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Signal relay during chemotaxis

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Summary

The ability of cells to migrate in response to external cues, a process known as chemotaxis, is a fundamental phenomenon in biology. It is exhibited by a wide variety of cell types in the context of embryogenesis, angiogenesis, inflammation, wound healing and many other complex physiological processes. Here, we discuss the signals that control the directed migration of the social amoebae *Dictyostelium discoideum* both as single cells and in the context of group migration. This multi-cellular organism has served as an excellent model system to decipher amoeboid-like leukocyte migration and has played a key role in establishing signalling paradigms in the chemotaxis field. We envision that *Dictyostelium* will continue to bring forward basic knowledge as we seek to understand the mechanisms regulating group cell migration.

Keywords

cAMP; chemotaxis; *Dictyostelium discoideum*

Dictyostelium discoideum as a model system to study basic cellular processes

D. discoideum is the most studied species of the social amoebae, which diverged early after the plant–animal split, and represents one of the basal clusters of the crown groups of eukaryotes (Kessin, 2001; Baldauf, 2003). Its small genome (34 Mbp) has been completely sequenced (Eichinger *et al.*, 2005). It is organized into six chromosomes that harbour ~12000 genes, many of which share homology with higher eukaryotes (Fey *et al.*, 2006). The strength of *Dictyostelium* as a model organism lies in its highly accessible genetics and biochemistry. The amoebae can be grown in shaking cultures, and 10^{12} clonal cells can be obtained in a few days for biochemical assays. Non-essential genes can be disrupted by homologous recombination or silenced by RNAi and insertional mutagenesis techniques allow the identification of novel genes (Landree & Devreotes, 2004; Kimmel & Faix, 2006; Kuhlmann *et al.*, 2006; Kuspa, 2006). Finally, transgenic cells expressing mutated genes or GFP fusions are readily obtained and have been invaluable to decipher protein function and the spatio-temporal distribution of proteins in live cells (Muller-Taubenberger, 2006). A genetic and literature database about *Dictyostelium*, Dictybase (<http://dictybase.org>), and a central repository for strains and plasmids, the *Dictyostelium* Stock Center, makes *Dictyostelium* research highly accessible (Chisholm *et al.*, 2006).

Dictyostelium cells live in two independent states. In the presence of nutrients, they live a solitary life, growing autonomously. However, upon starvation, the cells stop dividing and enter a developmental program where as many as 100000 cells aggregate and differentiate into

a multi-cellular organism – the fruiting body – made of spores atop a stalk of vacuolated cells (Fig. 1(A)). Return of favourable nutrient conditions leads to spore germination and re-initiation of the cycle (Williams & Harwood, 2003; Chisholm & Firtel, 2004; Kimmel & Firtel, 2004). Chemotaxis is essential throughout the life cycle of *Dictyostelium*. In their growth phase, the cells hunt and phagocytose bacteria (their favourite food source) by sensing and migrating towards folic acid, a byproduct of bacterial metabolism. During early development, cAMP becomes the primary chemoattractant and cells use chemotaxis to come together and form aggregates. Interestingly, as cells migrate they quickly organize in groups where individual cells align in a head-to-tail fashion to form chains of cells or streams that eventually form aggregates (Mahadeo & Parent, 2006) (Fig. 1(B)). In later developmental stages, cells continue to respond in groups to external cAMP cues, rotating and showing periodic polarization and surges in movement (Weijer, 2004). Since its discovery, *Dictyostelium* has provided a very useful and arguably unparalleled model for the study of motility and chemotaxis.

The signal relay loop

Pathways that have been conserved throughout evolution mediate the signals regulating the entire life cycle of *Dictyostelium*. Upon starvation, four distinct but highly related cAMP receptors (cAR1–4) are differentially expressed to mediate the effects of cAMP (Hereld & Devreotes, 1992; Kim *et al.*, 1998). These receptors are part of the large family of seven transmembrane receptor proteins that transduce their effects via heterotrimeric G proteins – the G protein coupled receptors (GPCRs) (Karnik *et al.*, 2003; Milligan & Kostenis, 2006; Prabhu & Eichinger, 2006). In mammalian cells, a large family of chemokines mediates chemotactic responses. Chemokines are small peptides of about 100 amino acids. They are classified as either CXC or CC according to the position of the first two cysteine residues in the chemokine sequence (Baggiolini, 2001). Much like cAMP in *Dictyostelium*, chemokines mediate their chemotactic activity via GPCRs, where the binding of the chemoattractant to their specific receptor leads to the dissociation of G proteins into α - and $\beta\gamma$ -sub-units and to the activation of a variety of conserved effectors (Bagorda *et al.*, 2006).

