Research Focus

Compartmentalization of the splicing machinery in plant cell nuclei

Zdravko J. Lorković and Andrea Barta

Max F. Perutz Laboratories, University Departments at the Vienna Biocenter, Department of Biochemistry, Medical University of Vienna, Dr. Bohrgasse 9/3, A-1030 Vienna, Austria

The cell nucleus is a membrane-surrounded organelle that contains numerous compartments in addition to chromatin. Compartmentalization of the nucleus is now accepted as an important feature for the organization of nuclear processes and for gene expression. Recent studies on nuclear organization of splicing factors in plant cells provide insights into the compartmentalization of the plant cell nuclei and conservation of nuclear compartments between plants and metazoans.

Most aspects of eukaryotic gene expression take place in the nucleus, a multifunctional cellular organelle. The multifunctionality of the nucleus is also reflected in its morphological complexity. In addition to the nucleolus (Box 1), a still increasing number of different nuclear compartments have been described in vertebrate nuclei [1,2]. Of these, the most prominent are speckles (Box 1), in which the majority of splicing factors are localized [1,3], and Cajal bodies (Box 1), which in addition to spliceosomal small nuclear ribonucleoprotein particles (snRNPs) (Box 2) contain components involved in transcription by RNA polymerase I, II and III and those involved in modifying rRNA and small nuclear (sn)RNAs [4–6]. Although they are often referred to as nuclear organelles, nuclear compartments are not separated from each other by membranes. However, the protein composition of each nuclear compartment seems to be highly specific. Furthermore, they can be morphologically identified by electron and fluorescent microscopy, and at least some of the nuclear compartments can be biochemically purified. In addition, some of the nuclear compartments are highly dynamic structures, as evident by the continuous exchange of protein and RNA-protein components between different domains [1–7].

The complexity of the plant cell nucleus is just beginning to emerge and so far not much is known about the compartmentalization of RNA-processing machineries in plant cells. Different approaches have been used to demonstrate that spliceosomal components in plant nuclei are localized in a diffuse nucleoplasmic network and in Cajal bodies [8,9]. By using fluorescent protein fusion technology in combination with confocal as well as electron microscopy, recent studies from several groups have clearly demonstrated that plant nuclei also contain speckles [10–14], which are dynamic structures like their mammalian counterparts [11–13].

Corresponding author: Zdravko J. Lorković (zdravko.lorkovic@univie.ac.at or zdravko.lorkovic@meduniwien.ac.at).

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Speckles in plant cell nuclei?

Although early studies with an antibody against the U2 snRNP-specific protein U2B″ revealed that splicing factors in plant nuclei localize to Cajal bodies and also to a diffuse nucleoplasmic network [8], the existence of

Box 1. Nuclear compartments

Nucleolus

This is the most prominent nuclear structure, clearly visible by bright-field microscopy. The nucleolus is the site of ribosomal RNA (rRNA) synthesis, processing and modification, and for the assembly of ribosomal subunits. It is organized into distinct regions, which in plant and animal cells reflect the step-wise process of ribosomal biogenesis. More recently, a variety of other putative functions such as biogenesis and transport of (m)RNAs and ribonucleoprotein particles (RNPs), cell cycle control and stress responses have been suggested for the nucleolus [27,29,30].

Speckle

In metazoan nuclei, speckles have been defined as compartments of variable size (0.5–1.8 μm) and irregular shape, seen as interchromatin granule clusters by electron microscopy. They are also called 'SC35 domain' or 'splicing factor compartment' because splicing factors are highly enriched in them. However, they are not primary sites of pre-mRNA splicing because most active genes are found at the periphery of speckles. It seems that speckles serve as storage or assembly sites of spliceosomal components. They are dynamic structures, and proteins and ribonucleoprotein complexes are recruited from them to sites of transcription [31]. Although a speckled localization pattern is highly diagnostic for proteins involved in splicing, some other proteins have been found in these domains, including some transcription factors, RNA polymerase II subunits, RNA 3′-end processing factors and lamin A. Also, poly(A)$^+$ RNA has been found in speckles to some extent [1,3,28].

Cajal body

Cajal bodies (also known as coiled bodies), are spherical structures ranging in size from 0.2–1.0 μm (up to 2.0 μm in plant cells) and localized in the nucleoplasm or close to the nucleolus. In plant nuclei, the majority of Cajal bodies are closely associated with nucleoli. Cajal bodies move within the nucleus in plant and animal cells. The function of Cajal bodies is not clear but recent experiments indicate multiple functions, including assembly of transcriptomes, small nuclear RNP assembly, modification of spliceosomal small nuclear RNAs and trafficking of small nucleolar RNPs involved in modifying rRNA in the nucleolus [4–6].

