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Hysteretic behavior and differential apparent stability properties of microtubule species emerge from the regulation of post-translational modifications of microtubules

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SUMMARY

At the epigenetic level, microtubule diversity is generated by several mechanisms of reversible post-translational modifications of tubulin subunits. In most cases, modification enzymes preferentially act on the tubulin subunits of microtubules, whereas the substrate of the enzymes which ensure the reverse reaction is preferentially the αβ-dimer of nonpolymerized tubulin. Most modifications identified to date appear to be nearly ubiquitous within the animal kingdom. Moreover, modifications are generally not mutually exclusive, so that cellular microtubules often bear several distinct biochemical alterations whose biological role is yet unknown.

Post-translational modifications often (but not always) occur on microtubule species with low turnover rate. However, in vitro comparison of the polymerization and depolymerization rates of modified or unmodified forms of tubulin did not reveal any significant difference between molecular species. Thus, post-translational modifications are thought to be the result rather than the cause of microtubule stability.

We re-examine this contention in the light of a regulated kinetic scheme for multiple and non-exclusive enzymatic modifications of microtubules. This study shows that different apparent stability properties of microtubule species emerge under such a kinetic regulation, although all the species were assumed to have identical intrinsic stability properties. This model can be used to reinterpret the results of well-known studies bearing on the relationship between microtubule stability and post-translational modifications. Another important finding is that the existence of a regulation loop in one of the multiple pathways of enzymatic differentiation of microtubules endows the system with hysteretic properties. These properties may be viewed, under restrictive conditions, as a buffering mechanism for the transitions between microtubule growing and shrinking phases during fluctuations in the regulation of centrosomal nucleating activity.

Key words: Microtubule, Post-translational modification, Hysteresis, Microtubule stability

FOREWORD

According to the reductionist approach, the global properties of any system are thought to be the sum of those of its components. However, most of the biological systems are dynamic and, in fact, new properties of these systems arise from the interactions between their components. These global properties cannot be reduced to those of any particular isolated element. For instance, we know that the suprachiasmatic nuclei of the hypothalamus comprise the primary pacemaker responsible for the generation of circadian rhythms in mammals. Although some of the biochemical components which are part of the system have been identified (particularly those which depend on *c-fos* and *c-jun* gene expression), a considerable work of fine biochemistry is still lacking to elucidate the molecular mechanisms underlying circadian clocks. However, searching for some putative molecule which could be the oscillator at the heart of the clock would be as fruitless as taking to pieces an old fob-watch in the hope of finding a quartz crystal. On the contrary, a reasonable hypothesis is that the circadian clock is based on autoregulatory loops in which the protein products of 'clock genes' are involved. The same dynamic dependence between constitutive elements exists at the molecular level. Consider for instance the signal-induced Ca^{2+} oscillations which occur through the regulation of the $InsP_3$-gated Ca^{2+} channel. In a great number of cell types, the key elements of this dynamic behavior are the regulatory properties of the $InsP_3$ receptor. However, the occurrence of Ca^{2+} periodicities cannot be reduced to the static properties of the receptor but also depends upon its ability to integrate several variables such as the level of second messengers and the concentration of cytosolic and intravesicular calcium. The existence of biological oscillations is the best evidence that biological systems are dynamic ones. This is probably why the dynamic approach, which explains such an emergent property, is so widely accepted in that specific case. However, oscillations (which are

the macroscopic sign of the existence of an unstable steady state) constitute only one of the possible manifestations of a dynamic behavior. One other possible dynamic property is the occurrence of hysteretic transitions between alternate steady states. The major finding of the last thirteen years of studies on microtubules is the discovery that these macromolecular assemblies are highly dynamic structures. Hence, significant progresses in the field may be expected from the use of the tools specifically developed for the study of dynamic systems. We previously showed (Laurent and Fleury, 1993) that the multistability property allows us to interpret the diversity of post-translational modifications of microtubules in a cell, without appealing to any putative compartmentalization of enzymatic activities responsible for the various modifications. The present paper extends our previous model and sheds new light upon the difficult question of the relationship between microtubule stability and post-translational modification processes.

INTRODUCTION

With the recent identification of a sixth type of post-translational modification of microtubules (Redeker et al., 1994; Bressac et al., 1995; Rüdiger et al., 1995), the recurrent question of the biological significance of all these enzymatic modifications must once again be raised. It is fair to say that we do not yet understand the role of any of the post-translational modifications of microtubules characterized so far. For example, Kozminski et al. (1993) showed that high-level expression of a transfected nonacetylable α-tubulin gene does not produce any major phenotypical change in the flagellate *Chlamydomonas reinhardtii*. More recently, Gaertig et al. (1995) showed that acetylation in α-tubulin is not essential in the ciliated protozoan *Tetrahymena thermophila*. Whatever the postulated function of post-translational modifications of microtubules, a crucial question remains as to how the repertoire of distinctly modified microtubules is generated. Gaining an insight into the mechanism of post-translational modifications might provide much-needed insights into the function of biochemical modifications of microtubules.

In a previous paper (Laurent and Fleury, 1993), we demonstrated how spatial and temporal differentiation of stabilized microtubules may be explained by the existence of a dynamic pathway separation ensured by biochemical switches between self-regulated mechanisms of differentiation. Such a scheme allowed us to interpret (Laurent and Fleury, 1995a) our experimental data obtained on the ciliate *Paramecium* (Fleury and Laurent, 1995) concerning the dynamics of a cortical network of acetylated microtubules with relation to the invariance of a morphogenetic field and the passage of a triggering calcium wave (Laurent and Fleury, 1995b). The goal of the present paper is to extend and generalize our previous model to a situation in which microtubules are able to be doubly post-translationally modified by two distinct, mutually nonexclusive enzymes. This situation appears to be very common in a biological context (Schulze et al., 1987; Bulinski et al., 1988). Moreover, we intend to go beyond the simple case in which modification enzymes act only on stabilized forms of microtubules. However realistic it may be for the acetylation and detyrosination processes of post-translational modification, this simplifying hypothesis does not seem to hold for at least one other type of modification, polyglutamylation, which was shown to occur also on unstabilized microtubules (Eddé et al., 1991).

Stability properties of microtubules

Post-translational modifications often (but not always) occur on 'stable' microtubules, i.e. on species which exhibit a low turnover rate. However, microtubules cannot simply be divided into 'stable' and 'dynamic' classes. Except in particular cases such as axonemes of ciliates and flagellates, all microtubules undergo subunit exchange, more or less slowly, with the tubulin pool. Hence, the adjectives 'stable' and 'dynamic' are ambiguous when applied to microtubules. 'Stable' cytoplasmic microtubules are completely renewed after several hours (Schulze et al., 1987). Thus, so-called stable microtubules are actually dynamic, but their dynamics is slow (Schulze et al., 1987; Webster et al., 1987; Kreis, 1987; Webster and Borisy, 1989). We shall see below how the question of microtubule stability is connected with post-translational modifications.

