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Distribution of mRNA Encoding the FA-CHIP Water Channel in Amphibian Tissues: Effects of Salt Adaptation

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Abstract. A water channel, the frog aquaporin-CHIP (FA-CHIP) was recently cloned from *Rana esculenta* urinary bladder. The 28.9 kDa encoded protein shows 78.8%, 77.4%, 42.4% and 35.6% identity with rat CHIP28, human CHIP28, rat WCH-CD and γ-TIP, other members of the new transmembrane water channel family (Aquaporin-CHIP). We have now studied membranes from different frog (*R. esculenta*) organs employing semiquantitative PCR using FA-CHIP specific primers and an internal standard to quantify the PCR products. The FA-CHIP mRNA was abundantly expressed in the frog urinary bladder, skin, lung and gall bladder, while a lower expression was detected in the colon, liver and oviduct. FA-CHIP mRNA was not detected in the frog kidney, erythrocytes and brain but its expression was observed in the toad (*Bufo arenarum*) urinary bladder and skin, showing that FA-CHIP is probably a general amphibian water channel. Salt acclimation is known to increase the water permeability of frog and toad epithelia. We have now observed that salt acclimation for 1, 3, 4 or 5 days markedly increased skin and urinary bladder FA-CHIP mRNA expression. It is generally accepted that water permeability is controlled in these tissues by the rate of water channel transfer from subapical vesicles (aggrephores) to the apical membrane. Our results indicate that water permeability is also regulated at the level of the FA-CHIP transcription.

Key words: Frog and toad urinary bladder and skin — Osmotic water permeability — Channel-forming integral protein (CHIP28) — Salt acclimation — RT-PCR

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Introduction

It has been recently demonstrated that water movements across cell membranes can occur via intrinsic membrane proteins that form channels for water molecules. One of these, described in mammalian tissues is the channel-forming 28 kDa integral protein, CHIP28, which is the major water channel of human erythrocytes (Preston & Agre, 1991). It is also present in the rat renal proximal tubule and thin descending limbs of the Henle's loop (Deen et al., 1992; Nielsen et al., 1993*c;* Zhang et al. 1993), and is found in several other rat water transporting tissues (Moon et al., 1993). An additional member of this family of water channel proteins, referred to as the aquaporin family (Agre et al., 1993), is expressed in the renal collecting duct, WCH-CD (Fushimi et al., 1993). On the basis of its localization, WCH-CD has been proposed to be the vasopressin-regulated water channel. Vasopressin (antidiuretic hormone) regulates body water balance by controlling renal collecting duct water permeability and a similar effect is found in two other vasopressin-sensitive epithelia: amphibian skin (Brown, Grosso & De Sousa, 1990) and amphibian urinary bladder (Chevalier, Bourget & Hugon, 1974).

A fully sequenced cDNA encoding a new member of the aquaporin-CHIP family was recently cloned from frog (*R. esculenta*) urinary bladder, a model for the kidney collecting duct (Abrami et al., 1994). The encoded protein, designated FA-CHIP (Frog Aquaporin-CHIP) corresponds to a predicted protein of 28.9 kDa with 78.8%, 77.4%, 42.4% and 35.6% identity with rat kidney CHIP28, human ertyhrocytes CHIP28, rat kidney WCH-CD and plant γ-TIP (Maurel et al., 1993), respectively. The water channel function of FA-CHIP was identified and characterized with the *Xenopus laevis* oocyte expression system.

Water permeable epithelia exist in several tissues

and it is not known whether a single set of channels could explain large water fluxes observed across these barriers. The distribution of FA-CHIP water channel in toad and frog tissues was examined in the present study using semiquantitative Polymerase Chain Reaction (PCR) with specific primers. Because some of the tested tissues showed a low expression of FA-CHIP, we developed a semiquantitative PCR assay, adapted to this experimental situation.

