1. Morphogenesis and cell adhesion-the thermodynamic model of cell interactions,
2. Concept of morphogen gradients ;a nd
3. Morphogenetic fields,
4. Cell adhesion molecules

Prepared By

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The formation of organized animal bodies is called *morphogenesis.*

How can matter alone construct the organized tissues *at* the embryo?

Following are the five questions that confront modern embryologists who study morphogenesis:

1. *How are separate tissues formed from populations of cells?* (For example, how do neural retina cells stick to other neural retina cells rather than becoming part of the pigmented retina or the iris cells next to them? How are the different cell types found in the retina (the three distinct layers of photoreceptors, bipolar neurons, and ganglion cells) arranged such that the retina is functional?)

2. *How are organs constructed from tissues?* (The retina of the eye forms at a precise distance behind the cornea and the lens. The retina would be useless if it developed behind a bone or in the middle of the kidney. Moreover, neurons from the retina must enter the brain to innervate the regions of the brain cortex that analyze visual information. All these connections must be precisely ordered.)

3. *How do organs form in particular locations, and how do migrating cells reach theirdestinations?* (What causes there to be two—and usually only two—kidneys, and how do their ducts form so that they can collect urine made by the filter-ing tissues of the nephron? Some cells—for instance, the precursors of our pigment cells, germ cells, and blood cells—must travel long distances to reach their final destinations. How are cells instructed to travel along certain routes in our embryonic bodies, and how are they told to stop once they have reached their appropriate destinations?)

4. *How do organs and their cells grow, and how is their growth coordinated throughout development?*

(The cells of all the tissues in the eye must grow in a coordinated fashion if one is to see. Some cells, including most neurons, do not divide after birth. In contrast, the intestine is constantly shedding cells, and new intestinal cells are regenerated each day. The mitotic rate of each tissue must be carefully regulated. If the intestine generated more cells than it sloughed off, it could produce tumorous outgrowths. If it produced fewer cells than it sloughed off, it would soon become nonfunctional. What controls the rate of mitosis in the intestine?)

5. *How do organs achieve polarity?*

(If one were to look at a cross section of the fingers, one would see a certain organized collection of tissues—bone, cartilage, muscle, fat, dermis, epidermis, blood, and neurons. Looking at a cross section of the forearm, one would find the same collection of tissues. But they are arranged very differently. How is it that the same cell types can be arranged in different ways in different parts of the same structure, and that fingers are always at the end of the arm, never in the middle?)

In the 1850s, Robert Remak (1852,1855) formulated the cell theory and showed that the fertilized egg divides to produce "tiny sensitive bodies"—cells—needed to form an embryo. In the mid-twentieth century, E. E Just (1939) and Johannes Holtfreter predicted that embryonic cells could have differences in their cell membrane components which would enable die formation of organs. In the late twentieth century, these membrane components—the molecules by which embryonic cells adhere to, migrate over, and induce gene expression in neighboring cells—began to be discovered and described. And presently, these pathways are being modelled to understand how the cell integrates the information from its nucleus and from its surroundings to take its place in the community of cells.

The cells of an embryo are either epithelial cells or mesenchymal cells. The epithelial cells can form tubes and sheets while remaining adhered to one another, whereas the mesenchymal cells often migrate individually and form extensive *extracellular matrices* that keep the individual cells separate.

CELL ADHESION

Differential cell affinity

The process of morphogenesis involve the properties of the cell surface. The cell surface looks pretty much the same in all cell types, and many early investigators thought that the cell surface was not even a living part of the cell. Each type of cell has a different set of proteins in its cell membrane, and that some of these differences are responsible for forming the structure of the tissues and organs during development. Observations of fertilization and early embryonic development made by E. E. Just (1939) suggest that the cell membrane differed among cell types, but **the** experimental analysis of morphogenesis began with the experiments of Townes and Holtfreter in 1955. Taking advantage of the discovery that amphibian tissues become associated into single cells when placed in alkaline solutions, they prepared single-cell suspensions from each of the three germ layers of amphibian embryos soon after the nrural tube had formed. Two or more of these single-cell suspensions could be combined in various ways. When the pH of the solution was normalized, the cells adhered to one another, forming aggregates on agar-coated petri dishes. By using embryos from species having cells of different sizes and colors, Townes and Holtfreter were able to follow the behavior of the recombined cells.

