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Review

Cell Adhesion: The Molecular Basis of Tissue Architecture and Morphogenesis

Barry M. Gumbiner
Cellular Biochemistry and Biophysics Program
Memorial Sloan–Kettering Cancer Center
New York, New York 10021

Introduction

Cell adhesion is crucial for the assembly of individual cells into the three-dimensional tissues of animals. Cells do not simply "stick" together to form tissues, but rather are organized into very diverse and highly distinctive patterns. A variety of cell adhesion mechanisms are responsible for assembling cells together and, along with their connections to the internal cytoskeleton, determine the overall architecture of the tissue. Thus, cell adhesion systems should be regarded as mechanisms that help translate basic genetic information into the complex three-dimensional patterns of cells in tissues.

The goal of this review is to illustrate the roles of adhesion mechanisms in the generation of tissue architecture. To understand tissue morphogenesis, it is essential to know the endpoint of the process, and therefore we will first consider the molecular basis of cell adhesion in fully formed tissues, that is, what maintains the structure at steady state once the tissue has developed. In the second part, we will consider how these cell arrangements arise during tissue development, which can occur either in embryos or in tissues undergoing development in adult organisms. It is important to appreciate, however, that distinguishing between stable adhesive interactions and dynamic adhesive events in developing tissues is somewhat arbitrary, because both often utilize the same sets of adhesion proteins and in many cases represent varying functional states of the same adhesive mechanism. For this reason, we must also understand the mechanisms that regulate the functional states of adhesion molecules and the dynamics of cell adhesion, a subject that will be discussed in the third part of the review. Of course, tissue morphogenesis requires an intimate cooperation between physical cell adhesion events and signaling processes that control the transfer of information between cells. There is overwhelming evidence that cell adhesion proteins both respond to cell signaling events and themselves transduce signals into the cell. Therefore, the last part of the review will be concerned with the relationship between physical cell adhesion mechanisms and intercellular signaling.

The functional units of cell adhesion are typically multiprotein complexes made up of three general classes of proteins; the cell adhesion molecules/adhesion receptors, the extracellular matrix (ECM) proteins, and the cytoplasmic plaque/peripheral membrane proteins. The cell adhesion receptors are usually transmembrane glycoproteins that mediate binding interactions at the extracellular (EC) surface and determine the specificity of cell–cell and cell–ECM recognition. They include members of the integrin, cadherin, immunoglobulin, selectin, and proteoglycan (for example, syndecans) superfamilies. At the EC surface, the cell adhesion receptors recognize and interact with either other cell adhesion receptors on neighboring cells or with proteins of the ECM. ECM proteins are typically large glycoproteins, including the collagens, fibronectins, laminins, and proteoglycans that assemble into fibrils or other complex macromolecular arrays. Owing to their binding to adhesion receptors, they can also be tightly associated with the cell surface. At the intracellular surface of the plasma membrane, cell adhesion receptors associate with cytoplasmic plaque or peripheral membrane proteins. Cytoplasmic plaque proteins serve to link the adhesion systems to the cytoskeleton, to regulate the functions of the adhesion molecules, and to transduce signals initiated at the cell surface by the adhesion receptors. These adhesion protein complexes will generally be considered as functional units in this review. The biochemical properties of these classes of proteins and the diversity of roles of the various families of adhesion receptors will not be described here in detail; for such information, the reader is referred to several excellent reviews (Bernfield et al., 1993; Gumbiner, 1993; Hynes, 1992; Hynes and Lander, 1992; Mosher et al., 1992; Springer, 1994; Turner and Burridge, 1991).

Stable Connections between Cells and the Maintenance of Tissue Structure

The adhesive elements that stably connect cells together play essential roles in overall tissue organization and the proper physiological function of the tissue and organ. Indeed, as we will see, the medical importance of these stable adhesion elements is well established. Although these adhesive contacts are generally stable, they should not be regarded as static, and in many cases the maintenance of stable connections requires active cellular processes. Numerous kinds of stable adhesion elements are found in an organism, but only a few of the most widespread and best-studied examples of cell–cell and cell–ECM contacts will be discussed here.

Cadherins and Cell–Cell Adherens Junctions

One of the most important and ubiquitous types of adhesive interactions required for the maintenance of solid tissues is that mediated by the classic cadherin adhesion molecules. Cadherins are transmembrane Ca^{2+}-dependent homophilic adhesion receptors that are well known to play important roles in cell recognition and cell sorting during development (Takeichi, 1991). However, they continue to be expressed at high levels in virtually all solid tissues. There are many members of the classic cadherin family (which is a subset of the larger cadherin superfamily), but E-cadherin in epithelial tissues has been the most studied in the context of stable adhesions. Continued expression and functional activity of E-cadherin are required for cells to remain tightly associated in the epithelium, and in its absence the many other cell adhesion and cell junction proteins expressed in epithelial cells (see below) are not capable of supporting intercellular adhesion. In its capacity to maintain the overall state of adhesion between epithelial cells,

E-cadherin is thought to act as an important suppressor of epithelial tumor cell invasiveness and metastasis (Birchmeier and Behrens, 1994; Takeichi, 1993). A loss of E-cadherin expression or function leads to enhanced cell invasiveness in cell culture, and E-cadherin deficiencies or mutations correlate with the invasiveness and metastasis of certain human tumors. E-cadherin gene knockouts in mice cause lethality at a very early stage (Laure et al., 1994), making it difficult to investigate its tumor suppressor role in whole organisms. This later finding is not very surprising, given the fundamental role for E-cadherin in the formation of epithelial tissues.

To exhibit functional adhesion activity, cadherins must form complexes with cytoplasmic plaque proteins, called catenins, and with the actin cytoskeleton (Gumbiner, 1993; Kemler et al., 1989). α-Catenin is required for cadherin-mediated cell adhesion, and since it has actin-binding activity, it probably functions to link the cadherins to the actin cytoskeleton (Rimm et al., 1995). Normally, β-catenin must also be required for adhesion, because it is a necessary intermediate in the linkage of α-catenin to the cadherin cytoplasmic domain. Surprisingly, however, β-catenin is experimentally dispensable for rudimentary cell adhesion, as long as α-catenin is fused directly to the cadherin cytoplasmic tail (Nagafuchi et al., 1994). This finding, along with observations that tyrosine phosphorylation of β-catenin correlates with diminished adhesion in response to growth factors and cell transformation (Kinch et al., 1995), has inspired the hypothesis that β-catenin acts as a regulatory component of the complex. β-Catenin also participates in signal transduction and developmental patterning, suggesting that it serves to couple physical adhesion to signaling events during morphogenesis (see below).

Cadherins are the major adhesion receptors of the zonula adhaerens junctions of epithelia, where they colocalize with a prominent actin filament bundle. Junctional localization is not necessary for cadherin function, however, as there are plenty of examples in which cadherins diffusely distributed over the cell surface mediate robust cell–cell adhesion. Nevertheless, it is generally assumed that junctional localization represents stronger points of intercellular attachment, perhaps analogous to the focal adhesion junctions associated with actin stress fibers and integrin–ECM contacts in fibroblasts (see below; see Figures 3B 4A). The zonula adhaerens junction and associated actin filament bundle, though not necessary for tight adhesion, may be needed in epithelia that experience strong contractile or mechanical forces at their apices, for example, in the digestive tract or in epithelia undergoing wound closure or invagination. Thus, dynamic regulation of the junctional state of cadherins could be important in epithelial physiology or morphogenesis.

