Cadherin adhesome at a glance

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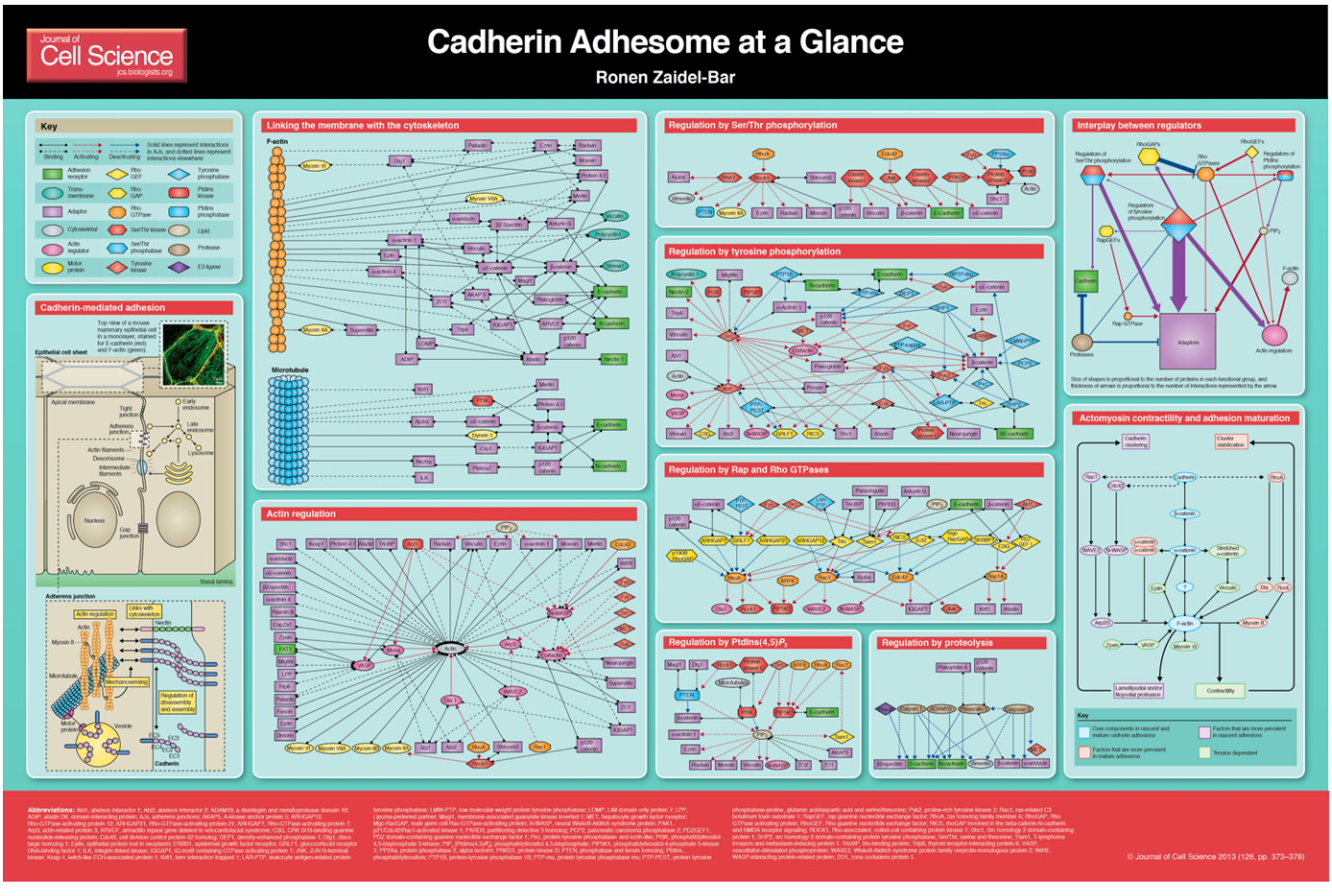
Multicellular life hinges on molecular ensembles that are able to mediate adhesion between cells (Abedin and King, 2010). One such conserved structure is the adherens junction (AJ); here, transmembrane glycoproteins, the cadherins, interact homophilically with cadherins that are located on apposing cells, on the outside, and are anchored through a conglomerate of adaptor proteins to the actomyosin cytoskeleton on the inside (Harris and Tepass, 2010; Niessen et al., 2011).

This Cell Science at a Glance article makes an inventory of the structural and regulatory components of AJs, and the interactions between them, collectively referred to as the cadherin adhesome, or cadhesome. Assembling this blueprint of building blocks and connections serves to highlight the molecular complexity and potential diversity of AJs, and offers an opportunity to address some important questions, such as: how do cadherins connect with the cytoskeleton? How are actin dynamics regulated at AJs? Which molecular switches regulate AJ dynamics? And what is the role of tension in the maturation of AJs?

Formation and function of AJs

Cell–cell adhesion in AJs is primarily mediated by a twofold symmetric interaction between the first extracellular (EC1) domains of two cadherins on juxtapositioned cells (reviewed by Brasch et al., 2012). This trans-interaction has been defined by crystallography as a 'strand swap', in which a conserved tryptophan side chain of one EC1 inserts into a hydrophobic pocket in the EC1 of the opposing cadherin (Harrison et al., 2011). In addition to binding in trans, cadherins can also form cis-interactions through an interaction between the EC1 and EC2 domains of two cadherins that are located on the same cell (Harrison et al., 2011). Despite our detailed understanding of the interactions at the structural level, we are still missing many details regarding the organization of cadherins on the surface of cells. Whether cis-interactions precede or follow trans-interactions, for example, is still an open question. Chemical cross-linking experiments suggested that cis-dimers exist on the cell surface (Takeda et al., 1999), and some *in vitro* experiments suggested that lateral dimerization is required for adhesion (Brieher et al., 1996). However, other experiments and models indicate that trans-dimers, with



Box 1. Assembly of the cadhesome

Components

Primary literature from the past 30 years was searched on PubMed in order to identify proteins that satisfy the following criteria: (1) they were found to colocalize with cadherin or one of the catenins in cells or in tissue and (2) they interact directly with one of the cadherins or one of the catenins, or their knockdown or overexpression was shown to affect the structure or dynamics of AJs. Although invertebrate model organisms, such as the fly and nematode, have been useful for the study of cadherin-mediated adhesion, this version of the cadhesome was limited to proteins characterized in mammalian models.

