The Schizosaccharomyces pombe cdc5 gene product is a cell cycle regulator that exerts its effects at the G2/M transition in fission yeast. We describe the cloning of a putative human transcription factor, pombe Cdc5-related protein (PCDC5RP), which bears significant homology to S. pombe Cdc5 and to expressed sequences in mouse, nematode, and budding yeast. PCDC5RP is expressed widely in normal adult human tissues and thus may have an important general function that has been preserved evolutionarily. PCDC5RP contains two tandem repeats of a helix-turn-helix DNA binding motif, four consensus nuclear localization signals, and a hydrophilic, proline-rich central region similar to the transcriptional activating domain in Myb family members. Remarkably, PCDC5RP moved rapidly from cytoplasm to nucleus upon serum stimulation of cultured cells. This movement correlated temporally with an increase in PCDC5RP phosphorylation. Thus, PCDC5RP is a presumed transcription factor that appears to transduce cytoplasmic signals to the nucleus upon serum stimulation.

The Schizosaccharomyces pombe cdc5 gene product is required for entry into mitosis (1, 2). Haploid yeast bearing a temperature-sensitive mutation in the cdc5 gene arrest with a diploid complement of DNA, single nucleus, and decondensed chromosomes without evidence of mitotic arrest or defective DNA replication (1, 2). In addition, S. pombe Cdc5 shares sequence similarity with the proto-oncogenic transcription factor, c-Myb (2). Thus, S. pombe Cdc5 appears to regulate entry into mitosis at the level of gene transcription.

We report the cloning and characterization of a cDNA encoding a novel human phosphoprotein with significant homology to S. pombe Cdc5 (2). This protein, designated PCDC5RP, contains two tandem repeats of a helix-turn-helix DNA binding domain similar to that seen in c-Myb and Myb-related proteins and a central, proline-rich, hydrophilic region that may confer transactivating ability. Widespread expression of PCDC5RP in normal human tissues suggests a general function. Homology with expressed sequence tags in normal human tissues suggests a general function. Homology with expressed sequence tags in normal human tissues and thus may have an important general function that has been conserved throughout evolution. Remarkably, PCDC5RP translocates from the cytoplasm to the nucleus of mammalian cells in response to stimulation with serum. These findings suggest that PCDC5RP may provide a novel pathway from the cytoplasm to the nucleus in mitogen-activated signal transduction.

**EXPERIMENTAL PROCEDURES**

**Cloning, Sequencing, and Site-directed Mutagenesis—**A novel 1.45-kb cDNA, clone 67, was isolated during a yeast two-hybrid screen of a HeLa cDNA library in pGADGH (3) (provided by R. Derynck, University of California San Francisco) with cytoplasmic domains (amino acids 775–799, 1094–1115, and 1274–1512) of the human thrombin receptor (4) in the GAL4 binding domain vector, pAS1-CYH (5) (provided by S. Elledge, Baylor College of Medicine). This was sequenced (6) and used to screen a HeLa cDNA library in Uni-ZAP XR (Stratagene) for full-length clones. The 1.45-kb cDNA insert from clone 67 was labeled using the ECL enhanced chemiluminescence system (Amer sham Corp.) according to the protocol provided, and plaques were screened using standard techniques (7) as modified in the ECL protocol. Eleven Uni-ZAP XR clones were excised in vivo into SK phagemid according to the protocol from Stratagene. The 2.8-kb insert from phagemid clone 67.1 was sequenced (6), and identity with pGADGH clone 67 was established. Clone 67.1 was modified after ultimate codon 802 to add an epitope (DYKDIDD) recognized by monoclonal antibody M2 (Kodak/IBI) using site-directed mutagenesis (8). The epitope-tagged insert was then excised from SK at NotI/ApaI restriction sites and subcloned into these same sites in pcDNA3 (Invitrogen) (7). This clone, pcD67F, was used in all further studies, as described below.

Related sequences were identified using the basic local alignment search tool (9) and sequence data bases available from the National Center for Biotechnology Information. Alignments and Pustell dot matrix homology analyses were performed using MacVector sequence analysis software (Oxford).