The intrinsic structural properties of the EC cadherin-binding domains also contribute to the assembly of cadherin-containing cell junctions. The EC domains of the classic cadherins are divided into five repeated subdomains, EC1 to EC5. High resolution structure determinations of N-terminal EC1 domains reveal that the homophilic adhesive binding region resides on a large external surface of the protein (Overduin et al., 1995; Shapiro et al., 1995). Furthermore, X-ray crystallographic studies

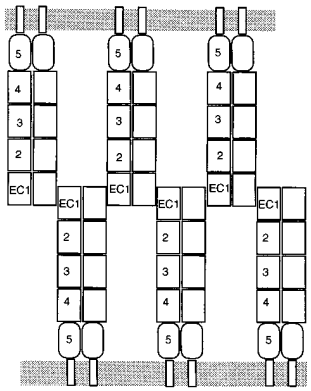

Figure 1. The Cadherin Zipper Model for the Structure of Cadherin-Mediated Adherens Junctions

Model proposed by Shapiro et al. (1995). Two dimerization interactions observed in the X-ray crystal structure of the N-terminal EC1 domain of N-cadherin serve as the basis of the model. A tight parallel strand dimer orients the two cadherin adhesion binding interfaces outward from the cell surface. An antiparallel dimer between adhesion binding interfaces of EC1 domains is proposed to represent the homophilic binding interface, leading to the interdigitation of adhesive elements from the two cell surfaces.

of the N-cadherin EC1 domain show that it forms a dimer, called the strand dimer, in which the monomers are oriented in parallel with their adhesive binding surfaces directed outward from the plasma membrane (Shapiro et al., 1995). In the crystal, each dimer unit interacts with another two dimers in an antiparallel orientation via their adhesive binding surfaces, forming a continuous linear ribbon structure (Figure 1). Assuming that the cadherin-binding activity resides solely in the N-terminal EC1 domain, the authors of this study propose a model in which the cadherin dimers from interacting cell surfaces are arranged as a linear "zipper" at the intercellular contact zone. If, however, the EC1 domains normally interact with one of the other EC domains in the adhesion interface, it would be possible to form a more complex two-dimensional lattice, as might be expected for the structure of a junctional contact (Hirokawa and Heuser, 1981). Regardless of the detailed model, a key concept is that the higher order junctional structure is based on the interdigitation of adhesive units emerging from both cell surfaces. An important problem will be to understand the relative contributions of the EC domains, the cytoplasmic catenin proteins and actin cytoskeleton, and the junctional localization of cadherins to the mechanisms that regulate cell adhesion.

Desmosomal Junctions

The desmosomes are the most conspicuous adhesive elements in epithelia and cardiac muscle. They are linked to the intermediate filament cytoskeletal network (cytokeratins in epithelia and desmin filaments in heart). Together the desmosomes and intermediate filament cytoskeleton form a contiguous network throughout the tissue that engenders it with high tensile strength. The adhesion receptors of the desmosomes are members of the cadherin superfamily, called desmogleins and desmocollins, for which there are a variety of isoforms with distinct tissue-specific patterns of expression (Garrod, 1993). The desmogleins and desmocollins are

linked to the intermediate filament network by several cytoplasmic plaque proteins, including the desmoplakins and plakoglobin. Desmoplakins share sequence similarity with intermediate filament proteins and seem to interact directly with them. Plakoglobin binds to the cytoplasmic tails of certain desmogleins and desmocollins and seems to be essential for the formation of the desmosomal plaque and attachment of cytokeratin filaments (Troyanovsky et al., 1993). Plakoglobin may have other important functions; it has high sequence similarity to β-catenin and can also transduce developmental signals (see below). It is also sometimes found in adherens junctions in association with cadherins, probably in place of β-catenin.

The role of the desmosomal–intermediate filament system in the maintenance of tissue integrity has been most definitively documented in the epidermis, owing to the incidence of several autoimmune and genetic blistering diseases. The epidermis is a multilayered epithelial tissue that undergoes constant cellular turnover throughout life (Fuchs and Byrne, 1994). Cells begin their journeys to the outer epidermal surface as relatively undifferentiated basal keratinocytes attached to the basement membrane. Movement to the surface is associated with a stereotyped program of differentiation. Epidermal cells contain the highest surface density of desmosomes, and the process of keratinization is an extreme state of intermediate filament production and assembly. Several autoimmune blistering diseases result from the disruption of desmosomes (Stanley, 1995). Pemphigus vulgaris is due to a loss of cell–cell adhesion deep in the epidermis, just above the basal layer. It is caused by autoantibodies to desmoglein-3. In another blistering disease, pemphigus foliaceus, the adhesion defect occurs in a more superficial layer, the granular layer. It is caused by autoantibodies to desmoglein-1. Furthermore, mutations in several epidermal cytokeratin genes have been found to cause a number of blistering diseases (Fuchs and Byrne, 1994). Thus, the desmosomes and cytokeratins operate together to provide the mechanical strength required to maintain the integrity of the epidermis. The desmosome–intermediate filament system probably plays similar roles in other tissues and is probably most significant in tissues that must withstand high levels of mechanical stress.

Occluding Junctions

One of the most physiologically important properties of tissues are their capacities to create selective permeability barriers. Cells are often organized into specialized structures that create interfaces between compartments, which serve to regulate the movements of cells, macromolecules, small solutes, and ions. A few common examples include the control of leukocyte traffic across endothelia and epithelia, the selective adsorption of nutrients by the epithelium of the gastrointestinal tract, the maintenance of proper electrolyte balance in the nervous system by the blood–brain barrier, and the electrical insulation of axons by myelin. The adhesive element most important for the formation of permeability barriers in tissues like epithelia and endothelia is the tight junction or zonula occludens. The tight junction actually serves two interrelated roles in these tissues: to regulate the permeability characteristics of the paracellular space between adjacent cells and to divide the surface of the cell into two functionally and biochemically distinct regions that interface with either one of the two physiological compartments (Gumbiner, 1987).

The importance of maintaining these occluding barriers for the well-being of the organism is obvious. Nevertheless, tight junctions are remarkably plastic and diverse structures. Their permeability properties vary from tissue to tissue, ranging from the exclusion of whole cells or macromolecules to the selective permeability to protons and ions. They are also often subject to rapid physiological regulation (Madara, 1988). The molecular basis of tight junction diversity and regulation is not well understood, but there has been significant progress in the elucidation of its molecular composition and structure. An integral membrane protein, called occludin, probably contributes to the formation of the EC contact and the occluding barrier (Furuse et al., 1994). Occludin interacts with two cytoplasmic plaque proteins, ZO-1 and ZO-2. Their functions are uncertain, but they may play a role in assembling occludin or localizing it to the specific site at the boundary between apical and basolateral cell surfaces. Several cytoskeletal-associated proteins, cingulin, the 7H6 antigen, and actin, also localize to the region of the tight junction.

Interestingly, ZO-1 and ZO-2 have significant sequence similarity to two proteins with demonstrated roles in tissue growth control and signal transduction, the Disks large (DLG) tumor suppressor in Drosophila and the *lin-2* gene product involved in vulval induction in Caenorhabditis elegans (Kim, 1995). Similar to ZO-1, DLG is a cytoplasmic plaque protein required for the formation of septate junctions in Drosophila. Although similar signal transducing functions have not yet been discovered for ZO-1 and ZO-2, their relatedness to DLG and LIN-2 raises the intriguing possibility that there is some important feedback mechanism between the permeability state of an epithelium and cellular growth control mechanisms. Such a system could be important, for example, in responses to wounding or developmentally in controlling growth of epithelium until it has acquired suitable physiological properties.

