Chapter 30. Motor protein assemblies: cargo transport, cytoskeletal anchoring and non-Newtonian viscous drag.

The motors that drive cytoplasmic flux and cargo transport consist of modular assemblies of heavy, intermediate and light chains. There are three major classes of motor protein: Myosin, Dynein and Kinesin. The Myosin and Kinesin heavy chain motor domains (Mhc and Khc) share a common protein fold, while the Dynein (Dhc) fold is unrelated. In general, Mhc and Khc motors deliver plus-end directed (anterograde) power strokes on actin microfilaments and microtubules, respectively. By contrast, Dhc motors deliver minus-end directed (retrograde) power strokes on microtubules [1]. The activity of the motor heads is regulated through their intermediate and light chains, which may be attached to different cargos via linkers such as Klarsicht, or the Dynactin complex [2 3 4]. Transported cargos include RNPs, lipid droplets, endocytotic vesicles and MVBs; while the distribution of mitochondria, Golgi and nuclei are regulated by direct motor coupling and labile cytoplasmic flux. Critically, all three classes of motor may transport cytoskeletal, and motor components, as cargos, allowing rapid cytoplasmic remodelling and cross-regulatory interactions.

In their active configurations the motor assemblies carry twin heads: the leading motor head providing a power stroke, while the trailing head maintains attachment to cytoskeletal filaments. The power stroke is triggered by mechanical stress transmitted from the trailing head, which determines both the motor run-length and cargo transport rate [5 6 7 8 9 10] (Roberts et al. 2013). In addition, some Mhcs may bind microtubules, and some Khcs may bind microfilaments, without providing power strokes; which may facilitate cargo transfer between cytoskeletal filaments [11 12]. Such transient mechanical coupling may also be critical for cytoskeletal filament alignment against the resistance of fluid drag. However, in consequence of the twin-headed clutch mechanism, single motor domains may form immobile, semi-permanent anchors for cytoskeletal filaments, particularly at the cortical periplasm/membrane interface. Cortical anchoring is regulated primarily through the motor light-chain assemblies. For example, the Calmodulin (Cam) light chain may anchor single, inactive Mhcs to FAs and AJs; while catalytically active Mhcs may also assemble as transient, single-headed anchors. In addition, twin Mhc motor assemblies may be coupled to the extracellular matrix through transmembrane linkages, *eg* Mhc/Dystrophin/Dystroglycan. Within the cytoplasmic volume actin filaments may also be coupled by braided microfilament assemblies, carrying multiple double MyoII motor heads, as in syncytial muscles. The periplasmic F-actin cytoskeleton may also template microtubule assembly via Shot/Patronin, see above **22**. Thus, mechanical tension may be transmitted through the cytoplasmic gel via transient motor coupling, with fibrillar molecules aligned against non-Newtonian fluid drag. The critical remodelling components may vary in different cell types, however, none of the motor components can function correctly without all (or most) of the others.

Myosin motors: the major contractile force in most cells is provided by myosin motor assemblies. The single muscle MyoII gene of *Drosophila* (*Mhc*, *CG17927*) encodes 21 protein isoforms; with short, tissue-specific, peptide substitutions [13 14]. Muscle Mhc assembles into braided, bipolar mini-filaments, with multiple motor heads that can template F-actin filament assembly. In contrast, the non-muscle Myo-II, Zipper (Zip) may assemble as a twin-headed motor complex with the Mlc-c essential light chain, which can drive cargo-transport along actin filaments. However, in combination with the Spagetti squash (Sqh) regulatory light chain, Zip/Mlc-c/Sqh braided, bipolar mini-filaments may assemble, like those of syncytial muscles. In this braided configuration, Zip/Mlc-c/Sqh mini-filaments may drive contractile sliding of cytoplasmic F-actin microfilaments [15]. However, the Mlc-c light chain

