Myosins in cell junctions

Katy C. Liu and Richard E. Cheney*

Department of Cell and Molecular Physiology; School of Medicine; University of North Carolina at Chapel Hill; Chapel Hill, NC USA

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The development of cell-cell junctions was a fundamental step in metazoan evolution, and human health depends on the formation and function of cell junctions. Although it has long been known that actin and conventional myosin have important roles in cell junctions, research has begun to reveal the specific functions of the different forms of conventional myosin. Exciting new data also reveals that a growing number of unconventional myosins have important roles in cell junctions. Experiments showing that cell junctions act as mechanosensors have also provided new impetus to understand the functions of myosins and the forces they exert. In this review we will summarize recent developments on the roles of myosins in cell junctions.

Introduction

Myosins are a superfamily of molecular motors that bind to actin filaments to generate force and movement. Myosins serve a growing array of functions within the cell, including cell motility, cell shape regulation, organelle trafficking and cell signaling. Myosins have a three-part structure consisting of a head, neck and tail. They are defined by the presence of a conserved myosin head domain that can bind to actin filaments, hydrolyze ATP and generate force. The neck domain consists of one or more IQ motifs, each of which provides a binding site for calmodulin or a calmodulin-like light chain. The tail domain varies dramatically between different classes of myosins, and endows each myosin class with specific functions such as binding to membranes or forming bipolar filaments. The human genome encodes heavy chains for 24 unconventional myosins, the human genome consists of one or more IQ motifs, each of which provides a binding site for calmodulin or a calmodulin-like light chain. The tail domain varies dramatically between different classes of myosins, and endows each myosin class with specific functions such as binding to membranes or forming bipolar filaments. The human genome encodes genes for 38 different myosin heavy chains (plus five named pseudogenes) that can be divided into 12 classes (see also the HUGO gene nomenclature website). Fourteen of these myosin heavy chain genes encode conventional (class II) myosins, the class that was first identified in studies of muscle contraction. The class II myosins are broadly and abundantly expressed in nonmuscle cells, and these nonmuscle myosin-IlS function as major cellular force generators in processes ranging from cell migration to cytokinesis.

In addition to the conventional myosins, the human genome encodes heavy chains for 24 unconventional myosins. These are divided into 11 distinct classes and have widely varying patterns of expression. In addition to two or three nonmuscle myosin-II genes, a typical cell is likely to express a dozen or more unconventional myosins. Although many different myosins are expressed in polarized epithelial cells, a metazoan-specific cell type with specialized cell junctions, only in recent times have we begun to elucidate the functions of the different myosins in polarized epithelial cells. In addition, until the past few years, most studies focused on investigating the roles of nonmuscle myosin-II. However, several recent studies have identified important functional roles for unconventional myosins in cell junctions in polarized epithelia. Here, we review the functions of myosins at epithelial cell junctions with particular emphasis on unconventional myosins (Fig. 1).

In vertebrate epithelial cells that line organs such as the gut and kidney, polarity is defined by the apical, lumen-facing domain and the underlying basolateral domain. At the intersection of these two domains, the apical junctional complex is comprised of two parts: the tight junction and the adherens junction (Fig. 2A). The tight junction (or zonula occludens) is apical-most, composed of a network of intramembranous strands that form a selective paracellular barrier between neighboring cells. Key components of the tight junction include the occludins, a family of transmembrane proteins responsible for forming the permeability barrier, and the ZO-1 family of proteins, large multidomain scaffolding proteins found on the cytoplasmic side of the tight junction. Key components of the adherens junction include the cadherins, a family of transmembrane proteins that mediate calcium dependent cell-cell adhesion.

The cytoplasmic tail of classical cadherins binds to β-catenin, a cytoplasmic protein that in turn binds to α-catenin, a scaffolding molecule that contains binding sites for many other proteins including actin filaments and actin binding proteins. A dense circumferential belt of actin filaments is located on the cytoplasmic face of the adherens junction and a loosened network of actin filaments is associated with the tight junction. Importantly, nonmuscle myosin-II is a well-known component of the circumferential actin belt. The apical junctional complex is essential to maintain the barrier function and integrity of the epithelial cell sheet.
The importance of myosins and forces at cell junctions is also highlighted by growing evidence that cell junctions are mechanosensitive.23 For example, application of tugging forces to endothelial cells recruits VE-cadherin (vascular endothelial cadherin) to junctions and increases junction size.24 At the molecular level, α-catenin can act as a tension transducer that undergoes force-dependent changes in conformation.25 These changes unmask its binding site for vinculin, a cytoskeletal protein that binds to actin filaments. Another actin-binding protein that binds to α-catenin is EPLIN (epithelial protein lost in neoplasm). 26 Although EPLIN localizes to linear adherens junctions (zonula adherens), it does not localize to punctate adherens junctions, which are small spot-like junctions thought to participate in the assembly of linear junctions; punctate adherens junctions are typically found at sites of cell-cell contact.