The cadhesome contains all 18 classic cadherins of type I and II (Oda and Takeichi, 2011), but most of the information found in the literature pertains to E-cadherin, and only to a lesser extent to N-cadherin and VE-cadherin; very little information, if at all, is known about other types of cadherin. Although some components, such as neurojungin, are expressed exclusively in non-epithelial cell types, the vast majority of proteins have been shown to be expressed in a type of epithelia.

Interactions

After compilation of the cadhesome components, the primary literature was searched to map all known direct interactions between them. An interaction was considered as direct if it had been shown to occur both *in vitro* (by yeast two-hybrid or pulldown experiments with purified proteins) and *in vivo* (by colocalization, FRET or co-immunoprecipitation). These stringent criteria should substantially decrease the number of false positives. However, it is likely that many true interactions have been excluded owing to the lack of *in vitro* or *in vivo* data confirming their existence.

Interactions between proteins have been classified here as binding, activating or deactivating. Binding refers to a non-enzymatic protein–protein interaction, whereas activating or deactivating implies that one protein modifies the other. Here, phosphorylation of tyrosine, serine/threonine and phosphatidylinositol, guanine nucleotide exchange, GTPase and [PtdIns(4,5)P_2] binding are considered activating, whereas tyrosine, serine/threonine and phosphatidylinositol dephosphorylation, GTP hydrolysis, cleavage by protease and ubiquitination are classifed as deactivating. Interactions were further distinguished as occurring either 'in AJs' or 'outside AJs', depending on whether there is evidence for an interaction within AJs or if the interaction was demonstrated in another context (see supplementary material Table S2).

their reduced molecular flexibility, are a prerequisite for the formation of cis-dimers and higher order assemblies (Brasch et al., 2012; Wu et al., 2010; Zhang et al., 2009).

Before contacting another cell, cadherin is present all over the cell surface, including at the tips of filopodia and along the leading edge of lamellipodia. Shortly after a cell makes contact with another cell, the concentration of cadherin at the adhesion site increases as they cluster and form higher order structures (Adams et al., 1998; Raich et al., 1999; Vasioukhin et al., 2000). The clustering of cadherin, which is driven by trans- and cis-interactions on the outside of the cell, has profound effects on the inside of the cell. The intracellular tail of cadherin binds β-catenin before arrival at the membrane (Hinck et al., 1994), but after cadherins cluster, they recruit a multitude of additional structural and regulatory proteins that make up the AJ 'plaque' (Baum and Georgiou, 2011; Hartsock and Nelson, 2008; Troyanovsky, 1999). By far the most studied and best understood of these 'plaque' proteins are the catenins (α-, β-catenin and p120), which have important structural and regulatory roles (Gumbiner and McCrea, 1993; Nelson, 2008; Shapiro and Weis, 2009), but over 160 proteins have been reported to associate with AJs (see below). Concomitant with the clustering of cadherins and plaque proteins, F-actin is also recruited to and/or polymerized at these sites (Adams et al., 1996; Ivanov et al., 2005; Kovacs et al., 2002; Vasioukhin et al., 2000).

Once formed, AJs constitute a physical link between the actin cytoskeleton of neighboring cells, providing the mechanical coordination needed for morphogenetic processes, such as gastrulation, neurulation, convergent extension, epithelial sheet-sealing and cell migration (Gumbiner, 2005; Halbleib and Nelson, 2006; Kardash et al., 2010; Lecuit, 2005; Lien et al., 2006). AJs also 'sense' their chemical and mechanical environment and serve as signaling centers in pathways controlling cell growth, differentiation and fate (Cavallaro and Dejana, 2011; Halbleib and Nelson, 2006; Lechler, 2012; Leckband et al., 2011). Importantly, AJs also regulate the homeostasis of adult tissues, and AJ dysfunction is involved in epithelial–mesenchymal transformation, a hallmark of cancer metastasis (Jeanes et al., 2008; Takeichi, 1993). Equally important as their assembly is the disassembly of AJs, a process that is tightly regulated by various signaling pathways (Baum and Georgiou, 2011; D'Souza-Schorey, 2005; Green et al., 2010); this way cells can modulate their adhesive properties in response to intrinsic or external cues in a temporally and spatially controlled manner.

Complexity and diversity of AJs

This short review and the accompanying poster are based on my compilation of information from primary literature from the last 30 years regarding AJ components and the direct interactions between them. The criteria used for assembling this dataset (the 'Cadhesome') are detailed in Box 1, and the complete annotated and referenced lists of components and interactions are available as supplementary material Tables S1 and S2.

Over 170 proteins have been reported to colocalize with cadherin or catenins in AJs, and either directly interact with them or affect AJ dynamics (see supplementary material Table S1). Broadly speaking, the cadhesome is inhabited by two types of proteins: structural and regulatory proteins. Structural proteins consist of other transmembrane receptors in addition to cadherin, including Ca^{2+}-independent immunoglobulin-like adhesion receptors of the nectin family (Sakisaka et al., 2007), cytoskeletal filaments, motors and over sixty adaptor proteins, many of which bind to cadherin or the cytoskeleton directly. For most of these adaptor proteins, it is not known whether they have a structural role or serve as a scaffold for signalling, or both. The presence of >70 regulatory proteins at AJs is not surprising, as a variety of cellular pathways regulate AJ dynamics, and signaling from AJs, in turn, controls important cellular behaviors (Braga, 2002; Cavallaro and Dejana, 2011; Lechler, 2012; McCrea et al., 2009; Stepniak et al., 2009). The largest groups of regulators are tyrosine kinases and phosphatases, and GTPases and their activators and inhibitors. These and other regulatory switches of the cadhesome will be discussed later.

