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Mini-review

Plant callose synthase complexes

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Abstract

Synthesis of callose (β-1,3-glucan) in plants has been a topic of much debate over the past several decades. Callose synthase could not be purified to homogeneity and most partially purified cellulose synthase preparations yielded β-1,3-glucan *in vitro*, leading to the interpretation that cellulose synthase might be able to synthesize callose. While a rapid progress has been made on the genes involved in cellulose synthesis in the past five years, identification of genes for callose synthases has proven difficult because cognate genes had not been identified in other organisms. An *Arabidopsis* gene encoding a putative cell plate-specific callose synthase catalytic subunit (CalS1) was recently cloned. CalS1 shares high sequence homology with the well-characterized yeast β-1,3-glucan synthase and transgenic plant cells over-expressing CalS1 display higher callose synthase activity and accumulate more callose. The callose synthase complex exists in at least two distinct forms in different tissues and interacts with phragmoplastin, UDP-glucose transferase, Rop1 and, possibly, annexin. There are 12 CalS isozymes in *Arabidopsis*, and each may be tissue-specific and/or regulated under different physiological conditions responding to biotic and abiotic stresses.

Introduction

Callose, a linear β-1,3-glucan with some 1,6 branches, differs from cellulose which is an exclusive β-1,4-glucan crystallized to form cellulose microfibrils. Structurally comparable β-1,3-glucans are found in the cell walls of yeasts, some filamentous fungi, as well as in bacteria (Stone and Clark, 1992; Kauss, 1996). Callose is synthesized by plants at many locations throughout development and in response to biotic and abiotic stress. How callose is made at various locations in response to different signals is not understood, due primarily to the difficulties in purification of callose synthase (CalS) and the lack of knowledge of genes encoding this enzyme.

β-1,3-Glucans are produced as helical chains which, upon heating, form a low-set gel. The gel-forming property of these glucans is exploited by plant cells to 'plug' and seal the plasma membrane from physical damage, such as that caused by fungal infection. Callose deposition has been associated with the pollen self-incompatability where it blocks the path of the growing pollen tube inside the stylar tissue (Dumas and Knox, 1983). Callose appears during cytokinesis in all multicellular green algae and higher plants (Cherp *et al*., 2001). Callose forms at the cell plate filling in the tubular network of fused vesicles (Verma, 2001) and consolidating it into a plate that expands in the center of the phragmoplast (Samuels *et al*., 1995). Callose is rapidly degraded and replaced by cellulose as the cell plate begins to mature and fuses with the parental cell wall (see Verma, 2001). Other specific functions of callose have been reviewed elsewhere (Stone and Clarke, 1992).

Various attempts to purify CalS from higher plants during the past decade have resulted in disagreements about the molecular mass and subunit composition of this enzyme. Partially purified CalS preparations have been shown to contain 6-9 major polypeptides ranging in size from 25 to 92 kDa (Kamat *et al*., 1992; Wasser-

man *et al*., 1992; Schlupmann *et al*., 1993; Dhugga and Ray, 1994; McCormack *et al*., 1997). Using various affinity labeling techniques, it has been reported that the presumptive 'catalytic subunit' of CalS from higher plants has a molecular mass between 32 and 57 kDa (Read and Delmer, 1987; Frost *et al*., 1990; Delmer *et al*., 1991; Li and Brown, 1993; Gibeaut and Carpita, 1994). One of the subunits that bind UDP-glucose was identified by labeling techniques with UDP-glucose or its analogues as probes. Recent data suggest that this is not the catalytic subunit but an associated protein acting as a UDP-glucose transferase (Hong *et al*., 2001b). CalS activity in *Nicotiana alata* pollen tubes is shown to be associated with a 190 kDa peptide as revealed by density fractionation of membranes followed by product entrapment (Turner *et al*., 1998). Several factors may have contributed to these discrepancies: CalS is likely to be a multi-subunit membrane-associated enzyme; extraction of the enzyme from membranes requires the use of detergents that may dissociate the complex or cause the loss of its activity; and the protein may be sensitive to protease degradation during purification. Moreover, the activity of this enzyme is highly regulated during plant development, and various biotic and abiotic factors may affect CalS activity. Identification of CalS gene (see below) showed that the CalS catalytic subunit is ca. 200 kDa, is distinct from cellulose synthases (CelS) and exists as a multi-subunit enzyme complex present in different tissues. An understanding of the machinery involved in *de novo* synthesis of β-glucans is of paramount importance for our ability to control callose synthesis at various locations in plants.

