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REVIEW

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Glycosyltransferases in secondary plant metabolism: tranquilizers and stimulant controllers

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Abstract. Plants are exposed to a wide range of toxic and bioactive low-molecular-weight molecules from both exogenous and endogenous sources. Glycosylation is one of the primary sedative mechanisms that plants utilise in order to maintain metabolic homeostasis. Recently, a range of glycosyltransferases has been characterized in detail with regard to substrate specificity. The next step in increasing our understanding of the biology of glycosylation will require information regarding the exact role of individual glycosyltransferases in planta, as well as an insight into their potential involvement in metabolon-complexes. Hopefully, this will answer how a large number of glycosyltransferases with broad, rather than narrow, substrate specificity can be constrained in order to avoid interfering with other pathways of primary and secondary metabolism. These and other topics are discussed.

Keywords Glycosylation · Glycosyltransferase · Secondary plant metabolism

Abbreviations. GT: glycosyltransferase · SA: salicylic acid

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Introduction

Plants are capable of synthesising several thousand different low-molecular-weight compounds (Wink 1999), defined as secondary plant metabolites. Part of this diversity arises from decoration with glycosyl-, carboxyl-, methyl- and hydroxyl-groups by glycosyltransferases, acyltransferases, methyltransferases and cytochrome P450s, respectively. The conjugation of endogenous and exogenous organic molecules with sugars is one such important tool employed by all organisms. The addition of a carbohydrate-moiety to endogenous and exogenous organic molecules has a wide range of effects including increased water solubility, improved chemical stability, reduced chemical reactivity and altered biological activity. Secondary plant metabolites are glycosylated to O (OH- and COOH-), N, S and C atoms by glycosyltransferases (GTs) using nucleotide-activated sugars as donor substrates (Fig. 1). Hydroxylated molecules are the most common acceptors, whilst UDP-glucose is the most common donor. A broad range of different carbohydrate moieties can be added employing all forms of sugars, independently (monoglycosides), in parallel or in chains (di-, tri-glycosides, etc.; Fig. 1). This gives rise to a broad spectrum of glycosidic structures for any given aglycone. For example, out of a total of 5,000 different flavonoids, 300 different glycosides of one single flavonol, quercetin, have already been identified (Harborne and Baxter 1999).

Individual plants accumulate a large range of glycosides. For example, berries of grapevine (*Vitis vinifera*) contain more than 200 different aglycones conjugated to glucose (Sefton et al. 1993, 1994). Foreign compounds originating from other organisms and man-made chemicals, defined as xenobiotics, are also glycosylated by plants (Pflugmacher and Sandermann 1998). Overall, the complement of all glycosylating activities in any given individual plant must therefore be regarded as broad. However, the number of expressed GTs involved in secondary plant metabolism and the substrate

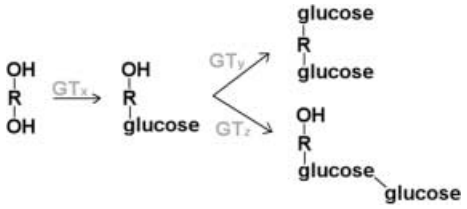

Fig. 1 The formation of mono and diglucosides by glycosyltransferases. GTx catalyzes the synthesis of a monoglucoside of R (the aglycone). GTy and GTz then synthesize two forms of diglucosides

specificities of these enzymes remain largely unknown. Results from the *Arabidopsis* genomic sequencing project indicate that more than 60 putative GTs, that might be involved in secondary plant metabolism, are present in the genome[1]. It is possible that a proportion of these are not expressed or involved in primary metabolism rather than secondary metabolism. It is important to note, however, that the border between primary and secondary metabolism is not clearly marked and many compounds defined as secondary metabolites, like sterols or hormones, play a crucial and conserved role in all plant species.

The diverse roles that glycosylation plays in plants are discussed first. Subsequently, it is examined how these various roles fit together, and how their interplay at the biochemical level is defined by the substrate specificity and organisation of individual GTs. The aim is to gain an insight into how glycosylation of a diverse range of aglycones is regulated and how the plant is able, with precise control, to regulate the biological activity of signaling molecules, whilst at the same time catering for a broad-spectrum coverage of xenobiotic substances that it is yet to encounter.

It is important to remember that the hydrolysis of glycosides by beta-glucosidases is also a very important and a complementary part of glycoside metabolism that will not be covered here (Warzecha et al. 1999; Cicek et al. 2000).

