Differential distribution of a glucuronyltransferase, involved in glucuronoxylan synthesis, within the Golgi apparatus of pea (*Pisum sativum* var. Alaska)

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The subcellular location of a glucuronyltransferase (GT) involved in glucuronoxylan synthesis in pea (*Pisum sativum*) has been investigated. Most of the GT activity was found in the Golgi fraction, but activity was also detected in the plasma-membrane fraction. Separation of Golgi membranes on a shallow continuous sucrose density gradient resulted in three distinct subfractions, with GT activity being confined to Golgi membranes of a density similar to that of smooth endoplasmic reticulum. The differential distribution of GT within the Golgi stack indicates that glucuronoxylan synthesis occurs in specific cisternae and that there is functional compartmentalization of the Golgi with respect to hemicellulose biosynthesis.

INTRODUCTION

The biosynthesis of the hemicellulose glucuronoxylan has been studied in some detail in pea (*Pisum sativum*) epicotyls (Waldron & Brett, 1983, 1987; Baydoun *et al.*, 1989a,b; Waldron *et al.*, 1989). The glucuronidation of a xylose backbone by a membrane-bound glucuronyltransferase (GT) that is stimulated by UDP-xylose (Waldron & Brett, 1983) has been found to occur within the Golgi apparatus (Waldron & Brett, 1987). GT activity was found over a range of densities after sucrose-density-gradient centrifugation, whereas the UDP-xylose stimulation appeared to be localized over a narrower range of densities (Waldron & Brett, 1987), suggesting a differential distribution of glucuronoxylan synthetases within the Golgi.

Glycosyltransferases have been found to be differentially distributed in Golgi isolated from animal cells, and this has been exploited to provide markers delimiting Golgi compartments (Dunphy & Rothman, 1985). With respect to oligosaccharide processing on transported glycoproteins, the Golgi stack consists of at least three functionally and compositionally distinct compartments (Dunphy & Rothman, 1983; Goldberg & Kornfeld, 1983; Chege & Pfeffer, 1990). In higher plants, two Golgi subfractions have been obtained from suspension-cultured cells of sycamore (Ali *et al.*, 1986) and from pea epicotyls (Brummell *et al.*, 1990). In the latter study (Brummell *et al.*, 1990) a difference in xyloglucan glycosyltransferase distribution within the Golgi was found, leading to the conclusion that some compartmentalization, with respect to polysaccharide synthesis, exists in the Golgi system of plant cells. In the present paper we report the subfractionation of Golgi membranes into three subfractions and the localization of GT within the lightest of these subfractions.

EXPERIMENTAL

Chemicals

UDP-D-[U-¹⁴C]glucuronic acid (11.6 GBq · mmol⁻¹) and UDP-D-glucose (12.9 GBq · mmol⁻¹) were purchased from Amersham International (Amersham, Bucks., U.K.). UDP-D-xylose was

purchased from Sigma (Poole, Dorset, U.K.). Peas (*Pisum sativum* var Alaska) were supplied by Sinclair McGill (Ayr, Scotland, U.K.).

Preparation of membranes

Peas were grown and harvested as described previously (Waldron *et al.*, 1989). All subsequent operations were performed at between 0 and 4 °C. Epicotyl tissue (100 g) was cut into 1–2 mm lengths with a razor blade before homogenization for 60 s with 35 cm³ of homogenization buffer [10 mM-Tris/HCl (pH 7.4)/10 mM-KCl/1.5 mM-MgCl₂/10 mM-dithiothreitol (DTT)] in a chilled pestle and mortar. The homogenate was strained through four crossed layers of muslin and compensation buffer [10 mM-Tris/HCl (pH 7.4)/0.72 M-KCl/28.5 mM-MgCl₂/10 mM-DTT/2 M-sucrose] added to give a sucrose density of 1.032 g · cm⁻³. The residue was re-homogenized for 60 s in 50 cm³ of homogenization buffer, strained, and compensation buffer was added to give a sucrose density of 1.032 g · cm⁻³ using an Atago refractometer. The filtrate was centrifuged at 13000 *g*_{av} for 10 min in a Sigma 3K20 centrifuge with a Sigma 12156 fixed-angle rotor. The pellet was resuspended in 5 cm³ of resuspension buffer (1 mM-Mes/NaOH, pH 6), using a 5 cm³ glass-on-glass homogenizer. The supernatant was loaded on to eight 5 cm³ sucrose (density 1.179 g · cm⁻³) cushions and centrifuged at 150000 *g*_{av} for 30 min in a Sorvall OTD-55B ultracentrifuge with a Sorvall T865 fixed-angle rotor. The resulting membrane fractions were collected and pooled before being adjusted to a sucrose density of 1.179 g · cm⁻³ with sucrose solution [10 mM-Tris/HCl (pH 7.4)/5 mM-MgCl₂/100 mM-KCl/10 mM-DTT/2.26 M-sucrose] of density 1.289 g · cm⁻³. The remaining solution was pooled, and an aliquot was diluted to 50% with resuspension buffer and pelleted at 150000 *g*_{av} for 30 min, with the resultant pellet being resuspended in 2 cm³ of resuspension buffer and termed 'R₁'. Four sucrose step gradients [see the Results section for details (each sucrose solution contained 40 mM-Tris/HCl, pH 7.4, 0.1 mM-MgCl₂, 1 mM-EDTA and 10 mM-DTT)] were formed on top of 10 cm³ of membranes and centrifuged at 150000 *g*_{av} for 3 h. Membranes from each interface were collected and pooled. The top interface

