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Orientation of chemotactic cells and growth cones: models and mechanisms

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SUMMARY

A model is proposed for an amplification step in chemotactically sensitive cells or growth cones that accounts for their extraordinary directional sensitivity. It is assumed that cells have an intrinsic pattern forming system that generates the signals for extension of filopods and lamellipods. An external signal such as a graded cue is assumed to impose some directional preference onto the pattern formed. According to the model, a saturating, self-enhancing reaction is coupled with two antagonistic reactions. One antagonist equilibrates rapidly over the whole cell, causing competition between different surface elements of the cell cortex for activation. It will be won by those cortical regions of the cell that are exposed to the highest concentrations of the external graded cues. The second antagonistic reaction is assumed to act more locally and has a longer time constant. It causes a destabilization of peaks after they have formed. While the total activated area on the cell surface is maintained, the disappearance of some hot spots allows the formation of new ones, preferentially at positions specified by the actual external guiding signal. Computer simulations show that the model accounts for the highly dynamic behaviour of chemotactic cells and growth cones. In the absence of external signals, maxima of the internal signals emerge at random positions and disappear after some time. Travelling waves or oscillations in counter phase can emerge on the cell cortex, in agreement with observations reported in the literature. In other ranges of parameters, the model accounts for the generation of a stable cell polarity.

Key words: Chemotaxis, Growth cone, *Dictyostelium*, Cell polarity, Pattern formation

INTRODUCTION

Concentration differences of a few per cent across a cell or a growth cone can be sufficient for their chemotactic orientation (Zigmond, 1977; Baier and Bonhoeffer, 1992). This raises the question of what mechanisms can be responsible for this extraordinary sensitivity. Time lapse studies suggest that this orientation is based on a highly dynamic process. Lamellipodia and filopodia are stretched out and retracted rapidly in a seemingly irregular way. Many cell types show such dynamic behaviour even in the absence of external signals (Euteneuer and Schliwa, 1984; Killich et al., 1993, 1994; Gerisch et al., 1995), arguing against a simple internal amplification of the external signal.

In some cases, the sensitivity of a cell towards external concentration differences is intimately connected with processes involved in the orientation of cell division. In yeast, for instance, mutants exist in which the chemotactic orientation towards a mating partner and the predictable orientation of the plane of cell division is abolished (Chant, 1995, 1996). In the brown alga *Fucus*, any slight asymmetric cue can orient the outgrowth of the rhizoid and thus the first cleavage plane of the egg. In the absence of such cues, the orientation will occur at random, although with some delay (for review see Goodner and Quatrano, 1993). These observations suggest the existence of a general mechanism underlying intracellular pattern formation. If certain conditions are met, the symmetry of a cell becomes unstable and minute external signals suffice for the orientation of the emerging pattern.

The primary event in directional movement is proposed to be the formation of local concentration maxima by an intracellular patterning system that, in turn, causes a rearrangement of the cytoskeleton and changes in the cell shape. The evidence for such a separation is circumstantial. If actin polymerization is blocked in *Dictyostelium* cells by latrunculin, the response of the cells visualized by the polarized CRAC-GFP binding is not diminished. The G-protein activation is still more strongly graded over the cell than the external signal (Parent et al., 1998; Parent and Devreotes, 1999), showing that an internal amplification mechanism exists and that actin polymerization is not a part of this amplification. In *Fucus*, a polarized current of Ca^{2+} ions is the first reliable indication for the forthcoming polarity of the cell (Jaffe, 1968). Blocking this current abolishes the emergence of this cell polarity. This early polarization phase is followed by a fixation step that depends on F-actin (see Goodner and Quatrano, 1993). If the second step is blocked, the determination of the axis is not stable and can be overridden by another external signal, in this case, unilateral illumination. The intimate coupling of the two systems is underlined by the observation that a stream stimulus of culture medium can direct the polarization of fish keratocytes or even fragments thereof (Verkhovsky et al., 1999). However, it is not yet clear whether this is due to the applied physical force or due to the change of the micro-environment of the cell.

After a brief outline of the general conditions that have to be satisfied in order that pattern formation can take place, the main part of the paper will be devoted to the question of how such a system must be constructed to obtain the following properties: (i) cells must be highly sensitive to minute asymmetries in the external signals. (ii) The sensitivity must be in a wide range independent of the absolute concentration of the external signal. (iii) For chemotactic cells, the polarization of a cell adapts to changes in the orientation of the external signal. (iv) The intracellular pattern formation must continue even in the absence of a signal. The minimum requirements that lead to this behaviour will be elaborated and possible molecular realizations will be discussed but the main emphasis will be devoted to the conceptual aspect of this process. It will be shown that the same mechanism can either lead to a stable cell pattern or to a highly dynamic appearance of 'hot spots' at changing positions on the cell.

