.

S1.1.4
Retrotransposition and the genetic identity of human neurons

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Retrotransposons are mobile genetic elements that spread via a germ line "copy-and-paste" mechanism. In humans, L1 retrotransposons comprise about 17% of the genome and contribute polymorphisms that impact our biology in a myriad of ways. Recent experiments suggest that L1 also mobilises throughout embryogenesis and later development, including in the somatic cells of the adult brain.
In this talk I will discuss recent developments in linking somatic genome mosaicism with phenotypic effects in the human brain. Using a high-throughput sequencing approach we mapped 4435 somatic L1 insertions in the hippocampus and caudate nucleus of two individuals. Surprisingly, we also found 6224 somatic Alu insertions. These events were heavily biased towards protein-coding genes differentially expressed in the brain and important for neurobiological function. The intriguing conclusion is that somatic retrotransposition generates populations of genetically distinct neurons and that these distinctions are likely to affect the functional output of the brain.

S1.1.5
Repetitive elements transcription and mobilization contribute to human skeletal muscle differentiation and duchenne muscular dystrophy progression

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See Abstract P01.7

S1.1.6
Non-canonical termination signal recognition by RNA polymerase III in the human genome

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See Abstract P01.13.

S1.2 Mechanisms controlling genome integrity

S1.2.1
Early events in eukaryotic DNA replication

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The eukaryotic cell cycle coordinates the accurate duplication and segregation of the genome during proliferation. The large genomes of eukaryotic cells are replicated from multiple replication origins during S phase. These origins are not activated synchronously at the beginning of S phase, but instead fire throughout S phase according to a pre-determined, cell type specific program. Only after the entire genome is completely replicated do cells proceed into mitosis.
Ensuring that each origin is efficiently activated once and only once during each S phase is crucial for maintaining the integrity of the genome. This is achieved by a two-step mechanism. The first step, known as licensing, involves the loading of the Mcm2–7 proteins into pre-replicative complexes (pre-RCs) at origins. We have recently reconstituted this reaction with purified proteins (Remus et al. Cell 2009 139: 719–30). In this reaction, Mcm2–7 are loaded as a head-to-head double hexamer around double stranded DNA. Mcm2–7 loading requires the Origin Recognition Complex (ORC) as well as Cdc6 and Cdt1. I will describe recent experiments showing that individual Mcm subunits play distinct roles during pre-RC assembly by interacting with different assembly factors. I will also show that the role of cyclin dependent kinases in promoting initiation has been conserved, at least in part, between yeast and humans.

S1.2.2
The ATM-mediated DNA damage response: the system and the pathways

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The DNA damage response (DDR) is a complex network of signaling pathways that is vigorously activated by DNA double strand breaks (DSBs). The primary transducer of the DSB response is the serine-threonine kinase ATM, which is missing in patients with the genomic instability syndrome ataxia-telangiectasia (A-T). We are exploring this complex network at the transcriptional and post-transcriptional levels using systems biology tools and proteomic and genetic high-throughput screens. Subsequently, in-depth analysis of novel pathways is carried out. Special attention is paid to the growing interface between the ubiquitin and the DDR arenas. Emerging pathways in this interface will be presented. An important meeting point combines players from the two arenas, as well as chromatin organization and DNA repair. The delicate interplay between these proteins, which finally leads to timely damage repair, is orchestrated mainly by protein phosphorylation and ubiquitylation. An interesting phenomenon is that protein machineries recruited to damage sites may act differently in stressed and in unstressed cells or may serve the same role. Examples of both cases will be presented.

S1.2.3
Telomeres and the challenges to chromosomal integrity

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Telomeres protect chromosome ends from degradation and fusion, in turn preserving genome stability. Our recent data challenge current ideas for the requisite building blocks of telomeres and expand the list of fundamental telomere functions.

Telomeres generally comprise repeated sequences and proteins that bind these sequences specifically. While the most terminal repeats are lost with each cell cycle via the end replication problem, they are replenished by telomerase. In the absence of telomerase, fission yeast can survive via telomeric recombination or chromosome circularization. We have found a third class of survivors called ''HAATI'' that lack telomeric DNA but do not harbor circular chromosomes. Rather, HAATI replace canonical telomeres with blocks of ''generic'' heterochromatin that acquire the ability to recruit specific end-protection factors. This discovery suggests a mode by which telomerase-minus cancer cells may achieve unlimited replicative potential.

Telomeres take on dramatically different roles in meiosis, when they gather at the nuclear membrane to form the so-called telomere ''bouquet''. While the bouquet is widely conserved among eukaryotes, its functional significance has not been understood. We find that the bouquet is required for meiotic spindle formation. In the absence of the bouquet, the γ-tubulin complex fails to localize to both spindle poles, suggesting that the gathered telomeres modify a pole protein that controls this localization. Finally, we present data leading to the provocative idea that the bouquet influences meiotic centromere assembly. Collectively, our data suggest an unforeseen degree of plasticity and functional diversity for telomeres.

S1.2.4
The structural basis of chromosome segregation

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Equational division of the genetic material during mitosis is based on the establishment of secure interactions of chromosomes with the mitotic spindle, a microtubule- and motor-based structure. The point of attachment of chromosomes to spindle microtubules is a complex protein scaffold (80–100 proteins) named the kinetochore. Kinetochores can be conceptually dissected into four modules: (i) a DNA-binding module that is built around a specialized nucleosome containing the Histone H3 variant CENP-A; (ii) a microtubule-binding module, that is physically tethered to the DNA-binding module, and that is based on a proteinaceous microtubule receptor that goes by the name of the KMN network; (iii) an attachment correction module, that removes improper attachments by activating microtubules ''saws'' such as MCAK and Aurora B; and (iv) a safety device known as the spindle assembly checkpoint, that coordinates the chromosome attachment process with a cell cycle oscillator consisting of cyclin-dependent kinases and associated cyclins. Our current challenge is to reduce the functional and structural complexity of kinetochores to a set of basic organizational principles. This requires the construction of an accurate topological map of the kinetochore's modules, an understanding of their points of contact, the availability of high-resolution structures of kinetochore components, and building a model of the dynamic regulatory steps that subtend to accurate segregation. We are therefore applying a combination of structural and functional investigations to unravel the architecture of the microtubule-kinetochore interface, and its interactions with the error correction mechanism and with the spindle assembly checkpoint. I will present our main results, and discuss them in the framework of an integrated model that explains many apparently contradictory aspects of kinetochore biology.

S1.3 Epigenetic control of cell fate

S1.3.1
Epigenetic challenges in centromere inheritance during the cell cycle

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Studies concerning the mechanism of DNA replication have advanced our understanding of genetic transmission through multiple cell cycles. Recent work has shed light on possible means to ensure the stable transmission of information beyond just DNA and the concept of epigenetic inheritance has emerged. Considering chromatin-based information, key candidates have arisen as epigenetic marks including DNA and histone modifications, histone variants, non-histone chromatin proteins, nuclear RNA as well as higher-order chromatin organization. Thus, understanding the dynamics and stability of these marks following disruptive events during replication and repair and throughout the cell cycle becomes of critical importance for the maintenance of any given chromatin state. To approach these issues, we study the maintenance of heterochromatin at centromeres, key chromosomal regions for the proper chromosome segregation. We wish to define a possible framework for an understanding of both the stability and reversibility of epigenetic marks and their dynamics at centromeres.

References

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S1.3.2
Genetic determinants of gene repression

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Chromatin and DNA modifications have emerged as a critical component for gene regulation in higher eukaryotes yet how these epigenetic variables are targeted to specific sites of the genome is still poorly understood.

We have generated global maps of DNA methylation, histone modifications and replication in higher eukaryotes using stem cell

differentiation as a dynamic cellular model for pluripotency, lineage commitment and terminal differentiation.
This comprehensive analysis allowed us to identify genomic sites that change their epigenetic status cell-state specific. Based on the resulting datasets we generate models how these epigenetic variables are targeted, which we test by genetic perturbation of involved modifiers and mutation of putative recruiting elements.
Our results suggest that DNA sequence of regulatory regions is the main determinant of dynamic chromatin states, a finding which will be discussed in the light of current models of the function of epigenetic restriction during development.

S1.3.3
Epigenetic reprogramming during tissue regeneration

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Mechanisms of transcriptional memory ensure that during proliferation cellular programs are faithfully transmitted to daughter cells. The chromatin proteins of the Polycomb (PcG) and Trithorax group (TrxG) play a major role in the epigenetic inheritance of gene expression patterns, by establishing repressed and active chromatin domains, respectively.
We combine tools of bioinformatics with chromatin analyses and deep sequencing to identify on a genome-wide scale epigenetic marks established by the PcG/TrxG system. We find that PcG/TrxG proteins have a preference for stalled promoter regions of annotated genes. In addition, we uncover many intergenic PcG binding sites coinciding with non-annotated transcription start sites.
Tissue regeneration induces considerable remodeling of gene expression patterns in the cells required to restructure the lost parts. By analyzing regeneration of imaginal discs in Drosophila we identified signaling cascades and epigenetic reprogramming events required for tissue repair. We observe that regeneration induces down-regulation of the PcG by the JNK signaling pathway. We established a continuous GFP-labeling system for tracing blastema cells in regenerating imaginal discs of Drosophila larvae. This technique enabled us to specifically isolate regenerating cells and subject them to expression profiling. We observed that ligands for several signaling cascades, Upd/JAK-STAT signaling, dpp/TGF-beta signaling, are up-regulated in a JNK-dependent manner. Repression of PcG silencing results in a spatially and temporally distinct reactivation of a diverse set of signaling cascades and developmental regulators, thereby, enabling cellular reprogramming at the site of tissue injury.

S1.3.4
Pluripotent stem cells and epigenetic reprogramming

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Reprogramming differentiated cells towards pluripotency can be achieved by at least three different routes – the forced expression of selected inducing factors (IPS), the transfer of differentiated nuclei into enucleated oocytes (nuclear transfer), and the fusion to pluripotent cells (to generate heterokaryons and hybrids). We have used epigenetic profiling of mutant ES cell lines in combination with experimental heterokaryon formation to investigate the chromatin events that are required to successfully reprogram differentiated cells towards pluripotency. We show that ES cells that lack Polycomb Repressor Complex (PRC) 1 or PRC2 activity fail to reprogram, although reprogramming is enhanced with cells lacking Jarid2, a recently described PRC2 subunit. Using elutriation to enrich ES cells at distinct stages of the cell cycle, we provide evidence that successful reprogramming can be enhanced or diminished depending on the availability of specific DNA binding and chromatin remodelling factors. These results may be important for optimising the conversion of uni-potent cells, such as lymphocytes, into multi-potent stem cells.

S1.3.5
Evidence for a dynamic role of the histone variant H2A.Z in epigenetic regulation of normal/carcinoma switch

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See Abstract P01.35.

S1.3.6
PcG complexes set the stage for inheritance of epigenetic gene silencing in early S phase before replication

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See Abstract P01.22.

S2 – Complexity in RNA biology

S2.1 Non-coding RNA: evolution, function

S2.1.1
Regulation of microRNA repression and microRNA turnover in mammalian cells

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MiRNAs regulate gene expression post-transcriptionally by causing translational repression, and mRNA deadenylation and degradation. miRNAs function as components of miRNPs, which are responsible for silencing of mRNA targets, but mechanistic details of how miRNPs repress protein synthesis are poorly understood. Proteins of the GW182 family represent effectors of the repression and deletion analysis of human and Drosophila GW182s identified regions responsable for the repression.
The miRNA-mediated repression is a reversible process in mammalian cells. In response to cellular stress, repression of CAT-1 mRNA by miR-122 in hepatoma Huh7 cells is largely alleviated. The effect requires binding to the mRNA 3′UTR of the HuR protein, which translocates from the nucleus to the cytoplasm upon stress. To better understand the mechanism of HuR action, we uncoupled the derepression from stress by using either HuR mutants or tumor cells which accumulate endogenous HuR in the cytoplasm. We will discuss *in vitro* experiments performed with recombinant miRNPs and HuR which allowed us to gain insight to the mechanism of HuR effect on miRNA repression
We are also investigating function and turnover of selected miRNAs in retinal and non-retinal rodent neurons. In collaboration with Botond Roska of the FMI, we found that levels of the sensory neuron-specific miR-182/183/96 cluster, and miR-204 and miR-211, are down-regulated in mouse retina during dark adaptation and up-regulated in light, with rapid miRNA decay and increased transcription being responsible for the respective changes. MiRNAs in non-retinal neurons also turn over much faster than in non-neuronal cells and miRNA turnover in neuronal cells is a subject of complex activity-dependent regulation. We will discuss factors potentially involved in regulated expression of the miR-183/96/182 cluster in retina and a potential role of the accelerated miRNA decay in neurons.

S2.1.2
Non-coding RNAs in the control of flowering time

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Due to its importance in determining reproductive success the timing of the transition to flowering in plants is tightly regulated. A central component controlling the timing of flowering is FLC, a gene encoding a MADS transcriptional repressor. We have been studying two pathways that independently repress FLC expression, both of which involve FLC antisense transcripts and chromatin regulation.
One of these pathways is vernalization, the acceleration of flowering through repression of FLC by prolonged cold. Central to the vernalization mechanism is a modified Polycomb Response Complex 2 associated with three different PHD proteins. An early step in the process is up-regulation of antisense transcripts to FLC, which appear to be involved in the initial transcriptional silencing. This is followed by a cold-induced accumulation of a PHD-PRC2 complex at one site and a progressive increase in H3K27me3 at that site. Once plants are moved back to warm the PHD-PRC2 complex spreads across the whole gene leading to very high H3K27me3 levels blanketing the locus. We are continuing to investigate the role of the antisense RNAs in the Polycomb mechanism and the link between initial cold silencing and accumulation of the epigenetic memory.
The second pathway regulates FLC developmentally and has been termed the autonomous floral pathway. This mechanism involves both alternative 3′ processing and splicing of the FLC antisense transcripts. This alternative processing triggers histone demethylation of the FLC locus through an Arabidopsis homologue of the mammalian LSD1 protein and results in transcriptional down-regulation of the gene. The talk will describe our latest understanding of these conserved mechanisms and how they intersect to give robust and quantitative regulation of this important developmental repressor.

S2.1.3
Functional analysis of Tdrd1 and Tdrd6 in the zebrafish Piwi pathway

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Piwi proteins function in a germ cell-specific RNAi pathway in animals, in which so-called Piwi-associated RNAs, or piRNAs guide them to their targets. Biogenesis of these piRNAs is poorly understood. Piwi mediated target cleavage has been implicated in this process, but no piRNA biogenesis intermediates or piRNA target cleavage products have been described. Besides Piwi proteins, many Tudor domain containing proteins have been implicated in the Piwi pathway. However, the biochemical functions of these proteins within the Piwi pathway is unknown. We have studied the Tdrd1 and Tdrd6 proteins in the zebrafish.
Tdrd6 binds rather specifically to Ziwi. Mutant analysis has not revealed strong fertility phenotypes thus far, but we have indications that Tdrd6 may be required for the proper subcellular localisation of Ziwi during oogenesis and early embryogenesis. More specifically, we find Tdrd6 localised to a conserved, oocyte specific structure called the balbiany body, and mass spectrometry analysis has that Tdrd6 interacts with many factors involved in RNA metabolism, including the core of the exon-junction complex.
In contrast to Tdrd6, Tdrd1 binds both Ziwi and Zili. Analysis of Tdrd1-bound piRNAs indicates that both Piwi proteins are bound in a roughly 1:1 ratio. Associated with Tdrd1 we find long RNA molecules that carry signatures of being piRNA targets. Using peptide-pulldown experiments we find that Tdrd1 can bind more than one Piwi protein at the same time and that these Piwi proteins bind relatively few piRNAs compared to Piwi protein isolated by straight immuno-precipitation, suggesting that some of the Piwi proteins bound by Tdrd1 are unloaded. In absence of

Tdrd1 the Piwi pathway is still active, but at a significantly lower level. Together, our results scetch a picture in which Tdrd1 binds loaded and unloaded Piwi proteins in the presence of piRNA targets and that these interactions facilitate the intermolecular interactions during the ping-pong cycle in the zebrafish.

S2.1.4
Functional role of ribosomal RNA methylation

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Translation is a key step in gene expression. Ribosome is not only responsible for synthesis of proteins, but also for correct function of all translation-related mechanisms of gene expression control in bacteria. We demonstrated that methylated nucleosides m2G966 and m5C967 of *Escherichia coli* 16S rRNA are necessary for function of several mechanisms for gene expression control. Experiments *in vivo* and *in vitro* demonstrated that loss of m2G966/m5C967 modification lowers efficiency of translation initiation, especially on AUU codon. This leads to disfunction of IF3 biosynthesis. Moreover, small decrease in the speed of translation initiation with the ribosomes lacking m2G966/m5C967 modification in the 16S rRNA leads to disruption of attenuation mechanism based on the correlation between the speed of RNA polymerase synthesis of mRNA and the speed of the translation of leader peptide. The data allow us to postulate the function of m2G966/m5C967 methylation to regulate at least two essential translation- related pathways of gene expression in bacteria.

S2.1.5
Role of microRNAs in duchenne muscular dystrophy and in muscle differentiation

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See Abstract YSF.14.

S2.1.6
The melanoma-upregulated long noncoding RNA SPRY4-IN1 modulates apoptosis and invasion

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See Abstract P02.14.

S2.2 Small RNA in disease

S2.2.1.
Selective inhibition of miRNA accessibility is required for p53 tumor suppressive activity

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Micrornas (miRNAs) interact with 3′-Untranslated Regions (3′UTRs) of messenger RNAs (mRNAs) to control the expression of a large proportion of the protein coding genome during normal development and cancer. RNA-binding proteins (RBPs) have been shown to control the biogenesis, stability, and activity of many different miRNAs. Functional impairment of the p53 pathway is instrumental for tumor progression. While the p53 pathway isinactivated in most, if not all, cancers, the p53 gene is generally mutated in about 50% of tumors. However, certain tumors, such as breast and prostate, show as low as 20–30% frequency of mutations in p53. In those tumors, other alterations in the p53 pathway occur that weaken p53 tumor suppressive activity. The results I will present demonstrate a novel layer of gene regulation by p53, which is required for its tumor suppressive function and involves the induction of an RBP to control miRNAs.

S2.2.2
Aptamer and dendrimer mediated delivery of therapeutics small RNAs

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A goal of our research is the application of small RNA based therapeutics for the treatment of HIV-1 infection. We demonstrate a novel dual inhibitory function anti-gp120 aptamer-siRNA delivery system for HIV-1 therapy, in which both the aptamer and the siRNA portions have potent anti-HIV activities. The envelope glycoprotein is expressed on the surface of HIV-1 infected cells, allowing binding and internalization of the aptamer-siRNA chimeric molecules. The Dicer-substrate siRNA delivered by the aptamers is functionally processed by Dicer, resulting in specific inhibition of HIV-1 replication and infectivity in cultured CEM T-cells and primary blood mononuclear cells.

A second approach uses a PAMAM G5 dendrimer for non targeted delivery of Dicer substrate small interfering RNAs in human $CD4^+$ T-lymphocytes. Our results show efficient nanoparticle formation of G5 dendrimers with our siRNAs, effective delivery to the target cells and the release of siRNAs that are processed by Dicer into functional 21-22mer siRNAs which are incorporated into the RNA induced silencing complex (RISC) and guide sequence specific degradation of the target transcripts.

The stringent tests for both the aptamer-siRNA and dendrimer-siRNA delivery systems was to test the effectiveness of these combination therapies in a humanized SCID mouse model that is reconstituted with human hematopoietic cells that are fully capable of infection by HIV. A group of humanized mice were treated with virus until they became viremic. Subsequently the animals were treated with either the aptamer-siRNA or the dendrimer-siRNA combinations by giving three to five weekly tail vein injections. We show that the *in vivo* applications of both the aptamer-siRNA and the dendrimer siRNAs resulted in three to six logs of inhibition of viral replication, siRNA mediated down regulation of the targeted mRNAs and protection of T-lymphocytes from HIV mediated depletion. These results represent the first

such small RNA applications for the successful treatment of HIV-1 infection, and either approach could potentially be used in HIV-1 eradication strategies.
We have extended our aptamer mediated delivery to B-cell lymphomas by developing an aptamer that selectively targets the BAFFR1 receptor on B-cells. The aptamer blocks Baff ligand mediated stimulation of cell proliferation and has no significant signaling effects as monitored by micro array analyses. Importantly, we have demonstrated that this aptamer can also internalize and deliver a dicer substrate to cells, which is effectively processed and enters RISC. This new strategy for treatment of lymphomas will be discussed as well.

S2.2.3
Oligonucleotide therapeutics for correcting defective rna splicing

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Spinal Muscular Atrophy (SMA) is a genetic disease characterized by progressive degeneration of motor neurons in the spinal cord, leading to muscle weakness and atrophy. SMA is caused by deletion or mutations in the Survival-of-motor neuron (SMN1) gene. The paralogous SMN2 gene, present in one or more copies in all SMA patients, attenuates SMA severity, but expresses low levels of full-length SMN protein, due to alternative splicing that results in inefficient inclusion of exon 7. Increasing SMN2 exon 7 inclusion to express more full-length, functional SMN protein in motor neurons is a promising approach to treat SMA.
Previously, we identified an optimal 2′-O-(2-methoxyethyl) (MOE) phosphorothioate 18mer antisense oligonucleotide (ASO) that targets a splicing-repressor binding site in intron 7. By preventing binding of the repressor (hnRNP A1), the ASO promotes efficient SMN2 exon 7 inclusion in liver and kidneys of transgenic mice after systemic administration.
Because ASOs do not cross the blood-brain barrier, we explored direct delivery to the mouse central nervous system to target motor neurons. Using a micro-osmotic pump, the ASO was delivered into cerebrospinal fluid through a lateral ventricle in adult Smn-null mice with four copies of a human SMN2 transgene, which have mild SMA. Intracerebroventricular (ICV) infusion of the ASO increased exon 7 inclusion in spinal cord to ~90%, compared to ~10% in control mice. This led to a robust and long-lasting increase of the transgenic SMN protein levels in spinal-cord motor neurons.
We have also used ICV bolus injection in embryonic, neonate, or adult mild or severe SMA mouse models to optimize the effectiveness of the ASO, characterize phenotypic improvement, and establish a time window for effective treatment. In addition, studies in non-human primates support IT bolus injection as a feasible route of delivery. Thus, this ASO is a promising drug candidate for SMA therapy.

S2.2.4
Co-transcriptional RNA checkpoints

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In eukaryotes, the production of mature messenger RNA that exits the nucleus to be translated into protein in the cytoplasm requires precise and extensive modification of the nascent transcript. Any failure that compromises the integrity of an mRNA may cause its retention in the nucleus and trigger its degradation. Multiple studies indicate that mRNAs with processing defects accumulate in nuclear foci or "dots" located near the site of transcription, but how exactly are defective RNAs recognized and tethered is still unknown. Using a combination of live-cell imaging and chromatin immunoprecipitation experiments, our recent results provide novel insight for coordination between splicing, dynamics of RNAPII and chromatin remodeling.

S2.2.5
Characterization of new small RNA populations in mouse embryonic stem cells

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See Abstract YSF.16.

S2.2.6
Sequence variants within the 3′-UTR of the COL5A1 gene alters mRNA stability: implications for musculoskeletal soft tissue injuries

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See Abstract P02.12.

S3 – Following the life of a protein

S3.1 Protein synthesis, traffic and turnover

S3.1.1
The ubiquitin proteolytic system – from basic mechanisms thru human diseases and onto drug development

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Between the 50s and 80s, most studies in biomedicine focused on the central dogma – the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins do turn over, the high specificity of the process – where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared – was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting.

S3.1.2
Structural diversity among cytoplasmic and organellar aaRSs may lead to incorporation of free-radical damaged amino acids into proteins

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The accumulation of proteins damaged by reactive oxygen species (ROS), having pathological potentials, is associated with age-related diseases such as Alzheimer's, atherosclerosis, and cataractogenesis. Exposure of the aromatic amino acid (aa) phenylalanine to ROS-generating systems produces multiple isomers of tyrosine: m-tyrosine (m-Tyr), o-tyrosine (o-Tyr), Levodopa, standard p-tyrosine (Tyr) etc. Previously it was established that exogenously supplied, oxidized aa could be incorporated into bacterial and eukaryotic proteins. It is, therefore, likely that in many cases, *in vivo*-damaged aa are available for de novo synthesis of proteins. Although the involvement of aminoacyl-tRNA synthetases (aaRSs) in this process has been hypothesized, the specific pathway by which ROS-damaged aa are incorporated into proteins remains unclear. The reason is that proofreading activity has been evolved by certain aaRSs to keep the fidelity of genetic code translation and to discriminate cognate amino acids from non-cognate ones. However, it turned out aaRSs catalyzing the same phenylalanylation reaction have considerably diverged in aa sequences, domain composition and subunit organization. Our results are indicative of differences in architecture between heterodimeric prokaryotic and eukaryotic cytosolic PheRSs and monomeric mitochondrial enzyme, that in turn leads to variation in tRNA(Phe) binding and recognition modes. As regards to proofreading activity associated with a distinct active site, where misactivated aminoacyl-adenylate or misaminoacylated tRNAP he have to be hydrolyzed, PheRSs from different compartments also vary substantially. We provide evidence that human mitochondrial and cytoplasmic PheRSs catalyze direct attachment of ROS-damaged phenylalanine (m-Tyr) and L-Dopa stably to tRNA(Phe) thereby opening up the way for delivery of the misacylated tRNA to the ribosome and incorporation of damaged amino acid into eukaryotic proteins.

S3.1.3
Quality control in the endoplasmic reticulum: removal of unwanted proteins

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Misfolded and otherwise unwanted proteins are removed from the membrane-delimited compartments in which they reside: in the case of the endoplasmic reticulum, such removal may involve extraction followed by cytoplasmic degradation. We have developed new tools with which to study this process, including the construction of dominant negative versions of ubiquitin-specific proteases and the generation of active variants of such enzymes that pre-emptively remove ubiquitin from substrates that would otherwise have been destroyed. Enzymatic interference in the ubiqutin proteasome pathway is likely to be of general applicability, and has allowed us to decipher the pathway via which misfolded proteins are extracted from the endoplasmic reticulum at unprecedented resolution. In addition to performing these experiments in tissue culture cells, we have generated mouse models in which the impact of these manipulations can be studied in a variety of primary cells.

S3.1.4
The proteasome and the ubiquitin system: the two faces of one enzyme

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Degradation of oxidant-damaged proteins or short-lived regulatory proteins requires the activity of the UPS. Similarly, antigenic peptides bound by MHC I molecules to be recognized by CTLs at the cell surface are generated in a proteasome dependent manner. The immunoproteasome (i-proteasome) is a specific proteasome isoform induced by IFNs. Its proteolytic function has been almost exclusively connected with the adaptive immune response and improved MHC class I antigen presentation.
Inflammation and IFN signaling represent a potent contribution to innate responses against pathogens. In infected tissues signalling cascades of the innate response rapidly induce the release of proinflammatory cytokines, thereby also triggering the production of

radicals in lymphocytes and target cells. These radicals affect infected cells and proteins derived from pathogens, but also proteins of non-infected cells also exposed to cytokines. Thus, in non-infected cytokine exposed cells i-proteasomes preserve cell viability by efficiently degrading DRiPs and preventing the accumulation of ALIS. On the other hand, infected cells must rapidly signal their infectious state to the adaptive immune system by presenting epitopes on MHC class I molecules at the cell surface, which is strongly improved by i-proteasome function. These each other not excluding functions locate the i-proteasome at the crossroad of the innate and the adaptive immune response. As part of the innate response response associated with oxidative stress i-proteasomes possess a more general protective role by maintaining cellular protein homeostasis.As part of the adaptive immune response i-proteasomes process nascent-oxidant damaged proteins from pathogenic sources thereby increasing the peptide supply for MHC class I antigen presentation.Here we discuss how immunoproteasomes protect cells against accumulation of toxic protein-aggregates and how i-proteasomes dysfunction associates with different diseases.

S3.1.5
Methionine oxidation induces amyloid fibril formation by apolipoprotein A-I

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See Abstract P03.59.

S3.1.6
The folding problem simplified: protein families, circular permutants and heteromorphic pairs

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See Abstract P03.54.

S3.2 Protein folding and binding

S3.2.1
Single-molecule FRET and transition paths in protein folding

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Both theory and simulations predict that protein folding is an extremely heterogeneous process with many microscopic pathways connecting the folded and unfolded states. All of the mechanistic information on protein folding and unfolding is contained in the transition path – the tiny fraction of an equilibrium molecular trajectory when the barrier separating the folded and unfolded states is actually crossed. The transition path is a uniquely single molecule property and has not yet been observed for any system. The first step toward the goal of using FRET to observe specific intramolecular distances changing during the transition path is to measure the transition path time. A photon-by-photon analysis of folding and unfolding transitions in single molecule FRET experiments on a slow folding protein yields an upper bound for the transition-path time of ˜10 microseconds, close to ˜2 microseconds estimated from the molecular-dynamics simulations of D.E Shaw and coworkers for a protein with a ˜10 microseconds folding-time (Shaw et al, Science 2010). These results show that an ultrafast and a slow-folding protein can take almost the same time to fold when it actually happens!

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S3.2.2
Folding approaching the speed limit

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The homeodmain family of proteins provides a paradigm that spans a continuum of mechanism from framework to nucleation-condensation and a time regime from sub-millisecond to sub-microseconds. I will describe how studies on the engrailed and the Pit1 homeodomain resolve kinetic processes from chain diffusion events, in tens to hundreds of nanoseconds, to docking and rearrangement processes in tens of microseconds.

