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Intraflagellar transport: a new player at the immune synapse

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Abstract

The assembly and maintenance of primary cilia, which orchestrate signaling pathways centrally implicated in cell proliferation, differentiation and migration, are ensured by multimeric protein particles in a process known as intraflagellar transport (IFT). It has recently been demonstrated that a number of IFT components are expressed in hematopoietic cells, which have no cilia. Here, we summarize data for an unexpected role of IFT proteins in immune synapse assembly and intracellular membrane trafficking in T lymphocytes, and discuss the hypothesis that the immune synapse could represent the functional homolog of the primary cilium in these cells.

The immune synapse: a common theme in the dialogue of immune cells

The immune synapse describes a highly organized interface that forms between a T cell and an antigen-presenting cell (APC) that bears cognate MHC-bound peptide (pMHC) [1,2]. It is characterized by partitioning of the T cell receptor (TCR), and a number of co-stimulatory receptors and integrins, in two concentric regions within the contact area, which are known as central and peripheral supramolecular activation clusters (cSMAC and pSMAC) [3]. Membrane receptors with bulky ectodomains that could hinder interaction of the TCR with pMHC are cleared from the T cell and APC contact surface into the distal SMAC. Formation of the immune synapse is orchestrated by reorganization of the cytoskeleton [4]. Moreover the centriole (See Glossary) and associated Golgi apparatus reorient and polarize to a site just beneath the immune synapse. The topological rearrangement of surface receptors leads to selective enrichment of a variety of signaling mediators at the cytosolic side of the immune synapse [1].

Although the immune synapse has been viewed as a stable signaling platform, a more complex picture has emerged regarding both the mechanism of immune synapse formation and its precise function as a signaling device (Box 1). Nevertheless, the importance of the immune synapse for orchestrating signals that drive T cell activation is clear. This has more recently been extended to functional contacts between other immune cells. The immune synapse allows polarized delivery of cytokines or lytic granules to target cells by effector T helper (Th) cells, cytotoxic lymphocytes (CTLs) and regulatory T cells [1,5]. Immune

synapses also form between natural killer (NK) cells and their targets, NKT cells and CD1d-expressing cells, as well as between B cells and subcapsular macrophages. Thus, it is important to understand how these structures assemble and the molecules that are involved.

Box 1

The immune synapse: facts and hypotheses

The initial contact between a T cell and an APC that carries a specific peptide ligand on the MHC complex triggers profound morphological changes in the T cell, which can be readily detected as the reorientation and movement of the centriole and associated Golgi apparatus towards the contact area with the APC [33]. The initial phase of immune synapse assembly is characterized by profound rearrangement of surface receptors, which culminates in a ‘bull’s eye’ configuration of the cSMAC, where the TCR and the costimulatory receptor CD28 are clustered, surrounded by a ring of LFA-1 bound to newly polymerized actin filaments by the cytoskeletal adaptor, talin, at the pSMAC [34,35]. Rapid polymerization of new actin filaments allows the initial clustering of TCR complexes in the cSMAC. These TCR complexes reach the cSMAC as small clusters (known as microclusters) that form in the distal SMAC (dSMAC), and are enriched in CD28. Following polarization of the centriole, the actin filaments retract to the pSMAC to form a ring that helps to stabilize the immune synapse by providing a physical barrier that prevents lateral diffusion of the molecules clustered at the cSMAC, while leaving room at the cSMAC for vesicular trafficking to and from the plasma membrane [3]. The immune synapse is stably maintained for several hours, which sustains signaling until the onset of proliferation of the activated T cell. During this time, engaged TCRs are internalized and continuously replaced through the assembly of new TCR microclusters at the dSMAC and their actin-dependent movement to the cSMAC. Moreover the intracellular TCR pool that is localized in recycling endosomes is mobilized to the cSMAC through the micro-tubule-dependent process of polarized recycling (Box 2) [25]. The mechanisms that regulate the breakdown of the immune synapse are less well understood, although PKC θ , which is enriched at the cSMAC, as been recently demonstrated to destabilize this structure [36].

