# Sorting inhibitors (Sortins): Chemical compounds to study vacuolar sorting in Arabidopsis

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# Sorting inhibitors (Sortins): Chemical compounds to study vacuolar sorting in *Arabidopsis*

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**Chemical genomics is an interdisciplinary approach that unites the power of chemical screens and genomics strategies to dissect biological processes such as endomembrane trafficking. We have taken advantage of the evolutionary conservation between plants and *Saccharomyces cerevisiae* to identify such chemicals. Using *S. cerevisiae*, we screened a library of diverse chemical structures for compounds that induce the secretion of carboxypeptidase Y, which is normally targeted to the vacuole. Among 4,800 chemicals screened, 14 compounds, termed sorting inhibitors (Sortins), were identified that stimulated secretion in yeast. In *Arabidopsis* seedlings, application of Sortin1 and -2 led to reversible defects in vacuole biogenesis and root development. Sortin1 was found to redirect the vacuolar destination of plant carboxypeptidase Y and other proteins in *Arabidopsis* suspension cells and cause these proteins to be secreted. Sortin1 treatment of whole *Arabidopsis* seedlings also resulted in carboxypeptidase Y secretion, indicating that the drug has a similar mode of action in cells and intact plants. We have demonstrated that screening of a simple eukaryote, in which vacuolar biogenesis is not essential, can be a powerful tool to find chemicals that interfere with vacuolar delivery of proteins in plants, where vacuole biogenesis is essential. Our studies were done by using a sublethal dose of Sortin1, demonstrating the powerful ability of the chemical to control the induced phenotype in a manner that would be difficult to achieve using conventional genetics.**

In plant biology, the isolation of T-DNA [portion of the Ti (tumor-inducing) plasmid that is transferred to plant cells] gene inactivation mutants has become a valuable tool for understanding gene function. However, the availability of viable and informative knockout mutants in genes involved in protein endomembrane trafficking is still limited. This constraint is due to the fact that many genes involved in trafficking have proven to be essential for gametophyte or embryo development (1–3) whereas others are in gene families containing members that are redundant functionally and can fully or partially relieve mutation effects (4). Although a method has been developed for the isolation of point mutants that may overcome the lethality associated with insertional gene knockouts (5), the development of additional tools that address the function of essential or redundant gene products would be extremely beneficial. Furthermore, plant biology would profit enormously from approaches that would yield both reversible and tunable plant responses, which are difficult or impossible to achieve using conventional genetic approaches.

Chemical genomics (genomics scale chemical genetics) offers such a powerful tool. This approach involves the screening of collections of synthetic compounds for those having desirable biological activities. The cognate targets of such compounds may identify novel pathways or novel interactions with known pathways. The cognate targets of biologically active compounds can then be determined by using the advanced genetics in plant systems such as *Arabidopsis thaliana* (6). This approach is in some ways analogous to that of classical forward genetics, in which collections of mutants are used to dissect pathways (for review, see refs. 7–10), except that chemical genomics offers distinct advantages. Because chemical libraries can be stored and screened in ordered arrays, one tremendous advantage is the ability to perform screening assays in a high-throughput or even automated mode. The design of such high-throughput screens that focus specifically on particular subcellular pathways may also pose challenges for plant biologists because plants are multicellular organisms with a highly complex developmental cycle. Nevertheless, for some pathways, in particular those that are evolutionarily conserved and cell autonomous, it may be possible to take advantage of simpler single-celled eukaryotes such as yeast. Although there are clear differences in protein endomembrane trafficking in plants and yeast (11), such as the fact that vacuoles are essential in plants but dispensable in yeast (3, 12), the machineries share similarities such as homologous genes and protein complexes (13, 14). Therefore, a potentially valuable approach to identify drugs that affect the endomembrane system of plants is to perform chemical screening employing *Saccharomyces cerevisiae*. In our assay, we focused on the identification of compounds that would alter the delivery of carboxypeptidase Y through the endomembrane system into the vacuole. Among the compounds identified, several drugs termed Sortins were biologically active in *Arabidopsis* plants and suspension cultures. Our results clearly demonstrate the power of this approach for identifying novel plant-active compounds. More importantly, we have discovered drugs to study the endomembrane system of plants, which has proven challenging to dissect by conventional genetics.

