Components of the SNARE-containing regulon are co-regulated in root cells undergoing defense

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ABSTRACT
The term regulon has been coined in the genetic model plant Arabidopsis thaliana, denoting a structural and physiological defense apparatus defined genetically through the identification of the penetration (pen) mutants. The regulon is composed partially by the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) syntaxin PEN1. PEN1 has homology to a Saccharomyces cerevisiae gene that regulates a Secretion (Sec) protein, Suppressor of Sec 1 (Sso1p). The regulon is also composed of the β-glucosidase (PEN2) and an ATP binding cassette (ABC) transporter (PEN3). While important in inhibiting pathogen infection, limited observations have been made regarding the transcriptional regulation of regulon genes until now. Experiments made using the model agricultural Glycine max (soybean) have identified co-regulated gene expression of regulon components. The results explain the observation of hundreds of genes expressed specifically in the root cells undergoing the natural process of defense. Data regarding additional G. max genes functioning within the context of the regulon are presented here, including Sec 14, Sec 4 and Sec 23. Other examined G. max homologs of membrane fusion genes include an endosomal bromo domain-containing protein1 (Bro1), syntaxin6 (SYP6), SYP131, SYP71, SYP8, Bet1, coatomer epsilon (ε-COP), a coatomer zeta (ζ-COP) paralog and an ER to Golgi component (ERGIC) protein. Furthermore, the effectiveness of biochemical pathways that would function within the context of the regulon have been examined, including xyloglucan xylosyltransferase (XXT), reticuline oxidase (RO) and galactinol synthase (GS). The experiments have unveiled the importance of the regulon during defense in the root and show how the deposition of callose relates to the process.

Introduction
The ability of eukaryotic membranes to fuse is important to many biological processes, including secretion.1 Owing to its importance, the genetically regulated process is ancient with gene homologs found in all eukaryotes.2 Subsequent biochemical experiments have determined the affinity that these proteins have for each other under various conditions.3,4 These experiments have provided foundational knowledge while also revealing new roles for some of the proteins. In A. thaliana, the secretory apparatus functions in defense responses.5 Furthermore, this secretion system has been expanded to include structural, biochemical and physiological components and is referred to as a regulon, a binary system composed of two parallel pathways converging on defense.6,7,8 Subsequent experiments have shown that the regulon components have the ability to coordinately regulate their expression (co-regulate) other regulon genes.7,9 In the review presented here, details are provided that describe the components of the regulon. The review examines how observations first made over a century ago regarding ecological plant variants capable of warding off pathogens have provided clues as to how the plant defense process functions.10 The review subsequently intercalates more recent genetic evidence that puts those original ecological observations into the context of the regulon.5,6,8-12 The review describes co-regulation of the regulon genes. The review then describes the identification of other genes expressed in the cells undergoing the process of defense that would be expected to function within the regulon platform.

Early observations of a genetic basis for the regulon
Observations made over a century ago identified the capability of certain ecological variants of the legume Trifolium repens to engage a successful defense response in their shoot that acts against various herbivores.10 This genetic system is defined by two loci.10-12 One diploid parent provides the dominant allele (Ac) driving α-hydroxynitrile glycoside production and one parent provides a dominant allele (Li) encoding for its hydrolyzing α-hydroxynitrile glycosidase.10-12 These observations implicate two different modes of secretion would converge during the process of defense because
α-hydroxynitrite glycosidases have a signal peptide allowing it to enter the secretion system while glycosides can be mobilized by ABC transporters.\textsuperscript{13,14} Subsequent experiments performed in the plant genetic model \textit{A. thaliana} have led to the identification of this genetic framework, referred to as a regulon.\textsuperscript{6} The framework of the regulon is defined by three genes including the syntaxin \textit{PENETRATION1} (\textit{PEN1}), \textit{β}-glucosidase (\textit{PEN2}) and an ATP binding cassette (ABC) transporter (\textit{PEN3}) (Fig. 1).\textsuperscript{5,6,15,16}

Knowledge of how regulon components function clearly implicated other genes would have roles within the context of the regulon. Using the \textit{Glycine max-Heterodera glycines} pathosystem as an experimental model, Klink et al.\textsuperscript{17} performed gene expression studies examining RNA isolated from \textit{G. max} root cells parasitized by \textit{H. glycines}. The experiments led to the determination of gene expression patterns in cells undergoing the process of defense. In those and related studies, Klink et al.\textsuperscript{17-21} identified that the defense apparatus may be quite extensive, possibly composed of hundreds to thousands of genes. Experiments then examined gene expression patterns occurring at the major \textit{H. glycines} resistance locus, \textit{resistance to heterodera glycines 1} (\textit{rhg1}).\textsuperscript{22,23} Transcriptional mapping studies of the \textit{rhg1} locus have identified \textit{α}-SNAP/\textit{Sec} 17 as a highly expressed component.\textsuperscript{23,24} \textit{α}-SNAP is homologous to the \textit{Saccharomyces cerevisiae} \textit{Secretion} (\textit{Sec}) gene, \textit{Sec} 17 (\textit{α}-SNAP/\textit{Sec} 17) that functions in secretion.\textsuperscript{1} In \textit{S. cerevisiae}, mutants of \textit{Sec} 17 (\textit{sec} 17), accumulate 50 nm vesicles that cannot fuse with a target membrane leading to the failure of the cells to transport the cargo protein carboxypeptidase Y.\textsuperscript{1,25} This result demonstrates that \textit{α}-SNAP/\textit{Sec} 17p functions in membrane fusion during anterograde transport. Invaluable to the study of the \textit{rhg1} locus in \textit{G. max} has been the availability of the genetic mapping data and its sequenced genome.\textsuperscript{22,26-29} Subsequent work has shown the \textit{rhg1} locus contains multiple copies of \textit{α}-SNAP/\textit{Sec} 17, but no clear understanding of a defense role had been obtained.\textsuperscript{30,31}

However, Matsye et al.\textsuperscript{23,24} and Sharma et al.\textsuperscript{9} demonstrated a clear role for \textit{Gm-α}-SNAP in defense of \textit{G. max} to \textit{H. glycines} parasitism. The identification of \textit{α}-SNAP/\textit{Sec} 17 as a resistance gene in \textit{G. max} strengthens the observation that identified SNARE functioning in defense in the plant genetic model \textit{A. thaliana}.\textsuperscript{5,9,24}

**Genetic identification of the importance of secretion**

Secretion is an orderly stepwise process that has been demonstrated through the genetic identification of the \textit{Sec} and related genes in \textit{S. cerevisiae} (Fig. 2).\textsuperscript{1,32-35} Functional equivalents (homologs) subsequently have been identified in all eukaryotes.\textsuperscript{2,36-43} A core set of proteins, known as Soluble NSF Attachment Protein REceptor (SNARE) is one macromolecular part of the membrane fusion apparatus (Fig. 2). The components of SNARE include syntaxin (\textit{SYP})/\textit{Suppressor of sec} 1 (\textit{SSO1}), a gene homologous to \textit{A. thaliana} \textit{PEN1}. 5 Other SNARE components include synaptobrevin (\textit{SYB})/\textit{YKT6}/\textit{SEC22} and \textit{SNAP-25/SEC9}.\textsuperscript{44-49} The \textit{SNARE} proteins tether the vesicle to the target membrane. Mammalian uncoordinated-18 (\textit{MUNC18/SEC1}), also known as SM, may inhibit or facilitate fusion.\textsuperscript{50,51} Synaptotagmin (\textit{SYT})/\textit{Tricalbin}-3 (\textit{TCB3}) is believed to serve as a calcium sensor. SNARE metabolism, including its disassembly is mediated by two additional proteins including \textit{α}-SNAP/\textit{Sec} 17p and the ATPase N-ethylmaleimide-sensitive factor (\textit{NSF})/\textit{Sec} 18p.\textsuperscript{1,3} The entire SNARE complex can be biochemically isolated as part a larger 20 S particle, including \textit{α}-SNAP/\textit{Sec} 17p and \textit{NSF}/\textit{Sec} 18p, that mediates secretion.\textsuperscript{52,53} Complimentary studies in animal systems investigating pathogenesis have identified botulinum and tetanus microbial neurotoxin effectors that target SNARE components and thus inhibit secretion.\textsuperscript{54-61} The effect of the neurotoxins is paralysis. Similar types of effectors are also being identified in plants leading to impaired functionality of 20 S components during defense, confirming its importance in the process of defense.\textsuperscript{62,63}
**α-SNAP/Sec 17 relates to plant defense by its association with SNARE during membrane fusion**

The observation that Gm-α-SNAP functions in G. max defense to H. glycines has introduced questions regarding its activity. The observations that have been made regarding α-SNAP/Sec 17p is that it possesses diverse roles based on the cellular milieu. Through biochemical experiments Söllner et al. have identified a number of SNARE-related proteins that are specific for a certain vesicle or target membrane, but requires α-SNAP/Sec 17p for fusion. This work has indicated α-SNAP/Sec 17p is a universal component of constitutive and regulated membrane fusion with variations in SNARE composition dictating specificity. In examining neuronal exocytosis, Barszczewski et al. have identified clusters of α-SNAP/Sec 17p at the plasma membrane revealing the addition of NSF/Sec 18p facilitates exocytosis. Schwartz and Merz have shown α-SNAP/Sec 17p can rescue a SNARE complex that is stalled in its ability to complete fusion, identifying the central role of α-SNAP/Sec 17p in membrane fusion. Lobinger et al. have examined α-SNAP/Sec 17p further. The experiments have demonstrated α-SNAP/Sec 17p, along with Sec 1p (Munc18) (SM), actually accelerates fusion, identifying the central role of α-SNAP/Sec 17p for fusion. This work has indicated α-SNAP/Sec 17p has multiple functions prior to, during and after the process of membrane fusion and that it works at all sites of fusion of membrane-bound structures that utilize SNARE.

