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*Hypothesis*

# A dynamical model for post-translational modifications of microtubules

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In many cases, post-translational modifications constitute indirect markers of non-dynamic microtubules. The correlation between stability of microtubular systems and post-translational modifications suggests that they may act as a signal in patterning mechanisms. However, a crucial question remains as to how the repertoire of distinctly modified microtubules is generated. We propose here an ubiquitous mechanism of spatial and temporal differentiation of microtubules. In this model, the diversity of post-translational modifications results from a dynamical pathway separation which is ensured by biochemical switches between self-regulated mechanisms of differentiation. Thus, it does not require any hypothetical subcellular compartmentalization of enzymatic activities responsible for the various post-translational modifications. These results are discussed in relation to experimental evidences for a temporal and spatial regulation of microtubule modifications.

Microtubule; Post-translational modifications; Dynamic systems; Bi-stability

## 1. INTRODUCTION

Eukaryotic cells possess several post-translational mechanisms whereby microtubules are enzymatically modified. Up to now, four distinct mechanisms have been identified: glutamylation [1], acetylation [2,3], phosphorylation [4] and detyrosination [5,6]. Although at least the three last reactions are found on stable microtubules, it has been demonstrated that post-translational modifications occur as a result, rather than as a cause of microtubule stability [7,8]. Microtubule stabilization is associated to a decrease in the turn-over of the macromolecular assembly and to the suppression of fluctuations of microtubule length associated with dynamic instability [9–12]. Stabilization probably results from interactions between microtubules and microtubules associated proteins (MAPs) and/or motors and from the formation of high-order assemblies such as microtubular bundles or complexes between microtubules and organelles [13,14].

Biochemical differentiation of microtubules leads to the elaboration of a polarized array of stable microtubules. Several experimental evidences indicate that the formation of this differentiated array of microtubules in specific locations at the onset of morphogenesis, plays an important role in generating cellular asymmetries [16,17]. More generally, it has been proposed [18] that the primary role of microtubule post-translational modifications is to mark the microtubules biochemically, enabling the cell to distinguish stable microtubules from dynamic ones. The multiplicity of types of modifications may also increase the functional repertoire of the cell so that different effector systems may utilize differentially the stable array of microtubules.

Whatever the postulated signalling function of post-translational modifications of microtubules, a crucial question remains as to how the repertoire of distinctly modified microtubules is generated. Any molecular mechanism must explain the establishment of location-specific and time-dependent differential post-translational modifications. In other words, at any time and cellular location, why does a subset of stable microtubules undergo one kind of modification whereas another subset goes through a different kind of modification? Also, why is only a subset of stable microtubules modified? Obviously, an hypothetical cellular compartmentalization of enzymes which carry out post-translational modifications does not suffice to answer both questions. In this paper, we show that a simple and generic model for the dynamics of post-translational modifications is able to ensure, both spatially and temporally, a segregation between two subsets of stable microtubules.

## 2. MODEL

Post-translational processes of modification are supposed to account for the cyclic mechanism of tyrosination/detyrosination of α-tubulin that has been thoroughly studied [19]. In this system, a tubulin-specific carboxypeptidase removes a tyrosyl residue from the C-terminal end of α-tubulin protomers within microtubules [20] while the reverse reaction of tyrosination is

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performed on the soluble $\alpha\beta$-dimer by another enzyme, namely the tubulin tyrosine ligase [21,22]. Thus, enzymatic activities of tyrosination/detyrosination are partitioned between separate pools of tubulin (protomeric and polymeric forms, respectively). This partitioning mechanism has to be thought of as a rather general process for post-translational modifications because it prevents a futile cycle [19,23] to occur (remember that one of the two processes, tyrosination in that case, is coupled to ATP-hydrolysis).

For the sake of simplicity, we assume that the stable microtubules (MT) have only two biochemical pathways of differentiation. Each of them, leading respectively to the MT-Y and MT-X forms of modified microtubules, corresponds to a distinct post-translational mechanism of modification. However, this model also account for a slightly different situation: only the MT-Y formation pathway can correspond to a post-translational mechanism of modification. The other formation pathway may represent either further stabilization of MT microtubules by MAPs or interacting organelles or, on the contrary, a shrinking process of stable microtubules. In the last case, the output $v_2$-reaction (see below) corresponds simply to the depolymerization process of stabilized MT species and MT-X represents unmodified tubulin monomer ($v_4$-reaction would have no meaning in this simplified scheme).