## Methods

**Phenotype Assays.** *S. cerevisiae* INVSc1 (*his3-Δ1*, *leu2*, *trp1-289*, *ura3-52*; Invitrogen) was used as a wild-type strain throughout this study and does not display secretion of carboxypeptidase Y (CPY) under normal conditions. A culture was grown in yeast-peptone-dextrose (YPD) medium at 28°C with constant shaking for 24 h. Yeast cells were then diluted 1,000 times in fresh media and plated in V-shaped 96-well polypropylene plates (Greiner Bio-One, Longwood, FL) at 100 $\mu$l per well, supplemented with chemical compounds in DMSO (4,800 from the DIVERSetE, Chembridge, San Diego) at a concentration of 10 mg/liter. Non-drug control cells were treated with DMSO only. After 2 days of incubation at 28°C with constant shaking, cells were centrifuged at 2,000 $\times$ *g*, and 60 $\mu$l of the growth media was transferred onto a nitrocellulose membrane by using a dot-blot apparatus (Bio-Rad). The membrane was then washed briefly with water and blocked with 5% milk in PBS. Secreted CPY was detected by monoclonal anti-CPY antibodies (Molecular Probes). The yeast vacuolar morphology was subsequently visualized by using a Leica TCS SP2/UV Confocal Microscope (Leica Microsystems, Wetzlar, Germany) and the dye MDY-64

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Abbreviations: Sortin, sorting inhibitor; CPY, carboxypeptidase Y; AtCPY, *Arabidopsis* homologue of CPY; EGFP, enhanced GFP; TIP, tonoplast intrinsic protein; ER, endoplasmic reticulum.

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(Molecular Probes). A *vps16*Δ deletion strain was used as a control yeast strain with altered vacuolar morphology (12). *Arabidopsis* seedlings expressing enhanced GFP (EGFP):δ-tonoplast intrinsic protein (TIP) chimeric protein (15) were germinated on Murashige and Skoog agar in the presence of chemicals. Vacuolar morphology was analyzed as described in ref. 16. To investigate the effect of the compounds at the molecular level, *Arabidopsis* and tobacco BY2 suspension cells (cultured as described in refs. 17 and 18) were exposed to drugs at various concentrations as indicated. Plant cell viability was visualized with fluorescein-diacetate (FDA) (19). In live, metabolically active cells, the nonfluorescent FDA is cleaved by esterases into a fluorescent product, whereas inactive, dead cells remain nonfluorescent (20).

**Identification of Secreted Proteins and Pulse–Chase Experiments.** To identify proteins that might be secreted after a chemical treatment, the growth medium was collected, concentrated, and analyzed by SDS/PAGE and immunoblotting. In addition, a pulse–chase experiment (21) was done to examine the secretion of an *Arabidopsis* homologue of CPY [AtCPY (22)] in the presence of Sortin1. Briefly, 1.2 ml of 4- to 5-day-old cells were distributed into 12-well microtiter plates and incubated with 132 μCi (1 Ci = 37 GBq) of Expre$^{35}$S$^{35}$S label (Perkin–Elmer) on an orbital shaker at 100 rpm. After 15 h, labeled proteins were chased by adding unlabeled methionine and cysteine to a concentration of 5 mM and 2.5 mM per well, respectively, and the cells were supplemented with either drug in DMSO (at 25 mg/liter, 57 μM) or DMSO (control). At the time points indicated, cells were pelleted by centrifugation for 30 s at 15,000 × *g*. The growth media were then subjected to immunoprecipitation as described in refs. 23 and 24 by using anti-AtCPY antibodies (22) and fractionated by SDS/PAGE, treated with a scintillant (Fluoro-Hance, Research Product International, Mount Prospect, IL), and fluorographed for 10 days.

**Immunoelectron Microscopy.** *Arabidopsis* seedlings were germinated in the presence of 57 μM Sortin1. Subsequently, sections of hypocotyls and roots were prepared (22) and used for all immunogold-labeling experiments as described in ref. 25. Controls were performed with the use of the corresponding preimmune serum substituted for the antisera. In all cases, the antisera demonstrated high specificity of the labeling.

