MINIREVIEWS

Initial Cell Type Choice in Dictyostelium

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Much remains to be understood about how a group of cells break symmetry and differentiate into distinct cell types. The simple eukaryote Dictyostelium discoideum is an excellent model system for studying questions such as cell type differentiation. Dictyostelium cells grow as single cells. When the cells starve, they aggregate to develop into a multicellular structure with only two main cell types: spore and stalk. There has been a longstanding controversy as to how a cell makes the initial choice of becoming a spore or stalk cell. In this review, we describe how the controversy arose and how a consensus developed around a model in which initial cell type choice in Dictyostelium is dependent on the cell cycle phase that a cell happens to be in at the time that it starves.

A central question in developmental biology is how cells from the same lineage can differentiate into different cell types (61). Early experiments, for instance, transplanting pieces of endoderm onto different types of mesoderm, showed that signals from one type of mesoderm might cause the endoderm to become a certain cell type, while signals from another type of mesoderm might cause the same endoderm to become a totally different cell type (22). This, however, did not explain how an early lineage initially broke symmetry and differentiated into endoderm and mesoderm, or into different types of mesoderm. Some of the first work on true symmetry breaking, done by observing early cell divisions in nematode embryos, showed that asymmetric cell division is a simple and elegant differentiation mechanism. Another way to break symmetry was found by observing yeast mating type switching, where a genetic cassette is exchanged at some point in the cell cycle (71). There are other potential ways to generate different cell types (12, 49, 76), and an example of a third type of a symmetry-breaking differentiation mechanism was found by observing the development of the social amoeba Dictyostelium discoideum.

Dictyostelium is a soil amoeba that feeds on bacteria (for a review, see references 45 and 51. Each group of cells forms a worm-like 1- to 2-mm-long slug, which crawls toward the soil surface. At the surface, the slug then rearranges itself to form a fruiting body consisting of a mass of spore cells held up off the ground by a thin column of dead stalk cells. So a developing Dictyostelium cell has two potential fates: to become a prestalk (presumptive stalk) cell and die or to become a prespore (presumptive spore) cell and have a chance at forming a new colony.

THE CONTROVERSY

For many years, there was a controversy over how Dictyostelium cells choose to become a prestalk or a prespore cell, and the controversy became somewhat heated (87). One camp argued that there is a morphogen gradient in aggregates (Fig. 1) and that cells use the local concentration of the morphogen to choose their initial fate (38, 44, 47, 60, 87). A compelling candidate for such a morphogen was found and designated differentiation-inducing factor (DIF) (38). The other group argued that the initial cell type choice occurs earlier and is dependent on the cell cycle phase that the cell happens to be in at the time of starvation (4, 25, 58, 62, 82, 93). There are several markers for distinguishing prestalk cells and prespore cells, and the differences in the two proposed mechanisms may have been due to the fact that different groups used different prestalk markers.

IDENTIFYING CELL TYPES: PRESPORE MARKERS

At the slug stage, prestalk and prespore cells are essentially indistinguishable by phase-contrast microscopy, so determining whether a given cell is prestalk or prespore is done by staining cells (8, 72). Prespore cells can be identified by staining with antibodies raised against spore cells or using an expression construct where a prespore promoter drives expression of a marker such as β-galactosidase, and there has been...
IDENTIFYING CELL TYPES: NEUTRAL RED STAINING

Essentially three different ways have been used to identify pre stalk cells, and the interpretation of these three different marker methods has in part led to the controversies in our understanding of the initial cell type differentiation mechanism used by Dictyostelium cells. The first marker used to identify prestalk cells was staining with the vital dye neutral red. When prestalk cells then become stalk cells (black arrowhead) in the fruiting body. Cells at the posterior of a slug (open arrowhead) become prespore cells, which become spores (open arrowhead) in the fruiting body.

