6 *Dictyostelium*

A Model Experimental System for Elucidating the Pathways and Mechanisms Controlling Chemotaxis

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1. INTRODUCTION

Chemotaxis, directed movement toward a chemoattractant agent, is involved in diverse biological responses, including wound healing in vertebrates, migration of tumor cells, axonal outgrowth to target cells, and aggregation leading to the formation of the multicellular organism in *Dictyostelium*. Chemotaxis is essential for the migration of polymorphonuclear leukocytes and macrophages to an inflammatory site. Amoeboid chemotaxis is also a key step in tumor metastasis, with morphological studies demonstrating that tumor cells exhibit leukocyte-like motility and psuedopod extension. The movement of cancer cells into and out of vascular channels has been shown to be stimulated and regulated by a number of cytokines, suggesting that chemotaxis is a key step in tumor metastasis. Axonal guidance in the developing nervous system may very well function via pathways that are analogous to chemotaxis of amoeboid cells. Higher concentrations of chemoattractant near target destinations of migratory neuroblasts appears to be involved in the regulation of the migration of neuroblasts while diffusible repulsive agents from a tissue may exclude axonal growth and help regulate the direction of the leading edge.

Chemotaxis is a fundamental process of cells and tissues and is based on the dynamics of the cytoskeleton. This process is activated by a variety of extracellular ligands. The binding of ligands to cell surface receptors leads to the directed reorganization of the actin and myosin cytoskeletons, pseudopod extension in the direction of the chemoattractant source, and cell movement via pathways that are thought to be highly conserved between mammals and *Dictyostelium. Dictyostelium* cells provide a powerful system to examine the role of cellular components to control coordinated cell movement because of the ability to apply genetic as well as cell biological approaches to study this evolutionarily conserved pathway. *Dictyostelium* cells, like neutrophils and macrophages, are motile and, in contrast to fibroblasts, lack stress fibers. In polymorphonuclear leukocytes, macrophages, and *Dictyostelium* cells, chemotaxis can be mediated through G protein-coupled cell surface receptors, and, in mammalian cells, the response is thought to function through G_i via release of Gβγ-subunits. Conserved signaling components involved in the regulation of chemotaxis in *Dictyostelium* and neutrophils is shown in Fig. 1.

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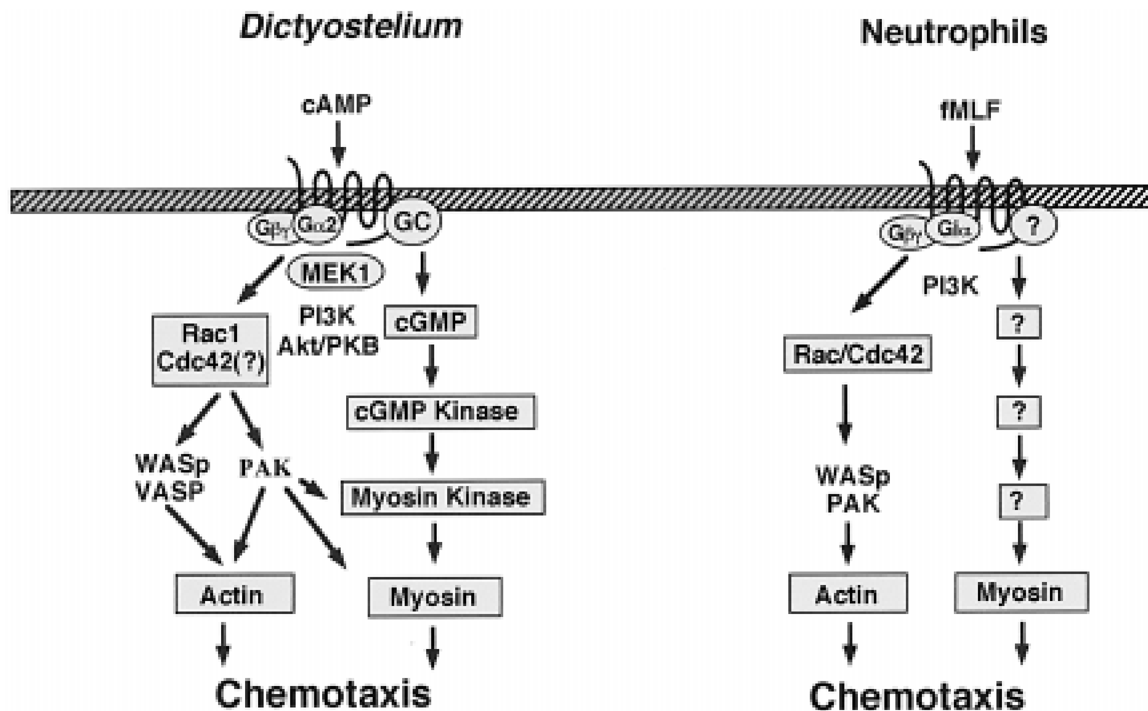

Fig. 1. Schematic diagram of signaling components involved in the regulation of chemotaxis in *Dictyostelium* and neutrophils. In both systems, chemotaxis can be mediated through G protein-coupled seven-transmembrane cell surface receptors. The binding of chemoattractants to the receptor activates a series of signaling events leading the reorganization of cytoskeleton. Directed reorganization of the actin and myosin cytoskeletons, pseudopod extension in the direction of the chemoattractant source, and cell movement via pathways are thought to be highly conserved between mammals and *Dictyostelium.*

Dictyostelium cells undergo a distinct developmental program in which up to 10^5 cells aggregate to form a multicellular organism. This is regulated by the chemoattractant cAMP, which is relayed from the center of an aggregation domain outward. Cells respond to the stimulus by chemotaxing up the cAMP gradient toward the cAMP source. This movement involves the activation of adenylyl cyclase and producing and secreting cAMP into the extracellular space, thus relaying the signal. Rises in intracellular cAMP, at the same time, activate PKA (cAMP-dependent protein kinase), whose function is also required for proper cell movement. Adaptation pathways make these responses transient so that a cell is responsive to the signal for only a short time. Cells closer to the aggregation center that have just responded to an outward moving wave of cAMP adapt and are not able to respond when cells produce and secrete cAMP. As a result, cells move inward while the cAMP wave moves outward, leading to the directionality of the cell movement. An extracellular phosphodiesterase hydrolyzes the cAMP, allowing cells to deadapt and become responsive to a new outward moving cAMP wave. At the height of aggregation, new cAMP waves are produced every 6 min. Approximately such 20 cycles are required to produce a mound.

After mound formation, the multicellular organism undergoes morphogenesis and cell-type differentiation, which is also controlled by extracellular cAMP functioning through the same receptor, can mediate both aggregation-stage pathways and those required for multicellular differentiation. Thus, for *Dictyostelium*, chemotaxis is an essential component of the developmental pathway. As such, it provides an excellent biological system for identifying genes required for chemotaxis, as such genes would be defective in the ability to form aggregates and thus are easily identified in mutant screens. Most of the analysis of gene function described in the following sections results from the physiological and biochemical analysis of null mutants created by homologous recombination. Through gene discovery and the analysis of gene function in *Dictyostelium*, significant progress has been achieved that is directly applicable to understanding these processes in humans. These findings provide an appropriate basis for understanding disease states.

