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Size control: Cell proliferation does not equal growth

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Abstract

Division subdivides mass without increasing it. So one should not expect that an increase in cell division would make an organism bigger. Both classic and recent experiments confirm this simple rationale: altering proliferation produces normally sized body structures with either especially small or exceptionally large cells.

Scientific progress is often marked by the realization that we are ready to address an old question that has become accessible. Two recent papers [1,2] use tools developed in studies of cell-cycle regulation to assess the effects of altering cell proliferation on growth in the context of the organism. The results suggest that we might profitably inquire how organismal size is regulated and consider the connection between growth — increase in mass — and cell proliferation — increase in cell number. Recognition of the connections relating growth and cell proliferation might alter our views of cancer, perhaps focusing attention on deregulation of growth, rather than cell proliferation.

Frequent use of the term growth control as a synonym for cell-proliferation control confuses issues that we would like to distinguish. Cells can grow without dividing. Mutations that block the cell cycle generally do not block growth [3], and some differentiated cell types grow without division — developing eggs and some neurons provide particularly dramatic examples of growth without cell division. Conversely, cells can sometimes divide without growing. For example, many eggs divide without growth as they partition their disproportionately large cytoplasm into smaller cells. Thus, growth and the cell cycle can be independently regulated, and control of the relative activities of the two processes produces the diversity of cell sizes that make up most metazoans.

Cells ordinarily must attain a minimal size to progress in the cell cycle. When growth of microorganisms or tissue culture cells is limited, proliferation arrests and coordinate growth and proliferation is observed upon addition of nutrients or growth factors. This parallel in growth and proliferation is the result of unidirectional coupling — growth must occur to satisfy a requirement for cell-cycle progression, but the cell cycle does not drive growth [4]. This hierarchical relationship is important to the growth of an organism or of a tumor. In neither case can growth be 'pushed' by driving the progress of the cell cycle (see below). Activation of cell-cycle regulators can drive the cell cycle, but if mass does not increase, these driven cycles will simply subdivide the same mass into smaller and smaller packets, a process that cannot continue indefinitely.

The control of growth is clearly important in development. For example, growth is controlled to ensure that your arms are the same length. Indeed, bilateral symmetry is a testament to the accuracy of the controls governing the sizes of organs, limbs and the organism itself. To dictate

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size, developmental programs must impose control on growth and cell proliferation. Among other things, we would like to identify the divergence of the regulatory pathways governing growth and those governing proliferation, and the imaginal discs of the fruitfly *Drosophila* provide an excellent system for investigating the developmental control of growth and proliferation.

Each imaginal disc is a flattened sac composed of a simple, folded epithelium that can develop autonomously into particular structures of the adult fly. The wing disc, composed of about 50 cells at the time that the embryo hatches, grows about a thousand fold in mass and cell number during larval growth. During pupal stages, the wing disc undergoes morphogenesis to form part of the central thoracic segment and the wing blade. While the morphology of the disc epithelium gives few indications of the complex events to come, molecular events prefigure pupal morphogenesis. Localized expression of developmental regulators and gradients of signaling molecules encode spatial information in the disc [5]. The impact of these developmental molecules is largely delayed until the patterns they dictate unfold during morphogenesis. The story is different, however, if one considers growth.

Each imaginal disc has a distinctive shape and size, indicating that its growth has been regulated before onset of morphogenesis [5]. Indeed, the patterning molecules, such as the Wingless or Dpp signaling molecules, are required for growth of the wing disc, and an ectopic focus of Wingless and Dpp expression can induce the formation of an additional wing blade. This remarkable duplication requires stimulation of growth, as well as *de novo* patterning. Although the mechanisms remain obscure, we should like to emphasize the point that developmental regulators govern both growth and cell proliferation in order to ensure development of appropriately sized structures upon pupal morphogenesis.

The connections between regulation of growth and proliferation in the disc have been explored in two recent studies [1,2]. In both these studies, experimental manipulations of cell-cycle regulators influenced cell size and cell number in complementary and reciprocal fashions, without altering disc growth (Figure 1). Weigmann *et al.* [1] cleverly interrupted cell division specifically in the anterior compartment of each disc by inactivating a temperature-sensitive form of the mitotic kinase Cdk1 [1]. Cells in the posterior compartment were protected by a wild-type *Cdk1* transgene expressed only in the posterior cells. As Cdk1 is essential for mitosis, proliferation ceased in the anterior compartment upon shifting to the restrictive temperature. Shifts early during the development of the disc produced an extraordinary discontinuity at or near the compartment border: the posterior cells, supported by expression of the wild-type transgene, appeared normal, whereas cells of the anterior compartment were greatly enlarged.

