

Adhesive Specificity and the Evolution of Multicellularity

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Most of the cells in a multicellular organism are connected to each other and/or attached to a substrate. Physically this is mediated by adhesion molecules exposed on the cell surface and attached to the cell membrane either by covalent linkage to the lipid bilayer or by a membrane spanning region.

Introduction

The step from unicellular to multicellular organisms was a major milestone in evolution. This was achieved by the generation of membrane-associated cell adhesion molecules. Cell adhesion is not only an essential component but sometimes even a driving force in the creation of the structural and functional complexity observed during metazoan evolution. The way multicellular organisms are morphologically organized, be it on the gross anatomical level or the microscopic histological level, results from highly regulated processes of specific cellular behaviour. These processes define how cells adhere to the substrate and to each other and how they communicate with their neighbours and with the (micro)environment. The focus of this chapter will be on intercellular adhesion, but we should keep in mind that essentially the mechanisms underlying cell–cell and cell–substrate adhesion are very similar.

How Cell Adhesion Is Observed and Studied

At the molecular level, cell adhesion is mediated by molecules that are exposed on the external surface of the cell and somehow physically linked to the cell membrane. In essence we can envisage three possible mechanisms by which membrane-attached adhesion molecules link cells to each other (**Figure 1a**). First, homophilic binding molecules on one cell could bind directly to similar molecules on the other cell. Second, heterophilic adhesion molecules on one cell could bind to other adhesion receptors on the other cell. Finally, in linker-mediated adhesion, two different adhesion molecules on two cells could both bind to a shared secreted multivalent ligand in the extracellular space. Cell–cell adhesion between two like cells is called homotypic cell adhesion, while heterotypic cell adhesion takes place between two different cell types.

Introductory article

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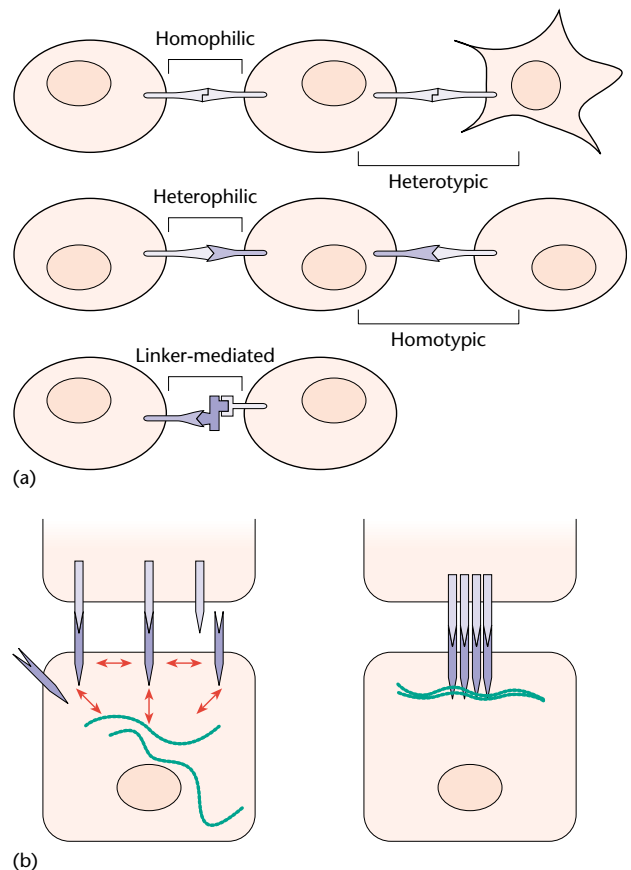


Figure 1 Different types of intercellular adhesion and mechanisms of strengthening. (a) Three mechanisms by which cell adhesion molecules can mediate intercellular adhesion. A cell surface molecule binds to an identical molecule (homophilic) on the opposing cell or to another adhesion receptor (heterophilic). Alternatively, cell adhesion receptors on two neighbouring cells bind to the same multivalent secreted ligand (linker-mediated adhesion). Independently of the adhesion molecules involved, intercellular adhesion can take place between identical cell types (homotypic) or between cells of different origin (heterotypic). (b) Intercellular adhesion is strengthened by intracellular linkage to the cytoskeleton and by lateral clustering.

