

Haas et al.

SUPPLEMENTAL DATA TWO

The role of ribosomal protein interactions in P6 nuclear import and nucleolar retention

P6 interacts with ribosomal proteins L13 and L18 via a region of the miniTAV domain overlapping with a putative dsRNA binding domain (dsRNA, Fig. 1A; Bureau et al. 2004). N-terminal GFP fusions of L13 and L18 (GFP-L13 and GFP-L18, respectively) show an exclusive nucleolar localization in BY-2 cells (Supp. Fig. 2 and data not shown). Upon co-bombardment of GFP-L13 and RFP-P6 $\Delta\Delta$, both proteins co-localized into the nucleolus but RFP-P6 $\Delta\Delta$ was additionally found in the nucleoplasm (Supp. Fig. 2, upper panel). Identical co-localization patterns were found between RFP-P6 $\Delta\Delta$ and GFP-L18 (data not shown). To test if the L13 or L18 association is involved in P6 nuclear import, we constructed RFP-P6 $\Delta\Delta$ Δ dsR, in which the putative dsRNA-binding domain lacks residues 132-191 required for L13/L18 interactions (Bureau et al. 2004). Upon co-bombardment of BY-2 cells, L13-GFP remained nucleolar, indicating that P6 $\Delta\Delta$ does not alter normal distribution of ribosomal proteins. By contrast, the red signal from RFP-P6 $\Delta\Delta$ Δ dsR had a nucleo-cytoplasmic distribution and was reduced, albeit not eliminated, in the nucleolus (Supp. Fig.2, lower panel). We conclude that a small part of P6 nuclear import occurs through its interaction with L13, L18, and presumably other ribosomal proteins. However, this process causes P6 retention within the nucleolar, as opposed to nucleoplasmic compartment.

REFERENCE

Bureau, M., Leh, V., Haas, M., Geldreich, A., Ryabova, L., Yot, P., and Keller, M. 2004. P6 protein of Cauliflower mosaic virus, a translation reinitiator, interacts with ribosomal protein L13 from *Arabidopsis thaliana*. *J Gen Virol* 85: 3765-3775.

