Combining polysaccharide biosynthesis and transport in a single enzyme: dual-function cell wall glycan synthases

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INTRODUCTION

Extracellular matrices (ECMs) are complex biopolymer mixtures produced by cells and deposited outside the cell membrane. Diverse eukaryotic and prokaryotic organisms throughout the biosphere produce ECMs, making the ECM a common feature of most living cells. ECMs play an integral role in many essential processes, from influencing the growth and development of cellular and organismal morphology to mediating interactions between cells and their environments.

The specific composition and structure of the ECM varies widely depending on species, cell type, developmental stage, and environmental conditions. Despite these differences, there are some aspects of ECM composition that are similar throughout most of biology. One common feature of the ECM produced by diverse species is the prevalence of polysaccharides as major structural components. Examples include pectin, hemicellulose, and cellulose polymers of the plant cell wall, chitin found in fungal, mollusk, and arthropod ECMs, chondroitin and hyaluronan synthesized in vertebrates, and peptidoglycan of bacterial cell walls.

Although there is wide variation in composition, structure, and function of ECM polysaccharides found in nature, there are certain required steps that must be accomplished during the biosynthesis of any extracellular polysaccharide. Essential processes include the translation of precursor mRNAs into polypeptides, the processing and modification of the polypeptides to form glycoprotein precursors, and the deposition of these glycoproteins into a polysaccharide polymer. In some cases, different sugar moieties incorporated into a polysaccharide may be transported across the membrane by different mechanisms. ECM polysaccharides whose activated sugar precursors are transported before polymerization include the glycan components of extracellular glycoproteins.

In contrast to transport that occurs before or after polymer synthesis, this review focuses on another mechanism of transport across the membrane that occurs at the same time as polymer synthesis and directly involves the biosynthetic enzyme. Enzymes that appear to processively synthesize ECM polysaccharides while transporting the polymer across a membrane are found in diverse species, from unicellular bacteria and Archaea to the largest multicellular plants and animals in the biosphere. Despite these differences, there are some aspects of ECM composition that are similar throughout most of biology.
Although we are just beginning to understand the fine structure of polysaccharide. An unsubstituted β-(1→4)-linked polymer of glucose (cellulose) is approximately 1 nm across in its widest axis. Because of the relatively high energy cost of partitioning glucose to a hydrophobic environment (Ha et al., 1991; Kollman, 1993; Mazzocchi et al., 2005), it is likely that a glucose polymer remains hydrated as it moves across the membrane. Therefore, the absolute minimum diameter for a GT transmembrane pore would be about 1.8 nm, allowing for water molecules associated with the polysaccharide. Assuming the outer diameters of standard alpha helices range from 1 to 1.4 nm, the minimum number of TMHs required to form a pore larger than 1.8 nm is between 10 and 18 helices.

Although useful as a starting point for modeling the number of TMHs required for a dual-function GT, the calculations above are necessarily based on assumptions about the minimum diameter of a polysaccharide transport pore and the diameter of TMHs. In addition, this estimate assumes that TMHs are oriented perpendicular to the plane of the membrane. In reality, it is likely that some pore-forming TMHs of a dual-function GT span the membrane at an angle, creating a larger pore. For example, one bacterial HS has five to six membrane domains, functions as a monomer, and transports a polysaccharide with a wider diameter than the β-(1→4)-linked glucan described above (Tlapak-Simmons et al., 1998). This is possible in part because one or more of the membrane domains of this bacterial HS appear to be reentrant loops, occupying more space parallel to the plane of the membrane than a TMH inserted perpendicular to the membrane plane (Heldermon et al., 2001).

Another likely structural requirement for a pore-forming membrane protein is the ability to restrict free diffusion of water and other small molecules across the membrane. If a pore large enough to accommodate a hydrated polysaccharide were to remain open in the absence of a polymer occupying the pore, the electrochemical potential of the membrane would be compromised. Therefore,
it is likely that dual-function GTs have a mechanism for pore gating. Gating could be mediated by the presence of an accessory protein, as occurs with the Sec61 translocon complex and the Hsp70 chaperone BiP (Alder et al., 2005). Alternatively, GTs may function as a pore gating mechanism, as occurs with the Sec61 translocon complex and gating. Gating could be mediated by the presence of an accessory protein by phosphorylation (Chaumont et al., 2005). In addition to the general requirement of cells to maintain membrane potential, this can be important for the function of dual-function GTs themselves, as evidenced by the ion potential requirement for the activity of CESA in plants and bacteria (Bacic and Delmer, 1981; Delmer et al., 1982).

