Journal of Cell Science 107, 659-667 (1994)
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JCS2017

The regulation of chemotaxis and chemokinesis in *Dictyostelium* amoebae by temporal signals and spatial gradients of cyclic AMP

Michael G. Vicker

Department of Biology, University of Bremen, 28334 Bremen, Federal Republic of Germany

SUMMARY

The tactic and kinetic locomotion of *Dictyostelium discoideum* amoebae were examined in cyclic AMP (cAMP) spatial gradient and temporal signal fields. The distributions of migrating cells were examined within 150 μm-thick micropore filters after incubation with different cAMP concentrations, [cAMP], applied in three ways across the fields: as positively or negatively developing gradients, generated either by increasing or decreasing the [cAMP] on one side of the filter, respectively, or as static, linear gradients after negative development. Chemotaxis was only induced by oriented, temporally increasing [cAMP]. Pulses propagated by molecular diffusion or mechanical flow were equally effective. Negatively developing cAMP gradients had no initial effect on cell accumulation. However, if the subsequent static spatial gradient was maintained by an infusion system, some gradients also induced cell accumulation, whose degree and direction depended on the gradient [cAMP]. The basis of this new effect was examined by tracking individual cells by computer-assisted videomicroscopy during locomotion in different [cAMP]. Cells produced a triphasic [cAMP]-dependent response, with optimal cell motility induced by 10-30 nM. The results demonstrate that cell accumulation either up-field or down-field in spatial gradients is governed by the field locations of the attractant concentrations that induce the relative locomotory maxima and minima in the gradient field. Cells perceive the ambient [cAMP], but cannot read the spatial gradient orientation in static or yet steeper regions of developing gradients. Accumulation in static spatial gradients is a function of klino- and orthokinesis, but chemotaxis requires an oriented cAMP pulse or impulse. A mechanism of tactic signal perception is proposed in terms of the recently discovered intracellular oscillator, which determines cell shape and movement.

Key words: actin, biological time, intracellular oscillator, cell tracking, computer-assisted video-microscopy, cell locomotion

INTRODUCTION

Investigations into the role of cell accumulation in development and pathogenesis usually focus on how cells might detect and respond to directional signal information, e.g. during inflammatory cell migration (Zigmond, 1978; Wilkinson et al., 1984), tumor cell metastasis (Folkman, 1985; Liotta and Schiffman, 1988) and morphogenetic movements (Vicker, 1993) such as neuronal targeting (Letourneau, 1978; Gundersen and Barrett, 1980; Gierer, 1987; Bixby and Harris, 1991; Baier and Bonhoeffer, 1992). Spatial concentration gradients of chemical attractants have been universally suggested to constitute the signal that maneuvers cells toward a specific location or direction (Pfeffer, 1884; Leber, 1888; McCutcheon, 1946; Gerisch et al., 1975; Zigmond, 1978; Devreotes and Zigmond, 1988; Fisher et al., 1989; van Duijn and van Haastert, 1992; Wessels et al., 1992). Two models are usually discussed in regard to how slow, crawling cells, especially the polymorphonuclear neutrophil leukocyte (PMN) or amoebae of *Dictyostelium discoideum*, might read these 'chemotactic gradients': (a) 'spatially', between receptors spaced across the cell's length (Zigmond, 1974a), or (b) 'temporally', as receptors carried upon randomly projecting pseudopods encounter increasing attractant concentrations, they will induce that pseudopod to persist and direct the cell (Gerisch et al., 1975). However, these assumptions leave unresolved the two major problems of taxis theory.

The first concerns the identification of specifically tactic behaviour. At present, 'taxis' is accepted to mean both accumulation and/or one of the cellular operations that get cells there. The temporal gradient reading mechanism (Gerisch et al., 1975) is formally identical to the 'random probing' movements known to guide bacterial accumulation. Yet, the bacterial reaction is klinokinetic (Fraenkel and Gunn, 1961) rather than tactic, because bacterial turning directions are 'random' (McNab and Koshland, 1972; Berg and Brown, 1974; Berg and Tedesco, 1975). Bacterial behaviour is significant, because it exemplifies the induction of cell accumulation by apparently random locomotion. Thus, rather than associate 'taxis' with accumulation irrespective of mechanism, I will use it here to specifically denote only the oriented turning of a motile cell in response to an oriented signal (Vicker, 1990a).

Likewise, klinokinesis (an effect on locomotory persist) and orthokinesis (an effect on cell speed) denote responses with no intrinsic oriented turning (see also Lapidus and Levandowsky, 1981). The problem of identifying tactic behaviour is symptomatic of the lack of a functional relationship between cell behaviour and the intended theories.

The second problem involves the nature of the orienting signal, particularly the role of temporal signals (TS). There has been almost no direct investigation of orienting signals, and the evidence for a role for spatial gradients has not been rigorously derived. Molecules diffusing from a localized high concentration will, in the absence of perturbation, initially distribute themselves in a spatial gradient in the course of time. But this fact alone provides no information about the signal quality, if any, possessed by the spatial gradient. Temporal concentration changes are intrinsic to spatial gradients developing by molecular diffusion and they appear even in mature, 'linear' gradients in low-viscosity media due to turbulence unless precautions are taken (Vicker, 1981). However, only a few reports (e.g. Futrelle and Berg, 1972; Zigmond, 1977; Letourneau, 1978; Gundersen and Barrett, 1980; Vicker, 1981; Fisher et al., 1989) have documentated the spatio-temporal form of the gradients applied to cells. In order to investigate the relative roles of both attractant TSs and spatial gradients, tactic and kinetic behaviour were examined in both *D. discoideum* and PMNs (Vicker et al., 1984, 1986; Vicker, 1989, 1990b). The results of these studies indicated that only pulses and impulses induce chemotaxis, but that spatial gradients are not perceived by either cell type.

