Models of the Collective Behavior of Proteins in Cells: Tubulin, Actin and Motor Proteins

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Abstract. One of the most important issues of molecular biophysics is the complex and multifunctional behavior of the cell's cytoskeleton. Interiors of living cells are structurally organized by the cytoskeleton networks of filamentous protein polymers: microtubules, actin and intermediate filaments with motor proteins providing force and directionality needed for transport processes. Microtubules (MT's) take active part in material transport within the cell, constitute the most rigid elements of the cell and hence found many uses in cell motility (e.g. flagella and cilia). At present there is, however, no quantitatively predictable explanation of how these important phenomena are orchestrated at a molecular level. Moreover, microtubules have been demonstrated to self-organize leading to pattern formation. We discuss here several models which attempt to shed light on the assembly of microtubules and their interactions with motor proteins. Subsequently, an overview of actin filaments and their properties is given with particular emphasis on actin assembly processes. The lengths of actin filaments have been reported that were formed by spontaneous polymerization of highly purified actin monomers after labeling with rhodamine-phalloidin. The length distributions are exponential with a mean of about 7 μm . This length is independent of the initial concentration of actin monomer, an observation inconsistent with a simple nucleation-elongation mechanism. However, with the addition of physically reasonable rates of filament annealing and fragmenting, a nucleation-elongation mechanism can reproduce the observed average length of filaments in two types of experiments: (1) filaments formed from a wide range of highly purified actin monomer concentrations, and (2) filaments formed from 24 mM actin over a range of CapZ concentrations. In the final part of the paper we briefly review the stochastic models used to describe the motion of motor proteins on protein filaments. The vast majority of these models are based on ratchet potentials with the presence of thermal noise and forcing due to ATP binding and a subsequent hydrolysis. Many outstanding questions remain to be quantitatively addressed on a molecular level in order to explain the structure-to-function relationship for the key elements of the cytoskeleton discussed in this review.

1. Cellular Biology Background

In order to understand the mechanisms underlying life-giving and life-sustaining processes biophysical models of the cell must be developed. Whether it be energy

Figure 1. A generalized animal cell during interphase depicting the major cellular organelles including the nucleus, centrosome and radiating microtubules. The endoplasmic reticulum is dotted with ribosomes.

production from food sources, DNA replication, protein folding, intracellular signaling, cellular locomotion, cellular division or a host of other essential functions; the cell has the responsibility of performing these tasks and it is a challenge to model them mathematically.

The fluid contents of a cell are known as the cytoplasm. Contained within the cytoplasm are certain smaller compartments known as organelles which are specialized to perform their respective functions. The cytoplasm is the liquid medium bound within a cell, while the cytoskeleton is the lattice of filaments formed throughout the cytoplasm. Figure 1 is a grossly simplified representation of an animal cell in cross-section, complete with common organelles such as the nucleus that contains the genetic material, the mitochondria that produce readily accessible energy for the cell, the ribosomes that build protein molecules and the elements of the cytoskeleton.

The cytoplasm provides the medium in which fundamental biophysical processes such as cellular respiration take place. Typical constituents of the cytoplasm are listed in Table I [1]. Most cells maintain a neutral pH and their dry matter is composed of at least 50% protein. The remaining dry material consists of nucleic

Table 1. Major constituents of cytoplasm in a typical mammalian cell

Ions	Concentration		
K ⁺	140 mM	protein	200–300 mg/mL
Na ⁺	10 mM	actin	2–8 mg/mL
Cl [−]	10 mM	tubulin	4 mg/mL
Ca ²⁺	0.1 μm	pH	~7.2
Mg ²⁺	0.5 mM		

acids, trace ions, lipids and carbohydrates. Most of the trace ions in the table are positively charged, however the cytoplasm cannot have any overall charge, the difference is made up of the other constituents such as proteins, bicarbonate (HCO₃[−]), phosphate (PO₄^{3−}) and other ions which are for the most part negatively charged.

There is mounting evidence for the existence of two phases of the cytoplasm [2]. These are the so-called liquid and solid phases, sol and gel, respectively. In the solid phase, the major constituents of the cell are rendered immobile while in the liquid phase, the cytoplasm's viscosity does not differ significantly from water [2]. Diffusion within the cytoplasm is affected mainly by macromolecular crowding. In the solid phase, diffusion is slowed by a factor of three relative to diffusive movement in water. Such properties of the cytoplasm seem to be regulated in some sense by the cytoskeleton, but the manner by which this regulation is accomplished is unclear. It is believed that it involves the tangling and detangling of a mesh of various protein filaments [3, 4].

The cytoskeleton is composed of three classes of filamentous protein: microfilaments, intermediate filaments and microtubules [1]. The cytoskeleton is a dynamic structure that undergoes continuous reorganization with the responsibility of defining the cell shape, protecting the cell from changes in osmotic pressure, organizing its contents, providing cellular motility and separating chromosomes during mitosis.

Cell shape is regulated by a complex balance of both internal and external forces exerted by the extracellular matrix. This balance has been described by Ingber [5] and is known as tensegrity. Contractile actin bundles act as molecular cables. These cables exert a tensile force on the cell membrane and the internal constituents of the cell, pulling them all towards the nucleus. Microtubules act as struts that resist the compressive force of the cables. In many cases, it is important to maintain the cell's shape to preserve its functionality. Chen et al. [6] have shown by experiment how cells switch between genetic programs when forced to grow into specific shapes, while King and Wu [7] have shown on theoretical grounds how the cell geometry changes the susceptibility of the cell to electromagnetic fields.

