

Determining cell fate and cell commitment

Reading: pp 109-119

Learning goals: Be able to:

1. Diagram the two major mechanisms of cell determination and explain their general molecular basis.
2. Distinguish (experimentally) between cell fate and cell commitment.
3. Explain what cytoplasmic maternally-contributed determinants are, and justify their importance during development.
4. Describe how the extracellular matrix (ECM) is involved in signaling and cell fate determination.

Cell Fate

An embryonic fate map describes for each cell in the embryo the structures and tissues it gives rise to in the adult animal. (Or conversely, it identifies the cells in the embryo from which every adult structure or tissue is derived.)

The ultimate fate map is the complete cell lineage (ancestry) of all cells in the adult (this is usually only known for simple organisms like *C. elegans*).

Fate maps can be constructed from observation only (if the embryo is transparent), or by ablation of cells in the embryo and observation of the results, or by marking early cells and observing where they end up. The normal fate of a cell is not necessarily the only fate that cell can take on. Many experiments in developmental biology are devoted to demonstrating that a cell's possible range of fates are larger than the fate it normally takes.

At some point in embryogenesis, many cells become committed to a particular cell fate, without changing their morphology or behavior. This point *cannot* be determined by observation only. Evidence for commitment can be obtained using techniques of experimental embryology; e.g. transplantation of cells or tissues from one location to another, or ablation of neighboring cells and observation of how the remaining cells develop. Lastly, a cell begins to differentiate. This is the actual production and use of function-specific proteins that allow a cell to do its job. Differentiation is not immediate: it can take days if not longer for a muscle cell, for example, to both take on its final shape and to express all the proteins required for its final functions.

Mechanisms of commitment:

Cells become committed by either of two general mechanisms.

1) Signaling: A signal from a neighboring cell triggers synthesis of new determinants: transcription factors, receptors, etc. ("American plan" determination). When a cell's fate is determined by signaling, it will not take on its normal fate unless in the presence of this other cell type or signal—ie, it cannot make its fate on its own! It requires a signal. Sometimes this mechanism is also called "non-autonomous".

2) Segregation: Asymmetric division of a precursor cell segregates pre-existing determinants (that were unevenly distributed in the precursor) to only one of two daughter cells, which thereby becomes committed ("European plan" determination). This mechanism is called autonomous, because the committed cell depends only on its own internal determinants and requires no information from an external source: if it is isolated away from other cells, it will still take on that fate, because it does not require information from other cells. From our previous discussions, you should be able to predict what kinds of proteins these determinants might be.

Progressive determination:

Commitment is generally not an all or none phenomenon. Instead, it occurs in stages, over several cell generations. For example, during embryogenesis, certain embryonic stem cells become committed as hematopoietic (blood-forming) stem cells. Later, some of these become committed as erythroid (red blood-forming) stem cells, and still later these are determined to become reticulocytes, which are committed to becoming only erythrocytes (red blood cells). The embryonic stem cells are totipotent (they can give rise to all

types of cells); the hematopoietic stem cells are pluripotent (they can give rise to either red or white blood cells); and the reticulocytes are unipotent: they have only one possible fate.

Morphogens

Any molecule that can lead to different cell fates when present in different concentrations is called a morphogen. Not surprisingly, many signaling molecules fall into this category, as well as some transcription factors (see *Drosophila* embryonic patterning, class 7). Whenever we mention a morphogen, we should think about how the concentration of that molecule can actually effect a change in the fate of the cell. Is there a threshold of ligand that must be bound to receptors in order to activate transcription? Is there a threshold of transcription factor required to synthesize enough of another transcript to convert a cell from one fate to another? There are many possibilities.

A look back to cell-signaling: roles of the ECM and adhesion molecules in cell signaling

One additional interesting component of signaling not yet mentioned is that cell signaling can actually occur in the absence of a the typical soluble ligand such as those described last time. You have undoubtedly heard about cell adhesion molecules like integrins, as well as cadherins and catenins. These important extracellular matrix molecules can play morphoregulatory roles, transmitting information that is important for morphogenesis as well as control of gene expression in developing tissues. There is still much to be learned in this area.

Integrin pathways

Ligands: ECM components such as fibronectins, laminin, and collagen.

Receptors: substrate adhesive molecules (SAMs) such as integrins. Members of the integrin receptor family are large transmembrane proteins, active as heterodimers of an α and a β chain, and present on the cell surface in 10- to 100-fold higher concentration than growth factor receptors. On their extracellular domains are relatively weak binding sites for ECM components.

