The intermediate filament-related system of higher plant cells shares an epitope with cytokeratin 8

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Summary

It is now apparent that plants possess intermediate filament (IF) antigens that exist either as cytoplasmic fibrillar bundles (FBs) or in a finer form, co-distributing with the microtubule arrays. In a previous study a monoclonal antibody (AFB) against the fibrillar bundles was shown to recognize representatives of what may be considered as a 'core' group of type III IFs. Here a monoclonal antibody is described that extends the range of similarity to cytokeratin. MAC322 stains the cytokeratin network in PtK_2 cells, unlike AFB that stained vimentin. Like other anti-IF antibodies successfully used against plants, it stains their microtubule-associated system but is also able to stain FBs, with or without methanol pretreatment. By immunoblotting, MAC322 does not recognize the acidic (type I) cytokeratins in MCF7 and TR146 epithelial cell lines but recognizes cytokeratin 8. This was confirmed by a positive cross-reaction against recombinant human cytokeratin 8. This type II polypeptide is known to be characteristic of simple epithelia, to occur in the earliest stages of amphibian development, and also in some non-epithelial and de-differentiated cells.

The sharing of a cytokeratin 8 epitope between higher plants and animals reinforces the idea that IF forms evolved before the two kingdoms diverged.

Key words: plant cells, intermediate filaments, cytokeratin 8, monoclonal antibody.

Introduction

There are now several lines of evidence to establish that higher plant cells contain intermediate filament-like antigens. Bundles of intermediate filament-like fibrils were reported in carrot suspension cells by Powell *et al.* (1982). Subsequently, these fibrillar bundles were isolated and immunoblotted with the universal monoclonal antibody anti-IFA (Pruss *et al.* 1981), by Dawson *et al.* (1985). Later, a monoclonal antibody (AFB) was raised against the carrot fibrillar bundles (Hargreaves *et al.* 1989*a*) and was shown to recognize desmin, vimentin and glial fibrillary acidic protein – the type III intermediate filaments (IFs) of animal cells. The isolated fibrillar bundles can be solubilized in 9 M urea/mercaptoethanol, which, when removed by dialysis, allows major polypeptides of 58 and $62\times10^3 M_r$ to self-assemble into 10 nm filaments and bundles of filaments (Hargreaves *et al.* 1989*b*). Since these filament-forming polypeptides contain the anti-IFA and AFB antigens, it seems reasonable to conclude that the fibrils contain proteins related to vertebrate intermediate filaments. Intermediate filament antigens do, however, occur in plants in another form that does not appear to be filamentous. Using anti-IFA (Dawson *et al.* 1985; Parke *et al.* 1987) or AFB (Hargreaves *et al.* 1989*a*; Goodbody *et al.* 1989) fibrous immunofluorescence staining patterns were obtained in onion root tip cells, and carrot and maize suspension cells – patterns that co-distribute with the four microtubule (MT) arrays. In addition, 34g5 (Klymkowsky, 1988) – a monoclonal antibody raised against *Chlamydomonas* basal bodies that recognizes vimentin – also stains MT arrays in suspension cells (Goodbody *et al.* 1989). It has been established by immunoblotting in these studies that the antibodies do not recognize plant tubulin *per se*; instead, immunogold labelling results (in the latter paper) indicate that electron-dense material alongside and between cortical MTs is being stained. Plants therefore contain intermediate filament-related antigens in two forms.

In the present article, we describe a monoclonal antibody raised against a crude cytoskeletal preparation from carrot suspension cells. A previous study with AFB (Hargreaves *et al.* 1989*a*) indicated similarities between plant IF antigens and what may be considered a core group of type III intermediate filaments. The present study now extends the relatedness to a particular cytokeratin. Cytokeratins are normally regarded as hallmarks of epithelial differentiation, except that the primary embry-

onic simple epithelial keratins 8 and 18 are expressed beyond the conventional epithelial differentiation programme of vertebrate cells, for they are found in oocytes (Franz *et al.* 1983), glial cells (e.g. see Rungger-Brandle *et al.* 1989) and embryonal or de-differentiated mammalian cells (e.g. see Stosiek *et al.* 1990). The finding of a CK8 antigen in plant cells indicates an even wider phylogenetic distribution than indicated by AFB alone and supports the concept of an archetypal form of intermediate filament-like protein (Rungger-Brandle *et al.* 1989).

Materials and methods

Plant cell lines

Suspensions of non-embryogenic carrot cells (*Daucus carota* L. cv. Scarlet Nantes) were maintained in modified Murashige and Skoog's medium (Flow Laboratories) supplemented with 1 mg l^{-1} 2,4-dichlorophenoxyacetic acid, 0.5 mg l^{-1} kinetin, 3% (w/v) sucrose and 5% (w/v) coconut milk, pH 5.8 (Powell *et al.* 1982), at 25°C in a 12 h/12 h, light/dark, cycle on a rotary shaker.

Suspensions of non-embryonic Black Mexican Sweetcorn (BMS; *Zea mays* L.) cells (courtesy of Dr E. Dennis, Canberra) were maintained in modified Murashige and Skoog's medium supplemented with 2 mg l^{-1}, 2,4-dichlorophenoxyacetic acid, 2% (w/v) sucrose, pH 5.7, at 27°C in the dark. Both lines were subcultured at weekly intervals.

