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Cajal bodies are developmentally regulated during pollen development and pollen tube growth in *Arabidopsis thaliana*

Dear Editor,

Cajal bodies (CBs) are sub-nuclear bodies first described in neurons by Ramon y Cajal in 1903 and subsequently characterized in both plant and animals (Gall, 2003). They appear close to the nucleoli as discrete foci whose size and number vary during the cell cycle (Strzelecka et al., 2010). They are dynamic structures that move, fuse and separate depending on the transcriptional status of the cell (Cioce and Lamond, 2005). CBs are involved in assembly and trafficking of small nucleolar ribonucleoproteins (snoRNPs) and in assembly and trafficking of spliceosomal small nuclear ribonucleoprotein (snRNPs) complexes that are involved in mRNA splicing (Cioce and Lamond, 2005; Gall, 2003). Cajal bodies also contain components of the machinery involved in siRNA-mediated silencing and in methylation of repetitive DNA (Li et al., 2006). Vertebrate Cajal bodies contain a high concentration of coilin, a protein that is widely used as a molecular marker for CBs (Cioce and Lamond, 2005). In Arabidopsis there is a distant homolog of vertebrate coilin (Collier et al., 2006). An Arabidopsis mutant, *ncb1* (*no cajal bodies 1*), lacks CBs due to a single base change at a splice site in Arabidopsis *coilin* (At1g13030), resulting in total disassembly of CBs (Collier et al., 2006; Fang and Spector, 2007). A tagged version of Arabidopsis coilin restored the formation of CBs in *ncb-1* (Collier et al., 2006). Despite the functional relevance of CBs in RNA processing, knock-out mutants for *coilin* have been obtained in mice, although they exhibit reduced viability (Walker et al., 2009), whereas Arabidopsis and Drosophila that lack CBs are completely viable and normal (Collier et al., 2006; Liu et al., 2009). These results therefore suggest that the processes that normally occur in the CBs can be carried out in the nucleoplasm.

During pollen development, four uninuclear microspores (UM) are formed after meiosis of each diploid sporogenous cell, then each microspore enlarges and undergoes an asymmetric mitosis to produce a bicellular pollen grain (BP) with a larger vegetative cell (VC) and a smaller generative cell (GC). The generative cell undergoes a symmetric mitosis to form the two sperm cells

typical of tricellular pollen (TP), which finally develops into a mature pollen grain (MP) (McCormick, 2004). The pollen grain extends a tube that specifically interacts with female tissues (Cheung and Wu, 2008). Numerous genes are expressed at precise times during the different stages of pollen development and during pollen tube growth (Borges et al., 2008; Honys and Twell, 2004; Qin et al., 2009). Thus pollen must have a robust system that controls gene expression and assures efficient maturation of the necessary RNA that is either stored or immediately processed and translated.

It is still debated whether the regulation of gene expression requires variation in the dynamics of CBs (Strzelecka et al., 2010). In order to investigate whether the number and size of the CBs vary during the cell divisions and cellular differentiation that occur during pollen development, we used *Arabidopsis thaliana* pollen. Here, we show that CB cellular distribution varies at different stages of pollen development. Furthermore, the size and number of CBs vary between vegetative and sperm cells of mature and germinated pollen. We suggest that these variations in size and number occur in order to respond to changing gene expression patterns throughout pollen development and germination.

To examine the dynamics of Cajal bodies during pollen development, we expressed a *coilin-YFP* reporter under the control of the Arabidopsis *coilin* promoter in the Col-0 background (Fang and Spector, 2007). Coilin-YFP localized to a single dot within the nucleus in unicellular microspores (Figure 1A, upper left panel; 3 images of the single dot pattern are shown). In bicellular pollen three patterns were identified (Figure 1A, upper right panel): 1) multiple intense dots in the vegetative cell nucleus (VCN) and a less intense single dot in the generative cell nucleus (GCN); 2) a much larger dot in the VCN and two or more smaller dots in the GCN; 3) a much larger dot in the VCN and a smaller single dot in the GCN. In tricellular pollen, there were three patterns of CBs (Figure 1A, lower left panel): 1) a single dot in the VCN; 2) multiple intense dots in the VCN and a less intense single dot in one sperm cell nucleus (SCN); 3) a larger single dot in the VCN with smaller dots in both SCN. In mature pollen, we found four patterns (Figure 1A, lower right panel): 1) a single dot in the VCN and one dot in only one SCN; 2) no signal in the VCN and a single dot in only one SCN; 3) no

