ELSEVIER

Cancer Letters 102 (1996) 85–90

CANCER LETTERS

A cDNA from the ovarian cancer critical region of deletion on chromosome 17p13.3

Nancy J. Phillips[a,*], Michelle R. Ziegler[a], Larry L. Deaven[b]

[a]*Department of Pathology, Saint Louis University School of Medicine, 1402 S. Grand Ave., St. Louis, MO 63110, USA*
[b]*Center for Human Genome Studies, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA*

Received 26 December 1995; revision received 19 January 1996; accepted 19 January 1996

Abstract

Chromosome 17p13.3 is frequently deleted in human ovarian carcinoma, and the 15 kb critical region of deletion may contain a tumor suppressor gene. A 2.3 kb cDNA has been identified which spans 17 kb of genomic DNA, including 8.1 kb within the critical region, and thus is a candidate tumor suppressor gene. This highly conserved gene has significant sequence similarity to a yeast gene of unknown function and to one of the yeast enzymes in the diphthamide synthetic pathway, DPH2, that has a role in global protein synthesis regulation. This gene, named DPH2L (diphthamide biosynthesis protein 2 -like), is expressed in multiple tissues and stages of development.

Keywords: Ovarian cancer; Chromosome 17; Tumor suppressor gene

1. Introduction

Tumor initiation in ovarian epithelium is not well understood. Relatively few consistent genetic alterations have been observed in ovarian tumors of low malignant potential and in non-metastatic (FIGO stage I) carcinomas. A region of chromosome 17p13.3 bounded by loci D17S28 and D17S30 is deleted in 80% of all ovarian epithelial malignancies, including 3 of 7 tumors of low malignant potential and 4 of 5 non-metastatic carcinomas [1]. This deletion precedes loss of known chromosome 17 tumor suppressor loci TP53 and BRCA1, which are deleted or mutated primarily in metastatic carcinomas [1–5]. The region of overlapping deletion between D17S28 and D17S30 is 15 kb in size and is GC-rich, with a CpG island located adjacent to D17S30 [6]. CpG islands are associated with the 5′ ends of constitutively expressed genes and tend to be found in regions of high gene density. One gene associated with the D17S30 CpG island has been cloned, HIC-1, and the coding sequences lie outside the ovarian carcinoma critical region of deletion [7]. In order to obtain a candidate tumor suppressor gene with coding sequences within the common region of deletion, we subjected genomic cosmids of the region to exon amplification and identified a novel cDNA clone using the amplified exon.

* Corresponding author. Tel.: +1 314 5778782; fax: +1 314 2685120; e-mail: phillinj@sluvca.slu.edu

0304-3835/96/$12.00
PII: S0304-3835(96)04169-9

2. Materials and methods

2.1. Contig assembly and exon amplification

A flow-sorted human chromosome 17 cosmid library (LA17NL01) constructed in the Center for Human Genome Studies at the Los Alamos National Laboratory [8] was probed with ^{32}P-random primed D17S28/pYNH37.3 and D17S30/pYNZ22.1 [9], yielding a 74 kb 3-cosmid minimal contig (Fig. 1A). Detailed mapping showed that the contig contained 34 kb adjacent to D17S28 and 24 kb adjacent to D17S30, in addition to the critical region between the two loci. Complete BamHI and EcoRI single digests of single cosmids were shotgun cloned into pSPL3 (BRL) [10], with gel analysis of resulting DNA preparations showing adequate representation of all cosmid fragments. Transfection and RT-PCR were performed according to the manufacturer's protocol. Subcloned inserts were ^{32}P-labelled and hybridized to blots of double restriction digests of the cosmid contig, confirming localization within the contig. Inserts were sequenced by the chain termination method using Sequenase 2.0 (Amersham).

