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Morphogenesis and Cell Specification at Embryonic Stage; Theoretical Analysis on Mosaic Eggs

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The organism builds up its architecture and specifies its cells through embryonic stage from the zygote. The skeletal structure of the embryo is represented by a graph. In the graph, the cell and the connectivity between them are reduced to the node and the edge, respectively. Along embryogenesis a sequence of graphs is obtained. I defined two types of graph-developmental system (GRADES) from the viewpoint of embryology; G-GRADES (defined from the skeletal architecture) and C-GRADES (mainly defined from the cell-fate). I analyzed the cleavage pattern of the ascidian eggs by them. The cleavage pattern is deterministic, then the ascidian G-GRADES is also deterministic, but the ascidian C-GRADES became non-deterministic. This means that the cell-fates (cell-determinants) cannot determine the architecture and the spatial pattern of cell division by themselves.

INTRODUCTION

An animal develops from the zygote to the adult. The zygote has the simple form as a cell compared with the form of the adult composed of many cells. So, the developing system of the animal makes its form more and more complex, and grows into the highly organized system.

Cells of the embryo divide and divide. Consequently, they are specified to produce specific kind of tissue, for example, neuron, intestine, muscle and so on, which compose the highly organized system. With regard to mosaic eggs, developmental biologists think that the cells have the specific determinants which determine their fates. The cells, for example, which develop into muscle have the determinants which determine that they differentiate into muscle. So, at embryonic stage the cell-determinants are segregated into cells by cell division.

Moreover, cells of the embryo construct the embryonic architecture (form). Development of the architecture is mainly defined by the spatial pattern of cell division, and the architecture becomes more complex through cell division. So, there may be significant mechanisms which control constructing the architecture and cell specification. I am interested in the mechanisms.

The architecture is roughly represented by a graph. In a graph, the cell is represented by a node, and the connectivity between cells is represented by an edge. Along cleavage history a sequence of graphs is obtained. ("Fig.1" shows the cleavage pattern of the ascidian egg (top) and the sequence of graphs representing it (bottom).) I defined a developmental system of graphs which produces a sequence of graphs. I call it graph-developmental system or GRADES (Doi; 1984, 1985). It is a kind of graph L-system composed of grammatical rules rewriting nodes and edges (Culik & Lindenmayer, 1976; Lindenmayer & Culik, 1979).

To investigate the mechanisms mentioned above I question that:

(Q1) Can cell-fates (cell-determinants) determine the embryonic architecture and the spatial pattern of cell division by themselves?

Mosaic eggs show invariant cleavage pattern, and the ascidian eggs are mosaic. I examined this question (Q1) on the cleavage pattern of the ascidian

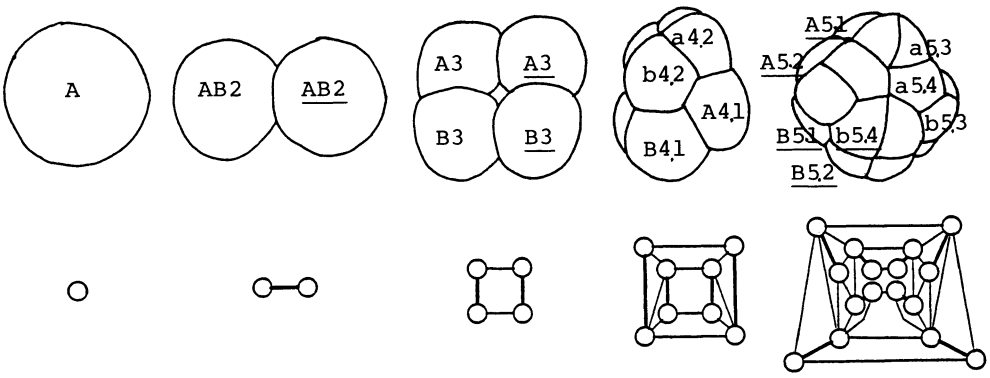


Fig.1 The cleavage pattern of the ascidian embryos up to the 16-cell stage observed by Satoh (1978) through scanning electron micrographs (top), and the sequence of graphs representing it (bottom).

