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MINIREVIEW

Submembraneous microtubule cytoskeleton: regulation of ATPases by interaction with acetylated tubulin

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The ATP-hydrolysing enzymes (Na^+,K^+)-, H^+- and Ca^{2+}-ATPase are integral membrane proteins that play important roles in the exchange of ions and nutrients between the exterior and interior of cells, and are involved in signal transduction pathways. Activity of these ATPases is regulated by several specific effectors. Here, we review the regulation of these P-type ATPases by a common effector, acetylated tubulin, which interacts with them and inhibits their enzyme activity. The presence of an acetyl group on Lys40 of α-tubulin is a requirement for the interaction. Stimulation of enzyme activity by different effectors involves the dissociation of tubulin/ATPase complexes. In cultured cells, acetylated tubulin associated with ATPase appears to be a constituent of microtubules. Stabilization of microtubules by taxol blocks association/dissociation of the complex. Membrane ATPases may function as anchorage sites for microtubules.

Among the huge number of membrane ATPases, those of the P-type (so termed because they undergo autophosphorylation during the ATP-hydrolysis cycle) have been extensively studied. Subunit composition, polypeptide sequence, spatial organization, molecular mechanism of enzyme activity and coding genes have been elucidated in detail for the (Na^+,K^+)-, H^+- and Ca^{2+}-ATPases. Energy generated by the ATP-hydrolysing activity of these enzymes is used in part to transport ions in to and out of cells. Regulation of the ATPase activity of these 'ion pumps' has been intensively studied because of their physiological importance. Regulatory mechanisms at the transcriptional level have been described for the P-type ATPases, and fast regulatory mechanisms at the membrane level seem to occur as well. One of these mechanisms involves interaction of the P-type ATPase with acetylated tubulin, apparently in the polymerized state.

Tubulin, the main component of microtubules, is a heterodimer comprised of α and β subunits. Multiple forms of α and β subunits have been identified as products of different genes [1,2], and as results of post-translational modifications including phosphorylation, tyrosination/detyrosination, acetylation, polyglycylation, polyglutamylation, and palmitoylation [3]. Microtubules are localized in cytoplasm, and are essential for cell structure and division. In proliferating cells, they form the mitotic spindle or, during interphase, a

Abbreviations
HDAC6, histone deacetylase 6; PMCA, plasma membrane calcium pump.

typical radial array. In differentiated cells, microtubules adopt diverse, cell-specific arrays. The functions of microtubules have been intensively studied, with emphasis on chromosome segregation and intracellular transport. Increasing evidence indicates that microtubules are also involved in cell polarity and in signal transduction processes. All microtubule functions are based on a delicate equilibrium between polymerized and nonpolymerized states. Although the integrity of microtubules is essential for their proper functioning, αβ-tubulin dimer in the nonassembled state can participate in other cell functions by interacting with different proteins. A tubulin 'pool' is present in membranes, and several integral membrane proteins, in addition to cytoplasmic proteins, have been reported to interact with assembled or nonassembled tubulin. Here, we review results indicating that the acetylated form of tubulin interacts with plasma membrane cation ATPases and regulates their enzymatic activities.

Membrane tubulin

The presence of tubulin in isolated membranes from various types of cells and tissues has been known since 1970 [4–10]. The facts that soluble tubulin can associate with isolated membranes [11,12], and that apparent tubulin content in membrane increases with the time spent for its isolation [13], made it difficult to rule out the possibility that membrane-associated tubulin is an *in vitro* artefact. However, more recent studies, using various approaches, demonstrate that tubulin binds to integral membrane proteins as the result of processes involved in normal cell functioning [14–20]. It is probable that other pools of tubulin are also linked to membranes through different mechanisms.

Association of tubulin with the sodium pump

Types of tubulin which were originally considered as integral membrane components are now viewed as peripheral proteins that remain associated with the membrane through interaction with integral proteins. When brain membranes are extracted at 4 °C with a solution containing 0.5–1% Triton X-114, and the preparation is partitioned (by increasing the temperature to 37 °C), part of total membrane tubulin appears in the detergent phase [21,22]. The hydrophobic behaviour of tubulin is due to its association with an integral membrane component that was identified as the α subunit of Na^+,K^+-ATPase [23]. When brain membranes are treated with 0.1 M Na_2CO_3 (pH 11), the associated tubulin molecule is released from membranes, and partitions into the aqueous phase rather than the detergent-rich phase [22]. Because this tubulin can be released without disrupting the lipidic bilayer [22], it was considered to be a peripheral rather than integral membrane protein [24–26].

The interaction of tubulin with ATPase also occurs *in vitro*; isolated membranes showed increased tubulin/ATPase complex content when incubated in the presence of cytosolic tubulin [27]. The nature of the forces that maintain the association of tubulin with ATPase is unclear. However, it seems unlikely that ionic bonds are involved, because the interaction of tubulin with membranes is not suppressed by treatment with 2 M NaCl [21]. The existence of a complex containing acetylated tubulin and Na^+,K^+-ATPase is supported on three observations: (a) Triton X-114 partition property of tubulin and Na^+,K^+-ATPase, as summarized above; (b) both proteins precipitate when detergent-solubilized membrane is incubated with Sepharose-linked anti-tubulin IgG [28]; and (c) ATPase contained in a detergent-solubilized membrane preparation was retained on a tubulin-linked Sepharose column [27]. The demonstration of a complex containing Na^+,K^+-ATPase and acetylated tubulin does not rule out the possibility that the complex contains additional components. For simplicity, the term 'tubulin/Na^+,K^+-ATPase complex' has been used in most studies.

Despite the lack of conclusive evidence, several observations suggest that tubulin interacts directly with ATPase. Commercial, purified Na^+,K^+-ATPase associates with microtubules *in vitro* [23]. The simplest explanation for the observed inhibition of Na,K-ATPase (as well as H^+- and Ca^{2+}-ATPase; see below) activity by purified tubulin is the association of the two molecules. Regardless of the type of ATPase, this observation suggests involvement of the catalytic subunit in the interaction with acetylated tubulin. SDS/PAGE analysis (Coomassie Brilliant Blue staining) of membrane components that associate with microtubules reconstituted from purified tubulin showed only one prominent protein band, which was identified as Na^+,K^+-ATPase [23]. If a third (or more) protein was acting as an intermediate, it should appear in the gel (considering 1 : 1 : 1 stoichiometry). We are currently producing recombinant polypeptides corresponding to cytosolic fragments of Na^+,K^+-ATPase in order to identify the domain that interacts with tubulin. Cross-linking experiments, and determination of co-localization of acetylated microtubules and Na^+,K^+-ATPase using immunogold, confocal and total internal reflection fluorescence (TIRF) microscopy, are also in progress.

