Plant Nucleotide Sugar Formation, Interconversion, and Salvage by Sugar Recycling*

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*Dedicated to Peter Albersheim for his inspiration and his pioneering studies in determining the structure and biological functions of complex carbohydrates.

Keywords

nucleotide sugar biosynthesis, nucleotide sugar interconversion, nucleotide sugar salvage, UDP-glucose, UDP-xylose, UDP-arabinopyranose mutase

Abstract

Nucleotide sugars are the universal sugar donors for the formation of polysaccharides, glycoproteins, proteoglycans, glycolipids, and glycosylated secondary metabolites. At least 100 genes encode proteins involved in the formation of nucleotide sugars. These nucleotide sugars are formed using the carbohydrate derived from photosynthesis, the sugar generated by hydrolyzing translocated sucrose, the sugars released from storage carbohydrates, the salvage of sugars from glycoproteins and glycolipids, the recycling of sugars released during primary and secondary cell wall restructuring, and the sugar generated during plant-microbe interactions. Here we emphasize the importance of the salvage of sugars released from glycans for the formation of nucleotide sugars. We also outline how recent studies combining biochemical, genetic, molecular and cellular approaches have led to an increased appreciation of the role nucleotide sugars in all aspects of plant growth and development. Nevertheless, our understanding of these pathways at the single cell level is far from complete.

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Carbohydrate: used interchangeably with sugar, a saccharide (monosaccharide, oligosaccharide, polysaccharide) or glycan

INTRODUCTION

Plants synthesize diverse carbohydrate-containing molecules (glycans) including glycoproteins, proteoglycans, glycolipids, and polysaccharides as well as a large number of low-molecular-weight molecules that exist as their glycosides. This diversity of structure is also reflected in a multiplicity of functions. Glycans including chloroplastic starch and cytosolic inulin serve as storage polysaccharides, whereas glycoproteins, proteoglycans, and glycolipids are typically present at the cell surface where their functions vary from catalytic activities to maintaining membrane integrity and recognition. Much of the glycan in a plant is present in the wall that surrounds each cell. The primary wall of growing plant cells is comprised predominantly of polysaccharides (cellulose, hemicelluloses, and pectin) together with smaller amounts of glycoprotein, proteoglycan, phenols, and minerals. In lignified secondary walls, glycans (cellulose and hemicellulose) account for up to 70% of a plant's biomass and are a potential source of sugar for the production of biofuels and renewable chemicals. These cell walls provide mechanical support to cells, tissues, and organs and also have a role in regulating plant growth and development. The cell wall also forms the interface between the plant and its environment and thus has an important role in a plant's interactions with symbionts, pathogens, and abiotic factors.

Understanding plant glycan structures and functions as well as developing technologies to increase the commercial value of these complex carbohydrates require knowledge of the enzymes and the corresponding genes involved in glycan synthesis and modification. In this article we review the current knowledge of the formation of nucleotide sugars, which serves as the primary building block for glycan synthesis.

THE BASIS FOR NUCLEOTIDE SUGAR AND GLYCAN DIVERSITY

Nucleotide sugars are activated sugar donors and the major precursors for glycan synthesis as they are "high energy bond" compounds ($\Delta G^{\circ\prime} > -7$ Kcal/mol) with a high group transfer potential that is used to form a glycosidic bond. Various types of nucleotide sugars exist in nature (**Figure 1*a***) with the majority of the

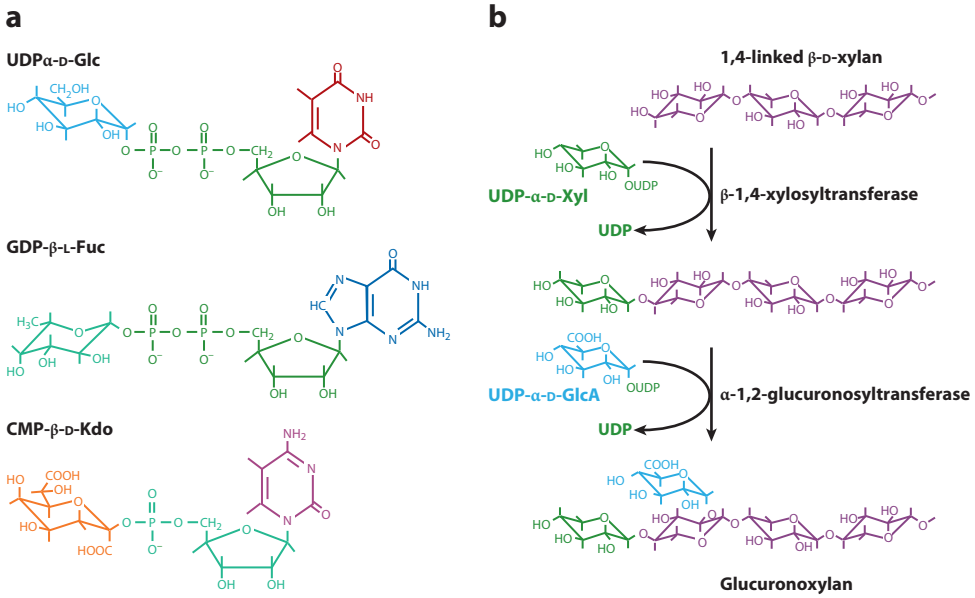

Figure 1

(*a*) Structures of a representative UDP-sugar (UDP-glucose, UDP-Glc), a GDP-sugar (GDP-fucose, GDP-Fuc), and an NMP-sugar (CMP-Kdo) that are formed by plants. (*b*) A schematic representation of the reactions involving UDP-sugars and glycosyltransferases in the synthesis of the plant polysaccharide glucurononxylan. One glycosyltransferase transfers a xylose moiety from UDP-Xyl to the xylan backbone acceptor and a different glycosyltransferase transfers a GlcA moiety from UDP-GlcA to the backbone. The mechanism shown depicts glycan extension by the addition of a new sugar to the nonreducing end of the backbone. However, other mechanisms including extension at the reducing end have been proposed (170).

sugars linked to a nucleotide-diphosphate (NDP-sugars). However, a limited number of activated sugars exist as the nucleotide-monophosphate (NMP-sugar) configuration. Other activated sugars including the polyisoprenyl phosphate-sugars and the polyisoprenyl di-phosphate-sugars exist but are not covered in this review.

The biosynthesis of glycans requires many different nucleotide sugars and the activity of a group of enzymes individually known as glycosyltransferases (GT). In general, a GT transfers a sugar from its activated donor to an appropriate glycan acceptor, leading to extension of the glycan polymer (**Figure 1*b***). The specificity of the individual GTs together with the diversity of activated sugar donors allows an organism to synthesize many different glycan structures. The ability to form structurally diverse glycans results in large part from the many ways monosaccharides can be linked together and the different forms and configurations in which a monosaccharide can exist:

- A glycose may exist in either of two absolute configurations (D or L).
- A glycose may exist in either a pyranose (*p*) or furanose (*f*) ring form.
- A glycose may have either of two anomeric configurations (α or β).
- A glycosidic linkage may be formed between the hydroxyl on C-1 of one sugar and any of the other hydroxyl groups (1→2, 1→3, 1→4, 1→6) on another sugar.
- A glycan may be linear or branched.
- A glycan may be modified with noncarbohydrate substituents (e.g., *O*-acetyl esters, *O*-methyl ethers, amino acids, sulfates, and phosphoesters).

Glycan: individual or chains of linked monosaccharides that may or may not be attached to another molecule (e.g., protein, lipid, flavonoid)

Glycoprotein: a protein containing sugars (<10%) linked to an asparagine (N-linked) or a hydroxy-amino acid (O-linked) in a polypeptide chain

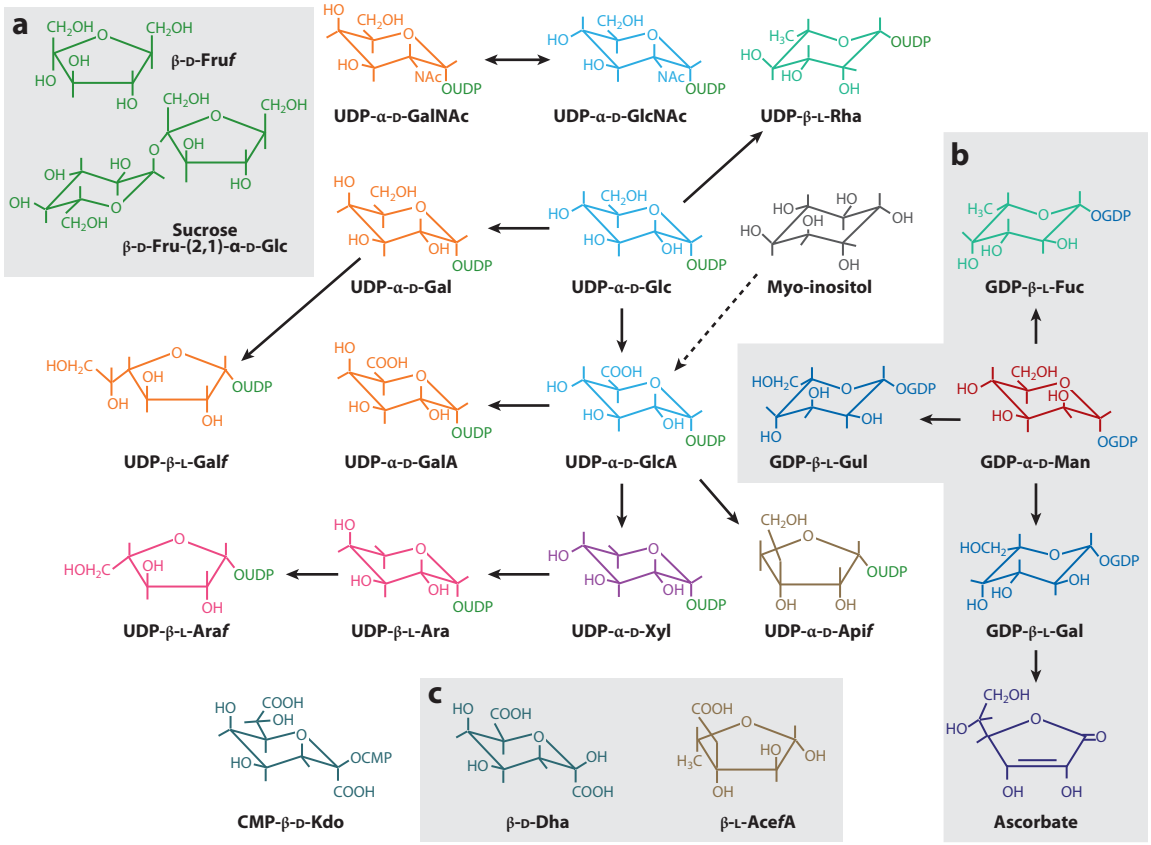

Figure 2

The nucleotide sugars used by plants for the synthesis of glycans. (*a*) Structures of fructose and sucrose that are the major carbon sources produced by photosynthesis. (*b*) Partial metabolic routes involving GDP-mannose. (*c*) Structures of the two sugars (Dha and aceric acid) for which no activated forms have been identified.

