



Characterization of the Chicken GCAP Gene Array and Analyses of GCAP1, GCAP2, and GC1 Gene Expression in Normal and *rd* Chicken Pineal

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Purpose: This study had three objectives: (1) to characterize the structures of the chicken GCAP1 and GCAP2 genes; (2) to determine if GCAP1, GCAP2, and GC1 genes are expressed in chicken pineal gland; (3) if GC1 is expressed in chicken pineal, to determine if the GC1 null mutation carried by the retinal degeneration (*rd*) chicken is associated with degenerative changes within the pineal glands of these animals.

Methods: GCAP1 and GCAP2 gene structures were determined by analyses of chicken cosmid and cDNA clones. The putative transcription start points for these genes were determined using 5'-RACE. GCAP1, GCAP2 and GC1 transcripts were analyzed using Northern blot and RT-PCR. Routine light microscopy was used to examine pineal morphology.

Results: Chicken GCAP1 and GCAP2 genes are arranged in a tail-to-tail array. Each protein is encoded by 4 exons that are interrupted by 3 introns of variable length, the positions of which are identical within each gene. The putative transcription start points for GCAP1 and GCAP2 are 314 and 243 bases upstream of the translation start codons of these genes, respectively. As in retina, GCAP1, GCAP2 and GC1 genes are expressed in the chicken pineal. Although the GC1 null mutation is present in both the retina and pineal of the *rd* chicken, only the retina appears to undergo degeneration.

Conclusions: The identical arrangement of chicken, human, and mouse GCAP1/2 genes suggests that these genes originated from an ancient gene duplication/inversion event that occurred during evolution prior to vertebrate diversification. The expression of GC1, GCAP1, and GCAP2 in chicken pineal is consistent with the hypothesis that chicken pineal contains a functional phototransduction cascade. The absence of cellular degeneration in the *rd* pineal gland suggests that GC1 is not critical for pineal cell survival.

The pineal glands of several lower vertebrates, including birds [1-4], fish [5,6], and reptiles [7], have been shown to be directly responsive to light stimulation. The light transduction mechanism in the pineal glands of these species is not known; however, immunocytochemical, biochemical, and physiological data suggest that pineal photoreception in lower vertebrates may involve a transduction cascade similar to that found in retinal photoreceptors [8].

In rod and cone photoreceptors, calcium and cGMP are internal transmitters that are essential for phototransduction and its regulation [9]. After photobleaching, and as a result of activation of the cascade, cGMP levels drop and cGMP-gated cation channels close. Closure of these channels reduces the cationic dark current and intracellular calcium levels drop from ~700 nM to less than 100 nM due to continued expulsion of calcium from the cell by light-insensitive Na⁺ / Ca²⁺-K⁺ exchanger. In the presence of low intracellular calcium, Ca²⁺-binding proteins, termed GCAPs, stimulate production of cGMP through interactions with photoreceptor guanylate cyclase (GC), a single subunit member of the particulate guanylate cyclase family. As cGMP levels increase, the cGMP-gated channels reopen and the dark current is reinstated. Thus,

the GCAP/GC regulatory system plays a key role in the recovery of the rod and cone photoreceptors in the retina following light stimulation.

Three GCAPs (GCAP1-3) [10-13] and one GCAP-like protein (GCIP) [14] have been characterized in the vertebrate retina. The diversity in calcium binding proteins in retina is matched by the presence of at least 3 particulate guanylate cyclases in this tissue [15-18], two of which are present in photoreceptors [19]. In chicken retina, two GCAPs (GCAP1 and 2) [20] and one particulate cyclase (GC1) [21] have been identified. Recent analyses of the *retinal degeneration (rd)* chicken confirm the importance of GC1 in maintaining the normal functioning of retinal photoreceptors. A re-arrangement in the GC1 gene that results in a null allele is postulated to underlie the absence of function in the photoreceptors of this retina at hatch, and the eventual degeneration of the photoreceptor cells [21].

If chicken pineal photoreception involves a transduction cascade similar to that observed in retina, then both GCAP and GC1 should be expressed in this tissue. Our analyses establish that GCAP1, GCAP2, and GC1 are expressed in normal chicken pineal, and that the *rd* pineal gland does not express functional GC1. No evidence of pineal degeneration was observed in 3-month-old *rd* chickens suggesting that, unlike the situation in retinal photoreceptor cells, the absence of GC1 in the pineal gland is not detrimental to pineal cell survival.

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METHODS

Isolation and Analyses of Genomic Clones: Random primer-labeled GCAP1 and GCAP2 cDNA probes were used to screen a chicken pWE15 cosmid library (Clontech, Palo Alto, CA). Colony filters were hybridized overnight at 42 °C in a solution containing 50% formamide, 5X SSC, 1X Denhardt's, and 0.2 mg/ml salmon sperm DNA. Filters were washed 3 times for 30 min each at 60 °C in a solution containing 0.1X SSC and 0.1% SDS. Several cosmid clones were isolated; one of these clones was selected for further analyses. The structure of the GCAP gene array was determined by sequencing restriction and PCR-generated subclones of the cosmid clone. All DNA sequencing was done using a Li-Cor Model 4000L automatic DNA sequencer (Li-Cor, Lincoln, NE) and Excel DNA polymerase (Epicentre, Madison, WI) for linear PCR amplification.