Figure 2. Schematic of an intermediate filament. The filamentous protein tetramer is non-polar and composed of two coiled-coil dimers.

The separation of chromosomes is accomplished by the cytoskeleton's largest constituents, the microtubules. During mitosis, microtubules connect to each of the chromosomes and align them along the cell's equatorial plate. A mysterious balance of forces prevents the microtubules from separating the chromosomes until all the chromosomes have become aligned and division may proceed in unison.

Intermediate filaments have a diameter of about 10 nm and are formed by the polymerization of elongated fibrous protein molecules such as vimentin, keratins, desmins and the nuclear lamins. These molecules are quite unlike the globular molecules, tubulin and G-actin, that form microtubules and microfilaments, respectively. These protein molecules form dimers through an anti-parallel association of two molecules which has a coiled-coil configuration. Consequently, intermediate filaments are non-polar structures. Two dimers associate to form a symmetrical tetramer as depicted in Figure 2. The overlap between dimers allows for the filaments to stretch and, as a result, intermediate filaments are able to withstand large stresses without breaking [1]. The main role of intermediate filaments seems to be related to their structural strength. An extensive network of intermediate filaments surrounds the nucleus forming what is known as the nuclear envelope. The formation and disassembly of the nuclear lamina is regulated by phosphorylation. This allows for the elimination of the nuclear envelope prior to mitosis. The filaments also extend out into the cell periphery where they act to maintain cell integrity and may connect with the cell membrane [1]. Specific cells, such as neurons, have their own distinctive intermediate filaments, known as neurofilaments.

2. Microtubules

Microtubules (MTs) are the filaments of the cytoskeleton with the largest diameter, i.e. 25 nm. They are found in nearly all eukaryotic cells and are polymers of tubulin protein. MTs serve as tracks on which motor proteins may carry materials about the cell and serve as scaffolding to maintain the cell shape since they are among the most rigid structures within a typical cell. Within the cell body, the majority of

the MTs emanate from a centriole. The negative ends of MTs are anchored at these microtubule organizing centers. The MTs *in situ* are interconnected and intraconnected by microtubule associated proteins (MAPs). MAPs have a stabilizing effect on the dynamics of MTs.

Ledbetter and Porter [8] were the first to describe these tubules found within the cytoplasm whose structure has since been well-established by light microscopy, immunofluorescence and cryoelectron microscopy. MTs are polymers formed from two largely homologous globular proteins, α -tubulin and β -tubulin. These two proteins are very closely structurally related and they bind together to form a heterodimer known as $\alpha\beta$ -tubulin. This dimer is the basic subunit which polymerizes to form the MT. The MT is a hollow tube with an outer diameter of 25 nm and an inner diameter of 15 nm (Figure 3). The tube is composed of strongly bound linear polymers, known as protofilaments, that are connected via weaker lateral bonds to form a sheet that is wrapped up to form a tube. Tubulin dimers are cylinders 5 nm in diameter and 8 nm in height. For simplicity, the dimer will be graphically represented by two equally sized spheres representing the α and β -monomers, respectively (Figure 4). The electron crystallography of Nogales et al. [9] has indeed shown that the α and β monomers are nearly identical. However, this small difference on the monomer level allows the possibility of several lattice types. In particular, the so-called MT A and B lattices. Moving around the MT in a left-handed sense, protofilaments of the A lattice [10] have a vertical shift of 4.92 nm upwards relative to their neighbors. In the B lattice [11], this offset is only 0.92 nm because the α and β monomers have switched roles in alternating protofilaments. This change results in the development of a structural discontinuity in the B lattice known as the seam.

In addition to lattice variation, it is also known from experimental observation that the number of protofilaments is variable from one MT to another. Although 13 protofilaments is by far the most common *in vivo*, Chrétien et al. [12] have observed that the protofilament number need not be conserved along the length of an individual MT. This leads to the emergence of a structural defect.

MTs are polar polymers formed by the association of tubulin dimers. When an individual MT is observed, it undergoes periods of almost steady growth interrupted by brief periods of very rapid shortening as illustrated in Figure 5. The transition from a growth period to a shortening period is known as a catastrophe event while the reverse is known as a rescue. The assembly dynamics at each end of the MT differ. The so-called plus end is between three and five times as dynamic as the negative end. That is to say that both the growth and shortening of the positive end of the MT occur at rates at least three times those of the negative end but both ends are dynamic in free MTs. Beyond the individual MT dynamics are the collective ensemble dynamics and the property of their assembly known as *dynamic instability* [13–15]. What is intriguing is how the stochastic individual behavior may result in smooth collective oscillations observed to take place at high tubulin concentrations [16, 17]. The answer is given simply by the application

Figure 3. A section of a typical microtubule demonstrating the hollow interior which is filled with cytoplasm, the helical nature of its construction. Each vertical column is known as a protofilament and the typical MT has 13 protofilaments.

of statistical mechanics to ensemble averaging with the inclusion of microtubule-microtubule interactions that result in nonlinearities. A complete explanation of the above requires an application of the master equation formalism [18, 19] or alternatively, the use of chemical kinetics [20].

The assembly dynamics for an individual MT are stochastic where the rate of shortening is about ten times the rate of growth [21, 22]. The probability of a single MT nucleating, growing, shortening and so on, depends on the local concentration of tubulin with bound GTP, tubulin with bound GDP and other molecules relevant to the assembly process which are found within the cytoplasm. Many theoretical models have successfully reproduced the single MT growth behavior such as that illustrated in Figure 5.

Figure 4. The 13A and 13B MT lattices: (a) in the A lattice, perfect helical patterns are formed. (b) in the B lattice, there is a structural discontinuity known as the seam.

