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Authors
Hynes, Richard O
Lander, Arthur D

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Contact and Adhesive Specificities in the Associations, Migrations, and Targeting of Cells and Axons

Review

Richard O. Hynes*†§ and Arthur D. Lander‡§
*Howard Hughes Medical Institute
†Center for Cancer Research
‡Department of Brain and Cognitive Sciences
§Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

What determines whether cells remain in one place, retaining their associations with their neighbors, or dissociate from these associations and move elsewhere? And, if they move, what determines where they go, where and when they stop, and whether or not they associate with like or unlike cells? These questions arise about many events during development, especially but not exclusively the development of the nervous system. The same questions arise when considering normal physiological processes, such as lymphocyte traffic, wound healing, and hemostasis, and pathologies such as invasion and metastasis, thrombosis, and inflammation. All these processes involve alterations in cell associations, many involve cellular (or axonal) migration, and all must be conducted with appropriate specificity to ensure proper development and function of the organism.

In recent years there have been major advances in deciphering the molecular bases for the cell adhesion events underlying these biological phenomena. Perhaps it is not surprising that there are many different adhesive molecules involved; at times there appears to be a bewildering variety. However, common themes are emerging, and many of the molecules fall into families whose members appear in the different processes performing similar roles. This means that insights obtained in one system can be applied in others, and this cross fertilization among disciplines is proving very fruitful. In this review we will discuss current ideas concerning the roles of cell adhesion molecules and cell interactions, both in development and in several physiological and pathological processes, attempting to highlight the general features and commonalities and presenting working hypotheses.

Fundamental Cellular Processes

Figure 1 diagrams several basic processes involving cell interactions, which play important roles during development and later. The early segregation of different groups of cells into separate germ layers and tissues (Figure 1a) involves changes in cell–cell adhesion, as does the dispersion of individual migratory cells (1b) from an initial coherent tissue (e.g., neural crest from neural tube) and the later aggregation (1c) of migratory cells into tissues (e.g., neural crest to sensory or sympathetic ganglia). Migration of individual cells (Figure 1c) and axonal outgrowth (1d) share many features. In each case, the migration must be promoted and must also be guided. Evidence exists for many different guidance mechanisms. These include chemotaxis (directional migration in response to a gradient of a diffusible signal), haptotaxis (directional migration in response to a gradient of substrate adhesivity), and contact guidance. The latter was originally proposed as guidance by physical features of the substrate, such as grooves or lines, but can also be considered to include guidance by specific chemical interactions with preexisting linear features, as in fasciculation of axons (see later). Migration and axonal outgrowth can be guided not only by these positive cues but also by inhibition, which prevents entry into particular regions. Induction, which is schematized in Figure 1e, can also involve cell–cell interactions via cell surface receptors, some of which are also adhesive, but the questions of induction are discussed elsewhere in this issue (reviewed by Gurdon, 1992; Jessell and Melton, 1992; Rubin and Greenwald, 1992) and will not be considered further here.

The involvement of many similar cellular processes in adult physiology and pathology can be seen in Figures 2 and 3, and these are often more accessible than those occurring in development. Figure 2 depicts the arrest, adhesion, and extravasation of leukocytes (neutrophils or monocytes), which then invade underlying tissues. These events must occur at the site of infection as a necessary part of the primary defense mechanisms, but excessive accumulation of leukocytes leads to inflammation and tissue damage. Therefore, leukocyte adhesion must be controlled very precisely (Osborn, 1990). The traffic of B and T lymphocytes to lymphoid and peripheral tissues involves very similar events, often referred to as "lymphocyte homing" (Gallatin et al., 1986; Stoolman, 1989). The behavior of malignant cancer cells has much in common with these cell adhesion and migration processes occurring in normal development and homeostasis (Figure 3), and alterations in tumor cells provide useful information about functions of normal cells.

Families of Cell Adhesion Receptors

A satisfying result of research on these diverse adhesion systems is that they often involve very similar molecules. Four major classes of cell surface adhesion receptors have been identified (Figure 4). There is not space to review these receptors thoroughly, but we will summarize their main properties and refer readers to review articles.

Cadherins (Takeichi, 1988, 1990, 1991) are calcium-dependent cell–cell adhesion molecules present on most cells. At least a dozen different cadherins are known (Suzuki et al., 1991). They mediate homophilic (like-with-like) adhesion between cells, and cells expressing different cadherins segregate from each other to form separate aggregates (Nose et al., 1988; Takeichi, 1988, 1990). The extracellular domains of cadherins contain four homologous repeats. The determinants for binding specificity have been mapped to the most distal repeat using site-specific mutagenesis and monoclonal antibodies that block function (Nose et al., 1990). The functions of the other three repeating units are unknown.

A second major class of cell–cell adhesion receptors are

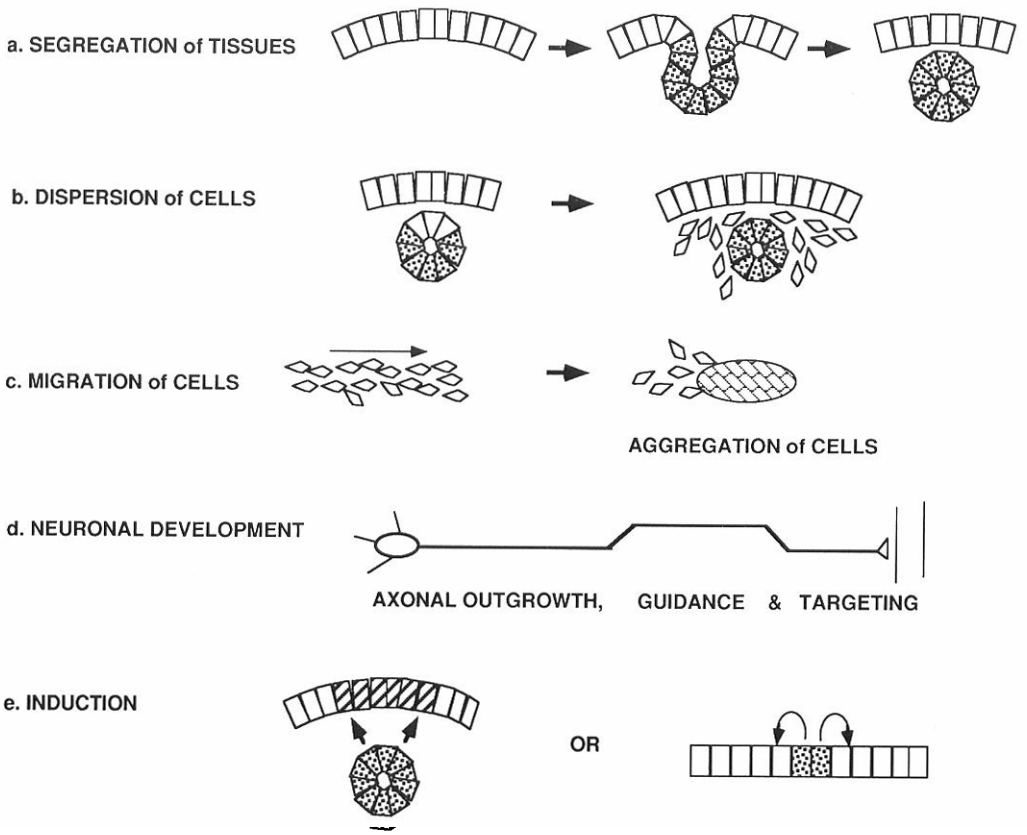

Figure 1. Morphogenetic Events Involving Changes in Cell–Cell or Cell–Substratum Interactions

Segregation of tissues, dispersion, and aggregation of cells involve changes in cell–cell adhesion; cell migration involves cell–matrix interactions; and neurite outgrowth, guidance, and targeting involve multiple interactions of both types. Induction events also involve cell interactions including paracrine signaling molecules, extracellular matrix interactions, and direct cell–cell contacts.

members of the *immunoglobulin superfamily* (Williams and Barclay, 1988; Jessell, 1988; Hunkapiller and Hood, 1989; Grumet, 1991). The first of these to be described was N-CAM (Cunningham et al., 1987; Santoni et al., 1989), but it is now clear that there is a large family of similar molecules, at least ten in addition to those on lymphocytes. The nonlymphocyte members of this family are typified by a number of repeats of immunoglobulin-related domains and, in many cases, by several membrane proximal repeats of a second type of protein module, known as a fibronectin type III repeat because it was first detected in the adhesive extracellular matrix protein, fibronectin (Hynes, 1990). Type III repeats also occur in cytokine receptors (Bazan, 1990) and in several extracellular matrix

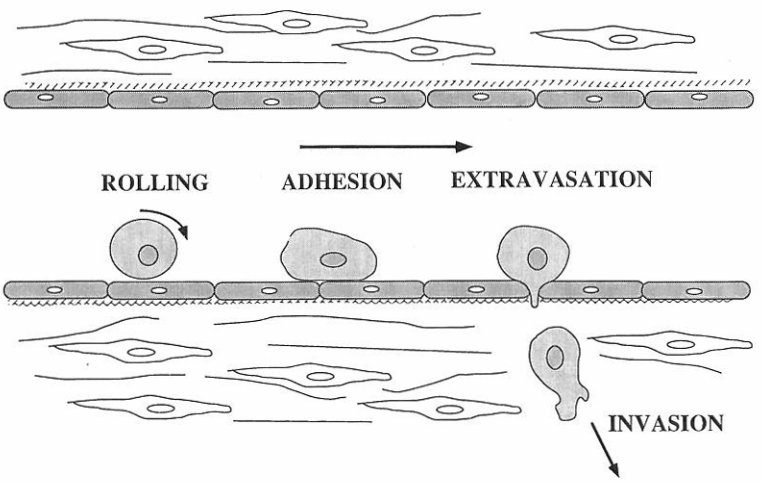

Figure 2. Interactions of Leukocytes with Endothelial Cells of the Blood Vessel Wall

Attachment, firm adhesion, extravasation from the blood vessel, and migration into the underlying tissue involve a variety of cell–cell and cell–matrix adhesion events. These must be precisely controlled so that adhesion occurs only at sites of inflammation.

