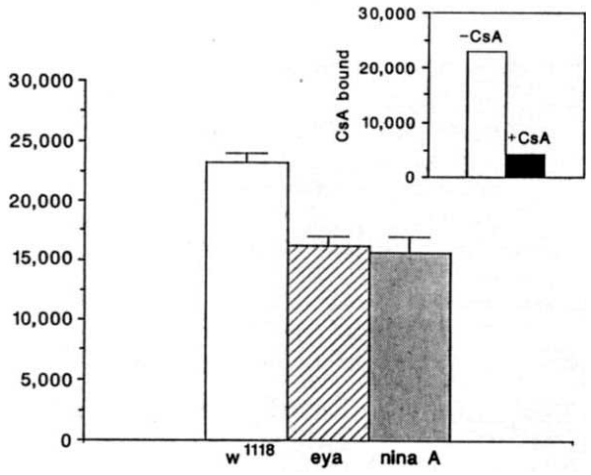

FIG. 4 *ninaA* flies have reduced levels of cyclosporin A-binding activity. Extracts prepared from heads of control w^{1118} flies, *eya* or *ninaA* flies were tested for CsA-binding activity[9]. The results indicate that *Drosophila* head extracts contain significant amounts of cyclophilin-like activity (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

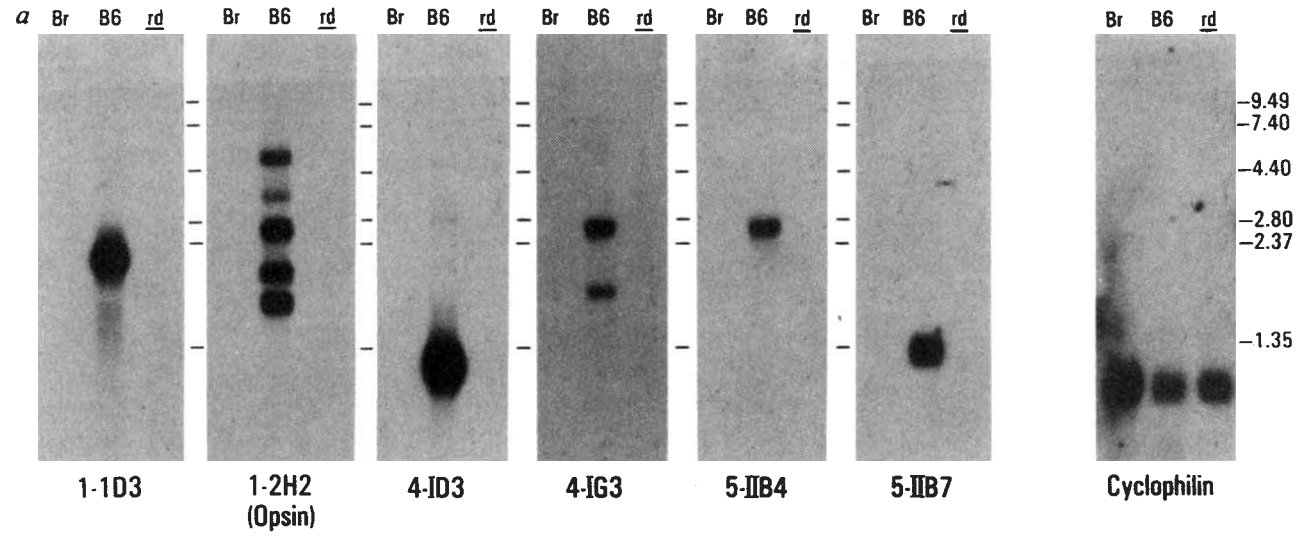

FIG. 1 Characterization of the *rds* mRNA. *a*, Northern blot hybridizations with six of the twleve clones of photoreceptor-specific mRNAs. Br, 6–7-week-old C57BL/6 total mouse brain; B6, 6–7-week-old C57BL/6 retina; rd, 6–7-week-old C3H/HeJ retina. Probes were prepared from cDNA clones derived from a C57BL/6 minus C3H/HeJ retinal subtractive screen. Ubiquitously expressed rat cyclophilin[18] was used as a control for equal loading of lanes. RNA size standards are indicated to the right in kb. *b*, The IG3.B9A mRNA has aberrant size and abundance in *rds/rds* retina. rd, 6–7-week-old C3H/HeJ retina; B/c, 4–8-week-old BALB/c retina; rds, 4–8-week-old *rds/rds* (020/cpb) retina. *c*, same northern blot as *b* re-probed with cDNA clone 2H2 of the mouse opsin mRNA.