The present investigation is aimed at clarifying these two problems. Previous studies have left the relevance of TS, spatial gradients and specific modes of cell behaviour undifferentiated. This study concerns the behaviour of *D. discoideum*, a classical model of chemotaxis (Bonner, 1947; Devreotes and Zigmond, 1988; Fisher et al., 1989). After about 6 hours of starvation, a few scattered 'pacemaker' cells begin stimulating neighbouring cells by autonomously secreting rhythmic pulses of cyclic AMP (cAMP) about each 8 minutes (Alcantra and Monk, 1974; Durston, 1974; Gerisch et al., 1975). A cascade of rhythmic, adaptive reactions (van Haastert, 1983; Dinauer et al., 1980b) follows each cAMP pulse or 'wave' (Tomchik and Devreotes, 1981) in each deadapted cell, including the synthesis and secretion of cAMP and cAMP phosphodiesterase, PDE, (Shaffer, 1975; Gerisch, 1976; Grutsch and Robertson, 1978; Darmon et al., 1978; Dinauer et al., 1980b), increased cohesiveness (Wurster and Kurzenburger, 1989), actin polymerization (McRobbie and Newell, 1984; Hall et al., 1988) and taxis, which occurs throughout early aggregation (Alcantra and Monk, 1974; Gerisch et al., 1975) and later pattern formation (Clark and Steck, 1979; Siegert and Weijer, 1991, 1992). Pulses of cAMP induce the immediately peripheral population to locomote tactically and to amplify the signal (Shaffer, 1975; Dinauer et al., 1980a) and relay it distally (Alcantra and Monk, 1974; Gerisch et al., 1975). PMNs demonstrate similar reactions to attractant pulses or impulses (O'Flaherty et al., 1977; Zigmond and Sullivan, 1979; Fechheimer and Zigmond, 1983; Jesaitis et al., 1984). Significantly, this TS-dependent series of reactions in both cell types is not induced by static spatial gradients of attractant. In *Dictyostelium*, taxis ceases if cells are exposed to decreasing [cAMP], which provides a 'back of the wave' paradox for gradient-sensing mechanisms, because cells have been expected to react to the gradient here as well and move accordingly (Shaffer, 1957; Futrelle et al., 1982; Wessels et al., 1992).

The results presented here provide evidence that cells treated with gradients developing by increasing [cAMP], i.e. a positive TS, express chemotaxis, but not if the gradient is generated by decreasing an isotropic [cAMP]. However, it is demonstrated here for the first time that: (1) accumulation evolves in some negatively developing gradients if the incubation times are long enough; and (2) that the reaction pattern to TS-free gradients depends only on stochastic cell motility, which corresponds to the spatial pattern of [cAMP] in the gradient. The role of the newly identified intracellular oscillator of *D. discoideum* (Killich et al., 1993), which is independent of the better known cAMP oscillator, is also discussed in terms of the mechanism of TS perception and taxis.

MATERIALS AND METHODS

Cell culture

Dictyostelium discoideum strain NC4(H) (wild-type) amoebae were cultured on agar with *Escherichia coli* bacteria as nutrient for 48 hours. The cells were then washed three times in potassium phosphate buffer (KK_2, pH 6.4) until free of bacteria, plated on KK_2 agar and placed at 6°C for 16 hours until they reached the early aggregative stage of development (Vicker et al., 1984; Sussman 1987). Cells were washed once more before experiments.

Spatial-gradient chambers

Micropore filters provide a good migration field, because cells may move relatively freely within a bounded territory, thin enough to reduce the time necessary for evolution of equilibrium (even or accumulated) cell distributions. They also allow maintainance of steep, defined, low-perturbation spatial gradients after gradient development. If the law of diffusion is to operate as the sole determinant of gradient development and turbulence is to be suppressed, the gradient requires support by, e.g., a high viscosity medium, a gel or a micropore filter (Vicker, 1981). Cells (0.5×10^6) in 0.5 ml KK_2 were seeded onto the upper surfaces of micropore filters held in chambers (Fig. 1) turned on one side. Excess fluid was gently vacuum aspirated from below. The chambers were inverted after 5 minutes and cells added to the other filter surface. Chambers were righted 5 minutes later. An even population distribution was promoted by infusing both compartments at 21°C for 30 minutes with KK_2 then for 30 minutes with a [cAMP] (Boehringer, Mannheim) in KK_2 containing 2 mM dithioerythreitol (DTE, Boehringer, Mannheim) to inhibit PDE (Pannbacker and Bravard, 1972; Devreotes and Steck, 1979; Klein, 1981; Vicker et al., 1984).

Signal propagation

A positively developing cAMP gradient, i.e. a pulse, was generated in cells evenly distributed throughout filters after the pre-incubation infusion solution (1 nM cAMP) was removed by 3 KK_2 washes. The cells were then incubated 20 minutes in DCB, consisting of KK_2 including 2 mM DTE and 2 mM caffeine (Sigma, Diesenhofen) to inhibit cAMP relay (Pannbacker and Bravard, 1972; Siegert and Weijer, 1989), to allow deadaptation to cAMP. The compartments were drained and 630 µl of fresh DCB was readded to each. Then 70 µl of DCB was added to the right compartment while 70 µl of cAMP in DCB was rapidly mixed into the left compartment. After 40 seconds, filters were washed thrice with KK_2 and incubated 7 minutes in DCB before fixation. To generate negatively developing gradients, evenly spread populations were pre-incubated with the isotropic [cAMP] used

