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Review

The cell-cell adhesion molecule E-cadherin

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Abstract. This review is dedicated to E-cadherin, a calcium-dependent cell-cell adhesion molecule with pivotal roles in epithelial cell behavior, tissue formation, and suppression of cancer. As founder member of the cadherin superfamily, it has been extensively investigated. We summarize the structure and regulation of the E-cadherin gene and transcript. Models for E-cadherin-catenin complexes and cell junctions are presented. The structure of the E-cadherin protein is discussed in view of the diverse functions of this remarkable protein. Homophilic and heterophilic adhesion are compared, including the role of E-cadherin as a receptor for pathogens. The complex post-translational processing of E-cadherin is reviewed, as well as the many signaling activities. The role of E-cadherin in embryonic development and morphogenesis is discussed for several animal models. Finally, we review the multiple mechanisms that disrupt E-cadherin function in cancer: inactivating somatic and germline mutations, epigenetic silencing by DNA methylation and epithelial to mesenchymal transition-inducing transcription factors, and dysregulated protein processing.

Keywords. E-cadherin, cell junction, catenin, structure-function relationship, signaling, morphogenesis, tumor suppressor.

Introduction to E-cadherin: Historical overview of its discovery and classic experiments

Cadherins comprise a large family of transmembrane or membrane-associated glycoproteins that mediate specific cell-cell adhesion in a Ca^{2+}-dependent manner, functioning as key molecules in the morphogenesis of a variety of organs (recently reviewed in [1–3]). The cadherin family consists of at least five major subfamilies, *i.e.*, 'classical' cadherins of type I, closely related cadherins of type II, desmosomal cadherins (desmocollins and desmogleins), protocadherins, and a variety of cadherin-related molecules [4, 5].

E-cadherin, a type-I cadherin, is generally considered the prototype of all cadherins because of its early identification and its thorough characterization, both in normal and in pathological conditions. As early as 1977, Takeichi [6] proposed that the adhesive properties of the V79 Chinese hamster lung cell line could be dissected into a Ca^{2+}-independent agglutination, and a more physiological Ca^{2+}-dependent cell-cell adhesion. By iodinating surface proteins, Takeichi discovered that a surface protein of about 150 kDa was protected by Ca^{2+} against iodination and trypsiniza-

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tion. This was probably the first report on E-cadherin and its Ca^{2+}-dependent adhesion potential. Initially, these studies seemed to be only marginally related to the findings of François Jacob's group in Paris, who in 1980 described an 84-kDa glycoprotein (gp84) that was identified by an immunological approach on the membranes of mouse embryonic carcinoma cells [7]. Antibodies reacting with gp84 perturbed cell-cell interactions and prevented compaction of preimplantation embryos. This glycoprotein could be extracted from cell membranes by trypsin treatment in the presence of Ca^{2+}. This same group generated a specific monoclonal antibody against gp84, and careful analysis allowed them to conclude that gp84 is synthesized as several short-lived precursors that are processed into a stable 120-kDa form. The latter is vulnerable to proteolysis by trypsin in the absence of Ca^{2+}, but in the presence of Ca^{2+} it yields gp84. Nowadays, we know that gp84 corresponds to the ectodomain of E-cadherin. However, back then this interesting adhesion molecule of 120 kDa was called uvomorulin (UM), whereas its gp84 fragment was called UMt. The term uvomorulin was based on the ability of anti-uvomorulin antibodies to convert a morula into a loose structure resembling a bunch of grapes (Latin *uva*) [8]. Immunoelectron microscopy revealed that uvomorulin was localized in the intermediate junctions or adherens junctions of intestinal epithelial cells [9]. Concurrently, the group of Damsky and Buck reported the identification and purification of a GP80 protein that is shed into serum-free medium by MCF-7 human breast cancer cells [10]. Anti-GP80 antibodies caused mouse epithelial cells to convert from an epithelioid to a fibroblastic morphology with disruption of cell-cell junctions. The same antibodies enabled characterization of the cell-surface form of the antigen as a glycoprotein of 120 kDa. This protein, which was found to be expressed by a variety of epithelial cell lines and tissues, was named cell-CAM 120/80. Complementary studies on embryonic chicken liver cells were meanwhile performed by Edelman's group [11]. Antibodies reactive with a 68-kDa polypeptide inhibited liver cell aggregation and histotypic patterning. Interestingly, these antibodies did not inhibit aggregation of retinal cells (which we now know express R-cadherin instead of E-cadherin). Further studies revealed that this major cell adhesion molecule in chicken liver, named L-CAM, is a 124-kDa glycoprotein, mainly converted into an 81-kDa protein by trypsinization in the presence of Ca^{2+}. All these pioneering studies were reconciled upon further characterization of the Ca^{2+}-dependent cell-cell adhesion molecule or molecules in epithelial cells with the help of specific antibodies. For instance, the monoclonal antibody ECCD-1, which was raised against teratocarcinoma cells by the Takeichi group [12], recognized mainly a 124-kDa protein in various epithelial cells but not in fibroblastic cells, and functionally inactivated cell-cell adhesion of cultured mouse hepatocytes or teratocarcinoma cells [13]. Interestingly, an antibody against gp84 [8] was shown to cross-react with ECCD-1. In 1984, Yoshida-Noro and Takeichi [13] introduced the name 'cadherins' as a more generic name for this important class of cell-cell adhesion molecules, and over time this name became generally adopted. E-cadherin obtained its prefix 'E' (for epithelial) when new antibodies produced by Takeichi's group revealed the existence of other related cadherins, like N- (neural) and P-(placental) cadherins, which have distinct spatio-temporal expression patterns. Other historic milestones in the E-cadherin story, which are overviewed below, include the following: (1) cloning of the E-cadherin cDNA and the compelling demonstration of the homophilic cell-cell adhesion function of its protein product [14, 15], (2) determination of the interaction of 'catenins' with the cytoplasmic domain of E-cadherin [16, 17], (3) demonstration of strong suppression of malignant invasion by E-cadherin [18, 19], (4) elucidation of the tertiary structure of its adhesion-mediating extracellular domain [20, 21], (5) understanding its role during embryonic development and morphogenesis as revealed by knockout mouse models [22], (6) detection of E-cadherin inactivating mutations in particular sporadic cancers and in heritable diffuse gastric cancers [23–25], and (7) identification of transcriptional silencers of the E-cadherin gene [26–28], and many more.

Basic structure of the E-cadherin protein and its encoding gene and transcript

The cloning of the mouse E-cadherin cDNA [14] led to the prediction that the E-cadherin protein precursor is a polypeptide with a short signal sequence for import into the ER, a propeptide of about 130 amino acid residues (AA), and a mature polypeptide of about 728 AA (Fig. 1). The mature E-cadherin contains a single transmembrane domain, a cytoplasmic domain of about 150 AA, and an ectodomain of about 550 AA comprising five tandemly repeated domains. Four of these domains are so-called extracellular cadherin repeats (EC1 to EC4), whereas the fifth, often called EC5, is characterized by four conserved cysteines. We suggested the name membrane proximal extracellular domain (MPED) instead of EC5 to emphasize its special structure [29]. Reduction of the disulfide bridges in that domain affects the formation of strong cell-cell contacts [30]. As other structurally related

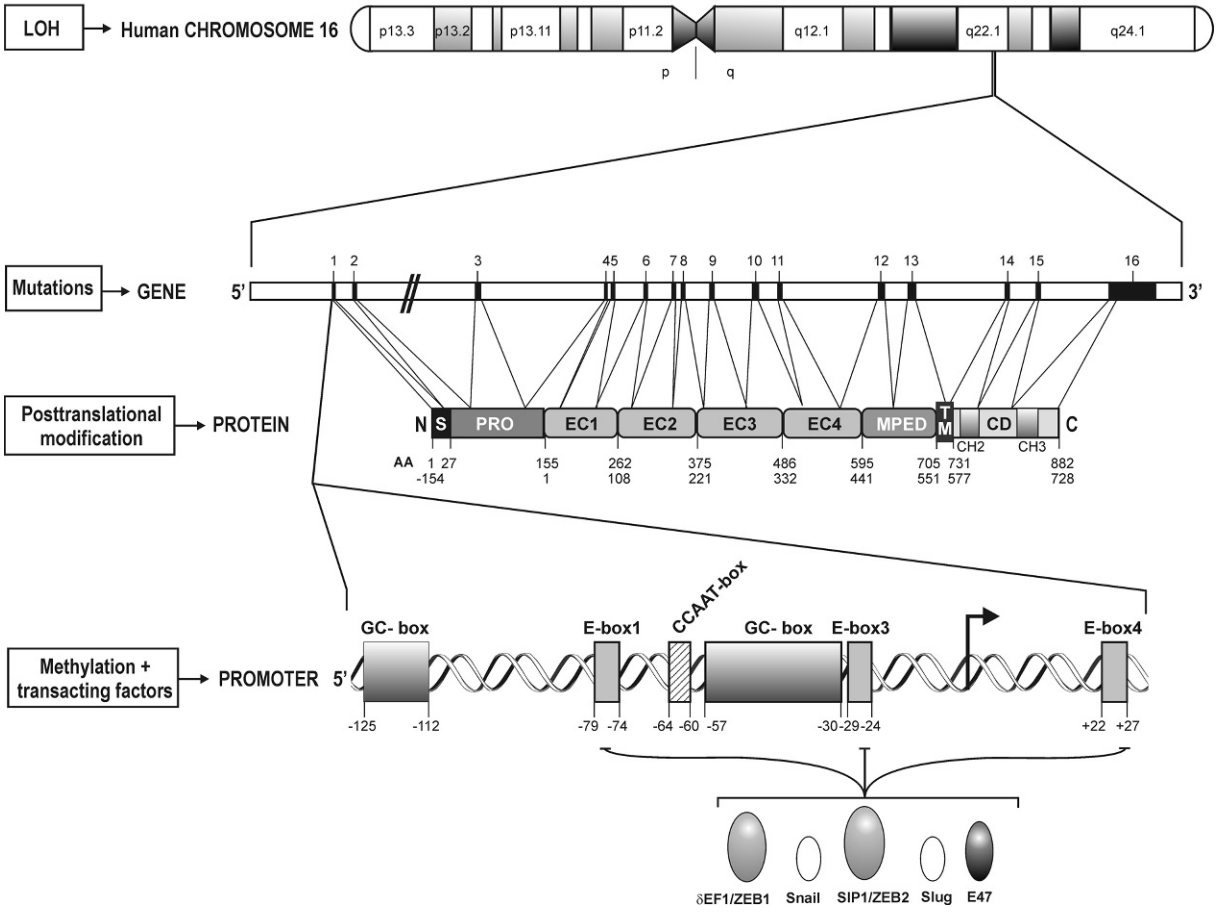

Figure 1. Structure of the human *CDH1* gene and promoter region, and the encoded E-cadherin protein. E-cadherin expression is dysregulated at various levels in human tumors, as indicated in the boxes at the left. The E-cadherin gene, *CDH1*, is located on chromosome 16q22.1, a region showing frequent loss of heterozygosity (LOH) in different types of human carcinoma. Specific inactivating mutations, scattered throughout the coding region, are particularly abundant in sporadic lobular breast cancer and diffuse gastric cancer. Germline mutations can also occur and are the cause of the hereditary diffuse gastric cancer syndrome. Furthermore, post-translational modifications, such as phosphorylation, glycosylation and other modifications can affect E-cadherin protein functionality. Epigenetic silencing has been associated with CpG methylation in the promoter region and with direct binding of specific transcriptional repressors to E-box sequences in the promoter. AA, amino acid position (numbering starts either at the start Met codon, or at the N terminus of the processed, mature protein); C, C-terminal; CD, cytoplasmic domain; CH, cadherin homology domain; EC, extracellular cadherin repeat; MPED, membrane-proximal extracellular domain; N, N-terminal; PRO, propeptide; S, signal peptide; TM, transmembrane region. The arrow points to the transcriptional initiation start.

cadherin members were cloned, it became clear that the presence of a few to numerous EC repeats is characteristic of all members not only of the small family of 'classic' cadherins, but also the ever growing superfamily of cadherins and cadherin-related proteins, such as desmosomal cadherins, type-II cadherins, protocadherins, FAT, and Dachsous [5, 29]. Meanwhile, many advances have been made in the structural analysis of EC repeats (see below).

Surprisingly, the structure of this multirepeat ectodomain was not at all reflected by the structure of the *CDH1* gene, encoding human E-cadherin [31] (Fig. 1). Indeed, each EC repeat is encoded by two to three exons and the EC boundaries do not correspond to exon boundaries. This apparent discrepancy is by no means solved, because the ectodomain of protocadherins, comprising six to seven cadherin repeats, is encoded by just one or two large exons [5]. A striking recurrent finding is the very long size of intron 2 (*i.e.*, the intron following the second protein-coding exon) in various E-cadherin genes (*e.g.*, [31]). This suggested the presence in that intron of gene regulatory sequences in addition to the promoter sequence. This was indeed demonstrated in studies on transgenic mice (see below).

An equally important component of E-cadherin is its cytoplasmic domain, which can be subdivided into two subdomains: the membrane proximal cytoplasmic/conserved domain (MPCD), often named juxtamembrane domain (JMD), and the (β-)catenin binding domain (CBD), each of which has a sequence motif of about 30–35 residues, respectively called CH2 and

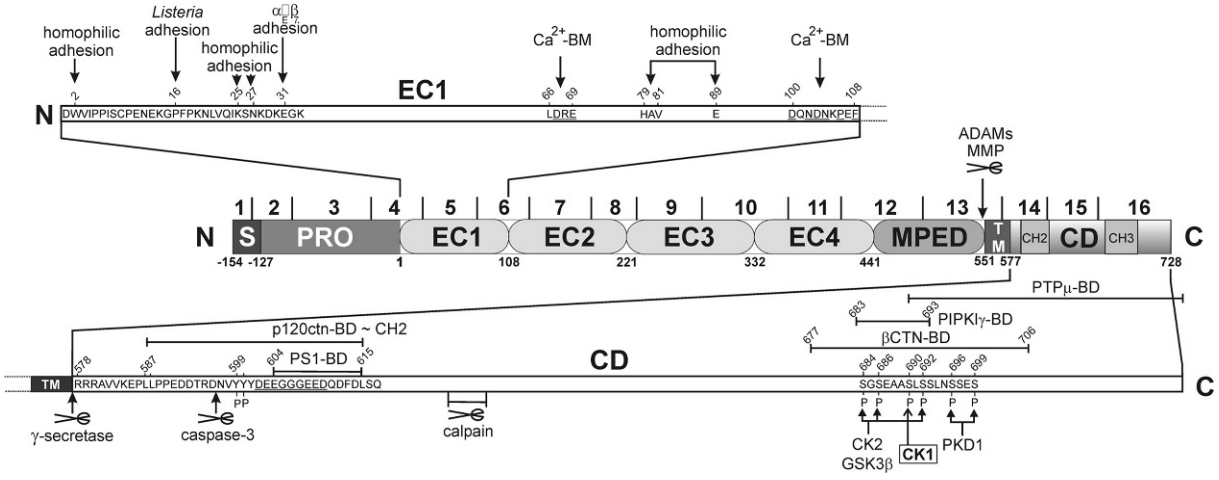

Figure 2. Functional domains and motifs of the human E-cadherin protein. The full-length protein is depicted in the center. Borders between the encoding exons and exon numbers are shown at the top. The EC1 domain and the cytoplasmic domain (CD), including selected AA sequences in single-letter code, are depicted at a larger scale at the top and the bottom of the figure, respectively. AA numbering starts at the N terminus of the processed, mature protein (total length of 728 AA). Cleavage sites of proteases are shown by the scissors symbol and the protease name. Phosphorylation sites are indicated by P letters and the responsible kinase. Casein kinase-1 (CK1) is boxed to indicate an inhibitory phosphorylation. ADAMs, disintegrin and metalloproteinases; βCTN, β-catenin; BD, binding domain; BM, binding motif; MMP, metalloprotease(s); PS1, presenilin-1. See legend of Figure 1 and the text for remaining symbols and acronyms.