The hypotheses about the origin and the mechanism of microtubule stability in vivo are complex and rather conflicting. Although a general consensus exists today about the dynamic instability model (Mitchison and Kirschner, 1984), the molecular mechanism leading to dynamic instability is yet controversial (lateral cap model, possible existence of stop proteins, etc). Moreover, several recent reports argue that a difference exists in the stability of microtubules under in vitro and in vivo conditions. In the traditional view (Sammak and Borisy, 1988; Schulze and Kirschner, 1988; Shelden and Wadsworth, 1993), microtubules are labile in interphase cells with dynamics which correspond to the behavior of microtubules assembled in vitro from purified tubulin preparations. On the contrary, recent data (Verde et al., 1990, 1992; Lieuvin et al., 1994) suggest that interphase microtubules would be intrinsically stable, whereas microtubule instability in the living cell would be conferred by regulatory mechanisms involving phosphorylation-dependent events.

A kinetic approach is needed

Up to now, surprisingly, little attention has been paid to the question of the dynamics of enzymatic post-translational modifications of microtubules. It is implicitly supposed that the dynamics of modified microtubules is a simple reflection of their intrinsic stability or instability. In other words, modification enzymes are supposed to act passively on the population of microtubules which are present during a significant time period but the modification itself is not supposed to influence the dynamics. The relationships between stability properties and post-translational modification processes are summarized in the current paradigm (for a review, see Bulinski and Gundersen, 1991): 'Post-translational modifications are a result rather than a cause of microtubule stability'. Paradoxically, this consensual view does not result from any direct experimental evidence. The rationality of the process is as follows: several experiments indicate that post-translational modifications do not seem to increase the intrinsic stability of microtubules, both under in vitro and in vivo conditions. Since post-translational modifications are often found on microtubules which have a low turnover rate, the increased content of modified tubulin in stabilized microtubules is thus thought as being the result of a microtubule lifetime long enough to allow the enzymatic modifications of tubulin. We shall discuss in this paper some of the limits of the interpretation of the above experiments.

From a thermodynamic point of view, the logic underlying the above reasoning relies on two implicit assumptions: that the modification enzymes are not regulated and that the concentration of the modifiable microtubules may be considered as constant. Obviously, neither of these assumptions is fulfilled in the case of post-translational modification of microtubules (see later). Hence, a kinetic approach is needed in order to understand the possible influence of these enzymatic processes on the apparent stability of the microtubular species.

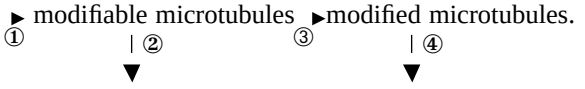
Principle and limits of the kinetic approach

A real difficulty exists for the development of a kinetic model: in a cell, only a global property (the apparent stability of the macromolecular systems) can be observed. On the contrary, for analytic purposes, we have to separate post-translational events from the dynamic instability phenomenon so as to study the possible influence of the former on the apparent stability property of modified microtubules. Experimental data (Argaraña et al., 1978; Barra et al., 1988; Raybin and Flavin, 1977a; Schroeder et al., 1985) show that a prerequisite for this separation is fulfilled in the case of the post-translational mechanisms of modification of microtubules: the enzyme which performs the reaction of modification acts on tubulin protomers within microtubules whereas the reverse reaction is performed on the αβ-dimer (or on tubulin monomers). It is noteworthy that this partitioning mechanism of enzyme activities prevents the occurrence of a futile cycle (Katz and Rognstad, 1976; Bulinski and Gundersen, 1991). How may the two processes be separated analytically? The most general scheme that should be considered is one which takes into account the rate at which modifiable microtubules appear in the system. The corresponding rate equation is generally unknown and probably quite complex. To overcome this difficulty, we can consider an open system in which this rate constitutes the input rate which will be firstly assumed as constant so as to give a description of the dynamic properties of the model (existence of multiple steady states). Then we consider it as a phenomenological variable, i.e. we describe the dynamics of the system when fluxes are changed as the concentration of modifiable microtubules varies (for instance as a consequence of the dynamic instability phenomenon). The method we use is a consequence of the meaning of a steady state in an open system: shortly after an input, species belonging to the system go into motion (in terms of concentration values for each species) until a time-independent stationary state is reached which lasts as long as the input species are present at some constant concentration. The steady state is commonly described by the profiles of the fluxes, overall kinetics and elementary constants. This means that steady-state concentrations of species are different when the parameter values such as the input value (the rate at which modifiable microtubules appear in the system) are modified. Hence, this method allows us to study specifically the effect of the post-translational modification events on the apparent stability of microtubules, independent of the complex mechanism of dynamic instability.

BIOLOGICAL BACKGROUND

Microtubule stability and post-translational modifications: the paradigm

Acetylation (L'Hernault and Rosenbaum, 1985; LeDizet and Piperno, 1987), phosphorylation (Eipper, 1972; Gard and Kirschner, 1985), detyrosination (Barra et al., 1974, 1988; Gundersen et al., 1984) and more recently polyglycylation (Adoutte et al., 1991; Redeker et al., 1994) were found to occur on 'stable' (or, more exactly, stabilized) microtubules. The current paradigm holds that post-translational modifications are a result rather than a cause of microtubule stability (for a review, see Bulinski and Gundersen, 1991). As a consequence, the ordered sequence: *unstabilized* microtubules→*stabilized* microtubules→*post-translationally modified* microtubules is widely admitted, at least for the detyrosination and acetylation mechanisms of post-translational modification of microtubules (Gundersen and Bulinski, 1987; Wehland and Weber, 1987; Barra et al., 1988; Bulinski and Gundersen, 1991). However, the observation that in some cell types all microtubules are acetylated even though turnover rate is very high could indicate that, in these cases, modification enzymes also act directly on unstabilized microtubules. The latter mechanism is also postulated for another post-translational modification, polyglutamylation (Eddé et al., 1990). Hence, the most general scheme for any post-translational modification could correspond to the following key sequence:



In this key sequence, steps 2 and 4 (depolymerization of the corresponding microtubule species) must have the same rate constant since experimental data indicate that the depolymerization rate of unmodified and post-translationally modified microtubules are not different (Raybin and Flavin, 1977b; Kumar and Flavin, 1982; Paturle et al., 1989). When post-translational modification (step 3) only occurs on the stablized form of microtubules, step 1 simply corresponds to the rate of stabilization of microtubules by extrinsic proteins such as MAPs. In the generic case, step 1 is the rate at which modifiable microtubules appear in the system. However, in both cases, this rate depends (at least indirectly) on the concentration of unstabilized microtubules and, consequently, on the parameters of the dynamic instability model. In the most general interpretation, no difference is assumed between the rate at which unstabilized and stabilized microtubules can be modified. This constitutes a simplifying assumption which is also consistent with the paradigm concerning the role of post-translational modifications: if such a difference must be assumed, post-translational modifications would no longer be only the consequence, but could also be the cause, of microtubule stability.

Post-translational modification pathways are regulated

No exhaustive, quantitative study on the kinetic properties of the enzymes which post-translationally modify microtubules has yet been performed. However, several lines of arguments indicate that most of these enzymes (as for a great number of metabolic enzymes) are regulated. In the case of acetylase, Maruta et al. (1986) have demonstrated the existence, in a cell body extract of *Chlamydomonas*, of a still-unidentified cytoplasmic inhibitor of tubulinyl acetyl transferase activity. Moreover, Ca^{2+} and coenzyme-A are known to inhibit this enzyme, although the postulated mechanism of calcium inhi-

bition seems unusual since Ca^{2+} is supposed to bind to the substrate and not to the enzyme (Maruta et al., 1986).