Proteoglycan: a protein containing large amounts of sugar (>80%) linked to an asparagine or hydroxyamino acid in a polypeptide chain

Glycolipid: a lipid O-linked to one or more glycan chains

Polysaccharide: a glycan composed of sequences of monosaccharides (sugars) linked together by a glycosidic bond

On the basis of the first five factors noted above, researchers (2, 84) calculated that more than one billion hexasaccharides can be formed using six different monosaccharides. This number does not include glycoses that are substituted with noncarbohydrate elements, which would increase the possible number of isomers to an even larger number. Living organisms, however, exploit only a limited portion of the potential glycan structures.

Numerous different nucleotide sugars exist in plants (**Figures 2**, **3**). These include the nucleotides linked to hexoses (D-glucose, D-galactose, D-mannose, and L-galactose), to the 6-deoxy hexoses (L-rhamnose and L-fucose), the pentoses (D-xylose, L-arabinopyranose, and L-arabinofuranose), to the hexuronic acids (D-glucuronic acid, D-galacturonic acid), to the keto sugar Kdo (3-deoxy-D-*manno*-octulosonic acid), to the branched-sugar D-apiose, and to the amino sugars *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine. Other sugar residues including Dha (3-deoxy-D-*lyxo*-2-heptulosaric acid) and aceric acid (3-C-carboxy-5-deoxy-L-xylose) are components of plant glycans (104) but their activated forms are not known.

Plants utilize the various nucleotide sugars to build glycans that vary considerably in their structural complexity. A glycan may be a linear polymer such as cellulose that is composed of 1→4 linked β-D-glucosyl residues or

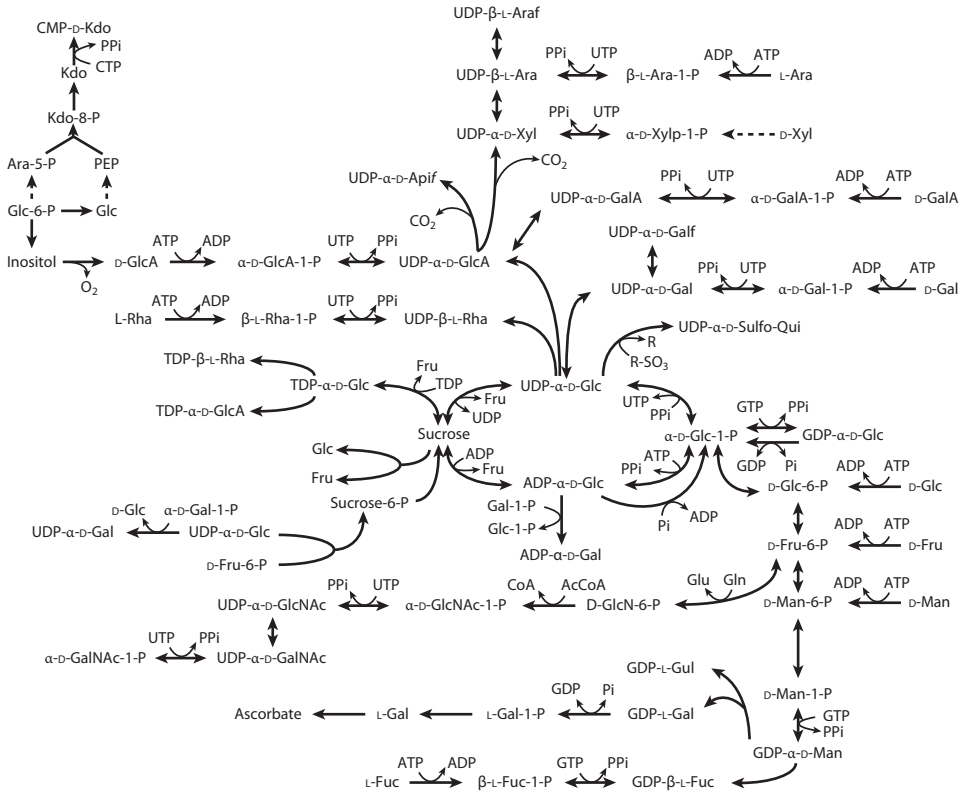

Figure 3

The major pathways for nucleotide sugar formation, salvage, and interconversion. Dashed lines indicate unresolved pathways.

homogalacturonan that is composed of 1→4 linked α-D-galactosyluronic acid residues. Alternatively, plant glycans may be highly branched structures such as the N-linked oligosaccharides of glycoproteins, the side chains of the pectin rhamnogalacturonan-I, and the arabinogalactan portions of arabinogalactan proteins. The pectic polysaccharide rhamnogalacturonan II that is present in the primary cell walls of all vascular plants is one of the most complex polysaccharides yet identified in nature given its composition of 12 different sugars linked together in various ways. Thus, for the assembly of RG-II, a plant must synthesize at least 12 different nucleotide sugars, at least 22 different GTs, as well as several *O*-acetyl and *O*-methyltransferases.

A BRIEF HISTORY OF NUCLEOTIDE SUGARS

Leloir and colleagues were the first to isolate and characterize UDP-glucose in the early 1950s. Subsequently, many other sugar nucleotides were isolated from bacteria, yeast, and plants, and some of the enzymes involved in their synthesis were identified. By the mid-1960s it was recognized that in all organisms glycan synthesis requires activated-sugar precursors. Evidence also began to accumulate that other forms of activated sugars including dolichol-linked sugars were donors for glycan synthesis and that glycan synthesis may occur by different mechanisms. For example, many glycans are synthesized by the sequential addition of sugar residues to the growing

Sugar: a generic term to refer to any low-molecular-weight carbohydrate (e.g., glucose, sucrose, and trehalose)

Monosaccharide: a 5-, 6-, 7-, 8-, or 9-carbon molecule (e.g., xylose, galactose, mannoheptulose, Kdo, and sialic acid)

Primary cell wall: the polysaccharide-rich matrix surrounding a growing plant cell

Secondary cell wall: the thick wall deposited once a plant cell has ceased to grow and divide

polymer, whereas others are first assembled as oligosaccharide subunits that are subsequently polymerized or transferred to a protein or lipid. This knowledge together with improved methods for structurally characterizing glycans led to an increased appreciation of the biological roles of glycans and some of the factors that control their synthesis and modification (159, 162). These advances are complemented by molecular studies that have begun to identify and functionally characterize many of the genes involved in the generation of activated sugars that are used in the synthesis of diverse glycans by living organisms (95, 126, 135). A greater understanding of glycan synthesis and assembly will ultimately require a detailed subcellular mapping of the enzymes involved in nucleotide sugar biosynthesis and understanding the molecular machinery that targets some of the cytosolic nucleotide sugar biosynthesis enzymes to membranes (see **Figure 4**).

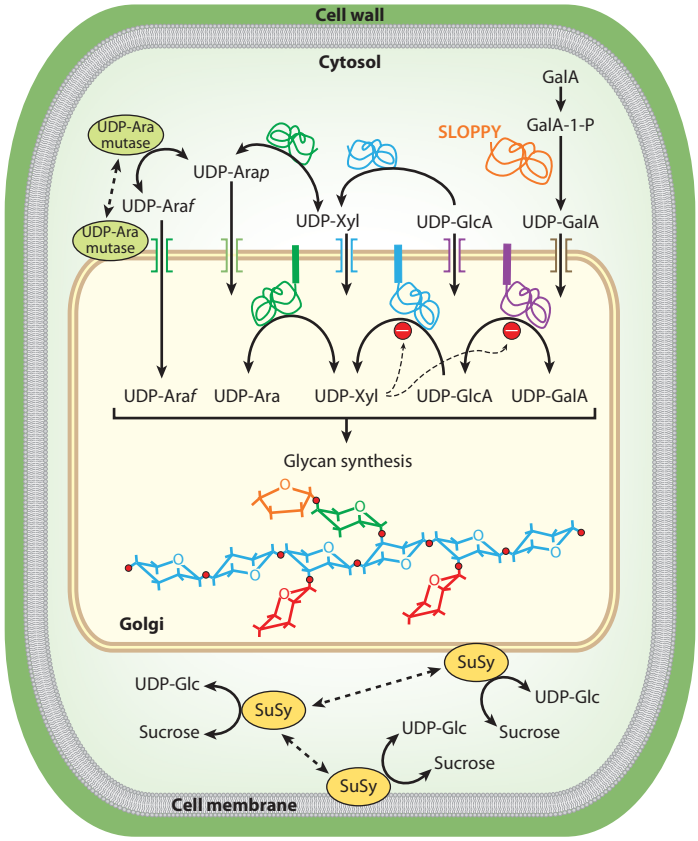

Figure 4

The biosynthesis of the same nucleotide sugar may occur in different cellular compartments. Several nucleotide sugars including UDP-GlcA, UDP-Xyl, and UDP-Ara are formed both in the cytosol and in the Golgi. The nucleotide sugars formed in the cytosol may be transported across the Golgi membrane for use in glycan formation. Some cytosolic enzymes including SuSy and UDP-Ara mutase (UAM) are present in the cytosol but can also undergo modification that targets them to membranes. Dashed lines indicate movement between various cellular locations.