may also assemble with MyoV, MyoVI, MyosVIIA and Abnormal spindle (Asp) (Franke 2006) with hybrid motor functions. Notably, GDP hydrolysis by Zip may be by triggered by tension transmitted between heterotypic motor heads, or transduced cross separate cytoplasmic filaments [16]. Thus, the tension developed between separated motor assemblies may straighten antiparallel F-actin filaments within the cortical periplasm, given that that outer actin filaments are anchored at the membrane interface. In consequence, Zip function affects many disparate morphogenetic processes, including AJ localisation, L/R (D/V) axis formation, dorsal closure, wound healing, ommatidial rotation and cell lineage restriction [17] [18] [19] [20] [21] [22] [23] [24] [25].

In addition to Zip, several unconventional Myosin heavy chains (uMhcs) contribute to cargo trafficking and the cortical membrane anchoring of F-actin filaments. The fly genome encodes twelve uMhcs (*ck*, *d*, *didum*, *jar*, *Mhcl*, *Myo10A*, *Myo28B*, *Myo31DF*, *Myo61F*, *Myo81F*, *Myo95E* and *NinaC*), most of which form active motor assemblies. Four of these (Myo10A, Ck, Myo28B and Myo81F) also contain microtubule-binding MyTH4-FERM domains, consistent with transient microfilament/microtubule coupling. The MyTH4-FERM domain is characteristic of vertebrate MyoX motors, where it binds α-tubulin, E-cadherin and the microtubule end-binding protein EB1 [26]. *Xenopus* MyoX couples cleavage plane alignment to cortical microfibrils (via RhoA) and directs spindle assembly from cortically-anchored centromeres [27] [28]. The *Drosophila* Myo10A (Myo XV, Sisyphus, Sis) is required for cargo transport of α-tubulin, Eb1, E-cadherin, Bap60, Cpsf160 (Cleavage and polyadenylation factor160) and mitochondria [29]. Sis localises to the tips of filopodia, with an essential function during dorsal closure [29]. MyoVIIA (Crinkled, Ck) is required for normal bristle and hair morphology, auditory transduction and Wg-dependent formation of embryonic denticles [30] [31]. The *Myo81F* gene is embedded in centric heterochromatin and transcribed from a 1.97 mb TU, deletions of which are embryonic lethal. Among the remaining uMhcs: Myo1D (Myo31DF) regulates cortical F-actin assembly, with PPIs including EB1, DAAM (Dishevelled Associated Activator of Morphogenesis) and Ds. Myo1D functions in conjunction with Myo1C (Myo61F) to regulate Par/Cdc42 partitioning, L/R (D/V) asymmetry and E-Cad localisation [32] [33] [34] [35]. MyoV (Didum) regulates Osk partitioning during A/P axial formation in the oocyte (see above, **22**). Notably, MyoV can assemble with either Mlc-c or the Dlc1 light chain (Ctp). By contrast to these active uMhcs, Dachs (D, MyoXX, Myo29D) and NinaC lack catalytic activity and only form single-headed assemblies. Thus, D enhances Zyx/microfilament binding at the periplasmic membrane interface, together with Pk and Ds; with related functions in cell migration and Pr/Dist limb growth [36] [37] [38] [39] [40] [41]. Similarly, the Mhc domain of NinaC may anchor its Kinase domain to the cortical F-actin/membrane interface, where it is required for photoreceptor activity [42].

Motor activity may also be engaged by tension transmitted between separated Mhcs attached to the same cargo. In particular, MyoV can increase Khc microtubule run lengths, and switch cargos between microtubule and actin filaments *in vitro* [11]. In this context, the MyoVI (Jaguar, Jar) is the only *Drosophila* Mhc that may reverse its normal (anterograde) procession and deliver a retrograde power stroke [43]. Jar may form active motor assemblies with Mlc-c and the vesicle adaptor, Dab2 [44]. By contrast, single Jar Mhcs may assemble with the Calmodulin (Cam) light chain to stabilise E-Cad localisation at AJs. Jar function is essential during Hh secretion, organelle transport, the Ap/Ba localisation of Miranda, the remodelling of the cortical blebs in the syncytial blastoderm, asymmetric Par/Cdc42 partitioning and spindle orientation (Mermall, 1995) [45] [46].

