Compensation: a key to clarifying the organ-level regulation of lateral organ size in plants

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Abstract

Leaves are ideal model systems to study the organ size regulation of multicellular plants. Leaf cell number and cell size are determinant factors of leaf size which is controlled through cell proliferation and post-mitotic cell expansion, respectively. To achieve a proper leaf size, cell proliferation and post-mitotic cell expansion should be co-ordinated during leaf morphogenesis. Compensation, which is enhanced post-mitotic cell expansion associated with a decrease in cell number during lateral organ development, is suggestive of such co-ordination. Genetic and kinematic studies revealed at least three classes of modes of compensation, indicating that compensation is a heterogeneous phenomenon. Recent studies have increased our understanding about the molecular basis of compensation by identifying the causal genes of each compensation-exhibiting mutant. Furthermore, analyses using chimeric leaves revealed that a type of compensated cell expansion requires cell-to-cell communication. Information from recent advances in molecular and genetic studies on compensation has been integrated here and its role in organ size regulation is discussed.

Key words: Cell proliferation, cell size, cell-to-cell communication, compensation, leaf size, organ size.

Introduction

A fundamental feature of the developmental process in multicellular organisms is the control of organ size. An organ will develop to an appropriate size depending on its developmental programmes. Organ size largely depends on the number and size of the constituent cells; hence, cell proliferation (recurring cycles of cell growth and division) and post-mitotic cell expansion should be tightly integrated during organogenesis. Plant leaves are an ideal organ to study the genetic control of organ growth because they have a flat shape and grow to a constant size under given growth conditions. After leaf primordia initiate at the flank of the shoot apical meristem as rod-shaped protrusions (Hanstein, 1868; Foster, 1936), they grow by cell proliferation. While cells divide actively throughout the whole leaf primordium during the early stages, as leaf development proceeds, cell division becomes restricted towards the junction between the leaf blade and the leaf petiole (Donnelly et al., 1999; Nath et al., 2003; White, 2006; Ichihashi et al., 2010, 2011; Kazama et al., 2010), making a proximal–distal gradient of cell proliferation activity, called the cell cycle arrest front in the leaf blade. Recent studies suggest that the arrest front does not progress gradually as previously thought, but remains at an almost fixed position during a certain period and then moves quickly towards the base of the leaf blade (Kazama et al., 2010; Andriankaja et al., 2012). After termination of cell proliferation, cells in the tip region start to undergo differentiation and expansion to increase their volume while cells in the base region continue proliferation, indicating that different cellular processes (cell proliferation and cell expansion) occur simultaneously in different regions within one organ (Fig. 1).
Finally, all cells stop proliferating and start to expand, ultimately reaching their final size. Molecular genetic studies using *Arabidopsis thaliana* (hereafter *Arabidopsis*) revealed that cell proliferation and post-mitotic cell expansion are controlled by transcription and protein degradation mechanisms, and also by small peptide and hormone signalling (Tsukaya, 2013b; Kalve et al., 2014). However, the developmental programmes responsible for the co-ordination of cell proliferation and post-mitotic cell expansion, as well as the regulation of final organ size, are poorly understood. Compensation is important to understand the co-ordination between cell proliferation and post-mitotic cell expansion (Tsukaya, 2002; Beemster et al., 2003). In this review, recent progress on the molecular mechanisms of compensation is referred to and its role in the co-ordination of cell proliferation and post-mitotic cell expansion during organ development is discussed.

Organ size regulation during animal development: insights from fly wings

To understand organ size control in animals and plants, the majority of studies have focused on the development of *Drosophila melanogaster* wings or *Arabidopsis* leaves (Day and Lawrence, 2000; Leevers and McNeill, 2005; Horiguchi et al., 2008). Although both leaves and wings are determinate, flat, multicellular units, the intrinsic regulation of proliferation and expansion of their cells are quite different. In animals, organ size is thought to be actively monitored at a level of total mass by a ‘total mass checkpoint’ mechanism that co-ordinates cell number and size (Potter and Xu, 2001). Mechanisms behind the total mass checkpoint have been well studied in *Drosophila* wing development (Potter and Xu, 2001). When cell proliferation is genetically enhanced during wing development, cell numbers increase in association with a cell-size decrease. By contrast, a defect in cell proliferation results in fewer but larger cells (Neufeld et al., 1998; Potter and Xu, 2001). In both cases, the wing develops to a constant size through the co-ordination of cell number and size.