Transcription sites

They appear as several thousands foci throughout the nucleoplasm of plant and animal cells and they are often localized on the periphery of interchromatin granule clusters or speckles. RNA polymerase II components co-localize with these sites. Although pre-mRNA splicing occurs co-transcriptionally, splicing factors do not strongly accumulate at these sites.

Box 2. Pre-mRNA splicing

Spliceosome
Large ribonucleoprotein particle in which pre-mRNA splicing takes place. It assembles in a step-wise manner from five small nuclear ribonucleoprotein particles (snRNPs; U1, U2, U4, U5 and U6) and numerous additional splicing factors.

snRNP
Small nuclear ribonucleoprotein particles. Each snRNP consists of a small U-rich snRNA, seven Sm core proteins and a set of snRNP-specific proteins.

SR proteins
A family of pre-mRNA splicing factors consisting of one or two N-terminally positioned RNA-recognition motif and a C-terminal domain containing many serine–arginine (SR) dipeptide repeats. Required for both, constitutive and alternative splicing. They are often used as markers for nuclear speckles.

speckles in plant nuclei was until recently a matter of debate. In four independent studies, localization of *Arabidopsis thaliana* SR proteins (Box 2) fused to fluorescent proteins was analysed either in stable *Arabidopsis* transgenic plants [11–13] or in transiently transformed protoplasts from tobacco (*Nicotiana tabacum*) leaf mesophyll cells and *Arabidopsis* cell suspension [14]. These studies clearly demonstrated that plant SR proteins [11–14] and at least some snRNP-specific proteins [14] localize into an irregular nucleoplasmic network comparable to speckles in mammalian cell nuclei. In addition, by using immunoelectron microscopy Yuda Fang *et al.* [13] showed that speckles, as they do in mammalian cells, correspond to interchromatin granule clusters (Box 1). Although expression of the fusion proteins in three of these studies [11,12,14] was driven by the strong 35S cauliflower mosaic virus (CaMV) promoter, the described patterns seem to be functionally relevant. First, Sarah Docquier *et al.* [12] reported similar patterns in root epidermal cells by using an antibody against SR protein RSp31 and in transgenic plants expressing the RSp31–green fluorescent protein (GFP) fusion protein; second, Lorković *et al.* [14] showed that snRNP-specific proteins correctly localize and incorporate into mature snRNPs when transiently expressed in plant protoplasts; third, few or no speckles were observed in meristematic root tip cells [11,12], which is consistent with previous reports of U2B″ immunodetection in pea root tips [8]. Moreover, expression of three SR proteins under the control of their endogenous promoters revealed speckled patterns similar to those obtained with the CaMV promoter [13,14]. Thus, the speckled patterns observed with fluorescent protein fusion proteins expressed from the CaMV promoter do not seem to be a consequence of protein overexpression. In addition, these data indicate that in spite of high expression levels driven by the CaMV promoter the fusion proteins are functional.

Finally, Lorković *et al.* [14] developed plasmids that express markers for two additional nuclear compartments fused to fluorescent proteins: Cajal bodies and nucleoli (Figure 1). They showed that transient expression in *Arabidopsis* cell suspension protoplasts is a suitable system for co-localization studies in living plant cells [14] (Figure 1). Although in this system, in contrast to transgenic *Arabidopsis* plants, only one cell type can be analysed, it provides a fast and simple way of analysing subnuclear localization of the protein of interest. An alternative to this system is the *Agrobacterium tumefaciens*-mediated transient transformation of plant explants used by Docquier *et al.* [12].

Properties of plant speckles

The appearance of speckles (their number and size) in plant nuclei depends on cell type, metabolic state and transcriptional activity of the cell [10–14]. For example, Docquier *et al.* [12] reported that in contrast to differentiated root epidermal cells, meristematic cells contain almost no speckles. Electron microscopy revealed interchromatin granule cluster-like structures in epidermal cells but not in meristematic ones, thus confirming results obtained with GFP-tagged RSp31 [12]. In addition, transient expression of SR proteins in protoplasts derived from tobacco leaf mesophyll cells and *Arabidopsis* cell suspension revealed significantly more pronounced speckles in tobacco nuclei [14]. Taken together, the authors concluded that higher transcriptional activity in meristematic and rapidly growing suspension culture cells results in recruitment of more splicing factors from speckles to transcription active sites (Box 1), which finally leads to a less speckled appearance [10,12–14]. Alternatively, the stronger speckled appearance in tobacco nuclei could reflect intrinsic differences in chromatin organization or condensation between tobacco and *Arabidopsis*.

