ARTICLE

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A mathematical approach to cytoskeletal assembly

Abstract The cytoskeleton is a fundamental and important part of cell's structure, and is known to play a large role in controlling the shape, function, division, and motility of the cell. In recent years, the traditional biological and biophysical experimental work on the cytoskeleton has been enhanced by a variety of theoretical, physical and mathematical approaches. Many of these approaches have been developed in the traditional frameworks of physicochemical and statistical mechanics or equilibrium thermodynamic principles. An alternative is to use kinetic modelling and couch the analysis in terms of differential equations which describe mean field properties of cytoskeletal networks or assemblies. This paper describes two such recent efforts. In the first part of the paper, a summary of work on the kinetics of polymerization, fragmentation, and dynamics of actin and polymers in the presence of gelsolin (which nulceates, fragments, and caps the filaments) is given. In the second part, some of the kinetic models aimed at elucidating the spatio-angular density distribution of actin filaments interacting via crosslinks is described. This model given insight into effects that govern the formation of clusters and bundles of actin filaments, and their spatial distribution.

Key words Cytoskeleton · Actin filaments · Actin bundles and networks · Filament length distribution · Mathematical modelling

1 Introduction

Actin is a biological polymer, and an important structural and functional component of the cytoskeleton. It is implicated in (animal) cell motility, chemotaxis, cell division, and other vital functions. Actin polymerizes into short, stiff, rod-like filaments which can elongate (via polymerization), shorten (by fragmentation) and/or form networks, bundles, gels, and other structures mediated by binding proteins. Polymer chemistry is itself a challenging and rich field of study. When compounded by the effects of a rich mixture of binding proteins, cross-linkers, enzymes, and intermediates that interact with both the monomers and the polymers, the result is a field of stunning diversity and complexity. It is little wonder that gaining an understanding by experimental techniques alone is difficult, whereas some help from theoretical directions can be beneficial.

The traditional theories for the behaviour of polymers (P.-G. deGennes 1979; Flory 1953; Doi and Edwards 1986) are formulated in the discipline of equilibrium thermodynamics. Some connections have been made to percolation theory (the formation of spanning clusters), and to the theory of visco-elastic networks, see, for example, Nossal (1988). However, many of these frameworks are not suitable for answering questions about time evolution of a process of polymerization, fragmentation, and interactions of the filaments. Similarly, many of these theories are not suited for investigating spatial distributions of polymer networks. Though there is very accurate predictability of when clusters, homogeneous gels, or bundles of filaments are expected, it is not possible to predict the spatial distribution of these structures. This suggests that alternative approaches can make a contribution to the overall goal of studying the complex process of cytoskeletal dynamics.

A fundamental problem in biophysics is how chemical signals received by the cell are translated into cell response, changes in cell function, and (in animal cells) purposeful motion of the cell such as *chemotaxis*. The **cytoskeleton**, consisting of microtubules, intermediate filaments, actin, and a host of smaller components is known to be a key mediator in the chain of events that take place (Lauffenburger and Horowitz 1996; Mitchison and Cramer 1996). The fundamental problem (above) is extremely complex. However, there are many sub-problems, of interest in their own right, that, if solved, may eventually aid our understanding of the big picture.

The cytoskeleton subproblems concerning actin in particular include: (1) Control and effect of polymerization,

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capping, and cutting of actin polymers, (2) association of filaments into structures such as bundles and gels (3) spatial distribution of actin, and the role that this plays in cell motility, cytokinesis, and other functions of the cell. Each of these problems has been studied extensively in an experimental setting, but it is only recently that theoretical, mathematical, and physical methods have been brought to bear on the problem. Some of these ideas and their outcomes are highlighted in this paper.

The results of the mathematical contributions can have several quite distinct goals. On one hand, by analyzing kinetics that are relatively well-established and understood by biologists, modelers can make specific quantitative predictions which are otherwise difficult to discover. This type of work simply uses mathematical techniques as tools to compute with precision the outcome of well-known events. A case in point is the prediction of **Actin filament length distributions** (Edelstein-Keshet and Ermentrout 1998; Ermentrout and Edelstein-Keshet 1998) discussed below.

A second approach is to treat qualitative phenomena which are not yet well understood, and to use mathematical concepts and models to shed light on possible causative mechanics. In this type of work, exemplified by the discussion of **actin filament associations**, qualitative ideas such as stability analysis, pattern formation, as well as numerical simulations can be used to test a variety of hypotheses (Spiros and Edelstein-Keshet 1998). This generally leads to kind of caricature of the real situation which not all biologists find relevant. However, though it may be only part of the picture, this approach can suggest new experiments or ideas to test.

A final approach is to use mathematical and/or physical methods to carefully dissect competing hypotheses for the mechanisms underlying complex phenomena such as cell motility. Examples of this type include recent work on the **polymerization ratchet** (Mogilner and Oster 1996).

2 Models for actin filament length distributions

Recent twin papers (Edelstein-Keshet and Ermentrout 1998; Ermentrout and Edelstein-Keshet 1998) were devoted to understanding the following problems: What can be said about the distribution of filament lengths for actin filaments given that they can polymerize and depolymerize at each end, and that a filament cutting agent (such as **gelsolin**) is added to interact with the polymers. Inputs into the model include assumed interactions of the participating intermediates and known values of biological parameters. Analysis using mathematical models (which are essentially classical reaction rate models) and computer simulations lead to the outputs, which include detailed filament size distribution, and average length.

