The Mechanical Role of Microtubules in Tissue Remodeling

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During morphogenesis, tissues undergo extensive remodeling to get their final shape. Such precise sculpting requires the application of forces generated within cells by the cytoskeleton and transmission of these forces through adhesion molecules within and between neighboring cells. Within individual cells, microtubules together with actomyosin filaments and intermediate filaments form the composite cytoskeleton that controls cell mechanics during tissue rearrangements. While studies have established the importance of actin-based mechanical forces that are coupled via intercellular junctions, relatively little is known about the contribution of other cytoskeletal components such as microtubules to cell mechanics during morphogenesis. In this review the focus is on recent findings, highlighting the direct mechanical role of microtubules beyond its well-established role in trafficking and signaling during tissue formation.

1. Introduction

The contribution of microtubules to cell mechanics, cell-shape changes and force coordination during morphogenesis is poorly understood. This is mainly due to the lack of quantitative approaches to study local microtubule dynamics in cells with high spatial and temporal precision, and the fact that many studies focused on global observation rather than investigating the relationship between physico/mechanical and molecular constituents.\(^1\)\(^-\)\(^4\) To unravel the precise mechanisms harnessed by the microtubule cytoskeleton during tissue development, improved approaches to probe microtubule mechanical properties in vivo with high spatiotemporal resolution are crucial. Recent studies in Drosophila that will be discussed in this review provide for the first-time insights into the intricate relation between microtubule organization, cell mechanics, and tissue level force patterning.

Microtubules are indispensable structural elements in living systems, and their mechanical properties are essential for a vast array of cellular functions, including cell shape, division, motility, intracellular transport, and the generation of diverse cellular shapes. The underpinning for their mechanical resilience is provided by their structure that makes them the most rigid intracellular cytoskeletal filaments. Microtubules are composed of alpha (\(\alpha\)) and beta (\(\beta\)) tubulin heterodimers that are assembled into a cylindrical, hollow structure. The uniform orientation of dimers within the microtubule gives them intrinsic polarity in which the \(\alpha\)-tubulin faces the minus end and \(\beta\) units the plus end. Microtubules are highly dynamic structures that display growth and shrinkage, the kinetics of which is regulated by microtubule-associated proteins (MAPs) and molecular motors.\(^5\)\(^-\)\(^6\) In order to fulfill a large diversity of functions, cells build microtubule arrays that differ in organization and dynamics (reviewed in ref. \([7,8]\)). In general, dividing cells contain radially organized microtubules that are anchored through their minus ends to the centrosome (Figure 1a). In contrast, most differentiated cells carry cell type specific, non-radial arrays (Figure 1b). As microtubules are central for properly execute various essential features in virtually every cell, failure to accurately organize the microtubule cytoskeleton leads to diverse pathologies including genomic instability, cancer, and various degenerative and developmental disorders (reviewed in ref. \([9–13]\)).

2. Microtubule Structure and the “Tubulin Code”

Despite fundamental breakthroughs in the regulation of microtubule dynamics and organization in vitro, the spatio-temporal regulation of microtubules in vivo—in particular during tissue morphogenesis—has remained poorly defined. Diversity in the organization and function of the microtubule cytoskeleton is primarily achieved through variations in the primary sequence of tubulin isotypes.\(^1\)\(^4\) These differences in amino acid sequences between individual tubulin isotypes affect two important aspects of tubulin function: First, they alter the global structure of individual tubulin protein and in consequence global microtubule properties. Second, such changes change the binding affinity of various microtubule associated proteins (MAPs).

