Overexpression of the Vimentin Gene in Transgenic Mice Inhibits Normal Lens Cell Differentiation

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Abstract. To investigate the role of the intermediate filament protein vimentin in the normal differentiation and morphogenesis of the eye lens fiber cells, we generated transgenic mice bearing multiple copies of the chicken vimentin gene. In most cases, the vimentin transgene was overexpressed in the lenses of these animals, reaching up to 10 times the endogenous levels. This high expression of vimentin interfered very strongly with the normal differentiation of the lens fibers. The normal fiber cell denucleation and elongation processes were impaired and the animals developed pronounced cataracts, followed by extensive lens degeneration. The age of appearance and extent of these abnormalities in the different transgenic lines were directly related to the vimentin level. Electron microscopic analysis revealed that the accumulated transgenic protein forms normal intermediate filaments.

Vimentin shows a unique pattern of expression relative to all known intermediate filament (IF) proteins. It is mainly expressed in cells of mesenchymal origin, but also in many undifferentiated cells and cultured cells (Lazarides, 1982; Osborn, 1982). It is known to be growth regulated in different cell types (Ferrari et al., 1986; Hirshchorn et al., 1984; Ritting and Baserga, 1987). Certain terminally differentiating cells replace vimentin partially (Tapskott et al., 1981) or completely (Tapskott et al., 1981; Granger and Lazarides, 1979) with their cell type specific IF protein. Finally, vimentin can be the sole IF subunit of the mature cell, as best exemplified in lens (Ramaekers et al., 1980), avian erythrocytes (Granger et al., 1982) and certain white blood cells (Dellagi et al., 1983). Despite this diversity of vimentin expression, its biological functions remain largely unknown. Vimentin seems to connect the nucleus with the plasma membrane (Lehto et al., 1978; Woodcock, 1980; Laurila et al., 1981; Granger and Lazarides, 1982; Georgatos and Blobel, 1987a,b; Georgatos and Marchesi, 1985; Georgatos et al., 1985; Goldman et al., 1986), possibly contributing in this structural way to the transport processes and signal transduction taking place between the cell surface and the nucleus. Indeed, recent biochemical studies suggest that purified vimentin binds to the nuclear envelope lamin B via its COOH terminus (Georgatos and Blobel, 1987a,b) and to the plasma membrane (via ankyrin) through its NH2 terminus (Georgatos and Marchesi, 1985; Georgatos et al., 1985). High binding affinity of vimentin, and other nonepithelial IF subunits to DNA has also been reported (Traub and Nelson, 1982; Traub, 1987; Shoeman et al., 1988; Cress and Kurath, 1988). However, to date, there is no evidence to support functional roles for any of the demonstrated structural interactions.

1. Abbreviations used in this paper: IF, intermediate filament; VXBK, vimentin construct.

We have chosen to investigate the function of vimentin using transgenic mice carrying intact or manipulated vimentin genes. We initially focused on the consequences of altering the normal pattern of vimentin during lens and erythrocyte development. The interest in these two tissues is because of the fact that these cells are anuclear in the mature form and they both show down regulation of the vimentin expression during mammalian cell terminal differentiation (Dellagi et al., 1983; Ngai et al., 1984; Ramaekers et al., 1980, 1982; Bradley et al., 1979; Granger and Lazarides, 1984). However, we have shown that during chicken erythropoiesis, the vimentin gene is highly induced (Capetanaki et al., 1983, 1984) in contrast to what is happening during mammalian erythroid development (Dellagi et al., 1983; Ngai et al., 1984). During terminal erythroid differentiation, chicken cells retain their nuclei. Therefore, vimentin might play an essential role in the terminal differentiation of these cells either by retaining the nuclei in chicken or facilitating enucleation by its disappearance during mammalian erythropoiesis. In lens cells, we have a parallel situation. Normal lens possesses a monolayer of mitotically active epithelial cells that lines the anterior part of the structure. These cells cease dividing at the equatorial region where they begin the differentiation process that results in formation of the fiber cells. They start elongating and eventually lose both nuclei and other organelles. Vimentin is mainly expressed in the epithelium of the lens (Ireland et al., 1978; Lo and Maisel, 1979; Bradley et al., 1979; Ramaekers et al., 1980; Ellis et al., 1984; Bagchi et al., 1985). When the epithelial cells initiate the differentiation pathway, vimentin filaments begin to decrease, become associated primarily with the outer cortex fibers and then vanish (Ramaekers et al., 1980; Bradley et al., 1979; Granger and Lazarides, 1984). We were interested in investigating the consequences of altered vimentin expres-
sion on terminal differentiation of these cells, including the denucleation process.

