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FOR THE RECORD

Developmentally expressed myosin heavy-chain kinase possesses a diacylglycerol kinase domain

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Abstract: In *Dictyostelium*, an ordered actin and myosin assembly–disassembly process is necessary for proper development, differentiation, and motility (Yumura S, Fukui F, 1985, *Nature 314*(6007):194–196; Ravid S, Spudich JA, 1989, *J Biol Chem 264*(25):15144–15150), and phosphorylation of myosin heavy chains has been implicated in the myosin assembly–disassembly process (Egelhoff TT, Lee RJ, Spudich JA, 1993, *Cell 75*(2):363–371). The developmentally expressed 84-kDa myosin heavy-chain kinase (MHCK) from *Dictyostelium* (Ravid S, Spudich JA, 1992, *Proc Natl Acad Sci USA 89*(13):5877–5881) is known to be a member of the protein kinase C (PKC) family. We have observed a rather striking homology between the large central domain of MHCK and the catalytic domain of diacylglycerol kinase (DGK), indicating that MHCK is in fact a gene fusion between a DGK and a PKC, possessing two separate kinase domains. The combined diacylglycerol kinase/myosin heavy-chain kinase (DGK/MHCK) may therefore have dual functionality, possessing the ability to phosphorylate both protein and lipid. We present a hypothesis that DGK/MHCK can antagonize both actin and myosin assembly, as well as other cellular processes, by coordinated down regulation of signaling via myosin heavy-chain kinase activity and diacylglycerol kinase activity.

Keywords: cell motility; dual specificity kinase; lipid-binding domain; myosin assembly–disassembly; protein kinase c

In the *Dictyostelium* growth cycle, developing cells migrate toward the chemoattractant cAMP via pseudopod formation (Gerisch & Malchow, 1976). The aggregated cells then develop into a pseudoplasmodium and behave as a single unit, undergoing further development. cAMP stimulation of movement is associated with increased intracellular diacylglycerol concentration (Janssens & Van Haastert, 1987) and reorganization of myosin II (conventional myosin) (Yumura & Fukui, 1985; Egelhoff et al., 1993). Myosin is transiently recruited from a soluble pool into cytoskeletal filaments and, after the cAMP response, myosin thick filaments disassemble back into the soluble pool (Yumura & Fukui, 1985).

The myosin reorganization process in *Dictyostelium* appears to be regulated by phosphorylation (Ravid & Spudich, 1989; Egelhoff et al., 1993). Myosin heavy-chain (MHC) phosphorylation inhibits filament formation. Two MHCKs have been purified and cloned from *Dictyostelium*, a 130-kDa isozyme (termed MHCK A) (Futey et al., 1995) and an 84-kDa isozyme (MHCK) that is homologous to the protein kinase c family (Ravid & Spudich, 1989). MHC truncation analysis indicates that both kinases phosphorylate within the same 34-kDa region (Egelhoff et al., 1993), and the phosphorylation sites for the MHCK A isozyme have been mapped to three threonines on MHC (Vaillancourt et al., 1988; Luck et al., 1990). Mutation of the three phosphorylated threonine residues to aspartate was shown to inhibit the ability of MHC to assemble into filaments and to drive contraction events in vivo (Egelhoff et al., 1993). In contrast, mutation of those same residues to alanine causes myosin thick-filament over-assembly in vivo, presumably because the mutant myosin lacks the ability to become phosphorylated (Egelhoff et al., 1993). MHCK is expressed only in developing *Dictyostelium*, whereas MHCK A is expressed in both developing and growth phase cells (Ravid & Spudich, 1992; Futey et al., 1995).

Diacylglycerol kinase (DGK) phosphorylates diacylglycerol, converting it into phosphatidic acid. There are eight known eukaryotic DGKs, ranging from 80 to 90 kDa. Most members of this family share a number of conserved domains, including an N-terminal EF-hand-like calcium-binding motif, a zinc-coordinating lipid-binding motif, and a C-terminal catalytic domain (Kanoh et al., 1993). The catalytic domain consists of approximately 350 residues and is solely responsible for diacylglycerol kinase activity (Kanoh et al., 1993; our unpubl. data). Although the first members of the DGK family were cloned only recently, there are already hints that these enzymes play a central role in many cell-signaling events. For example, in *Drosophila*, DGKs are known to show temporal and spatial patterns of expression during development (Masai et al., 1992; Harden et al., 1993). There is also evidence that DGKs are playing an important role

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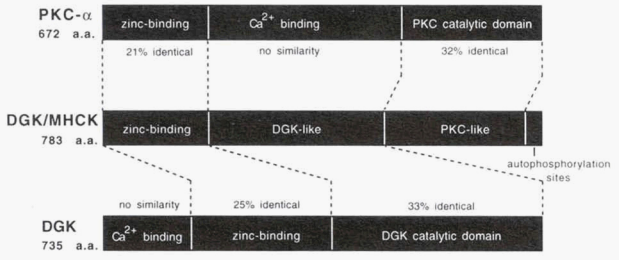

