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Plant nuclear bodies

Peter J Shaw¹ and John WS Brown²

Knowledge of the organization of transcription, RNA processing and transport, and the assembly of complexes such as the ribosome, spliceosome and other RNPs is essential to understanding gene expression. Over several years, the nucleolus and Cajal bodies have been examined in plants, and recently, various other sub-nuclear domains that are involved in RNA metabolism and hormonal responses have been discovered. These novel domains illustrate the complexity and subtlety of expression control and herald a new era of research on the molecular and cell biology of plant nuclei.

Addresses

¹ John Innes Centre, Colney, Norwich NR7 4UH, UK
e-mail: peter.shaw@bbsrc.ac.uk

² Scottish Crop Research Institute, Invergowrie, Dundee DD25DA, UK
e-mail: jbrown@scri.sari.ac.uk

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Abbreviations

AAPK	abscisic acid-activated protein kinase
AKIP1	AAPK-INTERACTING PROTEIN1
CB	Cajal body
cyp	cyclophilin
DFC	dense fibrillar component
GFP	green fluorescent protein
miRNA	microRNA
RNP	ribonucleoprotein particle
rRNA	ribosomal RNA
snoRNA	small nucleolar RNA
snRNP	small nuclear RNP
SR	serine-arginine

Introduction

The nucleus is a complex, highly structured organelle that is responsible for chromosome organization, replication and division, for gene activation, repression and expression, and for the integration of the multitude of activities that are required for cell and organism function. Ever since the nucleus was first observed microscopically, it has been clear that it is far from homogeneous and contains various sub-compartments. The most obvious compartment is the nucleolus, the site of ribosomal DNA (rDNA) transcription and ribosome biogenesis. The rest of the nucleus is organized into chromatin-rich regions,

which comprise condensed heterochromatin, and more dispersed euchromatin and interchromatin regions (Figure 1). The nucleus also contains numerous other structures or domains of different sizes, frequencies and functions [1,2] of which the best-studied are the Cajal bodies (CBs) [3–6]. With the advent of specific antibody probes, and more recently using fluorescent protein fusions, several other sub-nuclear structures have been identified, including speckles, paraspeckles, pro-myeloid leukaemia (PML) bodies and gemini of CBs (GEMS) [2,7–9,10*].

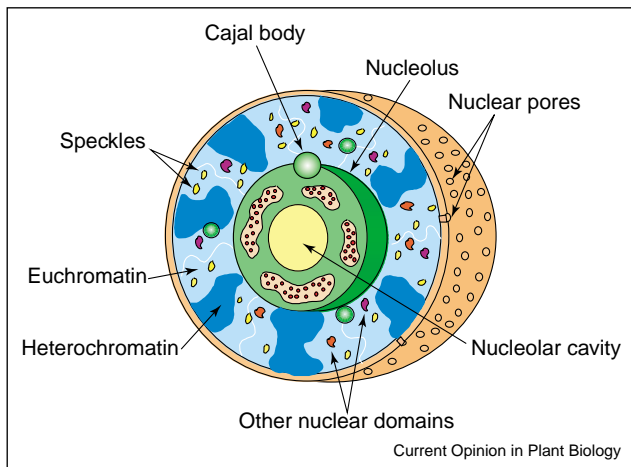
The characterization of the functional organization of plant nuclei lags significantly behind that of the nuclei of mammalian cells. Until recently, the only plant nuclear bodies to be in any way characterized were the nucleolus [11–13] and CBs [14–18]. However, current biochemical and cell biological approaches in plants are both extending our knowledge of nuclear bodies previously identified in animals and identifying novel plant nuclear bodies.

The nucleolus

In the transmission electron microscope, the structure of most mammalian nucleoli shows three different regions: small, lightly staining structures called fibrillar centres, which are surrounded by areas of densely stained material termed dense fibrillar component (DFC), which in turn is enveloped by a region that contains many particles, called the granular component (Figure 2). In typical plant nucleoli, the DFC is not as densely stained as that in animal nucleoli and occupies a much larger fraction of the volume of the nucleolus (up to 70%). In many plant nucleoli, there is a prominent central region called the nucleolar cavity. In plants, the nucleolus is very regular in its organization, often being close to spherical.

