The role of nectins in different types of cell–cell adhesion

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Summary

Mammalian tissues and organs are composed of different types of cells that adhere to each other homotypically (i.e. interactions between cells of the same cell type) or heterotypically (i.e. interactions between different cell types), forming a variety of cellular patterns, including mosaic patterns. At least three types of cell–cell adhesion have been observed: symmetric homotypic, asymmetric homotypic and heterotypic cell adhesions. Cadherins and nectins, which are known cell–cell adhesion molecules, mediate these cell adhesions. Cadherins comprise a family of more than 100 members, but they are primarily involved in homophilic trans-interactions (i.e. interactions between the same cadherin members) between opposing cells. By contrast, the nectin family comprises only four members, and these proteins form both homophilic and heterophilic trans-interactions (i.e. interactions between the same and different nectin members on opposing cells). In addition, heterophilic trans-interactions between nectins are much stronger than homophilic trans-interactions. Because of these unique properties, nectins have crucial roles in asymmetric homotypic cell–cell adhesion at neuronal synapses and in various types of heterotypic cell–cell adhesions. We summarize recent progress in our understanding of the biology of nectins and discuss their roles in heterotypic cell–cell adhesions, whose formation cannot be solely explained by the action of cadherins.

Key words: Nectin, Afadin, Cadherin, Cell adhesion molecule, Heterotypic cell adhesion, Mosaic pattern

Introduction

In multicellular organisms, cell–cell adhesion is essential for ontogenesis and for the regeneration and maintenance of tissues and organs. Dysfunction of cell–cell adhesion results in the progression of many diseases, such as cancers, psychiatric diseases and disorders of sensory and reproductive organs. Mammalian tissues and organs are composed of two or more cell types that can adhere homotypically (i.e. interactions between cells of the same type) or heterotypically (i.e. interactions between cells of different types). Cell–cell adhesions can be categorized into at least three groups: symmetric homotypic, asymmetric homotypic and heterotypic cell–cell adhesions (Fig. 1). Symmetric homotypic cell–cell adhesion refers to symmetric junctions that are formed between the same cell type, which is observed, for example, between intestinal absorptive epithelial cells, between vascular endothelial cells and between fibroblasts. Asymmetric homotypic cell–cell adhesion refers to the formation of asymmetric junctions between the same cell type, which is observed between the axons and dendrites of neurons. Finally, heterotypic cell–cell adhesions form between two different cell types, and they are observed, for example, between Sertoli cells and germ cells in the testis, auditory hair cells and supporting cells in the auditory epithelium of the inner ear, and neurons and glia cells in the brain. The typical cell–cell adhesion apparatus comprises adherens junctions (AJs), which contain the main cell–cell adhesion molecules (CAMs) cadherins (Takeichi, 1991). Cadherins are Ca^{2+}-dependent CAMs and the cadherin protein family comprises more than 100 members. Cadherins on neighboring or opposing cells trans-interact almost exclusively homophilically (i.e. interactions between the same cadherin members), which means that they do not account for the formation of asymmetric homotypic and heterotypic cell–cell adhesions that are found in a variety of tissues and organs (Fig. 1C).

Nectins, which were originally identified as virus receptors, are another family of CAMs (Takai et al., 2008a; Takai et al., 2008b). They are Ca^{2+}-independent immunoglobulin-like CAMs and the nectin family comprises four members, nectin-1, nectin-2, nectin-3 and nectin-4, which are encoded by the *PVRL1*, *PVRL2*, *PVRL3* and *PVRL4* genes, respectively. In contrast with cadherins, nectins are able to trans-interact both homophilically and heterophilically (i.e. interactions between the same and different nectin members). In addition, their heterophilic trans-interactions are much stronger than their homophilic trans-interactions. Because of these unique properties, nectins have crucial roles in mediating asymmetric homotypic and heterotypic cell–cell adhesions in addition to functioning in homotypic cell–cell adhesion in conjunction with, or independently of, cadherins. Examples of nectins mediating cell–cell adhesions can be found

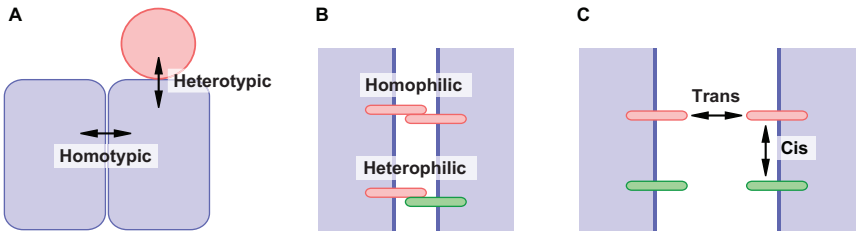

Fig. 1. Types of cell–cell adhesion and interactions between cell adhesion molecules. (**A**) Cell adhesion can occur homotypically (i.e. between the same cell types) or heterotypically (i.e. between two different cell types). (**B**) Along similar lines, cell adhesion molecules can interact with each other in a number of different ways. They can interact homophilically (i.e. forming interactions between molecules of the same type) or heterophilically (i.e. forming interactions between two different molecules). (**C**) Additionally, cell adhesion molecules can form interactions in cis (i.e. cell adhesion molecules on the surface of one cell interact) or in trans (i.e. cell adhesion molecules on the surface of one cell interact with adhesion molecules on the surface of an opposing cell).

in numerous tissues and cell types, such as mossy-fiber–CA3 synapses in the hippocampus (Mizoguchi et al., 2002), the contacts between commissural axons and floor plate cells in the neural tube (Okabe et al., 2004), the contacts between pigment cell and non-pigment cell layers of the ciliary epithelium in the eye (Inagaki et al., 2005), adhesions between Sertoli cells and germ cells in the testis (Inagaki et al., 2006; Mueller et al., 2003; Ozaki-Kuroda et al., 2002), the contacts between ameloblasts and stratum intermedium (SI) in the developing tooth (Yoshida et al., 2010), and the formation of a checkerboard-like mosaic pattern of auditory hair cells and supporting cells in the auditory epithelium of the inner ear (Togashi et al., 2011). In this Commentary, we will introduce general properties of nectins and summarize recent data that have contributed to progress in our understanding of the roles of nectins in asymmetric homotypic and heterotypic cell–cell adhesions.

