mechanism of NA release from rat cerebral-cortex synaptosomes. Because immunocytochemical studies have shown that B-50 is found in synapses throughout the brain[21,22], B-50 could be more generally involved in transmitter release. Three lines of evidence suggest that the phosphorylation of B-50 by PKC is essential for stimulus–secretion coupling during transmitter release: (1) phorbol esters that directly activate PKC enhance the release of a variety of neurotransmitters[1–4]; (2) by using an antibody-independent approach, we have previously shown that depolarization-induced neurotransmitter release from non-permeabilized synaptosomes and hippocampal slices is closely correlated with a PKC-mediated increase in B-50 phosphorylation[18,23]; and (3) here we have shown that anti-B-50 IgG inhibits B-50 phosphorylation as well as Ca^{2+}-dependent transmitter release. If B-50 phosphorylation by PKC is indeed involved in the mechanism of transmitter release, then a long-term increase in PKC-mediated B-50 phosphorylation[7,17] could be one of the mechanisms underlying the increase in the release of glutamate that occurs during long-term potentiation[5–7,24,25].

In view of the localization of B-50 at the inner leaflet of the plasma membrane[21,26], we suggest that B-50 is involved in the regulation of vesicle fusion with the plasma membrane, a process in which the vesicle-associated protein synapsin I (a substrate of calmodulin-dependent kinases) has also been implicated[27,28]. But the difference in the localization of phosphorylating enzymes of these two proteins indicates that they have distinct roles in the transmitter release process. It may be that the regulatory role of B-50 in vesicle fusion is not limited to transmitter release, but extends to membrane-fusion processes during neurite outgrowth[29,30]. It remains to be investigated to what extent calmodulin binding[14] and modulation of phosphatidyl inositol 4-phosphate kinate activity[12,13]—putative properties of B-50—are also involved in controlling neurotransmitter release. □

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Production of antibodies in transgenic plants

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COMPLEMENTARY DNAs derived from a mouse hybridoma messenger RNA were used to transform tobacco leaf segments followed by regeneration of mature plants. Plants expressing single gamma or kappa immunoglobulin chains were crossed to yield progeny in which both chains were expressed simultaneously. A functional antibody accumulated to 1.3% of total leaf protein in plants expressing full-length cDNAs containing leader sequences. Specific binding of the antigen recognized by these antibodies was similar to the hybridoma-derived antibody. Transformants having γ- or κ-chain cDNAs without leader sequences gave poor expression of the proteins. The increased abundance of both γ- and κ-chains in transformants expressing assembled gamma–kappa complexes was not reflected in increased mRNA levels. The results demonstrate that production of immunoglobulins and assembly of functional antibodies occurs very efficiently in tobacco. Assembly of subunits by sexual cross might be a generally applicable method for expression of heterologous multimers in plants.

The source of immunoglobulin mRNAs was a hybridoma cell line expressing a catalytic IgG_1 antibody (6D4) which binds a low molecular weight phosphonate ester (P3) and catalyses the hydrolysis of certain carboxylic esters. Constructs used for immunoglobulin expression in plants consisted of coding-length cDNAs of the 6D4 γ- or κ-chain with or without their leader sequences. These cDNAs were modified to contain terminal *Eco*R1 restriction enzyme digestion sites and were ligated into the constitutive plant expression vector pMON530 (ref. 2) to form pHi101 (kappa, no leader), pHi102 (kappa, leader), pHi201 (gamma, no leader) and pHi202 (gamma, leader). We transformed tobacco plants using Agrobacterium containing each of these four plasmids[3] and screened leaf extracts from regenerated transformants for the presence of immunoglobulin heavy or light chains by enzyme-linked immunosorbent assay (ELISA)[4]. Transformants expressing individual immunoglobulin chains were then sexually crossed to produce progeny expressing both chains. The results of the ELISA revealed high levels of kappa and gamma chains accumulating in individual plants containing DNA from both pHi102 and pHi202 (Table 1; Fig. 2*a*). We verified the expression of both heavy and light chains by western blotting (Fig. 1). From the ELISAs, we judged that virtually all the γ- and κ-chains in these plants were assembled into gamma–kappa complexes (Table 1). Western blots provided additional evidence for assembled antibodies in that, under non-reducing conditions, most of the immunoreactive γ- and κ-chains aggregated at a high molecular weight (Fig. 1).

