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Molecular cloning of a novel human cDNA homologous to CDC10 in Saccharomyces cerevisiae

Shuichi Nakatsuru[1,2], Kazunori Sudo[1] and Yusuke Nakamura[1*]

[1]Department of Biochemistry, Cancer Institute, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan

[2]Sanko Junyaku Co., Ltd., 1-10-6 Iwamoto-cho, Chiyoda-ku, Tokyo 101, Japan

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We isolated a novel human cDNA, termed hCDC10, whose predicted product showed a high degree of homology to the CDC10 protein of Saccharomyces cerevisiae. This cDNA contained an open reading frame of 1254 nucleotides encoding 418 amino acids, which included a GTP-binding motif, $GX_4GKS--DX_2G--KXD$. The predicted peptide sequence also revealed partial amino-acid identity (40-50%) with Diff 6 in Drosophila and with H5 in mouse. Each of these sequence homologues, including Saccharomyces cerevisiae CDC10, contains the GTP-binding motif. Northern blot analyses indicated that the hCDC10 gene is expressed ubiquitously in normal tissues. © 1994 Academic Press, Inc.

The mitotic cell-division cycle of the yeast Saccharomyces cerevisiae (S. cerevisiae) involves a variety of morphogenic events, that include cell-surface changes and specific positioning of intracellular organelles (1-3). As in other eukaryotes, cytoskeltal elements such as actin, 10-nm filament, and microtubles are thought to play important roles in these morphogenic changes (4-9). In S. cerevisiae the 10-nm filament lies directly inside the cytoplasmic membrane in the region of the mother-bud neck (5, 10); proteins encoded by the CDC3, CDC10, CDC11, and CDC12 genes probably constitute the ring of 10-nm filament (5, 11). Temperature-sensitive mutants defective in

* To whom correspondence should be addressed.
FAX: 03-3918-0342.

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any one of these four genes fail to form this filament, and display a pleiotropic phenotype that involves abnormal budding and an inability to complete cytokinesis (5, 10, 12, 13). Thus, these proteins are considered to be significant factors involving in cytokinesis of S. cerevisiae.

Here, we report isolation of a novel human gene whose predicted product is homologous to the CDC10 protein of S. cerevisiae.

Materials and methods

Identification of a cDNA clone homologous to yeast CDC10

Nearly 2,500 randomly selected clones from a human fetal lung cDNA library were sequenced by the dideoxy chain-termination method (K. Sudo, K. Chinen and Y. Nakamura, unpublished data). Searching for DNA sequence homologies between these cDNA clones and known genes in the public database by the FAST-A program revealed that one of them, 2.3 kb long, showed significant homology to yeast CDC10. We therefore designated it hCDC10. As Northern analysis indicated the original cDNA clone lacked a 5'-portion of the gene, we screened a cDNA library constructed from a fetal brain mRNA (12-16W) (purchased from Clonetech, CA) and isolated additional positive cDNA clones.

Northern analysis

Northern blots containing two micrograms each of polyadenylated RNA from various human tissues were purchased (Clonetech, Palo Alto, CA) and hybridized with the hCDC10 cDNA clone labeled by a random oligonucleotide priming method (16,17) as the probe. The hybridization and washing conditions were as follows; hybridization in a solution containing 50% formamide, 5X SSPE, 2X Denhardt's, 0.1% SDS and 0.1mg/ml salmon sperm DNA at 42°C for 18hr, followed by washing twice in 2X SSC and 0.1% SDS at room temperature for ten minutes and once in 0.1X SSC and 0.1% SDS at 50°C for 40min. The membranes were exposed to X-ray film (Kodak) at -70°C for 18 hour with intensifying screens.