Animal cell lines

PtK_2 cells were kindly provided by Drs R. Warn and A. Prescott, University of East Anglia. MCF7 and TR146 epithelial cell lines were also used.

Immunofluorescence

Carrot and BMS protoplasts were stained by the method of Goodbody *et al.* (1989) as follows: 3- to 6-day-old cells were converted to protoplasts with 1.5% (w/v) cellulase Onozuka RS (Yakult Honsha Co. Ltd, Mimato-Ku, Tokyo 105, Japan), 0.7% (w/v) hemicellulase (Sigma Chemical Co.) and 0.1% (w/v) pectolyase Y23 (Seishin Pharmaceuticals, Nihonbashi, Tokyo, Japan) in Mes buffer (0.5% (w/v) Mes, 80 mM $CaCl_2.2H_2O$, 0.3 M mannitol, pH 5.8) for 2.5 h at 25°C on a rotary shaker. Washed protoplasts were allowed to settle onto multi-test Flow slides coated with polylysine. They were fixed for 15 min at room temperature with 4% (w/v) formaldehyde, in a microtubule-stabilizing buffer (MTSB: 50 mM Pipes, pH 6.9, 5 mM EGTA, 5 mM $MgSO_4$) plus 0.3 M mannitol. This buffer, minus fixative, but containing 1% (v/v) Triton X-100 was then used to extract the protoplasts for 10 min. After washing, the cytoskeletons were immunostained as described by Clayton and Lloyd (1984). Carrot cells were prepared for staining as described by Hargreaves *et al.* (1989*a*). Three- to six-day-old carrot suspension cells were fixed in MTSB as above, containing 8% (w/v) formaldehyde, 1% (v/v) dimethyl sulphoxide, for 45 min at room temperature. Cells were then washed three times in MTSB but without formaldehyde and dimethyl sulphoxide. Cells were then resuspended in 2% (w/v) Driselase (Sigma Chemical Co.) in 5 mM EGTA, pH 6.9, and allowed to settle for 30 min. Cells were again washed in MTSB, then allowed to air dry on the wells of multitest slides (Flow Laboratories). After drying, slides were dipped quickly into methanol followed by Tris-buffered saline, pH 7.4 (TBS). Cytoskeletons were then immunostained as described by Clayton and Lloyd (1984).

Animal tissue culture cells grown for 3–4 days on coverslips were fixed in methanol at −20°C for 10 min. Coverslips were then washed in TBS, pH 7.4, twice, and then extracted in TBS with 1% (v/v) Nonidet P-40 for 5 min. After washing twice with TBS, cells were stained with first and second antibodies, with TBS washes in between. Slides were mounted in Citifluor PBS–glycerol anti-fade (City University, London).

Seeds of wheat (*Triticum aestivum* L. cv. Norman) and *Allium cepa* L. var. Bedfordshire Champion were germinated on moist filter paper and the terminal 3 mm of 4- to 6-day-old primary root tips was excised and the cells were prepared for staining using the method of Wick *et al.* (1981) as modified by Clayton and Lloyd (1984).

Electrophoresis and electroblotting

(1) SDS–PAGE was performed in 1 mm thick polyacrylamide gels, either 7.5% or 10% (w/v), according to Laemmli (1970). Electrophoretic transfer was performed according to Towbin *et al.* (1979). Nitrocellulose was blocked either with 3% (w/v) bovine serum albumin (Sigma) in TBS, pH 7.4, or in TBS plus 0.05% (v/v) Tween-20 (TBST), overnight at 4°C. The nitrocellulose was then incubated with the primary antibody at 4°C overnight, or at room temperature for 1–4 h. Higher-stringency blotting conditions were obtained where necessary by including a wash in 1.0 M sodium chloride in TBS, pH 7.4, with 0.1% (v/v) Tween-20 after antibody incubations. Cross-reactions between transferred proteins and monoclonal antibodies were detected using horseradish peroxidase or alkaline phosphatase-linked secondary antibodies (Dako Ltd, Slough, UK).

(2) NEpHGE was performed according to O'Farrell (1975) using Ampholines, pH 3.5–10 and pH 5–7, in a ratio of 3:1 (Pharmacia LKB, Sweden). First-dimension electrophoresis was for a total of 1000 Vh, and the second-dimension electrophoresis was as for SDS–PAGE on 1 mm 10% slab gels.

Cytokeratin antibodies and polypeptides

The following monoclonal anti-cytokeratins were used: LE41 (CK8), Lane (1982); M20 (CK8), Schaafsma *et al.* (1990); LP3K (CK8), Lane *et al.* (1985); CAM5.2 (CK8), Makin *et al.* (1984); LP2K (CK19), Stasiak *et al.* (1989); LP1K (CK7), Lane *et al.* (1985); LE61 (CK18), Lane (1982).

Apart from PtK_2 cells, cytokeratin samples containing CK8 were obtained from the epithelial cell lines, TR146 and MCF-7, by extracting in low salt and 0.1% (v/v) Triton X-100.

Recombinant human cytokeratin 8 was synthesized in *Escherichia coli* as a β-galactosidase fusion protein derived from human CK8 cDNA expressed in pUC-Rl expression vector (Waseem *et al.* 1990*a*,*b*).

