Accumulation Pattern of IgG Antibodies and F\textsubscript{ab} Fragments in Transgenic *Arabidopsis thaliana* Plants

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For the further optimization of antibody expression in plants, it is essential to determine the final accumulation sites of plant-made antibodies. Previously, we have shown that, upon secretion, IgG antibodies and F\textsubscript{ab} fragments can be detected in the intercellular spaces of leaf mesophyll cells of transgenic *Arabidopsis thaliana* plants. However, immunofluorescence microscopy showed that this is probably not their final accumulation site. In leaves, IgG and F\textsubscript{ab} fragments accumulate also at the interior side of the epidermal cell layers and in xylem vessels. These accumulation sites correspond with the leaf regions where water of the transpiration stream is entering a space impermeable to the proteins or where water is evaporating. In roots, plant-made F\textsubscript{ab} fragments accumulate in intercellular spaces of cortex cells, in the cytoplasm of pericycle and, to a lesser extent, endodermis cells, and in cells of the vascular cylinder. In other words, antibody accumulation occurs at the sites where water passes on its radial pathway towards and within the vascular bundle. Taken together, our results suggest that, upon secretion of plant-made antibodies or F\textsubscript{ab} fragments, a large proportion of these proteins are transported in the apoplastic of *A. thaliana*, possibly by the water flow in the transpiration stream.

Key words: Apoplastic water movement — *Arabidopsis thaliana* — *Nicotiana tabacum* — Recombinant antibodies — Secreted proteins — Transgenic plants.

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In recent years, plants have been engineered to synthesize full-size antibodies as well as antibody fragments such as F\textsubscript{ab} and single-chain variable fragments (scFv) (for a recent overview, see Smith 1996). Antibodies can be produced in plants and subsequently used for therapy (Ma et al. 1994, 1995), diagnosis, or immunopurification. Alternatively, plant-made antibodies can be envisaged as tools for the immunosuppression of an antigen present in planta. This approach has been used to suppress the activity of plant-specific antigens (Owen et al. 1992, Artsaenko et al. 1995) and pathogen-specific antigens (Tavladoraki et al. 1993, Voss et al. 1995).

The first report on the expression of full-size antibodies in plants (Hiatt et al. 1989) showed that for efficient assembly of the individual polypeptide chains a signal peptide is required. In general, upon endoplasmic reticulum (ER) targeting of the individual chains, plant-made antibodies become secreted (Hein et al. 1991, van Engelen et al. 1994, Voss et al. 1995, De Wilde et al. 1996). Recently, we demonstrated the exact subcellular localization of a full-size IgG and a derived F\textsubscript{ab} fragment (De Wilde et al. 1996). Using electron microscopy, we showed that IgG and F\textsubscript{ab} fragment are present within intercellular spaces of leaf mesophyll cells of transgenic *A. thaliana* plants. Analysis of leaf intercellular fluid confirmed that the major proportion of the microscopically detected proteins were integral and functional antibodies or F\textsubscript{ab} fragments. Therefore, these large, complex proteins must have crossed not only the plasma membrane but also the plant cell wall, a phenomenon that had not been expected (De Wilde et al. 1996).

For the study and optimization of antibody expression in plants, it is important to determine whether the local leaf intercellular spaces are the final accumulation sites of plant-made antibodies or antibody fragments as well as to determine the overall accumulation pattern in antibody (fragment)-producing plants. In particular, this is meaningful to ascertain the validity of approaches based on the use of plant-made antibodies, aiming at the inhibition of antigens present in the apoplastic compartment.

Here, we report the results of an immunofluorescence microscopic study. This technique, which is several orders of magnitude more sensitive than electron microscopy, allowed us not only to confirm the previously obtained results but also provided new data on the accumulation of IgG and F\textsubscript{ab} fragment in transgenic *A. thaliana* leaves. Moreover, we could localize IgG and F\textsubscript{ab} fragment in other tissues as well as the F\textsubscript{ab} fragment in leaves of transgenic *Nicotiana tabacum* plants. In addition, clear differences in the accumulation pattern between IgG and F\textsubscript{ab} fragment in particular cell types and cell type-specific accumulation phe...
nomens were revealed. These results are discussed in regard of our knowledge on the movement of water and solutes in the apoplastic compartment.