Notably, the apical junctional complex is not static, but is instead dynamic and constantly remodeling.13 In cell culture, junction assembly and disassembly are often modeled using the calcium-switch assay.14 In this assay, removal of extracellular calcium results in loss of cadherin-mediated cell-cell adhesion and disassembly of cell junctions, while subsequent re-addition of calcium triggers junction re-assembly (Fig. 2B). Importantly, junctional disassembly and assembly are accompanied by reorganization of the actin cytoskeleton. After junction disassembly, the now unpolarized cells often exhibit a ring of F-actin around their apical aspect.15 Then, during junction assembly, actin-rich protrusions that mature into radially oriented actin bundles are observed at sites of initial cell-cell contact.16 Using this model, it has been observed that nascent adherens-like junctions precede tight junction formation.12,17 As epithelial cells mature, junctional proteins and the circumferential actin belt organize at the cell junctions.

Indeed, the actin cytoskeleton is critical for the apical junctional complex, as disruption of actin dynamics affects the structural and functional integrity of both the adherens junction and the tight junction.12,15,18 Yet, how does the apical junctional complex physically interact with the actin cytoskeleton? Numerous proteins have been shown to interact with the tight and adherens junction cytoplasmic plaques, some of which are actin-binding proteins.19,20 Despite this, the identity of the linkages between the adherens junction and actin cytoskeleton, originally attributed to direct binding of α-catenin to F-actin, remains controversial.21,22 As actin-binding proteins that can generate force and movement, myosins are prime candidates to interact with the actin cytoskeleton at the apical junctional complex.

Figure 1. Bar diagrams of myosin heavy chains that have roles in cell junctions in vertebrates and/or Drosophila. See the text for descriptions of the different myosins and their key structural features.
The hexamers can, in turn, assemble into bipolar myosin filaments with each myosin minifilament containing a dozen or more myosin heads. The bipolar organization of these minifilaments endows class II myosins with their unique ability to exert contractile forces on antiparallel- and randomly-oriented arrays of actin filaments. The ATPase and motor activity of NMII is activated by phosphorylation of its regulatory light chain. Since the regulatory light chain is only found in class II myosins, this form of regulation occurs in NMII but not in unconventional myosins. Light chain phosphorylation can be mediated by several kinases, including myosin light chain kinases.

Mammals express three different genes for nonmuscle myosin-II,3 with most cells expressing two or more class II myosins.27,28 We will refer to these myosins here as NMII-A, NMII-B and NMII-C, but it should be noted that the official gene symbols for their heavy chains are MYH9, MYH10 and MYH14. Like all class II myosins, the tails of these heavy chains homodimerize by the formation of a parallel coiled coil, and the neck of each heavy chain binds to one essential light chain and one regulatory light chain.4 The native NMII hexamer thus consists of two heavy chains, two essential light chains and two regulatory light chains. The hexamers can, in turn, assemble into bipolar myosin filaments with each myosin minifilament containing a dozen or more myosin heads. The bipolar organization of these minifilaments endows class II myosins with their unique ability to exert contractile forces on antiparallel- and randomly-oriented arrays of actin filaments. The ATPase and motor activity of NMII is activated by phosphorylation of its regulatory light chain. Since the regulatory light chain is only found in class II myosins, this form of regulation occurs in NMII but not in unconventional myosins. Light chain phosphorylation can be mediated by several kinases, including myosin light chain kinases.

where actin bundles are oriented radially/ perpendicular to the plasma membrane. Importantly, the localization of EPLIN to linear adherens junctions is force-dependent and requires the activity of nonmuscle myosin-II.26

**Nonmuscle Myosin-II and Its Isoform Specific Roles at Cell Junctions**

All metazoan cells express one or more forms of nonmuscle myosin-II, a myosin which can constitute several percent of total protein and is thus likely to be the major force generator in most cells. Mammals express three different genes for nonmuscle myosin-II,3 with most cells expressing two or more class II myosins.27,28 We will refer to these myosins here as NMII-A, NMII-B and NMII-C, but it should be noted that the official gene symbols for their heavy chains are MYH9, MYH10 and MYH14. Like all class II myosins, the tails of these heavy chains homodimerize by the formation of a parallel coiled coil, and the neck of each heavy chain binds to one essential light chain and one regulatory light chain.4 The native NMII hexamer thus consists of two heavy chains, two essential light chains and two regulatory light chains. The hexamers can, in turn, assemble into bipolar myosin filaments with each myosin minifilament containing a dozen or more myosin heads. The bipolar organization of these minifilaments endows class II myosins with their unique ability to exert contractile forces on antiparallel- and randomly-oriented arrays of actin filaments. The ATPase and motor activity of NMII is activated by phosphorylation of its regulatory light chain. Since the regulatory light chain is only found in class II myosins, this form of regulation occurs in NMII but not in unconventional myosins. Light chain phosphorylation can be mediated by several kinases, including myosin light chain kinases.

