TABLE 3 Genetic variability at 12 loci

	Mean sample size per locus	Mean no. of alleles per locus	% Loci polymorphic	Mean heterozygosity
White Mountains populations				
Bishop	15.8	1.9	66.7	0.211
Coldwater Cyn	18.8	2.6	75.0	0.218
Gunter Cyn	16.3	2.3	75.0	0.194
Means	17.0	2.3	72.2	0.207
Last Chance Mountains populations				
Dedeckera Cyn (upper)	14.8	2.7	91.7	0.384
Dedeckera Cyn (lower)	16.7	3.3	91.7	0.293
Last Chance Mts	13.3	2.7	83.3	0.318
Means	14.9	2.9	88.9	0.332

Values in this table are derived from analysis of 12 loci—EST-2 (esterase), GSR-2 (glutathione reductase), IDH-2 (isocitrate dehydrogenase), MDH-1, MDH-2, MDH-4 (malate dehydrogenase), MDR-1 (menadione reductase), GPI-2 (glucose phosphate isomerase), PGM-1, PGM-2, PGM-3 (phosphoglucomutase) and SKDH (shikimate dehydrogenase)—that were polymorphic in at least one of the six populations. When five additional loci ADK-1 (adenylate kinase), GPI-1, GSR-1, LAP (leucine amino peptidase), and MDH-3], monomorphic for all populations, are included, the mean polymorphism is 56.8%. A locus was considered polymorphic if any allelic variant was detected. Mean proportion of heterozygotes (direct count) is less than the expected Hardy–Weinberg (unbiased estimate)[24]. The fixation index F[25] showed that only two loci (PGM-1 in the Gunter Cyn population and SKDH in the population of the Last Chance Mountains) had significant ($P < 0.001$) deviations in genotype proportions.

MDR-1 to 58.3% for GPI-2. These heterozygosity averages are underestimated because they include the zero values for those markers that are not polymorphic in the particular population sampled. This amount of variation is much higher than that reported for other species of desert shrubs[16], but is similar to other woody, outcrossing perennials[17–18].

Dedeckera is vegetatively vigorous and flowers profusely. Its longevity (at least 140 years and probably much more) may be correlated with heterozygosity as reported in *Liatris*[19] (Asteraceae). In *Dedeckera*, vegetative fitness may be largely dependent upon rare, highly heterotic (or possibly epistatic) genotypes. The uniquely heterozygous genotypes that do survive may, however, have low reproductive potential because of the excessively high segregational genetic load. Reproduction could be further compromised by the accumulation of recessive lethals in long-lived meristems[20] and chromosomal mutations[21].

The persistence of palaeoendemics may depend on rare multiple-locus heterotic genotypes that could arise as a result of tracking some protracted secular environmental change, for example, increasing aridity. The pace and extent of such change could easily exhaust the species' additive genetic variance relevant to adapting to that change. Heterosis may be the only survival strategy available to such species, in spite of the negative reproductive consequences. Thus the 13% increase in mean heterozygosity for the populations of *Dedeckera* in the Last Chance Mountains, as opposed to those in the White Mountains (Table 3), may be important because the former localities are both hotter and drier. In fact, many palaeoendemics may be ecologically 'out of place', in that they may not possess many of the adaptations typical of plants occupying that habitat—thus *Dedeckera* flowers in mid-summer when desert perennials are typically dormant.

Rabbits[22] and humans[23] have genetically mediated spontaneous embryo abortion rates of about 50% and 70%, respectively. The loss of reproductive capacity stemming from early embryonic genetic load should not be overlooked as a possible element in the decline of higher animal palaeoendemics. Such cases may be observed rarely because higher animals generally have much shorter life-spans, relatively limited reproductive potentials, and because early abortion is more difficult to detect than in plants.

