

Short Communication

OHMS: phytoplasmas dictate changes in sieve-element ultrastructure to accommodate their requirements for nutrition, multiplication and translocation**

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****OHMS** is an initialism for “on her majesty’s service” which is commonly printed on governmental correspondence of the UK.

Abstract

Phytoplasmas are among the most recently discovered plant pathogenic microorganisms so, many traits of the interactions with host plants and insect vectors are still unclear and need to be investigated.

At now, it is impossible to determine the precise sequences leading to the onset of the relationship with the plant host cell. It is still unclear how phytoplasmas, located in the phloem sieve elements, exploit host cell to draw nutrition for their metabolism, growth and multiplication.

In this work, basing on microscopical observations, we give insight about the structural interactions established by phytoplasmas and the sieve element plasma membrane, cytoskeleton, sieve endoplasmic reticulum, speculating about a possible functional role.

Key words

actin, plasma membrane, phytoplasma, phytoplasma adhesion, phytoplasma anchoring, sieve elements, sieve-element reticulum

TEXT

Phytoplasmas, phloem-residing biotrophic prokaryotes that belong to the *Mollicutes*, are among the latest discovered plant-pathogenic microorganisms.¹ They initially received modest attention so that many major traits of their interaction with host plants and insect vectors still require thorough investigation. Due to difficulties in cultivating phytoplasmas *in vitro*,² however, it has turned out to be impossible to date to determine the precise sequence and nature of the events that establish the relationship with the host plant.

Recently,³ we described an extensive ultrastructural re-organization of sieve elements (SEs) in tomato plants (*Solanum lycopersicum* L.) affected by stolbur, the disease associated with the 'Candidatus Phytoplasma solani' ('Ca. P. solani'). We showed the presence of membranous connections between phytoplasma and SE plasma membrane, and a close association of phytoplasma cells with the sieve-element reticulum (SER) and SE cytoskeleton.³ Based on further electron-microscopic observations using conventionally prepared sections (see³), we speculate here on the order of events in phytoplasma colonization and on the functional significance of the structural interaction between phytoplasmas and the SE components mentioned before.

Phytoplasma inoculation and initial floating in the SE lumen

After direct inoculation inside the SEs by the stylets of insect vectors,⁴ phytoplasmas probably float freely in the SE lumen having mostly round, sometimes elongated shapes (Figures 1A,B). Several of the phytoplasmas in the lumen exhibit actin labeling, which discloses unipolar actin fields (Fig. 1C and³). The presence of host-cell actin filaments on the phytoplasma membrane surface may enable the phytoplasmas to adhere to SE-structures and then anchor to the SE plasma membrane³ in analogy to events described for other pathogen/host cell interactions.^{5,6} We also observed free-floating dividing phytoplasmas in the lumen (Fig. 1A), of which it is unclear if the division occurs before or after phytoplasma anchoring.

Phytoplasma adhesion to host cell structures and anchoring to the SE plasma membrane

Bacterial adhesion to host cell structures is a fundamental step in the pathogenic process. Subsequent to inoculation by their vectors phytoplasmas supposedly initiate the infection cycle by anchoring to the host cell plasma membrane.⁷ The discovery of genes encoding putative membrane proteins carrying an adhesion motif on the surface of phytoplasmas⁸⁻¹² is supportive of phytoplasma adhesion to SE substructures. We highlight here that, in most cases, the “adhesion structure” that anchors the phytoplasma body to the SE plasma membrane is tubular, 30-40 nm in width and orientated perpendicularly to SE plasma membrane (Figure 2A). The central part appears mostly electron-translucent (Figure 2A). Sometimes, the tubular corridor shows a widened end towards the SE plasma membrane (Figures 2A,B,C) so that the structure is spatially funnel-like. However, differences in morphology of the “adhesion structure” may depend on the orientation of sectioning. Nothing is known regarding the molecular architecture or the functional significance of phytoplasma “adhesion structure”. In *Mycoplasmas* (*Mollicutes* phylogenetically related to phytoplasmas), in particular in *Mycoplasma pneumoniae*, a complex, distinct polar extension of the cell body that mediates both adherence to host cell and gliding motility, has been amply studied.¹³ Dense clusters of adhesion proteins at the tip of this polar extension (named terminal organelle) have been described.¹³ A putative membrane protein containing a *Mollicutes*-adhesion-motif (MAM) was recently identified in onion yellow phytoplasma¹² indicating a potential role in anchoring to the host SE plasma membrane. In addition to phytoplasma surface proteins, host-cell actin is likely engaged in anchoring of phytoplasmas. Phytoplasmas impose an intense remobilization of host-cell actin.³ This indicates that actin on the phytoplasma surface is probably derived from the actin network that has recently been identified in SEs.¹⁴ In support of a prominent role in anchoring, actin is present on the SE plasma membrane in the proximity of phytoplasma “adhesion structure” (Figure 2B).

