Review Article

Nectins and nectin-like molecules: Roles in cell adhesion, migration, and polarization

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Nectins are a family of Ca^{2+}-independent immunoglobulin-like cell-cell adhesion molecules consisting of four members, which homophilically and heterophilically *trans*-interact and cause cell-cell adhesion. Nectin-based cell-cell adhesion is involved in the formation of cadherin-based adherens junctions in epithelial cells and fibroblasts. The nectin-based cell-cell adhesion induces activation of Cdc42 and Rac small G proteins, which eventually regulate the formation of 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. Five nectin-like molecules (necls), which have domain structures similar to those of nectins, have recently been identified and appear to play different roles from those of nectins. One of them, named necl-5, which does not homophilically *trans*-interact, but heterophilically *trans*-interacts with nectin-3, regulates cell migration and adhesion. In this article, the roles and modes of action of nectins and necls in cell adhesion, migration, and polarization are reviewed. (Cancer Sci 2003; 94: 655–667)

In multicellular organisms, cell adhesion and migration are critical for many events, including tissue patterning, morphogenesis, and maintenance of normal tissues.[1–3] Adhesion and migration of non-transformed normal cells are dynamic and well regulated.[2, 3] Cells disrupt cell-cell adhesion and start to migrate in response to extracellular cues, such as growth factors, cytokines, and extracellular matrix molecules.[4, 5] Cells migrate as individuals or as groups: leukocytes, lymphocytes, and fibroblasts migrate as individuals, whereas epithelial cells and endothelial cells migrate as groups. Cell migration is divided into at least four mechanistically distinct steps: formation of protrusions, formation of new cell-matrix adhesions, contraction of the cell body, and tail detachment. The extracellular cues stimulate cell surface receptors to initiate intracellular signaling through second messengers, protein kinases, protein phosphatases, and heterotrimeric large and monomeric small G proteins, to regulate these multiple steps. When migrating cells contact other cells, they stop migration and proliferation and adhere to each other to become confluent.[5, 6] This phenomenon is known as contact inhibition of cell movement and proliferation. Transformation of cells causes disruption of cell-cell adhesion, increase of cell motility, and loss of contact inhibition of cell movement and proliferation, eventually leading transformed cells to uncontrolled cell proliferation, invasion into surrounding tissues, and finally metastasis to other organs.[5, 6] However, the molecular mechanisms underlying these physiological or pathological processes are not fully understood.

Cell-cell adhesion is mainly mediated by cell-cell adherens junctions (AJs) and tight junctions (TJs) in epithelial cells.[7] Formation and maintenance of TJs in epithelial cells generally depend upon formation and maintenance of AJs.[8] Cadherins are key Ca^{2+}-dependent cell-cell adhesion molecules at AJs.[1, 2, 8–12] E-Cadherin, a member of the cadherin family expressed in epithelial cells, is associated with the actin cytoskeleton through peripheral membrane proteins, including α- and β-catenins, vinculin, and α-actinin.[1, 13] This association strengthens the cell-cell adhesion of AJs.[4, 13] At TJs, claudins are key Ca^{2+}-independent cell-cell adhesion molecules.[14, 15] Occludin is another transmembrane protein at TJs, but its function has not been established.[14, 15] Claudins and occludin are associated with the actin cytoskeleton through peripheral membrane proteins, such as ZO-1, -2, and -3.[14, 15] Junctional adhesion molecules (JAMs) that belong to Ca^{2+}-independent immunoglobulin (Ig)-like cell-cell adhesion molecules also localize at TJs and interact with ZO proteins.[15] AJs play key roles not only in formation of TJs at the apical side of AJs, but also in gathering around many biologically active molecules, such as cell surface receptors, intracellular signaling molecules, and oncoproteins.[4, 12, 13, 16, 17] TJs also concentrate tumor suppressor proteins and cell polarity proteins.[14, 15, 18]

Transformation of normal cells results in loss of cell polarity and appearance of abnormal morphology.[5] Genetic studies in *Caenorhabditis elegans* and *Drosophila* have revealed a set of evolutionarily conserved polarity proteins that are required for asymmetric cell division during development and epithelial membrane polarity.[18, 19] One example is the set of proteins, including PAR-3, PAR-6, and atypical protein kinase C (aPKC), which form the PAR-3/PAR-6/aPKC complex and are involved in various biological contexts.[18, 19] This protein complex localizes at TJs by direct binding of PAR-3 to JAMs in epithelial cells.[18] A series of studies with cultured epithelial cells have established that the PAR-3/PAR-6/aPKC complex promotes the asymmetric development of the epithelial junctional complex and cell polarity, in which aPKC kinase activity plays a critical role.[18] However, it is not yet fully understood how TJs are formed at the apical side of AJs in normal epithelial cells or how transformation of normal cells loses cell polarity and induces abnormal morphology.

Mutations of the components of the cadherin-catenin system have been reported in some carcinomas with highly invasive and metastatic nature.[20] However, in carcinomas in which the cadherin-catenin system is intact, the causative gene mutations responsible for their highly invasive and metastatic nature have not been identified. We have recently found a novel cell-cell adhesion system consisting of nectins and afadin at AJs in epithelial cells and fibroblasts.[21] Nectins are Ca^{2+}-independent Ig-like cell-cell adhesion molecules, and afadin is a nectin- and actin-filament (F-actin)-binding protein that connects nectins to the actin cytoskeleton. This novel cell-cell adhesion system has roles in the formation of the E-cadherin-based AJs and subse-

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quent formation of the claudin-based TJs in epithelial cells.[21–26] This adhesion system induces activation of Cdc42 and Rac small G proteins, which regulate cell-cell adhesion through reorganization of the actin cytoskeleton, gene expression through activation of a mitogen-activated protein (MAP) kinase cascade, and cell polarization through cell polarity proteins.[27–29] Nectins play roles not only in formation of AJs and TJs, but also in the formation of a variety of cell-cell junctions in cooperation with, or independently of, cadherins, such as synapses in neurons and Sertoli cell-spermatid junctions in the testis.[21, 30] Nectins further serve as entry and cell-cell spreading mediators of viruses.[21, 30] On the other hand, five nectin-like molecules (necls), which have domain structures similar to those of nectins, have recently emerged and appear to play different roles from those of nectins (Table 1),[31–42] but in cooperation with nectins. One of them, named necl-5, regulates cell migration and adhesion.[42] This review deals with the roles and modes of action of nectins and necls in cell adhesion, migration, and polarization.

I. General properties of nectins

1. Molecular structures of nectins. Nectins comprise a family consisting of four members, nectin-1, -2, -3, and -4.[21] All the members of the nectin family have two or three splice variants, so that nectin-1α, -1β, -1γ, -2α, -2δ, -3α, -3β, and -3γ isoforms exist. Nectin-4 also has two splice variants. Nectin-1α and -2α were originally isolated as poliovirus receptor-related proteins and named PRR-1 and -2, respectively, although neither of them has subsequently been confirmed to serve as a poliovirus receptor. They were later shown to serve as receptors for α-herpes virus, facilitating its entry and cell-cell spread, and so were renamed HveC and HveB, respectively. All the members, except nectin-1γ, have an extracellular region with three Ig-like loops, a single transmembrane region, and a cytoplasmic tail region (Fig. 1A). Nectin-1γ is a secreted protein which lacks the transmembrane region. Furthermore, all of them, 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 binds the PDZ domain of afadin, an F-actin-binding protein. This binding of afadin to nectins links nectins to the actin cytoskeleton. Although nectin-4 lacks the conserved motif, it binds the PDZ domain of afadin at its carboxyl terminus.

Afadin has two splice variants, l- and s-afadin (Fig. 1C).[21] l-Afadin, the larger variant, binds nectins through its PDZ domain and F-actin through its F-actin-binding domain. Afadin

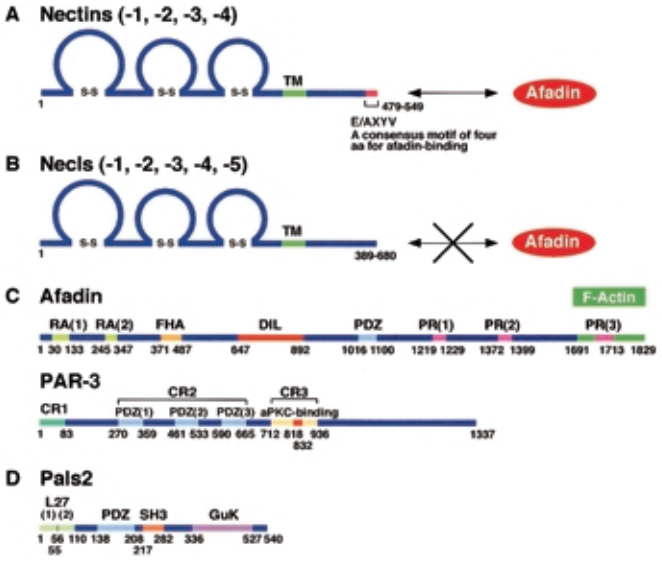

Fig. 1. Molecular structures of nectins, necls, and their direct binding proteins. (A) Nectins. Nectins have a conserved motif of four amino acid residues (Glu/Ala-X-Tyr-Val) at their carboxyl termini, and this binds the PDZ domain of afadin or PAR-3. (B) Necls. Necl-1 to -5 do not directly bind afadin. (C) Nectin-binding proteins. (D) Necl-2-binding protein. TM, transmembrane region; RA, Ras-associated domain; FHA, forkhead-associated domain; DIL, dilute domain; PDZ, PDZ domain; PR, proline-rich domain; CR, conserved region; L27, Lin-2/7 domain; SH3, Src homology domain 3; and GuK, guanylate kinase domain.

