The Polarity Protein Par1b/EMK/MARK2 Regulates T Cell Receptor-Induced Microtubule-Organizing Center Polarization

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The Polarity Protein Par1b/EMK/MARK2 Regulates T Cell Receptor-Induced Microtubule-Organizing Center Polarization

Joseph Lin,* Kirk K. Hou,* Helen Piwnica-Worms,†‡ and Andrey S. Shaw2*‡

Engagement of a T cell to an APC induces the formation of an immunological synapse as well as reorientation of the microtubule-organizing center (MTOC) toward the APC. How signals emanating from the TCR induce MTOC polarization is not known. One group of proteins known to play a critical role in asymmetric cell division and cell polarization is the partitioning defective (Par) family of proteins. In this study we found that Par1b, a member of the Par family of proteins, was inducibly phosphorylated following TCR stimulation. This phosphorylation resulted in 14-3-3 protein binding and caused the relocalization of Par1b from the membrane into the cytoplasm. Because a dominant-negative form of Par1b blocked TCR-induced MTOC polarization, our data suggest that Par1b functions in the establishment of T cell polarity following engagement to an APC. The Journal of Immunology, 2009, 183: 0000–0000.

Establishing and maintaining cellular polarity is critical for all organisms to grow, divide, and differentiate (1). The importance of polarity has been demonstrated in a variety of systems such as the initial cell division in Caenorhabditis elegans, bud site determination in yeast, apical/basal development of epithelial cells, and axon determination in hippocampal neurons (2–5). Cell polarity is also important in T lymphocytes (6). Following engagement of the T cell to an APC, several cell surface and cytoplasmic proteins are asymmetrically localized and enriched at the contact site called the immunological synapse (7). Another hallmark of T cell polarization, concomitant with immunological synapse formation, is the reorientation of the microtubule-organizing center (MTOC) toward the APC.

Although MTOC reorientation was observed over two decades ago, how signals from the TCR translate into MTOC polarization is still unknown (6). Previous studies have demonstrated that engagement of the TCR is sufficient for MTOC polarization and that the two Src kinases involved in proximal TCR signaling, Lck and Fyn, are required (8, 9). Other molecules known to play a role in proximal TCR signaling, such as the tyrosine kinase ZAP-70 and the adaptor proteins LAT and SLP-76, are also required (10). However, almost nothing is known about the molecules that convert signals generated through the TCR into movement of the MTOC. Presumably, proteins that regulate polarized localization of signaling molecules and proteins that regulate microtubule dynamics play a critical role in this phenomenon.

Searching for mutations that disrupt asymmetric cell division in Caenorhabditis elegans, Kemphues and colleagues identified several proteins that are essential for asymmetric cell division (2). This group of proteins is referred to as the partition defective (Par) family (2). The Par family consists of six proteins. Par1 and Par4 are Ser/Thr kinases, Par3 and Par6 are PDZ domain-containing adaptor proteins, Par2 is a RING finger protein, and Par5 is a 14-3-3 protein. All six Par proteins are conserved throughout evolution with the exception of Par2, which has no recognized ortholog in mammals (1). Also involved in polarity is protein kinase C (PKC) C, which, when mutated, displays a similar phenotype as that of mutations of the Par family (11).

All of these proteins not only regulate asymmetric cell division, but some are themselves asymmetrically localized within the cell. For example, in C. elegans, Par1 and Par2 polarize to the posterior pole whereas Par3, Par6, and PKC3 localize to the anterior portion of the cell (12, 13). A distinct pattern of localization has also been reported in polarized mammalian epithelial cells, with Par3 and Par6 localized to the junctional complexes that divide the apical and basolateral surfaces and Par1 localizing to the basolateral surface (14, 15). Recently, Par3 was shown to localize to the immunological synapse in T cells and PKCζ was shown to localize to the pole of the cell distal to the MTOC (16, 17).

Compared with C. elegans and Drosophila, significantly less is known about the function of the Par proteins in mammalian systems. Part of the difficulty in studying the Par proteins in mammalian systems is the existence of multiple orthologs and/or splice forms. For example, Par 6 has at least four homologues and Par3 has several alternatively spliced forms (18–20). The mammalian orthologs of PKC3 are the two atypical PKCs, PKCζ and PKCα. Par1 also has four homologues known by a variety of different names (Par1a/MARK3/C-TAK, Par1b/MARK2/EMK, Par1c/MARK1, and Par1d/MARK4) (21, 22). Interestingly, Par1 homologues have also been implicated in the regulation of microtubule dynamics. Microtubule-associated proteins (MAPs), which bind to and stabilize microtubules, have been demonstrated to be a substrate of Par1 (14, 21).

