Reviews

Adhesion molecules and animal development

H. Anderson

Department of Zoology, University of California, Davis (California 95616, USA)

Summary. In recent years considerable progress has been made in the identification and characterization of molecules that mediate cell adhesion during animal development. This review attempts to pick out from the vast amount of information in this rapidly expanding field some of the key features of adhesion molecules, to present ideas about their role in development, and to indicate the directions in which the field is now moving.
Key words. Adhesion molecule; animal development; morphogenesis; embryogenesis.

Introduction

Adhesive interactions between cells are important throughout the life of a multicellular organism. It is adhesive interactions that bind sperm to egg at the moment of conception, and that hold cells together in the differentiated tissues and organs of the adult. But it is also adhesive interactions that mediate many of the mysterious developmental processes that convert a fertilized egg into an adult: the movement of cells and the arrangement of cells into tissues and organs.

The first experimental evidence that embryonic cells differed in their adhesion to one another, and that this correlated with their developmental fate, was provided in 1955 by Townes and Holtfreter[144] in their classic experiments on amphibian embryos. They dissociated ectodermal, mesodermal, and endodermal cells from gastrulae and packed them together randomly in aggregates. The cells within the aggregates, identified by differences in size and pigmentation, sorted out into distinct homogeneous layers whose stratification corresponded to the normal germ layer arrangement in the intact embryo: the endodermal cells formed a compact ball, the ectodermal cells formed a surface epithelium, and between them the mesodermal cells formed a mass of loose mesenchyme.

As embryogenesis proceeds, many additional cell layers are formed and cells rearrange themselves into more complex patterns within them. But do such changes result from a change in the number or in the type of adhesion molecules on the cell surface? How many types of adhesion molecule are there? What properties do adhesion molecules have? These questions could not begin to be answered until the late 1970s when techniques were developed that permitted the isolation and characterization of individual adhesion molecules.

Most adhesion molecules have been identified using immunological methods: the cell types of interest are placed into a culture system in which adhesion can be quantified – by monitoring aggregation of cells in suspension or attachment of cells to a substratum for example. Antibodies are made against membrane fractions of these cells or against the substratum, added to the culture system, and tested for their ability to block cell adhesion. The antigens involved in adhesion are then identified by extracting components from solubilized cell membranes or the substratum and testing them for their ability to bind the antibodies. Since inhibition of adhesion by antibodies against a particular molecule is not direct proof of the adhesive function of that molecule, independent evidence of adhesive function has been sought by binding studies using the molecule as a tissue culture substratum[3,16,79,90,117,136,146], or conjugated to polystyrene beads[62,132], or inserted into liposomes[5,107,119]. Other tests have included the demonstration of acquired adhesive properties in normally non-adhesive cells transfected with cDNA that encodes adhesion molecules[45,58,91,94], a reduction in adhesive properties of cells transfected with antisense constructs of cDNA that encodes adhesion molecules[36], and the loss of adhesive properties in mutants defective in the adhesion molecule[98,136]. Many of these tests are open to several interpretations and a combination is really required for conclusive proof of function.

It is now clear that there are many different adhesion molecules expressed during development. Tables 1 and 2 list those identified to date; there are certainly more to be discovered. The extent to which they have been studied varies considerably. Some, such as NCAM and fibronectin, have been subject to intensive investigation for more than a decade while others are barely more than new protein sequences. Many of the adhesion molecules were discovered and named independently in different laboratories, some working with different species. Tables 1 and 2 list both the most commonly used name and other names used in the literature.

It is also clear that no single class of molecule is responsible for cell adhesion; glycoproteins, proteoglycans, and glycolipids may all act as adhesion molecules, binding by different mechanisms and with widely differing strengths. The complexity of animal development is certainly reflected in the diversity of adhesion molecules and mechanisms.

This review aims to extract from this diversity some of the key features of adhesion molecules involved in animal

Table 1. Cell adhesion molecules

Name and ref.	$M_r \times 10^3$	Name and ref.	$M_r \times 10^3$
+ NCAM [37,10]	180, 140, 120	+ csA [97]	80
BSP-2 [53]		+ F11 [20]	170, 130
+ E-cadherin [94]	124	+ contactin [109]	130
uvomorulin [113]		neurofascin [111]	185, 160
L-CAM [51]		R-cognin [59]	50
Cell CAM 120/80 [39]		+ MAG [80,107]	100
Arc-1 [12]		astrotactin [46]	100
+ P-cadherin [99]	118	+ fasciclin I [152]	70, 37
+ N-cadherin	127	+ fasciclin II [57]	95
N-cal CAM [35]		+ fasciclin III [102]	80, 66, 59, 54
A-CAM [148]		+ *l(2)gl* [70]	30
+ L1 [93]	180, 135, 80	+ neuroglian [153]	180, 167
NILE [11]			
NgCAM [55]			
8D9 [86]			
G4 [112]		+ primary sequence known	
69A1 [106]			
ASCS4 [137]			

Table 2. Matrix adhesion molecules

ECM Molecules Name and ref.	$M_r \times 10^3$	ECM Receptors Name and ref.	$M_r \times 10^3$
Glycoproteins		Glycoproteins	
+ fibronectin [75,133]	2 subunits, 250 each	+ fibronectin-integrin [8]	2 subunits 140 each
+ laminin [43,123,124,125]	3 subunits 440, 230, 220	laminin-integrin [52,143]	2 subunits 150, 120
+ tenascin [103]	2 subunits 220 each	tenascin-integrin [16]	2 subunits 145, 125
cytotactin [67]		fibronectin receptor [6]	47
hexabrachion [47]		# laminin receptor [54,87,88,110,149]	2 subunits 67 each
myotendinous antigen [47]		laminin receptor [74,135]	120 & 180
brachionectin [47]		laminin receptor [116]	
J1 [47]		discoidin I receptor [50]	67
GMEM [47]		Proteoglycans	
+ thrombospondin [85]	3 subunits 180 each	fibronectin receptor [84]	
+ nidogen/entactin [44,89]	150	adheron/purpurin receptor [126]	
+ discoidin I [108]	4 subunits 28 each	NCAM receptor [31,33]	
AMOG [5]	45–50	tenascin receptor [147,62]	
echinonectin [2]	2 subunits 230 each	Glycolipids	
+ purpurin [13]	20	fibronectin ganglioside receptor [24,25]	
+ amalgam [128]	333 amino acids	laminin sulphatide receptor [115]	
Complexes (from conditioned medium)		laminin ganglioside receptor [68]	
adheron [127]	15–20 nm particle		
neuronectin [34]	350	+ primary sequence known	
laminin/heparan sulphate proteoglycan complexes [40,81–83]		# partial sequence known	

development, and to indicate progress that is now being made with a new and more difficult set of questions: how do the adhesive properties of cells influence the initiation, execution, and regulation of developmental processes?

Functional categories of adhesion molecule

Adhesion molecules are generally placed into one of two categories according to their function. Molecules in one category mediate the adhesion of cells to one another and are commonly called cell adhesion molecules (or CAMs). Cell adhesion molecules are integral components of the cell membrane. They are anchored in place either through a stretch of hydrophobic amino acids or through a covalently bound hydrophobic phosphatidylinositol-containing glycolipid e.g.[60].

Molecules in the second category mediate the adhesion of cells to the extracellular matrix (ECM) and may be called matrix adhesion molecules or substratum adhesion molecules (or SAMs). They are likely to be of particular importance in the migration of cells over or through the ECM during development. ECM refers to the acellular material filling the spaces between mesenchymal cells and also to the specialized sheet of material called the basal lamina which is attached to the basal surface of epithelia, separating them from subjacent mesenchyme. ECM contains various components secreted by the surrounding cells including collagens, glycoproteins, and proteoglycans, some of which are adhesive to cells. The category of matrix adhesion molecules includes both adhesive ECM components and their receptors on the cell surface.

In practice, the distinction between the two categories of adhesion molecule is not always clear-cut. For example, in tissue culture many cell adhesion molecules, especially those associated with the cell surface via a phosphatidylinositol linkage, may be released into the medium and

bind to the tissue culture surface[59,60,137]. At this point a molecule formerly involved in cell-cell adhesion may participate in cell-substratum adhesion. For example, although NCAM is generally considered to be a cell adhesion molecule, some forms of NCAM are secreted and deposited in the ECM in vivo[29] and can act as matrix adhesion molecules through binding to cell surface NCAM and heparan sulphate proteoglycan[33].

Common structural features

Amino acid sequences

The properties of adhesion molecules have been studied in a variety of ways using the techniques of molecular biology, biochemistry, cell biology, and developmental biology. With each approach different properties are revealed. The most fundamental – the primary amino acid sequence – will be considered first.

Deduced amino acid sequences are now available for many of the adhesion molecules. Examination of the sequences shows that many contain internally repeating sequences, and a number of motifs or stretches of primary sequence are found in several different adhesion molecules. These observations support the view that the majority of proteins have evolved from a limited number of archetypal polypeptides through duplications, rearrangements, and divergence[42]. Figure 1 summarizes in simple form the arrangement of some of these motifs in those adhesion molecules for which a primary structure is now available. Study of these motifs may help in understanding the function of adhesion molecules.