Table 1. Five necls

Proposed nomenclature	Old nomenclature	Accession No.	Submitted year	Reference
Necl-1	human NECL1	AF062733	1998	Direct submission only
	mouse NECL1	AF195662	1999	Direct submission only
	human TSLL1	AF363367	2001	31)
	mouse TSLL1	AY059393	2001	Direct submission only
	mouse SynCAM3			32)
Necl-2	mouse NECL2	AF061260	1998	Direct submission only
	mouse RA175	AB021964–AB021966	1998	33)
	human NECL2	AF132811	1999	Direct submission only
	human IGSF4	NM_018770	1999	34)
	mouse SgIGSF	AB052293	2000	35)
	human TSLC1			36)
	mouse TSLC1	AF434663	2001	37)
	mouse SynCAM1	AF539424	2002	32)
Necl-3	human NECL3	AF538973	2002	Direct submission only
	mouse similar to NECL3	XM_139689	2002	Direct submission only
	mouse SynCAM2			32)
Necl-4	human TSLL2	AF363368	2001	31)
	mouse TSLL2	AY059394	2001	Direct submission only
	mouse SynCAM4			32)
Necl-5	rat Tage4	L12025	1993	38)
	mouse Tage4	MMU35836	1995	39)
	mouse similar to Tage4	BC013673	2001	Direct submission only
	human PVR/CD155	M24407, M24406	1989	40)
		X64116–X64123 (exon 1–8)	1990	41)

has two Ras-associated (RA) domains, a forkhead-associated (FHA) domain, a dilute (DIL) domain, and three proline-rich (PR) domains. Although l-afadin binds F-actin, it does not have a cross-linking activity. s-Afadin, the smaller variant, has the two RA domains, the FHA domain, the DIL domain, the PDZ domain, and the two PR domains, but lacks the F-actin-binding domain and the third PR domain. Human s-afadin is identical to the gene product of *AF-6*, a gene that has been identified as an *ALL-1* fusion partner involved in acute myeloid leukemias. Unless otherwise specified, afadin refers to l-afadin in this article.

2. Cell-cell adhesion activity of nectins. In contrast to cadherins, the cell-cell adhesion activity of nectins is Ca^{2+}-independent.[21] Cadherins first form *cis*-dimers, in which the monomers are aligned in a parallel orientation, and then *trans*-dimers, in which *cis*-dimers from opposing cell surfaces interact in an anti-parallel orientation (Fig. 2A). It has been suggested that cadherins can form *trans*-dimers through the EC1 region alone, but *trans*-dimers that are formed between the fully interdigitated proteins show the strongest adhesion activity (Fig. 2A).[10] Each nectin forms *cis*-dimers, followed by formation of *trans*-dimers (Fig. 2B). Each nectin forms homo-*cis*-dimers, but not hetero-*cis*-dimers. Each protein then forms homo-*trans*-dimers. Nectin-3, furthermore, forms hetero-*trans*-dimers with either nectin-1 or -2. Nectin-4 forms hetero-*trans*-dimers with nectin-1, but nectin-1 or -2 does not form hetero-*trans*-dimers. These hetero-*trans*-dimers show much higher affinity than the homo-*trans*-dimers. This property of nectin is different from that of cadherins, which form mainly homo-*trans*-dimers.[9] 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.[21, 43] 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.

3. Tissue distribution and subcellular localization of nectins. Nectin-1, -2, and -3 are ubiquitously expressed in a variety of cell types including epithelial cells, neurons, and fibroblasts.[21] Nectin-2 or -3 is expressed in cells where cadherins are not expressed, such as blood cells and spermatids. Human nectin-4 is mainly expressed in placenta. l-Afadin is ubiquitously expressed, whereas s-afadin is specifically expressed in neurons. In the absorptive epithelial cells of mouse small intestine, nectin-2 and afadin are highly concentrated at the E-cadherin-based AJs, which are undercoated with F-actin bundles. Neither nectin-2 nor afadin is found at the claudin-based TJs and desmosomes. This distribution pattern is different from that of E-cadherin; although E-cadherin is concentrated at AJs, it is widely distributed from the apical to basal side of the lateral membrane.[2, 16] Neither nectin-2 nor afadin is found at cell-matrix AJs. In cultured fibroblasts that lack TJs, such as L cells stably expressing E-cadherin, nectin-2 and afadin co-localize with E-cadherin at AJs. Nectin-3 and afadin co-localize at cell-cell adhesion sites in NIH3T3 fibroblasts.[27] In epithelial cells and fibroblasts, each nectin localizes symmetrically at both sides of the plasma membrane of the AJs.[21]

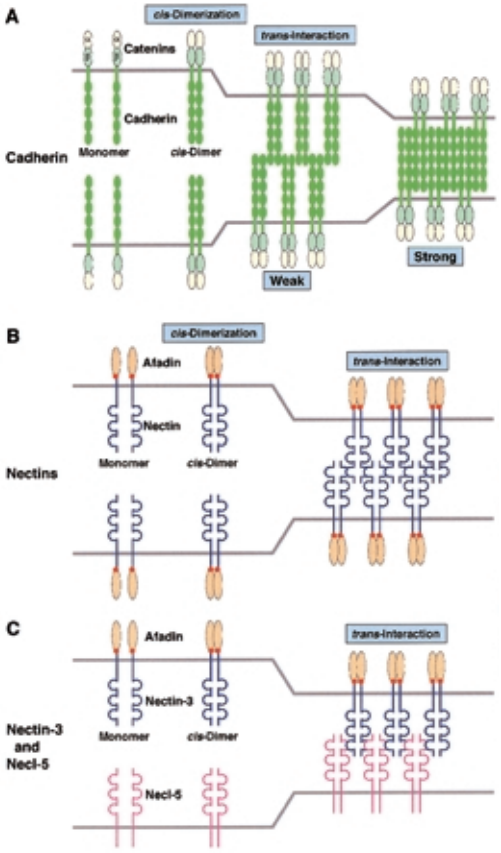

Fig. 2. Models for cell-cell adhesion activities of cadherins, nectins, and necl-5. (A) Cadherins. Cadherins first form homo-*cis*-dimers and then homo-*trans*-dimers, causing cell-cell adhesion. (B) Nectins. Nectins first form homo-*cis*-dimers and then homo- or hetero-*trans*-dimers, causing cell-cell adhesion. (C) Nectin-3 and necl-5. Nectin-3 and necl-5 first form homo-*cis*-dimers and then hetero-*trans*-dimers, causing cell-cell adhesion.

II. Roles of nectins in organization of AJs and TJs in epithelial cells

1. Regulation by nectins of the velocity of formation of AJs and TJs. When migrating epithelial cells meet and AJs and TJs are formed, primordial spot-like junctions are first formed at the tips of the cellular protrusions that radiate from adjacent cells.[12, 21] These primordial junctions fuse with each other to form short line-like junctions, which develop into more matured AJs. During and/or after this process, TJs are formed at the apical side of AJs to complete the junctional complex of AJs and TJs. It has been shown that E-cadherin, α- and β-catenins, and ZO-1 are first assembled to form spot-like junctions which are gradually matured to line-like junctions (AJs), followed by the assembly of claudin and occludin to form TJs.[12, 21] Nectins play a role in the formation of this junctional complex by regulating the velocity of the assembly of the components of AJs (Fig. 3A). Nectins and E-cadherin separately form *trans*-dimers that produce micro-clusters at cell-cell contact sites, when two migrating cells contact through protrusions such as filopodia and lamellipodia (Fig. 3A, a and b). Kinetically, nectins form micro-clusters more rapidly than E-cadherin, and therefore the nectin-based micro-clusters are mainly formed at the initial stage. The nectin-based micro-clusters then recruit E-cadherin, which results in the formation of a mixture of the nectin- and E-cadherin-based micro-clusters (Fig. 3A, c). The nectin and E-cadherin molecules in these micro-clusters are associated through afadin and catenins. E-Cadherin-based micro-clusters that form slowly and independently of the nectin-based micro-clusters rapidly recruit the nectin-afadin complex to form other primordial spot-like junctions. These primordial junctions fuse with each other to form short line-like junctions, which develop into more matured AJs (Fig. 3A, d). During and/or after the formation of AJs, JAMs are first assembled at the apical side of AJs (Fig. 3B, a and b), followed by the recruitment of claudins and occludin, which eventually leads to the establishment of the claudin-based TJs (Fig. 3B, c).

