Cadherins in development and cancer

Marc P. Stemmler*

Received 12th December 2007, Accepted 31st March 2008
First published as an Advance Article on the web 29th May 2008
DOI: 10.1039/b719215k

Proper embryonic development is guaranteed under conditions of regulated cell–cell and cell–matrix adhesion. The cells of an embryo have to be able to distinguish their neighbours as being alike or different. Cadherins, single-pass transmembrane, Ca^{2+}-dependent adhesion molecules that mainly interact in a homophilic manner, are major contributors to cell–cell adhesion. Cadherins play pivotal roles in important morphogenetic and differentiation processes during development, and in maintaining tissue integrity and homeostasis. Changes in cadherin expression throughout development enable differentiation and the formation of various organs. In addition to these functions, cadherins have strong implications in tumourigenesis, since frequently tumour cells show deregulated cadherin expression and inappropriate switching among family members. In this review, I focus on E- and N-cadherin, giving an overview of their structure, cellular function, importance during development, role in cancer, and of the complexity of *Ecadherin* gene regulation.

Introduction

For the proper development of metazoa coordinated cell–cell adhesion is essential for establishing the body plan and to maintain the integrity of the differentiated tissues. During the formation of complex structures it is important that differentiating cells are able to distinguish between cells with the same identity and other cell types. In pioneering studies Townes and Holtfreter in 1955 and Moscona in 1957 addressed the issue of selective adhesion of different cell types. After dissociation and mixing, the cells of amphibian embryos or of avian neural retina self-aggregate and reorganize into multilayered tissue based on germ-layer or cell-lineage origin.[1,2] Importantly, during the self-assembly of cells into complex structures some adhesion processes require the presence of Ca^{2+} ions.[3] The analysis of the underlying molecular mechanisms of cell sorting and reorganization during these processes led to the identification of the ever-growing superfamily of calcium-dependent adhesion molecules, the cadherins.[4] The founder member of these calcium-dependent adherins, E-cadherin (epithelial cadherin, also known as uvomorulin, *Cdh1*, *Ecadherin*) was identified using antibodies against mouse preimplantation embryos.[5] In a similar approach in chicken, the E-cadherin homologue, L-CAM, was found in embryonic liver cells[6,7] and N-cadherin (neuronal cadherin, formerly known as A-CAM, *Cdh2*, *Ncadherin*) was detected in the embryonic neural tube.[8] This started important research continuing over the last four decades on Ca^{2+}-dependent adhesion. Meanwhile, over 100 cadherin family members have been identified in vertebrates, arthropods, nematodes, and even cniderians. Their phylogenetic relationship was refined by comparative genomic analysis, and the results led to the current classification.[9,10] Cadherins can be subdivided into six subfamilies: the type I and type II "classical" cadherins,[11,12] the desmosomal cadherins,[13,14] the seven-pass transmembrane cadherins,[15] the large cadherins of the fat and dachsous group,[16,17] and the most recently identified subfamily, the protocadherins[18–20] (Table 1). Albeit a great variety of different cadherins have been analysed in the past, most of our knowledge about the superfamily was gained from type I classical cadherins. In this review, I want to give an overview of the function of this subfamily during normal

Department of Molecular Embryology, Max-Planck Institute of Immunobiology, Stuebeweg 51, D-79108 Freiburg, Germany. E-mail: stemmler@immunbio.mpg.de; Fax: +49 761 5108 474; Tel: +49 761 5108 477

Dr Marc Philippe Stemmler is currently a Junior group leader in the Department of Molecular Embryology at the Max-Planck Institute of Immunobiology, Freiburg (Germany). He did his Post-doc in the laboratory of Prof. R. Kemler at the same institution. His research is in the field of molecular and developmental biology using the mouse as a model system. His interest is based on the role of cadherin-mediated cell–cell adhesion and Wnt/β-catenin signaling during embryogenesis. The main research interest is the analysis of transcriptional regulation of Ecadherin during development.

development and their implication in cancer. Since E- and N-cadherin have been most intensively studied, I will concentrate on these two molecules.

The cadherins as transmembrane proteins

Classical cadherins: structure and function

The role of cadherins in cells and tissues in the formation of adherens junctions (AJs) at the plasma membrane is reflected in their molecular structure. Nearly all cadherins have a single transmembrane domain and are therefore classified as type I single-pass transmembrane domain proteins, with the exception being the seven-pass transmembrane cadherins (see Table 1). A common feature of all cadherins is the architecture of the extracellular domain. This domain is structured into tandemly arrayed blocks of extracellular cadherin domains (ECs, cadherin repeats) that are highly variable in number. The structure of these cadherin repeats is best studied for *Xenopus laevis* C-Cadherin.[21–23] Each contains about 110 amino acid residues (aa) that form a conserved β-barrel structure, albeit the amino acid composition is very different within the superfamily. Two opposing β-barrel structures form a binding pocket for three Ca^{2+} ions.[21,22,24] The term "classical" cadherin defines their ability to interact with β-catenin with the approximately 100 aa long cytoplasmic domain. The mature classical type I cadherins mediate strong cell–cell adhesion and have five ECs (1–5 numbering from the outermost domain; Fig. 1, Table 1) with a highly conserved Trp-residue (W) at position 2 as well as a His-Ala-Val (HAV) motif in the N-terminus. The first cadherins identified, E-cadherin, N-cadherin, P-cadherin and R-cadherin, belong to this classical type I subfamily and are named according to the tissue in which they were identified first: epithelial, neuronal, placental and retinal cadherin, respectively. The type II classical cadherins, such as the vascular enodothelial VE-cadherin, cadherin-7 and -8, lack this conserved HAV domain but have two conserved W-residues involved in dimerisation,[25] and are otherwise similar to type I classical cadherins.

Similar classical cadherin-like molecules have been identified in invertebrates, *D*E- and *D*N-cadherin in *Drosophila melanogaster* and Hmr-1a/b in *Caenorhabditis elegans*. However, in comparison to their vertebrate counterparts they differ in structure by having larger extracellular domains (7 EC domains in *D*E-cadherin, 17 in *D*N-cadherin and 3/19 in Hmr-1a/b).[10,15,26] In addition to the different number of cadherin repeats, their aa sequences contain unique segments at the proximal ends. These include EGF-like and laminin globular-like domains, which are found in seven-pass transmembrane cadherins but are absent in vertebrate classical cadherins. Nevertheless, *D*E-, *D*N-cadherin and Hmr-1a/b can be regarded as functional orthologues of their mammalian counterparts since they are able to bind to the β-catenin orthologues armadillo in *D. melanogaster* and Hmp-2 in *C. elegans*, respectively.[27–29] Furthermore, *D*E-cadherin is expressed in epithelial cells and is essential for the formation of AJs, whereas *D*N-cadherin is detectable in the nervous system.[15]

Cadherin assembly and junction formation

The cadherins are synthesized in the endoplasmatic reticulum (ER) as a propeptide, which includes a signal peptide for proper trafficking to the plasma membrane, an N-terminal prodomain, and a conserved subtilisin-like proprotein convertase (PC) proteolytic cleavage site, L(R/Q)RQKR-(D/E)WV(I/M)PPI.[30,31] The prodomain presumably functions in preventing precocious adhesion and aggregation by folding back to cap the EC1 domain.[32] In the *trans*-Golgi network proteolytic cleavage by furin and other PCs removes the prodomain to present the N-terminus of the mature protein, (D/E)WV(I/M)PPI,[30,31] followed by transport and integration into the plasma membrane. For proper function and stability, the protein has to be linked to β-catenin or plakoglobin, which already associate in the ER co-translationally and assemble to the cadherin–catenin–complex.[33–37] Cadherin-coupled β-catenin or plakoglobin binds to the actin-binding protein α-catenin (Fig. 1). Although indirect evidence led to the conclusion that α-catenin directly links the cadherin–catenin complex to actin, recent data strongly suggest that α-catenin cannot simultaneously bind cadherin-bound β-catenin and filamentous actin (Fig. 1). Moreover, monomeric α-catenin binds to β-catenin,

Table 1 The cadherin subfamilies

Subfamily	Characteristics	Examples	Refs.
Type I classical cadherins	Associated with actin cytoskeleton, conserved HAV motif in EC1, binds to β-catenin, 5 ECs	E-cadherin N-cadherin DE-cadherin	11, 12, 241
Type II classical cadherins	Associated with actin cytoskeleton, no HAV motif, binds to β-catenin, 5 ECs	VE-cadherin Cadherin-7 Cadherin-8	25, 44, 136
Desmosomal cadherins	Only found in vertebrates, present in desmosomes, connected to intermediate filaments *via* plakoglobin, desmoplakin and plakophilin, 4 ECs	Desmoglein-1 Desmoglein-2 Desmocollin-1 Desmocollin-2	13, 14
Seven-pass transmembrane cadherins	Found in vertebrates and invertebrates, seven-pass transmembrane domain, LG and EGF domain, flamingo box	Flamingo CELSR1	15, 26
Large cadherins of the fat and dachsous group	Large extracellular domain with up to 34 ECs, LG and EGF domains, conserved cytoplasmic domain	Fat Dachsous	16, 17
Protocadherins	Only found in vertebrates, subdivided into α-, β- and γ-protocadherins, 6 ECs, most members present in a gene cluster, whole extracellular domain is encoded by one exon	Pcdhγ-A3 PcdhX-C5 Protocadherin-4 XPAPC	18–20

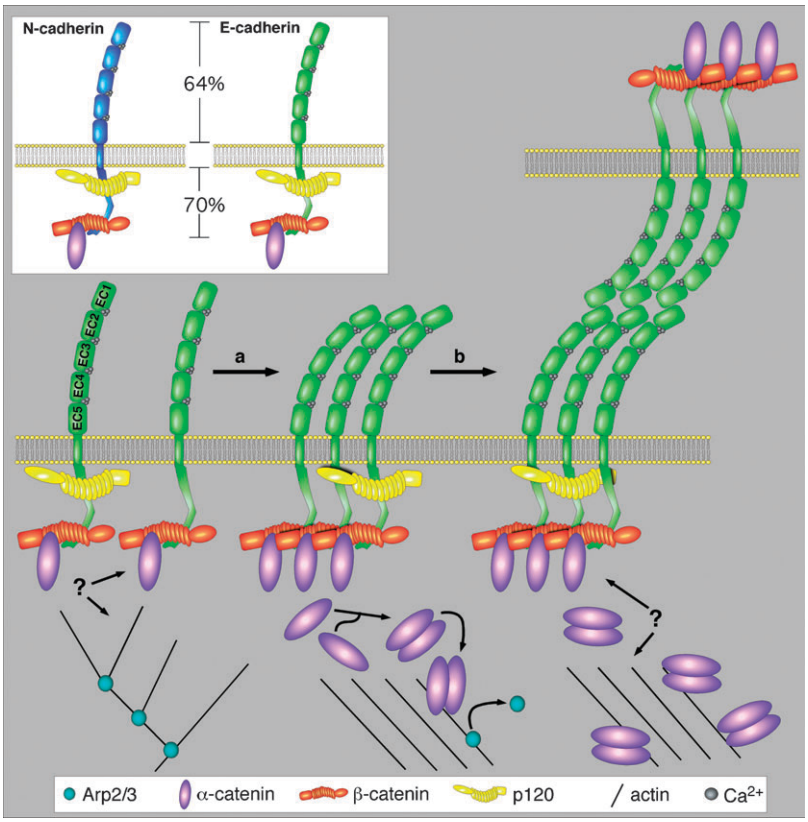

Fig. 1 The structure of the cadherin–catenin complex in the membrane and formation of adherens junctions: *cis* and *trans* homodimerisation. An example of the complex for E-cadherin (green) and N-cadherin (blue) is shown in the inset. For both, the C-terminal portion of the cytoplasmic domain binds to β-catenin, which then binds to α-catenin. p120 binds to the juxtamembrane domain. Percentages indicate sequence identity between E- and N-cadherin. The sequential formation of adherens junctions is shown in the lower part. On the extracellular side *cis* dimerisation involves a W-side chain of EC1 and a groove of EC2 and precedes *trans* interaction (a). The adhesive bond is then established *via* W2 and β-strand exchange of EC1–EC1 interaction (b). Intracellularly, at initial nascent contact sites (left), α-catenin is almost exclusively monomeric and bound to cadherins, allowing branching of the actin cytoskeleton involving the Arp2/3 complex. In mature strong contact sites cadherins cluster and increase the local concentration of α-catenin, which then also dissociates from the cadherin–catenin complex and dimerises (middle, right). α-Catenin homodimers then compete with Arp2/3 for binding to actin and in turn suppress actin polymerization and branching to reorganize the actin cytoskeleton to form actin bundles. Question marks indicate that the connection of the cadherin–catenin complex to the actin cytoskeleton is ambiguous. Abe and Takeichi recently suggested EPLIN as a candidate for the missing link.[65] Note that β-catenin and p120 bind as a monomer to cadherins even in mature contacts.

whereas α-catenin can only bind actin as a homodimer. Although the results are still being debated,[38,39] alternative mechanisms of cadherin anchoring to the cytoskeleton are possible, postulating the existence of a missing link between α-catenin and actin.[39,40]

