Myc, Cell Competition, and Compensatory Proliferation

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Abstract

The proto-oncogene Myc is already known to affect many cellular processes, but recent experiments in the fruit fly *Drosophila melanogaster* have revealed yet a new facet of Myc. Neighboring cells were shown to compare their Myc levels and the losers (cells with lower Myc activity) were actively eliminated. This phenomenon is called "cell competition," and it seems to be part of a developmental size and quality control program. Subversion of this mechanism may contribute to the transforming powers of Myc and possibly other oncogenes. (Cancer Res 2005; 65(15): 6485-7)

Background

In recent years, the modest fruit fly *Drosophila melanogaster* has become a popular model system for the analysis of cellular growth control and organ size determination during animal development. For their studies, many biologists have focused on the so-called wing imaginal discs, the precursor organs of adult wings and thoraxes. Wing imaginal discs originate from a group of 40 to 50 cells that are set aside at the end of embryogenesis. During the 4 days of larval development, these cells multiply 1,000-fold to form the mature imaginal disc, which consists mainly of a columnar epithelial monolayer (1). The cellular growth and cell cycle characteristics during this proliferative phase closely resemble those of vertebrate cells, inasmuch as similar regulatory proteins have been found to function in both situations. In contrast to cells cultured on plastic, however, imaginal disc cells are embedded in an intact tissue and subject to physiologic short- and long-range signals that cannot be observed *in vitro*. One phenomenon based on such signals was identified 30 years ago: cell competition. Cell competition was observed with a group of mutations called *Minutes* (*M*; ref. 2). *Minutes* are mutations in ribosomal protein genes (3) that are characterized by recessive lethality and by a dominant growth defect. Thus, heterozygous *M*+/− flies are delayed in their development and take longer to reach their normal size, a reflection of the slower growth rate of *M*+/− cells. Importantly, however, *M*+/− cells are viable and can give rise to almost normal-looking animals. In striking contrast, when clones of slow-growing *M*+/− cells are generated in an animal that otherwise consists of wild-type cells, the *M*+/− cells are actively eliminated—a process dubbed "cell competition"; although such clones can be observed shortly after they have been induced, within 2 days no more surviving *M*+/− cells can be seen (2).

Key Finding

A very similar growth defect was recently found to be associated with the sole *Drosophila* orthologue of the proto-oncogene Myc, dMyc. Whereas *dmyc* is an essential gene, hypomorphic *dmyc* mutations are viable and only characterized by a modest growth defect. However, when clones of cells carrying such a hypomorphic *dmyc* mutation are surrounded by phenotypically wild-type cells, they suffer from the same type of cell competition as seen for *M*+/− cells (4). Interestingly, this cell competition is not simply caused by cellular defects associated with the *dmyc* mutation: Even wild-type cells were shown to be competed when they are surrounded by cells overexpressing dMyc, suggesting that cells somehow compare their dMyc level to that of their neighbors and it is this relative dMyc level that determines whether a cell is competed out of existence (5, 6); importantly, as little as 2-fold differences in dMyc levels are already sufficient to trigger cell competition. This dMyc-dependent cell competition shares two characteristics with the competition of *M*+/− cells by wild-type cells. First, both only act over a short distance—wild-type cells are only competed up to eight cell diameters from dMyc-overexpressing cells. Second, this cell competition does not function across the boundary between the anterior and the posterior compartment of the wing disc (roughly corresponding to two halves of the wing disc; i.e., wild-type cells in the posterior compartment are not competed by dMyc-overexpressing cells situated in the anterior compartment). The molecular basis for these features is not clear.

