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The synthesis and transport of some plant glycoproteins

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Maize root slime is secreted by the outer root-cap cells. It is a complex of at least three polysaccharides, two of which are acidic and contain a high proportion of fucose. The polysaccharides are assembled and secreted by the endoplasmic reticulum and dictyosomes of the cells. It has been found that the synthesis of fucose-containing oligosaccharides takes place by an assembly of at least nine sugar residues on a polyprenyl diphosphate acceptor and this occurs at the endoplasmic reticulum. In addition, a glycoprotein carrying a large molecular mass carbohydrate portion containing fucose is synthesized mainly within the Golgi apparatus, although synthesis may be initiated in the endoplasmic reticulum. This glycoprotein could be an intermediate in the synthesis of slime polysaccharide, and the carbohydrate moiety of the glycoprotein may be assembled by transglycosylations from the polyprenyl diphosphate oligosaccharides.

Introduction

The outer root-cap cells of many plants secrete a slime that serves to keep the root moist and to lubricate and ease the passage of the growing root in the soil. These cells occur at the first few millimetres of the root tip in the root cap and in addition to the secretion of slime they are themselves continuously sloughed off and autolysed so that the root is covered not only with the secreted material but the products of the autolysed cells (Barlow 1975; Rougier 1981).

We have studied the structure and the mechanism of synthesis and secretion of the slime produced by the maize root tip. This material can be obtained as a droplet at the tip of sterile roots grown so that the tissue is suspended in air, and it can be washed off the root or collected on filter paper, or it can be collected from a mass of rootlets produced on a callus grown as a tissue culture on solid medium. It is also secreted into the liquid medium of a suspension culture of maize cells or into a liquid culture of maize primary roots (figure 1) (Wright & Northcote 1974). The slime collected direct from the intact root contains approximately 30 % protein and the rest of the dry matter of the material is carbohydrate, mainly polysaccharide (Wright & Northcote 1975).

Analysis and general structure of the slime

The polysaccharides in the material consist of at least three polymers that can be separated by electrophoresis on glass fibre paper (Wright & Northcote 1974). These are a neutral polymer, a weakly acidic polymer and an acidic polymer. The sugar composition of these polymers varies with the preparation, but the general nature of the materials as shown by measurement of their radioactive composition by feeding the roots [^{14}C]glucose is given in table 1.

The neutral polymer is mainly made up of glucose residues and consists of a β 1–4 glucan, whereas the acidic polymers contain fucose, arabinose, galactose and galacturonic acid residues with minor amounts of xylose, glucose and mannose. It is the variation in the uronic acid

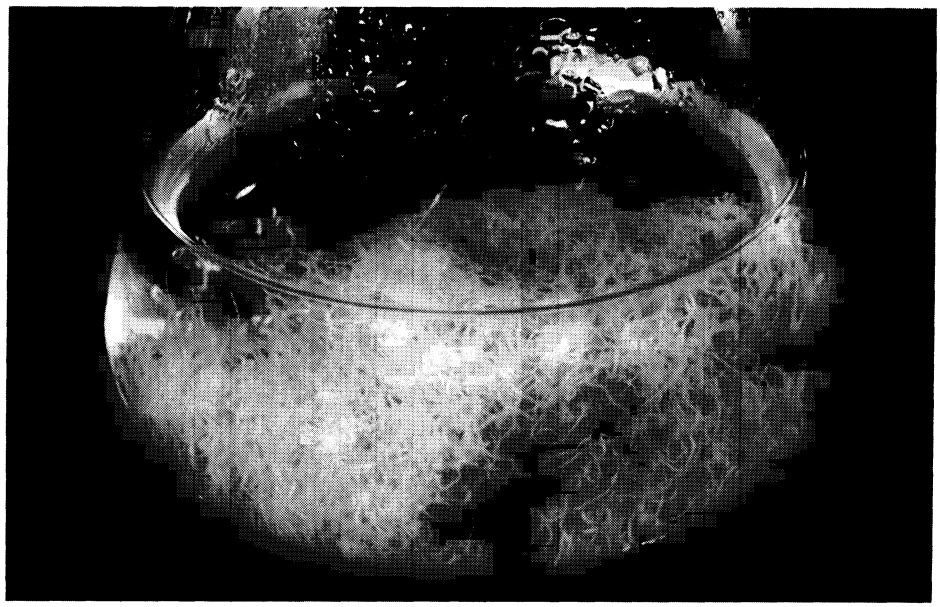

FIGURE 1. Maize roots grown in liquid culture. The roots originated on a solid callus derived from a shoot of a maize seedling. The callus was maintained on Hellers medium with coconut milk (20% by volume), sucrose (20 g l^{-1}) and 2,4-dichlorophenoxyacetic acid (6 mg l^{-1}) (Wright & Northcote 1974). The root culture was grown in half-strength Murashige & Skoog's medium (Murashige & Skoog 1962) with the addition of casamino acids(1 g l^{-1}), sucrose (25 g l^{-1}) and 3-naphthylacetic acid (2.5 mg l^{-1}).