Northern Blot Analyses: Total RNA was isolated from 1 to 3 day old normal and *rd/rd* chicken retina-pigment epithelium-choroid and pineal using a RNeasy total RNA kit (Qiagen, Valencia, CA). Care and handling of animals was conducted using procedures approved by the University of Florida Institutional Animal Care and Use Committee in accordance with guidelines published by the Institute for Laboratory Animal Research. Samples, each containing 10 µg of RNA, were electrophoresed in a 1.1% formaldehyde gel. Blots were prepared as previously described [22] and were probed sequentially using random-primed ³²P-labeled cDNA probes for chicken GC1, GCAP1, GCAP2 and 18S rRNA. Blots were exposed to Kodak BioMax film as follows: GC1, GCAP1, GCAP2 24 h at -70 °C; 18S rRNA 20 min at room temperature.

RT-PCR: Total RNA (0.5 µg for normal retina and pineal; 1.0 µg for *rd/rd* retina and pineal) was reverse-transcribed and amplified with primers specific for GC1 (178, 5-CCTTCC

CCC TGC CCT ACC AC; 156, 5-CTT GCA GAA GGC CAG CTT GG), GCAP1 (216, 5-CCA GTT TTG GCT GCA GAG TGA C; 215, 5-TCA CAG CCC ATT TCG TGT CAG), and GCAP2 (164, 5-TCA GAT AGA GGC GTG GAA CA; 59, 5-GAG CCA CAG CCA CAG TCT). RT-PCR was carried out using a GeneAmp RNA PCR kit (Perkin Elmer, Norwalk, CT) and the following cycle parameters: 95 °C for 2 min; 95 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min (35 cycles); 72 °C for 10 min; 4 °C soak. For each RT-PCR analyses, appropriate control reactions were run. The RT-PCR controls included reactions in which the reverse transcription step was omitted, reactions in which only one of the primers was included in the PCR reaction, and RT-PCR in the absence of template. To control for the presence of trace amount of genomic DNA in the samples, all primers were designed so that PCR products generated from genomic DNA would include intron sequences. All amplified products were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA) and sequenced to confirm their identities.

5'-RACE: The 5'-RACE protocol used to analyze both GCAP1 and GCAP2 was identical with the exception of the sequence-specific primers. Total RNA (1 µg extracted from normal chicken retina) was reverse transcribed using *rTth* DNA polymerase (Perkin Elmer). The final 20 µl reaction contained 1X *rTth* reverse transcriptase buffer, 1 mM MnCl₂, 5 U *rTth* DNA polymerase, 200 µM each dNTP, and 1 µM GCAP1 (5'-GAC GGG CTC AGG TTT TTC AAG) or GCAP2 (5'-TTT CCC CGT AAA ACA AGA TTC A) sequence-specific, antisense primer. The reaction was incubated at 65 °C for 15 min and was stopped by placing the tube on ice. Excess primer, dNTPs and buffer were removed from the reaction using a QIAquick PCR Purification kit (Qiagen) according to the recommended protocol. In the final step of the procedure, the

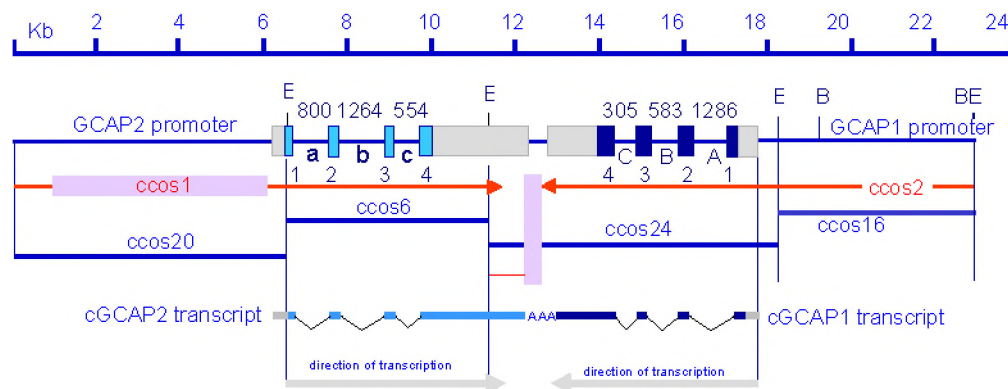


Figure 1. Physical map of the chicken GCAP gene array. The cosmid clone containing the GCAP2/GCAP1 gene array contained a 23 kb insert, which was subcloned into four *Eco* RI fragments (ccos 20, 6, 24, 16). GCAP2 and GCAP1 are each encoded by four exons that are depicted as boxes: the coding portions for GCAP2 are light blue and those for GCAP1 are dark blue. Non-coding regions of the exons are light gray for both GCAP2 and GCAP1. Introns and flanking sequences are depicted as lines. The GCAP2 and GCAP1 genes each contain 3 introns, the sizes of which are shown in bp above the lines indicating the relative positions of the introns. Each intron has been given a letter designation (GCAP2, a-c; GCAP1, A-C) which corresponds to those shown in Figures 2 and 3. Regions of the cosmid clone that were not sequenced are indicated by the light purple boxes. The direction of transcription is indicated for GCAP2 and GCAP1 genes with gray arrows.

