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Cardiomyocyte Microtubules: Control of Mechanics, Transport, and Remodeling

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Abstract

Microtubules are essential cytoskeletal elements found in all eukaryotic cells. The structure and composition of microtubules regulate their function, and the dynamic remodeling of the network by posttranslational modifications and microtubule-associated proteins generates diverse populations of microtubules adapted for various contexts. In the cardiomyocyte, the microtubules must accommodate the unique challenges faced by a highly contractile, rigidly structured, and long-lasting cell. Through their canonical trafficking role and positioning of mRNA, proteins, and organelles, microtubules regulate essential cardiomyocyte functions such as electrical activity, calcium handling, protein translation, and growth. In a more specialized role, posttranslationally modified microtubules form load-bearing structures that regulate myocyte mechanics and mechanotransduction. Modified microtubules proliferate in cardiovascular diseases, creating stabilized resistive elements that impede cardiomyocyte contractility and contribute to contractile dysfunction. In this review, we highlight the most exciting new concepts emerging from recent studies into canonical and noncanonical roles of cardiomyocyte microtubules.

Keywords

microtubule; cardiomyocyte; mechanics; trafficking; mRNA localization; mechanotransduction

INTRODUCTION

The cytoskeleton performs essential cellular functions such as the maintenance of cell shape, cell division, and organelle transport. It is comprised of three filament types: actin, microtubules, and intermediate filaments. Studies of actin, the initial cytoskeletal component discovered (1, 2), benefitted from its abundance in muscle and its visually striking, regular organization within myocytes. Less than 10 years after the discovery of actin, the sliding filament model of muscle contraction was proposed (3, 4), solidifying actin's indispensable role in striated muscle.

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Microtubules were discovered in the 1950s by electron microscopy as a component in cilia (5, 6). The ensuing decades of microtubule research were dominated by microtubules' roles in chromosome segregation and in cilia and flagella. Postmitotic cardiomyocytes were excluded from these studies, and nearly 15 years passed after the discovery of tubulin before microtubules were mentioned in relation to the heart (7).

Recent advancements have broadened our understanding of the microtubule network in nonmuscle cells and in the cardiomyocyte alike. This concurrent expansion of knowledge has under-scored both the conserved and specialized functions of microtubules in cardiomyocytes. Canonical functions include structural support, cargo transport, and chromosome segregation (Figure 1a, **subpanel *i***).

The mature cardiomyocyte contains rigidly structured contractile machinery comprised primarily of interdigitating actin and myosin filaments (Figure 1b). In response to changes in Ca^{2+}, the actinomyosin interaction results in shortening and lengthening of the cell during the contractile cycle. Thus, distinct pressures on this cell type (inability to divide, high energy consumption, constant contraction) require microtubules to perform additional specialized functions. In this review, we focus on three such areas. First, we discuss the role of microtubules and microtubule posttranslational modifications (PTMs) in contractile mechanics. Second, we discuss the essential role of microtubules in the localization of mRNA and translational machinery and how this pertains to cardiac growth. Finally, we examine how microtubules contribute to protein, membrane, and organelle transport within the cardiomyocyte. We aim to discuss the most exciting of these functions, rather than to provide an encyclopedic catalog, and to offer a framework for future research.

CARDIOMYOCYTE MICROTUBULE BASICS

Microtubule Network Formation

In all cells, microtubules are composed of obligate heterodimers of similar globular proteins, α- and β-tubulin. These tubulin subunits form a higher order assembly containing 11–14 protofilaments that arrange into a hollow polymer with an outer diameter of roughly 25 nm. Microtubules are nucleated from microtubule organizing centers (MTOCs) that contain γ-tubulin, which serves as a structural template for high efficiency nucleation. In mitotic cells, centrosomal MTOCs continually play a role in nucleating microtubules for the cell's entire lifetime. Yet when cardiomyocytes transform to postmitotic cells shortly after birth, they undergo a concomitant loss of centrosome integrity (8). Adult cardiomyocytes rely solely on noncentrosomal MTOCs decorating the nuclear envelope and associated Golgi and Golgi outposts for nucleation (Figure 1a).

Three distinct, yet overlapping, populations of microtubules exist in the cardiomyocyte. Early studies revealed an interfibrillar network running parallel to the long axis of the cell and closely associated with mitochondria and sarcoplasmic reticulum (SR), and a second dense population of perinuclear microtubules that surround and bridge the nuclei (9). Later research uncovered a third prominent population of cortical microtubules that wrap around the cardiomyocyte along its short axis (Figure 1). Interfibrillar, perinuclear, and cortical

microtubules can diverge in primary functions, tubulin isoforms, PTMs, and binding partners (Figure 1a).

Microtubules are highly dynamic structures. Tubulin dimers hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate (GDP) after addition to the growing microtubule lattice, weakening affinity for adjacent dimers and favoring disassembly (10, 11). This nonequilibrium behavior results in dynamic instability, or stochastic switching between a growing (addition of tubulin dimers) and shrinking (catastrophe, loss of tubulin) phase, primarily at the end emanating from the MTOC [hereafter referred to as the plus-end (12, 13)] (Figure 1c).

Tuning Microtubule Dynamics and Stability

Both extrinsic [microtubule-associated proteins (MAPs) and physical barriers] and intrinsic (tubulin concentration, isoforms, and PTMs) factors control microtubule dynamics, enabling both global and highly local tuning of microtubule growth and shrinkage. The tubulin code—the combination of tubulin isoforms and PTMs—influences the intrinsic biophysical properties of the microtubules and their interactions with associating proteins to confer unique functionality to distinct microtubule populations. In the heart, the most well-studied PTM is detyrosination, where the most C-terminal tyrosine of α-tubulin is removed. This tyrosination cycle is mediated by the enzymes tubulin tyrosine ligase (TTL), which adds the tyrosine to form tyrosinated tubulin, and the carboxypeptidase vasohibin/small vasohibin-binding protein (VASH/SVBP), which removes it (14) (Figure 1d). Detyrosination promotes the load-bearing capabilities of microtubules in the heart and is associated with increased microtubule stability (15), as further discussed below. Interestingly, TUBA4A is the only tubulin isoform translated in its detyrosinated form (16) and is one of the most highly expressed isoforms in the heart (17). Another PTM of interest is acetylation, catalyzed by the enzyme α-tubulin acetyltransferase 1 (ATAT1) within the microtubule lumen (18). Acetylation occurs on stable microtubules, but in turn, it also increases the lifetime of the microtubule by increasing flexibility and its ability to resist damage, which are beneficial properties for a polymer undergoing constant compression and relaxation (19). Many other tubulin PTMs exist, such as glutamylation, glycylation, methylation, phosphorylation, and ubiquitination. Insights into these PTMs are emerging as new biochemical techniques allow for the purification of modified tubulins or detection of endogenous PTMs (20, 21), but nearly all remain understudied in the heart.

In addition to changes to tubulin itself, tubulin-binding partners are potent regulators of microtubule dynamics. MAPs encompass a diverse interactome of proteins that associate with each other and/or the microtubule lattice, promoting or suppressing dynamics through functions such as recruitment of severing enzymes, linking microtubules to other structural elements, or acting directly as microtubule polymerases and depolymerases.

Microtubule Motors

The microtubule motors kinesin and dynein are a subclass of MAPs that form large multisub-unit complexes to transport cargo along microtubules. Microtubule-based transport of essential cargoes such as entire organelles, mRNA, or ribosome subunits is especially

important in large, polarized, or particularly structured cell types, where diffusion is slow and inefficient. The mammalian genome encodes more than 40 kinesins, which primarily facilitate anterograde (plus-end) directed trafficking; far fewer retrograde (minus-end) directed cytoplasmic dyneins exist. Additionally, several adaptor proteins interact with these motors to regulate their function and cargo binding. Notably, dynein requires binding to the essential cofactor dynactin for robust motor function. These motors directly bind to microtubules and convert chemical energy from the hydrolysis of ATP into mechanical work, thereby stepping along the lattice (Figure 1c). As this force is derived from hydrolysis, most single motors are unable to generate more than 10 pN of force (22). Thus, motors work in concert, with multiple kinesins and/or dyneins tethered to a single target to generate sufficient force. Such collective behavior also contributes to achieving cargo specificity and bidirectional movement (23, 24). In addition to directly changing microtubule stability, both microtubule PTMs and structural MAPs can modulate the binding and run length of motors, too, providing yet another layer of regulation (25, 26). Overall, a multitude of regulatory factors control microtubule structure and motor function, of which many require further investigation in the cardiomyocyte.