Given the potential role of Par1 in regulating asymmetric protein localization, as well as its role in regulating microtubule dynamics, we wanted to determine whether Par1 played a role in MTOC polarization during immunological synapse formation. We focused on Par1b, as this isoform has been previously implicated...
to play a role in T cell function (23). We found that TCR stimulation results in the phosphorylation of Par1b at two sites, S400 and T595. The phosphorylation of these sites resulted in 14-3-3 binding, allowing Par1b to come off the membrane and accumulate in the cytoplasm near the contact site. Strikingly, expression of a dominant-negative Par1b blocked MTOC polarization, supporting the notion that Par1b regulates TCR-induced MTOC polarization.

Materials and Methods

Cell culture and antibodies

Jurkat T cells and Daudi B cells were maintained in RPMI 1640 supplemented with 10% FBS, 2 mM glutamine, penicillin, and streptomycin. T cells isolated from OT-1 TCR transgenic mice and RMA-S T cells were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, non-essential amino acids, sodium pyruvate, 2-ME, penicillin, and streptomycin. TCR stimulation was performed with the anti-human TCR Vβ8 mAb (C305) or anti-mouse CD3 (2C11). Anti-phospho-T595 and anti-phospho-S400 Abs were generated by phosphopeptide immunization of rabbits (24, 25) and the polyclonal rabbit anti-14-3-3 has been previously described (26). The Ab for Par1b has also been previously described (23). The anti-PKCζ and anti-myc are from Upstate Biotechnology. Anti-Xpress is from Invitrogen. Anti-FLAG (M2) and anti-α tubulin mAbs are from Sigma-Aldrich. Goat anti-mouse IgG-Cy3, IgG-Cy5, and goat anti-human IgG-Cy3 were from Jackson ImmunoResearch Laboratories. PP2 was from Calbiochem.

Plasmids
cDNA was excised from pCDNA3.1-FLAG-Par1b (24) and cloned into pEGFP-C1 (Clontech) and pEF/4/myc-His (Invitrogen) for expression in Jurkat T cells and pMX-IRES-GFP for transduction into OT-1 T cells. Point mutations were generated by PCR and verified by sequencing. Myristoylated PKCζ and kinase dead (KD) PKCζ were previously reported (24). Constitutively active and KD PKCθ and constitutively active and KD PKCε were generous gifts from I. Roose (University of California, San Francisco, CA). Myc-tagged 14-3-3ζ in pCDNA3.1 was previously described (26). The control plasmid used for Fig. 5B (FLAG-C2DTM) has been previously described (27).

Cell transfections and transductions

Jurkat T cells were electroporated and lysed in 1% Nonidet P-40 and proteins were visualized by Western blotting as previously described (28). Primary T cells were transduced after a 48-h stimulation with anti-CD3 and anti-CD28, using the AMAXA mouse T cell Nucleofector kit following the protocol provided. OT-1 T cells were transduced with aviral supernatant produced in Plat E packaging cells. Briefly, bulk lymphocytes were stimulated overnight with peptide (1 μM SIINFEKL) and then spin infected for 20 min with viral supernatant two times, separated by 4 h. Cells were used for experiments 4 days after the initial stimulation.

Cellular fractionation

Cells were lysed in hypotonic lysis buffer (10 mM NaCl, 10 mM Tris (pH 7.6), and 2 mM EDTA) and Dounce homogenized. Cell lysates were then spun at low speed to remove insoluble fractions. Lysates were then spun at 100,000 rpm for 30 min. Supernatants were extracted and the membrane pellet was solubilized with sample buffer.

Microscopy

Jurkat-Daudi conjugates were made by loading Daudi B cells with 1 μg/ml staphylococcal enterotoxin E (SEE; Toxin Technologies) for 30 min before mixing 1:1 with Jurkat T cells. Cells were then pelleted and incubated at 37°C for 25 min. Conjugates were gently resuspended and allowed to settle on poly-l-lysine-coated slides before fixation in 3% paraformaldehyde (PFA). Cells were then permeabilized and stained with the indicated Abs. OT-1 T cell conjugates were prepared in a similar manner except that RMA-S cells loaded overnight with SINFEKL (1 μM) peptide were used as APCs. For polarization toward anti-TCR, coverslips were coated with anti-TCR and cells were allowed to settle onto the coverslips before fixation in 3% PFA. Polarization toward the coverslip was scored blinded. Cells were considered polarization positive if the MTOC was clearly visible, microtubules were radiating from the it, and it was centrally located within the cell in the focal plane adjacent to the coverslip. Images were collected on a LSM 510 (Zeiss Axiovert 200 microscope using a ×63/1.4 oil objective at room temperature). Images for the primary T cell polarization assays were collected using an Olympus FV1000 with a ×60 objective.

