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Biochemistry

Molecular cloning of the gene for the human placental GTP-binding protein G_p (G25K): Identification of this GTP-binding protein as the human homolog of the yeast cell-division-cycle protein CDC42

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ABSTRACT **We have isolated cDNA clones from a human placental library that code for a low molecular weight GTP-binding protein originally designated G_p (also called G25K). This identification is based on comparisons with the available peptide sequences for the purified human G_p protein and the use of two highly specific anti-peptide antibodies. The predicted amino acid sequence of the protein is very similar to those of various members of the ras superfamily of low molecular weight GTP-binding proteins, including the N-, Ki-, and Ha-ras proteins (30–35% identical), the rho proteins (≈50% identical), and the rac proteins (≈70% identical). The highest degree of sequence identity (80%) is found with the *Saccharomyces cerevisiae* cell-division-cycle protein CDC42. The human placental gene, which we designate CDC42Hs, complements the *cdc42-1* mutation in *S. cerevisiae*, which suggests that this GTP-binding protein is the human homolog of the yeast protein.**

GTP-binding proteins play key roles in a number of biological processes, including the hormonal regulation of adenylyl cyclase and phospholipases, visual transduction, protein synthesis, protein trafficking, secretion, and cell growth. The members of the growing class of low molecular weight GTP-binding proteins, with the *ras* oncogene products as prototypes, consist of only a single 21- to 28-kDa polypeptide (1). These proteins include the yeast YPT1 and SEC4 proteins, which have been implicated in transport through the Golgi and exocytosis (2, 3); the mammalian rho proteins, which are substrates for ADP-ribosylation by botulinum toxin (4, 5); the mammalian rap (6–8), rab (9), ral (10), and rac (10, 11) proteins; the bovine and yeast ARF proteins (12), which promote the cholera toxin-induced ADP-ribosylation of G_s in the Golgi compartment; and the human bovine G_p (G25K) proteins (13, 14).

The well-known transforming properties of the *ras* oncogenes (15) and the recent observation that the rap1A protein can counteract *ras* transformation (8) suggest that members of this protein family play key roles in cell growth pathways. Recently the G_p protein was shown to be an excellent substrate for the tyrosine kinase activity of the purified human placental epidermal growth factor (EGF) receptor (16), which suggests the possible involvement of this protein in the actions of the EGF receptor. We have now cloned a full-length cDNA for G_p from a human placental library. Analysis of the amino acid sequence and functional studies of the expressed protein indicate that G_p is the mammalian homolog of a yeast cell-division-cycle protein, CDC42.¶

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MATERIALS AND METHODS

Isolation of G_p cDNA Clones. A human full-term placental cDNA library in λgt11 was kindly provided by Evan Sadler (Washington University School of Medicine). Two oligonucleotide probes (P1 and P4; see *Results*) based on the peptide sequences of the purified human platelet and placental G_p (14) were labeled using [γ-^{32}P]ATP and T4 kinase. A third oligonucleotide probe (P1′) was prepared from a cDNA clone that was isolated from the placental cDNA library (see *Results*). Hybridization was carried out under low stringency at 42°C in 6× SSC (1× is 0.15 M NaCl/0.015 M sodium citrate, pH 7)/5× Denhardt's solution (1× is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin)/1% SDS/50 mM Tris·HCl, pH 7.5, containing denatured salmon sperm DNA at 50 μg/ml. Filters were washed in 2× SSC at 20°C and then in 0.2× SSC/0.1% SDS at 50°C. Hybridizing phages were plaque-purified and confirmed positive by Southern hybridization with the various probes. The cDNA inserts were subcloned into plasmid pUC19 and both strands were sequenced by the dideoxy chain-termination procedure (17). Sequence homology alignments were performed by the method of Lipman and Pearson (18).

