Guar Seed β-Mannan Synthase Is a Member of the Cellulose Synthase Super Gene Family
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skin wounding and tumorigenesis. Similarly, although not yet formally tested, DP-induced changes in Wnt signaling could explain why TOPGAL is transiently activated in the bulge at early anagen (29). If signaling pathways (e.g., Wnts) are generally important in SC self-renewal, as they are in hematopoietic SCs (33, 34), then differences in their status could have an impact on rates at which SCs divide and are mobilized from their niche.

In summary, we have uncovered a constellation of distinguishing features of bulge LRCs relative to related keratinocyte progeny, which, together with their localization, likely accounts for their special properties. Our findings suggest that the bulge SC niche is a growth and differentiation–restricted environment. The LRC-related changes that have thus far surfaced are already suggestive of a broader interaction between environmental stimuli and the SC niche.

References and Notes

30. T. Tumbar et al., data not shown.
35. We thank L. Degenstein, J. Fan, and L. Polak for help.

Guar Seed β-Mannan Synthase Is a Member of the Cellulose Synthase Super Gene Family

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Genes for the enzymes that make plant cell wall hemicellulosic polysaccharides remain to be identified. We report here the isolation of a complementary DNA (cDNA) clone encoding one such enzyme, mannan synthase (ManS), that makes the β-1, 4-mannan backbone of galactomannan, a hemicellulosic storage polysaccharide in guar seed endosperm cells. The soybean somatic embryos expressing ManS DNA contains high levels of ManS activities that localized to Golgi. Phylogenetically, ManS is closest to group A of the cellulose synthase–like (Csl) sequences from Arabidopsis and rice. Our results provide the biochemical proof for the involvement of the Csl genes in β-glycan formation in plants.

Cell walls define the shape and size of plant cells and make up the bulk of the renewable biomass on Earth. Of the two main cell wall polysaccharide fractions, cellulose is synthesized at the plasma membrane whereas matrix polysaccharides are made in the Golgi and then exported to the cell wall by exocytosis (1).

An unsolved problem in plant biology is the ability to identify genes for the enzymes that make structural and storage cell wall matrix polysaccharides (2–6). Mainly because polysaccharide synthases are labile upon isolation from the cell and because of subsequent solubilization in detergents, biochemical approaches have proven difficult in the identification of these corresponding genes (7, 8). The first plant cellulose synthase (CesA) gene was isolated from developing cotton fibers by rapid expression screened tag (EST) sequencing (9). Evidence supporting the role of the CesA genes in cellulose formation came from studies involving cellulose-deficient mutants of Arabidopsis (10, 11). A large number of sequences have been annotated in genomic databases as belonging to the CesA family based on sequence homology. A weakly related set of sequences has been referred to as cellulose synthase–like (Csl) (4). Both the CesA and Csl classes of sequences possess conserved motifs that are common to the polymerizing β-glycosyltransferases from a wide variety of organisms, including bacteria and fungi (12). On the basis of their divergence from the CesA sequences, Csl genes have been postulated to encode Golgi enzymes that make cell wall matrix polysaccharides (4–6). However, none of the Csl genes has yet been assigned to the synthesis of a specific wall polysaccharide, although mutations in some of them resulted in an impaired development of embryonic, pollen tube, or root hair (13–15).

Cell wall hemicellulosic polysaccharides assume the role of storage carbohydrates in the seeds of some plants, which is analogous to that of starch in cereal grains. For example, galactomannan is accumulated in the form of secondary wall thickenings in the endosperm of guar (Cyamopsis tetragonoloba), locust
bean (Ceratonia siliqua), and coconut palm (Cocos nucifera), as is xyloglucan in tamarind (Tamarindus indica) (2, 16). Some of the Csl genes have proven too labile to be amenable to biochemical purification (ferases have proven too labile to be amenable to biochemical purification (8)). Both the enzymes can be assayed in vitro in the particulate preparations derived from developing fenugreek, guar, or senna endosperm (18). The genes for the enzymes that add sugar residues to the backbones in galactomannan and xyloglucan have been isolated by enzyme purification and protein sequencing or by functional expression in a heterologous system (19–21). With the exception of callose synthase, however, polymerizing β-glycosyltransferases have proven too labile to be amenable to biochemical purification (8). We were successful in solubilizing the ManS activity from the particulate fraction derived from developing guar seed endosperm in digitonin and subsequently fractionating it by isopycnic density gradient centrifugation (data not shown). Proteomic analysis of the partially purified preparations did not yield any polypeptides that showed similarity to the known, predicted Csl proteins.

