On the crawling of cells

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TABLE OF CONTENTS

Summary
Introduction
The physical chemistry of cytogel
   Elastic properties of cytogels
   Osmotic properties of cytogels
   Ionic regulation of cytogels
   Solution and gelation
   Active contraction
   Regulation of cytoplasmic calcium concentration
A model for lamellipodia
   The effects of different modes of solation
Discussion
Conclusions
References

SUMMARY

Motile cells moving in vitro do so via a characteristic motile appendage, the lamellipod, a broad, flat region of the cell cytoplasm which extends ahead of the moving cell, and which is devoid of organelles. I describe here a mechanochemical model for how the lamellipod propels the cell forward. The propulsive force for the spreading of the lamella derives from the swelling pressure of the cytogel within the lamellipod. A local change in ionic conditions - caused by a leak through the leading membrane - activates solating factors, which upsets the equilibrium between the gel osmotic pressure and its elasticity. Subsequently, regelling and contraction pull the cell forward. The model is in accordance with a diverse array of experimental observations on cell motility, and suggests a common physical mechanism underlying a variety of cell motility phenomena.

I. INTRODUCTION

Despite an enormous body of observation and speculation, the mechanism of cell motion is still poorly understood. I shall propose here a model for cell locomotion which ties together many of the diverse properties of moving cells. This is not to say that the model 'explains' cell motion; the phenomenon of cell movement is sufficiently complicated that this task is probably beyond the power
of any single model. Rather, I will restrict my attention to the force generating mechanism for cell locomotion. That is, I address the question: what are the properties of the actomyosin cytogel that enable it to generate the mechanical forces that drive cell spreading and translocation, and how are these forces coordinated?

The ‘motile organs’ of moving cells fall into a few categories, according to their appearance. The structure I shall be concerned with is the lamellipod, a broad, flat sheet of cytoplasm that forms the leading edge of moving cells such as fibroblasts and translocating epithelial cells in vitro. It appears to be devoid of organelles, and composed almost exclusively of actin gel. Other cell protrusions of different morphology, such as microspikes, filopods, microvilli and blebs may also act as, or be converted into, motile appendages.

Almost everyone agrees that the contractile forces required for cell locomotion are generated by an actomyosin mechanism analogous to that in muscle. The difficulty arises in reconciling two aspects of cell motion: (a) How is protrusion accomplished? (b) How can directed motion arise from an apparently isotrophic contractile gel? The former question is especially vexing, for while it is easy to see how contraction forces are produced, it is less obvious how the force for extension is generated.

Numerous workers in the field of cell motility have proposed models and hypotheses which attempt to accommodate these difficulties, and I shall not attempt to review or comment on these efforts here (c.f. Middleton & Sharp, 1984; Sheterline, 1983; Bellairs, Curtis & Dunn, 1982; Abercrombie, 1980; Small & Isenberg, 1978; Dunn & Ebendahl, 1978; Trinkaus, 1984). The model I shall present here incorporates many of the suggestions of previous workers. It differs from these efforts in that it provides an integrated scheme for cell protrusion and contraction which can be formulated precisely in terms of mathematical equations based on the physical chemistry of gels. This is not just window dressing: in seeking to understand a complicated physical process, it is notoriously easy to advance verbal arguments that turn out to be specious when the calculations are actually carried out.

There are several issues which the model does not address. In particular, I do not discuss how the membrane is recycled, nor how the adhesions to the substrate are assembled and disassembled. To be sure, these are important aspects of cell locomotion, but they are peripheral to the issue of force generation.

The essence of the model is as follows. An ionic leak at the leading membrane leads to a local increase in calcium concentration. The elevated calcium causes the actin gel to partially solate in the vicinity of the leak. This allows the gel to swell osmotically, thus providing the force for spreading. Regelation occurs as the calcium is resequestered, and the gel commences to contract. The motion of the cell is thus driven by cycles of solation and expansion followed by gelation and contraction.

This article is organized as follows. In the following section I shall outline what
I feel are the most important aspects of the physical chemistry of actomyosin gels as they relate to cell motion. Then in Section 3 I construct a model for lamellipodial extension. In order to keep things as simple as possible my discussions will be mostly qualitative and heuristic; the Appendix contains the mathematical aspects of the model for the interested reader, as well as references to more detailed treatments. In Section 4 I discuss the model's properties as they relate to cell motion and morphogenesis.

2. THE PHYSICAL CHEMISTRY OF CYTOGEL

In order to discuss the force-generating machinery of actomyosin gels we must first address some aspects of their physical chemistry. I shall discuss here the elastic and osmotic properties of gels in general, and the ionic regulation of solation and contraction peculiar to cytogel.

Consider the situation shown in Fig. 1A: a block of gel is contained in a cylinder of unit cross-sectional area which is immersed in a bath of solvent. A piston which is completely porous to solvent is attached to the gel's upper surface so that the gel may be stretched or compressed without interfering with the passage of solvent.

If the gel is initially dry and we add solvent, the gel will swell by imbibing the

![Diagram of gel and piston](image)

Fig. 1. A gel is confined to a cylinder of unit cross-sectional area with a piston permeable to solvent. The gel consists of a network of crosslinked polymer chains. We use the term 'chain' to denote a polymer segment that joins two nodes in the network. The chain shown in the centre with only one end joined to a node cannot contribute to the elasticity of the gel. A force, $F$, is applied to the piston to stretch the gel, which is opposed by the elastic restoring force generated by the thermal motion of the network chains.
solvent. The force on the piston required to restrain the swelling is called the swelling pressure, which we denote by $P_s$. The swelling pressure will decrease as the piston rises until finally the gel reaches an equilibrium degree of swelling at a height $L$ as shown in Fig. 1. We can measure the deformation that accompanies swelling by defining the strain $\lambda = L/L_0$, where $L_0$ is the initial height of the gel.

Swelling ceases when the internal forces just balance each other. These internal forces derive from the elastic and osmotic properties of the gel. That is, we can break up the total swelling pressure into separate contributions:

$$P_s = P_{\text{elas}} + P_{\text{osm}}$$

At equilibrium, $P_s = 0$ (we no longer have to exert any pressure on the piston to maintain the gel volume constant) and $-P_{\text{elas}} = P_{\text{osm}}$. We can discuss each of these contributions to the swelling pressure individually.

(a) Elastic properties of cytogels

Suppose the gel is in equilibrium and we lift on the piston with a force $F$ so that the gel is in tension. The gel resists stretching with a force $F = -P_{\text{elas}}$. If we plot the displacement corresponding to a series of applied forces, $-P_{\text{elas}}$, we obtain a curve like that shown in Fig. 2.

The sign conventions physical chemists use to discuss gel swelling can be confusing: the elastic pressure, $P_{\text{elas}}$, is the negative of the applied force in this experiment ($-P_{\text{elas}} = F$); that is, a positive $F$ tends to stretch the gel, while the elastic component of the swelling pressure resists stretching.

We can divide the stress–strain curve into several domains. In region I the gel is in compression ($F < 0$ means we are pushing on the piston, thus putting the gel in compression). The curve must go to $-\infty$ as the strain, $\lambda \to 0$, since one cannot compress the gel to zero volume. In region II the gel is in tension ($F > 0$) and the curve rises, but with decreasing slope as the gel is stretched. In region III the curve bends upwards, and in region IV the curve reaches a maximum and commences to fall off. We can understand this behaviour by considering the molecular structure of the gel.

A gel consists of linear polymers, actin in our case, which are linked together into a network, as shown schematically in Fig. 1. The elasticity of the gel arises from the random thermal motion of the network chains: when the gel is stretched the fibres are extended beyond the length dictated by the thermal equilibrium between the chains and the solvent (Hill, 1960; Flory, 1953; Aklonis & MacKnight, 1983). Only network chains which connect two crosslinks are effective in generating elastic forces. The polymer segment with only one crosslink (i.e. with one free end) shown in Fig. 1 cannot contribute any mechanical effect. Thus we shall reserve the term ‘chain’ for a polymer segment which joins two nodes of the network.

The decreasing slope of the curve in region II can be derived from statistical mechanics (see Appendix, and Aklonis & MacKnight, 1983). It comes about
Crawling of cells

Fig. 2. The elastic response of the gel can be characterized by measuring the stress–strain curve. A force, \( F \), is applied to stretch the gel and the corresponding strain, \( \lambda = L/L_0 \) is measured. The force, \( F \), is opposed by the elastic component of the swelling pressure: \( F = -P_{\text{ELAS}} \). In region I (0 < \( \lambda < 1 \)) the gel is in compression, and falls off to \(-\infty\) as \( \lambda \to 0 \) since the gel cannot be compressed to zero volume. In tension, one can distinguish three regimes. In region II the elastic response is largely generated by the random thermal motions of the chains, and the stress–strain relationship rises, but with decreasing slope. In region III the stress–strain curve bends upward as the gel fibres align, and the effective modulus (i.e. the slope of the curve) increases. In region IV the network finally commences to tear as the fibres yield and crosslinks break, and the curve reaches a maximum and falls off. The form of the stress–strain curve in region I can be derived from statistical mechanics (c.f. Appendix): \( P_{\text{ELAS}} \propto N_c(\lambda - 1/\lambda^2) \). If the concentration of chains, \( N_c \), is decreased (i.e. solation of the gel), the elasticity of the gel decreases and the response curve falls as shown.

because, as the chains are stretched, the number of configurations they can achieve under thermal agitation becomes more restricted, and their elastic effect decreases.