The cadherin family. The cadherins are Ca^{2+}-dependent cell adhesion molecules. The E-, P-, and N-cadherins each consist of four domains which are homologous both within each molecule and between the three species identified so far. These adhesion molecules are considered to be members of a larger family with additional members remaining to be identified. The product of the *l(2)gl* gene of *Drosophila* is a homophilic cell adhesion molecule and its cDNA sequence has several regions of homology with the cadherins[70].

The integrin family. The integrins[65] are also a family of molecules with considerable sequence homology. They are all noncovalently-linked heterodimers with distinct alpha and beta subunits; there are at least 3 different beta subunits and more than 10 different alpha subunits, homologies existing within each group but not between alpha and beta subunits. Some alpha subunits are cleaved posttranslationally to give a heavy chain and a light chain linked by disulphide bonding. The integrins are cell surface receptors. Many are involved in adult physiological processes involving adhesive events, but several act as matrix adhesion molecules during development and are included in this review. These are the receptors for the ECM molecules fibronectin, laminin, and tenascin.

Immunoglobulin-like domains. Many of the cell adhesion molecules have several repeats of a sequence about 100 amino acids long containing two conserved cysteine residues which form a disulphide bond. This bond stabilizes the interaction between other conserved amino acids within the sequence to form two beta sheets folded together around a hydrophobic interior. This motif was first identified in immunoglobulin molecules and is called an immunoglobulin or Ig-domain and has been found in a variety of molecules involved in adhesive or binding functions[150]. Binding between Ig-domains can be homophilic, and may provide a form of weak interaction between cell surfaces. The domain has also been considered as providing a stable platform for the display of specific determinants for recognition reactions on the faces of beta sheets or at the bends between the beta strands. These determinants could be protein or carbohydrate in nature.

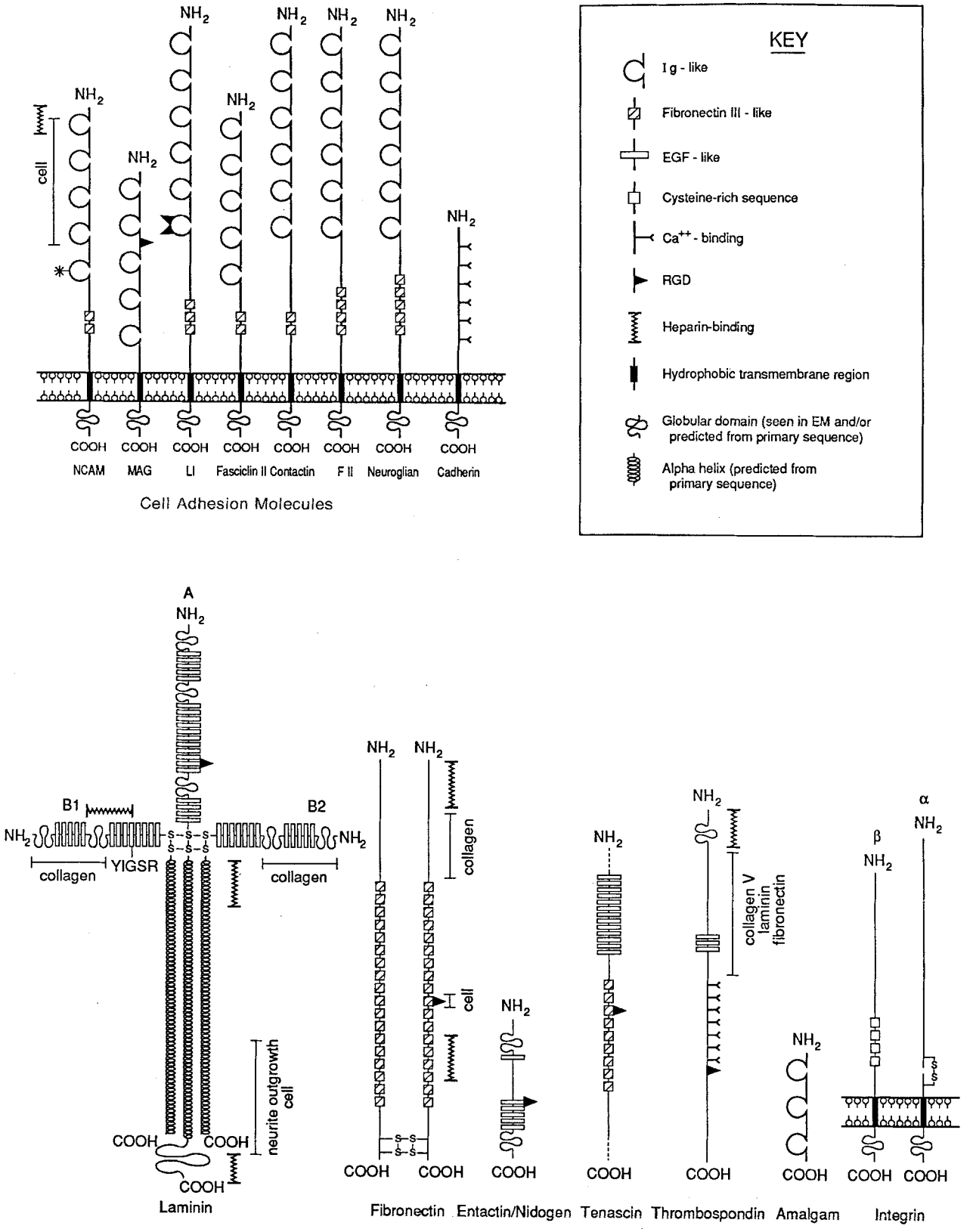
Fibronectin type III domains. This motif, which is about 100 amino acids long, was first identified in the ECM molecule fibronectin where it appears at least 15 times. It is present in several cell adhesion molecules (NCAM, L1, fasciclin II and contactin) and matrix adhesion molecules (fibronectin, tenascin). Little is known about its function.

EGF-like domains. A sequence about 40 amino acid residues long which has significant homology to Epidermal Growth Factor (EGF) has been found in the rod-like regions of several matrix adhesion molecules – laminin, nidogen/entactin, tenascin and thrombospondin. Its characteristic is the presence of 6 cysteines in highly conserved locations which form 3 disulphide bonds constraining the sequence to fold into several loops[7]. In general there are also several conserved turn-forming residues. The loops are thought to provide the ligand in receptor-ligand interactions, with the recognition sequence or binding site at the end of one of the loops, but with additional sequences contributing to the binding site to confer specificity or else maximizing binding. EGF-like domains could also form a scaffolding structure since they seem to be associated with the rod-like parts of molecules as indicated by EM. It is also possible that these regions confer a growth-stimulating function to the molecule. Both laminin and tenascin have been observed to have such a function under certain in vitro conditions[9,26].

The integrin beta subunits also contain four repeats of a 40 amino acid cysteine-rich sequence in their extracellular domain[8]. It does not have the same sequence as the EGF-like domain and its function is not known.

Ca^{2+}-binding sequences. The cadherins, thrombospondin, and the integrins require Ca^{2+} for their function and contain putative Ca^{2+}-binding regions which have clusters of Asp, Asn, Thr, and Ser residues in predicted loops at the surface of the protein[8,85,113].

The RGD sequence. The tripeptide Arg-Gly-Asp (RGD in letter code for amino acids) has been identified as the sequence in a number of ECM molecules which is recog-



Schematic diagrams of the primary sequence of cell adhesion molecules and matrix adhesion molecules that contain commonly-occurring motifs. The sequences are derived from information in references listed in tables 1 and 2. The sequences of only one thrombospondin subunit and one tenascin subunit are shown. *Amalgam* was thought to be a cell adhesion molecule but is now known to be secreted (Hortsch and Seeger, unpublished). The asterisk on NCAM indicates the location of polysialic acid. YIGSR on the laminin B1 subunit is a cell-binding sequence. For additional details, see text.

nized by their corresponding cell surface integrin receptor[118]. This sequence is present in many adhesion molecules: L1, MAG, laminin A chain, fibronectin, nidogen/entactin, tenascin, discoidin I and integrin. It may be contained within Ig-domains, EGF-like domains, fibronectin type III domains, or in regions that have no obvious homology with other sequences (fig. 1), suggesting an independent evolution of RGD and these motifs. It is unlikely that the RGD sequence serves as an adhesive binding site in most of these cases. Indeed, RGD is present in more than 120 proteins[151], most of which have no adhesive function. The RGD sequence has been shown to be a cell-binding site for fibronectin[118], discoidin I[136], nidogen/entactin[44,89], and tenascin[16]. It does not appear to be active in thrombospondin[49], nor in the binding of laminin to its integrin[52]. It seems that the presence of other amino acids around the RGD sequence may be important for the presentation of the sequence in the appropriate conformation for recognition by its receptor[105].

Heparin-binding regions. Heparin is a highly sulphated (and highly negatively charged) glycosaminoglycan, covalently bound to protein to form proteoglycan. Macromolecules that bear heparin chains and other glycosaminoglycans are widely distributed in the ECM and on cell surfaces and are likely participants in adhesive interactions. Indeed, several proteoglycans on the cell surface have now been identified as ligands for adhesion molecules (table 2).