Leaves are a simple model system for organ size control in plants

In contrast to the wing, leaf development exhibits distinct features. First, genetic enhancement of cell proliferation does not affect cell size. For example, when a positive regulator of cell proliferation such as *ANGUSTIFOLIA3/Arabidopsis thaliana GROWTH REGULATING FACTORS INTERACTING FACTOR 1* (*AN3/AtGIF1*) or *AINTEGUMENTA* (*ANT*) is over-expressed, cell numbers in leaves increase without affecting the cell size. Thus, the leaf size of *AN3* or *ANT* over-expressors (o/x) is larger than that in wild-type (WT) strains (Mizukami and Fischer, 2000; Horiguchi et al., 2005). Unexpectedly, leaves of *Arabidopsis grandifolia-D* (*gra-D*) mutants are 2–2.5-fold larger than WT leaves because of cell number increase due to chromosomal duplication in a specific region, including *ANT* and *CYCD3;1*, G1/S cyclin genes (Horiguchi et al., 2009). These results indicated that leaf size is not regulated by its total mass. In addition, post-mitotic cell expansion depends on a significant increase (often more than 50-fold) in vacuolar volume that is not observed in animal cells (Ferjani et al., 2007). Moreover, cell death does not participate in the determination of leaf size. Based on these results, leaf development is considered a simple and unique model to explore organ size control.
Co-ordination of cell proliferation and post-mitotic cell expansion in leaves

In Arabidopsis, cell proliferation is active throughout the early leaf primordium (Donnelly et al., 1999; Nath et al., 2003; White, 2006) and kept in a junction region between the leaf blade and the leaf petiole (Ichihashi et al., 2010, 2011); this meristematic region, a leaf meristem, is bordered by an ‘arrest front’ that stays at a particular distance from the junction (Kazama et al., 2010; Andriankaja et al., 2012). Cell proliferation and post-mitotic cell expansion occur simultaneously in the proximal and distal regions of the same leaf blade primordium (Fig. 1). Molecular genetics uncovered numerous genes responsible for cell proliferation and post-mitotic cell expansion during leaf development.

Cell proliferation in leaf primordia is actively regulated by AN3, a transcription factor GROWTH REGULATING FACTOR5 that interacts with AN3, a cytochrome P450 KLUH, an AP2/ERF family transcription factor ANT, and an auxin-inducible factor ARGOS that functions upstream of ANT (Mizukami and Fischer, 2000; Hu et al., 2003; Kim and Kende, 2004; Horiguchi et al., 2005; Anastasiou et al., 2007). By contrast, E3 ubiquitin ligase BIG BROTHER, ubiquitin receptors DA1 and DA1-related, a basic helix–loop–helix transcription factor SPATULA, ROTUNDIFOLIA4-LIKE/DEVIL family peptides, and multiple class II TCP (TEOSINTE BRANCHED1 CYCLOIDEA PCF) transcription factors are known to regulate cell proliferation negatively (Nath et al., 2003; Narita et al., 2004; Disch et al., 2006; Li et al., 2008; Ichihashi et al., 2010). Post-mitotic cell expansion is well known to be regulated by EXPANSIN through the modulation of cell wall extensibility in leaves (Pien et al., 2001). A cellulose synthase FRAGILE FIBER5/IRREGULAR XYLEM3 is also involved in post-mitotic cell expansion, possibly through the synthesis of secondary cell walls (Taylor et al., 1999; Horiguchi et al., 2006). In addition, mutations in AUXIN RESPONSE FACTOR7 and 19 cause a defect in post-mitotic cell expansion, suggesting that these two factors play a role in cell expansion under the control of auxin (Wilmoth et al., 2005; Horiguchi et al., 2006). These studies have advanced our understanding at the molecular level of cell proliferation or post-mitotic cell expansion, respectively, but further studies are required to explore the mechanism because compensation is suggestive of a co-ordinated regulation of these cellular processes for leaf size control (Tsukaya, 2002, 2008).