2.2. cDNA cloning

A 344 bp BglII-SalI fragment containing most of the 345 bp exon amplification insert derived from cosmid 66C4 was hybridized at high stringency to filters from a 9-week human fetal and placental cDNA library (FP4, ATCC 77434) [11], yielding 15 positive plaques from 5×10^5 plaques screened. After an additional round of hybridization screening, six clones were analyzed. Using 1 μM PCR primers 5′-ACCTTTGTTCCAGGGATTCTG-3′ and 5′-GCGG-ACCCCGACATCTC-3′ (designed from the exon amplification product sequence), 200 μM dATP, dCTP, dGTP, dTTP, 2.5 units TaqI DNA polymerase (Promega) and the supplied standard buffer with 1.5 mM $MgCl_2$, and 30 cycles of 94° for 1 min, 58° for 1 min and 72° for 1 min, the expected 256 bp PCR product was demonstrated in all clones. Gel analysis showed that inserts from the six clones

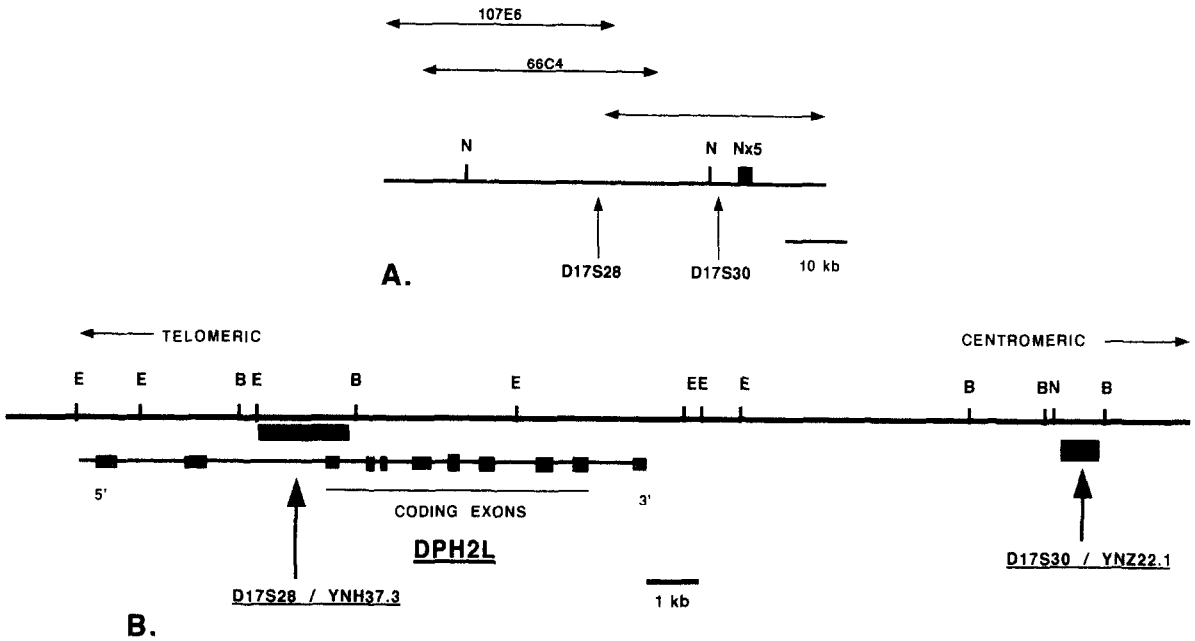

Fig. 1. (A) Cosmid contig containing ovarian cancer smallest region of deletion. Clone names are indicated. N, NotI site. (B) Detailed restriction map of D17S28–D17S30 region showing DPH2L exons localized to specific restriction fragments. Black boxes indicate exons with known intron-exon boundaries. Gray boxes indicate segments of the 5′ and 3′ non-coding sequence localized to specific restriction fragments by hybridization but with undetermined intron-exon boundaries. Restriction fragments containing D17S28 and D17S30 variable numbers of tandem repeats sequences are indicated with bars. (B) BamHI; E, EcoRI; N, NotI.

shared restriction fragments. The largest of these inserts (B2–3.1) was sequenced on both strands using exonuclease III nested deletion (Promega) and chain termination with Sequenase 2.0 (Amersham). The exon trap sequence was confirmed in this clone. 5′ and 3′ halves of the clone were hybridized to the cosmid digest blots to determine the genomic extent and to establish the transcriptional orientation relative to the loci D17S28 and D17S30 (Fig. 1B).

3. Results and discussion

The B2-3.1 clone insert is 2222 nucleotides in length exclusive of a 12 nucleotide poly-(A) tail, and contains a 1311 nucleotide open reading frame that encodes a 363 amino acid protein with a predicted molecular weight of 39 900 (Fig. 2). This predicted amino acid sequence has eight potential protein kinase C phosphorylation sites (residues 41, 87, 141, 206, 222, 278, 339, 352), six potential casein kinase II phosphorylation sites (residues 11, 20, 64, 132, 284, 352), and two potential N-myristoylation sites (residues 205 and 211) (Prosite 12.1).