**Northern Analysis—**Total multiple tissue Northern blot (Clontech) was prehybridized in 750 mM sodium chloride, 50 mM sodium phosphate, pH 7.4, 5 mM EDTA, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50% deionized formamide, 2% sodium dodecyl sulfate, 100 μg/ml sheared salmon sperm DNA (Sigma) at 42°C for 4 h, then hybridized in the same solution containing 1.2 × 106 cpm/ml labeled probe at 42°C for 24 h. Following hybridization, the blot was rinsed three times in 300 mM sodium chloride, 30 mM sodium citrate, pH 7, 0.05% sodium dodecyl sulfate at room temperature, then three times in the same solution at room temperature for 10 min, then two times in 15 mM sodium chloride, 1.5 mM sodium citrate, pH 7, 0.1% sodium dodecyl sulfate at 50°C for 20 min. The washed membrane was exposed to x-ray film for 24 h with one intensifying screen at ~70°C. The NotI/ApaI fragment of pcD67F or a human β-actin cDNA control (Clontech) was labeled to a specific activity of 3 × 108 cpm/μg using the Prime-It II random primer labeling kit (Stratagene) according to the manufacturer’s instructions. The blot was stripped between hybridizations by washing two times in 0.5% sodium dodecyl sulfate at 100°C for 10 min.

**Cell Culture and Transfection—**COS-7 and CV-1 cell lines were maintained in DMEM H-16 medium with 5 g/liter glucose (Life Technologies, Inc.) and 10% bovine calf serum (Life Technologies, Inc.). Transfections were performed using LipofectAMINE (Life Technologies, Inc.) according to the protocol provided by the manufacturer. Transiently transfected cells were allowed to recover in DMEM with serum for 12–18 h, then incubated an additional 18–24 h in serum-free DMEM containing 0.1% bovine serum albumin. Serum-deprived cells
**RESULTS AND DISCUSSION**

PCDC5RP was identified as an apparently false positive in a yeast two-hybrid screen intended to look for novel proteins that associate with the cytoplasmic domains of the cloned human thrombin receptor (4). Although subsequent studies failed to demonstrate either association or functional coupling of PCDC5RP and the thrombin receptor in mammalian cells, PCDC5RP’s ubiquitous expression and relatedness to a cell cycle control element in fission yeast (as described below) prompted further study.

The 1.45-kb partial cDNA identified during the yeast two-hybrid screen contained an open reading frame encoding a potential DNA binding domain. This cDNA was used to isolate a 2.85-kb cDNA from a HeLa cell cDNA library which contained a complete open reading frame encoding an 802-amino acid protein (Fig. 1A). The protein sequence contained two tandem repeats of a helix-turn-helix DNA binding motif (11), four consensus nuclear localization signals (12), a proline-rich, hydrophilic region similar to known activating domains (13, 14), and potential sites for phosphorylation by intracellular kinases (Fig. 1, A and B).

A search for related cDNA sequences revealed significant homology with thecdc5 gene product in S. pombe (2). The two proteins were 75% identical over the 223 amino acids comprising a DNA binding and nuclear localization domains and 17% identical over the subsequent 535 amino acids (Fig. 1A). These amino acids 4–56 and 57–107 of PCDC5RP represent two tandem repeats of a helix-turn-helix motif similar to the DNA binding domain of Myb-related proteins (Fig. 2B). This region was 83% identical to the analogous region (amino acids 2–103) of S. pombe Cdc5 (2) and 36–38% identical to the corresponding domains in the human Myb subfamilies (15, 16). Carboxyl to

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**Fig. 1.** Amino acid sequence of PCDC5RP contains two tandem repeats of a Myb-like DNA binding domain, a potential activating domain, consensus nuclear localization signals, and sites for phosphorylation by intracellular kinases. **Panel A.** A sequence of 802-amino acid open reading frame derived from HeLa cDNA clone 67.1. Thick solid underline, DNA binding domains; thin solid underline, nuclear localization signals; broken underline, activating domain. Shown are potential phosphorylation sites for casein kinase II (●), MAP kinase (□), protein kinase A (○), and protein kinase C (♦). **Panel B.** Diagram depicting the proposed functional domains of PCDC5RP, as detailed in Fig. 2. R2 and R3 refer to similar repeats of the helix-turn-helix motif seen in a-, b-, and c-Myb (see Fig. 2B). The amino acid number is indicated above the map. NLs, nuclear localization signals.

