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The Movement of Coiled Bodies Visualized in Living Plant Cells by the Green Fluorescent Protein

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Coiled bodies are nuclear organelles that contain components of at least three RNA-processing pathways: pre-mRNA splicing, histone mRNA 3′- maturation, and pre-rRNA processing. Their function remains unknown. However, it has been speculated that coiled bodies may be sites of splicing factor assembly and/or recycling, play a role in histone mRNA 3′-processing, or act as nuclear transport or sorting structures. To study the dynamics of coiled bodies in living cells, we have stably expressed a U2B″–green fluorescent protein fusion in tobacco BY-2 cells and in *Arabidopsis* plants. Time-lapse confocal microscopy has shown that coiled bodies are mobile organelles in plant cells. We have observed movements of coiled bodies in the nucleolus, in the nucleoplasm, and from the periphery of the nucleus into the nucleolus, which suggests a transport function for coiled bodies. Furthermore, we have observed coalescence of coiled bodies, which suggests a mechanism for the decrease in coiled body number during the cell cycle. Deletion analysis of the *U2B″* gene construct has shown that the first RNP-80 motif is sufficient for localization to the coiled body.

INTRODUCTION

The coiled body was first described by Ramon y Cajal (1903), who called it the "nucleolar accessory body" because of its association with the nucleolus. This nuclear organelle was later reidentified by electron microscopy and renamed the "coiled body" because of its appearance as loosely packed coiled fibrils (Monneron and Bernhard, 1969). Subsequent studies detected coiled bodies in animal and plant nuclei, showing that it is a conserved structure (Moreno Diaz de la Espina *et al.*, 1980; Seite *et al.*, 1982; Schultz, 1990).

Coiled bodies have been shown to contain splicing small nuclear ribonucleoproteins (snRNPs) and small nuclear RNAs (snRNAs), a subset of nucleolar components — including fibrillarin, Nopp140, NAP57, and U3 small nucleolar ribonucleoprotein (U3 snoRNP) — and the protein p80 coilin, which has been widely used as a marker for coiled bodies (Lamond and Earnshaw, 1998; Matera, 1998). The function of coiled bodies is still under debate, but several hypotheses have been proposed that are not necessarily mutually exclusive. Because coiled bodies do not contain DNA, nascent pre-mRNA, heterogeneous nuclear RNPs (hnRNPs), or the SC-35 splicing factor, which is required for splicing in vitro, it has been argued that they are not directly involved in transcription and pre-mRNA splicing (Lamond and Carmo-Fonseca, 1993). However, it has been speculated that coiled bodies may be sites of splicing factor assembly or recycling, may play a role in histone mRNA 3′ processing (Gall *et al.*, 1995; Lamond and Earnshaw, 1998), or may be involved in all these activities. As coiled bodies are frequently observed at the nucleolar periphery and also in the nucleoplasm and within nucleoli (Malatesta *et al.* 1994; Ochs *et al.*, 1994), they may also act as nuclear transport or sorting structures. It has been shown recently that coiled bodies are also involved in processing or transport of small nucleolar RNA (snoRNA) precursors in maize (Shaw *et al.*, 1998).

Coiled bodies have been shown to be dynamic structures, largely based upon immunofluorescence and in situ studies. When transcription is inhibited, splicing snRNPs no longer concentrate in coiled bodies but, instead, aggregate in large clusters thought to be storage sites of splicing factors (Carmo-Fonseca *et al.*, 1992). Changes have also been observed in the size

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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), with smaller and more coiled bodies during G_1 phase, and larger and fewer coiled bodies in S and G_2 phases of the cell cycle. Upon entry into mitosis, most coiled bodies disappear, reappearing in G_1 phase after reformation of nucleoli (Andrade *et al.*, 1993; Ferreira *et al.*, 1994). Changes in the number of coiled bodies as a function of the differentiation stage (Antoniou *et al.*, 1993; Santama *et al.*, 1996; Boudonck *et al.*, 1998), and after virus infection (Fortes *et al.*, 1995; Rebelo *et al.*, 1996), have been described. Exposure of mammalian cells to low levels of the specific Ser/Thr protein phosphatase inhibitor, okadaic acid, results in the accumulation of p80 coilin and splicing snRNPs within nucleoli, which indicates that protein dephosphorylation is required for normal formation of nucleoplasmic coiled bodies (Lyon *et al.*, 1997). Sleeman *et al.* (1998) expressed fusions of the spliceosomal proteins, SmE and U1A, and p80 coilin with green fluorescent protein (GFP) in human cell lines. This work confirmed earlier immunofluorescence and in situ studies that p80 coilin localizes to coiled bodies in a transcription-dependent manner and that a reversible protein phosphorylation mechanism is involved in regulating the interaction of snRNPs and coiled bodies with the nucleolus.

To investigate the dynamics of coiled bodies in living cells, we have generated a protein fusion between GFP and the U2B″ spliceosomal protein that is, among others, concentrated in coiled bodies. Although U2B″ is a component of the U2 snRNP complex, its precise role in pre-mRNA splicing is still unknown. It has been suggested that U2B″ may not be required for the splicing reaction itself but may have a role in U2 snRNP biogenesis (Mattaj and De Robertis, 1985; Pan and Prives, 1989). The full-length *U2B″* cDNA has been cloned from potato (Simpson *et al.*, 1991) and has been characterized at the molecular level (Simpson *et al.*, 1991, 1995), largely by analogy with the human *U2B″* cDNA (Scherly *et al.*, 1990; Bentley and Keene, 1991; Kambach and Mattaj, 1994). The potato U2B″ protein contains two RNP-80 motifs separated by a central putative nuclear localization signal (NLS). The RNP motifs consist of 80–90 amino acids containing two short, highly conserved sequences (RNP1 and RNP2) and are found in numerous proteins involved in RNA processing events (Birney *et al.*, 1993; Mattaj, 1993). It has been shown that only the N-terminal RNP motif of U2B″ is required for specific binding to U2 snRNA (Kambach and Mattaj, 1994; Simpson *et al.*, 1995) and that the interaction between U2B″ and U2 snRNA requires the presence of a second protein, U2A′ (Scherly *et al.*, 1990; Simpson *et al.*, 1995).

In the present work we show directly for the first time, by time-lapse confocal fluorescence microscopy, that coiled bodies can move within the nucleus and can coalesce. These findings suggest a transport function for coiled bodies and a mechanism for the observed decrease in coiled body number during the cell cycle. We have shown that overexpression of the U2B″–GFP fusion affects neither the cell cycle and the number of coiled bodies in tobacco BY-2 cells nor development of *Arabidopsis* plants. Deletion analysis of the *U2B″* gene fusion has identified the N-terminal RNP-80 motif as sufficient for localization to the coiled body and the nucleus.