CH3 (CH, cadherin homology region) [32] (Fig. 1). These sequences are conserved among all 'classic' type-I and type-II cadherins [29]. The deletion of the C terminus, including CH3, made the cell surface-exposed E-cadherin more soluble in nonionic detergent and abrogated Ca^{2+}-dependent cell aggregation [33]. This region was narrowed down to a core region of 30 AA that included up to eight well-conserved Ser residues that were highly phosphorylated [34] (Fig. 2). Substitution of all eight Ser residues by Ala abrogated catenin binding and cell-cell adhesion, whereas mutation of up to five Ser residues did not show this effect. The importance of differential phosphorylation of these residues is discussed further.

Overexpression in *Xenopus* of constructs that encode N-cadherin mutants lacking a large part of the ectodomain and various parts of the cytoplasmic domain (CD) resulted in dominant-negative inhibition of the endogenous cadherin(s) and hence in gastrulation failure [35]. The interaction of α-catenin with either endogenous cadherins or ectopically expressed wild-type E-cadherin was shown to be inhibited in this way. Interestingly, it was not only the C-terminal cadherin domain, known to interact with armadillo catenins β-catenin and plakoglobin, that could exert the dominant-negative effect, but also a membrane-proximal sequence of only 22 AA [35]. This short domain was later shown to be the p120ctn-interaction domain, indicating that also the binding of p120ctn to the CD is essential for functionality of E-cadherin and its relatives [36, 37].

The membrane-proximal conserved CH2 of E-cadherin has the core sequence motif DEEGGGEED (Fig. 2). Mutation of this sequence resulted in uncoupling of p120ctn from the E-cadherin/β-catenin/αE-catenin complex [37]. Transfection of p120ctn-uncoupled E-cadherin mutants in tumor cells lacking E-cadherin could not restore tight cell-cell contacts, in contrast to the rescue by wild-type E-cadherin [37]. This mutant phenotype could be ascribed to defective stabilization of mutant E-cadherin at the plasma membrane [38]. Indeed, p120ctn association with nascent E-cadherin prevents entry of cadherin into degrading endocytic membrane trafficking pathways [39]. Also, *via* interaction with kinesin motors, p120ctn accelerates the delivery or recycling of cadherins to the cell surface [40]. The above-mentioned core sequence (DEEGGGEED) in E-cadherin is preceded by a YYY sequence, but only the third Y of this sequence is perfectly conserved among classic cadherins [29]. Phosphorylation of the two nonconserved Y residues by kinases such as Src or the hepatocyte growth factor (HGF)-receptor Met triggers the E-cadherin-specific association of Hakai, a c-Cbl-like E3 ubiquitin-ligase, in competition with p120ctn binding [41] (Fig. 2). This leads to ubiquitination and endocytosis of the E-cadherin complex, which disrupts cell-cell junctions, as further elaborated below.

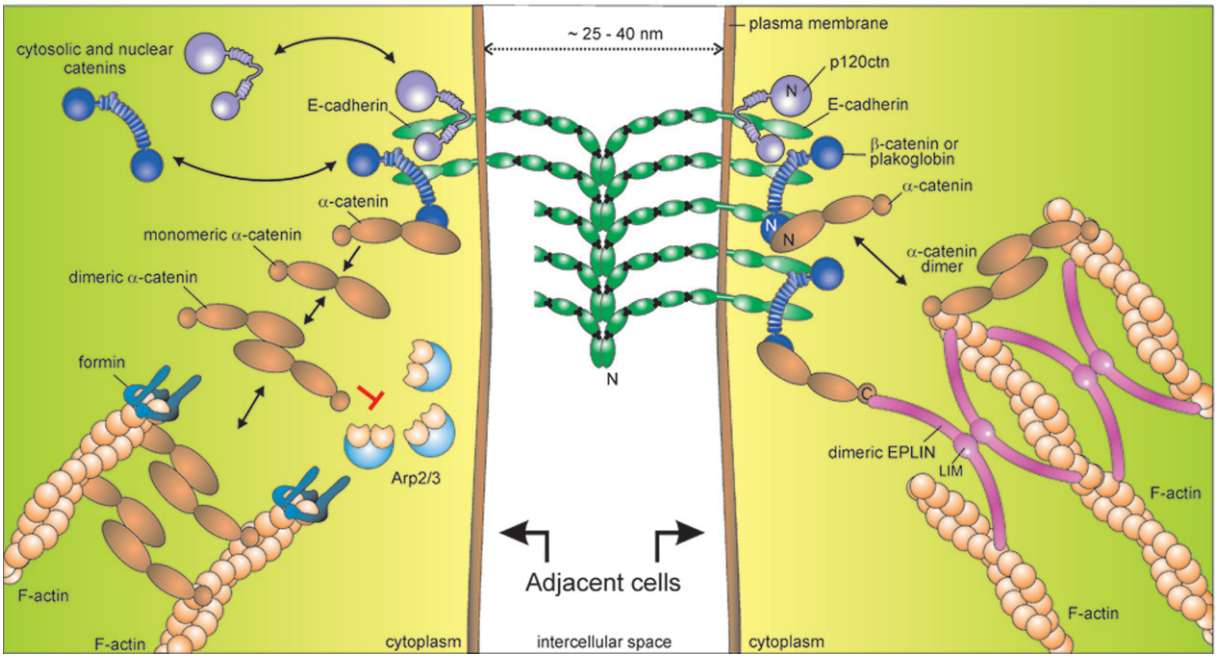

Figure 3. Schematic overview of the E-cadherin-catenin complex (CCC) at the junction between two neighboring epithelial cells (modified after [50, 233]). The armadillo catenins p120ctn and β-catenin/plakoglobin bind to, respectively, membrane-proximal and C-terminal halves of the cytoplasmic domain of E-cadherin. Left: Monomeric α-catenin binds to the CCC *via* β-catenin, whereas dimeric α-catenin cannot. Dimeric α-catenin inhibits the F-actin nucleation complex, Arp2/3. In addition to their specific functions in the CCC, both β-catenin and p120ctn have roles in the cytoplasm and in the nucleus, as depicted by double-headed arrows. Right: Dimeric α-catenin can bind and cross-link filamentous actin (F-actin), whereas the monomeric form cannot. On the other hand, EPLIN was recently reported as the 'missing' link that connects the CCC to F-actin [56]. LIM, LIM zinc finger domain; N, N-terminal end or domain. See text for more details and references.

Present models for the E-cadherin-catenin-cytoskeleton interactions

Early studies compiled compelling evidence for the existence of an E-cadherin/β-catenin/αE-catenin complex, also called CCC (Fig. 3). Within this complex the cytoplasmic domain of E-cadherin binds to the central Armadillo domain of β-catenin (or plakoglobin) (reviewed in [42]), while the binding between β-catenin and αE-catenin occurs between their respective N-terminal domains. The multifaceted roles of the various catenins (β-catenin, αE-catenin and p120ctn) have recently been reviewed [42–46], and so they will not be recapitulated here in depth.

It has long been assumed that the CCC binds the actin cytoskeleton, in this case the circumferential actin belt in polarized epithelial cells, *via* αE-catenin. This assumption was largely based on the presence of an actin-binding domain (named VH3 for vinculin homology domain 3) in the C-terminal part of αE-catenin [47]. However, the groups of Nelson and Weis recently used careful biochemical analysis and quantitative microscopy to challenge this hypothesis, with rather revolutionary results [48–50]. They confirmed the binding of αE-catenin to actin, but only for αE-catenin homodimers; on the other hand, αE-catenin could efficiently bind to E-cadherin/β-catenin complexes, but only in its monomeric form. The proposed CCC was shown to exist in the plasma membrane, but it was not directly linked to F-actin. So how can these findings be reconciled with the strong evidence that αE-catenin coupling to the cytoskeleton is indispensable for E-cadherin functionality? In particular, experiments with E-cadherin-αE-catenin chimeric proteins are worthwhile mentioning here, as these chimeras are fully active in tight coupling to the cytoskeleton despite the fact that they generate adhesion complexes lacking β-catenin [51]. However, conflicting data have also been published (reviewed in [50]). Some alternative explanations that have been critically assessed include the following. αE-catenin may continuously shuttle between an 'inactive' cadherin-bound pool and an actin-bound pool of dimers regulating the cytoskeleton (Fig. 3). The dimers are active in bundling actin filaments and in inhibiting the F-actin nucleation and branching activities of the Arp2/3 complex [48]. αE-catenin can also promote formin-mediated formation of linear actin cables [52], so that local actin remodeling will occur at sites of high αE-catenin concentrations generated by dissociation of αE-catenin from the CCC. However, this molecular switch model for αE-catenin still offers no solution for

the direct coupling of the CCC to the locally reorganized actin cytoskeleton, although such coupling is supported by different findings. One piece of evidence is the detergent-insolubility of the mature CCC. However, perhaps more convincing is the observed abrogation of CCC adhesive function by disruption of the circumferential cortical actin filament network but not by disruption of actin stress fibers or microtubules [53]. Moreover, a direct link between the CCC and actomyosin is required for particular morphogenetic changes during which active changes in cell shape occur in the presence of continued cell-cell adhesion [54]. As αE-catenin has been shown to bind to several other junction-associated, actin-binding protein partners, including ZO-1, spectrin, vinculin, α-actinin, vezatin, and the nectin-afadin complex (see also below), these molecular interactions might be responsible for the link of F-actin to cell junctions (reviewed in [44, 55]). To date, the strongest candidate for such a link between αE-catenin and F-actin is EPLIN, which stands for epithelial protein lost in neoplasm [56]. EPLIN localizes to the apical cortical actin cytoskeleton in epithelial cells and has at least two actin-binding sites, one on each side of a central domain, which by itself consists of twin zinc-finger domains [57] (Fig. 3). It cross-links and bundles actin filaments, and its lateral association with F-actin prevents depolymerization and Arp2/3-mediated secondary nucleation. Abe and Takeichi [56] demonstrated very convincingly that EPLIN is part of the CCC because it binds to a C-terminal domain of monomeric αE-catenin. This association turned out to be essential for linking the CCC to the apical actin belt. Interestingly, depletion of EPLIN resulted in disorganization of this actin belt but apparently did not affect non-junctional actin fibers. Moreover, cadherin-EPLIN chimeras were as efficient as cadherin-αE-catenin chimeras in recruiting F-actin to cell junctions.

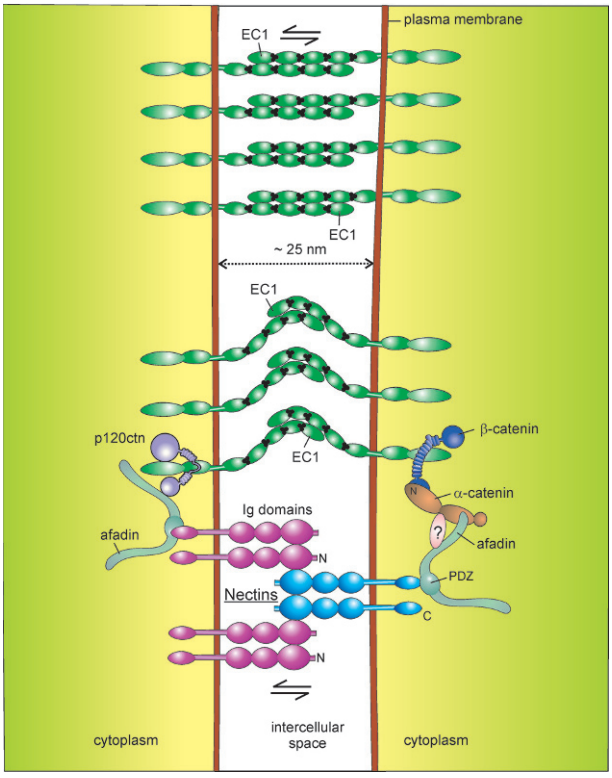

Figure 4. Alternative models for the homophilic adhesion between E-cadherin proteins; comparison with nectin interactions at the adherens junctions (AJ). Top: The interdigitation model. Center: A variant of the interdigitation model, taking into account a crescent form for E-cadherin. Bottom: Similar to E-cadherins, nectins are thought to form *cis*-homodimers and *trans*-homodimers. Here, heteropolymerization occurs, for instance between nectin-1 and nectin-3. The ectodomain of nectins comprises three immunoglobulin-like (Ig) domains. Their C-terminal ends bind to the PDZ domain of afadin. Afadin binds to a variety of proteins, including α-catenin and p120ctn. Other putative molecular links to the cadherin-catenin complex are symbolized by a question mark. Bidirectional arrows indicate that the interdigitation of either E-cadherins or nectins can be more or less extensive. EC1, N-terminal extracellular cadherin repeat. See text for more details and references.

