Teasing out function from morphology: Similarities between primary cilia and immune synapses

Tiphaine Douanne, Jane C. Stinchcombe, and Gillian M. Griffiths

Immune synapses are formed between immune cells to facilitate communication and coordinate the immune response. The reorganization of receptors involved in recognition and signaling creates a transient area of plasma membrane specialized in signaling and polarized secretion. Studies on the formation of the immune synapse between cytotoxic T lymphocytes (CTLs) and their targets uncovered a critical role for centrosome polarization in CTL function and suggested a striking parallel between the synapse and primary cilium. Since these initial observations, a plethora of further morphological, functional, and molecular similarities have been identified between these two fascinating structures. In this review, we describe how advances in imaging and molecular techniques have revealed additional parallels as well as functionally significant differences and discuss how comparative studies continue to shed light on the molecular mechanisms underlying the functions of both the immune synapse and primary cilium.

Introduction

Cytotoxic T lymphocytes (CTLs) play a critical role in adaptive immunity, recognizing and eliminating cells infected by intracellular pathogens as well as cancerous cells. Upon T cell receptor (TCR)–mediated recognition, CTLs form a zone of tight interaction with their target cells termed the immune synapse. CTLs undergo rapid rearrangements of both actin and microtubule cytoskeletons as the centrosome (the microtubule organizing center) polarizes toward the synapse, where it docks at the plasma membrane. Secretory granules loaded with perforin and granzyme move along the microtubules toward the docked centrosome that provides a focal point of secretion within the synapse. This exceptionally precise mechanism of polarized secretion enables CTLs to eliminate their targets without damaging surrounding healthy tissue (Golstein and Griffiths, 2018).

The primary cilium is a small microtubule-based organelle assembled by most eukaryotic cells in response to various developmental cues (Sorokin, 1962). Although historically regarded as a vestigial structure, it has become clear that primary cilia are essential signaling hubs, acting as "sensing antennae" to detect and integrate multiple extracellular cues (Pedersen et al., 2016). Dysfunctions of primary cilia are associated with a wide range of human inherited developmental degenerative diseases termed ciliopathies (Reiter and Leroux, 2017). The characterization and study of ciliopathy-associated phenotypes has improved our understanding of the cilium and the mechanisms regulating this enigmatic structure. Although lymphocytes are one of very few cell types that do not appear to form primary cilia (Hildebrandt and Otto, 2005; Wheatley, 1995), subsequent studies have revealed that immune cells appear to form "frustrated cilia" with centrosome docking but no cilia formation (Griffiths et al., 2010).

Striking parallels between the immune synapse and primary cilia were first noticed at the ultrastructural level, when images of centrosome polarization at the CTL immune synapse were compared with images of basal body docking during ciliogenesis (Stinchcombe et al., 2006; Stinchcombe and Griffiths, 2007). In both ciliogenesis and immune synapse formation, it was clear that docking of the centrosome at the plasma membrane also resulted in polarization of the Golgi apparatus and endocytic compartments toward the site of centrosome attachment, creating a focal point for endocytosis and exocytosis at a defined point on the plasma membrane (Griffiths et al., 2010; Stinchcombe and Griffiths, 2007). Many more molecular parallels between the immune synapse and primary cilia have emerged since these initial observations, and it is now clear that there are multiple molecular and functional similarities between these two seemingly disparate biological structures (Table 1).

Molecular parallels in centrosomal docking

The centrosome is composed of two centrioles, the mother centriole with distal and subdistal appendages and the daughter centriole that lacks appendages, surrounded by the pericentriolar material. Primary cilia formation is well described (Reiter

---

Cambridge Institute for Medical Research, Cambridge Biomedical Campus, University of Cambridge, Cambridge, England, UK.

Correspondence to Gillian M. Griffiths: gg305@cam.ac.uk.

Table 1. Common features are supported by shared molecular effectors at the immune synapse and the primary cilium

Shared mechanism between the immune synapse and primary cilia	Molecular effectors at the primary cilia	Molecular effectors at the immune synapse
Centrosomal docking at the plasma membrane	Distal appendages	Distal appendages
	CEP83, CEP164, CEP89, SCLT1, C2CD3, LRRC45	CEP83
Actin rearrangements	WASH, Arp2/3	WAVE2, WASp, Arp2/3
Trafficking machinery	IFT proteins, including IFT20, IFT52, IFT57, IFT88, and IFT54	IFT proteins, including IFT20, IFT52, IFT57, IFT88, and IFT54
	GMAP-210	GMAP-210
	GTPases and GEFs: Rabs, including Rab29, Rab11, and Rab8	GTPases and GEFs: Rab29, Rab11, Rab8
	ARL3, ARL13B	ARL3, ARL13B
	SNAREs: VAMP7	SNAREs: VAMP7
	Adaptors: MAL	Adaptors: MAL
Hh signaling pathway	PTCH, Hh ligands, SMO, Gli	PTCH, Hh ligands, SMO, Gli

and Leroux, 2017); in brief, it involves a series of well-orchestrated steps, initiated by the docking of the mother centriole, via its distal appendages, to what will become the ciliary membrane (Anderson, 1972; Mirvis et al., 2018; Sorokin, 1962). Microtubules extend from the docked mother centriole to form the ciliary axoneme composed of nine doublet microtubules, giving rise to the classical "antenna-like" cilium. In some cellular contexts, vesicles originating from the Golgi and recycling endosomes accumulate around the mother centriole and form a cap-like structure, termed the ciliary vesicle or cap, to which the mother centriole docks. This becomes the ciliary membrane following fusion with the surface (Lu et al., 2015; Reiter et al., 2012; Wang and Dynlacht, 2018; Yee and Reiter, 2015).

A combination of proteomic and super resolution microscopy approaches has led to the identification of several distal appendage proteins essential for ciliogenesis, including CEP164, CEP83, CEP89, SCLT1, C2CD3, and LRRC45 (Graser et al., 2007; Joo et al., 2013; Kurtulmus et al., 2018; Schmidt et al., 2012; Sillibourne et al., 2013; Tanos et al., 2013; Ye et al., 2014). Interestingly, the recruitment of distal appendage proteins to the mother centriole seems to be hierarchical. Use of direct stochastic optical reconstitution microscopy revealed that CEP83 is located at the root of the appendages, while CEP164 accumulates at their periphery (Bowler et al., 2019; Yang et al., 2018). This supports earlier observations that removal of CEP83 impairs the recruitment of CEP164, CEP89, and SCL1 to distal appendages and effectively disrupts their assembly (Tanos et al., 2013). Deletion of CEP83 through RNA interference prevented docking of mother centrioles at the plasma membrane (Tanos et al., 2013), with a subsequent study using CRISPR further demonstrating that CEP83 ablation impairs ciliary vesicle formation (Lo et al., 2019). The finding that mutations in CEP83 are associated with infantile nephronophthisis (NPHP18), a ciliopathy characterized by chronic renal failure (Failler et al., 2014), reinforces a critical role of CEP83 in ciliogenesis. Interestingly, the removal of distal appendages by CEP83 deletion affected subdistal appendage stability and localization, demonstrating a structural coupling of the distal and subdistal appendages (Chong et al., 2020). Of note,

it was recently reported that CEP83 is involved in centrosome membrane anchoring in radial glial progenitor cells, indicating an additional role for CEP83 distinct from ciliogenesis (Shao et al., 2020).

An important role for mother centriole distal appendages has also been described in immune cells. Transmission EM tomography revealed that the distal appendages of the CTL mother centriole associate with the plasma membrane upon killing, anchoring the centrosome at the immune synapse (Stinchcombe and Griffiths, 2014; Stinchcombe et al., 2015). The docking step organizes microtubules emanating from the subdistal appendages of the mother centriole to run underneath the synapse and thus guides microtubule-mediated granule transport toward the site of exocytosis (Stinchcombe et al., 2011, 2015; Fig. 1). Intriguingly, CEP83 seems to play an important part in this process in CTLs, as silencing it using RNA interference reduced granule secretion in CTLs (Stinchcombe et al., 2015).