The results of their experiments were striking. First, they found that reaggregated cells become spatially segregated. That is, instead of two cell types remaining mixed, each type sorts out into its own region. Thus, when epidermal ectodermal) and mesodermal cells are brought together

in a mixed aggregate, the epidermal cells move to the periphery of the aggregate and the mesodermal cells move to the inside (Fig: 1). In no case do the recombined cells remain randomly mixed; in most cases, one tissue type completely envelops the other. Second, the researchers found that the final positions of the re aggregated cells reflect their respective positions in the embryo.

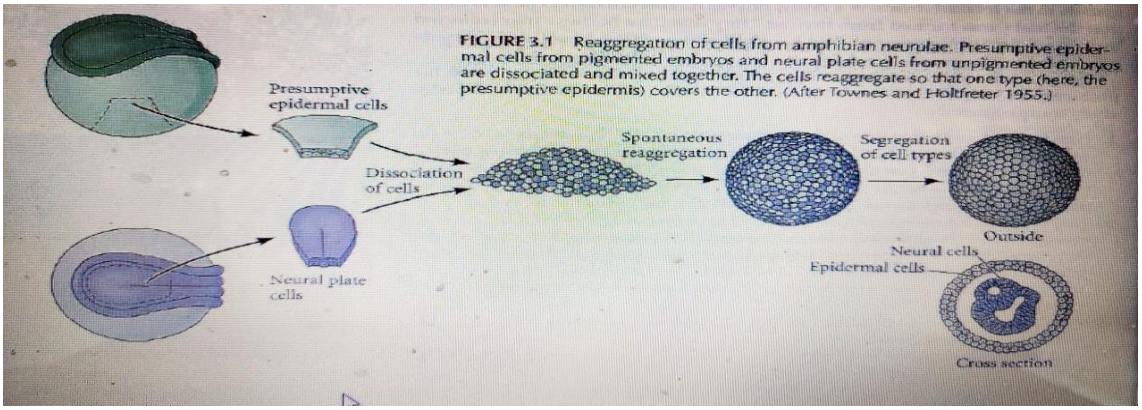

Fig: 1 **Reaggregation of cells from amphibian neurulae**

The reaggregated mesoderm migrates centrally with respect to the epidermis, adhering to the inner epidermal surface (Fig: 2A). The mesoderm also migrates centrally with respect to the gut or endoderm (Fig: 2B). However, when the three germ layers are mixed together, the endoderm separates from the ectoderm and mesoderm and is then enveloped by them (Fig: 2C). In the final configuration, the ectoderm is on the periphery, the endoderm is internal, and the mesoderm lies in the region between them. Holtfreter interpreted this finding in terms of selective affinity. The inner surface of the ectoderm has a positive affinity for mesodermal cells and a negative affinity for the endoderm, while the mesoderm has positive affinities for both ectodermal and endodermal cells. Mimicry of normal embryonic structure by cell aggregates is also seen in the recombination of epidermis and neural plate cells (Fig: 2D; also see Fig: 1). The presumptive epidermal cells migrate to the periphery as before; the neural plate cells migrate inward, forming a structure reminiscent of the neural tube. When axial mesoderm (notochord) cells are added to a suspension of presumptive epidermal and presumptive neural cells, cell segregation results in an external epidermal layer, a centrally located neural tissue, and a layer of mesodermal tissue between them (Fig: 2E). Somehow, the cells are able to sort out into their proper embryonic positions.

The third conclusion of Holtfreter and his colleagues was that selective affinities change during development. Such changes should be expected, because embryonic cells do not retain a single stable relationship with other cell types. For development to occur, cells must interact differently with other cell populations at specific times. Such changes in cell affinity are extremely important in the processes of morphogenesis. When tissues from later-stage mammalian and chick

embryos were made into single cell suspensions (using the enzyme trypsin, which split the proteins connecting the cells together), the cells reaggregated to form tissuelike arrangements.