The callose synthase genes in plants

While rapid progress has been made in the discovery of genes involved in cellulose synthesis in the past five years (Pear *et al*., 1996; Arioli, 1998; Taylor *et al*., 1999; Richmond and Somerville, 2000; Saxena and Brown, 2000), identification of genes for callose synthases has proven difficult (Delmer, 1999; Saxena and Brown, 2000). Since callose and cellulose are very similar polymers of glucan, it seems logical to assume that genes encoding respective synthases might be evolutionarily related. This was substantiated by the identification of a gene encoding a bacterial curdlan (a β-1,3-glucan) synthase (Stasinopoulos *et al*., 1999). Similarly, from cloning of the genes for hyaluronan (a polysaccharide containing both β-1,3 and β-1,4 linkages) synthases from bacteria and animals (Yoshida *et al.*, 2000), it was suggested that both β-1,3- and β-1,4-glucan synthases may have evolved from a common ancestor because they all contain D, D, D, and QXXRW motifs (Figure 1) identified by Saxena *et al*. (1995). However, cloning of the yeast β-1,3-glucan synthase (Douglas *et al*., 1994) showed that the primary structure of this enzyme is not similar to bacterial or plant CelSs, nor to curdlan or hyaluronan synthases.

The search for the plant CalS genes did not progress rapidly because there were no molecular probes available to identify the cognate gene. Moreover, the mechanism of callose synthesis in plants has been controversial as CalS could not be purified to homogeneity and most partially purified CelSs yielded β-1,3-glucans *in vitro*, leading to the interpretation that CalS and CelS are one and the same enzyme (Jacob and Northcote, 1985; Delmer, 1999). The debate was finally resolved by the isolation of a CalS gene and demonstration how this enzyme differs from CelSs and curdlan synthase in bacteria (Hong *et al.*, 2001a).

A cell plate associated protein that resembles dynamin GTPase, phragmoplastin, was identified by Gu and Verma (1996). This protein was found to interact with a novel UDP-glucose transferase (UGT1; Hong *et al*., 2001b). An open reading frame was discovered upstream from the *UGT1* gene, which showed homology with the catalytic subunit of the yeast β-1,3-glucan synthase and was termed AtCalS1, putatively encoding *Arabidopsis thaliana* callose synthase 1 (Hong *et al*., 2001a). Three independent lines of evidence suggested that AtCalS1 is, indeed, a catalytic subunit of the plant CalS. First, it is similar in sequence to FKS1, a well characterized β-1,3-glucan synthase in yeast. Second, when expressed in tobacco BY2 cells under the control of 35S promoter, the cells synthesized more callose at the cell plate (Figure 2C–D). Third, BY2 cells expressing CalS1 showed higher levels of CalS activity at the cytokinesis stage of synchronized cells (Hong *et al*., 2001a). That CalS1 is a cell plate-specific CalS was shown by the localization of the GFP-tagged CalS1 protein at the forming cell plate during cytokinesis (Figure 2B; see also Hong *et al*., 2001a). Putative CalS cDNAs from cotton and tobacco have also been cloned (Cui *et al*., 1999; Doblin *et al*., 2001). Together, the plant CalS and fungal FKS homologues have recently been classified into a new family (family 48) of the glycosyltransferase superfamily (http://afmb.cnrs-mrs.fr/~pedro/CAZY/gtf.html).