IDENTIFYING CELL TYPES: STAINING FOR THE CP2 ANTIGEN

In 1986, the Firtel lab showed that antibodies raised against part of the open reading frame encompassed by the prestalk cathepsin (pst-cathepsin) gene (now called CP2) stained cells at the anterior tip of the slug (24). The beginning of the controversy with CP2 started with CP2. Some groups argued that the CP2 mRNA is only marginally enriched in prestalk cells and thus cannot be used as a reliable marker for prestalk cells (35, 85). However, Mehdy and colleagues (59) showed that the CP2 mRNA is strongly enriched in prestalk cells, and β-glucuronidase expressed under the control of the CP2 promoter was observed only in the prestalk region of slugs (15). Clay and colleagues suggested that the antigen detected by staining with anti-CP2 antibodies is a viable marker for at least some prestalk cells (14). Using the expression patterns of CP2 and ecmA:β-galactosidase, they showed that expression of the CP2 antigen increases sharply at ~8 h of starvation and the number of CP2-positive cells reaches a plateau around ~10 h of starvation, whereas some ecmA:β-galactosidase-positive cells first appear at 16 h of starvation and the number of the positive cells steadily increases until 20 h of starvation. The ecmA:β-galactosidase-positive first appear as a subset of the CP2-positive cells, becoming a larger and larger subset. After 20 h of development, some CP2-negative cells then start expressing ecmA:β-galactosidase. This suggests that CP2 may be an early lineage marker for a subset of the ecmA:β-galactosidase-positive prestalk cells.

NULL CELLS

In Dictyostelium slugs and fruiting bodies, there are some cells that are not stained by either anti-CP2 or antiprespore (anti-SP70) antibodies (24). These cells are called null cells. In migrating slugs, null cells are mainly located in a region between the anterior (prestalk) and the posterior (prespore) region and also appear to be intermixed among the CP2-positive and the SP70-positive cells. Analysis of dissociated slugs showed that roughly 40% of the cells are null (25).

MORPHOGEN GRADIENT-DEPENDENT DIFFERENTIATION

One hypothesis regarding initial cell type choice in Dictyostelium was that the initial differentiation of prestalk and prespore cells is controlled by the concentration of a morphogen in the vicinity of a cell (19, 31, 42). One set of proposed morphogens, DIFs, are a set of chlorinated alky phenones which are dialyzable, lipid-like, and secreted by cells (31, 43, 77, 78). DIFs were identified as signal molecules which can induce isolated cells to differentiate into stalk cells in the presence of cyclic AMP (78). Among the different DIFs, DIF-1 [1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)hexan-1-one] is the main substance found in a purified DIF complex produced by prespore cells. DIF rapidly induces the expression of a subset of prestalk genes and represses the expression of all tested prespore genes (7, 11, 20, 21, 38-40, 43, 60, 86). DIF promotes differentiation of isolated amoebae into stalk cells and inhibits spore formation (44). Mutants (HM44 cells) that are impaired in DIF accumulation are able to form only pre-
spore cells (46). The HM44 cells are unable to make stalk cells in vitro and during normal development on agar, and this condition can be rescued by the addition of exogenous DIF.

Observing that a secreted factor (DIF) potentiates stalk cell formation and represses spore formation suggested that DIF might be part of a morphogen gradient that determines initial cell type choice, with cells that happen to be in a region of the aggregate containing high levels of extracellular DIF becoming prestalk cells, and cells in a region that contains low levels of DIF becoming prespore cells. However, there were at least two problems with this model. First, it was found that prespore cells tend to produce DIF, and that the highest levels of DIF are found in the posterior region of the slug (11), where the prespore cells are located, contradicting the hypothesis that high levels of DIF cause cells to become prestalk. Second, mutants lacking DIF still differentiate into prestalk and prespore cells (69, 70). DmtA methyltransferase is an enzyme that carries out the last step of DIF synthesis (75). In dmtA- mutants, both prespore and pstA cells appear, and the mutants form roughly normal fruiting bodies. However, pstO cells do not appear in the dmtA- mutants, suggesting that there are either multiple signaling molecules or different mechanisms for the induction of pstO and pstA cells (41, 65, 75), which agrees with the observations that mutants that do not accumulate DIF-1 still produce both prestalk and prespore cells (69, 70).

