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A null mutation in the photoreceptor guanylate cyclase gene causes the *retinal degeneration* chicken phenotype

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ABSTRACT **The retinas of the *retinal degeneration* (*rd*) chicken are fully developed and possess normal morphology at hatching but fail to respond to light stimulation. Analyses of retinal cGMP, the internal messenger of phototransduction, show that the amount of cGMP in predegenerate, fully developed *rd*/*rd* photoreceptors is 5–10 times less than that seen in normal photoreceptor cells. We show that the low levels of cGMP in *rd* chicken retina are a consequence of a null mutation in the photoreceptor guanylate cyclase (GC1) gene. Thus, the *rd* chicken is a model for human Leber's congenital amaurosis. Absence of GC1 in *rd* retina prevents phototransduction and affects survival of rods and cones but does not interfere with normal photoreceptor development.**

In photoreceptor cells, absorption of light leads to the hydrolysis of the internal transmitter of phototransduction, cGMP, by activated phosphodiesterase. During return to the dark state, cGMP levels are replenished by guanylate cyclase (GC), the activity of which is modulated by guanylate cyclase activating proteins (GCAPs) and calcium (1, 2). Two photoreceptor-specific GCs (GC1 and retGC2) (3–6) and two GCAPs (GCAP1 and GCAP2) have been cloned, all of which are expressed in rod and cone photoreceptor cells (for original references, see ref. 2; also see refs. 3–8). The phototransduction cascades in rods and cones are similar; however, their responses to different wavelengths and intensities of light are distinct. Disruption of cGMP metabolism in rod cells has been shown to severely compromise the development and function of these cells (9–11). The effects of abnormal cGMP metabolism on cone cell function and integrity are less clearly defined. This is due, in part, to the fact that cone photoreceptor cells represent only a small percentage (2–5%) of the total photoreceptor cell population in many mammalian retinas. Thus, animals possessing cone-enriched retinas, such as the ground squirrel and chicken, serve as valuable research models for studies of cone development and function.

Our research efforts have focused on a *retinal degeneration* (*rd*) chicken strain carrying an autosomal recessive mutation that produces blindness at hatch (12). Sections of 1-day-old *rd*/*rd* chicken retinas show no signs of degeneration. Pathology appears 7–10 days after hatch, is limited to the photoreceptor cells located in the central retina, and proceeds from central to peripheral regions (13, 14). At 115 days, very few cone photoreceptors remain in the central retina, and by 6–8 months, the photoreceptor cell layer is degenerated (14). Degenerative changes in the retinal pigment epithelium and inner retina become apparent only after photoreceptor degeneration is underway (15). The feature of this disease that distinguishes it from other animal models of recessive retinitis pigmentosa is that light fails to elicit measurable electroretinograms before any signs of rod or cone photoreceptor degeneration are detected (13, 16).

Preliminary studies have indicated that cGMP levels are significantly below normal levels in retinas of 1-day-old *rd* chickens. We have investigated several genes encoding phototransduction components (17–19); however, only GCAP1 was found to be down-regulated in the predegenerate, *rd* chicken retina (20). In human, GC1 frameshift and missense mutations (21) have been shown to cause Leber's congenital amaurosis (LCA), a severe autosomal recessive disease that causes blindness at birth. In this paper, we provide evidence that the chicken *rd* gene encodes photoreceptor GC1, and that the *rd* chicken is an animal model for human LCA. We propose that the presence of a deletion/rearrangement in the GC1 gene produces a null allele that leads to abnormally low levels of cGMP in the rod and cone cells, and to retinal degeneration.

MATERIALS AND METHODS

Animals. A breeding colony of *rd*/*rd* Rhode Island Red chickens is maintained at the University of Florida and is cared for in accordance with National Institutes of Health guidelines. The *rd* colony has been backcrossed with wild-type Rhode Island Red chickens (Morris Hatchery, Goulds, FL) every 3–4 years.

cGMP Assay. Levels of cGMP in either total retina or in retinal layers were determined by radioimmunoassay (RIA) (Biomedical Technologies, Stoughton, MA). To measure changes in cGMP in developing retina, embryonic and post-hatch chicken heads, frozen in liquid N_2, were transferred to a −35°C cryostat and tissue punches containing the posterior central retina (choroid, pigment epithelium, neural retina) were removed by using trephines. Punches were placed in 400 μl 1 M HCl, sonicated, boiled for 3 min, neutralized to pH 6–7 with 1.0 M NaOH, and assayed by RIA. Controls for assay specificity were done in the presence of excess PDE (Sigma). For measurement of cGMP in retinal layers, 7-μm sections of dark-adapted central retina were freeze-dried at −40°C and dissected (22). Layers of 10–30 sections were combined, extracted into 10% TCA, and centrifuged. The supernatant was assayed by RIA. Details of these procedures are given in ref. 23.

Western Blot Analyses. Membrane fractions of retinas from 1- to 3-day-old chickens (24) were separated by SDS/PAGE,

Abbreviations: GC1, photoreceptor guanylate cyclase; PDE, phosphodiesterase; GCAP, guanylate cyclase activating protein; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase–PCR; E, embryonic day; RIA, radioimmunoassay; LCA, Leber's congenital amaurosis.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF036942).