Although a few cadhesome components appear to be cell-type specific (e.g. neurojungin in neurons and KRIT1 in endothelia), the majority of cadhesome components have been observed in more than one cell type. This, however, does not necessarily mean that most components are present in every AJ. It has been shown that the composition of AJs can change over time (Green et al., 2010) in response to changes in tension (Taguchi et al., 2011), and varies between different cell types (Borrmann et al., 2000) and even between different AJs within the same cell (Peitsch et al., 1999). However, considerably more work remains before we can confidently classify subtypes of AJs according to their molecular composition and function.

The complexity of AJs is further compounded by the large number of possible interactions between their constituents. Close to 390 pairwise interactions have been reported among the 174 cadhesome components (see supplementary material Table S2), a subset of which are illustrated in the accompanying poster. Close to two thirds of these interactions are classified as binding, whereas just over one third are classified as either activating or deactivating, depending on the respective enzymatic reaction. The dataset and the poster distinguish between 230 interactions that have been shown within the context of AJs, and 158 interactions that were found to occur outside of AJs, and for which it is not yet known whether they also take place in AJs.

It is important to emphasize that although the poster depicts all of the possible interactions among cadhesome components, it is highly likely that only a subset of these interactions occur at the same time in the same AJ. Conceivably, every interaction is regulated and can be turned 'on' or 'off' under different conditions, thus allowing the AJ to respond to changes in the environment and signaling.

Linking cadherins with the cytoskeleton

Electron micrographs show a dense plaque of protein ~25 nm wide between the plasma membrane and F-actin at AJs (for example see Yonemura et al., 1995). So far, we know very little about the three-dimensional organization of proteins within this plaque. Super-resolution microscopy and tagging of proteins in electron tomograms has recently been performed on integrin adhesion sites (Kanchanawong et al., 2010; Patla et al., 2010), and applying similar techniques to AJs should substantially enhance our understanding of their ultrastructure. Until then, the map of protein interactions might provide clues on the structural organization of AJs.

The interaction between cadherins and the cytoskeleton has been the focus of intense research for the past 20 years, and yet the molecular details remain poorly understood (Yonemura, 2011). Two seemingly contradicting features characterize this interaction; (1) it is dynamic, as shown by fluorescence recovery after photobleaching (FRAP) experiments of cadherins, plaque proteins and actin (Yamada et al., 2005), and (2) it transmits tensile stresses, as observed in morphogenetic processes, such as apical constriction (Martin et al., 2009), and as directly demonstrated by cadherin adhesions that apply force on pillars (Ladoux et al., 2010), as well as by an intra-molecular stress sensor (Borghi et al., 2012). One model that is consistent with both characteristics hypothesises that cadherins are connected to F-actin by multiple transient links, with each link transmitting tension for the duration of its existence (Gates and Peifer, 2005).

For many years it was thought that the cadherin–F-actin connection is solely mediated by cadherin-bound β-catenin that binds to F-actin-bound α-catenin (Gates and Peifer, 2005), but in 2005 the Nelson and Weiss groups contradicted this notion by showing that α-catenin that is bound to β-catenin cannot bind F-actin directly (Drees et al., 2005; Yamada et al., 2005). Nevertheless, it still appears to be the case that α-catenin and β-catenin are central to the binding of cadherin to F-actin, if not directly, then through additional adaptors, such as vinculin, eplin or zona occludens protein 1 (ZO1, also known as TJP1) (reviewed by Yonemura, 2011). Other transmembrane cadhesome proteins, such as nectin and vezatin, can connect to F-actin through adaptors, such as afadin, or motor proteins, such as myo7A (Küssel-Andermann et al., 2000; Sakisaka et al., 2007). In addition, AJs contain a number of membrane-bound actin-binding proteins, such as the so-called ezrin, radixin and moesin (ERM) proteins, which link cortical actin to the plasma membrane (Neisch and Fehon, 2011).

There are also several lines of evidence to suggest an interaction between AJs and microtubules. The interaction between p120-catenin and the AJ components PLEKHA7 and Nezha (also known as CAMSAP3) anchors the minus ends of non-centrosomal microtubules to AJ, which could direct kinesin-driven vesicles towards AJ (Meng et al., 2008). Moreover, the interaction between β-catenin and dynein serves to capture the plus ends of microtubules at AJs and is important for the establishment of apico–basal cell polarity (Bellett et al., 2009; Ligon et al., 2001). In some specific cell types, such as in the lens or in dermal endothelial cells, there is evidence for a link between classical cadherins in AJ and the intermediate filament vimentin, mediated by γ-catenin and desmoplakin (Kowalczyk et al., 1998; Leonard et al., 2008).

Actin dynamics and F-actin structures at AJs

Cadherin adhesions are not simply tethered to a pre-formed actin network, but rather they have an active role in shaping the F-actin landscape that surrounds them (reviewed by Ratheesh and Yap, 2012). They do so by recruiting different actin nucleators and elongation factors, and a variety of actin dynamics regulators, cross-linkers and motors. At least three different actin structures can be found in association with AJs: F-actin bundles oriented parallel to and slightly separated from the plasma membrane; stress fibers oriented orthogonally to the plasma membrane with one end terminating at AJs; and a structurally unresolved pool of F-actin in close proximity to the membrane alongside cadherin clusters (reviewed by Niessen et al., 2011). The relationship between these different actin assemblies is not well defined. However, it has been shown that active actin polymerization that occurs adjacent to the membrane in AJs can contribute to the structure of circumferential actin bundles (Kovacs et al., 2011).