Several compounds are known to inhibit the carboxypeptidase activity responsible for the detyrosination of native tubulin within microtubules. For instance, Modesti and Barra (1986) showed that several polyanions, such as heparin, chondroitin sulfate, polyadenylic and polyglutamic acids, are potent inhibitors of this modification enzyme. Several polycations, such as polylysine and protamine also showed an inhibitory effect on carboxypeptidase activity (Modesti and Barra, 1986; Barra et al., 1988). It is worth noting that macromolecules such as proteoglycans, soluble RNA (Argaraña et al., 1981) and endogenous basic proteins (Kumar and Flavin, 1982; Modesti et al., 1986) are also able to inhibit this enzyme.

The theoretical prerequisite for a threshold behavior is the existence of at least one non-linear reaction. In the model developed below, the non-linearity will be introduced by assuming that one of the modification enzymes is inhibited by an excess of substrate. Although this mechanism may seem rather hypothetical in the present state of our knowledge, it conveniently accounts for the inhibitory effect several proteins exert on carboxypeptidase activity towards tubulin.

KINETIC MODELLING

According to the cyclic model for acetylation-deacetylation or tyrosination-detyrosination of α-tubulin, each microtubule species can be depolymerized to yield αβ-dimers of modified tubulin, in the general case, or unmodified tubulin, in the case of the unmodified MT species. In accord with the above considerations, the corresponding output processes (v_{out}) are assumed to correspond to first order rate equations with the same rate constant k_f. Similarly, the unregulated pathway of post-translational modification of microtubules (MT→MTY and MTX→MTXY steps with v_{unreg} rate) are assumed to correspond to a pseudo-first order kinetic law (k_y rate constant). Thus, the overall scheme of individual reactions which must be considered is given in Fig. 1. The biological significance of the input rate v_{in} is discussed above. The system is described by the following differential equations:

$$\frac{d[MT]}{dt} = v_{in} - v_{reg(MT)} - k_y[MT] - k_f[MT], \quad (1)$$

$$\frac{d[MTX]}{dt} = v_{reg(MT)} - k_y[MTX] - k_f[MTX], \quad (2)$$

$$\frac{d[MTY]}{dt} = k_y[MT] - v_{reg(MTY)} - k_f[MTY], \quad (3)$$

$$\frac{d[MTXY]}{dt} = k_y[MTX] + v_{reg(MTY)} - k_f[MTXY], \quad (4)$$

where the rate of the regulated processes (X-type post-translational modification) is:

$$v_{reg(Z)} = V_M[Z] / (K_m + [Z] + [Z]^2 / K_{si}),$$

where the Z variable refers either to the MT or MTY concentration and V_M, K_m and K_{si} are the maximum velocity, the Michaelis constant and the inhibition constant of the enzyme which catalyzes the X-type post-translational modification (MTX formation step and transformation from MTY to MTXY species).

Let us introduce dimensionless parameters and variables:

$$\alpha = \frac{[MT]}{Km} \quad \beta = \frac{[MTX]}{Km} \quad \gamma = \frac{[MTY]}{Km} \quad \delta = \frac{[MTXY]}{Km}.$$

The rate of the regulated process may be written as:

$$\Psi(\chi) = \frac{\chi}{1 + \chi + k\chi^2},$$

where $\kappa = K_m/K_{si}$ and χ refers either to α or γ dimensionless concentration.

We are thus led to the following dimensionless equations:

$$\frac{d\alpha}{d\tau} = f(\alpha) = \mu - \Psi(\alpha) - \rho\alpha - \Pi\alpha, \quad (5)$$

$$\frac{d\beta}{d\tau} = g(\alpha, \gamma) = \Psi(\alpha) - \rho\beta - \Pi\beta, \quad (6)$$

$$\frac{d\gamma}{d\tau} = h(\alpha, \gamma) = \rho\alpha - \Psi(\gamma) - \Pi\gamma, \quad (7)$$

$$\frac{d\delta}{d\tau} = j(\beta, \gamma, \delta) = \rho\beta + \Psi(\gamma) - \Pi\delta, \quad (8)$$

where $\tau = (V_M/Km)t$ is dimensionless time. The μ parameter represents the normalized input rate (v_{in} divided by V_M, with $0<\mu<1$ as a condition for steady state). The ρ and Π parameters have the following definitions:

$$\rho = \frac{k_y Km}{V_M} \quad \Pi = \frac{k_f Km}{V_M}.$$

It is worth noting that in the absence of accurate experimental determination of parameter values, we must focus our attention on the qualitative behavior that our model generates rather than attempting to fit quantitative parameter values.

RESULTS

Hysteretic behavior of the dynamic system of enzymatic modifications of microtubules

Steady states are defined by constant values of the flux and of the concentrations of all the species. For all intermediates the outflux equals the influx so that the net rate of production becomes zero. Steady state of the system is obtained for α and γ concentrations such that the conditions $f(\alpha)=0$ and $h(\alpha, \gamma)=0$ are simultaneously satisfied. The set of points (α, μ) which are the solutions of the equation $f(\alpha)=0$, forms a curve which is a trajectory of steady states (Fig. 2, upper curve): when μ varies, α varies; but any point defined by a given data pair (α, μ) necessarily lies on this trajectory. Its region of negative slope corresponds to unstable stationary states, whereas the regions of positive slope are associated with stable steady states. The correspondance between the stationary concentration values of α and γ is obtained by examining the nullcline $h(\alpha, \gamma)=0$ (Fig. 2, lower curve), i.e. the trajectory of steady states in the (α, γ) plane. In this representation, the stable branches of steady states correspond to the regions of negative slope. Since upper and lower curves

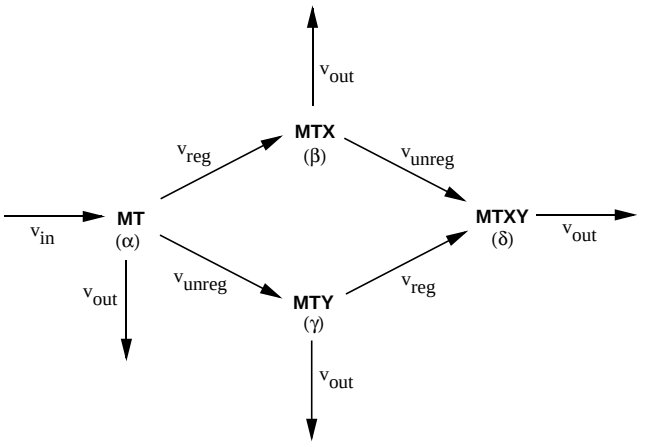


Fig. 1. Kinetic scheme for mutually non-exclusive post-translational modifications of microtubules. Only two modes of enzymatic modification of MT microtubules (leading to MTX and MTY species) are assumed; these are not mutually exclusive. Hence, MTX and MTY species may be transformed into a species with dual modification: MTXY. The X post-translational modification (MT→MTX and MTY→MTXY transformation steps) is catalyzed by an enzyme which is regulated (v_{reg}) whereas the Y-modification (MT→MTY and MTX→MTXY step) is not regulated (v_{unreg}). This system is an open system having one input (v_{in}) and one possible output (v_{out}) for each species (see text). Associated greek letter corresponds, for each species, to the name of the representative normalized variable used in the calculations.