## Results

**Screening for Biologically Active Compounds in Yeast.** In wild-type yeast strains, CPY is delivered to the vacuole by means of Golgi-to-endosome and endosome-to-vacuole pathways and cannot be detected outside the cell. Yeast *vacuolar protein sorting (vps)* mutants, by contrast, secrete a significant amount of CPY (26). This *vps* phenotype resulting from treatment with specific chemicals was the basis of our screen. We screened 4,800 compounds using an anti-CPY dot-blot immunoassay for the CPY secretion phenotype in the yeast strain INVSc1, which does not secrete CPY under normal conditions. The library chosen was available commercially and was composed of a diverse set of chemical structures (www.hit2lead.com). The library was supplied in a mg/liter format and has been used successfully by other researchers (27). As predicted for the screening of a library of diverse chemical structures, the preponderance of compounds (99%) had no detectable effect in initial screens. Nine compounds had fungicidal properties resulting in yeast lethality and were not examined further (data not shown). Forty compounds (0.8% of the total) were identified as "hits" in the primary screen. We confirmed that 14 (0.3% of the total) of these 40 compounds possessed biological activity at concentrations of 10 mg/liter and 100 mg/liter. The identification numbers of these 14 compounds according to the Chembridge database (www.hit2lead.com) and a qualitative description of the resulting CPY secretion under the primary screening concentration are presented in Table 1.

**Table 1. Effects of Sortins on the CPY secretion of yeast (*vps* phenotype) or the morphology of *Arabidopsis* or yeast vacuoles**

| Chembridge ID no. | *vps* phenotype | Vacuolar morphology |
|---|---|---|
| 6168516 (Sortin1) | Strong | *Arabidopsis* |
| 6239069 (Sortin2) | Strong | *Arabidopsis* |
| 6108321 (Sortin3) | Weak | Yeast |
| 5938112 | Weak | — |
| 6129791 | Weak | — |
| 6142844 | Weak | — |
| 6150489 | Weak | — |
| 6155208 | Weak | — |
| 6169464 | Weak | — |
| 5729136 | Weak | — |
| 6124558 | Weak | — |
| 6125186 | Weak | — |
| 6127923 | Weak | — |
| 6240429 | Weak | — |

Strong secretion is typified by Sortin1 and -2 (see Fig. 1 *D* and *E*), whereas weak secretion is typified by Sortin3 (see Fig. 1*F*).

Based on the primary screen phenotype, we named these drugs "Sortins" for protein sorting inhibitors. Two of them, Sortin1 (Fig. 1*A*) and Sortin2 (Fig. 1*B*) were considered high-priority hits based on the significant amount of secreted CPY (Fig. 1 *D* and *E*). Interestingly, doses of Sortin1 >25 mg/liter resulted in lesser, although still significant, stimulation of secretion. Although we do not understand the basis of this observation, the experiment was highly reproducible. The remaining 12

