CMLS, Cell. mol. life sci. 53 (1997) 1–12
1420-682X/97/010001-12 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Reviews

Transgenic mice carrying chimeric or mutated type III intermediate filament (IF) genes

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Received 4 November 1996; accepted 15 November 1996

Abstract. Mice carrying chimeric, truncated or mutated genes encoding intermediate filament (IF) proteins type III do not show any detectable severe pathology. However, upon (over)expression of the transgene in the eye lens all animals develop lens opacification (cataract). At the cellular level the loss of visual acuity is preceded by interference with the terminal differentiation of lens fibre cells, plasma membrane damage, distorted assembly of the IF cytoskeleton and perturbation of the cytoskeleton-membrane complex. The degree of expression is paralleled by the extent of the damages.
Key words. Transgenic mice; chimeric IF-genes; cataract.

Introduction

The skeleton of cells (cytoskeleton) consists of three major fibrillar networks: microtubules, intermediate filaments (IFs) and microfilaments. These filamentous structures can be distinguished on the basis of morphological features, composition and immunological properties (compare tables 1 and 2).

A wealth of data led to the notion that IF subunits consist of three distinct domains: (1) a central 'rod' of conserved length [approximately 310 amino acid residues (365 in case of type V IFs)], conserved secondary structure and partly conserved sequence; (2) a nonhelical amino-terminal domain ('head'); (3) a nonhelical carboxyl-terminal domain ('tail').

The two nonhelical domains flank the conserved central rod that has a helix structure, interrupted only by three short nonhelical linkers. The resulting four α-helical subdomains are named 1A (N-terminal end of the rod); 1B, 2A and 2B (C-terminal end of the rod) (fig. 1).

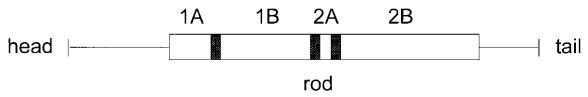

Figure 1. Schematic representation of the tripartite structural organization of an IF protein subunit. Single lines = non-helical flanking regions; white boxes = helices; black boxes = nonhelical linkers.

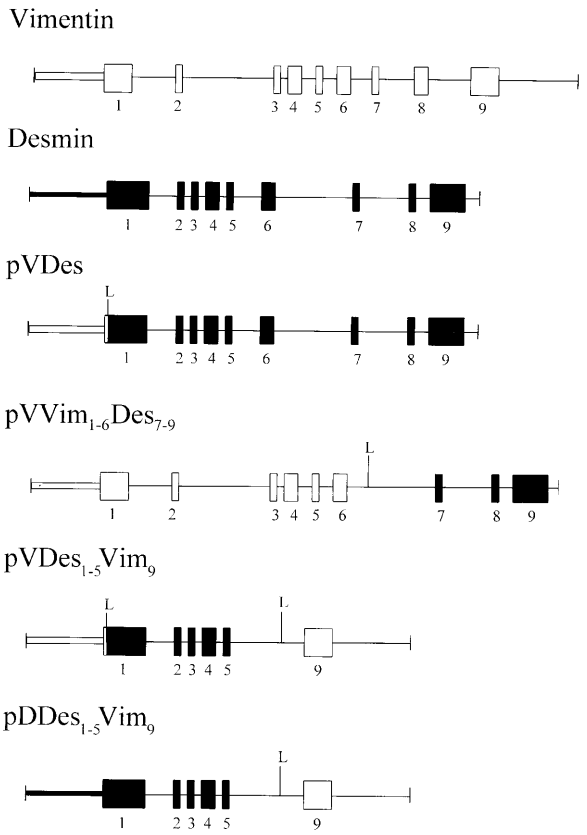

Figure 2. Schematic representation of the vimentin and desmin genes and the constructs derived thereof.

The amino acid sequence in the central helix follows the heptad rule, which means that this sequence comprises sets of seven amino acids with hydrophobic amino acid

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Table 1. Major constituents of the cytoskeleton.

Filament	Protein/subunit(s)	Diameter (nm)
Microtubuli	tubulin	25
Intermediate filaments	IF-protein*	10
Micro-filaments	actin	5

*See table 2.

residues at positions 1 and 4. This typical structure enables the formation of coiled-coil dimers.

It has been established that in the living cell microtubules and microfilaments are involved in important roles in processes such as mitosis, locomotion, anchorage and protoplasmic streaming. In contrast, the function of intermediate filaments is not yet fully understood. Apparently cytoplasmic IFs do not fulfil a typical 'household' function, as some cell lines and a few cell types in vivo do not contain an IF network. Recently, so-called knock-out experiments, in which a particular type-III IF gene has been removed from the genome in transgenic animals, led to controversial conclusions concerning the evidence of an abnormal phenotype [1–3]. Furthermore, various studies with cell lines or transgenic animals show that disruption of the IF networks does not affect mitosis, cell morphology or motility. However, there is a strong belief among workers in the field that IFs play a role in the cellular organization as a whole and that the networks provide mechanical strength and maintenance of the architecture of the living cell. Evidence for such a structural role stems from studies upon the expression of dominant negative, truncated human keratin-14 subunits in transgenic mice [4–7] that exhibit features of a serious autosomal human genetic skin disease called epidermolysis bullosa simplex (EBS). Expression of another truncated human gene, encoding keratin-10, causes a skin disease comparable to human epidermolytic hyperkeratosis [6]. Indeed patients suffering from those diseases reveal point mutations in the highly conserved amino carboxyl ends of the rod domain of a particular keratin.

A number of reviews dealing with expression, assembly and interaction of IFs with other cell components, particularly the plasma membrane, and putative functions have been published previously [8–34].

The present paper gives an overview of our previous and more recent work aimed to contribute to a better understanding of the function of vimentin and desmin IFs and the role of the membrane-cytoskeleton complex in cells and in the developing organism. With this goal in mind, several chimeric and truncated IF protein-encoding genes (fig. 2) were either transfected into cells in culture or microinjected into the murine germ line. Some of the resulting mutant IF subunits appeared to be assembly-incompetent and capable of disrupting pre-existing vimentin or desmin filaments in a dominant negative fashion. In all cases the expression of the gene constructs was tissue-specific in transgenic mice.

Strikingly, the disruption of endogenous vimentin or desmin IFs resulted in aberrations at the ultrastructural level but did not lead to obvious developmental or functional abnormalities with the exception of cataract formation in cases where the gene product was expressed in the eye lens.

Table 2. Classification of IF subunits.

