The tau of MARK: a polarized view of the cytoskeleton

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Microtubule-affinity regulating kinases (MARKs) were originally discovered by their ability to phosphorylate tau protein and related microtubule-associated proteins (MAPs), and thereby to regulate microtubule dynamics in neurons. Members of the MARK (also known as partition-defective [Par]-1 kinase) family were subsequently found to be highly conserved and to have key roles in cell processes such as determination of polarity, cell-cycle control, intracellular signal transduction, transport and cytoskeleton. This is important for neuronal differentiation, but is also prominent in neurodegenerative ‘tauopathies’ such as Alzheimer’s disease. The identified functions of MARK/Par-1 are diverse and require accurate regulation. Recent discoveries including the x-ray structure of human MARKs contributed to an increased understanding of the mechanisms that control the kinase activity and, thus, the actin and microtubule cytoskeleton.

The family of MARK

The microtubule-affinity regulating kinases (MARK; see Glossary)/partition-defective (Par-1) kinases belong to the adenosine monophosphate-activated protein kinases (AMPK)/sucrose non-fermenting 1 (Snf1) subfamily of adenosine monophosphate-activated protein kinases (AMPK)/group of kinases in the human kinome [1,2] (Figure 1a). In mammals the MARK family consists of four members (MARK1–MARK4), and further isoforms arise by alternative splicing [3] (Table 1). All isoforms have an N-terminal header (N), a conserved catalytic protein kinase domain (CAT), which is linked to a putative common docking domain (CD), followed by a ubiquitin-associated domain (UBA), a spacer domain, and a C-terminal tail domain, which includes the ‘kinase associated domain’ (KA1) (Figure 1b,c). The UBA and KA1 domains are conserved among the AMPK-related protein kinases and MARKs. Their functions are poorly understood but might be related to ubiquitin dependent signaling or supermolecular folding with influence on activity [4].

Given that the MARK/Par-1 kinases are involved in the regulation of essential pathways, it is important to understand the control of their activity. Because MARKs contain several domains, this offers multiple ways of regulation by post-translational modification (e.g. phosphorylation), interaction with scaffolding proteins, and interaction with other proteins. In addition to the diverse modes of MARK regulation we review the functions of MARK family members revealed by gene knockout mice, by loss of function or overexpression experiments emphasizing its role in neuronal differentiation, cell polarity, intracellular transport, cell migration and neurodegeneration.

MARK, tau and Alzheimer’s disease

The MARKs were originally discovered because of the ability of MARK1 or MARK2 to regulate the stability of microtubules (MTs), the ‘tracks’ used for intracellular transport. MARK/Par-1 kinases, such as MARK1, are involved in cell polarity, cell-cycle control and neuronal differentiation; but also in neuronal migration, cell morphology, intracellular signal transduction and transport. A new perspective on the family of MARKs (and Par-1) is highlighted by the identification of tau as a substrate of MARKs. Tau, a neuronal microtubule associated protein (MAP), was discovered in 1987 [5]. It is a highly conserved protein in invertebrates and vertebrates. In most multicellular organisms it is a component of the cytoskeleton (Figure 2), and in vertebrates, it forms core structures (neurofibrillary tangles) (NFT) in the human brain. Tau is localized in the axons of neurons, where it maintains the integrity of the axon, together with other MAPs. Tauopathies are a class of neurodegenerative diseases accompanied by pathological aggregation of tau protein in so-called neurofibrillary tangles (NFT) in the human brain.