The other aim of this work was to study the level of FA-CHIP mRNA in the epithelial cells in response to salt acclimation. Some amphibia can be acclimated to high salt-content media. This adaptation induces an enhanced water permeability in the skin of the toad *Bufo viridis* (Katz & Ben-Sasson, 1984) and in the urinary bladder of the frog *R. esculenta* (Verbavatz et al., 1992) together with an increase in the Intramembrane Particle Aggregates (IMPA) covering an unusually large surface area of cell apical plasma membrane.

Our study with salt-adapted animals made it possible to determine the increase of the FA-CHIP transcription during the acclimation.

Materials and Methods

Salt Acclimation of Amphibia

Female frogs (*R. esculenta*) or toads (*B. arenarum*) were kept unfed for one week in the laboratory at room temperature. Control animals were kept in running tap water. Those to be salt acclimated were kept for 5 days to 1 day in Ringer solution (in mM): 2.5 $NaHCO_3$, 5 KCl, 112 NaCl, 1 $CaCl_2$; 245 mOsm/kg H_2O, pH = 8.1, which was changed daily.

RNA Extraction

Total RNAs were extracted from different amphibian tissues using an adaptation of the method described by Chomczynski and Sacchi, 1987. *R. esculenta* or *B. arenarum* were pithed and perfused via the ventricular cavity with a fresh phosphate buffered saline solution. Urinary bladder and other tissues were rapidly transferred into 1 ml of the denaturing solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH = 7), 0.5% sarcosyl, and 0.1 mM β-mercaptoethanol. The suspension was immediately mixed using a glass homogenizer. Successively, 100 µl of 2 M CH_3CO_2Na (pH 4), 1 ml of water-saturated phenol, and 200 µl of chloroform were added, with 20 s vortexing after addition of each reagent. The final suspension was cooled on ice for 15 min and then centrifuged at 10,000 *g* for 20 min at 4°C. All the further steps were carried out at 4°C. The supernatant was transferred to a fresh tube and mixed with 1 ml of ice-cold isopropanol. Tubes were centrifuged at 17,000 *g* for 30 min and the RNA pellets were dissolved in 300 µl of the denaturing solution. After adding 100 µl of ice-cold isopropanol, centrifugation was again performed for 20 min. The RNA pellets were washed twice in 75% ethanol, sedimented, and vacuum dried. The final pellets were dissolved at 0.2 µg/µl in RNA dilution buffer (in mM): 10 Tris-HCl, 1 EDTA, pH = 7.6; 2 DTT; acetyl BSA, 100 µl/ml; RNAsin, 40 U/ml and stored at −80°C until RT-PCR. The amount of RNA was evaluated by optical density at 260 nm.

Semiquantitative PCR Assay

In tissues with a low expression of FA-CHIP mRNA, the RT-PCR assay requires a large number of PCR cycles to generate detectable amounts of DNA molecules. Under these conditions, exponential accumulation is not maintained throughout the PCR reaction, resulting in a lack of proportionality between the amount of targeting mRNA in the sample and the DNA products. To overcome these limitations, a semiquantitative assay was developed. To reduce the number of cycles and make the detection threshold of the method lower, a radiolabeled deoxynucleotide and an internal standard were introduced in the reaction to quantify the DNA products and thus to determine the amount of coamplified mRNA extracted from various amphibian tissues.

