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# Self-Organization of Polymer-Motor Systems in the Cytoskeleton

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# Self-organization of polymer-motor systems in the cytoskeleton

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**SUMMARY**

Microtubules and actin filaments are organized into dynamic arrays inside cells. In this paper I discuss in conceptual form the assembly mechanisms of three specific arrays: asters, spindles and leading edge structures. The role of energy transducing processes, particularly motor protein activity, in assembly is explored. I conclude that dynamic interaction between motor proteins and cytoskeletal polymers play a very general role in spatial organization of the cytoplasm.

## 1. INTRODUCTION

The cytoskeleton of eukaryotic cells consists of long, non-covalent polymers of the proteins tubulin and actin, called microtubules and actin filaments, together with intermediate filaments which I will not consider in this article. These polymers are responsible for shaping the cell and directing the movement of intracellular particles and organelles. To perform these functions the cytoskeletal polymers must be organized into specific spatial arrays. Examples of such arrays include the mitotic spindle which consists of microtubules and associated proteins arranged into a bipolar organization suited to segregating sister chromatids before cell division, and the leading edge which consists of actin filaments arranged to allow protrusive activity during cell locomotion. A major issue in trying to understand the cytoskeleton is the question of how these highly organized arrays assemble in the cell.

The polymerization of tubulin and actin into filaments occurs by mechanisms similar to the assembly of virus particles. Self-complementary interfaces on the protein subunits participate in forming non-covalent bonds, resulting into polymerization into a specific lattice structure (Holmes *et al.* 1990). This process is termed self-assembly and it is the fundamental mode by which protein complexes form inside cells (Inoue 1982). Simple self-assembly mechanisms cannot, however, account for the assembly of cytoskeletal arrays. When tubulin or actin polymerize in solution they form short, randomly arranged filaments. To make large-scale arrays requires additional levels of organization, and in fact the tendency for spontaneous polymerization must be suppressed, since it would lead to randomization of polymer distribution.

One clue to the assembly mechanisms of large cytoskeletal arrays comes from considering their energy transducing properties. There is a fundamental, thermodynamic difference between simple macromolecular assemblies such as virus coats or ribosomes and cytoskeletal arrays. The simple structures assemble to true equilibrium, which is the state of lowest free energy. Thus their assembly pathway is governed by the strict laws of equilibrium thermodynamics, as manifest in self-assembly pathways. Cytoskeletal assemblies in contrast are open, or steady-state systems in thermodynamic terms. They require a continuous energy input, which ultimately derives from ATP hydrolysis, to assemble and function. Such open systems assemble not to a state of minimum free energy, but to a state of minimum entropy production (Nicolis & Prigogine 1977). Because of this continuous energy input the assembly of the cytoskeletal assemblies can occur through pathways that are not familiar from simple, equilibrium model systems (Kirscher & Mitchison 1986). Also, the final structures will have dynamic properties which are more exotic than those of equilibrium structures, which are restricted to small thermal fluctuations. These dynamic properties of cytoskeletal assemblies may be used by the cell to perform work. In this article I will discuss potential mechanisms for the assembly of microtubule and actin filament arrays. I will highlight the role of energy transducing processes in assembly, which will require a brief review of polymerization dynamics and motor proteins. I will argue that dynamic interaction between motor proteins and cytoskeletal polymers represents a fundamental mode by which the cytoskeletal arrays assemble, and that considering such interaction provides unifying concepts for understanding the spatial organization of the cytoplasm.

## 2. POLYMERIZATION DYNAMICS

So far we know of two ways in which the energy of ATP hydrolysis is transduced by the cytoskeleton for organizational purposes. These are polymerization dynamics and the activity of motor proteins. Polymer-