The molecular mechanisms underlying cadherin binding in *cis* (lateral homodimers of molecules on the same cell) and in *trans* (homodimerization on opposing cells) are still not fully understood. NMR and X-ray crystallographic analyses provided models for the adhesive bonds.[21–24,32] Several observations suggest that in the mature cadherin protein the first steps of AJ assembly involve initial cadherin dimerization in *cis* to form an adhesive interface for the interaction in *trans* (Fig. 1). The current model implies that the *trans* homodimers of two approaching cells intercalate in a zipper-like fashion to form an AJ, but just which ECs contribute to *cis* and *trans* dimerisation remains controversial.[24,32] All data provide evidence that EC1 is essential for recognition of the homotypic binding partner and that the conserved W2 plays an essential role in binding (and in a similar fashion the two conserved W in type II cadherins[25]). This is evident from mutational analysis and EC1 swapping experiments.[41,42] In one model it is suggested that the W2 residue is involved in *cis* and in *trans* homodimerisation and that these states cannot occur simultaneously.[43] However, several lines of evidence indicate that the *cis* interaction is independent of the N-terminal W2. Rather, a β-sheet interaction of a conserved W-side chain on the opposite side of this first N-terminal β-strand in EC1 may be involved. This side chain binds to a corresponding groove in EC2 (Fig. 1).[21,25] This EC1–EC2 *cis* interaction then simultaneously allows β-strand swapping of the W2 chain in *trans*.[44] After the initial binding in *trans*, a full intercalation of the opposing ectodomains is suggested from force spectroscopy measurements of single cadherin domains or whole molecules. Hence, at least EC1 to EC3 are involved in establishing the adhesive bond.[24,45]

The forces that are generated by the adhesive bonds have been extensively investigated. Different techniques have been

used to measure the strength of binding between cell adhesion molecules.[46–48] In order to discriminate the adhesive forces of different classical cadherins, Chu *et al*. used a quantitative dual pipette assay.[49] Stronger adhesion was observed with classical type I cadherins, whereas seven-fold less force was detected with type II cadherins.[50] Similarly, the adhesive bond between two cells that express equal amounts of either *Ecadherin* or *Ncadherin* was determined to be 3–4 fold less for N-cadherin.[50]

Cytoplasmic interaction of the cadherin–catenin-complex

Cytosolic proteins associated with AJs either act as scaffold proteins to influence the stability of the AJ, modulate its adhesiveness and the turnover of cadherins, or are involved in signal transduction.

(a) Catenins. α- and β-catenin and plakogobin (γ-catenin) have been identified in immunoprecipitation experiments as binding partners of E-cadherin.[51] β-Catenin and plakoglobin both belong to the family of armadillo repeat proteins with an overall identity of 65%.[52,53] Both interact directly with the C-terminal 30 aa of the cytoplasmic tail of classical cadherins (Fig. 1).[54,55] Within β-catenin the E-cadherin interaction region spans over the entire armadillo repeat domain.[56] Each armadillo repeat contains approximately 40 aa that form a superhelix of 3 α-helices exposing a positively charged groove.[56] Interestingly, β-catenin has a dual role in the cell. Besides the important role in adhesion, β-catenin is, like its *Drosophila* homologue armadillo, the intracellular transducer of the canonical Wnt/Wg signalling pathway, acting as a transcriptional coactivator in the nucleus.[57–60]

The N-terminal portions of β-catenin (aa 120–151) and plakoglobin bind to α-catenin, and this protein assembly is supposed to be an essential component of the adhesion complex.[53,61] Although α-catenin cannot bind the cadherin–catenin complex and actin filaments simultaneously, it is very likely that an indirect interaction exists to anchor the complex to the cytoskeleton. Monomeric α-catenin associates to the adhesion complex and also interacts with α-actinin, vinculin or ZO-1, and other proteins. These α-catenin interaction partners bind to actin filaments independently.[62–64] Such indirect linking of the cadherin–catenin complex to the actin cytoskeleton may act *via* EPLIN (epithelial protein lost in neoplasia), a known actin-binding protein that has recently been shown to bind α-catenin associated to the complex.[65] Homodimeric α-catenin can bind to the actin cytoskeleton and inhibit Arp2/3 mediated actin branching. This favours actin bundle formation at sites of mature contacts (Fig. 1).[39] However, each member of the core cadherin–catenin complex (cadherin, β-catenin and α-catenin) is essential. Mutations affecting expression or binding of any of these proteins lead to dramatically reduced cell–cell adhesion.[54,66–68]

Yet another armadillo repeat family member p120-catenin (p120) associates with the juxtamembrane portion of type I and type II classical cadherins.[69] p120 is a substrate of the Src kinase and binds to cadherins independently of β- or α-catenin (Fig. 1).[70,71] However, the role of p120 is still not fully understood and is complicated by many different isoforms and closely related family members. These related molecules include ARVCF (armadillo repeat gene deleted in velocardiofacial syndrome), p0071 and δ-catenin, which presumably act in a manner similar to p120.[72,73] In contrast to β-catenin, p120 is not already co-translationally pre-assembled in the ER with cadherins. Rather it binds in a context-dependent manner after the complex has integrated into the plasma membrane.[74] However, the presence of cadherins is necessary and sufficient to recruit p120 to the membrane.[75] Dependent on the cellular context, p120 acts as a scaffold protein to regulate cadherin adhesive strength by clustering cadherins to specific sites on the cell surface.[69,75] Moreover, p120 regulates cadherin turnover by adapting the amount of cadherin available for adhesion on the surface.[76–78] The importance of p120 for stabilising cadherins was also demonstrated in mice. Genetic ablation of p120 from salivary gland epithelium or from the skin significantly reduced E-cadherin levels in these tissues.[79,80] Regulation of cell–cell adhesion is potentially controlled by intracellular crosstalk between p120 and receptor tyrosine kinases (RTKs), integrins and adhesion activities, that requires coordination of Rac activation and Rho inhibition.[81]

(b) Small GTPases. Small GTPases of the Ras superfamily regulate a variety of biological processes in the cell. They receive signals from growth factors and cytokines as well as from adhesion molecules to regulate cytoskeletal organization, organelle distribution, vesicle transport and gene expression.[82] They cycle from an active GTP-bound to an inactive GDP-bound form and the equilibrium is orchestrated by different proteins, like GEFs (guanine nucleotide exchange factors), GDIs (GDP dissociation inhibitors) and GAPs (GTPase activating proteins), that regulate Ras activity at different levels of cycling. Ras family members have been shown to be important for establishing and maintaining epithelial biogenesis including apical-basal polarity and cell–cell contacts (reviewed in ref. 83). In particular, Rho, Rac, Cdc42 and recently Rap1 were found to be associated with the cadherin adhesion complex, and inhibition of Rho and Rac interferes with the organization of AJs.[84–86] Upon cell–cell contact formation, small GTPases are activated and induce epithelial biogenesis. Adhesion is increased by stabilisation of AJs or *via* recruitment of actin to adhesive sites by activating Jun signalling.[83]

A small group of proteins has been identified with affinity to Rho GTPases and homology to GAPs. These proteins contain four IQ domains that are essential for binding to calmodulin and are consequently termed IQGAPs.[87] Three mammalian members are known, IQGAP1, IQGAP2 and IQGAP3, but none of these actually has GTPase activating properties. Nevertheless, IQGAP1 localises to basolateral membranes at cell–cell contact sites and is thought to regulate cadherin-based adhesion downstream of Rac1 and Cdc42.[88,89] IQGAP1 competes with α-catenin for binding to β-catenin. In overexpression experiments, it sequesters β-catenin from the cell-adhesion complex and thereby weakens adhesion.[88,89] In addition, the results of IQGAP1 RNAi knock-down in MDCK cells suggest that IQGAP1 may also enhance cadherin-based cell-adhesion since significant reduction in E-cadherin, β-catenin and actin filament protein levels is observed.[90] However, human IQGAP2 localises more to the apical membrane, suggesting distinct functions of both proteins.

Moreover, human IQGAP2 is involved in the activation of polarised acid secretion in gastric parietal cells.[91] Very recently, one of the *Xenopus* members, XIQGAP2, was inferred to play a role in cell–cell adhesion in early development since RNAi knock-down against XIQGAP2 caused ectodermal lesions in neural stage *Xenopus* embryos.[92] Hence, IQGAPs are able to modulate the establishment and stability of cadherin-based adhesion directly or as effectors of Rac1 and Cdc42 small GTPases.

(c) Receptor tyrosine kinases. Receptor tyrosine kinases (RTKs) play important roles in many different aspects of development and cancer. Upon ligand binding, oligomerisation and autophosphorylation, the receptors undergo a transition from an inactive to an activated state and subsequently phosphorylate target proteins. RTKs can interact with cadherins and the interaction alters both cell–cell adhesion and RTK signalling.[93,94] The interaction causes attenuation of the ligand-binding affinity and the ligand-dependent activation of the receptor as well as decreasing receptor mobility.[95] The epidermal growth factor receptor (EGFR), for example, controls different cellular decisions, including growth, differentiation, migration and transformation.[96] Activation of the EGFR induces rapid alterations in cell morphology, actin cytoskeletal rearrangements, and redistribution of the receptor. Overexpression of EGFR or constitutive activation has been observed in many neoplastic cells and tumours.[96] In HaCat keratinocytes, however, the E-cadherin–EGFR interaction can activate receptor signalling *via* the MAPK pathway even in the absence of a ligand.[94] Furthermore, it has been observed that in mammary epithelium EGFR tyrosine phosphorylation can be induced by E-cadherin which is clustered in AJs. This specifically requires the presence of the extracellular domain of E-cadherin and leads to a reduction in focal adhesion.[96] However, N-cadherin is incapable of activating the receptor, confirming the specific interaction of E-cadherin with the EGFR.

A second group of RTKs, the fibroblast growth factor receptors (FGFRs), mediates signalling essential for many developmental processes, including muscle differentiation, mesoderm induction and migration, neural induction, limb formation, and anterior–posterior patterning, and promotes angiogenesis.[97] Similar to EGFR, FGFRs are also implicated in cancer progression, especially in promoting epithelial–mesenchymal transition (EMT) events.[98] In contrast to E-cadherin, N-cadherin has been found to interact with members of the FGFR family. Mainly, an interaction was detected with FGFR1, but FGFR4 also binds to N-cadherin in special contexts.[99] Interestingly, analogous to the homophilic cadherin interaction, a HAV motif present in the CAM-homology domain of the FGFR is required for the interaction. In addition, the acid box binds to the sequence IDPVNGQ present in the EC4 domain of N-cadherin.[100–103] The interaction was analysed in detail in neuronal cells, where N-cadherin supports neurite outgrowth, axonal growth, and synapse plasticity.[104,105] Specifically, neurite outgrowth is activated by N-cadherin and inhibited by blocking FGFR signalling.[106] Furthermore, in a mouse pancreatic tumour model a multiprotein complex is formed that contains NCAM, N-cadherin and FGFR4. Complex formation leads to receptor activation, loss of cell–matrix adhesion and dissemination of tumour cells that have gained invasive properties.[99] Similarly, stimulation of breast cancer cells with FGF2 or overexpression of *Ncadherin* yields malignant phenotypes, indicating that N-cadherin and FGFR signalling act synergistically.[107] Mechanistically, interaction of ligand-bound FGFR1 with N-cadherin inhibits internalisation of the receptor and sustains signalling through the ERK–MAPK signalling pathway.

Posttranslational modifications of classical cadherins

During differentiation processes like gastrulation, neurulation or neurite outgrowth, cells have to rapidly adapt to fast morphogenetic changes or tissue rearrangements. The necessity is reflected by the complexity of mechanisms regulating cadherin dynamics and cell adhesion. For example fine-tuning of the amount of adhesion-competent cadherin molecules on the cell surface and thus adhesive strength can be regulated by protein turnover. Reversible posttranslational modifications can alter the affinity of cadherins to the different binding partners. The cadherin–catenin complex is substrate for phosphorylation and dephosphorylation events on several residues that modulate adhesiveness (reviewed in ref. 108 and 109). Phosphorylation of E-cadherin in a 30 aa portion of the cytoplasmic domain containing a cluster of eight serine residues enhances the binding affinity to β-catenin.[55] Four of these serine residues are embedded in consensus sites for casein kinase II (CKII) (S684, S697 and S699) and GSK3β (S693), respectively. *In vitro*, these sites have been shown to be phosphorylated, and mutation dramatically reduces binding to β-catenin and results in destabilisation of cadherin molecules at AJs.[55] Conversely, the phosphorylation of β-catenin on different residues can influence binding to E-cadherin. For example the phosphorylation of Y654 in armadillo repeat 12 by the EGFR or Src dramatically reduces β-catenin affinity to E-cadherin.[110] CKII phosphorylation of three N-terminal residues of β-catenin (S29, T102 and T112) increases binding to α-catenin and enhances the stability of the adhesion complex.[111] In contrast to CKII the activity of CKI weakens AJs. Phosphorylation at E-cadherin S690 enhances endocytosis of the protein with no ubiquitination or degradation observed.[112] Inhibition of CKI stabilizes AJs, whereas the overexpression of CKI or of a constitutively phosphorylated version of E-cadherin leads to decreased adhesion and to improper membrane localisation of cadherins.[112] Furthermore, the cadherin–catenin complex is destabilised by phosphorylation of β-catenin at Y142. This results in a dissociation of bound α-catenin and subsequently in a loss of adhesion.[113,114]

The extent and duration of cadherin-complex phosphorylation is regulated by the activity of phosphatases, like RPTPs, PTPμ, PTPκ, DEP-1, PTP-LAR and VE-PTP or the cytosolic phosphatases PTP1B and Shp-2.[108,109] PTPμ has been shown to localise to cell–cell junctions, to regulate junctional integrity and to interact with E-cadherin.[115] The absence of PTPμ in prostate carcinoma cells leads to loss of cadherin-based cell-adhesion although proteins of the cadherin–catenin complex are properly expressed.[116] The cytosolic tyrosine phosphatase