Cell type	IF protein	Sequence type	Size (kD)
Epithelial cells	acidic keratins	I	40–60
Epithelial cells	basic keratins	II	50–70
Mesenchymal cells Lens cells*	vimentin	III	55
Muscle cells	desmin	III	53
Peripheral nervous system	peripherin	III	57
Glial cells and astrocytes	GFAP (glial fibrillary acidic protein)	III	53
Retinal ganglion cells (goldfish)	plasticin	III	64
Most neurons	neurofilament proteins (NF-L, NF-M, NF-H)	IV	68, 145, 200
Neuroepithelial stem cells	nestin	IV	200
Central nervous system	α-internexin	IV	66
Nucleated cells	lamins (A/C and B)	V	60, 67, 70

*A 49-kDa and a 115-kDa lens protein termed *phakinin* and *filensin*, respectively, have been reported to be related to the family of IF proteins. These two proteins have a cytoskeletal structure which is distinct from classical 8–11 nm intermediate filaments, thus possibly comprising a novel form of intermediate filaments [49]. Evidence that the 49-kDa protein belongs to the keratin family has been provided by Orii et al. [52].

In the following sections the effects of various constructs are described briefly.

pVDes

pVDes comprising the vimentin promoter linked to the complete desmin coding region was microinjected into fertilized mouse eggs [35]. Immunofluorescence studies of eye lens cells derived from the resulting transgenic animals revealed a mosaic expression pattern of the transgene product [36]. This means that, in contrast to the endogenous vimentin, desmin cannot be detected in all cells of mesenchymal origin or in the eye lens (fig. 3). We observed that the number of cells expressing the transgene in the embryo is higher (fig. 3A) than in adult lens cells (fig. 3B). In cells where both intermediate filament proteins are coexpressed, hybrid IFs as well as amorphous desmin deposits are found (not shown).

The deviating assembly of IF networks induced by genetic manipulation is in some way correlated with abnormal lens cell differentiation. Immunofluorescence experiments show that lenticular cell nuclei, which are normally extruded from the elongating fibre cells, are now detectable in a number of inner fibres. The result of the distorted terminal differentiation process can also be demonstrated by electron microscopy (fig. 4).

Furthermore, marked aberrations of membranous and junctional domains can be observed that frequently appear as internalized structures, whereas a number of cells are fused as a consequence of membrane breakdown (fig. 5). The level of expression is paralleled by the extent of plasma membrane damage.

In lens fibre ghost preparations from transgenic mice impressive conchoidal whorls of IFs can be visualized on thin sections. These abnormal structures are frequently found near and in contact with the nuclear envelope (fig. 6). Eventually, the alterations of the architecture of the plasma membrane-cytoskeleton complex lead to opacification of the adult eye lens (cataract).

It appears that lens opacification is not the only aberration caused by the transgene. Alterations of the incisor tooth organ were seen in immunochemical and ultrastructural studies [37]. Apparently the expression of the

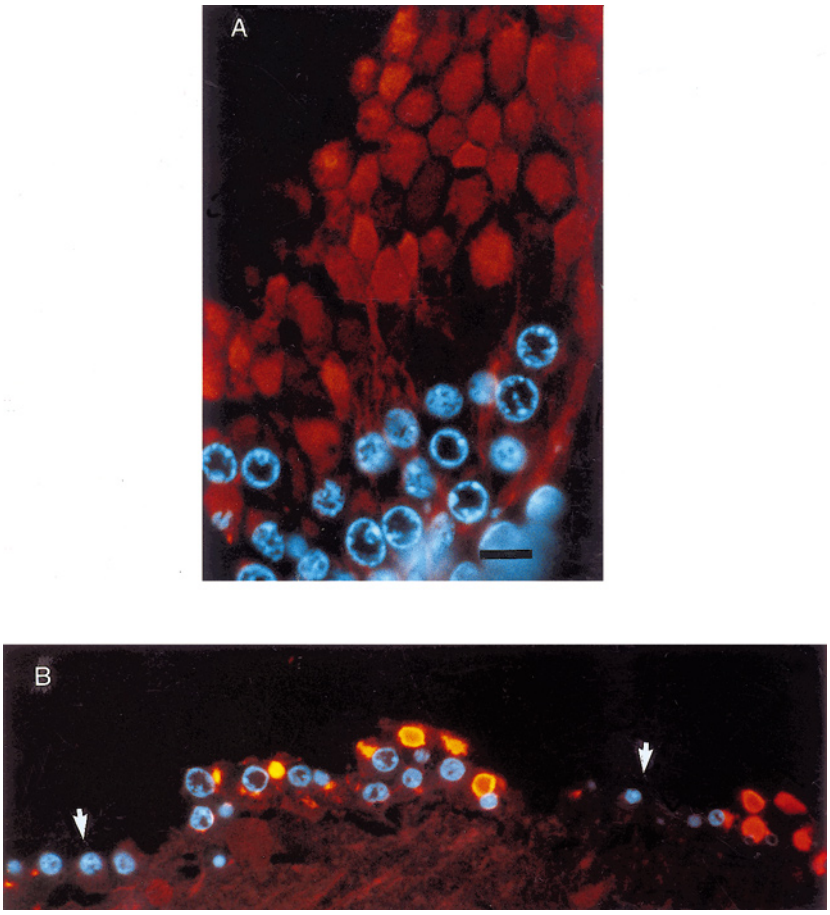

Figure 3. (*A*) Cryosection of an embryo lens (E_{14}) carrying the pVVim$_{1-6}$Des$_{7-9}$ transgene; staining with antidesmin and DAP1. The primary fibres appear virtually uniformly labelled. (*B*) Cryosection of the adult transgenic pVVim$_{1-6}$Des$_{7-9}$ lens. The mosaic mode of desmin expression is clearly shown; staining with antidesmin and DAPI. White arrows point to cells devoid of desmin expression.

