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Role of a Putative N-Glycosylation Site in Bovine Retinal Cyclic Nucleotide-Gated Channel

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Cyclic nucleotide-gated channels (CNGC's) contain a putative N-glycosylation site (Asn-X-Ser/Thr) in the linker regions connecting the fourth transmembrane domain (S4) and the ion coduction pore (P-region). This putative N-glycosylation site is highly conserved and thus found in many different CNGC in various organisms, from fruit fly to human. To reveal the role of N-glycosylation in channel function, we mutated the asparagine residue(Asn^{327} in bovine retinal CNGC) to a serine residue. Both wild type and the N327S mutant channel gene were transcribed *in vitro* and the mRNA's were micro-injected in *Xenopus* oocytes for a high-level expression. Using western blot analysis, we were able to show that the site is the only and a truely glycosylated site in the channel. When the channel currents were recorded using patch clamp methods, the mutant channel expresses cyclic nucleotide-dependent current robustly in oocytes. We found that the expression level and the overall macroscopic current characteristics (e.g. I-V relationship) are similar to those of the wild type channel. We are currently investigating the detailed permeation and gating properities of both the wild type and N327S mutant channels.