

Transgenic Expression of the Muscle-specific Intermediate Filament Protein Desmin in Nonmuscle Cells

Frank R. Pieper,* Gert Schaart,† Paul J. Krimpenfort,§ Jos B. Henderik,* Han J. Moshage,|| Annemiete van de Kemp,* Frans C. Ramaekers,‡ Anton Berns,§ and Hans Bloemendal*

*Department of Biochemistry, University of Nijmegen, 6500 HB Nijmegen; †Department of Pathology, University Hospital Nijmegen, Geert Grooteplein Zuid 24, 6525 GA Nijmegen; § Division of Molecular Genetics, The Netherlands Cancer Institute, and the Department of Biochemistry, University of Amsterdam, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX, Amsterdam; and || Division of Gastrointestinal and Liver Diseases, Department of Medicine, University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands

Abstract. The coding region of the hamster desmin gene was fused to the 5' flanking sequences of the hamster vimentin gene and introduced into the germ line of mice. The expression of this intermediate filament gene construct (pVDes) was analyzed at the RNA and protein level in transgenic mice as well as in fibroblast cell lines and primary hepatocyte cultures derived from these mice. In all transgenic mice, the pVDes-encoded protein was coexpressed with mouse vimentin in a tissue-specific fashion and was indistinguishable from normal hamster desmin. Culturing of transgenic hepatocytes induced desmin expression indi-

cating that 3.2 kbp of the vimentin gene 5' region regulates both tissue-specific and tissue culture-induced intermediate filament protein expression. Immunohistochemical staining and double-label immunoelectron microscopy of cultured transgenic fibroblasts showed that the pVDes protein assembled into intermediate filaments which colocalized with the mouse vimentin filaments. Endogenous vimentin RNA levels were not influenced by high-level pVDes expression. The coexpression of desmin and vimentin in nonmuscle cells did not result in detectable developmental, morphological, or physiological abnormalities.

INTERMEDIATE filaments (IFs)¹ represent a unique group of cytoskeletal structures that occur in the cytoplasm of virtually all mammalian cells. The expression of the different classes of IF subunits is regulated in a tissue-specific and developmentally regulated fashion. Generally, cytokeratins are expressed in cells of epithelial origin while neurofilaments are expressed in neuronal cells, glial fibrillary acidic protein in astrocytes, desmin almost exclusively in striated and most smooth muscle cells, and vimentin mainly in cells of mesenchymal origin (58, 59, 64). The expression of the nuclear lamins, which are part of the IF multigene family, is also cell type specific and developmentally regulated (12, 29, 61). The highly conserved specificity of IF subunit expression during development suggests that each type of subunit plays an important role in cellular differentiation.

On the basis of gene structure and sequence data, the IF subunits have been divided into four subfamilies. Vimentin (47), desmin (48), glial fibrillary acidic protein (40), and possibly a recently described neurofilament protein (39) display a high degree of homology and constitute one subfamily of proteins (type III subunits). Like the other IF proteins,

they have a conserved central α -helical domain of ~ 310 amino acid residues, flanked by nonhelical amino-terminal and carboxy-terminal domains of variable length and sequence (12, 22, 58, 59, 64). In view of the highly specific expression pattern of each IF subunit, it may be assumed that their variable terminal regions at least partly determine the specific properties that are needed in different cell types during various stages of development (23, 38, 58).

The vimentin expression pattern is relatively complex. During embryogenesis, vimentin is the first of the nonepithelial subunits to be expressed (18, 30). The appearance of the cell type-specific IF subunits is often preceded by vimentin expression, and in certain cases coexpression with the other types of IF is observed (9, 19, 37, 56, 57, 64, 65). When non-mesenchymal cells are dissociated from tissues and brought into culture, induction of vimentin synthesis often occurs (15, 64). This is mostly accompanied by the continued coexpression of the tissue-specific IF protein, but in some cases only vimentin expression remains (6, 17). The level of vimentin expression is growth regulated and can be stimulated by some growth factors (11, 54). This complex expression is mediated by multiple regulatory elements in the 5' flanking sequence of the vimentin gene (46, 54).

In cultured cells, the study of cell type-specific regulation and function of IF expression is necessarily limited. The

1. *Abbreviation used in this paper:* IF, intermediate filament.

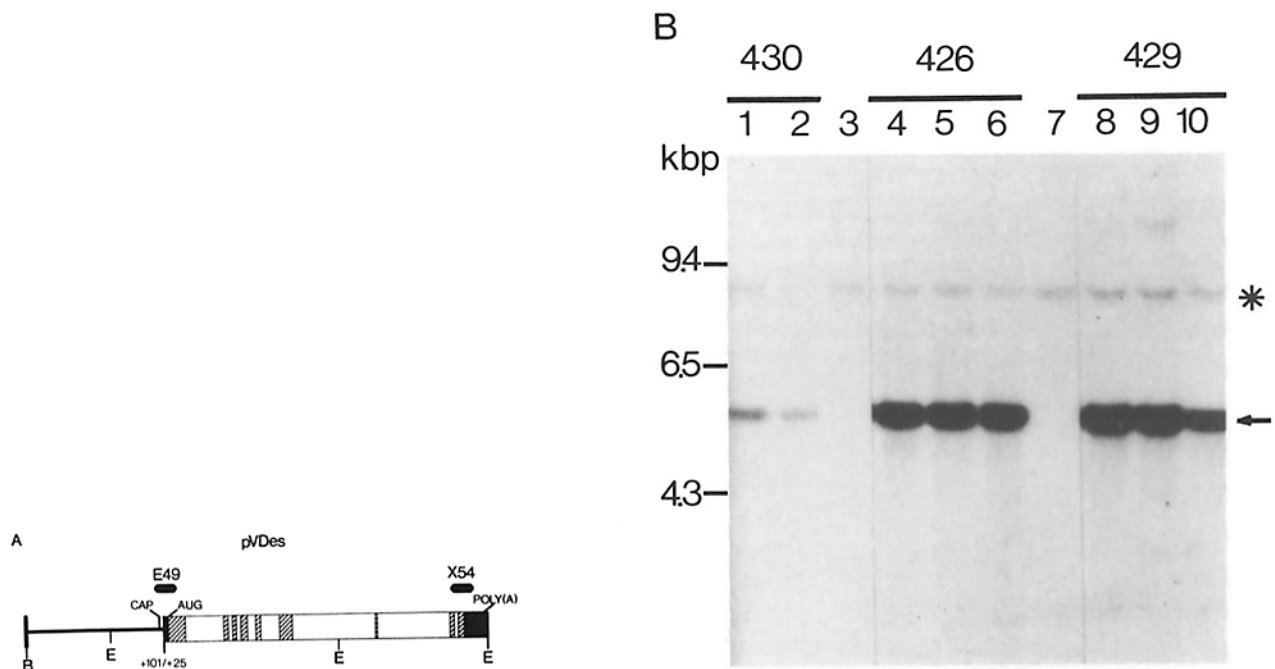


Figure 1. (A) Schematic representation of the IF gene construct pVDes (not drawn to scale). The line on the left represents the vimentin gene 5' flanking region (from $-3,100$ to $+101$ relative to vimentin cap site). Bar represents the desmin gene with black regions indicating the desmin 5' and 3' untranslated sequences, hatched regions indicating exons, and open regions indicating the introns. Positions of the vimentin-specific (E49) and desmin-specific (X54) probes are indicated. E, Eco RI restriction site; B, Bam HI restriction site. (B) Southern blot analysis of tail DNA from mice transgenic for pVDes. $\pm 10 \mu\text{g}$ of DNA was digested with Eco RI. Hybridization was performed with the vimentin probe E49. Asterisk indicates position of the 8.5-kbp Eco RI fragment from the single copy mouse vimentin gene. Arrow indicates position of the 5.15-kbp EcoRI fragment from pVDes. (Lane 1) DNA from offspring of founder 430; (lane 2) DNA from founder 430; (lanes 3 and 7) DNA from control mice; (lanes 4 and 5) DNA from offspring of founder 426; (lane 6) DNA from founder 426; (lanes 8 and 9) DNA from offspring of founder 429; and (lane 10) DNA from founder 429.

phenomenon of tissue culture-induced vimentin expression is not understood. The observations, that some cell lines do not express IFs (27, 41, 64) and that disruption of IFs does not affect growth or morphology of cultured cells (21, 34, 42), indicate that IFs—at least in vitro—do not fulfill an essential cellular function (13).

Transgenic mice, which have proven to be useful in the study of tissue-specific gene expression (for review, see reference 44), provide a better system to study IF gene regulation and function. Our approach was to change the normal IF expression pattern by introducing an IF gene construct into the germ line of mice composed of the 5' flanking region of the vimentin gene and the complete coding region of the desmin gene. Transgenic mice, primary cell cultures, and cell lines derived from these mice were analyzed for the pattern and levels of desmin and vimentin expression.

Materials and Methods

Plasmid Construction

For construction of the pVDes gene, the 0.7-kbp Hpa II–Bam HI fragment from the hamster desmin gene (from $+25$ bp to $+700$ bp relative to the cap site; reference 48) was subcloned into an Acc I–Bam HI-digested pUC19 plasmid. The 3.2-kbp Bam HI–Eco RI (from $+700$ to $+3,900$) and the 3.4-kbp Eco RI–Stu I (from $+3,900$ to $+7,300$) desmin fragments were ligated immediately 3' to this fragment, thereby generating a complete desmin gene (pDes) without 5' sequences upstream of the Hpa II site at $+25$. In this construct, 60 bp of 5' and 775 bp of 3' untranslated sequences (including the "poly A" signal) are present. The 3.2-kbp vimentin promoter region (ranging from $-3,100$ to $+101$ relative to the vimentin cap site) was isolated as a

Hind III fragment by ligating the 3.45-kbp Bam HI–Eco RI fragment from p3.1VimCAT (46) into an Eco RI–Bam HI-digested pUC19 plasmid, and subsequent partial Hind III digestion of this clone. This vimentin 5' region was ligated into the Hind III site of the pUC19 polylinker of pDes in the 5'–3' orientation. The resulting construct (pVDes) contains some additional base pairs from the pUC19 polylinker between the Hind III and the Acc I site. To facilitate removal of plasmid sequences before microinjection, the Bam HI site in the first intron of desmin was removed by filling in and subsequent blunt end ligation after partial Bam HI digestion.

Generation and Identification of Transgenic Mice

Transgenic mice were generated by pronuclear microinjection as described previously (36). Plasmid sequences were removed by Bam HI (5', complete) and Eco RI (3', partial) digestion, leaving a 10.5-kbp IF hybrid gene. This was isolated and purified by preparative gel electrophoresis and electroelution, dissolved in ultrapure water, and dialyzed against 10 mM Tris-HCl, 0.1 M EDTA (pH 7.4). The DNA concentration was adjusted to $4 \mu\text{g}/\text{ml}$, and ± 200 pVDes copies were injected. Several weeks after birth of animals that had developed from microinjected eggs, tail DNA was isolated and analyzed by Southern blotting. Briefly, equal amounts ($10 \mu\text{g}$) of total genomic DNA of each mouse was digested with the appropriate restriction enzyme(s), run on a 0.6% agarose gel, and transferred to nitrocellulose. The filter was hybridized to ^{32}P -labeled probes (see below) and washed as described (5). The number of integrated pVDes copies was determined by Southern blot analysis of serial dilutions of tail DNA, using the vimentin-specific probe E49, and subsequent densitometric scanning of the autoradiographs. pVDes hybridization signals were compared to those of the single copy mouse vimentin gene and of serial dilutions of the pVDes plasmid.