TABLE 1. COMPONENTS OF MAIZE-ROOT SLIME

(The analyses given in this table relate to the relative proportions of radioactive sugars in the components. The tissue had been exposed to radioactive glucose for 2.5 h and during this length of time the slime became extensively radioactive and the cytoplasmic pools of slime precursors became saturated with radioactivity. It is therefore probable that, although the analysis does not indicate an accurate chemical composition, it does reflect the differences between the components. The radioactive composition of the unfractionated material is very similar to that of the chemical composition of the slime (Harris & Northcote 1970).)

	radioactivity (%)			
sugar	unfractionated material	neutral component	weakly acidic component	strongly acidic component
uronic acid	16	—	19	57
galactose	21	—	31	18
glucose	23	91	4	0
mannose	1	—	4	3
arabinose	11	—	12	16
xylose	8	—	6	3
fucose	20	—	23	3

content that causes the separation of the weakly acidic from the acidic fractions of the slime polysaccharides. The overall composition of the slime compared with the composition of the separated fractions is shown in table 1.

It is probable that the complex is made up of a central core of fairly rigid β 1–4 glucan chains that are held together and are kept extended by intramolecular and intermolecular hydrogen bonds, not dissimilar from the chains in cellulose microfibrils but shorter. This central core is surrounded by a hydrophilic coat of the acidic polymers (Wright & Northcote 1976; Grant *et al.*

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1969). The acidic polymers do not seem to have continuous chains of galacturonic acid units as in pectin but to consist of either mixed chains of glucose and uronic acid residues carrying side chains of the neutral sugars, or chains of glucose units with the acidic and neutral sugar residues carried as side chains (Wright & Northcote 1975; Wright *et al.* 1976).

The large amount of fucose in the weakly acidic polymer is unusual and allows the metabolism of the polymer, which is only produced by the differentiated cells of the root tip, to be uniquely followed and it is this polymer that will be subsequently discussed in this work. The presence of such large quantities of fucose makes it possible to study the location and synthesis of this polysaccharide, because fucose applied to the roots is taken up and is not metabolized to carbon dioxide and water but is all incorporated into the polymers without breakdown or conversion into other sugars (Wright & Northcote 1976; Kirby & Roberts 1971; Paull & Jones 1975).

Location of synthesis

Radioautographic studies on the cells of wheat roots incubated with radioactive glucose (Northcote & Pickett-Heaps 1966) or in addition, in maize roots, radioactive fucose, have indicated that the slime is produced by the outer cap cells (Kirby & Roberts 1971; Rougier 1981). The endomembrane system is involved and the radioactive slime can be shown to be secreted across the plasma membrane by vesicle fusion and an exopinocytotic mechanism. The vesicles are derived from the Golgi apparatus and the radioautograms indicate that a concentration of the slime and possibly its active synthesis occur in this organelle (Rougier 1976; Paull & Jones 1976).

The work has been extended by membrane fractionation of the cells and the analysis of radioactive material located in characterized membrane fractions after feeding the roots a radioactive precursor such as [U-^{14}C]glucose (Harris & Northcote 1971; Bowles & Northcote 1972). This type of study has provided evidence for the function of both the endoplasmic reticulum and the Golgi apparatus for the production and transport of the slime from the cytoplasm. In these experiments maize roots were incubated with radioactive glucose and the membranes were isolated on a discontinuous sucrose gradient after breakage of the cells. The cells were fractionated into wall material and fractions enriched in microsomes, dictyosomes, mitochondria, smooth membranes and soluble polymers. The membranes were characterized by observations with the electron microscope and by their enzyme content. The composition of the radioactive polymers that they carried was determined (Bowles & Northcote 1972; Bowles & Northcote 1976).

Over the period of the incubation with the radioactive glucose, the type of polymer that is being incorporated into the cell wall or the slime can be determined. This varies with the position of the cell in the root. The cells that are differentiated into outer root-cap cells produce slime containing fucose. Further up the root the cells become differentiated to form vascular tissue and the walls are secondary-thickened, and there is an increase in the amount of polymers containing xylose that are secreted into the wall. At the root-tip, just behind the cap cells, the meristematic tissue is actively dividing and pectin that contains arabinose is laid down in relatively large amounts into the wall. These differences in polymer formation by the various differentiated cells are reflected in the type of polymer found in the membrane system (Bowles & Northcote 1972). The synthetic systems for oligosaccharide formation found at the membrane change, and these are the control steps whereby one aspect of the differentiation is manifested

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(Northcote 1982*a*). The information that is gained from such studies clearly shows that polymers that contain fucose are present both at the endoplasmic reticulum and the Golgi apparatus. It shows that the membranes at which these polymers are located and almost certainly formed occurs only in those cells in the first 1–2 mm of the root tip at the root cap (Bowles & Northcote 1972).

