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Review

Forces driving cell sorting in the amphibian embryo

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ABSTRACT

Adhesion differences are the main driver of cell sorting and related processes such as boundary formation or tissue positioning. In the early amphibian embryo, graded variations in cadherin density and localized expression of adhesion-modulating factors are associated with regional differences in adhesive properties including overall adhesion strength. The role of these differences in embryonic boundary formation has not been studied extensively, but available evidence suggests that adhesion strength differentials are not essential. On the other hand, the inside-out positioning of the germ layers is correlated with adhesion strength, although the biological significance of this effect is unclear. By contrast, the positioning of dorsal mesoderm tissues along the anterior-posterior body axis is essential for axis elongation, but the underlying sorting mechanism is not correlated with adhesion strength, and may rely on specific cell adhesion. Formation of the ectoderm-mesoderm boundary is the best understood sorting related process in the frog embryo. It relies on contact-induced cell repulsion at the tissue interface, driven by Eph-ephrin signaling and paraxial protocadherin-dependent self/non-self recognition.

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1. Introduction

Large-scale cell sorting is not a major morphogenetic mechanism in animal embryonic development. Historically, however, the observation that experimentally mixed cells of different origin and fate could spontaneously segregate again (Wilson, 1907; Townes and Holtfreter, 1955) led to important concepts. First of all, cells were apparently able to recognize each other as alike or different, probably by modulating cell-cell adhesion, an essential property of multicellular organisms. If so, the same force that would drive sorting under experimental conditions could prevent mixing across tissue boundaries in the living organism. Thus, sorting would provide an entry point into the study of cell adhesion and boundary formation. Indeed, small scale sorting has been implicated in the refinement of initially inaccurate and fuzzy boundaries between different cell populations, and in the maintenance of sharp boundaries despite the intermingling effects of cell division and random cell mobility (Dahmann et al., 2011; Batlle and Wilkinson, 2012). The extent to which such "maintenance" sorting occurs remains to be determined (Fagotto, 2014). Second, spontaneous sorting could be viewed as a self-assembly process which generated relatively complex tissue

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configurations and assured their stability. In this interpretation, cell sorting enabled a mechanistic, experimental approach to a range of morphogenetic processes. Work on amphibian embryos was at the origins of this eventually successful enterprise.

2. Principles of cell sorting

2.1. Holtfreter's tissue affinities

Holtfreter's influential work on cell sorting and tissue segregation in the amphibian embryo culminated in a now classical paper (Townes and Holtfreter, 1955) which laid out basic concepts that still shape our thinking in this field today. Starting from the simple observation that when dissociated cells from different tissues were mixed, they always reaggregated into a single cell mass, it was concluded that a general adhesion system common to all cell types must exist in the early embryo. Over time, however, cells sorted out according to their different origins, indicating the preference of like cells for each other and suggesting an additional, cell type specific adhesion component (Fig. 1A). Cells of different types showed various degrees of attraction or avoidance, and the resulting mutual attachment or separation between cell populations was also observed when whole pieces of tissues were combined (Fig. 1A). The respective tissue- and stage-specific, graded properties were summarized under the concept of tissue affinity (Holtfreter, 1939; Townes and Holtfreter, 1955). Autonomous, stage-specific changes in tissue affinities were thought to underlie the morphogenetic movements of gastrulation and neurulation (Holtfreter, 1939). Later, it was found that the same sequence – aggregation into a common cell mass followed by sorting out – could occur for the same tissue combination at different developmental stages, which led to the additional notion that sorting-related adhesion differences could be induced by heterologous cell contact (Townes and Holtfreter, 1955).

Sorting requires the movement of cells in a mixed aggregate in opposite directions to congregate with like cells. The eventual positions of cell populations were always the same, regardless of whether a mixture of cells or an experimental combination of tissue explants was the starting point, as expected from a system of tissue affinities that determined the final arrangement of cell types (Fig. 1A). For example, ectoderm always separated completely from endoderm, whereas mesoderm settled at the surface of an endodermal aggregate. Since mesoderm also attached to ectoderm, it could mediate the stable, spatially ordered combination of all three germ layers (Holtfreter, 1939; Townes and Holtfreter, 1955).