Protein sequences which bind heparin (or other negatively charged glycosaminoglycans) tend to have a relatively low (negative) value of hydropathy index and a relatively high number of the strongly positively charged amino acid residues lysine and arginine. But these are not absolute prerequisites; there is no consensus sequence established for heparin-binding. Biochemical studies have located heparin-binding regions on several ECM and cell adhesion molecules. Laminin has at least three heparin-binding sites[134], one of which has been sequenced[22]. Fibronectin, thrombospondin and NCAM also contain heparin-binding regions[30,49,85,117].

Transmembrane and cytoplasmic sequences. Many of the adhesion molecules are integral membrane proteins with a hydrophobic transmembrane region. The structure of the cytoplasmic domains has aroused a great deal of interest because it is through these domains that adhesion molecules could serve functions other than their adhesive function; an adhesive event could induce a change in the cytoplasmic domain which in turn could provide a signal to the cell to evoke a particular response. However, examination of the sequences of the cytoplasmic domains offers few clues about how or where these signalling interactions might occur. The avian fibronectin integrin cytoplasmic sequence has a sequence for tyrosine phosphorylation[8], which is in a region that binds the cytoskeletal component talin; phosphorylation in this cytoplasmic region inhibits talin binding to integrin[21]. The link between integrin and the cytoskeleton could provide the signalling mechanism for the change in cell shape observed when cells adhere to fibronectin and the abnormality of this response when cells are transformed by oncogenes which phosphorylate this region[21].

Unique protein sequences. The sequences of other adhesion molecules not depicted – csA, fasciclin I, fasciclin III, discoidin I, and purpurin – have no obvious homologies either with each other or with other proteins.

Carbohydrate moieties

All of the adhesion molecules contain carbohydrate, but in very few cases has the nature of the carbohydrate and its role in adhesion been determined. However, two carbohydrates have been found to be present on several adhesion molecules. A 3' sulphated glucuronic acid (recognized by L2 and HNK-1 antibodies) is present on some species of NCAM, L1, tenascin, MAG, the proteoglycan ligand for tenascin, and the fibronectin integrin[32,62,77,104]. An N-glycosidically linked carbohydrate epitope (exact identity unknown, but recognized by the L3 antibody) is found on some molecules of L1, MAG, AMOG, and the fibronectin integrin[78].

Diversity and complexity

Although examination of the primary sequence of many of the adhesion molecules reveals several interesting common features, biochemical and cell biological studies emphasize the diversity and complexity of their properties.

Multifunctional molecules. Electron microscopy of rotary-shadowed preparations has shown that many adhesion molecules are rather complex in structure and possess several different domains appearing as globular regions, stiff rods, and flexible threads. The different structural domains have different functions. Particular functions have been assigned to these domains through studies using peptide fragments generated after protease digestion, domain-specific antibodies, and synthetic peptides made to match the primary sequence of parts of domains. In addition to determining the location of binding sites for adhesion, these studies show that many adhesion molecules have domains that serve additional functions. Some of these regions are indicated in the schematic diagrams of adhesion molecules in figure 1.

Laminin serves as a good example to illustrate this point. It possesses domains that bind to other ECM components such as collagen, entactin/nidogen, and heparin and has at least two cell-binding domains. In addition to promoting cell adhesion, different regions of the molecules can stimulate cell migration, promote neurite outgrowth, stimulate differentiation of glial cells[90], and exert growth factor activity[9].

Adhesion molecules may also exert their effects as part of a complex. A complex containing laminin and heparan sulphate proteoglycan has been identified in vivo[27] and

its role in neurite regeneration studied[121]. Laminin is adhesive to neurons and promotes neurite outgrowth but the proteoglycan alone has no effect; the complex however is much more effective than laminin alone. Adhesive complexes are also known to be released by cells in tissue culture. Some, such as adherons[127], contain adhesion molecules such as fibronectin, NCAM, and purpurin as well as collagen, glycosaminoglycans, and other unidentified components. They exert their effects only on certain cell types. Whether adherons occur in vivo has not been demonstrated.

Multiple ligands. All of the cell adhesion molecules are membrane glycoproteins. Many show homophilic binding, i.e. like molecules on adjacent cell surfaces bind one another, and this appears to be mediated by the protein regions of the molecules. NCAM and the cadherins are well-documented cases of molecules showing homophilic binding[119,138]. For those that have been demonstrated to not bind in this manner, i.e. that show heterophilic binding such as L1 and cognin[55,145], the ligands have not yet been identified. NCAM also binds cell surface heparin. This binding may be modulatory in function, inducing a conformational change in the NCAM and permitting homophilic binding between NCAMs[31,33]. The heparin and homophilic sites are on different parts of the amino terminus[30].

The ECM adhesion molecules are also glycoproteins or glycoprotein-proteoglycan complexes (table 2). It is striking that each ECM molecule may bind (with different affinities) to many different molecules on the cell surface. Furthermore, these cell surface molecules may be of a wide range of types – glycoproteins such as the integrins, proteoglycans, or glycolipids. For example, laminin binds to a glycoprotein integrin[52,143], to a 67 kD glycoprotein through the YIGSR amino acid sequence on its B1 chain[54], to 110 and 180 kD glycoproteins[74,135], to a sialic acid-bearing glycolipid, i.e. a ganglioside[68], to a sulphated glycolipid, i.e. a sulphatide[115], and to a galactosyl transferase[116]. In this latter case the binding is between the transferase and N-linked oligosaccharides attached predominantly to the A chain of laminin[116]. Fibronectin binds to a glycoprotein integrin through its RGD sequence[8], a 47 kD glycoprotein[6], heparan sulphate proteoglycans[84], and to gangliosides[24,25]. Tenascin binds to an integrin through its RGD sequence[16] and to a chondroitin sulphate proteoglycan[62,147].

It is possible that some of the cell surface ligands act not as receptors directly but as modulators of binding to other receptors. The gangliosides may serve this function. Exogenous addition of gangliosides inhibits attachment of cells to various matrix proteins in culture[73], disialogangliosides are present in adhesive contacts of cells in tissue culture[23] and antibodies to the gangliosides disrupt this adhesion[24]. However, these are all indirect tests of an adhesive function for gangliosides. Conflicting results have been obtained from more direct tests; fluorescence polarization has indicated that fibronectin possesses a strong ganglioside-binding site which is localized in the amino terminal heparin-binding domain, separate from the integrin-binding region[142], and that the gangliosides do bind the ECM protein. However, fibronectin failed to bind to the gangliosides after their separation on thin-layer chromatography plates[24], suggesting that the gangliosides are not fibronectin receptors. The latter authors have suggested that the gangliosides, because of their known calcium-binding ability[1], instead play a modulatory role for Ca^{2+}-dependent adhesion molecules, such as the integrins, by chelating Ca^{2+} through their highly negatively-charged sialic acid component. The electrostatic environment created by the ganglioside-Ca^{2+} complex then stabilizes the interaction of the integrin with the ECM protein.

Heparan sulphate also acts as a modulator of fibronectin-mediated adhesion; when heparan sulphate binds to fibronectin it induces a configurational change in the fibronectin[101] which then binds to the cell surface with higher affinity[66]. It also appears that a second adhesive recognition site cooperates with the RGD site on fibronectin to produce full adhesive activity[100].

Multiple gene products. For several of the adhesion molecules the structure of the gene is now known and it has been shown that a single gene may produce a variety of different transcripts which produce different protein products. The best studied examples of this are the cell adhesion molecule NCAM and the ECM molecule fibronectin. Three major polypeptides are produced from the NCAM gene[37]. They have similar extracellular domains but one form lacks a transmembrane region and cytoplasmic domains and may be attached to the cell surface through phosphatidylinositol binding to lipid moieties, or may be a secreted molecule deposited in the ECM. The two other forms have transmembrane regions but differ in their cytoplasmic domains. These differences in cytoplasmic domains might reflect different linkages with the cytoskeleton or different phosphorylation capabilities, and might mediate profoundly different responses of the cell to a binding event at the extracellular domain of the molecule. In addition other variants are formed through alternative splicing in the extracellular region[41,122]. The significance of these differences in sequence for function is not yet known.

The fibronectin gene has three regions of alternative splicing and may generate at least 10 different polypeptides[117]. Two of the alternatively spliced segments lie next to a heparin-binding domain. One possible consequence of the alternative splicings may therefore be a change in the heparin-binding properties of fibronectin. The alternatively expressed segments also contain additional glycosylation sites which may affect function (see below).

Alternative splicing has also been demonstrated in tenascin[56], and in fasciclin I[152] and fasciclin III[102] and is probably widespread among the adhesion molecules.

An additional source of diversity is in the composition of adhesion molecules that are composed of subunits. Laminin, for example, is generally found as a complex of B1, B2 and A chains, but forms lacking the A chain are also present during kidney morphogenesis[64,71,72].