Glycosylation as a chemically modifying safety net

Glycosylation converts reactive and toxic aglycones into stable and non-reactive storage forms, thereby limiting their interaction with other cellular components (Fig. 2). Generally speaking, the attachment of carbohydrate moieties to nucleophilic molecules will reduce the possibility of electron transfer from the unglycosylated molecule to other cellular components, thereby lowering the reactivity, and consequentially improving the stability of that molecule. Since the nucleophilic sites are, in many cases, the particular part of the molecules that interact damagingly with other cellular components, the addition of sugars will block the interactive site and consequently reduce toxicity. Sugars are highly polar, and the addition of carbohydrate moieties to hydrophobic substrates will serve to increase the water solubility of the resultant glycoside. Thus, the main chemical roles for glycosylation are (i) stabilisation, (ii) detoxification (reduced reactivity), and (iii) solubilisation (increased polarity). The consequent outcomes of glycosylation, due to the combination of the above 'roles', are overlapping and may serve the same end purpose, depending on the chemistry of the individual secondary plant metabolites. Combined, glycosylation enables plants to store large amounts of toxic constituents.

Glycosylation is exerted upon molecules derived from exogenous and endogenous sources. Endogenous aglycones originate from both biosynthetic as well as degradative or turnover metabolism (Bak et al. 1999; Walter et al. 2000). Plants obviously have a glycosylating capacity for compounds that have exerted prior selective pressure in the evolution of GT substrate specificity. However, plants also have the capacity to glycosylate novel compounds that have not exerted any prior evolutionary selective pressure, derived either from exogenous (Tanaka et al. 1990; Lamoureux et al. 1991; Leah et al. 1992; Sandermann 1994; Pflugmacher and Sandermann 1998) or endogenous sources (Li et al. 1997; Bak et al. 1999).

Metabolic engineering has provided fine examples that plants have a substantial capacity to metabolise compounds of varying chemical structure (Bak et al. 1999) and with substantial quantitative differences (Li et al. 1997). As an example, the biosyntheses of cyanogenic glucosides and glucosinolates share many commonalties such as similar enzymes (cytochrome P450s and GTs) and similar intermediates (oximes) (Sibbesen et al. 1995; Bak et al. 1999; Jones et al. 1999; Wittstock and Halkier 2000). *Arabidopsis thaliana*, a plant that

Fig. 2 Glycosylation as a chemically modifying safety net

[1]Annotated sequences with a plant secondary product glycosyltransferase consensus sequence (Hughes and Hughes 1994) deposited in GenBank, June 2000.

accumulates tryptophan-, methionine- and phenylalanine-derived glucosinolates, was transformed with the cyanogenic CYP79A1 that forms *p*-hydroxyphenylacetaldoxime from tyrosine in *Sorghum* (Bak et al. 1999). The transgenic plants accommodated for the novel oxime by generating *p*-hydroxybenzylglucosinolate (not previously identified in *Arabidopsis*) and three new glucosidic metabolites of the oxime. Hence, *Arabidopsis* could accommodate for changes in oxime-structure and the accumulation of novel oxime intermediates. It is not unlikely that *Arabidopsis* with time could evolve a CYP79A1-like gene, given the high degree of similarity between CYP79A1 from *Sorghum* and the CYP79A2 from *Arabidopsis* involved in glucosinolate biosynthesis (Wittstock and Halkier 2000). Hence, this study illustrates a possible scenario in the eventual evolution of a CYP79A1-like gene in *Arabidopsis*. Another example was provided by Siebert et al. (1996) who transformed tobacco with *ubiC*, a bacterial gene encoding chorismate pyruvate-lyase which in a single reaction synthesis *p*-hydroxybenzoic acid from chorismate, resulting in more than a 1000-fold increase in *p*-hydroxybenzoic acid accumulation. Despite the substantial increase in the endogenous compound, the constitutive glucosyltransferase(s) present were able to conjugate all of the 'artificial' metabolite (Li et al. 1997). Hence, the above examples illustrate that plants contain a chemical safety net, with downstream enzymes capable of metabolising novel products, arising from xenobiotic products in the environment or as a result of changes in intermediate structures due to evolutionary modifications in upstream enzymes (Jones et al. 1999).

The following illustrates the different roles of glycosylation as a chemically modifying safety net (Fig. 2).

Anthocyanidins (anthocyanin aglycones), cyanohydrins (cyanogenic glucoside aglycones) and thiohydroximates (glucosinolate aglycones) are all examples of anabolic aglycones, which do not normally accumulate in plants. The thiohydroximates (Halkier and Du 1997) and the cyanohydrins (Møller and Seigler 1998) will without conjugation break down into other substituents. Glycosylation stabilises these molecules and thereby preserves their structural integrity to allow their accumulation. The anthocyanidins on the other hand will autocatalytically convert to colourless flavylium cations at either vacuolar or cytosolic pH (Brouillard 1982; Goto 1987). Studies with grapevine mutants void of berry anthocyanins have shown that the absence of vvUFGT (anthocyanidin-glucosyltransferase; Ford et al. 1998) results in anthocyanin-less berries (Boss et al. 1996). Hence, glycosylation at the C-3, C-5 or C-7 position of anthocyanidins will inhibit autoconversion into colourless forms, which are substrates for further catabolism (Calderon et al. 1992). In vivo, the catabolism of cyanohydrins, flavonols and anthocyanidins, but not of their corresponding glucosides, is facilitated by catabolic enzymes (Calderon et al. 1992; Morales et al. 1993; Wajant et al. 1994). The apparent improved stability of glycosides may therefore in some cases be more related to their lack of acceptance by catabolic enzymes rather than inherent aglycone instability.

