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Molecular Biology of Cadherins in the Nervous System

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Contents

Abstract

Cadherins are cell–cell adhesion molecules belonging to the Ca^{2+}-dependent cadherin superfamily. In the last few years the number of cadherins identified in the nervous system has increased considerably. Cadherins are integral membrane glycoproteins. They are structurally closely related and interspecies homologies are high. The function is mediated through a homophilic binding mechanism, and intracellular proteins, directly or indirectly connected to the cadherins and the cytoskeleton, are necessary for cadherin activity. Cadherins have been implicated in segregation and aggregation of tissues at early developmental stages and in growth and guidance of axons during nervous system development. These functions are modified by changes in type(s) and amount of cadherins expressed at different developmental stages. The regulatory elements guiding cadherin expression are currently being elucidated.

Index Entries: Adherens junctions; cadherins; cell adhesion; catenins; calcium; development; neurite outgrowth.

Abbreviations: CAM, cell adhesion molecule; DG, desmoglein; E, embryonic day; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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Introduction

Cadherins are Ca^{2+}-dependent cell–cell adhesion molecules (CAMs) (*see* reviews by Takeichi et al., 1988, 1990a, 1991; Bock, 1991; Edelman and Crossin, 1991). This article focuses on the neural cadherins, describing their molecular structure and functional roles during nervous system development.

The cadherin superfamily consists of cadherins, which were the first members to be identified, desmosomal glycoproteins (DG) I-III (DGI is also named desmoglein I, and DGII and DGIII are identical to desmocollins I and II; *see* review by Wheeler et al., 1991) and the closely related *Pemphigus vulgaris* antigen (PVA) (Amagai et al., 1991), and the *Drosophila fat* tumor suppressor gene, which contains 34 cadherin tandem domains (Mahoney et al., 1991). Cadherin function requires Ca^{2+}, which protects these molecules against proteolysis by low doses of trypsin (Takeichi, 1988). The cadherin cell–cell adhesion system only functions at physiological temperatures (Takeichi, 1977; Fischer and Schachner, 1988), implying that it depends on energy-dependent events.

Originally, Fab' preparations of adhesion blocking polyclonal antibodies were used to identify cadherins (Kemler et al., 1977; Dunia et al., 1979; Hyafil et al., 1981; Bertolotti et al., 1980; Takeichi et al., 1981; Yoshida and Takeichi, 1982; Grunwald et al., 1982; Damsky et al., 1983; Vestweber and Kemler, 1984). Later, adhesion blocking monoclonal antibodies were used (Gallin et al., 1983; Imhof et al., 1983; Yoshida-Noro et al., 1984; Hatta et al., 1985; Nose and Takeichi, 1986). Recently, novel cadherins have been identified by screening cDNA expression libraries with crossreactive antibodies (e.g., Geiger et al., 1990a; Napolitano et al., 1991) or DNA probes complementary to well-conserved sequences (e.g., Liaw et al., 1990; Walsh et al., 1990; Herzberg et al., 1991; Suzuki et al., 1991).

In the following, a short description of individual cadherins will be given. Table 1 summarizes the current literature regarding mRNA and protein expression of cadherins in various species. In Table 2, synonyms for various cadherins are given.

N-cadherin has been found in human, bovine, mouse, rat, chicken, and frog tissues; it is most abundant in brain and heart (Walsh et al., 1990; Liaw et al., 1990; Hatta et al., 1985; Chen et al., 1991; Hatta and Takeichi, 1986; Choi et al., 1990; Detrick et al., 1990; Fujimori et al., 1990). Chicken N-cadherin is immunologically indistinguishable from chicken NcalCAM (Grunwald et al., 1982; Crittenden et al., 1988; Lagunowich and Grunwald, 1989), and chicken A-CAM (Volk and Geiger, 1984; Duband et al., 1988) (*see* Table 2). The N-cadherin cDNA sequence has been determined for humans, ox, mouse, chicken, and frog (Walsh et at al., 1990; Reid and Hemperly, 1990; Salomon et al., 1992; Liaw et al., 1990; Miyatani et al., 1989; Hatta et al., 1987; Detrick et al., 1990; Ginsberg et al., 1991).

E-cadherin has also been found in many tissues in several animals; it is most abundant in liver, kidney, lung, intestine, and most epithelia (Hyafil et al., 1981; Yoshida and Takeichi, 1982; Damsky et al., 1983; Imhof et al., 1983; Behrens et al., 1985; Gumbiner and Simons, 1986; Nomura et al., 1988; Shimoyama et al., 1989a). In the nervous system it is expressed in a subset of cells in sensory ganglia and spinal cord (Takeichi et al., 1990b). The cadherin uvomorulin (Table 2) is identical to E-cadherin as demonstrated by cDNA analysis (Schuh et al., 1986; Nagafuchi et al., 1987; Ringwald et al., 1987; Mansouri et al., 1988). Canine Arc-1 and rr1-antigen and human Cell-CAM 120/80 are probably also E-cadherin interspecies homologs (Vestweber and Kemler, 1985; Wheelock et al., 1987). Whether L-CAM (Bertolotti et al., 1980; Gallin et al., 1983; Levi et al., 1987) belongs to this group is unclear, since there is a low overall amino acid sequence similarity between mouse E-cadherin and chicken L-CAM (Gallin et al., 1987; Takeichi, 1988) as opposed to the generally high sequence similarity between other interspecies cadherin homologs. L-CAM is also abundant in chicken liver, kidney, lung, and intestine (Thiery et al., 1984; Crossin et al., 1985), whereas no expression in the nervous system has been reported.

P-cadherin has been observed in human, bovine, and rodent tissues (Shimoyama et al., 1989b; Liaw et al., 1990; Nose and Takeichi, 1986; Nose et al., 1987). It was originally discovered in large amounts in mouse placenta, but it is not expressed in human placenta, or at least only in very low amounts (Nose and Takeichi, 1986; Shimoyama et al., 1989b). Neither is it expressed in the mouse, rat, or human nervous system (Nose and Takeichi, 1986; Suzuki et al., 1991; Shimoyama et al., 1989a).

