Proc. Natl. Acad. Sci. USA
Vol. 78, No. 9, pp. 5613–5617, September 1981
Cell Biology

Microfilament or microtubule assembly or disassembly against a force

(actin/tubulin/mitosis/cell shape change/sickle cell hemoglobin)

TERRELL L. HILL

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205

Contributed by Terrell L. Hill, May 5, 1981

ABSTRACT **Microtubules (tubulin) or bundles of microfilaments (actin) are thought to cause movement, in some instances, by disassembly or assembly of subunits. Possible examples are the pulling of a chromosome toward a pole in mitosis (anaphase) or the deformation of a cell membrane to change the shape of a cell. This paper examines the relevant elementary bioenergetic considerations when assembly or disassembly of an aggregate occurs against a resisting force. The problem is considered, in the first section, without NTPase activity. Sickle cell hemoglobin aggregation *in vivo* is an example. In the second section, the tubulin GTPase and actin ATPase activities are included in the analysis.**

In a previous companion paper (1), bioenergetic principles were considered which related to enzyme translocation on DNA and to the treadmilling of one-dimensional aggregates of DNA-binding proteins that are bound adjacent to a replicating fork. Here we examine possible motion that results from the lengthening or shortening of microfilaments (actin) or microtubules (tubulin).

Polymeric actin (2) and microtubules (3) are known to be involved in a number of examples of motility. In most of these cases the necessary force generation presumably arises from actin–myosin or from microtubule–dynein interactions, or variations on these. However, there have been suggestions (4) and there is evidence (2, 5) that assembly or disassembly of microtubules or of actin microfilaments might themselves be *directly* responsible for some kinds of motility. Examples are: the pulling of a chromosome toward a pole in mitosis (anaphase) by disassembly, at the pole, of microtubules that are attached to the kinetochore (4, 6); and protrusion of a cell membrane as a result of actin polymerization (5, 7). The former example is likely to be of more importance as a rate-determining process (6) than as a source of free energy to move the chromosome (4), but this is not definite yet. There are examples of the latter type that almost certainly involve actin only, and not myosin (2, 3). The *in vivo* aggregation of sickle cell hemoglobin is also an example of cell membrane distortion.

The object of this paper is to outline elementary theoretical principles that are pertinent for systems in which either disassembly or assembly of a filamentous aggregate can do work against a resisting force such as a chromosome or a cell membrane (i.e., a deformable surface), respectively.

A complication that will be included in the second section is the fact that microfilament and microtubule assembly or disassembly requires hydrolysis of ATP or GTP, respectively (2, 8). That is, in these processes, actin is an ATPase and tubulin is a GTPase. This NTPase activity leads, in solution, to steady-state treadmilling, also called head-to-tail polymerization, as is well known (9–13).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

An idealized aspect of the treatment to be given is that the pool of subunits or monomers used for the polymerization process is assumed, for concreteness, to be a dilute solution. In some cases the actual pool may be rather concentrated, or there may even be an intermediate bound state for the subunits (5). Such conditions would not alter the formal thermodynamics much but could change the kinetics significantly.

ASSEMBLY–DISASSEMBLY AGAINST A FORCE, WITHOUT NTP

Many of the essential features of interest here are not concerned with the NTPase activity of the aggregating subunits. Therefore we consider first, in this section, the much simpler case of assembly–disassembly without such activity.

Fig. 1 illustrates very schematically the kinds of systems we consider. Fig. 1*A* represents a microtubule (of 13 strands) or an actin microfilament (of 2 strands), or a bundle of microfilaments, under a total *compressing* force F, which we arbitrarily give a negative sign. Fig. 1*B* shows a microtubule under an *extending* force F (positive). In both cases there are binding (from solution) and release transitions of individual subunits at *one end* of the polymer, which can result in length changes in the polymer. These length changes have to contend with the force, F. The other end of the polymer is assumed (to simplify equations) to have an essentially permanent and nondynamic attachment (13). In Fig. 1*B*, we assume that a microtubule with 13 strands can still maintain attachment to some other nonrigid cellular structure at the top, under an extending force, even if several subunits out of a ring of 13 are missing (owing to the transitions referred to). The same assumption is plausible for a *bundle* of actin microfilaments, though not for a single microfilament (two strands). We do not propose an explicit mechanism for the sequence of subunit departures, and possible shifts in subunit lattice positions, that would allow simultaneous shortening and maintenance of contact with the other structure.

Fig. 1*C* illustrates the special case of Fig. 1*A* of most interest: the concentration c of subunits (also called monomers) in solution is large enough so that there is net aggregation (growth) at either end of a microfilament bundle, despite the compressing force $F < 0$ (from a deformable membrane) that resists the growth. Similarly, Fig. 1*D* is the special case of Fig. 1*B* of most interest: despite the extending force $F > 0$ from a chromosome that must be dragged through the solution, the microtubule shortens from a net loss of subunits at one end. We give a single treatment, below, that applies to both of these cases.