Expression of CDC42Hs in *Saccharomyces cerevisiae*. The conditions for the growth and maintenance of *S. cerevisiae* strain DJTD2-16A (*MATa cdc42-1 ura3 his4 leu2 trp1*) have been described (19). The permissive and restrictive temperatures for the growth of DJTD2-16A were 23°C and 36°C, respectively. The plasmid pBM272, which has the *URA3* yeast selectable marker and the *GAL10* promoter (provided by M. Johnston, Univ. of Vermont), was used to express CDC42Hs. The insertion of the coding region for the CDC42Hs cDNA into pBM272 and the results obtained from complementation studies are described in the legend to Table 1.

Expression of CDC42Hs in *Escherichia coli*. The *E. coli* pGEX plasmids express foreign DNA sequences as glutathione *S*-transferase (GST) fusion proteins (20). The pGEX-CDC42Hs plasmid was constructed by the ligation of a 900-base-pair (bp) *Bam*HI–*Eco*RI fragment of CDC42Hs cDNA into the pGEX-2T plasmid. The *Bam*HI site was created immediately upstream of the CDC42Hs start codon by use of the polymerase chain reaction (PCR) and the *Eco*RI site was in 3′ nontranslated cDNA. The fusion protein was produced in *E. coli* strain XL1-B and purified as described (21).

Abbreviations: EGF, epidermal growth factor; GST, glutathione *S*-transferase; GTP[γ-^{35}S], guanosine 5′-[γ-[^{35}S]thio]triphosphate; PCR, polymerase chain reaction.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M57298).

The CDC42Hs protein was also expressed in *E. coli* using the pET-3A expression vector under the control of the T7 promoter (22). The PCR was used to create an *Nde* I site on a 900-bp fragment of the CDC42Hs cDNA and the resulting fragment was cloned into the *Nde* I/*Bam*HI sites of the pET vector. After induction with isopropyl β-D-thiogalactopyranoside, the cells were harvested and lysed as described (21). The homogenate was centrifuged at 12,000 × *g* for 5 min and the supernatant was used for Western immunoblotting experiments. Western blot analyses of the recombinant CDC42Hs protein were performed as described by Polakis *et al.* (14), and binding of guanosine 5′-[γ-[^{35}S]thio]triphosphate (GTP[γ-^{35}S]) was measured as outlined by Hart *et al.* (16).

RESULTS

Cloning of the CDC42Hs cDNA. Recently, four different peptide sequences (p1–p4 in Fig. 1*A*) were identified in either the human placental or the human platelet G_p protein (14) by tryptic digestion and cyanogen bromide treatment of the purified proteins. Two of these peptide sequences (p1 and p4) were used to design oligonucleotide probes to screen a human placental λgt11 library for cDNA corresponding to G_p. One of these oligonucleotide probes coded for 10 of the carboxyl-terminal 11 amino acids of the p4 peptide and thus is designated as P4 in Fig. 1*B*. The second oligonucleotide probe (P1) coded for the amino-terminal 7 amino acids of p1.

The initial screening of the human placental cDNA library with the P1 probe yielded four positive clones. The longest of these clones was sequenced and found to code for the rac2 protein. Two other clones contained the Asn-Val-Phe-Asp sequence from the p1 peptide but did not code for any known proteins and are presently unidentified. The fourth clone coded for a sequence that was highly homologous to the p1 peptide (see P1′ in Fig. 1*B*). Specifically, the predicted sequence contained all of the amino acids of the p1 peptide; however, two amino acids (Thr-Gln) were inserted just prior to the last two amino acids (Pro-Lys) in the p1 sequence. The high degree of similarity between the predicted amino acid sequence of the P1′ clone and the sequence for the p1 peptide suggested that it might represent a partial clone coding for one form of the placental G_p protein.

The human placental library was rescreened with a probe prepared from the P1′ clone. This screening yielded four additional clones. Each of these clones was then screened with the original P1 and P4 probes. Two clones that hybridized with all three oligonucleotide probes (i.e., P1, P4, and P1′) were isolated. The longest of these [≈2.1 kilobases (kb)] was sequenced and found to contain an open reading frame of 573 bp that codes for 191 amino acids, yielding a predicted M_r of 21,258. This was followed by ≈1500 bp of 3′ nontranslated sequence. The restriction map and the sites used for the sequencing of this clone (designated CDC42Hs) are shown in Fig. 2*A*, while the nucleotide sequence and the deduced amino acid sequence are shown in Fig. 2*B*. The second clone that hybridized with the three probes was shorter (≈900 bp) but possessed an identical open reading frame.