S3.2.3
Intrinsically disordered proteins: a role in nervous system development

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Recent studies have identified a family of neural cell single-pass transmembrane adhesion proteins, with substantial sequence similarity to cholinesterases (ChEs), i.e. cholinesterase-like adhesion molecules (CLAMs)[1,2]. CLAMs are devoid of catalytic activity, as they lack residues of the catalytic triad. They appear to play key roles in the earliest stages of the development of the CNS and mutations in them have been associated with autism[3].
The cytoplasmic domains of CLAMs bear no sequence homology to any known protein, and physicochemical studies show that they are ''Intrinsically Disordered Proteins'' (IDP)[4] when expressed in *E. coli*[1,2]. It has been estimated that many cellular proteins exist in this disordered state; e.g. for mammals, about half of their total proteins are predicted to contain long disordered regions[4]. We developed FoldIndex© (http://bioportal.weizmann.ac.il/fldbin/findex)[5], which predicts regions of a protein sequence that are likely to be disordered and have used it to examine the CLAMs family. These ''*in silico*'' studies will be compared with our recent solution studies on CLAMs and their adhesion partners, as well as our studies on the life-time of IDPs *in vivo*[6,7] FoldIndex© is also being used in the ISPC (http://www.weizmann.ac.il/ISPC) to aid in crystallization of proteins by predicting which regions of a protein sequence are likely to be disordered. Examples of IDPs will be shown in a new web tool, Proteopedia, the collaborative 3D encyclopedia of proteins & other molecules[8] (http://www.proteopedia.org).

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S3.2.4
Unusual binding modes of intrinsically disordered proteins

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A primary aim in structural genomics is to determine the structures of "all" complexes and provide a complete picture of protein function at the cellular level. In some recent publications, however, we argue that interactions of intrinsically disordered proteins (IDPs [1]) might not conform to the classical rule that would suggest the formation of complexes of well-defined structures. The concept of moonlighting [2] suggests that a protein can fulfill more than one – often opposing – functions. Although structural data are sparse, biochemical studies suggest that different functions rely on alternative complexes of the same protein. Another manifestation of the malleability of IDPs in binding is fuzziness [3], which states that part(s) of the IDP remains disordered even in the bound state. Such fuzzy parts contribute to binding, as apparent in binding constants and/or the functional readout of the interaction. In addition, fuzziness may also underline the observed sequence-independence of binding, when interaction apparently does not require a defined sequence. Whereas IDPs often rely on short binding motifs [4, 5] that undergo folding upon binding, an additional deviation from our classical views is binding elicited by disordered domains [6]. By all these points it is suggested that the recognition phenomena of IDPs in many aspects contradict the classical view of strict correspondence between interactions, structures and complexes, which sets a natural limit to the identification and structural description of all protein-protein interactions in the living cell.

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S3.2.5
New insights into the coordination of protein export by the flagellar type 3 secretion system

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See Abstract P03.15.

S3.2.6
The signal peptides and the early mature domain cooperate for efficient secretion

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See Abstract YSF.15.

S3.3 NAD-dependent Post-translational modifications

S3.3.1
Macrodomains mediate NAD metabolite-dependent nuclear dynamics

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Chromatin packages DNA into an assembly that promotes genome stability. This packaging is an obstacle to the machines that read, copy or repair DNA. A key goal of the chromatin field has been to identify mechanisms through which DNA-modifying machines recruit to and remodel chromatin. The post-translational modification and recognition of histones have emerged as key mechanisms regulating chromosome dynamics and gene activity. Interestingly, many signaling-dependent modifications of chromatin rely on metabolite co-factors (e.g. acetyl-CoA, SAM, NAD). In some cases, notably the Sir2-family of deacetylases, this puts the activity of chromatin modifiers under metabolic control, providing a link between physiology and chromatin. Further, while modules recognizing acetylated or methylated proteins, including histones, have been described, little is known of how ADP-ribosylation is deciphered. Poly-ADP-ribosylation (PARylation) is a "historic" post-translational modification with roles in transcription, chromatin and DNA repair. Yet, only recently globular modules with specificity for this modification and that transduce the PARylation signal have been identified.
We discovered the first effector module for nuclear NAD metabolites, including the Sir2 product O-acetyl-ADP-ribose (AAR), as well as as poly-ADP-ribose (PAR), the product of DNA-damage activated PARP1, the so-called macrodomain. Structure & function analysis shows that macrodomains specifically bind small-molecule NAD metabolites, such as ADP-ribose or AAR, while others show selectivity for PAR. Further, life-cell imaging assays show how macrodomains readily sense nuclear PAR formation.
We will discuss two fundamental questions in the PARP field. First, we will present preliminary results on the mechanism of DNA-break recognition by PARP1. Second, we will show macrodomain behaviour upon DNA-damage-induced PARP1 activation *in vivo*.

S3.3.2
Novel developments in protein mono-ADP-ribosylation

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Mono-ADP-ribosylation (mono-ADPR) is a reversible post-translational modification of proteins catalyzed by ADP-ribosyltransferases (ARTs). The physiological role of the mono-ADPR is now recognized in processes such as membrane traffic, immune response and signalling. While the bacterial ARTs (such as pertussis, difteria, clostridium toxins) have been known for long time, the list of eukaryotic enzymes and relative substrates is still incomplete. For example, apart from the GPI-anchored, ecto-ART family, novel members of the PARP family and some

sirtuins are among the cellular enzymes for which mono-ADPR activities have been recently reported.
Moreover, a novel enzymatic process has been delineated by us, involving the mono-ADPR of the protein CtBP1-S/BARS (BARS), a target of the traffic-disrupting toxin brefeldin A (BFA) that is involved in the fissioning of membranes at several traffic steps of the secretory and endocytic pathways. The mechanism of this posttranslational modification involves the formation of an intermediate (BFA-ADP-ribose conjugate, BAC) that covalently binds BARS and abolishes its fission-inducing activity. The enzyme involved in this reaction belongs to the ADP-ribosyl-cyclase family.
In parallel, we are also investigating the modification of other cellular substrates by cellular enzymes such as the PARPs. In *in vitro* ADP-ribosylation assays, PARP12 turned out to be one of the more active of these enzymes. The substrates of PARP12 activity have been identified by a proteomic approach. From the functional point of view, in HeLa cells, PARP12 overexpression resulted in the loss of the classical ribbon organization of the Golgi complex, which appears fragmented. Thus, one possibility is that ADP-ribosylation catalysed by PARP12 (similar to the toxic reaction catalyzed by BFA) affects the structure of the Golgi complex and, as a consequence, membrane transport.

S3.3.3
NAD^+ – a key molecule in cellular signalling

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NAD is an important redox carrier in all cells. In energy metabolism, NAD^+ is reduced to NADH by accepting two electrons from metabolic intermediates. The electrons are eventually supplied to the mitochondrial respiratory chain to support ATP synthesis, thereby regenerating NAD^+.
It is now well established that NAD^+ also serves as a key component of signaling pathways. It is used for posttranslational protein modifications and is converted into potent calcium-mobilizing messengers such as cyclic ADP-ribose and ADP-ribose. The protein modifications include mono- and poly-ADP-ribosylation and NAD^+-dependent deacetylation by sirtuins. This multitude of conversions enables a wide range of regulatory functions. Indeed, NAD^+-dependent signaling controls critical cellular activities including DNA transcription and repair, epigenetic modifications, cell division, apoptosis and the biological clock. It also regulates key metabolic enzymes and is important for energy homeostasis. Since all these reactions involve the cleavage of NAD^+, the continuous regeneration of subcellular NAD pools is vital. Indeed, the pathways and regulation of NAD biosynthesis have moved into focus as their crucial functions to supply signaling processes have become apparent.
Mammalian NAD metabolism is far more complex than anticipated. This presentation will provide an overview over the known pathways. Moreover, results will be shown identifying NAD precursors and the generation of intracellular NAD pools. For the key step of NAD synthesis, three compartment-specific isoforms have been identified. The molecular mechanisms for their subcellular localization were found to be based on isoform-specific targeting and interaction domains. These unique domains contain subcellular targeting signals and can mediate interactions with other cellular factors. The presence of these isoforms in their specific compartments has direct impact on NAD^+-dependent protein modifications and cell viability.

S3.3.4
ARTD1/PARP1 ADP-ribosylates lysine residues of the core histone tails

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The chromatin-associated enzyme ADP-ribosyltransferase diphtheria toxin-like 1 (ARTD1; formally called PARP1) has previously been suggested to ADP-ribosylate histones, but the specific ADP-ribose acceptor sites have remained enigmatic. Here, we show that ARTD1 covalently ADP-ribosylates the amino-terminal histone tails of all core histones. Using biochemical tools and novel electron transfer dissociation mass spectrometric protocols, we identify for the first time K13 of H2A, K30 of H2B, K27 and K37 of H3, as well as K16 of H4 as ADP-ribose acceptor sites. Multiple explicit water molecular dynamics simulations of the H4 tail peptide into the catalytic cleft of ARTD1 indicate that two stable intermolecular salt bridges hold the peptide in an orientation that allows K16 ADP-ribosylation. Consistent with a functional cross-talk between ADP-ribosylation and other histone tail modifications, acetylation of H4K16 inhibits ADP-ribosylation by ARTD1. To further investigate ADP-ribosylation of histones *in vivo*, we established a workflow that allows the enrichment of ADP-ribosylated histones or peptides derived from their proteolytic digests. These fractions were subsequently analyzed by mass spectrometry to map the acceptor amino acids of ADP-ribose. Taken together, our computational and experimental results provide strong evidence that ARTD1 modifies important regulatory lysines of the core histone tails.

S3.3.5
High-resolution crystal structure of periplasmic Haemophilus influenzae NAD nucleotidase, lead to reveal a novel enzymatic function of human CD73

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See Abstract P03.53.

S3.3.6
Nicotinamide blocks proliferation and induces apoptosis of chronic lymphocytic leukemia cells through activation of the p53/miR-34a/SIRT1 tumor suppressor network

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See Abstract P11.145.

S4 – Cell-cell communication

S4.1 Intercellular trafficking of signal molecules

S4.1.1
Abscisic acid and cyclic ADP-ribose are first and second messenger in inflammatory cells, hemopoietic progenitors and pancreatic beta-cells

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Abscisic acid (ABA) is a hormone involved in pivotal physiological functions in higher plants, such as response to abiotic stress and control of seed germination. We recently identified ABA as a new hormone in humans and rodents and discovered its receptor and its second messenger.
Pro-inflammatory stimuli induce ABA production from human granulocytes and monocytes and nM ABA stimulates activities of granulocytes, monocytes and vascular smooth muscle cells involved in inflammation and atherogenesis.
ABA also stimulates the proliferation of human mesenchymal stem cells (MSC) and of uncommitted hemopoietic progenitors. On MSC, ABA stimulates several functional activities, including production of PGE2, release of several cytokines mediating the trophic and immunomodulatory properties of MSC and chemokinesis. ABA is produced and released by MSC stimulated by specific growth factors, by inflammatory cytokines and by lymphocyte-conditioned medium. Lymphocyte-stimulated MSC produce ABA at concentrations exerting growth-stimulatory effects on co-cultured $CD34^+$ cells. On $CD34^+$ cells, microM ABA induces transcriptional effects.
ABA is also produced and released by human and murine pancreatic beta cells in response to glucose and nM ABA stimulates glucose-dependent and -independent insulin release. The ABA concentration in human plasma increases in healthy subjects after glucose administration.
Thus, it appears that ABA is uniquely endowed with the capacity to affect activation of inflammatory cells and energy metabolism via insulin secretion.
These results provide a remarkable example of conservation of a stress-hormone and of its second messenger from plants to humans and open the way to a more in-depth investigation into the role of ABA and possibly of other plant hormones as well in human physiology and disease.

S4.1.2
New tools for activating and blocking the P2X7 ion channel – a key sensor of NAD and ATP released from cells

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P2X7, an ion channel gated by ATP and NAD released from injured cells, plays a key role in activation of the inflammasome. Extracellular ATP gates P2X7 directly by acting as a soluble ligand, extracellular NAD gates P2X7 indirectly via NAD-dependent ADP-ribosylation of P2X7 at R125 by the toxin-related ecto-ADP-ribosyltransferase ART2.2. Tools to activate or block P2X7 are being sought for therapeutic applications in inflammation-mediated clinical conditions. We hypothesized that single domain antibodies (nanobodies) derived from llama heavy chain antibodies might be particularly suited for this purpose, since these antibodies display a strong propensity to bind to functional crevices on proteins.
Llamas were immunized with a P2X7 cDNA expression vector or with stably transfected HEK cells expressing the cell surface receptor. After the last boost immunization, RNA and cDNA were prepared from blood and lymph node biopsies. The antibody repertoire was cloned into a phage display library and P2X7-specific nanobodies were selected by panning of phages on transfected cells. Recombinant nanobodies were tested for their capacity to block or enhance of P2X7-dependent shedding of CD62L and externalization of phosphatidyl serine in response to exogenous NAD or ATP. Two nanobodies blocked P2X7 activation with IC50 values of 5–50 nM. A distinct nanobody lowered the threshold ligand concentrations required for P2X7 activation. Recloning of these anti-P2X7 nanobodies into dimeric formats resulted in enhanced inhibitory and activating propensities. Within 30 minutes after intravenous injection, the nanobodies inhibited or activated the P2X7 on lymphocytes in blood, lymph node, spleen and liver.
Our findings provide a proof of principle that nanobodies can be used to block or activate an ion channel. These nanobodies provide new tools for specifically modulating P2X7 function *in vitro* and *in vivo* and pave the way for testing the therapeutic potential of P2X7-specific nanobodies in mouse models of inflammatory diseases.

S4.1.3
How sperm find the egg

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Fertilization requires diffusible chemical factors (chemoattractants) released from the egg's envelope to attract sperm. External fertilization in marine animals needs efficient search strategies to enhance sperm-egg encounter, as gamete dilution is enormous upon spawning. Speract, an egg-derived decapeptide, induces non-chemotactic motility responses in Strongylocentrotus purpuratus spermatozoa, while it is chemotactic in *Lytechinus pictus* sperm. Gradients of this decapeptide trigger a sequence of turning episodes that correlate with transient flagellar Ca^{2+} increases followed by periods of straighter swimming in both sperm species; yet only *L. pictus* spermatozoa swim towards the gradient source, as they can selectively undergo Ca^{2+} fluctuations while swimming along descending speract gradients. Contrary to this, *S. purpuratus* spermatozoa generate Ca^{2+} fluctuations in a spatially non-selective manner.
We derived a signaling network model from experimental results where nodes are discrete variables corresponding to the pathway elements and signal transmission takes place at discrete time intervals according to logical rules. This model, corroborated previous empirically determined responses and predicted the involvement of a high voltage activated Ca^{2+} channel as a regulator of

the delay in the onset of oscillations after activation of the signaling cascade, as well as the influence of a voltage-dependent Ca^{2+}-activated K^+ channel on the period of the $[Ca^{2+}]i$ fluctuations. These predictions were tested pharmacologically and proved to be consistent with the experimental results. Tuning of Ca^{2+} fluctuations and associated turning and straighter swimming episodes to the chemoattractant gradient shape is a central feature of sea urchin sperm chemotaxis, and may be a feature of sperm chemotaxis in general.

S4.1.4
The pathophysiological importance of NAMPT-mediated NAD biosynthesis in the regulation of metabolism and aging in mammals

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Nicotinamide phosphoribosyltransferase (NAMPT)-mediated NAD biosynthesis and the NAD-dependent deacetylase SIRT1 comprise a systemic regulatory network that maintains the robustness of our physiological system in response to a variety of nutritional and environmental stimuli, which we have termed the "NAD World" (Imai, BBA, 1804: 1584–90, 2010). NAMPT-mediated NAD biosynthesis functions as a pace maker that regulates circadian oscillatory NAD production and fine-tunes SIRT1 activity, whereas SIRT1 functions as a key downstream mediator that orchestrates metabolic responses to alterations in nutrient availability in multiple tissues. Indeed, we have previously demonstrated that NAMPT-mediated NAD biosynthesis drives a novel circadian clock feedback cycle through SIRT1 and CLOCK:BMAL1 (Ramsey, Yoshino, Brace, et al. Science, 324:651–4, 2009). Interestingly, NAMPT has intra- and extracellular forms (iNAMPT and eNAMPT, respectively), and eNAMPT is actively secreted from matured adipocytes. Significant amounts of eNAMPT exist in mouse and human blood circulation, and it has been proposed that eNAMPT contributes to extracellular biosynthesis of NMN that is distributed to all tissues and organs to promote NAD biosynthesis at a systemic level. We have recently found that deacetylation of NAMPT controls its secretion from adipocytes and that SIRT1 physically interacts with and deacetylates iNAMPT, resulting in the enhancement of eNAMPT secretion. These findings indicate another novel feedback loop regulating systemic NAD biosynthesis through SIRT1 and NAMPT. Administration of nicotinamide mononucleotide (NMN), a product of the NAMPT enzymatic reaction, dramatically ameliorates defects in NAD biosynthesis and improves glucose tolerance, hepatic insulin sensitivity, and lipid profiles in diet- and age-induced diabetic mice. Furthermore, our microarray analysis shows that expression profiles of genes involved in oxidative stress, inflammation, and circadian rhythm are significantly improved in NMN-treated diabetic livers, at least in part, through the activation of SIRT1. These findings provide important insight into the system dynamics of the NAD World and therapeutic and preventive interventions for age-associated metabolic complications.

S4.1.5
Cell-to-cell crosstalk between mesenchymal multipotent stromal cells and renal tubular cells in co-culture

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See Abstract P04.8.

S4.2 Regulation of cell functions by intercellular contact systems

S4.2.1
How integrins control breast development and function

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Cellular interactions with the extracellular matrix control the shape, migration, proliferation and differentiation of metazoan cells. Signals from the extracellular matrix are transmitted through integrins, which connect to cytoskeletal components, and to adaptors within adhesion complexes. Our laboratory has previously used a genetic approach to show that β1-integrins determine the differentiated function of mammary glands *in vivo*. We have now discovered that β1-integrins also control the orientation of epithelial polarity and thereby the formation of lumens in secretory alveoli. Once luminal mammary epithelial cells have made contact with the basement membrane, β1-integrins establish polarity and maintain it. The intracellular mechanism by which integrins control polarity is via endocytic internalization of apical components away from the basal surface, to create an opposing apical domain. This process involves recruitment of a specific adhesion complex component, integrin-linked kinase, and organisation of microtubules.

S4.2.2
Neural adhesion molecules as novel players in cancer progression

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Besides their established function in cell-cell and cell-matrix interactions, cell adhesion molecules (CAMs) play an important role in converting cues coming from the extracellular environment into intracellular signals that regulate fundamental aspects of cell physiology. We are interested in the functional properties of neural CAMs, a subset of immunoglobulin CAMs (Ig-CAMs) that have been initially characterized as important players in the central nervous system. However, they are also expressed in non-neural tissues where their function remains largely elusive. A prototypical example is provided by neural cell adhesion molecule (NCAM), which we have implicated in the pathophysiology of several non-neural cell types. In particular, NCAM forms a complex with fibroblast growth factor receptor (FGFR) on the surface of cancer cells. Such interaction results in an NCAM-induced, FGFR-mediated cellular response that is remarkably divergent from that elicited by FGF, the canonical ligand for FGFR.

NCAM expression is frequently aberrant in different tumor types, and our data provide clear evidence that NCAM enhances

the malignant phenotype of cancer cells, both *in vitro* and *in vivo*, and this effect requires the interaction with FGFR. Furthermore, interfering with the NCAM/FGFR interplay emerged as a suitable strategy to inhibit tumor progression and dissemination.
Our results, together with data from other groups, point to a novel signaling paradigm whereby neural adhesion CAMs can act as non-canonical ligands for receptor tyrosine kinases. This, on one hand, expands dramatically the spectrum of signaling pathways controlled by neural CAMs and, on the other hand, implies that an aberrant expression of these Ig-CAMs in cancer can lead to deregulated receptor tyrosine kinase signaling, thus opening new perspectives in the context of anti-cancer molecular therapies.

S4.2.3
Wnt/beta-catenin signaling in stem and cancer stem cells

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Abstract not received. Please see program and Late Abstract Addendum.

S4.2.4
Structural studies of connexin-26 gap junction channel

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Gap junctions consist of arrays of intercellular channels. Gap junction channels regulate the passage of ions and biological signaling molecules between adjacent cells and, therefore, are critically important in many biological activities.
The X-ray structure of human connexin-26 (Cx-26) gap junction channel has revealed structural details in its open state. The gap junction channel is formed by paired hemichannels of two adjacent cells, each of which consists of six protomers. The monomer folds in four transmembrane helices (TM1-TM4), two extracellular loops (E1, E2) and an N-terminal helix (NTH). A cytoplasmic loop (CL) between TM2 and TM3, and a C-terminal tail (CT) were not modeled yet. The NTH region folds in a short helix and is inserted into the lumen to form a funnel structure. The structure of amino-terminal region could explain gating mechanism of the channel.
The intracellular channel entrance is comprised of the cytoplasmic parts of TM2 and TM3. Positive charge residues are concentrated in this region and surround the entrance. The positively charged region around the entrance would be favorable to negatively charged permeate molecules that are accumulated at the entrance before the molecules enter the pore.
Both E1 and E2 domains contribute inter-hemichannel interactions and adhesive properties of Cx26 gap junction channel. The E1 and E2 loops of one hemichannel interact with the respective E1 and E2 loops of the other hemichannel. The interactions between two hemichannels make the gap junction channel to connect tightly two adjacent cells. Amino acid sequences of E1 loop are conservative among 21 Cxs, while those of E2 are variable. Amino acid residues in E2 that interacting with the other E2 loop through hydrogen bonds are critical residues to form both homotypic and heterotypic gap junction channels.

S5 – Membrane dynamics

S5.1 Membrane dynamics

S5.1.1
Control systems of the secretory pathway

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The secretory apparatus transports proteins and lipids from the endoplasmic reticulum (ER) to various cellular destinations. It controls the size and composition of most cellular membranes, as well as the secretion of thousands of cargo species; and it relies on an underlying molecular machinery of over 2000 proteins, most of which has been elucidated. In contrast, the question of how this apparatus coordinates its many compartments and maintains its homeostasis has been largely neglected. Here we analyze the cell-autonomous control systems that oversee intracellular membrane transport. Notably, sophisticated control systems have been described in other key areas of cell biology. For instance, the cell cycle and the unfolded protein response (UPR) are regulated by complex control mechanisms. For the secretory pathway, we have reported that when a pulse of traffic reaches the Golgi complex from the ER, it carries a signal (the ER chaperones), which is sensed at the Golgi by a molecular detector, the KDEL receptor. This receptor then activates a signalling cascade that includes the Src family kinases. These, in turn, activate anterograde transport through the Golgi, allowing the Golgi to complete the transport process and to maintain homeostasis. We have now extended the above findings to show that the activated KDELR binds and activates two G proteins, Gq and Gs, and that the structure of the KDELR is similar that of a G-protein-coupled receptor. Gq and Gs then activate distinct signalling pathways, which regulate, in turn, both anterograde and retrograde trafficking. In addition, this signalling can impinge upon and regulate other cell functions, such as cell motility and energy metabolism. We view this as a control system whose significance is two-fold: a) traffic is processed by shipping the incoming cargo proteins forward; and b) the system is activated when needed (rather than being constitutive).

S5.1.2
Protein sorting and packing along the secretory pathway

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A genome wide screen revealed a number of new components required for protein secretion (Bard et al., Nature 2006). One of these components called TANGO for Transport ANd Golgi Oragnization is anchored at the ER exit site and required for collagen VII export in mammalian cells (Saito et al., Cell. 2009). Solloway and colleagues (USA) have found that show that mice lacking are defective for the secretion of numerous collagens, from chondrocytes, fibroblasts, endothelial cells and mural cells. Collagen deposition by these cell types was abnormal, and extracellular matrix composition was compromised. Chondrocyte maturation and bone mineralization are severely compromised in TANGO1 null embryos, leading to dwarfism and neonatal lethality. This provides the proof of the involvement of TANGO1 in collagen secretion *in vivo*. We have identified a new protein, which, like TANGO1, contains two large coiled-coiled domains and a proline rich domain anchored to the cytoplasmic face of the ER exit site. This new protein TALI for TANGO 1 Like however, lacks the lumenal coiled-coiled and SH3 like domain of TANGO1. TALI does not bind collagen VII directly but dimerizes with the coiled-coiled domain of TANGO1. Knockdown of TALI by SiRNA in Hela cells did not inhibit general secretion but the bulky collagen VII was arrested in the ER. The TANGO1-TALI dimer, we suggest, assembles in a complex, which is necessary for the formation of mega vesicles at the ER exit site to permit export of bulky cargo such as collagen VII.

Another TANGO encodes the actin severing protein called Twinstar/Cofilin. Our data reveals the role of cofilin in cargo sorting at the TGN (von Blume et al., JCB. 2009; Dev.Cell. 2011).

I will describe the function of these TANGO's in protein sorting and cargo packing during protein secretion.

Introduction

How does a cell regulate the dimension of transport carriers depending on the size of cargo? Does transport of bulky cargoes such as the collagens require special c

S5.1.3
Systems analysis of endocytosis and signalling

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Endocytosis is an essential process serving multiple key cellular functions, such as nutrient uptake, signal transduction, and defence against pathogens. We have undertaken a broad systems biology analysis of endocytosis. We systematically profiled the activity of human genes with respect to Transferrin and EGF endocytosis by performing an image-based RNAi screening of HeLa cells in cooperation with the HT-TDS, the screening facility of the MPI-CBG. The genes were identified on the basis of a multi-parametric analysis quantitatively measuring uptake and intracellular cargo distribution. We uncovered novel regulators of endocytosis and endosome trafficking, including many signalling pathways (e.g. Wnt, Integrin, TGF-β, and Notch). A systems analysis by Bayesian networks further uncovered design principles regulating the number, size, concentration of cargo and intracellular position of endosomes. Further studies revealed novel principles whereby the endocytic pathway governs the sorting and signalling properties of receptor tyrosine kinases. These results have profound implications for our understanding of the mechanisms regulating organelle biogenesis and signalling at the cellular, tissue and organism level.

S5.1.4
Clathrin adaptors and polarized trafficking in epithelia

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Epithelial cells sort apical and basolateral plasma membrane (PM) proteins at the Trans Golgi Network (TGN) and Common Recycling Endosomes (CRE), located very close to each other in the perinuclear region. Apical routes are regulated by rab 11, microtubule motors (dynein and kinesins), actin, myosin 1 and dynamin 2. In contrast, basolateral routes require clathrin, protein kinase D, BARS, rab 6 and myosins 2 and 6. Clathrin and the epithelial-spe-

cific clathrin adaptor AP1B sort basolateral PM proteins at CRE (Deborde et al., Nature, 2008; Folsch et al., Cell 1999; Gan et al., Nature Cell Biol. 2002; Gravotta et al., PNAS, 2007; Cancino et al., Mol. Biol of the Cell 2007). Recent experiments, using single and double knock-down of AP1A and/or AP1B in MDCK cells, show that the ubiquitous clathrin adaptor AP1A complements AP1B in basolateral protein sorting. B-KD but not A-KD disrupted the steady-state localization of basolateral markers low density lipoprotein receptor (LDLR) and transferrin receptor (TfR). Biochemical assays demonstrated that AB-KD, but not A-KD or B-KD, disrupted polarized biosynthetic delivery of these proteins and that B-KD but not A-KD disrupted polarized recycling of TfR. CRE ablation experiments with Tf-HRP showed that A-KD but not B-KD rerouted LDLR and TfR from TGN to CRE. Yeast two-hybrid analysis demonstrated direct interactions between the medium subunits of both AP1A and AP1B with the basolateral sorting signal of TfR but not with those of LDLR or VSV G protein. Interestingly, TfR, missorted to the apical surface in the absence of AP1B, trafficked through Rab 11 endosomes. TfR normally recycles through rab 11 endosomes to the PM in non-polarized non-epithelial cells. Our experiments suggest that AP1A and AP1B co-regulate PM protein exit from the TGN and recycling endosomes into a specialized route branching out from the general rab 11 pathway for transport to PM. Supported by NIH, Research to Prevent Blindness and Dyson Foundation.

S5.1.5
Phosphoinositide 3-kinase-III is critical for recycling in apical receptor-mediated endocytosis by kidney proximal tubular cells

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See Abstract P05.3.

S5.1.6
Phosphatidylserine polarization is required for proper Cdc42 localization and for development of cell polarity

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See Abstract P05.5.

S5.2 Organelle dynamics

S5.2.1
Autophagy and mitochondrial elongation: sustaining cell viability under difficult conditions

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A plethora of cellular processes, including apoptosis, depend on regulated changes in mitochondrial shape and ultrastructure. The role of mitochondria and of their morphology during autophagy, a bulk degradation and recycling process of eukaryotic cells' constituents, is not well understood. Here we show that mitochondrial morphology determines the cellular response to macroautophagy. When autophagy is triggered, mitochondria elongate *in vitro* and *in vivo*. During starvation, cellular cyclic AMP levels increase and protein kinase A (PKA) is activated. PKA in turn phosphorylates the pro-fission dynamin-related protein 1 (DRP1), which is therefore retained in the cytoplasm, leading to unopposed mitochondrial fusion. Elongated mitochondria are spared from autophagic degradation, possess more cristae, increased levels of dimerization and activity of ATP synthase, and maintain ATP production. Conversely, when elongation is genetically or pharmacologically blocked, mitochondria consume ATP, precipitating starvation-induced death. Thus, regulated changes in mitochondrial morphology determine the fate of the cell during autophagy.

S5.2.2
Endosome dynamics in the biogenesis of lysosome related organelles

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The endosomal system comprises a complex network of organelles and membrane subdomains with important functions in signal transduction, nutrient uptake, pathogen destruction and other essential processes. Our work on the melanosome, the lysosome-related organelle of pigment cells has provided insights into mammalian endosomal membrane dynamics by revealing how specific trafficking events are exploited to generate tissue-specific organelles. Our recent studies have started to unravel how the endosomal system specializes to generate first unpigmented fibrillar melanosomes and secondly pigmented, mature melanosomes that can be transfered to keratinocytes. During early melanogenesis, sorting of the protein Pmel17 to intraluminal vesicles of multivesicular bodies precursors of melanosomes is concomitant with its cleavage and consequent formation of Pmel17-driven amyloid-like fibrils. Sorting of Pmel17 is independent of ubiquitylation and of the ESCRT (endosomal sorting complex required for transport) machinery. Our recent studies highlight a role for Tetraspanins in endosomal sorting and on the generation of amyloid-like fibrillar sheets *in vitro* and *in vivo* . Late melanogenesis requires the transfer of melanogenic enzymes from early endosomes to maturing melanosomes. Gene products mutated in different forms of albinism (such as the Hermansky Pudlak syndrom) encode proteins that regulate late melanogenesis (AP-3, BLOC complexes). Our recent studies have brought further knowledge on how these novel trafficking regulators operate in concert with additional adaptors, cytoskeletal motors and Rab GTPases to specialize endosomal sorting, endosome localization and positioning facilitating endosome-melanosome crosstalks required for the biogenesis of functional organelles. Our current studies aim to shed light on how the specialized trafficking events can be regulated within the integrated epidermal-melanin unit upon establishment of the pigmentation synapse.