Abbreviations used: GT, glucuronyltransferase; PM, plasma membrane; ER, endoplasmic reticulum; DTT, dithiothreitol; GSII, glucan synthase II.

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was termed 'endoplasmic reticulum' (ER) [approx. 18/25% (w/w)-sucrose interface], the middle Golgi (approx. 25/35% w/w sucrose interface) and the lower 'plasma membrane' [(PM) approx. 35/40% (w/w)-sucrose interface], and the remaining solution was termed 'R₂'. An aliquot of Golgi and the total volume of the other fractions were diluted to 50% with resuspension buffer, pelleted at 150 000 *g*_{av.} for 30 min and resuspended in 1 cm³ of resuspension buffer, using a 1 cm³ glass-on-glass homogenizer. The remainder of the Golgi fraction was adjusted to a sucrose density of 1.189 g·cm⁻³ with sucrose solution of density 1.289 g·cm⁻³, and two 24 cm³ linear sucrose gradients were formed on top of 14 cm³ of membranes. Gradients were centrifuged at 97 000 *g*_{av.} in a Sorvall AH629 swing-out rotor for 16 h. An ISCO model 185 density-gradient fractionator was used to collect 2 cm³ gradient fractions from the top of the gradient, the densities of which were determined with a refractometer. Fractions corresponding to the membrane sample were pooled and termed 'R₃'. Each fraction was adjusted to 30 cm³ with NaCl buffer [1 mM-Mes/NaOH (pH 6)/1 M-NaCl] and pelleted at 150 000 *g*_{av.} for 30 min before resuspension in 0.5 cm³ of resuspension buffer.

Enzyme assays

Glucuronyltransferase (GT). The incubation mixture consisted of 50 µl of membrane preparation, MnCl₂ (10 mM) UDP-D-xylose (1 mM) and UDP-D-[¹⁴C]glucuronic acid (0.14 nmol; 1.67 kBq) in a total volume of 100 µl. Incubations were performed at 25 °C for 4 h and were terminated by the addition of 1 cm³ of 96% (v/v) ethanol, followed by the addition of cellulose powder (to act as a carrier). Incorporation of radioactivity into hemicellulose was measured as described previously (Waldron & Brett, 1983), except that the pellet was only washed once with 1 cm³ of 70% (v/v) ethanol and three times with 1 cm³ of water, before estimation of radioactivity.

Glucan synthase II (GSII). This assay was carried out as described by Waldron & Brett (1987), with the addition that cellulose powder was added at the termination of the incubation.

Latent IDPase. The assay was as described previously (Waldron & Brett, 1987), except that the Triton X-100-activated IDPase incubation contained Triton X-100 at the start of the incubation.

NADH:cytochrome *c* reductase. Incubations contained 50 µl of membrane, 300 µl of sodium phosphate (0.2 M, pH 7.4), 150 µl of cytochrome *c* (5 mg·cm⁻³), 15 µl of KCN (10 mM), 7.5 µl of antimycin A (2 mg·cm⁻³) in a total volume of 852.5 µl. The assay was initiated by the addition of 150 µl of NADH (3 mg·cm⁻³), and the rate of increase in absorbance at 550 nm was measured over 2 min.