**Regulon components homologous to the SNARE component PEN1 exhibit co-regulation**

A number membrane-bound structures exist within the vesicle transport system. These structures include the endoplasmic reticulum (ER), conserved oligomeric Golgi (COG) complex, trans-Golgi network/early endosome (TGN/EE), homotypic fusion and protein sorting (HOPS) complex, the class C core vacuole/endosome tethering (CORVET), exocyst, trafficking protein particle (TRAPP) I–III complexes, Golgi-associated retrograde protein (GARP) complex, endosome-associated retrograde protein (EARP) complex, depends on SLY1-20 (Dsl1) complex and plasma membrane (PM). For details, please refer to Vukašinović and Zárský. These structures utilize different macromolecular protein complexes to facilitate their interactions and fusion events.

SNARE, benefitting from having the longest experimental history, has had its core components identified decades ago (Fig. 2). While the proteins function effectively to mediate fusion, comparatively little is understood regarding whether the genes influence each other’s expression (co-regulation). An early attempt made in S. cerevisiae attempted to understand whether the SNARE genes exhibited co-regulation. In this study, it has been shown that sec mutants did not exhibit coordinated suppressed transcriptional activity of the other Sec genes. However, technical limits of the time may have complicated such observation. In contrast, engineering SNARE components for constitutive induced expression has been shown to lead to induced expression of other SNARE components. Furthermore, experiments in other biological systems have shown α-COP overexpression can overcome a
temperature-dependent suppression of α-COP expression.\textsuperscript{76} Similar results have been shown for the ER to Golgi component (ERGIC).\textsuperscript{77} The observation of co-regulated gene expression of vesicle fusion components has held up to genomics-level scrutiny.\textsuperscript{78} These observations are consistent with those made in the \textit{G. max-H. glycines} pathosystem.\textsuperscript{79} The question in relation to SNARE became how extensive is co-regulated gene expression during plant defense?

\textbf{Regulon components homologous to \(\beta\)-glucoside PEN2 exhibit co-regulation}

Experiments have been presented showing that the co-regulation of defense-related genes is not limited to the vesicle transport system components. Work done in \textit{Lotus japonicus} has shown that \textit{LjBGD7}, a root expressed PEN2 \(\beta\)-glucosidase homolog, is related to \(\alpha\)-hydroxynitrile glucosidase.\textsuperscript{79} \textit{LjBGD7} acts to produce hydrogen cyanide (HCN), functioning effectively in defense.\textsuperscript{77} Furthermore, \textit{LjBGD7} is co-regulated with the cytochrome \textit{P450} protein \textit{LjCYP79D4}.\textsuperscript{79} In \textit{L. japonicus}, \textit{LjCYP79D4} has increased relative levels of expression exclusively in the roots where \textit{LjBGD7} occurs.\textsuperscript{79} In related studies, Morant et al.\textsuperscript{80} demonstrated the co-expression of \(\alpha\)-hydroxynitrile glucoside and their cognate hydrolyzing \(\alpha\)-hydroxynitrile glucosidase. Furthermore, the heterologous expression of a \textit{Manihot esculenta} (cassava) CYP79D2 in \textit{L. japonicus}, results in cyanogenic \(\alpha\)-hydroxynitrile glucoside accumulation.\textsuperscript{79} The experiments have clearly identified that genes functioning in \(\alpha\)-hydroxynitrile glucoside production and metabolism are co-regulated.

\textbf{Regulon components homologous to the ABC transporter PEN3 exhibit co-regulation}

The ATP binding cassette (ABC) transporter regulon component PEN3 delivers metabolites through various membranes. While comparatively little is known about ABC transporters in plants, work in animal systems have demonstrated they are capable of co-regulating the expression of other genes relating to their own function.\textsuperscript{81,82} However, while evidence exists for the ability of the regulon components to effect co-regulation the scope and breadth had not been examined in detail. The co-regulation of pathway components leading to the production of secondary metabolites is not limited to PEN2. For example, The \textit{Nicotiana benthamiana} terpene synthases \textit{TPS10} and \textit{TPS14} are co-regulated with the two cytochrome \textit{P450s} (CYP71B31 and CYP76C3) in \textit{flowers at anthesis}.\textsuperscript{83} Furthermore, the protein products are found in the endomembrane system resulting in monoterpenes alcohol linalool production.\textsuperscript{83} Therefore, it is likely that other secondary metabolite pathways are important to defense in the \textit{G. max-H. glycines} pathosystem (Fig. 3).

\textbf{Callose is a structural defense element whose genetic components exhibit co-regulation}

The recent identification of callose existing at sites of resistance in \textit{G. max} roots parasitized by incompatible \textit{H. glycines} is consistent with numerous observations of its presence at defense sites in many plant pathosystems.\textsuperscript{9,84-110} These studies have provided extensive support that callose performs an active role in defense. Consistent with these observations, other functional studies have demonstrated the active role callose plays as it participates in defense.\textsuperscript{111-117} However, in contrast, very limited studies have shown that callose may not accumulate to appreciable levels at defense sites or participate in defense at all.\textsuperscript{118,119}

Callose is a glucose-derived polysaccharide polymerized mainly by callose synthase (CaLS) or GLUTAN SYNTHASE–LIKE (GSL) enzymes, forming \(\beta\)-1,3- and lesser amounts of \(\beta\)-1,6-branches. Conversely, \(\beta\)-1,3-glucanases depolymerize callose. During defense, plants deposit a plate-like structure referred to as a cell wall apposition that is also known as a papillae. It is these papillae that contain callose, among other materials, that are thought to provide a physical barrier to the establishment of an invasion site for the pathogen.\textsuperscript{120} The development of papillae at the molecular level requires the vesicle transport system.\textsuperscript{115} For example, in \textit{A. thaliana}, papillae formation as a consequence of infection by \textit{Blumeria graminis} f.sp. \textit{hordei} is mediated by PEN1.\textsuperscript{115} The transport of callose has been suggested to occur through multivesicular bodies. However, conclusive evidence has not presented.\textsuperscript{114,115,120} These observations directly link vesicle transport to the delivery of callose at infection sites, consistent with the observations of Sharma et al. 9 in the \textit{G. max-H. glycines} pathosystem.

\textbf{Co-regulated gene expression occurring during defense is extensive}

The regulon is likely to be an apparatus having a broad structural, biochemical and physiological basis. This concept is supported through gene expression experiments. For example, gene expression experiments examining nematode-parasitized cells undergoing a defense response identified many expressed genes that would be defense candidates in \textit{G. max} that would be predicted to associate with the regulon (Fig. 4; Table 1; Table S1).\textsuperscript{23}
To examine this concept further, experiments have been done examining candidate defense genes functioning in different defense pathways. These genes include *G. max* homologs of Sec14-1, Sec4-6, Sec23-5, Bro1-1, SYP6-1, SYP71-6, SYP8-2, Bet1-1, ε-COP-1, ζ-COP-3 and ERGIC-3-3. Genetic pathways that associate with the regulon have also been examined, including those incorporating reticulon oxidase-40 (Fig. 5; Table S2), xyloglucan xylosyltransferase 2-5 (Fig. 6; Table S2) and galactinol synthase-3 (Fig. 7; Table S2). As a control to show the expression of any gene will not result in engineered resistance, we have selected a *G. max* homolog of the *A. thaliana* APETALA2 (*AP2*) Gm-Apetala2-1 (Glyma01g44130). In *A. thaliana*, APETALA2 (*AP2*) is the founding member of a family of transcription factors originally identified to regulate flower development. Further analysis has shown Gm-Apetala2-1 exhibits homology to the *A. thaliana* AP2 homolog TINY. Ectopic expression of *A. thaliana* TINY, caused by a semidominant dissociation insertion mutation, affects fertility, plant height and the elongation of hypocotyls. However, Gm-Apetala2-1 is not expressed within the control pericycle cells or syncytia undergoing the process of defense and therefore it would not be expected to contribute to the process (Table S1). A qPCR analysis shows the genetically engineered plants exhibit induced candidate gene expression (Fig. S1). An analysis of genetically engineered roots infected with *H. glycines* reveals suppressed parasitism as compared to control plants (Fig. 8; Table S3).

The regulon apparatus functions broadly in defense

It is not surprising that the presented genes, identified to be expressed within the syncytium undergoing the process of defense, have roles in defense. The *S. cerevisiae* Sec 14p is a cytoplasmic protein required for secretory vesicle formation from the Golgi apparatus and is encoded by a phosphatidylinositol (PtdIns) transfer protein (PITP). Sec 14p also functions as a sensor of the PtdIns/phosphatidylcholine (PtdCho) ratio in Golgi by directly regulating PtdCho biosynthesis. The *S. cerevisiae* Sec 4p is a Rab GTPase that functions to regulate the assembly of the exocyst through its interaction with Sec15p. The exocyst is a protein complex functioning in vesicle transport and membrane fusion between post-Golgi secretory vesicles to the plasma membrane. This interaction provides a regulatory function, occurring upstream of SNARE-mediated membrane fusion.

