COMMENTARY

Inositol phosphates, G-proteins and *ras* genes involved in chemotactic signal transduction of *Dictyostelium*

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Introduction

Chemotaxis requires some form of signal transduction system (or systems) to convert extracellular messages into intracellular actions. In the amoebal organism *Dictyostelium discoideum* two pathways of signal transduction have been found to operate, one involves adenylate cyclase for signal relay, and the other, which forms the subject of this review, involves inositol phosphates (Fig. 1).

In brief, the binding of the chemoattractant cyclic AMP to the cell surface receptors induces formation of inositol trisphosphate, which releases Ca^{2+} from (non-mitochondrial) intracellular stores. This ion can trigger rapid cytoskeletal actin polymerization and the formation of a pseudopodium within 5 s, and can also induce transient cyclic GMP accumulation (peaking at 10 s), which leads to association of myosin with the cytoskeleton and consequent cell elongation and movement.

Inositol phosphates

Evidence that formation of inositol trisphosphate ($InsP_3$) is involved in chemotactic signal transduction has been obtained from three types of experiment. First, when added to amoebae that had been permeabilized by treatment with saponin, it mimicked the action of chemoattractant added to non-permeabilized cells. At less than 5 μM, Ins(1,4,5)P_3 induced cytoskeletal actin polymerization and formation of cyclic GMP to a similar extent to that brought about by cyclic AMP (Europe-Finner & Newell, 1985, 1986*a*; McRobbie & Newell, 1984*a*). Second, when Ins(1,4,5)P_3 was added to permeabilized cells it rapidly released calcium from non-mitochondrial stores. This was demonstrated using amoebae that had taken up $^{45}Ca^{2+}$ in the presence of mitochondrial inhibitors and ATP plus an ATP-regenerating system. When Ins(1,4,5)P_3 was added to such amoebae, approximately 70% of the Ca^{2+} that had been taken up was rapidly released (Europe-Finner & Newell, 1986*b*). The degradation product of Ins(1,4,5)P_3, inositol(1,4)bisphosphate, had no effect. The third type of evidence came from using non-permeabilized amoebae labelled with [^{3}H]inositol. When labelled cells were stimulated with 50 nM cyclic AMP it was found that there was a rapid increase in the flux through the inositol phosphate pathway and the levels of $InsP_3$ and higher inositol polyphosphates rose to a value that was 80% higher than the pre-stimulus value within 5 s of stimulation (Europe-Finner & Newell, 1987*a*).

The role of Ca^{2+}

It was argued that, if $InsP_3$ operated by releasing Ca^{2+} from intracellular stores, its observed action of polymerization of actin and stimulation of cyclic GMP formation should be mimicked by addition of Ca^{2+} directly to permeabilized amoebae. This was found to be correct. Ca^{2+} added to amoebal suspensions caused polymerization of cytoskeletal actin ($K_{1/2} = 10\,\mu$M) and formation of cyclic GMP ($K_{1/2} = 60\,\mu$M) in the same manner as did Ins(1,4,5)P_3 (Small *et al.* 1986; Europe-Finner & Newell, 1986*a*; Newell *et al.* 1987). The mechanism of the Ca^{2+} action on actin polymerization is not currently understood but may be mediated by one of the Ca^{2+}-sensitive actin-binding proteins that are present (Fechheimer & Taylor, 1984; Fechheimer, 1987). How Ca^{2+} could mediate cyclic GMP formation, however, seems at present to be more of a mystery, as guanylate cyclase seems not to be affected by this ion *in vitro* (Padh & Brenner, 1984) so it seems probable that intermediary steps between Ca^{2+} release and guanylate cyclase are involved.