The curve turns upward in region III because the fibres of the network align as the gel is stretched, and the apparent modulus of the gel increases. This effect is the same as stretching a piece of wool: the longer it gets the stiffer it appears.

Eventually the network commences to yield as the fibres are torn and crosslinks broken under the applied tension; when the curve levels off and falls in region IV. In our subsequent discussion we shall focus on the behaviour of the
gel in region II, although the behaviour in other regions is important in other settings (Oster & Odell, 1984a,b).

One can show from statistical mechanics that the force required to stretch the gel in Fig. 1 from an initial length $L_0$ to a final length, $L$, is proportional to the concentration of chains in the network, which we denote by $N_C$ (Hill, 1960, Chap. 21; Aklonis & MacKnight, 1983, Chap. 6):

$$P_{ELAS} \propto N_C$$

Thus if some of the network chains were cut, so that $N_C$ decreases, then the elastic modulus of the gel would decrease and the stress–strain curve would fall, as shown in Fig. 2.

The stress–strain relationship shown in Fig. 2 describes the passive elastic properties of a block of cytogel. Its qualitative features can be derived from physical considerations; however, too little is known of the mechanical properties of cytogel to allow quantitative characterization, and so it is best to regard Fig. 2 as an empirical curve. Fortunately, the model depends only on the qualitative aspects of the curve; the key feature is that the elastic modulus is proportional to the concentration of chains in the network.

(b) The osmotic properties of cytogels

When a dry piece of gel is immersed in a solvent, the gel will imbibe solvent and swell (Fig. 3A). This is due to the chemical potential gradient between the interior of the gel and the solvent: the solvent molecules can lower their chemical potential by associating with the polymer (Hill, 1960; Tanaka, 1981). If the gel were not crosslinked into a network, then the swelling pressure – the pressure that must be exerted on the piston to prevent the gel from expanding – would derive entirely from the osmotic pressure, which we denote by $P_{OSM}$. $P_{OSM}$, in turn, is composed of three effects which can be treated separately (Tanaka, 1981; Hart & Farrell, 1971):

$$P_{OSM} = P_{MIX} + P_{INT} + P_{ELEC}$$

(i) $P_{MIX}$ is the contribution to the swelling pressure which arises from the free energy decrease which accompanies the mixing of the solvent in the polymer. This is an entropic effect: it depends on the configurational freedom of the polymer chains as they are buffeted by the solvent molecules and squirm through various twisted shapes. As the gel is stretched, the chains’ freedom to change configuration is restricted, and so $P_{MIX}$ decreases with strain. Conversely, if one cuts some network chains, their freedom to adopt various configurations is increased, and so $P_{MIX}$ rises.

(ii) $P_{INT}$ is pressure arising from the interaction between the polymer chains and the solvent. For actin gels under physiological conditions this will be a positive contribution to the swelling pressure.

(iii) $P_{ELEC}$ arises from the electrostatic repulsion of fixed charges on the fibres.
This is an important swelling force in polyelectrolyte gels such as the corneal stroma, articular cartilage and extracellular matrix rich in hyaluronate (Grodzinsky, 1983).

If $P_{\text{OSM}}$ is measured as a function of strain, one obtains a curve which decreases

![Diagram of swelling force](image)

**Fig. 3.** (A) A block of gel placed in a solvent bath will swell by imbibing fluid. The influx of fluid is driven by a chemical potential gradient, $\Delta \mu$, between the gel and the solvent bath. The fluid influx creates a swelling pressure in the gel proportional to $\Delta \mu$. The gel swells until the osmotic pressure, $P_{\text{OSM}}$, is counterbalanced by the elastic restoring forces of the network chains, $-P_{\text{ELAS}} = P_{\text{OSM}}$. (B) The qualitative dependence of the osmotic component of the swelling pressure, $P_{\text{OSM}}$, on strain and chain concentration. If the gel is stretched it absorbs more solvent and $P_{\text{OSM}}$ falls. If the gel solates, so that the chain concentration, $N_c$, decreases, then the osmotic pressure will overcome the elastic pressure and will cause the gel to expand further.
as the gel dilates, as shown in Fig. 3B. Presently, it is not possible to compute the contribution of these components from basic theory for actin gels. Therefore, we must regard the osmotic pressure curve shown in Fig. 3B as an empirical relationship. However, like the elasticity curve in Fig. 2, only its qualitative features are important for our purposes.

For most gels, there is only one ‘solute’ molecule, the network itself. However, gels like those formed by actin monomers are equilibrium structures; that is, monomers are constantly polymerizing onto and depolymerizing from the network. Adding to the complexity of the situation is the fact that actin is a polarized molecule: subunits appear to add more readily to the ‘barbed’ end (as indicated by binding of heavy meromyocin) and dissociate more easily from the ‘pointed’ end. The rate and degree of polymerization is regulated by actin-binding proteins which complex with monomers and chain fragments; I will say more about this later. Nevertheless, cytoplasmic gels generally consist of an actin network in equilibrium with subunits (monomers and network fragments). The concentration of subunits also contribute transiently to the local swelling pressure, but unlike the polymer they are free to diffuse away from the gel, and so do not affect the equilibrium swelling pressure.

If the gel is unrestrained by a piston, as shown in Fig. 3A, solvent will flow into the gel, swelling the gel. As we stated above, this swelling will continue until the elastic restoring forces in the gel are in equilibrium with the osmotic forces, whereupon the swelling pressure falls to zero: \( P_s = P_{ELAS} + P_{OSM} \rightarrow 0 \) at equilibrium. Fig. 4A shows how \( P_{ELAS} \) and \( P_{OSM} \) sum to form the swelling pressure:

\[
\begin{align*}
\text{Fig. 4. (A) The swelling pressure, } P_s, & \text{ is the sum of the elastic pressure, } P_{ELAS}, \text{ and} \\
& \text{the osmotic pressure, } P_{OSM}. \text{ The } P_{ELAS} \text{ curve is the negative of the stress–strain curve} \\
& \text{in Fig. 2, so in the region above the } \lambda = L/L_0 \text{ axis the gel is in compression. } \lambda_{eq} \text{ is the} \\
& \text{equilibrium strain where the elastic and osmotic forces just cancel. (B) The dependence of the swelling pressure, } P_s, \text{ on strain and chain concentration, } N_c. \text{ If the} \\
& \text{swelling pressure is held constant and the chain concentration decreased from } N_{c1} \text{ to} \\
& N_{c2}, \text{ then the equilibrium swelling of the gel increases from } \lambda_1 \text{ to } \lambda_2.
\end{align*}
\]
Crawling of cells

pressure curve. Fig. 4B shows, qualitatively, how the swelling pressure depends on the chain concentration, \( N_c \), and on the gel deformation, \( \lambda = L/L_0 \).

An unusual property of gels is that they are ‘distributed osmometers’: unlike a membrane osmometer, if a gel is punctured, it does not deflate. Thus a gel can support swelling pressure without a bounding membrane, a fact that has important consequences when discussing the turgor pressure within cells.

From our viewpoint, the important feature of the swelling pressure is this: if the network is disrupted by breaking chains and/or crosslinks, the gel will swell further until a new equilibrium is reached between the osmotic pressure and the elastic restoring pressure. This is shown in Fig. 4B: suppose that the swelling pressure is maintained constant – say, by placing a weight on the piston in Fig. 1 – and allowed to reach an equilibrium strain, \( \lambda_1 \). If we cut some chains, so that the chain number decreases from \( N_{c1} \) to \( N_{c2} \), then the gel will swell until a new equilibrium strain, \( \lambda_2 \), is reached.

The above properties of osmotic swelling and elastic response are characteristic of all gels, regardless of their constitution. However, actomyosin gels have two additional properties which give them a unique character: (i) cytogels can regulate their degree of gelation – that is, the number of chains – and (ii) they can generate active contractile forces. Both of these are accomplished by regulating ionic concentrations.

(c) Ionic regulation of cytogels

Actomyosin gels are regulated by local ionic conditions, particularly calcium, in a variety of ways (Korn, 1982; Weeds, 1982). This is a complicated, and as yet unresolved business; however, for the purposes of understanding the mechanical behaviour of cytogel we can treat the sol–gel transition and active contraction phenomenologically.