In fibroblasts, which lack TJs, nectins play a similar role in

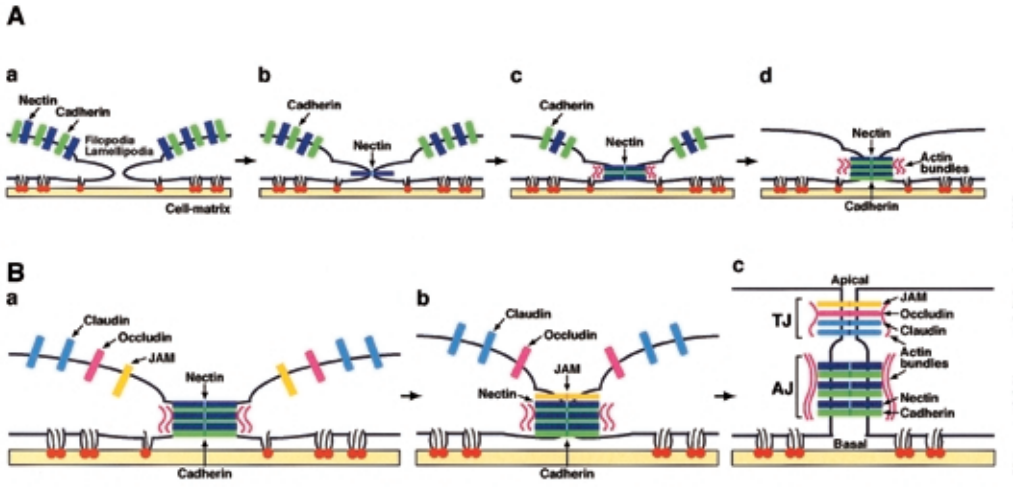

Fig. 3. A model for the role and mode of action of nectins in formation of the junctional complex in epithelial cells. (A) Formation of AJs. (a and b) When two migrating cells contact through their protrusions, nectins and E-cadherin separately form *trans*-dimers that generate micro-clusters at cell-cell contact sites. The nectin-based micro-clusters are mainly formed at the initial stage. (c and d) The nectin-based micro-clusters then recruit E-cadherin, which results in formation of a mixture of the nectin- and E-cadherin-based micro-clusters (primordial spot-like junctions). These primordial junctions fuse to form short line-like junctions, which develop into more matured AJs. Peripheral membrane proteins are not shown. (B) Formation of TJs. (a and b) During and/or after the formation of AJs, JAMs are first assembled at the apical side of AJs. (c) This is followed by the recruitment of claudins and occludin, which eventually leads to the establishment of the claudin-based TJs. Peripheral membrane proteins are not shown.

the formation of AJs in cooperation with cadherins. Because the formation and maintenance of TJs are generally dependent on the formation and maintenance of AJs,[8] nectins are most likely to regulate primarily the formation of AJs in epithelial cells. Many lines of evidence are available supporting this role of nectins in the formation of AJs.[21–26] Typical examples are that (i) overexpression of a nectin increases the velocity of the formation of AJs and TJs in epithelial cells and that of AJs in fibroblasts[21, 22, 25]; (ii) inhibition of the nectin-based cell-cell adhesion by a specific inhibitor, such as glycoprotein D (gD) or a recombinant extracellular fragment of a nectin fused to the Fc domain of IgG (Nef), conversely reduces the velocity of the formation of AJs and TJs in epithelial cells and that of AJs in fibroblasts.[21–25] gD is an envelope protein of herpes simplex virus type 1 which specifically binds to nectin-1 and inhibits its *trans*-interaction with nectin-1 or -3,[21] whereas Nef forms *trans*-dimers with a cellular nectin and thereby inhibits the formation of the nectin-based cell-cell adhesion[22]; and (iii) mutations in the *nectin-1* gene are responsible for cleft lip/palate-ectodermal dysplasia, Margarita island ectodermal dysplasia and Zlotogora-Ogür syndrome, which is characterized by cleft lip/palate, syndactyly, mental retardation, and ectodermal dysplasia.[21] It is unknown whether the nectin-based cell-cell adhesion regulates only the velocity of the formation of AJs or whether it is also essential for the formation and/or maintenance of AJs.

2. Mechanisms of association of nectins with cadherins and JAMs or claudins . It is not fully understood how nectins are physically associated with cadherins, but both afadin and α-catenin are essential for this association. Afadin directly binds α-catenin *in vitro*, but this binding is not strong.[21] The direct binding of these proteins may occur *in vivo*, but it is more likely that post-translational modification(s) of either or both proteins and/or an unidentified molecule(s) are required for the binding of α-catenin and afadin.[21] Two connector units for nectins and cadherins have been identified (Fig. 4, A and B). One is a ponsin-vinculin unit.[21] Ponsin is an afadin- and vinculin-binding protein and vinculin is an F-actin- and α-catenin-binding protein. The other is an afadin DIL domain-interacting protein (ADIP)-α-actinin unit.[44] ADIP is an afadin- and α-actinin-binding protein and α-actinin is an α-catenin-binding protein. In fibroblasts and epithelial cells, nectins and cadherins are associated through these connectors. The actin cytoskeleton is not involved in this association in fibroblasts.[27] However, in epithelial cells, the actin cytoskeleton is necessary for the formation of AJs and TJs (Fukuyama *et al*., unpublished results). Molecular mechanisms underlying this difference between fibroblasts and epithelial cells are unknown, but in epithelial cells, AJs are undercoated with F-actin bundles, forming belt-like structures, whereas in fibroblasts, AJs are less developed than those in epithelial cells and belt-like structures are not frequently observed at AJs. Therefore, it is likely in epithelial cells that the actin cytoskeleton is not necessary for the association of the nectin-afadin and cadherin-catenin systems, but is necessary for them to form matured AJs which are undercoated with F-actin bundles.

It is not known, either, how the nectin-afadin and cadherin-catenin systems regulate the formation of TJs in epithelial cells. ZO-1 is indirectly associated with the nectin-afadin system in a manner independent of the E-cadherin-catenin system.[21] During the formation of the junctional complex of AJs and TJs, JAMs are first recruited to the apical side of the nectin-, E-cadherin-based cell-cell adhesion sites, where ZO-1 colocalizes with afadin, followed by recruitment of claudins and occludin to the apical side of the nectin-, E-cadherin-based cell-cell adhesion sites (Fig. 4, C and D).[21] ZO-1 is also translocated from the nectin-, E-cadherin-based cell-cell adhesion sites to their apical side. Thus, the association of ZO-1 to the nectin-afadin system may play a role in recruiting the cell adhesion molecules of TJs to the apical side of AJs. The precise order of recruitment of the cell adhesion molecules and ZO-1 remains unknown. One possibility is that JAMs are first recruited to the apical side of the nectin-, E-cadherin-based cell-cell adhesion sites, and then the ZO-1 associated with the nectin-afadin system is translocated to the apical side to bind to JAMs. Alternatively, the ZO-1 associated with the nectin-afadin system is first translocated to the apical side, and then JAMs are recruited to the apical side by binding to ZO-1.

3. Activation of Cdc42 and Rac by nectins. The *trans*-interaction of E-cadherin induces activation of Rac, but not that of Cdc42, in epithelial cells and fibroblasts.[17, 45] The *trans*-interaction of E-cadherin does not induce activation of Rho in epithelial cells.[17, 45] The E-cadherin-induced activation of Rac is mediated

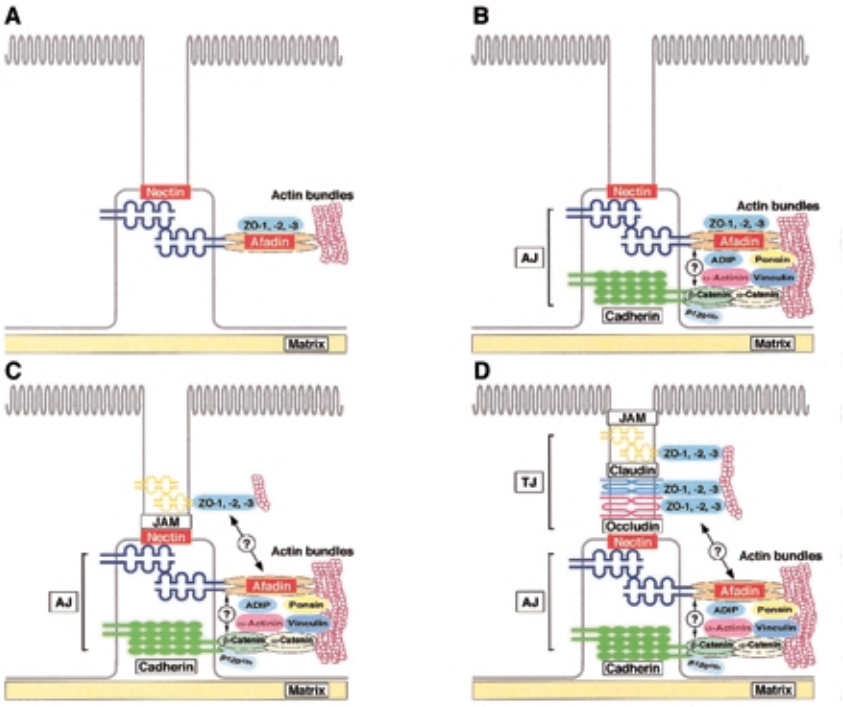

Fig. 4. Mechanism of association of the components of AJs and TJs. (A) The nectin-based cell-cell adhesions are mainly formed at the initial stage. The cytoplasmic region of nectins binds afadin that is directly associated with the actin bundles. ZO-1 is indirectly associated with the nectin-afadin system in a manner independent of the E-cadherin-catenin system. (B) 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. α-Catenin is associated with the actin bundles directly and indirectly. Afadin and α-catenin are associated with each other presumably through two connector units, a ponsin-vinculin unit and an ADIP-α-actinin unit. (C) During and/or after the formation of AJs, JAMs are first assembled at the apical side of AJs. The cytoplasmic region of JAM binds ZO-1, -2, and -3. ZO-1 and -2 are directly associated with the actin bundles and form a dimer with ZO-3. (D) Claudins and occludin are then recruited and eventually lead to the establishment of the claudin-based TJs. The cytoplasmic regions of claudins and occludin bind ZO-1, -2, and -3. Actin bundles and peripheral membrane proteins shown in the right-side cell are omitted in the left-side cell.