Northern Blot Analysis and Hybridization Probes

Total cellular RNA was isolated from tissues and cultured cells by the LiCl-urea method of Auffray and Rougeon (1). Primer extension analysis on total cellular RNA from ear fibroblast cell lines was performed as described (24,

procedure 2), using a 21-mer 5' desmin primer (5'-GGAGGCAGCGGG-CAGGCAGCCC-3'; from +25 to +46 relative to the transcription initiation site). For Northern blotting, 10-, 15-, or 20- μ g RNA samples were glyoxylated, fractionated on a 1.2% agarose gel, and transferred to Highbond-N (Amersham International, Amersham, UK). Hybridization was performed as described by Church and Gilbert (5). A 350-bp hamster desmin Sau3A fragment in M13 (X54), covering a region from 25 bp of exon 8 to 120 bp into the 3' untranslated region, was used as a desmin probe (46, 48). A 520-bp hamster vimentin Sau3A fragment in M13, ranging from -150 to +370 bp relative to the cap site, was used as a vimentin probe (46, 47). As an actin probe, we used a 1.25-kbp PstI hamster actin cDNA fragment (8), which hybridizes to α -, β -, and γ -actin. Densitometric scanning was performed on autoradiographs exposed for different times.

Cell Culture

Ear fibroblast cell lines were established and cultured as described (3).

Mouse hepatocytes were isolated and cultured using the hepatic portal perfusion method as described by Klaunig et al. (32, 33), with the following modifications. Instead of Hanks' solution, Williams E medium (Flow Laboratories, Inc., McLean, VA) supplemented with 2.5 mM EGTA was used. Hepatocytes were cultured in Williams E medium supplemented with 10% FCS, 2 mM L-glutamine, 20 mU/ml bovine insulin, 1 μ M dexamethasone, and antibiotics (25 μ g/ml fungizone; 100 μ g/ml vancomycin; 50 μ g/ml gentamycin).

Gel Electrophoresis and Immunoblotting

Cytoskeletal preparations of eye lens and cultured cells were obtained as described (35). One- and two-dimensional SDS gel electrophoresis and immunoblotting procedures have also been described previously (4).

Immunohistochemical Analysis

Single- and double-label indirect immunofluorescence analyses of frozen tissue sections and cultured cells were performed as described previously (36). In the underlying study, however, muscle tissue sections were pretreated with 0.5% Triton X-100 in PBS before incubation with the first antibodies. The following polyclonal and monoclonal antibodies were used: (a) an affinity-purified polyclonal rabbit antibody (poly-ker) raised against human skin keratins, which reacts with virtually all epithelial tissues (51); (b) an affinity-purified polyclonal rabbit antibody (poly-vim) to vimentin (51); (c) a polyclonal rabbit antibody (poly-des) to desmin (52); (d) the monoclonal antibody RV202 to vimentin (4); (e) the monoclonal antibody RD301 to desmin (4); and (f) the monoclonal antibody RCK 102 to human cytokeratins 5 and 8 (53).

Immunoelectron Microscopy

Transformed fibroblasts from control and transgenic mice were grown on Thermanox coverslips (Lux, Lab-Tek Div., Miles Laboratories Inc., Naperville, IL), coated with fibronectin by a 30-min incubation with a crude preparation from human serum. Cells at ~75% confluency were washed with double Hanks' buffer and converted to cytoskeletons by extraction for 2.5 min at room temperature with a buffer (pH 7.2) containing 0.5% Triton X-100 as described by Tölle et al. (63), and fixed in buffered 0.5% paraformaldehyde containing 0.3% Triton X-100 (pH 7.2). The cells were processed for immunogold labeling and electron microscopy essentially as described by De Mey (7) and as in AuroProbe EM product information (Janssen Pharmaceutica, Beerse, Belgium). To reduce background staining the coverslips were preincubated for 20 min with a TBS solution (pH 8.2) containing 10% normal goat serum and 0.1% BSA. The monoclonal and polyclonal antibodies were diluted in a similar buffer containing 1% normal goat serum and 0.1% Tween-20. Washing buffers also contained 0.1% Tween-20. Furthermore, the 5-nm and 10-nm antibody-coated colloidal gold preparations (Janssen Pharmaceutica) were preabsorbed extensively on fibroblast cytoskeletons and diluted in Tris buffer, pH 8.2, containing 1% BSA.

Results

Generation of Transgenic Mice

We have demonstrated previously that the structural gene for hamster desmin and modifications thereof can be expressed after gene transfer into different types of cultured nonmuscle

cells resulting in assembly of the newly synthesized protein into intermediate filaments (28, 48). Regulatory sequences which control desmin expression are located in a region between -89 and +25 relative to the transcription initiation site (46). We removed these regulatory elements by deleting the complete 5' flanking region of the desmin gene up to the HpaII site at position +25, and replaced it by 3.2 kbp of 5' flanking sequences (-3,100-+101) from the hamster vimentin gene (Fig. 1 A). This upstream region contains regulatory elements that confer high levels of vimentin expression in cultured cells and are instrumental in the down-regulation of vimentin expression during myogenesis (46). The resulting IF gene construct pVDes (10.5 kbp without plasmid sequences) consists of the complete coding region of the desmin gene and additional 5' and 3' untranslated regions, fused to 3.2 kbp of vimentin upstream sequences (Fig. 1 A; see Materials and Methods).

After removal of plasmid sequences the pVDes gene was injected into the most accessible pronucleus of fertilized mouse eggs. Southern blot analysis of tail DNA of mice born from these manipulated eggs showed that 5 of 32 mice had incorporated multiple copies of the construct into their genome in a head-to-tail tandem array. Three of these (strains 426, 429, and 430) produced offspring, all of which inherited the pVDes transgene (Fig. 1 B). Southern blot analysis revealed no detectable rearrangements in the pVDes sequences (data not shown). Offspring from founders 426 and 429 contained ~25 and 30 copies, respectively, whereas 430 and its offspring had incorporated only 3 pVDes copies. F₁ and F₂ analysis showed that integration had taken place into single loci.

Tissue-specific pVDes Expression

The desmin expression pattern in tissue sections from transgenic mouse strains 426, 429, and 430 and from control mice was analyzed by the indirect immunofluorescence technique, using polyclonal rabbit antisera directed against desmin (poly-des), vimentin (poly-vim), and cytokeratins (poly-ker) (51, 52). From transgenic mice 427 and 428 only tail sections were examined. From mouse strains 426, 429, and 430 a number of tissues was analyzed and for each of these strains at least two male and female mice were used.

Cells expressing vimentin but not desmin in tissues from control mice (e.g., fibroblasts, endothelial cells, cartilage, Kupffer cells, macrophages, Schwann cells) displayed intensive staining in transgenic tissues after incubation with poly-des and with poly-vim (Fig. 2). Occasionally, a filamentous staining pattern was observed, suggesting that the pVDes-encoded desmin had assembled into IFs (Fig. 2, *g* and *h*). Epithelial tissues stained with poly-ker but not with poly-des. In transgenic striated muscle tissue, connective tissue fibroblasts not only expressed vimentin (Fig. 2 *i*) but also desmin, both in skeletal (Fig. 2 *k*) and heart muscle (not shown). All five transgenic mice and their offspring (from 426, 429, and 430) expressed pVDes in a vimentin-specific fashion. Surprisingly, in testis from the three transgenic mouse strains Sertoli and Leydig cells, although expressing vimentin, deviated from all other tissues tested (listed in legend to Fig. 2) in that they did not contain detectable amounts of desmin (Fig. 2, *l-o*).

We did not observe any morphological effects of pVDes ex-

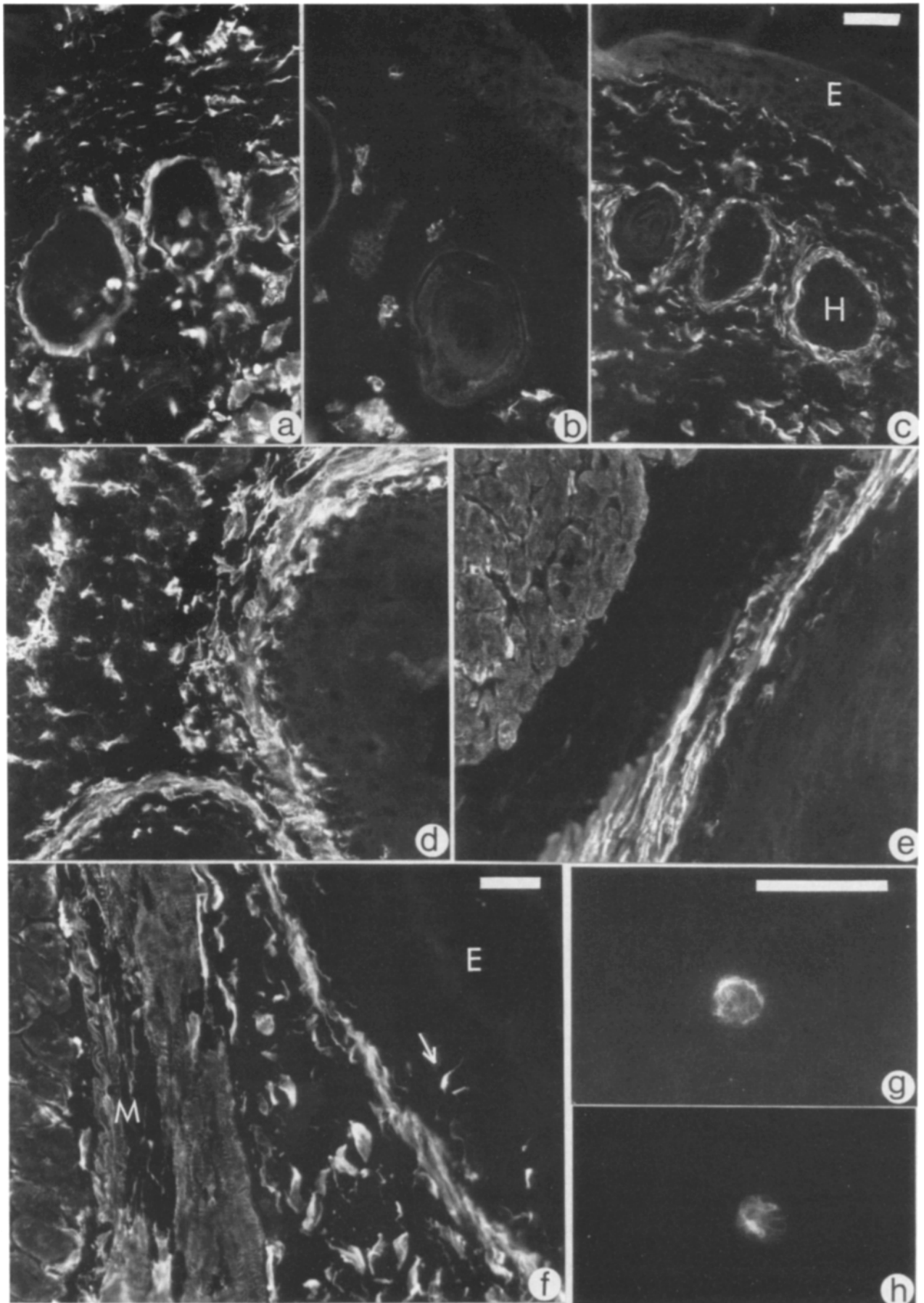
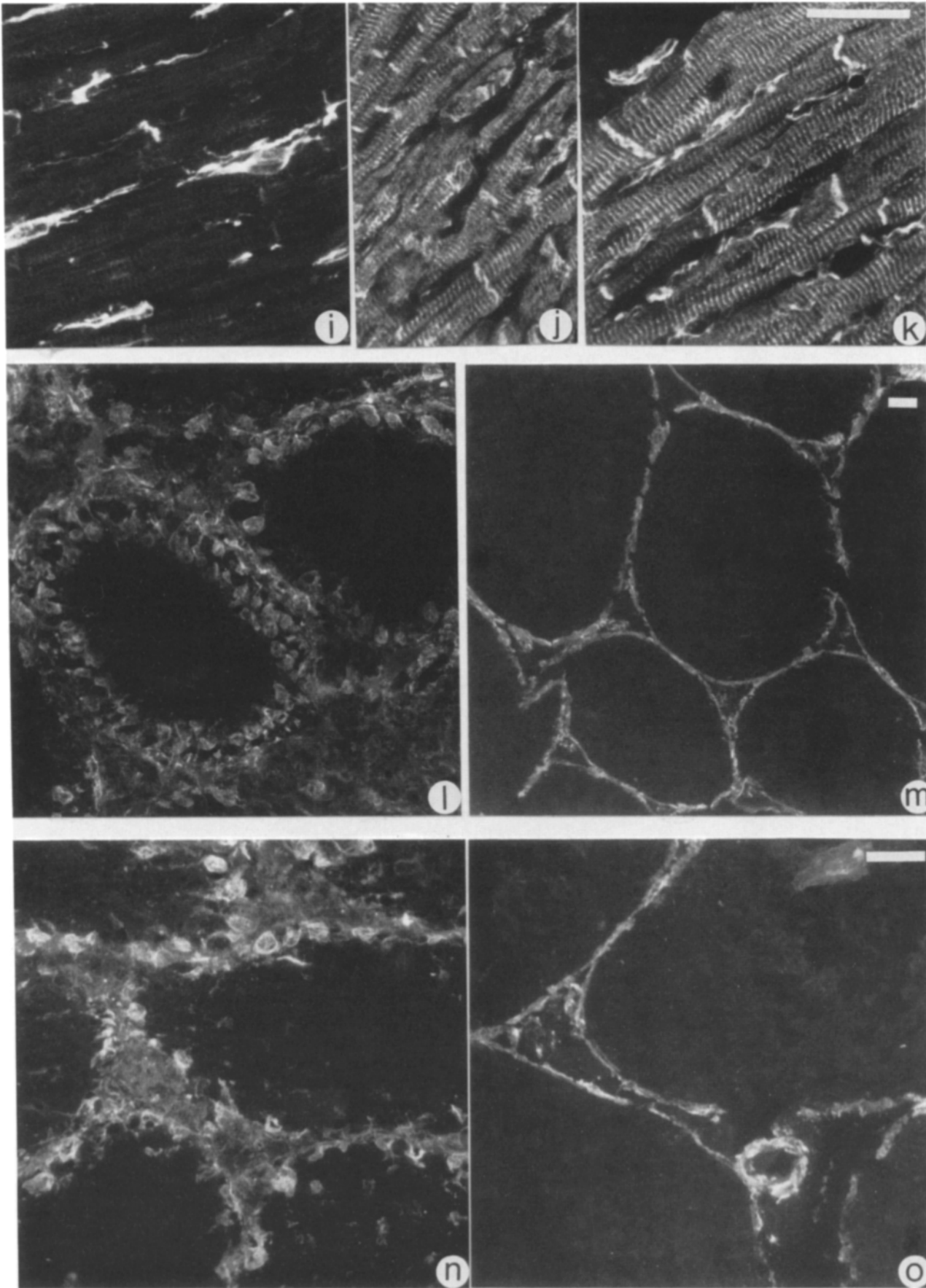