![Figure 2. The apical junctional complex and calcium-switch model of junction assembly.](image-url)

(A) The tight junction and adherens junction, and their associated actin cytoskeleton, comprise the apical junctional complex. The tight junction strands form a semi-permeable barrier, and ZO-1 functions as a tight junction scaffolding protein. At the adherens junction, the cadherin-catenin complex contributes to cell adhesion. The apical junctional complex separates the apical and basolateral domains. (B) In the calcium-switch model, junctions are disassembled by removing calcium (left, low Ca²⁺). Upon calcium re-addition (center), the junctions begin to assemble, and radial actin cables are observed at early cell-cell contacts. Cells are fully polarized in a mature monolayer (right).
kinase (MLCK), Rho kinase (ROCK), and myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK). Most research on NMII has focused on NMII-A and NMII-B (Fig. 1) rather than the more recently discovered NMII-C. NMII-A and NMII-B differ in their duty ratio, the proportion of time the motor spends binding actin during its ATPase cycle. Since NMII-B has a higher duty ratio, it remains bound to actin for a greater fraction of its ATPase cycle than NMII-A. Finally, all three NMII isoforms can be inhibited by blebbistatin, an ATPase inhibitor that targets class II myosins, trapping them in a state that only binds weakly to actin.

Since the circumferential actin belt forms a ring at the cytoplasmic face of the adherens junction that contains NMII and actin filaments, it has long been thought that minifilaments of NMII pull on antiparallel actin filaments in the actin belt to generate contractile forces in much the same way as a stress fiber or cytoskeletal furrow. NMII-mediated contraction of the circumferential actin would pull junctions inward, thus providing one mechanism to disrupt junctions and increase permeability. Alternatively, if the linkages between cells are strong enough to bear the load, NMII-mediated contraction of the actin belt could shrink the circumference of the apical domain, thus generating an apical contraction that could facilitate morphogenetic tissue movements such as gastrulation. NMII clearly has several central roles in junctions, and global inhibition of NMII with blebbistatin disrupts recruitment of E-cadherin (epithelial cadherin) to initial cell-cell contacts, junction assembly and disassembly, and paracellular permeability of the tight junction. Since these and other experiments on the global functions of NMII and actin in junctions have been previously reviewed, here we will focus on recent developments and the discovery of distinct roles for the different NMII isoforms.

Isoform-specific knockout studies indicate roles for both NMII-A and NMII-B in cell adhesion and polarized cell junctions. NMII-A knockout mice are lethal at embryonic day 7.5 (E7.5); the visceral endoderm in knockout embryos shows loss of polarized columnar morphology, whereas knockout embryoid bodies exhibit cell shedding, which suggests a cell adhesion defect. Interestingly, E-cadherin and β-catenin are reduced at cell-cell contacts in the absence of NMII-A. NMII-B is enriched in brain, particularly in the neuroepithelial layers. NMII-B mutants are also lethal (-E14.5) and show structural defects and discontinuities in the apical domain of the neuroepithelial layers that appear to lead to spinal canal obstruction, hydrocephalus and embryonic lethality.

In polarized epithelial cells, both NMII-A and NMII-B are present in the apical junctional complex (Fig. 3A). NMII-A clearly functions in the dynamics of junction assembly and disassembly. NMII-A knockdown cells show a delay in junction formation, measured by transepithelial resistance (TER) or by localization of tight junction and adherens junction markers. Despite the delay of several hours, apical junctions do eventually form. NMII-A knockdown also affects junction disassembly, as F-actin reorganization into actin rings was disrupted and the translocation of the tight junction protein occludin from cell-cell junctions was attenuated. Thus, NMII-A is required for the proper kinetics of junction assembly and disassembly.

Isoform-specific knockdown also reveals differential roles for NMII-A and NMII-B in E-cadherin-based cell-cell contacts. NMII-A is required for proper E-cadherin organization at the adherens junction, as NMII-A siRNA reduces the amount of E-cadherin at cell-cell contacts and decreases cadherin-based homophilic adhesion. Interestingly, an E-cadherin blocking antibody decreased NMII-A staining at cell-cell contacts. Thus, NMII-A activity is needed for E-cadherin localization to the junctions and vice versa. Furthermore, NMII-A knockdown results in increased and irregular oscillatory movement of E-cadherin at cell-cell contacts. In contrast, NMII-B maintains the integrity of the actin cytoskeleton at the adherens junction, as NMII-B knockdown decreases actin and its dynamics at cell junctions and fragments the localization of E-cadherin. The authors of this study suggest that the high duty ratio of NMII-B may allow this motor to generate tension to support dynamic actin at the cell junctions. Consistent with this, live cell imaging of NMII-B knockdown cells shows significantly less translational movement of actin and E-cadherin. Interestingly, double knockdown of NMII-A and B leads to disruption of the tight junction, visualized by zonula occludens-1 (ZO-1) localization. One potential link between the actin cytoskeleton and the tight junction complex is cingulin, a submembranous tight junction-associated phosphoprotein that binds to NMII and to ZO-1, ZO-2 and ZO-3. Recent work shows that simultaneous knockdown of ZO-1 and ZO-2 leads to a dramatic increase in F-actin, NMII-B, Rho kinase and phosphorylated regulatory light chain at the circumferential actin belt, indicating increased contractility at the adherens junction. This intriguing result indicates that ZO-1 and ZO-2, scaffolding proteins found at the tight junction, normally act to negatively regulate NMII-B contraction in the circumferential actin belt.