S5.2.3
Molecular insights into autophagy

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Autophagy is initiated by external stress including amino acid starvation, resulting in the sequestration or engulfment of cytosolic proteins, membranes, and organelles in a double membrane structure, called the autophagosome. The autophagosome then fuses with endosomes and lysosomes and delivers the sequestered material for degradation. A better molecular understanding of autophagy is an important goal as autophagy is implicated in a number of human diseases, many of which can either be characterized by an imbalance in protein, organelle or cellular homeostasis, ultimately resulting in an alteration of the autophagic response. We have been studying a set of Atg (autophagy related proteins) involved in starvation-induced autophagosome formation, in addition to novel proteins recently implicated in autophagy in our lab. We have shown that ULK1, a serine-threonine kinase, and mAtg9, a multi-spanning membrane protein, are required for autophagy. We now know that WIPI2, a PtdIns-3 P binding protein is also required for autophagy, and is recruited to autophagsomal membranes at early stages. Furthermore, our recent data from our study of the Atg proteins and our novel proteins suggests that autophagosome formation requires contributions from both the Golgi and recycling endosomes, and trafficking from these organelles contributes to autophagosome formation induced by amino acid starvation.

S5.2.4
Golgi biogenesis

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The Golgi lies at the heart of the secretory pathway, receiving the entire output of newly-synthesized proteins from the endoplasmic reticulum, processing them through modification of the bound oligosaccharides, and then sorting them to their appropriate destinations. As with all other cellular organelles, the Golgi undergoes duplication during the cell cycle and partitioning during mitosis, so as to ensure inheritance during successive generations. The process of duplication - making another copy of the Golgi – has been difficult to study since most cells have many, often hundreds, of Golgi, making it difficult to follow the appearance of new Golgi. We have solved this problem by focusing on protozoan parasites, which have only one Golgi that can be followed using GFP technology. Through studying the Golgi in Trypanosoma brucei (the causative agent of sleeping sickness in sub-Saharan Africa), we have been able to tackle the mechanism that ensures duplication and partitioning of this organelle. Our results implicate a novel bilobe structure comprising structural proteins (MORN1 & LRRP1) and centrins 2/4, calmodulin-like calcium-binding proteins. This bilobe appears to help determine the position at which a new Golgi is assembled and its size.

S5.2.5
Unconventional secretion of tissue transglutaminase involves phospholipid-dependent delivery into recycling endosomes

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See Abstract P03.20.

S5.2.6
Rab GTPases and mast cell exocytosis

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See Abstract YSF.91.

S5.3 Membrane dynamics and disease

S5.3.1
Proteostasis, folding and membrane traffic-protecting the proteome in human disease

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The cell exploits the emergent properties of proteostasis biology [Science (2008) 319:916; Mol. Memb. Biol. (2010) 27: 385; Curr. Opin Cell Biol (2010) 23: Epub ahead of print] to generate and maintain healthspan. Physical, pathological and inherited challenges to the energetics of the biological fold (its landscape) can compromise proteome balance [Science (2010) 367:766]. Our goal is to understand how inherited misfolding disease is managed by the proteostasis network (PN), a system of signaling pathways, folding chaperones and degradative pathways that direct protein folding in health and disease. By use of systems level proteomic, genomic and imaging tools we are building a dynamic, multi-layered view of the healthy biological protein fold in membrane trafficking pathways and the changes that occur in response to energetically compromised folding stress. It is becoming increasingly clear that chemical biology management of proteostasis can alter the composition of the local proteostasis program to restore protein folding and function. The discovery of tools that redirect the biological folding and membrane trafficking environment to mitigate disease highlights the potential value of the emergent properties of the PN to therapeutically rebalance function of the cellular and tissue proteome to benefit healthspan in human misfolding disease.

S5.3.2
Role of endosomes in virus entry

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Using cellular, molecular, and systems biology approaches in combination with live cell imaging and electron microscopy, we are investigating how animal viruses enter their host cells. We focus on cellular processes that the viruses take advantage of. In this lecture, we discuss so called late-penetrating viruses. They have to enter late endosomes (LE) or lysosomes for penetration into the cytosol. We analyzed influenza A virus, Uukuniemivirus (a bunyavirus), and LCMV (an arenavirus). Common to these is a low pH threshold for activation, a long delay between endocy-

tosis and penetration, and sensitivity to variety of perturbations that affect LE endosome maturation and function (such as ubiquitination, Rab5 and Rab7, the ESCRT complex, the switch from PI(3)P to PI(3,5)P2, the proteasome, microtubules, and an intact centrosome). Once the core has been delivered to the cytosol and early transcription has taken place, proteasomes are needed to uncoat the viral DNA, and ubiquitination and again the proteasome to start replication of the viral DNA.

S5.3.3
Membrane dynamics during phagocytosis

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Engulfment and elimination of microorganisms by macrophages, neutrophils and dendritic cells is an essential component of the innate immune response. This process, known as phagocytosis, involves extensive remodeling of the membrane and of the actin cytoskeleton. The resulting vacuole or phagosome undergoes multiple membrane fusion and fission events, collectively known as phagosome maturation. These responses are rapid, transient and highly localized, complicating their analysis by conventional biochemical means. We used digital imaging of live cells to analyze the spatio-temporal features of signal transduction and membrane remodeling during the formation and maturation of phagosomes. When monitored in live macrophages during the course of particle engulfment, phosphoinositide-specific probes revealed large, transient and highly localized changes at sites of phagocytosis and during the early stages of maturation. Remarkably, the changes differed depending on the type of phagocytic receptor engaged; the phosphoinositide profile observed during Fcγ receptor mediated phagocytosis was distinct from that observed during complement-receptor mediated engulfment. We also developed novel probes to track the distribution and dynamics of phosphatidylserine (PS) in live cells by non-invasive means. Because PS and inositides confer negative charge to the inner aspect of the plasma membrane, we developed genetically-encoded probes to measure surface potential in live cells. Expression of such probes in macrophages revealed acute changes in the surface charge of the cytosolic leaflet of the plasma membrane, which were restricted to sites of phagocytosis and maturing phagosomes. These changes were attributable to phospholipid metabolism, with little change in PS content. Importantly the local alterations in surface potential were accompanied, and were likely the cause of dissociation from the membrane of important signal transduction regulators, such as K-Ras and Rac1. We concluded that lipids and the charge they contribute to the membrane play a key role as determinants of protein targeting and activation during phagocytosis, and likely many other biological responses as well.

S5.3.4
Endocytic mechanisms at neuronal synapses

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At neuronal synapses, recycling of secretory vesicles is a complex, coordinated process required for the maintenance of neurotransmission and plasma membrane composition. Endocytosis occurs by various mechanisms: clathrin-independent forms are poorly understood, while clathrin-dependent endocytosis is well studied. It is initiated by clathrin adaptor proteins binding to membrane cargo proteins, triggering the formation of a clathrin coat. The progression of synaptic vesicle membranes through endocytic steps is controlled by the phospholipid PI(4,5)P2 and facilitated by accessory proteins recruited from the cytosol. These include the GTPase dynamin (vesicle scission), the PI(4,5)P2 phosphatase synaptojanin (vesicle uncoating) and the BAR family proteins (coupling membrane deformation to biochemical changes). Endophilin is a conserved BAR domain-containing protein that interacts directly with dynamin and synaptojanin and whose importance in synaptic vesicle recycling is supported by genetic and functional studies. Yet, the precise function of endophilin at the synapse is unclear. We have addressed this question in mice models by disrupting the genes encoding the three endophilins (1, 2 and 3), which are all present at the synapse. Mice lacking single endophilins had no pathological phenotype, while animals lacking endophilins 1 and 2 had recurrent seizures and failed to thrive. The absence of all three endophilins caused perinatal lethality. Mutant neurons showed strong defects in synaptic transmission and reduced synaptic vesicle number, consistent with synaptic vesicle recycling impairment. Importantly, further studies revealed that endophilin, unlike dynamin, is dispensable for vesicle fission but, like synaptojanin, is crucial for vesicle uncoating. These findings support a model in which the post-fission shedding of the clathrin coat cannot proceed without PI(4,5)P2 hydrolysis and requires the cooperative action of synaptojanin and the uncoating factors.

S5.3.5
Assigning a role to the dengue virus capsid protein during cellular infection

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See Abstract YSF.33.

S5.3.6
Quantitative proteomics analysis of secretome and secreted microvesicles of chronic myeloid leukemia cells using SILAC method.

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See Abstract YSF.95.

S6 – Molecular basis of development

S6.1 Stress adaptation and development

S6.1.1
Protein folding homeostasis in the endoplasmic reticulum

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The flux of newly-synthesized unfolded proteins into the endoplasmic reticulum is subject to considerable physiological variation. Such variation is especially pronounced in multi-cellular organisms that rely on secretion to maintain intra-cellular communication and possess cell types specialized in protein secretion. To meet this challenge, adaptive signal transduction pathways responsive to the unfolded protein load (also referred to as ER stress) have evolved. Here will be discussed certain aspects of the workings of these pathways that collective constitute an unfolded protein response in an attempt to relate them to the pathophysiology of protein misfolding.

S6.1.2
ERp44 acts as a pH-dependent chaperone to retrieve client proteins from the Golgi complex

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Newly synthesized secretory proteins undergo scrupulous quality control (QC) to avoid release of folding or assembly intermediates. High fidelity of secretion depends on retention in, or retrieval into the endoplasmic reticulum (ER), where immature cargoes are given another chance to complete folding and assembly 1,2,3,4. Exposure of unpaired cysteines is exploited in preventing secretion of intermediates that have undergone incomplete disulfide bond formation 5. Here we show that ERp44, a multifunctional chaperone of the PDI family 5,6,7, operates in a pH dependent manner, in synchrony with forward (ERGIC53)- and backward (KDEL-receptors) cargo transporters, to optimize secretion efficiency and fidelity. At ER-equivalent neutral pH, the ERp44 carboxy-terminal tail obscures the thiol-active cysteine and surrounding hydrophobic patches. At the cis-Golgi-equivalent, slightly acidic pH, however, the C-tail becomes flexible, unmasking both the active site to allow capture of client proteins and the RDEL motif to allow retrieval by KDEL receptors. Upon retrieval to the ER, the neutral pH ensures release of client proteins. Our results delineate a novel QC system, which works downstream the calnexin/calreticulin- and BiP-dependent cycles 1,4. The ERp44 cycle is paramount in the retrieval of orphan subunits of otherwise disulfide-linked oligomers such as IgM 8 or adiponectin 9,10,7, whose recognition depends on free thiols.

S6.1.3
BMP signaling and neurogenesis of developing spinal cord

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AbstractProliferation of the neural/neuronal progenitor cells (NPCs) at the ventricular zone of the dorsal spinal cord requires the stimuli of Wnt and BMP. However, how these two signaling pathways are regulated in the NPCs as they enter the intermediate zone to initiate differentiation is not known. Here, we show that Smad6, a negative regulator of BMP signaling, is expressed in the intermediate zone of the chick dorsal spinal cord. Knockdown and overexpression experiments show that Smad6 is necessary and sufficient to promote NPCs to exit cell cycle and differentiate into neurons. While we find that Smad6 inhibits the BMP signaling as expected, we also find that Smad6 unexpectedly inhibits the Wnt/β-catenin pathway. The inhibition of the Wnt/β-catenin pathway by Smad6 is independent of its effect on the BMP pathway. Rather, Smad6 through its N-terminal domain and link region enhances the interaction of C-terminal binding protein (CtBP) with the β-catenin/TCF complex and the TCF-binding element to inhibit β-catenin mediated transcriptional activation. Our study provides evidence that transition of NPCs from a proliferative state to a differentiating state is controlled by the dual inhibitory role of Smad6 to both BMP and Wnt signaling at the level of transcription.

S6.1.4
The stress of misfolded proteins in aging and disease

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The status of the proteome is constantly monitored by stress signaling pathways and proteostatic networks that ensure protein misfolding and aggregation does not comprise cellular function. The challenge to proteome stability, however, is substantial; in addition to the acute effects of environmental and metabolic stress, are the chronic effects of mutations, expressed polymorphisms, and intrinsic errors in gene expression. Moreover, many proteins utilize inherent metastability to achieve multiple functional properties, thus contributing to global protein instability. The inability to maintain protein quality control is costly and leads to an imbalance in chaperones, clearance machineries, and stress responses that places the cell and organism at risk for protein conformational diseases. We have shown that the chronic expression of aggregation-prone proteins i.e. polyglutamine and mutant SOD1, results in a global collapse of cellular proteostasis that interferes with the folding and stability of other essential metastable proteins. Upon aging and exposure to other forms of cell stress, this leads to an amplification of protein damage that results in a further disruption of signaling and regulatory path-

ways. Utimately, this leads to differential tissue dysfunction and organismal failure. This age-dependent collapse in the proteostasis network can be restored by the cell non-autonomous neuronal regulation of stress response activators such as Hsf1, resulting in up-regulation of molecular chaperones and other protective pathways to counter cell stress and to stabilize protein homeostasis. Collectively, these results reveal that the transmission of the environmental stress signal involves the balance of active neuronal activity, which serves to integrate temperature-dependent behavioral, metabolic, and stress-related responses that control proteome stability and as a consequence the healthspan of the cell and lifespan.

S6.1.5
Oxidative protein folding by an endoplasmic reticulum localized peroxiredoxin

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See Abstract P06.14.

S6.1.6
Crystal structure of schistosoma mansoni Peroxiredoxin I: insights into a general mechanism of assembly of stress-regulated chaperones

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See Abstract P03.122.

S6.2 Cell shape determination

S6.2.1
Cellular behaviors during renal branching morphogenesis

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Signaling by GDNF through the Ret receptor tyrosine kinase is required for the normal formation, growth and branching of the ureteric bud (UB) during kidney development. However, the precise role of GDNF/Ret signaling in this process, and the specific responses of UB cells to GDNF, remain to be elucidated. Recent studies provide new insight into the effects of Ret signaling on cell behavior, and the genes functioning downstream of Ret. Cell lineage studies show that the UB tip cells, which express Ret, are the progenitors for UB growth, while GDNF-expressing mesenchymal cells are the progenitors of nephron epithelia. Time-lapse studies of chimeric embryos reveal that the earliest role of Ret signaling is in the Wolffian duct, where it promotes cell movements within the epithelium, which give rise to the first ureteric bud tip. In chimeric embryos, Wolffian duct cells lacking Ret fail to migrate to the region that forms the UB tip; on the other hand, cells lacking the negative regulator Sprouty1 (which have elevated levels of Ret signaling) preferentially migrate to contribute to this domain. Thus, it appears that Wolffian duct cells compete with each other, based on the level of Ret signaling, to undergo cell movements. A number of genes whose expression is induced in the UB by GDNF has been identified, including the two ETS transcription factors Etv4 and Etv5. These genes are required downstream of Ret for the Wolffian duct cell movements that form the UB tip domain, as well as for later UB growth and branching.

S6.2.2
Cell and tissue mechanics in zebrafish gastrulation

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Tissue morphogenesis during embryonic development is brought about by mechanical forces which are generated by the specific biophysical and motility properties of its constituent cells. It has also been suggested that embryonic tissues behave like immiscible liquids with a given surface tension and that differences in surface tension between tissues determine their spatial configuration during embryogenesis. To understand how single cell biophysical and motility properties regulate tissue surface tension and how tissue surface tension controls tissue organization in development, we are studying the specific function of germ layer progenitor cell adhesion, cell cortex tension and motility in determining germ layer organization during zebrafish gastrulation. We found that the combinatorial activity of progenitor cell adhesion, cortex tension and motility determines germ layer tissue surface tension and that differences in germ layer tissue surface tension influence germ layer organization during gastrulation. We will discuss these findings in the light of different hypotheses explaining how single cell biophysical properties determine tissue morphogenesis in development.

S6.2.3
Adherens junctions and astrocyte polarity

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The relative position of the centrosome and the nucleus defines a cell polarity axis which dictates the spatial organization of intracellular organelles and is crucial for a correct polarization of cellular functions. During astrocytes migration, the centrosome localises in front of the nucleus in the direction of migration. We have investigated the contribution of cell-cell interactions to centrosome and nucleus positioning and cell polarity.
The role of adherens junctions in the regulation of cell polarity was first demonstrated in immobile primary astrocytes plated on adhesive micropatterns. In single cells, the centrosome localizes at the geometrical cell centre, in close proximity to the nucleus. In contrast, in cells engaged in cell-cell interactions, the nucleus and the centrosome are off-centred and localize near cell-cell contacts. Anisotropic cell-cell contacts influence the polarity axis as the centrosome is preferentially located in front of the nucleus in the direction of the free cell edge. Distinct and complementary roles of microfilaments, microtubules and intermediate filaments in the control nucleus positioning and cell polarization were identified.
Cadherin-mediated junctions also regulate cell polarity during astrocyte migration and decrease of N-cadherin level in astrocyte-derived tumours is associated with perturbations of cell polarity and persistent directed migration. Expression of N-cadherin in cadherin-depleted astrocytes as well as in gliomas rescues cell polarity and strongly inhibit cell migration, suggesting that change in cadherin expression is a key event leading to the abnormal migration of astrocyte-derived tumours.

S6.2.4
Polarity proteins in morphogenesis and metastasis

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The PAR polarity proteins play pivotal roles in many aspects of development, including the asymmetric divisions of stem cells, epithelial organization in organs such as the kidney, liver, intestine, and glands, and in neuronal differentiation and function. PAR proteins are components of a signaling network that regulates the intrinsically polar cytoskeleton, in addition to energy metabolism and the cell cycle. Some of the PAR proteins occupy distinct domains at the cell cortex, and maintain their respective territories by active exclusion of other PAR proteins.

S6.2.5
Turning stem cells into retina: possible strategies for the cure of retinal degenerations

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See Abstract P06.5.

S6.2.6
Polarity and coordinated cell division in epithelial morphogenesis

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See Abstract P07.3.

S6.3 Development of cognition and language

S6.3.1
Neural stem cells and the evolution of the cerebral cortex

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Our group studies the molecular and cellular mechanisms of neurogenesis in the developing neocortex in the context of mammalian brain evolution, specifically the various types of cortical stem and progenitors cells and their modes of division. In terms of their cell biology, two principal classes of cortical stem and progenitors cells can be distinguished. One class comprises stem/progenitor cells exhibiting bipolar morphology and apical-basal cell polarity that divide at the ventricular, i.e. apical, surface of the ventricular zone (VZ). These are the neuroepithelial cells and radial glial cells, which are collectively referred to as apical progenitors (APs). The other class comprises stem/progenitor cells dividing in a more basal, abventricular location, notably the subventricular zone (SVZ). These fall into two subclasses (i) radial glia-related progenitors exhibiting monopolar morphology and basal, but not apical, cell polarity, called outer SVZ (OSVZ) progenitors, outer radial glia or intermediate radial glia, and (ii) progenitors exhibiting nonpolar morphology and lacking overt apical-basal cell polarity, called basal progenitors (BPs) or intermediate progenitors. OSVZ progenitors are implicated in the expansion of the neocortex during evolution. They are thought to contribute to the inverted cone shape of radial units by being the founder cells of basal radial subunits. Their ability to self-renew appears to be linked to the retention of their basal process and the integrin-mediated signaling it provides. In gyrencephalic species such as human and ferret, OSVZ progenitors constitute a much greater proportion of the total SVZ progenitors than in mouse, a lissencephalic species. Analyses on the abundance of OSVZ progenitors in Marmoset, a near-lissencephalic primate, and on the cell biological mechanisms underlying the delamination of Aps, a process converting Aps into OSVZ progenitors, will be presented.

S6.3.2
From recognition molecules to cognition

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Neural recognition molecules and associated glycans mediate cell-to-cell and cell-to-extracellular matrix (ECM) interactions that are important for neural cell migration, survival, axon guidance and synaptic targeting. Deficiencies in cell adhesion or ECM molecules can impair these processes and disturb the fundamental excitation-inhibition balance, as found, for instance, in the olfactory bulb of mice deficient in NCAM or in the hippocampus of tenascin-R deficient mice. Strikingly, these mice show impaired olfactory discrimination and enhanced reversal learning, respectively. In addition to shaping ontogenesis, recognition and ECM molecules modulate neurotransmitter receptors and ion channels in the mature nervous system. These mechanisms are also crucial for synaptic plasticity and cognition. Our data revealed that polysialic acid, predominantly carried by NCAM, inhibits opening of GluN2B-containing NMDA receptors at low concentrations of glutamate. In hippocampal slices, deficits in NCAM/PSA increase GluN2B-mediated transmission and Ca^{2+} transients at extrasynaptic sites, and impair long-term potentiation (LTP) in the CA3-CA1 synapses. Behaviorally, NCAM deficient mice show impaired contextual fear conditioning. These defects in LTP and fear conditioning can be fully rescued by suppressing the activity of hippocampal GluN2B-containing receptors. These findings implicate glycans carried by adhesion molecules in modulating extrasynaptic signaling in the brain and demonstrate reversibility of cognitive deficits associated with abnormal cell adhesion. As NCAM is linked to schizophrenia and Alzheimer's disease, these data encourage development of neurocognitive enhancers by targeting mechanisms mediated by recognition molecules.

S6.3.3
Neural mechanisms for mapping of space

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Grid cells are a key component of the brain network for representating self-location in external space. These cells fire selectively at regularly spaced positions in the environment such that, for each cell, activity is observed only when the animal is at places that together define a repeating triangular pattern tiling the entire environment covered by the animal, much like the holes of a Chinese checkerboard. The scale of the grid map is topographically organized in that the spacing of the grid increases from the dorsal to the ventral end of medial entorhinal cortex. In the first part

of the talk, I will discuss fundamental properties of grid cells, I will show that the organization of the grid map is modular, that HCN channels contribute to the determination of grid scale, and that grid cells co-localize with other functional cell types such as head-direction cells and border cells, which each contribute to a dynamically updated metric representation of current location in the medial entorhinal cortex. Based on studies using a virus-mediated approach to selectively express microbial photoresponsive channel proteins in entorhinal cells with projections to the hippocampus, I shall conclude by presenting data suggesting that grid cells, head direction cells and border cells may all provide direct input to the hippocampus, suggesting that the place-cell code arises by combination of these inputs.

S6.3.4
Structural traces of learning and memory in the hippocampus

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Learning of new skills is correlated with formation of new synapses. These may directly encode new memories, but they may also have more general roles in memory encoding and retrieval processes. Here we investigated how mossy fiber terminal complexes at the entry of hippocampal and cerebellar circuits rearrange upon learning, and what is the functional role of the rearrangements.

We show that one-trial and incremental learning lead to robust, circuit-specific, long-lasting and reversible increases in the numbers of filopodial synapses onto fast-spiking interneurons that trigger feedforward inhibition. The increase in feedforward inhibition connectivity involved a majority of the presynaptic terminals, and correlated temporally with the quality of the memory. We then show that for contextual fear conditioning and Morris water maze learning, increased feedforward inhibition connectivity by hippocampal mossy fibers has a critical role for the precision of the memory and the learned behavior. In the absence of mossy fiber LTP in Rab3a−/ − mice, c-Fos ensemble re-organization and feedforward inhibition growth were both absent in CA3 upon learning, and the memory was imprecise. By contrast, in the absence of β-Adducin c-Fos re-organization was normal, but feedforward inhibition growth was abolished. In parallel, c-Fos ensembles in CA3 were greatly enlarged, and the memory was imprecise. Feedforward inhibition growth and memory precision were both rescued by re-expression of β-Adducin specifically in hippocampal mossy fibers. These results establish a causal relationship between learning-related increases in the numbers of defined synapses and the precision of learning and memory in the adult. The results further relate plasticity and feedforward inhibition growth at hippocampal mossy fibers, to the precision of hippocampus-dependent memories.

S6.3.5
Alterations of the melatonin pathway as a susceptibility factor to autism

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See Abstract P06.10.

S7 – Systems biology

S7.1 Omics and Bioinformatics

S7.1.1
Efficient bioinformatics approaches for large-scale data analysis

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Systems level understanding of complex diseases requires coordinated efforts to collect and share genome-scale data from large patient cohorts. However, translating genome-scale data into knowledge and further to effective diagnosis, treatment and prevention strategies requires effective computational approaches that allow analysis and integration of multidimensional data with clinical parameters and knowledge available in bio-databases.
The Cancer Genome Atlas (TCGA) is acoordinated effort to improve cancer diagnosis and treatment by providing genetics, genomics, epigenetics and clinical data for hundreds of cancer samples. In this presentation I introduce an efficient and scalable computational framework for the analysis and integration of the TCGA provided data for ˜500 glioblastoma multiforme and ˜600 ovarian cancer patients. The focus of our analysis is to identify genetics regions and transcripts that have a clear association to survival and drug response.

S7.1.2
Challenging our understanding of protein interaction networks

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In the context of the on-going efforts to create and validate protein interaction networks, I will introduce the approaches based on the analysis of protein families and their implications for the understanding of the molecular basis of protein interaction specificity. In particular, I will describe recent developments for study of concerted evolution (co-evolution) between interacting protein families (Juan et al., PNAS 2008), the detection of the residues potentially responsible of binding specificity (Rausell et al., PNAS 2010), and the potential implications of these and other developments for modeling protein complexes (Wass et al., MSB 2011).
In the second part of the talk I will describe the recent progress in the construction and validation of protein interaction networks with information extracted from primary publications, including the BiocreativeII.5 developments (Leitner et al., Nat Biotech 2010 and IEEE/ACM Trans Comput Biol Bioinfor 2010) and the BiocreativeIII results in the extraction from the primary literature of information on the experimental methods used to detect protein interactions.
Finally, I will introduce our proposal (Baudot et al., Genome Biology 2009) for the use of interaction networks to connect disease associated genetic variants with the biomedical meta-information, as well as the initial results in this area (Baudot et al., EMBO Rep. 2010, Glaab et al., BMC Bioinfo 2010) and their implementation in a personalize cancer treatment environment.

S7.1.3
Extracting phylogenetic and functional signals from metagenomics data

P. Bork
European Molecular Biology Laboratory

Although application of modern sequencing technologies to environmental sequencing (Qin, et al., 2010) enables a wealth of metagenomics data, our understanding of microbial community functioning remains limited, both in terms of internal interactions, but also in the context of environmental properties. Using a metagenomics pipeline, SMASH (Arumugam et al., 2010), we analyzed stool samples from individuals from six countries and identified three preferred community compositions, dubbed enterotypes. These are driven by networks of interacting genera and seem to be independent of a number of host properties studied such as nationality, age, gender or body mass index (Arumugam et al., 2011). However, we did find genes or pathways that correlate well with each of the latter properties. Similarly, we also observed adaptation of functional composition of ocean surface communities to various environmental properties related to climate and nutrition. Strong signals were found even for complex properties such as productivity (Raes et al., 2011), illustrating the potential of phylogenetic and functional biomarkers in various settings.

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S7.1.4.
Integrating phenotypic data from electronic patient records with molecular level systems biology

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Electronic patient records remain a rather unexplored, but potentially rich data source for discovering correlations between diseases. We describe a general approach for gathering phenotypic descriptions of patients from medical records in a systematic and non-cohort dependent manner. By extracting phenotype information from the free-text in such records we demonstrate that we can extend the information contained in the structured record data, and use it for producing fine-grained patient stratification and disease co-occurrence statistics. The approach uses a dictionary based on the International Classification of Disease ontology and is therefore in principle language independent. As a use case we show how records from a Danish psychiatric hospital lead to the identification of disease correlations, which subsequently are mapped to systems biology frameworks.

S7.1.5
Targeted mass spectrometry for quantitative proteomic analysis of energy metabolic pathways in breast cancer cells

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See Abstract P07.4.

S7.1.6
Systems biology approaches towards Neuronal ceroid lipofuscinoses interactome

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See Abstract P07.10.

S7.2 Networks and circuits

S7.2.1
System organization of mammalian fatty-acid metabolism

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Human metabolic diseases are typically network diseases. This holds not only for multifactorial diseases, such as metabolic syndrome and type II diabetes. Even when a single gene defect is the primary cause, the adaptive response of the entire network determines the severity of disease.
Fatty-acid metabolism is associated with numerous diseases and affects human ageing. Nevertheless, few biochemically rooted computational models exist of fatty-acid metabolism. This is probably due to the fact that the system is difficult to access experimentally. Many intermediate metabolites are present at low concentrations and difficult to quantify. Moreover, enzymes are often found in close association with each other, but we lack quantitative data about the properties of these complexes. We turned the argument around and started modelling fatty-acid oxidation in order to get a deeper insight into the general principles underlying pathway functioning and to develop a model-based experimental design.
Based on a detailed biochemical model of fatty-acid oxidation we will discuss the impact of substrate competition for a common set of enzymes, thermodynamic constraints, counterintuitive control of flux and potentially toxic intermediates, the function of metabolite channelling and the interplay between fatty-acid and carbohydrate metabolism. Based on fatty-acid oxidation as an example, we will discuss the relation between cell-based biochemical models and whole-body (dys)functioning of energy metabolism.

S7.2.2
Systems biology approaches to nuclear receptors signaling

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Transcription initiation is a complex, multi-step process, which involves coordinated action of numerous proteins. The 48 members of the nuclear receptor superfamily of ligand-dependent transcription factors play a multitude of essential roles in the development, homeostasis, reproduction and immune function. We use several genomic and systems biology approaches for investigating the role of the nuclear receptors vitamin D receptor (VDR), liver X receptors (LXRs) and peroxisome proliferator-activated receptor (PPARs) in health and disease:
(1) Studies on transcriptional dynamics of nuclear receptor association with its chromatin targets, DNA looping and mRNA accumulation of its up- and down-regulated target genes.
(2) Genome-wide investigation of the association of nuclear receptors, their partner proteins and chromatin marks using ChIP-Seq in the model systems of differentiating human monocytes and adipocytes.
(3) Identification of regulatory single nucleotide polymorphisms (SNPs) in nuclear receptor and other transcription factor binding sites that provide a functional explanation of SNPs in genome-wide association studies for traits, such as type 2 diabetes.

