human genome and thus could be considered recombinogenic in a manner similar to the long terminal repeat regions of retroviruses (*31*). Although these similarities could be due to functional constraints, viral or extrachromosomal origins for the VNTRs should be considered and explored.

Usefulness of VNTR loci for mapping disease loci. We expect the VNTR loci to be valuable as anchor points on the human genetic linkage map, and to be informative within the small sets of families that are available for mapping genetic disease loci, because the high frequency of heterozygosity both found and estimated in our study indicates that genotypic information will be obtained from most meioses in any sample set. Such genetic markers, made available to the research community, should contribute substantially to the construction of the human genetic linkage map and to the localization of genes that cause human genetic disease.

Note added in proof: Cosmids ascertained with the zeta-globin-, insulin-, and myoglobin-related oligonucleotides were rescreened with the oligonucleotides prior to testing for polymorphism. However, 212 cosmids ascertained with the HBV- and YNZ22-related oligonucleotides were tested for polymorphism without rescreening. Recently, we rescreened at random 106 of these cosmids and found that only 57 of the 106 in fact hybridized with the respective oligonucleotides. Eighteen of the 57 (32%) showed VNTR polymorphism, while only 2 (4%) of the 49 cosmids that failed to show oligonucleotide homology demonstrated VNTR polymorphism.

REFERENCES AND NOTES

1. J. F. Gusella *et al.*, *Nature (London)* **306**, 234 (1983).
2. J. M. Murray *et al.*, *ibid.* **300**, 69 (1982).
3. S. Reeders *et al.*, *ibid.* **317**, 542 (1985).
4. L.-C. Tsui *et al.*, *Science* **230**, 1054 (1985).
5. R. White *et al.*, *Nature (London)* **318**, 382 (1985).
6. R. Williamson *et al.*, *ibid.*, p. 384.
7. A. Jeffreys *et al.*, *Cell* **18**, 1 (1979).
8. S. E. Antonarakis, H. H. Kazazian, Jr., S. H. Orkin, *Hum. Genet.* **69**, 1 (1985).
9. A. Wyman and R. White, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6754 (1980).
10. G. I. Bell, M. J. Selby, W. J. Rutter, *Nature (London)* **295**, 31 (1982).
11. D. J. Capon, E. Y. Chen, A. D. Levinson, P. H. Seeburg, D. V. Goeddel, *ibid.* **302**, 33 (1983).
12. N. J. Proudfoot *et al.*, *Cell* **31**, 553 (1982).
13. A. Jeffreys, V. Wilson, S. Thein, *Nature (London)* **314**, 67 (1985).
14. R. White *et al.*, *ibid.* **313**, 101 (1985).
15. Z. Wong *et al.*, *Nucleic Acids Res.* **14**, 4605 (1986).
16. R. Koshy *et al.*, *Cell* **34**, 215 (1983).
17. H. Mizusawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 208 (1985).
18. K. Yaginuma *et al.*, *ibid.*, p. 4458.
19. R. Lawn *et al.*, *Cell* **15**, 1157 (1978).
20. A. Wyman L. Wolfe, D. Botstein, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2880 (1985).
21. Y.-F. Lau and Y. W. Kan, *ibid.* **80**, 5225 (1983).
22. M. Litt and R. White, *ibid.* **82**, 6206 (1985).
23. Family panels are in the archives of the Centre d'Etude du Polymorphisme Humain (CEPH), Paris.
24. Y. Nakamura *et al.*, in preparation.
25. J. Braman, D. Barker, J. Schumm, R. Knowlton, H. Donis-Keller, *Cytogenet. Cell Genet.* **40**, 589 (1985).
26. A. Silva, personal communication.
27. A. P. Jarman *et al.*, *EMBO J.* **5**, 1857 (1986).
28. A. Wyman, J. Mulholland, D. Botstein, *Am. J. Hum. Genet.* **39** (Suppl.), A-226 (1986).
29. T. J. Knott *et al.*, *Nucleic Acids Res.* **14**, 9215 (1986).
30. N. G. Stoker *et al.*, *ibid.* **13**, 4613 (1985).
31. T. Tamura and T. Takano, *ibid.* **10**, 5333 (1982); C. Shoemaker *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3932 (1980).

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Research Articles

Oncogenesis of the Lens in Transgenic Mice

KATHLEEN A. MAHON, ANA B. CHEPELINSKY, JASPAL S. KHILLAN, PAUL A. OVERBEEK, JORAM PIATIGORSKY, HEINER WESTPHAL

Neoplastic tumors of the ocular lens of vertebrates do not naturally occur. Transgenic mice carrying a hybrid gene comprising the murine αA-crystallin promoter (−366 to +46) fused to the coding sequence of the SV40 T antigens developed lens tumors, which obliterated the eye cavity and even invaded neighboring tissue, thus establishing that the lens is not refractive to oncogenesis. Large-T antigen was detected early in lens development; it elicited morphological changes and specifically interfered with differentiation of lens fiber cells. Both α- and β-crystallins persisted in many of the lens tumor cells, while γ-crystallin was selectively reduced. Accessibility, characteristic morphology, and defined protein markers make this transparent epithelial eye tissue a potentially useful system for testing tumorigenicity of oncogenes and for studying malignant transformation from its inception until death of the animal.

THE TRANSGENIC MOUSE PROVIDES AN EXPERIMENTAL SYSTEM to study the molecular genetics of malignant growth in the intact organism, from the earliest to the terminal stages of the disease. Both viral and cellular oncogenes have been introduced into the germline of mice, resulting in malignancies of various types (*1–7*). Although the introduced oncogenes were present throughout the organism, tumors were generally found only in cell types specified by the regulatory regions controlling oncogene expression. This indicates that oncogenesis can be targeted by the judicious use of DNA sequences regulating the tissue-specific expression of genes.

