Vesicle choreographies keep up cell-to-extracellular matrix adhesion dynamics in polarized epithelial and endothelial cells

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Abstract

In metazoans, cell adhesion to the extracellular matrix (ECM) drives the development, functioning, and repair of different tissues, organs, and systems. Disruption or dysregulation of cell-to-ECM adhesion promote the initiation and progression of several diseases, such as bleeding, immune disorders and cancer. Integrins are major ECM transmembrane receptors, whose function depends on both allosteric changes and exo-endocytic traffic, which carries them to and from the plasma membrane. In apico-basally polarized cells, asymmetric adhesion to the ECM is maintained by continuous targeting of the plasma membrane by vesicles coming from the *trans* Golgi network and carrying ECM proteins. Active integrin-bound ECM is indeed endocytosed and replaced by the exocytosis of fresh ECM. Such vesicular traffic is finely driven by the teamwork of microtubules (MTs) and their associated kinesin and dynein motors. Here, we review the main cytoskeletal actors involved in the control of the spatiotemporal distribution of active integrins and their ECM ligands, highlighting the key role of the synchronous (ant)agonistic cooperation between MT motors transporting vesicular cargoes, in the same or in opposite direction, in the regulation of traffic logistics, and the establishment of epithelial and endothelial cell polarity.

Highlights

- Apico-basally polarized cells exploit spatially oriented microtubule tracks to establish and maintain exo-endocytic traffic of integrins and ECM proteins

- Traffic logistics of vesicular cargos relies on sequential agonistic and synchronous antagonistic cooperation between microtubule motors

- Integrin-mediated cell adhesion to the ECM at the basal plasma membrane drives the formation of different protein complexes that capture microtubules +ends and tether exocytic vesicles

Introduction

The dynamic adhesion of progenitor and differentiated cells to extracellular matrix (ECM) proteins assembled in three-dimensional networks is crucial for the development, functioning, and repair of different tissues, organs, and systems of metazoans [1]. Moreover, abnormal cell-to-ECM adhesive interactions sustain the initiation and progression of several diseases, ranging from bleeding and immune disorders [2] to blistering diseases [3] and cancer [4]. Integrin αβ heterodimers are major ECM transmembrane receptors whose functions are regulated by both allosteric changes from inactive bent/closed to active extended/open conformation [5] and exo-endocytic traffic to and from the plasma membrane [1,6].

Integrin-mediated adhesion to the ECM occurs non homogeneously throughout the cell surface, being limited to discrete membrane domains [7] and micrometer-range points of contact [8]. Asymmetries in ECM-adhesion are key for cells to acquire polarized molecular and structural architectures that support their specialized biological functions [9]. In this regard, prominent examples are the organization of apical and basal sides in epithelial cells [7] or leading and trailing edges in directional migrating cells, such as leukocytes [10] or invading cancer cells [4]. It is known that epithelial and endothelial cells determine the polarized orientation of their apical–basal axis by sensing the ECM through integrin-mediated adhesion and signaling [9]. Once established, the over time maintenance of asymmetric matrix adhesion in polarized cells relies on continuous endo-exocytic cycles of ECM carrying vesicles [11]. Indeed, from basal adhesive contacts, active integrin-bound cleaved ECM is endocytosed and then replaced with freshly synthesized matrix proteins delivered by *trans* Golgi network (TGN)-derived secretory vesicles [6,12]. Notably, ECM adhesion sites physically associate with and spatially orient microtubule (MT) tracks [13], along which exo- and endocytic vesicles are coordinately trafficked [14,15] to control the spatiotemporal distribution of (active) integrins and their ECM ligands [6]. Furthermore, integrin-based adhesion sites are emerging as general spatial coordinators of MT-

dependent plasma membrane-associated cellular functions, such as epithelial apico-basal polarity [16], secretion of non-ECM proteins [17,18] and lysosome-dependent nutrient sensing [19–21].

Here, we will review the mounting evidence that the maintenance of asymmetrically localized ECM adhesion sites depends on and allows MT motor-driven vesicular traffic of adhesive cargoes in polarized epithelial and endothelial cells.

Microtubule motors control subcellular positioning of trafficking compartments

The coordinated regulation of spatial distribution strongly impacts on the delivery of key protein cargoes, such as integrins and ECM proteins, to adhesion sites [1,6]. In general, the localization in trafficking compartments, such as early endosomes (EE), late endosomes (LE), and lysosomes (LY), of receptors and their ligands significantly affects their traffic dynamics and function [22,23]. For instance, the spatial distribution of transmembrane cargoes in more immature and dynamic peripheral EEs, preferentially contribute to a faster turnover of cargoes, compared to the one observed for LEs or LYs [23].

