Review

Cite this article: Röper K. 2020 Microtubules enter centre stage for morphogenesis. *Phil. Trans. R. Soc. B* **375**: 20190557. <http://dx.doi.org/10.1098/rstb.2019.0557>

Accepted: 21 January 2020

One contribution of 15 to a discussion meeting issue 'Contemporary morphogenesis'.

Subject Areas:

developmental biology, cellular biology, biomechanics, genetics

Keywords:

morphogenesis, microtubules, actomyosin, cell shape

Author for correspondence:

Katja Röper
e-mail: kroeper@mrc-lmb.cam.ac.uk

Microtubules enter centre stage for morphogenesis

Katja Röper

MRC-Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Avenue, Cambridge CB2 0QH, UK

KR, 0000-0002-3361-766X

Cell shape changes are key to observable changes at the tissue level during morphogenesis and organ formation. The major driver of cell shape changes in turn is the actin cytoskeleton, both in the form of protrusive linear or branched dynamic networks and in the form of contractile actomyosin. Over the last 20 years, actomyosin has emerged as the major cytoskeletal system that deforms cells in epithelial sheets during morphogenesis. By contrast, the second major cytoskeletal system, microtubules, have so far mostly been assumed to serve 'house-keeping' functions, such as directed transport or cell division, during morphogenetic events. Here, I will reflect on a subset of studies over the last 10 years that have clearly shown a major direct role for the microtubule cytoskeleton in epithelial morphogenesis, suggesting that our focus will need to be widened to give more attention and credit to this cytoskeletal system in playing an active morphogenetic role.

This article is part of a discussion meeting issue 'Contemporary morphogenesis'.

1. House-keeping functions of microtubules that affect morphogenesis

The microtubule cytoskeleton is essential for normal cell function, in providing polarized tracks for transport of many cargoes, including carriers for membrane transport, and in assembling into a bipolar spindle for the faithful segregation of genetic information. As these processes clearly affect morphogenesis by serving such 'house-keeping' functions, microtubules are essential to morphogenesis. Furthermore, recent studies have revealed more specific aspects of such 'house-keeping' functions that are especially critical in supporting morphogenesis. This review does not aim to be exhaustive or complete in covering all aspects of microtubule involvement in morphogenesis, but rather aims to highlight certain aspects of microtubule function.

Trafficking of junctional components along microtubules is one area where a core microtubule function, providing tracks for polarized transport, directly intersects with an important aspect of epithelial morphogenesis: junctional remodelling. During the morphogenesis of the *Drosophila* tracheal system, junctional remodelling and control of E-Cadherin levels is key to the cell rearrangements that drive elongation of the smaller tracheal tubes. When microtubules are disrupted specifically in the tracheal cells only, the junctional levels of E-Cadherin and Par-3 are perturbed, and the cell rearrangements driving elongation are affected [1]. Microtubules in this case are required for the transport of carriers during E-Cadherin biosynthetic delivery as well as apical recycling. Similarly, apical trafficking along microtubules appears to be important during apical constriction of the cells of the salivary gland placode. Here, transported cargoes include E-Cadherin and Par-3, and the apical polarity transmembrane protein Crumbs as well as the ligand Fog [2]. An important function

