CLONING OF AN ANDROGEN RECEPTOR cDNA FROM *SQUALUS ACANTHIAS* TESTIS

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In all vertebrates studied, proliferation and development of male germ cells occurs in a steroid-rich environment generated by somatic elements of the testis (Sertoli cells, Leydig cells). Although several classes of steroids are involved in regulating spermatogenesis, the major focus of research has been on androgen. The actions of androgen in supporting male germ cell development are not direct but exerted via androgen receptors (AR) located in somatic cells. AR are ligand-activated transcription factors that bind to specific nucleotide sequences in the regulatory regions of target genes and positively or negatively regulate transcription. A program of research in this laboratory has established the utility of the shark testis model for the stepwise analysis of spermatogenesis (Callard et al., in: Bartke, A. (ed) Function of Somatic Cells in the Testis, Springer-Verlag, New York, pp. 27-54, 1994). To date, we have identified and characterized classical nuclear AR in shark testis by radiolabeled ligand binding analysis and have established that AR concentrations are stage-dependent: premeiotic (PrM) >> meiotic (M) > postmeiotic (PoM) stages (Cuevas, M.E. & G.V. Callard, *Endocrinology* 130(4):2173-2182, 1992). The goal of the present study was to isolate shark specific AR cDNA for use in defining the role of androgen in regulating specific stages of development, genes and physiological processes.

Total RNA was isolated from PrM-stage testicular tissue and reverse-transcribed (Superscript^{II}, Invitrogen). Synthetic oligonucleotide primers were designed to target highly conserved regions of known vertebrate ARs (dAR5', dAR3'; Fig. 1A) and used to amplify shark testis cDNA by polymerase chain reaction (PCR). A PCR product of the predicted size (501 bp) was isolated and sequenced (Fig. 1A). The deduced shark peptide (sharkAR) had 66% overall identity (79% similarity) when compared to AR from human prostate. Comparison of shark and human ARs showed high sequence identity in established functional domains: 89% in the DNA-binding domain (DBD) and 76% in the steroid-binding domain (SBD; Fig. 1B). The hinge region was more variable (33% identity), as has been reported across mammalian species. Semiquantitative RT-PCR of shark testis confirmed that AR mRNA was differentially distributed by stage and corresponded to AR protein as determined by binding analysis: PrM >> M > PoM. Isolation of a full-length AR cDNA is in progress.

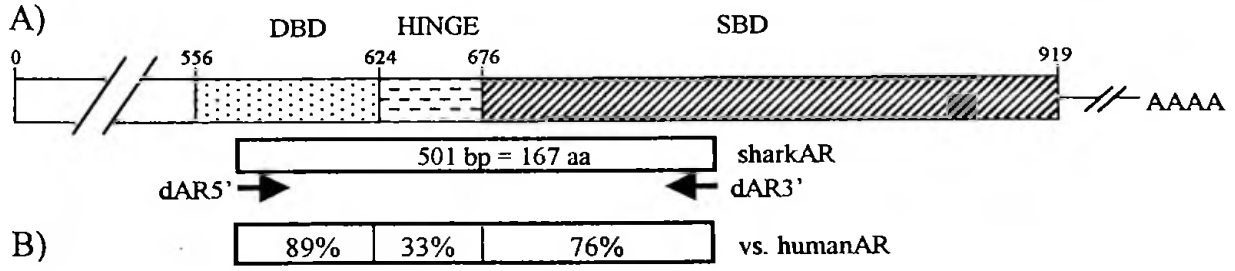

Figure 1: A) RT-PCR strategy for the isolation of AR from shark testis. B) Sequence identities of specific receptor domains between shark and human AR.

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