Succinate dehydrogenase. Incubations contained 50 µl of membrane, 1.2 cm³ of sodium phosphate (0.1 M, pH 7.5) containing KCN (1.7 mM), 50 µl of phenazine methosulphate (1%, w/v) and 50 µl of 2,6-dichlorophenol-indophenol (50 µg·cm⁻³). The assay was initiated by the addition of 150 µl of sodium succinate (0.4 M), and the rate of decrease in absorbance at 600 nm was measured over 2 min.

All assays, except where stated otherwise, were initiated by the addition of membrane.

Protein. Protein was assayed by the method of Peterson (1977), using BSA (Sigma; fraction V) as a standard.

All assays were performed in duplicate.

RESULTS

Isolation of membrane fractions on a step gradient

An initial membrane fractionation was carried out on a discontinuous sucrose-density gradient [10 cm³ of membrane at

Table 1. Specific activity (S.A.), yield and recovery of GT and marker enzymes during the initial fractionation of membranes on a discontinuous sucrose-density gradient

Etiolated pea epicotyls, 7 days old, were homogenized and treated as described in the text.

Fraction	Protein			GT			Latent IDPase			GSII			Cytochrome <i>c</i> reductase		
	Concn. (mg·ml ⁻¹)	Yield (mg)	Recovery (%)	S.A. (Bq·h ⁻¹ mg ⁻¹)	Yield (Bq·h ⁻¹)	Recovery (%)	S.A. (µmol·min ⁻¹ ·mg ⁻¹)	Yield (µmol·min ⁻¹)	Recovery (%)	S.A. (Bq·min ⁻¹ (mg ⁻¹))	Yield (Bq·min ⁻¹)	Recovery (%)	S.A. (µmol·min ⁻¹ ·mg ⁻¹)	Yield (µmol·min ⁻¹)	Recovery (%)
Homogenate	8.26	101	100	21.8	2206	100	0	0	—	0.82	82.3	100	198	20 × 10 ³	100
Pellet	9.26	46	46	15.3	704	32	0	0	—	4	182.9	222	207	9.5 × 10 ³	48
ER	2.20	2.2	2	284	624	28	8.5	18.6	—	6.5	14.3	17	172	378	1.9
Golgi	0.68	5.7	6	416	2370	107	19.5	111	—	0.15	0.87	1	25	144	0.7
PM	2.96	3.0	3	92	276	13	0.43	1.3	—	39.4	118.1	144	34	102	0.5
R ₁	3.06	13	13	21.3	277	13	0.07	0.87	—	1.3	17.9	22	32	411	2.1
R ₂	2.66	5.3	5	69.8	370	17	0.13	0.67	—	16.8	88.9	108	51	270	1.4

Fig. 1. Profiles of marker-enzyme separation after continuous-sucrose-density-gradient centrifugation of isolated Golgi membranes on a $1.115\text{--}1.165\text{ g}\cdot\text{cm}^{-3}$ density gradient (see the text for details)

▲, Protein; ■, GT activity; ◆, latent IDPase activity; ◇, GSII activity; ▼, NADH:cytochrome *c* reductase activity; ●, sucrose concentration in gradient fractions.

a density of $1.179\text{ g}\cdot\text{cm}^{-3}$, 10 cm^3 of sucrose at a density of $1.165\text{ g}\cdot\text{cm}^{-3}$, 7 cm^3 at $1.115\text{ g}\cdot\text{cm}^{-3}$ and 3 cm^3 at $1.074\text{ g}\cdot\text{cm}^{-3}$). The specific and total activities of marker enzymes and GT were determined for the homogenate, the 13000 g pellet and the membrane fractions resulting from discontinuous sucrose-density centrifugation (Table 1). Protein recovery was 75 %, with most in the 13000 g pellet. The Golgi fraction was the membrane fraction with the highest protein content. Succinate dehydrogenase activity was only detected in the homogenate and the 13000 g pellet, indicating that mitochondria were removed by the initial centrifugation. NADH:cytochrome *c* reductase activity was mainly located in the 13000 g pellet, whereas in the membrane fractions the ER contained the highest total activity. Latent IDPase activity was undetectable in the homogenate and in the 13000 g pellet. In the membrane fractions both total and specific latent IDPase activities were highest in the Golgi fraction. Total GSII activity was maximal in the 13000 g pellet, but the specific activity was highest in the PM fraction. GT activity was detected in all fractions, but was highest in the Golgi, where there was a 107 % recovery and a 19-fold purification.