The stringent theoretical requirement to observe a threshold behaviour is the existence of at least one non-linear reaction. The non-linearity can be introduced by considering that the enzyme which produces the MT-Y form of post-translationally modified microtubules is inhibited by an excess of its substrate. Although this hypothesis might appear as speculative in the present state of our knowledge of modification enzymes, we may remark, however, that the in situ regulatory properties of tubulinyl acetyl transferase are probably complex. Maruta et al. [24] have demonstrated the existence, in a cell body extract of *Chlamydomonas*, of a cytoplasmic inhibitor of the tubulinyl acetyl transferase activity yet unidentified. This inhibitory effect was shown to be distinct from that which would result from a tubulinyl deacetylase activity and, for that reason, might be tentatively modelized as an ante-inhibition loop. Note also that in the case of glutamylation, data of Fouquet et al. [25] suggest the existence of multiple tubulinyl glutamyl transferase isoenzymes. Thus, enzymatic processes of post-translational modification do not match necessarily simple Michaelis-Menten schemes.

According to the cyclic model for the tyrosination-detyrosination of $\alpha$-tubulin [19], MT-X and MT-Y species do not accumulate: they are involved in output reactions which correspond to the first step of depolymerization of the modified microtubules (in the case of the complete scheme). Thus, the overall scheme of individual reactions which has to be considered is given in Fig. 1.

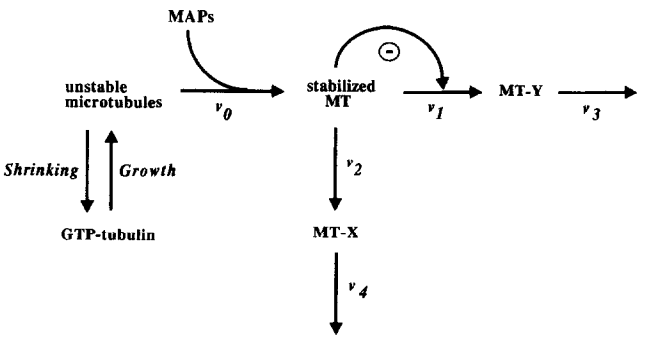


Fig. 1. Overall scheme for growth, shrinking, stabilization ($v_0$) and post-translational modifications ($v_1$ and $v_2$) of microtubules. Only two ways of differentiation (leading to MT-X and MT-Y species) are assumed for the stabilized form of microtubules (MT). MT to MT-Y transformation is catalyzed by an enzyme which is regulated by a feedback loop (anteinhibition process symbolized by the (−) curved arrow).

The system is described by the following differential equations:

$$d[MT]/dt = v_0 - v_1 - v_2 \quad \text{(I)}$$
$$d[MT\text{-}Y]/dt = v_1 - v_3 \quad \text{(II)}$$
$$d[MT\text{-}X]/dt = v_2 - v_4 \quad \text{(III)}$$

The kinetic processes described by $v_2$, $v_3$ and $v_4$ are supposed to correspond to first-order rate equations. At first, we consider that the rate of stabilization of microtubules ($v_0$) is a zero-order process. While this simplifying assumption will be overcome later, we have presently:

$$v_0 = k_f$$
$$v_2 = k_2[MT]$$
$$v_3 = k_3[MT\text{-}Y]$$
$$v_4 = k_4[MT\text{-}X]$$
$$v_1 = V_M[MT]/(K_m + [MT] + [MT]^2/K_{si})$$

where $V_M$, $K_m$ and $K_{si}$ are the maximum velocity, the Michaelis constant and the inhibition constant of the enzyme which catalyze the MT-Y formation step, respectively [26].