Materials and Methods

Plant material—Transgenic Arabidopsis thaliana Heynh. C24 and Nicotiana tabacum L. Petit Havana SR1 lines expressing the MAK33 IgG (a murine antibody directed against the human enzyme creatine kinase) or expressing its derived Fab fragment were obtained by double transformation with constructs encoding either the \(\gamma\) and \(\kappa\) chain or the \(\kappa\) and \(\kappa\) chain (De Neve et al. 1993). Each individual polypeptide chain was targeted to the ER using the signal sequence of the 2S2 gene of the 2S seed storage proteins of Arabidopsis thaliana (Krebers et al. 1988). De Neve et al. (1993) reported the accumulation of complete antibody and Fab fragment in transgenic plants and described the properties of the assembled entities. Here, we analyzed the progeny of the A. thaliana transformant RUG-kd 11/1 (seed stock RUG-kd 11/1-2, hemizygous for the \(\gamma\) chain-encoding T-DNA and homozygous for the \(\gamma\) chain-encoding T-DNA), which expressed the full-size antibody, and the progeny plants of the A. thaliana transformant RUG-kd 12 (seed stock RUG-kd 12/8-3-15, homozygous for the \(\kappa\) chain-encoding T-DNA and homozygous for the Fab fragment-encoding T-DNA), which expressed the Fab fragment. For N. tabacum, we analyzed the primary transformant 9V-kd 8A, which expressed the Fab fragment. The genetic and molecular characterization of the T-DNA integration patterns of the transformed lines has been described by De Neve et al. (1997). As a control, non-transformed A. thaliana C24 and N. tabacum Petit Havana SR1 were used.

Tissue processing for immunofluorescence microscopy—For A. thaliana, different organs were analyzed. Leaf tissue segments of approximately 6 mm\(^2\), intact young leaves, or root segments of approximately 2 cm length were isolated and fixed. Twenty-eight to 68-day-old seedlings, either grown axenically in the greenhouse, were also used. Axenically grown A. thaliana seedlings were fixed as a whole (3, 6, or 10 d after sowing). For N. tabacum, leaf tissue segments of approximately 6 mm\(^2\) of axenically grown plants were used. All samples were fixed overnight at 4°C, under rotation in a solution of 3% paraformaldehyde in H\(_2\)O (pH 7.2), 50% EtOH, and 5% CH\(_3\)COOH. Samples were dehydrated through graded ethanol series (100%, 90%, 70%, 50%) in water (100% EtOH overnight at 4°C, under rotation, in a solution of 3% paraformaldehyde in H\(_2\)O (pH 7.2), 50% EtOH, and 5% CH\(_3\)COOH). Samples were dehydrated through graded ethanol series (60%, 75%, 85%, and 95% EtOH in H\(_2\)O under rotation at 4°C, each step taking 2 h; 100% EtOH overnight at 4°C). Samples were gradually infiltrated using 10%, 20%, 30%, 40% (for 2 h each), 50% (overnight), 65%, 75%, 85%, 100% (for 2 h each), 100% (overnight), and 100% (for 3 h) polyethylene glycol 1550 (PEG) (Serva, Heidelberg, Germany) in EtOH, in a water bath at 36°C, under continuous shaking. Each PEG dilution was made fresh just before use. Samples were placed in plastic embedding moulds (Polysciences, Warrington, PA, U.S.A.) using fresh pure PEG that was allowed to solidify by putting the moulds on a mixture of ice and water. Solidified PEG blocks were sectioned at room temperature into 7-μm sections using a 2050 microtome (Reichert-Jung, Nußloch, Germany). Sections were collected on moist, antistatic slides.