Functional interplay between junctions at the cell surface

Epithelial cells are characterized by strong cell-cell adhesion mediated by particular junctions. These junctions are specialized adhesive sites at which a variety of transmembrane glycoproteins interface with the cytoskeleton. Cell-cell adhesion is mainly executed by three types of junctional complexes: tight junction types (TJs), adherens junctions (AJs) and desmosomes. Although each junction has its particular mechanism of formation, regulation and function, extensive communication between the different junctions mutually influences their dynamics and signaling properties. On the basis of pioneering experiments on E-cadherin blocking, it was thought for a long time that E-cadherin-dependent adhesion is a prerequisite for the assembly of other specialized cell-cell junctions [58]. *In vivo*, a particularly important role for E-cadherin in epithelial biogenesis can be deduced from knockout experiments ablating E-cadherin in the skin, which disturbs the functional assembly of TJs [59]. Disruption of cadherin-mediated adhesion delays and limits assembly of desmosomes [60]. On the other hand, in the absence of functional E-cadherin, protein kinase C activation can result in the formation of TJs and desmosomes [61]. Together, these examples indicate that E-cadherin-mediated adhesion could specifically regulate cellular signaling towards formation of other junctions. Further, accumulating evidence indicates that nectins create the initial cell-cell

adhesion sites and then recruit E-cadherin to these sites, facilitating in that way the initial transient cadherin contacts (reviewed in [55, 62, 63]). Nectins constitute a family of Ca^{2+}-independent immunoglobulin (Ig)-like cell adhesion molecules consisting of four members. In epithelial cells they are specifically present in AJs and connected to the actin cytoskeleton through afadin, which interacts directly *via* a PDZ domain with the four C-terminal AA of the nectins [64] (Fig. 4). Both afadin and α-catenin turned out to be essential for the mutual nectin-E-cadherin interactions [62]. The molecular mechanisms underlying the physical associations between nectins and E-cadherin are not completely understood, as many potential molecular interactions may be involved (reviewed in [63]). The most direct links appear to be through afadin binding to α-catenin and/or p120ctn [65, 66]. Some progress has been made using high-sensitivity force measurements [67, 68] (see also below). Upon *trans*-interaction of nectins in apposed cell surfaces, the activation of the small G proteins Rap1, Rac and Cdc42 is mediated by c-Src, Crk, C3G, Vav2 and FRG (reviewed in [69]). This particular signaling activity is dependent on integrin $\alpha_v\beta_3$, which associates with nectins. Activated Rac and Cdc42 reorganize the actin cytoskeleton, allowing recruitment of the cadherin-catenin system to the nectin-based cell-cell adhesion sites. Furthermore, the interaction of afadin with activated Rap1 also results in strengthened association of p120ctn with E-cadherin, resulting in reduced endocytosis of non-*trans*-interacting cadherins and, therefore, further establishment of cadherin-based and nectin-based AJs [66]. Subsequently, junctional adhesion molecules (JAMs), occludins and claudins are assembled at the apical side of AJs to form TJs.

Recent experimental evidence strongly indicates that components of TJs modulate AJs. Repression of the zonula occludens (ZO) proteins ZO-1 and ZO-2 resulted in the loss of TJ formation, but epithelial polarization of cells remained normal [70]. However, in a Ca^{2+} switch assay these cells were delayed in converting from point-like spots of E-cadherin-based adhesion to belt-like polarized epithelial AJs, indicating a crucial role for ZO proteins in the dynamic process of AJ maturation. This delayed cell polarization was due to impaired activation of Rac1 in primordial AJs, which is essential for cell-cell contacts mediated by E-cadherin and nectin [71]. Knockdown of PALS1/MPP5, a tight-junction component implicated in the establishment of cell polarity, perturbs both TJs and AJs [72]. Remarkably, under these conditions AJ components accumulate in E-cadherin-positive vesicles that are not efficiently exocytosed to the cell surface. Knockdown of the TJ component claudin-7 in esophageal squamous cell carcinoma cells led to decreased E-cadherin levels, increased cell proliferation and enhanced invasiveness, whereas overexpression of claudin-7 reversed this phenotype [73]. The underlying mechanism was not reported.

E-cadherin gene expression: Activation *versus* silencing

The spatio-temporal regulation of E-cadherin expression during embryonic development allows cell migration and morphogenesis. The first zygotic expression of the E-cadherin gene in the mouse begins at the two-cell stage [22]. During gastrulation this dynamic regulation is exemplified by decreased E-cadherin expression in delaminating epiblast cells at the primitive streak so that mesoderm is formed [74, 75]. Similarly, E-cadherin expression in the ectoderm is switched off at neurulation but remains strong at the ectoderm-neuroectoderm borders, where it plays an important role in neural tube closure [76]. Reciprocally, E-cadherin transcription is re-initiated in cells undergoing mesenchymal-epithelial transitions (MET) during kidney organogenesis [77].

Identification of the regulatory sequences of the mouse E-cadherin gene enhanced our understanding of E-cadherin transcriptional regulation [28]. Detailed analysis revealed a modular structure with different critical transcription factor binding regions that are highly conserved in mouse, dog and human (Fig. 1). Positive regulatory elements in the 5' regulatory sequence include a CCAAT box and a GC box. The transcription factor AP2 has been shown to bind the GC box in the mouse E-cadherin promoter and to induce E-cadherin transcription [78]. In addition, the retinoblastoma protein (Rb) and the proto-oncogene product c-Myc specifically activate transcription of the E-cadherin promoter by acting as co-activators of AP2 in epithelial cells [79, 80]. Another candidate transcription factor interacting with the GC box of the E-cadherin promoter is the zinc-finger protein WT1, encoded by the tumor suppressor gene *Wt1*, which is affected in Wilms' tumor of the kidney [81]. Binding of WT1 to the GC box has been shown to activate the E-cadherin promoter and to induce epithelial differentiation of fibroblasts upon ectopic expression of WT1. Other positive regulatory elements recently discovered in the E-cadherin promoter include four binding sites for hepatocyte nuclear factor-3 (HNF3) [82]. Exogenous HNF3 synergizes with AML-1 and p300 to stimulate E-cadherin transcription in metastatic E-cadherin-negative cell lines.

Besides the importance of these promoter elements for the regulation of E-cadherin expression, the

existence of additional *cis*-regulatory elements is suggested by the conserved genomic structure of the *CDH1/Cdh1* gene between different species that share a similar large intron 2 (Fig. 1). The presence of such a functional intronic enhancer sequence was first proven for the L-CAM gene [83]. Transgenic mice with the chicken L-CAM cDNA under control of a rat insulin promoter did not show any expression of L-CAM, whereas a similar construct using the L-CAM gene complete with intron 2 was expressed in the β-cells of the pancreas, as well as in extrapancreatic tissues. Therefore, the authors speculated on tissue-specific *cis*-acting elements in the L-CAM gene that could influence upstream heterologous promoter and enhancer sequences [83]. Moreover, from transient transcriptional reporter assays using E-cadherin-negative fibroblasts, it became clear that this intron 2 enhancer, in combination with the L-CAM promoter, was essential for stimulation of transcription by HNF-1 and HOXD9 [84]. Mapping in the *CDH1* locus of DNaseI hypersensitive sites, generally accepted as sites for the localization of *cis*-regulatory elements, predicted the presence of regulatory DNA elements located in introns 1 and 2 [78]. Indeed, an additional epithelial specific enhancer (ESE) exists in mouse intron 1. This regulatory sequence is recognized by AP2 factors, but this cannot explain the epithelial specific expression of the ESE [78]. These findings suggested that transcriptional regulation of the *CDH1* locus was by a more complex transcriptional tissue-specific mechanism than by *cis*-acting elements influencing upstream promoter and enhancer sequences. This was elegantly elaborated by the research group of Kemler [85], who generated a series of mouse reporter lines expressing a combination of *Cdh1* promoter and intronic fragments. This seminal work provided the genetic proof that intron 2 is essential for E-cadherin gene expression in embryonic stem (ES) cells and during early embryogenesis. Furthermore, in diverse differentiated epithelia, intron 2 fulfils a major role in regulation of transcriptional initiation and in maintenance of strong E-cadherin mRNA expression [85]. Nevertheless, no clear and conclusive information is available on the transcription factors that specifically interact with these intronic positive regulatory sequences. Indirectly acting regulatory factors, for which no intrinsic E-cadherin promoter binding activity has been demonstrated, include HNF4 [86] and PAX2 [87].

The isolation and analysis of the mouse E-cadherin promoter made it clear why non-epithelial tissues are largely E-cadherin negative [88]. The finding that inactivation of a tandemly duplicated palindromic E-box (E-pal box) resulted in E-cadherin transcriptional activity in mesenchymal cells suggested the existence of transcription factors that can down-regulate E-cadherin expression in non-epithelial cells. Only the 3' part of the E-pal box is conserved in the human and dog E-cadherin promoter, together with two more E-boxes located downstream [27, 28] (Fig. 1). The first evidence for such E-cadherin-specific transcriptional repressors was provided by the group of Cano [26, 89], who used one-hybrid screening with the E-pal sequence as bait. This screening identified transcription factors Snail, Slug and E12/E47, which were indeed subsequently proven to be potent repressors of E-cadherin expression. In addition, the two homologous deltaEF1/ZEB1 and SIP1/ZEB2 zinc-finger factors were both proven to be direct repressors of E-cadherin transcription [28, 90] (Fig. 1). Snail, Slug, SIP1 and deltaEF1 actively repress transcription by recruiting transcriptional corepressors, such as CtBP and mSinA [89, 91].

During epithelial bud development, the combined effect of WNT and Noggin signaling results in the formation of a β-catenin-Lef1/TCF complex that, surprisingly, represses transcriptional activity of the E-cadherin promoter in a manner dependent upon the Lef1/Tcf binding site [92]. This suggests that the transcriptional activity of Lef1/TCF is dependent on the context of the responsive promoter elements. The E-boxes in the E-cadherin promoter are located 3' from the Lef1/TCF binding site. Snail and Lef1/TCF act additively to repress E-cadherin promoter activity in keratinocytes. Interestingly, a direct interaction between β-catenin and Snail was reported recently [93]. Recruitment of Snail to the β-catenin-Lef1/TCF complex could endow this transcription factor complex with repressive activity [92].

In the meantime, still more putative E-cadherin repressors have been identified, including Twist, HOXB7, CBF-A and KLF8 (reviewed in [89, 94]). Most of these transcription factors participate in developmental epithelial to mesenchymal transition (EMT) and their expression results in a far-reaching reprogramming that represses not only E-cadherin but also multiple other adhesion and polarity genes [95–97]. The different E-cadherin repressors seem to act downstream of numerous signaling pathways, triggered by, *e.g.*, transforming growth factor (TGF)-β, nuclear factor (NF)-κB, fibroblast growth factor (FGF), HGF, epidermal growth factor (EGF), integrin engagement and hypoxia. Each of these pathways can induce both EMT and invasive cancer growth (reviewed in [89, 94, 98, 99]).

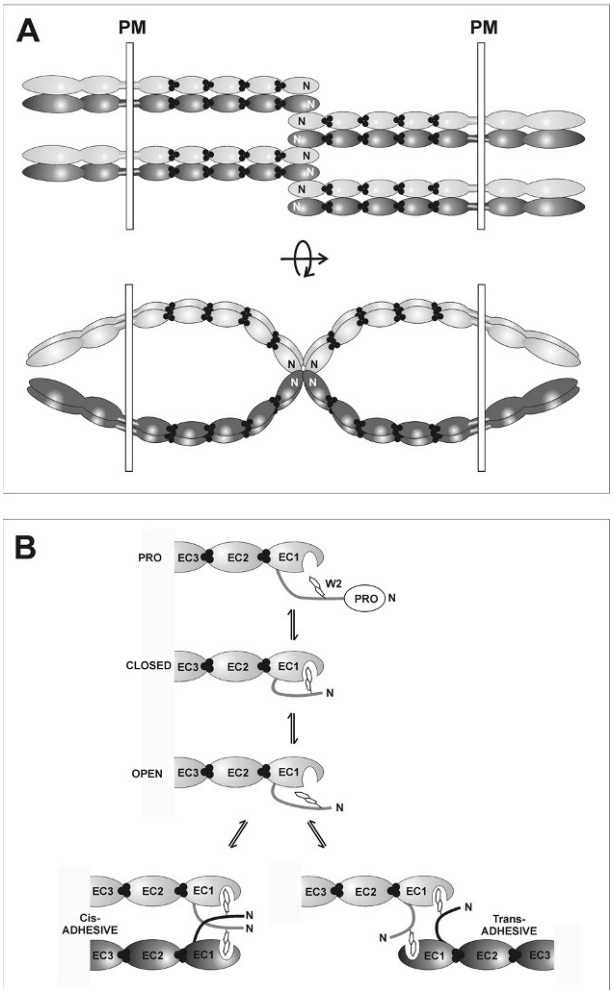

Figure 5. Models for *cis*-adhesive and *trans*-adhesive structures of E-cadherin. (*A*) Model for cadherin junctions based on cryo-electron tomography of desmosomes [121] in combination with the three-dimensional structure of *Xenopus* C-cadherin [21]. The bottom model corresponds to the model shown at the top rotated 90° and comprising four *cis*-interactions and three *trans*-interactions. All molecules are assumed to be identical and the different shading is only for display purposes. (*B*) Model for the mechanism of E-cadherin binding (modified after [110]). Emphasis is on the interaction of the tryptophan residue at position 2 of the mature protein (W2) with a hydrophobic pocket in the EC1 domain. The prodomain interferes with this interaction. After removal of the prodomain, intra-molecular docking results in a closed form that is unable to dimerize. This closed form is in equilibrium with an open form, which may engage with like molecules to form inter-molecular interactions, either in *cis* or in *trans* configuration. EC, extracellular cadherin repeat; N, N-terminal end or domain; PM, plasma membrane; Pro, prodomain. See text for more details and references.

Functional domains of E-cadherin in relationship to its three-dimensional structure

The ectodomain of classic cadherins, including that of the prototypic E-cadherin, is involved in homophilic cell-cell adhesion (between identical molecule types); this adhesion is often also homotypic (between identical cell types) [15]. The processing of the precursor form of E-cadherin was convincingly shown to be essential, not for cell surface exposition, but for the cell adhesive function [100]. The mature protein starts with a DWV sequence that is fully conserved among classical cadherins [29]. Pioneering studies by Blashuk et al. [101] identified the peptide sequence HAV at position 79–81 within EC1 (Fig. 2) as an essential cell adhesion recognition sequence in classic cadherins. A decapeptide comprising the tripeptide HAV could inhibit the E-cadherin-dependent compaction of mouse embryos. As HAV is conserved among all four classic cadherins [29], its occurrence cannot by itself explain the preferentially homophilic adhesion of each of these cadherins. Adhesion specificity studies with swapped chimeric constructs of E- and P-cadherin and with point-mutated versions of E-cadherin revealed that EC1 is critical for homophilic adhesion, and that the identities of the two HAV-flanking AA flanking (AA78 and AA83) codetermined the specificity [102]. Similar conclusions were drawn from peptide inhibition studies: only quite specific HAV-comprising decapeptides could inhibit E-cadherin-mediated cell-cell adhesion, and in this way induce invasion [103]. It was suggested that such E-cadherin fragments, released by tumor cells in an autocrine fashion, may induce and support cancer invasion. In obvious contrast with these findings is the more recent observation that inversion of the HAV sequence to VAH has no effect on E-cadherin-mediated cell-cell adhesion [104].