Dynein and Kinesin motors: in general, balanced anterograde (Khc) and retrograde (Dhc) motor activities regulate the assembly and maintenance of microtubule filaments, while also controlling cargo transport and cytoplasmic flux. Khc motor assemblies may drive the displacement of labile tubulin filaments, or the stable microtubule arrays in the shafts of cilia and flagella. Khc double-headed motor assemblies may also be linked tail-to-tail through their light chains, to form spoked, double-headed cogwheels. In particular, spoked Khc cogwheels drive the separation of microtubule filaments during spindle assembly, and may transport short microtubule oligomers, together with any attached cargos, along (more stable) microtubule bundles [47]. In particular, the selective removal of anti-parallel α/β Tub-oligomers is essential to establish (and maintain) polarised α/β microfilament bundles, during mitotic spindle assembly and neuronal outgrowth.

The major Khc motor, Kinesin-1, drives cargo transport of mRNAs, proteins and organelles. Kinesin-1 localises to the microtubule (+ end) actin cap, in axonal microtubules, dendrites and neuronal synapses. In combination with Milt and Miro, Khc motors drive the anterograde transport of mitochondria in the oocyte and along neuronal extensions. However, the Khc family in *Drosophila* includes 25 genes with only partially characterised functions. Among these, Kinesin-2 (Klp64D) forms a heterotrimeric motor assembly (Klp64D/Klp68D/Kap3) localised to extending microtubule (+ ends), which delivers a chiral, biased torque at dendritic branch points [48]. Kinesin-5 **(**Klp61F) separates antiparallel microtubule bundles to opposite the spindle poles, in a four-spoked cogwheel configuration [49]. In particular, Klp61F associates with spindle microtubule bundles, with run lengths of about 10 steps before detachment [50], which may favour the trafficking of short, antiparallel α/β Tubulin oligomers. This activity is in opposition to the Klp-14 motor (Non-claret disjunctional, Ncd), which forms the only minus end-directed Khc assembly. Klp-14 drives the right-handed helical sliding of antiparallel microtubules during kinetochore separation (Mitra et al. 2020). In addition, the Cana and Cmet kinetochore Kinesins have critical functions during metaphase chromosomal alignment. Centriole separation is compromised in Klp61F mutants, with the formation of predominantly monopolar mitotic spindles. Klp61F is activated by the Fj kinase and transports Wts (Wnt tumor suppressor) in the Golgi secretion pathway [51]. By contrast, a heterotrimeric cogwheel assembly of Klp64D and Klp68D drives transport of Arm, Dsh, β-Tubulin and α-Tubulin, and acts as a positive regulator of Wnt-TCF signalling [52]. Meanwhile, Hh-signalling components are transported by Klp (Costa, Cos), which also anchors Ci to the cortical periplasm before its proteolytic activation to form a Hh suppressor [53] [54] [55] [56]. Notably RNAi knockdown of MKlp1 (Pavarotti) blocks microtubule sliding, but not asymmetric cargo partitioning along the mitotic spindle [57]. Meanwhile, Klp10A binds EB1, triggers minus-end microtubule depolymerisation and supresses Patronin activity, see above **22**. By contrast Klp59D suppresses both + and – end depolymerisation and antagonises Klp10A activity [58].