Immunofluorescence labeling—Slides were soaked in water for 30 s to remove PEG and air-dried. Sections were rehydrated through graded ethanol series (100%, 90%, 70%, 50%) to water and phosphate buffered saline (PBS) solution (0.8% NaCl, 0.02% KCl, 0.14% Na\(_2\)HPO\(_4\)+2H\(_2\)O, and 0.02% KH\(_2\)PO\(_4\) in water) for 60 s each. After this step, sections were not allowed to dry. The slides were incubated overnight at 4°C with primary antibody (rabbit anti-mouse; Pierce, Rockford, IL, U.S.A.) diluted 1/100 or 1/500 in 0.5% bovine serum albumin (BSA) in PBS, in a moist environment to prevent drying of the sections. The primary antibody was drained off and slides were washed in PBS (10 s), 0.5% BSA in PBS (15 min), 0.01% Tween-20 in PBS (15 min), and PBS (15 min). The slides were subsequently incubated for 1 h at room temperature with fluorescein isothiocyanate (FITC)-coupled secondary antibody (anti-rabbit IgG (whole molecule) FITC conjugate, Fab fragment, of goat antibody) (Sigma, St. Louis, MO, U.S.A.) diluted 1/160 in 0.5% BSA in PBS, again in a moist environment. The secondary antibody was drained off and slides were washed in PBS (10 s), 0.5% BSA in PBS (15 min), 0.01% Tween-20 in PBS (15 min), and PBS (15 min). Sections were mounted with a drop of Vectashield (Vector Laboratories, Burlingame, CA, U.S.A.) and coverslips were sealed with nail varnish. Sections were examined with an Axioskop fluorescence microscope (Zeiss, Jena, Germany). When stored dry at 4°C, slides could be examined repeatedly for at least six months without major loss of signal. As a control, immunolabeling of sections of non-transformed A. thaliana C24 or N. tabacum SR1 grown under the same conditions was performed. Other controls included immunolabeling of sections of transgenic A. thaliana and N. tabacum plants with one of the detecting reagents being omitted.

Results

Immunofluorescence microscopy—For A. thaliana and N. tabacum plants expressing either the MAK33 IgG or its derived Fab fragment were processed and labeled for fluorescence microscopic immunolocalization as described in Materials and Methods. Fig. 1 and 2 show sections of leaves, roots, petioles, and stem tissue as well as of isolated cells prepared from these plants. The presence of antibodies or Fab fragments is visualized by a green fluorescence signal. The orange-to-red signal is caused by auto-fluorescence of the plant tissue. The results are described below and are summarized in Table 1. No signal was detected on control sections of nontransformed A. thaliana (Fig. 1c, h, k, Fig. 2c, d) or N. tabacum (Fig. 2h) plants or on control sections of transgenic A. thaliana or N. tabacum.
Fig. 1 Fluorescence micrographs of MAK33 IgG and F\textit{ab}
immunolocalization in \textit{A. thaliana}. (a) and (b) FITC-labeled sections through leaves of MAK33 F\textit{ab}
expressing progeny plants of seed stock RUG-kd 12/8-5-15; (c) FITC-labeled section through a leaf of an untransformed plant; (d) FITC-labeled section through a leaf of a MAK33 IgG-expressing progeny plant of seed stock RUG-kg 11/1-2; (e) and (i) FITC-labeled sections through roots of MAK33 F\textit{ab}
expressing progeny plants of seed stock RUG-kd 12/8-5-15; (f), (g), and (j) FITC-labeled sections through roots of MAK33 IgG-expressing progeny plants of seed stock RUG-kg 11/1-2; (h) and (k) corresponding control sections through roots of an untransformed plant. CC, cortex cell; EC, endodermis cell; EPC, epidermis cell; ICS, intercellular space; MC, mesophyll cell; PC, pericycle cell; S, stomata; XV, xylem vessel. Bar = 50 \mu m.
Accumulation of antibodies in plants

Accumulation pattern of the MAK33 F_\text{ab} fragment in leaf tissue of A. thaliana—In leaf tissue of transgenic A. thaliana, signal representing the F_\text{ab} fragment was almost exclusively detected in the apoplastic compartment (Fig. 1a, b). The major proportion of this signal was present in intercellular spaces (Fig. 1b), as it had been previously observed by using electron microscopy (De Wilde et al. 1996). Strikingly, the signal was gradationally distributed in the apoplast: the peripheric regions of the transversal leaf sections tended to show a more concentrated labeling than the interior part of the section (Fig. 1a). This labeling pattern most probably corresponds with F_\text{ab} accumulation in plants when one of the detecting reagents was omitted (data not shown).