Actin-mediated phytoplasma movement and spread

Adhesion and anchoring may have similarities with the action of diverse pathogenic bacteria,¹⁵ which induce early accumulation of actin cytoskeletal complexes at the plasma membrane of the host cell.¹⁶ Hence, actin-anchored corridors may play a role in active displacement of phytoplasmas,

in particular through the sieve pores, the cellular bottlenecks between SEs. Using molecular approaches, Galetto et al.¹⁷ and Boonrod et al.¹⁸ demonstrated that phytoplasmas are able to bind to vector and host-plant actin filaments.

As for the intracellular movements inside the host, it is known that intracellular pathogens have evolved very similar modes of motility, dependent on the unipolar polymerization of host actin.¹⁹ Many microbial pathogens have evolved the ability to exploit the host-cell actin cytoskeleton to disseminate within and through infected tissues.^{15,20} The contact between phytoplasmas and plant host actin has been recently demonstrated *in situ*.³ Actin is localized at one side of a large portion of the phytoplasma bodies floating in the SE lumen (Figure 1C). Actin is simultaneously present on the phytoplasma membrane and the SE plasma membrane,³ presumably to displace phytoplasmas against the mass flow (Figure 2B) and/or to facilitate passage of phytoplasmas through the sieve pores.³ As the phytoplasma diameter⁴ is appreciably larger than sieve pore diameter,²¹ phytoplasmas must modify their morphology when moving to the next SE. The elongation required to pass the narrow pores may also be dependent on actin activities (Figure 2D). Thus, the actin-binding capability probably has a multiple function in phytoplasma spread.

Other potential functions of the adhesion structure

It is still unclear, how phytoplasmas withdraw resources from the host for their metabolism, growth and multiplication. It seems obvious that anchoring of pathogen and host cell and the subsequent formation of cytoplasmic corridors (Fig. 2A,B,C) facilitate pathogen nutrition and the exchange of molecular signals responsible for pathogen multiplication and spread through the tissues, to ensure host colonization. Despite the apparent logic, it is hard to conceive how the corridors tethered to the plasma membrane (Fig. 2A,B) function in phytoplasma nutrition, since open connections to the SE for bulk flow seem to be absent and substances can be only absorbed from the nutrition-poor SE apoplast.

Phytoplasmas and sieve-element reticulum: nourishment and multiplication?

A stable parietal position of the ER, tethered by minute clamps, has been described for SEs in well-preserved sections of *Vicia faba* and *S. lycopersicum*.²² In stolbur-infected SEs of tomato, it was not easy to visualize the minute anchors attaching the distorted SER to SE plasma membrane. These minute structures are likely still present in infected SEs (Figure 3A,B, C), but in a few cases, SER was observed to be partially detached from SE plasma membrane (Figure 3D).

Apart from their contacts with the SE plasma membrane, phytoplasmas also establish relationships with the (distorted) SER,³ which is considered an important source of proteins and metabolites available for intracellular pathogens.²³ Like other pathogenic intracellular bacteria,^{3,24} phytoplasmas induce stress upon the ER followed by the unfolded protein response (UPR).³ The UPR induction could reflect a means through which pathogens gain nutrients from the strongly distorted ER of the host cell.²⁵

All in all, ER-stacks may be more serious candidates for phytoplasma nutrition stores than the SE apoplast. However, open physical contacts were not observed between phytoplasmas and SER stacks; phytoplasmas seem to solely rest close to the contorted ER³ (Figure 3D). Strikingly, actin labeling is always absent on the surface of phytoplasmas adhered to the SER³ (Buxa et al., 2015; Figure 3D). In conclusion, the proximity of the pathogens and host SER may facilitate the scavenging of nutrients, but the mechanisms of interaction between phytoplasmas and SER are unknown, and demand further in-depth investigation.