The heterogeneity of vesicular compartments is due to key specific membrane associated proteins, such as Rab5 or Rab7 small GTPases in the case of EE and LE/LY respectively, but it also depends on which subcellular compartment, *e.g.* the cell surface or the TGN, the trafficking vesicle had been generated from [24–26]. Furthermore, the cytoplasmic spatial distribution of transported vesicular cargoes is carried out by MTs along with their anterograde and retrograde associated motors, respectively moving towards MT polymerizing/plus (+) end and depolymerizing/minus (-) end [27,28]. Indeed, the movement of vesicles is strictly dependent on the competition between dynein and different kinesins, respectively driving MT -end and +end-directed motion [29,30] (**Fig. 1A, B and C**). In addition, the wide family of kinesins have also been reported to be in charge of scission of membrane tubules from MT-

associated endosomes, further modulating positioning dynamics [31]. Although extensive research has been carried out to understand the mechanisms driving dynein and kinesin function, how those two motor families are recruited and cooperate to deliver their cargoes in precise locations is still not completely understood. However, many questions have been so far addressed by *in vitro* reconstruction studies [32,33] and several biological evidences in living cells [29,34].

A key demonstrated aspect is that the loading activity and specificity between a molecular motor and the carried vesicle strictly depend on the adaptor exploited by the motor. Indeed, extensive work has been carried out to identify motor adaptors and their function in MT transport and cell behavior. It is known that dynein exists in an auto-inhibited state, which is released by the multiprotein asymmetric complex dynactin, whose assembly is stabilized by proteins, such as bicaudal D (BICD) cargo adaptor [35], the Rab11 family interacting protein 3 (FIP3), HOOK3 and the spindle apparatus coiled coil protein (SPDL1) [29,33,36] to prompt MT retrograde cargo traffic. On the other hand, kinesins can directly bind their cargoes or use molecular adaptors. For instance, KIF1A and KIF1Bβ bind to RAB3 proteins through the adaptor protein mitogen-activated protein kinase (MAPK) activating death domain (DeNN/MADD) [37]. Due to the complexity of the kinesin superfamily, more details have to be characterized to mechanistically describe the functioning and selectivity of these motors to its adaptors or cargoes in living cells. However, the discovery of molecular adaptors contributing to dynein movement processivity has provided useful explanation for the tug-of-war between a unique retrograde motor and multiple anterograde ones [38]. Moreover, some adaptors are shared by dynein and some kinesins, thus playing a key role in modulating the opposite polarity of MT-associated motors. Indeed, LIS1, BICD2, HOOK1 and HOOK3, are known to selectively recruit dynein or kinesins at cargo loading sites, thus significantly regulating their centripetal or centrifugal movement and cytoplasmic positioning [34,39,40].

In addition to the movement relying on motors proceeding to opposite MT ends, a key and widely accepted concept is the teamwork played by motors proceeding in the same direction (**Fig. 1D**). Groups of kinesins have been indeed shown to cooperate and drive the traffic of endosomes, but also of membranous organelles and mRNAs [41,42] towards the cell membrane. Interestingly, those motors can also compete for the binding to their cargo, resulting in a negative regulation of its motion. For instance, a mechanical competition has been demonstrated between two MT +end directed kinesins in *C. elegans* neuronal cilia formation [43]. Moreover, an unconventional subfamily of kinesins (such as KIF14), also called C-kinesins (KIFC1, KIFC2 and KIFC3), exists in mammals and drive cargo transport to MT – ends, oppositely to the direction of motion of the above described classic kinesins [37]. The existence of this specific class of kinesins has led us and others to speculate on an additional mechanism of cooperation between dynein and kinesins, proceeding to the same direction, in mammalian cells. We found that dynein and KIFC1, relying on HOOK1 and HOOK3 respectively, coordinately modulate the MT-end directed movement of EEs [26]. Our thorough analysis of physical parameters (such as size, distance from the nucleus, and velocity of movement in living cells) of two endocytic compartments, the larger and more centrally localized LEs versus the small and more dynamic EEs, identified a dual motor level of motion regulation for the latter only (**Fig. 1E**). Indeed, we demonstrated that the inhibition of dynein- or KIFC1-motor systems causes, differentially to LEs, the EE collapse around the nucleus. On the other hand, when both motor systems, or both their HOOK specific adaptors, are disrupted, the normal peripheral localization of EEs is restored, supporting a cooperative antagonism between dynein and KIFC1 in driving their MT -end-directed movement, potentially counterbalanced by a canonical MT +end-directed anterograde kinesin, such as kinesin 1 KIF5B. We indeed speculated that small endocytic vesicles, such as EEs, which, differently from large LEs, experience a relatively lower friction with the cytoplasm, may require the coupling of dynein and the cooperative, yet antagonistic, MT -end-directed motor KIFC1 to coordinately direct their typical peripheral localization. Additionally, the same

cooperative antagonism between dynein and KIFC1 has been also observed during ciliary protein exit from the Golgi during cilia formation [44]. Although the repertoire of cargoes transported by KIFC1 needs further investigation, this and our work support the notion of cooperative antagonism in motor-dependent cargo movement and shed light on how the specificity of motor systems for their cargo can be reached.