Fig. 2 Fluorescence micrographs of MAK33 IgG and F_\text{ab} immunolocalization. (a) FITC-labeled section through a MAK33 F_\text{ab}-expressing A. thaliana seedling grown from seed stock RUG-kd 12/8-5-15 processed 3 d after sowing; (b) and (c) FITC-labeled isolated leaf mesophyll cells of a MAK33 IgG-expressing plant grown from seed stock RUG-kd 11/1-2 and an untransformed A. thaliana plant, respectively; (d) FITC-labeled section through a petiole of an untransformed A. thaliana plant; (e) and (f) FITC-labeled sections through the stem and a petiole of a 73-day-old MAK33 IgG-expressing A. thaliana seedling grown from seed stock RUG-kd 11/1-2, respectively; (g) and (h) FITC-labeled sections through a leaf of a MAK33 F_\text{ab}-expressing GV-kd 8A and an untransformed N. tabacum plant, respectively. CC, cortex cell; CHL, chloroplast; EPC, epidermis cell; MC, mesophyll cell; PAC, parenchyma cell; XV, xylem vessel. Bar=50 \mu m.
Accumulation of antibodies in plants

Table 1 Summary of MAK33 IgG and Fab localization in transgenic A. thaliana and N. tabacum plants

<table>
<thead>
<tr>
<th>Organ</th>
<th>MAK33 IgG</th>
<th>MAK33 Fab</th>
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<tbody>
<tr>
<td><strong>Arabidopsis thaliana</strong></td>
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<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>Intercellular spaces</td>
<td>Intercellular spaces</td>
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<tr>
<td></td>
<td>Xylem vessels</td>
<td>Xylem vessels</td>
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<tr>
<td>Isolated leaf mesophyll cells</td>
<td>Mesophyll cells</td>
<td></td>
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<tr>
<td>Petioles</td>
<td>Cytosol</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Parenchyma cells</td>
<td>N.D.</td>
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<tr>
<td></td>
<td>Vascular bundle</td>
<td></td>
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<tr>
<td>Stems</td>
<td>Cortex cells</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Vascular bundle (xylem)</td>
<td></td>
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<tr>
<td>Roots</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Cortex cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vascular bundle</td>
<td>Intercellular spaces</td>
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<tr>
<td></td>
<td>Pericycle cells</td>
<td>Poles of the vascular bundle</td>
</tr>
<tr>
<td>Young seedlings</td>
<td>N.D.</td>
<td>Endodermal cells</td>
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<tr>
<td><strong>Nicotiana tabacum</strong></td>
<td></td>
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<tr>
<td>Leaves</td>
<td>N.D.</td>
<td>Xylem vessels</td>
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N.D., not determined.

at the interior side of the epidermal cell layers. Independent leaves and different regions of the same section of one leaf could show highly variable immunofluorescence. The former observation is in agreement with the variable amounts of Fab accumulation levels in different leaves as determined by ELISA (De Neve et al., manuscript in preparation). In every leaf section that was examined, signal representing the Fab fragment was also detected in xylem vessels (identified by the helically arranged lignifications showing orange autofluorescence) (Fig. 1a, b). In several sections, the Fab fragment could be detected almost exclusively in xylem vessels whereas intercellular spaces were hardly labeled (data not shown).

**Accumulation pattern of the MAK33 IgG in leaf tissue, petioles, and stem of A. thaliana**—In leaf tissue of transgenic A. thaliana, signal representing the IgG was, as for the Fab fragment, present in intercellular spaces (Fig. 1d) and in xylem vessels (data not shown). In contrast to the Fab fragment, signal representing the IgG could be detected not only in intercellular spaces of mesophyll cells but also in the cytoplasm of these cells. For sections of plants with high accumulation levels, the intensity of this intracellular signal was higher than that of the apoplastic signal. In order to examine the nature of the intracellular label, cells were isolated from leaf tissue of A. thaliana plants producing MAK33 IgG (Fig. 2b). The cytoplasmic signal within single cells was found not to be homogeneously distributed in the cytoplasm but to be present in a thread-like form, as it would be expected for proteins associated with the ER or other parts of the secretory pathway. Chloroplasts and other organelles visible within these labeled cells were always free of signal (Fig. 2b), indicating that no unexpected targeting events occurred.