MATERIALS AND METHODS

Construction of the U2B″–GFP Fusion Protein

The full-length 693-base pair (bp) sequence of the potato *U2B″* cDNA (Simpson *et al.*, 1991) was PCR amplified with specific primers introducing *Nco*I sites (underlined) at both termini for further cloning steps. At the same time the second methionine codon, the stop codon and an internal *Nco*I site near the 3′-end of the *U2B″* gene, were mutated (base changes shown in lowercase).

primer 1: 5′-AGCGCCCC ATG gTG CTT ACA GGA GAC A-3′

primer 2: 5′-GGTACGCCATggg TTT CTT GGC ATA GGT GAT TGC CAT tGG ATT TTG GG-3′

The amplified *U2B″* gene was fused in frame to the 5′-end of *sGFPS65T* (Chiu *et al.*, 1996) by use of the *Nco*I site. The *U2B″::GFP* cassette was cut out and inserted into the pRTL2 vector (Carrington *et al.*, 1991), creating the pRTL2::*U2B″::GFP* vector. The new *U2B″::GFP* construct was checked by sequence analysis. For stable transformation experiments, the expression cassette (2× En CaMV 35S promotor, TEV 5′-nontranslated sequence, *U2B″::GFP* coding sequence, and CaMV polyadenylation site) from the vector pRTL2::*U2B″::GFP* was excised, using *Hin*dIII, and inserted into the *Hin*dIII site of the vector pGreen0229, which carries the bar gene (http://intranet.jic.bbsrc.ac.uk/INFOSERV/DEPART/appgen/pgreen/a__cst__fr.htm by Roger Hellens), creating the pGreen0229::*U2B″::GFP* vector.

Construction of the U2B″del::GFP Deletion Cassettes

We constructed several *U2B″::GFP* fusions with different deletions in the *U2B″* gene (Figure 10). For a first deletion construct we PCR amplified the first 276 bp of the *U2B″* gene, corresponding to the N-terminal RNP-80 motif, introducing *Nco*I sites (underlined) at both termini for further cloning steps. Primers 1 and 3 were used for PCR amplification.

primer 3: 5′-GGTACGCCATGGG TGA CTT TGA TTT AGC-3′

For a second deletion construct we PCR amplified the first 453 bp of the *U2B″* gene, corresponding to the N-terminal RNP-80 motif plus a putative NLS, introducing *Nco*I sites at both termini for further cloning steps. Primers 1 and 4 were used for PCR amplification.

primer 4: 5′-GGTACGCCatgGG ATC TTG AGC ACT TGG-3′

For a third deletion construct, we PCR amplified nucleotide (nt) 286–453 of the *U2B″* gene, corresponding to a putative NLS, introducing *Nco*I sites at both termini for further cloning steps and introducing a new start codon at the 5′-end (lowercase). Primers 4 and 5 were used for PCR amplification.

primer 5: 5′-AGCGCCCC atg GTT GCT AAG GCA G-3′

The amplified *U2B″* deletion fragments were fused in frame to the 5′-terminus of *sGFPS65T* using the *Nco*I site in the pRTL2::*GFP* vector, creating vectors pRTL2::*U2B″del1::GFP*, pRTL2::*U2B″del2::GFP*, and pRTL2::*U2B″del3::GFP*, respectively. The three gene fusions were checked by sequence analysis. For stable transformation experiments, the expression cassettes (2× En CaMV 35S promotor, TEV 5′-nontranslated sequence, *U2B″del::GFP* coding sequence, and CaMV polyadenylation site) from the vectors pRTL2::*U2B″del::GFP* were excised using *Hin*dIII and inserted into the *Hin*dIII site of the vector pGreen0229,

which carries the bar gene, creating the pGreen0229::*U2B″del::GFP* vectors.

Stable Transformation of Tobacco BY-2 Cells and Arabidopsis thaliana

The pGreen0229::*U2B″::GFP* vectors were transformed into *Agrobacterium* strain C58C1 pGV3101 (pMP90) (Koncz and Schell, 1986), carrying the helper plasmid pJIC Sa__RepA. BY-2 tobacco cells were transformed by *Agrobacterium* essentially as described by Ito *et al.* (1998) and An (1985, 1987). After transformation, BY-2 cells were plated onto BY-2 medium (per liter: 4.3 g Murashige and Skoog salts; 30 g sucrose; 0.1 g inositol; 1 mg thiamine; 0.2 mg 2,4-dichlorophenoxyacetic acid (2,4-D); 0.2 g KH_2PO_4) + 0.4% Gelrite, containing carbenicillin (500 mg/l) to kill *Agrobacteria* and phosphinotricin (10 mg/l) to select transformed BY-2 cells. After selection for 3–4 wk, transformed BY-2 cells were maintained as suspension cultures in BY-2 medium and grown at 25°C in the dark.

Transgenic *Arabidopsis* plants, ecotype Columbia, were generated by *Agrobacterium* transformation using vacuum infiltration as described by Bechtold and Pelletier (1998). Transgenic T1 plants, carrying the bar gene, were selected on soil by spraying with the herbicide Challenge (2.5 ml Challenge/l water) (Duchefa, The Netherlands).

Time-Lapse Fluorescence Microscopy

For time-lapse confocal microscopy, cells from GFP-expressing BY-2 cultures, 5 d after subculture, were spun down and transferred onto BY-2 medium + 1% Difco Bacto agar in Petri dishes. A coverslip was placed on top. For confocal microscopy of GFP-expressing T2 *Arabidopsis* seedlings, seeds were germinated and grown as described by Boudonck *et al.* (1998). Three-day-old seedlings were mounted on slides in liquid AT growing medium (per liter: 4.4 g Murashige and Skoog salts, 30 g sucrose, 0.05 mg kinetin, 0.5 mg α-naphthaleneacetic acid (NAA), pH 5.8). A coverslip was placed on top and sealed with nail varnish. Optical sections were collected on living BY-2 cells or *Arabidopsis* seedlings using a confocal microscope (MRC 1024, Bio-Rad, Richmond, CA; or TCS SP, Leica, Deerfield, IL) with 488-nm excitation line. Images were transferred to a Macintosh computer and 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 Pictrography 3000 printer.