The second-order "on" rate constant for attaching subunits at the "dynamic" end, from solution, is α; the first-order "off" rate constant is α'. When $F = 0$, these rate constants are designated by α_0 and α_0'; α and α' depend on F. Even at $F = 0$, α_0 and α_0' presumably have somewhat different values than for the same polymer end when it is free and uninhibited in so-

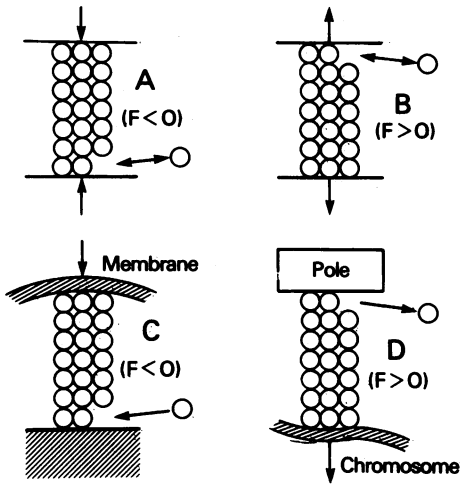

FIG. 1. (*A*) Exchange of subunits between a solution and one end of an aggregated filament or tubule that is under a compressing force, $F < 0$. (*B*) Same for an extending force, $F > 0$. (*C*) Special case of *A* in which net addition of subunits causes lengthening of an aggregate (e.g., a bundle of microfilaments), thus pushing back a resisting membrane. (*D*) Special case of *B* in which net loss of subunits in a microtubule causes shortening and the pulling of a chromosome toward a mitotic pole, against a resisting frictional force.

lution. A "capped" filament, for example, has $F = 0$.

The chemical potential of subunits in solution, at concentration c, is

$$\mu_s = \mu_s^0 + RT\ln c, \qquad [1]$$

where R is the gas constant and T is the absolute temperature. In the polymer, the subunit chemical potential is μ_0, which, for simplicity, we take to be independent of F or of length. That is, the polymer is considered incompressible and we neglect any thermodynamic end effects for finite polymers. The average length contributed to the polymer by one subunit is l. In a microtubule, for example, $l = 80\ \text{Å}/13 = 6.2\ \text{Å}$. When one subunit is removed from the solution and added to the polymer, the Gibbs free energy changes are $-\mu_s$ in the solution, μ_0 in the polymer, and $-lF$ in the "resistance" (membrane, chromosome, etc.). At equilibrium, $c \equiv c_e$, $\alpha c_e = \alpha'$ (detailed balance), and

$$\mu_0 - lF = \mu_s = \mu_s^0 + RT\ln c_e \qquad [2]$$

$$= \mu_s^0 + RT\ln(\alpha'/\alpha). \qquad [3]$$

These equations show how c_e and α'/α depend on F. When $F = 0$, $c_e \equiv c_e^0$, $\alpha_0 c_e^0 = \alpha_0'$, and

$$\mu_0 = \mu_s^0 + RT\ln c_e^0 = \mu_s^0 + RT\ln(\alpha_0'/\alpha_0). \qquad [4]$$

The separate dependences of α and α' on F can be written in a formal way as

$$\alpha = \alpha_0 e^{flF/RT} \qquad [5]$$

$$\alpha' = \alpha_0' e^{(f-1)lF/RT} \qquad [6]$$

where f is a dimensionless parameter that expresses the "split" of lF between the on and off rate constants. Of course, f drops out of the quotient:

$$\alpha/\alpha' = (\alpha_0/\alpha_0')e^{lF/RT}. \qquad [7]$$

The parameter f itself is a function of F. That is, the split would be expected to be different under compression ($F < 0$) and under extension ($F > 0$). Fig. 2 gives a hypothetical illustration, in which $f = \frac{1}{2}$ (symmetrical split) for large negative F and f

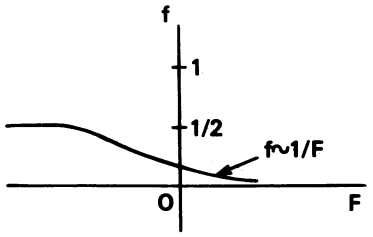

FIG. 2. Example of possible F dependence of kinetic parameter f. See text for further details.

$\sim 1/F$ for large positive F. The latter is meant to produce the diffusion-controlled limit for α, which ought to be larger than α_0 because of interference in the attachment process by the neighboring structure (Fig. 1*B*) when $F = 0$.

The subunit thermodynamic force, tending to produce aggregation, is $\Delta\mu \equiv \mu_s - \mu_0$. This is also equal to $RT\ln(c/c_e^0)$. At equilibrium, $\Delta\mu = -lF$ (Eq. 2), or $\Delta\mu + lF = 0$. This sum is the total thermodynamic force, for aggregation, including the resistance term. The total force has the value zero at equilibrium; at arbitrary c, it is equal to $RT\ln(c/c_e)$.