PTP1B directly interacts with N-cadherin at a position adjacent to the β-catenin-binding domain. The interaction is dependent on phosphorylation of PTP1B by Fer at residue Y152.[117,118] If phosphorylation of PTP1B is blocked, a strong reduction of cadherin-mediated adhesion is observed.[119] Presumably, activated PTP1B dephosphorylates β-catenin at position Y654, which enhances binding to N-cadherin and regulates cadherin junctional integrity.[117,118,120]

Aside from directly regulating dynamic protein–protein interactions in cell–cell adhesion, phosphorylation can also irreversibly target adhesion through cadherin degradation. Src activation leads to phosphorylation of E-cadherin at Y755 and Y756 and to recruitment of Hakai, an E3-ubiquitin ligase, which increases endocytosis of E-cadherin and targets it for degradation *via* the proteasome.[121,122]

Cadherins are also subject to N- and O-linked glycosylation. N-Linked glycosylation occurs at least at two sites in EC4 and 5 but does not affect the functional sites of cadherins. However, N-glycans alter the stability of E-cadherin adherens junctions and the interaction of the adhesion complex with vinculin and the actin cytoskeleton.[123,124] The O-linked glycosylation may have a role in cell surface transport in preventing cadherin transport to the membrane in order to destabilise adhesion in cells destined for apoptosis.[125]

Processing and signalling of cadherins

Signalling through cadherins can activate different transduction pathways including the Wnt/β-catenin pathway by sequestering or retaining β-catenin at the adhesion complex.[126,127] Aside from being targets of signal transduction events mediated by RTKs, Rho-GTPases, PTPs or cytoplasmic kinases, classical cadherins themselves may function as signal transducers (reviewed in ref. 19). Classical cadherins have been found to be substrates for metalloproteases and γ-secretases.[128,129] The extracellular cleavage of N-cadherin by ADAM10 produces a membrane-bound short-lived carboxy-terminal fragment (CTF1).[130] This is rapidly processed by a γ-secretase complex containing presenilin 1 to release a CTF2 into the cytoplasm.[128,129] By a mechanism that is still unknown, the CTF2 binds p300/CBP in the cytoplasm, which was translocated from the nucleus. This complex is then rapidly ubiquitinated and degraded by the proteasome, thereby modulating CBP/CREB-mediated gene expression.[129] Similarly, BMP4 signalling is able to trigger this process in neural tube cells undergoing EMT at the onset of neural crest cell delamination. BMP4 activity induces CTF2 generation and subsequently Wnt/β-catenin target gene activation. Forced expression of *Ncadherin* inhibits delamination by retaining neural crest progenitors in an epithelial state and attenuating Wnt/β-catenin signalling.[131]

In a seemingly analogous manner, ADAM10 and subsequent γ-secretase processing of E-cadherin leads to cytoplasmic accumulation of E-cadherin CTF2. This fragment is still bound to β-catenin in the cytoplasm and activates the Wnt/β-catenin target gene *CyclinD1* in the nucleus.[132] Interestingly, ADAM10 and γ-secretase also process members of the protocadherin gamma family.[133,134] Generated CTF2 fragments of certain Pcdhγ members translocate to the nucleus and are capable of transcriptional autoregulation by an unknown mechanism.[133,135]

Role of classical cadherins in cells and tissues during development

Cell sorting and adhesion

Individual members of the classical cadherin subfamily are expressed in a restricted, tissue-specific pattern, indicating specific functions for individual cell-types. Nose and colleagues showed sorting and aggregation of fibroblastic L cells transfected with either E- or P-cadherin, based on which cadherin molecule they expressed.[136] Hence, it was hypothesized that differential expression of cadherins plays an essential role in tissue segregation and cell-sorting during development.[136,137] Although the mode of action of cell separation induced by cadherins is still poorly understood, it is believed that the sorting is due to the property of classical cadherins of binding almost exclusively in a homophilic fashion. The EC1 domain is very important for homophilic interaction, and cell sorting can be reverted if the EC1 domain of E-cadherin is replaced with the EC1 of P-cadherin.[41] Heterotypic adhesion between P- and E-cadherin has been described *in vitro* in transfected L cells and can also be found for E- and N-cadherin in the embryo (Fig. 2B).[138,139] Additional experiments uncovered that cell sorting is not only achieved by different cadherin subtypes but also by distinctive expression levels of a single subtype expressed in two different subpopulations.[138,140] Based on differential interfacial tension, cells sort and segregate after initial random intermingling. Cells with higher tension and higher cadherin expression levels are found on the inside of a cell aggregate, whereas the cells with lower expression surround the cells in the centre.[138,140] The importance of proper cell sorting *in vivo* is demonstrated for example during gonad development and oogenesis in *Drosophila*.[141,142] Here, *D*E-cadherin expression is essential both in primordial germ cells and gonadal precursors in order to sort these cells from other mesodermal cells that do not express *D*E-cadherin. In addition, *D*E-cadherin mediates ensheathment of the primordial germ cells by somatic gonadal precursors.[142,143]

Another important role of cadherins is in establishing and maintaining apical–basal cell polarity, a hallmark of epithelial cells.[144] If *Ecadherin* cDNA is transfected into fibroblasts, a redistribution is observed of the diffusely located Na^+/K^+-ATPase protein to the cell–cell contact sites known from polarised epithelia. This indicates that E-cadherin plays an essential role in establishing polarity.[126] Polarity in wild-type cells is established *via* specific distribution of several proteins. E-Cadherin is present on the entire basolateral membrane, but higher concentrations are found in the zona adherens, a specific, electron-dense area present in the vicinity of the apical side. They are distinct from the more apically located tight junctions and the more basally located desmosomes.[145] The differential distribution of key molecules is achieved by sorting of apical *vs.* basolateral proteins into different vesicles of the *trans* Golgi network and subsequent targetted transport to their destination sites.[126,144,146] Molecular cues for this process in epithelial cells appear to be provided by E-cadherin and its

link to the underlying cytoskeleton. Vesicles destined to basolateral sites of the cell are transported along filaments connected to E-cadherin.[144,147]

Cadherins contribute to cell–cell adhesion, to maintenance of tissue-integrity and to programmed cell death. For example, in the intestinal epithelium or in endothelial cells the presence of cadherins is required to prevent apoptosis. If cadherin function is prevented by utilizing a dominant negative version of N-cadherin, a precocious entry into the cell death program is observed.[148,149] Taken together, these data demonstrate the beneficial role of cadherins in cell sorting and function in the cell.

Function of cadherins during embryogenesis

The presence of cadherins plays a pivotal role for the proper formation of a mammalian embryo. This is already evident in mouse preimplantation development. Here, E-cadherin mediates the process of morula compaction and blastocyst formation. *Ecadherin* null embryos are not able to develop to a proper blastocyst, do not hatch from the zona pellucida and die due to a failure in trophectoderm formation.[150,151] Nevertheless, compaction at the morula stage is still observed in *Ecadherin* null embryos.[150,152] If *Ecadherin* is depleted from the oocytes combined with the zygotic knock-out, the blastomeres are only held together by the zona pellucida, do not undergo compaction and die.[152] Since zygotic gene expression of *Ecadherin* is first detectable between the late 4- and the 8-cell stage at E2.0[152] the earlier functions of E-cadherin must be performed by maternally provided mRNA and protein.[153] However, morula compaction in *Ecadherin*-maternally depleted embryos can be rescued by *Ecadherin* expression from a wild-type paternal allele provided by the sperm and then embryos develop normally.[152] In a different study, morpholinos were used to knock-down *Ecadherin* maternal and zygotic expression, resulting in a more drastic phenotype: *Ecadherin* morpholino-treated embryos arrested in the 2-cell stage.[154]

During normal development cells of the trophectoderm and the parietal endoderm gradually lose *Ecadherin* expression upon implantation,[155,156] as is also observed when embryos are cultured for blastocyst outgrowth.[157] However, *Ecadherin* expression in the epiblast and in the visceral endoderm is maintained until gastrulation, before other classical cadherins start to be expressed.[137,156] During this process, cells in the epiblast downregulate *Ecadherin* expression in order to delaminate at the primitive streak and undergo EMT transition to populate the mesoderm as well as the definitive endoderm (Fig. 2A).[137,156] Cells of the definitive endoderm migrate to the fore- and hindgut diverticulum to subsequently line the gut tube. Whereas these cells re-express *Ecadherin*, the mesoderm starts to express *Ncadherin*.[137,156,158,159] Likewise, after specification of the neurectoderm on the dorsal side, expression of *Ecadherin* is turned off and is replaced by *Ncadherin* expression (Fig. 2B).[8] Interestingly, at the non-neural ectoderm–neurectoderm border *Ecadherin* expression remains high and is essential for neural tube closure.[137,160–162] Heterophilic cadherin interaction is observed on this border where cells of the neurectoderm are associated with non-neural ectoderm cells (Fig. 2B). How this heterophilic interaction is established and stabilized remains to be analysed. Later during development E-cadherin is mainly restricted to epithelia, whereas N-cadherin is present in the mesoderm and its derivatives like somites, the notochord, cardiac and skeletal muscles, as well as in neural tissues.[8,163]

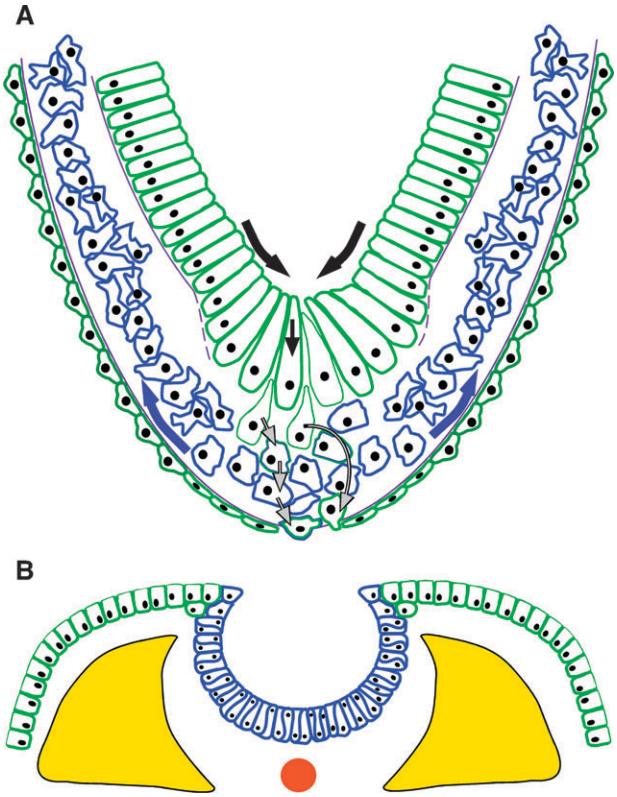

Fig. 2 *Ecadherin* and *Ncadherin* expression during gastrulation and neurulation. Classical cadherins are expressed in a mutually exclusive manner during development. (A) Schematic representation of a transverse section at the primitive streak region of a gastrulating E7.0 mouse embryo (anterior at the top and posterior at the bottom). Cells of the epiblast (upper green cell layer) undergo EMT and delaminate from the embryonic ectoderm, then migrate through the primitive streak laterally towards the anterior end of the embryo to populate the mesoderm (blue cells). During this process, *Ecadherin* expression is downregulated and is replaced by *Ncadherin* expression. Similarly, epiblast-derived cells give rise to definitive endoderm cells (flat green cells) that will line up along the gut tube and displace the visceral endoderm cells (lower layer with dark green cells). It is still unknown whether the definitive endoderm cells delaminate from the epiblast and integrate directly without a complete EMT into the endoderm layer (long grey arrow) or if they first become mesenchymal (loss of E-cadherin) and undergo mesenchymal–epithelial transition (MET) (gain of *Ecadherin* expression) shortly afterwards (short grey arrows). (B) Schematic transverse section at neurulation of an E8.0 embryo with dorsal at the top and ventral at the bottom. Cells from the surface ectoderm are specified to differentiate into neurectoderm to form the neural groove and later the neural tube. Accordingly, *Ecadherin* is downregulated in these cells and *Ncadherin* expression is activated. Only at the non-neural ectoderm/neurectoderm border are *Ecadherin*- and *Ncadherin*-expressing cells directly linked. Green cells express *Ecadherin*, and blue cells *Ncadherin*; in (A) thinner lines indicate lower expression levels; mixed blue and green lines represent cells that undergo switching of cadherin expression; purple lines, basal lamina; in (B): red, notochord; yellow, somites.

Whereas the straight knock-out led to early embryonic lethality, conditional knock-out mice helped to unravel the important and diverse functions of cadherins during later embryogenesis and organogenesis. *Ecadherin* has been genetically inactivated in many different tissues including skin, mammary gland and thyroid gland.[164–168] Depletion of *Ecadherin* from the lactating mammary gland by MMTV-Cre expression leads to premature apoptosis, which under normal conditions is only detectable during involution after weaning. As a result, alveolar structures cannot properly form, organ function is impaired, and the pups are not fed due to the lack of milk.[164] Genetic ablation of *Ecadherin* from the skin has been investigated by different groups with slightly different results. The most plausible explanation of the variable phenotypes is that they are due to differences in the spatio-temporal ablation of *Ecadherin* in the epidermis.[165,167,168] The strongest phenotype is obtained upon *Ecadherin* depletion already around E15.5.[165] This results in perinatal lethality because the barrier function of the skin is impaired. Absence of E-cadherin in the embryonic skin leads to loss of cell polarity and misguidance of zona occludens 1 (ZO-1) or claudin, resulting in improper tight junction formation. Concomittantly, *Pcadherin* and desmosomal cadherins are strongly upregulated in the basal layer of the interfollicular epidermis, providing an explanation for the lack of blistering observed upon *Ecadherin* depletion.[165] In contrast, when *Ecadherin* is depleted postnatally, mice occasionally survive until adulthood.[167,168] In this case, keratinocytes hyperproliferate but differentiation, including hair and whisker formation, is impaired, resulting in progressive loss of hair follicles. Taken together, these findings demonstrate the pivotal role of E-cadherin in organ function by prevention of apoptosis and maintenance of cell polarity.