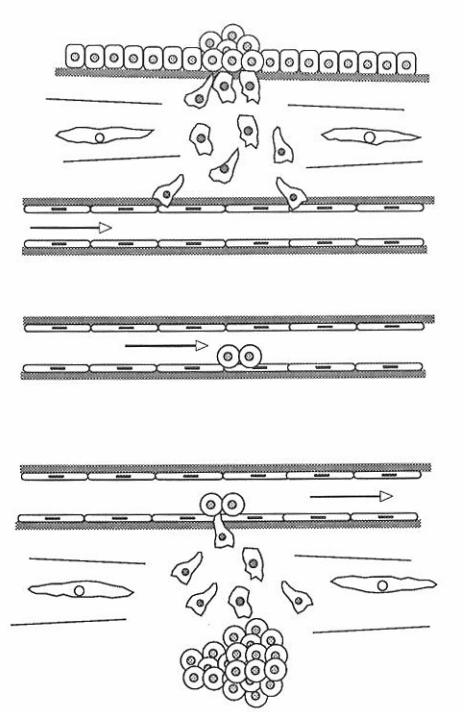

Figure 3. Commonalities of Development and Metastasis

Invasion and metastasis of tumor cells involve many of the same processes exhibited by normal cells during development and homeostasis. These include dissociation of individual cells from the primary tumor, invasion of underlying connective tissues and eventually of blood vessels, arrest of the circulating tumor cells, extravasation, and further invasion at the site of the metastatic lesion. These processes involve alterations in cell–cell adhesion, cell migration and possibly matrix degradation, and heterotypic cell interactions during arrest and extravasation. One might expect to see both the loss of cell–cell and/or cell–matrix adhesion contributing to initial release on invasion and the acquisition of new adhesive properties related to the various steps of invasion and to arrest during metastasis.

proteins (fibronectin, tenascin). The type III repeats are folded into a pair of β sheets apposed via hydrophobic faces, somewhat similar to an immunoglobulin-related domain.

The binding functions of the immunoglobulin superfamily receptors are divalent cation–independent. Mapping of the specificity determinants of N-CAM shows them to be in the two most distal immunoglobulin repeats (Frelinger and Rutishauser, 1986; Cole et al., 1986; Cunningham et al., 1987; Reyes et al., 1990). This is also true for the immunoglobulin-related adhesion receptor ICAM-1 (Staunton et al., 1990). The functions of the additional immunoglobulin domains, and of the fibronectin type III repeats when they occur, are unknown. Similar molecules also occur in insect nervous systems, such as fasciclin II and neuroglian in Drosophila (Harrelson and Goodman, 1988; Bieber et al., 1989).

Some of the immunoglobulin superfamily adhesion receptors are thought to be homophilic, particularly several that are expressed in the nervous system, such as N-CAM in vertebrates (Cunningham et al., 1987), although there is also clear evidence that N-CAM binds to heparan sulfate proteoglycans (Cole et al., 1986; Reyes et al., 1990). For many other receptors of this type, it is unclear whether they act in homophilic fashion or have a distinct counterreceptor. For yet others, notably three that are expressed on activated endothelial cells, ICAM-1, ICAM-2, and VCAM-1 (Staunton et al., 1988, 1989; Elices et al., 1990), it is clear that they function in a heterophilic fashion and bind to integrin receptors (see below) on adjacent cells. Thus, some immunoglobulin superfamily receptors mediate homotypic adhesion between like cells, whereas others mediate heterotypic adhesion between two different cell types. Some receptors of this class are transmembrane, as depicted in Figure 4. Others are attached to the membrane via glycosylphosphatidyl inositol tails, and several occur in both forms and with varying cytoplasmic domains derived via alternative RNA splicing (see, e.g., Cunningham et al., 1987; Santoni et al., 1989; Furley et al., 1990).

The third class of adhesion receptors, *selectins* (Bevilacqua et al., 1991; Lasky and Rosen, 1991), have also been called LECAMs as well as a variety of other names. Three selectins are known currently and are expressed on various blood cells and/or endothelial cells. Each selectin has a single Ca^{2+}-dependent C-type lectin domain, a single EGF-like repeat, and a number of repeats of a protein domain related to the consensus repeats of complement-binding proteins. Selectins bind to specific carbohydrate groups via their lectin domains, although the EGF-like repeat also contributes to binding. The functions of the complement-binding consensus repeats are unknown. Selectins typically occur as transmembrane proteins, although there are suggestions of PI-linked or secreted forms. As will be discussed later, selectins typically mediate heterotypic interactions between or among blood cells and endothelial cells during lymphocyte homing and leu-

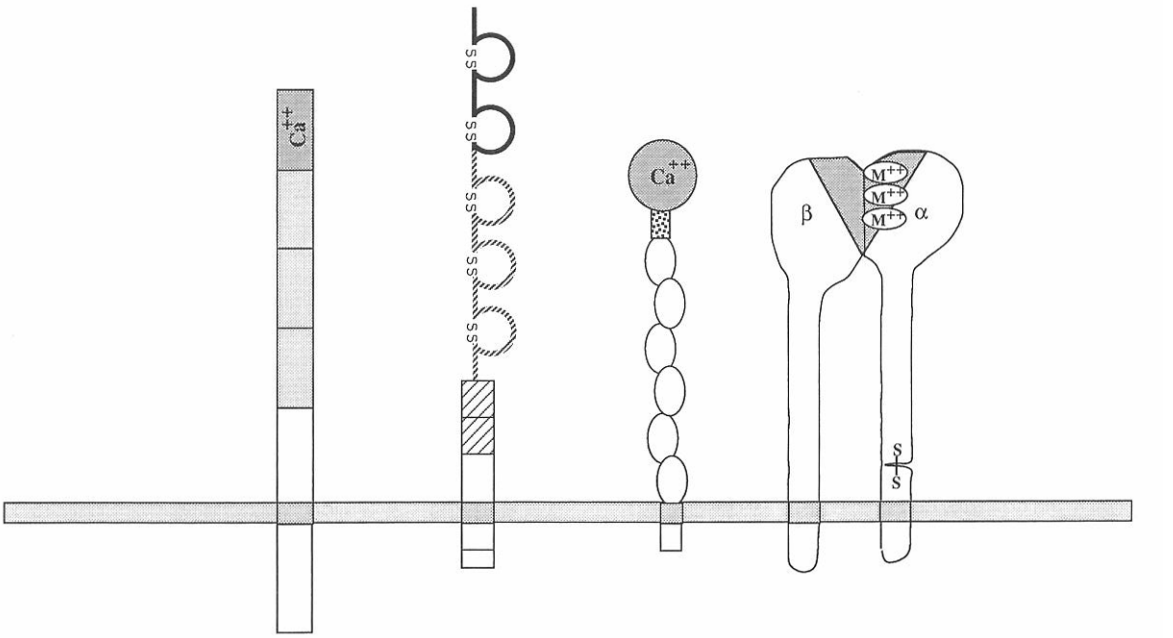

Figure 4. Major Families of Cell Adhesion Receptors

Cadherins are Ca^{2+}-dependent homophilic cell–cell adhesion molecules. Immunoglobulin superfamily adhesion receptors contain immunoglobulin domains (Ω) and, frequently, fibronectin type III repeats (cross-hatched boxes). These cell–cell adhesion receptors are Ca^{2+}-independent and participate in heterophilic and possibly also homophilic interactions (see text). Selectins contain a Ca^{2+}-dependent C-type lectin domain, a single EGF-like repeat (stippled box), and a series of repeats (ovals) related to those of complement-binding proteins. Selectins bind specific carbohydrate groups on adjacent cells. Integrins are heterodimeric receptors, some for extracellular matrix proteins and some for immunoglobulin superfamily counterreceptors, and therefore can be involved in both cell–matrix and cell–cell adhesion. Most receptors of all families are transmembrane proteins, although a few can be PI-linked (see text). Binding domains are indicated by the darkest colorings.

kocyte adhesion. Their roles in other tissues, if any, have been little investigated as yet.

The fourth major class of adhesion receptors are the *integrins*. Unlike the foregoing receptors, which have single subunits, integrins are αβ heterodimers. There are currently about 20 known integrin heterodimers, made up of various pairings of 8 β subunits and 13 α subunits (reviewed by Hynes, 1987, 1992; Hemler, 1990; Albelda and Buck, 1990). Although many of the theoretical pairings do not occur, it seems likely that there are more than 20 integrins. Each heterodimer has a distinct ligand specificity. A few integrins, notably those expressed on lymphocytes and leukocytes (Springer, 1990), mediate heterophilic, heterotypic cell–cell adhesion by binding to immunoglobulin superfamily molecules on the other cell (see later). However, the majority of the known integrins bind to various extracellular matrix molecules and mediate cell–matrix interactions during cell adhesion to basement membranes and other extracellular matrices and during cell migrations.

There is a large number of adhesive extracellular matrix (ECM) molecules, such as fibronectins (Mosher, 1989; Hynes, 1990), laminins (Martin and Timpl, 1987; Sanes et al., 1990), tenascins (Erickson and Lightner, 1988; Erickson and Bourdon, 1989), thrombospondin (Lawler and Hynes, 1987; Frazier, 1987), and others (Ruoslahti, 1988; Wagner, 1990). It is important to note that, although these ECM proteins are clearly adhesive, some, such as tenascin, thrombospondin, and laminin, can also act as anti-adhesive molecules in some cases (Chiquet-Ehrismann, 1991; Sage and Bornstein, 1991; Calof and Lander, 1991). The mechanism of these anti-adhesive effects is unknown. In contrast, many of the adhesive functions of ECM proteins are mediated via integrins. Frequently, several integrins can recognize a given matrix molecule, often at different sites. Most cells express several integrins and can therefore adhere to several adhesive ECM proteins. The selectivity of cells for different ECM proteins is thus controlled, at least in part, by their pattern of expression of integrins. The adhesive specificity and affinity of integrins can also be regulated posttranslationally by several mechanisms (Kieffer and Phillips, 1990; Springer, 1990; Phillips et al., 1991; Hynes, 1992; see also later). As diagrammed in Figure 4, integrins have a globular extracellular domain made up from both α and β subunits. This domain binds the ECM ligand or the immunoglobulin superfamily counterreceptor, and the ligand-binding function depends on divalent cations (Ca^{2+}, Mg^{2+}, or Mn^{2+}, depending on the integrin). Each subunit also has a transmembrane and a cytoplasmic domain.