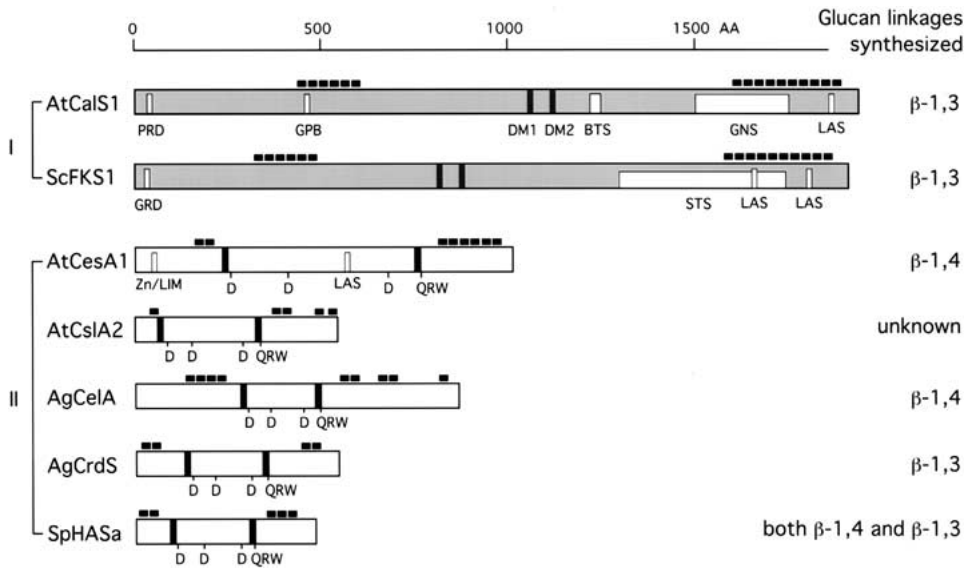

Figure 1. Schematic representation of the catalytic subunits of β-glucan synthases. Group I includes plant callose synthases and fungal FKS homologue proteins. Group II comprises plant cellulose synthases (CesA; Holland *et al*., 2000), CesA-like proteins (Csl; Richmond and Somerville, 2000), bacterial cellulose synthases, curdlan synthase (Stasinopoulos *et al*., 1999) and hyaluronan synthases (Yoshida *et al*., 2000). Proteins of Group II are characterized by the presence of conserved D, D, D, and QXXRW residues. Although there is no overall sequence similarity between Group I and II, domain 1 and 2 (DM1 and DM2; black bars; Kelly *et al*., 1996) of the catalytic site of cellulose synthases are present in all proteins including Group I. Open rectangles inside the sequences indicate conserved motifs. PRD, proline-rich domain (Prosite file, PS50099). GRD, glutamine-rich domain (PS50322). GPB, G-protein binding signature (PS00237). BTS, binding-protein-dependent transport system (PS00402). STS, sugar transporter signature (PF00083). GNS, yeast GNS1-homology region (PF01151). LAS, lipid attachment site (PS00013). Zn/LIM, zinc finger/LIM motif (PS50089). Solid black rectangle on the top of each sequence: predicted transmembrane domains. AtCalS1, *Arabidopsis thaliana* callose synthase 1 (AF237733); ScKFS1, *Saccharomyces cerevisiae* β-1,3-glucan synthase (SCU12893); AtCesA1, *Arabidopsis* cellulose synthase A1 (AF027172); AtCslA2, *Arabidopsis* cellulose synthase-like A2 (AB006699); AgCelA, *Agrobacterium tumefaciens* cellulose synthase A (L38609); AgCrdS, *Agrobacterium* sp. curdlan synthase (AF057142); SpHASa, *Streptococcus pyogenes* hyaluronan synthase A1 (L20853).