Tight polarization of the centrosome is an early hallmark of both ciliogenesis and immune synapse formation. However, subsequent events diverge, as centrosome docking at the synapse is transient, lasting only minutes, while primary cilia are stable structures persisting for several hours. Two proteins that regulate the stable association of the centrosome with the membrane during ciliogenesis are CP110 and its partner, CEP97, which dissociate from the distal end of the mother centriole, allowing centrosomal docking and axoneme growth. Loss of Cep97 or CP110 promotes primary cilia formation, while ectopic expression of CP110 thwarts it, suggesting the complex suppresses ciliogenesis (Spektor et al., 2007). This is thought to be mediated by the serine/threonine kinase Tau-tubulin kinase-2, which phosphorylates CEP83 to promote CP110 removal from the mother centriole (Bernatik et al., 2020; Goetz et al., 2012; Lo et al., 2019). In contrast, in CTLs the CP110–CEP97 complex is not lost from the mother centriole upon docking, and cilia formation does not occur (Stinchcombe et al., 2015). Interestingly, RNA interference of CP110 in Jurkat T cells was able to induce some primary cilia formation upon serum starvation, indicating immune cells have the capacity to make cilia when CP110 is
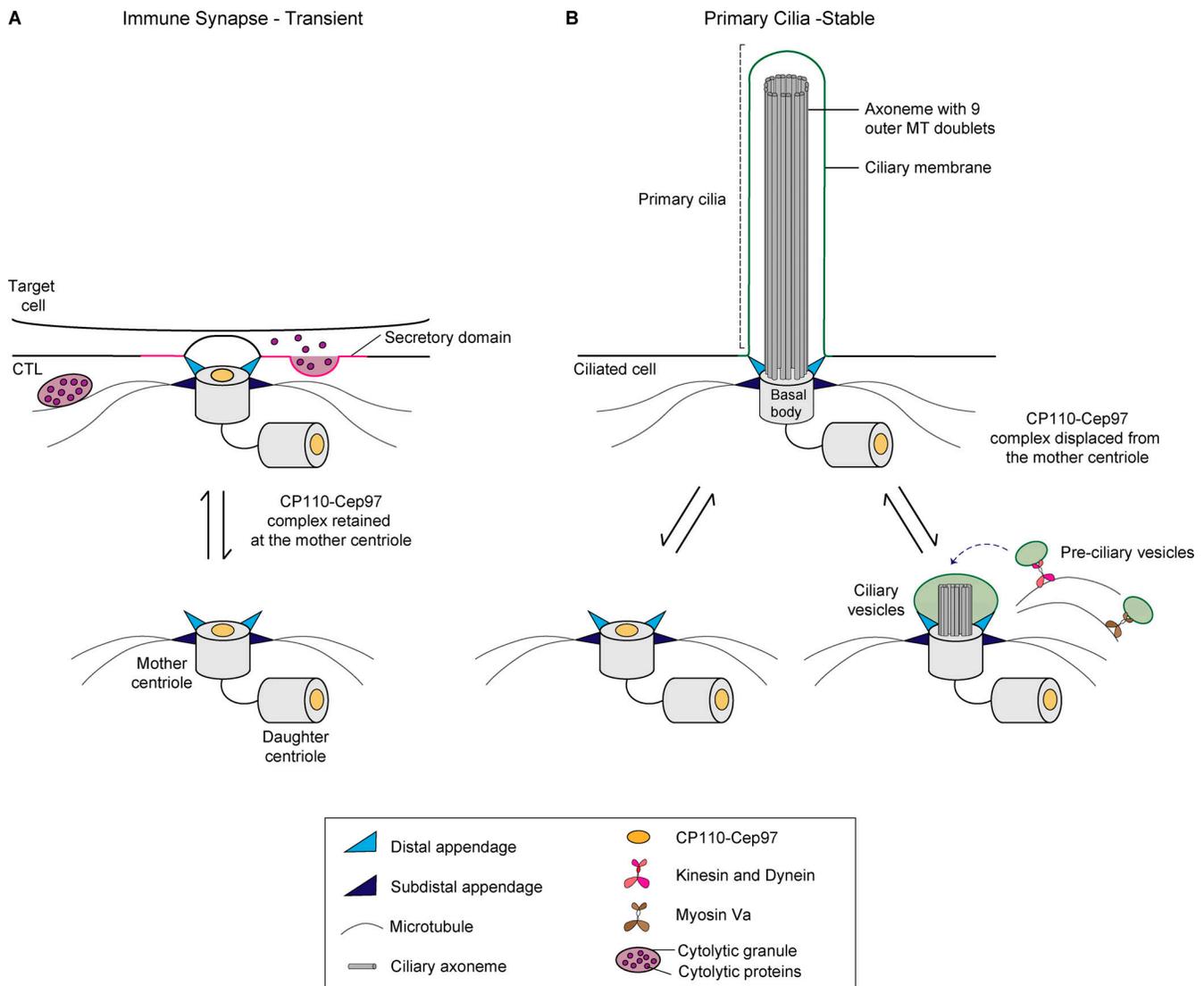
Figure 1. **Centrosomal docking at the immune synapse and primary cilia. (A)** In CTLs, upon target cell contact, the mother centriole docks at the plasma membrane through distal appendages. Microtubules radiate from the mother centriole through subdistal appendages, providing tracks for delivery of cytolytic granules to the secretory domain and target cell. During synapse formation, the CP110–Cep97 complex is maintained at the centrioles. This event is transient. **(B)** Upon environmental and developmental cues, the basal body, a differentiated form of the mother centriole, docks at the membrane through distal appendages, followed by axoneme extension. In some cases, there is a prior recruitment, docking, and fusion of ciliary vesicles at the distal appendage of the mother centriole, forming a nascent cilium that will later fuse with the plasma membrane. This is followed by axonemal microtubule extension. During ciliogenesis, the CP110–Cep97 complex is displaced from the mother centriole to allow axoneme extension. This event is relatively stable. MT, microtubule.

removed (Prosser and Morrison, 2015). Together, these findings suggest that the retention of CP110–CEP97 might explain why a cilium does not form at the immune synapse (Fig. 1).

To explore the role of centrosome homeostasis and assess the contribution of centrosome docking during CTL killing, one study used a Sas4/p53 deletion model (Tamzalit et al., 2020). As CTLs have been shown to contain multiple pairs of centrioles (Stinchcombe et al., 2015), this is a challenging problem to approach. While Sas4/p53 knockout lymphocytes displayed reduced killing, polarized secretion was unscathed probably due to the maintenance of a rudimentary microtubule organizing center, albeit with a less well-organized microtubule cytoskeleton. However, the Sas4/p53 CTLs were also disrupted in other ways, including alterations in actin remodeling and granule

content, both of which would have contributed to the defect in killing (Tamzalit et al., 2020). A recent study showing that pericentriolar material can lead to cilia formation in the absence of centrioles in *Caenorhabditis elegans* raises the intriguing possibility that centrosome function may also be retained in T cells in the absence of centrioles (Garbrecht et al., 2021).

Actin rearrangements shape both the primary cilium and immune synapse

In addition to changes in the microtubule organization, the formation of both the immune synapse and primary cilium involves rearrangement of the actin cytoskeleton.

Early interaction between a CTL and its target is mediated by actin-rich lamellipodial protrusions that form an interdigitated
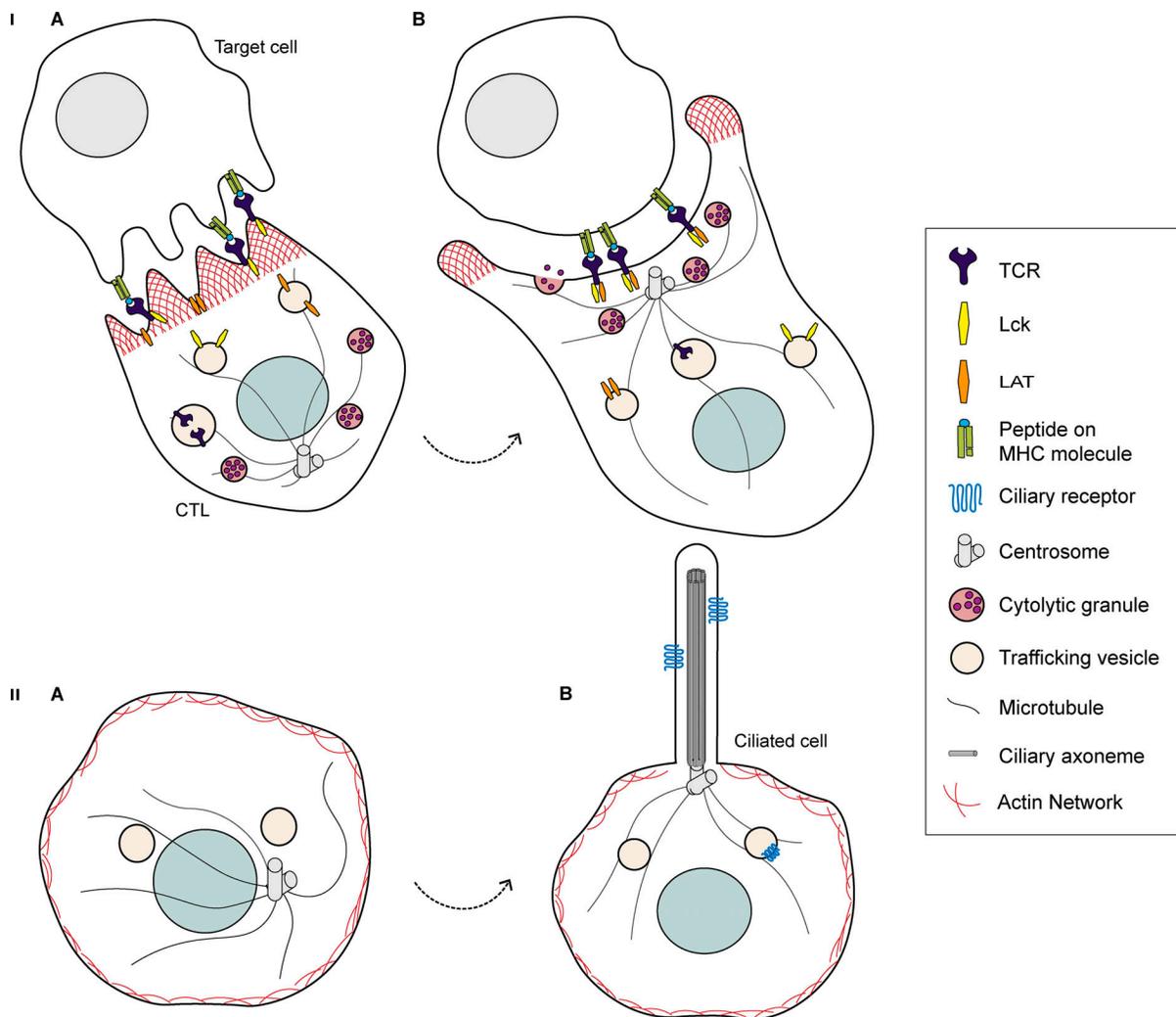
Figure 2. **Profound cytoskeleton rearrangements upon immune synapse formation and ciliogenesis. (IA)** Initial contact with CTLs is mediated through actin-rich filopodia and interdigitated contact sites with the target cell. At this stage, the centrosome is not polarized, and the cytolytic granules are dispersed within the cell. **(IB)** The mature synapse displays accumulation of actin at the peripheral lamellipodia, while cortical actin is depleted, and the CTL plasma membrane is flattened at the contact site. The mother centriole is docked at the plasma membrane through distal appendages. Cytolytic granules are delivered to the secretory domain. **(IIA)** Environmental and developmental cues initiate ciliogenesis. **(IIB)** The basal body docks at the plasma membrane through the distal appendage, and the cilium protrudes from the cell body and is enriched in signaling receptors.

contact site with the target cell and lead to a rapid accumulation of actin across the contact site (Jenkins et al., 2014; Ritter et al., 2015; Sanderson and Glauert, 1979). The initial actin accumulation at the synapse is quickly followed by its depletion across the structure, as the membrane reorganizes to form a tight contact during killing (Fig. 2). Actin then returns to the contact site before the CTL dissociates and moves on to further targets (Ritter et al., 2015; Stinchcombe et al., 2001). Loss of cortical actin is required for granule delivery, while its recovery drives secretion termination (Ritter et al., 2017). The rapid changes in actin distribution at the synapse are controlled by changes in the plasma membrane phospholipid composition in response to TCR signaling (Gawden-Bone et al., 2018; Ritter et al., 2015). In brief, TCR ligation activates PLCγ, triggering the depletion of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), a lipid known to interact with actin-regulating proteins (Rohatgi et al., 2000).

