IDENTIFICATION OF GENES EXPRESSED IN DEVELOPING NEPHRONS USING SUBTRACTIVE cDNA HYBRIDIZATION

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The kidney of elasmobranchs serves as a model system for kidney development, because they are able to develop new nephrons during their lifespan. By light and electron microscopy, Hentschel (Am. J. Anat.; 190 (4): 309 - 33, 1991) showed that the kidney in the adolescent dogfish revealed four developmental stages of nephrons as well as mature nephrons. These developmental zones express genes necessary for kidney development. Furthermore, the renal tissue shows morphological homology to the respective structures in mammals (Hentschel and Elger, Adv. Anat. Embryo. Cell Biol. 108: 1-151, 1987) suggesting that the same set of genes might be involved. The cell types of elasmobranch and mammalian glomeruli are identical (Lacy et al. Anat. Rec. 18 (3): 294 - 305, 1987). For molecular analysis we obtained tissue samples by microdissection from the developmental zone and from mature tissue. By subtractive hybridization we isolated candidate genes important for kidney development.

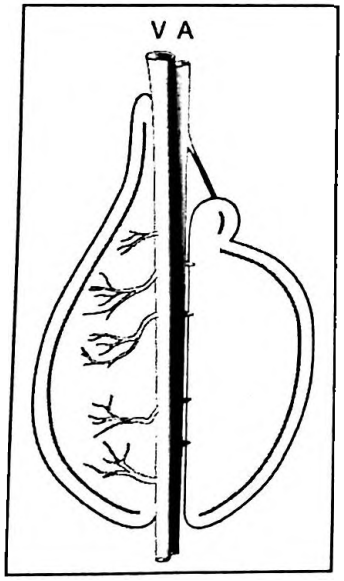

Fig. 1: Schematic view of ventral aspect of the kidneys of little skate, showing the zone of nephroneogenesis

The paired renal organs exhibit a zone of tissue growth by nephroneogenesis confmed to a narrow ventrolateral band located at the outer convex margin of the kidneys (for description of the developmental stages see Elger et al., this Bulletin). The dorsal aorta (A. shown in black) gives rise to multiple arteries (depicted for the left kidney) and the postglomerular blood as well as the blood of the renal portal system is drained by multiple renal veins to the cardinal vein (V. depicted in the right kidney). (For anatomical details of the blood vascular system, see Hentschel, H., Am. J. Anat.183: 130 - 147, 1988)

Total RNAs from shark (*Squalus acanthias*) or skate (*Leucoraja erinacea*) kidney developing zones and from differentiated zones were isolated using an established protocol (Qiagen) and cDNA-synthesis was performed. The cDNA subtraction kit (Clontech) allowed us to clone partial cDNAs involved in kidney development. From 600 clones, we sequenced 60 clones. Analysis were performed using BlastN and BlastX (database of National Institute of Health). Four cDNA-fragments involved in development were identified and described.

From skate developing nephrons we isolated the RNA-binding protein hermes. The cDNA-fragment contains an open reading frame of 161 aa and showed 95 % identity to the chicken hermes aa sequence. Gerber et al. (Mech. Dev. 80, 77-86, 1999) described the role of hermes in the embryonic heart development. In situ hybridization analysis indicate that hermes is expressed at highest levels in the myocardium of the heart and to a lesser extent in the ganglion layer of retina, the epiphysis and the pronephros. They speculated that hermes is involved in the processing of cardiac-specific splice variants. We suggest that hermes regulates a part of the differential splicing during the nephron development.

