ISOLATION OF GENES EXPRESSED IN DEVELOPING NEPHRONS IN THE ADULT KIDNEY OF SQUALUS ACANTHIAS

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Elasmobranches are able to develop new nephrons during their life span, and their kidney may serve as a model system for nephron development. We previously showed that the kidney in the adult dogfish revealed four developmental stages as well as mature nephrons. These developmental zones express genes necessary for kidney development. We used tissue dissection and subtractive hybridization to analyze gene expression in developing nephrons within the adult kidney. Total RNA from shark kidney developing and differentiated zones were isolated and cDNA synthesis was performed. Subtractive hybridization allowed us to clone partial cDNAs involved in kidney development. More than 1000 clones were amplified by PCR and the cDNA was spotted onto glass slides. The slides were hybridized with total RNA from developing and mature kidney tissue. The RNA was labeled with Cy 5 and Cy 3 fluorescent dyes as a flip dye experiment. Differentially expressed genes were sequenced and analyzed using public databases. From the subtractive hybridization, we found two candidate genes differentially expressed in developing nephrons.

The first cDNA fragment contains an open reading frame of 111 aa and shows 91% identity to the TAF-I of *Xenopus laevis*. Template activating factor I (TAF-I) was originally identified as a host factor required for DNA replication and transcription of adenovirus genome complexed with viral basic proteins. Purified TAF-I was shown to bind to core histones and stimulate transcription from nucleosomal templates. Human TAF-I consists of two acidic proteins, TAF-I alpha and TAF-I beta, which differ from each other only in their amino-terminal regions. TAF-I plays a role in remodeling higher-order chromatin structure as well as nucleosomal structure through direct interaction with chromatin basic proteins. (Matsumoto K et al., Mol Cell Biol 19, 6940-52, 1999) TAFs interact with transcriptional activators and mediate transcriptional activation. Innumerable transcription factors integrate cellular and intercellular signals to generate a profile of expressed genes that is characteristic of the cellular properties of the cell. Variants of the general transcription factors play specific roles in embryonic development, reflecting the requirements of the developmental gene regulation. (Veenstra and Wolffe, Trends in Biochem. Sciences 26, 665- 671, 2001)

A further cDNA fragment contains 209 base pairs and shows homologies (more than 50%) to the sec 61 of *Danio rerio*. Sec 61 codes for a protein translocation channel. The initial step in the biogenesis of most extra-cellular proteins in eukaryotic cells is their translocation into the endoplasmic reticulum. Translocation occurs through a hydrophilic channel that has been conserved throughout evolution. The channel itself has been proposed to be a passive conduit for polypeptides. Other associated proteins provide the driving force for translocation and determine its directionality. The Sec61ß subunit is required during Drosophila embryonic development. Homozygous mutant embryos die at the end of embryogenesis. (Valcarel et al., J. Cell Science 112, 4389 - 4396, 1999)

Using glass slide hybridization, we found cDNAs overexpressed in developing nephrons. A cDNA fragment translated into the corresponding protein shows 65% identity to the UDP-

glucuronosyltransferase of *macaca fascicularis*. Glucuronidation is the major detoxification pathway. The reaction is catalyzed by a family of UDP-glucuronosyltransferases and is involved in conjugation with many endobiotic and xenobiotic substances with glucoronic acid, forming inactive water-soluble intermediates. (Grancharov K et al. Pharmocology and The rapeutics 89, 171 - 186, 2001) Cubero et al. (Dig Dis Sci 46, 2762-2767, 2001) examined the bilirubin UDP-glucuronosyltransferase throughout rat fetal development. They observed an increase in conjugated billirubin within 90 days.

Furthermore, we isolated a 498 base pair cDNA fragment showing 57% identity to the human L-kynurenine aminotransferase. This enzyme catalyzes the formation of kynurenic acid. Kynurenic acid has become a standard tool for use in the identification of glutamate-releasing synapses and has been used as the parent for several groups of compounds now being developed as drugs for the treatment of epilepsy and stroke. Beal et al (Brain Res. Dev. Brain Res. 68, 136-139, 1992) observed that in rat fetal brain, kynurenic acid concentrations were increased 5 fold prenatally and reached adult levels at day 7 after birth.

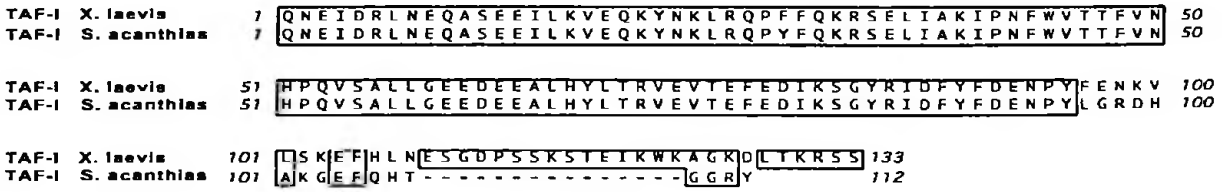

Fig. 1: Protein Alignment of the isolated protein sequence to the *Xenopus laevis* TAF-I sequence

In summary, we isolated cDNAs coding for Template activating factor I, Sec 61, UDP-glucuronosyltransferase and L-kynurenine aminotransferase. The cDNA fragments described play a role in developmental processes. Further experiments like quantitative RT-PCR and in situ-hybridization will confirm the role of the candidate genes and will give us a deeper insight into the molecular mechanisms of nephron development.