PAR-1/MARK: a Kinase Essential for Maintaining the Dynamic State of Microtubules

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ABSTRACT. The serine/threonine kinase, PAR-1, is an essential component of the evolutionary-conserved polarity-regulating system, PAR-aPKC system, which plays indispensable roles in establishing asymmetric protein distributions and cell polarity in various biological contexts (Suzuki, A. and Ohno, S. (2006). J. Cell Sci., 119: 979–987; Matenia, D. and Mandelkow, E.M. (2009). Trends Biochem. Sci., 34: 332–342). PAR-1 is also known as MARK, which phosphorylates classical microtubule-associated proteins (MAPs) and detaches MAPs from microtubules (Matenia, D. and Mandelkow, E.M. (2009). Trends Biochem. Sci., 34: 332–342). This MARK activity of PAR-1 suggests its role in microtubule (MT) dynamics, but surprisingly, only few studies have been carried out to address this issue. Here, we summarize our recent study on live imaging analysis of MT dynamics in PAR-1b-depleted cells, which clearly demonstrated the positive role of PAR-1b in maintaining MT dynamics (Hayashi, K., Suzuki, A., Hirai, S., Kurihara, Y., Hoogenraad, C.C., and Ohno, S. (2011). J. Neurosci., 31: 12094–12103). Importantly, our results further revealed the novel physiological function of PAR-1b in maintaining dendritic spine morphology in mature neurons.

Key words: PAR-1/Microtubule dynamics/Dendritic spine/MAPs/PAR-aPKC system

Mammalian PAR-1 Was Identified as a Microtubule (MT)-associated Protein (MAP)/MT Affinity-regulating Kinase (MARK)

PAR-1 was originally identified in C. elegans as a putative serine/threonine kinase essential for asymmetric division of the one-cell embryo (Kemphues et al., 1988). However, the first implication for its downstream targets was provided by a study investigating the pathogenesis of Alzheimer’s disease (Drewes et al., 1995). In the brain of Alzheimer’s patients, the microtubule (MT)-binding protein, tau, is hyperphosphorylated and forms large aggregates called neurofibrillary tangles. In the course of searching for the responsible kinases, Mandelkow’s group identified biochemically the mammalian homolog of PAR-1 as the kinase phosphorylating the Lys-Xaa-Gly-Ser (KXGS) repeats located in the MT-binding domains (Drewes et al., 1997). Because PAR-1 phosphorylates the corresponding sites in the other classical MAPs, MAP2 and MAP4, and commonly induced their detachment from MTs, the researchers termed mammalian PAR-1s as MARKs (MAP/MT affinity-regulating kinases; Ebneth et al., 1999). MTs are essential players in establishing cell polarity (Drewes et al., 1997), and indeed many phenotypes observed in PAR-1 mutants or PAR-1 depleted cells are tightly coupled with severe defects in MT organization (see below). Under these contexts, researchers have widely accepted the idea that PAR-1/MARK primarily regulates cell polarity by affecting MTs (Matenia and Mandelkow, 2009). However, although MAPs are one of the strong candidates linking PAR-1 activity to MT organization, the molecular mechanisms underlying the PAR-1-mediated MT regulation are still largely unknown (Doerflinger et al., 2003; Cohen et al., 2004).

PAR-1/MARK Exerts Diverse Effects on Subcellular MT Organization

Classical MAPs have been shown to facilitate tubulin assembly and stabilize polymerized MTs (Desai and
Drewes disrupted MT arrays when overexpressed in CHO cells (Cohen et al., 2001). Particular MT architectures (lateral MTs) in epithelial cells revealed intuitively contradicting activities of PAR-1 on MTs. For example, PAR-1/MARK overexpression increased MT plus-ends exhibiting over 2-fold less continuous growth (Akhmanova and Steinmetz, 2008). We found that neurite extension in neuronal cells (Timm et al., 2002) and inhibited MT-dependent neurite extension in neuronal cells (Timm et al., 2003). These apparently complicated results may be partially attributed to activities of PAR-1 other than MT destabilization through its MARK activity. However, we should consider an important caveat of these experiments, which analyzed static views of MTs using immunostaining techniques. In other words, as several authors reasonably pointed out by analyzing MT sensitivity to MT drugs or cold shock (Doerflinger et al., 2003; Cohen et al., 2004), these results suggest the intriguing possibility that PAR-1b increases microtubule dynamics, which could result in increased or decreased microtubule density, depending on the cell state such as free tubulin concentrations (Erickson and O’Brien, 1992). Because dynamic instability of MTs plays critical roles in various cellular functions including cell polarity, evaluation of this aspect of the PAR-1/MARK activities is highly important for understanding the molecular basis of the physiological functions of PAR-1/MARK. Nevertheless, owing to lack of direct evidence based on live imaging analysis, the positive role of PAR-1/MARK on microtubule dynamics has not yet been conclusively demonstrated. Lack of sufficient recognition of the roles of MAPs in MT dynamics has also made the situation ambiguous.