Cell–ECM Attachments and the Basement Membranes

The attachment of cells to the ECM is also crucial for the maintenance of tissue integrity. Cells attach either directly to components of the collagen-rich interstitial matrix or to the basement membrane, a more distinct sheath of the ECM that surrounds many kinds of tissues. Basement membranes cover the basal surfaces of virtually all epithelia, surround the surfaces of muscle fibers, and ensheath nerves. Basement membranes are comprised of two distinct layers. The basal lamina, immediately adjacent to the cells, contains a variety of adhesive ECM glycoproteins, including collagen IV, laminin, fibronectin, proteoglycans, and many others (Mosher et al., 1992). The reticular lamina is produced by fibroblasts of the underlying connective tissue and contains fibrillar collagens. Cells use a number of different adhesion receptors to attach to the ECM, including a family of cell surface proteoglycans called syndecans. Of course, the most prominent of the ECM adhesion receptors are the integrins, a large family of heterodimeric transmembrane proteins with different α and β subunits (see Hynes, 1992; discussed in depth below).

The importance of the ECM and the attachments of cells to the ECM is underscored by existence of many genetic and autoimmune diseases that perturb ECM structure or the adhesion of cells to the ECM in humans. For example, there are at least 13 known types of human genetic diseases resulting from collagen mutations or deficiencies (Olsen, 1995). Perhaps the most well-known diseases resulting from a defect in cell–basement membrane attachment are the muscular dystrophies (Campbell, 1995). The dystrophin gene that is mutated in Duchennes muscular dystrophy is a cytoplasmic plaque protein that links an adhesion receptor for the basement membrane, dystroglycan, to the actin cytoskeleton. Dystroglycan binds to laminin in the basement membrane, and when dystrophin is missing, the entire cell surface adhesion complex turns over and is lost from the surface. Mutations in the genes encoding other components of the dystroglycan complex also cause various forms of muscular dystrophy. In all cases, the loss of the functional attachment and anchoring of the muscle fiber plasma membrane to the basement membrane ultimately result in the deterioration of the muscle tissue. In this case the adhesion protein complex does not seem to be important for the development of muscle tissue, but rather provides a stabilizing role that makes the muscle fibers resistant to the mechanical stress produced by muscle contraction.

Perturbations of cell–basement membrane attachment also occur in autoimmune blistering diseases of the skin (Stanley, 1995). Bullous pemphigoid results from the disruption of the hemidesmosome, a cell–basement membrane junction with the morphology of a half-desmosome. Like the desmosome, the hemidesmosome is linked to the cytokeratin intermediate filament network, but its composition is completely different. The main adhesion receptor is the integrin $\alpha 6\beta 4$, which binds to laminin in the basement membrane. The cytoplasmic plaque proteins that link the hemidesmosome to the intermediate filaments are also unique, albeit related to desmosomal proteins. Thus, an intermediate filament–basement membrane attachment network is crucial for the maintenance of the mechanical integrity of the epidermis.

In addition to its mechanical roles, the basement membranes also contain information that influences the organization of the cells that attach to it. An important example is the development of epithelial cell polarity. Numerous different cues from the outside are needed for epithelial cells to exhibit all aspects of polarity (see Drubin and Nelson, 1996 [this issue of *Cell*]), but attachment of cells to the basement membrane triggers the formation of the apical–basal axis. The mechanism underlying this cellular response to the basement membrane is not well understood, but the adhesive ECM protein laminin has been implicated in epithelial polarization during the development of kidney tubules (Klein et al., 1988).

Localized adhesion components in basement membranes also provide spatial cues for the organization of contacting cells. For example, local regions of the muscle membrane guide the formation of the neuromuscular junction. The junctional basal lamina is a specialized region of the muscle basement membrane that is responsible for localization of acetylcholine receptors in the postsynaptic region and the differentiation of the presynaptic terminal (Hall, 1995). Agrin, an ECM glycoprotein secreted by the motorneurons, stimulates clustering of acetylcholine receptors, and s-laminin, a special isoform of laminin localized to the neuromuscular junction, contains a stop signal for motor neuron growth cones. Similar localized determinants in basement membranes may also be involved in the spatial differentiation of cell types in various epithelia and endothelia. In the kidney, for example, there is an extraordinary diversity of cell types distributed along the tubules within the same continuous epithelium. Similar spatial patterning of endothelial cell types occurs in subregions of the vasculature. The responses of cells to such localized cues in the basement membrane probably involve signaling mechanisms as well as physical cell attachment (see below).

Morphogenesis and the Dynamics of Cell Adhesion

Adhesion mechanisms are intimately involved in the dynamic changes in cell arrangements that give rise to various tissue architectures. Often, the adhesion processes and molecular components underlying dynamic changes in tissues are the same as, or very similar to, those that maintain the structures of formed tissues. Nevertheless, these dynamic processes emphasize important aspects of the functions of cell adhesion system, which are particularly relevant to developmental biology.

Cell Compaction and the Mesenchymal–Epithelial Transition

A prevalent morphogenetic transition mediated by cell adhesion is the process of cell condensation or compaction. In this transition, loosely organized mesenchymal-like cells surrounded by the ECM condense together and form extensive and intimate contacts along their surfaces (Figure 2A). Condensation or compaction occurs in many developing tissues and organ rudiments, but is best understood in the case of the mesenchymal–epithelial transition, in which the cells form tightly adherent polarized epithelial cell sheets with a full complement of epithelial junctions. Compaction is mediated by cadherins in a process that is mechanistically analogous to integrin-mediated spreading of fibroblasts on an ECM substrate (see Figure 4A; see below), with cells spreading against one another instead of spreading on an ECM substrate. Both involve forces generated by the actin cytoskeleton and the assembly of analogous cell junctions (zonula adhaerens and focal adhesions).

Dynamic regulation of the E-cadherin adhesion complex underlies the compaction of mesenchymal cells into a polarized epithelium. A good example is the compaction of the early mouse embryo at the 8- to 16-cell stage in which loosely adherent blastomeres form an epithelium called the blastocyst (Fleming and Johnson, 1988). This dramatic morphogenetic event entails the rapid activation of E-cadherin function at the cell surface rather than changes in its expression. Although the mechanism controlling E-cadherin activation in the mouse embryo is not well understood, it probably involves alterations in the catenin cytoplasmic plaque proteins, in the actin cytoskeleton, or in the interactions

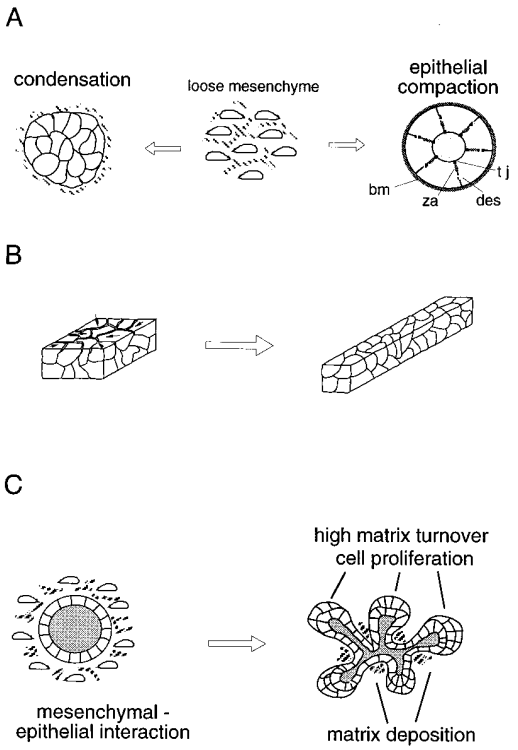

Figure 2. Three Kinds of Adhesion-Dependent Morphogenetic Process Discussed in This Review

(A) Condensation and compaction are mediated by cadherins, catenins, and the actin cytoskeleton and result in the development of extensive intimate adhesive contacts between adjacent cell surfaces. Compaction is the morphogenetic component of the mesenchymal to epithelial transition, which also involves changes in gene expression.

