

## Is there leaderless protein secretion in plants?

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**T**he sugar alcohol mannitol and its catabolic enzyme mannitol dehydrogenase (MTD), in addition to well-documented roles in metabolism and osmoprotection, may play roles in host-pathogen interactions. Research suggests that in response to the mannitol that pathogenic fungi secrete to suppress reactive oxygen-mediated host defenses, plants make MTD to catabolize fungal mannitol. Yet previous work suggested that pathogen-secreted mannitol is extracellular, while in healthy plants MTD is cytoplasmic. We have presented results showing that the normally cytoplasmic MTD is exported into the cell wall or extracellular space in response to the endogenous inducer of plant defense responses salicylic acid (SA). This SA-induced secretion is insensitive to brefeldin A, an inhibitor of Golgi-mediated protein transport. Together with the absence of MTD in Golgi stacks and the lack of a documented extracellular targeting sequence in the MTD protein, this suggests MTD is secreted by a non-Golgi, pathogen-activated secretion mechanism in plants. Here we discuss the potential significance of non-Golgi secretion in response to stress.

### Introduction

Because the cell wall is a major interface between plant cells and their environment, the rapid, regulated secretion of specific proteins into this extracellular space (the apoplast) is an important defense response.<sup>1</sup> Secretion of defense proteins in both plants and animals was originally thought to be solely via an endoplasmic reticulum (ER)/Golgi-mediated pathway, with an N-terminal signal peptide

directing the protein to the ER for routing, modification and subsequent secretion via the Golgi. However, the existence of alternate secretion mechanisms was suggested when Auron et al.<sup>2</sup> reported that interleukin 1 (IL1 $\beta$ ), a cytokine with no signal peptide, was secreted from human monocytes in response to *Staphylococcus*. Since then, numerous Golgi-independent or “leaderless” eukaryotic secretion mechanisms have been reported, and the importance of these pathways, particularly in response to stress, is well established.<sup>3</sup> Although non-Golgi secretion has been described in many other eukaryotes, our report that the normally cytoplasmic enzyme, mannitol dehydrogenase (MTD) is secreted by tobacco in response to salicylic acid (SA) is one of the few reports suggesting that non-classical secretion also occurs in plants.<sup>4</sup>

The regulated conversion of mannitol to mannose by MTD in the cytosol of plants such as celery (*Apium graveolens*) allows mannitol to be used as both a metabolite and an osmoprotectant.<sup>5</sup> There is now compelling evidence that mannitol and MTD also play roles in plant-pathogen interactions. Mannitol’s antioxidant activity<sup>6,7</sup> together with its secretion by fungal pathogens<sup>8-10</sup> allows these pathogens to use mannitol to quench reactive oxygen species (ROS) that signal plant defenses. In response, pathogen-induced plant MTD was hypothesized to catabolize this mannitol, thus protecting the host’s ROS-mediated defenses.<sup>5,11,12</sup> However, while fungal mannitol secreted during infection is found in the extracellular space,<sup>8,11</sup> MTD is cytoplasmic in uninfected celery.<sup>13</sup> If MTD catabolizes fungal mannitol, then mannitol and MTD must somehow become co-localized.

**Key words:** protein secretion, mannitol metabolism, plant-pathogen interaction, extracellular space, apoplast

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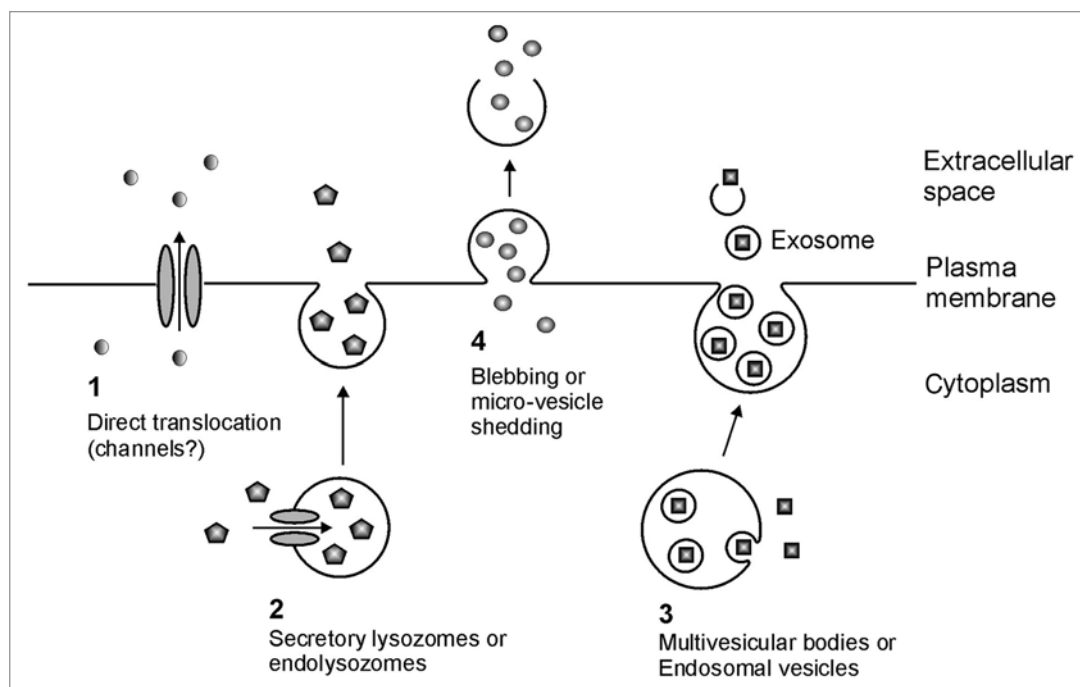
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**Figure 1.** Proposed mechanisms for non-Golgi secretion of normally cytoplasmic proteins in animals. (1) Translocation directly across the plasma membrane. (2) Endolysosomal or secretory lysosomal pathway. (3) Intra-endosomal vesicle or multi-vesicular body pathway. (4) Membrane blebbing or micro-vesicle shedding. Adapted from Nickel and Rabouille.<sup>3</sup>