through activation of phosphatidylinositol 3 (PI3) kinase,[17, 45] but it remains unknown how E-cadherin induces the activation of Rac through PI3 kinase. On the other hand, the *trans*-interactions of nectins induce activation of not only Rac but also Cdc42 in a PI3 kinase-independent manner in fibroblasts and epithelial cells (Fig. 5).[27, 28] It is unclear whether the *trans*-interactions of nectins induce 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 C-terminal four amino acids of nectins, which are necessary for their binding of afadin, are not essential for the activation of Cdc42 or Rac. Nectins, which do not *trans*-interact with other nectins (non-*trans*-interacting nectins), inhibit both the E-cadherin-induced activation of Rac and the formation of cell-cell AJs, and these inhibitory effects of non-*trans*-interacting nectins are suppressed by the activation of Cdc42 induced by the *trans*-interactions of nectins (Hoshino *et al*., unpublished results) (Fig. 6A). The C-terminal four amino acids of nectins are essential for the inhibitory effects 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).[46] GEPs enhance activation of the GDP-bound inactive form of the small G protein to the GTP-bound active form; GAPs enhance inactivation of the GTP-bound active form of the small G protein to the GDP-bound inactive form; and GDIs bind the GDP-bound inactive form of the small G protein, retrieve it from the membranes and keep it in the cytosol. E-Cadherin and nectins may induce either activation of GEPs, inactivation of GAPs, or both, but their precise molecular mechanism remains to be elucidated. GTP-Cdc42 and GTP-Rac thus formed then play at least three roles in cell-cell adhesion through reorganization of the actin cytoskeleton, gene expression through activation of c-Jun N-terminal kinase (JNK) pathway, and cell polarization through cell polarity proteins (Fig. 5).

4. Role of Cdc42 and Rac in cell-cell adhesion. The formation of AJs is enhanced by GTP-Rac and GTP-Cdc42 in epithelial cells,[12, 17, 45, 47] whereas the formation of AJs is suppressed by inhibition of activation of Rac or action of GTP-Cdc42 in epithelial cells[47] (Fukuhara *et al*., unpublished results). The formation of AJs is suppressed by inhibition of Rho.[17] The modes of action of these small G proteins appear to be different: expression of a dominant active mutant of Rac causes tight adhesion of the lateral plasma membrane from the apical to the basal area with interdigitation of the lateral plasma membrane in MDCK cells; expression of a dominant active mutant of Cdc42 causes similar tight adhesion of the lateral plasma membrane from the apical to the basal area, but without the interdigitation of the lateral plasma membrane in MDCK cells[17, 45]; and expression of a dominant active mutant of Rho does not affect the E-cadherin-mediated cell-cell AJs.[47] The precise modes of action of Rac and Cdc42 activated by the *trans*-interactions of nectins and/or that of E-cadherin during the formation of AJs remain unknown, but a plausible mechanism is as follows: when two migrating cells contact through their protrusions, nectins and E-cadherin separately form *trans*-dimers that form micro-clusters at cell-cell contact sites as described above (see Fig. 3A). At the initial stage, cell-cell contact sites are formed mainly by the *trans*-interactions of nectins (Fig. 6B), because nectins kinetically form micro-clusters more rapidly than E-cadherin. The *trans*-interactions of nectins induce activation of Cdc42 and Rac. GTP-Cdc42 formed in this way increases the

number of filopodia and cell-cell contact sites, like "fork initiation." GTP-Rac formed by either the *trans*-interactions of nectins and/or that of E-cadherin induces formation of lamellipodia and efficiently zips the cell-cell adhesion between the filopodia, like "zipper" (Fig. 6C). 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 (Fig. 6A), until the non-*trans*-interacting nectins *trans*-interact with other nectins and induce the activation of Cdc42 (Fig. 6B).

Many downstream effectors have been identified for Rac and Cdc42. Some of these effectors are F-actin-binding proteins: IQGAP1 and IRSp53/WAVE for Rac; and IQGAP1, NWASP, and WASP for Cdc42.[46, 48] Therefore, the nectin-afadin and E-cadherin-catenin systems regulate organization of the peripheral actin cytoskeleton in two different ways: one is organized by F-actin-binding proteins associated with nectins and E-cadherin, such as afadin, α-catenin, α-actinin, and vinculin; the other is organized by Cdc42 and Rac through their downstream effectors, the F-actin-binding proteins. The actin cytoskeleton reorganized in these ways facilitates the formation of AJs and strengthens their cell-cell adhesion (Fig. 7).

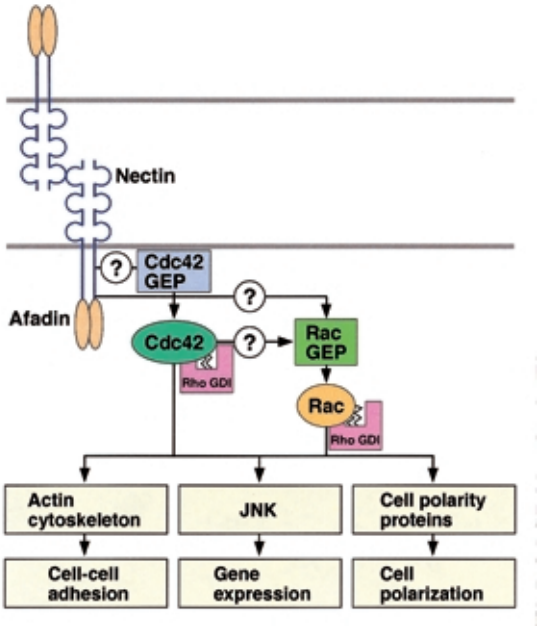

Fig. 5. Nectin-induced activation of Cdc42 and Rac and their roles. The nectin-based cell-cell adhesion induces activation of Cdc42 and Rac, which eventually regulate the formation of AJs through reorganization of the actin cytoskeleton, gene expression through activation of the JNK pathway, and cell polarization through cell polarity proteins. GAPs are not shown in this figure (see text).

5. Role of Cdc42 and Rac in gene expression. GTP-Cdc42 and GTP-Rac induce activation of MAP kinase cascades.[49] Three major cascades have been identified: extracellular signal-regulated kinase (ERK), p38 MAP kinase, and JNK pathways. Dominant active mutants of Cdc42 and Rac induce activation of the p38 MAP kinase and JNK cascades, but not 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 MKKs, MKK3 and MKK6.[50] It may be noted that GTP-Cdc42 and GTP-Rac formed by the *trans*-interactions of nectins selectively induce activation of JNK, but not p38 MAP kinase or ERK.[27] It remains unknown why GTP-Cdc42 or GTP-Rac formed by the *trans*-interactions of nectins does not induce the activation of p38 MAP kinase or ERK, but a possible reason is 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,[50, 51] it is important to clarify the role of the nectin-induced activation of JNK. On the other hand, the activation of ERK and p38 MAP kinase is induced upon the formation of the E-cadherin-mediated cell-cell adhesion in Caco-2/15 and HaCat cells, respectively,[17, 45] but it remains unknown whether activation of ERK and p38 MAP kinase is indeed induced by the *trans*-interaction of E-cadherin.

6. Roles of nectins and Cdc42 in cell polarization. Mechanisms of establishment of cell polarity have been studied most extensively in *C. elegans* and *Drosophila*.[19] PAR proteins, PAR-1 to

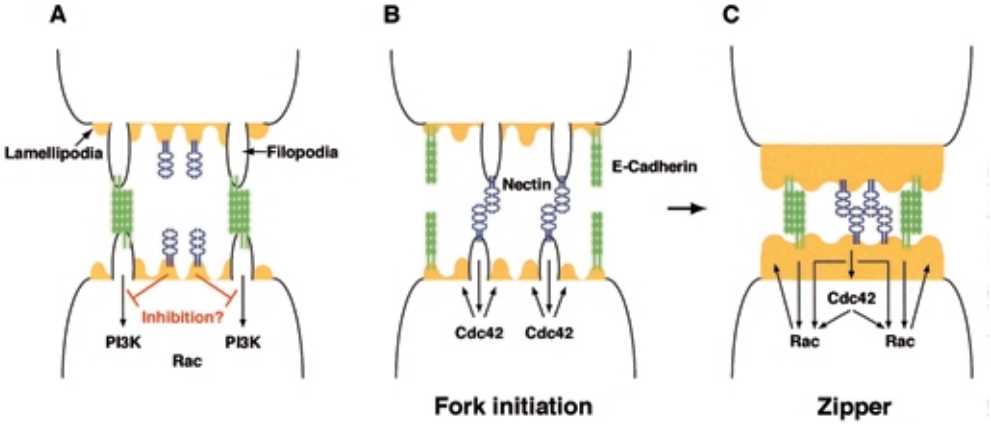

Fig. 6. A fork initiation and zipper model for the formation of AJs by nectins and E-cadherin in epithelial cells. (A) Nectins and E-cadherin are diffusely distributed on the free surface of the plasma membrane of migrating cells. When two migrating cells contact through their protrusions, nectins and E-cadherin separately form *trans*-dimers that generate 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*-interactions of nectins, resulting in an increase in the number of filopodia and cell-cell contact sites, like "fork initiation." (C) GTP-Rac formed by either the *trans*-interactions of nectins or those 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 (so-called "zipper").

