Who lives and who dies
Role of apoptosis in quashing developmental errors

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Apoptosis is essential for normal development. Large numbers of cells are eliminated by apoptosis in early neural development and during the formation of neural connections. However, our understanding of this life-or-death decision is incomplete, because it is difficult to identify dying cells by conventional strategies. Live imaging is powerful for studying apoptosis, because it can trace a death-fated cell throughout its lifetime.

The Drosophila sensory organ development is a convenient system for studying neural-cell selection via lateral inhibition. We recently showed that about 20% of the differentiating neuronal cells die during sensory organ development, which results in the characteristic spatial patterning of the sensory organs. The eliminated differentiating neurons expressed neurogenic genes and high levels of activated Notch. Thus, live imaging allowed us to document the role of apoptosis in neural progenitor selection, and revealed that Notch activation is the mechanism determining which cells die during sensory organ development.

Apoptosis is essential for normal development. The molecular mechanisms underlying apoptosis induction are highly conserved in worms, flies and mammals.1,2 In addition, the physiological significance of apoptosis has been revealed, especially in neural development.3 Severe brain malformation results when apoptosis is inhibited in flies or mammals,4,9 suggesting that apoptosis regulates the proper cell number by eliminating excess or aberrant cells during neural development. There must be a rule for determining which cells survive, and which die, but how the life-or-death fate is determined in neural development is still unclear. For example, it is uncertain whether the cells that die are selected randomly or whether the dying cells have specific characteristics. Whether a cell lives or dies is probably determined by multiple factors, including the cell's differentiation state, intercellular communications and microenvironment.

In neural-circuit formation, excess innervating post-mitotic neurons are eliminated by programmed cell death. This event is well accounted for by the neurotrophic theory, first proposed in the 1940s, in which limiting amounts of nerve growth factor (NGF) released by target tissues determines the fate of innervating neurons according to their connectivity; poorly connected neurons die from a lack of NGF10-12 (Fig. 1B). However, apoptosis is also critical during the early phase of neural-cell development, during which it regulates the number of neural progenitors13 and limits the amount of proliferation in the neuroblast lineages.14 However, because dying cells are mostly identified by the features of late-stage apoptosis, like nuclear fragmentation, destruction of the membrane structure and caspase activation, the properties of the cells that are fated to die have not been well defined. To elucidate the mechanisms determining the life-or-death fate in early neural development, we need to know the history of the dying cells, including their time of birth, state of differentiation and their interactions with surrounding cells. For such studies, live-imaging analysis has become a powerful tool for tracing the entire lifespan of cells eliminated through apoptosis.

Key words: caspase, apoptosis, drosophila, live-imaging, sensory organ development

Submitted: 04/04/11
Accepted: 04/05/11
DOI: 10.4161/cib.4.2.15739
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Addendum to: Koto A, Kuranaga E, Miura M. Apoptosis ensures spacing pattern formation of Drosophila sensory organs. Curr Biol 2011; 21:1–10; PMID: 21276725; DOI: 10.1016/j.cub.2011.0.
Moreover, the recent development of various fluorescent indicators has made it possible to detect the spatiotemporal dynamics of apoptotic signaling in live animals.

The developing Drosophila sensory organ is a convenient system for studying the process of neural cell-fate determination. The molecular mechanisms by which the sensory organ precursors (SOPs) develop from proneural cell clusters have been well studied. Notch/Delta-mediated lateral inhibition functions to generate a pattern of uniformly spaced SOPs. Through time-lapse imaging, we traced the development of sensory organ pattern formation. SOPs could be distinguished by their expression of the neurogenic gene neuralized. We observed the appearance of neuralized-positive cells, and the beginning of their differentiation into sensory organs. About 20% of the neuralized-positive cells died accompanied by high caspase activation and nuclear fragmentation, and were ultimately eliminated. We then characterized the neuralized-positive cells in detail, to understand how their life-or-death fate is determined.

Surprisingly, we found that the properties of the dying neuralized-positive cells are different from those of the surviving ones. They have characteristics intermediate between those of SOPs and epithelial cells; therefore, we named them, “SOP-like cells.” At their first division, the SOP-like cells divided symmetrically, and their daughter cells did not express other SOP markers. In contrast, normal SOPs divide asymmetrically, accompanied by the expression of neural markers like senseless or prospero. Importantly, the SOP-like cells showed high Notch activation, which was never observed in SOPs. Since Notch signaling functions to determine the non-neural cell fate in the surrounding epithelial cells, we speculated that Notch signaling also determines the cell-death fate of the SOP-like cells. In Notch heterozygous mutant flies, the proportion of SOP-like cells was decreased, and the number of adult bristles increased, indicating that the Notch activation level is a determinant for whether the neuralized-positive cells become SOPs. If a neuralized-positive cell fails to complete its neural differentiation, accompanied by inappropriate Notch activation, it is specifically eliminated via caspase-dependent cell death.
We also asked why excess neuralized-positive cells are produced in the normal developmental context. We examined whether the SOP-like cells could function as a reserve for SOPs when neighboring SOPs are lost. Contrary to our expectation, the fate of the SOP-like cells was irreversible, even when SOPs were ablated. Instead, an alternative SOP arose from the surrounding epithelial cells. This suggested that once Notch is activated in a neuralized-positive cell, the cell’s fate is invariant, and it must die to achieve the proper pattern of neural progenitors. We have not yet determined the detailed mechanisms underlying Notch activation in the SOP-like cells. One possibility is that the surrounding SOPs express Delta and activate Notch signaling in the SOP-like cells. Another is that a cis-interaction of Notch and its ligands determines Notch’s activation level in the SOP-like cells. A Notch indicator that allows to monitor Notch activity with high temporal resolution, which has already been applied to mammalian systems, would be useful for examining this unsolved issue.

Live imaging first made it possible to confirm the existence of SOP-like cells that never develop into sensory organs, by revealing the transient cell fate of neuralized-positive cells. Furthermore, by reversing the imaging movie, we could examine the detailed characteristics of these aberrant cells, like their birth timing, manner of cell division and gene-expression profile. From these analyses, we found that the excess neural progenitors do not die in a random manner during sensory organ development; instead, which neuralized-positive cells die follows a rule and depends on the level of Notch activation. Our recent report showed that apoptosis mediates the fine-tuning of SOP selection. Apoptosis-mediated cell selection has been proposed to eliminate harmful autoreactive lymphocytes in primary lymphoid tissues, suggesting that mammals are also equipped with an apoptosis-mediated error-correction system for eliminating aberrant cells, achieving normal development and maintaining homeostasis.

Acknowledgments

We thank E. Kuranaga for great contributions for this study, and all members of the Miura laboratory for technical support and helpful advice. We thank the University of Tokyo and Leica Microsystems Imaging Center (TLI) for imaging analysis.

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