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A class of plant glycosyltransferases involved in cellular homeostasis

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Many small lipophilic compounds in living cells can be modified by glycosylation. These processes can regulate the bioactivity of the compounds, their intracellular location and their metabolism. The glycosyltransferases involved in biotransformations of small molecules have been grouped into Family 1 of the 69 families that are classified on the basis of substrate recognition and sequence relatedness. In plants, these transfer reactions generally use UDP-glucose with acceptors that include hormones such as auxins and cytokinins, secondary metabolites such as flavonoids, and foreign compounds including herbicides and pesticides. In mammalian organisms, UDP-glucuronic acid is typically used in the transfer reactions to endogenous acceptors, such as steroid and thyroid hormones, bile acids and retinoids, and to xenobiotics, including nonsteroidal anti-inflammatory drugs and dietary metabolites. There is widespread interest in this class of enzyme since they are known to function both in the regulation of cellular homeostasis and in detoxification pathways. This review outlines current knowledge of these glycosyltransferases drawing on information gained from studies of plant and mammalian enzymes.

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Introduction

Glycosyltransferases transfer a sugar from an activated sugar donor to an acceptor molecule and as such are involved in the synthesis and modification of the multitude of glycoconjugates in existence in the biosphere. For a detailed analysis of glycosyltransferases and their classification into the 69 families known to exist, the reader is referred to the CAZy (carbohydrate-active enzymes) website (http://afmb.cnrs-mrs.fr/CAZY/). The classification system used depends both on the nature of substrates recognised by the enzymes and their sequence relatedness. Individual glycosyltransferases can therefore be classified either through biochemical studies to identify their substrates or through bioinformatic studies that reveal homology to genes encoding enzymes of known catalytic activity (Campbell *et al*, 1997; Coutinho *et al*, 2003).

To date, Family 1 contains >900 sequences with representatives in a wide range of prokaryotic and eukaryotic organisms. Substrates for these glycosyltransferases are small, lipophilic molecules in which single or multiple glycosylation can take place at –OH, –COOH, $–NH_2$, –SH and C–C groups (Sandermann and Pflugmacher, 1998; Ikan, 1999; Radominska-Pandya *et al*, 1999; Vogt and Jones, 2000; Jones and Vogt, 2001). Within Family 1, there is a class of enzymes defined by the presence of a consensus sequence of 44 amino acids. This class is expanding rapidly since individuals can be easily recognised in genome sequencing projects. Currently, 48.3% of Family 1 contains this consensus (Coutinho and Henrissat, personal communication). Figure 1 illustrates the relatedness of the different glycosyltransferases described in this review.

These enzymes have attracted very considerable interest because of their range of substrates, their potential role in developmental and metabolic homeostasis, and their function in detoxification processes of relevance to clinical and agricultural applications. The review will highlight some recent insights gained from studies of plant enzymes, in particular, those of *Arabidopsis thaliana*, and will discuss the findings in the wider context of related glycosyltransferases from other organisms.

Biomolecular studies of the enzymes

The consensus that defines the class of Family 1 enzymes discussed in this review was identified by Hundle *et al* (1992), when the sequence of a bacterial zeaxanthin glycosyltransferase was compared with those of a number of mammalian and plant enzymes. Hughes and Hughes (1994) first named the consensus as a 'PSPG' motif in plant enzymes, and the entire class was formally classified by the Glycosyltransferase Nomenclature Committee (Mackenzie *et al*, 1997). In the completed genome of *A. thaliana*, there are 117 sequences containing the consensus, scattered across all of the five chromosomes (Li *et al*, 2001; Ross *et al*, 2001; Paquette *et al*, 2003). Based on their alignment, a number of the sequences are considered pseudogenes due to interruptions in their open-reading frames caused by nucleotide substitutions, insertions or deletions. In the human genome, 27 sequences have been identified (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/; Bosio *et al*, 1996; Mackenzie *et al*, 1997; Tukey and Strassburg, 2000; Miners *et al*, 2004). The sequences are mapped only on two chromosomes (2 and

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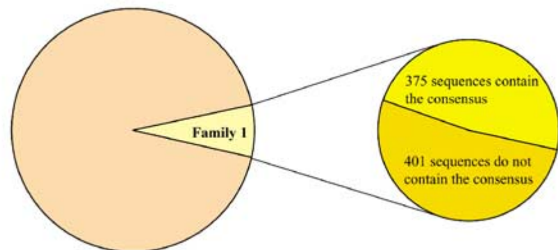