Actin structures at AJs can appear to be stable for many minutes, but FRAP experiments suggest that the actin filaments themselves turn over within seconds, indicating that actin at AJs is continuously being polymerized and depolymerized (Kovacs et al., 2002; Kovacs et al., 2011; Yamada et al., 2005).

In light of the variety of actin networks observed in AJs, it is not surprising to find in the cadhesome Arp2/3, which is responsible for nucleating and polymerizing a dendritically branched network, as well as the formin diaphanous and Ena/VASP proteins, which nucleate and elongate long

unbranched actin filaments (Carramusa et al., 2007; Kovacs et al., 2002; Scott et al., 2006). Branched networks push membranes and drive protrusions as well as endocytosis, and unbranched filaments can coalesce with myosin to form contractile bundles (reviewed by Chesarone and Goode, 2009). How the activity of the different factors is coordinated in space and time at AJs is not known. The cadhesome also contains the activators of Arp2/3, mDia1 (also known as DIAPH1) and VASP, which themselves need to be activated by Rho GTPases. Thus, the type of F-actin network that is polymerized is likely to be determined by which of the activators are recruited into AJs, and by the balance between active Rho GTPases. Once several F-actin filaments are polymerized, they can be arranged into different higher-level structures, and connect with the AJs through different actin-binding adaptors. The cadhesome contains several actin cross-linking proteins, such as α-actinin and filamin, and several myosin motors that can serve as cross linkers as well as force-generating machines (Knudsen et al., 1995; Maddugoda et al., 2007; Smutny et al., 2010; Wakamatsu et al., 2011).

Regulatory switches in AJs

During development and also in adults, cells regulate their AJs in response to changing needs (reviewed by Baum and Georgiou, 2011; Green et al., 2010; Lecuit, 2005). Long-term modulation of AJs, such as in epithelial–mesenchymal transition, is controlled at the levels of transcription, translation and trafficking, which determine the availability of cadhesome components at the membrane (Delva and Kowalczyk, 2009; van Roy and Berx, 2008). More rapid control of AJ dynamics is mediated by post-translational regulation of cadhesome proteins, which affects their activity and interactions (Bertocchi et al., 2012; van Roy and Berx, 2008). The types of post-translational regulation found within the cadhesome are: serine or threonine phosphorylation, tyrosine phosphorylation, GTPase binding, lipid binding and proteolysis.

I refer to the activity of regulatory proteins as ‘switches’, because in many cases their action results in switching ‘on’ or ‘off’ an interaction of a protein or its activity (see also Zaidel-Bar and Geiger, 2010). For example, tyrosine phosphorylation of E-cadherin creates a binding site for the cell polarity protein NUMB (Wang et al., 2009); phosphorylation of a threonine residue on ERM proteins keeps them in an active, actin-binding state (Yonemura et al., 2002); binding of RAP1 to afadin activates its capacity to stabilize AJs (Hoshino et al., 2005); and cleavage of E-cadherin by ADAM10 downregulates cell–cell adhesion (Maretzky et al., 2005).

Such regulatory switches are likely a general principle, although for most of the enzyme-substrate interactions in the cadhesome we do not know the functional implications of the modification for the target protein or for AJ dynamics. Nevertheless, grouping together all the known interactions between proteins from the same regulatory group helps to identify some design principles of AJ regulation. For example, it can be seen that regulators of actin dynamics are controlled by Rho GTPases, tyrosine phosphorylation and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], whereas cadherins are regulated by serine/threonine phosphorylation and proteases.

Interplay between AJs and tension

In a similar manner to integrin-mediated adhesions (Zaidel-Bar et al., 2004), cadherin-mediated adhesions undergo a process of maturation, during which their composition and associated actin structures evolve (Baum and Georgiou, 2011; Green et al., 2010; Troyanovsky, 1999). In recent years, it has become clear that tension has an important role in this process (Leckband et al., 2011; Papusheva and Heisenberg, 2010). However, it remains difficult to determine which of the differences between nascent and mature cadherin adhesions are actually driven by an increase in tension.

Cadherin ligation and clustering is thought to activate the small GTPases Rac1 and Cdc42, which promote lamellipodial and filopodial protrusions (Kim et al., 2000; Kovacs et al., 2002). These protrusions help to increase the contact area with the neighboring cell and thus positively feedback on cadherin ligation and clustering. At some point during AJ maturation, probably owing to the recruitment of RacGAPs and RhoGEFs (e.g. see Elbediwy et al., 2012; Ratheesh et al., 2012), there appears to be a switch from Rac1 and Cdc42 to RhoA activity (Yamada and Nelson, 2007). As the protrusive activity subsides, RhoA activates the formin mDia1 and Rho Kinase, whose joint activity leads to the formation of contractile actomyosin bundles (Watanabe et al., 1999; Smutny et al., 2010). Contractility driven by myosin II creates tension in the actin network, which pulls on the AJ plaque proteins (Borghi et al., 2012; Liu et al., 2010; Miyake et al., 2006; Shewan et al., 2005). Interestingly, tension appears to signal the recruitment of additional actin polymerization factors, namely Ena/VASP, which promote long, unbranched actin filaments (Kris et al., 2008). Ena/VASP might be recruited by zyxin, an adaptor that has been shown to be mechanosensitive in AJs, focal adhesions and stress fibers (Hoffman et al., 2012; Nguyen et al., 2010; Zaidel-Bar et al., 2003).