The generation of the primary internal signal by amplification of asymmetries is assumed to take place between signal reception and the rearrangement of the cytoskeleton. Only the generation of this signal is subject of the paper. Real shape changes based on physical forces are not considered (see e.g. Alt and Tranquillo (1995). Although at least two structurally different protrusions are formed, lamellipodia and filopodia, in this paper no distinction will be made between the two forms.

RESULTS AND DISCUSSION

Pattern formation requires local self-enhancement and long ranging antagonistic effects

Biological pattern formation has been proposed to depend on a local self-enhancing reaction that is coupled to a long range antagonistic reaction (Gierer and Meinhardt, 1972; Gierer, 1987; Meinhardt, 1982, 1998). For the sake of simplicity and generality, I will assume first that the self-enhancement is accomplished by an 'activator'. Its autocatalysis is balanced by the action of a long range 'inhibitor'. In a field larger than the range of the activator, a homogeneous distribution of both substances is unstable. For instance, a small local increase of the activator will be enhanced due to the autocatalysis. The surplus inhibitor diffuses into the surroundings, causing a decrease in the activator production there. At the incipient activated site, the activator production increases further until a dynamic equilibrium with the surrounding cloud of inhibition is reached. Instead of an inhibitor, the depletion of a diffusing factor used up in the self-enhancement is conceivable as well. The terms 'activator' and 'inhibitor' will be used as shorthand to denote the general process of self-enhancement and antagonistic reaction. Each of these processes can depend on several components. The possibility of generating patterns by the interaction of two substances with different diffusion rates was discovered by Turing (1952). Turing derived his equations by more abstract mathematical considerations but it can be shown that his equations also satisfy the conditions of local self-enhancement and long range inhibition.

The spatial localization of the self-enhancing reaction required for intracellular pattern formation can be realized by reactants attached to the cell cortex. In contrast, the required rapid redistribution of the antagonist suggests a spreading

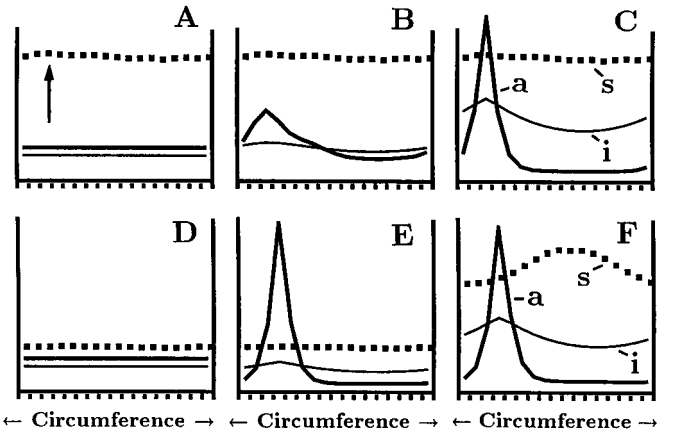

Fig. 1. Detection of minute asymmetries and the problem of reorientation. Assumed is a cell-internal pattern forming reaction consisting of a self-enhancing activator (a) and a long-ranging inhibitor (i). Sufficient for the localization of a strong internal signal is a noisy, slightly asymmetric external signal (s, black squares) that has a stimulating influence on the activator production. Simulations made on the circumference of a circle: the left and right elements are neighbours in reality. Shown is the initial (A), an intermediate (B) and the final stable distribution (C). A strong internal activator maximum appears at the position where the external signal is slightly above average (arrow in A). (D,E) As required for path-finding in a graded environment, orientation works also at a much lower absolute level of the external signal. The lower signal concentration is compensated by a lower inhibitor concentration. (F) The problem: after an incipient pattern has been formed, even a strong external asymmetry is unable to reorient the pattern.

mechanism in the cytoplasm. As shown below, to account for the dynamic shape changes of chemotactic cells and growth cones, it is necessary to introduce a second antagonistic reaction into the pattern forming system.

As shown in the simulation in Fig. 1, such a system is very convenient to detect minor external concentration differences and convert them into a pronounced intracellular pattern. This is even true if the external signal is blurred by random fluctuations. According to this model, the high sensitivity of a cell towards the external cue results from the positive feedback of the incipient internal pattern on its further accentuation. The final form of the pattern is nearly independent of the trigger. In particular, the absolute level of the external cue is, over a wide range, without effect. This is an obvious requirement to be met if a cell or growth cone should move upwards in a concentration gradient. In the model, an increased absolute level of the external signal becomes balanced by an elevated level of the antagonist. This keeps the activator concentration almost constant (Fig. 1C,E), accomplishing in this way an adaptation.