The common picture emerging from data on all these adhesion receptors is of a specific binding domain at the distal end of an extended integral membrane protein. The functions of those parts of these proteins connecting the

binding sites to the membrane are unknown, but their conservation argues that they serve purposes other than simple spacers. Perhaps they play a role in conformational changes and signal transduction events (see later). Integrins and cadherins have both been shown to bind through their cytoplasmic domains to the cytoskeleton via specific proteins (see reviews cited earlier). The same is suggested for some forms of N-CAM and is likely to be true for other immunoglobulin superfamily receptors. So far there are no data linking selectins to the cytoskeleton.

With this background we will now consider some of the roles played by these adhesion receptors (and a few others that do not fall into these families) in cell adhesion events during development.

Early Morphogenetic Events

The adhesion receptors implicated earliest in development are the cadherins (Takeichi, 1988, 1990). E-cadherin is expressed in morulae and, in the mouse embryo, plays a key role in compaction, a cell–cell adhesion event leading to polarization of the cells of the early blastocyst. Other cadherins appear at slightly later stages. For example, N-cadherin is first expressed at gastrulation in the region of the ectoderm that will form the neural plate and neural tube (cf. Figure 1a). Ectopic expression of N-cadherin in Xenopus embryos interferes with segregation of the neural tube from the ectoderm (Detrick et al., 1990; Fujimori et al., 1990), indicating that differential expression of cadherins (E- in the ectoderm, N- only in the prospective neural tube) plays a causal role in the segregation of these tissues (cf. Figure 1a). In contrast, although N-CAM is expressed in a pattern similar to that of N-cadherin at the time of neurulation (Thiery et al., 1982), ectopic expression of N-CAM does not interfere with segregation of the neural tube (Kintner, 1988), suggesting that, unlike N-cadherin, N-CAM does not play a role in defining tissue boundaries in this case.

Analyses of the expression of different cadherins during development show many examples of dynamic changes in expression. A common observation is that two parts of a cell sheet that are about to separate into two distinct cell layers or organ rudiments express different sets of cadherins (reviewed by Takeichi, 1988, 1990). The obvious model, that this differential cadherin expression leads to the segregation, has not yet been tested in most cases, although ectopic expression of N-cadherin in Xenopus embryos does lead to failures of segregation of ectoderm and to abnormal mesodermal condensations. Similarly, ectopic overexpression of N-CAM leads to defects in somite formation from mesoderm (Kintner, 1988). Thus, the idea is well established that tissue segregation events during development involve regulated changes in cadherins and possibly also in immunoglobulin superfamily adhesion receptors. Future work will test this postulate for individual cases.

Returning to earlier stages of development, it is clear that cell–matrix adhesion mediated by integrins plays a role in cell migration during gastrulation, neural crest migration, and elsewhere. In all vertebrates, gastrulation involves migration of mesodermal cells, which, to one degree or another, depending on the species, move as individual cells. The ECM protein fibronectin is strongly expressed in the regions where this cell migration occurs (reviewed by Thiery et al., 1989; Hynes, 1990). Antibodies to fibronectin block mesodermal migration in amphibians (Boucaut et al., 1984a) and birds (Harrisson, 1989), and in amphibians, gastrulation can also be blocked either by peptides (Boucaut et al., 1984b) containing the RGD sequence from fibronectin, which is recognized by $\alpha_5\beta_1$ integrin, or by antibodies against β_1 integrin (Darribère et al., 1988, 1990). The cells express integrins and will adhere to fibronectin in vitro. Thus, a reasonable model is that mesodermal cells are released from the ectodermal layers, perhaps by down-regulation of cell–cell adhesion receptors such as cadherins (although that has not been explicitly tested), and that these mesodermal cells acquire migratory properties, which include the expression of integrin receptors capable of recognition and migration on the fibronectin-rich matrix expressed beneath the ectoderm. Proper execution of these events requires coordinated spatial and temporal regulation of the adhesion receptors and of the deposition of the fibronectin-rich matrix.

A similar model applies to the migration of neural crest cells. These cells arise from the dorsal part of the neural tube (cf. Figure 1b). They lose expression of N-CAM and N-cadherin (Thiery et al., 1982; Duband et al., 1988; Hatta et al., 1987) and disperse into the spaces around the neural tube. These spaces are full of ECM, which is rich in fibronectin and laminin. Cultured neural crest cells adhere and migrate on these ECM proteins, particularly fibronectin, and can be blocked by antibodies to the ECM proteins or to integrins (e.g., Rovasio et al., 1983; Dufour et al., 1988; Perris et al., 1989; reviewed by Thiery et al., 1989; Hynes, 1990). Injection of RGD-containing peptides or anti-integrin antibodies into embryos effectively blocks cranial neural crest cell migration (Boucaut et al., 1984b; Bronner-Fraser, 1985, 1986a). Similarly, monoclonal antibodies directed against a laminin–proteoglycan complex also block cranial crest cell migration in vivo (Bronner-Fraser and Lallier, 1988).

These studies provide clear evidence that molecules of the ECM, particularly but not only fibronectin, promote neural crest migration in vivo, and that appropriate expression of integrins by the crest cells is important for this migration. This leads to the idea that these ECM pathways promote migration of the neural crest cells and, furthermore, might determine their eventual distribution. However, closer examination shows that this cannot be the whole story. Fibronectin and laminin are both expressed in adjacent regions into which neural crest cells do not migrate. For example, fibronectin is present in all parts of the somitic sclerotome, but neural crest cells migrate only into the rostral half (Bronner-Fraser, 1986a, 1986b; Erickson, 1988). One candidate ligand for promoting entry of crest cells into the rostral half is tenascin, an ECM molecule, which is expressed only in the rostral half late in crest migration (Tan et al., 1987; Mackie et al., 1988). Alternatively, an inhibitory molecule may be expressed in the caudal half of the somites. Candidates include a proteoglycan or high concentrations of laminin, both of which inhibit

neural crest migration in vitro (Perris et al., 1989). Another possibility is a novel glycosylphosphatidyl inositol-linked cadherin, T-cadherin, expressed in the caudal half of the somite prior to and independent of neural crest migration (Ranscht and Bronner-Fraser, 1991). One or more of these molecules may block entry of neural crest cells into the caudal half of each somite. Further experiments will be necessary to determine which of these or other molecules are necessary to define precisely the pathways of neural crest migration. A balance of positive and negative cues, appropriately displayed, seems necessary for correct guidance of cells to their eventual locations.

In the case of neural crest cells, among their final locations are the dorsal root ganglia, where they reaggregate (cf. Figure 1c) and differentiate into sensory neurons and glia. At the time of aggregation, both N-CAM and N-cadherin are reexpressed (Thiery et al., 1982; Duband et al., 1988). While no direct evidence as yet exists that either or both of these adhesion receptors cause the aggregation of neural crest cells into ganglia, this seems a plausible model.

Thus, the current picture is that cells that disperse as mesenchyme lose cell–cell adhesion receptors and acquire cell–matrix adhesion receptors, including integrins, which then mediate cell migration in response to ECM cues. Reassociation of mesenchyme cells may be mediated by reexpression of cell–cell adhesion receptors. This sequence of events requires temporal and spatial regulation of the expression and/or function of adhesion receptors during the various phases. Similar models apply to later morphogenetic processes (see reviews on cadherins: Takeichi, 1988, 1990, 1991; on N-CAM and relatives: Edelman, 1985, 1986; Rutishauser, 1986; Jessell, 1988; Grumet, 1991; and on matrix effects on cell migration: Thiery et al., 1989; Hynes, 1990). Rather than review each of them in detail, we will next consider pertinent data from tumor and blood cells before turning to the nervous system.

Insights from Tumor Cells

As diagrammed in Figure 3, tumor cells perform many of the same functions as do normal cells during development; dissociation to migratory cells, migration, and arrest. Given these biological similarities, one might hope to find some features in common between the molecular changes in tumor cells and those occurring during development, and indeed this is so.

We postulated above that loss of specific cadherins leads to dissociation of cells from coherent tissues to individual cells. Several studies have shown causal relationships between loss of cadherins and acquisition of an invasive phenotype by tumor cells. MDCK kidney epithelial cells express E-cadherin, and inhibition of its function by antibodies converts these cells to a migratory invasive phenotype in in vitro assays (Behrens et al., 1989). Furthermore, transformation by tumor viruses leads to loss of E-cadherin and acquisition of the invasive phenotype. A survey of carcinomas revealed a quantitative correlation between loss of E-cadherin and invasiveness, and transfection of E-cadherin cDNA into these cells blocked their invasive phenotype (Frixen et al., 1991; Chen and Obrink, 1991; Vleminckx et al., 1991). Furthermore, suppression of E-cadherin by expression of antisense RNA rendered cells more invasive (Vleminckx et al., 1991). Thus, cells expressing E-cadherin adhere to one another, whereas those that lose it separate as single migratory cells. The parallel with dispersion of neural crest cells is striking and suggests that loss of cell–cell adhesion receptors may play a general role in epithelial–mesenchymal transitions.

