

1 **Plant Endoplasmic Reticulum-Plasma Membrane Contact Sites (EPCS)**

2 Pengwei Wang¹, Chris Hawes² and Patrick J. Hussey^{1*}

3 1. Department of Biosciences, Durham University, South road, Durham, DH1 3LE, UK

4 2. Department of Biological and Medical Sciences, Oxford Brookes University, Oxford OX3 0BP, UK

5 *Corresponding author: p.j.hussey@durham.ac.uk

6

7 Key words: ER—PM contact sites, cytoskeletal organization, endoplasmic reticulum,
8 membrane trafficking, VAP27 proteins

9

10

1 **Abstract**

2 The endoplasmic reticulum (ER) acts as a superhighway with multiple side roads that
3 connects the different membrane compartments including the ER to the plasma membrane
4 (PM). ER—PM contact sites (EPCS) are a common feature in eukaryotic organisms, but have
5 not been studied well in plants, due to the lack of molecular markers, and the difficulty in
6 resolving the EPCS structure using conventional microscopy. Recently however, plant
7 protein complexes required for linking the ER and PM have been identified. This is a further
8 step towards understanding the structure and function of the plant EPCS. Here, we will
9 highlight some recent studies in this field and suggest a number of hypotheses that relate to
10 the possible function of EPCS in plants.

11

12 **Plant EPCS and the ER network**

13 The cortical endoplasmic reticulum (ER) in plants forms a dynamic geometrical network of
14 tubules and small cisternae underlying the plasma membrane [1]. The dynamics of this
15 network and to a certain extent its geometrical organisation are in part controlled by the
16 cortical actin network [2] and a number of members of the myosin XI family [3, 4]. However,
17 depolymerisation of the cortical actin by drugs such as cytochalasin B and latrunculin B,
18 although perturbing the ER network, do not result in its destruction [5]. The implication
19 being that perhaps ER tubules are anchored to other cellular components such as the
20 microtubule cytoskeleton, or the plasma membrane (PM).

21 *Cortical ER links with the PM through ER—PM contact sites*

22 Early electron microscopy studies identified sites of contact between the ER and PM; the so
23 called ER—PM contact sites (EPCS) [6]. More advanced light microscopy technologies using
24 persistency mapping of ER tubule dynamics further demonstrated the existence of the EPCS
25 [7, 8]. Although their structure and function in plants remains to be fully evaluated, here we
26 propose some possible models for the function of the EPCS that relate to their interesting
27 structures (Key Figure 1, A). Morphologically, EPCS can be defined as a region where the two
28 membranes are closely attached (less than 10 nm apart) and where ribosomes are excluded
29 (Key Figure 1, B). In mature plant cells, the central vacuole occupies most of the volume. As

1 a result, the cytoplasm is restricted to a thin layer at the cell cortex and organelles such as
2 the Golgi bodies and cortical endoplasmic reticulum reside very close to the PM [9].

3 The dynamic properties of the plant ER network are regulated by the cytoskeleton [10-12];
4 and the most obvious role of the EPCS would be to anchor the fast remodelling network.
5 However, the physiological functions of EPCS are most likely to be far more complex. Studies
6 conducted in yeast (*Saccharomyces cerevisiae*) have revealed that EPCS are sites of
7 phospholipid metabolism and signalling [13, 14]. For example, the EPCS localised oxysterol
8 homology-binding proteins (Osh) sense the level of PI4P at the PM, and facilitate the
9 interaction with an integral ER protein, Scs2p, and Sac1 (a PI phosphatase). These
10 interactions close the gap between cortical ER and the PM, allowing ER localised Sac1 to
11 convert PI4P to PI at the PM [13]. In addition, Scs2p (known as VAP or VAP27 in animals and
12 plants), is also required for multiple lipid synthesis and transport pathways [15, 16]. Studies
13 in animal cells revealed that calcium transport from the extracellular space is regulated
14 through a protein complex (STIM1 and Orai1) localised at the ER—PM junctions [17-21], and
15 such protein complexes are also associated with actin bundles at the leading edge of
16 migrating cancer cells [22].

17 *Proteins identified in the EPCS complex in plants*

18 A number of other proteins that localize to the EPCS have been identified and their
19 molecular function in yeast and animals is reasonably well established [18, 19, 23]. However,
20 their plant counterparts have not been well characterised (Table 1). Recently, a protein
21 complex forming an EPCS in plants has been identified. This complex is composed of VAP27,
22 NET3C, microtubules and actin filaments [24, 25]. NET3C belongs to the NETWORKED
23 super-family of actin binding proteins [26, 27]. Members of this family form links between
24 actin filaments and different membrane systems and they are specific to higher plants.
25 Using either VAP27 or NET3C as markers, further studies have revealed more components
26 of the EPCS in plants, such as synaptotagmin 1 that also confers mechano-tolerance (Key
27 Key Figure 1, C-E)[28,29]. Proteins of the EPCS could be spatially closely associated or
28 exactly co-locate at the same site (Key Figure 1,F-G) [30]. In this opinion article we evaluate
29 this step forward in our understanding of the biological relevance of the plant EPCS and

1 hypothesize on their possible functions which could include regulating membrane trafficking
2 pathways, cargo transport, calcium signalling and responses to biotic stress (Key Figure 1A).

3

4 **The formation of EPCS in different cell types and roles in lipid transport**

5 In our studies, when VAP27-GFP was stably expressed in arabidopsis (*Arabidopsis thaliana*)
6 under its endogenous promoter, the formation of VAP27 labelled EPCS largely depended on
7 the cell type [25]. This may reflect different requirements of EPCS in these cell types, such as
8 in their ER remodelling capacity.