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ization dynamics transduce energy by virtue of the GTP hydrolysis which accompanies polymerization of tubulin, and the ATP hydrolysis which accompanies polymerization of actin. In both cases the free energy of hydrolysis is used to destabilize the polymer relative to free subunits, and thus serves to promote subsequent depolymerization of the polymer. This is reflected by faster off rates for the NDP form of the subunit compared with the NTP form, an effect whose magnitude is much greater for tubulin than actin, at least for the pure proteins (Mitchison & Kirschner 1984; Pollard 1986*a*). The ways that NTP hydrolysis during polymerization is exploited by the cell are poorly understood, especially for actin filaments. For microtubules, both *in vitro* and inside cells, GTP hydrolysis is used to power dynamic instability (Mitchison & Kirschner 1984). Prolonged bouts of polymerization of individual microtubules are interspersed with bouts of depolymerization. The exact mechanism by which GTP hydrolysis powers dynamic instability is not known, but the polymerizing form of the microtubule is thought to be stabilized by a cap of GTP-liganded subunits (Mandelkow *et al.* 1991). The role of dynamic instability in spatial organization of microtubule arrays has been extensively explored by both theory and experiment (Kirschner & Mitchison 1986). It is remarkable that so simple a mechanism can drive relatively sophisticated organizational events such as chromosome capture during mitotic spindle assembly (Rieder & Alexander 1990). Much less is known about the role of ATP hydrolysis by actin filaments, or the role of GTP hydrolysis in arrays of stabilized microtubules. The lesson of dynamic instability has been that NTP hydrolysis during polymerization can be exploited by complex assembly pathways, and that the role of this form of energy transduction in the assembly of cytoskeletal arrays deserves further exploration.

## 3. MOTOR PROTEINS

Motor proteins bind to cytoskeletal polymers and ATP, and use ATP hydrolysis to produce force along the polymer. They include members of the kinesin, dynein and possibly dynamin gene families which are microtubule motors, and members of the myosin gene family which are actin filament motors (reviewed in Vallee Schpetner (1990); Pollard *et al.* (1991); Goldstein (1991); Sawin & Scholey (1991)). So far all motors which have been assayed move in one direction on the polarized polymer lattice. Defining the plus end of the polymer as that which polymerizes faster (which converts barbed ends to plus ends for actin filaments) dyneins are so far all minus-end directed and myosins all plus-end directed. Kinesin itself is plus-end directed, whereas the related protein ncd is minus-end directed (Walker *et al.* 1991). Thus within the kinesin gene family the same basic motor domain can move in either direction on the polymer, a fact which challenges our understanding of force generation mechanisms.

The polarity of force generation by motor proteins is central to their biological roles. The polymers in organized cytoskeletal arrays are arranged in the cytoplasm with specific polarities, for example microtubule plus ends towards the middle of the mitotic spindle (Euteneuer & McIntosh 1981), or actin filament plus ends forwards in the leading edge of motile cells (Small 1988). When motor proteins act to transport vesicles or other components through the cytoplasm, the movement direction will be determined by which motor is bound (or activated) on the vesicle surface, and the polarity of the polymers supporting transport. Less obvious is the possibility that the polarization of the filaments in an array is in fact a consequence of motor protein activity, as well as a cause of transport directionality. In this article I shall focus on tensioning and movement of the polymers themselves by motor proteins. When a motor protein is tethered to some stationary component and it exerts force on a polymer, two things can happen. The polymer can be tensioned but not moved, or it can be moved through the cytoplasm. In either case the activity of the motor protein will have important consequences for the organization and polarity of the polymer within an array. This aspect of motor protein biology is familiar for actin filaments, because it occurs during muscle contraction. Tensioning and moving of microtubules by motor proteins is less familiar, although it is responsible for the beating of cilia. In discussing possible assembly pathways for cytoskeletal arrays such as the spindle and leading edge I shall make frequent reference to polymer movement by motor proteins as this is likely to be a critical part of diverse assembly pathways.

## 4. MICROTUBULE ASTERS

The simplest organized microtubule array is an aster, which consists of microtubules of varying lengths radiating from a small central region with uniform polarity, always plus ends out. Microtubules in most small animal cells are arranged in an aster centred on a centrosome near the nuclear envelope, and asters form the pole structures of mitotic spindles. Thus understanding how the astral array assembles forms a starting point for consideration of other, more complex arrays. The conventional view of asters assembly is as follows (figure 1*a*): a microtubule organizing centre, usually a centrosome, lies at the centre of the aster. This structure contains a fixed number of nucleating sites which nucleate microtubule polymerization. Nucleated mirotubules elongate with their plus ends distal, and their minus ends held at the nucleating sites and blocked to dynamics. The biochemical nature of the nucleating site is unclear, but in the case of a centrosome it may involve gamma-tubulin molecules bound to a matrix of fibrous 'pericentriolar material' (Zheng *et al.* 1991, Stearns *et al.* 1991). This simple picture is complicated but not changed by dynamic instability, which causes individual microtubules to alternate between polymerization and depolymerization. Dynamic instability gives rise to rapid microtubule turnover at a rate which depends on cell-cycle state (Belmont *et al.* 1990). It plays a role in aster assembly by ensuring that