MICROTUBULES IN CARDIAC MECHANICS AND MECHANOSIGNALING

How does the microtubule network influence the mechanical properties of the cardiomyocyte? The first work to examine microtubules in contractile mechanics came from the lab of George Cooper in the early 1990s (27). Cooper and colleagues found that in cats subjected to chronic pressure overload, there was a stark proliferation of microtubules that impeded both the extent and velocity of myocyte shortening. Follow-up studies over the next decade supported three key conclusions (28): (*a*) microtubules could, in certain contexts, resist myocyte motion; (*b*) the resistance appeared to be primarily viscous (and not elastic) in nature; and (*c*) the degree of resistance was nominal in healthy myocardium and exacerbated in pathological states.

Yet others had difficulty replicating these findings (29, 30), and interest in cardiac microtubules waned. Recent studies utilizing modern tools have reinvigorated this area of study, and novel concepts have emerged regarding the microtubule contribution to myocyte mechanics. Emergent areas of insight include (*a*) the identification of mechanically distinct microtubule populations, (*b*) the rate and length dependencies of cytoskeletal mechanics, and (*c*) the drivers of cytoskeletal remodeling in cardiac pathologies.

Microtubules and the Myofilaments

The mechanical contribution of microtubules is highly context dependent and requires a brief discussion on the cytoarchitecture of the myocyte (Figure 1b). Myocyte contraction occurs by the shortening of sarcomeres, repeating contractile units that contain highly organized arrays of actin and myosin. A sarcomere spans from one protein-rich Z-disc to another, typically a distance of around 2 μm, and sarcomeres are aligned in both series and in parallel to form the myofilaments. During systole, a depolarization-dependent elevation in intracellular [Ca^{2+}] causes myosin to pull on actin, leading to force generation and sarcomere shortening. During diastole, repolarization and a return to basal [Ca^{2+}] causes

myosin to dissociate from actin and myofilament relaxation, which is followed by stretching of the myofilaments during ventricular filling.

The myofilaments are surrounded and supported by the nonsarcomeric cytoskeleton, specifically microtubules and desmin intermediate filaments (Figure 1b). Desmin bundles wrap around the Z-disc and are linked to α-actinin (31), the major structural component of the Z-disc. Interfibrillar microtubules run along the length of the myofilament, often squeezing into the gaps between sarcomeres and adjacent mitochondria. Together, longitudinal microtubules and transverse intermediate filaments form a lattice-like structure that wraps the force-generating myofilaments.

Because microtubules are by far the stiffest cytoskeletal element (32) and are aligned along the axis of myofilament motion, they have the potential to resist myofilament shortening and stretch. When considering the resistance microtubules may provide to systole versus diastole, it is important to note that microtubules behave quite differently upon compression and under tension. Upon compression, microtubules bear compressive loads until they reach a critical force that causes them to buckle (Figure 2a). The critical buckling force thus dictates how much resistance a microtubule provides to a compressive load. In vitro, an isolated microtubule will buckle into a single, long arc under very small loads (~1 pN; Figure 2a, **subpanel *i***), which would offer meaningless resistance to a powerfully contracting myofilament. Yet this critical buckling force can increase by 2–4 orders of magnitude in cells (Figure 2a, **subpanel *iii***). Lateral reinforcement along the microtubule length, typically provided by intermediate filaments, causes compressed microtubules to buckle at short wavelengths, a higher energy configuration than a long arc (Figure 2a, **subpanel *ii***). When combined with a viscous cytosol that resists buckling, this reinforcement can raise critical buckling loads to ~1 nN in densely packed cells (33-35). With a typical cardiomyocyte possessing hundreds of microtubules (15) and myofilaments that produce ~1 μN of force (36), microtubules could theoretically provide sufficient resistance to nearly stall contraction. But this would only occur if microtubules were extensively reinforced and rigidly coupled to the myofilaments, and cardiomyocytes clearly are not stalled! As such, a key determinant of their mechanical contribution is not just how many microtubules are present in the cell, but also how well they are reinforced and coupled to the contractile apparatus.

In diastole, microtubules have greater potential to resist myofilament motion. Microtubules inherently offer greater resistance to stretch than compression, and the tensile forces of diastole are weaker than the compressive forces of systole. Microtubules have an elastic modulus in the gigapascal range (34), orders of magnitude stiffer than a myocyte. As such, a microtubule can straighten, but it will not be stretched by diastolic forces. Thus, again, if microtubules were fixed to a myofilament, tension would escalate with minuscule excursions, and the Frank–Starling mechanism of the heart would be defeated. This does not occur under normal conditions, yet microtubules can still impede myofilament motion—so what is the nature of their relationship?

Mechanically Distinct Microtubule Subsets

In 2016, high spatiotemporal resolution imaging of microtubules in live, beating cardiomyocytes illuminated molecular determinants of microtubule–myofilament coupling (15). First, it was observed that microtubules repeatedly buckle at short wavelengths with consecutive heartbeats (Figure 2b). Importantly, not all microtubules buckle during each contraction, and they buckle at a variety of wavelengths. Yet the majority of buckling microtubules exhibit a wavelength of ~1.65 μm, the length of a single sarcomere in systole, and show inflections at the sarcomere Z-disc. A second population of microtubules buckles at ~3.3 μm, the length between two adjacent sarcomeres. This indicates that in a healthy cardiomyocyte, a population of microtubules is mechanically constrained by Z-disc interactions and that it bears compressive loads during contraction.

Two important determinants of this mechanical behavior have been identified: posttranslational tubulin detyrosination and desmin intermediate filaments. First, detyrosination (Figure 2b) determines both the likelihood that a given microtubule will buckle and the wavelength of buckling. When detyrosination is reduced, tyrosinated microtubules buckle less frequently, and if they do buckle it occurs at longer, nonsarcomeric wavelengths (15). The loss of sarcomeric buckling, combined with the general drop in buckling occurrence, indicates a tyrosination-dependent uncoupling of microtubules from the myofilament. This drops the incident force along the microtubule below the critical buckling force, and these non-load-bearing microtubules offer minimal resistance to myofilament motion. The remaining microtubules that buckle at longer wavelengths also offer less resistance, as this energetically preferred conformation inherently produces a lower buckling force. Combined, tyrosinated microtubules offer less resistance than their detyrosinated counterparts, and sarcomeres shorten more, and more rapidly, when microtubules are tyrosinated (15, 17, 37, 38).

Several pieces of evidence suggest desmin intermediate filaments (Figure 2b) laterally reinforce detyrosinated microtubules and provide at least one source of coupling to the myofilaments. Desmin associates with microtubules in a detyrosination-dependent manner (15), consistent with detyrosination promoting microtubule interaction with intermediate filaments (39). When desmin is absent, microtubule organization at the Z-disc is lost, as well as the detyrosination-dependent influence on myocyte mechanics (15).

While additional binding partners may mediate the interaction between detyrosinated microtubules, desmin, and the myofilaments, this link is likely to be dynamic. As outlined above, if microtubules were rigidly coupled to the myofilaments, they would form the dominant contributor to passive, elastic tension, which is not supported by evidence. Instead, elastic tension largely arises from the sarcomeric spring titin (40), whereas a dynamic cross-link between desmin and detyrosinated microtubules contributes to the viscoelastic (rate-dependent) properties of the myocyte (15, 41).

Rate Dependence of Microtubule Mechanics

Cytoskeletal filaments that interact in a dynamic, on-and-off fashion will confer a viscoelastic resistance to motion. For example, if a meshwork of transiently associating

microtubules and intermediate filaments is slowly strained, individual elements will slip past one another, producing minimal elastic tension. If, however, that same meshwork is rapidly strained, filaments will catch in a cross-linked configuration, providing a resistance to stretch that scales with the velocity of strain (viscoelasticity). If the stretch is held, those filaments will eventually dissociate, and tension will relax to a steady-state level (elasticity). Isolated myofilaments, cardiomyocytes, and myocardial tissue all display viscoelastic responses at diastolic rates of stretch, as illustrated in Figure 2c. The same is true for strain applied along the short (transverse) axis of a myocyte—tension increases robustly as the speed of deformation is increased.

Such experiments have been used to determine the rate dependence of the microtubule contribution to myocyte and myocardial mechanics. At low strain rates (either transverse or longitudinal), which probe myocyte elasticity, depolymerizing microtubules or reducing detyrosination has a modest or negligible effect (17, 37, 42, 43). Yet at physiological rates of strain, disrupting microtubules lowers myocyte tension (see the left side of Figure 2c). Importantly, simply reducing detyrosination confers a similar reduction in viscoelasticity as depolymerizing the entire network, indicating that this subset of microtubules dominates their mechanical contribution. Supporting the model that viscoelasticity arises from cross-linking to intermediate filaments, desmin depletion is sufficient to both reduce viscoelasticity and eliminate its dependence on detyrosination. In brief, speed matters when assessing the microtubule contribution to myocyte mechanics.

