Microtubules (MTs) serve as tracks for cellular transport, and regulate cell shape and polarity. Rapid transitions between stable and dynamic forms of MTs are critical to these processes. This dynamic instability is regulated by a number of cellular factors, including the structural MT-associated proteins (MAPs), which in turn are regulated by phosphorylation. MT-affinity-regulating kinases (MARKs) are novel mammalian serine/threonine kinases that phosphorylate the tubulin-binding domain of MAPs and thereby cause their detachment from MTs and increased MT dynamics. Molecular cloning of MARKs revealed a family of four closely related protein kinases that share homology with genes from the nematode Caenorhabditis elegans and fission yeast that are involved in the generation of cell shape and polarity. Hence, MARKs might play a role in the regulation of MT stability during morphogenesis.

**MAPs, MARKs and microtubule dynamics**

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Microtubules (MTs) serve as tracks for cellular transport, and regulate cell shape and polarity. Rapid transitions between stable and dynamic forms of MTs are critical to these processes. This dynamic instability is regulated by a number of cellular factors, including the structural MT-associated proteins (MAPs), which in turn are regulated by phosphorylation. MT-affinity-regulating kinases (MARKs) are novel mammalian serine/threonine kinases that phosphorylate the tubulin-binding domain of MAPs and thereby cause their detachment from MTs and increased MT dynamics. Molecular cloning of MARKs revealed a family of four closely related protein kinases that share homology with genes from the nematode Caenorhabditis elegans and fission yeast that are involved in the generation of cell shape and polarity. Hence, MARKs might play a role in the regulation of MT stability during morphogenesis.
The phosphorylation of tau at the Ser262 KXGS motif could be readily achieved in vitro by a mammalian brain tissue extract, but not by a number of known protein kinases that are predicted to exhibit the appropriate specificity. The purification of the major Ser262-phosphorylating activity from brain revealed that the activity was associated with a single catalytically active protein that has an apparent molecular mass of 110 kDa (Ref. 31). The kinase phosphorylated tau stoichiometrically on Ser262 as well as on the analogous KXGS motifs in the other repeats. The phosphorylated tau became rapidly detached from MTs, which in turn became highly dynamic (Fig. 2). We extended these findings to the dendritic processes (Fig. 3). The N-terminal catalytic domain contains a tyrosine residue as the C-terminal end of the catalytic sequence (the T region) that borders the catalytic domain is highly conserved (G. Drewes et al., unpublished). The overall homology shared by the four open reading frames is ~45%. The sequences contain a number of conserved features (Fig. 3). The N-terminal catalytic domain is highly conserved and contains the regulatory loop (G. Drewes et al., unpublished). The overall homology shared by the four open reading frames is ~45%. The sequences contain a number of conserved features (Fig. 3). The N-terminal catalytic domain contains a tyrosine residue as the C-terminal end of the catalytic sequence (the T region) that borders the catalytic domain is highly conserved (G. Drewes et al., unpublished). The overall homology shared by the four open reading frames is ~45%. The sequences contain a number of conserved features (Fig. 3).
motif. This region is followed by a ubiquitin-associated (UBA) domain, the presence of which suggests that the enzymes are regulated by ubiquitin—possibly through tagging for degradation or membrane recruitment. A prominent feature of all MARKs is the extended spacer region, which is hydrophilic and basic in character and might function in membrane localization. The spacer is the least-conserved region among the four MARK sequences and is predicted to contain little secondary structure. In each MARK, this region is followed by a strikingly conserved C-terminal domain that is predicted to contain two amphipathic helices.

Microtubule-affinity-regulating kinase is activated by phosphorylation

Active MARK isolated from brain is phosphorylated on serine, threonine and tyrosine residues. Dephosphorylation by the Ser/Thr protein phosphatase 2A abolishes catalytic activity. In a number of protein kinases, a regulatory segment in subdomain VIII is a target for activating phosphorylation, particularly at a Thr residue that is conserved in several Ser/Thr kinase families. Sequencing of the corresponding peptide from MARK revealed that the equivalent threonine residue (Thr215 in MARK1) and an adjacent serine residue (Ser219) within a Ser-Pro motif are phosphorylated. Mutational analysis showed that MARK activity was compromised when either or both of these residues were mutated. Activity of phosphatase-treated MARK cannot be fully restored by autophosphorylation, but dephosphorylated MARK can be partially reactivated by addition of crude fractions from brain tissue, which suggests that upstream activating kinases exist. Tyrosine phosphorylation has no effect on enzyme activity, but other functions (e.g., in protein–protein interactions) are possible.