**Fig. 1. (A) Mannan synthase activity in developing guar seeds and (B) Northern blot of different guar tissues probed with the ManS gene. Below the Northern blot is the ethidium bromide–stained portion of the gel corresponding to the large and small ribosomal RNA (rRNA) subunits. Gene abbreviations are as follows: R, root; S, stem; L, leaf; 10, 10 DAF seeds; 25, 25 DAF seeds; 30, 30 DAF seeds; E, endosperm; SC, seed coat; Em, embryo. Endosperm, seed coat, and embryo were derived from the 25 DAF seeds. The specific activity of ManS is 69 pmol min⁻¹ mg⁻¹ protein.**

**Fig. 2. Mannan synthase activity in soybean transgenic somatic embryos. (Top) Activity profile of the nontransgenic control and transgenic soybean somatic embryos and 25 DAF guar seeds. (Middle) Northern blot probed with the ManS gene, and (bottom) the ethidium bromide–stained portion of the gel corresponding to large and small subunits of rRNA.**

Csl gene expression correlated with the rate of galactomannan accumulation in developing guar seeds led us to conclude that it played a role in galactomannan formation, so possibly it was the gene encoding ManS protein. We have thus designated this Csl as CtManS.

Hydropathy analysis of the derived ManS protein sequence predicted five transmembrane (TM) domains, a result consistent with the ManS being a membrane-associated protein (18) (fig. S3). The aminoacyl residues that are conserved across the polymerizing β-glycosyltransferases are located in the hydrophilic region between the first and the second predicted TM domains (figs. S1 and S3). This region is believed to contain the catalytic center of the polymerizing β-glycosyltransferases (12). The region between the third and fourth TM domains in ManS is much longer than any of the corresponding inter-TM regions in CesA toward the C terminus (9) (fig. S3). This domain of ManS may be involved in interacting with α-galactosyltransferase, the enzyme that adds galactosyl residues onto the mannan backbone. It is predicted to be on the same side of the Golgi cisternae as the catalytic domain.

To determine whether the putative CtManS gene was functionally expressed in a plant system different from guar, we transformed it into embryogenic soybean suspension-culture cells under the control of a seed-specific promoter (22). The cells were allowed to develop into mature somatic embryos for gene expression analysis and enzyme assays. Soybean somatic embryos behave like developing seeds and have proven useful in the study of transgenes specifically expressed in the seed (22). The untransformed somatic embryo cells did not possess any appreciable ManS activity, which allowed us to assay the activity resulting from the transgene in a clean background. ManS activity was detected in only those transgenic events where Northern blot analysis confirmed that the ManS gene was expressed (Fig. 2) and the ManS protein was produced (fig. S4). A lack of correspondence between the gene expression level and enzyme activity...
for some events could result from differences in posttranslational modification and/or proper folding of the enzyme polypeptide. The specific activity of ManS in some of the transgenic events was as high as in the particulate fraction derived from the approximately 25 DAF guar seeds (Fig. 2). The Michaelis constant (Km) of the ManS enzyme in the transgenic events was the same as for the native enzyme from guar seed, ~20 μM, indicating that the enzyme is correctly processed in the transgenic soybean cells (22).

The other polymerizing glycosyltransferase activities that utilize different uridine diphosphate (UDP)-sugar substrates did not appear to be significantly affected in the transgenic soybean somatic embryosexpressing the ManS gene in comparison to the control embryos, indicating that the enzyme encoded by the transgene is specific to guar seed endosperm (GDP)-mannose (fig. S9). Thus, we conclude that the guar ManS gene does indeed encode ManS activity.

It has been widely speculated that the polysaccharide synthases consist of complex molecules derived from multiple, unrelated polysaccharides (7, 8). Our results suggest that the ManS polypeptide is functional by itself, which would place it in the class of chitin synthase and hyaluronan synthase enzymes that have been reported to be autonomously functional (23, 24). However, we cannot rule out the possibility that the ManS polypeptide acquired other polypeptide members from soybean to form a functional complex.