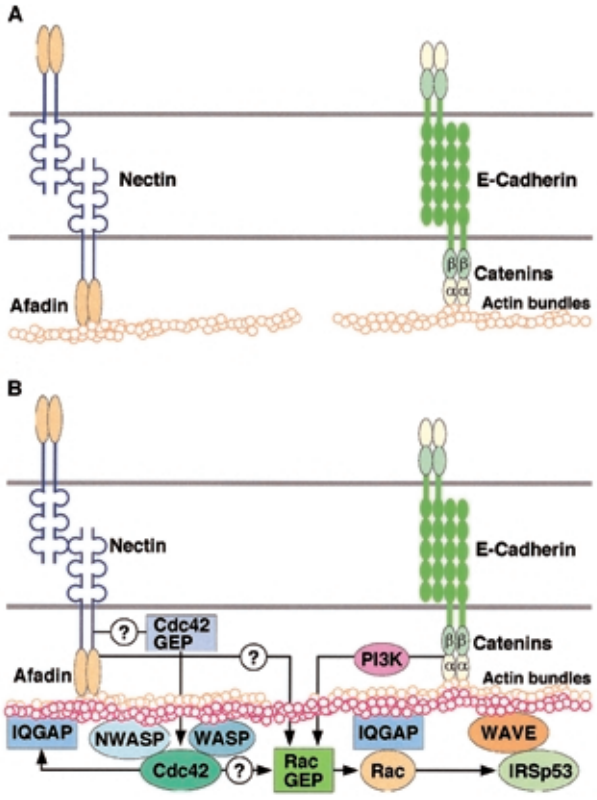

Fig. 7. Two different ways for nectins and E-cadherins to reorganize the peripheral actin cytoskeleton. The nectin-afadin and E-cadherin-catenin systems regulate organization of the peripheral actin cytoskeleton in two different ways. (A) One is organized by F-actin-binding proteins associated with nectins and E-cadherin, such as afadin, α-catenin, α-actinin, and vinculin. (B) The other is organized by Cdc42 and Rac through their downstream effectors, IQGAP1, IRSp53/WAVE, NWASP and WASP.

-6, were first identified in *C. elegans* as indispensable proteins that are involved in establishment of the anterior-posterior cell polarity of the one-cell embryo.[52] Subsequently, homologous proteins were identified in *Drosophila* and mammals.[18, 19] In mammals, PAR-3, a mammalian homologue of the *par-3* gene product, was first identified as an aPKCζ-binding protein (Fig. 1D).[18] PAR-3 localizes at TJs and forms a ternary complex with PAR-6 and aPKC in mammalian epithelial cells.[18] These three proteins directly interact with each other and play a critical role in the apico-basal polarization of mammalian epithelial cells.[18] This PAR-3/PAR-6/aPKC complex is an evolutionarily conserved cell polarization machinery that functions in a variety of biological contexts, from warm-blooded embryos to differentiated mammalian cells.[18] There are also other sets of proteins that establish both AJs and membrane polarity in *Drosophila*[53, 54]; these include Crumbs (Crb), Stardust (Std), and Discs lost (Dlt), which localize at the subapical region of epithelial cells, and Scribble (Scrib), Discs large (Dlg), and Lethal giant larvae (Lgl), which localize at the basolateral region. Mammalian homologues of Crb, Std, Dlt, Crumb, Pals1, and PATJ localize at TJs,[55, 56] whereas homologues of Dlg and Lgl localize at the basolateral region.[57]

A model for the mode of action of these polarity proteins in the formation of the junctional complex of AJs and TJs in epithelial cells has recently been proposed.[58, 59] 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 (Fig. 8B). The complex is inactive for the formation of TJs. Once aPKC is activated, it phosphorylates mLgl, resulting in release of mLgl from the PAR-6/aPKC complex (Fig. 8C). This triggers the formation of the active PAR-3/PAR-6/aPKC complex that promotes the formation of TJs. The dissociated mLgl molecule remains at the lateral region of the plasma membrane and is thought to contribute to the establishment of the basolateral plasma membrane. Although the mechanism of the activation of aPKC remains to be clarified, binding of GTP-Cdc42 to PAR-6 is likely to trigger its activation (Fig. 8, B and C). It has recently been shown that the Crumb/Pals1/PATJ complex interacts with the PDZ domain of PAR-6, and that this interaction is enhanced by binding of GTP-Cdc42 to PAR-6,[55] suggesting that the Crumb/Pals1 complex is also involved in the regulation of the interaction between mLgl and the PAR-6/aPKC complex, and that the PAR-6/aPKC complex, together with PAR-3, involves the Crumb/Pals1/PATJ complex to promote the formation of TJs (Fig. 8, E and F).[58, 59] Thus, the dissociation of mLgl from the PAR-6/aPKC complex is likely to trigger the interaction of the PAR-6/aPKC complex with the Crumb/Pals1/PATJ complex in addition to its interaction with PAR-3.[58, 59]

In this proposed model, Cdc42 functions as an initial trigger for the formation of TJs through these polarity proteins. GTP-Cdc42 formed by the action of nectins is likely to play this role (Fig. 8B). The roles of nectins in recruiting JAMs to the apical side of AJs and in activation of Cdc42 suggest that nectins operate in the following way in cell polarization: the *trans*-interactions of nectins recruit E-cadherin to form AJs and then recruit JAMs to the apical side of AJs (Fig. 8, A and E). On the other hand, they induce activation of Cdc42, which binds to PAR-6 (Fig. 8B). GTP-Cdc42-PAR-6 activates aPKC, which then phosphorylates mLgl, resulting in release of mLgl from the GTP-Cdc42/PAR-6/aPKC complex (Fig. 8C). The GTP-Cdc42/PAR-6/aPKC complex then forms a complex with PAR-3, resulting in association of this multiple complex with JAMs through PAR-3. It remains unknown where PAR-3 localizes before it binds to JAMs. Recent analysis indicates that PAR-3 binds to nectin-1 and -3, but not -2 (Fig. 1C and Fig. 8B).[29] The first PDZ domain of PAR-3 binds to the C-terminal four amino acids of nectin-1 and -3. Thus, nectin-1 and -3 bind both afadin and PAR-3. The precise role of this binding of PAR-3 to nectins remains unknown, but PAR-3 may bind to nectins before it binds to JAMs and the complex formation of PAR-3 with GTP-Cdc42/PAR-6/aPKC releases the PAR-3 with GTP-Cdc42/PAR-6/aPKC from nectins and translocates the complex to JAMs (Fig. 8, C, D, and E).

7. Role of nectins in disruption of cell-cell adhesion. Transformation of normal cells by expression of oncogenes or stimulation of normal cells by growth factors induces disruption of cell-cell junctions, followed by cell migration.[4] In cultured epithelial cells, for instance, scatter factor (SF)/hepatocyte growth factor (HGF) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a tumor-promoting phorbol ester, induce cell spreading, followed by disruption of cell-cell adhesion, subsequent cell-cell dissociation, and ultimately cell scattering.[4] Regulation of the cell-cell adhesion activity of E-cadherin has been shown to play a crucial role in rapid change of cell-cell adhesion and several different mechanisms have been proposed for this regulation: they include changes in the composition of the E-cadherin-catenin complex, interaction of α-catenin with the actin cytoskeleton, tyrosine phosphorylation of the E-cadherin-catenin complex, association of E-cadherin with p120 catenin, endocytosis of E-cadherin, ectodomain shedding of E-cadherin, and regulation by Rac, Cdc42, and Rho.[4] However, the mechanisms underlying the SF/HGF- and TPA-induced disruption of the E-cadherin-based cell-cell adhesion and the subsequent cell-cell dissociation in epithelial cells remain to be clarified. The role of the nectin-afadin system in the organization of AJs and TJs has raised the possibility that this cell-cell adhesion system is