The cDNA hybridized to genomic fragments totalling 17 kb, 8.1 kb of which, including most coding sequence, lie within the D17S28–D17S30 critical region of deletion. The transcriptional start site lies telomeric to D17S28, outside the critical region and 26 kb away from the CpG island just centromeric to D17S30 that contains the HIC-1 gene. Long-range mapping of the region by Ledbetter et al. [12] does not show a cluster of NotI, BssHII, or SfiI sites near the DPH2L transcriptional start site. The DPH2L cDNA itself has 17 CpG sites within 150 bases of the 5′ end and has an overall GC content of 60%, suggesting a high GC content of the critical region of deletion. The coding sequence has 8 exons, with an unknown number of exons 5′ to nucleotide 280 and 3′ to nucleotide 1398 (Fig. 1B). Three of the coding exons comprise the 345 bp exon amplification insert.

Comparison of the amino acid sequence with the Genbank non-redundant translated database and the dbest expressed sequence tag database was performed using the BLAST algorithms and NCBI default settings (BLOSUM 62 matrix). Three human expressed sequence tags had identity to portions of

```
   1 gcttgaattagcctgcgctctccgcgttcttccagcgctgtctttttagtaccacatgc
  60 gcaggcaggtgatggcggcgctggtcgtatccggggcagcggagcagggcgccgagacgg
 120 cctggcagaggtcgggccctcgggccgcgtggccaatcagatccccccgagatcctg
 180 aagaaccctcagctgcaggcagcaatccgggtcctgccttccaactacaacttttgagatc

   1                                                        MetPro
 240 cccaagaccatctggaggatccaacaagcccaggccaagaaggtggccttgcaaATGCCG

   3 GluGlyLeuLeuLeuPheAlaCysThrIleValAspIleLeuGluArgPheThrGluAla
 300 GAAGGCCTCCTCCTCTTTGCCTGTACCATTGTGGATATCTTGGAAAGGTTCACGGAGGCC

  23 GluValMetValMetGlyAspValThrTyrGlyAlaCysCysValAspAspPheThrAla
 360 GAAGTGATGGTGATGGGTGACGTGACCTACGGGGCTTGCTGTGTGGATGACTTCACAGCG

  43 ArgAlaLeuGlyAlaAspPheLeuValHisTyrGlyHisSerCysLeuIleProMetAsp
 420 AGGGCCCTGGGAGCTGACTTCTTGGTGCACTACGGCCACAGTTGCCTGATTCCCATGGAC

  63 ThrSerAlaGlnAspPheArgValLeuTyrValPheValAspIleArgIleAspThrThr
 480 ACCTCGGCCCAAGACTTCCGGGTGCTGTACGTCTTTGTGGACATCCGGATAGACACTACA

  83 HisLeuLeuAspSerLeuArgLeuThrProProProAlaThrAlaLeuAlaLeuValSer
 540 CACCTCCTGGACTCTCTCCGCCTCACCTTTCCCCCAGCCACTGCCCTTGCCCTGGTCAGC

 103 ThrIleGlnPheValSerThrLeuGlnAlaAlaAlaGlnGluLeuLysAlaGluTyrArg
 600 ACCATTCAGTTTGTGTCGACCTTGCAGGCAGCCGCCCAGGAGCTGAAAGCCGAGTATCGT

 123 ValSerValProGlnCysLysProLeuSerProGlyGluIleLeuGlyCysThrSerPro
 660 GTGAGTGTCCCACAGTGCAAGCCCCTGTCCCCTGGAGAGATCCTGGGCTGCACATCCCCC