Consequently the TCR-induced loss of PI(4,5)P2 reduces actin recruitment where TCR signaling occurs (Gawden-Bone et al., 2018). The conversion of PI(4,5)P2 to DAG also alters the membrane charge required for the electrostatic interactions for phosphatidylinositol 4-phosphate 5-kinase type I (PIP5K) membrane association, excluding PIP5K and ensuring actin depletion from the point of centrosome docking at the synapse (Gawden-Bone et al., 2018; Fig. 3).

The dynamic changes at the immune synapse produce a distinctive membrane specialization during centrosome docking. This resembles the membrane specialization described across the primary cilium, arising from changes driven by inositol polyphosphate 5-phosphatase (INPP5E), a phosphatase that dephosphorylates PI(4,5)P2 to generate phosphatidylinositol 4-phosphate (Dyson et al., 2012). Studies using lipid-specific antibodies (Chávez et al., 2015) or expression of phosphoinositide-binding domains
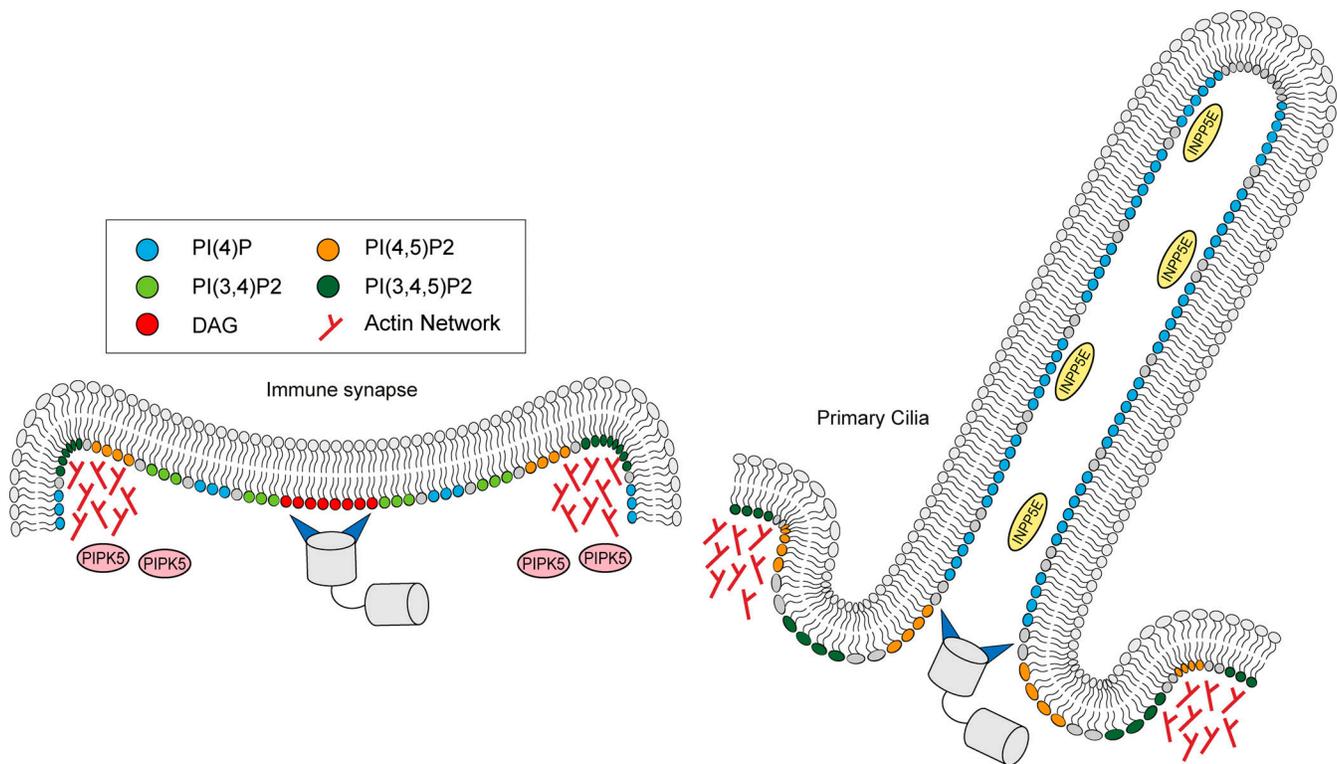
Figure 3. Phospholipid composition of membranes at the immune synapse and primary cilia. Composition of phospholipids at the membrane at the immune synapse and primary cilia when the centrosome is docked, highlighting the similarities that support their functions. Centrosome distal appendages dock at the plasma membrane, although whether they bind specific phospholipids is not known.

fused to fluorescent proteins (Garcia-Gonzalo et al., 2015) revealed that phosphatidylinositol 4-phosphate is enriched within the ciliary membrane, whereas PI(4,5)P2 and PI(3,4,5)P3 are localized at the base of the cilium, providing a very similar membrane architecture to that across the synapse, with actin depleted in both cases (Fig. 3). INPP5E, required for Hedgehog (Hh) signaling, is found along the cilia and marshals this compartmentalization (Chávez et al., 2015; Dyson et al., 2017; Garcia-Gonzalo et al., 2015; Nakatsu, 2015). Intriguingly, while TCR signaling displaces PIP5K from the immune synapse, growth signals displace INPP5E from the cilium, driving PI(4,5)P2 accumulation at the tip of the cilium, allowing intraciliary actin polarization, which plays a part in cilia shedding and disassembly (Phua et al., 2017). Thus, both the immune synapse and the primary cilium undergo dynamic remodeling of their membrane phospholipid composition, driving actin rearrangement and supporting their respective functions.

Studies in CD4^{+} T cells have shed some light on the roles of actin regulatory proteins in immune synapse function. Briefly, T cell stimulation leads to the activation of actin regulators WAVE2 and WASp that act in concert with Arp2/3 complexes to promote actin nucleation. While WAVE2 localizes at the lamellipodial protrusions, participating in their formation (Nolz et al., 2006; Pauker et al., 2014), WASp modulates the formation of TCR-rich actin foci (Comrie and Burkhardt, 2016; Kumari et al., 2015). Several studies have shown that CTLs lacking the Arp2/3 subunit ARPC1B are unable to form actin-rich lamellipodial protrusions, impairing synapse formation (Brigida et al.,

2018; Randzavola et al., 2019; Somech et al., 2017; Volpi et al., 2019). However, in CTLs, loss of ARPC1B also disrupted retromer-mediated recycling of receptors, including TCR and the glucose transporter GLUT-1, impairing signaling and glucose uptake required for cell proliferation (Randzavola et al., 2019). Intriguingly, it has been proposed that centrosomes act as actin organizing centers by nucleating actin filaments in a process mediated by the nucleation promoting factor Wiskott-Aldrich syndrome protein and SCAR homologue (WASH) in combination with the Arp2/3 complex (Farina et al., 2016). Actin reorganization at the centrosomal area seems to contribute to polarized secretion of multivesicular bodies from Jurkat T cells (Bello-Gamboa et al., 2020).

Actin rearrangements also participate in primary cilia formation (Copeland, 2020), and a number of proteomic screens have identified actin-binding proteins in the cilium (Gupta et al., 2015; Ishikawa et al., 2012; Kohli et al., 2017; Liu et al., 2007). Arp2/3 plays a role in primary cilia, with deletion of the Arp3 subunit leading to increased ciliogenesis and ciliary length (Kim et al., 2010; Yan and Zhu, 2013) as well as increased Hh signaling (Drummond et al., 2018), suggesting that F-actin nucleation inhibits primary cilia formation (Kim et al., 2010; Yan and Zhu, 2013). Moreover, actin dynamics at the centrosome also play a part in ciliogenesis, as preciliary vesicles seem to be transported from the pericentrosomal region to the distal appendages of the mother centriole to form the ciliary vesicle via an Arp2/3 branched actin network (Wu et al., 2018; Fig. 1).
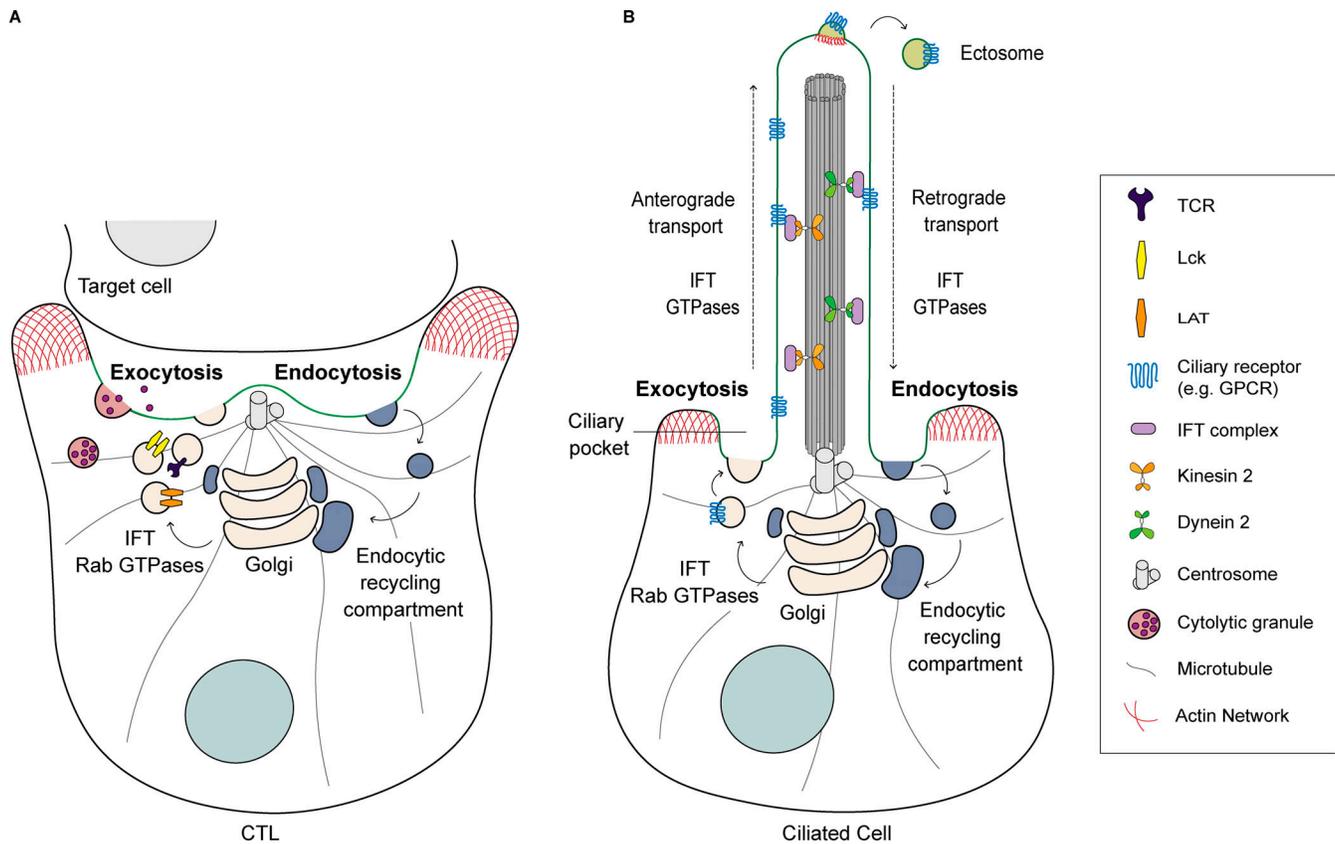
Figure 4. **Trafficking similarities between the immune synapse and primary cilia**. **(A and B)** Both the immune synapse (A) and primary cilia (B) display polarization of the Golgi apparatus, post-Golgi compartments, and endocytic compartments toward the membrane. Both structures are hubs of endo- and exocytosis and share common molecular modulators of trafficking. **(A)** In CTLs, IFTs and Rab GTPases control the rapid trafficking of crucial signaling molecules to the plasma membrane. **(B)** In the cilium, IFTs, Rab GTPases, and other GTPases participate in the sorting of proteins synthetized in the cytosol to the axoneme. IFT proteins are involved in the antero- and retrograde transport of signaling receptors within the axoneme. Primary cilia have been shown to shed ectosomes containing signaling receptors (e.g., GPCR) at their tips.