Fig. 1. Domain structure of DGK/MHCK. Regions of DGK/MHCK (Swiss Protein databank number P34125) were aligned to regions of PKC-α (Swiss Protein databank number P05696) or human DGK (Swiss protein databank number P23743). The zinc-binding domains were defined as ranging from the first zinc-coordinating residue in the first zinc-binding domain to the last zinc-coordinating residue in the second zinc-binding domain. By this definition, the zinc-binding domains consist of residues 38–151 in PKC-α, residues 18–139 in DGK/MHCK, and residues 206–319 in DGK. The PKC catalytic domain in PKC-α and the PKC-like domain in DGK/MHCK were defined by Ravid and Spudich (1989) and consist of residues 337–511 in PKC-α and residues 458–768 in DGK/MHCK. The catalytic domain of human DGK has been defined experimentally by deletion analysis and consists of residues 361–735 (unpubl. data). The DGK-like domain of DGK/MHCK was defined by sequence alignment of the human DGK catalytic domain with DGK/MHCK, excluding the PKC-like domain. By this definition, the DGK-like domain of DGK/MHCK extends from residues 193 to 457. Alignments were performed using the GAP program from the Genetics Computer Group (Madison, Wisconsin) using default parameters.

in oncogenic transformation. NIH/3T3 cells transformed with *ras* oncogenes all showed a significant decrease in DGK translocation to the membrane, resulting in an increased amount of DAG with consequent increased activation of PKCs (Kato et al., 1988). Confirmed regulators of individual DGKs include calcium and phosphatidylserine (Sakane et al., 1990, 1991) and there is evidence that their activity is modulated by epidermal growth factor receptor, cAMP-dependent protein kinase, and PKC (Schaap et al., 1990). Specific inhibitors of DGK activity have been shown to produce a wide variety of effects in many different cell types (Kanoh et al., 1990).

We have found that the catalytic domain of DGK shows high sequence similarity to the large central domain of the 84-kDa MHCK and we will therefore refer to the protein as DGK/MHCK in the following discussion. As outlined in Figure 1, we believe DGK/MHCK is composed of three distinct domains. (1) Ravid and Spudich (1992), upon cloning and sequencing DGK/MHCK from *Dictyostelium*, found a PKC-like catalytic domain at the C-terminus that is consistent with the known protein kinase activity of DGK/MHCK. (2) The large central domain is related to the catalytic domain of DGK, which is conserved in all known eukaryotic DGKs. DGK/MHCK and the catalytic domain of the 84-kDa human DGK (Schaap et al., 1990) share 33% sequence identity. To test whether residues important for DGK activity tend to be more strongly conserved than other residues, we aligned the catalytic domains of seven known eukaryotic DGK homologues to the putative DGK catalytic domain of DGK/MHCK (Fig. 2). Of 168 positions that are conserved in seven other known DGK homologues, 121 are also conserved in the more distantly related DGK/MHCK central domain. Thus, 72% of the residues that are likely to be important for DGK activity are conserved in DGK/MHCK. (3) The N-terminal region of DGK/MHCK contains two cysteine-rich, zinc-binding domains (Ravid & Spudich, 1992) that are related to the zinc-binding domains found in the PKC family, the DGK family, unc-13, the proto-oncogenes *raf* and *vav*, and the GTPase-activating protein neuronal chimaerin (Ahmed et al., 1991; Ghosh et al., 1994). The role of these domains in DGK has not yet been defined, but many of these domains are known regulatory elements and bind diacylglycerol (DAG), phorbol esters, or phosphatidylserine (Ono et al., 1989; Ghosh et al., 1994; Quest & Bell, 1994). The overall sequence identity is low in this region; however, the domains can be identified easily by the conserved Zn^{2+}-coordinating Cys and His residues (Ahmed et al., 1991). Ravid and Spudich (1992) found that MHCK is not activated by DAG or PS, thus, this domain's function in MHCK may be to