Together, these studies suggest specific roles for the different isoforms of NMII: NMII-A is required for normal kinetics of junction assembly and organizes E-cadherin at the adherens junction, whereas NMII-B supports the integrity and dynamics of the circumferential actin belt. In addition, recent studies with inhibitors show that NMII is largely responsible for generating the forces sensed by mechanosensory molecules at cell junctions.

It should also be noted that in addition to NMII-mediated contraction of the circumferential actin bundle, a more diffuse network of NMII and actin filaments in the apical region can also contract to power apical constriction, and this force is transmitted from cell to cell via junctions to drive gastrulation in Drosophila. Interestingly, during C. elegans gastrulation, apical constriction appears to be triggered by activation of a clutch-like linkage between the apical junction and an apically-directed actin flow generated by NMII contraction. As a major force generator and component of adherens junctions, NMII may also have yet to be discovered roles at the junction.

**Myo1e at Specialized Glomerular Junctional Complexes**

Class I myosins are single-headed motors with short tails that bind to lipid...
membranes. They are phylogenetically ancient and are found in amoebae, fungi and animals. Many organisms express several class I myosins; the slime mold Dictyostelium discoideum expresses seven and humans express eight class I myosins. Myo1a, one of the best known class I myosins, forms a link between the plasma membrane and the actin filaments of intestinal microvilli. Myo1e (initially called human myosin-1c or myr3) has a longer tail that contains both a membrane-binding domain and an SH3 domain (Fig. 1). Myo1e is ubiquitously expressed, with the highest levels in kidney, prostate, colon, liver and ovary. Notably, Myo1e in kidney is predominantly found in the glomerulus and its podocytes, which are epithelial cells that extend “foot processes” to wrap around glomerular capillaries.

Myo1e localizes to cell junctions in several cell types. Myo1e localizes with β-catenin at the adherens junction in intestine and kidney. In Caco-2 cells, a human colon carcinoma cell line used as a model for small intestine, Myo1e is enriched at the apical cell junctions in

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**Figure 3.** Functional roles of myosins in cell junctions and cell-cell contacts. (A) Nonmuscle myosin-II localizes to the adherens junction-associated circumferential actin belt, and knockdown on nonmuscle myosin-II disrupts E-cadherin-based cell-cell contacts. (B) Myo6 and vinculin have been suggested to tether E-cadherin to the perijunctional actin cytoskeleton. (C) In stereocilia, Myo7a binds cadherin-23 and protocadherin-15 in a stereocilia adhesive tip complex. (D) Myo9a is a RhoGAP that localizes to cell-cell contacts and suppresses Rho activity. Myo9a knockdown shows defects in formation and stabilization of early cell-cell contacts, resulting in a cell scattering phenotype. (E) Myo10 localizes to the tips of filopodia at nascent cell-cell contacts during junction assembly, and Myo10 knockdown delays junction assembly. (F) Dachs has a planar polarized distribution in Drosophila wing disc epithelia. Loss of Dachs disrupts orientation of the mitotic spindle and cell division along the proximal-distal axis.
spreading cells and mature monolayers. In cultured monolayers of mouse podocyte cells, Myo6 frequently localizes to cell-cell contacts and may be needed for proper actin organization. Renal glomeruli from Myo6 knockout mice show disrupted podocyte foot processes as well as thickened and disorganized glomerular basement membranes, leading to impaired renal function. Disruption in the cytoskeleton of the glomerular intercellular junctional complexes has also been shown to impair renal function. As a class I myosin, Myo6 could function to stabilize the actin cytoskeleton by binding the surrounding glomerular membrane.

In Myo6 knockout studies, Myo6-deficient mice exhibit podocyte injury and impaired renal function. Mutations in human Myo6 are associated with familial focal segmental glomerulosclerosis, an autosomal recessive disease of podocytes. Thus far, no extrarenal defects have been identified in knockout mice or patients with Myo6 mutations. Apart from Myo6, little is known regarding class I myosins and epithelial cell junctions. Do any of the other seven class I myosins localize to or function in cell junctions? With the Myo1a knockout mouse available, are there observable junctional defects? Important, loss of Myo6 causes deafness in both humans and the Snell's waltzer mouse. In the inner ear hair cells, Myo6 is enriched both in the vesicle-rich pericellular necklace and in stereocilia, which are mechanosensing actin-based protrusions on hair cells. In the Snell's waltzer mouse, the inner ear hair cells develop disorganized and fused stereocilia, and a similar phenotype is observed in intestinal microvilli. Furthermore, loss of function studies in Drosophila also indicate Myo6 is critical for epithelial morphogenesis. Drosophila Myo6 (Jaguar) deficiency disrupts dorsal closure, a process of epithelial sheet fusion at the dorsal midline in late embryogenesis. Drosophila Myo6 deficiency also disrupts border cell migration during oogenesis, where tethering of Myo6 to fixed integral membrane proteins could potentially provide the pushing force required to protrude cell extensions.

