ELSEVIER

Gene 215 (1998) 153–157

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

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

Elide Mantuano [a,b], Flavia Trettel [b], Anne S. Olsen [c], Greg Lennon [c], Marina Frontali [a], Carla Jodice [b,*]

[a] *Istituto di Medicina Sperimentale, CNR, Rome, Italy*
[b] *Dipartimento di Biologia, Università 'Tor Vergata', Via della Ricerca Scientifica-00133 Rome, Italy*
[c] *Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551, USA*

Received 2 February 1998; received in revised form 29 April 1998; accepted 29 April 1998; Received by E. Boncinelli

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 archaebacteria, 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.

Keywords: Hypusine biosynthesis; *DHPS* gene isoforms; Cell proliferation

1. Introduction

The unusual amino acid hypusine (N^{ϵ}-(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 deoxyhypusil 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 archaebacteria, 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

* Corresponding author. Tel.: +39 06 72594321; Fax: +39 4 2023500; E-mail: jodice@utovrm.it

Abbreviations: aa, amino acid(s); Asp, aspartic acid; BAC, bacterial artificial chromosome; bp, base pair(s); cDNA, DNA complementary to RNA; DHPS, deoxyhypusine synthase; *DHPS*, gene encoding DHPS; eIF-5A, eukaryotic translation initiation factor 5A; EST, expressed sequence tag; FISH, fluorescence in situ hybridization; I.M.A.G.E. Consortium, Integrated Molecular Analysis of Genome Consortium; *JUNB*, oncogene; kb, kilobase(s) or 1000 bp; Lys, lysine; *MANB*, gene encoding alpha mannosidase B; PCR, polymerase chain reaction; Ser, serine; Thr, threonine.

0378-1119/98/$19.00
PII: S0378-1119(98)00254-6

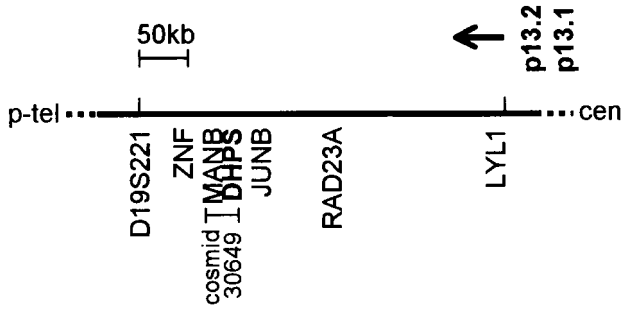

Fig. 1. Physical map of the region 19p13.2-distal 19p13.1. The *DHPS* gene maps between *MANB* and *JUNB* on cosmid 30649 (modified from Ashworth et al. (1995)).

eIF-5A is unknown, hypusine and eIF-5A appear to be vital for cell proliferation in eukaryotes (Park et al., 1993b; Kang and Hershey, 1994). In fact, inhibitors of hypusine biosynthetic enzymes exert antiproliferative effects (Jakus et al., 1993; Hanauske-Abel et al., 1994; Park et al., 1994; Chen et al., 1996; Shi et al., 1996). Therefore, DHPS represents a potential target for intervention in cell proliferation.

Three independent groups have recently cloned the cDNA of the human *DHPS* gene (Joe et al., 1995; Bevec et al., 1996b; 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 cosmid/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.

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 GenBank 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′-ACTCTGGGTTGTTGATCTCC-3′), *R5* (5′-ACGCCTTCTAAGATGATCGC-3′), *Fe* (5′-AACGATGTCCAGGACCAGGC-3′), *R7* (5′-CCACATCCCTGTGTTTAGTC-3′), *Fa* (5′-TGCCCCAGGAGACAGC-3′), *R4* (5′-ACCAGACGAGGCTGTCTC-3′), *Fc* (5′-GAAGGACTTCAGATACCATC-3′), and used to sequence cosmid 30649 with an ABI 310 automated sequencing apparatus with a Cycle Sequencing Perkin Elmer kit according to the manufacturer's instructions. Intron sizes were determined directly by sequencing or by exon–exon PCR amplification of cosmid and genomic DNA using the following primer pairs: *R1*/*Fd*, *R2*/*Fb*,

