Ho(a)*x*ing Autophagy to Regulate Development

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In this issue of *Developmental Cell*, Banreti et al. (2014) demonstrate that canonical autophagy is inhibited in the *Drosophila* fat body by Hox transcriptional factors. This regulation involves repression of the *Atg* genes. Programmed developmental autophagy is thus under Hox control and may influence cell fate determination.

Autophagy is an essential mechanism of degradation of several cellular components, from cytosolic portions to long-lived proteins, aggregates, or entire organelles (Mizushima and Komatsu, 2011). In normal conditions, autophagy is important in cell quality control: for instance, its activation regulates the homeostasis of healthy and functional mitochondria. Furthermore, autophagy is a mechanism of stress response, usually increased under specific stress conditions of the cell, such as nutrient deprivation. In recent years, increasing evidence has highlighted the pivotal role of autophagy in crucial mechanisms and pathophysiological conditions, such as cell proliferation and death, cancer, immunity, and neurodegeneration.

Another intriguing role for autophagy, clearly elucidated in lower eukaryotes, is its influence on organism development. As a cellular response in several developmental stages, it helps cells survive stressful steps ranging from yeast sporulation to insect metamorphosis to *Caenorhabditis elegans* dauer formation. Indeed, during these processes, the autophagic machinery is developmentally programmed and subtly regulated (Conradt, 2009). In *Drosophila*, autophagy is induced by food deprivation in the fat body, ovaries, and muscle, and it is activated in many tissues as physiological response to hormones (such as ecdysone during fly metamorphosis) in fat body, intestine, and salivary glands (McPhee and Baehrecke, 2009). In a developmental context, autophagy can hence be modulated genetically as well as through environmental alterations such as food starvation. A role for autophagy in the development of mammals has also been proposed (Cecconi and Levine, 2008; Mizushima and Levine, 2010), mostly based on the observations that many autophagy gene-knockout mice exhibit embryonic lethality at different stages of development. From these observations, it seemed that the more upstream in the autophagy machinery the targeted autophagy gene is positioned, the more severe would be the phenotype found in organism development. Indeed, in mice, knockout of two upstream autophagy regulators, such as Ambra1 and Beclin 1, causes embryonic lethality, whereas targeting of other autophagy-related genes (such as Atg5 and Atg7) by affecting autophagy progression induces perinatal death. Notably, however, until now in both insects and vertebrates, insight into the fine genetic regulation of autophagy during development has remained quite elusive.

Excitingly, the hypothesis that autophagy is genetically programmed in development has found solid experimental confirmation in this issue of *Developmental Cell*: Banreti and colleagues (2014) now elegantly show a link between autophagy and the highly conserved *Hox* genes that usually control the anteroposterior (A-P) axis determination in fly development. The *Hox* family is an evolutionary conserved group of genes responsible for the temporal and positional determination of the body segments' components. To achieve this, they are differentially expressed along the A-P axis based on the "posterior prevalence" rule (Duboule and Morata, 1994). Banreti et al. (2014) demonstrated that, other than their role in A-P determination, *Hox* genes are key temporal regulators of autophagy in *Drosophila* fat body cells. First, the authors observed that localization of *Hox* gene expression within the fat body was not paralleled by any functions in the organ's spatial organization. Thus, the *Hox* genes must also play an additional, still unappreciated role. Surprisingly, the authors observed that Hox proteins seem to show a crucial inhibitory activity on developmental autophagy (Figure 1). Indeed, imaging analysis clearly demonstrated that at stages when developmental autophagy was normally induced (in fly third-larval stage transition from feeding to wandering conditions), a concomitant temporal global downregulation of the *Hox* genes was detected. In contrast, by forcing the expression of Hox proteins during the wandering stages, these proteins were able to specifically block autophagy in this phase and to delay larvae development. Furthermore, Pontin, an accessory component of the Hox regulatory Brahma complex, is shown in this paper to be the key factor responsible for dictating the temporal modulation of *Hox* genes. Consistently, its expression is switched off in the feeding-wandering transition, impacting its ability to maintain *Hox* gene expression. Banreti et al. (2014) showed that downregulation of Pontin brings on a premature induction of autophagy, preceded by loss of *Hox* gene expression in feeding animals. However, Hox expression is sufficient to block autophagy, as shown in Pontin-deficient conditions by overexpression of any single *Hox* gene. Interestingly, not only is autophagy modulated in the fat body by ecdysone signaling (through temporal regulation of both Pontin and *Hox* gene expression that this developmental hormone exerts), as reported previously (Rusten et al., 2004), but also nutrient-starvation-induced autophagy needs downregulation of Hox