The adaptor α-catenin is thought to contribute to AJ maturation in two ways. In nascent adhesions, it is presumed to be primarily bound to cadherin (through β-catenin), but as the density of cadherin rises over a certain threshold, a subset of α-catenin could dimerize and bind to F-actin in a way that inhibits Arp2/3 from binding (Benjamin et al., 2010; Drees et al., 2005), thus promoting the shift from a protrusive activity to contractility. In the contractile phase, α-catenin that remains bound to cadherin has been hypothesized to be stretched, exposing a cryptic binding site for vinculin (Yonemura et al., 2010). The tension-dependent recruitment of the actin-binding protein vinculin serves to reinforce the link between cadherin and F-actin (le Duc et al., 2010). Other proteins, such as myosin VI and eplin, have been shown to be specifically recruited to AJs under tension (Maddugoda et al., 2007; Taguchi et al., 2011), facilitating a positive feedback between contractility and maturation of AJs.

Perspectives

The textbook model of AJ usually depicts cadherin only interacting with the three catenins and actin. By contrast, the complexity of the cadherin adhesome – assembled here from the published literature – is striking. It will be necessary to study as many cadhesome components as possible under various conditions in order to fully appreciate the diversity of AJs, and to help us define different subtypes of AJs in different cells, and also in different regions of the same cell and under different conditions.

Summarizing what we know about AJs also underscores the gaps in our knowledge. For example, the number of phosphorylation events described in the cadhesome literature is only a fraction of

the phosphorylation events detected experimentally by mass spectrometry on cadhesome proteins (see PhosphoSitePlus at http://www.phosphosite.org).

The literature-based integrin adhesome has close to 200 components (Zaidel-Bar and Geiger, 2010), and recent proteomic studies of isolated focal adhesions suggest that the actual number of components is fivefold higher (Geiger and Zaidel-Bar, 2012). However, assembling ever-longer lists of proteins will not enhance our understanding unless it is coupled with information on their molecular interactions and function. Although some cadhesome components have been extensively studied, the majority has hardly been examined. Thus, the cadhesome presented here should be regarded as a 'work in progress'. A combination of high-throughput and classic cell biological approaches will be needed to generate a cadherin adhesome that captures the salient features of AJs sufficiently well for it to be made into a model that can predict the dynamics of AJ assembly and disassembly in response to given cellular conditions. I strongly believe that the most interesting and important features of AJs, such as mechanosensing, do not arise from the activity of one type of protein, but rather are emergent properties of the network of interactions between cadhesome proteins. Therefore, to answer questions pertaining complex phenomena, such as morphogenesis, a systems-level approach will prove to be essential.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.111559/-/DC1

References

Abedin, M. and King, N. (2010). Diverse evolutionary paths to cell adhesion. *Trends Cell Biol.* **20**, 734-742.

Adams, C. L., Nelson, W. J. and Smith, S. J. (1996). Quantitative analysis of cadherin-catenin-actin reorganization during development of cell-cell adhesion. *J. Cell Biol.* **135**, 1899-1911.

Adams, C. L., Chen, Y. T., Smith, S. J. and Nelson, W. J. (1998). Mechanisms of epithelial cell-cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin-green fluorescent protein. *J. Cell Biol.* **142**, 1105-1119.

Baum, B. and Georgiou, M. (2011). Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *J. Cell Biol.* **192**, 907-917.

Bellett, G., Carter, J. M., Keynton, J., Goldspink, D., James, C., Moss, D. K. and Mogensen, M. M. (2009). Microtubule plus-end and minus-end capture at adherens junctions is involved in the assembly of apico-basal arrays in polarised epithelial cells. *Cell Motil. Cytoskeleton* **66**, 893-908.

Benjamin, J. M., Kwiatkowski, A. V., Yang, C., Korobova, F., Pokutta, S., Svitkina, T., Weis, W. I. and Nelson, W. J. (2010). AlphaE-catenin regulates actin dynamics independently of cadherin-mediated cell-cell adhesion. *J. Cell Biol.* **189**, 339-352.

Bertocchi, C., Vaman Rao, M. and Zaidel-Bar, R. (2012). Regulation of adherens junction dynamics by phosphorylation switches. *J. Signal Transduct.* **2012**, 125295.

Borghi, N., Sorokina, M., Shcherbakova, O. G., Weis, W. I., Pruitt, B. L., Nelson, W. J. and Dunn, A. R. (2012). E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. *Proc. Natl. Acad. Sci. USA* **109**, 12568-12573.

Borrmann, C. M., Mertens, C., Schmidt, A., Langbein, L., Kuhn, C. and Franke, W. W. (2000). Molecular diversity of plaques of epithelial-adhering junctions. *Ann. N. Y. Acad. Sci.* **915**, 144-150.

Braga, V. M. (2002). Cell-cell adhesion and signalling. *Curr. Opin. Cell Biol.* **14**, 546-556.

Brasch, J., Harrison, O. J., Honig, B. and Shapiro, L. (2012). Thinking outside the cell: how cadherins drive adhesion. *Trends Cell Biol.* **22**, 299-310.

Brieher, W. M., Yap, A. S. and Gumbiner, B. M. (1996). Lateral dimerization is required for the homophilic binding activity of C-cadherin. *J. Cell Biol.* **135**, 487-496.

Carramusa, L., Ballestrem, C., Zilberman, Y. and Bershadsky, A. D. (2007). Mammalian diaphanous-related formin Dia1 controls the organization of E-cadherin-mediated cell-cell junctions. *J. Cell Sci.* **120**, 3870-3882.

Cavallaro, U. and Dejana, E. (2011). Adhesion molecule signalling: not always a sticky business. *Nat. Rev. Mol. Cell Biol.* **12**, 189-197.

Chesarone, M. A. and Goode, B. L. (2009). Actin nucleation and elongation factors: mechanisms and interplay. *Curr. Opin. Cell Biol.* **21**, 28-37.