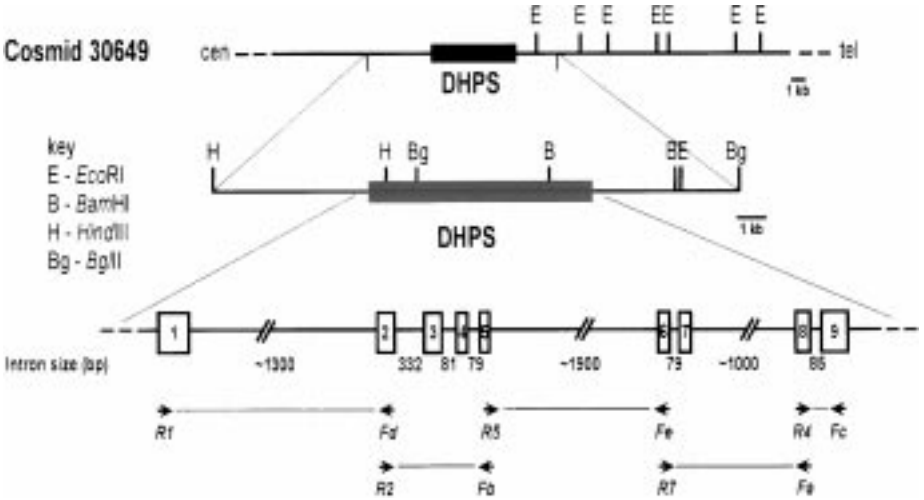

Fig. 2. A schematic representation of the genomic organization of the *DHPS* gene. The *Eco*RI LLNL restriction map of cosmid 30649 and its orientation is shown at the top of the figure (Ashworth et al., 1995). A restriction map around the gene is also shown.

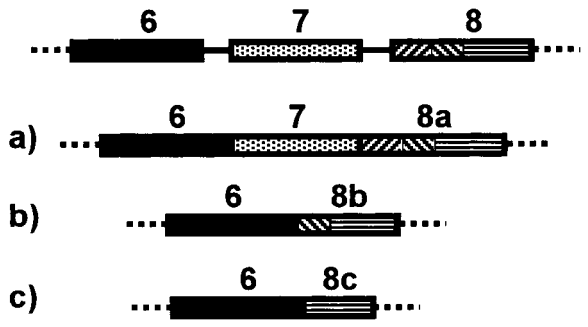

Fig. 3. Schematic representation of splicing events in the *DHPS* gene. At the top is the genomic structure in the region of exons 6–8. **(a)** Full-length mature transcript; **(b)** and **(c)** shorter isoforms by alternative splicing of exon 7 and part of exon 8.

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 μl 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 $MgCl_2$, 1.5 U Taq DNA 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 digestions with the restriction enzymes *Eco*RI, *Bam*HI, *Hin*dIII and *Bgl*II 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 corresponding to the 5′ and 3′ end of the gene, respectively, and with vector DNA.

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 (Brandriff et al., 1994).

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 *Eco*RI 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
Intron–exon boundaries of the human *DHPS* gene

Exon	Size	cDNA nt[a]	3′ splice acceptor	5′ splice donor
1	>244	−244		ATGCCATG **gt**gaggac
2	165	245–409	ttactcac**ag** ATCGAGAAGA	ACAACATG **gt**ggggac
3	122	410–531	ttggctgc**ag** GTGGACGTAT	ATCAATAG **gt**gagaac
4	97	532–628	ctgttctc**ag** GATCGGAAAC	ACACAGAG **gt**ggggct
5	87	629–715	cctctcct**ag** GGTGTAAAGT	CCCAGAAG **gt**gaggac
6	106	716–821	ttctctgc**ag** AACCACATCC	CGTTGAGG **gt**gaggcg
7	104	822–925	cttccccc**ag** ACCTGAGGCT	ACCTCATG **gt**gagtgg
8ab	126	926–1051	ctccccac**ag** CGGAACGGGG	CCGTCAAG **gt**aagcgc
8bb	89	963–1051	atcaacac**ag** CCCAGGAGTT	CCGTCAAG **gt**aagcgc
8cb	62	990–1051	tctgactc**ag** GTGCCCGACC	CCGTCAAG **gt**aagcgc
9	>226	1052–	ccctaccc**ag** GTCTATGCTG	