Electron microscopy of spread preparations of nucleolar transcription units showed classical ‘Christmas tree’ images, in which the transcribed gene is decorated with 50–100 ribosomal RNA (rRNA) molecules, increasing in length from the initiation site [19]. These ‘Miller spread’ preparations were produced by a detergent treatment that unravelled each active gene into a linear conformation 2–3 μm in length. Immunogold detection of exogenously added bromouridine that becomes incorporated into nascent transcripts suggests that transcription units are closely packed within the DFC ([20,21]; Figure 2). In a typical plant nucleolus, the transcription sites comprise many (200–400) small, elongated foci, of about 300 nm in length and 20–50 nm in width, that are spread throughout the DFC region and only occasionally associated with fibrillar centres. Thus, these foci are likely to be single

Figure 1

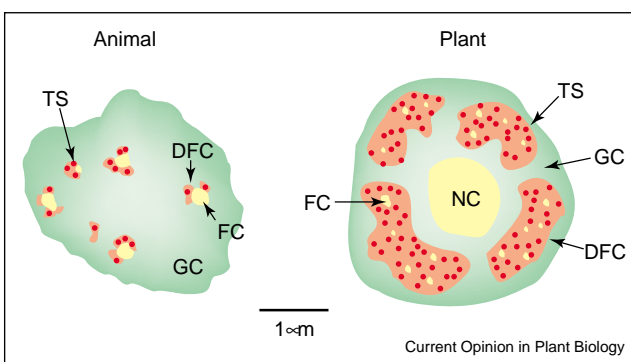


Schematic diagram of the plant nucleus, showing the heterochromatin and euchromatin regions and other nuclear domains and bodies, including the nucleolus, Cajal bodies, splicing-factor-containing speckles, cyclophilin-containing speckles and other speckle-like structures.

gene units, compacted (i.e. reduced in length along the axis of the DNA) by a factor of about 10 compared to the Miller spread preparations described above [20].

The major function of the nucleolus is in the transcription and processing of rRNA and ribosome assembly, and involves a large number of protein and small nucleolar RNA (snoRNA) components. SnoRNAs are involved in the cleavage and modification of pre-rRNAs. About 100 sites in the rRNAs are methylated by fibrillarlin, which is guided to each of these sites by box C/D snoRNAs. A similar number of uridine residues is converted to pseudouridine by Cbf5p/dyskerin, which is targeted by a family of box H/ACA snoRNAs [13,22]. In plant nucleoli,

Figure 2



Schematic comparison of animal and plant nucleolar structure. Fibrillar centres (FC), dense fibrillar component (DFC), granular component (GC), transcription sites (TS) and nucleolar cavities (NC) are labelled.

in-situ probes for the different transcribed spacers of the pre-rRNA show that the series of cleavages through which the 18S, 25S and 5.8S RNAs are derived from the precursor takes place in a series of layers that envelope the initial transcription sites [23]. This is confirmed by probes to many proteins and snoRNAs involved in this process, and suggests a layered or vectorial model for at least the early stages of ribosome biogenesis [11].

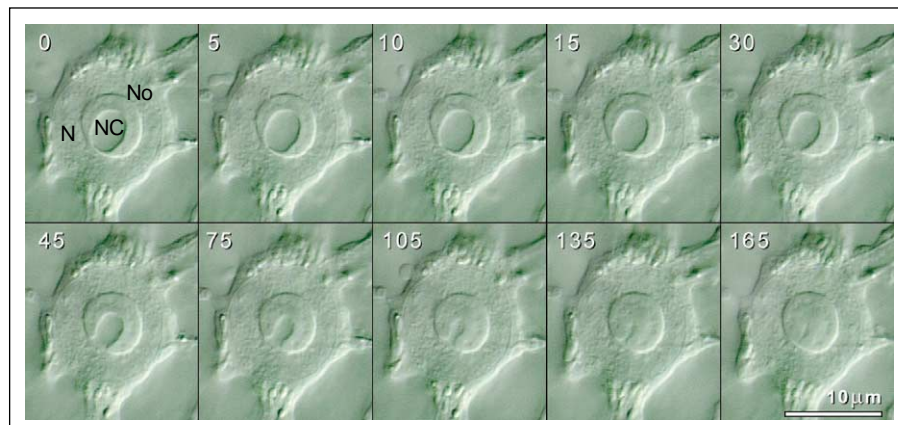
Proteomic approaches have recently been applied to purified nucleoli from human (AI Lamond *et al.*, unpublished; [24]) and *Arabidopsis* (PJ Shaw, JWS Brown, unpublished) cells. In the most-extensive current study, 692 proteins were identified in the human nucleolus. In the *Arabidopsis* study, 217 proteins have been identified to date. Many of the proteins that were identified were expected: known nucleolar proteins, ribosomal proteins, proteins that are involved in rDNA transcription, and other RNA-interacting proteins that are involved in ribosome biogenesis. However, many unexpected proteins were also found in the nucleolus; for example, spliceosomal proteins, small nuclear RNP (snRNP) proteins and translation factors. These studies reinforce the results of several previous studies, implicating the nucleolus in a variety of functions in addition to ribosome biogenesis. These functions include the biogenesis or transport of a range of RNAs and RNPs, and roles in mRNA maturation, cell cycle control and, very recently, stress responses [25–28].

