Journal of Cellular Biochemistry 61:514–523 (1996)

Cadherin-Catenin Complex: Protein Interactions and Their Implications for Cadherin Function

Hermann Aberle, Hillel Schwartz, and Rolf Kemler

Max-Planck-Institut für Immunbiologie, D-79108 Freiburg, Germany

Abstract Cadherins comprise a family of calcium-dependent glycoproteins that function in mediating cell-cell adhesion in virtually all solid tissues of multicellular organisms. In epithelial cells, E-cadherin represents a key molecule in the establishment and stabilization of cellular junctions. On the cellular level, E-cadherin is concentrated at the adherens junction and interacts homophilically with E-cadherin molecules of adjacent cells. Significant progress has been made in understanding the extra- and intracellular interactions of E-cadherin. Recent success in solving the three-dimensional structure of an extracellular cadherin domain provides a structural basis for understanding the homophilic interaction mechanism and the calcium requirement of cadherins. According to the crystal structure, individual cadherin molecules cooperate to form a linear cell adhesion zipper. The intracellular anchorage of cadherins is regulated by the dynamic association with cytoplasmic proteins, termed catenins. The cytoplasmic domain of E-cadherin is complexed with either β-catenin or plakoglobin (γ-catenin). β-catenin and plakoglobin bind directly to α-catenin, giving rise to two distinct cadherin-catenin complexes (CCC). α-catenin is thought to link both CCC's to actin filaments. The anchorage of cadherins to the cytoskeleton appears to be regulated by tyrosine phosphorylation. Phosphorylation-induced junctional disassembly targets the catenins, indicating that catenins are components of signal transduction pathways. The unexpected association of catenins with the product of the tumor suppressor gene *APC* has led to the discovery of a second, cadherin-independent catenin complex. Two separate catenin complexes are therefore involved in the cross-talk between cell adhesion and signal transduction. In this review we focus on protein interactions regulating the molecular architecture and function of the CCC. In the light of a fundamental role of the CCC during mammalian development and tissue morphogenesis, we also discuss the phenotypes of embryos lacking E-cadherin or β-catenin.

Key words: cadherin, catenin, plakoglobin, armadillo, APC, p120^{cas}, protein interactions, gene targeting

INTRODUCTION

Tissues and organs of multicellular organisms are formed during development by a strictly regulated integration and segregation of heterogeneous cell populations into an organized cell pattern. One of the major mechanisms to establish and maintain these organs during cell migration, proliferation, and differentiation is cell-cell adhesion, typically mediated by cell surface glycoproteins. Sequence comparisons allowed classification of most cell adhesion molecules in four major groups: integrins, immunoglobulins, selectins, and cadherins. Cadherins confer calcium-dependent adhesion through homophilic interactions with cadherin molecules on adjacent cells. They play a fundamental role in specific cell-recognition and cell-sorting processes during development [for review see Ranscht, 1994]. Their spatio-temporal expression pattern correlates well with morphogenetic events, suggesting that cadherins participate in the dynamic processes of histogenesis, such as germlayer formation and neural tube induction. On the cellular level, this correlates with cell migration, segregation, and terminal differentiation. Hence, members of the large cadherin superfamily seem to participate in homotypic cell recognition events leading to time- and position-dependent signaling cascades which could determine the fate of cells with respect to their tissue environment.

Cadherins

Most members of the cadherin superfamily are type I integral membrane proteins. Their extracellular domains contain a variable number of a repeated domain, approximately 110 amino acids in length, called cadherin-repeat.

Received September 25, 1995; accepted December 7, 1995.

Address reprint requests to Rolf Kemler, Max-Planck-Institut für Immunbiologie, D-79108, Freiburg, Germany.

Depending on the number of repeats and on sequence homologies in the conserved cytoplasmic domain, the cadherin superfamily can be subdivided into 6 gene families: classical cadherins type I (e.g., E-, N-, P-, R-cadherin), classical cadherins type II (cadherin-6 to -12), cadherins found in desmosomes (desmocollins, desmogleins), cadherins with a very short cytoplasmic domain or none (LI-, T-cadherin), protocadherins, and more distantly related gene products including the Drosophila *fat* tumor suppressor gene, the *dachsous* gene, and the *ret*-proto-oncogene [for review see Takeichi, 1994].

