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LETTER TO THE EDITOR

Reply: Escaping a Low-Security Prison OPEN

In his Letter to the Editor, Schulz (2017) raises some important issues regarding the composition of phloem exudate and the origin of macromolecules detected in transit. While he raises several points with which we agree, he raises others with which we do not.

First, he states that a given protein will be predisposed to escape and move long distances when it is overexpressed on free ribosomes in the companion cell (CC) cytosol. He suggests that retention rather than movement would be the default. This begs the question as to how the CC could retain all of its translated proteins when it is connected to the fast-flowing sieve element (SE) by numerous, large pore-plasmodesmata units (PPUs). Perhaps it cannot, and small amounts of proteins may leak constantly into the translocation stream. Indeed, it seems very unlikely that every CC protein is strongly anchored within the cytoplasm.

Second, Schulz discusses the possibility that a "lack of recognition at the PPUs" may contribute to the loss of proteins into the translocation stream. This is an interesting notion, but it lacks experimental support. To date, no direct evidence has been produced that CC proteins interact directly with PPUs. It is more likely that the high native size-exclusion limit of PPUs is the key factor that determines whether a protein will, or will not, enter the translocation stream. In the case of CC proteins (Paultre et al., 2016) and mobile mRNAs (Calderwood et al., 2016), simple abundance and size may determine the likelihood of macromolecular movement from CC to SE, the size-exclusion limit of PPUs determining the upper molecular cutoff. Intriguingly, almost all the examples of native, phloem-mobile macromolecules cited by Schulz (PP2, FT, and CmPP16) fit these criteria.

The possibility that protein overexpression from strong promoters may induce the translocation of proteins was discussed by Paultre et al. (2016), leading to their bioinformatic analysis of phloem exudate composition. However, these data are criticized by Schulz as unreliable since cutting the phloem may lead to pressure-induced surge artifacts, causing proteins to enter the translocation stream from the adjacent CCs. Not all reports on phloem composition have used phloem exudate. In the study by Deeken et al. (2008), microdissection of the phloem was used to avoid the use of phloem exudates. However, this approach is also criticized by Schulz because the dissected phloem samples may have contained companion cells and phloem parenchyma elements, leading to contamination. We cannot exclude the possibility that phloem samples contain a small level of contamination regardless of the collection method. However, analyzing the ultrastructure of cut castor bean (*Ricinus communis*) hypocotyls equally does not provide a robust picture of the prevalence and extent of phloem contamination without accompanying proteome data.

So what are we to believe? There have been numerous studies of macromolecular trafficking in the phloem of higher plants, and these have identified a host of mobile macromolecules in the translocation stream. Are all of these artifacts of sample preparation? Should the "escapees" have been housed exclusively in the CCs of source tissues by appropriate retention mechanisms? As pointed out by Schulz, the "gold standard" of macromolecular trafficking studies is the use of grafting, devoid of criticisms of cutting or microsampling. Here, a strong body of evidence has accumulated that many macromolecules are indeed moving over long distances. As noted, critical research in this exciting area is required to discriminate between systemic macromolecules with signaling functions from those that are merely unwanted passengers. Thus, the real experimental challenge is to "separate the wheat from the chaff."

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