For a cell or a navigating growth cone, it essential that the actual orientation of the internal signal is re-adjusted to the actual slope of the gradient encountered in the course of its movement. For a system as described above, the crucial problem is this reorientation, not the sensitivity. The system is sensitive to small asymmetries only as long as the system is near the (unstable) homogeneous steady state. Once the pattern is formed, the self-stabilization of the internal signal is so strong that minute external cues are unable to accomplish a reorientation (Fig. 1E,F). In the following, several mechanisms

will be discussed that render the system adjustable to changes in the orientation of the external signal.

Use of an oscillating pattern forming system for chemotactic sensitivity

One possibility to solve the problem of a permanent directional adjustment is the employment of an oscillating pattern forming system (Meinhardt and Gierer, 1974). Oscillations occur if the half life of the inhibitor is longer than that of the activator. Then, the system cycles through a phase where it is sensitive to external asymmetries, followed by a phase in which the imposed internal asymmetry is amplified, culminating in a strongly localized signal. The subsequent accumulation of the inhibitor leads to a collapse of the activation. After decay of the inhibitor, the system enters again into the sensitive phase in which a new direction can be chosen (Fig. 2). Such a model predicts that the measurement of the external gradient is not a continuous process but occurs in certain time intervals. A corresponding stop and go behaviour has been observed in retinotectal axons of living zebra fish embryos (Kaethner and Stuermer, 1992). During the resting periods, the growth cone becomes broader and develops filopodia. In the phases of rapid advancement, the growth cone has a long stretched shape. This periodicity does not depend on neural firing since it persists also after TTX treatment (Kaethner and Stuermer, 1994). Harrison (1910) observed such a pulsatile mode in his classical observation of nerve outgrowth in the tail bud of amphibian embryos, indicating that this is a rather general phenomenon. However, in *Dictyostelium*, for instance, exposing a cell to an external signal leads within seconds to a turning of the cell (Segall and Gerisch, 1989). This is independent of the phase of oscillation. Further below, a mechanism will be discussed that allows a permanent sensitivity.

Spacing of several filopodia

As a rule, a chemotactic cell or a growth cone extends several filopodia in diverse directions, exploring in this way a much larger region. The existence of multiple protrusions suggests that more than one signal to form a protrusion can be generated simultaneously. The formation of a pattern with several maxima that have a more or less regular spacing is one of the standard modes of pattern formation. This occurs if the range of inhibition does not cover the whole field (Fig. 3A-C). However, for the modelling of the semi-random extensions and retractions of multiple protrusions and their response to graded developmental cues, additional features must be introduced. For the detection of the most appropriate surface element, the range of inhibition has to cover the whole cell or growth cone, otherwise secondary internal signals can appear at inappropriate positions (Fig. 3D-F). This would abolish any chemotactic orientation. In other words, the patterning mechanisms for fingering and for the selection of the best surface element require conflicting parameters. If the fingering of a growth cone is based on a lateral inhibition mechanism, a separate mechanism would be required that decides which of the more or less evenly spaced protrusions will extend further. In this case, the orientation of filopodia would be independent of the orienting signal. The purpose of the fingering would be to enlarge the spatial extension over which the gradient can be detected. A second mechanism would be required to determine which of the protrusions should survive. However, at least in some cases, filopodia stretch out preferentially towards higher concentrations of an attractant (Zheng et al., 1996b). Further below, a mechanism will be elaborated that reconciles multiple protrusions and directional sensitivity.

Several activated regions with irregular spacing can emerge if the self-enhancement saturates

If the production rate of the activator has an upper bound, the local increase of activator concentration is limited. In this mode, the activated area becomes a certain fraction of the total field (Gierer and Meinhardt, 1972). More surface elements become activated until sufficient inhibitor is produced to balance the self-enhancement. The character of the activated region depends on the spread of the activator. If the activator has a considerable diffusion range, a tendency exists to form larger and coherent activated regions with a plateau-shaped activation profile (Fig. 3H). Such a broad maximum can change its position on the surface upon a change in the orientation of the external gradient for the following reason: if one of the flanking regions is in a better position in respect to the external signal, the activation will increase there. Since the total activation is restricted, this occurs at the expense of the opposite flanking region. Both changes together lead to an apparent movement of the activated region.

In contrast, if the self-enhancing component is almost non-diffusible, several activated regions with irregular spacing can coexist (Fig. 3I). The strength of the external signal and the extent of the fluctuations would be decisive as to where and how many maxima are formed. A stronger asymmetry of the external signal (resulting, for instance, from a steeper gradient) would lead to a more preferential localization (Fig. 3J). Without orienting cues, the activated regions would be randomly distributed over the cell surface (Fig. 3K). Thus, the model accounts for the smooth transition from random positioning based on some sort of noise to a polarized initiation of protrusions under the influence of orienting signals.