Identification of adhesion molecules

To study adhesion in a population of cells, we first have to dissociate the cells either by interfering with the chemical content of the environment (e.g. removing calcium ions) or by adding proteases or other enzymes (e.g. lipases, glycosidases) that remove adhesion molecules from the surface or disrupt the binding interface between two adhesion molecules. When the environment is then restored to its original state or when the disrupting enzymes are removed or neutralized, cells will restore boundaries and reaggregate to each other. The first adhesion molecules were identified by using isolated membrane fractions of adhesive cells as immunogen and screening the generated antibodies for their abilities to block reaggregation of cells. These antibodies were then used to purify and characterize the adhesion molecules against which they were generated. Another successful approach was to correlate specific proteolytic treatments (e.g. low trypsin in the presence of high calcium) that disrupt adhesion of certain cells, with the disappearance of specific membrane proteins from the cell surface.

Biochemical and biophysical characterization

Studies on cell adhesion molecules were advanced considerably by the cloning of their genes and the use of recombinant DNA technologies. This allows the production and purification of large quantities of the extracellular portions of adhesion molecules that can then be coupled to a substrate or beads to study the binding characteristics of these molecules. By doing these assays with only fragments of the adhesion molecules, the specific binding sites can be mapped, allowing the generation of synthetic neutralizing peptides that disrupt intercellular adhesion through competition. Production of large quantities of portions of soluble adhesion molecules also permits the investigation of their molecular structure by nuclear magnetic resonance (NMR). Similarly, crystallization and X-ray analysis of these extracellular fragments further allows the clarification of the molecular organization of the adhesion molecules and the atomic resolution of their binding interfaces. These types of studies contributed greatly to our current understanding of how the different adhesion molecules interact structurally and how, for instance, ions like calcium and magnesium induce conformational changes that influence the activity of the adhesion molecules.

Characterization *in vivo*

The biochemical and biophysical studies on adhesion molecules mentioned above need to be further complemented *in vivo* by investigating cultured cell lines, organ cultures or whole organisms. Besides neutralization studies with antibodies and peptides, two major strategies are used

to study genuine or potential adhesion molecules. In the first approach, exogenous expression of the genes encoding adhesion molecules in nonadhesive cells should induce their adhesion to the same cells (when the adhesion molecules are homophilic) or to cells expressing the receptor of the adhesion molecule (in the case of heterophilic cell adhesion). By introducing genes that have either point mutations or small deletions, the importance of single amino acids or limited domains for sustaining intercellular adhesion can be verified. In the second approach, we try to abrogate intercellular adhesion of adhesive cells or tissues by expressing dominant-negative variants of the adhesion molecule or by reducing or eliminating the expression of the endogenous adhesion molecule. This can be done by overexpressing antisense RNA, or by using DNA technology to create a cell line or a whole organism in which the gene in question has been mutated or genetically disrupted by homologous recombination. Of course, this approach will only work when a single adhesion mechanism is responsible for keeping the cells together. Nevertheless, with both criteria fulfilled (induction of intercellular adhesion when expressed in nonadhesive cells, and inhibition of cell–cell adhesion in normally adhesive cells upon interference with its expression or function), the molecule in question is a major candidate for being involved in cell adhesion. However, this does not necessarily mean that it is an adhesion molecule itself. It could, for instance, encode a signalling receptor or ligand that directly or indirectly influences the activity of the adhesion molecule(s) proper. Therefore, it is essential to integrate these *in vivo* experiments with biochemical and biophysical studies and vice versa.

Adhesion molecules can be associated with the cell membrane either by a glycosylphosphatidylinositol (GPI) anchor or by a membrane-spanning region. In the latter case the cytoplasmic part of the molecule often associates indirectly with components of the cytoskeleton (e.g. actin, intermediate filaments, submembranous cortex). This implies that adhesion molecules, which themselves establish the extracellular contacts, can be structurally integrated with the cytoskeleton and are often clustered in specific restricted areas in the membrane, the so-called junctional complex. This combination of linkage to the cytoskeleton and clustering can considerably strengthen the adhesive force of the adhesion molecules (**Figure 1b**). Another consideration we have to take into account is that expression of an adhesion molecule in a cell does not always guarantee its function. On the one hand, adhesion molecules can be stored in intracellular vesicles until they are required at the cell surface. On the other hand, exposed adhesion molecules can be in a conformation that does not support binding to its adhesion receptor. A signal within the cell can then induce a conformational change that activates the adhesion molecule. All these regulatory mechanisms allow a dynamic process of cell adhesion, which is required, for instance, during morphogenesis in

development, and during immunological defence (see later).