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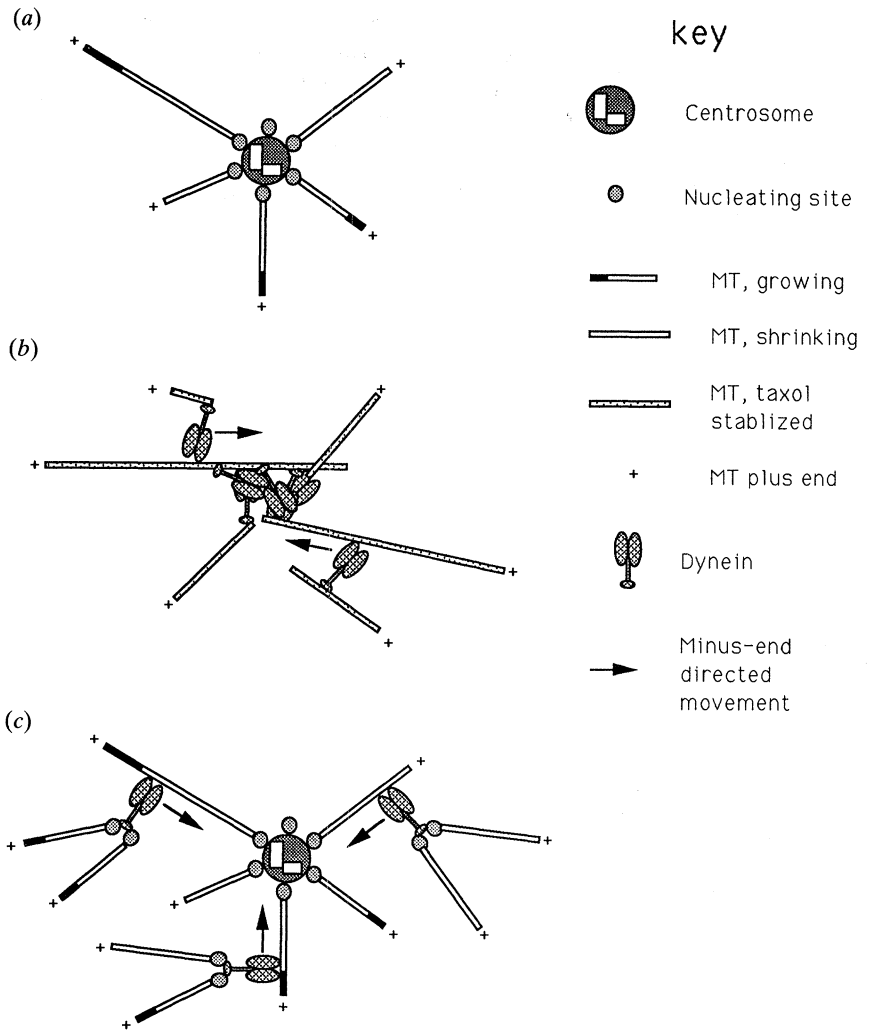


Figure 1. Assembly of astral microtubule arrays. (*a*) The conventional view of astral assembly. A fixed number of nucleating sites (perhaps made of gamma-tubulin) are attached to peri-centriolar material. These nucleate microtubules with plus ends distal, which then undergo dynamic instability. (*b*) A model for assembly of asters in taxol treated cytoplasmic extracts, from Verde *et al.* 1991. Dynein molecules bind microtubules by an ATP-insensitive site, and then drag these microtubules into the forming aster by their minus-end directed motor activity. (*c*) A model for astral array assembly incorporating elements of both (*a*) and (*b*). A conventional nucleating center initiates formation of an astral array. Next dynein molecules move inwards on this array, bringing with them attached nucleating sites. These new nucleating sites increase the size of the array, leading to explosive growth of the aster. This model can explain the rapid assembly of the cytaster around the sperm centrosome after fertilization in amphibians and sea urchins, and may be generally relevant.

centrosome-nucleated microtubules dominate over spontaneously nucleated ones at steady state. Centrosomal microtubules are rapidly re-nucleated after depolymerization, whereas spontaneously nucleated microtubules are not readily replaced because of the kinetic barrier to nucleation in solution (Kirschner & Mitchison 1986).