For this purpose, we have generated several transgenic mice bearing multiple copies of the chicken vimentin gene, as described elsewhere (Capetanaki et al., 1989). Analysis of RNA from different tissues of these animals demonstrated that the chicken vimentin gene was expressed qualitatively in the correct tissue specific pattern (Capetanaki et al., 1989). Chicken vimentin transcripts were properly processed into stable mRNA molecules that were efficiently translated into polypeptides that were modified posttranslationally and assembled into the mouse cytoskeleton (Capetanaki et al., 1989). In addition, vimentin produced from the transgene transcripts demonstrated correct spatial expression in the lens where, however, it tended to be overexpressed. In the present report, we demonstrate that the consequences of overexpression of the vimentin gene in lens cells include a striking inhibition of the normal fiber cell denucleation and elongation process as well as the appearance of pronounced lens cataracts that are always accompanied by extensive lens degeneration.

Materials and Methods

Generation and Screening of Transgenic Mice

The chicken vimentin construct (VXBK) used for microinjection has been described elsewhere (Capetanaki et al., 1989). Briefly, VXBK contains the entire 7.7-kb vimentin gene (Capetanaki et al., 1983; Zezher and Paterson, 1983; Zezner et al., 1987) plus 2.4-kb 5' and 2.6-kb 3' flanking sequences. It has been subcloned into puUC18 vector in a way that permits its isolation free of vector sequences by digestion with Xho I and Kpn I. Transgenic mice using this construct were generated as described elsewhere (Capetanaki et al., 1989; Brinster et al., 1985; Hogan et al., 1986; DeMayo and Bullock, 1987). 3-5-wk-old superovulated female donor mice (Inbred; Charles River Breeding Laboratories, Wilmington, MA [ICR]) were mated with 3-8-mo-old B6C3F1 males. Embryos were isolated from the oviduct of the donor mice and the chicken vimentin VXBK fragment was injected into the male pronuclei. Viable injected embryos were transferred to ICR pseudopregnant recipient females. Tail DNA screening for positive animals was performed as described elsewhere (Capetanaki et al., 1982; Hogan et al., 1986; Capetanaki et al., 1989).

Protein Analysis by Two-Dimensional Gel Electrophoresis and Western blot Analysis

Total lens protein from normal or different transgenic mice was analyzed by two-dimensional isoelectric focusing SDS-polyacrylamide gels essentially by the method of O'Farrell (1975). Separated polypeptides were transferred from SDS polyacrylamide slab gels to nitrocellulose for detection with chicken antivimentin and iodinated protein A as described elsewhere (Towbin et al., 1979) with some modifications (Glasser and Julian, 1986). The chicken vimentin antibodies were provided by Dr. R. Goldman and were previously characterized (Green and Goldman, 1986). For the immunoblot, 1:600 dilutions were used.

Histology

Whole eyes were fixed in 10% formalin, tissues were dehydrated and embedded in paraffin using routine procedures. Sections were cut 4 μm and stained with Harris’ hematoxylin and eosin. Light micrographs of Fig. 1 were taken in a microscope (Nikon Inc., Garden City, NY) at 4x. Micrographs of Fig. 3 (A-H) were taken under a microscope (Olympus Corporation of America, New Hyde Park, NY) and Figs. 3 (A-G) 5, and 6 on a microscope (Carl Zeiss, Inc., Thornwood, NY).

Electron Microscopy

Mouse eyes were fixed in 2.5% glutaraldehyde in PBS for 24 h, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Spurr's resin. 80-nm-thick sections were contrasted with uranyl and lead salts and photographed on an electron microscope (EM 410; Philips Electronic Instruments, Inc., Mahwah, NJ).