DNA/RNA was eluted from the column using 30 µl of 10 mM Tris-HCl, pH 8.5. A poly dATP tail was added to the single-stranded cDNA present in the sample using terminal deoxynucleotidyl transferase (Promega, Madison, WI). The 50 µl reaction mixture contained 30 µl of DNA/RNA, 1X terminal transferase reaction buffer, 200 µM dATP, and 25-50 U terminal transferase. The mixture was incubated at 37 °C for 10 min and the reaction was stopped by heating at 70 °C for 10 min. Excess dATP and buffer were removed from the reac-

tion as described above. Second-strand cDNA synthesis was carried out using AmpliTaq DNA polymerase (Perkin Elmer) and a poly d(T) anchor primer (5'-GCG GTA CCT CGA GAA TTC TTT TTT TTT TTT TTT). The final 100 µl reaction contained 30 µl of tailed cDNA, 5 U AmpliTaq, 2 mM MgCl₂, 200 µM each dNTP, 0.2 µM anchor primer, 1 X PCR buffer. The reaction was incubated at 40 °C for 5 min in a Perkin Elmer DNA Thermal Cycler. Following the 5 min incubation, the temperature of the sample was ramped to 72 °C and held at 72 °C for 2 min. The sample temperature was then increased to 80 °C and held at this temperature while the sequence-specific primer used in the RT step and a nested anchor primer (5'-GCG GTA CCT CGA GAA TTC TT) were added to the reaction (final concentration of these primers was 0.2 µM). The GCAP cDNA fragments present in the sample were then amplified using the following cycle parameters: 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min (30 cycles); 72 °C for 10 min; 4 °C soak. To complete the 5'-RACE, 1 µl of a 1:10 dilution of the PCR product was re-amplified using the nested anchor primer and a nested sequence-specific primer for GCAP1 (5'-GGG CAC TCC GTC ATG AAC TTC) or GCAP2 (5'-GGT TAT CCT GGA CGC CGA AGA A). The PCR cycle parameters were as follows: 94 °C for 1 min; 94 °C for 1 min, 65 °C for 1 min, 72 °C for 2 min (35 cycles); 72 °C for 10 min; 4 °C soak. The resulting product was run on an agarose gel, purified, and cloned into the pCR2.1 TOPO cloning vector (Invitrogen). Resulting clones were sequenced as described above.

Light Microscopy: The pineal glands of 3 to 4 day old and 79 day old normal and *rd/rd* chickens were fixed for several days in 4% paraformaldehyde in phosphate-buffered saline at 4 °C. The tissue was embedded in paraffin and 10 µm thick sections were processed and stained with cresyl violet. Stained sections were examined and photographed using a Zeiss Axioplan microscope.

RESULTS

Chicken GCAP1 and GCAP2 Gene Structures: Human [23] and mouse [24] GCAP1 and GCAP2 genes are arranged in a tail-to-tail array in which the regulatory sequences governing expression of the genes are located on opposite ends of the array. As a consequence of this arrangement, transcription of

