J. Plant Biol. (2009) 52:193–201
DOI 10.1007/s12374-009-9033-y

REVIEW

Plant Nucleolar Dynamics

Sang Hyon Kim

Received: 8 April 2009 / Accepted: 8 April 2009 / Published online: 15 May 2009

Abstract The nucleolus is the nuclear organelle for ribosomal DNA transcription, rRNA processing, and ribosome synthesis. However, abundant recent findings also have shown that the nucleolus is the site where virtually all eukaryotic RNA polymerase-transcribed RNAs are processed and where other important cellular functions are regulated. Although the number of nucleoli per cell and the nucleolar ultrastructures within animal and plant nucleoli differ, most nucleolar functions for those two organisms appear to be conserved. This review not only presents molecular details already known from yeast and animal systems, but also discusses recently published results about nucleolar functioning in plants, a research area still in its infancy.

Keywords Gene expression · Nucleolar dynamics · Ribosome biogenesis · Ribonucleoprotein processing · Subnucleolar domains

Abbreviations

rRNA	Ribosomal RNA
rDNA	Ribosomal DNA
NOR	Nucleolar organizer region
UBF	Upstream-binding factor
TIF-1B	Transcription initiation factor-1B
TTF-1	Transcription termination factor-1
pre-rRNA	Precursor ribosomal RNA
Pol I, II, and III	Eukaryotic RNA polymerase I, II, and III
FC	Fibrillar center
DFC	Dense fibrillar component
GC	Granular component
NC	Nucleolar cavity
snRNA	Small nuclear RNA
snoRNA	Small nucleolar RNA
scaRNA	Small Cajal body-specific RNA
RNP	Ribonucleoprotein
BrU	Bromouridine
MRP	Mitochondrial RNA processing small nucleolar RNA
tRNA	Transfer RNA
SRP RNA	Signal recognition particle RNA
SSU	Small ribosomal subunit
LSU	Large ribosomal subunit
ETS	External transcribed spacer
ITS	Internal transcribed spacer
Utp	U3-associated protein
RRM	RNA-recognition motif
GAR	Glycine and arginine-rich
TMG	2,2,7-trimethyl guanosine
NPC	Nuclear pore complex
IGC	Interchromatin granule cluster
pre-tRNA	Precursor transfer RNA
USE	Upstream element
TR	Telomerase RNA
TERT	Telomerase reverse transcriptase
EJC	Exon junction complex
NMD	Nonsense-mediated mRNA decay
PTC	Premature termination codon

S. H. Kim (✉)
Division of Biosciences and Bioinformatics,
College of Natural Science, Myongji University,
Yongin, Gyeonggi-do 449-728, South Korea
e-mail: kim6189@mju.ac.kr

The nucleolus, the largest nuclear domain, was originally identified as the site of a large rRNA precursor and its processing into 18S, 5.8S, and 25S mature forms. It is also

the starting point of the long journey to the cytoplasm through the nucleoplasm, by which active cytoplasmic ribosomes are assembled with rRNAs and ribosomal proteins. One of the most important cellular functions is integration of the organelle, which is largely dependent on the formation of ribosomes (Melese and Xue 1995).

Structure and Function of the Nucleolus

In eukaryotes, ribosomal DNA (rDNA) genes are present in hundreds to thousands of copies, clustered in a special region termed the nucleolar organizer region. This NOR is associated with the Pol I complex and includes an upstream-binding factor (UBF), core-binding factor TIF-1B (SL1 in humans), and a transcription termination and remodeling factor (TTF-1). *Arabidopsis* has two NORs, on Chromosomes 2 and 4, each of which comprises ~400 rDNA copies arranged in a continuous head-to-tail array that distally spans ~4,000 kb at the northern tips (Copenhaver and Pikaard 1996). Biogenesis of the nucleolus starts during the telophase in mitosis and continues to the early G1 phase. This nucleolar assembly may solely depend on Pol I transcription machinery that produces new precursor rRNA (pre-rRNA) and, subsequently, recruits complexes for rRNA processing (Scheer and Hock 1999). Researchers have provided an integrated view illustrating that this process benefits from machinery inherited from an earlier cell cycle. During mitosis the rRNA-processing complexes leave the nucleoli and are partially dispersed on the surface of all chromosomes; Pol I transcription remains active until the prophase in mitosis (Dousset et al. 2000). Pre-rRNAs generated in the prophase are, therefore, partially processed and stably maintained throughout mitosis to contribute to nucleolar assembly (Carmo-Fonseca et al. 2000; Boisvert et al. 2007).

As a subnuclear component, the nucleolus has long been considered the site of rDNA transcription, rRNA processing, and ribosome assembly. Three main sub-nucleolar domains (compartments) occur in both mammalian and plant nucleoli. As observed via electron microscopy, these include fibrillar centers (FCs), dense fibrillar components (DFCs), and granular components (GCs) (McKeown and Shaw 2009). FCs are found in a pale fibrillar region that is enriched in Pol I transcription factors such as UBF and DNA topoisomerase. The electron-dense fibrillar region of DFCs surrounds the FCs, either partially (in animals) or completely (in plants). Pre-rRNA-processing factors, including fibrillarin, a protein component of box C/D small nucleolar ribonucleoproteins (snoRNPs), are rich in DFCs. Granular regions of the nucleolus outside of the DFCs are covered by GCs enriched in assembly factors and ribosomal proteins (Fig. 1). Based on transmission electron microscopy, immunogold labeling of bromouridine (BrU) has revealed a cluster of intense signals in DFCs and some on FC/DFC borders. This suggests that pre-rRNAs are transcribed dominantly in DFCs and weakly on the FC/DFC borders in vertebrates (Koberna et al. 2002). However, BrU-labeled pea root tissues show nucleolar labeling with abundant foci (representing FCs) in the DFC, indicating that rDNA transcription occurs on the border between FCs and surrounding DFCs in plant cells (Thompson et al. 1997). Likewise, prolonged accumulation of pre-rRNA, processing in DFCs, and subsequent migration to surrounding GCs may explain why a cell produce up to 10,000 copies of ribosome per minute.

These findings support an evolutionary adaptation for increased efficiency of ribosome formation. That is, rDNA transcription occurs without the creation of nucleoli in prokaryotes including Archaea. Ribosome biogenesis is explained as a vectorial model in which pre-rRNAs migrate from fibrillar regions composed of FCs and DFCs to the granular region of GCs and, subsequently, to the nucleoplasm while maturing into pre-ribosomal particles (Tschochner and Hurt 2003).

