J. Biochem. **134**, 631–636 (2003)
DOI: 10.1093/jb/mvg198

Roles of the Intercellular Adhesion Molecule Nectin in Intracellular Signaling

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Received August 12, 2003; accepted August 19, 2003

The nectin family comprises four Ca^{2+}-independent immunoglobulin-like cell-cell adhesion molecules. Each nectin homophilically and heterophilically *trans*-interacts and causes intercellular adhesion, which organizes a variety of intercellular junctions in cooperation with, or independently of, cadherin. Nectin furthermore induces activation of Cdc42 and Rac small G proteins through c-Src, which eventually regulates formation of the cadherin-based adherens junctions through reorganization of the actin cytoskeleton, gene expression through activation of a mitogen-activated protein kinase cascade, and cell polarization through cell polarity proteins. We describe here the roles of nectin in intracellular signaling.

Key words: cadherin, Cdc42, c-Src, nectin, Rac, signaling.

Abbreviations: TJs, tight junctions; AJs, adherens junctions; DSs, desmosomes; Ig, immunoglobulin; JAM, junctional adhesion molecule; F-actin, actin filaments; MAP, mitogen-activated protein; PI3, phosphatidylinositol-3; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MKK, MAP kinase kinase.

In multicellular organisms, intercellular adhesion is critical for many events, including tissue patterning, morphogenesis, and maintenance of normal tissues (*1*, *2*). Molecular mechanisms of intercellular junctions have most extensively been investigated in polarized epithelial cells, in which intercellular adhesion is mediated through a junctional complex comprised of tight junctions (TJs), intercellular adherens junctions (AJs), and desmosomes (DSs) (*3*) (Fig. 1A). The formation and maintenance of TJs and DSs generally depend on the formation and maintenance of AJs (*4*). At AJs, E-cadherin is a key Ca^{2+}-dependent cell-cell adhesion molecule (*5*, *6*) (Fig. 1A). E-Cadherin forms *cis*-dimers and then *trans*-dimers (*trans*-interactions) through the extracellular region, causing cell-cell adhesion. The cytoplasmic tail of E-cadherin is linked to the actin cytoskeleton through many peripheral membrane proteins, including α-catenin, β-catenin, vinculin, and α-actinin, which strengthen the cell-cell adhesion activity of E-cadherin (*7*). At TJs, claudin is a key Ca^{2+}-independent cell-cell adhesion molecule that forms TJ strands (*4*). The cytoplasmic tail of claudin is linked to the actin cytoskeleton through ZO-1, -2, and -3. Junctional adhesion molecule (JAM), a Ca^{2+}-independent immunoglobulin (Ig)-like cell-cell adhesion molecule, also localizes at TJs and interacts with ZO proteins (*8*).

We have recently found a novel intercellular adhesion system consisting of nectin and afadin at AJs in a variety of cell types including epithelial cells and fibroblasts (*9*) (Fig. 1A). Nectin is a Ca^{2+}-independent Ig-like cell-cell adhesion molecule, and afadin is a nectin- and F-actin-binding protein that connects nectin to the actin cytoskeleton. This novel intercellular adhesion system has roles in formation of the E-cadherin–based AJs and subsequent formation of the claudin-based TJs in epithelial cells (*9*). Moreover, nectin recruits JAM to the apical side of the nectin-, E-cadherin-based cell-cell adhesion sites (*9*). Nectin plays roles not only in formation of AJs and TJs but also in formation of a variety of intercellular junctions in cooperation with, or independently of, cadherin, such as synapses in neurons, Sertoli cell-spermatid junctions in the testis (*9*), and the contacts formed between commissural axons and the processes of floor plate cells in the neural tube (Okabe *et al.*, unpublished results). The nectin-afadin and cadherin-catenin systems are thought to be connected through two connector units: a ponsin-vinculin unit (*9*) and an afadin DIL domain–interacting protein (ADIP)-α-actinin unit (*10*) (Fig. 1A). ZO-1 is indirectly associated with the nectin-afadin system in a manner independent of the E-cadherin–catenin system (*9*). The association of ZO-1 with the nectin-afadin system may play a role in recruiting the cell adhesion molecules of TJs to the apical side of AJs. Another line of evidence indicates that nectin serves as a receptor for α-herpes virus, facilitating its entry and cell-cell spread (*9*).

