Cell 62, 413–423
14 Weiss, R.B., Dunn, D.M., Shu, M., Atkins, J.F. and Gesteland, R.F. (1989) *New Biol.* 1, 159–169
15 Carter, C. and Zybarth, G. (1994) *Meth. Enzymol.* 241, 227–253
16 Mizrohki, L.J., Georgieva, S.G. and Ilyin, Y.V. (1988) *Cell* 54, 685–691
17 Pardue, M.L. (1994) *Curr. Opin. Genet. Dev.* 4, 845–850
18 Muller, H.J. (1938) *Collecting Net* 13, 182–198
19 Valgeirsdottir, K., Traverse, K.L. and Pardue, M.L. (1990) *Proc. Natl Acad. Sci. USA* 87, 7998–8002

M.L. PARDUE (mlpardue@mit.edu), O.N. DANILEVSKAYA, K. LOWENHAUPT, F. SLOT AND K.L. TRAVERSE ARE IN THE DEPARTMENT OF BIOLOGY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MA 02139, USA.

REVIEWS

Signaling through chemoattractant receptors in *Dictyostelium*

MEI-YU CHEN, ROBERT H. INSALL AND PETER N. DEVREOTES

Dictyostelium discoideum displays chemoattractant-directed cell migration typical of many higher cell types. Signaling through chemoattractant receptors involves a standard G-protein-linked pathway. Genetic analysis has distinguished essential and dispensable components and demonstrated that some signaling events are independent of G proteins. Genetic analysis has also led to the identification of additional genes involved in chemosensory transduction. Further studies on the newly discovered components and pathways should help in elucidating the molecular mechanisms of eukaryotic chemotaxis.

Chemotaxis is a fascinating phenomenon whereby motile cells sense and respond directionally to chemical gradients. Chemotactic phenomena have important functions in inflammation and diapedesis, wound healing, angiogenesis, metastasis and axonal targeting. Free-living cells of the simple eukaryote, *Dictyostelium discoideum*, provide an excellent subject for genetic analysis of this process. The chemotactic behavior displayed by these amoeboid cells is most similar to that observed in mammalian phagocytic cells, such as neutrophils and macrophages[1]. The amoebae respond to folic acid, platelet activating factor (PAF)[2], lysophosphatidic acid (LPA)[3] and cAMP. Leukocytes respond to PAF, LPA and a variety of polypeptide chemokines[4,5]. Remarkably, the spectrum of biochemical reactions elicited by these diverse attractants are quite similar in these evolutionarily distant cells. Moreover, both types of cells utilize molecular components of a G-protein-linked pathway to carry out the signal transduction (Box 1; reviewed in Ref. 1).

This observation might indicate that many of these signal transduction events are essential for chemotaxis. However, some of the reactions initiated by chemoattractants are involved in processes that are designed to serve particular physiological functions other than chemotaxis. For example, in leukocytes chemoattractants cause granular secretion and activation of superoxide production, which help to achieve the maximum efficiency in bactericidal and digestive activities[1]. Because chemoattractants elicit a range of physiological events, it is often difficult to determine which responses are essential for the oriented movement of the cells. To add to the quandary, a variety of paracrine hormones and growth factors (such as TGF-β, PDGF and FGF) that do not signal through G proteins, can act as 'chemoattractants' when presented in gradients[6–8].

When the nutrient source is depleted, the sensitivity of *Dictyostelium* amoebae to compounds that aid them in seeking bacteria disappears and a developmental program is initiated. At many stages in this program, extracellular cAMP acts not only as a chemoattractant but also as a mediator of cell–cell signaling.

Box 1. Chemoattractant receptors and G proteins

- cAR1–4 in *Dictyostelium* and the 18 cloned chemokine receptors in leukocytes are G-protein-linked receptors. The several hundred members of this superfamily, including many hormone and neurotransmitter receptors, all have a seven transmembrane helix topology. It is thought that the binding of ligands to sites on the extracellular loops, or within a pocket formed by the seven transmembrane domains, disrupts interactions between the helices and transmits a conformational change to the cytoplasmic loops.
- The excited receptors catalyze the exchange of GTP for GDP on the α-subunit of heterotrimeric G proteins. The α-subunit dissociates from the βγ-subunit complex; each component can act on a variety of effectors, which, in turn, pass the signal on and trigger a number of cellular events. A steady-state level of free α- and βγ-subunits is maintained as long as the excited receptor is present. Ligand-induced phosphorylation of the receptor by specific receptor kinases leads to its inactivation. The α-subunit then hydrolyses the bound GTP and the subunits reassociate to return to its resting state, thus, completing the cycle.
- In the absence of GTP, the receptor becomes locked to the heterotrimeric G protein and displays higher affinity for its ligand. Therefore, the capacity of GTP to decrease the affinity of agonist binding to the receptor is taken as a measure of G-protein–receptor coupling.