In petioles, signal representing the IgG was detected in the cytosol of virtually all parenchyma cells (Fig. 2f). In stem tissue, cytosolic labeling was present in a limited number of cortex cells (Fig. 2e). Both in petioles and stems, signal representing the IgG was present in the vascular bundle (Fig. 2e, f). In the case of stem tissue, this included labeling in mature xylem vessels (Fig. 2e).

**Accumulation pattern of the MAK33 IgG and Fab fragment in roots of A. thaliana**—Transverse and longitudinal sections through roots of transgenic A. thaliana plants showed signal representing the MAK33 Fab fragment to be concentrated in the intercellular spaces of cortex cells (Fig. 1e (arrows), i) and in the cytoplasm of pericycle cells (Fig. 1e). A faint cytoplasmic signal could be observed in cells of the endodermis (Fig. 1e). Also the cells of the vascular cylinder, protoxylem or protophloem poles, were labeled (Fig. 1e). For the IgG the labeling of the vascular cylinder was more uniform (Fig. 1g, j) than that for the Fab fragment and included the stellar parenchyma cells (Fig. 1g). In contrast to the Fab fragment, the MAK33 IgG accumulated abundantly in the cytoplasm of cortex and epidermis cells and to a lesser extent extracellularly (Fig. 1f, j). This accumulation pattern was similar to that in leaf tissue, where the IgG, but not the Fab, accumulated in the cytoplasm of mesophyll cells.

**Accumulation pattern of the MAK33 Fab fragment in A. thaliana seedlings at different developmental stages**—
Seedlings processed on the third day after sowing showed accumulation of apoplastic signal in the root (Fig. 2a). For many of the examined seedlings, the accumulation of signal continued from the root through the hypocotyl into the cotyledons (data not shown). At this stage, leaves are not yet developed. Seedlings processed 6 or 10 d after sowing showed Fab fragment-specific signal in intercellular spaces and in xylem vessels of leaf tissue (data not shown). Compared to sections of seedlings processed 6 d after sowing, transversal leaf sections of seedlings processed 10 d after sowing showed a higher signal intensity at their peripheral regions (data not shown). In general, the observed accumulation pattern in young and old leaves was independent from the developmental stage of the plantlet between 8 and 68 d after sowing.

Localization of the MAK33 Fab fragment in leaf tissue of N. tabacum—In leaf tissue of transgenic N. tabacum plants signal representing the MAK33 Fab fragment was exclusively found in xylem vessels (Fig. 2g), including the leaf main vein (data not shown), as it is the case in leaf tissue of transgenic A. thaliana seedlings with low accumulation levels. ELISA quantification of the MAK33 Fab fragment in N. tabacum leaf tissue had previously shown that its accumulation level was indeed relatively low. This is presumably the reason why we could not localize the MAK33 Fab in N. tabacum leaves by electron microscopy (De Wilde et al. 1996).

Discussion

For the efficient expression of full-size antibodies in plants, the individual immunoglobulin chains have to be targeted to the ER by means of a signal peptide (Hiatt et al. 1989, Voss et al. 1995). Upon translocation to the ER, the immunoglobulin chains are expected to assemble to a complete antibody. In the absence of additional targeting or retention signals and provided correct processing occurs, assembled antibodies or antibody fragments are expected to migrate through the secretory pathway to the cell surface (Denecke et al. 1990, Comord and Faye 1996, Kermode 1996), where they are secreted (Gomord and Faye 1996, Pedrazzini and Vitale 1996, Ponnambalan and Banting 1996). Glycosylation at acceptor sites starts in the ER lumen with the cotranslational addition of high mannos glycans and their subsequent trimming (Satiat-Jeunemaitre and Hawes 1993, Helenius 1994) and becomes completed in subsequent stacks of the Golgi compartment (Driouich et al. 1994). That, upon ER targeting of individual immunoglobulin chains, plant-made antibodies do become secreted has been demonstrated by showing their presence in leaf intercellular fluid or in the medium of cell suspension cultures (Hein et al. 1991, van Engelen et al. 1994, Voss et al. 1995, De Wilde et al. 1996).