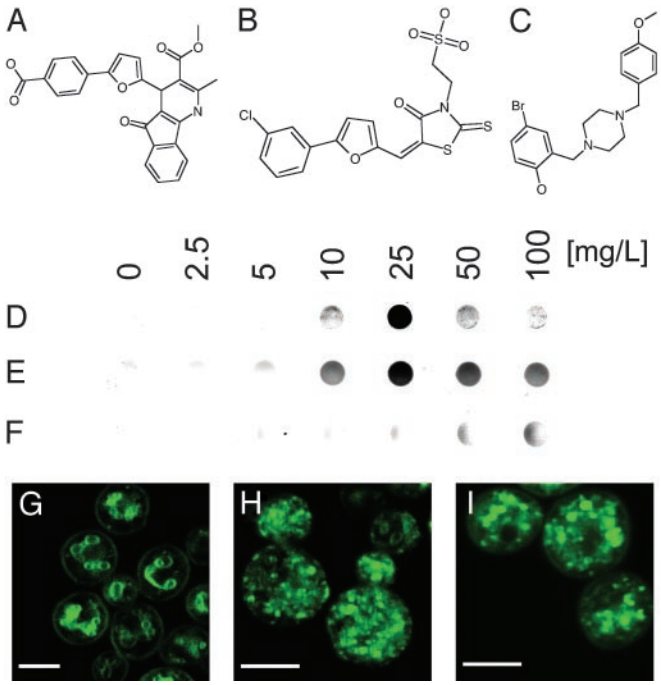


**Fig. 1.** Sortins trigger CPY secretion in yeast. Shown are chemical structures of Sortin1 (*A*, MW 441.44), Sortin2 (*B*, MW 429.92), and Sortin3 (*C*, MW 391.32). A dot-blot assay indicated that Sortin1 (*D*) and Sortin2 (*E*) generated strong *vps* phenotypes. In addition to a weak *vps* phenotype (*F*), Sortin3 induced a severe *vam* phenotype (*H*), similar to that of Class C *vps* yeast mutant *vps16*Δ (*I*). The tonoplast morphology of untreated yeast is shown (*G*). Concentrations are as indicated. (Scale bars = 5 μm.)

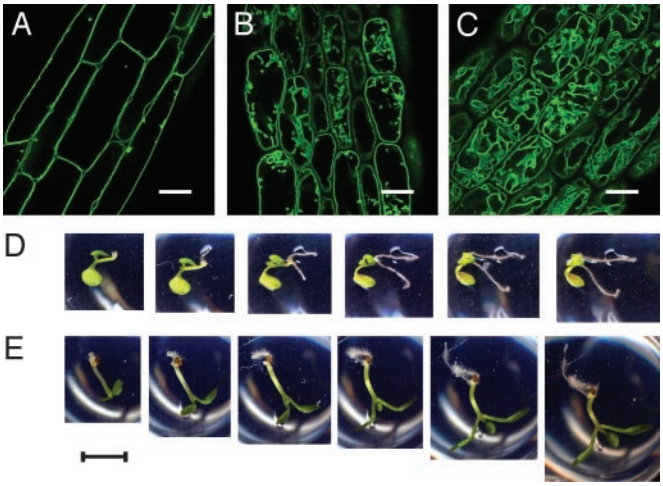


**Fig. 2.** Sortin1 and Sortin2 alter vacuolar morphology in *Arabidopsis* seedlings. Seedlings expressing EGFP:δ-TIP as a tonoplast marker were germinated in the absence of Sortins (*A*) or in the presence of 227 μM Sortin1 (*B*) or 233 μM Sortin2 (*C*). Vacuolar morphology in hypocotyls of 1-week-old seedlings was examined by confocal microscopy. Plants were then transferred onto non-drug medium to demonstrate reversibility of the phenotype induced by Sortin1 (*D*) and Sortin2 (*E*). Images in *D* and *E* (left to right) were taken at 0, 1, 2, 3, 4, and 5 days after transfer to non-drug medium. (Scale bars = 20 μm in *A–C* and 3 mm in *D* and *E*.)

compounds showed a weak degree of secretion enhancement compared with Sortin1 and -2, which was typified by Sortin3 (Fig. 1*F*). Untreated control cells showed little or no detectable secretion using the immunoblot assay (Fig. 1 *D–F*; 0 mg/liter). In addition to the *vps* phenotype, yeast cells were examined for an *altered vacuolar morphology* (*vam*) phenotype (28). When viewed by confocal microscopy using the specific vacuolar membrane dye MDY-64, 13 of the 14 compounds resulted in no obvious effect on yeast vacuolar morphology (Table 1). However, one of the compounds, Sortin3 (Fig. 1*C*), when compared with the untreated control cells (Fig. 1*G*), induced a dramatic and severe *vam* phenotype in yeast (Fig. 1*H*). This phenotype was similar to that of the control yeast mutant *vps16*Δ, which displays a *vam* phenotype (12) (Fig. 1*I*). In a search of the SciFinder database (Version 2004, American Chemical Society), we found no references to compounds with the structures of Sortin1, -2, and -3 having biological activity.

