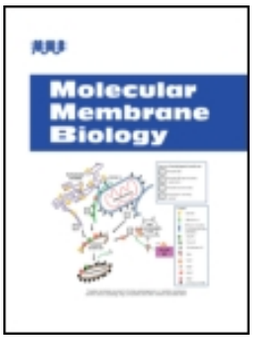

Molecular Membrane Biology

ISSN: 0968-7688 (Print) 1464-5203 (Online) Journal homepage: informahealthcare.com/journals/imbc20

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To cite this article: Orest W. Blaschuk & Tracey M. Rowlands (2002) Plasma membrane components of adherens junctions (Review), Molecular Membrane Biology, 19:2, 75-80, DOI: 10.1080/09687680210132467

To link to this article: https://doi.org/10.1080/09687680210132467

Molecular Membrane Biology, 2002, **19,** 75–80

Taylor&Francis healthsciences

Plasma membrane components of adherens junctions (Review)

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Summary

This review focuses on the three known plasma membrane components of adherens junctions: E-cadherin, nectin-2 and vezatin. The structures of these three components are discussed, with particular emphasis on the molecular mechanisms by which E-cadherin and nectin-2 promote cell adhesion.

Keywords: Cadherins, nectins, vezatin, adherens junction.

Introduction

The plasma membrane domain of epithelial adherens junctions is composed of E-cadherin (Volk *et al.* 1987), nectin-2 (Takahashi *et al.* 1999) and vezatin (Kussel-Andermann *et al.* 2000) (figure 1). E-cadherin and nectin-2 are calcium-dependent and calcium-independent cell adhesion molecules (CAMs), respectively (Takeichi 1990, Takahashi *et al.* 1999). It is not known whether vezatin can function as a CAM. This review discusses the structural and functional properties of E-cadherin, nectin and vezatin in adherens junctions.

Molecular mechanisms underlying classical cadherin-mediated cell adhesion

There are at least six sub-families of cadherins within the cadherin superfamily (Munro and Blaschuk 1996, Nollet *et al.* 2000). Epithelial (E)-cadherin was the first member of this superfamily to be discovered. This cadherin belongs to the classical or type I sub-family. Other members of this group include neural (N)-, retinal (R)- and placental (P)-cadherins. They have varying tissue distributions (Geiger and Ayalon 1992).

Classical cadherins are integral membrane glycoproteins that mediate intercellular adhesion in a calcium-dependent manner (Takeichi 1990). They are composed of five extracellular domains (designated EC1–5), each of 110 amino acids, a single transmembrane domain and two cytoplasmic domains (figure 1). Calcium ions are absolutely essential for cadherin function, as they bind to specific amino acid motifs within these CAMs, thereby maintaining the extracellular domains in a rod-like conformation (Shapiro *et al*. 1995, Nagar *et al*. 1996).

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Classical cadherins are anchored to the microfilament network of the cytoskeleton via a complex composed of multiple intracellular proteins including α- and β-catenin (Wheelock *et al.* 1996) (figure 1). β-Catenin binds directly to the carboxy-terminal cytoplasmic domain of E-cadherin (Nagafuchi and Takeichi 1988, 1989, Ozawa *et al.* 1990). Deletion of this domain abolishes E-cadherin function (Nagafuchi and Takeichi 1988, 1989, Ozawa *et al.* 1990). These results emphasize the need for E-cadherin to be anchored to the cytoskeleton in order to mediate adhesion. Another intracellular protein, designated p120, is also a key regulator of E-cadherin function (Anastasiadis and Reynolds 2001). This protein binds to a juxtamembrane site on the E-cadherin cytoplasmic domain (figure 1). Mutations that abolish the ability of E-cadherin to interact with p120 result in the failure of this CAM to promote strong cell adhesion (Thoreson *et al.* 2000). Studies have shown that p120 promotes E-cadherin clustering at the cell surface. Collectively, these observations suggest that E-cadherin/β-catenin interactions are necessary for initial adhesive events to occur, whereas E-cadherin/p120 interactions are essential to achieve strong and stable adhesive interactions characteristic of intercellular junctions (Anastasiadis and Reynolds 2001).