Classical cadherins are synthesized as precursor polypeptides, and intracellular processing seems to activate their adhesive properties. The extracellular domain of mature classical cadherins contains 5 tandemly arranged cadherin-repeats. The N-terminal repeat contains the adhesive domain mediating cadherin-specific adhesion. Five cadherin-repeats form four calcium binding pockets, each between two successive repeats (Fig. 1). Complex formation with calcium ions is necessary for cadherin function and confers resistance to extracellular proteases. Despite the sequence variety of the extracellular domain, all classical cadherins possess a highly conserved cytoplasmic domain which functions as a binding site for catenins, cytoplasmic proteins anchoring classical cadherins to the underlying cytoskeleton [for review see Kemler, 1993].

The recently published three-dimensional structures of the first repeat of both murine E- and N-cadherin provide a structural basis for understanding the repeat organization, the function of calcium and the homophilic interaction mechanism [Overduin et al., 1995; Shapiro et al., 1995a; Weis, 1995]. The N-terminal cadherin domain forms a barrel-like structure consisting mainly of anti-parallel β-strands. Unexpectedly, the folding topology shows remarkable similarity to the Ig-fold, found in the immunoglobulin superfamily [Wagner, 1995]. The similarity of the cadherin-repeat structure to the Ig-fold raises the possibility that these two superfamilies of calcium-dependent and -independent adhesion molecules are derived from a common ancestor [Overduin et al., 1995]. However, the sequence similarity between cadherins and immunoglobulins is low and their exon-intron structures are different, so it is more likely that this advantageous protein module has evolved from different origins [Shapiro et al., 1995b].

Primary structure based sequence alignments suggest that all five cadherin-repeats share a common folding topology [Shapiro et al., 1995a]. The N- and C-termini of each cadherin-repeat are located on opposite ends, such that a tandemly repeated array can form an elongated rod-shaped protein. The most N-terminal calcium binding pocket is formed by acidic residues at the C-terminal end of cadherin-repeat 1 together with acidic amino acids located at the N-terminus of repeat 2. Thus, calcium ions bridge and stabilize successive repeat domains through coordination of acidic residues of adjacent promoters, and align the domains into the essential rod shape [Shapiro et al., 1995a]. A reversible calcium-induced conformational change from a globular to a rod-like structure has been shown for the extracellular domain using electron microscopy [Pokutta et al., 1994]. Deletions or exon-skipping of calcium binding domains that most likely disrupt this rod structure are often found in diffuse-type gastritic carcinomas [Becker et al., 1994]. Furthermore, single amino acid substitutions in the N-terminal calcium binding motif abolished the adhesive function of E-cadherin in cell aggregation assays [Ozawa et al., 1990], again indicating that calcium is necessary to stabilize the active conformation.

The active site for homophilic recognition of anti-parallel oriented cadherin molecules on opposite cells, called the adhesive interface, is large and complex. Although it includes the conserved HAV-motif, several as yet unidentified residues may also contribute to the interaction [Overduin et al., 1995]. The crystal structure of the first repeat of N-cadherin, unlike the three-dimensional structure of soluble E-cadherin, suggests that parallel oriented N-cadherin molecules on the same cell surface dimerize along the so-called strand-dimer interface [Patel and Gumbiner, 1995]. Dimerization was found to be stabilized by conserved Trp and Phe residues buried in the hydrophobic core of the partner molecule. The crystal structure thus suggests that cadherin dimers of opposing cell membranes could aggregate into an extensive zipper-like superstructure. The linear *cadherin-zipper* could strengthen adhesion through cooperative effects of relatively weakly interacting monomers.