Microtubule-affinity-regulating kinase expression disrupts the microtubule cytoskeleton

Overexpression of MARK cDNAs in CHO (Ref. 33) and Neuro2a cells (A. Ebneth et al., unpublished) has dramatic morphological consequences: the MT cytoskeleton shows a varying degree of disorganization that correlates with the level of MARK expression. Cells that overexpress MARK at high levels are very small and rounded-up. Eventually, such cells become detached from the substratum and die. When expression levels are lower, cells appear relatively normal in shape and size. However, in many of these cells, the disruption of the MT network is still evident (Fig. 4). The observed disruption of the MT cytoskeleton could be a consequence of the reduced stability of MTs lacking MAPs. Such MAP-deficient MTs could be prone to disruption by catastrophe factors, for example. The observed effects are specific for MTs: we did not observe destruction of actin fibers (Fig. 4). MT disruption in these cells is mediated by the phosphorylation of MAPs.

A family of protein kinases involved in cell polarity

Members of the mammalian MARK family share striking similarities with several protein-kinase sequences from lower eukaryotes (Fig. 5). MARKs share significant homology with PAR-1, the product of one of a set of partitioning defective (par) genes, whose mutation disrupts the asymmetric cell divisions of C. elegans zygote. The par mutants exhibit abnormal symmetric divisions and, hence, non-functional embryo is formed. The PAR-1 kinase is localized at the posterior cortex, which develops into a germ cell (P cell) tunica. Mutations that cause the Par-1 phenotype were found at residues that are known to be critical for kinase activity. Correct enzymatic function of PAR-1 (but not its correct localization) is essential for the distribution of ribonucleoprotein particles (P granules), which are initially spread evenly throughout the cytoplasm but move to the posterior pole before the first
cleftage of the zygote. In subsequent asymmetric divisions, the P granules always end up in the germline. The use of cytoskeletal inhibitors showed that this process is dependent on F actin but not on MTs (Ref. 38). The process might be mediated by myosin 2, which has been shown to interact with a 200-residue C-terminal fragment of PAR-1 (Ref. 37). However, the N-terminal half of this region is not conserved in the mammalian MARKs and, thus, myosin binding might not be common to MARKs.

Recently, attempts to identify mammalian relatives of PAR-1 in epithelial cells resulted in the cloning of a MARK2 homolog, mPAR (Ref. 39). The function of the enzyme was studied in Madin-Darby canine kidney cells, which grow as epithelia and have apical-basal polarity. mPAR localized to the lateral membrane, below the apical tight junctions. Transient constructs lacking the mPAR kinase domain retained this localization; however, transfection caused loss of polarity and rounding up of the cells, which were eventually expelled from the monolayer and underwent apoptosis. The C-terminal mPAR fragment could have a dominant negative effect and inhibit the endogenous kinase – as in the C. elegans par-1 mutant.

Loss of epithelial polarity, induced by chemical carcinogenesis, in fetal pancreatic cells caused mislocalization and, finally, the loss of the p78 antigenic marker in the basolateral membrane (Ref. 40). This marker (GenBank accession number X07064) is homologous to a gene that we cloned recently and termed MARK1 (G. Drewes et al., unpublished). Taken together, these data suggest that correctly localized MARKs are markers for differentiating or differentiated cells. Protein kinases that share common structural motifs with MARK are present in organisms as evolutionarily distant from mammals as yeast (Fig. 5). In Schizosaccharomyces pombe, disruption of the kni-1 gene causes cells to become spherical rather than rod shaped; the cell-wall composition of these cells is altered, and they form abnormal filaments structures that are reminiscent of those in β-tubulin-mutant strains (Ref. 41). These data suggest that KIN1 plays a role in cytoskeletal polarization. The considerable degree of sequence conservation in members of the MARK–PAR–KIN family from organisms as evolutionarily distant as mammals, nematode and yeast suggests that essential mechanisms governing cellular polarity might also be conserved (Ref. 42).

Figure 4
Overexpression of microtubule-affinity-regulating kinase (MARK) in CHO cells causes microtubule (MT) breakdown. (a–c) The effect of overexpression of epitope-tagged wild-type MARK2. Cells were stained for the epitope tag (a), tubulin (b) and actin (c). (d–f) The effect of overexpression of an epitope-tagged inactive MARK2. Cells were stained for the epitope tag (d), tubulin (e) and actin (f). Bar = 20 µm.

Figure 5
Phylogenetic tree for MARK–PAR–KIN family from organisms as evolutionarily distant as mammals, nematode and yeast. Phylogenetic tree for MARK–PAR–KIN family from organisms as evolutionarily distant as mammals, nematode and yeast.
MAPs cause the inhibition of vesicle transport and organelle movement (Refs. 47, 48, and A. Ebneth et al., unpublished), which is consistent with the observation that the movement of the motor proteins kinesin and dynein is inhibited in vitro by the presence of MAP2 or tau [19,20]. In this sense, MAPK might function as a molecular switch that regulates vesicle and/or organelle transport. The depletion of MAPs along a particular MT might increase the net vesicle transport along MTs, caused by MARKs, might not only increase MT turnover but might also facilitate vesicle transport along an MT. Thus, MARK kinases might therefore play a role in cell polarity by regulating MT-based transport processes. Active MARKs are phosphorylated by as-yet-unknown upstream factors. The unraveling of this putative signaling cascade should provide insights into the molecular mechanisms that control cytoskeletal dynamics and, hence, cell polarity.

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