G-proteins

The involvement of G-proteins in this pathway was found using GTP and non-hydrolysable GTP analogues. When added to permeabilized amoebae, GTP

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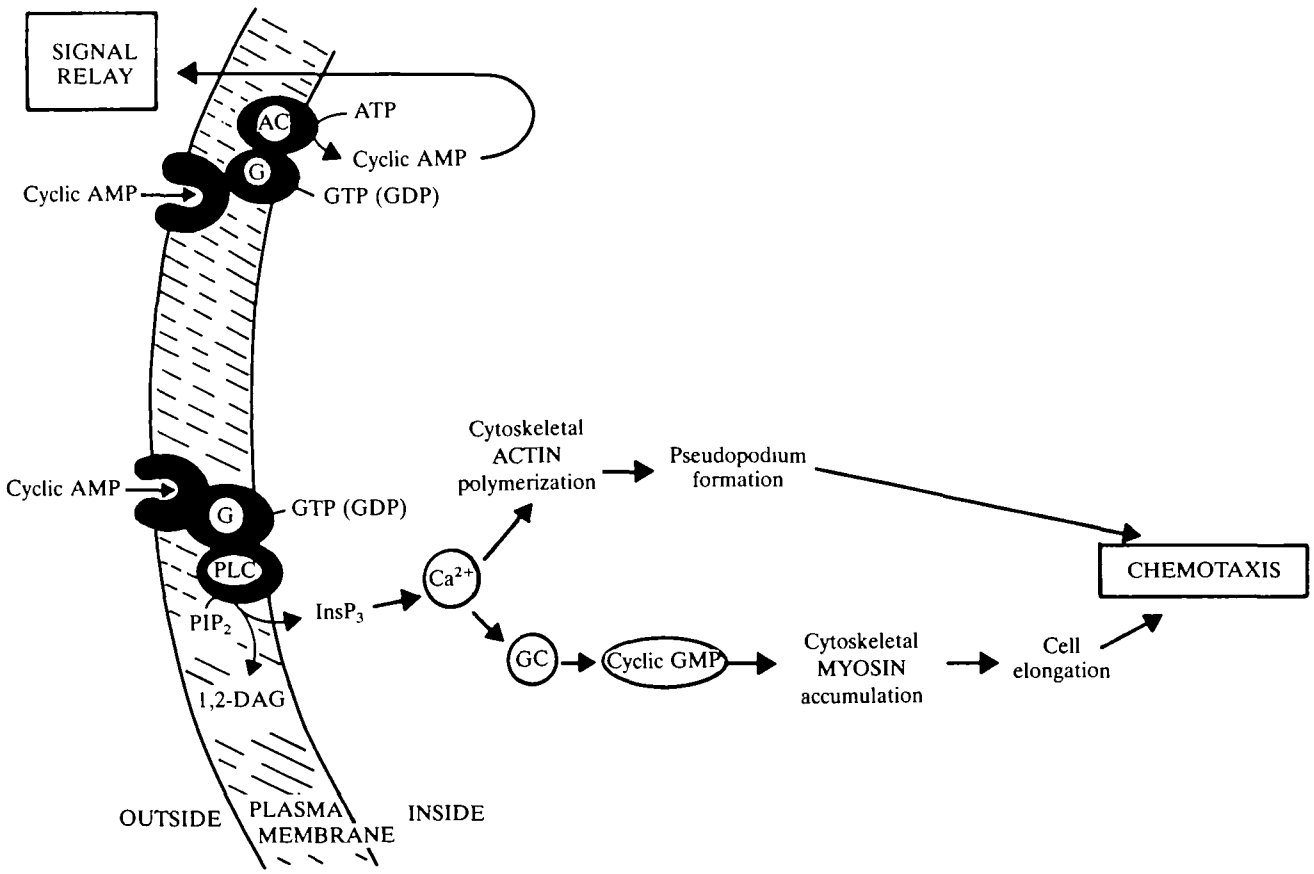

Fig. 1. Signal transduction pathways leading from cell surface cyclic AMP receptors to regeneration of the cyclic AMP signal (signal relay), and to events of chemotaxis such as pseudopodium formation and cell elongation. Evidence for the chemotactic pathway *via* inositol phosphates is summarized in this paper. Higher inositol polyphosphates such as $InsP_6$ are also involved in this signalling pathway (Europe-Finner *et al.* 1988) but their precise role and route of formation have not yet been elucidated. Evidence for the existence of different classes of cyclic AMP receptors for signal relay and for chemotaxis is from Van Haastert (1985). Abbreviations: G, GTP/GDP-binding protein; AC, adenylate cyclase; PIP_2, phosphatidylinositol 4,5-bisphosphate; 1,2-DAG, 1,2-diacyl glycerol; $InsP_3$, inositol(1,4,5)trisphosphate; GC, guanylate cyclase.