In addition to its intercellular adhesion activity, nectin induces activation of Cdc42 and Rac small G proteins, which regulate cell-cell adhesion through reorganization of the actin cytoskeleton (*11*), gene expression through activation of a mitogen-activated protein (MAP) kinase cascade (*12*), and cell polarization through cell polarity proteins (*13*) (Fig. 1B). In this review, we focus on the roles and the molecular mechanisms of the intracellular signaling mediated by nectin.

I. General properties of nectin

The nectin family consists of four members, nectin-1, -2, -3, and -4 (*9*), each of which has two or three splice variants. Nectin-1α, -1β, -1γ, -2α, -2δ, -3α, -3β, and -3γ isoforms have been identified, and nectin-4 also has two splice variants. Nectin-1α and -2α were originally iso-

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lated as the poliovirus receptor-related proteins and named PRR-1 and -2, respectively, although neither has subsequently been reported to serve as a poliovirus receptor. They were later shown to serve as a receptor for α-herpes virus, facilitating its entry and cell-cell spread, and so were renamed HveC and HveB, respectively. All nectins except nectin-1γ have an extracellular region with three Ig-like loops, a single transmembrane region, and a cytoplasmic tail region. Nectin-1γ is a secreted protein which lacks the transmembrane region. Furthermore, all nectins except nectin-1β, -3γ, and -4 have a conserved motif of four amino acid residues (Glu/Ala-X-Tyr-Val) at their carboxyl termini, and this motif binds the PDZ domain of afadin, an F-actin-binding protein. This binding of afadin to nectin links nectin to the actin cytoskeleton. Although nectin-4 lacks the conserved motif, it binds the PDZ domain of afadin at its carboxyl terminus.

Each nectin forms homo-*cis*-dimers and then homo-*trans*-dimers (*trans*-interactions), causing cell-cell adhesion (*9*). Nectin-3 furthermore forms hetero-*trans*-dimers with nectin-1 and -2. Nectin-4 also forms hetero-*trans*-dimers with nectin-1, but nectin-1 does not form hetero-*trans*-dimers with nectin-2. These hetero-*trans*-dimers show much higher affinity than the homo-*trans*-dimers. This property of nectin is different from that of cadherin, which forms mainly homo-*trans*-dimers. The second Ig-like loop of each nectin is necessary for the formation of the *cis*-dimers, whereas the first Ig-like loop is required for the formation of the *trans*-dimers, but not for the formation of the *cis*-dimers (*9*, *14*). The function of the third Ig-like loop is currently unknown. The interaction of each nectin with afadin is not essential for the formation of the *cis*-dimers or the *trans*-dimers. Whereas E-cadherin shows strong cell-cell adhesion activity that is accompanied by compaction, the nectin-mediated adhesion does not show such a property.

II. Roles of nectin in intracellular signaling

1. Activation of Cdc42 and Rac by nectin. Recent studies have demonstrated that the *trans*-interaction of E-cadherin induces activation of Rac, but not that of Cdc42, in several cell lines including epithelial cells and fibroblasts (*15–19*). Two studies (*15*, *18*) have shown that phosphatidylinositol-3 (PI3) kinase is required for the full activation of Rac by the *trans*-interaction of E-cadherin, whereas two studies (*17*, *19*) have shown that the activation of Rac by the *trans*-interaction of E-cadherin is independent of PI3 kinase, but dependent on epidermal growth factor receptor signaling or $p120^{ctn}$. It remains unknown whether the *trans*-interaction of E-cadherin induces activation of Rac through PI3 kinase, but there may be PI3 kinase-dependent and -independent pathways for the activation of Rac by the *trans*-interaction of E-cadherin. On the other hand, the *trans*-interaction of nectin induces not only activation of Rac but also that of Cdc42 in a PI3 kinase-independent manner in fibroblasts and epithelial cells (*11*, *12*) (Fig. 1B). It is unclear whether the *trans*-interaction of nectin induces activation of Rho. The nectin-induced activation of Rac requires the activation of Cdc42, whereas the nectin-induced activation of Cdc42 does not require the activation of Rac. The carboxyl-terminal four amino acids of nectin, which are necessary for it to bind afadin, are not essential for the activation of Cdc42 or Rac. Nectins that do not *trans*-interact with other nectins (non-*trans*-interacting nectins) inhibit both the E-cadherin-induced activation of Rac and formation of cell-cell AJs, and the inhibitory effect of non-*trans*-interacting nectins is suppressed by the Cdc42 activated by the *trans*-interaction of nectin (Fig. 2A) (*20*). The carboxyl-terminal four amino acids of nectin are essential for the inhibitory effect of non-*trans*-interacting nectins.