Glossary

- aPKC: atypical protein kinase C forms a complex with the scaffold proteins PAR-6 and PAR-3 and regulates cell polarity. This PAR complex is initially described in the nematode C. elegans and later in the fruit fly D. melanogaster and vertebrates.
- Microtubules: one of the components of the cytoskeleton consisting of tubulin polymers. They are involved in many cellular processes including mitosis and intracellular transport.
- Microtubule-associated proteins (MAPs): proteins that interact with microtubules and stabilize them.
- MAP/microtubule affinity regulating kinase (MARK): a family of four serine/threonine kinases in mammals that are homologues to yeast Kin1 and Par-1 in C. elegans and D. melanogaster.
- Notch: the Notch pathway is a highly conserved cell-signaling system present in most multicellular organisms and is important for cell-cell communication, which involves gene-regulation mechanisms that control multiple cell differentiation processes during embryonic and adult life. Notch is a transmembrane protein whose extracellular domain binds Notch ligands, followed by signaling into the cell.
- Notch-ligand: the Notch ligands are also single-pass transmembrane proteins and are members of the DSL (Delta/Serrate/LAG-2) family of proteins. In D. melanogaster, there are two ligands named Delta and Serrate. Ligand proteins bind to the extracellular domain of Notch induce proteolytic cleavage and release of the intracellular domain, which enters the cell nucleus to alter gene expression.
- Partitioning-defective (PAR) kinase family: six different proteins that are required for cytoplasmic partitioning in the early embryo of C. elegans. Par-1 and Par-4 are conserved protein kinases and homologous to the mammalian serine/threonine kinases MARK and LKB1.
- Tandem affinity purification (TAP): a technique for studying protein–protein interactions. The TAP method involves the fusion of the TAP tag to the C terminus of the protein under study. The TAP tag consists of calmodulin binding peptide (CBP) from the N terminal, followed by tobacco etch virus protease (TEV protease) cleavage site and Protein A, which binds tightly to IgG.
- Tau protein: a neuronal microtubule associated protein present in six main isoforms in the human central nervous system and additional isoforms (‘big tau’) in peripheral nerves. Tau is localized in the axons of neurons, where it maintains the integrity of the axon, together with other MAPs.
- Tauopathy: tauopathies are a class of neurodegenerative diseases accompanied by pathological aggregation of tau protein in so-called neurofibrillary tangles (NFT) in the human brain.
- Wnt signals: signals caused by Wnt proteins, which form a family of highly conserved secreted molecules that regulate cell-to-cell interactions during embryogenesis, but are also involved in normal physiological processes in adult animals.

14-3-3 binding motifs: an amino acid motif composed of phospho-Ser with proline at the +2 position and arginine or lysine at the +3 position.

14-3-3 proteins: a family of conserved regulatory molecules expressed in all eukaryotic cells. 14-3-3 proteins have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors.
Figure 1. Phylogenetic tree and domain structure of MARKs. (a) The phylogenetic tree shows the relationship between the four human MARK gene products and other members of the AMPK-related protein kinases [83]. (b) All MARK family members share a similar domain structure consisting of an N-terminal header domain (N, grey), followed by the catalytic domain (minor lobe, blue; major lobe, purple), the ‘common docking’ (CD) domain (orange) and linker (pale green), the ubiquitin-associated domain (UBA, red), the spacer-domain (dark green) and the C-terminal tail sequence, which contains the kinase-associated domain (KA, brown). For example, the catalytic domain of MARK2 can be activated by phosphorylation of the conserved Thr208 in the activation loop by MARKK (also known as thousand-and-one amino acids 1 [TAO1]) or LKB1 and inactivated by phosphorylation of the conserved Ser212 by GSK3β. For other determinants of activity see Figure 5. (c) The 3D structure of MARK2 as determined by X-ray crystallography [59], showing the catalytic domain, CD domain, linker and UBA domain (following the same color scheme as in (b)).
transport [1]. MTs are covered with microtubule-associated proteins (MAPs), which control their stability and dynamic behavior and thus contribute to cell shape and polarity. In addition, MAPs can compete with motor proteins for microtubule binding and thereby inhibit transport at elevated levels [5]. Because the binding of MAPs to MTs is regulated by phosphorylation, kinases that affect MAPs not only control the dynamics of MTs but can also counteract the transport inhibition by MAPs [6].

In mammals, tau belongs to the MAP family of proteins, which also includes MAP2, MAP2c and MAP4 [7]. Tau binds and stabilizes MTs, particularly in neuronal axons, and thus ensures microtubule-dependent axonal transport of vesicles and organelles by motor proteins [8]. Phosphorylation of MAP2K (or other MARK family members) at the Lys-Xaa-Gly-Ser motifs that are present in the repeat domain of tau causes the detachment and destabilization of the MT (Figure 2a). Enhanced phosphorylation of tau at multiple sites, including the Lys-Xaa-Gly-Ser motifs, is an early hallmark of Alzheimer’s disease (AD), followed by abnormal aggregation of tau protein to paired helical filaments (PHFs) and neurofibrillary tangles [9] (Figure 2a). Active MARK co-localizes with neurofibrillary tangles in the AD brain, and MARK sites on tau are elevated early in transgenic mouse models of tauopathy [10] emphasizing the importance of MARK in this disease (Figure 2b).