Modulation of Na^+,K^+-ATPase activity by association/dissociation of acetylated tubulin with the enzyme

Na^+,K^+-ATPase is an integral plasma membrane protein which uses energy from ATP hydrolysis to transport three Na^+ ions out of the cell and two K^+ into the cell, thereby generating an electrochemical gradient across the membrane. Proper functioning of this enzyme is essential for the maintenance of body fluid and electrolyte homeostasis [29,30]. Na^+,K^+-ATPase has two subunits: the catalytic α subunit (molecular mass ~ 110 kDa) and the glycosylated β subunit (protein mass ~ 31.5 kDa). In some tissues, the Na^+,K^+ pump has a small additional γ subunit (7.3 kDa) involved in regulation of enzyme activity [31,32]. Different isoforms of the Na^+,K^+-ATPase subunits are found in different tissues. The α subunit has 10 transmembrane segments, and its N- and C-termini are localized on the cytosolic side [33]. Na^+,K^+-ATPase is a member of the P-type ATPase superfamily, which is characterized by phosphorylation of an aspartyl residue localized within the highly conserved sequence DKTGS/T of the α subunit [34]. Many normal physiological processes, including nerve impulse transmission, nutrient uptake and pumping out of Ca^{2+}, depend on the electrochemical gradient resulting from activity of the sodium pump. Certain pathological processes such as arterial hypertension and altered excretion of Na^+ by the kidneys are related to disorders in the active transport of ions by the sodium pump, with involvement of cytoskeletal components.

Na^+,K^+-ATPase activity is regulated by a variety of factors including phosphorylation by protein kinase A and protein kinase C [35,36], cation occlusion [37,38], FXYD proteins [39–42], adducin [43,44] and L-glutamate [45].

After discovering that acetylated tubulin forms a complex with Na^+,K^+-ATPase, we investigated the effect of this association on enzyme activity and found that brain plasma membrane Na^+,K^+-ATPase was inhibited *in vitro* by acetylated tubulin [27]. The degree of inhibition was correlated with: (a) the concentration of acetylated tubulin isoform present in the tubulin preparation used, and (b) the amount of acetylated tubulin isoform associated with Na^+,K^+-ATPase. Inhibition was abolished by inorganic phosphate in a concentration-dependent manner, with a parallel decrease in the association of acetylated tubulin with the enzyme [27].

Involvement of acetylated tubulin in the regulation of Na^+,K^+-ATPase activity in intact cells was first demonstrated using cultured astrocytes [44]; stimulation of Na^+,K^+-ATPase activity by L-glutamate was correlated with a decreased quantity of acetylated tubulin/Na^+,K^+-ATPase complex. When the amount of complex was decreased by 50%, the enzyme activity was stimulated to double. This indicates that before stimulation, ~ 66% of the enzyme was associated with tubulin [46]. Glutamate mediates most excitatory synaptic transmissions in the brain by interacting with specific receptors [47]. By contrast, L-glutamate transporters facilitate the uptake of glutamate, thus lowering its concentration in the extracellular space and inducing activation of Na^+,K^+-ATPase activity. Glutamate uptake by transporters increases the sodium concentration within the cell. In astrocytes, three Na^+ ions (or two Na^+ and one H^+) accompany glutamate entry, whereas one K^+ is transported out accompanied by either one OH^- or one HCO_3^- [48].

We showed that the effect of glutamate on Na^+,K^+-ATPase activity is reversible. When astrocytes were treated with L-glutamate and subsequently maintained in glutamate-free medium containing 1 mM D-glucose, enzyme activity decreased and the level of acetylated tubulin/Na^+,K^+-ATPase complex increased [46,49]. The amount of acetylated tubulin associated with Na^+,K^+-ATPase was monitored by quantifying the tubulin partitioned by Triton X-114. One might expect that at maximal stimulation of enzyme activity by glutamate, acetylated tubulin would be absent in the detergent phase. However, the amount of tubulin appearing in the detergent phase was decreased by only ~ 50% [46,49]. This result suggests that some forms of acetylated tubulin–enzyme complex are not dissociated by L-glutamate, or that part of the acetylated tubulin pool was associated with membrane components other than Na^+,K^+-ATPase [23].

In astrocytes, the effect of L-glutamate was abolished by the glutamate transporter inhibitor DL-threo-β-hydroxyaspartate but was not affected by either specific agonists or antagonists of specific L-glutamate receptors [46]. The effect of L-glutamate appears to be mediated by Na^+ entry resulting from glutamate transport. This concept was supported by the finding that the Na^+ ionophore monensin increases Na^+,K^+-ATPase activity with concomitant dissociation of the complex.

Stimulation of Na^+,K^+-ATPase activity involving dissociation of acetylated tubulin/Na^+,K^+-ATPase complex was also found in COS, Hep-2, CHO, L6 and NIH3T3 cells [49]. In these non-neural cells, similarly to neural cells, the effect of L-glutamate was mediated by L-glutamate transporters, not by specific L-glutamate receptors. L-Glutamate -specific receptors and transporters have been reported in non-neural cells [50–54].

No effect of L-glutamate was observed when Na^+ was replaced by K^+ in the incubation medium.

Because Na^+ is a ligand of Na^+,K^+-ATPase, one possibility is that complex dissociation involves an interaction between the enzyme and Na^+ that accompanies the entry of L-glutamate. However, treatment of isolated membranes with sodium ions did not dissociate the complex or activate the enzyme [27]. L-Glutamate and the Na^+ ionophore monensin induced dissociation of acetylated tubulin/enzyme complex in living cells but not in isolated membranes. These findings indicate that the complex dissociation process requires ordered spatial organization of the enzyme, acetylated tubulin, L-glutamate transporter and possibly additional components. Directionality of the flux may also be an important factor in complex dissociation.

Tubulin must be acetylated at Lys40 of the α subunit to interact with Na^+,K^+-ATPase

Tubulin acetylation is a post-translational modification consisting of the reversible addition of an acetyl group on the ε-amino group of a conserved lysine residue at position 40 of the α subunit [55,56]. The enzyme that catalyses this reaction has not been identified. However, a tubulin acetyl transferase activity from *Chlamydomonas* flagella has been described [57]. Acetylation occurs preferentially on tubulin assembled into microtubules, which is remarkable because crystallographic data indicate that the acetyl group is located on the inside surface of the microtubule [58]. The acetyl group can be released by histone deacetylase 6 (HDAC6), a member of the histone deacetylase family [59–63]. In cultured cells, inhibition of HDAC6 activity leads to increased quantity of acetylated tubulin [64–66]. The acetyl group can also be released by SIRT 2, a NAD-dependent histone deacetylase which is a mammalian homolog of the yeast silent information regulator 2 (SIR2) [67,68].

