We have cloned a novel serine/threonine protein kinase (PK428) which is highly related (65%) within the kinase domain to the myotonic dystrophy protein kinase (DM-PK), as well as the cyclic AMP-dependent protein kinase (33%). Northern blots demonstrate that PK428 mRNA is distributed widely among tissues and is expressed at the highest levels in pancreas, heart, and skeletal muscle, with lower levels in liver and lung. Two PK428 mRNAs 10 and 3.8 kilobase pairs in size are seen in a number of cell lines, including hematopoietic and breast cancer cells. An antibody generated to a glutathione S-transferase-PK428 fusion protein detects a 65-kDa protein in these cell lines, and a similarly sized protein when the cloned cDNA is transiently expressed in Cos 7 cells. Immunoprecipitation of the transiently expressed PK428 protein and incubation with [γ-32P]ATP demonstrates that it is capable of autophosphorylation. In addition, immunoprecipitates of the PK428 protein kinase also phosphorylated histone H1 and a peptide encoding the corresponding mRNA is distributed widely among tissues and is expressed at the highest levels in pancreas, heart, and skeletal muscle, with lower levels in liver and lung. Two PK428 mRNAs 10 and 3.8 kilobase pairs in size are seen in a number of cell lines, including hematopoietic and breast cancer cells. An antibody generated to a glutathione S-transferase-PK428 fusion protein detects a 65-kDa protein in these cell lines, and a similarly sized protein when the cloned cDNA is transiently expressed in Cos 7 cells. Immunoprecipitation of the transiently expressed PK428 protein and incubation with [γ-32P]ATP demonstrates that it is capable of autophosphorylation. In addition, immunoprecipitates of the PK428 protein kinase also phosphorylated histone H1 and a peptide encoding a cyclic AMP-dependent protein kinase substrate. The gene corresponding to the 3.8-kb PK428 mRNA, and its corresponding 65-kDa protein, was isolated by polymerase chain reaction screening of a P1 phage human genomic library. Using this P1 phage clone as a probe, the PK428 gene was located on 1q41–42, a possible location for a human senescence gene, a gene associated with Rippling muscle disease, as well as a region associated with genetically acquired mental retardation.

Myotonic dystrophy (DM) is a systemic disease characterized by muscle weakness, myotonia, cardiac conduction defects, cataracts, testicular atrophy, insulin-resistant diabetes, and male baldness (1, 2), with an incidence of 1 in 8000 (1). This disease can present either as a slowly progressive form in adults or as a congenital form in childhood (1, 2). The molecular basis of DM involves the mutation and expansion of a trinucleotide sequence (CTG), located in the 3'-untranslated region of the corresponding mRNA (3, 4). The DM gene encodes a putative serine-threonine protein kinase (DM-PK) which is located in chromosome 19q13.3 (3–5). This protein kinase is predicted to contain a protein kinase catalytic domain near the amino terminus, a central α-helical coiled domain, and a potential carboxy-terminal transmembrane domain (3). Consistent with the systemic nature of this disease, transcripts of the gene are expressed in various tissues, including the heart, skeletal muscle, liver, and brain, both in human and mouse (6, 7).

Another protein kinase related to the DM-PK, p160ROCK, has been shown to bind the small GTP-binding protein Rho, and it contains a protein kinase domain that shares 44% similarity to the DM-PK, an amphipathic α-helix, a pleckstrin domain, and a cysteine-rich region (8–10). Since the activation of Rho regulates the actin cytoskeleton, it is possible that the DM-PK has a similar or parallel function. The warts protein kinase, which is found in Drosophila melanogaster, is also related to the DM-PK (46% identity); however, the biologic function of this protein is unknown. Deletion of the warts gene in Drosophila leads to the formation of cell clones that are fragmented, rounded, and greatly overgrown (11).

Signaling through the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor is mediated by two subunits, an α subunit, which binds GM-CSF and interacts with the β subunit containing the binding site for the JAK2 protein kinase. The JAK2 protein kinase has been shown to regulate at least some of the signaling mediated by this receptor. The α subunit has a short intracytoplasmic carboxyl-terminal tail (54 amino acids), which is essential for GM-CSF-mediated growth stimulation (12–15). Within this 54-amino acid tail is a short stretch of prolines, a sequence that is conserved in the α subunits of both the interleukin-3 and -5 receptors. Mutation of one or more of these proline residues blocks the ability of these hormones to signal and act as stimulators of growth (14, 15). Because the intracytoplasmic region of the α subunit is essential for the function of GM-CSF, we have used this portion of the receptor in a two-hybrid yeast interaction screen to search for proteins that may be essential for signal transduction by this hormone.