Sequence comparisons of the first cadherin-repeat indicate that different binding specificities among cadherins arise from the spatial varia-

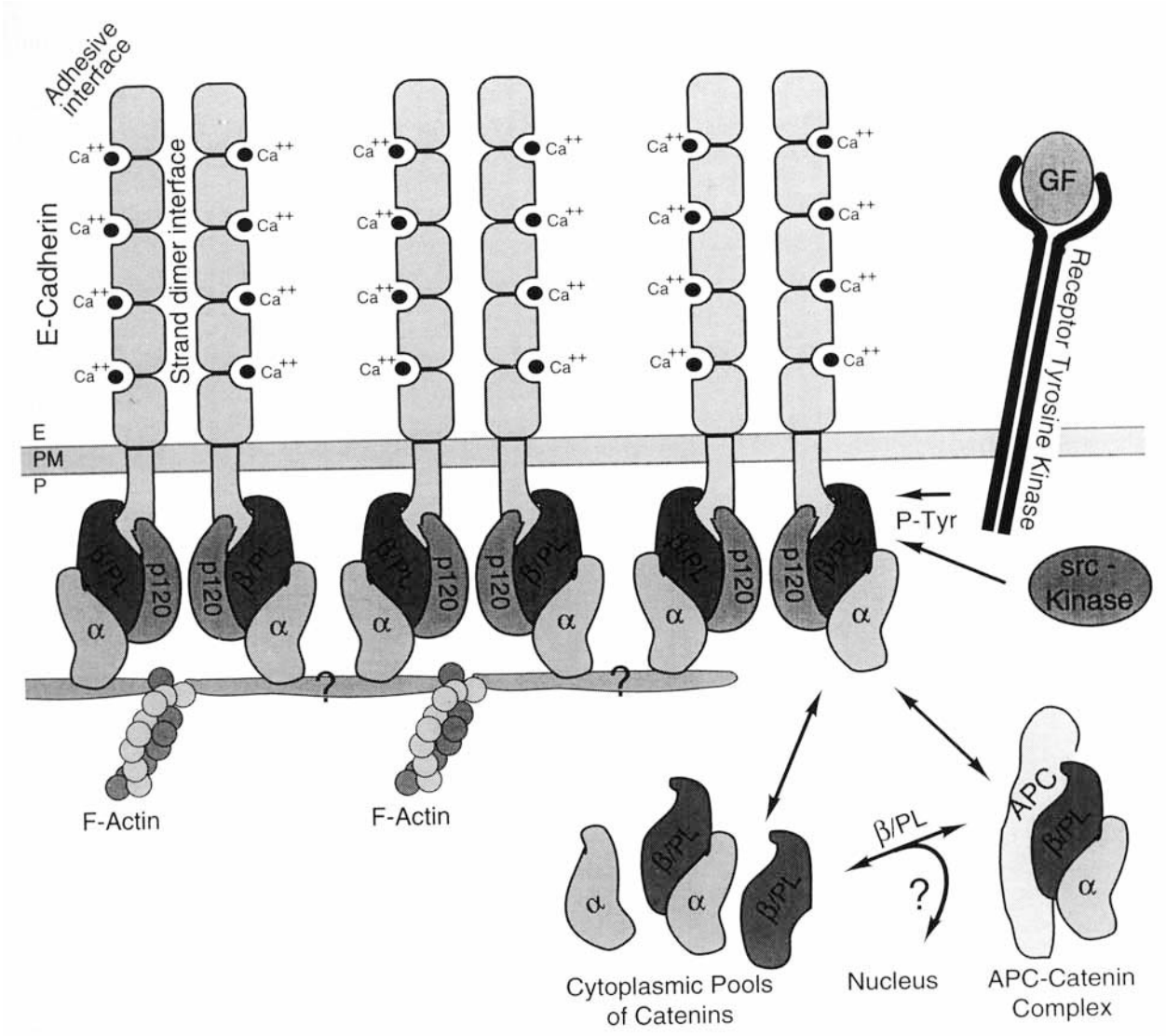

Fig. 1. Proposed model of cytoplasmic protein interactions regulating the functions of the cadherin-catenin complex. In the architecture of a single cadherin-catenin complex (CCC), either β-catenin or plakoglobin (β/PL) are central molecules linking α-catenin (α) to E-cadherin. So far there is no direct evidence for an oligomerization of individual complexes. However, the crystal structure of the first repeat of N-cadherin suggests that cadherins may aggregate into a cooperative supramolecular ribbon. A linear cadherin-zipper would have important consequences for the intracellular arrangement of the CCC, as catenins would then be integrated into an extensive meshwork. The cadherin adhesion system is thought to be linked to the actin-based cytoskeleton via α-catenin by an as yet unknown mechanism. The **right** part of the figure illustrates hypothetical protein interactions between different catenin pools, regulating the dynamic assembly and disassembly of the CCC and its function in signal transduction. The biological significance of β-catenin and plakoglobin localization in the nucleus remains to be investigated. GF, growth factor; P-Tyr, tyrosine phosphorylation; PM, plasma membrane; P, plasmatic; E, extracellular.

