Localization and genomic structure of human deoxyhypusine synthase gene on chromosome 19p13.2-distal 19p13.1

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Abstract

The amino acid hypusine is formed post-translationally in a single cellular protein, the eukaryotic translation initiation factor 5A, by two enzymes, namely deoxyhypusine synthase and deoxyhypusine hydroxylase. Hypusine is found in all eukaryotes and in some archaeabacteria, but not in eubacteria. The deoxyhypusine synthase cDNA was cloned and mapped by fluorescence in situ hybridization on chromosome 19p13.11-p13.12. Rare cDNAs containing internal deletions were also found. We localized the deoxyhypusine synthase gene on a high resolution cosmID/BAC contig map of chromosome 19 to a region in 19p13.2-distal 19p13.1 between MANB and JUNB. Analysis of the genomic exon/intron structure of the gene coding region showed that it consists of nine exons and spans a length of 6.6 kb. From observation of the genomic structure, it seems likely that the internally deleted forms of mature RNA are the result of alternative splicing, rather than of artifacts. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Hypusine biosynthesis; DHPS gene isoforms; Cell proliferation

1. Introduction

The unusual amino acid hypusine (N-(4-amino-2-hydroxybutyl)lysine) is formed post-translationally in a single cellular protein, the eukaryotic translation initiation factor 5A (eIF-5A) (Park et al., 1993a). Hypusine biosynthesis is one of the most specific post-translational modifications known to date. It occurs through two enzymatic steps. In the first step, deoxyhypusine synthase (DHPS) catalyzes the NAD-dependent transfer of the 4-aminobutyl moiety of spermidine to the ε-amino group of a specific lysine residue (Lys50) in the precursor of eIF-5A to produce the intermediate deoxyhypusyl residue. In the second separate enzymatic step, hydroxylation completes hypusine synthesis and eIF-5A maturation (reviewed in Park et al., 1993b). Hypusine is found in all eukaryotes and in some archaeabacteria, but not in eubacteria (Bartig et al., 1992; Park et al., 1993a). The amino acid sequence of eIF-5A is highly conserved in all eukaryotes examined, especially the stretch of 12 amino acids surrounding the hypusine residue, which is not found in any other known protein. This suggests an important, if not fundamental, function of this protein throughout eukaryotic evolution.

It has been suggested that eIF-5A plays a role in protein synthesis (Park et al., 1993a,b). However, recent data show that protein synthesis initiation is not significantly affected in eIF-5A-depleted yeast cells (Kang and Hershey, 1994). Recently, eIF-5A has also been identified as a cellular co-factor necessary for the HIV-1 regulatory protein Rev activity (Ruhl et al., 1993; Bevec et al., 1996a), which affects the generation of infectious virus particles. Although the actual cellular function of
between MANB and JUNB, and its genomic structure. We also discuss the possible presence of different isoforms of DHPS generated by exon skipping and alternative splicing.

2. Materials and methods

The I.M.A.G.E. Consortium (Lennon et al., 1996) cDNA No. 83125 corresponding to EST T68101 was mapped at the Lawrence Livermore National Laboratory (LLNL) to cosmid 30649. This cosmid belongs to a contig located in region 19p13.1-13.2 (Ashworth et al., 1995). A GeneBank search matched this EST sequence to the sequence of the human DHPS gene. EST T68101 represents a short form of the gene, differing from the long one by an internal deletion of 141 bp (Yan et al., 1996).

To determine the genomic structure of the DHPS gene, multiple primers were designed according to the known full-length cDNA sequence (Acc. No. L39068): R1 (5'-TATAGGCGCATGGAAGGTTC-3'), Fd (5'-TGAACTGATGAGGTTGGATG-3'), R2 (5'-ATCGAGAAGAAGCTGGAACC-3'), Fb (5'-ACTCTGGGTGTGTGATCTCC-3'), R5 (5'-ACGCCTTCTAAGATGATCGC-3'), Fc (5'-CCACATCCCTGTGTTTAGTC-3'), R7 (5'-TGCCCCAGGAGACAGC-3'), R4 (5'-ACCAGACGAGGCTGTCTC-3'), and used to sequence the full-length product, two clones with internal deletions of 168 and 141 bp (56 and 47 aa, respectively) were found (Joe et al., 1995; Yan et al., 1996). The deduced protein sequence, inferred from the full-length nucleotide sequence, consists of 369 amino acids. In addition to the full-length product, two clones with internal deletions of 168 and 141 bp (56 and 47 aa, respectively) were found (Joe et al., 1995; Yan et al., 1996). It is not clear whether these two deleted products are artifacts or if they represent different isoforms. DHPS was mapped on chromosome 19p13.11-p13.12 by FISH analysis (Jones et al., 1996).

Here we report the physical localization of the DHPS gene on a high resolution cosmide/BAC contig map of chromosome 19 to a region in 19p13.2-distal 19p13.1 between MANB and JUNB, and its genomic structure. We also discuss the possible presence of different isoforms of DHPS generated by exon skipping and alternative splicing.