A naturally occurring malignant tumor has never been reported in the ocular lens. However, abnormal proliferation of lens epithelium

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has been observed in certain strains of chicken (*8*) and in several types of cataracts (*9*), and a tumor-like invasive proliferation within the lens has been reported in trout exposed to the carcinogen thioacetamide (*9*). In contrast, tumors originating from other regions of the eye—such as the retina and iris—have been observed. Lens cells are not inherently refractive to neoplastic transformation, because cultured lens epithelial cells from several vertebrate species have been transformed in vitro (*10, 11*). These data suggest that there is something special about the intraocular environment or lens architecture which prevents spontaneous lens malignancy. We have directed the expression of a known oncogene, the SV40 T antigen, to the lens in transgenic mice in order to determine whether this tissue can be transformed in vivo.

Our experimental strategy was based on previous findings that a short 5′ flanking sequence (−366 to +46) of the αA-crystallin gene of mice can promote the expression of a foreign gene selectively in lens cells in primary explants (*12*) and in transgenic mice (*13*). αA-Crystallin is a structural protein expressed at high levels specifically in the eye lens (*14*). We thus linked this αA-crystallin gene regulatory sequence to the coding region of the gene for SV40 T antigen and used the hybrid gene to create transgenic mice. The SV40 T antigen causes various tumors in transgenic mice, and the types of tumors are dependent on the tissue specificity of the regulatory sequences controlling its expression. Mice harboring the entire SV40 early region, including the SV40 enhancers, developed tumors in the choroid plexus of the brain (*1, 15*). The insulin and elastase regulatory regions elicited tumors of the β and acinar cells of the pancreas, respectively, when linked to T antigen coding sequence (*3, 5*).

We report here that transgenic mice bearing the αA-crystallin–T antigen fusion gene synthesize large-T antigen in their lenses and develop heritable lens tumors. These results establish that the ocular lens is not intrinsically resistant to tumorigenesis. T antigen appeared at approximately the same time as the endogenous α-crystallin in the transgenic mice and had profound effects on the ontogeny of the lens and the differentiation of its fiber cells.

Lens pathology in transgenic mice. A chimeric gene was constructed by fusing 412 bp (−366 to +46) of the 5′ flanking sequence from the murine αA-crystallin gene to the coding sequence of the SV40 early region (Fig. 1). This construct (α-crys-Tag) was injected as a linear DNA fragment into the pronuclei of one-cell mouse embryos; the embryos were subsequently transferred into pseudo-pregnant foster mothers. Seven transgenic mice (F0) bearing independent insertions of the α-crys-Tag fusion gene were obtained. The copy number of the newly integrated DNA ranged from 1 to 20 copies per haploid genome. All of the transgenic mice were identifiable once they opened their eyes because their lenses appeared white and opaque—a stark contrast to the normally transparent lens (Fig. 2).

The α-crys-Tag transgenic mice died prematurely, generally before 4 months of age. Consequently, only one line was propagated. Within this line (line 7488), which bears two copies of the α-crys-Tag, the lens abnormality was inherited as a dominant Mendelian trait, and has been passed through five generations thus far.

Wild-type and transgenic eyes were examined histologically. In the wild-type lens (Fig. 3, A and E), a single layer of mitotically active epithelial cells lines the anterior part of the lens. At the equatorial region, these cells cease dividing and begin to differentiate into lens fiber cells, which elongate and ultimately lose their nuclei and other organelles. Continued growth of the lens pushes the more differentiated fiber cells toward the center or nucleus of the lens (*14*). The lens is enclosed in an extracellular collagenous capsule, and is avascular throughout the life of the animal.

The pathology seen in α-crys-Tag transgenic mice was consistent among the F0 mice and within the established line. In older animals (more than 2 months), the lens was replaced by a disorganized mass that had grown outside the lens capsule and become infiltrated by blood vessels (Fig. 3C). Invasive growth of the lens inevitably broke through the back of the eye or the cornea at the front of the eye.

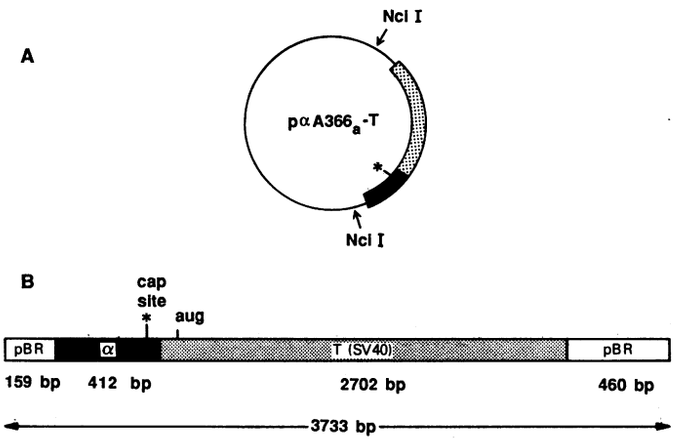


Fig. 1. The αA-crystallin–SV40 T antigen construct microinjected into fertilized mouse eggs. (**A**) The plasmid pαA366$_a$-T consists of the 5′ flanking region from the mouse αA-crystallin gene (solid bar) fused to an enhancerless-promoterless SV40 early region (stippled bar). The 412-bp αA-crystallin sequence (−366 to +46) contains the promoter and cap site (designated +1) (*12*). The SV40 early region sequences extend from the Alu I to the Bam HI site (nucleotides 5162 to 2716) (*17*), and contains 64 bp of sequence upstream from the ATG, the coding sequences for both large- and small-T antigen and polyadenylation signals. It does not contain the major initiation sites for SV40 early mRNA's. pαA366$_a$-T was constructed by introducing the 415-bp Bgl II–Bam HI DNA fragment from pMαACr1800 (*12*) into pEMP (*25*) at the Bam HI site. (**B**) The 3736-bp linear Nci I restriction fragment was purified by gel electrophoresis and electroelution, and microinjected into the pronuclei of one-cell mouse embryos. The zygotes were transferred into pseudopregnant female mice and allowed to develop. DNA was prepared from tail snips of live offspring and analyzed for the presence of the α-crys-Tag construct. All procedures were performed as described (*13, 26*). The inbred mouse strain FVB/N was the source of embryos and was used for all crosses in this study.