Figure 2. Expression of desmin and vimentin in tissues from control and transgenic mice as detected with the indirect immunofluorescence assay using poly-des and poly-vim. (a-c) Tail skin from control (a and b) and transgenic (c) mice incubated with poly-vim (a) and poly-des (b and c). *E*, epidermis; *H*, hair follicle. (d-f) Esophagus from control (d and e) and transgenic (f) mice incubated with poly-vim (d) and poly-des (e and f). Arrow indicates desmin-positive Langerhans cells. *M*, muscle tissue; *E*, epithelial tissue. (g and h) Cells from



blood smears of transgenic mice showing a filamentous staining pattern after incubation with poly-des. (*i-k*) striated muscle tissue of control (*i* and *j*) and transgenic (*k*) mice incubated with poly-vim (*i*) and poly-des (*j* and *k*). Note staining of transgenic muscle and nonmuscle cells with poly-des. (*l-o*) Testis tissue from control (*l* and *m*) and transgenic (*n* and *o*) mice incubated with poly-vim (*l* and *n*) and poly-des (*m* and *o*). Note presence of desmin only in myoid cells and blood vessel smooth muscle cells in both control and transgenic testis tissues. Other tissues investigated include kidney, liver, spleen, heart, brain, tongue, bladder, uterus, and prostate. Bars, 4 μ m.

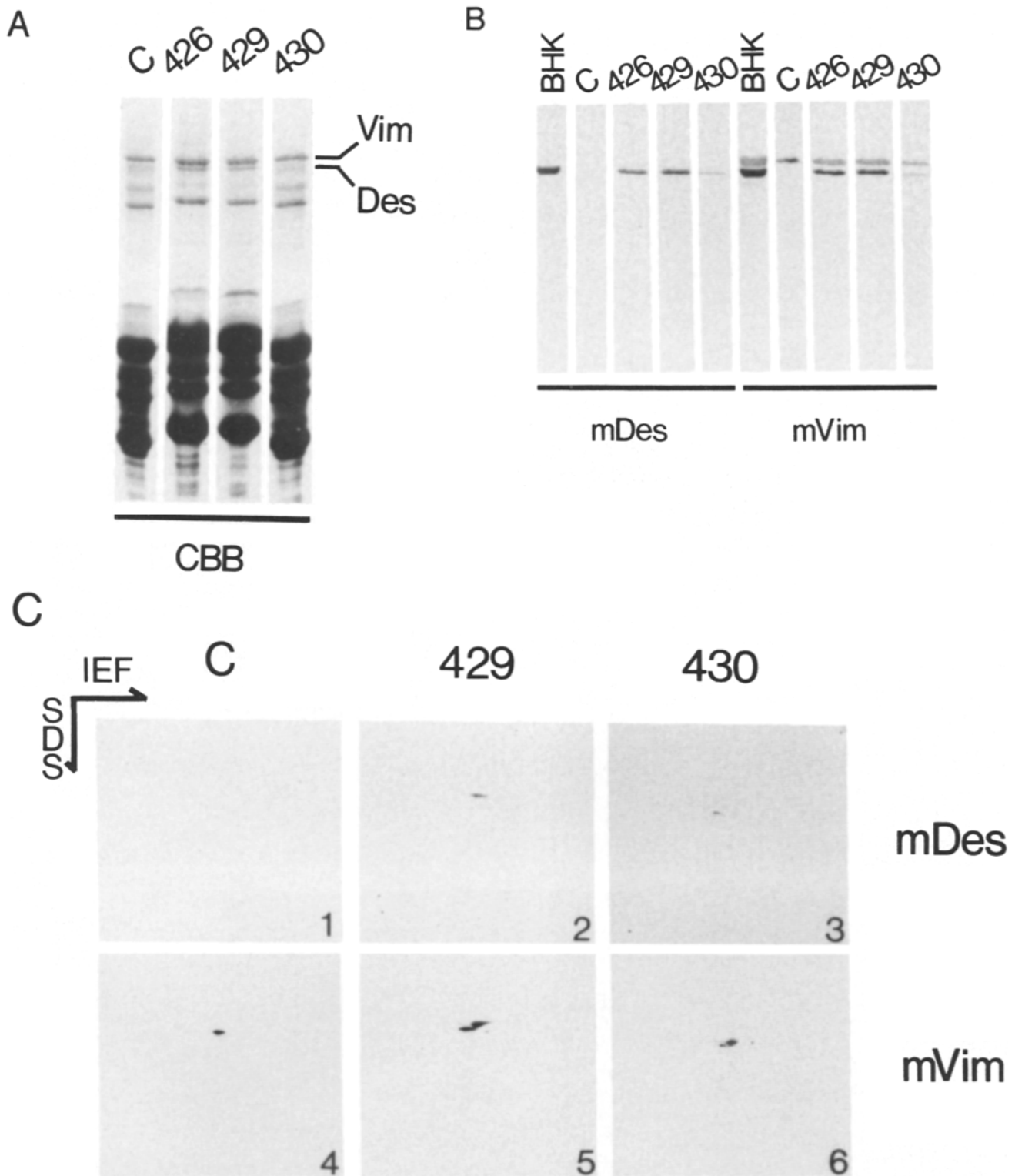


Figure 3. Characterization of pVDes protein from eye lens. (A) Coomassie Brilliant Blue (CBB)-stained SDS-polyacrylamide gels with equal amounts of total lens extracts from control (C) and transgenic mice (strains 426, 429, and 430). (B) Immunoblots of total lens extracts from control (C) and transgenic mice (strains 426, 429, and 430) incubated with the monoclonal antibody to desmin (RD301; *mDes*) and then with the monoclonal antibody to vimentin (RV202; *mVim*). Cytoskeletal proteins from BHK-21 cells were used as markers. (C) Total lens extracts of control (1 and 4) and transgenic mice from strains 429 (2 and 5) and 430 (3 and 6) analyzed by two-dimensional PAGE and immunoblotting. Blots were incubated with RD301 (*mDes*) and then with RV202 (*mVim*).

pression, nor did the transgenic mice show detectable developmental or physiological abnormalities.

Characterization of *In Vivo*-synthesized pVDes Protein

To identify biochemically and characterize the *in vivo*-synthesized pVDes protein, we analyzed eye lens extracts from transgenic mouse strains 426, 429, and 430. Eye lens tissue has the advantage of expressing high levels of vimentin in the absence of desmin (50). One-dimensional SDS-PAGE analysis of total lens extracts (Fig. 3 A) and cytoskeletal fractions (not shown), followed by immunoblotting with monoclonal antibodies to desmin (RD301; 4) and vimentin (RV202; 4), showed that the pVDes protein is expressed in transgenic lenses (Fig. 3, A and B). Immunoblotting of two-dimensional gels from total lens extracts (Fig. 3 C) confirmed the identity of the pVDes protein, which comigrated with desmin from BHK-21 hamster cells (not shown). Eye lenses from strains 426 and 429 contain comparable amounts of desmin. A relatively low concentration of hamster desmin was detected in strain 430 lenses (Fig. 3). As judged from Coomassie Brilliant Blue-stained gels and immunoblots of both total lens extracts and cytoskeletal fractions, lens vimentin expression levels were not influenced by pVDes expression.