Cyclical activation and inactivation of these small G proteins are regulated by three types of regulators: Rho GDP/GTP exchange proteins (GEPs), Rho GTPase-activating proteins (GAPs), and Rho GDP dissociation inhibitors (GDIs) (*21*). Nectin may induce either activation of GEPs, inactivation of GAPs, or both, but their precise molecular mechanism remains to be elucidated. It has recently been shown that c-Src is involved in the nectin-induced activation of Cdc42 and Rac, although it remains unknown how c-Src is activated by the *trans*-interaction of nectin (T. Fukuhara *et al*., unpublished results) (Fig. 1B). The Src family kinases play a positive role in formation of cell-cell adhesion in keratinocytes (*22*, *23*). Therefore, it is likely that nectin plays important roles in the intracellular signaling through c-Src, Cdc42, and Rac during formation of cell-cell adhesion.

2. Roles of nectin-activated Cdc42 and Rac in cell-cell adhesion. The Rho family small G proteins, Rho, Rac, and Cdc42, have all been reported to affect the formation and/or maintenance of AJs (*24–26*). The formation of AJs is suppressed by inhibition of Rho (*27*, *28*), although expression of a constitutively active mutant of Rho (V14Rho) does not affect the formation of the E-cadherin–mediated cell-cell AJs (*29*). Rac activity is necessary for the establishment of AJs in epithelial cells (*27–31*). It has been shown that the Cdc42 activated by the *trans*-interaction of nectin is involved in the formation of AJs and TJs and increases the velocities of their formation in epithelial cells (*32*, *33*). The precise modes of action of Rac and Cdc42 activated by the *trans*-interaction of nectin and/or that of E-cadherin during the formation of AJs remain unknown, but a plausible mechanism is as follows (Figs. 2 and 3): when two migrating cells contact through their protrusions, nectin and E-cadherin separately form *trans*-dimers that form micro-clusters at cell-cell contact sites as described above. At the initial stage, cell-cell contact sites are formed mainly by the *trans*-interaction of nectin, because nectin kinetically forms micro-clusters more rapidly than E-cadherin (*9*, *34*). The *trans*-interaction of nectin induces activation of Cdc42 and Rac. The Cdc42 activated in this way increases the number of filopodia and cell-cell contact sites, like the "fork initiation" described for DNA replication (*35*) (Fig. 2B). The Rac activated by either the *trans*-interaction of nectin and/or that of E-cadherin induces formation of lamellipodia, which efficiently zip the cell-cell adhesion between the filopodia, acting like a "zipper" (*31*) (Fig. 2C). On the other hand, at the cell-cell contact sites mediated by the *trans*-interaction of E-cadherin, non-*trans*-interacting nectins inhibit the E-cadherin-induced activation of Rac and formation of AJs, until eventually the non-*trans*-interacting nectins *trans*-interact with other nectin molecules and induce the activation of Cdc42 (*20*) (Fig. 2A).