Cadherin-related molecule in liver (CRM-L), is a chicken molecule closely resembling N-cadherin with regard to size and isoelectric point (Crittenden et al., 1988). Peptide mapping displays 69% shared peptides with N-cadherin, and only 20% with L-CAM. In two-dimensional SDS-PAGE, mouse E-cadherin comigrates with chicken CRM-L, and not with L-CAM, indicating that CRM-L might be the chicken homolog of mouse E-cadherin (Crittenden et al., 1988).

R-cadherin was originally discovered in chicken retina (Takeichi et al., 1990b; Inuzuka et al., 1991a), but has later been demonstrated in several areas of the brain and spinal cord (Inuzuka et al., 1991b; Redies et al., 1992). Since R-cadherin mRNA also has been demonstrated in rat liver (Suzuki et al., 1991), and R-cadherin and CRM-L have identical relative molecular sizes (Table 1), these two molecules may be identical.

Using the PCR-technique, Suzuki et al. (1991) were able to describe partial sequences of eight new cadherins (cad4–11) in rat brain and retina. The amplified DNA fragments were used as probes to isolate six of these cadherins from a human fetal brain cDNA library. Cad4 (Table 2) is probably the human homolog of chicken R-cadherin (Inuzuka et al., 1991a), since the various domains of the identified 2/3 of cad4 are 95–100% homologous to R-cadherin on the amino acid level.

Chicken B-cadherin, which is identical to K-CAM (*see* Table 2) with 99.5% amino acid sequence identity, was discovered in brain and exhibits approx 65% homology on the amino acid level to chicken L-CAM, and mouse P- and E-cadherin (Napolitano et al., 1991; Sorkin et al., 1991). It is expressed in certain areas in the optic tectum, in the ependymal cells, and the choroid plexus (Napolitano et al., 1991).

M-cadherin has been isolated from a mouse myogenic cell line cDNA library and sequenced (Donalies et al., 1991). This cadherin is less homologous to mouse E-, P-, and N-cadherin than these are to each other. M-cadherin has been proposed to play an important role in muscle differentiation (Donalies et al., 1991). M-cadherin expression has not been investigated in the nervous system (Donalies et al., 1991).

A truncated cadherin, T-cadherin, lacking the cytoplasmic domain, has also been identified in several chicken tissues, including brain and heart (Ranscht and Dours-Zimmermann, 1991; Ranscht and Bronner-Fraser, 1991). This cadherin is bound to the membrane via a glycosyl phosphatidylinositol anchor (Ranscht and Dours-Zimmermann, 1991; Vestal and Ranscht, 1992). The sequence identity on the amino acid level between T-cadherin and chicken N-cadherin and L-CAM is 47 and 40%, respectively (Ranscht and Dours-Zimmermann, 1991).

V-cadherin is a molecule only described in bovine aortic endothelial cells (Heimark et al., 1990). The expression of V-cadherin in the nervous system has not been investigated. Several cadherins in *Xenopus laevis* have been described, including homologs of N-cadherin (Choi et al., 1990; Detrick et al., 1990; Fujimori et al., 1990; Geiger et al., 1990a; Ginsberg et al., 1991), E-cadherin (Nomura et al., 1988; Choi and Gumbiner, 1989; Herzberg et al., 1990; Angres et al., 1991), and L-CAM (Levi et al., 1987), and novel cadherins such as XBcad (Herzberg et al., 1991) and EP-cadherin (Ginsberg et al., 1991; Herzberg et al., 1991), which both have been sequenced, and U-cadherin (Angres et al., 1991; Müller et al., 1992), and cadherin-like-protein (CLP) (Choi et al., 1990), whose cDNA sequences have not been determined. The difference in sequence at both the amino acid and DNA level between EP-cadherin and XBcad is only 8% (Herzberg et al., 1991). The relationship between EP-cadherin and CLP is unclear, but based on their relative molecular size and spatiotemporal distribution,

Table 1
Expression of Known Cadherins in Various Species[a]

Molecule	Species[b]	mRNA classes, kb	Tissue distribution of mRNA	Polypeptide size, kDa	Tissue distribution of polypeptide	References
N-cadherin	H	4.0/4.3/5.2	Embryonic brain and muscle	130, 135	Kidney, lens	Walsh et al., 1990; Maisel and Atreya, 1990; Biddlestone and Flemming, 1991
	O	1.7/4.1/5.0	Brain, kidney, heart, liver, placenta, endothelial cells	130, 140	Brain, heart, liver, lens	Liaw et al., 1990; Walsh et al., 1990; Maisel and Atreya, 1990
	M	3.5/4.3/5.2	Brain, heart, muscle, liver	130	Brain, heart, liver, eye	Walsh et al., 1990; Miyatani et al., 1989; Lagunowich et al., 1990
	R	3.5/4.3/5.2	Brain, lung, kidney, heart, muscle, liver	130	Brain, lung, kidney, heart, muscle, liver	Chen et al., 1991; Gaardsvoll et al., unpublished; Wagner et al., 1992
	C	3.3/3.8/4.3/4.7	Brain, heart	127–135	Brain, heart, muscle, skin, lens, testis	Duband et al., 1988; Hatta et al., 1988 Maisel and Atreya, 1990; Dalseg et al., 1990; Volk and Geiger, 1986a
	X	4.2	Heart, testis, embryo	135–140	Brain, embryo, tadpole brain and heart	Choi et al., 1990; Detrick et al., 1990; Crittenden et al., 1988; Ginsberg et al., 1991
E-cadherin	H	4.5	Keratinocytes, placenta, A431 carcinoma cells	120–130	Lung, kidney, liver, skin, intestine, placenta	Damsky et al., 1983; Shimoyama et al., 1989a,b; Eidelman et al., 1989; Nicholson et al., 1991