S7.2.3
A recruitment-reaction model for chromatin-associated regulatory processes

T. Höfer
German Cancer Research Center

Computational frameworks for gene regulation have focused on the sequence-specific binding of transcription factors and the subsequent recruitment of cofactors to DNA. Combining mathematical modeling and quantitative experimentation, we have developed kinetic models for gene regulation and DNA repair in mammalian cells. The experimental data forced us to include into these models biochemical reaction steps executed by the recruited proteins. I will show how the resulting recruitment-reaction models make testable predictions on rate, fidelity and memory of chromatin-associated regulatory processes.

S7.2.4
All our tomorrows: the science of human ageing

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Institute for Ageing and Health, Newcastle University

Life expectancy in developed nations is continuing to increase by 5 hours a day, presenting a profound challenge for the organization of society. At the same time, science is at last beginning to unravel the deep mysteries of the ageing process and creating new possibilities for translational research that might deliver innovative therapies for age-related disease. Evidence from many lines of research confirms that ageing is more malleable than was previously thought, since it arises not from a strict genetic programme but from the gradual accumulation of damage in cells and tissues of the body, which can be modulated in turn by many factors including nutrition, lifestyle and environment. A longstanding barrier to progress, however, has been the coexistence of multiple, seemingly competing hypotheses about causal mechanisms. Each

mechanism tends to be partially supported by data indicating that it has a role in the overall cellular and molecular pathways underlying the ageing process. However, the magnitude of this role is usually modest. New systems-biology approaches are needed that can combine (i) data-driven modelling, often using the large volumes of data generated by functional genomics technologies, and (ii) hypothesis-driven experimental studies to investigate causal pathways and identify their parameter values in an unusually quantitative manner. This enables the contributions of individual mechanisms and their interactions to be better understood and it allows for the design of experiments explicitly designed to test the complex factors contributing to ageing and health. Since age is the single biggest risk factor for a very wide spectrum of diseases, which individually attract major research effort, the prize of identifying exactly why aged cells are more vulnerable to pathology, and thereby how such pathology might be delayed or prevented, seems eminently worthwhile.

S7.2.5
The role of incoherent microRNA-mediated feedforward loops in noise buffering

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See Abstract P07.2.

S7.2.6
Unraveling the influence of endothelial cell density on VEGF-A signaling

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See Abstract P07.9.

S8 – Molecular engineering for medicine

S8.1 Synthetic biology for medicine

S8.1.1
Exploiting conformational change in biosensor design

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A ligand dependent change in the structure of a molecular receptor is a widely employed signal transduction mechanism in biology and we have sought to exploit the same effect in the design of biosensors.

Site specific labelling of the molecular receptor with suitably chosen probe species is the key to maximising the analyte dependent change in signal. This change can be either through a change in the probe's environment or in its distance from the sensor surface.

To illustrate this general principle I will describe our work with binding proteins and nucleic acid aptamers, employing both optical and electrochemical detection modalities.

S8.1.2
Bacterial hydroxylases and their potential to synthesize pharmaceutical products

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Besides playing an important role in drug metabolism, cytochromes P450 are crucial for the biosynthesis of steroid hormones. Steroids play an important role as hormones in mammals. In addition to this, many steroids are interesting pharmaceutical target substances for the production of different types of drugs. Since selective transformations of steroids are a difficult task, the pharmaceutical industry has rationalized the importance of microbial steroid transformations. Moreover, CYP106A2 from Bacillus megaterium ATCC 13368 is one of the few known bacterial steroid converting cytochromes P450 and hydroxylates many 3-oxo-Δ4-steroids mainly in 15β-position. Here we report on the creation of mutants of this enzyme with improved activity and changed selectivity of hydroxylation shifted from the 15 to the 11-position. To check mutants produced by directed evolution, an *E. coli* whole-cell screening system has been developed. We were able to select mutants with considerably improved activity and with changed selectivity of hydroxylation. This way, a system for creating libraries of steroid molecules with hydroxyl groups in different positions seems to be feasible.

In adition the CYPome of the Myxobeacterium Sorangium cellulosum So ce 56 was analyzed. Myxobacteria are due to their ability to produce antibiotics and cytostatica (such as epothilone) of high biotechnological impact. Sorangium cellulosum So ce 56 possesses with 13.1 Mb the largest of the so far sequenced bacterial genomes, containing a total of 22 ORFs for cytochrome P450 genes. Interestingly, nine completely new CYP families have been identified. Nearly none of the CYPs has been found in biosynthetic clusters thus making a prediction of their potential functions challenging. We have cloned and expressed all 21 CYPs in *E. coli* and identified functional endogenous redox partners among eight ferredoxins and three ferredoxin reductases. Several pharmaceutical interesting substrates have been identified.

S8.1.3
Ligand-binding interactions and quaternary association in human aromatase

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Cytochrome P450 aromatase (CYP19A1) catalyzes the biosynthesis of estrogens from androgens in vertebrates. The crystal structure of human placental aromatase complexed with androstenedione revealed an androgen-specific active site and provided new insight into the reaction mechanism [1,2]. Using X-ray diffraction, biochemical and computational data, we have analyzed the flexibility of the active site core of aromatase and its interaction with breast-cancer drugs and other ligands. The core structure of aromatase is found to be rigid. Nevertheless, some evidence of ligand-induced fit is observed at the catalytic cleft. Although the binding mode of the steroidal inhibitor exemestane resembles that of androstenedione, exemestane binds tighter and has a higher shape complementarity in the active site cleft than the substrate. Binding analyses with non-steroidal letrozole and anastrozole show that these molecules cannot be accommodated in the catalytic cleft without severe steric clashes or large structural changes, implicating possible alternative binding sites. We have also been investigating the structural and functional consequences of the observed higher order organization of the enzyme [3]. The oligomeric association between the aromatase molecules via the negative potential surface of the D-E loop region, located 39Å away from the active site, and the positively charged heme-proximal cavity is rather unique among the known P450 structures. Using a bacterial expression system for an amino terminus truncated enzyme, we have performed D-E loop mutations that disrupt the complementarity and the electrostatic property of the interface. The results demonstrate profound effect on the enzyme activity and the Soret absorption spectra, suggesting functional implications of the higher order organization of aromatase. Despite the absence of the amino-terminal transmembrane domain, the crystal structure of recombinant aromatase exhibits the same three-dimensional atomic arrangement and intermolecular association as the full-length placental enzyme. Computational analysis is also able to reproduce the intermolecular interface, indicating the specific nature of the interaction. This research is supported in part by grant R01GM86893 from the National Institutes of Health, U.S.A.

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S8.1.4
From flesh-eating bacteria to protein superglue

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Generating specific and high affinity interactions with proteins is an important challenge in biochemistry and medicine. The mammalian immune system usually addresses this challenge within a few weeks but the antibodies raised have modest affinity. We have worked to break this affinity limit by engineering protein interactions that undergo chemical reactions. For covalent binding to endogenous proteins, we have generated affibodies modified with an electrophile: initial non-covalent interaction by the affibody drives covalent reaction of the electrophile with the protein target. Covalent-binding affibodies/antibodies may have applications in blocking the activity of proteins such as cytokines active at femtomolar concentration and for lowering the detection threshold of serum markers of disease. For strong binding to peptides, we have harnessed a special feature of the human pathogen Streptococcus pyogenes, to engineer a peptide tag that spontaneously forms an amide bond upon binding its protein partner. Reaction of the peptide tag is robust to pH and temperature and reaches complete conversion in minutes. The reaction is specific for mammalian cell imaging and both partners are genetically encodable. Irreversible binding to peptide tags should have application in testing the role of force in cellular function and also for immobilization and assembly of new protein architectures for therapeutics.

S8.1.5
Ferritin: nanotechnology at the service of the new biomedicine

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See Abstract P08.10.

S8.1.6
Modulation of ROS production and cytosolic Ca^{2+} homeostasis by fullerenes C60 in oncotrasformed T-cells

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See Abstract P08.14.

S8.2 Biomedical application of nanotechnology

S8.2.1
Cell Membrane Penetrating Particles

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Abstract not received. Please see program and Late Abstract Addendum.

S8.2.2
Clinical status of lysosomotropic/endosomotropic polymer conjugates designed as nanomedicines

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It is two decades since we started first Phase I clinical trials involving synthetic polymer-drug conjugates designed as anticancer and gamma camera imaging agents (theranostics). A growing number of polymer therapeutics have entered clinical development or been approved as novel nanomedicines (reviewed in [1,2]). Such multifunctional nano-sized constructs (typically 5–25 nm) are rationally designed to use a ''biocompatible'' polymer platform, a polymer-drug linker that was stable in transit, EPR- or receptor-mediated tumour selective delivery and following endocytosis, lysosomotropic drug delivery [3]. Clinical data [2] have underlined the correlation between a pharmacokinetically-guided strategy for conjugate design and improved therapeutic index, and also the need for nanomedicine-specific biomarkers to guide selection of patients mostly likely to respond to such nano-sized therapeutics [4]. Growing understanding of the mechanisms of endocytosis and intracellular trafficking pathways in health and disease [5] is also bringing new opportunities for biomarker identification. Our recent studies have been developing novel polymer conjugates as anticancer agents and for promotion of tissue repair. Typically they require activation in specific intracellular compartments (e.g. lysosomes, endosomes), they must deliver combination therapy, and/or to localise to specific intracellular organelles (e.g. cytosol, nucleus, mitochondria, pathogen-containing vacuole etc.). To optimise design it is essential to first document their endocytic behaviour in the target cell type [6] and to use techniques (e.g. subcellular fraction [7]) that will quantitate intracellular fate. The opportunities and challenges of harnessing/circumventing the intracellular vesicular trafficking pathways will be discussed.

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S8.2.3
Multifunctional coating platform for the biomedical applications of magnetic nanoparticles

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Nanotechnology offers clear advantages over conventional techniques that can suppose a real breakthrough in biomedical research, and health care. It enables the development of multifunctional systems incorporating physical and biological functionalities in a single particle that could perform simultaneously several operations such as driving, sensing, imaging and therapy. The paper will provide an overall vision of these possibilities and wills present a core-shell multifunctional polymeric platform containing magnetic nanoparticles, luminescent centres and anchoring sites for biologically active molecules. The coated nanoparticles are stable in biological fluids, show low toxicity, excellent hemocompatibility, ability for cell internalization, anticoagulation properties, and very good performance in magnetic resonance imaging and hyperthermia. The magnetic properties of the nanoplatform (magnetic moment, susceptibility, blocking temperature, relaxivity, etc) can be tuned in the whole superparamagnetic range and further by changing the size of the magnetic nanoparticles from 2 to 25 nm. The total particle hydrodynamic diameter can be varied from 30 to 150 nm. The synthesis is based in a polymeric route, and all the components are biocompatible.

S8.2.4
Artificial organelles based on polymer nanoreactors

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Like conventional lipids, suitable amphiphilic block copolymers can self-assemble into supramolecular structures in aqueous media, their membranes mimicking biological membranes. The properties of such membranes can be extensively controlled via chemical composition, molecular weight and the hydrophilic-to-hydrophobic block length ratio of the polymers. Compared to conventional, low molar mass building blocks (e.g. lipids), membranes based on macromolecular self-assembly not only have the advantage of superior stability and robustness, but, because a single copolymer molecule can impart multifunctionality chemically, the tailoring of physical, chemical and biological properties is additionally possible. Other well-defined functions, such as molecular recognition, cooperation, and catalytic activity can be introduced by combining these polymeric superstructures with suitable biological entities.

We exploited the concept of bio-synthetic combination to develop polymer nanoreactors that encapsulated water-soluble enzymes in the aqueous cavities of vesicles generated by the self-assembly of amphiphilic copolymers. Channel proteins inserted into the polymer membrane selectively controlled the exchange of substrates and products with the environment, supporting the in situ activity of the enzymes.

By synthesizing appropriately functionalised polymers (e.g. biotin, antibody) we successfully immobilized the nanoreactors on solid support and created specifically decorated nanoreactors for targeting approaches. We used the immobilized polymer nanoreactors to follow the folding/unfolding of single proteins, and to monitor enzymatic reactions down to the scale of a few molecules. Model reactions have been used to demonstrate the potential of these structures in biosensing and for local production of bioactive compounds.

Nanoreactors with surfaces appropriately functionalized by specific entities involved in molecular recognition patterns have been targeted at predefined cells. After cellular uptake, the nanoreactors retained their function over extended periods of time, thus acting like artificial organelles that continuously exchanged molecular information with the host cell. This opens new avenues in protein therapy as well as intracellular sensing approaches.

S8.2.5
Near infrared fluorescent proteins

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See Abstract P08.34.

S8.2.6
Silk fibroin engineered 3D System for the study of megakaryocytes and functional platelet production

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See Abstract P08.6.

S9 – Probiotics as health-promoting agents

S9.1 Probiotics as health-promoting agents

S9.1.1
Moonlighting proteins as biomarkers for probiotic safety.

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Moonlighting proteins have been defined as polypeptides exhibiting two mechanistically unrelated functions; notably, some of these multifunctional proteins have been demonstrated to be invovled in bacterial prthogenicity. Prominent examples are (I) Aeromonas hydrophila enolase, an intracellular glycolytic enzyme, shown to be also expressed at the cell-surface where it facilitates activation of plasminogen to plasmin and (II) the cytoplasmic glyceraldehyde-3-phosphate dehydrogenase, which has been identified in the extracellular sub-proteome of the food-borne opportunistic pathogen Cronobacter turicensis and was speculated to contribute to the strains virulence by adhering to host blood proteins.
More recently, we tested different clinical and cheese-isolates of *Enterococcus faecalis* for their potential to express pathogenic traits and characterized the secretome of two representative strains by comparative two-dimensional gelelectrophoresis. *E. faecalis* is known as common nosocomial pathogens; however, it's also employed as cheese starter culture and has been discussed as probiotics due to its capacity to produce bacteriocins. Albeit all tested strains bore genes coding for virulence determinants such as gelatinase and serine protease, only the hospital-isolates were expressing these enzymes, thereby indicating an epigenetic control of pathogenicity genes. Interestingly, numerous moonlighting proteins were identified in the supernatant of the clinical strain but missing in the cheese-isolate's secretome, i.e. five glycolytic enzymes and the chaperone DnaK. It has been suggested that these proteins are able to bind plasminogen thereby rendering it more sensitive to host plasminogen activators and thus act as virulence factors. *E. faecalis* moonlighting proteins might serve as biomarkers to assess the risk potential of probiotic strains. Ongoing work therefore investigates, whether certain growth conditions might induce virulence factor production in the food-isolates.

S9.1.2
Bacteria-host dual communication uses molecular chaperones

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The enormous bacterial colonisation of vertebrates requires significant levels of communication between the bacteria and cells of the host. Most of our understanding of such communication comes from the study of exogenous and opportunistic pathogens. Moonlighting proteins play a significant role in such communication, including acting as selective adhesins. Analysis of the literature reveals that of bacteria employing moonlighting proteins as signals/adhesins, the following percentages use molecular chaperones for this purpose: 43% chaperonin (Cpn)60, 17% Hsp70 and 10% peptidylprolylisomerase. This compares with 29% using GAPD and 21% using enolase. These molecular chaperones are both present on the cell surface, where they can act as adhesins, and secreted into the medium, where they can act as potent signals to human cells inducing many different forms of cellular behaviour. Notably, signalling is a two way process with various human cells also expressing cell surface molecular chaperones (Cpn60, Hsp70, Hsp90) which can bind to bacterial components such as lipopolysaccharide, toxins, other moonlighting proteins and living bacteria. One example is Listeria monocytogenes which expresses a cell surface adhesin called Listeria adhesion protein (LAP). This turns out to be the alcohol acetaldehyde dehydrogenase of this organism. The receptor for LAP is human Cpn60. Thus one moonlighting protein is binding to another to allow L. monocytogenes to infect cells. Bacterial infection and colonisation is stressful for the bacteria and for the host, resulting in increased production of molecular chaperones which mainly function as cell stress proteins. It may not, therefore, be surprising that these proteins have also evolved to function as stress-regulated signals between the Prokaryotic and Eukaryotic Kingdoms. We now need to understand how important such signalling is for members of the human bacterial microbiota.

S9.1.3
Milk protein fragments induce bacteriocin biosynthesis in *Streptococcus macedonicus*: perspectives in food preservation and infection control

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Streptococcus macedonicus has been described as a new species in 1998 for *Streptococcus* thermophilus like strains isolated from naturally fermented Greek Kasseri cheese. Since then, strains of *S. macedonicus* have been isolated from many cheese varieties produced mainly in the Mediterranean basin, which is known for highest per capita cheese consumption in the world. Despite belonging to the well known pathogenic genus of *Streptococcus*, there is significant evidence that *S. macedonicus* may be the second non-pathogenic food compatible *Streptococcus* along with *S. thermophilus*. Several strains of *S. macedonicus* have been described to possess important technological properties, among them *S. macedonicus* ACA-DC 198, which produces the antimicrobial peptide Macedocin. The heat stable lantibiotic Macedocin inhibits a broad spectrum of food spoilage microorganisms, such as Clostridium spp. and Bacillus spp., as well as pathogenic bacteria, including *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Macedocin is produced only when *S. macedonicus* is grown in milk. In fact, Macedocin is produced when *S. macedonicus* is used either as starter or adjunct culture in pasta filata cheese preparation and it is active throughout cheese ripening. The biosynthetic gene cluster of Macedocin is chromosomally located and consists of ten open reading frames, which correspond to the genes involved in Macedocin biosynthesis, regulation and immunity. In contrast to the common auto-induction mechanism described so far for lantibiotics, it was recently shown that, in the case of Macedocin, milk proteins, and more specifically fragments of milk protein degradation by *S. macedonicus* ACA-DC 198, serve as induction factors. These physiological and technological features make *S. macedonicus* ACA-DC 198 a

multi-functional candidate culture for food preservation as well as for infection control.

S9.1.4
Interactions between Lactic acid bacteria and *Staphylococcus aureus*: an old story with new health perspectives

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Staphylococcus aureus is a Gram positive opportunistic pathogen and a major concern for both animal and human health worldwide. In some contexts where Lactic Acid Bacteria (LAB) are the normal dominant microbiota, such as in fermented food or in the vaginal ecosystem, *S. aureus* sometimes colonises, persists, expresses virulence factors and produces food poisoning or urogenital infections, respectively. Studies on the interactions between LAB and *S. aureus* began a few decades ago and were pursued to shed light on the inhibitory capabilities that LAB might have on *S. aureus* growth and/or enterotoxin production in fermented foodstuffs. These early studies had the aim of developing methods to prevent staphylococcal food poisoning, thus improving food safety. More recently, the concept of vaginal probiotic LAB has emerged as a promising way to prevent urogenital infections, *S. aureus* being one of the potential pathogens targeted. This talk provides an up-to-date look at the current hypotheses of the mechanisms involved in the inhibition of *S. aureus* by LAB in both the vaginal ecosystem and in fermented food ecosystems. We also emphasise that post-genomic approaches can now be envisioned in order to study these diverse and complex interactions at the molecular level. Further works in this field will open up new avenues for methods of biocontrol by LAB and/or for biotechnological uses of LAB-compounds to fight against the long-standing, yet incumbent menace of staphylococcal infection.

S9.1.5
The bacterial high affinity Zn-uptake system: a possible target for novel antibiotics.

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See Abstract P09.3.

S9.1.6
Characterization of proteins from *Pseudomonas aeruginosa* involved in c-di-GMP turnover

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See Abstract P09.26.

S9.2 Antimicrobial drug discovery: a new challenge for the future

S9.2.1
The quest for antibiotics and characterization of new inhibitors targeting the translational apparatus

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Since their introduction in therapy, antibiotics have played an essential role in human society, saving millions of lives, allowing safe surgery, organ transplants, cancer therapy. Antibiotics have also helped to elucidate several biological mechanisms and boosted the birth and growth of pharmaceutical companies, generating profits and royalties. The golden era of antibiotics and the scientific and economical drive of big pharma towards these molecules is long gone, but the need for effective antibiotics is increased as their pipelines dwindle and multi-resistant pathogenic strains spread.
Here we shall present the chemical, biological and topographical properties as well as the characterization of the mechanism of action of four novel natural products which inhibit the bacterial translational apparatus, and offer promising perspectives for their further development into antibiotics. Two of these molecules inhibit the translational initiation step which represents an underexploited antibiotic target, while the other two inhibit the elongation step with rather uncommon mechanisms.

S9.2.2
Inhibitors of bacterial cell division

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New antibiotics are urgently needed to treat the increasing number of life-threatening bacterial infections that are resistant to current therapies. In particular, the emergence and spread of drug-resistant staphylococci is of serious concern. The discovery and characterization of a novel class of small synthetic FtsZ inhibitors that have potent activity against staphylococci will be presented. The validation of FtsZ as an antibacterial target; the identification of 3-methoxybenzamide as a suitable starting point for expansion and optimisation; the characterisation of the mechanism of action of the series and the demonstration of *in vivo* efficacy in models of infection will be covered.

S9.2.3
Bypass the membrane barrier strategy in multidrug resistant Gram-negative bacteria

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The envelope of Gram-negative bacteria is a sophisticated biological structure comprising two membranes, the outer and the inner membrane that delineate the periplasmic space. The membrane permeability is a part of the early bacterial defence: the modification of permeability by decreasing the production of porins or increasing the expression of efflux pump systems has been char-

acterized in various clinical isolates showing a multidrug resistant (MDR) phenotype. This modification may confer low antibiotic susceptibility that contributes to the bacterial dissemination, the colonization of the patient and favours the acquisition of additional mechanisms of resistance. The bacterium manages the translocation process, influx and efflux, to control the intracellular concentration of various molecules. Antibiotics and biocides are substrates of these mechanisms and the continuing emergence of MDR isolates is a growing worldwide health concern. Different strategies could be proposed to bypass the bacterial membrane barrier, comprising influx and efflux mechanisms, in order to restore the activity of antibiotics against resistant bacteria. This presentation is focussing on the strategies that can improve the influx or modulate the efflux in order to increase the intrabacterial concentrations of antibiotic molecules.

S9.2.4
Inter-kingdom chemical signaling in host-bacterial associations.

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The worldwide challenge of antimicrobial resistance and the paucity of novel antibiotics underscore the urgent need for innovative therapeutics. The increasing understanding of bacterial pathogenesis and inter-cellular communication, when combined with contemporary drug discovery tools and technologies, provides a powerful platform for translating basic science into therapeutic applications to combat bacterial infections. Inter-kingdom chemical signaling bridges the communication between bacteria and their hosts. Many bacterial pathogens rely on a conserved membrane sensor, QseC, to sense and respond to host adrenergic signaling molecules and to bacterial signals to promote expression of virulence factors. Here we show that small molecule inhibitors of QseC-mediated signaling markedly inhibit the virulence of several pathogens *in vitro* and *in vivo* in animal models. Using a high throughput screen, we identified a potent small molecule, LED209, which inhibits binding of signals to QseC, preventing QseC's autophosphorylation, and consequently inhibiting QseC-mediated activation of virulence gene expression in enterohemorrhagic *E. coli* (EHEC), *Salmonella typhimurium* and *Francisella tularensis*. LED209 also prevented formation of lesions on epithelial cells by EHEC, and *F. tularensis* survival within macrophages. Remarkably, LED209 treatment protected mice from lethal *S. typhimurium* and *F. tularensis* infection. LED209 is not toxic and does not inhibit pathogen growth. Inhibition of microbial virulence without inhibition of growth may engender less selective pressure to promote the generation of resistance. As demonstrated herein, inhibition of inter-kingdom inter-cellular signaling constitutes a novel and highly effective strategy for the development of a new generation of broad spectrum antimicrobial agents.

S9.2.5
Purifcation, characterization and partial amino cid sequence of mesentericin W3, a new anti-Listeria bacteriocin

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See Abstract P09.7.

S9.2.6
Reduced phosphorylation of LPS decreases *E. coli* susceptibility to the human antimicrobial peptide LL-37

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See Abstract P09.10.

S10 – Metabolic control and disorders

S10.1 Nuclear receptors and lipid metabolism

S10.1.1
Decision-making by macrophages and dendritic cells using RXR heterodimeric receptors to sense their lipid environment

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A key issue in the immune system is to generate specific cell types often with opposing activities. The mechanisms of differentiation and subtype specification of immune cells such as macrophages and dendritic cells are critical in order to understand the regulatory principles and logic of the immune system. Besides cytokines and pathogens it is increasingly appreciated that lipid signaling also has a key role in differentiation and subtype specification.
We have explored how intracellular lipid signaling in immune cells via a set of transcription factors, regulates cellular differentiation, subtype specification, immune as well as metabolic homeostasis.
Peroxisome proliferator-activated receptor γ (PPARγ) is a lipid-activated transcription factor regulating lipid metabolism and inflammatory response in macrophages and dendritic cells (DCs). These immune cells exposed to distinct inflammatory milieu show cell type specification as a result of altered gene expression. We identified a mechanism how inflammatory molecules modulate PPARγ signaling in distinct subsets of cells. Proinflammatory molecules inhibited, whereas interleukin-4 (IL-4) stimulated PPARγ activity in macrophages and DCs. Furthermore, IL-4 signaling augmented PPARγ activity through an interaction between PPARγ and Signal Transducer and Activators of Transcription 6 (STAT6) on promoters of PPARγ target genes, including FABP4. Thus, STAT6 acts as a facilitating factor for PPARγ by promoting DNA binding and consequently increasing the number of regulated genes and the magnitude of responses. This interaction, underpinning cell type-specific responses represents a unique way of controlling nuclear receptor signaling by inflammatory molecules in immune cells.

S10.1.2
Nuclear receptor regulation of cholesterol metabolism

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The Liver X Receptors (LXRs) are nuclear receptors that play central roles in the transcriptional control of lipid metabolism. LXRs function as nuclear ''cholesterol sensors'' that are activated in response to elevated intracellular cholesterol levels in multiple cell types. Once activated, LXRs induce the expression of an array of genes involved in cholesterol absorption, efflux, transport and excretion. They also inhibit cholesterol uptake by inducing the ubiquitination and degradation of the LDL receptor. In addition to their function in lipid metabolism, LXRs modulate immune and inflammatory responses cell of both the innate and acquired immune systems. Synthetic LXR agonists promote cholesterol efflux and inhibit inflammation *in vivo* and inhibit the development of atherosclerosis in animal models. Loss of LXR expression in mice leads to pathologic lipid accumulation, atherosclerosis and the development of autoimmune disease. The ability of LXRs to integrate metabolic and inflammatory signaling makes them potentially attractive targets for intervention in human metabolic disease.

S10.1.3
Role of PPAR signalling in diabetic dyslipidemia

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Despite that statin treatment substantially reduces cardiovascular morbidity and mortality, many treated patients still experience a high residual risk. Statins lower LDL-cholesterol (LDL-C), with limited effects on other lipid parameters. A meta-analysis from 14 randomised trials conducted in high-risk patients reported that statin therapy is effective in reducing the proportional risk for major vascular events by 21% for each millimolar lowering of LDL-C. However, on average 14% of patients still experienced an event despite being allocated to statin. Beyond LDL-C, other factors, including triglycerides, non-HDL-C, HDL-C and apolipoprotein B and CIII, have been identified as factors determining residual risk, and normalization of these parameters may further decrease cardiovascular disease in patients treated with statins. PPARalpha activation improves atherogenic dyslipidemia characterized by high triglyceride and/or low HDL-C levels and elevated concentrations of small dense LDL particles, with or without high LDL-C levels. Data from fibrate trials indicate that these drugs are particularly effective in reducing cardiovascular morbidity in patients with atherogenic dyslipidemia and the metabolic syndrome. The ACCORD trial is testing the effect of fenofibrate and statin combination therapy on cardiovascular disease in diabetic patients. In addition, results from the FIELD trial have demonstrated a beneficial action of the PPARalpha activation on microvacular complications (retinopathy) and amputation in diabetic patients. Moreover, PPARdelta agonists improve dyslipidemia, glucose and energy homeostasis in preclinical animal models of (pre)diabetes. These observations provide a rationale to target PPARalpha and PPARdelta in the management of patients with high residual cardiovascular risk related to atherogenic dyslipidemia and persisting after single therapy.

S10.1.4
The transcriptional network of PPARgamma in adipocyte development and function

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Peroxisome proliferator-activated receptor (PPAR) γ plays a central role in adipocyte differentiation and function. We have previously shown that PPARγ binding is enriched in the vicinity majority of the genes that are upregulated during adipogenesis, indicating that this transcription factor is directly involved in the regulation of most adipocyte specific genes. Interestingly, we and others have also shown that there is a significant overlap between binding sites of PPARγ and another key adipogenic transcription factor, CCAAT/enhancer binding protein (C/EBP) α. Our data indicate that PPARγ and C/EBPα bind simultaneously to adjacent sites in the genome, sometimes in a cooperative fashion. Consistent with a high degree of interdependence between

PPARγ and C/EBPα binding, we have recently shown that conservation of binding sites of these two factors between mouse and human adipocytes is highly interdependent, i.e. interspecies conservation of shared binding sites is significantly higher than of binding sites where only one of the two factors bind. The majority of the shared PPARγ and C/EBPα binding sites are located in DNase I hypersensitive regions in mature adipocytes, and we propose that these constitute key transcriptional enhancers in adipocytes to which multiple adipogenic transcription factors bind.

Consistent with a major role of PPARγ in adipocyte specific gene expression, the potent agonist Rosiglitazone enhances the expression many of adipocyte specific genes associated with PPARγ binding. However interestingly, other adipocyte specific genes associated with PPARγ are non-affected or repressed. We have used genome-wide technologies to address the molecular basis underlying this difference.