The bulk of the transgenic lens was composed of small, mitotically active anaplastic cells (Fig. 3F). Morphologically normal epithelial cells were also present and secreted periodic acid Schiff (PAS)–positive capsular material, although they were often displaced into the interior of the lens. A multilayered epithelium was observed in many areas. The elongated fiber cells characteristic of the normal lens (see Fig. 3E) were absent. A few large abnormal, differentiated lens cells (balloon cells) were dispersed throughout the tissue. Balloon cells are sometimes observed in lens cell cultures and certain cataractous lenses, and are thought to arise from differentiating cells which have been unable to elongate into proper fiber cells.

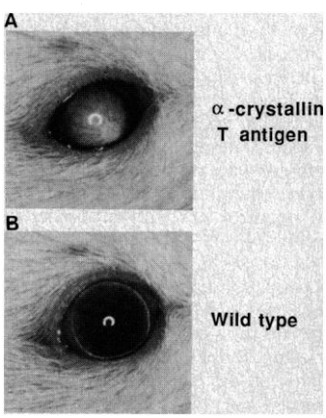


Fig. 2. Phenotype of mice bearing αA-crystallin–T antigen fusion genes. The lenses of all seven F0 mice bearing the αA-crystallin–Tag fusion gene were white and opaque. (**A**) The eye of F0 7478 at approximately 6 weeks of age. (**B**) Eye of a normal, nontransgenic sibling. In albino mice with no pigment and a transparent lens, the eye appears red because of the blood vessels in the retina.

The morphology and growth pattern of the lens masses were characteristic of proliferating, invasive tumors. Cells from the lens tissue of two different F0 mice were grown in culture and exhibited rapid growth and lack of contact inhibition, both criteria of transformation.

Tumor development in α-crys-Tag animals was monitored by histological analyses of eyes from younger animals. Around the time the eyes opened (10 to 15 days after birth), the lenses in all α-crys-Tag animals examined were approximately the same size and shape as those of normal controls, were still confined within the capsule, and were not yet vascularized (Fig. 3I). Despite the organization and the presence of a morphologically normal anterior epithelial cell layer (Fig. 3L), the differentiation of fiber cells was already affected: only the anaplastic cells present in the more advanced tumor were visualized in the posterior compartment of the lens (Fig. 3L).

Crystallin expression. Tissue sections were analyzed by immunostaining with antibodies against several lens-specific crystallin proteins. There are three classes of crystallins in the mouse lens—α, β, and γ—which are under differential temporal and spatial regulation (*14*). α-Crystallin is expressed in both epithelial and fiber cells. The β-crystallins are present only in the cortical and central fiber cells of the mouse lens. γ-Crystallin, the most restricted in its expression, is localized in the fiber cells in the central region of the lens. Thus, the types of crystallins synthesized characterize the differentiated state of the lens cells.

The lens neoplasms from α-crys-Tag mice were positively stained with antibodies to all three lens crystallins, confirming their lenticular derivation. However, the pattern of lens crystallin expression was aberrant. Immunoperoxidase staining with antibodies to α-crystallin shows that, in contrast to the uniform distribution of α-crystallin in the normal lens (Fig. 3, B and E), the α-crys-Tag lens had a blotchy labeling pattern (Fig. 3D). Interspersed among strongly labeled cells were large clumps of cells, clearly within the lens mass, which had low or undetectable levels of α-crystallin. A similar patchy labeling pattern was seen after immunostaining with β-crystallin antibodies. These patches may represent clonal subpopulations of cells indicative of an oligoclonal origin for the tumor. α-Crystallin was detected in the epithelial cells and balloon cells (Fig. 3G), whereas the β-crystallin antibodies did not stain the epithelial cells, but did stain the balloon cells (Fig. 3H).

In the advanced, highly disorganized tumors of 3.5-month-old mice, we could not distinguish how the different cells might be related. Consequently, the lenses of younger α-crys-Tag animals, which still had a capsule and were not yet vascularized (Fig. 3I), were stained with the crystallin antibodies. Again it is evident that there was differential synthesis of α-crystallin (Fig. 3J). Immunoreactive cells were present in the anterior epithelial layer and in the posterior half of the lens. Cells directly behind the epithelial layer, with a few exceptions, did not label with the α-crystallin antibody (Fig. 3, J and M).