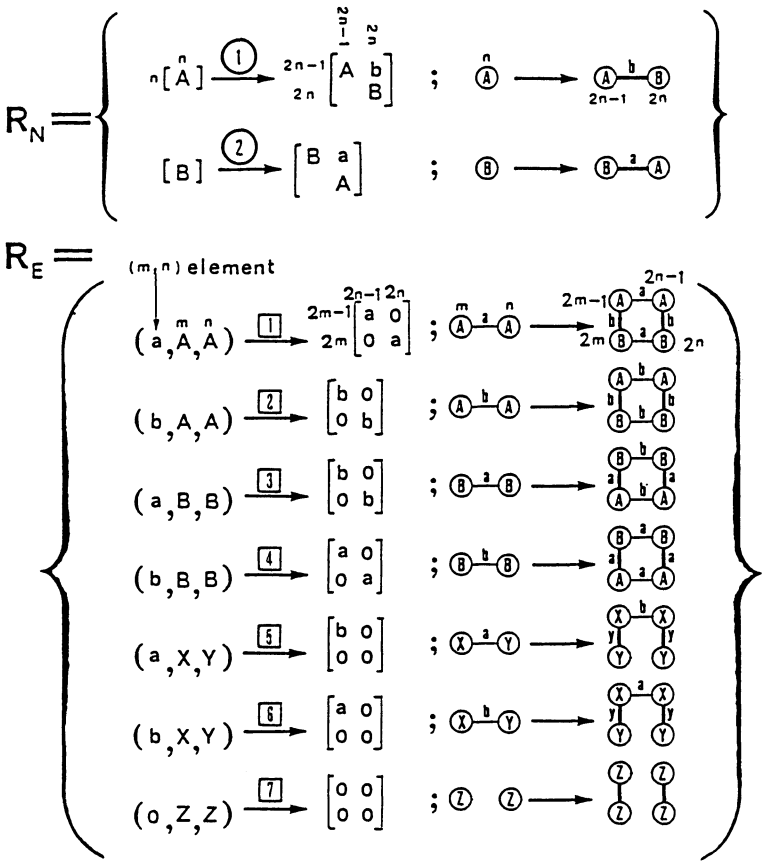


Fig.2 Example of rewriting rules of nodes (R_N) and renewal rules of edges (R_E).

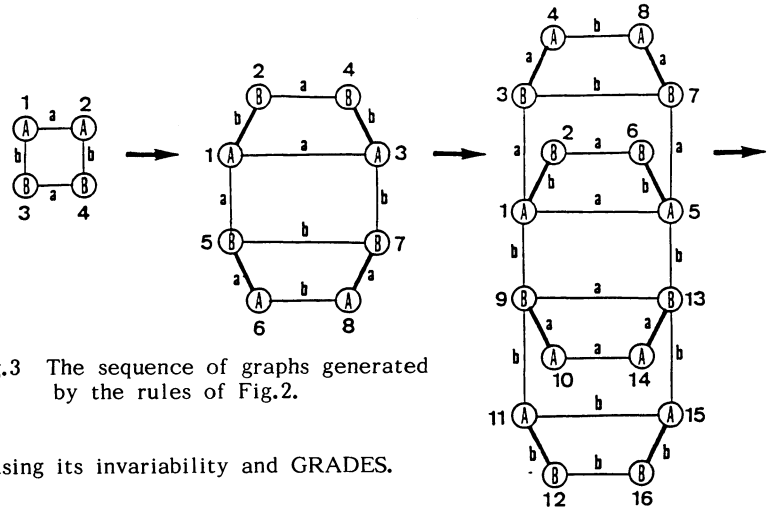


Fig.3 The sequence of graphs generated by the rules of Fig.2.

eggs using its invariability and GRADES.

GRADES AND ITS BIOLOGICAL MEANING

GRADES is composed of five factors:

1) node labels,
2) edge labels,
3) rewriting rules of nodes,
4) renewal rules of edges,
5) an initial graph.

The rewriting rules of nodes represent cell divisions, and the renewal rules of edges represent the developmental changes of the cell-connectivities.

"Fig.2" shows an example of the rules, and "Fig.3" shows the sequence of graphs generated by them iterated to the leftmost of the graphs of the figure.

