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# Myc: A Weapon of Mass Destruction

Minireview

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**Growth and proliferation potentiated by deregulated *myc* oncogene expression is balanced by *myc*-induced apoptosis. Abrogation of this apoptotic pathway in Myc overexpressing cells leads to cancer progression. Recent work has shown that cell clones in the *Drosophila* wing disc with higher dMyc expression levels act as supercompetitors to potentiate the programmed death of surrounding normal cells. Yet another paper identifies dE2F1 as a critical component of pathways that normally restrict the ability of growth perturbing genes like dMyc to cause organ overgrowth.**

In mammalian cells, the highly regulated expression of Myc family transcription factors is closely tied to cell growth and proliferation as well as inhibition of terminal differentiation and induction of apoptosis (Grandori et al., 2000). By contrast, deregulation of Myc expression drives progression of many different types of cancer. In general, Myc function has been viewed as an almost entirely cell autonomous phenomenon with most research focusing on essentially uniform populations of cells that overexpress or underexpress Myc. However, recent studies employing *Drosophila melanogaster* as a model system have shed light on the role of Myc-induced cell growth in organ size control mediated by competitive interactions with neighboring cells. These findings raise interesting questions regarding the role of Myc in sculpting tissues and in the etiology of cancer.

### *Myc Family Proteins Regulate Cell Growth*

*Drosophila* has a single *myc* gene, *dmyc*, encoded by the *diminutive* (*dm*) locus, that is functionally similar to mammalian *myc* genes (Gallant et al., 1996; Schreiber-Agus et al., 1997). Viable, hypomorphic *dmyc* alleles produce small adults, suggesting that dMyc is required for cell and/or organ growth control. Indeed, dMyc is likely to be rate limiting for cell growth as *dmyc* mutant cells are smaller than control cells and ectopic expression of dMyc results in larger cells (Johnston et al., 1999; Maines et al., 2004; Pierce et al., 2004). Despite its strong influence on cell size, cell division time is not appreciably affected by modulation of dMyc levels (Johnston et al., 1999). This aspect of Myc function is consistent with recent results from expression array analysis and genome-wide searches for Myc targets in *Drosophila* and mammalian cells (Levens, 2003). The majority of Myc target genes appear to be involved in cell metabolism, ribosome biogenesis, and translational control.

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### *dMyc-Induced Cell Competition*

The wing imaginal disc, the larval primordium of the adult wing, has proven a useful tool to study the processes required to generate adult structures of the correct size, shape, and pattern (Milan, 1998). The wing disc is an epithelial layer originating from a group of approximately 50 embryonic cells that divide rapidly during the larval stages to give rise to a mature disc containing ~50,000 cells. Cells that do not grow or divide as rapidly as other cells in the disc are quickly eliminated and replaced without affecting adult wing size. The process of sensing and eliminating slower growing cells within the wing disc is referred to as cell competition. This phenomenon was first described using a class of mutants, collectively referred to as *Minutes*, that cause reduced ribosome biogenesis when heterozygous, resulting in slow growth and small adults (Morata and Ripoll, 1975). Although *Minute* heterozygous cells do not suffer any disadvantage in a homotypic environment, clones of *Minute* cells are quickly eliminated from wild-type wing discs. In a similar manner, wing discs that are wholly mutant for the weak *dmyc* allele $dm^{P0}$ are viable and survive until adulthood, while clones of cells homozygous for $dm^{P0}$ generated in a $dm^{P0}$ heterozygous background are eliminated from the disc epithelium (Johnston et al., 1999).

Two papers in this issue of *Cell*, by de la Cova et al. (2004) and Moreno and Basler (2004), investigate the phenomenon of cell competition in response to altered dMyc levels and clearly demonstrate that cells with higher levels of dMyc out-compete adjacent lower dMyc-expressing cells which are eliminated by apoptosis. These papers address two key questions: what is the mechanism of dMyc-induced cell competition and what is its role in the regulation of organ size?