[FW]-x(2)-Q-x(2)-[LIVMYA]-[LIMV]-x(4-6)-[LVGAC]-[LVFYA]-[LIVMF]-[STAGCM]-[HNQ]-[STAGC]-G-x(2)-[STAG]-x(3)-[STAGL]-[LIVMFA]-x(4)-[PQR]-[LIVMT]-x(3)-[PA]-x(3)-[DES]-[QEHN]

Figure 1 Family 1 constitute 7% of all glycosyltransferases that have been classified to date. This review focuses on the 48% of Family 1 containing the consensus sequence. The statistical data of Family 1 glycosyltransferases with and without the consensus are provided by Dr Pedro Coutinho and Professor Bernard Henrissat (AFMB-CNRS, France). The reader is referred to the CAZy (carbohydrate-active enzymes) website (http://afmb.cnrs-mrs.fr/CAZY/) for the up to date information. The 44-amino-acid consensus sequence defining the class of Family 1 glycosyltransferases described in this review is illustrated.

4) and can be further grouped into three subsets: *UGT1* (consists of nine functional genes and four pseudogenes, located on chromosome 2), *UGT2* (consists of eight functional genes and five pseudogenes, located on chromosome 4) and *UGT8* (contains a single member, located on chromosome 4). Whereas >50% of the *Arabidopsis* genes do not have introns, all of the human glycosyltransferases are encoded by multiple exons. A particular feature of the human glycosyltransferases is alternative splicing. This occurs in the *UGT1* subset and leads to variability in the N-terminal regions of the proteins (Gong *et al*, 2001).

None of the plant glycosyltransferases identified to date have a signal sequence, nor any clear membrane-spanning or targeting signals (Li *et al*, 2001). This suggests the enzymes function in the cytosol, although within that compartment the proteins may associate as peripheral components of the endomembrane system, as suggested by Winkel-Shirley (1999). In contrast, the human glycosyltransferases have a signal sequence involved in cotranslational translocation into the rough endoplasmic reticulum (RER), as well as a transmembrane-spanning domain and an ER retention signal (Sprong *et al*, 1998; reviewed, Radominska-Pandya *et al*, 1999). The enzymes therefore clearly function in the ER, necessitating transport of nucleotide-sugar from the cytosol into the ER lumen for the transfer reaction (reviewed, Hirschberg *et al*, 1998).

To date, only 13 crystal structures of enzymes from the 69 glycosyltransferase families have been solved. Of these, two structural groups can be identified, GT-A and GT-B (Ünligil and Rini, 2000; Bourne and Henrissat, 2001; Tarbouriech *et al*, 2001). GT-A structures consist of parallel β-strands flanked on either side by α-helices. In contrast, GT-B structures consist of two Rossmann-fold-like domains separated by a deep cleft (Figure 2A). While it is clear that the primary sequences of glycosyltransferases are significantly different from one another, Hu and Walker (2002) have suggested that their three-dimensional shapes may be similar and reflect either the GT-A or GT-B structures. This suggestion is supported by a recent study of Zhang *et al* (2003) in which 262 representative glycosyltransferase sequences from different families were analysed using a 'fold recognition' strategy that revealed all of the sequences investigated adopted either the GT-A or GT-B structure.

In Family 1, two glycosyltransferases from the bacterium *Amycolatopsis orientalis* have been crystallised and shown to be GT-B structures (Mulichak *et al*, 2001, 2003). These enzymes, GtfA and GtfB, transfer sugar from NDP-sugar to the β-OH-Tyr_6 and 4-OH-$Phegly_4$ residues of vancomycin, respectively. The bacterial sequences are substantially different from those of plant and mammalian enzymes and are not classified in the same subset of Family 1, since they do not contain the 44-amino-acid consensus. Currently, no plant or mammalian glycosyltransferase has been crystallised. However, if the secondary structure of the bacterial enzyme GtfA is compared to a representative plant glycosyltransferase (UGT71C1) and a human glycosyltransferase (UGT1A1), a surprising similarity can be identified, particularly in the C-terminal region of the proteins (Figure 2B). It is possible therefore that the Family 1 class of enzymes described in this review will also adopt GT-B structures, containing the two Rossmann-fold-like domains.