 143 ArgLeuSerLysGluValGluAlaValValTyrLeuGlyAspGlyArgPheHisLeuGlu
 720 CGACTGTCCAAAGAGGTGGAGGCCGTTGTGTATCTTGGAGATGGCCGCTTCCATCTGGAG

 163 SerValMetIleAlaAsnProAsnValProAlaTyrArgTyrAspProTyrSerLysVal
 780 TCTGTCATGATTGCCAACCCCAATGTCCCCGCTTACCGGTATGACCCATATAGCAAAGTC

 183 LeuSerArgGluHisTyrAspHisGlnArgMetGlnAlaAlaArgGlnGluAlaIleAla
 840 CTATCCAGAGAACACTATGACCACCAGCGCATGCAGGCTGCTCGCCAAGAAGCCATAGCC

 203 ThrAlaArgSerAlaLysSerTrpGlyLeuIleLeuGlyThrLeuGlyArgGlnGlySer
 900 ACTGCCCGCTCAGCTAAGTCCTGGGGCCTTATTCTGGGCACTTTGGGCCGCCAGGGCAGT

 223 ProLysIleLeuGluHisLeuGluSerArgLeuArgAlaLeuGlyLeuSerPheValArg
 960 CCTAAGATCCTGGAGCACCTGGAATCTCGACTCCGAGCCTTGGGCCTTTCCTTTGTGAGG

 243 LeuLeuLeuSerGluIlePheProSerLysLeuSerLeuLeuProGluValAspValTrp
1020 CTGCTGCTCTCTGAGATCTTCCCCAGCAAGCTTAGCCTACTTCCCGAGGTGGATGTGTGG

 263 ValGlnValAlaCysProArgLeuSerIleAspTrpGlyThrAlaSerProLysProLeu
1080 GTGCAGGTGGCATGTCCACGTCTCTCCATTGACTGGGGCACAGCCTCCCCCAAGCCGCTG

 283 LeuThrProTyrGluAlaAlaValAlaLeuArgAspIleSerTrpGlnGlnProTyrPro
1140 CTGACACCCTATGAGGCGGCCGTGGCTCTGAGGGACATTTCCTGGCAGCAGCCCTACCCG

 303 MetAspPheTyrAlaGlySerSerLeuGlyProTrpThrValAsnHisGlyGlnAspArg
1200 ATGGACTTCTACGCTGGCAGCTCCTTGGGGCCCTGGACGGTGAACCACGGCCAGGACCGC

 323 ArgProHisAlaProGlyArgProAlaArgGlyLysValGlnGluGlySerAlaArgPro
1260 CGTCCCCACGCCCCGGGCCGGCCCGCGCGGGGGAAGGTGCAGGAGGGGTCCGCGCGTCCC

 343 ProSerAlaValAlaCysGluAspCysSerCysArgAspGluLysValAlaProLeuAla
1320 CCTTCGGCCGTGGCTTGCGAGGACTGCAGCTGCAGGGACGAGAAGGTGGCGCCGCTGGCT

 363 Pro *
1380 CCTctgacgcgctcccgggcctcagggtcctgccctccggaggagcagcctcgaggctggt
1440 ggttttcagagcaggaggccgacgttttctccgcattggaagagcccgccgctctgcaggg
1500 gcctggaggaatcactggggatggtggcacaggcactgaacaggctggggccttttgacg
1560 gccttcttggtttcagccaaggggctgcgctagcagcccttgtgtgtgccctgggccagg
1620 caggcgatcccccgcttcccctttgccacggttatcctcttggtgtctggtttctgtcccc
1680 ggggcattgggttcaaggaatccatcctgcaaaggcccttgtcattgccttcgctccatg
1740 tttttggggacactgacaaagtcatcccctctcaggagagtgtgcaactggccagccaat
1800 ttcccggagccatcacccctcacccactctggtggccacttcattccagcagctgcacccc
1860 agcgtcaggcctacctcaagttcttggaccagtttgcagagtgaaagatcaagaaatgtc
1920 tctgctcctacatccagctcctctaggggcagcctccgtcatccatgccctcccaggacc
1980 ctccactcactgctgtgagtgcgcctcaccagaaccagttaagagacaactatcaattct
2040 tgagacccaaattataagggccctgcctgtactgaagaaaaggggagcacaaggccttaa
2100 tggacattgacttgtgaaaacgcaaacatgaatatggttggagagccctggattaggagg
2160 gtgacatgggaaggcagaggctggcaccatggtgactgccacatAATAAAgtggtgatt
2220 ccaaaaaaaaaaaa
```