Cytoskeleton preparation for immunization

Carrot cells were used to make protoplasts using 2% (w/v) Driselase (Sigma) and 2% (w/v) Onozuka R10 cellulase in Murashige and Skoog's medium with 3% (w/v) sucrose and 0.4 M mannitol. Cells were incubated at 25°C on a rotary shaker for 2.5 h. After washing in protoplast medium (as above, omitting enzymes), the protoplasts were washed in double-strength MTSB, with 10% (w/v) dimethyl sulphoxide and 0.4 M mannitol. Cytoskeletons were then made by extracting protoplasts on ice in the above buffer plus 1% (v/v) Nonidet P-40 and 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg ml^{-1} leupeptin, 10 μg ml^{-1} benzamidine and 1 mg ml^{-1} α-macroglobulin. The sample was obtained by spinning the mixture at 150 ***g*** for 5 min at 4°C, then spinning the supernatant at 3000 ***g*** for 15 min at 4°C, and finally, the supernatant was spun at 100 000 ***g*** for 40 min at 4°C. Monoclonal antibodies were prepared as described by Hargreaves *et al.* (1989*a*). Supernatants were screened both by immunofluorescence, using carrot suspension cells, and by dot immunoblotting against the immunogen on nitrocellulose paper.

Results

Immunofluorescence of plant and animal cells

Monoclonal antibodies (mAbs) raised against the carrot cytoskeletal fraction were dot immunoblotted against purified fibrillar bundles, a preparation rich in actin bundles from carrot protoplasts, and a homogenate of carrot protoplasts. Positives were then screened by immunofluorescence against carrot protoplasts and PtK_2 cells. In this way, MAC 322 was found to immunostain PtK_2 cells (see Fig. 1) in a manner similar to LE41, a mAb that recognizes keratin 8 (Lane, 1982). MAC 322 also

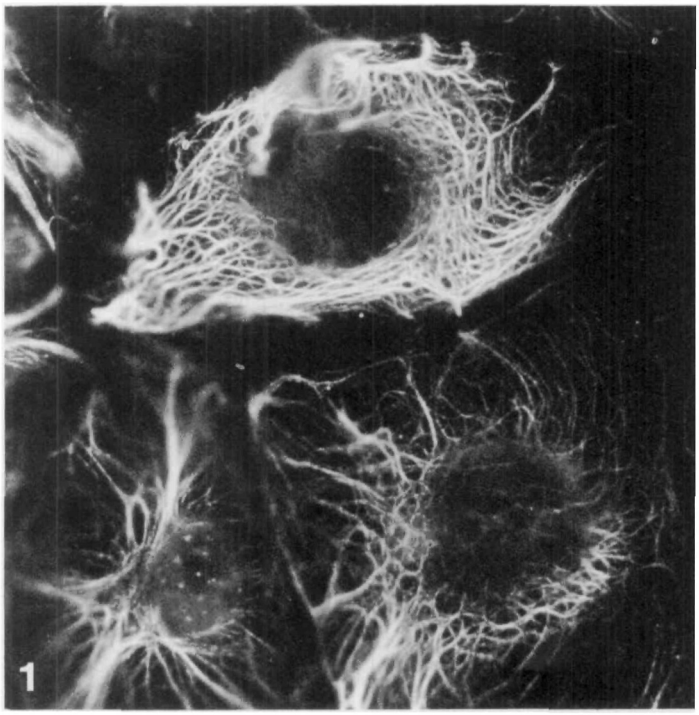

Fig. 1. By indirect immunofluorescence MAC 322 stains the cytokeratin network of PtK_2 cells fixed in cold methanol.

produced a dotted staining pattern over a large portion of the nuclei of the animal cells. The thick network of cytoplasmic filaments stained by this antibody is characteristic of the keratin system and is therefore quite distinct from the finer, perinuclear staining pattern of vimentin previously reported for the mAb raised against fibrillar bundles, AFB (Hargreaves *et al.* 1989*a*).

Both anti-IFA (Dawson *et al.* 1985) and AFB (Hargreaves *et al.* 1989*a*) have been found to immunostain material that co-distributes with all four microtubule arrays in cycling plant cells. Using confocal laser scanning microscopy, MAC 322 was found to produce identical patterns in wheat root tip cells. In Fig. 2A and B the antibody labels the interphase cortical microtubules, producing a pattern more coarse than that shown by anti-tubulin antibodies. During preprophase, MAC 322 stains the preprophase band (PPB) of microtubules (Fig. 2C). The same cell is shown at a deeper level of focus in Fig. 2D. In this case, the PPB can be seen end-on as bright dots at the cell cortex, but there is also clear staining around the nucleus. In Fig. 2E, MAC 322 can be seen to label the kinetochore bundles in a granular fashion. Later in the cell cycle, the monoclonal antibody stains the cytokinetic phragmoplast (Fig. 2F).