Another interesting example is provided by 2-*O*-glucosides of L-ascorbic acid, which are significantly more resistant towards chemical and enzymatic oxidation than either 6-*O*-glucosides or aglycones thereof (Yamamoto et al. 1990). Similarly, dhurrin (*p*-hydroxymandelonitrile-glucoside) is considerably more stable than gluco-*p*-hydroxymandelonitrile (Abrol et al. 1966). This illustrates the importance of the positioning of the carbohydrate moiety for chemical stability.

The reduced toxicity of certain glycosides compared to their respective aglycones is illustrated by the potato alkaloid solanidine. Moehs et al. (1997) utilised the differential toxicity of solanidine and solanidine-glucoside to isolate potato solanidine-glucosyltransferase using positive selection with a yeast expression library carrying a potato cDNA library. A similar approach may be useful in the search for enzymes involved in xenobiotic metabolism.

Flavonols (Wollenweber 1986), monoterpenoids (Sefton et al. 1994) and hydroxybenzoic acids (Klick and Herrmann 1988; Yazaki et al. 1995) accumulate as both aglycones and glycosides, whilst alkaloids rarely accumulate as glycosides (Warzecha et al. 1999). This pattern may reflect the varying stability or reactivity of secondary plant metabolite aglycones. For example, flavonols are much more stable than anthocyanidins at pH 8 (Ford et al. 1998). Since these aglycones also accumulate without glycosylation, there may be other reasons for glycosylation other than solubilisation and chemical stabilisation. Although glycosylation often precedes localisation to the vacuole or cell wall, it may not necessarily be a prerequisite for this localisation to take place. A prominent example is the violet betacyan betanidin, which, although usually glycosylated, may accumulate in high concentrations in the vacuole in flowers of the Ruschieae (Strack et al. 1990). It has been suggested that an increase in water solubility due to glycosylation may act as a vacuolar trapping mechanism for small lipophilic aglycones that otherwise may diffuse freely through the tonoplast (Wink 1998). However, this theory needs to be verified with transgenic plants with altered glycosylation patterns, since a large range of secondary plant products accumulate as both aglycones and glycosides. Solubilisation and stabilisation of labile and/or reactive molecules, although possibly important for inert vacuolar storage, may also be important outside the vacuole, particularly for reactive molecules in the presence of proteins.

Glycosylation as a biologically interpreted flag

The biological effect of glycosylation is characterised by a differential interaction between glycosylated versus non-glycosylated low-molecular-weight molecules and proteins involved in signal recognition, transport and metabolism. More specifically, the carbohydrate moiety

will inhibit (or in some cases allow for) the interaction between these particular molecules and signal receptors, transport proteins or degradative enzymes. The role of phytoanticipins, non-toxic glycosides which when degraded yield toxic catabolites, in plant defence has been reviewed recently (Osbourn 1996).

A glycosyl-flag which determines in vivo activity

Plant phenolic compounds are commonly detected by bacteria that are involved in plant-microbe interactions (reviewed by Peters and Verma 1990). The presence or absence of a glycosyl moiety on these phenolics may in several instances determine the activity of that signal. For example, a range of phenolic glycosides accumulated by *Prunus avium* is capable of inducing phytotoxin synthesis in *Pseudomonas syringae*, whilst their corresponding aglycones lack such activity (Mo et al. 1995). Similarly, the flavone luteolin acts as a *nod*-gene inducer in symbiotic *Rhizobium meliloti* (Peters et al. 1986). Luteolin-7-*O*-glucoside has less inducing activity than luteolin alone and most likely requires hydrolysis to the free aglycone for induction to take place (Hartwig and Phillips 1991). In the very same plant-microbe interaction, the reverse is true for the isoflavonoid malonylglucoside formononetin, as removal of either the malonyl or glycosyl group will delete the *nod*-gene-inducing activity (Dakora et al. 1993). Although not investigated, the nature of the directly sensed molecule may, however, still be the aglycone form. Whether these flavonoids are sensed directly by proteinaceous receptors, or simply induce nodulation due to indirect consequences following the elicitation of a general defence response would be interesting to know. A similar question should be asked about safener- and xenobiotic-inducible enzymes, including GTs, which are involved in the detoxification of xenobiotics (Farago et al. 1994; Robineau et al. 1998). Glycosylation also affects inter plant-to-plant signalling within the same species. For example, in maize and petunia, the flavonol aglycones kaempferol and quercetin are required for pollen germination whilst the corresponding glycosides are inactive (Mo et al. 1992).