9 The function of plant EPCS in lipid transfer is poorly understood. There is evidence to
10 suggest that the transport of oxysterol in plants is regulated by an interaction between
11 VAP27 proteins and the ORP (oxysterol-binding-related protein) family by a mechanism that
12 has been described in yeast [15]. There are 12 ORP genes identified in the arabidopsis
13 genome, and they have different intracellular locations [31]. The ER/Golgi localised ORP3a
14 binds to sterol *in vitro* [32] and a similar activity can be predicted for other ORP proteins
15 that contain oxysterol binding domains. The petunia version of ORP1 localises to distinct foci
16 that associate with the PM of pollen tubes [31], and this localisation is reminiscent of that of
17 the EPCS. We propose that oxysterol trafficking in plants is likely to be mediated by the ORP-
18 VAP27 complex, and such events could occur at the EPCS. In addition, the export of lipids
19 that form protective structures, such as waxes and the cuticle, may also occur at the EPCS.
20 The cuticle contains very-long chain fatty acids that are generated in the ER and transported
21 through the PM localized ABC transporter to the extracellular space. This process is
22 independent of the conventional Golgi dependent pathway, and is likely to be involved in
23 the direct communication of the ER and PM localised transporters [33]. However, no clear
24 evidence for EPCS mediated lipid transfer has yet been reported in plants. Future studies on
25 determining the localization of various lipid transporter/binding proteins in relation to EPCS
26 complex could provide valuable information in this aspect.

27 **EPCS acts as the hub for the organization of the cytoskeleton**

28 ER movement is regulated by the cytoskeleton and both actin filaments and microtubules
29 are found closely associated at the site where ER is anchored to the PM [24]. In fission yeast,

1 ER—PM contact sites regulate the formation of the actomyosin contractile ring, which is
2 made from dense actin filaments and is required for cell division [34]. In plants, a large
3 number of EPCS overlap with the site where cortical microtubules and actin intersect (Figure
4 2, A-C) [24, 25, 28].

5 NET3C is the best example of an actin binding protein that has been shown to be part of the
6 EPCS complex in plants, where it acts as an adapter between the actin cytoskeleton and the
7 PM. Other potential candidates for EPCS actin binding proteins are myosin VIII. A myosin
8 VIII tail domain deletion mutant of ATM1 localises to the PM as well as numerous stationary
9 puncta that sit closely to the three-way junctions of ER membrane [35]. This location is
10 reminiscent of known EPCS proteins. Further evidence is required to confirm this but it is
11 likely that actin-motor proteins participate in the EPCS where cargo transport and
12 membrane trafficking may occur [36]. Another potential candidate for an actin binding
13 EPCS component is FORMIN. For example, FORMIN 1 spans the PM [37], interacting with the
14 cytoskeleton on the cytoplasmic domain and is in contact with the cell wall through its
15 extracellular domain which constrains the movement of the protein in the PM [38]. A similar
16 constraint is observed for VAP27 at the EPCS and this is likely to be due to some indirect
17 binding involving a PM integral membrane protein [25]. Moreover, FORMINS are able to
18 interact with actin filaments and microtubules [39, 40], and this property makes them ideal
19 candidates for keeping the EPCS complex stationary as the dual association of VAP27 with
20 microtubules and the actin cytoskeleton has also been reported (Figure 3, A) [24, 25].

21 The SCAR complex regulates ARP2/3 mediated actin filament nucleation [41]. Previous
22 studies have shown that some SCAR complex components, are either PM or ER associated
23 [42-45]. Proteins from the SCAR complex (e.g. NAP1) and the Arp2/3 complex (ARP3)
24 localise to distinct puncta that associate with the ER surface as well as the cytoskeleton [45,
25 46]. Some of these NAP1 labelled puncta also overlap with EPCS that are labelled with
26 VAP27-1 [45]. **Taken together, these data suggest that functional ARP2/3 complexes or actin
27 regulatory proteins can be present at the EPCS, facilitating actin cytoskeleton dynamics and
28 remodelling.**

29 **Could EPCS be involved in pathogen and symbiont responses?**

1 Dramatic cytoskeletal rearrangements and membrane/organelle movement occur in
2 response to infection and symbioses [47-49]; dense ER membrane and F-actin cables are
3 normally found at the site where the pathogen touches the cell surface [50]. On the other
4 hand, such ER—PM associations have also been seen during symbioses, such as during
5 infection thread formation in root hairs and during nitrogen fixation and the formation of
6 mycorrhizal arbuscules [51]. Plasma membrane resident proteins localise to oomycete
7 haustoria, and EPCS resident proteins such as synaptotagmin 1 can also be found
8 concentrated at these sites (Figure 3, B) [52]. Therefore, could the EPCS function as hubs
9 that react to extracellular signals such as pathogen infection? This is an intriguing hypothesis,
10 which is supported by three lines of evidence: (i) Plant EPCS complexes have the capacity to
11 recruit cytoskeletal associated proteins and this function might be elevated during infection
12 where enhanced actin-membrane activity is required. (ii) Plant EPCS associate indirectly
13 with the cell wall (Figure 3, C) [25], and might be able to react to certain extracellular signals.
14 (iii) VAP27-1 interacts with a disease resistant protein (fungal pathogen protein), Cf-9, that
15 forms membrane associated complexes [53, 54]; it also interacts with a hypersensitive
16 response gene, ACD11 [55], all of which are required for the activation of plant defence
17 during fungal infection. Thus, future studies should address the behaviour of EPCS
18 complexes during infection and whether the susceptibility of plants changes when the
19 function of the EPCS complex is compromised.

20

21 **ER—PM contact sites and cell-cell communication**

22 Plasmodesmata (PD) are a unique feature of plant cells forming 50nm diameter channels
23 between cells. They are sites where the PM, ER and the actin cytoskeleton are known to
24 converge [56]. Therefore, PD can be regarded as specialised ER—PM junctions. However,
25 the number of EPCS on plasma membranes is greater than the number of PDs, especially at
26 the cortex of epidermal cells, which obviously do not have plasmodesmata in their outer
27 periclinal walls. At cell-cell borders, a large number of EPCS are found co-localised with PD,
28 suggesting that EPCS acts as a scaffold that supports the central desmotubules through the
29 PD [25]. More interestingly, recent studies also demonstrate that certain members of a
30 family of ER curvature inducing proteins, the reticulons, are found in the developing PD and

1 may be required for constricting desmotubules [57]. A proteomics screen, supported by *in*
2 *vivo* fluorescence resonance energy transfer experiments revealed that two of the PD
3 localised reticulons (RTN3 and RTN6) interact with the EPCS proteins, VAP27 and
4 synaptotagmins [58].