‘Compensation’ as a key to understanding of leaf size control

Notably, in leaf development, a severe defect in cell proliferation often triggers enhanced post-mitotic cell expansion (Fig. 2). This phenomenon is known as ‘compensation’ and occurs only in determinate organs such as leaves and cotyledons (Tsukaya, 2002, 2008; Beemster et al., 2003; Ferjani et al., 2007; Horiguchi and Tsukaya, 2011).

Compensation-like phenomena were first described by Haber (1962) on leaves of γ-irradiated wheat. Similar phenomena were then reported in various mutants and transgenic plants of Arabidopsis, e.g. loss-of-function mutants of ant, an3, fugul-5, erecta (er), strauwelpeter, and KIP-RELATED PROTEIN2 (KRP2) o/x (Hemerly et al., 1995; Mizukami and Fischer, 2000; De Veylder et al., 2001; Autran et al., 2002; Horiguchi et al., 2005; Ferjani et al., 2007). This phenomenon is not limited to Arabidopsis and has also been observed in Oryza sativa over-expressing KRP1 (Barróco et al., 2006), suggesting that compensation is widely observed in seed plants.

Is the defect in cell proliferation a cause of enhanced cell expansion? First, as described above, loss-of-function mutants of AN3 and AN3 show decreased numbers of leaf cells and enhanced cell expansion, but over-expression of these genes result in increased cell numbers but cause no changes in cell size (Mizukami and Fischer, 2000; Horiguchi et al., 2005). In addition, at some immature stages when cell proliferation and

Fig. 2. An example of a compensation-exhibiting mutant. (A, D) Pictures of first leaves of the wild type (WT; A) and a typical compensation-exhibiting mutant, fasciata1 (fas1; D) from plants 25 d after sowing. Bars=5 mm. (B, C) Palisade cells of the WT (B) and fas1 (C) in first leaves of plants 25 d after sowing. Typical cells are marked pale green. Bars=100 µm. Leaves of compensation-exhibiting mutants include fewer but larger cells.
cell expansion occur simultaneously in a leaf primordium, enhanced cell enlargement or compensated cell enlargement (CCE) is recognized at the distal region of the leaf primordium in mutants exhibiting compensation (Ferjani et al., 2007). Considering that cell proliferation temporally precedes cell expansion in a leaf primordium as described above, one may naturally think that the defect in cell proliferation is the cause and that the enhanced cell enlargement is the result.

It was then examined whether an opposite type of compensation occurred, i.e. enhanced cell proliferation caused by defective cell expansion. An opposite phenotype to compensation is observed in more and smaller celled mutants, in which cell numbers increase while cell size decreases (Usami et al., 2009). Detailed analysis revealed that these mutants show accelerated heteroblasty, i.e. juvenile leaves have various traits of the adult leaves (Kerstetter and Poethig, 1998; Tsukaya et al., 2000). Moreover, in Arabidopsis, adult leaves were revealed to have an increased cell number with a decrease in cell size compared with juvenile leaves (Usami et al., 2009). Therefore, this apparently ‘reverse compensation’ phenotype is not triggered by a defect in cell proliferation, but by a developmental phase change. Our consensus is that compensation occurs unidirectionally; i.e. a defect in cell proliferation triggers enhanced post-mitotic cell expansion, but not vice versa.

Compensation is a heterogeneous phenomenon

Is compensation, which is observed in many mutants and transgenics, governed by a common mechanism? The kinetics of cell size increases in compensation-exhibiting lines revealed three different modes of action for CCE (Ferjani et al., 2007, 2013b). In the an3, fugu2/fas1, and er mutants, the rate of post-mitotic cell expansion increases compared with the WT, but its period remains unaffected (class I; Fig. 3A).

