Polarization of Myosin II Heavy Chain-Protein Kinase C in Chemotaxing Dictyostelium Cells*

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Eukaryotic cells need morphological polarity to carry out chemotaxis (Parent, C. A., Blacklock, B. J., Froehlich, W. M., Murphy, D. B., and Devreotes, P. N. (1998) Cell 95, 81–91; Jin, T., Zhang, N., Long, Y., Parent, C., and Devreotes, P. N. (2000) Science 287, 1034–1036; Servant, G., Weiner, O. D., Herzmark, P., Balla, T., Sedat, J. W., and Bourne, H. R. (2000) Science 287, 1037–1040), but sensing direction does not require polarization of chemoattractant receptors. When cells are exposed to a gradient of chemoattractant, activation occurs selectively at the stimulated edge. Such localized activation, transmitted by the recruitment of cytosolic proteins, may be a general mechanism for gradient sensing by G protein-linked chemotactic systems. Here we show that in Dictyostelium discoideum cells exposed to a cAMP gradient the myosin II heavy chain kinase (MHC-PKC) and myosin II translocate to opposite ends of the cell. We further show that MHC-PKC C1 domain is responsible for the localization of MHC-PKC to the cell leading edge, but it is not sufficient to promote cell polarization. Our findings suggest a mechanism by which MHC-PKC regulates myosin II, allowing cell polarization and movement in the direction of the cAMP source.

CHEMOTAXIS IN EUKARYOTIC CELLS IS MEDIATED BY CHANGES IN THE ORGANIZATION AND FUNCTION OF CYTOSKELETAL STRUCTURES CONTAINING ACTIN AND MYOSIN II (6, 7). STUDIES ON THE ROLE OF MYOSIN II IN Dictyostelium discoideum CHEMOTAXIS SUGGEST THAT MYOSIN II MONOMERS UNDERGO TRANSIENT ASSEMBLY INTO BIPOLAR FILAMENTS THAT MAY PRECEDE RECRUITMENT INTO THE CYTOSKELETON AND THAT THE CYCLES OF MYOSIN II ASSEMBLY AND DISASSEMBLY MAY BE REGULATED BY PHOSPHORYLATION OF MYOSIN II HEAVY CHAIN (MHC)† (8, 9). INDEED, cAMP STIMULATION CAUSES MYOSIN II THAT EXISTS AS THICK FILAMENTS TO TRANSLATE TO THE CELL CORTEX. THIS TRANSLATION IS CORRELATED WITH A TRANSIENT INCREASE IN THE RATE OF MHC AS WELL AS LIGHT CHAIN PHOSPHORYLATION (8, 9). IN ADDITION, A NOVEL PROTEIN KINASE C (MHC-PKC), WHICH WE PURIFIED AND CLONED FROM D. DISCOIDEUM Cells exposing light chain phosphorylation (8, 9). In addition, a novel protein kinase C (MHC-PKC), which we purified and cloned from Dictyostelium discoideum cells, phosphorylates MHC in response to cAMP stimulation (10–12). In vitro phosphorylation of MHC by this kinase results in inhibition of myosin II thick filament formation (11, 13).

Morphological polarity is necessary for chemotaxis by eu-

Eukaryotic cells need morphological polarity to carry out chemotaxis (Parent, C. A., Blacklock, B. J., Froehlich, W. M., Murphy, D. B., and Devreotes, P. N. (1998) Cell 95, 81–91; Jin, T., Zhang, N., Long, Y., Parent, C., and Devreotes, P. N. (2000) Science 287, 1034–1036; Servant, G., Weiner, O. D., Herzmark, P., Balla, T., Sedat, J. W., and Bourne, H. R. (2000) Science 287, 1037–1040), but sensing direction does not require polarization of chemoattractant receptors. When cells are exposed to a gradient of chemoattractant, activation occurs selectively at the stimulated edge. Such localized activation, transmitted by the recruitment of cytosolic proteins, may be a general mechanism for gradient sensing by G protein-linked chemotactic systems. Here we show that in Dictyostelium discoideum cells exposed to a cAMP gradient the myosin II heavy chain kinase (MHC-PKC) and myosin II translocate to opposite ends of the cell. We further show that MHC-PKC C1 domain is responsible for the localization of MHC-PKC to the cell leading edge, but it is not sufficient to promote cell polarization. Our findings suggest a mechanism by which MHC-PKC regulates myosin II, allowing cell polarization and movement in the direction of the cAMP source.