***In Vivo* Expression Levels of pVDes**

To determine the pVDes mRNA expression levels in different tissues and its influence on endogenous vimentin and desmin expression, RNA from transgenic and control tissues was analyzed by Northern blotting. We also included RNA from transgenic mice which express the vimentin-desmin hybrid gene pVVim2 (36). In this construct, the last three exons of the vimentin gene have been replaced by the last three exons of the desmin gene, allowing the detection of the pVVim2 transcript with a desmin-specific probe. Blots were first hybridized to a vimentin-specific probe (E49; Fig. 1 A), which recognizes both endogenous vimentin, pVDes and pVVim2 transcripts, and subsequently to a desmin-specific probe (X54; Fig. 1 A). In control tissues only a 2.0-kb mRNA band was detected, representing the mouse vimentin messenger (e.g., Fig. 4 A, lane 4). In transgenic tissues an additional 2.4- or 2.3-kb band was observed that corresponds to the correctly sized pVDes transcript (e.g., Fig. 4 A, lanes 1-3) or the pVVim2 transcript, respectively (e.g., Fig. 4 A, lanes 5 and 6). Primer extension analysis showed that transcription started at the authentic initiation site (data not shown). Expression levels were determined by densitometric scanning of autoradiographs from blots containing equal amounts of RNA. Actin mRNA levels served as a standard. The levels of vimentin mRNA did not differ between control and transgenic tissues (Fig. 4). Relative to mouse vimentin mRNA, the amount of pVDes transcripts varied between different tissues. In skeletal muscle tissue, pVDes expression was relatively high, exceeding mouse vimentin transcript levels (Fig. 4 A). Both pVDes and pVVim2 mRNA levels were identical for skeletal muscle tissue from different parts of the body (Fig. 4 A, lanes 1, 2, 5, and 6). The lowest pVDes expression levels were detected in heart tissue (Fig. 4 B). Again, no pVDes expression was observed in testis from different transgenic strains (Fig. 4 C) indicating that the lack of expression in Sertoli and Leydig cells is caused at the level

of transcription or as a result of mRNA instability. In contrast, pVVim2 was expressed at high levels in testis of transgenic mice (Fig. 4 C, lane 4). Generally, pVDes expression levels were higher in tissues from strain 429 than in corresponding tissues from strain 426 (data not shown). An exception is skeletal muscle tissue, which in strain 426 contained almost twice the amount of pVDes mRNA found in strain 429 (Fig. 4 C). The relative levels of pVVim2 expression also varied between different tissues and were highest in skeletal muscle.

***In Vitro* Expression of pVDes**

Transgenic cell lines were established by immortalization of ear-shell fibroblasts from offspring of strains 426, 429, and 430 with SV40. Double-label immunofluorescence assays showed that in each of these cell lines both desmin and vimentin are expressed and assembled into filamentous structures (Fig. 5 A). In assays where the polyclonal vimentin antiserum and the monoclonal antibody to desmin were combined, vimentin and desmin staining intensities were about equal for cell lines 426 and 429, whereas cells from line 430 showed a much weaker desmin staining intensity. Upon incubation of line 430 fibroblasts with poly-des, this difference was not observed. Generally, there was a complete match of desmin and vimentin filaments (Fig. 5 A, c and d). Double-immunogold labeling of control and transgenic fibroblasts revealed that the pVDes protein coassembled into bona fide IFs together with endogenous vimentin (Fig. 5 B, a-e).

The pVDes and vimentin expression levels of the transgenic cell lines were analyzed by Northern blot analysis using desmin- and vimentin-specific probes (Fig. 6, A and B). The pVDes transcript was of the expected size (2.4 kb) and was detected in all three cell lines (Fig. 6 A). For cell line 430, the pVDes mRNA level was 20-fold lower than that of mouse vimentin mRNA. In contrast, cell lines 426 and 429 showed pVDes mRNA levels that were similar to those of the endogenous vimentin. The amount of vimentin mRNA (2.0 kb) was not influenced by pVDes expression (Fig. 6 B). This was also observed for a pVVim2 expressing ear-shell fibroblast cell line (strain 32; 36), which was cultured in parallel (Fig. 6 B). As in transgenic tissues, pVDes mRNA was less abundant than pVVim2 mRNA.

To identify the pVDes protein in the fibroblast cell lines and determine the correlation between transcript and protein levels *in vitro*, the relative amounts of IF protein in cytoskeletal fractions of the transgenic cell lines were analyzed by one-dimensional SDS-PAGE (Fig. 7). Desmin and vimentin were identified by immunoblotting with monoclonal antibodies to desmin (RD301) and vimentin (RV202), subsequently (Fig. 7 B). The pVDes protein has an apparent molecular mass of 54 kD and cannot be distinguished from hamster desmin of BHK-21 cells. Coomassie Brilliant Blue-stained gels reflected the levels of IF mRNA (Fig. 6) at the protein level (Fig. 7 A).

Induction of pVDes Expression in Tissue Culture

In liver tissue, hepatocytes express specific cytokeratins and completely lack vimentin (16). Culturing of rat hepatocytes induces vimentin expression (17). Indirect immunofluorescence assays with poly-vim, poly-des, and poly-ker on frozen sections of liver from control and transgenic mice confirmed

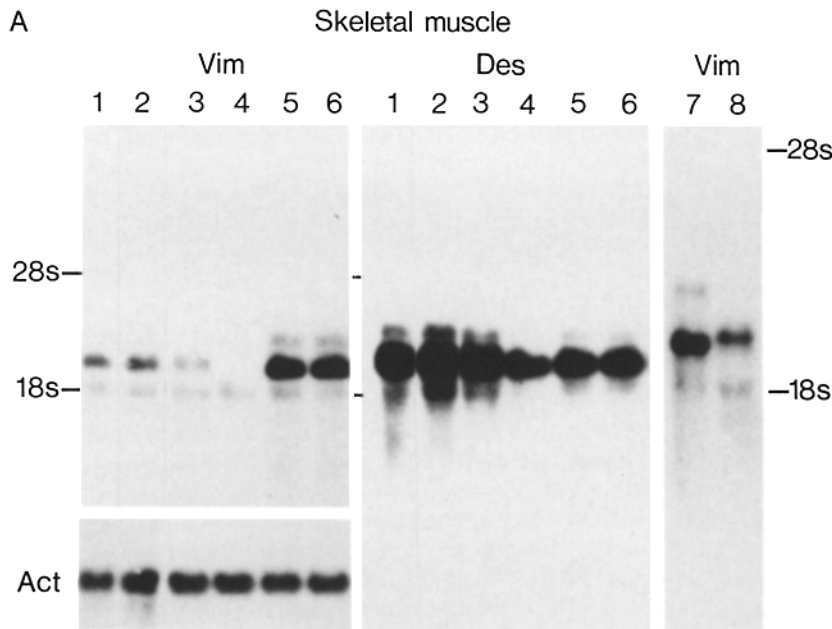


Figure 4. Northern blot analysis of 15 μ g of total RNA from skeletal muscle (A), heart (B), and testis (C) from control and transgenic mice. Blots were hybridized to a vimentin (E49; *Vim*) and then to a desmin-specific probe (X54; *Des*). The minor additional band above the desmin mRNA position was observed in all tissues and probably represents a splicing intermediate or is derived from a cryptic initiation site. After dehybridization the blots were hybridized to an actin probe (*Act*) that recognizes both α -actin (1.8 kbp) and β/γ -actin transcripts (2.1 kbp). A similar analysis was performed on RNA from kidney, liver, spleen, brain, and lens (results not shown). (A) RNA from skeletal muscle of abdomen (lanes 1, 3, 4, and 5) and thigh (lanes 2 and 6). (Lanes 1, 2, and 8) pVDes strain 426; (lanes 3) 429; (lanes 4) control; (lanes 5–7) pVVim2 strain 44. To illustrate the difference in length between pVVim2 and pVDes transcripts (2.3 and 2.4 kbp, respectively) RNA from strains 44 and 426 was run on a longer gel and hybridized to E49 (lanes 7 and 8). (B) RNA from heart tissue. (Lanes 1) pVDes strain 426; (lanes 2) 429; (lanes 3) control; (lanes 4) pVVim2 strain 44. To identify positively pVdes and endogenous desmin mRNA, longer exposure times, low stringency washes, and longer gels were used (lanes 2' and 3'). (Lane 2') As for lane 2, hybridized to E49; (lane 2'') as for lane 2', hybridized to E49 and X54, subsequently; (lane 3') as for lane 3 hybridized to E49 and X54, subsequently. (lanes 1–4) Exposure time and gel length as in Fig. 4, a and c. (C) RNA from testis. (Lanes 1) pVDes strain 426; (lanes 2) 429; (lanes 3) control; and (lanes 4) pVVim2 strain 44. After hybridization to X54 and long exposure times, a faint 2.3-kbp band was observed in lanes 1–3, representing endogenous desmin (not shown).

that hepatocytes express only cytokeratins, whereas Kupffer cells express vimentin in control mice and also desmin in transgenic mice.

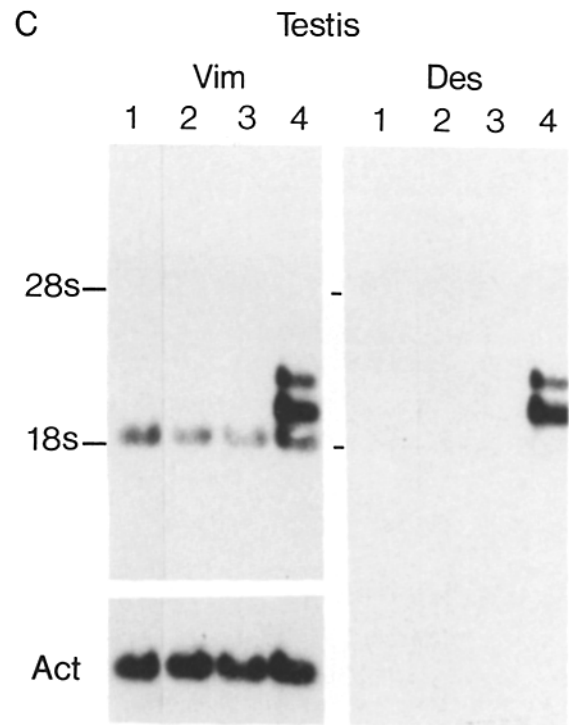
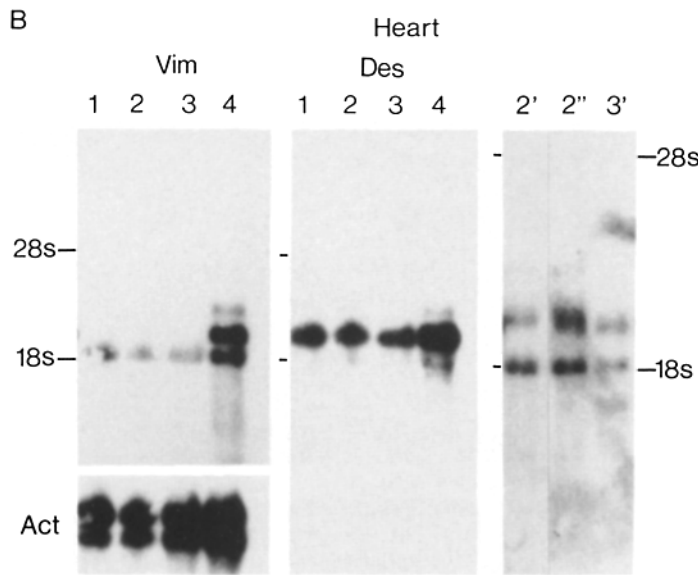
Hepatocytes from control and transgenic mice (strains 426 and 429) were isolated, cultured as a monolayer, and analyzed for IF expression by the indirect immunofluorescence assay during 4 d of culture. In all cases, the onset of vimentin synthesis was observed after 12–14 h (Fig. 8). At this point, vimentin expression occurred in only few cells and staining intensities were low. In addition, the filaments were not distributed uniformly throughout the cell but were located at the periphery (Fig. 8). Within the next 30–40 h, the number of vimentin-containing cells and the amount of vimentin in each cell increased until almost every cell was vimentin positive. Cytokeratins were expressed continuously and did not colocalize with vimentin filaments (Fig. 8, f and g). About 25 h after the earliest detection of vimentin in the immunofluorescence assays, hepatocytes from transgenic mice of strain 429 initiated expression of desmin (Fig. 9, a and b). Again, a gradual increase in expression over a 40-h period was observed until most cells were desmin positive. In control hepatocytes, desmin expression was never observed. Eventually, desmin and vimentin staining patterns were identical, suggesting copolymerization of these proteins. In cultured hepatocytes from four different transgenic mice of strain 426, desmin expression could not be detected (Fig. 9, e and f). Each of these mice, however, showed the tissue-specific expression pattern that is characteristic of all pVDes transgenic mice and, as described, pVDes levels in strain 426 were generally comparable to those of strain 429, both in tis-

sues and in the fibroblast cell lines. To extend our analysis, hepatocytes of the two different pVVim2 transgenic mouse strains (Nos. 32 and 44; reference 36) were also brought into culture. Hepatocytes from these mice displayed vimentin and cytokeratin expression patterns, which did not differ from those of control and pVDes hepatocytes. Initiation of pVVim2 expression was observed 1 d later than vimentin expression.