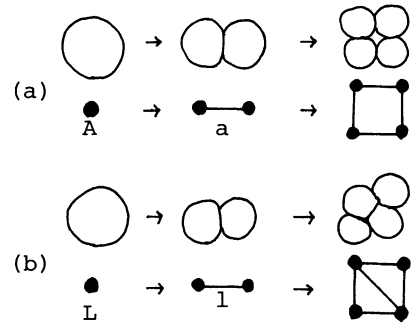


Fig.4 Procedure to label nodes (cells) and edges (connectivities) by Alphabet law.

Cells of the embryo construct the architecture, and are specified by cell division. So, I defined two types of GRADES. One is deifined by the development of cell-connectivity. I call it geometric GRADES or G-GRADES. The other is mainly defined by segregation of cell-determinants or cell-fates. I call it cell-fate GRADES or C-GRADES.

"Fig.4" shows the procedure to get labels of nodes and dges used in G-GRADES. Labeling nodes and edges to construct G-GRADES is, in other words, classifying cells and cell-connectivities by the development of connectivities.

To construct G-GRADES, first, cell-connectivities are classified. When a connectivity (edge) develops as shown in "Fig.4a", it is labeled "a". When another connectivity develops as shown in "Fig.4b", it is labeled "1".

With regard to the development of a connectivity, we have the following law. **"At the next time step, the possibilities of the development of a connectivity totals twenty six."** "Fig.5" shows the all possibilities. I assign the letters to these twenty six connectivities as shown in "Fig.5", and I call this law **Alphabet law.**

At the next step, cells are classified by the development of connections

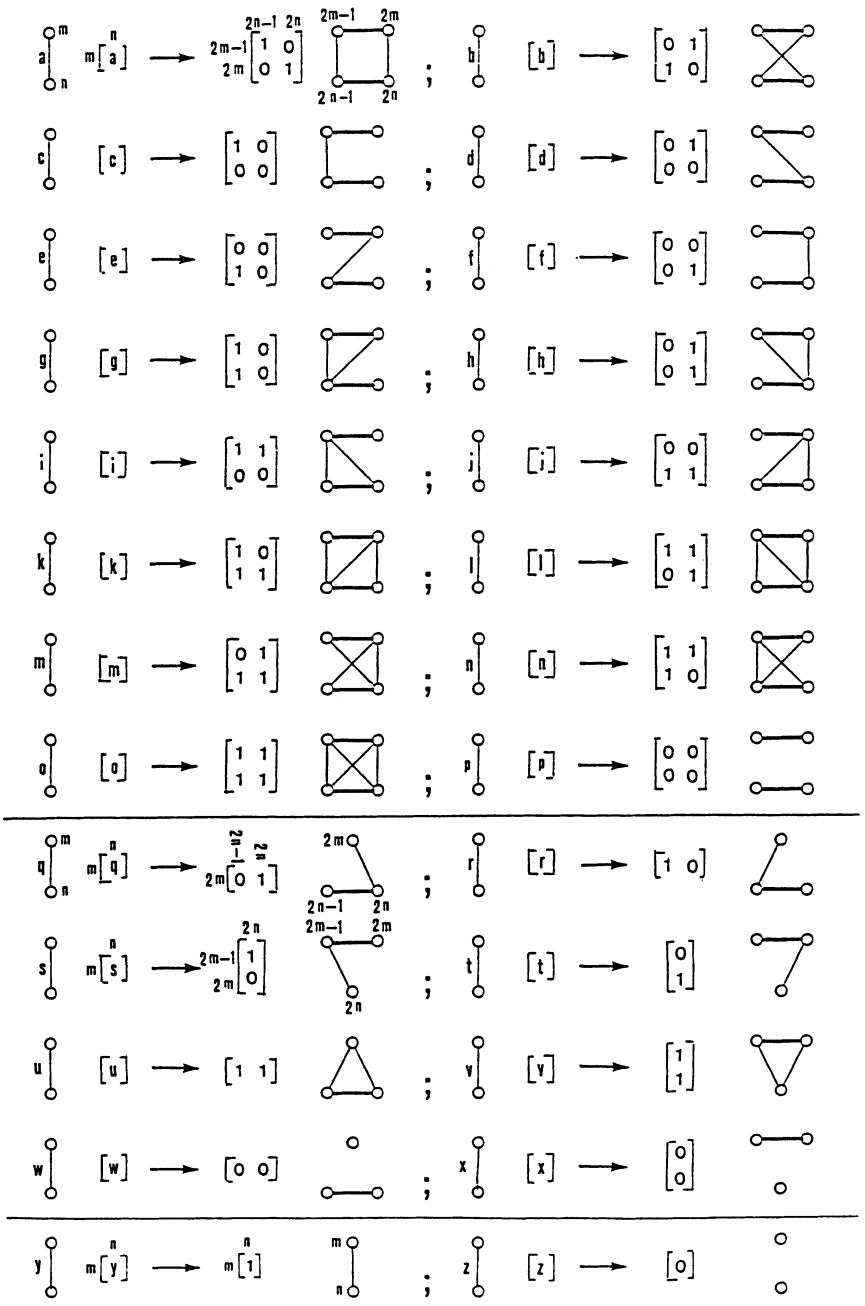
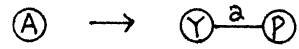