Saito et al. showed that DIF-1 is required to induce the basal disc, which is made up of pstO cells, and that DIF-1 is also partially required for formation of the lower cup, which is a mixed population of prestalk cells (65). In the absence of DIF-1, 30 to 40% fewer stalk cells were formed, suggesting that other prestalk cells can be formed without being exposed to DIF-1. In support of the idea that some aspects of Dictyostelium cell differentiation do not require DIF, it was found that some prestalk markers require DIF for expression (ecmA and ecmB), while other markers such as CP2, cAR2, and TagB do not require DIF for expression (65, 67, 68). Together, it appears that DIF-1 is required only for the differentiation of a subset of prestalk cells and that it is not a master control morphogen for stalk cell differentiation, suggesting that other factors are involved in cell type determination (52, 65, 89).

CELL CYCLE PHASE-DEPENDENT DIFFERENTIATION

As described below, several experiments suggested that the phase of the cell cycle that a cell happened to be in at the time of starvation could influence initial cell type choice (Fig. 2) (4, 5, 13, 23, 25, 57, 62, 83, 93). Dictyostelium cells growing in liquid culture have an ~8-h cell cycle with, as in some fungi, no detectable G1 phase (83). Therefore, most cells are in the G2 phase of the cell cycle, since S phase and M phase take only ~40 min to complete (45, 92).

In the first experiments that indicated that cell cycle phase affects initial cell type choice, cells were synchronized so that most of the cells in the population were at the same phase of the cell cycle. The cells were then labeled with dyes and mixed with unlabeled unsynchronized cells and allowed to develop. Cells in S and early G2 phase at the time of starvation sort out to the anterior regions of developing Dictyostelium slugs and become predominantly prestalk cells, whereas cells in late G2 phase at the time of starvation sort out to posterior regions and become predominantly prespore cells (4, 54, 62). Other workers starved cells in S or early G2 phase to show that the resulting slugs have unusually high percentages of prestalk cells (80, 82). Videomicroscopy using cells grown in a low-density submerged culture, and then starved, showed that cells that are in either the middle or the late G2 phase of the cell cycle at the time of starvation tend to form prespore cells, whereas cells in early G2, S, or M phases tend to become CP2-positive cells (25). Under these conditions, the cells were many cell diameters apart from each other while they were proliferating and developing, and the observations of prespore and CP2-positive cells randomly distributed in the field suggested that cells under essentially identical extracellular conditions could still differentiate in the absence of a morphogen gradient. This experiment also allowed an examination of the lineage of cells and showed that for each cell that was either prespore (SP70 positive) or CP2 positive, the sister cell became a null cell. Null cells sometimes differentiated into prespore cells when they came in contact with other cells, suggesting that cell-cell interactions affect the eventual fate of the null cells (25, 26).

Reinforcing the idea that the phase of the cell cycle that a cell happens to be in at the time of starvation affects the initial cell type choice, it was observed that cells differ in their intrinsic sensitivities to DIF-1 according to their position in the cell cycle at the time of starvation (74). This then suggests that cell cycle phase affects cell type choice before DIF-1-induced differentiation (41, 74).

POSSIBLE CELL CYCLE-DEPENDENT CELL TYPE CHOICE MECHANISMS IN OTHER SYSTEMS

Similar cell cycle phase-dependent mechanisms have subsequently been observed in other systems (3, 34, 64). The Notch signaling pathway plays an important role in the determination
of cell fate in many systems. In Caenorhabditis elegans vulva formation, Notch signaling occurs only during S phase (3). Cell cycle phase-dependent Notch signaling was also observed in asymmetric cell division during Drosophila melanogaster neurogenesis (3). In the formation of Drosophila bristles, cells are more prone to respond to Notch signaling during S phase (64). These results indicate that cell cycle phase may influence cell type choice in multiple systems.