There is a third possibility for how organisms with dual-function GTs function. In the formation of a pore, the GT protein is mature and competent to form a pore, it could be localized to a specialized vesicular compartment where free diffusion across the membrane has little consequence. Fusion of such vesicles to the appropriate membrane where polysaccharide biosynthesis occurs would be coupled with activation of the GT, followed by removal of the GT from the diffusion-sensitive membrane when synthesis is complete. If such a mechanism exists, it could provide a partial explanation for atypical vesicles observed in chitin (chitosomes) and cellulose biosynthesis (MASGs), and might relate to why CESA proteins spend such a short time at the PM (Barmacks-Garcia, 2006; Croswell et al., 2010; Wightman and Turner, 2010).

Many dual-function GTs function in enzyme complexes, so proteins other than the glycan synthase may participate in the formation of a pore. Cucurion et al. (2007) found that Pichia pastoris expressing both the xyloglucan glucan synthase CSLC4 and the xyloglucan xlyoholysyltransferase XXT1 accumulated insoluble, unsubstituted β-(1 → 4)-linked glucan. Much lower levels of this polymer accumulated in both wild-type Pichia and lines expressing only CSLC4, which produced shorter, soluble oligosaccharides that were absent in dual-expressing and wild-type lines. In other words, CSLC4 functions as an active glucan synthase in both transgenic yeast cell lines, but produces a product with a much higher degree of polymerization when co-expressed with XXT1. Interestingly, Pichia does not synthesize UDP-xylose, the substrate of XXT1, and the β-(1 → 4)-linked glucan produced is unsubstituted. One explanation for this observation could be that XXT1 (a type II membrane protein) participates in the formation of a pore with CSLC4.

At least some dual-function GTs may be dependent on non-protein membrane components. A major advance in our understanding of plant CESA biochemistry has been the development of techniques to measure cellulose and callose synthesis from diverse species, it seems likely that the dual-function mechanism provides an evolutionary advantage. One possibility is that dual-function GTs allow greater control over enzyme function from within the cell. For example, the predicted topology of CESA proteins places over 90% of the hydrophilic regions of these proteins facing the cytosol. This presents large surfaces of CESA proteins to the cytosolic milieu, providing ample opportunity for interactions with cytosolic factors. Multiple phosphorylation sites have been identified on CESA proteins using mass spectrometry-based approaches (Niibe et al., 2004; Taylor, 2007). Work by Taylor (2007) connected CESA7 protein phosphorylation at sites near the protein’s amino terminus with proteasome-dependent degradation. This study opens many potential avenues of exploration. For instance, under what circumstances does CESA7 target for phosphorylation, and which proteins...
control the phosphorylation status? Does phosphorylation target CESA7 for proteasome degradation directly? Alternatively, might phosphorylation regulate another process such as CSC assembly or motility, which subsequently results in proteasomal degradation?

Chen et al. (2010) investigated potential physiological roles of multiple phosphorylation sites on CESA1. The researchers expressed versions of CESA1 with mutations designed to eliminate or mimic protein phosphorylation at several sites. Depending on the phosphorylation site and modification, some transgenic plants exhibited altered cell expansion anisotropy. In the plants with altered cell expansion, movement of CSCs along microtubules was also affected. Though the molecular mechanisms are not understood, this work indicates that CESA phosphorylation can influence microtubule-directed translation of actively synthesizing CSCs, potentially through interactions with proteins connecting the CSC to microtubule “tracks.”

Recently, Gu et al. (2010) identified a protein that appears to be involved with physically connecting CSCs to cortical microtubules, highlighting another important interaction between the dual-function GT CESA proteins and cytosolic factors. Through a yeast two-hybrid approach, Gu et al. (2010) identified an armadillo repeat protein they termed cellulose synthase-interactive protein 1 (CSI1). Plants with disrupted CSI1 have reduced CSC motility and less uniformity of cellulose microfibril orientation (Gu et al., 2010). The CSI1 gene was later shown to be allelic with a previously identified cell expansion mutant POM-POM2 (Hauser et al., 1995) and further characterized by Bringmann et al. (2012). This work convincingly demonstrates that POM2/CSI1 protein is important for CESA protein association with microtubules (Bringmann et al., 2012). Considering the results of Chen et al. (2010) and the recent work on CSI1, it will be interesting to see whether CESA phosphorylation is related to CSI1 functions.