Loss of specific integrins can also produce reduced adhesion, in this case for ECM molecules. Several viral transformants of rodent cells show loss of $\alpha_5\beta_1$ integrin and reduced adhesion to fibronectin (Plantefaber and Hynes, 1989). Overexpression of transfected $\alpha_5\beta_1$ increases adhesion to fibronectin and reduces tumorigenicity in such cells (Giancotti and Ruoslahti, 1990). Therefore, loss of adhesion to "home base," either cell–cell or cell–matrix adhesion, can contribute to malignant transformation. However, invasion and metastasis are complex phenomena, involving not only loss of adhesion for the normal location but also requiring adhesion of the malignant cell to foreign matrices and heterologous cells (Figure 3). Therefore, one might also expect to see acquisition of new adhesive properties by tumor cells (reviewed by Hynes and Plantefaber, 1991). Indeed, novel expression of $\alpha_v\beta_3$ integrin correlates very well with the invasive vertical growth phase of melanomas (Albelda et al., 1990). $\alpha_v\beta_3$ integrin recognizes the RGD sequence in a variety of ECM proteins, and both in vitro invasion and experimental metastases of melanomas can be inhibited by RGD-containing peptides (Humphries et al., 1986; Gehlsen et al., 1988), consistent with involvement of this receptor. Finally, elevation of $\alpha_2\beta_1$ integrin (a collagen/laminin receptor) occurs in some tumor cells (Dedhar and Saulnier, 1990), and transfection of cDNA for α_2 into rhabdomyosarcoma cells leads to acquisition of metastatic potential (Chan et al., 1991). Clearly, changes in integrins and, therefore, in cell–matrix adhesion can affect the traffic of tumor cells. The parallel with neural crest and other migratory cells in embryos is again striking.

Insights from Leukocytes, Lymphocytes, and Platelets

White blood cells (neutrophils, monocytes, and lymphocytes) exhibit many of the same transitions from sessile to migratory and from nonadhesive to adhesive and also show selectivity in their adhesion. These cell types circulate around the body and "home" or "target" to specific locations. Information about the molecular basis for their selectivity offers useful insights into the basic principles of specific cell adhesion, which are applicable to considerations of the adhesive processes involved in development.

The task facing each of these cells (Figure 2) is to identify the appropriate place in the vessel wall to attach, to stick strongly enough to the endothelial lining of the vessel not to be swept away by the blood flow, and then to penetrate the endothelial layer (extravasate) and migrate into the underlying tissue (invasion). This process proceeds in

steps involving several different adhesion receptors. We will consider first the neutrophils, the first cells to arrive at an inflammatory site.

Neutrophils are known to use all of the families of adhesion receptors reviewed earlier, except, so far, the cadherins. The interplay among the adhesions mediated by selectins, immunoglobulin superfamily receptors, and integrins is very instructive. The initial targeting of neutrophils to the correct site is mediated by selectins. Within seconds of activation of endothelial cells by a variety of inflammatory agents, P-selectin (previously known as PADGEM, GMP-140, or CD62) is exocytosed from intracellular vesicles onto the surface of the endothelial cells (Geng et al., 1990; Patel et al., 1991). P-selectin is a receptor for a specific carbohydrate group, sialyl-Lewis^x, which is prevalent on neutrophil cell surfaces, perhaps attached to leukosialin (CD43), a large, highly glycosylated neutrophil surface protein (Cyster et al., 1991), or to other glycoproteins or glycolipids. Neutrophils attach to P-selectin in an interesting way. Surfaces coated with P-selectin cause neutrophils to adhere (Geng et al., 1990). Under flow conditions the neutrophils roll along the surface (Lawrence and Springer, 1991), that is, they are slowed down but not stopped. Neutrophil rolling is exactly what is seen in damaged vessels in vivo. Other selectins can also induce neutrophil rolling (see, e.g., von Andrian et al., 1991). Indeed, it currently appears that different selectins can recognize the same or similar carbohydrate groups, and a second selectin, E-selectin (or ELAM-1; Bevilacqua et al., 1989), is expressed by cytokine-activated endothelial cells with a slower time course (hours) requiring protein synthesis and can also cause neutrophil adhesion and rolling.

The selectin-induced rolling of neutrophils brings them close to the endothelial cells but does not cause firm adhesion. Full adhesion and extravasation both require β_2 integrins. The human genetic disease leukocyte adhesion deficiency (LAD) is characterized by failure of leukocytes to adhere and extravasate, leading to failure of the first line of defense against bacterial infections. LAD is caused by defects in the integrin β_2 subunit (Anderson and Springer, 1987). β_2 integrins are expressed by white blood cells, and their primary ligands are immunoglobulin superfamily molecules such as ICAM-1 and ICAM-2 (Staunton et al., 1988, 1989). ICAM-1 is also induced on the surfaces of activated endothelial cells with a slower time course like that of E-selectin. ICAM-2, on the other hand, is expressed constitutively but at lower levels on endothelial cells (Staunton et al., 1989). Why, then, do neutrophils not adhere to all endothelial cells? Because the β_2 integrins, particularly $\alpha_L\beta_2$ (LFA-1) in the case of neutrophils, require activation. Activation can be accomplished by various mediators, but in the case of neutrophil adhesion to activated endothelium, it is triggered by platelet activating factor (PAF), a phospholipid produced by the activated endothelial cells (Zimmerman et al., 1990). Thus, the sequence of events appears as follows: activation of the endothelial cells causes them to express P-selectin and PAF; the P-selectin hooks the passing neutrophils and causes them to roll along the endothelial surface, where they contact PAF and are themselves activated, so that their $\alpha_L\beta_2$ integrins become functional and bind to ICAM-2. Later expression of E-selectin and ICAM-1 in response to continued inflammation produces long-term activation of the endothelial surface for continued recruitment of leukocytes by cooperation between the selectin and β_2 integrin–ICAM interactions. Therefore, the selectivity of the adhesion event requires interplay between the two interacting cell types and among several different adhesion receptors (Figure 5).

The involvement of multiple adhesion receptors can also be seen in lymphocytes. The circulation of lymphocytes to various organs involves selective adhesion to endothelial cells in certain lymphoid organs. This phenomenon has been termed "lymphocyte homing," although "trafficking" might be a better term. A great deal of research has been devoted to finding adhesion receptors responsible for the specificity of lymphocyte trafficking (Gallatin et al., 1986; Stoolman, 1989). Several surface proteins have been described that appear to be involved in appropriate targeting (Stoolman, 1989). Among them are representatives of two of the families of adhesion receptors already discussed.

A selectin, L-selectin (previously known as LECAM-1, LAM-1, MEL-14, and a variety of other names), plays a role in homing of cells to peripheral lymph nodes (Lasky and Rosen, 1991). Like other selectins, this one binds carbohydrates, although its exact specificity has not yet been elucidated. L-selectin is rather widely expressed on blood cells, inducing neutrophils, and can promote their attachment to endothelial cells at inflammatory sites (Watson et al., 1991; von Andrian et al., 1991). Furthermore, activation of the neutrophils enhances the binding of a carbohydrate ligand by L-selectin without affecting surface levels of the selectin (Spertini et al., 1991). Therefore, leukocyte–endothelial adhesion mediated by L-selectin can be affected by the activation state of both cell types.

Lymphocyte trafficking to Peyer's Patch lymph nodes is promoted by an integrin, $\alpha_4\beta_p$ (LPAM-1; Holzmann et al., 1989), and another integrin, $\alpha_L\beta_2$ (LFA-1), plays a role in adherence of lymphocytes to a variety of lymph node endothelia (reviewed by Stoolman, 1989). The ligands for $\alpha_L\beta_2$ include ICAM-1 and ICAM-2, discussed previously; the ligand for $\alpha_4\beta_p$ is unknown.

Another class of adhesive molecules, known collectively as CD44, also plays some role in lymphocyte trafficking, particularly to peripheral lymph nodes (Stoolman, 1989). But CD44 is rather generally expressed and appears to be an extracellular matrix receptor as well as a cell–cell adhesion receptor (St. John et al., 1990; Aruffo et al., 1990; Miyake et al., 1990). Indeed, expression of a novel, alternatively spliced form of CD44 confers metastatic potential on carcinoma cells (Günthert et al., 1991).

Therefore, none of the molecules implicated in lymphocyte trafficking seems to be highly selective, and it is currently unclear exactly how precise is the selectivity of this trafficking. However, the involvement of several different molecules is reminiscent of the situation described for neutrophil targeting to inflamed endothelium. It seems likely that interplay between the two cell types (lymphocytes and

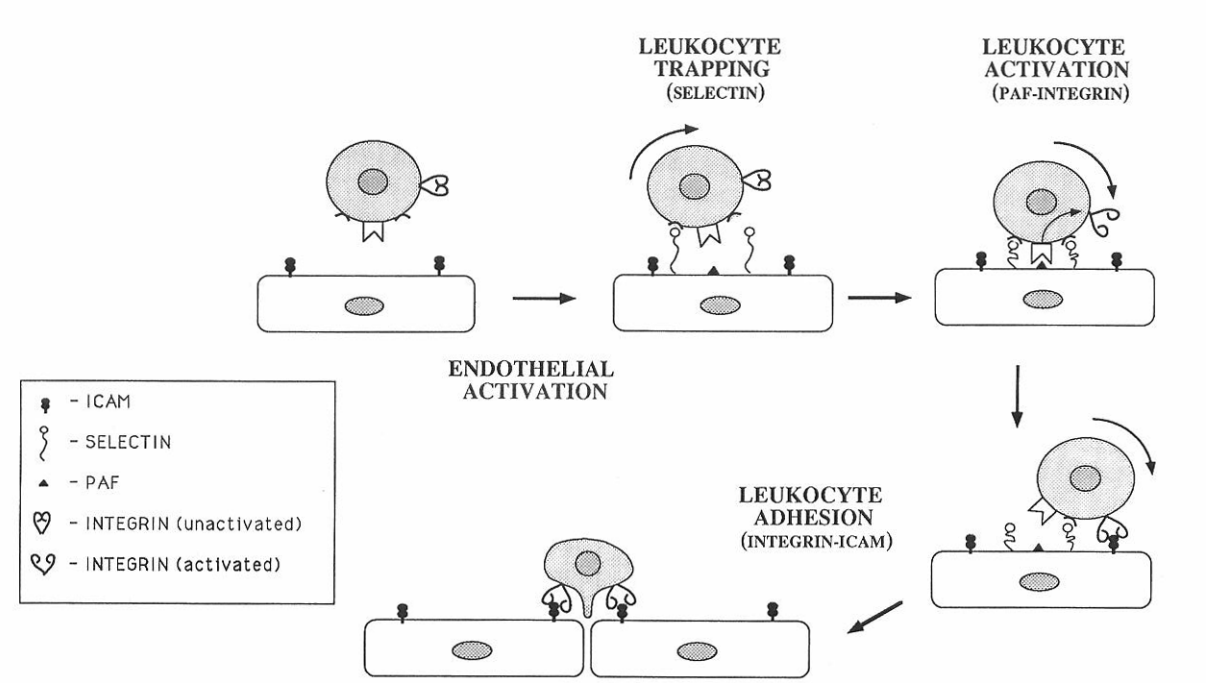

Figure 5. Interplay between Cells and among Receptors during Initial Arrest and Adhesion of Leukocytes to Endothelium

Activation of the endothelial cells leads to surface exposure of selectins and platelet activating factor (PAF). The selectins bind carbohydrates on the leukocytes, causing them to roll along the endothelial surface. Contact of the leukocyte with PAF causes activation of the β_2 integrins on the leukocyte. The activated integrins bind to ICAM counterreceptors on the endothelial cells.

endothelium) and crosstalk among the receptors play a role in refining the adhesive specificity. We have already mentioned activation of adhesion receptors in a couple of different contexts, and it is perhaps worth citing two more examples that have been fairly extensively studied.