Callose synthases are distinct from cellulose synthases

Arabidopsis genome analysis revealed that there are 12 *CalS*-related genes distributed over the five chromosomes (Hong *et al*., 2001a; see also http://www.plantcell.org/cgi/content/full/13/4/755/DC1/1). We named these genes AtCalS1–AtCalS12 based on the decreasing similarity to AtCalS1. Phylogenetic analysis suggests that cotton FKS1-like protein (CFL1) is most closely related to *Arabidopsis* CalS9 and is less similar to the cell plate-specific CalS1. CalS genes fall into two groups, one made up of genes containing 2–3 exons and the other up to 50 exons. In fact, the CalS genes are among the longest in the plant genomes characterized to date. They encode polypeptides of almost 2000 amino acids in length, a size twice as much as plant CelSs which are about 1000 amino acids long (Richmond and Somerville, 2000; Saxena and Brown, 2000; Holland *et al*., 2000). The plant CelSs (CesA) and their homologues (Csl) form the cellulose synthase superfamily, which contains several subfamilies (Richmond and Somerville, 2000; Saxena and Brown, 2000; Holland *et al*., 2000; Henrissat and Davies, 2000). However, none of the members of the CeSA superfamily resembles the CalS family in their primary amino acid sequences. The latter constitutes a new family of glycosyltransferases (see above).

One of the common features shared by both CeSA and CalS families is that they are membrane proteins with similar transmembrane topologies (Figure 1). They contain multiple transmembrane domains clustered into two regions, leaving a large hydrophilic central loop facing the cytoplasm. The hydrophilic loop contains the putative catalytic site which has been divided into two, the UDP-glucose binding domain (domain 1) and the glycosyltransferase domain (domain 2; Kelly *et al*., 1996). These domains are characterized by the presence of D, D, D, and QXXRW conserved residues in the CesA superfamily (Saxena *et al*., 1995). The fact that CrdS (curdlan synthase

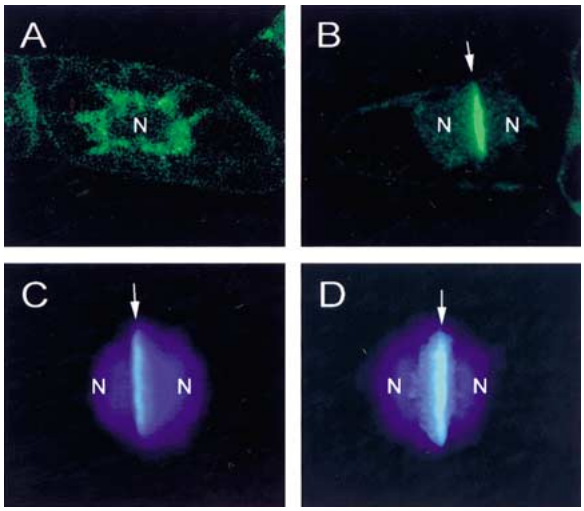

Figure 2. Subcellular localization of AtCalS1 and deposition of callose on the cell plate. *Arabidopsis* CalS1 was fused with GFP and expressed in tobacco BY2 cells under the control of the CaMV 35S promoter (Hong *et al*., 2001a). A, B. Fluorescent images of transgenic BY-2 cells expressing the GFP-CalS1 fusion protein at the G1 stage of cell cycle (A) and during cytokinesis (B). C, D. Callose deposition in the cell plate of control (C) and transgenic cells over-expressing GFP-CalS1 (D). The cells stained with aniline blue and 4′,6-diamidino-2-phenylindole (DAPI) were photographed with a fluorescence microscope with a UV filter set. Arrows indicate the cell plate. N, nucleus.

synthesizing β-1,3-glucan) and HAS (hyaluronan synthase synthesizing both β-1,3 and β-1,4 linkages) both have these conserved motifs reinforced the notion that CalSs should also contain these motifs. Such sequences, however, are neither present in yeast FKS1 (β-1,3-glucan synthase) nor in any of the plant CalS proteins. A detailed analysis of sequences in the domain 1 and domain 2 of bacterial and plant CelSs showed that residue E and Q in domain 1 and 2, respectively, are conserved between the CeSA and CalS families. However, domain 2 does not contain QXXRW, which is conserved in all CelSs.