NUCLEOTIDE SUGAR SYNTHESIS

To date, 30 different nucleotide sugars have been identified in plants, at least 70 are known in bacteria, 12 in humans, and up to 16 in fungi (47, 95). In this review we emphasize the formation and interconversion of nucleotide sugars in land plants. Where appropriate we draw from studies with other organisms that have provided insight that has benefited plant science. We outline six basic metabolic and catabolic pathways a plant cell utilizes to form diverse nucleotide sugars. These include the synthesis of NDP-sugar using the following pathways:

Carbon derived from photosynthesis
CO_2 → fructose-6-phosphate → NDP-sugar

Sucrose as a carbon source
Carbon mobilization (sucrose) → NDP-sugar

Storage carbohydrate as a carbon source
Storage polysaccharide (starch, glucomannan, etc.) → sugar → NDP-sugar

Recycling and salvage of sugar residues
Glycan (glycolipid, glycoprotein, 2° metabolites) → sugar → NDP-sugar

Primary and secondary cell wall restructuring and recycling
Polysaccharide → sugar → NDP-sugar

Sugar derived from plant-microbe interactions
Glycan → sugar → NDP-sugar

The seven basic biosynthetic routes to form activated sugars are as follows:

Glc +ATP ADP + Glc-6-P Glc-1-P + UTP **UDP-Glc** +PPi (photosynthesis)
Sugar + ATP ADP + Sugar-1-P Sugar-1-P + NTP **NDP-Sugar** +PPi (salvage)
Sugar + CTP **CMP-sugar**
NDP-sugarA NDP-sugarB (interconversion)
NDP-sugarD + sugarE-1-P sugar**D**-1-P + **NDP-sugar**E (transformation)
Sucrose +UDP **UDP-Glc** (mobilization?)
Glycan + Pi Sugar-1-P + NTP **NDP-Sugar** +PPi (recycling)

"SLOPPY": A PROMISCUOUS UDP-SUGAR PYROPHOSPHORYLASE

Plant cells can utilize diverse free sugars including Rha, Gal, Xyl, GalA, GlcA, Ara, and Fuc and incorporate them into polysaccharides (47). These monosaccharides are typically converted to their corresponding sugar-1-phosphate (sugar-1-P) by sugar-specific kinases. Pyrophosphorylases (also known as nucleotidyl transferases) can then convert individual sugar-1-phosphates to their corresponding NDP-sugars. Plants also contain a promiscuous pyrophosphorylase that in the presence of UTP converts at least six different sugar-1-phosphates to their corresponding UDP-sugars (81, 134, 168). An *Arabidopsis* gene (*At5g52560*) encoding this enzyme is expressed in all tissues examined. The recombinant *Arabidopsis* protein referred to as SLOPPY has a high affinity for GlcA-1-P and also catalyzes the conversion of Glc-1-P, Gal-1-P, Xyl-1-P, Ara-1-P, and GalA-1-P to their respective UDP-sugars in the presence of UTP (81, 168). However, the enzyme does not convert GalNAc-1-P or GlcNAc-1-P to their corresponding nucleotide sugars. SLOPPY is specific for the formation of UDP-sugars as it has no discernible activity in the presence of TTP, GTP, ATP, or CTP (80, 168).

The ability of plant cells to "recycle" free sugars into the nucleotide sugar pool has been described as a "salvage pathway" (47) and implies that there is a source of free sugars in plants. Such sugars may be generated by the turnover of glycans during plant growth and development, although it is not known how much these sugars contribute to the flux of NDP-sugars for glycan biosynthesis. It is also unclear if free sugars are generated by wall polysaccharide turnover in the apoplast and then transported across the plasma membrane or if the polysaccharides are endocytosed and then fragmented (35). The functional characterization of an *Arabidopsis* plasma membrane-localized sugar transporter (POLYOL TRANSPORTER5, AtPLT5) that is a member of a multigene family (75) suggests that plants do have the ability to transport glycoses from the apoplast to the cytosol.

FORMATION OF SPECIFIC NUCLEOTIDE SUGARS IN PLANTS

UDP-α-D-Glucose

Glucose is a quantitatively major component in many plant glycans including cellulose, callose, starch, xyloglucan, and glucomannan. However, the synthesis of Glc-containing glycans may involve ADP-Glc, GDP-Glc, or UDP-Glc. UDP-Glc is the precursor to UDP-Gal, UDP-Rha, and UDP-GlcA and is thus a key intermediate in nucleotide sugar interconversions (see **Figure 3**).

The main route of UDP-Glc formation is via fructose-6-P (Frc-6-P), a major product of photosynthesis. A reversible phosphoglucose isomerase converts Frc-6-P to Glc-6-P, which is then converted to Glc-1-P by a phosphomutase. Glc-1-P in the presence of UTP can then be converted into UDP-Glc by UDP-Glc pyrophosphorylase. Frc-6-P is also a precursor for the formation of other nucleotide sugars including ADP-Glc, UDP-GlcNAc, and GDP-Man. UDP-Glc is also formed from the products of starch and sucrose catabolism, and from galactose, glucose, and mannose recycled from other plant glycans.

UDP-Glc pyrophosphorylase (UGlcPP, also referred to as UTP glucose-1-P uridylyltransferase) is a key enzyme in carbohydrate

metabolism and is present in plants, animals, and micro-organisms. UGlcPP catalyzes the reversible formation of UDP-Glc and PPi from Glc-1-P and UTP. Two *Arabidopsis* genes (At5g17310 and At3g03250) encode proteins with 93% amino acid sequence identity to each other and >80% identity to potato and barley UDP-Glc PPases. A third *Arabidopsis* gene (At3g56040) has also been identified and reported to encode a chloroplastic UDP-Glc PPase that is required for sulfolipid biosynthesis (107).

Recombinant UGlcPP1 (At5g17310) utilizes Glc-1-P and UTP to form UDP-Glc; however, other sugar-1-phosphates, TTP, GTP, and ATP, are not substrates for this enzyme (L. Bar-Peled & M. Bar-Peled, unpublished data). The specificity of UGlcPP2 (At3g03250) has not been determined. Mutations in plant UDP-Glc PPases genes (*UGP*) have been reported to have various phenotypic and chemotypic affects. For example, studies of rice *ugp1* mutants suggest that during pollen development the production of UDP-Glc is critical for callose deposition (21). By contrast, some *Arabidopsis* plants carrying mutations in both UDP-Glc PPase genes (*ugp1* and *ugp2*) have been reported to be phenotypically comparable to wild-type plants (94), whereas other mutant alleles have a dwarfed phenotype and are male sterile (110). One explanation for these phenotypic discrepancies is that the *Arabidopsis ugp1* mutant described by Meng et al. (94) was leaky and the presence of residual amounts of UDP-Glc PPase1 likely contributed to the UDP-Glc pool of the *ugp1 ugp2* double mutant. It is also possible that other pyrophosphorylases, including SLOPPY whose gene is expressed in all plant tissues, contribute to the UDP-Glc pool in the *ugp* mutants. Another possibility that cannot be discounted is that the promiscuous *Arabidopsis* pyrophosphorylase UDP-GlcNAc PPase (GlcNac-UT2, At2g35020) can convert Glc-1-P and UTP to UDP-Glc (169), thus compensating for the lack of "normal" UDP-Glc PPase activity in the *ugp* mutants. Together these genetic and biochemical studies suggest that multiple mechanisms must exist to allow a plant to adapt to mutations that affect UDP-Glc PPase activity.

Early biochemical work established that UDP-Glc PPase is inhibited by UDP-Xyl (47). If this inhibition occurs in vivo, it would imply that UDP-Xyl regulates the UDP-glucose pool. Because many NDP-sugars are ultimately derived from UDP-Glc, this regulation likely also affects the NDP-sugar pool available for the synthesis of cell wall and other glycans.

Plants store glucose in the form of starch that is readily fragmented into glucooligosccharides and glucose by endo- and exo-α-1,4-glucanases. The liberated glucose can then enter the glycolysis and the pentose phosphate pathways. In addition, plants, animals, and certain bacteria contain phosphorylases that in the presence of inorganic phosphate release Glc-1-P from the reducing end of starch/glycogen (124). The reaction is reversible, but likely favors phosphorolysis of the terminal residue because of the relatively low amounts of Glc-1-P and high amounts of inorganic phosphate in plant cells (82). The released Glc-1-P can then be converted to UDP-Glc by SLOPPY or by UDP-Glc PPase. *Arabidopsis* has one gene (At3g29320) that encodes a chloroplastic starch phosphorylase and another gene (At3g46970) that encodes a cytosolic form of the enzyme. However, the plastidal enzyme in *Arabidopsis* leaves (173) and its diurnal regulation (160) may affect different metabolic pathways. In addition, the plasitidal enzyme in wheat endosperm (151) and *Arabidopsis* leaves (173) may be involved in other pathways. In rice endosperm the plastid phosphorylase (PHO1) has been proposed to synthesize starch oligosaccharides rather than generate Glc-1-P (65). The substrate specificity of the cytosolic phosphorylase has not been studied in detail. Thus, it is possible that the cytosolic enzyme can utilize other oligosaccharides including those generated by the turnover of cell wall polysaccharides as substrates to generate sugar-1-P.