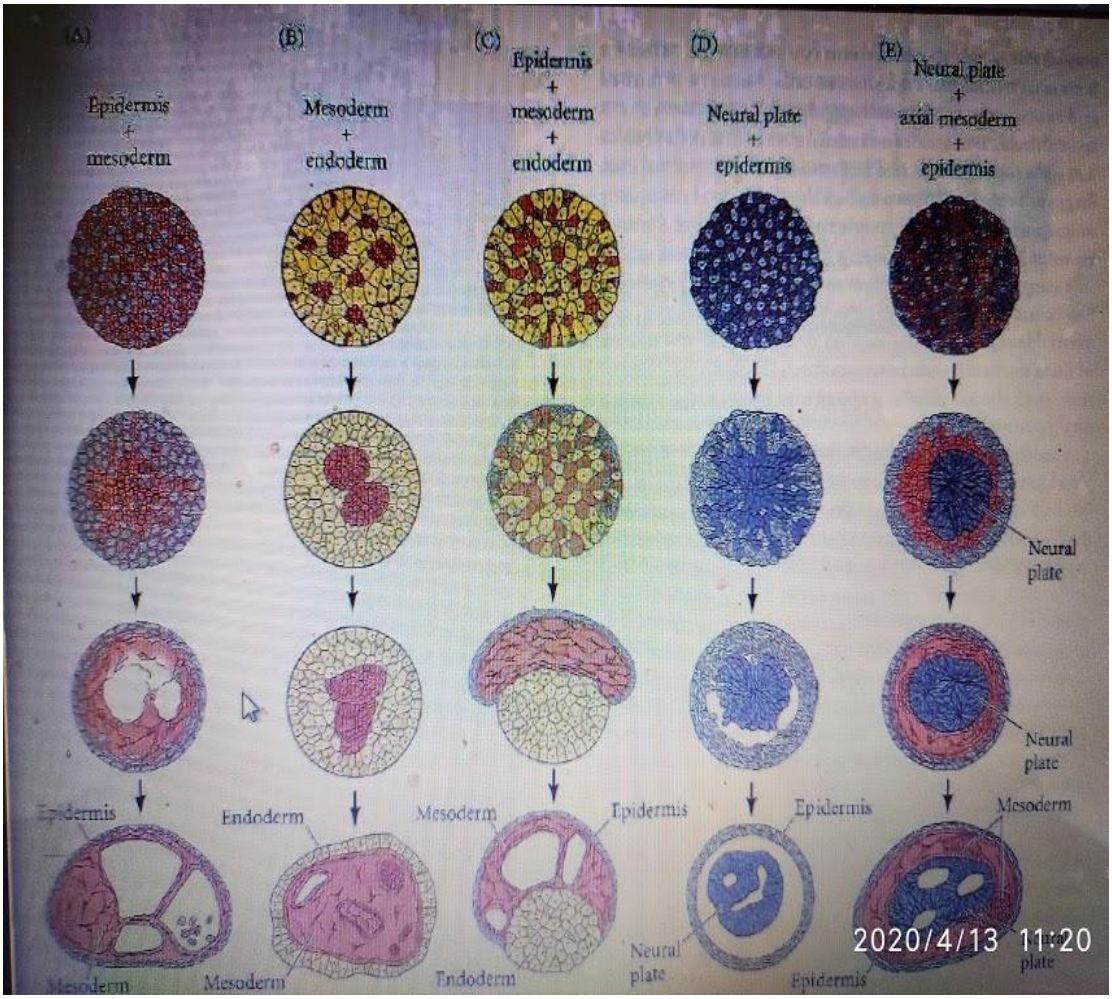

Fig 2A-B-C-D-E: **Sorting out and reconstruction of spatial relationships in aggregates of embryonic amphibian cells. (After Townes and Holtfreter 1955.)**

The thermodynamic model of cell interactions

Cells do not sort randomly, but can actively move to create tissue organization. There are certain forces that direct cell movement during morphogenesis. In 1964, **Malcolm Steinberg proposed the differential adhesion hypothesis,** a model that sought to explain patterns of cell sorting based on thermodynamic principles. Using cells derived from trypsinized embryonic tissues, Steinberg showed that certain cell types migrate centrally when combined with some cell types, but migrate peripherally when combined with others. **Fig:3** illustrates the interactions between pigmented retina cells and neural retina cells. When single cell suspensions of these two cell types are mixed together, they form aggregates of randomly arranged cells. However, after several hours, no pigmented retina cells are seen on the periphery of the aggregates, and after 2 days, two distinct layers are seen, with the pigmented retina cells lying internal to the neural retina cells. Moreover,