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transferred to membrane (7), and probed with two anti-retGC polyclonal antibodies (pAb UW28 and GC2) and an anti-rhodopsin monoclonal antibody (mAb 4D2). UW28 and GC2 were raised against the dimerization domain (I^{766}-K^{795}) and peptide H^{636}-T^{658} of bovine GC1, respectively. Blots were first incubated with the appropriate primary antibodies followed by incubation with secondary antibody conjugated with alkaline phosphatase (Promega).

cDNA Library Screening. A random primer-labeled 1,369-bp fragment of bovine ROS-GC (4) was used as a probe to screen a +/+ chicken retina-pigment epithelium-choroid Uni-ZAP XR cDNA library (17, 18). The primers used to generate the cDNA fragment were 5′-AAGGCGTGCTG-GCTGTGGTCT and 5′-TTGGGGATGGGTTTGTT-GAAG. Hybridization was carried out at 30°C in 40% formamide. The filters were washed 3 × 15 min in 1× SSC, 0.5% SDS at 30°C, followed by a single 25-min wash in the same solution at 35°C.

5′-Rapid Amplification of cDNA Ends (RACE), Reverse Transcriptase–PCR (RT-PCR), and DNA Sequence Analyses. 5′-RACE PCR (Marathon kit, CLONTECH) and RT-PCR (GeneAmp RNA PCR kit, Perkin–Elmer) techniques were used to clone +/+ and *rd/rd* chicken GC1. The following primers were used in this study: 60, 5′-GGICCIT-GGGCITG(C/T)GA(C/T)CC; 96, 5′-GCCGCCGTCCG TATTG; 97, 5′-TAGGCGTCCCCGATGGTCTCC; 98, 5′-GGGCTTCACCACCATCTC; 129, 5′-CAGCATTTGGGT-CAGCAGTTTATCC; 141, 5′-ACGTTTACGGCATCG-GCATCATTA; 149, 5′-AGGACACCTATTGGCTGGT; 155, 5′-GGICCIGA(C/T)CCI(T/A)(C/G)ITG(C/T)T GG; 156, 5′-CTTGCAGAAGGCCAGCTTGG; 162, 5′-TTTGAGTC-GGGCTCCATC; 163, 5′-GCCCG ACTCAAACCAGCACC; 165, 5′-CGTCCTCCTCATCTTCATCC; 166, 5′-ACIGCI CCICA(G/A)GA(T/C)(T/C)TITGG; 167, 5′-CAGCTGAT-GAAGGGACCC; 168, 5′-AGCACC GGGGACACCAAA; 175, 5′-AGCCCCCGTGCCCTCATCTC; 177, 5′-TAGGGC-AGGGGG AAGGTGAG; 178, 5′-CCTTCCCCCTGCCCT-ACCAC; 183, 5′-TCAGCTTGCAGAAGGCCA GC; 190, 5′-GTGTTGGGGCCGTGGCTGTG; 191, 5′-ACGGCGTC-CCACCAATAACC; 601, 5′-TA(C/T)GA(A/G)GGIGA(C/T)TGGGTITGG; and 603, 5′-GCCGATGCCGTAAACGTC GC. All PCR products were ligated into the pCR 2.1 TA cloning vector (Invitrogen). The inserts were sequenced in both directions by using a Li-Cor Model 4000L automatic DNA sequencer (Li-Cor, Lincoln, NE) (25) and Excel DNA polymerase (Epicenter) for linear PCR amplification.

Northern Blot Analyses. Total RNA was isolated from 1- to 3-day-old +/+, *rd/+*, and *rd/rd* chicken retina-pigment epithelium-choroid by using an RNeasy total RNA kit (Qiagen, Chatsworth, CA). For Northern blots (17), 20 μg total RNA was loaded per lane. Blots were probed sequentially with a 500 bp *Pst*I fragment of GC1 clone 35–6A and a 2.0 kb *Pst*I fragment of a chicken β-actin cDNA clone (26). The random primed cDNA probes (27) were labeled by using a Prime-it RmT labeling kit (Stratagene).

Southern Blot Analyses. Genomic DNA was extracted from +/+ and *rd/rd* blood samples (28). DNA (15 μg/sample) was digested with 40 units of either *Hin*cII or *Sau*3AI. The restriction fragments were separated on a 0.8% agarose gel, transferred to nylon membrane (Magnacharge, Micron Separations), and UV-crosslinked to the membrane (UV Strata-Linker, Stratagene). Blots were probed with a random primer-labeled 988-bp GC1 cDNA fragment that was amplified using primers 178 and 156 (Fig. 3*A*) and processed as described for the Northern blot.

RESULTS

cGMP Levels in *rd/rd* Photoreceptors Are Significantly Reduced. cGMP levels in *rd/rd* chicken retina were examined as a function of both development and of retinal cell type. Levels of cGMP in embryonic day 12 (E12) *rd/rd* and control retina were nearly identical (Fig. 1*A*); however, by E18, the