Fig. 2. cDNA nucleotide sequence and predicted amino acid sequence of the human DPH2L gene. The long underlined sequence is the exon trap product used to identify the full-length cDNA. The polyadenylation signal is also underlined. PCR primers used to confirm hybridization-positive cDNA clones are indicated above the sequence.

```
DPH2L       ----------------------------------------MPEGLLLFACTIVDILERFT-
PFA0163M    ------------------------------------------------------------
YIK3       <NDKELN-EAIKLLPSNYNFEIHKTVWNIRKYNAKRIALQMPEGLLIYSLIISDILEQFC-
CEC14B1_5   -------MITFQLPSNYTFEVPKTIWKIRSTESKYVALQFPEGLIMYACVIADILEKYT-
DPH2       <LGPCYNSDELMQLISAYYNVEPLVGYLEQHPEYQNVTLQFPDDLIKDSSLIVRLLQSKFP
CEC09G5_2  <SNTGYFENLPENDIHSFFEIDATSEWIKQG-NHQRIALQFPDSLLPYSEKVTKLIESRFR

DPH2L       ---EAEVMVMGDVTYGACCVDDFTARALGADFLVHYGHSCLIPMDTSAQDFRVLYVFVDI
PFA0163M    ------------------------------------------------------------
YIK3        ---GVETLVMGDVSYGACCIDDFTARALDCDFIVHYAHSCLVPIDVT--KIKVLYVFVTI
CEC14B1_5   ---GCDTVIMGDVTYGACCVDDYTAKSMGCDLLVHYGHSCLVPIQNT------------
DPH2        -HGKIKFWVLADTAYSACCVDEVAAEHVHAEVVVHFGDACLNAIQNLP----VVYSFGTP
CEC09G5_2   SESAKKTFVLADTSYRSCCVDEVAAAHADCTALVHFGEACHSAPTDK------------

DPH2L       RIDTTHLLDSLRLTFPPAT-ALALVSTIQFVSTLQAAAQELKAEY----RVSVPQCKPLS
PFA0163M    ------------------------------------------------------------
YIK3        NIQEDHIIKTLQKNFPKGS-RIATFGTIQFNPAVHSVRDKLLNDEEHMLYIIPPQIKPLS
CEC14B1_5   --------DGIAMLYVFGK-RLVVVSTVQFIPSLQTLRTTFNKDDSS-IRIDIPQCKPLS
DPH2        FLDLALVVENFQRAFPDLSSKICLMANAPFSKHLSQLYNILKGDLHYTNIIYSQVNTSAV
CEC09G5_2   -IDVKYVLGSMPIFIDQFRMEFQNVADQLTAEHIVLLMDSCFSHEQEKVAAVIKEVLPGN

DPH2L       PGEILGCTSPRLSK-EVEAVVYLGDG-----RF--HLESVMIANPNVPAYRY----DP--
PFA0163M    ------------------------------------------------------------
YIK3        RGEVLGCTSERLDKEQYDAMVFIGDG-----RF--HLESAMIHNPEIPAFKY----DP--
CEC14B1_5   PGEVLGCTSPRLDASKYDAIVYLGDG-----RF--HLESIMIHNPEIEAFQY----DP--
DPH2        EEKFVTILDTFHVPEDVDQVGVFEKNS---VLFGQHDKADNISPEDYHLFHLTTPQDPRL
CEC09G5_2   RHVECSLLPSEDVLKQNRQNIYLGREIPSCLRNNQPTDLIFCGFPNSPLLPIWLLSYPSC

DPH2L       -YSKVLSR--EHYDHQR---------MQAARQEAIATARSAKSWGLILGTLGRQGSPKIL
PFA0163M    ------------------------------------------------------------
YIK3        -YNRKFTR--EGYDQKQ---------LVEVRAEAIEVARKGKVFGLILGALGRQGNLNTV
CEC14B1_5   -YSRKLTR--EFYDHDL---------MRKNRIGSIEIARKCTTFGLIQGTLGRQGNLKVV
DPH2        LYLSTVFQSVHIFDPALPGMVTGPFPSLMRRYKYMHVARTAGCIGILVNTLSLRNTRETI
CEC09G5_2   STVSHFNPINKTIQHESTR----SSRLLRKRLFLVEKLKDADTVGLVVGSVGVDKHREAV

DPH2L       EHLESRLRALGLSFVRLLLS------------------EIFPSKLSLLP-EVDVWVQVA
PFA0163M    ----------------LLLS------------------EIFNEKLQLFQ-NVDLFIQIG
YIK3        KNLEKNLIAAGKTVVKIILS------------------EVFPQKLAMFD-QIDVFVQVA
CEC14B1_5   EELEAQLERKGKKFLRVLLS------------------EIFPEKLAMFP-EVDCWVQVA
DPH2        NELVKLIKTREKKHYLFVVG------------------KPNVAKLANFE-DIDIWCILG
CEC09G5_2   KRMREMCKKAGKKIYVISVGKVCLDIFWFFYLLSNCAKFQINVPKLSNFSTDIDVFVLLS

DPH2L       CPR--LSIDWGTASPKPLLTPYEAAVALR--DISWQQPYPMDFYAGSSLGPWTVNHGQDR
PFA0163M    CPR--LSIDWGNYNLKPLLNTYEAYVLLN--SVPYKDIYPMDYYS----------------
YIK3        CPR--LSIDWGYAFNKPLLTPYEASVLLKKDVMFSEKYYPMDYYEAKGYG----------
CEC14B1_5   CPR--LSIDWGTQFPKPLLYPFELAVALDNVSFKFRCLQITGQWTIIRMIPWVLGRIIMK
DPH2        CSQSGIIVDQFNEFYKPIITPYELNLALS-EEVTWTGKWVVDFRDAIDEIEQNLG-GQDT
CEC09G5_2   CPFG-VVLDSSDYFR-PVVSYFEAEIALN-----PAKTWAADFGWSAEFAAFLEDKIETE

DPH2L       RPHAPGRPA----------------------------RGKVQEGSARPPSAVACEDCSCR
PFA0163M    ------------------------------------------------------------
YIK3        ------------------------------------RGETPKHAIE--------------
CEC14B1_5   RTVRNGRNGNLILLSKPKIHSRELSYFNEEKAKRIGERFEGGKLAKKVHKSIEQLKRHDP
DPH2        ISASTTSDE-------------------PEFDVVRGRYTSTSRPLRALTHLELEAADDD
CEC09G5_2   VPDDKAAG-----------------------------DFSLISGKVRVQKTEEEKNGDGP

DPH2L       D-EKVAPLAP----------------------------------------------------
PFA0163M    ------------------------------------------------------------
YIK3        ------------------------------------------------------------
CEC14B1_5   D-VQISTEPTKYLLVSNSSIILCGVSLEELEEIFLPLDELAEFIVYPNKRSYSFVQCSSIE>
DPH2        DSKQLTTRHTASGAVIKGTVSTSASALQ---------NRSWKGLGSDFDSTEVDNTGADIE>
CEC09G5_2   S--SVAIYNPGYCN---------------------DRTWKGLNDGVSTAEDSTKMG--->
```

