

# Coiled body numbers in the *Arabidopsis* root epidermis are regulated by cell type, developmental stage and cell cycle parameters

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## SUMMARY

We have used whole mount immunofluorescence labelling with the antibody 4G3, raised against the human snRNP-specific protein U2B'', and whole mount in situ hybridization with an anti-sense probe to a conserved region of U2 snRNA, in combination with confocal microscopy, to examine the organization of spliceosomal components throughout the development of the *Arabidopsis thaliana* root epidermis. We show that the number of coiled bodies, nuclear organelles in which splicing snRNPs and snRNAs concentrate, is developmentally regulated in the *Arabidopsis* root epidermis. Firstly, there is a progression from a small number of coiled bodies in the quiescent centre and initial cells, to a larger number in the cell division zone, returning to a lower number in the cell

elongation and differentiation zone. Secondly, trichoblasts (root-hair forming epidermal cells) have on average 1.5 times more and often smaller coiled bodies than atrichoblasts (hairless epidermal cells). Moreover, we have shown that these differences in coiled body numbers are related to differences in cell cycle stage, cell type and developmental stage, but are not due to differences in nucleolar or general metabolic activity per se. We discuss possible explanations, including a model in which coiled bodies coalesce during interphase, for the developmental dynamics of coiled bodies.

Key words: Coiled body, snRNA, Root development, Confocal microscopy

## INTRODUCTION

Cell differentiation is accompanied by changes in nuclear structure and organization in many eukaryotic systems. Such reorganization reflects changes in the organization of transcription and transcript processing (spliceosomal) components during differentiation. For example striking reorganization of transcription and splicing components has been described for differentiating cells of the rat hippocampus (Santama et al., 1996). Changes in nuclear organization have also been described in plants, most notably during root cell differentiation in *Zea mays*, *Haemanthus katherinae* and *Cucurbita pepo* (Pachter et al., 1969; Kononowicz et al., 1976; Olszewska, 1976), during pollen development (Eady et al., 1995; Testillano et al., 1995) and during differentiation of caulonema of the moss *Funaria* (Kingham et al., 1995). To examine the role of these nuclear dynamic processes in the development of plants we are using the *Arabidopsis* root. The *Arabidopsis* root epidermis is a useful developmental system since it is composed of cells representing each stage of a developmental continuum which consists of a cell division, elongation and differentiation zone (Berger et al., 1998). Although the organization of spliceosomal components, including coiled bodies, round, nuclear organelles in which splicing components concentrate, has been studied in plant cells (Beven et al., 1995), coiled

body dynamics have not been examined in a progressively differentiating system in plants.

The function of coiled bodies is still under debate but a number of hypotheses have been proposed (for review see Lamond and Earnshaw, 1998). It has been speculated that coiled bodies may be sites of splicing factor assembly and/or recycling, or may play a role in histone mRNA 3' processing. Recently, it has been shown that coiled bodies are involved in processing or transport of small nucleolar RNA (snoRNA) precursors in maize (Shaw et al., 1998). The presence of small nuclear ribonucleoproteins (snRNPs), proteins complexed to small nuclear RNAs (snRNAs) involved in processing of pre-mRNA, in coiled bodies, is transcription or differentiation dependent (Carmo-Fonseca et al., 1992; Antoniou et al., 1993; Lafarga et al., 1995; Huang and Spector, 1996; Santama et al., 1996). When transcription is inhibited, snRNPs no longer concentrate in coiled bodies but instead aggregate in large clusters thought to be storage sites of splicing factors. Conversely, coiled bodies are more numerous in rapidly dividing cells, or in highly active cells and tumours (Lafarga et al., 1991; Brasch and Ochs, 1992; Carmo-Fonseca et al., 1992; Andrade et al., 1993; Alliegro and Alliegro, 1998). Changes are also observed in the size and number of coiled bodies during the cell cycle (Andrade et al., 1993; Carmo-Fonseca et al., 1993; Chan et al., 1994; Ferreira et al., 1994; Beven et al., 1995). During mitosis, coiled bodies are largely

disassembled and the splicing snRNPs are distributed throughout the cytoplasm. Coiled bodies are larger and fewer in S and G<sub>2</sub> phases of the cell cycle, smaller and more numerous during G<sub>1</sub> phase (Andrade et al., 1993; Chan et al., 1994). They contain spliceosomal snRNPs and snRNAs as well as several nucleolar proteins such as fibrillarin, SSB1 and NOPP140. A human autoantigen, p80 coilin, which is highly enriched in coiled bodies, has been widely used as a marker for coiled bodies. Coiled bodies are often seen adjacent to nucleoli, but they are also seen both in the nucleoplasm and within nucleoli (Malatesta et al., 1994; Ochs et al., 1994), and therefore may act as nuclear transport or sorting structures.

The morphology and development of the *Arabidopsis* root has been described by Dolan et al. (1993). Root development is a continuous process in which files of cells arise from a template of initial cells, surrounding 4 central quiescent centre cells located at the root tip (Clowes, 1953). Derivatives of the initials divide transversely in the meristematic region of the root to produce packets of cells that subsequently elongate and differentiate (Dolan et al., 1993). There are 2 classes of epidermal cells defined by their position relative to the underlying cortical cells (Dolan et al., 1994; Galway et al., 1994). Trichoblasts are epidermal cells that form root hairs and overlie the junction between two cortical cells (Cormack, 1947). Atrichoblasts form hairless epidermal cells and contact only one cortical cell. The epidermis therefore consists of alternating files of trichoblasts and atrichoblasts. The atrichoblasts are significantly longer than the trichoblasts. This size difference is apparent in the cell division zone before hairs form.