The importance of this problem stems from several aspects: (a) First, the length of the filaments influences the overall polymerization rate since ends act as nuclei. A single, long filament would thus contribute much less to actin polymerization than many short filaments (Theriot 1994; Zigmond 1993). (b) Second, the rheology of solutions containing filaments is sensitive to the lengths of the filaments. The longer the filaments, the likelier it is that entanglements occur, and the more viscous the solutions (Oster 1994; Janmey et al. 1986; Zaner 1995; Piekenbrock and Sackmann 1992; Tempel et al. 1996). (c) The mobility of the polymers, and their interactions with one another are highly sensitive to lengths. For example, filament rates of diffusion (and most particularly rotational diffusion) fall off rapidly with filament length (Doi and Edwards 1986). This then influences (d) the associations of filaments with one another into structures such as bundles or networks (Spiros and Edelstein-Keshet 1997; Civelekoglu and Edelstein-Keshet 1994), the formation of crystalline associations in vitro (Furukawa et al. 1993; Suzuki et al. 1991; Käs et al. 1996), and the interactions of the filaments with actin binding proteins (Wachsstock et al. 1993; Wachsstock et al. 1994; Coppin and Leavis 1992; Tempel et al. 1996). Actin filament length has also been identified as an important factor in other situations. Examples include the viscosity of secretions in the lungs of cystic fibrosis patients, and the propulsion speed of the intra-cellular parasitic bacterium, *Listeria* (Sechi et al. 1996; Marchand et al. 1995), etc.

2.1 Models for simple polymerization

In the actin filament, the **barbed end** grows much more rapidly than the **pointed end**. Kinetics and models of the process of polymerization have been given elsewhere (Korn et al. 1987; Frieden 1983; Tobacman and Korn 1983; Houmeida et al. 1995; Fesce et al. 1992), but it is not usually the case that the whole length distribution is considered. (Rather, the total amount in polymerized versus monomeric form, is followed; this makes for a much simpler treatment, but is then not always helpful in addressing the concerns discussed above).

The problem was approached in several stages in (Edelstein-Keshet and Ermentrout 1998; Ermentrout and Edelstein-Keshet 1998). First, the simple growth of actin polymers was modelled as follows. Aggregate rate constants, representing the combined polymerization and depolymerization at both ends were defined, $k_+ = k_+^{barbed} + k_+^{pointed}$, $k_- = k_-^{barbed} + k_-^{pointed}$. The polymerization reaction shown in Fig. 1, with these forward and reverse reaction kinetics was followed. Written in standard chemical reaction form, the reaction is

$$A_j + a \underset{k_-}{\overset{k_+}{\rightleftharpoons}} A_{j+1}$$
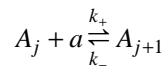
where a stands for a free monomer and A_j is a j-mer. The concentration of the various polymer length is then modelled by the set of **differential equations**

$$\frac{d[A_j]}{dt} = k_-[A_{k+1}] + k_+ a[A_{j-1}] - (k_- + ak_+)[A_j]. \qquad (1)$$

where $[A_j]$ represents the concentration of j-mers and a is now the concentration of monomers. Each of these differential equations keeps track of the disappearance of j-mers
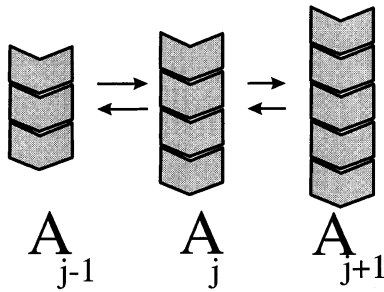
Fig. 1 An actin filament containing j monomers A_j can either grow by polymerization or lose a monomer and shrink. The free monomers are not explicitly shown here
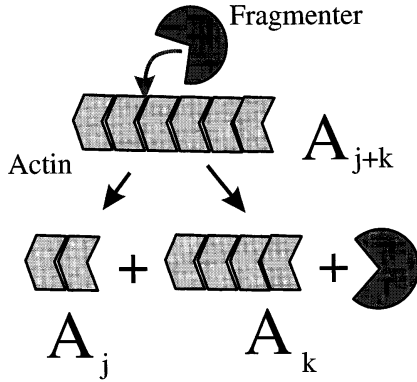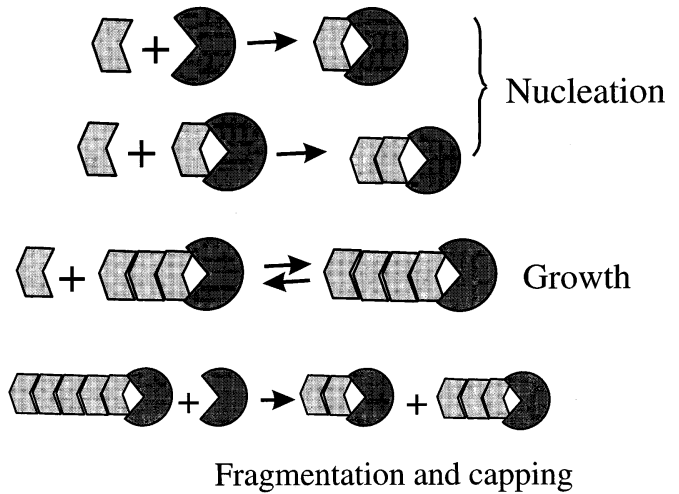

Fig. 2 A filament containing j + k monomers is cut by a fragmenter such as cofilin, producing a j-mer and a k-mer. This can happen in two possible ways, since the bond broken can be at either of two places on the original filament

Fragmentation and capping

Fig. 3 Gelsolin has multiple effects, including nucleation, capping, and fragmentation

2.2 Models for simple fragmentation

A more novel situation occurs when the filaments are broken or fragmented at their bonds – whether by mechanical action or by enzymatic activity. For example the actin binding protein **cofilin** results in the fragmentation reaction

$$A_{j+k} \xrightarrow{k_b} A_j + A_k,$$
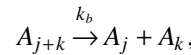
If the filaments are not polymerizing, the distribution of j-mers would follow a kinetic equation of the form

$$\frac{d[A_j]}{dt} = \begin{bmatrix} \text{Rate of formation} \\ \text{from larger pieces} \end{bmatrix} - \begin{bmatrix} \text{Rate of breakage} \\ \text{into smaller pieces} \end{bmatrix} \quad (3)$$

Since a filament can break up at any of its $j - 1$ bonds, and since the number of larger pieces is a sum of sizes bigger than j, the differential equation that describes this process is:

$$\frac{d[A_j]}{dt} = k_b \left(2\sum_{k=1}[A_{j+k}] - (j-1)[A_j] \right). \quad (4)$$

The factor of 2 in this equation results from the fact that each larger filament can fragment into a j-mer in two possible ways. Together with a suitable initial condition and description of what happens at the large and smallest sizes ("boundary conditions"), this equation can be simulated to predict the precise time behaviour of a length distribution of filaments. It can also be used to elucidate the equilibrium distribution under a given set of conditions.