	M	4.3,4.5	Placenta, F9, teratocarcinoma cells, PCC4 carcinoma cells	120–127	Lung, kidney, liver, intestine, tongue, uterus, gut, trachea	Shimoyama et al., 1989a; Nagafuchi et al., 1987; Ringwald et al., 1987; Inuzuka et al., 1991b; Peyriéras et al., 1983; Vestweber and Kemler, 1984
	R	4.3	Lung, kidney, liver	120	Pancreatic islets	Chen et al., 1991; Gaardsvoll et al., unpublished; Møller et al., 1992
	X	3.2/3.8/4.1/4.3	Brain, liver	138, 140	Brain, lung, kidney, heart, muscle, liver	Choi et al., 1990; Nomura et al., 1988; Hertzberg et al., 1990; Angres et al., 1991
P-cadherin	H	3.2	Keratinocytes, placenta, A-431 carcinoma cells	118	Skin, mammary and prostate gland, bronchus, tongue, esophagus, ureter, bladder, vagina	Shimoyama et al., 1989a,b; Nicholson et al., 1991
	O	3.3/3.7	Kidney, endothelial cells	—	—	Liaw et al., 1990;
	M	3.2	Placenta	118	Embryonic tissues: lung, heart, placenta, skin, intestine	Nose and Takeichi, 1986; Shimoyama, 1989a; Nose et al., 1987
	R	3.5	Lung, kidney, embryo	—	—	Chen et al., 1991
L-CAM	C	4.0	Embryonic kidney and liver	124	Lung, kidney, liver, intestine, skin, gallbladder	Gallin et al., 1983; Thiery et al., 1984; Crossin et al., 1985; Müller et al., 1985
	X	—	—	124	Lung, kidney, liver, intestine, skin	Levi et al., 1987
CRM-L	C	—	—	130	Liver	Crittenden et al., 1988

(continued)

Table 1 *(continued)*

Molecule	Species[b]	mRNA classes, kb	Tissue distribution of mRNA	Polypeptide size, kDa	Tissue distribution of polypeptide	References
R-cadherin	R	3.5–5	Brain, lung, kidney, liver	—	—	Suzuki et al., 1991
	C	4.6/5.4/7.4	Embryonic brain and retina	124	Embryonic tissues: brain, heart, muscle retina	Inuzuka et al., 1991a,b; Takeichi et al., 1990; Maisel and Atreya, 1990
Cad5	R	3.5–5	Brain, lung, kidney, liver	—	—	Suzuki et al., 1991
Cad6	R	3.5–5	Brain	—	—	Suzuki et al., 1991
Cad7	R	3.5–5	Brain	—	—	Suzuki et al., 1991
Cad8	R	3.5–5	Brain	—	—	Suzuki et al., 1991
Cad9	R	3.5–5	Brain	—	—	Suzuki et al., 1991
Cad10	R	3.5–5	Brain	—	—	Suzuki et al., 1991
Cad11	R	3.5–5	Brain, lung, liver	—	—	Suzuki et al., 1991
B-cadherin	C	3.0/4.0	Embryonic brain, heart, and liver	120–122	Embryonic tissues: brain, heart, muscle, liver, intestine, skin, eye, retina, bladder	Napolitano et al., 1991
M-cadherin	M	3.0	C2 Myoblast and myotube cells	—	—	Donalies et al., 1991
T-cadherin	C	3.2/7.5/10	Brain, lung, liver, kidney, heart, muscle, retina	90, 95	Brain, lung, kidney, heart, muscle	Ranscht and Dours-Zimmermann, 1991; Ranscht and Bronner-Fraser, 1991
V-cadherin	O	—	—	135	Aortic endothelial cells	Kemler and Ozawa, 1989
XB-cad	X	3.9	Oocytes	125	Brain, lung, kidney	Herzberg et al., 1991
U-cadherin	X	—	—	125	Egg, embryo	Herzberg et al., 1990; Müller et al., 1992
EP-cadherin	X	3.5	Lung, skin, egg, embryo, tailbud	120, 125	Egg, embryo, A6 kidney cell line	Ginsberg et al., 1991; Levi et al., 1991
CLP	X	—	—	120	Brain, egg, embryo, A6 kidney cell line	Choi et al., 1990

[a]The cadherins are described with regard to species examined, the size(s) of the mRNA and its tissue distribution, the polypeptide size in kDa and its tissue distribution and the relevant references.

[b]H = human; O = ox; M = mouse; R = rat; C = chicken, and X = *Xenopus laevis*. In this table, the postnatal tissue distribution is preferentially noted unless otherwise stated. The tissue distribution during development of some of the above cadherins has previously been reviewed by Takeichi, 1988.

Table 2
List of Synonyms

Molecule	Reference
N-cadherin	Hatta et al., 1985
A-CAM	Volk and Geiger, 1984
NcalCAM (GP 130/4.8)	Grunwald et al., 1982; Bixby et al., 1987
130 kDa glycoprotein	Heslip et al., 1986
E-cadherin	Yoshida and Takeichi, 1982; Yoshida-Noro et al., 1984
Uvomorulin	Hyafil et al., 1980; Peyiéras et al., 1983; Vestweber and Kemler, 1984
Cell-CAM 120/80	Damsky et al., 1983
Arc-1	Imof et al., 1983; Behrens et al., 1985
rr1 Antigen	Gumbiner and Simons, 1986
B-cadherin	Napolitano et al., 1991
K-CAM	Sokin et al., 1991
R-cadherin	Inuzuka et al., 1991a
Cad4	Suzuki et al., 1991

Molecules that are identical or interspecies homologs are grouped. With regard to L-CAM, this molecule has been suggested to be a chicken homolog of mammalian E-cadherin. However the relatively low homology between these two proteins compared to the high interspecies homology observed for other cadherins makes this assumption doubtful.

they may be identical. In *Xenopus* E- and N-cadherin, XB-cad, and CLP have all been shown to be expressed in the nervous system using immunochemical techniques. E-cadherin has also been demonstrated in brain using Northern blotting technique (Herzberg et al., 1990).

Genomic Structure

The genomic organization of several cadherins has been determined (Sorkin et al., 1988; Behrens et al., 1991; Hatta et al., 1991; Ringwald et al., 1991; Sorkin et al., 1991; Miyatani et al., 1992). The genes for chicken L-CAM (Sorkin et al., 1991), mouse E-cadherin (Ringwald et al., 1991), and mouse N-cadherin (Miyatani et al., 1992) contain 16 exons, whereas the mouse P-cadherin gene (Hatta et al., 1991) consists of only 15 exons, the first exon corresponding to the first two exons of L-CAM, E-, and N-cadherin. The sizes of these genes span from 10 kb for L-CAM to >200 kb for N-cadherin (Sorkin et al., 1988; Miyatani et al., 1992). Exon structure and sizes are conserved for all cadherins examined, except for the last exon, which varies considerably in length. Surprisingly, the exon structure does not seem to correspond to structural elements found in the cadherins, such as the internal repeats of the extracellular domains (ECI-III), the calcium-binding motifs, or the transmembrane domain (*see* Primary Protein Structure). The cytoplasmic domain is encoded by three exons. Here some correlation between exon structure and function is seen, since exon 15 of P-cadherin, which corresponds to E-cadherin and L-CAM exon 16, coincides roughly with the catenin-binding (*see* Cadherin-Binding Proteins, Catenins) region (Hatta et al., 1991). So far, only one case of alternative splicing has been observed; using the PCR-technique Liaw et al. (1990) found that in bovine P-cadherin an in-frame deletion of 225 bp exists, the sequence corresponding to mouse P-cadherin exon 11.