The sequences of the p1, p2, and p4 peptides derived from the purified G_p protein are present within the predicted amino acid sequence of CDC42Hs. That the carboxyl-terminal residues of CDC42Hs (residues 167–191) differ slightly from those coded for by P1′ (see Fig. 3) suggests that the latter codes for

```
A   p1:   NVFDEAILAALEPPEPK

    p2:   PFLLVGTQIDLR

    p3:   GIQTPETAEK

    p4:   GGEPYTLGLFDTAGQEDYDR

B   P1:   AATGTGTTTGATGAAGCCAT
            C C C G
    a.a.  N V F D E A I

    P4:   TTTGATACAGCTGGCCAAGAAGATTATGAT
            C C         G G C C C
    a.a.  F D T A G Q E D Y D

P1':  AATGTGTTTGATGAGGCTATCCTAGCTGCCCTCGAG-
a.a.  N V F D E A I L A A L E -
CCTCCGGAAACTCAACCCAAAAGGAAGTGCTGTATATTC
P P E T Q P K R K C C I F
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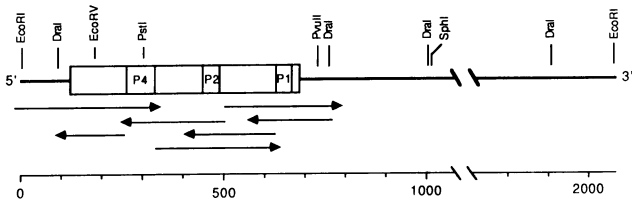
FIG. 1. (*A*) Amino acid sequences of peptides derived either from cyanogen bromide treatment of the human platelet GTP-binding protein (p4) or from tryptic cleavage of the human platelet and human placental GTP-binding proteins (p1–p3). (*B*) Oligonucleotide probes used to isolate the CDC42Hs cDNA clone and the corresponding amino acid (a.a.) sequences. P1 and P4 are degenerate probes corresponding to peptides p1 and p4. A sequence of the human placental clone P1′ also was used as a probe (see text).

A



B

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          -50                 -30                 -10
-59  agaagctgaggtcatcatcagatttgaaatatttaaagtggatacaaaattatttcagca    0

           10                  30                  50
  1  atgcagacaattaagtgtgttgttgtgggcgatggtgctgttggtaaaacatgtctcctg   60
  1  MetGlnThrIleLysCysValValValGlyAspGlyAlaValGlyLysThrCysLeuLeu   20

           70                  90                 110
 61  atatcctacacaacaaacaaatttccatcggaatatgtaccgactgtttttgacaactat   120
 21  IleSerTyrThrThrAsnLysPheProSerGluTyrValProThrValPheAspAsnTyr   40

          130                 150                 170
121  gcagtcacagttatgattggtggagaaccatatactcttggacttttttgatactgcaggg   180
 41  AlaValThrValMetIleGlyGlyGluProTyrThrLeuGlyLeuPheAspThrAlaGly   60

          190                 210                 230
181  caagaggattatgacagattacgaccgctgagttatccacaaacagatgtatttctagtc   240
 61  GlnGluAspTyrAspArgLeuArgProLeuSerTyrProGlnThrAspValPheLeuVal   80

          250                 270                 290
241  tgtttttcagtggtctctccatcttcatttgaaaacgtgaaagaaaagtgggtgcctgag   300
 81  CysPheSerValValSerProSerSerPheGluAsnValLysGluLysTrpValProGlu   100

          310                 330                 350
301  ataactcaccactgtccaaagactcctttcttgcttgttgggactcaaattgatctcaga   360
101  IleThrHisHisCysProLysThrProPheLeuLeuValGlyThrGlnIleAspLeuArg   120

          370                 390                 410
361  gatgacccctctactattgagaaacttgccaagaacaaacagaagcctatcactccagag   420
121  AspAspProSerThrIleGluLysLeuAlaLysAsnLysGlnLysProIleThrProGlu   140

          430                 450                 470
