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Jack of all trades: functional modularity in the adherens junction

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Adherens junctions, broadly defined as attachment sites in which cadherin adhesion receptors connect the actin cytoskeletons of neighboring animal cells, are multi-tasking by nature. In addition to mediating cell–cell adhesion and providing the tissue with mechanical continuity and barrier function, they maintain polarity, are sites of mechanosensing and signaling, and they regulate actomyosin dynamics and can thus generate forces to drive morphogenesis. Here we propose that the key to performing such diverse tasks is the integration within the cadherin adhesome of functional modules that evolved independently to perform other duties within the cell, and we discuss three such functional modules: force transmission, actin dynamics regulation, and contractile force generation. We compare each module to a more ancient cellular structure with similar function, identify shared components, and speculate on how the module was integrated into the cadherin adhesome.

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Introduction

Eukaryotic cells have been around for approximately 2 billion years [1] whereas metazoans only appeared around 600 million years ago [2]. Thus, when cells first came together to form the tissues of multicellular organisms, they had at their disposal molecular machineries developed during ~1.4 billion years of evolution. With this in mind it is not surprising that an evolutionary analysis of adherens junction (AJ) components, collectively referred to as the cadherin adhesome (or 'cadhesome'), showed that approximately 70% of cadhesome proteins, including cadherins and catenin-like proteins, are found in premetazoan unicellular organisms [3•].

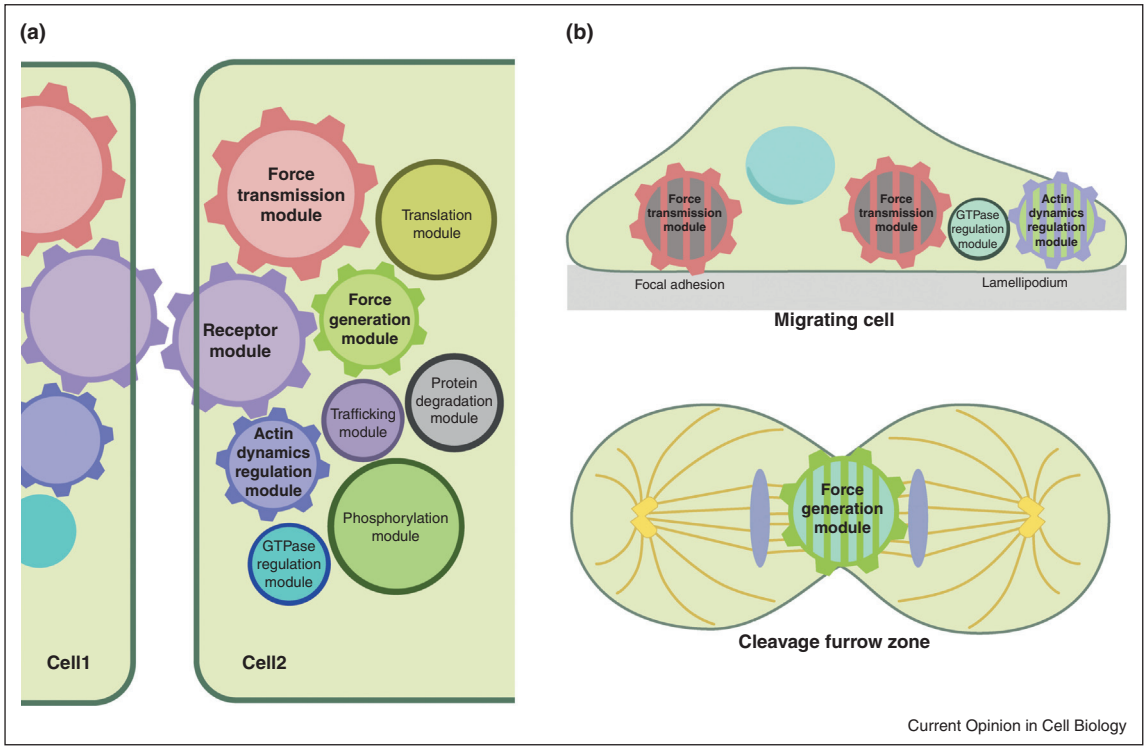
What sets metazoan cadherins apart from their premetazoan ancestors is the ability of their cytoplasmic tail to bind p120-catenin and β-catenin [3•]. The catenins in turn can directly interact with over 50 additional proteins, and given that each of those can bind one or more additional partners the number of proteins recruited to the cytoplasmic face of cadherin structures becomes, at least in theory, very large. Mining the AJ literature we previously identified approximately 170 proteins inter acting with cadherin or catenins at AJ [4]. Recently, proximity biotinylation (BioID) proteomics was employed to capture and identify all of the proteins closely associated with epithelial cadherin [5•,6•]. Based on these data it is now estimated that AJ can be home to over 400 different proteins, belonging to at least 20 different functional groups — including cytoskeleton regulators, scaffolding proteins, signaling kinases and phosphatases, transcription elements, endocytic machinery, and proteolytic enzymes — enabling AJ to perform a multitude of functions (reviewed in Refs. [7–12]).

