REVIEW

Signaling from adherens-type junctions

Noam Erez, Alexander Bershadsky, Benjamin Geiger*

Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

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The molecular identity of adherens junctions

The early 1980s brought a new focus to cell adhesion research. This field, which was dominated by structural characterization of different adhesion sites, their subcellular distribution and cytoskeletal association, became increasingly molecular. The adhesion receptors mediating the different forms of cell–matrix and cell–cell adhesion in a wide variety of cells were identified and their mode of interaction with the cytoskeleton was partially characterized. It was shown that this association is mediated via an electron-dense sub-membrane “plaque” that contains multiple components, including “adapter” proteins that participate in linking cytoskeletal filaments to the membrane, as well as signaling proteins, which are believed to partake in the generation and transduction of signaling cues.

In this context, adherens-type junctions (AJ) were defined in 1982 (Kartenbeck et al., 1982), named after their best-characterized prototype, namely the zonula adhaerens of simple epithelial cells. These junctions were shown to be associated with the actin cytoskeleton via a sub-membrane plaque structure, containing a set of “plaque proteins” (e.g. vinculin), which are distinct from those associated with other plaque-bearing junctions, such as desmosomes. Over the years, many additional proteins were shown to be associated with the various structural domains of AJ, and their functions were investigated.
Long-range signaling triggered by AJ

An important notion, triggered by the capacity to stimulate or block AJ formation, is that in addition to their mechanical role in tissue assembly, AJ also act as receivers and transmitters of signaling cues. Activation of pp60src, for example, was shown to induce tyrosine phosphorylation of AJ components triggering junction disassembly (Behrens et al., 1993; Fujita et al., 2002; Volberg et al., 1991). In turn, formation of cadherin-mediated adhesions can induce signaling events, affecting cell growth, survival, morphogenesis and locomotion. Such signaling most likely is responsible for the well-documented processes of contact inhibition of locomotion and growth that were described long ago by Abercrombie and others (e.g., Abercrombie, 1979). One of the early indications that such signaling activity can be specifically triggered by cadherin-mediated interactions, was proposed by Levenberg et al. (1998). In this study, it was shown that binding of beads, coated with N-cadherin extracellular domain, or with anti-N-cadherin antibodies, to appropriate cells, triggers the accumulation of AJ components not only locally but also in AJ with neighboring cells, tens of microns away from the attached beads. This recruitment process was accompanied by an increase in phosphoryrosine levels along AJ (Levenberg et al., 1998). It was further shown that N-cadherin stimulation induces growth arrest, most likely via elevation in the cyclin-dependent kinase inhibitor p27 (Levenberg et al., 1999). A similar phenomenon was described by St. Croix et al. (1998) showing that E-cadherin engagement results in dephosphorylation of the retinoblastoma protein, an increase in p27 levels and a late reduction in cyclin D1 levels.

The adhesion-signaling components of AJ

The molecular architecture of cadherin-mediated junctions is discussed in detail in several recent reviews (Gooding et al., 2004; Pokutta and Weis, 2002) and will thus not be addressed in detail here. We rather chose to focus here on a few AJ components that are directly or indirectly involved in specific signal transduction pathways (see Fig. 1). It should be, however, pointed out that all classical cadherins share highly conserved cytoplasmic domains that interact with a similar set of molecular partners. These include the armadillo family proteins: beta-catenin, plakoglobin (also known as gammacatenin), as well as p120 catenin (p120) and its relatives (see below). While p120 family proteins bind to the juxtamembrane domain of cadherins, beta-catenin and plakoglobin compete on the same site at the cadherin C-terminus. Both beta-catenin and plakoglobin bind alpha-catenin, which, in turn, can interact with actin filaments both directly and via intermediate links, such as vinculin, alpha-actinin, and, perhaps, ZO-1. Beta- and alpha-catenins, as well as p120, are multifunctional proteins that recruit to the cadherin-mediated junctions many other components (some of which are bona fide signaling molecules) and can also be directly involved in signaling. One particular example for AJ-related signaling involves beta-catenin, that in addition to its structural role in linking the actin cytoskeleton to the junctional membrane (Fig. 2), is also a key transcription factor, driving the wnt signaling pathway. The dual role of this protein in AJ and in the nucleus was amply described and discussed in recent years (Nelson and Nusse, 2004; Wheelock and Johnson, 2003).