Adhesive Interactions between Unicellular Organisms

While differential cell adhesion has its most dramatic effects and consequences in higher metazoa, it also defines the step from protozoan to metazoan life. Protozoa are in general unicellular although some can form colonies. All prerequisites to sustain life are present in such an organism. It has a functional barrier with the environment but nevertheless needs to interact with it, most notably to import chemical compounds; these are the building blocks for creating the molecular structures that make up the organism and may also be used for energy metabolism. This allows the organism to grow and multiply. The only time that cell adhesion *per se* is required in these organisms is during sexual reproduction when the gametes need to bind to each other and fuse.

In contrast to the definition, some primitive, often temporary interactions are made between unicellular organisms. Although the first organism that comes into mind when one thinks of multicellular protozoa is *Volvox*, it is a bad example since its multicellularity results from incomplete separation of the cells after cell division. Hence, the different cells are connected by cytoplasmic bridges. Genuine intercellular adhesion is, for instance, observed in the slime mould *Dictyostelium discoideum*. Because of their relative simplicity, these organisms have been the subject of many experiments studying cell–cell adhesion. I will use the example of *Dictyostelium* to illustrate the application of the different approaches to studying cell adhesion molecules.

The developmental saga of *Dictyostelium*

The developmental life cycle of the unicellular slime mould *Dictyostelium discoideum* is very peculiar. During vegetative growth, where cells divide regularly, the free living amoeboid cells feed on bacteria. When food becomes scarce, a developmental cycle is initiated. In the first hours the molecular content of the cell surface changes and finger-like extensions called filopodia are formed. When certain cells start to secrete cyclic AMP (cAMP), neighbouring cells are attracted and move towards the source of the chemoattractant. Cells will then aggregate through the involvement of cell–cell adhesion sites and are covered by a cellulose-protein sheath. This results in the formation of a slug in which patterning takes place. Prestalk and prespore cells become oriented in the anterior and the posterior end of the slug, respectively. Eventually the slug forms a fruiting body, a plant-like structure attached to the surface via a stalk and a footplate. The fruiting body contains the

spores, which can survive through hostile conditions for very long periods of time.

A prototype study on cell adhesion

During the first 8 hours of development, the *D. discoideum* cells adhere to each other by a Ca^{2+} -dependent mechanism involving a cell adhesion molecule called gp24. During the real aggregation stage of development, a Ca^{2+} -independent mechanism comes into play that involves several adhesion molecules, most notably gp80. These molecules were first identified by using purified fractions of *D. discoideum* to generate and purify antibodies that can interfere with Ca^{2+} -dependent and Ca^{2+} -independent aggregation. These antibodies were then used to isolate the adhesion molecules. Although this approach strongly suggests that gp80 is the adhesion molecule responsible for Ca^{2+} -independent adhesion, blocking cell adhesion with antibodies has the disadvantage that it might result from an indirect effect. The antibody may block adhesion because it binds to gp80 but inhibits the activity of the nearby real adhesion molecule, for instance, by steric hindrance or signalling. Consequently, other approaches were used to establish the role of gp80 as an adhesion molecule. (i) Purified soluble gp80 was labelled with ^{125}I and shown to bind to the surface of *D. discoideum* in a dose-dependent and saturable way. (ii) Beads conjugated with gp80 also bound to aggregation-stage *D. discoideum* cells. In addition the beads bound to each other, indicating that gp80-mediated adhesion is homophilic. In agreement with this, purified ^{125}I -labelled gp80 specifically bound to gp80 immobilized on nitrocellulose membranes. (iii) cDNA encoding gp80 was isolated and transformed into cells under control of a constitutive promoter. These cells then ectopically expressed gp80 in their vegetative growth phase and showed Ca^{2+} -independent aggregation. (iv) Transformed *D. discoideum* cells in which the gp80 gene was eliminated by homologous recombination showed a reduction in Ca^{2+} -independent adhesion.

Further biochemical studies were carried out to identify the gp80 homophilic interaction site. For this purpose, fusion proteins expressing different segments of gp80 were expressed in bacteria and assayed for cell binding activity, identifying the homophilic binding site within a stretch of 50 amino acids. Further competition experiments with small synthetic peptides recognized a YKLVNDS octapeptide sequence as the unique gp80 interaction site.