The most striking difference in subnucleolar structure between animal and plant nucleoli are that DFCs in the latter are typically much more extensive (up to 70% of the entire nucleolus) and do not stain more intensively than GCs. In addition, plants have a nucleolar cavity (NC), also known as a nucleolar vacuole (Fig. 1). Although the detailed functioning of the NC is yet largely unknown, it does accumulate some ribonucleoproteins (RNPs), e.g., small nuclear RNPs (snRNPs) and mitochondrial RNA processing (MRP) snoRNPs (Beven et al. 1996), as well as a Pol III-transcribed RNA-processing La phosphoprotein (Fleurdépine et al. 2007).

Processing of Non-coding RNAs in the Nucleolus

The nucleolus functions in regulating the biogenesis of most cellular RNPs, such as those associated with small nuclear RNAs (snRNAs), snoRNA, tRNA, signal recognition particle (SRP) RNA, telomerase RNA, and some mRNAs (i.e., in animals). Importantly, all eukaryotic non-coding RNAs transit through the nucleolus. Pol I-transcribed large precursor rRNA is processed and subjected to a series of base-specific nucleotide modifications in the DFC in the nucleolus. Afterward, mature-sized rRNAs are assembled with ribosomal proteins in the GC of the organelle. Meanwhile, all Pol III transcripts, including 5S rRNA and tRNA precursors, are transcribed in the nucleoplasm. These are immediately shuttled through the nucleolus for appropriate processing. Pol II transcripts are transcribed in the nucleoplasm and processed in various cellular compart-

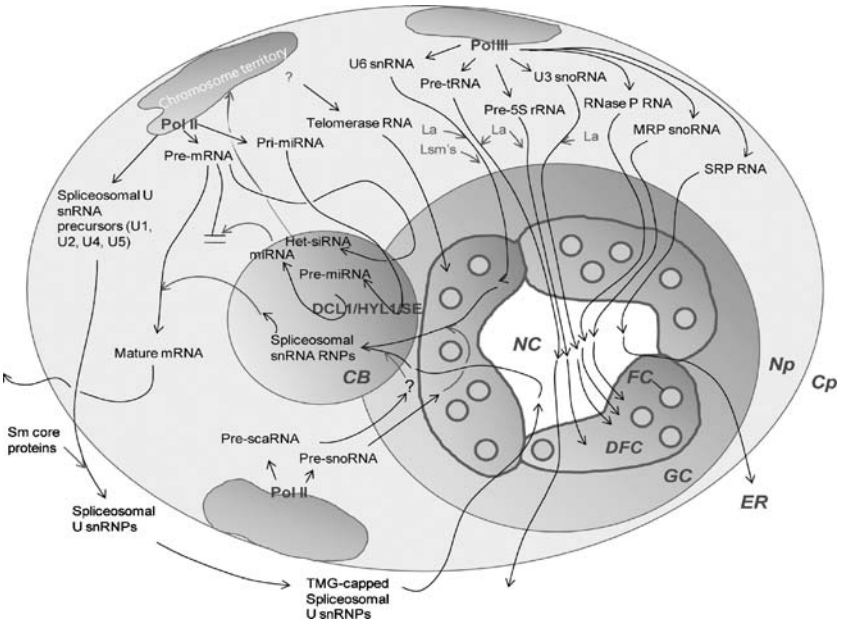

Fig. 1 RNA processing in the plant nucleus. Within the nucleolus, rDNA is transcribed by Pol I on border between fibrillar centers and dense fibrillar components. Pre-rRNA is processed in dense fibrillar components and pre-ribosome assembly is initiated in granular components (not shown). In addition to Pol I transcript, virtually all precursor RNAs transcribed by Pol II and Pol III transit through the nucleolus. *FC* fibrillar center, *DFC* dense fibrillar component, *GC* granular component, *NC* nucleolar cavity in the nucleolus, *Np* nucleoplasm, *Cp* cytoplasm, *ER* endoplasmic reticulum

ments, including the cytoplasm. However, at least later, their processing requires the nucleolar stage before transcripts of both Pol II and Pol III are transported for either functioning or further processing to other compartments, including the nucleolus itself. Recent RNomic analysis of *Arabidopsis* nucleoli has revealed the presence of a significant number of previously unexpected regulatory RNAs, e.g., all U-rich spliceosomal snRNAs, tRNAs, SRP RNA, and 7SL RNA, as well as many snoRNAs (Fig. 1; Kim and Brown, unpublished).

Processing of Pol I Transcript and Ribosome Synthesis

The ribosome assembly is a complex process initiated in the nucleolus. Pol III-transcribed 5S rRNA and Pol I-transcribed 45S (35S and 47S in yeast and animals, respectively) large precursor rRNA (pre-rRNA) require a great flux of ~170 non-ribosomal and ~80 ribosomal proteins from the cytoplasm into the nucleolus following the telophase, when a cell enters the G1 phase. Pre-rRNA processing involves cleavage, methylation, and pseudouridylation. The mature-sized RNA components (5.8S, 25S/28S, and 5S rRNA for the 60S large ribosomal subunit/LSU; 18S for the 40S small ribosomal subunit/SSU), are generated from pre-rRNAs by a complex pathway involving both endo- and exonucleolytic cleavage reactions. Pre-rRNAs are also extensively modified by ~200 different snoRNPs and are bound by ribosomal proteins before pre-60S and pre-40S particles leave the nucleolus. Pre-60S particles require further processing in the nucleus before they are exported to the cytoplasm to finalize their maturation. In contrast, processing of pre-40S particles is directly exported to the cytoplasm where maturation is completed (Tschochner and Hurt 2003).

Pol I-transcribed pre-rRNAs are cleaved in the 5′ and 3′ ETS (external transcribed spacer) and two ITS (internal transcribed spacer) sequences. This results in mature-sized 18S rRNA (SSU) and 5.8S and 25S rRNA (LSU). At least four snoRNPs—U3, U14, U18, and U22—that are accumulated in *Arabidopsis* nucleoli are responsible for early-stage cleavage reactions. In yeast, U3 snoRNP constitutes an SSU processome (>2 MDa) that is composed of ten known U3-associated proteins and as many as 17 unknown proteins; these are designated Utp1 to Utp17. Such nucleolar proteins are capable of immunoprecipitating U3 snoRNA (Dragon et al. 2002). Intriguingly, depletion of a subset of those SSU processomes leads to a decrease in pre-rRNA transcript accumulation. Because components referred to as t-Utps (Utps required for transcription) are also involved in rDNA transcription, this suggests that such transcription is not only closely associated with rRNA processing, but that synthesis and processing of pre-rRNA are also co-regulated (Gallagher et al. 2004).