Considering the cadhesome as a unified entity is appropriate, given that all its components can be found in one cellular location, and because the dynamics of AJ are the outcome of interactions between cadhesome proteins from different functional groups. However, to better understand the evolution and multitasking ability of AJ it is useful to think of the cadhesome as composed of functional modules (Figure 1a). This is not merely an exercise in deconstruction. We propose that the way the AJ acquired its complexity was by incorporating various signaling and structural modules from pre-existing cellular structures or processes (Figure 1b).

Force transmission module

The most basic function of the AJ, that is, to physically connect the cytoskeleton of neighboring cells, is carried out by a chain of structural proteins linked together by non-covalent interactions. Beginning with the extracellular domains of adhesion receptors, the chain continues with adaptors that bind the cytoplasmic tails of adhesion receptors, and ends with adaptors that bind filamentous (F-) actin. Due to contractile forces generated by the cytoskeleton and/or external stretching or shear stresses,

Figure 1



Functional modularity in the adherens junctions (AJ). **(a)** The protein network associated with cadherin-mediated cell–cell adhesion ('cadhesome') can be conceptually organized into distinct modules, each responsible for a unique function of the AJ. **(b)** Many of the functional modules of AJ can be found in other cellular structures, such as the leading edge and focal adhesions of a migrating cell or cytokinetic furrow of a dividing cell. A large number of components are shared between AJ modules and the other cellular structures, raising the possibility that the evolutionary origin of AJ modules was in more ancient modules found in single cells.

the chain of proteins is often under tension, and its function is to transmit the tensile forces between the outside of the cell and the actin cytoskeleton. The force transmitted by an entire AJ, measured in single cells on cadherin-coated pillars or inferred from the traction force imbalance of cell doublets, was found to be between 15 nN and 160 nN [13–15]. At the single protein level, a Fluorescence Resonance Energy Transfer (FRET)-based tension-sensor showed individual E-cadherin cytoplasmic tails to be under 1–2 pN tensile force [16]. Importantly, constitutive tension is a requisite for stable AJ, and inhibiting cellular contractility leads to AJ disassembly [15,17,18]. Recent work may explain, in part, the molecular basis of force-dependent AJ assembly: when α-catenin is in a complex with cadherin-bound β-catenin it can effectively bind F-actin only if the interaction is under tension [19•].

Importantly, the force transmission module is mechanosensitive and responds by reinforcing or downsizing the AJ when force is increased or decreased, respectively [15]. Force-induced reinforcement was demonstrated in monolayers [20••,21], in cell doublets [22,23•], and with twisting and pulling of cadherin-coated beads [21,24]. Presently, the leading molecular explanations for mechanosensing are conformational changes in adaptor proteins under tension that expose new protein binding sites or strengthen interactions through 'catch bonds' [25]. The most studied and best-understood example is the stretching of α-catenin and subsequent recruitment of vinculin to exposed vinculin binding sites. Such a mechanism was first proposed by Yonemura *et al.* [20••] and has since been demonstrated by single molecule stretching *in vitro* [26], and visualized dynamically in cells with an α-catenin FRET conformation sensor [27•]. Vinculin binding maintains α-catenin in the stretched conformation [26], provides an additional link with F-actin [21], and may also initiate other downstream signaling, such as recruitment of Arp2/3 or VASP [28,29].

Another cellular structure in which forces are transmitted from outside the cell to the actin cytoskeleton, and vice versa, through a series of adaptor proteins is the integrin-based cell–matrix junction, exemplified by focal adhesions. From the evolutionary perspective, cell–matrix adhesion preceded multicellularity [30], and so it is conceivable that parts of the cadhesome force transmission machinery were co-opted from cell–matrix adhesion.

