EXPRESSION OF AQP_{CIC} IN YEAST AND BACTERIA : SUBCELLULAR LOCALIZATION OF THE RECOMBINANT PROTEIN.

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Water crosses the plasma membrane of most cells by diffusion through the lipid bilayer, however particular cells exhibit high water permeability due to water selective membrane proteins. Such proteins have been recently identified and gathered in the aquaporin family. We have isolated and sequenced a full-length cDNA from a filter chamber library of *Cicadella viridis*, an homopteran insect. This cDNA encode for a 26 kDa protein whose sequence is 43% identical to the human water channel AQP1. Expression of this protein in Xenopus oocytes membranes led to a 15-fold increase of membrane water permeability. We concluded that this protein is the first insect aquaporin and called it AQPcic. In order to produce sufficient quantities of AQPcic for further functional and structural studies, we have cloned its cDNA in different expression vectors. Two systems have been used, bacteria (*Echerichia coli*) and yeast (*Saccharomyces cerevisiae*), and the expression have been analyzed by western blotting and immunocytochemistry. In bacteria, the data show that AQPcic is mainly produced in inclusions bodies. Indeed the protein was hard to solubilize and the product yield obtained was very low. In contrast, the expression of AQPcic in yeast gives a significant amount of protein (0.4 mg/l) and the protein is localized in all cell membranes. The recombinant AQPcic obtained using this procedure is currently functionally tested.

AQUAPORIN-RELATED PROTEINS IN THE FILTER CHAMBER OF HOMOPTERAN INSECTS.

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The filter chamber of homopteran insects is a water shunting complex in which water crosses plasma membranes through a transepithelial osmotic gradient. We previously purified and characterized from the plasma membranes of *Cicadella viridis* filter chamber a 25 kDa protein. Previous analysis showed that this protein belongs to the MIP family (major intrinsic family, a group of membrane channels for small solutes). We demonstrated that it functions as a water channel (or aquaporin) when present in the plasma membrane of xenopus oocytes. This protein was called AQPcic. In the present study, we used polyclonal antibodies raised against AQPcic for western blotting and immunocytochemical analysis of some homopteran insects intestinal tract. The species analyzed were : *Cercopis sanguinolenta, Philaenus spumarius, Aphrophora alni, Euscelidius variegatus* and *Scaphoideus titanus*. The latter insects are well known vectors for plant pathology through mycoplasma transmission during sap sucking process. Western blotting revealed that proteins immunologically related to the aquaporin from *Cicadella viridis* are found in all species. The molecular weights of these proteins are 24-28 kDa. This is consistent with the molecular weight values reported for known aquaporins from other biological systems (except for *Euscelidius variegatus* where a 15 kDa signal was obtained). Immunocytochemical studies on ultrathin sections revealed that the antibody systematically labelled the membrane microvilli of filter chamber epithelial cells. A good correlation thus exists between the presence of the aquaporin related proteins and the water transfer function of these cells.

THE LAMC1 PROMOTER CONTAINS TWO SEQUENCES, INTERACTING WITH THE LARGE SUBUNIT OF REPLICATION FACTOR C AND SP1, WHICH ARE INVOLVED IN LAMININ γ1 EXPRESSION IN FAZA 567 RAT HEPATOMA CELL LINE.

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Laminins, the major non-collagenous glycoproteins of all basement membranes are scarce in the normal adult liver. However, several laminin chains, particularly laminin γ1, can be expressed at high levels in various liver diseases, such as fibrosis and carcinogenesis, as well as in oth hepatocytes and hepatic stellate cells after a few days in culture and in hepatoma cell lines. We have investigated the molecular mechanisms involved in the expression of laminin γ1 in rat hepatoma cells. *cis* and *trans* acting elements were identified in the promoter region of LAMC1 gene coding for laminin γ1. Transfection of deletion mutants of the 5' flanking region of murine LAMC1 gene in the Faza 567 hepatoma cell line indicated that two enhancers and a silencer segment which contains GC-and CTC-containing sequences, were located between -594 bp and -94 bp. Two complexes were formed when nuclear extracts from hepatoma cells were used in gel-shift analyses with double-stranded synthetic oligonucleotides corresponding to the CTC- and GC-containing regions. Transactivation of LAMC1 promoter was achieved when cotransfected in *Drosophila* cells with a Sp1 expression vector. Immunocytochemistry, western-blotting and northern-blot analyses indicated that Faza 567 hepatoma cells expressed both Sp1 and A1p145, the large subunit of replication factor C (RFC), which interacted respectively with the GC- and CTC-motives in the silencer regulatory element of LAMC1 promoter. A combination of immunoprecipitation and western-blotting with Faza 567 cell extracts revealed that A1p145 was in complex with PCNA. These data show that LAMC1 gene expression in Faza 567 hepatoma cells is governed by nuclear factors interacting with CTC- and GC-rich regions within the promoter, including RFC and Sp1, thus suggesting that there is a relationship between laminin γ1 expression and the cell cycle.

OVEREXPRESSION OF MATRIX METALLOPROTEINASE-2 AND THE INHIBITOR TIMP2 IN LIVERS FROM PATIENTS WITH GASTROINTESTINAL ADENOCARCINOMAS AND NO DETECTABLE METASTASIS.

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We have investigated whether basement membrane remodeling may play a role in the invasion of metastatic cells in the target liver. The expression of both MMP2 and TIMP2 was analyzed in livers from patients with gastrointestinal adenocarcinomas and no detectable hepatic metastasis (n=12), in matching pairs of tumoral and non tumoral livers from patients with hepatic metastasis (n=9) and in control explanted livers (n=4). By semi-quantitative RT-PCR, the steady-state MMP2 and TIMP2 mRNA levels in livers from patients with hepatic metastasis were higher in tumoral areas compared to non-tumoral areas with a tumoral/non tumoral ratio of 6.2 ± 4 and 1.6 ± 0.8, respectively. By zymography, MMP2 activity was resolved as latent and active forms in the tumoral areas whereas in non tumoral areas, the latent form was predominant. In situ hybridization demonstrated MMP2 and TIMP2 transcripts in the stromal cells of liver metastases. In livers from patients with gastrointestinal adenocarcinomas and no detectable metastasis, MMP2 and TIMP2 mRNA levels were increased compared with those of either control livers (5-fold and 3.2-fold, respectively) or non tumoral areas of livers from patients with metastases (7.8-fold and 3-fold, respectively). MMP2 activity was detected by zymography mainly in latent form. In situ hybridization revealed no change in the localisation of MMP2 and TIMP2 transcripts, compared with non tumoral or control livers. These data suggest that primary gastrointestinal adenocarcinomas induce the overexpression of both MMP2 and TIMP2 mRNA in the target liver, before metastasis become detectable.

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