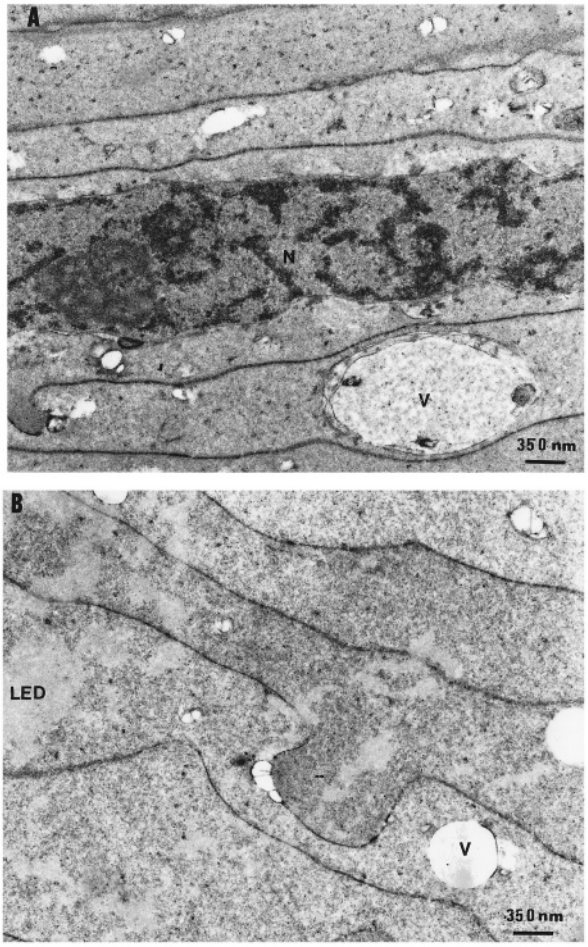

Figure 4. Thin sections of intact transgenic lens stained with uranyl acetate and lead citrate. (*A*) The lens fibres differ considerably in shape. Indentations and projections of the plasma membrane can be observed. The cytoplasm is occupied by remnants of endoplasmic reticulum and vacuoles (V) of different sizes wrapped by membrane profiles. Note the nucleus in a fibre cell. Bar = 350 nm. (*B*) The irregular framework comprising homogenous material of low electron density (LED). N = nucleus. (Reprinted with permission from the European Journal of Cell Biology)

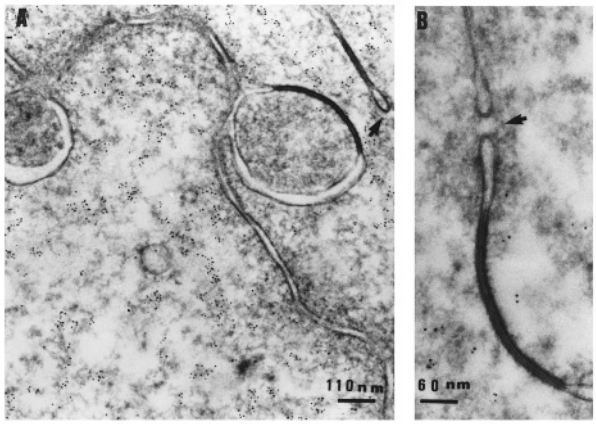

Figure 5. Ultrathin section of transgenic mouse lens showing the cortical region immunolabelled with antidesmin antibodies. Note the internalizations of the general plasma membranes and intercellular junctions. The arrows point to interruptions of the plasma membrane of adjacent fibre cells connected by a cytoplasmic bridge. The gold particles are associated with amorphous desmin. Bars = 110 nm (*A*), 60 nm (*B*). (Reprinted with permission from the European Journal of Cell Biology)

transgenic desmin in the odontoblasts modifies the composition and assembly of the endogenous IF network. Furthermore, our results show that alterations of odontoblast differentiation has a negative effect on the induction capacity of these cells in regulating the function of ameloblasts. Presumably odontoblast-ameloblast interactions are mediated by cytoskeleton-dependent polarization and by specialized domains of the plasma membrane generating specific signals between the two cell types.

$pVVim_{1-6}Des_{7-9}$

We also designed a construct that comprises the 5′ region of the hamster vimentin gene including the head and rod domain, and the 3′ region of the desmin gene, comprising the tail domain. This chimeric gene has been transfected into C_2C_{12} cells or microinjected into fertilized mouse eggs [38]. Several tissues of the transgenic animals were analysed by immunofluorescence in order to demonstrate the presence of the corresponding expression product (fig. 7).

Strong staining could be observed in all tissues of mesenchymal origin that normally express only vimentin. The fact that the fluorescence patterns often show the filamentous feature of the IF network strongly suggests that the chimeric Vim-Des protein assembles into pre-existing normal vimentin IF networks.

Since in addition to its occurrence in tissues of mesenchymal origin, vimentin is also expressed in the eye lens, it was not surprising that the chimeric protein could also be demonstrated in the water-insoluble protein fraction derived from the transgenic mouse lens. Obviously the extent of expression was high enough to allow detection even on a Coomassie Brilliant Blue-stained one-dimensional SDS-containing polyacrylamide gel (fig. 8).

From our experiments it may be concluded that the presence of appreciable amounts of $pVVim_{1-6}Des_{7-9}$ protein in all vimentin-expressing tissues does not interfere with normal development of the animal. However, the expression of this protein hampers normal lens cell differentiation by blocking the cell denucleation process and leads to cataract.

$pVDes_{1-5}Vim_9$

To further investigate the putative function of vimentin IFs in intact tissues and the developing organism, a

construct under direction of the vimentin promoter and encoding a truncated desmin subunit was injected into fertilized mouse eggs [39]. The expression product, a mutant desmin protein, appeared to be assembly-incompetent and capable of disrupting pre-existing IFs, both in transgenic mouse tissue and in cells derived thereof. Tissue specificity and levels of expression of the construct were analysed by Northern blotting in different tissues, including lens, heart, skeletal muscle, spleen, esophagus, tongue, brain, testis and ovary. Three independent transgenic mouse strains expressed the gene product to virtually the same extent (fig. 9). Western blots displaying the corresponding protein were in agreement with the Northern blotting patterns (not shown). Expression of the truncated protein caused disruption of the endogenous vimentin filament network, as can be visualized by indirect immunofluorescence of tail sections and blood smears derived from founders and offspring of the the transgenic mice (fig. 10). Instead of the normal filamentous structure, strongly fluorescent dots and clumps were seen in stromal cells. The disruption of the vimentin IF network has also been demonstrated on several tissues and cultured cells at the ultrastructural level. Electron microscopy was performed on thin sections of lens fibre ghosts from transgenic mice and compared with similar material from wild-type mice (fig. 11). The ultrastructural study demonstrated the presence of a disrupted IF network and the accumulation of amorphous transgene product.