Figure 1. Functions of microtubules in morphogenesis. (a) Polarized transport along microtubules in *Drosophila* pupal wings drives the planar cell polarity required for wing hair morphogenesis. (b) Cell divisions in the *Drosophila* pupal notum become oriented parallel to the cell's long axis and tricellular junction distribution anisotropy owing to the microtubule force generator Mud, which localizes to the tricellular junction. Oriented cell divisions drive oriented tissue expansion in morphogenesis. (c) A longitudinal non-centrosomal microtubule array helps to recruit and stabilize the apical-medial actomyosin driving apical area shrinkage in the salivary gland placodal cells in the fly embryo. (d) During mesoderm invagination in the fly embryo, an apical microtubule array assists repair of micro-tears in the interconnected apical-medial actomyosin network that spans all ingressing cells via cell-cell junctions. Without microtubules, large tears develop that do not heal. (e) During the formation of the amnioserosa in the fly embryo, the originally columnar epithelial cells transform into elongated squamous cells. This transition is driven by a reorientation of the microtubule cytoskeleton, pushing adherens junctions to elongate the apical area. (f) During germband extension in the fly embryo, apical RhoGEF2 activates the dynamic behaviour of apical-medial actomyosin. RhoGEF2 levels apically are modulated by microtubule plus ends and bound EB1 acting as a sink for RhoGEF2 owing to it binding to EB1. (g) During dorsal fold formation in early gastrulation in *Drosophila* a basal shift in polarity components including Par-1 leads to a basal relocalization of the minus-end-binding protein Patronin, thereby reducing microtubule density in the apical dome that the cells show and thus leading to collapse of this dome in the pre-fold cells.

for non-centrosomal microtubules in E-Cadherin trafficking has also been reported during *Caenorhabditis elegans* embryonic elongation in the epidermis [3].

In a variation of this theme, directed microtubule-based transport plays a crucial role in establishing and maintaining tissue-scale polarity in the form of planar cell polarity (PCP), both in the pupal wing in *Drosophila* and in the somatic follicle cells surrounding the germline in the fly. In pupal wings, PCP orients wing hairs uniformly across the tissue during wing development. Microtubules in pupal wings are required for the directional transport of one of the transmembrane receptors involved in setting up PCP in this tissue, Frizzled (figure 1a). Perturbation of microtubules in the pupal wings leads to a loss of the polarization of the distal components Frizzled and Dishevelled, concomitant with loss of hair polarity, thereby phenocopying loss of Frizzled function [4]. In this system, an initial bias in polarization is required to then polarize the complete microtubule array

[5]. In a related case of PCP depending on microtubules, the somatic follicle cells of the *Drosophila* female gonad undergo a dynamic rotational movement around the developing germline that is also coordinated by PCP. Here, a crucial membrane component is the atypical Cadherin Fat2, which is planar polarized. Fat2 polar localization and follicle cell PCP depend on an intact microtubule cytoskeleton [6].

Thus, transport of junctional cargo that allows junctions to remain dynamic, or that helps to polarize junctional components, is key to many morphogenetic processes, also beyond the examples listed above.

In addition to delineating polarized tracks for transport, microtubules also provide the core structure allowing cell division to occur, the mitotic spindle. Owing to their core role in this process, microtubules have been found to serve as a target in mitosis to influence cell divisions in ways that assist morphogenesis. Mitotic cells usually round up prior to division, and their mitotic shape thus does not reflect interphase cell

shapes. Recent work in the *Drosophila* notum, though, has illustrated that a 'memory' of interphase cells' shapes and connectivity does exist in the form of a cell's tricellular junctions and their positions (figure 1b). Force generators such as the protein Mud are placed at tricellular junctions, where they pull on astral spindle microtubules to orient cell divisions. This allows division orientation to be coupled to interphase shape, to cell elongation and to forces that reflect morphogenetic events [7].

Oriented mitoses or general orientation of proliferation have been shown to assist morphogenesis in various processes, including in the *Drosophila* wing disc [8,9] and the extending germband in the fly embryo [10]. And intriguingly, changes in physical properties of dividing cells have also been shown to assist tissue bending, in the case of the invagination of the cells of tracheal placodes in the *Drosophila* embryo. These cells undergo very defined divisions during the internalization, and the mitotic rounding accelerates tissue buckling [11].

Therefore, in addition to vital functions of microtubules required for cell and tissue survival, such as directed transport and cell division, aspects of these core functions have furthermore been co-opted to influence morphogenetic processes directly.