The immune synapse was initially proposed as a means to optimize signaling by TCR, coreceptors, costimulatory receptors, and integrins by maximizing and stabilizing the contacts with their ligands on the APCs, and by bringing into close proximity the components of the respective signaling cascades. The function of the immune synapse had to be reassessed when it was discovered that signaling by the initiating kinases Lck and ZAP-70 actually occurs in the distal TCR microclusters, and that it is terminated before they aggregate in the cSMAC [37,38]. This has led to the hypothesis that the function of the immune synapse might be to promote the effective degradation of these internalized TCRs, thereby attenuating signaling. This notion has been further modified with the finding that this only applies to high-affinity TCR ligands, and that the cSMAC actually does amplify signals when the TCRs are engaged by low-affinity ligands, which indicates that the immune synapse functions as a tuning device to optimize signaling during T-cell activation, by enhancing weak and dampening strong signals [39].

Box 2**Intracellular trafficking of the TCR**

The TCR is a long-lived receptor that cycles continuously between the plasma membrane and the cytosol in recycling endosomes [40]. This constitutive recycling regulates the levels of surface TCRs by generating a dynamic equilibrium between the surface and intracellular pools, where the TCR complexes internalized by endocytosis are readily replaced by exocytosis of an equal number of TCR complexes from the internal pool (which includes both recycled and *de novo* synthesized TCRs). Phosphorylation by PKC and a dileucine motif in the cytosolic tail of CD3 γ are required for this process [41]. Constitutive TCR recycling might have evolved as a mechanism of quality control of the receptor. To be functional and stable, the TCR complex requires all the components of its signaling module, the CD3 complex, to be intact. At variance with CD3 δ , γ and ϵ chains, CD3 ζ has a shorter half-life. Surface TCR recycling permits the identification and degradation of TCR complexes that have lost their integrity [42].

When T cells form an immune synapse with an APC that carries a cognate ligand, TCR triggering increases the rate of receptor internalization, which is followed by receptor degradation or polarized recycling to the cell surface [43]. TCR degradation is promoted by the TCR-dependent activation of the protein tyrosine kinases Lck and ZAP-70, which induce receptor ubiquitination and targeting to lysosomes with the assistance of Tumor susceptibility gene 101 (TSG101), a central component of the endosomal protein sorting complex (ESCRT) which co-clusters with the TCR at the cSMAC [44,45]. Recycling of the TCR to the plasma membrane is dependent on PKC-mediated activation of CD3 γ , which routes the internalized TCRs to the recycling compartment [46]. The TCR-containing recycling endosomes that contain internalized TCR complexes polarize to the immune synapse with the assistance of the microtubule cytoskeleton, which provides rails for their movement towards the polarized centriole. At the immune synapse, they dock through a target-SNARE/vesicle-SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) interaction and fuse with the plasma membrane, thereby delivering their TCR cargo [25]. Whether these TCRs form signaling-competent microclusters when they reach the plasma membrane is not known, however, this mechanism of TCR enrichment at the cSMAC is essential for the assembly of a stable immune synapse [25].

In addition to its function as an architectural framework for optimizing the flow of signals from the APC to the T cell, the immune synapse acts as a platform for focalized vesicular trafficking, which occurs at the actin-free membrane domain of the cSMAC [5,47]. The TCR and other receptors engaged at the immune synapse undergo endocytosis, which is instrumental for making space for incoming receptors during the long-lasting contact between T cells and APCs, and for dampening signaling. Moreover, the immune synapse is a site of focalized exocytosis. This not only applies to exocytosis of the TCR to the contact site with the APC through polarized recycling, but also to the focalized secretion of cytokines or lytic granules at the immune synapses formed between differentiated effector T cells and their targets [5]. The reorientation of the centriole is crucial to the process of focalized exocytosis, because it provides microtubular paths for

the directional movement of vesicles that contain cytokines or cytolytic mediators to the immune synapse. In cytotoxic T cells, the centriole is moreover directly implicated in the delivery of the lytic granules to the synapse [5].

