CLONING OF MULTIPLE G-PROTEIN ALPHA SUBUNITS AND CHARACTERIZATION OF A FULL LENGTH $G_{i\alpha 2}$ FROM THE SHARK (*SQUALUS ACANTHIAS*) RECTAL GLAND

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Heterotrimeric G proteins couple seven transmembrane (7TM) G-protein linked receptors to intracellular effectors in all cells. In the shark (*Squalus acanthias*) rectal gland, at least six G-protein coupled receptors regulate chloride secretion including adenosine, VIP, pituitary adenylate cyclase activating polypeptide (PACAP), somatostatin (SRIF), PYY and NPY receptors (Forrest, Kidney Int. 49:1557-62, 1996). We previously identified in the rectal gland a 41 kD protein that was ADP-ribosylated by pertussis toxin and two proteins (45 and 50 kD) ribosylated by cholera toxin which correspond to inhibitory and stimulatory G_{α} subunits, respectively (Kelley et al., Bull. MDIBL 25:104-107, 1985). Based on ADP ribosylation and ^{3}H-NECA binding studies, we presented evidence that G_i proteins are functionally coupled to adenosine receptors in the rectal gland (Kelley et al., Bull. MDIBL 25:104-107, 1985).

In mammals, at least 17 G_{α} genes have been identified. Some undergo alternative splicing to yield multiple gene products. Based on amino acid sequence, G protein alpha subunits are subdivided into four groups - G_s, G_i, G_q and G_{12} (Rodbell, Adv. Enzyme Reg. 37:427-35, 1997; Hildebrandt, Biochem. Pharm. 54:325-39, 1997). Although G proteins have been cloned from several species, none have yet been cloned from teleosts or elasmobranchs. To study the selectivity of receptor-G protein interactions and the molecular details of signal transduction in the shark rectal gland, we sought to clone the G-proteins involved in receptor regulation of Cl^- transport in this tissue.

We used a PCR based cloning strategy to design degenerate oligonucleotide primers to conserved regions of previously cloned inhibitory and stimulatory G_{α} proteins using sequences from Genbank. We succeeded in obtaining partial cDNA sequence of five distinct shark G protein subunits: $G_{i\alpha 1}$, $G_{i\alpha 2}$, $G_{i\alpha 3}$, and two novel forms of $G_{\alpha s}$. We report here the details of the full length cloning, cDNA sequencing and characterization of one of these proteins - $G_{i\alpha 2}$.

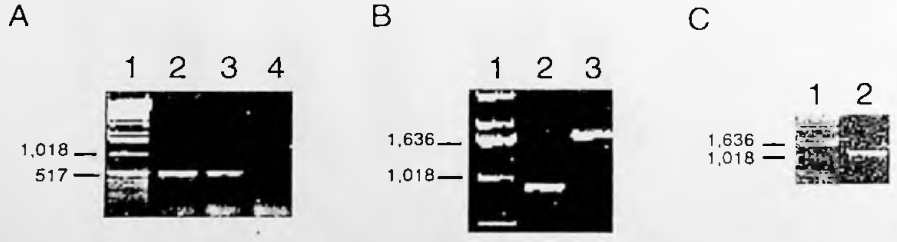

Figure 1. Gel electrophoresis of shark $G_{i\alpha 2}$ PCR products. Panel A displays PCR fragments obtained from degenerate $G_{i\alpha 2}$ primers: 1 kb ladder (lane 1), human control from placenta cDNA (lane 2), shark $G_{i\alpha 2}$ from rectal gland cDNA (lane 3), and negative control without cDNA template (lane 4). Panel B displays RACE-PCR products for shark $G_{i\alpha 2}$: 1 kb ladder (lane 1), 3' RACE product (lane 2) and 5' RACE product (lane 3). Panel C: 1 kb ladder (lane 1) and shark $G_{i\alpha 2}$ PCR product encoding the entire open reading frame obtained from shark $G_{i\alpha 2}$ specific primers (lane 2).

Using cDNA prepared from *Squalus acanthias* rectal gland total RNA, a 503 base pair PCR product was obtained using degenerate $G_{i\alpha 2}$ primers (Figure 1, panel A). This product was ligated

into the pCR2.1 vector (Invitrogen) and subjected to bi-directional automated sequencing. The sequence had highest homology (94%) with chicken $G_{i\alpha 2}$. To obtain the 5′- and 3′- sequence, RACE-PCR was performed using adaptor ligated cDNA and shark specific internal primers (Figure 1, panel B). Additional primers flanking the start and stop codons were used to obtain the complete open reading frame of shark $G_{i\alpha 2}$ (Figure 1, panel C).

