previously identified consensus motifs for ATP binding in DG kinase are not critical for nucleotide binding.

The question now remains where the true ATP-binding site(s) in 86 kDa DG kinase are located. Recently, cDNA clones of new DG kinases were identified, based on homology with the conserved C-terminal domain of 86 kDa DG kinase. These include two mammalian DG kinases: a 90 kDa rat DG kinase [12] and a 110 kDa human DG kinase (D. Schaap, T. Takenawa and W. J. van Blitterswijk, unpublished work), a highly conserved DG kinase from *Caenorhabditis elegans* that was identified while sequencing its chromosome 3 [13], and two distantly related DG kinases from *Drosophila* [14,15]. Since we expect that sites for ATP binding are evolutionarily conserved, we compared the primary structures of these DG kinases. Figure 2 shows the alignments of the two above-described motifs of 86 kDa DG kinase with the 90 and 110 kDa kinases and the *C. elegans* DG kinase (motifs 1 and 3). The *Drosophila* DG kinases are not shown because they are more distantly related, and either lack the double cysteine repeat or have a poorly conserved cysteine repeat. A third conserved $GxGxxGx_7K$ motif was recognized (Figure 2, motif 2) that is also located in the conserved C-terminal domain, roughly 30 amino acids upstream from motif 3. Comparison of the three $GxGxxGx_nK$ motifs reveals that none of them contains an absolutely conserved lysine residue at the position previously found to be invariable for protein kinases. Of note, Goto and Kondo [12] suggested different lysine residues in the first (Lys-294) and third motif (Lys-560) of 90 kDa DG kinase (Figure 2) to be essential for activity, but these lysines are not conserved with other DG kinase family members. Apart from these lysine residues and the ones that we mutated, three of the depicted DG kinases contain other conserved lysine residues more proximal to the GxGxxG sequence (shaded in Figure 2). However, none of these lysines are conserved in the 110 kDa DG kinase. We therefore conclude that DG kinase, being a lipid kinase and not a protein kinase, does not contain an ATP-binding site with a similar motif to that identified in protein kinases.

Finally, we compared the amino acid sequences of DG kinases with those of other nucleotide-binding proteins [16], such as adenylate kinases, thymidine kinases, phosphoglycerate kinases, *ras* proteins, elongation factors, ATP synthases and myosin heavy chains. Such a comparison did not reveal other candidate ATP-binding sites in DG kinases. Therefore, it seems likely that DG kinases contain their own unique motif for ATP binding, which remains as yet unknown.

We thank Dr. H. L. Ploegh and Dr. R. L. van der Bend for advice. This work was supported by the Dutch Cancer Society.

Dick SCHAAP, José van der WAL and Wim J. van BLITTERSWIJK

Division of Cellular Biochemistry, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

1 Berridge, M. J. and Irvine, R. F. (1984) Nature (London) **313**, 315–321
2 Hokin, L. E. (1985) Annu. Rev. Biochem. **54**, 205–235
3 Nishizuka, Y. (1984) Nature (London) **308**, 693–698
4 Gulbins, E., Coggeshall, K. M., Langlet, C., Baier, G., Bonnefoy-Berard, N., Burn, P., Wittinghofer, A., Katzav, S. and Altman, A. (1994) Mol. Cell. Biol. **14**, 906–913
5 Van Blitterswijk, W. J., Schaap, D. and van der Bend, R. (1994) Curr. Top. Membr. **40**, 413–437
6 Schaap, D., de Widt, J., van der Wal, J., Vandekerckhove, J., van Damme, J., Gussow, D., Ploegh, H. L., van Blitterswijk, W. J. and van der Bend, R. L. (1990) FEBS Lett. **275**, 151–158
7 Sakane, F., Yamada, K., Kanoh, H., Yokoyama, C. and Tanabe, T. (1990) Nature (London) **344**, 345–348
8 Goto, K., Watanabe, M., Kondo, H., Yuasa, H., Sakane, F. and Kanoh, H. (1992) Mol. Brain Res. **16**, 75–87
9 Hanks, S. K., Quinn, A. M. and Hunter, T. (1988) Science **241**, 42–52
10 Taylor, S. S., Knighton, D. R., Zheng, J., Ten Eyck, L. F. and Sowadski, J. M. (1992) Annu. Rev. Cell Biol. **8**, 429–462
11 Schaap, D., van der Wal, J., van Blitterswijk, W. J., van der Bend, R. L. and Ploegh, H. L. (1993) Biochem. J. **289**, 875–881
12 Goto, K. and Kondo, H. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 7598–7602
13 Wilson, R., Ainscough, R., Anderson, K., et al. (1994) Nature (London) **368**, 32–38
14 Masai, I., Hosoya, T., Kojima, S. and Hotta, Y. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 6030–6034
15 Masai, I., Okazaki, A., Hosoya, T. and Hotta, Y. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 11157–11161
16 Saraste, M., Sibbald, P. R. and Wittinghofer, A. (1990) Trends Biochem. Sci. **15**, 430–434