We have cloned a novel protein kinase, PK428, which is highly related to the DM-PK amino-terminal kinase domain, but which diverges in its carboxyl terminus. This novel protein kinase is highly expressed in skeletal muscle and heart, but it is also expressed in the brain, pancreas, and at lower levels in the lung. Northern blots demonstrate that this kinase is ex-
pressed as two separate mRNAs, 3.8 and 10 kb in size, in both leukemic and breast cancer cells, but not in HeLa (cervical carcinoma) cells. Transfection of this cDNA into Cos 7 cells results in the production of a 65-kDa protein, which is capable of autophosphorylation, as well as phosphorylation of histone H1 and a peptide substrate that contains a cyclic AMP-dependent protein kinase phosphorylation site. An antibody generated to the PK428 protein kinase immunoprecipitated a 65-kDa protein (corresponding closely to the predicted size of the open reading frame in a 3.8-kb PK428 mRNA) in both hematopoietic and breast cancer cells, as well as a larger 200-kDa protein (which presumably corresponds to a larger 10-kb PK428 mRNA). The chromosomal gene corresponding to the 3.8-kb PK428 mRNA was isolated by PCR screening of a human genomic P1 phage library. Two identical clones were isolated, and their identity was confirmed by partial DNA sequence analysis. Using fluorescence in situ hybridization (FISH) of the PK428 P1 phage clones, we found that the PK428 gene encoding the 3.8-kb mRNA and 65-kDa protein localizes to the long arm of human chromosome 1, specifically the q41–42 region. This area has previously been implicated in some forms of genetically acquired mental retardation (16), as a possible location for a second human senescence gene localized to the long arm of chromosome 1 (17), and most interestingly, as a location of a gene(s) involved in Rippling muscle disease (18).

**MATERIALS AND METHODS**

**Cloning of Full-length cDNA**—The PK428 cDNA fragment was identified using the yeast two-hybrid system (a gift of Dr. Steven Elledge). The 54-amino acid intracellular tail of the GM-CSF α subunit was cloned into plasmid pAS1-CYH2 as the bait. The yeast Y190 cells were cotransformed with a peripheral B lymphocyte library fused to the DNA activation domain of GAL4 that is contained within the plasmid pACT II. The two-hybrid screen was carried out as described previously (19). This screening yielded 10 positive cDNA clones, of which three were identified as PK428 cDNA clones. To obtain a full-length cDNA, the largest PK428 fragment was used as probe to screen a λ ZAP expression cDNA library constructed from five breast cancer cell lines (a gift of Dr. M. Ruppert, University of Alabama) using the ZAP Express™ cDNA synthesis kit (Stratagene). The prehybridization and hybridization were carried out in hybridization buffer (0.5 M NaCl, 3× SSC, 0.3% SDS) for 5 min at room temperature and 0.3× SSC, 0.3% SDS for 60 min at 65 °C. DNA sequence analysis was performed by the dyeoxy method using Sequenase 2.0 according to the manufacturer’s (U.S. Biochemical Corp.) specifications. Homology searches were performed with the BLAST network service at National Center for Biotechnology Information.

**Plasmid Construction**—The pcDNA3PK428/2.8 plasmid was constructed by digesting pBKCMV PK428, the longest clone from the PK428 clones, we found that the PK428 gene encoding the 3.8-kb mRNA and 65-kDa protein localizes to the long arm of human chromosome 1, specifically the q41–42 region. This area has previously been implicated in some forms of genetically acquired mental retardation (16), as a possible location for a second human senescence gene localized to the long arm of chromosome 1 (17), and most interestingly, as a location of a gene(s) involved in Rippling muscle disease (18).

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ing for 1 min, and a 72 °C extension for 30 s for 35 cycles. Two P1 phage clones containing the human PK428 gene (11499 and 11500) were isolated and analyzed further by direct DNA sequence analysis to confirm their identity (data not shown).