(B) Cellular rearrangements are common morphogenetic processes in developing embryos, which require that cells move within the tissue despite the fact that they are tightly adherent. There is evidence that cadherins participate in intercellular motility underlying cell rearrangements, but other factors are also likely to be important.

(C) Branching morphogenesis depends on complex dynamic interactions between mesenchymal and epithelial tissues. ECM turnover and cell proliferation occur at the tips of the buds, while ECM accumulates in the clefts where there is little growth.

between them. This is best illustrated by the effect of α-catenin expression in an α-catenin-deficient tumor cell lines (Watabe et al., 1994). Despite the expression of E-cadherin and a full complement of epithelial junctional and adhesion proteins, cells lacking α-catenin adhere very poorly. Introduction of α-catenin by cDNA transfection induces the cells to compact and form a polarized epithelium with a full complement of epithelial junctions. In addition to demonstrating the requirement for α-catenin association in epithelial compaction, these findings illustrate how cadherin-mediated adhesion controls the entire hierarchy of adhesive interactions in the epithelium. In this context, it is not surprising that the loss of E-cadherin expression or function can render epithelial tumors more invasive and metastatic.

Cell condensation and compaction are common features of the functions of many, if not all, cadherins. For example, N-cadherin mediates the condensation of mesenchymal cells during initial stages of chondrogenesis (Oberlender and Tuan, 1994), and N-cadherin is also important for the morphogenesis of somites, which involves the compaction of cells into epithelial-like tubes. This has important implications for the segregation of cells into distinct tissue layers or compartments. The expression of different cadherins in separate populations of cells is known to cause the cells to sort out from each other. Compaction reinforces the cell recognition and sorting process to cause the segregation of cells into physically distinct regions of tissue.

Despite the importance of cadherins for compaction, it is important to note that cadherins can also mediate "looser" noncompacted adhesions between cells, for example, the loose clustering of migrating neural crest cells into subpopulations mediated by chick cadherin 7 (Nakagawa and Takeichi, 1995). This suggests that cadherins may exhibit different functional states of adhesion, analogous to the functional states exhibited by leukocyte and platelet integrins (see below).

Cell Rearrangements

Another level of complexity beyond the basic process of compaction is the problem of how differing tissue structures arise. Why do some epithelia form almost spherical-shaped cysts (for example, thyroid), while some epithelia and blood vessels form elongated tubular structures, in some cases with complex branching patterns? A complete mechanistic explanation for this fundamental problem of morphogenesis is, of course, not yet possible. Nonetheless, we do know something about the contributions of certain adhesion systems to such complex processes.

Changes in the shapes of tissues frequently involve extensive cell migration. Cells can either migrate individually or as an adherent piece of tissue. Such long-range migrations usually occur along basement membranes or regions of the ECM and most likely entail motile processes involving integrin adhesion mechanism (see below; also see review by Lauffenburger and Horwitz, 1996 [this issue of *Cell*]). Morphogenetic changes in tissues also can be brought about by local cell rearrangements (Figure 2B) (Keller, 1987). Examples include the convergence and extension movements underlying germband elongation in gastrulating Drosophila embryos and dorsal surface involution and elongation in Xenopus embryos. Cell rearrangements are also observed during leg imaginal disk evagination in Drosophila and during neurulation and notochord formation in amphibian embryos. During these morphogenetic events, cells exchange neighbors despite the fact that they remain adherent throughout the process. Local cell rearrangements involve a process of intercellular motility, that is, the movement of cells with respect to one another.

The prominent roles of cadherins in intercellular adhesion and the similarity between cadherin-mediated compaction and integrin-mediated cell spreading suggest that cadherins may be intimately involved in generating intercellular movements. In fact, there is evidence that certain cadherins are involved in cell rearrangements and tissue morphogenesis. In the gastrulating Xenopus embryo, C-cadherin has been implicated in tissue elongation and in the convergence and extension movements associated with involution (Lee and Gumbiner,

1995). The adhesive activity of C-cadherin is regulated during tissue elongation, and subtle perturbations of C-cadherin function by a dominant negative mutant protein partially inhibit involution.

For epithelial tissues, cell rearrangements pose a particularly challenging problem, because rearranging cells have been found to maintain very tight occluding barriers and to retain their desmosomes for mechanical strength (Keller and Trinkaus, 1987). This indicates that tight junctions and desmosome are remarkably plastic and dynamic, despite their appearance as static structures. Desmosomes in particular look like spot welds and are very insoluble in high concentrations of guanidinium–HCl. Nevertheless, half-desmosomes observed on free surfaces of epithelial cells (called semidesmosomes) could represent intermediate structures during dynamic changes in desmosome formation (Duden and Franke, 1988). Given the extreme insolubility of isolated desmosomes, it is tempting to speculate that regulation of desmosomal adhesion requires a mechanism to switch between two discrete functional states, the semidesmosome and the fully formed desmosome. In this regard, a lattice- or zipper-like model for desmosomal cadherins (see below; see Figure 1) could provide a mechanism for a highly cooperative change to be propagated throughout the structure.

ECM and Branching Morphogenesis

The interactions of cells with the ECM play crucial roles in tissue morphogenesis. The most obvious roles are to provide substrates for cell migration and for epithelial cell attachment and polarization. In addition, cell interactions with the ECM also contribute to the patterns of tissue growth and branching. For example, kidney epithelial cells can be induced to form tubules in culture by treatment with scatter factor/hepatocyte growth factor, but only when they are imbedded within a collagen matrix; if otherwise cultured on top of the matrix, they simply migrate away from each other (Montesano et al., 1991). Similarly, for endothelial cells to undergo angiogenesis in culture, they must interact with the surrounding matrix via integrin adhesion receptors. The formation of such tubular structures in response to growth factors, such as scatter factor/hepatocyte growth factor, is a very complex process and probably involves combined effects on cell–cell adhesion, cell matrix adhesion, proteolytic remodeling of the matrix, and cell proliferation.

Branching morphogenesis of epithelial rudiments illustrates the interplay between various adhesion systems and cell proliferation (Figure 2C). In a classic paradigm, the pattern of branching depends upon the interaction of the epithelial cells with the surrounding mesenchyme (Bernfield et al., 1984). Regulation of the local synthesis, deposition, and turnover of ECM components controls the pattern of cell proliferation in the organ rudiment. While collagen fibrils accumulate in the clefts between outgrowing regions, the tip regions that exhibit the greatest proliferation and growth exhibit a high turnover of ECM proteins, especially proteoglycans. In fact, perturbing the turnover of matrix components, either in vivo or in vitro, inhibit branching morphogenesis. Although these epithelial mesenchymal interactions are quite complex and not yet understood in detail at the molecular level, they emphasize the exquisite coordination between adhesive interactions with the ECM and the control of cell proliferation during tissue morphogenesis (see below).

