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Current Opinion in Plant Biology

Sieve-element differentiation and phloem sap contamination

Michael Knoblauch[1], Winfried S Peters[1], Karen Bell[2], Timothy J Ross-Elliott[1,3] and Karl J Oparka[2]

Sieve elements (SEs) degrade selected organelles and cytoplasmic structures when they differentiate. According to classical investigations, only smooth ER, mitochondria, sieve element plastids, and, in most cases, P-proteins remain in mature SEs. More recent proteomics and immunohistochemical studies, however, suggested that additional components including a protein-synthesizing machinery and a fully developed actin cytoskeleton operate in mature SEs. These interpretations are at odds with conventional imaging studies. Here we discuss potential causes for these discrepancies, concluding that differentiating SEs may play a role by 'contaminating' phloem exudates.

Addresses
[1] School of Biological Sciences, Washington State University, Pullman, WA 99163, USA
[2] Institute of Molecular Plant Sciences, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, UK
[3] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Corresponding author: Knoblauch, Michael (knoblauch@wsu.edu)

Current Opinion in Plant Biology 2018, **43**:43–49

This review comes from a themed issue on **Physiology and metabolism**

Edited by **Noel Michele Holbrook** and **Michael Knoblauch**

https://doi.org/10.1016/j.pbi.2017.12.008

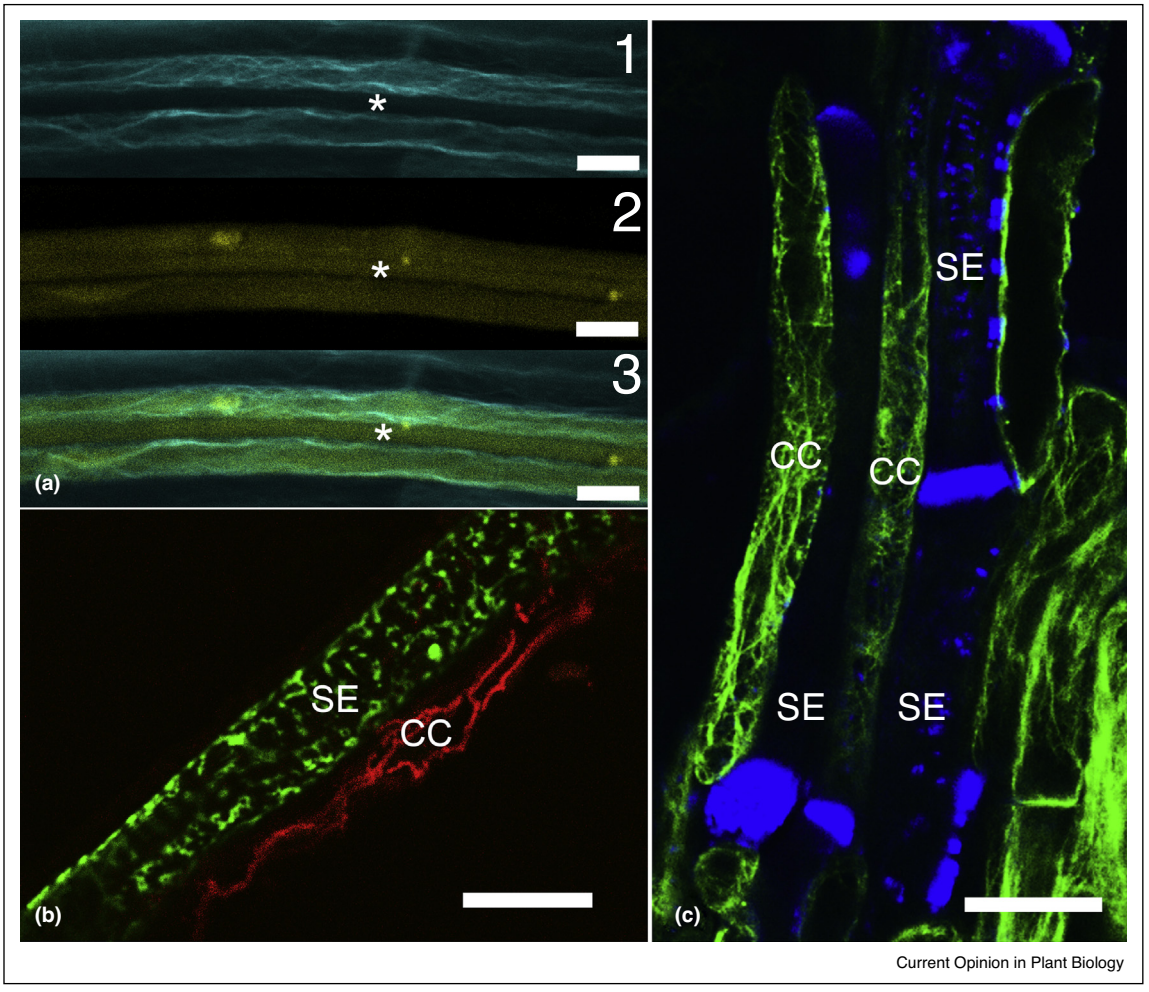
Introduction

In agreement with the function of sieve elements (SEs) in long-distance transport which favors an unobstructed tube with minimal flow resistance, differentiating SEs undergo partial autolysis [1]. It may take only minutes for a young SE to lose its nucleus, vacuole, and Golgi apparatus, as it connects to the existing sieve-tube system [1,2•]. Historically, extensive electron microscopy studies of mature SEs detected mitochondria, smooth ER, SE-plastids, and phloem-specific proteins (P-proteins), but no cytoskeleton. Except for forisomes, P-protein bodies involved in reversible sieve tube occlusion in legumes [3], the functions of P-proteins [4] and SE plastids are unknown. Application of correlative microscopy and super-resolution imaging [5–7] showed that, unlike P-proteins, membrane-bound organelles are restricted to a thin parietal layer in functional SEs. Gentle preparation and flash-freezing methods enabled the identification of protein clamps linking some of the SE organelles to each other and to the cell membrane [8,9]. Despite the wealth of evidence speaking against the presence of ribosomes and a cytoskeleton in sieve tubes (reviewed in [10]), a functional actin filament network [11,12] and protein synthesis machinery [13] were postulated to exist in mature SEs. Such uncertainties concerning SE structure are partially due to methodological challenges that derive from, first, the high turgor pressure in sieve tubes usually in the range of 1–2.5 MPa, second, the embedding of SEs in parenchyma and companion cells (CCs), hindering isolation of 'pure' sieve tubes, and third, the systemic nature of the sieve tube system in which local artefacts may be transmitted over long distances [7]. Here we discuss the contamination of sieve tubes with material from differentiating SEs as an additional factor potentially responsible for the contradictory findings.

Do mature SEs have an actin cytoskeleton?