RT-PCR with specific FA-CHIP primers K and E, giving an internal DNA fragment of 588-bp from frog urinary bladder cell mRNA, was performed (Fig. 1). The semiquantitative PCR assay we have developed requires a standard as close as possible to the DNA of interest. This assay was carried out using a mutant FA-CHIP cDNA: 81 bp were removed from pFA-CHIP between NarI (nt 418) and NciI (nt 499) restriction sites. The resulting mutant-507bp fragment given by primers K and E was used as an internal standard (Fig. 1). Reverse transcription was performed on 1 µg of RNA of different tissues using 10 pmol of downstream primer E for 45 min at 42°C with 400 µM of each dNTP and 200 U of Moloney Murine Leukemia virus Reverse Transcriptase (Gibco BRL). The temperature was raised to 96°C for 30 s to inactivate the enzyme. The mixture was PCI extracted and precipitated with ethanol in the presence of tRNA. The pellet was resuspended in 100 µl H_2O and a 4 µl fraction was used for PCR amplification. In order to compare the efficiency of each reaction tube and to minimize sample handling and contamination, wild-type FA-CHIP cDNA and a precise amount (10^5 molecules) of mutant FA-CHIP cDNA were coamplified in the same tube with the same primers. The PCR (23 cycles at 96°C, 30 s; 52°C, 30 s and 72°C, 75 s) was carried out using 50 µM of each dNTP, containing 1 µCi/nmol of [α-^{32}P] dCTP and 10 pmoles of each E and K primer. The number of cycles was minimized to avoid saturation of the reaction. Wild-type and mutant DNA fragments on each tissue were discriminated and evaluated by 2% agarose gel electrophoresis (10 µl of each reaction tube). The gel was fixed in 10% acetic acid, dried on 3 MM Whatman paper at 70°C and autoradiographs were obtained.

To study the effects of salt adaptation on FA-CHIP mRNA level, RT-PCR were performed on 1 µg of urinary bladder or skin RNA from frog (*R. esculenta*) or toad (*B. arenarum*). The reactions took place under the same conditions as those in the FA-CHIP distribution studies but without radiolabeled deoxynucleotides. In fact, the high level of FA-CHIP mRNA expression in the skin and urinary bladder of amphibia does not require radioactive analysis. Only 28 cycles are necessary to detect a signal with the sensitivity of ethidium-bromide staining, we thus avoid saturation of the PCR reactions. To test the efficiency of the reverse transcription with each RNA sample, we used specific *Xenopus* actin primers in the same RT-PCR conditions. To quantify the PCR products, 2% agarose gels were analyzed with an LKB scanner (Ultroscan 2202). The comparison of percentages of amplification between the PCR products of different salt acclimation times and the tap water animals PCR products was carried out using the internal standard PCR product intensity.

Results

FA-CHIP mRNA Expression in Amphibian Tissues: Detection by RT-PCR

Several frog (*R. esculenta*) organs were dissected from which RNA was isolated. The expression of FA-CHIP

in frog tissues was performed with an assay based on Reverse Transcription and Polymerase Chain Reaction (RT-PCR) using FA-CHIP specific primers and quantified by the addition of a truncated FA-CHIP cDNA as internal standard (Fig. 1).

A 507-bp PCR product specific for truncated FA-CHIP was detected in all lanes and the PCR product specific for FA-CHIP could be detected in the lanes corresponding to the frog tissues where FA-CHIP mRNA was expressed (Fig. 2*A*). The level of expression differed significantly between tissues. When normalized to the amount of RNA loaded (1 μg) and for the reaction efficiency per tube through the DNA product obtained with the truncated FA-CHIP cDNA, the amount of FA-CHIP mRNA was the highest for urinary bladder and the skin, while expression was half this level in lung and gall bladder. In the colon, liver and oviduct, the level of FA-CHIP mRNA expression was approximately fourfold lower, while in the lens and oocytes, FA-CHIP mRNA expression was hardly detectable and at least ten times lower than in the urinary bladders. In spite of longer autoradiographic exposure, FA-CHIP signals were not detected in the lanes corresponding to brain, kidney and erythrocytes. A summary of all RT-PCR experiments performed with frog tissues is given in Fig. 2*B*. The 588-bp band, specific for urinary bladder FA-CHIP, always had the same size, confirming the lack of PCR product heterogeneity. This indicates that FA-CHIP, in most urinary bladders, skins, gall bladders and lungs, is probably the product of the same gene and not homologous genes.