Since a non-diffusible self-enhancing reaction leads to a steep transition from activated to non-activated surface elements, these maxima cannot be shifted. A simple global oscillation as described in Fig. 2 would lead to a simultaneous extension and retraction of all filopods of a given cell, a behaviour incompatible with the observations.

Simultaneous pattern formation in space and time: the role of a second antagonistic reaction

To explain the simultaneous extensions of new and the retraction of existing filopodia, a second antagonist of the activator is assumed in the model. It acts locally and has a longer time constant than the activator. It causes a finite half life of a particular local activation. Since the total area of activation is maintained, the disappearance of some protrusions opens up the possibility of forming new ones, possibly at a more optimal position in respect to the actual external cue.

This view is supported by observations of chemotactic cells of *Dictyostelium discoideum*. In the absence of an external orienting signal, the dynamics of the internal pattern forming system become especially clear. Killich et al. (1993, 1994) have measured the shape changes of isolated amoebae and found that they are not random. Waves of pseudopod extensions can occur that travel around the circumference. In other cases, emission and retraction of pseudopods are found

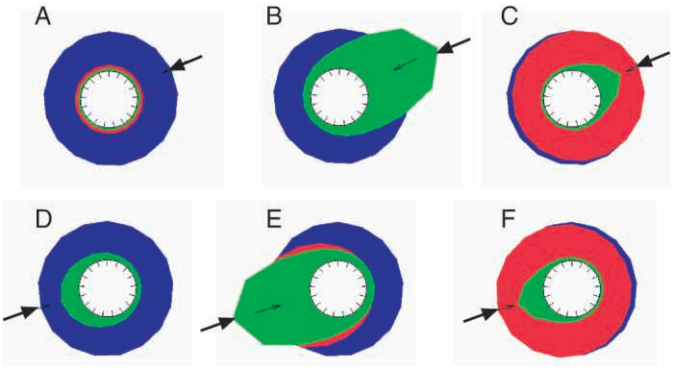

Fig. 2. Gradient detection by an oscillating activator/inhibitor system. (A-C) If the half life of the inhibitor (red) is longer than that of the activator (green), the pattern forming system oscillates. The maximum activator concentration (green) appears at the position where the external signal (blue) is slightly above average (arrow). The burst-like activation breaks down due to the accumulating inhibitor. (D-F) After the decay of the inhibitor, a new activation occurs. If the orientation of the external asymmetry (arrow) changes, e.g. during the movement of a cell, the activator maximum is formed in the subsequent cycle at the new optimum position. Thus, the system can detect and adapt to minute external asymmetries; the local concentrations are plotted as distance from the inner circle.

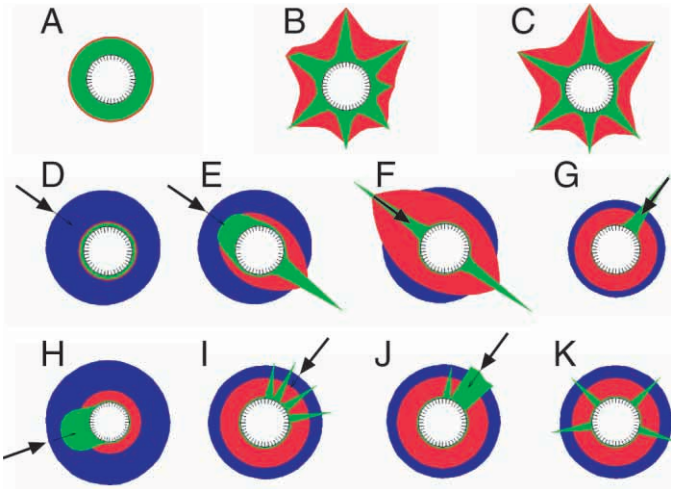

Fig. 3. Multiple peaks in an activator-inhibitor system. (A-C) If the range of the inhibitor is smaller than the total field, multiple maxima can emerge. They can be used as signals to form several protrusions. (D-F) Under these conditions, the influence of an external asymmetry may lead to a second maximum at the 'low' side. (G) With a very long inhibitor range (simulated by averaging, see Appendix), only one maximum can emerge. (H) Saturation of the activator autocatalysis and a moderate activator diffusion leads to a broad maximum (signal for a lamellipodium?). (I-K) With saturation but without diffusion, several sharp maxima can appear (signals for filopodia?). They form at positions where the external signal (2% asymmetry plus 1% random fluctuations) is above average. (J) A stronger external signal (5%) leads to a more coherent appearance of the maxima. (K) without an external signal, the maxima appear at random positions.