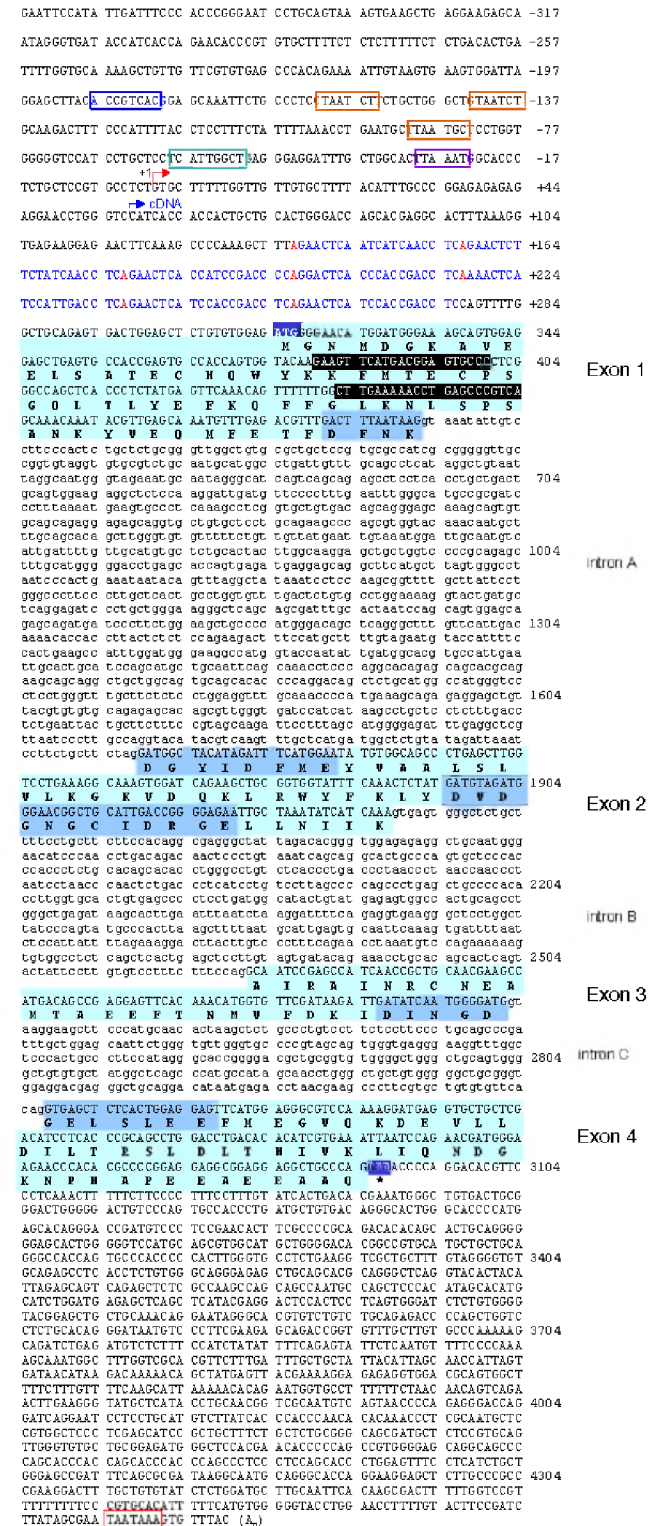


Figure 2. Sequence of the chicken GCAP1 gene. GCAP1 is encoded by 4 exons (shaded in light blue). Intron sequences are shown in lower case. The residues corresponding to the predicted EF hand Ca²⁺-binding domains are shaded in blue. The translation start (ATG) and stop (TAA) codons are shown in white on navy blue. A possible polyadenylation signal for GCAP1 is boxed in the 3'-UTR. The primers used in the 5'-RACE experiment are indicated in white on black backgrounds. A blue arrow labeled cDNA in the 5'-UTR indicates the 5' extent of the GCAP1 cDNA clones analyzed previously [20]. The blue text interrupted by red residues shows a repeated sequence that is present in the 5'-UTR. The positions of the putative tsp determined by 5'-RACE is indicated with a red arrow. A putative TATA box (+ strand, purple box), putative CAAT box (- strand, green box), Crx-like elements (+ strand, orange boxes), and a CREB/ATF-like element (- strand, blue box) are indicated in the proximal promoter region. The GCAP1 gene sequence is in GenBank as Accession number AF172707.

the GCAP genes proceeds along opposite strands of the DNA. Characterization of a single chicken cosmid clone encoding GCAP1 and GCAP2 revealed an identical gene arrangement in this species (Figure 1). The chicken GCAP array is contained in less than 11 kb of genomic DNA compared to the

human and mouse GCAP genes which each span more than 16 kb of DNA. The smaller size of the chicken array is due to smaller introns and a shorter intergenic region, which in chicken is less than 1 kb (human 4.5 kb). Existence of the GCAP gene array in chicken, mouse and human suggests that these genes originated from an ancient gene duplication/inversion event preceding vertebrate diversification.

As in mammals, the structures of the chicken GCAP1 (Figure 2) and GCAP2 (Figure 3) genes are identical to each other. In addition, the positions of the intron/exon splice junctions in both genes are conserved between chicken, mouse, and human. The GCAP1 and GCAP2 proteins are each encoded by 4 exons. In both genes, the first EF-hand Ca²⁺-binding domain is interrupted by the first intron, the second EF-hand domain is encoded by exon 2, and the last EF-hand domain is interrupted by the third intron.

Proximal Promoters of the GCAP1 and GCAP2 Genes:

We have tentatively assigned the transcription start point (tsp) of the GCAP1 gene to a position 314 bp upstream of the translation start point (ATG) by 5'-RACE. The sequences of all of the GCAP1 RACE clones analyzed (19 clones) matched the gene sequence of the cosmid clone, indicating that the 5' UTR is contiguous and that there are no introns in this region (Figure 2). Analysis of the GCAP2 RACE clones (11 clones) indicated that the putative tsp of the GCAP2 gene is located 243 bp upstream of the ATG (Figure 3). Both tsps are embedded within consensus cap signals (KCWBHYBY) [25] that are flanked at their 3'-ends with pyrimidine-rich sequences. Neither gene possesses a canonical TATA box; however, AT-rich sequences resembling TATA boxes are present at -29 (TTAAAT; GCAP1) and -20 (TATTATA; GCAP2) upstream of the predicted tsps. A putative CCAAT-box element is located on the antisense strand of GCAP1 at -50/-58 (AGCCAATGA) and on the sense strand of GCAP2 at -83/-75 (AGCCAAGAA). The locations of these putative RNA polymerase II promoter elements are consistent with the positions of the tsp sites identified using 5'-RACE.