5 Plasmodesmata mediate the cell-to-cell transport of macromolecules as well as virus
6 particles. Virus movement proteins (MP) localise to mobile punctate structures containing
7 RNA, and these particles are regarded as the viral replication complexes (VRCs). These VRCs
8 move on the cortical ER network, and they transiently pause at the cortical microtubule-
9 associated ER sites (C-MERs) [59], which are structurally very similar to the EPCS. Therefore,
10 viruses may use EPCS (or C-MERs) as a centre for the recruitment of membranes/proteins
11 (transported through the cytoskeleton) that are required for their replication. When the
12 formation of virus particles is complete, they can potentially be re-located using MPs to the
13 PDs that are adjacent to EPCS and therefore ready to infect neighbouring cells. This
14 hypothesis is supported from a few protein—protein interaction studies which suggest that
15 the EPCS protein, VAP27-1, is involved in the viral replicative cycle, and interacts with virus
16 proteins [60, 61]. In addition, a study using the EPCS localized synaptotagmin 1 and *Turnip*
17 *vein clearing virus* suggests that synaptotagmin 1 interacts with MPs and mediates the
18 trafficking of VRCs to the PD [29, 62].

19

20 **Could plant EPCS provide the route a junction for membrane trafficking?**

21 In plants, the Golgi and ER network are closely associated [1, 5]. Although the movement of
22 Golgi bodies is not required for cargo sorting and trafficking activities, Golgi stacks often
23 pause in association with stable ER junction associated microtubules, the cortical
24 microtubule-associated ER sites (C-MERs), which are structurally similar to the EPCS [59, 63]
25 (Figure 3, D). Thus, it could be postulated that such interactions are involved in transporting
26 specific cargos, such as delivering cellulose synthase proteins whose secretion require
27 pausing of Golgi bodies on the microtubules [64]. Alternatively EPCS could simply form a
28 structural barrier which Golgi bodies need to overcome during their transit around the ER
29 network.

1 EPCS localised proteins are also believed to regulate the endocytic pathway [62]. Studies
2 using arabidopsis SYT1 demonstrate that it co-localises with FM4-64 labelled endocytic
3 membranes, and over-expressing a dominant negative version of SYT1 inhibits the
4 formation of plasma membrane-derived endosomes [62]. In mammalian, fungal and plant
5 cells, endosomes are found closely associated with the ER network [65, 66]. Changes in the
6 ER network structure and homeostasis (by changing the expression of ER structural proteins,
7 e.g. reticulons, or RHD3) is critical for plant endosome streaming and endocytosis [65].
8 Direct interaction between endosome membrane proteins (e.g. StAR related lipid transfer
9 domain-3, STARD3) and VAP proteins has been observed in animal cells [67], and interaction
10 between VAP, retromer subunits and WASH proteins (ARP2/3 complex activators of actin
11 nucleation) controls the formation of endosomes [68]. A similar phenomenon might exist in
12 plants. For example, endocytotic vesicles form at the ER—PM site enriched in cytoskeletal
13 components where the actin cytoskeleton provides the force for endocytotic vesicle
14 formation and the closely associated ER membrane provides the track for endosome
15 movement and distribution (Figure 3, G).

16

17 **Concluding remarks**

18 The organelles and membranes in a cell are not structurally isolated. More and more
19 evidence supports the phenomenon that interactions and connections are found amongst
20 membrane bounded organelles and the ER network [69-71]. The ER—PM connection, a
21 conserved link observed across phylogeny, is one of the best known structures in this field.
22 Proteins involved in this ER—PM connection in plants have recently been discovered. Based
23 on the evidence we have to-date, we suggest that the plant EPCS are involved in
24 maintaining the ER network structure, involved in lipid transport and phospholipid signalling
25 using a pathway and molecular machinery that is similar to that in yeast and animal cells.
26 Because of the association with the cytoskeleton and possible involvement of actin
27 motor/regulator proteins, the EPCS in plant cells could play a central role in regulating
28 cortical membrane-cytoskeleton dynamics. Consequently, they may act as a hub that senses
29 various extracellular stimuli and signal [72], recruits various components that are required
30 for the response to pathogen infection and for viral movement and replication. Hopefully,

1 these hypotheses will be tested in the near future and the biological relevance of EPCS,
2 including their influence on membrane trafficking, cytoskeletal function, plant growth and
3 development will become clearer.

4 **Acknowledgements**

5 We thank Ulla Neumann and Angela Hay, MPIPZ, Cologne, for kindly providing the TEM
6 images of EPCS. This work was supported by a BBSRC grant (BB/G006334/1) and a
7 Leverhulme Trust grant (F/00382/G).

8 **References**

- 9 1. Hawes, C., et al. (2014) The endoplasmic reticulum: a dynamic and well-connected
10 organelle. *J Integr Plant Biol* 57, 50-62.
- 11 2. Boevink, P., et al. (1998) Stacks on tracks: the plant Golgi apparatus traffics on an
12 actin/ER network. *Plant J* 15, 441-447.
- 13 3. Sparkes, I., et al. (2009) Movement and remodeling of the endoplasmic reticulum in
14 nondividing cells of tobacco leaves. *Plant Cell* 21, 3937-3949
- 15 4. Avisar, D., et al. (2009) A comparative study of the involvement of 17 Arabidopsis
16 myosin family members on the motility of Golgi and other organelles. *Plant*
17 *physiology* 150, 700-709
- 18 5. Sparkes, I.A., et al. (2009) Grab a Golgi: laser trapping of Golgi bodies reveals in vivo
19 interactions with the endoplasmic reticulum. *Traffic (Copenhagen, Denmark)* 10,
20 567-571
- 21 6. Hepler PK., et al. (1990) Cortical endoplasmic reticulum in plants. *J Cell Sci* 96:355-
22 373.
- 23 7. Griffing, L.R., et al. (2014) ER network dynamics are differentially controlled by
24 myosins XI-K, XI-C, XI-E, XI-I, XI-1, and XI-2. *Frontiers in plant science* 5, 218
- 25 8. Stefano, G., et al. (2014) ER - the key to the highway. *Curr Opin Plant Biol* 22C, 30-38
- 26 9. Hughes, L., et al. (2013) Serial block face scanning electron microscopy--the future of
27 cell ultrastructure imaging. *Protoplasma* 251, 395-401
- 28 10. Wang, P., and Hussey, P.J. (2015). Interactions between plant endomembrane
29 systems and the actin cytoskeleton. *Frontiers in plant science* 6, 422.
- 30 11. Sparkes, I., et al. (2011) FrontiERs: movers and shapers of the higher plant cortical
31 endoplasmic reticulum. *Curr Opin Plant Biol* 14, 658-665
- 32 12. Hamada, T., et al. (2014) Microtubules contribute to tubule elongation and
33 anchoring of endoplasmic reticulum, resulting in high network complexity in
34 Arabidopsis. *Plant physiology* 166, 1869-1876
- 35 13. Stefan, C.J., et al. (2011) Osh proteins regulate phosphoinositide metabolism at ER-
36 plasma membrane contact sites. *Cell* 144, 389-401
- 37 14. Tavassoli, S., et al. (2013) Plasma membrane--endoplasmic reticulum contact sites
38 regulate phosphatidylcholine synthesis. *EMBO Rep* 14, 434-440