Cell Adhesion and the Evolution of Metazoans

Why evolution goes multicellular

Unicellular organisms have few limitations in colonizing new habitats under sometimes difficult conditions and they

probably represent the most versatile type of cell. Each unicellular organism has to be able to execute an enormous number of tasks: digest and absorb nutrients, sense the environment and move into it, sometimes actively catch prey, metabolize energy, reprogramme genes according to need, produce gametes, and so on. Despite this success, evolution has allowed the generation of multicellular organisms. The selective advantage of multicellular organisms is the ability of the different cells in the organism to specialize and cooperate. This allows them to significantly increase in size and exploit new resources. The fundamental basis for multicellular organization is that cells need to make stable contacts. For this purpose they need specific adhesion molecules.

Multicellularity calls for cell adhesion molecules

The most primitive metazoans are the sponges or *Porifera*. The sponges consist of a coherent multicellular sheet: a primitive epithelium. When these cells are mechanically separated by passing them through a sieve, they will spontaneously reaggregate and reassemble into an intact sponge. This behaviour is mediated by adhesion molecules. The specificity of the cell adhesion in sponges is illustrated when cells of different species are intermingled or pieces of two species are grafted onto each other. The reaggregated cells will sort out according to their species-origin. This allorecognition is based on cell–cell adhesion and appears to be, at least partially, mediated by cell surface proteoglycans. The underlying mechanism seems to be of the type where two surface receptors bind to the same extracellular linker, in this case a proteoglycan called aggregation factor. Interestingly, differential self-recognition through cell adhesion may have been a driving force in the divergence of species, at least in these early metazoans. As soon as two populations are unable to physically interact they will rapidly diverge from each other through genetic drift.

Next in the evolutionary ladder come the coelenterates, which include the jellyfish, the anemones, corals and a well-studied organism, *Hydra*. These organisms have two layers of epithelium: the ectoderm on the outside and the endoderm on the inside. The latter surrounds a cavity, the coelenteron, in which food is digested. Interestingly, the cells in the ectoderm form very tight cell–cell junctions that establish a structural barrier sealing the inside of the organism from the outside world. In this way it performs almost the same task as the cellular membrane in bacteria and protozoa. Certainly, new molecules come into play to fulfil this new and demanding designation. Another characteristic that is first observed in coelenterates is the formation of a simple nervous system. This also demands the formation of specific cell–cell contacts, and hence the expression of particular adhesion molecules.

Generation of complexity in organism requires dynamic cell adhesion

A next step in evolution is the formation of an additional layer of cells, the mesoderm that lies between the endoderm and the ectoderm. The mesoderm originates from the physical separation of cells either from the ectoderm or from the endoderm and, depending on the organism, is achieved by several morphogenetic processes: invagination, involution, epiboly, delamination and ingression. Whatever the exact mechanism for mesoderm formation, it is always accompanied by a change in cell adhesion. Proper mesoderm formation is first observed in the flatworms.

Further evolution is characterized by the formation of cavities, organization of cells in tissues, generation of a highly diversified neural network and a specialized immune system. All these new properties require the integration of cell adhesion. The major classes of cell adhesion molecules that are used in higher vertebrates are already observed early during metazoan evolution. However, the more complex the organism becomes, the more differential cell adhesion is required and the more new adhesion molecules are generated, mostly through gene duplication. Besides the formation of more adhesion molecules, embryos also use the same molecules at different times during development and for entirely different purposes. The involvement of cellular interactions in embryonic development will be discussed in more detail in the final section.

Adhesion Molecules in Multicellular Organisms

Now we have an idea of what cell adhesion involves and how it plays a role in animal evolution. The realization that the same families of adhesion molecules are being used by organisms as diverse as humans and insects or nematodes prompts us to give these molecules a face and a name. Based on their molecular structure and mode of interaction, five classes of adhesion molecules are generally distinguished: the immunoglobulin (Ig) superfamily, the cadherins, the integrins, the selectins and the proteoglycans (**Figure 2**). These are discussed in more detail below, but the limited extent of a single article necessitates some major generalization.