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this region was a hydrophilic stretch of 223 residues (amino acids 378–500), with some homology to the activating domains of α- and β-Myb (15, 16) and S. pombe Cdc5 (2) (Fig. 2C). This analysis suggests that PCDC5RP is conserved throughout evolution. Contiguous amino acids, as indicated by coordinates, were aligned using window size = 8, minimum percent score = 60, and hash value = 2. Panel B, sequence alignment demonstrating amino-terminal homology with S. pombe Cdc5 and the Myb family DNA binding domain suggests that PCDC5RP likely acts as a DNA-binding protein. Whereas α-, β-, and c-Myb contain three tandem repeats of the helix-turn-helix motif (R1, R2, R3), PCDC5RP and S. pombe Cdc5 contain two tandem repeats (R2, R3). R2 is shown. Identical amino acids are indicated by boxes. Gaps (−) were introduced to maximize alignment. Conserved tryptophan residues characteristic of the helix-turn-helix motif (11) are indicated in bold. The Val → Leu substitution in the R2 DNA binding cavity is indicated by ●. Panel C, sequence alignment showing the central, hydrophilic region of PCDC5RP and the activating domains of α- and β-Myb. Prolines are highlighted in bold. Gaps (−) were introduced to maximize alignment.
tagged version of PCDC5RP was transiently expressed in CV-1 cells. Cells grown in standard culture medium with 10% bovine calf serum demonstrated nuclear localization of PCDC5RP (data not shown), consistent with a role in transcriptional regulation. Remarkably, however, PCDC5RP was found exclusively in the cytoplasm in transfected CV-1 cells deprived of serum (Fig. 4A). When these serum-deprived cells were then stimulated with 10% bovine calf serum for 5, 15, or 60 min, PCDC5RP was found solely in the nucleus (Fig. 4B). This same phenomenon was observed in transiently transfected COS-7 cells (data not shown). To the extent that the results obtained with epitope-tagged PCDC5RP expressed in CV-1 or COS-7 cells accurately reflect the behavior of endogenous PCDC5RP, the rapid nuclear translocation of PCDC5RP in response to serum stimulation suggests a role in relaying signals from the cell surface to the nucleus.

Many transcription factors contain phosphorylation sites that regulate nuclear localization as well as DNA binding and transactivation (20). PCDC5RP contains 28 potential phosphorylation sites: 14 for recognition by casein kinase II (S/T-X-X-D/E), 9 for protein kinase C (S/T-X-R/K), 2 for protein kinase A (R/K-X-X-S/T), and 3 for MAP kinase (P-X-X-S/T-P or P-X-X-S/T-P) (see Fig. 1). Western blot analysis of whole cell lysates from COS-7 cells expressing epitope-tagged PCDC5RP demonstrated a single, transfection-dependent band at ~105 kDa (Fig. 5A). This is greater than the predicted size of 92.2 kDa, suggesting the possibility that PCDC5RP either maintains a structure that slows its mobility on gel electrophoresis or carries post-translational modifications.

To test the hypothesis that PCDC5RP is a phosphoprotein, immunoprecipitates of 32P-labeled COS-7 cells expressing epitope-tagged PCDC5RP were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Autoradiography revealed a transfection-dependent, 32P-labeled band of the appropriate molecular weight (Fig. 5B). The intensity of this band doubled within 10 min of serum stimulation, as quantitated by densitometry. Thus, although recombinant PCDC5RP was phosphorylated in non-stimulated COS-7 cells, the degree of phosphorylation or the amount of phosphoprotein available for

![Fig. 3. PCDC5RP mRNA is abundantly present in normal adult human tissues.](image)

Northern blot analysis of poly(A) mRNA probed with a 2.85-kb cDNA for PCDC5RP demonstrates a dominant 3.4-kb transcript in normal human heart, skeletal muscle, brain, placenta, liver, and pancreas (top). The membrane subsequently was probed with cDNA for β-actin as a control for the amount of mRNA loaded in each lane (bottom).