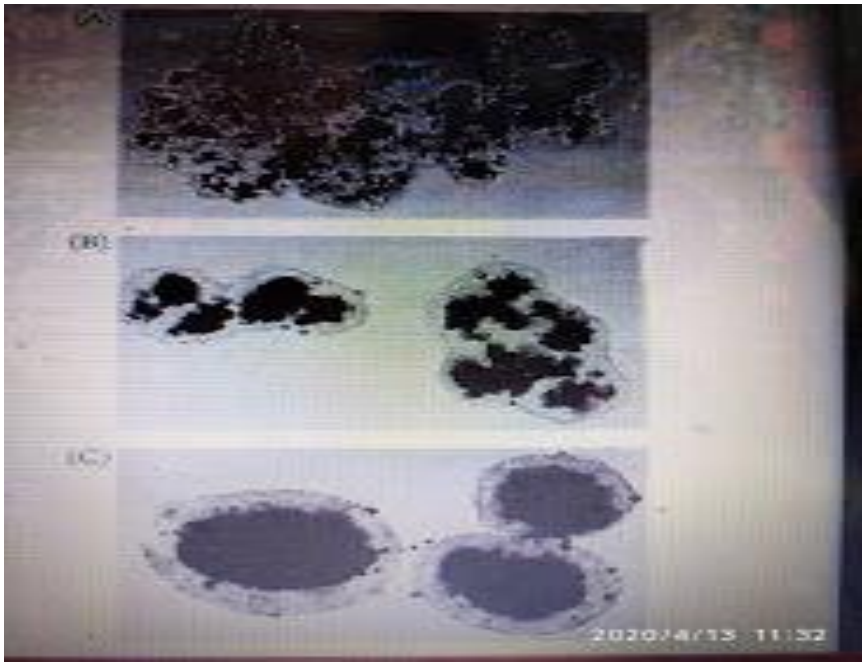

Fig 3: **Aggregates formed by mixing 7-day chick embryo neural retina (unpigmented) cells with pigmented retina cells.**
(A) Five hours after the single-cell suspensions are mixed, aggregates of randomly distributed cells are seen.
(B) At 19 hours, the pigmented retina cells are no longer seen on the periphery.
(C) At 2 days, a great majority of the pigmented retina cells are located in a central internal mass, surrounded by the neural retina cells. (The scattered pigmented cells are probably dead cells.) (From Armstrong 1989, courtesy of P. B. Armstrong.)

such interactions form a hierarchy . If the final position of cell type A is internal to a second cell type B, and the final position of B is internal to a third cell type C, then tire final position of A will always be internal to C. For example, pigmented retina cells migrate internally to neural retina cells, and heart cells migrate internally to pigmented retina cells. Therefore, heart cells migrate internally to neural retina cells.

This observation led Steinberg to propose that cells interact so as to form an aggregate with the smallest inter-facial free energy. In other words, the cells rearrange themselves into the most thermodynamically stable pattern. If cell types A and B have different strengths of adhesion, and if the strength of A-A connections is greater than the strength of A-B or B-B connections, sorting will occur, with the A cells becoming central. On the other hand, if the strength of A-A connections is less than or equal to the strength of A-B connections, then the aggregate will remain as a random mix of cells. Finally, if the strength of A-A connections is far greater than the strength of A-B connections— in other words, if A and B cells show essentially no adhesivity toward one another— then A cells and B cells will form separate aggregates. According to this hypothesis, the early embryo can be viewed as existing in art equilibrium state until some change in gene activity changes the cell surface molecules. The movements that result seek to restore the cells to a new equilibrium configuration. All that is required for sorting to occur is that cell types differ in the

strengths of their adhesion. In 1996, Foty and his colleagues in Steinberg's laboratory demonstrated that this was indeed the case: the cell types that had greater surface cohesion migrated centrally compared with those ceils that had less surface tension .In the simplest form of this model, all cells could have the same type of "glue" on the cell surface. The amount of this cell surface product, or the cellular architecture that allows the substance to be differentially distributed across the surface, could cause a difference in the number of stable contacts made between cell types. In a more specific version of this model, the thermodynamic differences could be caused by different types of adhesion molecules .When Holtfreter's studies were revisited using modem techniques, Davis and colleagues (1997) found that the tissue surface tensions of the individual germ layers were precisely those required for the sorting patterns observed both in vitro and in vivo.