421  actgctgaaaagctggcccgtgacctgaaggctgtcaagtatgtggagtgttctgcactt   480
141  ThrAlaGluLysLeuAlaArgAspLeuLysAlaValLysTyrValGluCysSerAlaLeu   160

          490                 510                 530
481  acacagaaaggcctaaagaatgtatttgacgaagcaatattggctgccctggagcctcca   540
161  ThrGlnLysGlyLeuLysAsnValPheAspGluAlaIleLeuAlaAlaLeuGluProPro   180

          550                 570                 590
541  gaaccgaagaagagccgcaggtgtgtgctgctatgaacatctctccagagccctttctgc   600
181  GluProLysLysSerArgArgCysValLeuLeu                              200

601  acagctggtgtcggcatcatactaaaagcaatgtttaaatcaaactaaagattaaaaatt   660
661  aaaattcgtttttgcaataatgacaaatgccctgcacctacccacatgcactcgtgtgag   720
721  acaaggcccatagggtatggccccccccttcccctcccagtactagttaattttgagtaa   780
781  ttgtattgtcagaaaagtgattagtactatttttttttgttgtttcaaaaaaaaaatttt   840
841  tgtgtgtctgtttttttttttttttttttttgttgtttaaaaggaaggcatgcttgtgga   900
901  tgactctgtaacagactaattggaattgttgaagctgctccctggttccactctggagag   960
961  taatctgggacatcttagtgttttgttttgttttttccctcctctttttttttgggggggg  1020
1021 agtgtgtgggggttttgttttttagtcttgttttttttaattcattaaccagtggttaagc  1080
1081 ccttaagggaggaggacggattgatt  1106
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FIG. 2. (*A*) Restriction map of the 2-kb cDNA of CDC42Hs. The sites of the p1, p2, and p4 peptides are indicated; arrows indicate the overlapping sequencing reactions. (*B*) Nucleotide and deduced amino acid sequences of the human placental CDC42Hs. As indicated by the breaks in the solid lines for the restriction map and nucleotide sequence, the 3′ noncoding sequence has not been sequenced in its entirety.

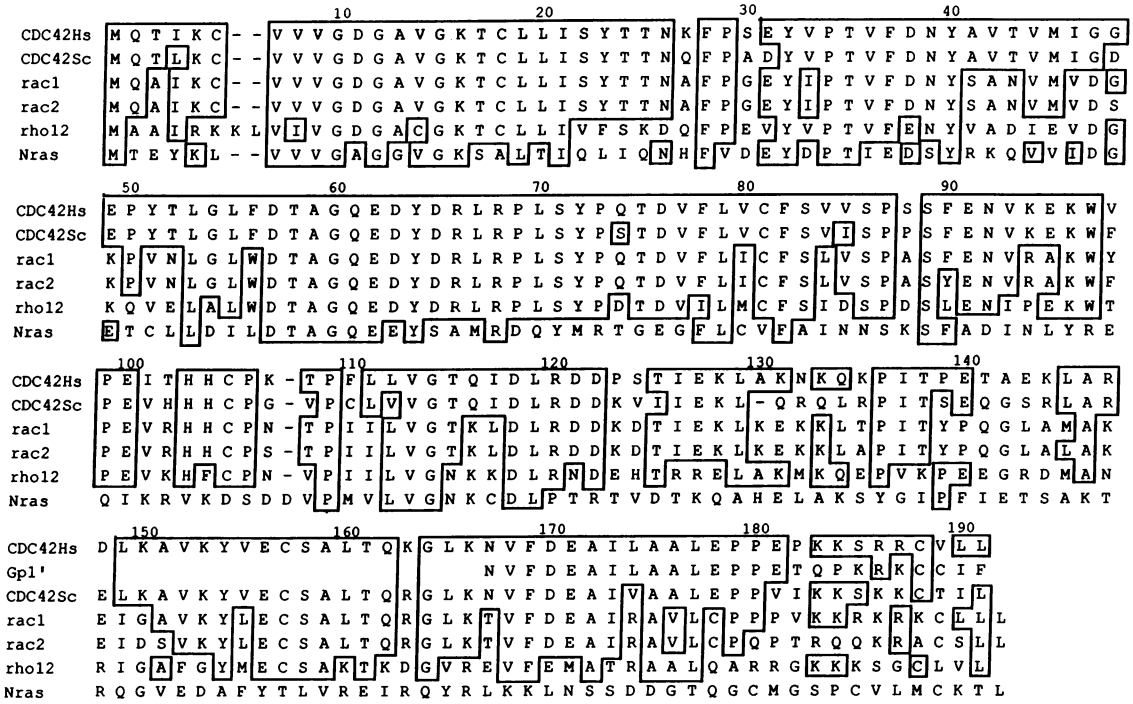

FIG. 3. Sequence similarities of the placental CDC42Hs to other low molecular weight GTP-binding proteins. Residues identical to those of the CDC42Hs protein are boxed.

a protein which is distinct from, albeit highly similar to, CDC42Hs.‖ Three amino acids (Gly-Ile-Gln) from the peptide fragment p3 are missing from the predicted amino acid sequence for CDC42Hs (i.e., residues 138–144).**

Sequence Comparisons Between CDC42Hs and Other Low Molecular Weight GTP-Binding Proteins. The predicted amino acid sequence of CDC42Hs identifies it as a member of the ras superfamily of low molecular weight GTP-binding proteins (Fig. 3). For example, the sequence of CDC42Hs is ≈30% identical to those of the Ha-ras, Ki-ras, and N-ras proteins and ≈50% identical to the sequences of the rho proteins. The predicted sequence of CDC42Hs contains many of the amino acids that have been shown to be essential for GTP-binding to the ras proteins, including Lys-16, Phe-28, and Thr-35. CDC42Hs also contains three amino acids that, when individually mutated in ras, result in oncogenic transformation—i.e., Gly-12, Ala-59, and Gln-61. As is the case for ras, as well as for many other members of this GTP-binding protein family, CDC42Hs contains a cysteine residue four positions upstream