(i) Solation–gelation

Free calcium affects the activity of a number of solation and gelation factors (Korn, 1982; Weeds, 1982; Taylor, Hellewell, Virgin & Heiple, 1979; Taylor & Condeelis, 1979; Yamamoto et al. 1982). While the details are still obscure, we need account only for the following aspect: when free calcium levels rise to the micromolar level, the actin gel commences to solate.

Solation amounts to breaking up the gel network, and this can be accomplished in at least three ways: (1) breaking of network chains, (2) breaking of crosslinks joining network chains, and (3) depolymerization of chains. Each of these affects the gel dynamics in somewhat different ways, as I shall discuss later. The qualitative features of the solation response are illustrated in Fig. 5A which shows how the number of network chains, \( N_c \), decreases as calcium levels increase. This decrease in chain concentration manifests itself as a decrease in measured viscosity of the gel (Stossel, 1981), as shown in Fig. 5B.
Fig. 5. (A) Solation of a gel corresponds to a decrease in the concentration of network chains, \( N_c \). At micromolar calcium concentrations the solation factors are activated initiating a gel–sol phase transition. (B) Solation results in a loss of gel elasticity, and a drop in measured viscosity corresponding to the decrease in chain concentration.

(ii) **Active contraction**

The chain of events by which free calcium ions trigger contraction is non-muscle actomyosin gels is not completely settled; however, it involves a number of intermediates, including calmodulin, myosin light-chain kinases and cAMP. For our purposes, I shall adopt the phenomenological viewpoint espoused in Oster & Odell (1984a, b) in modelling the plasmodial oscillations in *Physarum*. That is, I shall assume that calcium is the proximal trigger for actomyosin contraction.

The assumption that calcium initiates contraction of the actomyosin fibres is embodied in the qualitative features of the curve shown in Fig. 6, which shows that the active traction, \( T \), per chain increases monotonically with calcium concentration, \( c \). However, as I discuss below, calcium also solates the network, and so the number of chains capable of supporting tensile stress decreases. The result is that, in an experiment where calcium concentration is varied at constant strain, the active contractile stress of a piece of cytogel first rises as the contractile machinery is activated, then falls as the network solates. Thus there is a calcium ‘window’ for contraction, with a maximum traction stress in the micromolar region.

It is important to realize that the total traction curve in Fig. 6 must be measured in a ‘calcium clamp’ experiment. The reason is that there can be a considerable lag between the rise in calcium and the time contraction is manifest. Raising the calcium concentration to the micromolar region activates both solation and contraction kinetic sequences. However, it appears that the solation kinetics is faster: solation factors act immediately upon binding Ca++ , or in
near micromolar concentrations calcium activates the contractile machinery of the actomyosin system, thus the traction per chain increases. However, the solation factors are also activated, resulting in a drop in the chain concentration, $N_c$. Thus the total traction of a volume of gel first rises as active contraction commences, then falls as the gel solates. Note that in order to generate traction some solation is necessary to create free actin strands, to which myosin can bind and crosslink. Also, the time course of solation and contraction is such that there is usually a time lag between the rise of calcium, the solation and regelation, and the contraction. Thus Fig. 6 represents an experiment wherein the calcium concentration is 'clamped' at successively increasing values.

- Analysis of the model equations show that this temporal sequencing of solation and contraction can dramatically affect the dynamic behaviour of the cytogel. Qualitatively, this is easy to understand. First, a completely gelled network cannot develop much compressive stresses, both because it is too rigid, and because of the nature of actomyosin contraction. That is, contraction requires two counter-oriented actin strands to interdigitate with the myosin to create the sliding filament configuration. Therefore, creation of free chain ends is required before contraction can occur. Conversely, if too many chains are broken, then the network can no longer transmit tensile stresses, and the actomyosin contraction is ineffective.

Thus we see that a partial solation is most effective in creating a contractile network. One way to achieve this is by making the solation step fast, so that by the time contraction commences, the network is regelling.
(iii) Regulation of cytoplasmic calcium concentration

Finally, we must say something about how calcium levels are regulated by the cytogel. I shall assume that calcium is sequestered and released from membranous sites distributed throughout the cytogel.

A feature of this release–sequester system in Physarum, and a number of other systems, is the phenomenon of 'calcium stimulated calcium release' (Jaffe, 1981): introduction of a critical amount of free calcium, say $c^*$, into the cytogel triggers an autocatalytic release of calcium from the sequestering sites. The qualitative features of this release-sequestering system are shown in Fig. 7: small perturbations in calcium levels are buffered to their equilibrium levels, but too large a perturbation initiates the release cascade. I assume for simplicity that resequestration takes place according to first order kinetics.

To recapitulate, I have made the following assumptions about the physical chemistry of actomyosin gels:

1. With regard to its elastic and osmotic properties, cytogel behaves as an ordinary gel. In particular, the elastic modulus is proportional to the number of network chains, $N_c$, so that the gel will swell when network chains are severed.
2. Cytogel reversibly solates when exposed to micromolar levels of calcium.
3. Cytogel can contract actively when stimulated by micromolar levels of calcium.
4. Calcium is sequestered in membranous compartments distributed throughout the cytogel. This calcium can be autocatalytically released when exposed to a threshold level, $c^*$, of calcium.

3. A MODEL FOR LAMELLIPODIA

In the Appendix the above properties of cytogel are cast in mathematical form, in order to investigate the logical consequences of our assumptions (Oster & Perelson, 1984). In this section we investigate the implications of the model for cell motion.

In Fig. 8A I have constructed a model lamellipod, shown in cross section. It consists of a sheet of cytogel which is anchored to the substratum by elastic tethers (shown schematically as springs), and bound by a membrane. I shall not address the issue of where the extra membrane comes from as the lamellipod extends; it appears in the model only as a boundary condition for the cytogel.

The mechanochemistry of the lamellipod is described by the following four quantities (see the Appendix):

(a) $G$ is the gel concentration at each position along the lamella; i.e. the concentration of network chains.
(b) $S$ is the volume fraction of the sol-component. By 'sol' I mean all the
Calcium is assumed to be sequestered in sites distributed through the cytogel. When exposed to a concentration of calcium above a threshold value, $C^*$, the permeability of the sequestering vesicles, $P(c)$, increases and the internal stores of calcium are autocatalytically released ('calcium-stimulated calcium release'). After release, the calcium is resequestered by membrane pumps. The functional form of the calcium kinetics employed in the model is given in the Appendix.

(c) $c$ is the concentration of calcium at each point.
(d) $u$ is the displacement of each point in the gel from its initial position.

In order that the cell be able to move we must make some assumption about the cell adhesion sites. In the model equations I have assumed that the adhesion 'springs' are elastic 'tethers' attaching the gel to the substratum. Thus when the
gel solates the force of adhesion disappears, and reappears when regelling occurs. Thus the effective anterior tethers commence at the gel–sol front. In order to allow translocation, we must also allow the tethers to ‘yield’ when a critical stress is exceeded. Thus the contraction of the cytogel can pull the rear tethers free. In reality, the adhesions appear to fade out as they move centripetally, so the cell is attached primarily at its periphery. It is not clear why this happens, but a better model for the attachment sites would allow for this proximal attenuation of adhesive strength.

Now I make the critical assumption upon which all of the subsequent conclusions rest:

\textit{Lamellipodial extension is triggered by an ionic leak through the membrane near the leading edge.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig8a.png}
\caption{Fig 8A}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig8b.png}
\caption{Fig 8B}
\end{figure}
Crawling of cells

This is the simplest hypothesis which is consistent with observations; however, in Section 4 I shall briefly discuss an alternative mechanism for controlling calcium release which is known to operate in other cytogel systems.

This calcium leak may be triggered by a variety of stimuli, including binding of ligands to membrane receptors, electrical depolarization of the membrane, insertion of new membrane, or mechanical stress at adhesion sites, and so forth. I shall say more about this later; for now, I assume that such a leak is introduced at the right-hand edge of the model lamellipod in Fig. 8A.

Once the leak has been introduced, each volume of cytogel undergoes the cycle of events shown in Fig. 8B. The leak initiates release of calcium from internal membranous stores and/or leakage from the external medium. The elevated calcium levels in the cytogel activate solating factors (e.g. gelsolin, severin) which decrease the chain concentration in the vicinity of the leak. This allows the gel to swell until a new equilibrium is reached between the swelling pressure and the elastic pressure. As the calcium is resequestered, the chains commence to reform, increasing the gel elasticity once again.