Parallels in trafficking machinery

Both primary cilia and immune synapses can be viewed as specialized signaling hubs, with each relying on the correct spatial and temporal recruitment of proteins for signal transduction. Over the last decade, a large body of evidence has accumulated which suggests the cilium and synapse share common mechanisms to ensure polarized trafficking (Fig. 4). The best characterized of these are the intraflagellar transport (IFT) proteins. In cilia, IFT proteins associated with their cargo are transported bidirectionally along axonemal microtubules by the molecular motors kinesin 2 (base to tip) and dynein 2 (tip to base; Taschner and Lorentzen, 2016). IFTs participate in the active sorting and transport of cytosolic and membrane proteins destined for the cilium past the impermeable transition zone to the axoneme (Pedersen and Rosenbaum, 2008). Mutations in IFTs give rise to a number of human ciliopathies, supporting their critical role in ciliogenesis and cilium maintenance (Reiter and Leroux, 2017).

Interestingly, a number of IFT components have also been found to be expressed in hematopoietic cells and shown to participate in T lymphocyte activation (Finetti et al., 2009; Finetti et al., 2014). For instance, IFT20 is recruited to the immune synapse formed between a T lymphocyte and an antigen-presenting cell via association with the centrosome as well as the Golgi and post-Golgi compartments (Finetti et al., 2009). This echoes observations that IFT20 associates with the Golgi apparatus at the base of the cilium and functions in the sorting of proteins destined for the ciliary membrane (Follit et al., 2008; Follit et al., 2006). In addition, a study using CD4+ T lymphocytes derived from mice with a conditional defect in IFT20 highlighted a role for IFT20 in the recruitment to the immune synapse of linker of activated T cells (LAT), a key adaptor involved in T cell activation to the immune synapse (Vivar et al., 2016).

A proteomic analysis of the interactome of IFT20 in Jurkat cells using quantitative mass spectrometry identified binding partners for the protein (Galgano et al., 2017). These included IFT54 and the Golgin GMAP-210, which were previously described to associate with IFT20 in ciliated cells (Follit et al., 2008; Follit et al., 2009). IFT54 participates in IFT20-mediated TCR recycling, possibly through its role as a negative regulator of microtubule stability (Bizet et al., 2015). Although GMAP-210 was not required for TCR recruitment to the synapse (Galgano et al., 2017), it was found to mediate delivery of LAT-enriched vesicles. Furthermore, ectopic expression of LAT in ciliated cells led to its recruitment to the cilium in a GMAP-210–dependent manner, supporting the idea that similar molecular mechanisms are involved (Zucchetti et al., 2019). It is important to note that

the IFTs and particularly IFT20 seem to have general Golgi-related trafficking functions in several cellular contexts that are not restricted to the primary cilia or immune synapse. For example, IFT20 is essential for procollagen trafficking from the endoplasmic reticulum to the Golgi apparatus in central nervous system–derived osteoblasts (Noda et al., 2016).

Endosomal trafficking at the immune synapse and primary cilia share additional molecular effectors (Fig. 4). Indeed, IFTs recruit small Rab GTPases that orchestrate receptor recycling at both structures. Rab8, an essential player in cilium assembly and trafficking (Nachury et al., 2007; Westlake et al., 2011), colocalizes with IFT20 in Rab11+ endosomes in activated lymphocytes, and this interaction has been proposed to contribute to vesicle-mediated TCR targeting to the immune synapse (Finetti et al., 2015). The same group also identified Rab29 as an interactor of IFT20, Rab8, and Rab11 (Onnis et al., 2015).

In addition to Rab GTPases, the small GTPase ARL3 and its guanine exchange factor, ARL13B, appear to regulate membrane composition and recruitment of signaling molecules in both the cilium and synapse (Powell et al., 2021). The pivotal role of ARL3 in cilia is clear from the ciliary phenotypes associated with its mutations, including Joubert syndrome and dominant retinitis pigmentosa (Alkanderi et al., 2018; Holtan et al., 2019; Strom et al., 2016). Briefly, ARL3 and ARL13B were previously shown to play a role in mediating solubility of prenylated and myristoylated proteins, thereby controlling recruitment of several proteins to the axoneme (Powell et al., 2021). Intriguingly, the ARL3/ARL13B machinery has been shown to mediate transport of the downstream signaling effector protein Lck to the immune synapse of T cells, revealing an alternative use for this ciliary mechanism for targeting the delivery of signaling proteins in immune cells (Stephen et al., 2018).

Together with the IFTs and small Rab GTPases, ciliated and immune cells share trafficking pathway adaptors, including SNARE family proteins involved in the priming and fusion of vesicles to the plasma membrane. The SNARE VAMP7 is essential for cilium biogenesis in kidney cells (Szalinski et al., 2014) and has also been shown to be required for the recruitment of LAT-containing vesicles to TCR activation sites and consequent cell activation in CD4+ T lymphocytes (Larghi et al., 2013). In addition, VAMP7 localizes to cytolytic granules and was proposed to play a role in granule fusion at the synapse (Chitirala et al., 2019). Moreover, the myelin and lymphocyte (MAL) protein participates in primary cilia formation as well as modulating the sorting of Lck and LAT at the immune synapse (Antón et al., 2011; Takiar et al., 2012).

Together, these observations indicated that multiple components of the trafficking machinery, including IFTs, GTPases, and SNAREs, act in both ciliated and immune cells to ensure proper recruitment of signaling components to the axoneme and synapse, as well as their recycling, which is essential for signal termination (Fig. 4).

Shared pathways in signaling
Hh signaling
As part of its role as the "small antenna" of the cell, the ciliary membrane contains a high concentration of transduction receptors.

One of the best-described signaling pathways in the cilium is the Sonic Hh pathway (Goetz and Anderson, 2010). Briefly, upon binding of the transmembrane receptor Patched (PTCH) to one of the three Hh ligands (Sonic Hh, Desert Hh, or Indian Hh), Smo is transported to the axoneme by IFTs, where it can disrupt the repression of Gli1 at the tip of the cilia. Gli1 is then activated and free to translocate to the nucleus, where it acts as a transcription factor for Hh genes (Corbit et al., 2005; Rohatgi et al., 2007).

Interestingly, the study of knockout and transgenic models has established a role for Hh signaling in the development of T lymphocytes in the thymus as well as the differentiation of CD4+ T lymphocytes into T helper 2 cells (Crompton et al., 2007; Furmanski et al., 2013). Furthermore, Hh signaling has also been shown to be triggered upon TCR activation and contribute to the cytotoxic synapse as detected by Gli1 activation, while deletion or inhibition of Smo disrupted actin rearrangements and decreased CTL killing (de la Roche et al., 2013). These findings suggest a model where the Hh pathway modulates actin rearrangements, controlling CTL secretion (de la Roche et al., 2013).

Vesicle release
A growing body of evidence suggests that the primary cilium is a site of extracellular vesicle (EV) release. The studies of the unicellular alga *Chlamydomonas reinhardtii* and the nematode *C. elegans* have demonstrated that ectosomes can be released from flagella and primary cilia and mediate extracellular communication. In *C. reinhardtii*, early electron microscopy showed shedding of membrane microvesicles from the flagella tip (Bergman et al., 1975). Subsequent studies found that these 50- to 200-nm ectosomes contain proteases required for degradation of extracellular matrix and digestion of the mother cell wall required for daughter cell hatching (Wood et al., 2013; Wood and Rosenbaum, 2015). Vesicle fluorescence labeling and live and electron microscopy revealed that ciliated sensory neurons of *C. elegans* can release microvesicles into their environment (Wang et al., 2014). This process relies on several ciliary proteins and IFT components and participates in animal communication and mating behaviors. Interestingly, in this context, observations of microvesicle shedding have been made both at the base and the tip of the cilia, suggesting two distinct ciliary secretion sites (Wang and Barr, 2018). An alternative model suggests that EVs could be shed at the base of the cilium then transported along the ciliary membrane by the IFT to be released at the tip of the axoneme (Maguire et al., 2015). Exosome-like vesicles have been shown to accumulate around the cilium in the cells of patients suffering from autosomal dominant polycystic kidney disease, as well as in a mouse model recapitulating this human ciliopathy (Hogan et al., 2009). Subsequent studies have found that actin mediates vesicle shedding and ciliary disassembly, termed ectocytosis and decapitation, respectively (Nager et al., 2017; Phua et al., 2017). In one study, the shed vesicles were shown to carry ciliary G protein–coupled receptors (GPCRs), thereby modulating signaling (Nager et al., 2017), while another report focused on a role for these EVs in the disassembly of the cilium (Phua et al., 2017).