Copyright © 1996 Elsevier Science Ltd. All rights reserved. 0168-9525/96/$15.00

Propagated waves of extracellular cAMP coordinate the movements of up to 10^6 amoebae towards a common center. The resulting multicellular structure undergoes morphogenesis to form a fruiting body. The chemoattractant–receptor-mediated events, thus, also control differentiation and pattern formation (reviewed in Refs 9, 10).

The complex process of chemotaxis is not readily amenable to *in vitro* reconstitution; genetic analysis is, at present, the most useful tool for discovering the genes involved in this phenomenon. Because there appears to be an evolutionarily conserved 'package' of responses correlated with chemotaxis, studies of free-living amoebae, such as *Dictyostelium*, are applicable to the chemotactic behavior of a variety of eukaryotic cells. Considering that many of the known genes in the signal transduction pathways are conserved, the novel genes found by genetic analysis are expected to be present in higher eukaryotes also.

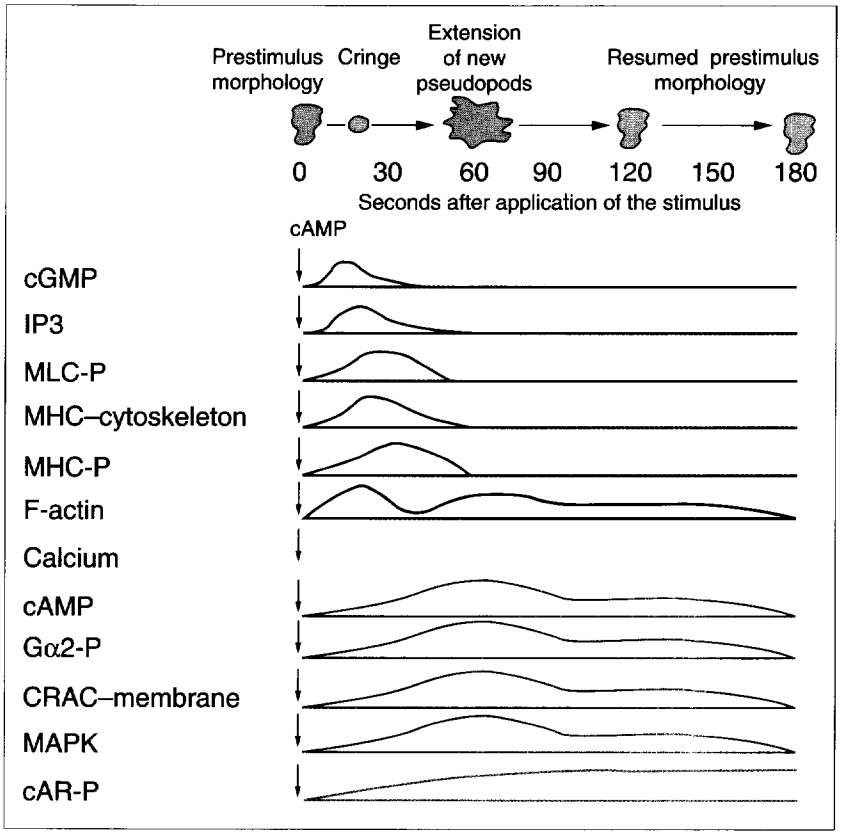


FIGURE 1. Responses to the application of cAMP. The morphological changes that cells undergo at given times after application of the stimulus are shown at the top. The profiles below the different morphological stages show the approximate time courses of different responses. Responses with similar kinetics are indicated by the same color. Abbreviations: cGMP, accumulation of cGMP; IP3, accumulation of IP_3; MLC-P, phosphorylation of myosin light chains; MHC–cytoskeleton, association of myosin heavy chain with the cytoskeleton; MHC-P, phosphorylation of myosin heavy chain; F-actin, the fraction of actin found as F-actin; calcium, intracellular calcium levels; cAMP, accumulation of cAMP or activation of adenylyl cyclase; Gα2-P, phosphorylation of Gα2 subunit; CRAC–membrane, association of CRAC with the membrane fraction; MAPK, activation of MAP kinase; cAR-P, phosphorylation of cAR1.