During positioning, the directional movement of single cells and of explants was different. Whereas cells in mixtures elongated, polarized and showed signs of individual “amoeboid” migration, aggregates seemed to “slip” as a whole over or between each other. To explain such aggregate movements, they were compared to the engulfment of liquids with different surface tensions. The directionality in both single cell and aggregate movements was speculatively explained not by chemotaxis, but by surface tension gradients which ensured the stereotypical final arrangement of tissues (Townes and Holtfreter, 1955). The eventual boundaries between cell populations were conspicuously straight or even cleft-like (Fig. 1B) (Townes and Holtfreter, 1955). Apparently, tissue affinity also ensured the formation of distinct tissue boundaries.

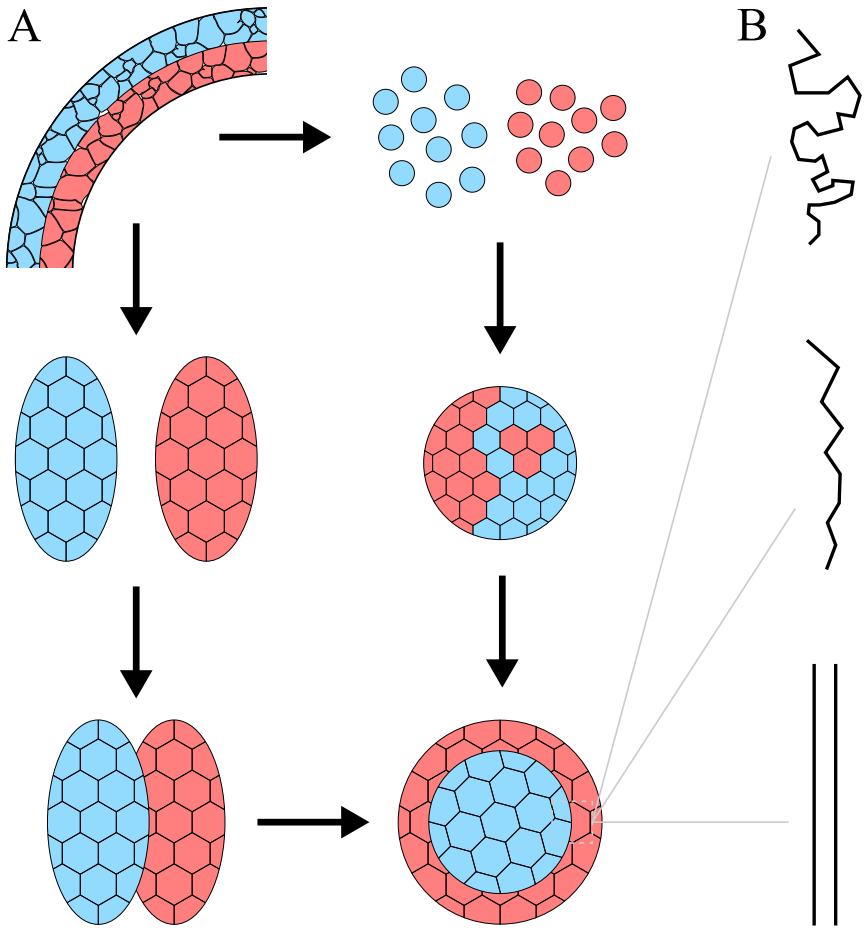

Fig. 1. Cell sorting, tissue positioning and boundary formation. (A) When ectoderm (blue) and mesoderm (red) are combined, either as explants (lower path) or dissociated cells (right path), they reproducibly form the same tissue arrays (lower right) either by tissue engulfment (lower path) or cell sorting (right path). (B) Sorting can lead to a coarse separation between tissues (top), a jagged but relatively straight boundary (middle), or a straight and cleft-like boundary characteristic of tissue separation (bottom).