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Cell-Context Signalling

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Cell-context signalling

ROGER I. PENNELL[1], QUENTIN C. B. CRONK[2]*, L. SCOTT FORSBERG[3], CHRISTINE STÖHR[4]‡, LARS SNOGERUP[4], PER KJELLBOM[4] AND PAUL F. McCABE[1]

[1] *Department of Biology, University College London, Gower Street, London WC1E 6BT, U.K.*
[2] *Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, U.K.*
[3] *Complex Carbohydrate Research Center, University of Georgia, 220 Riverbend Road Athens, Georgia 30602–4712, U.S.A.*
[4] *Department of Plant Biochemistry, Lund University, PO Box 117, S-221 00 Lund, Sweden*

SUMMARY

In plants, cells differentiate according to their position with relation to their cell neighbours. Monoclonal antibody (MAb) probes to polysaccharide epitopes, present at the surfaces of all plant cells, have defined a family of proteoglycan antigens which signify cellular position. These MAbs have been used to sort the single cells present in carrot somatic cell cultures on the basis of the presence or absence of specific polysaccharide epitopes. This sorting allows embryo initial cells to be cultured among different cell collectives (based on their polysaccharide epitope expression) and thus in altered contextual backgrounds. These experiments have shown that specific populations of embryo initial precursor cells induce and sustain the early development of the embryo initials, revealing that the populations of different cell collectives which are defined by different polysaccharide epitopes (cell-context) serves important regulatory function in early plant development. Somatic embryo initials deprived of the influence of the cell collective – defined by the presence of the polysaccharide epitope recognised by the MAb JIM8 – establish unorganised first divisions and develop as callus. However, in the presence of the JIM8-reactive cell collective, or medium conditioned by the collective, the initials develop into somatic embryos. This demonstrates that the cells defined by the JIM8 polysaccharide epitope are necessary to sustain the meristematic activity which drives the renewed development. Transfer of a cell-wall signal from the JIM8-reactive cells to cellular situations in carrot seedlings in which they would not normally occur (out-of-context signals) stimulates lateral root production, thus demonstrating that the inductive signal operative in suspension cultures can be reinterpreted by specific cells later in development and reinitiate meristematic activity. The communication between the precursor cells defined by JIM8 and embryo initials defines an early cell–cell interaction in developing carrot plants. Labelling of flower sections suggests that the same interaction exists between embryo apical and basal cells early in normal development.

1. INTRODUCTION

In plants, the stem cells which generate the soma occupy the apical meristems. Somatic cells cut off from the meristem flanks differentiate over specific periods of time so that different cell types with distinct functions appear in the elongating axis. The molecular events which fix the patterning of the differentiation are largely unknown, although clonal analysis of albino cells in shoot apical meristems has shown that inherited programmes play only minor roles in the process. Clonal and genetic analysis have shown that cell position influences cellular differentiation to a far greater extent (Poethig & Sussex 1985; Irish 1991), evidently through cell–cell interactions. However, the signals which communicate the cell–cell interaction are unknown. Plant hormones, although capable of influencing patterning (Liu *et al.* 1993), are not thought to be the signals which control cell fate decisions. Mosaic and genetic analysis has shown instead that cell–cell interactions communicate positional information between cell neighbours (Hake & Freeling 1986; Sinha & Hake 1990; Coen & Meyerowitz 1991; Szymkowiak & Sussex 1992; 1993; Weigel & Meyerowitz 1993; Carpenter & Coen 1995; Hantke *et al.* 1995).