It appears that plant CalS may not directly bind UDP-glucose; instead, this function may be complemented by UGT1 which contains a UDP-glucose-binding signature and has been shown to tightly interact with CalS1 (Hong *et al*., 2001a). Thus, UGT1 may act as a subunit of CalS in plants (Verma, 2001). This is consistent with the observation that a protein of 55 kDa was shown to bind to UDP-glucose (Dhugga and Ray, 1994) which may in fact be the UGT1 peptide. This needs to be directly demonstrated, however.

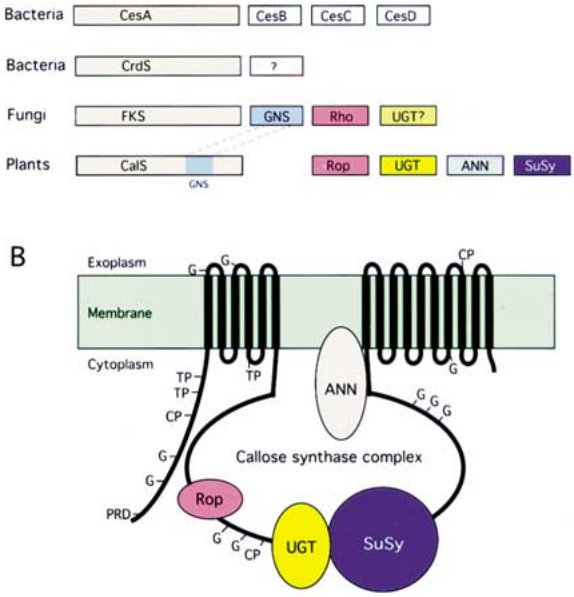

Figure 3. Putative regulatory and catalytic subunits of β-1,3-glucan synthases from bacteria, fungi and plants. A. Schematic representation of putative subunits of bacterial cellulose synthase (CesA), curdlan synthase (CrdS), yeast β-1,3-glucan synthases (FKS), and plant callose synthase (CalS). Bacterial *CesB*, *CesC* and *CesD* genes have been postulated to regulate cyclic di-GMP binding, pore formation in the membrane and crystallization of the cellulose fibers (Matthysse *et al*., 1995; Delmer, 1999). Yeast GNS1 and Rho act as regulators of β-1,3-glucan synthase. Rop1 and UGT1 have been shown to interact with CalS1 in *Arabidopsis* (Hong *et al*., 2001b) and annexin (ANN)-like proteins may modulate callose synthase activity in cotton (Andrawis *et al*., 1993; Shin and Brown, 1999), whereas sucrose synthase (SuSy) could be part of the CalS complex to provide substrate for callose synthesis. B. Hypothetical model of the callose synthase complex showing transmembrane domains and hydrophilic loop interacting with Rop1, annexin, UGT1 and SuSy. G, potential N-linked glycosylation sites; CP, cAMP- and cGMP-dependent phosphorylation sites; TP, potential tyrosine phosphorylation sites; PRD, proline-rich domain.

The need for a multi-subunit complex for β-glucan synthesis in plants

The synthesis of callose requires several steps, and hence a single peptide may not be able to perform all the necessary functions. Furthermore, the catalytic activity needs to be regulated to meet the requirements for synthesis of this polymer at different locations (see below) and hence participation of other proteins may be obligatory for a regulated production of this polysaccharide (Figure 3). *Arabidopsis* IRX1 and IRX3 CelSs, both essential for cellulose synthesis in secondary cell walls, were found to co-precipitate and are part of the same CelS complex (Taylor *et al*., 2000). Because the glucose residues