General properties of nectins

Nectin structure and protein interactions

Each nectin family member consists of two or three splice variants (Takai et al., 2008a; Takai et al., 2008b). All nectins, apart from the secreted nectin-1γ, contain an extracellular region with three immunoglobulin-like loops (one V type and two C2 types), a single membrane-spanning region and a cytoplasmic tail (Fig. 2A). Nectins directly bind afadin, an F-actin-binding protein, through their cytoplasmic tails. All nectins, with the exception of nectin-1β, nectin-3γ and nectin-4, contain a conserved motif (E/A-x-Y-V, where x represents any amino acid) at their C-termini, which serves as a binding motif for the PDZ (for PSD95–DLG1–ZO-1) domain of afadin. Despite lacking this conserved motif, nectin-4 is able to interact with the afadin PDZ domain through its C-terminus. Some, but not all, members of the nectin family are able to bind various cytoplasmic proteins in addition to afadin, including partitioning defective 3 homologue (Par-3, also known as PARD3 in mammals) (Takekuni et al., 2003), protein interacting with PRKCA1 (PICK1) (Reymond et al., 2005), multiple PDZ domain protein (MUPP1, also known as MPDZ), Pals1-associated tight junction protein (PATJ) (Adachi et al., 2009), membrane palmitoylated protein 3 (MPP3) (Dudak et al., 2011), zyxin (Gregory Call et al., 2011) and willin (Ishiuchi and Takeichi, 2012) (Fig. 2A).

Nectins first form cis-homo-dimers, which then undergo lateral cluster formation on the cell surface (Narita et al., 2011; Satoh-Horikawa et al., 2000) (Fig. 2B). The lateral clusters of the cis-homodimers have been suggested to interact in trans with those on the opposing cell surface. In this trans-interaction the nectin clusters can interact homophilically and heterophilically in trans with each other (Reymond et al., 2001; Satoh-Horikawa et al., 2000) (Fig. 2B). The V-type loop in the extracellular region of nectins is necessary for the formation of trans-dimers (Reymond et al., 2001). Initial studies suggested that the first C2-type loop in the extracellular region contributed to the formation of cis-dimers (Momose et al., 2002; Yasumi et al., 2003). However, recent crystallographic analysis has revealed that nectin-1 forms a V-shaped homophilic cis-dimer through the V-type loop (Narita et al., 2011). Structure-based site-directed mutagenesis of this domain has revealed that there are four essential residues that are involved in homophilic cis-dimerization. Thus, the V-type loop is involved in the formation of both trans- and cis-dimers. In addition, nectins on the surface of a given cell can interact in trans with other immunoglobulin-like transmembrane proteins, such as nectin-like molecules (Necls, also known as CADMs) (Ikeda et al., 2003; Kakunaga et al., 2004), tactile (also known as CD96) (Bottino et al., 2003), DNAX accessory molecule 1 (DNAM1, also known as CD226) (Bottino et al., 2003) and T cell immunoreceptor with Ig and ITIM domains (TIGIT) (Stanietsky et al., 2009) on the surface of opposing cells (Fig. 2C). In particular, nectin-1 can interact with nectin-3, nectin-4, Necl-1 (CADM3) and tactile; nectin-2 is able to interact with nectin-3, DNAM1 and TIGIT; and nectin-3 interacts with Necl-1, Necl-2 (CADM1) and Necl-5 (also known as PVR, Tage4 and CD155). Moreover, it has recently been reported that nectin-2 can interact with N-cadherin through their extracellular domains in *Xenopus*, although it remains unsolved whether they interact in trans or in cis (Morita et al., 2010).

Adhesive properties

As mentioned above, cadherins and nectins have different adhesive properties (Table 1). The heterophilic interactions between different nectin family members are stronger than the homophilic interactions. Ranging from the strongest to the weakest interaction, the interaction strength decreases as follows: nectin-1–nectin-3, nectin-2–nectin-3 and the homophilic nectin interactions. The K_d values for the heterophilic interactions between nectin-1 and nectin-3 and between nectin-2 and nectin-3 are 2.3 nM and 360 nM, respectively (Ikeda et al., 2003). These values are far smaller than those that have been measured for the trans-interaction between E-cadherin molecules (a value of around 80 μM) (Koch et al., 1997). On the other hand, the K_d values for the homophilic interactions between nectin-1–nectin-1, nectin-2–nectin-2, nectin-3–nectin-3 and nectin-4–nectin-4 are 17.5 μM, 0.4 μM, 228 μM and 153 μM, respectively (Harrison et al., 2012). Compared with the stronger trans-interaction