Polarized ECM secretion and integrin traffic in epithelial and endothelial cells

The ability of polarized cells, such as those of epithelial tissues [7] and vascular endothelium [45], to differentially transport cargos and solutes along their apico-basal axis crucially relies on their asymmetric integrin-mediated adhesion to the ECM. While, among ECM ligands, fibronectin prevails during embryonic development or post-natal wound healing, intact epithelial and endothelial cell monolayers instead adhere to laminin-containing basement membranes (BMs) in the adult organism [46–48].

The binding of integrins to BM proteins triggers signals that, by defining the basal domain, initiate and maintain the apico-basally oriented axis of epithelial cells [7,9,49]. BM-bound β1 integrins recruit the integrin linked kinase (ILK) adaptor that allows the capture and stabilization, close to adhesion sites, of non-centrosomal MT +ends, along which polarized apicobasal endocytic and exocytic vesicular cargoes are trafficked [16,50,51]. Indeed, thanks to its association with the scaffold IQ motif containing GTPase activating protein (IQGAP), which in turn interacts with multiple MT +end tracking proteins [52], ILK effectively recruits MTs at ECM adhesions [51] (**Fig. 2A**). In this context, ILK has been reported to promote the endocytosis of apical components from [50] and the exocytosis of caveolin at [51] the basal plasma membrane, thus fostering the biochemical and functional polarization of epithelial cells.

Albeit with a faster rate in embryonic than in adult tissues, epithelial BM undergo constant turnover [53], consisting in cycles of protease-mediated degradation of the existing ECM, followed by integrin-dependent endocytosis of ECM fragments and their replacement with new ECM proteins [11,53,54]. To keep promoting such dynamic apico-basal polarization of epithelial cells, secretory vesicles containing Golgi apparatus-synthesized fresh BM proteins must be directionally trafficked along MTs and released at the basal surface [16,49,50]. The Golgi-associated small GTPase Rab6, which is known to promote the anterograde transport of cargoes to the plasma membrane in different cell types [55], is required for laminin secretion and BM polymerization on the basal side of epithelial cells [56]. Similarly, 5.5 dpc *Rab6a* null embryos lack laminin^{+} BM in between the epiblast and the visceral endoderm [57]. In addition to Rab6, Rab8 [58] and Rab10 [59], which belong to same Rab subfamily [60], have also been involved in *trans*-Golgi network (TGN)-to-basolateral plasma membrane traffic in epithelial cells. Consistently, in *Drosophila* Rab10, which is enriched in vesicles localized near the basal surface of ovary epithelial follicle cells, promotes polarized laminin^{+} BM secretion [61], fibrillogenesis and normal egg chamber elongation [62].

Rab6^{+} post-Golgi carriers (PGCs) that bud from the *trans*-Golgi network (TGN) to deliver cargoes to the plasma membrane are processively transported towards the cell periphery along MTs by +end directed motors belonging to kinesin 1 and kinesin 3 families [14]. In HeLa cervical carcinoma cells kinesin 1 KIF5B and kinesin 3 KIF13B cooperate as predominant MT motors for Rab6^{+} PGC transfer to the cell periphery [63,64]. In this cell type, KIF5B prevails over KIF13B in the transport of Rab6^{+} PGCs along older and more central MTs, while KIF13B takes the lead on freshly polymerized peripheral MTs [64]. This may be due to the fact that, while more resistant to detachment under load [65] and capable of engaging in a tug-of-war with MT -end directed motor dynein, KIF5B recruitment on MT and activation strongly depends on MT associated protein 7 (MAP7), which does not effectively associate with growing MT +ends [66]. Of note, MT-dependent transport to the basal surface of ovarian follicle cells of type IV

collagen containing Rab10^{+} vesicles, together with the following polarized secretion of BM proteins and organized tissue architecture, rely on the synergy between Khc and Khc-73, the *Drosophila* orthologs of KIF5B and KIF13B respectively [67]. KIF5B is recruited to Rab6^{+} PGCs *via* the adaptor Dopey1, which simultaneously binds phosphatidylinositol 4 phosphate (PI4P) and KIF5B-associated kinesin light chain 2 (KLC2) and also connects with the dimeric adaptor Mon2, which further stabilizes the complex by interacting with phosphatidic acid (PA) [68]. While it is not known yet how KIF13B associates with PGCs [64], BICD adaptor protein directly links the -end MT dynein motor to Rab6 [69].