Received 11 July 1994

Identification of a new protein family which includes bovine protein kinase C inhibitor-1

A new protein family has been proposed in diverse eukaryotic and prokaryotic species, including mammals, plants, mycoplasmas, eubacteria and cyanobacteria The sequences for this family share regions which encode for a conserved novel zinc-binding site.

Bovine PKCI (Protein Kinase C Inhibitor-1) has been studied extensively by the group of M. P. Walsh. The bovine PKC inhibitor has an IC_{50} of 2.2 μM (McDonald and Walsh, 1985) and was isolated as a dimeric zinc-binding heat-stable protein (Pearson et al., 1990). Analysis of a synthetic peptide corresponding to the histidine-rich region of bovine PKCI (QSVYHVHLHVLGGRQ, in single-letter code for amino acids; Mozier et al., 1991) identified the zinc-binding site as HVHLH. A similar synthetic peptide (GHGHGHG) binds zinc in a 1:1 ratio (Iyer et al., 1981). The motif in this protein, where histidine residues alternate with hydrophobic residues (except at two positions) is distinct from any corresponding site on other known zinc-binding proteins (see Figure 1).

In certain neurons, noradrenaline is thought to activate PKC, causing the inhibition of voltage-dependent Ca^{2+} current. A patch-clamp technique has been used to introduce PKCI into the cell bodies of embryonic-chick sensory neurons (Rane et al., 1989). In that study, the inhibition of Ca^{2+} current was abolished, providing evidence *in situ* that PKCI inhibits PKC. However, in a study of the relative contribution of endogenous heat-labile and heat-stable proteins to the total kinase C inhibitor activity in mammalian brain, Fraser and Walsh (1991) reached the conclusion that mammalian PKCI may not be a physiological regulator of this kinase.

Using a probe for the 70 kDa protein component of the U1 ribonucleoprotein particle (Theissen et al., 1986), Simpson and co-workers analysed a λgt11 cDNA expression library of *Zea mays* (maize) A619 endosperm. A maize gene was cloned, sequenced and found to have high similarity to bovine PKCI (Simpson et al., 1994).

We have expressed this protein in *Escherichia coli* and shown that, like the bovine protein, it binds zinc. Antisera raised against bovine PKCI by the group of M. P. Walsh recognize the maize protein (K. Robinson and A. Aitken, unpublished work). However, it has very little activity as an inhibitor of mammalian brain PKC, although in the presence of another kinase C inhibitor protein, 14-3-3 (Robinson et al., 1994), the effects on PKC appear to be synergistic (K. Robinson and A. Aitken, unpublished work). The biological role for the maize protein in plant kinase regulation is therefore unclear.

