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Review of Cytology

EDITED BY

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ASSISTANT EDITOR
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I. Introduction

The cytoskeleton of all eukaryotic cells must have several special properties. *In vitro* the polymers that comprise the cytoskeleton are of indefinite length, while *in vivo* length and orientation are controlled in some manner. In the cell the cytoskeletal filaments interact with each other, the cell membrane, and other cytoplasmic organelles. These filaments are often under compressive or extensive forces owing to their involvement in the motility of the whole cell or of organelles within the cell. The ar-

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rangement of cytoskeletal filaments is often very dynamic. They must change their organization with cell growth, and undergo particularly major changes during cell division. The filament arrays are also drastically remodeled during cell differentiation, and there is a major reorganization of local parts of the cytoskeleton during cell movement and phagocytosis.

It is the goal of modern cell biology to explain the properties of the whole cell in terms of the biochemical properties of the individual components. In the case of the cytoskeleton this will be a difficult undertaking because many of the properties depend on a large number of specific interactions spanning large distances in the cell. Many of these interactions are mediated by specific proteins, of which more than 50 have been identified for the actin system alone. However, the very dynamic nature of the cytoskeleton encourages one to believe that the detailed history of each cell may not be necessary for describing the properties of the filamentous arrays within cells, and that much can be explained in terms of the energetics and kinetics of elementary processes of spontaneous assembly. This is demonstrated clearly by the ability of the microtubule system to regrow a normal array after drug-induced depolymerization (Brinkley *et al.*, 1976; Osborn and Weber, 1976), or the ability of actin arrays to reform cable patterns after trypsinization or viral induced disorganization (Lazarides, 1976; Pollack *et al.*, 1975). Although many of the detailed properties of these systems will require knowledge of the specific properties of many individual associated proteins, many important results can be obtained by looking at the pure polymers themselves. This is partially because the effects of associated proteins can be understood in terms of their modifying existing properties of the polymers in rather simple ways, such as by binding to one end or the other, binding to the monomer but not the polymer, binding to the polymer but not the monomer, or by cross-linking the polymer. Thus the rules for assembly of the polymer itself can be extended easily to include many of the properties of associated proteins. We have to expect, however, that effector molecules may be found that will alter considerably the chemistry of the polymers and which could require major changes in existing theoretical treatments.

Two of the major filamentous systems in the cell, actin filaments (microfilaments) and microtubules, share several interesting biochemical, physical chemical, and cellular properties. The third major filamentous system, intermediate filaments, is less well studied, but seems much less dynamic and may assemble by mechanisms different than actin and tubulin (Steinert *et al.*, 1978; Renner *et al.*, 1981). Both actin and tubulin assemble from globular subunits into helically ordered surface lattices in semiinfinite linear polymers (see reviews by Kirschner, 1978; Timasheff

and Grisham, 1980; Pollard and Weihing, 1974; Clarke and Spudich, 1977). Both show structural and kinetic polarity so that the two ends are different. Both bind and hydrolyze nucleoside triphosphates (ATP for actin, GTP for tubulin). The kinetics of assembly from purified subunits for both systems can be described roughly in terms of the condensation polymerization model of Oosawa and Kasai (1971a), with a lag phase for nucleation, an exponential phase for growth, and a slow phase for length redistribution.

In the cell both actin filaments and microtubules are found in many different locations. The ordered array of actin filaments in muscle is a special case, but the different spatial organization of actin in different regions of other cells is well documented (Lazarides, 1976; Heuser and Kirschner, 1980; Small, 1981). For microtubules, the highly ordered arrays in protozoa, neurons, and platelets are special examples and the dynamic and stereotyped arrays during mitosis of all cells are well known (see Weber and Osborn, 1979). In the case of microtubules, several well-known organizing centers have been identified such as the centriole, basal body, phragmoplast in plants, and the kinetochore of metaphase chromosomes. In actin the only known nucleating structure is a special structure in echinoderm sperm called the actomere (Tilney, 1976), but several proteins have been described which could serve to nucleate actin polymerization (for recent papers see 1981 Cold Spring Harbor Symposium).