Recently, the intraflagellar transport (IFT) protein IFT20 has been implicated in immune synapse formation in T cells [6]. In mammalian cells, IFT ensures the assembly and maintenance of primary cilia and is directly responsible for some of the sensory functions of these organelles [7]. Proteins are transported to and from the cilia by multimeric protein complexes, which are known as IFT particles, and move bidirectionally along the axonemal microtubules. IFT has also recently been implicated in exocytosis by the finding that IFT20 is associated with the Golgi apparatus [8,9]. The data linking IFT20 to immune synapse formation were unexpected, given that T cells do not have a primary cilium. Here, we summarize these findings and discuss the remarkable similarities between the cilium and the immune synapse.

The IFT system

Most vertebrate cells have a small, single appendage that projects from their surface, which is known as the primary cilium. These organelles consist of a microtubule-based axoneme that is templated from the basal body and surrounded by a specialized ciliary membrane that is biochemically distinct from, yet continuous with the plasma membrane [10]. Unlike motile cilia and flagella, primary cilia are non-motile, with the exception of the primary cilia on the nodal cells of the developing embryo, which control the establishment of the left–right asymmetry by regulating the direction of the flow of morphogens [11].

Primary cilia, which were discovered over a century ago, have been considered by many as vestigial appendages. This picture has recently changed, however, with the discovery that a number of genetic diseases, collectively known as ciliopathies, are associated with defects in primary cilia [12,13]. Primary cilia are now thought to sense a diverse range of environmental signals in the extracellular milieu, and through various surface receptors localized on the ciliary membrane, relay these signals to the cell body [14]. Primary cilia act as photosensors (rhodopsin signaling in cone and rod photoreceptors), chemosensors (odorant receptors on olfactory neurons), mechanosensors (signaling in response to urine flow in the kidney by voltage-dependent ion channels), and biochemical sensors (signaling by growth factors receptors). A number of cell surface receptors are specifically enriched in primary cilia, including platelet-derived growth factor receptor (PDGFR) α , the serotonin and somatostatin receptors, and the leptin receptor [15,16]. Moreover, signaling pathways involved in cell proliferation, differentiation, survival and migration are orchestrated in the cilium. These include the PDGFR α , Wnt and Shh signaling cascades [15,17,18]. Of note, primary cilia are coupled to the cell cycle. In vertebrates, cilia are only present on non-proliferating cells, and are resorbed during cell cycle progression to release the centrosome from the ciliary basal body, which makes it available for duplication and organization of the mitotic spindle. The ubiquitin conjugating system and the tubulin histone deacetylase (HDAC)6 have been implicated in the disassembly of cilia by promoting protein degradation and destabilization of the axonemal microtubules, respectively [19].

Cilia are assembled and maintained by a process known as IFT, which provides an explanation for how the growth of cilia and flagella occurs from the tip [20]. In this process, multimeric protein complexes move bidirectionally along the axonemal microtubules [21,22] (Figure 1a). Antero-grade movement, powered by kinesin-2, ensures transport and delivery of the building blocks and other ciliary membrane and axonemal proteins into the cilium; and retrograde transport of IFT particles and motors, powered by dynein 1b, returns turnover products back to the cell body. IFT particles are composed of two multimolecular complexes, complex A and complex B, which initially were genetically dissected in *Chlamydomonas*, but are highly conserved in all eukaryotes [7]. The two IFT complexes are stabilized during anterograde transport by an additional complex that consists of seven core components, which was originally discovered in a screen for the identification of the genetic defect in a human ciliary disease, Bardet–Biedl syndrome, and is therefore known as the BBSome [16].

