

# The Role of the Plant Nucleolus in Pre-mRNA Processing

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**Abstract** The nucleolus is a multifunctional compartment of the eukaryotic nucleus. Besides its well-recognised role in transcription and processing of ribosomal RNA and the assembly of ribosomal subunits, the nucleolus has functions in the processing and assembly of a variety of RNPs and is involved in cell cycle control and senescence and as a sensor of stress. Historically, nucleoli have been tenuously linked to the biogenesis and, in particular, export of mRNAs in yeast and mammalian cells. Recently, data from plants have extended the functions in which the plant nucleolus is involved to include transcriptional gene silencing as well as mRNA surveillance and nonsense-mediated decay, and mRNA export. The nucleolus in plants may therefore have important roles in the biogenesis and quality control of mRNAs.

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## Introduction

The nucleolus is the largest and most visible sub-nuclear compartment. Its main function is the transcription and processing of ribosomal RNA (rRNA) and ribosomal subunit production. In recent years, molecular and proteomic approaches have begun to dissect the pathways of ribosomal subunit assembly and transport from the nucleolus and examine the composition of protein complexes and RNPs involved in these processes (Grandi et al. 2002; Dragon et al. 2002; Schafer et al. 2003; Gallagher et al. 2004; Grannemann and Baserga 2004, 2005). In addition, many lines of evidence point to the nucleolus having a wide range of other functions in both RNA metabolism and cell growth (for reviews see Pederson 1998; Olsen et al. 2000; Olsen 2004; Raška et al. 2006; Boisvert et al. 2007). The multifunctional nature of the nucleolus has also been highlighted by proteomic approaches which have identified numerous unexpected proteins in the nucleolus, indicative of new interactions or functions (Andersen et al. 2002, 2005; Scherl et al. 2002; Pendle et al. 2005). The presence of non-ribosomal RNAs such as the RNA moieties of the signal recognition particle (SRP) and telomerase and some tRNAs suggests roles in processing and assembly of other RNAs and RNPs (Pederson 1998; Olsen et al. 2000; Olsen 2004; Boisvert et al. 2007). In this aspect, plants are of growing importance through the recent linking of the nucleolus to production of heterochromatic siRNAs and transcriptional gene silencing (Li et al. 2006; Pontes et al. 2006) and to mRNA export, mRNA surveillance and nonsense-mediated decay (Kim et al., unpublished). Finally, the nucleolus has been shown to be crucial to cell division regulation and senescence and as a sensor of cell stress (Rubbi and Milner 2003; Olsen 2004; Boisvert et al. 2007). In this chapter, we examine the multifunctionality of the eukaryotic nucleolus and describe recent data on novel functions of the plant nucleolus and, in particular, its potential roles in mRNA processing and biogenesis.

## The Dynamic Nucleus

The nucleus is the defining eukaryotic organelle. It is highly organised, with chromatin regions containing the genomic DNA and inter-chromatin domains containing the machinery for gene expression and regulation processes (Misteli 2005). The nucleus contains many sub-nuclear structures or bodies that vary in their components and functions (Lamond and Spector 2003; Cioce and Lamond 2005; Misteli 2005; Handwerger and Gall 2006). Such structures, rather than being fixed, unchanging entities, reflect the accumulation and steady-state flux of sub-populations of molecules and complexes, as has been clearly shown by fluorescence recovery after photobleaching (FRAP) studies. Their dynamic nature is also demonstrated by changes in size and distribution under different conditions and during differentiation and development reflecting the high mobility and trafficking of many components between the different bodies and nucleoplasm (Boudonck et al. 1998, 1999; Lamond and Spector 2003; Cioce and Lamond 2005; Misteli 2005; Handwerger and Gall 2006; Gorski et al. 2006; Raška et al. 2006). Although the functions of

many nuclear bodies have yet to be fully elucidated, clearly some nuclear bodies have multiple functions and some functions may overlap. Thus the spatial and temporal organisation of the nucleus is highly dynamic and is integral to the regulation and integration of networks of gene expression processes which ultimately determine cellular function. Consistent with these considerations, the nucleolus, already recognised as multifunctional, continues to add to its list of functions and emerges as a dynamic and major centre for RNA metabolism in the cell.

### *Nuclear Bodies in Animal Cells*

The nucleolus is not membrane-bound, has a high relative refractive index and thus appears under phase contrast microscopy as a dense region of the nucleus. It is formed around tens, hundreds or even thousands of tandemly repeated ribosomal RNA (rRNA) gene units and contains rRNA transcripts at different stages of transcription, processing and assembly into ribosomal subunits and the multitude of RNAs and proteins required for these processes. The observed nucleolar compartment therefore represents a snapshot of the steady-state import of components and export of the major products, the ribosomal subunits (Leung and Lamond 2003; Cheutin et al. 2004). The fundamental role of the nucleolus in production of ribosomes needed for translation is also reflected in the effects on rRNA transcription, ribosome production and nucleolar morphology of cell growth and environmental conditions (Leung and Lamond 2003; Leung et al. 2004; Mosgoeller 2004; Dove et al. 2006; Raška et al. 2006). This integral link between the nucleolus and normal functioning of the cell is further highlighted by its involvement in cell division, cell death and stress responses (Rubbi and Milner 2003; Olsen 2004; Raška et al. 2006; Boisvert et al. 2007). In addition to the factors required for ribosome biogenesis, the nucleolus also functions in aspects of the biogenesis of many other RNP complexes and contains proteins and protein complexes involved in a range of cellular functions as detailed below in this review.