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R5/Fe, R7/Fa and R4/Fc. All PCR amplifications were performed on 200 ng of genomic DNA and 10 ng of cosmid DNA in a 50 ml reaction volume containing 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 0.2 mM for each of dNTPs, 1.5 mM MgCl₂, 1.5 U Taq polymerase and 40 pmol of each primer. Thirty-five cycles (1 min 94 °C, 1 min 54 °C, 3 min 72 °C) were performed in a PTC-100 thermocycler (MJ Research, Inc., MA, USA). Furthermore, a panel of cosmid 30649 DNA single and double digests with the restriction enzymes EcoRI, BamHI, HindIII and BglII were blotted onto a nylon membrane and hybridized by standard procedures with probes obtained by PCR amplifications with R1/Fd and R4/Fc primer pairs coding region is fully contained in the 21.1-kb fragment of cosmid 30649 and the restriction map of the region allowed us to orient the gene with the 5’ end centromeric with respect to the 3’ end (Fig. 2).

3. Results and discussion

3.1. Physical localization of DHPS gene

The identity between the sequences of the DHPS cDNA and EST T68101, the latter physically mapped on a contig belonging to the high resolution cosmID BAC contig map of chromosome 19 (Ashworth et al., 1995), permitted the localization of the DHPS gene in the region 19p13.2-distal 19p13.1 between MANB and JUNB (Fig. 1). The localization reported here is different from the previous one (Jones et al., 1996) that mapped DHPS on chromosome 19p13.11-p13.12. The discrepancy may result from the different procedures that have been used. We assigned the DHPS gene a position based on that of cosmid 30649. The latter was mapped at LLNL by integrating data from a variety of distinct experimental sources including FISH, restriction mapping and STS screening (Brandri

3.2. Genomic structure of DHPS gene

The sequencing of the genomic DHPS gene and its comparison with the cDNA sequence revealed that the coding region was composed of nine exons encompassing approximately 6.6 kb (Fig. 2). The initiation and termination sites of translation were localized in exons 1 and 9, respectively. The poly-A signal was localized in exon 9. All junction sequences were in agreement with the consensus splice signal for the intron–exon junction (Padgett et al., 1986) (Table 1). (The data are available from the EMBL/GenBank data libraries under the following accession numbers: AJ001701–4.) The DHPS coding region is fully contained in the 21.1-kb EcoRI fragment of cosmid 30649 and the restriction map of the region allowed us to orient the gene with the 5’ end centromeric with respect to the 3’ end (Fig. 2).

3.3. Analysis of different cDNA isoforms produced by alternative splicing

Three different DHPS cDNA sequences have been found by two different groups (Joe et al., 1995; Yan

Table 1

<table>
<thead>
<tr>
<th>Exon</th>
<th>Size</th>
<th>cDNA nt</th>
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<tr>
<td>1</td>
<td>&gt;244</td>
<td>244</td>
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<tr>
<td>2</td>
<td>165</td>
<td>245–409</td>
</tr>
<tr>
<td>3</td>
<td>122</td>
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</tr>
<tr>
<td>4</td>
<td>97</td>
<td>532–628</td>
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<td>5</td>
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<tr>
<td>6</td>
<td>106</td>
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</tr>
<tr>
<td>7</td>
<td>104</td>
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<tr>
<td>8a</td>
<td>126</td>
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</tr>
<tr>
<td>8b</td>
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<tr>
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<td>926–1051</td>
</tr>
<tr>
<td>9</td>
<td>&gt;226</td>
<td>1052</td>
</tr>
</tbody>
</table>

Note: Upper- and lower-case letters represent exonic and intronic sequences, respectively. The 5’ splice donor of exon 8 is the same in all isoforms.

1 Nucleotide numbers correspond to the cDNA sequence Acc. No. L39068 (Joe et al., 1995).
have shown that the protein is a tetramer with four 47 aa, exhibited no detectable enzyme activity (Yan et al., 1996). Supporting these results, recent studies carried out on M15 cells, transfected with a plasmid expressing the shorter form of DHPS protein lacking the highly conserved region, including residues 277–330 of E355. Work by A.S.O. and G.L. was performed under the auspices of the U.S. DOE under contract No. W-7405-ENG-48.

Both deletions remove an important section of a 141 nucleotides, corresponding to the deletions of 47 amino acids (Asp – Thr – Ser). In this context, a highly frequent activation of cryptic splicing sites, resulting in the presence of shortened mRNAs in all three of the libraries analyzed, could not be excluded. On the other hand, one should consider the possibility that the proteins coded by the shortened forms of mRNAs play a yet unknown biological role. There are, in fact, many examples of the generation of proteins with diverse functions from a single gene by alternative splicing (for a review see Foulkes and Sassone-Corsi, 1992).

Moreover, the alternative spliced forms of DHPS do not seem to occur very frequently. In fact, only one internally deleted clone was found out of 25 assayed (Joe et al., 1995). In view of the present knowledge on the tetrameric structure of DHPS, the expression experiments mentioned above showed that a protein assembled from four short subunits did not exhibit the canonical function. It would be interesting to investigate the expression of the tetramer made up by different isoforms. Characterization of the genomic structure of the DHPS gene will aid in the detection of possible mutations and polymorphisms of the gene which can affect the production of ef-S-A and hence, indirectly affect cell proliferation and virus infection. As DHPS could represent a potential target for intervention in cell proliferation, further investigation regarding the possible function of these short DHPS isoforms would be of great interest. They could act as modulating factors of DHPS activity or have a different function, most likely correlated with cell proliferation.

Acknowledgement

This work was supported by TELETHERON grant No. E355. Work by A.S.O. and G.L. was performed under the auspices of the U.S. DOE under contract No. W-7405-ENG-48.

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