the B2-3.1 sequence: brain (T31191, M.D. Adams et al., unpublished), infant brain (R14587, Bento Soares et al., unpublished), and adult heart (T20070, Liew et al. [13]). Thus this cDNA species has been identified in several stages of development (early fetal, neonatal, adult) and tissues (brain, heart, mixed fetal tissues and placenta). Five additional sequences from three species, *Saccharomyces cerevisiae, Caenorhabditis elegans*, and *Plasmodium falciparum*, have highly significant sequence similarity (Fig. 3). Pairwise comparison, using the PAM 250 matrix and a similarity threshold of 0.5, and multiple sequence alignment (ClustalW) place these sequences into two groups. The more closely related group contains an expressed sequence tag from *P. falciparum* (T09561 and T09562; 55% identity and 72% similarity with B2–3.1) [14], yeast YIK3 chromosome IX sequence (sp:P40487, B.G. Barrell et al., unpublished) and corresponding expressed sequence tags (T37516 and T37605, K. Weinstock et al., unpublished) (52% identity and 69% similarity with B2-3.1), and *C. elegans* chromosome III genomic sequence hypothetical protein CEC14B1_5 (gp: Z37139, B. Harris, unpublished and R. Wilson et al. [15]; 48% identity and 66% similarity with B2-3.1). Among the members of this group, there is 46–55% identity and 65–72% similarity, and it is possible that the newly cloned human gene and these three genes from lower eukaryotes may be homologues. The less closely related group consists of the yeast diphthamide biosynthesis gene DPH2 (sp:P32461; 20% identity and 41% similarity with B2-3.1) [16] and *C. elegans* hypothetical protein CEC09G5_2 (gp: Z46791; 18% identity and 39% similarity with B2-3.1; 21% identity and 43% similarity with DPH2). Examination of the aligned sequences for conserved motifs shows that an N-myristoylation site is conserved among all members of the alignment, and two protein kinase C phosphorylation sites and one casein kinase II phosphorylation site are conserved only among the most closely related group of genes consisting of DPHL, YIK3, and CEC14B1_5 (Fig. 3). The human gene has been designated DPH2L, diphthamide biosynthesis protein 2-like), after the most similar protein of known biochemical function.