S10.1.5
Liver X receptor activation protects from diabetic neuropathy by restoring fatty acid biosynthesis

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S10.1.6
Glucose-induced increase of abscisic acid (ABA) levels in human plasma and ABA-stimulated glucose uptake by adipocytes

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See Abstract P10.6.

S10.2 Molecular perspectives for diabetes

S10.2.1
Transcriptional cofactors and NAD+ in the control of metabolism

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A century after the identification of a co-enzymatic activity for NAD^+, NAD^+ metabolism has come in the spotlight again due to the potential therapeutic relevance of a set of enzymes whose activity is tightly regulated by the balance between the oxidized and reduced forms of this metabolite. In fact, the actions of NAD^+ have been extended from being an oxidoreductase cofactor for single enzymatic activities to acting as a substrate for a wide range of proteins. These include NAD^+-dependent sirtuin protein deacetylase, poly(ADPribose) polymerases, and transcription factors that affect a large array of cellular functions.

Through these effects NAD^+ provides a direct link between the cellular redox status and the control of signaling and transcriptional events. Of particular interest within the metabolic/endocrine arena are the recent results, which indicate that the regulation of these NAD^+-dependent pathways may have a major contribution to oxidative metabolism and lifespan extension. I will provide an integrated view on how the control of NAD^+ production and cycling, as well as its cellular compartmentalization, alters transcriptional pathways via NAD^+'s commanding role on cofactor networks that involve and SIRT1, GCN5, and PGC-1α.

As such the modulation of NAD^+-producing and -consuming pathways have a major physiological impact and hold promise for the prevention and treatment of metabolic disease.

S10.2.2
Ectodomain shedding proteases acting at the interface of metabolic and vascular disorders

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The TNF-α Converting Enzyme (TACE), also called ADAM17 (A Disintegrin and A Metalloproteinase 17) is a type I transmembrane protein that belongs to a superfamily of Zn dependent metalloproteases. TACE plays a key role in the regulation of the proteolytic release from cellular membranes of some cytokines, chemokines, growth factors, and their receptors, including TNF-α, TNF receptors I and II, TGF-alpha, L-selectin, IL-6, and M-CSF receptor 1. The process is known as ectodomain shedding and affects downstream signaling and cellular responses. TACE knockout mice are not viable and TACE-deficient cells display impaired basal and stimulated cleavage of many target proteins indicating that TACE is a crucial regulator that controls the inflammatory cytokine and growth factor levels in the body. The tissue inhibitors of metalloproteinases 3 (TIMP3), a key endogenous inhibitor involved in regulation of the activity of MMPs and ADAMs, is the only known physiological inhibitor of TACE. Down-regulation of TIMP-3 increases TACE activity while up-regulation of TIMP-3 inhibits TACE activity. Moreover, TIMP-3 deficient mice have shown increased levels of TNF-alpha and severity of inflammation. Interestingly a genetic transmission of TIMP3 deficiency is able to impair glucose tolerance and, TIMP-3 was found to be downregulated in adipose tissue of genetic models of obesity.

The major pro-inflammatory cytokine processed by TACE is TNF-α which is a pleiotropic inflammatory cytokine produced by a number of cell types including macrophages, monocytes, T-cells, and plays a crucial role in the pathogenesis of inflamma-

tion. TNF-alpha acts either in a paracrine manner as a soluble protein through its p55 receptor (TNFR1) or in an autocrine fashion, via interaction of the membrane TNF with TNFR2 (p75). The two receptors may result in different activities being the TNFR1/p55 the dominant one and the responsible for most of the negative effects of TNF-α on metabolic and vascular homeostasis. The ratio between the soluble and membrane TNF forms is regulated by TACE. Increased TACE-mediated shedding of a number of inflammatory markers has been observed in a variety of diseases such as ischemia, heart failure, arthritis, atherosclerosis, diabetes, cancer, neurological and immune diseases and some of the TACE inhibitors are currently in the clinical trials for the prevention of rheumatoid arthritis and cancer.
Our most recent results in human studies and in several experimental models suggest that the TACE/TIMP3 dyad is a common target for cardiometabolic disorders promoted by insulin resistance and atherosclerosis.

S10.2.3
Regulation of cardiac energy metabolism – the role of long-chain fatty acids

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Cardiac substrate metabolism plays a fundamental role in the control of the proper function of the heart. Under resting conditions, the heart derives about 70% of its energy from the oxidation of lipids and about 30% of its energy from glycolysis and glucose oxidation. The elevated use of fatty acids (FA) in the heart has been implicated in a number of metabolic, morphological, and mechanical changes and, more recently, in ''lipotoxicity''. There is evidence that suggests that stearoyl-CoA desaturase (SCD), a rate-limiting enzyme in the biosynthesis of monounsaturated FA, is an important regulating enzyme in the regulation of cardiac substrate utilization and function. The lack of SCD1 expression decreases FA uptake and oxidation and increases glucose transport and oxidation in the heart. Disruption of the SCD1 gene improves the cardiac function in obese leptin-deficient ob/ob mice by correcting the systolic and diastolic dysfunction without affecting the plasma triglyceride and non-esterified FA levels. The rate of FA beta-oxidation is significantly lower in the hearts of ob/ob;SCD1$^{-/-}$ mice when compared to the ob/ob controls. The reduction in myocardial lipid accumulation and inhibition of apoptosis appear to be important factors in the improved left ventricle function in ob/ob mice caused by SCD1 deficiency. These data suggest that overexpression of cardiac SCD contributes to dysregulation of lipid and carbohydrate metabolism in the heart. Furthermore, feeding of high-stearate or high-oleate results in increased oxidation of mitochondrial FA and decreased cardiac glucose uptake. The results point to oleic acid (both dietary and de novo synthesized) as a potential regulator in cardiac substrate utilization. These data also show that endogenous synthesis of oleate in the heart can compensate for the deficiency of this fatty acid in a diet.

S10.2.4
Systems biology for diabetic biomarker discovery

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Type 2 Diabetes is a typical chronic disease that needs many years for progressing, and a typical systems-deficiency that involves metabolic dysfunctions of many different tissues of the body. Understanding the molecular properties of diabetic progression at systems-level is a big challenge in the systems-biology era. We reason that blood plasma proteome is an ideal ''window'' for observing global and dynamic progression of type 2 diabetes, since the circulation system is basic ''highways'' of the body for transporting all kinds of metabolic related molecules and, importantly, the plasma proteome is considered as the most comprehensive and largest version consisting of many different proteins that are secreted, or shed, or leaked from cells and tissues throughout the body. We have developed systematic approaches based on proteomics and bioinformatics to analyze human serum from normal and diabetes, in which a label-free analysis based on peptide spectral counts and a new bioinformatics tool has been applied to analyze the diabetes-associated proteins. In addition, we present a dynamic analysis of diabetic rat-plasma proteome from pre-diabetic stage to diabetic stage by combining mass spectrometric and mathematical techniques. Based on computing analysis of 553 overlapped plasma proteins between diabetic rat, Goto-Kakizaki (GK) rat, and non-diabetic rat, Wistar rat, we first time demonstrate that GK rats preserved a larger entropic values than Wistar rats at systems-level throughout time. Furthermore, using our newly developed computing approach, we characterize 14 featured plasma proteins either significantly overrepresented or underrepresented all the time as well 112 dynamic plasma proteins significantly differentiated from time to time during the diabetic progression.

S10.2.5
Class I histone deacetylases and energy metabolism: new players in ''diabesity''?

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See Abstract P10.10

S10.2.6
Evaluation of metabolic status in type 2 diabetes mellitus patients by proton nuclear magnetic resonance spectroscopy method (1H-NMR)

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See Abstract YSF.115.

S10.3 Redox balance and obesity

S10.3.1
Redox balance and adipogenesis

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Long considered only to be the main energy store of the body, white adipose tissue (WAT) now appears to be an endocrine organ able to interconnect all physiological functions to this energy store. For long time, the importance of the role of mitochondria in fat was only restricted on the investigations on brown adipose tissue mitochondria and Uncoupling Protein-1 although WAT oxidative potential is really significant. From increasing reports, an emerging hypothesis now considers mROS as an integrated physiological signal of the metabolic status of the cell. For adipose tissue, several studies provide significant clues to consider mROS as a basic fundamental and physiological signal including for its endocrine function and development in close link with its own energetic status. Thus, mROS and bioenergetics in adipose tissue appear as key elements not only to understand the physiopathology of metabolic diseases but also as interesting therapeutic targets to treat these diseases.

S10.3.2
Interaction between oxidative stress and inflammation in obesity

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A primary event in atherogenesis is the infiltration of activated inflammatory cells into the arterial wall. They there secrete reactive oxygen species and oxidize lipoproteins, inducing foam cell formation, and endothelial cell apoptosis, which in turn lead to plaque growth, erosion, and rupture. Importantly, this vicious circle between oxidative stress and inflammation does not only occur in the diseased arterial wall, but also in adipose tissues during obesity. This observation raised question what molecules are likely common regulators of these pathogenic processes in adipose and vascular tissues. We identified interleukin-1 receptor-associated kinase-3 as downregulated key inhibitor of NFκB-mediated chronic inflammation in blood monocytes and adipose tissue macrophages associated with oxidative stress and insulin resistance. Possible regulators of oxidative stress and inflammation are small, non-coding microRNAs (miRs). They control gene expression by inducing mRNA degradation or blocking translation. For example, let-7, miR-17, -21, -27b, -34a, -92, -125b, -130a, -132, -143, -150, -155, -210, -221, and -222 are deregulated in both tissues. Interestingly, they are at cross-roads with processes that play a role in the development of dyslipidemia, insulin resistance, atherosclerosis and plaque ruptures in association with obesity. MiRs are packaged in secreted microvesicles and contribute in the communication between cells. For example, high glucose reduces the miR-126 content of endothelium-derived vesicles resulting in increased VCAM-1 expression. In addition, adipocyte-related miRs (let-7b, miR-103, miR-146b and miR-148a) and gene transcripts (adiponectin) are packaged into microvesicles and delivered into vascular tissues where they are involved in atherogenesis. In conclusion, the miR-mediated natural ''gene silencing'' mechanism is important for regulating disease mechanisms associated with obesity-associated atherosclerosis and cardiovascular diseases.

S10.3.3
Mitochondria-targeted penetrating ions as inhibitors of the aging program and obesity

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There are indications that mitochondrial reactive oxygen species (ROS) operate as poisons inducing programmed death of organisms (phenoptosis). Two types of phenoptotic events can be distinguished: (i) fast ''biochemical suicide'' (e.g. sepsis caused by substances of bacterial or mitochondrial origin); (ii) aging. Antioxidants specifically targeted to mitochondria might be used to treat such pathologies. Recently we synthesized a compound called SkQ1 composed of plastoquinone and a decyltriphenylphosphonium cation. SkQ1 was shown to electrophoretically target into mitochondria. In isolated mitochondria *in vitro*, it was shown that nanomolar SkQ1 prevents peroxidation of cardiolipin. Higher SkQ1 concentrations were found to organize fatty acid cycling and ''mild'' uncoupling, an effect resulting in inhibition of the State 4 ROS formation. In human cell cultures, it was shown that (i) SkQ specifically accumulates in mitochondria; (ii) at nanomolar concentrations it arrests apoptosis initiated by added H2O2. Short-term *in vivo* treatment of animals with SkQ was shown to decrease tissue damage and to prevent death under conditions of experimental heart, brain, or kidney ischemia and rhabdomyolysis. Life-long treatment with SkQ1 was found to prolong lifespan of fungi, drosophila, fish, mice, mole-voles, and dwarf hamsters. Appearance of numerous traits of aging was found to be slowed down by SkQ1. These include osteoporosis, achromotrichia, balding, decrease in lymphocyte/neutrophil ratio, slow wound healing, disappearance of reproductive ability, retinopathies, cataract, glaucoma, etc. A group of 40 patients suffering from the age-linked disease ''dry eye'' were treated for 3 weeks with drops of SkQ1. A clear positive therapeutic effect and no adverse side effects were observed. A study of penetrating cations revealed that dodecylrhodamine 19 is efficient as a mild cationic uncoupler even without free fatty acids. It is promising as a tool to treat aging and obesity.

S10.3.4
p53 family, involvement of p73 in metabolism and senescence: why we need it?

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In the last ten years, p63 and p73 have been identified as the ancestral members of the p53 family. Despite the high sequence and structural similarity, the mouse knockouts revealed a crucial role in neural development for p73 and in epidermal formation for p63. We identified several transcriptional targets, the mechanisms of regulation of cell death, and the p63 isoform involved in epithelial development. Both genes are involved in female infertility and maternal reproduction as well as in cancer formation, although with distinct mechanisms.

p73 steady state protein levels are kept low under normal physiological conditions through degradation by the 26S proteasome, mediated by the HECT-containing E3 ubiquitin ligase ITCH, for which we are developing an inhibitor. We have also described additional mechanisms of degradation: (i) the orphan F-box protein FBXO45; (ii) the ring finger domain ubiquitin ligase PIR2 (p73-induced Ring Finger 2); (iii) the antizyme ubiquitin-independent, proteasome-dependent pathway, both specific for the ΔNp73 isoforms.

TAp73 knockout mice (TW Mak G&D 2008) show high tumor incidence with hippocampal dysegensis. Conversely, ΔNp73 knockout mice (TW Mak G&D 2010) show a very low incidence of cancer, with sign of moderate neurodegeneration with a significant loss of cellularity in the cortex. This indicate a tumor suppressor role for TAp73 and an oncogenic role for ΔNp73.
Here, we describe the involvement of p73 in senescence and metabolism. TAp73-null mice show a significant premature spontaneous aging phenotype at 12 months of age: alopecia, epidermal thinning, reduced subucutaneous fat, increased visceral fat TAp73, osteoporosis with scoliosis. This indicate a significant phenotype related to obesity and ageing. Both *in vivo* and *in vivo* TAp73-null mice show unbalanced redox defences. TAp73 is able to drive the expression of glutamiase type 2 (GLS2), acting on specific binding sites present on its promoter. In agreement with these *in vitro* data, TAp73-null cells show clear metabolic defects in the glutamine pathway affecting GSH and redox balance. In keeping, we show a role for TAp73 in the regulation of metabolic pathways.
Finally, we will speculate on why we need the p53 family at evolutionary level: it is the guardian of maternal reproduction.

S10.3.5
Hematopoietic heme oxygenase-1 impacts obesity-induced adipose macrophage infiltration and insulin resistance

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See Abstract P10.20

S10.3.6
Effects of exercise intensity on global hepatic mRNA expression in high fat-induced obese mice

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See Abstract P10.9.

S11 – Recent advances in cancer biology

S11.1 Genes and pathways in cancer

S11.1.1
Dissecting mechanisms of cancer drug resistance through functional genetics

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Unresponsiveness to therapy remains a significant problem in the treatment of cancer, also with the new classes of cancer drugs. In my laboratory, we use functional genetic approaches to identify biomarkers that can predict responsiveness to targeted cancer therapeutics; drugs that specifically inhibit molecules or pathways that are often activated in cancer. Nevertheless, it remains poorly explained why a significant number of tumors do not respond to these therapies. We aim to elucidate the molecular pathways that contribute to unresponsiveness to targeted cancer therapeutics using a functional genetic approach. This will yield biomarkers that may be useful to predict how individual patients will respond to these drugs. Furthermore, this work may allow the development of drugs that act in synergy with the established drug to prevent or overcome drug resistance.

To identify biomarkers that control tumor cell responsiveness to cancer therapeutics, we use multiple complementary approaches. First, we use genome wide loss-of-function genetic screens (with shRNA interference libraries) in cancer cells that are sensitive to the drug-of-interest to search for genes whose down-regulation confers resistance to the drug-of-interest (resistance screens). In addition, we use single well siRNA screens with a low dose of the drug to screen for genes whose inhibition enhances the toxicity of the cancer drug (sensitizer screens). As a third approach, we use gain of function genetic screens in which we search for genes whose over-expression modulates drug responsiveness. Once we have identified candidate drug response biomarkers in relevant cell line models, we ask if the expression of these genes is correlated with clinical response to the drug-of-interest. For this, we use tumor samples of cancer patients treated with the drug in question and whose response to therapy is documented.

In a fourth and distinct approach we perform high throughput sequencing of the ''kinome'' (some 600 genes) of tumor samples to identify connections between cancer genotype and drug responses.

Examples of these four approaches to identify mechanisms of resistance to different cancer drugs will be presented

S11.1.2
Rho GTPase signalling in invasion and metastasis

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Tumour cell migration is an essential component of invasion and metastasis. Rho-family GTPase signalling coordinates the dynamic cytoskeletal changes that are required for cell migration. To delineate Rho-family GTPase signalling in cell migration we are carrying out a systematic analysis of Rho-family GTPases their regulators and signalling pathways. Using RNA interference to target all Rho-family GTPases, guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) we have described pathways controlling two distinct forms of movement in melanoma cells. Elongated, mesenchymal-type movement is driven by Rac activation mediated by a pathway containing the adaptor protein NEDD9 and the exchange factor DOCK3. In contrast rounded/amoeboid movement is driven by Rho and Cdc42 signalling to actomyosin contractility and suppressed by Rac activation. To investigate how Rho GTPase signalling is regulated we have studied upstream signalling events regulating the activation of Rac and actomyosin contractility. These studies reveal new insights into transmembrane signalling to Rho GTPases and demonstrate a tight interplay between Rho and Rac signalling in determining modes of cell movement.

S11.1.3
Evolution of the cancer genome

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All cancers carry somatically acquired changes in their genomes. Some, termed ''driver'' mutations, are causally implicated in cancer development. The remainder are ''passengers'', and bear the imprints of mutational processes operative during cancer development. Following the advent of second generation sequencing technologies the provision of whole cancer genome sequences has become a reality. These sequences generate comprehensive catalogues of somatic mutations, including point mutations, rearrangements and copy number changes and provide insights into the evolutionary processes underlying the development of individual human cancers including the factors generating variation and the forces of selection. These insights will form the foundation of our understanding of cancer causation, prevention and treatment in the future.

S11.1.4
AMP-activated protein kinase, a tumor suppressor that opposes the metabolic changes in cancer cells

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AMP-activated protein kinase (AMPK) occurs in all eukaryotes as complexes comprising catalytic α subunits and regulatory β and γ subunits. The kinase is only active after phosphorylation of Thr172 on the α subunit by upstream kinases. The latter include LKB1, originally identified as a tumor suppressor mutated in the inherited cancer susceptibility, Peutz-Jeghers syndrome. LKB1 function is also lost in many spontaneous, non-inherited cancers. Although there are other protein kinases downstream of LKB1, AMPK is the only one known to cause cell cycle arrest and inhibition of cell growth, and it seems likely that it mediates many of the tumor suppressor actions of LKB1. LKB1 appears to be constitutively active and to phosphorylate Thr172 on AMPK continually. However, binding of ADP or AMP to the γ subunit of AMPK inhibits dephosphorylation of Thr172. Thus, increases in ADP and/or AMP during metabolic stress trigger a switch to the active, phosphorylated form. Once activated, AMPK effects a switch away from anabolism, growth and the cell cycle and towards catabolism and quiescence. In rap-

idly proliferating cells, the TCA cycle changes from being a catabolic pathway generating ATP to an anabolic pathway providing precursors for biosynthesis of cellular building blocks, particularly fatty acids for phospholipid synthesis. AMPK opposes these changes, for example by switching off fatty acid synthesis and switching on the catabolic function of the TCA cycle. The evidence that AMPK-activating drugs provide protection against cancer will be discussed. One might expect that rapidly proliferating cells would have a large demand for ATP and that AMPK would therefore be activated within them. However, since AMPK restrains growth and progress through the cell cycle, this would limit cell proliferation. Many tumors appear to have been selected for changes that circumvent this limitation by inactivating AMPK: the various mechanisms by which this might happen will be discussed.

S11.1.5
Characterization of amplicon junction sequences in genomic regions surrounding the MYCN gene in neuroblastoma tumors; Implications for clinical follow-up of high-risk patients

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See Abstract P11.75.

S11.1.6
Hydrogen sulfide promotes calcium signals and migration in tumor-derived endothelial cells

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See Abstract P11.120.

S11.2 Cancer stem cells and metastasis

S11.2.1
Circulating tumor cells and cancer micrometastasis

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The major problem in the clinical management of cancer patients is the development of metastases. Early spread of tumor cells is usually undetected by current imaging technologies. Therefore, sensitive methods have been developed to detect circulating tumor cells (CTC) in the peripheral blood and disseminated tumor cells (DTC) in the bone marrow of cancer patients without signs of overt metastases. These technologies can be classified into cytometric and/or immunological and molecular approaches. Recent data suggest that the bone marrow might be a common homing organ for cells derived from various epithelial tumors, and data from European and US groups have sustained the prognostic impact of DTC in the BM of breast cancer patients. However, a significant fraction of DTC survive in a ''dormant'' stage for years, and little is known about the conditions required for the persistence of dormancy or the escape from the dormant phase into the active phase of metastasis formation. Sequential peripheral blood analyses are much more convenient for patients than invasive BM analyses in patients with solid tumors and many research groups are currently assessing the clinical utility of CTC for assessment of prognosis and monitoring of systemic therapy. The molecular assessment of CTC among 1×10^6 ''normal'' blood cells is technically challenging and requires subtle and robust enrichment and analysis techniques. During the last years, advantages have been made in the development of such techniques and molecular characterization of single tumor cells becomes more and more possible. In view of the number of available prognostic indicators, monitoring of CTC during and after systemic adjuvant therapy might provide unique information for the clinical management of the individual cancer patient and may allow an early change in therapy years before the appearance of overt metastases as detected by current imaging techniques.

S11.2.2
Intravital imaging of cancer invasion and experimental therapy response: role of integrins

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The tumor microenvironment contributes to cancer invasion, growth and survival and thereby impacts tumor responses to therapy. Multiphoton microscopy (MPM) has become the method of choice for investigating cell structure and function in tissues and organs, including the invasion and progression of cancer lesions. Using a novel approach of infrared-excited (IR−)MPM at wavelengths above 1080 nm that enhances deep tissue microscopy in orthotopic fibrosarcoma xenografts, we here show deep collective invasion strands of several hundred connected cells. Invasion was fast (up to 200 μm/day), non-destructive and independent of β1 and β3 integrins. Despite normoxia, perivascular invasion strands were resistant to high-dose hypofractionated irradiation which otherwise was sufficient to induce regression of the tumor main mass. This invasion-associated radioresistance was sensitive to the simultaneous inhibition of β1 and β3 integrins by RNA interference or combined anti-β1/aV integrin antibody treatment caused byproliferation arrest, anoikis induction ablating both tumor lesion and invasion strands. Thus, collective invasion is an important invasion mode in solid tumors into a microenvironmentally priviledged perivascular survival niche which conveys radioresistance by integrin-dependent signals. Consequently, combining anti-integrin therapy with hypofractionated irradiation may be amenable to clinical cancer treatment of locally destructive and otherwise radioresistant tumor lesions.

S11.2.3
MET and invasive growth: a genetic program for stem and cancer stem cells

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Metastasis follows the inappropriate activation of a genetic programme termed invasive growth, which is a physiological process

that occurs during embryonic development and post-natal organ regeneration. Burgeoning evidence indicates that invasive growth is also executed by stem and progenitor cells, and is usurped by cancer stem cells. The MET proto-oncogene, which is expressed in both stem and cancer cells, is a key regulator of invasive growth. MET encodes the tyrosine-kinase receptor for ''Scatter Factor'', a sensor of adverse microenvironmental conditions (such as hypoxia) and drives cell invasion and metastasis through the transcriptional activation of a set of genes, including those controlling blood coagulation. In cancer cells the MET tyrosine kinase stimulates cell scattering, invasion, protection from apoptosis and angiogenesis, thereby acting as a powerful expedient for dissemination. In some cancers, MET has been genetically selected for the long-term maintenance oft he primary transformed phenotype, and those cancers appear to be dependent on (or ''addicted'' to) sustained MET activity for their growth and survival. Because of its dual role as an adjuvant, pro-metastatic gene for some tumour types and as a necessary oncogene for others, MET is a promising target for therapeutic intervention. Recent progress in the development of molecules that inhibit MET function will be discussed and their application in the subset of human tumours potentially responsive will be considered.

S11.2.4
Mechanisms of metastasis suppression

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Breast cancer is a heterogeneous disease, both biologically and clinically. An important parameter in the clinical management of breast cancer patients is the assessment of expression of steroid hormone receptors [estrogen (ER) and progesterone (PR) receptors] and HER2 overexpression/amplification; effective tailored therapies have in fact been developed for patients with hormone receptor-positive or HER2-positive diseases. In contrast, Triple Negative Breast Cancer (TNBC) is merely defined by lack of expression of ER, PR and HER2, and thus defines a category that combines tumors that are clinically and pathologically diverse, for which we strive to identify tumor-addicted molecular pathways. Indeed, understanding TNBC is of pivotal importance not only in light of the current lack of therapeutic options, but also because TNBC accounts for some of most aggressive types of breast cancers, marked by high rates of relapse, visceral metastases and early death. To shed light on some of these outstanding issues, we undertook this study by asking about the signaling networks and mechanisms promoting malignancy and metastastic spread of TNBC. Using bioinformatic tools, we interrogated Triple Negative Breast cancer (TNBC) clinical datasets about candidate signals and molecular players mediating the malignancy of this heterogeneous class of tumors. We found that the activities of p63 and Hypoxia-Inducible-Factors (HIFs), two master regulators of the invasive and metastatic cancer cell phenotype, are unanticipatedly linked in TNBC. The p63 target Sharp1 is at the center of this crosstalk. At the meeting I will present the underlying mechanism by which p63, Sharp1 and HIF regulate TNBC invasiveness and metastatic propensity.

S11.2.5
A genome-scale protein interaction profile of *Drosophila* p53 uncovers additional nodes of the human p53 network

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See Abstract YSF.25.

S11.2.6
Regulation of metabolism in health and disease by cancer genes: a new function for the promyelocytic leukemia protein

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See Abstract P11.18.

S12 – Cellular senescence and aging

S12.1 Plasticity of aging

S12.1.1
Sirtuin activating compounds, resveratrol and SRT1720, extend healthspan and lifespan of C57BL6 male mice

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Activation of SIRT1, and NAD^+-dependent deacetylase sensitive to nutrient availability, promotes longevity in animals ranging from yeast to mammals. Here, we present data on the treatment with resveratrol and SRT1720, activators of SIRT1. They both extend the mean and maximal lifespan of male mice fed a high fat diet (HFD) starting after 1 year of age. This lifespan extension is accompanied by numerous benefits to health including reduced liver steatosis, increased insulin sensitivity, enhanced locomotor activity and normalization of gene expression profiles and markers of inflammation and apoptosis, all in the absence of any observable toxicity. The ability of resveratrol or SRT1720 to increase cell survival and mitochondrial respiration (or oxygen consumption) were Sirt1-dependent, demonstrating the Sirt1-driven nature of these effects. These findings extend the known beneficial effects of resveratrol and SRT1720.
*Presenter.

S12.1.2
Nutrient-sensing pathways and ageing

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Genetic screens have revealed that healthy lifespan can be extended in laboratory animals. Furthermore, in at least some cases the mechanisms involved are conserved over large evolutionary distances. For instance, both reduced food intake itself and reduced activity of nutrient signalling network can increase lifespan in budding yeast, nematode worms, fruit flies and mice. In contrast, recent work with worms and flies suggests that the role of Sirtuins in ageing may be more restricted. Our work with *Drosophila* has elucidated the role of the key forkhead transcription factor dFOXO in extension of lifespan by reduced insulin signalling.

S12.1.3
TOR signaling, from yeast to human

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TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, cellular energy, and stress. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. The discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR is found in two structurally and functionally distinct multiprotein complexes, TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.
While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Data on the role of mTORC1 and mTORC2 in specific tissues will be presented. Recent results on the mechanism of mTORC2 regulation will also be presented.

S12.1.4
Caloric restriction and aging

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Life expectancy in the world has increased dramatically during the last century; the number of older adults is expected to rise while the number of youth will decline in the next future. This demographic shift has considerable public health and economic implications since aging is associated with the development of serious chronic diseases. Calorie restriction (CR) is the most effective nutritional intervention for slowing aging and preventing chronic disease in rodents. In non-human and human primates CR with adequate nutrition protects against abdominal obesity, diabetes, hypertension and cardiovascular diseases. Cancer morbidity and mortality are also diminished in CR monkeys, and data obtained from individuals practicing long-term CR show a reduction of metabolic and molecular factors associated with increased cancer risk and aging.

S12.1.5
Effects of dietary restriction and ageing on mitochondrial function in male C57BL/6 mice

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See Abstract P12.10.

S12.2 Cellular senescence

S12.2.1
Senescence and tumor suppression

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Human melanocytic nevi (moles) are benign neoplasms commonly harboring activating mutations in BRAF or NRAS. Although nevi are considered to be precursors of melanoma, a highly aggressive type of (skin) cancer, little is known about the mechanism underlying progression from nevus to melanoma. We and others have previously shown that, although initially, activated BRAF and NRAS oncogenes act mitogenically, eventually oncogene-induced senescence (OIS) ensues. This has led to the hypothesis that abrogation of OIS of nevus cells acts as a rate-limiting event in melanomagenesis. Favoring this model, nevi and melanomas are commonly, and significantly, histologically associated. Indeed, malignant melanoma can emerge within a nevus.
In addition to the activation of the ERK pathway, other frequent genetic events include the loss of the CDKN2A and ARF genes. Although the involvement of p16 in melanomagenesis is undisputed, there is little evidence to support a non-redundant role for p16 in BRAF(E600)- or NRAS(K61)-induced senescence, neither *in vitro* nor *in vivo*. Another common genetic event in melanoma is the activation of the PI3K pathway, which is seen in ~60% of cases. This is achieved by loss of PTEN expression, increased AKT3 activity or mutations in PIK3CA. Interestingly, some 20% of melanomas show concurrent mutation in BRAF and diminished expression of PTEN.
We have found that in cultured human melanocytes, BRAF(E600)-induced senescence is accompanied by suppression of AKT3. Activation of the PI3K pathway by ectopic expression of AKT or PIK3CA, or depletion of PTEN, abrogates senescence. Correspondingly, in a series of contiguous human nevus-melanoma specimens, we observed a decrease in PTEN and/or an increase in AKT3 in the melanoma relative to the adjacent nevus in >50% of the cases. In several of these, laser microdissection-guided genetic analysis revealed identical mutations in BRAF or NRAS in the nevus and contiguous melanoma, including a rare double mutation, which is in support of a nevus-to-melanoma progression model. These findings indicate that PI3K pathway activation serves as a rate-limiting event in human melanoma progression, acting at least in part by abrogating OIS. This provides an explanation for the frequent co-occurrence of mutations in BRAF and the PI3K pathway in melanoma.