![Fig. 4. PCDC5RP undergoes nuclear translocation in serum-stimulated CV-1 cells. Panel A, immunofluorescent staining of PCDC5RP-transfected CV-1 cells incubated in serum-free medium (“Experimental Procedures”). Representative fields are shown of immunofluorescence (top) and the corresponding phase-contrast images (bottom). Panel B, cells were treated as in panel A but incubated with serum for 5 min at 37 °C prior to fixation. Note that stimulation with serum causes rapid translocation of PCDC5RP to the nucleus.](image)
immunoprecipitation increased with serum stimulation.

Precedent for phosphorylation-dependent translocation of transcription factors is well established (20). Several potential phosphorylation sites flank the four consensus nuclear localization signals in PCDC5RP (Fig. 1). In addition, these nuclear localization signals and phosphorylation sites are conserved in S. pombe Cdc5. Whether phosphorylation regulates translocation, DNA binding, or activation for these putative transcription factors remains to be determined.

The relatedness of PCDC5RP to S. pombe Cdc5 prompts speculation about its possible function. The S. pombe molecule has been implicated in the regulation of cell division, specifically at the G2/M transition (1, 2). PCDC5RP is almost certainly a serum-regulated transcription factor. Taken together with its similarity to S. pombe Cdc5 and its ubiquitous expression, this suggests a possible role for PCDC5RP in cell cycle control.

Similarities and differences between PCDC5RP and c-Myb also are worthy of comment. The DNA binding ability of the helix-turn-helix motif seen in PCDC5RP has been well characterized (11). In contrast with the DNA binding domain seen in Myb, both PCDC5RP and S. pombe Cdc5 contain only two tandem repeats of the helix-turn-helix motif, whereas Myb family members possess three. Moreover, within this domain both PCDC5RP and S. pombe Cdc5 bear a valine to leucine substitution at a position critical for DNA binding specificity (21, 22). PCDC5RP and S. pombe Cdc5, therefore, may differ from Myb in their DNA binding properties. As noted above, the expression pattern of PCDC5RP differs from that of the Myb family. These observations suggest that although PCDC5RP and S. pombe Cdc5 share similarity with the Myb family, they are likely to have distinct biological roles.

In summary, we describe a novel putative human transcription factor, PCDC5RP, with significant homology to a cell cycle regulator in fission yeast, S. pombe Cdc5, as well as to related molecules in mouse, nematode, and budding yeast. PCDC5RP contains an amino-terminal DNA binding domain related to that seen in Myb family members but with several distinguishing features. PCDC5RP is widely expressed in adult human tissues, suggesting a general function. Remarkably, PCDC5RP undergoes rapid nuclear translocation in response to stimulation with serum.

Extracellular signals are transduced from the cell surface by a variety of schemes. Many growth factor–activated signaling pathways converge on members of the MAP kinase family (23, 24). For these pathways, MAP kinase represents the final element in a cytoplasmic signaling cascade, as stimulation of fibroblasts with serum results in MAP kinase translocation to the nucleus (25–29). In the nucleus, MAP kinase phosphorylates and thereby regulates the activity of transcription factors (30). Other strategies for relaying cytoplasmic information to the nucleus include the direct activation and nuclear translocation of cytoplasmic transcription factors by phosphorylation, as is the case for signal transducer and activator of transcription (STAT) proteins (31), and the release of transcription factors from cytoplasmic retention proteins, as exemplified by the Rel family of DNA-binding proteins (32). Although the precise nuclear function of PCDC5RP remains to be elucidated, its behavior in serum-stimulated mammalian cells suggests that this presumed transcription factor may provide a novel pathway from the cytoplasm to the nucleus in mitogen-activated signal transduction.

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