of CH_4. The redness of Pluto with respect to Charon probably arises from long-chain molecular products of the photolysis of CH_4 by solar ultraviolet insolation (*7*). By the same reasoning, the neutral color of Charon is further evidence of the absence of CH_4 on the surface of that body. Charon's geometric albedo falls within the range of the geometric albedos of the major Uranian satellites (0.18 to ~0.5), all of which are known to have water ice surfaces (*18*). The possible detection of H_2O ice on Charon by Marcialis *et al.* (*19*) therefore provides a reasonable explanation of its geometric albedo. The surface reflectance properties of Pluto and Charon are controlled by ices and cannot be classified to an asteroid taxonomy, which characterizes surface mineralogy.

REFERENCES AND NOTES

1. D. J. Tholen, *Int. Astron. Union Circ. 4403* (1987).
2. D. P. Cruikshank, C. B. Pilcher, D. Morrison, *Science* **194**, 835 (1976).
3. U. Fink, B. A. Smith, D. C. Benner, J. R. Johnson, H. J. Reitsema, *Icarus* **44**, 62 (1980).
4. L. Trafton and S. A. Stern, *Astrophys. J.* **267**, 872 (1983).
5. S. R. Sawyer, thesis, University of Texas at Austin (1986).
6. M. W. Buie and U. Fink, *Icarus* **70**, 483 (1987).
7. L. Trafton, S. A. Stern, R. Gladstone, paper presented at the Symposium on the Origin and Evolution of Planetary and Satellite Atmospheres, Tucson, AZ, 10 to 14 March 1987.
8. R. P. Binzel, D. J. Tholen, E. F. Tedesco, B. J. Buratti, R. M. Nelson, *Science* **228**, 1193 (1985).
9. D. J. Tholen, M. W. Buie, C. E. Swift, *Astron. J.* **93**, 244 (1987).
10. R. G. Tull, S. S. Vogt, P. W. Kelton, *Soc. Photo-Optical Instrum. Eng.* **172**, 90 (1979).
11. Because the entrance aperture of the spectrograph only projects onto about one-third of the width of the CCD, 100 spectra are produced (instead of 320).
12. Airmass is a term used to describe the thickness of atmosphere that light passes through before reaching the observer. It is defined to be one when the object is at the zenith (directly overhead) and increases as an object gets closer to the horizon. The airmass is approximately equal to the secant of the angle between the zenith and the line of sight to the object.
13. J. Hardorp, *Astron. Astrophys.* **63**, 383 (1978).
14. K. Horne, *Publ. Astron. Soc. Pac.* **98**, 609 (1986).
15. R. P. Binzel, personal communication.
16. Equivalent width is a measure of the amount of light that is absorbed in a spectral feature. It represents the width (in angstroms) of a feature with the same amount of absorption that has zero intensity across its entire width.
17. E. B. Jenkins *et al.*, *Astrophys. J.* **181**, L122 (1973).
18. D. P. Cruikshank and R. H. Brown, in *Satellites*, J. A. Burns and M. S. Matthews, Eds. (Univ. of Arizona Press, Tucson, 1986), pp. 836–873.
19. R. L. Marcialis, G. H. Rieke, L. A. Lebofsky, paper presented at the Symposium on the Origin and Evolution of Planetary and Satellite Atmospheres, Tucson, AZ, 10 to 14 March 1987.

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Genetic Ablation: Targeted Expression of a Toxin Gene Causes Microphthalmia in Transgenic Mice

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Lineage-specific regulatory elements can be used to direct expression of a variety of genes to specific tissues in transgenic mice. If the hybrid constructs contain a gene encoding a cytotoxic gene product, then genetic ablation of a specific cell lineage can be achieved. We have generated six transgenic mice by introducing into fertilized eggs the mouse γ2-crystallin promoter fused to the coding region of the diphtheria toxin A-chain gene. Three of these mice and all the transgenic offspring analyzed were microphthalmic. The lenses of these mice displayed considerable heterogeneity: some were almost normal morphologically but reduced in size, whereas others were grossly aberrant and deficient in nuclear fiber cells. These studies indicate that programmed ablation of specific cell types can be stably transmitted through the germ line.