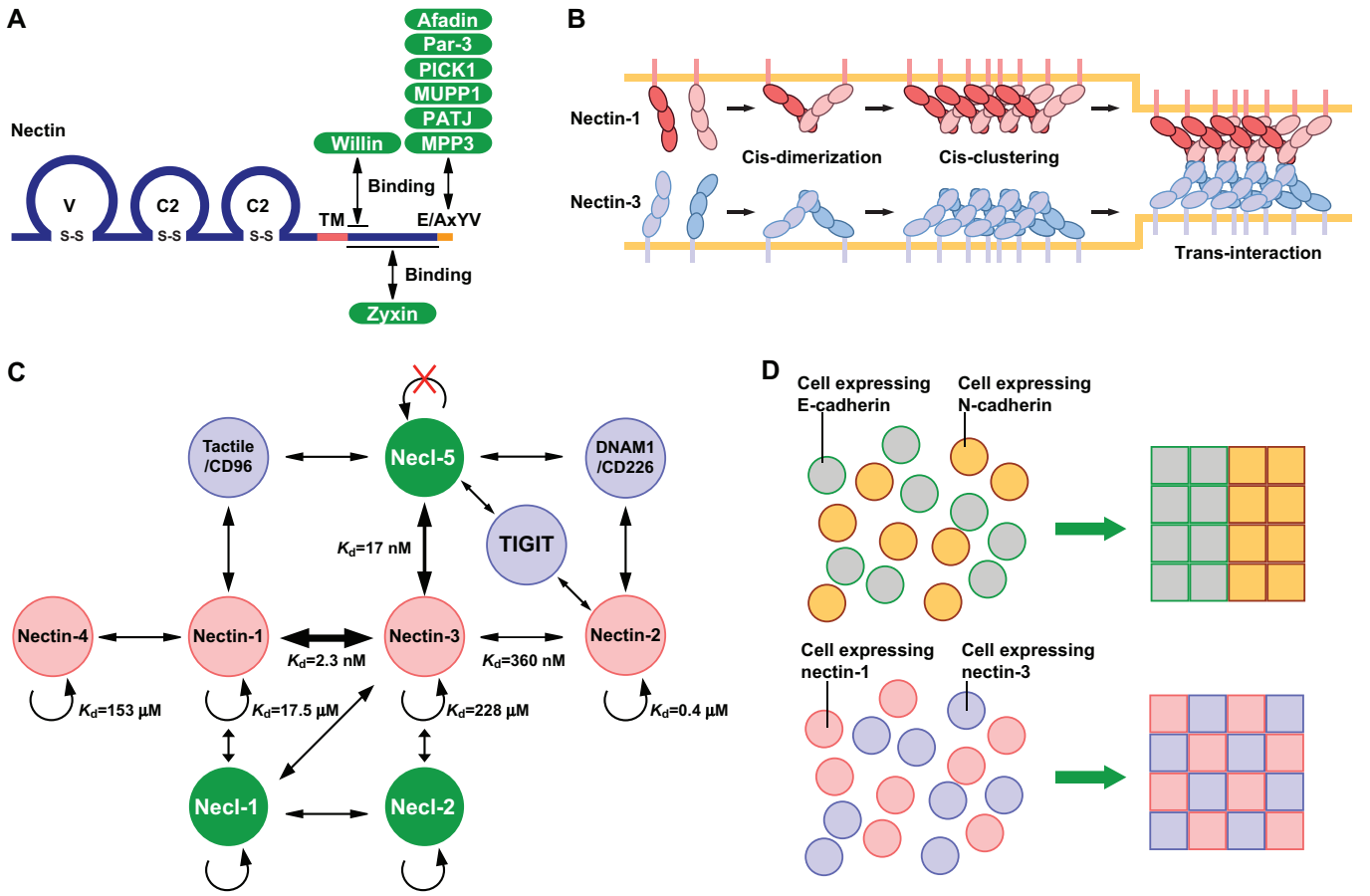

Fig. 2. Molecular structure of nectins and their interactions. (**A**) Nectins contain three immunoglobulin-like loops (one V-type and two C2-type loops) in their extracellular region, a single transmembrane (TM) segment and a cytoplasmic tail. The nectin C-terminus contains interaction motifs (E/AxYV, where x represents any amino acid) that allow interaction with afadin, Par-3, PICK1, MUPP1 and PATJ. Willin and zyxin interact with the juxtamembrane and cytoplasmic regions of nectins, respectively. (**B**) Nectins form V-shaped homophilic cis-dimers through the first immunoglobulin-like loop (the V-loop). They first form cis-homodimers, which then undergo lateral cluster formation on the cell surface. These lateral clusters have been suggested to interact in trans with clusters on the opposing cell surface. (**C**) Nectins on the surface of one cell can also form heterophilic trans-interactions with various other immunoglobulin-like molecules (blue and green) on the surface of opposing cells, besides forming homophilic trans-interactions between different nectin members (red). Known dissociation constants (K_d) are shown to indicate variations in affinity. (**D**) Cells expressing different cadherins tend to segregate, whereas cells expressing different nectins tend to be arranged in a mosaic pattern because of their likelihood to form heterophilic trans-interactions.

between E-cadherin molecules, which can facilitate long-term cell–cell adhesion, the weak trans-interaction between nectins is more suited to the formation of transient cell–cell adhesions and the repeated turnover of these adhesions. Trans-interaction strength depends at least on the plasmalemmal concentration of the adhesion molecules and their trans-interaction affinity. Cadherins show low affinity for trans-interaction with cadherins of opposing cell surfaces and slow lateral diffusion on the cell surface, whereas nectins show high affinity for trans-interaction and rapid lateral diffusion. Owing to these properties of nectins, cells expressing different nectins can be arranged in a mosaic pattern, whereas cells expressing different cadherins segregate separately (Fig. 2D).

Roles of nectins beyond cell–cell adhesion

Nectins regulate various cellular functions, such as cell movement, proliferation, polarization, survival and differentiation, besides cell adhesion, by interacting with various proteins (Takai et al., 2008a; Takai et al., 2008b).