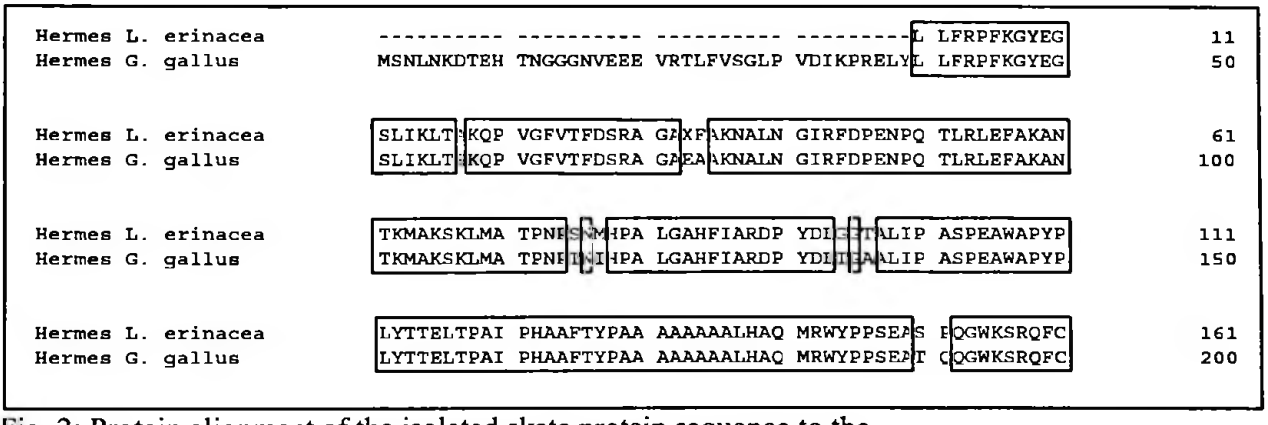

Fig. 2: Protein alignment of the isolated skate protein sequence to the *Gallus gallus* Hermes protein

A cDNA-fragment, isolated from shark developing nephrons, codes for the cytosolic aldehyde dehydrogenase. The corresponding protein showed 75 % identity to the human homologue. Bhat et al. (J. Histochem. Cytoch 46, 1025 - 32, 1998) reported that the ALDH functions as a retinal dehydrogenase. RALDH catalyzes the oxidation of retinal to retinoic acid, which regulates cell growth and differentiation. In situ-hybridization showed that RALDH mRNA expression is prominent in kidney of 2-day-old rats. In kidney the enzyme activity peaks two days after birth and decreases gradually until adulthood. During the postnatal development, (day 0 to day 6) hybridization is essentially concentrated within the marginal nephrogenic zone

of the cortex. These data suggest an important role for RALDH in modulating retinoic acid levels in different cell types during kidney development. Therefore, the cytosolic ALDH is a good candidate gene important in nephron development in the shark kidney.