In addition to the general eukaryotic promoter elements, we also searched the proximal promoter region of each gene for known consensus transcription factor binding sites using MatInspector at http://genomatix.gsf.de/cgi-bin/matinspector/matinspector.pl [26]. Both proximal promoters contain putative Crx sites (consensus C/TTAATCC) [27]. Three Crx-like

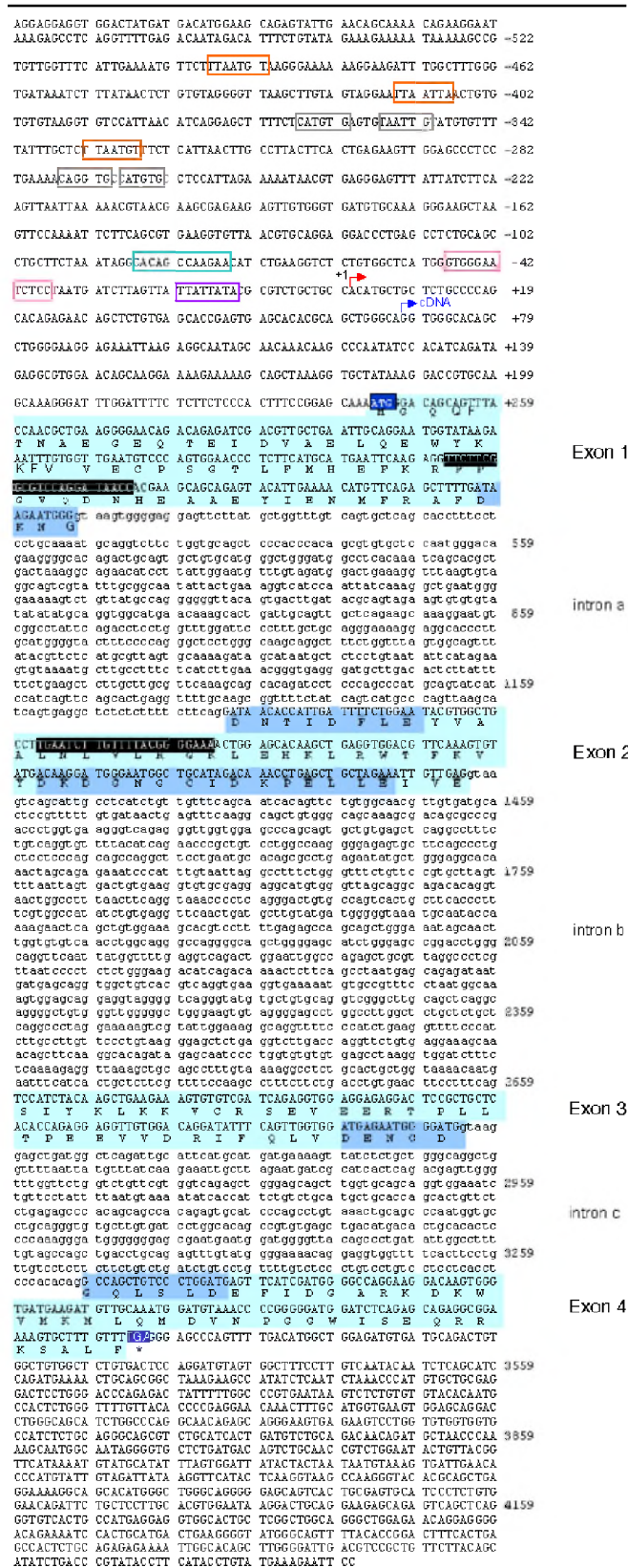


Figure 3. Sequence of the chicken GCAP2 gene. GCAP2 is encoded by 4 exons (shaded in light blue). Intron sequences are shown in lower case. The residues corresponding to the predicted EF hand Ca²⁺-binding domains are shaded in blue. The translation start (ATG) and stop (TAA) codons are shown in white on navy blue. The primers used in the 5'-RACE experiment are indicated in white on black backgrounds. A blue arrow labeled cDNA in the 5'-UTR indicates the 5' extent of the GCAP2 cDNA clones analyzed previously [20]. The positions of the putative tsp determined by 5'-RACE is indicated with a red arrow. A putative TATA box (+ strand, purple box), putative CCAAT box (- strand, green box), putative NF-kB site (+ strand pink box), paired E-box elements (+ strand, gray boxes), and Crx-like elements (+ strand, orange boxes) are indicated in the proximal promoter region. The GCAP2 gene sequence is in GenBank as Accession number AF172708.

cis DNA elements were identified within 200 bp upstream of the *tsp* of the GCAP1 gene (-84 to -90; -137 to -143; -154 to -161, see Figure 2) and within 500 bp of the *tsp* of the GCAP2 gene (-326 to -332; -408 to -414; -491 to -497, see Figure 3). Crx is an Otx-like photoreceptor-specific *trans*-acting factor that is expressed in retinal photoreceptors, as well as in pineal gland [27,28]. This factor has been shown to play a critical role in the regulation of photoreceptor development [28] and in the expression of several photoreceptor-specific genes [27]. The presence of Crx-like binding sites in the chicken GCAP1 and GCAP2 promoters suggests that Crx may play a role in regulating the expression of GCAP1 and GCAP2 in retina and pineal.