of cellulose are arranged in a way that each residue is oriented 180° to its neighbor, it has been predicted that two catalytic subunits exist in each enzyme complex to allow the simultaneous addition of two glucose residues (Carpita and Vergara, 1998). These subunits may interact with other proteins to form a large complex (see below). IRX1 and IRX3 may represent such a combination of two subunits to provide multiple catalytic sites for cellulose synthesis (Taylor *et al*., 2000). The CelS complex is a hexagonal rosette with each unit consisting of at least 6 CesA proteins presumably interacting via their zinc finger/LIM domains present at the N-terminus of CesA protein (Kimura *et al*., 1999). Formation of this rosette structure is apparently necessary for the production of crystallized cellulose sheet (Kudlicka and Brown, 1997; Saxena and Brown, 2000). Since callose does not form a sheet, CalS complexes may not form rosette structure and instead may be scattered in the membrane (Kudlicka and Brown, 1997). This is consistent with the observation that bacterial CesA does not synthesize crystalline cellulose and does not form a rosette structure (Matthysse *et al*., 1995). It is tempting to speculate that the scattered papillae observed in the study by Kimura *et al*. (1999) may represent CalS complexes. This is also evident with *in vitro* callose and cellulose synthesis studies which indicated the presence of two separate enzyme complexes (Kudlicka and Brown, 1997). Alternatively, it is possible that CalS is part of the rosette structure and uses a common UGT and SuSy mechinary for the rapid flux of substrate (Verma, 2001). With the availability of CalS antibodies, this question can now be answered.

Callose synthase complexes in plants

Bacterial curdlan synthase is controlled by a set of regulatory genes the function of which has not been established (Stasinopoulos *et al*., 1999). Such a regulation also occurs in cellulose synthesis in bacteria where *CesB*, *CesC* and *CesD* genes have been postulated to regulate cyclic di-GMP binding, pore formation in the membrane and crystallization of the cellulose fibers (Matthysse *et al*., 1995; Delmer, 1999). In the case of β-1,3 glucan synthesis in yeast, two genes (*FKS1* and *FKS2*) encoding the catalytic subunit of β-1,3-glucan synthase have been well characterized (Douglas *et al*., 1994; Mazur *et al*., 1995). In addition, it has been demonstrated that β-1,3-glucan synthase activity is regulated by Rho which forms a complex with the FKS protein (Qadota *et al*., 1996). Another gene, *GNS1*, has been demonstrated to regulate β-1,3-glucan synthase activity in yeast (el-Sherbeini and Clemas, 1995). During evolution in plants, a GNS1-like sequence and the pore forming ability (GNS and STS, Figure 1) may have been integrated inside the catalytic subunit of CalS1 (Figure 3A), a phenomenon similar to the formation of bifunctional enzymes, such as Δ^1-pyrroline-5-carboxylate synthase (P5CS, Hu *et al*., 1992). We have demonstrated that UGT1 (UDP-glucose transferase) binds, *in vivo* and *in vitro*, with CalS1 forming a complex that can be sedimented in a sucrose gradient. UGT1 binds to Rop1, a homologue of Rho, and interacts with phragmoplastin (Figure 3B). It is interesting to note that the binding of UGT1 with Rop1 only occurs in its GTP-bound state suggesting its functional significance (Li *et al*., 1999; Zheng and Yang, 2000; Hong *et al*., 2001b). Since Rho has been shown to regulate β-1,3-glucan synthase activity in yeast (Qadota *et al*., 1996), and plant CalS interacts with UGT which in turn binds with Rop1 (Hong *et al*., 2001b), it is possible that the plant Rop1 control CalS via UGT (Verma, 2001).

Based on the sedimentation of product-entrapped purified CalS, it seems that several other proteins may be associated with this complex (Hong *et al*., 2001b). The functions of some of these proteins may be involved in controlling CalS activity, particularly in response to biotic/abiotic signals. CalS has also been shown to interact with a membrane protein having similarity with annexin and protein kinase C (Andrawis *et al*., 1993). This annexin-like protein has GTPase activity which is inhibited by Ca^{2+} and stimulated by Mg^{2+} (Shin and Brown, 1999). However, addition of recombinant annexin to a CalS preparation did not influence the CalS activity. This suggests that annexin may be involved in Ca^{2+}-mediated switch from callose to cellulose synthesis, an activity which also requires Mg^{2+}. Rop1 may act as a spatial regulator of CalS while phragmoplastin may activate this enzyme by squeezing the vesicles into tubules (Gu and Verma, 1996, 1997; Zhang *et al*., 2000) at the forming cell plate (Verma, 2001). Binding of auxiliary proteins may occur by specific interactions with the hydrophilic loop of CalS or via the proline-rich domain located in the N-terminal region (PRD, Figure 1). Further dissection of different regions of the hydrophilic loop in the CalS protein is necessary in order to establish precise topology of this complex and to determine function of each domain and interacting protein.