The straight knock-out of N-cadherin causes embryonic lethality around E10 due to various malformations.[163] Although *Ncadherin* is expressed already after gastrulation, mesoderm formation and neurulation are normal. However, somites form in an irregular shape and the neural tube is undulated. The most striking cell–cell adhesion defect is detectable in the heart, where myocardiac cells dissociate and heart formation is impaired.[163] Interestingly, the heart phenotype can be rescued by ectopic expression of either *Ncadherin* or *Ecadherin* using muscle-specific promoters, but transgenic embryos still suffer from brain malformations.[169] Although the specific deletion of the *Ncadherin* gene in neural crest cells does not affect migration to the cardiac outflow tract, the cells are unable to undergo morphogenetic changes required for remodelling of the outflow tract and the truncus arteriosus persists.[170]

In the developing brain N-cadherin is involved in axonal guidance, in target recognition at the growth cone, and presumably in pathfinding decisions.[19,171] It accumulates at perisynaptic sites and later plays a role in stabilisation of the synapse.[19,172] A role for N-cadherin during long-term potentiation (LTP) has been suggested, since N-cadherin localisation at synapses is increased upon LTP. Conversely, when adhesion is blocked by pretreatment of hippocampal slices with anti-N-cadherin antibodies, LTP is significantly reduced.[173,174] Moreover, it has been shown that N-cadherin is also required for the control of short-term plasticity at glutamatergic synapses at the presynaptic site of ES-cell derived neurons.[175] Thus, N-cadherin plays fundamental roles in myocardial cell adhesion, proper differentiation and patterning of the mesoderm, as well as for neural crest cell rearrangements in the cardiac outflow tract, and for regulation of synapse formation in the central nervous system.

Additional important information about cadherin function was gained from experiments involving misexpression of cadherins. Premature expression of *Ncadherin* already at the 2-cell stage of a *Xenopus* embryo results in abnormal histogenesis, such as thickening, clumping or fusion of cell layers.[160,161] Similarly, misexpression in the ectoderm right before neural induction causes irregular formation of cell boundaries and severe morphological defects, indicating that N-cadherin is involved in controlling morphogenetic changes associated with early neurogenesis.[160] Although the ectopic expression of *Ecadherin* or *Ncadherin* driven by a muscle-specific promoter can rescue the *Ncadherin* knock-out myocardial heart phenotype, overexpression of either one of them causes dilated cardiomyopathy.[176] If *Ecadherin* is overexpressed in the crypts of the intestine, including the stem cell niche, in mice, malformations, altered proliferation and cell migration are observed. In this case cells divide less frequently and migrate more slowly to the villus region and undergo apoptosis more frequently.[177] If a dominant-negative mutant of *Ncadherin* is expressed in the crypt-villus epithelium in the intestine, cells lose their cell–cell and cell–matrix attachments. Enterocytes increase their rate of migration and drop their differentiated polarised phenotype. This causes inflammatory bowel disease and increased formation of adenomas.[148,178]

These experiments vividly demonstrate that cadherin expression is not only required to cluster cells of the same type together, but also that their expression has to be tightly controlled in a specific spatio-temporal manner to allow normal development. In addition, the control of cadherin quantity is of great importance to regulate how much cadherin protein reaches the cell-surface to maintain tissue-integrity and homeostasis. The idea that cadherins are not only responsible for cell sorting and separation of different tissues but also are actively involved in and required for tissue formation is based on two major findings. Firstly, *Ecadherin* null ES cells, unlike their wild-type counterparts, are incapable of differentiating *in vitro* and *in vivo* into derivatives of all three germ-layers.[179] When ES cells are injected under the kidney capsule or subcutaneously for teratoma formation, *Ecadherin* null ES cells do not form organised structures, whereas teratomas of wild-type ES cells are composed of completely differentiated cells of all three germ-layers.[179] The phenotype of *Ecadherin* null ES cells can be rescued by constitutive expression of *Ecadherin* or *Ncadherin* after transfection. Of note, overexpression of *Ecadherin* exclusively leads to the formation of epithelia, whereas *Ncadherin* expression generates predominantly neuroepithelia and cartilage but no epithelia.[179] Secondly, with a few exceptions like the lens, *Ecadherin* and *Ncadherin* are expressed in a mutually exclusive manner. Often cadherin expression is switched during important morphogenetic events, *e.g.* as observed during gastrulation or in neural induction. However, whether the switch is just a consequence

of the change of the morphogenetic program or whether it is required and precedes these events is still unknown. E- and N-cadherins are very similar in aa sequence with about 64% similarity in the extracellular domain, 70% in the entire cytoplasmic tail and 84% in the β-catenin binding domain (Fig. 1).[10] They both are complexed with catenins and mediate predominantly homophilic interactions. These facts might suggest that they are functionally identical and act redundantly. This issue has recently been addressed using a gene replacement strategy to express *Ncadherin* in the *Ecadherin* expression domain.[157] Interestingly, mice coexpressing both cadherins do not show any obvious phenotype. In homozygous mutant embryos, N-cadherin is able to functionally substitute for E-cadherin during morula compaction and in epithelia generated in teratomas. Surprisingly, N-cadherin cannot replace E-cadherin during the formation of a blastocyst, and *Ncadherin* knock-in embryos phenocopy the *Ecadherin* null phenotype. This strongly indicates that for trophectoderm formation special structural and/or signalling properties of E-cadherin are required.[157] Possibly, E- and N-cadherin differ in some intercellular binding partners and in adhesive strength, or else the forced switch in cadherin expression may alter intercellular signalling. The observation that E-cadherin preferentially interacts with the EGFR, while N-cadherin preferentially interacts with FGFR1 are in line with this hypothesis.[94,96,106,107] It will be very interesting to further characterise the special requirements and to see which part of the E-cadherin molecule is essential for trophectoderm formation, thereby elucidating the functional and molecular differences between these two related classical cadherins.

The cadherin-switch during cancer progression

The most frequent cancer types develop from epithelial cells of the endodermal lineage. During tumour formation, a small number of cells start to hyperproliferate and, after acquisition of a cascade of different mutations in the genes encoding p53, β-catenin or APC, Ras *etc.*, a full-blown tumour develops. Altered expression of cadherins plays a major part in the progression of tumourigenesis and influences invasion. Importantly, the transition from a well-differentiated benign adenoma to a dedifferentiated invasive carcinoma usually results in a poor prognosis for the patient. This malignant transformation is often correlated with strong reduction in cell–cell adhesion combined with alterations in signal transduction pathways.[180–182] Correspondingly, the expression of classical cadherins in the tumour cells is modified, which leads to loss of adhesion and enhances migration.[182] On a cellular level, the occurrence of an EMT is the driving force in these pathological situations towards malignancy.[98,183,184] EMT during tumourigenesis is a degenerated process also observed in physiological events in normal development (Fig. 2). During gastrulation, the downregulation of *Ecadherin* is a hallmark of EMT, and therefore E-cadherin is regarded as a tumour or invasion suppressor.[185,186] This is evident from the fact that carcinoma cells can be reverted to a normal epithelial phenotype by overexpression of *Ecadherin*.[186] Loss of *Ecadherin* expression can be due to transcriptional inactivation by hypermethylation of the promoter,[187–190] upregulation of repressors of the Snail, ZEB and basic helix–loop–helix families,[182] mutations in the coding region[191] and/or loss of heterozygosity. In many tumours, *Ecadherin* downregulation is correlated with *de novo* expression of *Ncadherin* or cadherin-11.[192,193] The presence of N-cadherin leads to increased cell motility and migration; hence, throwing the cadherin-switch induces an invasive phenotype.[193–197] If normal squamous epithelial cell lines are transfected to ectopically express *Ncadherin*, they acquire migratory properties and become more motile.[198] The domain of N-cadherin that confers motility has been mapped to a region of 80 aa within the EC4 that is also required for interaction with FGFR1.[101,102] In some tumour cell lines, *Ncadherin* expression is even dominant over E-cadherin. In these cases, despite the presence of E-cadherin, cells cannot be maintained in a polarised non-migratory state.[197] *Ecadherin* downregulation with *Ncadherin de novo* expression, is also observed in several human tumour specimens and in a Rip1-Tag2 mouse tumour model, in which tumour progression is accelerated by overexpression of Igf1r in pancreatic β-cells.[193,199] One possible explanation for the transition to a malignant phenotype after E-cadherin to N-cadherin switching could be that the cells that ectopically express *Ncadherin* try to migrate into tissue with a similar cadherin expression profile, hence away from the epithelial environment.[180] On the other hand, altered cadherin expression may change intracellular signalling, which then leads to depolarisation of cells and to gain of migratory properties.[180,200] This hypothesis is supported by the finding that specific interaction of N-cadherin with FGFR leads to ligand-independent activation of the receptor.[99,101,107] Also signalling pathways might change upon downregulation of *Ecadherin*, including modulation of RTK signalling, activation of the Wnt/β-catenin cascade, or RhoGTPase signalling.[180] However, the molecular mechanisms that govern the transition from benign to malignant tumours and the role of the cadherin-switch in these processes are still poorly understood. Analogous to the developmental setting it is of special interest to elucidate whether the cadherin switch is just a consequence of the EMT program or whether it is inducing signalling cascades that lead to EMT.

Transcriptional regulation of cadherins

As outlined in the previous paragraphs, E-cadherin is important for mammalian preimplantation development, organogenesis and morphogenetic processes, for proper tissue formation and for maintenance of its integrity. The pivotal role of E-cadherin in specific aspects of development and its function in preventing invasiveness during carcinogenesis require that the gene is tightly regulated. Besides the regulation during gastrulation and neurulation where expression declines, *Ecadherin* becomes upregulated during kidney organogenesis in cells of the metanephric mesenchyme that undergo mesenchymal–epithelial transitions (MET) in the uretic bud,[201] in restricted areas of the developing brain[202–204] and during melanocyte differentiation prior to entry into the epidermis.[205]

The gene that encodes E-cadherin (*Cdh1*, *Ecadherin*) is located in a cluster with other cadherins including P-cadherin (*Cdh3*, *Pcadherin*), in mouse on chromosome 8 and in humans on chromosome 16. The *Ecadherin* promoter lacks a TATA-

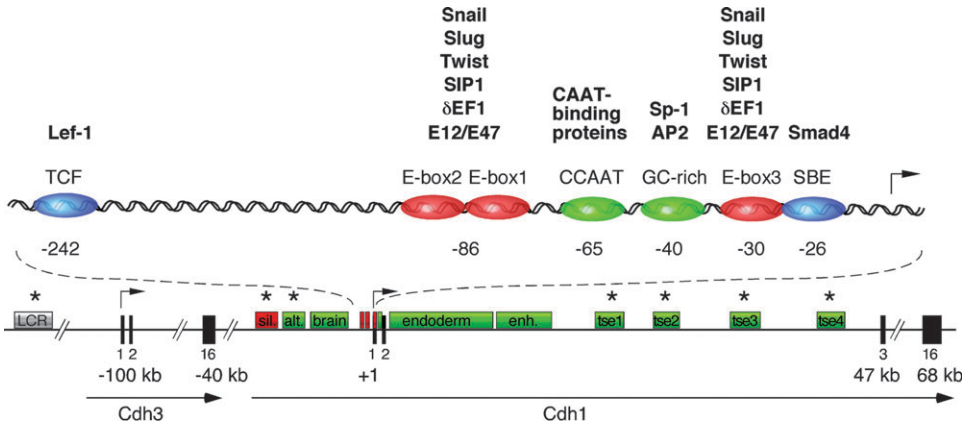

Fig. 3 Scheme of the *Ecadherin* locus and location of known *cis* regulatory elements. The blow-up shows the proximal promoter and factors that are known to bind there. The lower part shows the *Ecadherin* (*Cdh1*) locus located in a cluster with *Pcadherin* (*Cdh3*) and the *cis*-regulatory landscape of regions found to be essential for *Ecadherin* gene activation.[162,227] The results from reporter gene analysis suggest that additional *cis*-regulatory elements are located outside of the analysed region, possibly far upstream of *Pcadherin*. They may function as a locus control region (LCR) for the cadherin cluster. E-boxes are represented by red boxes, and sequences with enhancing activities by green boxes; alt., sequences that mediate alternative, intron 2-independent gene activation in late embryogenesis; brain, sequences that contribute to brain-specific expression; endoderm, sequences required for endoderm-specific expression; enh., sequences that generally enhance transcription; sil., brain-specific silencer; tse1-4, tissue-specific enhancers, including elements for ectoderm-specific expression. Note that the existence and the location of regions labelled with an asterisk are only postulated from results described in Fig. 4.