The role of plant hormone conjugation, including glycosylation, has been reviewed recently (Sembdner et al. 1994; Kleczkowski and Schell 1995). The interesting question about plant hormone glycosylation lies not only in the turnover or degradation of hormones, but also in the potential for temporary inactivation, since hydrolysis and conjugation may be involved in maintaining hormonal homeostasis without the need for synthesis de novo (Bandurski et al. 1995). Such a system would not only save energy by recycling intact molecules but also allow for rapid responses. Interestingly, a similar scenario has been shown for the synthesis and hydrolysis of the phytoalexins medicarpin and maackiain. At low levels of elicitation, the phytoalexins are synthesised and subsequently conjugated, whilst at a high level of elicitation, synthesis is paralleled by hydrolysis of preformed conjugates (Mackenbrock et al. 1993).

Recently, GTs with activity towards indole acetic acid (Szerszen et al. 1994), zeatin (Martin et al. 1999) and salicylic acid (Lee and Raskin 1999), and a beta-glucosidase possibly involved in the hydrolysis of cytokinins (Kristoffersen et al. 2000), have been isolated and cloned. The future employment of these genes in transgenic experiments, in the antisense and sense directions, should hopefully yield some information about the actual role of glycosylation in hormonal metabolism. A glucuronosyltransferase (PsUGT1) with high homology to the *p*-hydroxymandelonitrile-glucosyltransferase of *Sorghum bicolor*, which synthesises the cyanogenic glucoside dhurrin (Jones et al. 1999), was recently isolated from *Pisum sativum* (Woo et al. 1999). Although the acceptor substrate specificity of the protein encoded by PsUGT1 has not been identified yet, it was suggested that it might conjugate hormones or hormone-like signals, due to a possible involvement in the growth and development of alfalfa and pea (Woo et al. 1999).

Transportation

Several secondary plant products, including cyanogenic glucosides (Møller and Seigler 1998) and cardenolides (Christmann et al. 1993), are only transported within the plant in a glycosylated form. In the case of the cyanogenic glucoside linamarin in *Hevea brasiliensis*, where the diglucoside but not the monoglucoside or aglycone is the transport form, the substrate specificity of apoplastic beta-glucosidases restricts the extracellular transport of the monoglucoside (Gruhnert et al. 1994). The specificity of any membrane transport mechanism must, however, exert a primary restriction on monoglucoside mobility in order to avoid full-scale internal cyanogenesis.

It has been suggested that glycosylation may be required for vacuolar localisation in order to solubilise and inactivate hydrophobic and reactive molecules (Pridham 1965). Lately, however, there have been reports of vacuolar transporters that are differentially specific for flavonoid and xenobiotic glucosides, respectively (Klein et al. 1996, 2000). Furthermore, the transportation of the xenobiotic glucoside was enhanced by the application of safeners to barley (Gaillard et al. 1994). It is therefore most likely that the requirement of glycosylation for vacuolar deposition may involve more than the solubility, reactivity and stability of the aglycone. Unfortunately, in neither of these experiments did the authors compare the transport of these glucosides with their corresponding aglycones.

Soup or structure? – the organisation of glycosylation

Since plants have the capability to glycosylate a wide range of aglycones for a number of different reasons, it is

pertinent to ask how this task is accomplished. For example, how do plants distinguish between xenobiotic compounds or catabolites with widely different structures and endogenous molecules such as hormones or primary metabolites? It would not seem favourable if xenobiotic GTs were to interfere with primary or secondary metabolic pathways, or with the level of active signalling compounds. Hence, some form of restriction must exist to decide which compound is to be glycosylated and which is not. If all GTs and aglycones are present randomly in an intracellular soup, then this 'restriction' apparently will be determined by the individual substrate specificities of all constitutive GTs. Alternatively, a part of the entire complement of GTs must be 'restricted' by a structural segregation or a pre- or post-transcriptional regulation. In other words, a GT will only glycosylate those aglycones which it meets, and which it accepts.