and the analogues GTPγS and GppNHp provoked formation of inositol polyphosphates similar to that induced by cyclic AMP, whereas GDP had no significant effect (Europe-Finner & Newell, 1987*b*). A particularly interesting aspect of the data was that in synchronized cell populations, both cyclic AMP and GTPγS induced not just one inositol polyphosphate peak but an oscillation, with peaks at 10–15 s intervals. In the case of GTPγS these oscillations were persistent and after about 300 s (when synchrony had been lost) apparently resulted in an elevated basal level compared to the unstimulated control (Europe-Finner & Newell, 1987*b*). When the level of cyclic GMP was measured in such GTPγS-stimulated amoebae, peaks of cyclic GMP could be seen, which corresponded in their timing with the inositol polyphosphate peaks seen previously (Small *et al.* 1987). Evidence for a G-protein has also been reported by Van Haastert *et al.* (1986) who showed that binding of cyclic AMP to the fraction of cell surface receptors coupled to cyclic GMP formation is sensitive to guanine nucleotides.

The G-protein in this pathway has also been implicated in the phenomenon of adaptation (Small *et al.* 1987). Adaptation to chemotactic stimuli is observed as the failure of a second stimulus to evoke a second chemotactic response (such as cyclic GMP formation) unless the concentration of the second stimulus is higher than the first. Thus, two stimuli of the same concentration of cyclic AMP given 30 s apart, produce only a response to the first stimulus. With permeabilized amoebae it was possible to try various compounds such as Ins(1,4,5)P_3, GTPγS or Ca^{2+} for their ability to bypass the adaptation block and so trigger a second response. The results of these experiments showed that Ins(1,4,5)P_3, and Ca^{2+} were capable of eliciting such a second response in amoebae adapted to a saturating

dose of cyclic AMP and were therefore downstream of the adaptation block. However, GTPγS was unable to bypass the block and must, therefore, be upstream of it. As GTPγS does not cause adaptation (as demonstrated by its stimulation of multiple peaks of cyclic GMP formation) the phenomenon of adaptation cannot be explained by increased GTP binding to the G-protein but must be due to some other receptor–G-protein interaction that results from receptor occupation by the cyclic AMP.

The *Dictyostelium ras* gene

D. discoideum has been found to possess a *ras* gene that is highly homologous to the human *ras* gene (Reymond *et al.* 1984, 1985, 1986), and it codes for a protein (p23) that reacts with monoclonal antibodies to the $p21^{v\text{-}ras}$ protein of Harvey murine sarcoma virus (Pawson *et al.* 1985; Weeks & Pawson, 1987). In mammalian cells, an oncogenic form of the normal *ras* gene is produced if it is mutated so that it codes for a p21 protein with an amino acid such as threonine in place of glycine at position 12 (Seeburg *et al.* 1984). In the case of the *D. discoideum ras* gene, Reymond *et al.* (1986) used site-directed mutagenesis to mutate the gene to produce a threonine substitution at position 12 (*ras*-Thr_{12}) instead of the normal glycine at this position (*ras*-Gly_{12}) and transformed *D. discoideum* amoebae with plasmids bearing the mutant or normal genes. The phenotype of the resulting strains clearly showed that while multiple copies of the normal *ras*-Gly_{12} gene had no effect, similar transformation by the *ras*-Thr_{12} gene adversely affected chemotaxis. At the molecular level, it was found that the activity of the adenylate cyclase relay system was unaffected but the ability to produce cyclic GMP in response to chemotactic stimuli was reduced (Van Haastert *et al.* 1987). Experiments investigating the effect on phosphoinositol metabolism have recently indicated that, while amoebae transformed with *ras*-Gly_{12} are normal in their formation of inositol phosphates, those transformed with the mutant *ras*-Thr_{12} gene show a three- to fivefold increase in the basal level of $Ins(1,4,5)P_3$ and higher polyphosphates such as $InsP_6$ (Europe-Finner *et al.* 1988). Oscillations in $InsP_3$ and polyphosphates could be induced by cyclic AMP stimulation but were aberrant and started from the higher basal level. They were therefore probably less effective, which might explain the reduced cyclic GMP response.

