

Mechanisms of Compaction and Formation of the Inner Cell Mass

Compaction creates the circumstances that bring about the first differentiation in mammalian development: the separation of trophoblast from inner cell mass. How is this done? There is growing evidence that compaction is mediated by events occurring at the cell surfaces of adjacent blastomeres. In the first stage of compaction, each of the eight blastomeres interacts with its neighbors to undergo membrane polarization. Different components of the cell surface migrate to different regions of the cell (Ziomek and Johnson, 1980).

This polarity can be seen by tagging certain cell-surface molecules with fluorescent dyes. One such tag, which recognizes a class of glycoproteins, shows that at the 4-cell stage these glycoproteins are randomly distributed throughout the membrane. However, at the mid-8-cell stage, these molecules are found predominantly at the poles farthest away from the center of the aggregate. Membrane polarization is influenced by cell-cell interactions, because it takes place only when the cells are in contact with at least one other blastomere. If a blastomere is separated from the rest of the embryo, it loses its polarization.

Specific cell surface proteins play a role in compaction. One such molecule, E-cadherin (also known as uvomorulin), a 120-kDa adhesive glycoprotein, is synthesized at the 2-cell stage and is uniformly spread throughout the cell membrane. However, as compaction occurs, E-cadherin becomes restricted to those sites on cell membranes that are in contact with adjacent blastomeres. Antibodies to this molecule cause the decompaction of the morula (Peyrieras et al., 1983; Johnson et al., 1986). The carbohydrate portion of this glycoprotein may be essential to its function, as tunicamycin (a drug that inhibits the glycosylation of proteins) also prevents compaction.

The phosphatidylinositol pathway may also be important for initiating compaction. If 4-cell mouse embryos are placed into media containing drugs that activate protein kinase C, premature compaction occurs. Similarly, diacylglycerides can transiently cause these 4-cell embryos to undergo compaction. When this occurs, the E-cadherin accumulates specifically at the junctions between the blastomeres (Winkel et al., 1990). These results suggest that the activation of protein kinase C may initiate compaction by shifting the localization of E-cadherin.

Finally, the cell membrane may also be modified during compaction by cytoskeletal reorganization. Microvilli, extended by actin microfilaments, appear on adjacent cell surfaces and attach one cell to the other. These microvilli may be the sites where E-cadherin is functioning to mediate intercellular adhesion. The flattening of the blastomeres against one another may therefore be brought about by the shortening of the microvilli through actin depolymerization (Pratt et al., 1982; Sutherland and Calarco-Gillam, 1983).

Thus, there is growing evidence that compaction is caused by changes in the architecture of the blastomere cell surface. It is not certain, though, how these events relate to one another or how they are coordinated into the integrated network of events that causes compaction.

Formation of the Inner Cell Mass

The creation of an inner cell mass distinct from the trophoblast is the crucial process of early mammalian development. How is a cell directed into one or the other of these paths? How is a cell informed that it is either to give rise to a portion of the adult mammal or to give rise to a rather remarkable supporting tissue that will be discarded at birth? Observations of living embryos suggest that this momentous decision is merely a matter of a cell's being in the right place at the right time. Up through the 8-cell stage, there are no obvious differences in the biochemistry, morphology, or potency of any of the blastomeres. However, compaction forms inner and outer cells with vastly different properties. By labeling the various blastomeres, numerous investigators have found that the cells that happen to be on the outside will form the trophoblast, whereas the cells that happen to be inside will generate the embryo (Tarkowski and Wróblewska, 1967; Sutherland et al., 1990). The inner cells have been found to come most frequently from the first cell to divide at the 2-cell stage. This cell usually produces the first pair of blastomeres to reach the 8-cell stage, and these cells usually divide in such a way that they are inside the loosely aggregated cluster of blastomeres (Graham and Kelly, 1977).

Hillman and co-workers (1972) have shown that when each blastomere of a 4-cell mouse embryo is placed on the outside surface of a mass of aggregated blastomeres, the external, transplanted cells only give rise to trophoblast tissue. Therefore, it seems that whether a cell becomes trophoblast or embryo depends on whether it was an external or an internal cell after compaction.

How many cells form the inner cell mass?

If most of the cells of the blastocyst form the trophoblast, then how many cells of the compacted embryo actually form the inner cell mass? Beatrice Mintz (1970) solved this problem by making chimeric mice, wherein two mouse embryos are fused together during early stages of development. She took two 4-celled embryos, one from homozygous black-furred parents and one from homozygous white-furred parents, and she placed them together so that the cells integrated to form one eight-cell embryo. This embryo underwent compaction and was implanted into the uterus of a foster mother mouse. Each cell can become a part of the trophoblast or a part of the inner cell mass with equal frequency. Mintz performed this experiment hundreds of times. If only one cell formed the inner cell mass, then each mouse pup should be either all white or all black. Zero percent should be mixed. If the inner cell mass was composed of two cells, one-quarter of them should be white, one-quarter black and 50 percent should be mixed (1WW: 2WB : 1BB). If there were three cells in the ICM, the percentage of mixed colored mice should be 75 percent (1WWW; 3WWB; 3WBB: 1BBB); and if there were four cells, the frequency of chimeric coat patterns should be 87.5 percent. Her figures showed that her allophenic mice had mixed pigmentation 73 percent of the time. Therefore, it appears that three cells make the inner cell mass of the compacted embryo.

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