A particularly simple case occurs if filaments of size J (some arbitrarily large size) are constantly supplied to a fragmenter. It can be shown (Edelstein-Keshet and Ermentrout 1998) that in that case, the equilibrium distribution of lengths takes the form

$$[A_j] = C \frac{J(J+1)(J-1)}{(j^3 - j)}. \quad (5)$$

(by forming (j+1)-mers, by breaking down into (j–1)-mers), and the appearance of j-mers from the polymerization of the other sizes.

The equations shown above are relatively easy to handle mathematically as long as the level of monomer a is fixed. It was shown in Edelstein-Keshet and Ermentrout (1998) that the distribution of j-mers would settle into an equilibrium length distribution that was a simple exponential. This is not a surprising result, in view of similar findings (Oosawa and Kasai 1962; Kawamura and Maruyama 1970). When the nucleation reaction is taken into account (Fesce et al. 1992; Tobacman and Korn 1983; Korn et al. 1987; Frieden 1983; Lumsden and Dufort 1993), the result is

$$[A_j] = Cr^j = \frac{k_{init}}{k_-}(a_{crit})^n \left(\frac{a}{a_{crit}}\right)^j. \quad (2)$$

where $a_{crit} = k_-/k_+$ is the critical concentration of monomer at which polymerization just balances depolymerization, where k_{init} is the initiation rate constant, and where n the number of monomers (3 or 4 in various literature sources) required to initiate a filament. Since the equations describing the kinetics are linear differential equations, the mathematical treatment of this problem is quite simple, and it is rather easy to understand the results. As expected, the results are in complete agreement with those of the classic equilibrium thermodynamics treatments (Flory 1953).

Small pieces build up to an unlimited level unless they are also removed in this case. The number average length of the distribution can be calculated from the above and is found to be

$$L_n = (3J + 2)/(J + 2).$$ (6)

For large values of J, this ratio is roughly 3, which means that even if arbitrarily long filaments are supplied to a continual fragmenter, the number of tiny pieces will swamp out the distribution so that, on average, there will be 3 monomers per filament (regardless of the detailed kinetics). (In practice this limit may be uninteresting, since the supply of long filaments may end, the fragmenter may be incapable of breaking small fragments further, or other processes may interfere.)

2.3 The effect of gelsolin on the length distribution

A protein such as gelsolin interacts with actin in a number of ways. Although it is primarily a fragmentation protein, it is also caps the barbed end of a filament and acts as a nucleator for initiating new filaments. Thus, when the effect of gelsolin is inlcuded, the models become significantly more complicated. This is particularly true if the transient behaviour (i.e. behaviour leading up to the equilibrium situation) is of interest. If only the final outcome and the average filament length is of interest in a given situation, this modelling approach is superfluous: the number of actin filaments at steady state is known to be equal to the number of added gelsolin molecules (Janmey et al. 1986). We can also circumvent the detailed model of transient dynamics by appealing to the equilibrium thermodynamics Flory Model which would predict that the length distribution is exponential if we assume that each gelsolin-actin nucleus has the same probability of growth. However, it is not necessarily clear that this equilibrium would be established also in the case that gelsolin plays the role of a fragmenter (as well as a nucleator and a capper) so that investigating a fully dynamic model can confirm or reject this prediction.
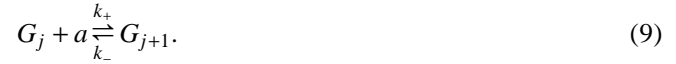
Detailed information about gelsolin is available (Ditsch and Wegner 1994; Schoepper and Wegner 1992; Ditsch and Wegner 1995) and this can be used in constructing detailed simulations about its effects. An interesting application of these ideas rests on the new treatment of cystic fibrosis using gelsolin. By fragmenting the long actin filaments in the airway mucal secretions of CF patients, gelsolin can help to reduce viscosity and alleviate symptoms of the disease (Vasconcellos et al. 1994). Gelsolin is being produced and tested by BIOGEN for this medical application.

The actions of gelsolin can be summarized as follows:

Gelsolin-mediated nucleation

$$G + a \xrightarrow{k_{init}} G_1.$$ (7)

$$G_1 + a \xrightarrow{k_{fast}} G_2.$$ (8)
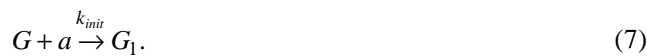
Growth at pointed end

$$G_j + a \underset{k_-}{\overset{k_+}{\rightleftharpoons}} G_{j+1}.$$ (9)

Gelsolin-caused fragmentation

$$G_{j+k} + g \xrightarrow{k_g} G_j + G_k.$$ (10)

In the first two nucleation reactions, gelsolin forms a complex with an actin monomer (rate k_{init}). This complex then reacts quickly with a second actin monomer to form a gelsolin:actin 1:2 complex (rate k_{fast}) (Schoepper and Wegner 1991). The third reaction treats polymerization at one end of the actin filament (the pointed end, since the barbed end is capped), and the fourth reaction is the fragmentation step. An interesting aspect of these kinetics is the considerably faster nucleation step which relies on a catalytic effect and no longer requires that 3 or 4 monomers participate. It was shown (Ermentrout and Edelstein-Keshet 1998) that this fact makes the system linear in the case that free gelsolin and actin monomer concentrations are artificially held constant. Although this is not a realistic situation, it does allow some great simplification to be made in the treatment of the problem.