The conventional view of aster assembly has been challenged by recent work using cell-free extracts from *Xenopus* embryos. When mitotic extracts are incubated with taxol many short microtubules are formed, and subsequent to polymerization they rearrange into normal-looking asters (Verde *et al.* 1991). This rearrangement is an accretion process, where individual foci capture more and more preformed microtubules from solution with time. Taxol aster formation in *Xenopus* extracts requires the motor protein dynein which is thought to drive aster formation by binding to one microtubule, and then dragging it into the forming aster by using its minus and directed motor activity (figure 1*b*). The polarity of microtubules in taxol-induced asters is thus a reflection of the directionality of dynein, and not the properties of a nucleating site. To drive taxol–aster formation the dynein must derive from mitotic extracts, presumably reflecting a requirement for a specific phosphorylation state. Phosphorylation may be required for dynein to bind to microtubules through a site distinct from its motor domain because mitotic but not interphase dynein remains attached to microtubules in lysed tissue culture cells (Pfarr *et al.* 1990; Steuer *et al.* 1990). Taxol-induced aster assembly is unlikely to be simply an artifactual aggregation because the taxol asters accumulate proteins characteristic of normal

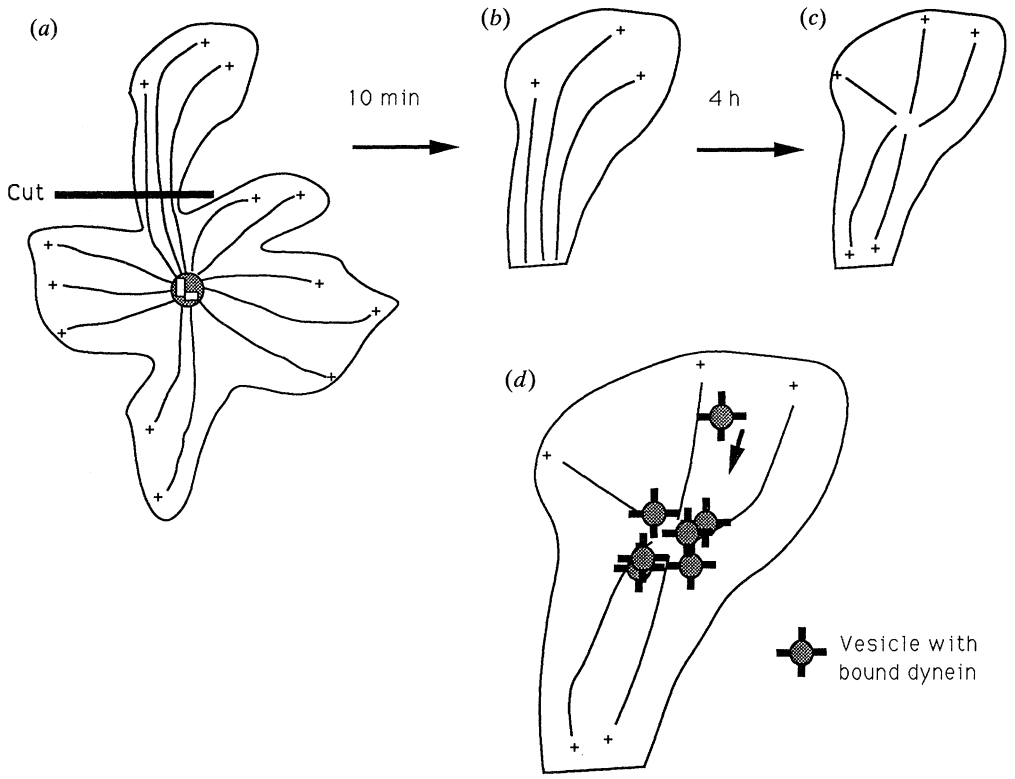


Figure 2. Astral microtubule array assembly in the severed arm of a fish scale melanophore cell, from McNiven & Porter (1984, 1988). (*a*) Melanophore cell showing typical astral microtubule distribution radiating from the centrosome. (*b*) Severed arm immediately after cutting. The microtubules are arranged as expected from severing the initial array. (*c*) Severed arm 4 h later. The microtubules have rearranged into an astral array, despite the lack of a centrosome. (*d*) Model for assembly of the astral array. Vesicles derived from elements of the endoplasmic reticulum, golgi, etc. have minus-end directed motor protein bound to their surface. By moving towards microtubule minus ends and aggregating, the vesicles drive aggregation of microtubule minus ends, and thus formation of an organizing centre. Inwards vesicle movement produces outwards force on the microtubules which pulls the organizing centre to the centroid of the severed arm.

