Site-directed mutagenesis of cDNA for serum- and glucocorticoid-regulated kinase (SGK) from *Fundulus heteroclitus*

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The euryhaline teleost *Fundulus heteroclitus* (killifish) adapts to rapid changes in environmental salinity and therefore represents an excellent model for studies on the regulation of salt transport. Marshall, et al.[1] reported that adaptation to seawater was accompanied by a transient increase in plasma cortisol levels, a sustained increase in CFTR chloride ion transporter expression, and increased Cl^- secretion by opercular epithelia. Stanton and colleagues[2] (and manuscript in preparation) found that killifish exposed to non-toxic doses of arsenic (5 umol/kg) for 24h exhibited significantly reduced CFTR-mediated opercular Cl^- secretion, reduced CFTR gene expression and were unable to adapt to seawater. Serum- and glucocorticoid-regulated serine/threonine kinase (SGK), which was originally discovered in rat mammary tumor cells[3], has been shown to stimulate the Cl^- transport activity of co-expressed CFTR molecules in *Xenopus* oocytes[4], and non-toxic concentrations of arsenic also transiently inhibit SGK expression in killifish gill tissue (unpublished results). These findings suggest that SGK is involved as a signaling intermediate in regulating CFTR activity in the adaptation of killifish to seawater.

In order to confirm a stimulatory effect of killifish SGK on CFTR activity and to investigate potential mechanisms by which SGK functionally interacts with CFTR we have cloned wild type and mutant killifish SGK cDNAs. We previously determined the coding region sequence of killifish SGK cDNA (GenBank accession number AY800243) through RT-PCR followed by 5'- and 3'-RACE reactions[5]. Based on those results, we used RT-PCR to amplify the 1,317 nucleotide coding region of killifish SGK cDNA (Fig. 1), and the product was cloned into the pCR2.1 topo-TA vector (Invitrogen). When sequenced, the cloned PCR product was found to contain three mutations relative to the reference cDNA sequence in GenBank. These mutations were corrected using the PCR-based QuikChange site-directed mutagenesis kit (Stratagene) to yield a wild type SGK cDNA clone.

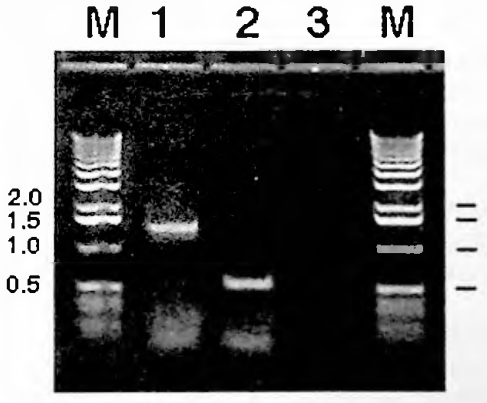

Figure 1. Amplification of killifish SGK cDNA coding region by RT-PCR. The 1.32 kb killifish SGK coding region (sample 1) was amplified from total liver cDNA by RT-PCR. A 0.6 kb fragment of the coding region was generated as a positive control (sample 2) while a negative control reaction contained no cDNA template (sample 3). PCR products were resolved by electrophoresis in a 1.2% agarose gel. M, 1kb DNA ladder.

The SGK protein has two identifiable functional domains: a highly conserved serine/threonine kinase domain[6]; and a carboxyl-terminal PDZ domain interaction sequence[7]. We have mutated both of

these domains in order to study the function of SGK. A cDNA clone encoding a kinase-dead version of killifish SGK was created by mutating the lysine residue in the ATP-binding pocket to asparagine (K. Karlson, data not shown). The QuikChange mutagenesis kit was used to create a cDNA clone lacking the DSYL PDZ domain interaction sequence: the TCC codon for serine was mutated to a TAA termination codon causing the deletion of the SYL tripeptide. The mutation was confirmed by DNA sequencing (Fig. 2).

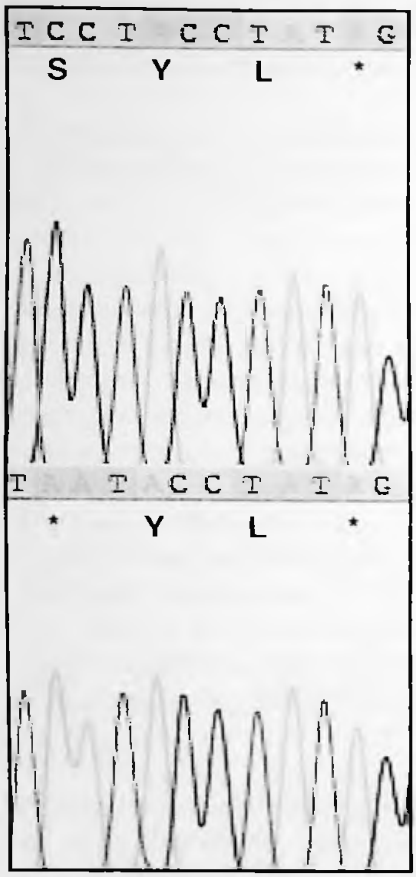

Figure 2. Confirmation of the mutation deleting the SGK PDZ domain interaction sequence. The upper sequencing chromatogram corresponds to the region of wild type cDNA encoding the terminal three amino acids (SYL) of killifish SGK. The lower chromatogram shows that the codon for serine (S) was changed to a stop codon in the mutant cDNA clone.

The wild type, kinase-dead and truncated forms of killifish SGK are being co-expressed with CFTR protein in *Xenopus* oocytes. Our preliminary results confirm that SGK stimulates CFTR Cl⁻ currents in this heterologous expression system. The mutant cDNAs generated here will help us determine whether protein-protein interactions other than that of enzyme and substrate are necessary for this effect.

We thank Christine Smith and the staff of the Marine DNA Sequencing Center for sequencing PCR products and plasmids generated in this research. JDS and PR were supported by grant P20-RR016463R from the National Center for Research Resources, and BAS, MCC, RB and KK were supported by grant P42-ES07373 from the National Institute of Environmental Health Sciences. SP was supported by the Hancock County Scholars Program. JDS and BAS are investigators of the Center for Membrane Toxicity Studies, which is supported by grant P30-ES03828 from the NIEHS.

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