Genetic analyses have already provided unequivocal evidence for isotype-specific functions in vivo.\(^1\)\(^5\) For example, the beta-tubulin isotype (\(\beta\)Tub85D) is required for meiosis and axoneme assembly in the Drosophila male germline,\(^1\)\(^6\) while the beta-tubulin isotype \(\beta\)VI (TUBB1) determines the final cell
shape of platelets,[17] and the beta-tubulin isotype βIII (TUBB3) yields neural migration in mice (reviewed in ref. [18]). While the molecular mechanisms of isotype-specific regulation of microtubule function is not fully understood, in vitro studies with purified recombinant tubulin demonstrated isotype specific polymerization dynamics.[19–21] Hence, as microtubule dynamics is essential for basic cellular processes (e.g., division, motility, and differentiation), the regulation of isotype expression directly influences these processes. Indeed, in various cancers the expression of tubulin isotype is altered, contributing to drug resistance and disease aggressiveness.[9]

Another principal feature of α- and β-tubulin variations is that sequence differences can be found predominantly in the C-terminal tails, where most posttranslational modifications occur (reviewed in ref. [22]). Enzymes that introduce posttranslational modifications are important for normal development and perturbation in these proteins have been linked to several diseases (reviewed in ref. [10,23]). This genetically encoded and posttranslational generated heterogeneity at the level of individual tubulin proteins—coined the “tubulin code”—has the potential to modulate structural properties and functions of microtubule such as the binding of MAPs and motor proteins.[24–28] The most common posttranslational modifications include acetylation, glutamylation, glycylation, phosphorylation, and polyamination, which are found at the C-terminal tails of both α and β-tubulin. In addition, detyrosination/tyrosination and Δ2- and Δ3-tubulin deletions occur, which only modify the C-terminal tail of α-tubulin (reviewed in ref. [10,24,28]).

Microtubules in motile cilia and flagella have high levels of glutamylation, acetylation, and glycylation.[29] Muscle microtubules, in contrast, are detyrosinated and acetylated,[30] while neurons contain axon-specific detyrosinated and acetylated microtubules.[31] Currently, we still lack a precise mechanistic understanding of the tubulin code. How exactly biochemical modification relate to microtubule requirements? Glutamylation, detyrosination, and acetylation are all enriched in microtubules subpopulations with long lifetimes that show resistance to drug-induced depolymerization.[12–14] In contrast, acetylation of a-tubulin lysine 40 (K40) increases their mechanical resilience, allowing microtubules to comply with deformative forces without breaking.[35] It was demonstrated that K40 acetylation alters interactions within the microtubule lattice, suggesting that biochemical modification of microtubules could play an important role in modulating global microtubule properties.[36] Moreover, it was demonstrated in cardiomyocyte that detyrosination increases the mechanical resistance to contraction via interactions with the sarcomere, indicating its role in the contraction–relaxation cycle of myocytes.[13,30] Finally, posttranslational modifications that affect binding of MAPs can also change the mechanical properties of microtubules.[28,37–39] Collectively, these studies demonstrate that the tubulin code controls microtubule cytoskeleton at many levels. Yet, how such regulatory mechanisms operate in vivo remains unknown and is a major challenge for the future.

3. Generation of Forces by Microtubules

Recent findings demonstrate that tissue remodeling requires physical forces generated either by the actomyosin or the microtubule cytoskeleton. While actomyosin is a well-established force generator, microtubules have traditionally not been viewed in this role (except during cell division). Yet, physiologically relevant forces are generated by the microtubule cytoskeleton. Microtubules can directly generate forces by polymerization and depolymerization. The addition of a GTP-tubulin dimer or the removal of GDP-tubulin dimer from the microtubule plus end releases free energy (ΔG) on the order of 10 k_BT (corresponds to ≈4 pN) that can be used to perform mechanical work.[40] When a growing microtubule encounters a barrier the addition of new tubulin dimers will exert a pushing force on the barrier, reminiscent of the Brownian ratchet described for actin.[41] Similarly, a depolymerizing microtubule that remains connected to a structure will pull the object (e.g., separations of chromosomes during cell division). It is estimated that a single microtubule (with 13 protofilaments) can generate a force of approximately 50 pN by polymerization or depolymerization,[3,42–44] respectively. This is in stark contrast to earlier studies suggesting that a single microtubule can only generate 4 pN.[45] In this study, microtubule buckling was used to quantify pushing forces against the barrier. While isolated microtubules buckle when compressed by 4 pN, this also limits the maximum force that can be generated by single isolated microtubules. Later studies,
however, showed that microtubules within the cytoplasm are reinforced by the meshwork of other cytoskeletal components, a property that allows them to resist compressive loads up to 100 pN.[46] This would allow to generate theoretical maximum force that is more than ten times higher in living cells. In the case of microtubule crosslinking/bundling, the generated force increases linearly with the microtubule number.[47] Moreover, it was measured that the spindle, a dense radial array of microtubules that is crosslinked with motor and non-motor proteins, can generate forces of up to 700 pN.[43,48,49] Thus, while we still lack precise measurements of forces generated by microtubules in vivo, these studies clearly establish them as relevant, yet understudied force-generating components of the cytoskeleton.