The challenge is to use the same model to explain ensemble dynamics and dynamic instability; and in addition, to explain changes in the dynamics when reaction conditions are altered. Suppose the length of our MT is given by the value of the variable, x , at an instant of time, t . Now, if we discretize the time variable, we want to write a recursion formula for the length of the MT. In the simplest model, there are only two possibilities, the addition of a subunit or the complete collapse of the structure. Addition of a subunit may be modeled as follows:

$$x_{t+1} = x_t + a \quad (2.1)$$

where a is the subunit length. Complete collapse of the microtubule is simply modeled using:

$$x_{t+1} = 0 \quad (2.2)$$

Figure 5. The growth of a single microtubule is erratic. Slow, steady growth is interrupted by large catastrophe events at about 8 s and 46 s while a rescue event occurs between two catastrophe events at about 26 s.

Now suppose that the probability of the addition of a tubulin subunit is p , then

$$x_{t+1} = r(x_t + a) \quad (2.3)$$

where

$$r = \begin{cases} 0 & \text{if } s > p \\ 1 & \text{if } s \leq p \end{cases} \quad (2.4)$$

and s is a random number between 0 and 1. Even a crude model such as that just described may successfully capture the essential dynamics of MT growth when compared by Hurst analysis or a recursive map technique [23]. However, it lacks any predictive power given that individual collapse events are random.

When an aggregation of MTs is studied, some collective properties develop. The most obvious is the phase transition behavior of the system in that certain conditions lead to overall disassembly and largely a pool of tubulin dimers, while other conditions promote self-assembly of tubulin in MTs. Phases of MT polymerization are determined largely by the temperature and concentration of tubulin. For a given temperature, some critical concentration of tubulin is required in order to keep MTs from disassembling. Fygenson et al. [24] have discussed this phase transition and Sept [23] has further classified the non-disassembly phase into one where MTs assemble but are not nucleated and one where MTs are both nucleated and assembled. For a collection of MTs in an assembly phase, dynamic instability is the term given to the observation that MTs are growing in the immediate vicinity of other MTs which are shortening. Dynamic instability has been observed *in vivo* and *in vitro* and highlights both the non-equilibrium nature of the problem

and the stochastic nature of individual MT growth. Despite these observations, ensembles of MTs show collective oscillations given suitable conditions. Specifically, when the concentration of assembled tubulin is measured, it is observed to undergo smooth oscillations which are damped out as the energy source of GTP, is depleted. Sept et al. [20] have modeled the assembly dynamics from a chemical reaction-kinetics standpoint and found good agreement with the experimental data.

The principal elements in the model of Sept et al. [20] can be summarized by the equations which follow. For simplicity, the MT is considered as a linear polymer rather than an object comprised of 13 protofilaments. We shall denote a MT of length n subunits by MT_n . Note that in solution, the free tubulin subunits may be bound to either GTP or GDP at their exchangeable nucleotide site and shall be denoted T_{GTP} and T_{GDP} , respectively, only tubulin bound to GTP is able to polymerize. The simple reaction set consists of addition, nucleation and catastrophic collapse:

For simplicity, we have again assumed that all collapses are complete and to keep the model of MT assembly simple, one normally selects a specific number of dimers, n , that will be required for nucleation. The exact value of this choice does not seem to have a large impact on the dynamics as long as it is relatively small e.g. six. The first of the preceding equations is reversible and a rate constant for the back reaction is considered in general. This is not required for this discussion. These equations can also be supplemented by the reactivation of tubulin, to make it assembly competent, which will occur when the concentration of GTP is high:

The free energy change associated with this reaction is less than the free energy change of GTP hydrolysis in solution and the difference is attributed to a structural change in the tubulin dimer. It is this conformational change which presumably makes assembly possible. The rate constants of these reactions, k_j , should also be in agreement with the probabilities assigned to corresponding reactions in the individual MT model. Furthermore, temperature dependence can be built into the rate constants using empirical data on the free energy of reactants and products. When at least one auto-catalytic reaction is added to the system, to provide a non-

linear element, the dynamics change significantly. Consider an induced catastrophe event,

incorporated into the model, this reaction has been able to reproduce oscillations observed *in vitro* caused by either temperature jumps or the injection of GTP into the system. It also identifies domains where tubulin is incorporated into MTs steadily and regimes where disassembly is favored. In addition, the model can reproduce the spatial pattern of MT assembly which are observed within cells simply by including a diffusive term in the equations of the assembly dynamics. This allows the model to be compared to the experiments performed independently by the Tabony [25] and Mandelkow [17] groups. Agreement has been found between this model and those empirical results. Generally, the statistical dynamics of MT assembly are now believed to be well understood except for the physical mechanism that triggers the catastrophes and the molecular origin of the rescue events.

3. Biochemistry of Tubulin

The tubulin that polymerizes to form MTs is actually a heterodimer of α -tubulin and β -tubulin. These two proteins are highly homologous and have 3D structures which are nearly identical. Although the similarity of α -tubulin and β -tubulin had long been suspected, the fact that tubulin has resisted crystallization for about 20 years prevented confirmation of this hypothesis until very recently. Nogales et al. [26] were able to perform cryoelectron crystallography on sheets of tubulin formed in the presence of zinc ion. Figure 6 has been produced from the Nogales data, via the protein data bank (PDB entry: 1tub), using MOLSCRIPT [27] and it makes clear the similarities between the two proteins. Each is composed of a peptide sequence more than 400 members long which is highly conserved between species. The amino acid sequences for these proteins may be compared given the data in Table II which lists the conventional one and three letter codes for the 20 naturally-occurring amino acids.

