

CLONING, CHARACTERISATION AND TISSUE DISTRIBUTION OF AN AQUAPORIN-3 CDNA FROM FISH (*SPARUS AURATA*)



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Introduction-The major intrinsic protein (MIP) family consists of several transmembrane channel proteins specific for water and neutral solutes. All proteins belonging to the MIP family evolved from two divergent bacterial paralogues, one giving rise to the CHIP group, functionally characterised as water channels and the other to the GLP group, specialised in glycerol transport. Three forms of MIP proteins belonging to the GLP group have been identified in mammals: aquaporin-3 (AQP-3), aquaporin-7 (AQP-7) and aquaporin-9 (AQP-9). We have recently cloned and characterised a GLP cDNA from the marine teleost sea bream (*Sparus aurata*) and studied its tissue distribution. Phylogenetic analysis revealed it was most like AQP-3 and further studies are now underway to determine its role in hydromineral balance.

Methods

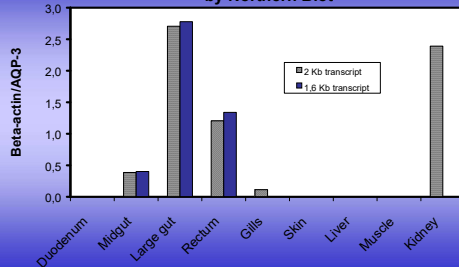
AQP-3 probe

Screening kidney cDNA library

Phylogenetic Analysis-was carried out by parsimony using PAUP. Mosquito AQP was used as an outgroup.

Northern Blot-10µg of mRNA from each tissue were separated on an agarose/formaldehyde gel, transferred to a nylon membrane and hybridised with sbAQP-3 and β-actin.

Tissue distribution of AQP-3 determined by Northern Blot



Quantification of Northern by phosphoimaging

Whole-mount in situ hybridization- was carried out on tissue slices, which were hybridised with the AQP-3 riboprobe labeled with digoxigenin. Note the intense signal in the hind gut.

Results

☺ Clone=1034bp
 ☺ 283 amino acids
 ☺ Two NPA motifs