Similar types of cell competition have also been described for other genes, e.g. Ras and the Ste20-like kinase Slik (7–9). As Ras has been shown to control dMyc protein levels posttranscriptionally, its effects on cell competition might be mediated by dMyc; for Slik, the relevant downstream effectors are unknown. It is important to note, however, that not all mutants affecting growth induce cell competition and a simple difference in growth rate between neighboring cells is not sufficient to trigger competition. In particular, mutations in the insulin-signaling pathway, one of the main controllers of growth and organ size in *Drosophila*, strongly reduce growth but do not induce competition; conversely, overexpression of phosphoinositide-3-OH kinase (which acts downstream of the insulin receptor) entails massive overgrowth but does not lead to elimination of neighboring wild-type cells. Similarly, overexpression of cyclin D in combination with Cdk4 promotes cell-autonomous overgrowth without affecting adjacent cells (5). What then distinguishes dMyc from other growth promoters and places dMyc in the same category as *Minutes*? An explanation may be found in the molecular function of Myc. Myc is a transcription factor that controls the expression of a wide variety of target genes, but most notably components of the ribosome and proteins involved in ribosome assembly transcribed by RNA polymerase II (10–13), rRNAs transcribed by RNA polymerase I (14–16), and the products of RNA polymerase III (17). Myc deregulation, therefore, strongly influences the activity of ribosomes, and whatever mechanism is impaired in *M*+/− cells is likely to be also affected in *dmyc* mutants or upon dMyc overexpression. Consistent with this assumption, the *Minute M(2)60E*, which disrupts the ribosomal

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protein gene *rpl19*, a transcriptional target of dMyc, dominantly suppresses the ability of dMyc-overexpressing cells to compete their neighbors away (6).

There is currently no definitive explanation for how relative differences in ribosome activity could lead to cell competition. According to one scenario, a reduced efficiency in transducing the signal of the morphogen *Decapentaplegic* (Dpp, the principal transforming growth factor-β family member in *D. melanogaster*) might play a role. It is not clear how impaired ribosome function might affect this process, but it has been shown that *M*+/− cells suffering from cell competition experience a lower Dpp response than their neighbors; both competed *M*+/− and *dmyc* mutant cells can be partially rescued by overstimulation of the endocytosis pathway that presumably also increases the efficiency of Dpp signal transduction. We do not know how the Dpp signaling activities are compared between neighboring cells, but a relative reduction of Dpp signal can then activate the stress-inducible Jnk pathway that ultimately triggers the execution of the apoptotic program (6, 18). The requirement for Jnk signaling for cell competition has been disputed, however, and it has been suggested that only the proapoptotic gene *hid*, but not *jnk*, is required for the induction of apoptosis (5). It should also be noted that cell competition has also been reported for cells with an inappropriately overactive Dpp pathway (19); furthermore, two other recent publications have shown that even cells completely lacking the ability to transduce the Dpp signal may be able to survive—although these cells are still forced to leave the epithelial monolayer and, thus, may experience at least some aspects of cell competition (20, 21).

Whatever the molecular mechanism of cell competition, it is generally assumed that this process provides a means of quality control whereby slowly proliferating (i.e., potentially damaged) cells are eliminated and replaced by their fitter neighbors. Cell competition also plays an important role in the regulation of organ size (5). When cell competition is reduced in the wing imaginal disc, by means of a mutation in the proapoptotic gene *hid*, the average size of adult wings is unchanged, but the distribution of wing sizes becomes much broader than in wild-type control (i.e., many more wings are either much smaller or much bigger than the average). Consistent with a role for cell competition in size control, overexpression of either