at stable positions but in opposite phases (Fig. 4E,F). An almost identical dynamic behaviour has been deduced from the pigment pattern on some tropical sea shells (Meinhardt and Klingler, 1987; Meinhardt, 1998). These patterns are time records of a one-dimensional patterning process along the growing edge. In the corresponding modelling it has turned out that spontaneous travelling waves (i.e. waves that are not organized by a pace-maker region) and oscillations of neighbouring regions in counter-phases emerge if two antagonists are involved. For instance, one inhibitor has a long range and enables a pattern in space. A second antagonist acts more locally but has a long time constant, causing a pattern in time. In application to the extension of filopodia, this mechanism would suggest that at first a local signal for the outgrowth is generated. Due to the action of the second antagonist, this position becomes 'poisoned', causing a retraction of the filopodium. Since the total fraction of activated regions is regulated, the de-activation of one region allows another region to become activated. As shown in the simulations in Fig. 4, the simulation of the travelling waves and the oscillations in counter-phase resemble closely the observation of Killich et al. (1993, 1994). Again, the second antagonistic reaction can be based on the depletion of a factor and it can be implemented far downstream of the reaction generating the primary polarity.

In the growth cones, the extension and retraction of filopods seems to be less co-ordinated. This is reproduced in the simulation in Figs 5 and 6 under the assumption that the range of the rapidly spreading antagonist covers the whole field and that saturation allows the formation of multiple peaks of activation (see Fig. 3I-K). The second antagonist with the longer time constant leads after some time to a de-activation. Not yet included in the model is the possibility that filopodia extending towards the source region may have a longer half life than those with other orientations. Although the external signal is assumed to be noisy, the unambiguous detection of an external asymmetry by the internal patterning system is obvious. At very low asymmetries in the external signal, the internal signal reflects a mixture of signal detection and noise (Fig. 5B). Also the somewhat irregular appearance and disappearance of internal signals in the absence of any external signal is clearly to be seen (Fig. 5C). Fig. 6 shows snapshots of the simulation, demonstrating that the model accounts for the dynamic nature of internal signal generation that leads to protrusions.

Molecular realization: possible involvement of G proteins and Ca^{2+} in intracellular pattern formation

According to the model, the generation of strong cell-internal signals under the influence of small external concentration differences requires the following components: (i) a self-enhancing feedback loop causes small deviations from the means to increase further. An upper bound of this reaction (saturation) allows several hot spots with irregular spacing. (ii) A long-ranging antagonistic component with a short time constant confines the self-enhancement to one or a few focal regions. These two components are sufficient to generate stable patterns. (iii) A more local antagonistic reaction with a longer time constant can destabilize hot spots in order to enable the formation of new ones, possibly at more appropriate positions. These elements characterize the simplest possible system that allows the simultaneous generation and withdrawal of signals for protrusions. Of course, a theoretical analysis cannot predict