Codes should be read from left to right and spaces are inserted each 10 residues for clarity. Based on the charge of the amino acid, the amino acids may be classified into 3 groups: those with a positive charge, those with a negative charge and the neutral residues. The size of the residue and its ability to react with other amino acids will affect protein folding and consequently function. The sequences of a few representatives samples of tubulin have been retrieved from the Swiss-Prot protein sequence database [28] and are shown in Table III.

In humans, six α isotypes and seven β isotypes of tubulin are found. Although the sequence of amino-acids is highly conserved overall, certain regions of α_1 -tubulin show divergence from α_2 -tubulin and so on. Recent studies have shown that the differences in α -tubulin are more subtle than those in β -tubulin [29]. Table IV

Figure 6. A diagram of the tubulin molecule produced from the Nogales et al. electron crystallography data [26] shows the similarity between the α -subunit (upper half) and β -subunit (lower half). The stick outlines near the base of each subunit indicate the location of GTP when bound.

Table II. The twenty naturally occurring amino-acids are listed along with their one and three letter codes as well as whether they have a polar character and whether they are charged. * Histidine has a pKa of 6.5 and consequently will be protonated and positively charged should the pH of the cytoplasm dip below this value

1 Letter	3 Letter	Amino acid	Polar	Charge
A	Ala	Alanine		
C	Cys	Cysteine	yes	
D	Asp	Aspartic Acid		-e
E	Glu	Glutamic Acid		-e
F	Phe	Phenylalanine		
G	Gly	Glycine		
H	His	Histidine		+e*
I	Ile	Isoleucine		
K	Lys	Lysine		+e
L	Leu	Leucine		
M	Met	Methionine		
N	Asn	Asparagine	yes	
P	Pro	Proline		
Q	Gln	Glutamine	yes	
R	Arg	Arginine		+e
S	Ser	Serine	yes	
T	Thr	Threonine	yes	
V	Val	Valine		
W	Trp	Tryptophan		
Y	Tyr	Tyrosine	yes	

gives a comparison between the main β -tubulin isotypes in cows. The location of cells expressing that particular variant of tubulin are given along with the homology in percent with β_1 which is derived from a comparison of primary sequences.

There exist at present several different studies which confirm the existence of different conformational states of tubulin; however there is little quantitative information on the structural changes. The first indication that there was more than a single conformational state of tubulin came simply from observing the assembly of MTs as shown in Figure 7. Tubulin bound to GTP, or assembly-ready tubulin, binds together and forms straight protofilaments. However, polymerized GDP-bound tubulin forms curved protofilaments that sometimes close up on themselves to produce oligomer rings. Another manifestation of the different conformations came when Hyman et al. [30] measured the energy released from a slowly hydrolyzable analogue of GTP known as GMPCPP. The energy released when the

Table III. The amino acid sequence of human α_1 - and β_1 - tubulin show a high degree of homology. The sequence is given along with the total number of amino acids and the molecular weight of the molecule

Human α_1 -tubulin amino-acid sequence (451 amino acids, 50157 Da)

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MRECISIHVG QAGVQIGNAC WELYCLEHGI QPDGQMPSDK TIGGGDDSFN TFFSETGAGK
HVPRAVFVDL EPTVIDEVRT GTYRQLFHPE QLITGKEDAA NNYARGHYTI GKEIDLVLVD
RIRKLADQCT RLQGFVLFHS FGGGTGSGFT SLLMERLSVD YGKKSKEFS IYPAPQVSTA
VVEPYNSILT THTTLEHSDC AFMVDNEAIY DICRRNLDIE RPTYTNLNL IGQIVSSITA
SLRFDGALNV DLTEFQTNLV PYPRIHFPLA TYAPVISA EK AYHEQLSVAE ITNACFEPAN
QMVKCDPGHG KYMACCLLYR GDVVPKDVNA AIATIKTKRT IQFVDWCPTG FKVGINYQPP
TVVPGGDLAK VQRAVCMLSN TTAIAEAWAR LDHKFDL MYA KRA FVHWYVG EGMEEGEFSE
AREDMAALEK DYEEVGHVSV EGE EEEEE EEE Y
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Human β_1 -tubulin amino-acid sequence (444 amino acids, 49759 Da)

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MREIVHIQAG QCGNQIGAKF WEVISDEHGI DPTGTYHGDS DLQLDRISVY YNEATGGKYV
PRAILVDLEP GTMDSVRSGP FGQIFRPDNF VFGQSGAGNN WAKGHYTEGA ELVDSVLDVV
RKEAESCDCL QGFQLTHSLG GGTGSGMGT LISKIREEYP DRIMNTFSV PSPKVS DTVV
EPYNATLSVH QLVENTDETY CIDNEALYDI CFRTLRLTTP TYGDLNHLVS GTMECVTTCL
RFPGQLNADL RKLAVNMVPF PRLHFFMPGF APLTSRGSQQ YRALTVPDLT QQVFDKNMM
AACDPRHGRY LTVAAVFRGR MSMKEVDEQM LNVQNKNSY FVEWIPNNVK TAVCDIPPRG
LKMAVTFIGN STAIQELFKR ISEQFTAMFR RKAFLHWYTG EGMDEMEFTE AESNMNDLVS
EYQQYQDATA EEEEDFGEEA EEEA
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Table IV. Localization and homology of bovine β -tubulin

Isotope	Localization	Homology	Abundance in brain (%)
β_1	everywhere, thymus	100.0	3
β_2	brain	95.0	58
β_3	brain, testis, tumours	91.4	25
β_4	brain, retina, trachea	97.0	11

analogue was bound to tubulin was reduced compared to the quantity of energy released by the free molecule. The speculation was that the difference must go into changing the conformation of the tubulin dimer. In addition, when GMPCPP was bound to tubulin in a MT, the energy release was further diminished.