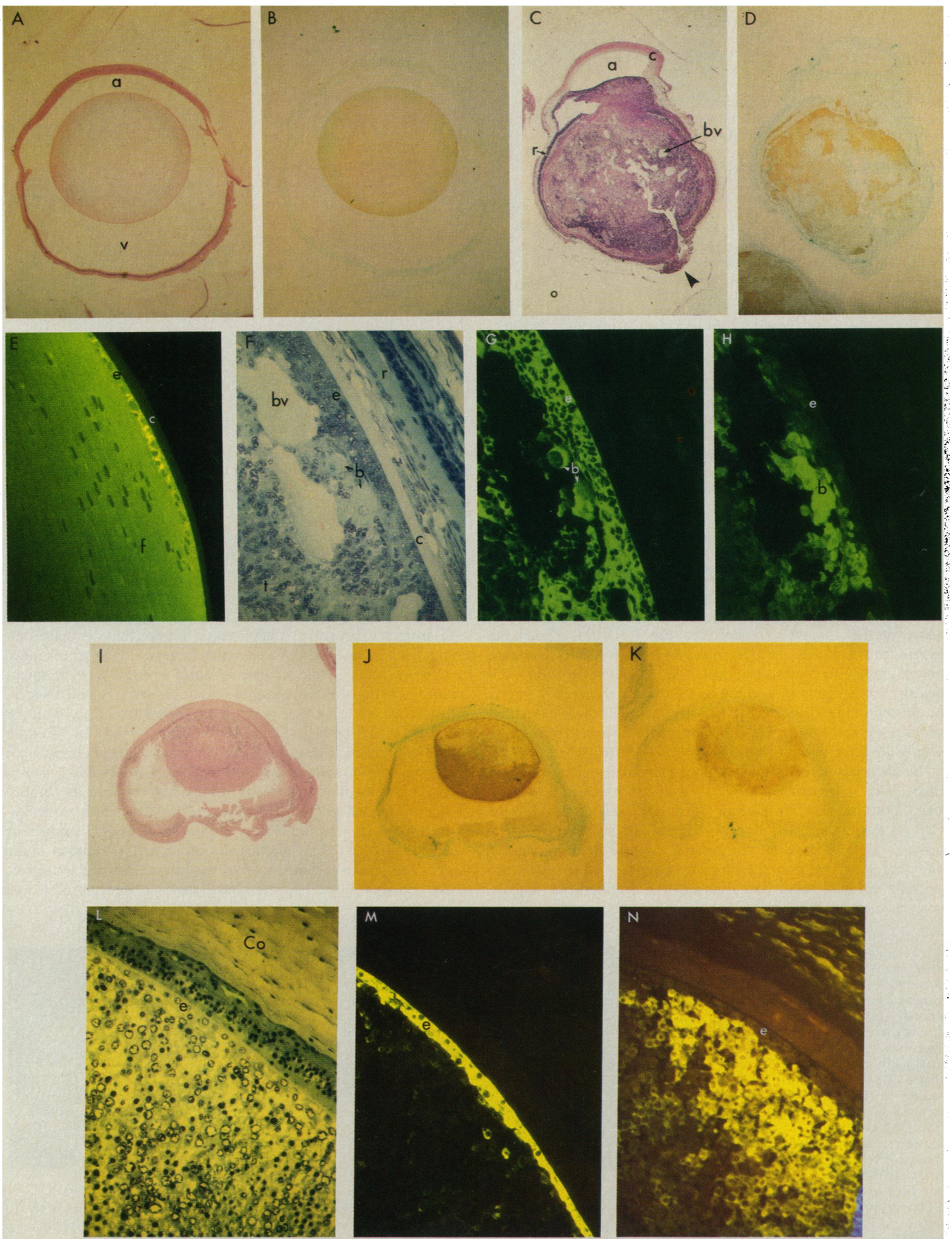
In contrast to the results with α-crystallin antibodies, the β-crystallin antibodies did not recognize the anterior epithelial cells of the 18-day-old α-crys-Tag lens (Fig. 3, K and N) and only began to label peripheral cells at the equatorial region (as in normal lenses), further substantiating that the morphologically typical epithelial cells in these lenses are equivalent to normal lens epithelial cells. β-Crystallin was present in many of the cells throughout the remainder of the lens, including the cells just posterior to the epithelial layer that were unlabeled by the α-crystallin antibody (Fig. 3N). Thus, many cells contained β-crystallin but not α-crystallin, unlike normal lens cells. Finally, γ-crystallin was detected in only a few cells by immunostaining. The ability of some α-crys-Tag lens cells to synthesize β- and γ-crystallin proteins characteristic of differentiated fiber cells indicates that, despite the lack of normal fiber cell elongation, some features of fiber differentiation were achieved. Similar results were obtained by the Western blotting procedure.

Ontogeny of α-crys-Tag lenses. In the mouse, α-crystallin is first detected in the developing lens between day 10.75 to day 11 of gestation (*16*). At this time, the embryonic lens consists of a hollow ball of cells, the lens vesicle. The cells at the posterior half of the lens vesicle cease dividing and elongate to become the primary fiber cells and obliterate the lumen of the lens vesicle by day 13 (*14*).

Previous results with transgenic mice carrying the αA-crystallin-CAT (chloramphenicol acetyltransferase) fusion gene indicated that initial expression of the introduced CAT gene is similar to that of the endogenous αA-crystallin gene (*13*). We thus examined the α-crys-Tag transgenic mice for signs of transformation during the initial differentiation of the lens.

The α-crys-Tag embryos and their wild-type littermates were examined for evidence of pathogenesis. Lens opacity in affected embryos was detected by external visual inspection by day 14 of embryogenesis. Histological analysis revealed that at day 11, just