2. Microtubules and apical constriction: the apical–medial 'hub'

The major driver of cell shape changes is the cortical actomyosin cytoskeleton present in each cell. In epithelial sheets of cells undergoing morphogenesis, actomyosin is usually highly concentrated in the apical region of cells, both near cell–cell adherens junctions, and also in the apical–medial domain of cells, just underlying the free apical plasma membrane. Over the last decade this apical–medial pool of actomyosin has been shown to be highly dynamic, displaying a pulsatile behaviour, and to be crucial for the apical constriction of whole cells or isolated apical cell–cell junctions [12–14].

Actomyosin does not seem to work in isolation, though, as work analysing the invagination of the embryonic salivary glands from a placode in *Drosophila* has shown. In this tissue primordium, a non-centrosomal microtubules cytoskeleton is in fact required for the assembly and/or maintenance of the apical–medial actomyosin pool and hence the apical constriction [15]. The non-centrosomal microtubules in these epithelial cells run the length of the cells and directly abut the apical–medial actomyosin (figure 1c). Disrupting the microtubule cytoskeleton severely alters or abrogates the dynamic pulsatility of the apical–medial actomyosin. Similar mechanisms might be at work in other tissues and organisms, as the microtubule cytoskeleton is also required for the formation of the indentation of the morphogenetic furrow in *Drosophila* larval eye discs [16] and for bottle cell formation and gastrulation in *Xenopus* [17], two processes that also rely on apical constriction initiated by apical–medial actomyosin.

In a variation of the above microtubule/apical–medial actomyosin interplay, recent data illustrate that microtubules also play an important role in *Drosophila* gastrulation during mesoderm invagination. This process requires coordination of apical constriction through a highly dynamic apical–medial actomyosin network linked up across several hundred cells [18]. In the constricting mesodermal cells, microtubules are required to assist the 'healing' and repair of this extended

actomyosin network, in positions where it connects between neighbouring cells at cell–cell junctions and where additive stresses lead to small tears in the network developing (figure 1d). In the wild-type, tears are repaired quickly, whereas in the absence of microtubules, the micro-tears develop into large-scale tears of the interconnected network, eventually leading to tissue and morphogenesis collapse [19].

The influence microtubules exert on apical actomyosin function in different processes illustrates the importance of cytoskeletal crosstalk during morphogenesis of epithelial sheets. Actin–microtubule crosstalk has been studied for many years, though mostly in the context of cell migration in tissue culture cells. The results summarized above illustrate how important such crosstalk is for many more cell- and tissue-level events.

3. Microtubules and cell shape change: mediating force transmission

The requirement for microtubules in the *Drosophila* mesoderm described above, a system in which they assist the efficient repair of a supracellular actomyosin meshwork that itself acts coordinately across many cells [19], is a prime example of microtubules facilitating force transmission during morphogenesis.

Microtubules have also been shown to be crucial for mechanical properties of cells in the morphogenesis of the *Drosophila* pupal wing. As mentioned above, microtubules align in a polarized fashion within the apical region of these cells and are important for PCP [4]. This polarized aligned non-centrosomal microtubule cytoskeleton within the wing cells also allows the cells to bear compressive forces during morphogenesis. Accordingly, the loss of these microtubules leads to cell shortening [20]. Microtubules have previously been shown to be able to bear compressive loads, suggesting this might be a more general function [21].

In part of the *Drosophila* embryonic epidermis, microtubules even take on a more active role in eliciting cell shape change. The cells of the forming amnioserosa in the embryo undergo a process of drastic cell shape change and elongation, going from a columnar epithelium with isotropic apical surfaces to highly squamous and elongated epithelial cells. Growing dynamic microtubules appear to actively push against apical junctions to increase the apical surface, reined in by cortical actin, which prevents precocious elongation (figure 1e) [22].

These results demonstrate that microtubules are not only passive structures involved in maintaining cell shape but actually play crucial functions during tissue morphogenesis, potentially acting as mechanosensors as assessed *in vitro* [23].