Meanwhile, the elevated calcium levels have begun to activate the actomyosin contractile machinery, and as the calcium levels fall the active contraction cycle commences. The contraction cycle completes, the gel relaxes and is now 'reset' to commence another cycle of solation, expansion, gelation and contraction (SEGC).

The behaviour of the model depends on the relative rates and amplitudes of the various processes: (i) the rate of calcium release and resequestration; (ii) the rate (and mechanism) of solation and gelation; (iii) the osmotic expansion rate; (iv) the rate of active contraction and relaxation, and (iv) the strength of

Fig. 8. (A) The model lamellipod: a gel sheet (shown in cross-section) is attached to the substratum by elastic tethers. The membrane acts only to wrap the gel, and contributes nothing to the mechanical force balance except a small restraining tension. The tethers attach to the gel, and so when the gel solates the attachments weaken and effectively disappear. The ion and gel profiles shown are snapshots at a particular time after the initiation of the ion leak at the leading edge. (B) Each volume of cytogel undergoes the cycle of events shown. 1. The ionic leak elevates the cytoplasmic concentration of calcium. This occurs by release from internal stores and/or influx from the surrounding medium. 2. If the calcium levels reach micromolar concentration, solating factors commence to reduce the chain concentration of the gel by severing and capping the actin fibres. The events leading to active contractile machinery is also set into motion, but contraction does not commence immediately. 3. The partially solated gel expands under its swelling pressure and the hydrostatic pressure created by contractile gel elsewhere. 4. As the calcium is resequestered and the cytoplasmic concentration falls below micromolar levels, regelling commences as the chains reform. 5. The gel now commences to contract, both from the increased elasticity arising from the increase in chain concentration, and from the active contractile machinery. 6. As the calcium is buffered back to normal levels (~10^{-7} M) the contractile machinery relaxes, and the sequestering vesicles emerge from their refractory period; the cytogel is ready for another cycle of solation, expansion, gelation and contraction.
adhesion to the substratum. A variety of situations can be described by varying these quantities; here I illustrate the possibilities by describing two cases which represent the extremes of the model’s behaviour.

(a) Consider the situation illustrated in Fig. 9A. An ionic leak at the lamella tip initiates a calcium wave which propagates rearward; this calcium wave partially solates the gel and so a front of solation follows the calcium wave. This solated region expands pushing the leading tip outward. This expansion is driven by the osmotic swelling pressure of the gel and the hydrostatic pressure generated by contraction of the gel elsewhere. Subsequently, the lamella regels, the new extension attaches to the substratum, and commences to contract actively. Thus a wave of solation and expansion follows the ionic wave, and following that a

(i) solation

(ii) expansion

(iii) gelation

(iv) contraction

Fig. 9A
Fig. 9. (A) An ionic leak at the right-hand boundary activates the solation factors and the tip region partially solates, (i). (ii) The solated region expands to the right until the swelling pressure is countered by the elastic pressure of the remaining chains. If the leak raises the internal calcium concentration too high, an autocatalytic release is initiated (calcium-stimulated calcium release) which propagates leftward as a calcium wave. Panels (i)–(iii) shows the calcium wave at three successive time snapshots. This wave leaves a trail of solation behind it, which expands under the gel swelling pressure. (iii) When the calcium level falls the expanded region regels, and reestablishes attachment sites to the substratum. The contraction wave which follows sweeps leftward; panel (iv) shows the contraction wave at a particular instant in time displayed as a graph of the displacement, \( u \), of points in the gel from their initial positions. Positive \( u \) denotes displacement to the right; the resulting traction exerted on the substratum by the cell is shown by the arrows below the substratum. Negative \( u \) indicates that gel is displaced to the left and thus exert traction forces to the left. This pulls the gel to the right, and if the traction is strong enough, the leftmost tethers are ruptured and the cell advances to the right. Note that the leftward directed tractions (those pulling the cell to the right) only win out if the net strength of adhesion of the distal (rightmost) attachments is stronger than the total of the adhesions to the left. This suggests that newly formed distal attachments must be stronger than older, more proximal attachments. (B) If the calcium stimulus is below the threshold for wave propagation, the solation can still swell to the right: (i)–(ii). The ionic profile is deformed by the water influx so that new gel subunits can diffuse into the solated region and polymerize onto the free fibre ends: (ii). Then regellation and contraction occurs: (iii). Finally, the ionic profile rebounds to its former shape and the newly formed gel solates and swells.
wave of gelation and contraction propagates towards the cell body.

It is worth noting that the phase lag between calcium induced solation and active contraction is also observed in other contractile systems. For example, the plasmodial oscillations in *Physarum* exhibit tension and calcium oscillations which are out of phase, the tension lagging the calcium by about a minute or so (Kamiya, Yoshimoto & Matsumura, 1982; Oster & Odell, 1984). Also, the surface waves observed in amphibian eggs exhibit a similar lag between ionic and contractile events and can be modelled by a similar cycle of solation–expansion–gelation–contraction (SEGC) (Cheer, Nuccitelli, Oster & Vincent, 1984).

The contraction wave depends on the local concentrations of myosin in the lamellipod. Assays show that myosin is concentrated back from the leading edge; however, these tests are not definitive since it is difficult to detect amounts of myosin which would nevertheless be mechanically significant. In any event, it is difficult to see how myosin could be prevented from diffusing or convecting to the tip, and so I have assumed that it is present everywhere. There is no difficulty, however, in modifying the model to account for a heterogeneous distribution of myosin, and this might change the dynamics somewhat.

This sequence of events appears to represent what happens during the forward motion of certain fibroblast cells (Trinkaus, 1984, p. 193–5): the front surges outward, then – if the lamella adheres to the substratum sufficiently strongly – a contraction wave pulls the cell forward. If adhesion is not strong enough, the lamella is retracted by the contraction. Time-lapse films of cell locomotion clearly reveal cycles of forward surging (which I ascribe to the solation–swelling cycle) and retraction (which I ascribe to the regelling and contraction cycle). It is also strikingly apparent from such films that a centripetally moving refractive front appears sporadically and follows the forward surge. This type of motion is mechanically akin to the crawling of a worm: forward extension and adhesion, followed by contraction and drawing up of the rear. The analogy is not too close, however, since the forward surge of a worm is caused by constriction of its circumferential muscles, instead of osmotic swelling. However, the rearward sweep of the contraction wave can create a hydrostatic pressure which will act as a peristaltic wave. This can augment the swelling pressure much like squeezing a tube of toothpaste (c.f. Trinkaus, 1984, p. 207–8). Such a mechanism has been suggested previously by several workers (Ambrose, 1961; Harris, 1973).

(b) In contrast to the intermittent motion of fibroblasts, certain epithelial cells in culture appear to move with a much smoother, continuous motion: the lamella appears to flow forward in a steady fashion (Euteneuer & Schliwa, 1984). This type of motion is characteristic of an operating regime as shown in Fig. 9B. Here the ion flux is not sufficient to excite the calcium wave, but yet strong enough to initiate the solation–expansion cycle near the tip. The fluid influx accompanying the expanding gel dilutes the calcium so that the ion profile is deformed downward as shown. This allows the gel front to advance into the expanded region, where new actin is polymerized and commences to contract. Gradually the ion
concentration restores itself towards its initial profile and the new gel commences to solate and expand, repeating the cycle. Here too, the cycle of solation–expansion, followed by gelation contraction propels the cell forward, although in a much smoother and less obviously pulsatile fashion. In these cells contraction waves can be observed periodically sweeping centripetally from the lamella to the cell body.

A second major difference between the pulsatile fibroblast motion and the smoother flowing epithelial cells is their pattern of substratum adhesion. The epithelia display fewer focal adhesion sites, and appear to adhere to the substratum by a more numerous distribution of weaker close contacts. The net effect from the viewpoint of the model is that the density of substratum tethers is higher, but the yield point is lower; this leads to a smoother peeling of the trailing edge from the substratum, and contributes to the more uniform advance of the leading edge.

It is worth noting that the contractile action of the gel can create a hydrostatic pressure gradient when the gel fibres are dragged forward through the liquid phase of the cytoplasm. This pressure gradient can be quite sizeable (Odell, 1977), and it can help force cytosol into the tip region, to align the gel fibres, and to exert tractions on the substratum via the adhesive junctions.

If the entire lamellipod were to solate then the swelling pressure would inflate it to a sphere. Several factors prevent this. First, the solation is only partial; enough gel elasticity remains to contain the swelling pressure except at the very tip of the lamellipod, perhaps in the last 0.5 µm or less, where solation is so extensive that swelling is appreciable. Second, the lamellipod is adherent to the substratum along its periphery, and lateral tensions generated by the contractions tend to maintain a flattened configuration. In the one-dimensional model described here these are irrelevant; however, they must be accounted for in the higher dimensional version.

