

Molecular characterization of phloem-mobile ribonucleoprotein complexes

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RNA-binding proteins (RBPs) are integral components of ribonucleoprotein (RNP) complexes and play a central role in RNA processing. Interestingly, in plants, some RBPs have been reported to function in a non-cell autonomous manner. In this regard, the angiosperm phloem translocation stream appears to contain a unique population of RBPs, but little is currently known regarding the nature of the proteins and mRNA species that constitute phloem-mobile RNP complexes. In this study, we identified and characterized a 50-kDa pumpkin (*Cucurbita maxima*) phloem RNA-binding protein (CmRBP50) that is evolutionarily related to animal polypyrimidine tract-binding proteins. In situ hybridization studies revealed that CmRBP50 transcripts were present only in companion cells (CCs), whereas immunolocalization experiments clearly detected CmRBP50 in both CCs and sieve elements (SEs). A comparison of the levels of CmRBP50 present in vascular bundles and phloem sap indicated that this protein is highly enriched in the phloem sap. Heterografting experiments established that CmRBP50 is indeed translocated from source to sink tissues. Collectively, these findings established that CmRBP50 functions as a non-cell autonomous RBP. A combination of protein overlay assays, coimmunoprecipitation and cross-linking experiments were used to identify the phloem proteins and mRNA species that comprised CmRBP50-based RNP complexes. Gel mobility-shift assays were used to demonstrate that specificity, with respect to the bound mRNA, is established by the presence of PTB binding motifs within such transcripts. These results will be discussed in terms of the role phloem RNP complexes play in long-distance signaling. This work was supported by grants DOE (DE-FG02-94ER20134), NSF (IBN0444725).