The extracellular cadherin repeat is an independently folding sequence of approximately 110 AA containing the conserved motifs DRE, DXNDNXPXF, and DXD (Fig. 2). The importance of these sequences became evident when single-point mutations were introduced: the mutated peptide lost calcium-binding activity, trypsin sensitivity was increased, and cell adhesion potential was abrogated [105]. These motifs are involved in calcium coordination as revealed upon resolution of the tertiary structure of the EC domains. Multidimensional heteronuclear magnetic resonance spectroscopy allowed resolution of the solution structure of the N-terminal 104 AA of mature mouse E-cadherin (domain EC1) in the presence of Ca^{2+} [20]. The EC protomer turned out to be a compact seven-stranded β-barrel structure with similarity to the well-known β-sandwich topology of immunoglobulin domains, despite the absence of sequence homology. A putative adhesion interface comprising the HAV sequence is indeed exposed on the surface, whereas the above-mentioned conserved calcium-coordinating AAs form calcium-stabilized linkage regions between successive EC protomers [20] (Fig. 3). This structure was fully confirmed by a high-resolution

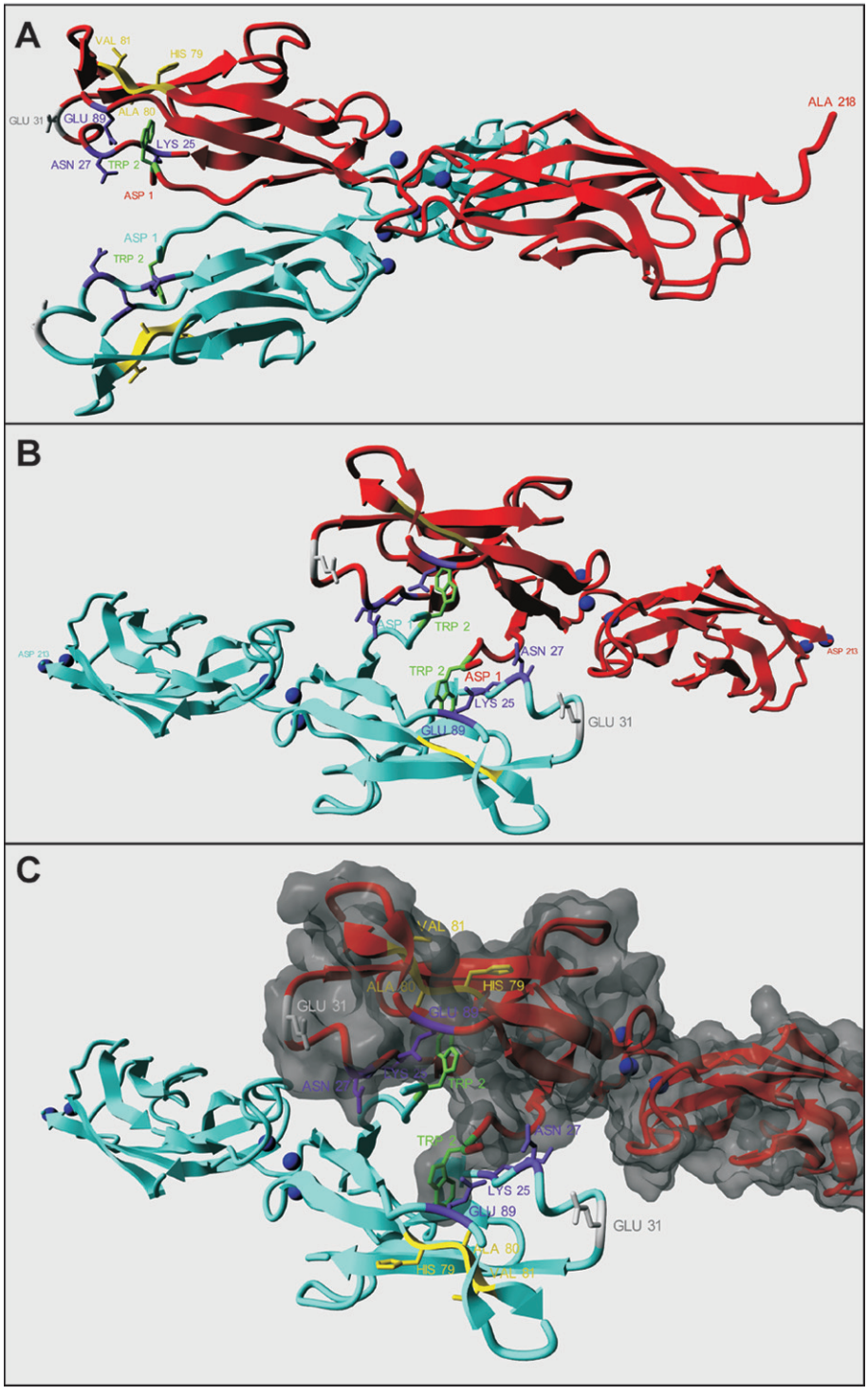

Figure 6. Three-dimensional models for the homophilic interactions between human E-cadherin proteins, based on the crystal structure of domains EC1 and EC2 in complex with calcium. The models were generated by use of the Yasara program (http://www.yasara.org). (*A*) Ribbon model of two mouse E-cadherin EC1–EC2 domains (218 AA) in 'closed' intra-molecular cis-interaction (PDB ID 1FF5) [303]. (*B; C*) Model of two human E-cadherin EC1–EC2 domains (213 AA) in mutual inter-molecular trans-interaction (PDB ID 2o72) [110]. In (*C*), the model is zoomed out and one of the EC1–EC2 domains is depicted in surface mode to emphasize the interaction of each Trp2 residue with the docking pocket in the apposing molecule. In each model, atom format has been used for residues with special importance for inter-molecular adhesion. Key residues for homophilic adhesion: Trp2 in green, Lys25, Asn27 and Glu89 in purple, the tripeptide His79-Ala80-Val91 in yellow. Glu31 (in gray) at the tip of EC1 is essential for specific heterophilic binding by the $\alpha_E\beta_7$ integrin. Calcium ions are depicted as purple balls (three per EC domain intersection). See text for more details and references.

(1.9–2.1 Å) crystal structure analysis of the EC1 domain of mouse N-cadherin [106]. Another intriguing finding was the formation of a linear zipper-like ribbon structure by the combination of so-called adhesion dimers and strand dimers, both with twofold symmetry. In this type of structure, adhesion dimers bridge the juxtaposed cell surfaces, whereas strand dimers arrange the cadherin molecules in a parallel orientation in the same cell surface in a way thought to mirror the linear structure of the intracellular actin belt [106]. The adhesive interface in this structure includes the HAV sequence as well as many other residues, which differ among various cadherin members and may contribute to homophilic adhesion

specificity. The proposed limited interdigitation of these structures in a linear way would thus generate a 'cell adhesion zipper', as depicted in the top panel of Figure 5A. Several research groups went on to progressively refine these basic findings (reviewed in [107, 108]). Important advances were based on three approaches: determining the structure (at 2.0-Å resolution) of the two N-terminal ECs of E-cadherin in the presence of calcium [109, 110]; determining the structure (at 3.1-Å resolution) of the full-size, functional ectodomain of EP/C-cadherin, a type-I cadherin in *Xenopus laevis* [21]; and comparing the type-I cadherin structure with those of other cadherins, including type-II cadherins [110, 111]. At the risk of oversimplification, these studies are summarized in Figures 5 and 6.

Type-I cadherins are presently thought to project from the cell surface as curved structures over the full length of their ectodomain. The curvature is such that the long axis of EC1 is approximately perpendicular to the long axis of the MPED (EC5) (Fig. 3). Within the MPED, two intramolecular disulfide bonds are formed. N- and O-glycosylation sites are used in EC3 and EC4, but their role is not clear. Three calcium ions are bound per EC connection (12 in total per ectodomain), in this way stretching and rigidifying the ectodomain. A Trp residue at position 2 in the mature protein (W2, also designated as W156 in the non-processed protein) (Fig. 2) is conserved among all members of the type-I and type-II cadherins, and plays a key role in *cis* and *trans* dimerization. The N-terminal residues form an 'adhesion arm' that interacts with an 'acceptor pocket' in the body of EC1 (Figs. 5B and 6). This pocket features the fully conserved Glu residue at position 89 in mature human E-cadherin (E89). Whereas W2 interacts in a complex way with the acceptor pocket, E89 forms a salt bridge with the positively charged N terminus of Asp1 (D1). If this docking occurs intra-molecularly, the molecule is 'closed'. If it occurs inter-molecularly by so-called strand exchange dimerization involving juxtaposed molecules, it mediates homophilic cell-cell adhesion. The formation of strand-swapped dimer interfaces (Figs. 5B, 6B and 6C) is apparently favored over intra-molecular docking corresponding to a closed non-swapped form (Figs. 5B and 6A). The same strand dimer interface mediates both adhesive and lateral dimerization [21, 112]. In the originally proposed linear zipper model, W2 is involved in *cis* interactions (between molecules presented in parallel on the same cell surface), and a large HAV-containing dimer interface is involved in *trans* adhesion [106]. However, these phenomena are now widely considered a crystallization artifact not occurring on the cell surface (reviewed in [108, 113]). In the present model for W2-mediated interactions, *cis* complex formation should be based on interactions between the front β-sheet of EC1 and the back β-sheet of EC2 of a neighboring ectodomain. This front-to-back arrangement results in a continuous line of crescent-shaped molecules rather than discrete zippering dimers (Fig. 3). The strand exchange model was confirmed for E-cadherin by studies combining solution NMR and X-ray crystallography of mature and N-terminally extended forms of the EC1–EC2 fragment [114]. A shift from a homo-associated, non-swapped form to an antiparallel curved dimer required high concentrations of the E-cadherin fragments as well as cleavage of the prodomain. Biochemical studies demonstrated that persistence of the prodomain interferes with the above-mentioned interaction of the adhesion arm, most notably with the salt bridge between the N-terminal amino group of one cadherin molecule and the acidic side chain of E89 of a second cadherin molecule [100, 115, 116].

Largely unresolved in this model is the attribution of homophilic specificity to members of type-I cadherins. Evidence has been provided for a more promiscuous adhesion pattern among these type-I cadherins ([117, 118] and references therein), and the specificity of homophilic binding is surely just one of several aspects that should be considered. Nevertheless, force measurements in a so-called 'dual micropipette assay' clearly demonstrated that after both short (4 min) and long (30 min) interactions the adhesion strength between a cell expressing E-cadherin and one expressing N-cadherin was zero, whereas it was high (up to 200 nNewton) for homophilic interaction between E-cadherin-expressing cells and somewhat less (50 nNewton) for N-cadherin homophilic adhesion [119]. An appealing model attributing homophilic specificity to classical cadherins with quite similar adhesion interfaces is based on the synergism between multiple weak interactions instead of strong, pseudo-specific interactions (see below and [120]). Further, type-II cadherins show several distinct sequence differences from type-I relatives [29, 111], and this is reflected in the clearly different structures of the adhesive dimers formed between EC1 domains [111]. The nonpolar adhesive interface is much larger in type-II cadherins than in type-I cadherins; two Trp side chains (W2 and W4) in type-II cadherins instead of one are involved in homophilic binding between EC1 domains, and the greater similarity among various type-II EC1 structures may explain their higher propensity to heterophilic interactions [111].

Several recent studies using cryo-electron tomography have further contributed to our present knowledge of the structure of cadherin-mediated junctions ([121] and references therein). Highly structured

desmosomes rather than AJs were analyzed, but ectodomains of desmosomal cadherins share significant sequence homology with the E-cadherin ectodomain [29]. The three-dimensional molecular architecture of desmosomal cadherins in close-to-native conditions of vitreous sections from human epidermis was in good agreement with the above-mentioned curved shape of the C-cadherin ectodomain [21]. Under these conditions, the organization of the adhesive interface in the junction appeared to be remarkably straight and densely packed. This favors the model of intrinsic weakness of the interaction between individual cadherins, which makes sense in view of the biological needs during dynamic morphogenesis, being strengthened by cooperativity of numerous cadherin molecules in the junction. Interestingly, the proposed model consists of building blocks of alternating V-shaped *cis* dimers and W-shaped *trans* dimers (bottom panel of Fig. 5A). In this model, the W2 interaction with a hydrophobic pocket of a neighboring EC1 domain plays a role first in the formation of *cis* homodimers, followed later, when the molecular aggregate grows, in formation of adhesive *trans* homodimers. In some way, this model reconciles the original 'cell adhesion zipper' model [106] with the more recent crescent arrangement on the basis of the C-cadherin ectodomain [21].

Several research groups have contributed to the biochemical and biological validation of the structural models for E-cadherin. One approach was flow chamber analysis of individual E-cadherin fragments [122]. Dimerization of the purified proteins depended on W2, and the duration of the intermolecular bond was found to be ~2 s. The weakness of the dimerization interaction of classical cadherins is thought to be due to the fact that the same adhesion interface is formed by the swapped domain in dimers and the homo-associated 'closed' domain in monomers [120]. Thus, the latter 'closed' conformation acts as competitive inhibitor of dimer formation. Stable cell adhesion was therefore proposed to result from the concerted homophilic interaction of clusters of thousands of individual cadherin pairs on apposed cell surfaces [120, 123]. Moreover, this mechanism of cell-cell adhesion, which is based on multiple weak interactions, was calculated to enhance selectivity provided that the cadherin concentration is rather low (below 25 000 cadherin molecules per cell surface). Clearly, higher concentrations will substantially increase the number of adhesive homophilic dimers, but also the number of heterophilic adhesion events. In line with this low-affinity model is the live behavior of GFP-tagged E-cadherin molecules, which showed continuous remodeling of the junctions (*e.g.*, [124]).

It remains a matter of debate whether the above-mentioned experiments have yielded a conclusive model for E-cadherin-driven cell junctions. First, it is conceivable that both AJs containing E-cadherin and E-cadherin-mediated cell-cell contacts outside morphologically discrete junctions are composed of much more loosely packed cadherin organizations than those predicted for desmosomes. Second, there is a growing body of evidence in favor of an active role of inner EC domains in cell-cell adhesion. The apparent conflict between the various models for homophilic E-cadherin interactions has been discussed in some depth [107, 108]. The so-called interdigitation model of Leckband and colleagues is noteworthy (reviewed in [125]) (Fig. 4, cartoon at the top). This model is based on a number of careful biophysical measurements using sets of apposed artificial lipid bilayers, as well as single cells of which the membranes have been manipulated to express various mutated and chimeric variants of classic cadherins, mainly *Xenopus* C-cadherin. Analysis of force measurements provided evidence for the following. The N-terminal EC1–EC2 segment possesses both adhesive and selectivity functions, but the full ectodomain and more specifically EC3 are required for strong adhesion, corresponding to high binding probability and slow dissociation kinetics. The strongest adhesion between single cadherin molecules is brought about by the full ectodomain, which exhibits interdomain and intradomain cooperativity. Coaggregation experiments using on the one hand cells expressing E-cadherin mutants lacking EC1, and on the other hand cells expressing either mutant or wild-type E-cadherin, revealed not only that EC1 is necessary for strong adhesive interactions, but also that EC1 can interact to some extent with the truncated E-cadherin ectodomain lacking EC1 [104]. Further complementary experiments, including bead aggregation and cell adhesion assays, revealed that increasing the distance of the EC1–EC2 segment from the cell surface by hooking it up to fibronectin repeats was not enough to increase the binding affinity and therefore cannot explain the need for the full ectodomain [123]. In the latter experiments, EC4 could substitute for EC3 to enhance homophilic binding. In essence, classic cadherins should be able to bind in two stages: a first fast-forming but low-probability state on the basis of mutual EC1–EC2 interactions, and a second slow-forming, high-probability state requiring the inner part of the ectodomains [126]. The cytoplasmic domain is not required for this kinetic behavior but is, of course, essential for *in vivo* cell-cell adhesion.

