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Mechanisms for Removal of Developmentally Abnormal Cells: Cell Competition and Morphogenetic Apoptosis

Takashi Adachi-Yamada[*,1,2,3] and Michael B. O'Connor[4,5]

[1]Department of Earth and Planetary System Sciences, Graduate School of Science and Technology, [2]Department of Sciences for Natural Environment, Faculty of Human Development, and [3]SORST, Japan Science and Technology Agency, Kobe University, Kobe 657-8501; [4]Department of Genetics, Cell Biology and Development, and [5]Howard Hughes Medical Institute, University of Minnesota, Minneapolis, Minnesota 55455, USA

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Various cell differentiation signals are tightly linked with apoptotic signals. For example, as a result of somatic mutations, cells within a developing field occasionally receive an altered level of morphogenetic signaling that gives rise to an abnormal cell type. However, these developmentally abnormal cells are frequently removed by activating apoptotic signals. Although such phenomena are crucial for assuring normal development and maintaining a healthy state of various organs, the molecular mechanisms that sense aberrant signals and activate the apoptotic pathway(s) have not fully been investigated. In this review, we discuss recent progress in this area. Cell competition and morphogenetic apoptosis are two kinds of cell death, both of which are mediated by abnormal signaling of Dpp, a member of the TGF-beta superfamily that functions in *Drosophila* as a morphogen, mitogen and survival factor. Cell competition results in autonomous apoptosis induced by reduced reception of the extracellular survival factor Dpp, while morphogenetic apoptosis is nonautonomous, and is induced by contact of cells receiving different levels of Dpp signaling.

Key words: cell autonomy, cell competition, *Drosophila*, JNK, morphogenetic apoptosis.

Abbreviations: Caps, capricious; Dad, daughters against Dpp; Dpp, decapentaplegic; FLP, flipper recombinase; FRT, flipper recombinase target; Fu, fused; GFP, green fluorescent protein; Hh, hedgehog; JNK, c-Jun N-terminal kinase; LRR, leucine rich repeat; Mad, mothers against Dpp; p-Mad, phosphorylated Mad; Puc, puckered; Sal, spalt; Tkv, thick veins; Trn, tartan; Wg, wingless.

Overview

There are many *in vivo* roles for apoptosis (*1*), including the removal of abnormal cells that occasionally occur during development (*2–4*). In many cases, abnormal differentiation signals in developing tissues directly or secondarily cause apoptosis. Here we describe apoptosis induced by abnormal Dpp signaling. Dpp provides several activities to cells within a developing field. It can act as a mitogen to promote growth and division, it can as a survival factor and it can act as a morphogen to specify different cell fates in a concentration dependent manner (*5–7*). Morphogens are thought to be required for early developmental steps in many animal tissues. They are produced in a restricted area of the tissue primordia, and then by either diffusion or transport a concentration gradient is produced throughout the tissue, which provides positional information crucial for proper differentiation of each cell (*8*). During *Drosophila* larval development, Dpp, Wg and Hh are known to act as morphogens within imaginal tissue. In this review we focus on Dpp and describe the cellular responses and significance of apoptosis resulting from abnormal Dpp signaling.

Cell competition induced by *Minute* mutations

In the genetically amenable organism *Drosophila*, cell competition is a phenomenon in which a clone of slowly proliferating cells is removed from a field of normal cells (*9*, *10*). Cell competition has been most frequently studied using *Minute* mutations. *Minute* is a generic name given to a large number of genes that exhibit similar loss-of-function characteristics. The relevant feature of these mutants with respect to cell competition is that heterozygosity for *Minute* leads to slow growth and subsequent elimination of these cells. A recent study of cells heterozygous for one of the *Minute* genes, *Minute(2)60E,* revealed that cell competition eliminates the slow growing cells as a result of autonomous apoptosis that is induced by reduced reception of Dpp (*11*). In this case, it appears that Dpp is a survival signal, and all cells require Dpp to live. Cells that do not receive enough Dpp activate JNK and undergo apoptosis. This role of Dpp may be analogous to that played by various growth factors during *in vitro* culture of certain cell types.