A number of studies now suggest that lymphocytes also release exosome-like vesicles at the synapse, in addition to secreted

proteins, although the origins of these vesicles is not always clear. Early observations suggested T cells shed vesicles containing the activation-induced cell death mediators FasL and APO2L (Alonso et al., 2011; Martínez-Lorenzo et al., 1999) and TCR–CD3–ζ complexes (Blanchard et al., 2002), as well as mRNA (Mittelbrunn et al., 2011). A more recent study has shown that FasL is localized on the intra luminal vesicles within cytolytic granules, revealing the origins of vesicle released FasL (Lee et al., 2018). Work using a TCR-triggering planar lipid bilayer synapse showed that microvesicles containing TCR were secreted in an endosomal sorting complexes required for transport (ESCRT)–dependent manner, and these could in turn activate surrounding cells (Choudhuri et al., 2014). In keeping with this, exosome-like vesicles isolated from CTLs stimulated with high-affinity ligands could activate bystander CTLs in the absence of antigen (Wu et al., 2019). However, whether these supernatant-isolated exosomes originated from the synapse remains to be determined.

Concluding comments

The initial observations of morphological similarities in transmission EM micrographs of centrosome polarization at the immune synapse and primary cilium (Stinchcombe et al., 2006; Stinchcombe and Griffiths, 2007; Stinchcombe and Griffiths, 2014; Stinchcombe et al., 2011) paved the way for what is now a large body of research, extending the range of structural, functional, and molecular similarities and so highlighting the relationship between the cilium and synapse.

One reason the similarity between the synapse and cilium appeared so intriguing in the early studies is that immune cells were known to lack primary cilia despite having a functional centrosome. While not an absolute rule, there seems to be a correlation between the presence of a centriole and the ability to form a cilium in most organisms. Organisms such as *Dictyostelium discoideum* and *Saccharomyces cerevisiae*, which lack cilia altogether, are devoid of centrosomes (Debec et al., 2010).

The observation that the CTL centrosome polarizes to the synapse and docks with the membrane via the mother centriole distal appendages upon target contact but fails to form a cilium was therefore particularly striking and indicated that centrosomal docking leads to different outcomes in ciliated and immune cells. This difference is likely critical for the distinct functional requirements of the transient immune synapse versus the stable cilium in ciliated tissues. The similarities in the docking process, nevertheless, suggested immune cells such as CTL might have "commandeered" mechanisms evolved for cilia formation to provide a fast mechanism for repolarizing the cell in response to target encounter (Stinchcombe et al., 2006). The growing number of key molecular players in the range of critical cellular processes that are shared between the synapse and the cilium, from microtubule and actin cytoskeletons rearrangements to membrane trafficking (e.g., IFTs, SNARE components, and GTPases) to signaling pathways (e.g., Hh), as well as common mechanisms (such as the release of EVs), supports the idea that lymphocytes have coopted ciliary machinery to fulfil their cytotoxic functions and hints at an evolutionary link between these structures. Much of our understanding of ciliogenesis came from the study of ciliopathies, a subset of human inherited

developmental degenerative diseases (Reiter and Leroux, 2017). Indeed, mutations in genes involved in ciliary function result in a variety of symptoms ranging from polydactyly to blindness to kidney diseases or neurodevelopmental defects (Waters and Beales, 2011). Whether some ciliopathies are accompanied by immune symptoms remains unclear and warrants further investigation. Comparisons between the immune synapse and ciliogenesis have yielded a multitude of molecular similarities between these seemingly disparate biological structures, illustrating how it is possible to tease out function from morphology.

References

Alkanderi, S., E. Molinari, R. Shaheen, Y. Elmaghloob, L.A. Stephen, V. Sammut, S.A. Ramsbottom, S. Srivastava, G. Cairns, N. Edwards, et al. 2018. ARL3 Mutations Cause Joubert Syndrome by Disrupting Ciliary Protein Composition. *Am. J. Hum. Genet.* 103:612–620. https://doi.org/10.1016/j.ajhg.2018.08.015

Alonso, R., C. Mazzeo, M.C. Rodriguez, M. Marsh, A. Fraile-Ramos, V. Calvo, A. Avila-Flores, I. Merida, and M. Izquierdo. 2011. Diacylglycerol kinase α regulates the formation and polarisation of mature multivesicular bodies involved in the secretion of Fas ligand-containing exosomes in T lymphocytes. *Cell Death Differ.* 18:1161–1173. https://doi.org/10.1038/cdd.2010.184

Anderson, R.G.W. 1972. The three-dimensional structure of the basal body from the rhesus monkey oviduct. *J. Cell Biol.* 54:246–265. https://doi.org/10.1083/jcb.54.2.246

Antón, O.M., L. Andrés-Delgado, N. Reglero-Real, A. Batista, and M.A. Alonso. 2011. MAL protein controls protein sorting at the supramolecular activation cluster of human T lymphocytes. *J. Immunol.* 186:6345–6356. https://doi.org/10.4049/jimmunol.1003771

Bello-Gamboa, A., M. Velasco, S. Moreno, G. Herranz, R. Ilie, S. Huetos, S. Dávila, A. Sánchez, J. Bernardino De La Serna, V. Calvo, and M. Izquierdo. 2020. Actin reorganization at the centrosomal area and the immune synapse regulates polarized secretory traffic of multivesicular bodies in T lymphocytes. *J. Extracell. Vesicles.* 9:1759926. https://doi.org/10.1080/20013078.2020.1759926

Bergman, K., U.W. Goodenough, D.A. Goodenough, J. Jawitz, and H. Martin. 1975. Gametic differentiation in Chlamydomonas reinhardtii. II. Flagellar membranes and the agglutination reaction. *J. Cell Biol.* 67:606–622. https://doi.org/10.1083/jcb.67.3.606

Bernatik, O., P. Pejskova, D. Vyslouzil, K. Hanakova, Z. Zdrahal, and L. Cajanek. 2020. Phosphorylation of multiple proteins involved in ciliogenesis by Tau Tubulin kinase 2. *Mol. Biol. Cell.* 31:1032–1046. https://doi.org/10.1091/mbc.E19-06-0334

Bizet, A.A., A. Becker-Heck, R. Ryan, K. Weber, E. Filhol, P. Krug, J. Halbritter, M. Delous, M.-C. Lasbennes, B. Linghu, et al. 2015. Mutations in

TRAF3IP1/IFT54 reveal a new role for IFT proteins in microtubule stabilization. *Nat. Commun.* 6:8666. https://doi.org/10.1038/ncomms9666

Blanchard, N., D. Lankar, F. Faure, A. Regnault, C. Dumont, G. Raposo, and C. Hivroz. 2002. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J. Immunol.* 168:3235–3241. https://doi.org/10.4049/jimmunol.168.7.3235

Bowler, M., D. Kong, S. Sun, R. Nanjundappa, L. Evans, V. Farmer, A. Holland, M.R. Mahjoub, H. Sui, and J. Loncarek. 2019. High-resolution characterization of centriole distal appendage morphology and dynamics by correlative STORM and electron microscopy. *Nat. Commun.* 10:993. https://doi.org/10.1038/s41467-018-08216-4

Brigida, I., M. Zoccolillo, M.P. Cicalese, L. Pfajfer, F. Barzaghi, S. Scala, C. Oleaga-Quintas, J.A. Álvarez-Álvarez, L. Sereni, S. Giannelli, et al. 2018. T-cell defects in patients with *ARPC1B* germline mutations account for combined immunodeficiency. *Blood.* 132:2362–2374. https://doi.org/10.1182/blood-2018-07-863431

Chávez, M., S. Ena, J. Van Sande, A. de Kerchove d'Exaerde, S. Schurmans, and S.N. Schiffmann. 2015. Modulation of Ciliary Phosphoinositide Content Regulates Trafficking and Sonic Hedgehog Signaling Output. *Dev. Cell.* 34:338–350. https://doi.org/10.1016/j.devcel.2015.06.016

Chitirala, P., K. Ravichandran, D. Galgano, M. Sleiman, E. Krause, Y.T. Bryceson, and J. Rettig. 2019. Cytotoxic Granule Exocytosis From Human Cytotoxic T Lymphocytes Is Mediated by VAMP7. *Front. Immunol.* 10:1855. https://doi.org/10.3389/fimmu.2019.01855

Chong, W.M., W.J. Wang, C.H. Lo, T.Y. Chiu, T.J. Chang, Y.P. Liu, B. Tanos, G. Mazo, M.B. Tsou, W.N. Jane, et al. 2020. Super-resolution microscopy reveals coupling between mammalian centriole subdistal appendages and distal appendages. *eLife.* 9:e53580. https://doi.org/10.7554/eLife.53580

Choudhuri, K., J. Llodrá, E.W. Roth, J. Tsai, S. Gordo, K.W. Wucherpfennig, L.C. Kam, D.L. Stokes, and M.L. Dustin. 2014. Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. *Nature.* 507:118–123. https://doi.org/10.1038/nature12951

Comrie, W.A., and J.K. Burkhardt. 2016. Action and Traction: Cytoskeletal Control of Receptor Triggering at the Immunological Synapse. *Front. Immunol.* 7:68. https://doi.org/10.3389/fimmu.2016.00068

Copeland, J. 2020. Actin-based regulation of ciliogenesis - The long and the short of it. *Semin. Cell Dev. Biol.* 102:132–138. https://doi.org/10.1016/j.semcdb.2019.12.005

Corbit, K.C., P. Aanstad, V. Singla, A.R. Norman, D.Y.R. Stainier, and J.F. Reiter. 2005. Vertebrate Smoothened functions at the primary cilium. *Nature.* 437:1018–1021. https://doi.org/10.1038/nature04117