Insights into the dynamical behaviour of the system will be first obtained grapho-analytically by examining this set of equations. Temporal and spatial evolutions will be described by numerical integration of differential Eqns. I–III. For numerical simulations, the constant values will be chosen such as the steady-state microtubule concentrations are of the same order of magnitude as those estimated by Mitchison and Kirschner [9] in fibroblasts, i.e about 30 $\mu$M. However that estimation was simply obtained by multiplying the average length by the average number of microtubules and might be rather imprecise. Moreover, in differentiated cells, concentrations would have to be calculated for

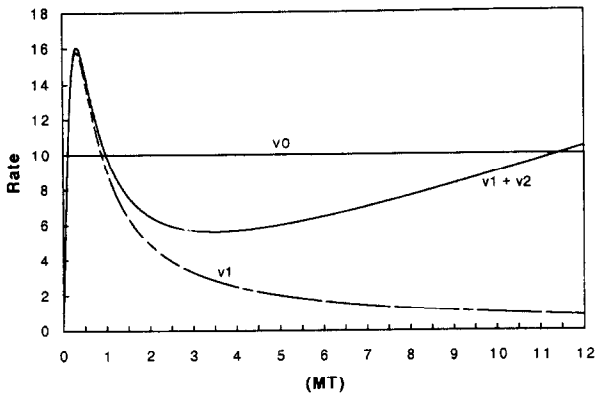


Fig. 2. Individual rates of production ($v_0$) and removal ($v_1+v_2$) processes of stabilized microtubules MT. Non-enzymatic production rate ($v_0$) of MT is assumed to be constant (independent of MT concentration). $v_2$ is assumed to correspond to a first-order rate process: $v_2 = k_2$[MT]. The kinetic law of disappearance of MT through the $v_1$-catalyzed reaction is given by: $v_1 = V_M$[MT]/($K_m$ + [MT] + [MT]$^2$/$K_{si}$), that is a reaction in which the enzyme is inhibited by an excess of its substrate. According to $v_0$ value, ($v_0$) and ($v_1 + v_2$) graphs may have one or three intercepts, i.e. the system has one or three steady-states. Exemplified curves were obtained using the following set of parameter values: $V_M = 10^4\ \mu M \cdot s^{-1}$, $K_m = 10^2\ \mu M$, $K_{si} = 10^{-3}\mu M$, $v_0 = 10\,\mu M \cdot s^{-1}$, $k_2 = 0.8\ s^{-1}$. Accordingly, reaction rates are expressed in $\mu M \cdot s^{-1}$ and MT concentrations in $\mu M$.

each compartment and not for the whole cell volume. Hence, in the absence of accurate quantitative experimental data, we must focus our attention on the qualitative behaviour that our model generates rather than attempting to fit quantitative parameter values.

## 3. RESULTS

Fig. 2 shows the MT-concentration dependence of kinetic processes involving unmodified stable microtubules MT. Obviously, the steady-state concentrations of MT correspond to the condition d[MT]/d$t$ = 0, i.e. MT-production rate $v_0$ equals the sum $v_1 + v_2$ of the rates of MT-removal processes. Over a wide range of $v_0$ values (Fig. 2), MT-input and -output graphs have three intercepts which define three steady-states for the system. In order to analyze the local stability properties of these steady-states, the net rate of MT-formation as a function of MT concentration has been reported on Fig. 3. The stability properties of each stationary state $SS_1$, $SS_2$ and $SS_3$ can be investigated by determining whether infinitesimal perturbations in [MT] away from the steady-state will decay or grow with time. Let us suppose that the system initially in $SS_1$ or $SS_3$ is perturbated by a slight positive fluctuation in MT concentration, d[MT] (right horizontal arrow). In these conditions, the net rate of MT-production becomes negative, i.e. rate of MT-consumption exceeds rate of MT-formation. Thus, the fluctuation is spontaneously eliminated and the system comes back to its initial steady-state. If d[MT] is negative (left horizontal arrow), the net rate of MT-production is positive: MT-production rate exceeds MT-consumption rate and once again the system will move back to its steady-state. Thus $SS_1$ and $SS_3$ are stable steady-states. On the contrary, small fluctuations around $SS_2$ will be amplified (Fig. 3) and $SS_2$ is thus instable.