Analysis reveals the following results, whose details are given in the cited references:

– If actin monomers and gelsolin monomers are kept at some constant levels, the total mass of polymerized actin will grow exponentially, but the average length of the filaments will be bounded due to fragmentation. The relative proportions of the various size-classes settles into a **stable size distribution**.
– If buffering is absent, and if the ratio of total actin to gelsolin is much larger than 1 (as is usually the case), then free gelsolin will be completely eliminated due to its irreversible capping of the filaments. When this happens, the process reverts to a simple polymerization process, but with only the pointed end of the actin filament nucleating growth (and thus a slower rate constant for polymerization). The length distribution will then eventually tend to an exponential, as predicted by the Flory Model (Flory 1953).
– The greater the initial concentration of gelsolin, the smaller the mean filament length, as expected. (The mean length of the filaments in monomer units is just the ratio of the total amount of actin to the total gelsolin.)
– If we consider the transient behaviour when a small amount of gelsolin is added to the mixture, the number of filaments will grow exponentially. This happens both due to nucleation of new filaments, and a result of breakage of large filaments forming pairs of smaller ones.

Although these general results give some new insight, it is also possible to use models such as the above to explore detailed questions concerning the action of gelsolin in the presence of ions or other factors that influence the dynamics, and thus also the length distribution of the actin filaments.

2.4 Comparison with experimental results

A variety of direct and indirect methods have been used to observe actin filament length distributions in experimental settings. Size distributions were measured from electron micrographs of actin solutions prepared in the presence of various molar ratios of gelsolin (Janmey et al. 1986). By comparing the ratio of weight average to number average length over various samples (and getting similar ratios over many samples), the authors concluded that the length distribution was an exponential one. They further suggested that this size distribution is as expected if gelsolin acts as a site of filament growth and monomers exchange at random from the uncapped end of each filament.

Indirect information about the size distribution of a solution of actin filaments can also be obtained by quasielastic light scattering, as done by Piekenbrock and Sackmann (1992). The authors studied actin networks polymerized with gelsolin and found several results whose relevance to this paper is direct. First, they noted that scattering intensity grows linearly with time in the case of gelsolin nucleation, whereas the growth is sigmoidal when actin is polymerized on its own. (This stems from the nucleation behaviour of the gelsolin-actin complexes, as discussed in Ermentrout and Edelstein-Keshet (1998): i.e. when gelsolin is present, the nucleation is more rapid). The authors also observed in electromicrographs that, in the presence of gelsolin, many filaments started to grow rapidly, whereas in the absence, only a few long filaments formed. It is interesting to comment that whether or not gelsolin also fragments filaments under the conditions of a given experiment, the fact that it binds to the filament fragmented means that eventually, the dominant actions are those of nucleation and capping. Eventually, each filament is associated with a single gelsolin cap, so that the dynamics converge on an equilibrium that is equivalent to the nucleation-capping equilibrium. Using a CONTIN analysis (essentially an inverse Laplace transform) of the dynamic structure factor measured experimentally, (Piekenbrock and Sackmann 1992) the length distribution of the filaments was shown to tend approximately to an exponential one. This is yet a further connection with the model results.

3 Model for filament interactions

3.1 Background and goals

The second class of models is geared less at the detailed kinetic steps in known reactions, and more at understanding possible phenomena stemming from the complex interactions of the cytoskeletal elements. In particular, such models address spatial and angular distributions of actin density. Work by Spiros and Edelstein-Keshet (1997) falls into this category. Again, some of the assumed interactions between actin and associated binding proteins, as well as values of known biological reaction kinetic parameters are
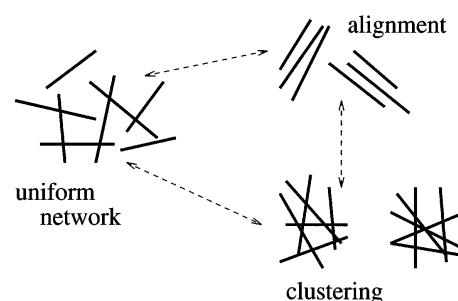
Fig. 4 A model for filament associations aims to predict transitions between various structures at the level of many filaments, or filament populations

inputs, whereas the mathematical modelling and analysis leads to insight about how filament length influences diffusion and interaction of actin filaments, and thus affects structures on the order of populations of filaments as shown in Fig. 4. Clusters are interpreted as sites of relatively high (but unaligned) actin filament density. Bundles are interpreted as sites of filament alignment.

Many of the detailed attributes of actin monomers and filaments are given in the literature. Some attributes are specific to actin, while others are generic to rod-like polymers. Of these, perhaps one of the most intriguing is the length-dependence of diffusion rates (Zaner 1995; Doi and Edwards 1986). This single factor – namely the very different behaviour of the rotational and translational diffusion rates as filament length increases – is already enough to explain some of the types of macroscopic patterns likely to occur in solutions of actin filaments. In a nutshell, as filaments get longer, their random rotation in solution is severely impeded (due to entanglement) though their motion parallel to the filament axis is only mildly affected. This means that alignment and clustering are favoured in different filament length regimes, as the model below illustrates.

In this modelling scenario, the basic aim is to understand how filaments and crosslinkers can give rise to three distinct types of structures at the level of many filaments: gel-like networks, bundles, and clusters. However, we are not interested merely in the transitions, but also in the way that filaments would be distributed over space and how their orientations would be correlated. The phenomena of interest include alignment to preferred directions and aggregation of certain sites. (Physically speaking, these phenomena are referred to as angular and spatial order). If we were merely interested in the phase transition itself, for example in predicting the state of the network at various concentrations of crosslinkers (i.e. at various degrees of crosslinking), other, preexisting theoretical approaches would be adequate. For example, the ideas of percolation theory have been used to advantage (Nossal 1988) to investigate the formation of a "gel cluster" beyond some critical crosslink fraction. However, if we want to have some prediction about relative spacing of clusters or bundles, a fully dynamic spatially-distributed model may be relevant.

In this model, the detailed chemistry is averaged in some sense, and phenomena at the level of many filaments are

studied. The steps involved in interactions between a given filament and cross-linkers that attach to it are considered in arriving at some global estimates of rates of attachment and detachment of filaments from the actin networks. We consider a large population of interacting components which can diffuse, bind, and unbind. The variables used to describe the situation are the densities $N(x, \theta, t)$, $F(x, \theta, t)$ of bound ("network") and free filaments at a given position, orientation, and time. The model, which describes the transitions between these and the rearrangement of the spatio-angular filament distributions is two-dimensional, but a three-dimensional version has been described by Mogilner and Edelstein-Keshet (1995).