Other palaeoendemic plants in North America, Africa and Australia also seem to have exceedingly low seed sets. Reproductive capacity should be given careful consideration in management decisions regarding rare and/or endangered species. □

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The *ninaA* gene required for visual transduction in *Drosophila* encodes a homologue of cyclosporin A-binding protein

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MUTATIONS of the *Drosophila melanogaster ninaA* gene affect phototransduction: *ninaA* mutant flies have a 10-fold reduction in the levels of rhodopsin in the R1–R6 photoreceptor cells[1,2]. The *ninaA* gene was isolated and found to encode a 237-amino-acid protein that has over 40% amino-acid sequence identity with the vertebrate cyclosporin A-binding protein, cyclophilin, a protein that seems to be involved in T-lymphocyte activation. The remarkable evolutionary conservation of cyclophilin in two phylogenetically distant organisms and its involvement in diverse transduction processes suggests that this protein plays an important role in cellular metabolism. Indeed, cyclophilin has recently been shown to be a prolyl *cis–trans* isomerase that catalyses, *in vitro*, rate-limiting steps in the folding of a number of proteins[3,23]. Here, we present evidence for the involvement of cyclophilin-like molecules in a defined cellular process. The availability of mutations in a cyclophilin gene provides a new model system for the study of cyclophilin and cyclosporin action.

Numerous mutations that affect visual transduction in *Drosophila* have been isolated (reviewed in refs 4, 5). To isolate genes encoding phototransduction-specific proteins, we used a subtractive hybridization protocol to identify genomic clones

containing sequences preferentially expressed in the adult visual system. Clones that tested positive in our screen (see Fig. 2 legend) were isolated and used in *in situ* hybridizations to polytene chromosomes to determine their cytogenetic map positions. The *ninaA* locus has been mapped to the second chromosome, position 21D3-E2 (ref. 1). Two of the 120 genomic clones we isolated, λ322 and λN4, were mapped to position 21D4-E2, within the cytogenetic map location of *ninaA*. The *ninaA* locus constitutes one of eight complementation groups of *Drosophila* mutants with drastically reduced rhodopsin levels[1,2]. *ninaA* flies have a severe reduction of rhodopsin (Rh1) levels in the R1–R6 photoreceptor cells (Fig. 1). The reduction of rhodopsin levels in the R1–R6 photoreceptors of *ninaA* mutant flies has recently been shown[6] not to be due to reduced expression of the R1–R6 opsin gene (*ninaE*) but may be the result of a defect in some aspect of post-translational processing of the *ninaE* opsin, or a secondary effect on the stability of rhodopsin due to a defect in phototransduction.

Characterization of clones λ322 and λN4 showed that they contain overlapping sequences and encode a 0.9 kilobase (kb) RNA (Fig. 2) that is present in preparations of poly $(A)^+$ RNA from wild-type heads, but not from wild-type bodies or heads from mutant flies lacking the compound eyes (*eya*; eyes absent[7]) (Fig. 2*b*). We used the large *Eco*RI restriction fragment of λ322 to screen a *Drosophila* head cDNA library and isolated several cDNA clones. Using M13 dideoxynucleoside triphosphate sequencing we have determined the DNA sequence of one of those cDNA clones and of the 1.2 kb *Hin*dIII–*Pst*I genomic fragment of λ322 (Fig. 2*a*). Figure 3*a* shows the nucleotide sequence and the deduced amino-acid sequence of the *ninaA* gene product. The structure of the RNA (Fig. 2*a*) was determined by analysing genomic and cDNA sequences. Confirmation that λ322 includes the *ninaA* gene was obtained by determining the nucleotide sequence of the $ninaA^{P228}$ mutant allele. Transcript size and levels are normal in these mutant flies (Fig. 2*b*). The mutant allele was isolated and cloned by a polymerase chain reaction. The $ninaA^{P228}$ allele has all the nucleotide sequence polymorphisms of the Oregon-R strain. Interestingly, however, this allele has a single coding nucleotide change at position 895, from TGG to TGA, causing a change from the encoded tryptophan to an opal translation termination codon (Fig. 3*a* arrow). The nature of this change is consistent with the chemical mutagenic origin of $ninaA^{P228}$ (ref. 1). Thus, the cytogenetic location, expression profile, and altered nucleotide sequence in mutant flies are all consistent with this being the *ninaA* gene.