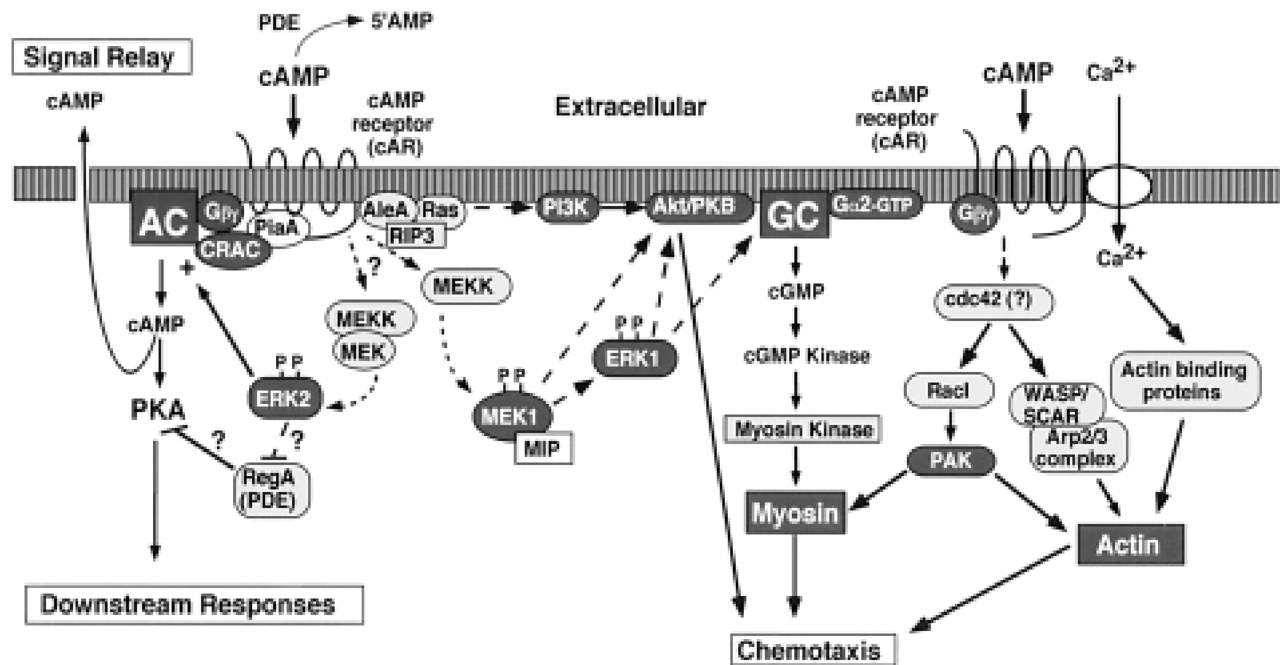

Fig. 2. Scheme of signal transduction involved in *Dictyostelium* chemotaxis. Binding of cAMP to the cell surface receptor induces **(1)** activation of adenylyl cyclase (ACA) resulting in the formation of cAMP for relay of the signal. cAMP activates cAMP-dependent kinase (PKA) leading cellular responses such as chemotaxis and developmental gene expression. The activation of ACA requires Gβγ, CRAC, Pianissimo (PiaA), and ERK2 activity. **(2)** Activation of guanylyl cyclase (GC) leading the formation of cGMP and the activation of cGMP-dependent kinase. Myosin II light and heavy chain kinases are regulated by cGMP, which regulates cytoskeletal organization during chemotaxis and morphogenesis. **(3)** Activation of Ras family proteins. Ras appears to activate PI3 kinases resulting in the activation of Akt/PKB which is required for the regulation of cytoskeleton and polarity during chemotaxis. Activation of Cdc42/Rac leads the change of cytoskeletal organization via WASP regulating F-actin organization and PAK controlling myosin II filaments assembly. **(4)** Uptake of Ca^{2+} across the plasma membrane. Activities of many actin crosslinking and binding proteins are regulated by the Ca^{2+} concentration.

2. SIGNALING COMPONENTS IN THE REGULATION OF CHEMOTAXIS

cAMP functions through the interactions of serpentine, G protein-coupled receptors designated cARs. cAMP binding results in the activation of a series of signaling pathways, including: activation of adenylyl cyclase, which produces the cAMP signal; guanylyl cyclase, which mediates chemotaxis; Akt/PKB; phospholipase C; Ca^{2+} influx; and gene expression. Regulation of signaling components and interactions between them are illustrated in Fig. 2. Transduction of the chemotactic signal to second messengers induces rearrangement of cytoskeletal components, including the polymerization of actin and myosin II and the relocalization of myosins. The first step in the directed movement of cells toward a chemotactic source involves the extension of pseudopodia initiated by the focal nucleation and polymerization of actin at the leading edge of the cell, which includes many actin-binding proteins. Myosin II is also assembled into filaments and relocalizes to posterior cortex. In this chapter, components involved in the transduction of chemotactic signals and their regulation is discussed. Changes and regulation of cytoskeletal components during chemotaxis are also reviewed.

2.1. Chemoattractant Receptors

Dictyostelium and neutrophils control chemotaxis through the activation of seven-transmembrane-domain G protein-coupled cAMP receptors. During the aggregation stage, chemotaxis that mediated the formation of the multicellular organism is controlled through cAMP receptors (cARs). Four cARs have been identified whose expression levels are tightly regulated throughout development. These receptors are approx 40% identical and 60% similar in sequence and share a great deal of homology within transmembrane and loop domains with metazoan G protein-coupled receptors. In addition to their expression pattern, the individual receptors also differ in their bind-

ing constant for cAMP. cAR1, which is preferentially expressed during aggregation and is required *in vivo* for aggregation-stage pathways, has the highest affinity and functions as the major receptor during aggregation and for the initial responses in the mound. *car1* null strains obtained by disrupting the cAR1 gene by homologous recombination do not aggregate normally but do show some response to cAMP due to the low level of expression of cAR3 at this stage. cAR3 is then maximally expressed during tight aggregate formation. cAR1 and cAR3 thus have partially overlapping functions. cAR2 and cAR4 are expressed in the prestalk cells in the multicellular stage of development program and appear to regulate pattern formation and cellular differentiation by controlling the expression of prestalk genes.

2.2. Heterotrimeric G Proteins

Early studies showing that the affinity of cAMP receptor was altered by guanine nucleotides and that cAMP increased GTPase activity strongly suggested the presence of heterotrimeric G proteins. Gα2 is a key player, as a null mutant of Gα2 does not aggregate. The *gα2* null mutant is unable to activate adenylyl or guanylyl cyclases and Akt/PKB, shows no chemotaxis, and does not respond to extracellular cAMP. Moreover, biochemical analyses strongly suggest that Gα2 may be the only Gα-subunit that interacts with any of the cAMP receptors. Seven other Gα-subunits have been identified and cloned, and deletion of most of these Gα-subunits results in subtle phenotypes. However, *gα4* null cells do not chemotax toward folic acid, the chemoattractant used by *Dictyostelium* cells during growth and early develop to identify and locate food (bacteria). *gα4* null cells also exhibit developmental phenotypes and the gene is expressed from two promoters: one expressed during growth and one expressed during the mound stage. A single Gβ-subunit has been identified and the deletion of gene encoding Gβ results in the inability of cells to chemotax to any chemoattractant and to activate adenylyl and guanylyl cyclases and Akt/PKB. It is expected that the deletion of the Gβ-subunit would abrogate all signal transduction via heterotrimeric G proteins.

2.3. Activation and Regulation of Adenylyl Cyclase

The aggregation-stage *Dictyostelium* adenylyl cyclase (ACA) has a similar structured to that of mammalian adenylyl cyclases. cAMP levels are controlled by the activation of ACA in response to receptor stimulation and its subsequent degradation extracellularly by a membrane-associated and -secreted cyclic nucleotide phosphodiesterase (PDE) and intracellularly by a cAMP-dependent PDE, RegA, which is a two-component system (histidine kinase) response regulator. The activation of ACA by extracellular cAMP is mediated through cAR1 acting through the G protein containing the Gα2-subunit. Cells lacking cAR1, Gβ, or Gα2 cannot activate ACA in vivo but ACA can be activated in vitro by GTPγS in *gα2* null but not *g*β null cells, suggesting that the pathways is mediated through Gβγ-subunits. In *gα2* null cells, Gβγ-subunits are presumably released from other heterotrimeric G proteins. ACA activation and cAMP accumulation also require the function of the mitogen-activated protein (MAP) kinase ERK2, as cAMP accumulation is absent in *erk2* null cells.

Two cytosolic proteins have been identified that are required for receptor activation of adenylyl cyclase, CRAC and pianissimo (PiaA). CRAC is a PH domain-containing protein that is proposed to interact with the Gß-subunit and has been shown to translocate from cytosol to the plasma membrane in a response to the chemoattractant. CRAC is required for the activation of adenylyl cyclase by Gβ, but its function in this process is not known. It is probable that the CRAC PH domain may interact with PtdIns(3,4,5)P_3 and/or PtIns(3,4)P_2 produced in the expected activation of phosphatidylinositol 3-kinase (PI3 kinase) in response to chemoattractants, and the PI3 kinases PI3K1 and PI3K2 are required for receptor activation of *Dictyostelium* Akt/PKB. The transient, receptor-mediated translocation of the PH domain-containing protein CRAC to binding sites on the inner face of the plasma membrane has been demonstrated by using the GFP–CRAC fusion protein, which is thought to reflect a localized activation of the G protein-linked signaling system. This localized and transient activation of ACA, controlled by the transient recruitment of CRAC, may be a paradigm for general mechanisms of gradient sensing by G protein-linked chemotactic systems. Pianissimo (PiaA) is a 130-kDa cytosolic protein that is also required for the chemoattractant receptor and G protein-mediated activation of the adenylyl cyclase. Both PiaA and CRAC are essential for activation of the adenylyl cyclase, as lysates of *crac- piaA-* double mutants require both proteins for reconstitution.