We show here that the number of coiled bodies changes as a function of development in the *Arabidopsis* root epidermis. We investigate the roles of cell cycle stage, metabolic activity and cell type in regulating this number. We provide some models to explain the developmental dynamics of coiled body numbers.

## MATERIALS AND METHODS

### Plant strains and growth conditions

*Arabidopsis thaliana* seeds were sterilized in 20% bleach and plated in Petri-dishes, on to Murashige and Skoog salt mixture supplemented with 1% agarose and 3% sucrose. Seeds were placed at 4°C for 48 hours and then grown upside-down, allowing roots to grow in the air, in a standard growth cabinet, 25°C, continuous light conditions. *Arabidopsis thaliana* ecotype 'Columbia' was used as wild-type. The *ttg* mutant, in the Landsberg background, was obtained from the Nottingham *Arabidopsis* Seed Stock Centre. 35S::R seeds, in the *ttg* mutant background, were kindly provided by Alan Tisier and Jonathan Jones (John Innes, Norwich).

### Whole mount immunofluorescence labelling

Three-day old seedlings were fixed for 1 hour in 4% (w/v) formaldehyde (freshly prepared by dissolving 8% (w/v) solid paraformaldehyde in water and then diluted with an equal volume of 2× PEM/0.4% NP40 (Nonidet P-40) buffer) (PEM: 50 mM Pipes, 5 mM EGTA, 5 mM MgSO<sub>4</sub>, pH 6.9). The fixed seedlings were rinsed two times in PEM/0.2% NP40, and finally washed in PEM. The root tips were laid down on to glutaraldehyde-activated APTES (γ-aminopropyltriethoxysilane) coated multiwell slides and allowed to dry. A permeabilization with 1% (w/v) driselase/0.5% cellulase/0.025% pectolyase Y23 in PEM, for 10 minutes, followed. The roots were then washed six times with PEM/0.2% NP40, and

allowed to dry. A blocking step for 1.5 hour, with PEM/0.2% NP40/3% BSA followed. The slides were incubated overnight at 4°C with a 1:10 dilution of primary antibody 4G3 (Euro-diagnostica B.V., Netherlands) in PEM/3% BSA. The primary antibody was detected by cy3-conjugated secondary antibody (Jackson ImmunoResearch). The nuclei were counterstained with 1 μg DAPI/ml PEM for 5 minutes. The slides were mounted in Vectashield (Vector laboratories).

### Whole mount in situ hybridization

Three-day old seedlings were fixed for 1 hour in 4% (w/v) formaldehyde (freshly prepared by dissolving 8% (w/v) solid paraformaldehyde in water and then diluted with an equal volume of 2× PEM/0.2% Tween-20 buffer), 0.1% (v/v) glutaraldehyde and 10% DMSO. The fixed seedlings were rinsed in methanol, twice, and subsequently in ethanol, four times, for 3 minutes each, at -20°C. The seedlings were then left in 1:1 (v/v) ethanol/xylene for 30 minutes and were finally washed in ethanol and methanol for 3 minutes each. The seedlings were next rinsed two times with PEM/0.1% Tween-20, and finally washed in PEM. The root tips were laid down on to glutaraldehyde-activated APTES coated multiwell slides and allowed to dry. A permeabilization with 1% (w/v) driselase/0.5% cellulase/0.025% pectolyase Y23 in PEM, for 10 minutes, followed. Roots were then washed six times with PEM/0.1% Tween-20, and allowed to dry. A limited proteinase K digestion followed (40 μg/ml in PEM/0.1% Tween-20) for 10 minutes at 37°C. The reaction was stopped by adding 0.2% glycine/0.1% Tween-20 for 5 minutes. After four washes with PEM/0.1% Tween-20, the roots were treated four times, for 15 minutes at room temperature, with freshly prepared sodium borohydride solutions (1 mg/ml in PEM) to reduce autofluorescence caused by glutaraldehyde. Four rinses with PEM/0.1% Tween-20, and finally with 0.1× SSC, followed. A probe solution containing 0.25 μl digoxigenin-labelled U2 snRNA anti-sense probe (~200 ng/μl) (for preparation see Beven et al., 1995), 0.25 μl unlabelled 5S RNA (~1,000 ng/μl), 2.5 μl deionised formamide, 1 μl 50% dextran sulphate, 0.5 μl buffer (100 mM Pipes, pH 8.0, 10 mM EDTA), and 0.5 μl 3 M NaCl, was added to each well. Slides were placed in a damp chamber at 37°C overnight, then washed in excess 0.1× SSC at 50°C for 1 hour. A blocking step for 1.5 hour, with PEM/0.1% Tween-20/3% BSA followed. Digoxigenin-labelled probe was then detected by an unlabelled primary antibody (Sigma Immuno Chemicals). The primary antibody was detected by cy3-conjugated secondary antibody (Jackson ImmunoResearch). The nuclei were counterstained with 1 μg DAPI/ml PEM for 5 minutes. The slides were mounted in Vectashield.

