[Cell Cycle 3:5, 564-566; May 2004]; ©2004 Landes Bioscience

Extra Views

To Differentiate or Not to Differentiate

Regulation of Cell Fate Decisions by Being in the Right Place at the Right Time

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Received 03/19/04; Accepted 03/22/04

Previously published online as a *Cell Cycle* E-publication: http://www.landesbioscience.com/journals/cc/abstract.php?id=870

KEY WORDS

competence, eye field, pax6, rx1, ephrinB1, FGF, *Xenopus*, cell movement, fate determination, retina

ABSTRACT

Cell fate decisions are influenced by intrinsic factors, expression of specific transcription factor genes, and cell-to-cell signaling. In addition, a recent paper by Moore et al. demonstrates that cell movements during development influence whether a cell becomes competent to express a particular fate. Interactions between the FGF and EphrinB1 signaling pathways regulate whether embryonic cells move into the eye field and ultimately contribute to the retina. This study illustrates the importance of being located in the right place at the right time during development.

Several years ago we were excited by the concept of "master-regulatory" genes—genes whose activity would cause naïve populations of cells to follow a restricted differentiation pathway to create a specific cell type. It was a simple concept to explain cell fate commitment by turning a single genetic switch. If an embryonic cell expresses MyoD it becomes a muscle cell, if it expresses Pit-1 it becomes a pituitary cell, if it expresses Pax6 it becomes a retinal cell. Likewise, if these genes are deleted, the specified cell types are missing. But not all cells can be converted to a pure population of a particular cell type simply by expressing a single master-regulatory transcription factor. This indicates that cells often require additional information or context to respond to these proteins as a genetic switch.

We know from studies of normal development that this context can be provided by where a cell is located in the embryo and what signals are present in the local environment. There are numerous examples from the classical literature demonstrating that the spatial location of a cell in the embryo places it in a unique signaling environment that influences what tissue it becomes. Eighty years ago Spemann and Mangold demonstrated that the dorsal lip of the blastopore is a signaling center that induces neural tissue. Thirty years ago Saunders and Reuss showed that the apical ectodermal ridge is a signaling center that influences the growth and anterior-posterior axis of the vertebrate limb bud.[1] Studies over the past two decades have revealed the identities of a plentitude of signaling factors that are expressed in restricted domains and influence the fate choices of the surrounding cells. For example, repression of BMP signaling by the dorsal lip tissue leads to a neural fate, and FGF and Shh signaling regulates limb bud growth. Much work also has elucidated the details of the intracellular signaling pathways for these factors, which can influence both individually and in combination the transcriptional pattern of the responding cell. Even more recently we have begun to appreciate that in addition to requiring the right combination of signals, cells also need to experience these signals in the right temporal sequence. For example, embryonic stem cells can only be converted to spinal motor neurons that innervate trunk muscle if exposed to the same sequence of signals as occurs in the intact embryo.[2] So it seems that master-regulatory genes really only act as masters when they are expressed in a receptive cellular context, a condition that embryologists long ago termed competence.

The term competence was first used to explain why certain tissues differentiated after embryonic rudiments were transplanted to novel locations in the embryo. For several embryonic structures there is a field of progenitor cells that normally gives rise to that structure (e.g., the limb field, the eye field), and there is an even larger domain of cells that can make that same structure if they are transplanted into the field. The cells in this larger domain are competent to make that particular structure, but they must be in the proper location to do so. There are other cells outside the competent domain that can not make the structure regardless of their position. It is now generally believed that master-regulatory genes have their impressive cell fate-determining effects primarily on competent cells that are already molecularly biased to respond to them. But a remaining question is how are

cells instructed to express those molecules that render them competent?

One of the best studied vertebrate systems for understanding the numerous influences on cell fate decisions is the eye. In both vertebrates and invertebrates it is one of the most cytoarchitecturally elegant structures, with different cell types recognizable by distinct morphologies and locations. Furthermore, the eyes are very accessible to experimentation throughout development, and both gain-of-function and loss-of-function mutations are relatively easy to identify by structural, physiological or behavioral anomalies. Genes that regulate eye development are conserved across vertebrates and invertebrates,[3] and in all animals studied to date a paired-domain gene (called *eyeless* in fly and *pax6* in vertebrates) is thought to act as a master-regulatory gene for eye fate. However, the efficacy of this "master-regulatory" quality in vertebrates also is influenced by the context of the responding tissue. Pax6 induces extra eye tissue best when the responding tissue has already been exposed to neural inducers, and it is much less potent when the responding tissue is biased toward an epidermal fate.[4,5] These results reinforce the idea that a master-regulatory gene relies upon the previous experience of the cell in which it is expressed, but how is this competence established?