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S12.2.2
Checkpoint responses to telomere dysfunction in stem cells

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Telomeres limit the proliferative lifespan of primary human fibroblasts by induction of cell cycle arrest (senescence) or p53-independent cell death (crisis). *In vivo* studies on telomerase deficient mice ($mTerc^{-/-}$) showed that telomere shortening affects the maintenance of organ systems with high-rates of cell turnover by impairing the function of stem and progenitor cells. Telomere dysfunction limits stem cell maintenance and differentiation by activation of cell intrinsic checkpoints and by induction of alteration in the cell environment (stem cell niche and circulatory environment). I will present new data on the role of p53-dependent apoptosis in telomere dysfunction induced aging of stem cells and tissues. Moreover, I will discuss novel approaches using functional genomics *in vivo* screens (RNAi) for the identification of novel checkpoints that are induced at stem cell level in response to telomere dysfunction. Given the pivotal role of stem cells in tissues aging and cancer formation, these studies will contribute to our understanding of both processes.

S12.2.3
Molecular mechanisms of cellular senescence

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Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. We have previously shown that expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Our most recent results on the interplay between DDR and heterochromatin formation, the differential repair of the human genome, the regulation of DDR in stem cells and our search for novel pathways regulating genome stability will be discussed.

S12.2.4
Cellular senescence links inflammation and aging

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Age is the largest single risk factor for developing a panoply of diseases. Most age-related diseases are degenerative in nature – that is, cells and tissues lose integrity and/or function. An exception is cancer, a hyperproliferative disease that entails the acquisition of new, albeit aberrant, cellular phenotypes and tissue structure. Is there, then, a common biology that links aging, degenerative disease and cancer? We propose that one common

link is the accumulation of senescent cells. Cellular senescence is a potent tumor suppressive response by which cells irreversibly arrest proliferation and acquire a robust pro-inflammatory secretory phenotype (the senescence-associated secretory phenotype or SASP). Cells undergo senescence in response to a wide range of potentially oncogenic stimuli. Moreover, senescent cells accumulate in aged tissues and at sites of age-related pathology, both degenerative and hyperproliferative. We and others have shown that senescent cells, and particularly the SASP, can disrupt normal tissue structure and function. Moreover, the SASP can fuel cancer progression. Suppressing the SASP, then, may be key to ameliorating both the degenerative diseases of aging and cancer. We have identified several pathways that, when dampened, suppress the SASP. These include the DNA damage response pathway, the p38MAPK-NF-kB pathway, and the evolutionarily conserved mTOR pathway.

S12.2.5
Does activation of DNA damage response in human T cells leads to cellular senescence?

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See Abstract YSF.56.

S12.2.6
Adoptive expression of a set of miRNAs induces cellular senescence in human fibroblasts

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See Abstract P12.7.

S13 – Rare diseases reveal new biochemical mechanisms

S13.1 Rare metabolic diseases

S13.1.1
The connectivity of genomic elements

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To understand the involvement of the genome in health and disease requires not only the elucidation of the function of each nucleotide of the genome, and of each variant of the genome, but also the physical and functional connectivity of the different genomic elements.
Three such examples of genomic connectivity from our laboratory will be presented: (i) the connectivity of protein-exons and the network of potentially functional transcipts; (ii) the connectivity of the conserved non-coding elements (CNC) using chromatin conformation experiments; (iii) the regulatory connectivity of eQTLs (expression quantitative trait loci) with gene expression variation of protein-coding genes and miRs. Studies on cell-specific eQTLs will be presented. In addition, the potential role of short sequence repeats as causative eQTLs will be discussed.
These illustrative examples provide evidence for the complexity of the genome function, and the challenges ahead for the understanding the pathophysiology of the myriad genomic disorders and phenotypic traits.

S13.1.2
Pathogenesis of diseases involving defects in Rab GTPase function and membrane traffic

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Several inherited human disorders have been associated with defects in Rab GTPase activity, either directly or indirectly. One of those is Choroideremia (CHM), an X-linked late-onset retinal degeneration characterised by progressive dystrophy of photoreceptors, retinal pigment epithelium (RPE) and the choroid. CHM is due to a defect in Rab Escort Protein 1 (REP1), a cofactor required for the prenylation of Rab GTPases. CHM is therefore a disease of intracellular membrane traffic but the events that trigger degeneration remain to be elucidated. We have generated mouse models for Choroideremia using conditional gene knockout (KO), cre-lox technology and studied them using a combination of techniques including histopathology, *in vitro* assays and functional analysis. We have shown that RPE disease accelerates photoreceptor degeneration, highlighting the central role of RPE in pathogenesis. Furthermore, in a RPE-restricted Rep1 KO, the RPE ages prematurely, accumulating pathological changes at 5–6 months of age, which are more exuberant than those observed in 2-year old controls. Extracellularly, we observed thickening of Bruchs membrane and accumulation of basal laminar deposits (BLamDs) and intracellularly, disorganisation of basal infoldings and accumulation of lipofuscin, the age pigment. This is accompanied by defects in intracellular membrane traffic pathways, including melanosome movement and phagosome processing. These phenotypes suggest that multiple chronic defects in membrane traffic pathways accelerate the ageing process in the RPE. Furthermore, the striking similarities between the present observations and those reported in age-related macular degeneration (AMD), a leading cause of blindness in the developed world, suggest that membrane traffic defects may contribute to the pathogenesis of AMD and could represent a new focus of therapeutic strategies.

S13.1.3
Nature and nurture in genetic diseases: the cases of hereditary fructose intolerance and hyperphenylalaninemias

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Some genetic diseases result from specific common nutrients, the metabolism of which is impaired by single gene alterations. Two examples are hereditary fructose intolerance (HFI) and pheylketonuria (PKU), in which, because of the gene alteration, some metabolites cannot be used by the organism: fructose in HFI, phenylalanine in hyperphenylalaninemias (HPAs). The deleterious effects of these alterations can be abolished by dietary elimination of the nutrients. Thus, early identification of the gene defect can be relevant in terms of starting an elimination diet.-Several years ago, we began studying HFI and contributed to the aldoB mutation spectrum. We also defined the functional-structural consequences of some natural missense variants on aldoB enzyme activity by in vitro assay and molecular modeling of recombinant mutated enzymes. The identification of only one aldoB mutation in symptomatic patients suggests that HFI symptoms can, albeit rarely, appear also in heterozygotes. HPAs result from PAH enzyme dysfunction. Specific alterations of the corresponding gene affect phenylalanine metabolism. In this field we studied some new missense mutations by analyzing the functional impairment of recombinant proteins; we also studied in vitro and in vivo the BH4 responsiveness of mutated enzymes. More recently, we reported that, in several mild mutations, conformational stability and oligomerization defects are the most relevant causes of HPAs. Lastly, we found that delivery of vector-dependent adenoviral vectors in mice caused complete normalization, reversal of coat hypopigmentation, restoration of spatial learning deficit and long-term potentiation, involving N-methyldiaspartate and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptor subunits. To conclude, analysis of functional-structural gene alterations and the study of variant proteins in monogenic diseases may reveal new biochemical mechanisms and the metabolic pathways deriving from the disease.

S13.1.4
Pathogenic mechanisms in Lafora's disease

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Lafora progressive myoclonus epilepsy (LD) is a fatal autosomal recessive neurodegenerative disorder characterized by the presence of glycogen-like intracellular inclusions called Lafora bodies (LB). LD involves at least two genes, EPM2A and EPM2B, encoding respectively laforin, a dual-specificity protein phosphatase, and malin, an E3 ubiquitin ligase. Functional characterization of LD-associated EPM2A and EPM2B mutations illustrated

that the enzymatic activities of laforin and malin, and their protein-protein interactions are critical in the pathogenesis of LD. In a series of recent papers, we and others have illustrated that the laforin-malin complex causes proteasome-dependent degradation of proteins implicated in glycogen metabolism and that laforin plays a crucial role in the dephosphorylation of glycogen and in the modulation of the intracellular proteolysis system. I will summarize these findings and recent studies in two LD mice models. As a whole these data provide insights into the process of LB formation and are beginning to delineate the relative contributions to the pathogenesis of LD of the different cellular processes that are altered by the absence of laforin or malin.

S13.1.5
Isolation and cloning of stimulatory anti-PDGF receptor auto-antibodies from the immunological repertoire of patients with systemic sclerosis

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See Abstract P13.11

S13.1.6
Aberrant splicing reverts a potentially lethal coagulation deficiency caused by A +1G/T splicing mutation

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See Abstract P13.14.

S13.2 Mitochondrial diseases

S13.2.1
OXPHOS-related mechanisms and pathways unraveled by new mitochondrial disorders

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The tremendous clinical, biochemical and molecular heterogeneity of mitochondrial disorders makes virtually each member of the whole mitochondrial proteome a candidate for disease. As a result, only 40% of adult-onset disorders are currently diagnosed at the molecular level, and much lesser so in infantile syndromes. However, new technological and biocomputational tools offer the possibility of rapid and affordable analysis of the exome, i.e. the coding regions of all genes in single individuals or small families. Mitochondrial disease proteins can then be selected by exploiting predictive softwares, dedicated databases, and *ex vivo* experiments. We have identified several new disease genes, including Surf1, Mpv17, ETHE1, FASTKD2, SDHAF1, AIF, and TTC19, each responsible of distinct defects of the respiratory chain, mtDNA metabolism, or both. Structural analysis based on blue-native gel electrophoresis has allowed us to identify the molecular consequences of the ablation or defects of these proteins, and their physical status in normal and disease conditions. To gain further insight on the functional role of these disease proteins, we have then created specific recombinant lines in yeast, flies, and mice. For instance, yeast SDHAF1 KO faithfully replicates the defect of complex II found in patients, allowing us to validate the corresponding mutations *in vivo*. Similar to human TTC19-less patients, a TTC19 KO fly displays profound complex III deficiency and an adult-onset neurological phenotype that mimics the neurodegenerative process observed in humans. However, complex III activity is normal in larval stages, suggesting a previously ignored development-dependent regulation of complex III assembly in animals. Finally, an Ethe1 KO mouse develops a disease similar to human Ethylmalonic Encephalopathy, and has allowed us to dissect out the pathophysiology of, and test an effective treatment for, this mitochondrial disorder of sulfur metabolism.

S13.2.2
Human diseases with impaired mitochondrial translation

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Mitochondrial respiratory chain deficiencies represent one of the major causes of metabolic disorders that are related to genetic defects in mitochondrial or nuclear DNA. The mitochondrial translation allows the synthesis of the 13 respiratory chain subunits encoded by mtDNA. Altogether, about 100 different proteins are involved in the translation of the 13 proteins encoded by the mitochondrial genome emphasizing the considerable investment required to maintain mitochondrial genetic system. Translation deficiency can be caused by mutations in any component of the translation apparatus including tRNA, rRNA and proteins. Mutations in mitochondrial rRNA and tRNAs have been first identified in various forms of mitochondrial disorders. Moreover abnormal translation due to mutation in nuclear genes encoding tRNA modifying enzymes, ribosomal proteins, aminoacyl-tRNA synthetases, elongation and termination factors and translational activators have been successively described. These deficiencies are characterized by a huge clinical and genetic heterogeneity hampering to establish genotype-phenotype correlations hampering an easy diagnosis. One can hypothesized that new techniques for gene identification, such as exome sequencing will rapidly allow to expand the list of genes involved in abnormal mitochondrial translation.

S13.2.3
Energyomics-energenomics: a mitochondrial etiology of common diseases

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Human genetics is rooted in three concepts: natural selection drives evolution, organ-specific symptoms result from tissue-specific defects, and disease genes are Mendelian. However, these paradigms cannot explain the common diseases. Life involves the interplay of anatomy, energy, and information. Biological information is generated by the energy flow through matter accumulated over the millennia in DNA.

In animals energy is generated through the mitochondrion and partial energy deficiency results in malfunction of the highest energy tissues: brain, heart, muscle, renal, and endocrine. The mitochondrial genome encompasses thousands of copies of the maternally-inherited mtDNA which encodes the core electrical elements of oxidative phosphorylation (OXPHOS) plus hundreds of nuclear DNA (nDNA)-encoded genes. The mtDNA has a very high mutation rate filtered by ovarian selection. The more deleterious mutations cause inherited diseases while the milder variants were originally adaptive but now can predispose to disease. mtDNA mutations also accumulate in somatic cells accounting for the aging clock and the delayed-onset and progressive course of "complex" diseases. Cellular phenotypes are influenced by the epigenomic modulation of the nDNA-encoded mitochondrial genes mediated by mitochondrial ATP, acetyl-CoA, and SAM modification of proteins. Thus, mtDNA mutations and epigenomic regulation nDNA energy genes are the primary risk factors for common diseases. For example, Alzheimer and Parkinson Disease (AD, PD) risk is influenced by mtDNA haplogroup (e.g., the H5a haplogroup with the tRNAGln A4336G variant accounts for up to 7% of AD-PD). Somatic mtDNA mutations are elevated in the brains of AD, PD, and Down Syndrome with dementia (DSAD) patients and the increased mtDNA mutation rates are systemic. Consequently, AD and DSAD brains have reduced L-strand transcription and mtDNA copy number. Thus, the etiology of "complex" diseases is mitochondrial energy deficiency.

S13.2.4
Understanding mitochondrial complex I assembly and misassembly in disease

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Mitochondrial Complex I (NADH:ubiquinone oxidoreductase) is an ~980 kDa multimeric enzyme composed of 45 subunits. The assembly of human Complex I is poorly understood, complicated by its large size and its regulation by two genomes, with seven subunits encoded by mitochondrial DNA and the remainder encoded by nuclear genes. We have built a model of Complex I assembly by monitoring the incorporation of radiolabeled mtDNA-encoded translation products and newly-imported nuclear-gene encoded subunits. We find that Complex I biogenesis involves two complementary processes – (i) de novo assembly seeded by synthesis of hydrophobic mtDNA encoded subunits; and (ii) dynamic exchange of pre-existing peripheral subunits with newly imported ones to maintain Complex I homeostasis. In addition, we have characterized a number of novel Complex I assembly factors involved at distinct stages of the assembly pathway. We also show that mutations in a number of genes encoding these assembly factors result in Complex I misassembly, leading to mitochondrial dysfunction and disease.

S13.2.5
Survey of genes involved in mitochondrial biogenesis in early development of zebrafish as candidates for mitochondrial pathologies

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See Abstract P13.13.

S13.2.6
Pathogenesis mechanisms in mitochondrial beta oxidation diseases: protein misfolding, function and small molecules

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See Abstract P13.4

S14 – Biochemistry of the brain and neurodegenerative disorders

S14.1 Recent advances in neurodegenerative disorders

S14.1.1
Astrocytes in neurodegeneration: potential target for Alzheimer's disease treatment

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The classic amyloid cascade hypothesis, which states that Alzheimer's disease (AD) is caused by accumulation of deposits of β-amyloid peptides (Aβ), has recently been revised to include the possibility that also reactive gliosis plays detrimental actions in the pathogenesis of the disease. Actually, although also A. Alzheimer himself in 1910 indicated glial cells may crucially participate in the pathogenesis of dementia, only in the last years a solid body of evidence has been provided to support the view that astrocytes are intimately implicated in the onset and progression of neurodegenerative disorders, either through the gain of damaging effects or the loss of their neurosupportive functions. Indeed AD brain astrocytes associated with the Aβ plaques show an hypertrofic phenotype, release a wide array of mediators of inflammation and oxidative stress, and display altered energy metabolism, a decreased ability to take up glutamate and a reduced oxidative defense. Therefore, besides cytotoxic mechanisms directly impacting on neurons, Aβ-induced glial activation promotes release of pro-inflammatory molecules that may act autocrinally to self-perpetuate reactive gliosis, and paracrinally to damage neighboring neurons, thereby amplifying neuropathological lesions. Moreover, when astrocytes adopt a reactive phenotype they neglect their supportive functions, thus rendering neurons more vulnerable to neurotoxins. Acute neuroinflammatory responses are beneficial to the central nervous system. However, when glial activation inappropriately persists long after the initial injury, the prolonged condition of neuroinflammation induces profound changes, so that reactive gliosis, from a defensive response, may inexorably turn into a detrimental process. Viewed from this perspective, astrocytes became a promising target for therapeutic interventions, if their compromised functions can be normalized with pharmacological agents able to return astrocytes to a normal phenotype.

S14.1.2
Parkinson's disease, a dying back pathology associated with synaptic alpha-synuclein aggregation

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Parkinson's disease (PD) is the most common movement disorder. Clinically is characterized by rigidity, resting tremore and bradikenisia that are associated to degeneration of neurones in the substantia nigra. Although the movement disorder is the main feature of Parkinson's disease, other non motor symptoms such as depression, disturbed sleep and intestinal problem can precede the motor dysfunction. Neuropathologically PD is characterized by the presence of intracellular protein aggregates known as Lewy bodies and Lewy neurites present in neurones of the substantia nigra and other brain regions. Braak et al. have shown that Lewy bodies can be present very early on in the gut of Parkinson's disease patients and concluded that the pathology progresses from the gut to the brain through neuronal networks. Genetic mutations and multiplications of the alpha-synuclein gene have been found to be the cause of familial forms of PD and alpha-synuclein has also been shown to be the major component of the filaments that form the Lewy bodies. These two findings clearly associate alpha-synuclein to the pathogenesis of the PD but the contributions of Lewy bodies to neurodegeneration and the mechanism leading to their formation remain unclear. We have produced transgenic mouse models that expresses truncated human alpha-synuclein in dopaminergic neurons. In these mice, both in the presence or absence of endogenous alpha-synuclein, truncated alpha-synuclein aggregates into granular and filamentous material and this aggregation is associated with progressive reduction in dopamine release, dopamine loss and appearance of motor impairment. The reduction of dopamine release is associated with alpha-synuclein aggregates at the presynaptic terminal and redistribution of the SNARE complex involved in neurotransmitter release. These mice represent a good model where to investigate the relationship between alpha-synuclein aggregation and dopaminergic system dysfunction.

S14.1.3
Aβ dimers, non-infectious prion and therapeutic antibodies

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The Alzheimer's disease (AD) brain is characterized by synaptic and neuronal loss, extracellular amyloid plaques and intra-neuronal neurofibrillary tangles. Synapse loss is an early and invariant feature of AD and its extent closely parallels severity of cognitive impairment. Accordingly, it has been proposed that synapse loss underlies the memory deficit evident in early AD and that persistent disruption of plasticity may precipitate the frank cell loss typical of later stages of AD. Diverse lines of evidence also indicate that the principal component of amyloid plaques, the amyloid β-protein (Aβ), plays a central role in pathogenesis. Until relatively recently it was assumed that Aβ had to be assembled into extracellular amyloid fibrils to exert its toxic effects, however, over the past decade, data have emerged to suggest that pre-fibrillar, diffusible assemblies of Aβ are also deleterious. In our studies of Aβ toxicity we have used Aβ extracted from human brain and biological readouts that directly parallel with pathological changes and symptoms typical of the early stages of AD, namely loss of synaptic form and function and impairment of episodic memory. Specifically, we find that brain extracts which contain SDS-stable Aβ dimers decrease synaptic density, alter long-term potentiation and impair memory consolidation in the rat. More recently we found that the prion protein (PrP) is required for this Aβ-mediated impairment of plasticity and that antibodies directed to two different sites on PrP can ameliorate synaptotoxicity. Furthermore, studies with synthetic peptides suggest that dimers may stabilize the formation of kinetically trapped pre-fibrillar intermediates and that these assemblies are the proximate mediators of Aβ toxicity. Together these results recommend that therapeutic targeting of Aβ dimers (or assemblies thereof) and their interaction with PrP may overcome the synaptic failure that characterizes AD.

S14.1.4
Amyloid-beta: from release to synaptic function

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A persistent challenge in unravelling mechanisms that regulate memory function is how to bridge the gap between inter-molecular dynamics of single proteins, activity of individual synapses and emerging properties of neuronal circuits. The prototype condition of disintegrating neuronal circuits is Alzheimer's disease. Although diverse lines of evidence suggest that the amyloid-β peptide (Aβ) plays a central role in synaptic dysfunctions in Alzheimer's disease, several key questions remain unresolved. First, endogenous Aβ peptides are secreted by neurons throughout life, but their physiological functions are largely unknown. Second, experience-dependent mechanisms that initiate the changes in Aβ composition in sporadic, the most frequent form of AD, are unidentified. And finally, molecular mechanisms that trigger Aβ-induced synaptic failure and memory decline remain elusive. To target these questions, we developed an integrative approach to correlate structure and function at the level of single synapses in hippocampal circuits. Our findings suggest that ongoing background synaptic activity critically determines Aβ composition and plasticity of synapses in hippocampal circuits.

S14.1.5
The Alzheimer's disease associated amyloid beta-peptide supports platelet adhesion and activation

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See Abstract P14.10.

S14.1.6
Anti-inflammatory effects of autotaxin on microglial cells

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See Abstract P14.33.

S14.2 Dopaminergic neurons and Parkinson disease

S14.2.1
Otx2 in adult meso-diencephalic dopaminergic neurons

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Mesencephalic and diencephalic dopaminergic (mdDA) progenitors generate two major groups of neurons corresponding to the A9 neurons of the Substantia Nigra pars compacta (SNpc) and the A10 neurons of the ventral tegmental area (VTA). MdDA neurons control motor, sensorimotor and motivated behaviour and their degeneration or abnormal functioning is associated to Parkinson's disease and psychiatric disorders. Although relevant advances have been made, the molecular basis controlling identity, survival and vulnerability to neurodegeneration of SNpc and VTA neurons remains poorly understood. Here, we will review recent findings on the role exherted by the transcription factor Otx2 in adult mdDA neurons. Otx2 expression is restricted to a relevant fraction of VTA neurons and absent in the SNpc. In particular Otx2 is prevalently excluded from neurons of the dorsal-lateral VTA, which expressed Girk2 and high level of the Dopamine transporter (Dat). Loss and gain of function mouse models revealed that Otx2 controls neuron subtype identity by antagonizing molecular and functional feautures of the dorsal-lateral VTA such as Girk2 and Dat expression as well as vulnerability to the parkinsonian MPTP toxin. Furthermore, when ectopically expressed in the SNpc, Otx2 suppresses Dat expression and confers efficient neuroprotection to MPTP toxicity by suppressing efficient DA uptake.

S14.2.2
Use of stem cells for cell replacement in Parkinson's disease

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Cell replacement therapy for Parkinson's disease (PD) is based on the idea that implanted dopamine (DA) neurons may be able to substitute for the lost nigrostriatal neurons. Studies in animal models of PD have shown that transplanted DA neuroblasts can re-establish a functional innervation and restore dopaminergic neurotransmission in the grafted striatum, that they are spontaneously active and release DA in an impulse-dependent manner, and reverse Parkinson-like motor impairments induced by damage to the nigrostriatal system. Open-label clinical trials in patients with advanced PD have shown that DA neuroblasts obtained from fetal human mesencephalic tissue can survive and function also in the brains of PD patients, restore striatal DA release, and ameliorate impairments in motor behavior.

The ethical and practical problems associated with the use of fetal tissue is today the most serious obstacle to further developments of this approach. Further progress, therefore, is critically dependent on the development of new procedures that will allow generation of DA neurons in large numbers from stem cells. The most promising results so far have been obtained using embryonic stem (ES) cells as starting material. Our own work has focused on a transcription factor, Lmx1a, that can function as an intrinsic determinant in the development of midbrain DA neurons. Expression of Lmx1a in mouse ES cells can drive ES cell-derived neurons with high efficiency into a fully differentiated midbrain dopaminergic phenotype. *In vivo*, the Lmx1a-transduced ES cells develop into fully mature DA neurons and grow to form an extensive axonal terminal network throughout the host striatum. The results indicate that genuine and fully functional midbrain DA neurons can be generated from mouse ES cells using this approach. With the differentiation protocol used so far, however, many transplants show some degree of tumor formation, suggesting that the differentiated ES cell cultures contain cells with tumorgenic properties. This problem should be possible to avoid by using either cell sorting, or by generating stable non-tumorgenic stem cell cultures from the Lmx1a-transduced ES cells.

S14.2.3
Engrailed Homeoproteins protect mesencephalic dopaminergic neurons in animal models of Parkinson disease

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Adult mice heterozygous for homeobox gene Engrailed-1 display progressive loss of mesencephalic dopaminergic (mDA) neurons and motor dysfunctions reminiscent of Parkinson disease (PD). When infused in the midbrain, exogenous Engrailed-1 and Engrailed-2 are internalized by live cells, including mesencephalic dopaminergic (mDA) neurons and halts their death. The same protective effect is observed, *in vitro* and *in vivo*, against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a mitochondrial complex-I toxin used to model Parkinson disease in animals. Protection by Engrailed is through the enhanced local translation of key mitochondrial complex I subunits, in particular Ndufs1. Our results demonstrate a direct link between Engrailed-1 expression, mitochondrial complex-I activity and protection against mDA cell death. In addition we find that in wild-type mice and in absence of any treatment placing mDA neurons in jeopardy, Engrailed-1 exerts an unexpected physiological activity by regulating striatal DA content and motor behavior. In conclusion Engrailed gene expression in the adult substantia nigra and ventral tegmental area is involved in the physiology and pathology of the mesencephalic dopaminergic systems. This allows us to consider Engrailed-1 (Engrailed-2 as well) as a potential therapeutic protein and a tool to find novel genetic pathways and therapeutic targets in diseases associated with the suffering of mDA neurons.

S14.2.4
A Pitx3 regulatory network in development and maintenance of mesodiencephalic dopamine neurons

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Development of meso-diencephalic dopamine (mdDA) neurons requires the combined actions of the orphan nuclear receptor Nurr1 and the paired-like homeobox transcription factor Pitx3. Whereas all mdDA neurons require Nurr1 for expression of Th and survival, dependence on Pitx3 is only displayed by the mdDA subpopulation that will form the substantia nigra (SNc). Previously, we demonstrated that Pitx3-/- embryos lack the expression of the retinoic acid (RA)-generating enzyme Ahd2, which is normally selectively expressed in the Pitx3-dependent DA neurons of the SNc. Restoring RA-signaling in $Pitx3^{-}/^{-}$ embryos revealed a selective dependence of SNc neurons on the presence of RA for differentiation into Th-positive neurons and maintenance throughout embryonic development. Whereas these data are suggestive of an important developmental role for RA in neurons of the SNc, the underlying mechanism has remained unclear. In this study we unravel the role of Pitx3 in driving gene expression in mdDA neurons. In addition, we have studied which downstream targets are affected through RA signaling. These data show that Pitx3 has RA-dependent and independent downstream targets in the mdDA dopaminergic system.

S14.2.5
Synphilin-1 inhibits alpha-synuclein degradation by the proteasome

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See Abstract YSF.2.

S14.2.6
Mitochondrial respiratory dysfunction in PARK6 and PARK2 familial Parkinsonism

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See Abstract P14.58.

S15 – Molecular basis of cardiovascular diseases

S15.1 Development of vascular system

S15.1.1
VEGF in control of vascular function

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Vascular endothelial growth factors (VEGFs) control vascular development during embryogenesis and the function of blood vessels and lymphatic vessels in the adult. There are five related mammalian ligands, which act through three receptor tyrosine kinases. Signaling is modulated through neuropilins, which act as VEGF co-receptors. Heparan sulfate and integrins are also important modulators of VEGF signaling. Therapeutic agents that interfere with VEGF signaling have been developed with the aim to decrease angiogenesis in diseases that involve tissue growth and inflammation, such as cancer.

VEGFR signaling has thus far mostly been studied *in vitro*. These studies have provided important knowledge on the impact of VEGF on endothelial cell function, which in turn has promoted the development of anti-angiogenic drugs. However, a number of parameters of *in vivo* vascular function cannot be accurately modeled in monolayer cultures of immortalized, transformed or even primary endothelial cells. Such parameters include (i) cell–cell junctions, which may not be properly organized *in vitro*; (ii) the vascular basement membrane and supporting cells which may be missing; (iii) the 3D context which may not be represented; and (iv) co-receptors, which may not be adequately expressed. Therefore, progress in research on VEGF receptor signaling requires not only tools for sensitive and accurate biochemical analyses but also ambitious *in vivo* models.

The presentation will outline the current understanding on VEGF receptor signaling and consequent biology with focus on molecular mechanisms in VEGF-induced vascular permeability *in vivo*.

S15.1.2
Angiogenesis: growth of new blood vessels and beyond

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Angiogenesis is the main process mediating the expansion of the blood vessel network during development, tissue regeneration or in pathological conditions such as cancer. The formation of new endothelial sprouts, a key step in the angiogenic growth program, involves the selection of endothelial tip cells, which lack a lumen, are highly motile, extend numerous filopodia, and lead new sprouts. Angiogenic sprouting is induced by tissue–derived, pro–angiogenic signals such as vascular endothelial growth factor (VEGF), which activates and triggers signaling by cognate receptor tyrosine kinases in the endothelium. However, this response is strongly modulated by intrinsic signaling interactions between endothelial cells (ECs). For example, expression of the ligand Delta-like 4 (Dll4) in tip cells activates Notch receptors in adjacent (stalk) ECs and is thought to downregulate VEGF receptor expression in these cells. Thus, the tip cell phenotype is suppressed in stalk cells and a balance between sprouting and the necessary preservation of existing endothelial tubes is established.

Our work is providing further insight into the regulation of sprouting angiogenesis. The Notch ligand Jagged1 is a potent pro-angiogenic regulator with the opposite role as Dll4. In contrast to current models, we found that Notch controls VEGFR2 only moderately whereas VEGFR3 is strongly regulated. Moreover, blocking of Notch enables angiogenic growth even in mutant animals lacking endothelial VEGFR2 expression.