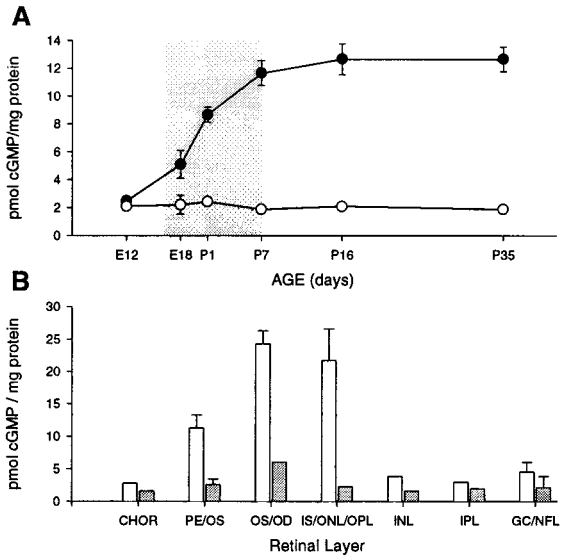

FIG. 1. cGMP levels in *rd/rd* retina. (*A*) Comparison of cGMP levels in *rd/+* control and *rd/rd* posterior central retina. Chickens were light adapted for 2 hr under laboratory illumination (1,600 lux) before sacrifice. The shaded area from embryonic day 18 (E18) to posthatch day 7 (P7) is the time during which photoreceptor outer segment development and elongation occurs. Each value represents the mean ±SD of 6–11 eyes. Filled symbols, control retina; open symbols, *rd/rd* retina. (*B*) cGMP levels in microdissected layers of retinas taken from 1- to 2-day posthatch chickens. Assays were carried out on seven retinal layers taken from central retina (CHOR, choroid; PE/OS, pigment epithelium plus outer segments; OS/OD, outer segments plus oil droplets; IS/ONL/OPL, inner segments plus outer nuclear layer plus outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GC/NFL, ganglion cells plus nerve fiber layer). Each value represents the mean ±SD of three to four eyes (10–30 sections per eye). All measurements were made in duplicate. Open bars, *rd/+* control retina; filled bars, *rd/rd* retina.

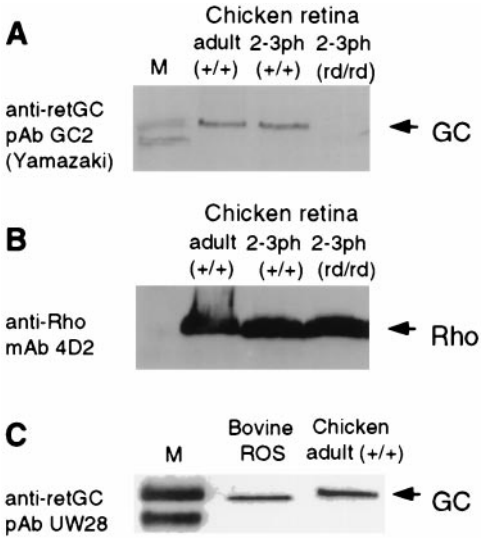

FIG. 2. Analyses of GC1 protein levels expression in normal and rd/rd retina. (*A*) Western blot probed with anti-retGC pAb GC2. (*B*) Western blot shown in *A* reprobed with anti-rhodopsin mAb 4D2. (*C*) Western blot probed with anti-retGC pAb UW28. The relative mobility of the chicken GC1 polypeptide is slightly slower than that of the bovine GC1 polypeptide. Lane M in *A* and *C* shows molecular mass markers, 115 and 80 kDa. 2–3ph, 2–3 days posthatch; ROS, rod outer segments.