tion of interaction-mediating residues in the adhesion interface. This implies that homophilic interactions could be specified by complementary pairs of cadherin-specific residues at the adhesion interface. In one case of known heterophilic interaction among cadherins, residues from N- and R-cadherin are almost identical in the adhesion interface [Weis, 1995]. The importance of this recognition mechanism for sorting heterogeneous cell populations into homotypic subpopulations is clearly illustrated by the formation of the neural tube and the migration of neural crest cells. During induction of the neural plate, ectodermal cells in this differentiating region change from expressing E-cadherin to expressing N-cadherin. This may allow segregation of neural precursor cells from other ectodermal cells [Hatta and Takeichi, 1986]. In con-

trast, migrating crest cells down-regulate both E- and N-cadherin but may initiate expression of other cadherins to mediate cell sorting. Indeed, the expression patterns of cadherin-6B and -7 suggest that subpopulations of migrating neural crest cells find each other by sequential and subgroup-specific expression of different cadherins [Nakagawa and Takeichi, 1995]. However, the signals determining the final cell fates of these migrating subpopulations remain unclear. In this respect, it is of interest that the sequences of the cytoplasmic domains are quite similar in classical cadherin. How can highly conserved cytoplasmic domains be involved in the determination of different cell fates? It seems likely that other position-specific signals are required to specify diverse kinds of cell fates.

During mammalian development, cadherin-mediated adhesion is probably best exemplified by the process of compaction in the early mouse embryo. At the 8-cell stage, uniformly distributed E-cadherin molecules become concentrated at cell-cell contacts of blastomeres. Clustering of E-cadherin is accompanied by a reorganization of the cytoskeleton and changes in cell morphology. Loosely attached blastomeres flatten and adhere to each other with maximal strength, forming a compact morula [Collins and Fleming, 1995]. The precise roles of E-cadherin during pre-implantation development were recently examined by targeting the murine E-cadherin gene [Larue et al., 1994]. E-cadherin negative embryos die at the time of implantation as a consequence of the failure to form a functional trophectoderm epithelium. Although E-cadherin is known to mediate the compaction process, E-cadherin null mutant embryos are able to compact. The presence of residual maternal E-cadherin is apparently sufficient to promote compaction and the initial formation of cellular junctions. While E-cadherin has been shown to be a key molecule for the induction of cell-cell junctions [Marrs et al., 1995], it appears not to be required for their maintenance, as blastomeres of E-cadherin-deficient embryos were still able to form tight junctions and desmosomes at sites of disturbed cell-cell contacts [Riethmacher et al., 1995]. Interestingly, despite the presence of tight junctions and desmosomes, mutant embryos are unable to generate an intact epithelial cell layer. The phenotype of E-cadherin-deficient embryos provides further evidence that E-cadherin is of central importance during early morphogenetic events of mammalian development and plays a fundamental role in the biogenesis of an epithelium.

Several reports have addressed the functions of cadherins at later stages of development by ectopic overexpression of mutant cadherins to interfere with the architecture of the CCC in a dominant negative manner [Dufour et al., 1994; Kintner, 1992; Levine et al., 1994]. If one component of the extracellular or intracellular anchorage of cadherins is missing or non-functional, cadherin-mediated adhesion is abolished. Overexpression of truncated cadherin molecules encoding only the extracellular and transmembrane domains in vertebrate embryos thus competes with the endogenous cadherin-specific homophilic interaction mechanism, leading to developmental abnormalities which are restricted to tissues expressing the respective cadherin. Thus, ectopic expression of E- but not N-cadherin molecules lacking the cytoplasmic domain leads to lesions in the E-cadherin expressing ectoderm in early Xenopus embryos [Levine et al., 1994]. In contrast, overexpressed cadherins lacking the extracellular domain compete for cytoplasmic components used by all classical cadherins. Injection of mRNA encoding for only the cytoplasmic and transmembrane domains of N-cadherin also inhibits E-cadherin-mediated adhesion in the ectoderm of Xenopus embryos [Kintner, 1992]. Thus, overexpression of the cytoplasmic domain but not the extracellular domain of N-cadherin inhibited E-cadherin specific cell adhesion in the embryonic ectoderm. This is consistent with current understanding of the extracellular and intracellular interactions of cadherins. Disruption of cadherin-mediated adhesion in a specific tissue was achieved by injection of XB- or N-cadherin lacking the extracellular domain into just the four animal dorsal blastomeres of Xenopus embryos at the 32-cell stage, targeting their expression to the prospective anterior neural territory [Dufour et al., 1994]. Interestingly, overexpression of these two proteins gave rise to different degrees of developmental defects in anterior structures. The various perturbations could reflect different affinities for intracellular ligands binding to the non-functional cadherins. These findings open up the possibility to inactivate classical cadherin-dependent adhesion in either a cadherin-specific, tissue-specific, or pan-cadherin fashion within an otherwise viable Xenopus embryo, by selecting the proper construct and locus of injection.

