CLONING AND SEQUENCING OF HISTONE H3.3 AND H3.1/2 FRAGMENTS FROM RECTAL GLAND OF DOGFISH SHARK *(SQUALUS ACANTHIAS)*

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Reverse-transcriptase PCR (RT-PCR) is a powerful and highly sensitive technique for the quantitative analysis of gene expression in different tissues. RT-PCR compares the expression of a gene of interest to an internal standard. Theoretically, an ideal standard gene has a constant ratio of its messenger RNA (mRNA) to total RNA in all cells in the organism. To perform RT-PCR in shark tissues, it was necessary to choose, clone and partially sequence a standard reference gene from the shark in order to design specific oligonucleotide primers for RT-PCR.

Genes such as actin, GAPDH, cytochrome B, ribosomal proteins and DNA binding proteins have served as standards for RT-PCR in mammalian and non-mammalian tissues. DNA binding proteins in eukaryotes are divided into two classes: the histones and the nonhistone chromosomal proteins that together form the complex chromatin. Histones are small abundant proteins that are rich in positively charged amino acids (arginine and lysine), and are crucial for the efficient packaging of chromosomal DNA in eukaryotic cells. Histones are among the most conserved proteins in nature. There is extreme conservation from plants (rice, soybean) and non-vertebrates (clam and fruit fly) to vertebrates (chicken, mouse and man). The synthesis of most histones is closely linked to DNA replication in the cell cycle. In higher eukaryotes, five histone subtypes have been described: the nucleosomal histones (H2A, H2B, H3 and H4) and the H1 histones. The H3.3 variant, which is cell replication-independent and constitutively expressed in all tissues, differs from the H3.1 and H3.2 (cell replication-dependent) variants by four amino acid substitutions (Table 1, modified from N. Chaubert, J. Mol. Biol., 255:569-574, 1992; Thatcher et al., Nucleic Acids Research 22:1994). We chose to examine the histone H3 subtype and found three somatic variants (H3.1, H3.2, H3.3) described in the literature (N. Chaubert, J. Mol. Biol., 225:569-574, 1992).

Table 1. Comparison of amino acid sequences of H3 histones

Amino acid position	31	87	89	90
H3.1/H3.2	Ala	Ser	Val	Met
H3.3	Ser	Ala	Ile	Gly

In order to clone and sequence a fragment of histone H3.3 from the dogfish shark, we first performed an amino acid alignment of the 136 amino acid residues encoding histone H3.3 from frog, fruit fly, sea squirt, three different sea urchins and the spoonworm, using sequences from Genbank. A computer program, written by S.G. Aller, was used to identify two separate strings of six amino acids that are coded by a relatively low number of codons. These amino acids were back translated into degenerate nucleotide sequence, sense and antisense oligonucleotides primers were synthesized, and RT-PCR was performed on cDNA prepared from *Squalus acanthias* rectal gland total RNA. A 380 base pair PCR product was cloned into the pCR2.1 T/A cloning vector (Invitrogen). High quality plasmid DNA was prepared from four different bacterial clones using the Promega Wizard Maxiprep kit. These four clones were sequenced using fluorescent nucleotide incorporation at the automated DNA sequencing facility at the University of Maine, Orono. Three clones were identical and identified as a shark histone H3.3 fragment. The fourth clone differed in four amino acids in positions 31 (Ser-Ala), 87 (Ala-Ser), 89 (Ile-Val), 90 (Gly-Met) and was therefore identified as a fragment of shark Histone H3.1/2.

Figure 1 presents the nucleotide alignment of the dogfish shark, fruit fly and human histone H.3.3. The shark nucleotide sequence is 78% similar to the human histone H3.3 and 80% to the fruit fly histone H3.3. The deduced amino acid translation of these sequences is 100% identical to the human H3.3 gene. Next, we designed shark specific sense and antisense histone H3.3 primers, performed PCR on shark rectal gland cDNA, obtained a band of the expected size of 255 bp (Figure 2) and confirmed the sequence as shark H3.3.

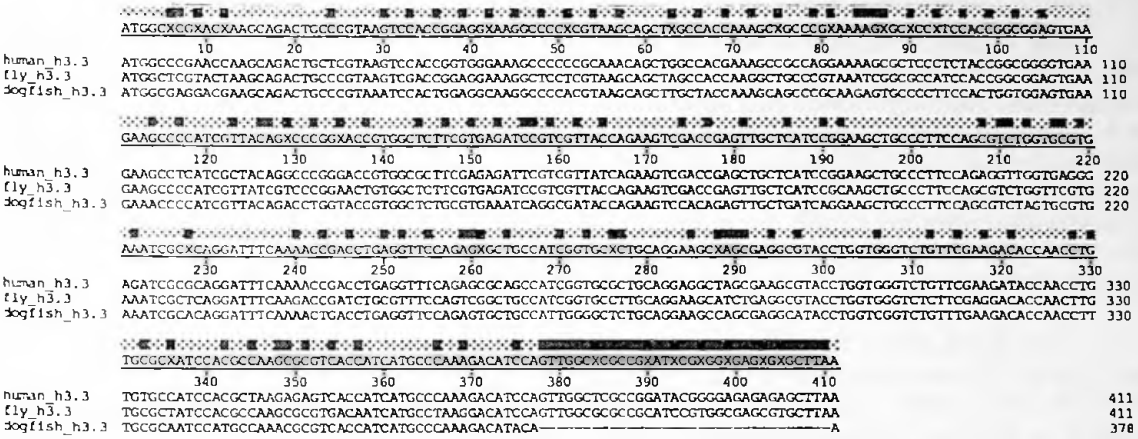
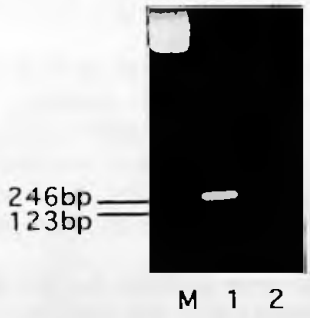


Figure 1. Nucleotide alignment of human, fruit fly and dogfish shark histone H3.3.
The shark nucleotide sequence is 78% similar to the human histone H3.3 and the deduced amino acid sequence is 100% identical to the human H3.3 gene.



Lane M: 123 bp marker
Lane 1: specific shark histone H3.3 primers
Lane 2: negative control (without cDNA)

Figure 2. PCR of shark rectal gland cDNA using shark specific histone H3.3 primers.

In summary, we have successfully cloned and sequenced a 380 bp fragment of the dogfish shark histone H3.3 gene and have designed primers that yield a 255 bp shark specific product which will serve as an internal standard for subsequent RT-PCR in this species. We have applied this technique to determine the gene expression of the shark A_0 adenosine receptor and shark CFTR in selected tissues of *Squalus acanthias*. (G. Hemminger et al., this Bulletin).

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