Fluorescence in Situ Hybridization—Bromodeoxyuridine-synchronized and phytohemagglutinin-stimulated peripheral blood lymphocytes from a normal male donor were used as a source of metaphase chromosomes. Purified DNA from the P1 phage clones containing the human PK428 gene (11499 and 11500) were labeled for FISH analysis by nick translation with digoxigenin-11-UTP (Boehringer Mannheim). To localize the human PK428 gene, one set of metaphase chromosomes was simultaneously hybridized with a control genomic centromeric clone corresponding to alphoid sequences of human chromosome 1, D1Z1 (Oncor, Inc.). Specific hybridization signals were detected by applying fluorescein-conjugated sheep antibodies to digoxigenin (Boehringer Mannheim) and avidin-Texas Red (Vector Laboratories, Inc., Burlingame, CA), followed by counterstaining in DAPI (Sigma). Fluorescence microscopy was performed with a Nikon microscope equipped with a cytovision image analysis system (Applied Imaging, Pittsburgh, PA) and a fluorescence filter wheel that sequentially captures the fluorescein, Texas Red, and DAPI images and electronically superimposes them.

RESULTS

Isolation of a Full-length cDNA Clone for PK428—The PK428 clone was isolated using the yeast two-hybrid screen designed to identify proteins that bind to the intracytoplasmic portion of the α subunit of the GM-CSF receptor. This 54-amino acid intracytoplasmic domain of the GM-CSF α subunit ap-
pears to play an important role in signal transduction, although the proteins that bind to this domain remain unknown (12, 13). We fused the 54-amino acid intracytoplasmic domain to the DNA binding portion of GAL4 as the bait for the two-hybrid analysis, and a B cell library fused to the GAL4 DNA transactivation domain was used as the prey. Ten positive clones were obtained, three of which encoded PK428. The PK428 cDNA fragment obtained from the yeast two-hybrid screening was used as a hybridization probe to isolate additional cDNAs from a human breast cancer cell cDNA library. Nine positive clones were isolated and analyzed by DNA sequencing. The PK428 insert contains a 2,818-base pair cDNA fragment (GenBank™ accession no. U59305) with a large open reading frame found from nucleotide 1289–2776, encoding a 496-amino acid protein (Fig. 1). The designated ATG is likely to be correct, since several stop codons in all three reading frames are found 5' of this ATG codon. Analysis of the amino acid sequence of PK428 demonstrates that it contains a complete protein kinase catalytic domain which conserves all 11 kinase subdomains (Figs. 1 and 2) (21). In addition to the high homology kinase region between DM-PK and PK428, the amino-terminal region of the deduced PK428 amino acid sequence is also related (to a lesser extent) to the DM-PK, suggesting that the two proteins are unique. Hydrophobicity analysis (22) shows that the PK428 amino acid sequence contains a helical region following the kinase domain, as well as a hydrophobic domain. Both of these regions, which are similar to those found in DM-PK, suggest that PK428 is most likely a protein kinase related to the DM-PK (Fig. 1).

The catalytic domain of the PK428 protein kinase corresponds to a 263-amino acid region that is located in the amino terminus of the protein. The GXGXXG sequence motif characteristic of many protein kinases is found in subdomain I. An invariant lysine at position 106 of the protein that is necessary for the ATP binding is also present (15), as well as the conserved amino acids DIKPEN in subdomain VI and GTPDYL-SPE in subdomain VIII. These features of the predicted PK428 open reading frame strongly suggest that this protein is a
serine/threonine protein kinase. A sequence homology search demonstrated that the kinase domain of PK428 was 65% homologous to the kinase domain of the myotonic dystrophy protein kinase (23), 50% homologous to the kinase domain of p160ROCK (10), 51% homologous to the rat ROK protein kinase (23), 50% homologous to the kinase domain of the myotonic dystrophy protein (8), and 46% to the D. melanogaster tumor suppressor protein kinase warts (11), 40% homologous to the Cot-1 protein kinase from Neurospora crassa (24), 43% homologous to the serine/threonine protein kinase from Mesembryanthemum crystallinum,2 and 33% homologous to the catalytic subunit of protein kinase A (25) (Fig. 2).

**Tissue and Cell Type Expression of PK428**—To examine the tissue expression of PK428 mRNA, Northern blot analysis of poly(A)⁺ RNA from different human tissues and cell lines was performed. A 10-kb mRNA was most abundant in the heart, brain, skeletal muscle, kidney, and pancreas, with little or no expression in the lung and liver (Fig. 3A). Northern blot analysis of various human cell lines demonstrates the presence of 3.8- and 10-kb hybridizing bands (Fig. 3B), and both are expressed in the hematopoietic cell lines U937, K562, and PLB985, the breast cancer cell line MDA-MB-231, A549 lung carcinoma cells, and the Wilms' tumor cell line, G-401. The 3.8-kb mRNA for PK428 was not found in HeLa cells, the T cell line MOLT-4, or the pre-B cell lines IE8 and OB5.