Database searches have revealed a further match between PKCI and an open reading frame (ORF1) upstream from a gene

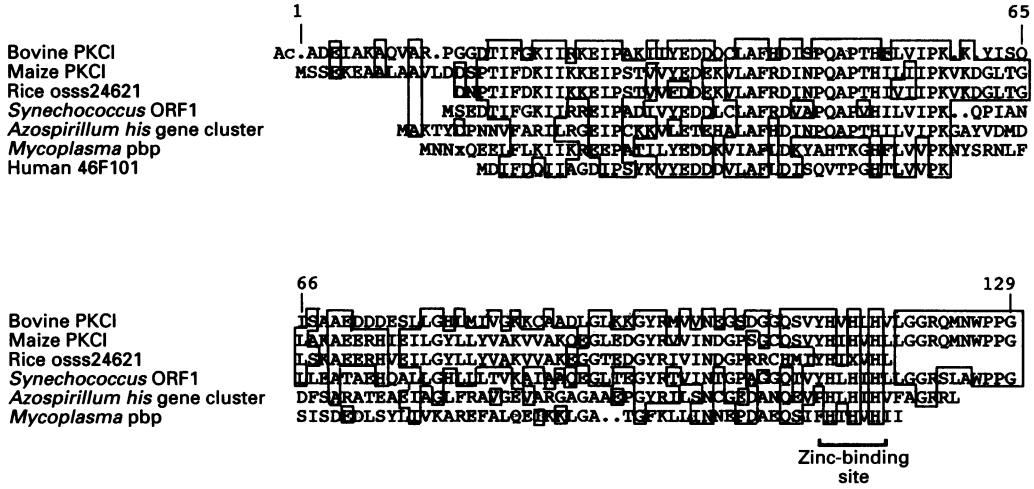

Figure 1 Alignment of proteins containing the novel zinc-binding domain

Bovine PKCI has been sequenced from the protein and is N-acetylated (Pearson et al., 1990). Maize PKCI amino acid sequence was deduced from the cDNA sequence. Rice osss24621 amino acid sequence is incomplete, and was translated from mRNA as follows: residues 15–82 use reading frame 3, residues 83–88 use reading frame 1 and residues 89–113 use reading frame 2. This shifting reading frame was presumably due to errors in DNA sequencing. *Synechococcus* ORF1 is the deduced amino acid sequence for an open reading frame upstream from the *psb*A gene cluster in the cyanobacterium *Synechococcus* sp. *Azospirillum his* gene cluster amino acid sequence is derived from an open reading frame downstream from a cluster of genes encoding histidine synthesis in nitrogen-fixing bacteria. *Mycoplasma* pbp is translated from a DNA sequence upstream from an operon which encodes proteins involved in periplasmic binding-protein-dependent transport. 'X' denotes a probable sequencing error. Human 46F101 is the amino acid sequence from an expressed sequence tag human DNA fragment. Identical residues are boxed. The zinc-binding site is emphasized.

(*psb*AII) encoding an integral thylakoid protein D1 of the photosystem II reaction centre in the cyanobacterium *Synechococcus* strain PCC 7942 (Bustos et al., 1990). The *psb*A genes encode two very similar forms of the D1 protein; *psb*AI codes for form I, and form II is the product of both *psb*AII and *psb*AIII. Although the level of a 1.6 kb *psb*AII transcript is unaffected by light intensity, 1.2 kb transcripts of the three genes respond as follows: *psb*AI mRNA level increases at low light intensity, and both *psb*AII and *psb*AIII mRNA are increased at high light intensity (Bustos et al., 1990). D1 protein may therefore serve a function in adaptation to varying light intensity (Schaefer and Golden, 1989). The function(s) of the ORF1 gene product is unknown. However, *Synechococcus* strain AMC084 in which the 1.6 kb transcript and ORF1 had been inactivated grew more slowly than the wild-type strain (Bustos et al., 1990). Cyanobacteria exhibit the oxygen-evolving chloroplast type of photosynthesis that is found in plants, and are thought to have given rise to chloroplasts via an endosymbiotic relationship with a eukaryotic organism (Aitken, 1976). The cyanobacterial gene may therefore code for a protein with a similar function to that of the plant protein.

Our further searches of the EMBL database show recent acquisitions of more sequences with similarity to these. This includes rice (*Oryza sativa*) mRNA (accession no. D21291; filename osss24621) coding for a protein of unknown function, but which is very similar to the maize sequence.

