



# Lymphocyte Polarization During Immune Synapse Assembly: Centrosomal Actin Joins the Game

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Interactions among immune cells are essential for the development of adaptive immune responses. The immunological synapse (IS) provides a specialized platform for integration of signals and intercellular communication between T lymphocytes and antigen presenting cells (APCs). In the T cell the reorganization of surface molecules at the synaptic interface is initiated by T cell receptor binding to a cognate peptide-major histocompatibility complex on the APC surface and is accompanied by a polarized remodelling of the cytoskeleton and centrosome reorientation to a subsynaptic position. Although there is a general agreement on polarizing signals and mechanisms driving centrosome reorientation during IS assembly, the primary events that prepare for centrosome repositioning remain largely unexplored. It has been recently shown that in resting lymphocytes a local polymerization of filamentous actin (F-actin) at the centrosome contributes to anchoring this organelle to the nucleus. During early stages of IS formation centrosomal F-actin undergoes depletion, allowing for centrosome detachment from the nucleus and its polarization towards the synaptic membrane. We recently demonstrated that in CD4<sup>+</sup> T cells the reduction in centrosomal F-actin relies on the activity of a centrosome-associated proteasome and implicated the ciliopathy-related Bardet-Biedl syndrome 1 protein in the dynein-dependent recruitment of the proteasome 19S regulatory subunit to the centrosome. In this short review we will feature our recent findings that collectively provide a new function for BBS proteins and the proteasome in actin dynamics, centrosome polarization and T cell activation.

**Keywords:** immune synapse, centrosome polarization, F-actin clearance, T lymphocytes, B lymphocytes

## INTRODUCTION

The immunological synapse (IS) is a specialized interface formed by T lymphocytes with antigen presenting cells (APCs) bearing a cognate peptide-major histocompatibility complex (pMHC) that allows for information exchange and execution of effector functions (1, 2). In its canonical configuration the IS is characterized by concentric Supra Molecular Activation Clusters (SMACs) differing in composition and function (3, 4). Ligand-bound antigen T cell receptors (TCRs) and associated signaling molecules occupy the central SMAC (cSMAC), which is surrounded by a peripheral SMAC (pSMAC) enriched in adhesion molecules, such as the integrin LFA-1, and an outer distal SMAC (dSMAC), where filamentous actin (F-actin) and CD45 are concentrated (4, 5).

Immune cell interactions rely on continuous cytoskeleton remodeling events, which not only shape the T cell-APC interface, but also asymmetrically distribute molecules and organelles within the lymphocyte, leading to the establishment of transient polarity (6, 7). Cytoskeleton remodeling is one of the earliest events induced by TCR signaling (8) and culminates in the formation of a synaptic F-actin ring, allowing the centrosome to polarize to the IS (9) together with the Golgi apparatus, endosomal and secretory compartments, multivesicular bodies (MBVs) and mitochondria (10–14). This polarized configuration is instrumental in the directional delivery of TCR<sup>+</sup> recycling endosomes to the synaptic membrane (15–17) that thus is refilled of signaling-competent receptors as exhausted TCRs are internalized to be sorted for recycling or lysosomal degradation (18, 19). Alternatively, post-endocytic TCRs are directed to MBVs and incorporated in intraluminal vesicles (ILVs), which are released into the synaptic cleft as exosomes and taken up by APCs (20). Mitochondria are also mobilized towards the APC contact site, where they contribute to IS formation and TCR signaling by providing a local source of ATP and modulating the concentration of intracellular calcium (13, 21, 22).

The rapid repositioning of the centrosome following F-actin depletion from the IS center highlights a spatiotemporal coordination between the actin and microtubule cytoskeletons during IS formation. Interestingly, recent studies have unveiled a new feature of the interplay between F-actin dynamics and centrosome repositioning during IS assembly, demonstrating that the balance between F-actin polymerization and depolymerization at the centrosome is crucial for its ability to untether from the nucleus and polarize to the IS. Here, we will summarize how actin dynamics at the synaptic and centrosomal areas regulate IS assembly. We will then describe the emerging role of the ubiquitin-proteasome system (UPS) in centrosomal F-actin remodeling, focusing on our recent findings that identify the ciliary protein Bardet-Biedl Syndrome 1 (BBS1) as a new regulator of T cell polarity during IS formation (23).