Weigmann *et al.* [1] observed that, even when the discordance in cell size between the two disc compartments was extreme, the anterior compartment was not much reduced in size. Furthermore, the anterior compartment often was normally shaped and exhibited normal patterns of localized gene expression. This result argues that the developmental controls of growth and patterning can operate independent of cell division.

Elimination of Cdk1 activity did more than block proliferation. Cdk1 is required to prevent DNA re-replication, and once deprived of its function, the anterior cells entered endocycles resulting in DNA amplification. As a modified cycle continued in these cells, it might have been argued that some aspect of cell cycle other than mitosis was important for growth. Newer experiments tested this possibility.

In more recent work, Neufeld *et al.* [2] used mitotic recombination to establish clones of cells lacking the cell-cycle regulators $Cdc25^{stg}$ or E2F. Although loss of the $Cdc25^{stg}$ phosphatase arrests cells in G2 phase of the cell cycle, and loss of the transcription factor E2F appears to block cells in G1, in both cases the arrested cells were found to grow. Furthermore, when

expression of the retinoblastoma protein Rb, an E2F inhibitor, was driven in the posterior compartment of the disc, proliferation was reduced and cell size increased.

In reciprocal experiments, increased expression of cell-cycle regulators in clones of disc cells drove steps of the cell cycle. While some conditions shifted the distribution of cells in different cell-cycle phases without accelerating the cycle, expression of both cyclin E and $Cdc25^{stg}$, or of E2F, drove cell proliferation. Increased proliferation resulted in reduced cell size, but the affected region, for example the posterior compartment, was found to be normal in size and shape (Figure 1).

These results argue that growth is regulated independently of proliferation, a conclusion that is in accord with older experiments in which changes in ploidy — the number of complete genome copies per cell — were used to alter cell size. Although the phenomenon is as yet unexplained, cell size correlates universally with ploidy. Thus, diploid yeast are larger than haploid yeast; but although diploid newt cells are similarly larger than haploid newt cells, diploid newts are not bigger than haploid newts. Indeed, the ploidy of newts has been changed from haploid to penta-ploid with parallel changes in cell size but no change in the size of the organs or the organism [6]. Manipulation of the ploidy of *Drosophila* has similar consequences [7].

New and old results thus argue that the developmental control of growth and patterning in a multicellular organism can occur independently of cell proliferation. Having arrived at a generalization, it might be important to point out some complications and exceptions. One complication is that reduced proliferation can create a growth disadvantage as a result of competition — cells of the wing disc slowly disappeared when their proliferation was compromised [2]. This disappearance was reduced when neighboring cells were genetically compromised for growth, or when non-dividing or slowly dividing cells were the only occupants of a disc compartment and so lacked competing wild-type neighbors. Hence, the loss of cells reflects an interaction with neighboring cells. Although the fate of the lost cells has not been resolved, one likely hypothesis is that they were eliminated from the epithelium. In any case, blocking proliferation can indirectly confer a growth disadvantage.

One dramatic observation of Neufeld *et al.* [2] violates the generalization that growth control is independent of proliferation. When E2F, Dp — E2F's partner in a heterodimeric transcription factor — and p35 were jointly expressed in a clone, the clone initially followed the generalization: it grew normally, but with increased proliferation and reduced cell size. At later stages, however, these clones failed to stop growing at the appropriate time, producing multiple layers of cells. The dysplasia associated with the late overgrowth of these cells is reminiscent of cancerous transformation. The disruption in morphology of the disc epithelium that accompanies overgrowth suggests that normal growth control depends on the architecture of the disc epithelium. This is consistent with known requirements for cell adhesion to limit growth of disc cells [8], and the involvement of spatial signals in the control of disc growth [5,9]. Accordingly, while accelerated proliferation can interfere with growth control, we suggest that this interference is indirect, and emphasize here the several cases where growth control works normally despite altered proliferation.

The diversity of developmental events requires exceptions to the generalization that the size of biological structures is independent of proliferation. When a cell lineage directs the development of a structure, such as a sensory bristle in *Drosophila* [10], each division produces daughter cells with different fates, and the final structure depends on the execution of the complete lineage. In these instances, development will require proliferation, and if the structural elements of the final organ are composed of individual cells, the structure will be bigger if the cells are bigger.