Fig. 3 (**opposite page**). Pathology and lens crystallin distribution in α-crys-Tag lenses. All sections are oriented with the anterior pole facing the top of the page. (**A**) Section of a normal eye from a 3.5-month-old FVB/N mouse stained with Giemsa. Aqueous humor (a), vitreous humor (v), lens (l) (×20). (**B**) Immunoperoxidase staining of a section of a control eye with antibodies to α-crystallin. (**C**) Giemsa-stained section of the eye from 3.5-month-old α-crys-Tag transgenic mouse (F0 10236) containing an advanced lens tumor. The lens mass, infiltrated by blood vessels (bv), has displaced the retina (r) and grown through the back of the eye (arrowhead). (**D**) Immunoperoxidase staining of a section through the α-crys-Tag eye with antibodies to α-crystallin. (**E**) Indirect immunofluorescence of the equatorial region of a control lens with antibodies to α-crystallin. Epithelial cells (e), fiber cells (f), and capsule (c) (×230). (F–H) Adjacent sections of the equatorial periphery of the tumor in (C) at higher magnification (×230). (**F**) Giemsa-stained section of the lens mass containing epithelial cells (e), dividing tumor cells (t), and balloon cells (b). The retina (r) has been displaced. Capsule (c), blood vessels (bv). (**G**) Adjacent section stained with antibodies to α-crystallin by indirect immunofluorescence. Epithelial cells (e) are labeled strongly. (**H**) Indirect immunofluorescence staining with β-crystallin antibodies. Balloon cells (b) are heavily labeled, whereas epithelial cells (e) are unlabeled. (**I**) A section stained, with hematoxylin and eosin, through the eye of an 18-day-old F0 (7532) α-crys-Tag mouse. In this mouse, normal differentiation of the iris and ciliary process has not occurred, an anomaly not observed in other animals (×20). (**J**) A neighboring section immunoperoxidase stained with antibodies to α-crystallin. (**K**) Immunoperoxidase staining with antibodies to β-crystallin. (L–N) Higher magnification (×230) of adjacent sections from the 18-day-old α-crys-Tag mouse shown in (I). (**L**) A Giemsa-stained section from the anterior region of the lens. The epithelium appears normal but the fiber region consists of small round cells. Cornea (Co). (**M**) Indirect immunofluorescence staining with antibodies to α-crystallin. Most of the cells behind the epithelium (e) are not stained. (**N**) An adjacent section immunostained with antibodies against β-crystallin. The epithelium (e) is not stained. Eyes were fixed in 4 percent paraformaldehyde or in ethanol and acetic acid (3:1), dehydrated through an ethanol series, and embedded in methacrylate (Polysciences) (*27*). Sections (1 to 2 μm) were cut on an ultramicrotome parallel to the optical axis and affixed to gelatin-coated (subbed) slides (*28*). Plastic was removed in xylene, the slides were processed through an ethanol series of decreasing concentration into water and then phosphate-buffered saline (PBS). Sections were blocked in 1 percent bovine serum albumin, 16 percent fetal calf serum, 0.05 percent Tween 20 for 10 minutes, then incubated with primary antibody for 30 minutes, and washed with PBS extensively. Either fluorescein isothiocyanate (FITC) or horseradish peroxidase conjugated goat antiserum to rabbit immunoglobulin G (1:100) (Cappel) was applied for 30 minutes at room temperature. Slides were washed extensively in PBS. All procedures were carried out at room temperature. FITC-labeled slides were mounted with cover slips, and viewed in a fluorescence microscope. Slides labeled with peroxidase were developed by incubation in a 1:100 dilution of saturated *o*-dianisidine in PBS containing 0.003 percent H_2O_2 for 15 minutes (*29*), and counterstained with 1 percent fast green.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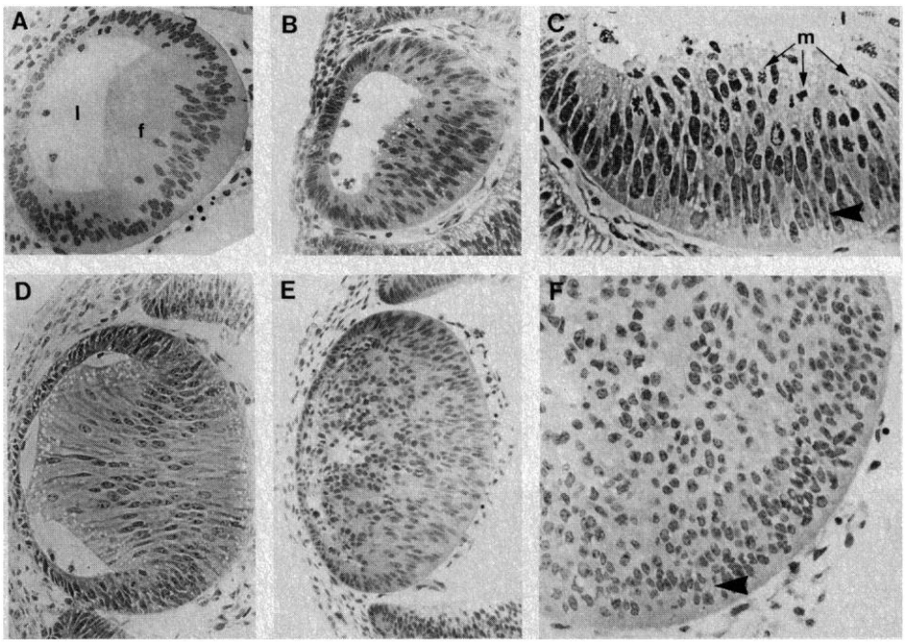

Fig. 4. Lens development in α-crys-Tag transgenic mice. A, B, D, and E are oriented with the anterior pole of the lens facing left. The anterior pole faces the top of the page in C and F. (**A**) Histological section through a wild-type lens at day 12 of embryogenesis. Primary fiber cells (f) have begun to elongate into the lumen (l) of the lens vesicle (×180). (**B**) A section through a lens from a slightly less developed day 12 α-crys-Tag embryo. The density of nuclei in the posterior region of the transgenic lens is greater than that of the wild-type lens in A (×180). (**C**) A higher magnification of the posterior region of the 12-day transgenic lens in B showing the elongating cells present at the posterior periphery (solid arrowhead) and the presence of numerous mitoses (m) as growth progressively fills the lumen (×285). (**D**) A wild-type lens at day 13 of embryogenesis. At this stage, elongating fiber cells have almost filled the lumen (×140). (**E**) An α-crys-Tag transgenic lens at day 13. The lumen has been filled by round cells. Slightly elongated cells are still visible at the posterior periphery (×140). (**F**) An enlargement of a more developmentally advanced day 13.5 transgenic embryonic lens. Elongated cells are no longer observed at the posterior pole (arrowhead) (×265). Transgenic embryos were third-generation progeny of FVB/N females that had been mated to heterozygous α-crys-Tag males from line 7488. Control embryos were wild-type littermates. The nominal age of the embryos was established by counting the day the vaginal plug was found as day 0.5 of embryogenesis. Eyes were fixed in 4 percent paraformaldehyde in PBS and embedded in methacrylate as described in Fig. 3. All sections were stained with hematoxylin and eosin.