Anti-IFA immunoblots but does not stain the large, paracrystalline fibrillar bundles in carrot cells (Dawson *et al.* 1985). In contrast, AFB does label fibrillar bundles (FBs), except that methanol treatment is essential to expose the epitope (Goodbody *et al.* 1989). In Fig. 3A, MAC 322 has been used to stain fibrillar bundles in aldehyde-fixed, methanol-treated carrot suspension cells, although the methanol step is not essential for this antibody. Black Mexican Sweetcorn suspension cells were also employed for immunofluorescence studies with MAC 322. Fig. 3B illustrates a fine, wispy cytoplasmic network that radiates from, and is concentrated around, the nucleus. Bursting protoplasts on coverslips is a useful way of exposing cortical MTs in a monolayer, free of the bulk of other cytoplasmic components. The linear–punctate staining of swirling microtubules (MTs) by MAC 322 – produced by bursting carrot protoplasts – is illustrated in Fig. 3C.

Immunoblotting

Immunoblots of one-dimensional (1-D) gels of carrot protoplasts indicate that MAC 322 recognizes several polypeptides (Fig. 4, lane a). Major cross-reacting polypeptides of apparent molecular mass 42, 50 and 55×10^3 M_r are also recognized by the anti-cytokeratin of monoclonal antibody LE41 (Fig. 4, lane b). In a comparison (unpublished) between MAC322, AFB and anti-IFA, immunoblotted against a fibrillar bundle preparation, the major reaction for all antibodies was with the 42×10^3 M_r band. It is possible that this is a breakdown product, since a range of other fainter cross-reactions was seen with polypeptides up to 110×10^3 M_r.

To confirm the cytokeratin cross-reaction observed in immunofluorescence of animal cells, and to establish whether type I (acidic) as well as type II (basic) cytokeratins are recognized, MAC 322 was tested against cell lines containing a range of polypeptides. MCF7 cells contain CKs 18 and 19 in addition to 8 (Moll *et al.* 1982). In Fig. 5, it can be seen that MAC 322 (lane a) recognizes a doublet of higher molecular mass than CK18 (which is stained with LE61 in lane b) and CK19 (which is stained with LP2K in lane c). The same higher molecular mass doublet is recognized by LE41 (not shown).

TR146 cells are very useful for screening anti-keratin cross-reactivity of antibodies, since they contain a broad range of cytokeratins: 5, 6, 8, 10, 14, 17, 18 and possibly 4, 13, 16 and 19 (Purkis *et al.* 1990). These were first separated by non-equilibrium pH gel electrophoresis and then by SDS–PAGE in the second dimension. The position of CK8 was established by immunoblotting with monoclonal antibodies LE41 (Lane, 1982) and M20 (Schaafsma *et al.* 1990) (and we have also used LP3K (Makin *et al.* 1984) and CAM5.2 (Lane *et al.* 1985) in confirmation). The relative position of CK8 within the constellation of the non-blotting cytokeratins was then determined by sensitive staining of the nitrocellulose with Pelikan ink. In Fig. 6C and E, M20 and LE41 are both shown to recognize the same triplet of approximately 52×10^3 M_r. MAC 322 (Fig. 6A) recognizes the right-hand (i.e. most basic) species of this keratin 8 triplet. There are faint cross-reactions with polypeptides at approximately 56×10^3 M_r (presumably CK5 and CK6) but these are also seen with the authentic cytokeratin antibodies, particularly with LE41 (Fig. 6E).

Finally, to confirm the CK8 cross-reaction, MAC 322 was tested against recombinant human CK8 expressed in a pUC-R1 plasmid vector in *E. coli* (Waseem *et al.* 1990). The position of the cross-reaction is shown in Fig. 7 (lane a) with a thinner, faint band just below it. No cross-reaction was obtained against the vector (lane b).

Discussion

Evidence presented in this paper indicates that the monoclonal antibody raised against a carrot cytoskeleton preparation recognizes a simple epithelial, or embryonic, type II keratin in animal cells. MAC 322 stains the cytokeratin network in PtK_2 cells and in MCF7 cells it immunoblots a doublet that migrates above cytokeratins