We also found that endothelial sprouting and proliferation extension depend on VEGF receptor endocytosis. Ephrin-B2, a ligand for Eph family receptor tyrosine kinases, is required for endothelial cell motility, VEGF receptor endocytosis and the activation of downstream signal transduction cascades. More recently, we have identified Disabled 2, a clathrin-associated sorting protein, and the cell polarity protein PAR-3 as novel interaction partners of ephrin-B2 and VEGF receptors. These results establish that regional VEGF receptor endocytosis, which is controlled by a complex containing Dab2, PAR-3 and ephrin-B2, play a key role in the spatial organization of angiogenic growth.

Finally, we have started to address the role of endothelial cells in the regulation of tissue morphogenesis and homeostasis. In the adult bone marrow, angiogenic signaling by the VEGF and Notch pathways controls sinusoidal endothelial growth as well as endothelial–mesenchymal interactions and tissue organization. Our work delineates a first structural and functional framework for bone marrow homeostasis.

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S15.1.3
Regulatory networks controlling cardiac cell fate

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Heart disease is a leading cause of death in adults and children. We, and others, have described complex signaling, transcriptional and translational networks that guide early differentiation of cardiac progenitors and later morphogenetic events during cardiogenesis. We found that networks of transcription factors and miRNAs function through positive and negative feedback loops to reinforce differentiation and proliferation decisions. Many of the same cues have been leveraged to control differentiation of pluripotent stem cells into cardiac, endothelial and smooth muscle cells that may be useful for regenerative purposes. Interestingly, we have shown that heterozygous mutations of these major regulatory proteins also result in human heart disease. We have generated induced pluripotent stem (iPS) cells from many such patients and differentiated the iPS cells into the appropriate cardiac cell to model their heart disease, revealing novel pathways by which human mutations may be causing disease. Finally, we utilized a combination of the major cardiac regulatory factors to induce direct differentiation of fibroblasts into beating cells with global gene expression and electrical activity similar to cardiomyocytes. Knowledge regarding the early steps of cardiac differentiation *in vivo* has led to effective strategies to generate large numbers of necessary cardiac cell types for disease-modeling and regenerative approaches, and promise to lead to new strategies for human heart disease.

S15.1.4
Regulation of integrin function in endothelial cells: a matter of conformation and traffic

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In multicellular organisms, the execution of complex morphogenetic events, such as vascular morphogenesis, depends on the dynamic modulation of adhesion. Guidance cues, such as chemokines, growth factors, and semaphorins control the attachment of cells to extracellular matrix proteins by regulating the conformational activation of integrin receptors. The endo-exocytic traffic of integrins back and forth from the plasma membrane represents another crucial regulatory aspect in cell adhesion and motility. Recent work added an additional layer of complexity by indicating that distinct molecular machineries are required for trafficking active and inactive integrins.

S15.1.5
Effect of cycloxygenase 2 in cardiac ischemia-reperfusion injury

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See Abstract P15.1.

S15.1.6
Exogenous hydrogen sulfide as a modulator of the mitochondrial permeability transition pore opening in rat heart

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See Abstract P15.29.

S15.2 Molecular basis of cardiovascular diseases

S15.2.1
Cardiac muscle microRNAs

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Muscle responds to a wide variety of stressors by hypertrophic growth of myocytes. In various muscular disorders, this hypertrophic response may temporarily serve a compensatory role but becomes detrimental when prohypertrophic stimulation persists. As such, cardiac hypertrophy has been identified as a risk factor for impaired cardiac function, future heart failure and life threatening arrhythmias.

Recently, several microRNAs (miRNAs) have been implicated in muscle disorders and in particularly in cardiac hypertrophy and failure. MiRNAs interact specifically with mRNAs by repressing their translation or inducing their degradation. They are thus, by their impact on gene expression, key factors in the development and maintenance of tissue, both in health and disease states. The majority of experiments have focussed on miRNAs whose cellular levels change under disease-causing conditions, based on the assumption that disease relevance and deregulation are tightly linked. In particular, hypertrophied cardiac cells or tissue have repeatedly been used as a source for microarray analyses and led to the identification of several miRNAs as potential disease modulators. A few of these miRNAs have been further characterized by experimentally induced alteration of their cellular concentration, and were thereby shown to either elicit or to prevent disease. We have identified miR-21 as the strongest upregulated microRNA in myocardial disease. We show that miR-21 regulates the ERK-MAPkinase signalling pathway in cardiac fibroblasts, which impacts on global cardiac structure and function.

However, focussing on the most strongly expressed and de-regulated miRNAs risks to neglect other disease-relevant miRNAs. This may be one reason why only few of the hundreds of miRNAs have so far been functionally characterized in the cardiovascular system. Taking these arguments into account, assays are highly desired that allow for a direct correlation of miRNAs with cellular effects, at best in an automated set-up that screens multiple miRNAs in parallel. A HCS technique designed to screen miRNA libraries for cellular effects has hitherto not been established in primary cells of the cardiovascular system. We have established a high content screening assay in neonatal rat cardiomyocytes (NRCM) and used this approach to identify hypertrophy-inducing miRNAs.

S15.2.2
Genetic modeling of PI3K inhibition

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Phosphoinositide 3-kinases (PI3K) are crucial elements needed for receptor-mediated signal transduction and modification of PI3K signaling is emerging as a key element in cancer, inflammation, metabolic disorders and cardiovascular diseases. PI3K consist of heterodimers of a 110 kD catalytic (p110) as well as a regulatory/adapter subunit and are required for the production of a membrane bound phosphorylated lipid (PIP3) that acts as a critical secondary messenger molecule. Class I p110s (p110α, β, γ and δ) share significant homology but studies using genetically engineered mice show that they all play non-redundant roles. Interestingly, modeling by genetic means of PI3K inhibition revealed that different isoforms can be distinctly involved in different pathologies. For example, we recently demonstrated that, while PI3Kγ is crucially involved in the establishment of inflammatory responses, PI3Kβ is a key determinant in the development of Erbb2-driven mammary gland cancer. In addition, we also recently found that PI3Kγ signaling also occurs in the heart where it can modulate the contractile response and contribute to the development of heart failure. While these genetic studies recently provided support for PI3K catalytic activity as a promising drug target they also unexpectedly revealed that these proteins not only work as kinases but also as scaffolds for protein-protein interactions. Despite this complex regulation, genetic modeling of PI3K inhibition clearly supports that selective targeting of different PI3K isoforms can represent a promising strategy to improve efficacy and reduce side effects. Efforts to produce and test such drugs are under way and clinical trials are foreseen for the next future.

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S15.2.3
Role of innate and adaptive immunity in atherosclerosis

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Atherosclerosis is a chronic disease of the arterial wall where both innate and adaptive immuno-inflammatory mechanisms are involved. Inflammation is implicated in the formation of early fatty streaks, when the endothelium is activated and expresses chemokines and adhesion molecules leading to monocyte/lymphocyte recruitment. It also acts at the onset of adverse clinical vascular events, when activated cells within the plaque secrete matrix proteases that degrade extracellular matrix proteins and weaken the fibrous cap, leading to rupture and thrombus formation. Recent advances in our understanding of the mechanisms of atherosclerosis provided evidence that the immuno-inflammatory response in atherosclerosis is modulated by regulatory pathways involving the two anti-inflammatory cytokines IL-10 and TGFβ, which play a critical role in counter-balancing the effects of pro-inflammatory cytokines. Interestingly, IL-10 and TGF-β are also the two cytokines that mediate the immune regulatory functions of regulatory T (Treg) cells, a sub-population of T cells that control autoimmune disease. We demonstrated that natural $CD4^+CD25^+$ Treg cells play an important role in the control of atherosclerosis in $apoE^{-/-}$ mice. Moreover, modulation of the peripheral immune response is achievable by transfer of regulatory T cells. It is therefore believed that atherosclerosis results from an imbalance between pathogenic T cells, mostly Th1, producing pro-atherogenic mediators, and Treg cells with immunosuppressive properties, and that promotion, expansion or exogenous administration of Treg cells, ideally specific of plaque-derived antigens, might limit disease development and progression. More recently, we showed that loss of suppressor of cytokine signaling (SOCS) 3 in T cells increases both interleukin IL-17 production, induces an antiinflammatory macrophage phenotype, and leads to unexpected IL-17-dependent reduction in lesion development and vascular inflammation.

S15.2.4
Adaptive responses in the ischemic myocardium

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A key energy-saving adaptation to chronic hypoxia that enables cardiomyocytes to withstand severe ischemic insults is hibernation, i.e. a reversible arrest of contractile function. hibernating cardiomyocytes represent the critical reserve of dysfunctional cells that can be potentially rescued. We developed a transgenic mouse system for conditional induction of long-term hibernation and, in turn, its rescue at will. The system is based on a myocardium-specific induction of a VEGF decoy receptor leading to escalating levels of vascular deficits. Importantly, ensuing ischemia is tunable to a level at which large cohorts of cardiomyocytes are driven to enter a hibernation mode, without cardiac cell death. Relieving the VEGF blockade even months later resulted in rapid revascularization and full recovery of contractile function. The system was used to elucidate the genetic program of hibernation; uncovering hypoxia-inducible factor target genes associated with metabolic adjustments to preserve maetabolic homeostasis and induced expression of several cardioprotective genes. Autophagy, specifically self-digestion of mitochondria, was identified as a key pro-survival mechanism.

A sustained, compromised cardiac output also leads to myocardial remodeling that enables the myocardium to withstand volume- and pressure overloads. However, because cardiac remodeling usually become maladaptive and contributes to heart failure, its intended reversal continues to be an urgent medical goal. Remodeling in our system was shown to be fully reversible upon re-vascularization during early- and late stages but not during the end stage of ischemic heart disease. Justifying the concern that adverse remodeling might reach a point-of-no-return, we show that while re-vascularization therapy can restore contractile function even when applied at advanced stages of ischemic heart disease, its utility for reversing maladaptive remodeling might indeed be restricted to earlier stages.

S15.2.5
Adrenomedullin 2 enhances macrovascular endothelial barrier function while it disrupts coronary microvascular barrier via differential regulation of Rac1

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See Abstract P15.2.

S16 – Biochemistry of immunity and inflammation

S16.1 Ectoenzyme network and diseases

S16.1.1
Ectonucleotidases, regulatory T cells and the conditioning of vascular and immune responses in inflammatory bowel disease

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Homeostatic and thromboregulatory mechanisms in the vasculature are dependent, at least in part, upon the biological activities of CD39 (the major vascular and immune cell ectonucleotidase) that scavenges extracellular nucleotides to generate adenosine. Hence, CD39 co-ordinates purinergic signaling responses that are triggered by both P2 (nucleotides) and P1 (adenosine) receptors. Furthermore, CD39, together with CD73, contributes to the suppressive machinery of T regulatory cells. Expression of these ectonucleotidases on either endothelial or immune cells allows for integration and control of vascular inflammatory and immune cell reactions in the liver and gastrointestinal tract. We note that ectonucleotidases mediate thrombosis and immunity in IBD. Acquired changes in ectonucleotidase activity with inflammatory stress, and/or genetic deficiencies of CD39 in mutant animals and certain patient populations, are associated with vascular perturbation and immune dysregulation in Crohn's disease and experimental colitis. Ongoing development of therapeutic strategies targeting ectonucleotidases offers promise for the management of hepatic and gastrointestinal inflammatory diseases.

S16.1.2
CD73 as a regulator of CNS inflammation

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CD73 is an ecto-nucleotidase enzyme which catalyzes the conversion of extracellular AMP to adenosine. It is abundantly expressed on endothelial cells, epithelial cells and on 10–15% of peripheral blood lymphocytes. The anti-inflammatory effects of adenosine are mediated via its binding to four adenosine receptors namely A1, A2A, A2B and A3 receptors which are widely expressed on a variety of cell types including immune cells and cells in the CNS.

Interferon-beta-treatment of multiple sclerosis (MS) improves the barrier function of CNS vessels partly via a mechanism mediated by CD73: interferon-beta treatment upregulates the expression of CD73 on brain microvascular endothelial cells and astrocytes, which leads to reduced transmigration of lymphocytes through the endothelial cell monolayer.

To investigate the role of CD73 in regulating CNS inflammation, EAE, an animal model for MS was induced in mice devoid of CD73. Because of the strong immunosuppressive and anti-inflammatory properties of adenosine, it was predicted that $CD73^{-/-}$ mice would develop severe EAE. Surprisingly, $CD73^{-/-}$ mice were resistant to EAE. Immunohistochemistry showed that $CD73^{-/-}$ mice had fewer infiltrating lymphocytes in their CNS compared with WT mice, suggesting that homing of lymphocytes into the CNS was impaired in the CD73-deficient mice. Importantly, blockade of adenosine receptor signaling with the A2a adenosine receptor-specific antagonist SCH58261 protected WT mice from EAE induction, suggesting that CD73 expression and adenosine receptor signaling were required for the efficient entry of lymphocytes into the CNS during EAE development.

Finally, preliminary PET (positron emission tomography)-imaging studies in patients with secondary progressive MS revealed increased ligand-binding to A2A receptors in the brain of the patients compared to age-matched healthy controls. We propose that A2A receptor signaling in the CNS has significance in MS pathogenesis.

S16.1.3
Nucleotide-metabolizing enzymes in tumor-host interactions: the chronic lymphocytic leukemia model

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Nicotinamide adenine dinucleotide (NAD) is an essential coenzyme that can be released in the extracellular milieu. Here, it may elicit signals through binding purinergic receptors. Alternatively, NAD may be dismantled to adenosine, in turn up-taken by cells and transformed to reconstitute the intracellular nucleotide pool. An articulated ecto-enzyme network is responsible for the nucleotide-nucleoside conversion. CD38 is the main mammalian enzyme that hydrolyzes NAD, generating $Ca2^{+}$-active metabolites. Evidence suggests that this extracellular network may be altered or used by tumor cells to (i) nestle in protected areas; and (ii) evade the immune response. We have exploited chronic lymphocytic leukemia as model to test the role of the ecto-enzyme network, starting to analyze the individual elements making up the whole picture.

S16.1.4
CD38 mediates social behavior in clinical and non-clinical subjects

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Increasing evidence suggests that the nonapeptide, oxytocin (OT), helps shape social and affiliative behaviors not only in lower mammals but also in humans. Recently, an essential mediator of brain OT release has been discovered, ADP-ribosyl cyclase and / or CD38. We have subsequently shown that polymorphisms across the CD38 gene are associated with autism spectrum disorders (ASD). Notably, CD38 expression in lymphoblastoid cells is reduced in cell lines derived from ASD subjects compared to parental cell lines. Intriguingly, a correlation was observed between CD38 expression and measures of social function in ASD. Finally, we have shown that all-trans retinoic acid (ATRA), a known inducer of CD38 transcription, can rescue low CD38 expressing LBC lines derived from ASD subjects and restore normal levels of transcription of this ectoenzyme providing "proof of principle" in a peripheral model that retinoids are potential therapeutic agents in ASD.

S16.1.5
Specific ER aminopeptidase 1 SNPs affect antigen processing *in vitro* and demonstrate substrate inhibition kinetics

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See Abstract YSF.29.

S16.1.6
Prolactin and *Staphylococcus aureus* inhibit nuclear factor kappa B activation in bovine mammary epithelial cells

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See Abstract P16.29.

S16.2 Structure and function of innate immunity receptors

S16.2.1
Toll-like receptor and NOD-like receptor signaling in inflammation and infection

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In the field of inflammation research, the most important advances in the past 10 years has been in the uncovering of multiple signalling pathways involved in innate immunity, and the continuing validation of signalling pathways activated by pro-inflammatory cytokines. There are now 7 distinct receptor families that sense microbial products and the products of inflamed tissues, and trigger the innate response. Most importantly this is the system primarily responsible for the induction of pro-inflammatory cytokines such as TNF, IL6 and IL17, as well as effector mechanisms in host defence. These advances have opened up a myriad of opportunities for drug development in diseases such as rheumatoid arthritis and psoriasis.
The best characterised reecptors are the Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). Genetic variation in several of these components has been linked to inflammatory diseases, notably in the TLR system and in the NLR protein Nlrp3 and associated proteins. The use of biologics that target the cytokines induced, notably against TNF but also IL-1, IL-6 and IL-17, is proving itself in the clinic, providing important new therapeutic options to clinicians. Newer targets are also being explored, which are yielding orally activate small molecule inhibitors of signalling. We now have a good understanding of the major components activated by these receptor systems, notably the TIR domain- containing adapters that initiate signalling following recruitment to TIR domains within the TLRs themselves, the IRAK family of protein kinases that are then recruited, and a series of ubiquitination and phosphorylation reactions that ultimately lead to the activation of transcription factors such as NF-kappaB and IRF family members. Other proteins kinases such as Bruton's tyrosine kinase and the JAK family of tyrosine kinases are being increasingly well-validated in inflammatory diseases and clinical progress in their targeting continues apace. As we continue to unravel the molecular details of these complex processes in inflammation, new therapeutic options will present themselves.

S16.2.2
Intracellular sensing of DNA by the innate immune system

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A central function of our innate immune system is to sense microbial pathogens by the presence of their nucleic acid genomes or their transcriptional or replicative activity. In mammals, a receptor-based system is mainly responsible for the detection of these "non-self" nucleic acids. Tremendous progress has been made in the past years to identify host constituents that are required for this intricate task. With regard to the sensing of RNA genome based pathogens by our innate immune system, a picture is emerging that includes certain families of the toll-like receptor family (TLR3, TLR7, TLR8) and the RIG-I like helicases (RIG-I, MDA5 and LGP2). Genetic loss of function studies implicate that the absence of these pathways can lead to a complete lack of recognition of certain RNA viruses. At the same time, intracellular DNA can also trigger potent innate immune responses, yet the players in this field are less clear. We and another group recently identified a role for RNA polymerase III in the conversion of AT-rich DNA into an RNA ligand that is sensed by the RIG-I pathway. However, at the same time other sensing pathways must exist that additionally operate to sense non-self DNA in cytoplasm or maybe even in the nucleus. This redundancy of DNA sensing pathways has tremendously complicated the search for the yet elusive innate immune DNA receptor(s).

S16.2.3
Human NK receptors: functional characteristics and clinical applications

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Natural Killer (NK) cells represent a major cell type in the innate immunity. They recognize MHC-class I molecules through surface receptors delivering signals that inhibit NK cell function (1). As a consequence, NK cells kill target cells that have lost (or underexpress) MHC class I molecules as it may occur in tumors and in virus-infected cells. NK cell triggering and target cell killing is mediated by various activating receptors and coreceptors (2). Normal cells are usually resistant to the NK-mediated attack, however, an exception is represented by dendritic cells (DC) (3). Thus, in their immature form (iDC), they are susceptible to NK-mediated lysis because of the expression of low levels of surface

MHC-class I molecules (DC editing). The efficiency of this phenomenon may profoundly affect both innate and adaptive immune responses. Regarding the possible exploitation of NK cells in therapy, an important example is the hemopoietic stem cell (HSC) transplantation to cure high risk leukemias. A major role for "alloreactive" NK cells (i.e. donor-derived NK cells that are not inhibited by the HLA-class I alleles of the patient) has been demonstrated in acute myeloid leukaemia of adult patients undergoing "haploidentical" HSC grafting (4). In these patients, donor's alloreactive NK cells not only mediated graft versus-leukemia (thus preventing leukemic relapses) but also inhibited graft-versus-host responses by killing DC of the patient (4). Similar results were obtained by our group in pediatric patients with high-risk acute lymphoblastic leukemias (5,6,7). FACS analysis of KIRs expressed by donor NK cells allows to define the size of the alloreactive NK subset. This subset is composed of cells expressing only KIRs specific for HLA-class I alleles absent in patient's haplotype. More recently we have shown that also the expression of activating KIRs, in particular the (C2-specific) KIR2DS1, may exert a beneficial effect and contribute to donor NK alloreactivity, provided that patient's cells express HLA-C alleles belonging to the C2 specificity. Importantly, we showed that the size of the alloreactive NK subset paralleles the level of NK cytotoxicity against leukemic cells (6,7). Thus, in the presence of two or more potential bone marrow donors, it is now possible to select the most appropriate one. We could also establish a correlation between the size of the alloreactive NK cell population in the donor and the clinical outcome. In this context, we showed that alloreactive NK cells derived from donor's HSC are generated and persist in patients for long time intervals (6,7). These studies and the high survival rates of patients undergoing haploidentical HSC transplantation highlight an important new reality in bone marrow transplantation to cure otherwise fatal leukemias.

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S16.2.4
Inflammasomes: IL-1beta cleavage and beyond

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Inflammasomes mediate the maturation of the pro-inflammatory cytokine proIL-1β, and have in recent years received a lot of attention because of their contribution to host defence as well as autoinflammatory disorders. On the molecular level, inflammasomes are cytoplasmic protein complexes that consist of a sensor molecule and Caspase-1, typically linked by the adapter protein ASC. Four different types of inflammasomes have been described, that differ in their sensor molecule. The Aim2 inflammasome is activated in response to the presence of DNA in the cytoplasm, while the Nlrp1- and Nlrc4-inflammasome respond to Anthrax lethal toxin and to certain enterobacteria including Salmonella sp., respectively. The most studied and at the same time the most elusive is the Nlrp3 inflammasome. While it is activated in response to a plethora of stimuli including live pathogens, bacterial toxins, environmental particles, and elevated levels of extracellular ATP, its actual mechanism of activation remains obscure. Despite its protective role against many infectious agents, hyperactivation of the Nlrp3 is also associated with numerous acquired and heritable autoinflammatory diseases. These include periodic fever syndromes, gout, diabetes, particle-induced lung inflammation (silicosis and asbestosis), and cancer. The connection to the disease is usually attributed to the ability of caspase-1 to cleave the inactive pro-form of IL-1β. However, it is becoming clear that the inflammasome has other functions in addition to IL-1β processing that are also relevant in host defence and disease.

S16.2.5
Ectoenzyme-generated extracellular adenosine creates local conditions favoring growth and survival of chronic lymphocytic leukemia cells

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See Abstract P16.34.

S16.2.6
Polymorphism in the TNF-alpha gene promoter at position -1031 is associated with increased circulating levels of TNF-alpha, myeloperoxidase and nitrotyrosine in primary Sjogren's syndrome

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See Abstract P16.10.

S16.3 Receptors and signal transduction

S16.3.1
T cell antigen receptor signalling

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Physiologic and pathologic changes of T cell fate are largely driven by TCR recognition of peptide-MHC and the ensuing signalling. The kinetics of TCR-peptide-MHC engagement, signal propagation and context are critical factors for T cells to develop, die, become tolerant or respond to foreign antigens. Mutations in genes controlling these processes often result in autoimmunity, immunodeficiency or allergy. Enormous progress has been made in this area, yet fundamental aspects of T cell activation remain unclear, such as the actual TCR signal triggering mechanism, the composition and connection of the signalling machinery and its change in time and context.
To approach these questions, we have applied quantitative proteomics analysis to the T cell activation process. In so doing, we have discovered unsuspected regulatory facets of TCR signal transduction as well as new TCR signaling effectors and connectivity to cellular functions.
These studies prompt a revision of commonly held concepts of cell signaling and will be discussed in the context of further understanding of immuno-pathological processes.

S16.3.2
Clinical and biological significance of CD157 in ovarian carcinoma

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Ovarian cancer is one of the most common and most lethal gynaecological malignancies. The poor prognosis of ovarian cancer is due to the difficulty of early diagnosis, and the lack of effective therapies for advanced-stage disease. Hence, there is a need for new therapeutic targets and for better understanding of the molecular mechanisms underpinning ovarian cancer cell invasion and dissemination.
CD157 is a cell surface NADase/ADP-ribosyl cyclase that mediates leukocyte adhesion to extracellular matrix proteins and diapedesis at site of inflammation. We demonstrated that CD157 is expressed in epithelial ovarian cancer primary cell cultures and tissues, and it is involved in interactions among tumor cells, extracellular matrix proteins, and mesothelium which ultimately control tumor cell migration and invasion. Using stable overexpression and knockdown in ovarian cancer cells, we demonstrated that CD157 promotes morphological and functional changes, characterized by cadherin switch, enhanced matrix metalloproteinases secretion, reduced intercellular cohesion and increased cell motility and invasiveness. The analysis of gene expression profile highlighted ~500 gene transcripts differentially expressed in CD157-positive versus CD157-negative tumor cells. Remarkably, several pathways and networks implicated in cell adhesion, migration, epithelial-to-mesenchymal transition and apoptosis were over-represented. Collectively, these data suggest a novel CD157-regulated pathway that could confer an aggressive, mesenchymal-like phenotype to ovarian cancer cells.
The results inferred *in vitro* were validated by clinical evidence. CD157 is expressed by >90% of ovarian cancers and high CD157 expression is associated with poor outcome in patients. Multivariable Cox regression showed that CD157 is an independent prognostic factor of survival and relapse shortly after surgical debulking of serous ovarian cancer. Overall these data suggest that CD157 may find clinical applications.

S16.3.3
Intracellular signalling pathways regulating epithelial homeostasis and inflammation

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Intracellular signal transduction pathways activated downstream of pattern recognition receptors and cytokine receptors play a critical role for the regulation of immune and inflammatory responses. The NF-κB pathway is one of the most important signalling cascades activated by cytokine and innate immune receptors. NF-κB regulates cellular responses to infection, injury and other stressful conditions requiring rapid reprogramming of gene expression. NF-κB activation has been implicated in the pathogenesis of many diseases, particularly chronic inflammatory conditions and cancer. NF-κB activation is mediated by the IκB kinase (IKK), which consists of two catalytic subunits, IKK1(IKKα) and IKK2(IKKβ), and a regulatory subunit named NF-κB Essential Modulator (NEMO) or IKKγ. *In vivo* studies in genetic mouse models provided experimental evidence for the fundamental functions of NF-κB signalling in inflammation. Using tissue specific ablation of IKK subunits we have studied the role of NF-κB for the maintenance of immune homeostasis in epithelial surfaces. Epidermal keratinocyte specific blockade of NF-κB signalling caused strong inflammatory skin lesions resembling human psoriasis. Moreover, epithelial cell specific blockade of canonical NF-κB activation caused severe chronic inflammatory colitis. These results revealed that NF-κB signalling in epithelial cells plays a critical role for the maintenance of immune homeostasis in epithelial tissues. Our current experiments investigate the mechanisms by which NF-κB signalling acts in epithelial cells to regulate tissue homeostasis and prevent the pathogenesis of chronic inflammation. The results of our most recent studies will be discussed.

S16.3.4
STAT3 in cancer inflammation and immunity

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Commensurate with their roles in regulating cytokine-dependent inflammation and immunity, signal transducer and activator of transcription (STAT) proteins are central in determining whether immune responses in the tumour microenvironment promote or inhibit cancer. Persistently activated STAT3 and, to some extent, STAT5 increase tumour cell proliferation, survival and invasion

while suppressing anti-tumour immunity. The persistent activation of STAT3 also mediates tumour-promoting inflammation. STAT3 has this dual role in tumour inflammation and immunity by promoting pro-oncogenic inflammatory pathways, including nuclear factor-kappaB (NF-kappaB) and interleukin-6 (IL-6)-GP130-Janus kinase (JAK) pathways, and by opposing STAT1- and NF-kappaB-mediated T helper 1 anti-tumour immune responses. Consequently, STAT3 is a promising target to redirect inflammation for cancer therapy.

S16.3.5
NLR expression and inflammasome activation in neutrophils

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See Abstract P16.38.

S16.3.6
Modular organization and glycosylation of the long pentraxin PTX3 dictate its biological functions

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See Abstract P16.15.

S17 – Biochemistry and molecular biology of malaria and tuberculosis

S17.1 Biochemistry and molecular biology of tuberculosis

S17.1.1
The biochemistry of activation of nitroimidazole antibiotics in *Mycobacterium tuberculosis*

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Bicyclic 4-nitroimidazoles have emerged as a promising class of compounds for the treatment of tuberculosis. PA-824 and OPC-67683, two members of this class that are currently in Phase II clinical trials, show activity against both rapidly replicating and hypoxic non-replicating *Mycobacterium tuberculosis* (Mtb). Both compounds are pro-drugs that are reductively activated by a deazaflavin (Coenzyme F420) dependent nitroreductase, Ddn. Using biochemical, computational and structural approaches we have made significant progress in unraveling the unique mechanism of activation of these molecules. In this lecture I will present an update on our progress including the three dimensional structure of the protein-F420 complex as well as mutagenesis and chemical data supporting a proposed binding mode for the drug. These results have led to significant improvements in our understanding of the features responsible for aerobic and anaerobic activity and have facilitated the synthesis of back-up candidates with improved characteristics.

S17.1.2
New medicines for tuberculosis: benzothiazinones

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The 1,3-benzothiazin-4-ones (BTZs), a new class of antimycobacterial agents, kill *Mycobacterium tuberculosis in vitro*, ex vivo, and in mouse models of TB. BTZ inactivate the enzyme decaprenylphosphoryl-b-D-ribose 2′-epimerase thereby abolishing the formation of decaprenylphosphoryl arabinose, a key precursor for cell-wall synthesis. Details will be presented of the mechanism of action and progression of BTZ043, a potential drug candidate for treating TB.

S17.1.3
ESX/Type VII secretion in tubercle bacilli – a key factor in virulence and protection

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It is now well established that pathogenesis of *Mycobcterium tuberculosis* depends on the secretion of key virulence factors, such as the 6 kD early secreted antigenic target ESAT-6 (EsxA) and its protein partner, the 10 kD culture filtrate protein CFP10 (EsxB) via the ESX-1 secretion system. ESX-1 represents the prototype system of the recently named type VII secretion systems that also exist in a range of other mycobacteria and actinobacteria. Indeed, three of the most well known attenuated strains in the history of tuberculosis research, Mycobacterium bovis BCG (BCG), Mycobacterium microti, and *M. tuberculosis* H37Ra (“a” stands for avirulent) are impaired for ESAT-6 and CFP10 secretion due to different genomic lesions or defects.
M. tuberculosis contains a total of five ESX systems that show similarity in gene content and gene order. While the genes conserved in at least four of the five systems were recently named as ESX conserved components (Ecc), these systems also contain genes coding for proteins defined as ESX-1 secretion-associated proteins (Esp). Research on type VII secretion systems has recently become a large and competitive research topic that is tightly linked to studies of host-pathogen interactions of pathogenic mycobacteria. Insights into this matter are of utmost importance for the improvement of current prevention strategies, diagnostics and therapy of tuberculosis as well as for a better understanding of the virulence mechanisms employed.