Chemoattractants elicit multiple transient responses

Eukaryotic cells employ several sensing mechanisms to orientate in a chemical gradient. Responses to chemoattractants are superimposed on a basal motility whereby cells extend pseudopods rhythmically at 30 s intervals. The chemoattractant elicits an immediate directional response at the side of a cell facing the higher concentration, rapidly establishing an orientation. The cell need not move in the gradient to determine the direction; it seems as if it can simultaneously monitor and compare concentrations at its ends. However, it is conceivable that undetected subcellular structures rapidly move in the gradient, sample the environment at two points and times, and make a comparison.

Chemotactic cells also have a capacity to keep track of previous chemotactic stimuli. Increments in attractant applied uniformly elicit a transient series of shape changes and accompanying biochemical reactions. Cells first 'cringe' or round up for about 20–30 s, then spread on the substrate by extending pseudopods in many directions. After a few minutes, the cells resume randomly motile behavior, even in the constant presence of the stimulus. Subsequent increments can elicit further responses; smaller increments induce briefer and smaller responses. These properties resemble those of the response regulators that provide a 'memory' for chemotaxis in bacteria[11], although the molecular basis is likely to differ. This system could serve to reinforce the initially weak pseudopod excursions in the appropriate direction and extinguish those in the wrong direction. The transient activity of a response regulator can be considered to result from a balance between a rapid excitation and a slower adaptation processes[1,9,11].

Within a few seconds of addition of extracellular cAMP to aggregation-competent amoebae, there occur a large number of physiological changes and biochemical reactions (Fig. 1) (Refs 1, 9, 12, 13; M. Maeda and R. Firtel, pers. commun.). Some responses peak at 5–30 s and return to baseline within 1 min, temporally correlating with the cringe response. These include the elevation of intracellular levels of cGMP and inositol 1,4,5-triphosphate (IP_3), the phosphorylation of the light chains of myosin I and II, translocation of myosin II to the cytoskeleton where its heavy chain is phosphorylated and a dramatic rise of the proportion of polymerized actin (F-actin). Chemoattractants induce a calcium influx, which begins after a 5 s lag and reaches a steady-state within 60 s; there is a transient rise in cytosolic calcium levels. Within 30 s of stimulation by cAMP, a talin homologue[12] has moved from cytosol to the tips of filopods. Some responses peak at 1–2 min and subside within 5 min after adding stimulus, correlating with the growth of new pseudopods and the transition from the cringe to

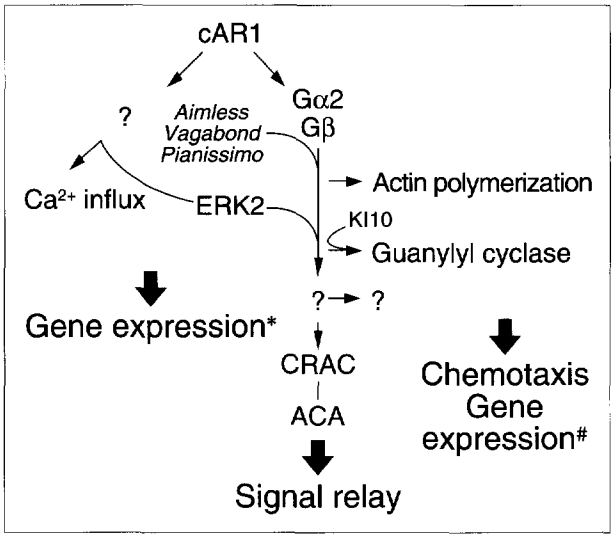


FIGURE 2. Signal transduction pathways in *Dictyostelium*. Shown are a standard G-protein-linked pathway, a cAR-mediated G-protein-independent pathway and several newly identified novel components that modulate the pathways. See text for details. Symbols: #, genes that respond to pulsatile condition of stimulation; *, genes that respond to continuous application of the stimulus.

a flattened amoeboid morphology. These include a second peak of F-actin content, the elevation of intracellular cAMP levels, the phosphorylation of a G-protein α-subunit, the recruitment of a cytosolic regulator of adenylyl cyclase to the membrane fraction[13] and the activation of a mitogen-activated protein kinase (MAP kinase) (M. Maeda and R. Firtel, pers. commun.). Some responses are persistent, including a proton release and the phosphorylation of the chemoattractant receptors.

The intensities of all of these responses depend on the increment in chemoattractant receptor occupancy. For responses that are transient, further increments induce subsequent responses; persistent responses are driven to higher steady-state levels by increased occupancy[1,9]. It appears that these transient responses are controlled by multiple excitation and adaptation processes because they have different time courses, and temperature and concentration dependencies. It is likely that certain of these events – for instance, actin polymerization – reflect the activity of the response regulator that controls the transient cell shape changes. Other events, such as the cGMP increases and myosin movements, might be part of the directional response that establishes cell orientation. Of course, a number of these responses could be expendable for chemotaxis.