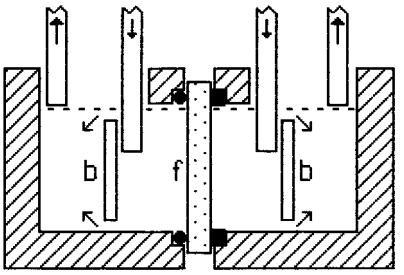

Fig. 1. A cross-section of a micropore-filter migration chamber made of two lucite blocks bolted flat together. The two compartments are separated by a 13 mm Ø nitrocellulose filter (f) of 5 μm pore size and nominal 150 μm thickness (Sartorius, Göttingen) between an O-ring and a flat gasket of neoprene. Glass baffles (b) guided pumped input flows across the filter surfaces. Input and output capillary glass tubing the maintained fluid volume, pressure and level and the [cAMP]. Developing spatial gradients, monitored spectroscopically using phenol red, stabilised at a steepness of >100:1 within 10 minutes (data not shown). Nominally, about 16 seconds is required for a static, linear gradient to develop under these conditions. A gradient steepness of 6.7% will develop across a cell of 10 μm length in such a filter, which is, thus, steeper then any other experimental static gradient field (Vicker, 1989, 1990b).

to produce the gradient (see below). After 30 minutes, the cAMP in the compartment on one side of the filter was replaced with DCB alone. Spatial gradient stability was maintained by infusing DCB or cAMP in DCB into each compartment by peristaltic pump at 0.5 ml/min and removing it at up to 2 ml/min from a single external pool connecting all compartments, to assure uniform fluid levels. Bulk media flow across the filter and within the compartments was suppressed by regulating the intra- and intercompartmental fluid levels and flow patterns, respectively. Even cell distributions were fostered and 'edge effects' avoided by the continuous infusion of fresh media into each compartment during pre-incubation and exposure to static gradients. Experiments were terminated by addition of 5% formalin for 16 hours.

Cell distributions

After fixation, filters were stained in Giemsa and mounted in Permount (Fisher, Philadelphia, USA) as before (Zigmond, 1974b; Vicker et al., 1984). Cell distributions were analysed using 18.75 μM optical sections, which terminated ~8 μM beneath each filter surface. Eight optical sections in each of 5-6 fields were examined per filter. The median position of all measured cells in each filter was calculated from the one-dimensional cell distribution in grouped form. Its significance was tested by applying the Wilcoxon signed rank test where Xnorm < –2.56 (>2.56) indicates a shift to the left, i.e. up-field, (right, i.e. down-field) at the 1% level of significance (Vicker et al., 1984).

Kinetic analysis

About 10^4 cells/ml in 2 μl KK_2, containing various [cAMP] to which the cells were adapted, were spread between a washed glass slide and coverslip separated by a ring of one parafilm sheet ~50 μm thick. A band of air was left around the droplet. Cell images were acquired every 6 seconds at 21°C with a CCD-500 video camera on a Zeiss Standard microscope (Oberkochen) with a ×20, 0.25 NA, F-LD phase-contrast objective at minimal illumination and a green filter. Between 5 and 15 cells in each video frame were tracked for 5 minutes. Cells were ignored if they remained immotile, left or entered the field or collided with another cell. The images were digitized using an AT computer with coprocessor and a FG-100-AT-1024 video digitizer (Imaging Technology; Woburn, Mass., USA) employing a program written by F. Siegert (Siegert and Weijer, 1989), which calculated the cell's grey-value centroid at each acquisition. A total of 33-47 cells were evaluated at each [cAMP] and each experiment was repeated at least 3 times with similar results. For each cell and ensemble, the reciprocal root mean square (rms) displacement per step was plotted versus the reciprocal step duration. The rms directional persistence time (P) and speed (S) were then calculated from a least-squares fit line to this plot. Its slope estimates $1/S$ and its x-intercept estimates $-1/(6P)$. The average rms displacement (R), or cell diffusion rate, was calculated from $R=2S^2P$ (Wilkinson et al., 1984; Vicker et al., 1986).

RESULTS

Cell behaviour in positively and negatively developing cAMP gradients

Evenly distributed cells were exposed to one of 4 different concentration gradients, which developed until static and linear either by increasing or decreasing the [cAMP] on one side of the filter field. The cells were then allowed to migrate briefly in DCB alone (Fig. 2). Gradients that developed by decreasing the [cAMP] induced no significant cell accumulation at any [cAMP] tested, in agreement with the results of others (Shaffer, 1957; Futrelle et al., 1982; Wessels et al., 1992). In contrast, one brief, oriented cAMP pulse induced cells to rapidly shift up-field throughout the filter. The signals applied here are comparable to the cAMP impulses delivered by microcapillary (Robertson et al., 1972; Swanson and Taylor, 1982; Futrelle et al., 1982; M. G. Vicker, unpublished observations) or pulses from native aggregation centers (Siegert and Weijer, 1989), which induce tactic behaviour.

The developmental kinetics and terminal spatial patterns of the gradients generated by increasing or decreasing the [cAMP] are identical, yet their effects on cells are qualitatively

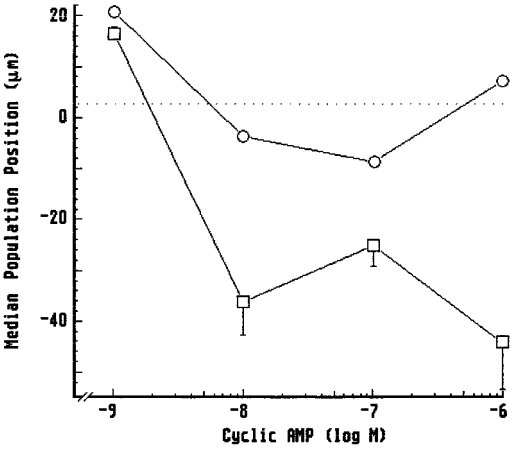

Fig. 2. Initial effects of developing cAMP spatial gradients on *Dictyostelium* cell accumulation. At 10 minutes after gradient development, either positively (squares) or negatively (circles), the cells were washed free of cAMP and incubated in DCB a further 10 minutes before fixation. The median positions of the populations from mid-filter are given together with the value and direction of Xnorm (vertical bars). Only the positively developing gradients between 10^{-8}-10^{-6} M produced significant accumulation, the Xnorm values of the other points being smaller than the symbols. The median position of cells in control filters with no cAMP is given as the dotted horizontal line (Xnorm = 0.94).