The differential adhesion hypothesis

*Cadherins and cell adhesion (*Cell adhesion molecules)

Recent evidence shows that boundaries between tissues can indeed be created by different cell types having both different types and different amounts of cell adhesion molecules. Several classes of molecules can mediate cell adhesion, but the major cell adhesion molecules appear to be the cadherins. As their name suggests, cadherins are oilcium-ifependent adhesion molecules. They are critical for establishing and maintaining intercellular connections, and they appear to be crucial to the spatial segregation of cell types and to the organization of animal form. Cadherins are transmembrane proteins that interact with other cadherins on adjacent cells. The cadherins are anchored inside the cell by a complex of proteins called catenins (Fig: 4A), and the cadherin-catenin complex forms the classic adherens junctions that help hold epithelial cells together. Moreover, since the cadherins and the catenins bind to the actin (microfilament) cytoskeleton of the cell., they integrate the epithelial cells into a mechanical unit. Interfering with cadherin function (by univalent antibodies against cadherin or morpholinos against cadherin mRNA) can prevent the formation of tissues and cause the cells to disaggregate (Fig:4B;).

Cadherin proteins perform several related functions.

1. their external domains serve to adhere cells together.
2. cadherins link to and help assemble the actin cytoskeleton, thereby providing the mechanical forces for forming tubes.
3. cadherins can serve as signaling molecules that change a cell's gene expression.

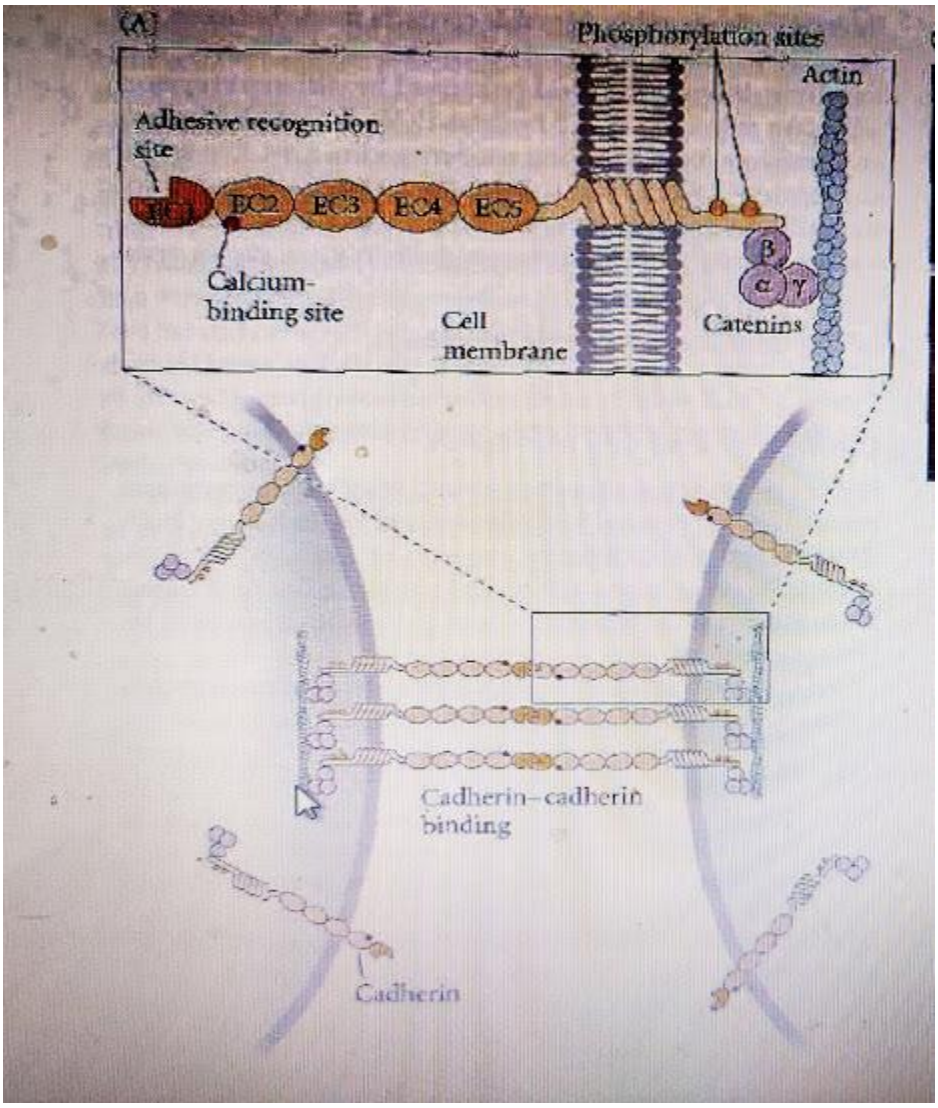

Fig 4: Cadherin-mediated cell adhesion.
(A) Simplified scheme of cadherin linkage to the cytoskeleton via catenins.
(B) When an oocyte is injected with an anlisensc oligonucleotide against a maternally inherited cadherin mRNA