Animal cells can make binary choices according to inherited programmes or molecular signals which are released by cell neighbours (Horvitz & Herskowitz 1992). Asymmetric divisions occur in, and are essential for, the correct differentiation of plant zygotes (Mayer *et al.* 1993; Berger *et al.* 1994) and one or two other kinds of cells later in development (Eady *et al.* 1995), but cellular interactions subsequently control differentiation. In *Arabidopsis*, for example, *Gnom* (Mayer *et al.* 1991) controls the asymmetric division of the zygote,

* Present address: Institute of Cell and Molecular Biology, University of Edinburgh, Wayfield Road, Edinburgh EH9 3JH, U.K.
‡ Present address: Technische Hochschule, Institüt für Botanik, Schnillspahnstrasse 3–5, D-64287, Darmstadt, Germany

but after this division cell–cell interactions seem to control subsequent cell fate decisions. *Gnom* may encode a protein necessary for cytoskeleton or cell wall function, as both can influence cell polarity (Mayer *et al.* 1993; Kropf *et al.* 1988). On the other hand, genes which affect cell patterning (Bowman *et al.* 1991; Coen & Meyerowitz 1991; Scheres *et al.* 1995), presumably participate in the regulatory communications between cell neighbours which determine cell fate. Several of these genes have been shown to encode transcription factors (Yanofsky *et al.* 1990; Coen & Meyerowitz 1991; Vollbrecht *et al.* 1991).

The signals which communicate cell–cell interactions within whole plants are unknown. The lipooligosaccharide Nod-factor signals released by host-specific *Rhizobium* bacteria that induce ectopic meristem development in legume roots (Fisher & Long 1992) are the only examples of defined signals involved with cell–cell interactions in which plant cells participate. In Nod factors, fine differences in structure such as the length and degree of unsaturation of the fatty acid and the presence of sulphations determine the host-specificity (Fisher & Long 1992), and so define a complex recognition system through which certain cells in host plants are able to establish species-specific interactions with particular strains of nitrogen-fixing bacteria. It is likely that Nod factors have evolved as molecular mimics of endogenous cell-specific signals involved with cellular differentiation in whole plants (Ganong 1901), thus suggesting that cell–cell interaction in plants is communicated through oligosaccharide or lipooligosaccharide signals. This in turn may explain the reported effects of cell wall 'oligosaccharins' on tobacco explants, which seem able to influence cellular redifferentiation when applied in out-of-context situations (Albersheim *et al.* 1983; Tran Thanh Van *et al.* 1985; Eberhard *et al.* 1989; Fry *et al.* 1993).

Oligosaccharide signals are difficult to study. Oligosaccharide signals involved with cellular differentiation are likely to be complex, non-obvious and non-abundant. MAb probes derived from immunizations with complex mixtures of plant antigens have identified cell surface polysaccharide epitopes which appear to participate in cell–cell interactions during cellular differentiation and plant development. Simple correlations between the appearances and disappearances of novel polysaccharide epitopes, with specific developmental events, such as the patterning of the protoxylem (Knox *et al.* 1989, 1991) and the late determination and differentiation of the sexual cells from the soma (Pennell & Roberts 1990; Pennell *et al.* 1991), have shown that the epitopes are regulated solely according to cell position relative to cell neighbours. The structures of the epitopes have not been resolved, although hapten inhibition (Pennell *et al.* 1989) and affinity interactions against a range of chemically defined *Acacia* gums (Q. C. B. Cronk and R. I. Pennell, unpublished results) have pointed to the presence of arabinosyl and rhamnosyl residues, probably in concert with glucuronosyl residues. The antigens themselves are plasma membrane surface hydroxyproline-rich glycoproteins and proteoglycans (Showalter & Varner 1989) termed arabinogalactan-proteins (AGPs) (Larkin 1977; Fincher *et al.* 1983; Pennell *et al.* 1989). Currently there is no direct evidence for a role for plasma membrane surface AGPs in cellular interaction.

The same AGP epitopes are exposed at the surfaces of single cells growing in embryogenic cell suspension cultures of carrot (Pennell *et al.* 1992) and other umbellifers, and plants from other families. Using these cells as simple models for disassembled whole plants, we have now used these epitopes for cell sorting experiments aimed at understanding the significance of developmental regulation in AGP polysaccharides, and have started to use the results from these experiments to extrapolate an AGP function in whole plants. Evidence from ectopic delivery experiments suggests that at least some of the cell culture-derived signals regulate meristematic activity in plant cells competent to respond to them.