By contrast, an extended period for post-mitotic cell expansion is observed only in fugu5 among compensation-exhibiting lines (class II; Fig. 3B). Another type of compensation, class III, is represented by KRP2 o/x, which has also been well characterized to show distinctive features for CCE (Fig. 3C). KRP2 is a cyclin-dependent kinase inhibitor that binds and inhibits A-type cyclin-dependent kinase (Verkest et al., 2005). Class III is unique in showing that the sizes of cells in the mitotic phase are already larger than the WT. Also, enhanced post-mitotic cell expansion in KRP2 o/x was reported to depend on its increased rate, but not the extended period (Ferjani et al., 2007). In addition, cell size increases in KRP2 o/x correspond to the extent of its over-expression; i.e. higher over-expression of KRP2 resulted in a stronger phenotype of compensation compared with a milder over-expression of KRP2 (Verkest et al., 2005). An increase in the ploidy level is not involved in the induction of compensation in KRP2 o/x because higher over-expression of KRP2 suppresses not only the mitotic cell cycle, but also the endoreduplication cycle (Verkest et al., 2005). Notably, genetic analysis using an3 over-expressing KRP2 demonstrated that KRP2 over-expression has an additive effect on the an3 mutation for cell proliferation activity (Kawade et al., 2010). These results suggest that class III compensation in KRP2 o/x is triggered by a different cue from that in an3 of class I. Therefore, an analysis of compensation in KRP2 o/x compared with an3 would increase our understanding regarding differences in the triggering mechanisms of CCE.

Some information on the mechanisms of CCE in KRP2 o/x is based on analyses of the de-etiolated3 (det3) mutant, which has a defect in the gene which encodes the C subunit of the vacuolar-type H+ ATPase (V-ATPase). Also, det 3 shows a decreased leaf cell size compared with the WT, suggesting that activity of the V-ATPase is required for post-mitotic cell expansion (Schumacher et al., 1999; Fukao and Ferjani, 2011; Fukao et al., 2011). A recent study performed a genetic analysis between det3 and compensation-exhibiting mutants to explore the involvement of V-ATPases in CCE (Ferjani et al., 2013a, b). These results revealed that the det3 mutation suppressed KRP2 o/x mediated (class III) CCE, but not other types of CCEs. They also found that activity of the V-ATPase in KRP2 o/x shoots is higher than in the WT. These results indicated that KRP2 o/x-mediated CCE occurs through increased V-ATPase activity, which enables vacuolar

![Fig. 3. Schematic presentation of three classes of compensation. (A–C) Kinematics of the cell expansion rate of class I (A), class II (B), and class III (C). In each panel, the horizontal axis indicates the leaf age and the vertical axis denotes the cell-size increase rate (μm² cell⁻¹ d⁻¹). Solid lines represent each class of compensation-exhibiting mutants and dotted lines indicate the wild type (WT). Class I mutants show an increased rate of cell expansion, while class II mutants show an increased duration of cell expansion. In class III mutants, proliferating cells are already larger than those of the WT, and the cell expansion rate also increases.](https://academic.oup.com/jxb/article-abstract/66/4/1055/594403)
Role of endoreduplication in compensation

In both plant and animal cells, the nuclear ploidy level increases through a process called the endocycle, a modified mitotic cell cycle in which DNA replication occurs without mitosis and leads to polyploidy (Sugimoto-Shirasu and Roberts, 2003). In Arabidopsis, not only trichomes but the majority of differentiated cells have polyploid nuclei; the ploidy level correlates with cell size in pavement cells in leaf and sepal epidermis (Melaragno et al., 1993; Roeder et al., 2010), suggesting that the ploidy level is important for cell size regulation in several organs. Mutants with lower ploidy levels such as brassinos- teroid insensitive 4 (bin4), hypocotyl 6 (hyp6), root hairless 1 (rhl1), and rhl2 often have smaller cells, whereas mutants having a higher ploidy level such as regulatory particle triple-a atpases 2a (rpt2a) and regulatory particle non-triple-a atpase I2 (rpt12) often have larger cells (Sugimoto-Shirasu et al., 2002, 2005; Breuer et al., 2007; Kurepa et al., 2009; Sonoda et al., 2009). Furthermore, the tetraploid Arabidopsis plants exhibit larger organ sizes caused by the larger cell size (Breuer et al., 2007; Tsukaya, 2013a).