Many of the aglycones recognised by human glycosyltransferases are also recognised by other enzymes and binding proteins. In some cases, the structures of these additional proteins are known and the amino acids involved in ligand binding have been identified. This has enabled a comparison to be made between sequences involved in aglycone glycosylation and other sequences that also recognise the same substrates. As reviewed by Radominska-Pandya *et al* (1999), conserved amino-acid residues in the N-terminal regions of the proteins have been identified that may be involved in substrate recognition. As yet, there are no similar studies on plant glycosyltransferases, but sequence alignment of the multigene family of enzymes in *Arabidopsis* has shown that there is much greater variability in the N-terminal regions of the proteins than in the C-terminal regions, which may reflect the diversity of aglycones recognised (Li *et al*, 2001).

Photoaffinity-labelling experiments have confirmed that residues in the consensus sequence of mammalian glycosyltransferases interact with UDP (reviewed, Radominska-Pandya *et al*, 1999). However, one study involving human UGT2B4 showed that there was also an interaction of the N-terminus with the sugar (Pillot *et al*, 1993). The role of this interaction in the catalytic mechanism will become clear when a structure for this enzyme class has been solved.

Glycosylation as a homeostatic mechanism of small molecule metabolism

In plants, enzymes of this class are known to recognise a great diversity of substrates including hormones, secondary metabolites and xenobiotics such as pesticides and herbicides (reviewed, Jones and Vogt, 2001; Ross *et al*, 2001). The sugar donor is generally UDP-glucose, although UDP-rhamnose, UDP-galactose and UDP-xylose have also been identified as activated sugars for the transfer reactions (Martin *et al*, 1999; Miller *et al*, 1999; Jones *et al*, 2003). There is considerable information available on the existence and diversity of glyco-

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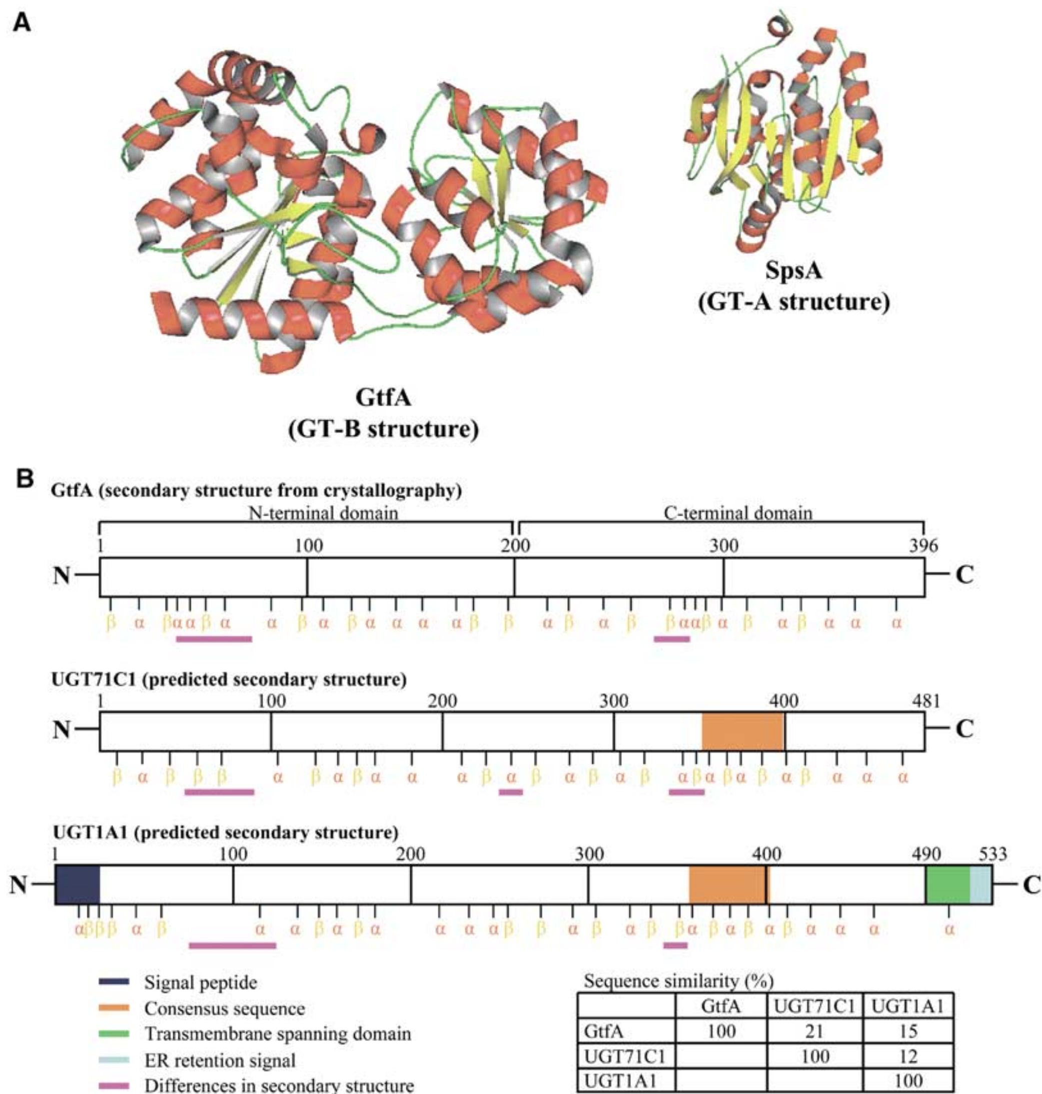