Several intriguing issues remain unanswered. Perhaps foremost among these is; How does slowing cell growth compromise reception of Dpp signaling. All *Minute* mutations characterized to date affect production of ribosomal proteins (*12*). Thus, many general cellular processes are likely to be affected. Perhaps the reduced ribosomal content differentially affects production of Dpp receptors or downstream signaling components such as the Mad and or Medea transcription factors. Alternatively, as sug-

[*]To whom correspondence should be addressed. Tel/Fax: +81-78-803-7743, E-mail: yamadach@kobe-u.ac.jp

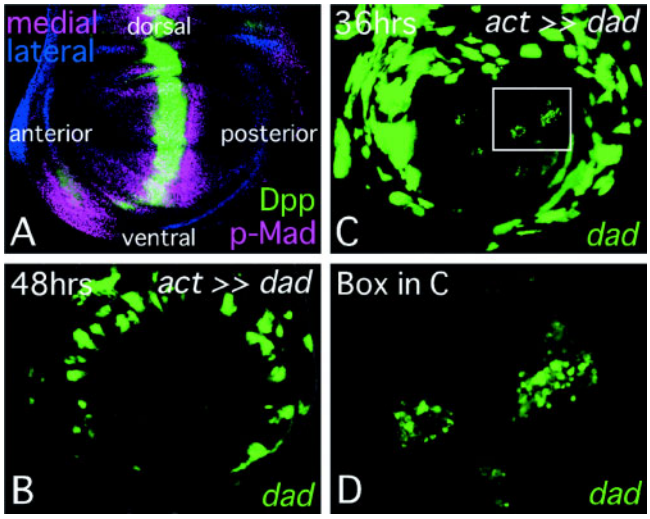

Fig. 1. **Elimination of cells with reduced Dpp signaling.** (A) Dpp expression (green) and p-Mad distribution (magenta) in the normal wing disc. Distribution of p-Mad is found in the medial wing region where high levels of Dpp signaling occur (*33*). Area in blue represents the lateral wing region where low levels of Dpp signaling occur. (B–D) Clones overexpressing *dad*, the gene encoding a repressor of Dpp signaling (*34*, *35*), are randomly induced by the flip-out technique using *hs-FLP* and *actin-FRT-stop-FRT-GAL4* (*36*). (B) Complete elimination of the clones from the central wing area at 48 h after induction. (C) Degeneration of the clones at 36 h after induction. (D) High power image of boxed area in D.

gested by Moreno *et al*. (*11*), perhaps these mutants alter the rate of receptor-ligand internalization which may be necessary for proper Dpp signaling. It will also be intriguing to determine if *Minute* mutations affect the reception of other extracellular survival factors such as Wnts and EGF-type ligands.

Very recently, a cell competition caused by cells overexpressing Myc was reported to involve a nonautonomous apoptosis in the surrounding normal cells, which may be related to the morphogenetic apoptosis discussed in later sections (*13*, *14*). The molecular mechanism of nonautonomy in the Myc-induced apoptosis remains unknown.

Autonomous cell death by mutation of *ras* and other genes

In addition to *Minute* mutations, cell competition can also be induced by mutations in other genes such as Ras, a small G-protein that transduces various cell growth signals (*15*). It is well known that activation of Ras provides both survival cues and growth stimulation to various types of cells (*16*, *17*). Therefore, it is not surprising that loss of *ras* can lead to autonomous cell death caused by loss of the survival signal. Signaling downstream of Ras is divided into two pathways. One employs the Raf-MEK-ERK pathway that controls cell size, affinity and differentiation, and the other utilizes the PI3K-PDK-S6K pathway and only regulates cell size (*18*). Accordingly, the Raf-MEK-ERK pathway is thought to play a greater role in regulation of cell survival. This is supported by the fact that the clones doubly mutant for JNK and Chico, a homolog of the insulin receptor substrate that relays the insulin receptor signal to PI3K, are not removed (*19*). In contrast, cells in which both the JNK and Raf pathways are blocked undergo cell death (*20*). The absence of a requirement for JNK activity in cell death by reduced Raf function is in direct contrast to the case of *Minute* mutations. In other examples, mutations in either Lace, which catalyzes sphingolipid biosynthesis (*21*) or Ste20-like kinase, which regulates cell proliferation (*22*), elicit an autonomous type of cell death without cell competition. Thus it is likely that there are multiple mechanisms for inducing autonomous cell death as a result of reduction in the levels of survival and /or proliferation cues that are independent of both JNK and reception of Dpp signals.