Myo6 has functional roles in cadherin-based cell-cell contacts and the perijunctional actin cytoskeleton. In vitro studies in MCF-7 cells show that Myo6 localizes to mature cell-cell contacts and co-localizes with E-cadherin. Immuno precipitation studies indicate Myo6 interacts with E-cadherin and β-catenin. Myo6 also binds Echinoid, a nectin-like cell adhesion molecule that cooperates with Drosophila epithelial cadherin (DE-cadherin). Interestingly, Myo6 and E-cadherin/β-catenin each appear to stabilize the other protein. In Myo6-null or mutant Drosophila border cells, E-cadherin and β-catenin levels are reduced. Conversely, Drosophila Myo6 protein levels are reduced in cells lacking either E-cadherin or β-catenin. Similar results were obtained in vitro by Myo6 knockdown in monolayers of vertebrate epithelial cells. Thus, Myo6 stabilizes E-cadherin at epithelial cell-cell contacts.

Given its high duty ratio, Myo6 may also stabilize the perijunctional actin cytoskeleton and/or tether membrane proteins, such as E-cadherin or β-catenin, to the perijunctional actin cytoskeleton. Myo6 has been previously reported to stabilize actin filament networks in unpolarized cells, and Myo6 serves a similar membrane anchoring role in stereocilia. In epithelial cells, loss of Myo6 leads to disrupted perijunctional actin as well as loss of tight junction markers at cell-cell contacts. Vinculin, a cytoskeletal protein found in focal adhesions and adherens junctions, is a downstream effector of Myo6, with Myo6 and vinculin acting together to stabilize perijunctional actin and cadherin-based cell-cell contacts (Fig. 3B). An important role for Myo6 at the adherens junction is also shown by recent work where Caco-2 monolayers were treated with HGF (hepatocyte growth factor/scatter factor), a growth factor that is secreted by many tumors and that induces epithelial to mesenchymal transition. HGF led to a rapid loss of F-actin from the adherens junction, and this loss was blocked by overexpression of Myo6. Given these in vitro roles for Myo6, it will be important to determine if the humans and mice that lack Myo6 exhibit in defects in their cell junctions, especially given that mammals only express a single Myo6 gene, and Myo6 is the only myosin known to move toward the pointed end.

**Myosin-VIIa Interacts with Junction-Associated Proteins at Epithelial Cell-Cell Contacts**

Myosin-VIIa (Myo7a) is a member of the “MyTH-FERM” family of myosins, an evolutionarily ancient family that is characterized by the presence of a tail containing a myosin tail homology 4 (MyTH4) domain located N-terminal to a protein 4.1, ezrin, radixin, moesin (FERM) domain. Functionally, these MyTH-FERM myosins often localize to actin-based extensions such as filopodia and stereocilia, and several MyTH-FERM myosins are implicated in cell adhesion.

The structure of the MyTH4-FERM domain from Myo7a was recently solved, revealing that the MyTH4-FERM domain forms a structural supermodule.
folding but appears to function as a processive dimer. Biochemical experiments indicate Myo7a has a high duty ratio. In mammals, Myo7a is predominantly expressed in epithelial cells with highest levels in the testis, cochlea and retina and lower expression in kidney.

Mutations in human Myo7a are responsible for Usher Syndrome 1B (USH1B), a disease of congenital deafness, vestibular dysfunction and pre-pubertal onset of retinitis pigmentosa leading to blindness. Mouse Myo7a is encoded by the shaker-1 locus, and homozygous shaker-1 mutants are characterized by deafness and vestibular dysfunction. In cochlear hair cells, loss of Myo7a function results in disruption in stereocilia structure and organization. Myo7a binds cadherin-23 (CDH23), an atypical cadherin present at the extracellular links between stereocilia that have been proposed to apply the tension needed for proper stereocilia organization (Fig. 3C). Myo7a has also been reported to interact with protocadherin-15 (PCD15), and protocadherin-15 in turn binds to cadherin-23 to form the stereocilia tip links required for hearing and balance. Finally, mutations in Drosophila Myo7a are responsible for crinkled, a fly mutant that is deaf and has defects in actin-based bristles and hairs. The shaker-1 and crinkled phenotypes both indicate a role for Myo7a in the formation of complex actin-based protrusions.