Again the assumption was simply that the energy difference is stored in the MT lattice and may be released upon disassembly or catastrophe events. More recently, Hyman et al. [31] reported on the observation of a structural change accompanying GTP hydrolysis. The change which was discovered was a length change in the monomer spacing from 4.05 nm to 4.20 nm. Thus energy may be stored locally as lattice deformation. This 4% change in tubulin's length results in a new moiré pattern when the MTs are imaged by electron cryomicroscopy and different pos-

Figure 7. The global conformational change that occurs between assembly and disassembly phases is illustrated. The once straight protofilaments become curved after the individual tubulin subunits undergo a structural change.

itions of equivalent peaks between the X-ray crystallographic diffraction patterns of GDP-MTs and GMPCPP-MTs. Tran et al. [22] argued that three conformational states exist with a metastable intermediary state between growing and shrinking conformations. Tran et al. seems to believe that these may be tubulin with GTP bound, with GDP $\cdot$ P_i bound and finally with GDP bound at the exchangeable site. This is similar to the hypothesis of Semenov in his review of MT research [32]. It therefore appears that in addition to the multitude of α and β isotypes and the numerous post-translational modifications, tubulin may also exist in several conformations.

Gittes et al. [33] have measured MT flexural rigidity by thermal fluctuations in the MT shape and found interestingly that it is increased when MTs are treated with MAP τ , the MTs are most stabilized by preventing GTP hydrolysis. Thus in addition to the already discussed fact that hydrolysis of GTP can result in destabilizing the MT with respect to catastrophic disassembly, it also makes the polymer more flexible. It is interesting to note that the application of taxol, that reduces MT assembly dynamics, actually reduces the mechanical rigidity of MTs [34].

The measured flexural rigidity for MTs corresponds to a Young's modulus of 1.4 GPa in normal MTs and can be raised by more than a factor of two to 3.4 GPa when hydrolysis is prevented [35]. In an independent study, a Young's modulus of 4.6 GPa was derived from the buckling of microtubules that required the application of a 10 pN force [36]. The Young's modulus of F-actin has also been measured and is of the same order of magnitude though conflicting measurements make actin both more and less rigid than MTs [37, 38]. This number is sufficiently large to

Figure 8. The 2-stranded helix of an actin filament. Each of the F-actin strands has a polarity indicated by the arrows (each sphere represents a monomer of G-actin).

imply that the cell must rely on depolymerization rather than deformation to effect a shape change.

This measured value of Young's modulus indicates that bending of a protofilament into an arc with radius of curvature of 20 nm, as observed by Mandelkow et al. [21], would require about 0.14 eV/dimer (3.2 kcal/mol) which is slightly less than the energy of GTP hydrolysis, 0.22 eV (5.1 kcal/mol). This is believed to explain the difference observed in the free energy release when free floating GTP is hydrolyzed compared to the hydrolysis of GTP bound to a MT.

4. Actin Filaments

Microfilaments have the smallest diameter of all the cytoskeletal fibers and are the most common. They are composed of a protein known as actin and consequently are often referred to as actin filaments. Actin is not only found within all eukaryotic species, but in many cells it is the most abundant protein [1]. Filamentous actin, or F-actin, is a polymer of the protein known as globular actin or G-actin. These strands are capable of forming both stable and labile components within cells. The polymerization of F-actin from G-actin is a largely monotonic process that is dependent on ATP. The F-actin assembles according to a standard nucleation, elongation mechanism. Once assembled, microfilaments have a diameter of about 8 nm (Figure 8).

Microfilaments are found linked together by actin-associated proteins and congregate into one of three major forms (Figure 9). The polymerization dynamics and filament organization of actin has been modeled by Edelstein-Keshet et al. [39, 40]. In the configuration of parallel strands, microfilaments often form the core of microvilli, while in an anti-parallel arrangement, actin may act in conjunction with myosin to bring about muscle contraction in the presence of ATP. Microfilaments are often found with the lattice configuration near the leading edge of growing or motile cells where they provide greater stability to the newly formed region. New actin filaments are nucleated at the leading edge of the cell's growth and trailing microfilaments are disassembled [1, 9].

The pioneering work of Oosawa and Asakura [41] established that spontaneous polymerization of actin monomers requires an unfavorable nucleation step followed by rapid elongation. Elongation is more accessible experimentally than

Figure 9. The three main arrangements of microfilament aggregation which form with the aid of actin-crosslinking proteins: (a) parallel bundles, (b) contractile bundles and (c) gel or lattice like arrangement.

nucleation, so it is much better understood, with a complete set of rate constants for association and dissociation of ATP-actin and ADP-actin subunits at both ends of the filament [42]. Nucleation has been studied by observing the complete time course of spontaneous polymerization as a function of actin monomer concentration and then finding a set of reactions and rate constants that fit these kinetic data [43–46]. These studies concluded that actin dimers are less stable than trimers, which are the nucleus for elongation.

In addition to nucleation and elongation, Oosawa et al. [41] established that actin filaments can break and anneal end to end. Inclusion of a fragmentation reaction improved the fit of nucleation-elongation mechanisms to the observed time course of polymerization under some conditions [47, 44, 48]. For annealing, there has been kinetic evidence both for [49–50] and against [51] its role in length redistribution after sonication, but the most direct evidence from electron micrographs supports rapid annealing [52].