Morphogenetic apoptosis

While reduction in the reception of Dpp signals during cell competition can trigger autonomous cell death, it has also been found that nonautonomous cell death can be triggered by alterations in the reception of Dpp signals. This type of apoptosis was referred to as morphogenetic apoptosis (*19*). The distinction between the two is that to induce nonautonoumous cell death there must be a juxtaposition of cells receiving high levels of Dpp signal next to those receiving low level signal. In this case, the nonautonomous activation of JNK begins in cells on either side of the boundary between the two cell types (Fig. 1 and 2). Morphogenetic apoptosis can also be induced when cells receiving abnormally high levels of Dpp signal as a result of overexpression of an activated receptor Tkv (Tkv^{CA}) are positioned next to cells that receive there normal level of Dpp (Fig. 2E). In this case there is no competition for the ligand itself and so the mechanism is distinctly different from that which induces apoptosis during cell competition. Since in normal tissues cells will receive different levels of morphogen signal depending on their position, it is not the absolute level of received signal that is important in determining whether apoptosis occurs. Rather it appears that cells compare their morphogen signal level with those of neighboring cells. Under normal conditions a morphogen such as Dpp might be expected to create a smooth slope and adjacent cells should therefore receive similar levels of morphogen signal. If however for some reason two adjacent cells receive vastly different levels of signal, then apoptosis is triggered. In this case, however, the cells may not be able to distinguish which one is abnormal. The morphogentic apoptosis mechanism deals with this problem by inducing nonautonomous cell death in order to assure removal of the abnormal cell. Once the cells die, the vacated space is immediately occupied by surrounding normal cells restoring the normal gradient.

Although the same Dpp signaling system is altered, two distinct responses, cell competition and morphogenetic apoptosis, are induced depending on which gene is manipulated. A possible explanation for this observation is that *Minute* mutations affect various survival pathways other than the Dpp pathway, as stated above. Alternatively, in the case of morphogenetic apoptosis, large discrepancies occur in signal reception between adjacent cells, while in the cell competition case, the signal reception discrepancy is likely to be significantly milder. However, variation in the strength of the discontinuity in signal reception does not seem to affect the choice of either mode of apoptosis, since clones with a mild *tkv* mutant