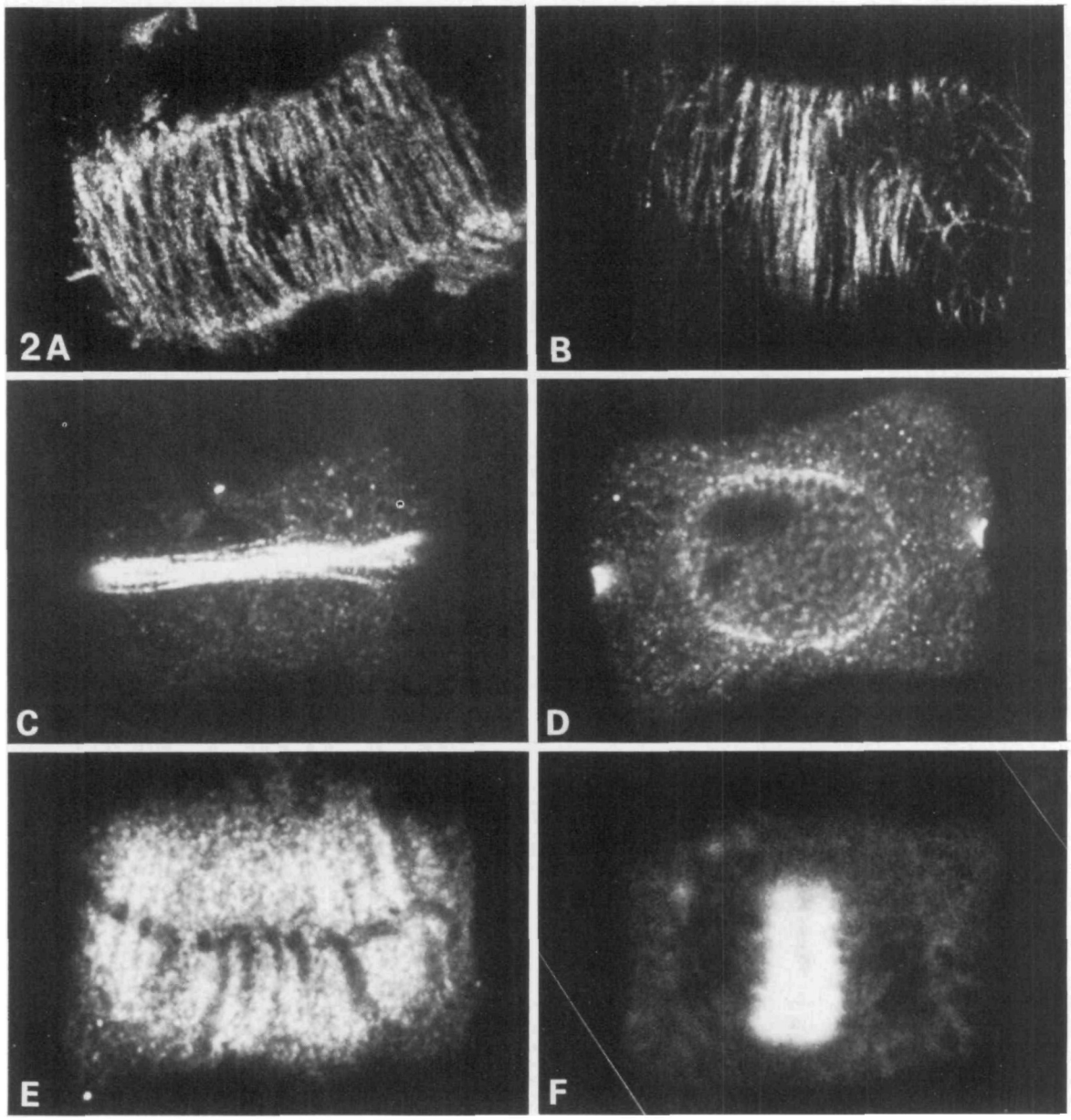

Fig. 2. Confocal laser scanning microscopy of formaldehyde-fixed wheat root tip cells, stained by indirect immunofluorescence with MAC 322. (A) and (B) The antibody is seen to label the cortical MTs. At preprophase (C) it labels the preprophase band at the cortex. The same cell, focussed at the nucleus (D) can also be seen to be labelled around the nucleus. At metaphase (E) the kinetochore bundles are strongly stained but the fibrogranular nature of the staining is pronounced. (F) Staining of the phragmoplast.

18 and 19, at around 52×10^3 M_r. Since the intermediate filament proteins of these cells consist of only keratins 8, 18 and 19 (Moll *et al.* 1982), it is probable that the doublet is CK8. The presence of two bands on SDS-containing gels appears to be characteristic of native or recombinant CK8. The band of smaller size may be a proteolytic fragment of the upper band, or an isoform in a different state of post-translational modification, since analysis with a broad range of well-characterized mAbs to CK8 could not distinguish between the bands (Waseem *et al.* 1990; unpublished observations).

The TR146 oral keratinocyte epithelial cell line is highly useful in screening anti-cytokeratin antibodies, since it expresses at least 11 cytokeratins (Purkis *et al.* 1990). On 2-D immunoblots MAC 322 recognizes a spot that a range of authentic anti-CK8 mAbs confirms to be CK8. There is also a faint, minor cross-reaction with a higher molecular weight doublet of similar pI, but this is also produced with the anti-CK8 mAbs LE41 and M20 under our blotting conditions. The major cross-reaction of MAC 322 is therefore with CK8 and no cross-reaction is observed in the region of blots where the more acidic cytokeratins are located.

To confirm that CK8 is specifically recognized by MAC 322, human recombinant CK8 was used to challenge the monoclonal. The positive cross-reaction is illustrated in Fig. 6. The monoclonal antibody raised against plant cytoskeletal proteins therefore recognizes a type II cytokeratin (CK8) rather than type I acidic cytokeratins.