For heat shock treatment of BY-2 cells, cells were imaged before heat shock. Petri dishes were then transferred to 42°C for 1 h. The same cells were then relocated and imaged over time. For inhibition of transcription in BY-2 cells by actinomycin D, 50 μl of 2 mM actinomycin D (Sigma Chemical, St. Louis, MO) was applied to the cells on the Petri dish. For treatment with okadaic acid, 50 μl of 100 nM okadaic acid were applied to the cells on the Petri dish.

Immunofluorescence on BY-2 Cells

Five-day old BY-2 cells were fixed for 1 h in 4% (wt/vol) formaldehyde in PEM buffer (50 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 5 mM EGTA, 5 mM $MgSO_4$; pH 6.9). The fixed cells were washed in PEM for 30 min followed by permeabilization with 2% (wt/vol) cellulase in TBS (25 mM Tris-HCl [pH 7.4], 140 mM NaCl, 3 mM KCl) for 1 h. Cells were washed four times with TBS and finally dried down on to glutaraldehyde-activated APTES (γ-aminopropyltriethoxysilane)-coated multiwell slides. A blocking step for 1 h, with PEM/0.2% NP40/3% BSA followed. The slides were incubated for 1.5 h at 37°C with a 1:10 dilution of primary antibody 4G3 (Euro-diagnostica B.V., Apeldoorn, Netherlands) or a 1:500 dilution of human autoantibody against p80 coilin (a gift from Professor Angus Lamond, University of Dundee, Scotland) in PEM/3% BSA. The primary antibody was detected by cy3-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). The slides were mounted in Vectashield (Vector laboratories, Burlingame, CA). GFP kept its fluorescence through this treatment.

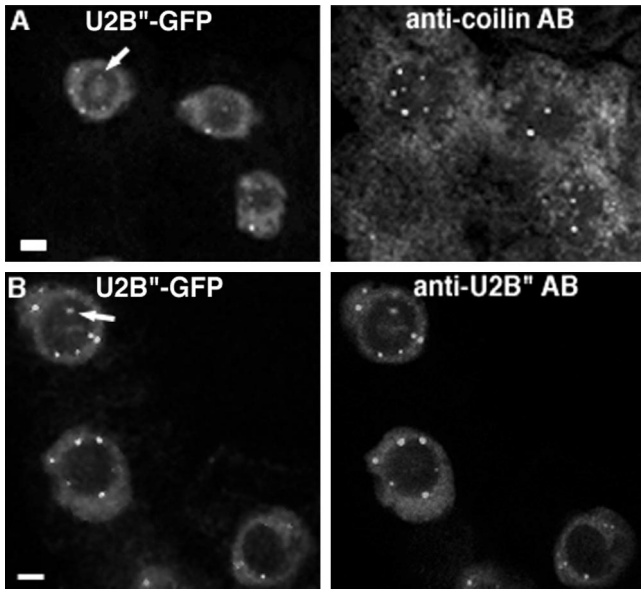

Figure 1. (A) Single confocal optical section of BY-2 cells expressing U2B″-GFP, double labeled with GFP (left panel) and autoantibody against p80 coilin (right panel). Three nuclei are shown, and the bright GFP spots colocalize with bright foci of anti-coilin labeling. There is some labeling of the cytoplasm by anti-p80 coilin. (B) Single confocal optical section of BY-2 cells expressing U2B″-GFP, double labeled with GFP (left panel) and 4G3 antibody (right panel). Three nuclei are shown. Most coiled bodies are in the nucleoplasm, but occasionally are seen in the nucleolus (arrows). All coiled bodies that contain U2B″ also express the U2B″–GFP fusion. Bars, 5 μm.

RESULTS

U2B″-GFP Is a Good Marker for the Coiled Body

No homologue of the p80 coilin gene has yet been identified in plants. However, the U2B″ protein is a well characterized plant spliceosomal protein that has been shown to be a good marker for plant coiled bodies (Beven *et al.*, 1995; Boudonck *et al.*, 1998). To study the dynamics of coiled bodies in detail, therefore, we generated a translational fusion between GFP and the full-length plant U2B″ protein and made stable transgenic lines of both BY-2 tobacco suspension culture cells and *Arabidopsis* plants expressing this fusion. BY-2 suspension culture cells have large nuclei, contain many prominent coiled bodies, and are particularly well suited for microscopy. To confirm that the U2B″–GFP fusion protein is expressed in coiled bodies in the BY-2 cells, we combined fluorescence imaging of the GFP with immunofluorescence labeling using an autoantibody against p80 coilin. Figure 1A shows that the bright GFP spots do indeed correspond to

Table 1. Coiled body numbers in tobacco BY-2 cells

	Mean CB number ± SEM (no. of cells counted)	Min. CB[a]	Max. CB[a]
Untransformed BY-2	8.88 ± 2.22 (60)	4	15
U2B″-GFP BY-2	8.97 ± 2.19 (60)	4	15

[a] Minimum and maximum coiled body number observed in these cells.

coiled bodies and demonstrates that all the coiled bodies contain the U2B″–GFP fusion protein. Figure 1B confirms our previous observations that the anti-U2B″ antibody, 4G3 (Habets *et al.*, 1989), is a good marker for coiled bodies in plants. In these cells, coiled bodies are most frequently found at the nucleolar periphery, but can also be found throughout the nucleoplasm and inside the nucleolus as well as in the central nucleolar cavity.

To determine whether overexpression of the U2B″–GFP protein fusion affected the numbers of coiled bodies in BY-2 cells, we labeled cells expressing U2B″-GFP, and untransformed BY-2 cells, with the 4G3 antibody. Table 1 shows that there is no difference in the number of coiled bodies between BY-2 cells transformed or not transformed with the *U2B″::GFP* fusion. To investigate whether the expression of the U2B″–GFP fusion affected the cell cycle duration, we set up fresh subcultures and measured the optical density (OD_{600}) for 8 successive days for BY-2 cells transformed or not transformed with the *U2B″::GFP* fusion. In five such time courses we found no significant difference between the growth curves for cells transformed with the *U2B″::GFP* construct and wild-type BY-2 cells (our unpublished results).

We also found no effect of the *U2B::GFP* transgene on development or fertility of *Arabidopsis* compared with wild type. The 1.5 times difference in coiled body number between root hair and hairless files in the *Arabidopsis* root epidermis, as described by Boudonck *et al.* (1998), was maintained in lines transformed with *U2B::GFP* (e.g., see Figure 10).