## REGULATION OF CENTROSOME POLARIZATION BY THE ACTIN CYTOSKELETON

### Synaptic F-Actin Controls Centrosome Repositioning to the IS and Microtubule-Driven Exocytosis

TCR engagement by cognate pMHC promotes profound cytoskeletal changes, achieved *via* the coordinated reorganization of the actin and microtubule cytoskeletons at the IS. Actin remodeling is triggered within seconds after TCR stimulation (8) and precedes centrosome translocation towards the IS (9). Imaging cortical actin at the T cell IS using super-resolution microscopy techniques has revealed the coexistence of distinct actin-based networks in the three concentric regions, or SMACs, featured by the IS: from the outer edge to the center, a

lamellipodial branched actin network (dSMAC), the lamellar acto-myosin network (pSMAC), a network consisting of actin foci spread throughout the dSMAC and pSMAC, and an hypodense actin network at the center (cSMAC) [synaptic actin networks are extensively reviewed in (24, 25)]. Another category of actin-based structures, the microvilli, has been described at the T cell surface (26). Although these microvillar extensions are a feature of resting T cells, their study has been recently extended to activated cells based on the observation that TCRs cluster at microvillar tips (27). T cell microvilli had been initially considered as sensors during antigen survey on APCs. However, the discovery of T cell microvillar particles (TMP) deposited on the APC surface has suggested the possibility that they may act as “immunological synaptosomes” that deliver a new class of membrane vesicles as a means of intercellular communication (28, 29).

TCR signaling is the main extrinsic cue for centrosome reorientation to the IS (30), with the second messenger diacylglycerol (DAG), which forms a gradient centered at the cSMAC, acting as a polarity determinant (31, 32). Multiple signaling pathways initiated at the IS by the TCR, the integrin LFA-1 and the co-stimulatory receptor CD28 coordinate the activation of several actin-regulatory proteins that promote F-actin polymerization, feeding back for optimal TCR signaling, integrin activation and T cell spreading over the APC (33, 34). Interestingly, both disruption of synaptic actin networks and depletion of F-actin nucleators (e.g. the formins diaphanous 1 and formin-like-1) result not only in impaired centrosome mobilization but also in defective TCR signaling, suggesting an indirect role of actin in centrosome repositioning. The physical interaction between microtubule (+)-ends and branched F-actin network at the IS periphery, which is mediated by molecular linkers that include the IQ domain-containing GTPase-activating protein, ezrin and Cdc42-interacting protein 4 (35–37), provides an additional function for actin in centrosome polarization by generating tension on microtubules.

Centrosome repositioning is considered a hallmark of T cell polarity during IS assembly. The reorientation of this organelle is accompanied by the polarization of other intracellular compartments, including secretory and recycling endosomes that require microtubule tracks for delivery to the IS and focalized exocytosis. Emerging evidence indicates an early participation of F-actin in polarized recycling. F-actin polymerizes at recycling endosomes through the assistance of the F-actin regulator Wiskott-Aldrich Syndrome protein and SCAR Homology (WASH) and its partner FAM21 to help membrane scission of nascent vesicles carrying recycling cargo, including the TCR, LFA-1, CD28 and the glucose transporter GLUT-1. These receptors exploit the recycling pathway to accumulate at the IS, where they participate in mature IS formation and maintenance, as well as in the metabolic reprogramming of activated T cells (38). The role of actin in the final steps of vesicle fusion and exocytosis at the IS is less clear-cut. Initially, cortical actin has been regarded as a barrier that prevents vesicle exocytosis. Consistent with this view, in T<sub>H</sub> cells the focalized release of IFN- $\gamma$  at the IS was found to be