The speckled patterns of SR proteins changed upon treating plant explants with phosphatase, kinase and transcription inhibitors or upon heat shock [11–13]. This, together with the time-lapse microscopy and fluorescence recovery after photobleaching (FRAP) analysis, revealed that plant speckles are highly dynamic structures [11–13].

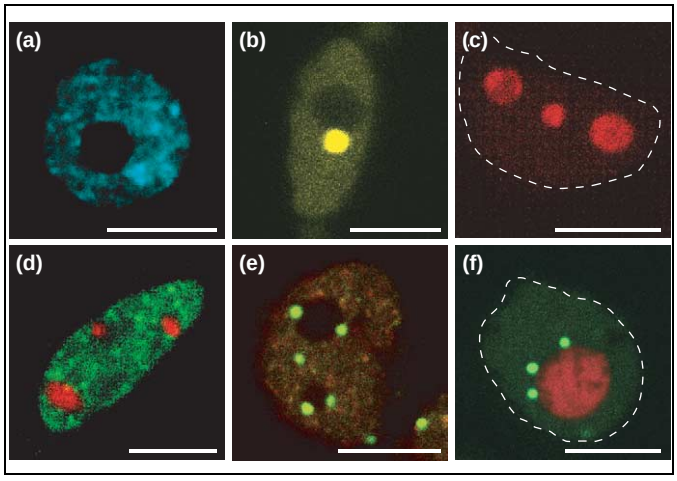

Figure 1. Nuclear compartments visualized by fluorescent microscopy. Fluorescent protein-tagged proteins were expressed in *Arabidopsis* cell suspension protoplasts and analysed by laser-scanning confocal microscopy [14]. **(a)** Speckles, SCL28–CFP fusion protein. **(b)** Cajal bodies, U2B″–YFP fusion protein. **(c)** Nucleoli, Nop10–mRFP fusion protein. **(d)** Nucleoli (red; Nop10–mRFP) and speckles (green; SRp30–GFP). **(e)** Cajal bodies (green; U2B″–GFP) and speckles (red; SCL30a–RFP). **(f)** Nucleoli (red; PRH75–RFP) and Cajal bodies (green; U2A′–GFP). All images shown are single confocal sections. Broken lines in (c) and (f) delineate the nucleus. Abbreviations: CFP, cyan fluorescent protein; GFP, green fluorescent protein; RFP, red fluorescent protein; YFP, yellow fluorescent protein. Scale bars = 6 μm.

Taken together, these data suggest that plant speckles are probably the functional equivalent of their mammalian counterparts.

Functional implications and future prospects

Recently, several plant proteins, including some that are not involved in any aspects of RNA metabolism, have been reported to localize to nuclear domains resembling speckles [15–18]. Although these authors termed these domains speckles it is not clear how they relate to true speckles, which are defined as irregular structures mostly containing splicing-related proteins (Box 1). Co-localization studies with speckle markers developed by the authors of Refs [10–14] should unambiguously show whether these proteins are indeed components of speckles and should reveal their specific significance in RNA processing or transcription.

The described higher complexity of plant SR proteins compared with those of humans raises the question of their specificity or redundancy in pre-mRNA splicing. It has been suggested that different SR proteins regulate splicing of specific subsets of pre-mRNAs, an idea that can be supported by the differential spatio-temporal expression of SR proteins in *Arabidopsis* [19]. In this respect it is interesting to note that members of different *Arabidopsis* SR protein subfamilies, in transiently transformed tobacco protoplast, localize into distinct subpopulations of speckles with no or little co-localization (Z.J. Lorković and A. Barta, unpublished). A possible explanation for these observations would be that gene-specific splicing factors (e.g. SR proteins) accumulate more strongly in speckle populations that are closer to their target genes. However, additional co-localization studies combined with FRAP and *in situ* hybridization are required to answer these questions.