Thus, the fibril-coupling and cargo transport functions of Dhcs complement, and antagonise, those of the Khc motor assemblies. Dhc64C transports RNPs, proteins, membrane-bound vesicles, lipid particles, Golgi, and other organelles along actin filaments, in motor assemblies with several different light chains and the Dynactin cargo linker. The Dhc64C/Sw assembly transports *bcd*-, *grk*-, *nos*-, *fs(1)k10*-, *h*-, *osk*-RNPs and the translational suppressor *nos*, in the oocyte. Similarly, the Ap/Ba (radial) localisation of *stardust* and *crumbs* mRNAs is dependent on Dhc64C in the syncytial blastoderm [59] [60]. While in the cellular blastoderm, Dhc64C drives the Ap localisation of *wg*, *ftz*, *h* and *run* mRNAs [61]; and transports Bicoid-D, Vasa, Hairy, Lissencephaly-1, Par3 and Rab5 proteins, in association with Dynactin [62] [4]. The *Drosophila* genome encodes eleven Dlics, including Roadblock,

Roadblock-like and Sw, with overlapping functions in neuronal pathfinding, dendrite morphology and mushroom body development [63].

Summary:

Myosin, Dynein and Kinesin motor assemblies drive cargo transport, cytoplasmic flux and cytoskeletal remodelling. In these double-headed motor assemblies, mechanical tension triggers a power-stroke from the leading motor head; while the trailing head maintains the attachment to cytoskeletal filaments. Single motor heads may also bind to F-actin filaments but are catalytically inactive. In particular, single Mhcs tend to form semi-permanent anchors at the cortical F-actin/cytoplasmic membrane interface. In addition, MyoII may assemble as braided bi-polar microfilaments, with entwined, light chains and multiple motor heads. These MyoII microfilaments can nucleate the assembly of antiparallel F-actin filaments and drive them apart. Similarly, Khcs may drive cargo transport as simple, double-headed motors, or combine tail-to-tail through their light chains, to form spoked cogwheel assemblies. Khc cogwheels drive antiparallel microtubule separation in mitotic spindles and the axonemes of cilia and flagella. In addition, Khcs may remove antiparallel Tubulin oligomers during the assembly, and maintenance, of unipolar microtubule arrays. Mechanical stress may be transmitted from cortically anchored actin filaments, with fibril alignment influenced by non-Newtonian, fluid drag through the cytoplasmic gel. Cytoplasmic remodelling may also be dependent on transient motor-protein coupling between F-actin and Tubulin filaments. Thus, none of these motor protein assemblies function correctly without all (or most) of the others. In particular, the motor protein sub-units may be transported (and recycled) as cargo components along labile cytoplasmic filaments. Thus, the combinatorial interactions between motor proteins and cytoskeletal filaments are self-organising, immensely complex, and only partially characterised. The regulatory algorithms that govern cytoskeletal remodelling, and the cell-cycle progression, have been assembled over geological timescales.

References:

1. Miki, H., Okada, Y. & Hirokawa, N. Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol.* **15**, 467–476 (2005).
2. Haghnia, M. *et al.* Dynactin is required for coordinated bidirectional motility, but not for dynein membrane attachment. *Mol. Biol. Cell* **18**, 2081–2089 (2007).
3. Gaspar, I. *et al.* Klar ensures thermal robustness of oskar localization by restraining RNP motility. *J. Cell Biol.* **206**, 199–215 (2014).
4. Chowdhury, S., Ketcham, S. A., Schroer, T. A. & Lander, G. C. Structural organization of the dynein-dynactin complex bound to microtubules. *Nat. Struct. Mol. Biol.* **22**, 345–347 (2015).
5. Oliver, T., Berg, J. & Cheney, R. Tails of unconventional myosins. *Cell. Mol. Life Sci. CMLS* **56**, 243–257 (1999).
6. Tzolovsky, G., Millo, H., Pathirana, S., Wood, T. & Bownes, M. Identification and phylogenetic analysis of Drosophila melanogaster myosins. *Mol. Biol. Evol.* **19**, 1041–1052 (2002).
7. Yang, Y. *et al.* Dimerized Drosophila myosin VIIa: A processive motor. *Proc. Natl. Acad. Sci.* **103**, 5746–5751 (2006).
8. Mao, Y. *et al.* Dachs: an unconventional myosin that functions downstream of Fat to regulate growth, affinity and gene expression in Drosophila. *Development* **133**, 2539–2551 (2006).