Sucrose, a major product of photosynthesis, is an important carbon source for growing cells and is readily transported from leaves to other

organs where it is hydrolyzed to Glc and Frc (see **Figure 3**). Sucrose can also be converted into UDP-Glc by sucrose synthase (SuSy), which catalyzes the reversible conversion of sucrose and UDP into UDP-Glc and fructose (120). In vitro, SuSy converts sucrose to TDP-Glc and ADP-Glc as well as GDP-Glc and CDP-Glc in the presence of the appropriate NDP-sugar (44, 120). However, it is not known if this conversion to glucose molecules with different nucleotide substituents occurs only in specific plant species or occurs throughout the plant kingdom. Moreover, if SuSy does generate different NDP-glucoses, how these activated sugars contribute to the NDP-sugar pool in different tissues remains to be determined.

In *Arabidopsis*, the tissue expression patterns of the six known SuSy isoform transcripts are complex (13). Biochemical characterization of each SuSy isoform and their relationships with UDP-Glc PPase isoforms are required to elucidate their role in regulating the flux of carbon to the cell wall or in carbohydrate storage. Moreover, there is evidence that distinct SuSy isoforms localize to the Golgi apparatus, the tonoplast, and the plasma membrane (46) and that some isoforms interact with the actin cytoskeleton and mitochondria (40, 61–63). One of the three SuSy isoforms in maize (SH1) has been reported to contain a mitochondrial targeting peptide (145). A mechanism has been proposed for regulating movement of SuSy between the cytosol and membranes by specific phosphorylation of amino acids at the N terminus region of the protein (62, 63).

Recent analyses of SuSy mutants suggest that individual members of this small multigene family may have specific functions and are not redundant with one another. Indeed, the three SuSy isoforms (Sus1–3) in pea have different kinetic properties. For example, only Sus1 is strongly inhibited by Frc (164), suggesting that each enzyme may be regulated in a different fashion in the cell. There are also reports that SuSy is a component of the cellulose synthase complex at the plasma membrane and may direct UDP-glucose to cellulose synthesis (4, 51). However, the role of SuSy, Suc-6-P synthase, and invertase in regulating carbon flux in plant growth and development remains a subject of considerable debate (11, 22, 23, 51).

Another route for formation of UDP-Glc in plants is via epimerization. UDP-Gal 4-epimerase (UGE) catalyzes the reversible interconversion of UDP-Glc and UDP-Gal (47). Studies of UGE isolated from numerous organisms have established that the enzyme requires NAD^+ as a cofactor to form the 4-keto sugar intermediate during catalysis (for review see Reference 49). Some UGEs tightly bind the cofactor whereas others do not. The avidity of UGE for NAD^+ may depend on the number of hydrogen bonds formed at the NAD-binding pocket. For example, bacterial UGE has 19 H-bonds and requires no added NAD, whereas human UGE, which requires added NAD^+, has only 11 (149).

UGEs have been isolated from *Arabidopsis* (38, 135) and barley (174), and multiple isoforms have been identified in the rice and maize genomes. Biochemical, genetic, and molecular studies on the five distinct UGE isoforms in *Arabidopsis* (128) established that they differ in their requirement for exogenous NAD^+, have different catalytic efficiencies, and differ in their cellular location (cytosol or Golgi). Barber et al. (10) also suggested that some of the isoforms may channel their product for the synthesis of a particular glycan. In addition, plant UGE isoforms may function in different metabolic situations with differences in enzymatic properties, gene expression patterns, and subcellular localization contributing to the isoform function (10). This suggestion is likely correct, not only for the UGE isoforms, but also for other isoforms of enzymes involved in the formation of NDP-sugars (64).

Independently isolated mutant alleles of one of the five ubiquitously expressed UGE genes (At1g64440, *UGE4*) including *reb1* (*root epidermal bulger 1*,) and *rhd1* (r*oot hair deficient 1*) (136) have altered cell wall compositions, indicating that this UGE may have a role in providing UDP-Gal for the galactosylation of arabinogalactan and xyloglucan. These results together with studies of other UGE

mutants (128) suggest that UGE2 and UGE4 affect the flux of galactose from UDP-D-Gal into different downstream products, and that substrate channeling has a role in regulating cell wall biosynthesis.

ADP-α-D-Glucose

ADP-Glc is the precursor for the synthesis of starch, a major storage polysaccharide in most plants. Adenosine 5′-diphosphate-glucose pyrophosphorylase (ADPGlc PPase) catalyzes the conversion of Glc-1-P and ATP to ADP-Glc and PPi in the first and rate-limiting step in starch biosynthesis. Cereal endosperms contain a cytosolic and a plastid-localized ADPGlc PPases (97, 98, 108). By contrast, ADPGlc PPase is located in the plastids of monocot and dicot leaves and in the heterotrophic organs of dicots. Plant ADPGlc PPase is composed of a small and a large subunit and is allosterically regulated by 3-phosphoglycerate and phosphate (8). The *Arabidopsis* genome contains six genes encoding ADPGlc PPase. Two genes encode small subunits (ApS1 and ApS2) and four genes encode the large subunits (ApL1–ApL4). On the basis of recombinant enzyme activities, mRNA expression, and the fact that recombinant Aps2 has no ADPGlc PPase activity, ApS1 has been proposed to be the catalytic isoform responsible for activity in *Arabidopsis* tissues (25, 26, 156). These authors also suggest that each isoform of the large subunits has a regulatory role. For example, ApL1 is expressed in source tissues, whereas ApL3 and ApL4 are the predominant isoforms expressed in sink tissues. Thus, in source tissues, ADPGlc PPase may be regulated by the 3-phosphoglycerate/phosphate ratios, whereas in sink tissues activity would be dependent on the availability of substrates (8). By contrast, in cereal endosperm the transport of ADP-Glc from the cytosol into the plastid may be the limiting factor. For example, the barley low-starch-content plastidial ADPGlc transporter (HvNst1) mutant (111) accumulates high levels of ADP-Glc in the developing endosperm, indicating that the cytosolic pool of ADP-Glc is not under metabolic control in this tissue. Furthermore, *Arabidopsis* leaves overexpressing SuSy contained more ADP-Glc and starch than wild-type leaves, whereas in leaves containing antisense SuSy the amounts of ADP-Glc and starch that accumulated were reduced (99). Such data suggest that in source leaves ADP-Glc produced by SuSy (outside the chloroplast) is directly linked to, and appears to control, starch biosynthesis. This implies that SuSy, but not ADPGlc PPase, controls the level of ADP-Glc in the cytosol in source leaves (25, 26). However, the metabolic fate of ADP-Glc in the cytosol may need to be re-evaluated following the identification of an *Arabidopsis* gene (At5g18200) that has been reported to encode an ADP-Glc phosphorylase (93). The authors suggest that the enzyme has adenylyltranferase activity and catalyzes the transfer of AMP from ADP-Glc onto Pi forming Glc-1-P and ADP. The ability of the enzyme to transfer AMP to Man-1-P and Gal-1-P is much lower than with Pi. Interestingly, the ADP-Glc phosphorylase cannot utilize UDP-Glc as a donor substrate unlike the human and fungal enzymes that transfer UMP from UDP-Glc onto Gal-1-P forming Glc-1-P and UDP-Gal. The ability of this enzyme to use other substrates including GDP-Glc has not been determined.

Understanding the relationship between the highly regulated ADPGlc PPase involved in ADP-Glc formation and the ADP-Glc phosphorylase involved in ADP-Glc catabolism to Glc-1-P may explain how a plant regulates the amounts of ADP-Glc in a specific cell type and shed light on the availability of ADP-Glc for different metabolic routes.

TDP- and GDP-α-D-Glucose

Extracts from numerous plants including alfalfa, soybean, mung bean, sugar beet, wheat, pea, and lily (for a review, see Reference 47) contain a thymidine-5′-diphosphoglucose pyrophosphorylase (TDP-Glc PPase) that is distinct from UDP-Glc PPase. This may account for the presence of TDP-GalA and TDP-Rha in some plants, although their biological

significance is not known. It would be of interest to determine if any of the functional UDP-Glc-PPases (see UDP-α-D-Glucose section above) can use TTP as a substrate for the formation of TDP-Glc in some plants.

GDP-Glc is a precursor for the synthesis of glucomannan (87, 119) and may also have a role in cellulose synthesis (33). Early reports suggested that plants contain pyrophosphorylase activity that converts GTP and Glc-1-P to GDP-Glc, but the enzyme was never purified nor were the corresponding gene(s) identified (47). Thus, for many years the mechanisms for the formation of GDP-Glc in plants remained enigmatic. However, a genetic screen for ascorbic acid–deficient plants identified two mutants, *vtc2* (At4g26850) and *vtc5* (At5g55120), which were subsequently shown to encode GDP-L-galactose phosphorylases by three independent groups (89). In addition to Pi-dependent interconversion of GDP-L-galactose and L-Gal-1-P, the recombinant enzymes interconvert GDP-D-glucose and D-Glc-1-P with catalytic efficiency similar to the formation of GDP-L-Gal (89, 90). Unlike typical pyrophosphorylases that utilize NTP and sugar-1-P (reaction 1), the VTC2 and VTC5 phosphorylases utilize GDP (catalyze reaction 2):

- GTP + Hexose-1-P ↔ GDP-hexose + PPi (reaction 1)
- GDP + hexose-1-phosphate ↔ GDP-hexose + Pi (reaction 2)

The recombinant *Arabidopsis* VTC2 and VTC5 enzymes do not utilize UDP-Glc or ADP-Glc (88, 90). Mutations in VTC2 affect ascobate accumulation; however, the roles of VTC2 and VTC5 in generating GDP-Glc for glucomannan synthesis have not been determined.