Fig. 3 summarizes six special cases that have to be considered, depending on the values of c and F. The ordinate in the figure is ln c; the abscissa is F. The heavy line shows the relationship between ln c and F *at equilibrium*. Explicitly, from Eqs. 2 and 4,

$$\ln c_e = \ln c_e^0 - (lF/RT). \qquad [8]$$

For all points in the plane above this line, we have $c > c_e$, $\Delta\mu + lF > 0$, and net aggregation (lengthening) occurs. For all points below this line, $c < c_e$, $\Delta\mu + lF < 0$, and disaggregation (shortening) occurs. To the right of the ordinate, $lF > 0$; to its left, $lF < 0$. The horizontal line that crosses the heavy line corresponds to $c = c_e^0$ (i.e., the subunit force $\Delta\mu$ is zero on this line). Above the horizontal line, $\Delta\mu > 0$; below the line, $\Delta\mu < 0$.

The cases a and d in Fig. 3 are relatively uninteresting because the two subforces ($\Delta\mu$ and lF) have the same sign as the total force. But in cases b, c, e, and f, the subforce with the *same* sign as the total force is larger in magnitude than the other subforce, which has the *opposite* sign. Consequently, there is free energy transduction in these cases, as we now explain.

For example, in case c (corresponding to Fig. 1*C*), the sub-

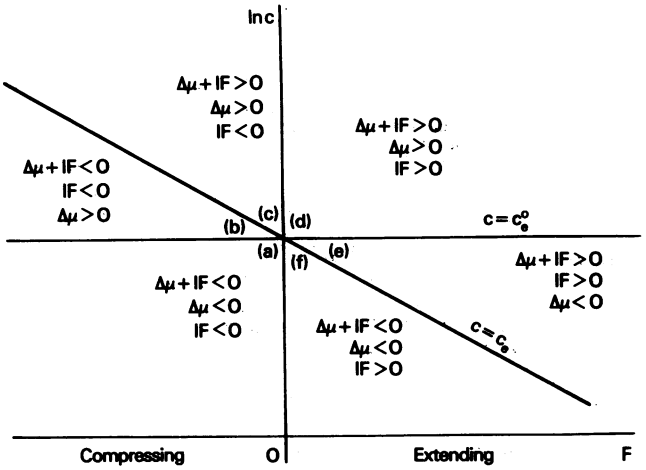

FIG. 3. The heavy line shows the relationship between ln c and a mechanical force F, at aggregation equilibrium, where c is the monomer concentration. The special cases a–f refer to nonequilibrium situations (off of the heavy line), discussed in the text.

unit concentration is large enough so that assembly and lengthening occur despite the compressing force ($F < 0$) which opposes lengthening. Part of the subunit free energy decrease $\Delta\mu$ (per subunit added to the polymer) is used to do an amount of work $-lF$ against the membrane (Fig. 1C). The efficiency of the transduction is $\eta = (-lF)/\Delta\mu$. The source of the free energy used is the subunit pool at high enough chemical potential μ_s. If c is decreased (keeping F constant) enough to cross the heavy line in Fig. 3, case c passes into case b. Here $\Delta\mu$ and lF have the same signs as in case c, but there is role reversal: the compressive force, the free energy source in this case, is large enough to drive subunits out of the polymer even though $\Delta\mu > 0$ (i.e., $c > c_e^0$). The efficiency is $\eta = \Delta\mu/(-lF)$.

If a free polymer, growing at $c > c_e^0$ and $F = 0$, encounters a rigid barrier, F will quickly decrease at constant c until growth stops ($c = c_e$ line).

Case f in Fig. 3 corresponds to Fig. 1D. Here the pool concentration c is sufficiently low so that disassembly and shortening occur despite the extending force $F > 0$. Part of the subunit free energy decrease $-\Delta\mu$ is used to do work, in the shortening process, against the resisting force F. The efficiency of transduction is $\eta = lF/(-\Delta\mu)$. Of course, in the example in Fig. 1D, the work against F is not stored (as it would be if, say, a weight were lifted) but rather it is dissipated as heat in the viscous medium through which the chromosome moves. Also, in this example, F itself would be proportional to the rate of shortening (see below). Incidentally, a chromosome, in anaphase, has a small velocity of only about 1 μm min^{-1} (6) or 0.2 Å msec^{-1} (the velocity in muscle contraction is of order 10 Å msec^{-1}).

If c is increased sufficiently, in case f, holding F constant, case f transforms into case e. Again $\Delta\mu$ and lF retain the same signs, and there is role reversal: the extending force is now large enough to induce aggregation of subunits even though $\Delta\mu < 0$ (i.e., $c < c_e^0$). The transduction efficiency here is $\eta = (-\Delta\mu)/lF$.