2. SINGLE CELL INTERACTIONS IN CELL CULTURES

The somatic embryogenesis which can be stimulated to occur in liquid cultures from artificially dedifferentiated cells provides an alternative to genetics for the analysis of cellular differentiation. In this process, low concentrations of auxins stimulate certain cells in certain tissue explants to dedifferentiate, divide and determine embryo initial cells which spontaneously redifferentiate in to clusters of meristematic cells termed proembryogenic masses. Transfer of these clusters to auxin-free medium then induces a somatic embryogenesis from surface meristematic cells (Steward *et al.* 1958; de Vries *et al.* 1988*a*; Dudits *et al.* 1991). Because proembryogenic masses and preglobular somatic embryos express several common genes (Wilde *et al.* 1988; Kiyosue *et al.* 1991; Sterk *et al.* 1991) it is generally thought that proembryogenic masses are auxin-arrested preglobular-stage embryos, and that both structures are clusters of meristematic cells in equivalent states of competence.

The somatic embryogenesis from proembryogenic masses is an indirect process which occurs adventitiously from initial cells which are currently unidentified. As an alternative route in which the somatic embryo initials and their cellular precursors can be studied, Nomura & Komamine (1985) demonstrated that single cells can be grown directly in to embryos. The direct pathway to somatic embryogenesis requires a cytokinin and a low level of auxin to sustain the early divisions of the single cells in the first week (see figure 1), and subsequently cytokinin without auxin (Nomura & Komamine 1985).

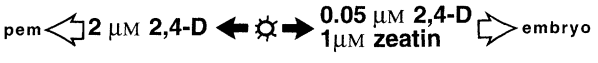

Figure 1. The smallest single cells in carrot embryogenic cultures contain the epitope recognized by the MAb JIM8. Cultured in 2 μM 2,4-D these cells develop into proembryogenic masses. However by culturing these cells in cytokinin and low levels of auxin for one week, followed by cytokinin alone, the cells can develop directly into somatic embryos.

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Table 1. *Glycosyl composition of the JIM8 antigen recovered from pectic cell wall polysaccharides*

residue	mole percent
arabinose	2.4
rhamnose	17.0
xylose	6.7
glucuronic acid	17.9
mannose	0.005
galactose	47.5
glucose	2.1

Using manual isolation and culture of the different kinds of single cells present in embryogenic carrot cell suspension cultures together with the culture in cytokinin-containing growth medium, Nomura & Komamine (1985) identified the 'type 1' cells as the unicellular precursors of the somatic embryos. These are the smallest single cells that occur in the suspension cultures, which are spherical in shape (Nomura & Komamine 1985). Like differentiating cells in whole plants, type 1 cells express regulated oligosaccharide epitopes at the cell wall surface which, in the absence of any kind of cell lineage control in the regeneration process, have been shown to signify a transitional cell state in the developmental pathway to carrot somatic embryogenesis (Pennell *et al.* 1992). Purification and glycosyl analysis of the antigen which contains these epitopes has revealed the presence of residues characteristic of AGP polysaccharides (see table 1). Labelling of the type 1 cells followed by immunofluorescence and cell tracking has demonstrated that asymmetric divisions, probably equivalent to those shown to occur by thymidine incorporation (Nomura & Komamine 1986), take place in the type 1 cells and give rise to somatic embryo initials. During this asymmetric division, the AGP epitopes become displaced from the embryo initials, so that the type 1 cells (JIM8-positive), and the initials (JIM8-negative), can be distinguished and sorted immunochemically. These results place the type 1 cells at the beginning of the redifferentiation pathway, and for the first time in plant biology make the first cells in developing plants biochemically separable and manipulable.