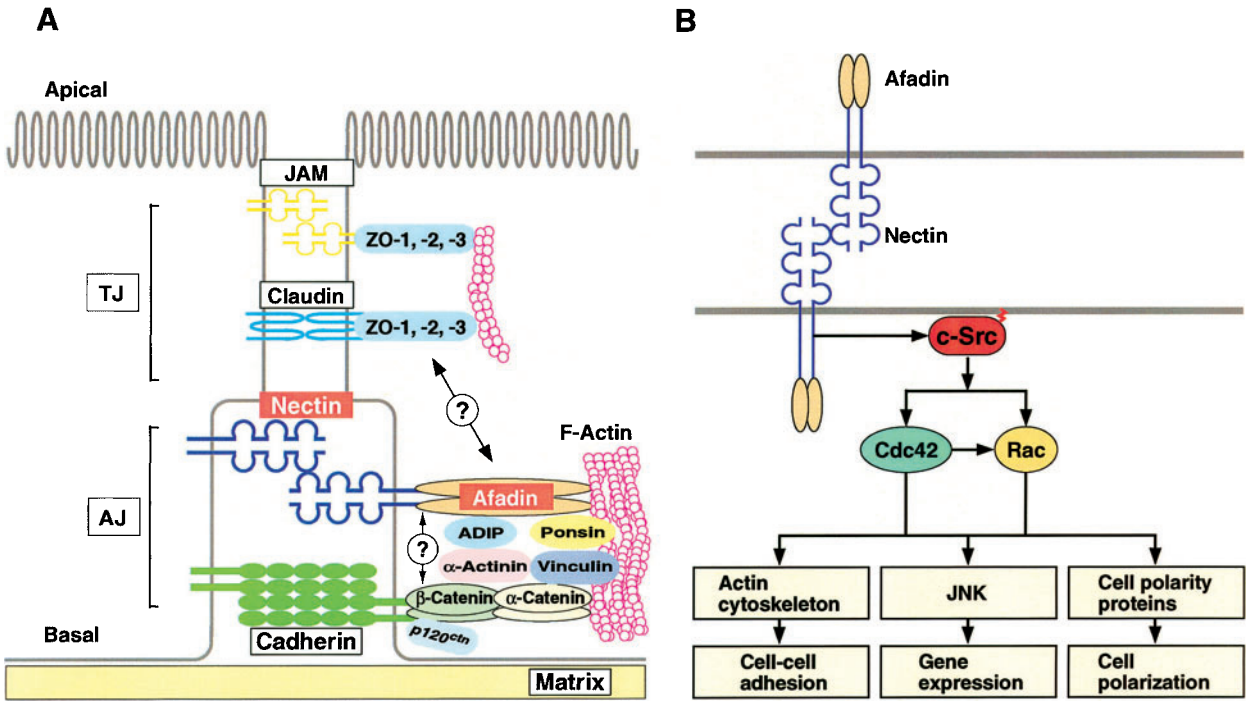

Fig. 1. **Intercellular adhesion and intracellular signaling mediated by nectin.** (A) The junctional complex of absorptive epithelial cells in the small intestine. The junctional complex, which consists of TJs and AJs, localizes at the lateral membrane and is undercoated with actin filaments (F-actin). The Ca^{2+}-independent immunoglobulin (Ig)-like cell-cell adhesion molecule, JAM, also localizes at TJs and interacts with ZO proteins. The nectin-afadin and cadherin-catenin systems are thought to be connected via two connector units: a ponsin-vinculin unit and an afadin DIL domain-interacting protein (ADIP)-α-actinin unit. The mechanism of the association of AJs and TJs remains unknown. (B) Nectin-induced activation of Cdc42 and Rac and their roles. The nectin-based cell-cell adhesion induces activation of Cdc42 and Rac through the activation of c-Src, which eventually regulates the formation of AJs through reorganization of the actin cytoskeleton, gene expression through activation of the JNK pathway, and cell polarization through cell polarity proteins.