Comparison of the deduced amino-acid sequence of *ninaA* with previously sequenced genes and proteins revealed very high homology to the bovine cyclosporin A-binding protein cyclophilin (Fig. 3*b*). These two proteins have more than 40% amino-acid identity throughout their length (shaded boxes); conserved

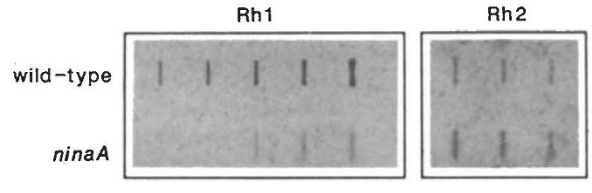

FIG. 1 *ninaA* mutants have a severe reduction of Rh1 opsin levels. Protein extracts were prepared from the heads of control and *ninaA* flies by digitonin extraction[23]. Serial dilutions (0.25, 0.5, 1, 2 and 4 μg) were slot-blotted onto nitrocellulose paper and processed for western analysis using a monoclonal antibody against the Rh1 opsin (kindly provided by Dr H. Gert de Couet). Mutant flies have a 8–10-fold reduction of the opsin present in the R1–R6 photoreceptors (Rh1). The right panel shows similar extracts (4, 2 and 1 μg) treated with a monoclonal antibody against the ocellar (Rh2) opsin. This monoclonal antibody was generated against a Rh2-specific synthetic peptide. Note the specificity of the mutant phenotype for the Rh1 opsin (see also refs 1, 2).

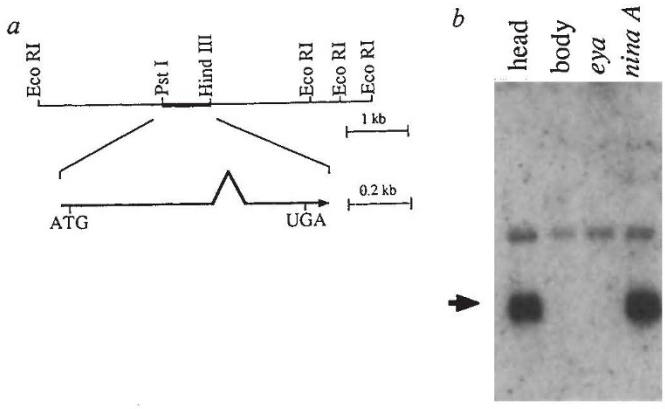

FIG. 2 λ322 encodes an eye-specific transcript. *a*, Restriction map of λ322 and structure of the RNA it encodes. A map of λ322 indicating the restriction sites for *Hin*dIII, *Pst*I and *Eco*RI (top map). The diagram below the map shows the structure of the RNA as deduced by comparison of the nucleotide sequence of a cDNA clone and the genomic sequence. *b*, Poly $(A)^+$ RNAs were extracted from adult heads of wild-type, *eya* and *ninaA* flies and from adult bodies. The RNAs (3 μg per lane) were gel-fractionated, blotted, and hybridized to a 1.2-kb λ322 radiolabelled *Pst*I–*Hin*dIII fragment. λ322 hybridizes to a 0.9-kb eye-specific transcript (arrow). The blot was also hybridized to a probe encoding 'common' RNAs to control for the amount of RNA loaded (λ9, unpublished observations). Hybridization to λ9 mRNA is seen as the band present in all lanes with a mobility significantly slower than λ322 mRNA. An RNA ladder (BRL) was used to provide size markers.

METHODS. λ322 was isolated by hybridization with eye-specific probes. Poly $(A)^+$ RNA, isolated from the heads of wild-type flies, was used to template the synthesis of cDNA. This cDNA was hybridized in solution to a 20-fold excess of poly$(A)^+$ RNA isolated from the bodies of adult flies. Single-stranded molecules were then separated from the double-stranded DNA:RNA heteroduplexes by hydroxylapatite chromatography[24]. Hybridizations were carried out in 30 μl of 0.5 M phosphate buffer (pH 6.9) containing 2 μg of head cDNA and 40 μg of body RNA. The reaction mixtures were incubated for 24 h at 65 °C. The resulting single-stranded cDNA, representing mostly 'head-specific' sequences, were used in a second round of subtraction with RNA isolated from heads of flies carrying the *eya* mutation[7]. Single-stranded molecules were fractionated by hydroxylapatite chromatography and used as templates for the synthesis of very high specific activity second-strand cDNA by two rounds of amplification with random primers (pN_5, Amersham). This radiolabelled cDNA, representing a probe highly enriched in eye-specific sequences, was then used to screen a genomic library. Subtractions with *eya* head poly$(A)^+$ RNA were carried out with 0.2 μg of head-enriched cDNA and 4 μg of *eya* RNA. About 10% of input cDNA was recovered after the first subtraction, and about 20% after the second. First and second-strand cDNA synthesis were carried out as described[25].