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2.4. cGMP and Guanylyl Cyclase

Stimulation of cells with extracellular cAMP leads to a transient 10-fold increase of the cGMP level within 10 s after stimulus and returns to a basal level within 30 s. The role of cGMP in *Dictyostelium* chemotaxis was proposed from the phenotype of Streamer F (*stmF*) mutant, which is defective in aggregation. Streamer F mutant strains are defective in cGMP-phosphodiesterase activity and their chemotaxis is not as efficient as that of wild-type cells. They exhibit a prolonged increase in intracellular cGMP in response to stimulation with chemoattractants and the rate of chemotactic movement of *stmF* mutant cells is significantly reduced. This decrease in speed correlates with the prolonged duration of high intracellular cGMP concentration, suggesting that cGMP concentration must return to basal level for efficient cell movement. During aggregation, guanylyl cyclase is regulated through cAR1, based on the observation that the cGMP response is greatly reduced in *car1* null mutant cells and absent in *car1/car3* double null mutant cells. cGMP is completely absent in *gβ* null cells, indicating the response functions through heterotrimeric G proteins. Gα2 and Gα4 mediate the cAMP- and folic acid-mediated activation of guanylyl cyclase, respectively, and disruption of either gene results in a loss of chemotaxis to the respective ligand. Guanylyl cyclase activity is strongly inhibited by Ca^{2+}, suggesting that the cGMP response can be controlled by Ca^{2+}-mediated inhibition of guanylyl cyclase. Analyses of the regulation of guanylyl cyclase in chemotactic mutants showed that a cGMP-binding protein mediates both stimulation and ATP-dependent inhibition of guanylyl cyclase. The positive and negative regulation of guanylyl cyclase by its product cGMP may explain how cells process the temporal and spatial information of chemotactic signals, which is necessary for sensing the direction of the chemoattractant.

2.5. Regulation by Ras

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There are at least five *Dictyostelium Ras* genes (*B, C, D, G,* and *S*) in *Dictyostelium*, with RasG and *RasD* being closely related to mammalian Ras proteins. *RasC* and *RasS* presumably function in aggregation, as these genes are developmentally regulated and maximum levels of their transcripts are detected during aggregation, suggesting that the encoded proteins have distinct functions during aggregation. Cells overexpressing an activated *RasG* are unable to activate adenylyl cyclase, causing an inability to aggregate. *RasD* and *RasG* appear to have several regulatory functions during aggregation. Recent studies indicate that *Ras* is a negative regulator of cAMP receptor-mediated activation of the *Dictyostelium* MAP kinase ERK2. ERK2 activation is delayed and the level of activation is significantly reduced in cells overexpressing activated $RasD^{Q61L}$, whereas the activation is enhanced in cells overexpressing dominant negative $RasD^{S17N}$ or Ras^{D57Y}. Activation of *Ras* is accomplished by guanine nucleotide exchange factors (*Ras*GEFs) which facilitate replacement of GDP bound to *Ras* with GTP. A *Dictyostelium* putative *Ras*GEF, Aimless (*AleA*), was identified and *aimless* null mutants were severely impaired in chemotaxis and activation of adenylyl cyclase. The motility of mutant cells appears normal, suggesting a defect in gradient sensing. Thus *aimless* null cells are defective in both the signal relay (activation of ACA) and chemotaxis pathways. ERK2 activation is enhanced in *aimless* null cells, which is consistent with enhanced ERK2 activity in cells expressing dominant negative *RasD*. Thus, *Ras* is involved in transduction signals from the cAMP receptor(s) to signaling pathways including activation of adenylyl cyclase and ERK2.

Another gene that is thought to function to control *Ras* regulation of aggregation is *RIP3* (*Ras*-interacting protein 3), which was identified in a two-hybrid screen. *RIP3* specifically interacts with *RasG* and *rip3* null cells show phenotypes that are indistinguishable from those of *aimless* null cells. These data strongly suggest a role for *Ras* pathways in the integration of the signal relay and chemotaxis pathways.

2.6. Role of Phosphatidylinositide 3-Kinases (PI3-kinases) and Protein Kinase B (Akt/PKB)

The *Dictyostelium* protein Akt/PKB (encoded by *pkbA*) is a structural homolog of mammalian Akt/PKB, having a conserved structure that includes an N-terminal PH domain, a kinase domain, and a C-terminal tail. During aggregation, *Dictyostelium* Akt/PKB is rapidly and transiently activated in response to chemoattractant signaling by cAMP with kinetics similar to receptor activation of guanylyl cyclase. Sites of phosphorylation that lead to Akt/PKB activation by upstream kinases in mammalian cells are conserved, and mutant analysis suggests that phosphorylation at these sites is required for activation. Akt/PKB activation is receptor and heterotrimeric G protein

dependent, as determined by the analysis of gene knockout strains. Pertinent to the subject matter of this chapter, *pkbA* null cells exhibit defects in chemotaxis. The cells are unable to properly polarize when placed in a chemotactic gradient and move slowly. More detailed analysis reveals that the cells produce multiple lateral pseudopodia as well as pseudopodia at the leading edge. Analysis of mutants suggests that the cells "tumble" rather than move smoothly in the direction of the cAMP source. This leads to a defective chemotaxis and the inability to form a multicellular aggregate when cells do not have direct cell–cell contacts.

The PH domain-containing protein CRAC, which is required for receptor activation of the aggregation-stage adenylyl cyclase ACA, translocates to the plasma membrane in response to cAMP signaling. In polarized chemotaxing cells, the localization is to the leading edge, suggesting that the activation of adenylyl cyclase is localized under these physiological conditions. Interestingly, a gene fusion of the Akt/PH domain with GFP also rapidly translocates to the plasma membrane in response to cAMP signaling and localizes to the leading edge in chemotaxing cells. This suggests a general principle: in chemotaxing cells in which there is an ~10% difference in the chemotactic gradient between the front and back of the cells, receptor-mediated pathways are activated at the front and not laterally around the entire perimeter of cells. This phenomenon indicates there is a spatially localized activation of pathways, possibly the underlying mechanism by which cells produce a pseudopod only in the direction of the chemoattractant source. Many of the pathways required for preferential actin polymerization at the leading edge may result from a specific localized activation of the necessary signaling pathways at this point in the cell rather than randomly around the cell.

PI3-kinases have been implicated in controlling cell proliferation, actin cytoskeleton organization, and the regulation of vesicle trafficking between intracellular organelles. There are three genes in *Dictyostelium discoideum, DdPIK1, DdPIK2,* and *DdPIK3,* encoding proteins most closely related to the mammalian 110-kDa PI–3 kinase. PI3K1 and PI3K2 are most related to p110α, while PI3K3 is a member of the PI3Kγ subfamily. A strain in which the genes encoding both DdPIK1 and DdPIK are disrupted (*pi3k1/pi3k2* null cells) is unable to activate Akt/PKB in response to cAMP stimulation. Moreover, these cells are impaired in pinocytosis and also almost completely devoid of large postlysosomal vacuoles. The mutant cells contain numerous filopodia projecting laterally and vertically from the cell surface, and fluorescent microscopy indicates that these filopodia are enriched in F-actin, which accumulates in a cortical pattern in wild-type cells. Together, these results suggest that *Dictyostelium* DdPIK1 and DdPIK2 gene products regulate multiple steps in the endosomal pathway, and function in the regulation of cell shape and movement perhaps through changes in actin organization. The inability of *pi3k1/pi3k2* null cells to polarize properly might reflect the improper localization of PKB/Akt which is expected to localize to leading edge by the interaction of InsP3 produced by PIKs with the PH domain of PKB.