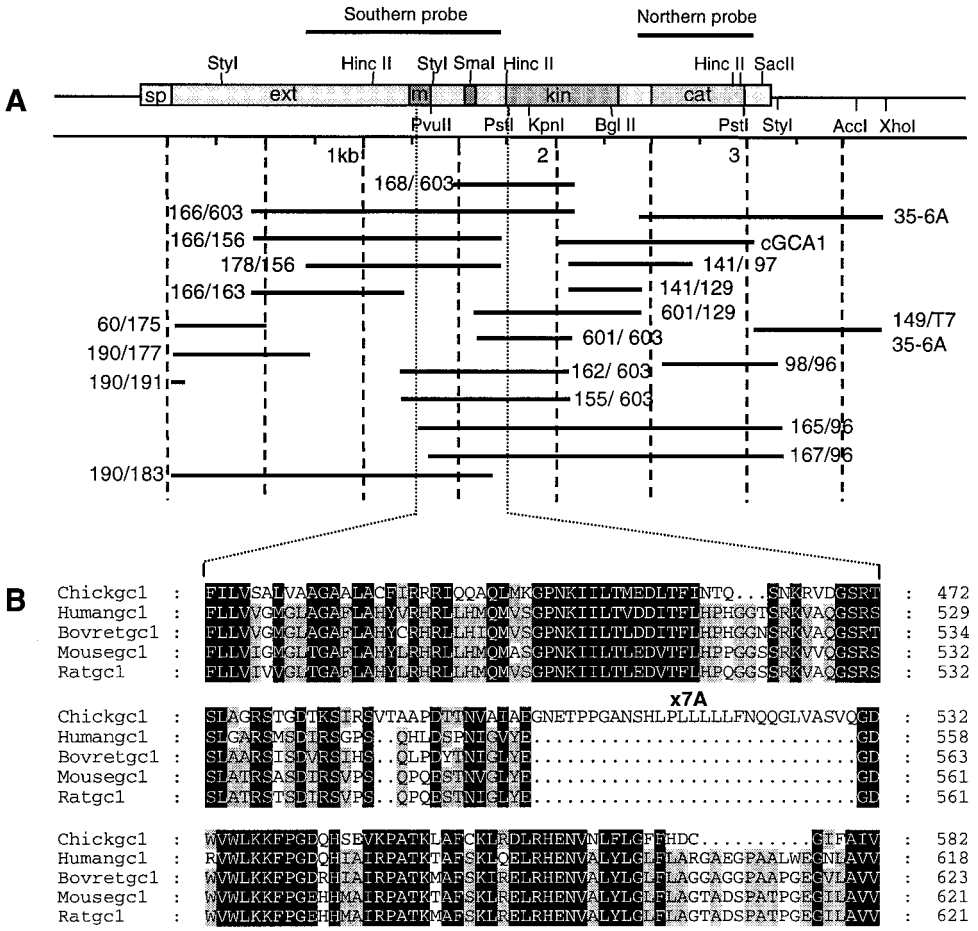

FIG. 3. Cloning and alignment of the deduced amino acid sequences of chicken GC1 with human, bovine, mouse, and rat GC1. (*A*) Schematic showing the 21 GC1 cDNA clones obtained by cDNA library screening, RT-PCR, and 5′ RACE. The 988-bp Southern and 500-bp Northern GC1 probes are shown above the GC1 domain map. The numbers used to identify the clones correspond to primers whose sequences appear in the *Methods* section. sp, signal peptide; ext, extracellular domain; m, membrane-spanning domain; kin, kinase-like domain; cat, catalytic domain. (*B*) Alignment of GC1 extending from the membrane-spanning domain to the kinase-like domain. The 29-aa peptide present in approximately one-half of the chicken GC1 transcripts (X7A) is shown. Residues printed in white are identical or represent conservative substitutions across all five sequences (L = I = V = M; E = D; S = T = A; *r* = K). Residues printed black on gray are identical or conserved substitutions in four of the five sequences. Nonconserved sequences are printed in black on white.

amount of cGMP present in control retina had nearly doubled whereas in *rd/rd* retina it remained near the E12 value. The amount of cGMP present in control retina continued to increase over the next 10 days, reaching a plateau around 7 days posthatch (P7). No increases were observed in *rd/rd* retina. By P7, levels of cGMP in control retina were six times greater than those found in *rd/rd* retina. This difference could not be attributed to cell loss because the initial signs of retinal degeneration in *rd/rd* retina do not become apparent until P7–P10. The increase in cGMP levels in control retina between E18 and P7 coincides with the development and elongation of the photoreceptor outer segments (29). To identify the cell type responsible for the observed differences in retinal cGMP levels, we measured cGMP levels in each of the layers of the retina. The results show that the amount of cGMP present in the photoreceptors of 1- to 2-day-old dark-adapted *rd/rd* retina is only 10–20% of that found in the photoreceptors of control retina (Fig. 1*B*). cGMP levels in the remaining layers of the control and *rd/rd* retinas were very low and comparable to each other. Thus, abnormal photoreceptor cGMP metabolism and photoreceptor dysfunction are concomitant phenotypes in *rd* chicken.

Photoreceptor GC1 Is Not Present in *rd/rd* Retina. Western blots of the membrane fraction of 2- to 3-day-old +/+ and predegenerate *rd/rd* retina were probed with polyclonal antibodies GC2 and UW28, both of which stained a polypeptide of

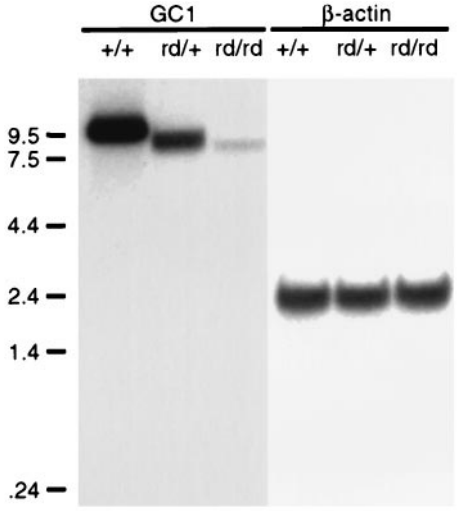

FIG. 4. Northern blot of +/+, +/*rd*, and *rd/rd* total retinal RNA. The normal GC1 transcript is approximately 9.5 kb in size (*Left*). The *rd/rd* transcript is 0.5–1.0 kb shorter than the normal transcript, and the amount of the transcript in predegenerate *rd/rd* retina is less than 10% of normal levels. Reprobing the blot with the chicken β-actin cDNA probe showed no difference in the amount of total RNA loaded per lane. The positions of RNA standards are shown on the *Left*.