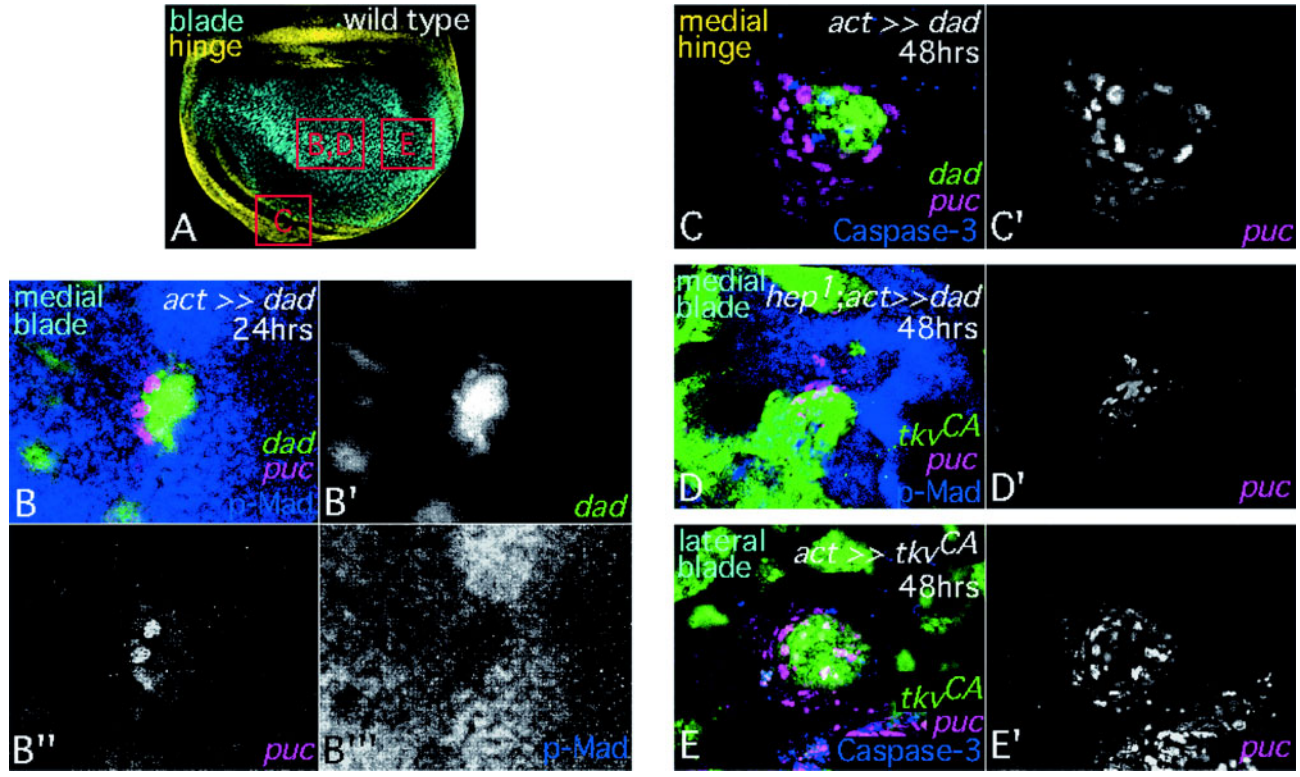

Fig. 2. **JNK activation monitored by expression of the JNK-responsive gene *puc-lacZ* (*23*, *37*) around the clones overexpressing *dad* or *tkv*^{CA}.** Green, GFP; Magenta, *puc-lacZ*; Blue, p-Mad. (A) A wing disc showing the blade (cyan) and hinge (yellow) subdomains. Rectangles in red indicate approximate position of the clones shown in B-E. (B-B‴) A *dad*-overexpressing clone at the medial area of the wing blade at 24 h after induction. A biased distribution of JNK activation around the boundary of the clone is found. JNK activation in the wing disc always results in apoptosis (*19*, *20*). (C and C′) A *dad*-overexpressing clone at the medial area of the wing hinge at 48 h after induction. The clones generated in the hinge grow to a relatively large size and then show clearer nonautonomy of JNK activation. (D and D′) A fused *dad*-overexpressing clone at the medial area of the wing blade in a mild *hep* mutant (*hep*^{1}) background at 48 h after induction. *hep*^{1} delays removal of the clone and induces a clearer nonautonomy of JNK activation. (E and E′) A *tkv*^{CA}-overexpressing clone at the lateral area of the wing blade at 48 h after induction.

did not show cell competition (Adachi-Yamada *et al.* unpubl.).

The cell competition by *Minute* has been reported to occur preferentially in the medial wing blade region close to the Dpp source, rather than in the lateral wing blade region far from the Dpp source (*11*). In contrast, morphogenetic apoptosis is more clearly observed in the lateral wing region, although it can be found in the medial wing region immediately after clone induction (Fig. 2B). Prolonged contact between mutant and wild type cells facilitates the induction of nonautonomous activation of JNK, since clearer nonautonomy can be seen in the medial wing region at the later stage when elimination of the clone is delayed by a mild mutation of *hep* (Fig. 2D), a gene encoding an activator of JNK (*23*). As another finding with regard to the blade/hinge subdomains, morphogenetic apoptosis is readily found in the wing hinge subdomain even in the medial region that is close to the Dpp source (Fig. 2C). Thus, we can regard the wing blade and hinge subdomains as representative tissue examples showing extremely different responses. The choice between cell competition or morphogenetic apoptosis may be defined in advance by genes that they have expressed to specify their tissue identities. In this case, the blade/hinge divergence may be determined by the homeodomain proteins Vestigial (Vg) and Homothorax (Hth), respectively. In addition, as has been shown in the abdominal histoblast, there are tissues that do not show any cell competition (*9*).