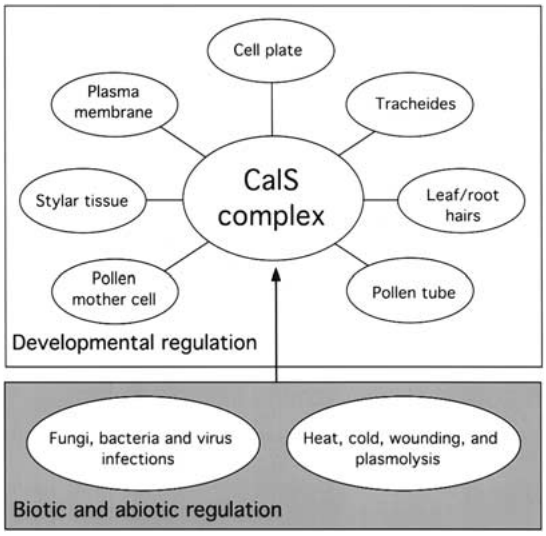

Figure 4. Callose synthases are present in many locations of the plant and their activities are regulated by developmental status and by biotic and abiotic signals.

Sucrose synthase (SuSy) also localizes to the forming cell plate (Amor *et al*., 1995). Attachment of SuSy to membranes is regulated by phosphorylation and precedes callose biosynthesis (Subbaiah and Sachs, 2001). SuSy generates UDP-glucose which can be transferred via UGT1 to the active site of CalS forming a substrate channel (Verma, 2001). The role of SuSy in supplying UDP-glucose to CalS is of paramount importance since a lag in the supply of substrate could affect polymer synthesis (Amor *et al*., 1995; Verma, 2001). The latter mechanism may also operate for the synthesis of cellulose. Over-expression of SuSy has been demonstrated to enhance cellulose synthesis in bacteria (Nakai *et al*., 1999). Whether the same UGT/SuSy provides the supply of substrate to both CelS and CalS needs to be determined. Because CelS contains a UDP-glucose binding motif, it may not need UGT as part of this complex. Although an interaction of UGT with phragmoplastin was observed (Hong *et al.*, 2001b), phragmoplastin may not be an integral part of all CalS complexes located at other sites besides the cell plate. Other possible proteins which may be associated with this complex may include β-1,3-glucanase. This is based on the fact that a β-1,4-glucanase is required for cellulose synthesis, to perform a proofreading function to ensure correct linkage (Delmer, 1999; Verma, 2001).

Multiple forms of callose synthases and their regulation

Since callose is synthesized in several locations in plants (Stone and Clarke, 1992) and CalS responds to developmental and environmental signals, including biotic and abiotic stresses, it is conceivable that different isoforms of CalS may constitute different complexes at each site (Figure 4). The spatial information, for example at the cell plate and during wound healing, may be mediated by Rho-like proteins shown to be an integral part of the CalS complex (Hong *et al*., 2001b). The fact that all plants including algae produce wound-induced callose (Scherp *et al*., 2001), suggests the conservation of a signaling pathway that regulates this type of CalS complex. The regulation of wound-induced CalS activity could be mediated by interaction with G-proteins for which a G-protein binding signature exists in the AtCalS1 sequence (Hong *et al*., 2001a). Also, several annexin-type molecules that are known to respond to Ca^{2+} levels interact with CalS (Andrawis *et al*., 1993). The level of transcription of each CalS isoform varies as suggested by the differences in the frequency of ESTs from different tissues (Hong *et al*., 2001a). Two genes from different CalS subfamilies have been shown to be preferentially expressed in *Nicotiana alata* pollen tubes (Doblin *et al*., 2001). On the other hand, AtCalS1 is expressed on the cell plate (Hong *et al*., 2001a). The exact level of each transcript needs to be determined in different tissues by northern blot analysis, quantitative PCR and by *in situ* hybridization. Finally, two types of CalS, one requiring Ca^{2+} and the other without the need for Ca^{2+} for its activity, exist as evident from the pollen localized enzyme (Li *et al*., 1997; Schlupmann *et al*., 1993) which is insensitive to Ca^{2+}. The fact that different CelS isoforms are expressed in different tissues (Wu *et al*., 2000; Holland *et al*., 2000; Doblin *et al*., 2001) suggest that CalS and CelS enzyme complexes are finely tuned to meet the structural and biochemical demands of each specialized plant tissue.