9. Liu, R. *et al.* Sisyphus, the Drosophila myosin XV homolog, traffics within filopodia transporting key sensory and adhesion cargos. *Development* **135**, 53–63 (2008).
10. Trybus, K. M. Myosin V from head to tail. *Cell. Mol. Life Sci. CMLS* **65**, 1378–1389 (2008).
11. Ali, M. Y., Lu, H., Bookwalter, C. S., Warshaw, D. M. & Trybus, K. M. Myosin V and Kinesin act as tethers to enhance each other's processivity. *Proc. Natl. Acad. Sci.* **105**, 4691–4696 (2008).
12. Ally, S., Larson, A. G., Barlan, K., Rice, S. E. & Gelfand, V. I. Opposite-polarity motors activate one another to trigger cargo transport in live cells. *J. Cell Biol.* **187**, 1071–1082 (2009).
13. Kronert, W. A., Dambacher, C. M., Knowles, A. F., Swank, D. M. & Bernstein, S. I. Alternative relay domains of Drosophila melanogaster myosin differentially affect ATPase activity, in vitro motility, myofibril structure and muscle function. *J Mol Biol* **379**, 443–456 (2008).
14. Trujillo, A. S. *et al.* Myosin dilated cardiomyopathy mutation S532P disrupts actomyosin interactions, leading to altered muscle kinetics, reduced locomotion, and cardiac dilation in Drosophila. *Mol. Biol. Cell* **32**, 1690–1706 (2021).
15. Vasquez, C. G., Tworoger, M. & Martin, A. C. Dynamic myosin phosphorylation regulates contractile pulses and tissue integrity during epithelial morphogenesis. *J. Cell Biol.* **206**, 435–450 (2014).
16. Grewe, J. & Schwarz, U. S. Mechanosensitive self-assembly of myosin II minifilaments. *Phys Rev E* **101**, 022402 (2020).
17. Dawes-Hoang, R. E. *et al.* folded gastrulation, cell shape change and the control of myosin localization. *Dev. Camb. Engl.* **132**, 4165–4178 (2005).
18. Doerflinger, H., Benton, R., Torres, I. L., Zwart, M. F. & St Johnston, D. Drosophila anterior-posterior polarity requires actin-dependent PAR-1 recruitment to the oocyte posterior. *Curr. Biol. CB* **16**, 1090–1095 (2006).
19. Fiehler, R. W. & Wolff, T. Drosophila Myosin II, Zipper, is essential for ommatidial rotation. *Dev. Biol.* **310**, 348–362 (2007).
20. Bertet, C., Rauzi, M. & Lecuit, T. Repression of Wasp by JAK/STAT signalling inhibits medial actomyosin network assembly and apical cell constriction in intercalating epithelial cells. *Development* **136**, 4199 (2009).
21. Franke, J. D., Montague, R. A. & Kiehart, D. P. Nonmuscle myosin II is required for cell proliferation, cell sheet adhesion and wing hair morphology during wing morphogenesis. *Dev. Biol.* **345**, 117–132 (2010).
22. Monier, B., Pélissier-Monier, A., Brand, A. H. & Sanson, B. An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in Drosophila embryos. *Nat. Cell Biol.* **12**, 60–69 (2010).
23. Pinheiro, D. *et al.* Transmission of cytokinesis forces via E-cadherin dilution and actomyosin flows. *Nature* **545**, 103 (2017).
24. Streichan, S. J., Lefebvre, M. F., Noll, N., Wieschaus, E. F. & Shraiman, B. I. Global morphogenetic flow is accurately predicted by the spatial distribution of myosin motors. *eLife* **7**, (2018).
25. Doerflinger, H., Zimyanin, V. & St Johnston, D. The Drosophila anterior-posterior axis is polarized by asymmetric myosin activation. *Curr. Biol.* **32**, 374-385.e4 (2022).
26. Yamashita, R. A., Sellers, J. R. & Anderson, J. B. Identification and analysis of the myosin superfamily in Drosophila: a database approach. *J. Muscle Res. Cell Motil.* **21**, 491–505 (2000).
27. Bement, W. M., Benink, H. A. & von Dassow, G. A microtubule-dependent zone of active RhoA during cleavage plane specification. *J. Cell Biol.* **170**, 91–101 (2005).