The effects of different modes of solation

I have used the term 'solation' in the model rather generically, to describe the melting of the actomyosin gel. However, the details of the solation–gelation kinetics, which are quite complex and as yet unresolved, are important for understanding the mechanism of cell movement.

There are at least three ways in which the gel network can be solated, according to the action of the calcium-activated solation factors: (a) cutting of network fibres, (b) breaking of crosslink sites, and (c) capping of the fast growing ('barbed') end of the fibres (c.f. Sheterline, 1983). The effect of each is somewhat different on the osmotic and elastic properties of the gel.

(a) Solation factors which only cut network fibres between crosslinks have several effects. First, since the elasticity depends on the number of chains, $N_c$, and the number of units per chain, cutting of chains reduces $N_c$, and thus the network elasticity decreases (Aklonis & MacKnight, 1983; Hill, 1960). The
osmotic component of the swelling pressure should not change much when chains are cut (although since cutting increases the freedom of the cut chains, there may be some increase).

However, cutting of a chain exposes two new growing tips a ‘barbed’ end and a ‘pointed’ end whose assembly–disassembly kinetics differ (Hill & Kirschner, 1982, 1983). Therefore, if no capping occurs cutting could actually cause the gel phase to grow. Thus if solation occurs primarily by severing fibres with little capping, then the protrusion mechanism can work as follows. The ionic leak at the tip activates the solation factors which cut the network and allow it to swell osmotically. The free ends can grow by addition of monomers from solution, and the gel interface will follow the advancing tip outward. This mechanism will operate below the threshold for calcium-stimulated calcium release, and produces a steady advance of the leading edge, in contrast to the intermittent mode of advance described above.

(b) Breaking of fibre crosslinks should not affect the osmotic pressure much, but for each link broken the number of chains, $N_c$, is reduced by one, and the number of links in that chain is doubled. Thus the net effect of breaking cross-links should be to decrease the network elasticity, leading to swelling.

(c) If a chain is severed, and the severing protein binds to the barbed end, then this leaves the pointed end free, and disassembly will commence, although at a rate less than the barbed end. If depolymerization proceeds past a network node, this would decrease the number of chains. Therefore, capping could decrease the network elasticity. Moreover, if fibres depolymerize from the sharp end, monomers are added to the total solute concentration in the gel, and there could be a substantial increase in the osmotic component of the swelling pressure (which depends on the number of solute particles). Thus capping of the barbed ends should enhance the local swelling of the gel, although this is a transient effect which is dissipated as the solute particles diffuse away.

4. DISCUSSION

The simple solation–expansion/gelation–contraction (SEGC) mechanism proposed here for propelling lamellipodia rests on the assumption that an ionic leak at the leading edge disturbs the swelling equilibrium of the cytogel. The model does not deal with the mechanism of this ionic leak: it enters as the boundary condition. The intermittent protrusive behaviour of fibroblast lamellae may indicate that the leak is stochastic, or at least intermittent; however, it may as well indicate the cyclic nature of the solation–contraction cycle (c.f. Oster & Odell, 1984a,b). In the absence of chemotactic agents which might induce leaks, one is led to suspect that the attachment sites might be the source of leaks since the membrane and cortical gel is under maximum tensile stress at these points. Moreover, plasma membrane glycoproteins are known to agglutinate at the site of contact between the cell and the substratum. It is
possible that a similar mechanism concentrates ion channels near adhesion sites. I shall explore the consequences of the various theories of membrane supply and leakage for lamellipodial movements in a subsequent publication.

Regardless of how the membrane leak is produced, it is important that this mechanism for directing extension be consistent with what is known about lamellipod locomotion. In order to establish this, I cite the following examples.

(1) **Chemotaxis.** The SEGC model suggests a common mechanism for chemotactic agents: binding of the attractant institutes a leak which ignites the SEGC cycle. This is an attractive mechanism for the following reason. The gel–sol phase transition curve upon which the motion depends is typically sigmoidal, as shown in Fig. 11. The steepness of this curve provides an ideal amplification mechanism for detecting small differences in bound attractant around the cell periphery (c.f. Zigmond, 1977; Lauffenburger, 1982; Zigmond, Sullivan & Lauffenburger, 1983). Furthermore, it is known that the binding of attractant to leukocytes is followed by a net influx of Ca++, and that blocking transmembrane calcium transport blocks locomotion (Zigmond, 1978; Snyderman & Goetzl, 1981).

(2) **Contact inhibition.** When the leading cell membrane contacts another cell, motion in that direction frequently ceases. In terms of the SEGC mechanism, this implies that membrane contact seals up the ionic leak, the lamella regels in that region, and swelling — and thus motion — in that direction halts. This is a different phenomenon than the rapid recoil that is observed when an lamellipod is touched with, say, a glass rod. Such a recoil I would ascribe to a rapid and large influx of calcium which solates the region around the contact site. Since the lamella is under tension everywhere, this local weakening results in a passive elastic recoil, wherein the surrounding cytogel pulls the solated region away. (In this regard, it is more like the elastic wound response observed when the yolk cytoplasmic layer is punctured in the *Fundulus* egg.)

(3) **Galvanotaxis.** It is known that most cells move toward the cathode of an externally imposed electric field (Cooper & Keller, 1984). Their interpretation is that the field depolarizes the cathode-facing membrane and hyperpolarizes the anode side. Hyperpolarization decreases the electrochemical barrier to external Ca++, thus the retraction of the anode-facing edge they attribute to an enhanced Ca++ influx which solates the edge region and degrades its adhesion and contractile capacities. In terms of the model I hypothesize that the depolarization of the cathode-facing membrane ignites the SEGC mechanism by inducing release by internal Ca++. 

(4) **Motility currents.** Numerous experiments have documented the presence of ionic currents associated with various protrusive activities of cells (Nuccitelli, 1983). From the viewpoint of the SEGC model this is easy to understand: the ionic influx which triggers the solation–swelling process must eventually be countered by ionic efflux from the cell; otherwise, the ionic strength of the cytoplasm would rise indefinitely. Thus we expect that any protrusive activity caused by the
solation–swelling mechanism must be accompanied by external current fluxes. Just what stimulates the membrane pumps into activity is not clear, but the rising ion content of the local cytoplasm beneath the membrane is a likely candidate.

(5) Osmotic effects. Osmolarity has a profound effect on cell protuberances of all kinds; typically, a hyperosmotic condition inhibits protrusion, in accordance with the SEGC mechanism (Trinkaus, 1984). Moreover, the effect of osmolarity on motility of leukocytes is in the direction consistent with the model: hypo-osmotic conditions enhance and hyperosmotic conditions inhibit motility (Bryant, Sutcliffe & McGee, 1972; Rabinovitch, de Stefano & Dziezanowski, 1980). However, it cannot be discounted that osmotic shock disrupts many cell functions, especially by upsetting the cell’s internal ionic milieu.

(6) Stress effects. Motile cells frequently (but not always) exhibit aligned actin fibres, either along the direction of cell motion or perpendicular to it (arcs) (c.f. Trinkaus, 1984, p. 199). According to the SEGC model, the region of contractility just posterior to the solation zone can nucleate such structures by stress alignment of the gel fibres. Moreover, the traction generated by this region also accounts for the stress wrinkles that accompany cell motion on deformable substrates – perpendicular to the cell axis beneath the cell, and radial to the cell in front and in back of the cell. This phenomenon has interesting morphogenetic consequences (Oster, Murray & Harris, 1983). Moreover, time-lapse cinemicrography almost always reveals refractive waves that move from the leading edge centripetally toward the cell body, a phenomenon intrinsic to the SEGC model.Arcs appear to be stress-aligned actin bundles, but their description requires a two- or three-dimensional model for the lamellipod.

(7) Ruffles. In a two-dimensional treatment of the lamellipod equations, it is physically clear that the stress distribution across the thickness of the lamellipod generally will not be uniform. Therefore, the lamella will buckle downwards toward the substratum, or upwards away from it. In the latter case, one would expect a stiff, gelled ruffle to form. The frequency of such structures depends on the location of the leak on the tip membrane, the density and strength of adhesion sites, and how far the leading edge protrudes before attachment occurs. The one-dimensional model analysed here cannot address these issues.

(8) Contact guidance. Motile cells will follow alignment cues in the substratum, such as fibres or grooves. The model provides no clues as to the mechanism except to suggest that these cues somehow affect the pattern of membrane leaks. Perhaps some sequence of events associated with the binding itself initiates the localized ion leak (Jacobson, 1983). One suggestion is the following. The membrane polarization provides an electrostatic barrier to anion entry. Since small radii of curvature concentrate charges, a fibre or groove might provide a local charge concentration which could lower the electrostatic barrier to anion influx. Another factor which may be important has to do with the stress distribution in the cortex of a cell which attempts to conform to a cylindrical surface. It can be shown that the circumferential stress in any such cell is twice the longitudinal
stress (this is why a puncture in a cylinder under pressure will gape more in the circumferential direction than along its length). Thus a cell subject to such an internal stress distribution might tend to protrude more in the direction of minimum stress, i.e. axially.