The toxic effect of tau on neurons is frequently ascribed to its aggregation [11], but this is preceded by the hyperphosphorylation of tau, mis-sorting into the somatodendritic compartment, the loss of synapses and mitochondrial dysfunction [12]. Therefore, it is not yet certain whether the aggregation of tau is the cause of its toxic effect or merely a consequence of it. Neurodegeneration seems to be accompanied by defects in transport, which could be due to an imbalance of motors, adaptors of cargo(s), or tracks and their modifiers such as tau [12,13].

**MARK target proteins and cellular polarization**

MARK2 (and its homologue Par-1) belongs to a set of conserved proteins in *Drosophila* and *Caenorhabditis elegans*, which are essential for cellular polarity, with roles in establishing the embryonic body axis and in maintaining cell differentiation [14,15] (reviewed in Refs [16–18]). The Par-dependent cell polarization is based on a tight network of cross-reactive and feedback interactions of the Par proteins, other regulators of polarity and the cytoskeleton [19]. The core of this network consists of the scaffold proteins Par-3 and Par-6, the serine/threonine kinases Par-1, Par-4 and atypical protein kinase C (aPKC), the adapter protein Par-5 (a 14–3–3 protein) and the ring domain protein Par-2 [17–19]. MARK/Par-1 is a central player in localization of these cell-polarity proteins. For example, phosphorylation of the scaffold Par-3 by MARK/Par-1 restricts Par-3 to the apical membrane of *Drosophila* photoreceptors. This is antagonized by the phosphatase PP2A, which removes MARK/Par-1-mediated phosphorylation and targets Par-3 and its binding partners to adhesion junctions. Thus, tightly regulated phosphorylation events are important regulatory steps during cellular development [20].

Studies in mammalian epithelial cells proved that MARK2/Par-1 is asymmetrically localized. Overexpression of inactive MARK2/Par-1 disturbs the polarity in this cell line suggesting a similar mechanism of governing polarization in *C. elegans* embryo and mammalian epithelial cells [21]. Microtubules are important determinants of cell polarity, and indeed some of the effects of MARK2/Par-1 on cell polarity are mediated by microtubules. MARK2/Par-1 has a role in polarized neurite outgrowth and maintenance of neuronal polarity in mammalian cells, which requires dynamic instability of microtubules [22,23]. A reduction of MARK2/Par-1 expression via RNA interference (RNAi) induces multiple axons in hippocampal neurons, whereas strong MARK2/Par-1 expression inhibits axon formation. This inhibition is counteracted by the Par-3–Par-6–aPKC-complex, which inhibits MARK2/Par-1 [23]. MARK2/Par-1 inhibits the development of dendrites via phosphorylation of MAP2 in hippocampal neurons [24]. Thus, elevated MARK/Par-1 activity or silencing MARK/Par-1 inhibits axonal and dendritic growth, whereas reduced MARK/Par-1 activity causes multiple axons; these features reflect the importance of a strict regulation of the kinase for neuronal development.

Another example of the key role of MARKs in neuronal differentiation is given by MARK4. It is absent from neuronal progenitor cells, but upregulated during neuronal differentiation [25]. Other kinases of the AMPK subfamily are also involved in neuronal polarization as recently shown for the synapses of amphids defective (SAD)-kinases [26,27]. Hippocampal neurons deficient in SAD A and SAD B showed a loss of polarity. SAD kinases act, at least in part, by locally regulating the phosphorylation of MAPs including tau, at Lys-Xaa-Gly-Ser motifs. Subsequent alterations in microtubule organization are critical for neuronal polarization [26].