Figure 2 (**A**) Ribbon diagrams of two representative GT structures. *A. orientalis* GtfA (Family 1, PBD accession code 1PNV) and *Bacillus subtilis* SpsA (Family 2, PBD accession code 1H7L) were selected to illustrate the GT-B and GT-A structures, respectively. (**B**) *Arabidopsis* UGT71C1 and human UGT1A1 were chosen as the representative glycosyltransferases containing the consensus. Their secondary structures were predicted using a web-based programme (http://cubic.bioc.columbia.edu/predictprotein/) and were compared to the secondary structure of GtfA (without the consensus) gained from the crystallographic study.

sides, the effect of glycosylation on the activity of the acceptor molecules, and the consequences in relation to cellular homeostasis. For example, it is well documented that glycosylation alters the bioactivity of plant hormones. This has been reviewed for auxins, cytokinins, gibberellins and abscisic acid (reviewed, Kleczkowski and Schell, 1995). The reason that conjugation inactivates these hormones is unclear, but could in principle arise directly from a change in recognition by the hormone's receptor(s), or indirectly from events enabled by the glycosylation status. In this context, glycosylation is known to provide access to membrane-bound transporters. Glycosides and glucose esters of small molecules, including hormones, secondary metabolites and xenobiotics, have been shown to accumulate in the vacuolar lumen (reviewed, Coleman *et al*, 1997; Rea *et al*, 1998; Martinoia *et al*, 2002). Transporters for some of these compounds have been identified in the vacuolar membrane, and there is evidence to suggest that different mechanisms function for glucosides of endogenous metabolites compared to those of xenobiotics (Klein *et al*, 1996). Therefore, addition of a sugar residue onto an aglycone can lead both to a change in bioactivity and a change in its cellular location. If the compound is hydrophobic and can diffuse across lipid bilayers, glycosylation of the aglycone can in principle be used to contain the glycoside in a specific hydrophilic compartment—whether intracellular, such as the vacuole and endomembrane system, or extracellular, such as the cell wall matrix. In terms of control of flux in pathways of secondary metabolism, local concentrations of substrates and products in the cytosol can therefore be affected by glycosylation and removal from the compartment within which the reaction is occurring. In this way, as suggested by Hösel (1981), glycosylation and deglycosylation can be a regulatory mechanism altering levels of metabolites along a pathway through controlling exit from the cytosol or re-entry into the reaction mix.

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In contrast to the diversity of sugar donors in plants, the mammalian UGT1 and UGT2 subset invariably use UDP-glucuronic acid. Known acceptors for these glucuronosyltransferases include endogenous substrates such as steroids, bilirubin and bile acids and exogenous xenobiotic substrates such as dietary flavonoids, and drugs such as morphine and naproxen. The reader is referred to recent reviews by Radominska-Pandya *et al* (1999), King *et al* (2000), Tukey and Strassburg (2000) and Miners *et al* (2004). UDP-glucuronosyltransferases represent the major class of enzymes involved in mammalian phase II detoxification pathways (reviewed, Bock, 2003). Glucuronides formed in mammalian cells are exported through multidrug resistance protein (MRP) transporters and are excreted from the body in the urine or bile (reviewed, König *et al*, 1999; Kruh and Belinsky, 2003). Unlike UGT1s and UGT2s, mammalian UGT8 transfers galactose from UDP-galactose to ceramide to form the glycosphingolipid galactosylceramide, which is the major component of the plasma membrane (reviewed, Kolter and Sandhoff, 1999).