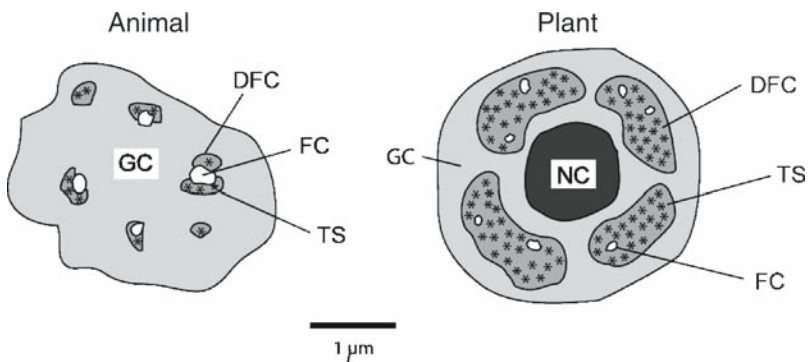
Besides the nucleolus, other nuclear bodies such as Cajal bodies (CBs) and speckles have been identified in animal and plant nuclei (Cioce and Lamond 2005; Handwerger and Gall 2006). In particular, CBs, which are often closely associated with nucleoli, contain a number of different proteins including coilin, a protein essential for CB formation, and fibrillarin and dyskerin, nucleolar proteins required for pre-rRNA processing and modification (Cioce and Lamond 2005). CBs are involved in the maturation of spliceosomal snRNPs and snoRNPs, with newly assembled snRNPs and snoRNPs trafficking through CBs before accumulating in splicing speckles and the nucleolus, respectively (Narayanan et al. 1999; Sleeman and Lamond 1999). The modification of specific nucleotides in snRNAs, guided by small CB-specific RNAs (scaRNAs), occurs in CBs (Darzacq et al. 2002; Jady et al. 2003). CBs also contain U7snRNA, which functions in the 3' end processing of some histone mRNAs, and are enriched in the RNA of telomerase RNP (Frey and Matera 1995; Jady et al. 2004; Zhu et al. 2004; Pillai et al. 2005). CBs are dynamic structures whose presence and abundance are linked

to the transcriptional activity of the cell and their association with the nucleolus is reflected in the trafficking of nucleolar proteins between the nucleolus and CBs. Thus nucleoli and CBs are both multifunctional and involved in RNP maturation.

### *Nuclear Bodies in Plant Cells*

In plants, the best-characterised nuclear bodies are the nucleolus and CBs. The plant nucleolus differs to some extent in organisation and structure from the animal nucleolus, although its major function in rRNA and ribosomal subunit production remains the same (Brown and Shaw 1998; Shaw and Brown 2004). The plant nucleolus contains a large proportion of dense fibrillar component (DFC), much more than in typical animal nucleoli, which is surrounded by the granular component (GC) (Fig. 1). The DFC regions contain rDNA transcription units which are being actively transcribed and have been visualised as ‘Christmas tree’ structures around 300 nm in length (Shaw and Jordan 1995; Gonzalez-Melendi and Shaw 2001). The localisation by fluorescence microscopy and in situ hybridisation of nucleolar proteins, rRNA gene regions and snoRNA species to sub-domains within plant nucleoli have been correlated with early and late events in rRNA processing (Beven et al. 1996; Brown and Shaw 1998). A distinctive feature of most plant nucleoli is the nucleolar cavity, whose function is unknown but contains spliceosomal snRNAs and accumulates snoRNAs (Beven et al. 1995, 1996) and contains centres for the production of heterochromatic small interfering RNAs (siRNAs) (Pontes et al. 2006). The relationship between these centres and CBs is not yet clear (Li et al. 2006; Pontes et al. 2006) but highlights the interaction between CBs and the nucleolus.

Plant CBs vary in number and size in different cells and under different conditions (Boudonck et al. 1998; Collier et al. 2006). Recently, plant coilin was identified and, despite the sequence divergence from animal coilin, was shown to be required for CB formation. Mutants in plant coilin will be invaluable in addressing the functions



**Fig. 1** Comparison of the structure of animal and plant nucleoli. *TS*, transcription sites; *FC*, fibrillar centres; *DFC*, dense fibrillar component; *GC*, granular component; *NC*, nucleolar cavity.

and properties of plant CBs (Collier et al. 2006). Plant CBs also contain snRNAs, snoRNAs and fibrillarin (Beven et al. 1995, 1996; Brown and Shaw 1998) and plant scaRNAs have been identified consistent with conserved roles in snRNP and snoRNP maturation (Marker et al. 2002). Moreover, the majority of plant snoRNAs are organised in gene clusters and expressed as polycistronic precursor snoRNAs (Brown et al. 2003). The detection of these polycistronic precursors in CBs and the nucleolus by in situ hybridisation suggests that processing occurs in both locations and/or that pre-snoRNAs traffic to the nucleolus via CBs (Shaw et al. 1998). The nucleolus is also involved in some virus infections (both animal and plant), with particular viral proteins localising to the nucleolus (Hiscox 2002, 2007). Although the function of the nucleolar localisation of many of these proteins remains to be established, the nucleolar accumulation of the ORF3 protein of the plant virus groundnut rosette virus (GRV) has been shown to involve interactions with CBs and fibrillarin and is essential for the formation of cytoplasmic RNPs and long-distance virus movement (Kim et al. 2007a, b). Finally, the Arabidopsis nucleolus contains exon junction complex proteins, involved in linking transcription and splicing to translation, export and mRNA surveillance (Pendle et al. 2005). Thus new functions in RNA metabolism have been demonstrated for both plant CBs and the nucleolus.