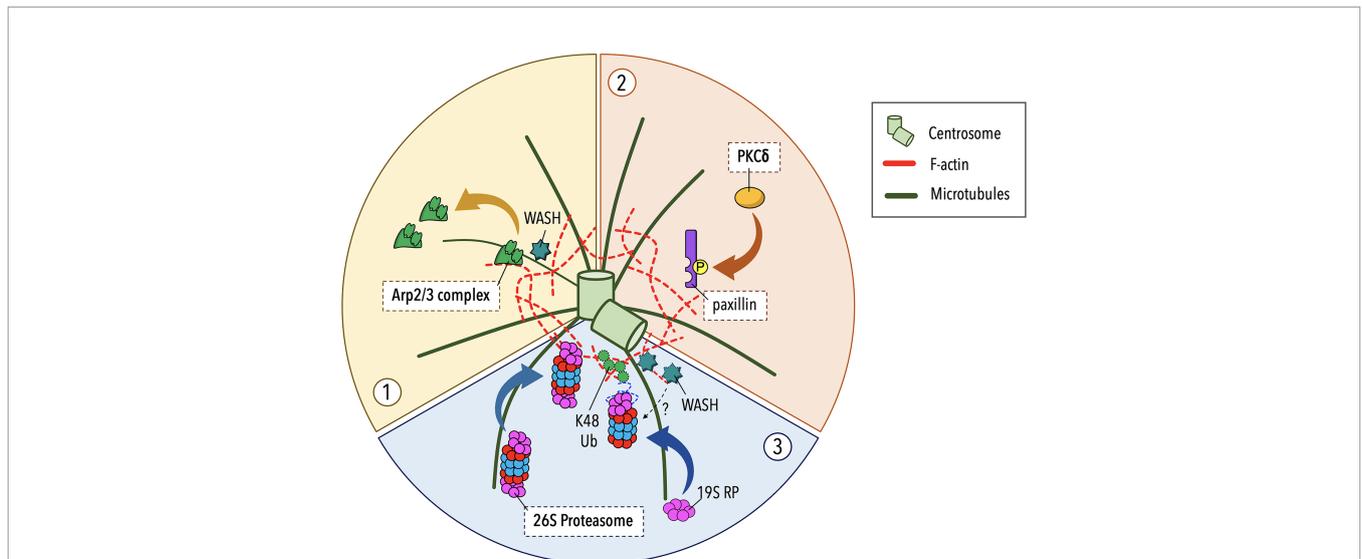
impaired in Cdc42-silenced T cells due to their failure to form a synaptic actin ring (39). Moreover, lattice-light-sheet microscopy of CTL-target cell conjugates showed that at mature synapses F-actin is cleared from the cSMAC before lytic granule secretion, and that lytic granule release triggers F-actin recovery at the lytic synapse blocking further lytic granule exocytosis and serial killing (40, 41). However, a more in-depth analysis of actin dynamics at the lytic synapse using super-resolution microscopy has revealed that the cSMAC is not entirely free from F-actin, but rather occupied by a hypodense actin network (42–44). Upon cell activation, holes of a size compatible with the access of lytic granules to the plasma membrane for docking and fusion have been observed (42, 44). Hence actin plays a dual role during secretion depending on the maturation stage of the lytic synapse: at immature synapses a dense actin cortex blocks secretion, while in mature, actin-hypodense synapses nanoscale actin filament dynamics fine-tunes regulated lytic granule exocytosis (45).

## A Centrosome-Associated F-Actin Pool Contributes to Lymphocyte Polarization

Although the centrosome is the major microtubule-organizing center, F-actin and microtubules coexist at the centrosomal area. Proteomic analyses have documented the presence of actin and actin-associated proteins at the centrosome (46–51) and different actin structures have been reported in association with the

centrosome and the nucleus in different cell types (52–55). Recent studies carried out on B lymphocytes have revealed that the centrosome is surrounded by a local, cloud-like meshwork of F-actin. A central function of this F-actin pool is to anchor the centrosome to the nucleus *via* the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex (51). Moreover, centrosome-associated F-actin can prevent microtubule growth from the centrosome, as supported by *in vitro* reconstitution assay on purified centrosomes (56), suggesting that synaptic F-actin clearance is not sufficient for centrosome polarization to the IS. Of note, the centrosome-associated F-actin network undergoes a dynamic turnover through repeated cycles of polymerization and depolymerization. Upstream centrosome polarization to the IS, the balance between F-actin assembly and disassembly is tilted towards the latter leading to a local depletion of centrosomal F-actin, which facilitates centrosome detachment from the nucleus and its subsequent translocation towards the IS. In B cells this occurs through a reduced recruitment of the actin nucleator Arp2/3 at the centrosome (Figure 1) in favor of its synaptic localization (51).