In addition to controlling motility and degradation of CESA, another potential advantage of a cytosolic active site involves substrate availability. As mentioned above, the activated sugars that are substrates for polysaccharide synthesis are made primarily in the cytosol. So cytosolic active sites allow GTs to access substrate without requiring an independent transport protein. This might be of particular advantage for PM-localized dual-function GTs, where relatively labile activated sugars might degrade or diffuse away from sites of synthesis if exported before polymer synthesis. Taking this idea a step further, a cytosolic active site might allow the development of protein interactions for metabolite channeling. Indeed, sucrose synthase (SUS), which converts sucrose into fructose and UDP-glucose, the substrate for cellulose biosynthesis, has been observed at the PM (Amor et al., 1995) directly associated with sites of cellulose synthesis and structures isolated from membranes that appear to be CSCs (Salnikov et al., 2000; Fujii et al., 2010).

Another potentially advantageous structural property of dual-function GTs involves the TMHs. If the model shown in Figure 1 is representative of the true structures of these proteins, the acceptor substrate (elongating polysaccharide) is situated among the TMHs during elongation. These regions may have other functions in addition to forming a pore for polymer transport across the membrane. It is possible that the TMHs could affect enzyme processivity by holding the product/acceptor substrate in place during catalysis. Recent work by Harris et al. (2012) showed that mutations within a TMH of a CESA protein can reduce cellulose microfibril crystallinity and alter the speed of CSC movement on the PM. The authors present evidence that the TMHs of dual-function GTs may have a role in promoting appropriate interactions between the polymer product and other molecules in the extracellular space. For example, the TMHs might participate in orienting the polysaccharide as it is extruded across the membrane, which could in turn affect cellulose crystallinity.

CONCLUSION
Dual-function GTs are fascinating biochemical machines that synthesize ECM polysaccharides in a remarkable variety of biological systems. We are just beginning to understand how these proteins function, and ongoing research is likely to produce exciting advances in the coming years. A better understanding of these enzymes will provide insight into ECM synthesis, one of the most fundamental biological processes in nature. Knowing how these enzymes function can also lead to the ability to modulate their activities, which could have tremendous impact on plant biotechnology and human health.

ACKNOWLEDGMENTS
This manuscript is supported by The Center for Lignocellulose Structure and Formation, an Energy Frontiers Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Award Number DE-SC0001990. The author also thanks Candace Haigler for assistance in editing the manuscript.

REFERENCES
Allday, N. N., Shen, Y., Broshsky, J. L., Henderson, S. M., and Johnson, A. E. (2005). The molecular mechanisms underlying BIP-mediated gating of the Sec61 translocon of the endoplasmic reticulum. J. Cell Biol. 168, 309–319.
Amor, Y., Haigler, C. H., Johnson, S., Wissounig, M., and Delmer, D. P. (1995). A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc. Natl. Acad. Sci. U.S.A. 92, 9353–9357.
Bartnicki-Garcia, S. (2006). Chitosome, part, present and future. J. Exp. Bot. 57, 349–353.
Bringmann, M., Li, E., Sampathkumar, A., Kratzip, T., Hauser, M. T., and Parson, S. (2012). POM-POM2/CESAL1 cellulose synthase-like C-family proteins function, and ongoing research is likely to produce exciting advances in the coming years. A better understanding of these proteins will provide insight into ECM synthesis, one of the most fundamental biological processes in nature. Knowing how these enzymes function can also lead to the ability to modulate their activities, which could have tremendous impact on plant biotechnology and human health.

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Amor, Y., Haigler, C. H., Johnson, S., Wissounig, M., and Delmer, D. P. (1995). A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc. Natl. Acad. Sci. U.S.A. 92, 9353–9357.
Bartnicki-Garcia, S. (2006). Chitosome, part, present and future. J. Exp. Bot. 57, 349–353.
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Davis, J., Brandizzi, F., Liepman, A., Crowell, E. F., Gonneau, M., Stier-Doblin, M. S., Pettolino, F. A., W. V., Bouvier-Navé, P., Carroll, A., Acab, A., Baskin, T. I., Persson, S., and Cobb, A., Carroll, A., Sampathkumar, V., and Keegstra, K. (2010). Proteomic identification of hyaluronan synthase CSLC4 have opposite orientation in the Golgi membrane. Plant Cell 22, 425–437.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

Received: 27 March 2012; accepted: 07 June 2012; published online: 22 June 2012 Citation: Davis JK (2012) Combining polysaccharide biosynthesis and transport in a single enzyme: dual-function cell wall glycosyltransferase. Front. Plant Physiol. 3:102. doi: 10.3389/fpls.2012.00138

This article was submitted to Frontiers in Plant Physiology, a specialty of Frontiers in Plant Science.

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"fpls-03-00138" — 2012/6/20 — 17:50 — page 5 — #5