Despite these complications, the new studies emphasize the importance of growth control as distinct from cell-cycle control. Recognition of this importance leads us to mysteries that should be the fodder of new investigation. What makes a disc or an organ or an organism grow to a particular size? How are the limbs and organs properly proportioned? Is there a ruler that measures our various body parts, and stops their growth at the appropriate size? Clearly, increasing proliferation is not the way to induce growth at the organ level. What does? And what goes wrong to produce the overgrowth characteristic of neoplastic growth and tumorigenesis? Moreover, the size of quiescent cells is remarkably stable. For example, in the absence of damage, endothelial cells can remain quiescent for ten years. During this time, the vast majority of their proteins will turn over many times, yet the cells neither grow nor shrink. This leads to the general question, what maintains the size of cells or organs once they have reached their specified value?

As size control is very much a part of the developmental program, it will be important to investigate it in its normal multicellular context, much as the recent papers have. The goal for the future will be to tease apart the steps by which known developmental regulators govern growth and, *secondarily*, proliferation.

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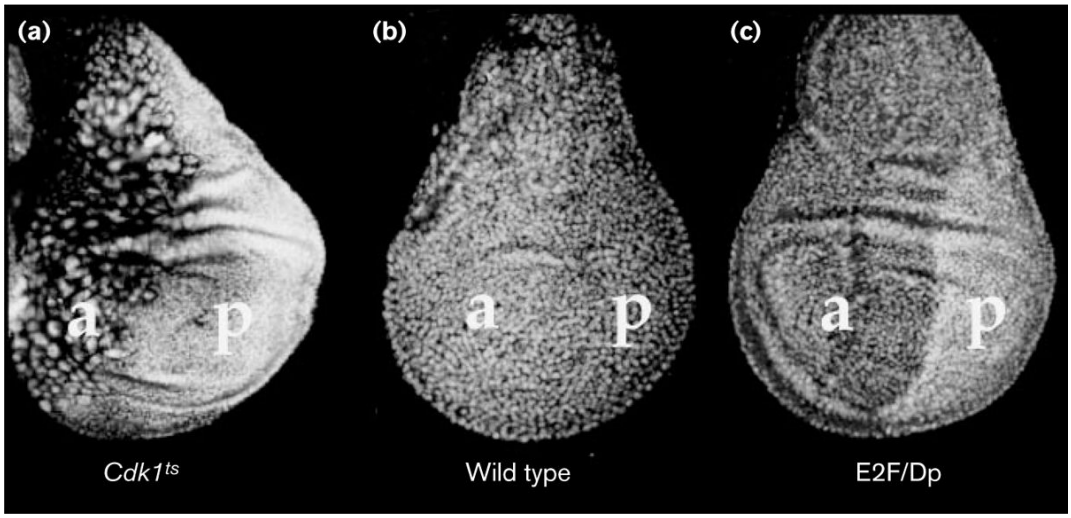

Figure 1.

Alterations in cell proliferation have little effect on organ growth. As described in the text, in two recent studies [1,2], compartment-specific expression of transgenes in wild-type or mutant *Drosophila* wing discs was used to modulate cell proliferation in the anterior and posterior compartments (labelled a and p, respectively). Differences in cell proliferation are seen here as differences in nuclear density when stained with a DNA dye. **(a)** A $Cdk1^{ts}$ mutant disc, in which a wild-type *Cdk1* transgene was expressed in the posterior compartment. The shift to the restrictive temperature stopped division in the anterior compartment, but not the posterior compartment [1]. Note the decrease in nuclear density in the anterior compartment in this disc. Nuclei enlarged in the anterior compartment as a result of endoreplication. **(b)** A wild-type disc shown for comparison; the nuclear density is similar in the anterior and posterior compartments. **(c)** A disc in which *Drosophila* E2F and Dp have been overexpressed in the cells of the posterior compartment. This expression increases cell number, visible as an increase in nuclear density, in the posterior compartment relative to the anterior compartment [2]. While the DNA stain detects nuclear size, which did not change in this case, cell size was reduced in proportion to the increased cell number (not visualized here). Despite the changes in cell proliferation in (a) and (c), the compartments and whole discs are of nearly normal size and morphology. (In part reproduced, with permission, from [1,2].)