Results

Nucleotide and deduced amino acid sequences of hCDC10 are shown in Fig. 1. This cDNA has an open reading frame of 1254 nucleotides encoding 418 amino acids. Fig. 2 shows a comparison of the predicted amino acid sequence of hCDC10 with those of S. cerevisiae CDC10, mouse H5, and Drosophila Diff6. hCDC10 revealed 39.2% identity with CDC10, and 56.0% and 53.7% identity with Diff6 and H5, respectively. These proteins seem to have been highly conserved during evolution; moreover, a $GX_4GKS--DX_2G--KXD$ motif (codons 38-45, 94-97, 176-178) that is characteristic of GTP-binding proteins is almost identical among them.

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AGTGCGAGATCCGCTGCTGCTGAGGAGAGGAGCGTCAACAGCAGCACCATGGTAGCTCAACAGAAGAACCTTGAAGGCTATGTGGGATTT   90
                                                M  V  A  Q  Q  K  N  L  E  G  Y  V  G  F

GCCAATCTCCCAAATCAAGTATACAGAAAATCGGTGAAGAGAGGTTTTGAATTCACGCTTATGGTAGTGGGTGAATCTGGATTGGGAAAG  180
 A  N  L  P  N  Q  V  Y  R  K  S  V  K  R  G  F  E  F  T  L  M  V  V  G  E  S  G  L  G  K

TCGACATTAATCAACTCATTATTCCTCACAGATTTGTATTCTCCAGAGTATCCAGGTCCTTCTCATAGAATTAAAAAGACTGTACAGGTG  270
 S  T  L  I  N  S  L  F  L  T  D  L  Y  S  P  E  Y  P  G  P  S  H  R  I  K  K  T  V  Q  V

GAACAATCCAAAGTTTTAATCAAAGAAGGTGGTGTTCAGTTGCTGCTCACAATAGTTGATACCCCAGGATTTGGAGATGCAGTGGATAAT  360
 E  Q  S  K  V  L  I  K  E  G  G  V  Q  L  L  L  T  I  V  D  T  P  G  F  G  D  A  V  D  N

AGTAATTGCTGGCAGCCTGTTATCGACTACATTGATAGTAAATTTGAGGACTACCTAAATGCAGAATCACGAGTGAACAGACGTCAGATG  450
 S  N  C  W  Q  P  V  I  D  Y  I  D  S  K  F  E  D  Y  L  N  A  E  S  R  V  N  R  R  Q  M

CCTGATAACAGGGTGCAGTGTTGTTTATACTTCATTGCTCCTTCAGGACATGGACTTAAACCATTGGATATTGAGTTTATGAAGCGTTTG  540
 P  D  N  R  V  Q  C  C  L  Y  F  I  A  P  S  G  H  G  L  K  P  L  D  I  E  F  M  K  R  L

CATGAAAAAGTGAATATCATCCCACTTATTGCCAAAGCAGACACACTCACACCAGAGGAATGCCAACAGTTTAAAAAACAGATAATGAAA  630
 H  E  K  V  N  I  I  P  L  I  A  K  A  D  T  L  T  P  E  E  C  Q  Q  F  K  K  Q  I  M  K

GAAATCCAAGAACATAAAATTAAAATATACGAATTTCCAGAAACAGATGATGAAGAAGAAAATAAACTTGTTAAAAAGATAAAGGACCGT  720
 E  I  Q  E  H  K  I  K  I  Y  E  F  P  E  T  D  D  E  E  E  N  K  L  V  K  K  I  K  D  R

TTACCTCTTGCTGTGGTAGGTAGTAATACTATCATTGAAGTTAATGGCAAAAGGGTCAGAGGAAGGCAGTATCCTTGGGGTATTGCTGAA  810
 L  P  L  A  V  V  G  S  N  T  I  I  E  V  N  G  K  R  V  R  G  R  Q  Y  P  W  G  I  A  E

GTTGAAAATGGTGAACATTGTGATTTTACAATCCTAAGAAATATGAAGATAAGAACACACATGCAGGACTTGAAAGATGTTACTAATAAT  900
 V  E  N  G  E  H  C  D  F  T  I  L  R  N  M  K  I  R  T  H  M  Q  D  L  K  D  V  T  N  N