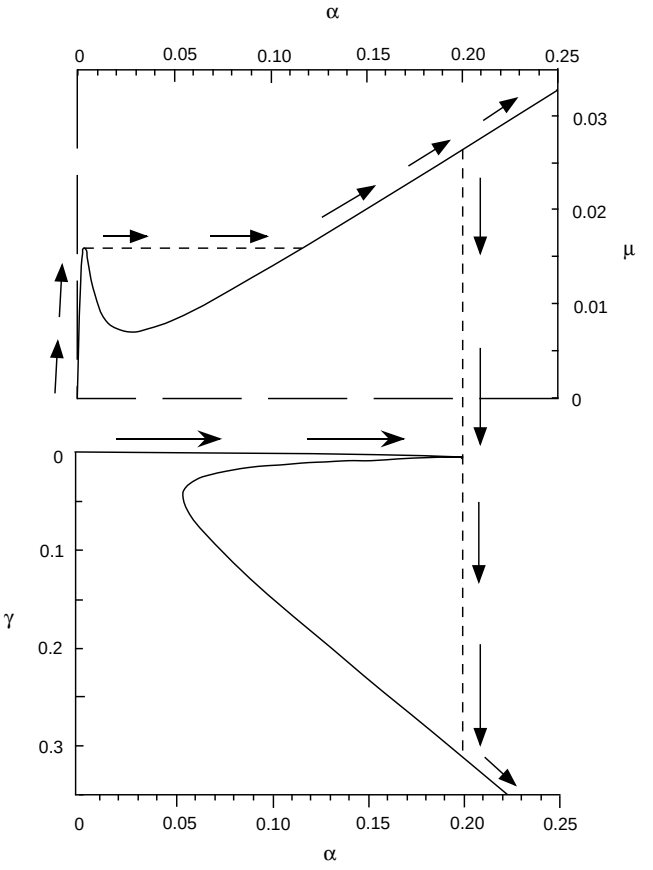


Fig. 2. Null isoclines and dynamic behavior of system (5)-(8) upon increasing the μ input value. The solid lines are the nullclines f(α)=0 (upper curve) and h(α, γ)=0 (lower curve) which were both calculated for the same set of parameter values: ρ = 0.008, Π = 0.005, κ = 10^5. Axes in the lower curve have been inverted (with respect to the upper curve) in order to have α as a variable in the abscissa on both graphs. Hence, stable branches of the nullcline on the lower graph correspond to the regions which have a negative slope whereas they correspond to regions of positive slope on the upper graph. Arrows illustrate the dynamic behavior of the system upon continuous increase in the μ input value, with initial conditions α=γ=0. Two consecutive jump-like transitions (broken lines) are observed between the stable branches of steady states (see main text).

both have α as a variable in the abscissa, we can visualize at once the γ_{SS} stationary concentration of γ which corresponds to a given α_{SS} value and, subsequently, to a given μ input value.

We will first discuss the dynamic behavior of the system upon increasing values of the input parameter μ (arrows in Fig. 2). Let us suppose that the system is initially at α=γ=0. Until the stationary state lies on the stable branches of both trajectories, α and γ concentrations are slightly readjusted, in accord with the solution trajectories. But when μ exceeds a first threshold value (which corresponds to the change in the sign of the slope of the f(α)=0 nullcline), the system moves to the right stable branch of this trajectory, and a strong discontinuity (jump-like transition symbolized by the horizontal dashed line on the upper graph in Fig. 2) appears for the steady-state concentration of α. However, this transition only produces a horizontal displacement of the system on the upper stable branch of the h(α,γ)=0 nullcline, i.e. a very slight adjustment in the stationary concentration of γ is observed as a result of the α-transition. As μ is continuously increasing, the system moves on the right stable branch of the f(α)=0 nullcline and on the upper stable branch of the h(α, γ)=0 nullcline until it reaches the extremity of the stable branch of the h(α,γ)=0 nullcline. Then, a second jump-like transition (symbolized by the vertical dashed line on the upper graph) occurs, but this time for the γ concentration, without any modification of the α_{SS} stationary value of α. Additional increase in the value of the μ input parameter does not produce any additional discontinuities, the system moving now on the right stable branch of the f(α)=0 nullcline and on the lower stable branch of the h(α,γ)=0 nullcline.

What now happens if the input parameter μ is decreasing? Such a situation is illustrated in Fig. 3, starting from high α and γ initial stationary concentrations (arrows). Upon decreasing μ value, a first threshold is observed (vertical dashed line) for the γ-transition (γ_{high} toward γ_{low}) followed by a second lower threshold value in μ (horizontal dashed line) corresponding to the α-transition (α_{high} toward α_{low}). The important point is that the μ values for which transitions between the alternate steady states occur are different depending on whether the value of the input parameter μ increases (Fig. 2) or decreases (Fig. 3). Such transitions are called hysteretic (Nicolis and Prigogine, 1977).

Variations in the steady-state concentrations of the microtubule species as a consequence of hysteretic transitions

Fig. 4 illustrates the relative distribution of microtubule species observed as the input parameter value increases (upper graph) or decreases (lower graph). We first examine the pattern

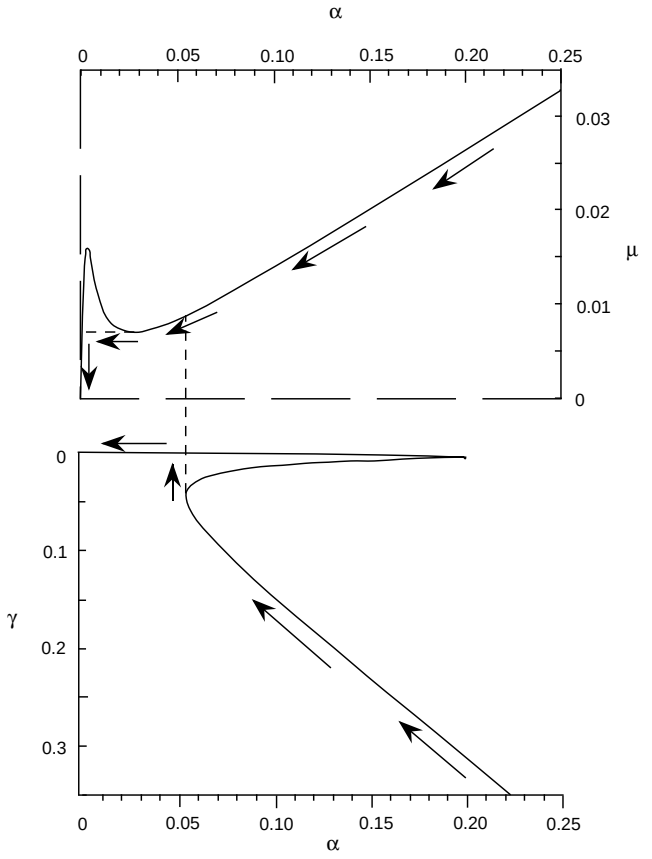


Fig. 3. Dynamic behavior of the system (5)-(8) upon decreasing μ input value. Same parameters as in Fig. 2. Hence, the nullclines f(α) = 0 (upper curve) and h(α, γ) = 0 (lower curve) are identical to those of Fig. 2. Arrows illustrate the dynamic behavior of the system upon a continuous decrease in the μ input value, from initial stable conditions in which the system lies on the right stable branch of the f(α)=0 nullcline and on the lower stable branch of the h(α,γ)=0 nullcline (i.e. high α and γ stationary concentrations). As in the case of Fig. 2, two consecutive jump-like transitions (broken lines) are observed between the stable branches of steady states (see main text), but the transitions occur for μ values which are different from those observed upon increasing the value of the input parameter μ (Fig. 2). Hence, these transitions are hysteretic.

