Post-Transcriptional Regulation of Plant Gene Expression

Involvement of the nucleolus in plant virus systemic infection

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Abstract

The nucleolus is a prominent subnuclear domain and is classically regarded as the site of transcription of rRNA, processing of the precursor rRNAs and biogenesis of pre-ribosomal particles. In addition to these traditionally recognized activities, the nucleolus also participates in many other aspects of cell function. The umbravirus-encoded ORF3 protein is a multifunctional RNA-binding protein involved in long-distance RNA movement, and protection of viral RNA from RNase attack, including possibly small interfering RNA-guided RNA silencing. In addition to its presence in cytoplasmic ribonucleoprotein particles containing viral RNA, the umbraviral ORF3 protein accumulates in nuclei, preferentially targeting nucleoli. The ORF3 protein domains involved in the localization of the protein to the nucleolus were identified. Functional analysis of the mutants revealed the correlation between the ORF3 protein nucleolar localization and its ability to form the cytoplasmic ribonucleoprotein particles and transport viral RNA long distances via the phloem. Possible mechanisms of the nucleolar involvement in systemic virus infection are discussed.

Structure and function of the nucleolus

The nucleolus is a dynamic non-membrane-bound subnuclear domain and is classically regarded as the site of transcription of rRNA, processing of the pre-rRNAs (precursor rRNAs) and biogenesis of pre-ribosomal particles (reviewed in [1–6]). Nucleolus formation is both transcription- and cell-cycle-dependent; the entire nucleolar structure breaks down and reforms during each mitotic cycle. A typical metazoan nucleolus contains three major morphological components: the fibrillar centres, the dense fibrillar component and the GC (granular component). It is generally accepted that there is a correlation between each of these components and spatial organization of ribosomal formation. Although the precise localization of transcription of rRNA genes remains unknown, it may be associated with fibrillar centres. The subsequent cascade of pre-rRNA processing progresses from the dense fibrillar component to the surrounding GC and is guided by small nucleolar RNAs that direct a series of specific nucleolytic cleavages as well as base modifications, such as site-specific methylation and pseudouridylation of rRNAs. Further assembly of the pre-ribosomal particles in the GC requires a constant influx of approx. 80 different ribosomal proteins from the cytoplasm into the nucleolus, as well as a large number of non-ribosomal proteins involved in the ribosome biogenesis pathway [7]. Finally, the completed ribosomal subunits are translocated in the opposite direction to the cytoplasm through the nuclear pores.

In addition to its major role in ribosome production, the nucleolus also participates in many other aspects of RNA/RNP (where RNP stands for ribonucleoprotein) and cellular functions. Thus because it is a site of transient sequestration and maturation of several factors and regulatory complexes, the nucleolus may be involved in the regulation of signal recognition particle biogenesis, small RNA processing, nuclear export of some mRNAs, telomerase activity, the cell cycle, cell growth and aging (see [1,2,4–6] for recent reviews).
Viral interactions with the nucleolus

A number of viruses interact with the nucleolus and its proteins. Certain viral proteins co-localize with, reorganize and re-distribute nucleolar antigens such as nucleolin, B23 and fibrillarin, which are the three most abundant and well-characterized non-ribosomal proteins (see [8] for a review). Many of these interactions are not restricted to any particular type of virus, with examples from retroviruses, DNA viruses and RNA viruses. It has been suggested that viruses may target the nucleolus and its components to favour viral transcription, translation and perhaps to alter cell growth and the cell cycle to promote virus replication [8]. For example, the nucleoprotein encoded by Infectious bronchitis virus, a member of the genus Coronavirus with a single-stranded positive-sense RNA genome, has been shown to localize to the nucleolus. It interacts with fibrillarin and nucleolin, reorganizes the distribution of fibrillarin within the nucleolus and as a result may disrupt the normal functions of these proteins in rRNA processing and modification and ribosome biogenesis to improve translation of virus mRNA [9].

The nucleoprotein also delays cell growth, possibly by interrupting cytokinesis [9]. Although the mechanism of such an inhibition of cytokinesis remains unknown, it can be suggested that coronaviruses might maintain cells in interphase to promote conditions for effective translation of viral proteins and production of virus particles.

There have been several reports of plant virus-encoded proteins targeting the nucleolus. Among them are the 3a MP encoded by Cucumber mosaic virus [10], the P3 protein with unknown function of Tobacco etch virus (a potyvirus; [11]) and the CP of Tomato yellow leaf curl virus (a begomovirus; [12]). In this last case, the CP acts as a nuclear shuttle to traffic viral DNA into and out of the nucleus, which is the site of its replication. However, the specific involvement of the nucleolus remains obscure.

Umbravirus genome: organization and expression

The genus Umbravirus comprises seven distinct virus species: CMoV (Carrot mosaic virus), CMoMV (Carrot mottle mimic virus), GRV (Groundnut rosette virus), LSMV (Lettuce speckles mosaic virus), PEMV-2 (Pea enation mosaic virus-2), TMoV (Tobacco mosaic virus) and TBTV (Tobacco bushy top virus). The past few years have brought remarkable progress in our understanding of the genome organization and expression of umbraviruses. At the same time, the recent findings, particularly the involvement of the nucleolus in umbravirus infection, have raised some new and fascinating questions related to basic molecular processes in plants.

The genomes of umbraviruses differ from those of most other viruses in that they do not encode a coat protein and thus no virus particles are formed in infected plants. Besides an RNA-dependent RNA polymerase (encoded by ORF1 and ORF2), umbravirus genomes encode two other proteins from almost completely overlapping open reading frames. One of these (encoded by ORF4) is a cell-to-cell movement protein that can mediate the transport of homologous and heterologous viral RNAs through plasmodesmata without the participation of a coat protein (see [13] for a review).