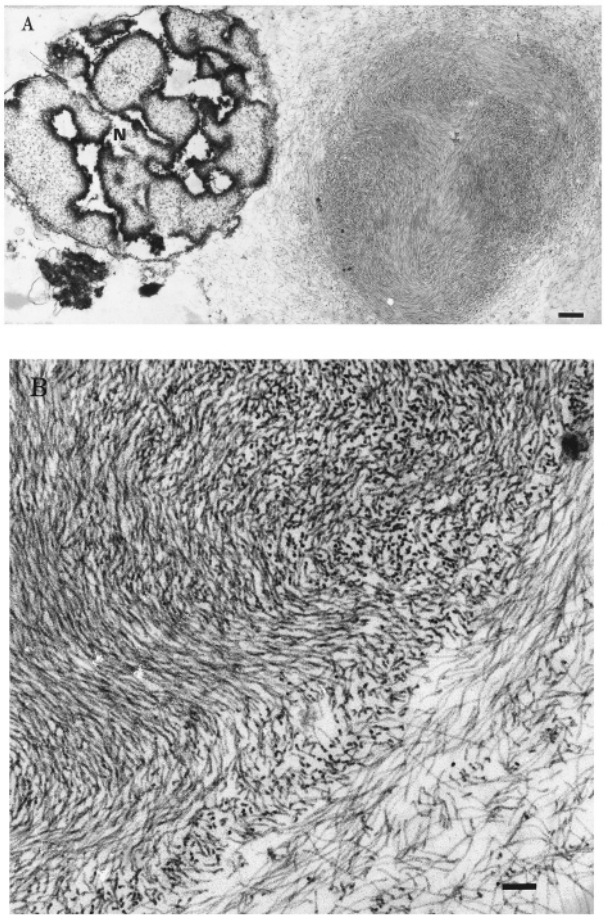

Figure 6. (*A*) Thin section of fibre ghosts prepared from the lens cortex of pVDes carrying transgenic mice. Note the giant conchoidal whorl of collapsed IFs still associated with the nucleus (N). Staining with uranyl acetate and lead citrate. Bar = 600 nm. (*B*) High magnification of a region of the conchoidal whorl showing irregular loops and compact IF bundles running in various directions. Bar = 100 nm. (Reprinted with permission from the European Journal of Cell Biology)

pDDes$_{1-5}$Vim$_9$

We also constructed a similar mutant desmin gene as described in the foregoing section, but driven by the desmin promoter [40]. Again, the first five exons of the desmin gene were linked to exon 9 of the vimentin gene. Transgenic mice were generated by pronuclear micro-injection. Like the previous mutant gene product, the expression product of the desmin promoter-driven gene was assembly-incompetent and capable of disrupting pre-existing vimentin and desmin filaments in a dominant negative fashion. Since the construct comprised the desmin promoter, expression occurred exclusively in transgenic mouse muscle tissue and cultured muscle cells. Visual inspection of the transgenic animals did not reveal gross morphological defects; however, severe damage was detected at the ultrastructural level of transgenic mice carrying the desmin promoter-driven truncated gene. The alterations were observed in sartorius muscle. Expression of the latter truncated gene induced radical changes of the myofibrillar organization, notably the disruption of the T system [transverse tubular system] (fig. 12). These findings suggest that alterations of the desmin IF network lead to collapse and damage not only of the T system but also to fragmentation of specialized membrane domains of the sarcoplasmic reticulum [40].

pV*MDR3*

As we observed considerable damage of the cytoskeleton-membrane complex in all cases of genetic manipulation described afore, we wondered whether or not a specific membrane protein gene, linked to the vimentin promoter, might cause similar effects in transgenic animals. We chose the human multidrug-resistant phosphoglycoprotein MDR3 Pgp [41]. This protein belongs to a class of highly conserved membrane proteins that can extrude hydrophobic drugs from mammalian cells [42–45]. The tissue distribution of this non-lens-specific protein has been described previously [46].

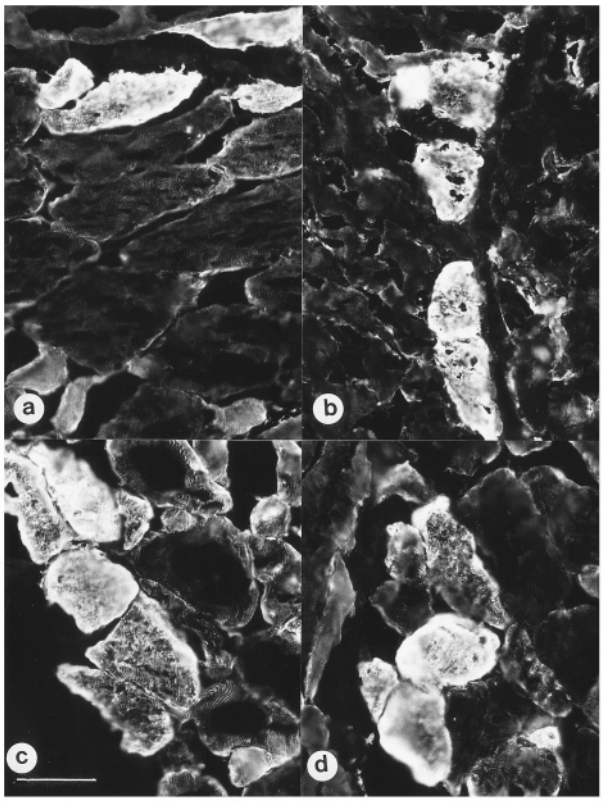

Figure 10. Indirect immunofluorescence of skeletal muscle (*a*, *b*) and tongue tissue sections (*c*, *d*) from $pVDes_{1-5}Vim_9$ transgenic mice strains 47 (*a*, *c*) and 50 (*b*, *d*), using poly-des. Note strongly fluorescent, diffuse staining pattern in a minority of muscle fibres. The striated pattern normally observed upon desmin staining also seemed to be present in the strongly fluorescent muscle fibres (not shown). Bar = 4 μm. (Reprinted with permission from the European Journal of Cell Biology)