Cell movement and proliferation

Necl-5 is localized at the leading edge of moving cells and forms a complex with integrin αvβ3 and either platelet-derived growth

Table 1. Comparison of the properties of cadherins and nectins

	Ca^{2+}-dependency	Trans-interaction	Relative binding strength	Affinity for trans-interaction	Lateral diffusion	Reference
Cadherins	Dependent	Homophilic	Strong	Low	Slow	(Takeichi, 1991)
Nectins	Independent	Homophilic Heterophilic	Weak	High	Rapid	(Takai et al., 2008)

factor receptor (PDGFR), in NIH3T3 cells (Amano et al., 2008; Ikeda et al., 2004; Minami et al., 2007), or vascular endothelial growth factor receptor (VEGFR), in human umbilical endothelial cells (Kinugasa et al., 2012). These complexes regulate the signals that are initiated by small G-proteins, including Rap, Rac and Rho, and thereby affect cell movement (Amano et al., 2008; Ikeda et al., 2004; Minami et al., 2007; Takahashi et al., 2008). By regulating another small GTPase, Ras, they can also affect cell proliferation (Kakunaga et al., 2004). Necl-5 thereby enhances the cell movement and proliferation induced by different growth factors.

The trans-interaction between nectin-3 and Necl-5 (Ikeda et al., 2003; Koike et al., 1990; Mendelsohn et al., 1989) is also involved in mediating contact inhibition of cell movement and proliferation (Fujito et al., 2005), which was originally identified as a phenomenon observed in epithelial cell and fibroblast cultures in vitro (Abercrombie and Heaysman, 1953). When moving cells collide with each other, Necl-5 on the surface of one cell interacts in trans with nectin-3 on the surface of the other cell. This leads to the downregulation of Necl-5 from the cell surface through clathrin-dependent endocytosis (Fujito et al., 2005), which subsequently results in the reduction of cell movement and proliferation. Necl-5 interacts with sprouty 2 (SPRY2), a protein that inhibits the Ras–mitogen activated protein kinase (MAPK) signaling pathway, and prevents the tyrosine phosphorylation and activation of SPRY2 (Kajita et al., 2007). Internalization of Necl-5 results in the release of SPRY2 from Necl-5, which leads to the activation of SPRY2 and the subsequent inhibition of Ras-mediated cell proliferation signals. By contrast, when nectin-3 becomes dissociated from Necl-5, Necl-5 is retained on the cell surface and subsequently interacts in trans with nectin-1. This results in the recruitment of the cadherin–catenin complex to the nectin-based cell–cell adhesion sites, and, finally, the establishment of AJs between the two cells.

Establishing cell polarity and cellular junctions

Following the formation of AJs, tight junctions (TJs) are formed on the apical side of AJs at the site of epithelial cell–cell adhesion. The formation of this apico-basal cell polarity is dependent on nectins (Fukuhara et al., 2002a; Fukuhara et al., 2002b; Ikeda et al., 1999; Komura et al., 2008; Sato et al., 2006). The C-terminal four amino acids of nectin-1 and nectin-3, but not of nectin-2, bind Par-3, a cell polarity protein that forms a complex with Par-6 (also known as PARD6A in mammals) and atypical protein kinase C (aPKC) (Takekuni et al., 2003). The Par-3–Par-6–aPKC complex regulates the formation of TJs, thereby leading to the establishment of epithelial cell polarity. Par-3 has also been shown to have a role in the formation of TJs and in establishing apico-basal polarity in cooperation with afadin in epithelial cells (Ooshio et al., 2007). In addition to being involved in the formation of AJs and TJs, nectins are involved in the formation and maintenance of desmosomes, the spot-like intercellular junction structures that anchor the cytoplasmic intermediate filaments (Barron et al., 2008; Yoshida et al., 2010). For instance, the heterophilic trans-interaction between nectin-1 and nectin-3 has been shown to be crucial for the formation of desmosomes during tooth development (Yoshida et al., 2010). Thus, nectins regulate cell polarity and cellular junction formation.

Cell survival

Once cells become confluent and cell–cell junctions have been established, they must survive without cell movement and proliferation. Nectin-3 is crucial for PDGF-dependent cell survival. It prevents apoptosis by regulating the activation of the phosphatidylinositol 3-kinase (PI3K)–Akt survival signaling pathway (Kanzaki et al., 2008). Nectin-3 is colocalized and physically associated with the PDGFR at cell–cell adhesion sites, and knockdown of nectin-3 attenuates the PDGF-induced phosphorylation of Akt in NIH3T3 cells (Kanzaki et al., 2008). Knockdown of nectin-3 enhances apoptosis induced by serum deprivation (Kanzaki et al., 2008). Collectively, these results indicate that nectin has an anti-apoptotic effect by regulating the PI3K–Akt signaling pathway.

Cell differentiation

Nectin-1 is expressed at cell–cell junctions in human and mouse epidermis (Matsushima et al., 2003; Wakamatsu et al., 2007). In mice lacking the gene encoding nectin-1 (*Pvrl1*$^{-/-}$ mice), the expression of loricrin, a differentiation marker and a major component of cornified cell envelopes in the epidermis, is downregulated and newborn pups have a shiny and slightly reddish skin (Wakamatsu et al., 2007). These observations suggest that there is an impaired differentiation of the epidermis in *Pvrl1*$^{-/-}$ mice and highlight that nectins also have a role in regulating cell differentiation during development.

Nectins in human diseases

As a result of their cell surface location, nectins can also act as mediators of virus entry: nectin-1 and nectin-2 for herpes simplex virus, nectin-4 for the measles virus (a paramyxovirus) and Necl-5 for the poliovirus (Geraghty et al., 1998; Koike et al., 1990; Mendelsohn et al., 1989; Mühlebach et al., 2011; Warner et al., 1998). Mutations in human *PVRL1* (which encodes nectin-1) are implicated in cleft lip or palate-ectodermal dysplasia syndromes, which includes Zlotogora–Ogur syndrome and Margarita Island ectodermal dysplasia (Sözen et al., 2001; Suzuki et al., 1998; Suzuki et al., 2000). Both of these are autosomal recessive disorders that are clinically characterized by unusual facial appearance, dental anomalies, hypotrichosis, palmoplantar hyperkeratosis and onychodysplasia, syndactyly, cleft lip or palate, and in some cases, mental retardation. Mutations in human *PVRL4* (which encodes nectin-4) that result in the failure of nectin-4 binding to nectin-1 cause an ectodermal dysplasia-syndactyly syndrome that is characterized by the combination of hair and tooth abnormalities, alopecia and cutaneous syndactyly (Brancati et al., 2010). Recent genome-wide association studies of various populations, including Japanese and African Americans, have shown a genetic association between single-nucleotide polymorphisms (SNPs) in *PVRL2* (which encodes nectin-2) and late-onset alzheimer's disease (Harold et al., 2009; Logue et al., 2011; Takei et al., 2009), and mutations in *PVRL3* (which encodes nectin-3) are associated with human ocular disease and congenital ocular defects (Lachke et al., 2012).