In this article, we would like to address other forms of signaling, triggered by AJ components, namely signaling systems that are physically associated with these junctions, and whose signaling activity can be directly triggered by AJ assembly.

It is noteworthy that besides cadherins, AJ contain other types of transmembrane proteins (Fig. 1), which are directly or indirectly associated with cadherins. These include Ig-type adhesion molecules known as nectins (Takai and Nakanishi, 2003), and several types of receptor tyrosine kinases (RTK) and phosphatases. The submembrane cytoplasmic domain of AJ contains small G-proteins of the Rho-family and possibly trimeric G-proteins (Fig. 1). Both the RTK and the G-proteins were shown to participate in adhesion-mediated signaling. The core proteins of AJ also participate in local, junction-related signal transduction pathways. Among the immediate partners of cadherin, p120 appears to play an important signaling role. Even alpha-catenin, a major structural link between the cadherin–catenin complex and the actin cytoskeleton, was shown to participate in some signal-tranduction events as a signaling molecule. Below, we discuss in more detail some of these systems, including: cadherin-mediated activation of RTK, cross-talk between cadherin signaling and G-proteins, and signaling functions of p120 and alpha-catenin.

AJ-associated activation of RTK

One mode of signaling from AJ involves a direct activation of classical RTK leading to specific tyrosine phosphorylation of these structures (Fig. 2). This notion was pioneered by studies demonstrating that neurite outgrowth can be triggered by interaction with N-cadherin on the surface of an appropriate feeder cell (Saffell et al., 1997; Utton et al., 2001; Walsh and Doherty, 1997) or attached to the tissue culture substrate (Utton et al., 2001). It was further shown that
this N-cadherin-dependent signaling requires the histidine–alanine–valine (HAV) site in the E1 domain of the cadherin molecule (Williams et al., 2000) and can be inhibited by a dominant negative mutant of fibroblast growth factor receptor (FGFR), implicating FGF signaling in the process.
N-cadherin activation was also shown to promote motility and invasiveness of human breast cancer cells (Hazan et al., 2000, 2004), involving a ligand (FGF)-dependent activation of FGFR, suggesting that junctional N-cadherin interacts with FGFR, stabilizes it on the plasma membrane and enhances its activation by FGF (Suyama et al., 2002). Another insight into the cadherin-FGFR signaling pathway was obtained by expressing N-cadherin in epithelial cells and showing enhancement of cell motility that can be blocked by inhibition of diacylglycerol lipase, a downstream target of FGFR (Nieman et al., 1999).

N-cadherin-dependent activation of FGFR is also involved in the generation of anti-apoptotic signals. It was shown that endothelial cells treated with an N-cadherin antagonist HAV peptide undergo massive apoptosis. Interestingly, this response to the HAV peptide was apparent only in dense cultures, and treated cells could be rescued by exogenous FGF. It was thus hypothesized that FGFR-mediated anti-apoptotic signaling is triggered in mature N-cadherin-containing AJ (Erez et al., 2004).

The cadherin-mediated activation of RTKs, appears to be a rather general phenomenon, involving different cadherins and growth factor receptors (GFR). While the signaling via FGFR is amply documented for N-cadherin, formation of E-cadherin-based AJ, for example, was shown to induce ligand-independent activation of epidermal growth factor receptor (EGFR) (Betson et al., 2002; Pece and Gutkind, 2000) and trigger MAP kinase (MAPK) activation (Pece and Gutkind, 2000). The interaction of RTKs with cadherins can be either direct (as shown for N-cadherin and FGFR), or indirect, via plaque proteins such as beta-catenin (Hoschuetzky et al., 1994). Furthermore, clustering of E-cadherin, using specific antibodies, induces EGFR-mediated activation of Rac1 (Betson et al., 2002). It was proposed that the mechanism underlying this activation involves clustering and transphosphorylation of EGFR, induced by its interaction with cadherin or with components of the AJ plaque.