The reversible acetylation of α-tubulin has been implicated in the regulation of microtubule stability and function [69]. Acetylated microtubules commonly resist drug-induced but not cold-induced disassembly [69]. Our studies revealed that acetylated tubulin is a requirement for the association with Na^+,K^+-ATPase, and consequent inhibition of enzyme activity. We observed during studies of the cytoskeleton of Cath a-differentiated (CAD) cells that this brain-derived cell line does not contain either acetylated tubulin or tubulin/ATPase complex [28]. L-Glutamate treatment of these cells did not stimulate Na,K-ATPase activity. However, when cells were treated with the deacetylase inhibitor Trichostatin A or tubacin [70], a significant amount of acetylated tubulin appeared and tubulin/ATPase complex was found in membranes. L-Glutamate treatment of cells containing acetylated tubulin induced dissociation of the complex with concomitant stimulation of enzyme activity (Fig. 1). Preparations containing acetylated tubulin (isolated from brain or from Trichostatin A-treated CAD cells) inhibited

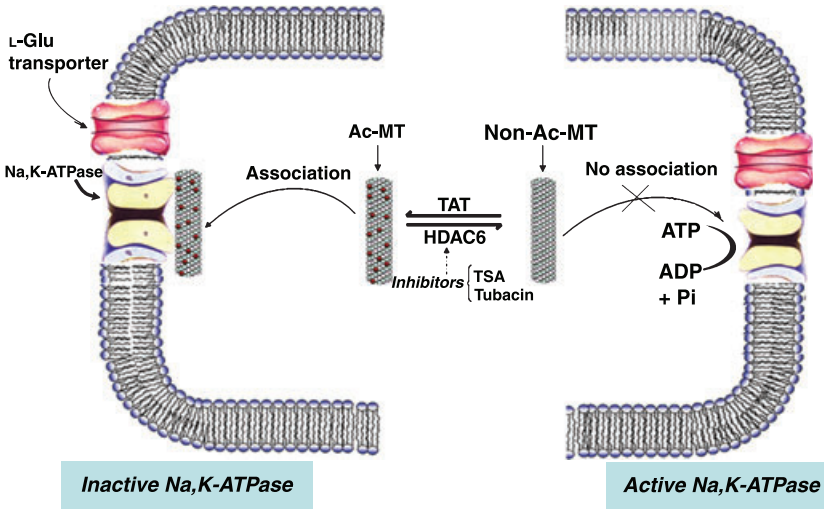

Fig. 1. Schematic representation of the association of microtubules with membrane Na^+,K^+-ATPase as determined by their acetylation state. Balance of activities of tubulin acetyl transferase (TAT) and HDAC6 determines the amount of acetylated and nonacetylated microtubules (Ac-MT and Non Ac-MT, respectively). Ac-MT associate with membranes through Na^+,K^+-ATPase and inhibit enzyme activity. Non Ac-MT cannot associate with membranes, and therefore Na^+,K^+-ATPase activity is not inhibited. Microtubules associated with Na^+,K^+-ATPase can be released by L-glutamate uptake by L-Glu transporters. The inhibitory action of trichostatin A (TSA) and tubacin on HDAC6 induces higher level of Ac-MT.

Na,K-ATPase activity of isolated membranes, whereas tubulin preparations lacking the acetylated isotype (tubulin from nontreated CAD cells), had no effect on enzyme activity. These results indicate that acetylated tubulin is necessary for complex formation. It remains to be determined whether this acetyl group is located in the interaction domain, or some other site of the molecule. The tubulin/Na,K-ATPase complex may contain either tyrosinated or detyrosinated tubulin isotypes. Involvement of other post-translational modifications in association/dissociation of the complex has not been studied.

Acetylation of the ε-amino group of Lys40 of the α-tubulin chain clearly gives tubulin the ability to interact with ATPases and consequently inhibit their enzyme activity. This is the first function of post-translational acetylation of tubulin demonstrated at the molecular level. Although we do not know the consequences of this interaction for cell functioning, there are several reports linking tubulin acetylation with microtubule dynamics and cell motility. Hubbert *et al.* [64] found that decrease of tubulin acetylation enhanced cell motility, and concluded that this was due to microtubule destabilization promoted by the reduced amount of acetylated tubulin. Along the same lines, Haggarty *et al.* [70] found that HDAC6 inhibition, which increases tubulin acetylation, reduced cell motility. The reduction in motility may be due to decreased cellular adhesion resulting from hyperacetylated microtubules [71]. Palazzo *et al.* [72] reported that microtubule stabilization is not promoted by tubulin acetylation, and suggested that the motility change observed by Hubbert *et al.* [64] resulted from alterations in the degree of tubulin acetylation, not from changes in formation of stable microtubules. Cabrero *et al.* [73] studied the role of HDAC6 in migration of T lymphocytes, and found that this deacetylase modulates lymphocyte chemotaxis independently of its enzyme activity. Serrador *et al.* [74] investigated the role of acetylated microtubules in the antigen-specific interaction of T helper and antigen-presenting cells. They found that HDAC6 plays a key role in this process, and suggested that a particular subset of acetylated microtubules is necessary for organization of immune synapse and activation of T cells. More recent studies showed that HDAC6 (and hence tubulin acetylation state) plays an important role in human immunodeficiency virus type 1 infection [75], and that microtubules containing acetylated tubulin are involved in motor-protein trafficking [76]. In addition, SIRT2-mediated tubulin deacetylation was shown to decelerate the differentiation/aging of oligodendroglia [77].

Polymerization state of acetylated tubulin that interacts with Na^+, K^+-ATPase

Biochemical and microscopic observations suggest that microtubules are anchored to the plasma membrane [78–80]. The finding that tubulin interacts with plasma membrane Na^+,K^+-ATPase prompted us to investigate the possible involvement of microtubules in the association of tubulin with Na^+,K^+-ATPase. Treatment of cultured cells with nocodazole (a microtubule depolymerizing agent) caused dissociation of acetylated tubulin/ATPase complex even in the absence of sodium in the culture medium, indicating a different dissociation mechanism compared with L-glutamate-induced dissociation [49]. Nocodazole also dissociated the complex in isolated membranes. In both whole cells and isolated membranes, dissociation was accompanied by increased Na,K-ATPase activity. Treatment of cells with L-glutamate following nocodazole did not increase enzyme activity, reinforcing the idea that complex dissociation is the cause of enzyme stimulation. Stabilization of microtubules with taxol prevented subsequent ATPase activation by L-glutamate. Taxol also suppressed the increase of ATPase activity and dissociation of tubulin/enzyme complex induced by monensin. This could be due to: (a) the requirement of microtubules to be depolymerized, or (b) a direct effect of taxol on the ATPase molecule inhibiting dissociation. An important point for future study is whether the acetylated tubulin that forms a complex with the enzyme is a constituent of microtubules, or an isolated tubulin dimer. Preliminary evidence supports the former concept: (a) taxol-stabilized microtubules formed with purified tubulin bind Na,K-ATPase from detergent-solubilized membranes or from commercial purified preparations; (b) when tissue is homogenized under microtubule-depolymerizing conditions in the absence of detergent, acetylated tubulin is found associated with membrane ATPase. Conversely, when tissue is homogenized under microtubule-stabilizing conditions in the presence of detergent, ATPase is found associated with acetylated tubulin constituent of native microtubules [21].