Proteins destined for the cilium are prevented from entering the cilium by the ciliary necklace, a membrane–cytoskeletal complex that acts as a diffusion barrier at the transition zone between cell and ciliary membranes [10]. Cytosolic proteins with the appropriate targeting sequence are sorted at the base of the cilium and transported into the cilium by IFT particles [7]. An unanswered question is how membrane proteins, including growth factor receptors, are sorted and targeted to the cilium. In addition to containing appropriate targeting sequences and a lipid moiety, membrane proteins must be sorted at some point during their transport to the plasma membrane from the Golgi, and targeted to the periciliary membrane to associate with IFT particles. IFT20, a complex B component, is implicated in this function [8]. IFT20 is associated with the basal body and cilia, as are other IFT proteins, and with the Golgi apparatus, to which it is tethered by the golgin GMAP120 [9]. According to the most plausible model, IFT20 marks vesicles that contain proteins destined for the ciliary membrane during their sorting in the Golgi. After delivery to the base of the cilium, IFT20 assembles with the other IFT components to form IFT particles, which, powered by kinesin, drag membrane-associated cargo through the transition zone into the cilium. As will be discussed, the discovery of the membrane trafficking-related function of IFT20 raises many new questions, and accumulating evidence indicates that the IFT/BBSome machinery functions in protein transport within cilia, and in exocytosis [16].

IFT and the immune synapse

Compared to the majority of vertebrate cells, hematopoietic cells are unusual in that they lack a primary cilium. There is, however, a striking similarity in the position of the centriole and Golgi apparatus at the base of the cilium in non-hematopoietic cells and the position of these organelles just below the immune synapse [23]. Moreover electron micrographs of the lytic immune synapse in cytotoxic T cells have revealed the frequent presence of a small bump in the membrane over the centriole [24], which has been referred to as a ‘frustrated cilium’ [5]. These structural observations have suggested to us that all or parts of the IFT system could be present and operational in T cells, even in the absence of a structured cilium; this hypothesis has turned out to be true. A number of IFT proteins, as well as the IFT-dependent motor kinesin-2, are expressed in lymphoid and myeloid cells; all of which lack primary cilia [6]. In T lymphocytes, IFT20 associates with the centriole and the Golgi,

similar to ciliated cells. It also colocalizes with post-Golgi membrane compartments (trans-Golgi network, recycling endosomes, early endosomes) that are implicated in protein trafficking to and from the cell membrane, which indicates that this IFT component plays a role at the immune synapse, where the centriole and associated Golgi apparatus polarize, and which is a site of intense vesicular trafficking [6] (Box 2).

When T cells are exposed to APCs loaded with antigen, IFT20 is recruited to the immune synapse in association with the Golgi apparatus and centriole [6]. Polarization of these organelles does not require IFT20, but TCR clustering to the immune synapse, which is essential for sustained signaling, is crucially dependent on IFT20. Consistent with this function, signaling at the immune synapse is severely impaired in IFT20 knockdown T cells, which results in impaired tyrosine phosphorylation at the immune synapse and impaired T cell activation [6].

Several mechanisms contribute to TCR clustering at the immune synapse. They include size exclusion to remove large molecules that could interfere with interaction of the TCR with its ligands on the APCs and passive, as well as cytoskeleton-driven, lateral mobility of the TCR [2]. An additional mechanism, known as polarized recycling (Box 2), contributes to a major extent to the accumulation of TCRs at the immune synapse. This involves the polarized delivery to this location of TCR complexes that are present in an intracellular pool associated with recycling endosomes [25]. The association of IFT20 with the Golgi apparatus and post-Golgi compartments suggests a role for IFT20 in TCR recycling. In support of this notion both constitutive and polarized TCR recycling are impaired in IFT20 knockdown T cells [6]. Hence IFT20 may contribute to T cell activation by promoting targeted delivery of TCR complexes localized in recycling endosomes to the immune synapse. Whether IFT20 interacts with the machinery that regulates the intracellular trafficking of the TCR, as well as the specific step of the recycling pathway controlled by IFT20, remain to be established. Neither constitutive endocytosis nor ligand-dependent internalization of the TCR requires IFT20 [6], therefore, it seems probable that IFT20 participates in TCR exocytosis.