The nucleolus is highly dynamic. There is an enormous flux of proteins and RNA complexes into and out of the nucleolus, and the internal nucleolar structure is itself dynamic [29]. A particularly striking and intriguing aspect of plant nucleolar dynamics is shown in Figure 3 [30]. In this time-course experiment, the nucleolar cavity empties its contents into the nucleoplasm. It is not known what accumulates in the cavity, and there is little evidence for the existence of pre-ribosomal particles in this region. The cavity could contain RNP complexes, however, as both small nuclear RNAs (snRNAs) and snoRNAs have been detected there [11,14].

Cajal bodies

Cajal bodies are found in nuclei across phylogeny from animals to plants. In plants, they are present in all species and in all cells of those species examined to date. In some mammalian cell types, they are always present whereas they may be absent in others. CBs frequently associate with the nucleolus. This linkage has been reinforced by the finding that many components are common to both structures. CBs are likely to be involved in snRNP and snoRNP maturation and transport, and snRNPs and snoRNPs accumulate in CBs before appearing in speckles or the nucleolus, respectively [3–6,31]. In particular, the modification of nucleotides in spliceosomal snRNAs is guided by small CB-specific RNAs (scaRNAs) [32,33].

Figure 3



Time-lapse series of the emptying of the nucleolar cavity of a tobacco BY-2 cell (reproduced with permission from Gunning [30]). The figures give time in seconds for each image (relative to an arbitrary zero time). N, nucleus; NC, nucleolar cavity; No, nucleolus.

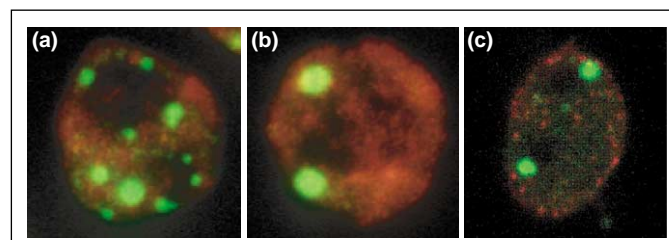
The structure or indeed the occurrence of CBs seems to be critically dependent on coilin, a protein that is generally considered to be diagnostic for CBs. CBs were absent in many cells in a mouse knockout line that expressed only an amino-terminal portion of coilin [34]. Thus, the presence of functional coilin seems to be required for the formation of CBs. The number of CBs per nucleus is under developmental control [15] and also changes through the cell cycle (Figure 4). Imaging of CBs in both living plant cells and *Arabidopsis* plants using a fusion between green fluorescent protein (GFP) and the spliceosomal protein U2B^{''}, and in human cells using a coilin–GFP fusion, has shown that CBs are very dynamic, moving within the nucleus, into the nucleolus, and fusing together [16,35]. Fluorescence recovery after photobleaching (FRAP) studies have shown a rapid flux of molecules and complexes into and out of CBs. Hence, CBs are currently thought to provide a location where components and sub-complexes can be

assembled before release to the site of function. Dynamic changes in nuclear bodies have been observed during viral infections, and recently, it has been shown in plants that the ORF3 protein from Groundnut rosette virus disrupts CBs and accumulates in the nucleolus[36].