TABLE 2. THE AMOUNTS OF ROOT-SLIME POLYSACCHARIDE COMPONENTS IN THE DICTYOSOME AND ROUGH ENDOPLASMIC RETICULUM FRACTIONS, AND THE RATE OF INCREASE IN ROOT SLIME OF MAIZE ROOTS

	amount of material/pmol		rate of increase into slime polysaccharide
sugar	rough endoplasmic reticulum	Golgi apparatus	pmol min^{-1}
galactose	5.7	0.3	0.87
glucose	6.0	0.3	0.91
mannose	1.6	0.09	0.25
arabinose	4.0	0.2	0.62
xylose	1.1	0.06	0.17
fucose	8.7	0.5	1.33
total	**27.1**	**1.5**	**4.15**

It is also apparent from the observations on the membranes, both *in situ* and by analysis of their contents, that the slime complex as a whole is synthesized and secreted probably as an organized unit with the core glucan surrounded by the acidic polymers. The secretion of skeletal scales in the alga *Chrysochromulina chiton* clearly illustrates that the membrane system of cells can secrete such polymers organized into a complex composite structure with cellulose-like microfibrils embedded in a matrix of polysaccharides and glycoprotein (Allen & Northcote 1975).

In the maize root it can be shown that, during an incubation period of the roots with radioactive glucose over a period of 30–40 min, the internal pools of polysaccharide-like polymers that are being formed and that are being continuously secreted by the cells, become saturated with radioactivity. It is thus possible to calculate the sizes of these pools and to estimate their rate of turnover to produce the amount of polysaccharide secreted (Bowles & Northcote 1974). Since the approximate composition of the slime is known and since this is the only polysaccharide that contains fucose, the rate of polysaccharide secretion can be subdivided into that for wall polysaccharide secretion and that for slime polysaccharide secretion. The rates of polysaccharide secretion and the estimated pool sizes are shown in tables 2 and 3. The values are suggested as an indication of the magnitude of the turnover because the membrane fractions from which the calculations were made were not pure but were enriched with the particular membranes. However, it can be seen that the rate of secretion of the slime by the comparatively few cells of the root cap is very high and that one aspect of their differentiation is the rapid turnover rate of polysaccharide synthesis and, connected with this, the rate of vesicle fusion at the membrane. The rate of turnover of the membranes in the root cap for slime production is approximately eight times as fast as that for the production of wall polysaccharides. It has been assumed in these calculations that the polymer material is secreted in the sequence from endoplasmic reticulum to Golgi apparatus to the extracellular space, and that there is no direct contribution from the endoplasmic reticulum to the outside of the cell.

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TABLE 3. THE AMOUNTS OF CELL-WALL POLYSACCHARIDE COMPONENTS IN THE DICTYOSOME AND ROUGH ENDOPLASMIC RETICULUM FRACTIONS, AND THE RATE OF INCREASE IN CELL-WALL POLYSACCHARIDES OF MAIZE ROOTS

	amount of material/pmol		rate of increase into cell-wall polysaccharide
sugar	rough endoplasmic reticulum	Golgi apparatus	pmol min^{-1}
galactose	38.7	1.8	1.1
glucose	19.2	0.56	0.8
mannose	2.8	0.01	0.0
arabinose	58.9	3.0	0.8
xylose	117.2	6.7	2.1
fucose	0	0	0
total	**236.8**	**12.1**	**4.8**

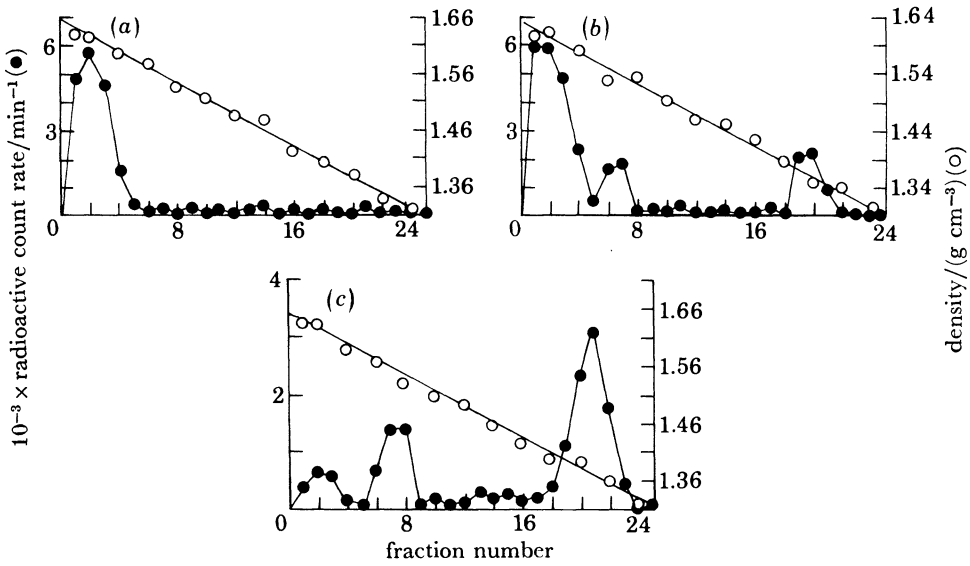

FIGURE 2. Distribution of radioactivity (●) in CsCl density gradients after centrifuging slime extracted from the intact roots (*a*) an aqueous extract of the broken cells of root tips (*b*) and an aqueous extract of sonicated membranes (*c*) prepared from the broken cells of root tips. The roots were incubated with L[1-^{3}H]fucose for 1 h at 20 °C in the dark before the slime and the aqueous extracts were prepared (Green & Northcote 1978).