In the light of exciting new reports on additional RNA-related nuclear activities, such as nonsense-mediated RNA decay, nuclear translation and heterochromatin silencing, compartmentalization of the cell nucleus seems to be an important issue in spatial regulation of RNA metabolism and consequently of gene expression. The demonstration of the existence of speckles and other nuclear compartments [20,21] not addressed here, together with recent advances in knowledge about plant Cajal bodies [9,10,22,23] and in understanding of chromatin organization of plant nuclei [24–26] should facilitate further experimentation in this field of plant cell biology. Although the data we discussed suggest that plant nuclei are, like their metazoan counterparts, highly compartmentalized, it is not clear yet to what extent these structures are conserved between plants and animals. Development of antibodies against plant components of specific nuclear compartments and development of protocols for biochemical purification of different compartments, successfully used in animal studies [27,28], would be of help in answering the obvious question – whether structural similarities between plant and animal nuclear compartments are also reflected in their similar protein and/or RNA composition. The first such study performed with isolated nucleoli from *Arabidopsis* nuclei by John W.S. Brown's and Peter J. Shaw's research groups (pers. commun.) gives us hope for future developments in this area.

The tools developed in Refs [10–14] should hopefully prompt further structural and biochemical analysis of compartmentalization of the plant cell nucleus. Fluorescent protein fusion technology will allow the analysis of dynamic properties of chromatin, RNA and proteins in living plant cells, which should provide a more complete picture of how nuclear processes are organized and coordinated in time and space.

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Unveiling the molecular arms race between two conflicting genomes in cytoplasmic male sterility?

Pascal Touzet[1] and Françoise Budar[2]

[1]Laboratoire de Génétique et Evolution des Populations Végétales, UMR-CNRS 8016, FR CNRS 1818, Université de Lille I, F-59655 Villeneuve d'Ascq cedex, France
[2]Station de Génétique et d'Amélioration des Plantes, Institut Jean-Pierre Bourgin, INRA route de Saint Cyr, 78026 Versailles, France

Cytoplasmic male sterility can be thought of as the product of a genetic conflict between two genomes that have different modes of inheritance. Male sterilizing factors, generally encoded by chimeric mitochondrial genes, can be down-regulated by specific nuclear restorer genes. The recent cloning of a restorer gene in rice and its comparison with restorer genes cloned in petunia and radish could be regarded as the beginning of a general molecular scenario in this peculiar arms race.

Gynodioecy is a mating system where both hermaphroditic and female individuals co-exist in a single population. This sexual polymorphism can be thought of as the outcome of a genetic conflict between two genomes that differ by their transmission: male sterilizing factors encoded by a cytoplasmic genome improve their transmission through resource allocation whereas the nuclear genome 'reacts' by re-establishing the male function through specific restorer alleles [1–4]. Frequently observed in wild and cultivated higher plant species, cytoplasmic male sterility (CMS) has been the object of extensive molecular investigation for the past decade. According to studies of numerous CMS systems in monocot and dicot species, the sterilizing factors are generally encoded by chimeric mitochondrial genes, probably as a result of intra-genomic recombination [5,6], a trait of the mitochondrial genome of plants [7]. Although extremely diverse, the CMS-related genes share common features such as containing parts of essential genes and/or being in the vicinity of genes enabling their 'opportunistic' transcription [8,9]. The restorer alleles generally act directly on the expression of the sterilizing factor at the post-transcriptional level. Until recently, the nature of restorer genes was completely unknown, apart from the possibility that they might code for proteins targeted to the mitochondrion and specifically interacting with the sterilizing factor expression. The recent publication by Toshiyuki Komori and colleagues [10], who cloned a restorer locus in rice, completes the picture that had already emerged from earlier studies on petunia and radish of the current understanding of the recruitment of restorer loci.

Restorer loci belong to the PPR gene family

Working on petunia, Stéphane Bentolila and colleagues [11] were the first to clone a restorer locus that is known to affect the expression of a CMS factor; earlier studies had identified *Rf2*, a 'metabolic' restorer locus in maize that might limit the effect of the CMS factor [12–14]. Bentolila *et al.* found that the petunia restorer belonged to a large gene family that codes for proteins characterized by tandem arrays of pentatricopeptide repeats (PPR) [15]. Subsequently, in radish [16–18], and recently in rice [10,19,20], restorer loci of unrelated CMS systems were shown to belong to the same family, which provided additional confirmation of the generality of the PPR feature, as judiciously foreseen in earlier studies [11,21]. Although the role of PPR genes was still hypothetical at the time, it appears now that the PPR genes are involved in organellar gene expression, probably by binding to specific transcripts, rather than being general unspecific RNA-binders, as suggested by the low redundancy of this huge family. Because obvious catalytic domains seem to be lacking in these proteins, they could be adaptors, directing the action of other factors [22]. Targeted to the mitochondrion through a transit peptide deduced in each restorer

Corresponding author: Pascal Touzet (pascal.touzet@univ-lille1.fr).
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