A crucial issue is whether, in non-ciliated cells, IFT20 works in concert with other IFT components. Although there is no clear evidence of canonical IFT particles in T cells, IFT20 forms a complex in an inducible manner with at least two other complex B components, IFT57 and IFT88, as well as with the TCR itself, in response to TCR engagement. Moreover, IFT57 colocalizes with IFT20, and clusters at the immune synapse together with IFT20 and the TCR in antigen-specific conjugates. Assembly of the IFT20-IFT57-IFT88 complex, its interaction with the TCR, and its recruitment to the immune synapse, all require IFT20, which suggests that this IFT component acts as a nucleation center for the assembly of the TCR-associated IFT complex [6]. Taken together, the data suggest that IFT20 tags TCRs associated with recycling endosomes, and promotes the assembly of an IFT complex, the full composition of which awaits elucidation. This complex might facilitate the polarized delivery of these vesicles to the immune synapse (Figure 1b).

IFT and intracellular transport

In ciliated cells, trafficking of membrane proteins to the primary cilium is dependent on IFT20, which marks Golgi-derived vesicles that contain proteins destined for the cilium, and directs them to the periciliary membrane by interacting with Rab8, a small GTPase that is required for Rab11 activation on recycling endosomes [26]. Rab8 assists vesicular transport by interacting with other small G-proteins implicated in cilia assembly and maintenance, such as Arl6/BBS3, or the Rab-like IFT components IFT27 and IFTA-2 [27]. Moreover, the small GTPases Arl-13 and Arl-3, as well as the Arl3 GAP RP2, have been recently implicated in the membrane trafficking-related coordination of IFT and ciliogenesis [28]. The pivotal role of vesicular transport in primary cilia formation and function is strikingly exemplified by the rod outer segment, where rhodopsin targeting to the cilium is regulated by Rab8, SNAP25 and syntaxin3 [16]. In addition to IFT20, other IFT proteins have been shown by immunoelectron microscopy to be localized in post-Golgi transport vesicles in photoreceptor cells, as well as in vesicle-like structures in the postsynaptic terminals and dendritic processes of the non-ciliated secondary neurons [29]. These findings suggest that IFT is a general regulator of polarized exocytosis [30]. The recently discovered role of IFT in exocytic trafficking at the immune synapse in the non-ciliated T lymphocytes [6] strongly supports this notion, and underscores the possibility that IFT is implicated in TCR trafficking and in the polarized delivery of cytokines or lytic granules at the immune synapse of effector T cells.

Is the immune synapse a functional homolog of the primary cilium?

Even though they lack a primary cilium, T cells appear to recapitulate certain functional properties of cilia at the immune synapse. First, the two structures are characterized by a polarized arrangement of the centriole and Golgi [23]. This positioning is essential for the growth of the cilium and assembly of the immune synapse, and is moreover, regulated by similar mechanisms that involve the actomyosin and tubulin cytoskeleton, dynein, Rho family GTPases, and the Par3/Par6/atypical protein kinase C (PKC) polarity complex (Table 1). Second, they both act as signaling platforms, with a specialized membrane enriched in lipid rafts, and with a protein composition enriched in receptors and signaling mediators, which include protein and lipid kinases and phosphatases, Ras and Rho GTPases and their regulators, molecular motors and polarity proteins, and ubiquitin ligases (Table 1). The latter are centrally implicated in the disassembly of both structures. Third, both cilia and immune synapses are sites of intense vesicular trafficking and targeted exocytosis, as also emphasized by others [5], and as witnessed by the presence of a number of Rab and Arf GTPases and respective regulators, as well as docking and fusion proteins (Table 1) in ciliated cells and cells that form an immune synapse. In both cases, this is crucially dependent on the microtubules, which provide tracks for the vectorial movement to and from the centriole/basal body.

Bioinformatic analysis of IFT proteins has revealed striking structural homologies with membrane coatomers, including COPI and COPII proteins, in the N-terminal β -propeller and C-terminal α -solenoid domains [31]. These proteins interact with membranes and induce a curvature that is essential for pinching off vesicles from specific membrane compartments,

which allows their transfer to the next compartment [32]. This has led to the hypothesis that IFT has developed as a specialized form of coated vesicle transport to a membrane domain, which has eventually evolved into a cilium through the growth of a microtubule-based axoneme from a polarized centriole/basal body. Our data [6] suggest that hematopoietic cells might have conserved the signaling and trafficking function of the primary cilium without the eventual development of a cilium, or despite the loss of the organelle. These cells transiently and inducibly assemble a specialized membrane patch at the immune synapse in response to extracellular cues, with the underlying polarized centriole and Golgi, to coordinate the rapid directional movement of specific membranous cargo towards the contact site with the APC.