Coiled Bodies Move and Coalesce in Plant Cells

We used time-lapse confocal microscopy of U2B″-GFP–expressing BY-2 cells to investigate the mobility of coiled bodies. We were able to collect three-dimensional (3-D) data sets consisting of ~15 focal sections at intervals of 30–60 min for periods up to 15 h, without unacceptable fading of the fluorescence. We examined ~60 cells in this way, and 70% of these cells showed movements of some or all of the coiled bodies over an average time range of 6 h. Figure 2 shows 5 of

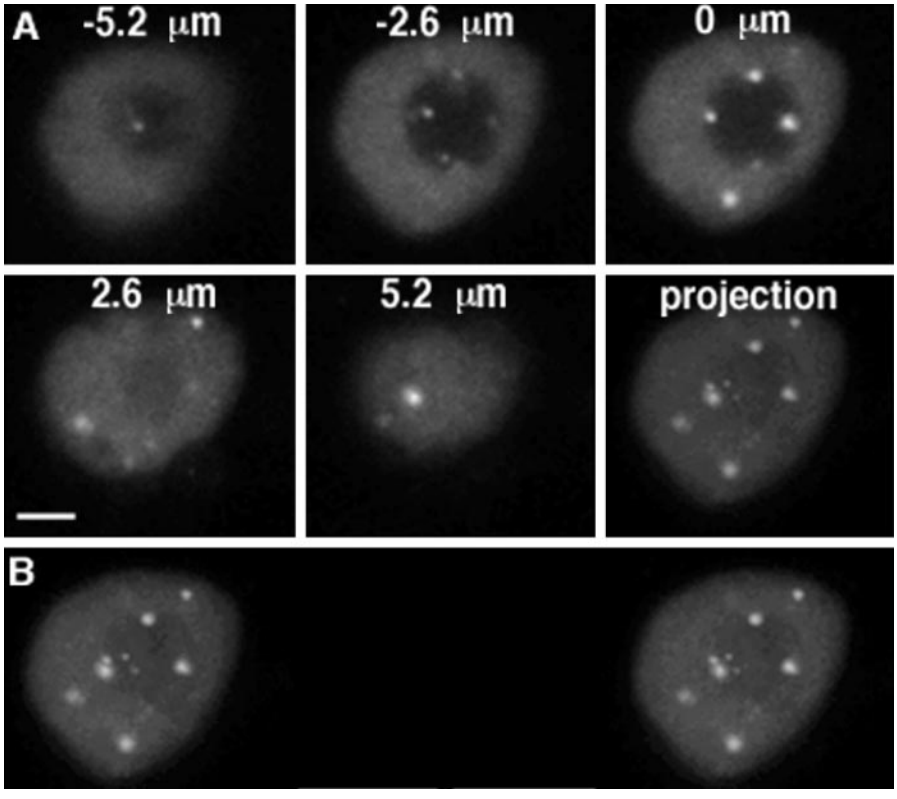

Figure 2. Confocal fluorescence microscopy of U2B″-GFP in a living BY-2 cell. (A) Single confocal optical sections of a BY-2 cell nucleus are shown, with 2.6 μm between consecutive sections, followed by a projection of the 3-D data stack. Coiled bodies can be found in the nucleoplasm and nucleolus, but most frequently just inside the nucleolar periphery. (B) Stereo projection of the confocal series shown in panel A. Bar, 5 μm.

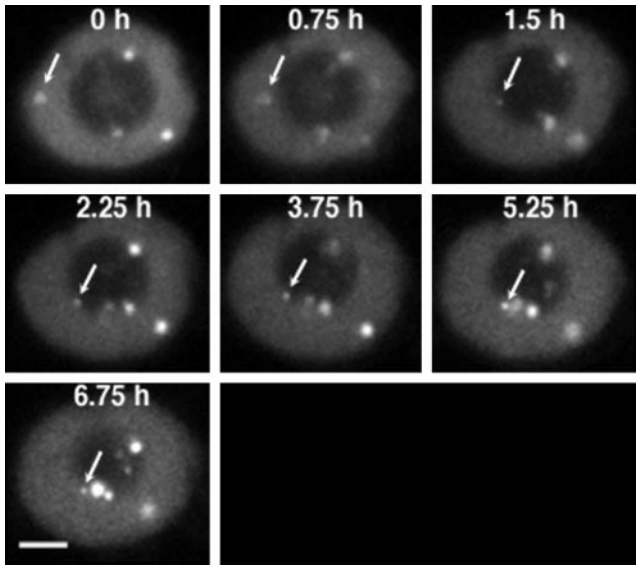

Figure 3. Time-lapse confocal microscopy of U2B″-GFP in living BY-2 cells. Projections of three or four confocal optical sections are shown at each time point. Coiled body marked with an arrow demonstrates that coiled bodies can move around in the nucleoplasm and nucleolus, and often move along the periphery of the nucleolus. Other, unmarked coiled bodies show smaller movements. Bar, 5 μm.

the 18 confocal sections through a single cell at one time point, together with projections of the entire confocal stack. In order to present four-dimensional data in most subsequent figures, we show only a projection of the 3-D data at each time point, but the interpretation of the data was made from the original 3-D image stacks.

The movements of coiled bodies that we observed in BY-2 cells can be categorized into a number of distinct classes. The most frequent were movements of coiled bodies within the nucleolus and the nucleoplasm, which occurred in 70% (42 cells) of the 60 cells examined. Coiled bodies were also seen to move along the boundary between nucleolus and nucleoplasm (Figure 3), in more than 20% of the 42 cells that showed coiled body dynamics. The most striking observations were unidirectional movements of coiled bodies from the nuclear periphery to the nucleolar periphery (Figure 4) in more than 25% of the 42 cells that showed coiled body dynamics. The velocity of these movements ranged from fractions of 1 μm/h to ~10 μm/h. We frequently observed very small coiled bodies within the nucleolus, which were also mobile, but were difficult to image clearly because of their small size and rapid photobleaching of GFP. In more than 20% of the 42 cells with coiled body dynamics, two or more coiled bodies moved toward one another and then fused together (Figure 4). Although we saw movements of coiled bodies toward one another in all regions of the nucleus, we observed fusions only at the nucleolar periphery.