from its carboxyl terminus. An interesting difference between CDC42Hs and the ras (or rho) proteins is that the latter contain the guanine nucleotide-binding consensus element Asn-Lys-Xaa-Asp (23), whereas the analogous region in CDC42Hs (residues 115–118) is Thr-Gln-Ile-Asp. There is a strong sequence similarity (≈70%) between CDC42Hs and the recently described rac1 and rac2 proteins (10, 11). This sequence similarity is most apparent in the amino-terminal halves of these molecules, where 36 of the first 40 amino acids for CDC42Hs, rac1, and rac2 are identical (Fig. 3).

The highest degree of sequence similarity (80%) exists between the predicted sequence for the CDC42Hs clone and that for the *S. cerevisiae* cell-division-cycle protein CDC42Sc. This protein is essential for yeast cell growth and plays an important role in the establishment of cell polarity and in the development of normal cell shape during the yeast cell cycle (19, 24). There is an especially strong degree of sequence similarity between the predicted amino-terminal sequences for the human and yeast proteins, where 74 out of the first 80 amino acids are identical.

Complementation of the *cdc42-1* Mutation in Yeast by CDC42Hs. Given the high degree of sequence similarity between the human GTP-binding protein (CDC42Hs) and the yeast cell-division-cycle protein (CDC42Sc), we have examined the ability of the CDC42Hs cDNA to complement the temperature-sensitive lethal *cdc42-1* mutation. Yeast strains containing *cdc42-1* bud normally at permissive temperatures. At restrictive temperatures, the nuclear cycle continues but bud formation is blocked, leading to a cell-cycle arrest (24). The CDC42Hs gene was inserted into the yeast centromere-containing plasmid pBM272, such that the expression of the human gene was galactose-inducible. The resulting plasmid, pGAL-CDC42Hs(+), along with the plasmid pGAL-CDC42Hs(−), which has the human gene inserted into pBM272 in the opposite orientation, were used to transform the *cdc42-1* strain DJTD2-16A to Ura$^+$ at the permissive temperature of 23°C. The Ura$^+$ transformants were then incubated at the restrictive temperature of 36°C on selective media containing either 2% glucose or 2% galactose as the sole carbon source. Cells containing pGAL-CDC42Hs(+) were able to grow at 36°C on plates with galactose but not on plates with glucose, while cells containing pGAL-CDC42Hs(−) were unable to grow at 36°C regardless of the carbon source (Table 1). Cells with YEp103(42), a plasmid containing the CDC42Sc gene (19), could grow at 36°C regardless of the carbon source, while cells containing the control plasmid YEp103 could not. Overall, these results indicate that CDC42Hs can complement the *cdc42-1* mutation in *S. cerevisiae*.

Expression of CDC42Hs in *E. coli*: Western Blot Analyses and GTP-Binding Activity. To examine some of the biochemical properties of CDC42Hs, we expressed this protein in *E. coli*. We first examined the ability of the recombinant *E. coli* CDC42Hs protein to react with two antibodies that were