Future perspectives

The finding that IFT controls a central process in cells that lack primary cilia, that is, formation of the immune synapse between naïve T cells and APCs, has revealed a new, unexpected player in the molecular machinery that controls T cell activation [6]. The mechanism whereby IFT regulates polarized TCR recycling needs now to be elucidated. This will involve identifying all the components of the IFT system in these cells, as well as their non-IFT interactors, and characterizing the composition and subcellular localization of the IFT complexes implicated in this function. The potential role of IFT in other processes that contribute to immune synapse assembly, such as formation of TCR microclusters and clustering of other membrane receptors to the immune synapse, will also have to be addressed. In this respect, lymphocyte function-associated antigen (LFA)-1, which, similar to the TCR, is a recycling receptor, appears as an attractive candidate. Moreover, the finding that IFT20 acts as a novel regulator of membrane trafficking at the immune synapse in naïve CD4⁺ T cells suggests a possible role of IFT in the focalized delivery of cytokines and/or lytic granules to effector immune synapses, which are sites of intense vesicular trafficking. Finally, although IFT20 is dispensable for the reorientation of the centriole during immune synapse formation, the function of the centriole-associated IFT20 needs to be elucidated; particularly as the basal body is the site of sorting of proteins destined for the cilium and their loading onto IFT particles in ciliated cells [16]. A mechanistic understanding of the IFT system in the assembly and function of immune synapses could provide new insight into T cell related immune dysfunctions. At a more general level, some of the defects ascribed to impaired ciliogenesis in cells with primary cilia might have to be revisited in the light of the emerging role of IFT in intracellular membrane trafficking.

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Glossary

α -solenoid a protein fold that consists of α -helices arranged in a curved pattern

β-propeller	a type of all- β protein architecture characterized by 4–8 blade-shaped β -sheets arranged around a central axis
Antigen-specific conjugate	a conjugate between a T cell and an APC that carries a specific antigen
Axoneme	a cytoskeletal structure that constitutes the central core of a cilium or flagellum. The axoneme of a primary cilium consists of nine pairs of microtubules (9 + 0 axoneme) which, in motile cilia, are arranged in a ring around a single central pair of microtubules (9 + 2 axoneme)
Basal body	a modified form of the centriole that has moved to the cell surface after cell division, which is involved in cilium or flagellum assembly
Centriole	a microtubule-based specialized organelle that is composed of nine bundles of microtubules
Centrosome	the major microtubule organizing centre that is composed of a pair of microtubule-based centrioles surrounded by a pericentriolar matrix
Coatomer protein	a complex of proteins implicated in both retrograde protein trafficking from the Golgi to the endoplasmic reticulum (ER) and in protein export from the ER
ESCRT proteins	a family of proteins implicated in targeting to lysosomes of proteins internalized into multivesicular bodies
SNARE	a large protein family that mediates vesicle fusion, which can be divided into vesicle-SNAREs that are localized at the vesicle membranes during budding, and target-SNAREs that are localized in the membranes of target compartments
Ubiquitin conjugating system	a protein system involved in targeting of cytosolic and nuclear proteins for degradation by the proteasome

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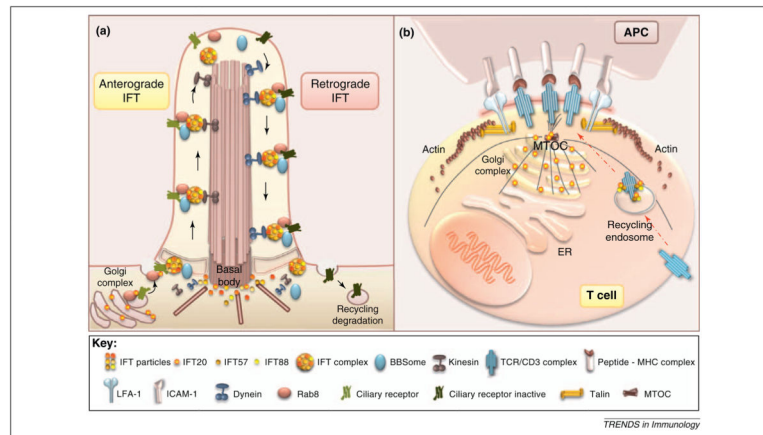


Figure 1.