These data support the concept that the nuclear ploidy level is important for cell size control, but the ploidy level is not an absolute determinant of cell size. The rpt2a mutants have larger cells in the leaf epidermis and petal epidermis than the WT. Flow cytometry analyses revealed that the ploidy level distribution is higher than in the WT in leaves but similar to the WT in petals, suggesting that an increase in cell size does not always correlate with increased ploidy (Kurepa et al., 2009). A comparative analysis in various cell size mutants in Arabidopsis also indicated that the effect of tetraploidy in terms of the increasing ratio of cell size from the diploid state varied among mutants (Tsukaya, 2013a). Note that polyploidy caused by the endocycle is found in limited species of annual herbal plants (Barow and Meister, 2003). Therefore, plants have several pathways to promote cell expansion: one is coupled with the ploidy level and another is uncoupled from the ploidy level. Indeed, Fujikura et al. (2007a) analysed the ploidy level distribution of a series of xs mutants of Arabidopsis, which are characterized by smaller cells in their leaves, and found that xs5 exhibits higher ploidy levels than the WT. Furthermore, RNAi knockdown of the replication licensing factor gene CDT1A exhibits similar phenotypes to xs5, i.e. smaller cells and higher ploidy, indicating that the ploidy level does not always correlate with cell size (Raynaud et al., 2005).

The relationship between endoreduplication and CCE is complex. In the case of rpt2a mentioned above, it seems to be negative (Kurepa et al., 2009). Moreover, this is not the case for the an3 mutant of the class I compensation or KRP2 o/x of class III, since these compensation syndromes do not accompany an increased endoreduplication level (Fujikura et al., 2007a). A recent study by Hisanaga et al. (2013) revealed a link between endoreduplication and a type of CCE. The fugu2fasl mutant is known to be a compensation exhibiting mutant (class I) with an increased ploidy level. An expression analysis revealed that fasl mutants suffer endogenous DNA damage, which activates the DNA damage response. Genetic analysis revealed that mutations in ATAXIA TELANGIECTASIA MUTATED (ATM) partially suppressed the decrease in cell number observed in fasl, and completely suppressed the increase in ploidy and CCE in fasl, while mutations in ATM AND RAD3 RELATED (ATR) suppressed increased ploidy and CCE without suppressing the decreased cell number phenotype in fasl. ATM and ATR are known regulators of the DNA damage response with different roles. These data suggest that the DNA damage response pathway may independently affect cell cycle arrest and CCE in fasl. Since the fugu2fasl mutant is in the same category as the an3 mutant in terms of the kinematics of cell size behaviours (class I), compensation is a very heterogeneous phenomenon.

The nature of an3-dependent compensation

AN3/GIF1 encodes a transcriptional co-activator that positively regulates cell proliferation in leaves (Kim and Kende, 2004; Horiguchi et al., 2005), possibly via chromatin remodelling (Vercruyssen et al., 2014). Active expression of AN3 is observed in the entire leaf primordium during the early stages and is kept in the proximal part of the leaf primordium for a certain period, as seen for meristematic activity of the leaf primordium. Indeed, the AN3-expressing domain in the leaf primordium overlaps the plate meristem in the primordium (Donnelly et al., 1999; Horiguchi et al., 2005). In leaves of the an3 null mutant, cell numbers decrease by more than 70% but cell size increases by 50% in terms of projected area to a leaf surface plane compared with the WT (Horiguchi et al., 2005).