box, but contains a CCAAT-box at −65 and a GC-rich region at −40, which are bound by the CAAT-binding proteins, and AP2 and Sp1, respectively (Fig. 3).[206–210] In addition, an E-pal element has been found in the murine promoter at −86 that consists of two E-boxes (E-box1 and 2), of which E-box1 is conserved in human and dog promoters.[209] Further analyses identified two additional E-boxes in the human promoter (E-box3 and 4), of which E-box3 is present in mouse and dog sequences as well at −30. The fourth E-box at position +22 in the human gene is not conserved. Additionally, a binding site for Lef/TCF factors has been found in the murine promoter at −242 (Fig. 3). Considerable progress has been made towards unravelling various aspects of *Ecadherin* gene regulation. Many transcriptional repressors have been identified that bind to the E-boxes in the promoter region during normal development as well as in the pathogenic situation in tumours and efficiently induce a downregulation of *Ecadherin* transcription (Fig. 3). The zinc-finger proteins of the Snail superfamily SNAI1 and SNAI2/Slug are key players in mediating EMT and integrate signals from different pathways including TGFβ, Wnt and EGF.[182,211] During gastrulation *Ecadherin* is downregulated in the mesenchyme, most likely due to the binding of Snail superfamily members to the various E-boxes in the *Ecadherin* promoter.[212–214] If Snail or Slug is overexpressed in epithelial cell lines, cells adopt a fibroblastoid phenotype and lose *Ecadherin* expression.[213,214] In Snail null embryos mesoderm cells do not delaminate from the epiblast and *Ecadherin* expression is not properly repressed.[158] Similarly, in various cancer cell lines an inverse correlation of Snail or Slug and *Ecadherin* expression has been demonstrated, indicating that these repressors are ectopically upregulated and induce EMT including *Ecadherin* downregulation.[212–215] In the context of tumourigenesis a variety of other factors have been identified that are upregulated in malignant tumours and correlate with poor prognosis and downregulation of *Ecadherin*. Inactivation of these factors changes the properties of the cell to a less migratory, less invasive phenotype due to re-expression of *Ecadherin*. These factors include the bHLH repressors Twist1, best studied in *Drosophila* where it is involved in mesoderm generation and differentiation,[216,217] the E2A gene product E12/E47,[218,219] and the multi-zinc finger proteins ZEB-1/ZFHX1A/δEF1/TCF8 and ZEB-2/ZFHX1B/SIP1 (Smad-interacting protein 1)[220–223] (Fig. 3). Similar to Snail, they all repress *Ecadherin* transcription upon binding to the E-boxes within the promoter. During gastrulation TGFβ signalling is active and can upregulate expression of δEF1 and SIP1 for EMT induction. This was demonstrated in NMuMG cells, where TGFβ treatment leads to activation of δEF1 and SIP1, resulting in loss of *Ecadherin* expression.[223] A different type of repression is observed at the Lef/TCF-site. This element binds Lef-1 in electromobility shift assays,[224] and it was proposed that Lef-1 together with β-catenin downregulates *Ecadherin* during gastrulation.[159] However, it has been shown that Lef-1 mediates this downregulation during hair follicle bud formation.[225] Here, inactivation of the *Ecadherin* locus is achieved independent of the E-boxes and does not require the action of Snail, but the presence of the Lef/TCF-site.[225] In a recent study, morphogenetic events during palate formation have been analysed with respect to the molecular players involved.[226] For proper craniofacial development, dissociation of the medial-edge epithelium (MEE) is important, and this depends on TGFβ3-mediated signalling, which promotes loss of *Ecadherin* expression.[226] During this morphogenetic event, Lef-1, Smad-2P and Smad4 form a complex and bind to the *Ecadherin* promoter *via* the Lef/TCF-binding site, the E-pal motif and an SBE (Smad-binding element) at −26 (Fig. 3).[226]

Since the majority of known regulatory events involve transcriptional repression, a recent model for *Ecadherin* regulation proposed that the gene is constitutively active due to

the presence of the positively acting CCAAT-box and the GC-rich elements. Furthermore, histone acetylation maintains *Ecadherin* promoter chromatin in a state accessible for the transcription machinery. Upon signalling events that induce EMT, several transcription repressors and co-repressors are recruited to the E-boxes. This in turn attracts histone deacetylases and specific histone methylases to bind, leading to epigenetic gene silencing.[182] Based on this model, proper regulation is achieved by the presence or absence of repressors. However, we have shown that the *Ecadherin* promoter alone is insufficient to properly reflect endogenous gene activity.[162,227] Transgenic analyses of mouse promoter fragments revealed that the *Ecadherin* promoter is silent or activates a β-galactosidase reporter gene in ectopic regions of the embryo. Mapping of DNAseI-hypersensitive sites (DHSs) has been done between −15 and +18 kb and revealed DHSs mainly located in intron 2 sequences (Fig. 4). E-cadherin-specific expression is only observed in the epithelium of the endoderm if the majority of mapped DHSs are included in the transgene.[227] Yet, the entire expression of *Ecadherin* was still not recapitulated with sequences between −6 and +16 kb (Fig. 4). When the entire 47 kb of intron 2 sequences were removed by homologous recombination at the endogenous locus, E-cadherin-specific expression was completely lost during early embryogenesis.[162] These results show that *Ecadherin* expression is complex and depends on multiple *cis*-regulatory elements located downstream of the promoter.[162,227] However, BAC transgenic mice that carry a similar *lacZ* reporter and cover the entire locus between −25 kb (close to the 3′ end of *Pcadherin*) and +140 kb fail to recapitulate *Ecadherin* expression completely (M.S., unpublished data), whereas mice carrying an E-cadherin-*lacZ* knock-in allele, faithfully recapitulate the *Ecadherin* gene expression pattern (Fig. 4).[162] Based on these results, and since *Ecadherin* is located in a cluster together with *Pcadherin*, *VEcadherin* and others, it is tempting to speculate that correct high level spatio-temporal expression of the cadherins within the cluster depends on a distantly located locus control region (LCR) (Fig. 3). This kind of gene regulation has been best analysed at the β-globin gene cluster, but is also known from other gene clusters, such as the *HoxD* genes.[228,229]

The rapid switch from the expression of one cadherin to another (Fig. 2) requires gene regulation beyond transcription and involves control of transcript and protein levels as well. This is even more plausible since the protein stability of E-cadherin is very high, with a half-life between 5 and 8 hours.[76,230,231] Therefore, in addition to the transcriptional regulation, it is important to tightly control protein amounts on the membrane. One mechanism involves regulation of E-cadherin protein turnover and an increase in endocytosis upon stimulation with growth factors as was demonstrated in cultured cells.[232,233] During gastrulation rapid E-cadherin

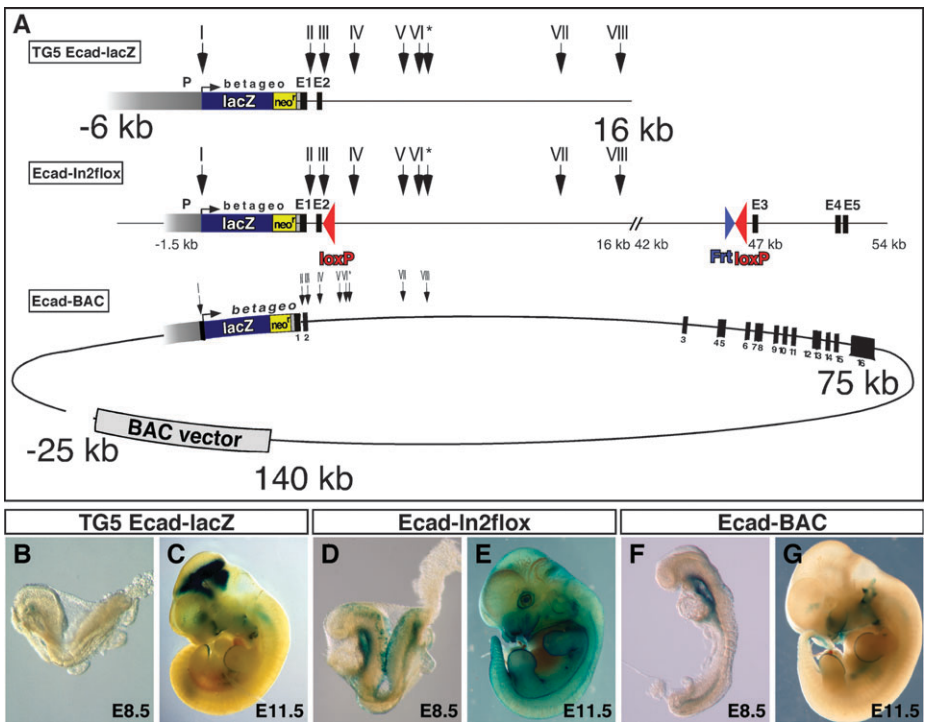

Fig. 4 Summary of reporter gene expression from different *Ecadherin* transgene and the Ecad-In2flox knock-in alleles. (A) Scheme of reporter alleles. Analyses with transgenic constructs were carried out with sequences of the *Ecadherin* gene between −6 and +16 kb (Ecad-*lacZ*);[227] the knock-in allele contains betageo sequences in the endogenous *Ecadherin* locus in addition to *loxP* sites flanking intron 2;[162] for transgenesis using BAC vectors, betageo was inserted at a similar position in a vector that contains *Ecadherin* sequences between −25 kb and +140 kb. Positions of DHSs are indicated by vertical arrows. (B–G) Reporter gene activity of representative embryos of all alleles as displayed by staining with X-gal is shown as indicated at E8.5 (B,D,F) and at E11.5 (C,E,G). Endogenous *Ecadherin* expression is faithfully recapitulated by the Ecad-In2flox knock-in allele, but with the TG5 Ecad-*lacZ* transgene only endoderm-specific expression and ectopic expression in the brain are found. Surprisingly, the Ecad-BAC transgene also cannot completely reflect the endogenous expression seen with the knock-in allele.

degradation is achieved by the activation of p38 MAPK and p38-interacting protein. If p38IP function is impaired, embryos die due to gastrulation defects in which migration of the mesoderm is impaired because E-cadherin is not properly downregulated.[234] This mutation phenocopies Snail null mice, which die at gastrulation and are incapable of shutting down *Ecadherin* transcription in the presumptive mesoderm.[158] In a recent study activin/nodal-signalling-mediated E-cadherin endocytosis has been described to modulate adhesion during gastrulation. The induced expression and interaction of Fibronectin Leucine-rich Repeat Transmembrane 3 (FLRT3) with the small GTPase Rnd1 control cell surface levels of cadherin through a dynamin-dependent endocytosis pathway.[235] Similarly, tyrosine phosphorylation of E-cadherin at residues Y755 and Y756 recruits Hakai, and targets E-cadherin to the degradation pathway (see above).[121,122]

The gene that encodes N-cadherin (*Cdh2*, *Ncadherin*) is located on chromosome 18 in mouse and in humans. A genomic organization of the gene identical with that of *Ecadherin* is observed, which includes a large intron 2. Unfortunately, very little is known about the transcriptional regulation of *Ncadherin* expression. A similarly complex mechanism as for *Ecadherin* is likely since the *Ncadherin* promoter alone is also insufficient to recapitulate *Ncadherin* expression.[236] Some lines of evidence led to the hypothesis that downstream sequences are involved as well, including intron 2. This idea is further supported by the identification of neural and placodal enhancers dispersed throughout large parts of the *Ncadherin* locus. In part, these enhancers contain SOX-binding sites, and misexpression of Group B1 Sox genes in chicken embryos generates ectopic placodes expressing *Ncadherin*.[237] One of the few transcription factors known to be involved in *Ncadherin* expression is GATA4. This factor is indispensable for high-level expression of *Ncadherin* in the heart. If GATA4 is knocked-down by siRNA, the expression of *Ncadherin* is reduced.[238] Interestingly, Twist1 also plays a role in *Ncadherin* control. Twist1 can induce *Ncadherin* expression in prostate cancer cells,[239] a fact that is interesting with respect to the cadherin-switch. Twist may be capable of simultaneously downregulating *Ecadherin* and upregulating *Ncadherin* during EMT, which might provide an explanation for the cadherin-switch observed in development and cancer.

Perspective

The initial concept of differential adhesion and homophilic interaction was largely elaborated on a limited number of different cadherin molecules. The fact that the expression patterns of the individual cadherins are restricted and are mutually exclusive supported the idea that their ability to separate cells is essential for morphogenesis.[41,136,137] Meanwhile the number of cadherin genes has risen to over 100 members. The large number of cadherin genes and the increasing experimental evidence suggest that homophilic and heterophilic interactions coexist and may exhibit functional differences during development. In early embryonic stages (preimplantation, gastrulation) homophilic interaction mediated predominantly by E- and N-cadherin is essential for strong cell adhesion and tissue segregation. During mid to late gestation the number of different cell types is increasing and requires fine-tuning of cadherin expression for proper morphogenesis. Hence, to fine-tune cadherin function at the plasma membrane heterophilic adhesion is used in parallel. Surprisingly, the 3D structure of the molecular surface for interaction is very similar between different cadherins and is likely incapable of allowing homophilic adhesion exclusively.[21–23,25,240] Based on these findings the homophilic adhesion concept needs to be reinvestigated. However, cell separation, aggregation and sorting can additionally be achieved by differential expression levels as demonstrated by Steinberg and colleagues.[138,140] As a consequence of the evolutionarily increased number of different cell types, an efficient mechanism was required to allow cell separation and sorting in the developing embryo. Differential expression levels may be generated by various gene duplications of an ancestor cadherin gene combined with individual modifications of gene regulation mechanisms. In that case the large number of cadherin genes reflects the increasing need for different cadherin concentrations on the plasma membrane to distinguish different cell types. Nonetheless, cell sorting during development is presumably based on a combination of all described processes. Differential gene expression may control the actual cadherin amount on the surface, combined with the variation of coexpressed subtypes. Small molecular differences in the adhesive surface of individual cadherins may contribute to shift the equilibrium from heterophilic to homophilic interaction. To which extent these mechanisms contribute to differential adhesion and sorting will be one focus of future studies.

