Hold on tightly, let go lightly: myosin functions at adherens junctions

Joshua C. Sandquist and William M. Bement

Adherens junctions, the sites of cadherin-dependent cell–cell adhesion, are also important for dynamic tension sensing, force transduction and signalling. Different myosin motors contribute to adherens junction assembly and versatility in distinct ways.

Adherens junctions (AJs) are major sites of cell–cell adhesion and attach actin filaments (F-actin) to the plasma membrane in epithelial cell layers. However, AJs are also critical for epithelial cell movement, tension sensing and signal transduction. The cellular and molecular mechanisms that lend AJs this versatility are not well understood, but the actin motor, myosin II, has previously been implicated in AJ assembly and function[1]. Now, on page 696 of this issue, Smutny *et al.* report that different myosin II isoforms make unique contributions to AJ assembly and function[2]. This study provides an intriguing glimpse into both the biological relevance of motor protein diversity as well as the molecular basis of AJ versatility.

AJ adhesion is mediated by the membrane-spanning cadherins, which cluster at the AJs and form homotypic interactions with cadherins from adjacent cells. The link to F-actin reinforces adhesion and is mediated by actin-binding proteins that interact directly or indirectly with the cadherins. The combination of cadherins and F-actin makes AJs strong, but nevertheless permits them remarkable plasticity. Within a typical epithelium, AJs form, change length, move up and down as well as side-to-side, and break, in response to intrinsic cues provided during morphogenesis, differentiation and extrusion of apoptotic cells[1]. AJs can also respond within seconds to extrinsic challenges such as wounding[3]. Furthermore, even in a 'resting' epithelium, AJs balance tension between neighbouring cells to maintain the size of the apical domain[4]. Although AJs are typically compared to focal adhesions (the structures that anchor cells to a substratum) an alternative and potentially useful analogy is provided by kinetochores, the structures that tether chromosomes to mitotic spindles[5] (Fig. 1). Like kinetochores, AJs must be able to sense forces from at least two different directions, and can respond to the forces imposed on them by maintaining a constant position, or by moving. AJs also resemble kinetochores in that both structures regulate, and are regulated by, the cytoskeletal system responsible for their movement — F-actin and microtubules, respectively.

At least some of the dynamic properties of AJs are driven by non-muscle myosin II. Myosin II motors form bipolar filaments, which permits them to both extensively crosslink and contract actin filaments. Smutny *et al.*[2] show that two myosin II isoforms, myosin IIA and myosin IIB, localize to the AJs of MCF7 cells, a human epithelial cell line. Strikingly, knockdown experiments showed that myosin IIA is required for cadherin clustering, cadherin concentration at the AJ and proper adhesion, whereas myosin IIB controls the continuous distribution of E-cadherin along the length of AJs and the normal levels of AJ-associated F-actin.

One immediate implication of these results is that the different myosins contribute to different stages of AJ assembly. Previous work indicated that, following homotypic cadherin adhesion, F-actin and myosin II (refs 6–8) are required for cadherin clustering and accumulation at the AJ. As the cadherin clusters become uniformly distributed along the length of the AJ it then matures, in a process that also depends on F-actin and myosin II. Thus, myosin IIA may control the initial stage of AJ assembly, whereas myosin IIB promotes AJ maturation and fine tuning. Such a model is consistent with results from mouse knockout studies; myosin IIA-deficient mouse embryos die very early in development, apparently as a result of widespread defects in cadherin-based adhesion, whereas some myosin IIB-null mice survive birth, exhibiting grossly normal development except for severe neuronal and cardiac defects[9,10]. Thus, myosin IIA is apparently sufficient to support at least a minimum level of functional, if not completely mature, cell–cell adhesion in most tissues of the developing mouse.

How do these myosins make different contributions to AJ assembly and function? One possibility is that the two myosins may differ in subcellular distributions, consistent with previous demonstrations that myosin IIA and myosin IIB are spatially separated in crawling cells[11]. Indeed, although both myosin IIA and myosin IIB are found at the AJ, distinct, non-overlapping regions in their distribution exist[2]. In particular, myosin IIA, but not IIB, is clearly enriched on the basolateral domain below the AJs. Because live imaging[8], as well as ultrastructural studies[12], indicate that the AJ is associated with pools of F-actin that differ with respect to both dynamics and distribution, it is reasonable to suppose that the subtle differences in myosin localization might contribute to their specific function. For example, myosin IIA may work with basolateral F-actin as a corral that promotes clustering