substitutions account for over 30% of the remaining amino-acid residues. Cyclophilin is a soluble protein with a relative molecular mass (M_r) of 17,000 with high binding affinity and stereospecificity for cyclosporin A (CsA)[8–11]. CsA is an 11-amino-acid cyclic peptide of fungal origin with potent immunosuppressive properties widely used to prevent graft rejection and for the treatment of autoimmune disorders (reviewed in refs 12–14). Recent data support the hypothesis that the action of CsA is mediated through cyclophilin[15–17]. Although the exact mechanism of CsA action is not known, there is good evidence from whole cell systems that CsA interrupts an early event in the antigenic activation of T helper cells that results in lymphokine production[18–20].

To determine whether *Drosophila* does indeed contain proteins with CsA-binding activity, we carried out [^{3}H]CsA binding assays on head extracts prepared from wild-type controls, *eya*, and $ninaA^{P228}$ flies. As shown in Fig. 4, head extracts have significant levels of cyclophilin-like activity (specific partition on sepharose LH-20 chromatography[8–11]); about 30% of the head binding is associated with the visual system in that it is removed by the *eya* mutation. The $ninaA^{P228}$ mutation leads to a similar decrease in CsA binding indicating that the *ninaA*

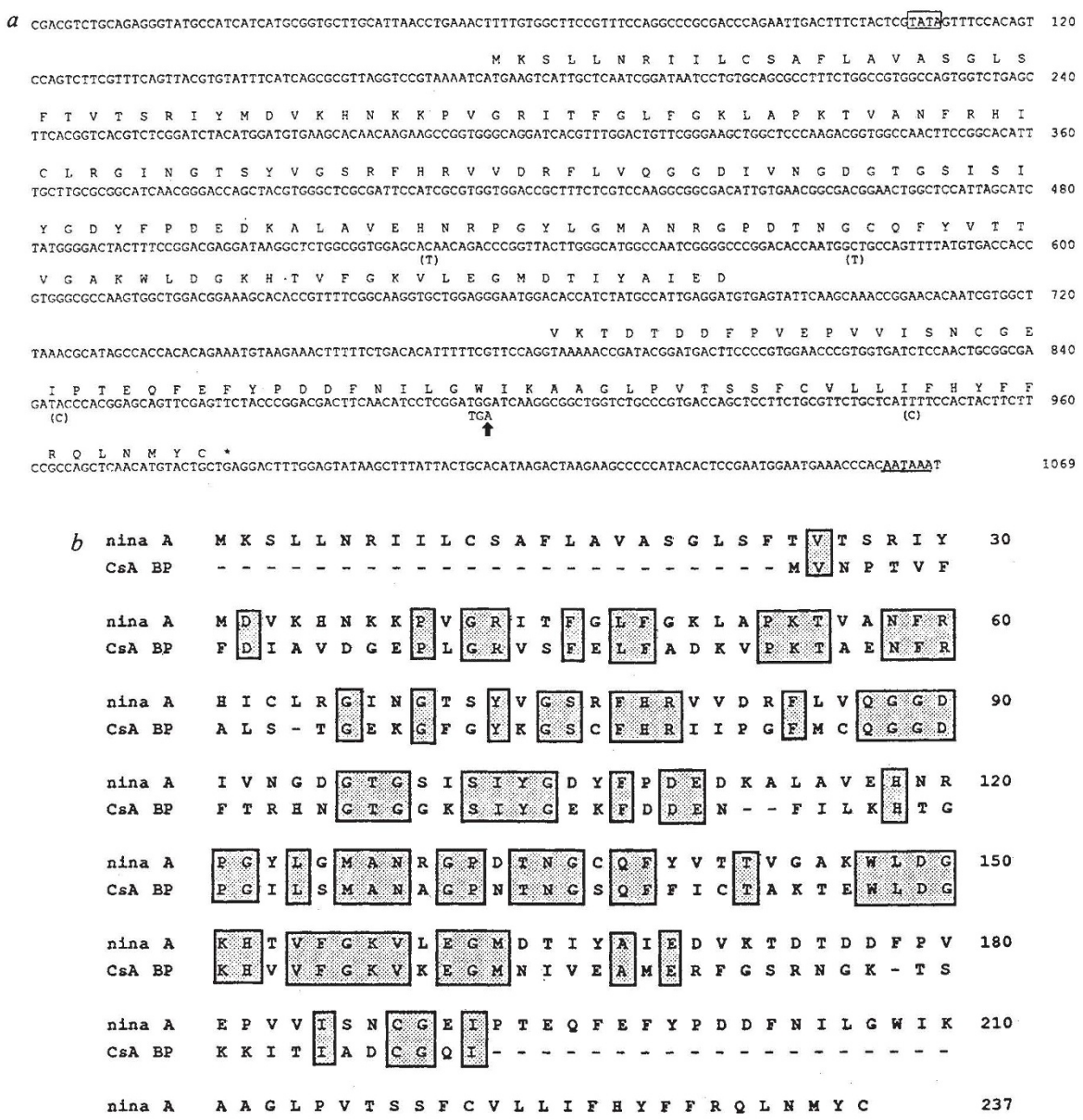

FIG. 3 Nucleotide sequence and deduced amino-acid sequence of the *ninaA* gene. *a*, The sequence shown was determined on both strands of the genomic and cDNA clones[26]. The boxed region at positions 106–110 shows the putative TATA box. The putative polyadenylation signal is underlined at positions 1,063–1,068. The deduced protein sequence is shown aligned under the nucleotide sequence. The initiator methionine was assigned as the first in-frame methionine in the sequence. The gap at nucleotides 686–782 indicate the presence of a 96-base pair (bp) intron. The arrow at position 895 highlights the single nucleotide change in the *ninaA*^{P228} mutant gene (G→A). This change creates a translation termination codon. The single nucleotides below positions 528, 579, 844 and 946 show the differences in the coding regions between the cDNA (Oregon R, P2 strain) and the genomic (Canton