Also in favor of a model implicating the inner EC domains is the intermembrane distance of about 25 nm measured at AJs [127]. Stretched cadherin

molecules with *trans*-interacting EC1 domains should extend over a distance of about 35–40 nm. Tilting the molecules at the level of the membrane passage could reduce this space [21], but an alternative explanation is an interaction of the inner EC domains, either with EC1 and each other in an interdigitation model (Fig. 4), or solely with each other in a model with a bent stretched *trans*-dimer configuration [67]. In the latter study, adhesive strengths for cadherins and nectins were measured with sub-picoNewton sensitivity by intermolecular force microscopy. As elaborated above, nectins are calcium-independent adhesion molecules specifically present in specialized junctions such as AJs and synapses (reviewed in [55, 62, 63]). In AJs of epithelial cells are found nectin-1, -3 and -4, each of which has three Ig domains in its ectodomain (Fig. 4). Just like cadherins, the nectins form *cis*-homodimers that can then interact between opposing cell surfaces to form *trans*-homodimers and *trans*-heterodimers. As different bound states were measured for paired nectins as well as paired cadherins, an interdigitation model was also proposed for nectins [67]. In view of the three-dimensional structure of the N-terminal Ig domain of the highly related nectin-like molecule 1 [128], the intercellular distance at nectin-nectin interaction sites is only about 20–30 nm for a stretched, non-interdigitated structure. AJ formation is thought to entail an early step involving nectin-nectin interactions, followed by recruitment of E-cadherin to these primary cell-cell adhesion sites. It is therefore conceivable, at least for AJs, that more extensive interactions involving the whole E-cadherin ectodomain in addition to initial EC1–EC1 interactions will be induced by the nectin activity (Fig. 4).

The structures of both the unphosphorylated and the phosphorylated mouse E-cadherin CD have been resolved in complex with the armadillo repeat region of β-catenin [129]. Without catenin binding, this CD appears to be intrinsically unstructured in solution [129]. Upon β-catenin binding, the last 100 AA of the 150-AA CD become ordered, and likely the first 50 AA become structured as well upon binding of p120ctn. As summarized below, specific phosphorylation events were found to enhance the structuring and increase the affinity for β-catenin up to 1000-fold [129, 130]. The CDs of many cadherins, including E-cadherin, contain PEST sequences, which are motifs associated with rapid protein turnover as they are recognized by ubiquitin ligases. The PEST motif in E-cadherin overlaps with the β-catenin-binding domain and is thought to be inaccessible in the complex with β-catenin [129].

Many researchers have studied the dynamics and stability of E-cadherin dimers. Considerable discrepancy arose between biophysical experiments with recombinant cadherin fragments and co-immunoprecipitation experiments on cultured cells (reviewed in [113]). In particular, it became debatable whether cell-cell adhesion really requires a cytoplasmic linkage with the cytoskeleton and whether cadherin dimerization is calcium dependent. Stable homodimeric cadherin complexes have been revealed in many experiments using cell adhesion assays on recombinant proteins, aggregation of specifically coated beads, and co-immunoprecipitation from living cells transfected with tagged, chimeric, or otherwise mutated cadherin molecules (*e.g.*, [116, 123, 131]). Such stable complexes contradict the low-affinity model for homophilic cadherin binding discussed above. Yap et al. [132] used the FKBP-FK1012 protein oligomerization system to drive cell-cell adhesion by EP/C-cadherin mutants lacking the CD, and found that the latter was indispensable for clustering of wild-type cadherins. This finding was later confirmed by Ozawa [116]. Ozawa also showed that lateral dimerization of the E-cadherin ectodomain is necessary but not sufficient for adhesive activity. Movement of wild-type and mutant E-cadherin on free cell surfaces was studied using single particle tracking and optical tweezers, and found to be regulated by a balance between strong tethering to actin microfilaments and a 'corralling' effect of the submembrane cytoskeletal network (reviewed in [133]). Corralled cadherin molecules may prompt the initial formation of small adhesion clusters at sites of cell-cell contacts, followed by formation of more coordinated, large-scale assemblies of cadherins into structural junctions. Formation of these assemblies requires cytoskeleton-driven cadherin oligomerization rather than dimerization [133].

The Troyanovsky group introduced site-specific cross-linking analysis and demonstrated in this way that E-cadherin strand dimerization indeed occurs in living cells, and that adhesive dimers are dominant over lateral dimers unless calcium is depleted [112]. The same interface is used in both dimer types. In more recent work, this group obtained intriguing data pointing to a mechanism for *in vivo* stabilization of E-cadherin ectodomain dimers [131]. Assays of *in vitro* bead aggregation showed that dimers formed under physiological conditions are weak unless they are 'activated' by destabilizing conditions (pH 5, cadmium ions, high temperature). On cell surfaces, however, only strong and stable dimers were found, and these dissociate only upon endocytosis. Even mutated molecules with deleted CDs and therefore lacking the cytoskeleton-mediated clustering effect, thought to strengthen weak intercadherin interactions, produce robust cell-cell adhesion *in vivo*. In a dual pipette assay with living cells, the link between E-cadherin and the actin cytoskeleton was dispensable

for initiation of cadherin-mediated adhesion, but contributed to further maturation and strengthening of the junction over time [119].

Heterophilic adhesion by E-cadherin

In addition to the homophilic adhesion mode of E-cadherin (identical molecules on the apposed surfaces of neighboring cells binding to each other), the ectodomain of E-cadherin binds in a heterophilic way with some specific molecules, such as the peculiar integrin $\alpha_E\beta_7$ and the internalin protein of the bacteria *Listeria monocytogenes*. Integrin $\alpha_E\beta_7$ is expressed by intraepithelial lymphocytes (IELs) and other mucosal leukocytes but rarely by peripheral blood lymphocytes. Initial findings showed that heterotypic adhesive interactions between epithelial cells and intraepithelial lymphocytes in culture are likely to be mediated by heterophilic binding between E-cadherin and the $\alpha_E\beta_7$ integrin [134]. These data were consolidated by binding studies on fusion or solubilized proteins [135]. The binding was much weaker for a P-cadherin ectodomain. Analysis of α_E(CD103)-deficient mice demonstrated the importance of this integrin chain for the diffuse distribution of T lymphocytes within epithelia [136]. Tumor-infiltrating lymphocytes (TILs) also often express the α_E(CD103)β_7 integrin, and interaction of this integrin with E-cadherin was demonstrated to be essential for cytolytic granule polarization and subsequent exocytosis at immunological synapses to kill epithelial tumor cells [137]. The binding site within the ectodomain of E-cadherin for the $\alpha_E\beta_7$ integrin has been carefully characterized [138] and found to reside in the BC loop at the tip of the EC1 structure. In particular, residue Glu31 (E31) was essential for specific binding (Figs 2 and 6). Interestingly, mutation of the nearby residues Lys25 (K25), Asn27 (N27) and Glu89 (E89) abrogated homophilic adhesion but not heterophilic integrin binding (Figs 2 and 6). This creates opportunities to design therapeutics for disrupting one of these two binding modes. Another target domain is the MPED (or EC5) of E-cadherin. Intramolecular deletion of this domain did not interfere with homophilic adhesion, in contrast to deletion of EC1, -2, -3 or -4, whereas heterophilic adhesion with $\alpha_E\beta_7$ was strongly affected by MPED loss [139].

A second interesting example of heterophilic binding to E-cadherin is its interaction with the surface protein internalin (also called InlA) of the food-borne pathogen *L. monocytogenes*, fully explored by Pascale Cossart's group. This Gram-positive bacterium can cross the intestinal, placental and blood-brain barriers in humans, causing listeriosis with an overall mortality rate of 30% [140]. Another *L. monocytogenes* protein, InlB, binds to several cellular receptors, including the receptor tyrosine kinase c-Met. Latex beads coated with either InlA or InlB enter cell types that are sensitive to *Listeria* entry. E-cadherin was identified by affinity chromatography as the internalin receptor [141]. Since then, both the interaction domain in E-cadherin and the molecular mechanisms for exploiting E-cadherin to mediate bacterial invasion have been elucidated. Mice cannot be infected efficiently by oral inoculation with *L. monocytogenes* because the bacterium cannot translocate across the intestinal barrier. This is due to the inability of mouse E-cadherin to allow InlA-dependent entry of *Listeria* [142]. This species specificity was traced to a difference of a single amino acid, residue 16, in the EC1 domain of E-cadherin: P16 in human and E16 in mouse (Fig. 2). Of two 30-mer peptides, corresponding to the N termini of human and mouse E-cadherin, only the human peptide comprising P16 could inhibit invasion of human intestinal and liver cells by *L. monocytogenes* [143]. Analysis of transgenic mice expressing human E-cadherin in enterocytes proved that *L. monocytogenes* requires the InlA-E-cadherin interaction to cross the intestinal barrier [144]. More recently, resolution of the crystal structure of InlA in complex with the EC1 of human E-cadherin made it possible to confirm the role played by P16 in the specificity of human E-cadherin binding [145]. Moreover, molecular modeling based on this structural information led to identification of two AA in the invasion protein InlA whose substitution increased binding affinity for E-cadherin 10 000-fold and extended binding specificity to include natural mouse E-cadherin [145].

The initial adhesion of the bacterium recruits E-cadherin to this contact site. This is followed by a complex phenomenon resulting in local rearrangements in the actin cytoskeleton, generating membrane extensions and leading to engulfment of the bacterium. The ectodomain of E-cadherin is sufficient for the initial adhesion [146]. However, the catenin-mediated link to the actin cytoskeleton was found to be essential for bacterial uptake: deletion of the β-catenin-binding domain of E-cadherin abrogates uptake, whereas a fusion protein consisting of the E-cadherin ectodomain and the C-terminal half of α-catenin can fully facilitate bacterial entry [146]. Regulatory proteins that have been demonstrated to play a role in local actin reorganization upon *Listeria* adhesion are activated Src kinase, cortactin, Rac1 and Arp2/3 [147]. These findings indicate that the mechanism of E-cadherin-controlled actin polymerization resembles that operating in AJ assembly, where activated cortactin recruits the Arp2/3 complex to

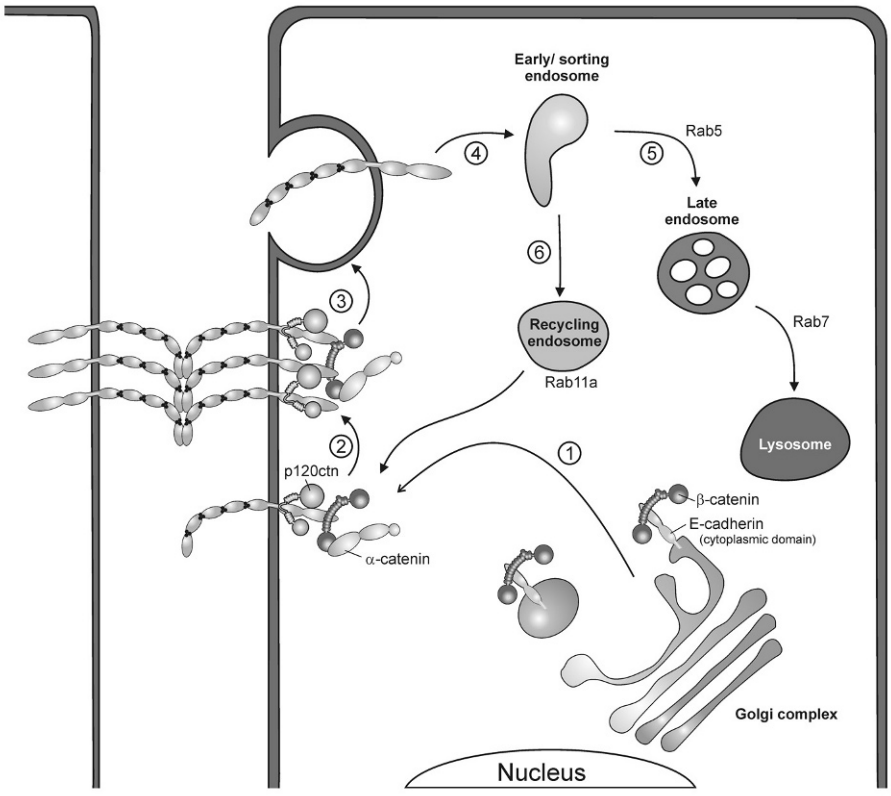

Figure 7. Synthesis, endocytosis and recycling of E-cadherin. β-Catenin associates with E-cadherin early in the biosynthetic pathway. These cadherin-catenin complexes bud from the Golgi complex (1) and are transported towards the basolateral cell surface, where they associate with p120ctn and α-catenin. At the plasma membrane assembly into functional E-cadherin-catenin adhesive complexes occurs (2). Upon endocytosis (3), E-cadherin is transported to the early sorting endosome (4), from where it will be recycled back *via* the recycling endosome (6), which is enriched in the Rab11a GTPase, or else directed towards the late endosome (5), which entails passage through compartments enriched in Rab5 and Rab7 for final degradation in the lysosome.

newly formed E-cadherin contacts [148]. This process is controlled by Rac1, which is activated by homophilic ligation of E-cadherin [148, 149]. A model has been proposed to explain the difference between bacterial contacts inducing E-cadherin-mediated phagocytosis and cellular contacts inducing cell-cell adherence: both the rounded form of the bacterium and the absence of counteracting forces from a subcortical cytoskeleton in the bacterium may be at the root of this difference from the contacts between adjacent epithelial cells [140, 146].

Other proteins shown to be required for InlA-dependent bacterial entry are the unconventional myosin VIIa, and vezatin, two actin-binding proteins associated with the cytoskeleton and localized at AJs, as well as ARHGAP10, a Rho/Cdc42-GAP protein isolated in a yeast two-hybrid screen as an interaction partner of α-catenin [150]. Both knockdown and overexpression of ARHGAP10 inhibit bacterial uptake, and a model has been proposed on the basis of transmembrane vezatin being linked to the myosin VIIa motor on the one hand, and on the other hand to α-catenin *via* Arf6 and ARHGAP10 [150].

Listeria is not alone in using E-cadherin as an entry receptor to invade the host. The receptor in human nasopharyngeal epithelial cells for pneumococcal surface adhesion A (PsaA) of *Streptococcus pneumoniae* also turned out to be E-cadherin [151]. Likewise, the adhesin Als3 of the fungus *Candida albicans* binds to E-cadherin on oral epithelial cells and to N-cadherin of endothelial cells [152]. The Als3 N-terminal domain appears to mimic the EC1–EC2 domain of classic cadherins, and so pseudo-homophilic binding may be the basis for specific cadherin recognition by *Candida.* This binding induces endocytosis of the fungus, and at the same time E-cadherin is progressively degraded by fungal and host proteases [153].