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2.7. Ca^{2+} Influx by Ca^{2+} Uptake and Activation of Phospholipase C

Signal transduction in *Dictyostelium* for oriented movement and differentiation involves a fine tuning of the cytosolic Ca^{2+} concentration. cAMP binding to cAR1 results in a rise in intracellular Ca^{2+} levels within approx 5 s. This occurs directly by stimulating Ca^{2+} influx and indirectly by activating a phospholipase C. Chemoattractant binding to cARs appears to generate intracellular signals that induce activation and adaptation of the Ca^{2+} uptake response. Additional analyses on signaling mutants demonstrates that Ca^{2+} entry is not regulated by heterotrimeric G proteins or by G protein-mediated changes in intracellular cAMP or cGMP.

Chemoattractants stimulate the activation of a phospholipase C activity leading to the transient generation of inositol (4,5)-trisphosphate (PIP_3) and diacylglycerol. A phosphatidylinositol-specific phospholipase C (Dd-PLC) has been identified and cloned in *Dictyostelium*. The C-terminal part of the protein shows strong homology with the mammalian PLCδ isoform. Expression of PLC in *Dictyostelium* cells results in increased basal levels of PIP_3 and enhanced PLC activity. Surprisingly, developmental gene regulation, cAMP-mediated chemotaxis, and activation of guanylyl and adenylyl cyclases are normal in Dd-PLC null cells. Although the cells lack PLC activity, PIP_3 is present at only slightly lower concentrations than in control cells, suggesting the presence of functional redundancy. PIP_3 induces Ca^{2+} mobilization from an NBD-Cl- and BHQ-sensitive compartment, which comprises the PIP_3 releasable pool. The role of diacylglycerol in chemotactic response and development of *Dictyostelium* is not known.

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2.8. Function and Regulation of MAP Kinase Cascades

The MAP kinase cascade is a universal signaling unit used by a wide number of eukaryotic signaling pathways. The archetypal MAP kinase cascade is composed of members of MEK kinase (MAP kinase kinase kinase, MEKK), MEK (MAP kinase kinase), and MAP kinase (or ERK) families, which carry out a sequential phosphorylation reaction thereby serving to relay, amplify, and integrate diverse signals. MAP kinase is a serine/threonine specific, proline-directed protein kinase. MAP kinase itself is activated by the upstream dual-specificity threonine and tyrosine protein kinase MEK via phosphorylation at tyrosine and threonine residues in the conserved TXY motif near the catalytic cleft of the kinase core. MEK in turn is activated by the further upstream serine/threonine protein kinase MEKK via phosphorylation at two serine or threonine residues in the activation lip.

A MAP kinase kinase (DdMEK1) required for proper aggregation in *Dictyostelium* has been identified. *mek1* null cells are unable to chemotax during aggregation in response to the chemoattractant cAMP. *mek1* null cells show highly impaired cAMP-mediated activation of guanylyl cyclase, suggesting that this may be the cause of the inability of *mek1* null cells to chemotax. The activation of the MAP kinase ERK2, which is essential for chemoattractant activation of adenylyl cyclase, is not affected in *ddmek1* null strains, indicating that DdMEK1 does not regulate ERK2, and at least two independent MAP kinase cascades control aggregation in *Dictyostelium*. Guanylyl cyclase is not constitutively active in *mek1* null cells expressing constitutively active MEK1 and can be activated in response to cAMP signaling. This suggests that MEK1 does not lie on a linear pathway between the receptor, G protein, and guanylyl cyclase, but its function is required for the ability of chemoattractants such as cAMP to activate guanylyl cyclase. MEK1 thus may function as a checkpoint in regulating chemotaxis.

Two MAP kinases, ERK1 and ERK2, have been identified. The function of ERK1 is not clear, but it may belong to a MAP kinase pathway including MEK1. ERK2 is required for aggregation. *erk2* null cells are defective in receptor-mediated cAMP production and thus do not produce sufficient cAMP for relaying the cAMP signal from cell to cell or the activation of PKA activity. ERK2 is rapidly and transiently activated in response to cAMP. This pathway requires cAR1, but neither Gα2 nor Gβ, suggesting that ERK2 is activated by a G protein-independent pathway. G protein-independent pathways also include Ca^{2+} influx and activation of STATa tyrosine phosphorylation and the transcription factor GBF. Thus, the cAR1 chemoattractant receptor is able to mediate distinct G protein-dependent and G protein-independent pathways. Activation of ERK2 by the chemoattractant appears to be negatively regulated by the Ras signaling pathway. Activated Ras results in reduced ERK2 activation, whereas disruption of putative RasGEF or expression of dominant negative Ras proteins have a more rapid, high, and extended activation. CRAC, a PH domain-containing protein required for adenylyl cyclase activation, is required for proper ERK2 adaptation. PKA overexpression results in a more rapid, high level of activation, whereas *pka* null cells show a lower level but more extended ERK2 activation. This suggests that PKA is required for both activation and adaptation of ERK2.

2.9. Protein Kinase C

Two protein kinase C-like enzymes have been suggested to exist in *Dictyostelium* due to the different cellular responses to two inhibitors specific for protein kinase C. One enzyme is preferentially sensitive to D-*erythro*-sphingosine, a diacylglycerol analog, and is required for growth. A second is preferentially inhibited by bisindolylmaleimide GF109203X and is required for chemotaxis. This activity is stimulated by diacylglycerol, an especially biologically relevant diacylglycerol species, and phosphorylates a peptide substrate that is an efficient substrate for mammalian protein kinase Cs. This activity is a candidate for the effector of diacylglycerol generated during the aggregative phase of *Dictyostelium* development and defines a role for diacylglycerol in the chemotactic response.

2.10. Control of Chemotaxis by Rho Family Members and Downstream Effectors

The Rho family of small G proteins are key regulators of changes in the actin cytoskeleton. There are five Racs identified in *Dictyostelium*. Rac1B appears to regulate cytoskeleton and cell movement during chemotaxis. Cells expressing constitutively active *DdRac1B*^{61L} (unable to hydrolyze GTP) exhibit an up-regulated assembly of F-actin with multiple actin-enriched crowns, suggesting aberrant regulation of cytoskeleton. In response to a chemoattractant,

Rac1B^{61L} cells polarize and move; however, the cells make many false turns and chemotaxis is inefficient. Cells expressing dominant negative *DdRac1B*^{17N} do not have prominent F-actin-rich lamellipodia and *DdRac1B*^{17N} cells do not polarize or move. Thus, DdRac1B regulates the general reorganization of the actin cytoskeleton. The activity of DdRac1B is negatively regulated by RacGAP (Rac GTPase activating protein). Cells lacking RacGAP show aberrant actin cytoskeletons and defective chemotaxis, very similar to cells expressing DdRac1B (Chung et al., submitted). The signaling pathway(s) activating Rac proteins are not known yet, although the activation of Rac proteins would be regulated by signaling pathways downstream from chemoattractant receptors. *Dictyostelium* DdRac1B is likely to regulate a signaling pathway downstream from chemoattractant receptors that control cell movement, migration, and directionality.

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PAKs have been implicated in the morphological changes resulting from changes in the actin cytoskeleton associated with Rac and Cdc42. A *Dictyostelium* Ste20/PAK (p21-activated protein kinase) family kinase that phosphorylates severin, a Ca^{2+}-dependent F-actin fragmenting protein, was purified and cloned recently, indicating a direct signal transduction from the plasma membrane to the cytoskeleton by phosphorylating actin-binding proteins. A *Dictyostelium* myosin I heavy chain kinase (MIHCK) homologous to PAK and Ste20 has also been cloned and proposed to provide a direct link between Cdc42/Rac signaling pathways and motile processes requiring myosin I molecules. Another PAK homolog, PAKa, has been identified. PAKa contains a long N-terminal targeting and regulatory domain that includes an acidic domain, a potential polyproline SH3 binding domain, and a CRIB domain, which we show binds DdRac1B^{GTP} and HsCdc42^{GTP}. *paka* null cells exhibit a defect in completing cytokinesis when they grow in suspension, suggesting a defect in the regulation of the actinomyosin cytoskeleton. In chemotaxis assays, *paka* null cells or wild-type cells expressing dominant negative PAKa produce many random, lateral pseudopodia, have a much higher frequency of making wrong turns than wild-type cells, and chemotax very inefficiently. PAKa appears to be required for the regulation of myosin II filament assembly in the posterior cortex, which is important to maintain polarity and restrict the formation of random, lateral pseudopodia. Genetic analyses suggest that PAKa may negatively regulate myosin II heavy chain kinase.