Genetic analysis of the pathways leading to these events

Which of the responses triggered by chemoattractants are required for the various aspects of chemotaxis? In the past several years, a combination of genetic and biochemical studies have led to an understanding of some of the gene products involved in the generation of these chemoattractant-elicited responses. Figure 2 summarizes the interactions of the essential components.

A cell-surface cAMP receptor (cAR) is required for every response to extracellular cAMP. There are four cARs (cAR1–4) whose mRNA and protein levels are tightly regulated, and are transiently expressed at specific stages in the developmental program[10]. In early development, all the cells express cAR1 and cAR3; these receptors are partially-overlapping in function. Because cAR1 is the earliest expressed and the highest affinity receptor, its deletion prevents spontaneous aggregation. However, *car1*$^-$ cells eventually express cAR3 and, in order to ablate all responses to higher concentrations of exogenous cAMP, it is necessary to delete both genes[14]. cAR2 and cAR4 are expressed in the prestalk cells in the newly formed multicellular structures[15,16]. Cells lacking either of these receptors aggregate normally and then arrest in the multicellular stages of the developmental program[16,17]. cAR1, cAR2 and cAR3 (as well as a variety of cAR1–cAR2 chimeras and cAR1 point mutants) expressed constitutively in the *car1*$^-$ *car3*$^-$ double mutant, are each capable of restoring all of the cAMP-mediated responses (J. Milne, M. Caterina, J-Y. Kim and P. Devreotes, unpublished). The dose–response curves of all of the elicited responses are shifted according to the affinity of the expressed receptor. These observations strongly suggest that all of the physiological responses, such as chemotaxis, cell–cell signaling and gene expression (as well as all of the known elicited biochemical reactions) can be mediated by a single receptor. The observed differential receptor expression appears to be designed to switch the cellular sensitivity and the cell-type responding to the external cAMP gradient.

Eight different G-protein α-subunits (Gα1–8) and a single β-subunit (Gβ) have so far been cloned in *Dictyostelium*[18–20]. There is presumably a γ-subunit but it has not been cloned. Gα2 and Gβ are required for most, but not all, cAR-mediated responses. In the absence of either subunit the cAMP-elicited responses in cGMP, cAMP or IP_3 production, actin polymerization and myosin phosphorylation, do not occur regardless of the type or level of the expressed cAR. The typical high affinity GTP-sensitive agonist-binding sites normally associated with these G-protein-coupled receptors (Box 1) are diminished or lost[21,22]. These mutants, of course, are unable to carry out cAMP chemotaxis or the cell–cell signaling response that underlies the propagation of cAMP waves. The *gα2*$^-$ cells, however, retain sensitivity to folic acid[21]. Deletion of the gene encoding Gα4 yields a reciprocal phenotype: responses to folic acid are lost and those to cAMP are retained[23]. These observations suggest that G2 and G4 are specifically associated with the cARs and folic acid receptors, respectively. In *gβ*$^-$ cells, chemotaxis to all chemoattractants is lost[22].

The significance of other G-protein α-subunits was also assessed by gene disruption using homologous recombination. Deletion of the genes for Gα1, Gα5, Gα7 and Gα8 each resulted in a cell line that has nearly wild-type characteristics of growth and development (Refs 20, 21, 24, 25; R. Firtel, pers. commun.). Often these genes are expressed in a single cellular subtype. Only subtle phenotypes and slight biochemical differences have been reported. These observations suggest either that each of these G-protein α-subunits is functionally redundant with another α-subunit or that the physiological role is too subtle to be easily detected under standard laboratory conditions. It is noted that, consistent with

the redundancy idea, overexpression of activated forms of Gα1 and Gα7 did result in organisms displaying abnormal morphogenesis or altered gene expression but this finding has not been pursued[24,25].

For the majority of the G2-mediated responses it is not known whether it is the activated α- or freed βγ-subunits that carries the signal downstream. That is, Gα2 might be required merely to couple the release of the βγ-complex to activation of the cARs and might not directly interact with effectors. For the adenylyl cyclase, ACA, the following evidence suggests that it is, indeed, the βγ-subunits that transmit the signal for activation of the enzyme. *In vivo*, Gα2 and Gβ are both required for cAMP-elicited production of cAMP. However, *in vitro*, GTPγS can elicit activation of ACA equally well in wild-type and *gα2*$^-$ cells, presumably by releasing βγ-subunits from other heterotrimeric G-proteins. Consistently, deletion of the unique β-subunit completely prevents GTPγS activation[22].