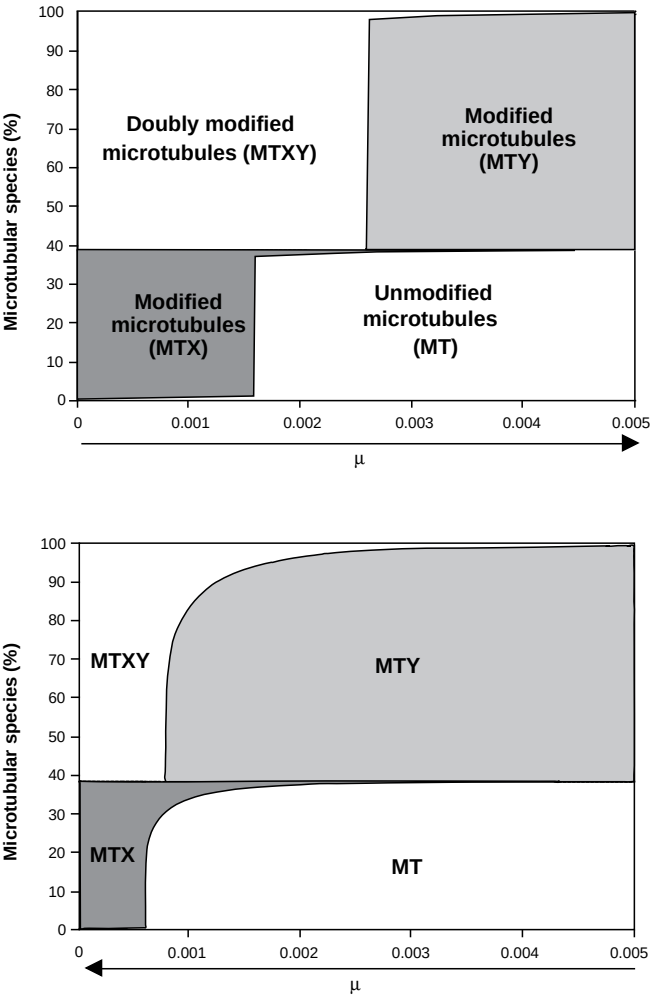


Fig. 4. Effect of hysteretic transitions on the relative steady-state distribution of microtubule species. Data were obtained by numerical integrations of equations 5-8 using the same values of the ρ, Π and κ parameters as in Fig. 2. The upper graph shows the relative steady-state distribution obtained when the μ-value is increasing whereas the lower graph shows the distribution obtained upon decreasing the value of the input parameter μ. It is noteworthy that when the μ parameter increases, the total microtubule concentration in the system also increases. Hence, for each μ value, the absolute concentration calculated for each species was normalized with respect to the total concentration of all microtubule forms, in order to obtain relative concentrations (%).

obtained upon increasing μ value. For lower α steady-state concentrations (corresponding to $\mu < 0.0016$), the net flux merely occurs through the $(v_{in})\rightarrow MT\rightarrow MTX\rightarrow MTXY\rightarrow(v_{out})$ pathway, although the stationary concentration of MT remains very low and MTX and MTXY steady-state concentrations are of about the same order of magnitude (at least for the values of the structural parameters ρ and Π used). When the first transition $\alpha_{low}\rightarrow\alpha_{high}$ has occurred, the regulated enzyme which transforms MT into MTX becomes inhibited and the net flux merely comes from the $(v_{in})\rightarrow MT\rightarrow MTY\rightarrow MTXY\rightarrow(v_{out})$ pathway. The stationary concentration of MTY is then sufficiently low to authorize the X-transformation from MTY into MTXY. Hence, for these intermediate conditions ($0.0016 < \mu < 0.0027$ on the upper graph of Fig. 4), only unmodified MT and doubly modified MTXY microtubule species are present in significant amounts. When the value of μ exceeds 0.0027, the second transition $\gamma_{low}\rightarrow\gamma_{high}$ has occurred, and the regulated enzyme which performs the X-post-translational modification in the transformation MTY→MTXY is also inhibited. Hence, the net flux essentially arises from the $(v_{in})\rightarrow MT\rightarrow MTY\rightarrow(v_{out})$ pathway, and only the unmodified MT and modified MTY microtubule species are found, namely the species not formed through the regulated pathways.

The same analysis holds for the pattern obtained upon decreasing μ value (Fig. 4, lower graph). However, due to the hysteretic nature of the transitions between alternate steady states upon μ variations, the corresponding threshold values of μ are lower than those observed in the previous case. It should also be noted that the transitions are less sharp in the lower graph. The hysteretic behavior means that, for a given set of other parameter values, knowledge of the value of the input parameter μ is not sufficient to determine the state of the

system, i.e. to predict the relative distribution between microtubule species. The other relevant parameter concerns the history of the system, more precisely the anterior values of the μ parameter.

Differential lifetime of microtubule species

Since post-translational modifications are often proposed 'to occur as a result, rather than as a cause of microtubule stability', it therefore seems interesting to determine, in our kinetic scheme, if the half-lifetimes of all microtubule species are identical. Half-lifetimes effectively depend on turnover rate of the system, and can be considered a quantitative measurement of the apparent stability of the related species.

Since, in accordance with experimental data, we chose the same value for the first order rate constant of the five output reactions (depolymerization process of each microtubule species), it is not surprising to observe (Fig. 5) that the mean half-lifetime for all the microtubule species actually present does not depend on the μ value, i.e. on the nature of these species (compare the half-lifetime for total microtubules in the upper and lower parts of Fig. 5, which correspond to distinct distribution profiles of microtubule species). It is noteworthy that the invariance of the mean half-lifetime for all microtubule species actually present also results from the fact that, at each transition, a species with a low (respectively a high) turnover rate is quantitatively replaced by another species which has also a low (respectively a high) turnover rate. This observation is not the consequence of a particular choice of parameter values; it results from the fact that our kinetic scheme does not transgress thermodynamic laws.

However, we note considerable differences for the half-lifetime of each microtubule species taken individually. The half-lifetime of the doubly modified species MTXY is more than fourfold greater than that of the MTX species (Fig. 5, upper part). About the same difference is observed between MTY and unmodified MT species, when these species are the main components in the system (Fig. 5, lower part). Hence, the regulation process in at least one of the enzymatic pathways of microtubule differentiation is sufficient to generate different apparent (i.e. kinetic) stability properties of microtubule species, although no particular intrinsic stabilty property was assumed for any modified or unmodified microtubule species.