Length Dependence of Microtubule Mechanics

Not simply the rate but also the magnitude of strain can influence the microtubule contribution to myocardial mechanics. Multiple factors contribute to myocardial stiffness, including myofilament tension (43), the nonsarcomeric cytoskeleton, titin, and the extracellular matrix (see the right side of Figure 2c). Work from Linke's group (44), Granzier's group (40), and others has elegantly demonstrated how skinned cardiomyocytes (which lack microtubules) show a gradual, nonlinear rise in passive tension attributable to extension of titin springs. In myocardial tissue, passive tension skyrockets as sarcomeres extend beyond 2.2 μm, suggesting that strain stiffening of the extracellular matrix dominates tissue mechanics at long excursions (44).

The length dependence of the microtubule contribution to mechanics has not been well examined in intact cardiomyocytes. This is partly because microtubules are not preserved when muscle is chemically skinned, the most common preparation used for mechanical testing. However, it has been examined in intact myocardial tissue. In pathologically remodeled myocardium, microtubule disruption reduces myocardial tension at physiologic (but not slow) rates of strain (37, 43) (see the left side of Figure 2c). Yet at least in failing human myocardium, the microtubule contribution to diastolic compliance diminished with increasing length. For example, at 5% length change, the low end of the physiological range, microtubule disruption robustly reduced passive tension. Yet at 18% stretch, just beyond the high end of the physiological range, the microtubule contribution was negligible (Figure 2c). Further, the microtubule contribution to diastolic mechanics at large excursions correlated with the extent of tissue fibrosis. In fibrotic myocardium from patients with

heart failure with reduced ejection fraction (HFrEF), the microtubule contribution to tissue viscoelasticity was lost by 18% strain. Yet in minimally fibrotic myocardium from patients with heart failure with preserved ejection fraction (HFpEF), the microtubule contribution was preserved even at large excursions (37). This implies that the extent of fibrosis in a failing heart may predict the contribution that myocyte microtubules confer to diastolic mechanics. Alternatively, this length dependence could be inherent to the cardiomyocyte, and dynamic cytoskeletal cross-links could fail at larger excursions. To discriminate between these hypotheses, the microtubule length dependence must be examined in isolated, intact cardiomyocytes—a challenging yet necessary experiment. Finally, microtubules have only been demonstrated to contribute meaningfully to tissue mechanics in pathologically remodeled myocardium; it remains to be determined if microtubules significantly influence myocardial viscoelasticity in healthy hearts.

Pathological Remodeling of the Cardiomyocyte Cytoskeleton

Studies consistently show that microtubules play a larger mechanical role in pathologically remodeled than in healthy myocardium. Proteomic assessments of human left ventricular tissue demonstrate a marked upregulation of intermediate filaments, microtubule associated proteins, and tubulin isoforms in advanced ischemic, hypertrophic, and dilated cardiomyopathy (17). In failing cardiomyocytes, detyrosinated microtubules occupy roughly twice the intracellular area of a nonfailing myocyte (17) (Figure 2b, **subpanel *ii***). In advanced heart failure, this proliferation of the nonsarcomeric cytoskeleton coincides with a loss in force-generating myofilaments (17, 45, 46), a double hit to mechanical efficiency through increased viscoelastic resistance concurrent with reduced force production.

Yet microtubule proliferation and detyrosination are not restricted to end-stage, failing hearts. Modified microtubules correlate with the severity of aortic stenosis in patients (47), and detyrosination is particularly enriched in myectomy samples from patients with hypertrophic cardiomyopathy associated with sarcomeric mutations (38). Detyrosination is also rapidly induced upon cardiac stress, either upon myocardial infarction (48), pressure overload (49, 50), or adrenergic stress (49).

It is unclear whether an isolated increase in detyrosination, in the absence of broader cytoskeletal remodeling, is sufficient to disrupt myocardial function. What is clearer, however, is that in pathologically remodeled myocardium, reducing detyrosination is sufficient to improve mechanics. In cardiomyocytes from hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), or ischemic cardiomyopathy (ICM) patients, reducing detyrosination increases the velocities of myocyte motion and reduces passive stiffness (17, 37, 51). Reducing detyrosination was also sufficient to normalize elevated transverse stiffness in a rodent model of myocardial infarction (48) and to restore contractile kinetics in a mouse model of HCM caused by sarcomeric mutations (38). These recent, detyrosination-focused studies complement earlier demonstrations of normalized cardiac function upon gross microtubule depolymerization in feline and canine models of pressure overload-induced remodeling (27, 50). Targeting detyrosination surprisingly confers a similar mechanical benefit as gross microtubule depolymerization (17, 41), presumably with less disruption of other cellular functions. As such, it holds significant potential to

improve myocardial mechanics with a more favorable safety profile than gross microtubule disruption. At the time of writing, several studies are underway to assess whether sustained modulation of VASH/SVBP or TTL can safely and stably improve cardiac function in models of heart disease.

Microtubules as Mechano-Responders

What drives the proliferation and posttranslational modification of microtubules in cardiac pathologies? While the reasons are likely multifactorial, the abovementioned studies suggest a common link between elevated mechanical stress and cytoskeletal remodeling. In pathologies characterized by increased myocardial loads, the severity of HCM (38, 52), aortic stenosis (47), and pressure overload (53) positively correlates with microtubule proliferation, detyrosination, and acetylation. This mechano-regulation of microtubules may also be reversible, as mechanical unloading partially reversed tubulin PTMs in both heart failure patients (15) and rats (48).

A plethora of mechanisms could lead to microtubule proliferation in stressed myocardium. These include, but are not limited to, (*a*) transcriptional or translational upregulation of tubulin, (*b*) inherent mechano-responses of the microtubule itself, and (*c*) altered interactions with MAPs or PTMs. Determining the relative contribution of each to remodeling in various stages of cardiac hypertrophy and failure is beyond our current reach, yet worthy of discussion, given the central role of microtubules in these processes.

In regard to transcriptional regulation of tubulin, evidence to date is mixed. First, it is important to note that in heart disease, tubulin transcripts may poorly correlate with tubulin protein. In myocardium from heart failure patients, the majority of tubulin isoforms are decreased at the transcript level (54) yet increased at the level of tubulin protein and polymerized microtubules (17). In a recent study that performed RNA sequencing and ribosomal profiling of cardiac tissue, there was no clear signature of tubulin transcriptional or translational upregulation upon pressure overload in mice (55).

There is better evidence for the mechano-regulation of MAPs that are implicated in microtubule stability. The transcript for MAP4, the predominant cardiac MAP, is robustly induced within hours of ventricular pressure overload (53), and MAP4 upregulation is sufficient to proliferate and stabilize cardiomyocyte microtubules (56). However, it is worth noting that MAP4 association with microtubules is tightly controlled by phosphorylation, and there is evidence for both MAP4 phosphorylation (57) and dephosphorylation (58) upon cardiac stress, with both linked to pathological remodeling.

Furthermore, studies throughout plant and animal biology suggest that the mechanoresponsive behavior of microtubules need not invoke any changes in transcription or translation (59, 60). For example, reconstituted microtubules moving randomly in vitro will align upon an axis of tension and orthogonal to an axis of compression, demonstrating that stress-dependent network remodeling requires minimal components (61).

In an intact cell, a host of posttranslational mechanisms are capable of remodeling the network. Stress-induced detyrosination alters microtubule interactions with a variety of

cellular factors that guide microtubule growth and stability (39, 62). This PTM is induced upon cardiac mechanical stress (53), but through an unclear mechanism. Recent work has more clearly defined how acetylation is both induced through mechanical stress and sufficient to remodel the network. In response to compression exerted on a microtubule wall, tubulin dimers can be displaced from the microtubule lattice (63). This damage not only compromises the flexural rigidity of the microtubules, but it can also increase the accessibility of the acetylating enzyme to lysine residues in the microtubule lumen. Acetylation of microtubules alters protofilament spacing in a manner that increases their ability to bend, but not break, thus protecting mechanically stressed microtubules from fragmentation (64). Further, the sites of lost tubulin on the microtubule lattice can be replaced by circulating, GTP-bound tubulin, a process known as microtubule repair. This and other factors that bind at sites of damage can convert this initial susceptibility into a stabilized patch that limits depolymerization beyond the site of repair (65). Through such a mechanism, damage from compressive stress may counterintuitively improve microtubule resiliency and the resistance they provide to compression, eventually leading to an increase in microtubule density through a bias against depolymerization.

Taken together, a hypothesis emerges for mechanical stress-dependent microtubule remodeling in the heart. Upon an elevation in load, increased detyrosination promotes microtubule interaction with the myofilaments, increasing the proportion of load-bearing, buckling microtubules. Microtubules also experience increased loading due to hypercontractility of the heart, as occurs in response to elevated hemodynamic load or actomyosin contractility. Increased compressive loads on buckling microtubules drive microtubule damage, acetylation, and repair, leading to long-lasting, stabilized microtubules. The intrinsic bias of the repaired network against depolymerization facilitates a gradual increase in microtubule network density. As longer-lasting microtubules are also a preferred substrate for detyrosination and acetylation, this promotes a positive feedback cycle that drives greater proliferation with sustained elevations in load.

