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Guanylyl Cyclase Activating Protein

A CALCIUM-SENSITIVE REGULATOR OF PHOTOTRANSDUCTION*

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Guanylyl cyclase activating protein (GCAP1) has been proposed to act as a calcium-dependent regulator of retinal photoreceptor guanylyl cyclase (GC) activity. Using immunocytochemical and biochemical methods, we show here that GCAP1 is present in rod and cone photoreceptor outer segments where phototransduction occurs. Recombinant and native GCAP1 activate recombinant human retGC (outer segment-specific GC) and endogenous GC(s) in rod outer segment (ROS) membranes at low calcium. In addition, we isolate and clone a retinal homolog, termed GCAP2, that shows ~50% identity with GCAP1. Like GCAP1, GCAP2 activates photoreceptor GC in a calcium-dependent manner. Both GCAP1 and GCAP2 presumably act on GCs by a similar mechanism; however, GCAP1 specifically localizes to photoreceptor outer segments, while in these experiments GCAP2 was isolated from extracts of retina but not ROS. These results demonstrate that GCAP1 is an activator of ROS GC, while the finding of a second activator, GCAP2, suggests that a similar mechanism of GC regulation may be present in outer segments, other subcellular compartments of the photoreceptor, or other cell types.

In vertebrate photoreceptor cells, the synthesis and hydrolysis of cyclic GMP (cGMP) are critical steps in phototransduction. In response to light, a cascade of reactions in the photoreceptor outer segment leads to the hydrolysis of cGMP and the closure of cGMP-gated cation channels in the outer segment plasma membrane. As a consequence, there is a reduction in the amount of calcium entering the cell. Calcium efflux owing to the Na^+:K^+, Ca^{2+} exchanger, however, is unaffected by light, resulting in a net decrease in the concentration of internal free calcium. This decrease in the calcium concentration leads to the activation of guanylyl cyclase (GC), which in part restores the dark conditions of the photoreceptor cell (reviewed by Lagnado and Baylor (1992)).

Photoreceptor GC, a member of the particulate GC family (Koch, 1991; Shyjan *et al.*, 1992; Goraczniak *et al.*, 1994; Umbarger *et al.*, 1992; Dizhoor *et al.*, 1994; Liu *et al.*, 1994), responds to an activator that senses changes in the calcium concentration (Lolley and Racz, 1982; Koch and Stryer, 1988). Recently, we proposed that a calcium binding protein isolated from rod outer segments (ROS),[1] guanylyl cyclase activating protein (GCAP, termed here GCAP1), mediates this process (Gorczyca *et al.*, 1994a), and its molecular properties were described (Palczewski *et al.*, 1994). GCAP1 restores the calcium sensitivity of GC in a reconstituted system, and it decreases the sensitivity, time-to-peak, and recovery time of the light response following its introduction into intact ROS (Gorczyca *et al.*, 1994a). The molecular cloning of GCAP1 from bovine, human, mouse, frog (Palczewski *et al.*, 1994), and chicken[2] retina and the genomic organization of mouse and human GCAP1 (Subbaraya *et al.*, 1994) demonstrate strong sequence conservation between species, conservation of three putative calcium binding loops, and relatedness to other neuronal calcium-binding proteins of the calmodulin superfamily. Transcripts encoding GCAP1 were localized to photoreceptor cells by *in situ* hybridization (Palczewski *et al.*, 1994; Subbaraya *et al.*, 1994), but the precise localization of the protein is not known. Independently, Dizhoor *et al.* (1994) proposed that another protein, p24, was responsible for the calcium sensitivity of photoreceptor GC.

In this paper, we describe the cellular localization of bovine GCAP1 by immunocytochemical and biochemical methods and provide further evidence that GCAP1 is a key element in the activation of photoreceptor GC. In addition, we show that the retina contains a second GC activator, GCAP2, that is identical with p24 and closely related to GCAP1.

MATERIALS AND METHODS

Purification of ROS and GCAP1—Fresh bovine eyes were obtained from a local slaughterhouse, and the retinas were dissected under dim red light. ROS were prepared according to Papermaster (1982). GCAP1 was purified as described previously (Gorczyca *et al.*, 1994a).