Effect of Salt Adaptation on FA-CHIP mRNA Level in Amphibian Urinary Bladder and Skin

To determine whether FA-CHIP mRNA in amphibian urinary bladder and skin was increased under antidiuretic conditions, frogs (*R. esculenta*) and toads (*B. arenarum*) were kept under salt acclimation for 0, 1, 3, 4 or 5 days. RT-PCR analyses of RNA isolated from urinary bladder or skin from salt-acclimated frog or toad were carried out. The first result from these experiments was that RT-PCR on amphibia showed the presence of FA-CHIP mRNA in the urinary bladder and skin of the toad (*B. arenarum*) indicating that FA-CHIP is a general amphibian water channel. Furthermore, the RT-PCR results (Fig. 3*A*) showed significant changes in the amount of FA-CHIP mRNA present under the various salt-adaptation conditions. The conclusion is that the expression of mRNA encoding FA-CHIP was increased in urinary bladder and skin from frogs and toads that were salt acclimated. Fig. 3*B* shows no change in *Xenopus* actin mRNA expression level between three different RNA preparations (5-, 3-, 0-day salt adaptation). This result demonstrates that in this experiment, we have the same

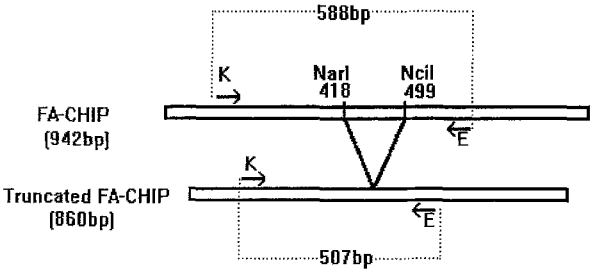


Fig. 1. Construction of an FA-CHIP mutant. FA-CHIP was obtained by a deletion of 81-bp between the restriction site NarI (nt 418) and NciI (nt 499) of the clone pFA-CHIP. The resulting 860-bp mutant given in PCR with sense primer K (5′-GCGAGATCTA ATG GCG AGC GAA TTC AAG AAG A-3′) and antisense primer E (5′-GTT CAT TCC ACA TCC AGT GTA GTC-3′) an internal standard of 507-bp whereas the wild type resulting fragment has a length of 588-bp.

efficiency of the reverse transcription and probably the same amount of actin mRNA in each sample. The internal standard was used to quantify the PCR products of the salt adaptation studies. In urinary bladder isolated from 5-, 3-, 1-day salt-acclimated frogs, the level of FA-CHIP mRNA expression was 4.5, 4.7 and 1.8 times higher, respectively, than in the bladder of frogs acclimated to tap water (Figs. 3*B* and 4). In the toad bladder, the level of FA-CHIP mRNA was also increased by salt acclimation. In 5- and 4-day salt-acclimated toad bladders, the FA-CHIP mRNA level was approximately 10 and 8 times higher, respectively, than in the bladder of toads maintained in tap water (Fig. 3*A*). These data are in agreement with the fact that in salt-acclimated frogs, water bladder permeability is markedly higher than in tap water-acclimated animals (Verbavatz et al., 1992) and with the in vitro experiments showing that isolated urinary bladder bathed in serosal hyperosmotic fluid increases its water permeability (Bentley, 1964; Ripoche, Parisi & Bourguet, 1969).

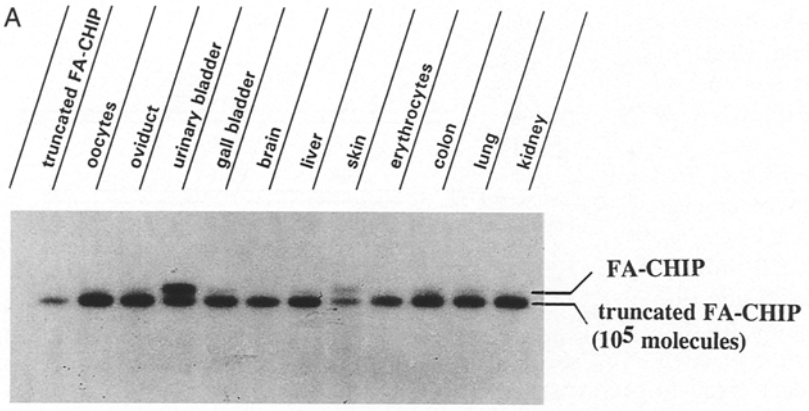
In frog skin, the level of FA-CHIP mRNA expression at 5- or 3-day salt acclimation is approximately the same, but 2.4 times higher than at 1-day adaptation or 3.6 times higher than in the absence of adaptation (Figs. 3*B* and 4). For toad skin, the level of FA-CHIP mRNA expression was weakly detectable in control animals but was approximately seven times higher after 5 or 4 days of salt acclimation (Fig. 3*A*). Thus, these results also demonstrate that the expression of the FA-CHIP gene is upregulated with respect to the number of days of salt adaptation of the amphibia for at least up to 3 or 5 days.