We define the flux J_m as the net rate of *adding* monomers to the polymer: $J_m = \alpha c - \alpha'$. The velocity of lengthening of the polymer is then $v = J_m l$. On using Eqs. 5 and 6, J_m becomes

$$J_m = \alpha_0' e^{(f-1)lF/RT}[e^{(\Delta\mu+lF)/RT} - 1]. \quad [9]$$

At equilibrium, both flux and total force are zero: $J_m = 0$ and $\Delta\mu + lF = 0$. Otherwise, J_m and $\Delta\mu + lF$ have the same sign. Near equilibrium, [ ] in Eq. 9 is replaced by $(\Delta\mu + lF)/RT$. In the absence of an outside mechanical force ($F = 0$), the rate of aggregation J_m is simply equal to $\alpha_0'(e^{\Delta\mu/RT} - 1)$. The remaining thermodynamic force here is $\Delta\mu$.

The rate of free energy dissipation in this system is

$$Td_iS/dt = (\alpha c - \alpha')(\mu_s - \mu_0 + lF) = J_m(\Delta\mu + lF) \geq 0. \quad [10]$$

Recall that J_m and $\Delta\mu + lF$ always have the same sign and that, in the transduction cases b, c, e, and f in Fig. 3, one of $\Delta\mu$ and lF is positive and the other is negative.

In Eq. 9, f is some function of F, as already mentioned. One would expect each particular system to have its own $f(F)$. Also, in the chromosome or any similar case (Fig. 1D), J_m and $\Delta\mu + lF$ are negative and $F = -\beta J_m l$, where $F > 0$ and β is the frictional coefficient. This connection between F and J_m converts Eq. 9, in such a case, into an implicit equation in J_m.

In summary: In the cases c (Fig. 1C) and f (Fig. 1D) of practical interest, there is no difficulty, in principle, with suggestions that subunit aggregation or disaggregation, respectively, can do work against an opposing mechanical force. The driving free energy for this work is a subunit chemical potential difference; NTPase activity is obviously not a required feature of the system in view of the fact that we have not yet included it in the model. The aggregation of sickle cell hemoglobin does not involve NTPase activity.

ASSEMBLY–DISASSEMBLY AGAINST A FORCE, INCLUDING NTP

We now reconsider systems of the type shown in Fig. 1, and include the NTPase activity that actin and tubulin actually show when they polymerize. The approach that we use is very similar to that in ref. 12, where the bioenergetics of steady-state treadmilling was examined. Here, only one end of the polymer can gain or lose monomers, the other end being blocked, and steady state (constant length) is of only incidental interest. Treadmilling (head-to-tail polymerization) is not possible. We take over the notation of ref. 12 except that $\Delta\mu_T$ (instead of X) is the NTP free energy of hydrolysis and c (instead of c_1) is the monomer concentration in solution. Explicitly, $\Delta\mu_T = \mu_T - \mu_D - \mu_P$ (T, ATP; D, ADP; P, P_i).

Fig. 4A shows a possible NTPase cycle (12, 14), where Λ represents a monomer, Λ_T a monomer with NTP bound, etc. There is only a partial NTPase cycle for monomers in solution and a complementary partial NTPase cycle for a terminal monomer of the polymer (a nonterminal monomer in the polymer is frozen in state Λ_D). The two partial cycles, however, form a complete cycle, as indicated in Fig. 4A. We make the usual assumption (11) that the two boxed species in Fig. 4A are dominant. The monomer concentration c thus refers to Λ_T. With this simplification, we can replace the six-state cycle in Fig. 4A by the two-state cycle in Fig. 4B. The latter figure includes the rate constant notation; α_1 and α_{-2} are second-order constants, whereas α_2 and α_{-1} are first-order constants. These rate constants refer to whichever end of the polymer (the two ends are intrinsically different) is able to exchange monomers with the solution. The rate constants α_1 and α_2 predominate in the kinetics; α_{-1} and α_{-2} are relatively small (but they are needed for thermodynamic purposes).

The chemical potential of a monomer (Λ_D) in the polymer is designated by μ_{AD}, a constant. The monomer (Λ_T) chemical potential in the solution is written

$$\mu_{AT} = \mu_{AT}^0 + RT\ln c. \quad [11]$$

Then if we consider a hypothetical equilibrium, including the mechanical force F, resulting from α_1, α_{-1} transitions only, we obtain the analogue of Eq. 3:

$$\mu_{AD} + \mu_P - lF = \mu_{AT}^0 + RT\ln(\alpha_{-1}/\alpha_1). \quad [12]$$

On rearrangement,

$$RT\ln(\alpha_1/\alpha_{-1}) = \mu_{AT}^0 - (\mu_{AD} + \mu_P - lF). \quad [13]$$