UDP-*N*-Acetyl-α-D-Glucosamine

N-acetyl-D-glucosamine (GlcNAc) is a component of the N-linked oligosaccharides of glycoproteins in plants and animals and is also present in glycolipids (83). In addition, posttranslational *O*-GlcNAc-modification of serine residues in certain cytosolic and nuclear proteins regulates metabolic processes in both plants and animals (109). In eukaryotes synthesis of UDP-GlcNAc is a multistep process. A single enzyme, GlcN-6-P synthase, transfers the amine from L-glutamine to Frc-6-P to form FrcN-6-P, which is then isomerized to glucosamine-6-P (GlcN-6-P). Glucosamine-6-P is then *N*-acetyled by GlcN-6-P acetyltransferase (GNA) using acetylCoA. The *N*-acetyl-glucosamine-6-P (GlcNAc-6-P) is then converted to GlcNAc-1-P by a phospho-*N*-acetylglucosamine mutase. Finally, the GlcNAc-1-P is converted to UDP-GlcNAc in a reversible reaction by a UTP:*N*-acetyl-glucosamine-1-P uridylyltransferase in the presence of UTP (42).

An *Arabidopsis* gene (At1g31070) that encodes an *N*-acetylglucosamine-1-phosphate uridylyltransferase (GlcNAc1pUT-1) has been identified (169). The enzyme requires divalent ions (Mg^{2+} or Mn^{2+}) and can also generate UDP-GalNAc. A second *Arabidopsis* gene (At2g35020) has been identified and encodes a protein (GlcNAc1pUT-2) with 86% amino acid sequence identity to GlcNAc1pUT-1 (169). Recombinant GlcNAc1pUT-2 also converts GlcNAc-1-P, GalNAc-1-P, and Glc-1-P to their corresponding UDP-sugars (169). There is evidence that plants accumulate UDP-GalNAc, (3) but it is not known if GalNAc is incorporated into glycans. Moreover, the ability of GlcNAc1pUT-2 to generate UDP-Glc suggests that multiple metabolic routes have evolved in plants to form this nucleotide sugar.

UDP-α-D-Galactose

Galactose is present in the pectic polysaccharides, arabinogalactan proteins, hydroxyproline-rich glycoproteins, xyloglucans, and galactomannans (20, 133). UDP-α-D-Gal is the sugar donor for the synthesis of these glycans and may be formed via the salvage pathway or through the epimerization of UDP-Glc.

The D-galactokinase that phosphorylates α-D-Gal at C-1 was first identified in mung

bean (100) and later cloned from *Arabidopsis* and functionally characterized (168). The galactokinase gene (At3g06580, GalK, Gal1) functionally complements yeast (71) and *Escherichia coli* mutants that cannot metabolize galactose. The *Arabidopsis* GalK belongs to the GHMP (galacto-, homoserine, mevalonate, and phosphomevalonate) family of kinases. By using NMR spectroscopy to monitor the enzyme reactions, unambiguous evidence was obtained that the recombinant *Arabidopsis* GalK only phosphorylates C-1 of α-D-Gal and that ATP cannot be substituted by other nucleotides (168). The kinase also phosphorylates 2-deoxy-Gal but cannot phosphorylate GalNAc. In the presence of SLOPPY and UTP, α-D-Gal-1-P and 2-deoxy-α-D-Gal-1-P can be converted to UDP-α-D-Gal and UDP-2-deoxy-α-D-Gal, respectively (168). GalK is likely widely distributed in green plants as homologs exist in rice, poplar, the moss *Physcomitrella patens*, the chlorophycean algae *Chlamydomonas reinhardtii*, and the prasinophytes *Ostreococcus lucimarinus* and *Micromonas pusilla*.

UDP-α-D-Glucuronic Acid

D-Glucuronic acid (GlcA) is a quantitatively minor constituent of plant glycans. β-D-GlcA is present in rhamnogalacturonans I and II and in arabinogalactans. However, the GlcA present in xylans is α-linked and is often methylated at O-4 (43). UDP-α-D-GlcA is a key intermediate in NDP-sugar metabolism as it is the precursor for UDP-D-xylose, UDP-L-Ara*p*, UDP-Api*f*, as well as UDP-D-GalA. Collectively these NDP-sugars contribute to synthesis of up to 40% of the glycans in cell wall polysaccharides.

Plant cells rapidly incorporate GlcA into pectin (74, 101). Membrane and soluble fractions from mung bean seedlings contain kinases that in the presence of ATP and Mg^{2+} specifically phosphorylate GlcA to form GlcA-1-P (47). These extracts also phosphorylate L-Ara and D-Gal but not GalA. Two *Arabidopsis* genes (At3g01640 and At5g14470) that encode GlcA kinases (GlcAK) were identified using peptide sequences obtained from a partially purified lily GlcAK (118). The recombinant *Arabidopsis* GlcAK requires Mg^{2+} for activity, is specific for GlcA, and has very low amino acid sequence identity with GalK or GalAK that belong to the GHMP kinase family. Once GlcA 1-P is formed it can be converted to UDP-GlcA by SLOPPY.

A second route to form UDP-GlcA is via UDP-Glc dehydrogenase (UDP-GlcDH, UGD, UGDH). This enzyme in the presence of NAD^+ catalyzes the oxidation of UDP-Glc to UDP-GlcA. UDP-GlcDH has been isolated from numerous organisms and characterized in detail. In plants, the gene encoding UGD activity was first described in soybean (76, 148). Subsequently, numerous UDP-GlcDH genes encoding proteins with high amino acid sequence similarity to one another were identified in *Arabidopsis*, poplar (68), tobacco (15), maize (72), and *Dunaliella* (122). Each plant has multiple UGD isoforms and their specific activities are of interest because studies with sugar cane revealed that CTP-glucose and TDP-glucose were also oxidized, albeit less effectively than UDP-Glc (154). The flux of UDP-Glc to UDP-pentoses may be feedback-regulated in part at the enzyme level as in vitro data shows that UDP-GlcDH is inhibited by UDP-Xyl (47).

The alcohol dehydrogenase (ADH) from *Phaseolus vulgaris* (127) and a recombinant tobacco ADH (15) are believed to be bifunctional enzymes that also convert UDP-Glc to UDP-GlcA. However, NADH production rather than UDP-GlcA formation was measured in these studies. Thus, the ability of ADH to catalyze the formation of UDP-GlcA remains to be confirmed. Moreover, maize mutants lacking ADH1 and ADH2 isoforms and wild-type plants have cell wall hemicelluloses with comparable glycosyl residue compositions (72). Additional studies of these ADH proteins are required to define their roles in NDP-sugar metabolism.

UDP-GlcA can also be formed via the myo-inositol oxidation pathway. *Myo*-inositol-1-P is formed by the cyclization of Glc-6-P in a reaction catalyzed by

myo-inositol-1-phosphate synthase. *Myo*-inositol-1-P is then dephosphorylated by *myo*-inositol monophosphatase (IMPase). Finally, the *myo*-inositol generated by IMPase is oxidized by inositol oxygenase (MIOX) to D-GlcA (91).

Two *Arabidopsis* genes (At4g39800 and At2g22240) encoding functional isoforms of *myo*-inositol-1-phosphate synthase have been identified (123). It was originally proposed that At3g02870 (*VTC4*) encoded a IMPase-like-protein, although biochemical and genetic data now suggest that this gene encodes a bifunctional enzyme with both L-Gal-1-P phosphatase (85) and IMPase activities (152). Other genes, At1g31190 and At4g39120, referred to as IMP-like (152) have been identified that may encode IMPase but biochemical evidence is lacking. *Arabidopsis* contains four MIOX isoforms (45) and mutants lacking MIOX1 and -2 isoform activities have comparable wall compositions to wild-type plants (70). Thus, the contribution of the *myo*-inositol oxidation pathway and the UDP-GlcDH pathway to the flux of UDP-GlcA is not clear, and these pathways may operate independently of one another. As *myo*-inositol is used for the production of ascorbate, it is possible that its contribution to UDP-GlcA formation is minor. However, the possibility cannot be discounted that the *myo*-inositol oxidation pathway provides UDP-GlcA for specific plant tissues.

The availability of UDP-GlcA for cell wall polysaccharide synthesis has been investigated using maize plants carrying mutations in UDP-Glc dehydrogenase isoforms A and B. Polysaccharides isolated from the walls of the isoform A mutant had lower Ara/Gal and Xyl/Gal ratios than wild-type plants, whereas the walls of the isoform B mutant and wild-type plants were comparable. Thus, isoform A of UDPGDH may provide NDP-sugars for wall polysaccharide synthesis (72, 73), whereas the isoform B mutant may provide a pool of NDP-sugars for the formation of other compounds including low-molecular-weight glycosides. However, the B isoform may generate NDP-sugars for the synthesis of a quantitatively minor cell wall component, and changes in the wall composition of the mutant may be small and not readily detected.

The factors that control the supply and flux of UDP-GlcA in plants is still a subject of debate, and it is possible that different plants adopt alternative mechanisms to regulate the formation of UDP-GlcA. For example, in maize the A isoform of UDPGDH may supply most of the UDP-pentoses and the *myo*-inositol oxidation pathway is unable to compensate for the altered formation of UDP-GlcA. Thus, it is likely that the major contributor for flux of NDP-sugars in plants is UDP-Glc.

UDP-α-D-Xylose

D-Xylose (Xyl) is a quantitatively major component of the polysaccharides present in both primary (xyloglucan) and secondary cell walls (xylan) (170). Small amounts of xylose are also present in the pectic polysaccharide xylogalacturonan (41, 67) and the N-linked oligosaccharides of plant glycoproteins (144). The xylose in the pectic polysaccharide RG-II is methylated at O-2 (104).