Results

Par1b is inducibly phosphorylated following TCR stimulation

We initiated our studies on the role of Par proteins in T cell polarization by focusing on Par1b, because the phenotype of the Par1b knockout mouse led to aberrant T cell function (23). Because TCR stimulation is sufficient to induce MTOC polarization, we hypothesized that if Par1b played a role in polarization, it would become phosphorylated in response to TCR stimulation. In Madin-Darby canine kidney cells and other adherent cell lines, Par1b has been shown to be basally phosphorylated at a conserved threonine (T595) (24, 29). To test whether this threonine is inducibly phosphorylated following TCR stimulation, Jurkat T cells were transfected with a FLAG-tagged Par1b. Following TCR stimulation with an anti-TCR mAb, cells were lysed and proteins immunoprecipitated with an anti-FLAG Ab. Proteins were then blotted with a phospho-specific Ab to phosphorylated threonine 595 (pT595) (24). Following TCR stimulation, Par1b was rapidly phosphorylated and remained phosphorylated for at least 20 min (Fig. 1A).

Recently, another phosphorylation site on Par1b, serine 400, was identified (25). A phospho-specific Ab toward the phosphorylated serine 400 (pS400) was used to test whether S400 was also inducibly phosphorylated following TCR stimulation. When Par1b-transfected T cells were stimulated, Western blotting showed that S400 was also inducibly phosphorylated following TCR stimulation with similar kinetics as T595 (Fig. 1A).

To ensure that the induced phosphorylation of Par1b was not due to overexpression, we immunoprecipitated endogenous Par1b from Jurkat T cells following TCR stimulation. Immunoprecipitates were then resolved and visualized using Abs specific for pS400, pT595, and Par1b. Endogenous Par1b was phosphorylated...
following TCR stimulation, supporting the finding obtained in the overexpression system (Fig. 1B). Furthermore, we detected two bands that correspond to the two previously reported splice forms of endogenous Par1b detected in lymphocytes (23). These data indicate that endogenous Par1b is also inducibly phosphorylated following TCR stimulation.

Because Src family kinases are the most proximal kinases to the TCR and are required for all known downstream signaling events from the TCR, we wanted to determine whether the inducible phosphorylation on Par1b was dependent on Src family kinases. Jurkat T cells were incubated with the Src family kinase inhibitor PP2 and then stimulated through the TCR. As expected, neither T595 nor S400 was phosphorylated in cells treated with PP2, indicating that the Src family kinases are required for Par1b phosphorylation induced by TCR stimulation (Fig. 1C).

**Phosphorylation of Par1b is mediated by PKCs**

We next wanted to determine which kinase or kinases are responsible for the phosphorylation of T595 in T cells. In other cell types, atypical PKCs (aPKCs) are known to phosphorylate T595 (24, 29, 30). To determine whether aPKCs were also responsible for the phosphorylation of T595 in T cells, cells were transfected with a constitutively active form of PKCζ (myristoylated PKCζ). Expression of the constitutively active PKCζ induced the phosphorylation of T595 in T cells. This was dependent on PKCζ kinase activity, as T595 phosphorylation was not observed when the kinase-inactive mutant was expressed (Fig. 2A). To determine whether the ability of PKCζ to phosphorylate T595 was specific, we tested active forms of two other PKCs, PKCa, a member of the conventional family of PKCs, and PKCb, a member of the novel family of PKCs (nPKC). Expression of either PKCα nor PKCθ induced the phosphorylation of T595, demonstrating that phosphorylation of this site was specific for aPKCs (Fig. 2B).

In the course of these experiments, we observed that although nPKC family members did not induce T595 phosphorylation, they did induce the phosphorylation of S400 in T cells. In cells expressing constitutively active PKCθ, but not the conventional PKC family member PKCα, we observed an increase in pS400 (Fig. 2B). This was dependent on the kinase activity of PKCθ, as S400 phosphorylation was not seen using a kinase-inactive form of PKCθ. Phosphorylation of S400 was also induced by PMA stimulation, supporting the notion that the phosphorylation is induced by a 1,2-diacylglycerol-responsive kinase such as PKCθ (data not shown). It was recently demonstrated, however, that S400 can be phosphorylated by PKD (25). Because nPKCs such as PKCe can activate PKD, we presume that the nPKC expressed in T cells, PKCθ, indirectly induces the phosphorylation at S400 by activating PKD (31).