Discussion

The Presence of the FA-CHIP Water Channel in Different Amphibian Epithelial Barriers

The recent description of the Frog Aquaporin-CHIP (FA-CHIP) in *R. esculenta* urinary bladder, an integral mem-

A



B

RT-PCR	Urinary Bladder	Skin	Gall Bladder	Oviduct	Brain	Lung	Blood	Liver	Colon	Kidney	Oocyte	Lens
Exp. 1	+++	+++	++	++	-	++	-	+	+	-	-	-
Exp. 2	+++	+++	++	+	-	++	-	+	+	-	+	-
Exp. 3	+++	+++	++	+	-	++	-	-	+	-	-	-
Exp. 4	+++	+++	++	++	-	+	-	+	+	-	-	+
Exp. 5	+++	+++	++	+	-	++	-	+	+	-	+	-
Exp. 6	+++	+++	++	+	-	++	-	+	+	-	+	+
Exp. 7	+++	+++	++	+	-	++	-	+	+	-	+	-

Fig. 2. Expression of the FA-CHIP water channel at the mRNA level in amphibian tissues. (*A*) Autoradiography of agarose gel electrophoresis of RT-PCR products for FA-CHIP mRNA on various frog tissues. Coamplification of FA-CHIP. cDNA and mutant FA-CHIP cDNA were done: different frog RNA samples (1 μg of each) and a constant amount (10^5 molecules) of truncated FA-CHIP cDNA as internal standard were amplified simultaneously using K and E primers (23 cycles at 96°C, 30 s; 52°C, 30 s and 72°C, 75s). Wild-type DNA fragment 588-bp and the mutant DNA fragment 507-bp were fractionated on a 2% agarose gel and detected by autoradiography. (*B*) Summary of each RT-PCR experiment on amphibian tissues to determine the distribution of FA-CHIP. All the experiments were done under the conditions described in 2*A*. The tissues were all extracted from the same frog (*R. esculenta*) and 1 μg of RNA of each tissue was used for the reactions. Only experiments 1 and 2 were done without the truncated FA-CHIP internal standard. The level of expression differed significantly among tissues. We can detect several levels of signals: The more intense (+++) was obtained with urinary bladder, the absence of signal was indicated (–).

brane protein of 28 kDa, and isolation of its cDNA made it possible to identify an amphibian water channel. This was achieved by expression of FA-CHIP in *Xenopus* oocytes. As in water channels in native membranes, FA-CHIP mediated water transport and was reversibly inhibited by p-chloromercuriphenylsulfonic acid, pCMBS (Abrami et al., 1994).