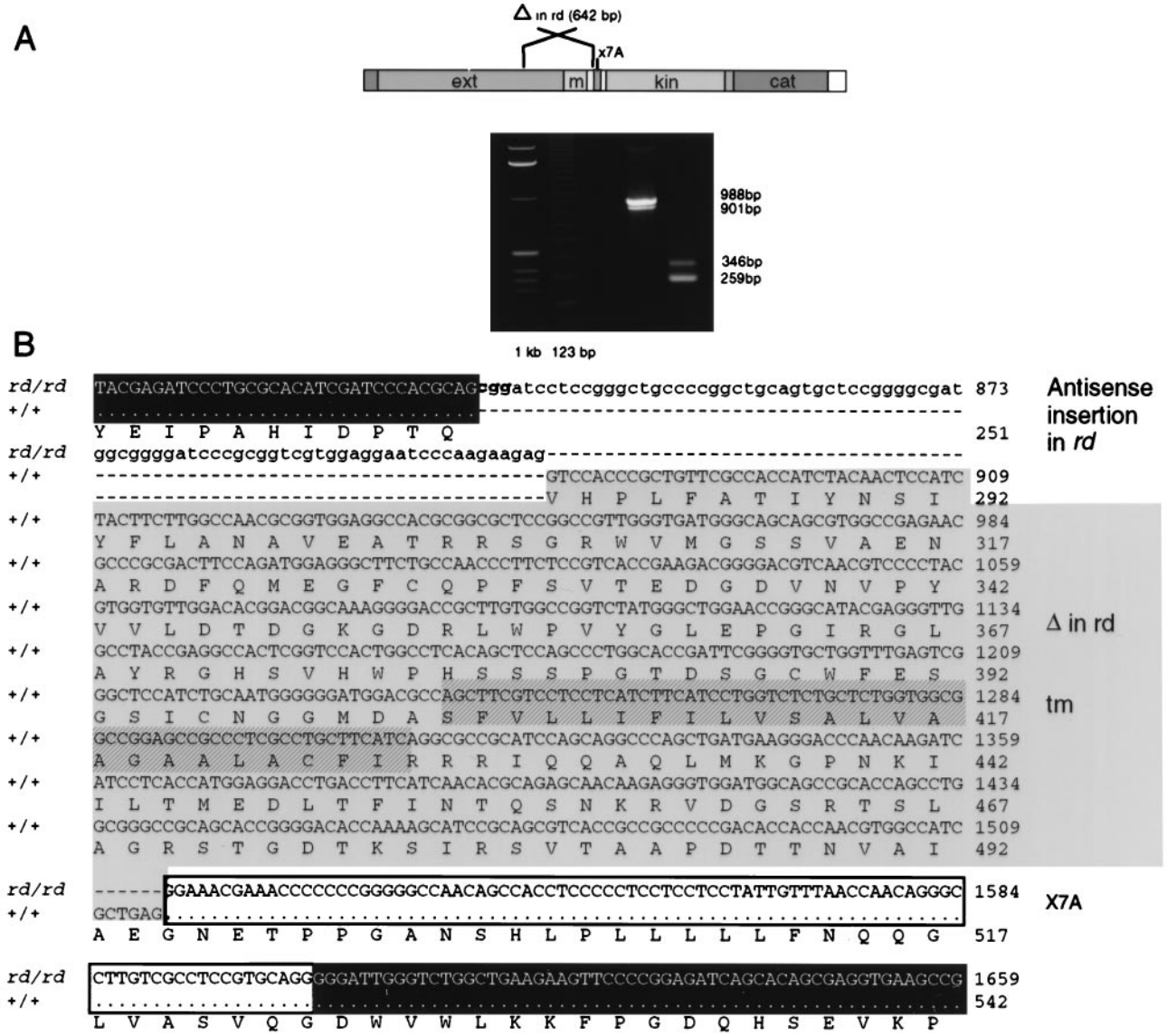

FIG. 5. Identification and analyses of the *rd* mutation. (*A*) RT-PCR analyses of +/+ and *rd*/*rd* total retinal RNA. The larger fragment in both the +/+ and *rd*/*rd* samples contains the alternatively spliced 87-bp nucleotide sequence. (*B*) Comparison of the +/+ and *rd*/*rd* GC1 cDNA sequences at the site of the *rd* mutation. Sequences shown in white letters on black are identical in +/+ and *rd*/*rd*. The 81-bp insert found in the *rd*/*rd* GC1 cDNAs is shown in lowercase letters. In some *rd*/*rd* GC1 clones, the 3 bases at the 5′ end of the fragment (CGG, bold text) were not present. The sequence corresponding to putative exons 4–7 in +/+, which is deleted in *rd*/*rd*, is shaded. The putative transmembrane domain is hatched. The boxed sequence, corresponding to putative exon 7A, is alternatively spliced in both *rd*/*rd* and +/+ GC1 transcripts. Residues identical in *rd*/*rd* and +/+ GC1 sequences are shown as dots; residues missing from either sequence are shown as hyphens.

approximately 110 kDa in normal adult and normal 2- to 3-day-old chicken retina samples (Fig. 2 *A* and *C*). No staining was observed in the *rd*/*rd* samples when using either antibody. Control staining by using the anti-rhodopsin antibody, mAb 4D2, showed no difference in signal intensity between the normal and *rd*/*rd* samples (Fig. 2*B*). These results show that GC1 is undetectable in *rd*/*rd* retina and suggest that the absence of cGMP accumulation in the photoreceptor cells is a result of a GC1 gene defect.

Cloning and Analyses of Normal Chicken GC1. To identify chicken GC1 clones, we screened more than 10^6 pfu of our Uni-ZAP cDNA library with a cDNA probe, but identified only a single clone, 35–6A (Fig. 3*A*). The insert of this clone encodes the catalytic domain of GC1 and a portion of the 3′ untranslated region. The paucity of GC1 clones in this library is most likely a result of the large size of the GC1 transcript (9.5 kb, Fig. 4) and use of oligo(dT) primers during library construction. The remaining portion of the ORF (minus the leader peptide and amino acids 1–4) was cloned by using 5′ RACE PCR and RT-PCR with degenerate primers. Comparisons of the predicted chicken GC1 amino acid sequence with human (3) (see also accession no. M92432), bovine (4), rat (6), and mouse (30) GC1 sequences revealed that chicken GC1 is 62% identical to mammalian GC1, and that the domain structures are identical. In approximately half of the chicken GC1 cDNAs, we found a unique 87-bp nucleotide sequence encoding 29 aa located between the membrane and putative kinase domains (Fig. 3*B*). Using RT-PCR, we were unable to identify a similar splice variant in rat retina, suggesting that this variation may be unique for chicken or avian species (data not shown).