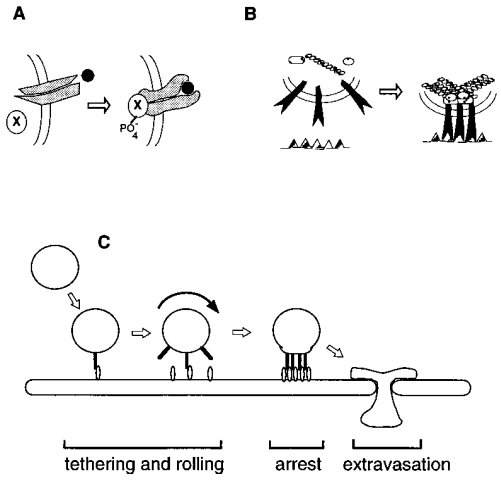

Figure 3. Mechanisms for Regulating the Binding Activities of Adhesion Receptors

(A) Affinity modulation. A conformational change in the EC adhesion-binding site occurs in response to intracellular signals. The conformational change is propagated from the cytoplasmic tail to the EC domain and is probably triggered by unknown cytoplasmic tail–binding proteins.

(B) Adhesion receptor clustering and cytoskeletal interactions are associated with the development of extensive tight adhesion, such as spreading and focal adhesion formation.

(C) Different adhesive states exhibited by β1 integrins during lymphocyte endothelial interactions under conditions of flow. In the inactivated state, the integrin can mediate tethering and rolling, which probably represents weaker attachments with high on and off rates. The same integrin can be activated to mediate arrest and tight binding.

Mechanisms of Adhesion Regulation

The dynamic aspects of cell adhesion described above underscore the need to regulate cell adhesions receptors at the cell surface. Many different adhesion systems are known to be subject to regulation, but the most-studied and best-understood class is the integrins. Integrins are known to be regulated at several levels (Gumbiner, 1993; Hynes, 1992). Modulation of the affinity of the adhesion receptor for ligand (called affinity modulation) is a well-documented mechanism for the activation of platelet aggregation and is thought to underlie activation of leukocyte adhesion (Figure 3A). Adhesive strengthening by the clustering of adhesion receptors or by cytoskeletal-dependent processes such as cell spreading (Figure 3B) is known to be crucial for strong cell attachment, the control of cell growth (see below), and cell motility. These regulatory changes occur either in response to intracellular events (hence, sometimes called inside-out signaling), as a result of EC ligand binding (often called postreceptor occupancy events), or in many instances from both. Although each physiological process tends to emphasize one aspect of integrin regulation, some integrins are subject to multiple levels of regulation (for example, lymphocyte–

endothelial interactions; see below). It remains to be seen whether there is a universal paradigm for adhesive states of most integrins (or, for that matter, of other classes of adhesion receptors).

Regulation of Integrin Binding

Affinity modulation is thought to be the major mechanism for activating the adhesion activity of the major platelet integrin αIIbβ3 and for stimulating adhesion mediated by leukocyte β2 subunit-containing integrins (Ginsberg et al., 1992; Schwartz et al., 1995). These molecules have been shown to undergo conformational changes during activation, as detected by antibody binding to activation-induced epitopes and by biophysical techniques. Moreover, ligand binding studies indicate that these conformational changes are associated with changes in binding affinity. In fact, many integrins can be activated by the binding of certain monoclonal antibodies, presumably because the antibodies favor binding to the activated conformations of the molecules.

During platelet activation the integrin αIIbβ3 is converted to a high affinity state for binding to soluble fibrinogen or von Willebrand factor. This regulatory event is very important, of course, to prevent circulating platelets from aggregating in the absence of an appropriate hemostatic stimulus. αIIbβ3 can, however, mediate the adhesion of inactivated platelets to immobilized fibrinogen, presumably to facilitate recruitment of platelets to a preexisting hemostatic platelet plug. This demonstrates an important point, that the inactivated αIIbβ3 is not an inactive or nonfunctional molecule, but rather that activation entails the conversion of a functional adhesion receptor to a different affinity state or binding specificity.

The molecular mechanisms underlying affinity modulation are only partially understood. Although activation by physiological signals must normally be initiated in the cytoplasm, the conformational change underlying the change in fibrinogen binding affinity can be elicited in the isolated αIIbβ3 molecule by binding of activating antibodies. The propagation of the conformational change can occur at long range over the length of the molecule, since the activating antibodies bind to the "stalk" region near the membrane anchor, while the ligand-binding region of αIIbβ3 is known to reside at the distal "head" of the protein. This is consistent with the hypothesis that structural alterations in the integrin cytoplasmic tails are somehow propagated across the membrane to induce conformational changes at the ligand-binding site. Indeed, there is abundant evidence that the cytoplasmic tails of αIIbβ3 and leukocyte β2 integrins control the affinities or the adhesive states (or both) of the EC domains (Sastry and Horwitz, 1993). Although Ser/Thr kinases are implicated in triggering activation, direct phosphorylation of integrin tails, which occurs during activation, does not seem to be required for activation. Rather, it is likely that specific cytoplasmic tail binding proteins are involved in regulation, since overexpression of isolated cytoplasmic tail domains specifically inhibit activation of αIIbβ3 (Chen et al., 1994). Unfortunately, the identity of such a protein is unknown; it is not clear whether the known cytoplasmic plaque integrin-binding proteins, such as talin, α-actinin, paxillin, or focal adhesion Tyr kinase (FAK) (see below), or novel unknown proteins are required for regulating affinity.

Regulation of integrin-mediated adhesion may involve conversions among several different states. For example, leukocytes exhibit several different adhesive behaviors as they interact with endothelial cells of vessel wall during homing or extravasation at sites of inflammation (Figure 3C). In the now classic three-step model (Springer, 1994), under the high shear forces present in flowing blood, leukocytes first become tethered and then roll along the vessel surface. When a local signal (for example, a cytokine) is released in their vicinity, they arrest, develop firm adhesion, and then migrate across the endothelium. Until recently, it has been thought that the rolling phase was mediated solely by the selectins, a family of carbohydrate-binding adhesion molecules implicated in leukocyte homing. Arrest and the tightening of adhesion are known to result from the activation of leukocyte integrins. However, it has been recently shown that a single type of integrin can mediate all adhesive phases, including the initial tethering and rolling. For example, α4β1 (VLA4) mediates tethering and rolling on vascular cell adhesion molecule 1 (VCAM-1), an endothelial integrin ligand belonging to the immunoglobulin superfamily (Alon et al., 1995b). As expected, this integrin can also become activated to bring about arrest and tight adhesion. Thus, prior to activation, the integrin exhibits binding properties that support tethering and rolling.

These two adhesive states are attributable to the specific binding properties of this integrin, because other integrins on these cells do not mediate tethering and rolling in shear even though they are functional in static cell adhesion assays. Furthermore, while the ligand VCAM supports tethering and rolling by α4β1, two other ligands for α4β1, fibronectin and intercellular adhesion molecule 1 (ICAM-1), do not. The molecular basis of the differences between these types of adhesive bonds is not known. However, the selectins are thought to have special ligand binding characteristics to permit rolling, such as very fast on and off rates for binding (Alon et al., 1995a). The arrest phase could be triggered either by affinity modulation or by clustering of the integrin, and the migration phase is probably analogous to the spreading and migration of fibroblasts (see below). The spectrum of behaviors exhibited by leukocytes under shear flow and in responses to physiological stimuli highlight the diversity of mechanism that cells can use to regulate the dynamics of adhesion. One wonders whether similar mechanisms could be relevant to the problems of cell motility and cell rearrangements in tissue morphogenesis.