Modulation of H^+-ATPase activity by association/dissociation of acetylated tubulin with the enzyme

Plasma membrane H^+-ATPase in yeast is encoded by the *PMA1* gene. This gene has been cloned and sequenced from *Saccharomyces cerevisiae* [81], *Schizosaccharomyces pombe* [82], *Neurospora crassa* [83,84]

and other fungal species. H^+-ATPase, a member of the P-type ATPase family often called the 'proton pump', uses energy from ATP hydrolysis to pump protons out of the cell, and to support H^+-coupled uptake of certain amino acids, carbohydrates and inorganic ions. H^+-ATPase is an integral membrane protein comprised of several polypeptides, most prominently a 100 kDa chain that is partially inserted in the plasma membrane. H^+-ATPase in yeast and fungi lacks a glycosylated β subunit of the type found in mammalian Na^+,K^+-ATPase and H^+,K^+-ATPase. Two small proteolipids, Pmp1p and Pmp2p, copurify with the 100 kDa catalytic polypeptide and have been proposed to play a regulatory role [85]. H^+-ATPase is activated when yeast cells are incubated in the presence of glucose [86], and this activation is regulated at the transcriptional and post-transcriptional levels [87–92]. Despite extensive investigation, the molecular mechanism of this glucose-mediated activation is not completely understood.

We showed recently that acetylated tubulin interacts with yeast H^+-ATPase to form a complex in which enzyme activity is inhibited, and that incubation of yeast with glucose dissociates the complex and restores enzyme activity as determined by ATP-hydrolysing capacity or H^+-pumping activity [93]. The association of acetylated tubulin with H^+-ATPase and consequent inhibition of enzyme activity was also demonstrated *in vitro* by incubating yeast membranes with purified tubulin. Alkaline treatment caused dissociation of the complex. Immunoprecipitation experiments using anti-(acetylated tubulin) and anti-(H^+-ATPase) IgG indicated a physical interaction between acetylated tubulin and this enzyme in membranes of glucose-starved cells [93]. The existence of the complex was also determined by the presence of acetylated tubulin in the detergent fraction after partition in Triton X-114. Double immunofluorescence, observed by confocal microscopy, indicated that H^+-ATPase and acetylated tubulin partially co-localize at the periphery of glucose-starved cells. Co-localization was not observed when the tubulin/(H^+-ATPase) complex was dissociated by glucose treatment. Dissociation of the complex by glucose was inhibited by 2-deoxy-D-glucose, a competitive substrate for glucose uptake, indicating that the dissociation involves glucose transporters. This idea was supported by the observation that complex in isolated membranes was not dissociated by glucose [93]. Dissociation of the complex may involve some biochemical modification that occurs in living cells but not in isolated membranes. Formation or dissociation of the acetylated tubulin-(H^+-ATPase) complex may require the presence of Snf3p (a glucose sensor), Gpa2 protein (a G protein) [94] and/or protein kinases [91], which were shown to participate in glucose-induced activation of plasma membrane H^+-ATPase.

Modulation of plasma membrane calcium pump activity by association/dissociation of acetylated tubulin with the enzyme

The main function of the plasma membrane calcium pump (PMCA) is to sustain a calcium gradient across the plasma membrane via ATP hydrolysis-driven expulsion of calcium ions from the cell. In humans, PMCA is encoded by four plasma membrane Ca^{2+}-ATPase genes whose transcripts can be alternatively spliced giving rise to numerous isoforms [95–99]. Because its polypeptide chain (130 kDa) is phosphorylated and dephosphorylated during the ion-transport cycle, PMCA belongs to the P-type ATPase family. The tissue distribution of different PMCA isoforms has been reviewed in detail previously [100]. Most of the PMCA mass protrudes into the cytoplasm, with three main domains. The calmodulin-binding domain is located in the C-terminal cytosolic protrusion. The biological activity of PMCA is regulated by several factors including calmodulin, acidic phospholipids and phosphorylation. The C-terminal domain of PMCA (the calmodulin-binding site) seems to be an internal inhibitor of the enzyme [101].

Membrane vesicles from synaptosomes isolated from rat brain were used to study PMCA interaction with acetylated tubulin. Results similar to those for Na^+,K^+-ATPase and H^+-ATPase were obtained. That is, acetylated tubulin interacts with PMCA to form a complex, resulting in inhibition of PMCA activity [102]. This complex is dissociated by ethanol or calmodulin at physiological concentrations, resulting in activation of PMCA activity. The enzyme activation induced by ethanol or calmodulin is additive, suggesting more than one mechanisms of action. The effect of ethanol and calmodulin on PMCA is altered by nocodazol or taxol treatment, suggesting that microtubules are involved in tubulin/ATPase interaction.

Conclusions and perspectives

The reversible interaction of acetylated tubulin with the sodium, proton and calcium pumps regulates their respective catalytic activities. This is the first demonstrated function for the acetylation of tubulin. It remains to be determined whether acetylated tubulin interacts with and regulates all ATPases, or only

P-type ATPases. It is interesting that three different ATPases can form a complex with the same molecule, acetylated tubulin. HDAC6 may play a crucial role by deacetylating tubulin and thereby preventing its association with ATPases. The external factors L-glutamate, glucose and ethanol induced dissociation of, respectively, (Na^+,K^+)-, H^+- and Ca^{2+}-ATPase/tubulin complexes (Fig. 2). There may be additional or complementary mechanisms leading to the dissociation of ATPase/tubulin complexes and modulation of ATPase activities. Complex disruption induced by endogenous compounds seems to depend on the spatial organization of the molecules involved. For example, dissociation of Na^+,K^+-ATPase from acetylated tubulin induced by L-glutamate requires flux of Na^+ ions into the cell. Because acetylated tubulin is a common partner in complexes formed with (Na^+,K^+)-, H^+- and Ca^{2+}-ATPases, dissociation of these complexes induced respectively by L-glutamate, glucose and ethanol/calmodulin should not proceed using acetylated tubulin as a target, rather using different pathways specific for each ATPase.