Both groups use genetic techniques to generate different dMyc levels in neighboring cells to investigate cell competition in wing discs. Cells with higher and lower levels of dMyc were juxtaposed either by generating cells homozygous for the hypomorphic *dmyc* allele $dm^{P0}$ in a $dm^{P0}$ heterozygous background or by generating cells with higher levels of dMyc using a duplication of the *dmyc* locus or UAS/Gal4-mediated overexpression in a wild-type background. In all cases, cells with higher levels of dMyc out-compete their lower dMyc-expressing neighbors, resulting in larger clones of the dMyc overexpressing cells relative to the cells expressing lower dMyc levels. Thus cells with a given dMyc level will out-compete neighboring cells with lower Myc levels but will themselves be out-competed if surrounded by cells expressing even higher dMyc. The abundance of dMyc relative to neighboring cells therefore determines the extent of competition. Interestingly, proximity to the cells expressing higher levels of dMyc dictates the degree of cell competition, such that cells within eight cell diameters of the dMyc-expressing cells suffer the strongest competitive disadvantage (de la Cova et al., 2004). It is also significant that dMyc-induced cell competition requires dMyc's ability to act as a transcription factor. dMyc-regulated activation of ribosomal protein genes is necessary, but not sufficient, for com-

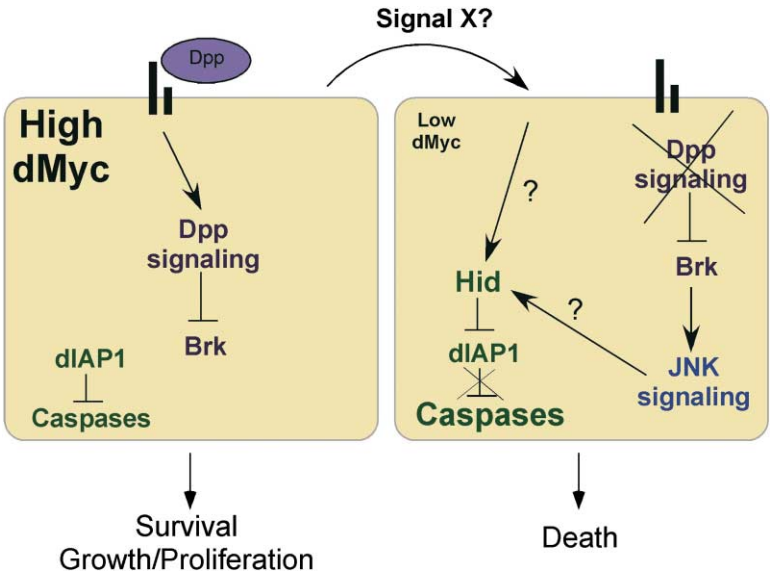


Figure 1. A Model for dMyc-Induced Cell Competition

When cells with differing levels of dMyc are juxtaposed, cells with lower dMyc are eliminated by apoptosis. At least two different mechanisms are implicated in the death of the low dMyc-expressing cell (see text). Cells with less dMyc relative to surrounding cells internalize less Dpp, by an unknown mechanism, leading to upregulation of the transcriptional repressor Brk and JNK pathway-mediated activation of caspases and apoptosis. In addition, JNK-independent induction of Hid expression and subsequent apoptosis of the low dMyc-expressing cell can also occur, perhaps in response to an as yet unidentified short-range signal (Signal X) from the high dMyc cell or to other changes in the cellular environment. Higher dMyc-expressing cells therefore survive and eventually replace lower dMyc-expressing cells that have died.

petition (Moreno and Basler, 2004) indicating that competition is likely to require dMyc's ability to coordinately regulate the expression of numerous growth regulatory genes.

***dMyc-Induced Cell Competition: A Race for Survival Factors?***

Surprisingly, Moreno and Basler (2004) and de la Cova et al. (2004) find that dMyc-mediated cell competition induces cell death in adjacent cells expressing lower levels of dMyc. Myc has long been known to sensitize cells to apoptosis, particularly when expressed at hyperphysiological levels and survival factors are limiting. This apoptosis is cell autonomous—i.e., it occurs in the Myc-overexpressing cells (Hueber and Evan, 1998). However in the wing disc, cells with higher dMyc levels thrive while cells with reduced dMyc levels relative to their neighbors display markers for apoptosis such as TUNEL labeling and expression of activated caspase-3 and the proapoptotic gene *head involution defective* (*hid*). Indeed, when apoptosis is inhibited by reducing the gene dosage of *hid* or by ectopically expressing the antiapoptotic proteins dIAP1 or p35, clones of cells expressing lower levels of dMyc grow much larger relative to surrounding cells, indicating that apoptosis is essential for the response to cell competition.