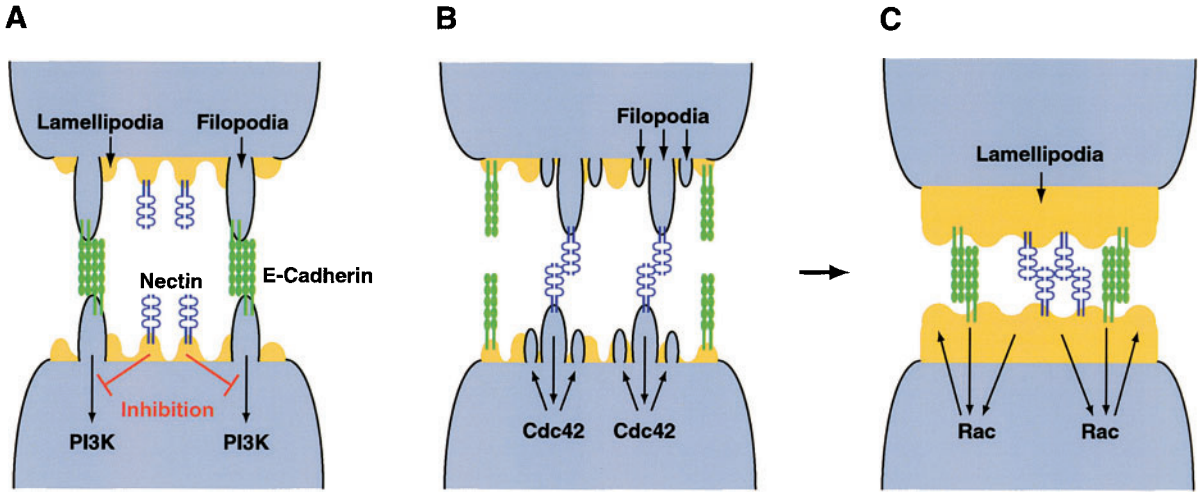

Fig. 2. **A fork initiation and zipper model for the formation of AJs by nectin and E-cadherin in epithelial cells.** (A) Nectin and E-cadherin are diffusely distributed on the free surface of the plasma membrane of migrating cells. When two migrating cells contact through their protrusions, nectin and E-cadherin separately form *trans*-dimers, which form micro-clusters at cell-cell contact sites. Non-*trans*-interacting nectins around *trans*-interacting E-cadherin inhibit the activation of Rac induced by the *trans*-interaction of E-cadherin and the Rac-mediated maturation of AJs. (B) The inhibitory effect of non-*trans*-interacting nectins is suppressed by the activation of Cdc42 induced by the *trans*-interaction of nectin, resulting in an increase in the number of filopodia and cell-cell contact sites, like the "fork initiation." (C) The Rac activated by either the *trans*-interaction of nectin or that of E-cadherin induces formation of lamellipodia. The lamellipodia efficiently expand the cell-cell adhesion between the filopodia, and efficiently zip the cell-cell contact sites, acting like a so-called "zipper."