Results

Generation of Transgenic Mice Overexpressing Vimentin in Lens

To alter the normal expression of vimentin and study the consequences on differentiation and development, we generated several transgenic mice bearing multiple copies of the chicken vimentin gene as described elsewhere (Capetanaki et al., 1989). Established lines include two homozygous lineages termed Tgl9 and Tg21 and two heterozygous lineages called Tg60 and Tg149. The characteristics of these lineages are summarized in Table I. Analysis of RNA from different tissues of these animals has previously demonstrated that the vimentin transgene was overexpressed in the lens of 75% of the obtained transgenic animals (Table I) Capetanaki et al., 1989). Immunofluorescence microscopy of lens frozen sections demonstrated that indeed the chicken gene product exhibits the expected localization in the mouse lens.

Development of Lens Cataracts in Transgenic Mice Overexpressing Vimentin

When mice homozygous for the vimentin transgene (Tgl9) reached 6–12 wk of age, a striking bilateral opacity of the lenses was observed (Fig. 1 B) in contrast to the normally transparent lens (Fig. 1 A). A similar situation was observed with mice from the Tg19 line that were heterozygous for chicken vimentin, but cataract formation began later around the fifth month. Tg60 mice are also heterozygotic, and again showed the same abnormality but even later and to a lesser extent than animals from the Tg19 line. Our other homozygous line, Tg21 has shown very low breeding capacity and survival. Consequently, the characteristics of lens cataracts from this line are not known. However, heterozygotes that have been derived from a subline of Tg21, develop cataracts between 6 and 7 mo. This Tg21 subline was most likely derived by loss of transgene copies during breeding (Capetanaki et al., 1989). The Tg149 line was the most recently obtained and the level of expression of the transgene was lower than that of the endogenous mouse vimentin gene. No lens opacity has been observed in these animals. Comparison of the levels of the transgenic vimentin RNA expression in these different lines indicated that the age of appearance and the degree of cataractous lens is proportional to the level of the transgenic vimentin mRNA (Table I) Capetanaki et al., 1989). To determine whether the above relationship held true at the protein level, total protein from lenses of the different transgenic animals was analyzed by two-dimensional gel electrophoresis. Fig. 2 demonstrates that the transgenic vimentin was very efficiently produced in all cases examined. The identity of the transgenic protein as chicken vimentin was confirmed by Western blot analysis of the two-dimensional gel protein patterns using chicken vimentin antibodies (Fig. 2 E). We have already shown that the transgenic chicken vimentin subunits are efficiently incorporated into the mouse cytoskeleton to form fibrillar configurations (Capetanaki et al., 1989). However, overexpression of this...
### Table I. Summary of Characteristics of Generated Chicken Vimentin Transgenic Mice

<table>
<thead>
<tr>
<th>Transgenic line</th>
<th>RNA expression (%)</th>
<th>Protein expression (%)</th>
<th>Age of cataract appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg21 heterozygous (F1)</td>
<td>70-90</td>
<td>1,000-1,100</td>
<td>ND</td>
</tr>
<tr>
<td>Tg21* heterozygous subline (F3)</td>
<td>40</td>
<td>500-600</td>
<td>400-500</td>
</tr>
<tr>
<td>Tg19 homozygous (F3)</td>
<td>4-6</td>
<td>900-1,000</td>
<td>1,200-1,300</td>
</tr>
<tr>
<td>Tg19 heterozygous (F3)</td>
<td>8-12</td>
<td>300-400</td>
<td>250-300</td>
</tr>
<tr>
<td>Tg60 heterozygous (F1)</td>
<td>3-4</td>
<td>10-20</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Tg21 subline has lost copies during breeding.
† Transgene copy number is given for the founder mice.
‡ Expression levels were determined by densitometric scanning on x-ray films (BioImage; Eastman Kodak Co., Rochester, NY) whole band electrophoresis analysis software. They are given in percentages relative to equivalent endogenous RNA (Capetanaki et al., 1989) or protein level (Fig. 2).
§ ND (as opposed to ND alone), cataract not detected because of early death of the animals for Tg21 or possibly because of low transgenic vimentin expression for Tg149.
¶ FI, first generation; F3, third generation.