Crompton, T., S.V. Outram, and A.L. Hager-Theodorides. 2007. Sonic hedgehog signalling in T-cell development and activation. *Nat. Rev. Immunol.* 7:726–735. https://doi.org/10.1038/nri2151

de la Roche, M., A.T. Ritter, K.L. Angus, C. Dinsmore, C.H. Earnshaw, J.F. Reiter, and G.M. Griffiths. 2013. Hedgehog signaling controls T cell killing at the immunological synapse. *Science.* 342:1247–1250. https://doi.org/10.1126/science.1244689

Debec, A., W. Sullivan, and M. Bettencourt-Dias. 2010. Centrioles: active players or passengers during mitosis? *Cell. Mol. Life Sci.* 67:2173–2194. https://doi.org/10.1007/s00018-010-0323-9

Drummond, M.L., M. Li, E. Tarapore, T.T.L. Nguyen, B.J. Barouni, S. Cruz, K.C. Tan, A.E. Oro, and S.X. Atwood. 2018. Actin polymerization controls cilia-mediated signaling. *J. Cell Biol.* 217:3255–3266. https://doi.org/10.1083/jcb.201703196

Dyson, J.M., C.G. Fedele, E.M. Davies, J. Becanovic, and C.A. Mitchell. 2012. Phosphoinositide phosphatases: just as important as the kinases. *Subcell. Biochem.* 58:215–279. https://doi.org/10.1007/978-94-007-3012-0_7

Dyson, J.M., S.E. Conduit, S.J. Feeney, S. Hakim, T. DiTommaso, A.J. Fulcher, A. Sriratana, G. Ramm, K.A. Horan, R. Gurung, et al. 2017. INPP5E regulates phosphoinositide-dependent cilia transition zone function. *J. Cell Biol.* 216:247–263. https://doi.org/10.1083/jcb.201511055

Failler, M., H.Y. Gee, P. Krug, K. Joo, J. Halbritter, L. Belkacem, E. Filhol, J.D. Porath, D.A. Braun, M. Schueler, et al. 2014. Mutations of CEP83 cause infantile nephronophthisis and intellectual disability. *Am. J. Hum. Genet.* 94:905–914. https://doi.org/10.1016/j.ajhg.2014.05.002

Farina, F., J. Gaillard, C. Guérin, Y. Couté, J. Sillibourne, L. Blanchoin, and M. Théry. 2016. The centrosome is an actin-organizing centre. *Nat. Cell Biol.* 18:65–75. https://doi.org/10.1038/ncb3285

Finetti, F., S.R. Paccani, M.G. Riparbelli, E. Giacomello, G. Perinetti, G.J. Pazour, J.L. Rosenbaum, and C.T. Baldari. 2009. Intraflagellar transport is required for polarized recycling of the TCR/CD3 complex to the immune synapse. *Nat. Cell Biol.* 11:1332–1339. https://doi.org/10.1038/ncb1977

Finetti, F., L. Patrussi, G. Masi, A. Onnis, D. Galgano, O.M. Lucherini, G.J. Pazour, and C.T. Baldari. 2014. Specific recycling receptors are targeted to the immune synapse by the intraflagellar transport system. *J. Cell Sci.* 127:1924–1937. https://doi.org/10.1242/jcs.139337

Finetti, F., L. Patrussi, D. Galgano, C. Cassioli, G. Perinetti, G.J. Pazour, and C.T. Baldari. 2015. The small GTPase Rab8 interacts with VAMP-3 to regulate the delivery of recycling T-cell receptors to the immune synapse. *J. Cell Sci.* 128:2541–2552. https://doi.org/10.1242/jcs.171652

Follit, J.A., R.A. Tuft, K.E. Fogarty, and G.J. Pazour. 2006. The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol. Biol. Cell.* 17:3781–3792. https://doi.org/10.1091/mbc.e06-02-0133

Follit, J.A., J.T. San Agustin, F. Xu, J.A. Jonassen, R. Samtani, C.W. Lo, and G.J. Pazour. 2008. The Golgin GMAP210/TRIP11 anchors IFT20 to the Golgi complex. *PLoS Genet.* 4:e1000315. https://doi.org/10.1371/journal.pgen.1000315

Follit, J.A., F. Xu, B.T. Keady, and G.J. Pazour. 2009. Characterization of mouse IFT complex B. *Cell Motil. Cytoskeleton.* 66:457–468. https://doi.org/10.1002/cm.20346

Furmanski, A.L., J.I. Saldana, M. Ono, H. Sahni, N. Paschalidis, F. D'Acquisto, and T. Crompton. 2013. Tissue-derived hedgehog proteins modulate Th differentiation and disease. *J. Immunol.* 190:2641–2649. https://doi.org/10.4049/jimmunol.1202541

Galgano, D., A. Onnis, E. Pappalardo, F. Galvagni, O. Acuto, and C.T. Baldari. 2017. The T cell IFT20 interactome reveals new players in immune synapse assembly. *J. Cell Sci.* 130:1110–1121.

Garbrecht, J., T. Laos, E. Holzer, M. Dillinger, and A. Dammermann. 2021. An acentriolar centrosome at the C. elegans ciliary base. *Curr. Biol.* https://doi.org/10.1016/j.cub.2021.03.023

Garcia-Gonzalo, F.R., S.C. Phua, E.C. Roberson, G. Garcia III, M. Abedin, S. Schurmans, T. Inoue, and J.F. Reiter. 2015. Phosphoinositides Regulate Ciliary Protein Trafficking to Modulate Hedgehog Signaling. *Dev. Cell.* 34:400–409. https://doi.org/10.1016/j.devcel.2015.08.001

Gawden-Bone, C.M., G.L. Frazer, A.C. Richard, C.Y. Ma, K. Strege, and G.M. Griffiths. 2018. PIP5 Kinases Regulate Membrane Phosphoinositide and Actin Composition for Targeted Granule Secretion by Cytotoxic Lymphocytes. *Immunity.* 49:427–437.e4. https://doi.org/10.1016/j.immuni.2018.08.017

Goetz, S.C., and K.V. Anderson. 2010. The primary cilium: a signalling centre during vertebrate development. *Nat. Rev. Genet.* 11:331–344. https://doi.org/10.1038/nrg2774

Goetz, S.C., K.F. Liem Jr., and K.V. Anderson. 2012. The spinocerebellar ataxia-associated gene Tau tubulin kinase 2 controls the initiation of ciliogenesis. *Cell.* 151:847–858. https://doi.org/10.1016/j.cell.2012.10.010

Golstein, P., and G.M. Griffiths. 2018. An early history of T cell-mediated cytotoxicity. *Nat. Rev. Immunol.* 18:527–535. https://doi.org/10.1038/s41577-018-0009-3

Graser, S., Y.D. Stierhof, S.B. Lavoie, O.S. Gassner, S. Lamla, M. Le Clech, and E.A. Nigg. 2007. Cep164, a novel centriole appendage protein required for primary cilium formation. *J. Cell Biol.* 179:321–330. https://doi.org/10.1083/jcb.200707181

Griffiths, G.M., A. Tsun, and J.C. Stinchcombe. 2010. The immunological synapse: a focal point for endocytosis and exocytosis. *J. Cell Biol.* 189:399–406. https://doi.org/10.1083/jcb.201002027

Gupta, G.D., É. Coyaud, J. Gonçalves, B.A. Mojarad, Y. Liu, Q. Wu, L. Gheiratmand, D. Comartin, J.M. Tkach, S.W. Cheung, et al. 2015. A Dynamic Protein Interaction Landscape of the Human Centrosome-Cilium Interface. *Cell.* 163:1484–1499. https://doi.org/10.1016/j.cell.2015.10.065

Hildebrandt, F., and E. Otto. 2005. Cilia and centrosomes: a unifying pathogenic concept for cystic kidney disease? *Nat. Rev. Genet.* 6:928–940. https://doi.org/10.1038/nrg1727

Hogan, M.C., L. Manganelli, J.R. Woollard, A.I. Masyuk, T.V. Masyuk, R. Tammachote, B.Q. Huang, A.A. Leontovich, T.G. Beito, B.J. Madden, et al. 2009. Characterization of PKD protein-positive exosome-like vesicles. *J. Am. Soc. Nephrol.* 20:278–288. https://doi.org/10.1681/ASN.2008060564

Holtan, J.P., K. Teigen, I. Aukrust, R. Bragadóttir, and G. Houge. 2019. Dominant ARL3-related retinitis pigmentosa. *Ophthalmic Genet.* 40:124–128. https://doi.org/10.1080/13816810.2019.1586965

Ishikawa, H., J. Thompson, J.R. Yates III, and W.F. Marshall. 2012. Proteomic analysis of mammalian primary cilia. *Curr. Biol.* 22:414–419. https://doi.org/10.1016/j.cub.2012.01.031

Jenkins, M.R., J.C. Stinchcombe, B.B. Au-Yeung, Y. Asano, A.T. Ritter, A. Weiss, and G.M. Griffiths. 2014. Distinct structural and catalytic roles for Zap70 in formation of the immunological synapse in CTL. *eLife.* 3:e01310. https://doi.org/10.7554/eLife.01310

Joo, K., C.G. Kim, M.S. Lee, H.Y. Moon, S.H. Lee, M.J. Kim, H.S. Kweon, W.Y. Park, C.H. Kim, J.G. Gleeson, and J. Kim. 2013. CCDC41 is required for ciliary vesicle docking to the mother centriole. *Proc. Natl. Acad. Sci. USA.* 110:5987–5992. https://doi.org/10.1073/pnas.1220927110

Kim, J., J.E. Lee, S. Heynen-Genel, E. Suyama, K. Ono, K. Lee, T. Ideker, P. Aza-Blanc, and J.G. Gleeson. 2010. Functional genomic screen for modulators of ciliogenesis and cilium length. *Nature.* 464:1048–1051. https://doi.org/10.1038/nature08895

Kohli, P., M. Höhne, C. Jüngst, S. Bertsch, L.K. Ebert, A.C. Schauss, T. Benzing, M.M. Rinschen, and B. Schermer. 2017. The ciliary membrane-associated proteome reveals actin-binding proteins as key components of cilia. *EMBO Rep.* 18:1521–1535. https://doi.org/10.15252/embr.201643846