Many other questions are still not entirely answered from analysis of classical cadherins. It still remains elusive how the cadherin molecules influence cellular morphology and properties. N-Cadherin in particular has a bipartite function in different cell types and manifests entirely different characteristics: *Ncadherin* expression in the neural tube results in cell–cell adhesion, cell polarization and establishment of adherens junctions whereas *Ncadherin* expression in mesodermal and invasive cancer cells enables migration accompanied by a depolarized phenotype. How this switching in molecular function of the same protein is achieved and controlled remains largely unknown. To elucidate these questions, more detailed analyses *in vivo* are necessary. Experimental fine-tuning of cadherin expression in cells or embryos will help toward understanding the structure, function and multimeric complex formation of the molecules on the surface of the cell. Specific switching of cadherin expression domains can unravel unique functions of individual members involved in morphogenesis, tissue function and cancer progression.

Glossary

aa, amino acid residues; ADAM, assignment of a disintegrin and metalloproteinase domain; AJ, adherens junction; APC, adenomatous polyposis coli; ARVCF, armadillo repeat gene deleted in velocardiofacial syndrome; BAC, bacterial artificial chromosome; bHLH, basic helix–loop–helix; BMP, bone morphogenetic protein; CAM, cell adhesion molecule; CBP, CREB-binding protein; CK, casein kinase; CREB, cAMP

response element-binding; CTF, c-terminal fragment; δEF1, delta-crystallin enhancer-binding protein; DEP-1, density-enhanced phosphatase-1; DHS, DNAseI-hypersensitive site; EC, extracellular cadherin domain; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; EPLIN, epithelial protein lost in neoplasia; ER, endoplasmatic reticulum; ERK, extracellular signal-regulated kinase; ES, embryonic stem; FGFR, fibroblast growth factor receptor; FLRT3, fibronectin leucine-rich repeat transmembrane 3; GAP, GTPase activating proteins; GDI, GDP dissociation inhibitors; GEF, guanine nucleotide exchange factor; GSK3β, glycogen synthase kinase 3β; Igf1r, insulin-like growth factor 1 receptor; IQGAP, IQ-domain containing GAP; kb, kilobase; LCR, locus control region; Lef, lymphoid enhancer factor; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MEE, medial-edge epithelium; MET, mesenchymal–epithelial transition; MMTV, mouse mammary tumour virus; p38IP, p38-interacting protein; PC, proprotein convertase; PTP, protein tyrosine phosphatase; PTP-LAR, protein tyrosine phosphatase-leukocyte antigen-related; RPTP, receptor tyrosine phosphatase; RTK, receptor tyrosine kinase; SBE, smad-binding element; SIP1, smad-interacting protein 1; TCF, T-cell factor; TCF8, transcription factor 8; TGFβ, transforming growth factor; VE-PTP, vascular endothelial tyrosine phosphatase; ZEB, zinc finger E-box binding homeobox; ZFHX, zinc finger homeobox; ZO-1, zona occludens-1.

Acknowledgements

I am grateful to Andreas Hecht, Rolf Kemler, Lenka Libusová and Verdon Taylor for helpful discussions. I thank Randy Cassada for critically reading the manuscript. This work was supported by the Max-Planck Society.

References

1. M. S. Steinberg and S. F. Gilbert, *J. Exp. Zool.*, 2004, **301**, 701–706.
2. A. Moscona, *Science*, 1957, **125**, 598–599.
3. M. Takeichi, *J. Cell Biol.*, 1977, **75**, 464–474.
4. F. Hyafil, C. Babinet and F. Jacob, *Cell*, 1981, **26**, 447–454.
5. R. Kemler, C. Babinet, H. Eisen and F. Jacob, *Proc. Natl. Acad. Sci. U. S. A.*, 1977, **74**, 4449–4452.
6. R. Bertolotti, U. Rutishauser and G. M. Edelman, *Proc. Natl. Acad. Sci. U. S. A.*, 1980, **77**, 4831–4835.
7. R. Brackenbury, U. Rutishauser and G. M. Edelman, *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 387–391.
8. K. Hatta and M. Takeichi, *Nature*, 1986, **320**, 447–449.
9. E. Hill, I. D. Broadbent, C. Chothia and J. Pettitt, *J. Mol. Biol.*, 2001, **305**, 1011–1024.
10. F. Nollet, P. Kools and F. van Roy, *J. Mol. Biol.*, 2000, **299**, 551–572.
11. M. Goodwin and A. S. Yap, *J. Mol. Histol.*, 2004, **35**, 839–844.
12. R. Kemler, *Semin. Cell Biol.*, 1992, **3**, 149–155.
13. M. D. Kottke, E. Delva and A. P. Kowalczyk, *J. Cell Sci.*, 2006, **119**, 797–806.
14. T. Yin and K. J. Green, *Semin. Cell Dev. Biol.*, 2004, **15**, 665–677.
15. M. Takeichi, *Nat. Rev. Neurosci.*, 2007, **8**, 11–20.
16. T. Tanoue and M. Takeichi, *J. Cell Sci.*, 2005, **118**, 2347–2353.
17. C. Redies, K. Vanhalst and F. Roy, *Cell. Mol. Life Sci.*, 2005, **62**, 2840–2852.
18. M. Frank and R. Kemler, *Curr. Opin. Cell Biol.*, 2002, **14**, 557–562.
19. D. Junghans, I. G. Haas and R. Kemler, *Curr. Opin. Cell Biol.*, 2005, **17**, 446–452.
20. Q. Wu and T. Maniatis, *Cell*, 1999, **97**, 779–790.
21. T. J. Boggon, J. Murray, S. Chappuis-Flament, E. Wong, B. M. Gumbiner and L. Shapiro, *Science*, 2002, **296**, 1308–1313.
22. O. Pertz, D. Bozic, A. W. Koch, C. Fauser, A. Brancaccio and J. Engel, *EMBO J.*, 1999, **18**, 1738–1747.
23. L. Shapiro, A. M. Fannon, P. D. Kwong, A. Thompson, M. S. Lehmann, G. Grubel, J. F. Legrand, J. Als-Nielsen, D. R. Colman and W. A. Hendrickson, *Nature*, 1995, **374**, 327–337.
24. D. Leckband and A. Prakasam, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 259–287.
25. S. D. Patel, C. Ciatto, C. P. Chen, F. Bahna, M. Rajebhosale, N. Arkus, I. Schieren, T. M. Jessell, B. Honig, S. R. Price and L. Shapiro, *Cell*, 2006, **124**, 1255–1268.
26. U. Tepass, K. Truong, D. Godt, M. Ikura and M. Peifer, *Nat. Rev. Mol. Cell Biol.*, 2000, **1**, 91–100.
27. H. Oda, T. Uemura, Y. Harada, Y. Iwai and M. Takeichi, *Dev. Biol.*, 1994, **165**, 716–726.
28. H. Oda, T. Uemura and M. Takeichi, *Genes Cells*, 1997, **2**, 29–40.
29. H. C. Korswagen, M. A. Herman and H. C. Clevers, *Nature*, 2000, **406**, 527–532.
30. M. Ozawa and R. Kemler, *J. Cell Biol.*, 1990, **111**, 1645–1650.
31. H. Posthaus, C. M. Dubois, M. H. Laprise, F. Grondin, M. M. Suter and E. Muller, *FEBS Lett.*, 1998, **438**, 306–310.
32. A. W. Koch, A. Farooq, W. Shan, L. Zeng, D. R. Colman and M. M. Zhou, *Structure*, 2004, **12**, 793–805.
33. S. Butz and R. Kemler, *FEBS Lett.*, 1994, **355**, 195–200.
34. L. Hinck, I. S. Nathke, J. Papkoff and W. J. Nelson, *J. Cell Biol.*, 1994, **125**, 1327–1340.
35. M. Ozawa, H. Baribault and R. Kemler, *EMBO J.*, 1989, **8**, 1711–1717.
36. Y. T. Chen, D. B. Stewart and W. J. Nelson, *J. Cell Biol.*, 1999, **144**, 687–699.
37. A. H. Huber, D. B. Stewart, D. V. Laurents, W. J. Nelson and W. I. Weis, *J. Biol. Chem.*, 2001, **276**, 12301–12309.
38. J. Gates and M. Peifer, *Cell*, 2005, **123**, 769–772.
39. W. I. Weis and W. J. Nelson, *J. Biol. Chem.*, 2006, **281**, 35593–35597.
40. J. M. Halbleib and W. J. Nelson, *Genes Dev.*, 2006, **20**, 3199–3214.
41. A. Nose, K. Tsuji and M. Takeichi, *Cell*, 1990, **61**, 147–155.
42. W. S. Shan, H. Tanaka, G. R. Phillips, K. Arndt, M. Yoshida, D. R. Colman and L. Shapiro, *J. Cell Biol.*, 2000, **148**, 579–590.
43. R. B. Troyanovsky, E. Sokolov and S. M. Troyanovsky, *Mol. Cell. Biol.*, 2003, **23**, 7965–7972.
44. C. P. Chen, S. Posy, A. Ben-Shaul, L. Shapiro and B. H. Honig, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 8531–8536.
45. E. Perret, A. Leung, H. Feracci and E. Evans, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 16472–16477.
46. W. Baumgartner, P. Hinterdorfer, W. Ness, A. Raab, D. Vestweber, H. Schindler and D. Drenckhahn, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 4005–4010.
47. E. Perret, A. M. Benoliel, P. Nassoy, A. Pierres, V. Delmas, J. P. Thiery, P. Bongrand and H. Feracci, *EMBO J.*, 2002, **21**, 2537–2546.
48. S. Sivasankar, B. Gumbiner and D. Leckband, *Biophys. J.*, 2001, **80**, 1758–1768.
49. Y. S. Chu, W. A. Thomas, O. Eder, F. Pincet, E. Perez, J. P. Thiery and S. Dufour, *J. Cell Biol.*, 2004, **167**, 1183–1194.
50. Y. S. Chu, O. Eder, W. A. Thomas, I. Simcha, F. Pincet, A. Ben-Ze'ev, E. Perez, J. P. Thiery and S. Dufour, *J. Biol. Chem.*, 2006, **281**, 2901–2910.
51. D. Vestweber and R. Kemler, *Cell Differ.*, 1984, **15**, 269–273.
52. S. Butz, J. Stappert, H. Weissig and R. Kemler, *Science*, 1992, **257**, 1142–1144.
53. R. Kemler, *Trends Genet.*, 1993, **9**, 317–321.
54. M. Ozawa, M. Ringwald and R. Kemler, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 4246–4250.
55. J. Stappert and R. Kemler, *Cell Adhes. Commun.*, 1994, **2**, 319–327.
56. A. H. Huber and W. I. Weis, *Cell*, 2001, **105**, 391–402.
57. K. M. Cadigan and R. Nusse, *Genes Dev.*, 1997, **11**, 3286–3305.
58. H. Clevers, *Cell*, 2006, **127**, 469–480.