Assembly of E-cadherin-based cell-cell contacts: Post-translational E-cadherin processing and maturation

Assembly of E-cadherin-based AJs is the most prominent way of bringing about specific cell-cell adhesion in epithelial cells, although non-junctional cadherin complexes may serve the basic needs for such adhesion (reviewed in [154]). Anyhow, the morphological and molecular assembly of the AJ has been scrutinized by the groups of Nelson, Fuchs, Yap, Stow and others. GFP-fused E-cadherin and advanced digital microscopy have been instrumental in the significant progress made in this field. Very briefly, at initial stages of calcium-induced intercellular adhesion, the E-cadherin/catenin complex organizes focal-

ly as nascent contacts or 'puncta' at the end of filopodial extensions (reviewed in [43]). In a second 'maturation' step, the actin cytoskeleton is reorganized and a zippered joining of the puncta occurs. Signaling and structural molecules that are involved include cytoskeleton-binding proteins such as α-catenin, vinculin, α-actinin, spectrin, ZO-1 and myosin-VI, actin nucleation proteins such as Arp2/3, formin 1 and Dia1, nucleation-promoting factors such as Ena/Vasp, Rac-WAVE and cortactin, components of the exocyst and of the 'lateral targeting patch', phosphatidylinositol lipids and the corresponding enzymes (reviewed in [43–45]) [72, 155–159]. The extensive actin dynamics during epithelial sheet formation appears to be under tight control of small GTPases and their numerous regulators (reviewed in [43, 160–163]). Below we discuss a few selected findings directly related to the E-cadherin protein.

Correct proteolytic cleavage of the prodomain of E-cadherin is required for adhesiveness [100]. Mutation of the preprotein cleavage site has no direct impact on E-cadherin integration into the cell membrane but such mutated E-cadherin molecules fail to confer adhesion. Two findings indicate that furin, a subtilisin-like convertase, might control cell polarization and the assembly of apical junctions, at least by promoting E-cadherin maturation [164]. First, there is a dibasic, furin-like recognition motif of four AA residues (Arg-Gln-Lys-Arg) preceding the defined proprotein cleavage site of E-cadherin; second, treatment of intestinal epithelial cells with the furin inhibitor decanoyl-RVKR-CMK results in a less polarized and differentiated phenotype. This suggests that other convertases with sequence specificity resembling that of furin also process pro-E-cadherin.

Upon formation of cell-cell contacts, presenilin-1 (PS1) is recruited to sites of cell-cell adhesion, where it forms complexes with E-cadherin and β-catenin at the cell surface and stabilizes the cadherin-based adhesion complex [165]. The membrane-proximal cytoplasmic sequence 604–615 of human mature E-cadherin was shown to directly bind PS1 (Fig. 2). This sequence is also required for the binding of p120ctn (see above). Under conditions stimulating cell-cell dissociation (apoptosis, Ca^{2+} imbalance), E-cadherin is cleaved by a PS1/γ-secretase activity at the membrane-cytoplasm interface (Leu577–Arg578), promoting disassembly of AJs and generating a soluble cytosolic E-cadherin fragment (CTF2) that is complexed with β-catenin and p120ctn [166]. The functional implications of these phenomena are not clear.

Physiopathological cleavage of E-cadherin is observed under various conditions. In view of the numerous proteases activated in the tumor microenvironment, it is not surprising that ectodomain shedding of several cadherins is regularly observed in tumors. The possible functional consequences of secreted cadherin fragments in cancer have been recently reviewed by De Wever et al. [167]. Also infectious agents can target E-cadherin. *Bacteroides fragilis* enterotoxin is a metalloprotease cleaving the ectodomain of E-cadherin and in that way changing the morphology and physiology of polarized epithelial cells [168]. *Porphyromonas gingivalis*, one of the causes of periodontitis, can invade epithelial cell layers and cleave several adhesion molecules, including the ectodomain of E-cadherin [169]. Transmigration of *C. albicans* through damaged intestinal epithelium is promoted by double cleavage of E-cadherin, both in the ectodomain by an unknown protease, and in the resulting 35-kDa intracellular fragment by a γ-secretase [153].

Transport of newly synthesized E-cadherin from the Golgi to recycling endosomes and eventually to the cell surface occurs *via* tubulovesicular carriers; the Golgi protein golgin-97, but not golgin-245, is essential for transporting E-cadherin out of the trans-Golgi network [170]. E-cadherin associates with β-catenin early in the biosynthetic pathway, and the two proteins show intracellular traffic to the plasma membrane as a complex [171] (Fig. 7). β-Catenin binds *via* its armadillo repeats directly to a C-terminal core region of 30 AA containing a cluster of eight Ser residues. Phosphorylation of three serine residues (Ser684, Ser686 and Ser692) by the protein kinases CK2 and GSK3β (Fig. 2) strongly increases the affinity of E-cadherin for β-catenin [129, 172]. α-Catenin and p120ctn seem to bind to the complex only after the cadherin proregion is removed and when the complex is close to the basolateral cell surface [171, 173]. In addition to other putative sorting signals, a highly conserved dileucine motif in the juxtamembrane region of E-cadherin (Leu587-Leu588; Fig. 2) was identified as being important for basolateral targeting of E-cadherin [174]. Indeed, inactivation of this motif resulted in missorting of the E-cadherin-β-catenin complex and consequent loss of cell polarity. The importance of this dileucine motif for basolateral targeting has been challenged by more recent reports, and a role for accumulation of adhesion-defective E-cadherin mutants in post-Golgi compartments and subsequent lysosomal targeting has been proposed instead [175, 176].

Recently, ankyrin-G was shown to bind to a sequence in the MPCD (JMD) of E-cadherin, overlapping both this Leu587–Leu588 sequence and the core of the p120ctn-binding domain [176]. At the same time, ankyrin-G can bind to β2-spectrin, and this ternary complex is required for the exit of E-cadherin from the trans-Golgi network in a microtubule-dependent

pathway, both in cultured epithelial cells and in preimplantation mouse embryos. Moreover, at sites of cell-cell contacts, an E-cadherin/ankyrin-G/spectrin/F-actin multimeric complex was detected and proposed to recruit other membrane-spanning proteins, such as the Na^+/K^+-ATPase ([176] and references therein). Binding of either p120ctn or ankyrin-G to the MPCD/JMD might also shield the PEST sequence in this domain from recognition by the endocytosis machinery.

At least one pool of cell surface E-cadherin is subjected, probably by a clathrin-mediated mechanism, to endocytosis and recycling to the cell surface *via* a post-Golgi endosomal pathway [177] (Fig. 7). This trafficking of E-cadherin seems to be regulated by cell-cell contacts, because the recycling pool is enlarged in the absence of cell-cell contacts. This regulated recycling pathway may provide a mechanism for the dynamic modulation of E-cadherin expression at the cell surface, cell-cell adhesion and morphogenesis [178]. The p120ctn protein is a well-known inhibitor of E-cadherin endocytosis and acts by blocking clathrin-mediated endocytosis of E-cadherin [39]. *O*-glycosylation of the E-cadherin CD has also been described as a mechanism for regulating E-cadherin cell surface transport and is used to rapidly down-regulate adhesion in some apoptotic pathways [179]. The reversible nature of *O*-GlcNAc addition permits dynamic regulation by distinct glycosyltransferases and glycosidases. Caspase-mediated inhibition of deglycosylation leads to accumulation of *O*-glycosylated E-cadherin that cannot be transported to the cell surface. *O*-glycosylated E-cadherin still binds to β-catenin and plakoglobin but not to p120ctn [179]. Once internalized, E-cadherin needs to be recycled back to the basolateral membrane of polarized epithelial cells or otherwise targeted for lysosomal degradation. Disassembly of AJs by treatment of cells with HGF or by v-Src activation was found to be dependent on activation of the small GTPase ARF6. This activation facilitates the recruitment of the nucleoside diphosphate kinase Nm23-H1 to the basolateral surface, thereby providing a source of GTP for dynamin-dependent fission of coated vesicles during endocytosis, and in this way promoting the disassembly of cell-cell contacts [180]. Furthermore, lysosomal targeting of E-cadherin seems to depend on the endosomal sorting activity of HGF-regulated tyrosine kinase substrate (Hrs), and on Src activation of the GTPases Rab5 and Rab7 [181] (Fig. 7). Indeed, expression of mutants of these three proteins introduces stage-specific blocks in E-cadherin trafficking to the lysosomes. Similarly, a c-Cbl-like E3 ubiquitin ligase, Hakai, induces mono-ubiquitination of E-cadherin in response to Src-mediated tyrosine phosphorylation of E-cadherin; this too results in lysosomal degradation of E-cadherin [41]. Interestingly, Hakai-mediated E-cadherin ubiquitination is also involved in endocytosis induced by Ca^{2+} depletion, with Cdc42 playing a critical role by activating EGFR and Src signaling [182]. On the other hand, E-cadherin is transported to the cell surface by recycling endosomes enriched in the small GTPase Rab11a (Fig. 7), and expression of mutant Rab11a caused apical mislocalization of E-cadherin [183]. The Rab11a-enriched endosomes and the exocyst complex are together involved in E-cadherin recycling.

Signaling activities of E-cadherin

It is logical that E-cadherin signals indirectly by sequestering the armadillo proteins p120ctn, β-catenin and plakoglobin at the cell surface, whereas the presence of these catenins in the cytoplasm or the nucleus influences cytoskeletal rearrangements and transcriptional activities. There is indeed ample evidence that E-cadherin suppresses signaling by this sequestration effect, although E-cadherin-mediated cell-cell adhesion turned out to be dispensable for this anti-signaling activity: E-cadherin mutants lacking the extracellular domain can keep β-catenin associated with the membrane and can suppress cell proliferation [184, 185]. The reciprocal mutation, removing the β-catenin binding site, did not reduce cell proliferation rate. Thus, inhibition of nuclear β-catenin signaling by E-cadherin may be based on a mechanism that is more complex than simple physical restraint. It was recently shown that E-cadherin facilitates formation of a complex between calveolin-1 and β-catenin at the cell surface, thereby precluding β-catenin/TCF-dependent transcription of the survivin gene [186]. The latter gene encodes an inhibitor of apoptosis and so its expression contributes to tumor formation and metastasis. Apparently, the caveolin-1-E-cadherin dependency is reciprocal, and both proteins are indeed known as tumor suppressors.

As elaborated below, protease-mediated cleavage of either the ectodomain or the cytoplasmic domain of E-cadherin (Fig. 2) appears to abolish the inhibitory effect of E-cadherin on β-catenin signaling (*e.g.*, [187]). Overexpression of p120ctn in the cytoplasm of fibroblasts lacking E-cadherin modulates small GTPases and results in a striking 'branching' phenotype, characterized by extreme arborization of cellular processes, as well as translocation of ezrin to the cytoplasm [188]. This p120ctn-induced signaling was counteracted by expression of wild-type E-cadherin but not by a p120ctn-uncoupled E-cadherin mutant.

In addition to its ability to inhibit signaling by sequestering catenins, E-cadherin also inhibits the ligand activation of the receptor tyrosine kinases (RTKs): EGF receptor (EGFR), Neu/ErbB2, the insulin-like growth factor receptor IGF-1R, and the HGF receptor c-Met [189]. In contrast, two tested G protein-coupled receptors were not regulated by E-cadherin. Indeed, several reports indicate that E-cadherin and receptor tyrosine kinases interact physically and colocalize at the lateral membranes of epithelial cells [190–197]. As for EGFR, formation of a complex with E-cadherin depended on the extracellular domain of E-cadherin [189, 198], but was independent of p120ctn and β-catenin binding [189]. This complex inhibited the binding of the EGF ligand at high cell density, but at low cell density or when E-cadherin was blocked with antibodies, ligand binding and EGFR signaling were readily detected [189, 193]. Inhibition of RTKs by E-cadherin seems to contradict the notion that activation of RTKs inhibits E-cadherin-dependent cell adhesion and induces EMT [199]. This discrepancy may be explained by the cell density effect, with high cell densities promoting the ligation of E-cadherin and therefore its inhibitory effect. The influence of E-cadherin homophilic ligation on EGFR signaling was studied by the Gumbiner group in an experimental system comprising recombinant E-cadherin protein attached to microspheres in confrontation with isolated single epithelial cells [200]. Under these conditions, where no contacts with other cells are involved, E-cadherin ligation was found to inhibit EGFR-mediated growth signaling. Generation of the growth-inhibitory signals required binding of β-catenin to E-cadherin, but it did not require E-cadherin binding to p120ctn, β-catenin binding to α-catenin, or the transcriptional activity of nuclear β-catenin/T cell factor [200]. Interestingly, when the E-cadherin germline missense mutations T340A and A634V within the ectodomain were expressed in CHO cells, the stability of the EGFR-E-cadherin complex was reduced, EGFR signaling was strengthened, and RhoA-GTP-dependent cell migration was increased [201]. On the other hand, expression of the missense mutations P799R and V832M of the CD did not affect EGFR inhibition as compared to wild type. This same study showed that EGFR interacted intracellularly with cytoplasmic β-catenin independently of E-cadherin, which explains some earlier observations of such interaction [190]. β-Catenin is a good substrate for the tyrosine kinase activity of the EGFR and interacts with the EGFR *via* its armadillo domain, which is also used to associate with E-cadherin. It is therefore unlikely that β-catenin can bind simultaneously to E-cadherin and to EGFR, and thereby may be able to connect these two transmembrane proteins.