The binding specificity of the assembled gamma–kappa complexes was studied in ELISAs in which a P3–bovine serum albumin conjugate was used as antigen. The antigen binding by antibody derived from plants was equivalent to antigen binding by the 6D4 hybridoma antibody. Incubation of plant extracts or the purified 6D4 antibody with 50 μmol l^{-1} P3 for 3 h at 25 °C before addition to the ELISA eliminated antibody binding to the P3–BSA conjugate, demonstrating that binding was specific for the P3 hapten. Half-maximal inhibition occurred with 10 μmol l^{-1} free P3 for both hybridoma and plant-derived antibodies.

Transformants derived from the leaderless constructs pHi101 and pHi201 contained very low levels of κ- and γ-chains respectively, but Southern and northern blots (Fig. 2) demonstrated the presence of transforming DNA and immunoglobulin transcripts. None of the plants expressing leaderless

immunoglobulin chains contained assembled gamma-kappa complexes (Table 1).

The increased recovery of immunoglobulin epitopes from transformants expressing full-length cDNAs was not reflected in increased mRNA transcript levels. Northern blots (Fig. 2*b*) comparing pHi201 and pHi202 transformants, for example, revealed nearly equivalent levels of heavy-chain transcripts, although ELISAs indicated a 40-fold increase in accumulation of heavy-chain protein in the pHi202 transformant. Likewise, immunoglobulin mRNA levels in a plant producing large amounts of assembled antibodies were not significantly different from the parental plants that accumulated low levels of immunoglobulin chains (Fig. 2*b*).

Our results show that individual cDNAs for immunoglobulin κ- and γ-chains can be efficiently expressed in tobacco to form functional antibodies. Assembly of immunoglobulin chains by sexual cross in plants represents a useful alternative to the expression by a single vector of both gamma and kappa cDNAs as in yeast or bacteria[5-7], or double transformation with vectors containing individual cDNAs[8,9]. Potentially, this method is applicable to the assembly of oligomers other than antibodies. The characterization of antibodies produced in plants (glycosylation, processing of leader sequences, cytolocalization and turnover) will be described in a later paper.

In B lymphocytes, immunoglobulin processing and assembly occurs in the endoplasmic reticulum/Golgi in a process that may be promoted by heavy-chain binding proteins present in the endoplasmic reticulum[10,11]. Plant cells may also have a system for multimer assembly in their endoplasmic reticulum/Golgi that can recognize immunoglobulin chains. Alternatively, assembly may occur spontaneously, given sufficient levels of each chain in the appropriate cellular compartment. Our results demonstrate that plants require a signal sequence for efficient assembly of γ- and κ-subunits. The presence of the mouse leader sequence clearly augments the accumulation of individual chains. This might be the result of an enhanced translation of the immunoglobulin messengers or an increased stability of each protein as a result of subcellular sequestering or secretion. The yield of each chain is increased in plants expressing both gamma and kappa, indicating that assembly of the gamma-kappa complex might enhance stability.

TABLE 1 Expression and assembly of immunoglobulin gamma and kappa chains in tobacco

Accumulation of γ- or κ-chains in transformed plants*

γ_{NL}	γ_L	$\gamma_L(\kappa_L)$	$\gamma_{NL}(\kappa_{NL})$
30 ± 16 (60)	1,412 ± 270 (2,400)	3,330 ± 2,000 (12,800)	32 ± 26 (60)
κ_{NL}	κ_L	$\kappa_L(\gamma_L)$	$\kappa_{NL}(\gamma_{NL})$
1.4 ± 1.2 (3.5)	56 ± 5 (80)	3,700 ± 2,300 (12,800)	6.5 ± 5 (20)