CONSIDERABLE PROGRESS HAS BEEN made in the understanding of the origins and interrelations of cell lineages during growth and development in invertebrates such as *Caenorhabditis elegans* and *Drosophila* (*1*). Lineage development in mammals is not as well understood, however, because of the indeterminate or stochastic nature of cell commitment during embryogenesis. Several experimental approaches have been applied to this problem, including microinjection of histochemical tracers, direct visualization of developing embryos, photoablation, and the generation of mosaic and chimeric animals (*2*). Recently, new genetic approaches to lineage analysis have been developed. These include the use of in situ hybridization to recognize cells of different genotypes in mouse chimeras or mosaics (*3*), the exploitation of retrovirus vectors as random insertional tags within both the hematopoietic system (*4*) and the developing mouse embryo (*5*), and the utilization of the bacterial *lacZ* gene as an in situ marker for visualizing lineage-specific gene expression (*6*) and cell-lineage relations (*7*). Here, we demonstrate that targeted expression of the gene for the A chain of diphtheria toxin (DT-A) in transgenic animals results in the death of a specific cell type during development, a method that we have termed "genetic ablation." Palmiter *et al.* (*8*) have independently described a similar approach to effect the ablation of cells within the pancreas.

To demonstrate the feasibility of this approach, we used the mouse γ2-crystallin promoter to target expression of the DT-A gene to the mouse eye lens. The DT-A gene encodes an adenosine diphosphate (ADP) ribosyltransferase that catalyzes the ADP-ribosylation of elongation factor 2, resulting in the inhibition of protein synthesis and subsequent cell death (*9*). As little as one molecule per cell of the DT-A chain is estimated to be cytotoxic (*10*).

The γ2-crystallin promoter was chosen to drive expression of the DT-A gene for the following reasons. (i) This promoter can direct expression of a *lacZ* reporter gene exclusively to the lens fiber cells of transgenic mice (*6*). (ii) Ablation of cells within the lens would be expected to result in a readily discernible phenotype (see below). (iii) The relation between crystallin gene expression and lens cell differentiation is well characterized. (iv) Many developmental mutants that affect the lens have been described, which provides a basis for interpreting lens phenotypes caused by ablation events. (v) Developmental aberrations within the lens are nonlethal, and thus would not complicate initial efforts to evaluate the potential of genetic ablation.

The lens consists predominantly of two cell types: undifferentiated epithelial cells, which make up the anterior surface layer, and terminally differentiated fiber cells, which constitute the major body of the lens. Because synthesis of γ-crystallins, as well as the activity of the mouse γ2 promoter in transgenic mice, is restricted to terminally differentiated fiber cells of the lens nucleus (*6, 11*), it was anticipated that expression of the DT-A gene from the mouse γ2 promoter in transgenic mice would result in a lens

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deficient in fiber cells. The predicted phenotype of such an event would be microphthalmia and possibly cataracts.

The γ2-crystallin/DT-A hybrid gene (γ2DT-A) was generated by replacing the human metallothionein IIA (MTIIA) promoter segment in pTH-1 (*12*) with mouse γ2-crystallin promoter sequences extending from −759 to +45 relative to the transcription start site (Fig. 1A).