Another classical cadherin which is involved in signaling is the vascular-endothelial cadherin (VE-cadherin), which plays an important role in VEGF signaling (Carmeliet et al., 1999; Dejana et al., 1999). It was shown that VEGFR forms a complex with VE-cadherin, beta-catenin and PI3K and thus enhances VEGF signaling (Carmeliet et al., 1999). It was further demonstrated that upon binding of VEGF to its ligand, VE-cadherin undergoes tyrosine phosphorylation and forms a complex with the adapter protein Shc. The authors also showed that Shc dephosphorylation occurs in VE-cadherin-expressing cells (Zanetti et al., 2002), suggesting that VE-cadherin promotes Shc dephosphorylation, probably via an interacting phosphatase.

Interestingly, cadherins can also down-regulate RTK signaling (Lampugnani et al., 2003; Qian et al., 2004). Expression of VE-cadherin can inhibit the activation of VEGFR-2 by VEGF (Lampugnani et al., 2003). VE-cadherin was also shown to attenuate the proliferating signals that are generated through VEGFR-2. It was demonstrated that the beta-catenin-binding domain of VE-cadherin is crucial for its interaction with VEGFR-2 and that this interaction is responsible for restraining growth factor signaling, probably via the activation of the high cell density-enhanced PTP 1 (DEP-1) (Lampugnani et al., 2003). E-cadherin can down-regulate the activity of EGFR and insulin growth factor receptor (IGF-1R) by weakening their interaction with their respective ligands (EGF and IGF1) (Qian et al., 2004). These observations provide a new insight into a possible role for cadherins in generating anti-proliferative signals.

**Involvement of G-proteins in cadherin-mediated signaling**

The first evidence of involvement of small Rho family GTPases (in particular, Rho and Rac) in the maintenance of cadherin-mediated junctions was obtained in experiments with dominant negative mutants of these proteins. However, it remains unclear whether the
observed effects (Braga et al., 1997; Kuroda et al., 1997; Takaishi et al., 1997) are indirect, due to the reorganization of the actin cytoskeleton, or attributable to direct effects on the junctions. Subsequent studies focusing on early stages of AJ formation showed that Rac and Cdc42 activation are rapidly induced in E-cadherin-containing cells following E-cadherin engagement (Betson et al., 2002; Kim et al., 2000; Kovaes et al., 2002; Nakagawa et al., 2001; Noren et al., 2001). The activation of different Rho-family GTPases appears, however, to be cell type and cadherin-type dependent. In CHO cells, ectopically expressing Xenopus C-cadherin, the onset of adhesion triggered the activation of Rac and Cdc42, but suppressed Rho activity (Noren et al., 2001); adhesion of endothelial cells, on the others hand involves VE-cadherin and activates Rac, without affecting Rho (Lampugnani et al., 2002). N-cadherin in C2C12 myoblasts activates RhoA and inhibits Rac1 and Cdc42 (Charrasse et al., 2002), while in other cellular system N-cadherin-mediated adhesion may activate Rac1. In particular, plating of bovine endothelial cells on an N-cadherin-coated surface induces fast cell spreading, manifested by extensive lamellipodial activity and accompanied by increased tyrosine phosphorylation at the cell periphery, and a time-dependent Rac1 activation (Fig. 3) (Erez and Geiger, unpublished data). It is noteworthy that the effect of adhesion on G-protein activation is a highly time-dependent process. For example, Rac activation, which is a common response to cadherin homophilic interaction, is a transient event that does not last longer than several minutes (Betson et al., 2002). Moreover, sustained Rac activation often leads to disassembly of AJ (Braga et al., 2000). Finally, modulation of the small GTPase activities can be a rather local event, leading to local recruitment of the specific G-protein into the contact zone, as demonstrated in experiments using GFP-Rac1 (Ehrlich et al., 2002). FRET-based techniques (Kraynov et al., 2000; Kurokawa et al., 2004; Mochizuki et al., 2001) currently being used for studying G-protein activation at a high spatial and temporal resolution may help to clarify this question.