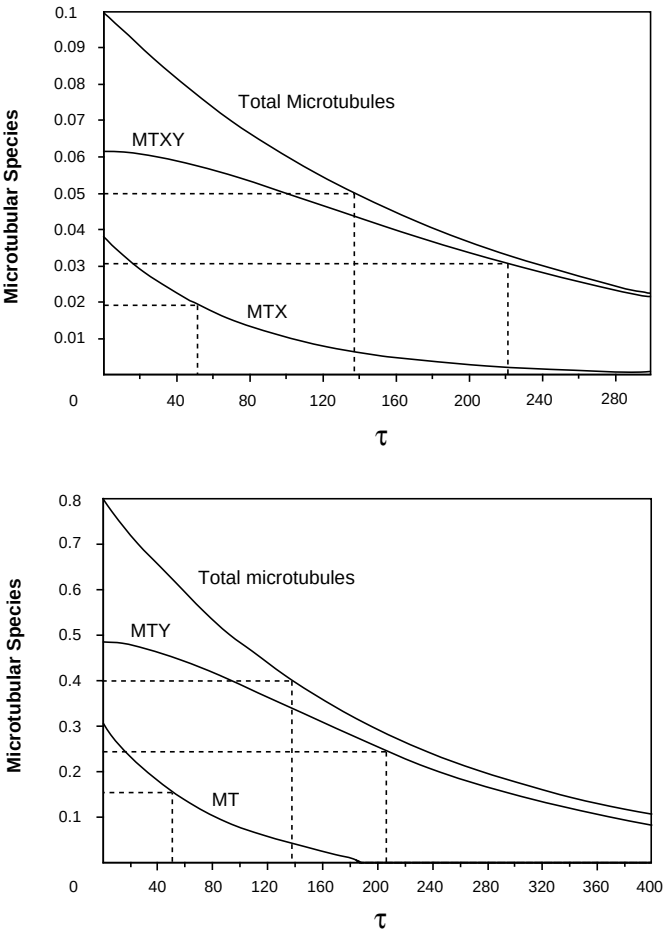


Fig. 5. Differential apparent stability properties of microtubule species as a kinetic consequence of the regulation of one of the two pathways of post-translational modification of microtubules. Initial steady-state concentrations for each microtubule species were calculated for μ=0.0012 (upper curve) or for μ=0.004 (lower curve) in a sequence of calculations in which the μ value was increasing. Other parameter values were as in Fig. 2. According to Fig. 4, MTXY and MTX species are largely predominant in the first conditions whereas only MTY and MT are essentially present in the second case. When the steady state was reached (the corresponding normalized time is defined as τ=0), input parameter μ was kept at 0 and numerical integrations of equations 5-8 were performed in order to examine the kinetics of disappearance for each microtubule species. Broken lines give the half-lifetime for each individual species. This parameter must be compared to the mean half-lifetime for all microtubule species which are actually present under these conditions.

DISCUSSION

Our results demonstrate that the existence of a regulation loop in one of the multiple pathways of enzymatic differentiation of microtubules generates at once a hysteretical behavior of the system and some differential apparent stability properties of the microtubule species. These results should be compared to available experimental data and discussed within the framework of the putative biological role(s) of post-translational modifications of microtubules.

Are post-translational modifications of microtubules the result rather than the cause of microtubule stability?

The contention that post-translational modifications are a result rather than a cause of microtubule stabilty is based on evidence first obtained from the detyrosination/tyrosination cycle and further extended to the acetylation process (Khawaja and Bulinski, 1988), merely because in both cases the enzymatic activities of modification/unmodification preferentially act upon separate pools of tubulin, i.e. polymeric and protomeric forms, respectively. According to Bulinski and Gundersen (1991), the salient points are: (1) in vitro comparison of polymerization and depolymerization characteristics of preparations enriched in either the tyrosinated or detyrosinated forms of tubulin did not show any significant difference (Raybin and Flavin, 1977b; Kumar and Flavin, 1982; Paturle et al., 1989). (2) Enzymatic detyrosination in cytoskeletons prepared from

permeabilized cells is not sufficient to increase the stability of microtubules modified in this manner (Khawaja and Bulinski, 1988). (3) If the tyrosinating enzyme (namely tubulinyl tyrosine ligase) is inhibited in vivo by microinjection of specific antibodies, the resulting detyrosinated (i.e. modified) microtubules of the injected cells are not more stable than tyrosinated (i.e. unmodified) microtubules present in uninjected cells (Webster et al., 1990).

Our model does not question the first set of in vitro experiments, since only the intrinsic properties of microtubule species and not the mechanisms of modification were experimentally studied. Our model does not assume any particular intrinsic stability property of modified microtubules, and we chose the same depolymerization rate for unmodified and post-translationally modified microtubules, in accord with these experimental observations. On the contrary, the two last points seem, a priori, in contradiction with our model. In fact, they are not at all. Analyses of experimental data obtained from cells often contain implicit conditions or hypotheses; opening the black box of the underlying mechanisms helps to shed new light on the related phenomena. The corresponding experiments were performed on TC-7 or 3T3 cells, in which microtubules are also acetylated (Bulinski et al., 1988; Webster et al., 1990) (and may be also modified in another, still unknown, manner). In addition, positive acetylation assays were performed independently in Webster's experiments, as controls on the stability properties of the microtubule species. Hence, the so-called 'tyrosinated microtubules' and 'detyrosinated microtubules' in these experiments are in fact (and at least) acetylated-tyrosinated microtubules and acetylated-detyrosinated microtubules, respectively. In terms of our model, these species correspond to MTY and MTXY species. We have shown that both species have the same half-lifetime, although the corresponding value is significantly different from that of unmodified microtubules MT.

Correspondingly, the half-lifetime of modified microtubules MTX is equal to the microtubules MT half-lifetime. But this value is different from that associated with the MTXY species. By comparing stability properties of the microtubules of an array in which X and Y post-translational modifications do not occur (MT species) with the properties of doubly modified microtubules (MTXY species) in which only the X-modification is detected, one could draw the erroneous conclusion that the X-modification stabilizes microtubules. This has some important methodological consequences. We are far from knowing all the post-translational modifications which affect microtubules (a new type of modification was reported last year; Redeker et al., 1994). A fortiori, the conditions in which these modifications are active remain unknown. Hence, classical measurements based on the specific immunodetection of a given modification may be hampered by the properties of another unsuspected or untested modification.

Comparison of our model with available experimental data in which doubly modified microtubules were studied

At least in metazoan cells, which often display a low level of cellular differentiation in controlled culture (otherwise they may undergo a process of differentiation but become unable to divide), studies of both acetylation and detyrosination processes have shown that when both modifications can be detected, as in human retinoblastoma (SKNSH) or African green monkey kidney epithelial (TC-7) cells, acetylated and detyrosinated microtubules represent the same subset of total cellular microtubules (Schulze et al., 1987; Bulinski et al., 1988), i.e. the corresponding modifications are not mutually exclusive. It should be noted that another subset of microtubules remains unmodified (Schulze et al., 1987). These cells are typically in physiological conditions such that the μ value corresponds to the intermediary range in which only MT and MTXY species are present (Fig. 4). Our predictions agree not only qualitatively but also quantitatively with data obtained on SKNSH cells by Schulze et al. (1987) which show that the two classes of microtubules were also kinetically different, with half-lives of about 1 hour for modified forms and about 5-10 minutes for unmodified ones.

On the contrary, in some cell types only one post-translationally modified form of tubulin (of the two modifications tested) was detectable in microtubules: the acetylated form in the case of human HeLa cells (Bulinski et al., 1988) and the detyrosinated form in marsupial PtK cells (Schulze et al., 1987; Bulinski et al., 1988). In these cases, one can assume either that the enzyme responsible for the second type of modification is absent, or that the conditions for expression of the corresponding activity are not fullfilled. If the second enzyme was indeed present, this would imply, in terms of our kinetic model, that under normal physiological conditions in the corresponding cells only the unmodified form MT and the modified form MTY can be detected (high μ value). In that case, the doubly-modified species could not be detected by increasing μ value (as we usually do by performing treatment with taxol, a microtubule-stabilizing drug) but by decreasing it. This constitutes a paradoxical prediction of our model and it may explain why taxol treatment of PtK cells does not reveal any acetylated-detyrosinated form of microtubules (Bulinski et al., 1988), if acetylase is indeed present in these cells.