The Amount and Size of the GC1 Transcript in *rd*/*rd* Retina Is Abnormal. Northern blots show that the GC1 transcript in normal chicken retina is approximately 9.5 kb in size (Fig. 4). Comparisons of the +/+ GC1 transcript to that found in *rd*/*rd* retina revealed that the *rd*/*rd* transcript is 0.5–1.0 kb smaller

than that observed in normal retina and that the amount of GC1 transcript in the predegenerate *rd/rd* retina is less than 10% of normal levels. Extended electrophoresis of *+/rd* samples revealed that the retinas of these animals contain both the normal 9.5-kb GC1 transcript and the shorter *rd/rd* mutant GC1 transcript (data not shown). The reduced amount and the size of the *rd/rd* GC1 transcript is consistent with a deletion in the GC1 gene.

The *rd/rd* Chicken GC1 cDNA Has a Deletion/Insertion Consistent with a Gene Rearrangement. *rd/rd* GC1 was cloned by using RT-PCR and primers specific for the normal chicken GC1 cDNA sequence. Amplification of normal chicken retina cDNA by using primers located within the kinase and extracellular domains of GC1 produced two fragments, 988 bp and 901 bp in size, the larger fragment containing the 87-bp insertion described above (termed X7A, Figs. 3*B* and 5*A*). Amplification of *rd/rd* retinal cDNA also produced two products, 346 bp and 259 bp, the sizes of which indicated the presence of a deletion (Fig. 5*A*). Sequence analyses of the *rd/rd* GC1 cDNA show that sequences corresponding to putative exons 4–7 were replaced by an 81-bp fragment with 89% sequence identity to a portion of putative exon 9 (21, 30) in reverse orientation (Fig. 5*B*). The deletion/insertion in *rd/rd* GC1 does not disrupt the reading frame of the transcript. The mutant GC1 is predicted to lack the membrane-spanning domain and the regions immediately flanking it, a region essential for proper folding and enzyme activity. Deletion of the transmembrane domain of recombinant human GC1 has been reported to completely inactivate the enzyme (31). The antibodies used in the Western blot (Fig. 2) recognize epitopes outside the deletion. Because no GC1 of the predicted size can be detected on Western blots of predegenerate *rd/rd* retina (Fig. 2), the mutant enzyme is likely to be unstable and rapidly degraded. Thus, the *rd* GC1 gene does not produce a functional enzyme and therefore is a null allele.

Southern Blot Analyses Confirm the Presence of a Deletion in the *rd/rd GC1 Gene*. Southern analyses (Fig. 6*A*) of normal and *rd/rd* genomic DNA were carried out to obtain an estimate of the size of the deletion within the *rd/rd* GC1 gene. The results revealed several restriction fragment-length polymorphisms, the sizes of which suggest that the deletion in the *rd/rd* GC1 gene is approximately 22 kb in size (Fig. 6*A*). These data are consistent with the deletion detected in our analyses of the *rd/rd* GC1 mRNA transcripts and cDNAs. The chicken GC1 gene structure[¶] has not been determined, but it is likely to be identical to that of mammalian GC1 genes (30) in the region of the deletion because the 5′ and 3′ borders of the deletion exactly match the borders of exons 4 and 7, respectively. A working model for the *rd* GC1 gene defect is shown in Fig. 6*B*.

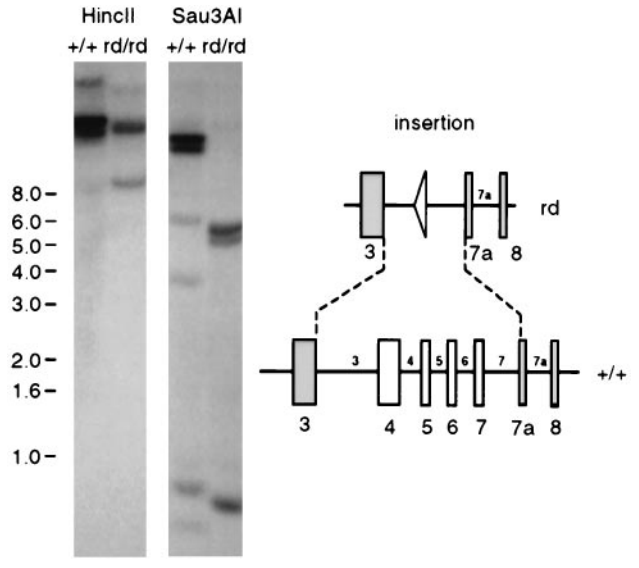

FIG. 6. Southern blot analyses. (*A*) *Hinc*II or *Sau*3AI DNA fragments were probed with a 988-bp chicken GC1 fragment (Fig. 4). Size markers are shown on the left. (*B*) Model of the *rd* gene defect based on the sequences of *rd/rd* cDNA clones. The splice variant found in both normal and *rd/rd* chicken GC1 transcripts is represented as a unique putative exon (X7A). The 81-bp sequence insert replacing exons 4–7 in the *rd/rd* transcripts is indicated by an arrowhead.