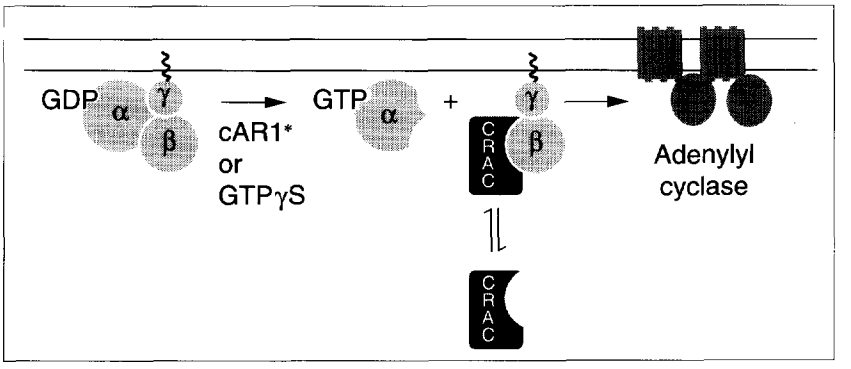


FIGURE 3. A model for CRAC-facilitated activation of ACA. The activated cAR1 (cAR1*) or GTPγS catalyzes the dissociation of βγ-subunits from the heterotrimeric G-protein complex. The cytosolic CRAC translocates to the membrane and interacts with the freed βγ-subunits. The βγ-subunits–CRAC complex then acts directly to activate adenylyl cyclase. Other models requiring both βγ-subunits and CRAC are also possible (see text).

The involvement of G-protein βγ-subunits in activation of ACA is analogous to the participation of βγ-subunits in activation of mammalian type II adenylyl cyclase[26]. However, the pathway linking the chemoattractant receptors to ACA displays unparalleled complexity. Activation requires the additional participation of the cytosolic regulator of adenylyl cyclase (CRAC)[27]. CRAC is soluble in lysates of resting cells but is recruited to the membrane fraction in response to brief pretreatment of the cells with cAMP or addition of GTPγS to the lysates (Fig. 3)[13]. This translocation requires the β-subunit but is independent of ACA itself. CRAC contains a pleckstrin homology (PH) domain[28] and several PH-domain-containing proteins have recently been reported to translocate to the membrane[29,30]. It remains to be seen whether there is a direct interaction of CRAC with the G-protein βγ-subunits.

Interestingly, there appears to be another layer of regulation that prevents the G protein from being activated or blocks between the activated G protein and the formation of the CRAC-binding sites on membranes. GTPγS does not stimulate the translocation of CRAC in lysates of cells that have been adapted by chemoattractant pretreatment for 10 min (Ref. 13). Therefore, the response regulator involved in the transient activation of ACA acts by controlling the transient appearance of CRAC binding sites. The regulation of the CRAC-binding sites might serve as an indicator of the activation of the G-protein-coupled pathway linked to cell motility and gene expression. Understanding the mechanisms involved in the transient appearance of these sites could provide an insight to the response regulator that drives many of the transient responses.

Chemoattractant-elicited changes of intracellular cGMP levels appear to play a significant role in chemotactic orientation (Fig. 2). In a mutant lacking a cGMP-specific phosphodiesterase (streamer F), cAMP elicits a large and prolonged peak of cGMP accumulation, which correlates with an exaggerated persistent polarization of the cells[31]. A series of mutants, obtained by chemical mutagenesis in a screen for defects in chemotaxis both to cAMP and to folic acid, also strongly point to cGMP as an intermediate in chemotaxis[32,33]. One of the mutants, designated KI-8, contains little or no cGMP; another, designated KI-10, does not elevate cGMP in response to chemoattractant stimulation. As a consequence, the stimulus does not trigger a translocation of myosin II to the cell cortex in these mutants. Both mutants elevate cAMP in response to cAMP and attain some of the properties of aggregation competence. Importantly, they generate a polymerization of actin in response to stimulation. Thus, the mutants appear to have a functional response regulator but this alone is not sufficient for chemotaxis. They might be defective in the directional response.

A single phospholipase C (PLC) that resembles mammalian PLC_δ has been cloned in *Dictyostelium*[34]. This PLC is apparently an effector of Gα2 (see above). Disruption of the *plcδ* gene resulted in cells that cannot elevate IP_3 in response to chemoattractants, yet there are no physiological consequences: the cells display an essentially wild-type developmental phenotype[34,35].