Nectins and afadin in asymmetric homotypic cell–cell adhesion

The synapse is the site of neurotransmission between the axons and dendrites of different neurons. In the synapses between mossy-fiber terminals of dentate gyrus granule cells and the dendrites of CA3 pyramidal cells, there are two types of junctions between axons and dendrites of neurons: synaptic junctions (SJs), which function as the sites of neurotransmission, and puncta adherentia junctions (PAJs), which function as the sites of

mechanical adhesion between axon terminals and their targets (Spacek and Lieberman, 1974) (Fig. 3A). The active zone, which is the site at which neurotransmission takes place, is formed at the presynaptic side of SJs, whereas the postsynaptic density (PSD), which contains the neurotransmitter receptors, is located below the postsynaptic membrane. PAJs are particularly developed in the CA3 region of the hippocampus. In the postnatal developmental stage, the synapses between mossy-fiber terminals of dentate gyrus granule cells and the dendrites of CA3 pyramidal cells are gradually remodeled into SJs and PAJs (Amaral and Dent, 1981). The active remodeling of these synapses after maturation has been implicated in the synaptic plasticity that underlies learning and memory (Yuste and Bonhoeffer, 2001). Whereas AJs are structurally and functionally symmetric, SJs are structurally and functionally asymmetric. In the CA3 region of the mouse hippocampus, cadherins, including N-cadherin, are localized at PAJs. The active zone and the PSD are surrounded by N-cadherin-based cell–cell adhesions (Uchida et al., 1996). Nectin-1 and nectin-3 are asymmetrically localized on the presynaptic and postsynaptic membranes, respectively, at PAJs, whereas afadin has been observed on both sides of PAJs in the mouse hippocampus (Mizoguchi et al., 2002) (Fig. 3A). At the stratum lucidum of the CA3 region in the mouse hippocampus, nectin-1 and nectin-3 are involved in the formation of PAJs (Honda et al., 2006), whereas afadin is required for synapse formation (Beaudoin et al., 2012; Xie et al., 2005). Similar to the formation of AJs during the establishment of cell polarity, nectin-based cell–cell adhesions are formed first. This is followed by the recruitment of N-cadherin-based cell–cell adhesions to form the synapse, which then results in the segregation into SJs and PAJs (D. Toyoshima, K.M. and Y.T., unpublished observations). In primary cultured hippocampal neurons, nectin-1 is preferentially present in axons, whereas nectin-3 is localized mostly in dendrites and partly in axons (Togashi et al., 2006). This asymmetric distribution of nectin-1 and nectin-3 is crucial for the specific interactions between axons and dendrites and proper formation of the synapse. However, the mechanisms that lead to the asymmetric distribution of nectin-1 and nectin-3 to axons and dendrites, respectively, have remained unknown so far.

Nectins in heterotypic cell–cell adhesion

Nectins have also been found to have roles in heterotypic cell–cell adhesion. In some of the cell–cell adhesion sites described below, cadherins are absent and nectins function in a cadherin-independent manner. As mentioned above, the weak cell–cell junctions that are formed by nectins might be favorable to dynamic regulation of cell–cell adhesion and rapid tissue remodeling. Several examples of heterotypic cell–cell adhesion, and the role of nectins in this process, are discussed below.

Heterotypic adhesion in the neural tube

In the neural tube, commissural axons grow towards the ventral midline, cross the floor plate, and then abruptly change their trajectory from the circumferential to the longitudinal axis (Fig. 3B). Contacts between commissural axons and basal processes of floor plate cells are involved in this guidance event (Fig. 3B). Whereas cadherins are not involved in this process, nectins have an important role in mediating the contacts between these two cell types. Nectin-1 and nectin-3 are asymmetrically localized at commissural axons and basal processes of floor plate cells, respectively (Okabe et al., 2004) (Fig. 3B). The commissural neuron axon guidance is regulated by the trans-interaction between nectin-1 and nectin-3. Impairing the formation of the contacts between commissural axons and floor plate cells by inhibiting the nectin-mediated heterotypic interaction results in the longitudinal turn of commissural axons in the contralateral sites of the rat hindbrain. Thus, the weak trans-interaction between nectins, instead of the strong adhesion mediated by cadherins, might be advantageous when commissural axons continuously elongate while being attached to floor plate cells. The weak attachment that is mediated by the trans-interaction between nectin-1 and nectin-3 is crucially involved in the determination of the axonal guidance because inhibition of the nectin-1- and nectin-3-based contacts by nectin inhibitors causes abnormal trajectories, such as abnormal turns and loss of the proper direction of the commissural axons (Okabe et al., 2004). Because commissural axons communicate with or transfer signals to floor plate cells through these contact sites (Stoeckli and Landmesser, 1998), both weak signal communication and weak mechanical contacts between commissural axons and floor plate cells that are mediated by the trans-interaction between nectin-1 and nectin-3 might be involved in the regulation of the axonal guidance.