Other nuclear bodies in plants have been defined by the presence of particular proteins such as splicing factors (see the chapter by G. S. Ali and A. S. N. Reddy, this volume) and cyclophilins, HYL1, phytochrome and AKIP1. At present, the components and functions of these bodies are not well characterised but SR proteins and cyclophilins are found in distinct splicing speckles and cyclophilin-containing bodies can interact and regulate functions in splicing (Lorković 2004). HYL1, involved in microRNA biogenesis, localises to a small number of nuclear bodies while phytochrome- and AKIP-containing bodies are involved in signalling pathways interactions and may regulate mRNAs at the transcriptional or post-transcriptional levels (Yamaguchi et al. 1999; Han et al. 2002; Kircher et al. 2002; Chen et al. 2003; Ang et al. 1998; Li et al. 2002; Shaw and Brown 2004; Song et al. 2007). Thus nuclear bodies are sites where different factors accumulate, allowing efficient RNA-protein and protein-protein interactions or RNP assembly, to influence or regulate a particular process. The bodies can form or disassemble or increase and decrease in size and number depending on different conditions or activity of the cell, again reflecting the dynamic interplay between nuclear bodies and the surrounding nucleoplasm.

## **Traditional Functions of the Nucleolus**

### ***rRNA Transcription and Processing***

The major recognised function of the nucleolus is transcription and processing of ribosomal RNA and assembly of ribosomal subunits. Eukaryotic ribosomal RNA genes are organised in large clusters, often containing hundreds or thousands of repeated genes, with each gene encoding one copy of the 18S, 5.8S and 25–28S

rRNAs. The coding regions are separated by internal transcribed spacer (ITS) regions and flanked by external transcribed spacer (ETS) regions. Genes are transcribed by RNA polymerase I as polycistronic precursor rRNAs which are processed to the mature 5.8S, 18S and 25–28S rRNAs. Processing of pre-rRNAs involves (a) cleavage of the transcript into precursors of the mature rRNAs and trimming of these precursors to their final size and (b) modification of specific rRNA nucleotides. The best-characterised pathway of pre-rRNA processing is that of yeast, in which the step-wise cleavage and trimming reactions required for production of mature rRNAs have been demonstrated and in which many of the protein and snoRNP components involved have been identified (Venema and Tollervey 1999; Fatica and Tollervey 2002). Initial cleavage of the pre-rRNA involves the U3, U14, MRP, snR10 and snR30 snoRNAs and the resultant precursors are trimmed by the action of exonucleases: Rat1p, Xrn1p and the exosome (Venema and Tollervey 1999; Fatica and Tollervey 2002). The pathway of processing and modification is poorly understood in plants but is likely to contain many processing steps which are conserved with other eukaryotes. Nevertheless, significant advances in understanding rRNA processing and the function of nucleolar proteins have been made recently. In particular, a multiprotein complex containing nucleolin, fibrillarin, U3 and U14 has been shown to cleave the pre-rRNA in the 5' external transcribed sequence (Saez-Vasquez et al. 2004). In addition, the absence of nucleolin expression causes disruption of the structural organisation of the nucleolus (Pontvianne et al. 2007).

Ribosomal RNAs contain numerous nucleotide modifications. The two major modifications in rRNA are 2'-*O*-ribose methylation and pseudouridylation. The sites of modification are determined by regions of complementarity to their cognate rRNA sequences. Box C/D and box H/ACA snoRNAs are responsible for generating 2'-*O*-ribose methylation and pseudouridylation of nucleotides, respectively (Kiss 2002). Thus snoRNAs act as guide sequences to define which nucleotides in rRNA are modified. In *Arabidopsis* and rice, snoRNA genes have been identified by both bioinformatics predictions and experimental approaches (Barneche et al. 2001; Brown et al. 2001, 2003; Qu et al. 2001; Marker et al. 2002). Many of the plant snoRNAs are conserved in yeast and/or human, in terms of the rRNA target sequences which are modified, but there are a number of plant-specific snoRNAs guiding modification of nucleotides in plant rRNAs which are unmodified in other organisms (Barneche et al. 2001; Brown et al. 2001; Qu et al. 2001; Marker et al. 2002). The fact that so many nucleotide modifications are conserved in eukaryotic rRNAs suggests important functions in rRNA processing or ribosome activity. The majority of nucleotide modifications are in the active sites of the ribosome and are thought to influence the efficiency of the ribosome and protein translation (Decatur and Fournier 2002).