**PAR-1 Is Required for Maintaining the Dynamic State of MTs**

To shed light on these problems, we recently performed live imaging analysis of +TIPs (EB3 or CLIP170) in PAR-1b knockdown cells, and provided novel evidence on the positive contribution of PAR-1 to microtubule dynamics (Hayashi et al., 2011). Because +TIPs localize MT plus-ends only during the growing state, the growing ends of microtubules can be traced by monitoring +TIPs movements (Akhmanova and Steinmetz, 2008). We found that MT plus-ends exhibited over 2-fold less continuous growing following PAR-1b knockdown in a neuroblastoma cell line and in dendrites of hippocampal neurons, without significant changes in growing velocity (Fig. 1A and B). Because the phases of MT dynamics are composed of three components: growing, shortening and attenuated (or pausing) phase (Tran et al., 1997; Carvalho et al., 2003), the results above indicate that PAR-1b suppresses the transition frequency of MT dynamics (measured by events/second) from the growing state to the pausing and/or shrinking state (Fig. 2). In our experiments, polymerized MT levels were not affected in PAR-1b knockdown cells, suggesting that tubulin polymerization was close to the steady state. Therefore, our results suggest that PAR-1 up-regulates MT dynamic instability (the net change in MT length over the time period) rather than affecting MT polymerization/dem polymerization activity.

**PAR-1 Plays Essential Roles in Maintaining Dendritic Spine Morphology by Regulating Microtubule Dynamics**

Recently, we further demonstrated that this regulation of MT dynamics by PAR-1 is essential for maintenance of dendritic spine morphology in mature hippocampal neurons (Hayashi et al., 2011). Spines are postsynaptic structures that receive excitatory synaptic input from presynaptic terminals. Previous in vivo (Trachtenberg et al., 2002; Yuste et al., 2002; K. Hayashi et al., 2004), the MAP-introduced cells were reported to show a decreased growth rate of MTs (Dhamodharan and Wadsworth, 1995; Bunker et al., 2004). Because the steady state condition is likely to most accurately reflect the conditions within cells such as mature neurons, these results indicate that endogenous classical MAPs suppress MT dynamic instability, and not simply facilitate MT polymerization (Dhamodharan and Wadsworth, 1995). Unlike our PAR-1b knockdown cells, the MAP-introduced cells were reported to show a decreased growth rate of MTs (Dhamodharan and Wadsworth, 1995; Bunker et al., 2004). However, this could be due to the reduction in free tubulin concentration caused by non-physiological concentrations of ectopic MAPs (Dhamodharan and Wadsworth, 1995; Bunker et al., 2004). Taken together, our live imaging data of PAR-1 knockdown cells may be consistent with its activity of inhibiting MT-binding of MAPs, although we cannot exclude the possibility that other downstream targets of PAR-1 are involved in its MT regulatory activity (Doerflinger et al., 2003).
**Fig. 1.** PAR-1b knockdown causes defects in continuous microtubule growth. (A) Representative images of CLIP170 comet trajectories (white lines) in differentiated Neuro 2a cells treated with the indicated RNAi. Scale bar, 10 \( \mu \)m. (B) Quantification of mean lengths of GFP-CLIP170 comet trajectories (left panel) and mean velocities of GFP-CLIP170 comet movements (right panel).

**Fig. 2.** Steady state diagram of MT polymerization. Under steady state conditions in which there is no net gain of MT mass, MTs are in either of the two different states: dynamic state (upper box) or static state (lower box). In the dynamic state, MTs are biased toward growing and/or shortening, whereas in the static state they are biased toward attenuation. Our results (Fig. 1) indicate that PAR-1 is required to maintain the dynamic state. Interestingly, classical MAPs are suggested to stabilize the static state (see text for details).
and Bonhoeffer, 2004) and in vitro (Matsuzaki et al., 2004) studies have shown that the shape and the number of spines alter dynamically by synaptic plasticity. Because immunofluorescence analysis did not detect MT signals in spines, it has been believed that actin filaments and their regulatory proteins play a central role in spine morphogenesis (Kaech et al., 2001). However, recent studies have revealed that MTs stochastically invade into spines in an activity dependent fashion (Kapitein et al., 2011; Gu et al., 2008; Hu et al., 2008). These studies also demonstrated that MT dynamics are essential for maintaining the shape of mature dendritic spines by regulating the dendritic localization of p140Cap, which is required for actin reorganization (Fig. 3; Gu et al., 2008; Hoogenraad and Bradke, 2009; Jaworski et al., 2009). In our recent paper, we demonstrated that PAR-1b knockdown in mature neurons induces conversion of mature mushroom-shaped spines into filopodia-like dendritic protrusions, and reduction of p140Cap accumulation in dendritic spines (Hayashi et al., 2011). Furthermore, p140Cap overexpression partially restored mature spines in PAR-1 knockout neurons, suggesting that PAR-1 regulates spine morphology by facilitating dendritic accumulation of p140Cap. Together with the above-mentioned results that PAR-1b knockdown in primary cultures of hippocampal neurons resulted in less continuous growing of MTs, we concluded that PAR-1b maintains mature dendritic spine morphology by regulating MT dynamics.

The functions of PAR-1 in developing neurons have been studied vigorously and shown to be involved in neurite elongation (Chen et al., 2006; Terabayashi et al., 2007; Uboha et al., 2007). Our results demonstrate that PAR-1 also plays crucial roles in mature neurons, and provide possible molecular mechanisms underlying the impaired spatial learning and memory observed in PAR-1b knockout mice (Segu et al., 2008). Intriguingly, previous studies have shown that aPKC, PAR-3 and PAR-6 exhibit indispensable roles in dendritic spine morphology by regulating the actin cytoskeleton via Rac and Rho small GTPases, respectively (Zhang and Macara, 2006, 2008). Dendritic spines are highly polarized local structures, maintained by intricate interactions between dynamic cytoskeleton reorganization and vesicle transport (Bourne and Harris, 2008). Taken together, our results indicate that dendritic spines are also one of the regulatory targets of the general cell polarity machinery, the PAR-aPKC system.

References
PAR1 and Microtubule Dynamics


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