To verify the identity and molecular weight of this putative protein kinase, a polyclonal antibody was generated using a GST-PK428 fusion protein. MDA-MB-231 and U937 cells were lysed, and PK428 protein was immunoprecipitated and Western blotted with this antibody (Fig. 4A). In both cell lines a protein with a apparent molecular mass of 65 kDa, as well as a less intense band at 78 kDa, were immunoprecipitated. In addition, in the U937 cells, a 200-kDa band was also seen. To determine which of these polypeptides was encoded by the cloned cDNA, and its molecular weight on SDS-PAGE gels, Cos 7 cells were transfected with each of two PK428 cDNA clones (2.0 and 1.5 kb), which contained the entire PK428 open reading frame and varying lengths of the 5'-untranslated region. A 65-kDa protein was immunoprecipitated from Cos 7 cells transfected with both of the PK428 cDNAs, suggesting that they both utilized an identical site for initiation of translation (Fig. 4B). These analyses demonstrated that the PK428 cDNA clones that were isolated corresponded to the 65-kDa protein species identified by the PK428 antibody in human cell lines.

**Protein Kinase Activity Associated with the PK428 Protein**—Since many protein kinases are capable of autophosphorylation, the in vivo phosphorylation state of the PK428 protein was examined. Cos 7 cells were transfected with the PK428 cDNA and labeled with [32P]orthophosphate, and the PK428 protein was immunoprecipitated with the PK428-specific antibody. A 65-kDa phosphorylated protein was detected in the Cos 7 cells transfected with the cDNA (Fig. 5A, lane 3), but it was not present in either preimmune cells (lane 2) or when this antibody was used for immunoprecipitation from the untransfected cells (lane 1). To demonstrate in vitro that this protein

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1. B. Baur, K. Winter, K. Fischer, and K. Dietz, unpublished GenBank accession numbers Z30329 and Z30330.

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FIG. 3. Northern blots of human tissues and cell lines. Panel A, human tissue Northern blot. The molecular sizes are shown in kilobase pairs. Panel B, Northern blot of RNA from cell lines. 20 μg of total RNA were electrophoresed on an agarose gel and transferred to a membrane (see “Materials and Methods”) and probed with the PK428 cDNA. The identical membrane was stripped and probed with tubulin. The cell lines used in this study are as follows: 1, U937 histiocytic lymphoma; 2, K562 chronic myelogenous leukemia; 3, MDA-MB-231 breast cancer; 4, A549 lung cancer; 5, G-401 Wilms' tumor; 6, Colo 320 colon cancer; 7, SK-OV-3 ovarian cancer; 8, MOLT-4 lymphoblastic leukemia; 9, HL-60 promyelocytic leukemia; 10, PLB 985 myelocytic leukemia; 11, HeLa epithelial cancer; 12, IE8 pre-B-cell leukemia; and 13, OB5 pre-B cell leukemia.

FIG. 4. Immunoprecipitation of PK428 from cell lines and transfected Cos 7 cells. Panel A, immunoprecipitation from cell lines. An antibody to a GST-PK428 fusion protein was raised (2188 antibody), and used to immunoprecipitate the PK428 protein from 10⁵ MDA-MB-231 cells (lanes 1 and 2) or the same number of U937 cells (lanes 3 and 4). The immunoprecipitated proteins were transferred to a membrane and then Western blotted with the 2188 antibody. Molecular mass markers are shown on the left side of the figure, and the 200- and 65-kDa immunoprecipitated proteins are indicated by the arrows. Panel B, immunoprecipitation from transfected cells. 6 × 10⁵ Cos 7 cells were transfected with either pcDNA PK428/1.5 (lanes 1 and 4), pcDNA3PK428/2.0 (lane 2), or pcDNA3 alone (lane 4). The cells were lysed, the PK428 protein was immunoprecipitated with either the 2188 antibody or preimmune serum, the proteins were transferred to a membrane, and a Western blot was performed with the 2188 antibody.
was capable of autophosphorylation, the PK428 protein from transfected and unlabeled Cos 7 cells was immunoprecipitated with the PK428 antibody, and the resulting protein was incubated in a kinase reaction buffer containing \( \gamma^{32} \text{P} \text{ATP} \) for the indicated time periods. This reaction was then run on an SDS-PAGE and the phosphorylated proteins identified by autoradiography. Panel C, phosphorylation of possible protein kinase substrates by PK428. Immunoprecipitation of transfected Cos 7 cells was either carried out with the 2188 antisera or a preimmune serum as a control (lanes 5 and 6). The immunoprecipitates were either added to the cyclic AMP fusion protein expressed as a GST fusion protein (GST-TK, lanes 1–4) or GST alone (lane 5), or histone H1 (lanes 5 and 6). The incubation time is shown at the top. At the completion of the reaction, the products were analyzed by SDS-PAGE, and the phosphorylated substrates were identified by autoradiography. The phosphorylated GST-TK and histone H1 are indicated by arrows.