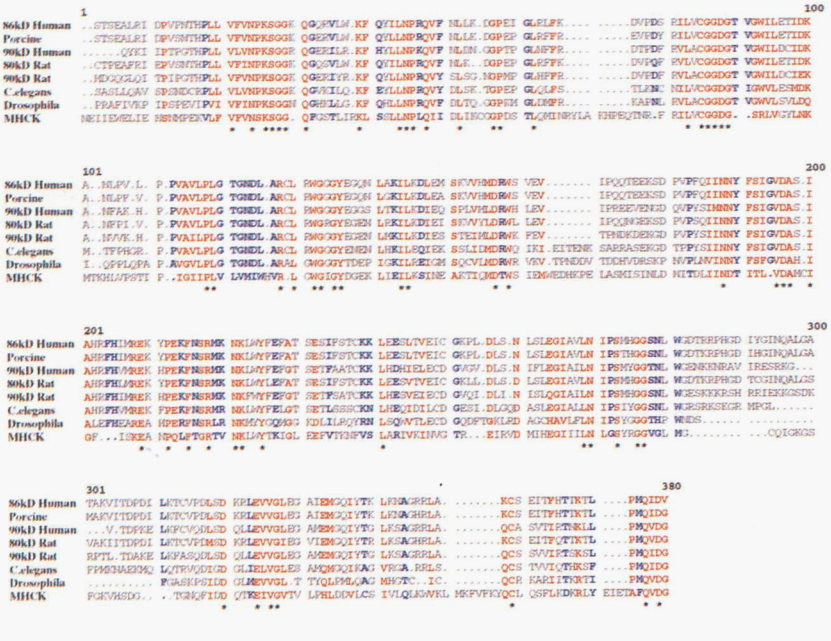

Fig. 2. Alignment of the catalytic domains of seven eukaryotic DGKs with the large central domain of MHCK. Alignments were performed using the program PILEUP from the Genetics Computer Group (Madison, Wisconsin). Positions that are conserved in all sequences are highlighted in red. Positions that are conserved in all sequences except DGK/MHCK are highlighted in blue. Positions that are invariant in all sequences are indicated by asterisks. A position was considered conserved in the multiple alignment if the PAM250 matrix score (Dayhoff & Schwartz, 1979) for every possible pair of residues at the position was greater than zero.

regulate the DGK domain rather than the protein kinase domain.

A regulatory protein with combined lipid and protein kinase activities is not unprecedented. For example, PI-3 kinase regulates its lipid kinase activity via auto-phosphorylation of its regulatory subunit (Dhand et al., 1994). However, it differs from the DGK/MHCK in that both kinase activities appear to originate from the same active site, whereas DGK/MHCK possesses a separate lipid kinase and protein kinase domain.

Why bundle both a diacylglycerol kinase and a protein kinase in the same polypeptide? As shown in Figure 3, combining both activities in a single protein would provide a simple way to coordinate the down regulation of cell motility in *Dictyostelium* in at least three ways. First, as stated above, the known protein kinase activity of DGK/MHCK may function to regulate myosin assembly-disassembly. Second, cAMP stimulation results in the generation of DAG (Janssens & Van Haastert, 1987), and it is now well accepted that most PKC isotypes are activated by the second messenger DAG (Nishizuka, 1992). The putative DGK activity of DGK/MHCK, converting DAG to phosphatidic acid, could attenuate cellular processes related to cell motility that are activated by PKC (Kanoh et al., 1990, 1993; Sakane et al., 1990, 1991; Schaap, et al., 1993). Finally, DAG has recently been implicated in the formation of actin nucleation sites by a process that is independent of PKC (Shariff & Luna, 1992). DGK activity could, therefore, play a role in antagonizing actin nucleation and thus actin assembly. In a related observation, a DGK isozyme was recently cloned that is localized in cerebellar Purkinje cells and is associated with the cytoskeleton (Goto et al., 1994).

The high degree of sequence identity between the 86-kDa DGK and the 84-kDa MHCK allows us to hypothesize that MHCK has a DGK activity. Because we have been unable to obtain the clone for DGK/MHCK, it has not been possible to verify the presence of DGK activity biochemically or to investigate the role of this domain in *Dictyostelium* development. Thus, it remains possible that the DGK catalytic domain in DGK/MHCK has been recruited for some unknown function. Nevertheless, our hypothesis can be readily tested experimentally. The biochemical demonstration of a DGK activity in a regulator of cell motility would add yet another role to the growing panoply of cellular functions that are regulated by DGK isozymes.

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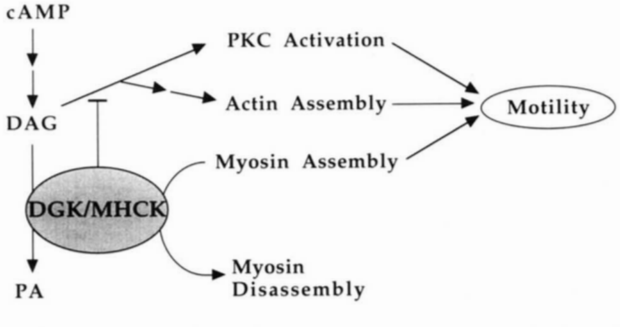

Fig. 3. A hypothetical model for coordinated regulation of cell motility by DGK/MHCK.

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