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3. REGULATION OF CYTOSKELETAL COMPONENTS IN CHEMOTAXIS

3.1. Components of Cytoskeletal Organization and Changes During Cell Migration

Work in *Dictyostelium* has been at the forefront in identifying and understanding the components of the cytoskeleton and their function in controlling cell movement. These analyses have taken advantage of the ability to do gene knockouts to obtain null mutants and the analysis of then mutants via biochemical and physiological assays. The change of the cytoskeleton in a *Dictyostelium* cell on stimulation by a chemoattractant relies mainly on the equilibrium between monomeric and filamentous actin and numerous actin-binding proteins controlling the rigidity of the cytoskeleton.

3.1.1. Actin

Actin represents approximately five of all cellular proteins in *Dictyostelium*. In the resting cell, G- and F-actin are present in approximately equal amounts. Actin cytoskeleton undergoes dramatic rearrangements after chemotactic stimulation. Within a few seconds of stimulation of cells with a chemoattractant, levels of F-actin increase by 50–60% and it incorporates into the cytoskeleton. The assembly of F-actin returns to the resting level by 20 s after the chemoattractant stimulation, which correlates with the shape change of cells to round. After 30 s, a longer lasting increase of F-actin polymerization starts and lasts for several minutes, which represents the extension of new pseudopodia.

3.1.2. Actin-Binding Proteins

The first step in the chemotaxis toward a chemoattractant gradient involves a regulated increase in actin nucleation activity that is correlated with an increase in actin polymerization occurring seconds after chemotactic stimulation at the leading edge of the cell. Actin-binding proteins regulate *in vitro* the assembly of actin into supramolecular structures.

3.1.2.1. Cofilin. Cofilin was identified and purified as an actin monomer binding protein of apparent molecular mass of 15,000 daltons from *Dictyostelium discoideum*. Cofilin has a depolymerizing activity of F-actin filaments in a pH-dependent manner and this activity is inhibited by phosphatidyl inositides. Two cofilin genes (*DCOF1* and *DCOF2*) were cloned. Gene disruption experiments suggest that *DCOF1* is

essential for cell proliferation, whereas the disruption of *DCOF2* did not show any phenotypes. Cofilin is distributed diffusely throughout cytoplasm in vegetative cells, but localizes in ruffling membranes of the leading edge where the actin cytoskeleton is dramatically reorganizing. The overexpression of cofilin induces the formation of actin bundles beneath ruffling membranes and stimulates cell movement and membrane ruffling. This result is rather unexpected, as cofilin has F-actin severing activity. These changes suggest that cofilin may sever actin filaments *in vivo* and create many nucleation sites that induce growth and bundling of the filaments in the presence of crosslinking proteins to generate contractile systems involved in membrane ruffling and cell movement.

3.1.2.2. Aginactin and Cap32/34. A chemoattractant-regulated capping activity, called aginactin, from *Dictyostelium* may regulate changes in actin nucleation activity. Aginactin is a barbed-end capping activity and inhibits the rate and final extent of actin polymerization. Immunofluorescence staining indicates the localization of aginactin in F-actin-rich regions of the cell cortex and cell protrusions. Later studies demonstrated that the capping activity of aginactin is attributed to cap32/34 contained in aginactin. Cap32/34 is a highly conserved heterodimeric barbed-end capping protein**.** It binds to the fast growing ends of actin filaments and effectively inhibits filament elongation. Exposure of free barbed ends results in actin assembly, followed by entry of free capping protein into the actin cytoskeleton, which terminates, not initiates, the actin polymerization transient.

3.1.2.3. ABP-50/EF1α. ABP-50 is the translation elongation factor 1 alpha (EF1α) and an actin filament binding and bundling protein. ABP-50 localizes with F-actin in surface extensions and exhibits a diffuse distribution throughout the cytosol in unstimulated cells. On addition of cAMP, ABP-50 becomes localized in the filopodia that are extended in response to stimulation within 90 s, suggesting that ABP-50 plays an important role in filopod extension. Crosslinked actin filaments bundled by ABP-50 are rotated by 90 degrees relative to each other, whereas other known crosslinking proteins require filaments to be parallel and unrotated. Bundles of actin ABP-50 would tend to exclude other actin bundling proteins. ABP-50 can thus regulate the state of the actin cytoskeleton as well as protein synthesis. At molar ratios present in the cytosol, the bundling activity of ABP-50 significantly blocks both polymerization and depolymerization of actin filaments and increases the final extent of actin polymer. The binding of ABP-50 to aminoacyl t-RNA is not pH dependent, but the interaction between ABP-50 and actin is dependent on cytoplasmic pH. Two pH-sensitive actin-binding sequences in ABP-50 are identified and are predicted to overlap with the aa-tRNA-binding sites. Thus, pH-regulated recruitment and release of ABP-50 from actin filaments in vivo may supply a high local concentration of ABP-50 to facilitate polypeptide elongation by the F-actin-associated translational apparatus.

3.1.2.4. ABP-120. The actin binding protein ABP-120 has been proposed to be required for crosslinking actin filaments in nascent pseudopods; this activity is important for the extension of pseudopodia in motile *Dictyostelium* amoebae. F-actin is incorporated into the Triton X-100-insoluble cytoskeleton at 30–50 s after cAMP stimulation, the time when ABP-120 is incorporated into the cytoskeleton and when pseudopods are extended after cAMP stimulation in wild-type cells. The *ABP-120* null mutant obtained by the homologous recombination exhibits profound defects in actin crosslinking, cytoskeletal structure, pseudopod number and size, cell motility, chemotaxis, and phagocytosis.

3.1.2.5. Talin. A *Dictyostelium* homolog of mammalian talin has been cloned. *Dictyostelium* talin is accumulated at the leading edge, where F-actin is enriched in polarized cells. Talin is cytoplasmic in unstimulated cells and translocates to the membrane cortex where chemoattractant receptors are strongly activated by local stimulation of cells with cAMP. The *talin* null mutant cells are capable of moving and responding to a chemoattractant, although they attach only loosely to a substrate via small areas of their surface, suggesting that talin might function as a membrane anchor.

3.1.2.6. Coronin. Coronin is an actin-binding protein identified in *Dictyostelium* and has an N-terminal domain with similarities to the β-subunits of G proteins (WD repeats) and a C-terminal domain with a high tendency for α-helical structure. Coronin is a cytoplasmic actin-associated protein that is enriched at the leading edge of the cells and in projections of the cell surface called crowns. Coronin is a member of the WD-repeat family of proteins and interacts with actin–myosin complexes, suggesting coronin contributes to the dynamics of the actin system. The mutant cells lacking coronin grow slowly

in suspension and become multinucleated. They also migrate more slowly than wild-type cells during chemotaxis.

3.1.2.7. Profilin. Profilin is a PIP_2-sensitive actin monomer sequestering protein. Two profilin isoforms (profilins I and II) have been purified and the genes encoding them cloned from *Dictyostelium*. Although both profilins contain a conserved lysine residue in the putative actin-binding region and can be crosslinked covalently to G-actin, the crosslinking efficiency of profilin II to actin is substantially higher than that of profilin I, resulting in a more efficient delay of the onset of elongation during the course of actin polymerization. Both profilin genes were disrupted and single mutants did not have any noticeable phenotype. However, cells lacking both profilins are 10 times larger than wild-type cells and cell motility is greatly impaired. Cells also show a broad rim of filamentous actin below the plasma membrane and the filamentous actin concentration in the cell is increased by about 60–70%, suggesting that profilin functions primarily as an actin-sequestering protein.

3.1.2.8. Ponticulin. Ponticulin, a 17-kDa integral glycoprotein, was purified as an F-actin binding protein. Ponticulin is abundant in the plasma membrane, constituting 0.4–1.0% of the total membrane protein. Ponticulin was proposed to be the major high-affinity link between the plasma membrane and the cortical actin network. Cells lacking ponticulin are deficient in high-affinity actin membrane binding. Even though cells are motile, *ponticulin* null cells are less efficient in chemotaxing to the chemoattractant due to the loss of positional stability of pseudopodia.