Since the PK428 protein was homologous to the protein kinase domain to the cyclic AMP-dependent protein kinase, the ability of the PK428 protein kinase to phosphorylate a cyclic AMP-dependent protein was examined. The phosphorylation of PK428 protein kinase activity was measured by the incorporation of \( \gamma^{32} \text{P} \) phosphate from \( \gamma^{32} \text{P} \text{ATP} \) into either histone H1 or a peptide substrate of cyclic AMP-dependent protein kinase encoded as part of a GST fusion protein (GST-TK) (20). PK428 immunoprecipitates phosphorylated the GST-TK fusion protein (Fig. 5C), but did not phosphorylate GST alone (Fig. 5C, lane 5). When the PK428 antiserum was replaced with normal rabbit serum, no phosphorylation of the substrate was seen (data not shown). Likewise, the PK428 immunoprecipitates phosphorylate histone H1 when it is used as a substrate (Fig. 5C), while no phosphorylation of histone H1 as seen if the preimmune serum was used as a control (Fig. 5C), or if histone H1 is not included in the reaction (data not shown).

FIG. 5. Protein kinase activity of PK428. Panel A, \( ^{32} \text{P} \) labeling of PK428. \( 6 \times 10^6 \) Cos 7 cells were transfected with either the pCDNA3 vector as a control (lane 1) or the pCDNA3PK428/1.5 expression construct (lane 2 and 3). The cells were labeled with \( ^{32} \text{P} \) orthophosphate, and PK428 was immunoprecipitated with either the 2188 antisera or preimmune serum. The phosphorylated PK428 is indicated with an arrow. Panel B, Autophosphorylation of PK428 in vitro. PK428 was immunoprecipitated from transfected COS 7 cells with the 2188 antiserum (lanes 1–7) or a preimmune serum as a control (lanes 8 and 9). The immunoprecipitated proteins were incubated in kinase buffer (see Materials and Methods) with \( ^{32} \text{P} \text{ATP} \) for the indicated time periods. This reaction was then run on an SDS-PAGE and the phosphorylated proteins identified by autoradiography. Panel C, phosphorylation of possible protein kinase substrates by PK428. Immunoprecipitation of transfected COS 7 cells was either carried out with the 2188 antisera (lanes 1–7) or a preimmune serum as a control (lane 6). The immunoprecipitates were either added to the cyclic AMP fusion protein expressed as a GST fusion protein (GST-TK, lanes 1–4) or GST alone (lane 5), or histone H1 (lanes 5 and 6). The incubation time is shown at the top. At the completion of the reaction, the products were analyzed by SDS-PAGE, and the phosphorylated substrates were identified by autoradiography. The phosphorylated GST-TK and histone H1 are indicated by arrows.

Isolation of the Human PK428 Gene and Its Chromosome Localization—Using oligonucleotide primers specific for a region of the 5'-untranslated region of the PK428 cDNA, nucleotides 870–1185, a 315-bp specific band was amplified from normal genomic DNA (data not shown). This confirmed the specificity and continuity of the selected cDNA sequences in genomic DNA, allowing the isolation of the PK428 gene by genomic PCR screening a gridded human P1 phage genomic library (Genome Systems). The corresponding human PK428 gene was isolated from this human P1 phage library (clone identities 11499 and 11500), verified by direct sequencing of the P1 DNA with the same primers, and then used to determine the location of the human gene by FISH. Both PK428 P1 phage clones localized to the same location, human 1q41–42 (Fig. 6). Previously, others have shown that this terminal region of the long arm of chromosome 1 is associated with certain forms of mental retardation (16) and with the possible location of a second human senescence loci (17). Of more interest, the gene(s) which is associated with Rippling muscle disease has also been localized to this same region of chromosome 1 (18).