### ***Ribosome Assembly***

The eukaryotic ribosome consists of a small (40S) and a large (60S) subunit containing the 5S, 5.8S, 18S and 26–28S rRNAs associated with around 80 ribosomal

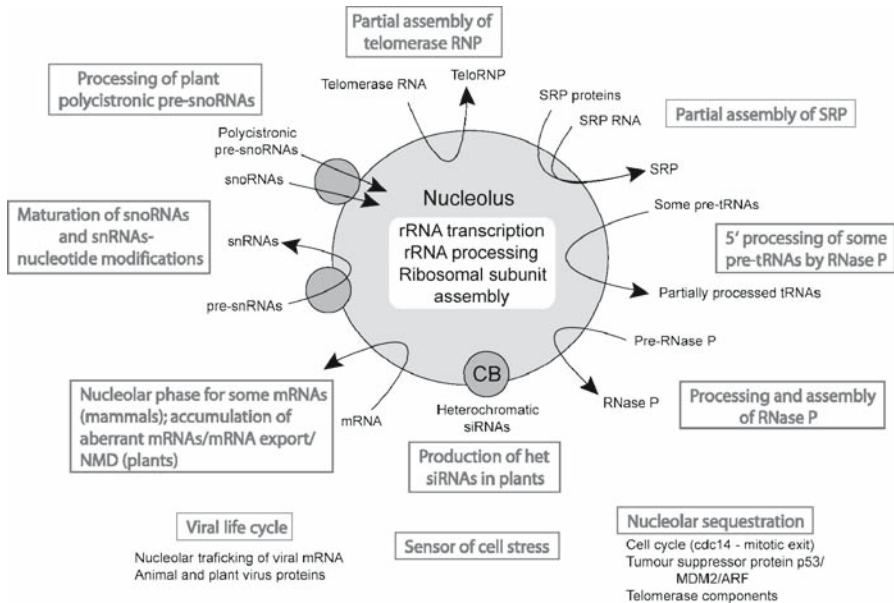
proteins (Tate and Poole 2004). As with other large RNP complexes such as the spliceosome, correct assembly occurs in a regulated and co-ordinated step-wise pathway involving non-ribosome factors and ribosomal proteins required for correct rRNA folding, protein association and ultimately export of the ribosomal subunits through the nuclear pore complex to the cytoplasm (Venema and Tollervey 1999; Fatica and Tollervey 2002; Grannemann and Baserga 2004). The pre-rRNA transcript is assembled into a 90S pre-ribosome containing factors required for formation of both ribosomal subunits. Cleavage of the primary transcript gives rise to pre-ribosomes which with further processing and assembly produce the 40S and 60S ribosomal subunits (Fatica and Tollervey 2002; Grannemann and Baserga 2004). One of these pre-ribosomal complexes includes the small subunit (SSU) processosome which contains the U3snoRNP along with ribosomal and non-ribosomal proteins including novel Utp proteins (Dragon et al. 2002; Grandi et al. 2002; Schafer et al. 2003). The SSU processosome complex is thought to be the RNP complex previously observed as knobs at the 5' regions of pre-rRNA transcripts (Miller and Beatty 1969). In plants, very little is known about ribosomal subunit assembly apart from detailed analyses of the ribosomal protein complement (Barakat et al. 2001) and the identification of orthologues of some yeast and human associated factors by, for example, nucleolar proteomics (Pendle et al. 2005). Thus the biochemistry of rRNA processing and ribosome assembly lags significantly behind that of yeast and animal systems.

## **Other Functions of the Nucleolus in RNA Metabolism**

Evidence for the nucleolus having functions other than rRNA transcription/processing and ribosome assembly continues to increase. These functions can be grouped into RNA processing activities, often associated with RNP assembly, mRNA metabolism, and regulatory cellular functions such as the control of the cell cycle and cell aging and as a sensor of cell stress (Fig. 2). In many cases, evidence currently comes from only one organism, making extrapolation to a general nucleolar function difficult. Nevertheless, the novel functions illustrate the potential complexity of the nucleolus. The various functions of the nucleolus have been reviewed previously (Pederson 1999; Olsen 2004; Boisvert et al. 2007) and here we briefly describe the roles of the nucleolus in RNA processing and RNP assembly.

### ***Signal Recognition Particle***

The signal recognition particle (SRP) is a small RNP involved in targeting the translation of specific proteins to the endoplasmic reticulum. SRP consists of the SRP RNA associated with specific proteins (Walter and Johnson 1994). At least part of the assembly of the SRP has been shown to occur in the nucleolus. Most, but not all, of the protein components of the mature cytoplasmic SRP (SRP19,



**Fig. 2** The multifunctional nucleolus. Functions of the nucleolus and associated Cajal bodies in RNA metabolism and cell processes.

SRP68 and SRP72 but not SRP54) have been localised to the nucleolus in vertebrates (Politz et al. 2000) and in yeast (Grosshans et al. 2001). SRP RNA injected into mammalian cultured cells rapidly localised to the nucleolus and then gradually relocated to the cytoplasm, suggesting that SRP was being assembled in the nucleolus and then exported (Jacobsen and Pederson 1998). Thus the assembly of the SRP has a nucleolar phase, but must be completed in the cytoplasm.

### *tRNA and RNase P*

Precursor tRNAs have been detected in the nucleolus by in situ hybridisation (Bertrand et al. 1998). Pre-tRNAs are trimmed at 5' and 3' ends and undergo base modifications such as pseudouridylation and 3' end processing (Hopper and Phizicky 2003). The 5' trimming of pre-tRNAs is catalysed by RNase P and both protein and RNA components of this RNP have been localised to the nucleolus (Jacobson et al. 1997; Jarrous et al. 1999), as has Cbf5p/Dyskerin which catalyses these and other pseudouridylation. The presence of components of the RNase P RNP also suggests that the assembly of this RNP could occur in the nucleolus. It has been shown that most tRNAs in *S. cerevisiae* are exported from the nucleus as aminoacyl tRNAs and that, in mutants defective in aminoacylation of tRNA, tRNAs accumulate in the nucleolus (Steiner-Mosonyi and Mangroo 2004), suggesting that the machinery for aminoacylation is also present in the nucleolus.