In *Dictyostelium* inter-cellular communication is essential to allow cells to spontaneously migrate directionally and form aggregates. To this end, the organism has developed an impressive machinery to exquisitely regulate the detection, synthesis, secretion and degradation of cAMP. Three distinct adenylyl cyclases are expressed throughout the life cycle of *Dictyostelium*, ACA, ACB and ACG (Saran *et al.*, 2002; Kriebel & Parent, 2004). During early aggregation, where the cells exhibit strongest chemotactic activity, ACA is maximally expressed. ACA is related to the mammalian G protein-coupled adenylyl cyclases and is composed of two sets of six transmembrane domains, each followed by a large cytosolic loop where the catalytic domains reside (Fig. 2(A)). The binding of cAMP to cAR1 leads to the activation of many effectors, including ACA, which converts ATP into cAMP. Although some of the cAMP produced will remain inside the cell to activate downstream pathways, the majority of cAMP is actually secreted to attract neighbouring cells (Dinauer *et al.*, 1980a; Mahadeo & Parent, 2006) (Fig.2(A)). This comprises a signal relay loop that is essential for cells to aggregate. Indeed, cells that are lacking any one of the components that leads to cAMP production cannot enter development and remain as smooth monolayers of cells when plated on non-nutrient surfaces (Pitt *et al.*, 1992; Insall *et al.*, 1994; Chen *et al.*, 1997).

Two distinct mechanisms allow cells to maintain responsiveness to cAMP. First, the chemoattractant-mediated activation of ACA is transient, showing an initial peak of activation followed by a phase of adaptation leading to a decrease in the amount of cAMP produced (Dinauer *et al.*, 1980b). The mechanisms regulating this essential adaptation response remain to be elucidated. It has been shown that ACA adaptation does not depend on cARs phosphorylation and appears to occur downstream of G proteins (Lilly & Devreotes, 1995;

Parent & Devreotes, 1996). Second, once generated, cAMP is readily degraded by a variety of intra-cellular and extra-cellular phosphodiesterases (Saran *et al.*, 2002; Bader *et al.*, 2007) (Fig. 2(A)). This allows the system to come back to basal levels and to respond to another round of activation.

Signal relay requires inputs from the PI3K and TOR pathways

The mechanisms that regulate the chemoattractant-mediated activation of ACA are complex (Fig. 2(A)). The cascade is initiated at the plasma membrane, where $G\beta\gamma$ -sub-units activate PI3K in a Ras-dependent manner (Sasaki *et al.*, 2004). This leads to the formation of phosphatidylinositol 3,4,5 phosphate (PIP₃) and the recruitment of the pleckstrin homology (PH) domain-containing protein CRAC (cytosolic regulator of adenylyl cyclase) (Parent *et al.*, 1998). CRAC was initially isolated from a chemical mutagenesis screen and shown to be essential for the chemoattractant-mediated activation of ACA (Theibert & Devreotes, 1986; Insall *et al.*, 1994; Lilly & Devreotes, 1994). More recently, we have shown that CRAC independently regulates chemotaxis and ACA activation downstream of PI3K (Comer *et al.*, 2005). The mechanisms by which CRAC activates ACA and chemotaxis remain to be determined. However, intermediate components are most probably involved to control ACA activity since the chemoattractant-mediated translocation of CRAC to the plasma membrane occurs a full 1 min before ACA activity peaks (Parent *et al.*, 1998).

The target of rapamycin complex 2 (TORC2) is also a key regulator of chemotaxis and ACA activity in *Dictyostelium* (Fig. 2(A)). TOR is a member of the phosphatidyl kinase family of serine–threonine protein kinases. It exists in two functionally distinct protein complexes (TORC1 and TORC2) (Sarbasov *et al.*, 2005; Wullschleger *et al.*, 2006; Yang & Guan, 2007). In mammalian cells, TORC1, which is sensitive to rapamycin, is composed of Raptor, LST8, PRAS40 and TOR and regulates protein synthesis and cell growth. TORC2 is insensitive to rapamycin. It is composed of Rictor, LST8, Sin1 and TOR and is primarily involved in regulating cytoskeletal organization. In *Dictyostelium*, homologues of TOR, Raptor, LST8, Rictor (called Pianissimo), and Sin1 (called Rip3) are expressed, and the role of TORC2 in chemotaxis and signal relay has been particularly well characterized (Chen *et al.*, 1997; Lee *et al.*, 1999; Lee *et al.*, 2005; Sasaki & Firtel, 2006). Cells lacking either Pianissimo (Pia), LST8 or Rip3 share a common phenotype showing strong defects in the chemoattractant-mediated activation of ACA as well as in chemotaxis. These defects are dependent on the presence of a pre-formed TORC2. Using reconstitution experiments, it has been shown that CRAC is not required for the formation of TORC2 (Lee *et al.*, 2005). Yet, experiments performed in cells lacking both Pia and CRAC established that ACA activity absolutely requires an input from both pathways (Chen *et al.*, 1997). Interestingly, work originally performed in *Dictyostelium* showed that TORC2 is also required for chemoattractant-mediated activation of Akt/PKB and the related PKBR1 (Lee *et al.*, 2005). Although cells lacking Akt/PKB and PKBR1 have been shown to have chemotaxis and polarity defects, it has not been determined if these effectors also regulate ACA (Meili *et al.*, 2000).