Fig.5 Alphabet law.
The posibilities of development of cell-connection totals 26.
Letters are assigned to the all posibilities.

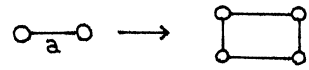
which they produce. For instance, when a cell divides into two cells connected by the connectivity "a", it is labeled "A" (see "Fig.4a"). When another cell divides into two connected by the connectivity "l", it is labeled "L" (see "Fig.4b"). In this way, the labels of cells and cell-connectivities are obtained.

In C-GRADES, at first cell-connectivities are labeled by Alphabet law in the same way as G-GRADES. At the next step, cells are labeled according to cell-determinants or cell-fates which they have. For example, when transparent cell 1 that has yellow and pink determinants divides into cell 2 and cell 3 that have yellow determinants and pink determinants, respectively, and that yields yellow cells and pink cells, cells 1, 2 and 3 are labeled "YP", "Y" and "P".

The rewriting rules of nodes are constructed as follows: For example, when cell "A" divides into two cells "Y" and "P" connected by "a", the rule representing this division is



The renewal rules of edges are constructed by Alphabet law. Connectivity (edge) "a", for example, develops like as the top of the left column of "Fig.5", then the rule is



I summarize the procedure to construct G-GRADES and C-GRADES. The first step is to translate the cleavage pattern into a sequence of graphs. The second step is to translate the sequence into a sequence of incidence matrices. I do not explain this step, but the matrix representation is very useful to treat embryogenesis by computers. The third step is to label the connectivities by Alphabet law. The forth step is to label cells by Alphabet law (G-GRADES) or by cell-determinants or cell-fates (C-GRADES). The fifth step is to define the rules.

Many mosaic eggs show unique cleavage pattern, namely, the celavage pattern of mosaic eggs is invariant. When the cleavage pattern is unique, the sequence of graphs which represent it is also unique, and G-GRADES which produces the sequence is unique. That is, the rules of G-GRADES are deterministic because of its construction manner. But we do not know which C-GRADES is unique or not unique (that is, which its rules are deterministic or non-deterministic?). The same cell-connectivity produced by cells with the same cell-fate means that C-GRADES is deterministic. So, this question is translated into the question (Q1) and the following question in biological term:

(Q2) Which do cells with the same cell-fate have the same cell-connectivity or the different cell-connectivities from each other in the following stages?

I think this type question is new one in embryology and is led by the concept of GRADES.

ANALYSIS OF ASCIDIAN EMBRYOS WITH GRADES

On these questions I analyzed the ascidian embryos. Because the ascidian eggs are mosaic, and the cleavage pattern is invariant. The cell lineage was closely observed by Conklin (1905) and Ortolani (1955, 1957, 1962). "Fig.6" shows the cell lineage observed by them. Moreover it is easy to extract the developmental change of cell-connectivity from the observation through scanning electron micrographs. The top of "Fig.1" shows the skech of the scanning electron micrographs of the cleavage pattern of the ascidian embryos up to the 16-cell stage observed by Satoh (1978).