In vertebrate embryos, several major cadherin types have been identified. E-cadherin is expressed on all early mammalian embryonic cells, even at the zygote stage. Later in development, this molecule is restricted to epithelial tissues of embryos and adults. P-cadherin is found predominantly on the placenta, where it helps the placenta stick to the uterus . N-cadherin becomes highly expressed on the cells of the developing central nervous system and it may play roles in mediating neural signals. R-cadherin is critical in retina formation . A class of cadherins called protocadherins lack the attachment to the actin skeleton through catenins. Expressing similar protocadherins is an important means of keeping migrating epithelial cells together; and expressing dissimilar protocadherins is an important way of separating tissues (as when the mesoderm forming the notochord separates from the surrounding mesoderm that will form somites).

Differences in cell surface tension and the tendency of cells to bind together depend on the strength of cadherin interactions . This strength can be achieved quantitatively (the more cadherins on the apposing cell surfaces, the tighter the adhesion) or qualitatively (some cadherins will bind to different cadherin types, whereas other cadherins will not bind to different types). The ability to sort cells based on the *amount* of cadherin was first shown when Steinberg and Takeichi (1994) collaborated on an experiment using two cell lines that were identical except that they synthesized different amounts of P-cadherin. When these two groups of cells, each expressing a different amount of cadherin, were mixed, the cells that expressed more cadherin had a higher surface cohesion and migrated internally to the lower-expressing group of cells. Foty and Steinberg (2005) demonstrated that this cadherin-dependent sorting directly correlated with the aggregate surface tension . The surface tensions of these aggregates are linearly related to the amount of cadherin they are expressing on the cell surface. The eel sorting hierarchy is strictly dependent on the cadherin interactions between the cells.

Moreover, the energetic value of cadherin-cadherin binding is remarkably strong—about 3400 kcal/mole, or some 200 times stronger than most metabolic protein-protein interactions. This free energy change associated with cadherin function could be dissipated by depolymerizing the actin skeleton. The underlying actin cytoskeleton appears to be crucial in organizing the cadherins in a manner that allows them to form remarkably stable linkages between cells

Qualitative interactions are also important. Duguay and colleagues (2003) showed, for instance, that R-cadherin and p-cadherin do *not* bind well to each other, and in these interactions the type of cadherin expressed becomes important. In another example, the expression of N-cadherin is important in separating the precursors of the neural cells from the precursors of the epidermal cells. All early embryonic cells originally contain E-cadherin, but those cells destined to become the neural tube lose E-cadherin and gain N-cadherin. If epidermal cells are experimentally made to express N-cadherin, or if N-cadherin synthesis is blocked in the prospective neural cells, the border between the nervous system and skin fails to form properly.

The timing of particular developmental events can also depend on cadherin expression. For instance, N-cadherin appears in the mesenchymal cells of the developing chick leg just before these cells condense and form nodules of cartilage (which are the precursors of the limb skeleton). X-cadherin is not seen prior to condensation, nor is it seen afterward. If the limbs are injected just prior to condensation with antibodies that block N-cadherin, the mesenchyme cells fail to condense and cartilage fails to form. It therefore appears that the signal to begin cartilage formation in the chick limb is the appearance of N-cadherin. During development, the many cadherins often work with other adhesion systems- For example, one of the most critical times in a mammal's life occurs

soon after conception, as the embryo passes from the oviduct and enters the uterus. If development is to continue, the embryo must adhere to and embed itself in the uterine wall. That is why the first differentiation event in mammalian development distinguishes the trophoblast cells (the outer cells that bind to the uterus) from the inner cell mass (those cells that will generate the embryo and eventually the mature organism). This differentiation process occurs as the embryo travels from the upper regions of the oviduct on its way to the uterus.

Trophoblast cells are endowed with several adhesion molecules that anchor the embryo to the uterine wall.

First, they contain both E- and P-cadherins and these two molecule types recognize similar cadherins on the uterine cells.

Second, they have receptors (integrin proteins) for the collagen and the heparan sulfate glycoproteins of the uterine wall ;

Third, trophoblast cell surfaces have a modified glycosyltransferase enzyme that extends out from the cell membrane and can bind to specific carbohydrate residues on uterine glycoproteins.

For something as important as the implantation of the mammalian embryo, it is not surprising that several cell adhesion systems appear to work together.

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