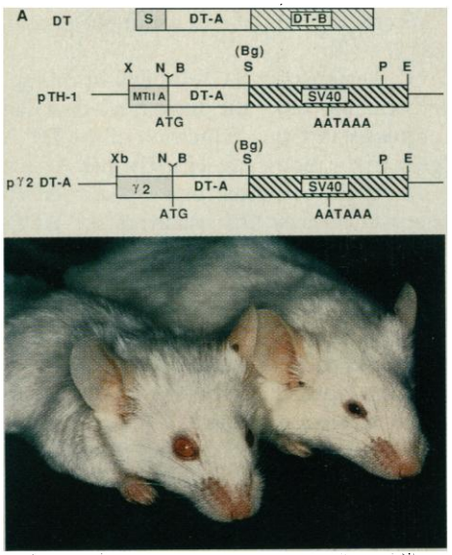

Fig. 1. (**A**) Diagram of the injected γ2DT-A fragment. The native DT gene is shown at the top. Indicated segments are the coding sequences for the 25–amino acid residue signal peptide (stippled) followed by the DT-A (white) and DT-B (slanted lines) domains of the mature polypeptide. The 2.98-kbp γ2DT-A Xba I–Eco RI fragment microinjected into fertilized eggs is shown at the bottom (*13*). Indicated segments of the γ2DT-A hybrid gene are: sequences −759 to +45 of the mouse γ2-crystallin gene (stippled), the DT-A gene of pTH-1 (*12*) (white), and SV40 sequences including the polyadenylation signal (slanted lines). Abbreviations are: X, Xma III; N, Nco I; B, Bam HI; S, Sau 3A; Bg, Bgl II; P, Pst I; E, Eco RI; Xb, Xba I. (**B**) Microphthalmic appearance of mouse A10 at 4 months of age (on the right) compared to an age-matched normal CD-1 strain mouse (on the left).

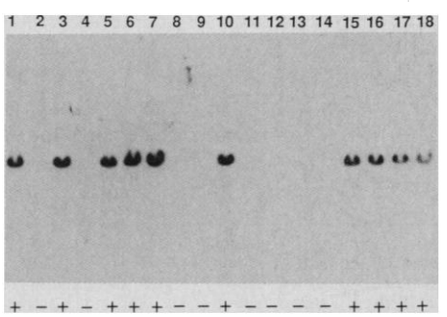

Fig. 2. Detection of γ2DT-A sequences in representative (A10 × CD-1) F_1 mice (*14*). The Bam HI–Eco RI fragment containing the DT-A coding region and SV40 splicing and polyadenylation sequences (Fig. 1) was used as a probe. Lanes 1 to 18: Sac I–digested tail DNA of F_1 animals. All mice carrying the transgene also manifested microphthalmia, as indicated in the figure.

Before implanting microinjected eggs it was first essential to establish that the γ2DT-A construct was not toxic as the result of a low level of nonspecific gene expression before integration. Of 308 eggs microinjected with the γ2DT-A hybrid gene, 228 (74%) developed to the two-cell stage. A comparable fraction (70%) survived to the two-cell stage after microinjection of other nontoxic constructs.

On the basis of these findings, microinjected eggs were transferred to pseudopregnant recipients. Six of the 111 live-born animals analyzed contained insertions of γ2DT-A sequences.

When these animals were examined as early as 3 weeks of age, it was readily apparent that three had microphthalmia (Fig. 1B). Two of the founder mice also had cataracts in at least one eye. At least one of the transgenic mice that did not display an obvious microphthalmia had a significant deletion in the transgene, consistent with the conclusion that the absence of any phenotypic abnormality in this mouse was due to a structural rearrangement in the γ2DT-A construct.

To study the microphthalmic phenotype further, and to determine whether it could be transmitted through the germ line, the offspring of one of the founder animals, mouse A10, were analyzed in detail. Sixteen out of 49 F_1 progeny of A10 × wild-type CD-1 mice manifested microphthalmia. Southern blot analysis of the F_1 mice established that expression of the phenotype completely cosegregated with the presence of the γ2DT-A construct (Fig. 2).

The eyes of these transgenic F_1 animals were compared with those of normal littermates by histological analysis of serial cross sections. There was considerable heterogeneity in the structure and organization of individual lenses (Fig. 3). Some were almost normal morphologically but reduced in size (Fig. 3B). These lenses contained organized lens fiber cells and intensely staining lens nuclear regions suggestive of significant crystallin accumulation. Others were clearly aberrant, with extensive vacuolation in the central nuclear region of the lens (Fig. 3C). The most extreme phenotype consisted of a