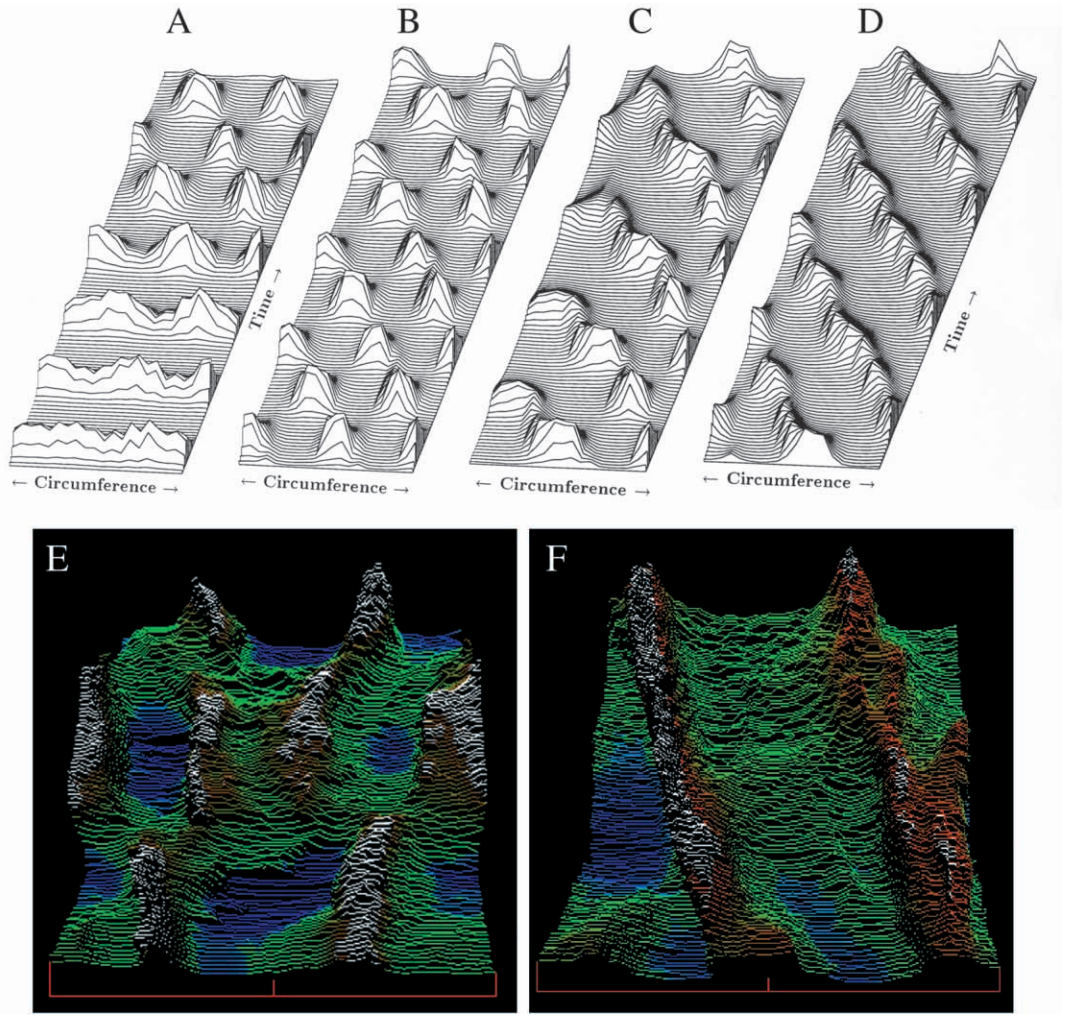

Fig. 4. Formation of travelling waves and of oscillations in counter-phase on the cell surface. A self-enhancing reaction is balanced by two antagonistic reactions. The first is an inhibitor with a short time constant and a long range. It causes a patterning in space. The second antagonistic reaction results from the depletion of a co-factor. Its short range and long time constant is responsible for the patterning in time. Shown is a simulation on a circle. Activator concentrations are given as function of time. The homogeneous oscillation becomes unstable (A). Via out-of phase oscillations (B) travelling waves around the surface are formed (C-D). (E,F) Dynamics of pseudopod formation on non-stimulated *Dictyostelium* cells as observed by Killich et al. (1993, 1994). Plotted is the distance of a surface element from the centre of the cell. Lines represent snapshots of a cell at 3 second intervals, a higher level indicates a larger protrusion. The observed oscillations out of phase and travelling waves around the circumference correspond closely to the simulations (E and F kindly supplied by Michael Vicker; for equations see Meinhardt, 1998).

how many components are actually involved in the realization of, e.g. the self-enhancement. The following discussion should merely illustrate that the expected elements have their counterparts with those experimentally observed.

In recent years, much progress has been made in the understanding of the role of G-proteins for signal transmission and the organization of the cytoskeleton (see Devreotes and Zigmond, 1988; Zigmond, 1996; Chen et al., 1996; Tapon and Hall, 1997; Parent et al., 1998; Parent and Devreotes, 1999, for review and references). Cdc42, for instance, is involved in the oriented outgrowth of growth cones and in the orientation of yeast cells (Vancura and Jay, 1998; Chant, 1995, 1996; Ziman et al., 1993; Yamochi et al., 1994). Since all surface elements must be able to become a hot spot, it is not expected that molecules that have a strong localization play a role in the primary selection of the activated site. According to Parent et al. (1998) in *Dictyostelium discoideum*, the receptors for chemotactic sensing are homogeneously distributed on the surface. The translocation of the protein CRAC to the correct site of the inner plasma membrane is a reliable indicator for receptor activation. Again, this requires a signal for where the CRAC protein must be translocated.

Much evidence exists that calcium is involved in the generation of cell polarity. Arguments for a role of calcium in the chemotrophic turning of growth cones has been reviewed by Zheng et al. (1996b). Asymmetric application of calcium ionophores can orient growth cones (Anglister et al., 1982) and the polarity of the egg of the brown alga *Fucus* (Robinson and Cone, 1980). Calcium plays a role in the chemotaxis of *Dictyostelium* (Malchow et al., 1996a,b). A local destruction of calcineurin, a protein phosphatase enriched in growth cones that depend on calcium ions and calmodulin causes a turning of growth cones (Chang et al., 1995). Also for branching of neurites, Ca^{2+} ions play a decisive role. bFGF is a potent neurotrophic factor for foetal rat neurons. The increased rate of branching is connected with the formation of clusters of L-type Ca^{2+} channels at the branching points, leading to locally increased Ca^{2+} concentrations (Shitaka et al., 1996). As mentioned, in the brown alga *Fucus*, a polarized current of Ca^{2+} ions is the first reliable indication for the forthcoming polarity of the cell (Jaffe, 1968; Goodner and Quatrano, 1993). Blocking this current abolishes the emergence of this cell polarity. Calcium influx leads in *Dictyostelium* to an actin depolymerization (Nebl and Fisher, 1997), demonstrating the