Microtubules as Mechanotransducers

Microtubules are not simply reactionary to mechanical stress but also transduce mechanical signals to activate second messenger signaling cascades. X-ROS signaling is one such mechanotransduction pathway that has been well studied in muscle. Initially described in cardiomyocytes (66), mechanical stretch was found to elicit a rapid (<1-s) increase in the NADPH oxidase 2 (NOX2)-dependent production of reactive oxygen species (ROS). An intact microtubule network is required for stretch-dependent ROS production, which in the cardiomyocyte leads to enhanced calcium release from the SR. Mechanotransduction containing the same key elements (rapid, stress-induced microtubule-dependent NOX-2 ROS production), but with different downstream targets, has since been described in skeletal muscle (67), bone (68), and cancer cells (69), suggesting a potentially conserved signaling mechanism. In cardiomyocytes, X-ROS is triggered by direct cell stretch (66), flow-induced shear (70), or substrate deformation (71), each of which may have a component of shear stress sensed by microtubules. Interestingly, the detyrosinated population of microtubules was also found to be essential for mechanotransduction in muscle (72), bone (68), and cancer cells (69), which may reflect their ability to bear and transduce mechanical loads.

A precise mechanism describing how microtubules activate transmembrane NOX2 is lacking. Activity could be mediated through a direct interaction of microtubules with NOX2 subunits such as Rac1 (66, 73) or through indirect shaping of membrane compartments that regulate NOX activity (74, 75). Further, multiple mechanosensitive channels (MSCs) have been implicated in X-ROS and related mechanotransduction in various cell types (67-69, 76), and such channels may directly interact with microtubules (77). Cardiomyocyte MSCs, including multiple isoforms of the transient receptor potential (TRP) channel family, are implicated in X-ROS, and Piezo1 (73) was also recently identified as a key component of X-ROS signaling in the heart. As MSC activity can be both regulated by ROS (67) and promote Ca^{2+} influx that stimulates ROS production (73), MSCs form potent potential amplifiers of ROS and Ca^{2+} signals in response to mechanical stress.

In the heart, a variety of physiologic and pathologic roles have been attributed to such signaling, which is perhaps unsurprising given the promiscuous second messengers involved. Several reports indicate that mechano-chemotransduction may be important for the myocytes' ability to augment Ca^{2+} signaling and contractile function in response to changing mechanical loads (66, 73, 78), or to promote synchronous contraction between neighboring myocytes in maturing myocardium (71, 79). When such mechanotransduction is exacerbated—whether from elevations in detyrosination and NOX2 expression in Duchenne muscular dystrophy (72), elevated Piezo1 or TRPV4 expression in heart disease (73) or aging (76), or simply excessive mechanical stress—altered mechanotransduction contributes to contractile dysfunction, aberrant calcium signaling, and the generation of arrythmias. Taken together, the data to date support a conserved, rapid, microtubule-dependent regulation of ROS and Ca^{2+} signaling in response to mechanical stress. The physiologic and pathologic ramifications of such signaling across cell biology, as well as the biophysical details of stress transmission, require further elucidation.

CONTROL OF RNA DISTRIBUTION AND LOCAL TRANSLATION

A canonical role of microtubules is the transport of cargo throughout the cell. In the latter half of this review, we explore how microtubule-based transport regulates essential cardiomyocyte processes such as membrane remodeling, ion channel trafficking, and cardiomyocyte hypertrophy.

A variety of signaling pathways involved in cardiac hypertrophy have been well defined, with a common end point of increased protein translation linked to growth of the cell. However, it is not immediately apparent how or why a cell would respond to more protein through growth (i.e., rather than increasing its degradation rate to maintain size, for example), and outstanding questions remain about how cardiomyocytes connect increased translation to new sarcomere formation, particularly in space and time. Previous studies established the necessity of microtubules for hypertrophy in vivo, but the mechanism was unclear (80, 81). Recent work demonstrates an essential role for microtubules in spatially coupling increased protein translation to productive growth of the heart through microtubule-based transport of mRNA and ribosomes (82).

mRNA Localization

Cardiomyocytes use multiple mechanisms to specifically localize protein in the cell. This includes canonical pathways of protein transport, and emerging evidence reveals that trafficking of the mRNA itself is a common mechanism to localize protein translation. Three main mechanisms have been proposed to achieve mRNA localization: diffusion-based entrapment, protection from degradation, and active, directed transport using the cytoskeleton. Early work postulated that because the cardiomyocyte is densely packed with rigid sarcomeres, diffusion-based localization may be difficult over long ranges, particularly for lengthy transcripts, and that mRNA may be associated with the cytoskeleton for proper localization (83).

Later studies implicated the cardiac microtubule network in proper mRNA localization, either indirectly through tracking of fluorescent exogenous RNA-binding proteins (RBPs) (84) or directly by relocalization of α-myosin heavy chain mRNA in the absence of microtubules (85). More recent studies have provided unprecedented visualization of this process at a subcellular resolution (82, 86) (Figure 3).

In adult cardiomyocytes, bulk mRNA and specific sarcomeric transcripts exhibit subcellular localization to a region near the Z-disc (87, 88), while mRNA encoding an intercalated disc protein localizes to the intercalated disc (82, 86). Consistent with localized translation, ribosomes and nascent peptides also exhibit a sarcomeric distribution, with additional enrichment at the intercalated discs (82, 86). However, the precise molecular makeup of this translational hotspot is still unclear.

Mechanisms of Subcellular Localization

Active transport along the cytoskeleton alone does not explain how mRNA (and thus, translation) becomes asymmetrically localized in the cardiomyocyte. Additionally, it is important to note that a random distribution of transcripts does not exclude directed transport as a mechanism of localization; in nonmuscle cells, data suggest that even some uniformly distributed transcripts rely on the cytoskeleton for transport (89, 90). Pioneering work in *Drosophila* embryogenesis established the role of the 3′ untranslated regions (UTRs) of some mRNAs as an essential *cis*-regulatory element in establishing proper mRNA localization (91, 92). These zip codes are generally short sequences that reside in the 3′ UTR that RBPs recognize and bind to in order to help direct mRNA in the cell (93, 94) (Figure 3). Moreover, these same RBPs that direct localization often also serve as translational repressors of the transcript, preventing premature translation until properly localized (95). More recent work has indicated that zip codes can also reside in the 5′ UTR or coding region, can be defined by complex secondary structure rather than primary sequence, or can include multiple discrete regions that govern stepwise localization (96-98). Thus, even with computational aid, defining and predicting mRNA zip codes has been challenging, and we still lack a set of predictive rules in any cell type, cardiomyocytes included.

Yet zip codes are not the only way cells can direct mRNA localization in the cell. Any modification that changes microtubule transport in general, such as changes to motors,

RBPs, or to the microtubule network itself, could conceivably alter mRNA transport. In nonmuscle cells, detyrosination of the microtubule network is sufficient to change mRNA localization (99), and for specific transcripts, localization depends on a minor subset of plus-end-oriented microtubules at the cortex (100). In neonatal cardiomyocytes, overexpression of MAP4 inhibits mRNA distribution throughout the cell (84). As such, there are likely many levels of integrated regulation to ensure proper mRNA localization in the heart. Few studies (with mixed results) have tested whether 3′ UTRs of mRNA in the cardiomyocyte govern localization (86, 101), and none have aimed to systematically define zip codes, including regions outside of the 3′ UTR, an important future area of study.

Regardless of the uncertainties surrounding zip codes, localization of mRNA appears to be the rule, rather than the exception. In *Drosophila* embryos, over 70% of transcripts detected using an in situ hybridization screen displayed subcellular localization (102), yet no studies have attempted a comprehensive view of the spatial organization of all mRNA in the cardiomyocyte. Recent developments in sequencing technology directly ligating biotin to mRNA have enabled the creation of high-throughput, high-resolution spatial atlases (103), which could be applied to the cardiomyocyte.

Local Translation and Cardiac Hypertrophy

Why might a cell choose to delay translation and invest in mRNA localization? Local translation could confer multiple advantages: reducing transport cost by translating multiple proteins from one transcript, spatially and temporally restricting a protein's function, and facilitating the assembly of multiprotein complexes through high local concentrations (104, 105). Vimentin, tropomyosin, titin, and α-myosin heavy chain all undergo some degree of cotranslational assembly in striated muscle, indicating that muscle cells already utilize this last mechanism to build higher-order structures (106-109). This mechanism could be useful for sarcomere formation, which has been proposed to occur through the assembly of preformed multimeric complexes rather than sequential assembly (110, 111) (Figure 3). In addition to assisting structure assembly, a constant pool of mRNA at sites of translation may help overcome nonstoichiometric amounts of sarcomeric mRNA, or so-called bursty transcription; the levels of sarcomeric mRNA in cardiomyocytes were recently found to be highly variable between cells (86, 112).