Three N-cadherin mRNAs of 3.5, 4.3, and 5.2 kb have been demonstrated by Northern blotting of mouse brain with the 4.3 kb message being the major component (Miyatani et al., 1989). For N-cadherin two copies of exon 16 have been demonstrated using genomic cloning (Miyatani et al., 1992). They are 100% identical in the coding region and 99% in the untranslated region of the shortest exon 16, which lies downstream of exon 15. It is possible that the extra exon 16 has a longer or shorter 3' untranslated region that through alternative splicing could account for one of the less abundant N-cadherin mRNAs (Miyatani et al., 1992).

While examining the upstream sequence of L-CAM, Sorkin et al. (1991) found a second closely related cadherin gene, which they called K-CAM, identical to B-cadherin (*see* Table 2). The presumed poly(A)$^+$ site of this gene is located <700 bp upstream of the translation initiation site of L-CAM. The sizes of the identified exons 4–15 of the B-cadherin gene are almost identical to

those in the L-CAM gene, and the exon/intron junctions occur at exactly equivalent positions in both genes (Sorkin et al., 1991). The proximity and overall structure of these two genes strongly suggest that they arose by gene duplication and raise the possibility that other cadherin genes also may be located in clusters. Indeed, this seems to be the case for mouse P- and E-cadherin. Both genes are located on chromosome 8 and have been shown to be linked (Eistetter et al., 1988; Hatta et al., 1991), whereas the mouse N-cadherin gene is located on chromosome 18 (Miyatani et al., 1992). In humans the N-cadherin and E-cadherin genes are located on chromosome 18 (Walsh et al., 1990) and chromosome 16, respectively (Mansouri et al., 1988). Thus, the cadherins have probably evolved from a common precursor gene that has undergone duplication and migration to other chromosomal locations (Walsh et al., 1990).

A few studies concerning the structure of the promoter region have been conducted (Behrens et al., 1991; Ringwald et al., 1991). For E-cadherin, several putative binding sites for transcription factors have been demonstrated, e.g., a GC-rich region harboring a potential Sp1 binding site and a CAAT box (Behrens et al., 1991). No TATA-box has been found, but surrounding the transcription initiation site, a sequence, 5'-CTCANTCT-3', similar to the initiator sequence found in the TATA-less promoter of the murine terminal deoxynucleotidyltransferase (TdT) gene, has been observed (Smale et al., 1989, 1990). A palindromic sequence, E-pal, highly homologous to binding sites for regulatory elements named KER-1 and KTF-1, present in keratin gene promoters of humans and *Xenopus laevis* (Leask et al., 1990; Snape et al., 1990), has also been identified (*see* Behrens et al., 1991). Ringwald et al. (1991) found putative binding sites for glucocorticoid and progesterone receptors. The latter is identical to functional *cis* acting elements in the promoter of the uteroglobin gene (Bailly et al., 1986). It has been shown, that cadherins can be regulated by steroid hormones (Blaschuk and Farookhi, 1989; Choi et al., 1990), and in light of the above mentioned findings the mechanism may be a direct transcriptional regulation by these hormones, at least in some cell types.

Analysis of various deletion fragments of transfected E-cadherin cDNA has led to the assumptions that the combination of the GC-rich region with the initiator element leads to transcriptional initiation at a single start site and that the general epithelial specificity of the E-cadherin promoter is produced by the combined interaction of Sp1 and other nuclear factors with the GC-rich region (Behrens et al., 1991).

Primary Protein Structure

Amino acid sequences are conserved among the various cadherins with homologies in the range of 43–65%, except between chicken N- and R-cadherin, where the similarity is as high as 74% (Inuzuka et al., 1991a).

Analyses of protein and cDNA sequences reveal that cadherins from as different species as *Xenopus laevis* and humans are very similar in their overall primary structure. The mature form consists of 723–748 amino acids. A single transmembrane domain separates the carboxy-terminal cytoplasmic domain of about 160 amino acids from the larger amino-terminal extracellular part (Fig. 1). The latter can be divided into three internal repeats, extracellular domains I-III (ECI-ECIII), of about 112-amino-acids starting ca. 27-amino-acids from the amino terminus (Gallin et al., 1987; Ringwald et al., 1987). ECI and ECII have the highest degree of internal homology, whereas ECIII has less homology to each of the other two. In the membrane proximal region of the extracellular part, four cysteine residues are conserved among all cadherins. These amino acid residues are also found in other cadherin superfamily members, such as the desmosomal glycoproteins II and III (*see* review by Wheeler et al., 1991) and the *Pemphigus vulgaris* antigen (Amagai et al., 1991). Several putative *N*-linked glycosylation sites are found, but they are generally not conserved among the cadherins (Takeichi, 1988; Donalies et al., 1991). Inhibition of glycosylation with *N*-linked oligosaccharides indicates that