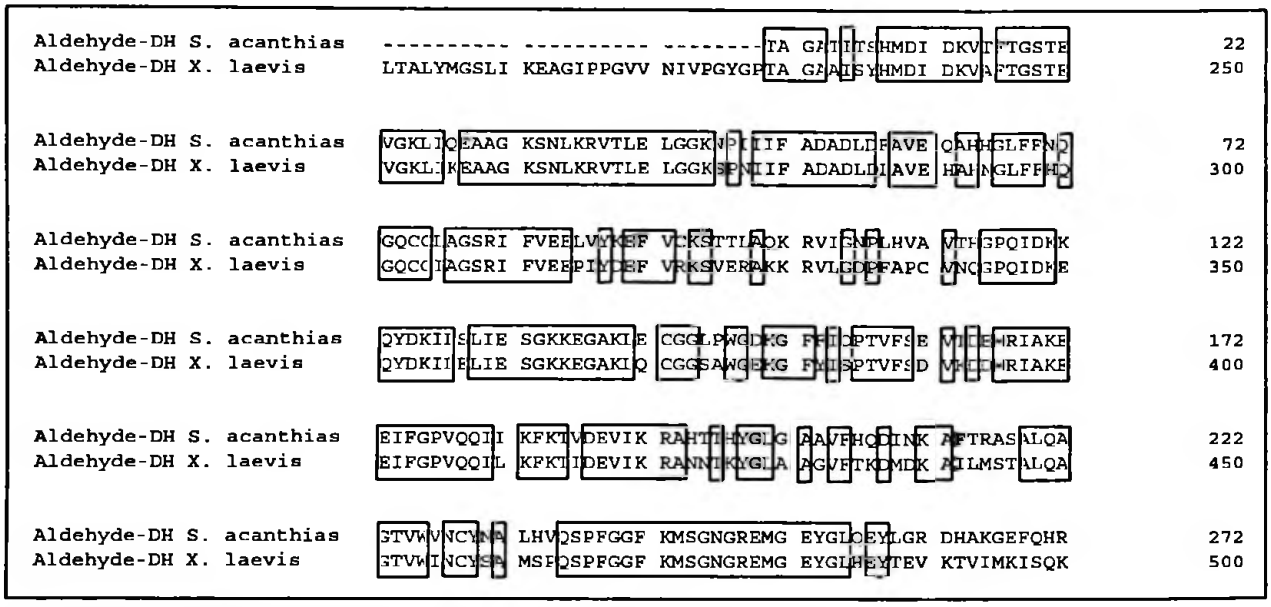

Fig. 3: Protein alignment of the isolated *S. acanthias* protein sequence to the *Xenopus laevis* aldehyde dehydrogenase

The third cDNA-fragment codes for an ubiquitin fused to ribosomal protein. The fragment shows 100 % identity over 54 aa to ubiquitin B from mouse. In eukaryotes, ubiquitin is encoded by a gene family whose primary translation products are rapidly processed fusion proteins. The released free ubiquitin is attached to certain proteins via the ubiquitin-conjugation system. Mezquita et al. (Gene 195, 313-319, 1996) postulated that the increase in the levels of histone ubiquitin conjugates during chicken spermiogenesis may participate in the mechanism of histone replacement by protamin. Furthermore, ubiquitin conjugation plays a role for further structural changes of chromatin. It seems to be possible that the ubiquitin fused to ribosomal protein plays a role in kidney development.

Fig. 4: Protein alignment of the isolated *Squalus acanthias* protein sequence to the *Mus musculus* Ubiquitin B

The fourth sequence isolated from unborn shark kidney, shows homology to the HoxA3 sequence from the horn shark *Heterodontus francisci* (Kim et al. PNAS 97, 4, 1655 - 1660, 2000). The Hox gene clusters, first described in *Drosophila melanogaster*, control pattern formation along the anterior-posterior axis in bilateral animals (McGinnis et al. Cell 68, 283 - 302, 1992). In contrast to invertebrates, which possess a single Hox gene cluster, multiple Hox gene cluster have been reported in vertebrates.The noncoding sequence motifs conserved over 800 million years may function as genetic control motifs essential for the developmental process.

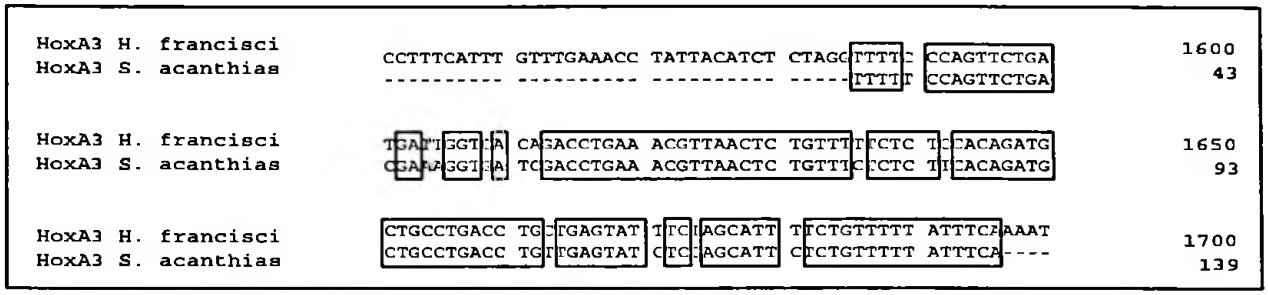

Fig. 5: DNA alignment of the isolated *S. acanthias* DNA sequence to the Hox A3 sequence from *Heterodontus francisci*

In summary, we isolated cDNAs coding for the skate hermes RNA-binding protein, for the shark aldehyde dehydrogenase and for the ubiquitin fused to ribosomal protein as well as a sequence containing a genetic control element HoxA3. All these sequences described play a role in developmental processes. Further experiments like quantitative RT-PCR, in situ-hybridization and differential screening will confirm the role of our candidate genes and will give us a deeper insight into the molecular biology of nephron development.