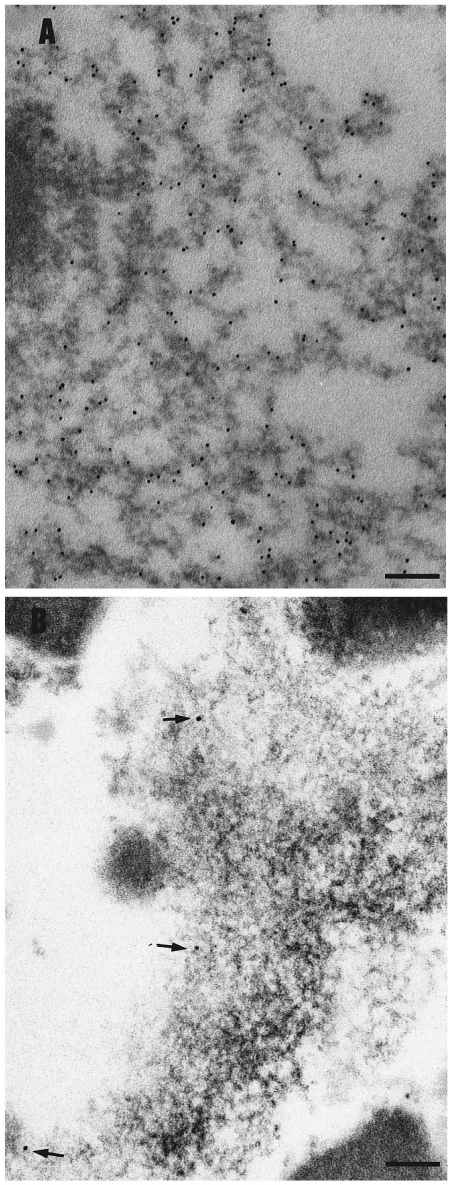

Figure 11. Thin sections of lens fibre ghosts from the cortical region of lens from transgenic mouse strain 55. Immunogold labelling with mono-vim (*A*) and poly-des (*B*). Note disruption of vimentin network (*A*) and poor accessibility of accumulated mutant desmin to the antibody (*B*). Gold particles (arrows) are 5 nm. Bars = 100 nm. (Reprinted with permission from the European Journal of Cell Biology)

transgenic mouse lens. Transgenes with a vimentin promoter linked to a heterologous IF coding region (complete, truncated or mutated) cause aberrations of the IF assembly process. Such abnormality is paralleled by impairment of membrane formation and membrane domain integrity. As far as the eye lens is concerned, the distortions lead to lenticular opacification independent of the type of IF coding sequence. Apparently the stringent prerequisite is the presence of a lens-specific promoter. It can be anticipated that strong viral promoters which drive the coupled gene into a variety of tissues in an aspecific way will act in a similar way as described here for IF or membrane protein-encoding genes. Actually, it appears from the literature that one of the obvious effects of viral promoter-driven transgenes is cataract, sometimes in addition to microphthalmia or other eye pathologies (table 3).

In our opinion earlier statements that (over)expression of IF protein-encoding sequences in transgenic mice does not lead to a defined phenotype are hardly tenable, as one may argue that the phenomenon cataract is a phenotypic feature.

Moreover, one should take into consideration that lens opacification is always preceded by subtle alterations which already are typical for an abnormal phenotype. The finding of the distorted differentiation process preceding incisor tooth formation demonstrates that introduction of the transgene may interfere with normal differentiation in any tissue where it is expressed.

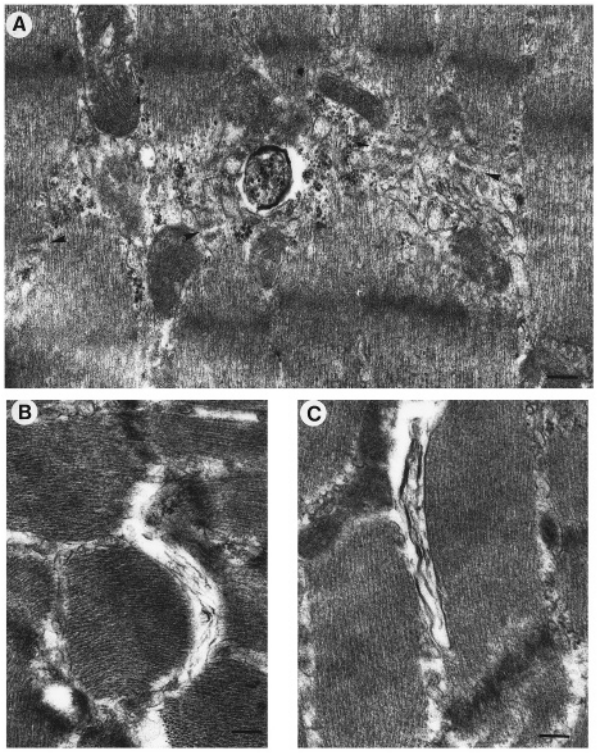

Figure 12. Longitudinal thin sections of sartorius muscle from transgenic mice stained with uranyl acetate and lead citrate. A. The T system at the A-I band junction is disrupted (arrowheads). Note the sequestration of sarcoplasmic areas wrapped by multilayered membrane profiles and disruption of the membrane vesicles of the sarcoplasmic reticulum longitudinal tubular system. (*B*, *C*) Transverse (*B*) and longitudinal (*C*) sections of myofibrils which are wrapped by fibrillar and membranous material. Bars = 200 nm. (Reprinted with permission from the European Journal of Cell Biology)