UDP-Xyl is formed primarily by the decarboxylation of UDP-GlcA in a reaction catalyzed by UDP-GlcA decarboxylase (UGlcA-DC). We typically refer to this enzyme as UDP-Xylose synthase (UXS) because some bacterial UDP-GlcA decarboxylases are involved in the formation of UDP-4-amino-4-deoxy-L-arabinose as well as UDP-xylose from UDP-GlcA (56, 163). UXS has a tightly bound NAD^+, which participates in the oxidation of UDP-GlcA to the UDP-4-keto-hexose intermediate that is then decarboxylated to form a UDP-4-keto-pentose (95). The enzyme-bound NADH then reduces the UDP-4-keto-pentose to UDP-Xyl, resulting in the regeneration of NAD^+ (64). Functional identification of plant UXS genes led to the realization that plants have multiple UXS isoforms (112). UXS genes have been identified in *Arabidopsis* (64, 112), rice, maize (57, 146), barley (175), and tobacco (15). In *Arabidopsis*, UXS isoforms act only on UDP-GlcA (64). UXS is active as a dimer and

is inhibited by UDP-Xyl (64, 112). Phylogenic analysis of *Arabidopsis* UXSs identified three distinct clades (64). Type A (At3g53520) and Type B (At3g62830, At2g47650) isoforms have an N-terminal extension (~120 amino acids long) that results in proteins with higher molecular masses than those of the cytosolic Type C (At5g59290, At3g46440, At2g28760) isoforms (112). Type A and B UXS isoforms are predicted type II membrane proteins with the catalytic domain facing the membrane lumen (112), whereas the three Type C UXS isoforms are cytosolic. Studies are now required to determine why certain NDP-sugar biosynthetic enzyme activities are partitioned in different cellular compartments (**Figure 4**). For example, why is UDP-xylose made in both the cytosol and inside the Golgi? Does this partitioning provide channeling of sugars to different glycan metabolic routes? Or does partitioning provide a mechanism to control, at the metabolite level (e.g., UDP-xylose), selected NDP-sugar biosynthetic enzymes (57), including UDP-Glc dehydrogenase and UDP-GlA 4-epimerase? Determining the roles of cytosolic and membrane-bound UXS isoforms and their role in supplying precursors for xylan and xyloglucan synthesis may shed light on the role of multiple isoforms of NDP-sugar interconverting enzymes in plants.

UDP-β-L-Arabinopyranose and UDP-β-L-Arabinofuranose

L-Arabinose (Ara) is a quantitatively major component of the pectic polysaccharides RG-I and RG-II, the arabinogalactan proteins, and the Hyp-rich glycoproteins. Arabinose is also present in the xylans of grasses and the xyloglucans of selected lycopodiophytes, pteridophytes, and solanaceous plants (114). Arabinose exists predominantly in the furanose ring form (Ara*f*) in most plant glycans, although some polysaccharides including RG-I and RG II and the xyloglucans of selected lycopodiohytes and pteridophytes also contain arabinopyranosyl (Ara*p*) residues (104, 114). UDP-Ara*p* but not UDP-Ara*f* has been detected in extracts from numerous plant species and its formation has been studied in detail (50). UDP-Ara*p* is formed by either epimerization of UDP-Xyl or by the arabinose salvage pathway (47). The interconversion of UDP-Ara*p* and UDP-Ara*f* is catalyzed by a mutase (78).

Membrane fractions from mung bean catalyze the phosphorylation of L-Ara to β-L-Ara-1-P (100). D-Ara is not a substrate for the arabinose kinase (AraK). The same membrane preparation, however, also converted D-Gal to α-D-Gal-1-P. Nevertheless, AraK and GalK differ because only the AraK requires Mg^{2+}or Mn^{2+} for activity. The membrane-bound AraK activity but not the GalK activity is solubilized by detergent (47). α-L-Ara-1-P can be converted to UDP-Ara by SLOPPY (80). The *Arabidopsis ara1* mutant (At4g16130) lacks Ara-1-P kinase activity and has reduced ability to metabolize arabinose (59, 140). Bioinformatic analysis suggests that ARA1 belongs to the GHMP family of kinases. However, the biochemical properties and substrate specificity of the ARA1 protein have not been determined. For example, it is not known if ARA1 can phosphorylate Ara*f* or if plants contain mutases that interconvert Ara*f* and Ara*p*.

A second route to form UDP-Ara*p* is via 4-epimerization of UDP-Xyl (47). An *Arabidopsis* mutant (*mur4*) that has reduced amounts of cell wall arabinose was indentified in a genetic screen of EMS-mutagenized plants (19). Subsequently the gene At1g30620 was identified and the recombinant protein UDP-Xyl 4-epimerase (UXE1) shown to interconvert UDP-D-Xyl and UDP-Ara. UXE1 is Golgi-localized and is predicted to be a type-II membrane protein with its catalytic domain facing the lumen (19). A second isoform (UXE2, At2g34850) that has at least 76% sequence identity to UXE1 has been identified in *Arabidopsis* (19). There are two UXE isoforms in rice and three UXE isoforms in barley (176).

Microsomes isolated from several plants contain glycosyltransferase activities that transfer the Ara*p* from UDP-Ara*p* onto endogenous and exogenous acceptors (66). This is somewhat surprising as Ara*p* is rarely present in

plant glycans. However, the possibility cannot be discounted that the transfers were catalyzed by galactosyltransferases given that UDP-β-L-Ara*p* differs from UDP-α-D-Gal*p* only by the absence of a primary alcohol (CH_2OH) attached to C-5.

The mechanism of UDP-Ara*f* formation remained enigmatic until Ishii's lab (78) demonstrated that rice plants contain a UDP-arabinopyranose mutase (UAM) complex that interconverts UDP-Ara*p* and UDP-Ara*f*. Amino acid sequences generated from the proteins in the complex allowed the identification of three polypeptides corresponding to rice genes *UAM1* (Os03g40270), *UAM2* (Os04g56520), and *UAM3* (Os07g41360). UAM1 and UAM3 have >80% amino acid identities but share only 50% identity with UAM2. Recombinant UAM1 and UAM3 interconvert UDP-Ara*p* and UDP-Ara*f* and to a lesser extent UDP-Gal*p* and UDP-Gal*f*. However, no activity was discernible with recombinant UAM2 (78). Somewhat unexpectedly, the rice UAMs had amino acid sequences virtually identical to plant proteins isolated from peas and *Arabidopsis* that were reversibly glycosylated (RGP) in the presence of UDP-Glc (30) and to a self-glucosylating protein from corn (141). Indeed, the rice UAM complex, the recombinant mutases UAM1 and UAM3, and the RGPs from pea and *Arabidopsis* all reversibly bind several UDP-sugars, thus UAMs/RGPs likely have dual functions: binding UDP-sugars and interconverting UDP-Ara*p* and UDP-Ara*f*. Four *Arabidopsis* genes (At3g02230, RGP1; At5g15650, *RGP2*; At3g08900, *RGP3*; At5g50750, *RGP4*) encode proteins homologous with UAM1 and UAM3. One *Arabidopsis* gene (At5g16510, *RGP5*) is homologous to rice UAM2 but the function of the encoded proteins remains elusive. Some RGPs have been reported to be cytosolic (36, 78) and to localize to the Golgi apparatus (29, 39) and to plasmodesmata (131, 172). Up to 80% of the rice UAMs have been reported to be cytosolic (78). However, the factors that control the distribution of RGPs/UAMs between the cytosol and membranes (**Figure 4**) are not understood nor is it known if the cytosolic and membrane-bound proteins have the same functions.

Highly conserved genes coding UAMs exist in the sequenced genomes of all land plants and in *C. reinhardtii* (78). Interestingly, *C. reinhardii* also contains a gene with sequence homology to the bacterial and fungal mutases that interconvert UDP-Gal*p* and UDP-Gal*f* (14). The existence of putative UDP-Ara*p* and UDP-Gal*p* mutases in this algae may explain the presence of both Ara*f* and Gal*f* residues in the hydroxyproline-rich glycoprotein of its cell wall (16, 48, 105). Early studies of the corn self-glucosylating protein indicated that the Arg residue in the sequence EGDAFV**R**GYP was glycosylated (141). Subsequently, it has been shown that replacing this Arg with Ala in rice UAMs results in no mutase activity (77). The evidence that UAMs (RGPs) interconvert UDP-Ara*p* and UDP-Ara*f* is compelling; nevertheless, UDP-Ara*f* accounts for only 10% of the products formed because of the preference of the reaction toward the pyranose product. This may explain why plant cells have to produce large amounts (1% of soluble protein) of UAM/RGP to generate UDP-Ara*f* in amounts sufficient for plant glycan synthesis. The UDP-Ara*f* formed is presumed to be the donor for the synthesis of Ara*f*-containing glycans. However, as far as we are aware no such arabinofuranosyltransferase activities have yet been demonstrated. Moreover, the biological significance of the ability of these proteins to reversibly bind a range of NDP-sugars remains to be determined. Nevertheless, it is clear that some of these proteins are essential for normal plant growth and development because knocking out both *RGP1* and *RGP2* is lethal in *Arabidopsis* (39).

UDP-α-D-Galacturonic Acid

Galacturonic acid (GalA) is a quantitatively major component of pectic polysaccharides and numerous plant gums and mucilages and is a minor component of the xyloglucans produced by mosses and liverworts (114). A GalA residue is also present in the glycosyl sequence

(4-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xyl*p*) present at the reducing end of dicot and gymnosperm xylans (115).