In polarized epithelial cells, endogenous Myo7a or the GFP-Myo7a tail localize to cell-cell contacts. In addition, in the testis, Myo7a is found in a dynamic adhesive structure called the ectoplasmic specialization. Interestingly, Myo7a interacts with several junction-associated proteins at epithelial cell-cell contacts. First, the FERM domain of Myo7a interacts with vezatin, a transmembrane protein at the adherens junction. Vezatin, which also binds E-cadherin and α- and β-catenin, has been proposed to bridge Myo7a to the cadherin-catenin complex at the adherens junction. Functionally, Myo7a and vezatin are needed for the bacterial entry of Listeria monocytogenes into epithelial cells. The MyTH4-ERM domain of Myo7a also mediates the interaction with Shroom2, a tight junction-associated protein. Thus, Shroom2 may link Myo7a and the actin cytoskeleton to the tight junction. Finally, Myo7a binds the actin-associated protein Keap1 at the specialized adhesion junctions in testis. Thus, Myo7a may link the apical junctional complex to perijunctional actin via its interactions with junction-associated proteins in epithelial cells. It should be noted, however, that the localizations of vezatin, Shroom2 and Keap1 are Myo7a-independent, as the localization of each Myo7a-binding protein in shaker-1 mutants is unchanged. Given that humans who lack Myo7a suffer from deafness and blindness, it will be important to determine if they also have defects in cell-cell junctions.

Class IX Myosins: Motorized Rho GAPS that Regulate Cell-Cell Junctions and Migration

Class IX myosins are metazoan-specific motor proteins which appear to act as single-headed myosins that can move processively on actin. Humans express two myosin IX genes, myosin-IXa (Myo9a, formerly known as myr 7) and myosin-IXb (Myo9b, formerly known as myr 5), and the two proteins share approximately 57% amino acid sequence identity in their motor domains. Unique among myosins, the tails of class IX myosins are Rho GTase activating proteins (GAPs) that negatively regulate Rho GTPases (Fig. 1). The RhoGAP domains of the class IX myosins are specific for Rho and Rho GTPase activity (RhoA) as cells contact and have little effect on Rac1 or Cdc42. Rho regulates many processes, including actin organization and cell migration, and a major Rho effector is Rho-kinase, which activates nonmuscle myosin-II. Importantly, Rho and its effectors are involved in epithelial junction assembly. Until recently, however, little was known about the functions of the class IX myosins and their Rho-GAP activity in epithelia and junctions.

Myo9a localizes at cell-cell junctions both in vivo and in vitro and it is ubiquitously expressed during development. In adult tissues, Myo9a is particularly abundant in the brain and testis, with lower levels of expression in the adrenal gland, kidney, lung and spleen. In the brain, Myo9a is highly expressed in the ciliated ependymal cells that form the epithelial layer lining the ventricles. In knockout studies, loss of Myo9a results in a distorted ependymal layer, blockage of the canals in the ventricular system and severe hydrocephalus. Remarkably, the hydrocephalus could be suppressed by pharmacologically inhibiting Rho-kinase, a major Rho effector. At the cellular level, Myo9a knockouts have defects in the localization of E-cadherin and β-catenin as well as loss of occludin localization to the tight junction. Myo9a in Caco-2 cells also reveals defects in junction assembly, although junctions do eventually form and the cells have normal apico-basal polarity.

A recent study by Omelchenko and Hall (2012) highlights the role of Myo9a in collective cell migration and the use of its RhoGAP activity at cell-cell junctions (Fig. 3D). In cultured human bronchial epithelial cells (16HBE), Myo9a is enriched at the leading edge and at nascent cell-cell contacts, and FRET experiments indicate that it negatively regulates Rho GTPase activity (RhoA) as cells contact one another. Similar to the knockout mouse, Myo9a siRNA knockdown leads to loss of ZO-1 and irregular E-cadherin staining at cell-cell contacts. In addition, actin at cell-cell contacts fails to reorganize into the radial actin bundles thought to stabilize contacts. Importantly, Myo9a knockdown leads to impaired wound healing and a dramatic cell scattering phenotype, as cells fail to stabilize initial cell-cell contacts.

Myo9b is of particular interest because Myo9b polymorphisms have been associated with Crohn’s disease, ulcerative colitis and celiac disease. Myo9b polymorphisms have been associated with Crohn’s disease, ulcerative colitis and celiac disease.
showed dramatically decreased staining of tight junction components including ZO-1, claudin and occludin. Knockdown cells also have defects in wound healing; rather than extending lamellipodia like control cells, the knockdown cells instead formed stress fiber-like arrays of actin and nonmuscle myosin-II along the leading edge. Myo9b thus appears to act as a motorized RhoGAP that transports itself to the leading edge, where it inhibits Rho activity and thus inhibits processes such as nonmuscle myosin-II-mediated contraction. Since Myo9b null mice have recently been generated and macrophages from these mice exhibit defects in spreading and chemotaxis in vitro and defective recruitment in vivo, it will be important to determine whether Myo9b null mice exhibit any defects in junction formation and if they provide a model for inflammatory bowel disease.

Myosin-X: A MyTH-FERM Myosin at the Basolateral Domain of Polarized Epithelial Cells

Myosin-X (Myo10) is a MyTH-FERM myosin best known for localizing to filopodial tips. It appears to have arisen just prior to the evolution of the metazoans, rather than extending lamellipodia like control cells, the knockdown cells instead formed stress fiber-like arrays of actin and nonmuscle myosin-II along the leading edge.121 Myo9b thus appears to act as a motorized RhoGAP that transports itself to the leading edge, where it inhibits Rho activity and thus inhibits processes such as nonmuscle myosin-II-mediated contraction. Since Myo9b null mice have recently been generated and macrophages from these mice exhibit defects in spreading and chemotaxis in vitro and defective recruitment in vivo,122 it will be important to determine whether Myo9b null mice exhibit any defects in junction formation and if they provide a model for inflammatory bowel disease.

