Embryo Development: Debate over Aggregation Factors

At certain times during the development of an embryo, particular kinds of cells aggregate, form patterns, and differentiate into specific tissues and organs. The process of morphogenesis seems linked to two properties of embryo cells: their ability to recognize and adhere to other cells and their ability to time their adhesions so that different tissues and organs are formed at different times. An explanation of how these properties are related and how specific adhesions occur has been a central problem in developmental biology.

In the early 1960's, A. A. Moscona and his colleagues at the University of Chicago suggested that specific macromolecules on or between cell surfaces are crucial to specific adhesions between cells. He proposed that these "aggregation factors" bind to receptors on the surfaces of adjacent cells and thereby link cells together. The new and substantial evidence that there are indeed aggregation factors raises the question of whether or not these factors are tissue specific and how their effects change as cell surfaces change during development.

Two hypotheses have been advanced to explain how aggregation factors may control specific adhesions between cells. According to one hypothesis, cells from each kind of tissue or organ make distinct aggregation factors. Specific adhesions between cells would then occur when the surfaces of a given kind of cell become receptive to the aggregation factor made by those cells. In the second hypothesis the emphasis is on temporal changes in cell surfaces, rather than distinct aggregation factors, as the key to specific adhesions. Thus a few aggregation factors may cause many kinds of cells to adhere as the surfaces of those cells become receptive to the factors.

Both hypotheses about aggregation factors have been supported by results obtained in experimental systems and, as many developmental biologists point out, these conflicting results may be due to differences in the ways that adhesion is measured. There are some indications that experiments with simple organisms such as slime molds may help to resolve this dilemma. Such organisms are now being used to determine the ways in which cells use aggregation factors to adhere, how aggregation factors relate to morphogenesis, and how these factors influence the pattern formation that occurs when cells adhere.

Moscona and several other investigators support the hypothesis that specific adhesions between cells arise when each kind of cell makes a distinct aggregation factor. Moscona has now purified an aggregation factor from embryonic chick retinas, which, he believes, is different from an aggregation factor that he previously isolated from cerebrum cells of chick embryos. He reports that the aggregation factor isolated from chick retinas will bind together retina cells from embryos of other animals but will not bind together cerebrum cells, or other kinds of cells from chick embryos. Likewise, the cerebrum aggregation factor is apparently tissue, but not species, specific.

Jack Lilien and Janne Balsamo of the University of Wisconsin in Madison also find that aggregation factors are tissue specific. They prepared aggregation factors from retinal cells of chick embryos that bind only to retinal cells. They also isolated aggregation factors from cerebral cells of chick embryos that bind only to cerebral cells. Moreover, they report that the binding of these factors depends on specific sugar molecules on the cell surfaces.

Those who disagree with the hypothesis that aggregation factors are absolutely tissue specific point out that the earlier studies did not include assays of temporal changes in adhesive properties. Thus it remains possible that a factor isolated from retinal cells when they aggregate may well cause other kinds of cells to stick together when they are due to aggregate.

Another objection is that the evidence on specificity was obtained when qualitative assays of aggregation were used. For example, Moscona measures aggregation by mixing cells together with an aggregation factor and looking for clumping of cells. More precise assays of cell adhesion, which have been developed in several laboratories, now make possible measurements better suited to answer the questions of whether and to what degree cells stick together.

At Rockefeller University, Gerald Edelman and his colleagues used a quantitative assay of cell adhesion to obtain support of the hypothesis that temporal changes in cell surfaces, rather than distinct aggregation factors, may be the key to specific adhesions between embryo cells. Edelman's group assayed cell adhesion mediated by aggregation factors by measuring binding between pairs of cells. They immobilized one group of cells on nylon fibers and then counted the number of free cells that stuck to immobilized cells under various experimental conditions.

The Edelman group used their assay of chick embryo retinal cell adhesion in experiments discussed by Urs Rutishauser at the ICN-UCLA winter conferences in Squaw Valley on 9 to 14 March 1975. They isolated a factor which is a protein that resembles, but may not be the same as, that described by Moscona; it also is present on the surfaces of a number of different embryonic tissues and apparently has some role in specific adhesions between cells from those tissues. Edelman and his colleagues demonstrated that the factor on surfaces of cells from chick embryos blocks aggregation by blocking its action with antibodies. Cells that would normally bind together would no longer adhere if they were first incubated with antibodies to the purified factor.

Although in all retinal cells, brain cells, and liver cells that they studied the aggregation factor was present at the cell surface, Edelman's group found that its ability to cause adhesion apparently depends on other properties of the surfaces of the cells, and that those properties change during development. For example, retinal cells from 9-day-old chick embryos stick to each other, but those from 5- or 12-day-old embryos do not. Similarly, 6-day-old cerebral cells stick to each other, but 9-day-old cerebral cells do not. Rutishauser said that adhesion is not absolutely tissue specific, since 9-day-old retinal cells bind to 6-day-old cerebral cells as well as to other 9-day-old retinal cells. From these results, Rutishauser suggests that temporal changes in the properties of cell surfaces can enable a single aggregation factor to account for at least some of the specificity observed when embryonic cells adhere.

The exact kinds of changes in cell surfaces that affect adhesion are not known, but evidence is accumulating that such changes can occur rapidly and exert dramatic effects. For examply, Ronald Merrell, David Gottlieb, and Luis Glaser at the Washington University School of Medicine in St. Louis isolated soluble components from membranes of 7-, 8-, and 9-day-old retinal cells from chick embryos, and found that each soluble component binds to retinal cells of the same age as that of its cells of origin and prevents those cells from binding to each other. In addition they found that a soluble component from an 8-day-old retinal cell will only partially block adhesions among 7-

or 9-day-old cells and, similarly, components from 7- and 9-day-old cells only partially block adhesions among cells of other ages. Thus they conclude that these components may affect temporal changes in the effects of aggregation factors on developing cells.