The *S. cerevisiae* Sec 23p is a cytosolic protein that functions in concert with Sar1p, Sec 24p, Sec 13p and Sec 14p as the minimal unit for COPII carrier formation. During vesicle formation, Sec12p-mediated activation of Sar1p results in its recruitment to the ER membrane which results in recruitment of Sec23p-Sec24p. This action results in the formation of the inner COPII prebudding complex which allows export protein capture.
Figure 5. Enzymes functioning in xyloglucan biosynthesis are xyloglucan glycosyltransferase (CSLCS4) (E.C. 2.4.1.168), xyloglucan 6 xylosyltransferase (XXT1, XXT2) (E.C. 2.4.2.39), xyloglucan 6 xylosyltransferase (XXT5) (E.C. 2.4.2.39), xyloglucan galactosyltransferase (KATMARI1) (no E.C. #), xyloglucan galactosyltransferase (no E.C. #), α-1,2 fucosyltransferase (E.C. 2.4.1.69). Xyloglucan terminology: X, glucose residues substituted by α-D-xylose; G, non-substituted glucosyl units within a xylosylglycan; L, glucose residues substituted by α-D-xylose and β-D-galactose; F, glucose residues substituted by α-D-xylose, β-D-galactose and α-L-fucose. Supplemental data is provided (Table S2).267-275

Figure 6. Enzymes functioning in reticuline metabolism. The biogenesis of (S)-reticuline begins with (L)-tyrosine. While a number of subsequent intermediates are made through different pathways, they converge on the production of (S)-norococlaurine via the enzymatic activity of noroclaurine synthase (E.C. 4.2.1.78). Subsequent enzymatic steps include norococlaurine 6-O-methyltransferase (E.C. 2.1.1.128), coclaurine N-methyltransferase (E.C. 2.1.1.140), the CYP80B1 N-methylcoclaurine 3′-monooxygenase (E.C. 1.14.13.71), 3′-hydroxy-N-methylcoclaurine 4′-O-methyltransferase (E.C. 2.1.1.116), berberine bridge enzyme tetrahydroprotoberberine synthase (reticuline oxidase) (EC 1.5.3.9). (S)-reticuline is an intermediate whose subsequent metabolism results in the generation of a wide number of isoquinoline alkaloids that are an important response to pathogen attack. Supplemental data is provided (Table S2).229-237
The S. cerevisae Bro1p is endosome-associated, functioning in the multivesicular body. Bro1p is a cytoplasmic protein named for its ~160 aa domain that interacts with the endosomal sorting complex required for transport complexes (ESCRT). Mutation of residues within this Bro1p domain interferes with its ability to localize to endosomes. An analysis of Bro1p has been performed, revealing it contains a tetratricopeptide repeat (TPR)-like structure that is known to function as a putative ESCRT-III binding site. Mutant analysis of Bro1p binds sucrose non-fermenting 7 protein (Snf7p) (ESCRT-III). 135 Bro1p-like proteins also function in plants.

A number of syntaxins that would function in related ways during membrane fusion function in defense. SYP6 has been shown to localize to the trans-Golgi network (TGN) and binds to α-SNAP. SYP131 is related to t-SNARE that functions in plant-arbuscular mycorrhizal interactions. SYP131 is closely related to SYPI32, shown to function in symbiotic interactions. SYP71 localizes mainly to the plasma membrane and also the cell plate, endosome and ER. SYP71 can also be subverted during virus infection to facilitate its pathogenicity. SYP8 can be accumulated to tonoplast and small prevacuolar compartments and co-localize with TGN markers and only partially with endocytic compartments with roles in vacuolar sorting, exocytosis and endocytosis.

The S. cerevisae Bet1p functions in anterograde transport from the ER to Golgi, found on Golgi membranes and is recruited to COPII vesicles by Sec 24p. Bet1p activates Bos1p with this interaction regulated by the small GTP-binding protein Ypt1p. Bet1p overexpression overcomes mutants of sec 35.

Sec 35p is novel, implicated in the tethering ER-derived vesicles. This tethering occurs at the Golgi apparatus. 170 Sec 35p binds to the hydrophilic Sec 34p to form a 480 kD complex that facilitates vesicle traffic. The ε-COP and ζ-COP proteins are part of the COPI coat, a 700 kD structure composed of α, β, β′, γ, δ, ε and ζ subunits that function in retrograde transport between the Golgi and ER. Furthermore, COPI functions in the maturation of endosomes and autophagy. Among these intermolecular interactions, α-COP (Sec 28p) has been shown to stabilize α-COP (Ret1p).

Secretion functions to drive a defense response

The demonstrated function of the secreted XTH and α-hydroxynitrile glucosidase proteins in defense indicated that the physiological needs of G. max resistance to H. glycines parasitism rank deep into the central metabolic processes of the parasitized cell. From genomics analyses performed in A. thaliana, a fundamental shift in metabolism during plant defense to pathogen attack has been long known and is consistent with observations made in the G. max-H. glycines pathosystem. In agreement with these observations is the demonstration of the involvement of the membrane fusion apparatus that would deliver XTH and α-hydroxynitrile glucosidase to the cell periphery and an ABC transporter that would likely deliver conjugated metabolites to the site of parasitism. Therefore, it appears that the defense of G. max to H. glycines parasitism involves altering the structure of the cell wall through the enzymatic activities of XTH, impeding the ability of H. glycines to make a functional syncytium. The defense process also involves the delivery of secondary metabolites to combat pathogen effectiveness. Furthermore, other metabolites are probably important to the defense process.

Hemicellulose: xyloglucan metabolism functions in defense in the root

Cytological observations of G. max undergoing the process of resistance to H. glycines have shown the cells engaged in the resistant reaction are limited in their ability to expand. These observations have indicated cell wall composition and modification are important to the defense process. A large proportion of the cell wall is composed of hemicellulose, a network that functions as a structural stabilizer. The majority of hemicellulose is composed of xyloglucan. Xyloglucan biosynthesis occurs by the stepwise activities of Golgi-localized enzymes and the delivery of these complex polysaccharides are generally believed to occur through bulk flow mediated by secretory vesicles (Fig. 5). The location of these materials in the Golgi, along with the secretion of these materials into the apoplast strengthens the importance of the vesicle transport system in defense. Upon polymerization, the hemicellulose strand can move within the cell wall to form resistant reaction are limited in their ability to expand. A G. max XTH has been shown to be highly expressed in H. glycines parasitized root cells undergoing defense and functions in the...
Figure 8. The overexpression of candidate defense genes in G. max leads to suppressed parasitism by H. glycines. (A) Sec 14-1; (B) Sec 4-6; (C) Sec 23-5; (D) Endosomal Targeting BRO1-Domain-1; (E) SYP6; (F) SYP131-1; (G) SYP71-6; (H) SYP8-2; (I) Bet1-1; (J) Coatomer ε; (K) Coatomer ζ; (L) ERGIC-3-3; (M) RO-40; (N) XXT 2-5; (O) GS-3; (P) AP2-1. The overexpression experiments present the outcome as the female index (FI) which is a percent of infection in relation to the control plants. wr, analysis examining cysts per whole root sample. pg, analysis examining cysts per gram of root tissue. * Statistically significant, p < 0.05; ** Not statistically significant, p > 0.05. Analyzed by Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test. Supplemental data is provided (Table S3).
defense process. XTHs function to restructure cell walls through cell wall loosening or intercalation of new xyloglucan. These two different functions of XTH are possible because it serves as a xyloglucan endo-transglucosylase (XET) where xyloglucan polymers are cleaved and joined to different xyloglucan chains. In contrast, the XEH activity of XTH hydrolyzes xyloglucan polymers. Regarding rapid cell wall expansion, auxin is known to regulate this process. XTH has a signal peptide and the protein can be N-glycosylated, indicating processing through the secretory pathway. The overexpression of XTH in *Populus* sp. results in the initial shortening of xyloglucan chain length, providing mechanistic insight into how plants can use XTH to limit cellular expansion during a defense reaction.

The demonstration that XTH is highly expressed during the *G. max* defense process toward *H. glycines* parasitism has indicated that the metabolic processes that lead to the generation of the xyloglucan would also be important. In examining this result further, we have identified that mRNA of 4 genes encoding the enzymes that function in xyloglucan biogenesis and metabolism are present in syncytia undergoing he process of defense (Table S2). The enzymes that function in xyloglucan biogenesis and metabolism have been shown to localize to the Golgi apparatus, clearly implicating the importance of a functional secretion system to defense. To test this hypothesis, we have cloned a *G. max* homolog of XXT and overexpressed it in a susceptible genotype, resulting in impaired parasitism (Fig. 7; Table S3). The results emphasize the importance of hemi-cellulose metabolism to defense. Furthermore, the importance of a functional secretion system is demonstrated since the proteins are processed through the secretory pathway with some functioning within the Golgi apparatus.

**Reticuline metabolism functions in defense in the root**

Plants produce an astonishing number of secondary metabolites, numbering over a hundred thousand in total. One of these important secondary metabolites, whose subsequent metabolism leads to the production of defense molecules, is (S)-reticuline. (S)-reticuline is a benzylisoquinoline alkaloid belonging to a family of secondary metabolites consisting of more than 2,500 different known small molecules. Some of the early biochemistry regarding reticuline has been worked out by understanding its basic structure. The biogenesis of (S)-reticuline begins with (L)-tyrosine. While a number of subsequent intermediates are made through different pathways, they converge on the production of (S)-nororclaurine. (S)-reticuline is an intermediate whose subsequent metabolism results in the generation of a wide number of isouquinoline alkaloids that are an important response to pathogen attack. A second pathway that leads to (S)-reticuline biosynthesis is also known to have its origins with (L)-tyrosine and leading to (S)-norlaudanosoline production, but the pathway has been described in mammalian cells.