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.
[a] Nucleotide numbers correspond to the cDNA sequence Acc. No. L39068 (Joe et al., 1995).
[b] Exon 8 of the *DHPS* transcript is alternatively spliced (see Section 3.3); 8a represents the major donor site.

et al., 1996). One of these corresponds to the full-length DHPS protein, the other two contain internal deletions of 141 nucleotides, corresponding to the deletions of 47 amino acids (Asp262–Thr308) and 168 nucleotides, corresponding to 56 amino acids (Asp262–Ser317), respectively. Comparisons between the genomic structure inferred by the full-length cDNA and the short cDNAs revealed that their deletions correspond to the skipping of exon 7 and part of exon 8. In one of them the first 37 nucleotides of exon 8 are lacking, and in the other, the first 64 nucleotides (Fig. 3). The acceptor splice sites of both deleted forms conform to consensus splice signals (Padgett et al., 1986).

Both deletions remove an important section of a highly conserved region, including residues 277–330 of the human enzyme (Joe et al., 1995). Experiments carried out on M15 cells, transfected with a plasmid expressing the shorter form of DHPS protein lacking 47 aa, exhibited no detectable enzyme activity (Yan et al., 1996). Supporting these results, recent studies have shown that the protein is a tetramer with four identical subunits and that the region corresponding to the deletion of the 47 aa contributes to shape the active site cavity in which spermidine and lysine side-chains of the eIF-5A precursor interact with the enzyme during cathalysis (Liao et al., 1998). However, the shorter forms of *DHPS* seem most probably due to natural mechanisms such as alternative splicing rather than to laboratory artifacts because: (1) both deletions start exactly at the end of exon 6; (2) both deletions end in a context corresponding to a consensus splice signal (Padgett et al., 1986); (3) both deletions express in-frame products; and (4) by PCR analysis, the presence of both short and long cDNAs in all three of the libraries analyzed was proved, although in different proportions (Yan et al., 1996). In this context, a highly frequent activation of cryptic splicing sites, resulting in the presence of shortened mRNAs in the different cDNA libraries, 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 with 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 eIF-5A 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 TELETHON 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.

References

Ashworth, L.K., Batzer, M.A., Brandriff, B., Branscomb, E., de Jong, P., Garcia, E., Garnes, J.A., Gordon, L.A., Lamerdin, J.E., Lennon, G., Mohrenweiser, H., Olsen, A.S., Slezak, T., Carrano, A.V., 1995. An integrated metric physical map of human chromosome 19. Nature Genet. 11, 422–427.

Bartig, D., Lemkemeier, K., Frank, J., Lottspeich, F., Klink, F., 1992. The archaebacterial hypusine-containing protein. Structural features suggest common ancestry with eukaryotic translation initiation factor 5A. Eur. J. Biochem. 204, 751–758.

Bevec, D., Jaksche, H., Himmelspach, M., Wöhl, T., Pacher, A., Schebesta, M., Koettnitz, K., Dobrovnik, M., Csonga, R., Lottspeich, F., Hauber, J., 1996a. Inhibition of HIV-1 replication in lymphocytes by mutants of Rev co-factor eIF-5A. Science 271, 1858–1860.

Bevec, D., Kappel, B., Jaksche, H., Csonga, R., Hauber, J., Klier, H., Steinkasserer, A., 1996b. Molecular characterization of a cDNA encoding functional human deoxyhypusine synthase and chromosomal mapping of the corresponding gene locus. FEBS Lett. 378, 195–198.