S17.1.4
Playing biochemistry with mycobacterial cell wall – an old good drug target

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The cell wall of *Mycobacterium tuberculosis* is one of the basic weapons that the pathogen uses to fight against the immune system of the human host during the course of infection. Not only it forms a highly impermeable, protective layer on the surface of bacteria, but also it releases specific molecules, which directly affect normally hostile macrophages, turning them to milieu, where the pathogen can thrive successfully. The essence of the mycobacterial cell wall is a tripartite structure composed of the covalently linked peptidoglycan, arabinogalactan and mycolic acids. Particularly the latter two components are characteristic for mycobacteria and their relatives from the order Actinomycetales; they are also the targets of several effective antituberculotics. In the talk I will focus on aspects of investigation of arabinogalactan biogenesis, which was initiated at Mycobacterial Research Laboratories at Colorado State University (CSU) led by Professor Brennan. The task was, indeed, rather challenging, taking into account the complex structure of the spectacular branched arabinogalactan heteropolysaccharide comprised of more than 120 carbohydrate units. One of the long-term goals of this research was identification of novel targets exploitable in TB drug discovery. Development of biochemical methods within the field, later supported with the information from the genome sequence of *M. tuberculosis* H37Rv published by Cole and coworkers in 1998 allowed functional characterization of a number of unique genes that are, indeed, interesting within this context. One of the successful examples is Rv3790 (dprE1) coding for the subunit of decaprenylphosphoryl ribose 2′-epimerase, the enzyme producing precursors for biosynthesis of the cell wall arabinans and a target of a new, extremely potential class of antituberculotic compounds – benzothiazinones.

S17.1.5
Muropeptides of mycobacterial peptidoglycan are the factors of dormant mycobacteria resuscitation

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See Abstract P17.7.

S17.1.6
Structural investigations on *M. tuberculosis* proteins involved in DNA repair

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See Abstract P17.9.

S17.2 Biochemistry and molecular biology of malaria

S17.2.1
A membrane biosynthesis enzyme of Plasmodium falciparum as a putative antimalarial target

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Plasmodium passage through the skin followed by infection of liver hepatocytes and, later, of blood erythrocytes are natural and sequential steps of Plasmodium life cycle in the mammalian host and should not be seen as independent entities. Importantly, in regions of high malaria transmission, infected individuals are constantly exposed to potential re-infection. Mosquito bites transmit liver-tropic sporozoites into subjects who are still infected from a previous mosquito bite. What is the impact of ongoing infection in the establishment of a secondary infection is unknown.

Recently, we have asked the question of what would be the impact of an ongoing blood stage infection on a subsequent liver infection. Using a rodent model of infection, we show that ongoing blood stage infections, above a minimum threshold, impair the growth of subsequently inoculated sporozoites such that they become growth arrested in liver hepatocytes and fail to develop into blood stage parasites. This effect can be mediated by the host iron regulatory hormone hepcidin, the synthesis of which is stimulated by blood stage parasites and which, by diverting iron away from hepatocytes, impairs the Plasmodium liver stage. We model this phenomenon and show that explains the epidemiological patterns of age-related risk and complexity of malaria infections seen in young children. Indeed, concurrent carriage of different parasite genotypes at low asymptomatic parasitaemias is frequently observed in older semi-immune children but not in the young. The interaction between these two Plasmodium stages and their host has thus broad implications on the current global efforts to reduce malaria transmission.

S17.2.2
Molecular events governing the lytic cycle in apicomplexa

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Apicomplexans lack normal appendages for locomotion such as cilia and flagella and instead the invasive stages of these parasites use gliding motility to power their migration across biological barriers, to invade host cells and to egress from infected cells. The conserved molecular machinery that generates motion, the glideosome, involves the action of signalling molecules, myosin motor complexes, regulators of actin dynamics, adhesins and proteases. The gliding associated protein GAP45 is an essential player of this machinery that recruits the motor at the pellicle. Our studies revealed that GAP45 recruit the myosin motor at the parasite pellicle. Moreover, the acylation of GAP45 and the length and rigidity of the molecule are critical for its function in maintaining the physical integrity of the pellicle. In the absence of ARP2/3, the formin homology 2 domain containing proteins, FRM1 and FRM2 orchestrate actin polymerization during host cell invasion. The distinct biochemical properties and subcellular distribution of the two FRMs highlight their non-overlapping contribution in glideosome function. Finally, recent findings on the parasite plasma membrane rhomboid protease, ROM4 and one of its substrate establishes a new concept in apicomplexan biology in which proteins involved in invasion may be concomitantly implicated in a checkpoint that signals the parasite to switch from an invasive to a replicative mode.

S17.2.3
Transporters as mediators of drug resistance in the human malaria parasite Plasmodium falciparum

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Drug resistance represents a major obstacle in the radical control of malaria. Drug resistance can arise in many different ways, but recent developments highlight the importance of mutations in transporter molecules as being major contributors to drug resistance in the human malaria parasite Plasmodium falciparum. I will concentrates on two transporters, namely the chloroquine resistance transporter PfCRT and the multi-drug resistance transporter 1 PfMDR1, and describe how polymorphisms within these entities contribute to resistance to antimalarial drugs. Our work is based on the functional studies of these transporters in *Xenopus laevis* oocytes and on genetic linkage analysis using the F1 progeny of genetic crosses between different *P. falciparum* strains. I will further discuss novel factors that synergistically interact with these two transporters in bringing about high levels of resistance.

S17.2.4
The study of antigenic variation in *Plasmodium falciparum*: mind games or a novel therapeutic approach

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Current malaria drug screening efforts target physiological processes vital to parasite survival in culture over the course of a

few days. Biological phenomena essential to immune evasion and pathogenesis in the host, but otherwise dispensable in *in vitro* culture, can be overlooked in whole parasite compound screens. Plasmodium parasites express proteins trafficked to the surface of infected red blood cells, resulting in parasite adhesion and sequestration from host clearance mechanisms. These surface proteins, called Erythrocyte Membrane Protein 1 (PfEMP1), are encoded by a 60-member family of var genes. Mono-allelic expression and subsequent switching of var genes, known as antigenic variation, is responsible for immune evasion and persistent infection. Like other eukaryotes, malaria parasites package their DNA around histones in chromatin structures. Post-translational modifications of histone tails can result in general activation or deactivation of their associated genes. We have previously determined the histone modifications associated with active and silent var genes. Specifically, histone 3 lysine 9 tri-methylation marks silent var loci, whereas histone 3 lysine 4 di- or tri-methylation is linked to silent but poised var loci, as well as to the general activation of genes. We are targeting the histone lysine methytransferases (HKMTs) that regulate var gene mono-allelic expression. This represents a novel target class in the treatment of malaria. A focused chemical library has been synthesized and compounds are being tested for target enzyme inhibition, for anti-malarial activity *in vitro* and for their effect on parasite histone methylation levels and var gene expression. We will report our progress on targeting the process of antigenic variation.

S17.2.5
Targeting a membrane biosynthesis enzyme of *Plasmodium falciparum* erythrocytic stages as a putative antimalarial target

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See Abstract P17.2.

S17.2.6
Role of malaria pigment hemozoin and hemozoin-generated 4-hydroxynonenal on inhibition of erythropoiesis in malaria anemia

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See Abstract P17.10.

S18 – Plant biochemistry for health and tomorrow's medicine

S18.1 Green Factory

S18.1.1 Engineering phenylpropanoid production in crops for healthy foods

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The past 20 years has seen an enormous rise in publicity about super foods that promote health and reduce the risk of cardiovascular disease, cancer and age-related degenerative diseases. These claims are supported by robust evidence from cell studies, animal feeding trials, human intervention studies and epidemiological studies. However, despite all the positive messages about the value of eating fruit and vegetables (the 5-aday program has been running for 25 years) the numbers of people meeting these dietary recommendations in the US remains below 25% of the population, numbers are falling, and chronic diseases, especially those associated with obesity and the metabolic syndrome, are reaching epidemic proportions in Western societies.

There is a need to engineer high levels of protective bioactives in the foods that people actually consume, to help combat this rise in chronic diseases. Most attempts at engineering the levels of bioactives have focused on increasing the activity of key, rate-limiting steps, but such strategies usually result in only modest improvements in bioactives. Use of transcription factors to up-regulate entire pathways of plant secondary metabolism is a far more effective strategy and results in food material with very significantly elevated levels of health-promoting bioactives. While such improvements may, in part, be achievable for some crops through selective breeding, genetic modification offers bigger improvements because it can overcome limits in the natural variation available in transcription factor specificity and activity. Use of genetically improved foods in animal feeding studies with models of tumorigenesis have revealed that protection is afforded by diets enriched in high bioactive foods. Such health-promoting foods will offer consumers tangible improvements in the products available to them, and have the potential for public approval of genetically improved plant varieties and foods derived from them, in Europe.

S18.1.2 Plant-made pharmaceutical antibodies

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Molecular farming has gained support and interest because plants, compared to traditional platforms for the production of recombinant proteins, are inexpensive, highly scalable and safe. The number of products in development has increased as tools and strategies have been developed to accumulate recombinant proteins with specific glycan modifications within storage organelles.

Antibody 2G12 is one of a small number of human IgG monoclonal antibodies exhibiting potent and broad HIV-1-neutralizing activity *in vitro*, and the ability to prevent HIV-1 infection in animal models. It could be used to treat or prevent HIV-1 infection in humans, although to be effective it would need to be produced on a very large scale. We have therefore expressed this antibody in tobacco and maize, which could facilitate inexpensive, large-scale production. The antibody was expressed along with the fluorescent marker protein DsRed, which helps to identify antibody-expressing lines and trace transgenic offspring.

The accumulation in ER-derived storage organelles of maize endosperm was confirmed by electron microscopy. In agreement with this localization, N-glycans attached to the heavy chain were mostly devoid of Golgi-specific modifications such. Most glycans were trimmed extensively, indicating that a significant endoglycanase activity was present in maize endosperm. In tobacco leaves the antibody contained mostly biantennary, Golgi-modified glycan structures, as expected.

Purification procedures were developed and compared for leaf and seed biomass. The specific antigen-binding function of the antibody purified from both plant species was verified by surface plasmon resonance analysis, and *in vitro* cell assays demonstrated that the HIV-neutralizing properties of the plant-produced antibody were equivalent to or better than those of its CHO-derived counterpart. Clinical studies with plant-derived antibody are being initiated.

S18.1.3 Domestication of wild unicellular algae for growth in photobioreactors

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Unicellular algae are a promising alternative to fossil fuels and terrestrial crops for the production of fuels. Advantages with respect to higher plants include (i) higher efficiency of light energy conversion; (ii) longer period of growth during the year; (iii) higher body fraction dedicated to photosynthesis; and (iv) no competition for agricultural land with food crops, making maximal biomass productivity of unicellular algae 1–2 orders of magnitude higher than crops. Nevertheless, economical sustainability of algal photobioreactor plants is still problematic. A major reason for lower-than-expected productivity of algae is the use of WT stains isolated from natural environment where growth conditions are strongly different from those experienced in natural environment. Domestication of crop plants has involved artificial selection by farmers and scientists making the present cultivars very different from WT strains. Examples of this general trend can be found with essentially any crop species including tomato [Baj and Lindhout Annals of Botany 2007 100**(5):**1085–94], corn (Wenke, J Robert. Patterns in Prehistory- Mankind's First Three Million Years. New York. Oxford University Press 1980, 267–327) and wheat (Heun et al. *Science* 1997, **278:**1312–14). This essential step in the way of using algae for biofuel production has yet to be accomplished due to the microscopic nature of the organisms which prevent direct manual selection of mutant strains with favourable characteristics and to the pressure of research grants on industrial applications skipping pre-industrial studies. Domestication of unicellular algae can be obtained in many aspects of their structure and metabolism among which the most urgent appear to be the following: (i) improvement of light use efficiency in photobioreactors; and (ii) increase of lipid versus carbohydrate content as energy-storage compounds. These targets can be obtained either by selection of naturally or artificially induced mutations or using transgenic approaches. The accept-

ability of transgenic algae for large scale photobioreactors, which cannot efficiently contain algal cells, require further levels bio safety to ensure that any transgenic material inadvertently released into the environment will not be able to become established in natural ecosystems nor to recombine in any manner with native algae. Most efficient containment (iii) technologies include modifications that preclude living outside the industrial bioreactors, whereas mitigation technologies are those that prevent either sexual or asexual recombination and viral-mediated horizontal gene flow. Algae with a reduced light-harvesting antenna size are likely to fulfil both the higher light use efficiency (i) and the containment (iii) requirements while can be further engineered for high lipid content.

S18.1.4
Metabolic engineering of terpenoids for green pharmaceuticals

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Plants produce a wealth of pharmaceutically active compounds and a particularly interesting class of metabolites are the terpenoids. An examples of terpenoid metabolites that have been developed into drugs are artemisinin, extracted from the annual plant Artemisia annua and the best anti-malarial drug currently available. In addition, there are hundreds and hundreds of additional pharmaceutically active terpenoids reported in plants that can potentially be developed into new drugs, for example in the sesquiterpene lactone and diterpene acid chemical groups.
We study the biosynthesis of these pharmaceutically active terpenoids in plants and isolate and characterise the genes that are involved in their biosynthesis. Hereto we use state-of-the-art technologies such as metabolomics, transcriptomics, high-throughput sequencing and bioinformatics. The genes we isolate are expressed in micro-organisms such as E. coli and yeast to characterise their biochemical function but also for microbial production of the corresponding metabolites. In addition, these genes are used for metabolic engineering in plants where we aim to increase or modify the production of pharmaceutically active terpenoids in homologous or heterologous plant hosts. For this work Nicotiana benthamiana is used as model. Our metabolic engineering work results in new insights into the function of enzymes, into subcellular compartmentation of terpenoid biosynthesis in plants and into the impact that endogenous enzymes of the engineered host may have on the results of the engineering. In addition to working with models, we also develop plant hosts adapted to agricultural production. An example of this is our work on the metabolic engineering of artemisinin production. In collaboration with the Belgian pharmaceutical company Dafra, we are engineering industrial chicory for the production of artemisinin. The aim of our work is to create a better and cheaper supply of this important anti-malarial drug.
Acknowledgements: We acknowledge funding by Dafra Pharma R&D.

S18.1.5
Flax produces biologically acitive cannabinoids

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See Abstract P18.33.

S18.1.6
The occurrence of ACC-dependent ethylene biosynthesis might have played important role in the speciation of β-cyanoalanine synthase (CAS) from the O-acetylserine sulfhydrylase (OASS) family proteins during land plant evolution

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See Abstract P18.14.

S18.2 Plant Innate Immunity

S18.2.1
How bacterial pathogen effector proteins manipulate the plant cell

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Pathogenicity of most Gram-negative plant-pathogenic bacteria depends on the type III secretion (T3S) system, a molecular syringe which injects effector proteins into the plant cell cytosol. In susceptible plants, type III effectors interfere with host cell processes to the benefit of the pathogen and allow its proliferation. In resistant plants, however, plant resistance genes mediate recognition of individual effector proteins usually resulting in a hypersensitive response (HR), a rapid and localized programmed cell death restricting pathogen ingress. Our lab studies the interaction between *Xanthomonas campestris* pv. *vesicatoria* (Xcv) and its host plants pepper and tomato. Xcv injects more than 20 different type III effectors into the plant cell. One of the best understood type III effectors is AvrBs3, which functions as transcription factor in the plant cell nucleus and affects both susceptible and resistant plants. Xcv strains expressing AvrBs3 induce the hypersensitive reaction HR in pepper plants carrying the resistance gene Bs3. In pepper plants lacking Bs3 and other solanaceous plants AvrBs3 induces a hypertrophy (cell enlargement) of mesophyll cells. AvrBs3 activity depends on a central region of tandem 34-aa repeats, its localization to the plant cell nucleus and the presence of an acidic activation domain. One of the direct targets of AvrBs3 is UPA20 (UPA, upregulated by AvrBs3) which encodes a transcription factor and is a key regulator of hypertrophy.

S18.2.2
Role of pathogen effectors in plant innate immunity

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Department of Plant and Microbial Biology

It has now well established that all classes of pathogens are able to deliver effector proteins directly to host plants often via specialized infection structures. Pathogen effector proteins are involved with the suppression or modulation of plant innate immunity and fundamentally control plant pathogenesis. Interestingly, the same proteins that modulate pathogen virulence are also involved in triggering genotype-specific plant disease resistance. In this presentation, I will highlight the original approaches that led to the discovery of pathogen effectors and how this information has shaped our current understanding of the ''dual'' role of effectors in both plant pathogenesis and the activation of disease resistance signalling pathways. Furthermore,

I will provide recent data from my laboratory in our attempts to employ pathogen effector proteins as molecular probes to identify host targets controlling plant innate immunity. Finally, I will present our recent data on elucidating the molecular events that are involved in effector recognition and the activation of plant disease resistance and how this knowledge can be employed to molecularly breed for durable resistance in agricultural crops.

S18.2.3
Discovering and validating plant pathogen effectoromes

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Plant disease resistance mechanisms are initiated by surface receptors and cytoplasmic receptors that respectively recognize conserved or variable pathogen components. To suppress defence, pathogens deliver "effector" molecules into host cells. Understanding these effectors is important to (i) identify new probes to host defence mechanisms; and (ii) develop durable resistance strategies. Although the effector complements of bacteria are becoming well defined, and the mechanisms of many bacterial effectors are quite well understood, the effectors of the fungal and oomycete pathogens that cause the most serious crop losses are poorly characterized. Recent advances in sequencing methods have rendered it feasible, though still challenging, to define genomes of such pathogens and to predict gene models.
We work with the downy mildew pathogen *Hyaloperonospora arabidopsidis* (Hpa) and two other related oomycete pathogens, *Albugo laibachii* and A candida. The Hpa genome was recently published. We have used Illumina paired read sequencing to assemble sequences of multiple races of *Albugo laibachii*, a pathogen that is particularly effective at shutting down host defence, and also of multiple A candida races. The analysis of its effectors is likely to provide very interesting new insights into host defence mechanisms; I will report on a novel class of effectors. In addition, we are using this system to investigate the molecular basis of pathogen/host specificity and non-host resistance. An update on recent progress will be presented.

S18.2.4
Healthy food for a healthy life: engineering resistance by constructing chimeric receptors for pathogen recognition

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An efficient sensing of danger and a rapid activation of the immune system are crucial for the survival of plants. Recognition by pattern recognition receptors (PRRs) of conserved pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) as well as of endogenous molecular patterns that are present only when the tissue is infected or damaged (damage-associated molecular patterns or DAMPs) leads to the activation of the plant immune response. The characteristics and the biological activity of a class of DAMPs, i.e. the oligogalacturonides (OGs), which are released from the extracellular matrix of the plant cell, and of Wall-Associated Kinase 1 (WAK1), an Arabidopsis PRR recently identified as a receptor of OGs, will be illustrated. The use of WAK1, PRRs and chimeric receptors to engineer resistance in crop plants will also be discussed.

S18.2.5
Bifunctional catalase-peroxidase is secreted by the rice blast fungus *Magnaporthe grisea* during oxidative burst

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See Abstract P18.30

S18.2.6
Novel antifungal defensins from seeds of wild plants

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See Abstract P18.23.

S19 – Molecular and cellular therapeutics

S19.1 Vectors for therapeutic and experimental applications

S19.1.1
Prospects and problems of gene therapy of haemoglobinopathies

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Haematologists are justifiably proud that a haemoglobinopathy (sickle cell anaemia) has been the first human disease explained at the molecular level, and that the globin genes have been the first human genes to be cloned and sequenced (circa 1978). Therefore it is not surprising that haemoglobinopathies have been also among the very first conditions for which the possibility of correction by gene transfer was seriously entertained. Early attempts carried out by using retroviral vectors were hampered by low levels of gene expression; but after the discovery in 1987 by Frank Grosveld's group of the upstream cis-regulatory element known as the locus control region (LCR), portions of the LCR were incorporated into vectors and β-globin gene expression increased. Nevertheless, only in year 2000 was the first successful pre-clinical experiment reported by Michel Sadelain's group through the use of a lentiviral vector which produced substantial haematological improvement in mice with severe β-thalassaemia.

Since then, the clinical use of vectors on whose integration sites we have no control has come under a shadow as a result of the development of leukaemia in a significant proportion of children with an X-linked form of severe combined immune deficiency (due to mutations in the gene encoding the gamma chain of IL2R), who had been otherwise successfully treated by gene therapy. Probably it is in part because of this that it took until 2007 before the first human patient with β-thalassaemia (more precisely, E/β-thalassaemia) received gene therapy. Since this patient remains to-date the only one thus treated any attempt at interpretation must remain cautious; but this patient can be regarded almost like a symbol of the prospects and of the problems still confronting us.

On one hand the prospects are good, because from updated follow-up data, kindly provided by Dr Leboulch, this patient has now been transfusion-free for 3 years, he has only moderate anaemia, and a good quality of life: within certain limits, one might say he is the first patient with a transfusion-dependent thalassaemia syndrome who has been cured by gene therapy. On the other hand, problems may be related to the fact that most of the Hb A in this patient is produced by a single haematopoietic clone in wich the β-globin-carrying lentiviral vector has integrated within HMGA2, a gene implicated in a number of non-malignant tumors. In spite of the patient's currently satisfactory clinical condition, this observation is causing some concern with respect to two issues: (i) could one cell of this clone undergo further transformation; and (ii) could it be that gene therapy by this approach will succeed only if integration takes place where it can cause clonal expansion. Neither of these two issues is likely to be solved definitively from the results on just one patient; and at any rate it must be admitted that uncontrolled vector integration is not ideal. In this regard, prospects arising from the use of induced pluripotent stem (iPS) cells seem to me highly attractive. In a mouse model, the sickle cell gene was corrected by Rudolf Jaenisch's group by homologous recombination in iPS cells obtained by re-programming skin fibroblasts. Also, in human iPS cells, Michel Sadelain's group has reported that when thousands of lentiviral integration events were analysed, 17% were in sites presumed to be "safe harbors": in the sense that, rather than within a gene, they are away from any gene. I think it is realistic to expect that one of these or a similar approach may overcome current problems and thus greatly improve prospects, until gene therapy can become accepted treatment for haemoglobinopathies.

S19.1.2
Hemophilia as trailblazer for gene therapy

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Hemophilia A and B are inherited bleeding disorders caused by deficiencies in coagulation factors VIII or IX in the blood plasma. The drawbacks of the classical protein substitution therapy fueled interest in alternative treatments by gene therapy. Hemophilia has been recognized as an ideal target disease for gene therapy because a relatively modest increase in clotting factor levels can result in a significant therapeutic benefit. Consequently, introducing a functional FVIII or FIX gene copy into the appropriate target cells could ultimately provide a cure for hemophilic patients. Several cell types have been explored for hemophilia gene therapy, including hepatocytes, muscle, endothelial and hematopoietic cells. Both nonviral and viral vectors have been considered for the development of hemophilia gene therapy, including transposons, γ-retroviral, lentiviral, adenoviral and adeno-associated viral vectors. Several of these strategies have resulted in stable correction of the bleeding diathesis in hemophilia A and B murine as well as canine models, paving the way towards clinical trials. Although clotting factor expression has been detected in hemophilic patients treated by gene therapy, the challenge now lies in obtaining prolonged therapeutic FVIII or FIX levels in these patients.

S19.1.3
Development and application of designer nucleases

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Designer nucleases have developed into powerful tools to edit the genomes of complex organisms *ad libitum*. The most widely used class of engineered nucleases are zinc-finger nucleases (ZFNs), which have been employed to trigger the targeted editing of genomes at over 50 different gene loci in more than 10 organisms, including model organisms, such as fruitfly, zebrafish, rat, mouse, and pig, as well as human stem cells. In my talk, I will summarize the technological innovations that have successfully catapulted ZFNs into the human gene therapy arena and provide an overview of parameters that determine ZFN activity and ZFN-associated toxicity, both key qualities in any therapeutic application involving designer nucleases. As a final point, I will present data that introduce TALe-based nucleases (TALeNs) as a valuable alternative to ZFNs.

S19.1.4
Targeting gene transfer to improve the efficiency and safety of gene therapy

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Abstract not received. Please see program and Late Abstract Addendum.

S19.2 Gene and cell therapy for genetic diseases

S19.2.1
Gene therapy strategies for genetic leukodystrophies

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We have developed gene therapy clinical protocols for genetic leukodystrophies, severe demyelinating diseases of the central nervous system. In X-linked adrenoleukodystrophy (X-ALD), cerebral demyelination can be stopped or reversed within 12–18 months by allogeneic HSC transplantation. The long-term beneficial effects of HCT transplantation are due to the progressive turn-over of brain macrophages (microglia) derived from bone-marrow cells. We have evaluated a gene therapy strategy based on the reinfusion of autologous HSC corrected with a lentiviral vector in four children with no compatible donor for allogeneic transplantation. Stable correction of peripheral leukocytes was demonstrated and stabilization of the demyelinating lesions up to the last follow up (48 months). Tests assessing vector-derived RCL and vector mobilization were negative. Integration of the vector was polyclonal as suggested by extensive integration profil analysis and high throughput sequencing of integration sites. In the two first treated patients with long-term follow-up, HSC gene therapy resulted in neurological effects comparable with allogeneic HSC transplantation. Long term results and perspectives will be discussed.
Metachromatic leukodystrophy, is characterized by a deficiency in arylsulfatase A (ARSA) enzyme and sulfatide accumulation. Given the rapid neurodegeneration and the delayed effect of hematopoietic stem cell transplantation, direct brain delivery of ARSA enzyme through gene therapy vector was evaluated as the most suitable therapeutic approach to stop disease progression. Intracerebral delivery of AAV5 encoding human ARSA corrected the phenotype of ARSA deficient mice and allowed significant ARSA overexpression in 60% of the injected hemisphere without any neuropathological and clinical signs of toxicity expression into the brain of normal non-human primates. AAVrh.10 AAVrh10-ARSA vector resulted in more rapid and complete neuropathological correction in MLD deficient mice. A non-human primate efficacy study and pivotal toxicology studies were performed to submitt clinical application in fall 2011 in patients with late infantile rapidly progressive forms of MLD.

S19.2.2
Gene Therapy for Primary Immunodeficiencies

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Abstract not received. Please see program and Late Abstract Addendum.

S19.2.3
New approaches for the gene therapy of inherited bone marrow failure syndromes: the fanconi anemia model

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Fanconi anemia (FA) is a rare inherited recessive (in all subtypes except FA-B) disease, mainly characterized by bone marrow failure and cancer predisposition. FA is generated by mutations in any of the fourteen FA genes that participate in the FA pathway. In contrast to the efficacy of gene therapy in other inherited diseases affecting the lympho-hematopoietic system (i.e. X1-SCID or ADA-SCID), no clinical benefits have been so far reported in FA, suggesting that conventional gene therapy approaches may not be directly applicable to FA. Aiming to develop a clinically efficient FA gene therapy protocol for FA-A patients, different lentiviral vectors carrying the FANCA gene have been generated, one of which has recently obtained the designation of Orphan Drug by the European Medicaments Agency (EMA). Because of the limited number of hematopoietic stem cells (HSCs) present in the BM of FA patients we have also investigated the possibility of producing high numbers of disease-free HSCs from FA mouse models and FA patients by means of the combined use of gene therapy and cell reprogramming. ES-like colonies, fulfilling all the criteria required for iPS cells were generated from somatic cells obtained from FA mice and FA patients. The *in vitro* differentiation of genetically corrected FA-iPS cells allowed us to generate hematopoietic progenitors that were resistant to otherwise cytotoxic concentrations of DNA cross-linking drugs. Finally, because of the self-renewal properties of iPS cells, cell reprogramming strategies have opened new perspectives for the gene therapy of inherited diseases, including FA, by means of homologous recombination strategies.

S19.2.4
Gene therapy for Parkinson's disease: strategies for local production of dopamine

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Three enzymes are necessary for efficient dopamine synthesis: tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC), and guanosine triphosphate cyclohydrolase I (GCH). A severe loss of nigrostriatal nerve terminals in individuals with advanced Parkinson's disease (PD) is associated with an 80–95% decrease in the activity of these enzymes, thus leading to a profound decrease in dopamine levels in the striatum. The main therapeutic strategy for PD is the replacement of dopamine to alleviate motor dysfunction. The dopamine precursor L-dopa is converted to dopamine by AADC, and has been the most effective drug for PD. However, as the disease progresses, loss of AADC activity and decreased capacity for dopamine storage in synaptic vesicles lead to failure of L-dopa therapy. Researchers including us have been developing gene therapies aimed at local

production of dopamine via the introduction of genes encoding dopamine-synthesizing enzymes into the putamen. Two phase I clinical studies have used recombinant adeno-associated virus (AAV) vectors to transfer the gene encoding AADC into the putamen to restore efficient conversion of orally administered L-dopa. Results of these studies have both confirmed the safety of AAV vectors and shown alleviation of motor symptoms associated with PD. Interestingly, motor performance in the "off" medication state was improved after gene therapy, suggesting long-term modulation of dopaminergic signals in striatal neurons. Delivery of the genes encoding TH and GCH in addition to AADC may help prevent motor fluctuations associated with intermittent intake of L-dopa by ensuring a continuous supply of dopamine in the putamen. A clinical study of such a triple gene transfer is presently underway using an equine infectious anemia virus (EIAV) vector. More recently, AAV vector-mediated AADC gene transfer was used to treat neurological symptoms in congenital AADC deficiency, a rare genetic disease in children.

S19.2.5
Treatment of lymphoid cells with the topoisomerase II poison etoposide leads to an increased juxtaposition of AML1 and ETO genes on the surface of nucleoli

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See Abstract P19.47.

S19.2.6
Antisense RNA-induced exon-skipping for the gene therapy of frontotemporal dementia and parkinsonism associated with chromosome 17 (FTDP-17)

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See Abstract P19.15