Impact of changing the level of expression of plant glycosyltransferases

There is now a variety of genetic approaches available to investigate the action of the glycosyltransferases in the plant and how their catalytic activities may be related to physiological functions. These include the use of different promoters to upregulate the expression of glycosyltransferase genes such that enzyme activities are increased and, conversely, the use of an antisense strategy, T-DNA knockouts or gene silencing methods to downregulate expression of the endogenous gene(s). Phenotypic mutants have also been characterised and there is one example in which the defect has been surprisingly discovered in a glycosyltransferase gene (Quiel and Benders, 2003). As yet, these studies are limited in number and few general conclusions can be drawn, but examples of the effects observed will be briefly discussed.

Disturbance of hormonal homeostasis

Two studies have involved glycosyltransferases of plant hormones. The gene encoding ZOG1, a *Phaseolus* enzyme shown to O-glycosylate *trans*-zeatin *in vitro*, was overexpressed in tobacco callus. The only metabolite change observed involved a massive accumulation of the corresponding glucoside, and the callus required much higher levels of supplementary *trans*-zeatin for induction of shoot differentiation (Martin *et al*, 2001a). The second study involved UGT84B1, a glycosyltransferase recognising indol-3-acetic acid (IAA) *in vitro* (Jackson *et al*, 2001). The gene was overexpressed in *Arabidopsis* plants and the resulting transgenic plants displayed a phenotype similar to auxin deficiency (Figure 3) (Jackson *et al*, 2002). Interestingly, the loss of gravitropism by the transgenic root system could be recovered by applying an auxin analogue not glucosylated by UGT84B1 *in vitro*. While IAA glucose ester levels substantially accumulated in the transgenic plants, IAA also increased, suggesting a complex regulation of free hormone levels *in planta*.

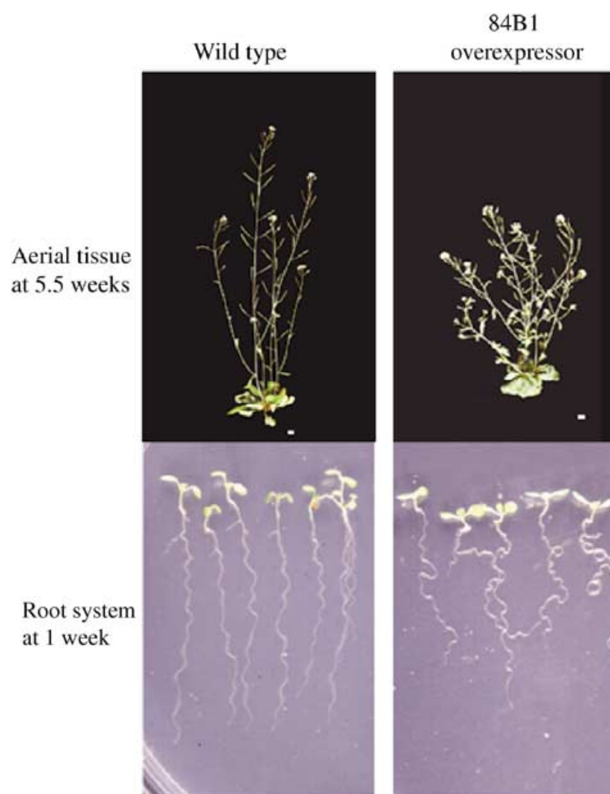

Figure 3 Phenotype of a transgenic line constitutively overexpressing an *Arabidopsis* gene *UGT84B1* encoding a glycosyltransferase of the auxin indole-3-acetic acid. The aerial tissues of the transgenic *Arabidopsis* plant (upper panel) showed a higher degree of branching and shorter stature compared to wild type. When seedlings (lower panel) were grown vertically, the transgenic root system displayed a phenotype of impaired gravitropism.

Effects on secondary metabolism

In the above studies, overexpression of the glycosyltransferase genes led to a significant increase in their respective glucosides, but two other recent studies have shown that this is not a general consequence (Fukuchi-Mizutani *et al*, 2003; Lim *et al*, 2003b). These studies involved glycosyltransferases that glycosylate plant secondary metabolites. Under conditions in which high levels of recombinant enzyme activity in the transgenic plants were demonstrated, levels of product only marginally increased above wild type (Lim *et al*, 2003b).