How and where new sarcomeres form in the cardiomyocyte are open questions (113-116). New sarcomere formation is believed to occur at discrete locations, suggesting that at least some components of the hypertrophic pathway must be specifically localized. Subcellular localization of mRNA appears to be a likely candidate for how cardiomyocytes might initiate sites of new sarcomere addition. In chick embryo fibroblasts, β-actin mRNA is highly localized to the leading edge, resulting in increased actin synthesis needed for cell motility; disruption of β-actin localization is sufficient to reduce cell motility, providing a link between mRNA localization and adaptive morphological change (117). Recent experiments in aligned, neonatal cardiomyocytes show that α-cardiac actin mRNA movement to the cell ends after a hypertrophic stimulus; this redistribution (as well as growth of the cardiomyocyte) requires microtubules (82). Moreover, kinesin-1 expression is increased upon hypertrophic stimulation in vitro (82) and in vivo (118), consistent with

the observed redistribution of mRNA under such conditions. Depletion of kinesin-1 is sufficient to prevent remodeling of neonatal cardiomyocytes, indicating that kinesin-based transport may play an important role in cardiac hypertrophy. In summary, microtubules appear to act as master regulators determining where translation and remodeling occur within cardiomyocytes. Many aspects of this regulation remain unknown and should serve as an exciting area of inquiry in the coming years.

Though not discussed in this review, both the ubiquitin-proteasome and autophagy pathways contribute meaningfully to the regulation of sarcomere remodeling and growth (for comprehensive discussion, see 119, 120). Cardiac microtubules mediate autophagy by transporting autophagosomes to lysosomes for fusion (for a review, see 42) and likely play an important role in regulating protein quality control during development and in disease.

REGULATION OF VESICLE TRANSPORT AND MEMBRANE REMODELING

Directed Trafficking of Cardiac Ion Channels

Cardiomyocytes are electrically driven force generators that are stimulated by a propagating action potential through membrane invaginations called t-tubules to drive calcium entry into the cell and induce calcium release from the SR. Elevated intracellular calcium causes the myofilaments to generate the force necessary to pump blood during systole, and the myofilaments will subsequently relax as calcium is pumped back into the SR. This process of excitation-contraction coupling occurs with each heartbeat and requires a complex, organized membrane system that concentrates macromolecular protein complexes within restricted domains to spatially control various processes. A separate membrane domain exists at the intercalated discs to mechanically and electrically couple adjacent cardiomyocytes to ensure that the action potential propagates throughout the myocardium. Microtubules play an important role in not only concentrating specific proteins to t-tubules and intercalated discs but also organizing the structure of the SR and sarcolemmal membranes themselves.

Microtubules regulate the cardiac action potential by modulating the surface expression of ion channels. Many ion channels have a half-life of a few hours, suggesting that there is a continual flux of channels arriving at and leaving from the surface membrane. The population of functional ion channels that are expressed on the sarcolemmal membrane is dynamic and can rapidly or chronically change in various contexts (e.g., exercise, pathophysiology). Many ion channels form macromolecular complexes targeted to specific sarcolemmal membrane domains and, as expected, disruption of trafficking to their respective domains can alter the electrical properties of cardiomyocytes and are often associated with arrhythmias (121-123). Microtubules form the infrastructure that directs the localization (and thereby function) of ion channels, ensuring the well-controlled rhythmic electrical activity of cardiomyocytes.

In contrast to cytosolic proteins, transmembrane proteins require specialized translational machinery on the endoplasmic reticulum (ER). Sarcolemmal ion channels are synthesized at the rough endoplasmic reticulum (RER) where an ER membrane-bound ribosome extrudes the nascent protein into the ER membrane through a translocon complex. The location and

structure of the cardiomyocyte RER is unclear and generally presumed to exist within the perinuclear region, although distal portions of the SR may represent an elusive component of the rough ER (124), similar to that observed in skeletal muscle (125), which would suggest that mRNA localization and localized translation would apply to a broader range of cardiomyocyte proteins beyond those translated in the cytoplasm. Upon proper folding, these nascent ion channels are sorted at the ER exit site where they are packaged into vesicles and transported to the Golgi. Vesicles containing these proteins are trafficked along microtubules from the Golgi to the sarcolemmal membrane for exocytosis (Figure 4a). The proportion of proteins that rely on mRNA trafficking versus protein transport to achieve proper localization, and the determinants that govern which pathway is utilized, remain undetermined in cardiomyocytes.

Trafficking of the voltage-gated potassium channel Kv1.5, which underlies the ultrarapid delayed rectifier current (I_{Kur}) that facilitates cardiomyocyte repolarization, is a well-characterized system that highlights the general principles of ion channel trafficking in cardiomyocytes (reviewed in 126). The surface expression of Kv1.5 is controlled by a balance of microtubule-dependent anterograde and retrograde trafficking (127). The current density of Kv1.5 is strongly augmented in heterologous cells overexpressing kinesin-1, and a dominant negative kinesin-1 blocks surface expression, suggesting that anterograde transport is mediated through kinesin-1 (128). During retrograde transport, Kv1.5 is internalized through clathrin-mediated endocytosis that is dependent on a direct interaction between the N-terminal SH3 domain of Kv1.5 and dynein (129). Disruption of either microtubules or the dynein–dynactin complex prevents internalization of Kv1.5, resulting in a significant augmentation of I_{Kur}. Generally, dynein-based retrograde trafficking appears to underlie recycling of several potassium channels in heterologous cells, as dynein inhibition leads to an increased surface expression of Kv2.1, Kv3.1, hERG, and Kir2.1 (130). Global microtubule disruption in cardiomyocytes leads to a profound decrease in the inward rectifier current (I_{K1}) in contrast to the augmentation of I_{Kur}, suggesting that different ion channels have different rates of anterograde and retrograde flux to and from the surface membrane. Surprisingly little is known about how microtubule-dependent trafficking of transmembrane proteins is influenced by the changes in MAPs or PTMs commonly observed in disease, an area ripe for future study.

Microtubule Search and Capture

How do microtubules determine where to lay the tracks for directed trafficking? Kirschner & Mitchison's (131) search and capture mechanism proposed that the inherent dynamic instability of microtubules allows for the growing end to search for interacting partners (132). Failure to find a stabilizing interaction results in catastrophe and growth that continue the search process, while a successful interaction with a binding partner stabilizes the microtubule. This process results in specific microtubule tracks that are directed to a specific destination (e.g., the kinetochore binding to microtubule bundles during mitosis). In postmitotic cardiomyocytes, the same basic principle drives the stabilization of microtubules at various sarcolemmal membrane domains to deliver sorted cargoes to their appropriate destinations (summarized in Figure 4b). Microtubules growing from noncentrosomal MTOCs have a plus-end that extends toward the sarcolemmal membrane. Various plus-end

binding proteins interact with targets at the membrane to stabilize microtubule tracks to the intercalated disc or t-tubules. Shaw et al. (133) initially proposed the targeted delivery model of connexin 43 (Cx43) trafficking to the intercalated disc, which has now been expanded to directed trafficking of the L-type calcium channels (LTCCs) to t-tubules as well (134).

At the intercalated disc, several targets likely bind the plus-end tracking protein EB1. The plus-end of the microtubule is thought to be anchored to the adherens junction via interaction with cadherin, its cytoplasmic binding partner β-catenin, dynein, and EB1 binding p150(glued), a subunit of the dynein/dynactin complex (133). Disruption of any of these interactions abolishes the accumulation of Cx43 at cell–cell junctions, suggesting the microtubule anchoring at the adherens junctions is required for targeting specific proteins to the intercalated disc. Similarly, the N terminus of the desmosomal protein desmoplakin can also interact with EB1 and stabilizes microtubules at desmoplakin hotspots (135). Mutations in this region of desmoplakin are associated with arrhythmogenic cardiomyopathy and cause defective trafficking of Cx43 to gap junctions. Interestingly, Cx43 itself may be an important regulator of directed trafficking of Nav1.5 to the intercalated disc. A C-terminal deletion of Cx43, which did not affect Cx43 localization to the intercalated disc, reduced the size of EB1 plaques at the intercalated disc and reduced the localization of Nav1.5 trafficking to the intercalated disc (136). These results raise the intriguing possibility that the formation of tracks for various intercalated disc cargoes follows a sequential assembly model, whereby the efficient trafficking of one protein is dependent on the targeted accumulation of a separate predecessor.

At the t-tubular membrane, the Bin/amphiphysin/Rvs (BAR) domain protein Bin1 is thought to anchor microtubules to direct trafficking of LTCCs (134). Compared to microtubules in other parts of the cell, a greater proportion of microtubules associated with Bin1 are paused and display a significantly reduced velocity, suggesting stabilization of dynamic microtubules at the Bin1 concentrated domains. Importantly, surface expression of Cav1.2 is correlated with Bin1 expression (134). The molecular components that underlie the interaction between Bin1 with microtubules has not been determined in cardiomyocytes, although cytoplasmic linker protein 170 (CLIP-170) has been proposed as a reasonable candidate (137) due to its direct binding to Bin1 (138, 139).