Differential glycosylation. The carbohydrate content of particular adhesion molecules can vary considerably. For example, NCAM bears the unusual carbohydrate polysialic acid (indicated with an asterisk on fig. 1[38]), and the amount present on the molecule is known to vary. Variation in the sialic acid content has been demonstrated to affect the strength of binding between NCAM molecules[61,120]. The L2/HNK-1 and L3 carbohydrate epitopes are found only on some examples within a population of adhesion molecules (see section Carbohydrate moities). These carbohydrates may also modulate the adhesive properties of the molecules on which they are found, although there is some evidence that the L2/HNK-1 carbohydrate has adhesive properties in its own right[114].

Glycosylation may have other more indirect effects on cell adhesion. For example, one carbohydrate moiety on the cell adhesion molecule csA of *Dictyostelium* is added as a posttranslational modification and this is blocked in *modB* mutants which show impaired adhesion. However, the requirement for this carbohydrate is not for adhesion itself, but for proper transport of the glycoprotein to the cell surface and for protection of the exposed glycoprotein against proteolytic cleavage[63]. Glycosylation of fibronectin also protects it against proteolysis[14].

In other cases glycosylation appears to be unnecessary for cell binding. For example, culturing cells in tunicamycin which blocks glycosylation does not affect E-cadherin activity[130].

Embryonic distribution

The immunocytochemical localization of adhesion molecules during embryogenesis has been an important first step in evaluating their possible roles in developmental processes. Surprisingly, most adhesion molecules are found on many different cell types and are present at many different stages of development, although it is possible that the variant forms discussed above have a more restricted distribution. For example, NCAM is expressed, sometimes transiently, on almost every cell type: notochord, neural crest, somites, placodes, epidermis, mesenchyme, mesonephros, neurons, glia, and muscle cells[141]. N-cadherin also has a broad tissue distribution at many stages of development[138]. The ECM molecules laminin and entactin/nidogen are ubiquitous basal lamina components and fibronectin is widespread. ECM receptors such as the avian fibronectin integrin are also widely distributed[76]. Other adhesion molecules may have a more limited distribution; E-cadherin is expressed only on nonneural epithelia[138], tenascin is present in only some regions of basal lamina[47], and some adhesion molecules associated with the nervous system are present on subpopulations of neurons[57,102,152]. But none of these adhesion molecules is exclusive to a particular cell type.

Rather it seems that the presence of particular adhesion molecules correlates with the occurrence of particular developmental events, such as the segregation of cells, the migration of cells, and the aggregation of cells. For example, after neural induction neuroepithelial cells switch from E-cadherin expression to N-cadherin expression at the time they segregate from surrounding E-cadherin-positive epithelial tissues[138]. Later, when neural crest cells separate from their N-cadherin-positive neighbors and migrate out of the neuroepithelium they lose N-cadherin expression, but when they aggregate to form sensory ganglia they reexpress it[138]. However, experimental studies are required to test the causal relationships suggested by these correlations.

These observations must also be considered in the light of another important point arising from immunocytochemical studies: cells usually have many different adhesion molecules on their surface. For example, ganglion cell axons from the retina bear on their surfaces the cell adhesion molecules NCAM, L1, neurofascin, F11, contactin, and N-cadherin as well as integrin matrix adhesion molecules[28,92,109,111,139,140]. In their immediate environment are ECM molecules, and the surface molecules of other ganglion cell axons, glia, and epithelial cells. The specificity of ganglion cell axon behavior (their precise navigation from particular regions of the retina to particular regions of the tectum) may arise not from the activity of a single type of adhesion molecule but from the expression of specific combinations of adhesion molecules, which can be regulated in number and modulated in activity, and which may interact cooperatively or competitively. Again, experimental studies are essential to elucidate the relative contributions of different adhesion molecules.

Testing roles in development

A number of experimental approaches have been developed to test the role of particular adhesion molecules in developmental events. Antibodies that block binding sites and peptides that compete for binding sites have been developed. Mutants that lack adhesion molecules or parts of them have been generated. Adhesion molecules have been expressed in cells that normally would not express them by the introduction of appropriate mRNA or by transfecting cells with cDNA that encodes the adhesion molecule.

Systems have also been developed in which to use these new probes and techniques. Tissue culture models have been favored because they are far more accessible to many procedures than are embryos, and because subsets of the participants in any developmental event may be studied in isolation, avoiding the sometimes overwhelm-

ing complexity of interactions in the embryo. To use an earlier example, retinal ganglion cell axons as they navigate to the tectum not only express many different adhesion molecules but also are in contact with different cell types, such as glial cells and other ganglion cells, and with the ECM. Tissue culture studies have permitted the study of these many possible interactions in isolation. Antibodies against NCAM, N-cadherin and integrins inhibit the outgrowth of retinal ganglion cell axons over glial cells[96] and retinal axons preferentially grow over cells expressing N-cadherin compared with the same cells not expressing it[91]. Antibodies against integrins inhibit their growth over laminin[28], and antibodies against G4, F11, and neurofascin inhibit their growth over one another[111,112] whereas those against N-cadherin do not inhibit fasiculation[91]. In order to establish a hierarchy of importance of these molecules which can each influence axon outgrowth when tested in isolation, more complex tissue culture systems or embryonic studies are required.

A considerable amount of effort has been put into developing in vivo systems. Synthetic transcripts for NCAM have been introduced into amphibian embryos to study the effect of its expression in cells that do not normally express it[69]. The use of insect embryos or larvae, especially *Drosophila*, in which the behavior of individually identified cells may be studied and in which sophisticated genetic techniques may be used[4,129], holds a lot of promise. Chick embryos have proved to be surprisingly accessible to perturbation by antibodies and peptides[15,17–19]. Injection of antibodies against NCAM in the developing visual system of the chick where NCAM is observed on the outgrowing retinal ganglion cells and on adjacent radial glial cells, results in the displacement of axons from the glial surface, a reduction of fasciculation of axons with one another, an overall distortion in the optic pathway, and, ultimately, the failure of axons to achieve their appropriate target locations[48,131,139]. Other antibodies, such as those used in the tissue culture experiments described earlier, have not yet been used in this in vivo system. There is also a caveat to be borne in mind when using antibodies: the binding of antibodies to the cell surface may sterically block access to molecules other than the specific antigen or may affect adhesion indirectly by perturbing a molecule that leads to other changes in the cell. Similar studies using genetically altered cells will provide useful corroborating evidence.

Adhesion molecules and differentiation

Adhesion molecules have generally been considered as acting in singular events that simply keep cells together or permit them to move apart. In the light of the many studies described above, it appears that adhesion molecules might be more appropriately viewed as participating in a sequence of events that lead to cell differentiation, and as participating in this process not only as adhesion molecules, but also as signalling molecules. For example, cells in culture initially bind to the substratum, change their morphology and spread out over the substratum, and then form focal contacts. Focal contacts are discrete specialized regions of the cell surface where cell surface adhesion molecules and the intracellular actin cytoskeleton and other cytoplasmic proteins are co-localized. Subsequently the cells, according to their type, might migrate over the substratum or remain stationary and form specialized cell junctions such as adherens junctions with one another. Each of these events might involve the participation of different adhesion molecules. For example, the galactosyl transferase receptor for laminin is required for cells to spread on laminin but not for their initial attachment[116], and N-cadherin and E-cadherin are associated with adherens junctions[113,148]. How is the appropriate sequence of expression of adhesion molecules coordinated for each pathway of differentiation?

It seems very likely that the cytoplasmic domains of the adhesion proteins themselves act as signalling sites for the expression of other surface adhesion molecules or for the clustering of existing surface molecules to sites where their combined activity is required. In addition, the cytoplasmic domains might act as receptors for intracellular signals and in turn regulate the function of the adhesion molecule. Truncation of the cytoplasmic domain of E-cadherin in cultured cells indeed blocks the binding ability of the extracellular domain and adherens junctions are not established between the cells[95]. Whether this results from an inability of the mutant molecules to interact with the cytoskeleton and a consequent failure of adhesion molecules to aggregate into focal contacts, or whether the functional state of the mutant molecules is impaired has yet to be resolved. What is clear is that adhesion molecules may have important intracellular functions and that research on adhesion molecules now needs to examine events taking place not only at the cell surface but also inside the cell.

Acknowledgments. I thank Drs Lois Abbott, Peter Armstrong, and Michel Roberge for advice on the manuscript, and the National Institutes of Health for support through grant NS 21355.

1 Abramson, M. B., Yu, R. K., and Zaby, V., Ionic properties of beef brain gangliosides. Biochim. biophys. Acta *280* (1972) 365–372.
2 Alliegro, M. C., Ettensohn, C. A., Burdsal, C. A., Erickson, H. P., and McClay, D. R., Echinonectin: a new embryonic substrate adhesion protein. J. Cell Biol. *107* (1988) 2319–2327.
3 Alstadt, S. P., Hebda, P. A., Chung, A. E., and Eaglstein, H., Effect of basement membrane entactin on epidermal cell attachment and growth. J. Invest. Dermat. *88* (1987) 55–59.
4 Anderson, H., Drosophila adhesion molecules and neural development. Trends Neurosci. *11* (1988) 472–475.
5 Antonicek, H., and Schachner, M., The Adhesion Molecule On Glia (AMOG) incorporated into lipid vesicles binds to subpopulations of neurons. J. Neurosci. *8* (1988) 2961–2966.
6 Aplin, J. D., Hughes, R. C., Jaffe, C. L., and Sharon, N., Reversible crosslinking of cellular components of adherent fibroblasts to fibronectin and lectin-coated substrata. Exp. Cell Res. *134* (1981) 488–494.