GTCCACTATGAGAACTACAGAAGCAGAAAACTTGCAGCTGTGACTTATAATGGAGTTGATAACAACAAGAATAAAGGGCAGCTGACTAAG  990
 V  H  Y  E  N  Y  R  S  R  K  L  A  A  V  T  Y  N  G  V  D  N  N  K  N  K  G  Q  L  T  K

AGCCCTCTGGCACAAATGGAAGAAGAAAGAAGGGAGCATGTAGCTAAAATGAAGAAGATGGAGATGGAGATGGAGCAGGTGTTTGAGATG 1080
 S  P  L  A  Q  M  E  E  E  R  R  E  H  V  A  K  M  K  K  M  E  M  E  M  E  Q  V  F  E  M

AAGGTCAAAGAAAAAGTTCAAAAACTGAAGGACTCTGAAGCTGAGCTCCAGCGGCGCCATGAGCAAATGAAAAAGAATTTGGAAGCACAG 1170
 K  V  K  E  K  V  Q  K  L  K  D  S  E  A  E  L  Q  R  R  H  E  Q  M  K  K  N  L  E  A  Q

CACAAAGAATTGGAGGAAAAACGTCGTCAGTTCGAGGATGAGAAAGCAAACTGGGAAGCTCAACAACGTATTTTAGAACAACAGAACTCT 1260
 H  K  E  L  E  E  K  R  R  Q  F  E  D  E  K  A  N  W  E  A  Q  Q  R  I  L  E  Q  Q  N  S

TCAAGAACCTTGGAAAAGAACAAGAAGAAAGGGAAGATCTTTTAAACTCTCTATTGACCACCAGTTAACGTATTAGTTGCCAATATGCCA 1350
 S  R  T  L  E  K  N  K  K  K  G  K  I  F  *

GCTTGGACATCAGTGTTTGTTGGATCCGTTTGACCAATTTGCACCAGTTTTATCCATAATGATGGATTTAACAGCATGACAAAAATTATT 1440
TTTTTTTTTGTTCTTGATGGAGATTAAGATGCCTTGAATTGTCTAGGGTGTTCTGTACTTAGAAAGTAAGAGCTCTAAGTACCTTTCCTA 1530
CATTTTCTTTTTTTATTAAACAGATATCTTCAGTTTAATGCAAGAGAACATTTTACTGTTGTACAATCATGTTCTGGTGGTTTGATTGTT 1620
TACAGGATATTCCAAAATAAAAGGACTCTGGAAGATTTTCATTGAGGAGAAATTGCCATAATATGATGCAAACTGTGCTTCTCTATGATA 1710
ATTACAATACAAAGGTTCCATTCAGTGCAGCATATACAATAATGTAATTTAGTCTAACACAGTTGACCCTATTTTTTGACACTTCCATTG 1800
TTTAAAAATACACATGGAAAAAAAAAAAACCCTATATGCTTACTGTGCACCTAGAGCTTTTTTATAACAACGTCTTTTTGTTTGTTTGTTT 1890
TGGATTCTTTAAATATATATTCTCATTTAGTGCCCTCTTTAGCCAGAATCTCATTACTGCTTCATTTTTGTAATAACATTTAATTTAGAT 1980
ATTTTCCACATATTGGCACTGCTAAAATAGAATATAGCATCTTTCATATGGTAGGAACCAACAAGGAAACTTTCCTTTAACTCCCTTTTT 2070
ACACTTTATGGTAAGTAGCAGGGGGGGAAATGCATTTATAGATCATTTCTAGGCAAAATTGTGAAGCTAATGACCAACCTGTTTCTACCT 2160
ATATGCAGTCTCTTTATTTTACTAGAAATGGGAATCATGGCCTCTTGAAGAGAAAAAAGTCACCATTCTGCATTTAGCTGTATTCATATA 2250
TTGTATTTCTGTATTTTTTGTTTGTATTGTAAAAAATTCACATAATAAACGATGGTTGTGATGT  2314
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Figure 1.
Alignment of nucleotide and deduced amino acid sequences of hCDC10. The initiation methionine (ATG) and polyadenylation signal (AATAAA) of this gene are underlined. The termination codon (TAA) is indicated by an asterisk.