E-cadherin is found in epithelial adherens junctions, whereas the other classical cadherins have been localized to other types of intercellular junctions (Volk *et al.* 1987, Geiger and Ayalon 1992, Fannon and Colman 1996, Uchida *et al.* 1996).

The mechanism by which the extracellular domains of classical cadherins interact to promote cell adhesion has been the subject of major investigation. Early studies showed that synthetic peptides whose amino acid sequences were identical to those found in a portion of the EC1 domain could inhibit cadherin-dependent cell adhesion (Blaschuk *et al.* 1990a, Noe *et al.* 1999). These peptides all contained the amino acid sequence His-Ala-Val (HAV), which is perfectly conserved in the EC1 domain of all classical cadherins (Blaschuk *et al.* 1990b) (table 1). This work led to the designation of the HAV motif as the classical cadherin cell adhesion recognition (CAR) sequence. Subsequent studies using antibodies directed to HAV-containing peptides (Alexander *et al.* 1993) and site-specific mutagenesis (Tamura *et al.* 1998, Pertz *et al.* 1999) provided supporting evidence for the role of this motif in directly mediating cadherin function. Most recently, the simple cyclic peptide, *CHAVC*, was shown to be capable of inhibiting classical cadherin function, underlying the importance of the CAR sequence in directly mediating cell adhesion (Williams *et al.* 2000a). Finally, structural biological studies demonstrated that the His and Val side chains of the CAR sequence protrude from the adhesive interface of classical cadherins, while the Ala side chain is a component of a hydrophobic pocket buried within the EC1 domain (Overduin *et al*. 1995, Shapiro *et al*. 1995) (figure 2).

Molecular Membrane Biology
ISSN 0968-7688 print/ISSN 1464-5203 online © 2002 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/09687680210132467

Initial three-dimensional structure analysis of E- and N-cadherin extracellular domain fragments (Overduin *et al.* 1995, Shapiro *et al.* 1995, Tamura *et al.* 1998, Pertz *et al.* 1999, reviewed in Koch *et al.* 1999, Troyanovsky 1999) revealed the potential ability of cadherins to form homodimers within the plane of the plasma membrane. These interactions have been referred to as either parallel, lateral or *cis* interactions. In addition, the studies suggested the potential for the homodimers to form adhesive complexes with one another between cells. Such interactions have been designated as anti-parallel or *trans* interactions.