INTERMEDIATES DURING THE SYNTHESIS OF THE SLIME POLYSACCHARIDE

Glycoproteins

Polymers can be extracted from the isolated membrane systems of the cells by breaking the membranes by homogenization and sonication to obtain water-soluble material. These solutions can be fractionated on a gradient of caesium chloride solution (Paull & Jones 1976) to give bands of polymers at different densities. In this way polysaccharide (density 1.6–2.0 g cm^{-3}) can be separated from protein density (1.3 g cm^{-3}) and glycoproteins (density between 1.6 and 1.3 g cm^{-3}) (Green & Northcote 1978) (figure 2).

The slime taken directly from the intact root tips contained only polysaccharide and protein; no glycoprotein could be detected. However, within the membrane system of the cytoplasm

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there were three polymers that incorporated radioactive fucose, two glycoproteins at densities of 1.55 and 1.37 g cm^{-3}, and a polysaccharide. There was in addition some free protein in the solutions. All the polymers could be made radioactive when the roots were incubated with radioactive glucose, whereas radioactive leucine was incorporated into those polymers that contained protein. The glycoproteins and polysaccharides were isolated from the gradient and

TABLE 4. RELATIVE AMOUNTS OF RADIOACTIVITY INCORPORATED FROM D-[U-^{14}C]GLUCOSE INTO THE POLYSACCHARIDE COMPONENTS OF DIFFERENT FRACTIONS OBTAINED FROM A CsCl DENSITY GRADIENT

	radioactivity of the isolated fractions (percentage of total)		
sugar	density 1.63 g cm^{-3}	density 1.55 g cm^{-3}	density 1.37 g cm^{-3}
uronic acid	15.6	15.0	12.5
galactose	21.4	17.5	20.2
glucose	23.2	27.5	22.8
mannose	1.0	0.8	0.7
arabinose	10.8	10.4	11.5
xylose	8.3	13.6	7.3
fucose	19.7	15.2	25.0
total radioactive count rate/min^{-1}	15300	8500	12200

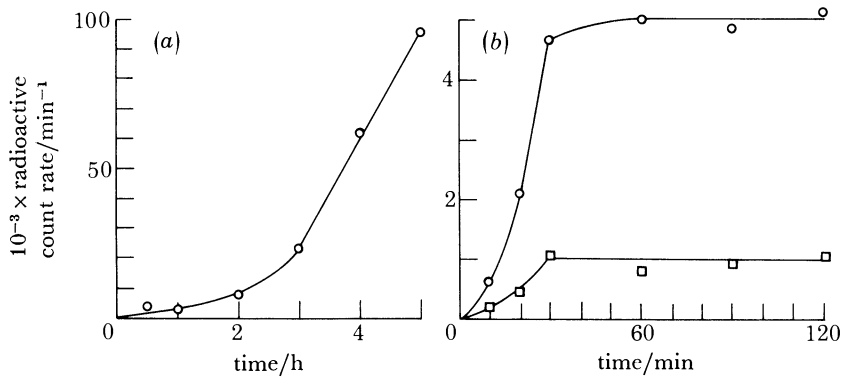

FIGURE 3. Time course of incorporation of L-[1-^{3}H]fucose into polysaccharide (*a*) (density 1.63 g cm^{-3}) and glycoprotein fractions (*b*) (densities 1.37 (○) and 1.55 (□) g cm^{-3}) of the maize root tip (Green & Northcote 1978).

their radioactive composition determined (table 4). It can be seen that the incorporation of radioactivity into the sugars of the glycoproteins resembles that of the slime polysaccharides. The polymers that were analysed were those that were isolated directly from the root tip. The glycoproteins were present in the material derived from the membrane fractions only.

The time courses for the incorporation of L-[1-^{3}H]fucose into polysaccharide and glycoprotein fractions are shown in figure 3. If the roots were incubated for 10 min in radioactive fucose and then left in a non-radioactive fucose solution for up to 150 min, the radioactivity was chased out of the glycoprotein and accumulated in the free polysaccharide (figure 4). The glycoproteins could therefore be intermediates and carriers of the carbohydrate that finally appeared as the free slime polysaccharides excreted from the root tip.

It was possible that the two glycoproteins were structural polymers of the membranes rather

than intermediates involved in the synthesis and transport of polysaccharide. However, the great size of the saccharide portion of the glycoproteins (larger than 10 kDa, a d.p. of at least 60 sugar residues) and the very low content of mannose together with the similarity of the sugar composition of the glycoprotein and polysaccharide, make this unlikely.