Downregulation of TOGT, a tobacco glycosyltransferase recognising multiple phenolic substrates *in vitro*, led to a decreased accumulation of scopoletin glucoside *in planta* and, in parallel, a reduced resistance to *Tobacco mosaic virus* (Chong *et al*, 2002). Importantly, the study demonstrated that TOGT functions *in planta* during viral infection, although its precise role in the process was not defined. In another example of glycosyltransferase downregulation, T-DNA insertion lines for UGTs 73C6 and 78D1 were analysed. The *Arabidopsis* glycosyltransferases recognised quercetin and kaempferol *in vitro*; in the knockouts, lower levels of the glycosides were observed compared to wild type (Jones *et al*, 2003).

Detoxification of mycotoxins and xenobiotics

Several studies have investigated the potential of plant glycosyltransferases for detoxification applications. For example,

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Table I Recombinant plant glycosyltransferases reported recently (2001–2004)

Substrate	Product	Organism	Reference
Hydroquinone	Arbutin	*Rauvolfia*	Arend *et al* (2001)
Indole-3-acetic acid	IAA glucose ester	*Arabidopsis*	Jackson *et al* (2001)
Phenylproponoids	Phenylpropanoid-4-*O*-Glc, glucose esters	*Arabidopsis*	Lim *et al* (2001)
Thiohydroximate	Glucosinolates	*Brassica*	Marillia *et al* (2001)
cis-Zeatin	*cis*-Zeatin-*O*-Glc	*Zea*	Martin *et al* (2001b)
Couramins	Coumarin-*O*-Glc	*Nicotiana*	Taguchi *et al* (2001)
Benzoxazinoids	Benzoxazinoid-*O*-Glc	*Zea*	von Rad *et al* (2001)
Benzoates	Benzoate-*O*-Glc, glucose esters	*Arabidopsis*	Lim *et al* (2002)
Betanidin	Betanidin-5-*O*-Glc, 6-*O*-Glc	*Dorotheanthus*	Vogt (2002)
Flavonoids	Flavonoid-3-*O*-Glc, 5-*O*-Glc	*Petunia*	Yamazaki *et al* (2002)
Flavonoids	Anthocyanin-3′-*O*-Glc	*Gentiana*	Fukuchi-Mizutani *et al* (2003)
Cyanohydrins	Dhurrin	*Sorghum*	Hansen *et al* (2003)
Flavonols	Flavonol-3-*O*-Rha; flavonol-3-*O*-Rha-7-*O*-Glc	*Arabidopsis*	Jones *et al* (2003)
Flavonoids	Flavonoid-3-*O*-Glc, 7-*O*-Glc, 4′-*O*-Glc	*Allium*	Kramer *et al* (2003)
Caffeic acid	Caffeic-3-*O*-Glc	*Arabidopsis*	Lim *et al* (2003a)
Coumarins	Coumarin-6-*O*-Glc, 7-*O*-Glc	*Arabidopsis*	Lim *et al* (2003b)
3,4-Dichloroaniline	3,4-Dichloroaniline-*N*-Glc	*Arabidopsis*	Loutre *et al* (2003)
Trichrolophenol	Trichrolophenol-*O*-Glc	*Arabidopsis*	Meßner *et al* (2003)
Deoxynivalenol	Deoxynivalenol-3-*O*-Glc	*Arabidopsis*	Poppenberger *et al* (2003)
Anthranilate	Anthranilate glucose ester	*Arabidopsis*	Quiel and Bender *et al* (2003)
Flavonoid/coumarin	Flavonol-7-*O*-Glc, coumarin-3-*O*-Glc	*Nicotiana*	Taguchi *et al* (2003)
cis-Zeatin	*cis*-Zeatin-*O*-Glc	*Zea*	Veach *et al* (2003)
Isoflavonoid	Formononetin-7-*O*-Glc	*Glycyrrhiza*	Nagashima *et al* (2004)
Quercetin	Quercetin-3-*O*-Glc, 7-*O*-Glc, 3′-*O*-Glc, 4′-*O*-Glc	*Arabidopsis*	Lim *et al* (2004)

screening of an *Arabidopsis* cDNA library in a yeast mutant hypersensitive to the mycotoxin deoxynivalenol revealed that colonies expressing a glycosyltransferase, UGT73C5, were protected against the effect of the mycotoxin. Overexpression of UGT73C5 in transgenic *Arabidopsis* led to mycotoxin resistance (Poppenberger *et al*, 2003). Glycosyltransferases capable of conjugating xenobiotics such as 2,4,5-trichlorophenol and 3,4-dichloroaniline *in vitro* have been identified (Loutre *et al*, 2003; Meßner *et al*, 2003), suggesting that transgenic plants overexpressing the respective glycosyltransferases may provide useful tools for phytoremediation. Interestingly, the enzymes that recognise the xenobiotics had previously been shown to glycosylate endogenous metabolites (Lim *et al*, 2001, 2002) suggesting that individual glycosyltransferases play multiple roles in the plant.