METHODS. Total cellular RNA was prepared from the indicated mouse retinas and brain[19]. Enrichment for poly(A)$^+$ RNA was performed on oligo(dT) cellulose[20]. Northern blots were prepared by electrophoresis in 1% agarose formaldehyde gels and transferring to nitrocellulose[21]. Each lane contained 2 μg of poly(A)$^+$ RNA. cDNA libraries were prepared from C57BL/6 and C3H/HeJ retina poly(A)$^+$ selected RNA in the plasmid vector pGEM4[13]. Plasmid DNA from the amplified C3H/HeJ library was used as a hybridization driver to subtract common sequences from a C57BL/6 ^{32}P-labelled cDNA probe as previously described[13]. The subtracted probe was used to screen the C57BL/6 library. Positive clones were checked for hybridization with unsubtracted C57BL/6 but not C3H/HeJ cDNA in a differential colony screen. Insert DNA from individual clones selected by the above screen was labelled with [α^{32}P]dCTP as described[22] and was used to probe northern blots. Final stringency wash conditions were 0.2 × SSC/0.2% SDS at 68 °C.

(Table 1). A full-length cDNA clone (IG3.B9A), containing an insert of approximately 2.7 kilobase (kb), was isolated by re-probing the C57BL/6 retinal cDNA library with clone IG3.

Clone IG3.B9A was used to probe northern blots containing RNA from the retinas of 1–2-month-old (pre-degenerate) *rds/rds* mice (BALB/c background) and age-matched wild-type (BALB/c) mice (Fig. 1*b*). Two major mRNA species of approximately 1.6 kb and 2.7 kb (estimated abundance, 0.05%) were observed in the BALB/c retina lane. These bands were not present in the *rds/rds* retina lane. A faint doublet band of approximately 12 kb was detected in the *rds/rds* lane however, which was not present in the BALB/c lane. Thus, the mRNAs detected with clone IG3.B9A in the retinas of *rds* mice are aberrant in size and abundance, suggesting that IG3.B9A is a clone of the wild-type *rds* mRNA.

When the same northern blot was reprobed with a cDNA clone of the opsin mRNA (clone 1-2H2 in Fig. 1*a*), the BALB/c and *rds/rds* retina lanes exhibited identical hybridization patterns, although the signal intensity in the mutant lane was slightly reduced (Fig. 1*c*), consistent with partial degeneration of photoreceptors. This experiment excluded the possibility that the observed difference in RNA hybridization patterns with the IG3.B9A probe in wild-type and *rds/rds* retina was due to premature degeneration of photoreceptors in the mutant, or to a generalized abnormality in RNA processing within photoreceptors in *rds/rds*.

To define the site of the mutation within the putative *rds* locus, five adjacent non-overlapping restriction endonuclease fragments spanning the full-length cDNA clone IG3.B9A were prepared (Fig. 2*a*). These fragments were used individually as probes to examine restriction digests of genomic DNA prepared from BALB/c and *rds/rds* mice on Southern blots. Restriction fragment length polymorphisms (RFLPs) were detected in the DNA of BALB/c relative to *rds/rds* mice with probe B9A-II (Fig. 2*b*), but not with probes B9A-I, -III, -IV or -V (data not shown).

Genomic libraries prepared with the DNA from BALB/c and *rds/rds* mice were probed with cDNA clone IG3.B9A. λ clone B7 was isolated from the BALB/c library and λ clone R24 was isolated from the *rds/rds* library. The λ-cloned DNAs gave similar hybridization patterns to their corresponding wild-type or mutant genomic DNAs on Southern blots probed with cDNA clone fragment B9A-II (Fig. 2*b*).