3.1.2.9. α-Actinin. α-Actinin is an actin-crosslinking protein. α-Actinin has been proposed to play a role in regulating actinomyosin-mediated contraction by promoting growth and/or crosslinking of actin filaments, based on the observation that it localizes with actin and myosin in newly formed projections filled with F-actin. Strains that are defective in α-actinin function chemotax and aggregate properly to cAMP gradient, suggesting that there might be another crosslinking protein that is functionally redundant.

3.1.2.10. Severin. Reorganization of the actin cytoskeleton requires the fragmentation of existing F-actin filaments. Severin is a principal severing protein in *Dictyostelium*. Severin fragments F-actin filaments and caps the newly formed barbed ends. A *severin* null strain does not exhibit major defects in cell motility, which may reflect a functional redundancy. The severing activity of severin appears to be controlled by the intracellular Ca^{2+} concentration. In the presence of micromolar Ca^{2+}, severin cuts actin filaments, binds tightly to the fast-growing ends, and nucleates actin assembly. The activity of severin is inhibited by PIP_2 and other negatively charged phospholipids. Thus, severin regulates the actin cytoskeleton in response to the chemoattractant stimulus. Recently, a kinase homologous to mammalian PAKs was found to have an activity to phosphorylate and regulate the function of severin.

3.1.2.11. WASp and SCAR. WAS (Wiskott–Aldrich syndrome) is an immunodeficiency syndrome caused by abnormal cytoskeleton organization in macrophages and neutrophils. The Wiskott–Aldrich syndrome protein, WASp, is an adapter protein implicated in the transmission of signals from tyrosine kinase receptors and small GTPases to the actin cytoskeleton. A gene homologous to mammalian WASp (Wiscott–Aldrich syndrome protein) was identified. *Dictyostelium* WASp has five distinct domains. At the N-terminus, WASp has a PH domain and a CRIB domain interacting with Rac/Cdc42. WASp also has polyproline repeats that bind to the SH3 domain of proteins such as Nck and Fyn. The ARPH domain sequence is homologous to verprolin, which binds to F-actin and has actin filament severing activity. Thus, WASp might also have actin filament severing activity. At the C-terminus, WASp has series of D/E repeats which might form a coiled-coil structure and serve as an oligomerization motif. SCAR, a member of a new family of proteins related to WASp, was identified as a suppressor of the cAR2 null phenotype. Cells lacking SCAR have reduced levels of F-actin staining during vegetative growth, and abnormal cell morphology and actin distribution during chemotaxis. A recent study indicates that WASp and SCAR interact with the Arp2/3 complex in mammalian cells. The actin-related proteins Arp2 and Arp3 are part of a seven-protein complex that is localized in the lamellipodia of a variety of cell types, and in actin-rich spots of unknown function. The Arp2/3 complex enhances actin nucleation and causes branching and crosslinking of actin filaments *in vitro*; *in vivo,* it is thought to drive the formation of lamellipodia and to be a control center for actin-based motility. Overexpression of SCAR or WASp in cells causes a disruption in the localization of the Arp2/3 complex and, concomitantly, induces a complete loss of lamellipodia and actin spots. These results suggest that WASp-related proteins may regulate the actin cytoskeleton through the Arp2/3 complex. Based on the sequence

similarity, *Dictyostelium* WASp is expected to interact with Arp2/3 complex and regulate the organization of the actin cytoskeleton.

3.1.3. Myosin II

Dictyostelium myosin II is the most abundant and best-studied myosin and is encoded by the *mhcA* gene. The molecular structure of *Dictyostelium* myosin II is very similar to that of mammalian myosin II. *Dictyostelium* myosin II exists as a hexameric complex composed of two 240-kDa heavy chains and two pairs (regulatory, 18 kDa and essential, 16 kDa) of light chains. The N-terminus of the heavy chain associates with a pair of light chains and forms a globular head domain that can be divided into the motor domain and neck domain. The motor domain contains Mg^{2+}-ATPase activity and actin-binding sites. The C-terminus tail region of myosin II is rich in sequences typical of α-helical coiled-coils. The coiled-coil domains of two heavy chains form a bipolar filament. Three threonine residues in the tail region of *Dictyostelium* myosin II heavy chain have been implicated in control of myosin filament formation. Alanine substitution of these sites causes substantial overassembly of myosin II in vivo. Similarly, aspartate substitution mimicking constitutive phosphorylation eliminates filament assembly in vitro and renders the myosin unable to drive any tested contractile event in vivo. These results demonstrate that heavy chain phosphorylation plays a key modulatory role in controlling myosin function in vivo.

Cells lacking functional myosin II were created by a gene knockout by homologous recombination and through the expression of antisense RNA. Neither strain can undergo cytokinesis and form large, multinucleated cells in suspension, which supports the idea that myosin II is a force-generating motor in the contractile ring or cleavage furrow. Surprisingly, these cells can chemotax and form aggregates. *myoII* null cells, however, are unable to develop past the mound stage. The weak aggregation defect suggests myosin II might not be required for pseudopodia protrusion. The ability of *myoII* null cells to complete cytokinesis on the substrate by traction-mediated cytofission reflects the functional motor system at the leading edge. However, based on the slower motility of *myoII* null cells on a substrate, it has been proposed that contractile forces generated by myosin II help the cell's rear edge to detach from the substratum and retract, allowing the cell to continue forward. Recent studies suggest that myosin II is required for retraction of the posterior end of the cell during cell movement. The regulation of myosin II polymerization occurs through receptor-mediated phosphorylation of myosin II heavy chain and myosin II light chain. Myosin II heavy chain is phosphorylated by two kinases, MHCKA and MHC–PKC, at threonine residues in the myosin II tail which lead to the disassembly of myosin filaments. Phosphorylation of residues in the myosin II tail is thought to lead to the disassembly of myosin II fibers, probably at the leading edge. In the posterior cortex, the phosphorylation of the myosin II tails might be inhibited by down-regulation of heavy chain kinase activity. Cells expressing a myosin II mutant in which the three mapped MHC-PKC phosphorylation sites are converted to Ala initially localize to the posterior cortex and form a C-shaped band, enabling the posterior of the cells to attain their wild-type shape. However, these cells do not maintain their shape and are unable to suppress the formation of lateral pseudopodia, suggesting that the assembly of myosin II at the posterior cortex might be important for maintaining cellular polarity and for preventing the formation of lateral pseudopodia. P21-activated protein kinase (PAKa) appears to be regulating the assembly of myosin II in the posterior cortex presumably via the regulation of myosin II heavy chain kinases.

The myosin essential light chain (ELC) was found to be required for myosin function in *Dictyostelium* from studies using cells expressing antisense RNA or a gene knockout. The *mlcE* null cells, when grown in suspension, exhibit the typical multinucleated phenotype observed in *myoII* heavy chain null cells. The aggregation of the *mlcE* null cells is delayed several hours and they never develop past the mound stage, similar to *myoII* null cells. The actin-activated ATPase activity of the myosin purified from the *mlcE* null cells is greatly reduced. This enzymatic defect of myosin probably results in the observed chemotactic defect of *mlcE* null cells. Despite the enzymatic defect, the localization of myosin in *mlcE* null cells is normal, suggesting that its phenotypic defects primarily arise from defective contractile function of myosin rather than its mislocalization. This supposition is strengthened by the observation that while *mlcE* null cells respond to chemoattractant with proper polarity, their movement is slower. During chemotaxis, the polarity toward the chemoattractant may depend primarily on proper localization of myosin II, while efficient motility requires proper contractile function of myosin II which can be regulated by the myosin light chain.

Phosphorylation of the 18-kDa regulatory light

chain (RLC) is required for the full activity of myosin II. A major RLC kinase (MLCKA) has been purified and cloned. *MLCKA* null cells undergo cytokinesis less efficiently than wild-type cells, but they undergo development and cap crosslinked surface receptors, processes that require the myosin heavy chain. A probable presence of additional light chain kinase(s) was indicated by the presence of phosphorylated regulatory light chain in *MLCKA* null cells.