Catenins

The catenins were first described as a set of three proteins, called α-, β-, and γ-catenin, that co-immunoprecipitated with E-cadherin [Ozawa et al., 1989]. Biochemical studies on cultured cells provided the first insights into the role of catenins in the architecture of the CCC. Pulse-chase experiments demonstrated that β-catenin first associates with the E-cadherin precursor, while α-catenin and γ-catenin (plakoglobin) join the complex afterwards [Ozawa and Kemler, 1992]. Immunoprecipitations with antibodies specific for β-catenin or for plakoglobin demonstrated that at least two CCC's exist in cells [Butz and Kemler, 1994]. The two complexes *both* contain E-cadherin and α-catenin but either β-catenin or plakoglobin in a mutually exclusive fashion [Fig. 1; Näthke et al., 1994]. Assembly of the CCC in vitro with recombinant proteins provided confirmation that β-catenin or plakoglobin is central to the architecture of each complex, linking α-catenin indirectly to E-cadherin [Aberle et al., 1994]. The catenin binding site in the cytoplasmic domain of murine E-cadherin was mapped to a conserved serine-rich region (AA 832-862) and is fully encoded by the last exon of the E-cadherin gene [Stappert and Kemler, 1994].

Primary structure analysis of α-catenin provided the first clues to its function. Three domains of α-catenin are 30% identical to vinculin. These homologous domains of vinculin have been shown to contain binding sites for talin, α-actinin, paxillin, and most likely actin. The higher order assembly of these vinculin binding proteins mediates the cytoplasmic linkage of integrins to actin filaments at focal contacts [Gumbiner, 1993]. Given the homology to vinculin, it is tempting to speculate that α-catenin may exhibit similar properties, being involved in connecting the cadherin adhesion system to the cortical cytoskeleton. Indeed, recent results suggest that α-catenin is able to link α-actinin to the CCC, apparently connecting the CCC to microfilaments [Knudsen et al., 1995]. α-catenin is required to form a functional CCC, as has been demonstrated in studies using the human lung carcinoma cell line PC9. Despite expressing high levels of E-cadherin, these cells do not express α-catenin and form only weak cell-cell contacts. Transfection with α-catenin cDNA resulted in strong intercellular adhesion, accompanied by a redistribution of the junctional marker proteins ZO-1, E-cadherin, and desmoplakin into the characteristic polarized pattern of epithelial cells [Watabe et al., 1994]. Correct assembly of the E-cadherin-catenin system is thus essential for the structural organization of epithelial cells.

The role of α-catenin for the intracellular anchorage of the CCC was studied by expressing E-cadherin-α-catenin fusion molecules in mouse fibroblastic L-cells, which do not normally express proteins of the CCC [Nagafuchi et al., 1994]. The chimeric molecules lack the catenin binding site and therefore do not associate with β-catenin or plakoglobin. Transfectants expressing either the C-terminal 397 amino acids or full-length α-catenin fused to E-cadherin showed a subcellular distribution and adhesion activity of the chimeric molecules identical to that of L-cells transfected with wildtype E-cadherin. In contrast, the N-terminal 508 amino acids of α-catenin fused to E-cadherin showed little adhesion activity, indicating that the C-terminus of α-catenin was sufficient to assemble a complex which makes E-cadherin fully adhesive. Although the adhesive properties of a cadherin complex circumventing β-catenin were indistinguishable from wildtype transfectants in this system, cell motility and down-regulation of adhesion during cytokinesis were significantly suppressed, suggesting that mechanisms regulating the adhesiveness of the CCC may require β-catenin as a regulatory element.