IFT in primary cilia and at the immune synapse. **(a)** Transport of cargo to primary cilia and back to the cell body is ensured by multimeric IFT particles in concert with the BBSome. Molecular motors (kinesin-2 for anterograde movement and dynein-1b for retrograde movement) assist the movement of particles along the axonemal microtubules. Ciliary proteins are prevented from entering the cilium by the ciliary necklace. Cytosolic proteins that are destined for the cilium are sorted by the IFT/BBSome particles at the base of the cilium based on specific targeting sequences, and are carried into the cilium as a complex with the particles. Ciliary membrane proteins are sorted by IFT20 at the Golgi and targeted to the periciliary membrane, from where they are carried to the ciliary membrane as a complex with an IFT particle. This process is assisted by the small GTPase Rab8. **(b)** When a T cell encounters an antigen-specific APC, the TCR/CD3 complex clusters in the central area of the immune synapse. The integrin LFA-1, which accumulates as a ring peripherally to the TCR, interacts with newly polymerized actin filaments through the adaptor talin, which results in stabilization of the immune synapse. One of the principal mechanisms of TCR clustering at the immune synapse is through polarized delivery of receptor complexes localized in recycling endosomes. Assembly of a stable immune synapse requires the translocation of the centriole close to the membrane patch at the immune synapse. In quiescent cells, IFT20 colocalizes with the centriole and Golgi, as well as with post-Golgi compartments, including recycling endosomes. Following TCR engagement, IFT20 interacts with the TCR and assembles in a complex with IFT57 and IFT88. This IFT complex promotes polarized recycling of the TCR to the immune synapse.

Table 1Similarities between primary cilium and the immune synapse^a

Primary cilium		Immune synapse	
Architectural similarities			
Centriole oriented at base of cilium	[10,14,48] ^b	Centriole polarized at the immune synapse	[1,2] ^b
Golgi polarized under the cilium	[10,14,48]	Golgi polarized at the immune synapse	[1,2]
Cilium surrounded by specialized membrane	[10,27,48]	Specialized membrane patch at the immune synapse	[1,2]
Transient lack of polarization	[10,14,48]	Transient polarization	[1,2]
Signaling platforms ^c			
Receptors: PDGFR α , SSTR ₃ , 5-HT ₆ R, leptin R, integrins $\alpha_{2,3,5}$ and β_1 , Ptch, Smo, MKS3	[14,16,49]	Receptors: TCR, CD4, CD28, LFA-1, CD2, CTLA-4	[1]
Kinases: PDGFR α , FGFR1-3, MEK1/2, Erk1/2, FAK	[14,15]	Kinases: Lck, ZAP-70, PKC θ , Itk, Erk	[1,25]
Phosphatases: inositol polyphosphate-5-phosphatase E	[50]	Phosphatases: SHP-1, SHP-2	[1]
Enzymes: PLC, PI-3K, AC3-6	[16,51]	Enzymes: PLC γ , PKA, PDE4, PI3K	[1,52]
Ion channels: PC1-2, TRPV4, fibrocystin, nephrocystin 4-5	[14]	Ion channels: STIM1, Orai1, Kv1.3, KCa3.1	[1]
Adaptors: β -arrestin, Radial spoke protein 3	[27,53]	Adaptors: Grb2, LAT, SLP76, ADAP, Nck, β -arrestin, ezrin	[1,54]
Small GTPases: Rab/Arl, rabin8	[26,27]	Small GTPases: Ras, Rab, Rap1, Rac1/cdc42	[1,5]
Polarity proteins: Dvl, Vangl2, Par6, aPKC, Par1, inversin, BBS4, IFT20, Fuz	[14,16,27]	Polarity proteins: Scribble, Crumbs, Par3, PKC ζ , Crtam	[1]
Ubiquitination: Aurora, HDAC6	[15–18,55]	Ubiquitination: Itch, Cbl-b, TSG101	[1,56]
Cytoskeleton: F-actin, microtubules, Rac1	[27]	Cytoskeleton: F-actin, talin, microtubules, Vav, WASp, WAVE, Arp2/3	[1,2,4]
Motors: kinesin-2, dynein	[7]	Motors: dynein, myosin IIA	[1]
Sites of polarized exocytosis ^c			
Rab8/Rabin8, Rabaptin5	[16,26,27]	TfR	[47]
IFT27/IFTA-2/BBS3: Rab/Arl GTPases	[27,57]	Rab27a/Munc 13-4	[58]
syntaxin3/SNAP25	[16]	Rab11/Unc 119	[5]
RP2	[59]	Rab35/GAP EPI64C	[5]
Rab11	[26]	syntaxin4/SNAP23	[47]
IFT20	[27]	IFT20	[6]