7 Appella, E., Weber, I. T., and Blasi, F., Structure and function of epidermal growth factor-like regions in proteins. FEBS Lett. *231* (1988) 1–4.

8 Argraves, W. S., Suzuki, S., Arai, H., Thompson, K., Piersbacher, M. D., and Ruoslahti, E., Amino acid sequence of the human fibronectin receptor. J. Cell Biol. *105* (1987) 1183–1190.

9 Baron-Van Evercooren, A., Kleinman, H. K., Ohno, S., Marangos, P., Schwartz, J. P., and Dubois-Dalq, M. E., Nerve growth factor, laminin and fibronectin promote neurite growth in human fetal sensory ganglion cultures. J. Neurosci. Res. *8* (1982) 179–194.

10 Barthels, D., Santoni, M.-J., Wille, W., Ruppert, C., Chaix, J.-C., Hirsch, M.-R., Fontecilla-Camps, J. C., and Goridis, C., Isolation and nucleotide sequence of mouse NCAM cDNA that codes for a Mr 79 000 polypeptide without a membrane-spanning region. EMBO J. *6* (1987) 907–914.

11 Beasley, L., and Stallcup, W. B., The Nerve growth factor-Inducible Large External (NILE) glycoprotein and neural cell adhesion molecule (N-CAM) have distinct patterns of expression in the developing rat central nervous system. J. Neurosci. *7* (1987) 708–715.

12 Behrens, J., Birchmeier, W., Goodman, S. L., and Imhof, B. A., Dissociation of Madin-Darby canine kidney epithelial cells by the monoclonal antibody Anti-Arc-1: mechanistic aspects and identification of the antigen as a component related to uvomorulin. J. Cell Biol. *101* (1985) 1307–1315.

13 Berman, P., Gray, P., Chen, E., Keyser, K., Ehrlich, D., Karten H., LaCorbière, M., Esch, F., and Schubert, D., Sequence analysis, cellular localization, and expression of a neuroretina adhesion and cell survival molecule. Cell *51* (1987) 135–142.

14 Bernard, B. A., Yamada, K. M., and Olden, K., Carbohydrates selectively protect a specific domain of fibronectin against proteases. J. biol. Chem. *257* (1982) 8549–8554.

15 Boucaut, J.-C., Darribère, T., Poole, T. J., Aoyama, H., Yamada, K. M., and Thiery, J.-P., Biologically active synthetic peptides as probes of embryonic development: a competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. J. Cell Biol. *99* (1984) 1822–1830.

16 Bourdon, M. A., and Ruoslahti, E., Tenascin mediates cell attachment through an RGD-dependent receptor. J. Cell Biol. *108* (1989) 1149–1155.

17 Bronner-Fraser, M., An antibody to a receptor for fibronectin and laminin perturbs cranial neural crest development in vivo. Devl. Biol. *117* (1986) 528–536.

18 Bronner-Fraser, M., Perturbation of cranial neural crest migration by the HNK-1 antibody. Devl. Biol. *123* (1987) 321–331.

19 Bronner-Fraser, M., and Lallier, T., A monoclonal antibody against a laminin-heparan sulfate proteoglycan complex perturbs cranial neural crest migration in vivo. J. Cell Biol. *106* (1988) 1321–1329.

20 Brümmendorf, T., Wolff, J. M., Frank, R., and Rathjen, F. G., Neural cell recognition molecule F11: homology with fibronectin type III and immunoglobulin type C domains. Neuron *2* (1989) 1351–1361.

21 Buck, C. A., and Horwitz, A. F., Cell surface receptors for extracellular matrix molecules. A. Rev. Cell Biol. *3* (1987) 179–205.

22 Charonis, A. S., Skubitz, A. P. N., Koliakos, G. G., Reger, L. A., Dege, J., Vogel, A. M., Wohlhueter, R., and Furcht, L. T., A novel synthetic peptide from the B1 chain of laminin with heparin-binding and cell adhesion-promoting activities. J. Cell Biol. *107* (1988) 1253–1260.

23 Cheresh, D. A., and Klier, F. G., Disialoganglioside GD2 distributes preferentially into substrate-associated microprocesses on human melanoma cells during their attachment to fibronectin. J. Cell Biol. *102* (1986) 1887–1897.

24 Cheresh, D. A., Piersbacher, M. D., Herzig, M. A., and Mujoo, K., Disialogangliosides GD2 and GD3 are involved in the attachment of human melanoma and neuroblastoma cells to extracellular matrix proteins. J. Cell Biol. *102* (1986) 688–696.

25 Cheresh, D. A., Pytela, R., Piersbacher, M. D., Klier, F. G., Ruoslahti, E., and Reisfeld, R. A., An Arg-Gly-Asp directed receptor on the surface of human melanoma cell exists in a distinct calcium-dependent functional complex with the disialoganglioside G-D2. J. Cell Biol. *105* (1987) 1163–1173.

26 Chiquet-Ehrismann, R., Mackie, E. J., Pearson, C. A., and Sakakura, T., Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell *47* (1986) 131–139.

27 Chiu, A. Y., Matthew, W. D., and Patterson, P. H., A monoclonal antibody that blocks the activity of a neurite regeneration-promoting factor: studies on the binding site and its localization in vivo. J. Cell Biol. *103* (1986) 1383–1398.

28 Cohen, J., Burne, J. F., McKinley, C., and Winter, J., The role of laminin and the laminin/fibronectin receptor complex in the outgrowth of retinal ganglion cell axons. Devl Biol. *122* (1987) 407–418.

29 Cole, G. J., and Glaser, L., A heparin-binding domain from N-CAM is involved in neural cell-substratum adhesion. J. Cell Biol. *102* (1986) 403–412.

30 Cole, G. J., Loewy, A., Cross, N. V., Akeson, R., and Glaser, L., Topographic localization of the heparin-binding domain of the neural cell adhesion molecule N-CAM. J. Cell Biol. *103* (1986) 1739–1744.

31 Cole, G. J., Loewy, A., and Glaser, L., Neuronal cell-cell adhesion depends on interactions on N-CAM with heparin-like molecules. Nature *320* (1986) 445–447.

32 Cole, G. J., and Schachner, M., Localization of the L2 monoclonal antibody binding site of chicken neural cell adhesion molecule (NCAM) and evidence for its role in NCAM-mediated cell adhesion. Neurosci. Lett. *78* (1987) 227–232.

33 Cole, G. J., Schubert, D., and Glaser, L., Cell-substratum adhesion in chick neural retin depends upon protein-heparan sulfate interactions. J. Cell Biol. *100* (1985) 1192–1199.

34 Coughlin, M. D., Grover, A. K., and Jung, C. Y., Determination of the molecular weight of neuronectin, a conditioned medium-derived, substrate-binding neurite-extension factor: comparison with laminin using radiation-inactivation analysis. J. Neurosci. *6* (1986) 1553–1559.

35 Crittenden, S. L., Pratt, R. S., Cook, J. H., Balsamo, J., and Lilien, J., Immunologically unique and common domains within a family of proteins related to the retina Ca^{2+}-dependent cell adhesion molecule, NcalCAM. Development *101* (1987) 729–740.

36 Crowley, T. E., Nellen, W., Gomer, R. H., and Firtel, R. A., Phenocopy of discoidin I-minus mutants by antisense transformation in *Dictyostelium*. Cell *43* (1985) 633–641.

37 Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Brackenbury, R., and Edelman, G. M., Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. Science *236* (1987) 799–806.

38 Cunningham, B. A., Hoffman, S., Rutishauser, U., Hemperly, J. J., and Edelman, G. M., Molecular topography of the neural cell adhesion molecule N-CAM: surface orientation and location of sialic acid-rich and binding regions. Proc. natl Acad. Sci. USA *80* (1986) 3116–3120.

39 Damsky, C. H., Richa, J., Solter, D., Knudson, K., and Buck, C. A., Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embronic and adult tissues. Cell *34* (1983) 455–466.

40 Davis, G. E., Manthorpe, M., Engvall, E., and Varon, S., Isolation and characterization of rat Schwannoma neurite-promoting factor: evidence that the factor contains laminin. J. Neurosci. *5* (1985) 2662–2671.

41 Dickson, G., Gower, H. J., Barton, C. H., Prentice, H. M., Elsom, V. L., Moore, S. E., Cox, R. D., Quinn, C., Putt, W., and Walsh, F. S., Human muscle neural cell adhesion molecule (N-CAM): identification of a muscle-specific sequence in the extracellular domain. Cell *50* (1987) 1119–1130.