Recent experiments in which the single cells present in embryogenic cell suspension cultures have been sorted in this way (i.e. JIM8-positive/negative) have confirmed that isolated type 1 cells determine somatic embryo initials (Nomura & Komamine 1985). However, negative-selected cell collectives obtained from cell sorting with MAb JIM8, which contain the somatic embryo initials (Pennell *et al.* 1992), are unable to sustain the further development of the embryos (P. F. McCabe and R. I. Pennell, unpublished results). It was found though that complementation of these initials by culture in conditioned growth medium (B5–0.2) obtained from the positive-selected cell collective (B5–0.2$^{\mathrm{JIM8+}}$), from which they had been sorted (see figure 2*a*) rescued the embryogenic development of the embryo initials. This formally demonstrates that the single carrot cells defined by the JIM8-polysaccharide epitope (see figure 2*b*), and nothing

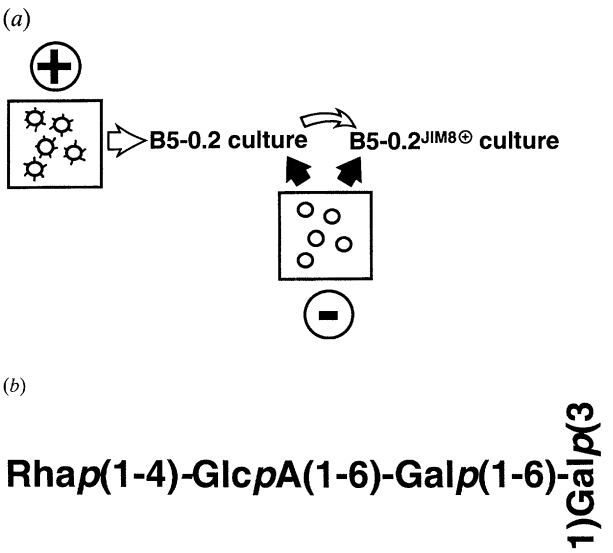

Figure 2. (*a*) Design of cell culture experiments, the cells labelled ⊕ are positive-selected, the cells labelled ⊖ are negative-selelcted. Negative-selected cells were cultured in B5–0.2 culture medium or B5–0.2 medium which had been conditioned by culturing the positive cells for one week. (*b*) Approximate model of the JIM8-reactive polysaccharide epitope used for the cell sorting.

else, control the development of the initials. Thus the asymmetric divisions which generate the somatic embryo initial cells set up inductive cell–cell interactions (Greenwald & Rubin 1992) which drive the first events in the regeneration of somatic plant cells. Asymmetric divisions in zygotes also partition the JIM8 polysaccharide epitope (Pennell *et al.* 1991), suggesting that equivalent cell–cell interaction between the apical and basal cells drive the first events in normal plant development as well. This might explain the embryogenesis failure in *Arabidopsis gnom* mutants (Mayer *et al.* 1991, 1993).

Cell wall composition has been shown before to influence somatic cell regeneration (David *et al.* 1994). Notably the cell wall is essential for fixation of the embryonic axis (Kropf *et al.* 1988) and cell fate thereafter (Berger *et al.* 1994) in *Fucus*, suggesting the cell–cell interaction described here for carrot is a fundamental induction at the beginning of plant development at all other evolutionary levels as well.

3. SIGNAL SECRETION IN CELL CULTURES

Rescue of sorted cell collectives by cell-conditioned growth medium demonstrates that cell-interactive signals are present in conditioned liquid growth medium. Previously, protein and glycoprotein signals involved with the late control of somatic embryo development have been described (de Vries *et al.* 1988*b*; Gavish *et al.* 1992). For example, a single isoperoxidase can rescue the effects of tunicamycin in carrot (de Vries *et al.* 1988*b*; Cordewener *et al.* 1991), and a chitinase can rescue the carrot variant culture *ts11* at the late globular-stage of development (de Jong *et al.* 1992). Extracellular AGPs (Knox *et al.* 1989; Pennell *et al.* 1989) recovered from embryogenic carrot-cell suspension cultures and AGPs washed out of

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Table 2. *Glycosyl composition of the JIM8 antigen which diffused from the cell wall into water*

residue	mole persent
arabinose	0.0
rhamnose	7.3
xylose	6.5
glucuronic acid	19.9
mannose	2.6
galactose	30.2
glucose	54.5

embryo sacs have also been shown to stimulate development in non-embryogenic cultures (Kreuger & van Holst 1993).