DISCUSSION

Our results show that absence of phototransduction in both rods and cones in the predegenerate *rd* chicken retina is a result of insufficient levels of cGMP. Using a combination of techniques, we have obtained evidence that the molecular basis for the observed cGMP phenotype is a null mutation in the gene encoding photoreceptor GC1, an enzyme involved in the synthesis of cGMP. Surprisingly, development of rod and cone photoreceptors is normal in the *rd* retina; they are indistinguishable from those found in normal age-matched retinas but fail to respond to light. Degeneration of the rods and cones begins approximately 7 days after hatching and is nearly complete by 6 months of age. The sequence of events leading to photoreceptor cell death in this mutant is not known. Based on our current understanding of phototransduction, we propose that the low levels of cGMP would lead to the permanent closure of the cGMP-gated cation channels located in the photoreceptor cell plasma membrane and elimination of the dark current. The photoreceptors would remain "chronically hyperpolarized," a condition mirroring that found in retinas exposed to constant light stimulation. Our hypothesis is supported by the observation that levels of glutamate, the neurotransmitter released by depolarized photoreceptor cells in the dark, are chronically elevated in *rd/rd* photoreceptors regardless of the light-adapted state of the retina (32). The constant or "equivalent light" hypothesis for a mechanism leading to photoreceptor degeneration (33) is consistent with our results.

Our results support the conclusion that phototransduction in both rod and cone cells is dependent on GC1. This view is also supported by recent studies of patients diagnosed with LCA. In this disease, rod and cone photoreceptor function is either absent or severely compromised at birth as evidenced by extinguished or barely detectable photopic and scotopic electroretinograms (34). In the early stages of the disease, the fundi of LCA patients frequently appear normal, suggesting that blindness is not caused by gross abnormalities in retinal development. Recently, LCA has been shown in some families to be linked to frameshift mutations in the GC1 gene located on 17p (21). Thus, LCA is an autosomal recessive retinal disease whose genetic and phenotypic attributes bear a striking resemblance to those found in the *rd* chicken. These two GC1 disease models represent naturally occurring GC1 gene knockouts, and, in both cases, the absence of GC1 abolishes phototransduction.

Examination of the *rd* chicken phenotype leads to several conclusions concerning the consequences of the absence of GC1 on photoreceptor development and function. First, cGMP levels too low to support phototransduction do not affect the development of photoreceptor cells. Photoreceptors in predegenerate *rd/rd* retina appear normal at the ultrastructural level (14), and the expression of several of the proteins

[¶]To identify the precise gene defect, we have made many unsuccessful attempts to clone the normal and *rd* GC1 gene, and to amplify genomic fragments in the area of the putative deletion. Genomic λ and cosmid libraries available to us do not contain GC1 clones, and none of the predicted 19 introns of the GC1 gene could be amplified by using exon-specific primers and a variety of *Taq* polymerases.

involved in the phototransduction cascade is normal (17, 18). This is in contrast to the situation found in *rd* mouse and *rcd1* Irish setter, two animal models of recessive retinitis pigmentosa, in which high levels of cGMP are associated with arrested photoreceptor development (35, 36).

Second, the inability of both the rods and cones to transduce light stimuli and their simultaneous degeneration is consistent with expression of the GC1 gene in both cell types. The presence of two GC1 splice variants in chicken retina that are present in nearly equal amounts raises the possibility that the GC1 gene may be spliced in a cell-dependent manner, one variant being present predominantly in rods, the other in cones. In contrast to mammalian retinas, which are rod-dominant, approximately 80% of the photoreceptor cells in the chicken retina are cones. Splice variants of the GC1 gene have not been identified in mammalian retinas, many of which contain only 2–5% cones.

Third, only GC1 contributes to the pool of cGMP essential to support phototransduction in rod and cone outer segments. RetGC-2, a second photoreceptor GC that has been localized to photoreceptor outer segments (8), is unable to compensate for the loss of GC1 in the *rd* chicken and LCA. Thus, either retGC-2 and GC1 are not present in the same subcellular compartment, or retGC-2's rate of cGMP synthesis is too low to replenish cGMP in the absence of GC1.

Fourth, levels of GCAP1 are down-regulated in the absence of GC1. In a recent study of GCAP1 and GCAP2 in the *rd* chicken retina, we found that GCAP1 levels in predegenerate *rd*/*rd* retinas are reduced by more than 90% relative to those found in age-matched control retinas, whereas levels of GCAP2 remain unchanged (20). Because transcript levels of both GCAP genes, which are arranged in a tail-to-tail array (S.S.R. and W.B., unpublished results), are near normal in the predegenerate retina, a regulatory mechanism at the gene level may be excluded. Recently, we have shown that GCAP1 is more susceptible to proteolytic degradation in the absence of calcium (37). Thus, the apparent down-regulation of GCAP1 in *rd* photoreceptors may be caused by permanent closure of the cGMP-gated channels that leads to a reduction in cytoplasmic levels of free calcium.

In summary, the *rd* chicken model of inherited retinal disease provides new insight into rod and cone photoreceptor transduction mechanisms. As a model for human LCA, the *rd* chicken allows detailed biochemical and physiological studies on the etiology of the disease. Moreover, it is now possible to begin to examine the feasibility of using viral expression vectors to restore synthesis of cGMP and phototransduction in *rd* retinas.