Golgi subfractionation

On a sucrose gradient of density $1.115\text{--}1.165\text{ g}\cdot\text{cm}^{-3}$. Golgi membranes from discontinuous sucrose-density gradients were subfractionated on a sucrose-density gradient ($1.115\text{--}1.165\text{ g}\cdot\text{cm}^{-3}$). As Fig. 1 shows, most of the protein, latent IDPase and GT activity was located at the top of the gradient, at densities of less than $1.134\text{ g}\cdot\text{cm}^{-3}$. There was a second peak of latent IDPase activity between densities of 1.134 and $1.146\text{ g}\cdot\text{cm}^{-3}$. GSII activity was mainly located at the bottom of the gradient, at densities greater than $1.145\text{ g}\cdot\text{cm}^{-3}$. There were two main peaks of NADH:cytochrome *c* reductase activity, at densities of $1.131\text{ g}\cdot\text{cm}^{-3}$ and $1.153\text{ g}\cdot\text{cm}^{-3}$.

The recovery of protein and GT from the linear gradient was

approx. 48 % of that applied (50 % of the Golgi membrane fraction was used for each linear gradient) (Table 2). This amounted to recoveries from the homogenate of 1.4 and 26 % for protein and GT respectively. In the most active fraction from the linear sucrose gradient, the GT specific activity was a factor of 2 greater than in the Golgi fraction, and this represented a total purification of 40-fold. The specific latent IDPase activity in the most active fraction was 2-fold greater than in the Golgi fraction, but the recovery was only 0.5 %.

On a sucrose gradient of density $1.083\text{--}1.155\text{ g}\cdot\text{cm}^{-3}$. The localization of GT was investigated further by using a shallower linear sucrose gradient, which was also of lower density, thus allowing the separation of activities found at the top of the previous linear gradient. As Fig. 2 shows, protein and latent IDPase fractionated into three peaks, with densities of 1.122 , 1.135 and $1.146\text{ g}\cdot\text{cm}^{-3}$. GT activity was located primarily at a density of $1.106\text{ g}\cdot\text{cm}^{-3}$, with a small amount of activity between densities of 1.136 and $1.155\text{ g}\cdot\text{cm}^{-3}$. In the most active GT fraction an overall recovery of 3.2 %, per gradient, of GT was achieved, with a purification of 345-fold. The Golgi-membrane fraction applied to the linear gradient was taken from a discontinuous sucrose gradient consisting of 10 cm^3 of membrane ($1.179\text{ g}\cdot\text{cm}^{-3}$), 6 cm^3 of sucrose at density $1.153\text{ g}\cdot\text{cm}^{-3}$, 8 cm^3 of $1.083\text{ g}\cdot\text{cm}^{-3}$ and 6 cm^3 of $1.074\text{ g}\cdot\text{cm}^{-3}$.

DISCUSSION

The separation of membranes on a discontinuous sucrose-density gradient resulted in three membrane fractions, corresponding to ER, Golgi and PM, with each showing enriched activity of the respective marker enzymes (i.e. NADH:cytochrome *c* reductase, latent IDPase and GSII respectively). The level of cross-contamination between membrane fractions varied among preparations. The greater than 100 % recovery of

Table 2. Specific activity (S.A.) yield, and relative specific activity (relative S.A.) and recovery compared with Golgi membranes after continuous sucrose-density centrifugation of Golgi membranes. Golgi membranes were isolated and subfractionated as described in the text.

Fraction	Sucrose density (g·cm ⁻³)	Protein			GT			Latent IDPase				
		Concn. (mg·ml ⁻¹)	Yield (mg)	Recovery (%)	S.A. (Bq·h ⁻¹ · mg ⁻¹)	Yield (Bq·h ⁻¹)	Relative S.A.	Recovery (%)	S.A. (μmol· min ⁻¹ · mg ⁻¹)	Yield (μmol·min ⁻¹)	Relative S.A.	Recovery (%)
Golgi	—	0.68	5.7	100	416	2370	1.00	100	19.5	111	1.00	100
1	1.1148	0.64	0.32	5.6	859	275	2.06	12	16.7	5.3	0.85	0.48
2	1.1290	0.41	0.21	3.7	405	85	1.00	3.6	19.0	4.0	1.00	0.36
3	1.1314	0.39	0.20	3.5	260	52	0.64	2.2	16.5	3.3	0.87	0.30
4	1.1338	0.21	0.11	1.9	336	37	0.84	1.6	24.6	2.7	1.30	0.24
5	1.1398	0.16	0.08	1.4	288	23	0.68	1.0	41.3	3.3	2.13	0.30
6	1.1411	0.18	0.09	1.6	311	28	0.75	1.2	36.7	3.3	1.89	0.30
7	1.1435	0.013	0.07	1.2	329	23	0.85	1.0	38.6	2.7	2.10	0.24
8	1.1460	0.17	0.09	1.6	200	18	0.50	0.8	7.8	0.7	0.40	0.06
9	1.1496	0.14	0.07	1.2	99	6.9	0.24	0.3	28.6	2.0	1.46	0.18
10	1.1534	0.12	0.06	1.1	40	2.4	0.10	0.1	11.7	0.7	0.57	0.06
11	1.1583	0.12	0.06	1.1	162	9.7	0.39	0.4	11.7	0.7	0.57	0.06
12	1.1633	0.06	0.03	0.5	253	7.6	0.60	0.3	23.3	0.7	1.14	0.06
R ₃	—	0	0	—	0	0	—	—	0	0	—	—