Recent progress on the biochemistry and structure of integrin ligand-binding sites provides some insights into the molecular mechanisms that control integrin-binding properties (Bergelson and Hemler, 1995). The X-ray crystal structure of a ligand-binding domain in the α subunit of the αMβ2 leukocyte integrin, called the insert domain (I domain), has been solved (Lee et al., 1995). The 200 amino acid I domain is present in seven different integrin α subunits. It contains a divalent cation-binding motif, called the metal ion-dependent adhesion site (MIDAS), that has been shown to be part of

the ligand-binding site and important for cell adhesion. Divalent cations are required for most integrin–ligand interactions, and mutagenesis of residues in the MIDAS motif interferes with ligand binding. In the X-ray structure of the I domain of αMβ2, the MIDAS motif is located at one end of the globular domain, in a position to interact with ligand. Moreover, one of the acidic residues that contributes to the metal ion coordination site comes from a neighboring molecule, and Lee et al. (1995) propose that this residue might normally come from the ligand. Many binding sites in integrin ligands do possess acidic residues, such as the Asp in the renowned RGD motif. Together the biochemical, mutagenesis, and structural data support a model in which divalent cations serve to bridge the integrin and ligand at the binding site.

This concept of a shared divalent cation and ligand-binding site may provide a paradigm for a great number of integrins. Similar domains that harbor MIDAS-like motifs are present in all integrin β chains, and some have been shown to harbor ligand-binding sites. In the platelet integrin αIIbβ3, ligand binding and cation binding both map to the same 13 amino acid segment of the β3 subunit, which includes a MIDAS-like motif (D'Souza et al., 1994). Moreover, ligand binding to this segment causes the displacement of the divalent cation. A model of integrin–ligand binding has been proposed in which cation, the ligand, and the MIDAS-like motif form an intermediate ternary complex, and cation displacement leads to a stable binding interaction. Such alterations in the structure of the ternary ligand–cation–receptor complex may be physiologically relevant to integrin function, because different divalent cations and changes in divalent cation concentration have been found to regulate the binding affinity or adhesive states (or both) of many integrins. Changes in the EC concentrations of divalent cations are not likely to be significant for physiological regulation, but these findings suggest that similar alterations in the conformation of the cation/ligand-binding sites may be important for the regulation of integrin-mediated adhesion.

Firm Adhesion, Spreading, and Migration

The formation of intimate, extensive adhesive contacts between cells or between cells and matrix (for example, cell spreading, migration, and epithelial compaction) results from a cooperation between adhesive systems and the actin cytoskeleton and the generation of force across regions of the cell. Cell migration entails the coordination of a cycle of cytoskeletal-mediated process extension (that is, filopodia and lamellopodia), formation of adhesive contacts at the leading edge of the cell, breaking adhesive contacts, and cytoskeletal -dependent retraction at the trailing edge (Figure 4B; see reviews by Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996 [this issue of *Cell*]). From the perspective of adhesion regulation, the mechanisms controlling the state of adhesive contacts with the substrate are the most important aspects to consider.

The focal adhesion is a common type of adhesive contact that cells make with the ECM. Focal adhesions are comprised of integrins as the major adhesion receptors (but integral membrane proteoglycans are also important) and associated cytoplasmic plaque proteins,

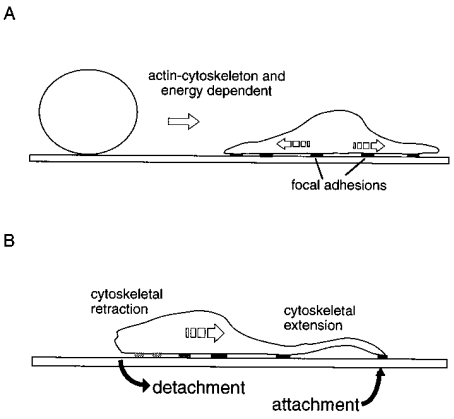

Figure 4. Cell Spreading and Motility on the ECM

(A) Cell spreading is associated with the assembly of focal adhesion junctions and depends on the actin cytoskeleton and the development of forces along the basal cell surface.

(B) A simple diagram to illustrate how cycles of cell–ECM attachment and detachment participate in cell motility. These must be coordinated with the spatial regulation of the actin cytoskeleton.

including talin, vinculin, α-actinin, tensin, paxillin, and a number of protein kinases (Gumbiner, 1993; Turner and Burridge, 1991). They are the major sites of actin filament attachment at the contact surface, and their formation is associated with the process of cell spreading. Thus, focal adhesions are thought to serve as sites for coordination between cell adhesion and cell motility. Actually, highly motile cells often lack easily distinguishable focal adhesions, probably because they are more transient, smaller, or less distinctively distributed. Nevertheless, the focal adhesions that are more prominent in adherent stationary cells probably represent a highly assembled state of the molecular complexes involved in cell migration.

The assembly of focal adhesions is regulated both by EC ligand binding events and by intracellular signaling events. Ligand binding controls the localization of β1- and β3-containing integrins into focal adhesions (Sastry and Horwitz, 1993). The cytoplasmic domains of the β subunits have intrinsic signals for focal adhesion localization, but incorporation of the integrins into focal adhesions is prevented by the α subunits of the heterodimers. Ligand binding, however, relieves this inhibition and allows the β subunit cytoplasmic tail signals to recruit the integrin dimer into the focal adhesion. For effective focal adhesion assembly, both receptor clustering and occupancy by ligand are required (Miyamoto et al., 1995). A combination of receptor occupancy and clustering triggers a synergistic response that includes the reorganization of the cytoskeleton and associated cytoplasmic plaque proteins and the activation of local signaling pathways.

Focal adhesion assembly and disassembly are also regulated by locally generated intracellular signals (Figure 5). The assembly of cell junctions cannot be explained by mass action and protein–protein interactions alone. The process is much too complex and needs to be spatially and temporally regulated in the cell and therefore requires control by biochemical modifications

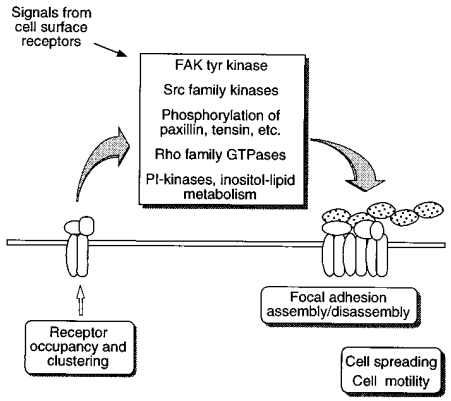

Figure 5. A Model for the Regulation of Focal Adhesion Assembly and Integrin-Mediated Adhesion by Locally Generated Signaling Pathways

Signaling events triggered by ligand binding and integrin clustering are also involved in the regulation of focal adhesion assembly. Similar signals are produced by traditional cell surface growth factor receptors, providing a potential mechanism to regulate focal adhesion assembly and adhesion by extrinsic signals.

and the production of soluble second messengers typical of signaling pathways. FAK, other nonreceptor Tyr kinases, and the tyrosine phosphorylation of focal adhesion proteins paxillin and tensin seem to be involved in focal adhesion assembly (Burridge et al., 1992; Schwartz et al., 1995). Because FAK activity and tyrosine phosphorylation of focal adhesion proteins are also triggered by integrin occupancy and clustering, these signaling events seem to link the assembly of the complete focal adhesion complex to the initial ligand binding event.