3.2 The model

Some of the assumptions that go into creating a model of the actin filament interactions are as follows:

1. Unbound crosslinkers diffuse rapidly, and have a roughly constant concentration everywhere.
2. The filament density is neither "too low" (where stochastic effects would dominate) nor "too high" (where Onsager's law would predict crystalline order).
3. The timescale of interest is greater than the average binding time of a filament to the network.
4. The average length of a filament, L is taken to be fixed. However, the influence of this length on the outcome forms an interesting theme.
5. Filaments are either **bound** to the network or **free**. (The degree of binding is not explicitly followed.)
6. A free filament can move by translational and rotational diffusion (in a length-dependent way to be described) but a bound filament is fixed spatially.

The overall equations of the model are essentially statements about "book-keeping" of filaments that change orientations and spatial positions. (We show the detailed mathematical form in the Appendix.) Essentially the equations state that

$$\frac{\partial N(\theta, x, t)}{\partial t} = \begin{bmatrix} \text{rate of attraction} \\ \text{of free filaments} \end{bmatrix} - \begin{bmatrix} \text{rate of loss of} \\ \text{network filaments} \end{bmatrix}$$

$$\frac{\partial F(\theta, x, t)}{\partial t} = - \begin{bmatrix} \text{rate of binding} \\ \text{to network} \\ \text{filaments} \end{bmatrix} + \begin{bmatrix} \text{rate of unbinding} \\ \text{of network} \\ \text{filaments} \end{bmatrix}$$
$$+ \begin{bmatrix} \text{rate of} \\ \text{random} \\ \text{reorientation} \end{bmatrix} + \begin{bmatrix} \text{rate of} \\ \text{spatial} \\ \text{diffusion} \end{bmatrix}$$

One of the central features of the model is the hypothesis that actin filaments interact not merely at a single point, but rather over some spatial and angular extent. This is equivalent to saying that:

– **The probability that a filament will bind to another filament depends on (a) how far apart they are and (b) their relative orientation**

binding possible   too far to bind   probablity of binding
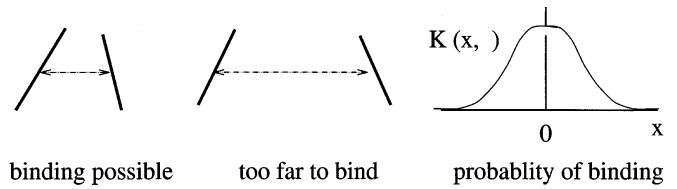
Fig. 5 Spatial binding probability. Filaments close together are more likely to bind than those farther apart. The spatial part of the binding probability is shown to the right

binding possible   angle too large   probablity of binding
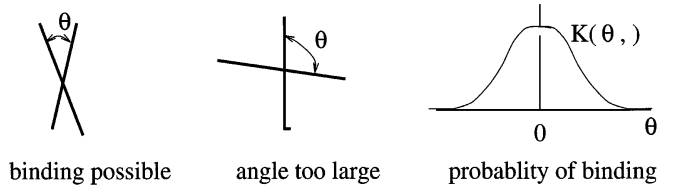
Fig. 6 Angular-binding probability. Depending on the binding protein, certain filament configurations favor binding over others. Here we show a case where filaments more nearly aligned are more likely to bind, but other possibilities are also of interest. The angular part of the binding probability is shown to the right

Mathematically, this is taken account in modelling the binding step. We assume that the probability that two filaments can interact and bind depends on their distance of separation and their relative orientations.

The probability of binding depends on the sizes of the filaments, as well as on the specific type of crosslinker that binds them. Figures 5 and 6 show how one can arrive at functions that encode a spatial and orientational preference. (Such functions are called the binding kernels, and they represent the angle and spatial dependence of the association between filaments. In practice, these would be specific to the crosslinker, but certain generic properties would be shared (Civelekoglu and Edelstein-Keshet 1994).) It is reasonable to assume that interactions can occur at distances which are up to $L/2$ from the "center" of a filament whose length is L. The optimal relative configuration of interacting filaments depends on the type of crosslinker that binds them. Some binding proteins favor filaments which are nearly parallel or antiparallel, while others favor the orthogonal configuration. This means that angles of 0 or π or $\pi/2$, respectively, might lead to greatest chances that the filaments will attach to a common crosslinker, though only the first situation is shown in Fig. 6. Typically, the rate of binding of one filament to any of the other filaments whose position is x and whose orientation angle is θ would be represented by

$$\text{Binding rate} = \beta \int_{-\pi}^{\pi} K(x - x', \theta - \theta') F(x', \theta') \, dx' \, d\theta'$$

where β is a parameter that describes the **maximal rate of binding at some optimal configuration**, x, x' are the positions of the filament binding and to be bound, and θ, θ' are the orientations of the two filaments.

It should be apparent to the reader that this model is formulated at a level of the "mean field", so that the outcome

of fluctuations is not addressed. However, random effects acting on the ensemble of filaments are incorporated via the assumed diffusion properties of the filaments. Further, the interesting point of contact between the model and the biology is the way that parameters in the system depend on properties of the participating reactants. For example, parameters (β, γ) for binding and unbinding rates depend on the kinetics of the particular actin-binding proteins, and on the number of sites available for interaction.

The mobility of the unbound filaments stems primarily from assumed translational μ_2 and rotaitonal μ_1 diffusion. The effect of length on rates of diffusion is rather dramatic, and has consequences that show up in the final structures formed. Indeed, entanglement is dominant in the so called **semi-dilute** regime, when the number of filaments per unit volume exceeds $(6/\pi)L^{-3}$, where L is filament length (Doi and Edwards 1986; Zaner 1995). In this regime, the **Rotational diffusion coefficient** μ_2 and the **Translational Diffusion Coefficient** μ_1 depend on filament length L as follows:

$$\mu_2(L) \approx C\frac{1}{L^7}, \quad \mu_1(L) \approx C\frac{1}{L} \tag{11}$$

where C is a constant (depending on temperature, viscosity, the shape of the filaments, and the total amount of actin). Thus, rotation is much more severely hampered for longer filaments than translational motion. This has interesting consequences for the structures that form as discussed below.