Reticuline oxidase (RO) has also been described as berberine bridge enzyme (BBE). RO originally had been shown to be associated with a particle within the cell. RO has a signal peptide, indicating it is targeted to the ER. In *Berberis wilsoniae* var. subcaulialata, RO has been shown to localize within vesicles with other enzymes involved in the biosynthesis and metabolism of (S)-reticuline. The RO-containing vesicles have been shown to compose a low number of detectable polypeptides (~20), indicating that vesicles with different types of cargo function in defense. In opium poppy (*Papaver somniferum* cv *Marianne*), RO has been shown to localize in the companion cells of phloem. Furthermore, the subcellular localization of RO is the ER. This observation is consistent with the presence of a signal peptide for the *G. max* RO-40 (Fig. S2). After elicitor treatment, the ER undergoes a major ultrastructural arrangement becoming dilated and producing vesicles that later fuse with the vacuole. Notably, a vacuolar sorting determinant is present in the N-terminus of the RO protein. The production of the metabolic intermediates leading to the production of (S)-reticuline and the structural requirements appear to be shared between different plant groups. We have identified *G. max* homologs of genes functioning in reticuline biogenesis and metabolism (Table S2). Further work using *Coptis japonica* as a model revealed that an ABC transporter was responsible for delivering berberine to its site of function. This observation provided mechanistic insight into the delivery of secondary metabolites synthesized by RO to the vacuole. As observed by Sharma et al., it is likely that a number of different ABC transporters function during the defense of *G. max* to parasitic nematodes.

**Stachyose metabolism functions in defense in the root**

Stachyose is produced in an enzymatic process beginning with galactinol synthase (E.C. 2.4.1.123) action involving myo-inositol and UDP-D-galactose. The process leads to the production of galactinol. The only known role for galactinol is for the production of larger soluble oligosaccharides such as raffinose, stachyose, and verbascose. However, galactinol has been shown to be induced in its production during a defense response in roots and functions in signaling during systemic acquired resistance. Subsequent enzymatic activities of raffinose synthase (E.C. 2.4.1.82) and stachyose synthase (E.C. 2.4.1.67) result in the production of stachyose (Fig. 7). The enzymes for the biogenesis of stachyose have been identified in *G. max* (Table S2).

**The framework of defense involving the co-regulation of regulon in roots**

The framework of defense as *G. max* protects itself from its major pathogen, *H. glycines* is based off of cytological observations and genetic experiments relating its *rhgI* locus. These results put into context the identification of an *H. glycines* effector that binds *G. max* α-SNAP/Sec 17 that would have the function of disarming its defense response. Evidence has been presented showing that the transcriptional activity of these genes influences each other, indicative of co-regulation. The co-regulation of components of a structural and biochemical element helps in explaining why so many genes are transcriptionally active in the cells destined to undergo the process of resistance. Additional experimental evidence is presented here to support those observations. The experiments show how the expression of these genes
recapitulates the natural defense process that leads to suppressed parasitism by *H. glycines* in *G. max*. Furthermore, the identification of callose at the site of defense expands the biochemical and structural environment pertaining to defense, unifying work done in other plant pathosystems. The work likely has identified a genetic program that is functional in other plants against other pathogens or could be altered to facilitate symbiotic interactions. As a polymer, callose has the capacity of rapid building block mobilization to infection sites with their subsequent polymerization into structures that can impede pathogenesis.

### Methods

The data that has been presented in this review has been obtained by the published methods in Sharma et al. Laser microdissection (LM) has been used to collect control cells (pericycle) at 0 days post infection (dpi) and *Heterodera glycines*-induced syncytia undergoing the process of resistance at 3 and 6 dpi. Uninoculated roots have been used to obtain pericycle cells which have served as the source of the control mRNA samples. Microarray hybridizations have been run in triplicate per replicate experiments. For robustness, the experiment scores, (3) **p**-value calculation using the Wilcoxon implementation of the standard Affymetrix DCM analysis consists of four steps, including (1) removal of saturated probes, (2) calculation of discrimination scores, (3) **p**-value calculation using the Wilcoxon rank test, and (4) making the detection call (present **[p < 0.05]**/marginal **[p = 0.05]**/absent **[p > 0.05]**). In the analysis presented here, the probe set is accepted as detecting probe if **p < 0.05**. The PCR and qPCR primers used in the studies are provided (Table S4). The qPCR data has been obtained using 2^−ΔΔC_T_ to calculate fold change (Fig. S2). The female index (FI), is the community-accepted method to determine the effect a condition has on *H. glycines* parasitism. The results have been presented as female cysts per whole root mass and female cysts per gram (Table S3).

### Disclosures of Potential Conflicts of Interest

The authors claim no potential conflicts of interest.

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### References

1. Novick P, Field C, Schekman R. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 1980; 21:205-15; PMID:6998832; http://dx.doi.org/10.1016/0092-8674(80)90128-2
2. Clary DO, Griff IC, Rothman JE. SNAREs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. Cell 1990; 61:709-21; PMID:211733; http://dx.doi.org/10.1016/0092-8674(90)90482-T
3. Jahn R, Fasshauer D. Molecular machines governing exocytosis of synaptic vesicles. Nature 2012; 490:201-7; PMID:23060190; http://dx.doi.org/10.1038/nature11320
4. Vukasinovi N, Zarsky V. Tethering complexes in the Arabidopsis endomembrane system. Front Cell Dev Biol 2016; 4:46; PMID:27243010; http://dx.doi.org/10.3389/fcell.2016.00046
5. Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu HJ, Hickelhoven R, Stein M, Freidenhoven A, Somerville SC, et al. SNARE-protein mediated disease resistance at the plant cell wall. Nature 2003; 425:973-7; PMID:14586469; http://dx.doi.org/10.1038/nature02076

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**Table 1.** A summary of *G. max* candidate gene expression.

| Gene | Accession | Time point (dpi) | 0' | 3 | 6 |
|------|-----------|-----------------|----|---|---|
| Sec14-1 | Glyma14g08180 | N/M | N/M | M |
| Sec4-6 | Glyma2g33210 | N/M | N/M | M |
| Sec23-5 | Glyma18g00670 | N/M | N/M | M |
| Bro1-1 | Glyma2g10910 | N/M | M | M |
| SYP1-6 | Glyma17g07100 | N/M | M | M |
| SYP131-1 | Glyma12g32100 | N/M | M | M |
| SYPP1-6 | Glyma19g36410 | N/M | N/M | M |
| SYPP2-2 | Glyma14g02120 | M | M | M |
| Bet1-1 | Glyma06g10370 | M | M | M |
| Cε-1 | Glyma09g03500 | M | M | M |
| Cɛ-3 | Glyma15g01150 | M | M | M |
| ERGIC-3-3 | Glyma13g01920 | M | M | M |
| RO-40 | Glyma15g14040 | N/M | N/M | M |
| XTT-25 | Glyma19g39760 | N/M | N/M | M |
| GS-3 | Glyma19g11550 | N/M | N/M | M |
| AP2-1 | Glyma06g44130 | N/M | N/M | M |

Footnote. Laser microdissection has been used to collect control cells (pericycle) at 0 days post infection (dpi) and *Heterodera glycines*-induced syncytia undergoing the process of resistance at 3 and 6 dpi. Uninoculated roots have been used to obtain pericycle cells which have served as the source of the control mRNA samples. M (red) probe has been measured. N/M (blue), probe has not been measured. For details, please see Table S1.
6. Humphry M, Bednarek P, Kemmerling B, Koh S, Stein M, Gobel U, Stubek K, Piselewska-Bednarek M, Loraine A, Schulze-Lefert P, et al. A regulon conserved in monocot and dicot plants defines a functional module in antifungal plant immunity. Proc Natl Acad Sci U S A 2010; 107:21896-901; PMID:21098265; http://dx.doi.org/10.1073/pnas.1003619107

7. Pauly SR, Matsu PE, McNeese BT, Sharma K, Krishnavajala A, Lawrence GW, Klink VP. Syntaxin 31 functions in Glycine max resistance to the plant parasitic nematode Heterodera glycines. Plant Mol Biol 2014; 85:107-21; http://dx.doi.org/10.1007/s11103-014-0172-2

8. Johansson ON, Fantozzi E, Fahlberg P, Nilsson AK, Buhot N, Tor M, Johansson ON, Fantozzi E, Fahlberg P, Nilsson AK, Buhot N, Tor M. Expression analysis of syncytial cells isolated from incompatible and compatible soybean (Glycine max) roots infected by the soybean cyst nematode. Plant Mol Biol 2009; 71:525-67; PMID:24889055; http://dx.doi.org/10.1007/s11103-012-9323-2

9. Sharma K, Pauly SR, McNeese BT, Lawrence GW, Klink VP. Co-regulation of the Glycine max soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE)-containing regulon occurs during defense to a root pathogen. J Plant Interactions 2016; 11:74-93; http://dx.doi.org/10.1007/s11359-015-9589-1

10. Armstrong HE, Armstrong EF, Horten E. Herbage studies. II. Varia-

tions in Lotus corniculatus and Trifolium repens: (cyanophoric plants). Proc R Soc Lond Ser B 1913; 86:262-9.