### In vitro transcription

For visualization of nucleolar transcription sites, three-day old seedlings were brought into physiological buffer (PB) (100 mM KAc, 20 mM KCl, 20 mM Hepes, pH 7.4, 1 mM MgCl<sub>2</sub>, 1 mM ATP, 1% thiodiglycol, 2 μg/ml aprotinin, 0.5 mM phenylmethylsulphonyl fluoride) for 5 minutes. They were incubated in 0.05% (v/v) Triton X-100 in PB for 5 minutes, followed by 1 wash in PB. The seedlings were incubated in transcription mix (500 μM CTP, 500 μM GTP, 250 μM BrUTP, 125 μM MgCl<sub>2</sub>, 100 units/ml RNA guard in PB) for 5 minutes, followed by 3 washes in PB. The roots were fixed and treated as mentioned in the whole mount immunofluorescence protocol. As primary antibody we used a 1:20 dilution mouse anti-BrdU antibody (Boehringer Mannheim). The primary antibody was detected by cy3-conjugated antibody (Jackson ImmunoResearch).

### Optical microscopy and image analysis

Confocal optical section stacks were collected using a Bio-Rad MRC-600 confocal scanning microscope as described previously (Rawlins and Shaw, 1990). Images were transferred to a PC or Macintosh computer, assembled into composite images using Adobe Photoshop and NIH Image, a program for the Macintosh available via <http://rsb.info.nih.gov/nih-image>. Images were printed on a Fujix Pictography 3000 printer.

Intensities of nucleolar transcription sites were measured using NIH Image. Firstly, confocal optical section series were projected into composite images using the mean value projection method (existing NIH routine). Transcriptional intensities were quantified then by drawing a wide boundary around every nucleus and measuring the number of pixels in the selected area \* (mean-background). Therefore we wrote a new subroutine: Area\*(Mean-Modal Value).

## RESULTS

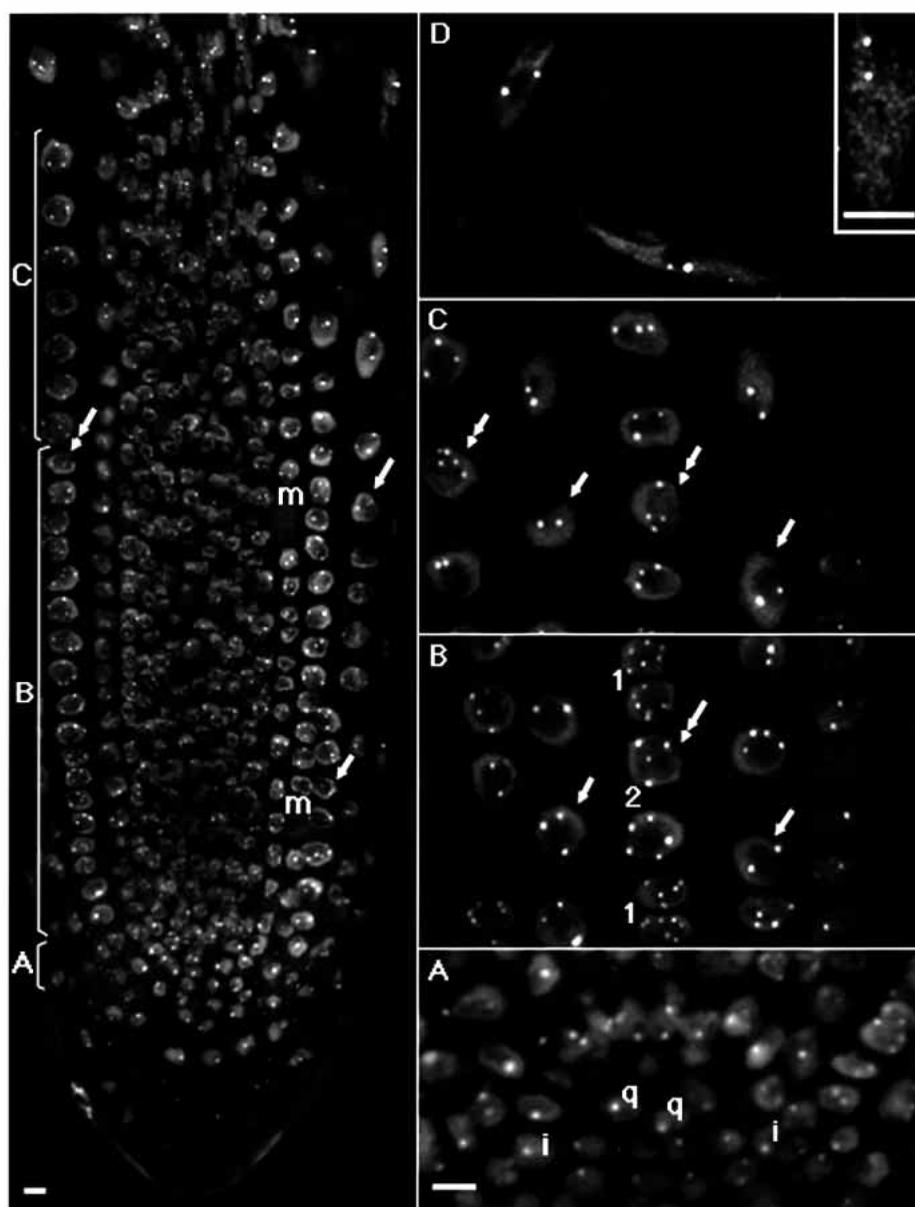
### U2B'' snRNP and U2 snRNA are present in coiled bodies and an interchromatin network in *Arabidopsis* roots