In this work, the expression of FA-CHIP in different amphibian species and tissues as well as the expression level under salt acclimation were studied. Epithelial cells of multiple organs were studied by semiquantitative PCR. The strongest expression of FA-CHIP was found in the urinary bladder and skin. There was also a clear expression in lung and gall bladder. The expression of FA-CHIP in the lung, like CHIP28 in rat lung (Nielsen et al., 1993*b*), suggests that water movements between blood and airways may be mediated by water channels. Lung water channels may be essential to maintain airspace fluid constant during evaporative losses that accompany amphibian respiration. FA-CHIP was also present in the colon, liver and oviduct. The expression in the colon, which was also observed in the mammalian colon (Hasegawa et al., 1994), could be explained by the fluid transport that is required for feces dehydration. Finally, a very low expression was detected in the lens and oocytes. FA-CHIP mRNA was not detected in the brain, erythrocytes and kidneys. The lack of FA-CHIP expression that is observed in kidneys probably results from the complete failure of frogs to form hypertonic glomerular filtrate. Thus, our results show that FA-CHIP is widely expressed in epithelial cells of frogs and toads, and it can be proposed that FA-CHIP is an important mediator of the transmembrane water transport in amphibian epithelia. Similar studies were previously reported for other members of the Aquaporin CHIP family. The CHIP28 water channel is widely expressed in many fluid transporting tissues in mammals, including kidney proximal tubules and thin descending limbs of Henle's loop, choroid plexus, ciliary body, airway epithelium and alveolus, sweat gland, pancreatic ducts, gall bladder, male reproductive tract (the efferent ducts) (Brown et al., 1993; Moon et al., 1993; Nielsen et al., 1993*b*). The divergent pattern of FA-CHIP expression in diverse amphibian tissues compared with mammalian tissue distribution and the lack of FA-CHIP in certain water permeable epithelia like kidneys, suggest that other water channels may exist at these locations in amphibian tissues.

In contrast, the WCH-CD water channel is expressed only in the mammalian kidney collecting duct (Fushimi et al., 1993; Nielsen et al., 1993*a*).

Effect of Salt Adaptation in the FA-CHIP Expression in Frogs and Toads

Frog and toad acclimation to a salt medium was found to upregulate the FA-CHIP mRNA expression, and expres-