MARK2/Par-1 is also involved in the control of neuronal migration through its dual activities in regulating cellular polarity and microtubule dynamics [28]. MARK2/Par-1 phosphorylates and regulates doublecortin (DCX) [29], a

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Table 1. MARK/Par-1/Kin kinases in different organisms [81,82]

<table>
<thead>
<tr>
<th>Organism</th>
<th>MARK/Par-1 family protein(s)</th>
<th>Alternative names</th>
</tr>
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<tbody>
<tr>
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<td>mPar-1c</td>
</tr>
<tr>
<td></td>
<td>MARK2</td>
<td>mPar-1b, EMK1</td>
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<tr>
<td></td>
<td>MARK3</td>
<td>mPar-1a, CTAK1, Kp78</td>
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<td>MARK4</td>
<td>mPar-1d, MARKL1</td>
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<td></td>
<td>MELK</td>
<td>pEG3, MPK38</td>
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<td>CTAK1-like</td>
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<td>C. elegans</td>
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<td>S. pombe</td>
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Figure 2. Pathological pathways to neurodegeneration involving MARK and tau. (a) In a normal axon, microtubules (green) form the tracks for the transport of vesicles and are stabilized by tau protein (purple), sparsely decorating the MT surface (i). Elevated tau bound to microtubules can lead to the inhibition of transport of vesicles and organelles. This is explained by interference between tau and microtubule motors (blue sphere and yellow lobes) and can be relieved by MARK [12] (ii). Phosphorylation of tau (red dot) by the MARKK/MARK cascade detaches tau from the microtubules, resulting in microtubule destabilization and disassembly (iii). In the pathological situation
microtubule-associated protein that is implicated in neuronal migration disorders and is highly enriched in the leading processes of migrating neurons. After MARK2 knockdown, neurons of the developing mouse cortex fail to migrate beyond the intermediate zone and fail to acquire a normal bipolar morphology, probably owing to a loss of microtubule dynamics [28].

Kinases are often involved in more than one signaling pathway. In flies and frogs, Par-1 can associate with and phosphorylate dishevelled (Dsh). This scaffold protein forms a branchpoint that links several signaling pathways. Wnt signals are transduced to Dsh leading to transcriptional activation of target genes (canonical pathway) or to cytoskeletal changes (planar cell polarity pathway [PCP]) [30]. Par-1 seems to be a positive regulator of the canonical pathway in Drosophila and mammalian cells [31]. Phosphorylation of Dsh by different Par-1 isoforms in Xenopus resulted in the transduction of either canonical or PCP signals. Notably, phosphorylation by the PCP-specific isoform of Par-1 was associated with the translocation of Dsh to the cell membrane, which is essential for PCP signaling [32]. These results show that the role of Par-1 in Wnt-signaling is complex and isoform dependent. Drosophila Par-1 phosphorylates the posterior determinant Oskar and hence increases the stability of Oskar at the posterior pole [33], which is required for embryonic development. In a similar way, Drosophila Par-1 is a key component of the postsynapse and influences a crucial event in synaptogenesis [34]. The tumor suppressor discs large (Dlg) is directly phosphorylated by Drosophila Par-1, which downregulates the mobility and targeting of Dlg. Thus, overexpression of Par-1 leads to decreased synapse formation and synaptic transmission at Drosophila neuromuscular junction [34].

Another example concerning MARK2/Par-1 and localization of target proteins is the regulation of LET-99, which is required for spindle positioning and asymmetric division in C. elegans embryos. Par-1 associates with and phosphorylates LET-99 thereby inhibiting the localization of LET-99 at the anterior and posterior poles respectively [35].