In summary, tissue culture-induced IF gene construct expression occurred in three out of four transgenic strains.

Discussion

Expression of desmin in adult mammalian tissues occurs almost exclusively in muscle cells. We have changed the tissue-specific distribution of this IF protein by creating transgenic mice, which express the IF gene construct pVDes, composed of the coding region of the hamster desmin gene and the 5' flanking region of the vimentin gene, in a vimentin-specific fashion. The pVDes protein was indistinguishable from hamster desmin. The levels of pVDes protein recovered in cytoskeletal fractions from transgenic cell lines correlated with the pVDes mRNA levels, supporting the notion that IF synthesis is determined primarily by the amount of mRNA present (43, 46, 54, 58). Double-label immunofluorescence assays and double-immunogold labeling of cultured transgenic ear fibroblast cells showed that the pVDes protein assembled into IFs that colocalized with the endogenous vimentin filaments. This is in agreement with previous reports concerning coexpression of desmin and vimentin in different types of muscle cells (49, 62). Taken together, the data indicate that



the pVDes-encoded desmin subunits are incorporated into the endogenous vimentin filaments.

The *in vivo* expression pattern of the pVDes protein was determined by immunohistochemical analysis of tissue sections from different transgenic strains. Each transgenic mouse and all offspring expressed hamster desmin in a vimentin-specific fashion. pVDes expression did not detectably affect cellular morphology or differentiation. Desmin synthesis in vimentin-negative cells was never observed. Surprisingly, no pVDes expression was detected in Sertoli and Leydig cells of all three lines of transgenic mice although these cells do express vimentin (12, 66, 67; Fig. 2). This lack of expression is either caused at the transcriptional level or by pVDes mRNA instability since no pVDes transcripts were detected in transgenic testis (Fig. 4 C). In contrast, an IF gene construct (pVVim2), consisting of the 5' flanking region and first six exons plus introns of the vimentin gene, fused to the last three exons and introns of the desmin gene is expressed in these testicular cells. Since regulatory elements have been identified within introns of some genes (2, 26, 35, 45, 55, 60), it is possible that such elements are located within the first six introns of the vimentin gene.

An important aspect of gene regulation in general is the establishment of the appropriate level of expression. For vimentin, questions concerning the mechanisms of this aspect of regulation cannot be addressed by the use of cultured cells only, since it is unclear whether or not expression levels are elevated upon culturing of vimentin-expressing cells, overruling regulatory mechanisms active *in vivo*. We show here that endogenous vimentin mRNA and protein levels were not influenced by pVDes expression. Obviously, the amounts of IF protein are not strictly regulated and can be elevated without deleterious effects.

The highest pVDes transcript levels were detected in skeletal muscle tissue (Fig. 4). Immunohistochemical staining of striated muscle tissue sections showed that nonmuscle cells, which are included in this tissue, express both vimentin and pVDes (Fig. 2). Transgenic myoblasts presumably also express these proteins. Since the pVDes encoded hamster desmin is indistinguishable from endogenous mouse desmin in these stainings, we cannot exclude the possibility that pVDes is also expressed in muscle cells of transgenic mice; e.g., as a result of the presence of muscle-specific regulatory sequences within the introns of the desmin gene in addition to those present in the desmin gene 5' region (46). However, pVVim2 mRNA levels were also highest in skeletal muscle tissue (Fig. 4), but it has been shown that the pVVim2 protein is not expressed in transgenic striated muscle cells (36). Furthermore, in transgenic heart tissue pVDes mRNA levels were low although endogenous desmin is expressed at high levels (Fig. 4 B).

Our studies on cultured hepatocytes establish that sequences in the 3.2 kbp of 5' flanking region of the vimentin gene confer tissue culture-induced vimentin expression. In hepatocytes from pVDes strain 429, desmin protein expression was first observed after 40–50 h of culture, and lagged ~25 h behind the initiation of vimentin synthesis. A similar time lag was observed in hepatocytes from pVVim2-expressing mice. pVDes and pVVim2 transcripts also were detected ~1 d later than vimentin mRNA (data not shown). This lapse may result from differences in mRNA stabilities. On the other hand, species differences in *cis* regulatory sequences of the hamster and mouse vimentin genes could explain the observed delay in desmin synthesis. Reports on vimentin expression during avian and mammalian hematopoiesis have shown that divergence of *cis*-linked sequences can result in

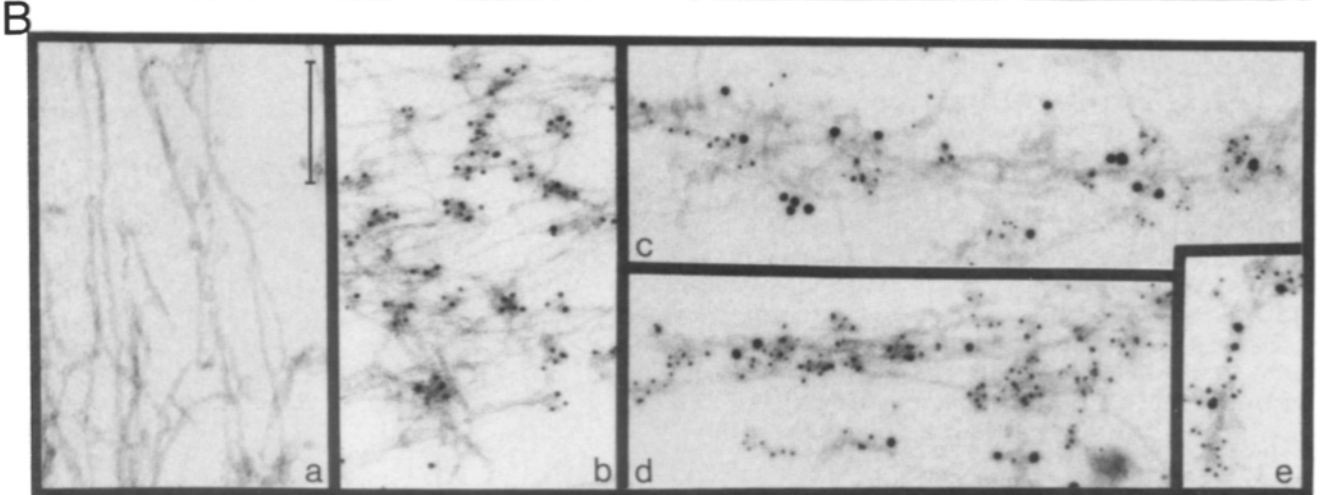
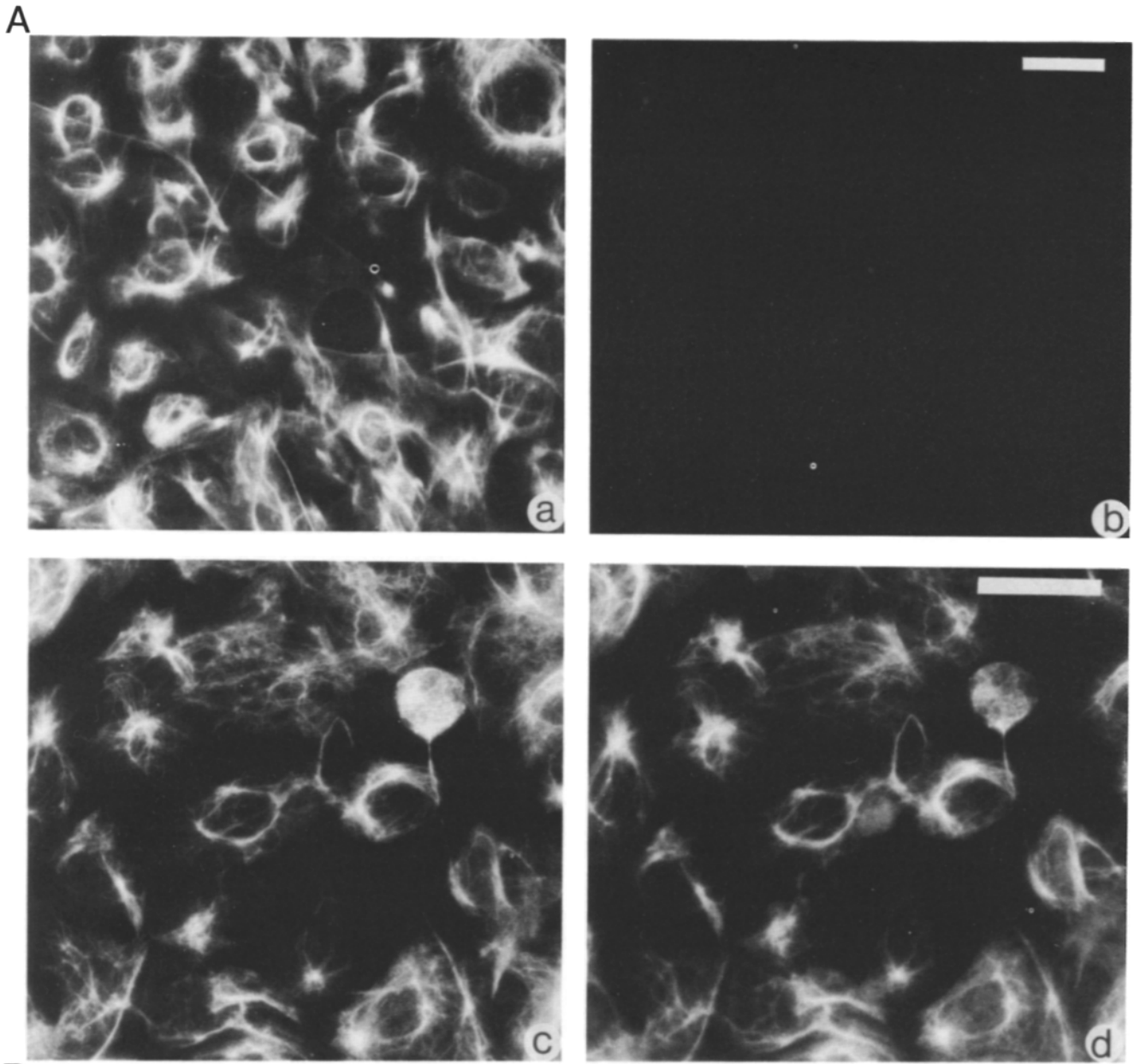


Figure 5. (A) Double-label immunofluorescence staining of control (a and b) and transgenic (c and d) transformed ear-shell fibroblasts incubated with poly-vim (a and c) and the monoclonal antibody to desmin RD301 (b and d). Note colocalization of filaments stained by both antibodies in transgenic fibroblasts. Bars, 4 μm . (B) Colocalization of desmin and vimentin in IFs of transgenic cultured fibroblasts as seen at the ultrastructural level after double-immunogold labeling. All preparations were incubated with a mixture of secondary antibody-coated colloidal gold probes in the detection step. (a) IFs from transgenic fibroblasts incubated with PBS instead of primary antibodies. (b and c) IFs from control (b) and transgenic (c) fibroblasts, incubated with poly-vim and RD301, detected with 5- and 10-nm gold particles, respectively. (d and e) IFs from transgenic fibroblasts, incubated with poly-des and RV202, detected with 5- and 10-nm gold particles, respectively. Bar, 200 nm.