During embryonic development, the binding of fibronectin to its major receptor α5β1 integrin induces apico-basal polarity of endothelial cells and the formation of the single lumen of blood vessels [70,71]. We [72] and others [73] revealed that, similarly to exocytosis in neuron presynaptic active zone [74], the polarized secretion of PGCs carrying freshly synthesized fibronectin at the basal surface of endothelial cells relies on the protein tyrosine phosphatase receptor type f polypeptide (PTPRF, also named LAR for leukocyte common antigen related) and its directly interacting adaptor PTPRF interacting protein α1 (PPFIA1), also known as liprin-α1. Of note both proteins were identified as components of integrin adhesome complexes in different cell types [75–78]. From the N- to the C-terminus, PPFIA1/ liprin-α1 comprises two coiled coil (CC) domains, a single α helical (SAH) domain, and three sterile α motif (SAM) domains [79]. Through SAM1-2 domains and CC1 domain, PPFIA1/liprin-α1 respectively binds the cytosolic D2 domain of PTPRF/LAR and co-oligomerizes with PTPRF/LAR at ECM adhesions [80] (**Fig. 2B**). The fact that in endothelial cells PPFIA1/liprin-α1 supports the localization of PGCs close to ECM adhesions [72] suggests that, similarly to its presynaptic function [74], PTPRF/LAR-bound PPFIA1/liprin-α1 may act as a local tether for ECM-loaded exocytic vesicles (**Fig. 2A**). Directly or indirectly PPFIA1/liprin-α1 interacting proteins known to bind PGC associated Rab GTPases, such as glutamine/leucine/lysine/serine-rich protein (ELKS) [81] or mammalian UNC13 (MUNC13) [82] respectively binding Rab6 and Rab11, may play a role in this

regard. Indeed, in addition to Rab6 [25], also Rab11 localizes at the TGN [83] and plays a role in secretory traffic [84,85]. Three different Rab11 family members exist [60] and interact with kinesin 3 KIF13A [86]. In epithelial cells, Rab11A controls the post-Golgi delivery of apical cargoes [87] and Rab11B, but not Rab11A, drives the polarized secretion of fibronectin at the basal plasma membrane of endothelial cells [11,12] and pleural mesothelial cells [88] through molecular mechanisms that have still to be definitively outlined. For example, similarly to presynaptic active zones [74], PTPRF/LAR-associated PPFIA1/liprin-α1 may indirectly promote the RAB11B-dependent mooring of fibronectin containing PGCs at endothelial ECM adhesions through MUNC13 [82]. We [12] and others [89] observed that in endothelial cells, upon internalization in early endosomes, conformationally active α5β1 integrins reach PGCs, where they, perhaps acting as secretory receptors, are recycled and released at the basal plasma membrane along with newly synthesized fibronectin. Thanks to its ability to bind PPFIA1/liprin-α1 [12], the β1 cytotail of active α5β1 integrins may also support the docking of fresh fibronectin-loaded PGCs at endothelial ECM adhesions.

Confirming and extending previous findings [63,90], Fourriere et al. [17] recently showed that TGN-derived Rab6$^+$ PGCs, transported along MT tracks to the cell periphery by KIF5B, dock *via* ELKS close to ECM adhesions, thus allowing the secretion of several cargoes, among which ECM proteins, such as type X collagen. The targeting of Rab6$^+$/ELKS$^+$ ECM containing PGCs [17] conceivably depends on the interaction of MT +ends with cortical MT stabilization complexes (CMSCs) that assemble at the rim of ECM adhesions [91]. Indeed, CMSCs are enriched with proteins, *e.g.* the kinesin-4 family member KIF21A [92] and CLIP-associating proteins (CLASPs) [90], that target and stabilize MT +ends. KIF21A and CLASPs are part of complexes respectively connected to talin [5] and filamin [93] actin cytoskeleton adaptor proteins known to modulate the conformational activation of integrins. KIF21A anchors CMSCs to ECM adhesions by interacting with KN motif and ankyrin repeat domain-containing proteins (KANKs) [94–96] that in turn bind the rod domain of the integrin activating protein talin [97,98]. Instead,