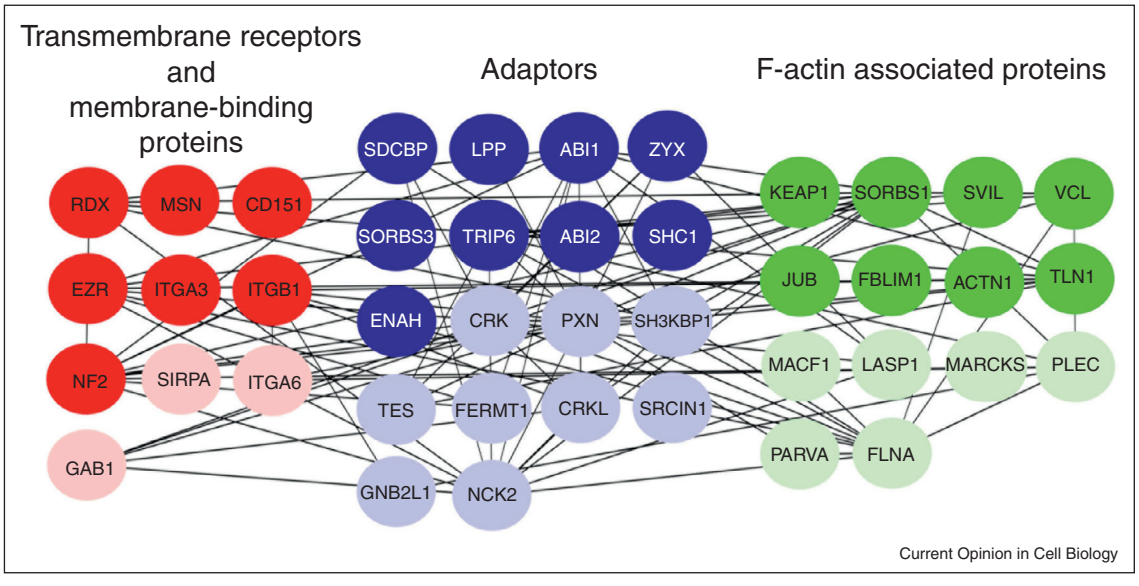
Supporting this hypothesis, a large number of proteins, including many structural adaptors, are found to be common to both integrin and cadherin adhesomes (Figure 2). Notably, the 'integrin-adaptor-F-actin' link is also under tension and focal adhesions are tension-dependent structures that display force-dependent reinforcement [31,32]. Interestingly, vinculin is also involved in the mechanoresponse of focal adhesions, in which stretching of talin exposes cryptic vinculin binding sites [33–35]. A proteolytic product of talin is essential for AJ formation [36], but whether it is stretched within AJ is not known. α-catenin and talin are not known to be related by sequence, but both form a tertiary structure of consecutive four or five alpha-helical bundles that when pulled apart reveal cryptic vinculin binding sites in the form of single-alpha helices with hydrophobic surfaces. Hence, it appears that each mechanosensitive switch evolved independently but arrived at a similar solution based on stretch-dependent vinculin recruitment.

Actin dynamics regulation module

Actin dynamics dictate to a large extent the dynamics of AJ. Even before AJ form, cortical F-actin, polymerized by the formin mDia1 and the Arp2/3 complex [37], delimits cadherin clusters in the plasma membrane [38••]. Depending on cell type and context, either Arp2/3-driven lamellipodial or formin-mediated filopodial protrusions are used to bring cell membranes into close contact, facilitating the formation of trans-ligated adhesive clusters [39,40••,41]. Beyond AJ establishment, lamellipodia also remodel existing junctions and maintain monolayer integrity [42]. Live imaging of cadherin both *in vitro* and *in vivo* has shown that isolated cadherin clusters are short lived and their stabilization in AJ is dependent on the formation of a more stable F-actin network [43•,44]. For instance, in epithelial cells neural Wiskott–Aldrich Syndrome Protein (N-WASP) works with WIRE to stabilize F-actin, though the mechanism is not yet understood [45]. Conversely, the actin severing proteins AIP1 and cofilin are needed for destabilization and remodeling of AJ [46].

From an evolutionary perspective, the cellular machinery regulating when and where G-actin will be polymerized into F-actin predates AJ as it is fundamental for many rudimentry cellular processes [47], independent of multicellularity. How then was this machinery co-opted by AJ? It is well-established that the Arp2/3 complex physically associates with E-cadherin [48], though the direct binding partner is not known. One potential candidate is vinculin, which is known to recruit Arp2/3 into focal adhesions [29,49•]. Another candidate is cortactin, which binds Arp2/3 and has known interactions with p120-catenin and ZO-1 [50–52]. Owing to its poor intrinsic nucleation ability, the Arp2/3 complex requires activation by nucleation-promoting factors, such as WASP and WASP-family verprolin-homologous protein (WAVE) [53]. N-WASP is associated with endothelial AJs via an interaction with p120-catenin [54]. WAVE2 is known to associate with E-cadherin in epithelial cells, but the binding partner is not known [55].