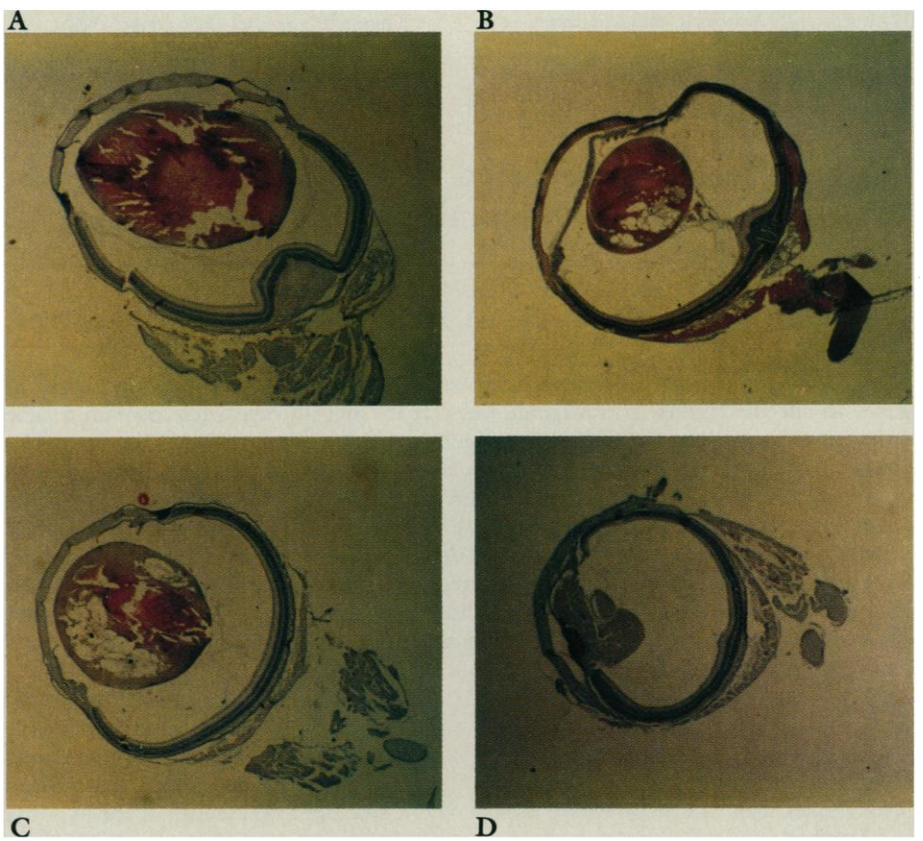

Fig. 3. Histological cross sections (7 μm) of normal and transgenic eyes from 3-week-old (A10 × CD-1) F_1 mice (*15*). (**A**) Wild-type control. (**B**) Eye from transgenic mouse showing almost normal lens morphology. (**C**) Eye from a second transgenic mouse showing extensive vacuolation in the central nuclear region of the lens. (**D**) Contralateral eye from the second transgenic animal, which shows a highly aberrant lens structure with no intense staining suggestive of significant crystallin accumulation. The stress fractures apparent in the control lens (A) are a consequence of the hardness of the lens, whereas the vacuoles evident in (C) were visible prior to imbedding.

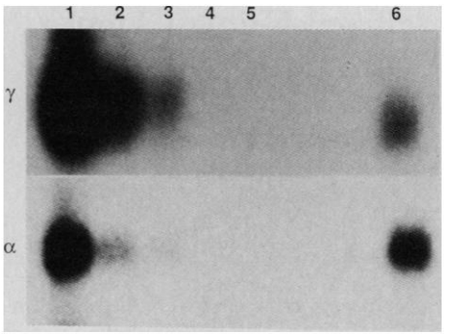

Fig. 4. Average relative levels of α- and γ-crystallin transcripts in eyes of 3-week-old normal and known transgenic (A10 × CD-1) F_1 mice (*16*). Mouse αA- and γ2-crystallin cDNAs were used as probes. Lanes 1 and 6: equal eye equivalents of wild-type and microphthalmic RNA, respectively. Lanes 2 to 5: fractions (1/5, 1/10, 1/50, and 1/100) of the wild-type eye RNA loaded in lane 1. The filter was probed with the mouse γ2 cDNA, deprobed, and then hybridized with the mouse αA cDNA.

highly disorganized lentoid structure in which no lens nucleus or differentiated fiber cells were apparent (Fig. 3D). Phenotypic variation occurred even between eyes of the same animals, suggesting that these differences were not due to genetic variation among outbred CD-1 mice.

