Introduction

A longstanding question in biology is how organ sizes are predetermined (Conlon and Raff, 1999; Tsukaya, 2008). What factors and mechanisms control the progress of organ or organism growth, halting growth when proper size is attained? Is size solely a function of the proliferative growth of individual cells, or is it instead determined by global control mechanisms that act at the level of the organ or organism? In searching for answers to these and related questions, we have sought to deepen our understanding of organ-size control in multicellular organisms.

Although environmental cues, such as light, temperature, and nutrient availability, affect organ size, intrinsic regulatory mechanisms present in developing organs, such as leaves, dictate proper size based on genotype. Accumulating evidence from intensive studies in the model plant Arabidopsis thaliana suggest that organ-wide size-control mechanisms interact in a complex manner that affects and is affected by the number and size of the constituent cells of the developing organ. The existence of an organ-wide control system is suggested by the “compensation” phenomenon, in which a decrease in cell number caused by a mutation triggers cell enlargement (Tsukaya, 1998, 2002, 2006; Beemster et al., 2003; Horiguchi et al., 2006a; Ferjani et al., 2008; Micol, 2009). At present, the molecular basis of the organ-wide size-control system remains a mystery. Here, we summarize recent advances in the understanding of size control and compensation with a focus on the Arabidopsis leaf as a model organ.

The phenomenology of size control

Persuasive evidence indicates that organ size in multicellular organisms is controlled genetically, although the underlying regulatory mechanisms differ substantially between plants and animals.
Organ-size control in animals: Is size itself the basis for control?

If the monitoring of organ size itself is the basis of size control, then the size of an organ will be at least partially independent of the number and growth of its constituent cells. The first evidence of an organ-size-monitoring system in animals came from amphibian studies; in salamanders, mature tetraploid animals closely resemble their diploid counterparts despite their having only half as many cells (Frankhauser, 1945). The great resemblance in the body size and proportion of diploid vs. tetraploid salamanders arises from the fact that the total cell number in tetraploid salamanders is decreased to compensate for increased cell size. In mammals, tetraploid fetuses also have fewer and larger cells than their diploid counterparts (Henery et al., 1992).

In the Drosophila fruit fly, on the other hand, the size and the shape of the adult wing is predetermined by the patterns of cell growth, division, and cell death in the wing disk, and these patterns are regulated primarily by intrinsic signals that rely on morphogen gradients (for reviews of wing growth and patterning, see Serrano and O’Farrel, 1997 and references therein). Thus, animal organ-size-control systems tend to ensure the constancy of the final organ size by coordinating cell number, cell size, and cell survival (Francisco et al., 2009).

Compensation, a key phenomenon in leaf-size control scenarios

The inverse relationship that exists between the two major determinants of leaf size, namely cell proliferation and cell expansion, was first described in the literature as “hosho-sayo” (in Japanese) or “compensation” by Tsukaya (1998; 2002). Over the last few years, the number of recognized compensation mutants has progressively increased, suggesting that compensation might reflect a general mechanism in plants. The intriguing compensation relationship between cell proliferation and cell expansion has convinced us of the importance of studying this phenomenon to gain insights into leaf size control (Tsukaya, 2002).

In 2005, we isolated and characterized an Arabidopsis mutant, angustifolia3 (an3), in which the leaves were unusually narrow (Horiguchi et al., 2005). Careful histologic analysis revealed that this mutant exhibited compensation, a phenomenon that had been described in several reports at the time. Because detailed analyses of the molecular bases of compensation and leaf-size control were lacking, we performed large-scale screening experiments that enabled us to isolate many additional Arabidopsis mutants with altered leaf size or shape (Horiguchi et al., 2006a; 2006b). Of these mutant lines, 147 were later classified into eight different histologic groups based on the number and size of palisade cells in the first leaf pair (Horiguchi et al., 2006b).

Next, to elucidate the mechanisms of compensation, we characterized mutants fugu1–fugu5 together with other mutants previously reported to exhibit compensation [an3, erecta (er), and KIP-RELATED PROTEIN2 (KRP2) overexpressor] (De Veylder et al., 2001; Torii et al., 1996; Horiguchi et al., 2005; 2006b). As a result, several fundamental aspects of compensation became clear for the first time. These aspects are briefly introduced in the following sections.

Organ-size control in plants: What is being monitored?