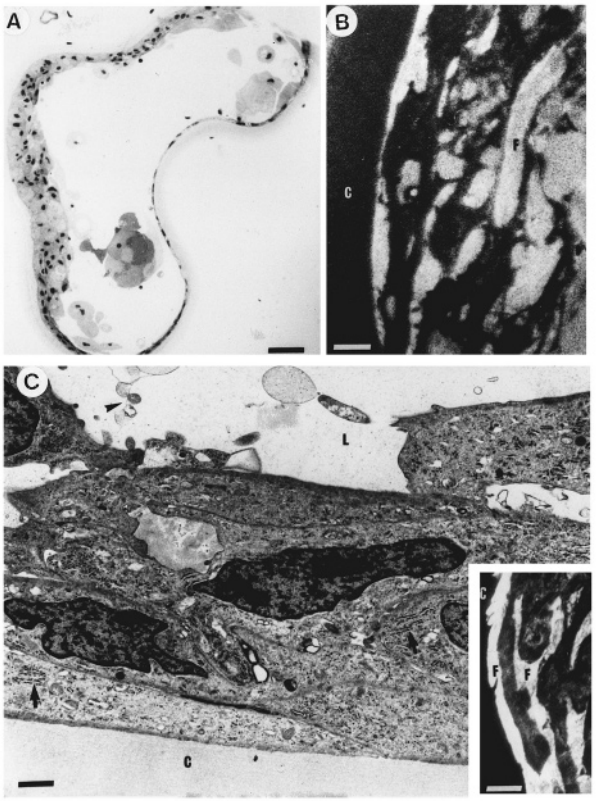

Figure 13. (*A*) Histological section of a cataractous adult transgenic mouse lens. The lens appears as a bladder surrounded by a thick capsule and formed by multilayered, round-shaped and elongated nucleated cells. The cavity is occupied by swollen cellular debris. Bar = 150 μm. (*B*) Confocal laser scanning microscopy on a cryosection of a cataractous adult transgenic mouse lens incubated with the MDR3 antibody. Note the mosaic distribution of the transgene product highly expressed in elongated multilayered cells. The capsule (C) is not stained. Bar = 60 μm. (*C*) Thin section of the wall of the lens bladder in an adult transgenic cataractous lens, stained with uranyl acetate and lead citrate. The cells are characterized by a well-developed rough endoplasmic reticulum (arrows). Note the presence of cytoplasmic vacuoles and shedded membranes (arrowheads). Bar = 2 μm. L = lumen; C = capsule. Inset: Cryosection of the same material incubated with antivimentin antibody showing the mosaic distribution of this protein. F = fibres; C = fragment of capsule detached from the lens during sectioning. Bar inset = 80 μm. (Reprinted with permission from the Journal of Cell Biology)

Finally, a phenomenon discovered several years ago [36] and which has to be clarified unequivocally is the mosaic expression of the transgene (fig. 3B). A straightforward interpretation might be that all lens cells actually do carry the transgene but do not have the capability of expressing the gene to a similar extent. Apparently the expression in some cells may be so low that it cannot be detected. From our results we may anticipate that this local phenotypic expression of the transgene indicated as 'mosaic' mode is not dependent on the level of transgene expression. An interesting feature is that the majority of cells expressing the transgene are actively dividing cells (fig. 3A). It cannot be excluded that, at least in a number of cells, the promoters of our constructs are downregulated as soon as the cells become post-mitotic (fig. 3B).

Our understanding of the structural and functional properties of IFs has been broadened by using transgenic mice expressing mutated IF proteins. With the availability of knock-out mice for specific cytoskeletal or membrane genes new issues can be addressed. One of the problems that has to be firmly established is the possibility that deletion of a single cytoskeletal or membrane constituent could be compromised by an alternative component capable of maintaining stable assembly of the membrane-cytoskeleton complex. Also, the elucidation of the various aspects of the aberrant differentiation will be necessary. We believe that the results of such studies may have important implications for forthcoming efforts in the field of gene therapy.

Table 3. Cataract in transgenic mice.

Promoter[1]	Coding region	Expression in lens and consequences	Ref.
1 αA (m)	E6-E7-SV40 polyA	inhibition of lens fibre denucleation inhibition of elongation, cataract, lens tumours	53
2 αA (m)	CRABP-SV40 polyA	ablated secondary lens fibre differentiation	54
3 αA (m)	stop-SV40 TAg	cataract, lens tumours	55
4 αA (m)	RAR-LacZ-pA	cataract, microphthalmia	56
5 αA (m)	huAR-SV40 polyA	galactose and diabetic cataract microphthalmia	57
6 αA (m)	H-2D^d	cataract, microphthalmia	58
7 αA (m)	HIV-1 protease	cataract, lens hydration	59
8 αA (m)	HIV-1 protease	cataract	60
9 γII (m)	tox 176	ablation of fibre cells, anophthalmia	51
10 vimentin (h)	desmin (h)	denucleation and elongation impaired, perturbed intermediate filament assembly, cataract	35, 62
11 vimentin (c)	vimentin (c) cataract	denucleation and elongation impaired,	63
12 vimentin (h)	P-glycoprotein	differentiation of primary fibres affected, terminal differentiation perturbed, microphthalmia, osmotic type cataract	41
13 MSV	murine NF-L	cataract	64
14 MSV-SV40	SV40 TAg	ablated secondary fibre differentiation, cataract	65
15 type I GT (hu)	rasT24 oncogene	cataract, microphthalmia	66
16 rho IF-γ	IFN-γ	cataract	67

αA (m) = mouse αA-crystallin; E6, E7 = oncoproteins from human papilloma virus (HPV); SV40 = simian virus; polyA (PA) = polyadenylic acid; CRABP = cellular retinoic acid-binding protein; stop = regulatory region composed of the C terminal sequence of yeast His 3 gene, 825 bp of SV40 polyadenylation signal region and a synthetic oligonucleotide; TAg = large tumor antigen; RAR = retinoic acid receptor; LacZ = β-galactosidase; γII = γ-crystallin II; tox176 = attenuated form of the diphtheria toxin-A gene; h = hamster; c = chicken; P-glycoprotein + ATP-dependent efflux pump; MSV = mouse sarcoma virus; NF-L = neurofilament low molecular weight subunit; IRBP = interstitial retinol-binding protein; rho = rhodopsin; IFN-γ = interferon-gamma.
[1]Lens-specific promoters: 1–9; non-lens-specific: 10–16.