Figure 2



Components of the force transmission modules common to both integrin and cadherin adhesomes. A comparison between the literature-based integrin adhesome [97] and the literature-based and mass spectrometry-based cadherin adhesome [4,5] reveals 42 shared structural proteins belonging to the force transmission module. The proteins are color coded as follows: transmembrane receptors and membrane-binding proteins in red; adaptors in blue; F-actin associated proteins in green. Dark colors indicate integrin adhesome proteins overlapping with both literature and mass spectrometry of cadhesome, whereas light colors indicate overlap with only the mass spectrometry data.

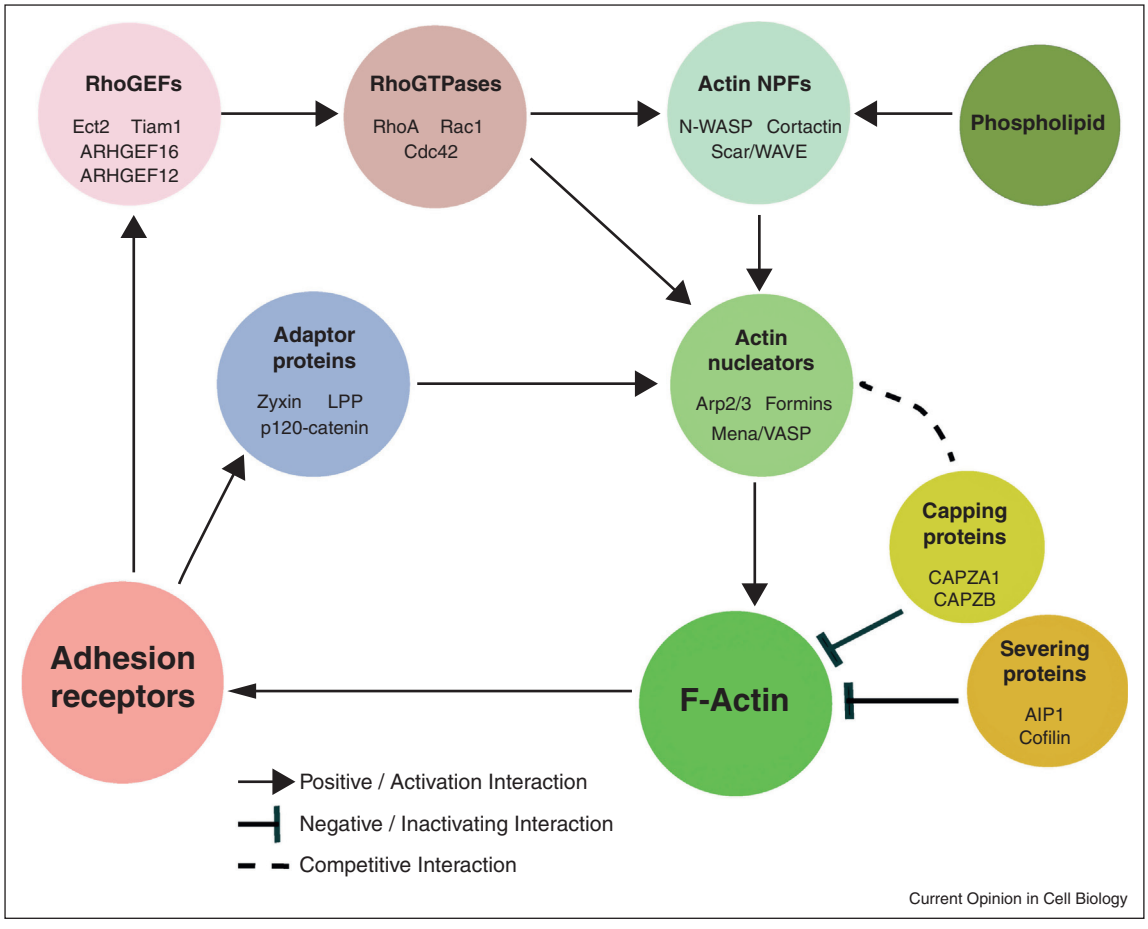
Other actin polymerization factors, namely the formin mDia1 and Mena/VASP, have also been localized to the AJ [56,57]. mDia1 may be recruited by the adaptor Abi1 [58], and Mena/VASP are likely recruited by LIM domain proteins zyxin and LPP [59,60]. Interestingly, Mena/VASP and Diaphanous formins (mDia2) were shown to cooperate in the regulation of filopodial morphology, dynamics and function [61]. Not much else is known about the interplay between the different actin polymerization factors at AJ. It has been shown, however, that α-catenin binding to F-actin inhibits the binding of Arp2/3 [62], and thus may shift the balance toward elongation of linear F-actin filaments by formins and Mena/VASP [63••,64••].