Similarly, for the α_2, α_{-2} pair, we find

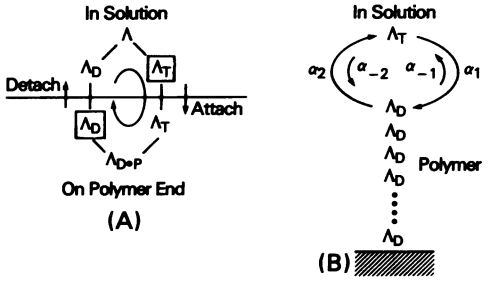

FIG. 4. (A) NTPase cycle in the attachment–detachment of subunits at one end of an aggregate. Λ represents one subunit. The boxed states dominate in the cycle. (B) Rate constant notation for two-state cycle, using "boxed" states only.

$$RT\ln(\alpha_2/\alpha_{-2}) = (\mu_{AD} + \mu_P - lF) - (\mu^0_{AT} - \Delta\mu_T). \quad [14]$$

Both α_1/α_{-1} and α_2/α_{-2} depend on F. The successive basic free energy levels (12, 15) in a cycle, starting from Λ_T in Fig. 4*B*, are μ^0_{AT}, $\mu_{AD} + \mu_P - lF$, and $\mu^0_{AT} - \Delta\mu_T$ (Eqs. 13 and 14). The middle level depends on F but the overall cycle thermodynamic force does not: on adding Eqs. 13 and 14, we have

$$\alpha_1\alpha_2/\alpha_{-1}\alpha_{-2} = e^{\Delta\mu_T/RT}. \quad [15]$$

The total thermodynamic force is $\Delta\mu_T$, of order 12 kcal mol^{-1} or more (1 kcal = 4.184 kJ). Thus $\alpha_1\alpha_2/\alpha_{-1}\alpha_{-2}$ is of order 10^9 or more. F drops out here because, in a cycle, a monomer is first added to the polymer, and then subtracted; NTP is hydrolyzed in a cycle but the polymer length is unchanged.

The gross free energy levels (12, 15) corresponding to the above basic levels are

$$\mu_1 \equiv \mu^0_{AT} + RT\ln c \quad [16]$$

$$\mu_2 \equiv \mu_{AD} + \mu_P - lF \quad [17]$$

$$\mu_3 \equiv \mu^0_{AT} + RT\ln c - \Delta\mu_T. \quad [18]$$

These will be needed below. In a complete cycle, $\mu_1 - \mu_3 = \Delta\mu_T$.

The net transition fluxes (15), in the main cycle direction (Fig. 4*B*), are

$$J_1 \equiv \alpha_1 c - \alpha_{-1}, J_2 \equiv \alpha_2 - \alpha_{-2}c. \quad [19]$$

The net rate of adding monomers to the polymer is then

$$J_m = J_1 - J_2 = (\alpha_1 + \alpha_{-2})c - (\alpha_2 + \alpha_{-1}) \approx \alpha_1 c - \alpha_2. \quad [20]$$

At steady state (constant length), $J_1 = J_2$, $J_m = 0$, and $c \equiv c_\infty$, where

$$c_\infty = (\alpha_2 + \alpha_{-1})/(\alpha_1 + \alpha_{-2}) \approx \alpha_2/\alpha_1. \quad [21]$$

When $c > c_\infty$, $J_m > 0$ (the polymer lengthens); when $c < c_\infty$, $J_m < 0$ (polymer shortens). Thus the condition $c = c_\infty$ for $J_m = 0$ replaces $c = c_e$ in the previous section (Fig. 3). Note that $c_\infty \approx \alpha_2/\alpha_1$, compared to $c_e = \alpha'/\alpha$ above. In c_∞, α_2 and α_1 belong to *different* transition pairs (Fig. 4*B*). The equilibrium concentrations for the *individual* transition pairs would be $c_e^{(1)} = \alpha_{-1}/\alpha_1$ (very small) and $c_e^{(2)} = \alpha_2/\alpha_{-2}$ (very large); $c_\infty \approx \alpha_2/\alpha_1$ is intermediate, of order 1 μM (10).

The rate constants in the above expressions depend on F. Corresponding to Eqs. 5 and 6, we write

$$\alpha_1 = \alpha^0_1 e^{f_1 lF/RT}, \alpha_{-1} = \alpha^0_{-1} e^{(f_1-1)lF/RT} \quad [22]$$

$$\alpha_2 = \alpha^0_2 e^{-f_2 lF/RT}, \alpha_{-2} = \alpha^0_{-2} e^{(1-f_2)lF/RT}, \quad [23]$$