At the 16-cell stage, b5.3 and b5.4 cells have the same cell-fate (see "Fig.1" and "Fig.6). These cells develops into only epidermis. The other cells have the different cell-fates from each other. "Fig.7" shows the developments of connections produced by b5.3 and b5.4. These cells have the same fate (epidermis), but they have the different connectivities from each other at the 64-

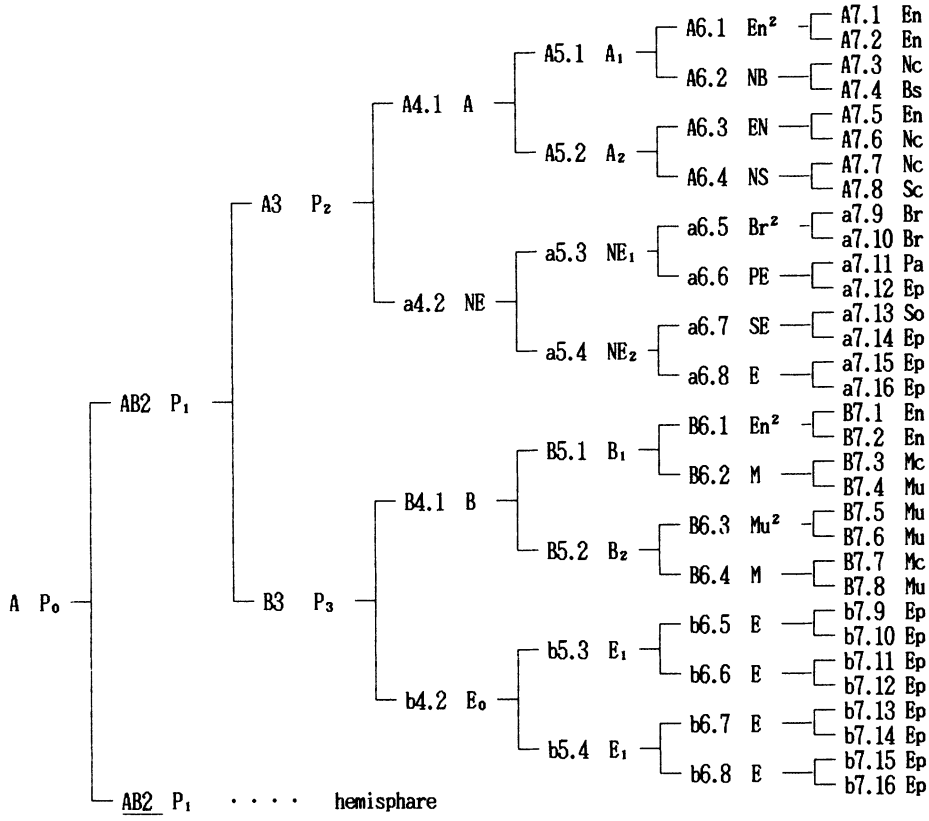


Fig.6 Cell lineage observed by Conklin (1905) and Ortolani (1955, 1957, 1962) and the node labels used in the ascidian C-GRADES.
A, AB2, A6.4, b7.1 and so on are the codes of cells assigned by Conklin. P_1, NE, E_1, En and so on are the node labels. The node labels at the 64-cell stage represent the cell-fates of the cells as follows: En, endoderm; Nc, notochord; Bs, brain stem; Sc, spinal cord; Br, brain; Pa, palps; So, sensor organ; Ep, epidermis; Mc, mesenchyme; and Mu, muscle.

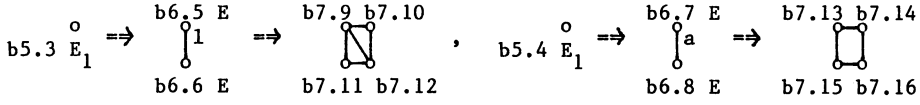


Fig.7 Developments of the connectivities produced by b5.3 and b5.4.
Conklin and Ortolani observed that these cells yield only epidermis, so in the ascidian C-GRADES b5.3 and b5.4 are labeled "E_1", and the daughter cells b6.5, b6.6, b6.7 and b6.8 are labeled "E". Recently Nishida and Satoh (1983, 1985) and Zalokar and Sardet (1984) observed that b5.3 produces epidermis, brain stem, spinal cord and muscle and b5.4 produces only epidermis. These cells produce the different connectivities.