### ***Telomerase RNP***

Chromosome replication in eukaryotes requires the addition of multiple tandem repeats of a short sequence at the ends of each chromosome (TTAGGG in humans). This is catalysed by a reverse transcriptase (TERT), which copies the repeated sequence from an associated RNA (TERC) (Cech 2004). Most somatic cells do not have active telomerase, which is proposed to limit the number of rounds of cell division that they can support and has been suggested to be a factor in aging and carcinogenesis (Maser and DePinho 2002). The localisation of the RNA and the reverse transcriptase catalytic subunit of human telomerase, hTERT, suggests that the telomerase RNP may be assembled in the nucleolus (Mitchell and Collins 2000; Etheridge et al. 2002). Telomerase RNA contains a box H/ACA domain and a CB localisation signal allowing the telomerase RNP to localise to both CBs and the nucleolus in its maturation (Mitchell et al. 1999; Lukowiak et al. 2001; Jady et al. 2004). The localisation of telomerase in the nucleolus may reflect its assembly pathway and/or its sequestration as part of the regulation of its activity. Telomerase resides in the nucleolus but is specifically redistributed in late S-phase, when telomere replication takes place, thereby avoiding inappropriate expression throughout the nucleus which could cause new telomeric structures to be nucleated on replication intermediates or double strand breaks (Wong et al. 2002).

### ***Small Nuclear RNPs***

Small nuclear RNAs (snRNAs) are involved in splicing and form the cores of the spliceosomal complexes. There is good evidence that U4/U6.U5 and U2 snRNAs are also in the nucleolus and undergo part of their maturation or assembly processing there, and that the individual snRNAs are transported independently to the nucleolus (Lange and Gerbi 2000; Yu et al. 2001; Gerbi and Lange 2002). A nucleolar phase for snRNPs is strongly supported by the proteomic analyses of the nucleoli in both human and plant cells (Andersen et al. 2002, 2005; Scherl et al. 2002; Pendle et al. 2005). These studies showed the presence of many snRNA-associated proteins, particularly the Sm class of proteins, pointing to a role in the maturation of snRNAs and the assembly of snRNPs. U6snRNA differs from the other spliceosomal snRNAs in its cap structure and maturation pathway. Factors involved in U6 nucleotide modifications are found in the nucleolus, suggesting a nucleolar phase for the production of mature U6snRNA (Ganot et al. 1999).

### ***Heterochromatic Small Interfering RNAs***

Plants contain a novel RNA polymerase (RNA polymerase IV, Pol IV) involved in production of transcripts which give rise to double-stranded RNAs and small interfering

RNAs (siRNAs) which guide DNA methylation. The protein components required for the production of such heterochromatic siRNAs include the large subunit of Pol IV (NRPD1b), AGO3, DCL3 and RDR2. These proteins co-localised with siRNAs in foci in the nucleolus or in CBs (Li et al. 2006; Pontes et al. 2006). Thus the nucleolus/CBs are involved in siRNA production and assembly of silencing complexes. This role in the transcriptional gene silencing pathway in plants raises the question of whether other RNA silencing pathways or the cross talk among pathways also involves the nucleolus.

## ***mRNA***

The nucleolus has previously been implicated in mRNA export or surveillance (Schneiter et al. 1995; Pederson 1998). In yeast, in mRNA transport-defective mutants and mutants of some nucleolar proteins, the nucleolus was disrupted and fragmented and accumulated poly(A)<sup>+</sup> RNA (Kadowaki et al. 1995; Schneiter et al. 1995). Similarly, in transport-defective mutants in *Schizosaccharomyces pombe*, an intron-containing transcript accumulated in the nucleolus, whereas transcripts from the intronless cDNA were exported (Ideue et al. 2004). Furthermore, heat shock of *S. pombe* cells resulted in accumulation of poly(A)<sup>+</sup> RNA in the nucleolus (Tani et al. 1995) while ethanol stress caused the accumulation of specific transcripts in the nucleolus (Brodsky and Silver 2000). In *S. pombe* mutants which undergo aberrant cytokinesis generating nuclei with and without a nucleolus, poly(A)<sup>+</sup> accumulated only in the latter, suggesting that poly A<sup>+</sup> mRNA associates transiently with the nucleolus during export (Ideue et al. 2004). However, recent work on mechanisms of RNA quality control which involve polyadenylation of aberrant RNA species by the TRAMP complex before their degradation may explain the accumulation of polyadenylated RNA species in some of the yeast mutants (Carneiro et al. 2007). In mammalian cells, some spliced mRNAs (e.g. *c-myc*) were localised to the nucleolus while their unspliced versions were found in the nucleoplasm (Bond and Wold 1993). Finally, the nucleolar targeting of some animal viral proteins regulates viral mRNA export and is essential to the virus life cycle, being required for viral replication or diverting normal host cell functions (Hiscox 2002, 2007). Whether the nucleolus is directly involved in mRNA export is still an open question.