Another layer of regulation on actin dynamics is afforded by a GTPase regulation module. Actin nucleation-promoting factors are usually autoinhibited, but can be activated by the active forms of Rho GTPases [65–67]. Rho GTPases are ubiquitous in the cell, but their activation is tightly regulated by guanine nucleotide exchange factors (GEFs). Two such factors, Tiam1 [68,69] and Ect2 [70••] have been shown to be localized at AJ and others, such as ARHGEF12 and ARHGEF16 have been identified in cadhesome proteomics [5]. Thus, actin dynamics regulate AJ and AJ regulate actin dynamics in a circular positive feedback loop, as illustrated in Figure 3.

Contractile force generation

Mature AJ in epithelial cells are often associated with a belt-like structure made of bundles of F-actin and myosin running parallel to the AJ and encircling the apical domain of the cell. Myosin dependent contractility along this actomyosin belt generates tensile forces that, acting like a corset, determine the shape of the cell and resists

Figure 3



Actin dynamics regulation module consists of a feedback loop between cell adhesion and actin dynamics. Engagement of adhesion receptors triggers downstream signaling that in turn promotes actin polymerization by actin nucleators. As discussed in the text, actin polymerization in filopodia and lamellipodia promotes adhesion receptor ligation thus completing the feedback loop. Activation and recruitment of nucleators is regulated by the action of RhoGEFs, RhoGTPases, phospholipids, and nucleation-promoting factors. In addition, actin nucleators may also be recruited to sites of adhesion via binding to adaptor proteins. Capping proteins bind to the barbed end of actin filaments and prevent filament growth, while severing proteins promote actin filament severing and disassembly. The general overview of actin dynamics regulation presented here holds true for both cadherin and integrin adhesions (for details of the participating proteins see [4,5]).

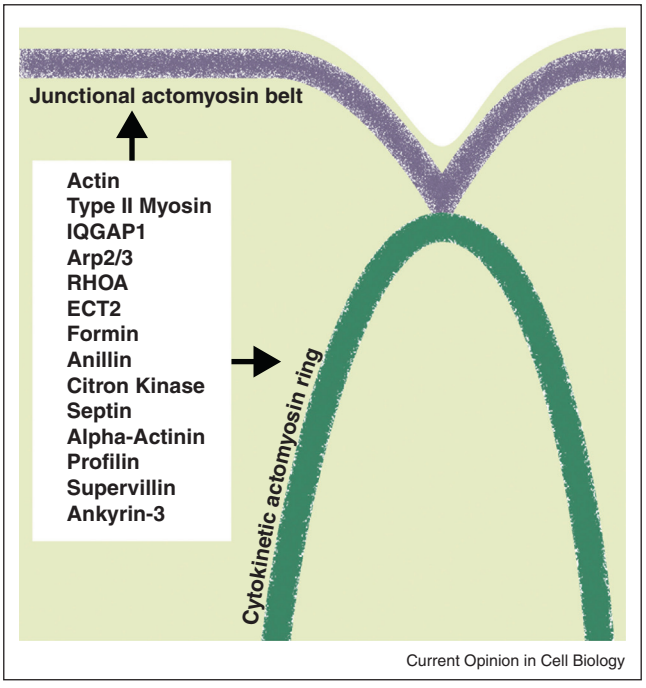
cellular deformation. This is possible because the contractile belt is linked to the AJ through the force transmission module described above. The magnitude of contractility in the apical belt is tightly regulated. For example, during gastrulation the apical belt functions like a ratchet in shrinking the apical domain, in concert with contraction of a medial actomyosin network [71]. Contractility can also be upregulated in a subset of AJ, resulting in shrinkage of specific junctions and neighbor exchange, as observed during convergent extension [72].