G-protein-dependent versus -independent pathways

Does all signaling via seven transmembrane receptors require G proteins? The long standing dogma (Box 1) suggests that it must. However, analysis of the G-protein-subunit mutants has shown that certain cAR-mediated responses occur in the absence of functional G proteins. The first of these responses was the chemoattractant-receptor-operated calcium influx discovered by Milne and Coukell[36]. Since then, several additional responses have been observed to occur in these mutants. For instance, cAR-mediated activation of a MAP kinase occurs in the *gα2*$^-$ and *gβ*$^-$ cells (M. Maeda and R. Firtel, pers. commun.). Several different receptor-mediated gene expression events are also relatively unimpaired. A recent study shows that two key genes expressed at the onset of development, encoding the phosphodiesterase that degrades cAMP and a protein that inhibits its activity, are regulated by cAMP in a similar way in wild-type, *gα2*$^-$ and *gβ*$^-$ cells[37]. Moreover, in cells constitutively expressing a multicellular stage

transcription factor, the G-box binding factor (GBF), continuous application of cAMP will elicit the precocious expression of the early intermediate genes. This response, which essentially bypasses the early stages of the developmental program, occurs in both the *gα2*$^-$ and the *gβ*$^-$ cells[38].

Among these phenomena, the response to the receptor-operated calcium influx is the most fully investigated[39,40]. The most compelling evidence for G-protein independence relies on the *gβ*$^-$cells. While most of the responses both to folic acid and to cAMP are ablated in these mutants, the agonist-activated calcium influx persists. Several lines of evidence suggest that the receptors are not coupled to G proteins in these mutants. First, as noted previously, there are no GTP-sensitive high affinity cAMP-binding sites in membranes. Second, the EC_{50} for the cAR-mediated calcium influx response closely matches the dissociation constant for the binding to the lower affinity sites. These observations suggest that it is the uncoupled form of the receptor that mediates the influx. It is important to note that the maximal responses are typically 50% lower in the absence of the Gα2 or Gβ, suggesting that there is some contribution from the G2 pathway. Nevertheless, these findings suggest that the activated cARs can act as transducers coupling directly, or through intermediates that are not typical heterotrimeric G proteins, to effectors. The activated state of the receptor that couples to both pathways appears to be the same because an extensive series of point mutations in the third intracellular loop of cAR1 has not produced a mutant that is specifically blocked either in calcium influx or in any G-protein-dependent response (Ref. 41; J. Milne, M. Caterina and P. Devreotes, unpublished).

Novel components of the signaling pathways

How can investigators make further progress in understanding chemotaxis in eukaryotic cells? The development of restriction-enzyme-mediated integration (REMI)[42] has allowed *Dictyostelium* geneticists to carry out direct phenotypic screens. In REMI, a linearized plasmid is co-electroporated into the cell along with a restriction enzyme, which enhances the transformation efficiency more than 20-fold and generates insertions of the plasmid into genomic restriction sites in an apparently random manner. The genomic DNA is digested with a different enzyme, circularized and transformed into *Escherichia coli*; only the regions flanking the integrated plasmid are cloned. These sequences serve as a probe to identify a cDNA that is then used to rescue the phenotype of the original mutant.

This approach has led to the discovery of some novel and surprising genes that appear to modulate or interact with the G-protein pathways. Because signal transduction events are involved throughout the developmental program, alterations in phenotypes are expected at many stages. Mutants that cannot aggregate or do so aberrantly are likely to be of interest. Such mutants are next examined for the expression of all of the known components of the signal transduction pathways, an indication of a relatively specific novel defect.

Four mutants, designated *erk2* (*erkB*$^-$), *aimless* (*aleA*$^-$), *vagabond* (*vagA*$^-$) and *pianissimo* (*piaA*$^-$) were isolated in a screen for the aggregationless phenotype (Fig. 2). Although the phenotypes of these mutants are quite similar – impaired both in the activation of adenylyl cyclase and in chemotaxis – these are completely independent loci. ERK2 is one of several *Dictyostelium* MAP kinase homologs[43]. Aimless is a homolog of Cdc25p, the *Saccharomyces cerevisiae* Ras exchange factor (R. Insall and P. Devreotes, unpublished). The genomic sequences flanking the REMI insertion sites in *vagA*$^-$ and *piaA*$^-$ are very similar to ORFs of novel genes in human and yeast, respectively (M-Y. Chen and P. Devreotes, unpublished).