Utility of glycosyltransferases as regioselective biocatalysts

Chemical synthesis of glycosides is notoriously difficult when regioselectivity is a requirement. A classic example is the synthesis of glycosides of the important medicinal compound quercetin, which has five hydroxyl groups that can act as sugar acceptors (Aherne and O'Brien, 2002). To synthesise any single monoglycoside, four other hydroxyl groups must be protected (Bouktaib *et al*, 2002; Li *et al*, 2002). Thus, chemical synthesis of glycosides often involves multiple blocking–deblocking steps before any product can be obtained. In this context, glycosyltransferases potentially offer a simple means for the synthesis of regiospecific glycosides.

Many studies have demonstrated the regioselectivity of glycosylation by plant enzymes, as illustrated by the recent examples given in Table I that describe the substrates recognised by enzymes from different plant species and the glycosides formed. The availability of an entire multigene family of glycosyltransferases from *Arabidopsis* enabled a systematic investigation of regioselectivity since many activities towards a single substrate could be directly compared (Figures 4A and B). Thus, for example, 48 of the 107 *Arabidopsis* enzymes recognised esculetin *in vitro* but glucosylated the hydroxycoumarin in a regioselective manner at either the 6-OH or the 7-OH position (Lim *et al*, 2003a). In another example of regioselectivity, UGTs 74F1 and 74F2 are highly homologous, have evolved from a common ancestor, but UGT74F1 recognises the hydroxyl group on the benzene ring of salicylic acid, whereas UGT74F2 recognises the carboxyl group (Lim *et al*, 2002).

Studies such as these provide a good foundation for exploring the structural determinants involved in governing regioselectivity and substrate recognition and can also inform the design of novel biocatalysts for glycoside synthesis through directed evolution. In this context, Hoffmeister *et al* (2002) have recently reported the use of gene shuffling as a tool to generate mutants of urdamycin glycosyltransferase possessing novel catalytic activity (Figure 4C).

A limitation in the use of these enzymes as biocatalysts is the perceived requirement of activated sugars such as UDP-glucose, and several studies have addressed the potential for regeneration of the sugar-nucleotides (Heidlas *et al*, 1992; Ichikawa *et al*, 1992; Wong *et al*, 1992). Given that UDP-glucose is abundant in bacterial cells (Ross *et al*, 1991), the possibility of using the endogenous metabolite to synthesise glycosides in a whole-cell biocatalysis process has been explored (Arend *et al*, 2001; Lim *et al*, 2004). The success of this approach has been demonstrated for the synthesis of hydroquinone glucoside and a variety of quercetin glucosides. Furthermore, bacterial cells expressing different glycosyltransferases or a range of other enzymes involved in the substrate modification can be co-cultured in a fermenter to synthesise a diverse range of products using an *in vivo* combinatorial biochemistry (Willits *et al*, 2004).