**Sortins Result in Aberrant Vacuoles in Intact Plants and Are Reversible.** All fourteen compounds were tested on *Arabidopsis* plants expressing EGFP:δ-TIP (15). Seedlings were germinated and grown in the presence of the drugs for 1 week and examined for vacuolar phenotype by confocal microscopy. Interestingly, compared with untreated control plants (Fig. 2*A*), the vacuolar membranes in hypocotyls of seedlings grown in the presence of 100 mg/liter (227 μM) Sortin1 or Sortin2 (233 μM), high-priority hits from the initial screen, seemed highly fragmented (Fig. 2 *B* and *C*). These findings suggest that the compounds likely target the vacuolar biogenesis machinery in plants. The remaining 12 compounds had no effect on vacuolar morphology at 100 mg/liter tested (data not shown).

In addition to a tonoplast morphology phenotype, Sortin1 also severely affected root development at 100 mg/liter. Despite this dramatic effect, the phenotype induced by Sortin1 was found to be reversible after transfer to non-drug medium (Fig. 2*D*). Sortin2 treatment also resulted in a dramatic inhibition of root development at 100 mg/liter and displayed reversibility similar to that of Sortin1 (Fig. 2*E*).

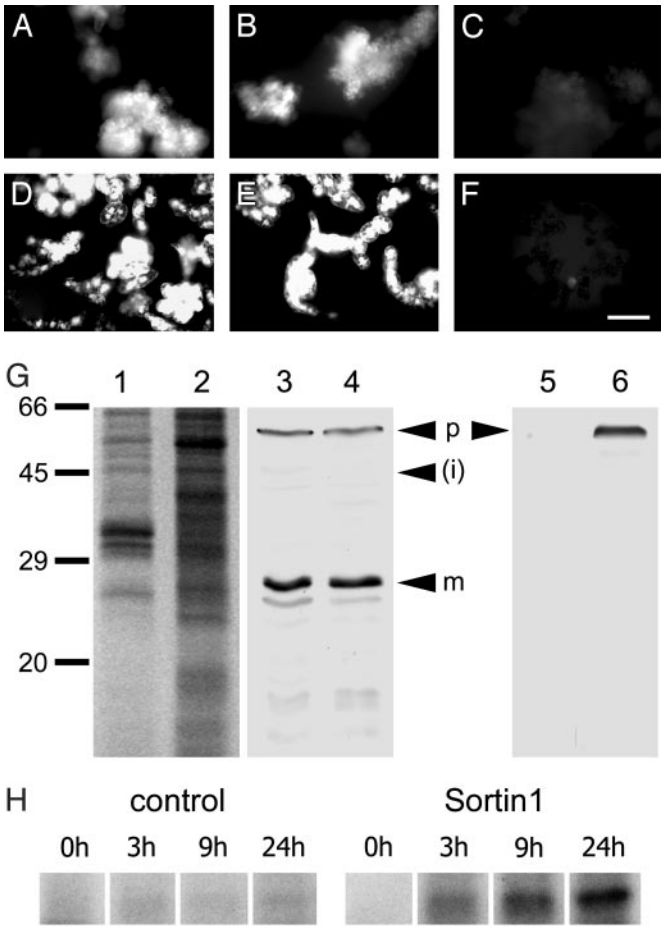


**Fig. 3.** Sortin1 induces secretion of the AtCPY precursor in *Arabidopsis* cell suspensions. The viability of *Arabidopsis* (*A–C*) and tobacco BY2 (*D–F*) suspension cells that were untreated (*A* and *D*) or treated with 57 μM Sortin1 (*B* and *E*) or 58 μM Sortin2 (*C* and *F*) was analyzed by using fluorescein-diacetate. (*G*) Proteins secreted into the growth media of *Arabidopsis* suspension cells that were untreated (lane 1) or treated with Sortin1 (lane 2) were concentrated and analyzed by SDS/PAGE. Growth medium samples were also analyzed by Western blot (control, lane 5; Sortin1, lane 6). The AtCPY processing pattern was examined in cell pellets (control, lane 3; Sortin1, lane 4). The positions of the 60-kDa precursor (p), 48-kDa intermediate (i), and 24-kDa mature (m) forms of AtCPY are indicated. (*H*) A pulse–chase experiment tracking the AtCPY precursor secretion in cells that were untreated (control) or treated during the chase period with Sortin1 (Sortin1). (Scale bar = 500 μm in *A–F*.)