To define the mutation at the nucleotide level, the 2.8 kb *Pst*I

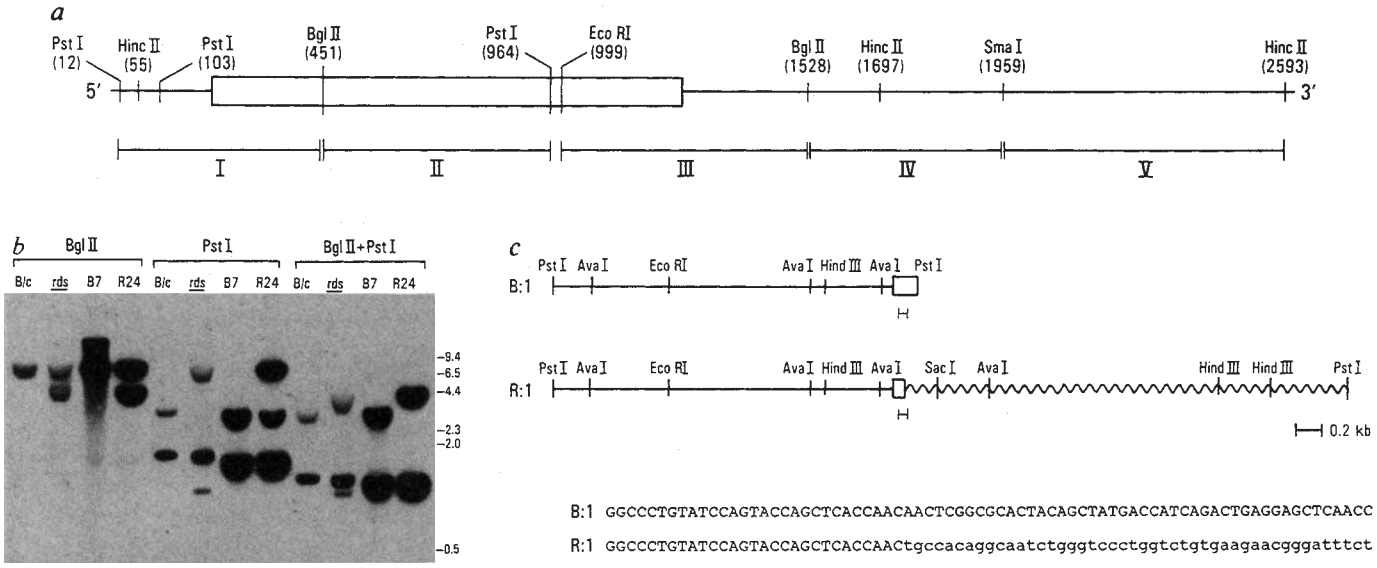

FIG. 2 *a*, Restriction map of the *rds* cDNA clone IG3.B9A derived from the nucleotide sequence (see Fig. 3). The open reading frame is indicated by the open box. Restriction fragments (B9A I–V) used as probes for Southern blot analysis are indicated below. *b*, Southern blot analysis of genomic and λ clone DNA with IG3.B9A fragment II probe. B/c, BALB/c DNA; rds, *rds/rds* DNA (10 μg genomic DNA per lane); B7, λ clone B7 from BALB/c library; R24, λ clone R24 from *rds/rds* library (0.5 ng λ clone DNA plus 10 μg sheared salmon sperm DNA per lane). DNA samples were digested with restriction endonuclease *Bgl*II, *Pst*I, or *Bgl*II plus *Pst*I as indicated. DNA size standards are indicated to the right in kb. Electrophoresis, transfer to nitrocellulose, preparation of probes and stringency wash conditions as described in Table 1. *c*, Restriction map of BALB/c and *rds/rds* genomic clones. B:1 is the 2.8 kb *Pst*I genomic subclone fragment from wild-type phage λ clone B7 detected with probe B9A-II. R:1 is the 6.3 kb *Pst*I genomic subclone fragment from *rds/rds* phage λ clone R24 detected with probe B9A-II. The open box indicates an exon which contains a protein-coding region (see Fig. 3). The wavy line indicates the insertion in the mutant locus. The nucleotide sequences of B:1 and R:1 in the region containing the divergence is indicated below. Capital letters in the nucleotide sequence correspond to the wild-type exon; lowercase letters correspond to the insertion. Bars underneath the restriction map show the origin of the indicated sequences.

TABLE 1 Segregation pattern of the mouse allele

Chromosome	Hybridization pattern +/+	−/−	+/−	−/+	Per cent discordance
1	4	5	4	1	35.7
2	5	3	3	3	42.9
3	2	4	3	0	33.3
4	3	3	5	2	53.8
5	0	6	8	0	57.1
6	3	4	5	2	50.0
7	4	0	4	6	71.4
8	3	5	5	0	38.5
9	1	5	7	1	57.1
10	1	6	7	0	50.0
11	0	4	6	0	60.0
12	4	1	2	3	50.0
13	3	2	5	3	61.5
14	1	5	7	1	57.1
15	6	0	0	4	40.0
16	2	4	5	1	50.0
17	8	6	0	0	0.0
18	3	2	4	3	58.3
19	2	2	6	3	69.2
X	4	5	4	1	35.7