In higher eukaryotes, one of the candidate proteins required for this linkage is nucleolin, which likely affects the co-transcriptional assembly of processing factors on the pre-rRNA (Roger et al. 2002, 2003). The 77-kDa vertebrate nucleolin protein contains three structurally distinct domains: an N-terminal acidic stretch of vigorous

phosphorylation sites, a central domain comprising four RNA-recognition motifs (RRMs), and the C-terminal glycine and arginine-rich domain. The multifunctional nucleolin protein ubiquitously localizes to the nucleolus, nucleoplasm, and cytoplasm, and even to the plasma membrane. *Arabidopsis* plants have two orthologues, AtNuc-L1 and AtNuc-L2, both of which have only two RRM motifs. AtNuc-L1 is highly expressed and localizes to the DFC in the nucleolus, whereas AtNuc-L2 expression is apparently repressed under normal conditions. An insertional mutant for AtNuc-L1, in which the nucleoli have loosely packed granules with numerous interstices, shows NOR de-condensation and accumulates unprocessed pre-rRNA (Pontvianne et al. 2007). Notably, this mutant leads to an increase in AtNuc-L2 transcript, implying that AtNuc-L1 controls AtNuc-L2 expression (Pontvianne et al. 2007). However, both AtNuc-L1 and AtNuc-L2 transcripts are induced by sugar or glucose. Interestingly, levels of RNP components for snoRNPs, as well as a subset of ribosomal proteins, are also enhanced by these metabolizable carbohydrates while snRNP protein levels are unaffected. Finally, the AtNuc-L1-insertional mutant impairs a sugar-induced rise in ribosomal protein transcripts (Kojima et al. 2007). These findings suggest that the roles the plant nucleolin orthologue AtNuc-L1 plays are not limited to rDNA transcription and, thereby, nucleolar biogenesis and pre-rRNA processing. Instead, it is also responsible for transcription of Pol II-specific genes involved in ribosome biogenesis by an as-yet unknown mechanism.

Processing of Pol II Transcripts

Spliceosomal snRNAs U1, U2, U4, and U5 are transcribed by Pol II then exported to the cytoplasm through the nuclear pore complex following formation of the export complex. In the cytoplasm, each snRNA is assembled into an snRNP core complex with a heteroheptameric ring composed of seven Sm core proteins (B/B', D1, D2, D3, E, F, and G). Their 5′ end is hypermethylated from a 7-methyl guanosine cap to a 2,2,7-trimethyl guanosine (TMG) cap. These two essential modifications provide snRNPs with their nuclear localization by forming a nuclear import complex that has three proteins containing importin-β. In the nucleus they enter Cajal bodies, where site-specific base modification is performed by small Cajal body-specific RNAs (scaRNAs). In HeLa cells, micro-injected snRNPs undergo their maturation cycles through Cajal bodies and the nucleoli and, later, to speckles (IGCs; Sleeman and Lamond 1999). Species-specific RNP proteins are imported to the nucleus independently of snRNPs, although their nuclear import also appears to be mediated by importin-β (Hieda et al. 2001). At this stage, those proteins are believed to be partially assembled into their cognate snRNPs in Cajal bodies (Kiss 2004).

Although the functioning of the nucleoli in the biogenesis of spliceosomal snRNPs is not yet understood, low levels of Pol II-specific snRNPs have been observed in the nucleoli of both mammals (Gerbi and Lange 2002; Sleeman et al. 2001) and *Arabidopsis* plants (Kim and Brown, unpublished). Using transient expression of functionally active U1snRNP-specific proteins—U1-70K, U1A, and U1C—in *Arabidopsis* cell cultures, Lorković and Barta (2008) have shown that all three are differentially localized to nuclear bodies, including the nucleolus. There, U1-70K is localized to speckles in 58% of analyzed cells compared with only 13% and 7% for U1A and U1C, respectively. These nucleolar and Cajal body localizations usually coincide when a single protein is being analyzed. However, when two proteins are co-transfected, particularly U1-70K with either of the other two, they are localized differentially in the nucleolus and Cajal bodies. Importantly, most speckled cells lack localization of either into the nucleolus or Cajal bodies. All of these results indicate that U1 snRNPs mature differentially in the nucleolus and Cajal bodies before they perform the splicing of pre-mRNAs. They are later recycled in speckles. Intriguingly, in many cases all three proteins are localized to the nucleolar cavity when their nucleolar retention is observed (Fig. 1; Lorković and Barta 2008). This implies that the snRNP biogenesis pathway in plants is likely to differ, at least in part, from the system in animals, where the nucleoli do not contain nucleolar cavities.

In Eukaryota and Achaea, the majority of snoRNAs determine the sites for 2′-O-ribose methylation and pseudouridylation of rRNA as guide RNAs. However, a few (see above) are responsible for essential cleavage reactions to generate mature 18S, 5.8S, and 25S rRNAs from pre-rRNA (Filipowicz and Pogačić 2002; Kiss 2002). These snoRNAs have two structurally distinct families, each of which has a differential function on rRNA or U6 snRNA for base modifications. Specifically, box C/D snoRNAs are involved in 2′-O-ribose methylation and box H/ACA snoRNAs act in pseudouridylation. A box C/D snoRNA contains two conserved sequences: boxes C (PuUGAUGA) and D (CUGA) near the 5′ and 3′ ends, respectively. The 9- to 13-nt region immediately upstream from box D is responsible for guiding methylation via complementary base-pairing with their cognate target rRNAs or U6 snRNA. A box H/ACA snoRNA folds into two stem-loop structures in the 5′ and 3′ halves of the RNA; these are followed by the conserved internal box H (ANANNA) and the 3′-terminal box ACA (ACANNN). The stem loops contain internal loops, where their sequences form specific base-pairings with those in cognate rRNA or U6 snRNA flanking a uridine residue that is targeted for modification

to pseudouridine. Each class of snoRNA is complexed with four different core proteins to form snRNP, a step apparently required for stability and functioning of the snoRNP (Terns and Terns 2002). There, fibrillarin for box C/D snoRNP and NAP57/Cbf5 (or dyskerin in humans) for box H/ACA snoRNP confer methylase and pseudouridylase activities, respectively. A related group of families is the scaRNAs, named for their target modification of Pol II-transcribed spliceosomal (U1, U2, U4, and U5) snRNAs in the Cajal bodies for the final stage of snRNP biogenesis (Fig. 1).