The first concerns the activation of lymphocyte adhesion. It is well known that the specificity of T cells for antigen-presenting cells comes from recognition by antigen-specific T cell receptors of antigen–MHC complexes. However, effective adhesion of T cells to their targets also requires interaction between $\alpha_L\beta_2$ integrin and ICAM-1 and can be blocked by antibody to either one (reviewed by Springer, 1990). Since these two counterreceptors are widely distributed and are not antigen specific, there must be some coupling between the antigen-specific T cell receptor and the major adhesion molecules. It turns out that crosslinking of the T cell receptor by antibody transiently activates the $\alpha_L\beta_2$ integrin, which then binds ICAM-1 on the target cell (Dustin and Springer, 1989; Springer, 1990). Thus, a specific but relatively weak adhesive interaction is coupled intracellularly to a less specific but stronger receptor to produce effective adhesion.

The second example comes from a consideration of platelet adhesion. Platelets, like lymphocytes, have many adhesion receptors (Kieffer and Phillips, 1990). A key one among them is the integrin $\alpha_{IIb}\beta_3$, or GPIIb–IIIa. Absence of this integrin in the genetic disease Glanzmann's thrombasthenia causes defective platelet adhesion and a bleeding disorder. On resting circulating platelets, this integrin is surface-exposed but inactive. However, activation of the platelets by a variety of agonists, including thrombin and collagen (for each of which there are receptors on the platelet), activates the $\alpha_{IIb}\beta_3$ integrin, leading to effective adhesion (Kieffer and Phillips, 1990; Phillips et al., 1991).

The parallels with the activation of integrins on neutrophils and lymphocytes discussed above are obvious and lead to a general proposition: there is no such thing as simple adhesion. Most adhesion events that have been well studied involve several different receptors acting cooperatively. Furthermore, this cooperation appears to be more than simply additive. There is frequently crosstalk among the receptors on a given cell and sometimes even between interacting cells, leading to activation of one or more of the adhesion receptors. Therefore, the adhesive specificity requires several different adhesion receptors. These are frequently individually not highly specific, but are activated locally to give specific cell adhesions. This sort of cooperative specificity is likely also to play a role in specific cell adhesion events in development. It is worth noting that communication among receptors could readily explain the anti-adhesive effects of some molecules.

Targeting in Nervous System Development

The most elaborate use of cell surface and extracellular matrix molecules in cellular targeting is seen in the developing nervous system. The highly specific synaptic connections characteristic of even simple nervous systems arise as a result of guided cell migration and axon growth, although subsequent "editing" (reshaping of axonal and dendritic arbors, axon retraction, and cell death) also plays an important role. In recent years, numerous examples of exquisite neuronal and axonal targeting have been de-

scribed, in both vertebrates and invertebrates. Many of these observations have been facilitated by recent technical advances. For example, the use of fluorescent, nontoxic lipophilic dyes to label migrating cells and growing axons in living preparations, in combination with the use of confocal fluorescence and other types of video-enhanced microscopy, makes possible the observation of cellular and axonal navigation with remarkable clarity and precision, even in living preparations. Genetic screens for mutations that affect nervous system targeting are being continually refined. Increasingly sophisticated tissue culture systems are permitting the reconstitution, in vitro, of tissue environments in which complex guidance decisions are made.

The recent activity in this area has increased our appreciation of the complexity and diversity of the targeting strategies employed by the nervous system. Whereas, at one time, recognition by axons of their correct synaptic partners may have seemed to be the pivotal problem in neuronal targeting, it is now clear that the path from neuron to target often consists of many discrete navigational steps, each of which may need to be specified, to some degree, by independent cues in the cellular microenvironment. Several recent studies highlight examples in which some of the cues necessary for achieving target specificity come not from target cells themselves but from cells along the pathway to the target (e.g., Yip, 1990; Lance-Jones and Dias, 1991).

What molecules provide these cues, and how do they work? The emerging picture suggests that many of the same types of molecules that control targeting in other organ systems—e.g., cadherins, immunoglobulin superfamily molecules, integrins, and integrin-binding extracellular matrix proteins—play related roles in the nervous system (selectins have so far not been detected in the nervous system). Many of the functions mediated by these molecules in nonneural tissues—e.g., cell adhesion, cell sorting, and haptotaxis—seem to apply for the nervous system as well. Where the nervous system distinguishes itself is in the diversity of expression of members of these protein families. For example, the majority of known immunoglobulin superfamily molecules involved in cell adhesion are expressed in the nervous system (Grumet, 1991), as are the majority of known cadherins (Napolitano et al., 1991; Suzuki et al., 1991) and several integrins (for detailed discussions of the structure and expression of these molecules in the nervous system, see reviews by Jessell, 1988; Dodd and Jessell, 1988; Lander, 1989; Takeichi, 1990; Reichardt and Tomaselli, 1991; Grumet, 1991). In addition to these molecules, the nervous system also appears to employ cell surface molecules of other types as guidance cues (see below; also see Tomaselli and Neugebauer, 1991), as well as making use of guidance information provided by diffusible molecules (reviewed by Tessier-Lavigne and Placzek, 1991) and possibly even electric fields (see, e.g., Patel et al., 1985).

Cell Migration in the Developing Nervous System

Nervous system targeting involves the guided migration of neuronal and glial cells, as well as the guidance of growing axons. Until recently, only a few types of neural cell migration could be subjected to experimental study, the most notable of which is the migration of neural crest cells, discussed earlier.

A second system for studying cell migration in the nervous system involves the developing mammalian cerebellum. In this tissue, large numbers of newly generated neurons (called granule neurons) undergo a directed migration from the surface of the cerebellum to a deep layer. As with many neurons in the vertebrate brain, granule neurons undergoing this migration do so in close apposition to "radial glia," cells with radial processes spanning the width of the neural tube. As first shown by Moonen et al. (1982), migration will occur in vitro within small chunks of cerebellar tissue, permitting easy observation and scoring of cell migration. More recently, Hatten and colleagues have reconstituted neuronal migration in vitro using only purified neurons and glia (cf. Hatten, 1990).

Several interesting conclusions can be drawn from experiments that have been done using these assays. For example, just before granule neurons begin to migrate, they express the immunoglobulin superfamily adhesion molecule L1/Ng-CAM. Antibodies directed against this protein have been found to disrupt granule cell migration in vitro (Lindner et al., 1983; Chuong et al., 1987). The appearance of L1 at the time of cell migration contrasts with what has been observed with neural crest cells, which lose expression of certain cell–cell adhesion molecules prior to migration. The difference could be explained by the fact that the substratum for granule neuron migration is a cell surface (of the radial glial cell), while the substratum used by crest cells is the extracellular matrix. However, it is probably an oversimplification to view the substratum used by granule cells as merely a cell surface, since extracellular matrix proteins are present throughout the cerebellum, and antibodies against at least two of them, tenascin and thrombospondin, can also disrupt migration (Chuong et al., 1987; O'Shea et al., 1990).

In addition to being useful for examining the roles of known adhesion molecules in neuronal migration, the cerebellar system has been helpful in identifying new adhesion molecules (e.g., astrotactin; Stitt and Hatten, 1990), as well as other, less conventional guidance molecules such as protease inhibitors and lectins (Lindner et al., 1986; Lehmann et al., 1990).

In recent years, the range of neuronal migrations that can be observed in vitro and in vivo has widened considerably (see, e.g., Gasser and Hatten, 1990; Gray and Sanes, 1991), and it seems likely that molecular analysis of these examples will yield new insights into molecules that guide neuronal targeting. One intriguing new system is the migration undertaken by neurons derived from the embryonic nasal epithelium into the vertebrate brain. As recently shown by Wray et al. (1989) and Schwanzel-Fukuda and Pfaff (1989), a certain subset of the neurons of the mammalian hypothalamus are not generated within that region of the brain, but rather migrate in from the nose, during embryonic life. In vitro, neurons derived from the nasal (olfactory) epithelium are highly motile on substrata treated with laminin or one of its isoforms (merosin), but

not on substrata treated with other extracellular matrix molecules (Calof and Lander, 1991). This behavior is quite unlike that of neural crest cells, which migrate on a wide variety of substrata. Although it is reasonable to suspect that laminin plays some role in guidance of these cells in vivo, some recent results in human genetics call attention to a new molecule. The hereditary condition known as Kallmann's syndrome (hypogonadotropic hypogonadism with anosmia) is characterized by defects in the olfactory system, as well as an absence of precisely those cells of the hypothalamus that derive from the nasal epithelium. It has therefore been suggested that defects in the migration of nasal epithelium–derived neurons underlie this condition. Two groups have recently identified a strong candidate for the gene affected by Kallmann's syndrome, and the predicted sequence of its gene product suggests that it is a secreted protein containing sequences related to fibronectin type III repeat motifs, especially the type III repeats found in the cell adhesion molecules NCAM, Tag-1, and contactin (F11) (Franco et al., 1991; Legouis et al., 1991).