Although sequence database analysis does not identify a definite homologue of known function, the amino acid similarity score of 66% in the 60 N-terminal amino acids (57% identity and 86% similarity for the sequence VMGDVTYGACCVDD) with the yeast diphthamide biosynthesis protein DPH2 suggests that DPH2L and yeast DPH2 could share a catalytic or recognition site. Yeast DPH2 represents one of five complementation groups involved in diphthamide synthesis, and it is possible that the yeast protein YIK3, the probable homologue of human DPH2L, could represent one of the other complementation groups. If human DPH2L is involved in diphthamide synthesis, it could participate in regulation of global protein synthesis. Diphthamide is a posttranslationally modified amino acid primarily seen in the eukaryotic protein synthesis factor EF-2 [17]. ADP-ribosylation of the diphthamide residue by bacterial exotoxins and cellular ADP ribosyltransferases [18] prevents EF-2 from translocating the growing peptide strand and thereby stops protein synthesis. The strict conservation of the EF-2 diphthamide residue, of the complex diphthamide biosynthesis pathway, and of the cellular ADP-ribosyltransferase activity suggests that diphthamide may be important in regulating the activity of the EF-2 pool, either by inactivation after ADP-ribosylation or by participation in the recognition site for calmodulin-dependent protein kinase III, which reversibly inactivates EF-2 [19]. Alternately, DPH2L may not participate in the human diphthamide synthesis pathway but may provide a DPH2-like interaction in a protein complex for an unidentified synthetic pathway.

Fig. 3. Sequence alignment of human DPH2L with similar proteins in *Saccharomyces cerevisiae* (YIK3 hypothetical protein and DPH2 diphthamide biosynthesis protein), *Caenorhabditis elegans* (CEC14B1.5 and CEC09G5.2 hypothetical proteins) and *Plasmodium falciparum* (PFA0163M expressed sequence tag). Accession numbers are listed in the text. Conserved potential protein kinase C phosphorylation sites (DPH2L TAR and SPR), casein kinase II phosphorylation site (DPH2L SPGE) and N-myristoylation site (DPH2L GLILGT) are indicated by underlining. Portions of the YIK3 sequence that have been found in expressed sequence tags are bracketed. < and > indicate additional residues not shown in this figure (N-terminal residues: 60 for YIK3, 29 for DPH2, 24 for CEC09G5.2; C-terminal residues: 496 for CEC14B1.5, 29 for DPH2, 13 for CEC09G5.2).

Acknowledgements

This study was supported by grant VM-115 from the American Cancer Society to N.J.P.

References

[1] Phillips, N.J., Ziegler, M.R., Radford, D.M., Fair, K.L., Steinbrueck, T., Xynos, F.P. and Donis-Keller, H. (1996) Allelic deletion on chromosome 17p13.3 in early ovarian cancer. Cancer Res., in press.

[2] Phillips, N.J., Ziegler, M.R., Saha, B. and Xynos, F.P. (1993) Allelic loss on chromosome 17 in human ovarian cancer. Int. J. Cancer, 54, 85–91.

[3] Kupryjanczyk, J., Thor, A.D., Beauchamp, R., Merritt, V., Edgerton, S.M., Bell, D.A. and Yandell, D.W. (1993) p53 gene mutations and protein accumulation in human ovarian cancer. Proc. Natl. Acad. Sci. USA, 90, 4961–4965.

[4] Kupryjanczyk, J., Bell, D.A., Dimeo, D., Beauchamp, R., Thor, A.D. and Yandell, D.W. (1995) p53 gene analysis of ovarian borderline tumors and stage I carcinomas. Hum. Pathol. 26, 387–392.

[5] Merajver, S.D., Pham, T.M., Caduff, R.F., Chen, M., Poy, E.L., Cooney, K.A., Weber, B.A., Collins, F.C., Johnston, C. and Frank, T.S. (1995) Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. Nature Genet., 9, 439–443.

[6] Ledbetter, D.H., Ledbetter, S.A., van Tuinen, P., Summers, K.M., Robinson, T.J., Nakamura, Y., Wolff, R., White, R., Barker, D.F., Wallace, M.R., Collins, F.S. and Dobyns, W.B. (1989) Molecular dissection of a contiguous gene syndrome: frequent submicroscopic deletions, evolutionarily conserved sequences and a hypomethylated "island" in the Miller-Dieker chromosome region. Proc. Natl. Acad. Sci. USA, 86, 5136–5140.