4. Microtubules as modulators of Rho/Rok activation

There is a further aspect of the dynamic nature of microtubules that allows them to influence morphogenesis. Microtubule plus ends undergo dynamic growth and shrinkage, termed dynamic instability. Plus ends, owing to structural differences from the rest of the microtubule lattice, also selectively recruit a class of protein, so-called (+)TIPs or end-binding (EB) proteins [24]. These dynamically bind to the growing and shrinking plus end. EB proteins serve as platforms for the recruitment

of a whole host of other proteins [25], and it is in this capacity that they have recently been shown to affect morphogenesis and cell shape changes.

EB1, a classic EB protein, has been shown to localize to dynamic microtubule plus ends in late embryonic epidermal cells in *Drosophila*. In these cells, microtubules are arranged into an aligned non-centrosomal network, with plus ends preferentially oriented towards the cell boundaries facing along the dorsoventral axis. EB1 can bind RhoGEF2 [26], and by binding, it provides a sink and thus depletes RhoGEF2 from nearby membrane locations [27]. In these epidermal cells, RhoGEF2 and downstream activated Rho-kinase usually reduce the mobility of E-Cadherin in the plasma membrane, thereby regulating neighbour exchange directionality during the final patterning and morphogenetic changes that the epidermis undergoes.

Earlier on in *Drosophila* embryonic development, during the process of germband extension, both apical–medial and junctional pools of actomyosin are involved in selective junction shrinkage during neighbour exchanges. A recent study revealed the specific requirement for different RhoGEFs for each pool of actomyosin, with RhoGEF2 controlling activity of the apical–medial pool, downstream of Rho activation via GPCR activation and the hetero-trimeric G-protein component $G_{\alpha_{12/13}}$ [28]. These ectodermal cells show a centrosomally nucleated microtubule cytoskeleton, with microtubule plus ends extending towards the apical and junctional region. RhoGEF2, the authors show, is sequestered via binding to EB1 at microtubule plus tips (figure 1f) and is usually only released to the apical–medial cortex upon $G_{\alpha_{12/13}}$ activation. Microtubule depletion via Colcemid injection leads to ectopic and increased release of RhoGEF2.

Thus, in addition to direct physical effects of the microtubule cytoskeleton as well as the microtubule–actomyosin crosstalk described above, the dynamic behaviour and direct binding of microtubules to important morphogenetic effectors constitutes yet another pathway in which microtubules affect morphogenesis.

5. Microtubules and bending without apical constriction

Interestingly, although as discussed above, tissue bending in many instances is driven by apical constriction of epithelial cells, this is not the sole mechanism that can induce tissue bending. During early gastrulation in the fly embryo, the

formation of the dorsal epidermal folds had previously been shown to be driven by a change in cell height rather than actomyosin activity. This change is initiated by down-regulation of Par-1 and a concomitant basal shift of adherens junctions in the cell forming the folds [29]. Recent data now show that the ensuing cell shortening is due to a repositioning of the microtubule minus-end-binding protein Patronin (figure 1g). Patronin prior to fold formation organizes an apical microtubule network stabilizing the apical dome of the cells through dynein-mediated pushing forces, but basal repositioning leads to weakening and descent of the dome, resulting in the observed cell shortening [30].

Thus, in the case of the forming dorsal folds, microtubule organization and dynamics seem to directly affect physical properties of the cell cortex, akin to functions usually attributed to cortical actomyosin only.

6. Conclusion

The above examples of evidence gathered over the last decade indicate that microtubules perform many important morphogenetic functions beyond core cell biological ones. As such, they deserve a very close look in future studies of morphogenetic processes.

A common theme that has emerged with regard to microtubule function during morphogenesis is the close interplay observed in many cases between actomyosin and the microtubule cytoskeleton. The proteins able to mediate such interplay will be important regulators to focus our studies on in more detail in the future. Finally, the way in which the microtubule cytoskeleton can influence morphogenetic processes depends crucially on the type of microtubule network, and in particular whether it is centrosomally or non-centrosomally generated and organized, and how minus and plus ends of microtubules are arranged within the cells. An understanding of how differently organized arrays are generated will therefore have to be another important focus in future research.

