Dispatches

Motor Proteins: Tightening Your Belt with Myosin VI

New work shows that the motor protein myosin VI, acting through vinculin, plays a key role in the maturation of cadherin-based adherens junctions in epithelial cells.

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Myosin VI, an actin-based motor, leads a complicated life. It is best-known for its role in the transport of endocytic vesicles [1,2], but it has a wider range of functions which all involve linking membranes to the actin cytoskeleton. For example, myosin VI maintains the separation between actin-filled stereocilia in sensory hair cells of the cochlea by anchoring the membrane between adjacent stereocilla to the underlying actin network [3,4]. Myosin VI has also been found to play a role in the internalization of endocytic vesicles [5,6]. The busy lifestyle of myosin VI is a reflection of its unique motor properties — it is processive and consequently capable of transporting cargo for long distances [7,8]; however, if subjected to tension, it can switch to serving as an anchor [9]. Myosin VI is also the only myosin that moves to the pointed (slow growing) end of the actin filament [10], allowing it to work in apposition to almost any other myosin, either by providing an opposing force or by moving organelles in the opposite direction of a plus-end myosin. Motor properties alone do not fully account for myosin VI's multitasking ability: this motor protein interacts with numerous enablers (binding proteins) which harness its activity at particular locations in the cell or on specific organelles [1]. Recent work has now shed light on one of the many, less-understood functions of myosin VI — its role in cell–cell adhesion — and has identified yet another new, and quite interesting potential binding partner in epithelial cells, vinculin [11].

Elegant studies of border cell migration in the fruit fly *Drosophila* first implicated myosin VI in adhesion and cell migration [12]. Border cells are recruited from the oocyte follicle to surround two polar cells, and this cluster of cells moves en masse to the border between the nurse cells and oocyte. Targeted depletion of myosin VI from border cells results in a significant drop in migration of the cell cluster, accompanied by a loss of protrusions observed in migrating cells. The development of myosin VI mutant embryos is also defective in processes requiring tight cell–cell contact, such as dorsal closure [13]. While these studies strongly suggested a role for myosin VI in cadherin-based adhesion, its contribution to this process in the fly is not yet clear.

The interesting findings in *Drosophila* prompted Maddugoda *et al.* [11] to explore the role of myosin VI in cadherin-based cell–cell adhesion using a cultured mammalian epithelial cell model. The formation of epithelial sheets from individual cells is a complex, multi-step process. Cadherins, calcium-dependent adhesion molecules, mediate the initial contacts between adjacent cells. These early contacts then expand and 'zipper' the two cells together at their apical borders. The cadherin links require, and are strengthened by, connections to the actin cytoskeleton through an anchoring complex, and the initial stages of adhesion are characterized by a loose belt of actin that becomes tighter as cell–cell connections mature (Figure 1). Myosin VI localizes only to mature, linear cell–cell junctions in an E-cadherin-dependent manner in epithelial cells and co-precipitates with E-cadherin.

Interestingly, depletion of myosin VI results in striking alterations in cadherin-dependent adhesion — the typical continuous E-cadherin distribution at sites of cell–cell contact in mature cells is lost and instead a serrated, punctate distribution is observed. In place of the continuous, tight belt of actin typical of mature contacts between control cells, the junctional actin in myosin VI-depleted cells has a frayed, disorganized appearance. Vinculin, an actin-binding protein that participates in linking adhesion receptors to the actin cytoskeleton, is lost completely from the remaining regions of cell–cell contact. All of these changes result in a loss of monolayer cohesion and fragmentation of contacts between neighboring cells, establishing a role for myosin VI in cadherin-based adhesion. These observations directly demonstrate a role for myosin VI in epithelial cell adhesion and suggest that it is part of the cytoskeletal adapter complex that interacts with cell adhesion receptors.