MAC 322 differs from previous anti-IF antibodies found

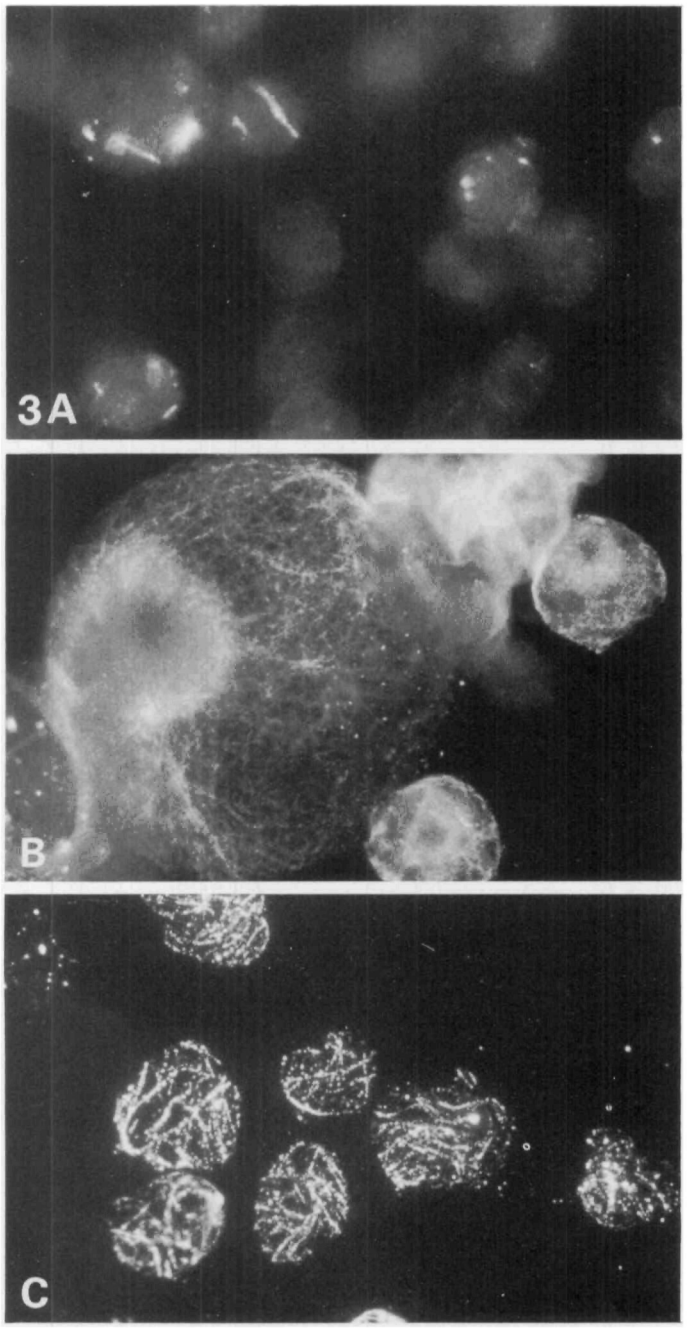

Fig. 3. Immunofluorescence of plant cells. (A) Formaldehyde fixed, methanol-treated carrot protoplasts can be seen to contain cytoplasmic fibrillar bundles of various sizes, when stained with MAC 322. (B) Formaldehyde-fixed Black Mexican Sweetcorn protoplasts stained with MAC 322 contain a fine cytoplasmic meshwork associated with increased peri-nuclear staining. (C) Burst carrot protoplasts leave adherent discs of plasma membrane ('footprints') bearing cortical MTs; these stain in a coarse, dotted manner with MAC 322.

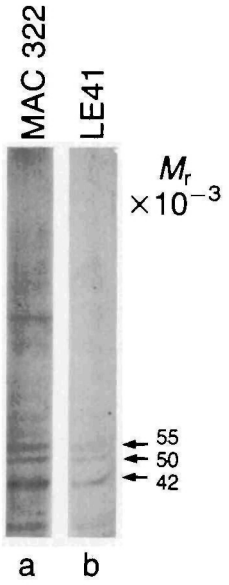

Fig. 4. Western blot of carrot protoplast polypeptides separated by SDS–PAGE. Lane A is blotted with MAC 322; lane b, with the anti-CK8 mAb, LE41.

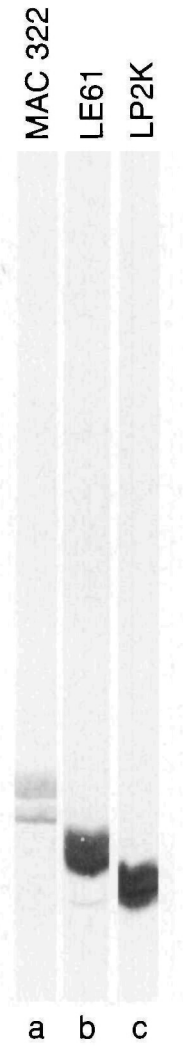

Fig. 5. Western blot of polypeptides from the epithelial cell line MCF7, separated by SDS–PAGE. This line contains cytokeratins 8, 18 and 19. Lane a is immunoblotted with MAC 322; lane b, with the mAb LE61, which detects CK18; lane c, with mAb LP2K, which detects CK19.