Data accessibility. This article has no additional data.

Competing interests. The author has no competing interests to declare.

Funding. This work is supported by the Medical Research Council (file reference number U105178780).

Acknowledgements. The author apologises to colleagues whose work could not be discussed owing to space limitations. The author thanks Ghislain Gillard for his input on the manuscript.

References

1. Le Droguen PM, Claret S, Guichet A, Brodu V. 2015 Microtubule-dependent apical restriction of recycling endosomes sustains adherens junctions during morphogenesis of the *Drosophila* tracheal system. *Development* **142**, 363–374. (doi:10.1242/dev.113472)
2. Le TP, Chung SY. 2019 Microtubule-dependent protein trafficking promotes apical constriction during tissue invagination. *bioRxiv* 827378. (doi:10.1101/827378)
3. Quintin S *et al.* 2016 Non-centrosomal epidermal microtubules act in parallel to LET-502/ROCK to promote *C. elegans* elongation. *Development* **143**, 160–173. (doi:10.1242/dev.126615)
4. Shimada Y, Yonemura S, Ohkura H, Strutt D, Uemura T. 2006 Polarized transport of Frizzled along the planar microtubule arrays in *Drosophila* wing epithelium. *Dev. Cell* **10**, 209–222. (doi:10.1016/j.devcel.2005.11.016)
5. Matis M, Russler-Germain DA, Hu Q, Tomlin CJ, Axelrod JD. 2014 Microtubules provide directional information for core PCP function. *eLife* **3**, e02893. (doi:10.7554/eLife.02893)
6. Viktorinova I, Dahmann C. 2013 Microtubule polarity predicts direction of egg chamber rotation in *Drosophila*. *Curr. Biol.* **23**, 1472–1477. (doi:10.1016/j.cub.2013.06.014)
7. Bosveld F *et al.* 2016 Epithelial tricellular junctions act as interphase cell shape sensors to orient