(9) Retraction-induced spreading. When the tail of a moving fibroblast pulls free there ensues an increase in the spreading velocity at the leading lamella (Trinkaus, 1984, p. 213). Several effects may conspire to produce this phenomenon. When the tail of the cell pulls free there is an elastic recoil of the cell body followed by an active contraction of the stress fibres. This will produce a hydrostatic pressure gradient directed toward the leading edge. Moreover, as I mentioned above, the contracting cytogel drags gel fibres through the fluid phase which can create a sizeable pressure gradient, for the coupling between fluid cytoplasm and the network is exceedingly strong at low Reynolds numbers (Odell, 1977; Nothnagel & Webb, 1982). The hydrostatic pressure gradient will augment the gel swelling pressure and increase the rate at which the leading edge spreads forward. In addition, the availability of new surface area that accompanies retraction of the tail decreases the retarding effect of the membrane tension on anterior spreading. In the model I have included membrane tension as a constant retarding force for lamellar extension which appears simply as additional gel elasticity. However, pulling loose of the tail would transiently decrease this tension, which amounts to a decrease in apparent gel elasticity and enhanced spreading velocity.

(10) Tension inhibition of spreading. When the edge of a cell is under tension, such as in the lateral sides of a moving fibroblast and the trailing edge of an epithelial cell, it appears that spreading and protrusive activity is inhibited in this region. In terms of the SEGC model we see this as the consequence of the alignment of the gel fibres in the region of edge tension. When the fibres are so aligned, their capacity to spread outward under the impetus of the swelling pressure is inhibited: that is, there is a geometrical antagonism between the isotropic swelling pressure and the anisotropy of the aligned fibres. Furthermore, close apposition of fibres may allow crosslinks to form between the actin fibres, which further inhibit swelling.

The above phenomena do not constitute a demonstration that the model I have proposed is correct; I list them only to show that they are consistent with the model, and can be interpreted within its conceptual framework.

There is, however, one alternative to the membrane leak hypothesis for initiation and maintenance of the SEGC cycle. In Physarum, and a few other systems cytogel contraction can be initiated by ‘stretch activation’ (Kamiya, 1981; Oster & Odell, 1984a,b; Odell et al. 1981). That is, a local release of calcium can be produced by mechanically stretching the cytogel. In Physarum, a local contraction stretches nearby regions, thus stimulating them to contract. This mechanical coupling is necessary for the synchronization of the plasmodial oscillations which drive shuttle streaming (c.f. Kamiya, 1981). If lamellipodial cytogel has the same
property of stretch-induced calcium release, then this could perpetuate a periodic sequence of contraction waves since the contraction wave itself could trigger the release of internal calcium (c.f. Oster & Odell, 1984a, b). However, since expansion requires extensive solation at the very tip of the lamellipod, the calcium levels must rise higher at the tip than elsewhere. This could be accomplished if the contraction wave stretched the membrane-cortex at the cell-substratum interface sufficiently to cause additional ion leakage from the outside. Nevertheless, this is an attractive hypothesis, for it allows the SEGC cycle to perpetuate itself without additional mechanisms; moreover, an analysis of the model equations shows that it will work. However, there is at this time no evidence for this mechanism in lamellipodia – although it should be easy to test for it – and so we shall adhere to the simpler alternative of a membrane leak.

The model does not address the issue of where and how membrane material is inserted in the leading edge of the lamella. During initial spreading it appears that membrane is recruited from elsewhere on the cell surface where it is ‘stored’ as microvilli and other protrusions (Trinkaus, 1984, p. 203). During locomotion there is evidence that new membrane is inserted from within at the leading edge of the lamella. It is also possible that surface redistribution of membrane material by diffusion is important, since the diffusion constant for lipids in the membrane is quite large.

The one-dimensional model presented in the Appendix is not sufficient to address several other aspects of cell motility, such as the extension of filopodia or the formation of blebs. These are important phenomena in cell locomotion; indeed they may be the dominant locomotory appendages in most in vivo situations. However, in order to treat these phenomena we must employ at least a two-dimensional model. This presents formidable mathematical difficulties, and I shall present the results of such models elsewhere. However, without delving too deeply I can point out some preliminary results of that investigation.

While I have not dealt with the role of the membrane here, it is clearly necessary to do so in order to understand the formation of blebs and filopodia. For example, the spherical morphology of blebs suggests that they are generated by internal turgor pressure. However, they do not contain the actin mesh characteristic of lamellae, suggesting that the attachment of the gel to the membrane has been disrupted. Moreover, the following simple considerations demonstrate that a smooth lamellar front should be stable against the formation of filopodia. Recall that the pressure that can be supported across a thin membrane depends on the curvature of the surface:

\[ \text{Pressure difference} = \text{surface tension} \times \text{mean curvature}. \]

If a protuberance, or bulge, forms at a leading edge, the surface at the base of the bulge is ‘saddle shaped’, and so its maximum and minimum curvatures have opposite signs. So the mean curvature is minimum there, and the ability of the surface to support a pressure difference is also minimum. Thus the bulge should
Crawling of cells

smooth itself out by spreading laterally, as it does on a soap bubble. The cell, however, does not always pay attention to such a calculation, as, for example, when filopodia form. This points out the necessity of accounting for lateral associations between the cytogel and the membrane in discussing such protuberances. However, the above argument may explain why the ‘webs’ frequently seen at the base of filopodia sometimes grow outward and ‘fill in’ the gap between the filopodia: if the membrane is detached from the cortex there, then because it has the smallest mean curvature, that is the weakest spot in resisting internal turgor. Preliminary considerations of a two-dimensional model indicate that the cortex is unstable to protuberances beyond a threshold distance, a result which depends on lateral interactions with the membrane.

5. CONCLUSIONS

Cell locomotion is a complicated process, involving the coordination of a large number of mechanoochemical events, and it is probably impossible to construct a comprehensive model of the entire phenomenon. However, by focusing on the mechanical aspects of cell locomotion we can begin to see how all of the chemical events can tie together. From the viewpoint of physical chemistry, actomyosin gels can do but three things: osmotically swell, elastically or actively contract, and solate. Somehow, these properties must be sufficient to pull the cell forward.

I have proposed here a simple mechanism by which these processes can generate cell motion. This mechanism is built on the assumption that an ionic membrane leak at some point on the cell periphery initiates the processes of solation and swelling, followed by gelation and contraction. According to the relative rates of these processes we can account for a variety of motile activities. Further, the model suggests that a diverse array of motile phenomenon, including contact inhibition and various kinds of taxes, can be understood in terms of how the membrane leak is initiated and/or sustained.

It is a common conviction amongst workers in cell motility that solation, gelation, actomyosin contraction and calcium regulation are essential features in cell locomotion. Aside from the inclusion of gel swelling pressure as a force for extension, most of the components of the model have been proposed before by others. I have simply integrated these processes into a unified scheme which shows how these various effects can be coordinated to create a motile organ.

Finally, it would, of course, be desirable to be able to estimate the numerical magnitude of the various effects we have discussed – especially the swelling pressure curve. Unfortunately, I am unaware of any experiments which address these issues for actomyosin gels, and so I have been forced employ qualitative features of the physical chemistry of cytogel. Therefore, the model equations can only demonstrate that the SEGC mechanism can work (on the cell type ‘fibroblastia mathematica’) – and I cannot assert that it does work in real cells.
Nevertheless, I hope that putting the various observations on cell movement into a unified theoretical framework will sharpen discussions on the subject and focus attention on the underlying physical chemistry. Perhaps this will stimulate cell biologists to propose alternative theories, or to attempt observations and experiments that might not otherwise have occurred to them.

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Crawling of cells


APPENDIX

In this Appendix I describe the mathematical model for how the lamellipod acts as the motile organ for a moving cell (Oster & Perelson, 1984). A model for protopod formation in leucocytes which is based on different physical assumptions has been presented by Schmid-Schoenbein & Skalak (1984).

We begin by considering the lamella in cross-section, as shown in Fig. 8A. I shall treat only variations in the x-direction, so that the model equations will be scalar. The generalization to higher dimensions will be described elsewhere. The model lamella consists of a gelled region extending from $x = 1$ to $x = L$, where $L = L(t)$ is the moving leading edge of the cell.

The model is described in terms of the following field variables:

- $u(x,t) = $ the displacement of a material point in the gel from its initial position.
- $G(x,t) = $ the concentration of gel at position $x$ and time $t$; i.e. the concentration of network chains.
- $S(x,t) = $ the concentration of the sol at position $x$ and time $t$.
- $c(x,t) = $ the concentration profile of the permeant ions.