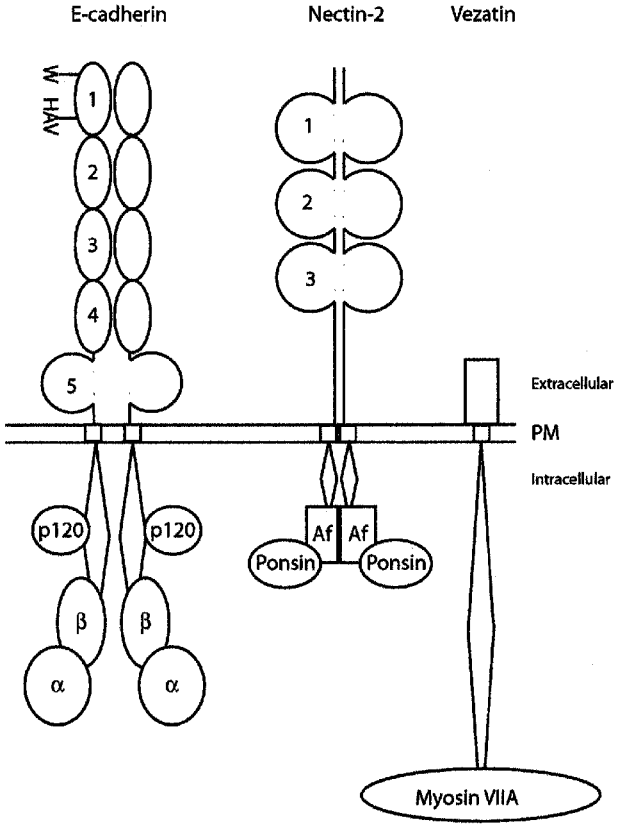

Figure 1. Diagram showing the plasma membrane (PM) components of epithelial adherens junctions. The extracellular domains of E-cadherin and nectin-2 are numbered. The first extracellular domain of E-cadherin contains a Trp (W) residue at the amino terminus and the classical cadherin CAR sequence, His-Ala-Val (HAV). The cytoplasmic domain of E-cadherin interacts with a supermolecular complex containing β-catenin (β) and α-catenin (α). The cytoplasmic domains of nectin-2 and vezatin interact with afadin (Af), which associates with ponsin, and myosin VIIA, respectively.

The molecular mechanism by which cadherin *cis* and *trans* homodimers are formed is still a subject of debate (Troyanovsky 1999). The initial crystallographic analysis of N-cadherin EC1 domain homodimers indicated that *cis* interactions were mediated by the interaction of a Trp residue located at the amino terminus of the EC1 domain and the Ala residue of the classical cadherin CAR sequence (figure 2). These data showed that the Trp residue of a cadherin monomer docked into a hydrophobic pocket containing the Ala residue on an adjacent monomer (Shapiro *et al.* 1995). In later crystallographic studies of an E-cadherin EC1-EC2 fragment, the EC1 domain was found to be structured such that the Trp residue of one monomer interacted within an Ala-containing hydrophobic pocket on the same molecule (Pertz *et al.* 1999). This interaction was driven by calcium-induced conformational changes, suggesting that the docking of Trp into the hydrophobic pocket within a single cadherin monomer is a key step in the establishment of the calcium-dependent cell adhesion interface. These divergent data can be reconciled, if one considers the possibility that the Trp residue directly participates in parallel cadherin monomer–monomer interactions (i.e. *cis* interactions) in non-adherent cells, and indirectly effects *trans* interactions in adhesive cells by docking to the Ala-containing pocket within the same monomer. This interaction would, thus, affect the conformation of the EC1 domain and orientation of the His and Val side chains protruding from the adhesive interface (Troyanovsky 1999, Shan *et al.* 2000) (figure 2).

The crystallographic studies discussed above have been complemented by the work of others concerning the formation of cadherin complexes in cell culture (Chitaev and Troyanovsky 1998, Tamura *et al.* 1998, Troyanovsky 1999, Klingelhofer *et al.* 2000, Shan *et al.* 2000). Mutation of either Trp or Ala residues has been shown to interfere with homodimer formation and abolish cell adhesion (Chitaev and Troyanovsky 1998, Tamura *et al.* 1998). Collectively, the

Table 1. Comparison of the amino acid sequences surrounding the terminal Trp residue and HAV sequence of the classical cadherins.

Classical	Cadherin	Amino acid sequences surrounding the terminal Trp residue							Amino acid sequences surrounding the HAV sequence								
Xenopus	C-cad	D	W	V	I	P	P	I	L	S	S	H	A	V	S	E	N	G
Human	E-cad	D	W	V	I	P	P	I	L	F	S	H	A	V	S	S	N	G
Mouse	E-cad	D	W	V	I	P	P	I	L	Y	S	H	A	V	S	S	N	G
Chicken	E-cad	D	W	V	I	P	P	I	L	L	S	H	A	V	S	A	S	G
Human	N-cad	D	W	V	I	P	P	I	L	R	A	H	A	V	D	I	N	G
Mouse	N-cad	D	W	V	I	P	P	I	L	R	A	H	A	V	D	V	N	G
Chicken	B-cad	D	W	V	I	P	P	I	L	Y	S	H	A	V	S	E	N	G
Xenopus	B-cad	D	W	V	I	P	P	I	L	L	S	H	A	V	S	E	N	G
Human	R-cad	D	W	V	I	P	P	I	L	R	A	H	A	V	D	M	N	G
Mouse	R-cad	D	W	V	I	P	P	I	L	R	A	H	A	V	D	M	N	G
Chicken	R-cad	D	W	V	I	P	P	I	L	R	A	H	A	V	D	M	N	G
Human	P-cad	D	W	V	V	A	P	I	L	F	G	H	A	V	S	E	N	G
Mouse	P-cad	E	W	V	M	P	P	I	L	Y	G	H	A	V	S	E	N	G