Distribution and assembly in crosses†

	γ only	κ only	$\gamma\kappa$	Null
$\kappa_{NL} \times \gamma_{NL}$	4	6	3 (0% assembly)	5
$\kappa_L \times \gamma_L$	3	10	11 (95 ± 16% assembly)	4

* Accumulation of individual gamma and kappa chains (in ng per mg total protein) was estimated by ELISA[4]. Microtitre wells were coated with a goat anti-mouse heavy or light chain-specific IgG (Fisher) in 150 mM NaCl, 20 mM Tris-HCl, pH 8.0 (TBS), followed by blocking with 5% non-fat dry milk in TBS. Plant leaves were homogenized in a mortar and pestle at 4 °C after removal of the midvein. To the supernatant a quarter volume of 5 × TBS was added, and 50 μl of 1 in 2 serial dilutions were added to each microtitre well. After 18 h at 4 °C, microtitre wells were washed with distilled water at room temperature. Bound γ- or κ-chains were reacted with goat anti-mouse heavy or light chain-specific antibodies conjugated to horseradish peroxidase for 2 h at 37 °C in TBS, and detected according to the manufacturer's instructions. Control microtitre wells contained extracts from plants transformed with pMON530 vector. Values given as mean (± s.d.) are derived from at least two determinations per plant and do not include transformants producing no detectable γ- or κ-chain. At least nine plants were assayed in each category. All values are given as ng per mg of total protein in the extract and are derived from the quantity of purified 6D4 antibody required to given an equivalent colour development in ELISA. Total protein in the extract was determined by the Bio-Rad Coomassie assay. Complementary DNAs containing no leader sequences are referred to as γ_{NL} and κ_{NL}; γ_L and κ_L refer to cDNAs with leader sequences; $\gamma(\kappa)$ refers to gamma chains in a plant that also expresses κ-chains, and vice versa. Numbers in parentheses are values for plants with the highest levels of accumulation.

† The number of plants expressing γ- or κ-chains among the progeny of a sexual cross. The ELISA for assembly used horseradish peroxidase-conjugated anti-κ-chain-specific antibodies to detect antigen bound to microtitre wells coated with unlabelled anti-γ-chain-specific antibodies, and vice versa. Values derived from these assays were used to calculate the per cent of assembly by comparison with the purified 6D4 antibody. This was determined at least three times for each $\gamma\kappa$ plant. The per cent assembly is expressed in parentheses as the mean ± s.d.