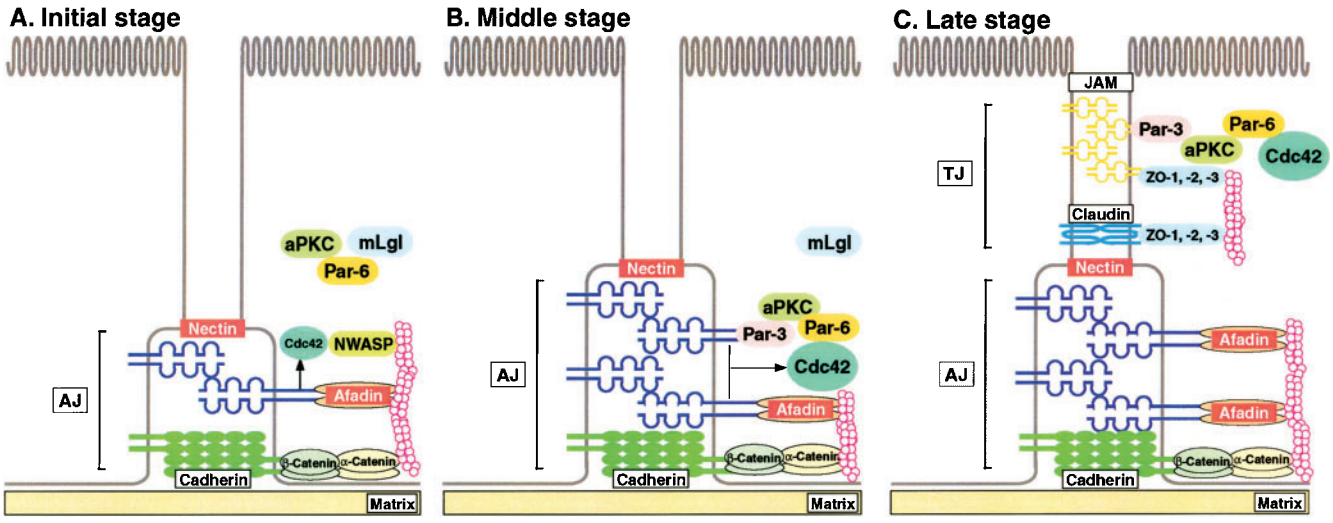

Fig. 3. **A model for the role and mode of action of nectin-activated Cdc42 in cell polarization in epithelial cells.** (A) The nectin-based cell-cell adhesions are mainly formed at the initial stage. The cytoplasmic region of nectin binds afadin. The nectin-based cell-cell adhesions then recruit E-cadherin, which results in formation of AJs. The cytoplasmic region of E-cadherin binds β-catenin, which in turn binds α-catenin. Moreover, the *trans*-interaction of nectin produces a lower concentration of activated Cdc42, which is enough for binding to NWASP/WASP but not to PAR-6, and this leads to the formation of AJs but not TJs. (B) At the middle stage, increasing *trans*-interaction of nectin produces a higher concentration of activated Cdc42, which is enough for binding to PAR-6 of the PAR-6/aPKC/mLgl complex. The activated Cdc42 activates aPKC, which phosphorylates mLgl, resulting in release of mLgl from the activated Cdc42/PAR-6/aPKC complex. PAR-3 binds to nectin. (C) At the late stage, claudin and JAM are recruited to the apical side of AJs. The complex formation of PAR-3 with the activated Cdc42/PAR-6/aPKC complex results in release of the PAR-3 with the activated Cdc42/PAR-6/aPKC complex from nectin and translocation of the complex to JAM, leading to establishment of TJs.

3. A role of nectin-activated Cdc42 and Rac in gene expression. Activated Cdc42 and activated Rac induce activation of MAP kinase cascades (*36*). Three major cascades have been identified: extracellular signal-regulated kinase (ERK), p38 MAP kinase, and c-Jun N-terminal kinase (JNK) pathways. Dominant active mutants of Cdc42 and Rac induce activation of the p38 MAP kinase and JNK cascades, but not that of the ERK cascade. These small G proteins induce activation of JNK through activation of two JNK kinases, MAP kinase kinase (MKK)4 and MKK7, and p38 MAP kinase through activation of two p38 MAP kinase kinases, MKK3 and MKK6 (*37*). The Cdc42 and Rac activated by the *trans*-interaction of nectin selectively induce activation of JNK, but not p38 MAP kinase or ERK (*12*) (Fig. 1B). It remains unknown why the Cdc42 or Rac activated by the *trans*-interaction of nectin does not induce the activation of p38 MAP kinase or ERK, but it may be due to the intracellular compartmentalization of the MAP kinase pathways. An overexpressed dominant active mutant of Cdc42 or Rac may be distributed randomly to many compartments, including the nectin-based micro-domains, and may induce the activation of p38 MAP kinase as well as that of JNK. However, the nectin-based micro-domain may be linked only to the JNK pathway. Since the JNK signaling pathway is involved in regulation of many cellular events, including growth control, transformation, and programmed cell death (*37*, *38*), it is important to clarify the role of the nectin-induced activation of JNK.