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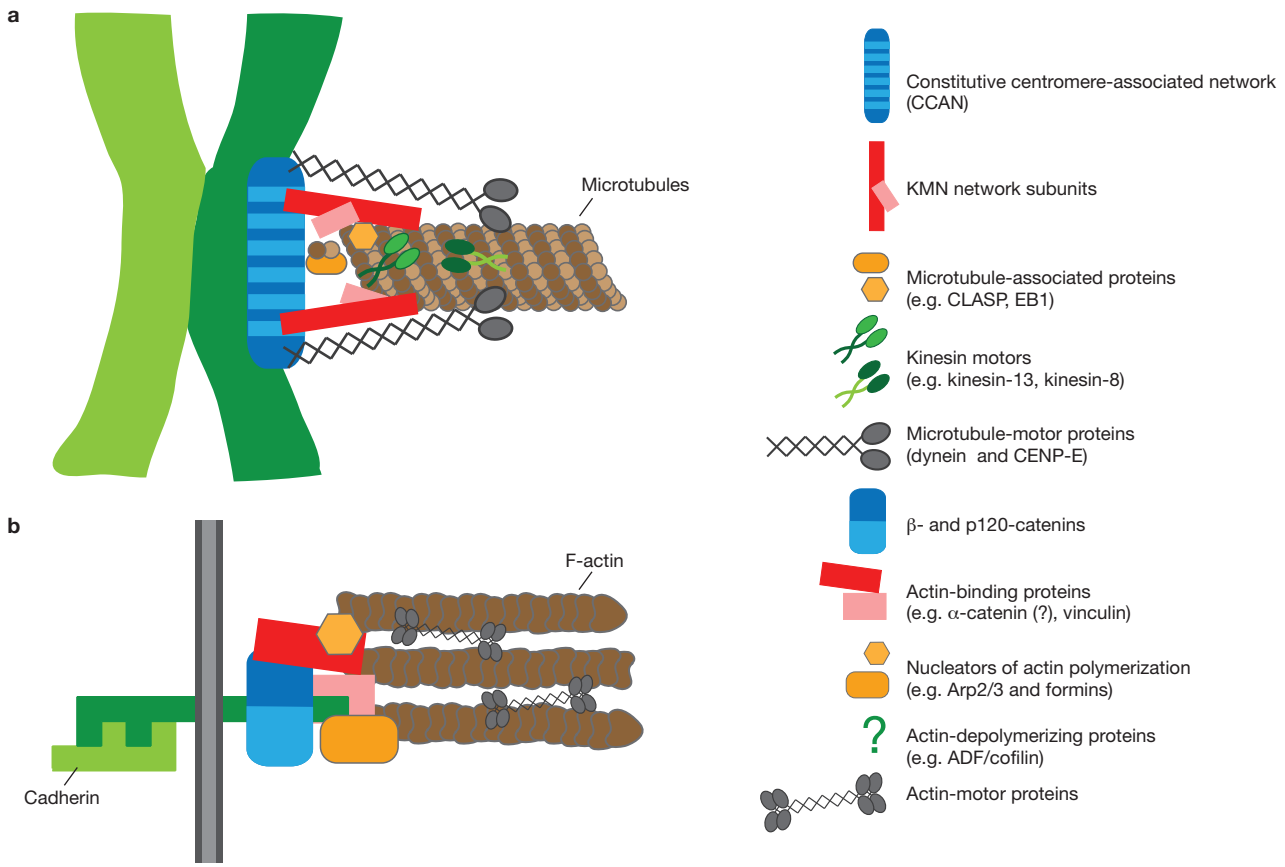

Figure 1 Adherens junctions may be functionally analogous to the kinetochore–sister chromatid complex. Both the kinetochore–sister chromatid complex (**a**) and the adherens junction (**b**) mediate attachment while simultaneously sensing and reacting to changes in tension exerted from opposing directions. In the AJ, the attachment is between cells and the tension changes are exerted primarily by the F-actin cytoskeleton, whereas in the kinetochore–sister chromatid complex the attachment is between sister chromatids and tension changes are exerted primarily by the microtubule cytoskeleton. Accordingly, AJs are equipped with proteins that link the cells (cadherins), tether the cadherins to F-actin (vinculin and α-actinin), sense and respond to tension (α-catenin), and impart force (myosins). The kinetochore–sister chromatid complex is respectively equipped with securins, the KMN network, and various microtubule-associated proteins and motors. Finally, both structures contain proteins that regulate the assembly and disassembly of their associated polymer system.

and accumulation of cadherin apically along the incipient AJ[8], whereas myosin IIB might preferentially localize near the ends of actin filaments that extend towards the cell interior, to promote lateral pulling on the AJ, a manipulation associated with increased cadherin accumulation[13].

Another possibility is that the differences in function of myosin IIA and myosin IIB reflect differences in their basic motor properties. Although myosin IIA and myosin IIB share many biochemical features, they differ strikingly in one respect — their duty ratio, a measure of the proportion of time they remain tightly bound to F-actin during the ATPase cycle. Specifically, the duty ratio of myosin IIB is calculated to be at least three times larger than that of myosin IIA[14,15]. This means that myosin IIB is much more likely to stay associated ('hold on tightly') with F-actin in the cell, a point supported by recent observations of the interaction between myosin IIB and F-actin *in vitro*[16]. In contrast, myosin IIA is more likely to bind and release F-actin quickly ('let go lightly') in the cell. Thus, myosin IIB is a strong candidate as a motor for the generation of sustained tension.