Affinity Chromatography—A soluble extract containing GCAP1 was prepared from either bovine ROS (equivalent to 50 bovine retinas) or from 25 bovine retinas with 25 ml of water, containing 1 mM benzamidine. The extract was separated from ROS or retinal particulates by centrifugation (48,000 × *g* for 30 min) and loaded onto an antibody-Sepharose column (mAb G-2; 6 mg of antibody per 1 ml of the CNBr-activated Sepharose; 1 × 2 cm) equilibrated with 10 mM 1,3-bis[tris(hy-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L43001.

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[1] The abbreviations used are: ROS, rod outer segment; BTP, 1,3-bis[tris(hydroxymethyl)methylamino]propane; HPLC, high performance liquid chromatography; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; pAb, polyclonal antibody; mAb, monoclonal antibody; GTPγS, guanosine 5′-*O*-(thiotriphosphate).

[2] S. Semple-Rowland and W. Baehr, unpublished results.

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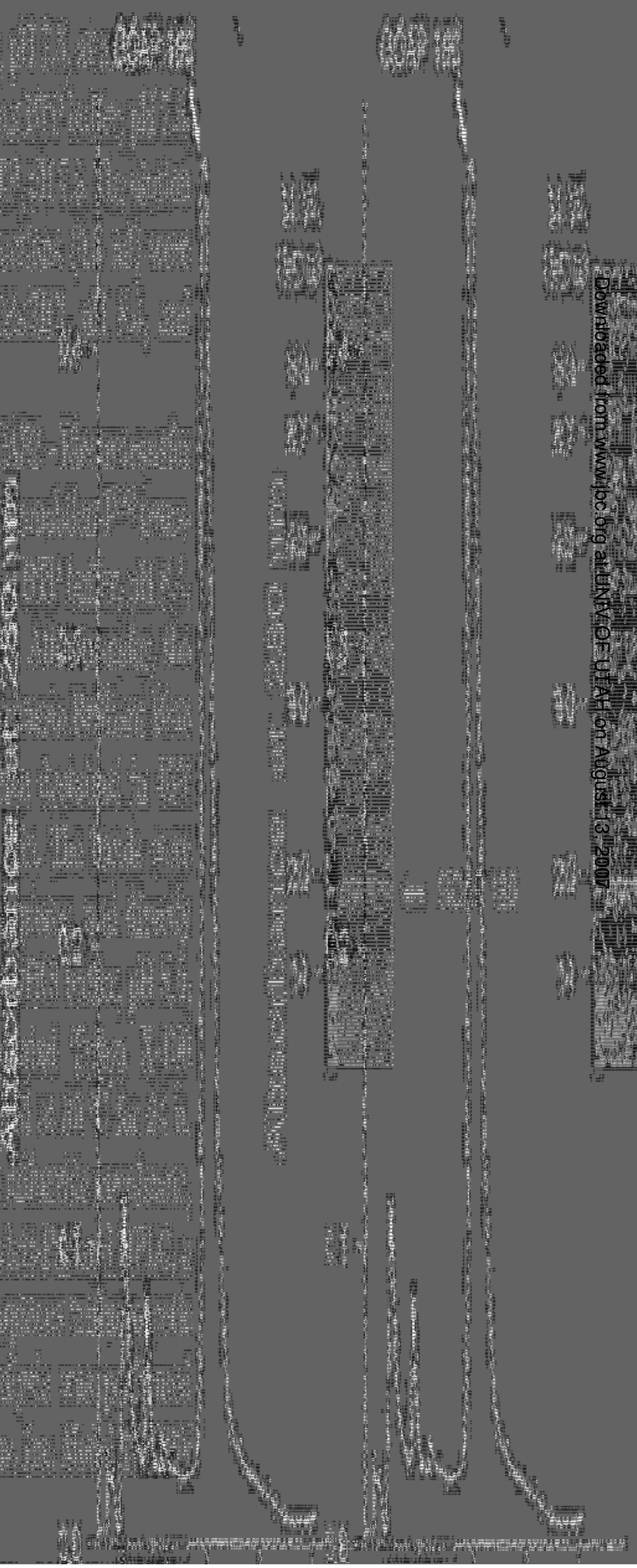