The position of the *ras* protein in the signal transduction chain is unknown at present, but, as has been found for human cells (Wakelam *et al.* 1986) it clearly plays an important and intriguing part in regulation of inositol metabolism.

The role of cyclic GMP

Precisely what function cyclic GMP has in the signal transduction chain is another intriguing problem that has stimulated much thought. Its involvement in chemotaxis has been inferred from its transient formation in response to various chemoattractants in several strains (Mato *et al.* 1977; Wurster *et al.* 1977) and its abnormal formation in certain 'streamer' mutants that show aberrant chemotaxis (Ross & Newell, 1981). However, its role has very recently been defined more precisely by work of Liu & Newell (unpublished results), which indicates that it has its effects by regulating the interaction of myosin with the cytoskeleton. In the wild type a cyclic AMP stimulus induces the association of myosin with the cytoskeleton in a characteristic pattern with a sharp peak of accumulation at 20–25 s. The streamer mutants used in this study (streamer F mutants) have a defect in the structural gene for the cyclic-GMP-specific phosphodiesterase. This defect causes them to form a peak of cyclic GMP in response to a cyclic AMP stimulus that persists approximately five times longer than in the wild-type strain (which shows a peak at 10 s and complete degradation by 25 s) (Ross & Newell, 1981; Van Haastert *et al.* 1982; Coukell & Cameron, 1985, 1986). When the level of myosin heavy chain associated with the Triton-insoluble cytoskeleton was determined in the wild type and streamer F mutants, it was found that their pattern of cytoskeletal myosin accumulation closely resembled the pattern of cyclic GMP formation formed in these cells, with the streamer mutants having a persistent myosin response that lasted about five times longer than in the wild-type strains (Liu & Newell, unpublished results). Such a link between cyclic GMP and cytoskeletal myosin helps explain the most obvious phenotype of the streamer mutants. Seen as monolayers on agar using dark-field optics, the mutants show a prolonged period of cell elongation visualized as a wide bright band lasting for up to 500 s in response to cyclic AMP signals from the aggregation centre, rather than the normal wild-type phenotype of a narrow bright band due to the phase lasting for only 100 s (Ross & Newell, 1981).

It is of interest that it is only the myosin that appears to be altered in the streamer F mutants, whereas the cytoskeletal actin is unchanged in these strains (McRobbie & Newell, 1984*b*; Liu & Newell, unpublished results). This difference confirms the dichotomy in the signal transduction chain after Ca^{2+} liberation indicated in Fig. 1.

A primitive eukaryotic system retained through evolution

An astonishing aspect of the studies described above is how similar the pathways found for this primitive

eukaryote are to those that have been discovered in mammalian cells. For example, the relay response with adenylate cyclase is like the β-adrenergic system, and the inositol phosphate pathway with its G-protein and intracellular Ca^{2+} liberation is much like the α-adrenergic system. The finding that the structure and function of the *Dictyostelium ras* gene are also so similar to those found in mammalian cells completes a picture of the pathways having been worked out in these amoebal cells a very long time ago and retained over a large evolutionary period. The primary purpose in the slime mould amoebae was probably as part of a food-seeking system using folate as chemoattractant (a system still used by *D. discoideum* in the growth phase) and this became modified for chemotactic aggregation of the cells by substitution of a cyclic AMP receptor coupled to the same transduction chain. In higher organisms this pathway has become modified for many purposes and the cell surface receptors changed accordingly. The finding that the transduction chains have largely remained intact suggests strongly that they were perfected long before mammalian cells and their complex interactions were envisaged.

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