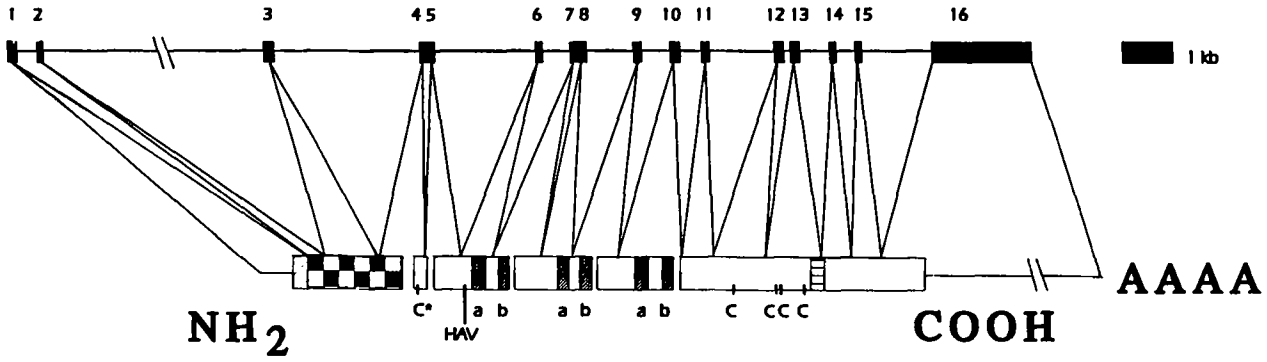

Fig. 1. Schematic drawing of E-cadherin genomic and protein structure. Genomic structure: The exons are numbered 1–16. Protein structure: Diagonal bars = signal peptide; check pattern = cleaved protein precursor; dotted areas marked "a" or "b" = calcium binding motifs; horizontal bars = transmembrane region; C = cystein residues observed in all cadherins; C* = cystein residue only found in E-cadherin; HAV = the His-Arg-Val-containing binding area. The untranslated 3'-end is also shown. Modified after Ringwald et al., 1991 and published by permission of Oxford University Press.

sugar moieties are not involved in the adhesive function of cadherins (Shirayoshi et al., 1986).

Both E- and N-cadherin have been shown to be phosphorylated (Lagunowich and Grunwald, 1991; Sefton et al., 1992). Phosphorylation in the intracellular part may lead to uncoupling of the catenin-cadherin complex from the cytoskeleton, thereby causing unstable cell–cell adhesion (Matsuyoshi et al., 1992) (*see* Cadherin-Binding Proteins, Catenins).

Ca^{2+}-Binding

Ringwald et al. (1987) have demonstrated direct binding of Ca^{2+} to a 84-kDa tryptic fragment of E-cadherin. They have also identified two sequence motifs, DQNDN (Asp-Gln-Asn-Asp-Asn) and DADDD (Asp-Ala-Asp-Asp-Asp) termed a and b, respectively, with putative Ca^{2+}-binding properties (Fig. 1). These motifs are present in each of the three extracellular domains (Ringwald et al., 1987). To investigate whether these elements actually bind Ca^{2+}, Ozawa et al. (1990a) synthesized a 13-mer peptide containing motif b. This peptide bound Ca^{2+}, but by changing the first Asp to Lys, Ca^{2+}-binding was lost (Ozawa et al., 1990a). The functional relevance of this for Ca^{2+}/trypsin susceptibility of the mature protein was tested using E-cadherin transfectants with mutations in the same Asp to either Lys or Ala. Not only were the mutant proteins far more susceptible to degradation than the wild-type, most likely owing to loss of Ca^{2+} protection caused by the amino acid substitution in the Ca^{2+}-binding site (Ozawa et al., 1990a), but this single amino acid substitution abolished the adhesive properties of E-cadherin (Ozawa et al., 1990a). This substitution is outside the *N*-terminal 113-amino-acid domain presumed to be responsible for adhesive specificity (Nose et al., 1990; *see* Binding Specificities), indicating that Ca^{2+} is not involved in this event but rather is directly or indirectly involved in the molecular mechanism of adhesion (Ozawa et al., 1990a).

Binding Specificities

Previous studies have shown that cells of different origin generally segregate after mixing. Many observations describing segregation or selective aggregation of cells can be explained in terms of cadherin subclass specificities (*see* review by Takeichi, 1988). A homophilic interaction between molecules on neighboring cells seems well documented for the cadherins. This is best

demonstrated in transfection studies, in which the expression of a particular cadherin induces specific cell adhesion (aggregation and segregation) that can be blocked by specific antibodies (Nagafuchi et al., 1987; Edelman et al., 1987; Hatta et al., 1988; Mege et al., 1988; Nose et al., 1988; Friedlander et al., 1989; Miyatani et al., 1989; Matsuzaki et al., 1990). However, heterologous interactions between cultures of N-cadherin expressing lens cells and L-CAM expressing liver cells have been described (Volk et al., 1987). By means of Fab' fragments of antibodies against either molecule, these seemingly heterologous interactions could be inhibited. On the other hand, this kind of interaction was not observed using N-cadherin and L-CAM transfected murine sarcoma S180 cells (Friedlander et al., 1989; Matsuzaki et al., 1990). Heterologous interaction between R-cadherin and N-cadherin transfected L-cells has also been reported, although the preference seemed to be for the homophilic binding (Inuzuka et al., 1991a). The heterologous interaction in the latter case is not unexpected, since the overall identity in amino acid sequence between N- and R-cadherin, as mentioned above, is 74%.

The EC1 domain of all identified cadherins contains the tripeptide sequence HAV (His-Ala-Val) located around amino acid residue 80 (Blaschuk et al., 1990a,b). A decapeptide containing this sequence in a concentration of 1 mg/mL completely inhibits compaction of mouse eight-cell-stage embryos and neurite outgrowth from dorsal root ganglia (DRG) grown on astrocytes by 40% (Blaschuk et al., 1990b), indicating the importance of this region in cadherin mediated cell adhesion.

In an effort to identify which region of the cadherins determines binding specificity, Nose et al. (1990) analyzed various chimeras of E- and P-cadherin. They found that the 113 amino-terminal residues are essential: For example, when this region of E-cadherin was replaced by the corresponding region in P-cadherin, the chimeric molecule displayed P-cadherin specificity. This region also represents the most highly conserved part of the extracellular domain. To determine which residues in ECI are important for the specificities of E- and P-cadherin, several nonconserved amino acid residues in E-cadherin were replaced by the corresponding P-cadherin residues (Nose et al., 1990). It was found that replacement of two amino acids at positions adjacent to the HAV-sequence altered the binding specificity of the molecule so that cells expressing mutant E-cadherin aggregated with both E- and P-cadherin, although still with a higher affinity for E-cadherin (Nose et al., 1990). The mutations in E-cadherin did not alter the binding specificity of E-cadherin to N-cadherin, indicating that the mutations at these two positions did not induce a general loss of cadherin specificity. This points to a central role for the residues surrounding the HAV sequence in determining the binding specificities of cadherins. The 113-amino-acid *N*-terminal region in E-, N-, and P-cadherin also harbors the epitopes of the adhesion-blocking monoclonal antibodies ECCD1, PCD1, and NCD2, respectively (Nose et al., 1990).