Figure 5 shows a pair of cells at late telophase/early G_1. As G_1 progressed, the size of the nuclei increased

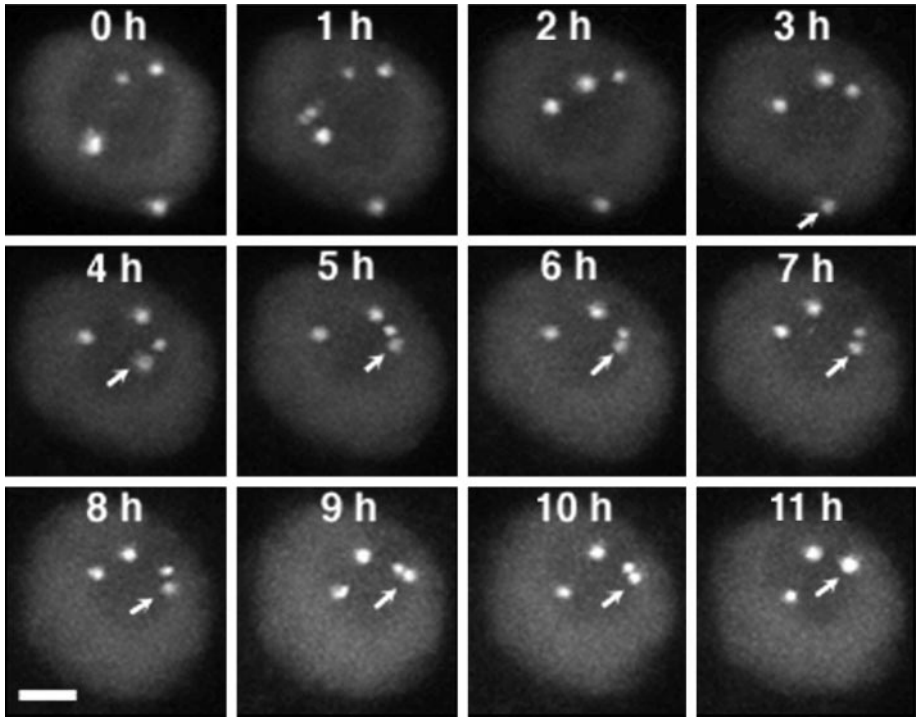

Figure 4. Time-lapse confocal microscopy of U2B″-GFP in living BY-2 cells. Projections of series of confocal optical sections are shown at each time point. Coiled body marked with an arrow moves from the periphery of the nucleoplasm to the periphery of the nucleolus, after which it moves along the periphery of the nucleolus and finally fuses with another coiled body. Other coiled bodies also show movements. Bar, 5 μm.

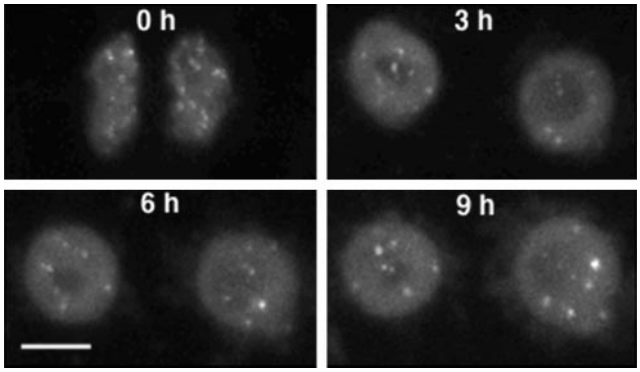

Figure 5. Time-lapse confocal microscopy of U2B″-GFP in living BY-2 cells. Projections of series of confocal optical sections are shown at each time point. Late telophase/early G_1 cells have many small coiled bodies. As the cells progress through the cell cycle, the nuclei enlarge and the number of coiled bodies decreases. Concomitantly, the size of the coiled bodies increases. Bar, 10 μm.

and the number of coiled bodies concomitantly decreased. This decrease in coiled body number was accompanied by an increase in the size of the coiled bodies. The number of coiled bodies and the complexity of their distribution made it impossible to follow their dynamics in detail.

Figure 6 shows that *Arabidopsis* root epidermal cells display coiled body dynamics similar to those of BY-2 cells. These images show movements of coiled bodies in the nucleoplasm and at the periphery of the nucleolus. Coiled bodies frequently coalesced, as we observed earlier for BY-2 cells (Figure 6, arrows). This image sequence also shows two cells undergoing mitosis (starred cells). In this sequence, mitosis, accompanied by redistribution of the U2B″-GFP throughout the cytoplasm, was followed by reformation of the coiled bodies in the G_1 daughter cells in the space of 2 h.

Dynamics of Coiled Bodies Are Affected by Regulators of Transcription and Phosphorylation in Plant Cells

Heat shock is known to inhibit transcription of most RNA pol II genes and disrupts pre-mRNA splicing in both plants and animals (Bond, 1988; Christensen *et al.*, 1992). Transfer of U2B″-GFP BY-2 cells to 42°C for 1 h resulted in the disappearance of all coiled bodies (Figure 7). After heat shock the coiled bodies reformed throughout the nucleus within hours. The heat shock could be repeated on the same cells for a second time, with subsequent second reformation of coiled bodies. There was an indication that some of the coiled bodies reformed near previous positions (Figure 7, arrows).

Treatment of U2B″-GFP BY-2 cells with 2 mM actinomycin D, a potent inhibitor of transcription, resulted in the redistribution of some coiled bodies as elongated structures around the nucleolus, while other coiled bodies remained in the nucleoplasm (Figure 8). The elongated structures were dynamic, moving around the nucleolus and changing in size.