- 1 15. Loewen, C.J. & Levine, T.P. (2005) A highly conserved binding site in vesicle-
2 associated membrane protein-associated protein (VAP) for the FFAT motif of lipid-
3 binding proteins. *J Biol Chem* 280, 14097-14104.
- 4 16. Mikitova, V. & Levine, T.P. (2012) Analysis of the key elements of FFAT-like motifs
5 identifies new proteins that potentially bind VAP on the ER, including two AKAPs and
6 FAPP2. *PLoS one* 7, e30455.
- 7 17. Jing, J., et al. (2015) Proteomic mapping of ER-PM junctions identifies STIMATE as a
8 regulator of Ca(2+) influx. *Nat Cell Biol* 17, 1339-1347.
- 9 18. Giordano, F. et al. (2013) PI(4,5)P(2)-dependent and Ca(2+)-regulated ER-PM
10 interactions mediated by the extended synaptotagmins. *Cell* 153, 1494-1509.
- 11 19. Carrasco, S. & Meyer, T. (2011) STIM proteins and the endoplasmic reticulum-
12 plasma membrane junctions. *Annu Rev Biochem* 80, 973-1000.
- 13 20. Deak, A.T. et al. (2013) The endocannabinoid N-arachidonoyl glycine (NAGly) inhibits
14 store-operated Ca²⁺ entry by preventing STIM1-Orai1 interaction. *J Cell Sci* 126, 879-
15 888.
- 16 21. Quintana, A. et al. (2015) TMEM110 regulates the maintenance and remodeling of
17 mammalian ER-plasma membrane junctions competent for STIM-ORAI signaling.
18 *Proc Natl Acad Sci U S A* 112, E7083-7092.
- 19 22. Dingsdale, H., et al. (2013) Saltatory formation, sliding and dissolution of ER-PM
20 junctions in migrating cancer cells. *Biochem J* 451, 25-32.
- 21 23. Manford, A.G., et al. (2012) ER-to-plasma membrane tethering proteins regulate cell
22 signaling and ER morphology. *Dev Cell* 23, 1129-1140.
- 23 24. Wang, P., et al. (2014) The plant cytoskeleton, NET3C, and VAP27 mediate the link
24 between the plasma membrane and endoplasmic reticulum. *Curr Biol* 24, 1397-1405.
- 25 25. Wang, P., et al. (2016) Plant VAP27 proteins: domain characterization, intracellular
26 localization, and role in plant development. *New Phyt*, DOI: 10.1111/nph.13857.
- 27 26. Hawkins, T.J., et al. (2014) The evolution of the actin binding NET superfamily.
28 *Frontiers in plant science* 5, 254
- 29 27. Deeks, M.J., et al. (2012) A superfamily of actin-binding proteins at the actin-
30 membrane nexus of higher plants. *Curr Biol* 22, 1595-1600.
- 31 28. Perez-Sancho, J., et al. (2015) The Arabidopsis synaptotagmin1 is enriched in
32 endoplasmic reticulum-plasma membrane contact sites and confers cellular
33 resistance to mechanical stresses. *Plant physiology* 168, 132-143.
- 34 29. Levy, A., et al. (2015) Synaptotagmin SYTA Forms ER-Plasma Membrane Junctions
35 that Are Recruited to Plasmodesmata for Plant Virus Movement. *Curr Biol* 25, 2018-
36 2025.
- 37 30. Siao, W., et al. (2016) Arabidopsis SYT1 Maintains Stability of Cortical ER Networks
38 and VAP27-1 Enriched ER-PM Contact Sites. *J Exp Bot*, doi: 10.1093/jxb/erw381.
- 39 31. Skirpan, A.L., et al. (2006) Identification and characterization of PiORP1, a Petunia
40 oxysterol-binding-protein related protein involved in receptor-kinase mediated

- 1 signaling in pollen, and analysis of the ORP gene family in Arabidopsis. *Plant Mol Biol*
2 61, 553-565.
- 3 32. Saravanan, R.S. et al., (2009) The targeting of the oxysterol-binding protein ORP3a to
4 the endoplasmic reticulum relies on the plant VAP33 homolog PVA12. *Plant J* 58,
5 817-830.
- 6 33. McFarlane, H.E., et al., (2010) Arabidopsis ABCG transporters, which are required for
7 export of diverse cuticular lipids, dimerize in different combinations. *Plant Cell* 22,
8 3066-3075.
- 9 34. Zhang, D., et al. (2015) ER-PM Contacts Define Actomyosin Kinetics for Proper
10 Contractile Ring Assembly. *Curr Biol*.
- 11 35. Golomb, L., et al. (2008) Different subcellular localizations and functions of
12 Arabidopsis myosin VIII. *BMC Plant Biol* 8, 3.
- 13 36. Amari, K., et al. (2014) Myosins VIII and XI play distinct roles in reproduction and
14 transport of tobacco mosaic virus. *PLoS pathogens* 10, e1004448
- 15 37. Martiniere, A., et al., (2011) Building bridges: formin1 of Arabidopsis forms a
16 connection between the cell wall and the actin cytoskeleton. *Plant J* 66, 354-365 .
- 17 38. McKenna, J.F., et al. (2014) Across the great divide: the plant cell surface continuum.
18 *Curr Opin Plant Biol* 22, 132-140.
- 19 39. Li, Y., et al. (2010) The type II Arabidopsis formin14 interacts with microtubules and
20 microfilaments to regulate cell division. *Plant Cell* 22, 2710-2726.
- 21 40. Deeks, M.J., et al. (2010) The plant formin AtFH4 interacts with both actin and
22 microtubules, and contains a newly identified microtubule-binding domain. *J Cell Sci*
23 123, 1209-1215.
- 24 41. Deeks, M.J. & Hussey, P.J. (2005) Arp2/3 and SCAR: plants move to the fore. *Nat Rev*
25 *Mol Cell Biol* 6, 954-964.
- 26 42. Dyachok, J., et al. (2008) Plasma membrane-associated SCAR complex subunits
27 promote cortical F-actin accumulation and normal growth characteristics in
28 Arabidopsis roots. *Molecular plant* 1, 990-1006.
- 29 43. Dyachok, J., et al. (2011) SCAR mediates light-induced root elongation in Arabidopsis
30 through photoreceptors and proteasomes. *Plant Cell* 23, 3610-3626.
- 31 44. Facette, M.R., et al. (2015) The SCAR/WAVE complex polarizes PAN receptors and
32 promotes division asymmetry in maize. *Nat. Plants* 1, 14024. doi:10.1038/nplants.
- 33 45. Wang, P., et al. (2016) Arabidopsis NAP1 Regulates the Formation of
34 Autophagosomes. *Curr Biol*, doi:10.1016/j.cub.2016.06.008.
- 35 46. Maisch, J., et al. (2009) Tobacco Arp3 is localized to actin-nucleating sites in vivo. *J*
36 *Exp Bot* 60, 603-614.
- 37 47. Inada, N. & Ueda, T. (2014) Membrane trafficking pathways and their roles in plant-
38 microbe interactions. *Plant Cell Physiol* 55, 672-686.
- 39 48. Henty-Ridilla, J.L., et al. (2013) The plant actin cytoskeleton responds to signals from
40 microbe-associated molecular patterns. *PLoS pathogens* 9, e1003290.