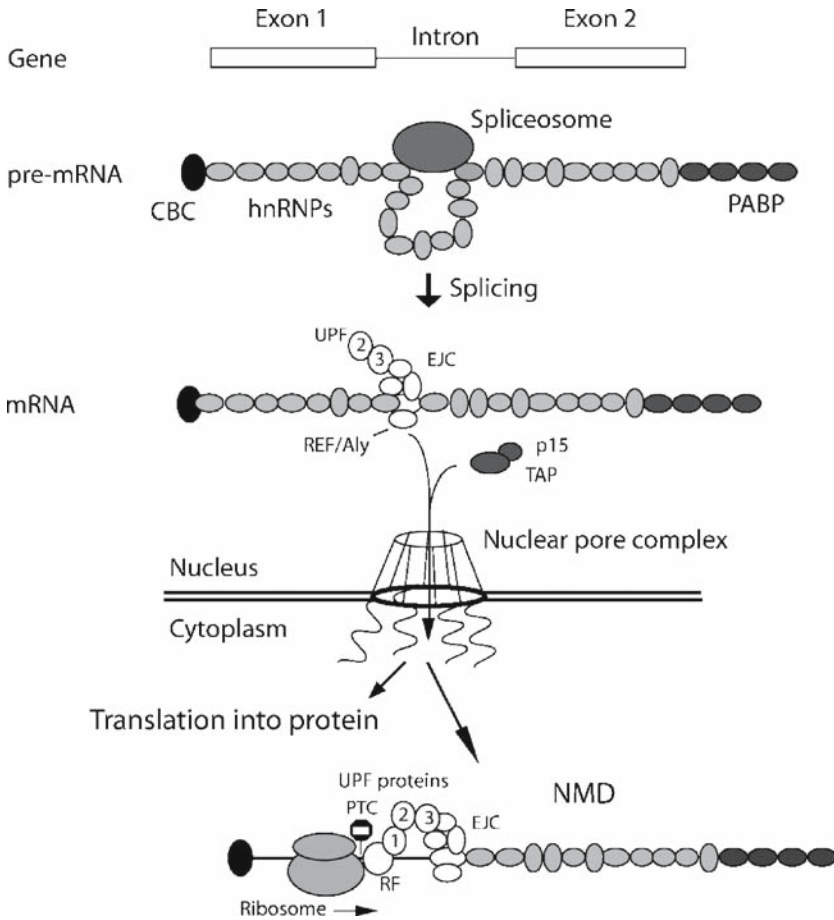
## **mRNA Biogenesis Proteins in the Nucleolus**

Recent proteomic and RNomic analyses of nucleoli have again raised the question of the function of the nucleolus in mRNA biogenesis. Proteomics of human and plant nucleoli identified almost 700 and 217 proteins, respectively (Andersen et al. 2002, 2005; Scherl et al. 2002; Pendle et al. 2005; Leung et al. 2006). The plant proteome contained many expected nucleolar, ribosome biogenesis and ribosomal

proteins as well as core snoRNP proteins and proteins involved in snoRNP biogenesis (Pendle et al. 2005). Unexpectedly, many components of mRNA splicing and translation were found in the plant nucleolus, an observation also made in the human nucleolar proteome (Andersen et al. 2002; Scherl et al. 2002). In particular, the plant nucleolar proteome contained exon junction complex (EJC) proteins which were also shown to be associated with nucleoli by localisation of GFP fusions (Pendle et al. 2005). In contrast, EJC proteins were detected in the human nucleolar proteome but have not been observed in the nucleolus (Custódio et al. 2004; Palacios et al. 2004), suggesting potential differences in EJC assembly, storage or function between animals and plants. The EJC is a multi-protein complex that is deposited upstream of splice junctions in mRNAs after intron splicing. It contains proteins that are recruited as part of the spliceosome, that interact with mRNP export adaptors, that enhance translation or that are involved in nonsense-mediated decay (Stutz and Izzuralde 2003; Maquat 2004; Lejeune and Maquat 2005; Tange et al. 2004; Andersen et al. 2006). As such, EJC proteins link gene transcription with splicing, export, translation and nonsense-mediated decay (Fig. 3). The discovery of EJC proteins, as well as splicing and translation factors, associated with the plant nucleolus suggested that aspects of mRNA biogenesis in plants involve the nucleolus.