signal in the VCN and a single dot in both SCN; 4) More than one dot in the VCN and a single dot in both SCN. In mature pollen the fluorescence intensity was weaker than at earlier developmental stages. This is consistent with *coilin* expression levels: high in unicellular microspores and bicellular pollen but basal levels in tricellular and mature pollen (Honys and Twell, 2004). Expression of Arabidopsis *coilin* was called absent in microarray experiments with sperm cells (Borges et al., 2008), suggesting that the *coilin* signal observed in SCN might derive from residual expression during earlier stages of pollen development.

In order to analyze whether pollen germination is accompanied by variation in the dynamics of CBs, we quantified the number and size of CBs in mature pollen and pollen tubes in a separate experiment. Figure 1B shows that in mature pollen CBs were larger in VCN than in SCN. However, there were more CBs in SCN than in VCN (Fig. 1B). The fewer and larger CBs in VCN might be equivalent to the more frequent and smaller CBs in SCN. In pollen tubes, although the difference in the number of CBs was maintained in the VCN and SCN (Fig. 1B), there were no changes in CB diameter when VCN and SCN of pollen tubes were compared (Fig. 1B). Representative pollen tubes are shown in Fig. 1C. In pollen tubes there was a further decrease in the size of CBs in the VCN (Fig. 1B) without an increase in their number (Fig. 1B), suggesting that there is less need for CBs in the vegetative cell after pollen germination. In mature and germinated pollen, respectively, sperm cells on average had 1.6 and 1.7 times more CBs than the vegetative cell. Also, the SCN of pollen tubes had more CBs than the SCN of mature pollen (Fig. 1B). Considering that CBs are more numerous in highly active cells (Cioce and Lamond, 2005), this suggests that sperm cells in pollen tubes are more transcriptionally active than sperm cells in mature pollen. Our results further suggest that CBs respond during pollen development to the differing physiological needs of the different types of male gametophytic cells. Therefore pollen is a good system to study post-transcriptional regulation in the nucleus.

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Figure Legend

Figure 1. Cajal bodies are developmentally regulated during pollen development.

(A) Representative images of Uninuclear Microspores (UM) (upper left panel), Bicellular Pollen (BP) (upper right panel), Tricellular Pollen (TP) (lower left panel) and Mature Pollen (MP) (lower right panel) from multiple flowers from at least 10 individual T1 plants harboring the *coilin-YFP* construct, acquired with a fluorescent microscope. Mature pollen percentages were calculated by combining results obtained from the T1 plants and from multiple flowers from four isogenic homozygous T3 plants (used to make Figure 1B). Percentages indicate the incidence of each pattern. At least 100 microspores or pollen grains were counted. Scale bar, 10 μm.

(B) Cajal body (CB) size and number vary between vegetative and sperm cells of mature and germinated pollen. Comparison of diameter (left panel) and number (right panel) of CBs in the nuclei of vegetative cells (VC) and sperm cells (SC) of mature pollen (MP) and germinated pollen (GP). In vitro germination was performed as described (Boavida and McCormick, 2007). Pollen from multiple flowers obtained from four isogenic homozygous T3 plants was incubated for 3 h without agitation at 22 ˚C, and then incubated with DAPI (5 ng/μl) for 2 h in the dark at 22˚C. At least 50 pollen grains and pollen tubes were counted. Bars

represent the standard error. Mean values are significantly different with $p<0.05$ (two tailed Student's t test). ns means not significant.

(C) Representative images from germinated pollen acquired with a confocal microscope. Scale bar, 10 μm. Arrows indicate sperm nuclei (SCN) while arrowhead indicates the vegetative nucleus (VCN).

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