CELL CYCLE PHASE-DEPENDENT DIFFERENTIATION AND CELL PHYSIOLOGY

Two physiological parameters, intracellular pH and calcium levels, have been linked to cell cycle-dependent initial cell type choice in Dictyostelium. In 1988, Gross and colleagues found that intracellular pH regulates differentiation (30). Using inhibitors of the plasma membrane proton pump to decrease the pH of intracellular vesicles, they were able to shift differentiation from the spore to the stalk pathway. Other researchers also found that cell cycle-dependent initial cell type choice is associated with intravesicular pH and cytosolic Ca2+ concentrations (6, 9, 10, 66). Starving Dictyostelium cells in high-Ca2+ medium induces stalk formation and inhibits spore formation (53). In addition, preaggregation and postaggregation Dictyostelium cells are heterogeneous with respect to Ca2+ levels, and cells that contain low Ca2+ levels have a prespore tendency while cells that contain high Ca2+ levels have a prestalk tendency (56, 66). Finally, cells in S and early G2 phase have relatively high levels of cellular Ca2+ and tend to differentiate into prestalk cells, and cells in mid- to late G2 phase have low Ca2+ levels and tend to differentiate into prespore cells (5).

Cytosolic pH also appears to vary during the cell cycle (29, 48, 81). Cells in M and S phases have a high cytosolic pH and have a prestalk tendency, whereas cells in mid- to late G2 phase have a low pH and a prespore tendency (2). In addition, starvation of cells in buffers with different pHs alters cell fate in accordance with the predicted cell fates (1, 2). Brazill et al. examined a mutant (rtoA+) with a randomized cell cycle-dependent cell type choice mechanism (prestalk and prespore cells originate from all phases of the cell cycle) and found that cytosolic pH is also randomized in rto+ cells (9). This suggests the possibility that during evolution, an ancestor of Dictyostelium may have originally used a stochastic mechanism, possibly based on stochastic variations in cytosolic pH, to choose the initial cell type and that RtoA evolved to connect this mechanism to the cell cycle, so that the larger cells with more nutrient reserves (cells in late G2) would become prespore and the smaller cells with fewer nutrient reserves (cells that had just emerged from a cell division) would tend to become stalk. Together, the data suggest that cell cycle-dependent changes in cytosolic or vesicular Ca2+ and/or pH may be a part of the cell cycle-dependent initial cell type choice mechanism.

CONCLUSION

The controversy surrounding the mechanism for initial cell type choice in Dictyostelium was likely caused by incomplete knowledge of the intricacies of prestalk subpopulations and by there not being enough markers to track those subpopulations in time and space. The expression of the ccm markers is dependent on DIF, and this then supported the idea that DIF regulates prestalk differentiation. Once prestalk markers that did not depend on DIF for their expression were discovered, the complexity of prestalk differentiation became apparent.

In Dictyostelium, the final (as opposed to the initial) cell type choice seems to involve both cell signaling and intrinsic biases present in the original growing cells (25, 50, 74). As with cells in many different organisms, Dictyostelium cell differentiation is not completely irreversible. The fate of at least some Dictyostelium cells during development is conditionally specified, meaning that cells can reversibly differentiate in response to external stimuli (for a review, see references 22, 45, 51, and 55). However, even if the fate of some cells in an organism during development is conditionally specified, it does not mean that every cell in the organism is conditionally specified, suggesting that there is room for autonomous specification. Together, these observations suggest that initial cell type choice can begin with cell-autonomous decisions which are later reinforced by a morphogen gradient-dependent mechanism.

ACKNOWLEDGMENTS

We thank Jonathan Phillips for helpful suggestions. This work was supported by the Dongguk University Research fund of 2006 (DRIMS 2006-1094-Z).

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