28. Weber, K. L., Sokac, A. M., Berg, J. S., Cheney, R. E. & Bement, W. M. A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature* **431**, 325–329 (2004).
29. Liu, R. *et al.* Sisyphus, the Drosophila myosin XV homolog, traffics within filopodia transporting key sensory and adhesion cargos. *Development* **135**, 53–63 (2008).
30. Gubb, D., Shelton, M, Roote, J, McGill, S, & Ashburner, M. The genetic analysis of a large transposing element of Drosophila melanogaster. The insertion of a w+ rst+ TE into the ck locus. : 54--64. *Chromosoma 91* 54–64 (1984).
31. Bejsovec, A. & Chao, A. T. crinkled reveals a new role for Wingless signaling in Drosophila denticle formation. *Dev. Camb. Engl.* **139**, 690–698 (2012).
32. Petzoldt, A. G. *et al.* DE-Cadherin regulates unconventional Myosin ID and Myosin IC in Drosophila left-right asymmetry establishment. *Development* **139**, 1874 (2012).
33. Magali et al. Coupling of Apoptosis and L/R patterning controls stepwise organ looping. *Curr. Biol.* **20**, 1773–1778 (2010).
34. Lebreton, G. *et al.* Molecular to organismal chirality is induced by the conserved myosin 1D. *Science* **362**, 949–952 (2018).
35. Chougule, A. *et al.* The Drosophila actin nucleator DAAM is essential for left-right asymmetry. *PLoS Genet.* **16**, e1008758 (2020).
36. Cho, E. *et al.* Delineation of a Fat tumor suppressor pathway. *Nat. Genet.* **38**, 1142 (2006).
37. Franke, J. D., Montague, R. A. & Kiehart, D. P. Nonmuscle myosin II is required for cell proliferation, cell sheet adhesion and wing hair morphology during wing morphogenesis. *Dev. Biol.* **345**, 117–132 (2010).
38. Brittle, A., Thomas, C. & Strutt, D. Planar polarity specification through asymmetric subcellular localization of Fat and Dachsous. *Curr. Biol.* **22**, 907–914 (2012).
39. Cao, Y., White, H. D. & Li, X. Drosophila Myosin-XX functions as an Actin-binding protein to facilitate the interaction between Zyx102 and Actin. *Biochemistry* **53**, 350–360 (2014).
40. Zhang et al.,. The novel SH3 domain protein Dlish/CG10933 mediates fat signaling in Drosophila by binding and regulating Dachs. *elife* (2016).
41. Arata et al.,. Difference in Dachsous Levels between Migrating Cells Coordinates the Direction of Collective Cell Migration. *Dev. Cell Vol. 42 Issue 5 479 - 497e10* **42**, 479–497 (2017).
42. Montell, C. & Rubin, G. M. The Drosophila ninaC locus encodes two photoreceptor cell specific proteins with domains homologous to protein kinases and the myosin heavy chain head. *Cell* **52**, 757–772 (1988).
43. Yu, C. *et al.* Myosin VI Undergoes Cargo-Mediated Dimerization. *Cell* **138**, 537–548 (2009).
44. Altman, D., Goswami, D., Hasson, T., Spudich, J. A. & Mayor, S. Precise positioning of Myosin VI on endocytic vesicles in vivo. *PLoS Biol.* **5**, e210 (2007).
45. Petritsch, C., Tavosanis, G., Turck, C. W., Jan, L. Y. & Jan, Y. N. The Drosophila myosin VI jaguar is required for basal protein targeting and correct spindle orientation in mitotic neuroblasts. *Dev. Cell* **4**, 273–281 (2003).
46. Finan, D., Hartman, M. A. & Spudich, J. A. Proteomics approach to study the functions of Drosophila myosin VI through identification of multiple cargo-binding proteins. *Proc. Natl. Acad. Sci.* **108**, 5566–5571 (2011).
47. Mitra, Aniruddha, M. *et al.* Kinesin-14 motors drive a right-handed helical motion of antiparallel microtubules around each other. *Nat. Commun.* **11**, (2020).
48. Mattie, F. J. *et al.* Directed microtubule growth, +TIPs, and Kinesin-2 are required for uniform microtubule polarity in dendrites. *Curr. Biol.* **20**, 2169–2177 (2010).