11. Ware WM. Experiments and observations on forms and strains of Trifolium repens. J Agric Sci 1925; 15:47-67.

12. Hughes MA. The cyanoenic polymorphism in Trifolium repens L. (white clover). Heredity 1991; 66:105-15; http://dx.doi.org/10.1038/s00037-011-1211-3

13. Kakes P, Linamarase and other beta-glucosidases are present in the cell walls of Trifolium repens L. leaves. Planta 1985; 166:156-60; PMID:24241426; http://dx.doi.org/10.1007/BF00397342

14. Francisco RM, Regalado A, Ageorges A, Burla BJ, Bassin B, Eisenach CF, Zarrouk O, Vialet S, Marlin T, Chaves MM, et al. ABCC1, an ATP binding cassette protein from grape berry, transports anthocyanidin 3-O-Glucosides. Plant Cell 2013; 25:1840-54; PMID:23723325; http://dx.doi.org/10.1105/tpc.105.038372

15. Kim M, Hyten DL, Song Q, Thelen JJ, Cheng J, et al. Genome sequence of the palaeopolyploid soybean. Nature 2010; 463:178-83; PMID:20075913; http://dx.doi.org/10.1038/nature08670

16. Grant D, Nelson RT, Cannon SB, Shoemaker RC. SoyBase, the USDA-ARS soybean genetics and genomics database. Nucl Acids Res 2010; 38:D843-4; PMID:20008513; http://dx.doi.org/10.1093/nar/gkp798

17. Klink VP, Hosseini P, Matsu PE, Alkarhour NF, Matthews BF. A gene expression analysis of synctia laser microdissected from the roots of the Glycine max (soybean) genotype PI 548402 (Peking) undergoing a resistant reaction after infection by Heterodera glycines (soybean cyst nematode). Plant Mol Biol 2009; 71:525-67; PMID:19787434; http://dx.doi.org/10.1007/s11103-009-9539-1

18. Klink VP, Hosseini P, Matsu PE, Alkarhour NF, Matthews BF. Synctium gene expression in Glycine max[PI 548402] roots undergoing a resistant reaction to the parasitic nematode Heterodera glycines. Plant Physiol Biochem 2010a; 48:176-93; PMID:20138530; http://dx.doi.org/10.1016/j.plaphy.2009.12.003

19. Klink VP, Overall CC, Alkarhour NF, MacDonald MH, Matthews BF. Microarray detection calls as a means to compare transcripts expressed within syncytial cells isolated from incompatible and compatible soybean (Glycine max) roots infected by the soybean cyst nematode (Heterodera glycines). J Biomed Biotechnol 2010b; 1-30; http://dx.doi.org/10.1155/2010/491217

20. Klink VP, Hosseini P, Matsu PE, Alkarhour NF, Matthews BF. Differences in gene expression amplitude overlie a conserved transcriptomic program occurring between the rapid and potent localized resistant reaction at the syncytium of the Glycine max genotype PI 548402 as compared to the prolonged and potent resistant reaction of PI 88788. Plant Mol Biol 2011; 75:141-65; PMID:21153862; http://dx.doi.org/10.1007/s11103-010-9715-3

21. Caldwell BE, Brim CA, Ross JP. Inheritance of resistance of soybeans to the soybean cyst nematode, Heterodera glycines. Agron J 1960; 52:635-6.

22. Matsu PE, Kumar R, Hosseini P, Jones CM, Tremblay A, Alkarhour NF, Matthews BF, Klink VP. Mapping cell fate decisions that occur during soybean defense responses. Plant Mol Biol 2011; 77:513-28; http://dx.doi.org/10.1007/s11103-011-9828-3

23. Matsu PE, Lawrence GW, Yousef RM, Kim KH, Matthews BF, Lawrence KS, Klink VP. The expression of a naturally occurring, truncated allele of an α-SNAP gene suppresses plant parasitic nemato-
de infection. Plant Mol Biol 2012; 80:131-5; http://dx.doi.org/
10.1007/s11103-011-9792-2

24. Stevens T, Esmon B, Schekman R. Early stages in the yeast secretory pathway are required for transport of carbamoylphosphate Y to the vac-

uole. Cell 1982; 30:439-48; PMID:6754086; http://dx.doi.
10.100109/1978.6478(82)90241-0

25. Concibido VC, Diers BW, Arell PR. A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci 2004; 44:1121-31;
http://dx.doi.org/10.2135/cropsci2004.11212

26. Grant D, Nelson RT, Cannon SB, Shoemaker RC. SoyBase, the USDA-ARS soybean genetics and genomics database. Nucl Acids Res 2010; 38:D843-4; PMID:20008513; http://dx.doi.org/10.1093/nar/gkp798

27. Kim M, Hyten DL, Bent AF, Diers BW. Fine mapping of the SCN resistance locus rhg1-b from PI 88788. Plant Genome 2010; 3:81-9; http://dx.doi.org/10.3835/plantgenome2010.02.0001

28. Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J, et al. Genome sequence of the pala-
opolyplipid soybean. Nature 2010; 463:178-83; PMID:20075913; http://dx.doi.org/10.1038/nature08670

29. Cook DE, Lee TG, Guo X, Melito S, Wang K, Bayless A, Wang J, Hughes TJ, Willis DK, Clemente T, et al. Copy number variation of multiple genes at Rhg1 mediates nematode resistance in soybean. Science 2012; 338:1206-9; PMID:23065905; http://dx.doi.org/10.1126/science.1228746

30. Novick P, Schekman R. Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of Saccaromyces cerevisiae. Proc Natl Acad Sci U S A 1979; 76:1858-62; PMID:377286; http://dx.doi.org/10.1007/s11103-011-9823-2

31. Cook DE, Bayless AM, Wang K, Guo X, Song Q, Jiang J, Bent AF. Distinct copy number, coding sequence and locus methylation pat-
terns underlie Rhg1-mediated soybean resistance to soybean cyst nematode. Plant Physiol 2014; 165:630-47; PMID:24738883; http://dx.doi.org/10.1104/pp.114.235952

32. Novick P, Schekman R. Conserved alpha-helical segments on yeast homologs of the synaptobrevin/VAMP family of v-SNAREs mediate exocytic fusion. J Biol Chem 1992; 267:12106-15; PMID:1601878

33. Gerst JE. Conserved alpha-helical segments in yeast homologs of the synaptothrein/VAMP family of v-SNAREs mediate exocytic fusion. J Biol Chem 1992; 267:12106-15; PMID:1601878
function. J Biol Chem 1997; 272:16591-8; PMID:9195971; http://dx.doi.org/10.1074/jbc.272.26.16591
38. Sanderfoot AA, Farhah F, Assaad FF, Raikhel NV. The Arabidopsis genome. An abundance of soluble N-ethylmaleimide-sensitive factor adaptor protein receptors. Plant Physiol 2001a; 124:1558-69; http://dx.doi.org/10.1104/pp.124.4.1558
39. Sabcofoot AA, Kowaleva V, Bashham DC, Raikhel NV. Interactions between syntaxins identify at least 5 SNARE complexes within the Golgi/prevacuolar system of the Arabidopsis cell. Mol Biol Cell 2001b; 12:3733-43; PMID:11739796; http://dx.doi.org/10.1091/mbc.12.12.3733
40. Payne WE, Kaiser CA, Bevis BJ, Soderholm J, Fu D, Sears IB, Glick BS. Isolation of Pichia pastoris genes involved in ER-Golgi transport. Yeast 2000; 16:979-93; PMID:10923200; http://dx.doi.org/10.1006/1097-0061(2000)011:67-93-AID-YEAS594-0.3.0.CO;2-C
41. Hong KaK, Chakravarti A, Takahashi JS. The gene for soluble N-ethylmaleimide sensitive factor attachment protein alpha is mutated in hydrocephaly with hop gait (hyh) mice. Proc Natl Acad Sci U S A 2004; 101:1748-53; PMID:14755058; http://dx.doi.org/10.1073/pnas.0308268101
42. Babcock M, Macleod GT, Leither J, Pallanck L. Genetic analysis of Drosophila reveals positive and negative secretory roles. J Cell Biol 1997; 138:1229-39; PMID:9195971; http://dx.doi.org/10.1083/jcb.138.4.1229
43. Rodríguez F, Bustos MA, Panetti MN, Ruete MC, Mayorga LS, Tomes CN. α-SNAP prevents docking of the acrosome during sperm exocytosis because it sequesters monomeric syntaxin. PLoS One 2011; 6:e21925; http://dx.doi.org/10.1371/journal.pone.0021925
44. Oyler GA, Higgins GA, Hart RA, Battenberg E, Billingsley M, Bloom B. Captive and release of partially zipped trans-SNARE paired membranes require lipids for progression to fusion. Elife 2014; 3:e01879; http://dx.doi.org/10.1021/jacs.5b08306
45. Bennett KM, Calakos N, Scheller RH. Syntaxin: a synaptic protein function in vesicular transport. EMBO J 1993; 12:4095-104; PMID:8223426
46. Bennett KL, Bennett KM, Tempst P, Rothman JE. A rab protein is required for the assembly of SNARE complexes on intact organelles. J Cell Biol 2009; 185:535-49; PMID:19414611; http://dx.doi.org/10.1083/jcb.200811082
47. Jin R, Rummel A, Binz T, Brunger AT. Botulinum neurotoxin B recognizes its protein receptor with high affinity and specificity. Nature 2006; 444:1092-5; PMID:17167421; http://dx.doi.org/10.1038/nature05387
48. Strotmeier J, Willjes G, Binz T, Rummel A. Human synaptotagmin-II is not a high affinity receptor for botulinum neurotoxin B and G: increased therapeutic dosage and immunogenicity. FEBS Lett 2012; 586:310-3; PMID:22265973; http://dx.doi.org/10.1016/j.febslet.2011.12.037
49. McNew JA, Segaard M, Lampen NM, Machida S, Ye RR, Lacomis L, Tempst P, Rothman JE. A prenylated SNARE-like protein and biotin are implicated in soybean cyst nematode virulence. PLoS One 2015; 10:e0145601; PMID:26714307; http://dx.doi.org/10.1371/journal.pone.0145601
50. Schwartz ML, Merz AJ. Capture and release of partially zipped trans-SNARE complexes on intact organelles. J Cell Biol 2009; 185:535-49; PMID:19414611; http://dx.doi.org/10.1083/jcb.200811082
51. Lobingier BT, Nickerson DP, Lo SY, Merz AJ. SM proteins Sly1 and Vps33 co-assemble with Sec17 and SNARE complexes to oppose SNARE disassembly by Sec18. Elife 2014; 3:e02272; PMID:24837546; http://dx.doi.org/10.1053/j.mb.e07-05-0498
52. Bekal S, Domier LJ, Gonfa B, Lakhsassi N, Meksem K, Lambert KN. A SNARE-like protein and biotin are implicated in soybean cyst nematode virus infection. Proc Natl Acad Sci USA 2013; 110:28047-52; PMID:24047598; http://dx.doi.org/10.1073/pnas.1305564110
53. Zick M, Stroupe C, Orr A, Douville D, Wickner WT. Membranes linked by trans-SNARE complexes require lipids prone to non-bilayer structure for progression to fusion. Elife 2014; 3:e01879; PMID:24596153; http://dx.doi.org/10.1053/j.mb.e07-05-0498
54. Yu H, Rathore SS, Chen C, Liu Y, Ouyang Y, Stowell MH, Shen J. Reconstituting intracellular vesicle fusion reactions: the essential role of macromolecular crowding. J Am Chem Soc 2015; 137:12873-83; PMID:26431309; http://dx.doi.org/10.1021/jacs.5b08306
55. Zick M, Stroupe C, Orr A, Douville D, Wickner WT. Membranes linked by trans-SNARE complexes require lipids prone to non-bilayer structure for progression to fusion. Elife 2015a; 4:e08843; http://dx.doi.org/10.1053/j.mb.e07-05-0498
56. Zick M, Stroupe C, Orr A, Douville D, Wickner WT. Correction: Membranes linked by trans-SNARE complexes require lipids prone to non-bilayer structure for progression to fusion. Elife 2015b; 4:e08843; http://dx.doi.org/10.1053/j.mb.e07-05-0498
57. Block MR, Glick BS, Wilcox CA, Wieland FT, Rothman JE. Purification of an N-ethylmaleimide-sensitive protein catalyzing vesicular transport. Proc Natl Acad Sci USA 1988; 85:7852-6.
71. Malhotra V, Orci L, Glick BS, Block MR, Rothman JE. Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the Golgi stack. Cell 1988; 54:221-7; http://dx.doi.org/10.1016/0092-8674(88)90544-5