Protein has strongly disturbed the normal morphogenesis of the lens. As shown in Fig. 2 D, Tg19 mice contained 6-7 times the normal endogenous level of vimentin in the lens. Tg21 mice (Fig. 2 C) achieved 4-5 times the normal level and Tg60 mice (Fig. 2 B) showed 50-60% lower expression than the subline of Tg21 mice. Comparison of the expression level of transgenic vimentin in the different lines shows that the age of appearance, and the degree of abnormality of the cataractous lens is indeed directly related to the vimentin level. This was further verified recently by demonstrating.

**Figure 1.** Cataract formation in the transgenic mouse. The striking lens opacity in the 17-wk-old transgenic homozygote Tg19 mouse (B) can be seen by comparing it to the normal mouse (A). (C) Histological section (4 μm) of the normal eye from A stained with Harris hematoxylin and eosin. (D) Histological section from the cataractous lens of the mouse in (B) stained as in (C). Notice the extent of degeneration of the lens cortex. The posterior half is missing completely (see text). L, lens; LC, lens cortex; LN, lenticular nucleus; RCE, region of cellular elongation (equatorial region); Cap, lens capsule; a, aqueous humor (anterior chamber); v, vitreous humor; R, retina; Cor, cornea; ON, optic nerve. Bar, 300 μM.
Figure 2. Overexpression of the chicken vimentin gene in the lens of transgenic mice. Analysis by two-dimensional gel electrophoresis. Total lens cell protein from 4–5-wk-old normal (A), transgenic heterozygote mouse Tg60 (F3) (B), heterozygote Tg21 (F3), a subline of the original line (C), heterozygote Tg19 (F3) (D), and 3-mo-old transgenic homozygote Tg19 (F3) with 2-wk-old cataract (Tg19++) (F) mice were separated by two-dimensional gel electrophoresis, and stained with Coomassie blue (A, B, C, D, and F) or transferred to nitrocellulose and probed with chicken vimentin antibodies and iodinated protein A (E). Note the overexpression of the chicken vimentin (ChV) versus the endogenous mouse vimentin (MV) in all cases. Also note the variation in the abundance of the transgenic protein. Tg19 expresses the most and Tg60 the least. The chicken vimentin from all transgenic lenses shows the known multiple isoelectric variants denoted with a horizontal bracket. The more acidic lower molecular weight spots denoted with arrows are degradation products of vimentin as verified by Western blot analysis similar to the one performed for Tg21 in E using chicken antibodies. Note the abundance of degradation products from cataractous lens (F) indicated by vertical bracket. A, actin.

that heterozygote Tg19 mice, generated by cross-breeding homozygous Tg19 with normal mice, exhibited normal lens development and phenotype. Even in 4-mo-old animals, cataract formation has not occurred. Similarly, heterozygous Tg19 mice have never developed cataracts before the fifth month of age. Together, these data indicate that the appearance and severity of lens abnormalities are directly linked to vimentin overexpression. It is interesting to note that when we compared the two-dimensional gel profiles of lens proteins obtained from normal and 5-wk-old Tg19 animals, it has not revealed any difference in the steady-state level of crystallins (data not shown).

Vimentin Overexpression Inhibits Normal Lens Cell Differentiation: Extensive Impairment of Denucleation Process