As expected, the levels of both α- and γ-crystallin RNA were reduced in the ablated lenses of microphthalmic mice (Fig. 4). The lenses of these mice had approximately one-half the wild-type level of αA-crystallin messenger RNA (mRNA) and one-seventh the level of γ-crystallin transcripts. The preferential reduction in γ mRNA is consistent with the observation that the ablation events were restricted to terminally differentiated lens fiber cells. The γ-crystallin probe cross-hybridizes with other members of the γ gene family. Because different γ genes may not all be expressed in the same set of fiber cells, the residual level of γ-crystallin mRNA detected in the lenses of γ2DT-A transgenic mice may correspond to γ transcripts present in fiber cells that are not subject to ablation. The residual levels of γ-crystallin mRNA could also reflect the possibility that the γ2DT-A transgene is transcriptionally active for a shorter period during development than the corresponding mouse γ2-crystallin gene.

In summary, we have shown that targeted expression of the DT-A gene with a γ-crystallin promoter can be used to ablate terminally differentiated cells within the lens lineage. Moreover, lines of transgenic animals can be generated that appear to be normal, aside from the ocular defect. Thus, we conclude that the γ2DT-A construct is controlled tightly enough to allow targeted cell ablation without compromising either embryological development or germ-line transmission.

An interesting and unexpected finding from these studies was the phenotypic heterogeneity among lenses of microphthalmic mice from the A10 line. There are several possible explanations for this observation. (i) Although one molecule of DT-A per cell is estimated to be cytotoxic (*10*), it is conceivable that threshold levels of the toxin do not accumulate in all lens fiber cells. (ii) There may be a stochastic relationship between the onset of DT-A expression and the early stages of lens morphogenesis. (iii) There is a finite probability that a cell within the undifferentiated lens epithelium has sustained a somatic mutation resulting in functional inactivation of DT-A expression. Explanations (i) and (iii) make certain testable predictions about the ablation phenotype in animals that are homozygous for the DT-A transgene.

The generation of a line of mice in which embryonic fiber cells within the lens have been genetically ablated should now allow detailed analysis of the precise relation between crystallin gene expression and lens morphogenesis, as well as the role these processes in turn play in the overall spatial organization of the developing eye. For such studies, precise fate mapping of ablation events within the lens should be facilitated through genetic crosses with γ2 *lacZ* transgenic indicator mice (*6*). In a broader context, our work demonstrates that it should be possible to effect ablation of any lineage or cell type for which a promoter of appropriate specificity is available. One potential problem with genetic ablation is that expression of many promoter/toxin gene combinations might be lethal to the early embryo or sufficiently toxic to prevent establishment of a transgenic pedigree. The microphthalmic mice described here, however, demonstrate that it is possible for programmed ablation of specific cell types within a lineage to be stably transmitted through the germ line.