Table 1. The behaviour of amoebae migrating within a bounded filter territory in static spatial gradients and after pulses of cAMP

cAMP field	N	Cells	Mean (μm)	Median (μm)	Xnorm
Isotropic	10	5242	–1.95 (5.26)	–0.566 (9.70)	–0.70 (2.08)
Gradient	13	6136	17.40 (8.29)	23.27 (8.57)	7.37 (3.38)
Pulse	5	2482	–15.55 (6.46)	–19.91 (9.49)	–7.47 (3.07)

Evenly distributed populations were treated, as in Fig. 2, with: (a) 30 nM cAMP for 30-120 minutes as isotropic fields or negatively developed spatial gradients, maintained by infusion; or (b) a 40 second pulse of cAMP, which was washed out before cells were allowed to migrate 7-20 minutes. The average mean and median cell positions from mid-filter (±s.d. in parentheses) stem from experiments (N) performed on 4 independent occasions for gradient and isotropic concentrations and 2 for pulses. Median positions were tested with the Wilcoxon signed rank test: Xnorm <2.56 (>2.56) indicates a shift to the left, i.e. up-field, (right, i.e. down-field) at the 1% significance level. Negative or positive signs indicate up-field or down-field accumulation, respectively.

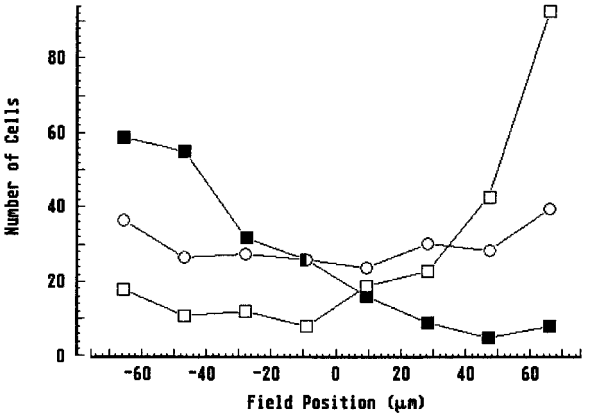

Fig. 3. A static cAMP spatial gradient and a temporal signal induce accumulation in different directions. Cell populations were migrating from an initially even field distribution in micropore filters. For pulse signals (closed squares), 10 nM cAMP was added to the up-field (left, negative by convention) compartment of the migration chamber and the cells were exposed to the developing gradient for 10 seconds. The cells were then washed free of cAMP and then allowed to migrate for 10 minutes before fixation. Static spatial gradients (open squares) were generated negatively across filters and then maintained by infusing the up-field (left) side with 10 nM cAMP and the other side with DCB alone for 60 minutes before fixation. The control population (circles) was fixed in formalin after 60 minutes of incubation in DCB alone. Median cell positions in μm from mid-filter (with Xnorm in parentheses) were: control 2.34 (0.71), static gradient 47.75 (4.16), pulsed cAMP –40.57 (–9.37). The control population demonstrates some 'edge' effect.

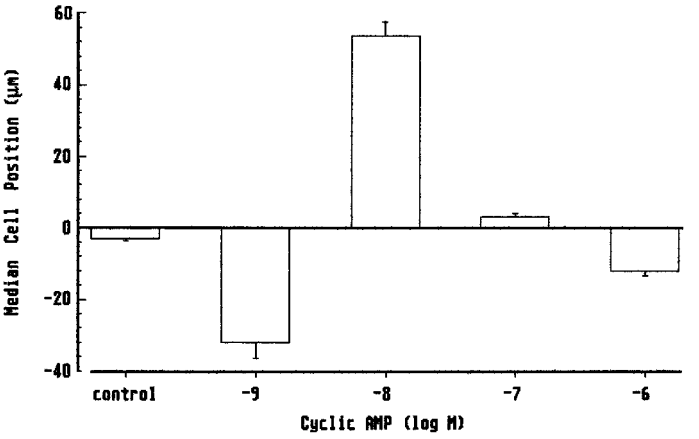

Fig. 4. The direction of cell accumulation in spatial cAMP gradients is cAMP-concentration-dependent. Negatively developing spatial gradients were generated in filter chambers after pre-incubation for 20 minutes in isotropic cAMP at the concentrations indicated on the abscissa. The cAMP in the down-field compartment was replaced by DCB, and the gradients were maintained by infusion. Cell populations were fixed after migrating 60 minutes in these static gradients. Their median cell position in the filter field is given together with their Xnorm as vertical lines. The control culture (Xnorm = 0.02) remained in isotropic 1 nM cAMP.

different. Nevertheless, although cells showed no detectable response to a brief, negatively developing gradient, accumulation may be induced if they are allowed more time to migrate and the static gradient is maintained (Fig. 3 and Table 1). Thus, after the negative development of a 10 nM cAMP gradient, the population median remains down-field as long as the gradient exists (45-120 minutes). The same [cAMP] delivered isotropically generates no effect, but a pulse induces accumulation up-field.