- 1 49. Takemoto, D., et al. (2003) GFP-tagging of cell components reveals the dynamics of
2 subcellular re-organization in response to infection of Arabidopsis by oomycete
3 pathogens. *Plant J* 33, 775-792.
- 4 50. Takemoto, D., et al. (2006) Re-organization of the cytoskeleton and endoplasmic
5 reticulum in the Arabidopsis *pen1-1* mutant inoculated with the non-adapted
6 powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei*. *Mol Plant Pathol* 7, 553-
7 563.
- 8 51. Genre, A., et al. (2008) Prepenetration apparatus assembly precedes and predicts
9 the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of
10 both *Medicago truncatula* and *Daucus carota*. *Plant Cell* 20, 1407-1420
- 11 52. Lu, Y.J. et al. (2012) Patterns of plant subcellular responses to successful oomycete
12 infections reveal differences in host cell reprogramming and endocytic trafficking.
13 *Cell Microbiol* 14, 682-697.
- 14 53. Chakrabarti, A., et al. (2015) A mutational analysis of the cytosolic domain of the
15 tomato Cf-9 disease-resistance protein shows that membrane-proximal residues are
16 important for Avr9-dependent necrosis. *Mol Plant Pathol*.
- 17 54. Rivas, S., et al. (2002) The Cf-9 disease resistance protein is present in an
18 approximately 420-kilodalton heteromultimeric membrane-associated complex at
19 one molecule per complex. *Plant Cell* 14, 689-702.
- 20 55. Petersen, N.H., et al. (2009) Identification of proteins interacting with Arabidopsis
21 ACD11. *J Plant Physiol* 166, 661-666.
- 22 56. Fitzgibbon, J., et al. (2010) Super-resolution imaging of plasmodesmata using three-
23 dimensional structured illumination microscopy. *Plant physiology* 153, 1453-1463.
- 24 57. Knox, K., et al. (2015) Putting the Squeeze on Plasmodesmata: A Role for Reticulons
25 in Primary Plasmodesmata Formation. *Plant physiology* 168, 1563-1572.
- 26 58. Kriechbaumer, V., et al. (2015) Reticulomics: Protein-Protein Interaction Studies with
27 Two Plasmodesmata-Localized Reticulon Family Proteins Identify Binding Partners
28 Enriched at Plasmodesmata, Endoplasmic Reticulum, and the Plasma Membrane.
29 *Plant physiology* 169, 1933-1945.
- 30 59. Pena, E.J. & Heinlein, M. (2013) Cortical microtubule-associated ER sites:
31 organization centers of cell polarity and communication. *Curr Opin Plant Biol* 16,
32 764-773.
- 33 60. Barajas, D., et al. (2014) Co-opted oxysterol-binding ORP and VAP proteins channel
34 sterols to RNA virus replication sites via membrane contact sites. *PLoS pathogens* 10,
35 e1004388.
- 36 61. Carette, J.E. et al. (2002) Characterization of plant proteins that interact with cowpea
37 mosaic virus '60K' protein in the yeast two-hybrid system. *J Gen Virol* 83, 885-893.
- 38 62. Lewis, J.D. & Lazarowitz, S.G. (2010) Arabidopsis synaptotagmin SYTA regulates
39 endocytosis and virus movement protein cell-to-cell transport. *Proc Natl Acad Sci U S*
40 *A* 107, 2491-2496.

- 1 63. Hamada, T., et al. (2012) RNA processing bodies, peroxisomes, Golgi bodies,
2 mitochondria, and endoplasmic reticulum tubule junctions frequently pause at
3 cortical microtubules. *Plant Cell Physiol* 53, 699-708.
- 4 64. Crowell, E.F., et al. (2009) Pausing of Golgi bodies on microtubules regulates
5 secretion of cellulose synthase complexes in *Arabidopsis*. *Plant Cell* 21, 1141-1154.
- 6 65. Stefano G., et al. (2015) ER network homeostasis is critical for plant endosome
7 streaming and endocytosis. *Cell Discovery* 1, 15033.
- 8 66. Guimaraes, S.C., et al. (2015) Peroxisomes, lipid droplets, and endoplasmic reticulum
9 "hitchhike" on motile early endosomes. *J Cell Biol* 211, 945-954.
- 10 67. Alpy, F., et al. (2013) STARD3 or STARD3NL and VAP form a novel molecular tether
11 between late endosomes and the ER. *J Cell Sci* 126, 5500-5512.
- 12 68. Dong, R., et al. (2016) Endosome-ER Contacts Control Actin Nucleation and Retromer
13 Function through VAP-Dependent Regulation of PI4P. *Cell* 166, 408-423
- 14 69. Griffing, L.R., et al. (2016) Plant ER geometry and dynamics: biophysical and
15 cytoskeletal control during growth and biotic response. *Protoplasma*.
- 16 70. Levine, T. & Loewen, C. (2006) Inter-organelle membrane contact sites: through a
17 glass, darkly. *Curr Opin Cell Biol* 18, 371-378.
- 18 71. Phillips, M.J., and Voeltz, G.K. (2016) Structure and function of ER membrane contact
19 sites with other organelles. *Nat Rev Mol Cell Biol* 17, 69-82.
- 20 72. Ho, C.M., et al. (2016) Modulators of Stomatal Lineage Signal Transduction Alter
21 Membrane Contact Sites and Reveal Specialization among ERECTA Kinases. *Dev Cell*
22 38, 345-357.