DISCUSSION

A novel protein kinase whose kinase domain is highly related, but not identical, to the DM-PK has been isolated by the two-hybrid analysis using a 54-amino acid region of the GM-CSF α subunit. Regions outside of the protein kinase domain demonstrate little amino acid homology with the DM-PK, although a short hydrophobic stretch of amino acids in the carboxyl-terminal portion of the protein is also conserved. In ad-
dition to these sequence differences between the DM-PK and PK428 protein kinases, additional data demonstrate that the PK428 protein is unique. The PK428 protein kinase described here has a molecular mass of 65 kDa and undergoes autophosphorylation as well as phosphorylating histone H1 and a cyclic AMP-dependent protein kinase substrate.

In the addition to the 65-kDa form of the PK428 protein, a 70–80-kDa protein was immunoprecipitated from breast and hematopoietic cells. The myotonic dystrophy protein kinase is 71–80 kDa in size (26), and it is possible that the PK428 antibody detects this protein as well. Furthermore, the PK428 antibody detects a 200-kDa protein in U937 cells. This larger protein could be another member of the DM-PK family. Recently, a 160-kDa protein with significant homology to the DM-PK kinase domain, p160ROCK, has been shown to interact with the small GTP-binding protein Rho, suggesting that there are larger members of this family of protein kinases (8–10). It is also possible that this 200-kDa protein is derived by alternative splicing of the PK428 gene, or that it corresponds to a PK428-related gene and protein. In fact alternative splicing of the DM-PK gene has been reported (27). The identity of these PK428-related mRNAs may be revealed by analysis of its corresponding gene and/or related genes.

Protein kinase activity of the PK428 protein was demonstrated by immunoprecipitation of PK428 produced by transient transfection. This kinase activity stimulated autophosphorylation and demonstrated that PK428 protein is phosphorylated in vivo. This PK428 protein kinase is also capable of phosphorylating a substrate for the cyclic AMP-dependent protein kinase and histone H1. However, we have not eliminated the possibility that another protein that coimmunoprecipitates with this protein kinase is responsible for some of the effects we have described. Although difficult to quantify, the phosphorylation of cyclic AMP-dependent protein kinase substrate that was seen with the PK428 immunoprecipitates was relatively weak when compared with the catalytic subunit of the cyclic AMP-dependent kinase (data not shown). These results suggest that “better,” more physiological, substrates for this protein kinase may exist.

Using FISH we have mapped this gene to human chromosome 1q41–42. A number of diseases have been localized to this region, including Usher’s syndrome type IIa, which is associated with hearing loss, and retinitis pigmentosa (28), a syndrome of mental retardation associated with trisomy 1q42 (16), arrhythmogenic right ventricular cardiomyopathy (29), and a Ripping muscle disease gene (18). Because of the similarity of PK428 with the DM-PK it is interesting to speculate that PK428 gene may also encode a protein which has an important role in muscle physiology.

Overexpression of the DM-PK has been shown to induce a skeletal muscle phenotype in BC3H1 cells (30). The neuromuscular junction location of the DM-PK protein (31) also suggests that it may be involved in signaling integrating extracellular events to cytoskeleton and the nucleus. Mutations in either the N. crassa Cot-1 or the D. melanogaster warts genes, which are related to DM-PK, result in abnormal cell growth and changes in cell morphology, supporting a possible role for this protein kinase in cytoskeletal morphology and signal transduction to the nucleus. In addition, the Rho protein regulates stress fibers and focal contacts in cells, presumably through its interaction with a protein kinase that shares sequence homology with the DM-PK. These signals appear to help to control cell shape. Furthermore, the DM-PK-like protein kinase that binds to Rho, p160ROCK, may coordinate responses to specific external stimuli such as lysosphosphatidic acid (32). Thus, it would appear that this family of protein kinases regulates responses to external stimuli that are involved in potential changes of the actin cytoskeleton and, subsequently, cell morphology. Identification of PK428 by two-hybrid interactive cloning, using the short intracytoplasmic tail of the GM-CSF α subunit as a bait, suggests that this protein kinase may be relevant to GM-CSF α subunit binding. The observation that deletion of the proline-rich segment or mutation of specific prolines to glycines blocks this interaction (data not shown) suggests that PK428 binds to a portion of this receptor, which is critical for hematopoietic signaling.

The addition of GM-CSF α subunit to hematopoietic cell lines induces marked changes in cell shape and changes in locomotion, cell division, and differentiation. However, although this PK428 protein kinase was isolated due to its ability to bind to the intracytoplasmic tail of the GM-CSF α subunit in yeast, demonstration of a strong interaction (i.e. coimmunoprecipitation) between the two proteins in hematopoietic cells has proven to be difficult. The exact role of PK428, if any, in hematopoietic signaling must await further analysis.

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