centrosomes (Buendia *et al.* 1990) which are thought to be carried inwards by dynein as multi-protein complexes.

In the artificial taxol aster systems, assembly of the aster is driven by polarity-specific transport, rather than polarity-specific nucleation. Does this system hold lessons for normal aster assembly, where microtubules undergo dynamic instability and nucleation is continuously required? One situation where accretion is thought to be important is the rapid assembly of a huge aster, termed the cytaster, around the sperm centriole following fertilization in amphibian and sea urchin eggs (Wilson 1928). Whether this interphase accretion depends on dynein is not known, but some motor protein is surely involved. A model for cytaster formation which combines nucleation and motor-dependent accretion is shown in figure 1*c*. The identity of nucleating material is not yet known, so this model is difficult to test, but it is interesting that gamma tubulin is present in large multi-protein complexes in *Xenopus* and *Drosophila* eggs (T. Sterns and J. Raff, personal communication). Conceivably these complexes also include minus-end directed motor proteins which will pull them into the accreting centrosome where they can act as new nucleating sites. The original sperm centrosome plays an important role in this model by nucleating an initial set of oriented microtubules. These serve as tracks for motor protein dependent accretion, initiating the accretion process and confining it to a single site.

A central role for motor proteins in aster assembly may also explain the results of a remarkable experiment in fish scale melanophore cells (McNiven & Porter 1984). These cells normally contain an extensive astral array of microtubules centred on a large centrosome which serves to support bidirectional transport of pigment granules (figure 2*a*). When one arm of this cell type was severed the microtubule distribution in the cut off piece was initially parallel, with minus ends at the cut site and plus ends towards the distal-most part of the arm, as expected for excision of one piece of an astral array (figure 2*b*). Over a few hours the microtubules in the severed arm rearranged into an astral organization, with minus ends at the centre of the arm, and plus ends towards the periphery (figure 2*c*). The array always centered on the centroid of the severed arm, and no centrosome or other morphologically distinct microtubule organizing centre was visible at this new centre of this astral array (McNiven & Porter 1988). However, many membranous organelles do accumulate at the new centre. The mechanism of this fascinating re-