72. Vivona S, Cipriano DJ, O’Leary S, Li YH, Fenn TD, Brunger AT. Disassembly of all SNAP receptors by N-ethylmaleimide-sensitive factor (NSF) is initiated by a conserved 1:1 interaction between alpha-soluble NSF attachment protein (SNAP) and SNAP complex. J Biol Chem 2013; 288:24984-91; PMID:23836889; http://dx.doi.org/10.1074/jbc.M113.489807

73. Zhao M, Wu S, Zhou Q, Vivona S, Cipriano DJ, Cheng Y, Brunger AT. Mechanistic insights into the recycling machine of the SNAP complex. Nature 2015; 518:61-7; PMID:25581794; http://dx.doi.org/10.1038/nature14148

74. Vahlensieck Y, Riezman H, Meyhack B. Transcriptional studies on yeast SEC genes provide no evidence for regulation at the transcriptional level. Yeast 1995; 11:901-11; PMID:8533466; http://dx.doi.org/10.1002/yea.320111002

75. Shanks SG, Carpp LN, Struthers MS, McCann RK, Bryant NJ. The Sec1/Munc18 protein Vps45 regulates cellular levels of its SNAP binding partners Tlg2 and Sec2 in Saccharomyces cerevisiae. PLoS One 2012; 7: e94928; PMID:23166732; http://dx.doi.org/10.1371/journal.pone.0049628

76. Duden R, Kajikawa L, Wuestehube L, Schekman R. v-COP is a structural component of coatamer that functions to stabilize alpha COP. EMBO J 1998; 17:985-95; PMID:9463377; http://dx.doi.org/10.1093/emboj/17.4.985

77. Otte S, Barlowe C. The Env41p-Env46p complex: multiple export signals and astrocytes of the CNS and regulate cholesterol homeostasis. Plant Cell 2013; 25:4640-57; PMID:24226400; http://dx.doi.org/10.1007/BF00403011

78. Kováts K, Binder A, Hohl HR. Cytology of induced systemic resistance of cucumber to Colletotrichum lagenarium. Planta 1991; 183:484-90; http://dx.doi.org/10.1007/BF00194268

79. Storti E, Latil C, Salti S, Bettini P, Bogani P, Pellegrini MG, Simeti C, Moltan A, Buiti M. The in vitro physiological phenotype of tomato resistance to Fusarium oxysporum f.sp. lycopersici. Theor Appl Genet 1992; 84:123-8; PMID:24203038; http://dx.doi.org/10.1007/BF00239991

80. Neill JS, Meins F, Jr. Physiological compensation in antisense transformants: specific induction of an “ersatz” glucon endo-1,3-beta-glucosidase in plants infected with necrotizing viruses. Proc Natl Acad Sci U S A 1993; 90:8792-6; PMID:8415609; http://dx.doi.org/10.1073/pnas.90.19.8792

81. Benhamou N, Roche, RM, Thomas M, Meins F, Jr. Decreased susceptibility to viral disease of [beta]-1,3-glucan-deficient plants generated by antisense transformation. Plant Cell 1996; 8:1001-11; PMID:12239410; http://dx.doi.org/10.1010/cpl.8.6.1001

82. Rodriguez-Galvez E, Mendgen K. Cell wall synthesis in cotton roots after infection with Fusarium oxysporum. The deposition of callose, arabinoxylans, xyloglucans, and pectic components into walls, wall appositions, cell plates and plasmodesmata. Plantanta 1995; 197:535-45; http://dx.doi.org/10.1007/BF00196676

83. Bestwick CS, Bennett MH, Mansfield JW. Hrp mutant of Pseudomonas syringae pv phaseolicola induces cell wall alterations but not membrane damage leading to the hypersensitive reaction in lettuce. Plant Physiol 1995; 108:503-16; PMID:12228488; http://dx.doi.org/10.1104/pp.108.2.503

84. Benhamou N, Rey P, Cherif M, Hockenhull J, Tirilý Y. Treatment with the mycoparasite Pythium oligandrum triggers induction of defense-related reactions in tomato roots when challenged with Fusarium oxysporum f. sp. radicis-lycopersici. Phytopathology 1997; 87:108-22; PMID:18945162; http://dx.doi.org/10.1094/PHYTO.1997.87.1.108

85. Benhamou N, Kloepper JW, Quadt-Hallman A, Tuzun S. Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol 1996; 112:919-29; PMID:12226427; http://dx.doi.org/10.1104/pp.112.3.919

86. Herbers K, Meuwly P, Frommer WB, Metraux JP, Sonnewald U. Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexaside sensing in the secretory pathway. Plant Cell 1996; 8:793-803; PMID:12239401; http://dx.doi.org/10.1101/cel.6793

87. Tang X, Xie M, Kim YJ, Zhou J, Klessig DF, Martin GB. Overexpression of Pto activates defense responses and confers broad resistance. Plant Cell 1999; 11:15-29; PMID:9878629; http://dx.doi.org/10.1101/tpc.11.2.177

88. McCormack BA, Gregory AC, Kerry ME, Smith C, Bolwell GP. Purification of an elicitor-induced glucan synthase (callose synthase) from suspension cultures of French bean (Phaseolus vulgaris L.): purification and immunolocalization of a probable M(r)-65,000 subunit of the enzyme. Planta 1997; 203:196-203; PMID:9362565; http://dx.doi.org/10.1007/s004250050182

89. Takahashi A, Kawashita T, Henmi K, Shil K, Kodama O, Satoh H, Shimamoto K. Lesion mimic mutants of rice with alterations in early signaling events of defense. Plant J 1999; 17:535-45; PMID:10205906; http://dx.doi.org/10.1046/j.1365-313X.1999.00405.x

90. Vleeshouwers VG, van Doornweijt W, Govers F, Kamoun S, Colon LT. The hypersensitive response is associated with host and nonhost pathogen-specific defense mechanisms in Arabidopsis by beta-amino- nitroacrylic acid. Proc Natl Acad Sci U S A 2000; 97:12920-5; PMID:10858166; http://dx.doi.org/10.1073/pnas.230416997

91. Stone JM, Heard JE, Asai T, Ausubel FM. Simulation of fungal-mediated cell death by fumonisin B1 and a selection of fumonisin B1-resistant (fbr) Arabidopsis mutants. Plant Cell 2000; 12:1811-22; PMID:11041878; http://dx.doi.org/10.1105/tpc.110.18111

92. Donofrio NM, Delaney TP. Abnormal callose response phenotype and hypersusceptibility to Peronospora parasitica in defence-
compromised Arabidopsis nim1-1 and salicylate hydroxylase-expressing plants. Mol Plant Microbe Interact 2001; 14:439-50; PMID:11310731; http://dx.doi.org/10.1094/MPMI.2001.14.4.439 

