Co-Expressing RCASBP (A) α7-nAChR and RCASBP (B) RIC-3 in embryonic chicken ciliary ganglia to test whether excess α7-nAChRs exacerbate cell death during development. Vanessa Ochoa, John Soltys, and Rae Nishi, Neuroscience Graduate Program and Dept. Anatomy and Neurobiology, University of Vermont

The mechanisms that regulate programmed cell death during the normal neural development are still not well understood. Previous studies in the avian ciliary ganglion have shown activation of alpha7-containg nicotinic acetylcholine receptors (α7-nAChR) induces ciliary ganglion (CG) neurons to die between the age of embryonic day (E) 8 and E14. If this is the case, overexpression of α7-nAChR should induce cell death. To test this experimentally, a chicken virus was constructed to express the α7-nAChR fused to a V5 epitope tag (RCASBP(A) α7-V5). A chicken fibroblast DF-1 cell line was infected with RCASBP(A) α7-V5. To determine whether the α7-V5 nAChRs are on the cell surface, cells were incubated with anti-V5 when alive, or after fixation and permeabilization. No V5-immunoreactivity was observed in live cells, however fixed and permeabilized cells did. The proper maturation and insertion of α7-nAChR may require the chaperone protein, RIC-3 (resistance to inhibitors of cholinesterase 3). The gene encoding RIC-3 was identified in 1995 as one of several genes conferring resistance to aceylcholinesterase inhibition in *Caenorhabditis elegans* (Nguyen et al., 1995). RIC-3 orthologs, have, been identified in *Homo sapiens*, *Drosophila*, and *Mus musculus* (Halevi et al., 2003 Lansdell et al., 2008). Since a cDNA clone for *Gallus* RIC-3 is unavailable, RIC-3 must first be amplified from chicken neural tissue and sub-cloned into chicken retrovirus RCASBP (B). The first attempt to amplify RIC-3 was unsuccessful. A new set of primers were designed from the databases: Ensembl Genome Browser, The Gene Indices (TIG) website, and (GenBank). Overlapping sequences from all three websites were combined and assumed to represent full-length RIC-3. The new primers were successful in amplifying RIC-3 from E13 tectum. We predict chickens infected with both α7-nAChRs and RIC-3 will over express functional α7-nAChRs on the cell surface, causing an increase in cell death.

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Ensembl Genome Browser (http://www.ensembl.org/index.html)

The Gene Indices (http://compbio.dfci.harvard.edu/tgi/)

GenBank (http://www.ncbi.nlm.nih.gov/guide/genes-expression/)