The mechanical equation of motion

A gel is fundamentally a 2-phase system consisting of the fluid solvent (the aqueous component of the cytosol) and the solid gel network. However, to avoid
Crawling of cells

dealing with the motion of the solvent directly, I shall assume that the motion of
the lamellipod is sufficiently slow that the fluid component of the cytoplasm can
be considered as essentially stationary (De Gennes, 1976; Johnson, 1982). The
equation for the mechanical balance of forces on the gel is
\[ \frac{\partial \sigma}{\partial x} + \sigma_v + F_s = 0 \]  
where
\( \sigma = \) the stress tensor for the gel,
\( \sigma_v = \) the viscous drag forces on the gel network arising from the relative motion
of the gel fibres and the solvent.
\( F_s = \) the forces exerted on the gel from the substratum.

The stress tensor for the gel, \( \sigma \), is composed of several parts:
(i) \( \sigma_A \), the active stresses generated by actomyosin fibres.
(ii) \( \sigma_{\text{ELAS}} \), the elastic stress due to bond deformation of the gel fibres and the
entropic contractile forces of the fibre network. The latter derives from the
random thermal motion of the gel fibres, and is usually referred to as ‘rubber
elasticity’ (Hill, 1960; Acklonis & MacKnight, 1983; Flory, 1953).
(iii) \( \sigma_{\text{OSM}} \), the osmotic pressure of the gel, \( \sigma_{\text{OSM}} \), which has two components,
   (a) The free energy of mixing of the solvent molecules (fluid cytoplasm and
   water) and the solute (gel fibres) creates an chemical potential gradient
   between the gel and the surrounding fluid. This arises from the polymer
   molecules excluding the solvent from certain regions of the solution, and
   from intermolecular interactions between the polymer molecules.
   (b) Fixed charges on the polymer will repel each other, and attract shielding
counterions.

The swelling pressure, \( P_s \), is the sum of the elastic and osmotic pressures
\[ P_s = P_{\text{ELAS}} + P_{\text{OSM}} \]

If a volume element of cytogel is to be in equilibrium with its surroundings, all
of these forces must balance. In particular, the osmotic pressure tending to
expand the gel must be counteracted by the contractile forces arising from elastic
and active stresses, along with the substrate forces. In order to implement the
force balance in equation [1], we must write expressions for each component of
the stress tensor.

Thermodynamics treats the gel–solvent system as a 2-component mixture
whose constitutive relations are derived from the composite Helmholtz free
energy difference, \( \Delta A \), between the mixture and the separate components. An
expression for \( \Delta A \) can be derived from statistical mechanics (Hill, 1960, p. 412),
from which the chemical potential difference between the solvent and the gel can
be obtained:
\[ \mu_0(\phi_2) - \mu_0(0) = P_S\dot{V}_s = (P_{OSM} + P_{ELAS})\dot{V}_s \]
\[ = kT[\ln(1 - \phi) + \phi + \chi\phi^2] + [(1/\lambda - \phi/2)/M] \]

where the subscript \( s \) refers to the solvent, \( \dot{V} \) is the partial molar volume of the solvent, \( \phi \) is the volume fraction of the gel, \( \lambda = L/L_0 \) is the stretch ratio, \( M \) is the number of subunits that comprise a chain, and \( \chi \) is the Flory parameter which measures the strength of interaction between the polymer and the solvent.

The first term in brackets derives from the free energy of mixing of the polymer and solvent, and the second term is the elastic free energy due to dilatation of the network by the solvent.

From [2] one can derive the uniaxial force–length relationship, \( F(\lambda) = (\partial\Delta A/\partial\lambda)_T \):
\[ F = E(\lambda - V/V_0) \]

where \( V/V_0 \) is the swelling ratio, and the elastic modulus, \( E \), is (Aklonis & MacKnight, 1983, Chap. 6):
\[ E = (N_cRT + a)\frac{T_0^2}{r_f^2}(1 - \frac{2M_c}{M}) \]

Here \( N_c \) is the chain concentration, \( a \) is a measure of the chain entanglements, \( M_c \) is the average relative molecular mass of a chain, \( M \) is the relative molecular mass of an entire polymer fibre, and the middle factor is the ratio of the mean-square chain length before and after stretching.

Various modifications of [2] have been proposed (e.g. Tanaka, 1978). However, since most of the quantities have yet to be measured for cytogel, we will make certain simplifying assumptions to yield a tractable mathematical problem. In particular, we shall view the gel+solvent mixture as a composite substance whose stress tensor can be modelled as the sum of contributions from the active traction, the network elasticity, and the osmotic pressure. Thus we write the stress tensor per chain, \( \sigma_c \), as
\[ \sigma_c = \sigma_A - P_S = \sigma_A + P_{ELAS} - P_{OSM} \]

We model the active traction forces, \( \sigma_A \), generated by the actomyosin as
\[ \sigma_A = \tau(\varepsilon, c) \]

where \( \tau \), the traction stress per chain (dyne/cm²) is a decreasing function of strain, \( \varepsilon, (\partial\tau/\partial\varepsilon < 0) \) and, as we shall see below, an increasing function of calcium concentration, \( c, (\partial\tau/\partial c > 0; Oster & Odell, 1984a, b) \). A typical functional form for \( \tau \) is
\[ \tau(\varepsilon, c) = \tau_1(c)/(1 + \tau_2\varepsilon) \]

The passive elastic stress is modelled by the relation:
Crawling of cells

\[ P_{\text{ELAS}} = E \varepsilon \]  \[7b\]

where \( E \) is the elastic modulus per chain, and \( \varepsilon = \partial u / \partial x \) is the strain. For rubber-like materials \( E \) is given by equation [4]; in general, \( E \) is a function of strain. However, I shall treat the gel as if it is a linear, homogeneous, elastic substance; the generalization to nonlinear elasticity is straightforward.

The osmotic pressure, \( P_{\text{OSM}} \), is also an increasing function of the polymer volume fraction, and the chain concentration, and a decreasing function of strain (see Fig. 3B). An expression for this can also be derived from statistical considerations (c.f. Tanaka, 1978); however, the parameters characterizing cytogels are not available. Therefore, we shall write \( P_{\text{OSM}} \) as

\[ P_{\text{OSM}} = P(\varepsilon, G, \phi) \]  \[8\]

where \( P(\cdot) \), is an increasing function of gel concentration \( (\partial P_{\text{OSM}} / \partial G > 0) \) and a decreasing function of strain \( (\partial P_{\text{OSM}} / \partial \varepsilon < 0) \). For example,

\[ P_{\text{OSM}} = P_0 G / (1 + P_1 \varepsilon) \]  \[9\]

The viscous forces acting on the network can be modelled as

\[ \sigma_v = -f \frac{\partial u}{\partial t} \]  \[10\]

where \( f \) is the frictional drag coefficient per unit volume of the network. With solation, the gel viscosity drops precipitously, as shown in Fig. 5B; however, for simplicity, I shall assume that \( f \) is a constant.

The body forces, \( F_s \), model the attachment of the gel to the substrate. I shall model these as simple elastic restoring forces

\[ F_s = G \kappa u \]  \[10\]

where \( \kappa \) is the elasticity of the attachment site, and the gel concentration enters since we assume that the attachment is between the substrate and the gel. \( \kappa \) should decrease with distance from the leading tip, since the attachments appear to fade away toward the cell body. In the simplified model, however, I shall assume \( \kappa \) is a constant.

With the above expressions, we can write the force balance equation for the gel [1] as:

\[ f \frac{\partial u}{\partial t} = \frac{\partial}{\partial x} \left\{ GE \frac{\partial u}{\partial x} + G \tau(\varepsilon, c) - P_{\text{OSM}}(G, \varepsilon, c) \right\} + G \kappa u \]  \[11\]

Typical boundary conditions for equation [11] are:

\[ \sigma = 0 \text{ at } x = L(t), \text{ i.e. the lamella is stress free at the moving boundary } [12a] \]

\[ u = 0 \text{ at } x = 0, \text{ i.e. the lamella is fixed at the cell body, } [12b] \]

Condition [12b] assumes that the cell body is stationary, and so the model would apply only to the initial extension of the lamellipod. A boundary condition
appropriate for continuous cell motion plastic yield stress, $\sigma_Y$, allowing them to be pulled free at the left end, providing the traction generated by the gel is sufficient:

$$GEu_x(x = l,t) = \sigma_Y \text{ if } GEu_x > \sigma_Y, \text{ a yield stress}$$

$$= xGu(x = l,t) \text{ if } GEu_x < \sigma_Y$$

[13a]

The initial condition is

$$u(x,t = 0) = 0$$

[13b]

Equation [11] describes how a material point on the gel is displaced under the action of the various forces it experiences. If the gel concentration, $G$, remained constant, then the equation predicts the ultimate equilibrium dilation of the gel under the influence of osmotic swelling. However, the gel is not a static material, as I discuss next.