[7] Makos Wales, M., Biel, M.A., El-Deiry, W., Nelkin, B.D., Issa, J.-P., Cavenee, W.K., Kuerbitz, S.J. and Baylin, S.B. (1995) p53 activates expression of HIC-1, a new candidate tumor suppressor gene on 17p13.3. Nature Med., 1, 570–577.

[8] Deaven, L.L., Van Dilla, M.A., Bartholdi, M.F., Carrano, A.V., Cram, L.S., Fuscoe, J.C., Gray, J.W., Hildebrand, C.E., Moyzis, R.K. and Perlman, J. (1986) Construction of human chromosome-specific DNA libraries from flow-sorted chromosomes. Cold Spring Harbor Symp. Quant. Biol., 51, 159–186.

[9] Williamson, R., Bowcock, A., Kidd, K., Pearson, P., Schmidtke, J., Ceverha, P., Chipperfield, M., Cooper, D.N., Coutelle, C., Hewitt, J., Klinger, K., Langley, K., Beckmann, J., Tolley, M. and Maidak, B. (1991) Human gene mapping 11. Report of the DNA committee and catalogues of cloned and mapped genes, markers formatted for PCR and DNA polymorphisms. Cytogenet. Cell Genet., 58, 1548–1569.

[10] Church, D.M., Stotler, C.J., Rutter, J.L., Murrell, J.R., Trofatter, J.A. and Buckler, A.J. (1994) Isolation of genes from complex sources of mammalian genomic DNA using exon amplification. Nature Genet., 6, 98–105.

[11] Swaroop, A. and Xu, J. (1993) cDNA libraries from human tissues and cell lines. Cytogenet. Cell Genet., 64, 292–294.

[12] Ledbetter, S.A., Wallace, M.R., Collins, F.S. and Ledbetter, D.H. (1990) Human chromosome 17 NotI linking clones and their use in long-range restriction mapping of the Miller-Dieker chromosome region (MDCR) in 17p13.3. Genomics, 7, 264–269.

[13] Liew, C.C., Hwang, D.M., Fung, Y.W., Laurenssen, C., Cukerman, E., Tsui, S. and Lee, C.Y. (1994) A catalogue of genes in the cardiovascular system as identified by expressed sequence tags. Proc. Natl. Acad. Sci. USA, 91, 10645–10649.

[14] Reddy, G.R., Chakrabarti, D., Schuster, S.M., Ferl, R.J., Almira, E.C. and Dame, J.B. (1993) Gene sequence tags from *Plasmodium falciparum* genomic DNA fragments prepared by the "genease" activity of mung bean nuclease. Proc. Natl. Acad. Sci. USA, 90, 9867–9871.

[15] Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., Coulson, A., Craxton, M., Dear, S., Du, Z., Durbin, R., Favello, A., Fraser, A., Fulton, L., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Laisster, N., Latreille, P., Lightning, J., Lloyd, C., Mortimore, B., O'Callaghan, M., Parsons, J., Percy, C., Rifken, L., Roopra, A., Saunders, D., Shownkeen, R., Sims, M., Smaldon, N., Smith, A., Smith, M., Sonnhammer, E., Staden, R., Sulston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson-Sproat, J. and Wohldman, P. (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature, 368, 32–38.

[16] Mattheakis, L.C., Sor, F. and Collier, R.J. (1993) Diphthamide synthesis in *Saccharomyces cerevisiae* : structure of the DPH2 gene. Gene, 132, 149–154.

[17] Bodley, J.W. and Veldman, S.A. (1990) Biosynthesis of diphthamide: ADP-ribose acceptor for diphtheria toxin. In: ADP-Ribosylating Toxins and G Proteins, pp. 21–30. Editors: J. Moss and M. Vaughn. American Society for Microbiology, Washington, DC.

[18] Iglewski, W.J. (1994) Cellular ADP-ribosylation of elongation factor 2. Mol. Cell. Biochem., 138, 131–133.

[19] Bagaglio, D.M., Cheng, E.H.C., Gorelick, F.S., Mitsui, K.-I., Nairn, A.C. and Halt, W.N. (1993) Phosphorylation of elongation factor 2 in normal and malignant rat glial cells. Cancer Res., 53, 2260–2264.