Both actin and microtubules are involved in motility and must undergo either extensive or compressive forces that could affect the properties of the filaments themselves. This is again clear for actin in muscle but also in the contractile ring of dividing cells. For microtubules the best examples are in mitosis where the poles are moved apart relative to each other and the chromosomes are moved relative to the poles. In some cases movement in these filamentous systems may be explained solely by the forces of polymerization and depolymerization (Inoue and Ritter, 1975). However, in other cases where other proteins act on the filaments (e.g., myosin on actin filaments or dynein on microtubules), it follows that these external forces should cause changes in the polymerization of the filaments. (We are *not* referring here to muscle or cilia.) These forces, for example, could deform or compress the filament and alter the association of the filament with free subunits.

Until recently no general theoretical treatment of the polymerization of microtubules and actin filaments had been given that takes into account two very important properties: forces acting on the polymers and simple interactions of the ends of the filaments with other components. However, recently such problems have become even more interesting with the further experimental and theoretical investigations of the role of nucleoside

triphosphate hydrolysis in assembly. This added chemistry of tubulin and actin complicates considerably the energetics and kinetics of assembly, but more importantly allows for several interesting and unique properties of the system that may be very important in specifying the position of these filaments in the cell and their capacities for doing work.

Although it was recognized early that tubulin and actin bind and hydrolyze nucleoside triphosphates (Weisenberg *et al.*, 1968; Straub and Fever, 1950; Oosawa and Kasai, 1971b) it was unclear until recently what function this hydrolysis might have. It was clearly demonstrated that nucleotide hydrolysis was not required for assembly (Cooke and Murdoch, 1973; Penningroth and Kirschner, 1977; Arai and Kaziro, 1977), since rapid and efficient polymerization would occur with nonhydrolizable ATP and GTP analogs. However it was also demonstrated that when the natural triphosphates were used, the stoichiometry of hydrolysis was approximately 1 mole per each mole of subunit assembled (Oosawa and Kasai, 1971b; David-Pfeuty *et al.*, 1977), suggesting that hydrolysis was coupled to assembly.

A striking theoretical and experimental paper by Wegner (1976) argued that nucleoside triphosphate hydrolysis could be used to drive head-to-tail polymerization of actin at steady state. This property, now also called "treadmilling," involves the net assembly of subunits of the filaments at one end and the net disassembly at the other end, at steady state (i.e., when the polymer mass remains unchanged). This is a consequence of the ATP free energy being utilized to make the effective affinity of the two ends of the polymer for the monomer different. Margolis and Wilson (1978) then demonstrated that treadmilling also exists in microtubules by using a direct method for measuring the flux.

In their initial studies Margolis and Wilson (1978) assumed that there was an exclusive addition of subunits to one end and exclusive loss at the other. However, the measured flux of 0.28 dimers s^{-1}, or 0.31 dimers s^{-1} (Terry and Purich, 1980), was small compared to measured dissociation rates under pre-steady state conditions of 154 dimers s^{-1} (Karr *et al.*, 1980) and thus was inconsistent with exclusive assembly at one end and loss at the other, as pointed out by Zeeberg *et al.* (1980). In microtubule protein preparations containing associated proteins, the flux owing to treadmilling measured from pre-steady-state rates was 1.5 dimers s^{-1} (Bergen and Borisy, 1980). Zeeberg *et al.* (1980) also measured a flux of 2 dimers s^{-1} at steady state in a microtubule system where the dissociation rate at steady state was found to be 119 dimers s^{-1}, which is somewhat high compared to other measured values (Johnson and Borisy, 1977). Thus, although treadmilling was demonstrable, it was inefficient, and questions were even raised by Zeeberg *et al.* (1980) as to experimental and theoretical problems

in showing it. However, recently in a very complete study, Cote and Borisy (1981) measured a treadmilling flux of 28 dimers s^{-1} for microtubules, depleted of most of the associated proteins, which have dissociation rates measured under the same steady-state conditions of about 100 dimers s^{-1}. Thus, under these conditions, treadmilling occurs to an appreciable extent and amounts to more than one translocation event for every four association or dissociation events at steady state. As expected from the theoretical treatments of Wegner (1976) and Hill (1980a), treadmilling does not occur with nonhydrolyzable analogs that support microtubule polymerization (Terry and Purich, 1980; Margolis, 1981; Cote and Borisy, 1981). For a recent review of experimental studies of treadmilling, see Margolis and Wilson (1981); see also Pollard and Mooseker (1981).