Substrate specificity of GT – accepting the substrate

Is there one GT that glycosylates all aglycones or one GT for each and every substrate? The answer is most likely somewhere in between, although we cannot exclude that there is a great variation in the range of substrates that different GTs are able to accept. It has been suggested that GTs are highly regiospecific (or selective) rather than substrate-specific (Vogt et al. 1997). In other words, acceptance is strictly based on a certain substructure, with little regard for the entire structure of the acceptor molecule. In this regard it is interesting to compare the studies performed by Tanaka et al. (1990) and Marcinek et al. (2000). Marcinek et al. (2000) purified an indoxyl-GT 863-fold from *Baphicacanthus cusia* and found that it only accepted 4-OH-, 5-OH-, 6-OH- and 7-OH-indole out of 60 different aglycones. On the other hand, Tanaka et al. (1990) provided evidence to suggest that three different GTs were present in cell-suspension cultures of *Mallotus japonicus* with activities towards *O*-hydroxybenzoic acid, *p*-hydroxybenzoic acid and *m*-hydroxybenzoic acid, respectively.

Most knowledge about GT substrate specificity is based on assays performed on enzymes that have been purified from their native source. Unfortunately it is seldom possible to guarantee that purified GTs are isolated and not just partially purified together with other GTs (Ford et al. 1998; Jones et al. 1999). It is therefore vital to analyse recombinant GTs that are expressed in a heterologous system and subsequently isolated in order to ensure the validity of the in vitro substrate-specificity profiles. A range of GT-encoding genes have been expressed in heterologous systems and verified to accept particular substrates: indole acetic acid (IAA) (Szerszen et al. 1994), anthocyanidins (Tanaka et al. 1996), solanidine (Moehs et al. 1997), anthocyanins (Yamazaki et al. 1999), zeatin (Martin et al. 1999), and limonoate A-ring lactone (Kita et al. 2000). Alas, these recombinant proteins were not tested against a range of other substrates in order to obtain a substrate-specificity profile. To date this has only been done for the six different GTs listed in Fig. 3. We can only draw some preliminary conclusions from this small set of substrate-specificity profiles: It is not clear what the native substrate of the salicylic acid (SA)-inducible TOGT (profile 4, Fraissinet-Tachet et al. 1998) or the second SA-inducible GT (profile 5, Lee and Raskin 1999) is. These glucosyltransferase-encoding genes were isolated from tobacco. Their gene products conjugate both the hydroxyl- and carboxyl-groups of SA, as well as a range of other compounds, suggesting a rather broad substrate specificity. Interestingly, SA is only weakly accepted by either of the two enzymes and their in vivo substrates may therefore be other compounds. Are they related and do they have a similar in vivo function? Other GTs, like the anthocyanidin-3-*O*-GT from grapevine, are almost exclusively specific for their in vivo substrate (Ford et al. 1998). The GTs from *Sorghum*, *Dorotheanthus* and petunia also accept substrates that are not known to be conjugated by these respective enzymes, although at a lower maximal rate than their preferred substrates. According to their substrate specificity we might group these six GTs into broad (tobacco: Fraissinet-Tachet et al. 1998; Lee and Raskin 1999), intermediate (*Sorghum*: Jones et al. 1999 and *Dorotheanthus*: Vogt et al. 1999) and narrow (grapevine, Ford et al. 1998; petunia: Miller et al. 1999) substrate specificity. However, a more exhaustive range of GTs and substrates needs to be tested in order to confirm this preliminary classification.

It is also important to consider the specificity of the overall complement of glycosylating activities, in order to understand the regulation of this modification. For example, it may facilitate the task to 'tightly' regulate glycosylation of IAA if only one GT, i.e. *Iaglu* in maize (Szerszen et al. 1994), accepts this substrate. The complement of glycosyltransferase activities towards xenobiotic substances may on the other hand not need such a 'tight' regulation. Ford and Høj (1998) conducted a thorough study on the complement of GT activities in grapevine and their results corroborated this hypothesis. Protein extracts were separated by ion exchange and selected fractions assayed using geraniol, biochanin A, gentisic acid and SA. Whilst multiple fractions accepted biochanin A, geraniol and gentisic acid, only one fraction displayed low activity towards SA. Similarly, Kowalczyk and Bandurski (1991) only found a single enzyme with activity towards IAA in maize kernels. The recombinant GTs from *Sorghum* (Jones et al. 1999), petunia (Miller et al. 1999) and TOGT from tobacco (Fraissinet-Tachet et al. 1998) were tested and found to have no activity towards IAA. It is somewhat confusing, however, that the recombinant SA-inducible GT from tobacco surprisingly accepted both SA and IAA at a 10:3 ratio (Lee and Raskin 1999).