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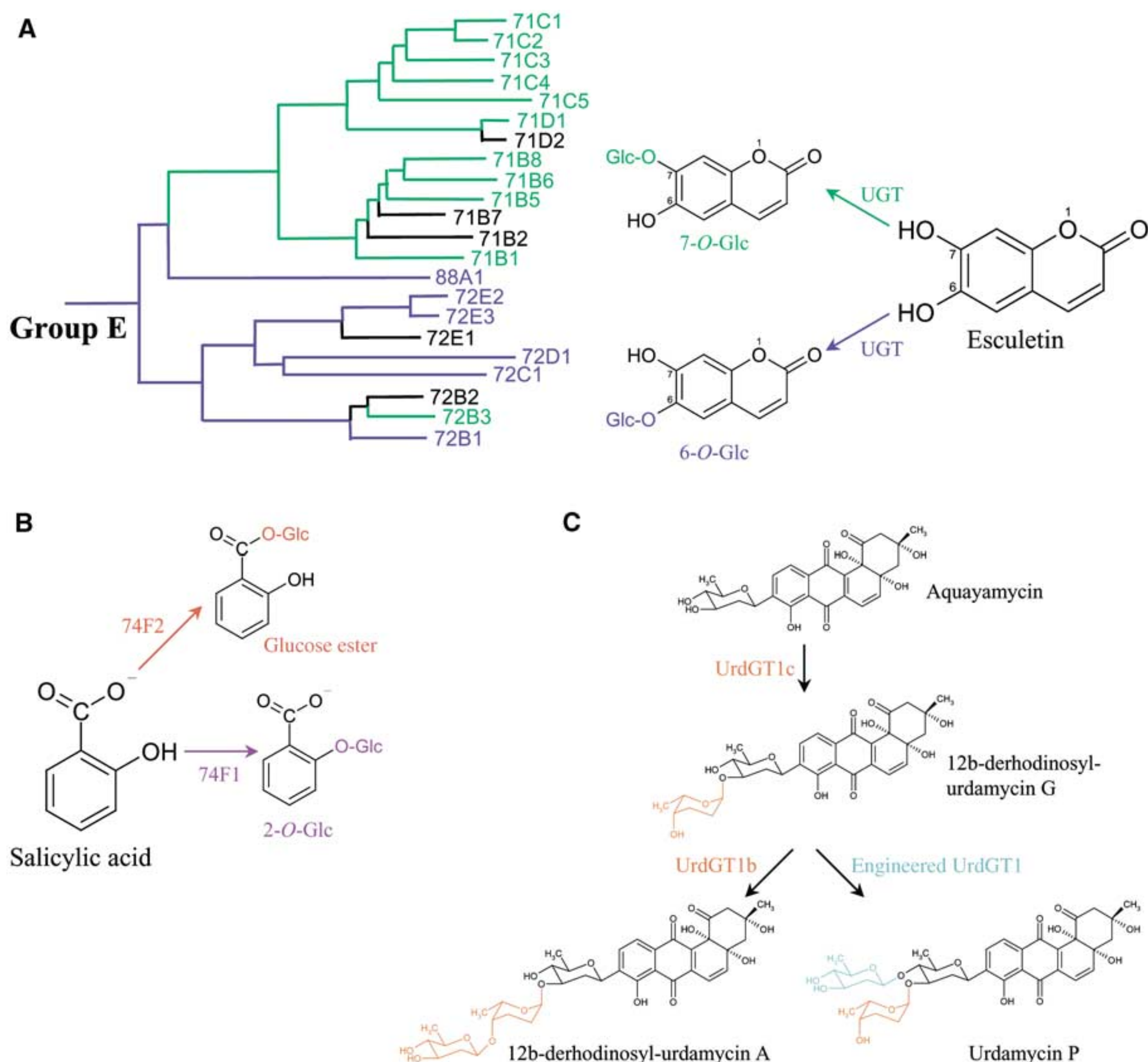

Figure 4 (**A**) The sequences illustrated are those of the phylogenetic group E of *Arabidopsis* glycosyltransferases (Li *et al*, 2001). Regioselective glycosylation of esculetin falls into two distinct subsets in Group E, with the exception of UGTs 72B1 and 72B3, which suggests that a switching event in regioselectivity has occurred during evolution (Lim *et al*, 2003a). Sequences not analysed are labelled in black. (**B**) UGTs 74F1 and 74F2 are 82% identical at the amino-acid sequence level, but display different regioselectivity towards salicylic acid. (**C**) UrdGT1b and UrdGT1c are *Streptomyces fradiae* glycosyltransferases involved in the biosynthesis of the antibiotic urdamycin. Gene shuffling using the DNA sequences encoding these two glycosyltransferases enabled the generation of an engineered protein (cyan) with a catalytic activity different from the parental enzymes (Hoffmeister *et al*, 2002).

Concluding remarks

The number of glycosyltransferases classified in this subset of Family 1 is likely to increase in the coming years through the increased availability of gene sequences from pro- and eukaryotic organisms. Studying these enzymes highlights a common problem in postgenomic science—how to relate gene sequence to cellular function. Catalytic activity can be screened *in vitro*, but the precise role of the glycosyltransferases in the cell continues to remain elusive. In plants, several tens of thousands of small molecule glycosides are known to exist, suggesting that the enzymes will recognise multiple substrates. The availability of the entire multigene family of enzymes in *Arabidopsis* has also shown for the first time that multiple enzymes of a plant are capable of recognising the same substrate. In addition, since glycosyltransferases are known to be involved in detoxification pathways in plant and mammalian cells, it is probable that the same enzymes will recognise both endogenous metabolites and xenobiotics. Genetic studies are now beginning to examine the metabolic and developmental consequences of silencing glycosyltransferases. In this context, it will be interesting to determine the effects of silencing individuals compared with silencing groups of sequences that share common substrates since the enzymes may well function in a network of complementary activities.

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