D'Souza-Schorey, C. (2005). Disassembling adherens junctions: breaking up is hard to do. *Trends Cell Biol.* **15**, 19-26.

Delva, E. and Kowalczyk, A. P. (2009). Regulation of cadherin trafficking. *Traffic* **10**, 259-267.

Drees, F., Pokutta, S., Yamada, S., Nelson, W. J. and Weis, W. I. (2005). Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* **123**, 903-915.

Elbediwy, A., Zihni, C., Terry, S. J., Clark, P., Matter, K. and Balda, M. S. (2012). Epithelial junction formation requires confinement of Cdc42 activity by a novel SH3BP1 complex. *J. Cell Biol.* **198**, 677-693.

Gates, J. and Peifer, M. (2005). Can 1000 reviews be wrong? Actin, alpha-Catenin, and adherens junctions. *Cell* **123**, 769-772.

Geiger, T. and Zaidel-Bar, R. (2012). Opening the floodgates: proteomics and the integrin adhesome. *Curr. Opin. Cell Biol.* **24**, 562-568.

Green, K. J., Getsios, S., Troyanovsky, S. and Godsel, L. M. (2010). Intercellular junction assembly, dynamics, and homeostasis. *Cold Spring Harb. Perspect. Biol.* **2**, a000125.

Gumbiner, B. M. (2005). Regulation of cadherin-mediated adhesion in morphogenesis. *Nat. Rev. Mol. Cell Biol.* **6**, 622-634.

Gumbiner, B. M. and McCrea, P. D. (1993). Catenins as mediators of the cytoplasmic functions of cadherins. *J. Cell Sci*. **Suppl. 17**, 155-158.

Halbleib, J. M. and Nelson, W. J. (2006). Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev.* **20**, 3199-3214.

Harris, T. J. and Tepass, U. (2010). Adherens junctions: from molecules to morphogenesis. *Nat. Rev. Mol. Cell Biol.* **11**, 502-514.

Harrison, O. J., Jin, X., Hong, S., Bahna, F., Ahlsen, G., Brasch, J., Wu, Y., Vendome, J., Felsovalyi, K., Hampton, C. M. et al. (2011). The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. *Structure* **19**, 244-256.

Hartsock, A. and Nelson, W. J. (2008). Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim. Biophys. Acta* **1778**, 660-669.

Hinck, L., Näthke, I. S., Papkoff, J. and Nelson, W. J. (1994). Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J. Cell Biol.* **125**, 1327-1340.

Hoffman, L. M., Jensen, C. C., Chaturvedi, A., Yoshigi, M. and Beckerle, M. C. (2012). Stretch-induced actin remodeling requires targeting of zyxin to stress fibers and recruitment of actin regulators. *Mol. Biol. Cell* **23**, 1846-1859.

Hoshino, T., Sakisaka, T., Baba, T., Yamada, T., Kimura, T. and Takai, Y. (2005). Regulation of E-cadherin endocytosis by nectin through afadin, Rap1, and p120ctn. *J. Biol. Chem.* **280**, 24095-24103.

Ivanov, A. I., Hunt, D., Utech, M., Nusrat, A. and Parkos, C. A. (2005). Differential roles for actin polymerization and a myosin II motor in assembly of the epithelial apical junctional complex. *Mol. Biol. Cell* **16**, 2636-2650.

Jeanes, A., Gottardi, C. J. and Yap, A. S. (2008). Cadherins and cancer: how does cadherin dysfunction promote tumor progression? *Oncogene* **27**, 6920-6929.

Kanchanawong, P., Shtengel, G., Pasapera, A. M., Ramko, E. B., Davidson, M. W., Hess, H. F. and Waterman, C. M. (2010). Nanoscale architecture of integrin-based cell adhesions. *Nature* **468**, 580-584.

Kardash, E., Reichman-Fried, M., Maitre, J. L., Boldajipour, B., Papusheva, E., Messerschmidt, E. M., Heisenberg, C. P. and Raz, E. (2010) A role for Rho GTPases and cell-cell adhesion in single-cell motility in vivo. *Nat. Cell Biol*. **12**, 47-53.

Kim, S. H., Li, Z. and Sacks, D. B. (2000). E-cadherin-mediated cell-cell attachment activates Cdc42. *J. Biol. Chem.* **275**, 36999-37005.

Knudsen, K. A., Soler, A. P., Johnson, K. R. and Wheelock, M. J. (1995). Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J. Cell Biol.* **130**, 67-77.

Kovacs, E. M., Goodwin, M., Ali, R. G., Paterson, A. D. and Yap, A. S. (2002). Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr. Biol.* **12**, 379-382.

Kovacs, E. M., Verma, S., Ali, R. G., Ratheesh, A., Hamilton, N. A., Akhmanova, A. and Yap, A. S. (2011). N-WASP regulates the epithelial junctional actin cytoskeleton through a non-canonical post-nucleation pathway. *Nat. Cell Biol.* **13**, 934-943.

Kowalczyk, A. P., Navarro, P., Dejana, E., Bornslaeger, E. A., Green, K. J., Kopp, D. S. and Borgwardt, J. E. (1998). VE-cadherin and desmoplakin are assembled into dermal microvascular endothelial intercellular junctions: a pivotal role for plakoglobin in the recruitment of desmoplakin to intercellular junctions. *J. Cell Sci.* **111**, 3045-3057.

Kris, A. S., Kamm, R. D. and Sieminski, A. L. (2008). VASP involvement in force-mediated adherens junction strengthening. *Biochem. Biophys. Res. Commun.* **375**, 134-138.

Küssel-Andermann, P., El-Amraoui, A., Safieddine, S., Nouaille, S., Perfettini, I., Lecuit, M., Cossart, P., Wolfrum, U. and Petit, C. (2000). Vezatin, a novel transmembrane protein, bridges myosin VIIA to the cadherin-catenins complex. *EMBO J.* **19**, 6020-6029.