Brandriff, B.F., Gordon, L.A., Fertitta, A., Olsen, A.S., Christensen, M., Ashworth, L.K., Nelson, D.O., Carrano, A.V., Mohrenweiser, H.W., 1994. Human chromosome 19p: a fluorescence in situ hybridization map with genomic distance estimates for 79 intervals spanning 20 Mb. Genomics 23, 582–591.

Chen, Z.P., Yan, Y.P., Ding, Q.J., Knapp, S., Potenza, J.A., Schugar, H.J., Chen, K.Y., 1996. Effects of inhibitors of deoxyhypusine synthase on the differentiation of mouse neuroblastoma and erythroleukemia cells. Cancer Lett. 105, 233–239.

Foulkes, N.S., Sassone-Corsi, P., 1992. More is better: activators and repressors from the same gene. Cell 68, 411–414.

Hanauske-Abel, H.M., Park, M.H., Hanauske, A.-R., Popowicz, A.M., Lalande, M., Folk, J.E., 1994. Inhibition of the G1-S transition of the cell cycle by inhibitors of deoxyhypusine hydroxylation. Biochim. Biophys. Acta 1221, 115–124.

Jakus, J., Wolff, E.C., Park, M.H., Folk, J.E., 1993. Features of the spermidine-binding site of deoxyhypusine synthase as derived from inhibition studies. J. Biol. Chem. 268, 13151–13159.

Joe, Y.A., Wolff, E.C., Park, M.H., 1995. Cloning and expression of human deoxyhypusine synthase cDNA. J. Biol. Chem. 270, 22386–22392.

Jones, T., Sheer, D., Kapetanopoulos, A., Hauber, J., Bevec, D., Steinkasserer, A., 1996. The gene coding for human deoxyhypusine syn-

thase (DHPS) maps to chromosome 19p13.11-p13.12. Genomics 35, 635–636.

Kang, H.A., Hershey, J.W., 1994. Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae*. J. Biol. Chem. 269, 3934–3940.

Lennon, G.G., Auffray, C., Polymeropoulos, M., Soares, M.B., 1996. The I.M.A.G.E. Consortium: an integrated molecular analysis of genome and their expression. Genomics 33, 151–152.

Liao, D.-I., Wolff, E.C., Park, M.H., Davies, D.R., 1998. Crystal structure of the NAD complex of the human deoxyhypusine synthase: an enzyme with a ball-and-chain mechanism for blocking the active site. Structure 6, 23–32.

Padgett, R.A., Grabwski, P.J., Kornaska, M.M., Seiler, S., Sharp, P.A., 1986. Splicing of messenger RNA precursors. Annu. Rev. Biochem. 55, 1119–1150.

Park, M.H., Wolff, E.C., Folk, J.E., 1993a. Hypusine: its post-translational formation in eukaryotic initiation factor 5A and its potential role in cellular regulation. Biofactor 4, 95–104.

Park, M.H., Wolff, E.C., Folk, J.E., 1993b. Is hypusine essential for eukaryotic cell proliferation? Trends Biol. Sci. 18, 475–479.

Park, M.H., Wolff, E.C., Lee, Y.B., Folk, J.E., 1994. Antiproliferative effects of inhibitors of deoxyhypusine synthase. Inhibition of growth of Chinese hamster ovary cells by guanyl diamines. J. Biol. Chem. 269, 27827–27832.

Ruhl, M., Himmelspach, M., Bahr, G.M., Hammerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Probst, H., Bevec, D., Hauber, J., 1993. Eukaryotic initation factor 5A is a cellular target of the HIV-1 rev activation domain mediating trans-activation. J. Cell. Biol. 123, 1309–1320.

Shi, X.-P., Yin, K.-C., Ahern, J., Davis, L.J., Stern, A.M., Waxman, L., 1996. Effects of N1-guanyl-1,7-diaminoheptane, an inhibitor of deoxyhypusine synthase, on the growth of tumorigenic cell lines in culture. Biochim. Biophys. Acta 1310, 119–126.

Yan, Y.P., Tao, Y., Chen, K.Y., 1996. Molecular cloning and functional expression of human deoxyhypusine synthase cDNA based on expressed sequence tag information. Biochem. J. 315, 429–434.