Sarcoplasmic Reticulum and T-Tubule Remodeling

In addition to the delivery of smaller cargo-containing vesicles, microtubules may direct the broader reorganization of the membrane systems critical for Ca^{2+} handling. The SR and t-tubule networks are dynamic structures that form stabilized dyads to support rapid, synchronized Ca^{2+} release (and thus contraction) in cardiomyocytes.

In cardiomyocytes, the SR forms a cross-striated network of contiguous membranes that extends throughout the cell. The SR has several structural domains, including the network SR that surrounds myofibrils, junctional SR that closely apposes the t-tubule membranes to form dyads, corbular SR boutons, and the terminal cisternae where the SR network ends near the I-band and Z-disc. The structure of the SR is dynamic, consistent with the dynamic microtubule-driven tubulation of ER membranes observed in other cell types (140, 141). The Santana lab (142) observed that SR boutons travel with an average speed of ~0.02 μm/s,

consistent with microtubule-based motility. Similar dynamic movement of the junctional SR was observed by tracking fluorescently tagged triadin (143). Indeed, disruption of kinesin and knockdown of dynein both reduce the dynamic SR movement, supporting the notion that microtubules are involved in reshaping the SR (142, 143). In skeletal muscle, the SR transmembrane protein CLIMP-63 can bind the junctional SR protein triadin through its luminal domain and link to microtubules through its cytosolic domain (144). This interaction presents a plausible mechanism to allow for junctional SR to be anchored to microtubules to shape the terminal ends of the SR. Furthermore, the disruption of microtubule-dependent SR remodeling may have consequences for calcium handling, as expression of dominant negative kinesin-1 results in a decreased Ca^{2+} transient amplitude. The authors suggest that the mobile jSR may represent the biogenesis of dyads prior to the stabilization by structural proteins such as junctophilin 2 (JP2).

Proper localization of the junctional SR is critical for the stabilization of t-tubules. JP2 is a junctional SR transmembrane protein with N-terminal domains that bind the sarcolemmal membrane and LTCCs to form a structural link between the SR and t-tubule membranes (145, 146). Dyad formation is disrupted in various forms of cardiovascular disease and frequently manifests as t-tubule disorganization and loss of t-tubule segments (for reviews, see 147, 148). The loss of dyad structure results in orphaned ryanodine receptors, dyssynchronous Ca^{2+} release, and weakened contractions (149). T-tubule loss shows a strong correlation with JP2 mislocalization and, importantly, knockdown of JP2 leads to the disruption of t-tubule organization, calcium handling defects, and contractile dysfunction (150), demonstrating the importance of a stabilized dyad in maintaining the overall organization of the Ca^{2+} handling network.

The disruption of the cardiomyocyte t-tubule network may in part be due to the microtubule densification that occurs during decompensated hypertrophy and heart failure. In pressure overload-induced heart failure in mice, colchicine treatment could preserve t-tubule organization and improve cardiac function (151). The loss of t-tubules in this model is correlated with a disruption of JP2 localization. A similar maintenance of t-tubule organization and JP2 expression were observed with colchicine treatment in a model of monocrotaline-induced pulmonary arterial hypertension and right ventricular dysfunction (152). In cultured cardiomyocytes, which show a gradual loss of t-tubules over 2–3 days in culture, microtubule destabilization preserved while microtubule stabilization accelerated the disruption of t-tubule organization (151, 152), again demonstrating a correlation between microtubule density and t-tubule loss. Interestingly, JP2 demonstrates a preference for accumulating at the cell periphery under conditions when microtubules are stabilized and a t-tubule localization when microtubule depolymerization occurs (151). It remains unclear how microtubules regulate the differential localization of JP2 and whether this process plays a critical role in vivo.

T-tubule biogenesis is a critical process that occurs during development and likely hypertrophy. T-tubules that are lost during pathological conditions are not permanently lost, as removal of the underlying mechanical stress (153-155) leads to recovery of t-tubule density. This t-tubule regeneration raises the possibility that promoting t-tubule biogenesis may be a viable therapeutic strategy to improve cardiac function, although the

mechanisms underlying biogenesis remain poorly understood. Microtubules may directly regulate de novo biogenesis of t-tubules. In cell types that lack t-tubules, tubulated surface membrane invaginations can be formed with exogenous expression of Bin1 (156). An intact microtubule network is necessary to stabilize these tubulated membranes, as nocodazole treatment leads to the vesiculation of Bin1-induced membrane tubules (157). The microtubule plus-end associated protein CLIP-170 directly interacts with Bin1, and knockdown of CLIP-170 can significantly disrupt the ability of Bin1 to form tubules (138), suggesting that microtubule polymerization may drive the formation of these tubulated membranes. Live imaging of t-tubule formation in zebrafish skeletal muscle demonstrated that t-tubules grow perpendicularly inward from the lateral membrane (158). During formation, these nascent t-tubules are associated with Bin1 along with many endocytic markers (such as Cavin, Cav3, EHD1, and dynamin), leading to the endocytic capture model of t-tubule formation in which the sarcolemmal membrane undergoes incomplete endocytosis and the resulting tubulated membrane is stabilized by an as-yet-unknown interaction at the sarcomere. Although the force that pulls the sarcolemmal membrane remains undetermined in striated muscle, microtubules form a putative candidate driving this initial tubulation during t-tubule biogenesis.

Taken together, microtubules play essential transport roles necessary to maintain cardiomyocyte homeostasis and respond to stimuli. Hypertrophic growth is directed by mRNA trafficking to sites of active translation, and electrical remodeling is dictated by the transport of the active ion channels expressed on the sarcolemmal membrane or by the restructuring of membranous organelles critical for excitation-contraction coupling itself. Many of the specific details that underlie microtubule regulation in cardiomyocytes remain unexamined, and microtubule regulation represents an exciting frontier of cardiomyocyte biology open for novel discoveries.

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Compression:

a pushing force, as occurs during systole

Tension:

a pulling force, as occurs during diastole

Strain:

change in length relative to the initial length

Viscoelasticity:

rate dependent on stiffness

Elasticity:

rate independent of stiffness

Stiffness:

stress divided by strain, which describes the rigidity of a material in response to a deformation

Stress:

force per unit cross-sectional area

SUMMARY POINTS

1. Microtubules are hollow tubes formed from the polymerization of tubulin heterodimers from microtubule organizing centers that are distributed throughout the cardiomyocyte.
2. The microtubule network is dynamic, undergoing periods of growth and shrinkage, and the stability and function of microtubules are dependent on posttranslational modifications and microtubule-associated proteins (MAPs).
3. Microtubule proliferation and detyrosination are prevalent in cardiovascular diseases. Generally, mechanical stress is associated with posttranslational modifications and MAP decoration that increase the stability of microtubules.
4. Detyrosinated microtubules associate with desmin intermediate filaments and confer viscoelastic resistance to motion in a rate- and length-dependent manner. While resistance is modest in healthy cells, it increases significantly in disease and presents an attractive therapeutic target to improve cardiac function.
5. Microtubules control mRNA and ribosome transport through densely packed cardiomyocytes to coordinate local translation and drive cardiac growth.
6. Transport of ion channels on microtubules regulates the cardiac action potential and susceptibility to arrhythmias.
7. Anchoring of microtubules to macromolecular complexes on the specialized membrane systems of the cardiomyocyte leads to the formation of stabilized tracks that deliver specific cargo to those membrane domains. Similar microtubule interactions can also drive remodeling of the sarcoplasmic reticulum (SR) and t-tubule membrane systems.

FUTURE ISSUES

1. How do microtubules influence the full length-tension relationship of healthy cardiomyocytes and myocardium?
2. What leads to increased microtubule density in the diseased heart? Assuming multiple mechanisms, do the relative contributions vary by type of heart disease?
3. What percentage of transcripts in the cardiomyocyte are localized? What are the molecular determinants of translational hotspots throughout the cell?
4. How do cardiomyocyte microtubules direct specific mRNA to specific locations in the cell? Are these mechanisms changed in heart disease?
5. How do microtubules coordinate the sorting and trafficking of different ion channel complexes? To what extent do MAPs contribute to this specificity?
6. How do microtubules regulate localization of junctophilin-2 and contribute to both SR and t-tubule structure and function? Could targeting microtubules prevent adverse t-tubule remodeling and defective excitation-contraction coupling in disease?