Several other findings point at alternative, more complex interactions between E-cadherin and RTKs. These interactions might be related, at least in part, to a low cell density during reestablishment of junctions in a calcium switch experiment, or during active migration. In these situations, RTKs may negatively affect E-cadherin functionality, or otherwise E-cadherin may positively affect RTK activity (reviewed in [195, 202]). The first effect can be understood on the basis of tyrosine phosphorylation of E-cadherin and catenins by RTKs, the second effect on the basis of co-recruitment of RTKs by E-cadherin to the cell surface, and also on co-endocytosis (see below). For instance, IGF-1R, the receptor for insulin-like growth factor 2 (IGF-II) was shown in various cells to localize at cell-cell contacts and to form a supramolecular complex by interaction with the CD of E-cadherin [191]. Addition of IGF-II induced internalization of the cadherin-catenin complex, degradation of E-cadherin, and nuclear translocation of β-catenin, thus leading to EMT. The complexity of these interactions is demonstrated by the finding that forced expression of high levels of IGF-1R in MCF-7 cells promotes rather than inactivates E-cadherin-mediated cell-cell adhesion [203]. Cross-linking studies suggested a ligand-independent interaction between the ectodomains of E-cadherin and the HGF receptor c-Met [192]. In this case, E-cadherin recruited intracellular c-Met to the cell surface, but c-Met was primed to ligand reactivity rather than inactivated. In MDCK cells, HGF binding triggered co-endocytosis of E-cadherin and c-Met, whereas in calcium switch conditions both proteins were exocytosed [194]. The mammary epithelial cell lines MCF-7 and MCF-10 were found to behave differently with respect to HGF-triggered c-Met association with E-cadherin and to the downstream signaling events [197]. Binding of FGF to epithelial MCF-7 cells triggers co-endocytosis of the FGFR1 receptor and E-cadherin into early endosomes, followed by nuclear translocation of FGFR1 [196]. Overexpression of E-cadherin blocks these phenomena, and so does stabilization of surface-exposed E-cadherin by overexpression of p120ctn. The assembly of E-cadherin junctions in normal keratinocytes undergoing a calcium switch induces rapid recruitment of EGFR, followed by a remarkable ligand-independent activation of this receptor, and activation of the MAPK pathway and the small GTPase Rac1 [204, 205]. E-cadherin engagement induced transient activation of EGFR also in MCF-10A mammary epithelial cells [198], whereas homophilic E-cadherin ligation in a CHO-derived experimental system was also able to recruit Rac to nascent adhesive contacts, which specifically stimulates Rac signaling [149]. More recently, EGF treatment of MCF-7 cells was

shown to lead to internalization of the E-cadherin-catenin complex by Rac1-modulated macropinocytosis into endosomal compartments [206]. In this process, the sorting nexin SNX1 turned out to be required for efficient recycling of internalized E-cadherin to the cell surface, in that way rescuing it from a degradative endosomal pathway. On the other hand, the combination of TGF-β and activated Raf-1 induces EMT in mouse mammary epithelial cells by enhancing lysosomal degradation of E-cadherin in combination with increased endocytosis and ubiquitination [207]. EMT induced by v-Src activation in MDCK cells or by calcium depletion in MCF-7 cells provoked similar phenomena: E-cadherin underwent tyrosine phosphorylation by c-Src, followed by Hakai-mediated ubiquitination (see above), endocytosis and degradation in the lysosomes [181, 182]. It has been demonstrated in MCF-7 cells that the small GTPase Cdc42 plays an intriguing role in the calcium deprivation phenotype [182]. Under these conditions Cdc42 is rapidly activated, and then it activates EGFR, which in turn activates c-Src, which phosphorylates E-cadherin, allowing the formation of a ternary complex between the tyrosine-phosphorylated E-cadherin CD, activated Cdc42 and Hakai. This binding of activated Cdc42 to E-cadherin was found to be critical for dissolution of the AJ [182].

This brings us to the complex interactions between E-cadherin and c-Src. The Yap group provided evidence for a delicate functional interplay between cadherin adhesion and c-Src signaling in MDCK cells [208]. Ligation of E-cadherin stimulated the c-Src signaling pathway in a biphasic way: lower signal strengths were supportive of E-cadherin-based cell-cell contacts, whereas higher signal strengths inhibited E-cadherin functionality. Phosphatidylinositol-3-kinase (PI3-kinase) signaling was implicated in the positive effect of E-cadherin-activated c-Src signaling on E-cadherin function. This is congruent with the finding that PI3-kinase, upon tyrosine phosphorylation by c-Src, is recruited to E-cadherin-containing junctions [209, 210]. There, PI3-kinase is activated, leading to Akt kinase stimulation and aggregation-dependent cell survival [149, 209]. Recruitment of PI3-kinase to the E-cadherin/catenin complex is observed also in epidermal keratinocytes undergoing calcium-induced differentiation [211]. In addition to the need for E-cadherin, both p120ctn and β-catenin were needed to achieve this recruitment and the resultant phospholipase-C-γ1 activation, intracellular calcium release and keratinocyte differentiation. Recently, another lipid kinase, type Iγ PI phosphate kinase (PIPKIγ), was found to directly bind to the CD of E-cadherin [212]. PIPKIγ binding occurred preferentially with dimerized E-cadherin and in a region that corresponded to the N-terminal half of the β-catenin-binding domain (Fig. 2). PIPKIγ associated also with the μ subunits of clathrin adaptor proteins (AP), and this dual interaction was thought to mediate formation of the scaffold between AP complexes and E-cadherin, and in this way to facilitate intracellular E-cadherin trafficking [212]. At the same time, the enzymatic activity of PIPKIγ generates $PI4,5P_2$ locally at sites where E-cadherin is present. $PI4,5P_2$ serves as a substrate for signaling phospholipases and for phospholipid kinases. The E-cadherin germline mutant V832M, which cannot mediate cell-cell adhesion or suppress invasion [213], shows reduced PIPKIγ binding but normal β-catenin binding [212].

Other physical interactions with E-cadherin worth mentioning include the interactions with phosphotyrosine phosphatases [214, 215] and with TGF-β receptor II (TβRII) [216]. The phosphatase PTPμ was observed to interact with several cadherins, including the 38-AA C terminus of E-cadherin [214] (Fig. 2). Conditions that result in tyrosine phosphorylation of E-cadherin are associated with dissociation of PTPμ. Expression of functional PTPμ is essential for functions of E-cadherin, such as neurite outgrowth of chicken retinal ganglion cells, possibly because PTPμ recruits signaling proteins such as RACK1 and PKCδ to the cadherin-catenin complex [217]. In addition, the phosphatase PTP1B has been found to directly interact with the E-cadherin CD [215]. Again, this binding was significantly reduced by tyrosine phosphorylation of E-cadherin. This release of PTP1B from the cadherin-catenin complex results in increased tyrosine phosphorylation of β-catenin, which weakens its interaction with E-cadherin and consequently leads to loss of homophilic E-cadherin-mediated cell-cell adhesion. A totally different situation arises when the E-cadherin ectodomain binds to the ectodomain of TβRII [216]. This interaction in epithelial cells also recruits TβRI. In esophageal squamous cell carcinomas, E-cadherin and TβRII are often coordinately lost but their expression is maintained in adjacent normal tissue. In a physiological microenvironment, the growth-inhibiting effect of TGF-β1 appeared to be dependent on full E-cadherin functionality [216].

Role of E-cadherin in development and tissue morphogenesis

The role of E-cadherin in mammalian development has been assessed by various means: (1) detailed expression analysis in embryos and tissues at various stages; (2) overexpression *versus* inactivation experiments in models of tissue and organ development

(inactivation can be achieved by blocking antibodies, blocking peptides, overexpression of dominant negative mutants, or knockdown experiments); (3) total or tissue-specific transgenesis in the mouse, leading to ectopic overexpression, gene knockout or gene knockin; (4) studies of the role of E-cadherin-related molecules in model organisms like the fruit fly, the worm, the sea urchin, zebrafish and *Xenopus*. Unfortunately, the latter investigations fall beyond the scope of this review.

E-cadherin is expressed in all mammalian epithelia. As the key molecule of the cadherin-catenin-cytoskeleton complex, it is important for establishing and maintaining apicobasal polarity, preserving epithelial cell survival, and controlling proliferation (reviewed in [3]). The so-called cadherin switching occurs during embryonic development, tissue morphogenesis and cancer progression (reviewed in [2, 218]). Cell sorting based on the preference of classic cadherins for homophilic binding could be an important aspect of the morphogenetic role of cadherins, but it is certainly not the only one. The relative expression levels of cadherins and the size of the shearing forces on the cells have been shown to be extremely important for cell sorting phenomena [219]. All this has led to the differential adhesion hypothesis as a biophysical basis for morphogenetic phenomena [118, 220].

E-cadherin-blocking antibodies and expression of dominant-negative E-cadherin mutants induced anoikis in Ewing sarcoma cells grown under anchorage-independent conditions but it did not affect survival of adherent cultures [221]. This anoikis sensitivity correlated with decreased activation of the ErbB4 RTK, which led to reduced Akt kinase activation. Efficient knockdown of E-cadherin was recently achieved in MDCK cells [222]. Surprisingly, reduction of E-cadherin levels in confluent cell monolayers had little effect on the localization or function of AJs or TJs, in contrast to αE-catenin silencing. However, the effects of E-cadherin silencing on establishment of cell polarity became evident during reassembly of junctions in a calcium switch experiment. To our knowledge, *in vivo* silencing of E-cadherin has not been reported.

Embryos of the total E-cadherin (*Cdh1*$^{-/-}$) knockout mouse die at about the time of implantation (embryonic day 4 or E4) and have defects in cell junctional and cytoskeletal organization, resulting in failure to form trophectoderm, the first polarized epithelial layer in the mouse embryo [22]. Initially, compaction of the blastula proceeded normally due to the presence of residual maternal E-cadherin but compaction was not maintained. The contribution of maternal E-cadherin was shown by combining a conditional, 'floxed' *Cdh1* allele with an oocyte-specific *Zp3-Cre* transgene [223]: blastomeres did not adhere to each other but were kept near each other by the surrounding zona pellucida. Delayed compaction at the morula stage occurred upon *de novo* expression from the paternal *Cdh1* allele. The tissue-specific role of E-cadherin was demonstrated by transfection of either E-cadherin or N-cadherin cDNA in the *Cdh1*$^{-/-}$ ES cells [224]. Teratomas that formed upon subcutaneous injection of *Cdh1*$^{-/-}$ ES cells did not form organized epithelial tissues unless E-cadherin was reintroduced. In contrast, forced N-cadherin expression in these mutant ES cells induced formation of neuroepithelium and cartilage but not epithelia. The Kemler group [225] scrutinized these findings by an *in vivo* gene replacement approach: N-cadherin cDNA was introduced by non-random insertion in the *Cdh1* genomic locus. Homozygous N-cadherin knockin mutant embryos phenocopied *Cdh1*$^{-/-}$ embryos by failing to form an intact trophectoderm, although they could achieve morula compaction even upon removal of maternal E-cadherin. Moreover, upon teratoma formation, these N-cadherin knockin ES cells generated different epithelium-like structures.

Further, the floxed *Cdh1* allele was combined with several other tissue-specific *Cre* transgenes. Conditional knockout of E-cadherin in postnatal epidermis and hair follicles by use of a *Cre* gene under the control of the *Krox20 cis*-regulatory elements induces loss of AJs, altered epidermal differentiation and progressive loss of hair follicles [226]. Moreover, β-catenin was not up-regulated and skin tumors did not appear. Similar observations were made upon prenatal ablation of *Cdh1* by use of keratin-14-Cre (K14-*Cre*) [227]. Intercellular adhesion between keratinocytes was maintained because P-cadherin was up-regulated. However, terminal differentiation was impaired, hair follicles lost their integrity, and progressive hyperplasia developed. Somewhat surprisingly, a much more severe phenotypic abnormality was described later for what appears to be an identical K14-*Cre* x floxed *Cdh1* mouse: the *in vivo* epidermal barrier function was lost due to defects in TJ formation, and mice died perinatally [59]. The difference in phenotype might be explained by earlier activation of Cre in the latter study. MMTV-Cre was used to knock out *Cdh1* in the differentiating alveolar secretory cells of the mammary gland [228]. The absence of E-cadherin did not influence gland development up to day 18 of pregnancy, but at the time of parturition terminal differentiation into lactating cells was severely impaired. Extensive cell death occurred, as it normally should during involution. The absence of tumor formation indicates that the frequent loss of E-cadherin in epithelial cancers, including breast carci-

nomas (see below), must be accompanied by oncogenic anti-death mechanisms. This was elegantly demonstrated by Jonkers' group [229], who combined a floxed *Cdh1* gene with the K14-*Cre* gene, which is expressed at low and stochastic levels in the mammary epithelium. No abnormalities were seen in these mice, but combined loss of E-cadherin and p53 resulted in accelerated development of malignant mammary carcinomas resembling human infiltrative lobular carcinomas. Compared to tumors formed upon loss of p53 alone, metastatic spreading, anoikis resistance and higher vascularization were induced by the combinatorial ablation of the two tumor suppressors. As E-cadherin was originally identified as L-CAM in chicken liver (see above), it is surprising that it is not required for establishment of the hepatic epithelium and formation of the various cell junctions in the liver [230]. One explanation for this is the strong expression of N-cadherin in the liver [231]. Conditional inactivation of *Cdh1* at E15 in mouse thyroid follicular cells by use of Cre under the control of the thyroglobulin promoter did not markedly affect cell-cell junction formation, which may not be surprising in view of the continued expression of cadherin-4 (R-cadherin) and cadherin-16 (Ksp-cadherin) in thyrocytes [232]. Nonetheless, morphological abnormalities were observed in thyroid follicles of glands in which E-cadherin was ablated.

Dysregulation of the E-cadherin gene in cancer

It has been well documented that epithelial tumors lose E-cadherin partially or completely as they progress towards malignancy [233]. Seminal studies have demonstrated both strong anti-invasive and anti-metastatic roles for E-cadherin [18, 19, 234]. Different mechanisms for E-cadherin inactivation in malignant tumors include mutation, epigenetic silencing, endocytosis, and increased expression of non-epithelial cadherins, as outlined below and summarized in Figure 1.

Loss of heterozygosity and inactivating mutations in cancer

Important hints of a role for E-cadherin in human cancer development came from studies on loss of heterozygosity (LOH) of chromosome 16q21–22. A few years after the human E-cadherin gene was mapped to chromosome 16q22.1 [31], a series of published studies demonstrated frequent LOH of 16q in gastric, prostate, hepatocellular and esophageal carcinomas (reviewed in [233]). LOH at 16q is a very frequent somatic genetic event particularly in breast cancer, occurring in about 50% of all ductal carcinomas [235], and even more frequently in lobular breast cancer [236].

E-cadherin-inactivating mutations were first described in diffuse gastric cancer [237]. In sporadic diffuse gastric cancer, somatic mutations preferentially cause skipping of exons 7 and 9, which corresponds to in-frame deletions. Several truncation mutations have also been reported for this histological subtype of tumors [23, 238]. Promoter hypermethylation, rather than LOH, accounts for biallelic *CDH1* silencing [239]. By contrast, no mutation hotspots have been identified in sporadic lobular breast carcinomas because E-cadherin inactivating mutations in this cancer type are scattered along the gene [236]. Most mutations found in infiltrating lobular breast cancers are out-of-frame mutations predicted to yield secreted truncated E-cadherin fragments or no stable protein at all. E-cadherin expression is silenced completely in these breast carcinomas because the mutations are accompanied by *CDH1* promoter methylation or by LOH [236, 238]. Missense mutations are infrequent in both subtypes of cancer, but they were found to be frequent in monophasic synovial sarcomas [240]. E-cadherin mutations are reportedly rare in carcinomas of bladder, colon, endometrium, lung, esophagus, ovary and thyroid and in intrahepatic cholangiocarcinoma [236, 241–244].

Familial aggregation of gastric cancer has been known for many years. These familial cancers can be classified by histopathological subtype into hereditary diffuse gastric cancer (HDGC), familial diffuse gastric cancer (FDGC), and familial intestinal gastric cancer. The criteria for identifying HDGC families have been well defined by the International Gastric Cancer Linkage Consortium (IGCLC), and are based on the incidence and onset of diffuse gastric cancer in families [245]. Gastric cancers in families with high incidence of these malignancies and with an index case with diffuse gastric cancer, but not fulfilling the IGCLC criteria for HDGC, are classified as FDGC [246]. Germline mutations in the E-cadherin gene were described for the first time in 1998 by Guilford et al. [25], who identified *CDH1*-inactivating mutations in three Maori families with early-onset diffuse gastric cancer. Since then, 68 different families carrying germline *CDH1* mutations have been identified worldwide [247]. *CDH1* mutations were found in 30.5% of HDGC families and 13.8% of FDGC families (reviewed in [246]). These mutations are similar to sporadic mutations in that most of them are predicted to cause premature stop codons as a consequence of nonsense, splice-site and frameshift mutations. Only a minority of these *CDH1* mutations were missense mutations [248]. Germline mutations are scattered over the entire length of the gene.