Mellersh DG, Heath MC. Plasma membrane-cell wall adhesion is required for expression of plant defense responses during fungal penetration. Plant Cell 2001; 13:413-24; PMID:11226194; http://dx.doi.org/10.1105/tpc.1.10.431.1343 

Rate DN, Greenberg JT. The Arabidopsis aberrant growth and death2 mutant shows resistance to Pseudomonas syringae and reveals a role for NPR1 in suppressing hypersensitive cell death. Plant J 2001; 27:203-11; PMID:11532166; http://dx.doi.org/10.1046/j.1097-4636.2001.00887.x 

Iwano M, Che FS, Goto K, Tanaka N, Takayama S, Isogai A, Electron microscopic analysis of the H(2)O(2) accumulation preceding hypersensitive cell death induced by an incompatible strain of Pseudomonas aeruginosa in cultured rice cells. Mol Plant Pathol 2002; 3:1-8; PMID:120569303; http://dx.doi.org/10.1104/tpc.100.00087.x 

Veit S, Wörl JM, Nürnberger T, Koch W, Seitz HU. A novel protein elicitor (PaNie) from Pythium aphanidermatum induces multiple defense responses in carrot, Arabidopsis, and tobacco. Plant Physiol 2001; 127:832-41; PMID:11706166; http://dx.doi.org/10.1104/pp.010350 

Yang L, Qin L, Liu G, Peremyslov VV, Dolja VV, Wei Y. Myosins XI effector suppresses cell wall-based extracellular defense in susceptible Arabidopsis plants. Proc Natl Acad Sci U S A 2001; 98:1364-9; PMID:11226194; http://dx.doi.org/10.1105/tpc.6.9.1343 

Fridborg I, Smyth DR, Meyerowitz EM. Genes directing flower development in Arabidopsis. Plant Cell 1989; 1:37-52; PMID:2535466; http://dx.doi.org/10.1105/tpc.1.1.37 

Jofuku KD, den Boer BG, Van Montagu M, Okamura JK. Control of Arabidopsis flower and seed development by the homeotic gene APETALA2. Plant Cell 1994; 6:1211-25; PMID:7919989; http://dx.doi.org/10.1105/tpc.6.9.1211 

Wilson K, Long D, Svinburne J, Coupland G. A Dissociation insertion causes a semidominant mutation that increases expression of TINY, an Arabidopsis gene related to APETALA2. Plant Cell 1996; 8:659-71; PMID:8624440; http://dx.doi.org/10.1105/tpc.6.9.1211 

Stockinger EJ, Gilmour SJ, Thomashow MF. Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci U S A 1997; 94:10350-40; PMID:9023378; http://dx.doi.org/10.1107/tpas.94.3.1035 

Bankaitis VA, Malehorn DE, Emr SD, Greene R. The Saccharomyces cerevisiae SEC14 gene encodes a cytosolic factor that is required for transport of secretory proteins from the yeast Golgi complex. J Cell Biol 1989; 108:1271-81; PMID:2466847; http://dx.doi.org/10.1083/jcb.108.4.1271 

Bankaitis VA, Aitken FR, Cleves AE, Dowhan W. An essential role for a phospholipid transfer protein in yeast Golgi function. Nature 1990; 347:561-2; PMID:2215682; http://dx.doi.org/10.1105/tpc.1.1.37 

Guo W, Roth D, Walch-Solimena C, Novick P. The exocyst is an effector for Sec 4p, targeting secretory vesicles to sites of exocytosis. EMBO J 1999; 18:1071-80; PMID:10022848; http://dx.doi.org/10.1093/emboj/18.4.1071 

Mizuno-Yamasaki E, Rivera-Molina F, Novick P. GTPase networks in membranes traffic. Annu Rev Biochem 2012; 81:637-59; PMID:22463690; http://dx.doi.org/10.1146/annurev-biochem-051110-143651 

Jahn R, Scheller RH. SNAREs—engines for membrane fusion. Nat Rev Mol Cell Biol 2006; 7:631-43; PMID:16912714; http://dx.doi.org/10.1105/tpc.1.1.37 

Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N, Rexach MF, Ravazzola M, Amherdt M, Schekman R. COPII: a coat mediated vesicle budding and regulates cell death. Cell 1994; 77:895-907; PMID:8004667; http://dx.doi.org/10.1103/physiol.2002.5822.2006.00683.x 

Lord C, Ferro-Novick S, Miller EA. The highly conserved COPII coat complex sorts cargo from the endoplasmic reticulum and targets it to the golgi. Cold Spring Harb Perspect Biol 2013; 5:a013367; http://dx.doi.org/10.1101/cshperspect.a013367 

Chung KP, Zheng Y, Jiang L. COPII paralogs in plants: functional redundancy or diversity? Trends Plant Sci 2016; 21:758-69; PMID:27317658; http://dx.doi.org/10.1016/j.plants.2016.05.010 

Oodorizzi G, Katzmann DJ, Babst M, Audhya A, Emr SD. Bro1 is an endosome-associated protein that functions in the MVB pathway in Saccharomyces cerevisiae. J Cell Biol 2003; 116:1893-903; PMID:12668726; http://dx.doi.org/10.1242/jcs.00395
Watson RT, Pessin JE. Functional cooperation of 2 independent targeting domains in syntaxin 6 is required for its efficient localization in the trans-Golgi network of 3T3L1 adipocytes. J Biol Chem 2000; 275:1261-8; PMID:10625671; http://dx.doi.org/10.1074/jbc.275.2.1261

150. Dulubova I, Yamaguchi T, Gao Y, Min SW, Huryeva I, Sudhof TC, Rizo J. How Tlg2p/syntaxin 16 'snare' Yps45. EMBO J 2002; 21:3620-31; PMID:12110575; http://dx.doi.org/10.1093/emboj/cdf381

151. Choudhury A, Marks DL, Proctor KM, Gould GW, Pagano RE. Regulation of caveolar endocytosis by syntaxin 6-dependent delivery of membrane components to the cell surface. Nat Cell Biol 2006; 8:317-28; PMID:16565709; http://dx.doi.org/10.1038/nclb1380

152. Burkhardt P, Hattendorf DA, Wei W, Fasshauer D. Munc18a controls SNARE assembly through its interaction with the syntaxin N-peptide. EMBO J 2008; 27:923-33; PMID:18337752; http://dx.doi.org/10.1038/emboj.2008.37

153. El Kasmi F, Hou H, Sanfacon H, Wang A. The SNARE protein Syp71 is essential for turnip mosaic virus infection by mediating fusion of virus-induced vesicles with chloroplasts. Plos Pathogens 2013; 9:e1003378; PMID:23696741; http://dx.doi.org/10.1371/journal.ppat.1003378

154. Pan H, Oztas O, Zhang X, Wu X, Stonoha C, Wang E, Wang B, D. A symbiotic SNARE protein generated by alternative termination of transcription. Nat Plants 2016; 2:15197; PMID:27249189; http://dx.doi.org/10.1038/nplants.2015.197

155. Suwastika IN, Uemura T, Shiina T, Sato MH, Takeyasu K. SYP71, a plant-specific Qc-SNARE protein, reveals dual localization to the plasma membrane and the endoplasmic reticulum in Arabidopsis. Cell Struct Func 2008; 33:185-92; PMID:18827404; http://dx.doi.org/10.1247/cssf.080024

156. El Kasmi F, Krause C, Hiller U, Sterzhof YD, Mayer U, Conner L, Kong L, Reichardt I, Sanderfoot AA, Jürgens G. SNARE complexes of different composition jointly mediate membrane fusion in Arabidopsis cytokinesis. Mol Biol Cell 2013; 24:1593-601; PMID:23515225; http://dx.doi.org/10.1099/mcb.0.12-00747-0

157. Wei T, Zhang C, Hou H, Sanfacon H, Wang A. The SNARE protein Syp71 is essential for turnip mosaic virus infection by mediating fusion of virus-induced vesicles with chloroplasts. Plos Pathogens 2013; 9:e1003378; PMID:23696741; http://dx.doi.org/10.1371/journal.ppat.1003378

158. De Benedictis M, Bleve G, Faraco M, Stigliano E, Grieco F, Piro G, Dalessandro G, Di Sansebastiano GP. ATsYP51/52 functions diverge in the post-Golgi traffic and differently affect vacuolar sorting. Mol Plant 2013; 6:916-30; PMID:23087325; http://dx.doi.org/10.1093/mps/sns117

159. Wuestehube LJ, Duden R, Eun A, Hamamoto S, Korn P, Ram R, Schekman R. New mutants of Saccharomyces cerevisiae affected in the transport of proteins from the endoplasmic reticulum to the Golgi complex. Genetics 1996; 142:393-406; PMID:8852839

160. Hay JC, Hirling H, Scheller RH. Mammalian vesicle trafficking proteins of the endoplasmic reticulum and Golgi apparatus. J Biol Chem 1996; 271:5671-9; PMID:8621431; http://dx.doi.org/10.1074/jbc.271.10.5671

161. Hay JC, Chao DS, Kuo CS, Scheller RH. Protein interactions regulating vesicle transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. Cell 1997; 89:149-58; PMID:9094723; http://dx.doi.org/10.1016/S0092-8674(00)81091-9

162. Zhang T, Wong SH, Tang BL, Xu Y, Peter F, Subramaniam VN. Wuestehube LJ, Duden R, Eun A, Hamamoto S, Korn P, Ram R, Schekman R. New mutants of Saccharomyces cerevisiae affected in the transport of proteins from the endoplasmic reticulum to the Golgi complex. Genetics 1996; 142:393-406; PMID:8852839