Monomeric actin and various actin-binding proteins are found routinely in phloem exudates [13–17]. The debate about their significance in SEs was stimulated greatly by Hafke *et al.* [11], who were cited as having 'unequivocably shown that SEs contain a fully developed actin network' [18]. Unsurprisingly, this fostered new hypotheses on the actin cytoskeleton in relation to phloem function and plant-pathogen interactions [12,19,20]. Hafke *et al.* [11] provided two lines of evidence for an actin network in mature SEs. First, microinjection of fluorescent phalloidin into sieve tubes resulted in labeling of an extensive meshwork in the periphery of the SEs. Unfortunately, tagged phalloidin fluoresces independently of any association with actin and may bind to unrelated targets including forisomes, as noticed in [11]. Thus, phalloidin labeling lacks actin-specificity in sieve tubes. Moreover, the structure of the putative, phalloidin-labeled actin cytoskeleton looked remarkably similar to the sieve-tube ER and P-protein meshworks described by other authors (compare Figure 1 in [11] with our Figure 1b, Figure 2 in [9], or Figure 2 in [21]). Second, immunocytochemical TEM images showed labeling in the periphery of SEs (Figure 2 in [11]). However, the putative actin filaments lack the constant diameters and distinct fibrillar structure that

Figure 1



Lack of actin cytoskeleton in mature sieve elements (SEs). **(a)** *In situ* imaging of a transgenic *Arabidopsis* root expressing the actin-binding-domain-protein tagged with cyan fluorescent protein (blue; panel 1). Carboxyfluorescein (yellow; panel 2) loaded into the sieve tube system marks an SE (asterisk) from where it has moved into neighboring companion cells (CCs). Typical actin filaments are visible in CCs but not in the SE (combined image; panel 3). **(b)** Confocal micrograph of transgenic *Nicotiana tabacum* expressing GFP tagged to the ER under the control of the SE-specific SEOR promoter (green). The section was immunolabelled with the anti-actin antibody C4 (red). Actin filaments and tagged ER are seen in the CC and SE, respectively; there is no overlap of the signals. **(c)** Confocal micrograph of a transgenic *N. tabacum* line expressing GFP fused to the actin-binding protein fimbrin (green). Aniline blue stains callose in SEs (blue). Well-developed actin filaments are visible in parenchyma and CCs but not in SEs. Scale bars: *A* = 10 μm; *B* = 20 μm; *C* = 20 μm.

usually characterize actin filaments. The labeled, inhomogeneous structures [11] resembled the parietal ER meshwork reported by others (e.g. Plate 3 in [22]). Therefore, rather than indicating the presence of a functional actin cytoskeleton, the immuno-labeling probably identifies monomeric actin that was translocated in the sieve tubes at the time of fixation. Our interpretation is influenced by our complete failure to detect actin filaments in mature SEs using two fluorescent actin probes, actin-binding-domain-CFP (Figure 1a) and fimbrin-GFP (Figure 1c), both of which labeled actin filaments clearly in CCs. Similarly, we detected actin in CCs but not in SEs by immuno-fluorescence using the same antibody as Hafke *et al.* [11] (Figure 1b). Our data support the conventional wisdom that differentiating SEs dissolve their cytoskeleton. Breakdown products such as monomeric actin may then enter the translocation stream (as suggested in [15]), potentially causing confusion when detected in mature SEs.

Do immature SEs 'contaminate' exudates collected from mature SEs?

Sieve tube exudates can be collected by a variety of methods and are the primary source for analyses of SE contents [23]. The collected sap is subjected to proteomics, metabolomics or RNA analysis. Long-distance signaling by sap