Kumari, S., D. Depoil, R. Martinelli, E. Judokusumo, G. Carmona, F.B. Gertler, L.C. Kam, C.V. Carman, J.K. Burkhardt, D.J. Irvine, and M.L. Dustin. 2015. Actin foci facilitate activation of the phospholipase C-γ in primary T lymphocytes via the WASP pathway. *eLife.* 4:e04953. https://doi.org/10.7554/eLife.04953

Kurtulmus, B., C. Yuan, J. Schuy, A. Neuner, S. Hata, G. Kalamakis, A. Martin-Villalba, and G. Pereira. 2018. LRRC45 contributes to early steps of axoneme extension. *J. Cell Sci.* 131:223594. https://doi.org/10.1242/jcs.223594

Larghi, P., D.J. Williamson, J.-M. Carpier, S. Dogniaux, K. Chemin, A. Bohineust, L. Danglot, K. Gaus, T. Galli, and C. Hivroz. 2013. VAMP7 controls T cell activation by regulating the recruitment and phosphorylation of vesicular Lat at TCR-activation sites. *Nat. Immunol.* 14:723–731. https://doi.org/10.1038/ni.2609

Lee, J., N.M.G. Dieckmann, J.R. Edgar, G.M. Griffiths, and R.M. Siegel. 2018. Fas Ligand localizes to intraluminal vesicles within NK cell cytolytic granules and is enriched at the immune synapse. *Immun. Inflamm. Dis.* 6:312–321. https://doi.org/10.1002/iid3.219

Liu, Q., G. Tan, N. Levenkova, T. Li, E.N. Pugh Jr., J.J. Rux, D.W. Speicher, and E.A. Pierce. 2007. The proteome of the mouse photoreceptor sensory cilium complex. *Mol. Cell. Proteomics.* 6:1299–1317. https://doi.org/10.1074/mcp.M700054-MCP200

Lo, C.H., I.H. Lin, T.T. Yang, Y.C. Huang, B.E. Tanos, P.C. Chou, C.W. Chang, Y.G. Tsay, J.C. Liao, and W.J. Wang. 2019. Phosphorylation of CEP83 by TTBK2 is necessary for cilia initiation. *J. Cell Biol.* 218:3489–3505. https://doi.org/10.1083/jcb.201811142

Lu, Q., C. Insinna, C. Ott, J. Stauffer, P.A. Pintado, J. Rahajeng, U. Baxa, V. Walia, A. Cuenca, Y.S. Hwang, et al. 2015. Early steps in primary cilium assembly require EHD1/EHD3-dependent ciliary vesicle formation. *Nat. Cell Biol.* 17:228–240. https://doi.org/10.1038/ncb3109

Maguire, J.E., M. Silva, K.C. Nguyen, E. Hellen, A.D. Kern, D.H. Hall, and M.M. Barr. 2015. Myristoylated CIL-7 regulates ciliary extracellular vesicle biogenesis. *Mol. Biol. Cell.* 26:2823–2832. https://doi.org/10.1091/mbc.E15-01-0009

Martínez-Lorenzo, M.J., A. Anel, S. Gamen, I. Monle n, P. Lasierra, L. Larrad, A. Piñeiro, M.A. Alava, and J. Naval. 1999. Activated human T cells release bioactive Fas ligand and APO2 ligand in microvesicles. *J. Immunol.* 163:1274–1281.

Mirvis, M., T. Stearns, and W. James Nelson. 2018. Cilium structure, assembly, and disassembly regulated by the cytoskeleton. *Biochem. J.* 475:2329–2353. https://doi.org/10.1042/BCJ20170453

Mittelbrunn, M., C. Gutiérrez-Vázquez, C. Villarroya-Beltri, S. González, F. Sánchez-Cabo, M.A. González, A. Bernad, and F. Sánchez-Madrid. 2011. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat. Commun.* 2:282. https://doi.org/10.1038/ncomms1285

Nachury, M.V., A.V. Loktev, Q. Zhang, C.J. Westlake, J. Peränen, A. Merdes, D.C. Slusarski, R.H. Scheller, J.F. Bazan, V.C. Sheffield, and P.K. Jackson. 2007. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell.* 129:1201–1213. https://doi.org/10.1016/j.cell.2007.03.053

Nager, A.R., J.S. Goldstein, V. Herranz-Pérez, D. Portran, F. Ye, J.M. Garcia-Verdugo, and M.V. Nachury. 2017. An Actin Network Dispatches Ciliary GPCRs into Extracellular Vesicles to Modulate Signaling. *Cell.* 168:252–263.e14. https://doi.org/10.1016/j.cell.2016.11.036

Nakatsu, F. 2015. A Phosphoinositide Code for Primary Cilia. *Dev. Cell.* 34:379–380. https://doi.org/10.1016/j.devcel.2015.08.008

Noda, K., M. Kitami, K. Kitami, M. Kaku, and Y. Komatsu. 2016. Canonical and noncanonical intraflagellar transport regulates craniofacial skeletal development. *Proc. Natl. Acad. Sci. USA.* 113:E2589–E2597. https://doi.org/10.1073/pnas.1519458113

Nolz, J.C., T.S. Gomez, P. Zhu, S. Li, R.B. Medeiros, Y. Shimizu, J.K. Burkhardt, B.D. Freedman, and D.D. Billadeau. 2006. The WAVE2 complex regulates actin cytoskeletal reorganization and CRAC-mediated calcium entry during T cell activation. *Curr. Biol.* 16:24–34. https://doi.org/10.1016/j.cub.2005.11.036

Onnis, A., F. Finetti, L. Patrussi, M. Gottardo, C. Cassioli, S. Spanò, and C.T. Baldari. 2015. The small GTPase Rab29 is a common regulator of immune synapse assembly and ciliogenesis. *Cell Death Differ.* 22:1687–1699. https://doi.org/10.1038/cdd.2015.17

Pauker, M.H., B. Reicher, N. Joseph, I. Wortzel, S. Jakubowicz, E. Noy, O. Perl, and M. Barda-Saad. 2014. WASp family verprolin-homologous protein-2 (WAVE2) and Wiskott-Aldrich syndrome protein (WASp) engage in distinct downstream signaling interactions at the T cell antigen receptor site. *J. Biol. Chem.* 289:34503–34519. https://doi.org/10.1074/jbc.M114.591685

Pedersen, L.B., and J.L. Rosenbaum. 2008. Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling. *Curr. Top. Dev. Biol.* 85:23–61. https://doi.org/10.1016/S0070-2153(08)00802-8

Pedersen, L.B., J.B. Mogensen, and S.T. Christensen. 2016. Endocytic Control of Cellular Signaling at the Primary Cilium. *Trends Biochem. Sci.* 41:784–797. https://doi.org/10.1016/j.tibs.2016.06.002

Phua, S.C., S. Chiba, M. Suzuki, E. Su, E.C. Roberson, G.V. Pusapati, S. Schurmans, M. Setou, R. Rohatgi, J.F. Reiter, et al. 2017. Dynamic Remodeling of Membrane Composition Drives Cell Cycle through Primary Cilia Excision. *Cell.* 168:264–279.e15. https://doi.org/10.1016/j.cell.2016.12.032

Powell, L., Y.H. Samarakoon, S. Ismail, and J.A. Sayer. 2021. ARL3, a small GTPase with a functionally conserved role in primary cilia and immune synapses. *Small GTPases.* 12:167–176. https://doi.org/10.1080/21541248.2019.1703466

Prosser, S.L., and C.G. Morrison. 2015. Centrin2 regulates CP110 removal in primary cilium formation. *J. Cell Biol.* 208:693–701. https://doi.org/10.1083/jcb.201411070

Randzavola, L.O., K. Strege, M. Juzans, Y. Asano, J.C. Stinchcombe, C.M. Gawden-Bone, M.N. Seaman, T.W. Kuijpers, and G.M. Griffiths. 2019. Loss of ARPC1B impairs cytotoxic T lymphocyte maintenance and cytolytic activity. *J. Clin. Invest.* 129:5600–5614. https://doi.org/10.1172/JCI129388

Reiter, J.F., and M.R. Leroux. 2017. Genes and molecular pathways underpinning ciliopathies. *Nat. Rev. Mol. Cell Biol.* 18:533–547. https://doi.org/10.1038/nrm.2017.60

Reiter, J.F., O.E. Blacque, and M.R. Leroux. 2012. The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep.* 13:608–618. https://doi.org/10.1038/embor.2012.73

Ritter, A.T., Y. Asano, J.C. Stinchcombe, N.M. Dieckmann, B.C. Chen, C. Gawden-Bone, S. van Engelenburg, W. Legant, L. Gao, M.W. Davidson, et al. 2015. Actin depletion initiates events leading to granule secretion at the immunological synapse. *Immunity.* 42:864–876. https://doi.org/10.1016/j.immuni.2015.04.013

Ritter, A.T., S.M. Kapnick, S. Murugesan, P.L. Schwartzberg, G.M. Griffiths, and J. Lippincott-Schwartz. 2017. Cortical actin recovery at the immunological synapse leads to termination of lytic granule secretion in cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 114:E6585–E6594. https://doi.org/10.1073/pnas.1710751114

Rohatgi, R., H.Y. Ho, and M.W. Kirschner. 2000. Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4, 5-bisphosphate. *J. Cell Biol.* 150:1299–1310. https://doi.org/10.1083/jcb.150.6.1299

Rohatgi, R., L. Milenkovic, and M.P. Scott. 2007. Patched1 regulates hedgehog signaling at the primary cilium. *Science.* 317:372–376. https://doi.org/10.1126/science.1139740

Sanderson, C.J., and A.M. Glauert. 1979. The mechanism of T-cell mediated cytotoxicity. VI. T-cell projections and their role in target cell killing. *Immunology.* 36:119–129.