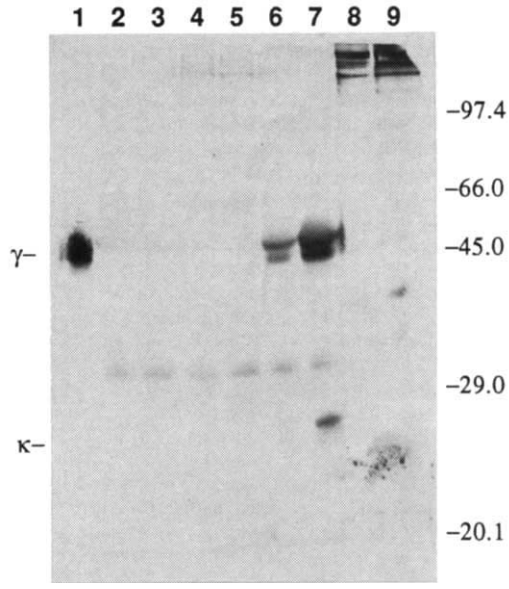

FIG. 1 Western blot of leaf proteins from transgenic tobacco plants expressing immunoglobulin chains. Leaf segments (1 g) from mature plants were homogenized in a mortar and pestle with 1 ml 0.05 M Tris-HCl, pH 7.5, 1 mM phenylmethanesulphonyl fluoride. Extracts were boiled in 4 M urea, 1% SDS, with or without 2 mM dithiothreitol (DTT) as indicated, for 3 min. SDS-PAGE in 10% acrylamide[16] and blotting of the proteins to nitrocellulose[17] were performed as described. Blots were preincubated for 6 h at 4 °C in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% Tween 20 (TBST) containing 5% BSA, and 0.5% non-fat dried milk before the addition of antibodies. A biotinylated goat anti-mouse whole IgG antibody (Cappel), diluted 1:500 in TBST was used to probe the blots at 4 °C for 24 h. A variety of commercially available antibodies (anti-mouse IgGs) were used in other experiments with similar results. Antibody binding was visualized after binding of streptavidin-conjugated alkaline phosphatase (25 °C, for 2 h) by incubation in 300 μg ml^{-1} nitroblue tetrazolium and 150 μg ml^{-1} 5-bromo-4-chloro-3-indolyl phosphate. In lanes 1–7, 40 μl of each extract containing DTT; lanes 8 and 9, 40 μl extract without DTT. Lane 1, 100 ng purified antibody from the 6D4 hybridoma; lane 2, 15 μg wild-type plant-extract protein; lane 3, 15 μg protein from a plant transformed with truncated κ-chain cDNA (pHi101) containing no leader sequence; lane 4, 15 μg from plant transformed with truncated γ-chain cDNA (pHi201); lane 5, 15 μg from a full-length kappa cDNA transformant (pHi102); lane 6, 15 μg from a full-length γ-chain cDNA transformant (pHi202); lane 7, 15 μg from an F1 plant derived from the cross between a kappa and a gamma producer; lane 8, 100 ng 6D4 antibody (no DTT); lane 9, same as lane 7, except no DTT. Gamma and kappa on the left refer to the positions of the 6D4 heavy and light chains; positions of molecular weight (given in thousands) markers are shown on the right. By ELISA, extracts in lanes 3–5 contained very low levels of κ- or γ-chains (<0.008% of total protein, Table 1), whereas extracts in lanes 6, 7 and 9 contained 0.24, 1.3 and 1.3% immunoglobulin respectively.