### **mRNAs in the Plant Nucleolus**

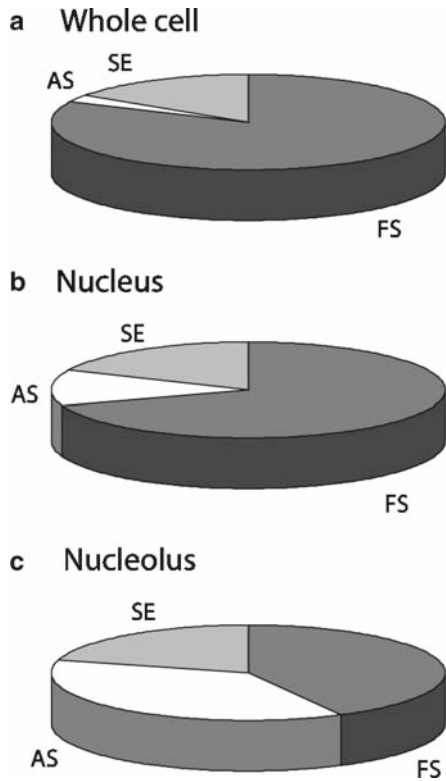
Whether mRNAs are present in the plant nucleolus was addressed by sequencing clones from cDNA libraries made from cell cultures, isolated nuclei and purified nucleoli. Not only were mRNAs detected in the nucleolar cDNA library but the abundance of different classes of mRNAs was significantly different between the three libraries (Fig. 4; Kim et al., unpublished). The mRNAs fell into three classes: fully spliced transcripts, transcripts from single exon genes (i.e. that had not undergone splicing) and aberrantly spliced transcripts. Aberrantly spliced transcripts include transcripts with potential splicing errors, incompletely spliced transcripts (where one or more introns is unspliced) and alternatively spliced transcripts. Although it is difficult to distinguish among these possibilities, the aberrant nature of this class of the transcripts in the nucleolus was demonstrated by the fact that over 90% contained premature stop codons. The proportion of fully spliced transcripts decreased from 82% in the whole cell library to 68% and 42% in the nuclear and nucleolar libraries, respectively. In contrast, the proportion of aberrantly spliced transcripts increased from 2% in the whole cell library to 13% and 38% in the nuclear and nucleolar libraries, respectively, while those of single exon genes remained around the same levels (16%–20%) (Fig. 4; Kim et al., unpublished). Thus the nucleolus contained enhanced levels of aberrant mRNAs. The unexpected discovery of mRNAs in the nucleolus suggests that mRNAs can move freely between the nucleoplasm and nucleolus and may point to a nucleolar mRNA export function. The majority of aberrant mRNAs contained premature termination codons, further



**Fig. 3** mRNA biogenesis in mammalian cells—the exon junction complex links transcription, splicing, export, surveillance, translation and nonsense-mediated decay. As genes are transcribed, the pre-mRNAs are bound by hnRNP protein factors. After capping and polyadenylation, the cap and poly(A)+ tail are bound by the cap binding complex (CBC) and poly(A)-binding proteins (PABP). Introns and splice sites are recognized co-transcriptionally. Intron removal by the spliceosome deposits the exon junction complex (EJC) circa 24 nt upstream of the exon-exon junction. The EJC contains around 20 proteins including the UPF proteins UPF3 (3) and UPF2 (2) and the export factor Aly/REF. The latter interacts with the export adaptor dimer TAP/p15 which, in turn, interacts with the nuclear pore complex, allowing the mRNP to be exported. Once exported, mRNAs are translated into proteins by ribosomes. If the mRNA contains a premature termination codon (PTC), pausing of the ribosome recruits a complex containing release factors (RF) and UPF1 (1). Phosphorylation of UPF1 is a key step in targeting the aberrant transcript for degradation.

suggesting a role in mRNA surveillance and nonsense-mediated decay. This is supported by the localisation of the NMD proteins UPF2 and UPF3 in the nucleolus (Kim et al., unpublished). Thus the plant nucleolus may function in mRNA export, surveillance and NMD.

**Fig. 4 a-c** mRNAs in the plant nucleolus. Distribution of the relative proportions of different classes of mRNAs from cDNA libraries of *Arabidopsis* whole cells, nuclei and nucleoli showing the decrease in fully spliced and increase in aberrantly spliced transcripts in the nucleolus. *SE*, single exon gene transcripts; *FS*, fully spliced transcripts; *AS*, aberrantly spliced transcripts.



### A Role in mRNA Export?

In eukaryotes, export of mRNPs from the nucleus to the cytoplasm is mediated by Aly/REF and UAP56 (Yra1p and Sub2p, respectively, in yeast) as part of the mRNP complex which interacts with the export receptor protein TAP (Mex67p in yeast) (see the chapter by V. Chinnusamy et al., this volume). This, in turn, interacts with components of the nuclear pore complex (Reed and Hurt 2002; Stutz and Izaurralde 2003; Aguilera 2005; Reed and Cheng 2005). In yeast, all three proteins (Mex67p, Yra1p and Sub2p) are required for export, but in *Drosophila* and *Caenorhabditis elegans* Aly/REF is non-essential (Gatfield and Izaurralde 2002; Longmann et al. 2003; MacMorris et al. 2003). This may reflect the ability of arginine-serine-rich (SR) protein splicing factors to interact with TAP and act as alternative export adaptors (Reed and Cheng 2005). Little is known about mRNA export components and pathways in plants. In particular, it is not known whether Aly/REF or UAP56 is needed for mRNA export and only recently has a possible orthologue of TAP been identified (Hernandez-Pinzon et al. 2007). Nevertheless, three of the four *Arabidopsis* Aly/REF proteins and UAP56 localise strongly to the nucleolus (Pendle et al. 2005) and both *Arabidopsis* and tobacco Aly proteins shuttle

between the nucleolus/nucleus and cytoplasm (Uhrig et al. 2004; Canto et al. 2006). In addition, besides EJC proteins, some splicing factors (including SR proteins), spliceosomal snRNP proteins and hnRNP proteins were identified in the Arabidopsis nucleolar proteome (Pendle et al. 2005) and the Arabidopsis SR protein RSZ22 has recently been shown to shuttle between the nucleoplasm, nuclear speckles and the nucleolus (Tillemans et al. 2006). These observations are consistent with the involvement of the nucleolus with mRNA export.