Axonal Targeting—Growth Cones Make Many Kinds of Decisions

Many of the efforts to understand targeting in the nervous system have focused on axon guidance. Axons accomplish remarkable feats of navigation and can easily be studied in vitro. Certain simplifying assumptions about growing axons constrain the possible mechanisms that can be involved in their guidance. Most notably, growing axons are effectively separated from the genetic and protein synthetic machinery of the cell by a variable but significant time delay. Navigational decisions are made at the growth cone, the growing tip of the axon, which must communicate with the cell body (site of all transcription and translation) by the relatively slow process of axonal transport.

Many insights into how growth cones work have come from watching them. Typically, as axons grow, their growth cones extend and retract filopodial and lamellipodial processes, in apparent exploration of the microenvironment. Recent observations in living preparations obtained from grasshoppers (O'Connor et al., 1990), Drosophila (Halpern et al., 1991), Xenopus (O'Rourke and Fraser, 1990), and mammalian visual pathways (Sretavan, 1990, Soc. Neurosci., abstr.) indicate that growth cones in vivo engage in such behaviors at least as vigorously as they do in vitro. The notion that growth cone activity is exploratory in purpose is supported by observations in many systems that growth cone activity and the complexity of growth cone morphology increase significantly at locations where growth cones must make navigational decisions (Bovolenta and Mason, 1987).

Some of the results from in vitro systems have tended to support simple models of growth cone steering, e.g., that pathfinding can be explained in terms of single, global properties of growth cones, most notably the adhesion of the growth cone or its component parts to the substratum (Letourneau, 1985), and the levels of free intracellular calcium within the growth cone cytoplasm (Kater and Mills, 1991). Although growth cone adhesion and calcium regulation are likely to exert large effects on growth cone behavior, recent data raise doubts that such hypotheses can explain all, or even most, of what growth cones do. Some of the cell surface or extracellular matrix molecules that have been found to be most active in steering growth cones in vitro appear to do so by mechanisms that have little to do with adhesion per se (Gundersen, 1987, 1988; Lemmon et al., 1991). In at least some cases, dramatic changes in growth cone motility can be elicited by physiologically relevant stimuli, without causing any detectable changes in levels of intracellular calcium (Ivins et al., 1991).

The fact that no single unifying hypothesis manages to explain growth cone behavior may just be a consequence of the fact that growth cones steer in more than one way. For example, in the grasshopper, growth cones of the Ti1 neurons follow a highly stereotyped pathway through the developing limb bud, obtaining guidance information from various different kinds of structures (O'Connor et al., 1990). At some navigational choice points, axon growth proceeds by extension of multiple lamellipodia, consolidation of one or a few lamellipodia into the nascent axon, and retraction of inappropriate filopodial and lamellipodial processes. This behavior is similar to that of many growth cones in vitro (cf. Goldberg and Burmeister, 1986). At other choice points, however, single filopodia encounter specific "guidepost cells" and rapidly expand in diameter, filling in with axoplasm and abruptly reorienting the entire growth cone (O'Connor et al., 1990). Growth cone collapse (see below) is yet another abrupt change in growth cone behavior that can be mediated through contacts made by one or a few filopodia.

The growth cone is clearly a highly specialized and versatile navigational machine. The molecular mechanisms that underlie different kinds of growth cone responses remain to be identified, although recent dramatic advances in the observation of cytoskeletal components in actively motile growth cones are likely to lead to important new insights (Sabry et al., 1991; Tanaka and Kirschner, 1991). It is interesting to point out that orchestrating the rapid response of an entire growth cone to an interaction occurring at the tip of a single filopodium is not likely to be a trivial feat of cellular engineering. How are a sufficient number and diversity of receptors deployed at the tip of such a thin (~100 nm), elongated structure? How is an appropriate message conveyed back to the body of the growth cone? How do filopodial contacts withstand the powerful contractile forces produced by filopodia (cf. Heidemann et al., 1990)? Answers to these questions are not likely to become available for some time.

Extracellular Matrix and Cell Adhesion Molecules in Axon Targeting

Most of what has been learned about how molecules guide growth cones comes from studies of the molecules of the extracellular matrix and molecules that mediate neural cell adhesion. Detailed descriptions of these molecules and their effects on neurons may be found in other reviews (Jessell, 1988; Dodd and Jessell, 1988; Sanes, 1989; Rog-

ers et al., 1989; Lander, 1989, 1990; Takeichi et al., 1990; Grenningloh et al., 1990; Reichardt and Tomaselli, 1991; Grumet, 1991). It will suffice here to discuss functional categories into which these molecules may be grouped.

Of the extracellular matrix molecules, laminin is well known to promote the outgrowth of neurites from a wide variety of neurons. The effects of merosin, an isoform of laminin (Ehrig et al., 1990), are similar. Fibronectin has a similar, if weaker, effect on a smaller variety of neurons (Rogers et al., 1989). Recently, vitronectin and thrombospondin have been added to the list of neurite outgrowth–promoting molecules (Neugebauer et al., 1991; O'Shea et al., 1991). Thus, a wide variety of extracellular matrix molecules may provide permissive substrata for the growth of various types of axons in vivo. Laminin also exerts a guidance effect on neurons: growth cones will accurately follow substratum pathways along which laminin has been deposited. It is not yet clear whether the same can be said for other extracellular matrix molecules (Gundersen, 1987). In addition to providing a nondirectional pathway for axon growth, laminin, if present in a concentration gradient, might also be expected to guide axons in a directional manner (i.e., haptotaxis). So far, however, attempts to demonstrate such an activity for laminin in vitro have produced convincingly negative results (McKenna and Raper, 1988).

The mechanism by which extracellular matrix molecules exert neurite growth–promoting and guiding effects has been the subject of much discussion. Early studies argued that these molecules might function simply by being adhesive, i.e., binding growth cones tightly to the substratum. More recent work suggests that laminin, at least, is unlikely to work in that manner, neither in guiding neurites nor for migrating cells (Gundersen, 1987, 1988; Calof and Lander, 1991). These results suggest that matrix molecules signal cells in other ways. Possibly the same conclusion can be drawn from the evidence, mentioned in an earlier section, that some extracellular matrix molecules actually antagonize adhesion. The most notable of these is tenascin, which can markedly interfere with the adhesiveness of fibronectin substrata (Chiquet-Ehrismann et al., 1988), but recently even laminin has been added to the list of anti-adhesive molecules (Calof and Lander, 1991).

What are the potential consequences of anti-adhesion in the nervous system? The answer is still unclear. Both tenascin and the extracellular matrix glycoproteins J1–160/180 (which are immunochemically related to tenascin) produce in vitro substrata unsuitable for neurite outgrowth by certain types of neurons (Pesheva et al., 1989; Faissner and Kruse, 1990). For some neurons, however, neurite outgrowth may actually be stimulated by tenascin, provided that artifical means are used to ensure adequate attachment of the cell body (not necessarily the growth cone) to the substratum (Wehrle and Chiquet, 1990). Thus, the true effect of tenascin on growth cones, if there is a consistent one, is not yet clear. Nonetheless, the temporal and spatial localization of tenascin in the developing mammalian nervous system suggests a certain amount of correlation with "boundaries" through which axons do not penetrate (Steindler et al., 1989).

The roles played by cell–cell adhesion molecules in nervous system development are likely to be at least as varied and complex as those played by the extracellular matrix (Grumet, 1991). Molecules such as NCAM and the cadherins not only appear to be involved in holding the cells of the nervous system together, but also serve as substrata that promote the outgrowth of neurites. Molecules such as L1 are widely distributed on axons and appear to play a widespread role in axon bundling, or fasciculation. Many other members of the immunoglobulin superfamily are more restricted in their distribution and, most likely, mediate the specific fasciculation of certain groups of axons. Nowhere has the importance of selective fasciculation in the guidance of axons been better illustrated than in the developing insect central nervous system (CNS) (Goodman et al., 1984; Grenningloh et al., 1990). In Drosophila and grasshopper, cell adhesion molecules known as fasciclins mark selected axonal pathways and, in at least some cases, are essential for the guidance of axons along those pathways (Jay and Keshishian, 1990; Grenningloh et al., 1990). In the vertebrate spinal cord, Tag-1 (Dodd et al., 1988; Furley et al., 1990) and DM-GRASP/SC-1 (Burns et al., 1991; Tanaka et al., 1991) mark restricted sets of axons during development. Interestingly, axons that express Tag-1 switch to expressing L1 just as they reach a critical navigational choice point, suggesting that dynamic changes in the fasciculation preferences of axons may play an important role in guidance (Dodd et al., 1988).

Guidance by Inhibition—An Important Mechanism for Shaping the Nervous System?

The number of cell surface and extracellular matrix molecules known to stimulate axon growth has increased dramatically in recent years. Concomitantly, a large amount of data has emerged suggesting that specific inhibitors of axon growth also exist. In addition to extracellular matrix molecules that may be anti-adhesive, as described above, cell surface molecules have been found that cause the collapse and complete paralysis of growth cones.

The best characterized growth cone–collapsing activity was identified as a component of CNS myelin (the glial membranes that ensheath and insulate many types of axons). Myelination usually occurs in each part of the vertebrate CNS at about the time that axon growth ceases, and in higher vertebrates (mammals and birds), the onset of myelination usually corresponds to the time when axons stop responding to injury by attempting to regenerate. Experiments in which fetal tissue is transplanted into adult animals strongly argue that white matter (myelin-rich tissue) is a uniquely unfavorable environment for axon growth. In vitro, growth cones strongly avoid growing in contact with purified CNS myelin, or with mature oligodendrocytes, the cells that make such myelin (Schwab and Caroni, 1988). Interestingly, peripheral nervous system myelin, as well as CNS myelin from lower vertebrates, has no such in vitro inhibitory effect, nor are these types of myelin associated with any detectable barrier to axonal regeneration in vivo (cf. Bastmeyer et al., 1991).