The clear demonstration of the phenomenon of treadmilling *in vitro* prompted an evaluation of the cellular consequences of having the two ends of the filament different and the possible role of treadmilling to do work. Margolis *et al.* (1978) described a model for mitosis where differential polymerization and depolymerization at the two ends played a key role but treadmilling itself played a minor one. Various experimental observations from Inoue's laboratory have long suggested that force generation could be achieved by polymerization and depolymerization (Inoue and Ritter, 1975), but treadmilling at this time could not be considered. The apparent stable steady-state distribution of organized filaments within cells led to the proposal that an important consequence of treadmilling could be that the cell could use this property to selectively stabilize filaments attached at one end in nucleating structures. Treadmilling could then be used as a mechanism of suppressing spontaneous filament assembly (Kirschner, 1980). This focused attention on the theoretical effects of proteins or structures which might cap one end of a filament at the same time that such proteins were being described in the actin system. Finally, it was possible to show that treadmilling could actually be made to do work under conditions which might be expected to exist in cells (Hill and Kirschner, 1982).

The need to examine the kinetics and energetics of linear polymerizing systems while at the same time taking into account nucleotide hydrolysis, external forces acting on the polymer, and fluctuations has led to a reformulation of polymerization theory in terms of general models utilized previously to explain other metabolic and mechanochemical cycles that use ATP hydrolysis (Hill, 1977a). In this article we will consider the kinetics and bioenergetics of polymers like actin filaments and microtubules that utilize ATP and GTP hydrolysis and, for comparison, we will also consider the kinetics and bioenergetics of those that do not. We will also

consider important boundary conditions such as forces acting in various ways on the filaments and the effect of materials that interact at the ends of filaments. We will be mostly concerned with the biological implications of these properties in terms of capacity to do work, regulate length, and regulate spatial distribution. Although this article reviews the material given in five previous articles (Hill, 1980a, 1981a,b; Kirschner, 1980; Hill and Kirschner, 1982) we go into these problems here in much greater depth, with more examples, and with more biological commentary. In addition, much of the material, including that dealing with the effects of capping and specific properties of the ends of filaments, has not been previously published. It is hoped that this unified and comprehensive format will clarify some of the characteristics of actin and microtubule assembly and stimulate further studies of the way other cellular materials interact with and modify these systems.

II. Polymer with Free Ends

In this and in each of the remaining sections we consider first an "equilibrium polymer," or aggregate, by which we mean a rod-shaped, linear polymer comprised of physically aggregated monomers (subunits) with no enzymatic activity. We then turn, in each section, to the more complicated problem of a "steady-state polymer," which refers to polymers whose subunits contain bound nucleotide diphosphate (NDP) and whose *terminal* subunits are enzymatically active (see Section II,B for details). Sickle-cell hemoglobin (HbS) is an example of an equilibrium polymer while microtubules (tubulin) and microfilaments (actin) are steady-state polymers. In addition to possible application to HbS, etc., the prior equilibrium treatment in each section provides necessary background for the steady-state problem.

In Sections II through V, only macroscopic thermodynamics and the corresponding kinetics are used. This treatment is applicable to very long polymers. Topics that relate to statistical mechanical partition functions, fluctuations, stochastics, and finite systems (polymers) are all reserved for Section VI. This will make it convenient for readers so inclined to omit the subjects included in Section VI.