Phosphorylation is a common mechanism to change the cellular localization of proteins. 14–3-3 proteins are phospho-serine/phospho-threonine binding proteins that interact with a diverse array of binding partners thereby regulating essential biological processes. MARK kinases phosphorylate the 14–3-3 binding motifs on Cdc25, KSR1, HDACs, PTPH1 and PKP2 [36-39] (Figure 3a). Moreover, 14–3-3/Par-5 proteins were identified as interactors of Drosophila Par-1 [40]. Par-1 binds to a domain of 14–3-3 distinct from the phosphoserine binding pocket and could, therefore, directly target substrates to 14–3-3. Tandem affinity purification with TAP–14–3-3γ as a bait confirmed an interaction with MARK2 and MARK3. Further studies indicated a binding specificity between different MARK family members and 14–3-3 isotypes pointing to individual regulatory mechanisms and functions of each isoform [41]. For example, MARK2 phosphorylates the cell-cycle regulatory phosphatase Cdc25 resulting in a complex of Cdc25 and 14–3-3, which blocks the cell cycle [36]. In a similar manner, MARK3 regulates Ras signaling by phosphorylating KSR1 (kinase suppressor of ras) to generate a 14–3-3 binding site [37]. Non-phosphorylated KSR1 is localized at the plasma membrane where it acts as a scaffold that positions proteins important for Ras–Raf signaling. Thus, phosphorylation of KSR1 by MARK3 retains KSR1 in the cytosol and downregulates Ras signaling. Class IIa histone deacetylases (HDACs) are found both in the cytoplasm and in the nucleus where they repress genes involved in several major developmental programs. MARK2 and MARK3 phosphorylate HDACs on one of their multiple 14–3-3 binding sites and neutralize their repressive activity by 14–3-3-mediated nuclear exclusion [38]. Furthermore, the tyrosine phosphatase PTPH1, a key regulator of many aspects of cellular signaling events, is phosphorylated by MARK3, which could provide a link between serine/threonine and tyrosine phosphorylation-dependent signaling pathways. This phosphorylation on a single residue enhances the association of PTPH1 and 14–3-3 and thus might regulate the activity of PTPH1 [39]. Plakophilins (PKPs) are armadillo family members related to the classical cadherin-associated protein, a crucial scaffold for PKCα that regulates intercellular-junction assembly, and are localized in desmosomes [42]. Plakophilin 2 (PKP2) was identified as a novel MARK3 substrate that generates a 14–3-3-binding site and thus relocalizes PKP2 to the nucleus (summarized in Figure 3a) [36].

Analogous studies in Madin-Darby canine kidney (MDCK) cells suggest that MARK2 is involved in the regulation of protein targeting in polarized mammalian cells as well [43]. This cell line uses two different routes to target new synthesized luminal proteins to the cell surface. MARK2 promotes the establishment of the indirect transcytotic route probably by affecting the generation of post-Golgi transport carriers and MT-based targeting. Interestingly, recent evidence demonstrates that the yeast Par-1 homologues Kin1 and Kin2 regulate the exocytic apparatus, implicating a role in the secretory pathway [4]. Kin1 and Kin2 directly associate with the t-SNARE Sec9, a membrane-bound component of the exocyst, and mediate a phosphorylation of Sec9. This triggers its release from the cell membrane and, presumably, promotes incorporation of Sec9 into novel SNARE complexes increasing the exocytic activity.

A molecular connection exists between a human pathogen and MARK because the CagA protein of Helicobacter pylori associates with MARK2/Par1, recruits it to the plasma membrane, and inhibits the activation of the kinase [44]. This leads to polarity defects and destroys the epithelial architecture. Another example is the human Kp78/MARK3, which is found on the cell surface of differentiated human pancreas cells but is absent in transformed populations, suggesting a link to human pancreas carcinogenesis [45]. A genetic study in families carrying the Peutz-Jeghers syndrome (PJS), an autosomal-dominant disorder characterized among others by
increased cancer risk, did not identify mutations in the MARK/Par-1 genes [46]. Other results suggest an association between MARK1 dysregulation and autism because MARK1 is overexpressed in the prefrontal cortex of patients with autism and causes changes in the function of cortical neuronal dendrites [47]. These findings suggest broad pathological consequences of a dysregulation of MARK family kinases in various cell types.

**Functional clues from model organisms**

Members of the MARK/Par-1 kinase family have been studied in several model organisms and the identified functions...
affect microtubule stability, cell polarity, protein stability, intracellular signaling and cell-cycle control (Figure 3b). MARK2/Par-1 knockout mice have no obvious neuronal phenotype, which might be due to genetic redundancy with the other three MARKs. But these mice display growth retardation and immune-cell dysfunction suggesting that MARK2 is essential for maintaining immune system homeostasis [48]. MARK2/Par-1 is required for regulating glucose metabolism because mice null for MARK2/Par-1 are lean, insulin hypersensitive, resistant to high-fat-diet-induced weight gain, and hypermetabolic. These data indicate that MARK2/Par-1 regulates metabolic rate, adiposity and insulin sensitivity [49].