^aThe primary cilium and the immune synapse display remarkable similarities at multiple levels: (i) both are characterized by the polarized arrangement of the centriole and Golgi apparatus, as well as by a membrane with a specialized protein and lipid composition; (ii) both act as signaling platforms, and are enriched in the same categories of both positive and negative signaling mediators; and (iii) both are sites of polarized exocytosis, and are enriched in the same types of regulators of exocytic trafficking.

^bAs a result of space limitations, references to original research articles have been limited to the past 2 years. In all other instances, reviews have been used.

^cAbbreviations: SSTR₃, somatostatin receptor type 5; 5-HT₆R, 5-Hydroxytryptamine₆ receptor; Ptch, Patched; Smo, Smoothened; MKS3, meckelin; FGFR1-3, Fibroblast growth factor receptor 1-3; MEK1/2, mitogen-activated protein kinase 1/2; Erk1/2, Extracellular Signal-Regulated Kinase 1/2; FAK, Focal adhesion kinase; PLC, phospholipase C; PI-3K, Phosphatidylinositol-3-Kinase; AC3-6, Adenylate cyclase 3-6; PC1-2, polycystin 1-2; TRPV4, transient receptor potential channel 4; Rab, Ras gene from rat brain; Arl, Arf-like; Dvl, Dishevelled; Vangl2, Van Gogh 2; Par, partitioning-defective protein; aPKC, atypical protein kinase C; Fuz, fuzzy protein; Rac1, Ras-related C3 botulinum toxin substrate 1; IFTA-2, IFT associated-protein 2; SNAP25, Synaptosomal-associated protein 25; RP2, retinitis pigmentosa protein; CTLA-4, Cytotoxic T-Lymphocyte

Antigen 4; Lck, lymphocyte-specific protein tyrosine kinase; ZAP-70, Zeta-chain-associated protein kinase 70; PKC, Protein kinase C; Itk, IL2-inducible T-cell kinase; SHP, Src homology 2 domain containing protein tyrosine phosphatase; PKA, protein kinase A; PDE4, phosphodiesterase 4; STIM1, stromal interaction molecule 1; Kv1.3, voltage-gated potassium channel 1.3; KCa3.1, calcium-activated potassium channel; Grb2, Growth factor receptor-bound protein 2, LAT, Linker of Activated T cells; SLP76, SH2 domain containing leukocyte protein 76; ADAP, adhesion and degranulation promoting adapter protein; Nck, noncatalytic tyrosine kinase; cdc42, Cell Division Control Protein 42; Crtam, class I-restricted T cell-associated molecule; Itch, E3 ubiquitin-protein ligase Itchy homolog; Cbl-b, Casitas B-lineage lymphoma proto-oncogene b; WASp, Wiskott–Aldrich syndrome protein; WAVE, WASP family Verprolin-homologous protein; Arp2/3, actin related protein; TfR, transferrin receptor; Munc 13-4, Protein unc-13 homolog; Unc 119, Uncoordinated 119; SNAP23, Synaptosomal-associated protein 23.