Stephen Roth of Johns Hopkins University points out that interpretations of studies of adhesion among embryo cells are not only complicated by the use of different assays of aggregation by different investigators and the paucity of results on temporal changes in cell surfaces during development but they are also complicated by the existence of different kinds of cells in a given tissue. According to Roth, specific tissues, such as retina or cerebrum consist of several cell types, and there is no reason to believe that each type of cell behaves the same way.

In attempting to skirt the problems associated with studies of adhesion among embryo cells some investigators are studying slime molds, which are much simpler in structure than embryo cells. Slime molds are useful model systems for studying development because they share with higher organisms many of the features observed during embryogenesis. Slime molds live as individual and identical cells until they no longer have bacteria to ingest, whereupon the cells come together, adhere, and develop into a multicellular organism.

Although students of chick embryo cells have often assumed that these cells synthesize aggregation factors when they adhere, only in slime molds has this phenomenon been conclusively demonstrated. Steven Rosen who works at Samuel Barondes' laboratory at the University of California at San Diego reported at the ICN-UCLA conferences that a carbohydrate-binding protein isolated from aggregating cells of the slime mold *Polysphondylium pallidum* is present on the surfaces of aggregating cells but not on the surfaces of cells living as individuals. When this protein is added to isolated cells that are ready to aggregate, it promotes cell adhesion. This adhesion, as well as the native adhesiveness of developed cells, can be blocked by sugars that react with the aggregation protein and, apparently, prevent it from binding to sugars on the surfaces of slime mold cells. Aggregation factors isolated from several slime mold species are blocked by different sugars. Richard Reiterman, also of Barondes' laboratory reported at the ICN-UCLA conferences that, for two slime mold species, the carbohydrate-binding protein from one species binds to cells from the other species, but binds more strongly to cells from its species of origin.

Investigators who study cell adhesion in higher organisms have not yet approached the question of whether there are preferred sites of adhesion. Gunther Gerisch of the Max-Planck-Gesellschaft in Tubingen, Germany, has, however, found preferred directions of adhesion among slime mold cells; namely, side-to-side and end-to-end adhesion. He has fractionated the antibodies to cell surfaces and obtained two kinds of univalent antibodies, which distinguish between these two directions of adhesion. One kind of antibody blocks side-to-side adhesion and the second antibody blocks end-to-end adhesion. He reports that slime mold cells can adhere side-to-side before they ever begin to aggregate but when they aggregate to form a multicellular organism they also adhere end-to-end. Gerisch has not determined whether this end-to-end adhesion is affected by the protein Rosen and his colleagues isolated from aggregating cells. Rosen notes, however, that the aggregation protein is made at the same time that end-to-end adhesion occurs.

Gerisch's experiments are interesting to developmental biologists because the existence of preferred sites of adhesion may be a basis for pattern formation among aggregating cells, as in the formation of a tubule. Although biologists emphasize aggregation factors as a means to randomly bind cells together, they find that random aggregations are not sufficient for development in that cells must form specific patterns.

Moscona has noted the importance of pattern formation in his experiments with retinal cells. He finds that cells from retinal tissue, which form distinct patterns, can be induced by hydrocortisone to produce glutamine synthetase. But retinal cells in culture do not respond to hydrocortisone before they aggregate or if they are dispersed after they aggregate. In fact, when Moscona dispersed aggregated cells from retinal tissue and prevented pattern formation by cultivating them in a monolayer, they did not respond to hydrocortisone, even though they made contact with each other and even though they had hydrocortisone receptors on their surfaces.

Pattern formation thus appears to be a key piece in solving the puzzle of embryonic development. Many investigators are now confident that further study of aggregation factors and cell adhesion during development will lead to increased understanding of how patterns are formed and, eventually, of how cell surfaces help to control development.

—GINA BARI KOLATA

Fermilab Flexes Its Muscle

The huge proton synchrotron at the Fermi National Accelerator Laboratory near Batavia, Illinois, recently completed an extended run at energies well above its normal level of 300 billion electron volts (Gev). During the month-long run, protons were accelerated to 380 Gev without major problems and without diminution of normal intensity, currently about 10^{13} protons per pulse. The demonstration of improving capability is timely in view of the continuing ferment among high energy physicists over the nature and significance of the psi or J particles (*Science*, 6 December 1974, p. 909). Indeed, a primary purpose for the high energy run was to extend the range of an experiment being conducted by a team of scientists from Columbia University, the University of Illinois, the University of Hawaii, Cornell University, and Fermilab which bears on the new particles. The experimenters studied psi particles produced by photon collisions (photoproduction) with a beryllium target. Early results indicated that the psi is indeed made up of "charmed" quarks or something like them, and by going to higher energies the experimenters hope to observe still other new particles. A second experiment at 380 Gev with neutrinos was also to look for new particles.

The Fermilab accelerator is thus beginning to establish itself as the powerful research tool its designers had hoped and planned for. More than 70 experiments have been completed and another 36 are under way. Improvements for which the hardware has already been built but has not yet been integrated into the operating system are expected to increase the already remarkable intensity by a factor of 2 later this year—to 2×10^{13} protons per pulse. A peak intensity of 1.5×10^{13} has already been produced, but not as a sustained beam. The beam is split among three experimental areas, with about 75 percent of the current normally going to neutrino and muon experiments, for which intensity is an important constraint on the rapidity with which data can be collected.

Routine operation at energy levels near 400 Gev is still some time off, because of problems in procuring the needed transformers. As the recent experiment demonstrates, however, the capability of the Fermilab accelerator is continuing to evolve toward both higher energies and higher intensities.—A. L. H.