How does myosin VI contribute to the maturation of epithelial cell adhesions? The answer appears to lie in the relationship between myosin VI and vinculin. Depletion of vinculin from epithelial cells causes a disruption in E-cadherin localization quite similar to that observed in myosin VI-depleted cells. E-cadherin immunoprecipitates contain both vinculin and myosin VI, but vinculin is missing from the immunoprecipitate in the absence of myosin VI, consistent with the observed loss of vinculin from regions of cell–cell contact in cells with reduced levels of myosin VI. Vinculin is composed of two major domains, an amino-terminal head domain that can associate with various cytoskeletal proteins involved in adhesion, and a tail region that binds actin and acidic phospholipids [14]. Interaction
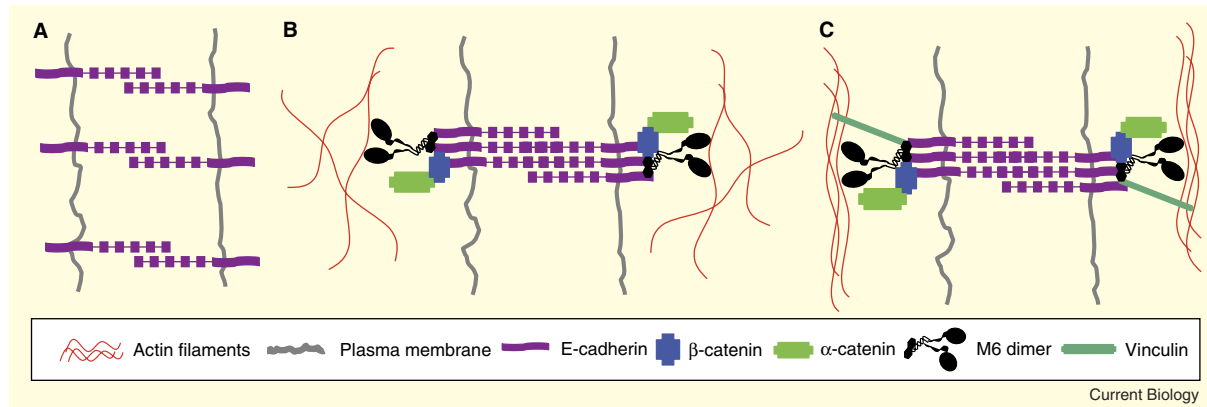
Figure 1. A model of myosin VI-mediated adherens junction maturation.

(A) Transmembrane cadherin monomers begin to form dimers with cadherins in neighboring cells. (B) As dimers form they cluster and recruit additional junction members. Only β-catenin, α-catenin and myosin VI are shown for simplicity. The perijunctional actin network is still loose and disorganized. (C) Vinculin is recruited by myosin VI and aids in tightening of the circumferential actin belt, further stabilizing the anchoring complex.

between these two domains prevents vinculin from binding to its partners (including actin) and must be disrupted to activate vinculin [14,15]. The vinculin head and tail regions were fused individually to the β-catenin transmembrane domain to target them to the cadherin adhesion complexes and the fusions expressed in the myosin VI-depleted cells [11]. Surprisingly, both chimeras rescued the adhesion defect of these cells and unexpectedly the head fusion promoted greater rescue than the actin-binding tail region fusion. Finally, overexpression of myosin VI in vinculin-depleted cells does not restore mature adhesions. These findings demonstrate that vinculin acts downstream of myosin VI to mediate the maturation of cadherin-based adhesions but they do leave open the question of vinculin's precise role.

Many questions about the relationship between E-cadherin, myosin VI and vinculin remain. For example, do these proteins interact directly with each other? Does myosin VI provide a direct link, via vinculin, between cadherin and the actin cytoskeleton or are other binding proteins involved (Figure 1)? In follicular epithelial cells of the ovary *Drosophila* myosin VI binds directly to β-catenin and the levels of both *D*E-cadherin and β-catenin are dependant on myosin VI (the reciprocal is also true) [12]. While mammalian myosin VI does not stabilize E-cadherin or β-catenin