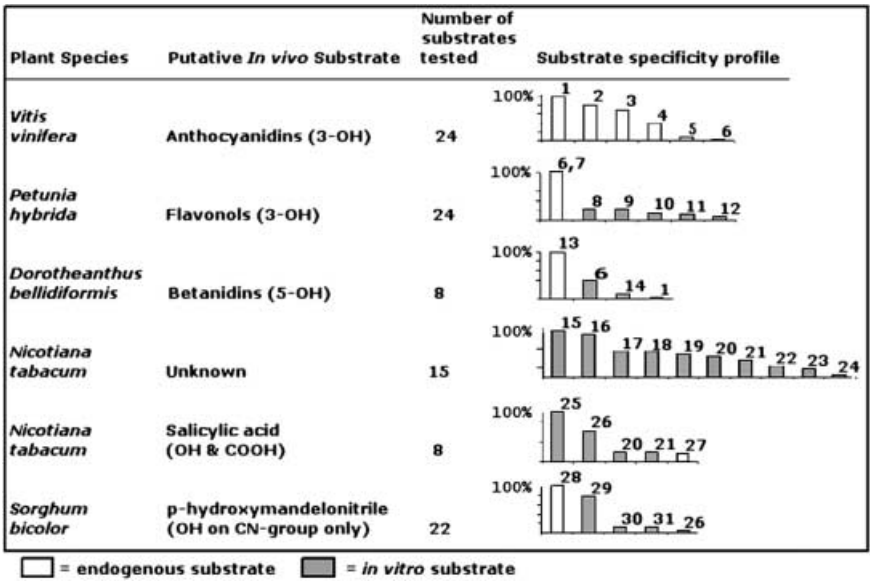

Fig. 3 The substrate-specificity profiles of six recombinant GTs. The profiles display relative activity against that substrate which has the highest activity (100%) as determined in the following papers: Ford et al. (1998), Fraissinet-Tachet et al. (1998), Jones et al. (1999), Lee and Raskin (1999), Miller et al. (1999), and Vogt et al. (1999). The substrates are: *1*, cyanidin; *2*, delphinidin; *3*, peonidin; *4*, pelargonidin; *5*, malvidin; *6*, quercetin; *7*, kaempferol, *8*, myricetin; *9*, fisetin; 10, galangin; *11*, iso-rhamnetin; *12*, rhamnetin; *13*, betanidin; 14, luteolin; *15*, esculetin; *16*, scopoletin; *17* *p*-coumaric acid; *18*, caffeic acid; *19*, *O*-coumaric acid; *20*, *p*-hydroxybenzoic acid; *21*, cinnamic acid; *22*, umbelliferone; *23*, coniferyl alcohol; *24*, ferulic acid; *25*, *m*-hydroxybenzoic acid; *26*, benozoic acid; *27*, salicylic acid; *28*, *p*-hydroxymandelonitrile; *29*, mandelonitrile; *30*, benzyl alcohol; *31*, geraniol

Transcriptional control of glycosylation – meeting the substrate

There are several ways in which plants can regulate the temporal and spatial distribution of plant glycosylation. For example, enzymes involved in xenobiotic metabolism in the liver of mammals, including glucuronosyltransferases, are known to be induced by xenobiotic agents (Tephly and Burchell 1990). A possible analogy to this may be found in plants, where several enzymes thought to be involved in xenobiotic metabolism are known to be inducible (Farago et al. 1994; Robineau et al. 1998). Several putative and verified GT-encoding genes have been found to be induced by methyl jasmonate (Imanishi et al. 1998), SA (Horvath and Chua 1996; Fraissinet-Tachet 1998; Lee and Raskin 1999) and wounding (O'Donnell et al. 1998). The induction was both rapid and transient in several of these cases (Horvath and Chua 1996; Fraissinet-Tachet 1998; O'Donnell et al. 1998; Lee and Raskin 1999). Tanaka et al. (1990) found evidence for substrate-inducible glucosyltransferases in a very interesting study on the glucosylation of isomeric hydroxybenzoic acids by *M. japonicus* cell suspension cultures. The three isomeric hydroxybenzoic acids were all glycosylated by three different substrate-inducible GTs with different induction patterns. Only one of the GTs was constitutive, and although they were not separated and examined individually, they are clear candidates for further studies of inducible plant xenobiotic metabolism.

Several plants show differential glycosylation in light compared to dark (Bandurski et al. 1977; Leah et al. 1992) and this has also been demonstrated at the mRNA level (Gong et al. 1997; Yamazaki et al. 1999). Glycosyltransferases are also known to be developmentally regulated (Sparvoli et al. 1994; Boss et al. 1996; Miller et al. 1999; Woo et al. 1999; Kita et al. 2000) and restricted in tissue localisation (Schmid et al. 1982; Vogt and Taylor 1995; Boss et al. 1996; Gong et al. 1997; Ford and Høj 1998; Martin et al. 1999; Miller et al. 1999).