Further characterization of the first two mutants has been carried out (Ref. 43; M. Maeda and R. Firtel, pers. commun.; R. Insall and P. Devreotes, unpublished). Both in *erkB*$^-$ and *aleA*$^-$ cells, CRAC activity is similar to that in the wild type, suggesting that the lesion is earlier in the pathway. GTPγS stimulation of ACA in lysates is severely impaired, a defect that cannot be corrected by the addition of excess CRAC. This suggests that the mutants are defective in the generation of CRAC-binding sites or in the capacity of membrane associated CRAC to stimulate the enzyme. Interestingly, in wild-type cells, ERK2 is acutely activated by chemoattractants. As noted above, this stimulation is a G-protein-independent response: it still occurs in *gα2*$^-$ and in *gβ*$^-$ cells. Thus, appropriate activation of the adenylyl cyclase might require dual inputs: one G-protein-dependent and the other G-protein-independent through the MAP kinase. However, it is not yet known whether these novel genes are integral components of the signal transduction pathway. It is conceivable that the effects of the mutations on adenylyl cyclase activation and chemotaxis are because of a failure to express another essential component, for instance, an enzyme required for the modification of the β- or γ-subunit.

Several conclusions can be drawn from the phenotypes of these recently isolated mutants. First, the mechanism of regulation of ACA appears to be more complex than the current views of regulation of the mammalian adenylyl cyclases. However, it is worth pointing out that there are many types of mammalian cyclases and all of the modes of regulation are not yet known. Second, although intracellular cAMP is not necessary for chemotaxis, the pathways leading to activation of adenylyl cyclase and chemotaxis appear to be intertwined – multiple genes affect both. This could be because these gene products regulate the activity of common G-protein subunits that, in turn, regulate effectors involved in each response. In effect, the same response regulator could drive both responses. Third, because many of the novel genes are developmentally regulated, it is unlikely that they are also involved in chemotaxis to the growth stage attractants, such as folic acid.

Conclusions

The G-protein-linked signal transduction paradigm appeared early in the evolution of eukaryotic cells. Components of these pathways are essential for chemotaxis, cell–cell signaling and gene expression processes that mediate morphogenesis and pattern formation during the developmental program of *Dictyostelium*. The general features of chemotaxis in these amoeboid cells are similar to those in mammalian leukocytes. This

genetically tractable system, thus, offers the possibility of using random mutagenic approaches to study the structure and function of receptors, G-protein subunits and effectors, as well as to discover novel genes and linkages that might not readily present themselves in biochemical studies. For example, these genetic analyses have led to the discovery of G-protein-independent signaling pathways, and several novel and ubiquitous genes involved in chemotaxis. Further genetic and biochemical dissection of components of the chemoattractant receptor-mediated signaling pathway promises new insights into this interesting process of directed cell migration.

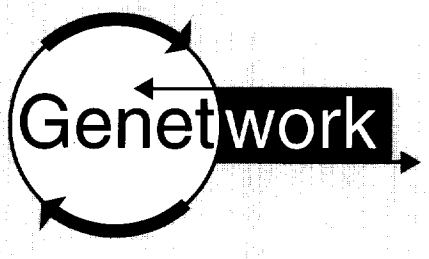
Acknowledgements

We thank Dr Dale Hereld for design and construction of Fig. 3. We thank Dr Sally Zigmond for stimulating discussions. This work was supported by GM28007 to P.N.D. and a Merck predoctoral fellowship to M.Y.C.