One of the glycoproteins (density 1.3 g cm^{-3}) we have investigated in more detail to determine the linkage between the carbohydrate and protein. The glycoprotein can be β-eliminated if it is

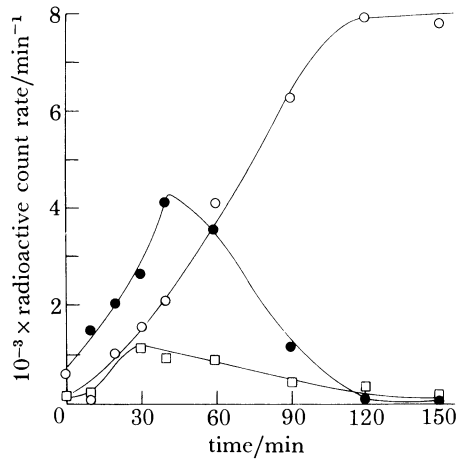

FIGURE 4. Incorporation of L-[1-^{3}H]fucose into polysaccharide (density 1.63 g cm^{-3} (○) and glycoprotein (density 1.37 (●) and 1.55 (□) g cm^{-3}) fractions of the maize root tip during a pulse–chase experiment (Green & Northcote 1978).

treated with 0.5 M sodium hydroxide at 20 °C for 24 h so that the protein and oligosaccharide are separated. The material after β-elimination could be reduced with sodium[^{3}H] borohydride and, after hydrolysis of the radioactively labelled product, radioactive α-aminobutyric acid was detected. Similarly when the glycoprotein was made radioactive by incubating roots in L-[U-^{14}C]threonine and the glycoprotein was β-eliminated and hydrolysed, radioactive α-aminobutyric acid was again detected in the hydrolysate. The sugar at the attachment point between the protein and oligosaccharide was detected by carrying out the β-elimination in the presence of [^{3}H]sodium borohydride and hydrolysis of the products. Radioactive xylitol was the only sugar alcohol that was found. Thus a xylose–threonine linkage was present in this glycoprotein; no evidence for an attachment to the hydroxy group of serine was found (Green & Northcote 1978). The other glycoprotein, at a density of 1.55 g cm^{-3}, was found not to be susceptible to β-elimination.

GLYCOLIPIDS PRESENT IN THE MEMBRANE SYSTEM

Two types of experiments were carried out. Either maize roots were incubated in L-[1-^{3}H]-fucose and the membranes were then isolated and analysed, or membranes were prepared from root tips and these were incubated with GDP-L-[U-^{14}C]fucose or UDP-D-[U-^{14}C]glucose (Green & Northcote 1979*a*). The radioactively labelled lipids that were synthesized *in vivo* and *in vitro* were extracted and separated into polar and neutral component. It was significant that no lipids containing fucose were found in parts of the root away from the root-tip in the experiments *in vivo*, or in membranes obtained from higher up the root in the experiments *in vitro*.

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The polar lipids had the characteristics of polyprenyl diphosphate fucose, and glucose derivatives and possibly some polyprenyl monophosphate glucose derivatives were present. The neutral lipids were fucose or glucose sterol glycosides; the sterol fucosides were again only found in the preparations from the root tip.

Membranes were fractionated into those sedimenting at 20000 *g* and those at 100000 *g*, and it was shown by enzyme characterization that the 100000 *g* pellet concentrated the endoplasmic reticulum and the 20000 *g* pellet concentrated the membranes of the Golgi apparatus. Most of the transglycosylases that gave rise to the polar lipids for both glucose and fucose were located in the 100000 *g* pellet. The sterol fucosides were mainly synthesized by the 20000 *g* pellet and the activity was probably associated with the Golgi apparatus, whereas the sterol glucosides were synthesized by both membrane fractions.

A more detailed study of the polar lipids derived by labelling *in vivo* and extraction of the dried membranes with mixtures of chloroform and methanol was made. The polar lipids were chromatographed on a column of DEAE-cellulose. The fractions from the column were hydrolysed by 0.1 M hydrochloric acid in tetrahydrofuran (80 % by volume) and the oligosaccharides analysed on a Biogel P-2 column to determine their size. A complete acid hydrolysis to determine their composition was also carried out. Some of the oligosaccharides were built up of at least nine sugar units. The composition of the unfractionated oligosaccharides resembled that of the slime polysaccharide and those of the carbohydrates of the glycoproteins. Some of the oligosaccharide contained at least four different sugars including galacturonic acid, glucose, arabinose and fucose; however, some did not contain fucose. The oligosaccharides were present as polyprenyl diphosphate derivatives (Dixon & Northcote 1983).

Transglycosylases

There are at least two types of fucosyl transglycosylase found on the membrane system of the cells of the root tip. One transfers fucose to polyprenyl phosphate and the other transfers fucose to polysaccharide or glycoprotein. These were investigated by experiments *in vivo* in which L-[1-^{3}H]fucose was incubated with intact roots and the membranes were isolated and characterized, and fucosyl derivatives extracted from them. They were also studied by isolation of the membranes from the cells and incubation of the membranes with GDP-L-[U-^{14}C]fucose. The membranes were carefully isolated and characterized from a discontinuous sucrose gradient into fractions at the interfaces of 8–18 % sucrose, 18–25 %, 25–29 %, 29–33 %, 33–39 %, 39–45 %, greater than 45 %, and also a supernatant fraction. Membranes were prepared in the presence of Mg^{2+}, which maintained the attachment of ribosomes to the endoplasmic reticulum, and also in the presence of EDTA, which removed most of the ribosome complex. In this way the membranes of the endoplasmic reticulum were displaced in the density gradient and could be unambiguously identified (Green & Northcote 1979*b*).