In the nitrogen-fixing bacterium *Azospirillum brasilense* a 4.5 kb DNA fragment has been identified which encodes the histidine-biosynthetic genes. This fragment contains an open reading frame (ORF2) that has similarity to PKCI, and which is downstream from *hisB*, *hisH*, ORF1, *hisF* and *hisE* genes but upstream from the translational terminator of this gene cluster (Fani et al., 1989). Although ORF2 is located near these histidine-synthesizing genes, its function is as yet undetermined. Since ORF2 contains the conserved zinc-binding site, one could speculate that its function may be to regulate histidine (and so zinc-binding-protein) synthesis when intracellular zinc concentrations are high.

Mycoplasma hyorhinis contains a putative operon encoding p37, p29 and p69 proteins (Dudler et al., 1988). When fibroblast cell lines are infected with this mycoplasma, p37 is expressed on the cell surface and may increase fibroblast invasivity (Schmidhauser et al., 1990). Both p29 and p69 are similar to proteins of the periplasmic binding-protein-dependent multicomponent transport systems for Gram-negative bacteria. An open reading frame upstream from the p37 gene codes for a protein with similarity to PKCI and which retains the zinc-binding domain. This open reading frame could code for an as yet unidentified protein which has a metal-transporting function in the periplasmic binding-protein-dependent transport system. A partial *Homo sapiens* cDNA from an expressed sequence tag (accession no. HSA46F101) has similarity to the N-terminus of PKCI. The function of this sequence in humans is unknown, but may be the human equivalent of bovine PKCI.

Although the physiological role(s) for this diverse family is not yet clear, the conserved zinc-binding site may provide a clue to a common functionality.

Karen ROBINSON and Alastair AITKEN

Laboratory of Protein Structure, National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

Aitken, A. (1976) Nature (London) **263**, 793–796
Bustos, S. A., Schaefer, M. R. and Golden, S. S. (1990) J. Bacteriol. **172**, 1998–2004
Dudler, R., Schmidhauser, C., Parish, R. W., Wettenhall, R. E. H. and Schmidt, T. (1988) EMBO J. **7**, 3963–3970
Fani, R., Bazzicalupo, M., Damiani, G., Bianchi, A., Schipani, C., Sgaramella, V. and Polsinelli, M. (1989) Mol. Gen. Genet. **216**, 224–229
Fraser, E. D. and Walsh, M. P. (1991) FEBS Lett. **294**, 285–289
Iyer, K. A., Laussac, J.-P. and Sarkar, B. (1981) Int. J. Pept. Protein Res. **18**, 468–477
McDonald, J. R. and Walsh, M. P. (1985) Biochem. Biophys. Res. Commun. **129**, 603–610

Mozier, N. M., Walsh, M. P. and Pearson, J. D. (1991) FEBS Lett. **279**, 14–18
Pearson, J. D., DeWald, D. B., Mathews, W. R., Mozier, N. M., Zurcher-Neely, H. A., Heinrikson, R. L., Morris, M. A., McCubbin, W. D., McDonald, J. R., Fraser, E. D., Vogel, H. J., Kay, C. M. and Walsh, M. P. (1990) J. Biol. Chem. **265**, 4583–4591
Rane, S. G., Walsh, M. P., McDonald, J. R. and Dunlap, K. (1989) Neuron **3**, 239–245
Robinson, K., Jones, D. H., Patel, Y., Martin, H., Madrazo, J., Martin, S., Howell, S., Elmore, M., Finnen, M. and Aitken, A. (1994) Biochem. J. **299**, 853–861
Schaefer, M. R. and Golden, S. S. (1989) J. Biol. Chem. **264**, 7412–7417
Schmidhauser, C., Dudler, R., Schmidt, T. and Parish, R. W. (1990) J. Cell Sci. **95**, 499–506
Simpson, G. G., Clark, G. and Brown, J. W. S. (1994) Biochim. Biophys. Acta **1222**, 306–308
Theissen, H., Etzerodt, M., Reuter, R., Schneider, C., Lottspeich, F., Argos, P., Luhrmann, R. and Philipson, L. (1986) EMBO J. **5**, 3209–3217

Received 3 August 1994