Histological examination of lens tissue was performed initially in 16–17-wk-old Tg19 homozygous mice. As shown in Fig. 1 D, 60–70% of the cortical fibers are completely missing from the posterior half of the lens, but the lens nucleus looks normal. The rest of the lens cortex shows numerous vacuoles of degenerating tissue. Fiber cells cannot easily be identified at this stage. The whole eye was observed to be smaller and more fragile, with the remainder of the lens being pushed close to and occasionally touching the retina. In most cases, the retina appears undulated and thicker than normal with a high degree of detachment in transgenic eyes. We examined the development of this abnormal lens phenotype by comparing histological sections of cataractous lenses from different ages of transgenic mice (Fig. 3). Lens obtained from a 28-wk-old heterozygous Tg60 mouse shows the earliest stage of abnormality we have observed (Fig. 3 B). It is less extensive than those from homozygous Tg19 mice that were 4–6-wk-old (Fig. 3, C and C'), or 8–22-wk-old (Fig. 3, D–H), probably because of the lower levels of vimentin expression (Fig. 2). The initiation of these dramatic changes was observed mainly in the outer cortex of the equatorial region (Figs. 1 E, 3, B and B'), and partially in the epithelium. As revealed by electron microscopic analysis, typically, the epithelium of the transgenic mice in the equatorial region had two to three times the normal density of nuclei (Fig. 4, a and b). It seems that the lens fiber cell differentiation is normal up to a certain stage during development and then it is strongly disturbed (Table I). Lens fiber elongation becomes insufficient and reaches only short dis-
Figure 3. Stages of development of the lens cataract in transgenic mice. Histological sections of a normal (14-wk-old) (A) and several cataractous lenses from different stages of development (6-wk-old to 22.5-wk-old) (C–G) of the homozygous Tg19 (F3) mice (Bar, 500 μM). (B) 28-wk-old heterozygote Tg60 that represents the earliest stage of lens degeneration (Bar, 500 μM). (A′–G′) higher magnification (Bar, 200 μM) of the regions denoted by asterisks in A–G, respectively. Ba is a higher magnification (Bar, 50 μM) of the area of B′ where initiation at lens degeneration (DL) occurs at the beginning of the posterior half of the lens. Compare A′ and B′ bow areas to notice the persistence of nuclei and their spread to mouse posterior regions as well (B′ small area at top). (See Fig. 6 for better visualization of nuclei persistence.) Arrows in E′ denote a structure similar to capsule separating the lenticular nucleus from the degenerating cortex. L, lens; LN, lenticular nucleus; RE, equatorial region (region of elongation); DL, degenerating lens; Ep, epithelium (in G, it is also degenerating); A, aqueous humor; V, vitreous humor; R, retina; Cap, lens capsule; Ir, iris.

tance from the equator (Fig. 5 B). The posterior capsule becomes very thin and eventually can be ruptured (Fig. 3 C). In all cases examined, degeneration starts by the formation of numerous small vacuoles in the equatorial region that eventually fuse creating larger cysts (Fig. 3, B, B′, and Bα′). Persistent retention and scattering of nuclei throughout the lens cortex is the most readily observed early abnormality (Fig. 6, B and C). As mentioned above, normally only the
epithelial cells and the young fiber cells are nucleated. In a normal lens cross section (Fig. 6 A), fiber nuclei are seen only at the equatorial region. This is obviously not the case in the cataractous transgenic lens (Fig. 6, B and C). In all cases examined, nuclei are present in areas between intact and degenerating lens tissue at positions where they do not normally exist. Although this is clear in Fig. 6, it cannot be easily visualized in Fig. 3 (C'-H'), because of the extensive size reduction. It seems that inhibition of normal fiber cell denucleation is one of the earliest processes that is disturbed since it occurs at earlier stages where cataracts have not yet formed and fiber cell elongation seems normal (data not shown).

Chicken Vimentin Forms Normal IF Filaments in the Lens of the Transgenic Mice

To show that the chicken vimentin was assembled into normal filaments in the mouse cells, we examined thin sections of transgenic lenses by EM. Fig. 7 a shows the typical appearance of 10-nm-diam vimentin filaments in the perinuclear region of normal mouse lens epithelial cells. By contrast, the lens epithelial cells of 15-d-old homozygous transgenic (Tgl9) mice have unusually high numbers of intermediate filaments (Fig. 7 b). These filaments are morphologically identical to the 10-nm-vimentin filaments of control cells; this taken together with the biochemical data in Fig. 2 leads us to conclude that the transgenic chicken vimentin is being assembled into normal filaments. As outlined in Table I, cataract formation has not been observed at this early stage (15-d old). On the contrary, cataract formation was at an advanced stage in 7-mo-old mice of the same transgenic line (Fig. 8). The most obvious correlates of cataract formation seen at the EM level were first the increased density of filaments, similar to that in earlier age (Fig. 7 b), and second, a significant number of cells showing degenerative changes. Fig. 8 b is an epithelial cell from the same lens seen in Fig. 8 a. This cell has abnormal mitochondria that may indicate an early phase of the degenerative process.