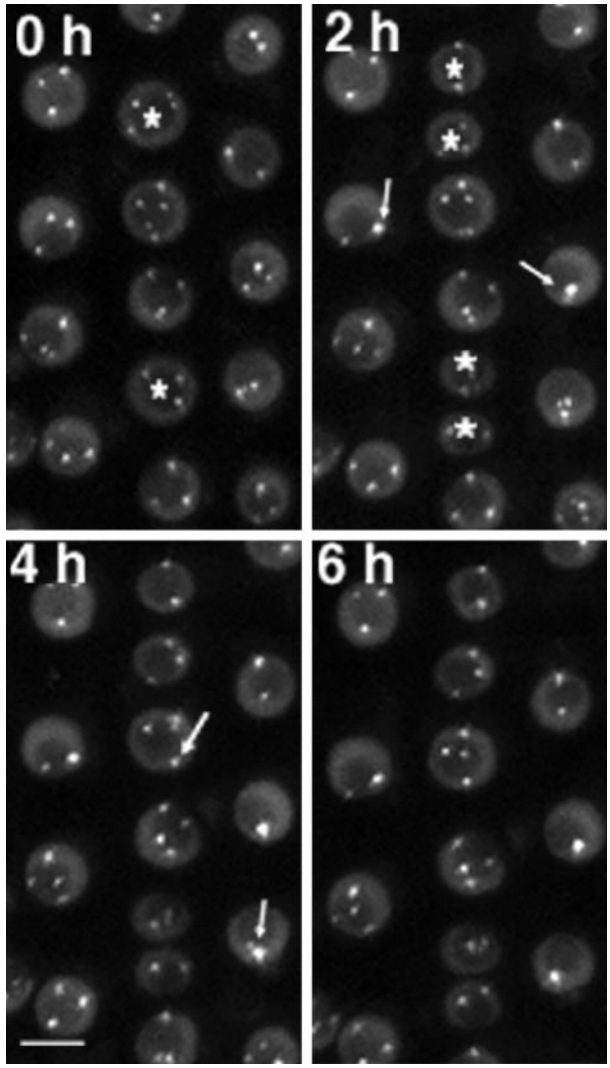

Figure 6. Time-lapse confocal microscopy of U2B″-GFP in stable *Arabidopsis* transformants. Projections of series of confocal optical sections through the root epidermis are shown at each time point. Many coiled bodies are clearly mobile. Several coiled bodies coalesce (arrows). Two cells underwent division during this sequence, and the newly divided cells decreased their coiled body numbers quickly (asterisk). Bar, 10 μm.

We tested the effect of okadaic acid, a specific Ser/Thr protein phosphatase inhibitor that has been shown to result in the accumulation of p80 coilin and splicing snRNPs within nucleoli in mammalian cells (Lyon *et al.*, 1997; Sleeman *et al.*, 1998). Figure 9 shows that 19 h after addition of 100 nM okadaic acid to

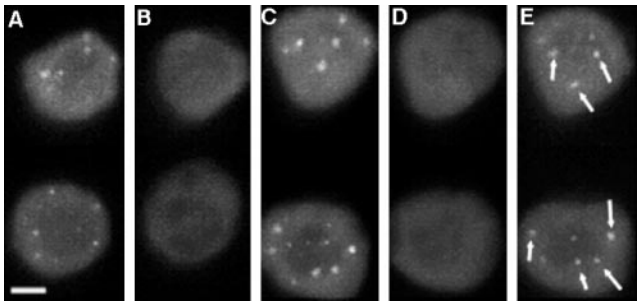

Figure 7. Heat shock on U2B″-GFP–expressing BY-2 cells. Projections of series of confocal optical sections are shown at each time point. (A) Cells before heat shock. (B) After 1 h heat shock, at 42°C, all coiled bodies have disappeared. (C) 19.5 h after heat shock, coiled bodies have reformed. (D) A second heat shock, at 42°C, for 1 h, results in the disappearance of all coiled bodies. (E) 2.5 h after heat shock, coiled bodies are already present. Several coiled bodies reappear near places where they were before heat shock (arrows). Bar, 5 μm.

U2B″-GFP BY-2 cells, all the coiled bodies in most cells were located within the nucleolus. This indicates that one possible mechanism for nucleolar localization of coiled bodies is through a phosphorylation/dephosphorylation mechanism.

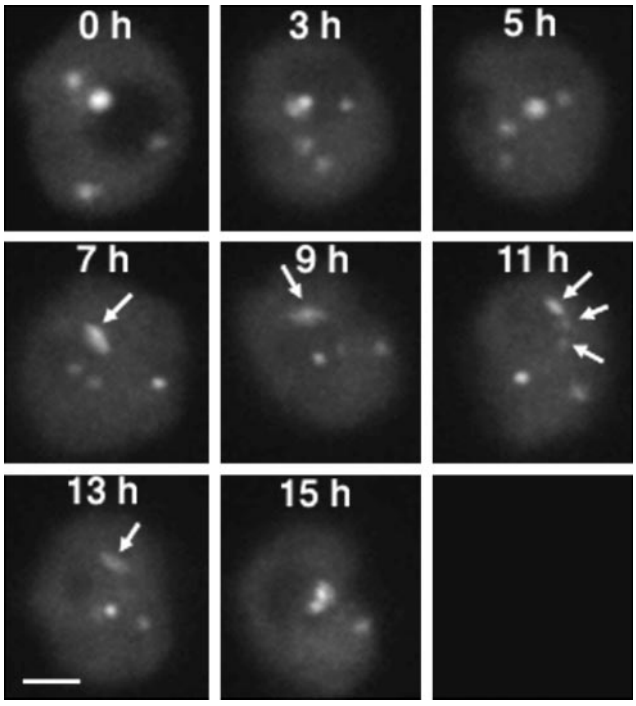

Figure 8. Actinomycin D treatment of U2B″-GFP–expressing BY-2 cells. Projections of series of confocal optical sections are shown at each time point. After addition of 2 mM actinomycin D, at time point 0 h, U2B″-GFP forms elongated structures around the nucleolus (arrow). These elongated structures change their relative positions around the nucleolus and can increase or decrease in size. Bar, 5 μm.

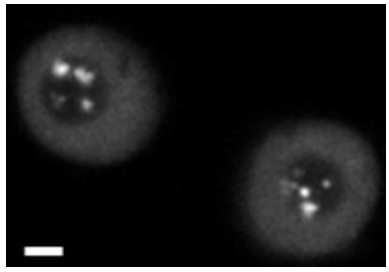

Figure 9. Okadaic acid treatment of U2B″-GFP–expressing BY-2 cells. A single confocal section is shown. After 19 h treatment with 100 nM okadaic acid, coiled bodies are mainly found in the nucleolus in BY-2 cells. Bar, 5 μm.

We also tested the effect of melittin and mastoparan, which have been shown to activate the plant phosphoinositide-signaling system (Drobak and Watkins, 1994). Addition of these drugs did not affect the dynamics or location of coiled bodies, which suggests that the phosphoinositide-signaling pathway is not involved in the movements of coiled bodies (our unpublished results).

N-terminal U2B″ RNP-80 Motif Is Sufficient for Localization to the Coiled Body

Stable expression of the full-length *U2B″::GFP* fusion in *Arabidopsis thaliana* resulted in nucleoplasmic localization with clear GFP expression in coiled bodies (Figure 10A). This localization is the same as described previously using antibodies against U2B″ (Boudonck *et al.*, 1998). To investigate which part of the U2B″ protein is required for localization to the coiled body, we made three additional stably transformed *Arabidopsis* lines using three deletion constructs: *U2B″del1::GFP*, *U2B″del2::GFP* and *U2B″del3::GFP*. U2B″del1-GFP, which contains the first U2B″ RNP-80 motif, was localized to the coiled bodies (Figure 10B) and also to fluorescent strands emerging from the nucleus and to plastid-like structures in the root cap and in the hypocotyl (our unpublished results). U2B″del2-GFP, which contains the RNP-80 motif plus the putative NLS sequence, showed the same localization pattern as the full-length U2B″-GFP protein fusion (Figure 10C). U2B″del3-GFP, which only contains the putative NLS from U2B″, was distributed throughout the cytoplasm and within the nucleoplasm, with no labeling of coiled bodies and weaker labeling of the nucleolus (Figure 10D). All the transformant seedlings examined showed GFP fusion protein expression in roots, hypocotyl, and cotyledons. In summary, these data demonstrate that the U2B″–GFP fusion protein is localized to coiled bodies throughout the plant and that the first part of the U2B″ protein is sufficient for localization to the coiled body.