Signal relay is spatially restricted

Through the use of the GFP technology, it has been possible to visualize the distribution of most of the cAMP signalling components in live chemotaxing cells (Fig. 2(B)). Although both receptors and G proteins remain uniformly distributed on the plasma membrane during this process, Ras, PI3K, PKB and CRAC are found to specifically and dynamically translocate from the cytosol to the leading edge of chemotaxing cells (Xiao *et al.*, 1997; Parent *et al.*, 1998; Meili *et al.*, 1999; Jin *et al.*, 2000; Funamoto *et al.*, 2002; Sasaki *et al.*, 2004). Concurrently, the phosphatase responsible for dephosphorylating PIP₃, PTEN, is also dynamically associated with the plasma membrane at the sides and back of cells (Iijima &

Devreotes, 2002). This highly regulated, and complementary cellular distribution of PI3K and PTEN allows the restricted production of PIP₃ at the leading edge of cells, where CRAC and other PH domain-containing proteins redistribute (Comer & Parent, 2002). It was therefore proposed that PH domain-containing proteins act as nucleation factors, spatially directing where signalling cascades are active during chemotaxis.

Surprisingly, we found ACA to be highly enriched at the back of chemotaxing cells as well as on dynamic intra-cellular vesicles (Fig. 2(B)) (Kriebel *et al.*, 2003). We further showed that this cellular distribution is required for cells to align in a head-to-tail fashion and stream during chemotaxis. In this context, we proposed that the spatial restriction of ACA at the back of cells provides a compartment from which cAMP is released and attracts cells specifically at the back of cells in front of them. The mechanism that regulates cAMP secretion has yet to be identified, although it has been established that ACA itself is not the cAMP transporter (Pitt *et al.*, 1992). We envision, like others before us, that vesicular exocytosis may regulate cAMP secretion (Maeda & Gerisch, 1977). We have shown that although PI3K and CRAC are both essential for the activation of ACA, they are dispensable for its cellular distribution (Kriebel *et al.*, 2003; Comer & Parent, 2006). These findings therefore suggest that distinct mechanisms independently regulate the activity and the cellular distribution of ACA. As there is significant lag between CRAC translocation and ACA activation (see above), it remains possible that the activation of ACA is initiated at the front of cells and that additional factors, such as TORC2, are involved later in the process.

Perspectives

Studies performed in chemotaxing *Dictyostelium* cells have provided invaluable insight into how cells transduce apparently simple signals, such as increases in cAMP levels, into complex biological responses. These cells do so by carefully restricting the cellular distribution of key components in the signalling cascade. Remarkably, much of this spatial organization is conserved as human neutrophils also redistribute PH domain-containing proteins to their leading edge (Servant *et al.*, 1999; Servant *et al.*, 2000; Li *et al.*, 2003; Xu *et al.*, 2003; Lacalle *et al.*, 2004; Nishio *et al.*, 2007). We envision that signal relay will also be spatially restricted in higher eukaryotes. Indeed, in human neutrophils, chemoattractants also relay the signal to surrounding cells by stimulating the production and release of more attractants, such as LTB₄ and interleukin 8 (IL-8), which act in an autocrine and paracrine fashion to spread the chemotactic response to surrounding cells (Kuhns & Gallin, 1995; Kuhns *et al.*, 2001; Yokomizo *et al.*, 2001). Yet, very little work has been done to understand how complex signal relay pathways regulate neutrophil migration and homing. It has recently been shown that LTB₄-mediated neutrophil degranulation protects against cytomegalovirus infection, but the signal transduction mechanisms that regulate this remain to be determined (Gaudreault & Gosselin, 2007). Future work will undoubtedly reveal novel and unexpected findings in the complex regulation of signal relay during cell migration and development.

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Fig. 1.

The life cycle of the social amoebae *Dictyostelium*. (A) Montage of images depicting the developmental program of starving *Dictyostelium* cells. Cells were plated on non-nutrient agar plates, and pictures were taken at the indicated time after the initiation of starvation. Bar represents 1 mm. (B) Images showing cells migrating in streams during chemotaxis. The image on the left depicts several aggregation centres in streams. The picture on the right presents a higher magnification image of cells migrating in streams to a micro-pipette containing 10 μ M cAMP. The white arrow indicates the position of the micro-pipette. Bar represents 1 mm for the right image and 250 μ m for the left image.

Fig. 2.

The cAMP signal transduction pathway in *Dictyostelium*. (A) Cartoon representing the cascade of events leading to the activation of ACA. (B) Cartoon depicting the cellular distribution of various signal transduction components during chemotaxis. Actual images of cells expressing CRAC-GFP or ACA-YFP are presented in the last two panels. See text for details.