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Dual chemotaxis signalling regulates *Dictyostelium* development: Intercellular cyclic AMP pulses and intracellular F-actin disassembly waves induce each other

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Abstract

Aggregating *Dictyostelium discoideum* amoebae periodically emit and relay cAMP, which regulates their chemotaxis and morphogenesis into a multicellular, differentiated organism. Cyclic AMP also stimulates F-actin assembly and chemotactic pseudopodium extension. We used actin-GFP expression to visualise for the first time intracellular F-actin assembly as a spatio-temporal indicator of cell reactions to cAMP, and thus the kinematics of cell communication, in aggregating streams. Every natural cAMP signal pulse induces an autowave of F-actin disassembly, which propagates from each cell's leading end to its trailing end at a linear rate, much slower than the calculated and measured velocities of cAMP diffusion in aggregating *Dictyostelium*. A sequence of transient reactions follows behind the wave, including anterior F-actin assembly, chemotactic pseudopodium extension and cell advance at the cell front and, at the back, F-actin assembly, extension of a small retrograde pseudopodium (forcing a brief cell retreat) and chemotactic stimulation of the following cell, yielding a 20 s cAMP relay delay. These dynamics indicate that stream cell behaviour is mediated by a dual signalling system: a short-range cAMP pulse directed from one cell tail to an immediately following cell front and a slower, long-range wave of intracellular F-actin disassembly, each inducing the other.

Keywords: Actin; Autowave signalling; Cell aggregation; Chemotaxis; Cyclic AMP; Development; *Dictyostelium discoideum*; Gradient; Reaction–diffusion wave; Self-organisation; Streaming

Introduction

Chemotaxis governs the different developmental stages in the cellular slime mould *Dictyostelium discoideum*, from dispersed amoebae to a multicellular, differentiated, patterned and motile organism. The first morphological signs of development — enhanced cell polarity and chemotaxis – become evident about 6 h after starvation as *Dictyostelium's* specific attractant cyclic 3′,5′ AMP (cAMP) is periodically synthesised and emitted by cells in response to a pulse of cAMP relayed from other cells (Shaffer, 1975; Tomchik and Devreotes, 1981). Cellular adaptation to the cAMP signal induces their transient refractoriness to further signalling, which is thought to gate the frequency and direction of signal relay and chemotactic cell locomotion. Consequently, cAMP is believed to propagate periodically as a chemical wave – a developing gradient – through the cell population, inducing self-organised waves of cells to

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migrate tactically in the opposite direction toward an aggregation centre (Tyson and Murray, 1989). As the population's age and density increase, it breaks symmetry and coalesces into radial streams (Van Oss et al., 1996).

Intercellular communication and cell responses in *Dictyostelium* streams depend on three factors, which are linked throughout development:

The first concerns chemotaxis, which is believed to result from each cell reading across its length a positive, static spatial "chemotactic gradient" and thereby polarizing and directing itself towards higher cAMP concentrations, even in a cell stream (Alcantra and Monk, 1974; Gerisch et al., 1975; Wessels et al., 1992; Dormann et al., 2002; Myers et al., 2005; Affolter and Weijer, 2005; Xu et al., 2005; Levine et al., 2006; Chen et al., 2007; Keizer-Gunnink et al., 2007). The spatial gradient is usually illustrated as spanning several cells and each cell is thought to reflect gradient direction by generating an intracellular chemical gradient through the redundant activities of three intracellular enzymes (the "molecular compass") consisting of phosphoinositide-3-kinase (PI3K), $PI(_{3,4,5})P_3$-phosphatase (PTEN) and phospholipase A_2 (PLA_2) (Chen et al., 2007; Keizer-Gunnink et al., 2007; van Haastert et al., 2007). Thus, about 10 s after stimulation, phosphatidylinositol-3,4,5-trisphosphate (PIP_3) synthesis becomes restricted to the cell's leading plasma membrane (Loovers et al., 2006). About 10 s later, pleckstrin homology (PH) domain-containing proteins, e.g. CRAC, bind to the PIP_3 there, inducing the reactions thought to lead to cell polarity, cAMP synthesis and chemotactic locomotion, driven by filamentous (F-)actin assembly (Condeelis, 1993; Westphal et al., 1997; Parent et al., 1998; Dormann et al., 2002, 2004; Iijima et al., 2003; Ridley et al., 2003; Xu et al., 2005).