Focal adhesions and actin–membrane interactions are also regulated by the Rho subfamily of GTP-binding proteins (Nobes and Hall, 1995). Cdc42, Rac, and Rho stimulate the assembly of structures resembling focal adhesions in association with filopodia, lamellopodia, and actin stress fibers, respectively. These GTPases are thought to act sequentially in a pathway to regulate the various types of actin–membrane associations. Interestingly, integrins can regulate the levels of phosphatidylinositol (4,5)bisphosphate (PIP_2) in a pathway that is dependent on Rho, and PIP_2 can promote actin filament polymerization by interacting with actin-binding proteins (Schwartz et al., 1995). These data suggest that Rho and other related GTPases may also function in the local signaling pathway coupling integrin–ligand binding to focal adhesion assembly. Given the roles of Cdc42, Rac, and Rho in the regulation of different actin–membrane interactions, this pathway could provide a mechanism for coordinating the cycle of cell process extension, adhesion, and detachment that is implicated in cell motility.

Signals generated externally from traditional growth factor receptors also exert rapid effects on cell adhesion and motility. These receptors trigger signaling pathways that are very similar to the local signals generated for focal adhesion assembly. For example, Cdc42, Rac, and Rho are stimulated by serum factors, and PI metabolism is linked to the function of many cell surface receptors. This overlap between locally generated signals and signaling pathways triggered by growth factor receptors provides one way for extrinsic signals to regulate the state of focal adhesion assembly and disassembly (Figure 5). Other signal transducing proteins, including protein kinase C (PKC) and phospholipase A2 can also stimulate focal adhesion assembly and cell spreading. Also, Ca^{2+} and the Ca^{2+}-regulated phosphatase calcineurin have been implicated in the regulation of cell detachment in migrating neutrophils (Hendey et al., 1992). An important challenge will be to understand how these various signals, extrinsic and locally generated, are coordinated to regulate cell adhesion, cell spreading, and cell motility.

Cell Adhesion and Signal Transduction

There is abundant evidence that adhesion molecules participate in a large variety of signal transduction events important for regulating cell adhesion and cell motility (see above), cell growth, apoptosis, and specific gene regulation (Juliano and Haskill, 1993; Ruoslahti and Reed, 1994). For the integrins in particular, many biochemical pathways involving protein phosphorylation and the generation of cytoplasmic second messengers are now well known. Delineation of these pathways and the mechanisms by which they are triggered and controlled is a major area of current research that has been extensively covered in several recent reviews (Clark and Brugge, 1995; Schwartz et al., 1995). Here we will focus on biological roles of adhesion protein–mediated signaling and, in particular, on the relationship between the physical adhesion interactions in tissues and the signaling events. Only a few well-defined examples in this burgeoning area will be discussed in order to illustrate a few basic points.

An important issue to address is why there is a need for signaling by cell adhesion molecules, which are especially suited to mediate physical interactions between cells, when there exist plenty of traditional cell surface receptors dedicated to signal transduction. Although there may be instances in which adhesion molecules have simply been adopted by nature to perform both tasks, it is apparent that there are functional reasons to couple certain signaling processes to specific cell adhesion events. In this regard it is useful to distinguish between two types of signaling events (though not necessarily mutually exclusive): signals that control local cytoplasmic processes and signals that influence cell growth and differentiation, in particular those that impact on cell cycle regulation and gene transcription.

Local regulation of cytoplasm processes, including alterations of the cytoskeleton, secretion, and the control of adhesion itself, is a prominent feature of integrin-mediated signaling. This is most dramatically illustrated in the platelet, a cell fragment with no nucleus and no cell cycle control. Yet most of the major known integrin-associated phosphorylation events and components, including kinases and substrates as well as systems for the generation of second messengers, occur in the platelet (Shattil et al., 1994). These signaling reactions regulate postaggregation processes, including platelet spreading (analogous to cell spreading), platelet contraction for clot retraction, and secretion via exocytosis of platelet granules. The reason that these signals are

dependent on integrin-mediated adhesion (by αIIbβ3) is obvious; they are only needed, and should only occur, after platelet aggregation. Similarly, much of the signaling that occurs at focal adhesions in spreading or motile fibroblasts, probably represents local regulation of focal adhesion assembly and cell motility, as described above, although such signals can also be utilized to control aspects of cell growth and differentiation (see below).

Another important reason adhesion molecules are utilized for signal transduction is to localize the signal to a specific region of the cell surface or ECM. The guidance of migrating cells depends on the presence of local cues in the environment. This is best exemplified in the nervous system, where a number of adhesion proteins participate in axonal guidance and neuronal pathfinding. Although adhesion molecules do help guide axons by physical adhesion mechanism, such as axon fasciculation mediated by N-CAM and L1 (members of the immunoglobulin superfamily) (Sonderegger and Rathjen, 1992), it has become quite evident that many of the cues that guide growth cones are not even adhesive in nature. The old concept that migrating cells and growth cones simply follow adhesive gradients (haptotaxis) is no longer held to be valid, as there is no simple correlation between direction of migration and adhesion strength. In fact, many of the cues important for neuronal pathfinding provided by adhesion molecules produce signals that inhibit migration or growth cone motility (Kapfhammer and Schwab, 1992). An example is s-laminin localized in the synaptic region of the muscle fiber basement membrane (see above). Although s-laminin and laminin can both support motoneuron adhesion and growth cone motility, s-laminin provides a specific stop signal that determines the site of synapse formation (Porter and Sanes, 1995). Other important examples include the semiphorins, members of the immunoglobulin superfamily of cell adhesion molecules that induce growth cone collapse (Kolodkin et al., 1993). When growth cones encounter these adhesion receptors at the surface of a cell, they retract from the cell and then migrate in a different direction. In all of these examples, it is clear that the ECM proteins and adhesion molecules act primarily to provide localized signals rather than to support physical attachment.

Nevertheless, signaling pathways can be intimately coupled to physical cell adhesion events in order to control aspects of cell growth and differentiation. A classic example is the anchorage dependence of cell proliferation, a common cellular property that is lost during malignant cell transformation. Strong integrin-mediated attachment to a substrate serves as a checkpoint for cell cycle progression, and there is evidence that signals arising from focal adhesions directly communicate with pathways that regulate cell proliferation (Figure 6). Adhesion of fibroblasts to fibronectin leads to the activation of the Ras/MAP kinase pathway, which is known to be activated by mitogenic growth factors (Schlaepfer et al., 1994; Zhu and Assoian, 1995). One biochemical mechanism for the activation of the Ras/MAP kinase pathway by integrins and focal adhesion signaling complexes has been described. The focal adhesion kinase FAK, which associates with focal adhesion proteins, interacts directly with the GRB2 adapter protein. Since GRB2 is linked to Ras via the SOS protein, this establishes a link between FAK activation and a well-established mitogenesis pathway.