Like other higher eukaryotes, plants contain about 200 snoRNAs. Although animal and yeast snoRNA genes are generally intronic (Filipowicz and Pogačić 2002; Kiss 2002), most plant snoRNA genes (and some yeast genes) are intergenic, comprising either a single or a polycistronic cluster of closely related snoRNA genes. This means they are transcribed from their own promoters. However, at least 20 intronic snoRNAs are also present in *Arabidopsis*, either singly, or in dicistonic or tricistonic organizations (Kim and Brown, unpublished). Once transcribed with host genes, precursor snoRNA (pre-snoRNA) requires splicing of pre-mRNA, followed by exonucleolytic trimming of RNP-bound intermediate snoRNA. Endonuclease activity is, in turn, likely involved in the processing of polycistronic pre-snoRNAs. An RNase III-like endonuclease (Rnt1p) in yeast is responsible for this cleavage reaction, acting on the bulged nucleotides in a spacer region containing stem loops (Chanfreau et al. 1998). Processing of plant polycistronic snoRNA also requires endonuclease ahead of exonuclease trimming, but the enzyme(s) responsible for this activity is/are still unknown (see review by Brown and Shaw 2008).

Conserved box C/D and H/ACA sequences are the main determinants for nucleolar retention of two snoRNA species (Terns and Terns 2002). Most, if not all, box C/D snoRNAs have a Cajal-body stage for their biogenesis. For example, 3′-end trimming and 5′-TMG (2,2,7-trimethyl guanosine) capping of U3 snoRNP occurs in Cajal bodies from yeast and mammalian cells (Verheggen et al. 2002). The box H/ACA scaRNA contains a putative *cis*-acting sequence, CAB box, for localization to Cajal bodies, which explains why this snoRNA species targets to this organelle even though it carries an H/ACA box (Richard et al. 2003). However, it must still be elucidated why box C/D scaRNA localizes to Cajal bodies.

Maturation of Pol III-specific RNAs

All Pol III-transcribed RNAs, including U6 snRNA, tRNA, RNase P RNA, MRP snoRNA, SRP RNA, U3 snoRNA, telomerase RNA, and 5S rRNA, are synthesized in the nucleoplasm before they enter the nucleolus (Fig. 1). For example, the La protein binds to the 3′ U-stretch of pre-5S rRNA (Preiser et al. 1993), tRNA precursors (Yoo and Wolin 1997), and the U6 snRNA precursor (Xue et al. 2000) and U3 snoRNA (Kufel et al. 2000) to prevent nibbling by 3′-5′ exonuclease activity and, thereby, to promote precise endonucleolytic processing. Essentially, the La protein localizes to the nucleoplasm. However, this protein apparently shuttles through the nucleolus together with its associated Pol III transcripts. *Arabidopsis* has an orthologue of La protein—AtLa1—and the distantly related AtLa2 (Fleurdépine et al. 2007). The former binds to a 3′-terminal UUU motif of Pol III transcripts and restores all nuclear functioning of a yeast mutant that lacks Lhp1, the *Saccharomyces cerevisiae* La protein. Insertional mutants for the AtL1 gene display embryonic-lethal phenotypes arrested at the early globular stage of development. Interestingly, AtLa1 localizes to the NC in the nucleolus (Fleurdépine et al. 2007), suggesting that processing of Pol III-transcribed RNA in plants apparently differs from that within vertebrates that do not contain this ultrastructure.

U6 snRNA is transcribed by Pol III in the nucleoplasm. The 3′ U-stretch is recognized by and bound to La protein that facilitates a heteromeric ring of seven Sm-like proteins (Lsms). Assembly with Lsm proteins is required for subsequent targeting to the nucleolus (Gerbi and Lange 2002). In the nucleolus, the snoRNAs responsible for all eight methylations and pseudouridylations of animal U6 snRNA are localized. A novel box H/ACA snoRNA that targets to U6 snRNA also has been found in the *Arabidopsis* nucleoli (Kim and Brown, unpublished). This indicates that the nucleolus is the site of final U6 snRNP maturation before it migrates to Cajal bodies for assembly with Pol II-specific snRNPs (Fig. 1).

RNase P RNA (M1 RNA in *Escherichia coli*) is the RNA component of RNase P involved in 5′-end processing of pre-tRNA. It is found in the nucleolus as well as in the nucleoplasm of humans (Jacobson et al. 1997). A yeast RNase P mutant affecting pre-rRNA processing leads to an assumption that eukaryotic RNase P in the nucleolus plays a role in pre-rRNA processing (Chamberlain et al. 1996). This is in contrast to nucleoplasmic RNase P, which is possibly involved in pre-tRNA processing. However, it does not explain why a subset of tRNAs is accumulated in the nucleoli of both animals and plants. It is probable that further maturation of at least a subset of pre-tRNAs might also occur in the nucleoli before being exported to the cytoplasm. MRP snoRNA, also known as RNase MRP, plays at least two roles in two separate subcellular compartments. MRP snoRNAs localize mainly to the nucleolus, where the A_3 site of pre-rRNA cleavage involves the snoRNA to generate 5.8S rRNA (Lygerou et al. 1996). A minor portion of MRP snoRNAs localize to the mitochondria to process RNA transcripts, which then serve as RNA primers for mitochondrial DNA replication (Lee

and Clayton 1998). Despite their limited primary sequence homology, both Pol III-transcribed MRP snoRNA and RNase P RNA that accumulate in the nucleolus are similar in secondary structure, folding into caged pseudoknots (Schmitt and Clayton 1993) and sharing common protein components in both mammalian (Liu et al. 1994) and yeast cells (Salinas et al. 2005). Mammalian RNase P contains up to 340 nt RNase P RNA whereas the size of its plant counterpart is unknown. In contrast, 7-2 MRP snoRNAs (~261 nt) have been identified in the *Arabidopsis* nucleoli (Kim and Brown, unpublished) and tobacco (Kiss et al. 1992); these are dominantly accumulated in the nucleolus and show a caged pseudoknot structure similar to human 7-2 MRP snoRNA and RNase P RNA (Kiss et al. 1992).