It is well known that many embryonic cells do not simply stay in their place and passively respond to whatever signals waft their way. During gastrulation many cells move great distances, or simply rearrange and reorder themselves, which provides them the opportunity to interact with new neighbors and new signaling environments. We recently found that this cellular reorganizing makes embryonic cells competent to become retinal. Our studies were done in *Xenopus* because a fate map of the early cleavage blastomeres delineates which cells produce retina, which ones are competent to produce retina and which ones are not.[6] First, we tested whether transcription factors that are associated with retinal fate specification can transform non-retinal lineages to produce eye tissues, and uncovered the first clue that being in the right place at the right time is important. Progeny derived from a blastomere far distant from the normal progenitors of the retina could express markers characteristic of retinal cells when we induced these cells to express Pax6 or Rx1, another eye-specifying transcription factor.[5] But most interesting, not every Pax-6 expressing cell became retinal, only those that had moved into the eye competent domain. This suggested that the ectopically expressed transcription factors caused cells to migrate from their initial ventral position into the dorsally located eye field. In fact, an analysis of cell movements during gastrulation demonstrated that the ventrally derived cells expressing these exogenous genes dispersed in a manner characteristic for eye field cells and quite different from that for ventral epidermal cells. This was a surprising finding because there is very little evidence that these transcriptional regulators affect cell motility genes.

These studies raised several additional questions. How do cells detect that they need to move and what signals cue them to the correct locations? We pursued the idea that cell movement into the eye field is an important aspect of acquiring the competence to become retinal by studying two signaling pathways.[7] We were interested in the FGF pathway because it is well known to affect cell movements during gastrulation.[8] Constitutive activation of the FGF receptor 2 (FGFR2) stopped retinal cell progenitors in their tracks during gastrulation; they clumped at the midline, did not enter the eye field or express eye field genes. In situ hybridization studies showed that *fgfr2* mRNA is normally expressed just lateral to the eye field, as though forming a boundary zone. We investigated the EphrinB-Eph pathway because activated FGFRs can phosphorylate the intracellular domain of members of the EphrinB family and modulate their signaling activity. Because Ephrin-Eph signaling also has been strongly implicated in morphogenesis,[9] and because *ephrinB1* mRNA is highly expressed in the eye field, we hypothesized that EphrinB1 signaling might act as an antagonist to FGFR2 signaling in the formation of the eye field. In fact, overexpression and ectopic expression of EphrinB1 promoted cell dispersal and movement into the eye field, whereas FGFR2 had the opposite effect. Furthermore, EphrinB1 reversed the effects of FGFR2 activation in normal retinal progenitors and EphrinB1 knock-down reduced the number of retinal progenitors that gained access to the eye field. Thus, these two signaling pathways coordinately regulate access to the eye field, showing that movement into this field is a critical step in retinal cell fate. Being able to get to the right place defines which competent progenitor cells will receive the appropriate signals to then express the appropriate transcription factors and become retinal.

An important question not resolved by this study is how or whether the FGFR2/Ephrin signaling that controls cell movement into the eye field interfaces with the master-regulatory transcription factors that promote a retinal fate. Although we found no evidence for either FGFR2 or EphrinB1 regulation of Pax6 or Rx1 transcription, both of these latter genes rescued the effects of FGFR2 in retinal progenitors. These results indicate that Pax6 and Rx1 act downstream of the FGFR2/EphrinB1 signaling, but we do not know how far downstream or how many intervening signaling or transcriptional pathways there may be. Another question to be addressed is whether the control of the cell cycle influences these retinogenic cell movements. There is evidence that cell cycle genes are regulated by Pax6 (e.g., ref. 10), and that regulation of the cell cycle during gastrulation movements is critical for normal embryonic patterning (e.g., ref. 11).

The elucidation of the cellular and molecular mechanisms that influence the fate choices of embryonic cells is an extremely important task. We would like to determine how stem cells (embryonic, hematopoietic or adult) can be manipulated to produce replacement cells for congenital, degenerative and traumatic tissue loss, and how to reverse the de-differentiation and migration of tumorogenic cells. These goals will require an understanding of which signaling and transcriptional factors are needed for the required cell types, but also which processes are required to produce cells that are competent to respond to these factors. The study by Moore et al. demonstrates that the process that controls where cells move in their embryonic environment is critical to produce competent cells. Being in the right place at the right time modulates the signals to which cells are exposed and makes them optimally competent to respond correctly to the necessary master-regulatory genes. Elucidating how these different influences on cell fate decisions are interwoven to produce an elegantly arranged, complex tissue like the retina is an important next step.

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