59. K. Willert and K. A. Jones, *Genes Dev.*, 2006, **20**, 1394–1404.
60. S. Hoppler and C. L. Kavanagh, *J. Cell Sci.*, 2007, **120**, 385–393.
61. H. Aberle, S. Butz, J. Stappert, H. Weissig, R. Kemler and H. Hoschuetzky, *J. Cell Sci.*, 1994, **107**(Pt 12), 3655–3663.
62. M. Itoh, A. Nagafuchi, S. Moroi and S. Tsukita, *J. Cell Biol.*, 1997, **138**, 181–192.
63. K. A. Knudsen, A. P. Soler, K. R. Johnson and M. J. Wheelock, *J. Cell Biol.*, 1995, **130**, 67–77.
64. E. E. Weiss, M. Kroemker, A. H. Rudiger, B. M. Jockusch and M. Rudiger, *J. Cell Biol.*, 1998, **141**, 755–764.
65. K. Abe and M. Takeichi, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 13–19.
66. V. Vasioukhin, C. Bauer, L. Degenstein, B. Wise and E. Fuchs, *Cell*, 2001, **104**, 605–617.
67. T. Oyama, Y. Kanai, A. Ochiai, S. Akimoto, T. Oda, K. Yanagihara, A. Nagafuchi, S. Tsukita, S. Shibamoto, F. Ito, M. Takeichi, H. Matsuda and S. Hirohashi, *Cancer Res.*, 1994, **54**, 6282–6287.
68. A. Nagafuchi, S. Ishihara and S. Tsukita, *J. Cell Biol.*, 1994, **127**, 235–245.
69. A. S. Yap, C. M. Niessen and B. M. Gumbiner, *J. Cell Biol.*, 1998, **141**, 779–789.
70. A. B. Reynolds, J. Daniel, P. D. McCrea, M. J. Wheelock, J. Wu and Z. Zhang, *Mol. Cell. Biol.*, 1994, **14**, 8333–8342.
71. A. B. Reynolds, D. J. Roesel, S. B. Kanner and J. T. Parsons, *Mol. Cell. Biol.*, 1989, **9**, 629–638.
72. P. Z. Anastasiadis and A. B. Reynolds, *J. Cell Sci.*, 2000, **113**(Pt 8), 1319–1334.
73. A. B. Reynolds and A. Roczniak-Ferguson, *Oncogene*, 2004, **23**, 7947–7956.
74. K. C. Miranda, S. R. Joseph, A. S. Yap, R. D. Teasdale and J. L. Stow, *J. Biol. Chem.*, 2003, **278**, 43480–43488.
75. M. A. Thoreson, P. Z. Anastasiadis, J. M. Daniel, R. C. Ireton, M. J. Wheelock, K. R. Johnson, D. K. Hummingbird and A. B. Reynolds, *J. Cell Biol.*, 2000, **148**, 189–202.
76. R. C. Ireton, M. A. Davis, J. van Hengel, D. J. Mariner, K. Barnes, M. A. Thoreson, P. Z. Anastasiadis, L. Matrisian, L. M. Bundy, L. Sealy, B. Gilbert, F. van Roy and A. B. Reynolds, *J. Cell Biol.*, 2002, **159**, 465–476.
77. M. A. Davis, R. C. Ireton and A. B. Reynolds, *J. Cell Biol.*, 2003, **163**, 525–534.
78. K. Xiao, D. F. Allison, K. M. Buckley, M. D. Kottke, P. A. Vincent, V. Faundez and A. P. Kowalczyk, *J. Cell Biol.*, 2003, **163**, 535–545.
79. M. Perez-Moreno, M. A. Davis, E. Wong, H. A. Pasolli, A. B. Reynolds and E. Fuchs, *Cell*, 2006, **124**, 631–644.
80. M. A. Davis and A. B. Reynolds, *Dev. Cell*, 2006, **10**, 21–31.
81. G. A. Wildenberg, M. R. Dohn, R. H. Carnahan, M. A. Davis, N. A. Lobdell, J. Settleman and A. B. Reynolds, *Cell*, 2006, **127**, 1027–1039.
82. L. Van Aelst and H. T. Cline, *Curr. Opin. Neurobiol.*, 2004, **14**, 297–304.
83. V. M. Braga and A. S. Yap, *Curr. Opin. Cell Biol.*, 2005, **17**, 466–474.
84. A. L. Knox and N. H. Brown, *Science*, 2002, **295**, 1285–1288.
85. V. M. Braga, A. Del Maschio, L. Machesky and E. Dejana, *Mol. Biol. Cell*, 1999, **10**, 9–22.
86. V. M. Braga, L. M. Machesky, A. Hall and N. A. Hotchin, *J. Cell Biol.*, 1997, **137**, 1421–1431.
87. L. Weissbach, J. Settleman, M. F. Kalady, A. J. Snijders, A. E. Murthy, Y. X. Yan and A. Bernards, *J. Biol. Chem.*, 1994, **269**, 20517–20521.
88. M. Fukata, S. Kuroda, M. Nakagawa, A. Kawajiri, N. Itoh, I. Shoji, Y. Matsuura, S. Yonehara, H. Fujisawa, A. Kikuchi and K. Kaibuchi, *J. Biol. Chem.*, 1999, **274**, 26044–26050.
89. S. Kuroda, M. Fukata, M. Nakagawa, K. Fujii, T. Nakamura, T. Ookubo, I. Izawa, T. Nagase, N. Nomura, H. Tani, I. Shoji, Y. Matsuura, S. Yonehara and K. Kaibuchi, *Science*, 1998, **281**, 832–835.
90. J. Noritake, M. Fukata, K. Sato, M. Nakagawa, T. Watanabe, N. Izumi, S. Wang, Y. Fukata and K. Kaibuchi, *Mol. Biol. Cell*, 2004, **15**, 1065–1076.
91. R. Zhou, Z. Guo, C. Watson, E. Chen, R. Kong, W. Wang and X. Yao, *Mol. Biol. Cell*, 2003, **14**, 1097–1108.
92. S. Yamashiro, H. Abe and I. Mabuchi, *Dev. Biol.*, 2007, **308**, 485–493.
93. H. Hoschuetzky, H. Aberle and R. Kemler, *J. Cell Biol.*, 1994, **127**, 1375–1380.
94. S. Pece and J. S. Gutkind, *J. Biol. Chem.*, 2000, **275**, 41227–41233.
95. X. Qian, T. Karpova, A. M. Sheppard, J. McNally and D. R. Lowy, *EMBO J.*, 2004, **23**, 1739–1748.
96. M. Fedor-Chaiken, P. W. Hein, J. C. Stewart, R. Brackenbury and M. S. Kinch, *Cell Commun. Adhes.*, 2003, **10**, 105–118.
97. R. T. Bottcher and C. Niehrs, *Endocr. Rev.*, 2005, **26**, 63–77.
98. J. P. Thiery, *Nat. Rev. Cancer*, 2002, **2**, 442–454.
99. U. Cavallaro, J. Niedermeyer, M. Fuxa and G. Christofori, *Nat. Cell Biol.*, 2001, **3**, 650–657.
100. E. Sanchez-Heras, F. V. Howell, G. Williams and P. Doherty, *J. Biol. Chem.*, 2006, **281**, 35208–35216.
101. E. J. Williams, G. Williams, F. V. Howell, S. D. Skaper, F. S. Walsh and P. Doherty, *J. Biol. Chem.*, 2001, **276**, 43879–43886.
102. J. B. Kim, S. Islam, Y. J. Kim, R. S. Prudoff, K. M. Sass, M. J. Wheelock and K. R. Johnson, *J. Cell Biol.*, 2000, **151**, 1193–1206.
103. S. D. Skaper, L. Facci, G. Williams, E. J. Williams, F. S. Walsh and P. Doherty, *Mol. Cell. Neurosci.*, 2004, **26**, 17–23.
104. L. D. Derycke and M. E. Bracke, *Int. J. Dev. Biol.*, 2004, **48**, 463–476.
105. P. Doherty, G. Williams and E. J. Williams, *Mol. Cell. Neurosci.*, 2000, **16**, 283–295.
106. E. J. Williams, J. Furness, F. S. Walsh and P. Doherty, *Neuron*, 1994, **13**, 583–594.
107. K. Suyama, I. Shapiro, M. Guttman and R. B. Hazan, *Cancer Cell*, 2002, **2**, 301–314.
108. J. Lilien and J. Balsamo, *Curr. Opin. Cell Biol.*, 2005, **17**, 459–465.
109. J. L. Sallee, E. S. Wittchen and K. Burridge, *J. Biol. Chem.*, 2006, **281**, 16189–16192.
110. S. Roura, S. Miravet, J. Piedra, A. Garcia de Herreros and M. Dunach, *J. Biol. Chem.*, 1999, **274**, 36734–36740.
111. S. Bek and R. Kemler, *J. Cell Sci.*, 2002, **115**, 4743–4753.
112. S. Dupre-Crochet, A. Figueroa, C. Hogan, E. C. Ferber, C. U. Bialucha, J. Adams, E. C. Richardson and Y. Fujita, *Mol. Cell. Biol.*, 2007, **27**, 3804–3816.
113. H. Aberle, H. Schwartz, H. Hoschuetzky and R. Kemler, *J. Biol. Chem.*, 1996, **271**, 1520–1526.
114. M. Ozawa and R. Kemler, *J. Biol. Chem.*, 1998, **273**, 6166–6170.
115. S. M. Brady-Kalnay, T. Mourton, J. P. Nixon, G. E. Pietz, M. Kinch, H. Chen, R. Brackenbury, D. L. Rimm, R. L. Del Vecchio and N. K. Tonks, *J. Cell Biol.*, 1998, **141**, 287–296.
116. C. B. Hellberg, S. M. Burden-Gulley, G. E. Pietz and S. M. Brady-Kalnay, *J. Biol. Chem.*, 2002, **277**, 11165–11173.
117. G. Xu, C. Arregui, J. Lilien and J. Balsamo, *J. Biol. Chem.*, 2002, **277**, 49989–49997.
118. G. Xu, A. W. Craig, P. Greer, M. Miller, P. Z. Anastasiadis, J. Lilien and J. Balsamo, *J. Cell Sci.*, 2004, **117**, 3207–3219.
119. J. Rhee, J. Lilien and J. Balsamo, *J. Biol. Chem.*, 2001, **276**, 6640–6644.
120. J. Balsamo, T. Leung, H. Ernst, M. K. Zanin, S. Hoffman and J. Lilien, *J. Cell Biol.*, 1996, **134**, 801–813.
121. J. Behrens, L. Vakaet, R. Friis, E. Winterhager, F. Van Roy, M. M. Mareel and W. Birchmeier, *J. Cell Biol.*, 1993, **120**, 757–766.
122. Y. Fujita, G. Krause, M. Scheffner, D. Zechner, H. E. Leddy, J. Behrens, T. Sommer and W. Birchmeier, *Nat. Cell Biol.*, 2002, **4**, 222–231.
123. A. Liwosz, T. Lei and M. A. Kukuruzinska, *J. Biol. Chem.*, 2006, **281**, 23138–23149.
124. Y. Shirayoshi, A. Nose, K. Iwasaki and M. Takeichi, *Cell Struct. Funct.*, 1986, **11**, 245–252.
125. W. Zhu, B. Leber and D. W. Andrews, *EMBO J.*, 2001, **20**, 5999–6007.
126. M. J. Wheelock and K. R. Johnson, *Annu. Rev. Cell Dev. Biol.*, 2003, **19**, 207–235.
127. W. J. Nelson and R. Nusse, *Science*, 2004, **303**, 1483–1487.
128. P. Marambaud, J. Shioi, G. Serban, A. Georgakopoulos, S. Sarner, V. Nagy, L. Baki, P. Wen, S. Efthimiopoulos, Z. Shao, T. Wisniewski and N. K. Robakis, *EMBO J.*, 2002, **21**, 1948–1956.