Notably, processive molecular motor proteins associated with microtubules can use the free energy that is released by hydrolysis of ATP to generate forces to push or pull microtubules. The sliding of microtubule pairs was demonstrated for plus end and minus end motor proteins. Kinesin-5 will push apart two microtubules that are aligned antiparallel when walking towards their plus ends.[50] Similar observations were made for the minus end directed motor proteins dynein[51] and members of the kinesin-14 family.[52] Dynein can also pull on microtubule by moving laterally on microtubule lattice, while being attached to the cell cortex, thus generating pulling forces that are crucial for spindle and centrosome positioning in cells.[53] Individual motor proteins generate forces ranging from 1 to 10 pN. Hence, as longer overlaps can bind multiple motor proteins, the final pushing and pulling forces will scale correspondingly.[54] Finally, force generation was also attributed to diffusible microtubule crosslinkers lacking intrinsic motor activity. In this motor independent force-generating mechanism, the diffusion within a confined space between two microtubules generates a pressure that yields microtubule sliding,[55] establishing a rich variety of mechanisms exerting microtubule-based forces via interaction to auxiliary proteins.

Curiously, not only the active generation of forces, but also the mechanical properties of microtubules play a role in cell mechanics. Microtubules have the same elastic modulus as actin filaments (on the order of 1 GPa), however they are 100 times stiffer than actin with a bending rigidity of $\approx 2 \times 10^{-23}$ Nm$^2$.\textsuperscript{[56,57]} This corresponds to a persistence length of the order of millimeters, what makes them crucial structural elements in a vast array of cellular functions. As mentioned earlier, the measured microtubule rigidity in vitro differs significantly to in vivo, since it depends on their diameter. In addition, microtubules contain a varying number of protofilaments, ranging from 8 to 17, which not only changes their diameter, but also alters their stiffness (stiffness scales with diameter).[56-60] Finally, they can also be bundled by other proteins, further increasing their stiffness. For example, for tightly cross-linked bundles that do not slide, the stiffness would scale with the square of the microtubule number.[61] Strikingly, the stiffness of two cross linked microtubules will increase 4-fold, a drastic change that is consistent with a significant contribution of microtubules to cell mechanics.

4. Microtubules in Cell Mechanics

Despite their favorable biophysical properties and their widespread deployment in a large variety of cellular contexts, our understanding of the mechanical contribution of microtubules to tissue formation is only now emerging. In the past years, several studies demonstrated their mechanical contribution to cell mechanics\textsuperscript{[46,62-69]} and a tensegrity model was proposed, suggesting that the ability of microtubules to withstand compression balance tensional forces in vivo (reviewed in ref. [70]). Yet, to exert forces, or resist compression, requires not only rigid microtubules but also a mechanical coupling to the cell boundary. Microtubules generally grow persistently in the cytoplasm, but once they hit the cell cortex they transition from growth to shrinkage. Moreover, microtubule plus ends are sensitive to compression-induced catastrophes, which limits the load bearing capacity necessary for their direct mechanical function.[71] It was thus proposed that catastrophe rescue factors, which promote assembly, could increase stability of microtubule plus ends at load-bearing microtubule sites.[72,73] In vivo, microtubules are indeed associated with a number of plus-end-tracking proteins (+TIPs) that accumulate at the growing end, where they regulate microtubule dynamics in various ways (reviewed in ref.[6,73]). End-binding proteins (EBs), for instance, form a tip-tracking complex that promotes MT dynamics and growth, while cytoplasmic linker proteins (CLIPs) and CLIP-associated proteins (CLASPs) increase microtubule polymerization rates and promote rescues. Similarly, members of the kinesin-4 family,[6] and the recently identified +TIP protein SLAIN2 inhibit catastrophe.[74] These rescue factors are thus a pivotal part of the mechanism controlling microtubule dynamics. Understanding the full extent of their interaction with microtubules will be essential for deciphering the direct role of microtubules in cell mechanics.