S) sequence; none of these polymorphisms would result in a change in the encoded amino acid. *b*, A co-linear alignment of the deduced amino-acid sequence of the *ninaA* gene and the bovine cyclophilin protein[10]. Cyclophilin from mammalian species analysed so far have over 95% sequence identity, and the physical properties of the CsA binding proteins from other vertebrate species are remarkably similar[10,27,28]. Amino acids are designated by the single-letter code. The alignment has been optimized for the largest number of identities with the least number of gaps. Boxed areas indicate the amino-acid identities between the two proteins.

METHODS. The mutant allele was cloned by selective amplification of the *ninaA* gene by polymerase chain reaction using protocol and reagents supplied by Perkin-Elmer Inc. (PCR kit N801-0043). Oligonucleotides complementary to positions −8 to +11 and +986 to +1,005 were used as primers.

locus encodes a cyclophilin-like protein that accounts for most of the visual system-specific CsA-binding activity (Fig. 4). The binding activity remaining in the *eya* and *ninaA* extracts probably represents the presence of non-eye-specific cyclophilin-like proteins.

The *ninaA* R1–R6 photoreceptors have about 10% of wild-type levels of rhodopsin[1,2]. Zuker and co-workers[6] have previously shown that the Rh1 opsin gene is expressed at normal levels in *ninaA* mutants. The reduction of rhodopsin must therefore be a post-transcriptional or a post-translational event. The discovery that cyclophilin encodes a prolyl *cis-trans* isomerase[3] suggests that it may be required for the correct folding of proline-containing polypeptides. We propose that this isomerase activity is necessary for the correct folding and the stability of rhodopsin in *Drosophila* R1–R6 photoreceptors. The requirement for large amounts of rhodopsin in these cells may explain the existence of a photoreceptor cell-specific form of this enzyme. Moreover, the existence of a cell-specific isoform suggests that there are specific substrates for these proteins and explains the presumed diversity of cyclophilin-like molecules (M.A.S. and C.S.Z., unpublished observations and refs 3, 10). It is therefore possible that, in the immune system, CsA blocks