Another interesting area in relation to cadherin specificity and binding is the extracellular region close to the membrane. Several monoclonal antibodies react with epitopes in this area and block adhesion (Ozawa et al., 1991; Volk et al., 1990a; Volk and Geiger, 1986b; Vestweber and Kemler, 1985), indicating that this region also may be involved in the adhesive mechanism. As mentioned above, four cysteine residues are located in the extracellular membrane-proximal region of cadherins. When Ozawa et al. (1991) treated dissociated cells with a reducing agent, the cells aggregated but in a more loose manner than control cells; that is, the cells did not compact (Ozawa et al., 1991). Not only the type of cadherin on a cell, but also the number of cadherin molecules affect binding (Friedlander et al., 1989). Cells expressing high amounts of either N-cadherin or L-CAM segregate from cells expressing low amounts of the identical CAM.

Transmembrane Interactions

Cadherins constitute the transmembrane component of the cell-to-cell adherens junction called *zonula adherens*. In these junctions, actin filaments

are associated with the plasma membrane through a well-developed undercoat (Itoh et al., 1991 and reviews by Geiger, 1989; Geiger et al., 1990b; Tsukita et al., 1990). Some unique proteins have been demonstrated to be concentrated here, among them vinculin, α-actinin, tenuin, plakoglobin, radixin, and several kinases, which most likely are implicated in signal transduction (Geiger et al., 1990b; Tsukita et al., 1990; Itoh et al., 1991). Cadherins have been found to colocalize with some of these proteins as well as with cortical actin bundles (Boller et al., 1985; Geiger et al., 1987; Hirano et al., 1987; Heimark et al., 1990; Matzusaki et al., 1990). Three proteins, termed catenins, form complexes with cadherins in immunoprecipitation experiments (Kemler and Ozawa, 1989).

Cadherin-Binding Proteins, Catenins

Using various anticadherin antibodies, protein analysis has revealed that by immunoprecipitation additional polypeptides of about 102, 88, and 80 kDa are coprecipitated. These have been termed catenins α, β, and γ, respectively (Peyriéras et al., 1983, 1985; Yoshida-Noro et al., 1984; Vestweber and Kemler, 1984, 1985; Ozawa et al., 1989, 1990b; Herrenknecht et al., 1991; McCrea and Gumbiner, 1991; Wheelock and Knudsen, 1991; Hirano et al., 1992; Ozawa and Kemler, 1992). Conversely, an α-catenin antibody has been shown to immunoprecipitate complexes containing various cadherins (Herrenknecht et al., 1991), demonstrating a strong affinity between the two groups of molecules. The cadherin–catenin association is controlled by a cadherin domain containing the carboxy-terminal 72-amino-acid residues (Nagafuchi and Takeichi, 1989; Ozawa et al., 1990b). Deletion of the carboxy-terminal half of the cytoplasmic domain leads to loss of the ability to coimmunoprecipitate any of the catenins (Ozawa et al., 1989, 1990b; Nagafuchi and Takeichi, 1989).

Ozawa and Kemler (1992) analyzed the structure and assembly of an E-cadherin–catenin complex and found that β-catenin is strongly and directly associated to E-cadherin, whereas the binding of α-catenin is weaker than that of β-catenin but stronger than γ-catenin binding (Ozawa and Kemler, 1992). This is in agreement with other studies demonstrating the presence of α- and β-catenin or β-catenin alone after stringent washings of the E-cadherin immunoprecipitate (McCrea and Gumbiner, 1991; Nagafuchi et al., 1991).

α-Catenin is probably identical to an adherens junction protein of 102 kDa (CAP102), which has been shown to be a vinculin homolog (Nagafuchi et al., 1991). L-cells deficient in cadherin molecules express the mRNA for α-catenin, but exhibit only trace amounts of the protein. After transfection with cDNAs for either E-, N-, or P-cadherin, the production of the α-catenin protein was shown to be greatly enhanced (Nagafuchi et al., 1991). This effect is not observed for E-cadherin transfectants lacking the catenin-binding area (Nagafuchi et al., 1991). Thus, cadherin molecules may stabilize α-catenin turnover by reducing degradation through the specific binding to this molecule or alternatively by increasing the translational activity of α-catenin mRNA through a feedback mechanism by decreasing the free form of the molecule (Nagafuchi et al., 1991).

Based on cDNA sequencing *Xenopus laevis*, β-catenin has been shown to be closely related, but not identical, to human plakoglobin and to the plakoglobin homolog in *Drosophila melanogaster*, the product of the segment polarity gene *armadillo* (McCrea et al., 1991). The amino acid homology between mouse β-catenin and human plakoglobin is 68% (Butz et al., 1992; Peifer et al., 1992). The fact that plakoglobin and β-catenin both are found in adherent-type junctions suggests that these proteins may be involved in related cellular processes (Butz et al., 1992). In analogy with the direct interaction of β-catenin with cadherins, plakoglobin has been shown to interact directly with desmosomal glycoprotein I, which is a major glycoprotein component of the desmosomes and, again, also a member of the cadherin superfamily (Korman et al., 1989).

γ-Catenin has not been isolated, but it has been suggested that this catenin is identical to plako-

globin (Knudsen and Wheelock, 1992; Peifer et al., 1992). These two molecules migrate identically in SDS-PAGE and plakoglobin coimmunoprecipitates and colocalize with both E- and N-cadherin (Knudsen and Wheelock, 1992; Peifer et al., 1992). As previously mentioned, deletion of the carboxy-terminal half of the cytoplasmic domain of a cadherin leads to loss of catenin binding, and also to loss of adhesive function and cytoskeletal anchoring, indicating that the catenins are essential for these functions (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989, 1990b). Thus, one may suggest that these two functions are coupled. What is the purpose of this coupling? Several theories exist: For example, during neural growth and guidance there has to be a transduction of extracellular signals to the cytoskeleton to direct axons properly (*see* Morphogenetic Role of Cadherins in the Nervous System). This signal transduction may be mediated by the cadherin–catenin complex. Cadherins may also act as inducers of cell surface polarity by a catenin-mediated redistribution of the membrane cytoskeleton, as demonstrated for E-cadherin by McNeill et al. (1990). This study demonstrated that when fibroblasts were transfected with E-cadherin a redistribution of Na^+,K^+-ATPase took place to sites of E-cadherin-mediated cell–cell contacts, similar to that in polarized epithelial cells. At the same time, a reorganization of the membrane cytoskeleton could be observed (McNeill et al., 1990). The cadherin–catenin complex has also been implicated in the organization of multicellular structures: An α-catenin deficient E-cadherin expressing cell line that grows as isolated cells can be transformed into growing in aggregates showing epithelial arrangements and occasionally cystic spheres by transfection with an α-catenin variant, αN-catenin (Hirano et al., 1992). Finally, it has been suggested that the cadherin–catenin protein complex may participate in transducing developmental information between neighboring cells, since this seems to be the case for the product of the segment polarity gene *armadillo* (McCrea et al., 1991; Peifer et al., 1992).