Ladoux, B., Anon, E., Lambert, M., Rabodzey, A., Hersen, P., Buguin, A., Silberzan, P. and Mège, R. M. (2010). Strength dependence of cadherin-mediated adhesions. *Biophys. J.* **98**, 534-542.

le Duc, Q., Shi, Q., Blonk, I., Sonnenberg, A., Wang, N., Leckband, D. and de Rooij, J. (2010). Vinculin potentiates E-cadherin mechanosensing and is recruited

to actin-anchored sites within adherens junctions in a myosin II-dependent manner. *J. Cell Biol.* **189**, 1107-1115.
Lechler, T. (2012). Adherens junctions and stem cells. *Subcell. Biochem.* **60**, 359-377.
Leckband, D. E., le Duc, Q., Wang, N. and de Rooij, J. (2011). Mechanotransduction at cadherin-mediated adhesions. *Curr. Opin. Cell Biol.* **23**, 523-530.
Lecuit, T. (2005). Adhesion remodeling underlying tissue morphogenesis. *Trends Cell Biol.* **15**, 34-42.
Leonard, M., Chan, Y. and Menko, A. S. (2008). Identification of a novel intermediate filament-linked N-cadherin/gamma-catenin complex involved in the establishment of the cytoarchitecture of differentiated lens fiber cells. *Dev. Biol.* **319**, 298-308.
Lien, W. H., Klezovitch, O. and Vasioukhin, V. (2006). Cadherin-catenin proteins in vertebrate development. *Curr. Opin. Cell Biol.* **18**, 499-506.
Ligon, L. A., Karki, S., Tokito, M. and Holzbaur, E. L. (2001). Dynein binds to beta-catenin and may tether microtubules at adherens junctions. *Nat. Cell Biol.* **3**, 913-917.
Liu, Z., Tan, J. L., Cohen, D. M., Yang, M. T., Sniadecki, N. J., Ruiz, S. A., Nelson, C. M. and Chen, C. S. (2010). Mechanical tugging force regulates the size of cell-cell junctions. *Proc. Natl. Acad. Sci. USA* **107**, 9944-9949.
Maddugoda, M. P., Crampton, M. S., Shewan, A. M. and Yap, A. S. (2007). Myosin VI and vinculin cooperate during the morphogenesis of cadherin cell cell contacts in mammalian epithelial cells. *J. Cell Biol.* **178**, 529-540.
Maretzky, T., Reiss, K., Ludwig, A., Buchholz, J., Scholz, F., Proksch, E., de Strooper, B., Hartmann, D. and Saftig, P. (2005). ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and beta-catenin translocation. *Proc. Natl. Acad. Sci. USA* **102**, 9182-9187.
Martin, A. C., Kaschube, M. and Wieschaus, E. F. (2009). Pulsed contractions of an actin-myosin network drive apical constriction. *Nature* **457**, 495-499.
McCrea, P. D., Gu, D. and Balda, M. S. (2009). Junctional music that the nucleus hears: cell-cell contact signaling and the modulation of gene activity. *Cold Spring Harb. Perspect. Biol.* **1**, a002923.
Meng, W., Mushika, Y., Ichii, T. and Takeichi, M. (2008). Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. *Cell* **135**, 948-959.
Miyake, Y., Inoue, N., Nishimura, K., Kinoshita, N., Hosoya, H. and Yonemura, S. (2006). Actomyosin tension is required for correct recruitment of adherens junction components and zonula occludens formation. *Exp. Cell Res.* **312**, 1637-1650.
Neisch, A. L. and Fehon, R. G. (2011). Ezrin, Radixin and Moesin: key regulators of membrane-cortex interactions and signaling. *Curr. Opin. Cell Biol.* **23**, 377-382.
Nelson, W. J. (2008). Regulation of cell-cell adhesion by the cadherin-catenin complex. *Biochem. Soc. Trans.* **36**, 149-155.
Nguyen, T. N., Uemura, A., Shih, W. and Yamada, S. (2010). Zyxin-mediated actin assembly is required for efficient wound closure. *J. Biol. Chem.* **285**, 35439-35445.
Niessen, C. M., Leckband, D. and Yap, A. S. (2011). Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation. *Physiol. Rev.* **91**, 691-731.
Oda, H. and Takeichi, M. (2011). Evolution: structural and functional diversity of cadherin at the adherens junction. *J. Cell Biol.* **193**, 1137-1146.
Papusheva, E. and Heisenberg, C. P. (2010). Spatial organization of adhesion: force-dependent regulation and function in tissue morphogenesis. *EMBO J.* **29**, 2753-2768.
Patla, I., Volberg, T., Elad, N., Hirschfeld-Warneken, V., Grashoff, C., Fässler, R., Spatz, J. P., Geiger, B. and Medalia, O. (2010). Dissecting the molecular architecture of integrin adhesion sites by cryo-electron tomography. *Nat. Cell Biol.* **12**, 909-915.
Peitsch, W. K., Grund, C., Kuhn, C., Schnölzer, M., Spring, H., Schmelz, M. and Franke, W. W. (1999). Drebrin is a widespread actin-associating protein enriched at junctional plaques, defining a specific microfilament anchorage system in polar epithelial cells. *Eur. J. Cell Biol.* **78**, 767-778.
Raich, W. B., Agbunag, C. and Hardin, J. (1999). Rapid epithelial-sheet sealing in the Caenorhabditis elegans embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* **9**, 1139-1146.
Ratheesh, A. and Yap, A. S. (2012). A bigger picture: classical cadherins and the dynamic actin cytoskeleton. *Nat. Rev. Mol. Cell Biol.* **13**, 673-679.