A more recent study on Jurkat T cell-APC conjugates has demonstrated that centrosome-associated F-actin remodeling is a mechanism controlling centrosome polarization also in T lymphocytes (57). A pathway involving protein kinase C- $\delta$  (PKC $\delta$ ), which is activated downstream TCR engagement, has



**FIGURE 1** | Regulation of centrosomal F-actin clearance in lymphocytes. Recent studies carried out on B and T lymphocytes have implicated three discrete pathways in centrosomal F-actin clearance and centrosome polarization during early stages of IS assembly. **(1)** In B lymphocytes the recruitment of the branched F-actin nucleator Arp2/3 to the IS upon BCR activation leads to a local depletion of centrosome-associated Arp2/3 that results in reduced F-actin polymerization at the centrosome, allowing for centrosome untethering from the nucleus and its repositioning to the B cell IS (51). **(2)** In T lymphocytes protein kinase C- $\delta$  (PKC $\delta$ ) has been identified as a novel regulator of centrosomal F-actin remodeling, beyond its role in cortical actin reorganization at the IS. Following TCR triggering, PKC $\delta$  phosphorylates the scaffold protein paxillin, which localizes at the centrosome where it contributes to centrosome translocation to the T cell IS by promoting a local F-actin clearance through an unknown mechanism (57). **(3)** An alternative pathway, based on the proteolytic activity of a centrosome-associated proteasome, controls F-actin clearance from the centrosome to enable its dissociation from the nucleus and polarization to the nascent IS. This pathway is exploited by both B and T lymphocytes with some cell type-dependent features. In B lymphocytes the intracellular distribution of the proteasome is regulated by the proteasome adapter and scaffold protein Ecm29 during B cell IS formation, allowing a sequential recruitment of the proteasome to the centrosome and then to the IS, which is crucial for F-actin reorganization at both locations (58, 59). In T lymphocytes proteasome-mediated degradation of unknown targets at early stages of IS assembly is dependent on the transport of the 19S regulatory particle (RP) to the centrosome, which is paralleled by an active degradation of K48-linked polyubiquitylated proteins (K48 Ub) (23). The contribution extent as well as the sequential implication of these pathways to the process remain to be elucidated.

been proposed to regulate centrosomal F-actin in T cells (**Figure 1**). The PKC $\delta$ -mediated phosphorylation of the cytoskeleton adaptor protein paxillin, which is associated with the centrosome under resting and stimulating conditions, has been related to F-actin clearance around the centrosome and its polarization to the IS (57), however the underlying mechanism remains to be elucidated. Since paxillin interacts with several signaling and cytoskeletal proteins (60, 61), it is likely that paxillin directly or indirectly binds to one or more actin regulators. Furthermore, paxillin associates with the microtubule cytoskeleton (62, 63), suggesting that it might play a role in the actin-microtubule interplay that drive centrosome repositioning to the IS. An interesting aspect in the model proposed by Bello-Gamboa et al. is that the reorganization of the actin cytoskeleton at the centrosome occurs in a coordinated fashion with synaptic F-actin remodeling. In fact, PKC $\delta$  was found to phosphorylate another substrate, the formin-like molecule FMNL1 $\beta$ , that is recruited to the IS (57), where membrane-bound formins generate bundles of linear actin filaments across the dSMAC (64, 65).