‖Recently, Susan Munemitsu, Paul Polakis, and their colleagues at the Cetus Corporation have isolated and sequenced a full-length cDNA clone from a fetal human brain cDNA library that contains the complete sequence for our P1′ clone (see ref. 26).

**This also is the case for the cDNA clone isolated by Munemitsu, Polakis, and their colleagues (described in footnote ‖).

Table 1. Complementation of the *cdc42-1* mutation by the human CDC42Hs gene

Plasmid	Growth at 36°C on selective medium	
	Glucose	Galactose
YEp103	−	−
YEp103(42)	+	+
pGAL-CDC42Hs(+)	−	+
pGAL-CDC42Hs(−)	−	−

The human CDC42Hs gene, contained on a 1.9-kb *Eco*RI fragment isolated from a λgt11 human placental cDNA library, was inserted in both orientations into the unique *Eco*RI site of pBM272. pGAL-CDC42Hs(+) contains the fragment in the proper orientation so that expression of the gene is under the control of the galactose-inducible *GAL10* promoter, whereas pGAL-CDC42Hs(−) has the fragment in the opposite orientation. YEp103(42) contains the *S. cerevisiae CDC42* gene under the control of its own promoter and YEp103 contains no *cdc42*-complementing sequences (19). Plus sign indicates the ability of cells to grow at 36°C on selective medium with the indicated carbon source (2% final concentration); − indicates the inability of cells to grow at 36°C.

raised against the p1 and p3 peptides (Fig. 1*A*) of the G_p protein (14). These antibodies do not crossreact with any other members of the low molecular weight GTP-binding protein family that presently have been characterized. A third polyclonal antibody (25), raised against the purified human platelet G_p, also was examined. Each of these antibodies reacts strongly with the recombinant CDC42Hs protein (Fig. 4). That the anti-p3 antibody shows a strong reaction with the recombinant CDC42Hs protein, despite the fact that this protein lacks the first three amino acids of the p3 sequence, indicates that the epitope for anti-p3 resides in the sequence Thr-Pro-Glu-Thr-Ala-Glu-Lys.

We also expressed CDC42Hs as a GST fusion protein (20). This protein could be readily purified and was capable of binding guanine nucleotides. Fig. 5 shows that the purified 55-kDa GST–CDC42Hs protein was able to react with anti-G_p antibodies and bound GTP[γ-^{35}S] with stoichiometries approaching 0.8–0.9 mol per mol of protein.

DISCUSSION

In this report, we describe the molecular cloning of the cDNA for a low molecular weight GTP-binding protein from a human placental library. The cloning was accomplished using two oligonucleotide probes prepared from peptide sequences for a human placenta/platelet GTP-binding protein, originally designated G_p (G25K). The G_p protein, which was the first low molecular weight GTP-binding protein to be identified aside from the ras proteins, was found to be present in high