Splitting up the global process into its component parts (as shown in Fig. 2) allows to understand what is the parametric dependence of this multistable behaviour. For instance, for a given $v_0$ value and a given $v_1$ curve (i.e. for a set of $V_M$, $K_m$ and $K_{si}$ parameter values), a decrease in the $k_2$ value would only increase the MT concentration corresponding to the $SS_3$ steady-state. On the contrary, an increase in $k_2$ would bring $SS_3$ closer to $SS_2$ on the [MT] axis. However, beyond a limit in $k_2$ values, the median branch of the ($v_1 + v_2$) curve would disappear and the system would have only one steady-state. But this limit has no physiological sense because such a situation corresponds to a case in which the fluxes would always occur through the MT-X pathway. Hence MT-Y species would never be detected, whatever the experimental conditions. Note also that $V_M$ parameter is not a fundamental property of the enzyme which catalyze $v_1$-reaction since it depends upon enzyme concentration ($V_M = k_{cat}$[$E_T$]). Hence, the greater the concentration of this enzyme, the larger the range of $v_0$ values for which the system has three steady-states.

Since the system has two stable steady-states and not one, it is clear that their stability must be local rather than global. If the system is in one stable steady-state and is then acted on by an external influence (an increase in $v_0$ input value for instance) which is sufficient to bring it above the threshold $SS_2$, it will then settle into

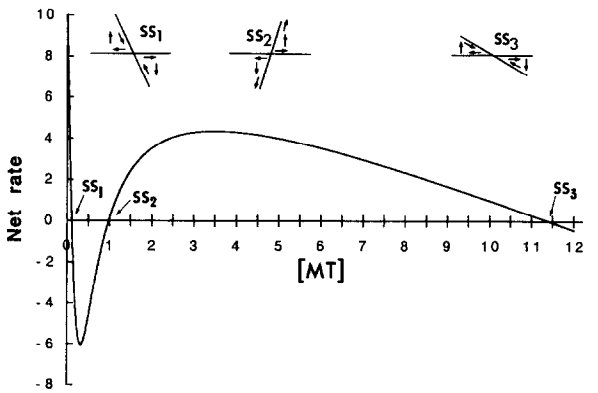


Fig. 3. Net rate of MT-production as a function of MT concentration. Analysis of the local stability properties of the three steady-states $SS_1$, $SS_2$ and $SS_3$. Net rate of MT-production (in $\mu M \cdot s^{-1}$) represents the algebraic difference between production and removal processes for MT species, as shown in Fig. 2. Steady-state conditions correspond to MT concentrations (expressed in $\mu M$) for which the net rate of MT-production is null. The local stability properties of the system are examined (inserts) by producing slight perturbations in MT concentration around each of the three steady-states. As discussed in the text, small fluctuations disappear around $SS_1$ or $SS_3$, while they are amplified around $SS_2$. Thus, $SS_1$ and $SS_3$ are stable steady-states whereas $SS_2$ is unstable. (Same parameter values as in Fig. 2).

the other stable steady-state and remain there even when the fluctuation is removed.

Although the in vivo conditions of microtuble stabilization by MAPs are not yet known, we may suppose that it is not necessarily a zero-order process as hypothetized above. Thus, if $v_0$ is now considered as a phenomenological variable, the dynamical behaviour of the system will be described by considering the $(v_1 + v_2)$ curve in Fig. 2 as the trajectory of steady-states. Its region of negative slope corresponds to unstable stationary states whereas the regions of positive slope are associated to stable steady-states ($SS_3$ for the branch located on the right of the unstable domain and $SS_1$ for the branch lying on the left of this domain). Let us suppose that the system is initially on the $SS_1$ branch and that a continuous increase in $v_0$ input value occurs. Until the stationary state lies on the $SS_1$ branch, MT concentration is slightly readjusted, in accord with the solution trajectory. But when $v_0$ exceeds the threshold value (which corresponds to the change in the sign of the slope of the solution trajectory), the system moves to the $SS_3$ branch and a strong discontinuity (jump-like transition) appears for the steady-state concentration of MT. The same conclusion holds for the reverse transition from $SS_3$ to $SS_1$ as $v_0$ decreases, but the jump-like transition occurs for a threshold value which is different from the previous one. Thus the transitions between the alternate stable stationary states upon $v_0$ variations are hysteretic [27].