The Ig superfamily

Of all the classes of adhesion molecules discussed here, the immunoglobulin superfamily is probably the most diverse. The most prominent representatives are the NCAMs, L1, Po, CD2 and the VCAMs. As the name suggests, the members of this family all contain an extracellular domain consisting of different immunoglobulin-like domains (**Figure 2**). Interestingly, genes encoding Ig-like adhesion

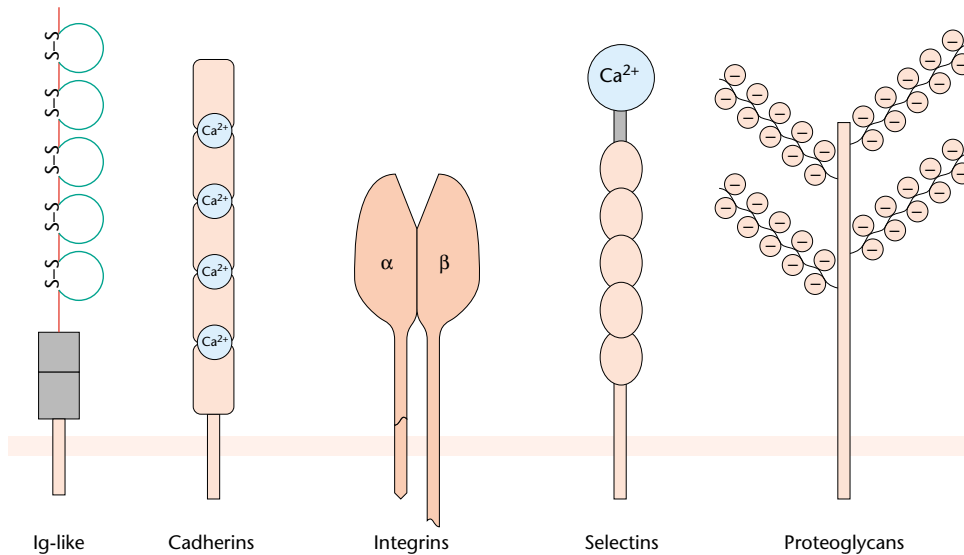


Figure 2 The five major classes of cell adhesion molecules. The immunoglobulin superfamily (Ig-like) is characterized by a various number of immunoglobulin-like domains (open circles) and more membrane-proximal, often fibronectin type III repeats (grey boxes). Cadherins are Ca^{2+} -dependent adhesion molecules consisting of a varying number of cadherin repeats (five in case of the classical cadherins) whose conformation is highly dependent on the presence of Ca^{2+} -ions. Integrins are functional as heterodimers consisting of an α and β subunit. Selectins contain an N-terminal Ca^{2+} -dependent lectin domain (circle), a single EGF-like repeat (grey box) and a number of repeats related to those present in complement-binding proteins (ovals). Proteoglycans are huge molecules consisting of a relatively small protein core to which long side-chains of negatively-charged glycosaminoglycans are covalently attached. See text for details.

molecules have already been isolated from the lowest metazoan organisms, the sponges.

The neural cell adhesion molecules (NCAMs) and L1 and the *Drosophila* orthologue, neuroglian, sustain homophilic and heterophilic interactions that play a central role in regulation and organization of neural networks, specifically in neuron–target interactions and in fasciculation. The basic extracellular structure consists of a number of Ig-domains responsible for the homophilic interaction, followed by a discrete number of fibronectin type III repeats. This structure is linked to the membrane, either by a GPI-anchor or a transmembrane domain. A peculiar cell–cell adhesion molecule is Po. It is localized in the myelin sheaths of nerve cells and is responsible for holding the individual wraps together. Hence, one could consider Po to be an autotypic cell adhesion molecule. It contains a single Ig-domain (which can form tetramers) and a transmembrane and a cytoplasmic domain.

The V(ascular)CAM subgroup, including the I(nter-cellular)CAMs and the mucosal vascular addressin adhesion molecule (MAdCAM), are involved in leukocyte trafficking (or homing) and extravasation. They consist of membrane-linked Ig-domains that form heterophilic contacts with integrins (see below). CD2 molecules are found on cytotoxic and helper T cells and enhance their binding to antigen-presenting cells. CD2 binding is pseudohomophilic, to highly homologous adhesion receptors.

The cadherins

Cadherins and protocadherins also form a very large and diverse group of adhesion receptors. They are Ca^{2+} -dependent adhesion molecules, involved in various adhesive events in both the embryo and the adult. Cadherins play most fundamental roles in the metazoan embryos, from the earliest gross morphogenetic events (e.g. the separation of the germ layers during gastrulation) to the most delicate tunings later in evolution and/or development (e.g. the molecular wiring of the neural network). The vertebrate classical cadherins are the best studied. Their extracellular part contains a number of cadherin repeats whose conformation is highly dependent on the presence or absence of calcium ions (Figure 2). Only in the presence of calcium can homophilic interactions be made, usually by the most distal cadherin repeat. Invertebrate cadherins contain some extra domain structures proximal to the cadherin repeats but it is presently not clear what the function of these domains is. Classical cadherins are generally exposed as homodimers and their cytoplasmic domain is tightly associated with the actin cytoskeleton. It is not surprising that cadherins are the major adhesion molecules in tissues that can come under high mechanical stress, such as epithelia (E-cadherin) and endothelia (VE-cadherin). Recently, however, cadherins have also been implicated in more refined and elegant intercellular interactions such as synaptic contacts.