Upon isopycnic centrifugation of the particulate fraction derived from the transgenic soybean cells, the ManS activity comigrated with that of inosine diphosphatase (IDPase), a Golgi marker (fig. S6). Similar results were obtained with the particles obtained from the developing guar seed endosperm (data not shown). An antibody was raised in rabbits against the region between the first and the second TM domains of the ManS protein (figs. S3 and S7). This antibody recognized a polypeptide of approximately 55 kD in correspondence with the ManS activity (figs. S4 and S6). These findings led us to conclude that the guar ManS enzyme was correctly targeted to the Golgi in functional form in the transgenic soybean cells. These results also suggest that the Golgi targeting information could be contained in the primary sequence of a protein, as was originally suggested by Wee et al. who found that a mammalian sialyltransferase introduced into Arabidopsis cells was targeted to the same Golgi compartment as in the native cells (25).

The product made from the in vitro reaction of the particles derived from the soybean transgenic events was hydrolyzed by endo-β-mannanase but not by cellulase or β(1-3, 1-4)-glucanase (lichenase) enzymes (Fig. 3). Similar results were obtained with the product derived from the guar seed endosperm particles, which are in agreement with previous observations (18). The specificity of the mannanase toward mannose, an epimer of glucose, is obvious from these results because cellulose and mannan chains have identical chemical linkage but the latter was preferentially degraded by mannanase and not by cellulase. Acid hydrolysis of the mannan product formed in vitro from the particulate preparations obtained from the transgenic somatic embryos or guar seed endosperm yielded only mannose, indicating that no detectable conversion of mannose to other sugars occurred in the course of the reaction (fig. S8). The product made in vitro has a high molecular mass with the degree of polymerization exceeding 1000 (fig. S9). These results show that the product made by the enzyme encoded by the guar ManS gene in soybean cells is a polymer made of β-1, 4-linked mannosyl residues. Mature seeds from the first generation of transgenic events contained 2% or more mannan than the control seeds, suggesting that the ManS gene was functional in developing soybean seeds (table S2).

Phylogenetic analysis using CesA and Csl sequences from Arabidopsis and rice revealed that the ManS was most closely related to the CesA class (Fig. 4). Only two CslA sequences each from Arabidopsis and rice grouped with the ManS protein. The remaining CslA sequences formed species-specific clades distinct from the one containing ManS. Addition of the CesA and remaining classes of the Csl sequences did not alter the overall topology of the phylogenetic tree (not shown). Although the Csl genes are believed to be involved in making different cell wall matrix polysaccharides, none of them has yet been associated with a particular type of linkage formation and no specificity has been established for the sugar nucleotide substrates (4–6, 13–15). It is possible that the Arabidopsis and rice sequences that group with the ManS protein form (gluc)omannan in dicot and monocot plants, respectively. Mannans are present in the walls of a wide variety of plant cells (3). The ManS protein is fairly divergent from its nearest homolog, AtCslA9, showing 67% identity at the amino acid level, suggesting that it has evolved to make the storage form of mannan in the seed tissue. The other CslA sequences that form species-specific clades distinct from the one containing ManS might be involved in the formation of the glucan backbone of xyloglucan and xylan backbone of arabinoxylan in the cell walls of dicot and monocot plants, respectively. Like galactomannan, both these polysaccharides are branched with β-1, 4-linked backbones.

Gums are most commonly used as food additives to provide stiffness and texture, prevent ice crystal formation, maintain crispiness, and retain moisture (26). They have many other nonfood applications such as drying and printing aids in the textile industry, thickeners for shampoos and conditioners, binders and hardeners for paper, rheological facilitators for concrete, and drilling agents for oil and gas wells (26). Most of guar and all of locust bean gums or the raw materials to process them are currently imported from South Asia or the Mediterranean. Prices fluctuated in the past...
tuate because of seasonal variations in crop performance (16). Production of galactomannan gums in high-yielding commercial crops, such as soybean, should help stabilize their supply and thus price.

Degree of galactosyl substitution on the mannan backbone determines the quality of naturally occurring galactomannans independent of $\alpha$-galactosidase (19, 29), it has become possible to reconstitute the galactomannan synthase machinery in other plant species. An attractive scenario is to produce gums of high value, like those of locust bean, as well as novel gums.

References and Notes
21. We thank K. Kollipara for help with phylogenetic analysis; D. Schmidt and M. Vogt for field support; C. Hainey for cDNA library construction; T. Harp for sugar composition analyses; and the members of the Pioneer Hi-Bred International Discovery Research committee for supporting this work.

Supporting Online Material
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Materials and Methods
Tables S1 and S2
Figs. S1 to S9
References
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