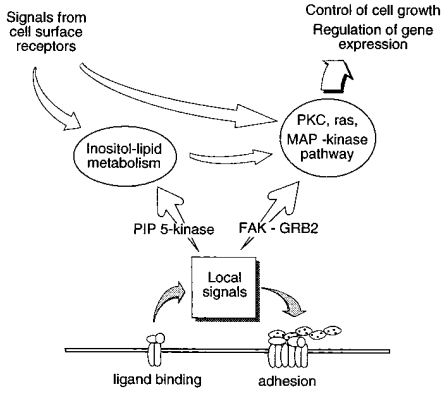

Figure 6. A Model for the Integration of Adhesion Dependent Signals with the Signaling Pathways That Control Cell Growth and Differentiation

Locally generated signals involved in the regulation of focal adhesion assembly and integrin-mediated adhesion can also turn on the Ras/MAP kinase pathway. Also, signals arising from focal adhesions signals can synergize with growth factor stimulated pathways. For example, adhesion can modulate the cellular sensitivity to a growth factor (PDGF) by stimulating the synthesis of 4,5-PIP_2, which, as a substrate for PLCγ, leads to the generation of DAG and IP_3 and to the activation of PKC and Ca^{2+} mobilization.

Synergy between cell adhesion–mediated and growth factor–triggered signals may be an even more significant mechanism for regulating various cell behaviors (Figure 6). A particularly illustrative example is the synergy between adhesion to fibronectin and platelet-derived growth factor (PDGF) in stimulating signaling pathways in fibroblasts (McNamee et al., 1993; Schwartz et al., 1995). In nonadherent cells, PDGF cannot trigger downstream events in the phospholipase C (PLC) pathway (that is, production of diacylglycerol [DAG] and inositol 1,4,5-trisphosphate [IP_3] and activation of PKC) even though it is capable of stimulating the tyrosine phosphorylation of PLCγ. This deficiency occurs because the levels of the substrate for PLC, 4,5-PIP_2, are very low in nonadherent cells. Adhesion to fibronectin stimulates the activity of PIP 5-kinase via the GTP-binding protein Rho, which results in the synthesis of 4,5-PIP_2. Thus, the growth factor receptor regulates the activity of an upstream signaling component, PLCγ, while cell attachment via integrins controls the cellular sensitivity to growth factor. This and other instances of synergy between signals generated by cell adhesion receptors and growth factor receptors help to explain the anchorage dependence of the cellular response to growth factor–initiated mitogenesis.

Adhesion-dependent signaling is important in developing tissues, because highly localized signals in the ECM are needed to control the patterns of morphogenesis. As described above, basement membranes are thought to contain highly regionalized signals to induce a diverse range of endothelial cell types in small vascular regions and epithelial cell types along the length of a continuous epithelium, such as the kidney tubules. In branching morphogenesis (see above; see Figure 2C), it is crucial for proliferation to be controlled locally so

as to generate the intricate and stereotyped patterns of growth needed for each tissue. Localization of developmentally significant signals to regions of the ECM or basement membrane is probably quite common, but in most cases the molecules and adhesion systems involved in generating the signal have not been determined.

Cell adhesion proteins also participate in signaling pathways that control long-range developmental patterning processes in embryos. The cadherin-associated cytoplasmic plaque protein β-catenin is an essential component of a WNT signaling pathway that controls developmental patterning in both Drosophila and Xenopus embryos (Gumbiner, 1995; Peifer, 1995). β-Catenin and the related protein plakoglobin have very high sequence similarity to the product of the Drosophila segment polarity gene *armadillo* (*arm*). ARM protein mediates a late step in a signaling pathway initiated by Wingless (WG), a member of the WNT growth factor family. In Xenopus embryos, β-catenin is involved in an early signaling event that induces the dorsal–ventral and anterior–posterior body axes, and, like ARM, it transduces an intracellular step in a WNT signaling pathway. β-Catenin and ARM are functional homologs, both with respect to the formation of cadherin-mediated cell junctions and the transduction of the WNT signal. The signaling pathways mediated by both proteins are virtually the same, as the known components of the WG pathway in Drosophila (Dishevelled [DSH], Zeste-White-3 kinase) also participate in the Wnt axis induction pathway in Xenopus. These findings raise the interesting possibility that there is a coordination between events controlling tissue morphogenesis and processes that control long-range embryonic patterning.

Despite the well-established role of β-catenin/ARM in cadherin-mediated adhesion, a body of evidence indicates that its signaling activity in Xenopus and Drosophila embryos takes place in the cytoplasm or the nucleus, independent of its role in cadherin-mediated cell adhesion. In Drosophila, the upstream signaling steps mediated by WG, DSH, and Zeste-White-3 lead to increased cytoplasmic levels of ARM, which, in some as yet unknown way, then regulates target gene expression. If the cytoplasmic pool of β-catenin/ARM is responsible for signal transduction, what then is the relationship between β-catenin/ARM-dependent cell adhesion and signaling or embryonic patterning? These two functions of β-catenin/ARM function operate within the same cell. Furthermore, high levels of cadherin expression actually inhibit β-catenin signaling activity (Heasman et al., 1994). Therefore, it seems likely that cadherin-mediated cell adhesion is linked in some important way to these signaling and developmental patterning events.

From the available data, there are several hypotheses to explain the relationship between adhesion and signal transduction by β-catenin/ARM. Cadherins could antagonize the WNT pathway by sequestering β-catenin/*arm*, resulting in a reciprocal relationship between cell adhesion and signaling. The level of cadherin expression could set a threshold level over which β-catenin/*arm* must accumulate to transduce the signal. Alternatively, cadherins could play a more active role in signaling, and like the putative WNT receptor, they could control the release of β-catenin/ARM into the cytoplasm. So far, there is not yet any evidence that this can occur. Finally, it is possible that both a cytoplasmic signal and an increase in cadherin-mediated adhesion constitute a coordinated response to β-catenin/ARM accumulation. Indeed, WNT expression in certain cultured cell lines leads to elevated β-catenin or plakoglobin levels and increased cadherin-mediated cell adhesion (Bradley et al., 1993; Hinck et al., 1994). Such a dual response could help to create a tight collective of similar cells to function as a highly localized signaling center, which could be important for the behavior of an embryonic organizer. Cadherins would be ideally suited for such a role, since they are well known to underlie the sorting out and segregation of cell types within tissues. Regardless of the correct relationship between β-catenin/ARM signaling and cadherin-mediated adhesion, the existence of this novel signaling pathway suggests that there is a coordination between local tissue morphogenesis and long-range patterning in development.

Summary and Conclusions

A variety of cell adhesion mechanisms underlie the way that cells are organized in tissues. Stable cell interactions are needed to maintain the structural integrity of tissues, and dynamic changes in cell adhesion participate in the morphogenesis of developing tissues. Stable interactions actually require active adhesion mechanisms that are very similar to those involved in tissue dynamics. Adhesion mechanisms are highly regulated during tissue morphogenesis and are intimately related to the processes of cell motility and cell migration. In particular, the cadherins and the integrins have been implicated in the control of cell movement. Cadherin-mediated cell compaction and cellular rearrangements may be analogous to integrin-mediated cell spreading and motility on the ECM. Regulation of cell adhesion can occur at several levels, including affinity modulation, clustering, and coordinated interactions with the actin cytoskeleton. Structural studies have begun to provide a picture of how the binding properties of adhesion receptors themselves might be regulated. However, regulation of tissue morphogenesis requires complex interactions between the adhesion receptors, the cytoskeleton, and networks of signaling pathways. Signals generated locally by the adhesion receptors themselves are involved in the regulation of cell adhesion. These regulatory pathways are also influenced by extrinsic signals arising from the classic growth factor receptors. Furthermore, signals generated locally by adhesion junctions can interact with classic signal transduction pathways to help control cell growth and differentiation. This coupling between physical adhesion and developmental signaling provides a mechanism to tightly integrate physical aspects of tissue morphogenesis with cell growth and differentiation, a coordination that is essential to achieve the intricate patterns of cells in tissues.

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