The primary sequence of β-catenin shows significant homology to the desmosomal component plakoglobin and to the product of the Drosophila segment polarity gene *armadillo* [Butz et al., 1992]. The central region of these homologous proteins contains a 42 amino acid motif repeated 12–13 times, originally identified in armadillo. These "armadillo (arm) repeats" are present in a variety of proteins with diverse cellular functions [Peifer et al., 1994]. Proteins with arm-repeats have been grouped together as the armadillo repeat family. This gene family can be subdivided into true armadillo homologs (armadillo, β-catenin, plakoglobin) and more distantly related proteins ($p120^{cas}$, band-6-protein, APC, smgGDS, SRP1).

The α-catenin binding site in homologs of armadillo is located in a conserved 29 amino acid domain overlapping the border between the N-terminal domain and the first arm-repeat [Aberle et al., submitted]. In vitro binding assays using proteins carrying point mutations in the α-catenin binding site identified crucial amino acids necessary for binding α-catenin and

pointed to a hydrophobic interaction mechanism between armadillo homologs and α-catenin. Based on the results with point mutants, it is worth noting that the part of the binding site within the first arm-repeat cannot be replaced by any other known arm-repeat sequence to reconstitute a functional α-catenin binding site. Although highly conserved in homologs of armadillo, the sequence motif of the α-catenin binding site is absent in more distantly related proteins containing arm-repeats. This fact renders it unlikely that family members such as APC, $p120^{cas}$ or band-6-protein interact with α-catenin by the same mechanism as homologs of armadillo. Indeed, it has been demonstrated that APC and $p120^{cas}$ do not interact directly with α-catenin [Fig. 1; Daniel and Reynolds, 1995; Hülsken et al., 1994]. The first arm-repeat has been shown to be necessary but not sufficient for binding α-catenin in vitro. Similarily, the N-terminally truncated β-catenin expressed in the human gastritic carcinoma cell line HSC-39 is unable to interact with α-catenin in vivo, although it retains an almost complete copy of arm-repeat 1 [Kawanishi et al., 1995]. In contrast, exon 3 of both β-catenin and plakoglobin fully encodes the α-catenin binding site, indicating that the exon-encoded protein domain rather than the first arm-repeat harbors the functional protein module [Aberle et al., submitted]. If so, arm-repeats could represent structural domains providing a scaffolding for protein interactions rather than being independent functional units. The picture emerging from the mapping of protein-protein interaction sites within the CCC is that mutations in these protein domains are likely to influence the architecture of the multiprotein complex, with potentially important consequences for the tight adhesion of epithelial cells and the progression of carcinomas in the course of tumorigenesis.

Initial insights into the function of β-catenin during mouse development have recently been obtained from targeting the *β-catenin* gene locus [Haegel et al., 1995]. Embryos lacking β-catenin developed normally into the egg-cylinder stage. At day 7 p.c., the development of the embryonic ectoderm was affected. Cells detached from the ectodermal cell layer and were dispersed into the proamnionic cavity. In contrast, the formation of the first embryonal epithelium, the trophectoderm, was not affected. The proper adhesion of trophectodermal cells is most likely assured by maternally supplied β-catenin. With respect to cell adhesion, a similar phenotype is encountered in zygotic mutations affecting the β-catenin homolog armadillo. In Drosophila, the armadillo and wingless proteins are components of the wingless signaling pathway establishing pattern formation within embryonic segments [Peifer, 1995]. Mutations in each of these segment polarity genes lead to an identical phenotype, the replacement of the posterior part of each embryonic segment by a mirror-image duplication of the anterior part. Zygotic null mutations of *armadillo* primarily affect segmental patterning and not cell adhesion. Maternally supplied armadillo appears to be sufficient for junction function but not for cell fate determination by the wingless signaling pathway [Peifer, 1995]. By analogy to Drosophila, lack of the mammalian homologs of wingless or armadillo, the product of the proto-oncogene *wnt-1* or β-catenin, should result in a similar phenotype. Since the phenotypes of wnt-1 and β-catenin deficient mice are in fact distinct [Haegel et al., 1995; Thomas and Capecchi, 1990], it could well be that in embryonic ectoderm of vertebrates β-catenin is the target for another member of the family of wnt signaling molecules or that β-catenin is additionally involved in another, earlier morphogenetic signaling cascade.