## THE UBIQUITIN-PROTEASOME SYSTEM (UPS) AND BBS1 COOPERATE IN CENTROSOMAL F-ACTIN CLEARANCE IN T CELLS

In recent years new, unexpected players have been identified in the mechanisms that regulate centrosomal F-actin clearance and centrosome polarization during IS assembly. We and Ibañez-Vega et al. have implicated the ubiquitin-proteasome system (UPS) in this process (23, 58, 59) (**Figure 1**). The UPS is a major degradation pathway in eukaryotic cells and is responsible for proteolysis of cytosolic proteins regulating a variety of cellular processes. This system consists of ubiquitin ligases that target proteins for degradation by covalently adding ubiquitin to proteasome substrates, allowing for their recognition by the 26S proteasome. The 26S proteasome is a multisubunit complex, composed by the 19S regulatory particle (RP) and the 20S core particle (CP), which identifies, unfolds, and degrades ubiquitinated proteins in an ATP-dependent manner (66). Accumulating evidence suggests a role for the proteasome in centrosome proteostasis (67), and thus in centrosome-related functions, including cell polarity in neurons (68, 69), growth and signaling function of the primary cilium in ciliated cells (70–74), and differentiation and metabolic profile of CD8<sup>+</sup> T cells (75, 76).

Although the proteasome is largely cytosolic, a proteasomal pool is associated with the centrosome, as witnessed by a local accumulation of proteasome subunits and proteasomal substrates around the centrosome (67). Recently, it has been reported that in B cells proteasome relocation from the centrosome to the IS is required for the spatiotemporal coordination of centrosomal and synaptic F-actin depolymerization (58, 59). In T lymphocytes a proteomic analysis of centrosomes purified from activated cells has revealed a local enrichment of proteasomal components (77).

Consistent with this evidence, we observed that the centrosomal 19S RP pool increases early during IS formation, to progressively return to baseline with IS maturation (23). Additionally, we found that the centrosome failed to polarize to the IS in T cells pre-treated with proteasome inhibitors and that the mislocation of the centrosome in these cells is paralleled by F-actin accumulation at the centrosome, supporting a role for the centrosomal proteasome in IS formation. The mechanisms linking the centrosomal proteasome to the local F-actin pool remain unknown. While actin depletion is regulated by the balance in the depolymerization and *de novo* synthesis of actin filaments, proteasome-regulated and centrosome-associated actin nucleators, such as Arp2/3 and its activator WASH [assembly and activity of the WASH complex are extensively reviewed in (78)], which become depleted during IS assembly (23), may represent potential targets of the centrosomal proteasome. Particularly interesting candidates are the proteasome-regulated E3 ligase TRIM27, which activates WASH through K63 mono-ubiquitination (79), and the centriolar satellite protein PCMI (80), which recruits Arp2/3 and WASH to the T cell centrosome (50). Whether these are actual targets of the centrosomal proteasome, and the relative contribution of degradation versus changes in subcellular localization to the centrosomal depletion of Arp2/3 and WASH as well as to the resulting local decrease in F-actin during IS formation, are important issues to be addressed.