after the lens vesicle had formed, there was little apparent difference between the lens vesicles of α-crys-Tag embryos and those of their normal control littermates. By early day 12, coincident with primary fiber cell elongation, morphological differences became apparent. First, the nuclear packing density in the posterior region of the vesicle was higher in the transgenic lens (Fig. 4, B and C) than in the control lens (Fig. 4A). Second, many mitotically active cells were present in the fiber region, especially near the lumen. Normally, cell division in this region is absent. The cells closest to the optic cup at the posterior boundary of the lens retained a slightly elongated morphology very similar, but not identical, to normal primary fiber cells, while those near the lumen were round in shape.

This aberrant morphology was more pronounced in early day 13 lenses. The longitudinally ordered array of primary fiber cells conspicuous in the normal lens (Fig. 4D) was replaced by a relatively disorganized accumulation of small mitotically active cells in the α-crys-Tag lenses of comparable age (Fig. 4E). Elongated cells were still present at the posterior periphery of the lens, but generally did not persist past this stage of development. Slightly more developed 13- or 13.5-day embryonic lenses contained only small round cells in the posterior compartment (Fig. 4F). Our interpretation is that elongation of the primary fiber cells initiated in α-crys-Tag lens vesicles at approximately the right time, but that this differentiation ceased shortly thereafter, and was followed by the resumption of cell division.

T antigen expression. It has been established that the transforming activity of the SV40 early region is provided by the large-T antigen (*17, 18*). Tissue sections of lens neoplasms were immunostained with two different monoclonal antibodies to large-T antigen. Most of the nuclei in transgenic lenses showed reactivity with the monoclonal antibodies (Fig. 5A), a result verified by Western blotting. Similarly, most of the lens epithelial cells and almost all of the small cells invading the lumen in day 14 embryonic lenses of transgenic mice showed positive immunofluorescence for large-T antigen. The cell lines derived from α-crys-Tag lenses from 3.5-month-old mice were stained by antibodies to large-T antigen as well (Fig. 5B). We conclude that the αA-crystallin promoter in the α-crys-Tag construct functioned in the lens cells of the transgenic mice, and that T antigen was synthesized and transported to the nucleus.

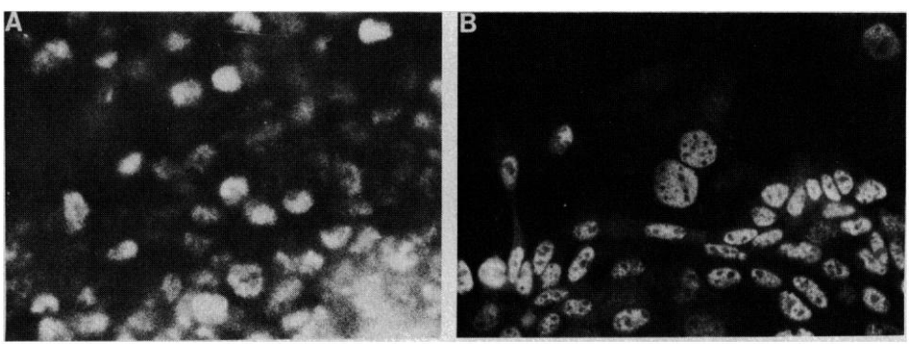

Fig. 5. Large-T antigen expression in α-crys-Tag lenses. (**A**) A section of a lens from a 12-day-old F1 animal from the transgenic line 7488 stained by indirect immunofluorescence with monoclonal antibody (pAb412) to SV40 large-T antigen. Most of the nuclei are stained. (**B**) Lens cells from a 3.5-month-old F0 (10236) cultured for one passage and stained with the antibody to large-T antigen (×380). Tissue was flash frozen in isopentane at liquid nitrogen temperature and mounted in tissue embedding media (TISSUE-TEK OCT). Sections (7 μm) were cut on a cryostat at −18°C and mounted on subbed slides. Tissue culture cells were grown on glass slides and washed in PBS. Slides with both cells and sections were fixed in either 4 percent paraformaldehyde in PBS or ice-cold acetone for 10 minutes, and washed in PBS several times before staining. Samples were blocked and labeled with antibodies as described for Fig. 3. Primary antibody was either pAb412 or pAb419 (*30*), both monoclonal antibodies to SV40 large-T antigen. Secondary antibody was FITC-labeled goat antibody to mouse immunoglobulin G (1:200; Cappel).

Non-lens pathologies. We have found that oncogenesis is well progressed in the lenses of all α-crys-Tag transgenic mice by 2 to 3 months. In addition, several animals from line 7488 surviving to 3 to 5 months of age developed non-lenticular tumors at various sites throughout the body; these tumors were derived from more than one cell type and consequently were not due to metastasis from the lens tumor. Cultured cells from several tumors gave positive fluorescence when immunostained with antibodies to large-T antigen. Other transgenic mice (previously described) bearing the α-crys-CAT fusion gene showed no evidence for CAT expression outside the lens under conditions in which CAT activity three orders of magnitude lower than that of the lens would have been detectable (*13, 19*). Possible explanations for ectopic expression of T antigen in this line include chromosomal position effects, generalized low-level ectopic expression of crystallin genes (*20*), or secondary somatic mutation (such as gene amplification or chromosomal rearrangements) activating the α-crys-Tag construct in one or a few cells in these tissues.