Accumulation direction in different gradients

Striking differences appeared in the degree and direction of cell accumulation in static gradients depending on the [cAMP] applied (Fig. 4). A 'top', 1 nM cAMP up-field induced accumulation up-field, as might be expected of tactic behaviour. But 100 nM and 1 μM gradients produced no significant shift, confirming the results of previous investigations (Vicker et al., 1984); 1 μM is the nominal [cAMP] in the natural tactic pulse (Tomchik and Devreotes, 1981). A 10 nM gradient induced a particularly strong accumulation down-field. These results are the first report of such behaviour and they cannot be explained by any present gradient reading theory of taxis.

In order to determine the basis of these accumulation patterns, different isotropic [cAMP]s were tested for their effect on cell motility parameters. The [cAMP]-dependent pattern of rms cellular speed, S, and displacement (diffusion), R, (and locomotory persistence, $P=R/2S^2$) indicated a complex, triphasic response with an optimum of each parameter falling between 10 and 30 nM cAMP in cells adapted to the [cAMP] (Fig. 5). These data indicate that the degree of cell motility, R, at each point across a gradient field depends on the ambient [cAMP]. Thus, gradients are 'structured' in that they contain graded zones of different cell motility amplitudes, and 'complex' in the sense that the motility in one zone may be greater or less than that in an adjacent zone depending on the gradient's top [cAMP]. For example, a linear gradient of 10

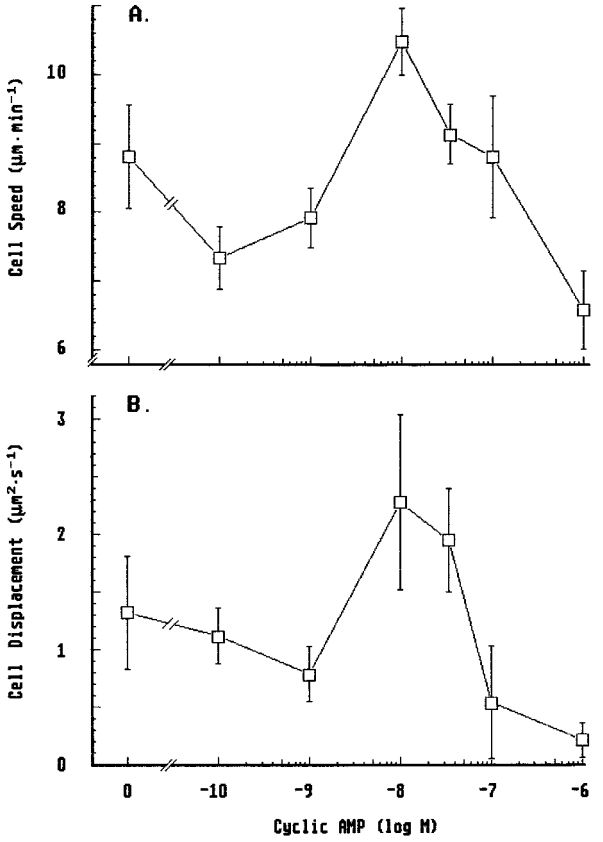

Fig. 5. Cell motility is sensitive to the cAMP concentration. The root mean square cell (a) speed and (b) displacement ($R=2S^2 \cdot P$) both demonstrate local minima and optima for between 33-47 aggregative-stage cells locomoting on glass at each different isotropic cAMP concentration. The values are given together with the s.e. as vertical bars.

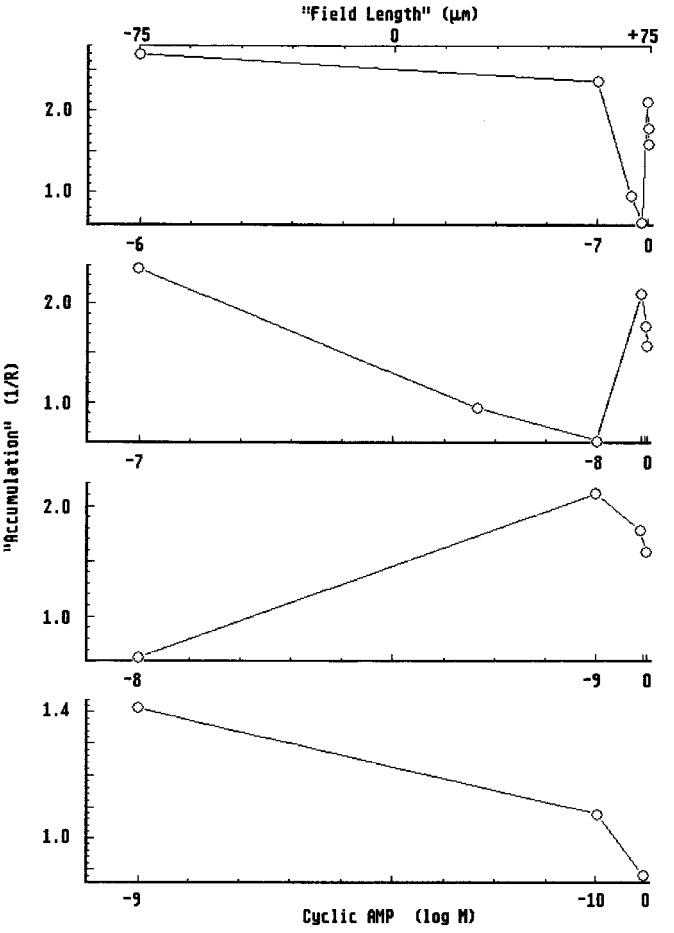

Fig. 6. Simulated cell accumulation in static cAMP spatial gradients from the cell motility data in Fig. 5b. The abscissa of Fig. 5b was 'cut' at 4 cAMP concentrations and each was replotted as 4 'top' cAMP concentrations of 4 linear spatial gradients: each falling linearly from one of the 'top' concentrations to 0 M cAMP. The plots are presented as linear gradients, with the original direction of the abscissa in Fig. 5 reversed, so that the gradient appears similarly oriented to that in Fig. 3. The gradient field is also set arbitrarily to 150 μm (upper abscissa) for comparison. The mobility data in Fig. 5b at and below each top concentration was inverted ($1/R$) upon the 4 ordinates here to simulate accumulated cell number.

nM cAMP would exhibit relatively high cell motility up-field. In keeping with precedents that regions of lower motility trap cells (Wilkinson et al., 1984), this anisotropic, [cAMP]-dependent motility pattern would drive accumulation down-field.