How global the morphogenic apoptosis response is in terms of whether it can be induced by discontinuities in the reception of other morphogenic signals is not clear although some data suggesting that both autonomous and nonautonomous apoptosis occur when reception of signals from another morphogen**,** Wg**,** are altered in *Drosophila* wing discs (*19*, *24*, *25*). Also in the case of Wg morphogen, morphogenetic apoptosis is more clearly observed in the hinge subdomain (Adachi-Yamada *et al.* unpubl.).

Short range cell-cell communication prior to cell removal

A recent report describes a phenomenon similar to morphogenetic apoptosis and a hypothetical mechanism to regulate it (*26*). *sal* is a target gene on the Dpp signaling pathway and its transcription is induced by a high level of Dpp signaling (*6*). Thus *sal* is expressed in the medial wing area, where a high level of Dpp signal is present. If clones ectopically expressing *sal* are generated in the lateral region of the wing disc, most of them disap-

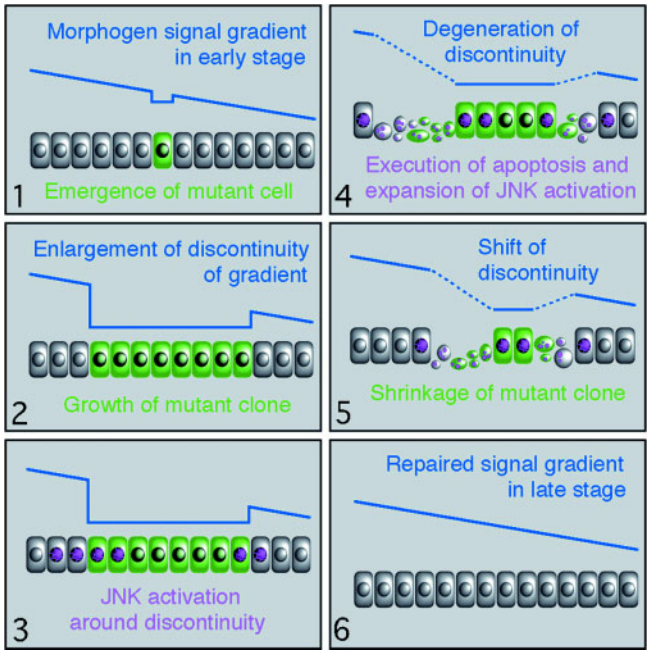

Fig. 3. **Model for the regeneration of a smooth gradient after detection of a discontinuity in a morphogen signal gradient.** (1) A somatic mutation that affects reception of a morphogen signal arises in a field with a smooth morphogen signal gradient in an early developmental stage. (2) The discontinuity in the gradient is initially enlarged by proliferation of the mutant clone. The degree of enlargement likely depends on the position of the clones. A greater discontinuity may induce the apoptotic response more obviously. (3) JNK is activated in the cells on either side of the discontinuity boundary. (4) Apoptosis by JNK activation results in loss of cells on both sides of the discontinuity and degeneration of the discontinuity. (5) The empty area is immediately occupied by surrounding normal cells. Loss of the mutant cells creates an even larger discontinuity in the remaining cells, which may help propagate JNK activation to cells in the interior of the clone. (6) A smooth gradient is recovered by ligand diffusion or transport once the nonreceptive cells are removed.

pear later in development. In these *sal*-overexpressing clones, expression of the *caps* and *trn* genes, both of which encode LRR family transmembrane proteins, is decreased. When expression of either *caps* or *trn* is restored by coexpression with *sal*, these clones can survive. Accordingly, Caps and Trn seem to convey information about the identity of a lateral cell to its neighbors to regulate apoptosis. Since clones doubly mutant for *caps* and *trn* and those overexpressing *caps* or *trn* are rounded, it is believed that these LRR proteins mediate cell affinity in some as yet undefined way. It is likely that nonautonomous cell death is linked to alterations in cell affinity because most clones with abnormal positional information become rounded prior to cell death (*27*–*30*). At present there is no evidence that the LRR proteins are homophilic adhesion molecules.