The GDP fucose: polyprenylphosphate transfucosylase occurred in the endoplasmic reticulum, and the fucosyl transferase that transfers fucose to polymer occurred mainly in the Golgi apparatus, but there was a significant amount of activity associated with the endoplasmic reticulum. Whether the transfucosylase that transfers fucose to the polymers uses a lipid intermediate as a substrate at either location is not known.

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Membrane fusion and secretion by the membrane system

To investigate the possible mechanisms of membrane fusion an *in vitro* system was devised in which the membranes from the root tip were isolated into four membrane fractions and soluble material on a discontinuous sucrose gradient. The membrane fractions enriched in endoplasmic reticulum (14–25 % sucrose interface), Golgi apparatus (25–34 %), plasma membrane (34–39 %) and mitochondria (39–45 %) were isolated and characterized by their enzymic properties

Table 5. Transfer of radioactivity between membrane fractions one of which was non-radioactive and the other made radioactive with D-[U-^{14}C]glucose

(Roots were incubated with D-[U-^{14}C]glucose *in vivo*. Membrane fractions were prepared from radioactive roots (*) and from non-radioactive roots (^{0}). These were mixed at 30 °C for 40 min in the presence of 1 mM Ca^{2+} and then refractionated by centrifugation. The distribution of radioactivity in the fractions was determined. Recovery was calculated by comparing total radioactive count rate with the initial count rate obtained by pelleting a radioactive fraction.)

	radioactivity (%) from refractionated membranes after mixing			
fraction	G.a.* with p.m.^{0}	G.a.* alone	G.a.^{0} with p.m.*	p.m.* alone
Golgi apparatus-rich (G.a.)	47.9	89.1	32.8	4.0
plasma membrane-rich (p.m.)	49.4	8.3	47.8	75.1
mitochondria-rich	0.9	0.9	10.7	12.0
remainder fraction	1.8	1.7	8.7	8.9
total count rate obtained from all the fractions/min^{-1}	14950	13650	7180	6350
recovery (%)	90.0	83.0	94.8	83.8

and ultrastructural appearance (Baydoun & Northcote 1980*a*). Maize root tips were incubated with radioactive glucose or choline and membranes were prepared from radioactive and non-radioactive roots. The membranes were then mixed, for example radioactive Golgi apparatus with the non-radioactive plasma membrane fraction at 30 °C for 40 min, and then refractionated. Radioactivity was transferred between the membrane fractions with all the radioactive markers used. Control experiments without mixing showed that the membranes could be recovered at the appropriate densities. The transfer of radioactivity between mixed membrane fractions has enabled a quantitative system to be developed to study membrane fusion *in vitro* between a Golgi apparatus-rich fraction and one enriched in plasma membranes (tables 5 and 6) (Baydoun & Northcote 1980*b*).

Membrane fusion was found to be dependent on time, temperature, Mn^{2+} and Ca^{2+}. Mn^{2+} was as effective as Ca^{2+} in promoting membrane fusion, but other divalent cations including Mg^{2+} had a moderate effect, or none.

Trypsin treatment of mixed membrane fractions before the addition of Ca^{2+} inhibited their ability to fuse. It also resulted in a selective and progressive elimination of a characteristic intense polypeptide band seen when the membrane proteins were displayed by electrophoresis on a sodium dodecyl sulphate (SDS) polyacrylamide gel. Thus, the fusion of the membranes that was enhanced by the presence of Ca^{2+} was probably dependent on the presence of this particular membrane protein. It was not removed by chymotrypsin or thermolysin. It appeared to be an integral membrane protein with an exposed portion of the peptide chain to the outside of the membrane, and it was this that was degraded by the trypsin.

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The results indicated that Ca^{2+} could probably form bridges between mixed membrane vesicles through negative charges on surface proteins or glycoproteins, or that there were present specific polypeptides that bind Ca^{2+} ions. The bridges would bring membranes into close contact, a prerequisite for membrane fusion. Trypsin treatment did not completely abolish membrane fusion in the presence of Ca^{2+}. This could be due to the presence of other proteins that were not removed by trypsin or that Ca^{2+} could bind to acidic phospholipids to bring membranes in contact.

TABLE 6. TRANSFER OF RADIOACTIVITY BETWEEN MEMBRANE FRACTIONS ONE OF WHICH WAS NON-RADIOACTIVE AND THE OTHER MADE RADIOACTIVE WITH [Me-^{14}C]CHOLINE CHLORIDE

(Roots were incubated with [Me-^{14}C]choline chloride *in vivo*. Membrane fractions were prepared from radioactive roots (*) and from non-radioactive roots (°). These were mixed at 30 °C for 40 min in the presence of 1 mM Ca^{2+} and then refractionated by centrifugation. The distribution of radioactivity in the fractions was determined.)