genes in order to be activated. The autophagy pathway involved is thus the canonical autophagy pathway, primed by food deprivation and by the consequent inhibition of the nutrient/energy sensor TOR complex, but functioning earlier than at the feeding larvae stage. Hence, Pontin-regulated Hox proteins are general strong inhibitors of canonical starvation-induced autophagy, thus indicating a more general role for this gene family on autophagy regulation. How do *Hox* genes shut autophagy down? Banreti and colleagues (2014) show that the Hox proteins can repress the expression of key autophagy modulators, such as the autophagy genes (i.e., *Atg1*, *Atg7*, and *Atg8b*). Because the transcriptional factor TFEB (Mitf in *Drosophila*) has been shown to directly and globally activate *Atg* genes upon sensing the nutrient content of the cells at the lysosome (Settembre et al., 2013), it could be hypothesized that, during specific developmental stages, the comprehensive autophagy transcriptional regulation is hoaxed by the *Hox* control and somehow distracted from TFEB regulation even in the presence of nutrients.

These interesting findings open an entire set of crucial questions: What is the role of autophagy modulation during this specific developmental stage in the fly larvae? Is it controlling cell death? Is any cellular metabolic switch, dependent on autophagy, crucial in the homeostasis or function of the fat body? Does this downregulation halt a sort of cellular resetting that autophagy can control by digesting specific transcriptional factors, signalers, or cell fate determinants? Although the effect of *Hox* genes on autophagy seems to occur regardless of the Hox gene identity, is this regulation of autophagy by *Hox* genes typical of the fat body, or does it take place in an orchestrated way along the A-P axis? Because this regulatory pathway, according to the results obtained by Banreti and colleagues (2014), is evolutionary conserved, what is the role played by vertebrate-specific autophagy factors, such as the proautophagic molecule Ambra1, in this context?

Developmental autophagy may represent a reservoir of novel cellular activities and molecular networks that need to be strictly temporally and/or spatially regulated. The observations of Banreti et al. (2014) provide insights into this complex interplay between cell proliferation, death, and differentiation.

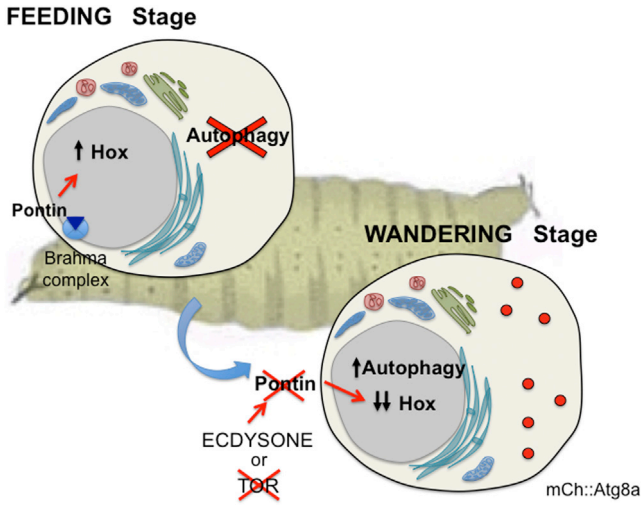

Figure 1. *Hox* Genes Modulate Autophagy in the *Drosophila* Fat Body

Schematic representation of the molecular mechanisms regulating the switchoff of *Hox* genes and the consequent increase on autophagy during the transition from feeding stage 3 to wandering stage 3 of fruit fly larvae in a fat body cell. Autophagy was detected by mCherry::Atg8a-marked (mCh::Atg8a) granules (Banreti et al., 2014).

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