In fact even truncated forms of Caps and Trn, in which the cytoplasmic tails are lacking, still possess the ability to suppress *sal*-induced apoptosis (*26*). These truncated forms may act as ligands rather than receptors and stimulate neighboring normal cells to send back a survival signal or prevent them from sending a death signal to the *sal*-overexpressing cells. Although these results suggested an attractive direction for future research aimed at deciphering how nonautonomous apoptosis is triggered, various unresolved questions remain. For example, the clones doubly mutant for *caps* and *trn* and the clones overexpressing *caps* or *trn* show a round shape indicating differences in cell affinity but do not undergo apoptosis. Likewise coexpression of *sal* together with *caps* or *trn* can suppress *sal*-induced apoptosis but does not suppress cell affinity differences at least as measure by clone shape. Lastly morphogenetic apoptosis induced by Tkv^{CA} is not suppressed by Caps coexpression (Adachi-Yamada *et al.* unpubl.). Thus, the connection, if any, between these particular LRR proteins and morphogenetic apoptosis is unclear. It should be noted however, that there are several other LRR genes encoded in the *Drosophila* genome whose functions may overlap with *caps* and *trn*.

Another mechanism to detect abnormal levels of morphogen signal

Another way for cells to detect abnormal levels of morphogen signaling is to measure the ratio of signaling between multiple morphogens that are generally present in developing tissues (*19*). For example, in a mild mutant of *tkv*, all of the cells throughout the wing disc receive relatively low levels of Dpp signal. Thus there is no sharp discontinuity generated in reception of Dpp signal in these mutant discs. Nevertheless, some cells in the wing tip area just anterior to the anteroposterior compartment boundary, undergo apoptosis by activating JNK. In wild type, the cells in this area receive high levels of both Hh Dpp signaling, and no cells receive high levels of Hh signaling with low levels of Dpp signaling, because Hh induces Dpp production (*8*). Thus it appears that an imbalance between signaling levels of the two morphogens can also be used to trigger apoptosis. If this imbalance is partly corrected by making a double mutant for *tkv* and *fu*, a gene encoding the intracellular transducer of Hh signaling, JNK activation is drastically suppressed. Accordingly, cells receiving an abnormal level of the morphogen signals can partly undergo apoptosis autonomously, even when all of the cells in the tissues are mutant, as well as when a mutant clone is locally generated (*19*).

Other nonautonomous apoptosis

At least two other cases of nonautonomous cell death in the wing disc have been reported. In one case, overexpression of activated Ras leads to nonautonomous death at a distance (*31*), while in another, autonomous death by expression of the toxic protein Ricin in the posterior compartment leads to induction of nonautonomous death in the anterior compartment (*4*, *32*). In the former case, the nonautonomous death might be related to cell competition, because local activation of Ras generates a large population of relatively slowly growing cells in the surrounding region. In the latter case, the purpose of nonautonomous death in the anterior compartment appears to be to match the size of the anterior compartment to that of the posterior compartment. As a result, the relative sizes of the anterior and posterior compartments remain constant. In this case the involvement of JNK in mediating the death response has not been examined and the

trigger mechanism is likely to be quite distinct from morphogenetic apoptosis since it occurs over long range and does not linking involve cell affinity issues. This nonautonomous effect was shown to be mediated by cell cycle arrest (*32*).

Concluding remarks

Recent studies have revealed that cells with an abnormal fate due to altered levels of morphogen signals are either autonomously removed during development by cell competition or nonautonomouly removed by morphogenetic apoptosis. At present it is not clear how frequently nonautonomous apoptois mechanism is used to remove cells with abnormal fates. Furthermore, which kinds of cell-cell interactions are required to activate the cell death pathway is an interesting but poorly resolved question. Further study aimed at elucidating the molecular mechanism(s) that induce the process may provide novel insights for development of new diagnostic and therapeutic tools for the detection and treatment of various pathological states caused by somatic mutations.

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