129. P. Marambaud, P. H. Wen, A. Dutt, J. Shioi, A. Takashima, R. Siman and N. K. Robakis, *Cell*, 2003, **114**, 635–645.
130. K. Reiss, T. Maretzky, A. Ludwig, T. Tousseyn, B. de Strooper, D. Hartmann and P. Saftig, *EMBO J.*, 2005, **24**, 742–752.
131. I. Shoval, A. Ludwig and C. Kalcheim, *Development*, 2007, **134**, 491–501.
132. T. Maretzky, K. Reiss, A. Ludwig, J. Buchholz, F. Scholz, E. Proksch, B. de Strooper, D. Hartmann and P. Saftig, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 9182–9187.
133. I. G. Haas, M. Frank, N. Veron and R. Kemler, *J. Biol. Chem.*, 2005, **280**, 9313–9319.
134. K. Reiss, T. Maretzky, I. G. Haas, M. Schulte, A. Ludwig, M. Frank and P. Saftig, *J. Biol. Chem.*, 2006, **281**, 21735–21744.
135. B. Hambsch, V. Grinevich, P. H. Seeburg and M. K. Schwarz, *J. Biol. Chem.*, 2005, **280**, 15888–15897.
136. A. Nose, A. Nagafuchi and M. Takeichi, *Cell*, 1988, **54**, 993–1001.
137. M. Takeichi, *Development*, 1988, **102**, 639–655.
138. R. A. Foty and M. S. Steinberg, *Dev. Biol.*, 2005, **278**, 255–263.
139. C. M. Niessen and B. M. Gumbiner, *J. Cell Biol.*, 2002, **156**, 389–399.
140. M. S. Steinberg and M. Takeichi, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 206–209.
141. D. Godt and U. Tepass, *Nature*, 1998, **395**, 387–391.
142. D. Godt and U. Tepass, *Curr. Biol.*, 2003, **13**, R683–685.
143. A. B. Jenkins, J. M. McCaffery and M. Van Doren, *Development*, 2003, **130**, 4417–4426.
144. H. McNeill, M. Ozawa, R. Kemler and W. J. Nelson, *Cell*, 1990, **62**, 309–316.
145. M. Perez-Moreno, C. Jamora and E. Fuchs, *Cell*, 2003, **112**, 535–548.
146. K. K. Grindstaff, C. Yeaman, N. Anandasabapathy, S. C. Hsu, E. Rodriguez-Boulan, R. H. Scheller and W. J. Nelson, *Cell*, 1998, **93**, 731–740.
147. C. Yeaman, K. K. Grindstaff and W. J. Nelson, *Physiol. Rev.*, 1999, **79**, 73–98.
148. M. L. Hermiston and J. I. Gordon, *J. Cell Biol.*, 1995, **129**, 489–506.
149. N. Erez, E. Zamir, B. J. Gour, O. W. Blaschuk and B. Geiger, *Exp. Cell Res.*, 2004, **294**, 366–378.
150. L. Larue, M. Ohsugi, J. Hirchenhain and R. Kemler, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 8263–8267.
151. D. Riethmacher, V. Brinkmann and C. Birchmeier, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 855–859.
152. W. N. De Vries, A. V. Evsikov, B. E. Haac, K. S. Fancher, A. E. Holbrook, R. Kemler, D. Solter and B. B. Knowles, *Development*, 2004, **131**, 4435–4445.
153. M. Ohsugi, L. Larue, H. Schwarz and R. Kemler, *Dev. Biol.*, 1997, **185**, 261–271.
154. B. Kanzler, A. Haas-Assenbaum, I. Haas, L. Morawiec, E. Huber and T. Boehm, *Mech. Dev.*, 2003, **120**, 1423–1432.
155. A. Nose and M. Takeichi, *J. Cell Biol.*, 1986, **103**, 2649–2658.
156. S. Butz and L. Larue, *Cell. Adhes. Commun.*, 1995, **3**, 337–352.
157. N. G. Kan, M. P. Stemmler, D. Junghans, B. Kanzler, W. N. de Vries, M. Dominis and R. Kemler, *Development*, 2007, **134**, 31–41.
158. E. A. Carver, R. Jiang, Y. Lan, K. F. Oram and T. Gridley, *Mol. Cell. Biol.*, 2001, **21**, 8184–8188.
159. O. Huber, C. Bierkamp and R. Kemler, *Curr. Opin. Cell Biol.*, 1996, **8**, 685–691.
160. R. J. Detrick, D. Dickey and C. R. Kintner, *Neuron*, 1990, **4**, 493–506.
161. T. Fujimori, S. Miyatani and M. Takeichi, *Development*, 1990, **110**, 97–104.
162. M. P. Stemmler, A. Hecht and R. Kemler, *Development*, 2005, **132**, 965–976.
163. G. L. Radice, H. Rayburn, H. Matsunami, K. A. Knudsen, M. Takeichi and R. O. Hynes, *Dev. Biol.*, 1997, **181**, 64–78.
164. O. Boussadia, S. Kutsch, A. Hierholzer, V. Delmas and R. Kemler, *Mech. Dev.*, 2002, **115**, 53–62.
165. J. A. Tunggal, I. Helfrich, A. Schmitz, H. Schwarz, D. Gunzel, M. Fromm, R. Kemler, T. Krieg and C. M. Niessen, *EMBO J.*, 2005, **24**, 1146–1156.
166. G. Cali, M. Zannini, P. Rubini, C. Tacchetti, B. D'Andrea, A. Affuso, T. Wintermantel, O. Boussadia, D. Terracciano, D. Silberschmidt, E. Amendola, M. De Felice, G. Schutz, R. Kemler, R. Di Lauro and L. Nitsch, *Endocrinology*, 2007, **148**, 2737–2746.
167. C. L. Tinkle, T. Lechler, H. A. Pasolli and E. Fuchs, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 552–557.
168. P. Young, O. Boussadia, H. Halfter, R. Grose, P. Berger, D. P. Leone, H. Robenek, P. Charnay, R. Kemler and U. Suter, *EMBO J.*, 2003, **22**, 5723–5733.
169. Y. Luo, M. Ferreira-Cornwell, H. Baldwin, I. Kostetskii, J. Lenox, M. Lieberman and G. Radice, *Development*, 2001, **128**, 459–469.
170. Y. Luo, F. A. High, J. A. Epstein and G. L. Radice, *Dev. Biol.*, 2006, **299**, 517–528.
171. C. Redies, *Prog. Neurobiol.*, 2000, **61**, 611–648.
172. A. M. Fannon and D. R. Colman, *Neuron*, 1996, **17**, 423–434.
173. O. Bozdagi, W. Shan, H. Tanaka, D. L. Benson and G. W. Huntley, *Neuron*, 2000, **28**, 245–259.
174. L. Tang, C. P. Hung and E. M. Schuman, *Neuron*, 1998, **20**, 1165–1175.
175. K. Jungling, V. Eulenburg, R. Moore, R. Kemler, V. Lessmann and K. Gottmann, *J. Neurosci.*, 2006, **26**, 6968–6978.
176. M. C. Ferreira-Cornwell, Y. Luo, N. Narula, J. M. Lenox, M. Lieberman and G. L. Radice, *J. Cell Sci.*, 2002, **115**, 1623–1634.
177. M. L. Hermiston, M. H. Wong and J. I. Gordon, *Genes Dev.*, 1996, **10**, 985–996.
178. M. L. Hermiston and J. I. Gordon, *Science*, 1995, **270**, 1203–1207.
179. L. Larue, C. Antos, S. Butz, O. Huber, V. Delmas, M. Dominis and R. Kemler, *Development*, 1996, **122**, 3185–3194.
180. U. Cavallaro and G. Christofori, *Nat. Rev. Cancer*, 2004, **4**, 118–132.
181. G. Christofori, *EMBO J.*, 2003, **22**, 2318–2323.
182. H. Peinado, D. Olmeda and A. Cano, *Nat. Rev. Cancer*, 2007, **7**, 415–428.
183. G. P. Gupta and J. Massague, *Cell*, 2006, **127**, 679–695.
184. J. P. Thiery and J. P. Sleeman, *Nat. Rev. Mol. Cell Biol.*, 2006, **7**, 131–142.
185. W. Birchmeier, *Bioessays*, 1995, **17**, 97–99.
186. K. Vleminckx, L. Vakaet, Jr, M. Mareel, W. Fiers and F. van Roy, *Cell*, 1991, **66**, 107–119.
187. G. Strathdee, *Semin. Cancer Biol.*, 2002, **12**, 373–379.
188. J. R. Graff, E. Gabrielson, H. Fujii, S. B. Baylin and J. G. Herman, *J. Biol. Chem.*, 2000, **275**, 2727–2732.
189. S. J. Nass, J. G. Herman, E. Gabrielson, P. W. Iversen, F. F. Parl, N. E. Davidson and J. R. Graff, *Cancer Res.*, 2000, **60**, 4346–4348.
190. T. Kanazawa, T. Watanabe, S. Kazama, T. Tada, S. Koketsu and H. Nagawa, *Int. J. Cancer*, 2002, **102**, 225–229.
191. G. Berx, F. Nollet and F. van Roy, *Cell Adhes. Commun.*, 1998, **6**, 171–184.
192. G. Li and M. Herlyn, *Mol. Med. Today*, 2000, **6**, 163–169.
193. K. Tomita, A. van Bokhoven, G. J. van Leenders, E. T. Ruijter, C. F. Jansen, M. J. Bussemakers and J. A. Schalken, *Cancer Res.*, 2000, **60**, 3650–3654.
194. R. B. Hazan, R. Qiao, R. Keren, I. Badano and K. Suyama, *Ann. N. Y. Acad. Sci.*, 2004, **1014**, 155–163.
195. R. B. Hazan, G. R. Phillips, R. F. Qiao, L. Norton and S. A. Aaronson, *J. Cell Biol.*, 2000, **148**, 779–790.
196. G. Li, K. Satyamoorthy and M. Herlyn, *Cancer Res.*, 2001, **61**, 3819–3825.
197. M. T. Nieman, R. S. Prudoff, K. R. Johnson and M. J. Wheelock, *J. Cell Biol.*, 1999, **147**, 631–644.
198. S. Islam, T. E. Carey, G. T. Wolf, M. J. Wheelock and K. R. Johnson, *J. Cell Biol.*, 1996, **135**, 1643–1654.
199. A. K. Perl, P. Wilgenbus, U. Dahl, H. Semb and G. Christofori, *Nature*, 1998, **392**, 190–193.
200. U. Cavallaro, B. Schaffhauser and G. Christofori, *Cancer Lett.*, 2002, **176**, 123–128.
201. D. Vestweber, R. Kemler and P. Ekblom, *Dev. Biol.*, 1985, **112**, 213–221.
202. H. Matsunami and M. Takeichi, *Dev. Biol.*, 1995, **172**, 466–478.
203. K. Shimamura, S. Hirano, A. P. McMahon and M. Takeichi, *Development*, 1994, **120**, 2225–2234.

204. K. Shimamura and M. Takeichi, *Development*, 1992, **116**, 1011–1019.
205. E. K. Nishimura, H. Yoshida, T. Kunisada and S. I. Nishikawa, *Dev. Biol.*, 1999, **215**, 155–166.
206. J. Behrens, O. Lowrick, L. Klein-Hitpass and W. Birchmeier, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 11495–11499.
207. M. L. Faraldo, I. Rodrigo, J. Behrens, W. Birchmeier and A. Cano, *Mol. Carcinog.*, 1997, **20**, 33–47.
208. G. Hennig, J. Behrens, M. Truss, S. Frisch, E. Reichmann and W. Birchmeier, *Oncogene*, 1995, **11**, 475–484.
209. H. Peinado, F. Portillo and A. Cano, *Int. J. Dev. Biol.*, 2004, **48**, 365–375.
210. M. Ringwald, H. Baribault, C. Schmidt and R. Kemler, *Nucleic Acids Res.*, 1991, **19**, 6533–6539.
211. H. W. Lo, S. C. Hsu, W. Xia, X. Cao, J. Y. Shih, Y. Wei, J. L. Abbruzzese, G. N. Hortobagyi and M. C. Hung, *Cancer Res.*, 2007, **67**, 9066–9076.
212. E. Batlle, E. Sancho, C. Franci, D. Dominguez, M. Monfar, J. Baulida and A. Garcia De Herreros, *Nat. Cell Biol.*, 2000, **2**, 84–89.
213. A. Cano, M. A. Perez-Moreno, I. Rodrigo, A. Locascio, M. J. Blanco, M. G. del Barrio, F. Portillo and M. A. Nieto, *Nat. Cell Biol.*, 2000, **2**, 76–83.
214. V. Bolos, H. Peinado, M. A. Perez-Moreno, M. F. Fraga, M. Esteller and A. Cano, *J. Cell Sci.*, 2003, **116**, 499–511.
215. K. M. Hajra, D. Y. Chen and E. R. Fearon, *Cancer Res.*, 2002, **62**, 1613–1618.
216. J. Yang, S. A. Mani, J. L. Donaher, S. Ramaswamy, R. A. Itzykson, C. Come, P. Savagner, I. Gitelman, A. Richardson and R. A. Weinberg, *Cell*, 2004, **117**, 927–939.
217. Y. Kang and J. Massague, *Cell*, 2004, **118**, 277–279.
218. M. A. Perez-Moreno, A. Locascio, I. Rodrigo, G. Dhondt, F. Portillo, M. A. Nieto and A. Cano, *J. Biol. Chem.*, 2001, **276**, 27424–27431.
219. H. Peinado, F. Marin, E. Cubillo, H. J. Stark, N. Fusenig, M. A. Nieto and A. Cano, *J. Cell Sci.*, 2004, **117**, 2827–2839.
220. J. Comijn, G. Berx, P. Vermassen, K. Verschueren, L. van Grunsven, E. Bruyneel, M. Mareel, D. Huylebroeck and F. van Roy, *Mol. Cell*, 2001, **7**, 1267–1278.
221. A. Eger, K. Aigner, S. Sonderegger, B. Dampier, S. Oehler, M. Schreiber, G. Berx, A. Cano, H. Beug and R. Foisner, *Oncogene*, 2005, **24**, 2375–2385.
222. M. L. Grooteclaes and S. M. Frisch, *Oncogene*, 2000, **19**, 3823–3828.
223. T. Shirakihara, M. Saitoh and K. Miyazono, *Mol. Biol. Cell*, 2007, **18**, 3533–3544.
224. O. Huber, R. Korn, J. McLaughlin, M. Ohsugi, B. G. Herrmann and R. Kemler, *Mech. Dev.*, 1996, **59**, 3–10.
225. C. Jamora, R. DasGupta, P. Kocieniewski and E. Fuchs, *Nature*, 2003, **422**, 317–322.
226. A. Nawshad, D. Medici, C. C. Liu and E. D. Hay, *J. Cell Sci.*, 2007, **120**, 1646–1653.
227. M. P. Stemmler, A. Hecht, B. Kinzel and R. Kemler, *Dev. Dyn.*, 2003, **227**, 238–245.
228. F. Spitz, F. Gonzalez and D. Duboule, *Cell*, 2003, **113**, 405–417.
229. P. P. Levings and J. Bungert, *Eur. J. Biochem.*, 2002, **269**, 1589–1599.
230. D. B. Stewart, A. I. Barth and W. J. Nelson, *J. Biol. Chem.*, 2000, **275**, 20707–20716.
231. D. Meyer zum Buschenfelde, H. Hoschutzky, R. Tauber and O. Huber, *Peptides*, 2004, **25**, 873–883.
232. D. M. Bryant, M. C. Kerr, L. A. Hammond, S. R. Joseph, K. E. Mostov, R. D. Teasdale and J. L. Stow, *J. Cell Sci.*, 2007, **120**, 1818–1828.
233. D. M. Bryant, F. G. Wylie and J. L. Stow, *Mol. Biol. Cell*, 2005, **16**, 14–23.
234. I. E. Zohn, Y. Li, E. Y. Skolnik, K. V. Anderson, J. Han and L. Niswander, *Cell*, 2006, **125**, 957–969.
235. S. Ogata, J. Morokuma, T. Hayata, G. Kolle, C. Niehrs, N. Ueno and K. W. Cho, *Genes Dev.*, 2007, **21**, 1817–1831.
236. B. Li, N. E. Paradies and R. W. Brackenbury, *Gene*, 1997, **191**, 7–13.
237. M. Matsumata, M. Uchikawa, Y. Kamachi and H. Kondoh, *Dev. Biol.*, 2005, **286**, 601–617.
238. H. Zhang, T. Toyofuku, J. Kamei and M. Hori, *Biochem. Biophys. Res. Commun.*, 2003, **312**, 1033–1038.
239. N. R. Alexander, N. L. Tran, H. Rekapally, C. E. Summers, C. Glackin and R. L. Heimark, *Cancer Res.*, 2006, **66**, 3365–3369.
240. D. Haussinger, T. Ahrens, T. Aberle, J. Engel, J. Stetefeld and S. Grzesiek, *EMBO J.*, 2004, **23**, 1699–1708.
241. M. Takeichi, *Annu. Rev. Biochem.*, 1990, **59**, 237–252.