It is further imperative to realize that companion cells, providing all compounds needed for sieve-element maintenance *via* pore-plasmodesmata units,²⁶ must largely contribute to the supply of metabolites and macromolecular building blocks to phytoplasmas.

Concluding remarks

To acquire host-derived nutrients, obligately intracellular pathogens must exploit the internal stores of host cells by establishing relationships with the host-cell compartments. The distribution of phytoplasmas inside the SE and the connection to the SE substructures allowed us to speculate on the involvement of SE organelles in the pathogen life cycle. The number of phytoplasma bodies

found in each SE compartment is reported in Table 1. Phytoplasmas were counted and recorded according to their position: attached to the plasma membrane, located in the proximity of SER and floating in the lumen (Table 1). Quantitative analyses have been performed on leaves, sampled from fully-symptomatic plants. The majority of phytoplasmas (approx. 75%) occurs in the SE lumen, some (approx. 20%) are attached to the plasma membrane and few (approx. 5%) are in the proximity of the SER. The possible relationships of phytoplasmas with SE organelles are summarized in a scheme (Figure 4). Even if the events that determine the dynamics of nutrition, proliferation and spread of phytoplasmas within their host are still not understood, bacterial distribution inside the infected SE may give a first glimpse on the interaction of the phytoplasmas with different host-cell compartments in keeping with the pathogen life cycle. The exact order of events is uncertain (Figure 4), but it seems that part of the phytoplasmas is equipped with a unipolar actin field that enables to adhere to SE membranes. It is unclear if such contacts to the plasma membrane are compulsory. Part of the phytoplasmas may move on to the sieve pore to become elongate, other may release the contact tubule and move to the ER, where they withdraw nutrition from the host (contact/relation with SER) in order to multiply eventually.

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Table 1 Subcellular distribution of phytoplasmas in infected sieve-elements of tomato.

Fields	SE plasma membrane	SE reticulum	SE lumen	Total number of phytoplasmas counted
12	3,83 ± 1,95 (a)	1,00 ± 0,85 (a)	14,75 ± 11,33 (b)	235

Fields are defined as the cross sections of four sieve elements observed in three non-serial sections.

Phytoplasmas in each field were counted manually and screened for the attachment to the sieve-element

plasma membrane, for the proximity to the sieve-endoplasmic reticulum (SER) and for floating in the

*lumen*²⁷. *The data represent the average number of phytoplasma bodies per entire compartment in a cross-*

section. Different letters next to each standard deviation represent significant differences. ANOVA test, P-

values < 0,001

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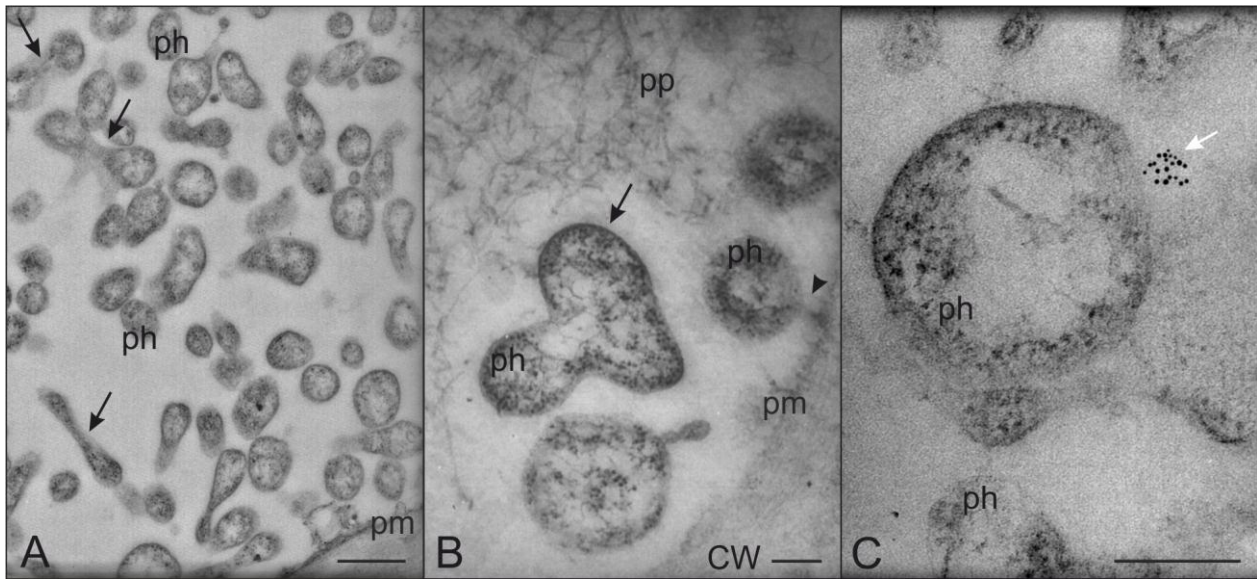