The basis of the standard nucleation-elongation model is one or more unfavorable nucleation steps followed by more favorable elongation [41]. Earlier models for actin polymerization showed that the critical size for the nucleus is somewhere between a dimer and a trimer, and that the number of explicit nucleation steps does not affect the results of the model [43–46]. It was also found that choosing a critical nucleus larger than three or four monomers did not affect the results of the model. With these considerations in mind, a simple five-step model was proposed [75]:

where A represents the concentration of actin monomers, A_i for a filament with i actin monomers and N represents the concentration of all longer filaments. The rate constants for the last reaction have been experimentally measured [42] and lead to the correct critical concentration ($C_c > 0.1 \mu m$), but the other rate constants are only approximations from kinetic simulations, chosen to reproduce the time course of polymerization over a limited range of actin monomer concentrations. Such polymerization curves have already been published [43–48]. Filaments longer than 4 subunits are assumed to be stable and the back-reaction rate k_{-4} is set to zero. This is appropriate, since most filaments are much longer than four monomers. The coupled first-order differential equations that arise from the set of reactions above are ‘stiff-equations’ due to the large differences in the forward and back reaction rates. Because of this, a semi-implicit scheme was used to solve the system of equations. This set of equations produces correct polymerization curves, but the average length as a function of actin concentration is completely incorrect. The mean lengths of the observed filaments are almost independent of the initial concentration of actin monomers, while this simple model predicts a mean length with quite a different behavior, especially at high concentrations of actin (see Figure 10). To solve this problem, additional processes are added to the model below.

The average length is simply given by the total amount of polymer divided by the total number of filaments formed. Since the reaction above produces the correct time course and extent of polymerization but not the correct average length, the simple model produces the incorrect number of filaments. The average length is too low, so the actual mechanism must produce fewer filaments. The number of filaments in the system are represented as the number of filaments that are formed by the addition of a monomer onto a polymer A_4 . Since there is no back reaction rate for this process, the equation for the change in N , the filament number concentration, is simply

$$\dot{N} = k_{+}AA_4 \quad (4.2)$$

Figure 10. Dependence of the number average length (L_n) of actin filaments on the purity and monomer concentration during polymerization. The actin monomer concentration during polymerization was varied as indicated [75]. The filaments formed during each 30 msec interval were allowed to elongate over the succeeding course of the reaction. (●) Singly gel filtered actin. (▲) Doubly gel filtered actin. Theoretical lengths calculated by kinetic simulation using the nucleation-elongation model and the rate constants in the text without annealing/fragmenting (---) and with both annealing and fragmenting (—).

Addition of monomers to existing filaments increases the concentration of polymerized actin, but does not increase N . To include filament annealing, we should also consider the breakup of a filament or fragmentation. To include filament annealing and fragmenting in the reaction scheme, the following reaction is added:

where k_a represents the annealing rate and k_f the fragmentation rate of a filament. Since two filaments are joined to form a new filament the annealing rate has a quadratic dependence and the new equation for the change in N is

$$\dot{N} = k_+ A A_4 - k_a N^2 + k_f N \quad (4.4)$$

The addition of a monomer to the fast growing barbed end of a filament is a diffusion limited process, so it is reasonable to assume that annealing of two filaments

is also limited by diffusion, since similar bonds are formed and filaments diffuse more slowly than monomers. Murphy et al. [52] performed ultrasonification experiments to estimate the rate of annealing. Following sonification, they found an initial rate constant for annealing of $k_a = 10 \mu\text{M}^{-1} \text{s}^{-1}$ for very short filaments, but as annealing progressed and the filaments became longer, the rate fell off rapidly with time. Kinoshita et al. [49] found the rate of annealing after sonication value of $2.2 \mu\text{M}^{-1} \text{s}^{-1}$. If k_a is diffusion limited, the diffusion that we need to consider is the relative diffusion of the two filaments. It is difficult to write an accurate expression for the movement of an individual polymer embedded in a polymer gel. The most common treatment uses the reptation idea [54–55]. In fact, actin filaments above 40 nM filament concentration, have been shown to exhibit reptation motion [56, 57]. The transverse diffusion is controlled by the mass of the polymer and the density of the polymer network, but the diffusion constant along the tube is inversely proportional to the length of the filament [57] having the form

$$D_{//} = \frac{k_B T}{\zeta L} \quad (4.5)$$

where ζ is the friction coefficient. The annealing rate constant k_a is therefore chosen to be proportional to $D_{//}$, namely

$$k_a = k'_a / L \quad (4.6)$$

where all of the constants are absorbed in the variable k'_a .

Erickson [58] estimated the fragmentation rate to be in the neighborhood of $k_f \approx 10^{-8} \text{s}^{-1}$. The rate is very low, since it involves breaking more than one bond in the filament, but since it is equally probable between any given pair of monomers, the rate should be proportional to the length of the filament. The gel network that is formed may also affect the amount of fragmentation. Within the gel, each individual filament is constrained by its neighbors or, in reptation terms, by the filaments that make up the reptation tube. Doi [55] showed that the number of rods within a distance b of a given rod is given by bL^2N where L is the filament length and N is still the filament concentration. Choosing an additional fragmentation rate proportional to this quantity results in:

$$k_f = k'_{f1}L + k'_{f2}L^2N \quad (4.7)$$

where once again all the constants are absorbed into the two factors k'_{f1} and k'_{f2} . Subsequently, the constant k'_{f2} is treated as a free parameter in a minimization scheme.