Using JIM8 to track a cell wall antigen through a classical purification, we have been able to produce and analyse a molecule which sorts with the activity that rescues the negative-selected cell population (see table 2). Unlike the cellular target for JIM8 (see table 1), this antigen washes from the cell wall within seconds of transfer to water or fresh growth medium, demonstrating that it is a cell-derived component of the cell-conditioned growth medium. Chemical analysis of this antigen revealed carbohydrate and lipid, the glycan residues being components of a rhamnosylated but non-arabinosylated AGP polysaccharide (see table 2). Previously, AGPs applied to ectopic sites in liverwort shoots have caused altered phyllotaxy (Basile 1990); extrapolating to carrots, this cell wall antigen has been bioassayed by delivery to ectopic sites in germinating carrot and *Arabidopsis* seeds, in which JIM8 antigen occurs only in the developing protoxylem. At picogram concentrations, the JIM8 cell wall antigen stimulated the production of lateral root meristems able to sustain a partial development. This suggests that the cell wall AGP signal which controls the first events in carrot somatic embryo development exerts a similar function in the regulation of meristematic activity, and may argue that oligosaccharide signals regulate cellular differentiation in plants (Fry *et al.* 1993).

4. PLASMA MEMBRANE AGP COMPOSITION

Biochemical analysis of the plasma membrane AGPs in whole sugar beet plants has revealed a family of glycoproteins (see figure 3) which are differentially glycosylated during cellular differentiation (Pennell *et al.* 1989, 1991). However, these seem not to be separate proteoglycan species but mixtures of 2 monomers and up to 3 dimers that are generated by oxidative cross-linking (presumably aa, ab and bb dimers) where in sugar beet a is an 84 kDa proteoglycan and b is a 68 kDa proteoglycan. This raises the interesting possibility that H_2O_2-driven dimerizations involving a basal family of only 2 AGP proteoglycans somehow regulates AGP function. Also, glycosyl analysis has revealed the presence of *N*-acetyl glucosamine residues (Komalavilas *et al.* 1991). This is interesting because *N*-acetyl glucosamine is the unusual amino sugar from

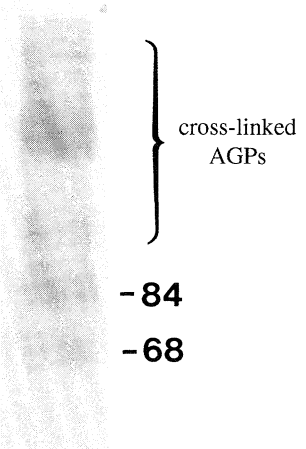

Figure 3. Western blot of sugar beet leaf AGPs. M_r is molecular mass of the proteoglycans determined by SDS-PAGE.

which *Rhizobium* Nod factors are synthesised (Lerouge *et al.* 1990; Fisher & Long 1992) and constitutive expression of a *Rhizobium* chitooligosaccharide deacetylase in tobacco also alters axis and leaf development (John *et al.* 1993; Schmidt *et al.* 1993), suggesting that Nod-factor-like signals participate in the control of cellular differentiation in whole plants. The *N*-acetyl glucosamine residues in the plasma membrane AGPs may therefore mean that the polysaccharide side-chains are developmentally regulated substrates for cell-wall enzymes (Fry *et al.* 1993) and release signalling oligosaccharides which function like Nod factors when glycosylated with enzyme targets. This could explain why chitinases and Nod factors are able to rescue carrot *ts11* embryo development mutants (de Jong *et al.* 1992, 1993).

5. CONTEXT CONTROLS IN CELLULAR DIFFERENTIATION

In tissue culture the involvement of single cells, defined only by the presence of oligosaccharide AGP epitopes, in a cell–cell interaction suggests that the cells in whole plants which express the same epitopes participate in cellular interactions with one-another as well. This has been proposed based on correlative evidence which links the appearance and disappearance of plasma membrane AGP epitopes with specific developmental events in pea, carrot and oilseed rape (Knox *et al.* 1989, 1993; Pennell & Roberts 1990; Pennell *et al.* 1991). The essential findings in this respect are: (i) that all plant cells express multiple epitopes which are independently regulated (Pennell *et al.* 1990; 1991) and probably rapidly turned over (Herman & Lamb 1992); and (ii) that AGP epitopes occur on cell collectives that are related to one another positionally (Pennell *et al.* 1990; Pennell *et al.* 1991; Knox *et al.* 1991) and that combinations of AGP epitopes occur on cell sub-collectives that are related to one another positionally and nothing else (see figure 4).