Heterotypic adhesion in the ciliary epithelium

The heterophilic trans-interaction between nectin-1 and nectin-3 also has a key role in the apex–apex adhesion between the pigment cell and non-pigment cell layers of the ciliary epithelium in mice. The ciliary body is a structure in the eye, which is located immediately behind the iris and in front of the choroids. It secretes the aqueous humor, and is the source of the zonules that support the lens (Bishop et al., 2002). The ciliary body also contains the ciliary muscle, which changes the shape of the lens when the eye focuses on an object. Ciliary epithelia have two layers, the pigment cell and non-pigment cell layers (Raviola and Raviola, 1978) (Fig. 3C). These appose and adhere to each other through apex–apex junctions, which consist of PAJs and gap junctions. Nectin-1 and nectin-3 are localized at the PAJs between the pigment cell layer and the non-pigment cell layer of the ciliary epithelia (Inagaki et al., 2005) (Fig. 3C). Impairment of the trans-interaction between nectin-1 and nectin-3 in *Pvrl1*$^{-/-}$ or *Pvrl3*$^{-/-}$ mice (which lack nectin-1 and nectin-3, respectively) causes separation of these two cell layers and results in the disruption of the ciliary body (Inagaki et al., 2005). Thus, the heterotypic apex–apex adhesion between the pigment cell and non-pigment cell layers that is mediated by nectin-1 and nectin-3 is required for the formation of the ciliary body, as well as the subsequent folding of these two cell layers (Inagaki et al., 2005). By contrast, this type of adhesion might not be involved in survival or differentiation of the ciliary epithelial cells (Inagaki et al., 2005).

Sertoli-cell–germ-cell junctions in the testis

The weak trans-interactions between nectins also appear to be important for the interaction between Sertoli cells and spermatids during spermatid differentiation in the testis. In spermatocytogenesis, a spermatogonium divides into two spermatocytes, which will later divide again into spermatids, which finally undergo maturation into spermatozoa (Cheng and Mruk, 2002) (Fig. 3D). Spermatogenic cells are embraced and cultivated by Sertoli cells during spermatogenesis. During the later stages of spermatogenesis, spermatids interact with Sertoli cells through Sertoli-cell–spermatid junctions, which do not contain AJs or TJs. On the other hand,

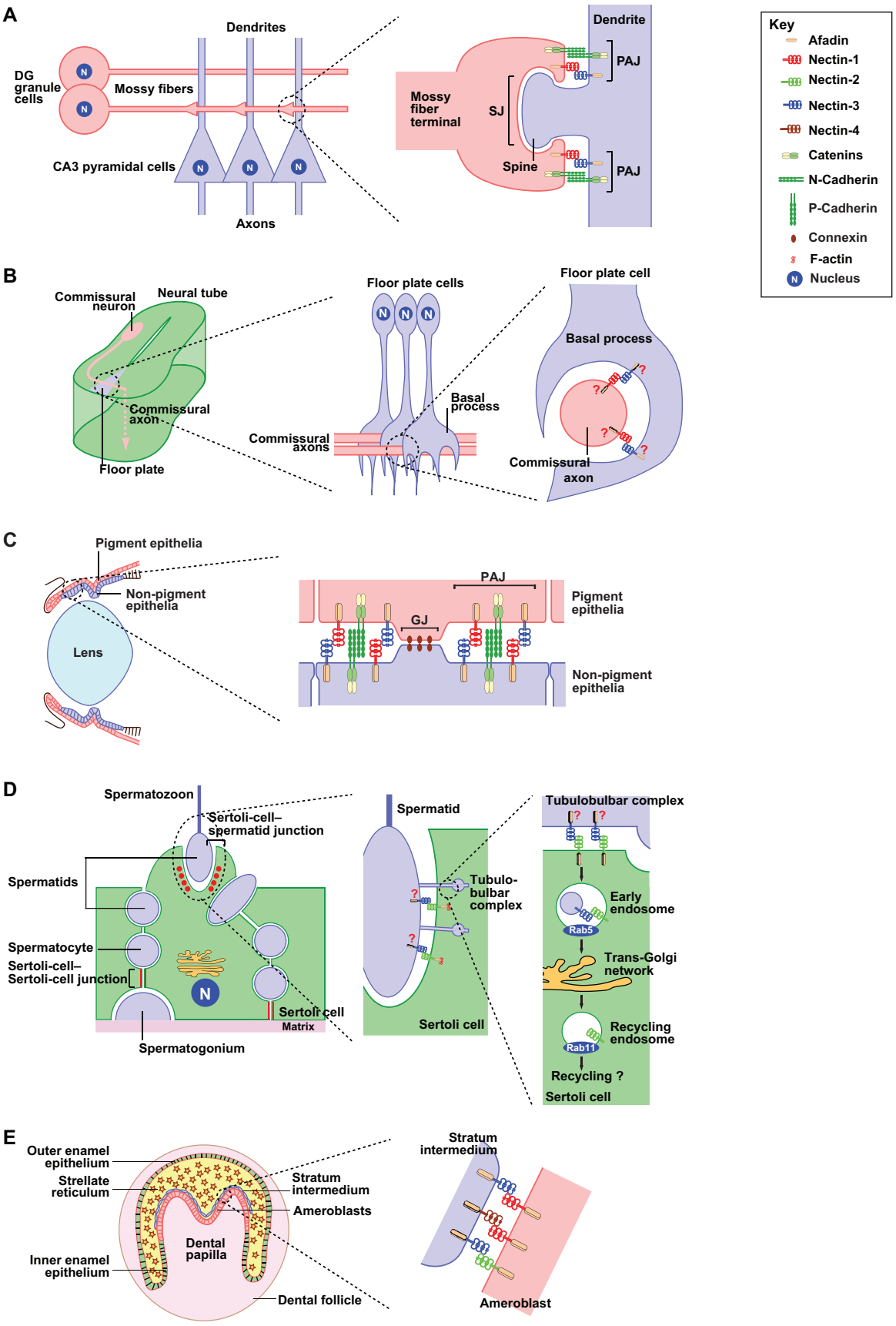

Fig. 3. See next page for legend.