where f_1 and f_2 are parameters that themselves depend on F (see, e.g., Fig. 2), and α^0_1, etc., are the rate constants at $F = 0$. Substitution of Eqs. 22 and 23 in Eq. 21 gives c_∞ as an explicit function of F. At $F = 0$, $c_\infty \equiv c^0_\infty$. If $c_\infty(F)$ is plotted as $\ln c_\infty(F)$, the curve (Fig. 5) would in general not be linear (compare Fig. 3). But if α_{-1} and α_{-2} can be neglected (one-way cycle), we have

$$\ln c_\infty = \ln c^0_\infty - [(f_1 + f_2)lF/RT], \quad [24]$$

which *is* linear if $f_1 + f_2$ is constant. We also obtain a linear relation, as in Eq. 8, for the *two*-way cycle, if $f_1 + f_2 = 1$ (the special case $f_1 + f_2 = 1$ simulates an equilibrium). If the transitions in Fig. 4*B* referred to elementary processes, we might expect, for example, f_1 and $1 - f_2$ for the two "on" processes (α_1 and α_{-2}) to be equal. But these are actually *composite* transitions (Fig. 4*A*); no such special relationship is to be expected.

The net assembly flux J_m can be put in the form

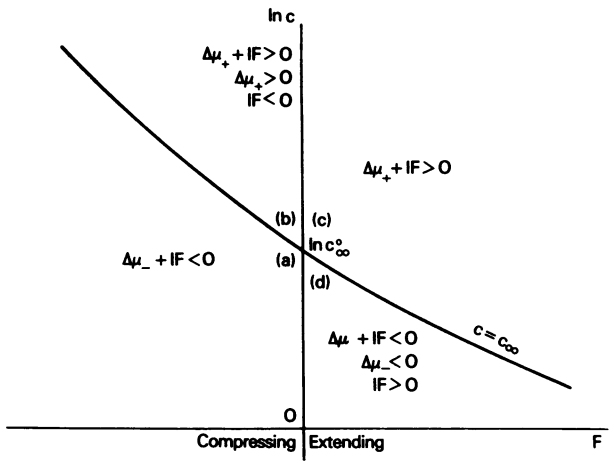

FIG. 5. Modification of Fig. 3 with NTPase subunits. The curve shows, schematically, ln c as a function of mechanical force F at steady state ($c = c_\infty$). For $F > 0$, $\Delta\mu_+ > 0$ above $c = c^0_\infty$, but a categorical statement about $\Delta\mu_+$ cannot be made below c^0_∞. For $F < 0$, $\Delta\mu_- < 0$ below $c = c^0_\infty$, but a categorical statement about $\Delta\mu_-$ cannot be made above c^0_∞. See text for further details.

$$J_m = (\alpha_2 + \alpha_{-1})[c/c_\infty) - 1]. \quad [25]$$

The quantity $(c/c_\infty) - 1$ is an apparent thermodynamic force defined about the steady state; but c_∞ is a *kinetic* property (Eq. 21), so that this is not a legitimate thermodynamic force. Substitution of Eqs. 21–23 in Eq. 25 gives J_m as an explicit function of F.

The expression for the rate of free energy dissipation provides the most insight into the nature of this system. We start with the "transition" relationship (15)

$$Td_iS/dt = J_1(\mu_1 - \mu_2) + J_2(\mu_2 - \mu_3) \geq 0, \quad [26]$$

where the detailed expressions are given in Eqs. 16–19. This single formal equation applies to all cases. But, for conceptual advantage, we rearrange the terms here in two different ways depending on whether $c > c_\infty$ (polymer lengthens) or $c < c_\infty$ (polymer shortens).

When $c > c_\infty$, we also have $J_m = J_1 - J_2 > 0$, so that $J_1 > J_2$. The two transition fluxes J_1 and J_2 are represented schematically in Fig. 6*A*. Because $J_1 > J_2$, J_1 is subdivided into two parts (arrows): an amount J_2 to match the other transition flux; and the excess $J_1 - J_2$. J_2 now represents a *complete cycle* flux (Fig. 6*A*); in a complete cycle one molecule of NTP is hydrolyzed but the polymer remains unchanged. We therefore define the NTP flux as $J_T^{(+)} \equiv J_2$ (the + refers to the lengthening case, $c > c_\infty$); the associated complete cycle thermodynamic force is $\Delta\mu_T$. The excess flux $J_1 - J_2 = J_m$ ("on") is the net rate of addition of monomers, occurring via α_1, α_{-1} transitions (Fig. 6*A*). We break down the total thermodynamic force $\mu_1 - \mu_2$ (Eqs. 16 and 17), associated with this excess α_1, α_{-1} flux, into the subunit thermodynamic force and the "resistance" force:

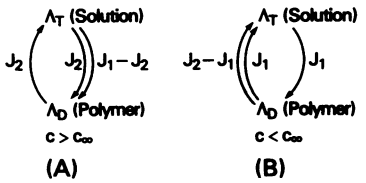

FIG. 6. Reclassification of the two end fluxes J_1 and J_2 in order to separate the cyclic NTPase activity (J_2 in *A*; J_1 in *B*) from the excess addition to (J_m in *A*) or loss of ($-J_m$ in *B*) subunits from the aggregate.