49. Sharp, D. J., Yu, K. R., Sisson, J. C., Sullivan, W. & Scholey, J. M. Antagonistic microtubule-sliding motors position mitotic centrosomes in Drosophila early embryos. *Nat. Cell Biol.* **1**, 51–54 (1999).
50. Cheerambathur, D. K., Brust-Mascher, I., Civelekoglu-Scholey, G. & Scholey, J. M. Dynamic partitioning of mitotic kinesin-5 cross-linkers between microtubule-bound and freely diffusing states. *J. Cell Biol.* **182**, 429–436 (2008).
51. Kwon, Y. *et al.* The Hippo signaling pathway interactome. *Science* **342**, 737–740 (2013).
52. Swarup, S. & Verheyen, E. M. Wnt/Wingless Signaling in Drosophila. *Cold Spring Harb. Perspect. Biol.* **4**, a007930 (2012).
53. Sisson, J. C., Ho, K. S., Suyama, K. & Scott, M. P. Costal2, a novel kinesin-related protein in the Hedgehog signaling pathway. *Cell* **90**, 235–245 (1997).
54. Lum, L. *et al.* Hedgehog signal transduction via Smoothened association with a cytoplasmic complex scaffolded by the atypical kinesin, Costal-2. *Mol. Cell* **12**, 1261–1274 (2003).
55. Chen, Y. & Jiang, J. Decoding the phosphorylation code in Hedgehog signal transduction. *Cell Res.* **23**, 186–200 (2013).
56. Shi, Q., Li, S., Jia, J. & Jiang, J. The Hedgehog-induced Smoothened conformational switch assembles a signaling complex that activates Fused by promoting its dimerization and phosphorylation. *Development* **138**, 4219 (2011).
57. McLaughlin, C. N. *et al.* Single-cell transcriptomes of developing and adult olfactory receptor neurons in Drosophila. *eLife* **10**, e63856 (2021).
58. Rath, U. *et al.* The Drosophila Kinesin-13, KLP59D, impacts Pacman- and flux-based chromosome movement. *Mol. Biol. Cell* **20**, 4696–4705 (2009).
59. Horne-Badovinac, S. & Bilder, D. Dynein regulates epithelial polarity and the apical localization of stardust A mRNA. *PLoS Genet.* **4**, e8 (2008).
60. Lerit, D. A. & Gavis, E. R. Transport of germ plasm on astral microtubules directs germ cell development in Drosophila. *Curr. Biol. CB* **21**, 439–448 (2011).
61. Wilkie, G. S. & Davis, I. Drosophila wingless and pair-rule transcripts localize apically by Dynein-mediated transport of RNA particles. *Cell* **105**, 209–219 (2001).
62. Dix, C. I. *et al.* Lissencephaly-1 promotes the recruitment of dynein and dynactin to transported mRNAs. *J. Cell Biol.* **202**, 479–494 (2013).
63. Bowman, A. B. *et al.* Drosophila roadblock and Chlamydomonas Lc7: A conserved family of Dynein-associated proteins involved in axonal transport, flagellar motility, and mitosis. *J. Cell Biol.* **146**, 165–180 (1999).