Localized Rho activity along the AJs drives the assembly of the junctional actomyosin belt and expansion of AJs [73]. Recruitment of centralspindlin to AJ in interphase cells results in junctional localization and activation of the RhoGEF Ect2 [70••] and its activity is balanced by the RhoGAP p190GAP [74]. Active RhoA serves to assemble the contractile belt through activation of the formin mDia1 and the serine/threonine kinase ROCK, which phosphorylates and activates non-muscle myosin II [17,56,75,76]. Additionally, various actomyosin regulators such as anillin [77•], α-actinin [78], IQGAP1 [79], Profilin1 [5] and septins [5], have been shown to localize to AJs, regulating junctional actomyosin dynamics and contractility.

A primordial cellular structure with many of the same features and components as the contractile module of AJ is the cleavage furrow zone involved in cell division. At anaphase onset, interaction with the centralspindlin complex leads to Ect2 activation and relocalization to the equatorial cortex, where it triggers local RhoA activation. [80,81]. Downstream of RhoA, formin-mediated polymerization of linear F-actin filaments is required for cleavage furrow formation [82,83]. Additionally, RhoA activity is also required for the recruitment and assembly of scaffolding proteins such as anillin and citron kinase, which in turn brings together RhoA, F-actin, non-muscle myosin type II and IQGAP [84–86]. Septin and profilin are also required for the cytokinetic cleavage furrow zone, as loss of their function results in defective cytokinesis [87,88]. In addition to these striking similarities between the assembly of the cleavage furrow zone and the apical belt, a comparison of the parts list of cytokinetic furrow components with the list of E-cadherin associated proteins reveals an extensive overlap of components between the two structures (Figure 4). This leads us to hypothesize that the cadhesome contractility module has its evolutionary origins in the cytokinesis machinery.

If so, how might have the contractile machinery been integrated into the cadhesome? A direct recruitment mechanism has been shown for several core components: the centralspindlin complex is recruited by α-catenin [70], ROCK interacts with p120-catenin [76], and IQGAP can directly bind β-catenin and E-cadherin [89]. IQGAP in turn can recruit the myosin essential light chain as well as Rho GTPases and calmodulin [90], all key components of the contractile module. The integration of the contractility module is obviously more complicated than this, as different isoforms of myosin II have been shown to be recruited to distinct contractile structures within the AJ [75]. However, the mechanism for this differential recruitment is not yet known.

Figure 4



Shared components between the junctional actomyosin belt and the cleavage furrow zone. Junctional actomyosin belt (purple) and cytokinetic cleavage furrow zone (green) are two contractile structures the cell assembles to carry out cell–cell adhesion and division, respectively. Although different in their cellular functions, the contractility of both structures is achieved by a common cellular contractility module. A comparison of the parts list of cytokinetic ring components (assembled mostly from studies in yeast [98,99]) with the list of E-cadherin associated proteins, revealed by mass spectrometry [5], suggests an extensive overlap of components between the two structures (proteins listed in box).

Conclusions and future perspectives

The striking similarities of the AJ functional modules we described here to other cellular structures is not surprising, given that evolution often works as a tinkerer, retooling and reusing the same proteins and pathways for different purposes [91]. Regardless of their histories, the significant number of shared components between AJ modules and other cellular structures raises important questions relating to how these components function within the cell: Is a given polypeptide dedicated to a specific structure? Can it shuttle between them? if so, how is its affinity regulated? finally, how do the shared

components affect the interplay between structures? It is likely that posttranslational modifications play an important role in targeting of shared proteins to specific structures. This has been shown to be the case for vinculin, wherein a specific tyrosine phosphorylation affects vinculin function specifically in AJ [92•]. To what extent, if at all, the same protein shuttles between AJ and other cellular structures has yet to be examined in detail. The possibility of local translation must also be considered.

One can imagine the interplay between AJ modules and other cellular structures taking the form of cooperation based on similar signaling pathways or antagonism based on competition for shared components, and examples for both of these scenarios have been described. Src and PI3K activation downstream of either integrin or cadherin ligation upregulated contractility and increased both cell–matrix and cell–cell adhesion forces [93,94]. Conversely, in the case of planar division in *Drosophila* epithelial cells, the cytokinetic cleavage furrow zone is anchored to the AJ and its constriction leads to local disengagement of AJ [95,96]. The concept of functional modularity in the AJ that we put forward here is useful for identifying primordial structures that may have been integrated into the cadhesome and helps in understanding the complexity and multi functionality of AJ.

Acknowledgments

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