

**P<sup>19</sup> DETECTED IN THE RAT RETINA AND PINEAL GLAND IS A  
GUANYLYL CYCLASE ACTIVATING PROTEIN (GCAP)**

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A reduced retinal tissue concentration of Ca<sup>2+</sup> ions resulting from illumination of the photoreceptor cell is the signal for the resynthesis of cGMP by retina-specific guanylyl cyclases (retGC1 and/or retGC2). This Ca<sup>2+</sup>-dependent activation of retGCs is mediated by Ca<sup>2+</sup>-binding proteins named GCAPs (guanylyl cyclase activating proteins) and contributes to the recovery of the photoreceptor cell to the dark state, thus playing an important role in vision. Three different GCAPs (GCAP1, GCAP2 and GCAP3) have been identified in vertebrate retina to date. All activate retGCs at low concentrations of Ca<sup>2+</sup> ( $\leq 50$  nM  $\Rightarrow$  [Ca<sup>2+</sup>]<sub>L</sub>), and inhibit the enzyme(s) at high concentrations ( $\geq 500$  nM  $\Rightarrow$  [Ca<sup>2+</sup>]<sub>H</sub>). Although the retina is the primary site where GCAPs are expressed, GCAP1 was also detected in the bovine pineal gland, and the expression of genes encoding GCAP1 and GCAP2 was reported for the chicken pineal gland. The presence of retGC1 in the pineal glands of both species indicates that synthesis of cGMP in this organ is also regulated in a Ca<sup>2+</sup>-dependent manner but the physiological significance of this mechanism is unknown.

The aim of our studies was to verify whether the 19 kDa protein (p<sup>19</sup>), which we detected in rat retina and pineal gland extracts using an antibody specific to bovine GCAP1, belongs to the GCAP subfamily. Using an assay of guanylyl cyclase (GC) activity, we showed that, at [Ca<sup>2+</sup>]<sub>L</sub>, extracts obtained from the rat retina and rat pineal gland markedly stimulate the synthesis of cGMP in washed membranes of bovine rod outer segments (wROS). We also showed that p<sup>19</sup>, purified from rat retinal extracts by means of immunoaffinity chromatography, activates the GCs present in bovine wROS and in the membrane fraction of rat retinas at [Ca<sup>2+</sup>]<sub>L</sub>. At the same time, there is no stimulation of GC activity at [Ca<sup>2+</sup>]<sub>H</sub>, indicating that the main enzyme in membrane fractions of ROS and rat retina regulated by the extracts as well as purified p<sup>19</sup> is the retGC. Moreover, purified p<sup>19</sup> is recognized by the antibody against bovine GCAP1, and displays Ca<sup>2+</sup>-dependent changes in electrophoretic mobility, characteristic for all known GCAPs. All these results clearly show that p<sup>19</sup> is a rat guanylyl cyclase-activating protein (GCAP), most likely GCAP1.