Myosin-X (Myo10) is a MyTH-FERM myosin best known for localizing to filopodial tips. It appears to have arisen just prior to the evolution of the metazoans, but was apparently lost in the lineages leading to flies and worms.3 The tail of Myo10 has several unique domains. These include three pleckstrin homology (PH) domains, one of which binds inositol phospholipids with high affinity for phosphatidylinositol (3,4,5)-triphosphate (PIP3).123-125 In addition, a C-terminal MyTH-FERM domain can bind microtubules126 and β-integrins89 (Fig. 1). Myo10 is ubiquitously expressed, with relatively high levels of expression in epithelial tissues such as kidney, but it appears to be a low abundance myosin that is orders of magnitude less abundant than nonmuscle myosin-II.127 Notably, Myo10’s IQ motifs can bind either calmodulin or calmodulin-like protein (CLP), which is an epithelia-specific light chain.128

In unpolarized cells such as fibroblasts, Myo10 localizes to tips of filopodia and is required for the formation of filopodia.129 In polarized MDCK cells, Myo10 localizes to the lateral membrane during junction assembly50 while in kidney it localizes basolaterally130 and fractionates with basolateral membranes.78 The basolateral targeting of Myo10 is likely to be mediated by interaction of its PH domains with PIP3, since inositol phospholipids in epithelial cells are segregated with PIP3 enriched basolaterally and PIP2 enriched apically.130 Consistent with this, the PH domains of Myo10 localize to the basolateral membrane in MDCK cells.125

Recent work reveals that Myo10 has important roles in junction assembly and epithelial morphogenesis.130 As observed with knockdown of key junctional components, ZO-1 and E-cadherin,132,133 Myo10 knockdown results in a delay in junction assembly, as measured by a delay in the localization of tight junction and adherens junction markers to cell-cell contacts and a delay in peak transepithelial resistance (TER).130 Importantly, the delay in junction assembly following Myo10 knockdown does not appear to be due to a defect in epithelial polarity since apico-basal polarity of several markers was normal, as was the TER of mature monolayers. Although Myo10 has been reported to interact with VE-cadherin and to undergo co-transport with it in filopodia during early stages of junction formation in endothelial cells,134 no interaction between Myo10 and E-cadherin has been detected thus far.130 Filopodia linked to radial actin cables are theorized to form and stabilize initial cell-cell contacts at the early stages of junction formation.16 Since GFP-Myo10 shows localization to the tips of dynamic filopodia-like structures at the basal surface of MDCK cells during junction assembly,130 Myo10 is likely to function at the initial stages of cell-cell contact (Fig. 3E).

In three-dimensional culture, Myo10 knockdown cells form cysts with multiple lumens rather than the normal, single lumen cysts in MDCK cells. Since defects in spindle orientation can result in multilumen cysts,135-137 and Myo10 is required for normal spindle positioning and orientation,126,130,138,139 Myo10 knockdown may lead to multiple lumens because of defects in spindle orientation. Consistent with this, knockdown of Myo10 disrupts the normally horizontal orientation of spindles in MDCK monolayers.130 Although the mechanisms by which Myo10 orients spindles remain unclear, in epithelial cells of Xenopus embryos, Myo10 is reported to be present at both the cell cortex and the mitotic spindle.140 Intriguing new data indicates that the vertical position of the spindle in this system is determined by the balance between a basally directed force that depends on Myo10 and microtubules and an apically directed actin flow that depends on nonmuscle myosin-II and actin.141

Myosin-XVa in Stereocilia and Drosophila Sisyphus in Dorsal Closure

Myosin-XVa (Myo15a) is another member of the MyTH-FERM family of myosins, and in mammals it exists as two alternatively spliced isoforms with or without a large N-terminal extension122,123 (Fig. 1). Expression of Myo15a in mammals appears to be limited to only a few cell types, including the neurosensory cells of the inner ear.142

Human mutations in Myo15a are responsible for human non-syndromic autosomal recessive deafness (DFNB3).143 In mice, Myo15a mutations (shaker-2) result in deafness and vestibular dysfunction.145,146 Myo15a localizes to the tips of stereocilia in the inner ear,143 and shaker-2 mice have short and disorganized stereocilia that fail to form their characteristic staircase structure.140 Therefore, Myo15a is necessary for the graded elongation of stereocilia. Moreover, Myo15a interacts with whirlin,147 a scaffolding protein associated with Usher syndrome type 2,148 and with Eps8, an actin capping protein, to form a stereocilia tip complex.149