3.3 Some mathematical results

It was shown in Spiros and Edelstein-Keshet (1998) that the density of actin will become nonuniform whenever a certain inequality, containing the parameters of the model is satisfied. (See Appendix.) The inequality can be stated in terms of the number of clusters formed per unit distance (n_2) and how many orientations favored (n_1) when alignment occurs. Briefly, a condition which must be met for clustering and/or bundling is, roughly speaking, that there are integers n_1, n_2 satisfying:

$$0 < \frac{n_1^2}{a^2} + \frac{n_2^2}{b^2} < 1 \tag{12}$$

where the parameters a, b depend on the binding (β), unbinding (γ) rates, the diffusion coefficients (μ_1, μ_2) and the total mass of actin (M), as follows:

$$a = \frac{\beta M}{\sqrt{\gamma\mu_1}}, \quad b = \frac{\beta M}{\sqrt{\gamma\mu_2}}$$

When this condition is just satisfied, we would expect to see, on average, n_2 clusters per unit distance and/or structures with n_1 prefered directions of alignment. ($n_1 = 0$ for an isotropic nonaligned network, $n_1 = 1$ for filaments aligned all in parallel, $n_1 = 2$ for filaments with both parallel and antiparallel alignment, and $n_1 = 4$ if there are or-
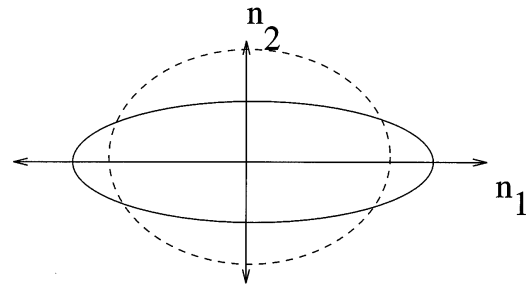

Fig. 7 The number of clusters (n_2) and the number of dominant directions of alignment (n_2) are integer values restricted to fall inside an elliptical region. If the parameter values do not favor structures like clusters and bundles, the ellipse contains no nonzero integer values. Changes in filament length will change the relative sizes of the semi-major and semi-minor axes of this ellipse (e.g. from the *dotted* to the *solid figure*) since the parameters μ_1, μ_2 are affected differently by filament lengths. This, will then affect whether clusters or bundles will form

thogonal structures, such as those favoured in the presence of ABP; the units of distance for n_1 are determined by the units used for the diffusion coefficient and the time constant in the expression for a). Note that a, b depend on, respectively, the rotational and translational diffusion of filaments, and this is one of the major ways that filament length can affect the possibility of structures. (The actual condition is slightly more stringent that this, and involves that assumed probability kernels, but the point can be made here with this simpler necessary condition.) This prediction stems from a linear stability analysis calculation, and holds close to the transition to pattern. However, nonlinear effects may eventually result in more complex structures or patterns, and these must be deduced from full numerical simulation of the equations.

From Fig. 7 we can see that the condition shown here is equivalent to restricting the integers n_1, n_2 to an elliptical region in the n_1, n_2 plane. The larger the ellipse, the more likely it is that structures consisting of clusters and/or bundles of actin would form. No structure at all can form (i.e. the mixture remains a uniform network or gel) if the ellipse is too small to enclose any points with integer coordinates. Since the parameters a, b determine the major and minor axes of this ellipse, any influence which tends to increase a, b would increase the probability of heterogeneous and anisotropic structures. For example, if β is large or γ is small (as would be true if the molar ratio of crosslinker to actin is sufficiently high – see section 4.4) then formation of such structures is more likely. If M is large (which means that the total amount of actin – i.e. total number of filaments of some given average length – is large) then structures are also likely to form.

Further, changes in the filament length, which affect μ_1, μ_2 differently, lead to a change in the aspect ratio of the elliptical instability region; this would result in a bias towards one type of structure (bundles) or another (clusters) because it means that one of the two integers n_1, n_2 can take on larger values than the other. For example, the rotational diffusion, $\mu_1(L)$ varies more sharply with L than
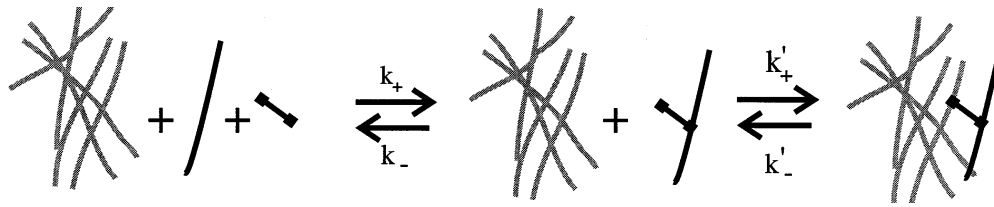
Fig. 8 This diagram shows steps in the binding of a free filament to the actin network with the help of one of the actin-binding proteins. In principle, it takes only one double-link to convert the free filament to a network filament. By considering the reactions involved in binding of a crosslinker to a filament, and then to a second filament, we can estimate the "effective filament binding rate" for a given concentration of the crosslinking proteins

does the translational diffusion, $\mu_2(L)$. As filament length increases, this would cause a to increase more dramatically than b, making the ellipse "long and skinny" in the direction of the n_1 axis, and thus increasing the likelihood of structures which have net alignment of filaments. For shorter filament lengths, the reverse is true: the ellipse may be "fatter" in the direction of the n_2 axis, since b is larger, and thus the likelihood of clusters forming is higher. If the ellipse is "fat enough" to contain points (n_1, n_2) where both coordinates are integers, we expect localized aggregations which have aligned filaments (i.e. bundles), and the spacing between these structures can be predicted from the value of n_2. (However, it should be kept in mind that the details of the pattern will depend both on the effect of the binding kernel, which we have not described in detail here, and on non-linear effects which can only be predicted from full simulations (Spiros and Edelstein-Keshet 1997), or more detailed non-linear analysis.)