42 Doolittle, R. F., Similar amino acid sequences: chance or common ancestry? Science *214* (1981) 149–159.

43 Durkin, M. E., Bartos, B. B., Liu, S.-H., Phillips, S. L., and Chung, A. E., Primary structure of the mouse laminin B2 chain and comparison with laminin B1. Biochemistry *27* (1988) 5198–5204.

44 Durkin, M. E., Chakravarti, S., Bartos, B. B., Liu, S.-H., Friedman, R. L., and Chung, A. E., Amino acid sequence and domain structure of entactin. Homology with epidermal growth factor precursor and low density lipoprotein receptor. J. Cell Biol. *107* (1988) 2749–2756.

45 Edelman, G. M., Murray, B. A., Mege, R.-M., Cunningham, B. A., and Gallin, W. J., Cellular expression of liver and neural cell adhesion molecules after transfection with their cDNAs results in specific cell-cell binding. Proc. natl Acad. Sci. USA *84* (1987) 8502–8506.

46 Edmondson, J. C., Liem, R. K. H., Kuster, J. E., and Hatten, M. E., Astrotactin: a novel neuronal cell surface antigen that mediates neuron-astroglial interactions in cerebellar microcultures. J. Cell Biol. *106* (1988) 505–517.

47 Erickson, H. P., and Lightner, V. A., Hexabrachion protein (tenascin, cytotactin, brachionectin) in connective tissues, embryonic brain and tumors. Adv. Cell Biol. *2* (1988) 55–90.

48 Fraser, S. E., Murray, B. A., Chuong, C.-M., and Edelman, G. M., Alteration of the retinotectal map in Xenopus by antibodies to neural cell adhesion molecules. Proc. natl Acad. Sci. USA *81* (1984) 4222–4226.

49 Frazier, W. A., Thrombospondin: a modular adhesive glycoprotein of platelets and nucleated cells. J. Cell Biol. *105* (1987) 625–632.

50 Gabius, H. J., Springer, W. R., and Barondes, S. H., Receptor for the cell binding site of discoidin I. Cell *42* (1985) 449–456.

51 Gallin, W. J., Sorkin, B. C., Edelman, G. M., and Cunningham, B. A., Sequence analysis of a cDNA clone encoding the liver cell adhesion molecule, L-CAM. Proc. natl Acad. Sci. USA *82* (1987) 2789–2793.

52 Ghelsen, K. R., Dillner, L., Engvall, E., and Ruoslahti, E., The human laminin receptor is a member of the integrin family of cell adhesion receptors. Science *241* (1988) 1228–1229.

53 Goridis, C., Deagostini-Bazin, H., Hirn, M., Hirsch, M.-R., Rougon, G., Sadoul, R., Langley, O. K., Gombos, G., and Finne, J., Neural surface antigens during nervous system development. Cold Spring Harbor Symp. Quant. Biol. *48* (1983) 527–537.

54 Graf, J., Ogle, R. C., Robey, F. A., Sasaki, M., Martin, G. R., Yamada, Y., and Kleinman, H. K., A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67 000 laminin receptor. Biochemistry *26* (1987) 6896–6900.

55 Grumet, M., and Edelman, G., Heterotypic binding between neuronal membrane vesicles and glial cells is mediated by specific cell adhesion molecule. J. Cell Biol. *98* (1984) 1746–1756.

56 Gulcher, J. R., Nies, D. E., Marton, L. S., and Stefansson, K., An alternatively spliced region of the human hexabrachion contains a repeat of potential N-glycosylation sites. Proc. natl Acad. Sci. USA *86* (1989) 1588–1592.

57 Harrelson, A. L., and Goodman, C. S., Growth cone guidance in insects: fasciclin II is a member of the immunoglobulin superfamily. Science *242* (1988) 700–708.

58 Hatta, K., Nose, A., Nagafuchi, A., and Takeichi, M., Cloning and expression of cDNA encoding a neural calcium-dependent cell adhesion molecule: its identity in the cadherin gene family. J. Cell Biol. *106* (1988) 873–881.

59 Hausman, R. E., and Moscona, A. A., Isolation of retina-specific cell-aggregating factor from membranes of embryonic neural retina tissue. Proc. natl Acad. Sci USA *73* (1976) 3594–3598.

60 He, H.-T., Barbet, J., Chaix, J.-C., and Goridis, C., Phosphatidylinositol is involved in the membrane attachment of NCAM-120, the smallest component of the neural cell adhesion molecule. EMBO J. *5* (1986) 2489–2494.

61 Hoffman, S., and Edelman, G. M., Kinetics of homophilic binding by embryonic and adult forms of the neural cell adhesion molecule. Proc. natl Acad. Sci. USA *80* (1983) 5762–5766.

62 Hoffman, S., and Edelman, G. M., A proteoglycan with HNK-1 antigenic determinants is a neuron-associated ligand for cytotactin. Proc. natl Acad. Sci. USA *84* (1987) 2523–2527.

63 Hohmann, H.-P., Bozzaro, S., Merkl, R., Wallraff, E., Yoshida, M., Weinhart, U., and Gerisch, G., Post-translational glycosylation of the contact site A protein of *Dictyostelium discoideum* is important for stability but not for its function in cell adhesion. EMBO J. *6* (1987) 3663–3671.

64 Holm, K., Risteli, L., and Sariola, H., Differential expression of the laminin A and B chains in chimeric kidneys. Cell Diff. *24* (1988) 223–228.

65 Hynes, R. O., Integrins: a family of cell surface receptors. Cell *48* (1987) 549–554.

66 Johansson, S., and Höök, M., Substrate adhesion of rat hepatocytes: on the mechanism of attachment to fibronectin. J. Cell Biol. *98* (1984) 810–817.

67 Jones, F. S., Burgoon, M. P., Hoffman, S., Crossin, K. L., Cunningham, B. A., and Edelman, G. M., A cDNA clone for cytotactin contains sequences similar to epidermal growth factor-like repeats and segments of fibronectin and fibrinogen. Proc. natl Acad. Sci. USA *85* (1988) 2186–2190.

68 Kennedy, D. W., Rohrbach, D. H., Martin, G. R., Momoi, T., and Yamada, K. M., The adhesive glycoprotein laminin is an agglutination. J. Cell Physiol. *114* (1983) 257–262.

69 Kintner, C., Effects of altered expression of the neural cell adhesion molecule, N-CAM, on early neural development in Xenopus embryos. Neuron *1* (1988) 545–555.

70 Klämbt, C., Müller, S., Lützelschwab, R., Rossa, R., Totzke, F., and Schmidt, O., The *Drosophila melanogaster l(2)gl* gene encodes a protein homologous to the cadherin cell-adhesion molecule family. Devl Biol. *133* (1989) 425–430.

71 Klein, G., Langegger, M., Timpl, R., and Ekblom, P., Role of laminin A chain in the development of epithelial cell polarity. Cell *55* (1988) 331–341.

72 Kleinman, H., Ebihara, I., Killen, P., Sasaki, M., Cannon, F. B., Yamada, Y., and Martin, G. R., Genes for basement membrane proteins are coordinately expressed in differentiating F9 cells but not in normal adult murine tissues. Devl Biol. *122* (1987) 373–378.

73 Kleinman, H. K., Martin, G. R., and Fischman, P. H., Ganglioside inhibition of fibronectin-mediated cell adhesion to collagen. Proc. natl Acad. Sci. USA *76* (1979) 3367–3371.

74 Kleinman, H. K., Ogle, R. C., Cannon, F. B., Little, C. D., Sweeney, T. M., and Luckenbill-Edds, L., Laminin receptors for neurite formation. Proc. natl Acad. Sci. USA *85* (1988) 1282–1286.

75 Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K., and Baralle, F. E., Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene. EMBO J. *4* (1985) 1755–1759.

76 Krotoski, D. M., Domingo, C., and Bronner-Fraser, M., Distribution of a putative cell surface receptor for fibronectin and laminin in the avian embryo. J. Cell Biol. *103* (1986) 1061–1071.

77 Kruse, J., Mailhammer, R., Wernecke, H., Faissner, A., Sommer, I., Goridis, C., and Schachner, M., Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. Nature *311* (1984) 153–155.

78 Kücherer, A., Faissner, A., and Schachner, M., The novel carbohydrate epitope L3 is shared by some neural cell adhesion molecules. J. Cell Biol. *104* (1987) 1597–1602.

79 Lagenaur, C., and Lemmon, V., An L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension. Proc. natl Acad. Sci. USA *84* (1987) 7753–7757.

80 Lai, C., Watson, J. B., Bloom, F. E., Sutcliffe, J. G., and Milner, R. J., Neural protein 1B236/Myelin-associated glycoprotein (MAG) defines a subgroup of the immunoglobulin superfamily. Immun. Rev. *100* (1987) 129–151.

81 Lander, A. D., Fujii, D. K., Gospodarowicz, D., and Reichardt, L. F., Characterization of a factor that promotes neurite outgrowth: evidence linking activity to a heparan sulfate proteoglycan. J. Cell Biol. *94* (1982) 574–585.

82 Lander, A. D., Fujii, D. K., and Reichardt, L. F., Laminin is associated with the "neurite-promoting factors" found in conditioned medium. Proc. natl Acad. Sci. USA *82* (1985) 2183–2187.