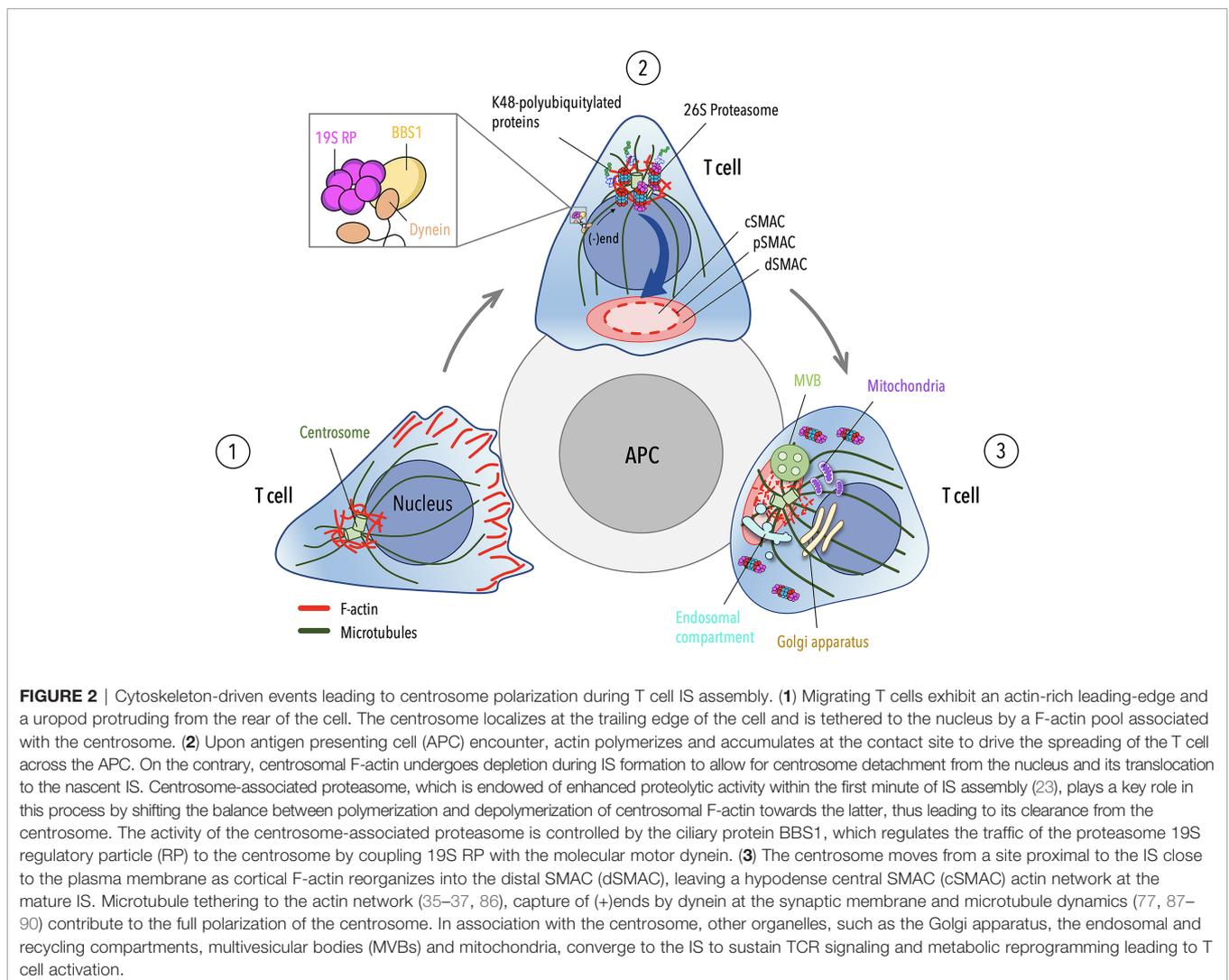
An expected twist in the proteasome-dependent regulation of centrosomal F-actin was the identification of the ciliopathy-related protein BBS1 as a novel player in T cell IS assembly (23). The BBS complex, or BBSome, is an evolutionary conserved octameric complex that regulates cilia-based signaling pathways by acting as an adaptor between the Intraflagellar transport-B (IFT-B) complex and membrane proteins, of which activated G protein-coupled receptors (GPCRs) are the main group, for their ciliary exit (81, 82). Several ciliogenesis proteins and ciliary signaling pathways are important regulators of different steps in T cell IS assembly (83, 84) and the BBSome core component BBS1 is no exception. In our recent work we demonstrated that BBS1 controls a key event in IS formation, i.e. centrosome polarization, on which the synaptic recruitment of endosomal TCRs and accumulation of tyrosine phosphoproteins depend. Having ruled out an early TCR signaling defect as the cause of the inability of the centrosome to polarize to the IS in BBS1-depleted T cells, we hypothesized alternative explanations. Centrosomal F-actin clearance appeared an interesting possibility, since in BBS4-, BBS6- or BBS9-depleted cells ciliogenesis is compromised due to increased F-actin polymerization (85). Indeed, we found that F-actin failed to be cleared from the centrosome in the absence of BBS1 and that the persistence of a F-actin meshwork at the centrosome was paralleled by a local accumulation of WASH (23). Our results identify a novel cilium-independent function for BBS1 in the clearance of centrosomal F-actin, due at least in part to local depletion of WASH. This is expected to result in impaired F-actin nucleation that is not able to counterbalance the continuous depolymerization of pre-existing filaments,