This description of the model for filament interactions and it linear stability condition leads to the speculation that **by changing the state of polymerization of actin, (i.e. the average filament length), it is possible to control transitions in the overall actin structures, and influence whether bundles or clusters will dominate**. Further, small refinements can also determine how closely spaced the clusters are, or how many angles of alignment occur.

Fig. 9 A filament is freed from the network in a sequence of steps in which all the links between the given filament and the rest of the network are severed. By considering the reactions involved in binding and unbinding of a crosslinker from a filament, we can estimate the "effective filament unbinding rate" for a given concentration of the crosslinking proteins
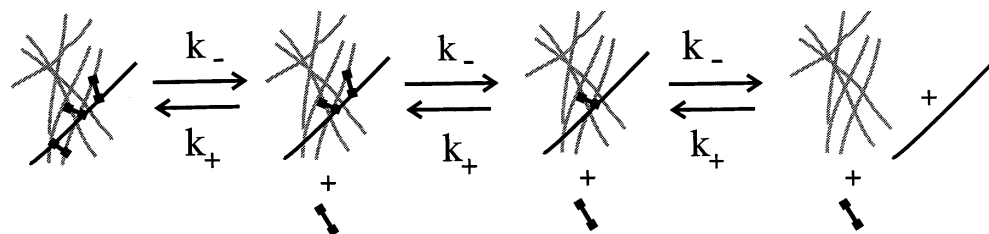
3.4 Estimating the parameters

Even in a caricature model such as this one, making contact between parameters in the model and those actually measured or known in the biological system is of the essence, and this can be a nontrivial undertaking. Some comments about how this was done are given below.

3.4.1 Binding rates: estimating β

In Spiros and Edelstein-Keshet (1998), rate constants k_+, k_- for the attachment of the crosslinker α-actinin (Wachsstock et al. 1994; Meyer and Aebi 1990) were used to arrive at an estimate for the parameter β. Figure 8 shows how several binding steps together lead from a free to a network (or bound) filament. The parameter β can be related to the rate constants and to the concentration of the crosslinker, α. For example, it is found that (Spiros and Edelstein-Keshet 1998)

$$\beta_1 \approx \frac{k_+^2 [\alpha]}{k_-}. \tag{13}$$

3.4.2 Unbinding rates: estimating γ

For a filament to unbind from a network of other filaments, all its links (which may be numerous) must be severed as shown in Fig. 9. To estimate this rate, one needs the average degree of binding, and the mean transit time from fully bound to fully unbound. This multi-step process is not explicitly modelled in the equations at the level of filament populations, but rather, used as an aggregate or average unbinding parameter γ which can be computed mathematically from the process shown in Fig. 9. For a given cross-linker abundance and reaction rate constants, eigenvalue analysis is used to determine a numerical value for γ by methods outlined in Spiros and Edelstein-Keshet (1998).

4 Results and predictions

4.1 Summary of the main results

Aside from the analysis that leads to predictions such as those already discussed, the equations of the model, (as shown in the Appendix) were simulated numerically using methods developed to handle the combination of integral terms (convolutions), the spatial diffusion, and the range of variability of the rates of diffusion from small to large filament lengths. Technical details are given in Spiros and Edelstein-Keshet (1998). Values of the parameters in these equations were based on the values developed from arguments described briefly above, using information from the literature. The results, which were spatial and angular distributions of actin filament density, can be summarized as follows:

– If the values of the parameters are outside the appropriate ranges (corresponding to the situation in which the elliptical region in the $n_1 n_2$ plane is too small), the filaments form a uniformly dense network, with no tertiary apparent structure – i.e. no clusters or bundles form, and no alignment of filaments into preferred directions occurs.
– The simulations of the longer filaments $(1, 0.8\mu)$ reveal a tendency of alignment into bundle-like structures.
– Short filaments tend to freeze into clusters and will not align.
– The number of clusters that form is greater for the smallest filament lengths.
– Increasing the amount of crosslinker tends to accelerate the formation of nonuniform actin distribution.

4.2 Comparison with experimental work

A study of the sol-gel transitions in actin networks crosslinked with a α-actinin has appeared recently (Tempel et al. 1996). The authors were able to systematically vary the association-dissociation kinetics of the crosslinker by varying the temperature. They also explored the behaviour at various molar ratios of α-actinin to actin. By measuring creep compliance and shear modulus of actin solutions, they characterised various rheological regimes and identified the point at which the transition to a gel occurs. They were able to infer three types of regimes: (1) a homogeneous gel, (2) a gel which is locally heterogeneous ("clustering") and finally (3) a regime in which bundle-like structures of actin coexist with a few free filaments. A percolation model of the sol-gel transitions was discussed in the context of their observations. The phase diagram (for actin molar fraction vs fraction of crosslinkers) shows the transition from (1) to (2) to (3) as the degree crosslinking is increased. This would correspond to increase of the parameter β discussed in Section 4.3 or decrease of γ, and would agree with the general prediction of our model that a transition to the formation of structures would be favoured. Similarly, the prediction that an increase in the ac-

tin molar fraction (or mesh size) of the network results in favoured transition to clusters and bundles (for a constant degree of crosslinking) matches with the observation, in our model, that increasing M tends to enlarge the region corresponding to such structures.

A systematic variation of the length of the filaments was not part of the above study, so that this aspect of the predictions can not be directly compared. However, it has been noted in separate studies and various experimental conditions including more concentrated actin concentrations (Furukawa et al. 1993; Suzuki et al. 1991) that increased filament length tends to favour long-range alignment of filaments (liquid crystals) over clusters. While this fits the general results of the model, it should be remarked that the conditions of such experiments are probably outside of the range of applicability of this charicature model.

Although the model has many simplifications and aspects that make is primitive, it does point to phenomena which may not have been previously recognized, and suggests a possible new mechanism for the control of structure in actin filament networks: by controlling the average length of the filaments (as well as any one of the other factors involved in the crosslinking dynamics), the cell would be able to locally adjust the transitions from homogeneous gel to clusters and to bundles of actin. Clearly, some caution is needed in interpreting the predictions. As indicated, this model is a gross charicature and has certain features that make it unrealistic. These include: (1) Failure to consider changes in filament length due to polymerization or fragmentation; (2) Failure to distinguish between filaments at the edge versus the center of a cluster or to consider the binding state of a filament (one bond, versus multiple bonds to the network); (3) Mechanical and fluid dynamic effects are not included in the model. This reduces its relevance to in vitro actin dynamics.