Figure 2.
Comparison of deduced hCDC10 amino acid sequence with related proteins. Identical residues are marked by shading. Alignments among the four sequences were generated with the "pile-up" program of the GCG format. A putative GTP-binding motif, GXXXXGKS--DXXG--KXD, is indicated in bold type above the sequences. CDC10, CDC10 protein in S. cerevisiae ; H5(M), H5 protein in mouse; D6(D), Diff6 protein in Drosophila.

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hCDC10  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 CDC10  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 H5 (M) MDHSLGWQGN SVPEDGTEAG IKHFLEDSSD DAELSKFVKD FPGSEPYHSA ESKTRVARPQ ILEPRPQSPD LCDDDVEFRG SLWPQPSDSQ QYFSAPAPLS  100
 D6 (D) .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                                                GXXXXGKS
hCDC10  .......... ...MVAQQKN LEGIVGPAML PNQVYRRSVK RGFEFTLMVV GESGLGKSTL INSLFLTDLY S.PEYPGPSH .RIKKTVQVE QSKVLIKEGG   85
 CDC10  .......... ..MDPLSSVQ PASYVGFDTI PNQIEHRLLK KGFQFNIMVV GQSGLGKSTL INTLFASHLI DSATGDDISA LPVTKTTEMK IETHTLVMDR   88
 H5 (M) PSSRPRSPWG KLDPYDSSED DKEYVGFATL PNQVHRKSVK KGFDFTLMVA GESGLGKSTL VNSLFLTDLY RDRKLLGAEE .RIMQTVEIT KHAVDIEEKG  199
 D6 (D) ........MA DTKGF.SSIE TPGYVGFANL PNQVHRKSVK KGFEFTLMVV GRSGLGKSTL VNSLFLTDLY PERIIPDAIE .KQNQTVKLE ASTTEIEERG   90

                DX XG                                                                                        KXD
hCDC10  VQLLLTIVDT PGFGDAVDNS NCWQPVIDYI DSKFEDYLNA ESRVNR.RQM PDNRVQCCLY FIAPSGHGLK PLDIEFMKRL HEKVNIIPLI AKADTLTPEE  184
 CDC10  VRLNINVIDT PGFGDFIDNS KAWEPIVKYI KEQHSQYLRK ELTAQRERFI TDTRVHAILY FLQPNGKELS RLDVEALKRL TEIANVIPVI GKSDTISLDE  188
 H5 (M) VRLRLTIVDT PGFGDAVNNT ECWKPVAEYI DQQFEQYFRD ESGLNR.KNI QDNRVHCCLY FISPFGHGLR PLDVEFMKAL HQRVNIVPIL AKADCLTPPE  298
 D6 (D) VKLRLTVVDT PGFGDAIDNS NSFGAILEYI DEQYERFLRD ESGLNR.RNI VDNRIHCCFY FISPFGHGLK PLDVEFMKKL HSKVNIVPVI AKADCLTKKE  189

hCDC10  CQQFKKQIMK EIQEHKIKIY EFPETDDEEE NKL...VKKI KDRLPLAVVG SNTIIEVNGK RVRGRQYPWG IAEVENGEHC DFTILRNMKI KTHMQDLKDV  281
 CDC10  RTEFRELIQN EFEKYNFKIY PY.DSEELTD EELEL.NRSV RSIIPFAVVG SENEIEINGE TFRGRKTRWS AINVEDINQC DFVYLREFLI KTHLQDLIET  286
 H5 (M) VDRKECKIRE EIEHFGIKIY QFPDCDSDED EDFKLQDQAL KESIPFAVIG SNTVVEARGR RVRGRLYPWG IVEVENPGHC DFVKLRTMLV RTHMQDLKDV  388
 D6 (D) ILRLECRIMQ EIESHGIKIY PLPDCDSDED EDYKEQVKQL KEAVPFAVCG ANTLLEVKGK KVRGRLYPWG VVEVENPDHC DFIKLRTMLI .THMQDLQEV  288