CLASPs interact with LL5β [90] that also connects to the Rab6 adaptor ELKS [90] and the integrin inhibiting protein filamin [99,100]. In addition to binding talin, KIF21A-associated KANK binds PPFIA binding protein 1 (PPFIBP1)/liprin-β1 to recruit the LL5β-CLASP complex at CMSCs [97] (**Fig. 2A**). Through their SAM domains PPFIBP1/liprin-β1 and PPFIA1/liprin-α1 can heterodimerize [101]. However, the SAM domain-mediated binding of PPFIA1/liprin-α1 to PPFIBP1/liprin-β1 and PTPRF/LAR is mutually exclusive [80] (**Fig. 2B**). Therefore, two distinct PPFIA1/liprin-α1-PTPRF/LAR and PPFIA1/liprin-α1-PPFIBP1/liprin-β1 complexes are expected to exist. Furthermore. PPFIA1/liprin-α1 was found to bind the same region [102] through which ELKS associates with LL5β [90], suggesting that binding of ELKS to PPFIA1/liprin-α1 and LL5β may be mutually exclusive as well [103]. Altogether, these findings hint a model in which three distinct complexes may be at work to coordinate the delivery and exocytosis of PGCs at ECM adhesions. The liprin-β1-interconnected KANK-KIF21A and LL5β-CLASP complexes would promote the stabilization of MT+ ends close to adhesion sites, thus allowing the long-range MT-dependent transport of PGCs carrying fresh ECM and recycled active integrins. Similarly to its function at the neuronal presynaptic active zone [74], the PTPRF/LAR-PPFIA1/liprin-α1 complex would instead promote the docking and ensuing fusion of PGCs with the plasma membrane surrounding the ECM adhesions.

Conclusions

Apico-basally polarized cells exploit spatially oriented MT tracks to establish and maintain, *via* exo-endocytic traffic, the asymmetric distribution of their intracellular, transmembrane, and secreted proteins. Sequential agonistic (*e.g.*, KIF5B and KIF13B) and synchronous antagonistic cooperation between MT motors transporting vesicular cargoes in the same (*e.g.*, dynein and KIFC1) or in opposite (*e.g.*, dynein and KIF5B) directions is crucial to control the underpinning traffic logistics. Integrin-mediated adhesion

to the ECM defines the basal plasma membrane domain by driving the formation of different protein complexes that, capturing MT +ends (ILK-IQGAP, KANK-KIF21A, and LL5β-CLASP complexes) and tethering exocytic vesicles (PTPRF/LAR-PPFIA1/liprin-α1 complex) at the plasma membrane, allow directional endo-exocytic traffic and protein secretion. In addition, the traffic of PGCs carrying fresh ECM proteins and recycling active integrins give rise to a key positive feedback that allows the dynamic maintenance of basal ECM adhesions. Further work is needed to pinpoint and thoroughly characterize the roles played by different PGC associated GTPases (Rab6, Rab8, Rab10 and Rab11), anterograde and retrograde MT motors, ECM adhesion-associated MT capturing and vesicle tethering complexes in the delivery of the panoply of secreted and recycling cargoes in polarized epithelial and endothelial cells.