3.1.4. Unconventional Myosins (Myosin Is)

Movement of a eukaryotic cell occurs by protrusion of lamellipodia and pseudopodia at the anterior and retraction at the posterior retraction of the cell body. Unconventional myosin Is appear to play roles in the protrusion of lamellipodia and pseudopodia. There are at least six distinct myosin Is, myoA–F. Each member of the myosin I family has a head domain at the N-terminus that is followed by a single IQ motif, a sequence implicated in the binding of light chains. C-terminal tails of myoB, C, and D have a polybasic domain, a GPA domain (rich in glycine, proline, and alanine or glutamine), and a Src homology domain (SH3) domain. myoA, E, and F lack the GPA and SH3 domains. Myosin IB was the first unconventional myosin identified and it has been shown by immunofluorescence microscopy that nonfilamentous myosin IB localizes at the leading edges of the lamellipodial projections of migrating *Dictyostelium* amoebae, which are devoid of myosin II, whereas filamentous myosin II is concentrated in the posterior of the cells. Myosin IC and ID also appear to colocalize in the actin-rich pseudopodia at the leading edge of migrating cells. Based on their locations and biochemical properties, actinomyosin I may contribute to the forces that cause extension at the leading edge of a motile cell, while the contraction of actinomyosin II at the rear squeezes the cell mass forward.

3.2. Regulation of Cytoskeletal Organization in Chemotaxis

A dramatic rise of the proportion of polymerized actin (F-actin) is one of the most striking changes after addition of extracellular cAMP to aggregation-competent *Dictyostelium* cells. A schematic diagram for the establishment of the leading edge by redistribution of actin filaments and components involved in the regulation of actin filaments is presented in Fig. 3. Localized actin assembly is required for the psuedopod formation during chemotaxis. Sites of actin filament assembly in the cell are regulated by the actin-binding proteins that control the localization, length, and stability of actin filaments. These actin-binding proteins are regulated by phosphorylation, Ca^{2+}, and phospholipids and thus are targets for the intracellular changes that occur upon stimulation of a cell with a chemoattractant. Chemotactic stimulation leads to an enrichment of ABP-120 with actin crosslinking activity in extending pseudopodia, suggesting that ABP-120 is involved in psuedopod extension. Talin stays in the cytoplasm and, within 30 s after chemotactic stimulus, translocates to the membrane cortex, where chemoattractant receptors are strongly activated by local stimulation of cells with cAMP. ABP-50 becomes localized in the filopodia that are extended in response to stimulation within 90 s, suggesting that ABP-50 plays an important role in filopod extension. The recruitment of ABP-50 at leading edge seems to be regulated by the change of cytoplasmic pH. The activity of cap32/34 may be regulated by anionic phospholipids as the preincubation of cap32/34 with phosphatidylinositol 4,5-bisphosphate (PIP_2) inhibits its actin binding and capping activities. The severing activity of a major severing protein, severin, appears to be controlled by the intracellular Ca^{2+} concentration. In the presence of micromolar [Ca^{2+}], severin cuts actin filaments, binds tightly to the fast-growing ends, and nucleates actin assembly. The activity of severin is inhibited by PIP_2 and other negatively charged phospholipids. Scanning electron microscopy indicates that the double mutant disrupted in PI3K1 and PI3K2 contains no F-actin enriched pseudopodia, but had numerous filopodia projecting laterally and vertically from the cell surface. Fluorescent microscopy indicates that these filopodia are enriched in F-actin which accumulates in a cortical pattern in control cells. These results suggest that PI3K1 and PI3K2, possibly through modulation of the levels of $PI(3,4,5)P_3$ and $PI(3,4)P_2$, regulate the organization of actin filaments necessary for the extension of pseudopodia and the aggregation of cells into streams.

Immunofluorescence studies demonstrate that cytoskeletons composed of actin and myosin II rapidly reorganize in *Dictyostelium* cells that have been stimulated with the chemoattractant cAMP. The amounts of F-actin increase in the cortical region of cells after 5–10 s of stimulation by cAMP (the first peak). After a transient decrease in the amount of F-actin in the cortical region, the amounts of both actins increase in the cortical region after 25–35 s of stimulation (the second peak). A schematic diagram of signaling components involved in the regulation of myosin II filaments is presented in Fig. 4. Filaments of myosin II become associated within the cell membrane with

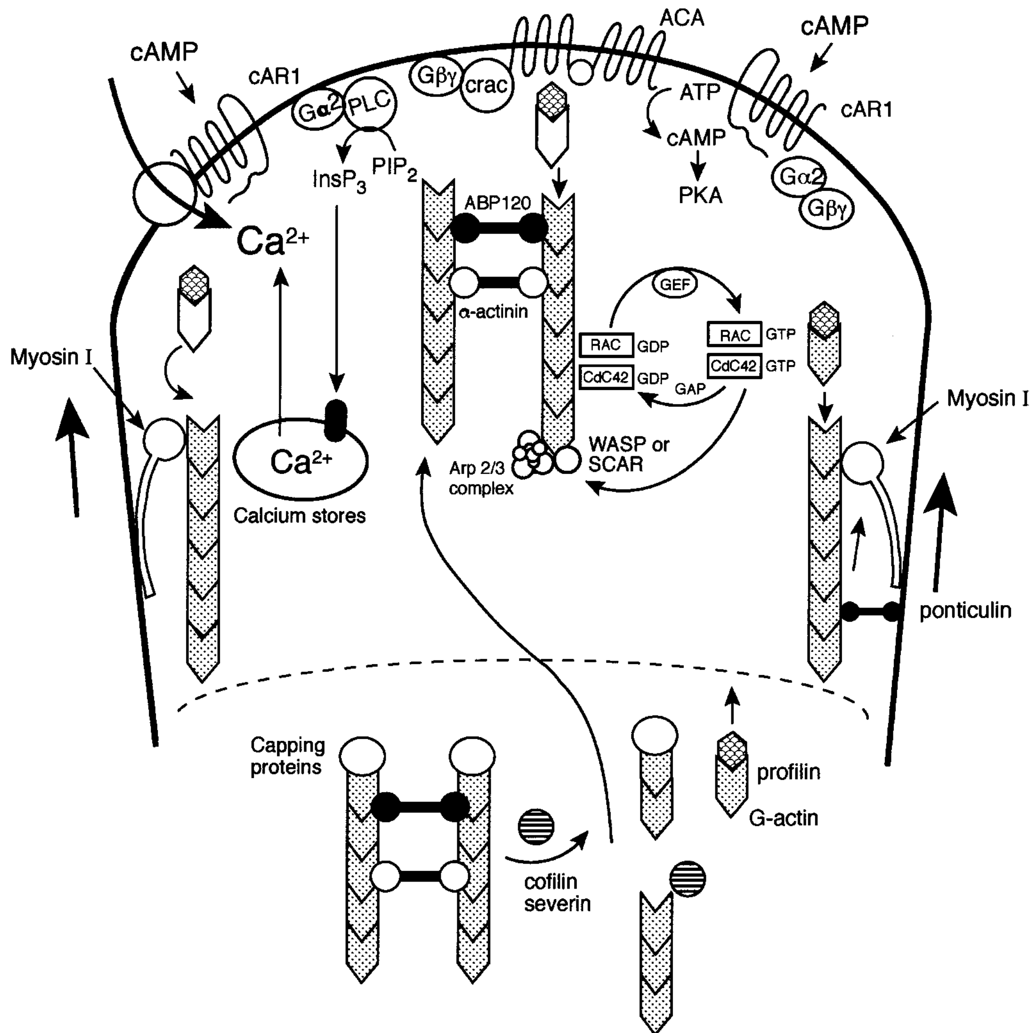

Fig. 3. Scheme for the establishment of the new leading edge. At the leading edge, the polymerization of actin filaments is stimulated by profilin. The interaction between between WASP or SCAR and Arp2/3 complex has been suggested to be important for nucleation of actin filaments. New nucleation site also can be created by severing existing actin filaments by severing protein such as cofilin and severin. Rac/Cdc42 proteins appear to regulate the activity of WASP. Actin crosslinking proteins stabilize the actin filaments into networks and they are regulated by intracellular pH and/or Ca^{2+} concentration. Pnticuling and talin are thought to link actin filaments to the leading edge plasma membrane. Myosin I function as a link between actin filaments and membrane and as a motor protein that is required for the extension of pseudopods.