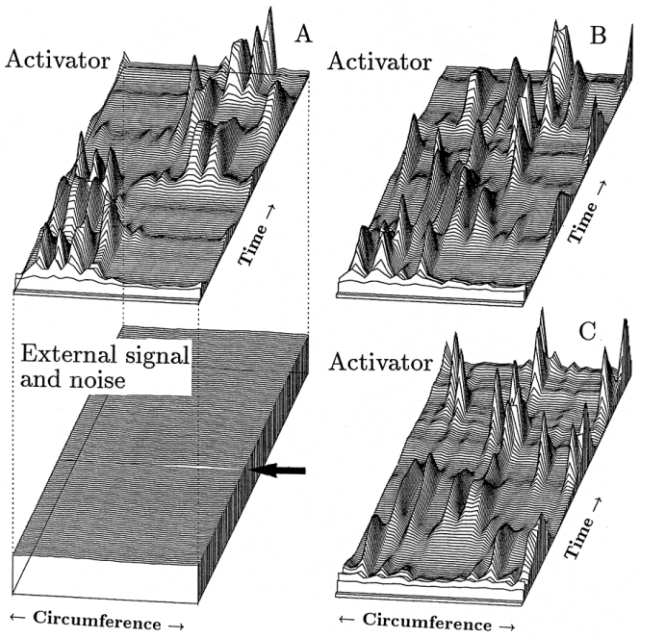

Fig. 5. (A) Continuous adaptation of internal maxima to a changing external environment. Assumed is an autocatalytic activator (top) and two antagonists (not shown), one with a range covering the whole cell and one with a short range but a longer time constant. Due to the latter, maxima once formed (see Fig. 3I,J), disappear after a certain time interval. New maxima can emerge, possibly at a more appropriate position. The external signal (bottom) is assumed to have 2% difference across the cell and to be blurred by 1% random fluctuation. After a change of its orientation at the time indicated by the arrow, the system detects this new optimal position. (B) Simulation with 1% signal and 1% noise and same signal change as in A: the system is at the borderline to detect the optimum position. (C) The dynamic generation of maxima is maintained even in the absence of an external asymmetry. In contrast to the simulation in Fig. 4, it is assumed that the rapidly spreading antagonist can be regarded as being homogeneously distributed (details of the simulation are given in the Appendix).

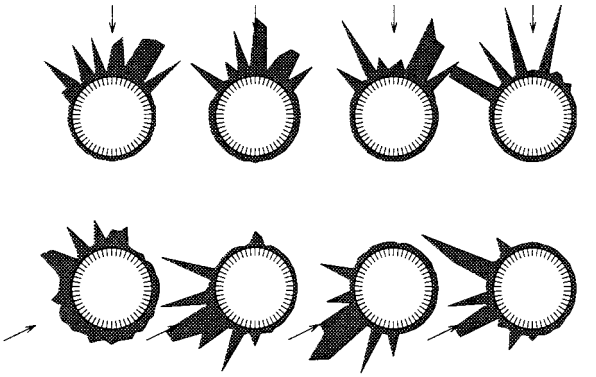

Fig. 6. Simulation of signal formation on a cell. Calculated as in Fig. 5A on a ring (see Appendix); For the external signal 2% concentration difference over the cell and random fluctuations smaller 1% are assumed. The internal signal is plotted. Upper row: although the external signal remains unchanged, local maxima to extend a protrusion are built up and disappear. They point preferentially to the region of highest concentration of the external signal (arrow). Lower row: after a change in the orientation of the external signal, the internal signal adapts rapidly to the new position.

intimate coupling between calcium and the cytoskeleton. A relative increase of chemoattractand cAMP leads to a change in the cytoplasmic free calcium concentration. This change is fast (maximum is obtained after about 25 seconds). Whether it depends only on the extracellular calcium in the medium is controversial (see Schaloske et al., 1998). On the other hand, Marks and Maxfield (1990) found no local increase of Ca^{2+} in the chemotaxis of neutrophils.