**Sortin1 Stimulates Protein Secretion in *Arabidopsis* Suspension Cells.** To examine the effect of Sortin1 and Sortin2 on secretion at the biochemical level, we treated plant suspension cells with the drugs. Cell viability was first examined at several concentrations over time in *Arabidopsis* and tobacco BY2 cells by using the vital dye fluorescein-diacetate (data not shown). When compared with untreated control cells (Fig. 3 *A* and *D*), Sortin1 was well tolerated at a concentration of 25 mg/liter (57 μM) for >16 h by both *Arabidopsis* and tobacco cells (Fig. 3 *B* and *E*). To the contrary, Sortin2 was highly toxic, and the cells were found without metabolic activity at a concentration of 25 mg/liter (58 μM) after 8 h (Fig. 3 *C* and *F*). Cell death was also apparent at doses of Sortin2 as low as 10 mg/liter (23 μM) after only 4 h (data not shown). The similar results from *Arabidopsis* and tobacco cultured cells indicated that the toxic effect of the drugs was not specific to cells from a single plant species. Due to the high toxicity of Sortin2 on cultured cells, and thus the possibility of cell lysis, we focused on Sortin1 to ask whether a sublethal dose would result in a significant increase in overall protein secretion.

After treatment with 57 $\mu$M Sortin1 for 16 h, we concentrated the growth media and separated the proteins by SDS/PAGE. The protein profiles in Coomassie blue-stained gel lanes from untreated and treated *Arabidopsis* cells (Fig. 3*G*, lanes 1 and 2, respectively) differed significantly, suggesting that Sortin1 triggered a general increase in protein secretion. AtCPY is known to undergo processing after delivery to the vacuole (22), so we assayed for intracellular and extracellular mature and precursor forms of AtCPY to provide direct evidence of altered fidelity of protein sorting. Indeed, when we analyzed the concentrated growth media by immunoblots with anti-CPY antibodies, we identified a precursor of AtCPY in medium from cells treated with Sortin1 (Fig. 3*G*, lane 6) that was absent in an untreated control sample (Fig. 3*G*, lane 5). Our data indicated that a notable amount of AtCPY precursor was diverted to the secretion pathway as a result of Sortin1 treatment. The lack of mature AtCPY in the medium indicated that AtCPY detected in the medium was probably the result of active secretion and not cell and vacuole lysis. A similar analysis of cell pellets did not reveal any obvious difference in intracellular processing of AtCPY (Fig. 3*G*, lanes 3 and 4). This finding indicated that, at the sublethal dose examined, not all AtCPY was secreted. However, the proportion that was secreted was diverted at a step before proteolytic processing. This finding also pointed to the fine level of control that was possible with Sortin1 derived from the chemical screen.

To demonstrate conclusively that Sortin1 induced secretion of the AtCPY precursor in metabolically active cells, we performed a pulse–chase experiment in *Arabidopsis* suspension cells in the presence of Sortin1. Cells were labeled with $^{35}$S-amino acids and chased with unlabeled amino acids in the presence or absence of Sortin1. The AtCPY that was secreted into the media was then immunoprecipitated by using anti-CPY antibodies and analyzed by SDS/PAGE and fluorography. Cells treated with Sortin1 displayed a rapid accumulation of a polypeptide corresponding to the unprocessed precursor of AtCPY. Compared with an untreated control in which little or no precursor was detected even after 24 h (Fig. 3*H*, control), this accumulation was detectable in 3 h or less after Sortin1 treatment (Fig. 3*H*, Sortin1). These results clearly established that Sortin1 stimulated secretion from cells that were metabolically active and intact.