Speckles

The majority of plant and animal genes contain introns that must be removed following transcription. This removal is mediated by the spliceosome, which consists of five snRNAs and more than 200 splicing factors [37–40]. SR proteins are a family of splicing factors that contain RNA-binding motifs and serine-arginine (SR)- or arginine-serine (RS)-rich regions. They are required for intron recognition, exon definition and spliceosome assembly [41,42], and they are often involved in determining splice-site selection in alternatively spliced transcripts. *Arabidopsis* contains a family of 19 SR proteins,

Figure 4



Cajal bodies and speckles in plant nuclei. The nuclei of pea roots are labelled by an immunofluorescent 4G3 antibody to the spliceosomal protein U2B^{''} (green). Chromatin is labelled by 7-amino actinomycin D (red). (a) The nucleus in G₁, showing many Cajal bodies. (b) The nucleus in late G₂ showing two large Cajal bodies. (c) The nucleus of an *Arabidopsis* cell suspension protoplast labelled with U2B^{''}–GFP (green) and with a fusion between the SR protein SCL28 and red fluorescent protein (RFP) (red). The U2B^{''} fusion protein is localized to the Cajal bodies, and the SCL28 fusion to spliceosomal speckles. (c) Courtesy of Andrea Barta and Zdravko Lorkovic.

which include both putative homologues of human SR proteins and plant-specific SR proteins [43**].

In mammalian cells, some splicing factors and snRNP proteins localise to irregular nuclear domains or bodies called speckles as well as to the nucleoplasm, and in some cells, they also accumulate in CBs [3,4,9,10*]. SR proteins are relocalised from speckles to regions of active transcription, suggesting that speckles are sites of storage and assembly of spliceosomal components. Recently, plant SR proteins have also been shown to localize to speckles [44–46,47*]. Inhibition of transcription by drugs or heat shock, or inhibition of kinase and phosphatase activity, caused loss of nucleoplasmic localisation of plant SR proteins and the accumulation of these proteins in larger speckles or nuclear bodies [44,45]. These observations are consistent with plant SR-protein-containing speckles having similar functions to their mammalian counterparts.

The complexity of SR-protein-containing speckles in plants may be greater than originally expected; SR proteins from different sub-families localize to different populations of nucleoplasmic speckles (Z Lorkovic, A Barta, unpublished). This raises the possibility that different SR proteins interact with different sub-sets of pre-mRNAs or mRNAs to effect the splicing, alternative splicing, or transport of pre-mRNAs or mRNAs.

Novel plant nuclear domains

Recently, several other plant proteins have also been shown to localize to the nucleus, in nuclear bodies or speckle-like domains: cyclophilins, HYL1, phytochrome and abscisic acid-activated protein kinase (AAPK)-INTERACTING PROTEIN1 (AKIP1). Although the term 'speckles' has been used to describe some of these domains, with the exception of the cyclophilin-containing bodies, these bodies do not co-localise with splicing factors. Hence, it is not known whether these nuclear domains coincide with splicing-factor-containing speckles or whether they represent novel functional nuclear domains. We reserve the term 'speckles' for nuclear domains that contain splicing factors, SR and snRNP proteins, and that correspond to electron micrograph structures called interchromatin granule clusters [10*]. The proteins found in the novel nuclear domains have putative functions in splicing or other RNA metabolism pathways, or are involved in signalling pathways. Below, we refer to these domains by the proteins that define them.

Cyclophilin-containing domains

Cyclophilins are thought to function in protein folding or as chaperones by binding to proline-rich sequences and catalyzing structural rearrangements [48]. Recently, two proteins (CypRS64 and CypRS92) that contain cyclophilin and RS/serine/proline (SP) domains have been identified in plants by their ability to interact with SR proteins

[43**]. Besides SR proteins, these proteins interact with the U1snRNP-specific protein U1-70k and with the U11snRNP-specific protein U11-35k, suggesting that they function in the early stages of spliceosome assembly, possibly in the recruitment of U1snRNP and U11snRNP to the 5' splice [43**]. CypRS64 localized to a small number of novel nuclear bodies, which, although reminiscent of CBs, were clearly distinct from CBs in co-localisation studies. More interestingly, when co-expressed with the SR proteins that directly interact with CypRS64, the cyclophilin relocalised to SR-containing speckles. This suggests that the cyclophilin-containing nuclear bodies are sites of storage for cyclophilins that relocate to speckles to effect a modification or chaperone role for the SR proteins, perhaps allowing the accessibility of phosphorylation sites [43**].