In Cheng et al.<sup>4</sup> we demonstrated that MTD is exported to the apoplast in response to SA, an endogenous inducer of many plant defense responses. This is of special significance because MTD lacks a recognized ER/Golgi signal sequence, and its SA-induced secretion is not inhibited by an inhibitor of Golgi-mediated protein transport, brefeldin A. Thus, contrary to the model that PR-protein secretion occurs only via the ER/Golgi pathway, secretion of MTD appears to be by a non-Golgi mechanism.

### Types of Non-Golgi Secretion

The existence of leaderless secretion in animals is well accepted, and four general mechanisms have been proposed (Fig. 1): (1) *Translocation directly across the plasma membrane*: proteins such as fibroblast growth factor 1 (FGF-1)<sup>14</sup> and IL1 $\alpha$ <sup>15</sup> are translocated directly across the plasma membrane via the chaperonin-like action of hydrophobic “release complexes” or through transmembrane channels. (2) *Endolysosomal pathways*: normally cytoplasmic proteins such as IL-1 $\beta$ <sup>16</sup> and HMGB1,<sup>17</sup> are transported into intracellular vesicles called endolysosomes via

protein-conducting channels such as ATP-binding cassette (ABC) transporters. These endolysosomes then fuse with the cell membrane to release the proteins into the apoplast. (3) *Exosome-mediated secretion*: first intracellular vesicles called endosomes are generated by inward budding from the plasma membrane. Cytosolic proteins, such as HSP90 are then packaged into the endosomes by further inward budding to form multivesicular bodies. These next fuse with the plasma membrane to release the included, formerly cytosolic components.<sup>18</sup> (4) *Membrane blebbing or microvesicle shedding*: cytosolic secretory transglutaminase and galectins<sup>19</sup> are proposed to be packaged directly into membrane-derived blebs, or protrusions. The blebs detach and the formerly cytosolic components are released when the blebs rupture.

Non-classically secreted proteins appear to be first modified in response to a stress mediated signal, enabling them to interact with the relevant secretion machinery.<sup>20</sup> These proteins are then apparently transported across the membranes in a folded or native configuration like FGF2.<sup>21</sup> As a result, the “targeting signal” is an integrated feature of the protein’s

three-dimensional structure rather than a simple linear amino acid sequence.

### Why Non-Golgi Secretion?

Proteins might be secreted by nonclassical mechanisms for a number of reasons. For instance, non-Golgi secretion would be necessary if the presence of a protein in the ER/Golgi would disrupt ER functioning. For example, in animal cells the non-Golgi secretion of galectin 1 keeps this  $\beta$ -galactose-binding lectin from binding glycolipids and glycoproteins in the lumen of the Golgi/ER.<sup>22</sup> Non-Golgi secretion could also be desirable if a protein has multiple functions, with each occurring in a different cellular compartment. HMGB1 (High mobility group box 1), for example, is normally found in the nucleus, where it mediates DNA-binding complex assembly. Upon bacterial induction of monocytes, HMGB1 is secreted into the extracellular space where it acts as an endotoxin mediator or a cell differentiation signal.<sup>23</sup> As previously documented,<sup>5</sup> MTD has normal roles in central metabolism and osmoregulation in the cytoplasm. When mobilized during pathogen attack, however, MTD has

a very different role in the extracellular space. As MTD's normal role in central metabolism requires cytoplasmic localization, a nonclassical mechanism is needed to mediate its subsequent secretion in response to pathogen attack.

There are a growing number of indications that non-Golgi mechanisms are involved in the secretion of proteins other than MTD in plants. For instance, although extracellular SODs (ecSOD's) containing a classical signal peptide are well known in animals, plant SODs lack such a signal sequence, and were long thought to be absent from the apoplast. This raised questions about how superoxide was converted to H<sub>2</sub>O<sub>2</sub> in the apoplast during the early stages of defense responses. In fact, many researchers are now reporting that SOD activity is present in the apoplast of stress or pathogen-induced plants.<sup>24-28</sup>

Subsequent mass spectrophotometric analyses of SA-induced protein secretion have not only confirmed the presence of SOD in the secretome,<sup>29-31</sup> but suggest that a large number of proteins whose regulated secretion occurs soon after SA treatment lack a classical signal peptide.<sup>31</sup> Finally, this is consistent with the report that pathogen-induced apoplastic H<sub>2</sub>O<sub>2</sub> production in *Arabidopsis* is insensitive to BFA.<sup>32</sup> Taken together, the preponderance of data suggests the existence of a novel, pathogen-activated, protein secretion mechanism that mediates pathogen-induced secretion of normally cytoplasmic enzymes in plants.

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