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Article Addendum

FZR2/CCS52A1 mediated endoreduplication in Arabidopsis development

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Plant organogenesis generally involves three basic processes: cell division, cell expansion and cell differentiation. Endoreduplication, a process of genome replication without intervening mitosis, often occurs during cell expansion and cell differentiation. The switch from the mitotic cell cycle to the endocycle, however, is still poorly understood in plants. We have recently demonstrated that FIZZY-RELATED2 (FZR2) is a factor controlling endoreduplication in Arabidopsis. *fzr2* mutants lacked gross morphological defects but showed a general decrease of endoploidy level in trichomes and other leaf cells, while expression of *FZR2* under constitutive or tissue specific promoters induced extra or ectopic endoreduplication in all tissues examined. We also showed that decrease of leaf cell size in *fzr2* mutants could be compensated by increased cell proliferation. In this addendum, we discuss additional phenotypes of *FZR2* misexpression, including apparent mosaic leaf sectors in which local cell overexpansion due to *35S::FZR2* appears to be compensated by reduced cell expansion in neighboring tissues.

Plants begin vegetative development as single zygotes and reach final sizes million-fold bigger, with complex tissues and cell types. During development, plants undergo three basic processes: cell division, cell expansion and cell differentiation. While cell division is dependent on mitotic cell cycle, cell expansion and cell differentiation are often coupled with a modified cell cycle called endoreduplication.[1] Endoreduplication enables a cell to increase its ploidy by replicating its genome without subsequent chromosomal and cellular division. This endocycle, widespread in eukaryotes but especially common in plants, may provide individual cells with the gene-expression capacity to reach larger sizes.[2] In the well-studied dicotyledon model *Arabidopsis thaliana*, endoreduplication occurs in most of the differentiated cell types, such as trichoblasts and trichomes, or cells with very high metabolic activity, such as the endosperm.[3] The switch from mitotic cycles to endocycles requires cells to start another round of DNA replication without intervening mitosis. Therefore, a cell must induce re-entry into S-phase after G_1-phase while inhibiting M-phase. The regulatory mechanisms mediating the G_1 to S transition in endocycles share components of the mitotic pathways,[4] with the CDK/CYCLIN B complex influencing DNA replication.[5] Much evidence in yeast, fly and plants has pointed to the involvement of a WD-40 protein, Fizzy-Related/Ccs52, in triggering the switch to endoreduplication by controlling the degradation of Cyclin B.[6,7]

Using reverse genetics in Arabidopsis, we investigated *FIZZY-RELATED2* loss-of-function mutants.[8] *fzr2* plants showed reduced endoreduplication and cell size in both pavement cells and trichomes.[8] When *FZR2* was misexpressed with the CMV *35S* promoter, transgenic plants showed a range of phenotypes such as retarded growth, supernumerary trichome branches and distorted roots, with ectopic endoreduplication induced in all examined tissues. When expressed under control of the petal- and stamen-specific *APETELA3* promoter, FZR2 caused great increases in the cell and nuclear sizes of petal and stamen cells, which normally endocycle little or not at all in Arabidopsis.[8]

Since *AP3* also drives gene expression in pollen, and pollen mother cells undergo two rounds of meiosis to generate haploid sperm cells,[9] the effects of *FZR2* expression on male gametogenesis seemed particularly interesting. Microscopic analysis showed larger pollen grains in *AP3::FZR2* plants relative to wildtype, whereas DAPI staining revealed a concomitant increase in sperm cell nuclear size (Fig. 1A–D). These results suggested that endoreduplication had been induced in these pollen grains. Although these polyploid sperm cells proceeded through double fertilization, the corresponding embryos failed to complete development. Examination of cleared embryos with Nomarski microscopy showed that about half of them stopped growth at the torpedo stage (Fig. 1G and H), possibly due to abnormal endosperm development. When endosperm cellularization was completed in wildtype seeds (Fig. 1E), there were only 2 to 3 bubble-like structures at the chalazal