HYL1-containing domains

HYL1 is a double-stranded RNA-binding protein that is involved in microRNA (miRNA) metabolism. Mutation of HYL1 caused the decreased accumulation of miRNAs and increased levels of target mRNAs [48]. HYL1 shows nucleoplasmic labelling and its location includes a small number of nuclear bodies that are reminiscent of CBs and ring-like structures, suggesting that compartmentalization is involved in miRNP biogenesis or function [49**].

Phytochrome-containing domains

Phytochromes are a small family of plant red/far-red light photoreceptors that regulate many aspects of plant development by transducing light signals into changes in gene expression. Light induces the import of phytochromes into the nucleus, where they form protein complexes and accumulate in speckle-like nuclear domains [50,51*,52**]. The biological function of these domains is unknown but their formation is related to function and light response. For example, the domains vary in size and content of the active phytochrome conformer (Pfr) in a light-dependent and phytochrome isoform-dependent manner, and they are not formed in non-functional phytochrome mutants [51*,52**]. Other proteins that are involved in light responses also localise to phytochrome-containing domains [53], suggesting that these domains represent dynamic regions of the nucleus where signalling molecules and effector molecules (such as transcription factors or inhibitors) can associate into complexes that determine transcriptional and cellular activity. The differential formation of phytochrome-containing domains that contain different phytochrome isoforms under different conditions may generate the degree of subtle regulation required for plant responses to light. Transcription is clearly one level of regulation, but with an increasing number of plant genes known to be alternatively spliced, and in particular, the existence of genes whose alternative splicing is controlled by light, it is also possible that regulation may be post-transcriptional. In this context, it will be intriguing to discover

whether any SR proteins co-localize with different phytochrome-containing domains.

AKIP1-containing domains

AAPK and AKIP1 are found in the nuclei of guard cells. AKIP1 contains RNA-binding motifs that have homology to heterogeneous nuclear RNA (hnRNP)-binding protein A/B. Phosphorylation of AKIP1 by AAPK is required for AKIP1 to bind to mRNAs that encode dehydrins (which are involved in stress responses), and treatment with abscisic acid promotes the relocalisation of AKIP1 into speckle-like domains [54^{••}]. Thus, phosphorylation of the AKIP1 RNA-binding protein may determine its specificity in binding to particular mRNAs. The specific function of the AKIP1-containing domains is unknown but they may be regions where mRNAs bound by hnRNPs accumulate as protection against cellular stress.

Conclusions

Several novel plant nuclear domains have recently been discovered and it is likely that more will ultimately be discovered. The localization of proteins to these domains may reflect either sites at which components interact, the location of pathways in which complexes are assembled *en route* to their final site of function, or sites for the sequestration of components or complexes. The purification and determination of components that are present in these domains will provide substantial clues to the biochemical processes that occur there and to the dynamic interactions between these domains and the nucleoplasm.

A fundamental, but difficult question is why nuclear bodies exist — are they a necessary consequence of the biochemical processes? All eukaryotic cells appear to have a nucleolus in some form, although it is possible to build ribosomes without a nucleolus [55]. Similarly, some animal cell types lack CBs altogether, and there are animal and plant mutants in which CBs are either lacking or substantially altered without major effects on growth and development ([56]; PJ Shaw, L Dolan, S Wastell, unpublished). This suggests that CBs are not absolutely required, but their conservation across phyla points to a selective advantage, perhaps in increasing the efficiency of a process or processes. The most clearly defined activities of nuclear bodies are in ribosome and spliceosome biogenesis. Both the ribosome and spliceosome are very large molecular machines that are required in very large numbers by the cell. Thus, even small increases in the efficiency of processes involved in their biogenesis could have significant evolutionary advantages, and could explain the widespread occurrence of specialized structures that are involved with these processes. It can be argued that concentrating factors together within a smaller domain will increase the rates of processes simply by increasing the probability of interactions. It is also necessary that all of the interacting factors can move freely into and through the nuclear bodies, and dynamic studies in

living cells are indeed showing this to be the case. Finally, the presence of at least some nuclear bodies across such a wide phylogenetic range from plants to animals suggests that they may have been present in the earliest eukaryotes or even their predecessors.

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