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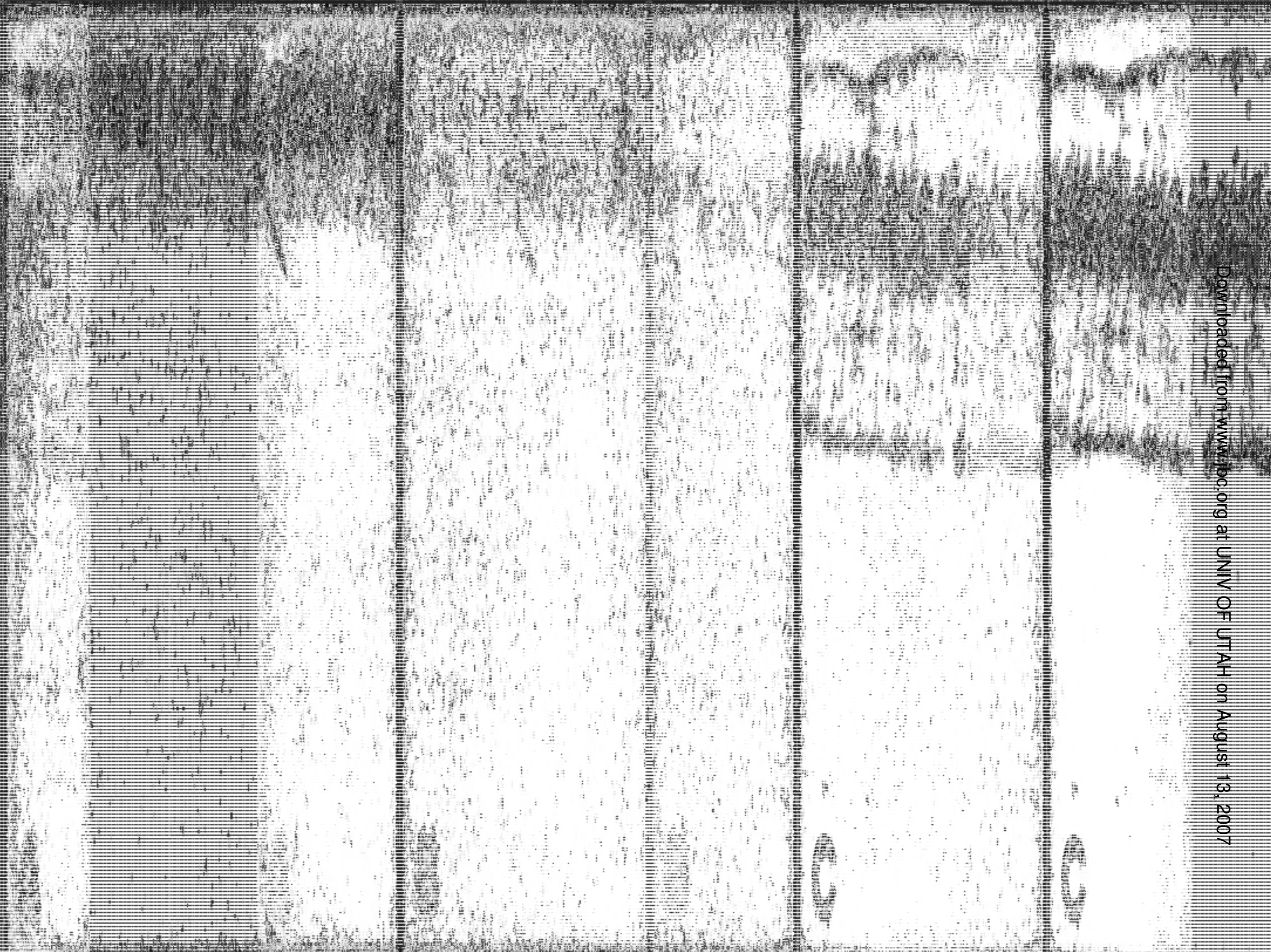

FIG. 2. Anti-GCAP1 antibody staining of bovine retina fixed in paraformaldehyde and cryoprotected with ... and cryoprotected with ... and Methods." ...