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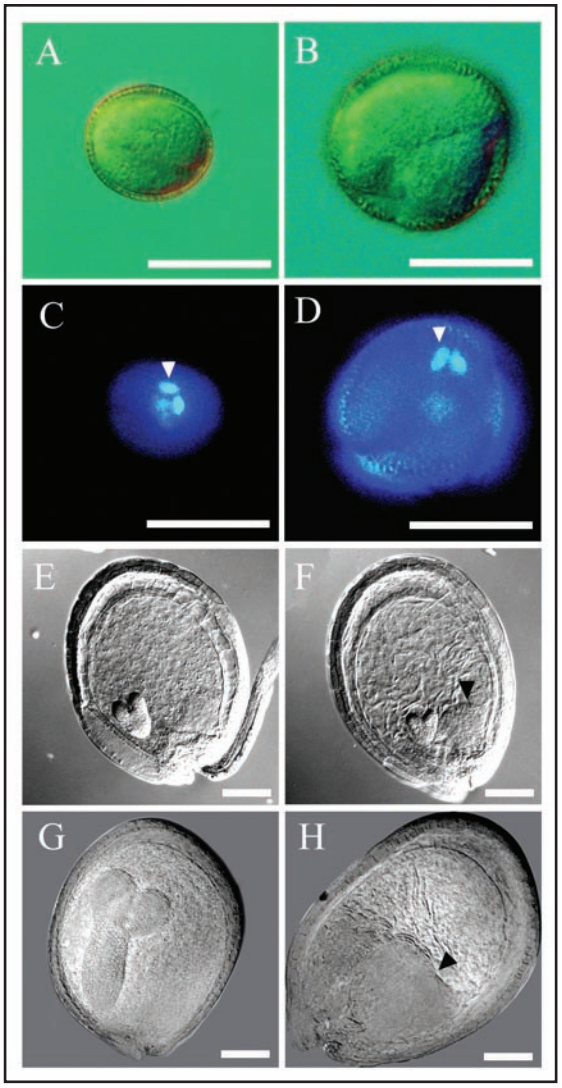

Figure 1. Comparisons of pollen grain sizes, nuclear sizes and embryo development among wildtype (WT, left: A, C, E and G) and *AP3::FZR2* lines (right: B, D, F and H). (A and B) Micrographs of representative pollen grains. (C and D) DAPI staining of representative pollen grain. Arrowheads in (C and D) indicate the enlarged nuclei of sperm cells. (E and F) Micrographs of heart-stage embryos. (G and H) Micrographs of torpedo-stage embryos. Arrowheads in (F and H) indicate the abnormal endosperms. In (E–H), seeds were cleared with Hoyer solution and viewed using Nomarski optics. Scale bars represent 10 μm in (A–D), and 100 μm in (E–H).

poles of developing *AP3::FZR2* seeds (Fig. 1F). This phenotype was similar to that of developing seeds derived from fertilization of a diploid plant with pollen from an hexaploid plant,[10] further supporting the conclusion that *AP3::FZR2* sperm cells underwent endoreduplication.

Another interesting result of this study was the different manner in which stamens and petals were altered by *AP3::FZR2* expression. While petal cells showed extreme increases in size and decreases in numbers, the organs became disrupted, losing their characteristic laminar shape. Conversely, *AP3::FZR2* stamens maintained their cylindrical shape, despite becoming wider at the organ level and