What is the meaning of this bi-stable behaviour in terms of MT-X and MT-Y concentrations, i.e. in terms of post-translational modifications? As shown in Fig. 2, the stable steady-state $SS_1$ obtained at the lowest MT concentration corresponds to conditions in which $v_1$ dominates over $v_2$. On the contrary, $v_1$ is almost negligible (with respect to $v_2$) when the system is in its stable steady-state $SS_3$ (corresponding to highest MT concentration). According to Eqns. II and III, this means that the transition between $SS_1$ and $SS_3$ corresponds to a switch between MT-Y and MT-X formation pathways. This alternative behaviour is illustrated by the simulations of Fig. 4 (obtained by numerical integration of Eqns. I–III) in which a shallow linear gradient in $v_0$ values is assumed to take place at time 0 along the space axis. Only those positions exposed to a $v_0$ value above a threshold, switch from one stable steady-state to the other, i.e. from one pathway of microtubule differentiation to the other. Despite the shallowness of the variable signal, the system responds unambiguously in an all-or-none manner. As expected from the switching process and the values of the kinetic parameters in the model ($k_2 = k_3$ in these simulations), variations in the three-dimensional concentration profiles of MT-X and MT-Y distributions are complementary.

Our model also allows to interpret different situations whose interest is particularly obvious if only the MT-Y formation pathway corresponds to a post-translational mechanism of modification, MT-X pathway corresponding to the depolymerization of unmodified stable microtubules (see section 2). Since Eqn. I does not include the $v_3$ rate, the $k_3$ parameter value determines solely the absolute concentrations of MT-Y in $SS_1$ and $SS_3$ steady-states (Table I) but does not modify the dynamical properties of the system. When $k_2 = k_3$ (line 1 in Table I), the switch between $SS_1$ to $SS_3$ steady-states produces a switch between the stationary concentrations of MT and MT-Y without any significant modification of the total microtubule concentration ([MT] + [MT-Y]). That means that, in these conditions, a microtubular unmodified network is quasi-quantitatively replaced by a post-translationally modified network. This situation is of course reminiscent of that of Fig. 4, for a slightly different interpretation of the model. On the contrary, if $k_2$ is significantly greater than $k_3$ (line 2 in Table I), the residual flux through the 'switched-off' pathway in the steady-state $SS_3$ is significantly greater than 0. Thus, only a subset of stable microtubules MT may be modified, even if the corresponding modification pathway is switched-on. Finally, strong differences between $k_2$ and $k_3$ values (line 3 in Table I) lead to a situation in which concentrations of total microtubules ([MT] + [MT-Y]) in $SS_1$ and $SS_3$ steady-states are not of the same order of magnitude. However, such an occurrence may be questionable from a physiological point of view since it seems unlikely that the depolymerization rate of unmodified and modified microtubules can be so different.

Table I

Concentrations ($\mu$M) of MT and MT-Y species in $SS_1$ and $SS_3$ steady-states as a function of the $k_3$ parameter value ($s^{-1}$)

| $k_3$ | [MT]$ss_1$ | [MT-Y]$ss_1$ | [MT]$ss_3$ | [MT-Y]$ss_3$ |
|---|---|---|---|---|
| 0.4 | 0.23 | 37.4 | 49.5 | 0.5 |
| 0.08 | 0.23 | 187 | 49.5 | 22 |
| 0.008 | 0.23 | 1871 | 49.5 | 22 |

Steady-state concentrations were calculated by numerical integration of Eqns. I and II with the following set of parameter values: $V_M = 10^4$ $\mu M \cdot s^{-1}$, $K_m = 10^2$ $\mu$M, $K_{si} = 10^{-3}$ $\mu$M, $k_2 = 0.4\ s^{-1}$ and $k_f = 10$ (for $SS_1$ steady-state) or 20 $\mu M \cdot s^{-1}$ (for $SS_3$ steady-state).