A threshold decrease in cell number or cell proliferation activity induces CCE in the an3 mutant (Fujikura et al., 2009). In AN3-silenced lines, in which their transcript levels are reduced by 60%, the decrease in cell number is relatively mild (20–30% decrease) and CCE is not observed. However, when AN3 transcripts are strongly suppressed up to 30%, cell numbers decrease by more than 50%, resulting in CCE (Fujikura et al., 2009). This threshold mechanism in compensation is also supported by an analysis using a subset of oligocellulida (oli) mutants. In the leaves of oli2, 5, and 7, cell numbers decrease mildly (<20–30% reduction) and CCE is absent. By contrast, the double mutants oli2 oli5 and oli2 oli7 exhibit strong defects in cell proliferation (40–60% reduction) in leaves, as well as the induction of CCE (Fujikura et al., 2009).

However, the gra mutation, which results in a 3-fold increase in the number of leaf cells, does not suppress CCE in the an3 background that decreases cell numbers by one-third (Horiguchi et al., 2009; G Horiguchi et al., unpublished data). Thus, it is not the total cell number in a leaf primordium but rather the proliferation pattern or activity that seems to be important for triggering the CCE. These observations
indicate that CCE in the \textit{an3} mutant is not simply a passive result of a defect in cell proliferation, but rather an active mechanism that triggers compensation, as discussed below.

The intense expansion of post-mitotic cells in \textit{an3} is completely suppressed in a group of \textit{xs} mutants (\textit{xs}1, 2, 4, and 5) specifically defective in post-mitotic cell expansion with various levels of endoreduplication (Fujikura \textit{et al}., 2007a,b). These results indicate that \textit{an3}-dependent compensation is a result of the modulation of a subset of the normal pathway for post-mitotic cell expansion involved both in ploidy-dependent and -independent pathways.

A role of cell-to-cell communication in compensation

Although our understanding of compensation has increased, how cell proliferation activity is monitored during early leaf development and how compensation is triggered remain unclear. Subsequently, the intermediary step links the status of cell proliferation with the extent of post-mitotic cell expansion through an unknown signal. If this signal acts in a non-cell-autonomous manner, it has a positive or negative effect on post-mitotic cell expansion when \textit{an3} or WT cells, respectively, generate the signal. Alternatively, in a cell-autonomous case, cell proliferation activity is memorized in each cell and the extent of post-mitotic cell expansion depends on this memorized signal at a single cell level. However, until recently, how these cellular processes are co-ordinated was unknown, especially in terms of cell autonomy in the intermediary step.

\textbf{Kawade \textit{et al}. (2010)} addressed this question by analysing chimeric leaves for AN3 or KRP2 expression using the Cre-lox system (Fig. 4). When AN3-expression was chimerically induced or shut down under the \textit{an3} mutant background, all cells in the chimeric leaf showed typical CCE at the same level as the \textit{an3} mutant leaves, irrespective of the presence or absence of AN3 expression (Kawade \textit{et al}., 2010; Fig. 4D). Moreover, \textit{an3} cells seemed to generate and transmit an intercellular signal that enhanced post-mitotic cell expansion. They also found a unique feature of the effect of signalling that acts within one-half of a leaf partitioned by a midrib. Whether this signalling is transmitted through symplastic or apoplastic transport could be explored, for example, by modulating the size of plasmodesmata through callose accumulation (Simpson \textit{et al}., 2009).

In contrast to the above case of \textit{an3} (class I), compensation in \textit{KRP2} \textit{o/x} (class III) was found to be a cell-autonomous process in the Cre-lox chimera system (Kawade \textit{et al}., 2010; Fig. 4E). Since KRP2 directly inhibits cell cycling (Verkest \textit{et al}., 2005), the cell-autonomous action in \textit{KRP2} \textit{o/x} is induced possibly through a defect in cell cycling itself. Again, the nature of compensation is shown to be heterogeneous.