**Phosphorylation mediates 14-3-3 binding and localization to the contact site**

Because previous studies had demonstrated that pT595 is a binding site for 14-3-3 proteins, we tested whether phosphorylation of S400 was also able to recruit 14-3-3 proteins. Site-directed mutagenesis of T595 and S400 was performed to substitute alanine at each site, individually, or in combination. Each of the constructs was then tested for its ability to bind to 14-3-3 proteins. Wild-type (wt) Par1b coimmunoprecipitated 14-3-3, whereas mutation of T595 substantially reduced the ability of Par1b to bind 14-3-3 proteins. Wild-type (wt) Par1b coimmunoprecipitated 14-3-3, whereas mutation of T595 substantially reduced the ability of Par1b to bind 14-3-3 proteins (Fig. 3A). Whereas mutation of S400 only modestly decreased the association of 14-3-3, the double mutation almost completely abrogated 14-3-3 protein binding to Par1b.

We next confirmed that TCR engagement induces the association of 14-3-3 proteins with Par1b. Jurkat T cells transfected with mutated or wt Par1b were stimulated through the TCR, and immunoprecipitates were prepared with Abs to Par1b. Consistent with the induced phosphorylation of T595, TCR stimulation augmented the association of 14-3-3 proteins with wt Par1b. In contrast, cells expressing the double-mutant form of Par1b demonstrated very little 14-3-3 association (Fig. 3B). These data demonstrate that TCR stimulation induces the association of 14-3-3 proteins to Par1b and that the major binding sites are pT595 and pS400.
Par1b REGULATES T CELL POLARITY

In general, 14-3-3 protein association functions to change subcellular localization of the bound protein. To determine whether this was true in the case of T cells for Par1b, cell fractionation experiments were performed. In resting cells, Par1b is mainly associated with membranes. Following TCR stimulation, greater amounts of Par1b were detected in the cytoplasmic fraction (Fig. 3C). In contrast, the double mutant form of Par1b (S400A, T595A) was only detected in membrane fractions before and after TCR stimulation.

GFP-Par1b is localized to the site of T cell-APC contact

We next examined Par1b localization. A Par1b-GFP fusion protein was generated and imaged following T cell stimulation with Daudi B cells loaded with the superantigen SEE. After 20 min, cells were fixed and visualized by confocal microscopy. Unconjugated T cells displayed mainly a membrane-bound distribution of GFP-wt Par1b, consistent with its localization to the plasma membrane. In contrast, T cells that had formed conjugates with the superantigen-loaded APCs demonstrated a clear accumulation of GFP-wt Par1b near or at the immunological synapse (Fig. 4A). Interestingly, the ST/AA double mutant and the kinase-inactive mutant did not demonstrate any preferential localization near or at the synapse. To determine whether the accumulation of wt Par1b was at the membrane or in the cytoplasm, we co-stained with Abs to the TCR to label the membrane. Although there was some membrane-bound Par1b at the immunological synapse, there was also significant cytoplasmic localization of Par1b (Fig. 4B). Importantly, there was little if any cytoplasmic Par1b seen with either the double ST/AA mutant or with the kinase-inactive mutant. Localization of GFP-wt Par1b and GFP-ST/AA Par1b within conjugates was scored negative if the localization was primarily membrane associated or positive if there was cytoplasmic accumulation at the contact site. The samples were quantitated in a blinded manner and the results are graphed in Fig. 4C. These data together suggest that the accumulation of Par1b at the immunological synapse requires its phosphorylation and suggest that the enrichment is mainly due to the accumulation of Par1b in the cytoplasm adjacent to the immunological synapse.

Confocal images of T cells stained with an anti-FLAG Ab to determine the localization of Par1b near the synapse occurred in primary T cells. OT-1 TCR transgenic T cells were transduced with a retrovirus encoding a FLAG-tagged Par1b. Conjugates were formed with APCs for 30 min. Cells were then fixed, permeabilized, and stained with an anti-FLAG Ab to determine the localization of Par1b in T cell-APC conjugates. Par1b localized near to the T cell-APC contact site in transduced OT-1 T cells, confirming that the localization of Par1b is similar in both Jurkat T cells and primary T cell conjugates (Fig. 4D).