Par-1 is expressed throughout Drosophila development, but it is difficult to characterize its function because of oocyte lethality of null mutants [50]. Flies have only a single Par-1 kinase gene, whereas mammalian MARKs have probably redundant functions. Nevertheless, reduced levels of Drosophila Par-1 disrupts oocyte microtubule network [51]. Null mutants in Drosophila follicle epithelium cells show increased density of MTs and polarity defects consistent with the function of mammalian MARK2 [52,53]. Drosophila Par-1 RNAi embryos display a neural lateral inhibition manifested in a hyperplasia of central and peripheral nervous system [54]. Overexpression of Par-1 disrupts embryonic eye disc cell polarity. Further experiments reveal that Par-1 acts synergistically with Notch to stimulate cell growth within the eye disc. This depends on the kinase activity of Par-1, which functions upstream of Notch and is crucial for localization of the Notch-ligand delta [54]. Notch signaling is important for the development of cell polarity during gastrulation. Xenopus Par-1 is also essential for gastrulation and is dependent on 14–3-3 and aPKC [55]. Eye degeneration analysis of tau toxicity in Drosophila confirms that kinases and phosphatases control the tau-induced neurodegeneration. Overexpression of MARK/Par-1 leads to elevated tau phosphorylation and enhanced toxicity, whereas removing MARK/Par-1 function abolishes tau toxicity [56]. Interestingly, a screen for genetic modifiers of tau-induced neurodegeneration in Drosophila identifies MARK/Par-1 as a suppressor of tau toxicity [57]. Overexpression of tau in Drosophila might be toxic not only because of aggregation but also because of transport defects. MARK/Par-1 expression could rescue these defects, confirming the importance of a tight regulation of the kinase.

Regulation of MARK
MARK kinases are relatively large proteins that contain several domains and are regulated by multiple pathways. X-ray analysis yields clues to the regulatory function of particular residues in the MARK-activation loop. Phosphorylation events in the catalytic domain of MARK can enhance or reduce kinase activity. Additionally, MARK activity can be positively or negatively modulated by interaction with other proteins. Because of this diversity of regulation and the large number of modulators of MARK activity, we first discuss the structural and molecular aspects of regulation followed by a summary of MARK regulating kinases and participating signaling pathways.

Structural and molecular principles
MARK2/Par-1 isolated from porcine brain is in part phosphorylated at both Thr208 and Ser212 (Figure 1) in the activation loop [58]. Phosphorylation of Thr208 is needed for activation, but unphosphorylated Ser212 must be present to enable the activity of MARK2/Par-1. Phosphorylation of Ser212 or mutating it to Ala or Glu abolishes the kinase activity, despite the activating phosphorylation of Thr208 [58]. X-ray analysis of the catalytic domains of MARK1 and MARK2 [59,60] confirm the important function of this serine in stabilizing the activation loop. In the inactive state of the kinase, the activation loop folds into the catalytic cleft, blocking the entry of the ATP and the substrate peptide (e.g. tau). Upon activation, the activation loop folds away, opening the catalytic cleft [61].

MARK activation by phosphorylation
Two types of upstream activating kinases have been identified in mammals, MARK kinase (MARKK) [58] and the tumor suppressor LKB1 [62]. MARKK is equivalent to the kinase TAO-1 [63] and LKB1 is the homologue of Drosophila melanogaster and C. elegans Par-4. Both kinases phosphorylate Thr208 in the activation loop of MARK2/Par-1 and corresponding residues in other MARK isoforms [58]. In cells the activity of MARKK or LKB1 enhances microtubule dynamics through the activation of MARK and leads to phosphorylation and detachment of tau or equivalent MAPs from microtubules. Forced expression of LKB1 suppresses MT regrowth, whereas LKB1 knockdown accelerates it, confirming the pathway of regulation of MT dynamics through MARK2/Par-1 and subsequent phosphorylation of tau at the Lys-Xaa-Gly-Ser motifs [61]. The LKB1–MARK2 signaling pathway is thought to enhance proteasome-mediated degradation of phospho-tau [64], but this is at variance with other recent results [65]. Akt is a key signaling kinase for cell survival, proliferation, tumor growth and angiogenesis. It has a role in the phosphoinositide 3-kinase signal transduction pathway and has several substrates, including glycogen synthase kinase 3β (GSK3β), which becomes inactivated. It has been proposed that Akt and MARK2 interact directly so that Akt enhances the activity of MARK2 to promote tau hyperphosphorylation at Lys-Xaa-Gly-Ser motifs [65]. This would be reminiscent of the cases of MARKK or LKB1 that we mentioned before. A more likely alternative is that Akt operates indirectly by inhibiting GSK3β (p-Ser9), which enables the activation of MARK2/Par-1 [66], see later. In the Akt pathway, the resulting phosphorylation of tau at Lys-Xaa-Gly-Ser motifs is thought to protect against proteasomal degradation (because it interferes with ubiquitination of tau). This view is in conflict with the conclusions derived from the LKB1–MARK2 pathway [64]. Further work is required to resolve these issues.