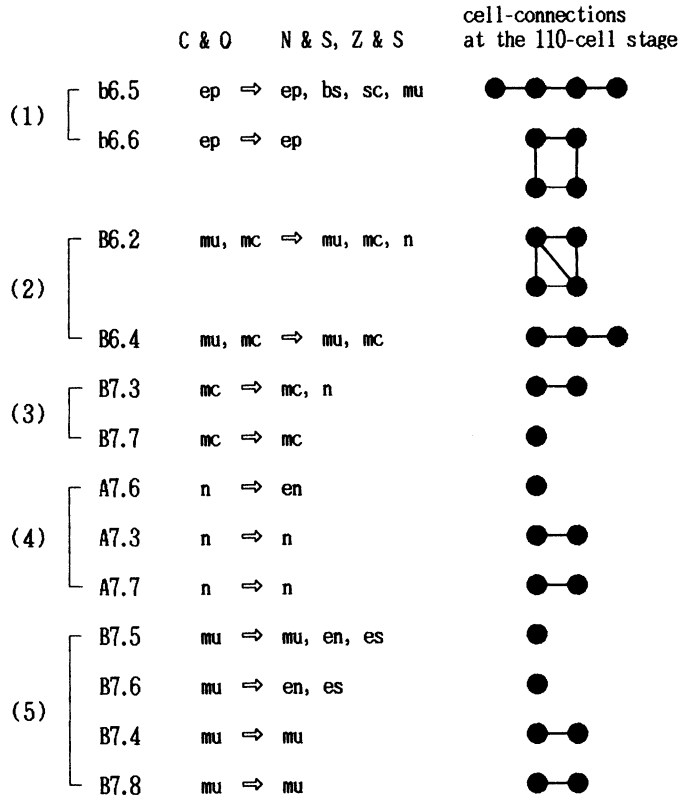


Fig.8 Five groups whose each cells were observed having the same cell-fate by Conklin (1905) and Ortolani (1955, 1957, 1962) but have the different connections at the 110-cell stage.
The abbreviations are: C & O, cell-fates observed by Conklin and Ortolani; N & S, Z & S, cell-fates corrected by Nishida and Satoh (1983, 1985) and Zalokar and Sardet (1984); en, endoderm; bs, brain stem; n, notochord; sc, spinal cord; mu, muscle; mc, mesenchyme; ep, epidermis; and es, endoderm stem.

cell stage. The ascidian C-GRADES of which the node labels are defined from the old observation of Conklin and Ortolani is not unique (i.e., non-deterministic). Recently, Nishida & Satoh (1984, 1985) and Zalokar & Sardet (1984) corrected the old observations of Conklin and Ortolani. They observed that b5.3 produces epidermis, brain stem, spinal cord and muscle, on the other hand b5.4 produces only epidermis. So, the following conclusion is obtained: Cells which have the different connectivities in the following stages have the different fates from each other. The contraposition of this conclusion is, that is the answer to the question **(Q2)** is; **cells with the same cell-fate have the same cell-connectivity in the following stages.** (The ascidian C-GRADES of which node labels are defined from the new data is unique, that is, deterministic.) Or to the question **(Q1)**, this conclusion is; **cell-determinants or cell-fates cannot determine the spatial pattern**

of cell division and the embryonic architecture by themselves. That is, the answer to (Q1) is "no".

Moreover, at the 32- and the 64-cell stages, the ascidian embryo has five groups like as the group of b5.3 and b5.4 cells (see "Fig.8"). Conklin and Ortolani observed that each cells of the groups have the same cell-fate ("O & C" in "Fig.8"). But they have the different connectivities from each other at the 110-cell stage. And Nishida and Satoh (1983, 1985) and Zalokar and Sardet (1984) also corrected the old observations, that is, they observed that some cells of the groups have the different fates ("N & S, Z & S" in "Fig.8"). With regard to these cells, the same conclusion in the above is obtained.

CONCLUSION

Cells of the embryo have two roles; one is to construct the embryonic architecture by cell division, and the other is to be segregated cell-determinants by cell division and produce specific kind of tissue. These roles are very significant to construct the highly organized system from the zygote. The embryo might have the mechanisms filling them. To represent the roles, I defined newly graph-developmental system (GRADES), which is not the one to analyze the mechanisms. But the concept of GRADES gave new questions (Q1) and (Q2) in embryology and led the conclusions mentioned here. From the conclusions I think the mechanisms are directly coupled with each other, and cannot be separated from each other by any methods. And I think the concept of GRADES may give us new viewpoints in developmental biology.

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