Discussion

During normal lens development, the mitotically active epithelial cells cease dividing at the equatorial region and begin to differentiate and form the fiber cells which elongate and eventually lose their nuclei (Piatigorsky, 1981). The objective of the present study was to alter the expression level of vimentin and investigate the consequences on the normal differentiation and development of the lens. We have now shown that overexpression of the chicken vimentin transgene in the mouse inhibits normal fiber cell differentiation (Figs. 1 and 3-6) and leads to cataract formation. There was a direct correlation between the amount of the expressed transgenic protein and the age of appearance of the cataract (Table I) that strongly suggests the direct causal association between vimentin overexpression and differentiation defects.

At present, we do not know the exact mechanism by which the excess vimentin has contributed to abnormal lens cell differentiation and, in particular, the increased nuclei density in the equatorial epithelium, the extensive impairment of the denucleation process and the insufficient cell elongation. We also do not yet know how the expression of the foreign vimentin is linked to the formation of the cataract. The present electron microscopic analysis has demonstrated that the excess transgenic vimentin forms normal IF filaments. This suggests that whatever the exact mechanism is, the effect is most possibly because of a high number of vimentin filaments. We do not know, however, how this excess of filaments interferes with the normal cell differentiation. Vimentin is believed to interlink the nucleus to the plasma membrane via binding of its -COOH terminus to lamin B (Georgatos and Blobel, 1987a,b), and its -NH2 terminus to ankyrin (Georgatos and Marchesi, 1985; Georgatos et al., 1985). Indeed, careful analysis of Fig. 8 a shows areas of nuclear membrane have been in very close connection to intermediate filaments. It has also been postulated that in conjunction with microfilaments and microtubules, IF might participate in other cell functions such as the maintenance of spatial organization of cytoplasmic organelles (Jimbow and Fitzpatrick, 1975; Wang and Goldman, 1978; David-Ferreira and David-Ferreira, 1980; Geiger and Singer, 1980) and intracellular transport (Jimbow and Fitzpatrick, 1975; Goldman and Naipe, 1972; Aubin et al., 1980; Zieve et al., 1980). It is possible that extra vimentin filaments tightly connecting the nucleus to the plasma membrane interfere directly or indirectly with the proper cell elongation and denucleation process. This could be because of a mechanical effect or an interruption of the normal communication from cell surface to nucleus.
or both. Microtubules are believed to play a role in the elongation of the lens cell body (Piatigorsky et al., 1972a,b). Comparison of two-dimensional electrophoresis patterns of lens proteins from normal and transgenic mice, however, has not revealed any major difference in the tubulin expression (Fig. 2 and similar gels after silver staining, not shown).

Disturbance of the normal processes because of possible formation of abnormal mouse-chicken vimentin heteropolymers of intermediate filaments cannot be excluded. Electron microscopic immunolabeling investigations would be a way of approaching this issue. Our present electron microscopic analysis cannot easily answer these questions because of the lack of specific mouse antibodies. We have recently sequenced the mouse vimentin cDNA and determined the full protein primary structure. Comparison of the mouse and the chicken proteins reveals 80% overall amino acid sequence homology. In some regions believed to be crucial for filament assembly and function, the homology reaches 90-100%. The amino
Figure 6. Impairment of the lens cell denucleation process in the cataractous transgenic mice overexpressing vimentin. Sections of 8-wk-old normal and cataractous homozygote Tg19 (Tg19w) lenses. A, equatorial region of normal lens. B, equatorial region of Tg19w lens. C, anterior cortex of Tg19w, from the same section as B. Notice the striking persistence of the nuclei both in B and C. Bar, 25 μM.
Figure 7. Chicken vimentin forms normal 10-nm filaments in the lens of transgenic mice. (a) Electron micrographs of thin sections through lens epithelial cells. (a) A normal mouse showing the typical appearance of 10-nm filaments (arrows) in the perinuclear regions. (b) This lens was obtained from a 15-d-old homozygote Tgl9 mouse that had not developed any cataract. All the filaments seen in the cytoplasm are of the expected 10-nm diameter as shown in longitudinal (arrow) and cross section (circle). Notice the abundance of these filaments. Bar, 0.5 μm.
Figure 8. (a) Electron micrograph of a thin section of a lens epithelial cell of a 7-mo-old transgenic mouse (Tgi9). This mouse had a severe cataract and histologically is comparable to the lens shown in Fig. 3 G. The cytoplasm is almost totally filled with 10-nm-diam filaments. Bar, 0.5 μm. (b) An adjacent region of the same lens showing a cell with early signs of degeneration. The mitochondria (m) have lost cristae, and the cytoplasm is less electron dense. Bar, 0.5 μm.
terminus shows around 8% divergence, but we do not know if this is enough to disturb the normal vimentin filament function. If heteropolymers can be formed but are not functional, what we have created is a dominant mutant for functional vimentin filaments that is vimentin dosage-dependent and the vimentin threshold concentration is inversely proportional to the age of the animal. Alternatively, the abnormalities are related simply to the overabundance of the filaments.