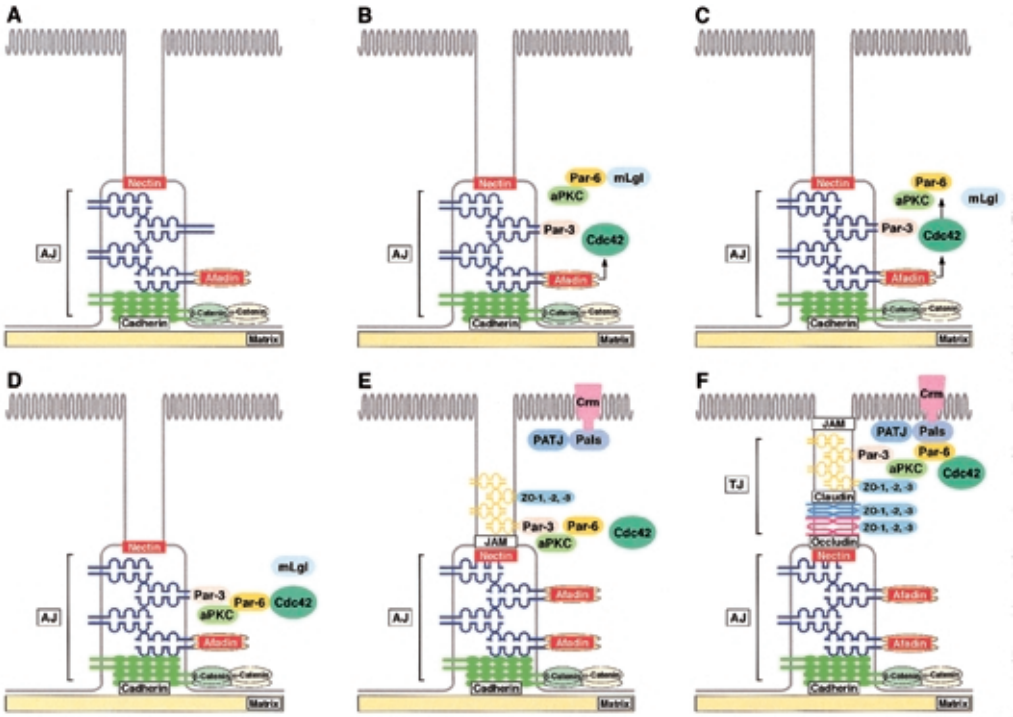

Fig. 8. A model for the role and mode of action of nectins in cell polarization in epithelial cells. (A) The nectin-based cell-cell adhesions are mainly formed at the initial stage. The cytoplasmic region of nectins binds afadin. The nectin-based cell-cell adhesions then recruit E-cadherin, which results in formation of AJs. The cytoplasmic region of E-cadherin binds β-catenin, which in turn binds α-catenin. (B) The *trans*-interactions of nectins induce activation of Cdc42, which binds to PAR-6 of the GTP-Cdc42/PAR-6/aPKC/mLgl complex. Nectins bind both afadin and PAR-3. (C) GTP-Cdc42/PAR-6 activates aPKC, which phosphorylates mLgl, resulting in release of mLgl from the GTP-Cdc42/PAR-6/aPKC complex. (D) The GTP-Cdc42/PAR-6/aPKC complex then forms a complex with PAR-3 bound to nectins. (E) The *trans*-interactions of nectins recruit JAMs to the apical side of AJs. The GTP-Cdc42/PAR-3/PAR-6/aPKC complex translocates from nectins and binds to JAMs. The Crumb/Pals1/PATJ complex also localizes at the apical side of AJs, but the order of recruitment of the Crumb/Pals1/PATJ complex and the GTP-Cdc42/PAR-3/PAR-6/aPKC complex remains unknown. (F) Claudins and occludin are recruited and eventually lead to establishment of TJs. The cytoplasmic regions of JAMs, claudins, and occludin bind ZO-1, -2, and -3. The Crumb/Pals1/PATJ complex interacts with the GTP-Cdc42/PAR-3/PAR-6/aPKC complex through the direct interaction between Pals1 and PAR-6. Actin bundles and peripheral membrane proteins are omitted in this figure.

involved in the SF/HGF- and TPA-induced disruption of the E-cadherin-based cell-cell adhesion and the subsequent cell-cell dissociation. It has recently been shown that SF/HGF and TPA induce proteolytic cleavage of nectin-1α in the ectodomain, resulting in generation of the 80-kD extracellular fragment and the 33-kD fragment composed of the transmembrane and cytoplasmic domains, in MDCK cells.[60, 61] It has also been reported that nectin-1α serves as a substrate for presenilin/γ-secretase.[60] This shedding of nectin-1α is inhibited by metalloprotease inhibitors. One possible role of the ectodomain shedding of nectin-1α is that it may be directly associated with the cell-cell dissociation induced by TPA and SF/HGF. Disruption of the nectin-based cell-cell adhesion may function as a trigger for the disruption of the E-cadherin-based cell-cell adhesion and the subsequent cell-cell dissociation. Another possible role is that the ectodomain shedding of nectin-1α may somehow transduce a signal. The 80-kD extracellular fragment of nectin-1α, which is released into the culture medium, may *trans*-interact with cellular nectin-1 or -3 and induce cell spreading and scattering through activation of Cdc42 and Rac. The 33-kD fragment composed of the transmembrane and cytoplasmic domains, which is anchored to the plasma membrane, may also transduce an intracellular signal as suggested for PECAM-1.[62]

III. Nectin-like molecules (Necls)

1. General properties. Five molecules with domain structures similar to those of nectins have been identified (Table 1). All of these molecules have an extracellular region with three Ig-like loops, a single transmembrane region, and a cytoplasmic tail region (Fig. 1B). As to NECL1/TSLL1/SynCAM3, NECL1 was directly reported in GenBank (1998); *TSLL1* was identified as a *TSLC1*-like gene[31]; and SynCAM3 was identified as a member of the SynCAM family.[32] In the case of NECL2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1, NECL2 was directly reported in GenBank (1998); *IGSF4* was identified as a candidate tumor suppressor gene in the loss of heterozygosity (LOH) region of chromosome 11q23.2[34]; RA175 was identified as being highly expressed during neuronal differentiation of embryonic carcinoma cells[33]; SgIGSF was identified as being expressed in spermatogenic cells during early stages of spermatogenesis[35]; TSLC1 was identified as a tumor suppressor in human non-small cell lung cancer[36]; and SynCAM1 was identified as a brain-specific synaptic adhesion molecule.[32] *TSLL2* and SynCAM4 were identified as a *TSLC1*-like gene and a member of the SynCAM family, respectively.[31, 32] As for NECL3/Similar to NECL3/SynCAM2, human NECL3 was directly reported in GenBank (2002); mouse Similar to NECL3 was directly reported in GenBank (NCBI annotation project, 2002); and SynCAM2 was identified as a member of the SynCAM family.[32] Human NECL3 and mouse Similar to NECL3 are derived from a common ancestor gene. The human poliovirus receptor (PVR)/CD155 was originally identified as a human poliovirus receptor.[40, 41] The *PVR/CD155* gene has thus far been found only in primates, and Tage4 has thus far been found only in rodents.[63] The sequence of Tage4 shows 42% amino acid identity to that of PVR/CD155. Thus, the *Tage4* gene may be the ortholog of the *PVR/CD155* gene,[63] but phylogenetic analysis of nectins and necls has not led to any definite conclu-

sion as to whether *Tage4* and *PVR/CD155* are derived from the same or different ancestor genes. However, because both genes localize on homologous chromosomes (rat Tage4, 1q22; mouse Tage4, 7A2-B1; and human PVR/CD155, 19)[63] and the functional properties of Tage4 and PVR/CD155 are similar (see below), we tentatively propose here that they are derived from a common ancestor gene.

The existence of many nomenclatures for the same molecule is confusing. We have proposed that the group of proteins with structures similar to those of nectins, but without the ability to directly bind afadin, should be called necls. NECL1/TSLL1/SynCAM3, NECL2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1, NECL3/Similar to NECL3/SynCAM2, and TSLL2/SynCAM4 have a consensus motif with four amino acids for binding to a PDZ domain at the extreme carboxyl termini, and their cytoplasmic regions show high levels of similarity. Tage4 or PVR/CD155 does not have this motif. Tage4, NECL1/TSLL1/SynCAM3, and NECL2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1 do not bind afadin[42, 64] (Kakunaga *et al*., unpublished results). It remains to be examined whether NECL3/Similar to NECL3/SynCAM2, TSLL2/SynCAM4, and PVR/CD155 directly bind afadin, but we assume that they do not. Thus, we propose the following nomenclatures[42]: necl-1 for NECL1/TSLL1/SynCAM3, necl-2 for NECL2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1, necl-3 for NECL3/Similar to NECL3/SynCAM2, necl-4 for TSLL2/SynCAM4, and necl-5 for Tage4/PVR/CD155.

The roles of necl-2 and -5 have been partly elucidated, but those of other necls are not known, except that the necl-1/TSLL1 mRNA is expressed exclusively in adult and fetal human brain,[31] that the necl-1 protein is expressed in the brain (Kakunaga *et al*., unpublished results), and that the necl-4/TSLL2 mRNA is expressed in several specific human tissues.[31] Therefore, we focus below on the roles of necl-2 and -5.