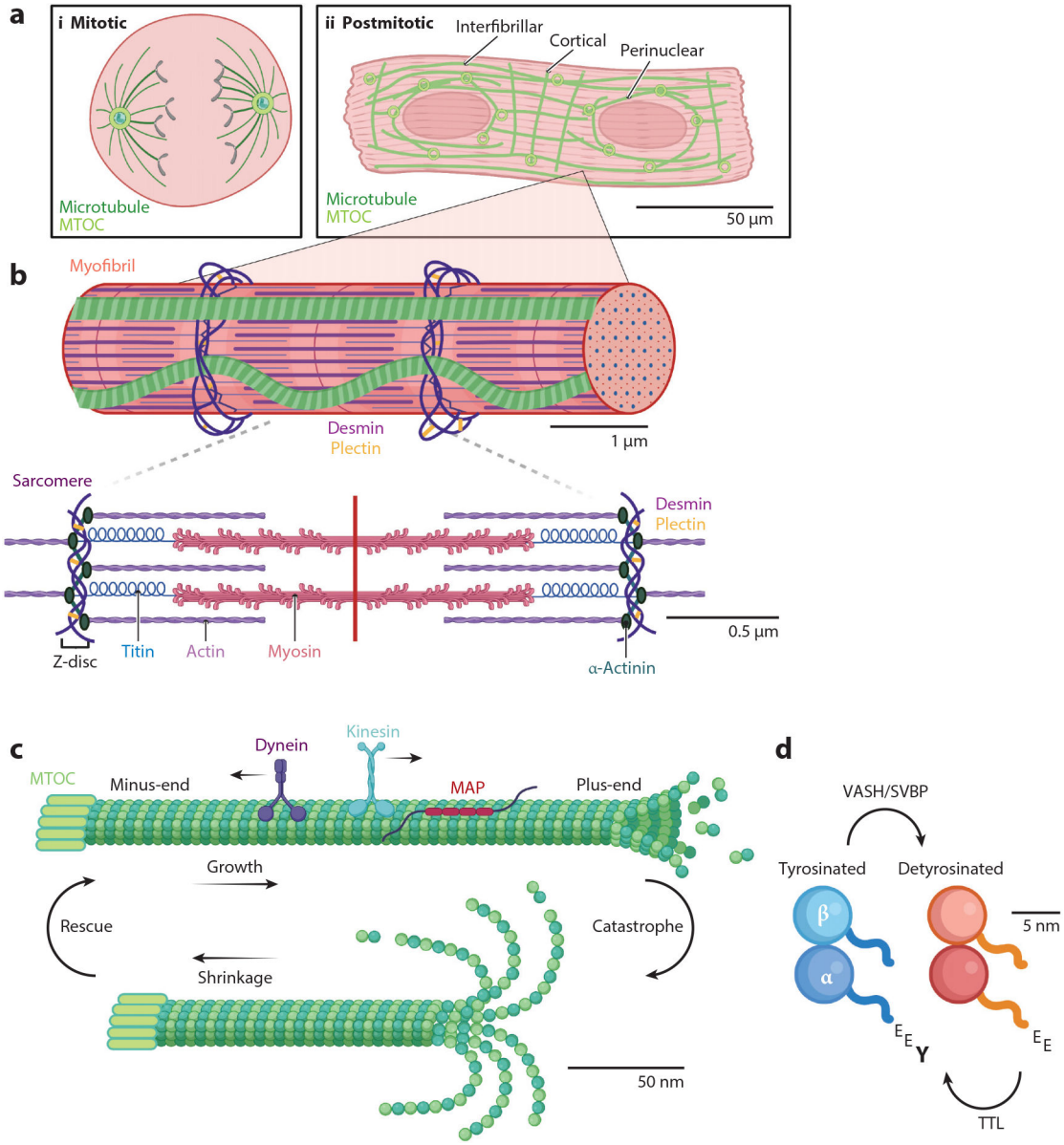


Figure 1.

Microtubules in the cardiomyocyte. (*a*) Cardiomyocytes transition from mitotic (*i*) to postmitotic (*ii*) shortly after birth, changing the localization of microtubule nucleation factors, such as microtubule organizing centers (MTOCs) and, thus, the polarity of the network. Mature cardiomyocytes are comprised of three microtubule populations (all in *green*): interfibrillar, cortical, and perinuclear. (*b*) Each myofibril in the cardiomyocyte contains many individual units called the sarcomere. The sarcomere is composed primarily of actin (*purple*) and myosin (*pink*). α-Actinin (*dark teal*) serves to anchor actin at the Z-disc. Desmin (*dark purple*), cross-linked by plectin (*yellow*), is also present at the Z-disc and can reinforce microtubules at this location. (*c*) Microtubules undergo dynamic instability, meaning they can grow or shrink and transition between these modes through rescue and catastrophe. Additionally, the microtubule on top is decorated with microtubule-associated

proteins (MAPs; *red*) and the motor proteins kinesin (*blue*) and dynein (*purple*), which step along the microtubule lattice toward the plus-end or minus-end, respectively. (*d*) One of the most-studied tubulin posttranslational modifications in the cardiomyocyte is detyrosination, the removal of the distal tyrosine on the C-terminal tail of α-tubulin (Y; *bottom left*). The tyrosination cycle is mediated by the enzymes tubulin tyrosine ligase (TTL), which adds the tyrosine to form tyrosinated tubulin (*blue*) and vasohibin/small vasohibin-binding protein (VASH/SVBP), which removes the tyrosine to form detyrosinated tubulin (*orange*).

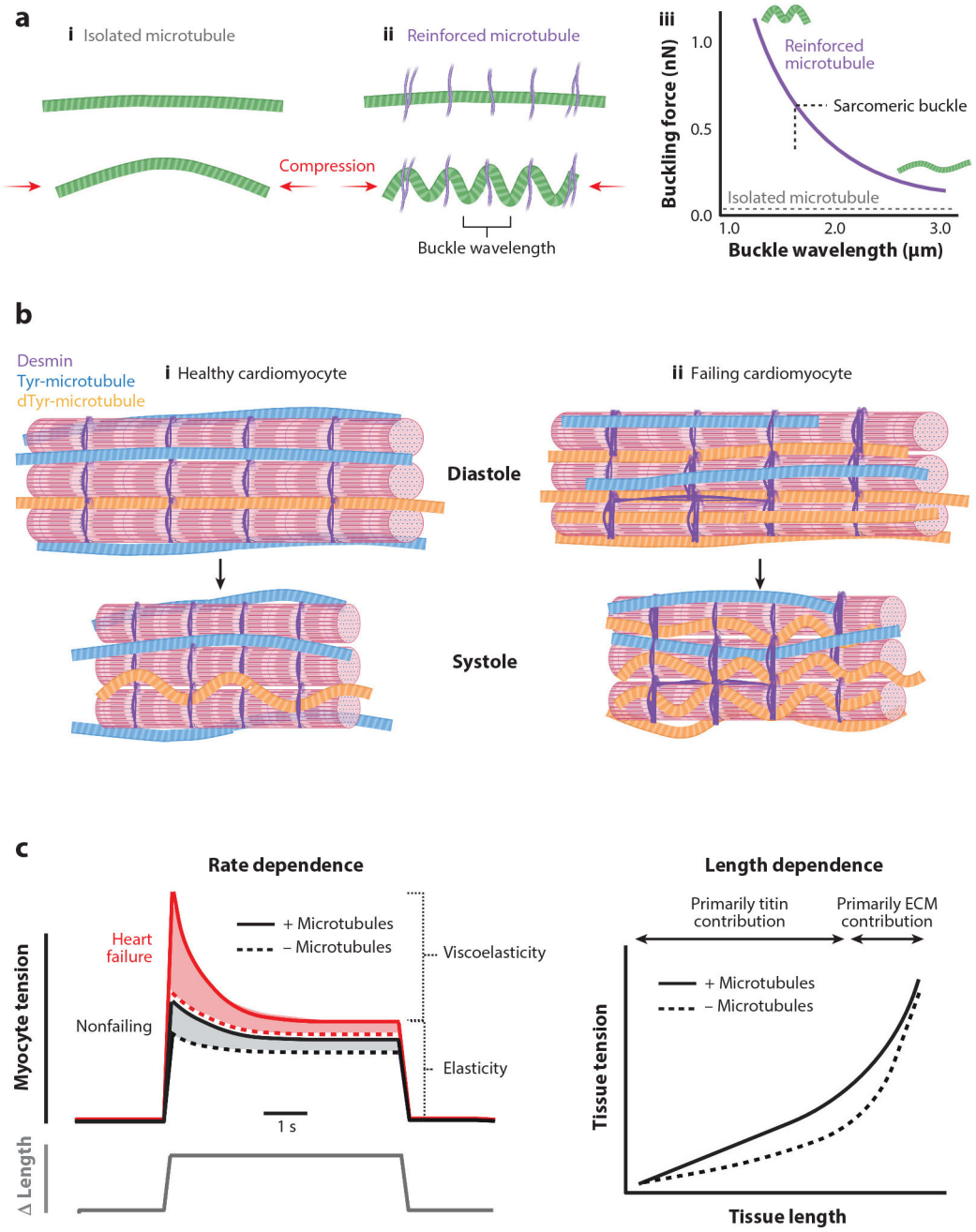


Figure 2.