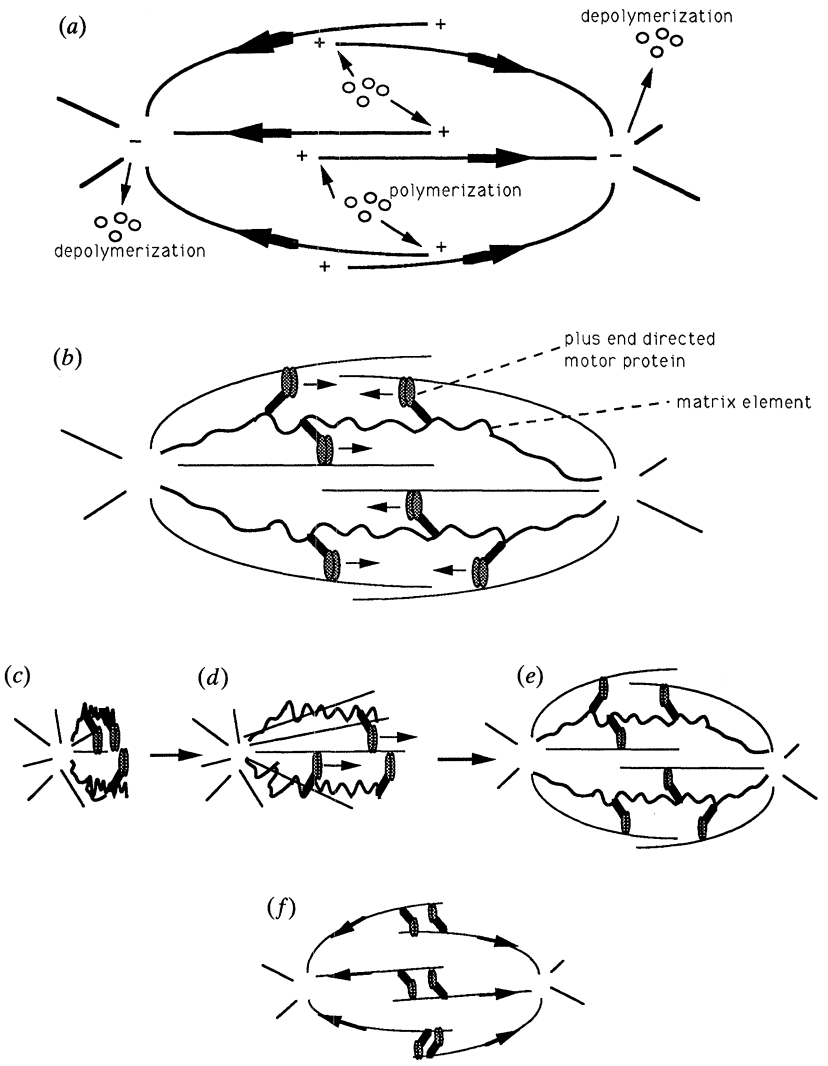


Figure 3. Role of plus-end directed motor proteins in mitotic spindle assembly. (*a*) Dynamic behaviour of spindle microtubules as inferred from photoactivation marking experiments (Mitchison 1989; Sawin & Mitchison 1991). Microtubules in each half spindle move continually polewards, denoted by the heavy arrows. In the steady-state metaphase spindle this movement is balanced by polymerization onto plus ends in the center of the spindle, and depolymerization of minus ends at the poles. (*b*) Model for spindle structure based on results from spindle dynamics and genetic experiments. Microtubules are held in place by plus-end directed motors, probably members of the kinesin family related to *Bim*C. These motors drive continual microtubule sliding, but their main role is structural. The motors are drawn attached to a non-microtubule matrix component. (*c–e*) Model for assembly of the structure shown in (*b*); (*c*) shows an initially astral array of microtubules; (*d*) motor driven extension of the matrix begins to shape the half-spindle; (*e*) two half-spindles merge by fusion of their matrix components. (*f*) Alternative view of how plus-end directed motors are arranged in the spindle. Instead of a matrix attachment, motors may directly cross-link adjacent, antiparallel microtubules. Motors in this position would be ideally suited to hold together the two half-spindles. For a more extensive discussion of motor proteins in spindle assembly see (Sawin & Scholey 1991).

arrangement are not known. Dynamic instability is almost certainly involved, if only to allow microtubules to explore different configurations before arriving at the most stable. However, polymerization dynamics alone cannot explain the assembly of an astral array in the absence of conventional nucleating sites, and I suspect that inwards movement of minus-end directed motors plays a key role. Vesicle accumulation at the cell centre presumably occurs by motor dependent movement. If these vesicles tend to aggregate, they could drive astral assembly by the mechanism proposed for taxol asters above (figure 2*d*). Minus-end directed motors distributed through the cytoplasm may also explain how the astral array centres itself in the cytoplasm of the cut arm. The number of such motors which can engage on a given microtubule increases with its length. Thus longer microtubules would experience a stronger force pulling them towards the cell periphery. Under these conditions the aster tends to centre itself in the cytoplasm, as this position equalizes the force vectors pulling it in different directions. This mechanism was

proposed to account for the centring of the cytaster in the cytoplasm of sand-dollar eggs (Hamaguchi & Hiramoto 1986).