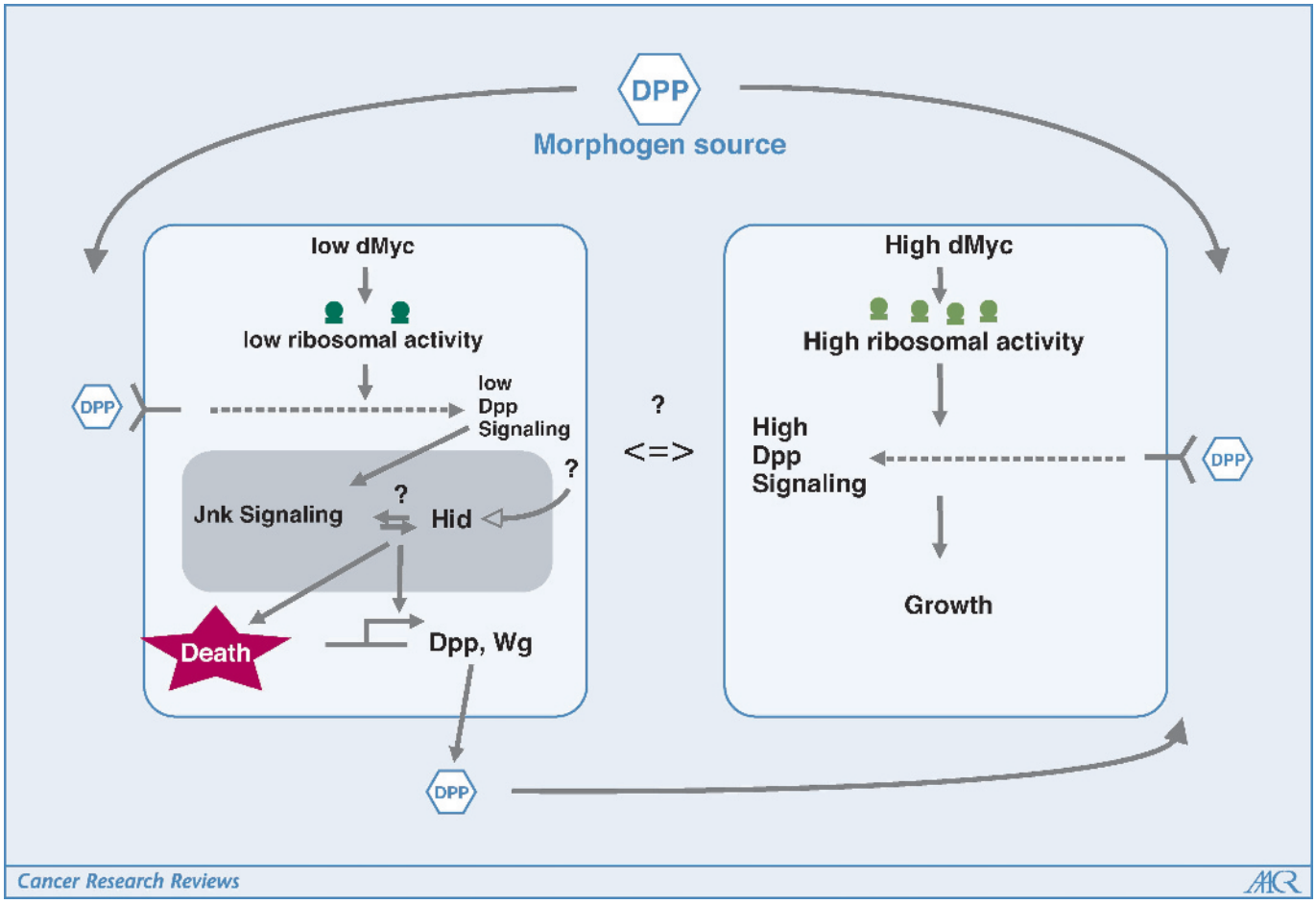

Figure 1. A speculative model for cell competition and compensatory proliferation. Ribosomal activity (i.e., the general ability of a cell to synthesize proteins) defines the "fitness" of a cell; this fitness is strongly influenced by Myc, because Myc plays a central role in controlling the levels of ribosomal components. Mutations in any component of the ribosome assembly line (e.g., in *myc*) reduce the fitness of a cell compared with its neighbor; this reduced fitness in turn impairs the ability of the mutant cell to transduce Dpp signals (by some unknown mechanism). Dpp signaling levels are (somehow) compared between neighboring cells; a cell autonomous reduction in Dpp signal transduction (compared with the neighbors) then triggers an apoptotic program through the activation of the Jnk pathway and/or the *hid* gene, which has two ultimate consequences: the elimination of the faulty cell and the emission of a Dpp or Wg signal to the neighborhood (which hereby is incited to replace the dying cell). The imminent death of the signal-sending cell ensures that this proproliferative signal is very short-lived and does not self-amplify, and, furthermore, that the overall distributions of the morphogens Wg and Dpp (which are important to shape the wing) are not grossly disturbed (25).