Similarly, activated CaMKI binds and phosphorylates MARK2 within its kinase domain at novel sites. Mutation of Thr294 and Ser90–92 together were necessary to completely abolish MARK2/Par-1 phosphorylation. Coexpression of both kinases in N2a or primary hippocampal neurons promotes neurite outgrowth, indicating that MARK2/Par-1 is active [67]. This study pointed to a coupling of calcium signaling to MT rearrangement via
MARK2/Par-1. Interestingly, analogous serine residues in the catalytic domain of MARK3 are phosphorylated by Pim1, which is also a member of the CaMK superfamily [68]. But in this case the phosphorylation is thought to cause an inhibition of MARK3. This is another unresolved issue that needs clarification.

**MARK inhibition by phosphorylation**

aPKC and MARK2/Par-1 are required for cell polarity in various contexts. Mammalian aPKC functions upstream of MARK2/Par-1 and promotes the establishment of epithelial cell polarity by phosphorylating MARK2/Par-1 at Thr595 in the spacer domain and enhancing its binding to 14-3-3/Par-5. This phosphorylation downregulates kinase activity and promotes the dissociation of MARK2/Par1 from the lateral membrane in polarized cells [69,70]. aPKC phosphorylates the equivalent site of MARK3 in vitro, indicating that the MARK isoforms might have redundant functions in the cell [70].

The proline-directed kinase GSK3β phosphorylates MARK2/Par-1 at Ser212 and inhibits its activity, even when it is activated by MARKK at Ser208 [66]. This recent result is in contrast to an earlier claim that GSK3β activates MARK2 by phosphorylating Ser212 [71]. In this earlier study only point mutations of MARK/Par-1 were tested to identify the responsible residue phosphorylated by GSK3β without checking the actual kinase activity of the MARK mutants. However, some mutants are not active because of structural alterations and not because the mutated residue cannot be phosphorylated by GSK3β anymore; however, coexpression of GSK3β and MARK2 stabilizes the microtubule network so that cells cotransfected with MARK2 and GSK3β do not form neurites. Endogenous inactive GSK3β and active MARK2 colocalize in the neurite tips of PC12 cells and in the growth cones of rat cortical neurons. Similarly, active MARK and inactive GSK3β are involved in the growth of an axon and are predominantly found at the axon tips where the highest dynamics of MTs is needed for growth and retraction [66].

Another kinase whose activity is regulated by LKB1 and GSK3β is the salt-inducible kinase-1 (SIK1) [72]. SIK1 also belongs to the AMPK family of protein kinases and shares a similar primary structure of the kinase domain with...
MARK2/Par-1. SIK1 is activated by metabolic or energy stress and hence represses the transcription of steroidogenic enzymes. This involves the phosphorylation of Thr182 in the activation loop of SIK1 by LKB1, emphasizing potential parallels between SIK and MARK2/Par-1 regulation.

As mentioned earlier, the human PIM1 kinase, a downstream effector of many cytokine-signaling pathways, associates with and phosphorylates MARK3 (also known as CTAK-1), which leads to a substantial decrease in MARK3 kinase activity [68].