Regulation of ATPase activity by the association/dissociation of acetylated tubulin/enzyme complex is still at the early stage of study. However, we can suggest some topics of interest for future investigation. Studies to date on tubulin/ATPase interaction have used membranes isolated after homogenization under microtubule-depolymerizing conditions. We therefore do not know whether the tubulin molecule that forms a complex with ATPase was originally (a) part of a microtubule, or (b) associated with ATPase as an individual dimer. If possibility (a) is found to be correct, it would indicate that ATPases function as 'anchorages' for microtubules. Anchorage of microtubules to membrane has been deduced from biochemical experiments or microscopic observations, but has been never demonstrated at the molecular level. Such anchorage could be a point of interaction between signals from outside and inside the cell. Completely stabilized microtubules are not adequate for establishment of interaction with Na^+,K^+-ATPase, or sensitivity of the complex to glutamate treatment [49]. Microtubules which are at least partially dynamic are required. Thus, acetylated microtubules, which are less dynamic than nonacetylated microtubules (though still not completely stable structures) are associated with ATPases. An interesting question arises: is the increased stability of acetylated microtubules due to their binding to membrane through ATPase? This possibility is supported by the finding of Bershadsky and Gelfand [103] that disassembly of microtubules is an ATP-dependent process, and demonstration by Infante *et al.* [104] that microtubules are stabilized by a plus-end cap that includes ATPase activity.

There are other intriguing questions. Perhaps modulation of the association/dissociation of tubulin with ATPase controls the flow of information from inside to outside the cell. For example, a signal originating from the nucleus or cytosol, whose transmission out of the cell requires active ATPase, could be blocked at the membrane level by association with acetylated tubulin. By contrast, information can flow from outside to inside the cell. Import of certain substances (ions, trophic factors, hormones, amino acids, carbohydrates, neurotransmitters) into the cell could dissociate the complex, with consequent activation of ATPase and transmission of a signal into the cytosol.

ATPase activities are crucial elements in reception/transmission of signals at the membrane level. Endogenous activators of these cation pumps, e.g., adducin in the case of the sodium pump [44], are therefore important factors in regulation of signalling. In this context, acetylated tubulin is the first described endogenous ATPase inhibitor.

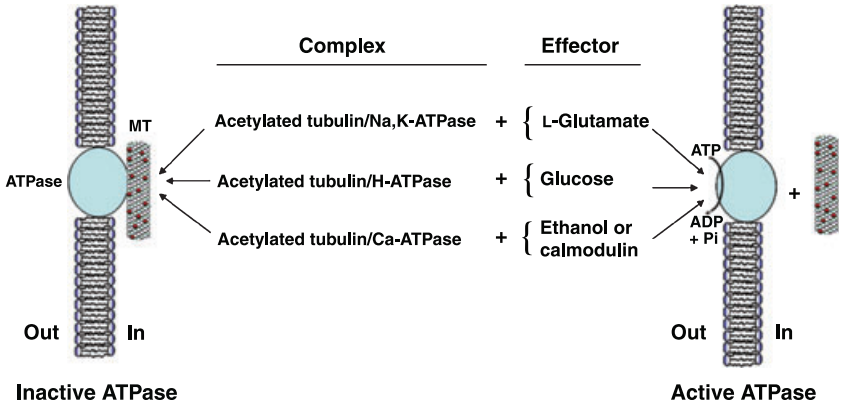

Fig. 2. Schematic representation of the dissociating effect of various compounds on microtubules anchored to sodium, proton and calcium pumps. Na^+,K^+-ATPase of neural and non-neural cells, H^+-ATPase of yeast and Ca^{2+}-ATPase of brain membrane vesicles do not show enzymatic activity when they are associated to acetylated microtubules. Microtubules are released from membranes upon treatment with the indicated effector resulting in increased enzyme activity.

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References

1 Cleveland DW & Sullivan KF (1985) Molecular biology and genetics of tubulin. *Annu Rev Biochem* **54**, 331–365.
2 Villasante A, Wang D, Dobner P, Dolph P, Lewis SA & Cowan WJ (1986) Six mouse α-tubulin isotypes: testis-specific expression of two sister genes. *Mol Cell Biol* **6**, 2409–2419.
3 Westermann S & Weber K (2003) Post-translational modifications regulate microtubule function. *Nat Rev Mol Cell Biol* **4**, 938–947.
4 Feit H & Barondes SH (1970) Colchicine-binding activity in particulate fractions of mouse brain. *J Neurochem* **17**, 1355–1364.
5 Bhattacharyya B & Wolff J (1975) Membrane-bound tubulin in brain and thyroid tissue. *J Biol Chem* **250**, 7639–7646.
6 Kornguth SE & Sunderland E (1975) Isolation and partial characterization of a tubulin-like protein from human and swine synaptosomal membranes. *Biochim Biophys Acta* **393**, 100–114.
7 Zenner HP & Pfeuffer T (1976) Microtubular proteins in pigeon erythrocyte membranes. *Eur J Biochem* **71**, 177–184.
8 Estridge M (1977) Polypeptides similar to the alpha and beta subunits of tubulin are exposed on the neuronal surface. *Nature* **268**, 60–63.
9 Gozes I & Ritcher-Landsberg C (1978) Identification of tubulin associated with rat brain myelin. *FEBS Lett* **95**, 169–172.
10 Zisapel N, Levy M & Gozes I (1980) Tubulin: an integral protein of mammalian synaptic vesicles membrane. *J Neurochem* **34**, 26–32.
11 Sherline P, Schiavone K & Brocato S (1979) Endogenous inhibitor of colchicines–tubulin binding in rat brain. *Science* **10**, 593–595.
12 Rodriguez JA & Barra HS (1983) Tubulin and tubulin–colchicine complex bind to brain microsomal membrane *in vitro*. *Mol Cell Biochem* **56**, 49–53.
13 Carlin RK, Grab DJ & Siekevitz P (1982) Postmortem accumulation of tubulin in postsynaptic density preparations. *J Neurochem* **38**, 94–100.
14 Klopfenstein DR, Kappeler F & Hauri HP (1998) A novel direct interaction of endoplasmic reticulum with microtubules. *EMBO J* **17**, 6168–6177.
15 Yan K, Popova JS, Moss A, Shah B & Rasenick MM (2001) Tubulin stimulates adenylyl cyclase activity in C6 glioma cells by bypassing the beta-adrenergic receptor: a potential mechanism of G protein activation. *J Neurochem* **76**, 182–190.
16 Wang N, Yan K & Rasenick MM (1990) Tubulin binds specifically to the signal-transducing proteins, Gsα and Giα1. *J Biol Chem* **265**, 1239–1242.
17 Bifulco M, Laezza C, Stingo S & Wolff J (2002) 2′,3′-Cyclic nucleotide 3′-phosphodiesterease: a membrane-bound, microtubule-associated protein and membrane anchor for tubulin. *Proc Natl Acad Sci USA* **99**, 1807–1812.
18 Carré M, André N, Carles G, Borghi H, Brichese L, Briand C & Braguer D (2002) Tubulin is an inherent component of mitochondrial membranes that interacts with the voltage-dependent anion channel. *J Biol Chem* **277**, 33664–33669.
19 Goswami C, Dreger M, Jahnel R, Bogen O, Gillen C & Hucho F (2004) Identification and characterization of a Ca^+-sensitive interaction of the vanilloid receptor TRPV1 with tubulin. *J Neurochem* **91**, 1092–1103.
20 Chae YC, Lee S, Lee HY, Heo K, Kim JH, Kim JH, Suh P-G & Ryu SH (2005) Inhibition of muscarinic receptor-linked phospholipase D activation by association with tubulin. *J Biol Chem* **280**, 3723–3730.
21 Beltramo DM, Nuñez M, Alonso A del C & Barra HS (1994) The relationship of hydrophobic tubulin with membranes in neural tissue. *Mol Cell Biochem* **141**, 57–63.
22 Nuñez-Fernandez M, Beltramo DM, Alonso A del C & Barra HS (1997) Conversion of hydrophilic tubulin into a hydrophobic compound. Evidence for the involvement of membrane proteins. *Mol Cell Biochem* **170**, 91–98.
23 Alonso A del C, Nunez-Fernandez M, Beltramo DM, Casale CH & Barra HS (1998) Na^+,K^+-ATPase was found to be the membrane component responsible for the hydrophobic behaviour of the brain membrane tubulin. *Biochem Biophys Res Commun* **253**, 824–927 (erratum appears in Biochem Biophys Res Commun **257**, 642).
24 Fujiki Y, Hubbard AL, Fowler S & Lazarow PB (1982) Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplamic reticulum. *J Cell Biol* **93**, 97–102.
25 Kuhn DM, Arthur R Jr, Yoon H & Sankaran K (1990) Tyrosine hydroxylase in secretory granules from bovine adrenal medulla. *J Biol Chem* **265**, 5780–5786.
26 Niehrs C, Stinchcombe JC & Huttner WB (1992) Two membrane-bound forms of tyrosylprotein sulfotransferase as revealed by phase partitioning in Triton X-114. *Eur J Cell Biol* **58**, 35–43.