The inference from these studies is that plant cells differentiate according to the AGP polysaccharide profiles that characterize the cells and define cell-

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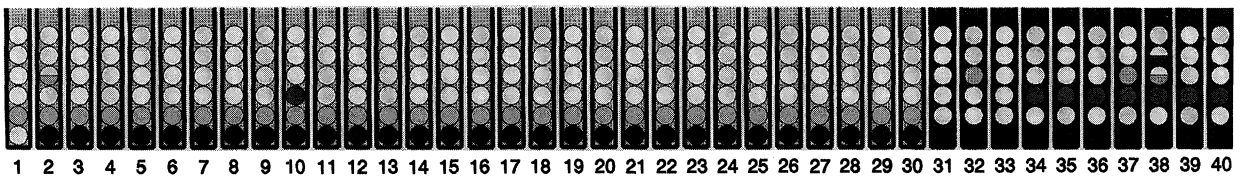

Figure 4. Schematic representation of plasma membrane AGP epitope distributions in *Arabidopsis* adults. The cell types are numbered 1–40; the somatic cells are 1–30 and the reproductive cells are 31–40. The JIM14-reactive epitope is first row up and the JIM8-reactive epitope is third row up, for example, and the cell type 10, for example, is protoxylem.

context accordingly. Presumably, differentiation is controlled by sequential cell-context changes that are signalled in this way. Cell culture experiments are unlikely to be much use for cell–cell interaction studies in whole plants because the removal of cells from tissues and organs leads to loss of context interactions and cellular dedifferentiation. Genetic analysis supplemented with cellular and biochemical studies seems to be a more appropriate approach, and for this the primary root of *Arabidopsis* is a better model system (Benfey *et al*. 1993; Dolan *et al*. 1993; Scheres *et al*. 1994, 1995).

6. CONCLUSIONS

The polysaccharide side-chains in AGPs at the surfaces of plant cells contain complex sets of epitopes that have evolved in register with plant form. Combinations of independently regulated epitopes specify cell type and probably identify each of the transitional cell states through which they pass during cellular differentiation. Sorting according to epitope profiles has demonstrated that plant cells defined in this way participate in cell–cell interactions; in whole plants, the patterns of appearance and disappearance of the epitopes suggests that the interactions involve cell neighbour collectives. This predicts that regulated AGP polysaccharide epitopes signal the patterning information which drives cellular differentiation in plants according to cell position. Although the mechanism for the cell–cell interaction is not yet known, the effects of Nod factors on specific cells in legume roots suggests that AGPs may be sources of cell–cell signals.