Integrins

The integrins are another group of important players in the field of cell adhesion both in the embryo and in the adult. They are involved in various processes such as morphogenesis and tissue integrity, haemostasis, immune response and inflammation. Integrins are a special class of adhesion molecules, not only because they can mediate both cell–cell and cell–substrate interactions (with components in the extracellular matrix (ECM) like laminin, fibronectin and collagen) but also because they are functional as heterodimers consisting of an α and a β subunit (Figure 2). To date, 16 α subunits and 8 β subunits are known and of the theoretical 128 heterodimeric pairings at least 21 are known to exist. While most integrin heterodimers bind to ECM components, some, most notably those expressed on leukocytes, are heterophilic adhesion molecules binding to members of the immunoglobulin superfamily (see earlier). The α subunit mostly contains the ligand-binding domain and requires the binding of divalent cations for its function (Mg^{2+} , Ca^{2+} , Mn^{2+} , depending on the integrin). Integrins can be present on the cell-surface in a nonfunctional and a functional configuration and the cytoplasmic domain appears to be responsible for the conformational change activating the integrin.

Selectins and proteoglycans

These types of adhesion molecules depend on carbohydrate structures for their adhesive interaction. Selectins have a C-type lectin domain that can specifically bind to discrete carbohydrate structures present on cell surface proteins (Figure 2).

Intercellular interactions mediated by selectins are of particular interest in the immune system, where they play fundamental roles in trafficking and homing of leukocytes (see later). Proteoglycans are very big extracellular proteins consisting of a relatively small protein core to which long chains of glycosaminoglycans are attached. Although poorly documented, proteoglycans may bind to each other or may be the attachment sites for other adhesion molecules.

Cellular Interactions in the Immune System

Dynamic cellular interactions play a cardinal role in the immune defence of a metazoan organism. While more and more has been learned in recent years about the immune system of lower metazoa, most of the research has focused on higher vertebrates and the mammalian immune system in particular has been extensively studied. Interestingly, almost all the classes of adhesion molecules discussed earlier are somehow involved in the immune system.

Migratory behaviour of leukocytes

To be able to fulfil their immense task, leukocytes migrate through the body and specifically traffic and home to the sites where they are needed. In general, three forms of migration are observed in the immune system. (i) Dendritic antigen-presenting cells are found in peripheral organs like the skin and the intestinal epithelia, where they make strong contacts with the surrounding cells and the ECM. When they capture and process antigens, they become highly mobile and migrate to the lymphoid organs, where they present their antigens to the lymphocytes. This migratory behaviour requires a change in cell adhesion molecules, e.g. reduction of E-cadherin expression in the case of Langerhans cells. (ii) T and B lymphocytes patrol the body, scanning for infectious pathogens, and for this purpose they constantly circulate in the vascular and lymphatic compartments. Lymphocytes can leave the blood vessels at the lymph nodes, where they are confronted with the antigen-presenting cells, after which they proliferate and differentiate, traverse the lymphatic system and then return to the vascular system. (iii) Granulocytes and monocytes circulate in the blood and extravasate into the surrounding tissue in response to inflammatory stimuli.

Leukocyte migration involves a highly regulated adhesive mechanism, also known as the ‘multistep adhesion cascade’. For simplicity, only the homing and extravasation of neutrophils will be discussed, but the mechanism can easily be extrapolated to leukocyte trafficking.

The multistep adhesion cascade

The multistep adhesion cascade starts with the selective and local expression of selectins on the cells of the vessel wall in response to inflammatory stimuli. These selectins can bind to carbohydrates expressed on the neutrophils that pass by in the bloodstream. This interaction, known as tethering, is of low affinity and is transient and easily disrupted by the continuous blood flow. As a result, the neutrophils roll along the surface of the endothelium. The neutrophils express integrins on their cell surface, but these are in a nonfunctional state. However, chemokines released from the endothelial cells on which the neutrophils are rolling induce a G protein-mediated conformational change in the integrins of the neutrophil. As a result, these integrins are activated and can bind their targets, which are ICAMs on the endothelial cells. The neutrophils are arrested, attach firmly to the endothelium, and migrate through it. If we consider that passage through the endothelial cell layer requires VE-cadherin-mediated cell–cell contacts to be disrupted, we can see that this ‘multistep adhesion cascade’ of leukocytes involves all the major families of adhesion molecules discussed earlier.