abundance in placenta and platelets (hence the name G_p) (13). The full-length cDNA that we have isolated contains the complete sequences for three of the peptides of the G_p protein: p1, p2, and p4 (Fig. 1*A*). These peptides are located in different regions throughout the coding sequence (Fig. 2*A*). Each of these peptides is unique to the G_p protein and have not been found in any of the other members of the ras superfamily. Moreover, the sequence Thr-Gln-Ile-Asp (residues 115–118), which is found within the p2 peptide of G_p and in the yeast CDC42Sc protein, replaces the Asn-Lys-Xaa-Asp sequence that appears to be highly conserved in many other members of the low molecular weight GTP-binding protein family.

Seven out of 10 amino acids of a fourth peptide, designated p3 in Fig. 1*A*, also are present within the predicted sequence of our clone. The first 3 amino acids of p3 (i.e., Gly-Ile-Gln)

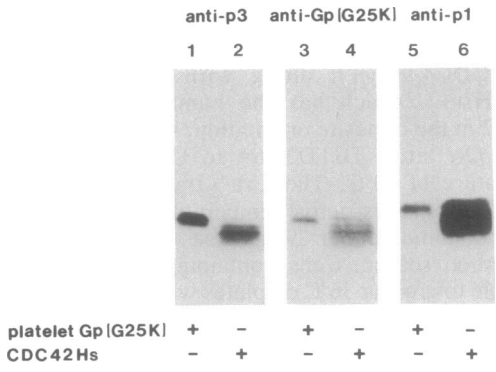

FIG. 4. Reactivity of the *E. coli*-expressed CDC42Hs protein with G_p-specific antibodies. The human placental CDC42Hs protein was expressed in *E. coli* with the pET-3a vector (21), and a crude bacterial supernatant was prepared. Aliquots of the supernatant (30 μg of protein) (lanes 2, 4, and 6) and of the purified human platelet G_p (1 μg) (lanes 1, 3, and 5) were subjected to SDS/PAGE. The gel was immunoblotted with two different anti-peptide antibodies specific for human G_p (designated as anti-p3 and anti-p1; see ref. 14) and a rabbit polyclonal antibody against the purified human platelet G_p protein (anti-G_p; ref. 25). The immunoreactive bands were visualized with ^{125}I-labeled protein A and autoradiography.

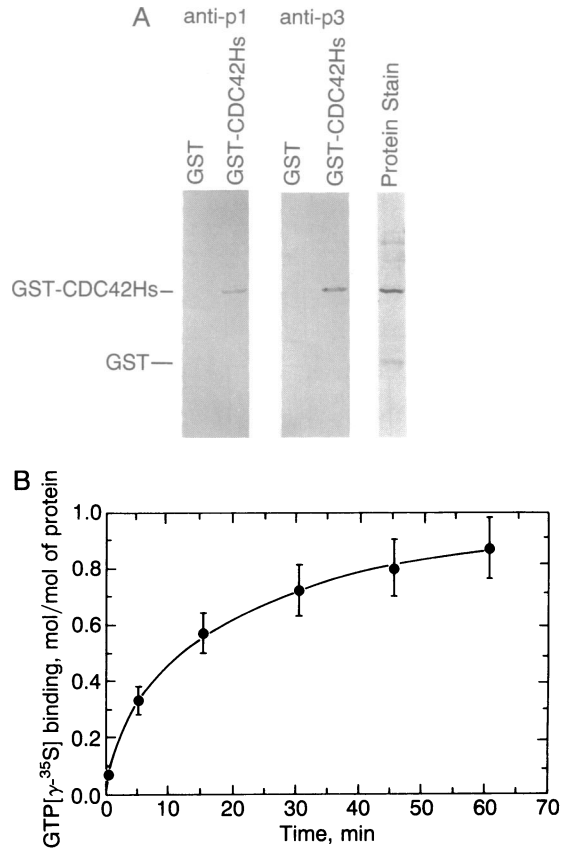

FIG. 5. Western blot analyses and GTP[γ-^{35}S] binding studies using a purified GST–CDC42Hs fusion protein. (*A*) The human placental CDC42Hs clone was expressed as a GST fusion protein in *E. coli* using the pGEX-2T vector, purified by glutathione-agarose chromatography, and subjected to Western blot analysis using two different anti-peptide antibodies specific for the human G_p protein. In each case a control lane is shown in which the GST protein was examined alone. After blotting, the proteins were visualized by reacting the antibodies with a second antibody conjugated to alkaline phosphatase. The lane at the far right shows the silver-stain profile of the purified GST–CDC42Hs protein. (*B*) The GST–CDC42Hs protein (25 ng) was assayed for GTP[γ-^{35}S] binding in the presence of 1 mM $MgCl_2$ and 2 μM GTP[γ-^{35}S] as described (16). The data shown represent one experiment that was repeated twice with essentially identical results (each data point was performed in triplicate).