	radioactivity (%) from refractionated membranes after mixing			
fraction	G.a.* with p.m.°	G.a.* alone	G.a.° with p.m.*	p.m.* alone
Golgi apparatus-rich	48.1	83.0	32.8	6.7
plasma membrane-rich	46.3	10.6	53.3	83.1
remainder fraction	5.6	6.4	13.9	10.2
total count rate obtained from all the fractions/min^{-1}	18880	18700	8520	8150
recovery (%)	99.0	98.1	97.8	93.5

Sodium deoxycholate was used to solubilize the proteins of mixed membrane fractions. The extracted proteins were analysed by non-SDS polyacrylamide gel electrophoresis: at least four bands were formed that could be cut from the gel. One of these bands had the same mobility as the polypeptide removed by trypsin when it was re-run electrophoretically in a gel with SDS. The molecular mass of this band was 36 kDa. The non-SDS polyacrylamide gels were stained for various enzymic activities, and the position at which the trypsin-sensitive polypeptide band ran showed that there was a protein present that was a Ca^{2+} and Mg^{2+} activated ATPase (Baydoun & Northcote 1981).

SUMMARY OF THE MECHANISM OF SYNTHESIS OF SLIME AND ITS POSSIBLE CONTROL

The slime secreted from the roots of maize is a complex of at least three polysaccharides. It is assembled and secreted by the membrane system of the outer root cap cells. One of the polysaccharides is acidic, containing galacturonic acid and also large quantities of fucose. Glycoproteins and polyprenyl diphosphate oligosaccharides of similar sugar composition to this polysaccharide have been identified in the membrane system of the cells. In addition, radioactive pulse–chase experiments have indicated that the glycoproteins could be intermediates during the assembly and synthesis of the polysaccharides.

It is therefore possible that the polysaccharide is assembled by the transfer of a regular sequence of sugars in the form of an oligosaccharide of at least nine sugar residues synthesized on to a polyprenyl acceptor. This type of synthesis has recently been shown to occur during the biosynthesis of an acidic polysaccharide secreted by *Acetobacter xylinum* (Couso *et al.* 1982) and also during the formation of xanthan gum secreted by *Xanthomonas campestris* (Ielpi *et al.* 1981 *a*, *b*).

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In the maize root tip the synthesis of the lipid-oligosaccharide probably starts in the endoplasmic reticulum, and transfer onto glycoprotein occurs in the Golgi apparatus. However, the early stages of this polymerization process, such as the initial transfer of an oligosaccharide or sugar to the protein to give the nascent glycoprotein, probably occur at the endoplasmic reticulum.

Work that we have carried out with suspension cultures of sycamore cells show that these cells secrete polysaccharides into the growth medium. These polysaccharides are similar to those present in the matrix material of the wall and synthesized and secreted into the wall by exopinocytotic vesicles derived from the Golgi apparatus. With this system it can be shown that the application of Ca^{2+} immediately stimulates the secretion within 10 s from the addition of the divalent ion (Morris & Northcote 1977). Thus for secretion at the plasma membrane there is an immediate control, and a rate-limiting factor in secretion is the fusion of the vesicles at the cell surface. Such a control at the cell surface to increase or decrease the rate of secretion of the polysaccharides must be correlated with their rate of synthesis within the membrane systems. We have shown that the rate of synthesis of a number of polysaccharides produced by plant cells and secreted into the wall is controlled at the synthetase steps, and during differentiation of the cell these synthetases are induced or repressed at the various developmental stages. With the polymers secreted in the slime of maize root tips and maybe in the synthesis of other polysaccharides where lipid or protein acceptors could be involved and on which the polymers are in part assembled, control could be exerted at the several transglycosylates involved at each stage (Northcote 1982*b*).

References

Allen, D. M. & Northcote, D. H. 1975 The scales of *Chrysochromulina chiton*. *Protoplasma* **83**, 389–412.

Barlow, P. W. 1975 The root cap. In *The development and function of roots* (ed. J. G. Torrey & D. T. Clarkson), pp. 21–54. London: Academic Press.

Baydoun, E. A. H. & Northcote, D. H. 1980*a* Isolation and characterization of membranes from the cells of maize root-tips. *J. Cell Sci.* **45**, 147–167.

Baydoun, E. A. H. & Northcote, D. H. 1980*b* Measurement and characteristics of fusion of isolated membrane fractions from maize root-tips. *J. Cell Sci.* **45**, 169–186.

Baydoun, E. A. H. & Northcote, D. H. 1981 The extraction from maize (*Zea mays*) root cells of membrane-bound protein with Ca^{2+}-dependent ATPase activity and its possible role in membrane fusion *in vitro*. *Biochem. J.* **193**, 781–792.

Bowles, D. J. & Northcote, D. H. 1972 The sites of synthesis and transport of extracellular polysaccharides in the root tissue of maize. *Biochem. J.* **130**, 1133–1145.

Bowles, D. J. & Northcote, D. H. 1974 The amounts and rates of export of polysaccharides found within the membrane system of maize root cells. *Biochem. J.* **142**, 139–144.

Bowles, D. J. & Northcote, D. H. 1976 The size and distribution of polysaccharides during their synthesis within the membrane system of maize root cells. *Planta* **128**, 101–106.