Signal recognition particles are composed of SRP RNA (formerly 7SL RNA), with six protein components (SRP54, SRP19, SRP68, SRP72, and an SRP14/SRP9 heterodimer). These deliver a subset of polypeptides to specific sites on the cytoplasmic side of the endoplasmic reticulum. *Arabidopsis* and rice plants have nine and seven SRP RNA genes, respectively, and both have mixed promoter elements composed of USE (upstream element) and TATA in their upstream regions, as well as intragenic boxes A and B (Yukawa et al. 2002). SRP RNA localizes to the nucleolus immediately following nuclear microinjection then moves to the cytoplasm (Jacobson and Pederson 1998). Two isoforms of *Arabidopsis* SRP RNA have been found in the nucleolus (Kim and Brown, unpublished), suggesting that it is processed in the nucleoli of both plants and animals. Except for SRP54 and possibly the SRP14/SRP9 heterodimer, all SRP proteins are found in the nucleolus, suggesting that partial assembly occurs there (Politz et al. 2000). All SRP protein components are well conserved in *Arabidopsis*. Nevertheless, it is noteworthy that, whereas most proteins are expressed from single genes in mammalian cells, SRP54 has ten gene variants in addition to two for SRP72. This raises the possibility that differences exist between animal and plant nucleolar functions and that more than one complex might be produced to perform differential functions in plants.

Because a CAB box is present, the RNA component of human telomerase (telomerase RNA: TR) localizes to Cajal bodies (Jady et al. 2004). However, no direct evidence has been found for any available TR in plants, possibly because of the rapid change in sequences and the shorter telomere arms with unique sequences and high tolerance to telomerase null mutation (Fitzgerald et al. 1999). In contrast, telomerase RNP processing has been studied more extensively than other RNP species in *Arabidopsis*. Intriguingly, NAP57/Cbf5, the most prominent protein component of box H/ACA snoRNP, appears to be essential to telomerase RNP, from both animals (Cohen et al. 2007) and plants (Kannan et al. 2008). The *Arabidopsis* orthologue of NAP57/Cbf5 is dominantly localized to the nucleolus together with a catalytic subunit of telomerase (TERT, telomerase reverse transcriptase). Immune complexes from both FLAG-NAP57 and TERT contain telomere repeats and a heterozygous plant (a NAP57 insertional mutant line) that is not haplo-sufficient has apparent variability in its telomere length. Moreover, overexpression of the dominant negative mutant T66A, whose position is associated with a defect in telomere maintenance in humans, causes shorter telomeres (Kannan et al. 2008). These results support the idea that NAP57/Cbf5 proteins (in animal and plants) are components of box H/ACA snoRNPs plus the telomerase RNP complex, both of which are integral to cellular function, and that the nucleolus is the site of plant telomerase RNP functioning.

The Nucleolus as a Center of Plant Gene Expression

In mammalian cells the nucleolus serves as a sequestering compartment for essential cellular functions, such as cell-cycle regulation and stress responses (see review by Carmo-Fonseca et al. 2000). Recent proteomic analyses of the nucleoli from humans (692 proteins; Lamond and Mann, unpublished) and *Arabidopsis* (217 proteins; Pendle et al. 2005) have shown that their functions are highly conserved. In fact, 69% of the *Arabidopsis* set matches the human nucleolar proteome. As expected, these profiles contain many known nucleolar proteins, non-ribosomal and ribosomal, for ribosome biogenesis, including snoRNP proteins. In contrast, many unexpected proteins/protein components have been identified from both proteomes, such as those involved in transcription, splicing, and translation; protein export; nucleotide binding; and chaperones. Almost 20% of both proteomes has unknown function, suggesting that much of the nucleolus remains to be analyzed. Intriguingly, *Arabidopsis* proteomic analysis has identified six protein orthologues that build up the exon junction complex (EJC). In addition, subsequent analysis of subcellular localization, using GFP-fusion constructs, has shown that the *Arabidopsis* nucleoli accumulate most of those EJC components identified so far (Pendle et al. 2005). Following pre-mRNA splicing, mature mRNA deposits an EJC, at 22 nt upstream of the exon–exon junction, that then contributes to quality control (surveillance) of the mRNA. Therefore, the EJC is essentially composed of protein factors involved in splicing, translation, and mRNA export. Aberrant mRNA containing premature termination codons (PTC) are subjected to degradation by an ordered process called nonsense-mediated mRNA decay (NMD). These aberrants are produced through transcription and splicing errors so that they normally comprise PTCs that are recognized by ribosomes in translation process in the

cytoplasm. The PTC signal is transferred to the EJC, which then recruits so-called UPF proteins that utilize decapping and depolyadenylation enzymes for mRNA turnover (see reviews by Chang et al. 2007; and Isken and Maquat 2007). The presence of most EJC components in the *Arabidopsis* nucleoli (Pendle et al. 2005) suggests that mRNA surveillance of NMD occurs there.

Whereas pre-miRNAs from mammalian cells are separately processed in the nucleoplasm by Drosha/Pasha and in the cytoplasm by Dicer, plant pre-miRNAs are generated by a single complex composed of DICER-Like 1 (DCL1), HYPONASTIC LEAVES 1 (HYL1), and SERATED (SE) proteins (Dong et al. 2008). Recent protein localization analyses have shown that all these components and pri-miRNA accumulate at the nucleolar peripheries, within dicing bodies or nucleolar-associated bodies (Fang and Spector 2007; Song et al. 2007). These proteins also are co-localized with either of two proteins of seven core proteins of spliceosomal snRNPs, which suggests that those bodies are Cajal bodies (Fujioka et al. 2007). Therefore, these findings support the idea that plant miRNA biogenesis is nucleolus-driven (Fig. 1).