We examined the distribution of the U2B'' splicing protein by whole mount immunofluorescence using the 4G3 antibody on *Arabidopsis* roots and confocal microscopy. In situ hybridization using a probe against U2 snRNA showed an identical localization. Labelling was localised to small,

prominent spherical structures, corresponding to coiled bodies (Beven et al., 1995), and to a diffuse interchromatin network (Fig. 1). There was little or no cytoplasmic labelling, and the label was excluded from the nucleolus, apart from some labelling of the central nucleolar cavity (not shown). Upon entry into mitosis the labelling was redistributed throughout the cytoplasm (Fig. 1), although occasionally a few coiled bodies remained visible. At telophase many small bodies reappeared. Furthermore, putative G<sub>1</sub> nuclei had more and often smaller coiled bodies than putative S/G<sub>2</sub> nuclei (Fig. 1B).

### Coiled body number varies as a function of development in *Arabidopsis* roots

Coiled body numbers were examined in the different developmental regions of the root (Fig. 1; Table 1). A single large coiled body was present in each of the four quiescent centre cells (Fig. 1A). 1-5 coiled bodies were present in the initial cells of the epidermis/lateral root cap that lie next to the



**Fig. 1.** Labelling of *Arabidopsis* root epidermal files with 4G3. In each case a projection of a series of confocal images is shown. The nuclear interchromatin network is brightly labelled, the nucleolus is unlabelled except from the centre of the nucleolus. The round bright spots, often associated with the nucleolus, are coiled bodies. Cells in mitosis show diffuse cytoplasmic labelling (m). (A) Quiescent centre and initials zone; quiescent centre (q) and epidermis/lateral root-cap initials (i) show a few big coiled bodies. Number and size of coiled bodies are variable in the cell division zone (B). Trichoblast files (double arrows) have on average more and smaller coiled bodies than atrichoblast files (single arrows). Nuclei become more widely spaced and enlarge in the slow elongation zone (C). Coiled body numbers decrease in elongation zone. Most nuclei have 1 or 2 coiled bodies in the differentiation zone (D). Inset shows a speckled network in the nucleoplasm of a root-hair nucleus. Putative G<sub>1</sub> cells (1) have lots of small coiled bodies while putative S/G<sub>2</sub> cells (2) have fewer and bigger coiled bodies. Bars, 5  $\mu$ m.

**Table 1. Coiled body numbers in epidermal files of *Arabidopsis* roots**

|  | Number of nuclei counted | Average no. coiled bodies $\pm$ s.e.m. | Min. CB* | Max. CB‡ |
|--|--------------------------|--|----------|----------|
| Quiescent centre                       | 18                       | 1.00 $\pm$ 0.00                        | 1        | 1        |
| Epidermal/lateral root-cap initials    | 14                       | 1.79 $\pm$ 1.12                        | 1        | 5        |
| Trichoblasts (root-hair forming cells) |                          |  |          |          |
| Cell division zone                     | 450                      | 4.89 $\pm$ 1.91                        | 1        | 20       |
| Slow elongation zone                   | 122                      | 3.78 $\pm$ 1.06                        | 1        | 6        |
| Fast elongation zone                   | 32                       | 3.16 $\pm$ 1.05                        | 1        | 5        |
| Early+mid differentiation zone         | 161                      | 1.70 $\pm$ 0.60                        | 1        | 3        |
| Late differentiation zone              | 74                       | 1.00 $\pm$ 0.00                        | 1        | 1        |
| Atrichoblasts (hairless cells)         |                          |  |          |          |
| Cell division zone                     | 276                      | 3.22 $\pm$ 1.05                        | 1        | 6        |
| Slow elongation zone                   | 95                       | 2.72 $\pm$ 0.90                        | 1        | 5        |
| Fast elongation zone                   | 22                       | 2.09 $\pm$ 0.75                        | 1        | 5        |
| Early+mid differentiation zone         | 185                      | 1.23 $\pm$ 0.44                        | 1        | 3        |
| Late differentiation zone              | 20                       | 1.00 $\pm$ 0.00                        | 1        | 1        |

\*Min. CB means minimum coiled body number observed in these cells.  
‡Max. CB means maximum coiled body number observed in these cells.

central cells (Fig. 1A). Initial cells give rise to a rapidly dividing population of meristematic cells which had between 1 and 20 coiled bodies (Fig. 1B). The files of meristematic epidermal cells were clearly distinguishable as trichoblasts or atrichoblasts, since nuclei of atrichoblast files are spaced further apart than those of the trichoblast files (Fig. 1B). We determined the number of coiled bodies in the two epidermal cell types in the cell division zone (meristem) (Table 1). The coiled body frequency distributions were clearly different (Fig. 2). Coiled body number in trichoblasts ranged from 1-20 with a mean value of 4.9 while coiled body number ranged from 1-6 with a mean of 3.2 in atrichoblasts. Trichoblasts therefore have on average 1.5 times more coiled bodies than atrichoblasts in the cell division zone.