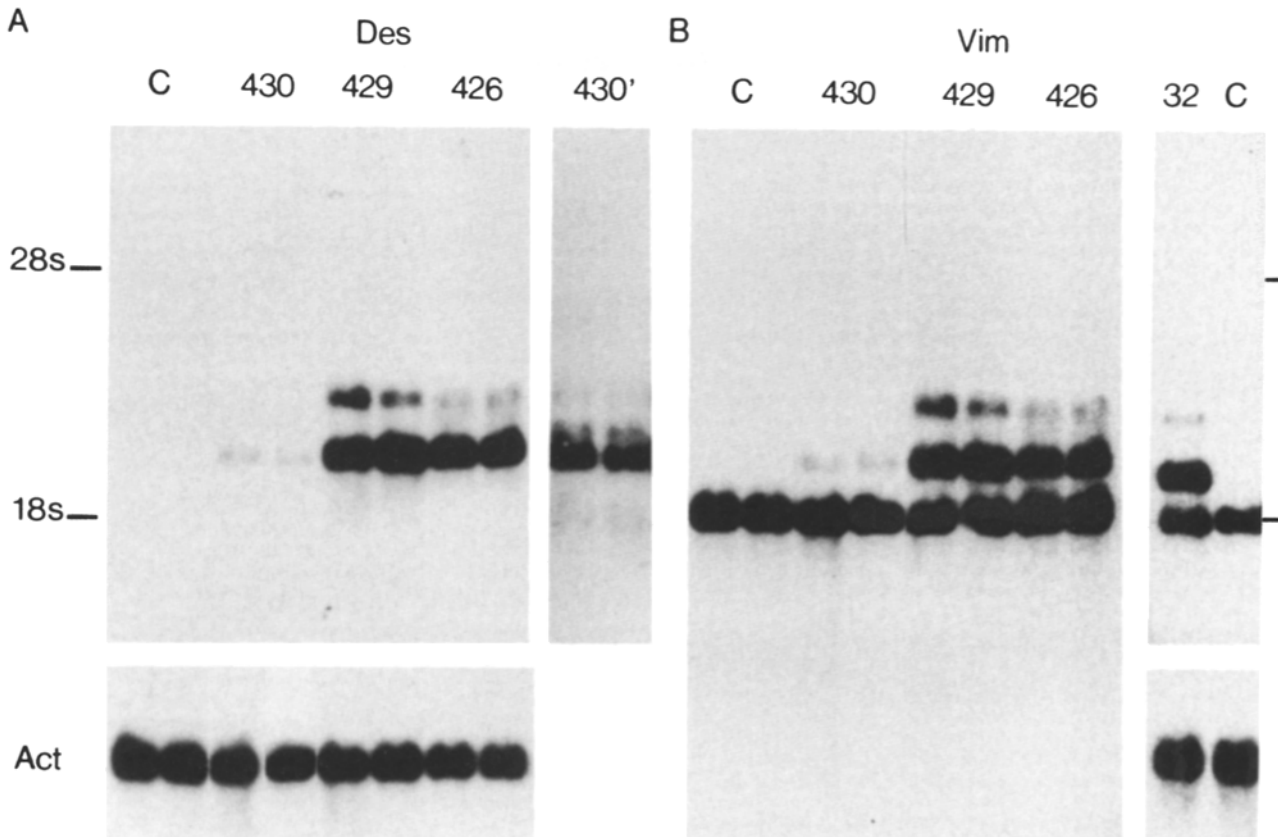


Figure 6. Northern blot analysis of 10 μg of total RNA from exponentially growing control (C) and transgenic transformed ear fibroblast cell lines (pVDes strains 426, 429, and 430; pVim2 strain 32). To exclude clonal variation and influences of growth conditions on expression levels, RNA was isolated from two independent cell lines for each pVDes strain. Blots were hybridized to a desmin-(A, X54, *Des*) and subsequently to a vimentin-specific probe (B, E49, *Vim*). After dehybridization the blots were hybridized to an actin-specific probe (*Act*). Fibroblasts from strain 430 contained relatively little pVDes transcripts, which were readily detected, however, after longer exposure times (lanes 430').

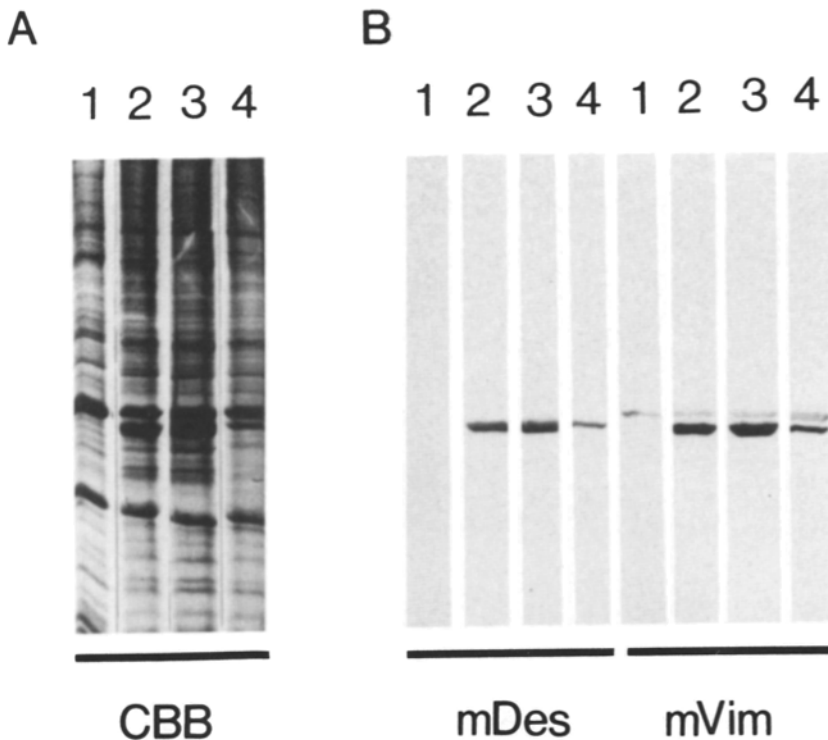
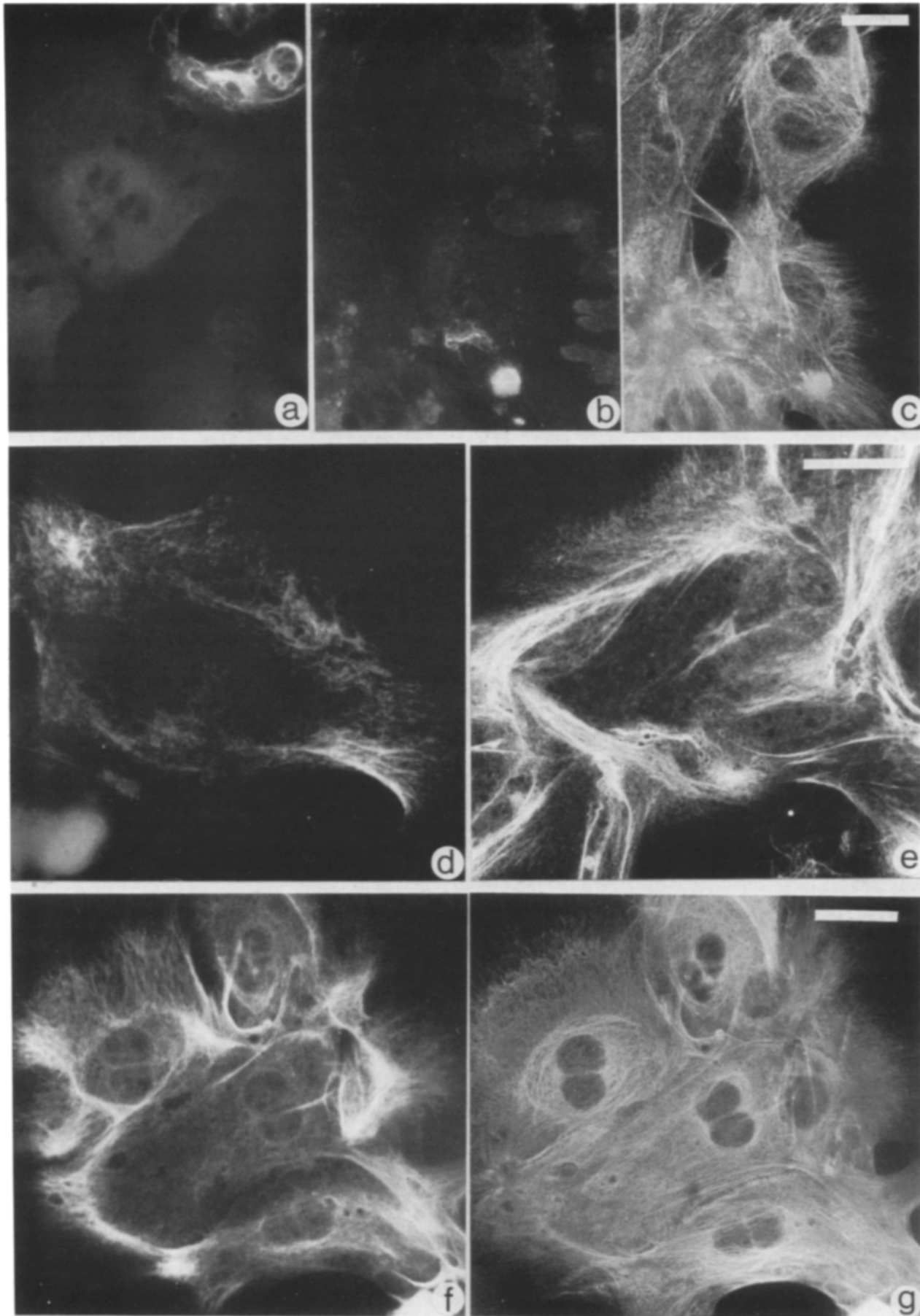


Figure 7. Cytoskeletal fractions of control (lanes 1) and transgenic (lanes 2-4) transformed fibroblast cell lines, analyzed by one-dimensional SDS-PAGE and immunoblotting. (A) Coomassie Brilliant Blue (CBB) staining of SDS-polyacrylamide gel. (B) Immunoblots, incubated with the monoclonal antibody to desmin (RD301, *mDes*) and subsequently with the monoclonal antibody to vimentin (RV202, *mVim*). (Lane 1) Control fibroblasts; (lane 2) strain 426; (lane 3) strain 429; (lane 4) strain 430.



species-specific regulation of expression (43). Timing of human neurofilament (NF-L) gene expression in transgenic mice also differed from endogenous NF-L expression (31).

Cultured hepatocytes from pVDes strain 426 expressed vimentin, but no pVDes expression could be detected by indirect immunofluorescence (Fig. 9) and Northern blotting (data not shown). However, mice from this strain displayed tissue-specific pVDes expression at similar levels as strain 429. The site of integration of the pVDes copies in strain 426 might not allow transcriptional activation under in vitro conditions. At the same time, regulatory processes in control of tissue-specific and tissue culture-induced vimentin expression may not be identical. We did not detect major rearrangements in the vimentin promoter region of the pVDes copies of strain 426. It remains to be established exactly which 5' sequences mediate induction of vimentin synthesis in cultured cells.