Heterochromatic gene silencing is an epigenetic regulation in plants that is guided by 24-nt small interference RNA (het-siRNA), thereby explaining RNA-directed DNA methylation (reviewed by Pikaard 2006). Biogenesis of het-siRNA, which occurs in the nucleolus, is triggered by RNA-dependent RNA polymerase 2, by producing double-strand RNA from mostly Pol II-transcribed single-strand RNA. The resultant double-strand RNA is then macerated to 24-nt small RNA by DCL3, the Hua enhancer 1 target for methylation. Afterward, that methylated het-siRNA trigger is subjected to amplification by plant-specific RNA polymerase IV. This Pol IV has two forms, Pol IVa and Pol IVb, whose largest subunits are NRPD1a and NRPD1b, respectively (Onodera et al. 2005). Together with NRPD2a, which is commonly required for both subunits at different stages of het-siRNA biogenesis, Pol IVa is involved in amplifying the het-siRNA trigger from a lightly methylated DNA template. Likewise, Pol IVb is required for methylating repetitive DNA targets, guided by Argonaute 4 (AGO4) and/or AGO6. All of these processes occur in the nucleolus (Pontes et al. 2006) and nucleolus-associated Cajal bodies (Fig. 1; Li et al. 2006).

Conclusion

In eukaryotic cells, the nucleoli play pivotal roles not only in ribosome biogenesis, but in controlling other essential cellular functions (see reviews by Carmo-Fonseca et al. 2000 and Boisvert et al. 2007) and in processing and modifying all types of RNA. A growing number of data regarding *Arabidopsis* proteins localized to the nucleoli suggest that the nucleolus has abundant as-yet unidentified functions. For example, an F-box domain containing actin-related protein 8 (ARP8) is ubiquitously expressed in plant organs and is localized to the nucleoli (Kandasamy et al. 2008). Transcription coactivators for stress-inducible multi-protein bridging factor 1 (MBF1) are accumulated in the nucleoplasm and nucleolus (Sugikawa et al. 2005). ASYMMETRIC LEAVES (AS) 1 and 2 proteins that repress the expression of a Class 1 KNOTTED1-like homeobox (KNOX) gene for initiating leaf primordia are localized in the nucleolus and nucleoplasm, respectively (Zhu et al. 2008). These possible transcription factors might play roles in rDNA transcription as modulators, e.g., chromatin remodelers. Alternatively, they might be regulated by sequestration in a similar way in mammalian nucleoli (Carmo-Fonseca et al. 2000). That former possibility for ARP8, however, is not likely because those proteins are dominantly localized to the GC in the nucleolus. A functional complex composed of SIN-related G-protein 1 and the mitogen-activated protein kinase ε1 (MAPKε1) complex, as well as the downstream target MAPKα2, are primarily found in the nucleolus, predicting a possible role for it in cytokinesis (Champion et al. 2004). The disruption of several nucleolar proteins—Rab28 (responsive to abscisic acid; Borrell et al. 2002), DOMINO1 (Lahmy et al. 2004), SLOW WALKER 1 (SWA1; Shi et al. 2005), AGAMOUS-LIKE 80 (AGL80; Portereiko et al. 2006), SISTER CHROMATID COHESION 1 PROTEIN 3 (SYN3; Jiang et al. 2007), and TORMOZ (TOZ; Griffith et al. 2007)—results in lethality at different stages of embryogenesis. A putative RNA chaperone protein, COLD SHOCK DOMAIN PROTEIN 2, is also predicted to work in the *Arabidopsis* nucleoli (Sasaki et al. 2007). The proteins listed above have not been found in the previous *Arabidopsis* proteome (Pendle et al 2005), suggesting multiple roles and the possibility of discovering many more nucleolar proteins. Therefore, as a site for regulating gene expression, researchers now agree that the plant nucleolus is the control center of the cell.

Acknowledgements The author would like to thank Prof. Sang Bong Choi for insights on rRNA processing and critical review of the manuscript and Dr. Seung Hwan Yang and Jun Yong Lee for reviewing the manuscript. This work was supported by a Korea Research Foundation Grant (KRF-2007-313-C00699) funded by the Korean Government (MOEHRD, Basic Research Promotion Fund).

References

Beven AF, Lee R, Razaz M, Leader DJ, Brown JWS, Shaw PJ (1996) The organization of ribosomal RNA processing correlates with the distribution of nucleolar snRNAs. J Cell Sci 109:1241–1251

Boisvert FM, van Koningsbruggen S, Navascués J, Lamond AI (2007) The multifunctional nucleolus. Nat Rev Mol Cell Biol 8:574–585

Borrell A, Cutanda MC, Lumbreras V, Pujal J, Goday A, Culiáñez-Macià FA, Pagès M (2002) *Arabidopsis thaliana* atrab28: a nuclear targeted protein related to germination and toxic cation tolerance. Plant Mol Biol 50:249–259

Brown JWS, Shaw PJ (2008) Intronic noncoding RNAs and splicing. Trends Plant Sci 13:335–342

Carmo-Fonseca M, Mendes-Soares L, Campos I (2000) To be or not to be in the nucleolus. Nat Cell Biol 2:E107–E112

Chamberlain JR, Pagán-Ramos KDW, Engelke DR (1996) An RNase P RNA subunit mutation affects ribosomal RNA processing. Nucleic Acids Res 24:3158–3166

Champion A, Jouannic S, Guillon S, Mockaitis K, Krapp A, Picaud A, Simanis V, Kreis M, Henry Y (2004) AtSGP1, AtSGP2 and MAP4K alpha are nucleolar plant proteins that can complement fission yeast mutants lacking a functional SIN pathway. J Cell Sci 117:4265–4275

Chanfreau G, Legrain P, Jaquier A (1998) Yease RNase III as a key processing enzyme in small nucleolar RNA metabolism. J Mol Biol 284:975–988

Chang YF, Imam JS, Wilkinson MF (2007) The nonsense-mediated decay RNA surveillance pathway. Ann Rev Biochem 76:51–74

Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR (2007) Protein composition of catalytically active human telomerase from immortal cells. Science 315:1850–1853

Copenhaver GP, Pikaard CS (1996) RFLP and physical mapping with an rDNA-specific endonuclease reveals that nucleolus organizer regions of *Arabidopsis thaliana* adjoin the telomeres on chromosome 2 and 4. Plant J 9:259–272

Dong Z, Han MH, Fedoroff N (2008) The RNA-binding proteins HYL1 and SE promote accurate in vitro processing of pri-miRNA by DCL1. Proc Natl Acad Sci U S A 105:9970–9975

Dousset T, Wang C, Verheggen C, Chen D, Hernandez-Verdun D, Huang S (2000) Initiation of nucleolar assembly is independent of RNA polymerase I transcription. Mol Biol Cell 11:2705–2717