Replacing k_a and k_f in Eq. (3.4) yields

$$\dot{N} = k_+AA_4 - k'_a \frac{N^2}{L} + k'_{f1}LN + k'_{f2}L^2N^2 \quad (4.8)$$

The amount of polymerized protein in the system is given by the expression

$$P = A_0 - A - 2A_2 - 3A_3 - 4A_4 \quad (4.9)$$

where A_0 represent the initial actin concentration. Using this expression, the average length of a filament is given simply by $L = P/N$ and we get

$$\dot{N} = k_+ A A_4 - k'_a \frac{N^3}{P} + k'_{f1} P + k'_{f2} P^2 \quad (4.10)$$

Note that as the actin concentration is increased and more protein polymerizes, the rate of annealing decreases while the fragmentation rate increases. The values for the rate constants depend on the actin concentration and the filament density and length.

When actin is purified from cells such as skeletal muscle, some actin-associated proteins remain in the sample, including the capping protein CapZ. A reaction for capping filaments can be written as

where N^* now represents a filament that is capped at the barbed end preventing monomer addition or dissociation as well as annealing. The constants for the above reaction are $k_{z+} \approx 3.5 \mu\text{M}^{-1} \text{s}^{-1}$ and $k_{z-} \approx 3 \times 10^{-4} \text{s}^{-1}$ [59]. With this added reaction, the total number of filaments in the system is $N + N^*$ and the average length is now given by

$$L = \frac{P}{N + N^*} \quad (4.12)$$

A capped filament can still fragment, but a capped filament can only anneal with an uncapped filament, since this requires at least one free barbed end. Since two uncapped filaments can anneal in two ways, but capped and uncapped filaments have only one method of annealing, the rate of annealing is half the original value. With these additions, the equations for the change in N and N^* become

$$\dot{N} = k_+ A A_4 - k'_a \frac{N(N + \frac{1}{2}N^*)}{L} + k'_{f1} P + k'_{f2} P^2 - k_{z+} N + k_{z-} N^* \quad (4.13)$$

$$\dot{N}^* = k_{z+} N - k_{z-} N^* \quad (4.14)$$

where L is given by Eq. (4.12)

During the polymerization of actin monomers, the rates for annealing and fragmentation will change with time, since they depend on the filament length and density. The rate of annealing immediately following sonication has been measured in the range $2.2\text{--}10 \mu\text{M}^{-1}\text{s}^{-1}$ [49, 52]. Since $k_a = k'_a/L$ and $L > 30$ (subunits) following sonication, we chose $k'_a = 300 \mu\text{M}^{-1}\text{s}^{-1}$ so that $k_a = 10 \mu\text{M}^{-1}\text{s}^{-1}$ for $L = 30$. The factors k'_{f1} and k'_{f2} were treated as free parameters for fitting the two curves for mean polymer length vs. actin concentration for singly and doubly

filtered actin. The rate constants for the nucleation-elongation steps were fixed at the values previously used to reproduce polymerization curves. Singly filtered actin has been estimated to contain about one part in 50000 CapZ, and other proteins, such as severing proteins, could also be present at higher concentration in the singly gel filtered than doubly gel filtered actin. These latter proteins cut actin filaments, and since they act with equal probability along the length of a filament, the severing rate is proportional to L . Thus, the presence of low concentrations of severing proteins results in a larger value for k'_{f1} for singly filtered actin. It is also possible that the CapZ that is present in the single filtered actin in some way increases the fragmentation rate, perhaps by changing the structure of a filament when it binds. With these points in mind, the values chosen were: $k'_{f2} = 1.8 \times 10^{-8} \mu\text{M}^{-1}\text{s}^{-1}$, and $k'_{f1} = 2.0 \times 10^{-7} \text{s}^{-1}$ and $1.1 \times 10^{-8} \text{s}^{-1}$ for the single and doubly gel filtered actin respectively [75]. The value of k_a will change as the average filament length increases. In agreement with the experimental observations of Murphy et al. [52], the annealing rate constant falls off rapidly with time following sonication (see Figure 11). For k_f , the term with k'_{f1} only contributes about 2% towards the value of k_f in the doubly filtered actin. In the singly filtered case, perhaps due to the presence of other severing proteins, k'_{f1} and k'_{f2} contribute roughly equally to the fragmentation rate. This model including annealing and fragmentation agrees with the observed lengths over a wide range of starting actin monomer concentrations (Figure 10). In these calculations [75] the ratio of CapZ to actin in the singly filtered actin is about 1:50 000 and zero in the doubly filtered actin. Note that a 1:50 000 concentration ratio corresponds to about one CapZ molecule for every twenty filaments. It is interesting to see the influence such a small amount of CapZ has on the average filament length. This effect was predicted by Casella et al. [60].

5. Motor Proteins

Two of the principal motor proteins that attach to MTs are kinesin and dynein. While kinesin moves towards the plus end of a MT, dynein is negative end directed. Each of these proteins consists of a globular head region and an extended coiled-coil tail section as shown in Figure 12. The study of motor proteins including myosin, which has a similar structure, has shown that while the long tail is able to increase the force generated by the molecular motor, the essential components for force generation are located within the globular head. Models of motor protein movement can essentially be divided into two different mechanisms, those with diffusion and those with a *power stroke* [61]. The efficient propagation of these proteins, often with pairs such as kinesin and dynein moving in opposite directions simultaneously and seemingly avoiding collisions led to the proposal that they may be directed by electrostatic interactions with the MT [62] and it is interesting to note that the binding of kinesin to MTs has since been shown to be primarily electrostatic [63].