To examine whether this behaviour might account for the accumulation patterns in static cAMP gradients, the data in Fig. 5b may be illustrated as 4 gradients of each of the [cAMP] used in Fig. 4. Thus, the motility axis of Fig. 5b was inverted to represent 'accumulation' ($1/R$) on the ordinate. The 'gradients' were made by successively plotting the [cAMP] linearly as 10^{-6}-0 M, 10^{-7}-0 M, 10^{-8}-0 M and 10^{-9}-0 M cAMP to simulate 4 gradients from left-to-right on each abscissa. The results (Fig. 6) depict complex patterns of simulated accumulation. Thus, the regions of maximal accumulation (minimal motility from the data) appear in the 10^{-6}-0 M 'gradient' everywhere except in one small zone far down-field, in 10^{-7}-0 M at up- and down-field extremes, in 10^{-8}-0 M as a peak down-field and in 10^{-9}-0 M far up-field. These patterns may be quantitated as median cell shifts (Fig. 7) and they qualitatively agree with the data in Fig. 4, except for the simulated gradient of 100 nM cAMP. However, this particular gradient is unique in that it contains the optimum for cell motility, ~10-30 nM cAMP, roughly in mid-field. Consequently, compared to the other gradients, its accumulation pattern would be especially sensitive to the actual field position and extent of this putative low-accumulation region relative to those of its two, flanking high accumulation zones. Fig. 6 indicates that the direction and degree of accumulation in spatial gradients may be determined by the ambient [cAMP] and its spatial distribution, which effects klino- and orthokinesis (the components of R) at each point in the field.

Mechanical cAMP pulses

One way to test the role of spatial gradients in taxis is to propagate a cAMP pulse across a cell population swifter than the rate of diffusion so as to reduce the spatial components of the gradient to a minimum. Thus, by mechanically drawing a sample of cAMP across the filter field by suction, and then

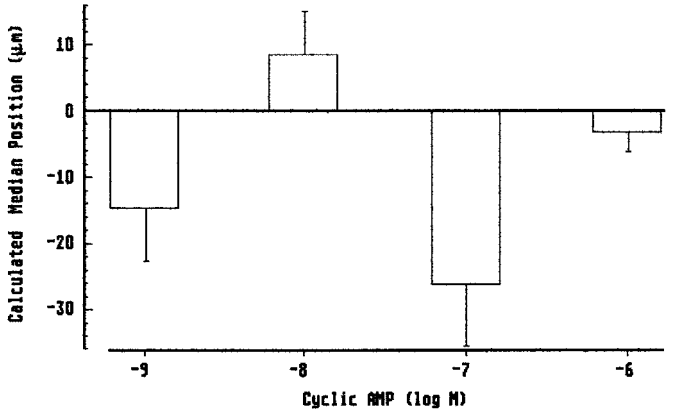

Fig. 7. Shifts in the median cell position calculated from the data in Fig. 6. The simulated gradient abscissa was arbitrarily normalized to 150 μm and the 1/*R* amplitudes of the 4 plots in Fig. 6 were evaluated each 18.75 μm as in Fig. 3. The values are given together with the Xnorm as vertical lines. Compare this plot to Fig. 4.

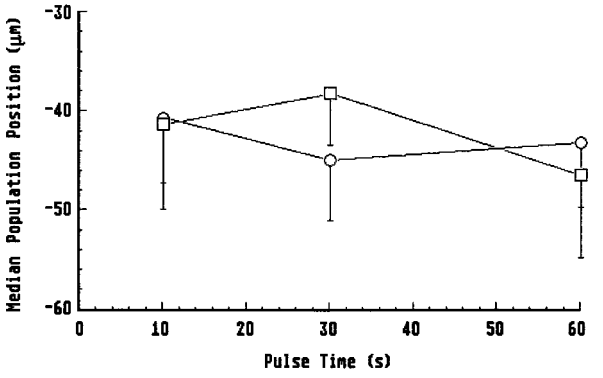

Fig. 8. A single cAMP pulse of 1 nM induces cell accumulation whether delivered by molecular diffusion (circles) or by mechanical flow (squares). The latter was propagated across the filter in less than 1 second, but calculations show that the former requires 4-16 seconds for development to be completed and the TS to cease (Vicker, 1989). The abscissa indicates the incubation time in cAMP before it was replaced with DCB. Subsequently, cell migration was allowed for 10 minutes in this buffer. The median cell position values are given together with the Xnorm as vertical bars. A control population treated by mechanical flow of DCB, but without cAMP, produced an insignificant median shift of –6.87 (Xnorm = –1.13).

washing it away, a sharp [cAMP] step-up/down may be produced. The effect of this TS was comparable to that induced by a gradient developing positively by diffusion (Fig. 8). Interestingly, the exposure to a TS demonstrated no time dependence between 10 and 60 seconds. Short exposure times should decrease any putative gradient signal quality, but not that of a TS.