Recently, we illustrated the fate of IgG antibodies and Fab fragments following their exit from the secretory pathway (De Wilde et al. 1996); electron microscopy showed that these proteins accumulate in intercellular spaces of leaf mesophyll cells of transgenic A. thaliana plants. We ascertained that the microscopically detected proteins are intact full-size immunoglobulins or Fab fragments by analysis of leaf intercellular fluid (De Wilde et al. 1996). Here, we demonstrate the pattern of protein accumulation at the level of tissues, organs, and intact young plantlets. This yields information on the final accumulation sites of the IgG and the Fab fragment as a result of expression occurring within the cells and subsequent transport in the extracellular compartment.

A striking feature is the difference between the accumulation patterns of IgG and Fab fragment. In general, the full-size antibody is additionally located intracellularly in different cell types, including leaf mesophyll cells and root cortex cells (Table 1). The thread-like accumulation of the IgG in isolated cells suggests that the cytoplasmic signal for the IgG is associated with the components of the secretory pathway (Denecke et al. 1995, Satiat-Jeunemaitre et al. 1996). Several reasons could be at the basis of this observation, the most important being that the IgG is a molecule with complex folding requirements. It is built up of four polypeptide chains of two different types, making up a molecule of 146 kDa. The Fab fragment, on the other hand, is made up of only two polypeptide chains with a total molecular mass of 48 kDa. This different folding requirement could impose a substantial delay of the IgG in assembly, folding, and transit through the ER.

If proteins, following secretion, are transported in the apoplastic compartment, this can be expected to occur by the natural water flow in the transpiration stream. Water, taken up by the roots, is transported through the stem and the petioles to the leaves by way of the xylem vessel elements. Although it is clear that the transpiration stream transports low molecular weight components such as inorganic ions, soluble carbohydrates, organic and amino acids, and various phytohormones (Canny 1993, Lösch 1995), the transport of proteins is not well documented. The observation that both IgG and Fab fragment can always be detected in leaf xylem vessel elements, even when the accumulation levels are low, seems to support the model that antibodies, synthesized and secreted within the leaf itself are transported by the transpiration stream to accumulate at preferential sites. These preferential accumulation sites could be the regions in the leaf where the water of the transpiration stream is entering a space impermeable to the proteins, as it is observed for xylem-loaded dyes (Canny 1995), or where the water of the transpiration stream is evaporating. It is known that apoplastic water and solutes, when leaving the xylem vessel elements, move through the intercellular spaces of the mesophyll cell layers towards the epidermis (Lösch 1995). Evaporation of the water can then
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occur not only through stomata but also through the epidermal apoplast. Because proteins are not expected to permeate the anticlinal epidermal cell walls and the cuticle, it is not surprising that both IgG and F\textsubscript{ab} fragment become concentrated in the peripheries of the leaf. The predominant accumulation of IgG and F\textsubscript{ab} fragment in leaf xylem vessels can also be explained using the model of transpiration water-mediated protein flow; accumulation is expected at the sites where water is leaving the vessel faster than it is moving forward (Canny 1995). In addition, the antibody (fragments) could be immobilized, in planta, or during tissue processing, in the vessels through interactions with organic or inorganic ions (which are relatively abundant; Canny 1995). Also interactions with the vessel walls themselves are probable because MAK33 IgG and F\textsubscript{ab} are expected to have a slightly positive charge at apoplastic pH. In addition, proteins could enter xylem vessels as a result of protoxylem maturation, but this does probably not account for their high content of antibody fragments. Previously, electron microscopy has revealed that the MAK33 F\textsubscript{ab} fragment accumulates in leaf xylem vessels and is associated with electron-dense material surrounding the lignified cell wall thickenings (De Wilde et al. 1996). That the signal representing the F\textsubscript{ab} fragment in xylem vessels is the result of specific accumulation is indicated by the localization of the F\textsubscript{ab} in \textit{N. tabacum}. Indeed, from gene fusions with the \textbeta-glucuronidase gene (\textit{uidA}) (Mitsuhara et al. 1996) it can be deduced that the CaMV 35S promoter is probably not substantially more active in the leaf vascular bundles than in the surrounding mesophyll of \textit{N. tabacum}. Irrespective of the mechanism, antibodies accumulating at preferential apoplastic sites could be more stable, an important feature when the production of plant-made antibodies for ex situ applications is envisaged.