Radiolabeled GalA fed to plants is readily incorporated into pectic polysaccharides (47). Soluble enzyme preparations from germinating mung bean seeds have GalA kinase (GalAK) activity (47). An *Arabidopsis* gene (At3g10700) encoding a functional GalAK activity was recently described and the recombinant enzyme was shown by ^{1}H NMR spectroscopy to phosphorylate α-D-GalA in the presence of ATP (168). The GalA-1-P can then be converted to UDP-GalA by SLOPPY (168). However, the contribution of this salvage pathway to wall biosynthesis and the amounts of GalA that are recycled to UDP-GalA in vivo is not known.

UDP-GalA is also formed from UDP-GlcA in a reaction catalyzed by a UDP-GlcA 4-epimerase (UGlcAE, GAE). There are six different genes in *Arabidopsis* that encode isoforms of UGlcAE (54) that are all predicted to be membrane-localized proteins. Indeed, UGlcAE activity has been reported to cofractionate with Golgi markers on sucrose gradients (143). We have obtained data using protease protection assays suggesting that the UGlcAEs are type-II membrane proteins with their catalytic domain facing the lumen and that UGlcAE1-GFP is targeted to the Golgi (X. Gu & M. Bar-Peled, unpublished data). Multiple UGlcAE isoforms, also described in the literature as GAE (126), are also present in other plants including poplar, rice, and maize. The *Arabidopsis*, maize, and rice enzymes generate UDP-GlcA and UDP-GalA in the ratio of 1:2 and are inhibited by UDP-Ara and UDP-Xyl (55). Interestingly UDP-Xyl inhibits maize UGlcAE more than *Arabidopsis* UGlcAE2. Whether such an affect may in part account for the differences in the amounts of xylan and pectin present in the primary walls of grasses and dicots remains to be determined.

There is now compelling evidence that enzymes involved in the formation of UDP-GalA are present in both the cytosol (GalAK and SLOPPY) and in the Golgi (UGlcAE) (53, 168). Moreover, based on a variety of transcriptome data, it appears (**https://www.genevestigator.com/gv/index.jsp**) that the genes encoding these enzymes are expressed in all *Arabidopsis* tissues. How this compartmentalization (**Figure 4**) provides the UDP-GalA for the synthesis of specific glycans remains to be determined.

UDP-β-L-Rhamnose

L-Rhamnose (6-deoxy-L-mannose, Rha) is present in the backbone of RG-I and in the side chains of RG-II (104). A Rha residue is also present in the reducing-end component of dicot and gymnosperm xylans (115). Rhamnose is also a major component in numerous glycosides of secondary metabolites. UDP-β-L-rhamnose (UDP-Rha) is used for the synthesis of flavonoids (9). However, the activated form of Rha used for pectin synthesis has not been identified with certainty.

UDP-Rha is likely formed from UDP-Glc by a UDP-Rha synthase in the presence of NAD(P)H and possibly by a salvage pathway involving the formation of Rha-1-P (**Figure 3**). The synthesis of UDP-Rha from UDP-Glc was initially believed to require three separate enzymes based on the mechanism of conversion of TDP-Glc to TDP-Rha in bacteria (47). However, this notion proved to be incorrect when an *Arabidopsis* gene (At1g6300) was shown to encode a protein (NRS/ER, also annotated as UER) with both 3,5-epimerase and 4,6-keto-reductase activities (158). Interestingly, in vitro NRS/ER uses TDP- and UDP-4-keto-6-deoxy-Glc as substrates to form TDP-Rha and UDP-Rha, respectively. Although TDP-Glc is found in plants (47) and several enzymes can generate TDP-Glc in vitro, the physiological significance of the ability of NRS/ER to generate TDP-Rha is unclear.

The *Arabidopsis* genome contains three genes (At1g78570, *RHM1*; At3g14790 *RHM3*; At1g53500, *RHM2*) that encode proteins (~670 amino acids long) with an N-terminal domain (~330 amino acids long) that shares amino acid

sequence similarity to a 4,6-dehydratase and a C-terminal domain (~320 amino acids long) that shares more than 80% sequence identity to NRS/ER. Indeed, the C-terminal domain of At1g78570 and NRS/ER have comparable enzyme activities (158). *Arabidopsis* plants overexpressing *RHM1* have cell walls that contain more rhamnose and galactose and less glucose than wild-type walls, suggesting an increase in the amounts of rhamnogalaturonan (157). However, silencing of *RHM1* had no discernible effect on cell wall composition or plant phenotype. *Arabidopsis mum4* and *rhm2* plants carry mutations in At1g53500 (6, 155, 161) and have seed mucilage that contains decreased amounts of Rha and GalA. By contrast, the seed mucilages of the *rhm1* knockdown mutant and wild-type plants were indistinguishable (157). Nevertheless, these studies together with the data showing that recombinant MUM4/RHM2 forms UDP-L-Rha (106) provide strong evidence that *MUM4/RHM2* encodes a UDP-Rha-synthase.

UDP-α-D-Apiose

D-apiose (Api) is a branched-chain sugar present in RG-II, polysaccharides isolated from seagrasses (52) and from *Lemna*, and numerous secondary metabolites including apigenin synthesized by parsley and cyanidin (5). Early studies established that parsley contains an enzyme that in the presence of NAD^+ generated UDP-Api and UDP-Xyl from UDP-GlcA. The enzyme was proposed to decarboxylate UDP-GlcA, thereby forming a UDP-4-keto-pentose intermediate that could then undergo ring rearrangement to form UDP-Api or be reduced to UDP-Xyl. However, the formation of UDP-apiose was not confirmed as it is readily converted to the cyclic apiose 1,2-di-phosphate (58).

Numerous studies have led to the identification and functional characterization of genes from *Arabidopsis* (96), tobacco (1), and potato (58) that encode proteins that convert UDP-GlcA to UDP-Api. Two isoforms exist in *Arabidopsis* (At1g08200, At2g27860) and are predicted to be located in the cytosol. Guyett et al. (58) used real-time NMR spectroscopy to demonstrate unambiguously the production of UDP-Api by the recombinant potato enzyme. These authors also showed that the ratio of UDP-Api and UDP-Xyl formed was 2:1 and that in vitro UDP-Xyl synthesis lags behind UDP-Api formation. Apiose is critical in plant growth and development (103), and mutations in UDP-Api synthase are lethal in *Nicotiana benthamiana*, most likely as a consequence of the lack of RG-II (1).

GDP-α-D-Mannose

D-Mannose (Man) is present in many plants as a component of storage polysaccharides that include mannan, glucomannan, and galactoglucomannan. Mannose is also a major component of the N-linked oligosaccharides of plant glycoproteins. Glucomannans are present in the secondary walls of gymnosperms. Mannose-containing polysaccharides have also been reported to be major components in seedless plant cell walls (121) as well as in woody plant cell walls (69, 167).

GDP-α-D-Man is the source of mannose for the synthesis of glycoproteins, polysaccharides, and ascorbic acid in plants. GDP-Man is also the precursor for GDP-β-L-Fuc and GDP-L-Gal. GDP-Man pyrophosphorylase (GDP-Man PPase also referred to as GTP: α-D-mannose-1-phosphate guanylyltransferase) catalyzes the conversion of α-D-Man-1-P and GTP to GDP-Man and pyrophosphate. Work from our laboratory (M. Echole & M. Bar-Peled, unpublished data) has identified an *Arabidopsis* gene (At2g39770) that encodes a GDP-Man PPase that is specific for Man-1-P. The *Arabidopsis* cell wall mutant *cyt1* (92) and the ozone-sensitive/ascorbate deficient mutant *vtc1* both carry a mutation in At2g39770 (24, 142). The *vtc1* mutation is not lethal as the homologous gene At4g30570 may compensate for loss of activity. However, *cyt1* mutants arrest at the embryo stage. Mutations that affect GDP-Man formation are likely to be pleiotropic (12) as they also affect the formation

Glycosylation: the transfer of a sugar from an activated sugar donor to another molecule (e.g., lipid, polysaccharide, polypeptide, flavonoid)

of GDP-Fuc and GDP-L-Gal, the formation of ascorbate, the glycosylation of proteins, and the formation of storage and cell wall glycans including cellulose (92). Thus, it is difficult to assess how each of these pathways contributes to the phenotypes of *vtc1* and *cyt1* and to normal plant growth and development.

GDP-β-L-Fucose

L-fucose (6-deoxy-L-Gal; Fuc) is present in numerous plant cell wall polysaccharides including pectins, root and seed mucilages, arabinoglactan proteins (166), and xyloglucan as well as plant glycoproteins (138, 153). The donor GDP-β-L-Fuc is derived from GDP-α-D-Man by enzymes that have been characterized and the corresponding genes identified in plants, animals, and bacteria. In addition, GDP-Fuc can be formed via a salvage pathway with a bifunctional protein that converts L-fucose to Fuc-1-P, which subsequently in the presence of GTP forms GDP-Fuc and PPi (79). Plants carrying a null mutation in the *AtFKGP* gene had no visible phenotype, although chemotypically they accumulated up to 40-fold more (~45 μg/g fresh weight) free fucose than wild-type plants [~1 μg/g fresh weight (79)]. The gene encoding this bifunctional enzyme is expressed in all *Arabidopsis* tissues indicating that the Fuc salvage pathway is important but apparently, under lab conditions, is not essential for GDP-Fuc formation as this GDP sugar is also formed from GDP-Man. Given that fucose accounts for ~4% of the *Arabidopsis* leaf cell wall (117, 132) we estimate that the accumulation of free fucose in the *fkgp* mutant requires that ~10% of the fucose contained in wall glycans is turned over to account for the presence of 45 μg/g free fucose in the mutant.