## 5. SPINDLES

The assembly of mitotic spindles is a very complex problem, of which the astral assembly pathways involving dynein discussed above are one component (McIntosh & Koonce 1989). One view of the spindle is to consider it as two astral arrays that interact with each other through an overlap zone. Thus the question of how anti-parallel microtubules interact in the overlap zone is central to the assembly process. Experiments in which spindle microtubules are marked by photoactivation of fluorescence have revealed that they slide slowly and continuously polewards, balanced by polymerization at their plus ends, and depolymerization at their minus ends (figure 3*a*) (Mitchison 1989; Sawin & Mitchison 1991). Sliding is blocked by AMPPNP, an analogue known to induce rigor binding of kinesin to microtubules. This suggests a key role for plus-end directed motor proteins in driving sliding, and thus in spindle structure and assembly. Results from both genetic approaches also suggest a key role motors of the kinesin family in spindle assembly. Mutations in the kinesin-related genes *Bim*-C in *Aspergillus* and *Cut*-7 in *S. pombe* prevent mitotic spindle assembly (reviewed in Sawin & Scholey 1991). The sequence of the motor domain of these genes suggest they are related to each other, and also to the *Xenopus* protein Eg-5. Antibodies to both *Cut*-7 and Eg-5 decorate spindle fibres in their respective species (I. Hagan and K. Sawin, personal communication). Combining the functional and genetic data we proposed a model in which plus-end directed motor proteins, perhaps of the *Bim*C – Eg 5 family, mediate interaction between microtubules in the two half-spindles, and at the same time drive microtubule sliding. One version of this model is shown in figure 3*b*. This version invokes a non-microtubule 'matrix' component of the spindle which binds the motors. Matrix-bound motors play a key role in the assembly process in this model. They stretch the matrix outwards along astral microtubules, and thus cross-link microtubules in one half spindle (figure 3*c–d*). Interaction between matrix elements at the periphery of each half spindle then results in generation of the overlap zone (figure 3*e*). As well as driving the assembly pathway and steady-state sliding, matrix attached motors may also drive spindle expansion during anaphase B (Mitchison & Sawin 1990). Figure 3*f* shows an alternative version of the model in which plus end directed motors directly bridge anti-parallel microtubules. Distinguishing the detailed role of microtubule-motor protein interaction in spindle assembly will require additional experiments. The requirement for a minus-end directed kinesin family member, ncd, in meiotic spindle assembly in drosophila (Walker *et al.* 1991) indicates that the analysis will be complex. However, our recognition that polymer-motor interactions are a key organizing principle (see also Sawin & Scholey 1991; Karsenti 1991) will help guide future approaches.

## 6. LEADING EDGES

The organization of dynamic actin filament arrays is poorly understood, despite rapid progress in the biochemistry of actin filaments and actin binding proteins (Pollard 1986*b*; Holmes *et al.* 1990). Dynamic arrays include leading edges, cortices, cleavage furrows and stress fibres which are all formed in association with the plasma membrane. To use our knowledge of actin biochemistry to understand the assembly of such arrays, we first need more information about their structure and dynamics in living cells.

The leading edge has been one of the most studied actin filament assemblies. This is a very thin part of the cytoplasm which protrudes from the front of motile cells, either in the form of sheets known as lamellipodia, or rods known as filipodia or microspikes. Both these organizational forms of the leading edge are packed with actin filaments, aligned preferentially with their plus ends forwards (Small 1988). The filaments are cross linked by binding proteins including members of the alpha-actinin and fimbrin families (Matsudaira 1991). The biological role of the leading edge is to protrude forwards, the necessary first step in cell locomotion. Leading edge structures are not thought to be contractile, and they lack myosin-II which is present in other contractile assemblies such as cleavage furrows and stress fibres. However, they do contain at least some forms of myosin-I (Fukui *et al.* 1989), alhough its biological role is not yet known. Myosin-I, or other motor proteins, are likely to play a central role in the assembly and dynamics of the leading edge.

The dynamic behaviour of actin filaments in lamellipodia has been probed by fluorescence photobleaching, photoactivation, and DIC observation (Wang 1985; Forscher & Smith 1988; Fischer *et al.* 1988; Theriot & Mitchison 1991). These techniques reveal that actin filaments continually flow backwards with respect to the front edge of the cell, and similar dynamics are suspected to occur in filipodia. In all cell examined except the rapidly moving fish scale keratocyte filaments also flow backwards relative to the substrate (Theriot & Mitchison 1991). As with polewards microtubule sliding in the spindle, backwards movement of leading edge actin filaments must be balanced by polymerization at the tip, and depolymerization at the base of the structure. To understand the assembly of the leading edge two questions it seems particularly important to answer two questions: how are filaments transported backwards, and what is the mechanism of polymerization at the tip of the leading edge? In *Aplysia*, growth cones treated with cytochalasin actin filaments are released from the leading edge, but they continue to move backwards (Forscher & Smith 1988). After withdrawal of the drug they appear to nucleate at the tip of the leading edge, and to immediately resume their backwards movement. These results suggest the model for leading edge organization shown in figure 4*a*, in which actin