The co-localisation of GTs with secondary metabolic pathways

Living organisms apparently employ enzymatic complexes ("metabolons") for many metabolic pathways, including primary and secondary metabolic pathways such as the phenylpropanoid pathway (Winkel-Shirley 1999). It is easy to envision the advantage of including GTs in such complexes given that many aglycones are highly labile and toxic. In contrast to mammalian GTs (Meech and Mackenzie 1997), most plant GTs are soluble and are therefore thought to be cytosolic, with only few exceptions (Leah et al. 1992; Warnecke and Heinz 1994; Vogt and Taylor 1995). The intracellular localisation of GTs has not been investigated thoroughly and opinions vary. Two studies made with cellular fractionation have found GT activities to be cytosolic (Blume et al. 1979; Yazaki et al. 1995). A third study by Anhalt and Weissenböck (1992) found that mono- and di-GT activities of luteolin were cytosolic, whilst a third

tri-GT was found to be vacuolar. Immunolocalisation has been employed in two instances. Schmid et al. (1982) showed a cytosolic localisation of a coniferyl-GT in spruce seedlings, whilst Latchinian-Sadek and Ibrahim (1991) presented evidence of a vesicle-like and cytoplasmic membrane localisation of a flavonol-B-ring specific GT in *Chrysoplenium americanum*. Ibrahim (1992) subsequently suggested that these results were indicative of a very loose association between the GT and the endoplasmic reticulum.

Several pathways would benefit from a co-localisation of GTs with upstream enzymes, including the biosynthesis of anthocyanins, cyanogenic glucosides and glucosinolates. Clustering of GTs together with cytochrome P450s in the metabolism of xenobiotic substances may also be beneficial, particularly in circumstances where the hydroxylated intermediates are more toxic than their precursors (Plewa and Wagner 1993). In certain instances it is difficult to conceive of the absence of such an arrangement, as with cyanogenic glucoside biosynthesis in *Sorghum* (Fig. 4). The microsomal part of the biosynthetic pathway of cyanogenic glucosides is channelled in vitro, except for the final glucosylating step (Møller and Seigler 1998). The aglycone precursor, *p*-hydroxymandelonitrile, forms an equilibrium with its toxic degradation products at cellular pH (Reay and Conn 1974). In the event of such a breakdown, *p*-hydroxybenzaldehyde-GT activities would shift the equilibrium towards free cyanide by the removal of free *p*-hydroxybenzaldehyde (Reay and Conn 1974). Furthermore, alpha-hydroxynitrilases, which catalyse the breakdown of the cyanohydrin, are present in the cytosol of biosynthetic tissue in *Sorghum* (Wajant 1994). Despite this, Møller and Conn (1980) were unable to cross-link the GT with the microsomal component. If there is any association between the GT and the cytochrome P450s, it must therefore be weak. In the phenylpropanoid pathway, however, there is evidence that several soluble components, including GTs, are weakly associated with the microsomal components (Wagner and Hrazdina 1984; Hrazdina and Wagner 1985; Hrazdina et al. 1987; Burbulis and Winkel-Shirley 1999).

Conclusion on soup or structure

Even if a given GT is co-localised in a macromolecular complex with upstream enzymes, will this hinder potential interference with other pathways which do not have such an organisation? We can envision that it is possible to either (i) compartmentalise the GT away from sensitive molecules or (ii) restrict the localisation of the substrate. The former approach may need to rely on transcriptional control or strict subcellular compartmentation whilst macromolecular complexing in addition to compartmentation would seem beneficial for the latter. For example, assuming that the tobacco SA-inducible GT, which has in vitro activity towards both SA and IAA (Lee and Raskin 1999), does not glycosylate both of these signalling compounds in vivo, how is this regulated? Although the GT is transcriptionally inducible it is still constitutively present in tobacco (Lee and Raskin 1999). Certainly, this is an area of plant research that demands more attention.

The above information highlights which components we need to consider in order to understand how all glycosylating events are organised in parallel. However, it does not answer what role any of the verified individual GTs may have in planta. For example, do GTs