Multiple cases of lobular breast cancer (including mixed ductal and lobular histology) have been reported in families with HDGC [245, 249–251]. The estimated cumulative lifetime risk of breast cancer in women from HDGC families with germline *CDH1* mutations is 39 % [247]. Interestingly, recent findings indicate that *CDH1* germline mutations can be associated with invasive lobular breast cancer in the absence of diffuse gastric cancer [252]. Most of these hereditary tumors are E-cadherin-negative, pointing to a double inactivating mechanism.

Epigenetic silencing of the E-cadherin locus in cancer

Promoter hypermethylation has been identified as an important epigenetic event associated with the loss of E-cadherin gene expression during cancer progression. A large CpG island in the 5' proximal promoter region of the E-cadherin gene shows aberrant DNA methylation in at least eight different human carcinoma types and correlates with reduced E-cadherin protein expression [31, 253–256]. In cancer cell lines, the E-cadherin gene shows a heterogeneous pattern of promoter methylation that is dynamic and unstable, with allele-to-allele variability [254, 257, 258]. This is compatible with the heterogeneous loss of E-cadherin protein expression, which is believed to be influenced by the tumor microenvironment. CpG island methylation in the *CDH1* gene seems to increase during malignant progression of breast and hepatocellular carcinomas [259, 260]. Causal involvement of hypermethylation in E-cadherin repression is supported by the reactivation of E-cadherin in certain cancer cell lines upon treatment with the demethylating agent 5-aza-2'-deoxycytidine (5AzaC) [253, 254]. This 5AzaC-induced E-cadherin expression in dedifferentiated breast cancer cells correlated with increased *in vitro* cell aggregation, reduced motility, and suppression of metastasis [261]. The methylated CpG island of the repressed E-cadherin promoter is bound by the methyl-CpG binding proteins MeCP2 and MBP2. Binding of these nuclear factors results in recruitment of HDACs to the methylated E-cadherin promoter area, leading to histone-3 (H3) deacetylation, which is essential for suppressing the methylated E-cadherin gene [262]. Interestingly, generation of somatic breast cell hybrids made from E-cadherin-positive cells and cell lines with a methylated inactive E-cadherin promoter indicated that the loss of E-cadherin expression can be linked to a dominant transacting pathway [263]. Indeed, the expression of E-cadherin transcriptional repressors has been associated with promoter hypermethylation in esophageal squamous cell carcinoma [264]. Furthermore, the methylated *CHD1* promoter status in breast cancer cell lines seems to be part of a general transcriptional program that conforms with EMT and increased invasiveness but diverges from the specific consequence of E-cadherin mutational inactivation [265].

Different repressors of E-cadherin transcription have already been associated with progression of multiple cancer types (Fig. 1). Increased Snail expression is common in ductal breast carcinomas and is strongly associated with reduced E-cadherin gene expression [266]. High-grade breast tumors and lymph node-positive tumors consistently show strong Snail expression [267]. A new role for Snail in tumor recurrence has been inferred from a reversible HER-2/neu-induced breast cancer mouse model [268]. Also abnormal expression of Slug has been associated with disease aggressiveness in metastatic ovarian and breast carcinoma [269]. Twist, another EMT-regulating transcription factor, is involved in breast tumor cell metastasis [270], and its expression was found to rise as nodal involvement increased (tumor-node-metastasis status) [271]. Strong expression of SIP1/ZEB2, which is associated with loss of E-cadherin expression, was reported in gastric cancer of the intestinal type, but Snail does not seem to be involved in these tumors [272]. In contrast, Snail is upregulated in diffuse gastric cancer, a tumor subtype frequently affected by E-cadherin inactivating mutations [272]. Interestingly, the transcription factor deltaEF1/ZEB1 seems to be downstream of Snail expression [273]. Knockdown of deltaEF1/ZEB1 in dedifferentiated human epithelial colon and breast cancer cell lines results in the re-expression of E-cadherin and other epithelial differentiation markers [90, 97]. Although there are extensive data showing that expression of E-cadherin repressors is inversely correlated with expression of E-cadherin, care should be exercised in interpreting these results because many data are based on RT-PCR and on the use of antibodies with poorly defined specificity.

Recently, the induction of expression of transcriptional repressors of E-cadherin has been inversely linked with the expression status of the von Hippel-Lindeau (VHL) tumor suppressor. VHL is a component of SCF (Skp1-Cdc53-F-box)-like ubiquitin ligase complex that targets the alpha subunits of the hypoxia-inducible transcription factor (HIFα) for proteasomal degradation. Loss of VHL is an early and requisite step in the pathogenesis of clear-cell renal cell carcinoma (CC-RCC) [274]. Activation of HIFα proteins in cells devoid of VHL, including CC-RCC cells, has been shown to induce transactivation of several E-cadherin repressors, such as SIP1/ZEB2 and Snail, which contributes to the particularly malignant character of this tumor type [275–277]. Thus, inactivation of VHL in CC-RCC results in the loss of E-cadherin in an HIF-dependent manner.

Endocytosis and proteolytic processing of E-cadherin in cancer

Besides the genetic and epigenetic silencing of E-cadherin, many other mechanisms could serve as alternative ways for disturbing or inhibiting normal E-cadherin function under pathological conditions. As described above, E-cadherin is removed from the plasma membrane by endocytosis and subsequently recycled to sites of new cell-cell contacts (Fig. 7). Abnormal activation of proto-oncogenes, such as c-Met, Src and EGFR results in increased phosphorylation of tyrosine residues in the CD of E-cadherin, which leads to recruitment of the E3-ubiquitin ligase Hakai and subsequently mediates internalization and ubiquitin-dependent degradation of E-cadherin [41, 182]. MDM2, an E3-ubiquitin ligase known as a major p53 antagonist, was also recently reported to induce E-cadherin degradation [278]. The β-catenin-binding CD of E-cadherin includes a number of serine and threonine residues that are putative phosphorylation sites for casein kinase 1 (CK1), casein kinase 2 (CK2), glycogen synthase kinase 3β (GSK3β) and protein kinase D1 (PKD1 or PKCμ) (Fig. 2). Phosphorylation of these sites leads to altered binding of β-catenin to E-cadherin, either reducing it [279] or enhancing it [172, 280, 281]. The enhancing effect of the phosphorylation of residues Ser684 (consensus site for CK2), Ser686 and Ser692 (consensus sites for GSK3β) has been explained at the structural level, as only the phosphorylated form of E-cadherin acquires the appropriate structure and is able to bind β-catenin [282]. A similar situation may exist for Ser696 and Ser699, consensus sites for PKD1. In contrast, phosphorylation of Ser690 (Ser846 in the mouse precursor protein) by CK1 negatively regulates E-cadherin-based cell-cell contacts [279]. A pseudophosphorylated E-cadherin mutant (S690D) showed decreased interaction with β-catenin and enhanced endocytosis, in contrast to the nonphosphorylatable mutant S690A. It is noteworthy that the kinases acting *in vivo* on E-cadherin remain poorly defined, although PDK1 has been shown to colocalize and co-immunoprecipitate with E-cadherin, and to be down-regulated in advanced human prostate cancer [281]. In addition, β-catenin itself also acts as a substrate for tyrosine phosphorylation as well as serine/threonine phosphorylation (reviewed in [283, 284]). This regulates its affinity for either E-cadherin or α-catenin and ultimately leads to disruption of AJs [285, 286].

Considering the role of p120ctn in stabilizing the cadherin-catenin complex (see above), it is not surprising that many cancer types are characterized by loss or dislocalization of p120ctn (reviewed in [287]). We recently showed that p120ctn interacts with hNanos1, the human ortholog of the *Drosophila* zinc-finger protein Nanos [288]. Transcription of hNanos1 mRNA is suppressed by E-cadherin expression, and this makes sense in view of the invasion suppressor role of E-cadherin. Indeed, conditional expression of hNanos1 in human colon DLD1 cancer cells induces cytoplasmic translocation of p120ctn, up-regulates expression of membrane type 1-matrix metalloproteinase (MT1-MMP) at the mRNA and protein levels, and increases the migratory and invasive abilities [288, 289].

Moreover, matrix metalloproteinases, including stromelysin-1 (MMP3), matrilysin (MMP7), MMP9, and MT1-MMP (MMP14), cleave the E-cadherin ectodomain near the plasma membrane (reviewed in [167]) (Fig. 2). Comparably, plasmin and the disintegrin and metalloproteinase ADAM10 mediate E-cadherin shedding, which affects normal cell-cell adhesion as well as cell migration [187, 290, 291]. Several other proteases, such as the serine protease kallikrein 6 (Klk6), are up-regulated in human squamous skin carcinomas. Ectopic expression of Klk6 in keratinocytes induces E-cadherin ectodomain shedding in parallel with remarkably increased levels of mature ADAM10 proteinase [292]. Pancreatic adenocarcinomas often overexpress kallikrein 7, which is also able to generate soluble E-cadherin fragments [293]. Such fragments may function as pseudoligands that block normal E-cadherin interactions and promote invasion [167, 293]. In the case of epithelial ovarian carcinomas, the tumor cells maintain direct contact with ascites, which accumulates high concentrations of the solubilized E-cadherin ectodomain in that way promoting disruption of cell-cell junctions and metastatic dissemination. Also ADAM15, which is associated with progression of breast and prostate cancers, has been demonstrated to generate a soluble E-cadherin ectodomain [294]. Remarkably, this E-cadherin fragment appeared to stabilize heterodimerization of the HER2 (ErbB2) receptor tyrosine kinase to HER3. This leads to Erk signaling, which stimulates both cell proliferation and migration.

Other enzymes, such as calpain and caspases, cleave E-cadherin upstream of the β-catenin binding domain in its cytoplasmic part (Fig. 2), thereby blocking the formation of stable AJs [295, 296]. The resultant intracellular fragment disturbs β-catenin subcellular localization and stimulates cyclin D1 expression in human keratinocytes, thereby stimulating migration and proliferation and reducing cell-cell adhesion [187]. The ectodomain of E-cadherin was also identified as target substrate of secreted cathepsins B, L and S, but not of cathepsin C [297]. This correlated quite well with impaired malignant invasion upon ablation of any of these three cathepsins in the mouse pancreatic islet cell carcinogenesis model, RIP1-Tag2,

whereas tissue-specific cathepsin C knockout had no effect on either tumor formation or progression. Cathepsins are often secreted by various cells in the tumor microenvironment [298].

Cadherin switching during cancer progression

Loss of E-cadherin expression in cell lines and tumor tissues is often associated with induced expression of mesenchymal cadherins, such as N-cadherin and cadherin 11, a phenomenon generally referred to as cadherin switching (reviewed in [218, 299]). E-cadherin repressors, such as Snail and SIP1, can induce N-cadherin expression during EMT, suggesting that this cadherin switch is part of a transcriptional reprogramming of dedifferentiating epithelial cells [26, 95]. Overexpression of N-cadherin in epithelial breast tumor cells induces a scattered morphology even in the presence of E-cadherin, and provides these cells with a more motile, invasive and metastatic capacity when they are injected in nude mice [218, 299]. Mechanistically, N-cadherin is believed to functionally interact with the FGF receptor, causing sustained downstream signaling by phospholipase Cγ, PI3-kinase and MAPK-ERK, and thereby promoting cell survival, migration and invasion [300]. N-cadherin homophilic interactions between tumor cells and N-cadherin-positive tissues, including stroma and endothelium, might facilitate penetration and survival of tumor cells in secondary organs [299]. Shedding of N-cadherin by proteases might stimulate FGFR signaling on neighboring cells. Furthermore, presenilin 1 (PS1)/γ-secretase cleaves N-cadherin in the cytoplasmic part to release a free C-terminal 35-kDa fragment that translocates to the nucleus, where it binds the transcriptional coactivator CBP (CREB binding protein) [301]. CBP is thereby targeted for degradation, and this represses CBP/CREB-mediated transcription. Nonetheless, mammary gland tumors arising in a bi-transgenic mouse model overexpressing both N-cadherin and ErbB2/HER-2/neu in a tissue-specific manner are not pathologically different from N-cadherin-negative tumors [302]. In contrast, co-expression of polyomavirus middle-T antigen and N-cadherin in the mammary epithelium produces breast cancers with greater potential for metastasis to the lung [218]. N-cadherin did not enhance tumor onset but affected tumor progression by potentiating ERK oncogenic signaling involving MMP-9 up-modulation. The above-mentioned studies suggest that the effects of cadherin switching could be late events in tumor progression and that the impact of abnormal cadherin expression can depend on the cellular context: additional events, such as overexpression of FGF(R), loss of E-cadherin or up-regulation of MMPs might be required to act in concert with N-cadherin to promote mammary tumor invasion and metastasis *in vivo*.

Concluding remarks

Our strict focus on E-cadherin in this review springs from our belief that it deserves exclusive treatment, not only because it is a paradigm for the numerous other cadherins and cadherin-like molecules, but also because its functions have a major impact on many important processes in multicellular animals. Of course, the cadherin family members and in particular the E-cadherin-associated catenins do deserve to be discussed in depth, but here we had to resort to referring the reader to several excellent reviews on these other molecules. The reason is that many established concepts of E-cadherin have been challenged during recent years, and the E-cadherin literature has grown considerably. For instance, the homophilic binding mechanism of E-cadherin is widely accepted but may be less dogmatic after all; the three-dimensional structure of E-cadherin remains a matter of debate despite the impressive amount of appealing data produced by many recent experiments; the link of the cadherin-catenin complex to the cytoskeleton has recently been critically revisited; studies in various experimental settings and model organisms revealed that the cell-sorting mechanism used *in vivo* during morphogenesis is far more complex than formerly believed; the AJ, once thought to be quite rigid, now appears to be subjected to many regulators and other influences, and so must be very dynamic; interactions of E-cadherin with various signaling pathways and modifying enzymes turned out to be very diverse, which means that they are regulated by finely tuned mechanisms. As for the roles of E-cadherin in pathological conditions, it came as a surprise that E-cadherin increases the vulnerability of epithelial tissues to certain infections; however, its role as tumor and invasion suppressor has withstood critical examination. The way in which cancers can disrupt the normal functions of E-cadherin functions can only be described as ingenious, and so the development of an anti-cancer therapy based on restoring E-cadherin, although an attractive concept, is quite challenging. It is understandable that during the 30 years since the discovery of E-cadherin, research activity has shifted towards the various catenins. It is equally logical that the study of cadherins has been expanding to include the numerous other members of the cadherin superfamily. There is no doubt, however, that the continued scrutiny of E-cadherin is generating new ideas, models, and concepts, and that these fruits of research have major and sometimes surprising influences on our knowledge of cells, tissues and organisms.

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