163. Stone S, Sacher M, Mao Y, Carr C, Lyons P, Quinna AM, Ferro-Novick S. Bet1p activates the v-SNARE Bos1p. Mol Biol Cell 1997; 8:1175-81; PMID:9243499; http://dx.doi.org/10.1091/mcb.8.7.1175

164. Springer S, Schekman R. Nuclaeation of COPII vesicular coat complex by endoplasmic reticulum to Golgi vesicle SNAREs. Science 1998; 281:698-700; PMID:9685263; http://dx.doi.org/10.1126/science.281.5377.698

165. Sacher M, Jiang Y, Barrowman J, Scarpa A, Burston J, Zhang L, Schietz D, Yates JR, 3rd, Abeliovich H, Ferro-Novick S. TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. EMBO J 1998; 17:2494-503; PMID:9564032; http://dx.doi.org/10.1093/emboj/cd7.9.2494

166. Rowe T, Dascher C, Bannik Y, Patter H, Balch WE. Role of vesicle-associated syntaxin 5 in the assembly of pre-Golgi intermediates. Science 1998; 279:696-700; PMID:9445473; http://dx.doi.org/10.1126/science.279.5351.696
167. Kuehn MJ, Herrmann JM, Schekman R. COPII-cargo interactions direct protein sorting into ER-derived transport vesicles. Nature 1998; 391:187-90; PMID:9428766; http://dx.doi.org/10.1038/34438

168. Van Rheenen SM, Cao X, Lushpashin VV, Barlowe C, Waters MG. Sec35, a novel peripheral membrane protein, is required for ER to Golgi vesicle docking. J Cell Biol 1998; 141:1107-19; PMID:9608204; http://dx.doi.org/10.1083/jcb.141.5.1107

169. Adolf F, Rhiel M, Reckmann I, Wieland FT. Sec 24D/isoform-specific sorting of the preassembled ER-Golgi Q-SNARE complex. Mol Biol Cell 2016; 27:2677-70; PMID:27413010; http://dx.doi.org/10.1091/mbc.E16-04-0229

170. Kim DW, Sacher M, Scarpa A, Quinn AM, Ferro-Novick S. High-affinity transport in the COPII pathway. J Cell Sci 2006; 119:4730-40; PMID:17077122; http://dx.doi.org/10.1242/jcs.03250

171. Lorente-Rodriguez A, Barlowe C. Entry and exit mechanisms at the cis-face of the Golgi complex. Cold Spring Harb Perspect Biol 2011; 3:a005207; PMID:21482742; http://dx.doi.org/10.1101/cshperspect.a005207

172. Cosson P, Demolle C, Hennecke S, Duden R, Letourneur F, Y., and Sec23- and Sec24-dependent recruitment of coatomer to membranes. Cell 1999; 10:3317-29; PMID:10512869; http://dx.doi.org/10.1016/s0092-8674(99)80275-6

173. Razi M, Chan EY, Tooze SA. Early endosomes and endosomal coat proteins are involved in ER retrieval. EMBO J 1996; 15:1792-8; PMID:8617224

174. Beck R, Rawet M, Wieland FT, Cassel D. The COPI system: Molecular mechanisms of the COPI complex in higher plants. Mol Cells 2015; 38:866-74; PMID:2606030a

175. Yu X, Breitman M, Goldberg J. A structure-based mechanism for direct protein sorting into ER-derived transport vesicles. Nature 1992; 360:603-5; PMID:1461285; http://dx.doi.org/10.1038/360603a0

176. Presley JF, Ward TH, Pfeifer AC, Siggia ED, Phair RD, Lippincott-Schwartz J. Dissection of COPI and Arf1 dynamics in vivo and role in Golgi function. Mol Biol Cell 2006; 17:487-93; PMID:16723730; http://dx.doi.org/10.1091/mbc.E05-07-0538

177. Yu, M, Chen EY, Tooze SA. Early endosomes and endosomal coat protein. Nature 2002; 417:187-93; PMID:12375429; http://dx.doi.org/10.1038/nature00675

178. Welsh LM, Tong AH, Boone C, Jensen ON, Otte S. Genetic and molecular interactions of the Erv41p-Erv46 complex involved in transport between the endoplasmic reticulum and Golgi complex. J Cell Sci 2006; 119:4730-40; PMID:17077122; http://dx.doi.org/10.1242/jcs.03250

179. Hosobuchi M, Kreis TE, Schekman R. SEC21 is a gene required for arf1-dependent recruitment of coatomer to membranes. Cell 1992; 70:1493-8; PMID:613821

180. Shibuya A, Margulis N, Christiano R, Walther TC, Barlowe C. The Erv41-Erv46 complex serves as a retrograde receptor to retrieve escaped ER proteins. J Cell Biol 2015; 208:197-209; PMID:25583996; http://dx.doi.org/10.1083/jcb.201408024

181. Endo BY, Veech JA. Morphology and histochemistry of soybean roots infected with Heteroder a glycines. Phytopathology 1965; 55:375-81.

182. Endo BY. Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by Heterodera glycines. Revue Nematology 1991; 14:73-84.

183. Scheideler M, Schlaich NL, Fellenberg K, Beissbarth T, Hauser NC, Tschorsch C, Deme molle C18, Emr60:1, and C19 emrol4 induce differentially expressed genes in lung adenocarcinoma: ERGIC3 is a novel lung cancer-related gene. BMC Cancer 2013; 13:44; PMID:23374247; http://dx.doi.org/10.1186/1471-2407-13-44

184. Breuza L, Gaynor EC, Hennecke S, Duden R, Emr SD, Riezman H, Cosson P. Coatomer is essential for retrieval of dilysocephalin in Golgi membrane transport. Nature 2002; 417:187-93; PMID:12375429; http://dx.doi.org/10.1038/nature00675

185. Ben-Tekaya H, Miura K, Pepperkok R, Hauri HP. Live imaging of bidirectional traffic from the ERGIC. J Cell Sci 2005; 118:357-67; PMID:15632110; http://dx.doi.org/10.1242/jcs.001615

186. Welsh LM, Tong AH, Boone C, Jensen ON, Otte S. Genetic and molecular interactions of the Erv41p-Erv46 complex involved in transport between the endoplasmic reticulum and Golgi complex. J Cell Sci 2006; 119:4730-40; PMID:17077122; http://dx.doi.org/10.1242/jcs.03250

187. Lorente-Rodriguez A, Barlowe C. Entry and exit mechanisms at the cis-face of the Golgi complex. Cold Spring Harb Perspect Biol 2011; 3:a005207; PMID:21482742; http://dx.doi.org/10.1101/cshperspect.a005207

188. Driouich A, Follet-Gueye ML, Bernard S, Kousar S, Chevalier L, Grifﬁths T, Berti B, Stambulova E, Navez P, Otte S, Barlowe C, Hong W, Hauri HP. Characterization of matrix polysaccharides of the primary cell wall of higher diophyte Selaginella kraussiana suggests that XTH sequence characterization and function are highly conserved during the evolution of
270. Cavalier DM, Keegstra K. Two xyloglucan xylosyltransferases catalyze the addition of multiple xylosyl residues to cellohexaose. J Biol Chem 2006; 281:34197-207; PMID:16982611; http://dx.doi.org/10.1074/jbc.M606379200

271. Cocuron JC, Lerouxel O, Drakakaki G, Alonso AP, Liepman AH, Keegstra K, Raikhel N, Wilkerson CG. A gene from the cellulose synthase-like C family encodes a beta-1,4 glucan synthase. Proc Natl Acad Sci U S A 2007; 104:8550-5; PMID:17488821; http://dx.doi.org/10.1073/pnas.0703133104

272. Tamura K, Shimada T, Kondo M, Nishimura M, Hara-Nishimura I. KATAMARI1/MURUS3 is a novel Golgi membrane protein that is required for endomembrane organization in Arabidopsis. Plant Cell 2005; 17:1764-76; PMID:15863516; http://dx.doi.org/10.1105/tpc.105.031930

273. Zabotina OA, Avci U, Cavalier D, Pattathil S, Chou YH, Eberhard S, Danhof L, Keegstra K, Hahn MG. Mutations in multiple XXT genes of Arabidopsis reveal the complexity of xyloglucan biosynthesis. Plant Physiol 2012; 159:1367-84; PMID:22696020; http://dx.doi.org/10.1104/pp.112.198119

274. Zabotina OA, Avci U, Cavalier D, Pattathil S, Chou YH, Eberhard S, Danhof L, Keegstra K, Hahn MG. Mutations in multiple XXT genes of Arabidopsis reveal the complexity of xyloglucan biosynthesis. Plant Physiol 2012; 159:1367-84; PMID:22696020; http://dx.doi.org/10.1104/pp.112.198119

275. Chou YH, Pogorelko G, Young ZT, Zabotina OA. Protein-protein interactions among xyloglucan-synthesizing enzymes and formation of Golgi-localized multiprotein complexes. Plant Physiol 2012; 159:1355-6; PMID:22665445; http://dx.doi.org/10.1104/pp.112.199356

276. Mann HB, Whitney DR. On a test of whether one of 2 random variables is stochastically larger than the other. Annals Mathemat. Statistics 1947; 18:50-60; http://dx.doi.org/10.1214/aoms/1177730491

277. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 2011; 8:785-6; PMID:21959131; http://dx.doi.org/10.1038/nmeth.1701