Why does that matter? As mentioned above, AJs somehow sense tension, and it was recently shown that α-catenin, a protein that interacts indirectly with cadherins, is likely to be an important component of the tension-sensing mechanism[17]. In brief, myosin-II-dependent imposition of tension unfolds α-catenin, unmasking a binding site for vinculin, a protein that binds to F-actin. Vinculin recruitment to α-catenin increases the amount of AJ-associated F-actin, which in turn results in recruitment of more cadherin, α-catenin and so forth. Although it remains to be shown directly that myosin IIB is specifically responsible for providing the pulling force that opens the catenin, this model is consistent with the observations that the loss of myosin IIB, but not myosin IIA, results in reduced vinculin recruitment and F-actin accumulation at AJs[2,7,17]. Further, Smutny *et al.* now show that although motor function — the ability to translocate F-actin with respect to the myosin as opposed to simply crosslinking F-actin — is dispensable for the ability of myosin IIA to promote cadherin clustering and accumulation, it is essential for myosin IIB's ability to promote junction fine-tuning. Thus, the available data are consistent with a model in which myosin IIB exerts a pulling force on AJs that results in catenin-dependent recruitment of vinculin, a function for which myosin IIB is uniquely suited because of its ability to produce a tensile, rather than contractile force.

It is also probable that differential regulation contributes to the different roles of myosin IIA and myosin IIB. Smutny *et al.* find that AJ localization of myosin IIA depends on signalling by the small GTPase, Rho, whereas localization of myosin IIB depends on another small GTPase, Rap1. These findings are consistent with previous observations that Rho-dependent kinase and other pathways show isoform-specific regulation of myosin II in migrating cells (for example, in ref. 18). They are also consistent with the observation that AJs are sites of localized Rho and Rap1 signalling[3,19].

One of the fascinating aspects of this work is that Smutny *et al.* also addressed whether the two myosins can functionally compensate for one another. They found that excess myosin IIB can compensate for loss of myosin IIA but not *vice versa*, clearly demonstrating that myosin IIB does indeed have a unique role. Why then, if myosin IIB can perform the functions of myosin IIA do the cells express both isoforms and, indeed, express approximately four times more myosin IIA than myosin IIB? One possibility is that although myosin IIB is capable of doing whatever myosin IIA can do, it does so more slowly. If this is the case, it will be of considerable interest to subject cells expressing no myosin IIA and excess myosin IIB with challenges that normally result in rapid junction reorganization, such as wounding, or transmigration of leukocytes, and determine whether AJs respond with normal kinetics.

In summary, AJs are associated with two myosin-II isoforms, each of which has different biochemical properties and each of which have different but linked roles in AJ function. Add to the presence of multiple AJ motors the identification of α-catenin as a promising candidate as a tension sensor[17], and work indicating that AJs are sites of local signalling by small GTPases[3,19], and a return to the analogy proposed above is warranted: these findings in the AJ neatly parallel the known features of kinetochores, which have at least four microtubule motors, a tension-sensing mechanism, and a variety of signalling molecules[5]. It will thus be fascinating to see if these two structures, both of which can mediate adhesion, also share other key properties.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Rab6 and myosin II at the cutting edge of membrane fission

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Rab GTPases regulate the dynamics of transport carriers by participating in their translocation across the cytoplasm, and in their docking and fusion with acceptor compartments. An interaction between Golgi-associated Rab6 and myosin II has now been shown to regulate the fission of Rab6-positive carriers, illuminating a previously unappreciated role for Rab6 and the actomyosin system in carrier biogenesis.

Long-range transport of newly synthesized or endocytosed cargo between membrane-bound compartments in eukaryotic cells relies on transport carriers that form from the membrane of a donor compartment, move along cytoskeletal tracks, and then dock and fuse with an acceptor compartment. The Rab and Arf GTPases control the budding, movement and docking of transport carriers in multiple trafficking pathways[1]. Arf proteins are generally thought to control carrier biogenesis by regulating budding, cargo sorting and vesicle fission, whereas Rab GTPases control the trafficking of secretory and endocytic carriers. These properties of Rab proteins are mediated through interactions with effector proteins, including processive molecular motors. On page 645 of this issue, Miserey-Lenkei *et al.* now report that two splice variants of Rab6, Rab6A and Rab6A′, interact with the non-processive motor protein, myosin II, to regulate the fission of transport carriers from the Golgi complex[2].

Rab6A and Rab6A′ are associated with tubular-vesicular structures that bud from the Golgi complex, and regulate the motility of these vesicles as they move towards the cell periphery[3]. Miserey-Lenkei *et al.* now show that Rab6 also controls the generation of these structures at the Golgi complex. Depletion

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