Other adhesive interactions in the immune system

Other adhesive interactions are involved in the immune system. It is found, for instance, that immature thymocytes require intimate interactions with the epithelial cells in the thymus. These contacts seem to be established by homophilic but heterotypic E-cadherin adhesion. Interestingly, E-cadherin can also form heterophilic interactions with a $\alpha_E\beta_7$ integrin on certain T lymphocytes. Aggregation of platelets also involves adhesion receptors; in this case members of the integrin family. These integrins need to be activated by agonists like thrombin in order to induce effective adhesion. Finally, T cells interact with antigen-presenting cells through binding of the T-cell receptor with the antigen–MHC complex. However, this binding is of very low affinity. Efficient interaction between the T cell and the antigen-presenting cell requires the cooperation of adhesion molecules, most likely a heterophilic interaction between an integrin and ICAM-1 or a pseudohomophilic interaction between CD2 adhesion molecules (see earlier).

Adhesive Cell Interactions Involved in Embryonic Development

The first studies on cell adhesion in embryos date from long before the identification of adhesion molecules. Pioneering studies with dissociated sponges later provided the basis for reconstitution experiments with tissues from vertebrates. These studies mostly dealt with sorting and segregation of cells. Later, when the existence of adhesion molecules began to be realized and workers learned how to manipulate them, several other adhesive processes in the embryo came under close investigation. Again the format of a single article does not allow much detail, but some fundamental morphogenetic processes that are observed throughout development and in which adhesion is involved will be mentioned.

Tissue separation and sorting out

It has long been observed that when tissues from different origins are dissociated, intermingled and allowed to reaggregate, they will eventually sort out from each other. Separation of tissues is often observed in development, for instance in the early embryo when the neural plate separates from the ectoderm to form the neural tube (Figure 3a). It is now clear that the molecular mechanism underlying this separation is differential cell adhesion. Sorting out, or tissue separation, is a result of either quantitative or qualitative differences in cell adhesion. Quantitative differences in cell adhesion imply that two populations of cells express the same adhesion molecule but in different amounts. When qualitative differences are

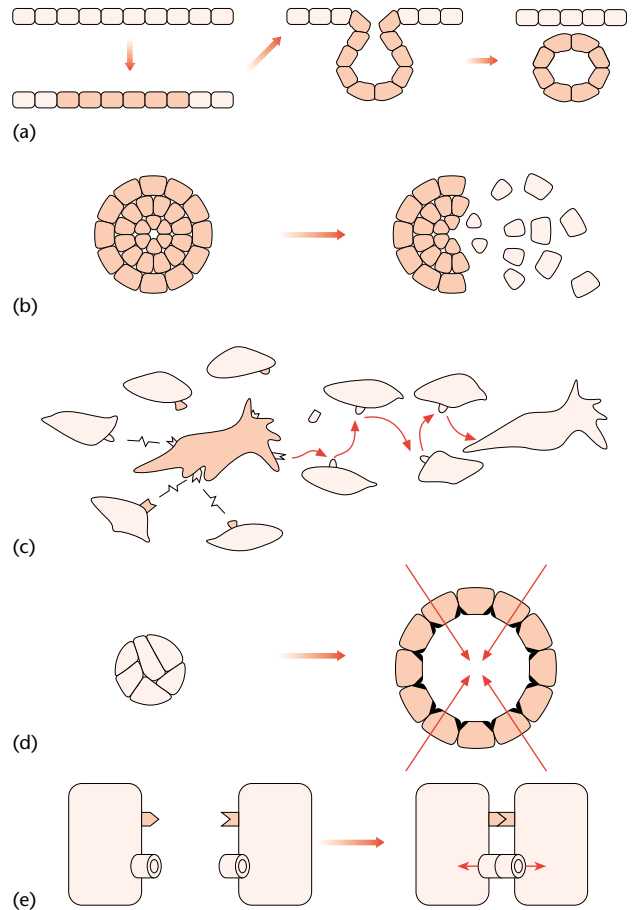


Figure 3 Embryonic processes involving cell adhesion. (a) Segregation of tissues during neural tube formation resulting from different expression of adhesion molecules (light pink versus dark pink cells). (b) Dispersion of cells from a solid tissue resulting from a decrease in cell–cell adhesion. (c) Migration of cells along adhesive guidance cues. (d) Cavity formation requires a combination of intercellular sealing by tight junctions (black triangle) and vectorial ion and water transport (arrows). (e) Cell–cell communication through gap junctions (barrels) is dependent on cell–cell adhesion. See text for details.