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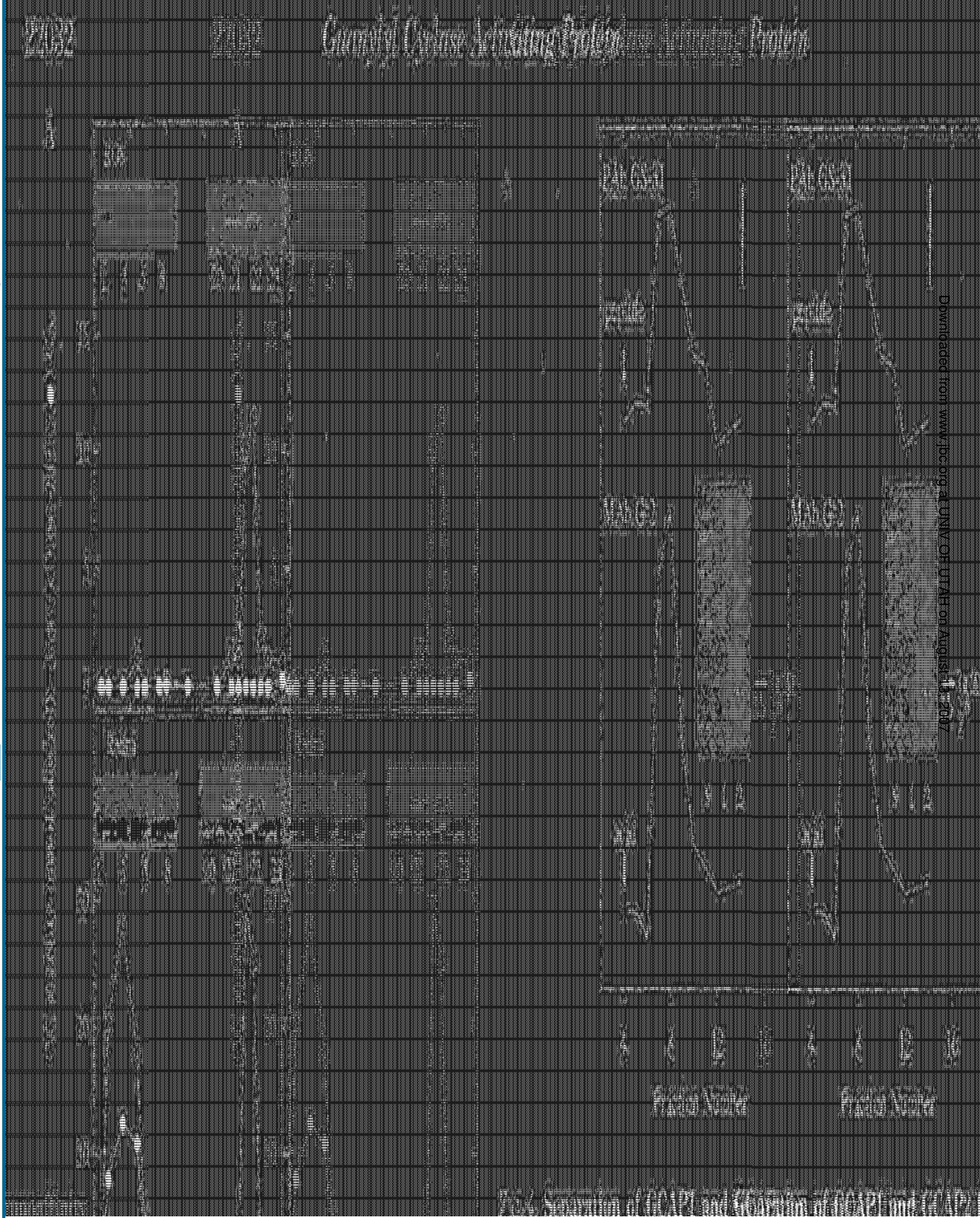

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