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1. Koutalos, Y. & Yau, K.-W. (1996) *Trends Neurosci.* **19,** 73–81.
2. Polans, A., Baehr, W. & Palczewski, K. (1996) *Trends Neurosci.* **19,** 547–554.
3. Shyjan, A. W., de Sauvage, F. J., Gillett, N. A., Goeddel, D. V. & Lowe, D. G. (1992) *Neuron* **9,** 727–737.
4. Goraczniak, R. M., Duda, T., Sitaramayya, A. & Sharma, K. (1994) *Biochem. J.* **302,** 455–461.
5. Lowe, D. G., Dizhoor, A. M., Liu, K., Gu, Q., Spencer, M., Laura, R., Lu, L. & Hurley, J. B. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5535–5539.
6. Yang, R.-B., Foster, D. C., Garbers, D. L. & Fülle, H.-J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 602–606.
7. Otto-Bruc, A., Fariss, R. N., Haeseleer, F., Huang, J., Buczylko, J., Surgucheva, I., Baehr, W., Milam, A. H. & Palczewski, K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4727–4732.
8. Yang, R.-B. & Garbers, D. L. (1997) *J. Biol. Chem.* **272,** 13738–13742.
9. Farber, D. B. & Lolley R. N. (1976) *J. Cyclic Nucleotide Res.* **2,** 139–148.
10. Aguirre, G., Farber, D., Lolley, R., Fletcher, R. T. & Chader, G. J. (1978) *Science* **201,** 1133–1134.
11. Ulshafer, R. J., Garcia, C. A. & Hollyfield, J. G. (1980) *Invest. Ophthalmol. Vis. Sci.* **19,** 1236–1241.
12. Cheng, K. M., Shoffner, R. N., Gelatt, K. N., Gum, G. G., Otis, J. S. & Bitgood, J. J. (1980) *Poultry Sci.* **59,** 2179–2182.
13. Ulshafer, R. J., Allen, C., Dawson, W. W. & Wolf, E. D. (1984) *Exp. Eye Res.* **39,** 125–135.
14. Ulshafer, R. J. & Allen, C. (1985) *Exp. Eye Res.* **40,** 865–877.
15. Ulshafer, R. J. & Allen, C. (1985) *Curr. Eye Res.* **4,** 1009–1021.
16. Dawson, W. W., Ulshafer, R. J., Parmer, R. & Lee, N. R. (1990) *Clin. Vision. Sci.* **3,** 285–292.
17. Semple-Rowland, S. L. & van der Wel, H. (1992) *Biochem. Biophys. Res. Commun.* **183,** 456–461.
18. Semple-Rowland, S. L. & Green, D. A. (1994) *Invest. Ophthalmol. Vis. Sci.* **35,** 2550–2557.
19. Semple-Rowland, S. L. & Green, D. A. (1994) *Exp. Eye Res.* **59,** 365–372.
20. Semple-Rowland, S. L., Gorczyca, W. A., Buczylko, J., Helekar, B. S., Ruiz, C. C., Subbaraya, I., Palczewski, K. & Baehr, W. (1996) *FEBS Lett.* **385,** 47–52.
21. Perrault, I., Rozet, J. M., Calvas, P., Gerber, S., Camuzat, A., Dollfus, H., Chatelin, S., Souied, E., Ghazi, I., Leowski, C., Bonnemaison, M., Le Paslier, D., Frezal, J., Dufier, J. L., Pittler, S., Munnich, A. & Kaplan, J. (1996) *Nat. Genet.* **14,** 461–464.
22. Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis* (Academic, New York), pp. 221–260.
23. Lee, N. (1991) Ph.D. thesis (University of Florida, Gainesville, FL).
24. Palczewski, K. & Smith, W. C. (1996) *Exp. Eye Res.* **63,** 599–602.
25. Surguchov, A., Bronson, J. D., Banerjee, P., Knowles, J. A., Ruiz, C. C., Subbaraya, I., Palczewski, K. & Baehr, W. (1997) *Genomics* **39,** 312–322.
26. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) *Cell* **20,** 95–105.
27. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
28. Thompson, C. B. & Neiman, P. E. (1987) *Cell* **48,** 369–378.
29. Meller, K. & Tetzlaff, W. (1976) *Cell Tissue Res.* **170,** 145–159.
30. Yang, R.-B., Fülle, H.-J. & Garbers, D. L. (1996) *Genomics* **31,** 367–372.
31. Laura, R. P., Dizhoor, A. M. & Hurley, J. B. (1996) *J. Biol. Chem.* **271,** 11646–11651.
32. Ulshafer, R. J., Sherry, D. M., Dawson, R., Jr., & Wallace, D. R. (1990) *Brain Res.* **531,** 350–354.
33. Fain, G. L. & Lisman, J. E. (1993) *Exp. Eye Res.* **57,** 335–340.
34. Franceschetti, A. & Dieterle, P. (1958) *Conf. Neurol.* **14,** 184–186.
35. Farber, D. B. & Lolley, R. N. (1974) *Science* **186,** 449–451.
36. Aguirre, G., Farber, D., Lolley, R., Fletcher, R. T. & Chader, G. J. (1978) *Science* **201,** 1133–1134.
37. Rudnicka-Nawrot, M., Surgucheva, I., Holmes, J. D., Haeseleer, F., Sokal, I., Crabb, J. W., Baehr, W. & Palczewski, K. (1998) *Biochemistry* **37,** 248–257.