## 4. DISCUSSION

Although it accounts for the experimental data on the best-known molecular mechanism of post-translational modification of microtubules, our model of spatial and temporal differentiation will have to be somehow modified as further information accumulates on the different steps of the enzymatic mechanisms of post-translational modifications. For instance, this model could be easily adapted to the possible existence of MT species having several sites of post-translational modifications. To a large extent, however, the present analysis is independ-

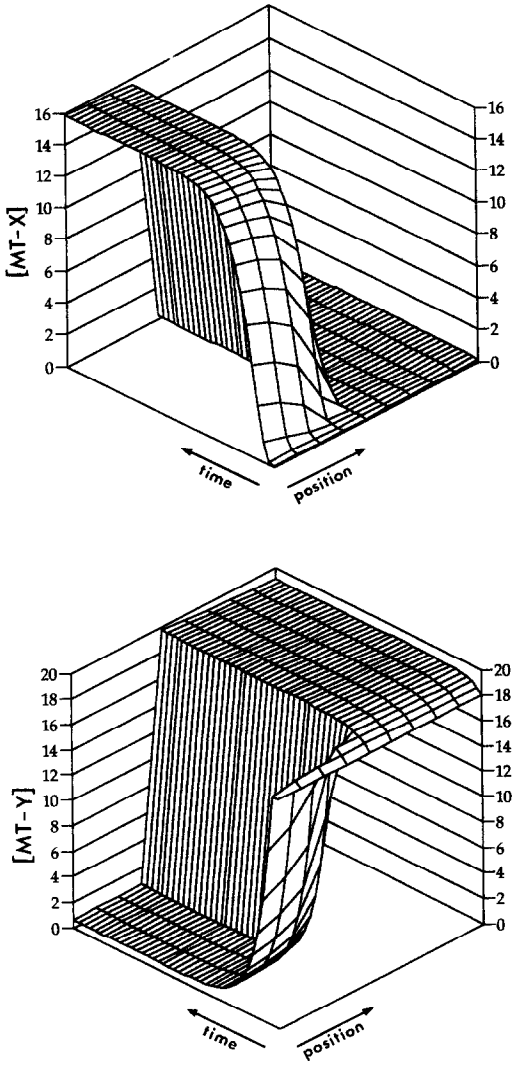


Fig. 4. Complementary pattern formation of MT-X and MT-Y species as a function of time and space, according to the model of Fig. 1. Starting from an uniform distribution for each of the MT-X and MT-Y species (in the vicinity of the stable steady-state $SS_1$ in which MT-Y formation pathway is predominant), a shallow linear gradient in $v_0$ values (from 16.5 to 15.5 $\mu M \cdot s^{-1}$ for the ten discrete positions calculated on the figure) is applied at time 0 along the space axis. For positions which are above a threshold, the system is switched to $SS_3$ steady-state which corresponds to high MT-X and low MT-Y concentrations. The curves were calculated by numerical integration of Eqn. I to III with the same set of kinetic parameter values as in Fig. 1 and the following initial conditions (at time 0): $[MT]_0 = 0.2$ $\mu M$; $[MT\text{-}X]_0 = 0.1$ $\mu M$; $[MT\text{-}Y]_0 = 18$ $\mu M$. Accordingly, [MT-X] and [MT-Y] scales are expressed in $\mu M$ and overall time-scale represents 5 min. For each position, 400 steps of time-dependent integrations were performed (but one out of ten was plotted).

ent of the details of the individual processes. In fact, the skeleton of our model consists of an open system in which MT species have at least two ways of determination, one of these ways being regulated by an anteinhibition process. However, the molecular nature of the feedback loop is to some extent irrelevant to the dynamics of the system: according to Franck [28], we can show that anteinhibition or retroactivation mechanisms both lead to the same global characteristic of reaction (Fig. 3) which has two stable stationary states around another unstable steady-state.

In our model, the diversity of post-translational modifications of microtubules results from a dynamical pathway separation. Here the expression 'pathway separation' has the following meaning: different biochemical processes share the same intermediate (here unmodified stable microtubules) serving as substrates for distinct enzymatic modifications. The separation of the different processes is ensured by environmental conditions under which only one of the processes occurs at a time and in a particular location and an alternation of the activities of the other processes is realized in time and in space. A spatial separation is commonly thought of as being achieved by the localization of distinct processes in different cellular compartments. For instance, distinct enzymatic activities of post-translational modification of microtubules would be partitioned between membraneous and cytosolic compartments. According to this idea, one kind of modification of microtubules would affect microtubules in contact with or in the vicinity of cellular membranes while an other kind of modification would affect cytosolic microtubules. Although not irrealistic in some cases [29], the major drawback of such a static view is that it does not explain why, in the same location, a multiplicity of distinct modifications may occur on subsets of microtubules. Moreover, it supposes that cellular responses to environmental perturbations depend on a strict local determinism (governed, for instance, by the cellular location of enzymatic activities). Experiments performed on a model system made of cells migrating with known polarity in a wounded monolayer system [15], does not favour such a conception. These studies show that cells immediatly adjacent to the wound acquire an extensive array of post-translationaly modified microtubules oriented towards the direction of impending migration (these cells will further migrate into the empty space).