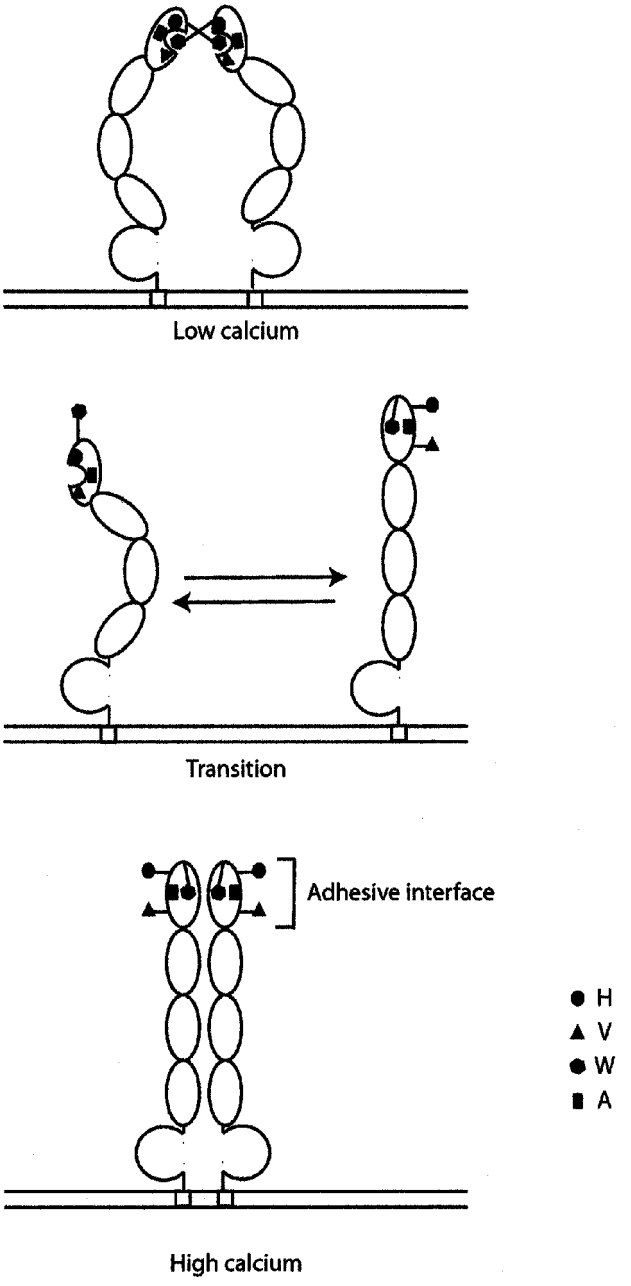

Figure 2. Diagram illustrating the effects of calcium on the conformation of classical cadherin extracellular domains. In the presence of high calcium levels, the extracellular domains adopt a rigid, rod-like conformation, whereas the structure is relaxed in low calcium. Under low calcium conditions, the amino terminal Trp (W) residue of a cadherin monomer is buried within a hydrophobic pocket containing the Ala (A) residue of the classical cadherin CAR sequence on an adjacent monomer. In addition, the His (H) and Val (V) side chains are not exposed on the surface of the monomer. In high calcium, the Trp residue becomes buried within the Ala-containing pocket of the same monomer resulting in the exposure of the His and Val side chains on the adhesive interface. A transition between these states is hypothesized in which adjacent monomers dissociate from one another as a consequence of alterations in the conformation of the EC1 domain. The transition state would be the most unstable structural situation, since the Trp residue is released from its hydrophobic pocket.

observations obtained from structural, biological and cell culture studies suggest that HAV-containing peptides are capable of inhibiting both *cis* and *trans* cadherin interactions. These peptides likely interfere with the docking of the Trp residue to the Ala-containing pocket, as well as anti-parallel (*trans*) interactions involving the His and Val side chains protruding from the adhesive interface.