These limitations make the model oversimplified if the detailed in vitro dynamics are of interest, but sufficient if a global view is desired of how actin filaments and crosslinkers, on their own, can interact to produce bundles, gels, and or networks.

5 Other mathematical approaches

To some extent, mathematical ideas or tools have appeared in many of the well-known papers in the literature, though not always as the centerpiece. We give a few examples below.

The flexibility, mobility, and reptation of individual actin filaments (longer that 1μ) were described in (Käs et al. 1996; Janmey et al. 1986). The mechanical properties of actin at the level of multiple filaments and gels, were investigated in the works of (Zaner 1995; Nossal 1988; Janmey et al. 1994). The effects of crosslinks, including generic and specific cases were also given by (Nossal 1988; Wachsstock et al. 1993; Wachsstock et al. 1994). Percolation models, in which the degree of crosslinking is related to the formation of macroscopic clusters can be found in

several references, including (Nossal 1988; Tempel et al. 1996) and others. Such models can serve as predictors of phase transitions in the gel, though they do not explicitly address the spatial distribution or the orientational preferance in the resultant structures. The effects of the shapes and sizes of a crosslinking protein on its ability to bundle actin filaments was discussed in Meyer and Aebi (1990). Other theories for actin bundle formation include those that place greater emphasis on ionic interactions (Tang and Janmey 1996). Most of the above-mentioned papers are at least partly, if not dominantly experimental, and the physical and mathematical tools are used to complement experimental measurements.

Many of the papers in which chemical kinetics are of interest also make contact with mathematical arguments. These include the papers on actin polymerization and nucleation (Pollard 1986; Korn et al. 1987; Ditsch and Wegner 1994; Oosawa and Kasai 1962; Tobacman and Korn 1983; Selve and Wegner 1986; Bonder et al. 1983) and interactions with gelsolin (Schoepper and Wegner 1991; Schoepper and Wegner 1992). A recent and much more detailed treatment of polymerization of actin in the context of signal transduction and ligand-receptor kinetics is given by Adams et al. (1997).

A few works in the literature, including those on which this paper was based, (Edelstein-Keshet and Ermentrout 1998; Ermentrout and Edelstein-Keshet 1998; Spiros and Edelstein-Keshet 1998) are more heavily theoretical. Other examples include detailed simulations of the cytoskeleton developed in Lumsden and Dufort (1993), which, unlike Spiros and Edelstein-Keshet (1998) dealt at the level of individual filaments, binding proteins, etc. Mathematical work which had its motivation in the cytoskeleton include (Geigant and Stoll 1996; Geigant et al. 1997;Ladizhansky 1994), though the main thrust of these papers is mathematical.

Finally, some recent papers narrow the gap between theory and experiment by working closely with the known biology and with advanced and powerful tools of mathematics. An example includes Mogilner and Oster (1996), in which the polymerization of actin is linked to the mechanism of cell motility. It is hoped that by the combined efforts of experimentalists, biophysicists, and mathematicians, working at various levels of detail and with various combinations of experimental and theoretical tools, the secrets of the cytoskeleton, its dynamics, and its functions in the cell will gradually be revealed.

Appendix: model for filament rearrangements

The equations of the model, as given in Spiros and Edelstein-Keshet (1998) are:

$$N_t(x,\theta,t) = \underbrace{\beta_1 F\,K*F + \beta_2 N\,K*F}_{\substack{\text{filament}\\\text{association}}} \quad \underbrace{-\gamma N}_{\substack{\text{filament}\\\text{dissociation}}}$$

$$F_t(x,\theta,t) = \underbrace{-\beta_1 F\,K*F - \beta_2 F\,K*N}\ \ \underbrace{+\gamma N}$$

$$+ \underbrace{\mu_1 F_{\theta\theta}}_{\substack{\text{rotational}\\\text{diffusion}}} + \underbrace{\mu_2 F_{\theta\theta}}_{\substack{\text{translational}\\\text{diffusion}}}$$

where $N(x,\theta,t)$, $F(x,\theta,t)$ are the densities of actin filaments bound in the network and free at the given location and directions. Free filaments may diffuse rotationally μ_1 and translationally μ_2. Unbinding of filaments from the network is assumed to take place at rate γ. Binding of filaments (convolution terms) is assumed to occur at a rate that depends on relative configurations $K*F$ where

$$K*F = \int_{-\pi}^{\pi}\int_{\Omega} K(\theta-\theta', x-x')F(x',\theta')\,d\theta'\,dx'. \tag{14}$$

This makes such models nonlocal in that filaments can interact over some spatial and angular ranges. The kernels (K) considered in some of the previous models of actin dynamics have typically had the form

$$K(\theta,x) = K_1(\theta)\,K_2(x), \tag{15}$$

where

$$K_i(u) = \frac{1}{\sigma_i\sqrt{2\pi}}\exp\!\left(-\frac{u^2}{2\sigma_i^2}\right). \tag{16}$$

K_1 describes the angular part of the way filaments interact in presence of a cross-linker, and K_2 the spatial part. The parameter σ_1 is the angular range over which filaments can interact and σ_2 is the spatial range. The kernel $K(\theta,x)$ can be adapted to a variety of cases, including interactions that tend to create parallel or orthogonal configurations, that tend to bunch filaments together, etc. (Civelekoglu and Edelstein-Keshet 1994).

The condition for instability, in its fuller form is:

$$\mu_1 n_1^2 + \mu_2 n_2^2 < \left(\frac{\beta_1\beta_2}{\gamma^2}\right)\tilde{M}^2\,\hat{K}(1-\hat{K}) \tag{17}$$

where $\hat{K}$ is the Fourier Transform of K, and is a function of n_1, n_2. This function is never larger than 1. $\tilde{M}$ is a parameter which can be considered as an "effective total average density of actin" (number of filaments per unit volume).

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