phosphoinositide-3-OH kinase or cyclin D plus Cdk4 in a subset of cells in the wing imaginal disc result in an increased adult wing size; in contrast, dMyc overexpression in the same system only induces cell autonomous overgrowth, but thanks to cell competition the resulting adult wings have wild-type size. However, when ectopic dMyc expression is extended to all cells of the wing imaginal disc, no more cells with relatively lower dMyc levels are left that could be competed away, and as a result of the growth-promoting effects of dMyc the adult wings are significantly larger than wild type (5).

Thus far, we have discussed the story of dMyc and cell competition. A recent set of publications has now added an interesting twist to the interaction between slow- and fast-growing cells. These studies began with the observation that normal adult structures are developed from *Drosophila* imaginal discs, even if half of all cells in the imaginal disc are artificially eliminated (e.g., by X-irradiation; ref. 22). To find out how imaginal discs could cope with such a massive loss, three groups used different means to initiate apoptosis and then prevented the execution of downstream processes by ectopic expression of the viral protein p35 (which blocks downstream executor, but not the upstream initiator caspases; refs. 23–25). Whereas the publications disagree with respect to the relative importance of different triggers to induce apoptosis (*diap1* inactivation versus Dronc activation), they all agree that the resulting "undead" cells then synthesize some growth factors, which stimulate proliferation of the surrounding wild-type cells. Ryoo et al. (25) go on to show that the generation of this stimulatory signal requires the activation of the Jnk pathway, and both Perez-Garijo et al. (23) and Ryoo et al. (25) identify the secreted growth factors as Wg and Dpp. How exactly these growth factors promote proliferation in the recipient cells was not analyzed any further, but it is conceivable that part of the effects of Dpp is mediated by dMyc, as Dpp has previously been reported to positively regulate *dmyc* expression in the wing imaginal disc (26).

Meaning and Implication

This brings us back to cell competition. Based on the data discussed above, the close collaboration of cell competition and compensatory proliferation is likely to be important for the proper development of different fly tissues (see Fig. 1 for a graphical summary of the data). At present, we can only speculate about the possible relevance of these processes for non-insects. It is an intriguing possibility, however, that mutations in genes involved in cell competition or compensatory proliferation might contribute to deregulated growth and cancer. Mutational activation of *myc* genes is certainly widely encountered in human tumors. Myc overexpression contributes in many ways to cellular transformation, but the ability of Myc-overexpressing cells to kill their normal neighbors (by means of cell competition) would certainly add to their selective advantage (6). On the other hand, Myc overexpression has long been known to induce apoptosis cell autonomously, and Myc-overexpressing cells need to acquire secondary mutations to avoid an untimely death (27). It is well conceivable that such an antiapoptotic mutation might lock a cell in a state similar to that of the "undead" wing imaginal disc cells overexpressing p35—and as a consequence such cells might permanently synthesize (self-stimulatory) growth factors (25).

None of these possible mechanisms have been experimentally examined. It should be noted, however, that recent experiments with murine embryonic stem cells carrying a mutation in the gene coding for the ribosomal protein RpL24 have revealed the existence of a phenomenon similar to cell competition in vertebrates (28). No doubt, we will soon hear more about the influence of cell competition and compensatory proliferation on vertebrate development and disease.