Transcript mapping. To ascertain whether expression of T antigen in the α-crys-Tag lenses and non-lens tumors was directed by the αA-crystallin promoter, primer extension analysis, with an oligonucleotide primer complementary to T antigen sequence, was conducted to map the 5′ end of the T antigen messenger RNA (mRNA). The eyes of 25-day-old heterozygous α-crys-Tag mice contained T antigen transcripts that initiated at the same nucleotides as the endogenous αA-crystallin gene (Fig. 6, lane 1). Similar initiation sites were observed in an epithelioma of the thymus and from cultured cells from two different non-lens sarcomas (lanes 2 and 3) obtained from an α-crys-Tag mouse (F3 12256).

These results indicate that transcription of the crys-Tag construct in all lens and non-lens tumors examined resulted from appropriate initiation directed by the crystallin promoter and was not the result of readthrough or aberrant transcription from adjacent cellular sequences. This does not exclude the possibility that adjacent cellular enhancers or sequences within the T antigen coding region itself influenced transcription from the αA-crystallin promoter.

Course of tumorigenesis. We have shown that lens tumors, or phakomas, can be generated in vivo by directed expression of the SV40 large-T antigen. This result indicates that the absence of reported lens tumors is not due to an absolute cellular resistance of lens tissue to malignant transformation. The molecular and cellular basis for the apparent lack of lens tumors remains unclear, although several features of the lens potentially reduce the frequency of tumor formation. Metabolic constraints, particularly the low oxygen requirement of the lens, and the physical barrier imposed by the lens capsule could limit neoplastic growth or protect the lens from a variety of tumor producing assaults. The role of these and other factors was considered as early as 1948 (*21*). It is intriguing that T antigen is synthesized in mid-gestation embryonic lenses, causing cellular dysplasia and increased cell division, and yet it generally takes 2 to 3 months for invasive growth of the lens mass to extend outside the lens capsule. The lack of vascularization of the lens may present the greatest block to tumor proliferation, since it is known that tumors cannot exceed a certain size without sufficient blood supply (*22*), and suggests that the ability of the transformed lens cells to induce angiogenesis may be the rate limiting step in invasive tumor formation.

In transgenic mice bearing the SV40 early region or insulin or elastase SV40 T antigen fusion genes, T antigen was usually detected immunocytochemically prior to overt tumorigenesis, although its appearance was sometimes accompanied by morphological changes such as hyperplasia (*3, 5, 23*). In all cases, tumors did not develop until the mice were 3 to 7 months of age, implying that T antigen is necessary but not sufficient for tumorigenesis. Our α-crys-

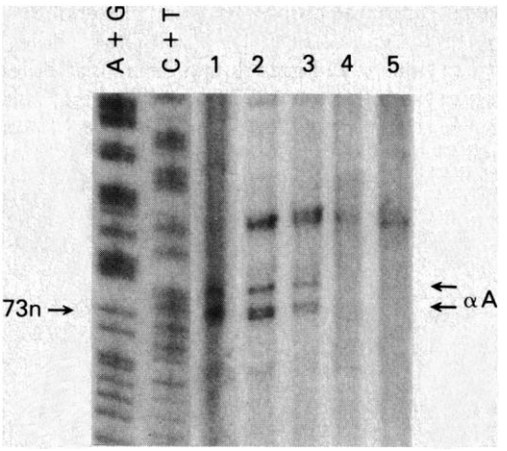

Fig. 6. Primer extension analysis of T antigen mRNA from lens tumors and tumor cell lines. A synthetic oligonucleotide primer (5′-GCCTCCTCACT-ACTTCTGG-3′) complementary to SV40 T antigen sequence was annealed to 40 μg of RNA and extended with reverse transcriptase (*31*). Extension products were resolved on an 8*M* urea–10 percent polyacrylamide gel. Template RNA's were obtained from eyes of 25-day-old heterozygous α-crys-Tag transgenic mice (lane 1); cell lines were derived from a peritoneal sarcoma (lane 2), and a sarcoma from the sternum (lane 3) excised from a tumor-bearing 5.5-month-old heterozygous α-crys-Tag mouse (12256). Liver RNA (lane 4) and brain RNA (lane 5) from αA-crystallin-CAT transgenic mice (*13*) served as control templates. The arrows indicate accurate transcription start sites for RNA initiating at the αA-crystallin cap site or sites (*12*). The T antigen primer was extended 54 bases from its 3′ end, generating fragments with a total length of 73 nucleotides. A larger band, observed with RNA from α-crys-Tag cell lines (lanes 2 and 3), maps approximately 15 bp upstream from the major αA-crystallin initiation site. This band was also seen in control tissues (lanes 4 and 5). Sequencing reactions of a DNA fragment of known length, lanes (A + G) and (C + T) were used as size markers. RNA was purified as described (*13, 32*). All α-crys-Tag transgenic mice were from line 7488.

Tag transgenic mice are distinctive in that morphological changes indicative of a predisposition for malignancy are evident less than a day after T antigen is first synthesized in the lens, provided that the fusion gene is activated at the same time as the endogenous crystallin gene (*13, 16*). This does not exclude the possibility that additional factors prevail upon this incipient neoplasm before it becomes the vascularized phakoma seen in adult animals.

Normal differentiation of the lens fiber cells is not achieved in α-crys-Tag lenses. In the transgenic embryonic lens, elongating cells are only observed transiently, at approximately the same time α-crystallin (and presumably T antigen) is first synthesized. In contrast, morphologically normal epithelial cells are seen throughout lens development and even in well progressed tumors. Since immunologically detectable T antigen is present in most of these epithelial cells as well as in the small, dividing "transformed" cells it is possible that the T antigen specifically transforms lens cells as they begin to differentiate into fiber cells at the equatorial region of the epithelium. An alternative explanation is that epithelial cells do not synthesize enough T antigen to elicit phenotypic changes, whereas differentiating presumptive fiber cells do. Analysis of the distribution of T antigen mRNA in α-crys-Tag lenses by cytohybridization may differentiate between these two alternatives.