eventually leading to centrosome disengagement from the nucleus and its polarization to the IS. A more in-depth investigation of the molecular mechanism by which BBS1 controls centrosomal F-actin dynamics in T cells revealed that, consistent with the BBSome function in dynein-dependent retrograde transport of ciliary cargo to the base of the cilium (82), BBS1 acts as a dynein adaptor coupling the 19S RP with dynein allowing for its transport to the centrosome. Consistent with this function, a lesser recruitment of 19S RP to the centrosome and an increased centrosomal accumulation of K48-polyubiquitylated proteins were observed in conjugates of BBS1-deficient T cells (23).

Taken together, our findings indicate that, similar to B cells, T cells exploit a proteasome-mediated pathway for centrosomal F-actin clearance, allowing for centrosome polarization to the IS. In this context, the ciliary protein BBS1 regulates the local activity of a centrosome-associated proteasome by coupling the 19S RP to dynein to allow for its retrograde transport to the centrosome (**Figure 2**).

## CONCLUSIONS AND FUTURE DIRECTIONS

While cortical F-actin remodeling has been long considered sufficient for centrosome polarization (40), the existence of a F-actin meshwork around the centrosome, the clearance of which is required for centrosome polarization during IS assembly, has added a new level of complexity to the regulation of this process. Currently, three major pathways have been implicated in the depletion of the centrosome-associated F-actin pool and centrosome polarization in lymphocytes. Despite these recent advances, major questions remain to address. For example, whether at some point these pathways intersect converging on shared regulators, or whether they are distinct pathways that sequentially participate in centrosome repositioning to the IS. Imaging studies of the kinetics of centrosome polarization suggested that this process consists of two steps: a relative fast reorientation of the centrosome towards the IS, followed by a slower approach of



the centrosome to the synaptic membrane (87). One possibility might be that the dynein- and -BBS1-mediated centrosomal recruitment of either the proteasome or its regulatory component occurs early during centrosome polarization promoting a partial centrosomal F-actin depletion, concomitant with centrosome mobilization to the IS. When the centrosome is in the correct orientation relative to the IS, the synaptic recruitment of the Arp2/3 complex or the PKC $\delta$ -dependent phosphorylation of paxillin might complete centrosomal F-actin clearance allowing a full polarization of the centrosome towards the synaptic membrane. Since centrosome polarization can be triggered both by TCR and non-TCR signals (91, 92), we can speculate that a rapid translocation of the centrosome to the IS is driven by TCR-independent signals or minimal TCR signals, while a slower movement of the centrosome towards the maturing IS is tightly controlled by the TCR signaling pathway.

Another major challenge due to the tight squeezing of organelles around the centrosome in the cytoplasm-poor lymphocyte is to image centrosomal F-actin in T cell-APC conjugates at a super-resolution level to confirm that the changes observed in the centrosomal F-actin meshwork are restricted to the centrosome without any involvement of membrane-bound organelles, such as the pericentrosomal endosomal compartment, which is also a site of active actin dynamics. Additionally, super-resolution live-cell imaging is expected to elucidate the interplay of centrosomal F-actin and microtubules during their coordinated reorganization. These considerations suggest novel, exciting directions to be explored in order to better characterize the role

of F-actin in centrosome polarization and to identify new regulators of cell polarity during IS formation. Our implication of a BBSome component in IS assembly in the non-ciliated T cell, which further supports the homology of this structure with the primary cilium (83, 84) opens the possibility that other BBS proteins or ciliogenesis proteins functionally related to the BBSome may participate in these processes.

## AUTHOR CONTRIBUTIONS

CC and CB wrote the manuscript and conceptualized the figures. CC prepared the figures. All authors substantially, directly, and intellectually contributed to the article and approved the submitted version.

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