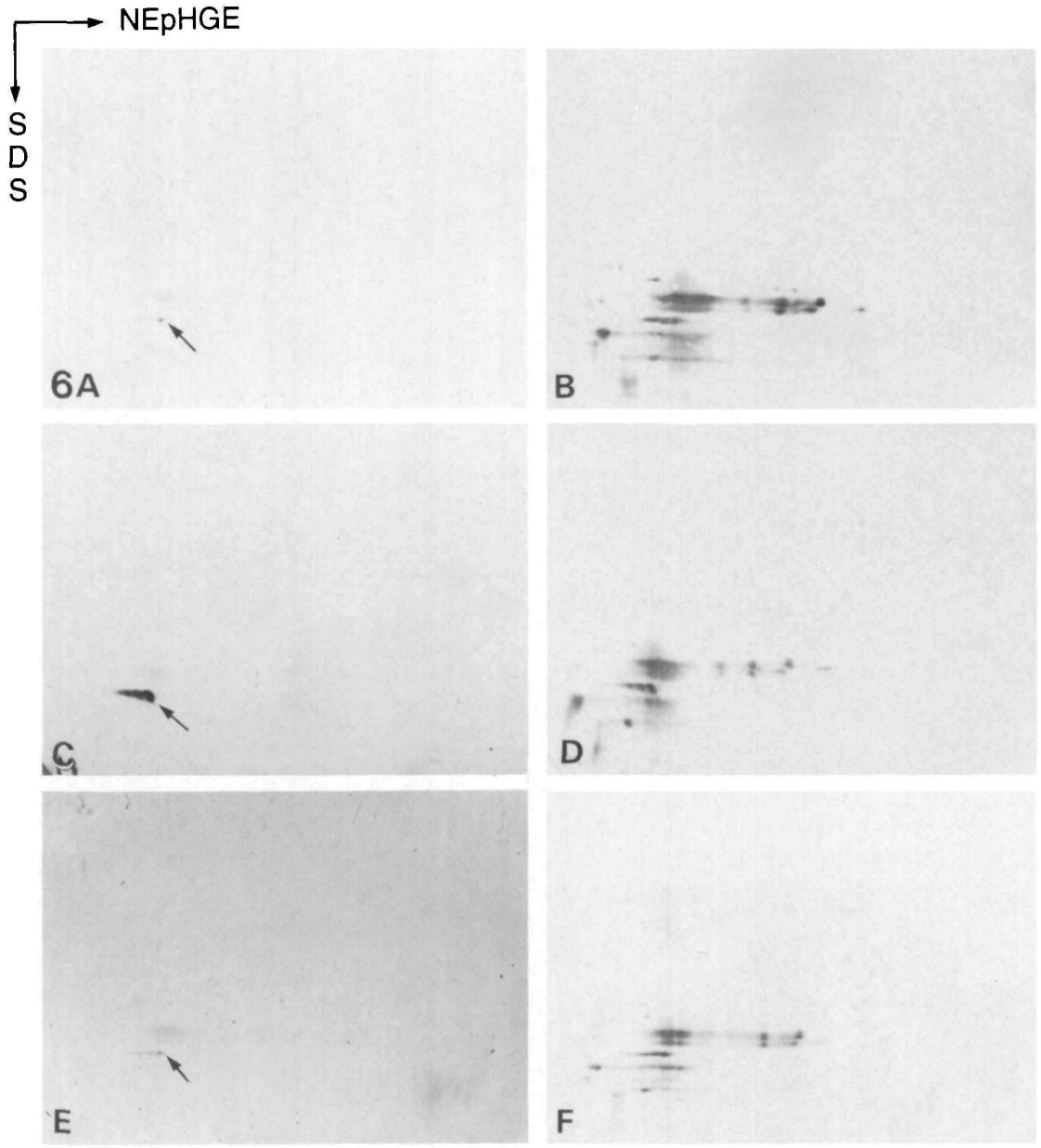

Fig. 6. Immunoblotting 2-D gels of TR146 cytoskeleton preparations with MAC 322 (A,B); an anti-CK8 mAb, M20 (C,D) and another CK-8 mAb, LE41 (E,F). The NEpHGE dimension is horizontal, the SDS–PAGE dimension vertical; basic proteins to the left, acidic to the right. The left-hand column contains the immunoblots; the right-hand column contains the same sheets of nitrocellulose stained with Pelikan ink to reveal total polypeptides. The arrows point to CK8.

to stain the plant cytoskeleton. For instance, anti-IFA immunostained the MT-associated system but not fibrillar bundles (Dawson *et al.* 1985). AFB, however, immunostained fibrillar bundles in carrot cells only when cells were treated with methanol to unmask the epitope (Hargreaves *et al.* 1989*a*); otherwise AFB stained the MT-associated system but never both under the same set of conditions (Goodbody *et al.* 1989). MAC 322, on the other hand, is able to stain FBs whether or not methanol is used and is likely, therefore, to be recognizing different epitopes from those recognized by the other two antibodies. The immunostaining of wheat root tip cells by MAC 322 is particularly clear. This improvement is partly due to the confocal microscopy but, nevertheless, the cortical arrays – not always recognized by immunofluorescence (Parke *et al.* 1987) – are clearly stained. The fibrogranular immunostaining is characteristic of the anti-IF antibodies successfully applied to plants. The granularity is especially evident in mitotic cells (e.g. Fig. 2E). Previous immunogold studies have established that this is due to the fact that electron-dense material along and between the MTs, rather than MTs themselves, is being recognized. It is not known for certain, therefore, whether there is a fine but collapsed fibrous network that co-distributes with MTs or whether the IF antigens in plants (other than the paracrystalline FBs) are non-filamentous. However, the fine, wispy staining pattern around the nuclei of BMS protoplasts (e.g. Fig. 3B) suggests a filamentous network.