One of the distinguishing features of the GCAP2 proximal promoter is that it contains two putative paired E-box elements (consensus CANNTG) located at position -263 to -275 and at position -351 to -366 (Figure 3). These elements bind transcription factors belonging to the basic helix-loop-helix (bHLH) family, a family of transcriptional activators and repressors that regulate several key events during neurogenesis and differentiation [29,30]. GCAP1 and GCAP2 proteins exhibit very similar functional characteristics *in vitro* [10-12]; based on the differences noted in the promoters of these genes and the tail-to-tail arrangement within the genome, it seems likely that the specific roles that these proteins play in retina and pineal may be quite different. Formal analyses of the promoters of these genes will be required to positively identify functionally relevant *cis*-elements and to determine how these

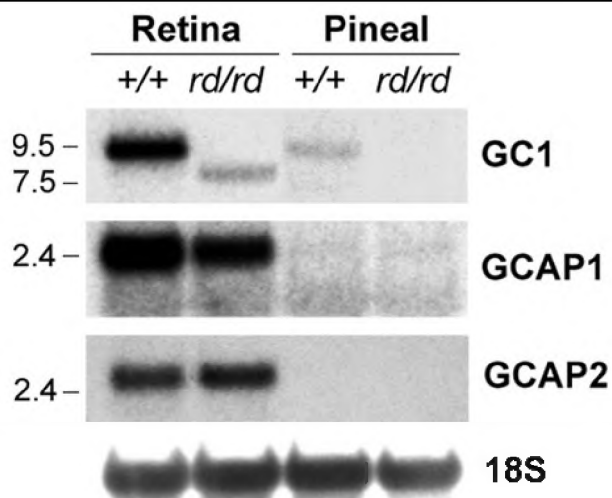


Figure 4. Northern blot analyses of GC1, GCAP1, and GCAP2 transcripts in normal and *rd/rd* retina and pineal. Each of the four lanes shown contains 10 μ g of total RNA that was isolated from the retinal-pigment epithelium-choroid or pineal of 1 to 3 day old normal or *rd/rd* chickens. The blot shown was hybridized sequentially with probes for chicken GC1, GCAP1, GCAP2 and 18S rRNA. The hybridization signals shown for GC1, GCAP1 and GCAP2 were obtained following 24 h exposure of Kodak BioMax film at -70 °C. The signal for 18S rRNA was obtained after a 20 min exposure at room temperature. Longer exposures of the GCAP2 blots (72 h exposure at -70 °C) did not produce detectable GCAP2 signals. The normal GC1 transcript is approximately 9.5 kb in size. The GCAP1 and GCAP2 transcripts are approximately 2.4 and 2.6 kb in size, respectively.

elements influence the temporal and cellular expression patterns of these proteins.

Expression of GCAP1, GCAP2, and GC1 in the chicken pineal: We have previously shown that GC1 and the GC regulatory proteins, GCAP1 and GCAP2, are expressed in normal chicken retina [20,21]. In the present study, we examined total RNA extracted from the pineal glands of normal and *rd/rd* chickens using Northern blot and RT-PCR techniques to determine if GC1, GCAP1, and GCAP2 are also expressed in