83 Lander, A. D., Fujii, D. K., and Reichardt, L. F., Purification of a factor that promotes neurite outgrowth: isolation of laminin and associated molecules. J. Cell Biol. *101* (1985) 893–913.

84 Laterra, J., Silbert, J. E., and Culp, L. A., Cell surface heparan sulfate mediates some adhesive responses to glycosaminoglycan-binding matrices, including fibronectin. J. Cell Biol. *96* (1983) 112-123.

85 Lawler, J., and Hynes, R. O., The structure of human thrombospondin, an adhesive glycoprotein with multiple calcium-binding sites and homologies with several different proteins. J. Cell Biol. *103* (1986) 1635–1648.

86 Lemmon, V., and McLoon, S. C., The appearance of an L1-like molecule in the chick primary visual pathway. J. Neurosci. *6* (1986) 2987–2994.

87 Lesot, H., Kühl, U., and Von der Mark, K., Isolation of a laminin-binding protein from muscle cell membranes. EMBO J. *2* (1983) 861–865.

88 Malinoff, H. L., and Wicha, M. S., Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. J. Cell Biol. *96* (1983) 1475–1479.

89 Mann, K., Deutzmann, R., Aumailley, M., Timpl, R., Raimondi, L., Yamada, Y., Pan, T.-L., Conway, D., and Chu, M.-L., Amino acid sequence of mouse nidogen, a multidomain basement membrane protein with binding activity for laminin, collagen IV and cells. EMBO J. *8* (1989) 65–72.

90 Martin, G. R., and Timpl, R., Laminin and other basement membrane components. A. Rev. Cell Biol. *3* (1987) 57–85.

91 Matsunaga, M., Hatta, K., Nagafuchi, A., and Takeichi, M., Guidance of optic nerve fibres by N-cadherin adhesion molecules. Nature *334* (1988) 62–64.

92 Matsunaga, M., Hatta, K., and Takeichi, M., Role of N-cadherin cell adhesion molecules in the histogenesis of neural retina. Neuron *1* (1988) 289–295.

93 Moos, M., Tacke, R., Scherer, H., Teplow, D., Fruh, K., and Schachner, M., Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature *334* (1988) 701–703.

94 Nagafuchi, A., Shirayoshi, Y. Okazaki, K., Yasuda, K., and Takeichi, M., Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. Nature *329* (1987) 341–343.

95 Nagafuchi, A., and Takeichi, M., Cell binding function of E-cadherin is regulated by the cytoplasmic domain. EMBO J. *7* (1988) 3679–3684.

96 Neugebauer, K. M., Tomaselli, K. J., Lilien, J., and Reichardt, L. F., N-cadherin, NCAM, and integrins promote retinal neurite outgrowth on astrocytes in vitro. J. Cell Biol. *107* (1988) 1177–1187.
97 Noegel, A., Gerisch, G., Stadler, J., and Westphal, M., Complete sequence and transcript regulation of a cell adhesion protein from aggregating *Dictyostelium* cells. EMBO J. *5* (1986) 1473–1476.
98 Noegel, A., Harloff, C., Hirth, P., Merkl, R., Modersitzki, M., Stadle, J., Weinhart, U., Westphal, M., and Gerisch, G., Probing an adhesion mutant of *Dictyostelium discoideum* with cDNA clones and monoclonal antibodies indicates a specific defect in the contact site A glycoprotein. EMBO J. *4* (1985) 3805–3810.
99 Nose, A., Nagafuchi, A., and Takeichi, M., Isolation of placental cadherin cDNA: identification of a novel gene family of cell-cell adhesion molecules. EMBO J. *6* (1987) 3655–3661.
100 Obara, M., Kang, M. S., and Yamada, K. M., Site-directed mutagenesis of the cell-binding domain of human fibronectin: separable, synergistic sites mediate adhesive function. Cell *53* (1988) 649–657.
101 Österlund, E., Eronen, I., Osterlund, K., and Vuento, M., Secondary structure of human plasma fibronectin: configurational changes induces by calf alveolar heparan sulfates. Biochemistry *24* (1985) 2661–2667.
102 Patel, N. H., Snow, P. M., and Goodman, C. S., Characterization and cloning of Fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. Cell *48* (1987) 975–988.
103 Pearson, C. A., Pearson, D., Shibahara, S., Hofsteenge, J., and Chiquet-Ehrismann, R., Tenascin: cDNA cloning and induction by TGF-β. EMBO J. *7* (1988) 2677–2981.
104 Pesheva, P., Horwitz, A. F., and Schachner, M., Integrin, the cell surface receptor for fibronectin and laminin, expresses the L2/HNK-1 and L3 carbohydrate structures shared by adhesion molecules. Neurosci. Lett. *83* (1987) 303–306.
105 Piersbacher, M. D., and Ruoslahti, E., Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. J. biol. Chem. *262* (1987) 17294–17298.
106 Pigott, R., and Davies, A. M., The monoclonal antibody 69A1 recognizes an epitope found on neurones with axons that fasciculate but not on those with non-fasciculating processes. Development *100* (1987) 489–500.
107 Poltorak, M., Sadoul, R., Keilhauer, G., Landa, C., Fahrig, T., and Schachner, M., Myelin-associated glycoprotein, a member of the L2/HNK-1 family of neural cell adhesion molecules, is involved in neuron-oligodendrocyte and oligodendrocyte-oligodendrocyte interaction. J. Cell Biol. *105* (1987) 1893–1899.
108 Poole, S., Firtel, R. A., and Lamar, E., Sequence and expression of the discoidin I gene family in *Dictyostelium discoideum*. J. molec. Biol. *153* (1981) 273–289.
109 Ranscht, B., and Dours, M. T., Sequence of contactin, a 130-kD glycoprotein concentrated in areas of interneuronal contact, defines a new member of the immunoglobulin supergene family in the nervous system. J. Cell Biol. *107* (1988) 1561–1573.
110 Rao, N. C., Barsky, S. H., Terranova, V. P., and Liotta, L. A., Isolation of a tumor cell laminin receptor. Biochem. biophys. Res. Commun. *128* (1983) 804–808.
111 Rathjen, F. G., Wolff, J. M., Chang, S., Bonhoeffer, F., and Raper, J. A., Neurofascin: a novel chick cell-surface glycoprotein involved in neurite-neurite interactions. Cell *51* (1987) 841–849.
112 Rathjen, F. G., Wolff, J. M., Frank, R., Bonhoeffer, F., and Rutishauser, U., Membrane glycoproteins involved in neurite fasciculation. J. Cell Biol. *104* (1987) 343–353.
113 Ringwald, M., Schuh, R., Vestweber, D., Eistetter, H., Lottspeich, F., Engel, J., Dolz, R., Jahnig, F., Epplen, J., Mayer, S., Muller, C., and Kemler, R., The structure of cell adhesion molecule uvomorulin. Insights into the molecular mechanism of Ca^{2+}-dependent cell adhesion. EMBO J. *6* (1987) 3647–3653.
114 Riopelle, R. J., McGarry, R. C., and Roder, J. C., Adhesion properties of a neuronal epitope recognized by the monoclonal antibody HNK-1. Brain Res. *367* (1986) 20–25.
115 Roberts, D. D., Rao, C. N., Magnani, J. L, Spitalnik, S. L., Liotta, L. A., and Ginsburg, V., Laminin binds specifically to sulfated glycolipids. Proc. natl Acad. Sci. USA *82* (1985) 1306–1310.
116 Runyan, R. B., Versalovic, J., and Shur, B. D., Functionally distinct laminin receptors mediate cell adhesion and spreading: the requirement for surface galactosyltransferase in cell spreading. J. Cell Biol. *107* (1988) 1863–1871.
117 Ruoslahti, E., Fibronectin and its receptors. A. Rev. Biochem. *57* (1988) 375–413.
118 Ruoslathi, E., and Piersbacher, M. D., New perspectives in cell adhesion: RGD and integrins. Science *238* (1987) 491–497.
119 Rutishauser, U., Hoffman, S., and Edelman, G. M., Binding properties of a cell adhesion molecule from neural tissue. Proc. natl Acad. Sci. USA *79* (1982) 685–689.
120 Sadoul, R., Hirn, M., Deagostini-Bazin, H., Rougon, G., and Goridis, C., Adult and embryonic mouse neural cell adhesion molecules have different binding properties. Nature *304* (1983) 347–349.
121 Sandrock, A. W., and Matthew, W. D., An in vitro neurite-promoting antigen functions in axonal regeneration in vivo. Science *237* (1987) 1605–1608.
122 Santoni, M. J., Barthels, D., Vopper, G., Boned, A., Goridis, C., and Wille, W., Differential exon usage involving an unusual splicing mechanism generates at least eight types of NCAM cDNA in mouse brain. EMBO J. *8* (1989) 385–392.
123 Sasaki, M., Kato, S., Kohno, K., Martin, G. R., and Yamada, Y., Sequence of cDNA encoding the laminin B1 chain reveals a multidomain protein containing cysteine-rich repeats. Proc. natl Acad. Sci. USA *84* (1987) 935–939.
124 Sasaki, M., Kleinman, H. K., Huber, H., Deutzmann, R., and Yamada, Y., Laminin, a multidomain protein. J. biol. Chem. *263* (1988) 16536–16544.
125 Sasaki, M., and Yamada, Y., The laminin B2 chain has a multidomain structure homologous to the B1 chain. J. biol. Chem. *262* (1988) 17111–17117.
126 Schubert, D., and LaCorbière, M., Isolation of a cell surface receptor for chick neural retina adherons. J. Cell Biol. *100* (1985) 56–63.
127 Schubert, D., LaCorbière, M., Klier, F. G., and Birdwell, C., A role for adherons in neural retina cell adhesion. J. Cell Biol. *96* (1983) 990–998.
128 Seeger, M. A., Haffley, L., and Kaufman, T. C., Characterization of amalgam: a member of the immunoglobulin superfamily from Drosophila. Cell *55* (1988) 589–600.
129 Sémériva, M., Naidet, C., Krejci, E., and Gratecos, D., Towards the molecular biology of cell adhesion in Drosophila. Trends Genet. *5* (1989) 24–28.
130 Shirayoshi, Y., Nose, A., Iwasaki, K., and Takeichi, M., N-linked oligosaccharides are not involved in the function of a cell-cell binding glycoprotein E-cadherin. Cell Struct. Funct. *11* (1986) 245–252.
131 Silver, J., and Rutishauser, U., Guidance of optic axons in vivo by a preformed adhesive pathway on neuroepithelial endfeet. Devl. Biol. *106* (1984) 485–499.
132 Siu, C. H., Cho, A. S., and Choi, A., Mechanism of action of the contact site A glycoprotein during development of *Dictyostelium discoideum*. J. Cell Biol. *103* (1986) 3a.
133 Skorstengaard, K., Jensen, M. S., Sahl, P., Petersen, T. E., and Magnusson, S., Complete primary structure of bovine plasma fibronectin. Eur. J. Biochem. *161* (1986) 441–453.
134 Skubitz, A. P. N., McCarthy, J. B., Charonis, A. S., and Furcht, L. T., Localization of three distinct heparin-binding domains of laminin by monoclonal antibodies. J. biol. Chem. *263* (1988) 4861–4868.
135 Smalheiser, N. R., and Schwartz, N. B., Cranin: a laminin-binding protein of cell membranes. Proc. natl Acad. Sci. USA *84* (1987) 6457–6461.
136 Springer, W. R., Cooper, D. N. W., and Barondes, S. H., Discoidin I is implicated in cell-substratum attachment and ordered cell migration of *Dictostelium discoideum* and resembles fibronectin. Cell *39* (1984) 557–565.
137 Sweadner, K. J., Post-translational modification and evoked release of two large surface proteins of sympathetic neurons. J. Neurosci. *3* (1983) 2504–2517.
138 Takeichi, M., The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development *102* (1988) 639–655.
139 Thanos, S., Bonhoeffer, F., and Rutishauser, U., Fiber-fiber interaction and tectal cues influence the development of the chicken retinotectal projection. Proc. natl Acad. Sci. USA *81* (1984) 1906–1910.
140 Thiery, J.-P., Delouvée, A., Grumet, M., and Edelman, G. M., Initial appearance and regional distribution of the neuron-glia cell adhesion molecule in the chick embryo. J. Cell Biol. *100* (1985) 442–456.
141 Thiery, J.-P., Duband, J.-L., Rutishauser, U., and Edelman, G. M., Cell adhesion molecules during early chicken embryogenesis. Proc. natl Acad. Sci. USA *79* (1982) 6737–6741.
142 Thompson, L. K., Horowitz, P. M., Bentley, K. L., Thomas, D. D., Alderete, J. F., and Klebe, R. J., Localization of the ganglioside-binding site of fibronectin. J. biol. Chem. *261* (1986) 5209–5214.
143 Tomaselli, K. J., Damsky, C. H., and Reichardt, L. F., Purification and characterization of mammlian integrins expressed by a rat neuronal cell line (PC12): evidence that they function as α/β het-