As in animals, organ size in plants is essentially determined by the number and the size of constituent cells. Therefore, leaf-size regulation has been intensively studied with respect to the fundamental processes of cell proliferation and expansion (Donnelly et al., 1999; Ferjani et al., 2007; Usami et al., 2009). To this end, several key genes that affect organ size by acting on these processes have been identified. These aspects of leaf development have been intensively reviewed by Ingram and Waites (2006) and Anastasiou and Lenhard (2007) and are therefore not discussed here. Because organogenesis in plants, unlike that in animals, does not involve cell migration and cell death, the leaf provides an ideal system for the investigation of size control in higher plants.

Post-mitotic induction of compensation: Cell-autonomous or non-cell-autonomous?

Because decreased cell number is followed by excessive cell enlargement in compensation-exhibiting
mutants, compensation was generally considered to be a simple result of delayed cell cycling and continued growth. However, our kinematic analyses of leaf development revealed that cell expansion in the *fugu2-1*, *fugu5-1*, *an3-4*, and *er-102* mutants is enhanced only after exit from the mitotic cell cycle (Ferjani et al., 2007), indicating that cell enlargement in these mutants is not a simple result of the uncoupling of cell division from cell growth. Because these analyses also confirmed that decreased cell proliferation is the triggering factor for enhanced cell enlargement, we refer to enhanced cell expansion hereafter in this review as “compensated cell enlargement.” Our analysis of cell expansion rates revealed that in each of the mutants, either the rate or duration of cell expansion was selectively enhanced (Ferjani et al., 2007). Together, these findings suggest that cell cycling and post-mitotic cell enlargement are integrated in leaf primordia in either a cell-autonomous or a non-cell-autonomous manner (Ferjani et al., 2008).

To examine the validity of the above hypothesis, Kawade et al. have established a heat shock-inducible system for chimeric analysis of *an3*, a typical compensation-exhibiting mutant (unpublished). In this system, *an3* mutant sectors are artificially created early in leaf primordia development, and the behavior of mesophyll cells in neighboring wild-type and *an3* sectors is carefully analyzed with respect to cell size at leaf maturity. If compensated cell enlargement in the *an3* mutant occurs in a non-cell-autonomous manner, the cells in both the wild-type and *an3* sectors should be comparable in size. On the other hand, if compensated cell enlargement in *an3* mutant is induced cell-autonomously, then only the cells in the *an3* sectors should become enlarged. This analysis, which is presently ongoing, may provide the first direct evidence that cell–cell communication pathways are involved in compensated cell enlargement.

Compensation is induced in determinate organs

Growth within organs with determinate fate, such as leaves and floral organs, occurs for a given period of time and ceases when the organ reaches its characteristic final shape and size. Compensation in leaves and in petals has been reported, and we recently demonstrated that compensated cell enlargement occurs in cotyledons with dynamic characteristics similar to those observed in leaves (Ferjani et al., 2007). In contrast, cell expansion is not enhanced in roots of compensation-exhibiting mutants, even though they contain fewer cells than do wild-type roots (Ferjani et al., 2007). Thus, compensation has been shown to occur only in determinate organs.

Compensated cell enlargement does not necessarily correlate with increased ploidy

Leaf cell proliferation appears to stop in a basipetal manner through a process involving the so-called “cell-cycle arrest front” (Donelly et al., 1999; White, 2006). After exiting mitotic cell cycling, leaf cells enter the expansion phase, which in plants is generally accompanied by an increase in ploidy resulting from endoreduplication (Sugimoto et al., 2002; 2005). Ploidy is an important factor, and cell size is usually proportional to the ploidy level for a given cell type (Melaragno et al., 1993). To examine the possible involvement of endoreduplication in compensated cell enlargement, we measured the ploidy distribution in mature first leaves and cotyledons of wild-type and the compensation-exhibiting mutants (Ferjani et al., 2007); our flow cytometric analyses revealed that compensation can be triggered in the absence of an increase in ploidy. In addition, some of the extra small sisters (*xs*) mutations, which cause a reduction in cell size without affecting cell number, completely suppress compensated cell enlargement in *an3* mutants (Fujikura et al., 2007). Interestingly, the *xs* mutants demonstrate reduced, normal, or increased ploidy (Fujikura et al., 2007). Taken together, these findings suggest that compensation is only partially mediated by ploidy-dependent processes (Ferjani et al., 2007).

Organ-size homeostasis is not the primary function of compensation

Several mutants that exhibit compensation have been isolated and analyzed in detail (Horiguchi et al., 2005, 2006b; Ferjani et al., 2007). With few exceptions (such
as fugu2 and fugu3-D, which have leaves comparable in size to those of wild-type plants), these mutants have leaves that are both smaller than and differently shaped from wild-type leaves (Figure 1). On the other hand, the overexpression of AN3 increases cell number without affecting cell size (Horiguchi et al., 2005). Similar cellular phenotypes have been reported for plants overexpressing AINTEGUMENTA (ANT) (Mizukami and Fisher, 2000). These results indicate that compensation occurs only when cell number is decreased and not vice versa (i.e., no decrease in cell size when cell number is increased); this phenomenon thus differs fundamentally from those found in animal systems and appears to be unique to plants.