DISCUSSION

Dictyostelium's response to a developing gradient of cAMP is the sum of its different reactions to only two components of the field: the ambient [cAMP] and the TS. An oriented, positive TS is required to induce chemotaxis, but cells do not perceive the spatial gradient in either developing or mature gradients. Rather, in a spatial gradient, scalar aspects of cell locomotion are sensitive to the ambient [cAMP], which alone bears signal quality, not the gradient in itself. In the absence of a significant TS, the [cAMP] pattern in a gradient will determine the population's accumulation pattern by effecting non-oriented cell motility (Vicker et al., 1984) through effects on locomotory persistence (klinokinesis) and speed (orthokinesis). A locally optimal [cAMP] for motility at one region will induce cells there to accumulate elsewhere in the field, a conclusion supported by observations of cell behaviour (Wilkinson et al., 1984) and simple computer models of kinesis (Vicker et al., 1984).

The range of cell speeds reported here is comparable to those found by others (Potel and MacKay, 1979; Futrelle et al., 1982; Fisher et al., 1989) as is the value for the [cAMP] optimum (25 nM, Fisher et al., 1989). Varnum and Soll (1984) found no such optimum, perhaps because they measured speed by comparing initial and final cell positions 20 minutes apart. *Dictyostelium* locomotion is more sensitive to the [cAMP] of a pulse than to that in a gradient. For accumulation, the pulse concentration becomes broadly optimal at and above 1 nM (Vicker, 1990b), which further differentiates the responses to cAMP pulses and gradients.

Effects of gradients on cell accumulation and adaptation to cAMP

Chemotaxis has been thought to depend on spatial gradient steepness. However, numerical simulations comparing the dynamic spatio-temporal steepness patterns of cAMP gradients developing by diffusion with the patterns of cell movement in developing and static gradients do not support this view (Vicker, 1989, 1990b). In adaptive systems, it is reasonable to consider the possible role relative steepness might play in taxis. The simulations show that the pattern of relative steepness, $\partial C/\partial x/C$ (where C is [cAMP] and x is distance) has no apparent relationship to the pattern of cell movement in static gradients. Alternatively, the steepness of a gradient may be considered as $\partial C/\partial x$, which has a constant value across the field in mature linear gradients. Neither this gradient nor the far steeper one generated briefly during gradient development are perceived by *Dictyostelium* cells. The ephemeral evolution of high steepnesses during gradient development is restricted to but one region appearing only up-field from mid-filter for the first few seconds of development. However, an oriented cAMP pulse immediately stimulates taxis throughout the entire field.

The failure here to detect taxis in spatial gradients cannot be explained by the inhibition of spatial gradient perception by signal adaptation during a [cAMP] decrease (Futrelle et al., 1982) or in static gradients. The observation that taxis only occurs during the [cAMP] increase has been consistently emphasized (Futrelle et al. 1982; van Duijn and van Haastert, 1992; Fisher et al., 1988; Wessels et al., 1992), and has nurtured the 'back of the wave' paradox (Shaffer, 1957; Futrelle et al., 1982). Most of these authors conclude that a decreasing [cAMP] inhibits cAMP perception due to adaptation to the preceding high [cAMP] in the wave. But no physiological correlate has been put forward to support this view. Is

the 1-2 minutes elapsing during the wave's increasing-[cAMP] phase sufficient to induce complete signal adaptation? *Dictyostelium* cells washed free of 10 nM cAMP require about 10 minutes to recover full sensitivity to a pulse of the same concentration (Dinauer et al., 1980a), and there is no evidence that cAMP perception is completely refractory to a [cAMP] increase (Futrelle et al., 1982). *Dictyostelium* cells in static spatial gradients are also adapted to the ambient [cAMP], perhaps more so than in passing waves, because their velocity, and thus the rate of increasing [cAMP] perceived while ascending the gradient, is slower than the intrinsic rate of their adaptive reactions, unlike swifter bacteria.

Perhaps tactic and kinetic reactions have different adaptive behaviours. Indeed, two classes of cAMP perception system have been demonstrated in *Dictyostelium* (Wurster and Butz, 1983; van Ments-Cohen et al., 1991). However, consideration is owed the fact that only a TS stimulates the non-linear adaptive reaction cascade referred to above, e.g. the quadriphasic oscillation of actin polymerization following a cAMP pulse (McRobbie and Newell, 1984; Hall et al., 1988).

Gradient-sensitivity models

Several observations do not support the temporal and spatial gradient-reading hypotheses. Thus, cells stimulated by a positively developing gradient immediately project a pseudopod (i.e. turn) toward the signal direction (Gerisch and Keller, 1981; Swanson and Taylor, 1982; Wessels et al., 1992) rather than test the gradient's orientation with trial pseudopods, as required by the temporal gradient reading model (Gerisch et al., 1975). Observations of high frequency, 'random-directional' pseudopod projection are invariably associated with intersignal periods during natural rhythmic cell aggregation in *D. discoideum* (e.g. Wessels et al., 1992), and thus seem unlikely to have any relationship with gradient perception (Futrelle et al., 1982). Problems with the spatial gradient reading model appear in both PMNs (Sullivan et al., 1984) and *Dictyostelium* (Wang et al., 1988), in which the centripetal redistribution of attractant receptors on the cell surface seems incompatible with gradient reading. Receptor accumulation, e.g. capping, decreases signal sensitivity (Berg and Purcell, 1977). The tendency of cells to contract and reduce their dimensions after TS reception (Futrelle et al. 1982; Hall et al., 1988; Killich et al., 1993) would also reduce their ability to resolve a gradient signal.