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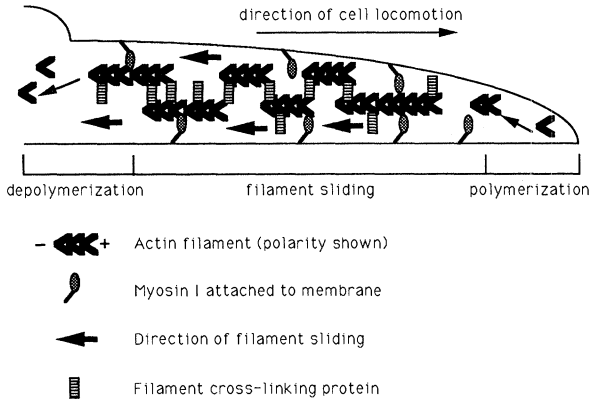


Figure 4. Dynamic organization of the leading edge. The leading edge of motile cells are packed with actin filaments, and dynamics observations show that these slide continually inwards. At steady state this movement must be balanced by polymerization at the tip of the leading edge, and depolymerization at the base. Recent results from photoactivation marking experiments (Theriot & Mitchison 1991) suggest that the tip must nucleate filaments and then release them, and that the filaments move backwards as a cross-linked meshwork. Filament movement may be driven by myosin-I molecules attached to the plasma membrane. Assembly of this array is dependent on the interaction between the myosin and the cross-linked filaments. Note the organizational similarities between this model and the models for half-spindles in figure 3.

filament-myosin-I interactions play a key role. The assembly pathway for this structure is not known, but it will be determined by the properties of the key components: The actin filaments themselves, and the effect of ATP hydrolysis on their polymerization dynamics, the motor protein, the filament cross-linking protein, and the nucleating site. The model shown in figure 4 is closely related to that proposed for half-spindles in figure 3 in terms of overall organization and the role of motor proteins, despite the enormous differences in protein constituents and biological function. This is an encouraging result in our search for unifying principles for understanding the organization of the cytoskeleton.

Recent results in fish keratocytes suggest that the leading edge tip must release filaments after nucleation (Theriot & Mitchison 1991). Thus the substrate for the membrane-attached motors is a cross linked web of short filaments. It is not clear how filament polarity can be established or maintained in such a meshwork, so figure 4 must be regarded as a very preliminary model. I recently addressed the polarity issue in a related system, retraction fibres in PtK2 cells (Mitchison 1991). Cytochalasin addition and withdrawal lead to formation of local domains in the actin-rich cortex, which could have normal or reversed polarity of filament transport. Reversed polarity was inferred from the unusual inwards movement of actin-rich nodules in the fibres, in contrast to their normal outwards movement. This result suggests that the dynamic actin filament assembly could locally self-organize and self-polarize, presumably by interaction between motor proteins and newly polymerizing filaments. This result is significant in that assembly occurs in the absence of any obvious unique organizing centre: reminiscent of astral self-organization in severed melanophore arms discussed above. Interestingly the retraction fibres eventually all reverted to the normal polarity of filament transport, indicating global control of polarity by an unknown mechanism.

## 7. CONCLUSION

In discussing the assembly of some simple microtubule and actin arrays I have invoked key roles for interaction between motor proteins and filaments, and in particular for movement of filaments through the cytoplasm by motor proteins. This movement continues in the steady-state structures, and indeed one could consider that structures such as the spindle and leading edge are continually assembling themselves. Motor proteins provide a flexible way of binding a filament to an adjacent structure. By utilizing ATP hydrolysis to escape the constraints of equilibrium thermodynamics motor proteins can make strong, transient bonds without suppressing the turnover of the polymer by NTP consuming polymerization dynamics. Such bonds can tolerate, or even drive, movement of the polymer, and these movements play a key role in both the assembly and function of the array. The idea that microtubules and actin filaments are held in place in dynamic arrays by interaction with motor proteins is a powerful concept for future thoughts and experiments, both in the spindle, the leading edge and elsewhere.

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