Sertoli-cell–Sertoli-cell junctions are equipped with AJs and TJs and serve as the blood–testis barrier (Cheng and Mruk, 2002). Nectin-2 and nectin-3 are found on the surface of Sertoli cells and spermatids, respectively, at Sertoli-cell–spermatid junctions (Ozaki-Kuroda et al., 2002) (Fig. 3D). At these junctions, each nectin-2-containing adhesive membrane domain colocalizes with one F-actin bundle. The trans-interaction between nectin-2 and nectin-3 is likely to be essential for the formation of Sertoli-cell–spermatid junctions, because *Pvrl2*$^{-/-}$ and *Pvrl3*$^{-/-}$ mice (which lack nectin-2 and nectin-3, respectively) show male-specific infertility as a result of spermatogenesis defects (Inagaki et al., 2006; Mueller et al., 2003; Ozaki-Kuroda et al., 2002).

Tubulobulbar complexes, which consist of a finger-like process of the spermatid plasma membrane that protrudes into the adjacent Sertoli cell plasma membrane and a bulb-like swelling at the end of the tubular process, are formed at junctions between Sertoli cells and spermatids in the apical region of the seminiferous epithelium (Russell and Clermont, 1976). Nectin-2 and nectin-3 are localized at the tubulobulbar complexes and appear to participate in adhesion of spermatids to the plasma membranes of Sertoli cells (Guttman et al., 2004) (Fig. 3D). When spermatids are released from Sertoli cells as spermatozoa, the plasma membranes of the Sertoli cell and the spermatid that contain nectin-2 and nectin-3, respectively, become internalized. This process occurs through the association of the early endosomal marker Rab5 with tubulobulbar complexes, and leads to the release of the late spermatids and Sertoli-cell–spermatid junction disassembly (Fig. 3D). Nectin-2 and the late recycling endosome marker Rab11 have also been shown to localize to junctions in early spermatids that are found deeper in the epithelium (Young et al., 2011). Some of the internalized junction proteins might be recycled to form junctions with the next generation of spermatids.

Heterotypic adhesions in the developing tooth

In the developing tooth, ameloblasts that are derived from the oral epithelium deposit tooth enamel. The epithelial compartment of the developing tooth is composed of the peripheral inner and outer enamel epithelia, and the stellate reticulum cells in the core (Lesot and Brook, 2009) (Fig. 3E). The inner enamel epithelial cells are differentiated into ameloblasts. The stellate reticulum cells facing the preameloblasts form the stratum intermedium – a transient and thin epithelial layer that supports the differentiation and function of ameloblasts to allow them to form tooth enamel. In the mouse tooth, nectin-1 and nectin-2 are expressed in ameloblasts, and nectin-3 and nectin-4 are expressed in the neighboring stratum intermedium cells at postnatal stage P10 (Yoshida et al., 2010) (Fig. 3E). Nectin-1 and nectin-3 are involved in the formation of desmosomes between ameloblasts and SI cells and in the localization of TJs and cytoskeletal proteins in ameloblasts. Besides the trans-interaction between nectin-1 and nectin-3, the trans-interactions between nectin-1 and nectin-4 and/or between nectin-2 and nectin-3 might also have roles in tooth morphogenesis because *Pvrl1*$^{-/-}$, *Pvrl3*$^{-/-}$ mice (which lack both nectin-1 and nectin-3) show more severe dental abnormalities than *Pvrl1*$^{-/-}$ or *Pvrl3*$^{-/-}$ mice, which only possess a single mutation (Yoshida et al., 2010). Thus, nectins are involved in the formation of cell–cell junctions between ameloblasts and stratum intermedium cells.

Nectins in the formation of the checkerboard-like mosaic pattern

The cochlea in the inner ear is the hearing organ in mammals (Fig. 4A). There are three cavities inside the cochlea: the scala vestibuli, scala media and scala tympani (Fig. 4B). The so-called Reissner's membrane separates the scala vestibuli and the scala media. The auditory sensory epithelium, the organ of Corti, is located in the scala media and contains sensory hair cells and supporting cells. Hair cells convert sound impulses from the outer ear into electrical signals, which are, in turn, transmitted to the brain through the auditory nerve. Hair cells and supporting cells are arranged into highly ordered rows and are interdigitated to form a checkerboard-like mosaic pattern (Kelley, 2006). A number of genes or molecules have been implicated in the formation of this highly ordered structure in the cochlea (Chen et al., 2002; Lanford et al., 1999; McKenzie et al., 2004; Montcouquiol and Kelley, 2003; Montcouquiol et al., 2003). A mathematical model predicted that the checkerboard-like pattern could be generated from a mixture of two cell types when their heterotypic cell–cell adhesions dominated over their homotypic ones (Honda et al., 1986). However, it has remained unclear how the cells become arranged in a mosaic pattern. In the mouse organ of Corti, nectin-1 and nectin-3 are localized in hair cells and supporting cells, respectively, and nectin-2 is expressed in both hair cells and supporting cells. The trans-interaction between nectin-1 and nectin-3 mediates the heterotypic adhesion between these two cell types, because the checkerboard-like mosaic pattern is disrupted in *Pvrl1*$^{-/-}$ and *Pvrl3*$^{-/-}$ mice as a result of aberrant attachment (Togashi et al., 2011) (Fig. 4C). The aberrant attachment between hair cells occurs much more frequently in *Pvrl3*$^{-/-}$ than in *Pvrl1*$^{-/-}$ mice. When cells expressing either nectin-1 or nectin-3 are co-cultured and allowed to move ad libitum, they arrange themselves into a mosaic pattern (Togashi et al., 2011). Thus, the heterophilic trans-interaction between nectin-1 and nectin-3 is crucial for the formation of the checkerboard-like mosaic pattern in the auditory epithelium of the organ of Corti. In *Pvrl1*$^{-/-}$ or *Pvrl3*$^{-/-}$ mice, the position of the kinocilium and the orientation and shape of stereociliary bundles in hair cells are changed, which additionally suggests that nectins could regulate planar cell polarity (K. Kominami, T. Fukuda and Y. T., unpublished observations).