A candidate for the expected self-enhancement mechanism could be the calcium-induced-calcium release based on voltage-gated channel opening (see Berridge, 1993, for review). Calcium can bind to the channels causing a further opening. Another mechanism that could contribute to a local self-enhancement has been proposed by Fromherz (1988): ion channels can move on the cell surface. Any local fluctuation in channel density leads also to a change of the local potential. This, in turn, can lead to an electrophoretic movement of the charged channel molecules, increasing in this way the local accumulation of channels, and so on. Since any local accumulation of channel molecules leads to their depletion on the remaining surface, a long ranging antagonistic reaction would be straightforward. A spatially and temporally correlated redistribution of channels and an increased local concentration of intercellular calcium has been observed in *Fucus* (Shaw and Quatrano, 1996).

An influx or release of free calcium ions would also suggest a possible mechanism for the expected long ranging antagonistic reaction. Since the cells have to remain electrically neutral, influx of Ca^{2+} ions can be accompanied by an efflux of H^+ ions. In other words, Ca^{2+} influx can lead to a change of the pH within a cell. Such a link between pH changes and Ca^{2+} influx has been observed in *Dictyostelium* cells after stimulation with the chemoattractant cAMP (Malchow et al., 1978). In agreement with the expectation from the model for the antagonistic reaction, there is no pH-pattern within the cell. The pH in pseudopods is the same as in the remaining cytoplasm (Yumura and Fukui, 1998). This is reasonable since the equilibration of H^+ ions within cells is expected to occur rapidly, in contrast to the localization of Ca^{2+} ions due to the many binding sites. According to the model, although this antagonist has no cell-internal pattern, it plays an important role in the formation of the pattern. If sufficient hot spots are present, it suppresses the formation of additional ones elsewhere on the cell cortex, otherwise new hot spots will emerge until a steady state is reached. If this antagonistic reaction was non-functional, e.g. due to a mutation, a non-patterned activation all around the cell would be expected. However, as the given example of pH change illustrates, this antagonistic reaction may not be based on a single substance that can be mutated.

The last component required would be a local antagonistic reaction. At least for the calcium-induced-calcium-release such a mechanism has been shown to exist (Iino and Endo, 1992). The inositol 1,4,5-trisphosphate receptor ($InsP_3R$) is a widely used Ca^{2+} channel for this release. The type I $InsP_3R$ has the property to open at low and to close at high Ca^{2+} concentrations (Hagar et al., 1998). Therefore, both the self-enhancing reaction and the subsequent antagonistic reaction could be

accomplished by the same receptor and have thus the same range.

In conclusion, a genuine pattern forming mechanism within a cell provides a realistic molecular basis for its extraordinary chemotactic sensitivity. A stable polarity within a cell, for instance to orient cell division, can be generated in this way. Due to a second antagonistic reaction, the system can permanently remain sensitive to external signalling and produce hot spots in a highly dynamic way. This dynamic behaviour can remain even in the absence of an external signal, or if the transduction of the external signal is abolished due to a mutation. Although the molecular interpretation is certainly tentative, I hope that the model helps in the unravelling of forthcoming observations.

APPENDIX: DETAILS OF THE COMPUTER SIMULATION

For the simulations in Figs 5 and 6, the following interaction between an autocatalytic activator a, the rapidly distributed inhibitor b and a local inhibitor c was assumed:

$$\frac{da_i}{dt} = \frac{s_i(a_i^2/b + b_a)}{(s_c + c_i)(1 + s_a a_i^2)} - r_a a_i$$

$$\frac{db}{dt} = r_b \sum_{i=1}^{n} a_i/n - r_b b$$

$$\frac{dc_i}{dt} = b_c a_i - r_c c_i$$

i=1...n denotes the surface elements (‘cells’). The following constants have been used: decay rate of the activator a, r_a=0.02; basic production of the activator, required for initiation of the activator autocatalysis b_a=0.1; saturation of the activator autocatalysis to enable the coexistence of several maxima, s_a= 0.005; production and decay rate of the rapidly equilibrating inhibitor b, r_b=0.03; production and decay rate of the non-diffusible inhibitor c, b_c=0.005 and r_c=0.013, Michaelis-Menten constant s_c=0.2; external asymmetry and random fluctuations are integrated in the factor s_i=r_a(1+$dy\ cos$(6.283(i–j)/n)) (1+dr RND); external asymmetry dy=0.02 (Fig. 5A) or 0.05 (Fig. 5B), random fluctuation dr=0.01; RND=random numbers, 0<RND<1, direction of the external asymmetry j, 1≤j≤n. Fig. 5: n=30; between each plotted line 40 iterations are calculated, 4800 iterations in total. Fig. 6: n=50; 300 iteration between each plot. Further computational details for pattern forming reactions in general, for pattern formation by one inhibitor and one depleted substrate (Fig. 4) and PC software can be found elsewhere (Meinhardt, 1998).

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