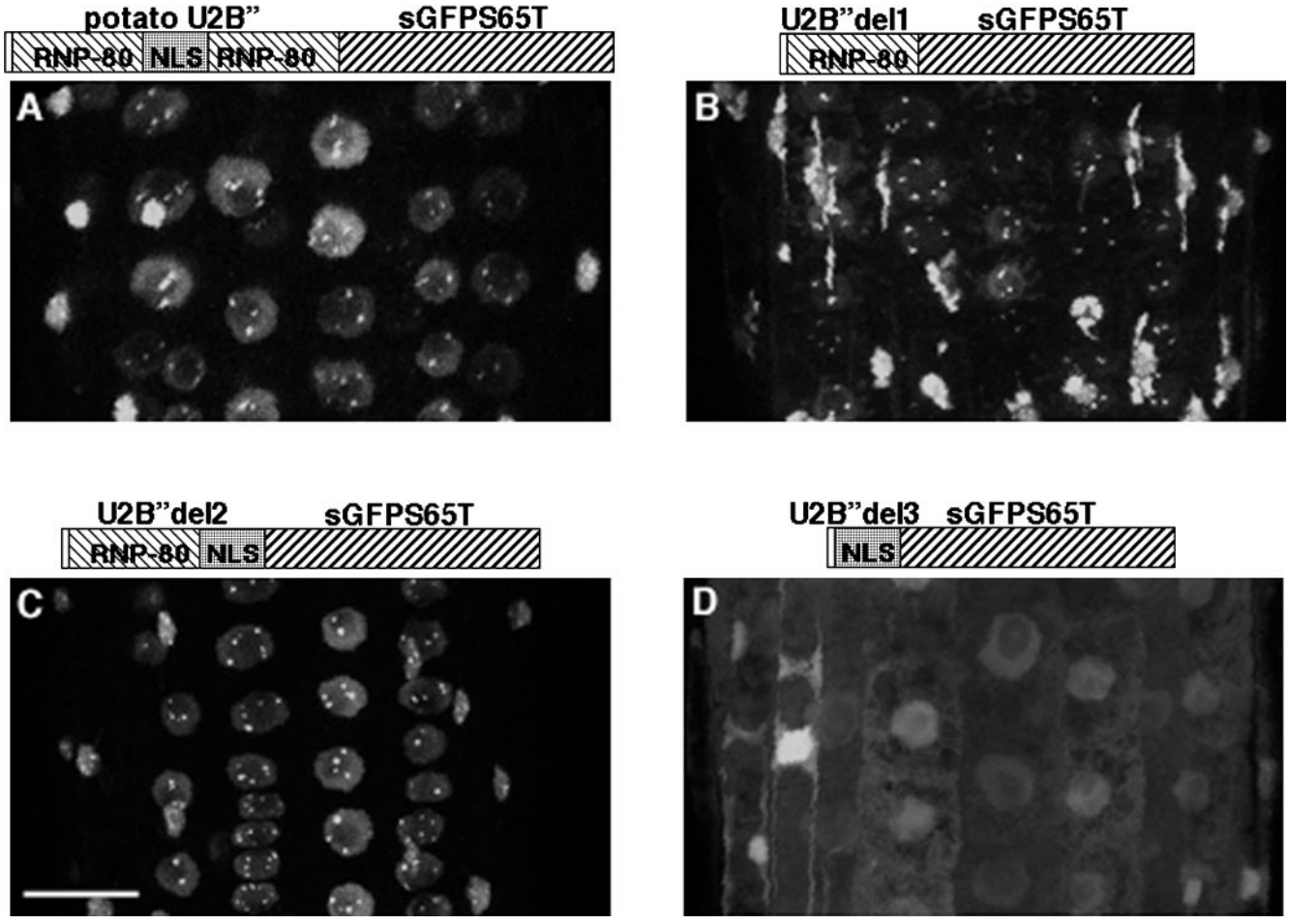

Figure 10. Stable *U2B″::GFP* expression in *Arabidopsis* roots. Projections of series of confocal sections through the root meristem are shown. (A) The full-length *U2B″::GFP* fusion shows expression in the nucleoplasm and in the coiled bodies (bright spots) in all root nuclei in the epidermis. The nucleolus is largely devoid of labeling (dark centers). Root cap nuclei are labeled very brightly (small nuclei). (B and C) Deletion constructs *U2B″del1::GFP* and *U2B″del2::GFP* label the nucleoplasm and the coiled bodies. In addition, cytoplasmic strands emerging from the nucleus and plastid-like structures are clearly labeled in the root meristem of *U2B″del1::GFP* transformants. (D) Fusion of the U2B″ putative NLS to GFP results in labeling of the cytoplasm and somewhat brighter labeling of the nucleoplasm. There is no labeling of coiled bodies. Bar, 20 μm.

DISCUSSION

We have shown, using lines stably expressing the *U2B″::GFP* fusion construct, that coiled bodies are mobile organelles in living plant cells. We have observed both dramatic movements of coiled bodies from the nuclear periphery to the nucleolus and also smaller movements within both the nucleoplasm and nucleolus. Furthermore, we have seen frequent events in which coiled bodies fuse together. We have demonstrated that overexpression of the U2B″–GFP fusion does not affect the cell cycle duration or viability of BY-2 cells or the growth and development of *Arabidopsis* plants. Therefore, the observed movements of coiled bodies in cells transformed with *U2B″::GFP* are likely to faithfully reflect the natural movements in untransformed cells.

Although other researchers have observed coiled bodies in living cells, our results are the first to show clear movements over time. Almeida *et al*. (1998) detected no movements of coiled bodies in HeLa cells after injection of fluorescent-labeled antibodies to coilin. This inconsistency with our results could be due to the immobilization of coiled bodies by antibody binding, or inhibition of movement by phototoxicity. Sleeman *et al*. (1998) also visualized coiled bodies in living human cells by fusing a number of snRNP proteins and p80 coilin to GFP, but only observed small movements of coiled bodies (Sleeman, personal communication). It is entirely possible that we have been able to observe more and larger coiled body movements because plant cells are more resistant to photodamage. How-

ever, the possibility remains that coiled bodies are less mobile in animal than plant cells.