1 Collucci-Guyon E., Portier M. M., Dunia I., Paulin D., Pournin S. and Babinet C., (1994) Mice lacking vimentin develop and reproduce without an obvious phenotype. Cell **79:** 679–694
2 Weitzer G., Milner D. J., Kim J. U., Bradley A. and Capetanaki, Y. (1995) Cytoskeletal control of myogenesis: a desmin null mutation blocks the myogenic pathway during embryonic stem cell differentiation. Develop. Biol. **172:** 422–439
3 Galou M., Colucci-Guyon E., Ensergueix D., Ridet J.-L., Gimenez y Ribotta M., Privat A., Babinet C. and Dupouey P. (1996) Disrupted glial fibrillary acidic protein network in astrocytes from vimentin knockout mice. J. Cell Biol. **133:** 853–863
4 Coulombe P. A., Hutton M. E., Letai A., Hebert A., Paller A. S. and Fuchs E. (1991) Mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. Cell **66:** 1301–1311
5 Fuchs E. and Coulombe P. A. (1992) Of mice and men: genetic skin diseases of keratin. Cell **69:** 899–902.
6 Fuchs E., Esteves R. A. and Coulombe P. A. (1992) Transgenic mice expressing a mutant keratin-10 gene reveal the likely genetic basis for epidermolytic hyperkeratosis. Proc. Natl. Acad. Sci. USA **89:** 6906–6910
7 Vassar R., Coulombe P. A., Degenstein L., Albers K. and Fuchs E. (1991) Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human skin disease. Cell **64:** 365–380.
8 Aebi U., Haner M., Troncosco J., Eichner R. and Engel A. (1988) Unifying principles in intermediate filament (IF) structure and assembly. Protoplasma **145:** 73–81
9 Bloemendal H. and Pieper F. R. (1989) Intermediate filaments: known structure, unknown function. Biochim. Biophys. Acta **1007:** 245–253
10 Carmo-Fonseca M. and David-Ferreira J. F. (1990) Interactions of intermediate filaments with cell structures. Electron Microsc. Rev. **3:** 115–141
11 Franke W. W. (1987) Nuclear lamins and cytoplasmic intermediate filament proteins: a growing multigene family. Cell **48:** 3–4.
12 Fuchs E. and Hanukoglu I. (1983) Unraveling the structure of the intermediate filaments. Cell **34:** 332–334
13 Fuchs E., Marchuk D. and Tyner E. (1986) Differential expression of the genes encoding the keratins of cultured human epidermal cells. In: Cell and Molecular Biology of the Cytoskeleton, pp. 85–101, Shay J. W. (ed.), Plenum Press, New York
14 Geisler N. and Weber K. (1982) The amino acid sequence of chicken muscle desmin provides a common structural model for intermediate filament proteins. EMBO J. **1:** 1649–1656
15 Geisler N. and Weber K. (1986) Structural aspects of intermediate filaments. In: Cellular and Molecular Biology of the Cytoskeleton, pp. 41–64, Shay J. W. (ed.), Plenum Press, New York
16 Goldman R. D., Goldman A. E., Green K. J., Jones J. C. R., Jones S. M. and Yang H. Y. (1986) Intermediate filament networks: organization and possible functions of a diverse group of cytoskeletal elements. J. Cell Sci. **5:** 69–97
17 Klymkowsky M. W., Bachant J. B. and Domingo A. (1989) Functions of intermediate filaments, Cell Motil. Cytoskel. **14:** 309–331.
18 Lazarides E. (1982) Intermediate filaments: a chemically heterogenous, developmentally regulated class of proteins. Ann. Rev. Biochem. **51:** 219–250
19 Liem R. K. H. (1990) Neuronal intermediate filaments. Curr. Opin. Cell Biol. **2:** 86–90
20 Osborn M. and Weber K. (1982) Intermediate filaments: cell-type specific markers in differentiation and pathology. Cell **31:** 303–306
21 Osborn M. and Weber K. (1986) Intermediate filament proteins: a multigene family distinguishing major cell lineages. Trends Biochem. Sci. **11:** 469–472
22 Raats J. M. H. and Bloemendal H. (1992) The role of protein domains in the assembly process of intermediate filaments. Progr. Nucleic Acid Res. Molec. Biol. **43:** 67–86