Figure 1 Transmission electron microscopy (TEM) micrographs of phytoplasmas floating in the SE lumen. (A,B) Phytoplasmas are mostly roundish, sometimes elongated; a few are dividing (black arrows). (C) Aggregates of SE actin form unipolar fields on the phytoplasma surface in the SE lumen (white arrow). The arrowhead in (B) indicates the attachment of a phytoplasma to the SE plasma membrane. In (A), the bar corresponds to 500 nm; in (B) and (C) the bars correspond to 200 nm. CW: cell wall; ph: phytoplasma; pm: plasma membrane; pp phloem protein

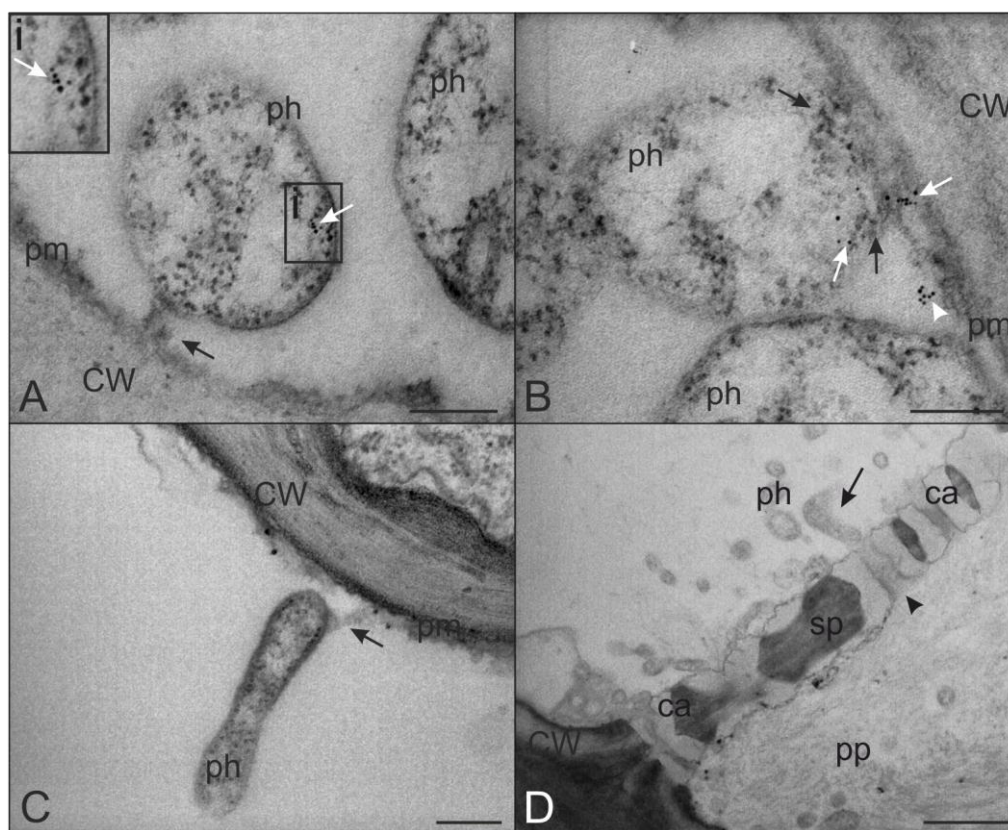


Figure 2 TEM micrographs showing phytosomas and the adjoining SE plasma membrane.