The lack of sequence similarity between CalS and CrdS proteins (both synthesizing β-1,3-glucans) is an indication of convergent evolution such as that observed for β-glucan hydrolases. The (1→3,1→4)-β-glucan hydrolases from plants (Hoj and Fincher, 1995) are distinct from those from bacteria (Heinemann *et al*., 1996) but their specificities and catalytic mechanisms are identical (Hoj and Fincher, 1995). Both CesA and CalS protein families share simi-

lar membrane topologies which may be required for the synthesis and deposition of the structurally similar polymers, cellulose and callose. To accommodate the site for the interaction with various regulatory proteins, the length of the plant CalS peptide, in particular, the hydrophilic loop, is significantly increased. There are several glycosylation and phosphorylation sites in the hydrophilic loop as well as in the N-terminal region which is also hydrophilic (Figure 3). It is interesting to note that a mannose-1-phosphate guanylyl transferase mutant required for N-liked glycosylation significantly reduces cellulose synthesis (Lukowitz *et al*., 2001), making glycosylation a potential regulator for both CelS and CalS enzymes. Many putative glycosylation sites also exist on UGT which acts as a subunit for CalS. Since UGT1 and SuSy do not have any transmembrane domains, they may be associated with CalS via specific interaction with the hydrophilic loop. Neither of these proteins contain meristylation/isoprenylation motifs for membrane attachment but Rop1 could use this mechanism to become membrane-associated, in addition to interacting with UGT1 (Hong *et al*., 2001b). Since there are both Ca^{2+}-requiring and non-Ca^{2+}-requiring CalSs in plants and Ca^{2+} also plays a role in inhibiting cellulose synthesis, a switch from callose synthesis to cellulose synthesis during cell plate formation (Samuels *et al*., 1995; Verma, 2001) and wound healing would necessitate involvement of a Ca^{2+}-sensitive protein. Whether annexins could perform such a function or other proteins such as synaptotagmin homologues to bring about this control (Verma, 2001) needs to be established.

Conclusion

The identification of *CalS* and *CelS* genes and demonstration that these enzymes are encoded by distinct families of genes, members of which may constitute tissue-specific enzyme complexes, is a major step towards understanding the complexity of biosynthesis of these cell wall polysaccharides in higher plants. The regulation of these enzyme complexes in response to development and biotic/abiotic stresses awaits further dissection of the enzymatic machinery and elucidation of the role of each component of signal transduction systems. Having now available various proteins of the CalS complex and their antibodies, a precise mechanism and role of each protein in the biosynthesis of this polysaccharide can be elucidated. Furthermore, post- and co-translational modifications such as phosphorylation and glycosylation may contribute to the dynamics of CalS complexes. Finally, the question of the primer that initiates the synthesis of callose needs to be addressed. It is also important to determine whether cellobiose synthase is localized to the Golgi or at the nascent cell plate and is activated after the fusion of vesicles along with CalS. An understanding of the machinery responsible for callose/cellulose synthesis is important in order to manipulate cell wall biosynthesis and to alter the quality and quantity of callose/cellulose for modification of plant architecture and for improving material for the pulp and paper industries.

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