$$\mu_1 - \mu_2 = \Delta\mu_+ + lF \qquad [27]$$
$$\Delta\mu_+ \equiv (\mu^0_{AT} + RT\ln c) - (\mu_{AD} + \mu_P).$$

Here $\Delta\mu_+$ is analogous to $\Delta\mu$ in the previous section. With these definitions of $J_T^{(+)}$ and $\Delta\mu_+$, Eq. 26 becomes

$$Td_iS/dt = J_m(\Delta\mu_+ + lF) + J_T^{(+)}\Delta\mu_T \qquad (c \geq c_\infty). \qquad [28]$$

Compared to the analogous Eq. 10, there is a new term here representing the dissipation of NTP free energy. When $c = c_\infty$, the first flux–force expression on the right of Eq. 28 drops out (because $J_m = 0$), but $J_T^{(+)}\Delta\mu_T$ remains. In this case ($c = c_\infty$, steady state), NTP is being used but the polymer does not change; no work is being done by the system. Incidentally, $c = c_\infty$ is not, as might be expected, the value of c at which Td_iS/dt is minimized (1).

Eq. 28 applies on or above the curve in Fig. 5. Case c in this figure is not very interesting, but case b involves free energy transduction: the filament or polymer lengthens against a compressing (negative) force F, as in Fig. 1C. The efficiency of the transduction is

$$\eta = J_m(-lF)/(J_m\Delta\mu_+ + J_T^{(+)}\Delta\mu_T) \qquad (c \geq c_\infty). \qquad [29]$$

The efficiency is reduced by the presence of the NTPase term. Without this term, the efficiency would be $-lF/\Delta\mu_+$. In fact, the NTPase activity serves no obvious purpose here (see the preceding section). However, there could, conceivably, be some kinetic advantage to the use of the NTP cycle in Fig. 4 rather than simple assembly–disassembly transitions.

If a free polymer, growing at $c > c^0_\infty$ and $F = 0$, encounters a rigid barrier, F will quickly decrease at constant c until growth stops ($c = c_\infty$ line). If both ends can exchange subunits, treadmilling will occur.

In Eq. 10, both J_m and $\Delta\mu + lF$ are equal to zero on the $c = c_e$ line (Fig. 3). In the present *steady-state* case, $J_m = 0$ on the $c = c_\infty$ curve (Fig. 5), but $\Delta\mu_+ + lF > 0$. This can be seen as follows. We have, by definition,

$$\Delta\mu_+(c_\infty) + lF = \mu_1(c_\infty) - \mu_2, \qquad [30]$$

where c_∞ is given by Eq. 21. If we replace c_∞ on the right by $c_e^{(1)} = \alpha_{-1}/\alpha_1$, the right side becomes equal to zero (equilibrium). Thus the right side in Eq. 30, as it stands, is positive if $c_\infty > \alpha_{-1}/\alpha_1$, which is the case if $\alpha_2/\alpha_{-2} > \alpha_{-1}/\alpha_1$ (Eq. 21). But this is true in view of Eq. 15. Of course, as c increases above $c = c_\infty$, $\Delta\mu_+ + lF$ also increases (Eq. 27). Thus, above the $c = c_\infty$ curve, both flux–force products in Eq. 28 have two positive factors; both contribute to free energy dissipation.

We turn now to the opposite case, $c < c_\infty$, in which $J_m < 0$ (polymer shortens) and $J_2 > J_1$. In view of Fig. 6B, we define $J_T^{(-)} \equiv J_1$ (complete cycle), with associated force $\Delta\mu_T$, while the excess subunit flux (positive) $J_2 - J_1 = -J_m$ ("off") has the conjugate force $\mu_2 - \mu_3$ (positive). We subdivide $\mu_2 - \mu_3$ into subunit and "resistance" terms, as in Eq. 27:

$$\mu_2 - \mu_3 = -\Delta\mu_- - lF \qquad [31]$$
$$\Delta\mu_- \equiv (\mu^0_{AT} + RT\ln c - \Delta\mu_T) - (\mu_{AD} + \mu_P).$$

Again $\Delta\mu_-$ corresponds to $\Delta\mu$ in the previous section, but $\Delta\mu_-$ is negative for values of c of interest. In fact,

$$\Delta\mu_+ - \Delta\mu_- = \Delta\mu_T. \qquad [32]$$

Because $\Delta\mu_T$ is of order 12 kcal mol^{-1}, $\Delta\mu_+$ and $-\Delta\mu_-$ might both be of order 6 kcal mol^{-1}. With these definitions of $J_T^{(-)}$ and $\Delta\mu_-$, Eq. 26 becomes

$$Td_iS/dt = J_m(\Delta\mu_- + lF) + J_T^{(-)}\Delta\mu_T \qquad (c \leq c_\infty). \qquad [33]$$