2. Possible role of necl-2 in the localization of another transmembrane protein at the extra-junctional regions of the plasma membrane. TSLC1, SynCAM1, and necl-2 show Ca^{2+}-independent homophilic cell-cell adhesion activity.[32, 64, 65] Moreover, necl-2 shows Ca^{2+}-independent heterophilic cell-cell adhesion activity with necl-1/TSLL1/SynCAM3 and nectin-3.[64] SynCAM1 is specifically expressed in mouse brain as estimated by Western blotting,[32] but TSLC1, SgIGSF, and necl-2 are expressed ubiquitously as estimated by Northern blotting and Western blotting.[35, 37, 64] This inconsistency appears to be due to the specificity of the antibody used for SynCAM1. In epithelial cells, necl-2 localizes at the basolateral plasma membrane.[64] Immunoelectron microscopic analysis in epithelial cells of mouse gall bladder demonstrated that necl-2 localizes uniquely at the basolateral plasma membrane, but not at specialized cell-cell junctions, such as TJs, AJs, and desmosomes. SynCAM1 has been shown to form synapses between HEK293 cells expressing exogenous SynCAM1 and primary cultured hippocampal neurons *in vitro*.[32] TSLC1 has a band 4.1-binding motif at the juxtamembrane region and binds tumor suppressor DAL-1, one of the band 4.1 family members, which connects TSLC1 to the actin cytoskeleton.[66] Necl-2 does not bind afadin but binds pals2 (Fig. 1D),[64] a membrane-associated guanylate kinase family member known to bind Lin-7, of which the *C. elegans* homologue is implicated in proper localization of the Let-23 protein, the homologue of a mammalian EGF receptor.[67] The exact role of necl-2 is currently unknown, but the unique localization of necl-2 and its ability to bind pals2 suggest that necl-2 is involved in localization of other transmembrane protein(s), such as growth factor receptors, through pals2, at the extra-junctional regions of the basolateral plasma membrane of epithelial cells.

Because necl-2 does not bind afadin, it does not recruit E-cadherin to the necl-2-based cell-cell adhesion sites and may not be directly involved in formation of AJs. However, it heterophilically *trans*-interacts with nectin-3, and this heterophilic *trans*-interaction may recruit E-cadherin through nectin-3 to the cell-cell adhesion sites formed between necl-2 and nectin-3 and thereby may be indirectly involved in the formation of AJs, at least partly (Fig. 9). Because the affinity of nectin-3 for necl-2 is far less than that for nectin-1 or -2, the necl-2 molecule *trans*-interacting with nectin-3 may be replaced by nectin-1 or -2, resulting in formation of the heterophilic *trans*-interaction of nectin-3 with nectin-1 or -2 and segregation of necl-2 from the nectin- and E-cadherin-based cell-cell adhesion sites (Fig. 9, C, D, and E). In this way, a transmembrane protein(s) associating with necl-2 through pals2 may be distributed to the extra-junctional region of basolateral plasma membrane (Fig. 9F).

3. Roles of necl-5 in cell migration and adhesion. Tage4 was originally identified as a gene overexpressed in rat and mouse colon carcinoma.[38, 39] PVR/CD155 has also been shown to be overexpressed in human colorectal carcinoma.[68] Necl-5 is up-regu-

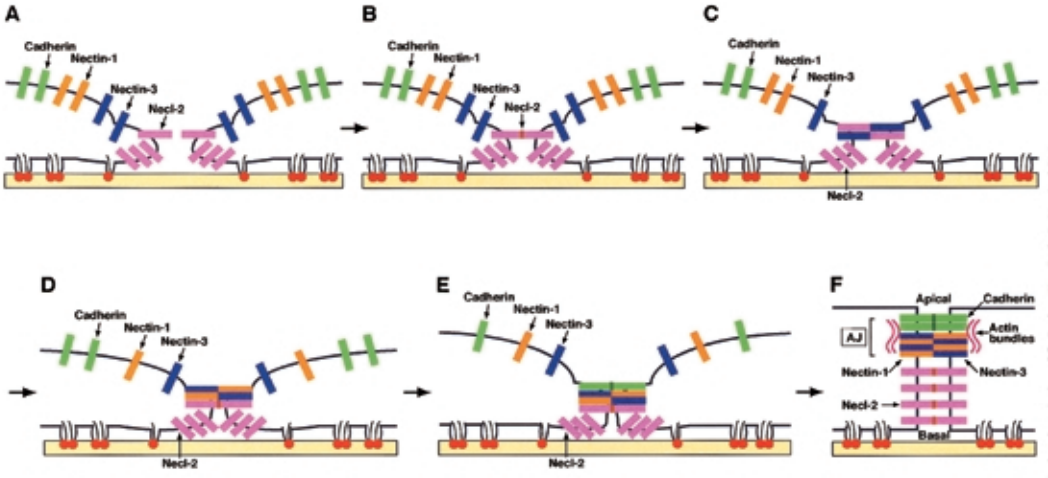

Fig. 9. A possible role of necl-2 and its mode of action in formation of cell-cell adhesion. (A and B) When two migrating cells make contact through their protrusions, necl-2 forms *trans*-dimers that generate micro-clusters at cell-cell contact sites. (C) Necl-2 heterophilically *trans*-interacts with nectin-3. (D) The necl-2 molecule *trans*-interacting with nectin-3 is replaced by nectin-1 or -2, resulting in formation of the heterophilic *trans*-interaction of nectin-3 with nectin-1 or -2 and segregation of necl-2 from the nectin-based cell-cell adhesion sites. (E) The nectin-based cell-cell adhesions then recruit E-cadherin, which results in formation of a mixture of the nectin- and E-cadherin-based cell-cell adhesion.

lated in NIH3T3 cells transformed by an oncogenic Ras (V12Ras-NIH3T3 cells).[42] Northern blot analysis has revealed that the Tage4 and necl-5 mRNAs are expressed to small extents in normal adult rat and mouse tissues[38, 39] (Ikeda *et al*., unpublished results), and Western blot analysis indicates that the necl-5 protein is undetectable in normal mouse tissues.[42] However, FACS analysis clearly indicates that the necl-5 protein is expressed in a variety of cell lines. Thus, necl-5 is expressed in normal cells, but to only a small extent. PVR/CD155 or necl-5 does not show homophilic cell-cell adhesion activity, but shows heterophilic cell-cell adhesion activity selectively with nectin-3 (Fig. 2C).[42, 69, 70] This property of necl-5 is different from those of nectins, which both homophilically and heterophilically *trans*-interact.[21, 42] Because necl-5 does not bind afadin, it does not recruit cadherins to the cell-cell adhesion site formed by the *trans*-interaction of necl-5 with nectin-3. However, because necl-5 heterophilically *trans*-interacts with nectin-3, this heterophilic *trans*-interaction may recruit E-cadherin through nectin-3 to the cell-cell adhesion sites formed between necl-5 and nectin-3 and thereby may be indirectly involved in formation of AJs, at least partly (see Fig. 11).

On the other hand, evidence is accumulating that necl-5 regulates cell migration[42] (Ikeda *et al*., unpublished results). The heterophilic *trans*-interaction of necl-5 of V12Ras-NIH3T3 cells with nectin-3 of L cells expressing nectin-3 (nectin-3-L cells) enhances the motility of V12Ras-NIH3T3 cells on a confluent culture of nectin-3-L cells.[42] Stable interaction of necl-5 of V12Ras-NIH3T3 cells with Nef-3 precoated on dishes inhibits their motility. The mechanism of the inter-nectin-3-L cell motility of V12Ras-NIH3T3 cells is not known at present, but dynamic attachment and detachment between V12Ras-NIH3T3 cells and neighboring nectin-3-L cells are necessary for the motility. In addition to the intercellular motility-enhancing activity of necl-5, it localizes at the leading edges of singly migrating V12Ras-NIH3T3 cells and mediates serum-dependent cell motility without interaction with nectin-3 (Ikeda *et al*., unpublished results). Studies on the mechanisms of the cell-migrating activity of necl-5 using L cells expressing various mutants have revealed that the extracellular region of necl-5 is necessary for directional cell migration, but not for random cell motility. The cytoplasmic region is necessary for both random and directional cell movement. It is unknown which factor(s) in serum enhances cell motility through necl-5, but proteins or lipid factors, such as platelet-derived growth factor, fibroblast growth factor, lysophosphatidic acid, and sphingosine-1-phosphate, are possible candidates. The serum-dependent, necl-5-mediated motility of L cells expressing necl-5 (necl-5-L cells) is inhibited by echistatin, an inhibitor of integrin $\alpha_V\beta_3$, and necl-5 co-localizes with this integrin at the leading edges of migrating necl-5-L cells, suggesting that the integrin is also involved in the cell motility-enhancing activity of necl-5. The serum-dependent, necl-5-mediated cell motility is inhibited by precoating dishes with the extracellular matrix molecule vitronectin, an integrin $\alpha_V\beta_3$- and $\alpha_V\beta_5$-binding protein, but vitronectin shows this inhibitory effect on L cells expressing necl-5 with deletion of the extracellular region, suggesting that it does not inhibit the cell motility by direct binding to necl-5. The inhibitory effect of vitronectin may be due to physical hindrance of cells that stick to the substratum through the stable interaction of the cellular integrins with vitronectin fixed on the dishes. This result also supports the involvement of the integrin in the cell-motility-enhancing activity of necl-5. As to the cell-motility-enhancing activity of PVR/CD155, it has not been systematically investigated, but PVR/CD155 is physically associated with CD44 on human monocyte cell surfaces.[71] CD44 is known to be a transmembrane protein which is involved in cell migration and metastasis of cancer cells.[72] PVR/CD155 co-localizes with integrin $\alpha_V\beta_3$ on the surface of transfected mouse fibroblasts and at amniotic epithelial cell junctions.[70] The extracellular region of PVR/CD155 binds vitronectin.[73] This result is apparently inconsistent with the result that vitronectin inhibits the motility of L cells that express necl-5 with the extracellular region deleted (Ikeda *et al*., unpublished results), but the reason for this inconsistency is unknown. Available evidence thus far obtained indicates that necl-5/Tage4/PVR/CD155 regulates the motility of at least transformed cells in cooperation with a cell surface receptor for a serum factor and integrins.