Finally, the ability to use the αA-crystallin regulatory region to target specific gene expression to the ocular lens provides a potentially useful system to assay the efficacy of other oncogenes in transgenic mice. The lens is not an essential tissue for viability, and tumors can be removed for study without losing valuable breeding animals. The lens is a relatively simple tissue composed of epithelial cells derived from the ectoderm. The differentiation of fiber cells is specialized, and very precise cell-cell interactions must be rigidly

maintained to ensure the proper shape and clarity of the lens. The intricate cellular architecture of the lens allows the detection of subtle perturbations in cell growth and differentiation, even those not leading to bona fide tumors. Moreover, the crystallins whose genes have been characterized (*24*), provide discrete markers for lens cell differentiation. Use of the ocular lens for determining the physiological consequences of oncogene expression, not only addresses tumorigenic potential, but may also provide valuable information for intermediate steps involved in malignant transformation.

REFERENCES AND NOTES

1. R. L. Brinster *et al.*, *Cell* **37**, 367 (1984).
2. T. A. Stewart, P. K. Pattengale, P. Leder, *ibid.* **38**, 627 (1984).
3. D. Hanahan, *Nature (London)* **315**, 115 (1985).
4. J. M. Adams *et al.*, *ibid.* **318**, 533 (1985).
5. D. M. Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 399 (1985).
6. M. Lacey, S. Alpert, D. Hanahan, *Nature (London)* **322**, 609 (1986).
7. R. D. Palmiter and R. L. Brinster, *Annu. Rev. Genet.* **20**, 465 (1986).
8. R. M. Clayton, *Genet. Res. Cambridge* **25**, 79 (1975); R. M. Clayton *et al.*, *J. Embryol. Exp. Morphol.* **35**, (1976); P. G. C. Odeigah, R. M. Clayton, D. E. S. Truman, *Exp. Eye Res.* **28**, 311 (1979).
9. L. von Sallmann, J. E. Halver, E. Collins, P. Grimes, *Cancer Res.* **26**, part 1, 1819 (1966).
10. D. M. Albert, A. S. Rabson, P. A. Grimes, L. von Sallmann, *Science* **164**, 1077 (1969); H. Bloemendal *et al.*, *Exp. Eye Res.* **31**, 513 (1980).
11. Y. Courtois, L. Simonneau, J. Tassin, M. V. Laurent, E. Malaise, *Differentiation* **10**, 23 (1978); G. G. Miller, D. G. Blair, E. Hunter, G. Y. Mousa, J. R. Trevithick, *Develop. Growth Differ.* **21**, 19 (1979); R. E. Jones, D. DeFeo, J. Piatigorsky, *Vision Res.* **21**, 5 (1981).
12. A. B. Chepelinsky, C. R. King, P. S. Zelenka, J. Piatigorsky, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2334 (1985); A. B. Chepelinsky, B. Sommer, J. Piatigorsky, *Mol. Cell. Biol.*, in press.
13. P. A. Overbeek, A. B. Chepelinsky, J. S. Khillan, J. Piatigorsky, H. Westphal, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7815 (1985).
14. J. Piatigorsky, *Differentiation* **19**, 134 (1981); J. W. McAvoy, in *Mechanisms of Cataract Formation in the Human Lens*, G. Duncan, Ed. (Academic Press, New York, 1981), pp. 7–46.
15. R. D. Palmiter, H. Y. Chen, A. Messing, R. L. Brinster, *Nature (London)* **316**, 457 (1985).
16. J. Zwann, *Dev. Biol.* **96**, 173 (1983).
17. J. Tooze, Ed., *Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1980), part 2.
18. C. E. Clayton, D. Murphy, M. Lovett, P. W. J. Rigby, *Nature (London)* **299**, 59 (1982); W. A. Colby and T. Shenk, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5189 (1982).
19. H. Westphal *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 411 (1985).
20. R. M. Clayton, I. Thomson, D. I. de Pomerai, *Nature (London)* **282**, 628 (1979); D. J. Bower, L. H. Errington, D. N. Cooper, S. Morris, R. M. Clayton, *Nucleic Acids Res.* **11**, 2513 (1983); A. A. Moscona, L. Fox, J. Smith, L. Degenstein, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5570 (1985); K. Agata, K. Yasuda, T. S. Okada, *Dev. Biol.* **100**, 222 (1983).
21. E. Sachs and R. L. Larsen, *Am. J. Ophthalmol.* **31**, 561 (1948).
22. J. Folkman, E. Merier, C. Abernathy, G. Williams, *J. Exp. Med.* **133**, 275 (1971); J. Folkman, *Adv. Cancer Res.* **19**, 331 (1974).
23. T. VanDyke, C. Finlay, A. J. Levine, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 671 (1985).
24. J. Piatigorsky, *Cell* **38**, 620 (1984); *Invest. Ophthalmol. Vis. Sci.* **28**, 9 (1987).
25. C. Benoist and P. Chambon, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3865 (1980).
26. B. Hogan, F. Costantini, E. Lacy, *Manipulating the Mouse Embryo—A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986).
27. M. Jamrich, K. A. Mahon, E. R. Gavis, J. G. Gall, *EMBO J.* **3**, 1939 (1984).
28. J. G. Gall and M. L. Pardue, *Methods Enzymol.* **21**, 470 (1971).
29. C. M. Gorman, P. W. Rigby, D. P. Lane, *Cell* **42**, 519 (1985).
30. E. R. Gurney, R. O. Harrison, J. Fenno, *J. Virol.* **34**, 752 (1980); E. Harlow, L. V. Crawford, D. C. Pim, N. M. Williamson, *ibid.* **39**, 861 (1981).
31. S. P. Eisenberg, D. M. Coen, S. L. McKnight, *Mol. Cell. Biol.* **5**, 1940 (1985).
32. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979).

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