5. Microtubules as Driving Force of Epithelial Morphogenesis

Over the last decade, the importance of the actin cytoskeleton in morphogenesis has been shown for many tissues. The picture that has emerged from these studies is that actin, together with myosin, forms contractile arrays that are key constituents of different morphogenetic processes, ranging from epithelial folding to cell intercalation and tissue convergence (reviewed in ref.[75,76]). While these studies have established the importance of actin-based mechanical forces, which are coupled across cell boundaries via intercellular adherens junctions, the role of microtubules in cell shape changes and cell mechanics has only recently emerged.[77-79] In the following section, I focus on recently published works revealing new insights into the mechanical role of microtubules in 2- and 3-dimensional remodeling of epithelial tissues during morphogenesis.

5.1. Microtubule Mechanics is Required for Epithelial Folding

Epithelial folding is one of the fundamental morphogenetic processes that shape organs and tissues (reviewed in ref. [80]). Folding is driven by coordinated cell shape changes, normally starting with the apical constriction, followed by cell shortening and basal expansion. If cells preserve their volume, then these changes cause global folding of the tissue. Both these steps have been
Viodynein was used to further increase microtubule-generated forces. Moreover, they showed that sliding of anti-parallel microtubules is necessary for apical cell shape (Figure 2a). Instead, the authors tested whether microtubules control cell shortening needed for tissue folding. Strikingly, they demonstrated that the cell shape relies on stiff microtubules that generate pushing forces to shape the apical cell surface (Figure 2a). Moreover, they showed that sliding of anti-parallel microtubules via dynemin was used to further increase microtubule-generated forces. Notably, microtubules that line the inner apical side of cells are non-centrosomal microtubules, patterned by the minus-end stabilizing protein Patronin (homolog of the mammalian CAMSAP proteins), which is localized to the apical cortex just prior to emergence of the apical dome. Together, these findings are consistent with the tensile model, whereby microtubule cytoskeleton initially generates forces that shape epithelial cells and consequential remodeling of microtubules that disturbs force balance along the apico-basal axis leading in cell shortening during gastrulation.

Figure 2. Organization of microtubules during epithelial cell reshaping. a) Schematic drawing of the Drosophila gastrula indicating the organization of non-centrosomal meshwork (blue) along the apical/basal axis (A/B) supporting the apical surface of the cell (red arrows). Patronin (green dots), which stabilizes microtubule minus ends, is localized to the apical cortex just prior to emergence of the apical dome. b) Schematic of Drosophila wing epithelium showing patterning of the microtubule cytoskeleton (blue) along the proximal/distal axis (P/D) in developing pupae and the direction of microtubule-based forces (red arrows) from ref. [78].

5.2. Microtubule Mechanics Directs Epithelial Elongation

Remodeling within the plane of the tissue, which is essential for epithelial elongation, is another fundamental morphogenetic process controlled by microtubule dynamics. Tissue elongation can be achieved by different cell behaviors, including cell shape changes, polarized cell intercalation, oriented cell division, and collective cell migration. As above, recent study demonstrate a direct mechanical role of microtubules in this process. Using the developing Drosophila wing the study revealed that patterned non-centrosomal microtubules generate pushing forces to elongate individual cells in a coordinated manner. During pupal wing development, apical junctions become the major non-centrosomal microtubule-organizing center that organize microtubules into a planar polarized apical microtubule network. The junctional microtubule organizing centers are located on the proximal and distal sides of each columnar epithelial cells, and are globally aligned along the evolving proximal-distal (PD) axis (Figure 2b). The resulting trans-cellular microtubule network undergoes coordinated rearrangements comitant with tissue remodeling including cell elongation, suggesting a role in this process. Strikingly, additional experiments demonstrated that during epithelium elongation individual cells stayed mechanically autonomous and did not rely on tissue extrinsic forces for cell shape changes. These findings are relevant, as they demonstrate that forces required for initial cell elongation are generated within individual cells. Interestingly, the distribution and contractility of myosin in these cells is low, like in dorsal cells during gastrulation. Hence, the study revealed that microtubules in these cells are stiff and bear compressive forces that originate from cell-endogenous microtubule polymerization. Together, these results provide evidence that physical forces based on global patterning of microtubules contributes to cell mechanics to coordinate cell elongation during tissue rearrangements.