Of interest is the fact that related abnormal lens differentiation has been described in some hereditary mouse cataracts (Brown et al., 1970; Hamai et al., 1974) and in transgenic mice overexpressing the oncogene c-mos in lens (Khillian et al., 1987). Since cells transfected with other oncogenes, like H-ras, not only fail to down regulate but also increase vimentin expression (Olson and Capetanaki, 1989), it may be of importance to study the pattern of vimentin expression in those other cases. In addition, it should be noted that there are several examples of cataract development in transgenic mice expressing either the transforming SV40 T antigen gene (Maillon et al., 1987) or a toxin producing gene (Breitman et al., 1987; Landel et al., 1988). In all these cases, there are major abnormalities in the growth and development of the lens cells that eventually lead to cataract formation. There is no obvious correlation between all these cases. Furthermore, it should be mentioned that lower levels of hybrid hamster vimentin-desmin protein have been expressed in the lens of transgenic mice (Krimpenfort et al., 1988) with no reported cataract development. On the other hand, in preliminary results, ectopic expression of the small neurofilament gene in transgenic lens is linked to cataract formation (Monteiro and Cleveland, personal communication). Since the levels of extratransgenic intermediate filaments are not known, no direct conclusion can be made of the relevance of these other studies to our data with transgenic vimentin.

When excised lens is bathed with a Ca²⁺-containing medium, there is a loss of transparency and this experimental system has been used as a suitable biological model for cataract formation (Clark et al., 1980; Hightower and Reddy, 1982; Hightower and Dering, 1984). It has been reported by Lorand et al. (1985) that in lenses bathed with Ca²⁺, vimentin is extensively degraded. This might be because of the already reported Ca²⁺-dependent protease (Nelson and Traub, 1981, 1982). Interestingly, vimentin also shows a high degree of degradation but only at later stages after cataract formation (Fig. 2F). We do not yet know if this degradation is the consequence of lens degeneration or plays an active role in this process. Comparison of the protein profile in Fig. 2F with the corresponding profile from normal lens of the same age (not shown here) does not favor the former possibility since other proteins do not show any such extensive degradation. Of great importance and interest is the correlation of the transgenic vimentin abundance and the age of cataract appearance. The more protein present, the earlier the appearance of the cataract formation. This could be explained by three different mechanisms. First, vimentin is normally expressed at higher level during earlier stages of development when differentiation has not been completed. When the growth rate is higher, the cell needs and can probably tolerate higher quantities of vimentin. The lens epithelium proliferates faster at earlier ages and as the mouse ages, slows down. Second, if we assume that the chicken protein inhibits normal cellular differentiation by competing in the formation of functional filaments, then the lower levels of endogenous protein in later stages would give a pronounced effect. Third, it is established that aging plays a significant role in cataractogenesis, but it is not known why. It may be because lens becomes more susceptible to insult as it grows older or the calcium content changes as well as many other factors. The availability of mouse lines that show temporal differences in the onset of cataract formation affords the opportunity to study the molecular mechanisms involved in this serious age-related process.

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