(A) A tubular “adhesion structure”, connecting the phytosoma body to the SE plasma membrane is visible (black arrow). SE actin (white arrow in inset i, upper left) is aggregated on phytosoma cell membrane at the opposite side of the “adhesion structure” (black arrow). (B) Actin (white arrows) is also located in the proximity of the “adhesion structure” (black arrows) and close to the SE plasma membrane (white arrowhead). (C) Elongate phytosomas attached (black arrow) to the plasma membrane. (D) Phytosomas near the sieve pores. The elongate shape of the phytosoma body (black arrow), similar in dimension to the sieve pore (black arrowhead) diameter, is required to move from one SE to the next one. In (A), (B) and (C) the bars correspond to 200 nm; in (D), the bar corresponds to 1 μ m. Ca: callose; CW: cell wall; ph: phytosoma; pm: plasma membrane; pp: phloem protein; sp: sieve plate

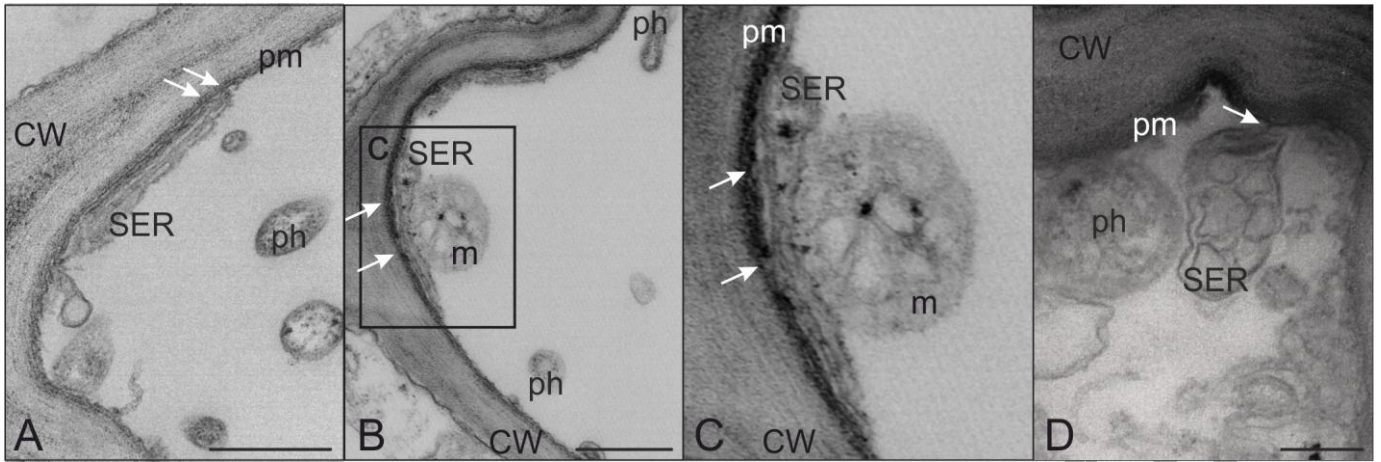


Figure 3 TEM micrographs showing sieve endoplasmic reticulum (SER) and phytoplasmas.