The mechanism of the cell-motility-enhancing activity of necl-5 remains unknown, but serum induces activation of Cdc42 and Rac through the cytoplasmic tail region of necl-5, resulting in formation of filopodia and lamellipodia, respectively, which are necessary for cell motility (Ikeda *et al*., unpublished results). The cytoplasmic tail region of PVR/CD155 binds to Tctex-1, a subunit of the dynein motor complex,[74] which may also be involved in regulation of cell motility in cooperation with microtubules.[75] On the basis of these observations, our current model for the role and mode of action of necl-5 in cell motility is as follows (Fig. 10): a serum factor binds to its specific cell surface receptor and the activated receptor transduces a signal to the cytoplasmic tail region of necl-5, resulting in formation of filopodia and lamellipodia through activation of Cdc42 and Rac, respectively, while integrins are activated by vitronectin and may induce activation of these events. Many other signaling molecules in addition to Cdc42 and Rac may be activated by the action of the cell surface receptors and integrins, and all of them cooperatively enhance cell motility. It should be emphasized here that necl-5 localizes at the leading edges of migrating cells, although it remains unknown how it does so, because it has been shown that cell surface receptors, such as the G protein-coupled chemoattractant receptors, are distributed along the cell surfaces of migrating cells and not concentrated at leading edges,[76] and that PI3 kinase and phosphatidylinositol 3,4,5-triphosphate (PIP_3) formed by the action of PI3 kinase are highly concentrated at leading edges, while phosphatase and tensin homolog (PTEN), which hydrolyzes PIP_3, is highly concentrated at the rear of migrating cells.[76, 77]

Although necl-5 by itself has no potency to recruit E-cadherin because it does not bind afadin, the heterophilic *trans*-interaction of necl-5 with nectin-3 may recruit E-cadherin through nectin-3 as described above (Fig. 11). Because necl-5 localizes with integrins at the leading edges of migrating cells,

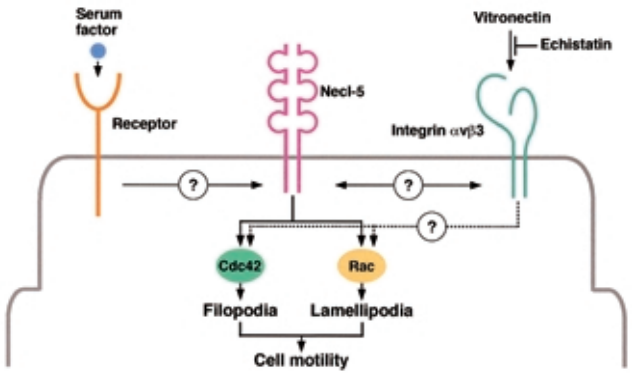

Fig. 10. A possible role of necl-5 and its mode of action in cell migration. A cell migrating factor in serum binds to its specific cell surface receptor and transduces a signal to the cytoplasmic tail region of necl-5, resulting in formation of filopodia and lamellipodia through activation of Cdc42 and Rac, respectively, while integrins are activated by vitronectin and induce activation of these events. Necl-5 co-localizes with integrin $\alpha_V\beta_3$ at the leading edges of migrating cells, and the serum-dependent, necl-5-mediated cell motility is inhibited by echistatin, an inhibitor of integrin $\alpha_V\beta_3$.

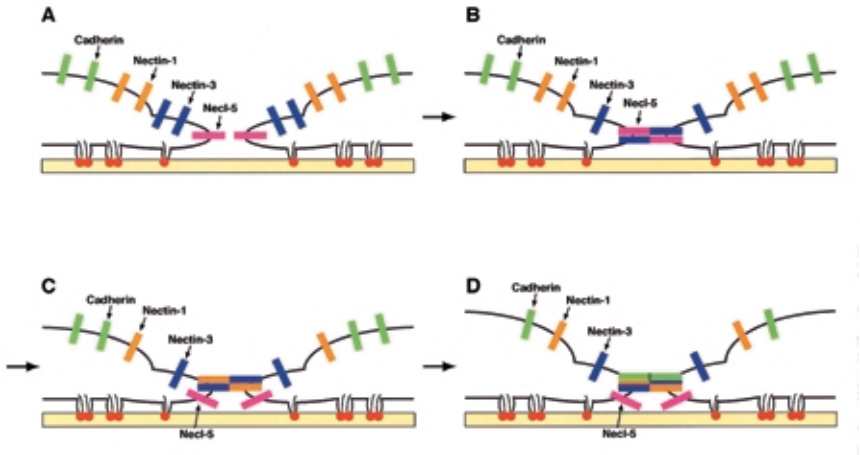

Fig. 11. A possible role of necl-5 and its mode of action in cell-cell adhesion. (A and B) When two migrating cells make contact through their protrusions, necl-5 heterophilically *trans*-interacts with nectin-3 to form micro-clusters at cell-cell contact sites. (C) The necl-5 molecule *trans*-interacting with nectin-3 may be replaced by nectin-1, resulting in the heterophilic *trans*-interaction of nectin-3 with nectin-1 and segregation of necl-5 from the nectin-based cell-cell adhesion sites. (D) The nectin-based cell-cell adhesions then recruit E-cadherin, which results in formation of a mixture of nectin- and E-cadherin-based cell-cell adhesion.

necl-5 may first recognize nectin-3, which may be diffusely distributed along the free surface of the plasma membrane, and heterophilically *trans*-interacts with it, causing formation of necl-5- and nectin-3-based micro-clusters (Fig. 11, A and B). These micro-clusters are formed more rapidly than those formed by nectins by themselves, but may be transient, because the affinity of nectin-3 for necl-5 is far less than that for nectin-1, and the necl-5 molecule *trans*-interacting with nectin-3 may be replaced by nectin-1, resulting in formation of heterophilic *trans*-interaction of nectin-3 with nectin-1 (Fig. 11, B and C). The nectin-3 molecule recruited by its heterophilic *trans*-interaction with necl-5 or the nectin-1 molecule recruited by its heterophilic *trans*-interaction with nectin-3 recruits E-cadherin at the cell-cell adhesion sites of both sides of protrusions of migrating cells, resulting in formation of AJs (Fig. 11D). Thus, necl-5 may play key roles in not only cell motility but also formation of cell-cell adhesion at the initial stage.

Conclusions and perspectives

We have described here how nectins play roles in the formation of AJs in cooperation with cadherins in fibroblasts and in the formation of AJs in cooperation with cadherins, and the subsequent formation of TJs in cooperation with JAMs, claudins, and occludin in epithelial cells. Furthermore, we have described how the nectin-based cell-cell adhesion induces activation of Cdc42 and Rac, which eventually regulate the formation of AJs through reorganization of the actin cytoskeleton, gene expression through activation of a MAP kinase cascade (the JNK pathway), and cell polarization through cell polarity proteins. We have finally reviewed possible roles of necls, particularly focusing on necl-2 and necl-5. Necl-5 is up-regulated in transformed cells and regulates cell motility and adhesion in cooperation with nectin-3, integrins, and cell surface receptors. Transformation of cells causes disruption of cell-cell adhesion, increase of cell motility, and loss of contact inhibition of cell movement and proliferation, eventually leading transformed cells into uncontrolled cell proliferation, invasion into surrounding tissues, and finally metastasis to other organs. It still remains to be elucidated how nectins are associated with cadherins or the components of TJs, and how nectins induce activation of Cdc42 and Rac. It has not been clarified, either, how necl-5 is up-regulated by transformation, which serum factor induces activation of necl-5, how the cell surface receptor of such a serum factor induces activation of necl-5, how necl-5 induces activation of Cdc42 and Rac, or how necl-5 is functionally associated with integrins. However, the roles of nectins and necls thus far clarified suggest that abnormality of known or unidentified components of the nectin and necl-5 systems may be important molecular mechanisms underlying the abnormal phenotypes of cancer cells. The cross-talk between cell-cell and cell-matrix adhesion processes has long been known to play important roles in the regulation of cell migration, adhesion, and proliferation, but the molecular mechanisms remain to be established. The direct interaction of necl-5 and nectin-3 may serve to connect integrins and cadherins. Further studies of nectins and necls should provide deeper insights into the molecular linkage between cell-cell junctions and various other physiological and pathological functions, such as morphogenesis, differentiation, proliferation, migration, and transformation.

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