The reasons for the existence of the highly specific expression patterns of desmin and vimentin are unknown. Although the functional differences between these proteins are obscure, some different properties have been noted. Comparison of hamster vimentin and desmin in vivo phosphorylation patterns have shown that desmin contains an additional, subunit-specific phosphorylation site in the COOH-terminal "tail" region (10, 20). This might allow a differential response to exogenous stimuli and may reflect subunit-specific functions. In addition, it has been described that the "tail" domains of desmin and vimentin both associate with lamin B at the nuclear envelope but with different affinities (25). In double immunofluorescence assays on transgenic ear-shell fibroblast cells and cultured hepatocytes we observed a small percentage of cells that displayed large differences in desmin and vimentin staining intensities, suggesting that some filaments contained predominantly desmin or vimentin subunits. The same observation has been made for pVim2-expressing cultured fibroblasts (36). This might reflect differences in assembly characteristics.

The coexpression of desmin and vimentin in tissues and cultured cells from transgenic mice did not result in detectable abnormalities. Future experiments, aimed at interfering with IF expression in vivo will hopefully shed some light on the function of these cytoskeletal structures.

We thank Dr. H. Theo Cuypers for helpful discussions and ideas. Wilma Vree Egberts, Olof Moesker, and Hennie Roelofs are acknowledged for excellent technical assistance. We also thank Dr. S. H. Yap for making available to us the laboratory facilities at the division of gastrointestinal and liver diseases.

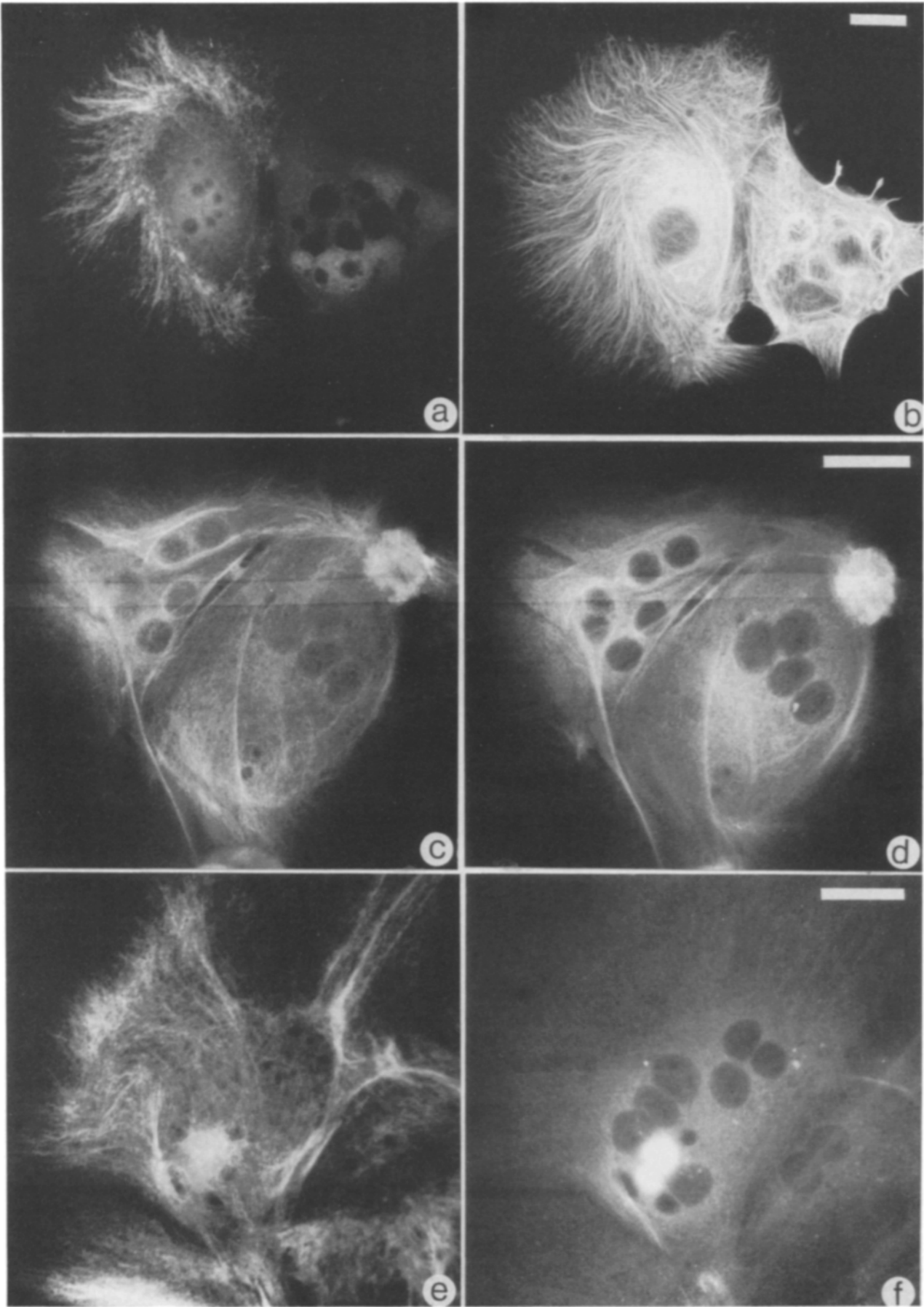
This work was supported by the Netherlands Organization for Scientific Research (NWO) through the Foundation for Chemical Research (SON; F. R. Pieper) and the Foundation of Medical Research (MEDIGON; P. J. Krimpenfort), and by the Netherlands Cancer Foundation (KWF; G. Schaart and F. Ramaekers).

Received for publication 9 September 1988.

References

1. Auffray, C., and F. Rougeon. 1980. Purification of mouse immunoglobulin heavy chain mRNAs from total myeloma tumor RNA. *Eur. J. Biochem.* 107:303-314.
2. Banerji, J., L. Olson, and W. Schaffner. 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell.* 33:729-740.
3. Bloemendal, H., J. H. Lenstra, H. Dodefont, F. C. S. Ramaekers, A. A. Groeneveld, I. Dunia, and E. L. Benedetti. 1980. SV40-transformed hamster lens epithelial cells: a novel system for the isolation of cytoskeletal messenger RNAs and their translation products. *Exp. Eye Res.* 31:513-525.
4. Broers, J. L. V., D. N. Carney, M. Klein Rot, G. Schaart, E. B. Lane, G. P. Vooy, and F. C. S. Ramaekers. 1986. Intermediate filament proteins in classic and variant types of small cell lung carcinoma cell lines: a biochemical and immunological analysis using a panel of monoclonal and polyclonal antibodies. *J. Cell Sci.* 83:37-60.
5. Church, G. M., and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA.* 81:1991-1995.
6. Connell, N. D., and J. G. Rheinwald. 1983. Regulation of the cytoskeleton in mesothelial cells: reversible loss of keratin and increase in vimentin during rapid growth in culture. *Cell.* 34:245-253.
7. De Mey, J. 1986. The preparation and use of gold probes. In *Immunocytochemistry: Modern Methods and Applications*. 2nd ed. J. M. Polak and Van Noorden, editors. John Wright and Sons Ltd., Bristol. 115-145.
8. Dodefont, H. J., P. Soriano, W. J. Quax, F. Ramaekers, J. A. Lenstra, M. A. Groenen, G. Bernardi, and H. Bloemendal. 1982. The genes coding for the cytoskeletal proteins actin and vimentin in warm-blooded vertebrates. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:167-171.
9. Dräger, U. C. 1983. Coexistence of neurofilaments and vimentin in a neurone of adult mouse retina. *Nature (Lond.)* 303:169-172.
10. Evans, R. M. 1988. The intermediate-filament proteins vimentin and desmin are phosphorylated in specific domains. *Eur. J. Cell Biol.* 46:152-160.
11. Ferrari, S., R. Battini, L. Kaczmarek, S. Rittling, B. Calabretta, J. Kim de Riel, V. Philiponis, J.-F. Wei, and R. Baserga. 1986. Coding sequence and growth regulation of the human vimentin gene. *Mol. Cell. Biol.* 6:3614-3620.
12. Franke, W. W. 1987. Nuclear lamins and cytoplasmic intermediate filament proteins: a growing multigene family. *Cell.* 48:3-4.
13. Franke, W. W., and R. Moll. 1987. Cytoskeletal components of lymphoid organs. I. Synthesis of cytokeratins 8 and 18 and desmin in subpopulations of extrafollicular reticulum cells of human lymph nodes, tonsils, and spleen. *Differentiation.* 36:145-163.
14. Franke, W. W., C. Grund, and E. Schmid. 1979. Intermediate-sized filaments present in Sertoli cells are of the vimentin-type. *Eur. J. Cell Biol.* 19:269-275.
15. Franke, W. W., E. Schmid, S. Winter, M. Osborn, and K. Weber. 1979. Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp. Cell Res.* 123:25-46.
16. Franke, W. W., H. Denk, R. Kalt, and E. Schmid. 1981. Biochemical and immunological identification of cytokeratin proteins present in hepatocytes of mammalian liver tissue. *Exp. Cell Res.* 131:299-318.
17. Franke, W. W., D. Mayer, E. Schmid, H. Denk, and E. Borenfreund. 1981. Differences of expression of cytoskeletal proteins in cultured rat hepatocytes and hepatoma cells. *Exp. Cell Res.* 134:345-365.
18. Franke, W. W., C. Grund, C. Kuhn, B. W. Jackson, and K. Illmensee. 1982. Formation of cytoskeletal elements during mouse embryogenesis. III. Primary mesenchymal cells and the first appearance of vimentin filaments. *Differentiation.* 23:43-59.
19. Franke, W. W., M. Hergt, and C. Grund. 1987. Rearrangement of the vimentin cytoskeleton during adipose conversion: formation of an intermediate filament cage around lipid globules. *Cell.* 49:131-141.
20. Gard, D. L., and E. Lazarides. 1982. Analysis of desmin and vimentin phosphopeptides in cultured avian myogenic cells and their modulation by 8-bromo-adenosine 3',5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. USA.* 79:6912-6916.
21. Gawlitta, W., M. Osborn, and K. Weber. 1981. Coiling of intermediate filaments induced by microinjection of a specific monoclonal antibody. *Cell.* 24:185-193.
22. Geisler, N., and K. Weber. 1982. The amino acid sequence of chicken mus-

Figure 8. Indirect immunofluorescence microscopy of primary hepatocyte cultures from control mice. Staining was performed with monoclonal and polyclonal antibodies to desmin, vimentin, and cytokeratins after different periods of culture. (a) Hepatocytes cultured for 8 h, poly-vim stained. Note absence of vimentin filaments in hepatocytes and strong, filamentous staining of a contaminating fibroblast. (b and c) Double-label immunofluorescence staining for vimentin and cytokeratin (poly-vim and RCK102, respectively) after 8 h of culture. Hepatocytes are vimentin negative (b) but contain cytokeratin filaments (c). (d and e) Hepatocytes cultured for 18 and 40 h, respectively, and poly-vim stained. Note gradual increase in vimentin expression. (f and g) Double-label immunofluorescence staining for vimentin and cytokeratin (poly-vim and RCK102, respectively) after 40 h of culture. Hepatocytes contain both vimentin (f) and cytokeratin filaments (g), which do not seem to colocalize. Bars, 4 μ m.