both GT and GSII activity may have resulted from reduced activity in the homogenate caused by substrate competition, proteinase action or by the action of other soluble factors contained within the homogenate. GSII activity might also be affected by the amount of manipulation that the sample is subjected to, with activity increasing with increased manipulation, due to the involvement of this enzyme in wound responses (Brett, 1978; Delmer, 1987). As was found previously (Waldron & Brett, 1987) the GT activity was chiefly in the Golgi fraction. When GT activity was detected in the ER fraction it probably resulted from Golgi cross-contamination, since the ratio of total latent IDPase activity in the Golgi to that in ER was similar to that for total GT activity in Golgi and ER (0.17 and 0.26 for latent IDPase and GT respectively). The contamination of the ER fraction with Golgi membranes is supported by the localization of GT in a Golgi subfraction that has a density of 1.122 g·cm⁻³, which is the reported density of ER membranes (Bertho *et al.*, 1989). GT activity was also detected in the PM, but in this case the ratio of total latent IDPase in Golgi to PM was more than 10-fold greater than the ratio of GT in Golgi to PM (0.012 and 0.22 for latent IDPase and GT respectively). The presence of GT activity in the PM fraction was not found by Waldron & Brett (1987), but this may be due to differences in homogenization and centrifugation between the two studies.

Waldron & Brett (1987) found that latent IDPase activity appeared in two peaks with densities of 1.06 g·cm⁻³ and between 1.12 and 1.17 g·cm⁻³, the latter containing GT activity. Subfractionation of Golgi membranes on a shallow gradient of 1.115 to 1.165 g·cm⁻³ resulted in the separation of the GT-associated latent IDPase peak reported previously (Waldron & Brett, 1987) into two peaks of latent IDPase activity, with the peak of lower density also containing the GT activity. The NADH:cytochrome *c* reductase activity associated with the least dense peak of latent IDPase activity is due to smooth ER, which has a density of 1.11 to 1.12 g·cm⁻³ (Quail, 1979; Bertho *et al.*, 1989). The more dense NADH:cytochrome *c* reductase peak corresponds to the density of rough ER (Quail, 1979), and, although EDTA was present in the sucrose-gradient media, it is possible that this did not lead to the dissociation of all ribosomes from the ER (Sabatini *et al.*, 1966). The small peak of NADH:cytochrome *c* reductase activity associated with the more dense latent IDPase peak is unlikely to be due to ER contamination, but may be due to NADH:cytochrome *c* reductase activity in the Golgi membranes (Hino *et al.*, 1978a,b,c; Bergeron, 1979).

To study further the localization of GT, Golgi membranes were separated on a linear sucrose gradient of lower density (1.083–1.155 g·cm⁻³). Three Golgi subfractions were obtained, as determined by latent IDPase activity and protein content, and the lightest of these contained most of the GT activity. With respect to glycoprotein oligosaccharide processing, in animal Golgi three distinct Golgi compartments, termed *cis*-, *medial*- and *trans*-Golgi, have been identified (Dunphy & Rothman, 1985; Rothman & Orci, 1990). In plants, Golgi membranes have been separated into two subfractions (Ali *et al.*, 1986; Sturm *et al.*, 1987; Brummell *et al.*, 1990). In Golgi subfractions from bean (*Phaseolus vulgaris*) there was no evidence of differential localization of glycosyltransferases involved in the processing of *N*-linked oligosaccharides (Sturm *et al.*, 1987). Ali *et al.* (1986) found some evidence for the differential distribution of glycosyltransferases, although it is not clear whether these were involved in glycoprotein oligosaccharide processing or polysaccharide synthesis. However, the differential distribution of glycosyltransferases involved in the biosynthesis of the hemicellulose xyloglucan has recently been reported (Brummell *et al.*, 1990). It was proposed that the enzymes involved in the synthesis of the glucan backbone and the xylose side chains are located at the *cis*-