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References

1. Cohen SM. Imaginal disc development. In: Bate M, Martinez Arias A, editors. The development of *Drosophila melanogaster*. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1993. p. 747–841.
2. Morata G, Ripoll P. Minutes: mutants of *Drosophila* autonomously affecting cell division rate. Dev Biol 1975; 42:211–21.
3. Lambertsson A. The *minute* genes in *Drosophila* and their molecular functions. Adv Genet 1998;38:69–134.
4. Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P. *Drosophila* myc regulates cellular growth during development. Cell 1999;98:779–90.
5. De La Cova C, Abril M, Bellosta P, Gallant P, Johnston LA. *Drosophila* myc regulates organ size by inducing cell competition. Cell 2004;117:107–16.
6. Moreno E, Basler K. dMyc transforms cells into super-competitors. Cell 2004;117:117–29.
7. Prober DA, Edgar BA. Ras1 promotes cellular growth in the *Drosophila* wing. Cell 2000;100:435–46.
8. Karim FD, Rubin GM. Ectopic expression of activated Ras1 induces hyperplastic growth and increased cell death in *Drosophila* imaginal tissues. Development 1998; 125:1–9.
9. Hipfner DR, Cohen SM. The *Drosophila* sterile-20 kinase slik controls cell proliferation and apoptosis during imaginal disc development. PLoS Biol 2003;1:E35.
10. Eisenman RN. Deconstructing myc. Genes Dev 2001; 15:2023–30.
11. Orian A, van Steensel B, Delrow J, et al. Genomic binding by the *Drosophila* Myc, Max, Mad/Mnt transcription factor network. Genes Dev 2003;17:1101–14.
12. Levens DL. Reconstructing MYC. Genes Dev 2003;17: 1071–7.
13. Hulf T, Bellosta P, Furrer M, et al. Whole-genome analysis reveals a strong positional bias of conserved dMyc-dependent E-boxes. Mol Cell Biol 2005; 25:3401–10.
14. Grandori C, Gomez-Roman N, Felton-Edkins ZA, et al. c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. Nat Cell Biol 2005;7:311–8.
15. Arabi A, Wu S, Ridderstrale K, et al. c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. Nat Cell Biol 2005;7:303–10.
16. Grewal SS, Li L, Orian A, Eisenman RN, Edgar BA. Myc-dependent regulation of ribosomal RNA synthesis during *Drosophila* development. Nat Cell Biol 2005;7: 295–302.
17. Gomez-Roman N, Grandori C, Eisenman RN, White RJ. Direct activation of RNA polymerase III transcription by c-Myc. Nature 2003;421:290–4.
18. Moreno E, Basler K, Morata G. Cells compete for decapentaplegic survival factor to prevent apoptosis in *Drosophila* wing development. Nature 2002;416: 755–9.
19. Adachi-Yamada T, O'Connor MB. Morphogenetic apoptosis: a mechanism for correcting discontinuities in morphogen gradients. Dev Biol 2002;251:74–90.
20. Gibson MC, Perrimon N. Extrusion and death of DPP/BMP-compromised epithelial cells in the developing *Drosophila* wing. Science 2005;307:1785–9.
21. Shen J, Dahmann C. Extrusion of cells with inappropriate Dpp signaling from *Drosophila* wing disc epithelia. Science 2005;307:1789–90.
22. Milan M, Campuzano S, Garcia-Bellido A. Developmental parameters of cell death in the wing disc of *Drosophila*. Proc Natl Acad Sci U S A 1997;94:5691–6.
23. Perez-Garijo A, Martin FA, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in *Drosophila*. Development 2004;131:5591–8.
24. Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the *Drosophila* wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. Curr Biol 2004;14: 1262–6.
25. Ryoo HD, Gorenc T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Dev Cell 2004;7: 491–501.
26. Prober DA, Edgar BA. Interactions between Ras1, dMyc, and dPI3K signaling in the developing *Drosophila* wing. Genes Dev 2002;16:2286–99.
27. Green DR, Evan GI. A matter of life and death. Cancer Cell 2002;1:19–30.
28. Oliver ER, Saunders TL, Tarle SA, Glaser T. Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse Minute. Development 2004;131:3907–20.