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Conflict of interests

All authors declare that they have no competing interests

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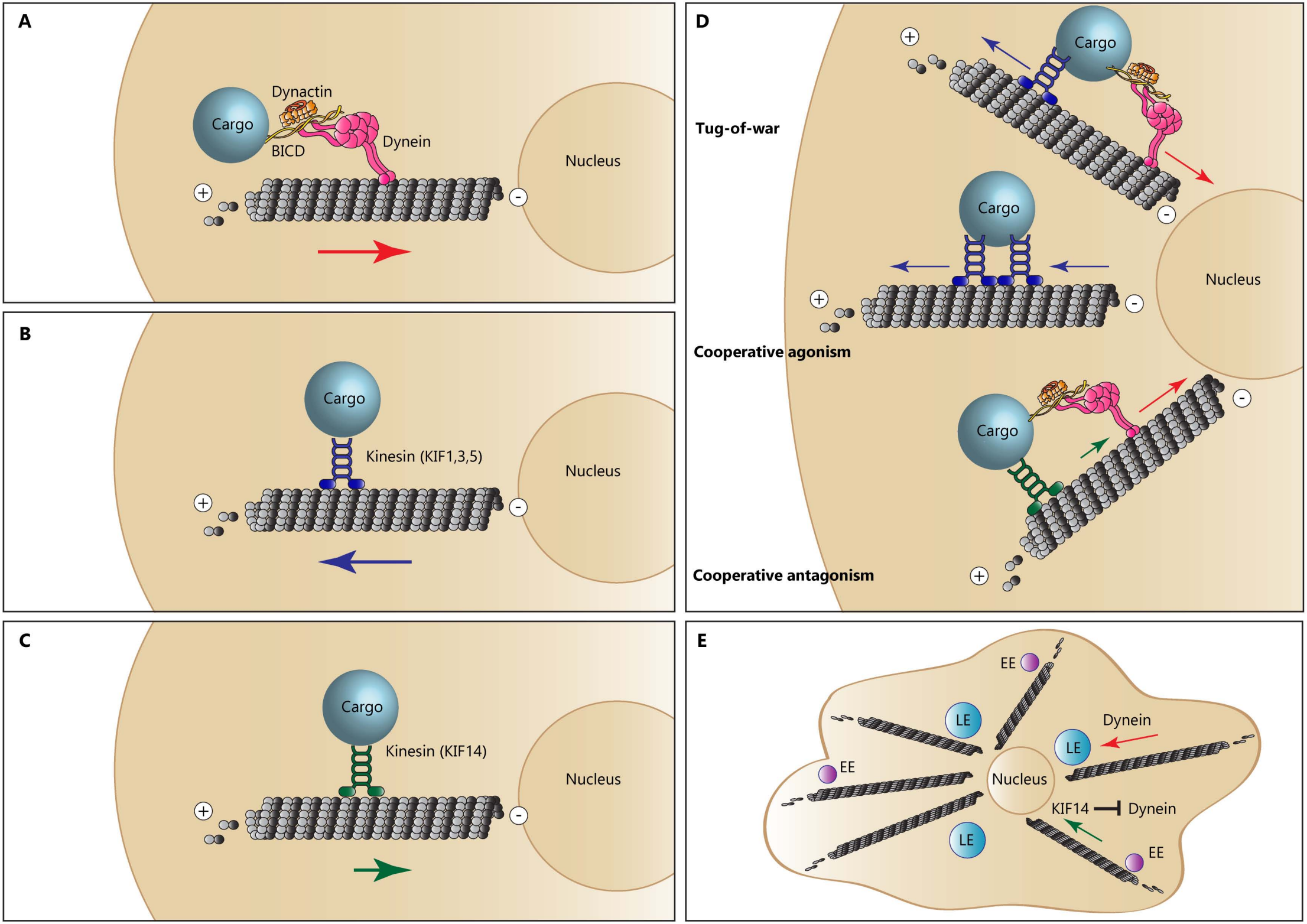
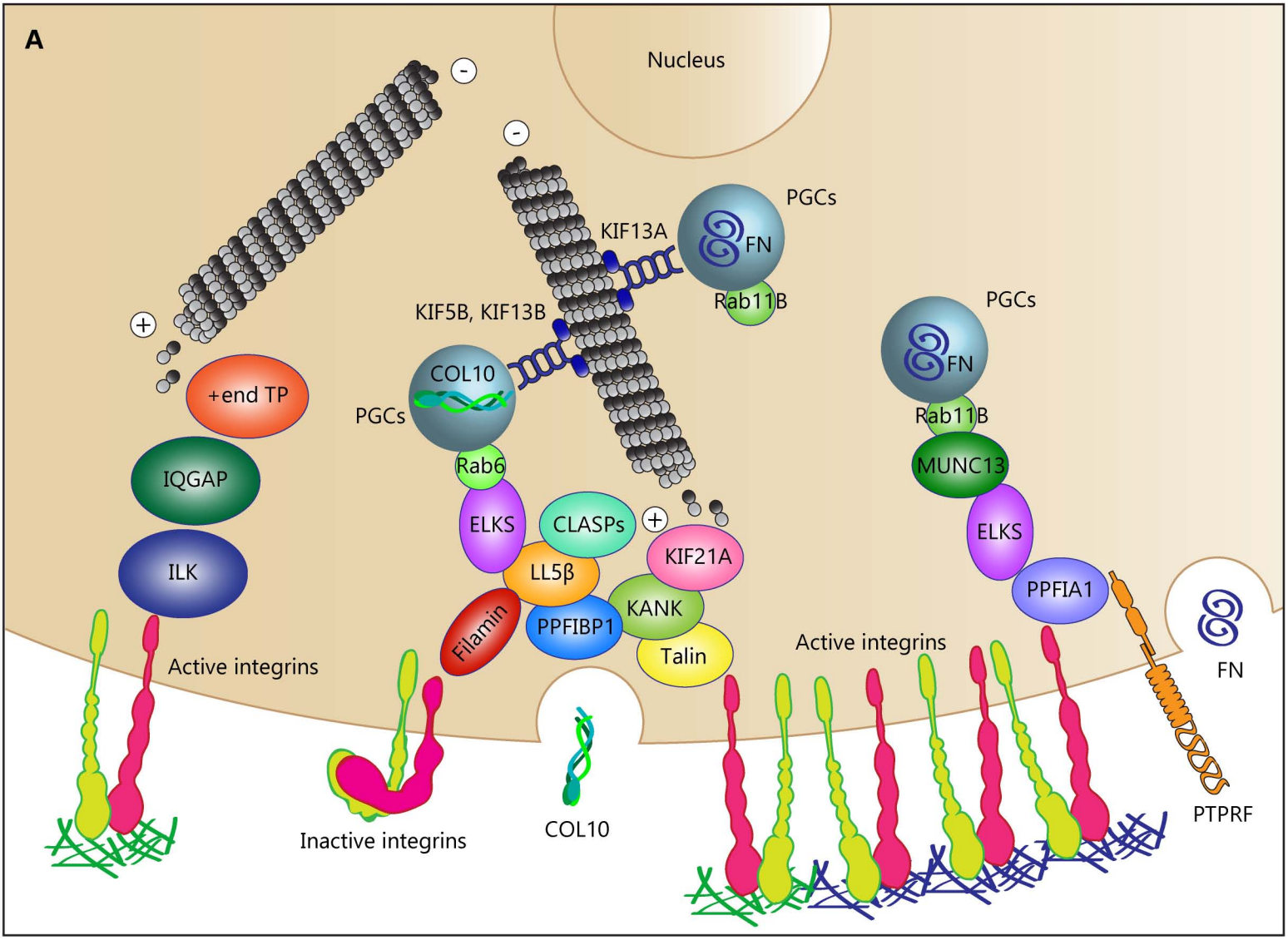
Figure legends