23

24

1

2 **Figure legends**3 **Table 1. Known ER—PM contact site proteins in yeast, animals, and plants.**

Known ER—PM proteins in different species			Notes	Refs
Yeast	Animal	Plant		
n/a	STIM1/Orai1	n/a	ER localised STIM1 interact with a PM calcium channel, Orai1, regulating calcium influx	[17,19,20,22]
n/a	STIMATE/TMEM110	n/a	Interact with STIM1 and regulate calcium signalling	[18,21]
Scs2,Scs22	VAP-A,VAP-B	VAP27-1,VAP27-3	Interact with various lipid interacting proteins and mediate lipid transport	[14,16,24,25]
Osh	ORP	ORP	It binds to oxysterol., and also regulates PI4P metabolism	[13,15,31,32]
Tcb1-3	E-Syt	Syt1	It brings ER close to the PM in response to Calcium signal	[23,28-30,62]
Ist2p	n/a	n/a	Cortical ER protein involved in ER—PM tethering, and regulates cellular ion homeostasis	[23]
n/a	n/a	NET3C	Plant specific NET family, and interact with actin cytoskeleton	[24-27]

4

5 **Key Figure 1. Summary of possible functions of the plant ER—PM contact sites in plants,**
6 **and the localization of known EPCS candidates. (A)** Multiple functions of plant EPCS are
7 proposed. EPCS may be involved in various cellular processes, such as membrane trafficking.
8 Specific cargos may be transported through EPCS via Golgi bodies (1), and EPCS could also
9 provide a route for endocytosis (2). EPCS are also likely to be involved in regulating the
10 calcium signalling pathway (3), controlling viral movement through EPCS-PD interactions (4)
11 and mediating responses to extracellular stimuli such as biotic stresses (5). **(B)** Transmission
12 electron micrograph depicting a 70nm section of an outer integument cell of a developing
13 *Cardamine hirsuta* seed (fruit development stage 15) after high pressure freezing and freeze

1 substitution. Numerous ER-plasma membrane contact sites can be seen all around the cell
2 circumference. (1, 2) High magnification images show that ER and plasma membrane are in
3 close physical contact and that contact sites can stretch over several hundreds of
4 nanometers. Note the absence of ribosomes on the ER surface facing the plasma membrane.
5 Images courtesy of Ulla Neumann and Angela Hay, MPIPZ, Cologne. **(C-E)** Subcellular
6 localization of selected EPCS proteins in plants is illustrated. These include VAP27-1 (B),
7 NET3C (C), and synaptotagmin, all of which label persistent ER nodules known to be ER—PM
8 contact sites [24, 25, 30]. **(F-G) Images showing the co-localization of NET3C with SYT1 and**
9 **VAP27-1 respectively; NET3C and VAP27 localize to the same sites, whereas there is only**
10 **partial over-lap of NET3C and SYT1 [30]** (Scale Bar = 10µm for confocal; 0.5 µm for TEM).

11

12 **Figure 2. Plant EPCS associate with the cytoskeleton. (A-C)** EPCS are found closely
13 associated with the cytoskeleton. The localization of VAP27-1 labelled EPCS often follows
14 the pattern of microtubules especially in mature trichome cells (A). Co-alignment between
15 EPCS and the actin cytoskeleton is also significant (B), and a number of EPCS locate to the
16 point where actin filaments and microtubules intersect (arrow, C) [26].

17

18 **Figure 3. Suggested models and possible functions of plant EPCS. (A)** Diagram illustrating
19 the interaction of plant EPCS and the cytoskeleton. EPCS complexes interact with
20 microtubules directly where they may recruit certain actin regulatory proteins and regulate
21 actin organization. **(B)** EPCS localise to the oomycete haustorium, as a consequence, dense
22 ER and actin filaments are found at these sites. **(C)** ER/PM contact sites (VAP27-1-YFP, red)
23 have been found within the hechtian strands (green) after plasmolysis, most of which were
24 located at the tips of the strands indicating they are connected to the cell wall. **(D)** In plants,
25 Golgi bodies can interact with EPCS transiently, and this process may be involved in
26 transporting specific cargos. Furthermore, endocytosis could also occur around EPCS in
27 plants. The EPCS associated actin filaments provide the force for PM invagination and the ER
28 membrane provide the track for endosome movement (Scale Bar = 10µm).

29

Glossary

ER—PM contact sites (EPCS)

Also known as ER—PM junctions or ER—PM anchor sites, they are close appositions between the two structures. They exist in different species, and their main functions studied so far are lipid transport and calcium homeostasis.

VAP27 Protein

It is known as Scs2 in yeast and VAP in animal cells, all of which localise to EPCS. There are 10 homologues of VAP27 in arabidopsis, and all contain a Major Sperm Domain, which is believed to interact with proteins containing a FFAT motif.

Synaptotagmins (SYT)

They were first known as a Ca^{2+} sensor in the membrane of the pre-synaptic axon terminal in animals. They contain a C-terminal C2-domain, which binds to phospholipids in response to calcium signals. There are 5 homologues found in the arabidopsis genome, and SYT1 has been shown to localise to EPCS.

NET proteins

NETWORKED super-family of actin binding proteins, found specifically in plants. Members of this family form links between actin filaments and different membrane systems.

STIM1/Orai1

This protein complex is required for Ca^{2+} transport in animal cells. The ER localized STIM1 interacts with the PM Ca^{2+} channel, Orai1, when the Ca^{2+} levels in the ER is low. However, these proteins do not exist in plants.

SCAR and ARP2/3 complexes

ARP2/3 complex mediates actin nucleation, and its activity is regulated by the SCAR complex that contains multiple proteins, such as NAP1 and PIR121. This machinery is conserved in most eukaryotes.