The second factor concerns F-actin dynamics. Reversible F-actin assembly propagates in the cytoplasm of *Dictyostelium* and vertebrate cells as a dissipative structure, a self-organised chemical reaction–diffusion autowave (Vicker, 2002), which may arise in systems far from equilibrium. F-actin waves generate the self-organised, oscillatory cell surface shape modes, which determine the size and distribution of pseudopodial extensions and, thus (along with cytoplasmic flow), cell locomotion in *Dictyostelium* amoebae, neutrophil leukocytes, keratinocytes and melanoma cells (Killich et al., 1993, 1994; Hartman et al., 1994; Alt et al., 1995; Ballestrem et al., 1998). F-actin and Arp2/3 complex associate in wave formation (Bretschneider et al., 2004; Weiner et al., 2007), which apparently facilitates F-actin functions near the plasma membrane, e.g. locomotory extension (DesMarais et al., 2004). An artificial pulse of 10^{-7} M cAMP induces the rapid global assembly and accumulation of F-actin at the plasmalemma cortex, beginning within about 2.6 s and dissipating 3 s later (Etzrodt et al., 2006). This damping oscillatory excursion – a prominent feature of signal reaction in *Dictyostelium* – induces the extinction of most other F-actin structures within the cell and is related to the transient inhibition of cell locomotion and retraction of locomotory extensions, noted by Futrelle et al. (1982) as cell "cringing". The F-actin distribution returns to its nominal equilibrium level at least 20 s before the induction of a local wave of F-actin assembly, which induces the first locomotory pseudopodium (Vicker et al., 1997; Postma et al., 2003; Chen et al., 2003).

The third factor involves the kinematics of the cAMP signal itself. Cyclic AMP signalling is understood as a signal adaptation model (Martiel and Goldbeter, 1987; Laub and Loomis, 1998; Lit¸canu and Velázquez, 2006). In a developing gradient of 1 μM cAMP and a diffusion coefficient of $2.5 \times 10^2\ \mu m^2/s$, the diffusion velocity of a threshold, taxis-activating cAMP concentration – 2.5×10^{-9} M (Grutsch and Robertson, 1978) – is about 2400 μm/min across a 10 μm distance, as yielded from calculations by Rappel et al. (2002). Thus, the bulk of the cAMP released by one cell, about 1.2×10^7 mol/cell per min (Kriebel et al., 2003), will diffuse beyond one cell length within milliseconds after its emission. In realistic simulations of cell aggregation, the inclusion of additional factors (e.g., cAMP phosphodiesterase (PDE) activity, a cAMP relay threshold concentration and a signal relay delay time) results in lower signal velocities, ranging from ca. 160–300 μm/min (MacKay, 1978; Lit¸canu and Velázquez, 2006). However, intra- and extracellular PDE concentrations were found to decrease in denser cell populations (Grutsch and Robertson, 1978).

Data on actual cAMP signalling parameters in *Dictyostelium* cultures vary widely. Signal wave frequency and velocity are inversely related during development (frequency increasing and velocity falling): the dispersion relation (Tyson and Murray, 1989; Siegert and Weijer, 1989). Optical density waves, propagating along aggregating streams, demonstrate frequencies of 1–0.5/min and speeds of about 100 μm/min (Gottmann and Weijer, 1986). Dormann et al. (2002) reported a signal speed of ca. 90 μm/min and incidentally demonstrated cAMP signalling in early streams. Early aggregation-stage cultures display frequencies as low as 0.2–0.1/min (Durston, 1974). Gerisch (1965) measured the signal wave velocity in such cultures at 43 μm/min. Cyclic AMP wave fronts were recorded fluorographically and estimated at ca. 1.0 μM maximal concentration in 300–1000 μm-wide bands, which passed across a cell in 1–3 min (coincident with the cell locomotion step phase) and propagated at 300 μm/min (Tomchik and Devreotes, 1981). Gingle (1976) found that the signal ranged up to 45 μm (3–4 cell lengths). Alcantra and Monk (1974) reported a 12 s signal relay time delay and calculated the signal range at about 57 μm (4–6 cell