The situation observed in human HeLa cells is much more complex. The acetylated microtubules found under normal conditions represent only a minor subset of the total microtubule complement (Bulinski et al., 1988). Treatment of these cells with taxol results in a dramatic increase in the level of both detyrosinated and acetylated microtubules. A possible explanation is that the major, previously unmodified set of microtubules becomes doubly modified after the drug treatment. This agrees with the interpretation that, in interphase HeLa cells, two distinct enzymatic machineries for post-translational modification of microtubules are present but only one of them is active in normal conditions. However, it is not clear (the corresponding data were not shown) whether the taxol-induced microtubules were actually present prior to administration of the drug. Moreover, one must keep in mind that, in real cellular conditions, more than two distinct chemical modifications are probably present; the distribution of the molecular species might be more complex than the typical diagram shown in Fig. 4.

Complete experimental test of our model would require the ability to vary the μ parameter in both directions, under controlled cellular conditions. This is not a simple task; for example, both cold and nocodazole treatments (which typically decrease the μ parameter) have generally drastic effects. In fact, we have observed that extended treatment of *Paramecium* cells with nocodazole results in complete depolymerization of

the internal network of microtubules (Fleury and Laurent, 1995). Under normal interphase conditions, this network is unacetylated. However, when the corresponding microtubules slowly begin to regrow (low μ value) after depolymerizing treatment, they appear decorated with anti-acetylated tubulin antibodies (unpublished results). The occurrence of other post-translational modifications of microtubules under these conditions remains to be studied.

On the possible biological meaning of the hysteretic properties of microtubules

One of the advantages of the analysis presented here is that it permits the examination of the intrinsic effect(s) of the mechanisms of post-translational modification of microtubules, independent of the complex process of dynamic instability: when the total microtubule concentration changes, v_{in} (and it normalized counterpart μ) changes, and our model describes what occurs in terms of post-translational modification events. However, strictly speaking, the v_{in} parameter represents the rate at which modifiable microtubules appear in the model. This rate is a complex function of multiple factors: parameters of the dynamic instability model, rate of stabilization of microtubules by extrinsic proteins such as MAPs, etc. However, one of the main factors which may affect the value of v_{in} is the nucleating activity of centrosomes, and its regulation (at least for centrosome-nucleated microtubules). Regulated fluctuations in the nucleating activity of centrosomes tend to induce dramatic transitions between microtubule growing and shrinking phases. Hence, hysteretic properties of microtubules can be viewed as a buffering mechanism for these fluctuations. However, such a putative biological role of hysteresis assumes that a particular molecular function, other than their differential stability properties, must be assumed for the differentially modified microtubules, since we have shown that the mean turnover rate of the global microtubule population was not modified at any of the transitions.

CONCLUSION

Although the role of post-translational modifications of microtubules remains an open question, the paradigm which assumes that post-translational modifications are the result rather than the cause of microtubule stability should be questioned, and is not applicable to multiple non-exclusive post-translational modification schemes having at least one regulation loop. Apparent stability of microtubule species does not necessarily result from particular intrinsic properties of the species, but depends also upon the mechanism and the kinetics of these modifications, as established experimentally by Kirschner's group (Schulze et al., 1987). In the case of tubulin, as for any post-translational modification of other proteins (Krishna and Wold, 1993), the main limitation is that one knows, at best, only the original, message-specified, complete sequence and the final, fully modified, product characterized (Laurent, 1995). All the reactions of interest take place between these endpoints and involve unknown, transient intermediates arising in the 'black box' of protein biosynthesis. Opening this black box requires the elucidation of the kinetic schemes and structure-function relationships of the enzymes involved in the modification processes. At present, there is no evidence that: (i) all post-translational modifications have a specific molecular-type function; (ii) all the enzymes ensuring the same type of modification in different species have the same structural, functional and regulatory properties; (iii) all cellular effectors of modification enzymes are identified. We are presently attempting to improve our view of the black-box contents, along both experimental and theoretical lines.

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REFERENCES

Adoutte, A., Delgado, P., Fleury, A., Levilliers, N., Lainé, M. C., Marty, M. C., Boisvieux-Ulrich, E. and Sandoz, D. (1991). Microtubule diversity in ciliated cells: evidence for its generation by post-translational modification in the axonemes of Paramecium and quail oviduct cells. *Biol. Cell* **71**, 227-245.

Argaraña, C. E., Barra, H. S. and Capputo, R. (1978). Release of [^{14}C]tyrosine from tubulinyl[^{14}C]tyrosine by brain extract. Separation of a carboxypeptidase from tubulin:tyrosine ligase. *Mol. Cell. Biochem.* **19**, 17-22.

Argaraña, C. E., Barra, H. S. and Capputo, R. (1981). Inhibition of tubulinyl-tyrosine carboxypeptidase by brain soluble RNA and proteoglycan. *J. Biol. Chem.* **256**, 827-830.

Barra, H. S., Arce, C. A., Rodríguez, J. A. and Caputto, R. (1974). Some common properties of the protein that incorporates tyrosine as a single unit and the microtubule protein. *Biochem. Biophys. Res. Commun.* **60**, 1384-1390.

Barra, H. S., Arce, C. A. and Argaraña, C. E. (1988). Posttranslational tyrosination/detyrosination of tubulin. *Mol. Neurobiol.* **2**, 133-153.

Bressac, C., Bré, M. H., Darmanaden-Delorme, J., Laurent, M., Levilliers, N. and Fleury, A. (1995). A massive new posttranslational modification occurs on axonemal tubulin at the final step of spermatogenesis in Drosopila. *Eur. J. Cell Biol.* **67**, 346-355.

Bulinski, J. G., Richards, J. E. and Piperno, G. (1988). Posttranslational modifications of α tubulin: detyrosination and acetylation differentiate populations of interphase microtubules in cultured cells. *J. Cell Biol.* **106**, 1213-1220.

Bulinski, J. C. and Gundersen, G. G. (1991). Stabilization and post-translational modification of microtubules during cellular morphogenesis. *BioEssays* **13**, 285-293.

Eddé, B., Rossier, J., Le Caer, J. P., Desbruyères, E., Gros, F. and Denoulet, P. (1990). Posttranslational glutamylation of α-tubulin. *Science* **247**, 83-85.

Eddé, B., Rossier, J., Le Caer, J. P., Berwald-Netter, Y., Koulakoff, A., Gros, F. and Denoulet, P. (1991). A combination of posttranslational modifications is responsible for the production of neuronal α-tubulin heterogeneity. *J. Cell Biochem.* **46**, 134-142.

Eipper, B. A. (1972). Rat brain microtubule protein: purification and determination of covalent bound phosphate and carbohydrate. *Proc. Nat. Acad. Sci. USA* **69**, 2283-2287.

Fleury, A. and Laurent, M. (1995). Microtubule dynamics and morphogenesis in Paramecium. I. Deployment and dynamic properties of a cortical network of acetylated microtubules in relation to the invariance of a morphogenetic field. *Eur. J. Protistol.* **31**, 190-200.