In early studies, Takeichi and colleagues performed aggregation assays using classical cadherin tranfected cell lines to obtain data suggesting that cadherins acted in a homophilic manner to promote cell adhesion (Nose *et al.* 1988, 1990). Furthermore, they showed that mutating the Ser residue immediately adjacent to the His residue of the HAV sequence affected the ability of the classical cadherins to interact in an homophilic manner (table 1). Subsequently, Troyanovsky and colleagues used a domain-swapping approach to analyse the formation of homophilic and heterophilic interactions between classical cadherins. This work confirmed that the EC1 domain determines the specificity of adhesive interactions (Klingelhofer *et al.* 2000). Other studies utilizing linear and cyclic HAV containing peptides are in agreement with these findings (Noe *et al.* 1999, Williams *et al.* 2000a). For example, the linear peptide LFSHAVSSNG (whose amino acid sequence is identical to that found in human E-cadherin; table 1) is capable of inhibiting human E-cadherin-mediated cell aggregation, whereas the linear peptides LRAHAVDING and LFGHAVSENG (whose amino acid sequences are identical to those found in human N- and P-cadherin, respectively) have no effect on this process (Noe *et al.* 1999). Similarly, the cyclic peptide *CHAVDC* (which contains the amino acid sequence found in N-cadherin; table 1) is an effective disruptor of N-cadherin function, whereas cyclic peptides such as *CSHAVC* and *CHAVSC* (which contain amino acid sequences found in E-cadherin) have no effect on the function of this cadherin (Williams *et al.* 2000a). Collectively, these observations suggest that the amino acids flanking the classical cadherin CAR sequence play an important role in modulating the specificity of cadherin interactions.

Other studies have shown that classical cadherins can interact with one another in a heterophilic manner in certain assay systems (Volk *et al.* 1987, Murphy-Erdosh *et al.* 1995, Niessen and Gumbiner 2002). This is likely due to the fact that classical cadherins possess a significant degree of similarity in their EC1 domain, which is the most well conserved domain among these CAMs (Nollet *et al.* 2000). All classical cadherins contain the HAV sequence, as well as the terminal Trp residue (table 1). Therefore, heterophilic interactions between the EC1 domains of different classical cadherins would be expected to occur under certain conditions *in vitro*. However, it should be noted that such heterophilic interactions are usually weaker than homophilic interactions (Murphy-Erdosh *et al.* 1995). The physiological relevance of heterophilic binding is uncertain. Indeed, if heterophilic cadherin interactions occurred and were of equal strength to homophilic interactions *in vivo*, then cell sorting and other cadherin-driven events would not occur.

A number of studies have suggested that the EC1 domain of classical cadherins is solely involved in mediating *trans* adhesive interactions (Shapiro *et al.* 1995, Koch *et al.*

1999, Pertz *et al.* 1999). Several amino acid sequences that are capable of interacting with the His and Val side chains of the classical cadherin CAR sequence to promote *trans* interactions are located in the EC1 domain (Shapiro *et al.* 1995, Williams *et al.* 2000b). For example, according to the data obtained from the analysis of N-cadherin EC1 domain crystals, the amino acid sequence INP interacts with the Val residue of the HAV sequence, as well as with the Asp residue immediately adjacent to this sequence (Shapiro *et al.* 1995, Williams *et al.* 2000b). The cyclic peptide *CINPC*, as well as a linear peptide containing this sequence (INPISGQ) have been shown to inhibit N-cadherin function (Williams *et al.* 2000b). These results indicate that the N-cadherin EC1 domain adhesive interface reported by Shapiro *et al.* (1995) indeed represents a biologically relevant interface, as peptides whose amino acid sequences are identical to those found at this interface antagonize N-cadherin activity.