27 Casale CH, Alonso A del C & Barra HS (2001) Brain plasma membrane Na^+,K^+-ATPase is inhibited by acetylated tubulin. *Mol Cell Biochem* **216**, 85–92.
28 Santander VS, Bisig CG, Purro SA, Casale CH, Arce CA & Barra HS (2006) Tubulin must be acetylated in order to form a complex with membrane Na^+,K^+-ATPase and to inhibit its enzyme activity. *Mol Cell Biochem* **291**, 167–174.
29 Skou JC & Esmann M (1992) The Na,K-ATPase. *J Bioenerg Biomembr* **24**, 249–261.
30 Jorgensen PL, Hakansson KO & Karlish SJ (2003) Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annu Rev Physiol* **65**, 817–849.
31 Therien AG, Pu HX, Karlish SJ & Blostein R (2001) Molecular and functional studies of the gamma subunit of the sodium pump. *J Bioenerg Biomembr* **33**, 407–414.
32 Arystarkhova E, Wetzel RK, Asinovski NK & Sweadner KJ (1999) The gamma subunit modulates Na^+ and K^+ affinity of the renal Na,K-ATPase. *J Biol Chem* **274**, 33183–33185.
33 Antolovic R, Bruller HJ, Bunk S, Linder D & Schoner W (1991) Epitope mapping by amino-acid-sequence-specific antibodies reveals that both ends of the α subunit of Na^+/K^+-ATPase are located on the cytoplasmic side of the membrane. *Eur J Biochem* **199**, 195–202.
34 Lutsenko S & Kaplan JH (1995) Organization of P-type ATPases: significance of structural diversity. *Biochemistry* **34**, 15607–15613.
35 Chibalin AV, Vasilets LA, Hennekes H, Pralong D & Geering K (1992) Phosphorylation of Na,K-ATPase alpha-subunits in microsomes and in homogenates of *Xenopus* oocytes resulting from the stimulation of protein kinase A and protein kinase C. *J Biol Chem* **31**, 22378–22384.
36 Yudowski GA, Efendiev R, Pedemonte CH, Katz A, Berggren PO & Bertorello AM (2000) Phosphoinositide-3 kinase binds to a proline-rich motif in the Na^+, K^+-ATPase alpha subunit and regulates its trafficking. *Proc Natl Acad Sci USA* **97**, 6556–6561.
37 Beaugé L, Berberian G & Campos M (1990) Potassium occlusion in relation to cation translocation and catalytic properties of Na^+,K^+-ATPase. In *Regulation of Potassium Transport Across Biological Membranes* (Reuss L, Russell JM & Szabo G, eds), pp. 29–63. University of Texas Press, Austin.
38 Glynn IM & Karlish S (1990) Occluded cations in active transport. *Annu Rev Biochem* **59**, 171–205.
39 Crambert G, Fuzesi M, Garty H, Karlish S & Geering K (2002) Phospholemman (FXYD1) associates with Na^+,K^+-ATPase and regulates its transport properties. *Proc Natl Acad Sci USA* **99**, 11476–11481.
40 Sweadner KJ, Arystarkhova E, Donnet C & Wetzel RK (2003) FXYD proteins as regulators of the Na^+,K^+-ATPase in the kidney. *Ann NY Acad Sci* **986**, 382–387.
41 Geering K (2005) Function of FXYD proteins, regulators of Na,K-ATPase. *J Bioenerg Biomembr* **37**, 387–392.
42 Garty H, Lindzen M, Füzesi M, Aizman R, Goldshleger R, Asher C & Karlish SJD (2003) A specific functional interaction between CHIF and Na^+,K^+-ATPase: role of FXYD proteins in the cellular regulation of the pump. *Ann NY Acad Sci* **986**, 395–400.
43 Tripodi G, Valtorta F, Torielli L, Chieregatti E, Salardi S, Trusolino L, Menegon A, Ferrari P, Marchisio PC & Bianchi G (1996) Hypertension-associated point mutations in the adducin alpha and beta subunits affects actin cytoskeleton and ion transport. *J Clin Invest* **97**, 2815–2822.
44 Ferrandi M, Salardi S, Tripodi G, Barassi P, Rivera R, Manunta P, Goldshleger R, Ferrari P, Bianchi G & Karlish SJ (1999) Evidence for an interaction between adducin and Na^+,K^+-ATPase: relation to genetic hypertension. *Am J Physiol* **277**, H1338–H1349.
45 Pellerin L & Magistretti PJ (1997) Glutamate uptake stimulates Na^+,K^+-ATPase activity in astrocytes via activation of a distinct subunit highly sensitive to ouabain. *J Neurochem* **69**, 2132–2137.
46 Casale CH, Previtali G & Barra HS (2003) Involvement of acetylated tubulin in the regulation of Na^+,K^+-ATPase activity in cultured astrocytes. *FEBS Lett* **534**, 115–118.
47 Gasic GP & Hollmann M (1992) Molecular neurobiology of glutamate receptors. *Annu Rev Physiol* **54**, 507–536.
48 Bouvier M, Szatkowski M, Amato A & Attwell D (1992) The glial cell glutamate uptake carrier countertransports pH-changing anions. *Nature* **360**, 471–474.
49 Casale CH, Previtali G, Serafino JJ, Arce CA & Barra HS (2005) Regulation of acetylated tubulin/Na^+,K^+-ATPase interaction by L-glutamate in non-neural cells: involvement of microtubules. *Biochim Biophys Acta* **1721**, 185–192.
50 Kanai Y, Nussberger S, Romero MF, Boron WF, Hebert SC & Hediger MA (1995) Electrogenic properties of the epithelial and neuronal high affinity glutamate transporter. *J Biol Chem* **270**, 16561–16568.
51 Cooper B, Chebib M, Shen J, King NJC, Darvey IG, Kuchel PW, Rothstein JD & Balcar VJ (1998) Structural selectivity and molecular nature of L-glutamate transport in cultured human fibroblasts. *Arch Biochem Biophys* **353**, 356–364.
52 Sato K, Inaba M, Suwa Y, Matsuu A, Hikasa Y, Ono K & Kagata K (2000) Inherited defects of sodium-dependent glutamate transport mediated by glutamate/aspartate transporter in canine red cells due to a decreased level of transporter protein expression. *J Biol Chem* **275**, 6620–6627.