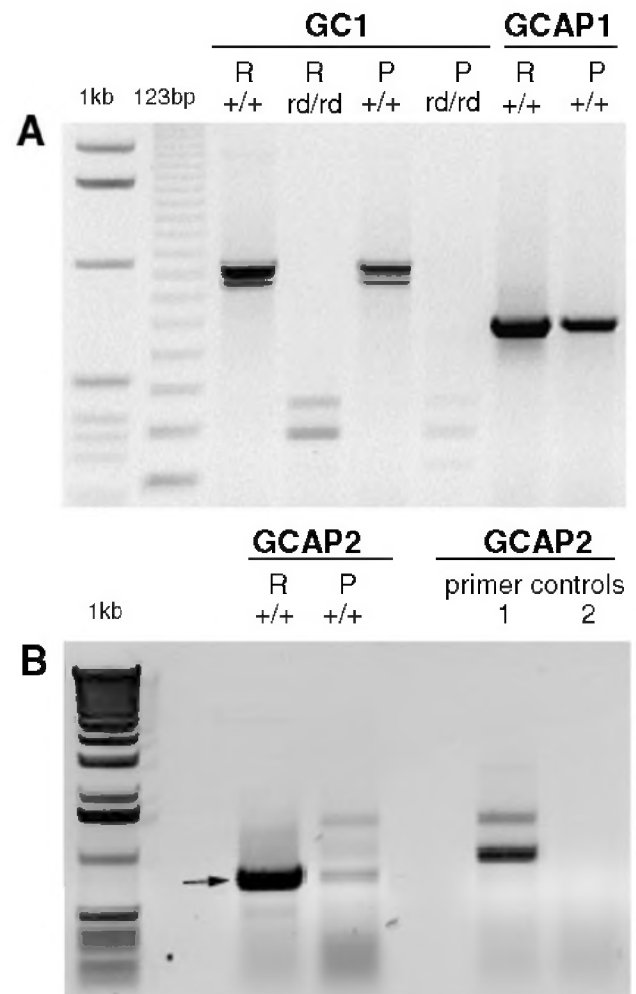


Figure 5. RT-PCR analyses of GC1, GCAP1, and GCAP2. **A.** Analyses of GC1 and GCAP1 in normal and *rd/rd* retina and pineal. Primers used to amplify GC1 flank the deletion previously described in GC1 in the *rd* chicken. Two GC1 products were amplified in both retina and pineal, the larger representing an alternatively spliced form of the GC1 transcript present in chicken [21]. **B.** Analyses of GCAP2 in normal retina and pineal. The arrow indicates the GCAP2 product, the identity of which was verified by sequence analyses. GCAP2 RT-PCR control experiments indicated that the additional products in the pineal sample were due to non-specific priming activity of GCAP2 primer 59 (GCAP2 primer control, lane 1). No products were obtained using GCAP2 primer 164 alone (GCAP2 primer control, lane 2). Each lane is labeled with either an R (retina) or P (pineal) and +/+ (normal chicken) or *rd/rd* (mutant chicken) indicating the source and type of tissue from which the sample was obtained. DNA size ladders (1 kb and 123 bp) are shown in the left most lanes of each gel.

chicken pineal. Using Northern blot methods, GC1 and GCAP1 transcripts were detected in normal pineal, albeit at levels lower than those observed in retina. The sizes of these transcripts match those present in normal retina (Figure 4). The presence of GC1 mRNA in chicken pineal is consistent with previous reports of GC1 transcripts in rat [31] and bovine pineal [32]. A faint GC1 mRNA signal was detected in *rd/rd* pineal when the blot was exposed to film for 48 h (data not shown), the size of which was identical to that present in the retinas of these animals [21]. Comparable levels of GCAP1 mRNA were detected in normal and *rd/rd* pineal. No GCAP2 transcript was detected in either normal or *rd/rd* pineal using this method. Extension of the film exposure period to 72 h did not produce a detectable GCAP2 signal.

RT-PCR was used to confirm the presence of the mutant GC1 transcript in *rd/rd* pineal and to verify the GCAP1 and GCAP2 Northern blot results obtained for normal chicken pineal. The results of the RT-PCR analyses of normal chicken retina and pineal (Figure 5A) confirmed the GC1 Northern blot data and revealed that the alternatively spliced GC1 transcripts that are present in chicken retina [21] are also present in the pineal gland. As expected, the GC1 transcripts in *rd/rd* pineal possessed the same deletion that we previously characterized in *rd/rd* retina [21]. RT-PCR analyses confirmed the GCAP1 Northern data (Figure 5A) and also revealed that GCAP2 transcripts are present in pineal, albeit at very low

levels (Figure 5B). All RT-PCR control reactions were negative except for the single primer PCR control that was run for GCAP2 primer number 59. The results of the single primer control reactions for GCAP2 are shown in Figure 5B.

Morphology of normal and rd/rd pineal glands: The presence of the GC1 null mutation in the *rd* chicken results in degeneration of the retinal photoreceptor cells, a process that begins approximately 10 days after the birds hatch and is nearly complete in 8-month-old birds [33,34]. In the present study, we were interested in determining if cellular degeneration occurs in pineal as a result of expression of the mutant GC1 gene in this tissue. The pineal glands of 3-days-old *rd/rd* chickens (Figure 6B) were found to be histologically indistinguishable from those of normal chickens at this age (Figure 6A). In both animals, the glands are comprised of several clearly defined follicles, each possessing a large lumen surrounded by numerous cells. In the older, 79 day old chickens, there was a notable decrease in the density of cells and in the size of the follicular lumens in both normal and *rd/rd* pineal glands (Figure 6C,D). These changes are consistent with previous descriptions of the development of chicken pineal morphology [35]. Electron microscopic analyses of chicken pineal have shown that photoreceptor-like cells possessing whorls of lamellar membrane are among the three cell types (ependymal, secretory, and sensory) that border the lumen of each follicle [36]. Although we were unable to distinguish among these three cell types using light microscopy, the absence of cellular degeneration in *rd/rd* pineal suggests that the GC1 null mutation in *rd* pineal does not have a significant impact survival of cells in this gland.