Dominant-negative Par1b blocks MTOC polarization in T cells

Because Par1b was responsive to TCR-dependent stimuli, we next asked whether MTOC reorientation, a hallmark of T cell polarization, was regulated by Par1b. Because Par1 has four homologues in the mammalian systems, we chose to use a dominant-negative approach. Overexpression of a construct lacking either the kinase domain or kinase activity is known to exhibit potent dominant-negative effects (14, 15, 32). Therefore, we tested whether the KD-Par1b could block TCR-induced MTOC reorientation.

T cells were transected with KD-Par1b and conjugates were prepared using superantigen-coated Daudi cells. Conjugates were then fixed and stained for epitope-tagged KD-Par1b to detect transfected cells and α-tubulin to visualize the MTOC. In many conjugates, the MTOC was not polarized properly in T cells expressing the KD-Par1b, suggesting that inhibition of Par1b blocks MTOC polarization in T cells (Fig. 5A).

To quantify polarization, we used anti-TCR-coated coverslips to measure MTOC polarization as previously described (10). Cells were transfected with KD-Par1b, fixed, stained for KD-Par1b and α-tubulin, and imaged by confocal microscopy. Cells were scored as positive for polarization only if they had a centralized MTOC with microtubules emanating in a radial pattern. The majority of cells expressing KD-Par1b failed to display the characteristic astral pattern of a polarized MTOC (Fig. 5B). After TCR stimulation, the number of polarized MTOCs increased to 63 ± 3%. In contrast, expression of the KD-Par1b reduced this number to 33 ± 5%. This was similar to the percentage of polarized MTOCs seen in unstimulated cells (25 ± 3%) and reflects a random pattern of MTOC polarization.

To determine whether the dominant-negative effects of Par1b could be seen in primary cells as well, we transfected primary T cells with a GFP-tagged KD Par1b construct and scored transfected cells for MTOC polarization toward an anti-TCR Ab as before. Confirming our data using Jurkat cells, the dominant-negative Par1b inhibited MTOC polarization in primary T cells (Fig. 5C). This supports a role for Par1b in the regulation of MTOC polarization in T cells.
The data presented here demonstrate that Par1b plays a critical role in T cell polarity. The T cell in the lower conjugate (denoted by arrowhead), expressing KD-Par1b, shows defective MTOC polarization. B, KD-Par1b transfected Jurkat T cells were stimulated on anti-TCR-coated coverslips and allowed to polarize for 20 min before fixation in 3% PFA. Cells were then stained with anti-FLAG to identify transfected cells and anti-tubulin to visualize the position of the MTOC. Arrowheads denote cells expressing KD-Par1b. Confocal sections adjacent to the coverslip were visualized and quantitated. No anti-TCR on the coverslip (No Stim) and a control transfection of an unrelated FLAG-tagged protein (Control) were negative for polarization, respectively. Five experiments in which >100 cells were scored is shown. Error bars represent SD.

Discussion

The data presented here demonstrate that Par1b plays a critical role in the regulation of T cell polarity. Par1b becomes phosphorylated by PKC/PKD family members during T cell activation inducing 14-3-3 protein binding to Par1b. PKCζ, PKCθ, and PKD are all known to be recruited to the immunological synapse and/or lipid rafts following TCR stimulation (33–35). This suggests a simple model where the activation of these kinases in the synapse results in the phosphorylation of Par1b, the binding of 14-3-3, and the movement of Par1b away from the contact membrane and into the cytoplasm (Fig. 6).

Previously, Russell and coworkers showed that Par3 localizes to the immunological synapse (17). Using Abs to Par3, we confirmed that Par3 is localized to the synapse in our system (data not shown). Because Par3, Par6, and atypical PKCs localize opposite to Par1 in the C. elegans zygote, we were initially surprised to find Par1b localized to the immunological synapse in our experiments. However, a closer examination using biochemical and imaging techniques showed that the localization of Par1b near the synapse was most likely due to cytoplasmic accumulation. The fact that the double ST/AA and the KD mutants are unable to become efficiently phosphorylated and bind 14-3-3 is consistent with the idea that the accumulation near the synapse requires the ability to leave the membrane. It follows that the accumulation near the synapse is due to accumulation in the cytoplasm and not at the membrane. It is interesting to speculate that the exclusion of Par1b from the plasma membrane may be important for the accumulation of Par3 to the immunological synapse. Par3 is known to be phosphorylated by Par1, inducing 14-3-3 protein binding to Par3 (36). Without Par1b at the membrane, Par3 is no longer phosphorylated and free to accumulate at the immunological synapse.