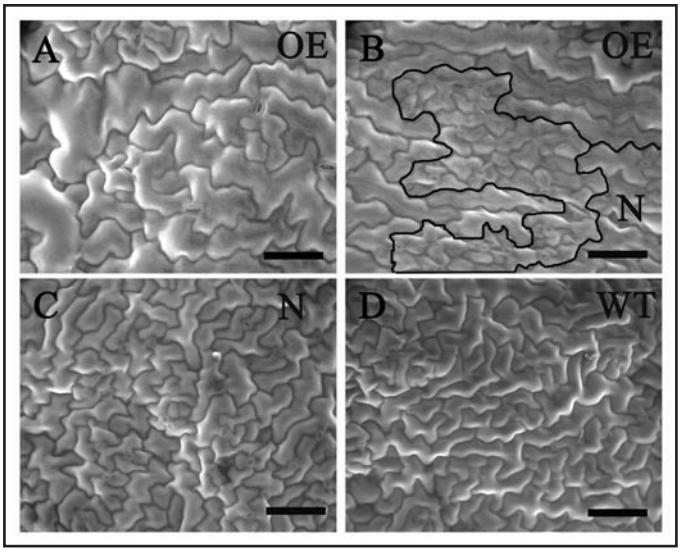

Figure 2. Comparisons of cell sizes inside and outside of a mosaic sector. (A) Scanning electron microscope graphs of epidermal cells from a mosaic sector of *35S::FZR2*. (B) Epidermal cells at boundary region between mosaic sector (OE) and surrounding normal cells (N). Black lines highlight the band of smaller cells. (C) Normal epidermal cells outside the mosaic sector in the same leaf (N). (D) Epidermal cells from wildtype plants (WT). Scale bar represents 100 μm.

composed of larger cells.[8] This discrepancy in the severity of petal and stamen organ-level phenotypes may be because the two tissues respond differently to *FZR2* misexpression, or because the shapes of these two organs place unique constraints on the effects of cell overgrowth. Like these stamens, roots and stems of *35S::FZR2* plants also retained normal shape despite severe distortion of internal tissue architecture.[8] Perhaps a cylindrical organ is maintained more easily due to the dynamics of biophysical forces. It is also possible that the morphogenesis of a filamentous structure makes more use of intercellular communication than a laminar structure, so the cell proliferation and cell expansion are more strictly regulated by non-cell autonomous signals such as protein movement via plasmodesmata to provide additional positional information.[11] The regulatory contribution of these additional signals may override the effects of *FZR2* ectopic expression.

Finally, the most intriguing phenotype found in *fzr2-1* mutant was that the overall leaf size showed no significant difference compared with wildtype, although the average cell was smaller. This suggests that proliferation is enhanced to generate more cells in response to the decreased average cell size. A mechanism called compensation is postulated to coordinate cell proliferation and cell expansion to attain proper organ size.[12] For example, mutations or transgenes that cause decreases in leaf cell proliferation can be compensated by extra leaf cell expansion, such that the organ approaches normal size.[13] Little is known, however, about how organs and cells respond to local perturbations of cell sizes. In a subset of *35S::FZR2* transgenic plants, the expression of *FZR2* was silenced at the whole plant level, but some groups of cells escaped silencing. These sectors showed *FZR2* overexpression phenotypes such as over-branched trichomes and giant pavement cells, whereas

nearby sections of the same leaf contained normal-sized pavement cells and 3- or 4-branch trichomes. These mosaic sectors provided an opportunity to observe how compensation works even within an organ. Inside the sectors were overgrown pavement cells typical of some *FZR2* overexpression lines (Fig. 2A). Away from the sectors, the pavement cells were wildtype in appearance (Fig. 2C and D). At the sector boundary, however, a strip of very small cells formed (Fig. 2B). The smaller cell size at the border may have came about to compensate for the abnormally large cells within the sector, although it is unclear whether this decrease in cell size was followed reduced endoreduplication or simple space limitation.

By studying *fzr2* mutants and misexpression lines, we showed that *FZR2* is necessary and sufficient to induce endoreduplication in various cell types. Our observation that cells increase proliferation to compensate the decreased cell size in *fzr2* mutants provides important evidence that cell proliferation and cell expansion are closely interconnected to regulate organ development in Arabidopsis. Further experiments such as mosaic analysis are needed to further elucidate the compensation mechanism.

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