Recently, we reported a new class of mutants, the more and smaller cells (msc) mutants, which exhibit a cellular phenotype opposite that of compensation syndrome; in these mutants, cell number is increased and cell size is decreased (Usami et al., 2009) as a result of accelerated heteroblasty. However, compensation syndrome is not necessarily caused by heteroblasty-associated genetic pathways, as most compensation-exhibiting mutants do not show delayed heteroblasty. Thus, cell number and cell size must be integrated by at least two distinct genetic pathways, one of which is associated with heteroblasty and the other of which is related to compensation syndrome.

Taken together, these findings indicate that compensated cell enlargement is a specific response that occurs within leaf lamina with impaired cell proliferation activity. Thus, the primary function of compensated cell enlargement is not to maintain a constant leaf area; rather, compensated cell enlargement is the result of an intrinsic, organ-wide mechanism that integrates cell proliferation and cell enlargement.

Compensation is threshold-dependent

Theoretically, the compensation phenomenon is divisible into two distinct stages: induction and response. The induction stage coincides with the decrease in cell proliferation activity, and the response stage coincides with the resulting compensated cell enlargement. However, in oli mutants, which have a modestly reduced cell number, the latter response stage does not occur, whereas it does occur in double mutants between different oli loci, which have more drastically reduced cell number (Fujikura et al., 2009). This finding strongly suggests that compensation is triggered when cell proliferation is compromised in a threshold-dependent manner. In addition, when compensation-exhibiting mutants, which by definition exceed this threshold, are crossed to create double mutants, the cell number decreases further, and compensated cell enlargement is strongly enhanced.

Figure 1. Morphology of aerial parts and first leaf areas of compensation-exhibiting mutant Arabidopsis plants. (A) Images of shoots of wild-type and mutant 25-day-old plants. Bar: 10 mm. (B) Images and average areas (mm²) of mature first leaves of wild-type and mutant 25-day-old plants. Data shown represent mean areas ± SD for eight leaves. Bar: 5 mm. [Figure slightly modified with permission from Ferjani et al. (2007); www.planphysiol.org, "Copyright American Society of Plant Biologists"].
These observations indicate that cell number is regulated by different compensation-related genes that belong to different genetic pathways. Thus, compensated cell enlargement appears to be mediated by some as-yet-unidentified cell expansion pathways that respond differently in organs containing different numbers of cells.

Future outlook and open questions

Although huge strides have been made in the understanding of several aspects of plant development, one of the most fundamental aspects of development, size control, remains a mystery (Tsukaya, 2008). In light of recent work on compensation syndrome by our and other groups, we believe that the mystery of size control is just beginning to be illuminated.

As argued in this short review, initial studies using several compensation-exhibiting mutants have taught us that leaf size can be regulated independent of the absolute cell number, at least to some extent. The accumulating evidence strongly suggests that an organ-wide mechanism controls leaf size. Future experiments using chimeric mutants may provide the first demonstration of the existence of mobile factors that move between cells and act at the whole organ level to coordinate growth.

If such molecules that move from cell to cell to regulate growth do exist, are they the same in an3, fugu2, and other compensation-exhibiting mutants? How far do these mobile factors move within a particular leaf tissue layer? How do they move from cell to cell, and what are their targets? Although the nature of these postulated mobile factors is still unknown, Fujikura et al. (2007) have emphasized that the xs mutations are able to block compensated cell enlargement in an an3 mutant background without affecting cell number. These recent findings have opened up new paths of investigation that

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may lead to a better understanding of the molecular basis of the amazing array of plant organ sizes in nature.

Although several features of compensation have been characterized, the identity of the underlying driving force for compensated cell enlargement remains unknown. Vacuoles can occupy up to 96% of the total cellular volume (in extreme cases) in differentiated plant cells. During the post-mitotic stage, cell expansion largely relies on an increase in vacuolar volume, controlled cell wall modification, and de novo synthesis. Therefore, we believe that vacuolar volume control is fundamentally important for proper size control. Our future attempts to identify the driving force for cell enlargement in compensation will focus on this important organelle.

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References


Micol JL (2009) Leaf development: time to turn over a new leaf?