Downstream components: Integrin intracellular domains are linked to the actin cytoskeleton by linker molecules that provide a direct link from the cell surface to the cytoskeleton (6.32). Because these signals can affect cytoskeletal organization as well as gene expression, they are important for *both* tissue morphogenesis *and* differentiation. Integrins also mediate reciprocal interactions between the actin cytoskeleton of cells and the surrounding ECM. For example, oriented cells can orient fibronectin molecules in the ECM, which can then orient other cells, thereby propagating the ordered addition of cells in tissue formation (6.34). Lastly, integrins provide biochemical connections to the cell cortex. Phosphorylation of either Ser or Tyr residues on the intracellular domain by cytoplasmic kinases can decrease the binding affinity for extracellular ligands. Also, in response to extracellular ligand binding, clustered integrin receptors (such as hemi-desmosomes) are thought to assemble an intracellular signaling complex of kinases that can activate *ras* signaling pathways. These connections allow multiplexing with other signaling pathways that result in phosphorylation of target proteins to affect gene expression and other cellular functions. Some of these kinases may be activated in some mechanical manner, through changes in the actin cytoskeleton.

These biochemical connections account for some behaviors of vertebrate cells in culture. For example, cultured cells often will not proliferate in response to a growth factor unless attached through integrins to ECM molecules as a substrate. This must involve multiplexing of the above pathways.

Cadherin pathways (3.28)

Cell adhesion molecules (CAMs), which hold cells together in a tissue, can also have signaling functions. The most important molecules for cell-cell adhesion are cadherins, N-CAMs and E-CAMs. They hold cells together by homophilic interactions that also mediate intercellular signals. They are indirectly important for maintaining the association required for juxtacrine interactions like Notch/Delta signaling (more on this pathway later), but also signal directly.

Cadherins: Transmembrane proteins expressed on almost all cells, with sites for Ca^{++} -dependent binding on the cell exterior and connections to the actin cytoskeleton inside the cell via α , β , and γ -catenins. Specific cadherin types are expressed in different cell types: e.g. N-cadherins on neurons and E-cadherins in epithelial

cells. In the absence of Ca^{++} they change conformation and are rapidly degraded. Wnt signaling, which regulates the stability of β -catenin, may influence the functions of cadherins, and vice-versa.

Other CAMs. The CAMs have immunoglobulin-like extracellular domains that hold cells together by homotypic binding that is not Ca^{++} -dependent. They form weaker associations than the cadherins, and may be responsible for fine tuning cell associations during morphogenesis. Evidence for direct functions in cell signaling is so far sparse, but some N-CAM interactions appear to regulate the opening of Ca^{++} channels. Like the cadherins, there are tissue specific CAMS—N-CAM and E-CAM.

Summary: Multiplexing and combinatorial control of responses to cell signaling

Growth factors and other developmentally important signaling ligands, acting via the signaling pathways described above, control the activation and repression of specific transcription factors in the nucleus as well as the functions of cytoskeletal and membrane components. In doing so, they are crucially important in determining cell fates and cell behaviors (e.g. adhesion, shape changes, and movements) during embryonic development. Cells during development often interact simultaneously with ligands for two or more pathways, which then may interact intracellularly such that each pathway modulates the output of the other(s). Sometimes two pathways, or an entire signaling network *must* function together to produce a particular output (6.37).

Different cells can respond differently to the same ligand, for any of several reasons. First, to respond at all to this ligand, the target cell must have already synthesized a) the appropriate cell-surface receptors, and b) the appropriate adaptors, protein kinases, and other pathway components that respond to receptor activation and deactivation. Second, the response to this ligand will depend on which of the possible regulatory targets of the pathway, transcription factors or other proteins, the cell has already synthesized. Third, the response will depend on the *combination* of signaling pathways that are simultaneously active as the result of stimulation by this ligand and others. Therefore, the principle of *combinatorial control* we discussed last week is also crucial to understanding the roles of signaling pathways.

Review Questions

- 1) Define and explain the differences between cell fate, cell determination, and cell differentiation.
- 2) What is a fate map of an embryo? How can a fate map be made?
- 3) Why is it difficult to make a clear distinction between determination and differentiation?
- 4) What are the two fundamental classes of mechanisms by which cells become determined?
- 5) What kinds of experiments can distinguish between these mechanisms?
- 6) What is a cell-autonomous determinant? What is meant by “induction” as applied to embryos?
- 7) What kinds of experimental evidence show whether or not the fate of a particular blastomere or tissue is already determined?
- 8) What kinds of experimental evidence indicate that the fate of a particular blastomere or tissue is determined by subsequent induction?