Morphological polarity is necessary for chemotaxis by eukaryotic cells, but it does not require receptor polarization (5). Dictyostelium discoideum cells exposed to a cAMP gradient translocate myosin II in a gradient correlated manner, with the leading edge of the cell accumulating myosin II-GFP tagged with MHC-PKC and myosin II translocating to the posterior part of the cell. These findings indicate a mechanism whereby MHC-PKC and myosin II contribute to cell polarization and chemotaxis.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors—All DNA manipulations followed standard methods (20). A MHC-PKC-GFP expression plasmid was constructed as follows. A 1.8-kb fragment of MHC-PKC was isolated from pBluescript-MHC-PKC (12) by digestion with BglII-BamHI and cloned into pDxHy-7 (21) to create pDxH-MHC-PKC. To isolate the GFP, the plasmid pGFP10.1 containing the GFP cDNA (22) was digested with BamHI and cloned in frame in front of the MHC-PKC to create the expression vector pDxH-MHC-PKC-GFP. The pDxH-C1-GFP expression plasmid was created by restricting the pDxH-MHC-PKC-GFP with SspI and rendered blunt. This was followed by restriction with XhoI, resulting in the deletion of a 1.4-kb fragment from the 3′ prime. The expression plasmid was then ligated resulting in GFP fused in frame to a 0.4-kb fragment from the 5′ prime of MHC-PKC encoding the C1 domain. The expression plasmid pDxH-MHC-PKC-C1-GFP was created by restriction of pDxH-MHC-PKC-GFP with SspI and EcoRI rendered it blunt. This plasmid was ligated. The restriction resulted in the deletion of the C1 domain.

Growth of Dictyostelium Cells—Dictyostelium cells were grown in HL-5 medium (23), supplemented with 60 units of penicillin and 60 μg of streptomycin/ml at 21 °C. Transformations were performed using the calcium phosphate procedure as described previously (24). The mhc-pkc null cells were transformed with either pDxH-MHC-PKC-GFP, pDxHA-C1-GFP, or pDxH-MHC-PKC-C1-GFP to create mhc-pkc-gfp, c1-gfp, and c1-c1-gfp cell lines respectively. The myosin II null cell line HIS1 (25) was transformed with pBigGFPmyo to create myosin II-gfp cell lines (26). Transformed cells were selected and grown in the presence of 5 μg/ml (Geneticin, Invitrogen).

Cell Development—Amoeba of Dictyostelium discoideum strains were grown in HL-5 medium (23), harvested at a density of 2 × 10⁶ cells/ml, washed twice in MES buffer (20 mM MES, pH 6.8, 0.2 mM CaCl₂, 2 mM MgSO₄), and resuspended in MES buffer at a density of 2 × 10⁶ cells/ml. Cells were shaken at 100 rpm at 22 °C for 3.5 h. 5 μM caffeine was added to the suspension 30 min prior to the addition of cAMP.

Western Blot Analysis—Cells were developed for 4 h as described

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† The abbreviations used are: MHC, myosin II heavy chain; PKC, protein kinase C; GFP, green fluorescent protein; MES, 4-morpholineethanesulfonic acid.

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MHC-PKC Localization in Chemotactic Cells

FIG. 1. Western blot analysis of mhc-pkc null cells expressing MHC-PKC-GFP and C1-GFP, and myosin II null cells expressing myosin II-GFP fusion proteins. Extracts from the different cell lines were electrophoresed in 10% SDS-PAGE. The proteins were transferred to a nitrocellulose membrane and probed with antibodies specific to GFP.

above, washed in 10 mM Tris-HCl (pH 8.0) and 150 mM KCl, and lysed in 50 mM Tris-HCl (pH 8.0), 20 mM sodium pyrophosphate (pH 6.8), 5 mM EDTA, 5 mM EGTA, 0.5% Triton X-100, and protease inhibitor mix (Sigma). Protein concentration was determined by the method of Peterson (27), and the lysates were electrophoresed on SDS-PAGE (28). Western blots were probed with MHC-PKC polyclonal antibody (11) or with GFP antibody (Santa Cruz Biotechnology, Inc.). The blots were developed using a horseradish peroxidase-coupled secondary antibody (Bio-Rad). ECL was performed using a kit from Amersham Biosciences.

RESULTS AND DISCUSSION

To define the spatiotemporal dynamics of MHC-PKC that may lead to cell polarization and directed movement we expressed GFP-tagged MHC-PKC (MHC-PKC-GFP, Fig. 1) in mhc-pkc null cells (12). MHC-PKC-GFP rescued all mhc-pkc null cell defects. mhc-pkc null cells exhibit substantial myosin II overassembly in vitro, as well as aberrant cell polarization, chemotaxis, and morphological differentiation (12). However, expression of MHC-PKC-GFP resulted in cells showing similar chemotaxis and differentiation properties to wild-type cells (data not shown). These results indicate that tagging the MHC-PKC with GFP did not affect its properties.

To study the localization properties of MHC-PKC during chemotaxis toward cAMP, mhc-pkc-gfp cells were subjected to a cAMP gradient using a Zigmond chamber (29). The chemotactic response of mhc-pkc-gfp cells was observed at 5-s intervals 10 min after placing the coverslips containing cells in the chamber (Fig. 2). Cells freshly placed in the chamber (time, 0 s) were round, and the MHC-PKC-GFP was distributed evenly throughout the cytoplasm. As time progressed the cell sent lamellipodia toward the region of high cAMP concentration and became polarized. The localization of MHC-PKC was also altered together with the changes in cell morphology. In a cell first subjected to a cAMP gradient, the MHC-PKC-GFP was distributed evenly throughout the cytoplasm (Fig. 2, 0–20 s), but after 25–30 s it was concentrated at the leading edge of the cell. These results indicate that a cell moving up a cAMP gradient achieves cell polarization as well as polarization of MHC-PKC.