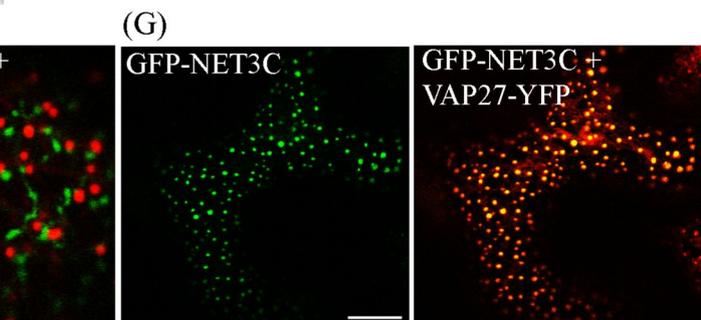
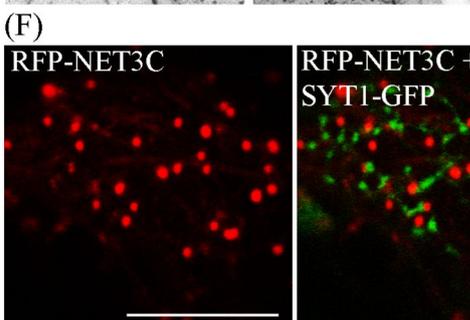
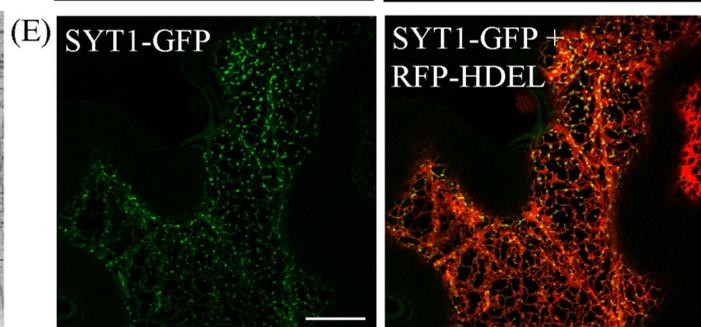
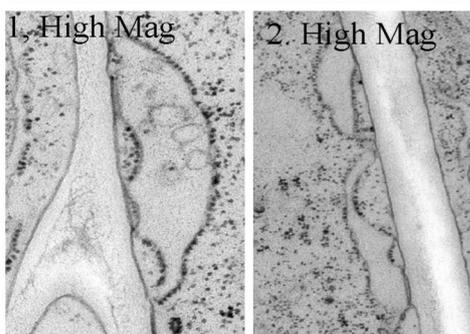
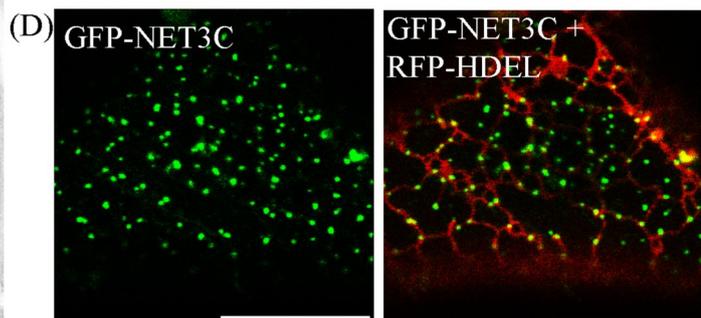
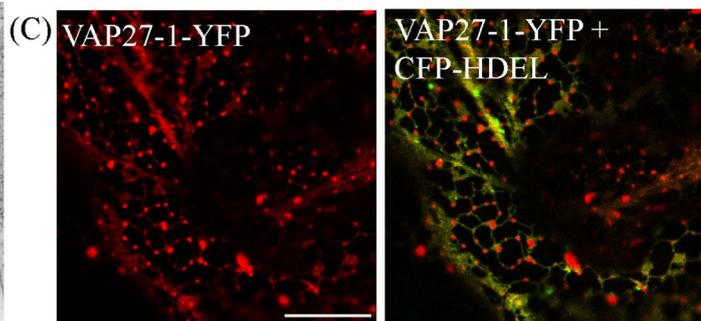
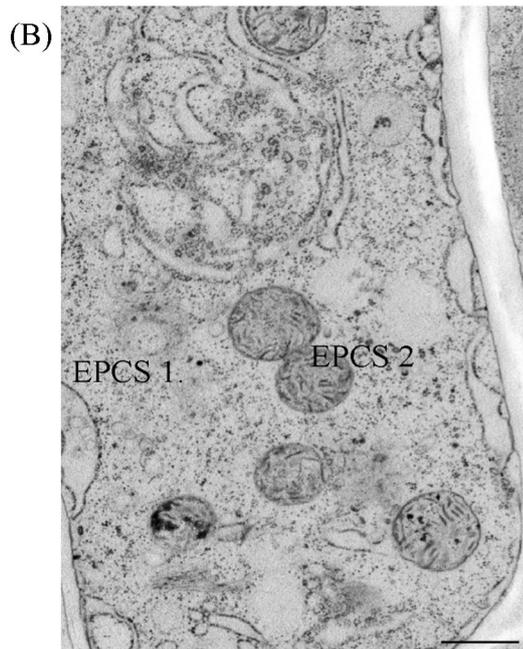
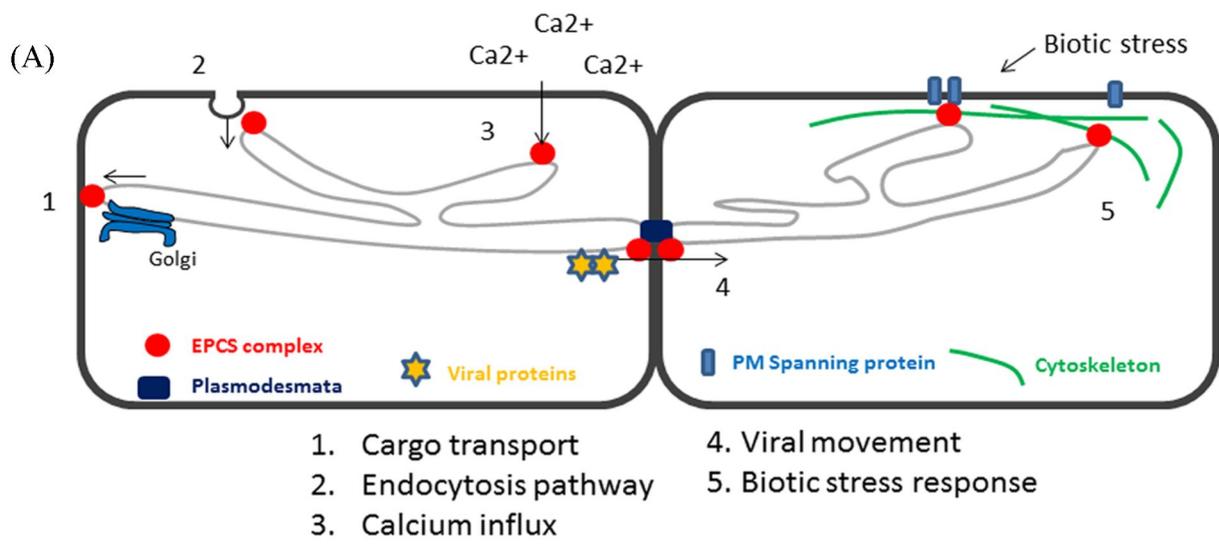
Plasmodesmata