[11], it is clearly linked to them and could perhaps act to tether the cadherin–catenin complex to the underlying actin cytoskeleton via interactions with β-catenin and vinculin. It is tempting to speculate that incorporation of myosin VI into an adhesion complex imposes tension on the motor that switches it into an anchor, contributing then to linking junctional actin filaments to the cadherin adhesion complex. Consistent with this possibility are the observations that expression of the myosin VI tail in the myosin VI-depleted cells does not rescue the adhesion defect, and ectopic expression of the tail in control cells disrupts adhesion [11]. Interestingly, the tail is no longer detected at the periphery of the cell but this delocalization is most likely due to the loss of the integrity of the cadherin adhesion complex. The diffuse cytosolic localization of the tail in the myosin VI depleted cells and its ability to co-precipitate with both E-cadherin and vinculin suggests that the tail is in a cytosolic complex with both of these molecules, perhaps even on membrane vesicles, as a result of the disintegration of peripheral myosin VI docking sites.

The finding that the motor domain of myosin VI is required for proper organization of an actin-based structure suggests a tethering or cross-linking role for this motor. Precedent for such a role comes from an analysis of sperm individualization in *Drosophila*. A beautiful recent

study [16] revealed that the myosin VI motor region is essential for localization of this myosin to the specialized actin-rich individualization cone, and that it resides in these structures for long periods of time. Furthermore, the actin cone is less dense in the absence of myosin VI. These findings are consistent with myosin VI functioning either as an actin filament cross-linker or as an adaptor molecule for other proteins that direct formation of the proper actin network. The observations in epithelial cells are consistent with the latter model where myosin VI that resides at the cadherin adhesion sites recruits vinculin that, in turn, is required for the formation of a tight belt of actin that is linked to the adhesions [11].

One wonders how the activity of the exact same motor can be modulated to allow it to function in two extremely different manners — anchor or translocator — given the variety of roles myosin VI can play. The kinetic properties of myosin VI, most likely dictated by inserted sequences unique to this myosin [17], undoubtedly play a role in its functional flexibility and tension clearly alters its kinetic cycle [9]. However, it remains to be seen if other regulatory modifications (for example, phosphorylation) or interactions with other binding partners can also influence myosin VI's mode of action. The potential importance of these latter interactions is highlighted by the

finding that myosin VI association with endocytic vesicles promotes dimerization, a switch likely to be essential for processive translocation of these organelles [18]. The cultured epithelial cell model used by Maddugoda et al. can now be exploited to further dissect the molecular details of myosin VI's contribution to cadherin-based adhesion and how its activity is modulated. Such studies are likely to also provide valuable insight into the contribution of myosin VI to the aggressive migration of metastatic ovarian and prostate cancer cells that express an excess of this motor protein [19,20].

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Human Evolution: Neandertal Gene Speaks out

An analysis of Neandertal DNA indicates that they shared with living humans a form of the FOXP2 gene, the absence of which impairs speech and cognitive processing related to human language.

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The study of human origins since the 19th century has focused on purportedly uniquely human biological and behavioral characteristics. The list of such characteristics is a long one: expanded brains, fine manipulation, tool-use, bipedalism, reduced teeth and faces, language, social caring, clothing, disposal of the dead, ornamentation, art, and hunting, among others. In all of this, there is the assumption that there is a silver bullet which identifies us as 'human' and which can be used to identify past human forms as more or less 'human'. It should be obvious that there is no Rubicon in human evolution that made us us. Nonetheless, this particularist approach to human evolution persists, with the current focus on the past several hundred thousand years during which modern human anatomy emerged. This has produced a plethora of articles regarding the antiquity of human 'modernity', concerning both the antiquity of modern human behavior and what constitutes 'modernity' behaviorally. Recent work on Neandertals [1], published in this issue of Current Biology, sheds some new, direct light on that ongoing issue.

One human characteristic is persistently used to define us: language. In this, I am using language as an open-ended communication system with an expandable symbol set (vocabulary) and a structure which imparts meaning (grammar). Human language is normally transmitted vocally, through speech, although it can be exchanged through a variety of auditory, visual and tactile forms. It is also a social entity, and meanings conveyed by language are extensively modified by context and non-verbal communication.

There have been many attempts to determine the antiquity of