**Sortin1 Stimulates Secretion in Whole Plants.** To investigate the effect of Sortin1 in intact plants, we performed immunoelectron microscopy of AtCPY in 1-week-old *Arabidopsis* seedlings grown in the presence of the drug at a concentration of 57 $\mu$M. Compared with untreated controls (Fig. 4 *A* and *G*), immunogold labeling detected a significant amount of AtCPY in the apoplast of hypocotyl (Fig. 4*B*) and root (Fig. 4*H*) tissue of treated seedlings. Consistent with the results of the immunoblots, AtCPY was also detected in vacuoles of untreated and treated seedlings (Fig. 4 *D* and *E*, respectively), again indicating that, at sublethal doses, redirection to the secretion pathway was not complete. We also performed immunolocalization of the vacuolar invertase AtFruct4 (22) in root tissue of Sortin1-treated *Arabidopsis* seedlings. In young seedlings, AtFruct4 was previously localized to endoplasmic reticulum (ER)-derived precursor protease vesicles (22) and thus utilizes a different pathway than AtCPY for vacuolar targeting. Labeling of AtFruct4 was clearly observed in precursor protease vesicles of 1-week-old seedlings treated with Sortin1 (Fig. 4*I*). Interestingly, no AtFruct4 labeling in the apoplast was detected (Fig. 4*I*), suggesting that Sortin1 probably targets specific protein sorting pathways. In addition, when we analyzed the concentrated growth medium from cultured *Arabidopsis* cells treated with Sortin1, no vacuolar invertase was detected, which is consistent with our immunolocalization and cell viability assays (data not shown). Several drugs known to affect endomembrane trafficking have significant impacts on Golgi morphology (reviewed in ref. 29). To further characterize the effect of 57 $\mu$M Sortin1 on the endomembrane system, we examined the morphology of the Golgi apparatus (Fig. 4*J Left*) and ER (Fig. 4*J Right*) in hypocotyls of 1-week-old seedlings by electron miscroscopy. Sortin1 treatment did not affect Golgi structure or trigger loss of cisternae. Additionally, 57 $\mu$M Sortin1 did not alter the morphology of either the ER or the central vacuole in seedlings (Fig. 4*K*) or *Arabidopsis* suspension cells (Fig. 4*L*).

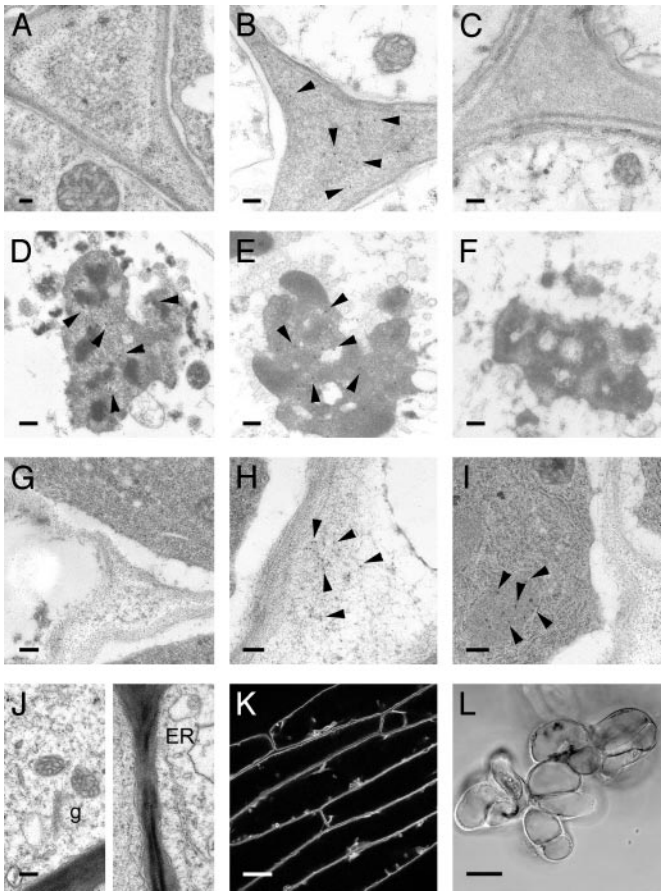


**Fig. 4.** AtCPY is secreted into the apoplast in Sortin1-treated 1-week-old *Arabidopsis* seedlings. Immunolocalization of AtCPY was performed in sections of control plants (hypocotyl, *A* and *D*; root, *G*) or Sortin1-treated plants (hypocotyl, *B* and *E*; root, *H*). A preimmune serum was used as a control (*C* and *F*). Immunolocalization of invertase AtFruct4 was performed in Sortin1-treated roots (*I*). *A–C* and *G–I* show apoplast whereas *D–F* are of vacuoles. Arrowheads indicate the position of gold particles. Golgi (g) (*J Left*) and ER (*J Right*) morphology was examined in 1-week-old Sortin1-treated seedlings by electron microscopy. Tonoplast morphology in 1-week-old seedlings expressing EGFP:$\delta$-TIP and treated with Sortin1 was analyzed by confocal microscopy (*K*). *Arabidopsis* suspension cells were treated with Sortin1 for 16 h and examined by brightfield light microscopy (*L*). (Scale bars = 200 nm in *A–J* and 20 $\mu$m in *K* and *L*.)