The c-*J*un *N*-terminal *k*inase (JNK) pathway has been shown to be required for cell competition-associated cell death using *Minute* mutations (Moreno et al., 2002). dMyc-induced cell competition is likely to act through a similar pathway since components of the JNK pathway are induced in low dMyc-expressing cells and expression of the JNK pathway inhibitor Puckered (Puc) efficiently blocks competition-associated cell death (Moreno and Basler, 2004). To explain the observation that cells expressing lower levels of dMyc die only when located adjacent to higher dMyc-expressing cells, Moreno and Basler (2004) propose that cells with higher levels of dMyc out-compete their neighbors for a limiting extracellular survival signal. Consistent with this, enhancing endocytosis of a variety of extracellular signaling molecules by overexpressing Rab5 specifically in the lower dMyc-expressing cells allows their survival. While other signaling pathways may also be involved, Moreno and Basler (2004) focus on the role of the Decapentaplegic (Dpp) pathway that is required for cell growth and survival in wing discs (Burke and Basler 1996). Alterations in expression of the Dpp downstream genes *spalt* and *brinker* (*brk*) suggest that Dpp signaling is compromised in lower dMyc-expressing cells. In addition, survival of cells expressing lower relative levels of dMyc can be rescued by overexpression of Dpp itself or of a constitutively active form of the Dpp receptor Thickveins ($Tkv^{QC}$), strongly suggesting that it is reduced Dpp signaling in cells under competitive stress that leads to apoptosis.

Under competitive stress induced by *Minutes*, Brk is likely to mediate JNK-dependent apoptosis due to insufficient Dpp signaling (Moreno et al., 2002), although the mechanism by which this would occur remains to be determined. While Brk levels have not been shown to be critical for dMyc-mediated cell competition, it remains likely that dMyc-induced competition relies on the relative abilities of higher and lower dMyc-expressing cells to endocytose a limited supply of the Dpp extracellular ligand (Figure 1). While the experiments presented by Moreno and Basler (2004) provide a convincing model for the involvement of Dpp signaling in dMyc-induced cell competition, the mechanism by which reduced dMyc levels leads to downregulation of the Dpp-signaling pathway remains unclear. One possibility is that under conditions of competitive stress, dMyc may directly regulate the transcription of genes required to bind or internalize Dpp. Cells with higher dMyc would therefore more effectively sequester and utilize limiting Dpp than cells with relatively lower levels of dMyc. However, expression of genes known to be involved in these processes is not significantly altered in response to dMyc overexpression, nor have they been identified as direct dMyc targets (Orian et al., 2003). Thus, while dMyc may regulate subtle transcriptional changes in these genes or regulate their expression posttranscriptionally, it is more likely that an indirect

mechanism links dMyc levels with Dpp signaling in cells under competitive stress.

In contrast to Moreno and Basler (2004), de la Cova et al. (2004) did not detect changes in expression of components of either the JNK or Dpp pathways during cell competition. de la Cova et al. (2004) do find, however, that genetically eliminating JNK signaling using a *hemipterous* (*hep*) mutant reduces cell competition-associated apoptosis. This argues that, while changes in expression of JNK pathway components were not detected, JNK-mediated apoptosis does play a role in their cell competition assays. Interestingly, the reduction in cell competition-associated cell death observed in a *hep* mutant was less striking than that observed by reducing levels of the proapoptotic gene *head involution defective* (*hid*). Cell competition-associated cell death is therefore likely to be mediated by both JNK-dependent and -independent pathways.

### *What Is the Role of dMyc-Induced Cell Competition during Wing Development?*

Overexpression of dMyc promotes cell growth and induces cell competition in adjacent cells expressing lower dMyc. However, an imbalance in cellular growth rates is not sufficient to induce cell competition as cooverexpression of cyclin D with its kinase partner Cdk4 or activation of the insulin/PI3K pathway results in increased cell growth but does not induce cell competition (de la Cova et al., 2004). Correlating with this, expression of cyclin D/Cdk4 or activation of the insulin/PI3K pathway leads to overgrowth of the adult wing, while overexpression of dMyc does not. Based on these observations, de la Cova et al. (2004) propose that cell competition is required to generate appropriately sized adult appendages. This model predicts that eliminating overt cell competition by overexpressing dMyc throughout the entire disc, as opposed to the mosaic expression described above, would allow overgrowth and result in a larger adult wing. Indeed, larger wings are observed upon ubiquitous overexpression of dMyc during wing development. Reestablishing cell competition by "inserting" clones of cells with wild-type dMyc levels reverses this overgrowth effect, even though the majority of the adult wing is comprised of dMyc-overexpressing cells. Thus, even a small number of cells expressing wild-type levels of dMyc is sufficient to induce cell competition and generate a normal-sized wing. Given that cell competition leads to apoptosis of cells expressing lower dMyc, inhibiting the death of these cells would then be expected to lead to wing overgrowth. Consistent with this prediction, overexpression of dMyc in animals heterozygous for the proapoptotic gene *hid* results in a dramatic reduction in cell competition and produces larger than normal wings. Competition-induced cell death is therefore required for regulation of wing size in response to overexpression of dMyc.