We discuss in the present section polymers (aggregates) in solution with free ends. That is, the ends are not in contact with cellular barriers nor are they capped with foreign substances or structures: the terminal subunits of the polymer have direct and uninhibited access to the solution. The above-mentioned cases that are excluded here are treated in Sections III, IV, and V.

A. Equilibrium Polymer

We consider a long (essentially macroscopic) rod-shaped aggregate in solution, in equilibrium with dilute free monomers at concentration c_e (Oosawa and Asakura, 1975). For simplicity, we do not include the solvent explicitly in the thermodynamics nor do we include pressure–volume effects (but see Hill, 1964, for a treatment). The polymer, then, can be characterized thermodynamically by the temperature T and by the chemical potential $\mu_0(T)$ of the monomers in the polymer. Because the polymer is open with respect to addition of monomers and its ends are unrestrained, its length L and number of monomers N are thermodynamically indeterminate at $c = c_e$ (Hill, 1964). That is, any L (if large enough) is consistent with μ_0 and c_e. But if c is just less than c_e, the polymer has a definite mean finite length, $L(c)$ (Hill, 1980a).

Monomers in solution at an arbitrary concentration c have a chemical potential

$$\mu_s = \mu_s^0(T) + kT\ln c, \tag{1}$$

where $\mu_s^0(T)$ is a standard free energy. (μ_s is the chemical potential *per molecule*, and k is the Boltzmann constant.) Because c is of order 1 μM for the tubulin and actin cases of interest, we omit an activity coefficient in Eq. (1). However, this would not be a good approximation for HbS (Ross and Minton, 1977). Because of the assumed equilibrium at $c = c_e$,

$$\mu_0(T) = \mu_s^e = \mu_s^0(T) + kT\ln c_e \tag{2}$$

The polymer can be considered to be a one-dimensional crystal with solubility c_e. The concentration c_e is also referred to as the critical concentration of monomer: starting with monomer at $c \ll c_e$, if c is increased, linear aggregates begin to form as c nears c_e and essentially infinite polymers are produced at the "critical" concentration $c = c_e$ (Hill, 1964; Oosawa and Asakura, 1975). If we denote the monomer by Λ, then c_e is also the equilibrium constant for the process Λ(polymer) $\rightarrow$ Λ(solution). The equilibrium constant c_e is related to the standard free energy change for this process in the conventional way by

$$\mu_s^0(T) - \mu_0(T) = -kT\ln c_e \tag{3}$$

That is, on a per mole basis, the right-hand side is $-RT\ln K$. The more stable the monomers are in the one-dimensional crystal (e.g., from strong intermolecular attractive forces), the lower $\mu_0(T)$ and, consequently, from Eq. (2), the lower c_e.

Turning now to related kinetic aspects, we assume that monomer exchange between solution and polymer occurs only via the two polymer

ends and not through the bulk of the polymer. It should be noted that, in the macroscopic thermodynamic discussion above, the number and nature of the polymer ends are of no consequence and were not mentioned. However, the ends control the aggregation kinetics.

The polymers of interest have a polarity (are not isotropic). This is demonstrated structurally by the directional binding of a fragment of myosin to actin filaments (Huxley, 1963) and by the directional binding of dynein or tubulin ribbons to microtubules (Heidemann and McIntosh, 1980). Because the polymer is polar, the two ends are different. In general, then, the rate constants for the addition or loss of subunits at the two ends will be different. The on and off rate constants at one end are denoted α and α', respectively, and at the other end, β and β' (α and β are second-order constants, α' and β' are first-order). This is shown schematically in Fig. 1A.