hCDC10  TNNVHIENYR SRKLAAVTYN GVDNNKNKGQ LTKSPLAQME EERREHVAKM KKMEMEMEQV FEMKVKEKVQ KLKDSEAELQ RRHEQMKKNL EAQHKELEEK  381
 CDC10  TSYIHYEGFR ARQLIALKEN A..NSRSSAH MSSNAIQR.. .......... .......... .......... .......... .......... ..........  322
 H5 (M) TRETHYENYR AQCIQSMTR. LVVKERNRNK LTRESGT... .......... ...DFPIPAV PPGTDPETEK LIREKDEELR RMQEMLHK.I QRQMKETH..  468
 D6 (D) TQEVIYENYR S...DRLAK. GIKGKENGVK AERDSSS... .......... ...Q....VV SNSVLGEKDR ILQEKEAELR RMQEMLAQ.M QARMQAQQ..  361

hCDC10  RRQFEDEKAN WEAQQRILEQ QNSSRTLEKN KKKGKIF  418
 CDC10  .......... .......... .......... .......
 H5 (M) .......... .......... .......... .......
 D6 (D) .......... .......... .......... .......
```

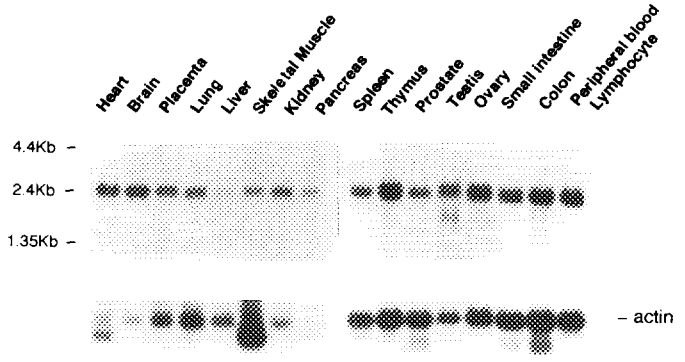

Figure 3.
Northern-blot analysis of hCDC10 in various human tissues. Expression of the actin gene was used as an internal control.

The result of Northern hybridizations using hCDC10 as the probe is shown in Fig. 3. Ubiquitous expression of a 2.4-kb hCDC10 transcript was seen in various human tissues. It is notable that two smaller transcripts, about 1.0 and 2.0 kb long, were detected in testis, although the level of expression of these two transcripts was low.

Discussion

We have reported here the isolation of a novel human cDNA, termed hCDC10, whose predicted amino acid sequence is highly homologous to yeast CDC10. CDC10 is predicted to bind GTP, and it is considered to be a component of the 10-nm neck filament in the bud site (13). Disruption of the CDC10 gene results in abnormal bud growth and inability of the yeast to complete cytokinesis (5). The FtsZ protein of Escherichia coli, which also specially binds and hydrolyzes GTP, is localized in a ring at the site of septum formation, and is also considered to play an important role in cytokinesis (14). This body of evidence suggests that cytokinesis in many organisms is mediated by filaments formed from GTP-binding proteins.

We have shown here that the predicted protein product of hCDC10 contains a putative GTP binding motif, $GX_4GKS--DX_2G--KXD$, which is conserved in many GTP-binding proteins (15) and that hCDC10 is ubiquitously expressed in normal human tissues. The sequence similarities across widely divergent species suggest that hCDC10 may be one of the components required for cytokinesis in human cells.

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