23 Robson R. M. (1989) Intermediate filaments. Curr. Opin. Cell Biol. **1:** 36–43
24 Skalli O. and Goldman R. D. (1991) Recent insights into the assembly, dynamics and function of intermediate filament networks. Cell Motil. Cytoskel. **19:** 67–79
25 Steinert P. M., Steven A. C. and Roop D. R. (1985) The molecular biology of intermediate filaments, Cell **42:** 411–420.
26 Steinert P. M. and Roop D. R. (1988) Molecular and cellular biology of intermediate filaments, Annu. Rev. Biochem. **57:** 593–625.
27 Steinert P. M. and Liem R. K. H. (1990) Intermediate filament dynamics. Cell **60:** 521–523.
28 Stewart M. (1990) Intermediate filaments: structure, assembly and molecular interactions. Curr. Opin. Cell Biol. **2:** 91–100
29 Tokutake S. (1990) On the assembly mechanism of neurofilaments. Int. J. Biochem. **22:** 1–6
30 Traub P. (1985) Intermediate Filaments: A Review. Springer Verlag, Berlin
31 Van de Klundert F. A. J. M., Raats, J. M. H. and Bloemendal, H. (1993) Intermediate filaments: regulation of gene expression and assembly. Eur. J. Biochem. **214:** 351–366
32 Wagner, A. P. (1989) On the relationships between the rate of cytoskeletal stable assemblies turnover, stability of the differentiated state, development and aging. J. Theor. Biol. **138:** 175–184
33 Weber, K. and Geisler, N. (1987) Biochemistry and molecular structure of intermediate filaments. Fortschritte der Zoölogie **34:** 251–260
34 Inagaki, M., Matsuoka, Y., Tsujimura, K., Ando, S., Tokui, T., Takahashi et al. (1996) Dynamic property of intermediate filaments: regulation by phosphorylation, BioEssays **18:** 481–487
35 Pieper, F. R., Schaart, G., Krimpenfort, P. J., Henderik, J. B., Moshage, H. J., Van der Kemp et al. (1989) Transgenic expression of the muscle-specific intermediate filament protein desmin in nonmuscle cells. J. Cell Biol. **108:** 1009–1024
36 Dunia I., Pieper F., Manenti S., Van de Kemp A., Devilliers G., Benedetti E. L. et al. (1990) Plasma membrane-cytoskeleton damage in eye lenses of transgenic mice expressing desmin. Eur. J. Cell Biol. **53:** 59–74
37 Berteretche M. V., Dunia I., Devilliers G., Van der Kemp A., Pieper F., Bloemendal H. et al. (1993) Abnormal incisor-tooth differentiation in transgenic mice expressing the muscle-specific desmin gene. Eur. J. Cell Biol. **62:** 183–193
38 Krimpenfort, P. J., Schaart, G., Pieper, F. R., Ramaekers F. C., Cuypers H. T., Van den Heuvel R. M. et al. (1988) Tissue-specific expression of a vimentin-desmin hybrid gene in transgenic mice. EMBO J. **7:** 941–947
39 Pieper F. R., Raats J. M. H., Schaart G., Dunia I., Van der Kemp A., Benedetti E. L. et al. (1995) Disruption of vimentin intermediate filaments in transgenic mice by expression of a dominant negative mutant desmin subunit. Eur. J. Cell Biol. **68:** 355–368
40 Raats J. M. H., Schaart G., Henderik J. B. J., van der Kemp A., Dunia I., Benedetti, E. L. et al. (1996) Muscles-specific expression of a dominant negative desmin mutant in transgenic mice. Eur. J. Cell Biol. **71:** 221–236
41 Dunia I., Smit J. J. M., Van der Valk M. A., Bloemendal H., Borst, P. and Benedetti E. L. (1996) Human multidrug resistance 3-P-glycoprotein expression in transgenic mice induces lens membrane alterations leading to cataract. J. Cell Biol. **132:** 701–716
42 Devine, D. E., Ling, V. and Melera, P. W. (1992) Amino acid substitutions in the sixth transmembrane protein of eye lens fibers. J. Cell Biol. **105:** 1679–1689
43 Gros P., Neriah Y. B., Croop J. M. and Housman D. E. (1986) Isolation and expression of a cDNA (*mdr*) that confers multidrug reistance. Nature **323:** 728–731
44 Lincke C. R., van der Bliek A. M., Schuurhuis G. J., van der Velde-Koerts T., Smit J. J. M. and Borst P. (1990) Multidrug resistance phenotype of human BRO melanoma cells transfected with a wild-tye human mdr1 complementary DNA, Cancer Res. **50:** 1779–1785
45 Ueda K., Cardarelli C., Gottesman M. M. and Pastan I. (1987) Expression of a full-length cDNA for the human 'MDR1' gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc. Natl. Acad. Sci. USA **84:** 3004–3008
46 Smit J. J. M., Schinkel A. H., Mol C. A. A. M., Majoor D., Mooi W. J., Jongsma A. P. M. et al. (1994) The tissue distribution of the human MDR3 P-glycoprotein. Lab. Invest. **71:** 638–649
47 Smit J. J. M., Schinkel A. H., Oude Elferink R. P. J., Groen A. K., Wagenaar E., van Deemter L. et al. (1993) Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell **75:** 451–462
48 Smith A. J., Timmermans-Hereijgers J. L. P. M., Roelofsen B., Wirtz K. W. A., van Blitterswijk W. J., Smit J. J. M. et al. (1994) The human MDR3 P-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice. FEBS Lett. **354:** 263–266
49 Ruetz, S. and Gros, P. (1994) Phosphatidylcholine translocase: a physiological role for the mdr2 gene. Cell **77:** 1071–1081
50 Bloemendal H., Benedetti E. L. and Dunia I. (1996) Transgenic mice: models for the study of cataractogenesis. Ophthalmic Res. **28:** 1–7
51 Hess J. F., Casselman J. T. and Fitzgerald P. G. (1993) cDNA analysis of 49kDa lens fiber cell cytoskeletal protein: a new, lens-specific member of the intermediate filament family. Curr. Eye Res. **12:** 77–88
52 Orii H., Agata K., Sawada K., Eguchi G. and Maisel H. (1993) Evidence that the chick lens cytoskeleltal protein CP-49 belongs to the family of intermediate filament proteins. Curr. Eye Res. **12:** 583–588
53 Pan H. and Griep A. E. (1994) Altered cell cycle regulation in the lens of HPV-16 E6 or E7 transgenic mice: implications for tumor suppressor gene function in development. Gen. Dev. **8:** 1285–1299
54 Perez-Castro A. V., Tran V. T. and Nguyen-Huu M. C. (1993) Defective lens fiber differentiation and pancreatic tumorigenesis caused by ectopic expression of the cellular retinoic acid-binding protein I. Development **119:** 363–375
55 Lakso M., Sauer B., Mosinger B. Jr., Lee E. J., Manning R. W., Yu S-H. et al. (1992) Targeted oncogene activation by site-specific recombination in transgenic mice. Proc. Natl. Acad. Sci. USA **89:** 6232–6236
56 Balkan W., Klintworth G. K., Bock C. B. and Linney E. (1992) Transgenic mice expressing a constitutively active retinoic acid receptor in the lens exhibit ocular defects. Dev. Biol. **151:** 622–625
57 Lee A. Y. W., Chung S. K. and Chung S. S. M. (1995) Demonstration that polyol accumulation is responsible for diabetic catract by the use of transgenic mice expressing the aldose reductase gene in the lens. Proc. Natl. Acad. Sci. USA **92:** 2780–2784
58 Martin W. D., Egau R. M., Stevens J. L. and Woodward J. G. (1995) Lens-specific expression of a major histocompatibility complex class I molecule disrupts normal lens development and induces cataracts in transgenic mice. Invest. Ophthalmol. Vis. Sci. **36:** 1144–1154
59 Bettelheim F. A., Zeng F. F., Bia Y., Tumminia S. J. and Russell P. (1995) Lens hydration in transgenic mice containing HIV-1 protease linked to the lens aA-crystallin promoter. Arch. Biochem. Biophys. **324:** 223–227
60 Tumminia S. J., Jonak G. J., Focht R. J., Cheng Y. S. and Russell P. (1996) Cataractogenesis in transgenic mice containing the HIV-1 protease linked to the lens αA-crystallin promoter. J. Biol. Chem. **271:** 425–431
61 Breitman M. L., Rombola H., Maxwell I. H., Klintworth G. K. and Bernstein, A. (1990) Genetic ablation in transgenic mice with an attenuated diphtheria toxi A gene. Mol. Cell Biol. **10:** 474–479
62 Dunia I., van der Kemp A., Pieper F., Bloemendal H. and Benedetti E. L. (1991) Eye lens structural abnormalities in mice expressing transgenes affecting the organization of intermediate filaments. In: Eye Lens Membranes and Aging: Topics in Aging Research in Europe, vol. 15, pp. 145–159, Vrensen, G. F. J. M. and Clauwaert, J. (eds), EURAGE, Leiden

63 Capetanaki Y., Smith S. and Heath J. P. (1989) Overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation. J. Cell Biol. **109:** 1653–1664
64 Monteiro M. J., Hoffman P. N., Gearhart J. D. and Cleveland D. W. (1990) Expression of NF-L in both neuronal and nonneuronal cells of trangenic mice: increased neurofilament density in exons without affecting caliber. J. Cell Biol. **111:** 1543–1557
65 Götz W., Theuring F., Favor J. and Herken R. (1991) Eye pathology in transgenic mice carrying a MSV-SV 40 large T-construct. Exp. Eye Res. **52:** 41–50
66 Chevez-Barrios P., Schaffner D. L., Barrios R., Overbeek P. A., Lebovitz R. M. and Lieberman M. W. (1993) Expression of the ratTG oncogene in the ciliary body pigment epithelium results in hyperplasia, adenoma and adenocarcinoma. Am. J. Pathol. **143:** 20–28
67 Geiger K., Howes E., Gallina M., Huang X. J., Travis G. H. and Sarvetnick N. (1994) Transgenic mice expressing IFN-γ in the retina develop inflammation of the eye and photoreceptor loss. Invest. Ophthalmol. Vis. Sci. **35:** 2667–2681

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