Chemokinesis induction by spatial gradients

Consider, as a thought experiment, cells spreading from a local accumulation, mid-way up a static spatial gradient of cAMP. Let the optimal [cAMP] for cell motility, and thus maximal speed and locomotory persistence, be up-field. A klinokinetic mechanism operating alone, with no effect on speed, will induce a transient, initial up-field accumulation or 'pseudochemotaxis' (Lapidus, 1980; Futrelle, 1982; Vicker et al., 1984), but the cells will eventually become randomly distributed if the field is a bounded one. An orthokinetic mechanism operating alone will induce accumulation down-field. Klinokinesis, but not orthokinesis, will induce some degree of cell orientation simply as a consequence of the anisotropic cell flux. Thus, considering both klino- and orthokinetic mechanisms operating together in this field, accumulation will eventually occur down-field if the field is bounded, but if it is unbounded, cells will continually drift up-field (M. G. Vicker, unpublished observations), where their turning will be increasingly suppressed.

This is essentially the behaviour Fisher et al. (1989) observed in a comprehensive tracking study of *Dictyostelium* in static cAMP gradients. The authors argued that taxis depends on gradient reading after observing cells at 1.5 minute intervals in a static spatial gradient about 2 mm across. However, the exclusion of cells entering and leaving the ~1 mm^2 filming area from the analysis would produce, de facto, an unbounded field: cell would be relatively less likely to leave and enter it down-field than up-field. The exclusion of entering cells from the analysis generated a slight artifactual drift. The parameters measured by Fisher et al. (1989) are shared by populations accumulating by tactic and kinetic mechanisms, except for the tendency to turn up-field, which is, nominally, specifically tactic. The authors reported that about 2% of the total turning activity in a spatial gradient was non-random in character and, thus, considered to contribute to taxis. They suggested that half of this turning was due to 'error correction', defined as movement, which if too far left or right of the up-field direction, is subsequently followed by a turn in the opposite direction. However, zig-zagging is a constitutive element of the stimulated locomotion of PMNs (Nossal and Zigmond, 1976) and *Dictyostelium* (Killich et al., 1993). The other half of the non-random turns was attributed to tactic turn suppression. However, the figure of 2% of all turns is a decidedly low value, unexpected of tactic behaviour as defined by Fraenkel and Gunn (1961) and far weaker than that observed in positively developing gradients (e.g. Swanson and Taylor, 1982; Gerisch et al., 1975; Wessels et al., 1992). The responses observed in spatial gradients by Fisher et al. (1989) appear qualitatively different from those induced by natural and artificial pulse signals and may, therefore, be described as non-oriented cell locomotion: i.e. chemokinesis, rather than gradient reading.

Taxis based on the intracellular oscillator

Nevertheless, a mechanism of taxis need account for the puzzling fact that a TS induces oriented pseudopodium projection and motility although the signal's quality remains virtually constant as it sweeps across a cell. The reactions to cAMP are characterized by its binding to specific receptors on the cell surface and a subsequent chain of intracellular reactions. These involve chemical reactions and molecular diffusion, and it seems unlikely that they might transit the cell faster than the signal itself. In order for the tactic reaction to stem primarily from the proximal side of the cell first contacted by the oriented TS, subsequent distal reactions need to be briefly suppressed. This suggests that a mechanism of taxis by TS perception rests on intrinsic dynamical behaviour of the cell (biological time) to limit the cell's response, i.e. pseudopodium extension, to the peripheral region first responding to the signal (Vicker et al., 1986; Vicker, 1989, 1990b).

Recently, we have described a new oscillator in *Dictyostelium* cells, based on rotating oscillatory waves in the cell, and suggested its role in TS perception and chemotaxis (Killich et al., 1993). The oscillator governs pseudopodial activity, cell shape and movement and appears associated with the dynamics of actin polymerization. Although the oscillator, found initially in preaggregative cells, is not identical with the better known

cAMP oscillator (Gerisch and Hess, 1974), it may interact with it. Thus, a few seconds after a cAMP pulse, similar to that delivered mechanically in Fig. 8, aggregative cells contract and round-up (Futrelle et al., 1982) as most of the intracellular actin begins the first of two episodes of enhanced polymerization-depolymerization within the sub-plasmalemmal cortex (McRobbie and Newell, 1984; Hall et al., 1988). We have proposed that the initial, ~10 second-long response constitutes a phase resetting of the intrinsic intracellular oscillator, which abruptly terminates cell locomotion and pseudopod activity (Killich et al., 1993).

A mechanism of chemotaxis based on TS perception requires this phase transformation to extend from the site of signal impact and progress across the cell. Once nearly all the cell's actin is polymerized, its dynamic-instability character will reverse the reaction (Schleicher and Noegel, 1992; Dufort and Lumsden, 1993). The first site that completes this reaction will enter the next phase of actin polymerization ahead of other intracellular regions and, thus, will form the first pseudopod during the second polymerization peak, i.e. at the original site of signal impact and phase resetting. This site will briefly dominate the cell by creating an actin monomer shortage elsewhere. Thus, tactic pseudopodium expression will be determined by principles of chemical waves and dynamic instability.

Systems gain advantages and reduce the errors associated with signal reading by communicating periodically rather than by molecular concentration (Li and Goldbeter, 1992; Harrison and Lacalli, 1993). Thus, hormonal communication in many systems depends on temporal signalling (Belchetz et al., 1978; Dietl et al., 1993). Non-linear signalling mechanisms based on biological time assure that cells bear a full complement of sensitive receptors coordinated with the secretion of efficient signal concentrations. Biological time means that cellular reactions are affected by the intrinsic dynamics of intracellular processes, rather than only by receptor-ligand binding kinetics. The role of intracellular oscillations is being further investigated in chemotaxis and developmental interactions.

I am indebted to Florian Siegert and Kees Weijer (Munich) for their gift of the cell tracking and analysis programs.

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(Received 21 August 1993 - Accepted 15 October 1993)