The distribution of the MAK33 IgG in leaf tissue should be compared to that of the secretory immunoglobulin complex components in \textit{N. tabacum} leaf mesophyll (Ma and Hein 1995, Ma et al. 1995), which are most probably localized in the luminal apoplastic space between the cell wall and the plasma membrane. This accumulation pattern is different from that of an IgG or F\textsubscript{ab} fragment, which cross the plant cell wall to accumulate predominantly in intercellular spaces. Most probably, retention by the cell wall is a result of the consistently higher molecular mass of the secretory immunoglobulin complex compared to that of an IgG or F\textsubscript{ab}.

In roots of \textit{A. thaliana}, the MAK33 F\textsubscript{ab} fragment accumulates in intercellular spaces of cortex cells and in the cytoplasm of pericycle cells; a faint cytoplasmic signal can also be observed in endodermis cells. It is tempting to assume that the intense signal in pericycle cells could be the result of formerly apoplastic antibody fragments that were secreted by the cortex and epidermis cells and that are subsequently transported from the cortical intercellular spaces towards the vascular bundle. Because the apoplastic spaces on the cortical and stellar sides of the endodermis are not connected, the only effective way to pass from the cortical apoplast to the pericycle cells is through the cytosol of the endodermal cells (Lösch 1995, Clarkson 1996, Moreshet et al. 1996). This mechanism would assume symplastic loading of proteins into the endodermal cells, a phenomenon that has not been described yet. Symplastic transport of such large proteins from endodermis to pericycle could be questioned because plasmodesmata have relatively small exclusion limits, at least in leaves (Lucas et al. 1996, McLean et al. 1997). No data are available on the properties of root plasmodesmata. However, the unique morphology (Leisner and Turgeon 1993) and altered gating capacities (Kempers et al. 1993) of plasmodesmata connecting sieve elements with their supporting companion cells show that different types of plasmodesmata exist. Also plant proteins that behave as viral movement proteins (Lucas et al. 1995) can be involved in symplastic transport in the root. An argument against radial transport is that the intercellular spaces of cortex cells, at least in small roots, are not interconnected radially, but longitudinally (Moreshet et al. 1996), and mostly air-filled (Clarkson 1996), two factors impeding radial flow or diffusion. On the other hand, water flow through discontinuities in the Casparian strips of the endodermis cells has been shown to support a major part of the total transpiration (Sanderson 1983), indicating that cortex and vascular bundle need not to be always strictly separated.

Alternatively, intense signal in pericycle cells could simply be the reflection of high stability of the antibodies in the pericycle cells or of high activity of the CaMV 35S promoter within these cells.

In conclusion, the final accumulation sites of plant-made antibodies and F\textsubscript{ab} fragments in transgenic plants are shown. The observed accumulation pattern, in particular in leaves, allows us to suggest that the secreted proteins are transported by the fluid of the transpiration stream. It will be interesting to analyze whether transpiration-mediated transport of proteins is an important factor in the accumulation of secreted, heterologous proteins in general and to unravel its physiological significance for secreted proteins of plant origin. The final accumulation sites of secreted plant-made antibodies largely determine which antigens will be available as target molecules. Thus, our results are of particular importance for approaches that make use of plant-made antibodies in order to obtain immunomodulation of apoplastic antigens.
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