Once cells have stopped dividing they continue to elongate, and the zone of elongation can be divided into an initial slow and subsequent fast zone. The numbers of coiled bodies decreased in all epidermal cells in the elongation zone but there were still 50% more coiled bodies in trichoblasts than atrichoblasts (Fig. 1C; Table 1). Cells then enter the differentiation zone where elongation ceases and root hairs develop. This progression, from elongation to differentiation zone, was accompanied by a further decrease in both mean and maximum numbers of coiled bodies (Fig. 1D; Table 1) in both trichoblast and atrichoblast files.

We also examined coiled body numbers in the *ttg* mutant

**Table 2. Coiled body numbers in cell division zone in wild-type, *ttg* mutant and 35S::R roots**

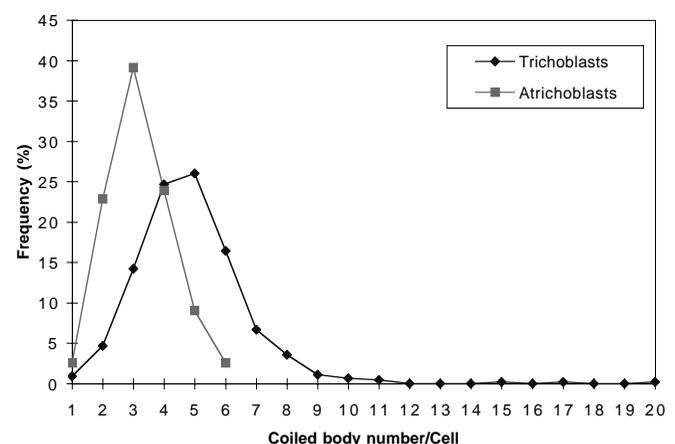
|               | Number of nuclei counted | Average no. coiled bodies $\pm$ s.e.m. | Min. CB* | Max. CB‡ |
|---------------|--------------------------|--|----------|----------|
| Wild type     |                          |  |          |          |
| Trichoblasts  | 450                      | 4.89 $\pm$ 1.91                        | 1        | 20       |
| Atrichoblasts | 276                      | 3.22 $\pm$ 1.05                        | 1        | 6        |
| <i>ttg</i>    |                          |  |          |          |
| Trichoblasts  | 225                      | 4.39 $\pm$ 1.32                        | 2        | 9        |
| 35S::R        |                          |  |          |          |
| Atrichoblasts | 110                      | 3.41 $\pm$ 1.21                        | 1        | 7        |

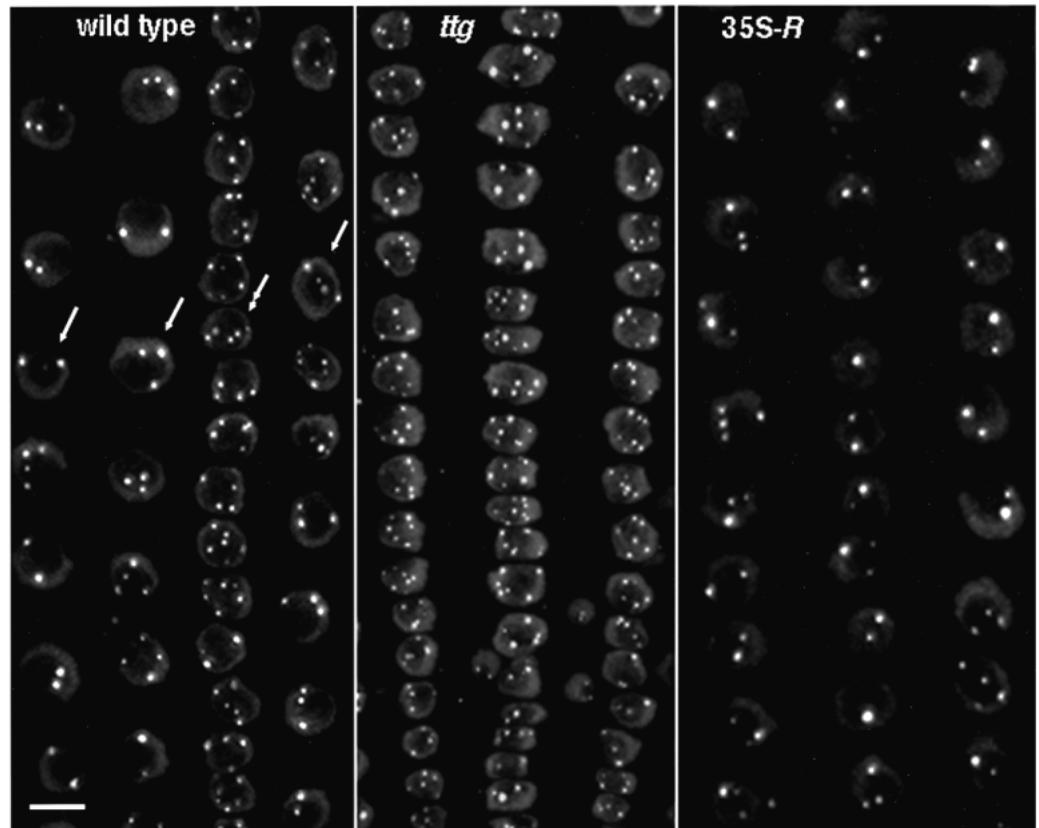
\*Min. CB means minimum coiled body number observed in these cells.  
‡Max. CB means maximum coiled body number observed in these cells.