Observation of growth cones as they contact fragments of myelin or the surfaces of oligodendrocytes indicates

that the contact of one or a few filopodia with myelin results in a collapse of growth cone structure (loss of most filopodia and lamellipodia), as well as a profound and sometimes long-lasting loss of motility (Bandtlow et al., 1990). Caroni and Schwab (1988a, 1988b) were recently able to purify two components of myelin possessing collapsing activity. A single monoclonal antibody, IN-1, recognizes both proteins and blocks their activity. In vivo and in vitro experiments using this antibody argue that much of the nonpermissiveness of CNS myelin for axon regeneration can be reversed by blocking the activity of the identified proteins (Caroni and Schwab, 1988b; Schnell and Schwab, 1990). More recently, Schwab and colleagues have argued that myelin "barriers" may serve during development to prevent intermingling of actively growing axons with already established axon tracts. Experiments in which myelination is reduced by irradiation (to kill oligodendrocytes), or in which IN-1 is introduced into the developing spinal cord, have produced some axonal misrouting (Schwab and Schnell, 1991), although experiments with hypomyelinating mutant mice have failed to produce the same result (Stanfield, 1991). Whether or not the myelin-associated inhibitors of axonal growth play a role in development, the likelihood that they at least play a major role in limiting axon regeneration in adults has created much excitement about possible new therapies for nervous system injury.

Since the initial description of the myelin-associated inhibitors, growth cone–collapsing activities have been identified in several other tissues. For example, Raper and Kapfhammer (1990) found such an activity in embryonic chick brain; the biochemical characteristics of the active factor suggest that it is different from the active molecules in myelin. Davies et al. (1990) found a similar activity in chick embryo somites, the condensations of mesodermal tissue not only through which neural crest cells migrate, but also through which axons emerging from the spinal cord and spinal ganglia must grow. Interestingly, the segmental pattern of spinal nerves and ganglia observed in all vertebrates appears to result from the restriction of axon growth to the anterior half of each somite. The collapsing activity purified by Davies et al. may, in fact, derive primarily from the posterior halves of somites, since the active factor bears carbohydrates recognized by the lectin peanut agglutinin, a specific marker for the posterior half-somite (Davies et al., 1990).

"Unconventional" Molecules and Receptors in Neuronal Guidance

Although integrins, cadherins, and immunoglobulin superfamily molecules account for many of the adhesive and targeting interactions of cells, other families of molecules are likely to join this list in the near future. At present, there are several less "conventional" molecules and receptors that seem likely to mediate targeting interactions in the nervous system. Proteoglycans, for example, have long been suspected to play some sort of ancillary role in the functions of many extracellular matrix and cell surface molecules. Indeed, most extracellular matrix attachment proteins and at least two immunoglobulin superfamily adhesion molecules (NCAM and myelin-associated glycoproteins) possess domains that bind to either the heparan sulfate or chondroitin sulfate glycosaminoglycans that are found on most proteoglycans (Lander, 1989). Nonetheless, the actual functions of proteoglycans remain obscure. Recent work in the nervous system suggests that at least some proteoglycans exert inhibitory influences on neural cell migration (Pettway et al., 1990), axon growth (Oohira et al., 1991; Snow et al., 1990a), and the ability of extracellular matrix proteins to promote axon growth (Muir et al., 1989). This possibility is supported by the localization of certain glycosaminoglycan epitopes to brain regions that act as barriers to axonal elongation, such as the roofplate of the spinal cord and midbrain (Snow et al., 1990b) and the posterior half-somite (Oakley and Tosney, 1991).

Lectins also may play an important role in nervous system targeting. Lactose-binding lectins are found in subsets of vertebrate peripheral sensory neurons, although the function of these molecules is not yet known (Hynes et al., 1990). A recent study suggests that another lectin plays a role in the migration of cerebellar granule cells (Lehmann et al., 1990). Functionally similar to the lectins are the cell surface glycosyltransferases. These enzymes recognize specific carbohydrate side chains on proteins and will, in the presence of an appropriate nucleotide-sugar, catalyze the addition of an additional monosaccharide. In the absence of a nucleotide-sugar, the enzyme remains bound to its substrate, acting much like a lectin. The cell surface glycosyltransferases that have been identified in the nervous system include galactosyltransferase and N-acetylgalactosaminylphosphotransferase. The former enzyme binds to terminal N-acetylglucosamine residues, and may participate in the responses of neurons and neural crest cells to laminin (Runyan et al., 1986; Begovac and Shur, 1990). Studies with a neuronal cell line suggest that cell surface galactosyltransferase interacts with the E8 domain of laminin (a region also recognized by several integrins) and is involved in laminin's ability to stimulate the initiation of neurites (Begovac and Shur, 1990; Begovac et al., 1991). The latter enzyme associates with and apparently glycosylates N-cadherin on the surface of chick retinal neurons (Balsamo and Lilien, 1990). The fact that antibodies directed against this enzyme inhibit calcium dependent cell adhesion suggests that it may play a role in modulating cadherin function (Balsamo et al., 1991). Interestingly, this enzyme is specifically localized to neuromuscular junctions (motor nerve synapses) in vivo (Scott et al., 1990).

Yet another example of the involvement of carbohydrates in regulating neural cell adhesion and axon growth comes from the examination of the role of $\alpha(2\rightarrow8)$ polysialic acid chains in the functioning of NCAM. This unusual polysaccharide moiety appears abundantly on NCAM molecules during development, and in much lower amounts in adulthood. NCAM is virtually the only major nervous system protein known to bear this structure. It has been proposed for many years that electrostatic repulsion between the polysialic acid chains on interacting NCAM molecules should weaken the homophilic interaction of these pro-

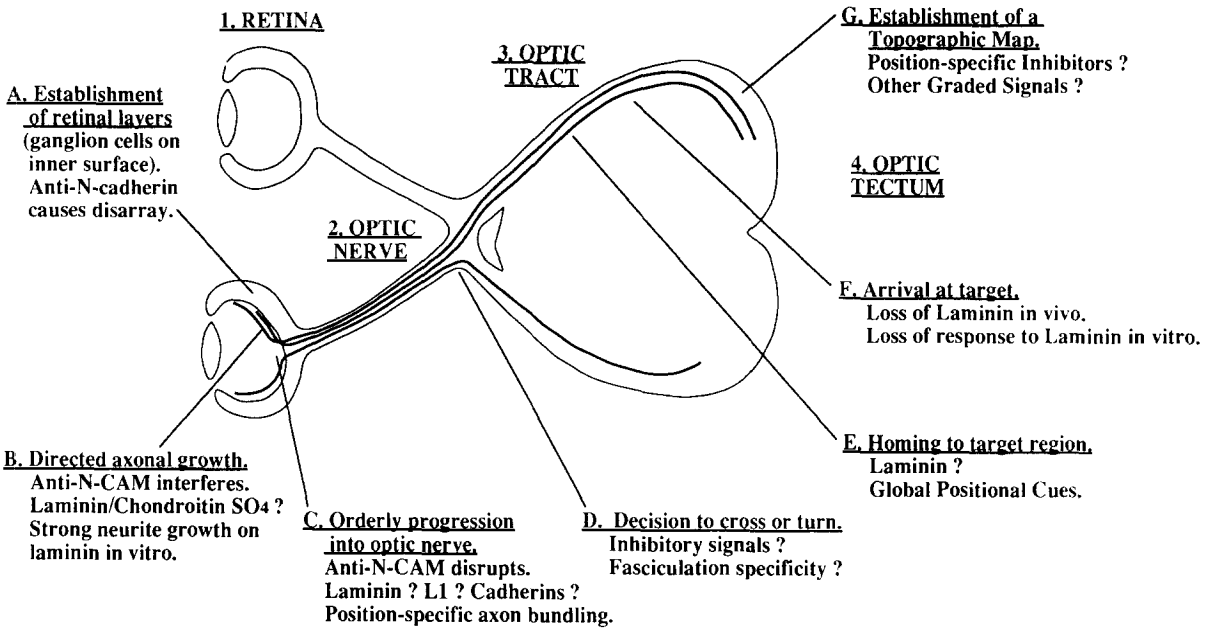

Figure 6. Sequential Directional Cues for Guidance of Retinal Ganglion Cell Axons from the Retina to the Optic Tectum

Evidence exists for involvement of N-cadherin in organization of the retinal layers (A) and for NCAM and integrin–laminin interactions in axonal guidance (B–E), although it is also clear that other cues contribute both then and later (F and G). Details are given in text.

teins. More recently, the proposed role of polysialic acid has been broadened to include the possibility that polysialic acid on two interacting cell surfaces may, by the same mechanism, interfere with many or all interactions that involve close membrane–membrane apposition, not just those involving NCAM. This hypothesis is supported by several observations (e.g., Acheson et al., 1991), and the plausibility of the proposed mechanism is strengthened by the fact that NCAM is a very abundant cell surface component, and that polysialic acid chains are predicted to extend a considerable distance beyond the membrane.

The Retinotectal Pathway: An Example of Stepwise Navigation

The classes of molecules discussed in preceding sections seem likely to provide many of the cues that are present on complex pathways along which neurons and growth cones travel. Just how these cues are strung together to delineate a pathway is poorly understood. Some insights can be obtained by focusing on a particularly well-studied navigational route, the one leading axons from the retina of the vertebrate eye to the optic tectum. The tectum, a region of the brain involved in processing certain kinds of visual information, is one of several major targets for vertebrate retinal neurons.

This pathway is illustrated schematically in Figure 6. The earliest steps occur within the retina itself (labeled "A"). Somewhere around the time that ganglion cells (the neurons destined to send axons out of the retina) first differentiate, cell-sorting events occur that place these cells along the inner margin of the retina. Dynamic changes in cadherin expression in the retina occur around this time and suggest a possible mechanism for the cell sorting (Matsunaga et al., 1988; Inuzuka et al., 1991). Subsequently, ganglion cell axons grow along the inner surface of the retina and follow relatively straight trajectories leading them to a single location, the optic nerve head ("B," Figure 6). These axons grow in contact with a laminin-containing basal lamina, which may play a role in promoting their growth. It is unlikely, however, that laminin provides the information to guide these axons to the optic nerve head, since laminin, even in relatively steep gradients, does not appear to confer directionality on axon growth in vitro (McKenna and Raper, 1988). Preliminary evidence suggests that an inverse gradient of an inhibitory molecule, the glycosaminoglycan chondroitin sulfate, may help guide axons at this early stage in their growth (Snow and Letourneau, 1991, Soc. Neurosci., abstr.).