**The material composition of the cytogel**

The cytogel is a dynamic material, being constantly polymerized and depolymerized according to the local conditions. The characteristics of the gel–sol transition of cytogel have not been completely determined yet; however, it is known that ambient levels of calcium ions regulate the cross-linking state of actin gels. This is accomplished via a number of solation and gelation proteins whose activity is controlled by calcium. For the purposes of understanding the physics of lamellae we need not deal with the complexity of actin gel regulation; rather we employ a simplified model for the interconversion of gel and sol under the control of local ionic conditions.

In the steady state there is an equilibrium between the sol (S) and gel (G) components of the cytoplasm: $S \rightleftharpoons G$. We write this reaction as

$$\frac{dS}{dt} = -\frac{dG}{dt} = -k_G(c)S + k_S(c)G$$

[14], [15]

where the solation rate, $k_S$, and gelation rate, $k_G$, depend on the local ionic concentration, $c(x,t)$. This dependence has the sigmoidal shape typical of phase transitions, as shown in Fig. 10. Treating the gel→sol transition kinetically is a considerable simplification over the usual treatments (e.g. Flory, 1953); however, it captures the essential features of the process for the purposes of the model.

In order for the gel–sol front to move we must add transport terms to the above equations. I assume that the sol (i.e. actomyosin ‘monomers’ and network fragments) can diffuse, but the gel can only move by deformation:

$$\frac{\partial S}{\partial t} = D \frac{\partial^2 S}{\partial x^2} - k_G(c)S + k_S(c)G$$

[16a]

$$\frac{\partial G}{\partial t} = -\frac{\partial}{\partial x}(G\frac{\partial u}{\partial t}) + k_G(c)S - k_S(c)G$$

[17a]

The boundary conditions for the gel–sol material balances are:
Crawling of cells

\[ S(x = 0, t) = S_0; \] a constant reservoir of sol components in the cell body [16b]

\[ \frac{\partial S}{\partial x} \bigg|_{x = L(t)} = 0; \] the lamella tip is impermeable to sol [16c]

\[ S(x, t = 0) = S_0; \] initially, the lamella has the same sol composition as the cell body [16d]

\[ G(x = 0, t) = G(x, t = 0) = G_0; \] the gel state of the unperturbed cytoplasm [17]

Finally, since the gel–sol state of the cytoplasm is regulated by the ionic concentration we must write a balance law for \( c(x, t) \) as well:

\[ \frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + R \] [18]

where \( R \) is the rate calcium is released and resequestered within the cytoplasm, and \( D_c \) is the diffusion constant for the ion.

The model operates somewhat differently depending on the form of the calcium kinetics, \( R \), we assume. For example, it is known in some systems, such as Physarum, that the cytogel contains calcium-sequestering membranous vesicles. These vesicles are capable of releasing their contents in response to small calcium perturbation (‘calcium-stimulated calcium release’) (c.f. Oster & Odell, 1984a, b). I shall model the release kinetics by a calcium-dependent permeability, \( P(c) \) and first order resequestration kinetics as in Oster & Odell (1984a, b):

\[ R(c) = P(c)(C - c) - rc \]

where \( P(c) \) is the sigmoidal autocatalytic curve shown in Fig. 11, which we model as

\[ P(c) = \frac{ac^2}{(1 + \beta c^2)} \]

\( C \) is the concentration inside the sequestering vesicles, and \( r \) is the resequestration rate.
In order to model the calcium-stimulated-calcium-release mechanism, we require the calcium kinetics to be 'triggerable', (in the same sense as the FitzHugh-Nagumo equations for the nerve impulse). Therefore, we must introduce a second kinetic equation. It turns out that the resequestration of calcium requires the presence of cAMP. Moreover, the production of cAMP from ATP is accomplished via the enzyme adenylate cyclase, which itself requires calmodulin – and thereby calcium. Thus the calcium kinetics can be modelled by the pair of equations:

\[
\frac{dc}{dt} = D \frac{d^2c}{dx^2} + \frac{a c^2}{(1 + \beta c^2)} - rac \\
\frac{da}{dt} = k_1 c - k_2 a
\]

The boundary condition for [19] is the crux of the model. We assume that at \( t = 0 \) the membrane at the tip of the leading lamella becomes permeable to ions:

\[
\left. \frac{\partial c}{\partial x} \right|_{x = L(t)} = P_m [C_0 - c(L, t)]
\]

or

\[ P_m c(L, t) + \frac{\partial c}{\partial c} (L, t) = P C_0 \]

where \( P_m \) is the permeability of the membrane to c, and \( c_0 \) is the external concentration of the ion.

The other boundary conditions for [18] are:

\[ c(x = 0, t) = c(x, t = 0) = c_i \]
Crawling of cells

where $c_t$ is the (buffered) cytoplasm ionic concentration.

Finally, the velocity of lamellipod extension is

$$\frac{dL}{dt} = \frac{\partial u}{\partial t} \bigg|_{x=L} = \left(1/fL\right)[\sigma]_{x=L}$$

where $[\sigma]$ indicates the jump in stress across the moving boundary (Tayler, 1974). Thus the moving boundary is given by

$$L(t) = L_0 + \int_0^t \frac{\partial u}{\partial t}(L(t),t)/\partial t$$

where $L_0$ is the initial position of the leading edge.

In addition to controlling the gel ⇔ sol equilibrium of the cytoplasm, calcium ions also regulate the active contraction of the actomyosin fibres (Oster & Odell, 1983). Thus the active stress, $\sigma_A = G(t,c)$, as a function of $c$ will have the general shape shown in Fig. 4; hence $\sigma_A$ will have a maximum at a calcium level somewhat lower than the solation point.

The role of intermediate kinetics

The kinetic network that controls solation and contraction is much more complicated than we have so far assumed, and there is one aspect that appears to be important in the mechanics of cytogels.

The solation factors are directly calcium activated, and so solation commences very soon after calcium levels rise to the micromolar level. The contraction cycle, however, appears to commence much more slowly, reflecting the longer kinetic pathway required to activate the contraction machinery. This pathway proceeds from calcium to calmodulin which binds to the myosin light chain kinase, which then phosphorylates the myosin. A phosphatase is present in many systems, which reverses the phosphorylation, thus effectively slowing down the phosphorylation step. Moreover, myosin assembly and binding to actin is also calcium dependent. Finally, the actual contraction is retarded by internal and fluid friction. Thus there is considerable delay between the release of calcium and the actual mechanical contraction of the network. This delay is evident, for example, in the plasmodial oscillations of Physarum, where the tension oscillations lag the calcium oscillations by nearly a minute (Oster & Odell, 1984a,b).

One way of including this delay into the model is to simply redefine $\tau$ and the kinetic coefficients $k_G$ and $d_s$ to be delayed functions of calcium:

$$\tau = \tau[c(t - T_c)], \quad k_G = k_G[c(t - T_s)], \quad k_s = k_s[c(t - T_s)]$$

where $T_c$ and $T_s$ are the contraction and solation delays, respectively, and $T_c > T_s$. Simulating delays on a computer, unfortunately, is rather expensive, and so we adopt a simpler approximation.
The best method, of course, would be to include all of the kinetic steps known to be involved in solation and contraction. Even if all of these steps were known and their rate constants measured, such a model would be too unwieldy. Therefore, I shall proceed by defining two composite intermediate quantities as follows:

\[
\frac{dp}{dt} = k_1c - k_2p \tag{25}
\]

\[
\frac{dq}{dt} = k_3c - k_4q \tag{26}
\]

where \( p \) represents all of the phosphorylation steps leading to contraction, and \( q \) represents the solation kinetics. By adjusting the kinetic constants in these equations we can approximate the relative delays involved in the solation and contraction reaction sequences.

If solation and gelation proceeded at the same rate, then the maximum traction generated by the actomyosin fibres could not be transmitted through the gel because the network would be so disrupted. However, because of the delays approximated by equations \(25\) and \(26\) the contraction does not reach its maximum strength until the network is regelling.

Thus the model equations consist of \(11\) governing the mechanical balance of forces, \(16\) and \(17\) which describe the solation–gelation process, \(19\) and \(20\) which describe the calcium kinetics, and \(26\) and \(27\) which approximate the relative delays involved in the solation and contraction reaction sequences.

By carrying out the differentiations in \(11\), we see that it has the formal appearance of a diffusion equation for the displacement, \(u\). Therefore, the model consists of five parabolic conservation laws and two linear kinetic equations. The principle difficulty in simulating them numerically is the presence of the moving boundaries; however, this can be handled by standard techniques. A complete numerical study of these equations will be presented elsewhere.