One way of regulating the binding of cadherins to catenins, and thereby presumably to the cytoskeleton, may be through an uncoupling, for example by a protease, of the cadherins from the catenins. Indeed, Covault et al. (1991) have demonstrated that N-cadherin is a substrate for an intracellular calcium-activated neutral protease, most likely μ-type calpain. Another regulatory mechanism is probably phosphorylation of the cadherin–catenin system itself, leading to an uncoupling of the cadherins from the cytoskeleton (Lagunowich and Grunwald, 1991; Matsuyoshi et al., 1992; Behrens et al., 1993). This has been demonstrated by transforming nonmetastasizing cadherin-expressing cell lines with v-*src*, whose oncogene product, $p60^{v\text{-}src}$ is a tyrosine kinase (Matsuyoshi et al., 1992; Behrens et al., 1993). This led to acquisition of a metastatic potential and looser contacts between cell aggregates. α-Catenin, and to a lesser degree P- and E-cadherin, where phosphorylated in the transformed cells (Matsuyoshi et al., 1992; Behrens et al., 1993). Thus, tyrosine phosphorylation of the cadherin–catenin system itself might affect its function, causing unstable cell–cell adhesion, which again could be a way of regulating cadherin function during development.

Morphogenetic Role of Cadherins in the Nervous System

N-Cadherin is the most abundant, although not the only, neural cadherin. Expression and function of N-cadherin during development has been determined, and its importance in neurite outgrowth in various in vitro systems has been evaluated.

N-Cadherin is first observed at the primitive-streak stage (Hatta and Takeichi, 1986). Mesodermal cells separating from the ectoderm, which until then has been expressing L-CAM, start expressing N-cadherin and downregulate L-CAM. The same phenomenon is observed during formation of the neural tube, where ectodermal cells of the neural plate gradually lose L-CAM and acquire N-cadherin expression (Thiery et al., 1984; Crossin et al., 1985; Hatta and

Takeichi, 1986; Hatta et al., 1987; Duband et al., 1988). Does N-cadherin play an active role in these morphogenetic events? Following neural induction in *Xenopus laevis* embryos, N-cadherin mRNA is expressed in the ectoderm prior to the morphogenetic events, indicating a possible role in these processes (Detrick et al., 1990). Furthermore, ectopic expression of injected N-cadherin in the ectoderm leads to severe morphological defects (Detrick et al., 1990; Fujimori et al., 1990).

At the apposition of the neural folds, N-cadherin staining increases in the neural epithelium, whereas it disappears from the premigratory neural crest cells just prior to migration away from the neural tube and aggregation into, among other derivatives, the dorsal root ganglia (DRG) (Hatta et al., 1987; Duband et al., 1988). Neural crest cell migration takes place in the rostral part of the sclerotome (Teillet et al., 1987; Serbedzija et al., 1989). At the time of migration, T-cadherin expression is turned on in the caudal part of the developing sclerotome (Ranscht and Bronner-Fraser, 1991). This polarized fashion of expression is maintained during neural crest cell migration. Thus, T-cadherin expression and neural crest cell migration is topographically inversely correlated. This may reflect a role for T-cadherin in the process of segmental orientation of the peripheral nervous system in vertebrates (Ranscht and Bronner-Fraser, 1991): T-cadherin may exert its effect by making the cells in the caudal part inhibitory or less permissive than cells in the rostral part of the sclerotome for migrating neural crest cells.

N- and R-cadherin are found in chicken DRG, sympathetic, enteric, trigeminal, and ciliary ganglia (Duband et al., 1987, 1988; Hatta et al., 1987; Inuzuka et al., 1991b; Redies et al., 1992), although R-cadherin is only transiently expressed in some of these ganglia (Inuzuka et al., 1991b). E-cadherin expression in the nervous system is confined to a subset of neurons in the DRG and trigeminal ganglia from embryonic d 11.5 (E11.5) (Takeichi et al., 1990b). It is possible that E-cadherin is added or substituted for N-cadherin in these ganglia (Takeichi et al., 1990b).

In the chicken spinal cord, R-cadherin is observed in the motor column and the associated ventral root, the lateral marginal zone, and the ventricular zone at E3.5 (Inuzuka et al., 1991b). This distribution changes gradually, and at around E6.5–E8.5 R-cadherin is mainly expressed in the midline region (Inuzuka et al., 1991b; Redies et al., 1992). In E6–E11, chicken embryos N-cadherin is expressed by the descending trigeminal tract in pons and medulla oblongata, which is contiguous with the N-cadherin positive zone of the dorsal funiculus of the spinal cord (Redies et al., 1992). At the same time R-cadherin is expressed by the visceral motor system of the vagus and glossopharyngeal nerves (Redies et al., 1992), again demonstrating the topographically restricted expression of cadherins during development.

In the chicken cerebrum N-cadherin is expressed uniformly at high levels in the cerebral and optic lobes already at E7 (Lagunowich et al., 1992). At the time of hatching, N-cadherin expression in the forebrain is much reduced with high levels restricted to the choroid plexus and the ependymal cells lining the ventricles (Lagunowich et al., 1992). The same distribution is observed for B-cadherin at E13 in addition to expression in the optic tectum layers VI and VIII (Napolitano et al., 1991). The relatively high expression of N- and B-cadherin in the ependymal cells and choroid plexus suggests that these molecules may play a role in the formation of junctional complexes that function as selective barriers between components of the cerebrospinal fluid and brain (Napolitano et al., 1991; Lagunowich et al., 1992).