erodimeric receptors for laminin and type IV collagen. J. Cell Biol. *107* (1988) 1241–1252.
144 Townes, P., and Holtfreter, J., Directed movements and selective adhesion of embryonic amphibian cells. J. exp. Zool. *128* (1955) 53–120.
145 Troccoli, N. M., and Hausman, R. E., Retina cognin does not bind to itself during membrane interaction in vitro. Cell Differ. *22* (1988) 225–232.
146 Tuszynski, G. P., Rothman, V., Murphy, A., Siegler, K., Smith, L., Smith, S., Karczewski, J., and Knudsen, K. A., Thrombospondin promotes cell-substratum adhesion. Science *236* (1987) 1570–1573.
147 Vaughan, L., Huber, S., Chiquet, M., and Winterhalter, K., A major six-armed glycoprotein from embryonic cartilage. EMBO J. *6* (1987) 349–353.
148 Volk, T., and Geiger, B., A-CAM: a 135 kd receptor of intercellular adherens junctions. I. Immunoelectron microscopic localization and biochemical studies. J. Cell Biol. *103* (1986) 1441–1450.
149 Wewer, U. M., Liotta, L. A., Jaye, M., Ricca, G. A., Drohan, W. N., Claysmith, A. P., Rao, C. N., Wirth, P., Coligan, J. E., Albrechtsen, R., Mudry, M., and Sobel, M. E., Altered levels of laminin receptor mRNA in various human carcinoma cells have different abilities to bind laminin. Proc. natl Acad. Sci. USA *83* (1986) 7137–7141.
150 Williams, A. F., and Barclay, A. N., The immunoglobulin superfamily – domains for cell surface recognition. A. Rev. Immun. *6* (1988) 381–405
151 Yamada, K. M., Fibronectin domains and receptors, in: Fibronectin, pp. 47–121. Ed. D. F. Mosher. Academic Press, New York 1989.
152 Zinn, K., McAllister, L., and Goodman, C. S., Sequence analysis and neuronal expression of Fasciclin I in grasshopper and Drosophila. Cell *53* (1988) 577–587.
153 Bieber, A. J., Snow, P. M., Hortsch, M., Patel, N. H., Jacobs, J. R., Traquina, Z. R., Schilling, J., and Goodman, C. S., Drosophila neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1. Cell (1989) in press.

0014-4754/90/010002-12$1.50 + 0.20/0

Steroid hormones and the cardiovascular system: Direct actions of estradiol, progesterone, testosterone, gluco- and mineralcorticoids, and soltriol [vitamin D] on central nervous regulatory and peripheral tissues

W. E. Stumpf

Departments of Cell Biology and Anatomy, and Pharmacology, University of North Carolina, Chapel Hill (North Carolina 27599, USA)

Summary. Knowledge of steroid hormone sites of action and related effects in cardiovascular and neural regulatory tissues is reviewed. Evidence for nuclear receptor sites is derived mainly from autoradiographic studies with relatively intact tissues and some biochemical studies with tissue homogenates.
In the heart and in the walls of blood vessels, estradiol, dihydrotestosterone, corticosterone, aldosterone, dexamethasone, and soltriol (vitamin D) show nuclear binding. In the brain and spinal cord, neuronal regions associated with cardiovascular regulation contain nuclear receptors in specific patterns for each steroid hormones, including progesterone and soltriol. These data indicate that all steroid hormones exert direct actions on the cardiovascular system at its different levels of organization, thus enabling adjustment to the changing demands during reproduction (gonadal steroids), stress (adrenal steroids), and solar seasons (vitamin D-soltriol).
Key words. Estradiol; progesterone; dihydrotestosterone; adrenal steroids; soltriol; vitamin D; cardiovascular system; brain; spinal cord.

Introduction

Blood flow varies with the needs of the organism and its individual tissues. Changes in blood flow can be accomplished by adjusting the functions of cardiovascular tissues through peripheral messengers and neural factors, 'regulated according to certain pre-programmed priorities'[16] for different physiological conditions of procreation and survival. Such vital conditions include procurement of food, competition for partner, nest building and territorial defense, estrus and mating, pregnancy, lactation, and maternal care. Induction and control of these conditions require actions of sex steroid hormones, adrenal steroid hormones, and the seasonal steroid hormone soltriol[97].
Steroid hormone regulation of cardiovascular functions is exerted at different levels of organization. Receptors for steroid hormones can be demonstrated in neural regions of the brainstem and spinal cord, and in the heart and in walls of blood vessels including capillaries. In addition to direct effects, indirect effects on cardiovascular functions may be exerted through steroid hormone actions on endocrine and metabolism controlling organs, such as pituitary, adrenal, liver and kidney. Effects of steroid hormones on the cardiovascular system, thus, appear to be extensive and complex, and to affect all phases of life. Such conclusions can be supported through existing information, albeit incomplete, on steroid hormone receptor distribution, steroid hormone effects on glucose, protein, and lipid metabolism, on monoamine and peptide messenger related receptor production, on liver, kidney and pituitary-endocrine func-