Fig. 3. Nectins are involved in various types of heterotypic cell–cell adhesion. (**A**) Localization and role of nectin-1 and nectin-3 at the synapse between mossy-fiber terminals and dendrites of pyramidal cells in the CA3 region of the hippocampus. In this synapse, SJs function as the site of neurotransmission and are associated with synaptic vesicles that are docked at the presynaptic active zone, where Ca^{2+} channels are localized, and with postsynaptic densities, where specific neurotransmitter receptors are localized. PAJs are regarded as the site of mechanical adhesion between axons and their target dendrites. Here, nectin-1 and nectin-3 localize to the pre- and post-synaptic side, respectively, whereas N-cadherin localizes to both sides. DG, dentate gyrus. (**B**) Localization and role of nectin-1 and nectin-3 at the contact site between commissural axons and floor plate cells in the neural tube. (**C**) Localization and role of nectin-1 and nectin-3 at the contact site between the pigment cell and non-pigment cell layers of the ciliary epithelium in the eye. GJ, gap junction. (**D**) Localization and role of nectin-2 and nectin-3 at Sertoli-cell–spermatid junctions in the testis. (**E**) Localization and role of nectins at the contact site between ameloblasts and stratum intermedium in the developing tooth at postnatal stage P10 in mice. F-actin is not shown in A–C and E.

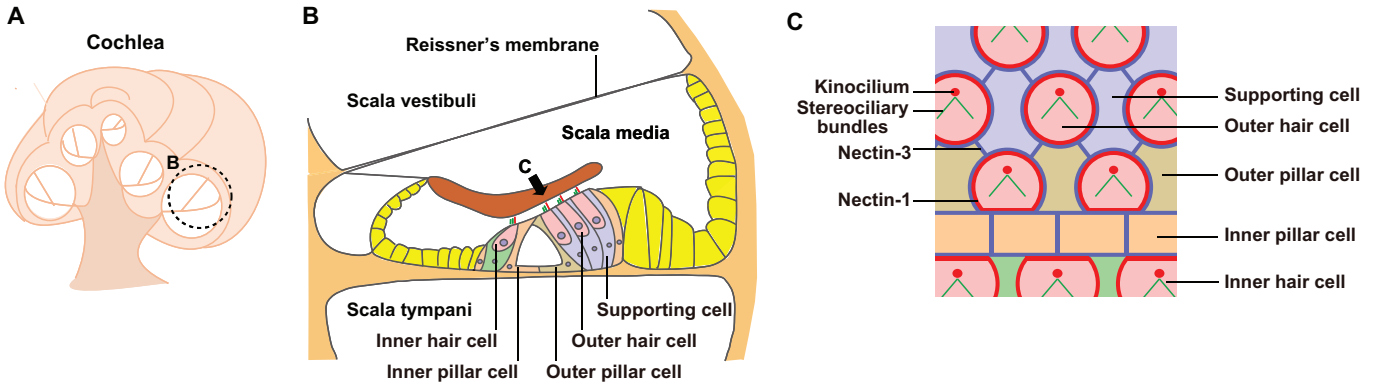

Fig. 4. Involvement of nectins in the formation of the checkerboard-like mosaic pattern in the organ of Corti. (**A**) Schematic illustration of the cochlea. (**B**) Cross-sectional illustration of the organ of Corti (enlarged image of the area encircled by dashed dots in A). The cochlea includes three chambers and the organ of Corti is located in the scala media. A single row of inner hair cells is located on the medial side of the epithelium, whereas three rows of outer hair cells are located more laterally. The inner hair cell and outer hair cell regions are separated by the tunnel of Corti, which is surrounded by single rows of inner pillar and outer pillar cells. An arrow indicates the direction of view shown in C. (**C**) Luminal surface illustration of the auditory epithelium in the organ of Corti. Hair cells (outer hair cells and inner hair cells) and various types of supporting cells are arranged in a checkerboard-like mosaic pattern in the auditory epithelium. Nectin-1 and nectin-3 are expressed exclusively by hair cells and supporting cells, respectively. Therefore, the trans-interaction between nectin-1 and nectin-3 occurs at the boundaries between these cells.

Conclusions and perspectives

In this Commentary, we have reviewed the role of nectins in various kinds of asymmetric homotypic and heterotypic cell–cell adhesions. Other types of heterotypic cell–cell adhesion exist in addition to those mentioned above. For example, the olfactory epithelium in the nose exhibits the characteristic arrangement of olfactory receptor cells and supporting cells. Concomitantly, dynamic changes occur in the arrangement of olfactory receptor cells and supporting cells. Unlike hair cells in the auditory epithelium, which are not capable of regeneration, olfactory receptor cells in the olfactory epithelium can be regenerated after birth. Nectins might be expressed and might have a role in the characteristic arrangement in the olfactory epithelium of mice during development and regeneration. Heterotypic cell–cell adhesion is also crucial for the interaction between stem cells and their niche, which regulates various aspects of stem cell behavior, including symmetric and asymmetric cell divisions. It is well known that cadherins and integrins are required for interactions in the stem cell niche in many systems. Given that nectins function cooperatively with cadherins and integrins in cell–cell adhesion (Sakisaka et al., 2007), it is tempting to speculate that nectin deficiency could cause disturbance of cell–cell adhesion and lead to dysreguation of division and differentiation of stem cells. In addition, future studies will provide further insight into how the dysfunction of nectin-mediated cell–cell adhesion could contribute to the pathogenesis of human diseases, such as cancers, psychiatric diseases and disorders of sensory and reproductive organs.

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