Figure 11. The rate of annealing as a function of time in a sonication simulation, with an initial actin monomer concentration of $24 \mu\text{M}$. The system was allowed to reach equilibrium at which point the filament concentration N was manually set so that the average length L was equal to 30 subunits, a value consistent with what is observed in sonication experiments, and the system was again allowed to equilibrate [75].

The motion of the motor protein in either model is accomplished through the hydrolysis of ATP. The diffusion models require an oscillating potential that is presumably driven by a conformation change of the motor-MT bond. Activation of the complex by ATP leads to a potential that is relatively flat. Diffusion occurs in this state and once the potential reverts to its asymmetric form, the geometry of the potential is such that forward propagation of the motor protein is favored. A review of such schemes may be found thanks to Jülicher et al. [64]. In the power stroke models by contrast, it is the motor protein whose structure changes. Sometimes such models are envisioned as models of proteins walking since one imagines the protein to stretch and bind at a second location before relaxing to its original conformation when the 'back leg' releases its grip on the MT to start the process anew once additional ATP has arrived. The motor protein has two or more distinct states where at least one conformational change occurs and is driven by ATP hydrolysis as has been experimentally demonstrated for myosin by Spudich et al. [65]. Phosphorylation of the motor protein such as in the case of myosin

Figure 12. A motor protein is shown 'walking' along a MT protofilament.

may lead to subsequent conformational changes. Thus the protein may be viewed as walking along the MT powered by ATP. It is interesting to note that the use of GTP to control MT dynamics and ATP to control motor protein motion along MTs, allows a cell to have control over both the cars (motor proteins) and the track (MTs) individually.

M. Buttiker [66] and R. Landauer [67] proposed a model for molecular motors which results in uni-directional motion of a molecule along a quasi-one-dimensional protein fiber. This model was essentially a generalization of the thermal ratchet model due to R. Feynman [68]. The key ingredient of this mechanism is an inhomogeneous temperature distribution with the periodicity of the filamentous structure to which the motor binds. However, it is well known from the theory of thermal conductivity that temperature variations over tens of nanometers are equilibrated on time scales of microseconds. Therefore, models of this type appear unrealistic. Recent attempts at explaining the motor protein behavior use isothermal ratchets in which the motor is subjected to an external potential that is periodic and asymmetric. In addition, a fluctuating force $F(t)$ is acting on the motor. A recent review article on this topic by F. Jülicher [64] distinguishes three classes of such models, namely:

(i) A Langevin-based approach [69] with the equation of motion given by

$$\xi \frac{dx}{dt} = -\partial_x W(x) + F(t) \quad (5.1)$$

where x is the direction along the protofilament axis, ξ is the friction coefficient, $W(x)$ is the potential due to the fiber and $F(t)$ is the fluctuating force whose time average is zero, i.e.:

$$\langle F(t) \rangle = 0 \quad (5.2)$$

This fluctuating force is typically related to the kinetics of ATP binding to the motor protein and a subsequent hydrolysis of ATP into ADP that provides the excess energy allowing the protein to unbind from the filament.

(ii) An approach in which the potential fluctuates in time [70]

$$\xi \frac{dx}{dt} = -\partial_x W(x, t) + F(t) \quad (5.3)$$

where the Gaussian (white) noise term $F(t)$ satisfies the fluctuation-dissipation theorem and the typical conditions are imposed:

$$\langle F(t) \rangle = 0 \quad (5.4)$$

$$\langle F(t)F(t') \rangle = 2\xi T \delta(t - t') \quad (5.5)$$

(iii) A generalized model with several internal states of the particle which is described by the Langevin equation that depends on the state $i=1, \dots, N$, i.e.

$$\xi_i \frac{dx}{dt} = -\partial_x W_i(x, t) + f_i(t) \quad (5.6)$$

The Gaussian noise term also satisfies the fluctuation-dissipation theorem via:

$$\langle f_i(t) \rangle = 0 \quad (5.7)$$

$$\langle f_i(t)f_j(t') \rangle = 2\xi T \delta(t - t')\delta_{ij} \quad (5.8)$$

A more convenient and elegant approach is through the Fokker-Planck formalism [71] for the probability distribution function $P(x, t)$ that describes the motion of the motor in a statistical manner. Here, $D^{(1)}$ is the drift coefficient and $D^{(2)}$ the diffusion coefficient.

$$\frac{\partial P}{\partial t} = \left[-\frac{\partial}{\partial x} D^{(1)}(x) + \frac{\partial^2}{\partial x^2} D^{(2)}(x) \right] P(x, t) \quad (5.9)$$

So far, models of the above types have been fairly successful in representing the gross features of the experimental data obtained [72]. However, the use of a hypothetical potential and a number of arbitrary parameters casts some doubt on the usefulness or even correctness of such models.

So, can this model of motor protein movement be successfully applied to the problem of chromosome separation? The prevailing model of how the MTs that form the mitotic spindle separate chromosomes is that motor proteins attach to the chromosomes and the distal ends of spindle MTs. The force generation is thereby developed locally by the motor proteins and cannot depend on the length of the MT. However, the force attracting the chromosome to the pole has been shown to increase as the spindle shortens [73]. Only a repulsive force from the pole or generated by the tubulin subunits of the microtubule would solve this difficulty. However, if it is the motor proteins that are responsible for the force generation, it

must be explained how it is that the chromosome movement does not require ATP [74]. Therefore if the motor proteins are important for more than simply binding the chromosome, this has not been shown to date and the relationship between force and the length of the spindle MTs must still be explained.

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