REFERENCES AND NOTES

1. J. Sulston and J. G. White, *Dev. Biol.* **78**, 577 (1980); J. E. Kimble, *ibid.* **87**, 286 (1981); W. J. Gehring, *Cell* **40**, 3 (1985).
2. J. Rossant, *Curr. Top. Dev. Biol.*, in press.
3. J. Rossant, M. Vijh, L. D. Siracusa, V. M. Chapman, *J. Embryol. Exp. Morphol.* **73**, 179 (1983); C. Lo, *J. Cell Sci.* **81**, 143 (1986).
4. J. E. Dick, M. C. Magli, D. Huszar, R. A. Phillips, A. Bernstein, *Cell* **42**, 71 (1985); G. Keller, C. Paige, E. Gilboa, E. F. Wagner, *Nature (London)* **318**, 149 (1985); I. R. Lemischka, D. H. Raulet, R. C. Mulligan, *Cell* **45**, 917 (1986).
5. P. Soriano and R. Jaenisch, *Cell* **46**, 19 (1986).
6. D. R. Goring, J. Rossant, S. Clapoff, M. L. Breitman, L.-C. Tsui, *Science* **235**, 456 (1987).
7. J. R. Sanes, J. L. Rubenstein, J. Nicholas, *EMBO J.* **5**, 3133 (1986); J. Price, D. Turner, C. Cepko, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 156 (1987); D. L. Turner and C. L. Cepko, *Nature (London)* **328**, 131 (1987).
8. R. D. Palmiter *et al.*, *Cell* **50**, 435 (1987).
9. A. M. Pappenheimer, Jr., *Annu. Rev. Biochem.* **46**, 69 (1977).
10. M. Yamaizumi, E. Mekada, T. Uchida, Y. Okada, *Cell* **15**, 245 (1978).
11. J. W. McAvoy, *J. Embryol. Exp. Morphol.* **44**, 149 (1978).
12. I. H. Maxwell, F. Maxwell, L. M. Glode, *Cancer Res.* **46**, 4660 (1986).
13. To construct pγ2DT-A, the Xma III site of plasmid pTH-1 (see Fig. 1) was first converted to an Xba I site by digestion of pTH-1 with Xma III, filling in with the Klenow fragment of *Escherichia coli* DNA polymerase I, ligating of Xba I linkers, followed by digestion with Xba I. The resulting plasmid was then digested with Xba I and Nco I to remove the 144-bp metallothionein gene promoter and ligated to a Xba I–Nco I fragment containing promoter sequences from the mouse γ2-crystallin gene from −759 to +45 and derived from a γ2-CAT plasmid [S. Lok *et al.*, *Mol. Cell. Biol.* **5**, 2221 (1985)].
14. Three-week-old animals were screened for the insertion of the γ2-crystallin–diphtheria toxin hybrid gene by Southern blot analysis [E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975)] of tail DNA digested with Sac I, a noncutter for the test construct. The probe was a 2.2-kb Bam HI–Eco RI fragment spanning the DT-A coding region and SV40 splicing and polyadenylation sequences (see Fig. 1), and labeled with the random-priming procedure [A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983)]. Digested DNA (10 μg) was separated by electrophoresis through 0.6% agarose gels, transferred to Zetabind filters (AMF Cuno), and prehybridized and hybridized as described [G. M. Wahl, M. Stern, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979)]. Filters were washed at room temperature in 2× standard saline citrate (SSC) and 0.1% SDS twice, then at 50°C, four times, alternately with 2× SSC and 0.1% SDS and 0.2× SSC and 0.1% SDS. Dried filters were exposed to Kodak XAR-5 film for 1 to 3 days at −70°C with one Dupont Lightning Plus intensifying screen.
15. Whole eyes from known transgenic offspring of mouse A10 and eyes from age-matched normal siblings were fixed in a solution containing 10% formalin, 10% glacial acetic acid, and 30% ethanol. After dehydration and clearing, eyes were imbedded in Paraplast (Monoject Scientific), sectioned at 7 μm, and stained with hematoxylin and eosin.
16. Average relative levels of α- and γ-crystallin transcripts in eyes of 3-week-old normal and known transgenic (A10 × CD-1) F_1 mice were assessed by Northern blot analysis [P. S. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980)] of total eye RNA. Probes were mouse αA- and γ2-crystallin complementary DNAs (cDNAs), kindly provided by J. Platigorsky, and labeled by random priming. Total RNAs prepared, respectively, from 4 and 12 eyes of normal and microphthalmic mice, were run in equal eye equivalents, or various fractions thereof, through 1.5% formaldehyde-agarose gels and transferred to a Zetabind filter. Conditions for prehybridization and hybridization were as described (*14*). After hybridization with the γ2 cDNA probe, the filter was immersed in H_2O and 0.1% SDS at 100°C for 10 minutes and then reprobed with the mouse αA cDNA. Dried filters were exposed to Kodak XAR-5 film with one Dupont Lightning Plus intensifying screen for 1 to 5 days at −70°C.

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