The concept that the EC1 domain of the classical cadherins is solely responsible for adhesive interactions has recently been challenged by a series of biophysical studies (Sivanskar *et al.* 1999, 2001). In these experiments, the homophilic interactions between the extracellular domains of C-cadherin were investigated utilizing a surface force apparatus (Sivanskar *et al.* 1999, 2001). The results suggest that the EC1 domain of C-cadherin can interact with multiple cadherin domains including EC5. This is a surprising result, as the EC5 domain is the most poorly conserved of all classical cadherin domains (Hatta *et al.* 1988), suggesting that it is not functionally important. Furthermore, the EC5 domain is not likely to be readily available to interact with the EC1 domain (as EC5 is immediately proximal to the plasma membrane) due to steric hindrance caused by other membrane glycoproteins and glycolipids (see also Garrod *et al.* 2002, for further discussion). Therefore, the physiological significance of these observations remains to be determined.

Nectin-2

Nectins comprise a sub-family of the immunoglobulin superfamily (Takahashi *et al.* 1999). They promote cell adhesion in a calcium-independent manner (Takahashi *et al.* 1999, Miyahara *et al.* 2000). It has recently been shown that nectin-2 is a component of the plasma membrane domain of adherens junctions (Takahashi *et al.* 1999). Nectin-2 is composed of three extracellular immunoglobulin domains (designated EC1–3), a membrane domain and a single cytoplasmic domain (figure 1). It is linked to the microfilaments of the cytoskeleton via the intracellular protein, l-afadin (Mandai *et al.* 1997, Takahashi *et al.* 1999). Preliminary observations indicate that l-afadin interacts with α-catenin, a component of the supramolecular complex that links E-cadherin to the cytoskeleton (Wheelock *et al.* 1996, Tachibana *et al.* 2000). 1-Afadin is also associated with the cytoplasmic protein ponsin (Mandai *et al.* 1999). The precise molecular details concerning the interactions between the nectin/afadin and cadherin/catenin systems remain to be resolved, but this research will be crucial to achieve an understanding of adherens junction assembly.

Nectin-2 monomers are thought to form dimers within the plane of the plasma membrane (*cis* interactions) in a similar manner to E-cadherin, prior to establishing homophilic *trans* interactions between cells (Miyahara *et al.* 2000). Antibodies directed against either the EC1 or EC3 domains of nectin-2 block cell adhesion (Aoki *et al.* 1997). Furthermore, a point mutation in the EC1 domain abolishes *trans*, but not *cis* interactions (Miyahara *et al.* 2000). Collectively, these observations have led Miyahara *et al.* (2000) to suggest that the EC3 domain of nectin-2 is involved in promoting *cis* interactions, whereas the EC1 domain is directly involved in facilitating *trans* interactions.

Vezatin

Vezatin is a newly discovered plasma membrane component of adherens junctions (Kussel-Andermann *et al.* 2000). It has a short extracellular domain, transmembrane domain and long intracellular domain (figure 1). The intracellular domain of vezatin binds to myosin VIIA. Co-immunoprecipitation studies also suggest that vezatin/myosin VIIA system can interact with E-cadherin/catenin system, although the precise details of this interaction remain to be determined. Furthermore, studies are needed to reveal whether vezatin is capable of acting as a CAM.

Conclusions

The molecular architecture of adherens junctions continues to grow in complexity. Three plasma membrane components of epithelial adherens junctions have been identified: E-cadherin, nectin-2 and vezatin. All three of these components are thought to be capable of indirectly interacting with one another via supramolecular complexes attached to their respective cytoplasmic domains. Resolution of the molecular mechanisms underlying adherens junction assembly will require a detailed analysis of the interactions between the intracellular proteins comprising these complexes. A key step in adherens junction assembly appears to be the formation of E-cadherin and nectin-2 homodimers within the plane of the plasma membrane. This process is followed by the assembly of adhesive complexes between cells. The EC1 domains of E-cadherin and nectin-2 appear to be directly involved in promoting cell adhesion. In particular, the classical cadherin CAR sequence HAV has been identified as playing a pivotal role in mediating both *cis* and *trans* cadherin interactions.

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Received 14 December 2001, and in revised form 4 February 2002.