4. Roles of nectin-activated Cdc42 in epithelial cell polarity. In epithelial cells, the basolateral and apical domains are segregated by TJs, which is essential for establishing and maintaining their polarized morphology (*4*). The formation and maintenance of TJs depend on the formation and maintenance of AJs (*4*). The cell adhesion molecules of TJs, claudin and JAM, are recruited to the apical side of the nectin-, E-cadherin–based AJs (*9*). The Cdc42 activated by the *trans*-interaction of nectin is required for the formation of not only the E-cadherin-based AJs but also the claudin-based TJs, and a higher concentration of activated Cdc42 is required for the formation of the claudin-based TJs than for that of the E-cadherin-based AJs (*33*). Many downstream effectors have been identified for Cdc42, including IQGAP1, NWASP/WASP, and PAR-6 (*39*–*41*). IQGAP1 and NWASP/WASP are F-actin-binding proteins (*39*, *40*), whereas PAR-6 is a key intracellular molecule for the formation of TJs (*41*, *42*). The K_d values for binding of activated Cdc42 to the Cdc42 and Rac interactive binding domains of WASP and PAR-6 are 1.6 nM and 50 nM, respectively (*43*).

A model has recently been proposed for the mode of action of PAR-6 in formation of the junctional complex of AJs and TJs in epithelial cells (*44*, *45*). Cell-cell contact initially stimulates the localization of the protein complex containing PAR-6, aPKC, and mammalian Lgl (mLgl) at the cell-cell contact sites. The complex is inactive for the formation of TJs. Once aPKC is activated, mLgl is phosphorylated and dissociates from the PAR-6/aPKC-containing complex. This triggers the formation of the active PAR-3/PAR-6/aPKC complex that promotes the formation of TJs. Although the mechanism of the activation of aPKC remains to be clarified, it is highly likely that the binding of activated Cdc42 to PAR-6 activates aPKC, which then phosphorylates mLgl, resulting in release of mLgl from the activated Cdc42/PAR-6/aPKC

complex (Fig. 3B). The activated Cdc42/PAR-6/aPKC complex then forms a complex with PAR-3, resulting in association of this multiple complex to JAM, which is recruited to the apical side of the nectin-, E-cadherin-based AJs, through PAR-3 (Fig. 3C). It remains unknown where PAR-3 localizes before it binds to JAM. Recent analysis indicates that PAR-3 directly binds to nectin-1 and -3, but not -2 (*13*) (Fig. 3B). The precise role of this binding of PAR-3 to nectin remains unknown, but PAR-3 may bind to nectin before it binds to JAM, and the complex formation of PAR-3 with the activated Cdc42/PAR-6/aPKC complex results in release of the PAR-3 with the activated Cdc42/PAR-6/aPKC complex from nectin and translocation of the complex to JAM (Fig. 3C).

On the basis of these observations, our current model for the modes of action of the Cdc42 activated by the *trans*-interaction of nectin is as follows: at the initial stage of the formation of the cell-cell adhesion, the *trans*-interaction of nectin produces a lower concentration of activated Cdc42, which is enough for binding to NWASP/WASP but not to PAR-6, and this leads to the formation of AJs but not TJs (Fig. 3A); and at the middle and late stages, increasing *trans*-interaction of nectin produces a higher concentration of activated Cdc42, which is enough for binding to PAR-6 (Fig. 3B) and leads to the formation of TJs (Fig. 3C). In this way, the Cdc42 activated by the *trans*-interaction of nectin may be involved in establishment of polarized morphology of epithelial cells.

CONCLUSIONS

We have revealed that the intercellular adhesion molecule nectin plays key roles in organization of a variety of intercellular junctions in cooperation with, or independently of, cadherin. In this review, we have described that nectin furthermore plays important roles in intracellular signaling. Nectin induces activation of Cdc42 and Rac through the activation of c-Src, which eventually regulates the formation of AJs through the reorganization of the actin cytoskeleton, gene expression through the activation of a MAP kinase cascade, the JNK pathway, and the cell polarization through cell polarity proteins. Furthermore, nectin negatively and positively regulates the E-cadherin-induced activation of Rac. However, it remains unknown how nectin regulates these intracellular signaling molecules. The role of the nectin-induced activation of JNK also remains unknown. Clarifying the molecular mechanisms of these events will extend our understanding of tissue patterning, morphogenesis, and maintenance of normal tissues.

This work was supported by Grants-in-Aid for Scientific Research and for Cancer Research from Ministry of Education, Culture, Sports, Science, and Technology, Japan (2002, 2003).

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