Dragon F, Gallagher JE, Compagnone-Post PA, Mitchell BM, Porwancher KA, Wehner KA, Wormsley S, Settlage RE, Shabanowitz J, Osheim Y, Beyer AL, Hunt DF, Baserga SJ (2002) A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. Nature 417:967–970

Fang Y, Spector DL (2007) Identification of nuclear dicing bodies containing proteins for microRNA biogenesis in living *Arabidopsis* plants. Curr Biol 17:818–823

Filipowicz W, Pogačić V (2002) Biogenesis of small nucleolar ribonucleoproteins. Curr Opin Cell Biol 14:319–327

Fitzgerald MS, Riha K, Gao F, Ren S, McKnight TD, Shippen DE (1999) Disruption of the telomerase catalytic subunit gene from *Arabidopsis* inactivates telomerase and leads to a slow loss of telomeric DNA. Proc Natl Acad Sci U S A 96:14813–14818

Fleurdépine S, Deragon JM, Devic M, Guilleminot J, Bousquet-Antonelli C (2007) A bona fide La protein is required for embryogenesis in *Arabidopsis thaliana*. Nucl Acids Res 35:3306–3321

Fujioka Y, Utsumi M, Ohba Y, Watanabe Y (2007) Location of a possible miRNA processing site in SmD3/SmB nuclear bodies in *Arabidopsis*. Plant Cell Physiol 48:1243–1253

Gallagher JE, Dunbar DA, Granneman S, Mitchell BM, Osheim Y, Beyer AL, Baserga SJ (2004) RNA polymerase I transcription and pre-rRNA processing are linked by specific SSU processome components. Genes Dev 18:2506–2517

Gerbi SA, Lange TS (2002) All small nuclear RNAs (snRNAs) of the [U4/U6.U5] tri-snRNP localize to nucleoli: Identification of the localization element of U6 snRNA. Mol Biol Cell 13:3123–3137

Griffith ME, Mayer U, Capron A, Ngo QA, Surendrarao A, McClinton R, Jürgens G, Sundaresan V (2007) The TORMOZ gene encodes a nucleolar protein required for regulated division planes and embryo development in *Arabidopsis*. Plant Cell 19:2246–2263

Hieda M, Tachibana T, Fukumoto M, Yoneda Y (2001) Nuclear import of the U1A spliceosomal protein is mediated by importin a/β and Ran in living mammalian cells. J Biol Chem 276:16824–16832

Isken O, Maquat LE (2007) Quality control of eukaryotic mRNA: Safeguarding cells from abnormal mRNA function. Genes Dev 21:1833–1856

Jacobson MR, Pederson T (1998) Localization of signal recognition particle RNA in the nucleolus of mammalian cells. Proc Natl Acad Sci U S A 95:7981–7986

Jacobson MR, Cao L-G, Taneja K, Singer RH, Wang Y, Pederson T (1997) Nuclear domains of the RNA subunit of RNase P. J Cell Sci 110:829–837

Jady BE, Bertrand E, Kiss T (2004) Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. J Cell Biol 164:647–652

Jiang L, Xia M, Strittmatter L, Makaroff CA (2007) The *Arabidopsis* cohesin protein SYN3 localizes to the nucleolus and is essential for gametogenesis. Plant J 50:1020–1034

Kandasamy MK, McKinney EC, Meagher RB (2008) ACTIN-RELATED PROTEIN8 encodes an F-box protein localized to the nucleolus in *Arabidopsis*. Plant Cell Physiol 49:858–863

Kannan K, Nelson AD, Shippen DE (2008) Dyskerin is a component of the *Arabidopsis* telomerase RNP required for telomere maintenance. Mol Cell Biol 28:2332–2341

Kiss T (2002) Small nucleolar RNAs: an abundant group of noncoding RNAs with diverse cellular functions. Cell 109:145–148

Kiss T (2004) Biogenesis of small nuclear RNPs. J Cell Sci 117:5949–5951

Kiss T, Marshallsay C, Filipowicz W (1992) 7–2/MRP RNAs in plant and mammalian cells: association with higher order structures in the nucleolus. EMBO J 11:3737–3746

Koberna K, Malinsky J, Pliss A, Masata M, Vercerova J, Fialova M, Bernar J, Raska I (2002) Ribosomal genes in focus: New transcripts label the dense fibrillar components and form clusters indicative of "Christmas trees" in situ. J Cell Biol 157:743–748

Kojima H, Suzuki T, Kato T, Enomoto K, Sato S, Kato T, Tabata S, Sáez-Vasquez J, Echeverría M, Nakagawa T, Ishiguro S, Nakamura K (2007) Sugar-inducible expression of the nucleolin-1 gene of *Arabidopsis* thaliana and its role in ribosome synthesis, growth and development. Plant J 49:1053–1063

Kufel J, Allmang C, Chanfreau G, Petfalski E, Lafontaine D, Tollervey D (2000) Precursors to the U3 small nucleolar RNA lack small nucleolar RNP proteins but are stabilized by La binding. Mol Cell Biol 20:5415–5424

Lahmy S, Guilleminot J, Cheng CM, Bechtold N, Albert S, Pelletier G, Delseny M, Devic M (2004) DOMINO1, a member of a small plant-specific gene family, encodes a protein essential for nuclear and nucleolar functions. Plant J 39:809–820

Lee DY, Clayton DA (1998) Initiation of mitochondrial DNA replication by transcription and R-loop processing. J Biol Chem 273:30614–30621

Li CF, Pontes O, El-Shami M, Henderson IR, Bernatavichute YV, Chan SW, Lagrange T, Pikaard CS, Jacobsen SE (2006) An ARGONAUTE4-containing nuclear processing center colocalized with Cajal bodies in *Arabidopsis* thaliana. Cell 126:93–106

Liu M-H, Yuan Y, Reddy R (1994) Human RNase P RNA and nucleolar 7–2 RNA share conserved 'To' antigen-binding domain. Mol Cell Biochem 130:75–82

Lorković ZJ, Barta A (2008) Role of Cajal bodies and nucleolus in the maturation of the U1 snRNP in *Arabidopsis*. PLoS ONE 3:1–8

Lygerou Z, Allmang C, Tollervey D, Séraphin B (1996) Accurate processing of a eukaryotic precursor ribosomal RNA by ribonuclease MRP in vitro. Science 272:268–270