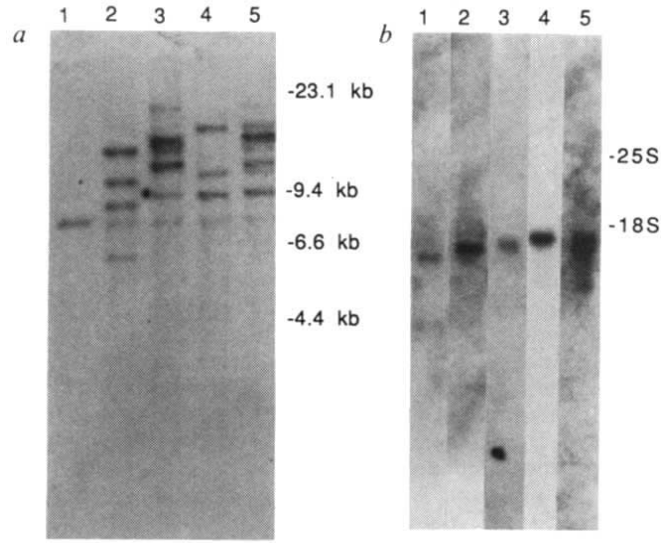

FIG. 2 Southern and northern blots of leaf DNA and RNA from transgenic plants expressing immunoglobulin cDNAs. *a*, DNA was extracted from 1 g mature leaf tissue after freezing the fresh segments in liquid N_2. Homogenization into urea mix[18] was in a mortar and pestle. The homogenate was extracted with phenol:$CHCl_3$ (1:1) and the nucleic acids precipitated by addition of one volume of isopropyl alcohol. The resuspended DNA (20 μg each) was cut with *Hind*III and Southern-blotted as described[19]. The probe used for both Southern and northern blots was ^{32}P-labelled pMON530 plasmid[2] containing either a kappa cDNA or a gamma cDNA. Both cDNAs were used in the hybridization shown. Lane 1, DNA from a transformant expressing a light-chain cDNA without a leader sequence (pHi101); lane 2, DNA from a heavy-chain cDNA transformant, no leader (pHi201); lane 3, DNA from a transformant expressing full-length light chain with leader (pHi102); lane 4, DNA from a transformant expressing heavy chain with leader (pHi202); lane 5, DNA from an F_1 plant derived from a cross between plants expressing full-length gamma or kappa cDNAs (pHi102 × pHi202). *b*, Extraction of RNA from 1 g fresh leaf tissue was by homogenization in 10 ml 0.1 M Tris-HCl, pH 9.0, and 10 ml phenol saturated with this buffer, using a Polytron at high speed. Nucleic acids were precipitated by addition of one tenth volume 3.0 M sodium acetate, pH 5.0, and 1.5 volumes isopropyl alcohol. Resuspended RNA was electrophoresed in gels containing formaldehyde and northern blotted onto nylon membranes (Amersham) as described[19]. Lane 1, RNA from a transformant expressing a light-chain cDNA without a leader sequence (pHi101); lane 2, RNA from a heavy-chain cDNA transformant, no leader (pHi201); lane 3, RNA from a transformant expressing full-length light chain with leader (pHi102); lane 4, RNA from a transformant expressing heavy chain with leader (pHi202); lane 5, RNA from an F_1 plant derived from a cross between plant expressing full-length gamma or kappa cDNAs (pHi102 × pHi202). Total RNA (20 μg) was loaded in each lane. Lanes from separate hybridizations were aligned with respect to the 18S (1,900 base pairs) and 25S (3,700 base pairs) ribosomal RNA bands on the blots, detected by methylene-blue staining.

Expression of functional antibodies from transcripts that do not contain signal sequences may require modifications to yield alternative antigen-binding structures (such as single-chain antigen-binding proteins[6,7]) that do not need to be assembled. Thus binding of constituents of metabolic pathways involved in morphogenesis, stress responses or plant–pathogen interactions could be used to further our understanding of these processes in a way analogous to the blocking by microinjected antibodies of specific protein functions in mammalian cells[12,13].

As large macromolecules such as protein multimers do not pass through plant cell walls[14,15], the binding by antibodies of small organic molecules (toxins, herbicides, plant hormones, organic chelates, for example) that are permeable to the cell wall might result in the net uptake and retention of these molecules in the plant. Accumulation of functioning antibodies may provide new options for the recovery of an array of environmental contaminants, as well as other biologically significant organics. Catalytic processing of small molecules within cell wall boundaries by antibodies may also become a generalized strategy for the elimination or modification of permeable organic compounds and could introduce new catalytic properties into existing metabolic pathways. □

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Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor

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THE human pan-leukocyte antigen Leu-8 has attracted wide interest because its presence or absence identifies suppressor-inducer and helper-inducer CD4$^+$ T-lymphocyte subsets respectively. We report here that Leu-8 is the human homologue of the mouse Mel-14 homing receptor, a molecule that promotes the initial adhesion of blood-borne lymphocytes to the specialized post-capillary endothelium of peripheral lymph nodes. We also show that Leu-8 can adopt both conventional and phospholipid anchored forms, a finding that may have relevance in the context of antigen shedding following activation or homing. The assignment of lymphocytes to different functional classes based on lymph node homing potential may represent a more general association between lymphocyte function and tissue distribution.

Two complementary DNA clones encoding Leu-8 determinants were isolated from a human T-cell library by methods previously described[1,2]. DNA sequence analysis showed that the longer insert of the two contains 2,350 residues, whereas the shorter lacks 436 internal residues but is otherwise identical (Fig. 1). The predicted polypeptide sequences were found to diverge at their C-termini.

The protein encoded by the larger insert bears a strongly hydrophobic putative membrane spanning domain near its C terminus, followed by several positively charged residues resembling a cytoplasmic anchor sequence. The protein is closely related to the recently described murine Mel-14 homing receptor[3,4] (Fig. 1) and the corresponding cDNA sequence shares