**MARK Inhibition by Interaction**

Recently we identified PAK5 as a MARK2/Par-1 inhibitor [73]. PAKs are members of Rac/Cdc42-associated Ser/Thr protein kinases, which regulate death and survival signaling, cell-cycle progression and the coordination of the actin- and microtubule-cytoskeleton dynamics. PAKs are divided into an N-terminal regulatory domain containing the p21-binding domain and a C-terminal kinase domain [74]. PAK5 is expressed mainly in the brain and induces filopodia, neurite outgrowth and dendritic spines [75]. The interaction between PAK5 and MARK2/Par-1 is based on their catalytic domains independently of its state of phosphorylation so that the binding of PAK5 is sufficient to inhibit MARK2/Par-1, whereas the activity of PAK5 is not affected. PAK5 eliminates the effect of MARK2/Par-1 on the cytoskeleton, that is, the destabilization of microtubules. Active PAK5 has two independent effects on the microtubule and actin cytoskeleton. First, it stabilizes microtubules by binding and inhibiting MARK2/Par-1 and, second, it makes actin filaments dynamic by dissolving stress fibers and focal adhesions, inducing the formation of filopodia (Figure 4a) [73,76].

A similar antagonistic regulation of the microtubule and actin cytoskeleton can be observed on the level of upstream activators. A search for regulators of MARKK identified TESK1 and Spred-1 as interaction partners, suggesting that a three-way interaction between these proteins affects both the microtubule and actin microfilament dynamics [77]. TESK-1 belongs to the LIM kinase family and influences actin organization by phosphorylating cofilin (Figure 4b). Cofilin is a ubiquitous actin-binding factor, which disassembles actin filaments, thereby preventing their reassembly. Cofilin is regulated via inhibitory phosphorylation. Spred-1 exerts regulatory functions in different pathways. It binds to MARKK, but it has no effect on MARKK activity. However, the interaction of Spred-1 and TESK1 blocks the activity of TESK1, decreases the phosphorylation of cofilin and renders the actin network more dynamic. These effects are prevented by expression of TESK1, which leads to inactivation of MARKK by binding. Spred-1 could serve as a scaffold for these two kinases. Thus, this three-protein complex of MARKK–TESK1–Spred-1 regulates the microtubule and actin cytoskeleton in an antagonistic manner in analogy with PAK5 and MARK2/Par-1, such that stable microtubules correspond to labile actin microfilaments and vice versa (Figure 4).

**Other modes of MARK Regulation**

Besides the regulation of the catalytic domain, MARK/Par-1 can be controlled by a variety of other interactions.

For example, the adaptor protein 14–3–3 (Par-5) can interact in a non-phosphorylation-dependent manner with the catalytic domain of MARK2 [53]. Alternatively, aPKC can phosphorylate the spacer domain of MARK2 and creates a 14–3–3 binding motif (see earlier) [70]. This leads to relocation and inactivation of MARK2, probably by stabilizing the inhibitory interaction of the tail domain of MARK2/Par-1 (KA1) with the N-terminal header of the catalytic domain. Similar results were shown for MARK3 [78], that is, 14–3–3 binding to MARK3 can be achieved by phosphorylation of multiple sites and serves to anchor MARK/Par-1 in the cytoplasm. Ubiquitin might exert some regulation by binding to the UBA domain [59], and upstream or downstream regulators could bind to the CD motif, in analogy to MAP kinases [79]. An interaction between the KA1 domain and the N-terminal header or the catalytic domain has been suggested to generate a folded and inactive conformation of MARK [4] (Figure 5).

**Concluding remarks and future perspectives**

Kinases are often involved in more than one signaling cascade, and, therefore, an accurate regulation of the crosstalk between kinase pathways is necessary. A proteomic approach involving tandem affinity purification (TAP) has revealed several proteins expected to interact with MARK/Par-1 kinases during the development of cell polarity, notably Ced42, ARH-GEF2, aPKC and the scaffold proteins Par-5, Par-3 and Par-6 [80]. In addition, more than 50 novel interactors have been identified, suggesting a highly interconnected network. Protein kinases of the MARK/Par-1 family have crucial roles in the establishment of cell polarity, neuronal differentiation, intracellular
transport, neuronal migration, neurodegeneration and more (Figure 3b). Many of these are related to the dynamics and polarity of the cytoskeletal microtubule and actin networks. An analysis of the interplay between the different modes of regulation will be essential to determine the signaling mechanisms of MARK and to utilize this knowledge for developing therapeutic approaches for neurodegeneration and other disease processes where this family of kinases is implicated. Examples are compounds that alter the level of MARK regulators, inhibit the activity of MARK, or interfere with the interaction between MARK and its cellular partners.

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