and 35S::R transgenic line, in which all cells in the root epidermis are trichoblasts or atrichoblasts, respectively. Most epidermal cells in *ttg* roots produce root hairs in the differentiation zone while 35S::R roots show a hairless phenotype. In all epidermal cells of *ttg* roots the number of coiled bodies was similar to wild-type trichoblast cells (Fig. 3; Table 2). In 35S::R roots, the number of coiled bodies in all epidermal cells was approximately the same as in wild-type atrichoblast cells (Fig. 3; Table 2). These results support the correlation between coiled body number and cell type in the meristem.

### Coiled body number and nucleolar transcription

Several reports have suggested a functional relationship between coiled bodies and nucleoli, based on the presence of nucleolar proteins in the coiled body, the frequent association of coiled bodies with the nucleolus, the appearance of coiled bodies in the nucleolus under certain conditions and the increase in the number of coiled bodies after stimulation of nucleolar transcription (Lafarga et al., 1991; Malatesta et al., 1994; Ochs et al., 1994; Bohmann et al., 1995a,b; Lyon et al., 1997). To examine directly the relation between nucleolar

**Fig. 2.** Frequency distributions of coiled body numbers in trichoblasts and atrichoblasts in the cell division zone.



**Fig. 3.** Labelling of wild-type, *ttg* and 35S::R roots with 4G3 antibody. In each case a projection of a series of confocal images is shown. In wild-type, cells in trichoblast files (double arrows) have on average more and smaller coiled bodies than cells in atrichoblast files (single arrows). All cells in files of *ttg* roots have a trichoblast fate and show a coiled body pattern which is similar to wild-type trichoblast cells. 35S::R roots, in which all files have an atrichoblast fate, show a coiled body pattern which is similar to wild-type atrichoblast files. Bar, 5  $\mu$ m.

transcriptional activity and coiled body number in the *Arabidopsis* root, we visualized nucleolar transcription sites by incorporation of BrUTP.

We observed low levels of nucleolar transcription in the quiescent centre and initial cells (Fig. 4A) which correlated with the low number of coiled bodies in these cells. Higher

levels of nucleolar transcription were observed in cells of the meristematic region (Fig. 4B). This increase in nucleolar transcription was correlated with an increase in nucleolar volume and accompanied by an increase in the numbers of coiled bodies. There was variation between different roots in the transcription ratio between trichoblasts and atrichoblasts

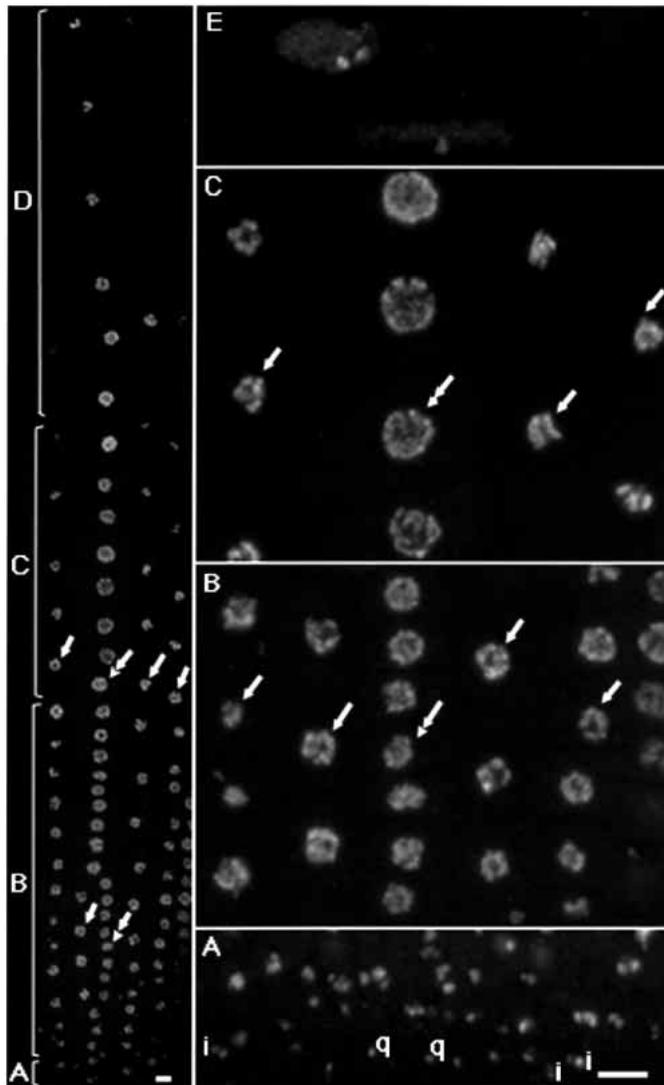
**Table 3. Nucleolar transcription intensities in trichoblasts and atrichoblasts**