involved, the two cell populations express different types of adhesion molecules. The principle of sorting out was nicely demonstrated when cells were transfected with the homophilic adhesion molecule cadherin and grown in suspension. Cells expressing E-cadherin separated from cells expressing N-cadherin. Similarly, cells expressing low amounts of E-cadherin sorted out from cells expressing high amounts of the same molecule. Remarkably, in the embryo, when the neural tube separates from ectoderm, it is found that the first cell layer expresses N-cadherin and the latter E-cadherin.

Dispersion and condensation

In the embryo it is often observed that cells disperse from a coherent tissue and migrate away from it. This process is usually accompanied by reduced expression of cell–cell adhesion molecules and increased cell–substrate adhesion. Sometimes, a complete area of tissue can undergo such an epithelial–mesenchymal transition and disperse into the surroundings (**Figure 3b**). Interestingly, the opposite is also frequently observed in the embryo; this is called cell condensation, in which loose cells increase their inter-cellular adhesion and start to form aggregates. Such processes are very often under control of secreted factors that change the cellular behaviour. During kidney formation, the epithelial ureteric bud induces the surrounding mesenchyme to condense and form an epithelial structure, the collecting duct. Other examples where condensation is observed include formation of somites, bones and hair follicles.

Guided migration and target recognition

At different points in development, cells migrate away from where they originated and colonize new areas. This is a highly regulated process and on their migratory path cells such as neural crest cells are guided by several environmental cues that keep them on the right track. These guidance cues can be locally secreted signalling molecules but also factors in the ECM and on the surface of cells encountered on route (**Figure 3c**). The migrating cells need to make several adhesive contacts, but these have to be temporary. A similar phenomenon is observed when neuronal axons migrate through tissues in search of their target (e.g. a muscle cell). Once the migrating cell or the axonal growth cone has arrived on the right spot, migration has to stop. Again this is likely to involve an adhesive recognition event between two cells or with a matrix component.

Cavity formation

It is clear that the animal body does not consist of a solid mass of cells. An essential feature of all higher organisms is the presence of cavities, e.g. the blood vessels, the ducts in the kidneys, the mammary gland, the lungs. The cavities are often generated by directional secretion of fluids and as such require that the epithelial-like cell layer that borders the cavity be perfectly sealed (**Figure 3d**). This demands a unique adhesive structure, the tight junction, where specialized adhesion molecules bring the membranes of bordering cells into very close proximity.

Cell-to-cell communication

A less structural or morphogenetic aspect of cell adhesion that is nevertheless of cardinal importance during embry-

ogenesis is the direct communication between two neighbouring cells. This includes juxtacrine signalling, which involves an interaction of a cell surface receptor on one cell with a ligand bound to the cell membrane of another cell. Because this type of signalling requires the two interacting cells to be in close proximity, and since the receptor–ligand binding by itself is not of sufficient strength, juxtacrine signalling is greatly aided by the function of adhesion molecules expressed on the two cells. Also in gap junctional communication, where neighbouring cells form cytoplasmic contacts through special proteins called connexins, cell membranes have to be brought into close contact by use of adhesion molecules (**Figure 3e**). A similar intimate contact between cell membranes is found at the synapse when neurons make contact with their target cells. This is again achieved with the aid of cell adhesion molecules that also serve to seal the synaptic cleft. Finally, there is also increasing evidence that adhesion molecules can signal themselves, but this goes beyond the scope of this article.

Summary

Cell adhesion defines the step from unicellular to multicellular life in the animal kingdom. We have seen how cell adhesion is studied and how specific adhesion molecules were identified, isolated and characterized. This has led to the realization that a limited number of cell adhesion molecules originated early in metazoan evolution. Through gene duplication and divergence, each family of adhesion molecules has expanded considerably in the different organisms. With the generation of more complexity in the course of evolution, the functional requirements for the adhesion molecules have become more diverse and demanding. This has resulted in higher specialization and dynamic functional regulation of the various adhesion molecules. Cell adhesion is an essential and integral component of many biological processes, ranging from morphogenesis, growth, neural development and immunological defence, to wound healing and fertilization.

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