Plasmodesmata (PD) are a unique feature of plant cells forming channels that mediate cell-to-cell transport.

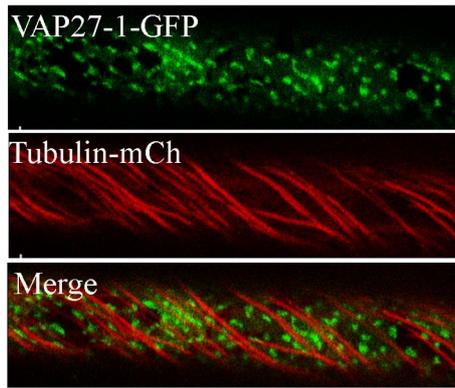
C-MERs

Cortical microtubule-associated ER sites, they intersect with the ER-actin network and mark the position of pausing organelles. Most of them are stable and structurally similar to EPCS. The association of microtubules at the C-MER places it adjacent to, but not directly on the EPCS.

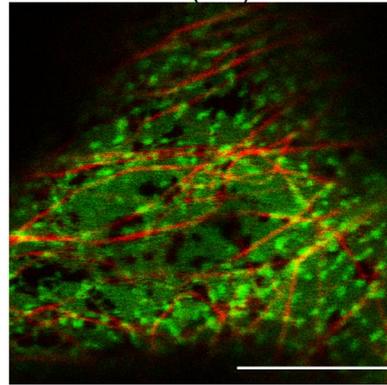
1
2
3
4



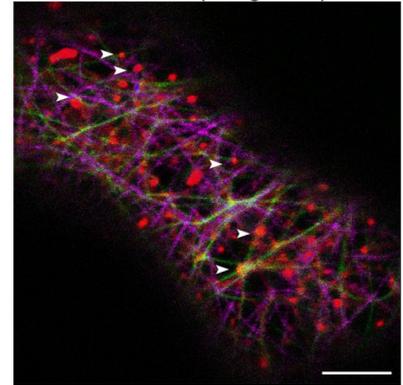
(A)

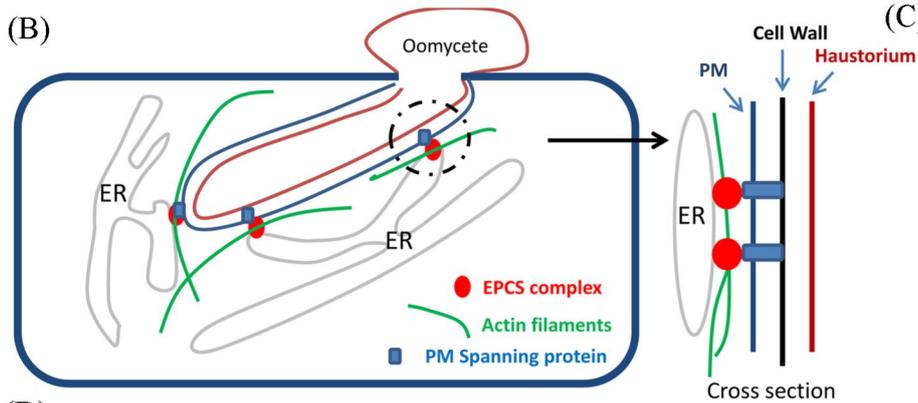
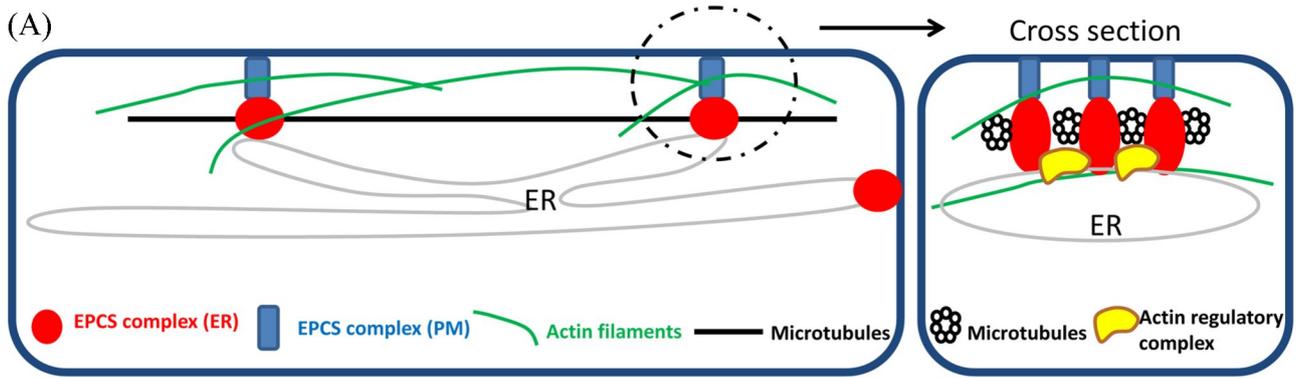


(B) VAP27-1-GFP (green)
+ RFP-Lifeact (red)



(C) VAP27-1 (red) + F-actin (green)
+ Microtubules (Magenta)





(C) PIP2-CFP (Hechtian strands, green) + VAP27-1-YFP (EPCS, red)