The second enzymatic system that generates GDP-Fuc in plants is via GDP-Man. GDP-Man is first converted to a GDP-4-keto-6-deoxy-Man intermediate by GDP-Man-4,6,-dehydratase (GMD). This intermediate is then converted to GDP-Fuc by GDP-4-keto-6-deoxymannose-3,5-epimerase-4-reductase (GER1) (126). *Arabidopsis* has two GMD isoforms encoded by At5g66280 (GMD1), and At3g51160 (GMD2), also referred to as *MUR1*, which have 92% amino acid sequence identity. In addition, two GER isoforms encoded by At1g73250 (GER1) and At1g17890 (GER2) have 88% sequence identity. The GMD isoforms are coexpressed in some but not all plant tissues. For example, GMD2 is expressed in most root cells but not in the root tip, where strong expression of GMD1 is observed in the root meristem (17). Within shoot organs, GMD2 is expressed in most cells, whereas GMD1 expression is restricted to stipules and pollen grains. The lack of GMD2 in the above-ground portions of the *Arabidopsis mur1* mutant results in an almost complete lack of fucose in the cell wall polysaccharides (113, 171) and glycoproteins (125). Surprisingly, many of the glycans that would normally be fucosylated are substituted with L-Gal in *mur1*. This may result from the increased availability of GDP-L-Gal and the ability of a fucosyltransferases to transfer L-Gal to the polymers (86).

GDP-L-Galactose

L-galactose (L-Gal) is a component of RG-II (104) and the xyloglucans of certain plants (60). This L-Gal is likely derived from GDP-L-Gal. However, in plants GDP-L-Gal is used predominantly for the formation of ascorbic acid (89). GDP-Man 3′,5′-epimerase (165) converts GDP-D-Man to GDP-L-Gal. Studies of recombinant GDP-Man 3′,5′-epimerase from *Arabidopsis* [At5g28840, (165)] and rice (157a) demonstrated that the enzyme converts GDP-D-Man to both GDP-L-gulose (GDP-Gul) and GDP-L-Gal. These results led to the suggestion that GDP-L-Gal and GDP-L-Gul are used for the formation of ascorbate. However, it is also possible that GDP-L-Gul is a precursor for the formation of other NDP-sugars, although the biological significance of these in vitro metabolites remains to be determined.

CMP-D-Kdo

The eight-carbon sugar 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) is present in numerous

bacterial glycans but in plants is present only in RG-II (104). Most of the information for the synthesis of CMP-Kdo has been obtained with bacterial enzymes (129) and confirmed to some extent in plants.

Kdo-8-P synthase (KdsA) catalyzes the condensation of phosphoenolpyruvate and D-Ara-5-phosphate (129). Genes encoding functional Kdo-8-P synthase have been identified in several plants and have ~50% amino acid sequence identity with their bacterial counterparts (18, 31). In *Arabidopsis*, two genes (AtkdsA1, At1g79500 and AtkdsA2, At5g09730) with high sequence identity have been identified. AtkdsA1 is predominantly expressed in shoots, whereas AtkdsA2 transcripts accumulate predominantly in roots. The activity of the recombinant plant KdsA toward other phosphorylated sugars, including D-erythrose-4-phosphate (E-4-P) was not tested. However, studies with a KdsA partially purified from potato indicated that the enzyme could use E-4-P and ribose-5-P, albeit much less effectively than Ara-5-P. The potato KdsA was also reported to have a weak 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase activity (37). Once formed, Kdo-8-P is dephopshorylated, presumably by a specific Kdo-8-P phosphatase or nonspecific sugar phosphatase, to form Kdo. These phosphatases have not been isolated nor have their gene(s) been identified. CMP-Kdo synthase (KdsB) then catalyzes the transfer of the cytidylyl group (CMP) from CTP to Kdo to yield CMP-β-Kdo. The maize KdsB gene homolog was functionally identified (130), and the homologous *Arabidopsis* protein is encoded by At1g53000. Bioinformatic analysis suggests that the plant KdsB is a type I-b transmembrane protein with the catalytic domain facing the cytosol. However, such topology and the subcellular location of the plant protein have not been determined.

Arabidopsis plants with mutations in either of the KdsA genes are indistinguishable from wild-type plants. However, attempts to obtain the double knockout were unsuccessful (32). Thus, it would appear that the formation of Kdo and RG-II is required for normal plant growth and development. However, the same group has reported that a putative Kdo transferase resides in the mitochondria and suggests that these organelles have a Kdo-containing glycan (139). Studies are now required to biochemically characterize plant KdoTs and their specific acceptors. Recently, putative nucleotide sugar transporters have been identified in *Arabidopsis* (At5g41760) (7) and rice [OsCSTLP1 (Os06g0523400), OsCSTLP2 (Os07g0573700)] and one (OsCSTLP1) is capable of transporting CMP-sialic acid (147). Heterozygous mutants of the homologous *Arabidopsis* genes (At5g41760 and At4g35335) were identified, but Takashima et al. (147) were unable to generate a homozygous mutant for either loci. The presence of sialic acid in plants is a subject of debate (137); thus Takashima et al. (147) concluded that OsCSTLP1 may be a CMP-KDO transporter. The keto sugar Dha is structurally related to Kdo; however, nothing is known about its formation in plants nor has the activated form of Dha been described.

Rare and Modified Nucleotide Sugars

Numerous NDP-sugars including ADP-L-Ara, GDP-L-Ara, ADP-ribose, GDP-Xyl, ADP-Gal, GDP-D-Gal, ADP-D-Man, UDP-Fructose, ADP-D-Fructose, UDP-D-digitoxose (2,6 dideoxy-D-ribohexose), TDP-GalA, UDP-2-deoxy-2-acetamido-D-Glc, UDP-2-deoxy-2-acetamido-D-Gal, and UDP-cellobiose have been identified in plants (47) but their function remains unknown.

Aceric acid, as far as we are aware, is present only in the pectic polysaccharide RG-II. Nothing is known about its formation in plants nor has its activated form been described. The sugar moieties of many plant glycans are often modified by *O*-methylation, *O*-acetylation, or *O*-sulfation. In sulfolipid biosynthesis in chloroplasts sulfation occurs at the nucleotide sugar level because the activated donor is UDP-Glc-6-sulfonate. However, it is not known if *O*-acetylation or *O*-methylation occurs at the NDP-sugar level or after the sugar has been incorporated into the glycan.

REGULATION OF NUCLEOTIDE SUGAR BIOSYNTHESIS

Pioneering studies by Northcote and colleagues demonstrated that during the transition from primary to secondary wall formation there is a corresponding change in the types of NDP-sugar biosynthetic enzymes formed in the cell (27, 28, 102, 150). For example, the enzymes that form UDP-GalA decreased and those that form UDP-Xyl increased. Such changes in activities are correlated with a change in the amounts of pectin and xylan synthesized. Some of the factors that regulate expression of genes involved in the transition from primary to secondary walls are being investigated (34). However, surprisingly little is known about the factors that regulate the expression of nucleotide sugar biosynthetic genes during plant growth and development. Studies are required to determine if NDP-sugar biosynthetic enzymes are regulated at the gene level or by metabolic feedback or a combination of both.

THE FATE OF NDP AFTER SUGAR TRANSFER

Most GTs are inhibited by the NDP released after the sugar is transferred to the acceptor. To avoid this inhibition, the NDP is first converted to the nucleotide monophosphate and inorganic phosphate. The NMP is then transferred back to the cytosol by NMP-specific transporters. The NMP can be used in a variety of metabolic routes (177). For example, UMP can be recycled back into carbohydrate metabolism, used for the formation of RNA, or salvaged to form β-alanine.

FUTURE CHALLENGES

Our current knowledge of the metabolism of nucleotide sugars exceeds our understanding of other metabolic routes involved in the molecular and biochemical processes that are required to assemble and modify diverse glycans. Many unresolved questions related to NDP-sugar biosynthesis and the regulation of the genes involved in their synthesis and transport across membranes remain (see Future Issues, below).

Future research is required to elucidate, at the level of the single cell, the interactions among nucleotide sugar biosynthetic enzymes, the proteins that transport nucleotide sugars across various membranes, and the GTs that assemble glycans. Such information will contribute to a complete molecular description of the cellular machinery that assembles the glycan-rich surfaces of plant cells and how these surfaces are involved in the formation of plant tissue and organs.

FUTURE ISSUES

1. Does the transport of each NDP-sugar require a specific transporter?
2. How are the NDP-sugar biosynthetic enzymes and glycosyltransferases required for the synthesis of a specific glycan organized within the plant Golgi?
3. Why are there so many isoforms of the enzymes that interconvert NDP-sugars?
4. What were the evolutionary, metabolic, and energetic driving forces that resulted in some of these isoforms being localized in the cytosol and some in the Golgi?
5. Why are the different isomers regulated differently in a single cell?
6. What are the contributions of the salvage and de novo NDP-sugar synthesis pathways to glycan synthesis?
7. Do the mechanisms that regulate NDP-sugar synthesis differ between cells, tissues, and plant species?

8. Were changes in the regulation of genes for UDP-Xyl and UDP-Ara formation one of the factors that resulted in grass cell walls containing more xylan than dicot walls?
9. Is the formation of precursors for the synthesis of storage glycans independent of the formation of precursors for cell wall glycan synthesis?
10. Why do plants generate so many different NDP-glucoses?
11. Does the formation of ADP-Glc, UDP-Glc, TDP-Glc, and GDP-Glc allow plants to partition Glc into different polymers?
12. Why do plants preferentially generate UDP-Rha, whereas bacteria form TDP-Rha?
13. Did the formation of UDP-rhamnose provide the first green plant cell with a selective advantage over microbes?
14. Does the supply of NDP-sugars, the activities of glycosyltransferases, or the availability of acceptor molecules control plant glycan synthesis?
15. How is the amount of a specific glycan regulated?
16. Can in silico models of NDP-sugar flux explain the formation and relative amounts of specific glycans?
17. What are the limits to engineering plant cell walls with new properties—can we "Arabidopsize" rice and build a plant with hybrid walls containing dicot polysaccharides?
18. Were specific combinations of NDP-sugars and cell surface glycans required for the first multicellular plants to appear?

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