Southern blots containing DNA from mouse × hamster somatic cell hybrids were probed with cDNA clone IG3. Column designations: +/+, chromosome and IG3 hybridization signal both present; −/−, chromosome and hybridization signal both absent; +/−, chromosome present and hybridization signal absent; −/+, chromosome absent and hybridization signal present. A hybridization signal corresponding to the hamster allele was present in all cell lines. 10 μg DNA samples from 16 mouse × hamster hybrid cell lines containing different mouse chromosomes[14] were digested with restriction endonuclease *Pst*I and resolved by electrophoresis on 0.7% agarose. The DNA was transferred to nitrocellulose[17] and then probed with DNA from clone IG3 (see Fig. 1). Final stringency wash conditions were 0.2 × SSC/0.2% SDS at 68 °C.

fragment from (wild-type) λ clone B7 and the 6.3 kb *Pst*I fragment from (*rds/rds*) λ clone R24 detected by B9A-II were both subcloned. Restriction endonuclease mapping of these subcloned *Pst*I fragments (B:1 and R:1) revealed that the 5′ 2.7 kb of each were indistinguishable. After the third *Ava*I site (Fig. 2*c*), the restriction maps of B:1 and R:1 diverged. The 0.25 kb *Ava*I–*Pst*I 3′ fragment from the wild-type subclone B:1, and the corresponding 0.95 kb *Ava*I–*Ava*I fragment from the mutant subclone R:1 were isolated and their nucleotide sequences determined. The sequences were identical up to the position corresponding to nucleotide 899 in the mRNA (see below and Fig. 3). At this point, a foreign sequence appears in the mutant gene, disrupting the protein-coding exon (Fig. 2*c*). As the mutation occurs within an exon, this foreign sequence is probably transcribed in *rds/rds*. The observed increase in the size of the transcripts detected with clone IG3.B9A in the retinas of *rds/rds* mice is consistent with the insertion of approximately 10 kb of foreign DNA into an exon of the *rds* gene.

In an attempt to identify this foreign insert, we used Southern blots to analyse genomic DNA isolated from BALB/c and *rds/rds* using the 0.4 kb *Sac*I–*Ava*I restriction fragment from the inserted element of R:1 (Fig. 2*c*) as a probe. About 30 bands were detected in both the wild-type and *rds/rds* lanes, with two additional bands present in the *rds/rds* lanes that were absent from the wild-type lanes (data not shown). A computer database search was performed with the nucleotide sequence from the 0.4 kb *Sac*I–*Ava*I fragment of R:1 (data not shown). No significant similarity to any eukaryotic or viral sequences was detected. Based upon its size (approximately 10 kb) and its copy number in the mouse genome (10–30 copies), this inserted DNA may represent an unidentified retrovirus-like element.

Sequence analysis of the IG3.B9A cDNA revealed an insert containing 2,632 base pairs (bp) followed by a poly(A) tail (Fig. 3). A single long open reading frame began at nucleotide 213, encoding a putative protein of 346 amino acids. A common