In the chicken cerebellum there is an increase in N-cadherin expression through hatching. The spatiotemporal expression pattern in this part of the brain is different from other regions, most likely owing to the relatively late maturation of the cerebellum (Lagunowich et al., 1992). The lens is formed by invagination of the ectoderm. N-cadherin is observed in the lens placode but is almost totally absent from the surrounding ectoderm (Hatta and Takeichi, 1986). This expression continues into adulthood (Maisel and Atreya, 1990). R-cadherin is not observed in the lens (Inuzuka et al., 1991b). In the retina of the chicken embryo, N-cadherin is evenly distributed at early

developmental stages, whereas around hatching it is confined to the outer limiting membrane (Matsunaga et al., 1988a). The expression pattern of R-cadherin in the retina during development varies from that of N-cadherin. R-cadherin expression appears only after a certain degree of neuronal differentiation has taken place and persists in several regions of the retina at least until hatching (Inuzuka et al., 1991a). The distribution of N- and R-cadherin is complementary in many regions of the retina and optic stalk (Inuzuka et al., 1991a). This suggests the existence of some regulatory mechanism for the differential expression of these two molecules; e.g., that R-cadherin suppresses N-cadherin expression or vice versa. This might be an efficient mechanism for the immediate switching of cadherin expression from one type to another during development (Inuzuka et al., 1991a).

The development of neurons depends on interactions with molecules in their environment. These include chemotropic and trophic factors, cell adhesion molecules, and molecules anchored in the extracellular matrix. Several molecules involved have been identified in recent years (*see* reviews by Reichardt et al., 1990; Edelman and Cunningham, 1990; Bixby and Harris, 1991; Hynes and Lander, 1992).

Neurite growth in relation to cadherins has mainly been studied using one of three in vitro approaches:

1. Inhibition of neurite growth either by antibodies to N-cadherin (Bixby et al., 1987, 1988; Neugebauer et al., 1988; Tomaselli et al., 1988; Letourneau et al., 1990, 1991; Drazba and Lemmon, 1990; Chuah et al., 1991) or by synthetic cadherin-peptides containing the tripeptide HAV (Blaschuk et al., 1990b; Chuah et al., 1991; Doherty et al., 1991a);
2. Enhancement of neurite-promoting activity by means of cells transfected with N-cadherin cDNA as cellular substratum for the neurite extending cells (Matsunaga et al., 1988b; Doherty et al., 1991a, 1992; Redies et al., 1992);
3. Induction of neurite growth using purified N-cadherin as substratum (Bixby and Jhabvala, 1990; Bixby and Zhang, 1990; Lemmon et al., 1992; Payne et al., 1992).

The various studies are somewhat difficult to compare, since the neurons and the cells used for support vary between studies, as do their developmental stages. For example, whereas N-cadherin antibodies inhibit neurite growth from E8 chicken ciliary ganglia on astrocytes by 75% (Tomaselli et al., 1988), neurite growth from the same cells on myotubes can only be inhibited by 37% and only when a combination of antibodies against N-cadherin, N-CAM, and several extracellular matrix receptors are used (Bixby et al., 1987). Low Ca^{2+} can also mimic the effects of antibodies against N-cadherin (Letourneau et al., 1991).

Using cadherin-peptides containing the tripeptide HAV inhibition of about 80% is found in cultures of olfactory mucosa neurons grown on astrocytes and in cultures of retinal ganglion cells grown on N-cadherin transfected 3T3 cells (Chuah et al., 1991; Doherty et al., 1991a), whereas outgrowth from DRG neurons on astrocytes is suppressed by 40% (Blaschuk et al., 1990b). Redies et al. (1992) cultured chicken E6 DRG explants on N-cadherin transfected neuroblastoma cells and observed strong defasciculation that could be inhibited by anti-N- cadherin IgG, again suggesting that axons can use N-cadherin as a cue for directed growth along preformed, molecularly defined pathways.

Doherty et al. (1991b) showed that the mechanism involved in N-cadherin stimulated neurite outgrowth from PC12 cells grown on N-cadherin-transfected nonneuronal cells seem to be an activation of L- and N-type neuronal calcium channels via a pertussis toxin-sensitive G-protein. The same investigators have also shown that exogenously added ganglioside GM1 can become stably associated with PC12 cells and significantly enhance both NCAM- and N-cadherin-dependent neurite outgrowth (Doherty et al., 1992). These data suggest that gangliosides may promote axonal growth in vivo by promoting cell adhesion molecule-induced calcium-influx into neurons (Doherty et al., 1992). It has also been demonstrated that substrate-bound purified N-cadherin is a potent factor for induction of neurite outgrowth from chicken ciliary ganglia, more so

than L1 and comparable to laminin (Bixby and Zhang, 1990).

A factor that guides as opposed to one that simply promotes neurite outgrowth can be defined as an activity that is actually responsible in vivo for steering growth cones along their proper pathways (Bixby and Harris, 1991). One feature indicating a guidance function is correlation between the in vivo spatio-temporal expression pattern of the molecule in question with morphogenetic changes, implying a guidance event, e.g., axon growth (Bixby and Harris, 1991). T-cadherin is expressed on motor neurons, on the spinal cord floor plate, and on the caudal sclerotomes of the chicken embryo, and this restricted distribution therefore makes it a candidate for a guidance molecule most likely inhibitory in nature (Ranscht and Bronner-Fraser, 1991). N-cadherin is, as mentioned, also expressed in a restricted manner in, for example, the spinal cord dorsal funiculus and the dorsal root fibers (Redies et al., 1992). This is also true for R-cadherin, which, for instance, is expressed in the motor nuclei of the vagus and glossopharyngeal nerves and their processes (Redies et al., 1992).

Perspectives

One of the more intriguing problems that some researchers have started addressing is the question of how the spatio-temporal expression of the various cadherins is regulated. Another interesting area, in which some progress has been made, is the elucidation of the structure of the transmembrane interactions and the involvement of secondary messengers in the adhesion reaction "cascade." When it comes to cadherins in the nervous system, it will be of importance to know more about their spatio-temporal expression pattern to better understand their roles during development in processes such as neurite growth and guidance. Hopefully, the use of transgenic animals will prove an important tool in revealing the role of cadherins in vivo during development. Finally, one important subject, which is beyond the scope of this review, is the role of cadherins, especially E-cadherin, in invasion and metastasis of cancer. This is a rapidly growing area, and interested readers are referred to the latest reviews (Mareel et al., 1992; Van Roy and Mareel, 1992).

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