53 Danbolt NC (2001) Glutamate uptake. *Prog Neurobiol* **65**, 1–105.
54 Skerry TM & Genever PG (2001) Glutamate signalling in non-neuronal tissues. *Trends Pharmacol Sci* **22**, 174–181.
55 L'Hernault SW & Rosenbaum JL (1985) *Chlamydomonas* α-tubulin is posttranslationally modified by acetylation on the ε-amino group of a lysine. *Biochemistry* **24**, 473–478.
56 LeDizet M & Piperno G (1987) Identification of an acetylation site of *Chlamydomonas* α-tubulin. *Proc Natl Acad Sci USA* **84**, 5720–5724.
57 Maruta H, Greer K & Rosenbaum JL (1986) The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. *J Cell Biol* **103**, 571–579.
58 Nogales E, Whittaker M, Milligan RA & Downing KH (1999) High resolution model of the microtubule. *Cell* **96**, 79–88.
59 Grozinger CM, Hassig CA & Schreiber SL (1999) Three proteins define a class of human histone deacetylases related to yeast Hda1p. *Proc Natl Acad Sci USA* **96**, 4868–4873.
60 Verdel A & Khochbin S (1999) Identification of a new family of higher eukaryotic histone deacetylases. *J Biol Chem* **274**, 2440–2445.
61 Verdel A, Curtet S, Brocard M-P, Rousseaux S, Lemercier C, Yoshida M & Khochbin S (2000) Active maintenance of mHDA2/mHDAC6 histonedeaceylase in the cytoplasm. *Curr Biol* **10**, 747–749.
62 Guardiola AR & Yao T-P (2001) Molecular cloning and characterization of a novel histone deacetylase HDAC10. *J Biol Chem* **277**, 3350–3356.
63 Grozinger CM & Schreiber SL (2002) Deacetylase enzymes: biological functions and the use of small-molecule inhibitor. *Chem Biol* **9**, 3–16.
64 Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang X-F & Yao T-P (2002) HDAC6 is a microtubule-associated deacetylase. *Nature* **417**, 455–458.
65 Matsuyama A, Shimazu T, Sumida Y, Saito A, Yoshimatsu Y, Seigneurin-Berny D, Osada H, Komatsu Y, Nishino N, Khochbin S *et al.* (2002) *In vivo* destabilization of dynamic microtubules by HDAC6-mediated deacetylation. *EMBO J* **21**, 6820–6831.
66 Zhang Y, Li N, Caron C, Matthias G, Hess D, Khochbin S & Matthias P (2003) HDAC-6 interacts with and deacetylates tubulin and microtubules *in vivo*. *EMBO J* **22**, 1168–1179.
67 North BJ, Marshall BL, Borra MT, Denu JM & Verdin E (2003) The human Sir2 ortholog, SIRT2, is an NAD^+-dependent tubulin deacetylase. *Mol Cell* **11**, 437–444.
68 Dryden SC, Nahhas FA, Nowak JE, Goustin AS & Tainsky MA (2003) Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol Cell Biol* **23**, 3173–3185.
69 Piperno G, LeDizet M & Chang X (1987) Microtubules containing acetylated α-tubulin in mammalian cells in culture. *J Cell Biol* **104**, 289–302.
70 Haggarty SJ, Koeller KM, Wong JC, Grozinger CM & Schreiber SL (2003) Domain-selective small molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin. *Proc Natl Acad Sci USA* **100**, 4389–4394.
71 Tran AD, Marmo TP, Salam AA, Che S, Finkelstein E, Kabarriti R, Xenias HS, Mazitschek R, Hubbert C, Kawaguchi Y *et al.* (2007) HDAC6 deacetylation of tubulin modulates dynamics of cellular adhesions. *J Cell Sci* **120**, 1469–1479.
72 Palazzo A, Ackerman B & Gundersen GG (2003) Cell biology: tubulin acetylation and cell motility. *Nature* **421**, 230.
73 Cabrero JR, Serrador JM, Barreiro O, Mittelbrunn M, Naranjo-Suarez S, Martin-Cofreces N, Vicente-Manzanares M, Mazitschek R, Bradner JE, Avila J *et al.* (2006) Lymphocyte chemotaxis is regulated by histone deacetylase 6, independently of its deacetylase activity. *Mol Biol Cell* **17**, 3435–3445.
74 Serrador JM, Cabrero JR, Sancho D, Mittelbrunn M, Urzainqui A & Sanchez-Madrid F (2004) HDAC6 deacetylase activity links the tubulin cytoskeleton with immune synapse organization. *Immunity* **20**, 417–428.
75 Valenzuela-Fernández A, Alvarez S, Gordon-Alonso M, Barrero M, Ursa A, Cabrero R, Fernández G, Naranjo-Suárez S, Yáñez-Mo M, Serrador JM *et al.* (2005) Histone deacetylase 6 regulates human immunodeficiency virus type 1 infection. *Mol Biol Cell* **16**, 5445–5454.
76 Reed NA, Cai D, Blasius TL, Jih GT, Meyhofer E, Gaertig J & Verhey KJ (2006) Microtubule acetylation promotes kinesin-1 binding and transport. *Curr Biol* **16**, 2166–2172.
77 Li W, Zhang B, Tang J, Cao Q, Wu Y, Wu C, Guo J, Ling E & Liang F (2007) Sirtuin 2, a mammalian homolog of yeast silent information regulator-2 longevity regulator, is an oligodendroglial protein that decelerates cell differentiation through deactylating α-tubulin. *J Neurosci* **27**, 2606–2616.
78 Murray JM (1983) Three-dimensional structure of a membrane–microtubule complex. *J Cell Biol* **98**, 283–295.
79 Sonobe S & Takahashi S (1994) Association of microtubules with the plasma membrane of tobacco BY-2 cells *in vitro*. *Plant Cell Physiol* **35**, 451–460.
80 Waterman-Storer CM, Sanger JW & Sanger JM (1993) Dynamics of organelles in the mitotic spindles of living cells: membrane and microtubule interactions. *Cell Motil Cytoskel* **26**, 19–39.
81 Serrano R, Kielland-Brandt MC & Fink GR (1986) Yeast plasma membrane ATPase is essential for growth