increases in the mesh of actin filaments on the cell membrane at the time of the second peak. The amount of myosin II decreases thereafter, but F-actin stays localized in pseudopodia. The translocation of myosin II is suggested to be achieved by decreasing filament assembly in the cytoplasm and a concomitant increase of elongation and/or nucleation at the cortex. Many studies demonstrated that phosphorylations of three threonines near the C-terminus of myosin by heavy chain kinase inhibits myosin assembly. From the observation of cells expressing three myosin II mutants that cannot be regulated by the phosphorylation on the mapped light chain site, phosphorylation of the light chain is not required for the localization of myosin II to the furrow region of dividing cells and in the tail region of migrating cells. However, myosins that are deficient in heavy chain phosphorylation are distributed only in the cortical region of interphase cells and cells expressing this mutant myosin have a very slow rate of chemotactic migration. Cells expressing a myosin II mutant in which the three mapped MHC-PKC phosphorylation sites are converted to Ala initially localize to the posterior cortex and form a C-shaped band, enabling the posterior of

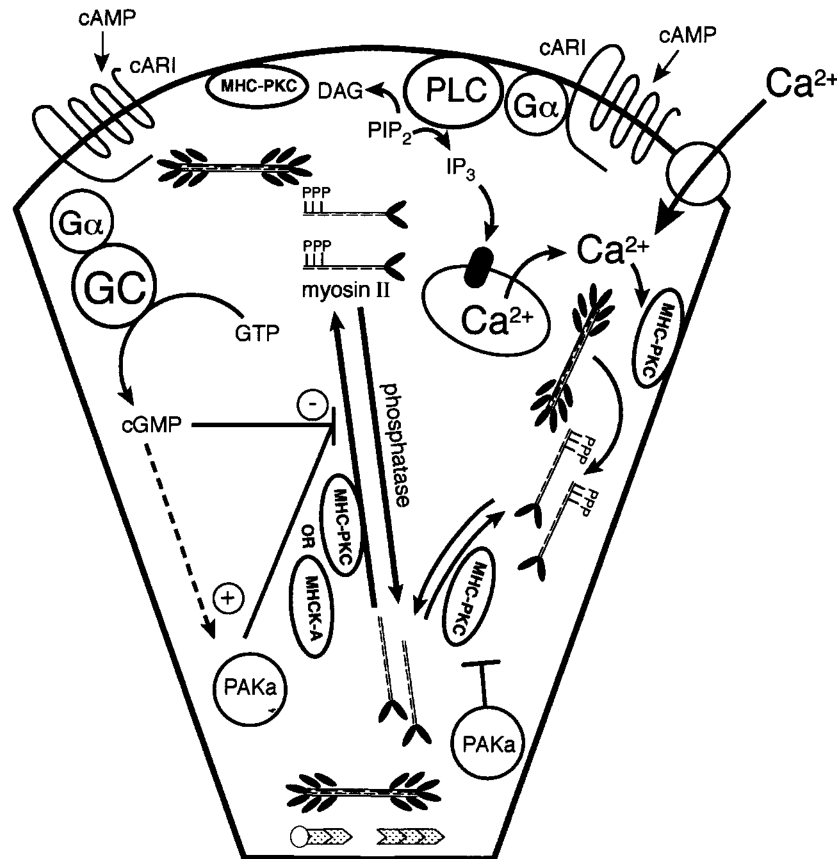

Fig. 4. Schematic diagram of the regulation of myosin II filaments assembly. The myosin II phosphorylated by MHC–PKC or MHCKA exist as a monomer while the myosin II molecules dephosphorylated by a phosphatase are in the form of parallel fibers that form thick filaments. Increase of intracellular Ca^{2+} concentration at the leading edge presumably recruits and activates the MHC–PKC, inducing the phosphorylation of heavy chains and disassembly into myosin II monomers. cGMP produced by the activation of GC inhibits heavy chain phosphorylation, thereby inducing an assembly of myosin II filaments and association of myosin II filaments into cytoskeleton. Localized activation of PAKa also appears to be important for the regulation of the assembly of myosin II in the posterior part of the cell. The inhibition of phosphorylation is thought to be localized in the posterior cell body, resulting in the polarization of the migrating cell.

the cells to attain their wild-type shape. However, these cells do not maintain their shape and are unable to suppress the formation of lateral pseudopodia.

Binding of extracellular cAMP to cAR1 causes a transient phosphorylation of myosin heavy chain and light chain. Phosphorylation of myosin heavy and light chains is a key process regulating dynamic rearrangement of myosin thick filament in response to the chemoattractant. Two distinct myosin II heavy chain kinases have been identified. MHC–PKC was purified from the membrane fraction of developing cells and cloned. Sequence analysis of the cDNA revealed that the *Dictyostelium* MHC-PKC possesses all of the domains characteristic of members of the protein kinase C family. Cells lacking MHC–PKC exhibit substantial myosin II overassembly, as well as aberrant cell polarization and chemotaxis. Cells overexpressing the MHC–PKC contain highly phosphorylated MHC and exhibit impaired myosin II assembly and localization, no apparent cell polarization, and chemotaxis. From these observations, MHC–PKC appears to play an important role in the regulation of myosin II localization during chemotaxis. MHC–PKC has regulatory domains involved in interaction with Ca^{2+}, phospholipid, and diacylglycerol. Therefore, MHC–PKC is likely to be activated by Ca^{2+}, whose concentration rises in response to the chemoattractant. Another myosin II heavy chain kinase, MHCK-A, was purified and the gene was cloned. Analysis of the primary sequence reveals that the N-terminal amino acids form an α-helical coiled-coil domain and 300 residues of the C-terminus (residue 1146) form a WD repeat similar to the WD repeat of the β-subunit of heterotrimeric G proteins. Even

though no part of the MHCK-A sequence displays similarity to the catalytic domain of conventional eukaryotic protein kinases, it autophosphorylates and phosphorylates *Dictyostelium* myosin II. Both growth phase and developed *mhckA* null cells show substantially reduced MHC kinase activity in crude lysates, as well as significant overassembly of myosin into the Triton-resistant cytoskeletal fractions. PAKa might phosphorylate and thereby inhibit the activity of MHC–PKC in the posterior cortex, as the assembly of myosin II filaments in the posterior cortex is absent in the *paka* null cell. This inhibition would occur in the posterior of cells where PAKa is localized, possibly allowing MHC–PKC to promote disassembly of myosin II fibers at the leading edge. Thus, the ability to sequentially polymerize and depolymerize myosin, possibly in response to the activation and adaptation of PAKa kinase activity, appears to be critical for cell movement.

cGMP regulates the association of myosin II with the cytoskeleton via inhibition of the phosphorylation of the myosin II heavy chain via a PKC activity [Liu, 1991 #1905; Dembinsky, 1996 #260]. A mutant (KI-10) that is defective in chemotaxis and lacks the normal cAMP-induced cyclic GMP response exhibits a normal cAMP-induced cytoskeletal actin response but the cytoskeletal myosin II heavy chain response is abolished probably due to no phosphorylation of myosin II heavy chain in response to cAMP [Liu, 1991, #1905]. Cyclic GMP may play a role in the proper regulation of myosin function. Also, cGMP may be involved in regulating light chain phosphorylation in response to the chemotactic stimulus [Liu, 1994 #579].

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4. CONCLUDING REMARKS

How cells respond to environmental stimuli such as circulating hormones, growth factors, lymphokines, neurotransmitters, and stress (osmotic shock, UV, heat shock) has been a major question in understanding cell development, differentiation, and oncogenesis. Chemotaxis, a directed cell movement toward the chemoattractant gradient, plays an important role in responses to changes of environment. Chemotaxis involves the recognition of the concentration difference of the chemoattractant across the length of a cell, a relay of signal through signaling pathways, and directional movement. Directed reorganization of the actin and myosin cytoskeletons, pseudopod extension in the direction of the chemoattractant source, and cell movement via pathways are thought to be highly conserved between mammals and *Dictyostelium.* G protein-coupled cAMP receptors are important for sensing the chemoattractant gradient. Relay of the signal through signaling pathways downstream of cAMP receptors induces a variety of changes in cellular components including the actinomyosin cytoskeleton, and these changes, in turn, induce chemotactic movement of cells. It is remarkable that these biochemical reactions including G protein-coupled receptors are quite similar in evolutionarily distant organisms. *Dictyostelium* cells thus provide a powerful system in which to examine the role of cellular components in controlling coordinated cell movement because of the ability to apply genetic and cell biological approaches to study this evolutionarily conserved path. A number of outstanding questions are still to be answered.

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