The full length sequence of shark $G_{i\alpha 2}$ and a comparison to chicken and human counterparts are shown in figure 2. The dogfish shark $G_{i\alpha 2}$ is 94% identical to both the chicken and human G-protein alpha subunits. There are 30 residues which are not identical in all three species. Shark $G_{i\alpha 2}$ has seven non-conserved residues compared to chicken and human $G_{i\alpha 2}$ at positions 9, 14, 92, 99, 112, 296, and 299.

In human $G_{i\alpha 2}$, four protein domains (A, C, E, & G) combine to form the internal guanine nucleotide binding site. Regions forming the internal guanine nucleotide binding site are crucial in defining tertiary structure of GTP-binding proteins and therefore exons encoding for these regions are highly conserved throughout evolution. Regions located at the amino and carboxy-termini and between the domains A and C vary considerably. These regions are believed to be involved in interactions with receptors, beta-gamma subunits and effector proteins. The $G_{i\alpha 2}$ promoter also contains possible DNA binding domains for AP-1, a factor that mediates the transcriptional effects of phorbol esters, and AP-2, a factor that may mediate the transcriptional effects of cAMP and phorbol esters (Weinstein et al., FEBS Lett. 232:333-340, 1988).

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                                                           A                A                         80
   Shark  MGCTISAENK AAAKRSRMID KNLREDGEKA AREVKLLLLG AGESGKSTIV KQMKIIHEDG YSEEECRQYK AVVYSNTIQS
 Chicken  ....V...D. ...E...... R......... .......... .......... .......... .........K ..........
   Human  ....V...D. ...E...... K......... .......... .......... .......... .........R ..........

                                                                                             160
   Shark  IMAIIKAMGN LKVDFGESGR ADDARQLFAL SGTAEEQGIL PEDLANVIRR LWADAGVQAC FGRSREYQLN DSAAYYLNDL
 Chicken  ....I..... .QI..GDSS. .......... AC......IM .E..AN.... ....H..... .N........ ..........
   Human  ....V..... .QI..ADPS. .......... SC......VL .D..SG.... ....H..... .G........ ..........

                                S1                                  S2                      S3 240
   Shark  ERIARSDYIP TQQDVLRTRV KTTGIVETHF TFKDLHFKMF DVGGQRSERK KWIHCFEGVT AIIFCVALSA YDLVLAEDEE
 Chicken  ....RA.... .......... .......... .......... .......... .......... .......... ..........
   Human  ....QS.... .......... .......... .......... .......... .......... .......... ..........

          C/E                                                          G                             320
   Shark  MNRMHESMKL FDSICNNKWF TDTSIILFLN KKDLFEEKIT KSPLTICFPE YSGANEYDAA AGYIQTKFED LNKRKDTKEI
 Chicken  .......... .......... .......... .........V H......... .T...K..E. .G...S.... ..........
   Human  .......... .......... .......... .........T H......... .T...K..E. .S...S.... ..........

                                              *355
   Shark  YTHFTCATDT KNVQFVFDAV TDVIIKNNLK DCGLF
 Chicken  .......... .......... .......... .....
   Human  .......... .......... .......... .....
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Figure 2. Alignment of the deduced amino acid sequence of dogfish shark, chicken, and human $G_{i\alpha 2}$. Residues that are non-conserved differences in all three species are in bold. Domains A, C, E and G are overlined. Switch 1, Switch 2 and Switch 3 are overlined and indicated as S1, S2 and S3. The ADP-ribosylation site is indicated with an asterisk.

Of the 30 different amino acids between the shark, chicken and human $G_{i\alpha 2}$, 7 are distinctly different (non-conserved changes) in the shark compared to both the human and chicken $G_{i\alpha 2}$. The unique shark sequence at positions 9 and 14 are in the putative receptor binding region and could confer differences in functional binding of shark $G_{i\alpha 2}$ to receptors compared to human and chicken (Lambright et al., Nature 379:311-319, 1996). Indeed, these different amino acids in the binding domain are specifically not present in 10 other species examined (including human, lobster, and snail) and are not different in other inhibitory G proteins. The ADP ribosylation site and the switch regions are 100% conserved in all three species. The additional shark unique amino acids

(positions 92, 112, 135, 296 and 299) occur in regions of the protein whose function is not defined.

In summary, we have cloned and sequenced the full length gene for the dogfish shark rectal gland $G_{i\alpha 2}$ and obtained partial sequence for four additional G_{α} subunits. The sequences are presently being confirmed by screening a rectal gland cDNA library. We plan to purify and co-express these G protein subunits to define receptor-G protein interactions at a structural level in this elasmobranch epithelium.

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