The homology to armadillo suggests a role for β-catenin in the morphogenesis of embryonic tissues. This point of view is supported by the observation that β-catenin negative embryos failed to express the mesodermal marker gene *T-brachury,* indicating that no mesoderm was formed in mutant mouse embryos [Haegel et al., 1995]. Studies of Xenopus embryos also provided evidence that β-catenin is implicated in mesoderm formation and axis patterning. Ectopic overexpression of β-catenin by injection of mRNA into the ventral blastomeres of Xenopus embryos led to an induction of a second Spemann organizer and duplication of the anterior-posterior axis [Funayama et al., 1995]. Axis duplication could also be induced by overexpression of plakoglobin [Karnovsky and Klymkowsky, 1995] and was mediated by the conserved arm-repeat domain of these homologous proteins. Interestingly, all overexpressed deletion mutants of β-catenin or plakoglobin that induced a second anterior axis accumulated in the nucleus. In contrast, down-regulation of β-catenin with antisense oligonucleotides inhibited dorsal mesoderm formation [Heasman et al., 1994]. These

observations clearly point to a role for β-catenin in embryonic patterning, especially mesoderm formation.

Another interesting finding on the β-catenin mouse mutant is that β-catenin could not be functionally substituted by its homolog plakoglobin [Haegel et al., 1995], although each protein forms similar complexes with E-cadherin and APC (Fig. 1). A possible explanation for this observation is that amino acid sequences required for complex formation are present in both proteins, most likely in the highly conserved core region, but that sequences in the N- and C-termini, which are less conserved, may possess unique functions. This view is supported by results obtained from studies in Drosophila. Armadillo proteins lacking the C-terminus could carry out junction function but not wingless signaling [Peifer et al., 1993]. Given the homology to armadillo, it is tempting to speculate that the C-terminus of β-catenin, which lacks significant homology to plakoglobin, may be important for a β-catenin-specific function which cannot be carried out by plakoglobin. Elucidating catenin functions specific for β-catenin or plakoglobin could lead to better understanding how catenins act to allow crosstalk between cell-cell adhesion and signal transduction.

Catenin Complexes Connecting Cell Adhesion and Signal Transduction

Accumulating evidence suggests that junctional integrity is regulated by tyrosine phosphorylation. An elevated level of phosphotyrosine, generated by inhibition of phosphotyrosine phosphatases or activation of tyrosine kinases, rapidly but reversibly induces junctional disassembly. The catenins are target molecules of this apparently phosphotyrosine regulated disassembly [Fig. 1; Behrens et al., 1993]. For example, treatment of epithelial cells with EGF leads to cell rounding and scattering of confluent cell layers. Within minutes of EGF stimulation, β-catenin and plakoglobin are phosphorylated on tyrosine residues. Furthermore, the co-immunoprecipitation of the EGFR with the CCC, together with in vitro binding assays, indicates that the EGFR can directly associate with β-catenin [Hoschuetzky et al., 1994]. However, the physical interaction of catenins with E-cadherin was found to be not disrupted after stimulation of tyrosine phosphorylation. Instead, complete CCC's were dispersed around the cell membrane. This redistribution suggests that tyrosine phosphorylation causes the dissociation of the CCC from the cytoskeleton.