We have shown that coiled bodies can move unidirectionally from the nucleoplasm to the nucleolar periphery. This shows that nucleoplasmic and nucleolar coiled bodies are one and the same. Ochs *et al.* (1994) reported that nucleolar coiled bodies differed in composition from those found in the nucleoplasm. This suggests that some components of a coiled body can be modified or replaced and may be either the result or the cause of differential localization of coiled bodies. So far, we have observed unidirectional movement of coiled bodies only from the nucleoplasm into the nucleolus. Such a directional movement might indicate a transport function for coiled bodies (Lamond and Earnshaw, 1998). It could provide a mechanism for the import of factors involved in nucleolar transcript processing, such as fibrillarin, into the nucleolus, or it might provide a mechanism for shuttling of proteins such as Nopp140 and NAP57 between the nucleoplasm and nucleolus. Alternatively, the coiled body might be the initial site for assembly of processing complexes, which are then sorted to other locations in the nucleus or nucleolus where the actual RNP processing takes place, as suggested earlier by Gall *et al.* (1995).

While most coiled bodies in a given nucleus undergo constant small movements, a coiled body will occasionally undergo a sudden dramatic movement over longer distances. This suggests that these large movements of coiled bodies are triggered by a biochemical modification. We currently have no detailed quantitative data on the maximum speed and the time scale of such sudden movements, as we cannot predict them, and imaging at time points too close together causes bleaching of the GFP. The accumulation of coiled bodies in the nucleolus upon addition of okadaic acid shows that phosphorylation is likely to be an important factor for localization (Lyon *et al.*, 1997; Sleeman *et al.*, 1998) and that the mobile coiled bodies may differ in phosphorylation state from the more static bodies. Addition of actinomycin D, an inhibitor of transcription, results in the accumulation of U2B″–GFP protein into elongated structures around the nucleolus, which are mobile and change in size. This suggests a link between transcriptional activity and snRNP location, which may either be a direct effect or the result of changes in chromatin or other nuclear organization.

Previous studies have shown that the nucleus is organized into chromosome territorial domains and interchromosomal channels (Cremer *et al.*, 1988; Lichter *et al.*, 1988; Abranches *et al.*, 1998). It will be instructive to investigate whether coiled bodies move through interchromosomal channels, or along an underlying nuclear matrix, and whether specific motor proteins are required. Alternatively, as some coiled bodies have been found associated with specific chromosomal loci, including gene clusters that encode U snRNAs and histone mRNAs (Frey and Matera, 1995; Smith *et al.*, 1995), and as there is direct evidence that chromosome movements take place in several cell types (Li *et al.*, 1998; review by Zink and Cremer, 1998), it is possible that the movements of coiled bodies are related to underlying movements of chromosome loci to which they are attached. On the other hand, as Frey *et al.* (1999) showed that the frequency of colocalization of coiled bodies with artificial U1 and U2 snRNA genes depends on the transcriptional activity of the gene array, the movement of a coiled body might be the result of the termination of transcription and the subsequent release of the coiled body from a particular chromosome locus.

We have also shown directly that coiled bodies can coalesce. This fusion mechanism explains the observed decrease in coiled body number and the increase in size through the cell cycle that has been reported for several organisms (Andrade *et al.*, 1993; Chan *et al.*, 1994; Boudonck *et al.*, 1998). It is noteworthy that all coiled body fusions that we observed took place at the nucleolar periphery. The elongated structures observed at the nucleolar periphery on treatment with actinomycin D might be the result of the fusion of several coiled bodies into elongated structures. Other nuclear bodies may also coalesce. Boddy *et al.* (1997) demonstrated that nuclear matrix-associated promyelocytic leukemia bodies are often located together and linked in pairs, as though in the process of fusing. However, pairing of the promyelocytic leukemia nuclear bodies could also be explained as budding or replication, since no studies have been carried out in living cells to date.

The biogenesis of coiled bodies remains an open question. It has been suggested that coiled bodies are assembled at the nucleolar periphery (Bohmann *et al.*, 1995), and coiled bodies are often found adjacent to nucleoli or physically attached to nucleoli, as though in the process of emergence or fusion (Lafarga *et al.*, 1983; Ferreira and Carmo-Fonseca, 1995; Matera, 1998). In most BY-2 cells, we observed very small coiled bodies inside the nucleolus, which were either too small or photobleached too quickly to follow during time-lapse experiments. It is possible that these small coiled bodies are newly formed in the nucleolus; however we saw no sign of such nucleolar coiled bodies emerging into the nucleoplasm. On the other hand, the reappearance of U2B″–GFP in coiled bodies in similar locations on recovery from heat shock shows that coiled bodies can reform in the nucleoplasm. However, it is possible that heat shock leaves a remnant of the coiled body structure and U2B″–GFP and other coiled body components simply reaccumulate in preexisting coiled body structures when transcription restarts after the heat shock. Supporting ev-

idence for a biogenesis model in which coiled bodies form throughout the nucleus comes from the study of coiled bodies in late telophase/early G_1 cells. Coiled bodies are first visible as several tens of coiled bodies or their precursors located all over the nucleus (Beven *et al.*, 1995; present study). We have seen no evidence of any concentration of coiled bodies around the nucleolus at this stage, as might be expected if it was the site of coiled body biogenesis.

Mutation analysis of the human *U2B″* gene has previously shown which part of the sequence localizes U2B″ to the nucleus (Scherly *et al.*, 1990; Bentley and Keene, 1991; Kambach and Mattaj, 1994), but not which part of the sequence is responsible for localization to the coiled body. We have shown that fusion of the first N-terminal RNP-80 motif of the potato U2B″ protein to GFP is sufficient for localization of the fusion protein to the coiled body. As Simpson *et al.* (1995) showed that the plant N-terminal RNP-80 motif is responsible for binding to the U2 snRNA and that this binding is mediated by the U2A′ protein, our deletion analysis results demonstrate that localization of the U2B″ protein to the coiled body is mediated by binding of the U2B″ protein to the U2A′ protein and U2 snRNA, and that the central NLS domain of the U2B″ protein alone is not sufficient for localization to the coiled body.

As we have produced stable transgenic *Arabidopsis* lines that express the *U2B″::GFP* fusion in the coiled bodies of all cells, we are now able to screen for coiled body mutants with defects in their size, number, or organization. Such studies should shed new light on the function of these intriguing nuclear organelles.

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