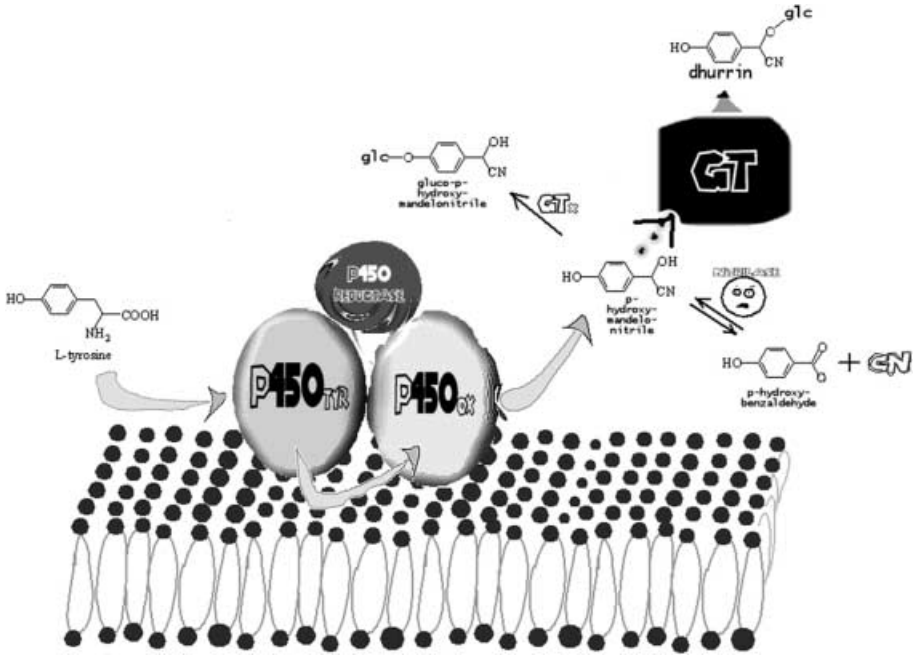

Fig. 4 Cyanogenic glucoside biosynthesis and catabolism. Microsomal cytochrome P450s convert tyrosine into *p*-hydroxymandelonitrile, which subsequently can be glycosylated to dhurrin or *p*-glucosyloxymandelonitrile, or degraded to cyanide and *p*-hydroxybenzaldehyde (Møller and Seigler 1999)

utilise all of their "specificity"? The next step towards understanding this will require the modulation of individual GTs in vivo. To date there are only few examples of modified GT expression in planta. Mutants of maize and grapevine with changes in anthocyanin accumulation have been linked with changes in anthocyanidin 3-*O*-GT activity and gene transcript levels (Dooner and Nelson 1977; Larson and Coe 1977; Dooner 1979; Boss et al. 1996). Although grapevine anthocyanidin 3-*O*-GT is able to conjugate flavonols it is still not clear whether this enzyme or the encoded maize homologue, *Bronze*-1, glucosylate flavonols in vivo (Ford et al. 1998). Transgenic lisianthus overexpressing a putative *Anthirrhinum majus* flavonoid-GT showed evidence of both 3-*O*-anthocyanidin and 3-*O*-flavonol glucosylation (Schwinn et al. 1997). The unidentified putative GT from *Pisum sativum* (PsUGT1) was also overexpressed in both sense and antisense orientation in *Pisum* and alfalfa, and future experiments may there give an insight into the exact in vivo role of this gene product (Woo et al. 1999). It is a fair conclusion that more of these experiments are required in conjunction with a thorough analysis of plant physiological aspects and natural product glycosylation.

Concluding remarks

The preceding discussion has described some of the different roles that glycosylation may play in plants and what components are involved in regulating this process. Although this review has not answered how glycosylation is organised in planta, principally due to a shortage of available information, it has highlighted which components need to be considered. More knowledge is needed regarding the diversity of GTs within plants and their specific biological roles. Further biochemical characterisation of isolated GTs, coupled with experiments in which individual GT expressions are modulated will be fundamental for progress. We need to increase our understanding of how secondary plant metabolic pathways are organised inside the cell. Recent results certainly point towards a high degree of organisation as opposed to a random soup (Burbulis and Winkel-Shirley 1999). More knowledge is also needed regarding the role of complex glycosylation, since many secondary metabolites accumulate as di- and tri- glycosides with glucose moieties innermost (Crouzet and Chassagne 1999).

It may also be possible to categorise GTs into different classes depending on their biological roles, which may facilitate our interpretation of future experimental data. From the above discussion we can differentiate at least three candidate GT-"types": (i) anabolic, (ii) regulatory and (iii) xenobiotic and endogenous metabolism. Examples of potential candidates would include (i) *Sorghum p*-hydroxymandelnitrile-GT (Jones et al. 1999), grapevine anthocyanidin-3-*O*-GT (Ford et al. 1998) and *Dorotheanthus* betanidin-5-*O*-GT (Vogt et al. 1999), and (ii) maize IAA-GT (Szerszen et al. 1994), the two zeatin-GTs from *Phaseolus lunatus* (Martin et al. 1999) and petunia flavonol-3-GT (Miller et al. 1999). There are no clear candidates for xenobiotic/catabolic GTs, although the two tobacco GTs with unknown in vivo substrates (Fraissinet-Tachet et al. 1998; Lee and Raskin 1999) warrant consideration. It is not inconceivable that GTs with such different roles may also be grouped according to differential localisation, regulation and substrate-specificity profiles.

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Note added in proof. The *Arabidopsis thaliana* IAA-GT (*UGT84B1*, Database accession number AC002391) has been identified recently based on sequence similarity search, cloning and heterologous expression in *E. coli* (Jackson et al. 2001, J Biol Chem, in press).

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