The potential export of mRNAs via the nucleolus parallels studies of viral RNA export in mammals. The export of herpesvirus saimiri (HVS) mRNA involves the expression of the viral ORF57 protein which causes Aly, UAP56 and other human export factors to be redistributed to the nucleolus (Boyne and Whitehouse 2006). ORF57 is a nucleocytoplasmic shuttling protein which binds viral intronless mRNAs and, by interacting with Aly and the transcription/export complex (TREX), accesses TAP-mediated export (Williams et al. 2005). Nucleolar trafficking of ORF57 is essential for efficient export of the viral mRNAs (Boyne and Whitehouse 2006). Similarly, the human immunodeficiency virus type 1 (HIV-1) exports unspliced and singly spliced viral mRNAs via the nucleolus for replication (Michienzi et al. 2000; Hiscox 2007). The virus-encoded protein Rev is a nucleocytoplasmic shuttling protein which accumulates in the nucleolus. It interacts with the export factor CRM1 and particular nucleoporins, causing their relocalisation to the nucleolus. By binding a specific sequence in viral unspliced mRNAs and interacting with CRM1 and the nucleoporins, it is thought to assemble an export-competent complex in the nucleolus (Hope 1999). Thus these viral mRNAs are targeted to the nucleolus, where they associate with export factors and are exported. These pathways may represent an inherent eukaryotic nucleolar mRNA export pathway, which is exploited by these viruses. The importance of mRNA export in abiotic stress responses is reviewed in the chapter by V. Chinnusamy et al. (this volume).

### **A Role in mRNA Surveillance and NMD?**

Eukaryotic systems utilise NMD as a quality-control mechanism to recognise and destroy transcripts containing PTCs (PTC+ transcripts) (Maquat 2004; Lejeune and Maquat 2005) preventing the formation of truncated polypeptides which could be potentially harmful for the cell by acting as dominant-negative mutations (see the chapter by D. A. Belostotsky, this volume). The NMD pathway has been widely studied in yeast, *Caenorhabditis elegans*, *Drosophila* and humans and there are differences in components and mechanisms by which PTCs are discriminated (Culbertson and Leeds 2003; Conti and Izzurde 2005). In mammals, PTC-containing transcripts have classically been thought to be targeted for NMD by the interaction of a paused ribosome at the PTC with a downstream EJC (Maquat 2004; Lejeune and Maquat 2005). However, recent results suggest that the distance between the termination codon and the poly(A) tail is a major determinant of NMD such that premature translation termination in the wrong mRNP context, possibly through interaction with poly(A)-binding proteins, triggers NMD (Bühler et al. 2006).

An EJC downstream of a premature termination codon potentially acts as an enhancer of NMD in this model (Bühler et al. 2006). Thus the underlying mechanism of NMD in mammals may be more related to those of yeast and *Caenorhabditis elegans*, which are EJC-independent and rely on the distance between the PTC and 3' UTR, although, in yeast, this may also involve specific sequences being recognised by *trans*-acting factors.

More than 90% of the aberrant transcripts found in plant nucleoli are putative NMD substrates based on NMD determinants in plants (Kim et al., unpublished; Kertész et al. 2006). The majority of reported examples of NMD in plants derive from intron-containing genes and, for the most part, the positions of PTCs and exon junctions would be consistent with an EJC-dependent or -enhanced effect. However, it is also clear that EJC-independent NMD occurs in plants with reduced mRNA stability dependent on the position of the PTC in PTC-containing intronless transcripts (Van Hoof and Green 2006). Recently, long 3' UTRs and introns in the 3' UTR were shown to trigger NMD in plants (Kertész et al. 2006). In terms of the protein components of plant NMD, orthologues of UPF1, UPF2 and UPF3 have been identified (Pendle et al. 2005; van Hoof and Green 2006). Arabidopsis gene orthologues of *UPF1* and *UPF3* have also been shown to function in NMD. UPF3 suppresses the levels of aberrantly spliced, PTC-containing transcripts (Hori and Watanabe, 2005), and Arabidopsis *UPF1* is indispensable (Arciga-Reyes et al. 2006) and causes decreased levels of mRNAs when tethered in different positions in mRNAs (Kertész et al. 2006). Thus the rapid degradation of PTC-containing transcripts from both intron-containing and intronless genes, and the overall conservation of EJC and NMD proteins (Pendle et al. 2005; Kertész et al. 2006; van Hoof and Green 2006), suggests that both EJC-dependent and EJC-independent mechanisms are utilised in plants. The link between the nucleolus and NMD in plants, seen with the presence of aberrant mRNAs in the nucleolus, is further reflected by the localisation of UPF2 and UPF3 to the nucleolus (Kim et al., unpublished). Other aspects of mRNA decay are covered in the chapter by D. A. Belostotsky.

## **The Nucleolus as a Major Centre for RNA Metabolism and Regulation of Gene Expression**

Over the last 40 years and particularly in the last 10–15 years, the nucleolus has grown in importance as a highly dynamic centre of RNA metabolism and RNP assembly. It has the major function in ribosomal subunit assembly needed to supply the cell with ribosomes for translation, and is involved in the processing and assembly of many other RNAs and RNPs. Not surprisingly, therefore, the nucleolus is involved in the regulation of key cellular processes and responds to growth conditions and external stresses. The recent links to transcriptional silencing, mRNA export and NMD in plants not only add to the multiple functions of the nucleolus but also enhance the concept of a role for the nucleolus in co-ordinating different RNA metabolism pathways.

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