At this time, the entity of the intercellular signal remains unknown, and various molecules responsible for intercellular signals during development have recently been identified in plants (Hirakawa \textit{et al}., 2010). Molecular elucidation of the intercellular signals would increase our understanding regarding the spatio-temporal regulation of the co-ordination of cell proliferation and post-mitotic cell expansion in leaves.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Cell-autonomous or non-cell-autonomous mode of compensation. (A–E) Schematic presentation of leaf cell size in the wild type (WT; A), \textit{an3} (B), \textit{KRP2} \textit{o/x} (C), \textit{an3} chimera (D), and \textit{KRP2} \textit{o/x} chimera (E). In each panel, circles represent leaf palisade cells. Smaller circles are for the WT size cell and larger circles represent compensated cell enlargement (CCE)-exhibiting cells. The colour of the circle indicates the genotype of each cell. Light blue indicates the WT, magenta shows \textit{an3} and light green represents \textit{KRP2} \textit{o/x}. In \textit{an3} chimeric leaves, not only \textit{an3} genotype cells but also WT genotype cells exhibit CCE, indicating a non-cell-autonomous mode of compensation. In \textit{KRP2} \textit{o/x} chimeric leaves, only \textit{KRP2} \textit{o/x} genotype cells exhibit CCE, indicative of a cell-autonomous mode of compensation.}
\end{figure}
Subcellular aspects of compensation

At this time, cellular phenotypes have been intensively investigated in the study of compensation, but how subcellular status and cytosolic components are affected by the enhanced cell expansion during compensation remains largely unknown. Chloroplasts are essential for the appropriate photosynthetic activity of mesophyll cells. Anatomical studies suggest that chloroplast number is often tightly correlated with cell size (Pyke and Leech, 1992, 1994). However, little is known regarding changes in chloroplast number in response to the enhanced expansion of post-mitotic cells. Analysing subcellular changes in association with compensation would be worthwhile to clarify the yet-to-be-investigated aspect of this phenomenon.

Recently, Kawade et al. (2013) investigated how compensation affects chloroplast numbers. They found that the number of chloroplasts increased in compensation-exhibiting mutants such as an3, fugu5, and KRP2 o/x, suggesting that chloroplast number is regulated in a cell size-dependent manner, and that not only vacuole size, but increases in chloroplast number, sustain the CCE. Furthermore, ploidy-level measurements of these mutants revealed that the effects of their ploidy levels on chloroplast numbers are variable, and that tetraploidization of these mutants doubles their ploidy level but not always their chloroplast numbers. These data suggest that nuclear ploidy levels are not an important factor for determining chloroplast numbers. In addition, the chloroplast is a photosynthetic organelle; thus, compensation may have a physiological role for the photosynthetic function of leaves. Many studies have investigated the mechanisms of chloroplast division; among them, the expression level of two paralogous genes, PLASTID DIVISION1 (PDV1) and PDV2 is known to be a determinant factor of chloroplast proliferation (Miyagishima et al., 2006; Okazaki et al., 2009, 2010). However, the expression levels of these genes in compensation-exhibiting mutants were not up-regulated, suggesting that the promotion of chloroplast proliferation in these mutants does not depend on the expression level of PDV's (Kawade et al., 2013).

Concluding remarks

In this review, the current understanding of compensation has been described in which cell proliferation is linked to post-mitotic cell expansion during leaf development. Although each process has been well studied, their interaction remains unclear. Kinematics studies have indicated that compensation is a heterogeneous phenomenon and molecular genetic studies have revealed key players of cell proliferation and post-mitotic cell expansion in each pathway. Whether these different pathways act independently or are integrated during organogenesis remains unclear. In addition, an3-mediated CCE has been shown to require cell-to-cell communication. At this time, the molecular entities of these signals remain unclear, but future studies will explore this signalling and expand our understanding of organ size regulation. Furthermore, CCE has been shown to affect the regulation of chloroplast proliferation. As the chloroplast is a photosynthetic organelle, leaf size regulation through compensation should have a physiological impact on the mesophyll cell function in leaves. These findings would increase our understanding of leaf development.

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