Recently, a new protein associated with the CCC has been identified. The novel protein, termed p120^{cas} for cadherin associated src-substrate, was originally characterized as a major substrate of activated forms of the src tyrosine kinase [Reynolds et al., 1992]. p120^{cas} is also tyrosine phosphorylated in response to growth factor induced activation of receptor tyrosine kinases, including EGFR and PDGFR. These facts, together with the observation that p120^{cas} is expressed in a variety of different cell types, suggest a fundamental role for p120^{cas} in signal transduction. However, p120^{cas} lacks structural motifs such as SH2 and SH3 domains, found in most tyrosine kinase substrates involved in mitogenic signaling. The predicted amino acid sequence contains 11 arm-repeats. The arm-repeats, together with the co-localization and co-immunoprecipitation with the CCC classify p120^{cas} as a new catenin [Reynolds et al., 1994]. Using the yeast two-hybrid system, it has been shown that p120^{cas} interacts directly with E-cadherin but not with catenins [Daniel and Reynolds, 1995]. These results, together with the observation that p120^{cas}-specific antibodies coprecipitated E-cadherin, β-catenin, and plakoglobin [Shibamoto et al., 1995], suggest that p120^{cas} interacts with a domain of E-cadherin distinct from the catenin binding site (Fig. 1). However, the role of p120^{cas} in the assembly of the CCC is not yet understood, as a large proportion of p120^{cas} is not associated with the CCC and four isoforms have been identified which are expressed in a cell-type specific manner. Nevertheless, the association of p120^{cas} isoforms with the CCC, together with its signal-dependent tyrosine phosphorylation indicates that p120^{cas} could be a modulator of cadherin-mediated adhesion.

The association of catenins with the product of the adenomatous polyposis coli gene *APC* raises new questions about catenin function [Rubinfeld et al., 1993; Su et al., 1993]. The tumor suppressor gene *APC* encodes a very large protein with 2843 amino acids. Mutations in *APC* occur early in the progression of dominantly inherited and spontaneous colorectal carcinomas to malignancy and usually result in C-terminal protein truncations, indicating that the C-terminus is important for APC function [Polakis, 1995]. The phenotype of mice heterozygous for a mutated *APC*-allele indicates that

truncated APC proteins cause mild overproliferation of crypt cells rather than adhesion defects in the intestinal epithelium [Oshima et al., 1995]. Most carcinogenic truncations of APC retain the capability to interact with catenins. The β-catenin binding sites in APC (AA 1013–1174) are not within the arm-repeat region [Su et al., 1993], arguing against the possibility that arm-repeats are homophilic interaction domains. α-catenin binds to APC only indirectly, through β-catenin or plakoglobin (Fig. 1). Cadherins and $p120^{cas}$ are not contained within the APC-catenin complex, as β-catenin or plakoglobin form mutually exclusive complexes with APC or cadherins [Daniel and Reynolds, 1995; Hülsken et al., 1994]. This is consistent with the finding that $p120^{cas}$ interacts directly with cadherins.

The existence of a second, probably cytoplasmic, catenin complex implies that APC is involved in the functions of the CCC. Transfection experiments indicate that full-length APC functions in the down-regulation of cytoplasmic β-catenin pools [Munemitsu et al., 1995]. In contrast, accumulation of β-catenin and plakoglobin was observed in wnt-1 responsive cells upon expression of wnt-1 [Bradley et al., 1993; Hinck et al., 1994]. This effect appears to be similar to the accumulation of armadillo after the wingless signal. In Drosophila, there is a clear correlation between the accumulation of cytoplasmic armadillo and the fate adopted by cells [Peifer et al., 1993]. In the context of a conserved signal transduction pathway, it would be interesting to investigate whether the Drosophila homolog of APC is involved in the transduction of the wingless signal. By regulating the pool size of β-catenin, the APC-catenin complex could be a component of the hypothetical β-catenin signaling pathway downstream of the cadherin adhesion system. Taken together, catenin-complexes seem to be at the convergence of several signal transduction pathways, supporting the hypothesis that catenins are key molecules connecting cell adhesion and signal transduction.

CONCLUSION AND FUTURE PROSPECTS

During recent years, much progress has been made in defining the protein interactions regulating cadherin-mediated cell-cell adhesion. The biochemical characterization of functional protein domains has led to an increased understanding of the molecular architecture of the CCC. However, much remains to be learned about how the catenins interact with the actin-based cytoskeleton and how they regulate the adhesive activity of cadherins. The existence of similarly organized adherens junctions in vertebrates and invertebrates involving homologous proteins suggests that these organisms could also share a common signaling pathway to modulate cell-cell adhesion. It is a challenge for the future to investigate the role of the CCC in the transduction of signals helping to regulate proliferation, pattern formation, and cell fate determination.

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