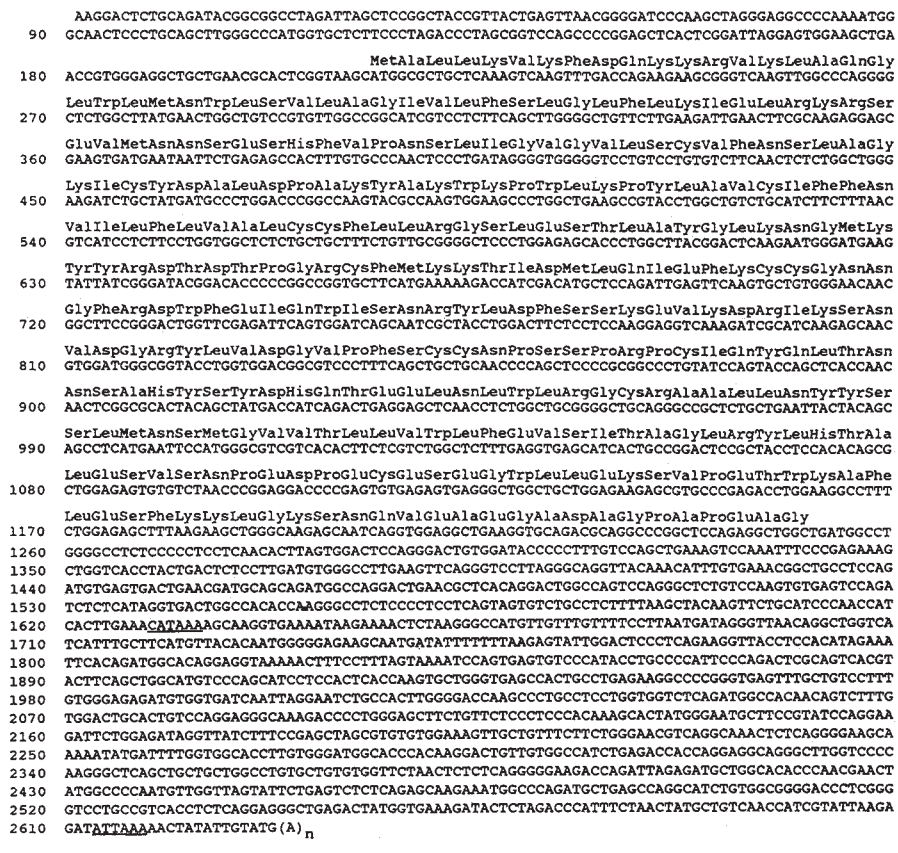

FIG. 3 Nucleotide sequence of the full-length *rds* cDNA clone IG3.B9A. Nucleotide numbering is shown on the left. The single long open reading frame has the corresponding amino-acid sequence translated above. The polyadenylation signal CATAAA for the 1.6 kb mRNA (IG3.D6), and ATTAAA for the 2.7 kb mRNA (IG3.B9A) are underlined.
METHODS. The sequence was determined by a modified version[23] of the chemical degradation method of Maxam and Gilbert[24]. The complete sequence was determined for both DNA strands of clones IG3.B9A and IG3.D6 (see below). Primer extension analysis was performed on clone IG3.B9A as described[25] using a synthetic deoxyoligonucleotide corresponding to the antisense strand of IG3.B9A from position 64 to 41 (data not shown). cDNA clone D6 of the small, wild-type *rds* transcript was isolated by screening for clones that hybridized with full-length clone IG3.B9A but failed to hybridize to a truncated clone containing 0.6 kb from the 3′ end of IG3. The sequence of clone IG3.D6 was identical to IG3.B9A except for the use of an alternative polyadenylation signal.

variant (AUUAAA)[15] of the consensus polyadenylation signal (AAUAAA)[16] was found 20 nucleotides upstream from the poly(A) tail. Primer extension analysis showed that IG3.B9A was only six nucleotides short of being a full-length clone of the 2.7 kb mRNA (see Fig. 3 legend). A cDNA clone of the 1.6 kb *rds* transcript (IG3.D6) was isolated by further screening (Fig. 3 legend). Sequence analysis of IG3.D6 showed that the 1.6 kb mRNA was identical to the 2.7 kb mRNA except that the poly(A) tail began 24 bases downstream from the alternative polyadenylation signal CAUAAA[15], at position 1,651 rather than 2,633.

A computer search of a protein sequence database (Protein Identification Resource, 31 March 1988) with the predicted protein encoded by the *rds* mRNA revealed no significant similarities to any known protein sequences. The *rds* protein (relative molecular mass, M_r 39,259) does not seem to have an N-terminal secretion signal sequence. It does contain three uncharged regions (amino acids 16–41, 100–122 and 252–275) which may represent membrane-spanning domains. Four sets of tandem basic residues (amino-acid residues: 11–13, 46–48, 178–179 and 324–325) are possible candidates for proteolytic cleavage signals. The protein also contains 13 cysteine residues which are potential sites for intra- or inter-chain disulphide linkages. As the *rds* mRNA has been shown here to be photoreceptor-specific within the retina and is not detectable in brain, it is possible that the *rds* protein function relates to some photoreceptor-specific process, possibly as an unidentified member of the visual transduction cascade. As the insertion occurred within a protein-coding exon, the resulting protein will be aberrant, lacking its normal C-terminal 87 amino-acid residues if translation of the aberrant *rds* mRNA does occur in *rds/rds* retina.

From the evidence presented here, we concluded that we have cloned the *rds* gene and characterized its normal mRNA products. This is the first molecular description of a gene determining neuronal degeneration in a mammalian system where nothing was known in advance about the gene product. It remains a formal possibility that the gene identified here is not *rds*: if loss of function of the identified gene product is not lethal to photoreceptors and if another gene on chromosome 17 which is also expressed in photoreceptors bears an independent second mutation. Final proof that the identified gene is indeed *rds* will await correction of the phenotype by introducing DNA from the cloned wild-type allele into the *rds/rds* background in a transgenic experiment. Elucidation of the mechanism of photoreceptor degeneration in *rds/rds* will need studies of the *rds* gene product. The first steps towards this involve immunocytochemical localization of the rds protein within photoreceptors, and the identification of other proteins which may be physically associated. □

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