Initial investigations of class XV myosins in epithelial cells have been performed by studying Sisyphus, a Drosophila MyTH-FERM myosin that shares similarity to Myo15a, but that is expressed in many tissues.150 Liu et al. found that Sisyphus is required for epithelial morphogenesis and functions in cell adhesion. As epithelial sheets close during dorsal closure, Sisyphus accumulates in contact-making filopodia and newly formed junctions. Sisyphus-deficient mutants show defects in epithelial alignment, and delay and/or failure in fusion of epithelial sheets. Sisyphus was reported to co-localize with and bind Drosophila E-cadherin, and
Sisyphus-deficient embryos show reduced E-cadherin at the dorsal side of leading edge cells during dorsal closure.\textsuperscript{150}

It thus appears that Myo15a and Sisyphus have distinct roles. In mammals, Myo15a functions in the inner ear to regulate stereocilia length and organization, while in Drosophila, Sisyphus interacts with E-cadherin and is critical for epithelial sheet alignment and adhesion during dorsal closure. This raises the question of whether other MyTH-ERM myosins expressed in mammals such as Myo10 might fill the roles played by Sisyphus in Drosophila.

**Dachs: A Drosophila Unconventional Myosin is Planar Polarized at Apical Junctions**

Dachs is an unconventional myosin in Drosophila\textsuperscript{153-155} that lacks a clear mammalian homolog\textsuperscript{156} (Fig. 1). The Dachs sequence includes an N-terminal extension preceding the head and neck, and a relatively short tail that shows no sequence similarity to other proteins. Dachs mRNA is broadly expressed throughout embryonic and wing disc development.\textsuperscript{153} Recent investigations reveal that Dachs is required for proper orientation of cell division and tissue growth.\textsuperscript{154} In the wing disc epithelium, Dachs protein is planar polarized along the proximal-distal axis, localizing to the distal side of each cell near the adherens junction.\textsuperscript{153,155}

Loss of Dachs results in reduced tissue growth, shortened legs and wings and often fused tarsal segments.\textsuperscript{153,156} In dachs mutants, wing discs are rounded and shortened in the proximal-distal axis due to tissue undergrowth and disrupted wing disc elongation.\textsuperscript{154} Importantly, dachs mutants show mitotic spindle misorientation\textsuperscript{154} (Fig. 3F), a defect that can arise in several ways, including inhibition of junctional proteins such as E-cadherin.\textsuperscript{157} Interestingly, dachs mutants have dilated apical cell surfaces, which suggests that the Dachs myosin is required for exertion of a polarized contractile force that constricts apical junctions and orients the mitotic spindle along the proximal-distal axis to produce net elongation and tissue growth along this axis.\textsuperscript{154}

Dachs is a component of the Fat signaling pathway,\textsuperscript{153} a pathway that involves two large protocadherins, Dachsous and Fat.\textsuperscript{158} Dachsous on a given cell appears to act as a ligand for Fat on the adjacent cell, and the planar polarized distribution of Dachs is disrupted in the absence of Fat or other Fat signaling regulators.\textsuperscript{153} Recent work indicates that Dachsous recruits Dachs to one side of a junction and that Fat signaling on the other side of the junction inhibits Dachs localization there.\textsuperscript{153} This leads to anisotropic tension across the junction and oriented cell rearrangements that generate extension in the proximal-distal axis. Almost nothing is known about the structural and motor properties of Dachs, and it would be of interest to learn whether Dachs generates tension on junctions directly by linking the protocadherin Dachsous to actin or if it regulates tension indirectly by regulating nonmuscle myosin-II. Since virtually all of the components of the Fat signaling pathway are conserved in vertebrates and several are tumor suppressors, it would also be of great interest to determine if a different myosin takes the place of Dachs in humans.

**Conclusions**

It is now clear that many different myosins function at cell-cell junctions. This includes the different forms of nonmuscle myosin-II and an increasing number of unconventional myosins. As knowledge of these actin-based motors continues to grow, several themes begin to emerge. The different forms of nonmuscle myosin-II are major components of the circumferential actin belt and clearly have central roles in junction assembly and disassembly. One of the most exciting developments in the field has been the growing recognition that cell-cell junctions are mechanosensitive, and nonmuscle myosin-II exerts much of the force that is sensed by junctions. Several myosins act at the early stages of junction formation by facilitating the formation or stabilization of actin-based protrusions and initial cell-cell contacts. Myosins can also function by facilitating cell adhesion, organizing actin, tethering junctional proteins and orienting the mitotic spindle. Another important function for myosin motors is cargo transport, which raises the question of whether there are specific unconventional myosins that deliver molecules to cell junctions. A major challenge in the field is that we currently lack drugs analogous to blebbistatin that specifically target the different unconventional myosins. In addition, cell junctions are so fundamental to metazoan physiology that the molecular mechanisms underlying their function may be highly redundant. Despite these challenges, recent studies have revealed important roles at cell-cell junctions for several unconventional myosins, and there are several others whose functions remain unknown. In the next few years, it is likely that the growing numbers of myosin knockout models, along with large scale sequencing of human patients, will reveal even more roles for myosins in cell junctions and human health.

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