(*a*) Microtubule mechanics. (*i*) Under compression (*red arrows*), isolated microtubules tend to buckle in one single long arc. (*ii*) When reinforced by other structural elements in the cell (*purple*), microtubules tend to buckle between sites of reinforcement, leading to a shortened buckle wavelength. (*iii*) Isolated microtubules will buckle under very small loads (~1 pN; *gray dotted line*). When reinforced, increased force is required to cause microtubule buckling, with higher forces leading to shorter buckling wavelengths. In the cardiomyocyte, microtubules tend to buckle with a wavelength of ~1.65 μm, the length of a sarcomere in systole (*black dotted line*). (*b*) Microtubules during myocyte shortening. Healthy

cardiomyocytes (*i*) have a mix of tyrosinated (Tyr) microtubules (*blue*) and detyrosinated (dTyr) microtubules (*orange*). During systole (*bottom*), the laterally anchored detyrosinated microtubules buckle and provide a small resistive element against myocyte motion. In failing cardiomyocytes (*ii*), there is an increase in the number of stable detyrosinated microtubules. Additionally, there are more desmin intermediate filaments (*purple*) that provide lateral stabilization to detyrosinated microtubules. Together, these modifications result in more buckling and resistance to contraction during systole and relengthening during diastole, contributing to contractile dysfunction. (*c*) Microtubules during myocyte stretch. (*Left*) Rate dependence. In response to a rapid increase in strain (*bottom line*), there is a transient increase in tension (*top line*) in nonfailing (*black*) cardiomyocytes that dissipates to a steady-state level slowly over time. The transient component reflects the viscoelastic resistance to stretch, while the steady-state component reflects the elastic resistance. In cardiomyocytes from failing hearts (*red*), the viscoelastic contribution to tension is exacerbated. In the absence of microtubules (*dotted lines*), the viscoelastic component is largely diminished, while the elastic component is largely unaffected. (*Right*) Microtubule length dependence. Microtubules (*solid black line*) cause pathologically remodeled myocardium to resist stretch and increase tissue tension more than in myocardium with disrupted microtubules (*dotted black line*). At high strains, the traces converge as the tissue tension is largely dominated by the contribution of the extracellular matrix (ECM). The viscoelastic resistance imposed on cardiomyocytes and myocardium by detyrosinated microtubules contributes to impaired diastolic function in failing hearts.

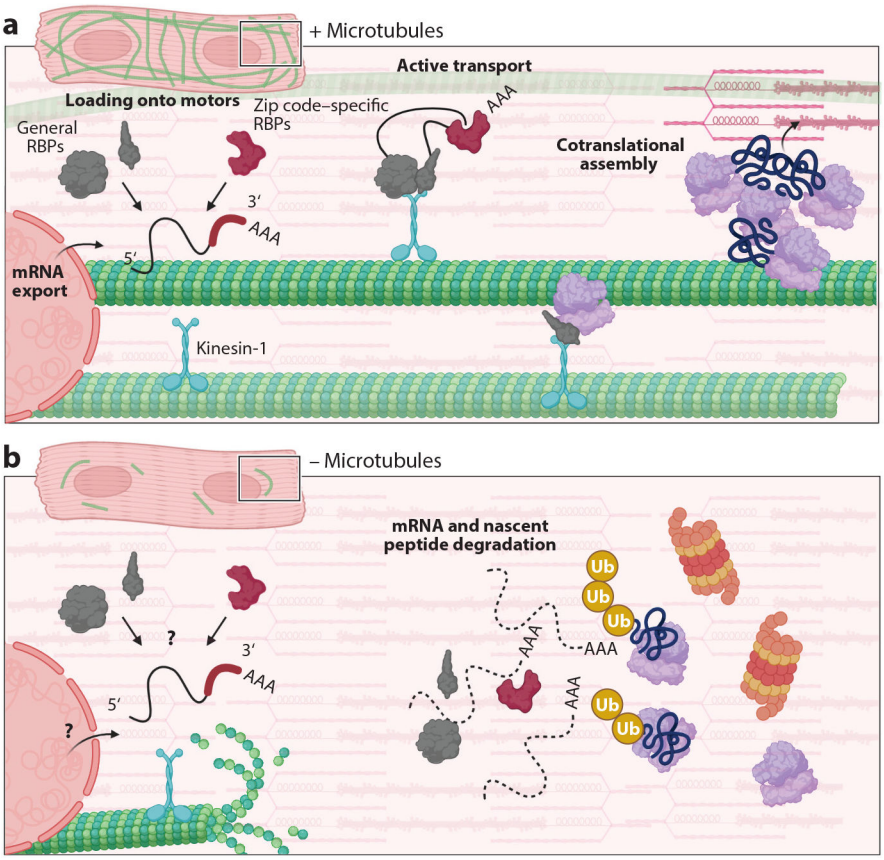
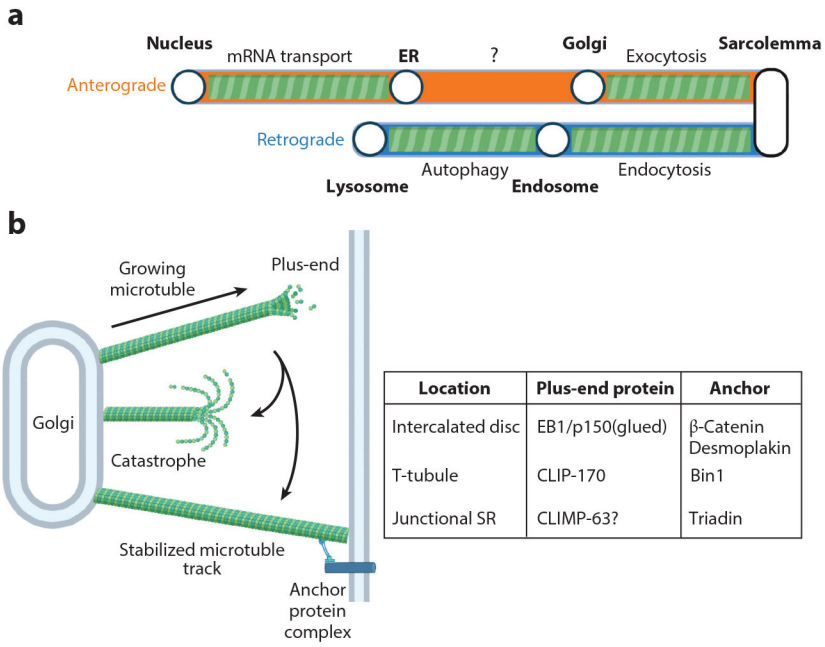


Figure 3.

mRNA transport in the cardiomyocyte. (*a*) Cardiomyocytes with an intact microtubule network export mature mRNA (as indicated by the polyA tail, …AAA) that forms a complex with a variety of general RNA-binding proteins (RBPs; *gray*) and zip code–specific RBPs (*red*), forming a heterogeneous ribonucleoprotein complex to be loaded onto kinesin-1 (*blue*). This particle is then actively transported on the microtubule. Ribosome subunits (*purple*) are also actively transported via motors. mRNAs and ribosomes are trafficked to both the Z-disc and intercalated disc to form a translational hub (*top right*). Here, sarcomeric mRNAs are cotranslated to facilitate assembly of structures and incorporation into existing sarcomeres. (*b*) In the absence of microtubules, both mRNA and ribosomes are mislocalized in the cardiomyocyte. It is unknown whether mRNA export, and/or the assembly of RBPs onto the mRNA, is inhibited in this scenario (*bottom left*). This perinuclear collapse of mRNA results in mislocalized translation and the degradation of mRNA and new peptides (indicated by addition of ubiquitin, Ub, to peptides; *bottom right*). Under these circumstances, the cardiomyocyte is unable to grow due to its inability to localize and add new sarcomeric protein.



Location	Plus-end protein	Anchor
Intercalated disc	EB1/p150(glued)	β-Catenin Desmoplakin
T-tubule	CLIP-170	Bin1
Junctional SR	CLIMP-63?	Triadin

Figure 4.

Cardiomyocyte-directed transport. (*a*) Subway map of microtubule-based transport in cardiomyocytes. Anterograde pathways are displayed in orange and retrograde pathways in blue. Nodes represent specific organelles, and green microtubules represent segments where microtubules are reported to be involved in trafficking. (*b*) Search and capture of microtubules in cardiomyocytes. Microtubules growing from microtubule organizing centers have a plus-end that extends outward. If the plus-end-associated proteins fail to find an interaction partner, the microtubule is susceptible to catastrophe and will repeat the growth process. If the microtubule finds a binding partner, the microtubule is protected from depolymerization and forms a stable, long-lived microtubule track. The table lists known/putative plus-end proteins and their interaction partner that forms an anchor for the microtubule for different cardiomyocyte membrane domains. Abbreviations: ER, endoplasmic reticulum; SR, sarcoplasmic reticulum.