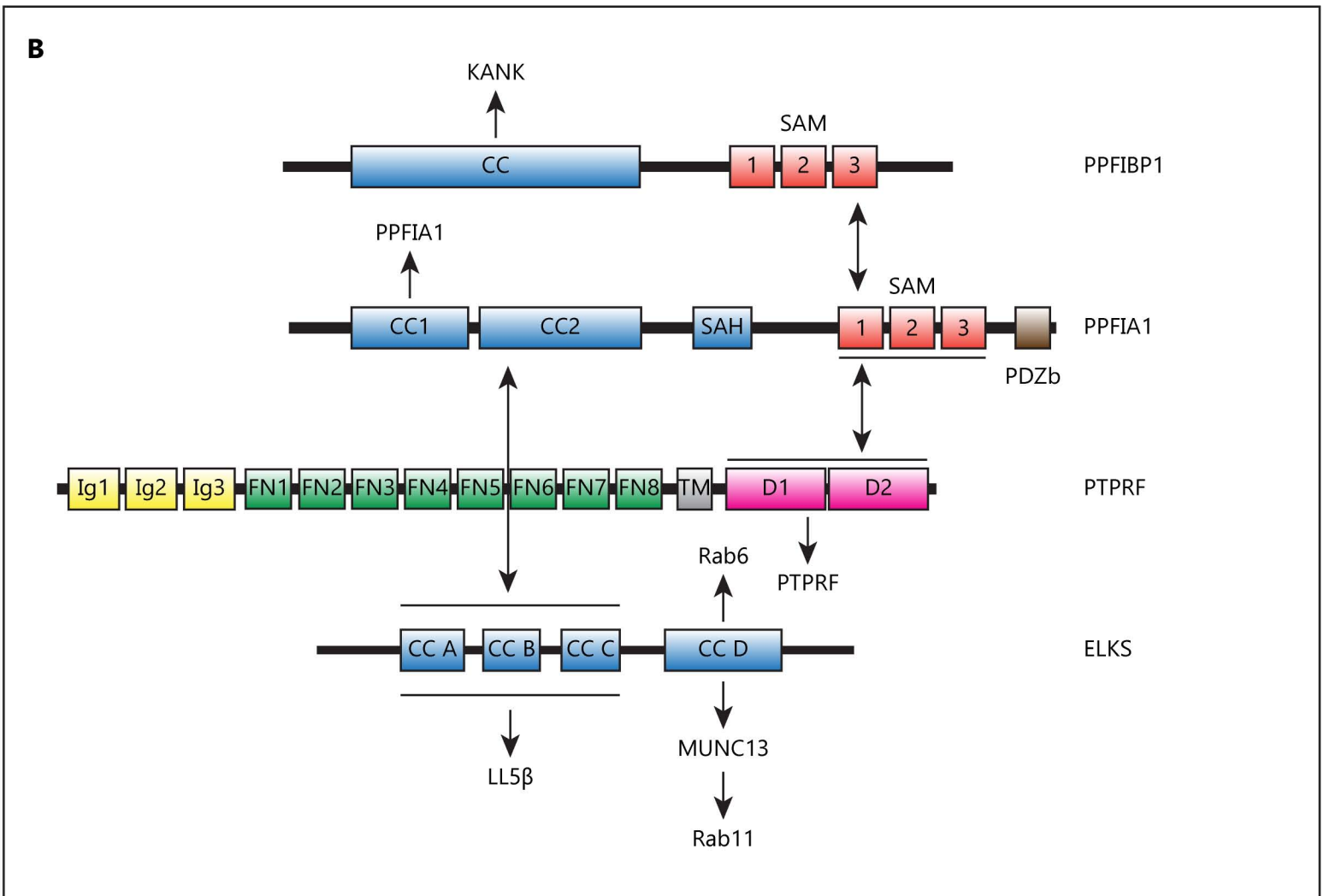
Figure 1. The movement of cargoes relies on cooperating or competing MT -end and +end-directed motors. A. The Dynein-Dynactin complex, with its stabilizing adaptor BICD, drives the MT –end directed motion of cargoes driving retrograde traffic. **B.** Classical Kinesins (such as KIF1, 3, 5) move cargoes towards MT +ends driving their anterograde traffic. **C.** Unconventional Kinesins (such as KIF14) translocate cargoes towards MT –ends driving their retrograde traffic. **D.** Cargo movement results from the combination of those different motor machineries in: a tug-of-war between two motors proceeding in two opposite directions (top drawing), a cooperative agonism between two motors proceeding in the same direction (middle drawing) and a cooperative antagonism between two motors proceeding in the same direction but inhibiting each other (bottom drawing). The shorter green arrow in the bottom drawing highlights the described antagonism. **E.** Distinct MT motor sets drive specific cargo motion in mammalian cells. The motion of larger and more centrally localized late endosomes (LE in blue) relies on the MT –end directed Dynein machinery only, whereas that of small and more dynamic early endosomes (EE in purple) depends on the cooperative antagonism between Dynein and KIFC1. Indeed, the identified dual motor level of motion inhibition maintains EEs in their typical peripheral localization.