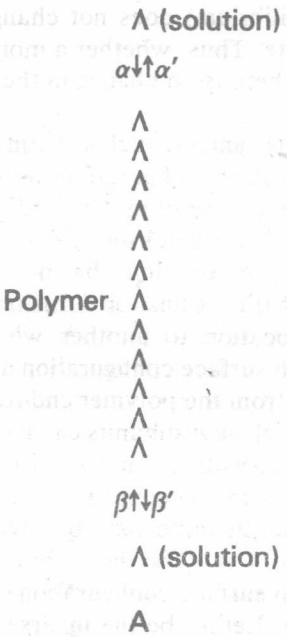
In any system at a true equilibrium, there can be no net flux or flow in *any* process, even at the most elementary level. This is the principle of detailed balance at equilibrium (i.e., balance, or equality, of inverse rates). If the polymer is in equilibrium with free subunits at concentration c_e, the on rate must equal the off rate at *both* ends of the polymer

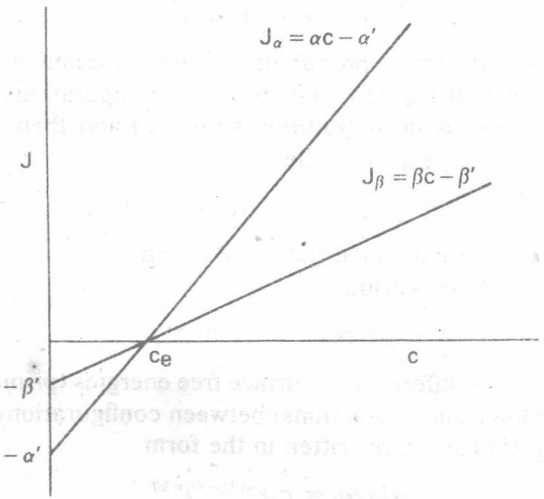
$$\alpha c_e = \alpha', \qquad \beta c_e = \beta', \qquad \text{or} \qquad c_e = \alpha'/\alpha = \beta'/\beta \tag{4}$$

In general $\alpha \neq \beta$ and $\alpha' \neq \beta'$, but the ratios must be equal [Eq. (4)]. As we shall see later, in steady-state polymers, where detailed balance is not required, it is possible to have zero total flux of subunits onto the polymer with nonzero flux at both ends (one flux negative, the other positive).

The significance of detailed balance for this system can also be examined thermodynamically. The addition, at equilibrium, of a monomer to the polymer at a particular end does not in any way alter the equilibrium state of the end itself but rather simply has the effect of increasing the number of bulk (nonterminal) monomers in the polymer by one: the polymer free energy increases by μ_0 and the solution free energy decreases by $\mu_s^e(=\mu_0)$. After the addition of the monomer, the polymer would be in exactly the same state regardless of which end the addition was made to, even though the ends are different. Thus the equilibrium constant for monomer addition ($1/c_e$) must be the same at the two ends. This alternative argument confirms Eq. (4).

Although there are obvious similarities between ligand binding and polymer aggregation, there is also a fundamental difference. When a ligand is bound on a site on another molecule or on a surface, the state of the site itself is changed by the binding. An empty site becomes an occupied site, and is no longer available for binding. But when a monomer is added to the end of a polymer (made of the same monomers), the state of





B

FIG. 1. (A) Equilibrium polymer in solution with ends that are different and with on–off transitions at the ends. (B) Net rate of adding monomers at the two ends as a function of free monomer concentration c.

the polymer end (at equilibrium) does not change. The attachment site remains an attachment site. Thus, whether a monomer adds to the α or β end of a linear polymer, there is no change in the capacity of the polymer to add or lose subunits.

Polymers that are multistranded, such as actin, tubulin, and HbS, have many possible detailed surface configurations at their ends. Some of these have been seen during the course of assembly of microtubules *in vivo* and *in vitro* (Kirschner *et al.*, 1974; Erickson, 1974; Dentler and Rosenbaum, 1977). The surface configurations may be in dynamic equilibrium with each other via on and off transitions, or diffusion transitions, where subunits move from one location to another while still attached to the polymer. Included in each surface configuration are several nonequivalent subunits that can escape from the polymer end to the solution and several nonequivalent sites to which new subunits can be added from the solution. Thus the observable rate constants α and α' (also, of course, β and β') are really composites of more microscopic rate constants. This can be expressed formally, as would be important for any theoretical analysis of α and α' in a particular case. Let α_{ij} be the on rate constant for the addition of a subunit to that site in surface configuration i that converts configuration i into configuration j. Let α'_{ji} be the inverse off rate constant. There must be detailed balance in this elementary process at equilibrium:

$$p_i^e c_e \alpha_{ij} = p_j^e \alpha'_{ji}, \tag{5}$$

where p_i^e is the equilibrium probability of surface configuration i, etc. If we sum both sides of Eq. (5), first, over all configurations j that can be reached from i (i.e., over all addition sites in i) and then over all i, we obtain $\alpha c_e = \alpha'$, as in Eq. (4), where

$$\alpha = \Sigma_{i,j} p_i^e \alpha_{ij}, \qquad \alpha' = \Sigma_{i,j} p_j^e \alpha'_{ji} \tag{6}$$

This exhibits the more detailed nature of α and α'.

We also have, at equilibrium,

$$p_i^e / p_j^e = e^{-(G_i - G_j)/kT}, \tag{7}$$

where $G_i - G_j$ is the difference in surface free energies (primarily owing to different intermolecular interactions) between configurations i and j. Consequently, Eq. (5) can be rewritten in the form

$$\alpha'_{ji} / \alpha_{ij} = c_e e^{-(G_i - G_j)/kT}, \tag{8}$$

where c_e may be replaced here by α'/α, if desired. This is the microscopic equilibrium constant for the release of a subunit from the polymer to the solution, from configuration j to give configuration i. A simple explicit example of the above analysis is included in Section III,A.

At an arbitrary free monomer concentration c,

$$J_\alpha = \alpha c - \alpha' \qquad \text{and} \qquad J_\beta = \beta c - \beta' \tag{9}$$

are the net rates of addition of monomers to the two ends, per polymer molecule. The total rate of addition is $J_{on} \equiv J_\alpha + J_\beta$. At equilibrium, $J_\alpha = J_\beta = 0$ and $c = c_e$. An example of $J_\alpha(c)$ and $J_\beta(c)$ is shown in Fig. 1B in which the α end is more active than the β end (i.e., $\alpha > \beta, \alpha' > \beta'$). The two lines necessarily cross at $c = c_e$ [Eq. (4)]. This type of diagram (Bergen and Borisy, 1980) is very useful in more complicated cases (below). If a large number of these (Fig. 1) polymers are present in a relatively small volume of solution with, initially, $c > c_e$, aggregation will occur at *both* ends of the polymers ($J_\alpha > 0, J_\beta > 0$) but, as a result of loss of free monomers from the solution to the polymers, c will steadily decrease. This will continue until c reaches c_e, at which point growth of polymers will cease. Similarly, if $c < c_e$ at the outset, polymers will lose monomers from *both* ends to the solution ($J_\alpha < 0, J_\beta < 0$) causing c to increase until, again, the stable value c_e is finally reached. An explicit, but more complicated, example of this kind of behavior will be presented in Section III,B.

In writing Eqs. (9), one usually assumes the rate constants are independent of c. This in turn implies that the equilibrium averaging in Eq. (6) is valid at any c. That is, we are assuming that an internal equilibrium among the many surface configurations is maintained even under conditions of steady subunit gain or loss ($c \neq c_e$). This would require subunit surface diffusion transitions that are relatively fast compared to on–off transitions. The simple example treated in Section III,A shows that without surface diffusion we would expect the rate constants themselves to be functions of c.

Because the distribution among surface configurations at the ends would depend on the relative rates of subunit addition, which depends on c, and subunit diffusion, which does not, this problem may also arise in the case of proteins that interact with the subunits in the polymer. Such proteins are well known for both actin filaments and microtubules. Since all the known proteins bind substoichiometrically, the exact ratio of these proteins to the monomers at the ends can vary. If transitions among configurations of bound proteins are slow compared to the rates of assembly, the observed average rate constants can again be dependent on the rate of subunit addition and hence on the monomer concentration.

Incidentally, the above is a special case of a general problem in biochemical kinetics (Hill, 1980b): whenever rate constants are assigned to transitions between pairs of discrete states in a biochemical cycle, the implicit assumption is made that the individual states of the cycle are all in