DISCUSSION

Our finding that GCAP1 and GCAP2 transcripts are present in chicken pineal is the first evidence that these proteins are expressed in pineal. In retinal photoreceptor cells, GC1 activity is modulated by changes in intracellular $[Ca^{2+}]$ that are largely the consequence of closure of the cGMP-gated cation channels located in the plasma membrane of these cells. Light-induced decreases in intracellular $[Ca^{2+}]$ result in activation of GC1 through interactions with GCAP. The presence of GC1, GCAP1, and GCAP2 in chicken pineal suggests that GC1 activity in pineal may be regulated in a manner similar to that found in retinal photoreceptors. That such a mechanism may be present in chicken pineal is supported by two additional observations. First, addition of EGTA to cultured chicken pineal glands fosters accumulation of cGMP in both the light and in the dark [37]. Second, cGMP-gated cation channels possessing response characteristics similar to those found in the plasma membrane of retinal photoreceptor cells have been identified in chicken pineal [38,39].

What might the role of the phototransduction cascade be in chicken pineal? Studies of light effects on circadian function in chicken pineal suggest that chicken pineal contains at least two independent pathways for light transduction, one of which resets the rhythms of the circadian oscillators intrinsic to the pinealocytes and one that inhibits melatonin secretion [40,41]. The details of these pathways are not known; however, the observation that pertussis toxin fails to block light-

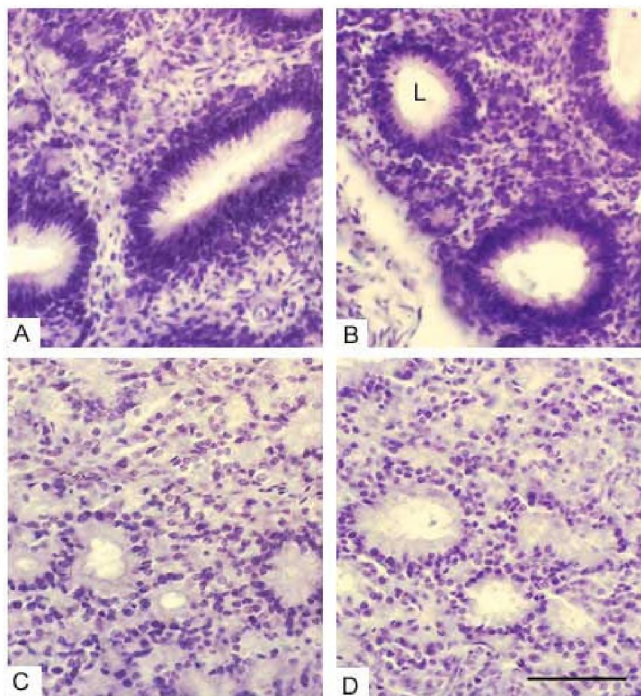


Figure 6. Light microscopic comparisons of normal and *rd/rd* pineal morphology. Pineal glands were obtained from 3 day old (A, B) and 79 day old (C, D) chickens. No apparent morphological differences were observed between the normal and *rd/rd* tissues at either age. Note that the size of the lumens of the follicles (L) decreases with age in both normal and *rd/rd* pineal suggesting that tissue differentiation is normal in the mutant pineal. Sections were stained using cresyl violet. The bar shown in panel D equals 20 μ m. All panels were photographed using the same magnification.

induced phase shifts of the pineal circadian oscillators [40] suggests that if present, the phototransduction cascade may not be directly involved in entrainment of these oscillators to light. The results of a study of the *rc* chicken, a chicken that carries the same GC1 null mutation that is carried by the *rd* chicken (Semple-Rowland and Cheng, unpublished observation), suggest that the phototransduction cascade may not play a direct role in controlling melatonin levels in chicken pineal either. Analyses of pineal glands of 4 to 10 week old carrier *+rc* and *rc/rc* chickens revealed that melatonin levels in the glands of these animals are not significantly different, a result consistent with our observation that the GC1 null mutation in *rd* chickens does not lead to pineal degeneration [42]. Of particular interest was their finding that melatonin levels in the pineal glands of *rc/rc* birds housed under a 12 h light:12 h dark cycle exhibit a light/dark rhythm similar to that observed in pineal glands of *+rc* birds housed under identical conditions. This result suggests that the light transduction pathway that controls melatonin levels in chicken pineal is not dependent upon GC1.

Currently, there is no direct evidence that a fully functional retinal phototransduction cascade is present in chicken pineal. The identification of several key components of the phototransduction cascade in chicken pineal [43], together with the observation that pinopsin, a photopigment present in chicken pineal [44,45], is capable of activating rod transducin in a light-dependent manner [46] support the hypothesis that this cascade is present and functional in chicken pineal. Further study of the *rd* chicken provides a unique opportunity to determine what role the phototransduction cascade plays in pineal function. Clearly, the role that GC1 plays in pineal function is not as critical to pineal cell survival as it is to the survival of retinal photoreceptor cells.

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