|                      | Average nucleolar<br>transcr. act. in<br>trichoblasts* $\pm$ s.e.m. | Average nucleolar<br>transcr. act. in<br>atrichoblasts* $\pm$ s.e.m. | Ratio transcription<br>trich./atrich. ‡ |
|----------------------|---|--|---|
| Cell division zone   |   |  |   |
| Root 1               | 30,889 $\pm$ 8,520 (8)  | 43,131 $\pm$ 6,108 (5)   | <b>0.72 (-3.01)</b>                     |
| Root 2               | 24,151 $\pm$ 12,564 (24)  | 25,105 $\pm$ 8,203 (13)  | 0.96 (-0.28)                            |
| Root 3               | 5,294 $\pm$ 2,336 (33)  | 2,679 $\pm$ 573 (17)   | <b>1.98 (6.08)</b>                      |
| Root 4               | 4,718 $\pm$ 3,253 (35)  | 4,593 $\pm$ 3,213 (24)   | 1.03 (0.15)                             |
| Root 5               | 76,816 $\pm$ 15,538 (8)   | 55,741 $\pm$ 17,308 (9)  | <b>1.38 (2.65)</b>                      |
| Root 6               | 7,497 $\pm$ 3,868 (22)  | 8,030 $\pm$ 3,218 (28)   | 0.93 (-0.52)                            |
| Root 7               | 8,924 $\pm$ 4,164 (13)  | 13,445 $\pm$ 5,627 (13)  | <b>0.66 (-2.33)</b>                     |
| Root 8               | 32,386 $\pm$ 8,886 (12)   | 26,446 $\pm$ 6,269 (8)   | 1.22 (1.75)                             |
| Root 9               | 39,881 $\pm$ 12,246 (18)  | 46,080 $\pm$ 12,689 (19)   | 0.87 (-1.51)                            |
| Slow elongation zone |   |  |   |
| Root 2               | 47,904 $\pm$ 17,545 (6)   | 12,682 $\pm$ 416 (2)   | <b>3.78 (4.91)</b>                      |
| Root 4               | 14,478 $\pm$ 4,779 (13)   | 8,745 $\pm$ 4,806 (10)   | 1.66 (2.84)                             |
| Root 5               | 118,739 $\pm$ 0 (1)   | 48,179 $\pm$ 2,973 (2)   | <b>2.46 (33.57)</b>                     |
| Root 9               | 37,892 $\pm$ 15,811 (12)  | 14,569 $\pm$ 8,315 (4)   | <b>2.60 (3.78)</b>                      |

\*Between brackets are the number of cells the data are based on.

‡Between brackets are z values for testing of significant differences between trichoblast and atrichoblast transcription levels using the two-tailed test (M. Spiegel, 1992). Transcription levels between the 2 cell types are significantly different at the 0.05 level if z lies outside the range -1.96 to 1.96. Significant differences are marked bold.

Note: Average transcr. activity numbers (units=number of pixels) cannot be compared between different roots because of different labelling intensities between roots.



**Fig. 4.** Visualization of nucleolar transcription sites in the *Arabidopsis* root epidermis. In each case a projection of a series of confocal images is shown. (A) Quiescent centre and initial zone; nucleolar transcription sites are small in the quiescent centre (q) and initial cells (i) and become larger in the cell division zone (B). Trichoblast files (double arrows) show roughly the same transcription intensities as atrichoblast files (single arrows). Nucleolar transcription increases in trichoblast files but decreases in atrichoblast files in the slow elongation zone (C). Nucleolar transcription decreases in trichoblast files in fast elongation zone (D). In root-hair cells there is clear extranucleolar transcription, and nucleolar transcription sites (bright spots) are very small (E). Bars, 5  $\mu$ m.

(Table 3). Two roots out of 9 showed a significantly higher transcription level in the trichoblasts compared to the atrichoblasts and two roots showed a significantly lower level. The other 5 roots showed similar transcription levels in both cell types. However, in all roots examined, we always found a higher average number of coiled bodies in trichoblasts than in atrichoblasts. Therefore the difference in coiled body number in this zone, between trichoblasts and atrichoblasts, cannot be ascribed to differences in nucleolar transcription levels.

**Table 4. Nucleolar transcription ratios upon progression from cell division to elongation zone**

|                            | Trichoblasts       | Atrichoblasts       |
|----------------------------|--------------------|---------------------|
| Ratio nucleolar transcr.   |                    |                     |
| slow elong./cell div. zone |                    |                     |
| Root 2                     | <b>1.98 (3.12)</b> | <b>0.51 (-5.41)</b> |
| Root 4                     | <b>3.07 (6.80)</b> | <b>1.90 (2.51)</b>  |
| Root 5                     | <b>1.54 (7.63)</b> | 0.86 (-1.23)        |
| Root 9                     | 0.95 (-0.37)       | <b>0.32 (-6.21)</b> |

For every root the average transcription activity of  $\pm 6$  cells in slow elongation zone was divided by the average transcription activity of  $\pm 15$  cells in cell division zone. Between brackets are z values for testing of significant differences. Significant differences are marked bold.

Upon progression from cell division to slow elongation zone, 3 roots out of 4 showed an increase in nucleolar transcription in trichoblasts while there was more variability in atrichoblasts (Fig. 4C; Table 4). However, coiled body number decreased in both cell types upon progression from the meristematic to the elongation zone in all roots. This supports the earlier observation that coiled body number is regulated independently of nucleolar transcription levels in the *Arabidopsis* root developmental context.