Experiments of Bulinski et al. [30] may be reinterpreted in light of our model. They show that in human HeLa cells, only the acetylated form of tubulin is detectable as post-translationally modified microtubules. However, treatment of these cells with taxol (a microtubule-stabilizing drug) results in a dramatic increase in the level of both acetylated and detyrosinated forms of microtubules. Moreover, the cellular pattern of both forms in taxol-treated cells are indistinguishable from the total microtubule pattern. This agrees with the interpretation that in interphase cultured HeLa cells, two distinct enzymatic machineries of post-translational modification of microtubules are present but only one of them is expressed in normal conditions.

Examination of microtubule behaviour in the course of cell differentiation provide experimental evidences for a temporal and spatial regulation of microtubule

modifications. For instance, the staining pattern obtained with an anti-acetylated tubulin antibody on quail oviduct cells [31] may be interpreted as a change in the regulative properties of tubulinyl acetyl transferase during the course of differentiation. In undifferentiated cells, the enzyme is active in the cytoplasm whereas in cells undergoing ciliogenesis, in which microtubules reorganize to assemble in cilia, the enzyme is only active in the ciliary compartment. In the same way, in proliferating rat $L_6$ myoblasts, as in other types of proliferating cells, the level of detyrosinated microtubules is very low when compared with the level of unmodified microtubules. However, when $L_6$ cells are shifted to differentiation media, a rapid accumulation of detyrosinated tubulin is observed in cellular microtubules [17]. Acetylation of microtubules also increases during $L_6$ myogenesis but, when compared to the detyrosination process, this event is delayed in the course of differentiation. Another striking example of a temporal and spatial regulation of microtubule modification is given by the studies of Fouquet et al. [25] on the differential distribution of post-translationally modified microtubules during spermatogenesis in mamalian testis. In germ and somatic cells, there is, for each post-translational modification, a differential distribution of microtubular species among the different structures (axoneme, centriole, manchette, spindle midbody and Sertoli cells). Moreover, each microtubular structure exhibits a particular combination of the diverse isoforms of microtubules (detyrosinated, tyrosinated, acetylated and glutamylated). This subcellular sorting is not related to compartmentalization of defined isotypes since, for instance, two isotypes which were shown to be present both in the axoneme and in the manchette are glutamylated in the former but not in the latter.

However, metazoan cells often display low level of cellular differentiation in controlled culture or else they may undergo a process of differentiation but become unable to divide. This is not the case for ciliates which thus provide a suitable model for the study of microtubule diversity. Moreover, these species contain very little if any genetic diversity of tubulin genes. Hence, the molecular diversity essentially results, in that case, from post-translational modifications. Adoutte et al. [31] have previously shown that there exists, in *Paramecium*, an initial stage for all newly assembled microtubular arrays during which, although they are well identified by anti-sequence tubulin antibodies, they are unreactive or weakly reactive with the anti-PA tubulin one (this antibody is directed against a not yet well characterized post-translational modification). Moreover, studies on the dynamics of paratene-associated microtubules (a subcortical array of acetylated microtubules) in the course of the cell cycle of *Paramecium*, show that this system behaves as a bi-stable system, the transient inhibitory effect of $Ca^{2+}$ on tubulinyl acetyl transferase having a triggering action on the dynamics of these microtubules (Laurent and Fleury, manuscript in preparation). This last example shows that in order to understand the processes of pattern formation at the molecular level, the language of dynamical systems theory seems a natural one to use [32]. In this way, post-translational modifications of microtubules which correspond to biochemical switches between different pathways of differentiation, must simply be seen as a trigger behaviour caused by hysteretic transitions between alternate stable steady-states.

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