- mitosis. *Nature* **530**, 495–498. (doi:10.1038/nature16970)
8. Legoff L, Rouault H, Lecuit T. 2013 A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing *Drosophila* wing disc. *Development* **140**, 4051–4059. (doi:10.1242/dev.090878)
9. Mao Y, Tournier AL, Hoppe A, Kester L, Thompson BJ, Tapon N. 2013 Differential proliferation rates generate patterns of mechanical tension that orient tissue growth. *EMBO J.* **32**, 2790–2803. (doi:10.1038/emboj.2013.197)
10. da Silva SM, Vincent JP. 2007 Oriented cell divisions in the extending germband of *Drosophila*. *Development* **134**, 3049–3054. (doi:10.1242/dev.004911)
11. Kondo T, Hayashi S. 2013 Mitotic cell rounding accelerates epithelial invagination. *Nature* **494**, 125–129. (doi:10.1038/nature11792)
12. Blanchard GB, Etienne J, Gorfinkiel N. 2018 From pulsatile apicomedial contractility to effective epithelial mechanics. *Curr. Opin. Genet. Dev.* **51**, 78–87. (doi:10.1016/j.gde.2018.07.004)
13. Martin AC, Kaschube M, Wieschaus EF. 2009 Pulsed contractions of an actin–myosin network drive apical constriction. *Nature* **457**, 495–499. (doi:10.1038/nature07522)
14. Rauzi M, Lenne PF, Lecuit T. 2010 Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature* **468**, 1110–1114. (doi:10.1038/nature09566)
15. Booth AJ, Blanchard GB, Adams RJ, Röper K. 2014 A dynamic microtubule cytoskeleton directs medial actomyosin function during tube formation. *Dev. Cell* **29**, 562–576. (doi:10.1016/j.devcel.2014.03.023)
16. Corrigan D, Walther RF, Rodriguez L, Fichelson P, Pichaud F. 2007 Hedgehog signaling is a principal inducer of Myosin-II-driven cell ingression in *Drosophila* epithelia. *Dev. Cell* **13**, 730–742. (doi:10.1016/j.devcel.2007.09.015)
17. Lee JY, Harland RM. 2007 Actomyosin contractility and microtubules drive apical constriction in *Xenopus* bottle cells. *Dev. Biol.* **311**, 40–52. (doi:10.1016/j.ydbio.2007.08.010)
18. Yevick HG, Miller PW, Dunkel J, Martin AC. 2019 Structural redundancy in supracellular actomyosin networks enables robust tissue folding. *Dev. Cell* **50**, 586–598. (doi:10.1016/j.devcel.2019.06.015)
19. Ko CS, Tserunyan V, Martin AC. 2019 Microtubules promote intercellular contractile force transmission during tissue folding. *J. Cell Biol.* **218**, 2726–2742. (doi:10.1083/jcb.201902011)
20. Singh A, Saha T, Begemann I, Ricker A, Nusse H, Thorn-Seshold O, Klingauf J, Galic M, Matis M. 2018 Polarized microtubule dynamics directs cell mechanics and coordinates forces during epithelial morphogenesis. *Nat. Cell Biol.* **20**, 1126–1133. (doi:10.1038/s41556-018-0193-1)
21. Brangwynne CP, MacKintosh FC, Kumar S, Geisse NA, Talbot J, Mahadevan L, Parker KK, Ingber DE, Weitz DA. 2006 Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. *J. Cell Biol.* **173**, 733–741. (doi:10.1083/jcb.200601060)
22. Pope KL, Harris TJ. 2008 Control of cell flattening and junctional remodeling during squamous epithelial morphogenesis in *Drosophila*. *Development* **135**, 2227–2238. (doi:10.1242/dev.019802)
23. Hamant O, Inoue D, Bouchez D, Dumais J, Mjolsness E. 2019 Are microtubules tension sensors? *Nat. Commun.* **10**, 2360. (doi:10.1038/s41467-019-10207-y)
24. Reid TA *et al.* 2019 Structural state recognition facilitates tip tracking of EB1 at growing microtubule ends. *eLife* **8**, e48117. (doi:10.7554/elife.48117)
25. Akhmanova A, Steinmetz MO. 2010 Microtubule + TIPs at a glance. *J. Cell Sci.* **123**, 3415–3419. (doi:10.1242/jcs.062414)
26. Rogers SL, Wiedemann U, Hacker U, Turck C, Vale RD. 2004 *Drosophila* RhoGEF2 associates with microtubule plus ends in an EB1-dependent manner. *Curr. Biol.* **14**, 1827–1833. (doi:10.1016/j.cub.2004.09.078)
27. Bulgakova NA, Grigoriev I, Yap AS, Akhmanova A, Brown NH. 2013 Dynamic microtubules produce an asymmetric E-cadherin–Bazooka complex to maintain segment boundaries. *J. Cell Biol.* **201**, 887–901. (doi:10.1083/jcb.201211159)
28. Garcia De Las Bayonas A, Philippe JM, Lellouch AC, Lecuit T. 2019 Distinct RhoGEFs activate apical and junctional contractility under control of G proteins during epithelial morphogenesis. *Curr. Biol.* **29**, 3370–3385. (doi:10.1016/j.cub.2019.08.017)
29. Wang YC, Khan Z, Kaschube M, Wieschaus EF. 2012 Differential positioning of adherens junctions is associated with initiation of epithelial folding. *Nature* **484**, 390–393. (doi:10.1038/nature10938)
30. Takeda M, Sami MM, Wang YC. 2018 A homeostatic apical microtubule network shortens cells for epithelial folding via a basal polarity shift. *Nat. Cell Biol.* **20**, 36–45. (doi:10.1038/s41556-017-0001-3)