Fig. 2. Profiles of marker-enzyme separation after continuous-sucrose-density centrifugation of isolated Golgi membranes on a 1.083–1.155 g·cm⁻³ density gradient (see the text for details)

▲, Protein; ■, GT activity; ◆, latent IDPase activity; ●, sucrose concentration in gradient fractions.

end of the Golgi stack, whereas enzymes involved in the elaboration of the basic xyloglucan polymer are localized at the *trans*-end of the Golgi and in the *trans*-Golgi network (Brummell *et al.*, 1990). The localization of GT in a Golgi subfraction with a density similar to that of the ER suggests that this glycosyltransferase is located in *cis*-Golgi membranes, since the *cis*-side of the Golgi stack is closely related to the ER (Mollenhauer & Morré, 1980). As GT glucuronidates a β 1–4-xylose backbone, and since GT activity is influenced by a xylosyltransferase (Baydoun *et al.*, 1989a), it is probable that the xylosyltransferase is also located in the *cis*-Golgi, or to a lesser extent in the ER. If the synthesis of glucuronoxylan and xyloglucan are analogous, it is possible that the basic polysaccharide structure is synthesized in the *cis*-Golgi and elaborated, via methylations, acetylations etc. in the *medial*- or *trans*-Golgi. The synthesis of a basic core polymer, which is then modified, would be similar to the case of glycoprotein oligosaccharide processing in the Golgi (Tartakoff, 1983). Studies of the localization of the xylosyltransferase, methyltransferase and acetyltransferases involved in glucuronoxylan synthesis are needed to confirm the present view of the subcellular localization of hemicellulose biosynthesis.

The localization of GT at the *cis*-face of the Golgi stack and the formation and release of secretory vesicles, bound for the PM, from the *trans*-face (Mollenhauer & Morré, 1980) provides evidence of the transit of cell-wall hemicelluloses through the Golgi in a *cis*-to-*trans* direction. Membrane flow in animals has been found to occur via repeated rounds of budding and fusion of transport vesicles from one cisternae to another, such that the cisternae do not turn over in their entirety (Morré, 1987; Pfeffer & Rothman, 1987). Only a small amount of the GT activity in the PM could be accounted for by Golgi contamination, since the latent IDPase activity in the PM was lower than would be expected if all the GT activity was due to Golgi contamination. GT activity in the PM may be a consequence of bulk membrane flow (Karrenbauer *et al.*, 1990), since in rapidly growing cells there is a high rate of vesicle formation and re-formation as material rapidly passes through the Golgi stack (Mollenhauer & Morré, 1980). The high rate of vesicle formation and re-formation may result in the co-transport of small amounts of GT, which finally accumulate in the PM, since the PM of rapidly growing cells may be regarded as an end point of membrane flow.

In the *cis*-Golgi fraction, where the GT activity was maximal, a 345-fold purification of GT was achieved. The isolation of a membrane with a high specific GT activity represents an ideal starting point for the solubilization and subsequent purification of GT (van Renswoude & Kempf, 1984). However, the recovery of GT was only 6.4%, thus a more realistic starting point for

solubilization may be the initial Golgi fraction, where the total activity was slightly greater than in the homogenate. The instability of GT in particulate form (Waldron & Brett, 1983) was probably the cause of the loss of activity during the linear-sucrose-density-gradient centrifugation.

The results presented here provide further evidence for the existence of subcompartmentalization of the Golgi system of higher plants. There is also an indication that membrane flow through the Golgi stack and finally to the PM is slightly 'leaky', since, within the Golgi, GT activity is localized chiefly at the *cis*-end, yet there is also activity in the PM. The control of membrane flow within the Golgi and from the Golgi to the PM, in animal cells, is receiving much attention at present, "... yet remains one of the outstanding mysteries of cell biology." (Rothman & Orci, 1990). The possible existence of a 'leaky' system of membrane flow in plant cells may provide an opportunity to unravel some of the mechanisms involved in membrane flow within the Golgi stack.

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