Next, axons enter the optic nerve ("C," Figure 6) and grow toward the brain. The first axons traverse an environment consisting of neuroepithelial cells and early glia, where molecules such as laminin (Cohen et al., 1987) and cell–cell adhesion molecules are expressed. In vitro studies suggest that interactions mediated by integrins, cadherins, and NCAM may all be involved in supporting axon growth in such an environment (Neugebauer et al., 1988). Later axons bundle closely with earlier ones, and the morphologies of their growth cones support the conclusion that they are engaging in a fasciculated mode of growth (Bovolenta and Mason, 1987). Because axons arrive at the optic nerve head at different times and locations (depending upon the part of the retina they derive from), there is an ordering of axons going into the optic nerve, an ordering that is at least partly maintained throughout the journey from retina to tectum. NCAM appears to play some role in establishing or maintaining this arrangement, since introduction of antibodies against NCAM into the eye causes axons to enter the optic nerve in a disorderly fashion, which

in turn leads them to emerge at positions in the tectum abnormal for their starting points in the retina (Thanos et al., 1984).

Before entering the brain, retinal axons encounter a critical crossroads, the optic chiasm ("D," Figure 6), where some axons grow straight across to the opposite side of the brain, while others turn 90° and head for the side of the brain on which they started. Whether an axon crosses or turns at the chiasm is strictly a function of the position of its cell body in the retina. Because this occurs simultaneously for both eyes, axons reassort so that, for the most part, those that will carry information about the left visual world go to the right side of the brain, and those that will carry information about the right visual world go to the left side of the brain, regardless of which retina they came from. Remarkably, axons nearly always make correct navigational choices at the optic chiasm (Sretavan, 1990). Observations of growth cones at the chiasm suggest that noncrossing axons may be repulsed by a structure at the midline of the chiasm, while crossing axons traverse this structure by interacting with the noncrossing axons (from the other eye) on the other side of it (Godement et al., 1990). Nothing is currently known about the molecules involved in this process, although it is reasonable to expect that molecules involved in inhibiting axon growth and molecules involved in axon–axon interactions may be involved.

After the optic chiasm, retinal axons grow along the surface of the brain as the optic tract ("E," Figure 6), where they remain closely associated with each other, glial cells, and significant amounts of laminin. The presence of laminin all along the retinal pathway so far has attracted interest because its appearance is transient, corresponding to the time when retinal axons are navigating this route, and because laminin is absent from most other brain regions (Cohen et al., 1987). Whether laminin actually guides retinal axons in this location has yet to be established. Considerable evidence suggests that there are strong directional cues along this pathway, which orient retinal axons toward their targets; as in the retina itself, these directional signals are unlikely to come from laminin. Curiously, cues that direct retinal axon growth toward the optic tectum seem to be present at locations all over the surface of the brain (i.e., where retinal axons do not normally go) (Harris, 1986). Such a situation suggests that the tectum may be releasing a soluble chemoattractant, but this appears not to be the case. Instead, directional cues appear to be intrinsic to the pathway itself (Harris, 1989). It seems likely, therefore, that retinal axons are channeled into a pathway by growth-promoting molecules with a restricted distribution, such as laminin, and given orientation along that pathway by a fairly global system of positional information.

At the end of the optic tract lies the target region, the optic tectum ("F," Figure 6). To enter the tectum, axons must leave the laminin-containing territory within which they have been growing and arborize within a tissue lacking any detectable laminin (except in association with blood vessels). The ability of retinal axons to abandon a laminin-containing environment may result from a developmental change they undergo. In vitro, retinal neurons taken from stages at which axons have not reached the tectum are stimulated to extend neurites by laminin. In contrast, retinal neurons from stages after the tectum has been reached appear unresponsive to laminin (Cohen et al., 1986). In part, this phenomenon seems to be related to the down regulation by retinal ganglion cells of the integrin subunit α_6, which in combination with β_1 forms a laminin receptor (de Curtis et al., 1991). However, some retinal neurons that do not lose α_6 expression still lose the ability to grow neurites on laminin, suggesting that the activation state of laminin-binding integrins may also be developmentally regulated. Consistent with this view, the loss of retinal neurons' ability to respond to laminin can be quickly reversed by application of a monoclonal antibody that binds to a non–ligand-binding site of the integrin subunit β_1 (Neugebauer and Reichardt, 1991).

Once axons grow into the tectum ("G," Figure 6), two additional targeting problems remain. One is finding the correct classes of neurons on which to form synapses (little is known about this process), and the other is forming a topographic map over the tectal surface. Specifically, retinal fibers distribute themselves across the tectum so that there is point-to-point continuous mapping of retinal position (and, therefore, position in the visual world) onto tectal position. Topographic maps are characteristic of many axonal projections in vertebrates and some invertebrates. Although much of the precision of the map arises not through axon targeting, but through later editing (retraction, sprouting, and rearrangement of axon terminals), which may be driven in part by visual signals received by the retina, at least the broad outline of the map appears to be specified by intrinsic, position-dependent molecular properties of retinal axons and tectal neurons (e.g., Thanos et al., 1984; Fraser and O'Rourke, 1990). From the evidence, hints of the types of molecules involved are beginning to emerge.

In particular, several cell surface or extracellular molecules have been identified that are expressed in a graded or discontinuous fashion across either the retina or the tectum (see, e.g., Trisler et al., 1981; Trisler and Collins, 1987; Schlosshauer et al., 1988; McLoon, 1991). It is not yet known whether these molecules are involved in axon guidance or are merely correlates of positional differences among cells. On the other hand, a body of in vitro work, largely by Bonhoeffer and colleagues (e.g., Bonhoeffer and Huf, 1982, 1985; Walter et al., 1987), implies that multiple types of position-specific molecules are involved in guiding axons to form a map. For example, the mapping of the naso-temporal (i.e., medial-to-lateral) axis of the retina onto the posterior-to-anterior axis of the tectum seems likely to involve both position-specific axon fasciculation (i.e., the preferential bundling of temporal axons with other temporal axons [Bonhoeffer and Huf, 1985]) and position-specific inhibition of growth (i.e., the avoidance by temporal axons, but not nasal axons, of growth over posterior tectal cells or membranes prepared from them [Walter et al., 1987]). Recent evidence suggests that the position-specific inhibition of growth in this system is controlled by a posteriorly enriched tectal membrane protein that triggers complete or partial collapse of retinal growth

cones, an effect to which temporal axons are especially sensitive (Cox et al., 1990). A candidate for the molecule responsible for this activity has recently been identified (Stahl et al., 1990).

Thus, it can be seen that the pathway from eye to tectum sequentially confronts growing axons with several important navigational choices. Much the same conclusion has been drawn from examination of the development of the vertebrate spinal cord (Dodd and Jessell, 1988) and the sensory projections of the grasshopper limb (O'Connor et al., 1990). In the retinotectal system, this process appears to depend on many different types of guidance molecules, acting either in concert or sequentially. It seems likely that similar paradigms are used throughout the nervous system.

Conclusions

We have seen that the final arrangements of cells and connections of neurons, which arise during development, involve a great variety of cell–cell and cell–matrix interactions. It is not surprising that these processes involve a fair number of different receptors. We have largely omitted, for lack of space, discussion of the additional diversity introduced by alternative splicing of many of these molecules. While the full spectrum of these receptors is not yet known and will undoubtedly rise from the current count of a few dozen, it is already clear that the same receptors (and particularly the same families of receptors) appear in many different processes during development and homeostasis. Some generalizations are becoming clear.

Most adhesion events involve several receptors of different types. Specificity arises not so much from high selectivity on the part of individual receptors as from the coupling of multiple receptors in a variety of ways. This combinatorial approach can be seen at several levels. For example, the projection of an axon from its parent cell to its final target frequently involves several different cell adhesion processes in sequence, and may involve cell–matrix interactions as well as a variety of different cell–cell interactions. Each of these cellular interactions can involve several different receptors cooperating to provide specificity. This comes not only from combinatorial specificity but can also involve crosstalk among the receptors on individual cells and between interacting cells, leading to activation of certain receptors. This is currently best understood in blood cells (see above) but very likely plays a role in other cells as well. The nature of "activation" of receptors is not understood, although evidence exists for conformational changes in some receptors as a consequence of activation and/or ligand occupancy. We have mentioned the likelihood of a balance between positive and negative cues. Crosstalk among receptors can accommodate both, and the nature of signaling events among receptors and the modulation of their activities requires much more investigation. We have barely touched on the intracellular consequences of receptor occupancy, which also include effects on cytoskeletal organization and other signaling events. Both cell–cell and cell–matrix adhesion affect cell polarization, metabolism, and gene expression, issues which we have not had space to discuss here. It seems evident that future research will shed further light on these various intracellular consequences of cell adhesion.

One point that is clear is that the orderly arrangements of cells during development require detailed control of the temporal and spatial expression of the various adhesion receptors and their ligands. Rather little is known about the genetic control of adhesive molecules. The elegant analyses of the hierarchies of control genes in generating asymmetries and patterns in embryos (see other reviews in this issue) lead eventually to control of morphogenetic events. Among the gene products that execute morphogenesis must be the adhesion receptors and their ligands. The spatial and temporal control of expression of these molecules by homeobox proteins, retinoid receptors, and other transcription factors will be a fruitful line of research over the next several years.

Research in the last 5–10 years has uncovered the diversity of cell adhesion receptors and provided us with cDNA clones and probes for many of them. Future work along these lines should reveal the full extent of this diversity, as well as the many subtle variations introduced by alternative splicing and regulatory events. More importantly, the use of recombinant DNA and genetic methods to modify the expression and function of adhesion receptors in vivo, coupled with sophisticated methods for following the behavior of individual cells, promises to reveal many of the secrets of how, when, and where cells attach, detach, migrate, target, and arrest with such precision and specificity.

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