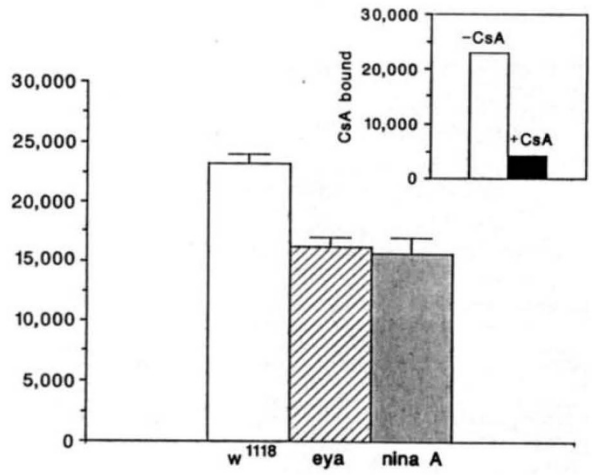

FIG. 4 *ninaA* flies have reduced levels of cyclosporin A-binding activity. Extracts prepared from heads of control w^{1118} flies, *eya* or *ninaA* flies were tested for CsA-binding activity[9]. The results indicate that *Drosophila* head extracts contain significant amounts of cyclophilin-like activity (approximately 30 ng CsA binding mg^{-1} extract) and that all eye-associated binding is reduced in *ninaA* flies (compare *eya* and *ninaA* extracts). Bars above the graph indicate standard errors (w^{1118}, $n=10$; *eya*, $n=8$; $ninaA^{P228}$, $n=7$). *Drosophila* extracts were prepared from heads of wild-type or mutant individuals exactly as described[9]. Binding assays were carried out with ^{3}H-cyclosporin A (17 Ci $mmol^{-1}$ Amersham) at either 30 °C or 37 °C and the products separated by partition chromatography on a Sephadex LH-20 column[8]. The inset shows the specificity of the binding assay as determined by competition with unlabelled CsA.

the activity of a cyclophilin required for the proper functioning of the antigen-mediated transduction pathway.

In the visual system, the interconversion between the active (metarhodopsin) and inactive (rhodopsin) states of the visual pigment molecule involves significant conformational changes. In the invertebrate visual cascade, these two forms are thermally stable and photoconvertible. It would therefore be very interesting to determine whether the isomerase encoded by *ninaA* is important in this event during the transduction cycle. The availability of *Drosophila* lines carrying mutations in the endogenous *ninaA* gene, and the use of P-element-mediated germline transformations[21,22], may allow for the functional expression of wild-type and modified alleles in their normal cellular and organismal environment. A combined biochemical, physiological and molecular genetic dissection will help assign specific roles to the *ninaA* gene product in the phototransduction process. □

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Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (*rds*)

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MUTANT mice homozygous for 'retinal degeneration slow' (*rds/rds*) are characterized phenotypically by abnormal development of photoreceptor outer segments in the retina, followed by gradual degeneration of photoreceptors[1–3]. This process of degeneration is complete by one year, with preservation of all other retinal cells[4]. The biochemical defect that leads to the mutant phenotype is not known. Our strategy for cloning the *rds* gene was based upon three previously reported observations. First, the *rds* locus maps to chromosome 17[5,6]. Second, experimental *rds/rds* ↔ +/+ and *rds*/+ ↔ +/+ tetra-parental mice manifest patchy photoreceptor changes in the retina[7,8], suggesting that the wild-type *rds* locus is expressed within cells of the photoreceptor lineage. Finally, the process of degeneration is specific to photoreceptors. On the basis of these observations, we predicted that the *rds* mRNA is encoded by a gene on chromosome 17 and is normally expressed exclusively within photoreceptors in the retina. We here present evidence that this is the case.

Given our predictions, a cDNA representing a photoreceptor-specific mRNA encoded by a gene on chromosome 17 would be a candidate clone of the *rds* mRNA. To isolate cDNA clones of photoreceptor-specific mRNAs, we took advantage of the unrelated mouse mutant, retinal degeneration (*rd/rd*)[9,10]. Mice homozygous for this mutation manifest rapid degeneration of photoreceptors, a process that is virtually complete by four weeks, with the preservation of all other retinal cell types[11,12]. Therefore, an mRNA present in wild-type (C57BL/6) but absent from fully degenerate *rd/rd* (C3H/HeJ) retina is photoreceptor-specific. cDNA clones of twelve different photoreceptor-specific mRNAs were isolated from an adult C57BL/6 mouse retina library by subtractive and differential colony screening[13] of 6–7-week-old C57BL/6 minus 6–7-week-old C3H/HeJ retina. Northern blot hybridization patterns for six of these clones are shown in Fig. 1*a*.

The chromosome assignments for the genes encoding each of the 12 photoreceptor-specific mRNAs were made by probing a panel of mouse × hamster hybrid cell-line DNAs[14] with a representative clone of each of the photoreceptor-specific mRNAs. Clone IG3 mapped to chromosome 17 with 100% concordance