McKeown P, Shaw PJ (2009) Chromatin: linking structure and function in the nucleolus. Chromosoma 118:11–23

Melese T, Xue Z (1995) The nucleolus: an organelle formed by the act of building a ribosome. Curr Opin Cell Biol 7:319–324

Onodera Y, Haag JR, Ream T, Nunes PC, Pontes O, Pikaard CS (2005) Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. Cell 120:613–622

Pendle AF, Clark GP, Boon R, Lewandowska D, Lam YW, Andersen J, Mann M, Lamond AI, Brown JWS, Shaw PJ (2005) Proteomic analysis of the *Arabidopsis* nucleolus suggests novel nucleolar functions. Mol Biol Cell 16:260–269

Pikaard CS (2006) Cell biology of the *Arabidopsis* nuclear siRNA pathway for RNA-directed chromatin modification. Cold Spring Harb Symp Quant Biol 71:473–480

Politz JC, Yarovoi S, Kilroy SM, Gowda K, Zwieb C, Pederson T (2000) Signal recognition particle components in the nucleolus. Proc Natl Acad Sci U S A 97:55–60

Pontes O, Li CF, Nunes PC, Haag J, Ream T, Vitins A, Jacobsen SE, Pikaard CS (2006) The *Arabidopsis* chromatin-modifying nuclear siRNA pathway involves a nucleolar RNA processing center. Cell 126:79–92

Pontvianne F, Matía I, Douet J, Tourmente S, Medina FJ, Echeverria M, Sáez-Vásquez J (2007) Characterization of AtNUC-L1 reveals a central role of nucleolin in nucleolus organization and silencing of AtNUC-L2 gene in *Arabidopsis*. Mol Biol Cell 18:369–379

Portereiko MF, Lloyd A, Steffen JG, Punwani JA, Otsuga D, Drews GN (2006) AGL80 is required for central cell and endosperm development in *Arabidopsis*. Plant Cell 18:1862–1872

Preiser P, Vasisht V, Birk A, Levinger L (1993) Poly(U)-binding protein inhibits *Drosophila* pre-5S RNA 3-exonuclease digestion. J Biol Chem 268:11553–11557

Richard P, Darzacq X, Bertrand E, Jady BE, Verheggen C, Kiss T (2003) A common sequence motif determines the Cajal body-specific localization of box H/ACA scaRNAs. EMBO J 22:4283–4293

Roger B, Moisand A, Amalric F, Bouvet P (2002) Repression of RNA polymerase I transcription by nucleolin is independent of the RNA sequence that is transcribed. J Biol Chem 277:10209–10219

Roger B, Moisand A, Amalric F, Bouvet P (2003) Nucleolin provides a link between RNA polymerase I transcription and pre-ribosome assembly. Chromosoma 111:399–407

Salinas K, Wierzbicki S, Zhou L, Schmitt ME (2005) Characterization and purification of *Saccharomyces cerevisiae* RNase MRP reveals a new unique protein component. J Biol Chem 280:11352–11360

Sasaki K, Kim MH, Imai R (2007) *Arabidopsis* COLD SHOCK DOMAIN PROTEIN2 is a RNA chaperone that is regulated by cold and developmental signals. Biochem Biophys Res Commun 364:633–638

Scheer U, Hock R (1999) Structure and function of the nucleolus. Curr Opin Cell Biol 11:385–390

Schmitt ME, Clayton DA (1993) Nuclear RNase MRP is required for correct processing of pre-5.8S rRNA in *Saccharomyces cerevisiae*. Mol Cell Biol 13:7935–7941

Shi DQ, Liu J, Xiang YH, Ye D, Sundaresan V, Yang WC (2005) SLOW WALKER1, essential for gametogenesis in *Arabidopsis*, encodes a WD40 protein involved in 18S ribosomal RNA biogenesis. Plant Cell 17:2340–2354

Sleeman JE, Lamond AI (1999) Newly assembled snRNPs associate with coiled bodies before speckles, suggesting a nuclear snRNP maturation pathway. Curr Biol 9:1065–1074

Sleeman JE, Ajuh P, Lamond AI (2001) snRNP protein expression enhances the formation of Cajal bodies containing p80-coilin and SMN. J Cell Sci 114:4407–4419

Song L, Han MH, Lesicka J, Fedoroff N (2007) *Arabidopsis* primary microRNA processing proteins HYL1 and DCL1 define a nuclear body distinct from the Cajal body. Proc Natl Acad Sci U S A 104:5437–5442

Sugikawa Y, Ebihara S, Tsuda K, Niwa Y, Yamazaki K (2005) Transcriptional coactivator MBF1s from *Arabidopsis* predominantly localize in nucleolus. J Plant Res 118:431–437

Terns MP, Terns RM (2002) Small nucleolar RNAs: versatile trans-acting molecules of ancient evolutionary origin. Gene Expr 10:17–39

Thompson WF, Beven AF, Wells B, Shaw PJ (1997) Sites of rDNA transcription are widely dispersed through the nucleolus in Pisum sativum and can comprise single genes. Plant J 12:571–581

Tschochner H, Hurt E (2003) Pre-ribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol 13:255–263

Verheggen C, Lafontaine DL, Samarsky D, Mouaikel J, Blanchard JM, Bordonne R, Bertrand E (2002) Mammalian and yeast U3 snoRNPs are matured in specific and related nuclear compartments. EMBO J 21:2736–2745

Xue D, Rubinson DA, Pannone BK, Yoo CJ, Wolin SL (2000) U snRNP assembly in yeast involves the La protein. EMBO J 19:1650–1660

Yoo CJ, Wolin SL (1997) The yeast La protein is required for the 3′ endonucleolytic cleavage that matures tRNA precursors. Cell 89:393–402

Yukawa Y, Matousek J, Grimm M, Vrba L, Steger G, Sugiura M, Beier H (2002) Plant 7SL RNA and tRNA(Tyr) genes with inserted antisense sequences are efficiently expressed in an in vitro transcription system from Nicotiana tabacum cells. Plant Mol Biol 50:713–723

Zhu Y, Li Z, Xu B, Li H, Wang L, Dong A, Huang H (2008) Subcellular localizations of AS1 and AS2 suggest their common and distinct roles in plant development. J Integr Plant Biol 50:897–905