Schmidt, K.N., S. Kuhns, A. Neuner, B. Hub, H. Zentgraf, and G. Pereira. 2012. Cep164 mediates vesicular docking to the mother centriole during early steps of ciliogenesis. *J. Cell Biol.* 199:1083–1101. https://doi.org/10.1083/jcb.201202126

Shao, W., J. Yang, M. He, X.Y. Yu, C.H. Lee, Z. Yang, A.L. Joyner, K.V. Anderson, J. Zhang, M.B. Tsou, et al. 2020. Centrosome anchoring regulates progenitor properties and cortical formation. *Nature.* 580:106–112. https://doi.org/10.1038/s41586-020-2139-6

Sillibourne, J.E., I. Hurbain, T. Grand-Perret, B. Goud, P. Tran, and M. Bornens. 2013. Primary ciliogenesis requires the distal appendage component Cep123. *Biol. Open.* 2:535–545. https://doi.org/10.1242/bio.20134457

Somech, R., A. Lev, Y.N. Lee, A.J. Simon, O. Barel, G. Schiby, C. Avivi, I. Barshack, M. Rhodes, J. Yin, et al. 2017. Disruption of Thrombocyte and

T Lymphocyte Development by a Mutation in *ARPC1B*. *J. Immunol.* 199: 4036–4045. https://doi.org/10.4049/jimmunol.1700460

Sorokin, S. 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* 15:363–377. https://doi.org/10.1083/jcb.15.2.363

Spektor, A., W.Y. Tsang, D. Khoo, and B.D. Dynlacht. 2007. Cep97 and CP110 suppress a cilia assembly program. *Cell.* 130:678–690. https://doi.org/10.1016/j.cell.2007.06.027

Stephen, L.A., Y. ElMaghloob, M.J. McIlwraith, T. Yelland, P. Castro Sanchez, P. Roda-Navarro, and S. Ismail. 2018. The Ciliary Machinery Is Repurposed for T Cell Immune Synapse Trafficking of LCK. *Dev. Cell.* 47: 122–132.e4. https://doi.org/10.1016/j.devcel.2018.08.012

Stinchcombe, J.C., and G.M. Griffiths. 2007. Secretory mechanisms in cell-mediated cytotoxicity. *Annu. Rev. Cell Dev. Biol.* 23:495–517. https://doi.org/10.1146/annurev.cellbio.23.090506.123521

Stinchcombe, J.C., and G.M. Griffiths. 2014. Communication, the centrosome and the immunological synapse. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369:20130463. https://doi.org/10.1098/rstb.2013.0463

Stinchcombe, J.C., G. Bossi, S. Booth, and G.M. Griffiths. 2001. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity.* 15:751–761. https://doi.org/10.1016/S1074-7613(01)00234-5

Stinchcombe, J.C., E. Majorovits, G. Bossi, S. Fuller, and G.M. Griffiths. 2006. Centrosome polarization delivers secretory granules to the immunological synapse. *Nature.* 443:462–465. https://doi.org/10.1038/nature05071

Stinchcombe, J.C., M. Salio, V. Cerundolo, D. Pende, M. Arico, and G.M. Griffiths. 2011. Centriole polarisation to the immunological synapse directs secretion from cytolytic cells of both the innate and adaptive immune systems. *BMC Biol.* 9:45. https://doi.org/10.1186/1741-7007-9-45

Stinchcombe, J.C., L.O. Randzavola, K.L. Angus, J.M. Mantell, P. Verkade, and G.M. Griffiths. 2015. Mother Centriole Distal Appendages Mediate Centrosome Docking at the Immunological Synapse and Reveal Mechanistic Parallels with Ciliogenesis. *Curr. Biol.* 25:3239–3244. https://doi.org/10.1016/j.cub.2015.10.028

Strom, S.P., M.J. Clark, A. Martinez, S. Garcia, A.A. Abelazeem, A. Matynia, S. Parikh, L.S. Sullivan, S.J. Bowne, S.P. Daiger, and M.B. Gorin. 2016. De Novo Occurrence of a Variant in ARL3 and Apparent Autosomal Dominant Transmission of Retinitis Pigmentosa. *PLoS One.* 11:e0150944. https://doi.org/10.1371/journal.pone.0150944

Szalinski, C.M., A. Labilloy, J.R. Bruns, and O.A. Weisz. 2014. VAMP7 modulates ciliary biogenesis in kidney cells. *PLoS One.* 9:e86425. https://doi.org/10.1371/journal.pone.0086425

Takiar, V., K. Mistry, M. Carmosino, N. Schaeren-Wiemers, and M.J. Caplan. 2012. VIP17/MAL expression modulates epithelial cyst formation and ciliogenesis. *Am. J. Physiol. Cell Physiol.* 303:C862–C871. https://doi.org/10.1152/ajpcell.00338.2011

Tamzalit, F., D. Tran, W. Jin, V. Boyko, H. Bazzi, A. Kepecs, L.C. Kam, K.V. Anderson, and M. Huse. 2020. Centrioles control the capacity, but not the specificity, of cytotoxic T cell killing. *Proc. Natl. Acad. Sci. USA.* 117: 4310–4319. https://doi.org/10.1073/pnas.1913220117

Tanos, B.E., H.J. Yang, R. Soni, W.J. Wang, F.P. Macaluso, J.M. Asara, and M.F. Tsou. 2013. Centriole distal appendages promote membrane docking, leading to cilia initiation. *Genes Dev.* 27:163–168. https://doi.org/10.1101/gad.207043.112

Taschner, M., and E. Lorentzen. 2016. The Intraflagellar Transport Machinery. *Cold Spring Harb. Perspect. Biol.* 8:a028092. https://doi.org/10.1101/cshperspect.a028092

Vivar, O.I., G. Masi, J.-M. Carpier, J.G. Magalhaes, D. Galgano, G.J. Pazour, S. Amigorena, C. Hivroz, and C.T. Baldari. 2016. IFT20 controls LAT recruitment to the immune synapse and T-cell activation in vivo. *Proc.*

Natl. Acad. Sci. USA. 113:386–391. https://doi.org/10.1073/pnas.1513601113

Volpi, S., M.P. Cicalese, P. Tuijnenburg, A.T.J. Tool, E. Cuadrado, M. Abu-Halaweh, H. Ahanchian, R. Alzyoud, Z.C. Akdemir, F. Barzaghi, et al. 2019. A combined immunodeficiency with severe infections, inflammation, and allergy caused by ARPC1B deficiency. *J. Allergy Clin. Immunol.* 143:2296–2299. https://doi.org/10.1016/j.jaci.2019.02.003

Wang, J., and M.M. Barr. 2018. Cell-cell communication via ciliary extracellular vesicles: clues from model systems. *Essays Biochem.* 62:205–213. https://doi.org/10.1042/EBC20170085

Wang, L., and B.D. Dynlacht. 2018. The regulation of cilium assembly and disassembly in development and disease. *Development.* 145:151407. https://doi.org/10.1242/dev.151407

Wang, J., M. Silva, L.A. Haas, N.S. Morsci, K.C. Nguyen, D.H. Hall, and M.M. Barr. 2014. C. elegans ciliated sensory neurons release extracellular vesicles that function in animal communication. *Curr. Biol.* 24:519–525. https://doi.org/10.1016/j.cub.2014.01.002

Waters, A.M., and P.L. Beales. 2011. Ciliopathies: an expanding disease spectrum. *Pediatr. Nephrol.* 26:1039–1056. https://doi.org/10.1007/s00467-010-1731-7

Westlake, C.J., L.M. Baye, M.V. Nachury, K.J. Wright, K.E. Ervin, L. Phu, C. Chalouni, J.S. Beck, D.S. Kirkpatrick, D.C. Slusarski, et al. 2011. Primary cilia membrane assembly is initiated by Rab11 and transport protein particle II (TRAPPII) complex-dependent trafficking of Rabin8 to the centrosome. *Proc. Natl. Acad. Sci. USA.* 108:2759–2764. https://doi.org/10.1073/pnas.1018823108

Wheatley, D.N. 1995. Primary cilia in normal and pathological tissues. *Pathobiology.* 63:222–238. https://doi.org/10.1159/000163955

Wood, C.R., and J.L. Rosenbaum. 2015. Ciliary ectosomes: transmissions from the cell's antenna. *Trends Cell Biol.* 25:276–285. https://doi.org/10.1016/j.tcb.2014.12.008

Wood, C.R., K. Huang, D.R. Diener, and J.L. Rosenbaum. 2013. The cilium secretes bioactive ectosomes. *Curr. Biol.* 23:906–911. https://doi.org/10.1016/j.cub.2013.04.019

Wu, C.T., H.Y. Chen, and T.K. Tang. 2018. Myosin-Va is required for preciliary vesicle transportation to the mother centriole during ciliogenesis. *Nat. Cell Biol.* 20:175–185. https://doi.org/10.1038/s41556-017-0018-7

Wu, S.W., L. Li, Y. Wang, and Z. Xiao. 2019. CTL-Derived Exosomes Enhance the Activation of CTLs Stimulated by Low-Affinity Peptides. *Front. Immunol.* 10:1274. https://doi.org/10.3389/fimmu.2019.01274

Yan, X., and X. Zhu. 2013. Branched F-actin as a negative regulator of cilia formation. *Exp. Cell Res.* 319:147–151. https://doi.org/10.1016/j.yexcr.2012.08.009

Yang, T.T., W.M. Chong, W.J. Wang, G. Mazo, B. Tanos, Z. Chen, T.M.N. Tran, Y.D. Chen, R.R. Weng, C.E. Huang, et al. 2018. Super-resolution architecture of mammalian centriole distal appendages reveals distinct blade and matrix functional components. *Nat. Commun.* 9:2023. https://doi.org/10.1038/s41467-018-04469-1

Ye, X., H. Zeng, G. Ning, J.F. Reiter, and A. Liu. 2014. C2cd3 is critical for centriolar distal appendage assembly and ciliary vesicle docking in mammals. *Proc. Natl. Acad. Sci. USA.* 111:2164–2169. https://doi.org/10.1073/pnas.1318737111

Yee, L.E., and J.F. Reiter. 2015. Ciliary vesicle formation: a prelude to ciliogenesis. *Dev. Cell.* 32:665–666. https://doi.org/10.1016/j.devcel.2015.03.012

Zucchetti, A.E., L. Bataille, J.M. Carpier, S. Dogniaux, M. San Roman-Jouve, M. Maurin, M.W. Stuck, R.M. Rios, C.T. Baldari, G.J. Pazour, and C. Hivroz. 2019. Tethering of vesicles to the Golgi by GMAP210 controls LAT delivery to the immune synapse. *Nat. Commun.* 10:2864. https://doi.org/10.1038/s41467-019-10891-w