To study the localization properties of myosin II in chemo-taxing cells, we expressed myosin II-GFP in myosin II null cells (Fig. 1). Moores et al. (26) have shown that purified myosin II-GFP protein displays wild-type myosin II properties in vitro. Furthermore, expression of myosin II-GFP fully complements the myosin II null cell phenotype. To follow myosin II dynamics, myosin II-gfp cells were subjected to a cAMP gradient similar to that described above. As shown in Fig. 3, 30 s after exposing the cell to the cAMP gradient, the cell became polarized sending out lamellipodia along with thin filopodia toward the region of high cAMP concentration. At the same time most of the myosin II-GFP became localized to the posterior part of the cell. These results indicate that, in a cAMP gradient, Dicyostelium cells send their MHC-PKC-GFP and myosin II-GFP to opposite ends of the cell. These findings are consistent with experiments in polarized Dicyostelium cells in which immunofluorescent stained myosin II was found to be localized at the posterior part of the cell (30–32).

Expression of C1 from mammalian PKC results in translocation of this domain to the cell membrane (33). This observation together with biochemical evidence indicates that PKC binds to the cell membrane through its C1 domain (for reviews see Refs. 34 and 35). To define the MHC-PKC domain responsible for binding MHC-PKC to the cell membrane and thus enabling it to concentrate at the anterior part of chemotaxing cells, we expressed two MHC-PKC truncation proteins tagged with GFP in mhc-pkc null cells. The first protein was the C1 domain of MHC-PKC, and the second was MHC-PKC in which the C1 domain had been deleted (ΔC1-GFP) (Fig. 1). Both cell lines were subjected to a cAMP gradient in the Zigmond chamber (29) as described above. In contrast to MHC-PKC-GFP, which at first appeared diffusely distributed throughout the cytoplasm and then gradually concentrated at the leading edge of the cell (Fig. 2), the C1-GFP appeared as small spherical aggregates that concentrated at the cell region facing the cAMP high concentration (Fig. 4). These results suggest that similar to mammalian PKC, MHC-PKC binds to the cell membrane through its C1 domain. The ability of C1 domain to localize to the cell leading edge indicates that C1 domain possessed the information required for the localization of MHC-PKC to this region in a cAMP gradient. Even though C1 domain localized to the cell leading edge, the c1-gfp cells did not undergo cell

FIG. 2. Localization of MHC-PKC-GFP in live cells in a cAMP gradient. Chemotactic response of mhc-pkc-gfp cells was observed under Nomarski optic and the fluorescence microscope (see "Experimental Procedures"). Six Nomarski and fluorescent images were captured every 5 s, 10 min after the glass coverslips containing the cells were placed in the Zigmond chamber. Arrows indicate the position of the high cAMP concentration.
polarization or chemotaxis; this may be because of the absence of MHC-PKC catalytic domain that is required for cell polarization and chemotaxis (12).

Deletion of the C1 domain (i.e. ΔC1-GFP) also resulted in spherically shaped proteins, but in contrast to C1-GFP, the ΔC1-G distributed evenly throughout the cytoplasm regardless of cAMP stimulation (Fig. 5). These results further indicate that MHC-PKC concentrates at the cell membrane in response to cAMP stimulation using its C1 domain, and when the C1 domain is absent, the protein remains in the cytoplasm. Similar to c1-gfp cells, Δc1-gfp cells did not undergo cell polarization and chemotaxis; this can be because of the absence of C1 that is required for MHC-PKC binding to the cell membrane, a step necessary for MHC-PKC activation (36). The appearance of C1-GFP and ΔC1-GFP in aggregates may be because of the expression of truncated MHC-PKC proteins.

The results described above indicate that exposure of Dictyostelium cells to the cAMP gradient results in cell polarization and localization of MHC-PKC and myosin II to opposite sites of the cell. Furthermore, the MHC-PKC C1 domain is required for the localization of MHC-PKC to the cell leading edge of chemotaxing cell; however the C1 protein is not sufficient for cell polarization and chemotaxis. These results fit with our previous observations in which we biochemically followed the localization and activation of MHC-PKC and of myosin II stimulated with cAMP (36). Based both on these previous results and the current results, we suggest the following model for the involvement of MHC-PKC and myosin II in cell polarization and chemotaxis. The unstimulated cell is rounder in shape because of a contractile shell formed by an actin-myosin II network in the cortex. This network presumably inhibits events necessary for pseudopodial projection. cAMP stimulation of one edge of the cell results in activation of the cytosolic MHC-PKC, causing it to translocate to the membrane. Here it concentrates in an active form at the site of cAMP stimulation (i.e. the cell’s leading edge). Active MHC-PKC phosphorylates the cortical MHC at the anterior part of the cell, causing disassembly of myosin II thick filaments. The disassembled myosin II molecules reassemble at the posterior part of the cell, thus providing a force that moves the cell forward.
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