The list of antibodies known to label this MT-associated system in higher plants is now reasonably extensive: anti-IFA (Dawson *et al.* 1985; Parke *et al.* 1987; Goodbody *et al.* 1989); mAbs against *Chlamydomonas* basal bodies (Parke

Fig. 7. Cross-reaction between MAC 322 and recombinant human CK8, expressed in the pUC-R1 PL16 plasmid vector in *E. coli* (lane a). Lane b contains protein of *E. coli* transformed with vector alone, without the inserted keratin sequence.

et al. 1987); 34g5 (Goodbody *et al.* 1989), which is a different mAb raised against *Chlamydomonas* basal bodies, and which recognizes vimentin (Klymkowsky, 1988); AFB (Hargreaves *et al.* 1989*a*; Goodbody *et al.* 1989); and now MAC 322. MAC 322, AFB and anti-IFA immunoblot or stain carrot fibrillar bundles, as does an anti-C tektin (Goodbody *et al.* 1990), which was characterized by Chang and Piperno (1987). These bundles can be solubilized in strong urea and upon dialysis against salt have been shown to reconstitute 10 nm filaments and bundles of filaments that immunoblot with anti-IFA and AFB (Hargreaves *et al.* 1989*b*). In this characteristic, they resemble animal IFs.

The detection of the anti-IFA epitope in plants (Dawson *et al.* 1985; Parke *et al.* 1987) emphasizes the broad basis of the relationship between plant and animal IF-like proteins, for this epitope on the highly conserved helix termination peptide (Geisler *et al.* 1983) occurs in all classes of cytoplasmic IFs as well as the lamins (Lebel and Raymond, 1987; Osborn and Weber, 1987). AFB (raised against carrot fibrillar bundles) indicates a relationship to type III animal IFs (Hargreaves *et al.* 1989*a*). The fact that MAC 322 is now found to recognize a type II cytokeratin is not at odds with this, since the relationship between plant and animal IF-like proteins is obviously an ancient one that predates the tissue-related speciation demonstrated by vertebrate IFs. Because keratin I and II genes contain some introns at homologous positions to those in desmin, vimentin and GFAP genes, it was suggested that type I, II and III genes share a common ancestral origin (Tyner *et al.* 1985). The sequencing of *Ascaris lumbricoides* IFs indicates a more primitive relationship. Weber *et al.* (1989) found that the nematode filament-forming proteins contain additional heptads in the coil IB segment that are characteristic of nuclear lamins from vertebrates and invertebrates and are not found in vertebrate IF proteins.

More recently, Doering and Stick (1990) compared the lamin LIII gene of *Xenopus laevis* with the gene structure of vertebrate and invertebrate type I-III IF genes. They provided further evidence that the tail domains of lamins and invertebrate IF proteins, but not those of vertebrate IF proteins, were homologous. Similarly, in an accompanying paper, Dodemont *et al.* (1990) compared the structure of the gastropod *Helix aspersa* IF gene with those of vertebrate and invertebrate IFs. These authors pointed out the identical exon/intron pattern in snail and mammalian type III IFs, some variation between snail and vertebrate type II IFs, and the quite different pattern of organization of vertebrate neurofilament genes. They suggest that the archetype IF gene arose from a lamin-like progenitor and, because plants contain cytoplasmic IFs (Hargreaves *et al.* 1989*b*), that the lamin/IF divergence had already occurred at that stage of metazoan evolution.

The expression of specifically different subsets of up to 40 cytokeratins is regulated in a highly differentiation-specific manner (Cooper *et al.* 1985; Moll *et al.* 1982) but embryonic cytokeratins behave differently. The first cytokeratins to be expressed in embryogenesis, CKs8 and 18, are also the primary simple epithelial keratins of adult tissues and are expressed across a significantly broader range of cell types, albeit at a lower level than in typical simple epithelial cells. They have been detected in oocytes (Franz *et al.* 1983), astroglial cells (Rungger-Brandle *et al.* 1989), smooth muscle cells (Norton *et al.* 1987; Brown *et al.* 1987) and certain other mesenchymal cell types (Khong *et al.* 1986; Jahn *et al.* 1987), as well as a wider range of tissue during embryogenesis (Viebahn *et al.* 1988; Page, 1989), regeneration and dedifferentiation (Ferretti *et al.* 1989; Stosiek *et al.* 1990). Expression of these two cytokeratins is often elevated, or appears anomalously, in malignant carcinomas (Schaafsma *et al.* 1990; Angus *et al.* 1988; Smedts *et al.* 1990). The DNA and amino acid sequence of CK 18 clearly distinguish it from the rest of the family of type 1 cytokeratins (Kulesh and Oshima, 1989), and the recent discovery that the genes for cytokeratins 8 and 18, alone amongst all the other coexpressed pairs of cytokeratin genes, are localized on the same human chromosome (Waseem *et al.* 1990*a*,*b*), although type I and type II cytokeratin gene clusters are otherwise on different chromosomes, has strengthened the concept of these embryonic cytokeratins being closer to an archetypal keratin gene than the others (Blumenberg, 1988). Thus, if any keratin were related to intermediate filament-like molecules within another kingdom, then one might anticipate such a relationship to exist with one of the simple epithelial keratins. However, how specific cytokeratin epitopes found in plants, type III IFs and lamins are related is unknown but should emerge from structural studies on plant IF genes.

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