Gaertig, J., Cruz, M. A., Bowen, J., Gu, L., Pennock, D. G. and Gorovsky, M. A. (1995). Acetylation of Lysine 40 in α-tubulin is not essential in *Tetrahymena thermophila*. *J. Cell Biol.* **129**, 1301-1310.

Gard, D. L. and Kirschner, M. W. (1985). A polymer-dependent increase in phosphorylation of β-tubulin accompanies differentiation of a mouse neuroblastoma cell line. *J. Cell Biol.* **100**, 765-774.

Gundersen, G. G., Kalnoski, M. H. and Bulinski, J. C. (1984). Distinct population of microtubules: tyrosinated and non-tyrosinated tubulins are distributed differently in vivo. *Cell* **38**, 779-789.

Gundersen, G. G. and Bulinski, J. C. (1987). Post-polymerization detyrosination of α-tubulin: a mechanism for subcellular differentiation of microtubules. *J. Cell Biol.* **106**, 251-264.

Katz, J. and Rognstad, R. (1976). Futile cycles in the metabolism of glucose. *Curr. Top. Cell. Regul.* **10**, 238-289.

Khawaja, S. G. G. and Bulinski, J. C. (1988). Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level. *J. Cell Biol.* **106**, 141-150.

Kozminski, K., Diener, D. R. and Rosenbaum, J. L. (1993). High level expression of nonacetylable α-tubulin in *Chlamydomonas reinhardtii*. *Cell Motil. Cytoskel.* **25**, 158-170.

Kreis, T. E. (1987). Microtubules containing detyrosinated tubulin are less dynamic. *EMBO J.* **6**, 2597-2606.

Krishna, R. and Wold, F. (1993). Post-translational modification of proteins. *Advan. Enzymol.* **67**, 265-298.

Kumar, N. and Flavin, M. (1981). Preferential action of a brain detyrosinolating carboxypeptidase on polymerized tubulin. *J. Biol. Chem.* **256**, 7678-7680.

Kumar, N. and Flavin, M. (1982). A new tubulin-binding protein. *Biochem. Biophys. Res. Commun.* **106**, 704-710.

Laurent, M. and Fleury, A. (1993). A dynamical model for post-translational modifications of microtubules. *FEBS Lett.* **336**, 1-7.

Laurent, M. (1995). Pour une approche dynamique du fonctionnement cellulaire. *Medecine/Sciences* **11**, 119-123.

Laurent, M. and Fleury, A. (1995a). Microtubule dynamics and morphogenesis in Paramecium. II. Modelling of the conversion of a transient molecular signal into a morphogenetical process. *Eur. J. Protistol.* (in press).

Laurent, M. and Fleury, A. (1995b). A model with excitability and relay properties for the generation and the propagation of a Ca^{2+} morphogenetic wave in *Paramecium*. *J. Theor. Biol.* **174**, 227-236.

LeDizet, M. and Piperno, G. (1987). Identification of an acetylation site of *Chlamydomonas* α-tubulin. *Proc. Nat. Acad. Sci. USA* **84**, 5720-5724.

L'Hernault, S. W. and Rosenbaum, J. L. (1985). *Chlamydomonas* α-tubulin is post-translationally modified by acetylation on the ε-amino group of a lysine. *Biochemistry* **24**, 473-478.

Lieuvin, A., Labbé, J. C., Dorée, M. and Job, D. (1994). Intrinsic microtubule stability in interphase cells. *J. Cell Biol.* **124**, 985-996.

Maruta, H., Greer, K. and Rosenbaum, J. L. (1986). The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. *J. Cell Biol.* **103**, 571-579.

Mitchison, T. and Kirschner, M. W. (1984). Dynamic instability of microtubule growth. *Nature* **312**, 237-242.

Modesti, N. M., Argaraña, C. E., Barra, H. S. and Capputo, R. (1986). Inhibition of brain tubulinyl-tyrosine carboxypeptidase by endogenous proteins. *J. Neurosci. Res.* **12**, 583-593.

Modesti, N. M. and Barra, H. S. (1986). The interaction of myelin basic protein with tubulin and the inhibition of tubulin carboxypeptidase activity. *Biochem. Biophys. Res. Commun.* **136**, 482-489.

Nicolis, G. and Prigogine, I. (1977). Self-organization in non-equilibrium systems. Wiley, New-York.

Paturle, L., Wehland, J., Margolis, R. L. and Job, D. (1989). Complete separation of tyrosinated, detyrosinated and nontyrosinatable brain tubulin subpopulations using affinity chromatography. *Biochemistry* **28**, 2698-2704.

Raybin, D. and Flavin, M. (1977a). Enzyme which adds tyrosine to the α-chain of tubulin. *Biochemistry* **16**, 2189-2194.

Raybin, D. and Flavin, M. (1977b). Modification of tubulin by tyrosylation in cells and extracts and its effects on assembly *in vitro*. *J. Cell Biol.* **73**, 492-504.

Redeker, V., Levilliers, N., Schmitter, J. M., Le Caer, J. P., Rossier, J., Adoutte, A. and Bré, M. H. (1994). Polyglycylation of tubulin: a posttranslational modification in axonemal microtubules. *Science* **266**, 1688-1691.

Rüdiger, M., Plessmann, U., Rüdiger, A. H. and Weber, K. (1995). β tubulin of bull sperm is polyglycylated. *FEBS Lett.* **364**, 147-151.

Sammak, P. J. and Borisy, G. G. (1988). Direct observation of microtubule dynamics in living cells. *Nature* **332**, 724-726.

Schroeder, H. C., Wehland, J. and Weber, K. (1985). Purification of brain tubulin:tyrosin ligase by biochemical and immunological methods. *J. Cell Biol.* **100**, 276-281.

Schulze, E., Asai, D. J., Bulinski, J. C. and Kirschner, M. (1987). Posttranslational modification and microtubule stability. *J. Cell Biol.* **105**, 2167-2177.

Schulze, E. and Kirschner, M. (1988). New features of microtubule behavior observed *in vivo*. *Nature* **332**, 724-726.

Shelden, E. and Wadsworth, P. (1993). Observation and quantification of individual microtubule behavior *in vivo*: microtubule dynamics are cell-type specific. *J. Cell Biol.* **120**, 935-945.

Verde, F., Labbé, J. C., Dorée, M. and Karsenti, E. (1990). Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of *Xenopus* eggs. *Nature* **343**, 233-238.

Verde, F., Dogterom, M., Stelzer, E., Karsenti, E. and Leibler, S. (1992). Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.* **118**, 1097-1108.

Webster, D. R., Gundersen, G. G., Bulinski J. C. and Borisy G. G. (1987). Differential turnover of tyrosinated and detyrosinated microtubules. *Proc. Nat. Acad. Sci. USA* **84**, 9040-9044.

Webster, D. R. and Borisy, G. G. (1989). Microtubules are acetylated in domains that turnover slowly. *J. Cell Sci.* **92**, 57-65.

Webster, D. R., Wehland, J., Weber, K. and Borisy G. G. (1990). Detyrosination of α-tubulin does not stabilize microtubules *in vivo*. *J. Cell Biol.* **111**, 113-122.

Wehland, J. and Weber, K. (1987). Turnover of the carboxy-terminal tyrosine of α-tubulin and means of reaching elevated levels of detyrosination in living cells. *J. Cell Sci.* **88**, 185-203.

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