are absent from our clone as well as from a related clone recently isolated by Munemitsu and colleagues.[‖] However, an antibody that was raised against p3 and has been shown to react specifically with G_p also reacts strongly with recombinant forms of the GTP-binding protein obtained by the expression of our cDNA in *E. coli*. Using the PCR, we have not yet been able to find the complete sequence for p3 in any clone from various cDNA libraries including human placenta, human epidermoid carcinoma cells, and bovine retina, which suggests that it either arose from a protein sequencing error or represents another form of this GTP-binding protein present in very low abundance. Several lines of evidence suggest the existence of multiple forms of G_p (14, 16). During the course of these studies, we isolated a partial clone that appeared to code for a distinct form of G_p. This clone coded for an amino acid sequence (P1′ in Fig. 3) that contained the first 18 amino acids of the p1 sequence, followed by a 2-amino acid insert (Thr-Gln) prior to the last 2 amino acids of p1 (Pro-Lys). The P1′ clone, which differs in 8 out of 25 amino acids at positions 167–191 (see Fig. 3) of the full-length clone that we describe in this study, is likely to represent a partial version of the clone isolated by Munemitsu and colleagues.[‖]

Recently, it was shown that the yeast cell-division-cycle protein CDC42Sc contains sequence similarity to the p1, p2, and p4 peptides of G_p (19). A high degree of sequence identity is maintained throughout the entire coding region for the human placental protein described here and, to our knowledge, represents the highest degree of homology between any yeast and human GTP-binding proteins. We found that the human cDNA could completely complement the *cdc42-1* mutation in *S. cerevisiae*. The ability of the gene that codes for the human G_p protein to complement the *cdc42-1* mutation appears to be very specific. Plasmids that contain the cDNAs for the rac1 or rac2 proteins (which are 70% identical to the CDC42Sc protein) do not complement the *cdc42-1* strain. Thus, we suggest that G_p represents the human homolog of the yeast CDC42 cell cycle protein, and have proposed renaming the human gene CDC42Hs.

An important question concerns the physiological function of the CDC42Hs protein. G_p is an excellent *in vitro* substrate for the EGF receptor (16). In addition, the EGF receptor will phosphorylate the purified, recombinant CDC42Hs (data not shown). Given the high degree of phosphorylation observed (approaching 2 mol of $^{32}P_i$ incorporated per mol of G_p; ref. 16), and the apparent specificity of the phosphorylation, we have speculated that this GTP-binding protein may function in the signaling pathway of the EGF receptor or related growth factor receptor tyrosine kinases. It is likely that further insights into the function of the CDC42 protein will come from studies of *S. cerevisiae*. A number of yeast gene products that functionally interact with CDC42Sc have been identified, and homologs of these proteins may have important roles in mammalian signal transduction.

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