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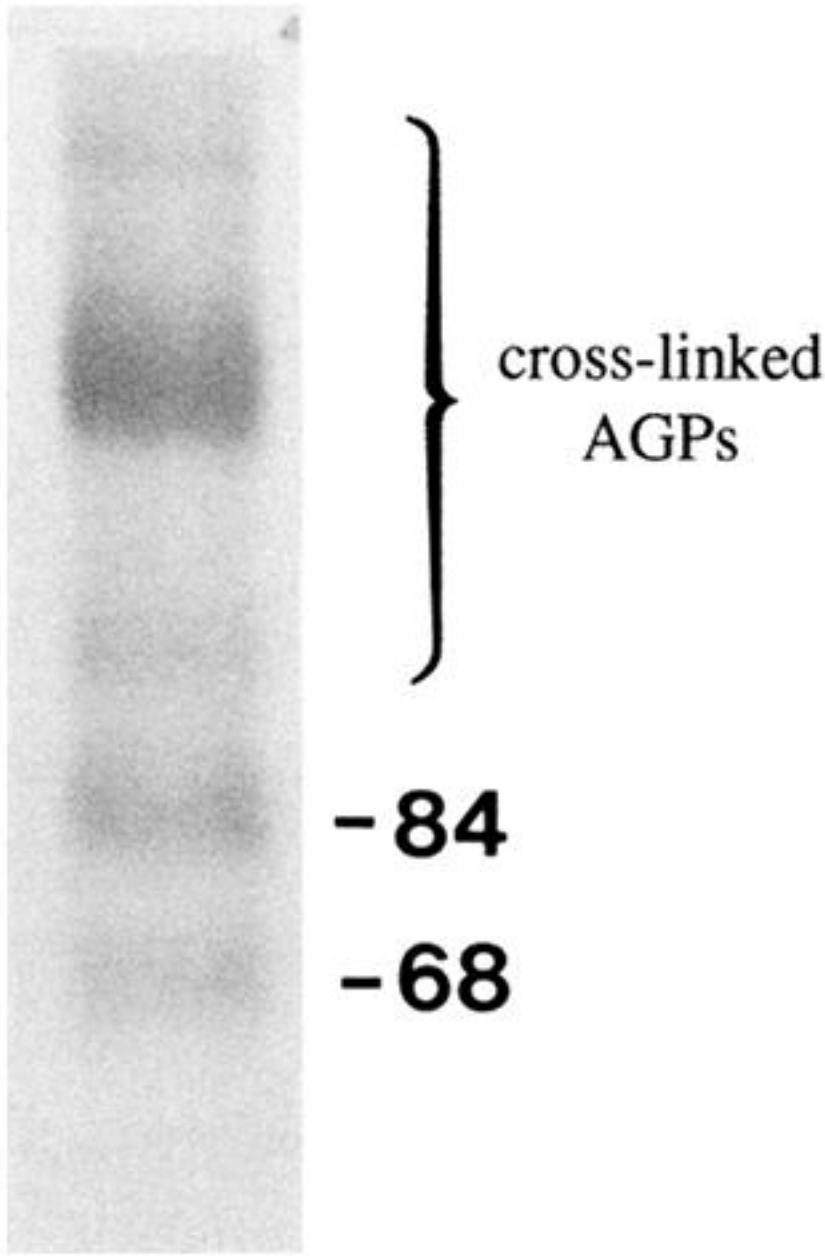

gure 3. Western blot of sugar beet leaf AGPs. M_r is olecular mass of the proteoglycans determined by SDS-AGE.

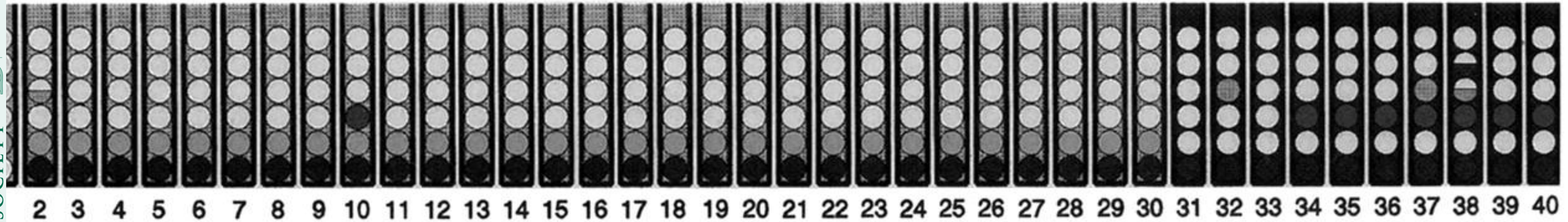

Figure 4. Schematic representation of plasma membrane AGP epitope distributions in *Arabidopsis* adults. The cell types are numbered 1–40; the somatic cells are 1–30 and the reproductive cells are 31–40. The JIM14-reactive epitope is first row up and the JIM8-reactive epitope is third row up, for example, and the cell type 10, for example, is protoxylem.