and has homology with Na^+/K^+-, K^+-, and Ca^{2+}-ATPases. *Nature* **319**, 689–693.
82 Ghislain M, Schlesser A & Goffeau A (1987) Mutation of a conserved glycine residue modifies the vanadate sensitivity of the plasma membrane H^+-ATPase from *Schizosaccharomyces pombe*. *J Biol Chem* **262**, 17549–17555.
83 Hager KM, Mandala SM, Davenport JW, Speicher DW, Benz EJ Jr & Slayman CW (1986) Amino acid sequence of the plasma membrane ATPase of *Neurospora crassa*: deduction from genomic and cDNA sequences. *Proc Natl Acad Sci USA* **83**, 7693–7697.
84 Addison R (1986) Primary structure of the *Neurospora* plasma membrane H^+-ATPase deduced from the gene sequence. Homology to $Na+/K+$-, Ca^{2+}-, and $K+$-ATPase. *J Biol Chem* **261**, 14896–148901.
85 Navarre C, Catty P, Leterme S, Dietrich F & Goffeau A (1994) Two distinct genes encode small isoproteolipids affecting plasma membrane H^+-ATPase activity of *Saccharomyces cerevisiae*. *J Biol Chem* **269**, 21262–21268.
86 Serrano R (1983) *In vivo* glucose activation of the yeast plasma membrane ATPase. *FEBS Lett* **156**, 11–14.
87 Capieaux E, Vignais ML, Sentenac A & Goffeau A (1989) The yeast H^+-ATPase gene is controlled by the promoter binding factor TUF. *J Biol Chem* **264**, 7437–7446.
88 Eraso P & Portillo F (1994) Molecular mechanism of regulation of yeast plasma membrane H^+-ATPase by glucose. Interaction between domains and identification of new regulatory sites. *J Biol Chem* **269**, 10393–10399.
89 García-Arranz M, Maldonado AM, Mazon MJ & Portillo F (1994) Transcriptional control of yeast plasma membrane H^+-ATPase by glucose. Cloning and characterization of a new gene involved in this regulation. *J Biol Chem* **269**, 18076–18082.
90 De la Fuente N, Maldonado AM & Portillo F (1997) Glucose activation of the plasma membrane H^+-ATPase requires the ubiquitin-proteasome proteolytic pathway. *FEBS Lett* **411**, 308–312.
91 Goossens A, de La Fuente N, Forment J, Serrano R & Portillo F (2000) Regulation of yeast H^+-ATPase by protein kinases belonging to a family dedicated to activation of plasma membrane transporters. *Mol Cell Biol* **20**, 7654–7661.
92 Lecchi S, Allen KE, Pardo JP, Mason AB & Slayman CW (2005) Conformational changes of yeast plasma membrane H^+-ATPase during activation by glucose: role of threonine-912 in the carboxy-terminal tail. *Biochemistry* **44**, 16624–16632.
93 Campetelli AN, Previtali G, Arce CA, Barra HS & Casale CH (2005) Activation of the plasma membrane H-ATPase of *Saccharomyces cerevisiae* by glucose is mediated by dissociation of the H^+-ATPase–acetylated tubulin complex. *FEBS J* **272**, 5742–5752.
94 Souza MA, Tropia MJ & Brandao RL (2001) New aspects of the glucose activation of the H^+-ATPase in the yeast *Saccharomyces cerevisiae*. *Microbiology* **147**, 2849–2855.
95 Shull GE & Greeb J (1988) Molecular cloning of two isoforms of the plasma membrane Ca^{2+}-transporting ATPase from rat brain. *J Biol Chem* **263**, 8646–8657.
96 Greeb J & Shull GE (1989) Molecular cloning of a third isoform of the calmodulin-sensitive plasma membrane Ca^{2+}-transporting ATPase that is expressed predominantly in brain and skeletal muscle. *J Biol Chem* **264**, 18569–18576.
97 Strehler EE & Zacharias DA (2001) Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol Rev* **81**, 21–50.
98 Adamo HP & Penniston JT (1992) New Ca^{2+} pump isoforms generated by alternative splicing of rPMCA2 mRNA. *Biochem J* **283**, 355–359.
99 Grover AK & Khan I (1992) Calcium pump isoforms: diversity, selectivity and plasticity. *Cell Calcium* **13**, 9–17.
100 Guerini D, Garcia-Martin E, Zecca A, Guidi F & Carafoli E (1998) The calcium pump of the plasma membrane: membrane targeting, calcium binding sites, tissue-specific isoform expression. *Acta Physiol Scand Suppl* **643**, 265–273.
101 Enyedi A, Vorherr T, James P, McCormick KJ, Filoteo AG, Carafoli E & Penniston JT (1989) The calmodulin binding domain of the plasma membrane Ca^{2+} pump interacts both with calmodulin and with another part of the pump. *J Biol Chem* **264**, 12313–12321.
102 Monesterolo NE, Campetelli AN, Previtali G & Casale CH (2005) Acetylated tubulin inhibits the activity of PMCA that is recovered by addition of ethanol. *Biocell* **29**, ES–P19. 10th congress of the Panamerican Association for Biochemistry and Molecular Biology, Pinamar (Argentina).
103 Bershadsky AD & Gelfand VI (1981) ATP-dependent regulation of cytoplasmic microtubule disassembly. *Proc Natl Acad Sci USA* **78**, 3610–3613.
104 Infante AS, Stein MS, Zhai Y, Borisy GG & Gundersen GG (2000) Detyrosinated (Glu) microtubules are stabilized by an ATP-sensitive plus-end cap. *J Cell Sci* **113**, 3907–3919.