Assays such as the ones described above raise the important question of whether cell competition occurs during normal development. Cell competition may be an adaptation to prevent overgrowth caused by misregulation of genes such as *dmyc*. Alternatively, it may allow discs to respond to and compensate for local variations in nutritional conditions or mitogenic signaling that might otherwise adversely affect adult appendage size. If so, preventing competition by eliminating cell death during wing development should lead to disc overgrowth. However, the mean wing disc size of animals that overexpress the apoptosis inhibitor p35 remains unchanged (de la Cova et al., 2004). Nonetheless, these discs exhibit significantly more size variation than control discs, suggesting a defect in size regulation. Thus, in contrast to the notion that cell competition simply prevents disc overgrowth, cell death, presumably linked to cell competition, is required during normal development for reproducibility of disc size.

### *Factors that Constrain dMyc's Ability to Cause Overgrowth*

Cell competition ensures that an appropriately sized adult wing is formed even in response to overexpression of growth regulators such as dMyc. The ability of dMyc to cause overgrowth is also restricted by the phenomenon of cell cycle compensation. Overexpression of dMyc in wing disc cells accelerates the G1 to S phase transition, but the total cell cycle time is unchanged because cells compensate by increasing the length of their G2 phase (Johnston et al., 1999; Prober and Edgar, 2000). This phenomenon is not restricted to overexpression of dMyc; in general, induced lengthening or shortening of one phase of the cell cycle results in a compensatory change in another (Neufeld et al., 1998; Reis and Edgar, 2004). In this issue of *Cell*, a paper by Reis and Edgar (2004) investigates the mechanism of cell cycle compensation, demonstrating that regulation of the cell cycle transcription factor dE2F1 is critical for compensation to occur. While dE2F1 is not essential for expression of cell cycle genes per se, it is important for modulating their expression. dE2F1 can transcriptionally activate both *cyclin E* and *string*, which are rate limiting for the G1-S and G2-M transitions, respectively. Regulating dE2F1 levels therefore provides a direct means of coordinating the lengths of these two phases. Shortening the G1 phase by activating *C*yclin *d*ependent *k*inase *2* (Cdk2) reduces dE2F1 levels, delaying expression of *string* and lengthening the G2 phase (Reis and Edgar, 2004). Similarly, shortening the G2 phase by activating Cdk1 also reduces dE2F1 levels and lengthens G1 by delaying *cyclin E* expression. Significantly, the converse is also true: inhibiting Cdk2 or Cdk1 activity elevates dE2F1 levels, increasing transcription of *cyclin E* and *string* and accelerating the G1 or G2 phase, respectively. Consistent with dE2F1 being critical for the crosstalk between G1 and G2, wing disc cells in which dE2F1 function is eliminated by mutating the dE2F1 coactivator dDP fail to compensate when Cdk2 activity is inhibited and have a significantly longer cell cycle. dE2F1 function is therefore critical for regulating total cell cycle length in response to aberrant cell cycle controls.

If dE2F1 function is required for compensatory lengthening of G2 in response to dMyc-mediated shortening of G1, cells would be expected to divide faster in response to overexpression of dMyc in a *dDP* mutant background. Unfortunately, the cell doubling time of dMyc-overexpressing cells in a *dDP* mutant background could not be determined because a majority of these cells undergo apoptosis (Reis and Edgar, 2004). Precisely why these cells die is unclear, although forcing cells to enter S phase in a background in which transcription of genes required for DNA replication is compromised by the loss of dE2F1 activity may result in

genomic instability and apoptosis. It is, however, clear that regulating cell cycle length in the larval wing disc is important, presumably to allow sufficient time to ensure that preparations are made for the subsequent cell cycle phase while preventing delays that would slow development. Perhaps the ability of dE2F1 to adjust the cell cycle in response to the perturbation of cell growth by dMyc abrogates genetic instability or apoptosis within dMyc-overexpressing cell clones, thereby permitting their competitive success.