## DISCUSSION

We have shown that the number of coiled bodies is determined in the *Arabidopsis* root epidermis by the cell cycle stage, the cell type and the developmental stage. Although previous studies have shown a link between nucleolar transcriptional activity and coiled body number, we have shown that these parameters can be uncoupled in the root epidermis. Our results illustrate that the regulation of the coiled body number in the *Arabidopsis* root epidermis is a complex interaction between different factors.

### Cell type is a key regulator of coiled body number in the *Arabidopsis* root epidermis

While coiled body number is influenced by a diverse set of factors such as general metabolic activity, cell cycle, drugs, etc., we show that cell type is a major determinant of coiled body number in the *Arabidopsis* root epidermis. As shown previously (Andrade et al., 1993; Carmo-Fonseca et al., 1993; Chan et al., 1994; Ferreira et al., 1994; Beven et al., 1995), there was a decrease in coiled body number upon progression from G<sub>1</sub> to S/G<sub>2</sub> cell cycle stage. Nevertheless the coiled body number in G<sub>1</sub> nuclei from the two epidermal cell types is different, indicating that the initial number of coiled bodies formed in post-mitotic cells is cell type dependent. The number of coiled bodies subsequently decreases in each cell type as cells progress through the cell cycle. Consequently the variation in coiled body number between the epidermal cell types might be considered to be independent of cell cycle regulation. Such cell type regulation of coiled body number has only been described in humans by Raska et al. (1991) who showed that human blood lymphocytes contain a single coiled body while most other human cell types contained 3-5 coiled bodies. Our analysis shows that while cell cycle stage/progression is clearly an important factor in determining

coiled body number, cell type is an independent factor involved in coiled body number regulation.

### Coiled bodies and nucleolar activity can be decoupled

Previous studies from both plants and animals have shown that coiled bodies are more numerous in highly active cells and that upon inhibition of transcription, splicing components no longer concentrate in coiled bodies but instead aggregate in interchromatin granule clusters, thought to be storage sites for splicing factors (Lafarga et al., 1991; Brasch and Ochs, 1992; Carmo-Fonseca et al., 1992; Andrade et al., 1993; Antoniou et al., 1993; Jiménez-García and Spector, 1993; Malatesta et al., 1994; Beven et al., 1995; Lafarga et al., 1995; Huang and Spector, 1996; Santama et al., 1996; Alliegro and Alliegro, 1998). The overall progression from low coiled body number in the QC, to higher numbers in the cell division zone, returning to lower numbers in the differentiation zone mirrors the overall progression in metabolic activity of the respective cells as judged by the nucleolar volume, which is considered to be a good marker for metabolic activity (for review see Goessens, 1984). To examine, in detail, whether the differences in coiled body numbers in the *Arabidopsis* root epidermis reflect differences in nucleolar or metabolic activity, we visualized nucleolar transcription sites by BrUTP incorporation. Our results show that while coiled body numbers reflect the gross metabolic status of cells in the root, the coiled body number does not always mirror the nucleolar transcriptional activity of any particular cell. For example nucleolar transcription levels increase in trichoblasts in the slow elongation zone while the coiled body number decreases in the transition from the cell division zone to the slow elongation zone. This suggests that transcription and coiled body number are not obligatorily coupled. Other studies have also provided indirect evidence for a partial uncoupling between transcription and coiled body numbers. Nucleolar transcription levels have been shown to increase from G<sub>1</sub> to G<sub>2</sub> phase (Moreno Díaz de la Espina et al., 1980; Hochhauser et al., 1981; de la Torre and Giménez-Martín, 1982), while coiled body numbers show the opposite dynamics. Furthermore, Ferreira and Carmo-Fonseca (1995, 1996) have shown that the biogenesis of coiled bodies in animal embryonic cells is independent of the onset of transcriptional activity.

### Models for coiled body dynamics

Coiled body number decreases through the cell cycle and the number formed after mitosis at early G<sub>1</sub> clearly depends on the cell type. These observations are consistent with a model in which coiled bodies, initiated in early G<sub>1</sub>, coalesce during the subsequent period of the cell cycle. Such a coalescing coiled body model is plausible as recently Boddy et al. (1997) have also observed coalescence of PML nuclear bodies in mammalian cells. Other evidence comes from observations that coiled bodies are occasionally linked together in pairs, as though in the process of fusing (not shown). An alternative hypothesis is that small coiled bodies lose components to larger coiled bodies during cell cycle progression. A prediction of both of these models is that coiled body number decreases with time from the last mitosis. This is consistent with the one or two coiled bodies seen in the slowly cycling QC cells or in the non-dividing cells of the differentiation zone. However, these

models do not take into account cell type factors involved in specifying the number of coiled bodies in the post-mitotic cell. It is likely that such factors include regulators of cell fate. Moreover, we have observed coiled bodies as cap-like structures located at the nucleolar periphery (not shown), which indicates that coiled bodies might bud from or fuse with the nucleolus. To distinguish between different models it will be necessary to observe coiled bodies in living cells. To this end we are currently generating GFP-U2B' fusions in transgenic plants.

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