Ratheesh, A., Gomez, G. A., Priya, R., Verma, S., Kovacs, E. M., Jiang, K., Brown, N. H., Akhmanova, A., Stehbens, S. J. and Yap, A. S. (2012). Centralspindlin and α-catenin regulate Rho signalling at the epithelial zonula adherens. *Nat. Cell Biol.* **14**, 818-828.
Sakisaka, T., Ikeda, W., Ogita, H., Fujita, N. and Takai, Y. (2007). The roles of nectins in cell adhesions: cooperation with other cell adhesion molecules and growth factor receptors. *Curr. Opin. Cell Biol.* **19**, 593-602.
Scott, J. A., Shewan, A. M., den Elzen, N. R., Loureiro, J. J., Gertler, F. B. and Yap, A. S. (2006). Ena/VASP proteins can regulate distinct modes of actin organization at cadherin-adhesive contacts. *Mol. Biol. Cell* **17**, 1085-1095.
Shapiro, L. and Weis, W. I. (2009). Structure and biochemistry of cadherins and catenins. *Cold Spring Harb. Perspect. Biol.* **1**, a003053.
Shewan, A. M., Maddugoda, M., Kraemer, A., Stehbens, S. J., Verma, S., Kovacs, E. M. and Yap, A. S. (2005). Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. *Mol. Biol. Cell* **16**, 4531-4542.
Smutny, M., Cox, H. L., Leerberg, J. M., Kovacs, E. M., Conti, M. A., Ferguson, C., Hamilton, N. A., Parton, R. G., Adelstein, R. S. and Yap, A. S. (2010). Myosin II isoforms identify distinct functional modules that support integrity of the epithelial zonula adherens. *Nat. Cell Biol.* **12**, 696-702.
Stepniak, E., Radice, G. L. and Vasioukhin, V. (2009). Adhesive and signaling functions of cadherins and catenins in vertebrate development. *Cold Spring Harb. Perspect. Biol.* **1**, a002949.
Taguchi, K., Ishiuchi, T. and Takeichi, M. (2011). Mechanosensitive EPLIN-dependent remodeling of adherens junctions regulates epithelial reshaping. *J. Cell Biol.* **194**, 643-656.
Takeda, H., Shimoyama, Y., Nagafuchi, A. and Hirohashi, S. (1999). E-cadherin functions as a cis-dimer at the cell-cell adhesive interface in vivo. *Nat. Struct. Biol.* **6**, 310-312.
Takeichi, M. (1993). Cadherins in cancer: implications for invasion and metastasis. *Curr. Opin. Cell Biol.* **5**, 806-811.
Troyanovsky, S. M. (1999). Mechanism of cell-cell adhesion complex assembly. *Curr. Opin. Cell Biol.* **11**, 561-566.
van Roy, F. and Berx, G. (2008). The cell-cell adhesion molecule E-cadherin. *Cell. Mol. Life Sci.* **65**, 3756-3788.
Vasioukhin, V., Bauer, C., Yin, M. and Fuchs, E. (2000). Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell* **100**, 209-219.
Wakamatsu, Y., Sakai, D., Suzuki, T. and Osumi, N. (2011). FilaminB is required for the directed localization of cell-cell adhesion molecules in embryonic epithelial development. *Dev. Dyn.* **240**, 149-161.
Wang, Z., Sandiford, S., Wu, C. and Li, S. S. (2009). Numb regulates cell-cell adhesion and polarity in response to tyrosine kinase signalling. *EMBO J.* **28**, 2360-2373.
Watanabe, N., Kato, T., Fujita, A., Ishizaki, T. and Narumiya, S. (1999). Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nat. Cell Biol.* **1**, 136-143.
Wu, Y., Jin, X., Harrison, O., Shapiro, L., Honig, B. H. and Ben-Shaul, A. (2010). Cooperativity between trans and cis interactions in cadherin-mediated junction formation. *Proc. Natl. Acad. Sci. USA* **107**, 17592-17597.
Yamada, S. and Nelson, W. J. (2007). Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. *J. Cell Biol.* **178**, 517-527.
Yamada, S., Pokutta, S., Drees, F., Weis, W. I. and Nelson, W. J. (2005). Deconstructing the cadherin-catenin-actin complex. *Cell* **123**, 889-901.
Yonemura, S. (2011). Cadherin-actin interactions at adherens junctions. *Curr. Opin. Cell Biol.* **23**, 515-522.
Yonemura, S., Itoh, M., Nagafuchi, A. and Tsukita, S. (1995). Cell-to-cell adherens junction formation and actin filament organization: similarities and differences between non-polarized fibroblasts and polarized epithelial cells. *J. Cell Sci.* **108**, 127-142.
Yonemura, S., Matsui, T., Tsukita, S. and Tsukita, S. (2002). Rho-dependent and -independent activation mechanisms of ezrin/radixin/moesin proteins: an essential role for polyphosphoinositides in vivo. *J. Cell Sci.* **115**, 2569-2580.
Yonemura, S., Wada, Y., Watanabe, T., Nagafuchi, A. and Shibata, M. (2010). alpha-Catenin as a tension transducer that induces adherens junction development. *Nat. Cell Biol.* **12**, 533-542.
Zaidel-Bar, R. and Geiger, B. (2010). The switchable integrin adhesome. *J. Cell Sci.* **123**, 1385-1388.
Zaidel-Bar, R., Ballestrem, C., Kam, Z. and Geiger, B. (2003). Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. *J. Cell Sci.* **116**, 4605-4613.
Zaidel-Bar, R., Cohen, M., Addadi, L. and Geiger, B. (2004). Hierarchical assembly of cell-matrix adhesion complexes. *Biochem. Soc. Trans.* **32**, 416-420.
Zhang, Y., Sivasankar, S., Nelson, W. J. and Chu, S. (2009). Resolving cadherin interactions and binding cooperativity at the single-molecule level. *Proc. Natl. Acad. Sci. USA* **106**, 109-114.