The mechanism underlying the cross-talk between cadherin ligation and the modulation of Rho GTPases activity is still unclear. Typical regulators of Rho GTPases are guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs like Tiam-1 may participate in the cadherin-mediated Rac1 activation (Malliri et al., 2004). A prominent RhoA GAP known as p190RhoGAP, was proposed to be responsible for the cadherin-mediated inactivation of RhoA (Noren et al., 2003). One attractive mechanism for cadherin effect on G-proteins may involve a local activation of phosphatidylinositol 3-kinase (PI-3K) by cadherin ligation (Kovacs et al., 2002; Pecce et al., 1999) creating docking sites for pleckstrin homology (PH) domains on GEFs, such as Tiam-1 and VAV-2 which, in turn activate the specific G-proteins (Welch et al., 2003). It was shown, however, that cadherin-mediated adhesion can activate Rac also in a PI-3K-independent manner (Kovacs et al., 2002), possibly via p120 catenin (Gavard et al., 2004a; Goodwin et al., 2003, and see below). This, rather complex signaling cross-talk is further complicated by additional cadherin-activated pathways (e.g EGFR) that can affect cadherin-mediated Rac activation (Betson et al., 2002).

What is the function of small Rho GTPases in the assembly of AJ and in cadherin signaling? Obviously, these molecules are involved in the cytoskeletal reorganization that might affect AJ formation. Activation of Rac, for example, promotes extension of lamellipodial protrusions that might facilitate development of new cadherin contacts in sub-confluent cultures. It was recently shown that transient association of the actin-nucleating complex, Arp2/3, with cadherin is critical for AJ formation (for a review, see Bershadsky, 2004). Rac participates in several pathways involved in the activation of Arp2/3-driven branching polymerization of actin (Etienne-Manneville and Hall, 2002). In addition, both active Rac and Cdc42 interact with a common effector molecule, IQGAP1, which binds to beta-catenin, and interferes with its interaction with alpha-catenin. Binding of Rac or Cdc42 to IQGAP prevents its interaction with beta-catenin and thus releases AJ from the IQGAP-mediated negative regulation (Fukata et al., 1999). Moreover, IQGAP1, activated by Rac or Cdc42 binds to CLIP-170, a protein that captures microtubule growing ends and possibly affects their interactions with the actin cortex (Fukata et al., 2002). This effect might

Fig. 3. N-cadherin induces bovine endothelial cell spreading and Rac1 activation. BCAP cells were plated on an N-cadherin-coated surface. Cells display high lamellipodial activity accompanied by strong tyrosine phosphorylation at the cell periphery. Rac1 is activated in a time-dependent manner and reaches maximal activity after 30 min.
also be related to the stabilization of AJ, since microtubules were implicated in the transport of cadherins to the junction area (Mary et al., 2002). Finally, Rac is involved in cadherin endocytosis and may regulate junction stability affecting cadherin turnover (Akhtar and Hotchin, 2001).

In addition to Rho family GTPases, other small G-proteins may participate in the cadherin-mediated signaling. Arf6 is a major regulator of endocytosis, and in its constitutively active form it leads to disassembly of adherens junctions due to excessive internalization of E-cadherin. Dominant negative Arf6, on the other hand, stabilizes AJ and interferes with their disassembly, induced by the motogenic scatter factor (Palacios and D’Souza-Schorey, 2003; Palacios et al., 2001). An interesting relationship has been found recently also between catenin and the small G-protein Rap1. The cytoplasmic domain of E-cadherin interacts with C3G, a GEF for Rap1, while Rap1 itself interacts with afadin 6, a cytoplasmic partner of the AJ molecule nectin (Hogan et al., 2004). Rap1 activity is enhanced when cells are incubated with E-cadherin-coated beads, and suppression of Rap1 activity interferes with formation and maintenance of AJ (Hogan et al., 2004; Price et al., 2004). Rap1 activity is also required for cell adhesion to recombinant E-cadherin extracellular domains (Price et al., 2004). Thus, Rap1 is a positive regulator of E-cadherin recruitment to cell-cell junctions and its involvement in AJ formation has been demonstrated genetically in Drosophila (Knox and Brown, 2002). Finally, Cdc42 appears to function downstream of Rap1 in AJ formation, since its activation depends on Rap1 while its constitutively active mutant rescued the inhibitory effect of Rap1 deficiency (Hogan et al., 2004).

Several recent studies suggest that not only small (Ras superfamily) GTPases, but also trimeric G-proteins might be involved in cadherin-dependent signaling. In particular, hetero-trimeric G proteins of the G12 subfamily comprised of G-alpha12 and G-alpha13 can interact with the cytoplasmic domain of cadherin (Meigs et al., 2001, 2002) as well as with p120 ((Krakstad et al., 2004) and see below). G12 proteins negatively regulate E-cadherin-mediated AJ formation and suppression of cell migration (Meigs et al., 2002).