Figure 2. ECM secretion and peripheral MT targeting in polarized cells. A. The integrin linked kinase (ILK) adaptor is recruited by basement membrane-bound β1 integrins at adhesion sites and allows the capture and stabilization of non-centrosomal MT +ends, along which polarized apicobasal vesicular cargoes are trafficked. ILK association with the scaffold IQ motif containing GTPase activating protein (IQGAP), which in turn interacts with multiple MT +end tracking proteins (+end TP), effectively ensures MT recruitment at ECM adhesions. Type X collagen-containing $Rab6^{+}$ PGCs are transported along MT tracks to the cell periphery by KIF5B or KIF13B motors and dock *via* glutamine/leucine/lysine/serine-rich protein (ELKS) close to ECM adhesions. Interaction of MT +ends with cortical MT stabilization

complexes is crucial for vesicle targeting. KIF21A and CLIP-associating proteins (CLASPs) stabilize MT +ends and are connected with integrin interacting proteins filamin and talin via a protein complex containing LL5β, PPFIA binding protein 1 (PPFIBP1) and KN motif and ankyrin repeat domain-containing protein (KANK). Protein tyrosine phosphatase receptor type f polypeptide (PTPRF) and its interactor PTPRF interacting protein α1 (PPFIA1) reside at integrin adhesion complexes and tether fibronectin (FN)-loaded, $Rab11B^{+}$ post-Golgi carriers (PGCs), thanks to MUNC13, ELKS and PPFIA1 interaction. **B.** Schematic representation of PPFBP1, PPFIA1, PTPRF and ELKS domains. Black arrows indicate direct interactions between the indicated proteins/protein domains. CC coiled coil domain, SAM sterile-alpha motif domain, SAH single α helical domain, PDZ-b PDZ binding motif, Ig immunoglobulin domain, FN fibronectin type III domain, TM trans-membrane region, D1 phosphatase domain, D2 phosphatase–like domain (catalytically inactive).


A
Cargo
Dynactin
BICD
Dynein
Nucleus
B
Cargo
Kinesin (KIF1,3,5)
Nucleus
C
Cargo
Kinesin (KIF14)
Nucleus
D
Tug-of-war
Cargo
Cargo
Nucleus
Cooperative agonism
Cargo
Cooperative antagonism
E
EE
LE
Dynein
LE
EE
Nucleus
KIF14
Dynein
LE
EE


A
Nucleus
PGCs
KIF13A
FN
Rab11B
KIF5B, KIF13B
COL10
PGCs
Rab6
+end TP
IQGAP
ILK
ELKS
CLASPs
KIF21A
LL5β
KANK
PPFIBP1
Filamin
Talin
Active integrins
Inactive integrins
COL10
PGCs
FN
Rab11B
MUNC13
ELKS
PPFIA1
Active integrins
FN
PTPRF


B
KANK
SAM
CC
1
2
3
PPFIBP1
PPFIA1
SAM
CC1
CC2
SAH
1
2
3
PPFIA1
PDZb
Ig1
Ig2
Ig3
FN1
FN2
FN3
FN4
FN5
FN6
FN7
FN8
TM
D1
D2
PTPRF
Rab6
PTPRF
CC A
CC B
CC C
CC D
ELKS
MUNC13
LL5β
Rab11