(A,B and inset C). In infected SEs, the SER stays in parietal position by means of minute anchoring structures (arrows). (D) The SER shows swollen, enlarged and distorted cisternae and a partial detachment from the SE plasma membrane (arrow). In (A) and (B) the bars correspond to 500 nm; in (D) the bar corresponds to 200 nm. CW: cell wall; m: mitochondrion; ph: phytoplasma; pm: plasma membrane

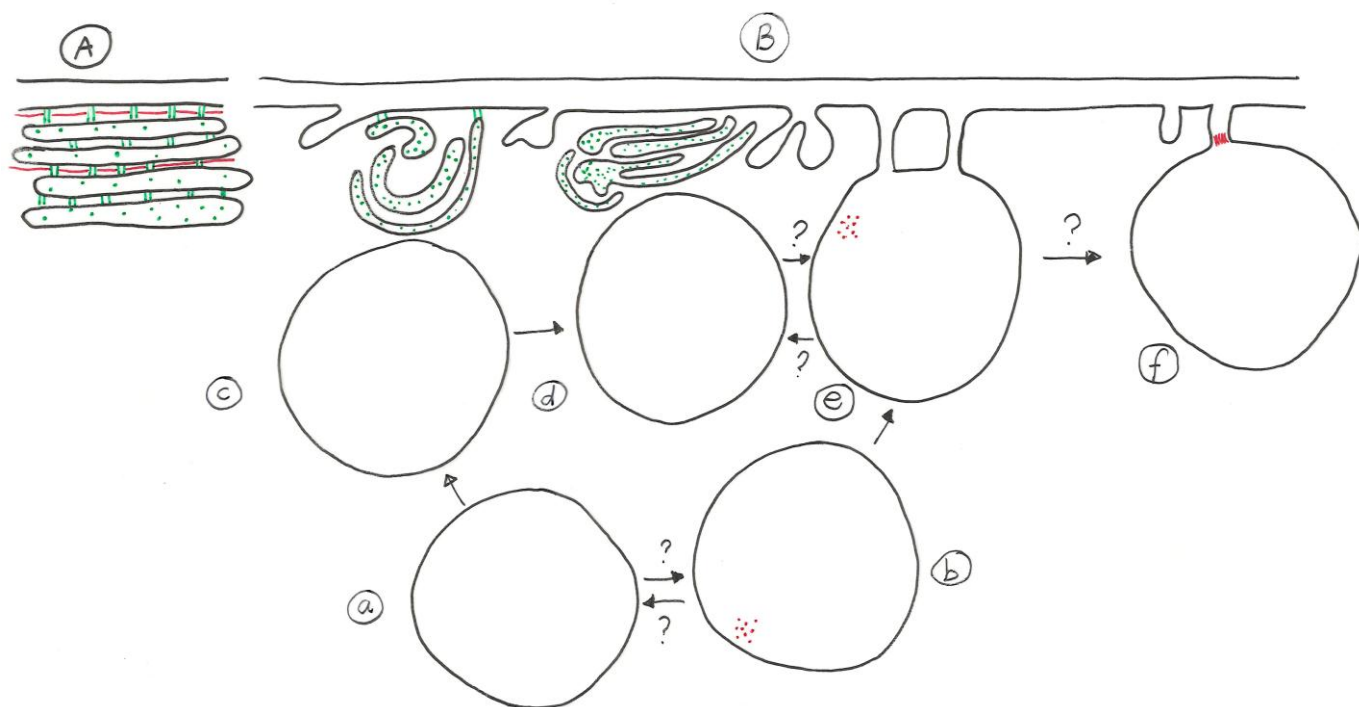


Figure 4 (A) Schematic representation of plasma membrane, actin location (red) and SER in non-infected SEs. The plasma membrane is appressed to the SE wall. SER stacks ordered parallel to the plasma membrane, regular in shape, are connected to each other and to plasma membrane by tiny anchors and are accompanied by longish actin filaments (in red: actin, in green: SER proteins). (B) Possible interrelationships of phytoplasmas with SE substructures. The order of “phytoplasma processing” is represented by arrows. The SE plasma membrane, SER stacks and actin filaments are re-arranged following phytoplasma infection. The SE plasma membrane becomes undulated or invaginated. After injection into the SE by insect vectors (a), some of the phytoplasmas locate to the surface of the distorted SER (c,d) that is partially detached from the plasma membrane. Others become equipped with a unipolar SE actin field (b) that enables them to attach to the plasma membrane by “tubular adhesion corridors”. The corridors may be involved in nutrition and signaling and active displacement of phytoplasmas along the plasma membrane (e) and through the sieve pores (f). As indicated by several question marks, the sequence of events is unknown as well as whether the various stages occur in a compulsory order.