Couso, R. O., Ielpi, L., Garcia, R. C. & Dankert, M. A. 1982 Biosynthesis of polysaccharides in *Acetobacter xylinum* sequential synthesis of a heptasaccharide diphosphate prenol. *Eur. J. Biochem.* **123**, 617–627.

Dixon, W. T. & Northcote, D. H. 1983 Glycolipids and glycoproteins during maize-root slime synthesis. In preparation.

Grant, G. T., McNab, C., Rees, D. A. & Skerrett, R. J. 1969 Seed mucilages as examples of polysaccharide denaturation. *Chem. Commun.*, pp. 805–806.

Green, J. R. & Northcote, D. H. 1978 The structure and function of glycoproteins synthesised during slime-polysaccharide production by membranes of the root-cap cells of maize (*Zea mays*). *Biochem. J.* **170**, 599–608.

Green, J. R. & Northcote, D. H. 1979*a* Polyprenyl phosphate sugars synthesised during slime-polysaccharide production by membranes of the root-cap cells of maize (*Zea mays*). *Biochem. J.* **178**, 661–671.

Green, J. R. & Northcote, D. H. 1979*b* Location of fucosyl transferases in the membrane system of maize root cells. *J. Cell Sci.* **40**, 235–244.

Harris, P. J. & Northcote, D. H. 1971 Polysaccharide formation in plant Golgi bodies. *Biochim. biophys. Acta* **237**, 56–64.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ielpi, L., Couso, R. & Dankert, M. 1981*a* Lipid-linked intermediates in the biosynthesis of xanthan gum. *FEBS Lett.* **130**, 253–256.

Ielpi, L., Couso, R. & Dankert, A. 1981*b* Xanthan gum biosynthesis. *Biochem. biophys. Res. Commun.* **102**, 1400–1408.

Kirby, K. G. & Roberts, R. M. 1971 The localized incorporation of ^{3}H-L-fucose into cell wall polysaccharides of the cap and epidermis of corn roots. Autoradiographic and biosynthetic studies. *Planta* **99**, 211–221.

Morris, M. R. & Northcote, D. H. 1977 Influence of cations at the plasma membrane in controlling polysaccharide secretion from sycamore suspension cells. *Biochem. J.* **166**, 603–618.

Murashige, T. & Skoog, F. 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Pl.* **15**, 473–496.

Northcote, D. H. 1982*a* Macromolecular aspects of cell wall differentiation. In *Encyclopedia of plant physiology* (new series), vol. 14A (ed. D. Boulter), pp. 638–655. Berlin: Springer-Verlag.

Northcote, D. H. 1982*b* Control of enzyme activity during plant cell development. In *Differentiation* in vitro. (*Br. Soc. Cell Biol. Symp.* vol. 4) (ed. M. M. Yeoman & D. E. S. Truman), pp. 49–64. Cambridge University Press.

Northcote, D. H. & Pickett-Heaps, J. D. 1966 A function of the Golgi apparatus in polysaccharide synthesis and transport in the root-cap cells. *Biochem. J.* **98**, 159–167.

Paull, R. E. & Jones, R. L. 1975 Studies on the secretion of maize root-cap slime. II. Localization of slime production. *Pl. Physiol.* **56**, 307–312.

Paull, R. E. & Jones, R. L. 1976 Studies on the secretion of maize root-cap slime. IV. Evidence for the involvement of dictyosomes. *Pl. Physiol.* **57**, 249–256.

Rougier, M. 1976 Sécrétion de polysaccharides dans l'apex radiculaire de mais: étude radioautographique par incorporation de fucose tritié. *J. Microsc., Paris* **26**, 161–166.

Rougier, M. 1981 Secretionary activity of the root-cap. In *Encyclopedia of plant physiology* (new series), vol. 13B (*Plant carbohydrates II*) (ed. W. Tanner & F. A. Loewus), pp. 542–574. Berlin: Springer-Verlag.

Wright, K. & Northcote, D. H. 1974 The relationship of root-cap slimes to pectins. *Biochem. J.* **139**, 525–534.

Wright, K. & Northcote, D. H. 1975 An acidic oligosaccharide from maize slime. *Phytochemistry* **14**, 1793–1798.

Wright, K. & Northcote, D. H. 1976 Identification of β1→4 glucan chains as part of a fraction of slime synthesized within the dictyosomes of maize root caps. *Protoplasma* **88**, 225–239.

Wright, K., Northcote, D. H. & Davey, R. H. 1976 Preparation of rat epididymal α-L-fucosidase free from other glycosidases: its action on root-cap slime from *Zea mays* L. *Carbohydr. Res.* **47**, 141–150.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 1. Maize roots grown in liquid culture. The roots originated on a solid callus derived from a shoot of a maize seedling. The callus was maintained on Hellers medium with coconut milk (20% by volume), sucrose (20 g l^{-1}) and 2,4-dichlorophenoxyacetic acid (6 mg l^{-1}) (Wright & Northcote 1974). The root culture was grown in half-strength Murashige & Skoog's medium (Murashige & Skoog 1962) with the addition of casamino acids(1 g l^{-1}), sucrose (25 g l^{-1}) and 3-naphthylacetic acid (2.5 mg l^{-1}).

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