References

1 Devreotes, P.N. and Zigmond, S.H. (1988) *Annu. Rev. Cell Biol.* 4, 649–686
2 Sordano, C. *et al.* (1993) *J. Cell Sci.* 104, 197–202
3 Jalink, K., Moolenaar, W.H. and Van Duijn, B. (1993) *Proc. Natl Acad. Sci. USA* 90, 1857–1861
4 Murphy, P.M. (1994) *Annu. Rev. Immunol.* 12, 593–633
5 Chettibi, S., Lawrence, A.J., Stevenson, R.D. and Young, J.D. (1994) *FEMS Immunol. Med. Microbiol.* 8, 271–281
6 Adams, D.H. *et al.* (1991) *J. Immunol.* 147, 609–612
7 Shure, D., Senior, R.M., Griffin, G.L. and Deuel, T.F. (1992) *Biochem. Biophys. Res. Commun.* 186, 1510–1514
8 Grant, M.B. *et al.* (1992) *Invest. Ophthalmol. Vis. Sci.* 33, 3292–3301
9 Caterina, M.J. and Devreotes, P.N. (1991) *FASEB J.* 5, 3078–3085
10 Devreotes, P.N. (1994) *Neuron* 12, 235–241
11 Springer, M.S., Goy, M.F. and Adler, J. (1979) *Nature* 280, 279–284
12 Kreitmeier, M., Gerisch, G., Heizer, C. and Muller-Taubenberger, A. (1995) *J. Cell Biol.* 129, 179–188
13 Lilly, P. and Devreotes, P. (1995) *J. Cell Biol.* 129, 1659–1665
14 Insall, R.H., Soede, R.D.M., Schaap, P. and Devreotes, P.N. (1994) *Mol. Biol. Cell.* 5, 703–711
15 Yu, Y. and Saxe, C.L., III *Dev. Biol.* (in press)
16 Louis, J.M., Ginsburg, G.T. and Kimmel, A.R. (1994) *Genes Dev.* 8, 2086–2096
17 Saxe, C.L., III *et al.* (1993) *Genes Dev.* 7, 262–272
18 Wu, L. and Devreotes, P.N. (1991) *Biochem. Biophys. Res. Commun.* 179, 1141–1147
19 Lilly, P., Wu, L., Welker, D.L. and Devreotes, P.N. (1993) *Genes Dev.* 7, 986–995
20 Wu, L. *et al.* (1993) in *Handbook of Experimental Pharmacology, Vol. 108/II GTPase in Biology II* (Dickey, B.F. and Birnbaumer, L., eds), pp. 335–349, Springer-Verlag
21 Kumagai, A., Hadwiger, J.A., Pupillo, M. and Firtel, R.A. (1991) *J. Biol. Chem.* 266, 1220–1228
22 Wu, L., Valkema, R., Van Haastert, P.J.M. and Devreotes, P.N. (1995) *J. Cell Biol.* 129, 1667–1675
23 Hadwiger, J.A., Lee, S. and Firtel, R.A. (1994) *Proc. Natl Acad. Sci. USA* 91, 10566–10570
24 Dharmawardhane, S., Cubitt, A.B., Clark, A.M. and Firtel, R.A. (1994) *Development* 120, 3549–3561
25 Wu, L. *et al.* (1994) *Mol. Biol. Cell.* 5, 691–702
26 Tang, W-J. and Gilman, A.G. (1991) *Science* 254, 1500–1503
27 Lilly, P.J. and Devreotes, P.N. (1994) *J. Biol. Chem.* 269, 14123–14129
28 Insall, R. *et al.* (1994) *J. Cell Biol.* 126, 1537–1545
29 Pitcher, J.A., Touhara, K., Payne, E.S. and Lefkowitz, R.J. (1995) *J. Biol. Chem.* 270, 11707–11710
30 Li, T. *et al.* (1995) *Immunity* 2, 451–460
31 Newell, P.C. and Liu, G. (1992) *Bioessays* 14, 473–479
32 Kuwayama, H., Ishida, S. and Van Haastert, P.J.M. (1993) *J. Cell Biol.* 123, 1453–1462
33 Liu, G., Kuwayama, H., Ishida, S. and Newell, P.C. (1993) *J. Cell Sci.* 106, 591–596
34 Drayer, A.L. and Van Haastert, P.J.M. (1992) *J. Biol. Chem.* 267, 18387–18392
35 Drayer, A.L., Van der Kaay, J., Mayr, G.W. and Van Haastert, P.J.M. (1994) *EMBO J.* 13, 1601–1609
36 Milne, J.L. and Coukell, M.B. (1991) *J. Cell Biol.* 112, 103–110
37 Wu, L. *et al.* (1995) *Dev. Biol.* 171, 149–158
38 Schnitzler, G.R., Briscoe, C., Brown, J.M. and Firtel, R.A. (1995) *Cell* 81, 737–745
39 Milne, J.L. and Devreotes, P.N. (1993) *Mol. Biol. Cell* 4, 283–292
40 Milne, J.L.S., Wu, L., Caterina, M.J. and Devreotes, P.N. (1995) *J. Biol. Chem.* 270, 5926–5931
41 Caterina, M.J., Milne, J.L.S. and Devreotes, P.N. (1994) *J. Biol. Chem.* 269, 1523–1532
42 Kuspa, A. and Loomis, W.F. (1992) *Proc. Natl Acad. Sci. USA* 89, 8803–8807
43 Segall, J.E. *et al.* (1995) *J. Cell Biol.* 128, 405–413

M-Y. CHEN (mychen@welchlink.welch.jhu.edu) AND P.N. DEVREOTES (pnd@welchlink.welch.jhu.edu) ARE IN THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE, 725 N. WOLFE STREET, BALTIMORE, MD 21205, USA; R.H. INSALL IS IN THE MRC LABORATORY FOR MOLECULAR BIOLOGY AND DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF LONDON, GOWER STREET, LONDON, UK WC1E 6BT.



Genetwork is a regular column of news and information about Internet resources for researchers in genetics and development (see pp. 76–78). Genetwork is compiled and edited with the help of Steven E. Brenner (MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK CB2 2QH), and Jeremy Rashbass (Department of Histopathology, Addenbrooke's Hospital, Hills Road, Cambridge, UK CB2 2QQ).

If you would like to announce or publicize an Internet resource, please contact: TIG@elsevier.co.uk