## Discussion

We have taken advantage of similarities in the secretion pathways in yeast and plants to screen for chemicals that stimulate secretion. Of the 14 confirmed compounds from our initial yeast screen, two were found to be active in plants and to affect vacuole biogenesis and root development in *Arabidopsis* seedlings. The severe yet reversible effects of Sortin1 and -2 on root development may be due to the essential nature of vacuole biogenesis in plants. For example, the *vcl1* mutant of *Arabidopsis* lacks proper vacuole development, missorts vacuole proteins to the extracel-

lular space, and is embryo lethal (3). Thus, it is reasonable that drugs that disrupt these processes would lead to growth defects. The severity of root phenotype was likely enhanced by direct contact of the developing roots with the drug-containing growth media.

One of the compounds, Sortin1, was examined in detail and found to stimulate protein secretion in general and AtCPY in particular in both cell cultures and the whole plants. We have demonstrated the validity of using a simple eukaryote to screen for drugs that affect the plant endomembrane machinery, which reflects evolutionary similarities. However, the fact that not all of the chemicals were biologically active points to significant differences between yeast and multicellular plants. Such differences could be in uptake, intercellular transport, or metabolism of the drugs due to the complex multicellular nature of plants compared with yeast and other evolutionary differences in the sorting machineries. Thus, the use of yeast must be viewed as a complement to plant-based screens.

The few drugs that have been available that affect the endomembrane system including the Golgi-disturbing drugs brefeldin A and monensin (reviewed in ref. 29), the N-glycosylation inhibitor tunicamycin (reviewed in ref. 30), and the recently identified Exo1 (27) have been extremely useful in increasing our understanding of the endomembrane system even though all of their cognate targets are still unclear (27, 31). Recognizing the power of such reagents, we have used a chemical genomics approach to identify several sorting drugs termed Sortins. In contrast to brefeldin A and Exo1, which interfere with exocytosis, Sortins seem to stimulate secretion of AtCPY and other proteins but not proteins delivered to the vacuole by means of protease precursor vesicles. The ionophore monensin has been shown to stimulate protein secretion in cotelydons (32, 33). However, it is unlikely that a mode of action of Sortin1 is analogous to that of monensin. At the cellular level, the most dramatic phenotypes resulting from monensin treatment are swollen Golgi cisternae. We examined the Golgi structure by electron microscopy after Sortin1 treatment and found that the cisternae stack was intact. This result indicates that Sortin1 probably does not act as a monensin-like ionophore and functions through a different mode of action. We examined the activity of Sortin1 at a sublethal dose. Given that vacuole biogenesis is essential in plants (3), the ability to "fine tune" the biological response is a critical advantage over the use of conventional genetics in which such adept control is seldom possible.

Although we do not yet know the cognate target of Sortin1, the general stimulation of protein secretion and the secretion of AtCPY precursor, as well as aberrant vacuole biogenesis, suggest that the drug acts at a step before proteolytic processing and may interfere with vesicle budding or fusion or other essential process in vacuole biogenesis. One of the major challenges of the chemical genomics approach is target identification. *Arabidopsis* is very well suited for identification of putative targets by isolation of either resistant or hypersensitive mutants to a particular compound (10). *Arabidopsis* has also proven to be a useful organism for the identification of a potential target for a specific bioactive compound (6). A powerful advantage of chemical genomics not often recognized is the ability to challenge characterized mutants with drugs like Sortin1 to look for interactions within a pathway or with interacting pathways. In addition, the use of chemical analogues with altered or no biological activity could provide correlations between structure and activity. Such analyses will facilitate target identification and provide additional insights into the mechanics of endomembrane transport.

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