### *Myc-Induced Cell Competition and Cancer*

dMyc-induced cell competition in *Drosophila* suggests a mechanism for driving preneoplastic clonal expansion that has implications for our understanding of cancer etiology. There is strong evidence that the ability of Myc-overexpressing cells to abrogate apoptosis and maintain proliferation in a cell autonomous manner is an important step in tumor progression (Evan and Vousden, 2001). However, the papers by Moreno and Basler (2004) and de la Cova et al. (2004) suggest that apoptosis coupled to cell competition could work to facilitate expansion of high Myc-expressing preneoplastic clones, particularly in tissue compartments with limiting amounts of survival factors. Some tumors, such as lymphomas, may have only modest increases in Myc abundance while many others, such as neuroblastomas and lung and breast carcinomas, have significantly elevated levels of Myc. The cell competition experiments in the *Drosophila* wing disc would predict that as little as a 2-fold increase in Myc levels are sufficient to induce cell competition. Because the surrounding normal cell population can suppress the proliferation of cells transformed by oncogenes, such as activated *ras*, (Land et al., 1983) the apoptotic elimination of surrounding cells may be a particularly important consequence of cell competition upon Myc activation and may explain aspects of oncogene cooperation. Moreno and Basler (2004) raise the possibility that expansion of a clone of preneoplastic Myc-overexpressing cells through competition may conform to the well known notion of "field cancerization" (Braakhuis et al., 2003), which posits a patch or field of genetically altered cells from which multiple clonally related primary tumors arise through accumulation of additional genetic lesions. This prompts the somewhat counterintuitive idea that inhibition of apoptosis in the cells surrounding a nascent tumor might at least partly suppress cell competition and permit more effective containment of the tumor. These papers provide further support for the principle that experiments in small eukaryotes, such as fruit flies, can inform our thinking about human biology.

#### Selected Reading

Burke, R., and Basler, K. (1996). Development *122*, 2261–2269.

Braakhuis, B.J.M., Tabor, M.P., Kummer, J.A., Leemans, C.R., and Brakenhoff, R.H. (2003). Cancer Res. *63*, 1727–1730.

de la Cova, C., Abril, M., Bellosta, P., Gallant, P., and Johnston, L. (2004). Cell *117*, 107–116.

Evan, G.I., and Vousden, K.H. (2001). Nature *411*, 342–348.

Gallant, P., Shiio, Y., Cheng, P.F., Parkhurst, S.M., and Eisenman, R.N. (1996). Science *274*, 1523–1527.

Grandori, C., Cowley, S.M., James, L.P., and Eisenman, R.N. (2000). Annu. Rev. Cell Dev. Biol. *16*, 653–699.

Hueber, A.-O., and Evan, G.I. (1998). Trends Genet. *14*, 364–367.

Johnston, L.A., Prober, D.A., Edgar, B.A., Eisenman, R.N., and Gallant, P. (1999). Cell *98*, 779–790.

Land, H., Parada, L.F., and Weinberg, R.A. (1983). Nature *304*, 596–601.

Levens, D. (2003). Reconstructing MYC. Genes Dev. *17*, 1071–1077.

Maines, J.Z., Stevens, L.M., Tong, X., and Stein, D. (2004). Development *131*, 775–786.

Milan, M. (1998). Bioessays *20*, 969–971.

Morata, G., and Ripoll, P. (1975). Dev. Biol. *42*, 211–221.

Moreno, E., and Basler, K. (2004). Cell *117*, 117–129.

Moreno, E., Basler, K., and Morata, G. (2002). Nature *416*, 755–759.

Neufeld, T.P., de la Cruz, A.F., Johnston, L.A., and Edgar, B.A. (1998). Cell *93*, 1183–1193.

Orian, A., van Steensel, B., Delrow, J., Bussemaker, H.J., Li, L., Sawado, T., Williams, E., Loo, L.W., Cowley, S.M., Yost, C., et al. (2003). Genes Dev. *17*, 1101–1114.

Pierce, S.B., Yost, C., Britton, J.S., Loo, L.W.M., Flynn, E.M., Edgar, B.A., and Eisenman, R.N. (2004). Development, in press.

Prober, D.A., and Edgar, B.A. (2000). Cell *100*, 435–446.

Reis, T., and Edgar, B.A. (2004). Cell *117*, this issue, 253–264.

Schreiber-Agus, N., Stein, D., Chen, K., Goltz, J.S., Stevens, L., and DePihno, R.A. (1997). Proc. Natl. Acad. Sci. USA *94*, 1235–1240.