How Par1b is held at the membrane is still unclear. Recently, it has been shown that Wnt signaling or Dishevelled expression can induce Par1b membrane recruitment via phosphorylation of T324 on Par1b. When T324 is mutated to Glu, the mutant Par1b accumulates more readily at the membrane, suggesting that the phosphorylation of T324 induces the binding of Par1b to a membrane-associated protein (37, 38). Other groups have mapped the segment required for membrane association to the C-terminal spacer region between residues 511–661 (29, 39). Further investigation is needed to determine what Par1b is binding to at the plasma membrane.

One of the functions of the immunological synapse may be tofacilitate polarization of the T cells. The recruitment of PKCθ, PKD, and atypical PKCs to the synapse helps to enrich several molecules at the contact site and at the same time induces the removal of Par1b from this area. This suggests that the localization of Par1b to the plasma membrane is constitutive and simply reflects the normal propensity of Par1b to bind to membranes. We suspect that the residual Par1b that we see at the immunological synapse is secondary to the overexpression of Par1b.
This raises interesting issues regarding what it means to be localized to the immunological synapse. In general, localization to the immunological synapse implies membrane association; however this distinction is not always easy to make using standard confocal imaging. In the case of Par1b, it seems that the removal of Par1b from the synaptic membrane results in its accumulation just under the membrane in the cytoplasm. That this is functional was shown by the phenotypic effect of dominant-negative Par1b, which was unable to leave the plasma membrane. Thus, the distinction between localization in the membrane vs localization in the cytoplasm is an important one. Given the small volume of cytoplasm in T cells and the movement of the cytoplasmic space adjacent to the apical surface, the accumulation of any cytoplasmic protein could be confused with immunological synapse localization.

Because Par1b-deficient mice develop immunologic abnormalities (23), we wanted to address whether these defects correlated with defects in T cell polarization. Primary T cells isolated from Par1b-deficient mice and assayed for their ability to polarize toward anti-CD3-coated coverslips did not show any obvious deficiencies in MTOC polarization (data not shown). In addition, Par1b-deficient CD8 T cells did not show any defects in cytolytic killing (data not shown). We attempted to cross the knockouts of Par1b and Par1a; however, the resulting double knockout resulted in embryonic lethality. Because transcripts of all four Par1 homologues can be detected in primary T cells, it seems likely that other Par family members can compensate for the loss of Par1b for MTOC polarization in T cells. This is ultimately why we chose to pursue the dominant-negative approach. As with any dominant-negative experiment, there are always concerns regarding off-target effects. However in the case of kinases it is common to use kinase-deficient forms to block function. Also, other groups have used a kinase-deficient form of Par1b as a dominant negative (14, 15, 32). Because proximal TCR signaling events are required for MTOC polarization, we also tested whether the dominant-negative Par1b was simply disrupting proximal signaling events, leading to the block in MTOC polarization. We did not detect any effects of the dominant-negative Par1b on proximal events in TCR signaling (data not shown), supporting the notion that the dominant-negative Par1b blocks a specific mechanism involved in MTOC polarization.

At least two possible models could explain how KD-Par1b blocks MTOC polarization. One possible mechanism is that Par1b may be regulating the asymmetric distribution of proteins responsible for the establishment of polarity within the cell. In the original identification of the Par family, loss of one Par protein altered the localization of other Par family members (2). Mutation of Par1 is known to alter the distribution of other Par family members such as Par3 (36, 40). Because Par3 had been previously shown to localize to the immunological synapse (17), we also investigated whether the overexpression of KD-Par1b would result in the mislocalization of Par3 in T cells. We, however, did not observe changes in Par3 localization in cells expressing KD-Par1 (data not shown).

Another possible effect of KD Par1b expression could be on microtubule dynamics. One of the previously identified functions for the mammalian homologues of Par1 is that they phosphorylate MAPs. MAPs have been shown to bind and stabilize microtubules; however phosphorylation of the MAPs prevents this function. Overexpression of MAPs has been previously shown to increase microtubule stability and to induce a more stable, hyperacetylated state of polymerized tubulin (41). Whether the effects induced by KD-Par1b are on MAP phosphorylation and therefore microtubule stability or other as-of-yet undefined pathways will require further investigation. However, these results demonstrate that Par1b plays an important role in regulating TCR-induced MTOC polarization.

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Disclosures

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