Yet how are microtubules aligned on the tissue level, a core requirement to coordinate forces during morphogenesis? Notably, patterning of microtubules in the wing tissue depends on the conserved Fat planar cell polarity (Ft–PCP) signaling pathway. The Ft–PCP consists of the atypical cadherins Fat (Ft) and Dachsous (Ds), as well as the Golgi resident protein Four-jointed (Fj). Ft and Ds localize to adherens junctions, where they form transcellular heterodimers. As Fj and Ds are expressed in opposing gradients across tissues, they orient the polarization of Ft–Ds heterodimers along the same tissue axis.
(reviewed in ref. [98–102]). Ft–PCP signaling in wing cells is required for both, polarized nucleation of microtubule minus ends and stabilization of microtubule plus ends at junctions oriented along the extended cell axis.[78] The dual function of the Ft–PCP can be explained by polarized distribution of Ft–Ds heterodimers, where one cadherin acts as a microtubule minus end organizing protein while the other serves as effector of plus end proteins. Importantly, the increased stability of microtubule plus ends at the adherens junctions is a key factor for increased microtubule load bearing capacity, a potential mechanism by which microtubules contribute to cell mechanics.[71] Moreover, Ft–PCP mutant animals, with constant microtubule number but perturbed organization, show impaired cell elongation and an abnormal rounded wing shape.[5,78,103,104] thus further supporting the central role of microtubule mechanics in tissue elongation.

6. Conclusions and Outlook

The last decade led to a sharp rise in evidence for microtubules as central structural components that counter compression,[30,46,62,64–69,77,105] thus contributing to the mechanical state of epithelial tissues.[77,78] Yet, to date our understanding of microtubule mechanics is strongly influenced by insights gained from in vitro models that do not reveal the complexity of mechanisms controlling the interplay between microtubules and their effectors (e.g., motor proteins, MAPs, modification enzymes). Accordingly, a large number of fundamental questions remain unanswered. For instance, while current work has established microtubules as force generators, it does not accommodate the fact that biochemical differences exist between microtubules, leading to different tempor-spatial distributions of microtubule mechanical properties within the same tissue. Moreover, we still lack the knowledge on contribution of MAPs and motor proteins to generation of forces within microtubule cytoskeleton during morphogenesis. Also, how are microtubules organized within cells, and how do they switch between different types of architecture—especially in developing tissues that are still proliferating? How do these mechanisms crosstalk during morphogenesis? For instance, the recent insight into the mechanical properties of the epithelium tissue during Drosophila cellularization revealed that it undergoes microtubule depending softening.[79] Yet how this is achieved remains elusive. These modifications are relevant, as changes in tissue mechanical properties could significantly impact the sequential remodeling of the tissue, as in softer environment the propagation of the strain would be weakened, limiting the deformation to local side and not affecting other process within tissue. Lastly, the hallmark of tissue development is collective cell behavior, yet it remains elusive how the accurate integration of locally produced mechanical forces into a global tissue force pattern is accomplished.[106]

To tackle these fundamental questions requires the continued development of new biological, chemical, and physical approaches that will allow accurate quantification of mechanical parameters of the microtubule cytoskeleton in vivo. While a challenging task, these future studies will provide new concepts that can uncover novel functions for microtubules and explain both normal development and physiology, and disease states where force coordination has gone awry.

Acknowledgements

The author would like to thank the members of the Matis lab for critical reading of the manuscript. This work was supported by funds from the German research foundation DFG (EXC-1003, SPP-1782, MA 6726/3) and from the IZKF (Mat2/019/16).

Conflict of Interest

The author declares no conflict of interest.

Keywords

cell mechanics, cell shape changes, microtubules, tissue development

Received: December 12, 2019
Revised: February 12, 2020
Published online: April 3, 2020

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