**Signaling roles of p120 catenin and alpha catenin**

p120 catenin (also known as p120ctn or p120cas) is an armadillo family protein that binds, at the juxtaembrane position, to the cytoplasmic portion of classical cadherins (Anastasiadis and Reynolds, 2000; Thoreson et al., 2000). Its knockdown by RNA interference, destabilizes AJ in mammalian cells (Davis et al., 2003; Xiao et al., 2003). Cells with low levels of p120 or impaired junctional localization of this protein form unstable AJ, with a rather fragmented morphology. Such cells also fail to spread on surfaces coated with cadherin extracellular domain (Davis et al., 2003; Gavard et al., 2004b; Goodwin et al., 2003; Thoreson et al., 2000).

The mechanism of p120 involvement in the stabilization of AJ and in cadherin-mediated signaling is still poorly understood, but several ideas are being considered. First, it was shown that p120 overexpression affects the activation of small Rho family GTPases and consequently the organization of the actin cytoskeleton (Anastasiadis et al., 2000; Grosheva et al., 2001; Noren et al., 2000). The activities of Rac and Cdc42 increase (Grosheva et al., 2001; Noren et al., 2000), while the activity of Rho changes in a cell-type specific manner (Anastasiadis et al., 2000; Cozzolino et al., 2003; Grosheva et al., 2001). Thus, cadherin contact-dependent activation of Rac and consequent contact-zone extension seems to depend on the association of p120 with cadherin (Gavard et al., 2004a; Goodwin et al., 2003). Most probably, p120 is responsible for the PI3-K-independent pathway of cadherin-induced activation of Rac (Gavard et al., 2004a; Goodwin et al., 2003). Interestingly, p120 interacts also with heterotrimeric G-proteins of the G12 family, which, as mentioned, also participate in the regulation of cadherin-mediated signaling (Krakstad et al., 2004).

Recent publications have attracted attention to the possible role of p120 as a master regulator of cadherin turnover. It was shown that in many cell types down-regulation of p120 by siRNA-mediated silencing leads to a pronounced decrease in cadherin levels (Davis et al., 2003; Xiao et al., 2003). It is not clear yet whether p120 controls cadherin internalization and degradation (Xiao et al., 2003), or facilitates its microtubule-dependent transport to intercellular junctions (Chen et al., 2003), or both.

These diverse activities depend on interactions of p120 with a variety of cytoplasmic partners. In view of the fact that p120 is a stable protein one may propose that its concentration in the cytoplasm, as well as its signaling activity can be altered when changes in AJ assembly occur. This possible mechanism for density-dependent signaling is yet to be explored. Moreover, similarly to beta-catenin that plays an important role in transcription regulation, some splicing isoforms of p120 were reported to be localized also to the nucleus, where they interact with a transcriptional repressor known as Kaiso and probably modulate its transcription repression activity (Kelly et al., 2004). The significance of these observations is yet to be determined.
Somewhat less well documented, yet quite compelling is the evidence that alpha catenin, besides its role as a major link between AJ and the actin cytoskeleton, also participates in signaling events. This is inferred, from studies showing that mice with conditional knockout of this protein in the skin (Vasioukhin et al., 2001) display a sustained activation of the MAPK pathway and augmented proliferation. This effect on proliferation was not a consequence of AJ destabilization and was independent of beta-catenin-mediated transcription regulation (Vasioukhin et al., 2001). Alpha-catenin was also shown to rescue deficient cells from sphingosine-induced apoptosis (Matsubara and Ozawa, 2001) and this effect does not depend on the restoration of cell–cell junction integrity.

Conclusion
As outlined in this review, AJ, that were formerly considered primarily for their roles in cell adhesion and tissue formation, appear now to be important signaling organelles. Similar to matrix adhesions, AJ “report” to cells on the “cellular environment” around them by activating specific signaling pathways. These signaling events can affect many cellular functions including cell proliferation, viability, locomotion and morphogenesis, and involve a variety of junctional and signaling systems. We are still at early stages of gathering information on the signaling pathways triggered by cadherins in AJ, and already we sense that we cannot see the forest for the trees. Understanding this complexity will be a major challenge for future research.

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