This equation is the companion of Eq. 28. In this case, J_m and $\Delta\mu_- + lF$ are both negative, while $J_T^{(-)}$ and $\Delta\mu_T$ are both positive. On the curve $c = c_\infty$, $J_m = 0$ but $\Delta\mu_- + lF < 0$; the NTPase term remains at steady state. The case in Fig. 5 of interest is d: the polymer shortens despite the opposing extending force $F > 0$, as in Fig. 1D. Some subunit free energy is used to do work against the resisting force F. The efficiency of the transduction is

$$\eta = (-J_m)lF/[(-J_m)(-\Delta\mu_-) + J_T^{(-)}\Delta\mu_T] \qquad (c \leq c_\infty). \qquad [34]$$

Again the NTPase activity reduces the efficiency. Without the NTPase term, the efficiency would be $lF/(-\Delta\mu_-)$.

Quite aside from the wasteful NTPase dissipation terms in Eqs. 28 and 33, the thermodynamic forces $\Delta\mu_+$ and $-\Delta\mu_-$ (of order 6 kcal mol^{-1}) seem unnecessarily large for the purpose at hand. These forces are related to the ratios α^0_1/α^0_{-1} and α^0_2/α^0_{-2}, respectively. But the quantity of practical interest here (rate of assembly or disassembly) is $J_m \approx \alpha_1 c - \alpha_2$, which depends essentially only on the rate constants α_1 and α_2 and not on α_{-1} and α_{-2}. In the previous section (no NTPase activity), on the other hand, $J_m = \alpha c - \alpha'$ and $\Delta\mu$ is related to α_0/α'_0: flux and force depend on the same (inverse) rate constants, which is more efficient (there is less free energy dissipation).

On the curve $c = c_\infty$, there is a discontinuity between $\Delta\mu_+$ and $\Delta\mu_-$ (but $J_m = 0$). On the other hand, $J_T^{(+)} = J_T^{(-)}$ on this curve, because $J_1 = J_2$. There are, of course, no *real* discontinuities across $c = c_\infty$; we have merely, for conceptual reasons, changed our classification scheme (Fig. 6) at this boundary.

In summary, Eq. 26 has been rearranged in Eqs. 28 and 33 in order to separate pure (complete cycle) NTPase activity from the residual monomer activity (addition to or subtraction from the polymer). This monomer activity is analogous to that in the previous subsection (NTPase absent); the NTPase activity here is superimposed, and is wasteful. Although inefficient, there is no difficulty, in principle, in the use of assembly or disassembly to work against an outside mechanical force, as in Figs. 1C (cell shape changes) and 1D (chromosome movement).

I am much indebted to Dr. Marc Kirschner for a helpful discussion.

1. Hill, T. L. & Tsuchiya, T. (1981) *Proc. Natl. Acad. Sci. USA* 78, 4796–4800.
2. Korn, E. D. (1978) *Proc. Natl. Acad. Sci. USA* 75, 588–599.
3. Roberts, K. & Hyams, J. S., eds. (1979) *Microtubules* (Academic, New York).
4. Inoué, S. & Ritter, H. (1975) in *Molecules and Cell Movement*, eds. Inoué, S. & Stephens, R. E. (Raven, New York), pp. 3–29.
5. Tilney, L. G. (1975) in *Molecules and Cell Movement*, eds. Inoué, S. & Stephens, R. E. (Raven, New York), pp. 339–386.
6. McIntosh, J. R. (1979) in *Microtubules*, eds. Roberts, K. & Hyams, J. S. (Academic, New York), pp. 381–441.
7. Goldman, R. D., Schloss, J. A. & Starger, J. M. (1976) in *Cell Motility*, Cold Spring Harbor Conferences on Cell Proliferation, eds. Goldman, R. D., Pollard, T. D. & Rosenbaum, J. L. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 3, Book A, pp. 217–245.
8. Jacobs, M. (1979) in *Microtubules*, eds. Roberts, K. & Hyams, J. S. (Academic, New York), pp. 255–277.
9. Margolis, R. L. & Wilson, L. (1977) *Proc. Natl. Acad. Sci. USA* 74, 3466–3470.
10. Bergen, L. G. & Borisy, G. G. (1980) *J. Cell Biol.* 84, 141–150.
11. Wegner, A. (1976) *J. Mol. Biol.* 108, 139–150.
12. Hill, T. L. (1980) *Proc. Natl. Acad. Sci. USA* 77, 4803–4807.
13. Kirschner, M. W. (1980) *J. Cell Biol.* 86, 330–334.
14. Hill, T. L. (1981) *Biophys. J.* 33, 353–371.
15. Hill, T. L. (1977) *Free Energy Transduction in Biology* (Academic, New York).