The other umbravirus-encoded protein, the ORF3 protein, is a multifunctional RNA-binding protein involved in phloem-associated long-distance RNA movement [14], and protection from RNase attack, including possibly small interfering RNA-guided RNA degradation (RNA silencing) [13–15]. Localization studies showed that the ORF3 protein encoded by GRV accumulated in cytoplasmic granules [16,17]. These granules consisted of filamentous RNP particles, contained viral RNA and the ORF3 protein. The granules were detected in all types of cells and were abundant in phloem-associated cells. It is suggested that these RNP particles serve to protect viral RNA and may be the form in which it moves through the phloem. Formation of the cytoplasmic RNP complexes may also be involved in the protection of viral RNA from the plant’s defensive RNA silencing response, although it is not a ‘classical’ suppressor of RNA silencing machinery and is unable to suppress post-transcriptional silencing of non-viral mRNA in plants [13].

Functional link between nucleolar activities and development of umbravirus systemic infection

The studies of localization of the GRV ORF3 protein also provided another quite unexpected finding; in addition to the cytoplasmic granules containing RNP particles described above, the ORF3 protein labelled with GFP (green fluorescent protein) was also found in nuclei, preferentially but not exclusively targeting nucleoli (Figure 1; [17]).

Database searches with the sequence of the umbraviral 26–29 kDa ORF3 proteins revealed no significant similarity
with any other viral or non-viral proteins, except the corresponding proteins encoded by different umbraviruses [18], suggesting that there are no analogous proteins encoded by other viruses. Further analysis revealed that the most conserved central region of these proteins consists of a rather basic and highly hydrophilic domain (amino acids 108–130), which seems to be exposed on the protein surface. This sequence includes a highly basic arginine-rich sequence containing conserved arginine residues (Figure 2) that resembles a nuclear localization signal and, moreover, is not unlike some of the nucleolar localization signals listed in [8]. Another conserved region (amino acids 151–180) of the umbravirus ORF3 protein is hydrophobic and contains invariant leucine residues in a motif LXXLL (Figure 2) that resembles a NES (nuclear export signal). Mutagenesis studies confirmed that the arginine-rich domain is a nuclear localization signal and the leucine-rich domain functions as a NES [19]. Thus the presence of these sequences may explain the accumulation of ORF3 protein in the nucleolus. The putative NES may be conserved among the ORF3 proteins to ensure that they can be exported back to the cytoplasm and prevent them being trapped in the nucleus.

The most intriguing finding of the functional analysis of the ORF3 mutants is a correlation between nucleolar localization of the ORF3 protein and its ability to transport viral RNA long distances via the phloem (S.H. Kim and M. Taliansky, unpublished work), which indicates a possible link between nucleolar function(s) and phloem-associated long-distance transport of viral RNA. Moreover, taking into account that the viruses may exploit and modify pre-existing endogenous pathways for macromolecular movement, it can be suggested that the nucleolus may be involved in control of long-distance transport of RNA and possibly other macromolecules even in normal healthy plants. The probable pathways taken by ORF3 protein in an infected plant cell are illustrated in Figure 3.

Although the umbraviral ORF3 proteins lack significant sequence similarities to proteins encoded by other viruses, they may possess functional similarities. All viruses with negative-sense RNA genomes encode an RNA-binding nucleoprotein that encapsidates the virus genome to form RNP particles, which somewhat resembles the RNP particles formed by the umbravirus ORF3 proteins. However, the nucleoprotein of Influenza A virus (a well-studied example) is much more than just a structural RNA-binding protein; it also functions as a key adapter molecule between virus and host cell processes. Similar to the umbraviral ORF3 proteins, it is a nucleocytoplasmic shuttling protein and is involved in intracellular trafficking of the virus genome [20]. Another Influenza A virus-encoded protein, the non-structural NS1 protein, also has functional similarity to the umbraviral ORF3 proteins. NS1, similar to the ORF3 proteins, is a
multifunctional protein, and both the ORF3 proteins and the NS1 protein protect viral RNA. Whereas the ORF3 proteins protect viral RNA from RNase attack and possibly from the plant's defensive RNA-silencing response, the NS1 protein blocks the α/β interferon defensive system by binding and sequestering dsRNA, which is the inducer of the interferon system [21]. It is noteworthy that the ORF3 proteins also bind to dsRNA [16]. The Influenza A virus NS1 protein also targets the nucleus, where it plays a key role in the modulation of virus and host gene expression by blocking mRNA processing, splicing, polyadenylation, nuclear export and suppression of RNA silencing (see e.g. [22,23]), processes which may also be affected by the umbraoviral ORF3 proteins.

The distribution of GFP-labelled GRV ORF3 protein within the nucleolus is not uniform [17] but resembles that of the GC, which is the site for later stages of ribosome biogenesis [24]. However, the ORF3 protein-associated RNP structures identified in the cytoplasm (see above) were not found in ultrathin sections of nuclei [16]. Thus the ORF3 protein in nuclei is apparently in a different form from that in the cytoplasm, and the role of its nuclear and nucleolar localization is still enigmatic.

These findings raise a whole series of interesting questions. Does the ORF3 protein modify nucleolar activities and if so how? Does it interact with RNA components of the nucleolus, such as rRNAs or small nucleolar RNAs to modify RNA metabolism, or does it bind to one or more nucleolar proteins, altering or inhibiting their enzymic or other properties? Does the ORF3 protein modify the translation machinery (ribosomes) to favour viral accumulation? Does it have an effect on nucleolar sequestration of the cell growth and cell-cycle regulators? What is the connection between ORF3 nucleolar localization and long-distance RNA movement? Is the nucleolus involved in RNA silencing? Future research will address these questions and attempt to use the ORF3 protein to open up our understanding of nucleolar functions.

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References

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