- cle desmin provides a common structural model for intermediate filament proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1649-1656.
23. Geisler, N., and K. Weber. 1986. Structural aspects of intermediate filaments. In *Cell and Molecular Biology of the Cytoskeleton*. G. Shay, editor. Plenum Publishing Corp., New York. 41-64.
 24. Gelieter, J., R. A. Zeff, R. W. Melvold, and S. G. Nathenson. 1986. Mitotic recombination in germ cells generated two major histocompatibility complex mutant genes shown to be identical by RNA sequence analysis: K^{bm9} and K^{bm6} . *Proc. Natl. Acad. Sci. USA.* 83:3371-3375.
 25. Georgatos, S. D., K. Weber, N. Geisler, and G. Blobel. 1987. Binding of two desmin derivatives to the plasma membrane and the nuclear envelope of avian erythrocytes: evidence for a conserved site-specificity in intermediate filament-membrane interactions. *Proc. Natl. Acad. Sci. USA.* 84:6780-6784.
 26. Gillies, S. D., S. L. Morrison, V. T. Oi, and S. Tonewaga. 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell.* 33:717-728.
 27. Hedberg, K. K., and L. B. Chen. 1986. Absence of intermediate filaments in a human adrenal cortex carcinoma-derived cell line. *Exp. Cell Res.* 163:509-517.
 28. Heuvel, R. M. M. van den, G. J. M. M., van Eys, F. C. S. Ramaekers, W. J. Quax, W. T. M. Vree Egberts, G. Schaart, H. T. M. Cuypers, and H. Bloemendal. 1987. Intermediate filament formation after transfection with modified hamster vimentin and desmin genes. *J. Cell Sci.* 88:475-482.
 29. Houlston, E. M.-N. Guilly, J.-C. Courvakian, and B. Maro. 1988. Expression of nuclear lamins during mouse preimplantation development. *Development (Camb.)*. 102:271-278.
 30. Jackson, B. W., C. Grund, S. Winter, W. W. Franke, and K. Illmensee. 1981. Formation of cytoskeletal elements during mouse embryogenesis. II. Epithelial differentiation and intermediate sized filaments in early postimplantation embryos. *Differentiation*. 20:203-216.
 31. Julien, J.-P., I. Tretjakoff, L. Beaudet, and E. Peterson. 1987. Expression and assembly of a human neurofilament protein in transgenic mice provide a novel neuronal marking system. *Genes and Development*. 1:1085-1095.
 32. Klauinig, J. E., P. J. Goldblatt, D. E. Hinton, M. M. Lipsky, J. Chacko, and B. F. Trump. 1981. Mouse liver cell culture. I. Hepatocyte isolation. *In Vitro (Gaithersburg)*. 17:913-925.
 33. Klauinig, J. E., P. J. Goldblatt, D. E. Hinton, M. M. Lipsky, and B. F. Trump. 1981. Mouse liver cell culture. II. Primary culture. *In Vitro (Rockville)*. 17:926-934.
 34. Klymkowsky, M. W., R. H. Miller, and E. B. Lane. 1983. Morphology, behaviour, and interaction of cultured epithelial cells after the antibody-induced disruption of keratin filament organization. *J. Cell Biol.* 96:494-509.
 35. Konieczny, S. F., and C. P. Emerson. 1987. Complex regulation of the muscle-specific contractile protein (troponin I) gene. *Mol. Cell. Biol.* 7:3065-3075.
 36. Krimpenfort, P. J., G. Schaart, F. R. Pieper, F. C. S. Ramaekers, H. T. Cuypers, R. M. van den Heuvel, W. T. Vree Egberts, G. J. van Eys, A. Berns, and H. Bloemendal. 1988. Tissue-specific expression of a vimentin-desmin hybrid gene in transgenic mice. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:941-947.
 37. Lane, E. B., B. L. M. Hogan, M. Kurkinen, and J. I. Garrels. 1983. Coexpression of vimentin and cytokeratins in parietal endoderm cells of early mouse embryo. *Nature (Lond.)*. 303:701-704.
 38. Lees, J. F., P. S. Schneideman, S. F. Skuntz, M. J. Carden, and R. A. Lazzarini. 1988. The structure and organization of the human heavy neurofilament subunit (NF-H) and the gene encoding it. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1947-1955.
 39. Leonard, D. G. B., J. D. Gorham, P. Cole, L. A. Greene, and E. B. Ziff. 1988. A nerve growth factor-regulated messenger RNA encodes a new intermediate filament protein. *J. Cell Biol.* 106:181-193.
 40. Lewis, S. A., J. M. Balcarek, V. Krek, M. Shelanski, and N. J. Cowan. 1984. Sequence of a cDNA clone encoding mouse glial fibrillary acidic protein: structural conservation of intermediate filaments. *Proc. Natl. Acad. Sci. USA.* 81:2743-2746.
 41. Lilienbaum, A., V. Leganeux, M.-M. Portier, K. Dellagi, and D. Paulin. 1986. Vimentin gene: expression in human lymphocytes and in Burkitt's lymphoma cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2809-2814.
 42. Lin, J. J.-C., and J. R. Feramisco. 1981. Disruption of the in vivo distribution of intermediate filaments in living fibroblasts through the microinjection of a specific monoclonal antibody. *Cell.* 24:185-193.
 43. Ngai, J., G. Bond, B. J. Wold, and E. Lazarides. 1987. Expression of transfected vimentin genes in differentiating murine erythroleukemia cells reveals divergent cis-acting regulation of avian and mammalian vimentin sequences. *Mol. Cell. Biol.* 7:3955-3970.
 44. Palmiter, R. D., and R. L. Brinster. 1986. Germ-line transformation of mice. *Annu. Rev. Genet.* 20:465-499.
 45. Picard, D., and W. Schaffner. 1983. A lymphocyte-specific enhancer in the mouse immunoglobulin gene. *Nature (Lond.)*. 307:80-82.
 46. Pieper, F. R., R. L. Slobbe, F. C. S. Ramaekers, H. T. Cuypers, and H. Bloemendal. 1987. Upstream regions of the hamster desmin and vimentin genes regulate expression during in vitro myogenesis. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3611-3618.
 47. Quax, W., W. Vree Egberts, W. Hendriks, Y. Quax-Jeuken, and H. Bloemendal. 1983. The structure of the vimentin gene. *Cell.* 35:215-223.
 48. Quax, W., L. van den Broek, W. Vree Egberts, F. Ramaekers, and H. Bloemendal. 1985. Characterization of the hamster desmin gene: expression and formation of desmin filaments in non-muscle cells after gene transfer. *Cell.* 43:327-338.
 49. Quinlan, R. A., and W. W. Franke. 1982. Heteropolymer filaments of vimentin and desmin in vascular smooth muscle tissue and cultured baby hamster kidney cells demonstrated by chemical cross-linking. *Proc. Natl. Acad. Sci. USA.* 79:3452-3456.
 50. Ramaekers, F. C. S., M. Osborn, E. Schmid, K. Weber, H. Bloemendal, and W. W. Franke. 1980. Identification of the cytoskeletal proteins in lens-forming cells, a special epithelioid cell type. *Exp. Cell Res.* 127:309-327.
 51. Ramaekers, F. C. S., J. J. G. Puts, O. Moesker, A. Kant, A. Huysmans, D. Haag, P. H. K. Jap, C. Herman, and G. P. Vooijs. 1983. Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumours: an overview. *Histochem. J.* 15:691-713.
 52. Ramaekers, F. C. S., R. H. M. Verheijen, O. Moesker, A. Kant, G. P. Vooijs, and C. J. Herman. 1983. Mesodermal mixed tumor: diagnosis by analysis of intermediate filament proteins. *Am. J. Surg. Pathol.* 7:381-385.
 53. Ramaekers, F. C. S., A. Huysmans, G. Schaart, O. Moesker, and G. P. Vooijs. 1987. Tissue distribution of keratin 7 as monitored by a monoclonal antibody. *Exp. Cell Res.* 170:235-249.
 54. Rittling, S. R., and R. Baserga. 1987. Functional analysis and growth factor regulation of the human vimentin promoter. *Mol. Cell. Biol.* 7:3908-3915.
 55. Rossi, P., and B. de Crombrugge. 1987. Identification of a cell-specific transcriptional enhancer in the first intron of mouse α_2 (type I) collagen gene. *Proc. Natl. Acad. Sci. USA.* 84:5590-5594.
 56. Schmid, E., M. Osborn, E. Rungger-Braendle, G. Gabbiani, K. Weber, and W. W. Franke. 1982. Distribution of vimentin and desmin filaments in smooth muscle tissue of mammalian and avian aorta. *Exp. Cell Res.* 137:329-340.
 57. Schnitzer, J., W. W. Franke, and M. Schachner. 1981. Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. *J. Cell Biol.* 90:435-447.
 58. Steinert, P. M., and D. R. Roop. 1988. Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem.* 57:593-625.
 59. Steinert, P. M., A. C. Steven, and D. R. Roop. 1985. The molecular biology of intermediate filaments. *Cell.* 42:411-419.
 60. Sternberg, E. A., G. Spizz, W. M. Perry, D. Vizard, T. Weil, and E. N. Olson. 1988. Identification of upstream and intragenic regulatory elements that confer cell-type-restricted and differentiation-specific expression on the muscle creatine kinase gene. *Mol. Cell. Biol.* 8:2896-2909.
 61. Stewart, C., and B. Burke. 1987. Teratocarcinoma stem cells and early mouse embryos contain only a single major lamin polypeptide closely related to lamin B. *Cell.* 51:383-392.
 62. Tokuyasu, K. T., P. A. Maher, and S. J. Singer. 1985. Distributions of vimentin and desmin in developing chick myotubes in vivo. II. Immunoelectron microscopic study. *J. Cell Biol.* 100:1157-1166.
 63. Tölle, H.-G., K. Weber, and M. Osborn. 1986. Microinjection of monoclonal antibodies to vimentin, desmin, and GFA in cells which contain more than one IF type. *Exp. Cell Res.* 162:462-474.
 64. Traub, P. 1985. Intermediate Filaments. A Review. Springer-Verlag,

Figure 9. Indirect double-label immunofluorescence staining of primary hepatocyte cultures from pVDes transgenic mice, strains 429 (a-d) and 426 (e and f). (a and b) Staining for desmin (a) and cytokeratin (b) (poly-des and RCK102, respectively) after 35-40 h of culture. Note filamentous desmin staining of one hepatocyte with poly-des while an adjacent hepatocyte is completely desmin negative (a). Both cells express cytokeratins in large amounts (b). (c and d) Staining for vimentin (c) and desmin (d) (poly-vim and RD301, respectively). Note expression of both vimentin (c) and desmin (d). (e and f) Staining for vimentin (e) and desmin (f) (poly-vim and RD301, respectively) of strain 426 hepatocytes after 70 h of culture. Note complete absence of desmin staining (f), while vimentin is expressed abundantly (e). Bars, 4 μ m.

Berlin.

65. Viebahn, C., E. B. Lane, and F. C. S. Ramaekers. 1987. The mesonephric (Wolfian) and paramesonephric (Muellerian) ducts of golden hamsters express different intermediate filament proteins during embryogenesis. *Differentiation*. 34:175-188.
66. Virtanen, I., R. A. Badley, R. Paasivuo, and V. P. Lehto. 1984. Distinct cytoskeletal domains revealed in sperm cells. *J. Cell Biol.* 99:1083-1091.
67. Vorstenbosch, C. J., B. Colenbrander, C. J. Wensing, F. C. S. Ramaekers, and G. P. Vooijs. 1984. Cytoplasmic filaments in fetal and neonatal pig testis. *Eur. J. Cell Biol.* 34:292-299.