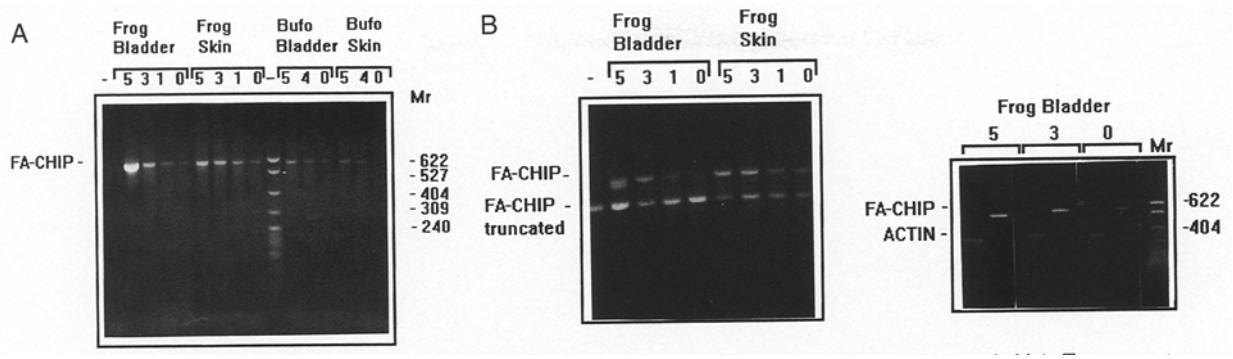


Fig. 3. Effect of salt adaptation on FA-CHIP mRNA level. (*A*) RT-PCR on frog and toad mRNA (bladder and skin). Two-percent agarose gel electrophoresis of RT-PCR products for FA-CHIP mRNA on urinary bladder and skin from frog (*R. esculenta*) and toad (*B. arenarum*). The amphibia were kept under salt acclimation conditions for 5, 4, 3, 1 or 0 days. In the first lane, the RT-PCR reaction was carried out in the absence of RNA but in the presence of Reverse Transcriptase and TAQ polymerase. In the other lanes, a single band is detected at 588-bp corresponding to the FA-CHIP fragment obtained with K and E primers (*see* Fig. 1). (*B*) Semiquantitative PCR on frog bladder and skin mRNA. Left: 2% agarose gel electrophoresis of RT-PCR products for FA-CHIP mRNA on urinary bladder and skin from frogs (*R. esculenta*). In the first lane, the RT-PCR reaction was carried out in the absence of RNA but in the presence of Reverse Transcriptase, TAQ polymerase and FA-CHIP truncated cDNA. In the other lanes, two bands are detected at 588-bp and 507-bp corresponding to the FA-CHIP fragment obtained with K and E primers and the mutant FA-CHIP fragment obtained with the same primers. The frogs were kept under salt acclimation conditions for 5, 3, 1 or 0 days. Right: 2% agarose gel electrophoresis of RT-PCR products for actin or FA-CHIP mRNA on frog urinary bladder under various salt acclimation conditions (5, 3 or 0 days). The RT-PCR reactions were carried out in the first lines with specific *Xenopus* actin primers (sense: 5′-CCTGAAGAACACCCCACCCTGCTC-3′ and antisense: 5′-TGCCATCTCATTCTCAAAGTCCAA-3′) or in the second lines with specific FA-CHIP K and E primers (*see* Fig. 1).

sion of the gene increased during salt adaptation. Several authors have reported (Katz & Ben-Sasson, 1984; Verbavatz et al., 1992) that in salt-acclimated frogs or toads, skin and bladder water permeability was markedly higher. The salt medium was found to induce several modifications of water transport properties in the urinary bladder: (i) the bladder surface and urine volume were increased, (ii) the relative surface area of the epithelial apical plasma membrane covered by intramembrane particle aggregates (IMPA) was also larger. The hyperosmotic body fluids of salt-acclimated frogs seem to induce the insertion of extra water channels into the bladder apical plasma membrane. A similar effect was found in other vasopressin-sensitive epithelia, the amphibian skin (Brown et al., 1990). However, from these experiments it was not possible to know whether this insertion of IMPA, probably corresponding to water channels, requires the neosynthesis of additional channels or just the fusion of a larger quantity of the channels available in the cytoplasmic vesicles (aggrephores) into the apical plasma membranes (Wade, 1980).

Our data indicate that salt acclimation increases the transcription of FA-CHIP in the skin and bladder of amphibia. The upregulation of the rat collecting duct water channel measuring WCH-CD transcript level after dehydration (Fushimi et al., 1993; Nielsen et al., 1993*a*) has also been demonstrated and the increase of mRNA encoding WCH-3, a new specific rat kidney water channel (Ma et al., 1993), was also found when animals were dehydrated by 14–21 weight percent. This common property suggests that FA-CHIP could be intramembrane particles of aggregates (IMPA) in amphibian tissues.

The Aquaporin Family and the FA-CHIP: Structure Similarities and Possible Functions

The transcriptional upregulation of FA-CHIP salt acclimation supports the hypothesis that the FA-CHIP water channel could be considered to be the ADH-sensitive one. While the homology with WCH-CD is low, we cannot exclude this possibility. Indeed, the criteria of sequence homologies in the MIP family that reflect a functional specificity appear to be inadequate. A better homology was found between MIP26 (Gorin et al., 1984), which is not a specific way for water, and constitutive water channel (CHIP28) than between different water channels (CHIP28, WCH-CD or γ-TIP).

The FA-CHIP tissue localization is similar to the CHIP28 distribution. As CHIP28, high FA-CHIP mRNA expression was found selectively in epithelial cells that are believed to be involved in fluid absorption and/or secretion, and in selected endothelia that are probably involved in transcapillary water movements. FA-CHIP amino-acids sequence is related more to the constitutive rat CHIP28 water channel (78.8%) than to the ADH-sensitive rat WCH-CD water channel (42.4%). In addition, FA-CHIP has functional properties similar to those found in kidney proximal tubules and in erythrocytes, where the CHIP28 was described (Deen et al., 1992; Preston et al., 1992; Nielsen et al., 1993*c;* Zhang et al., 1993). All these facts agree with the second hypothesis that FA-CHIP water channels could be, as CHIP28, a constitutive water channel, responsible for water transport across basolateral membrane channels that are permanently present in these membranes (Van Der Goot,

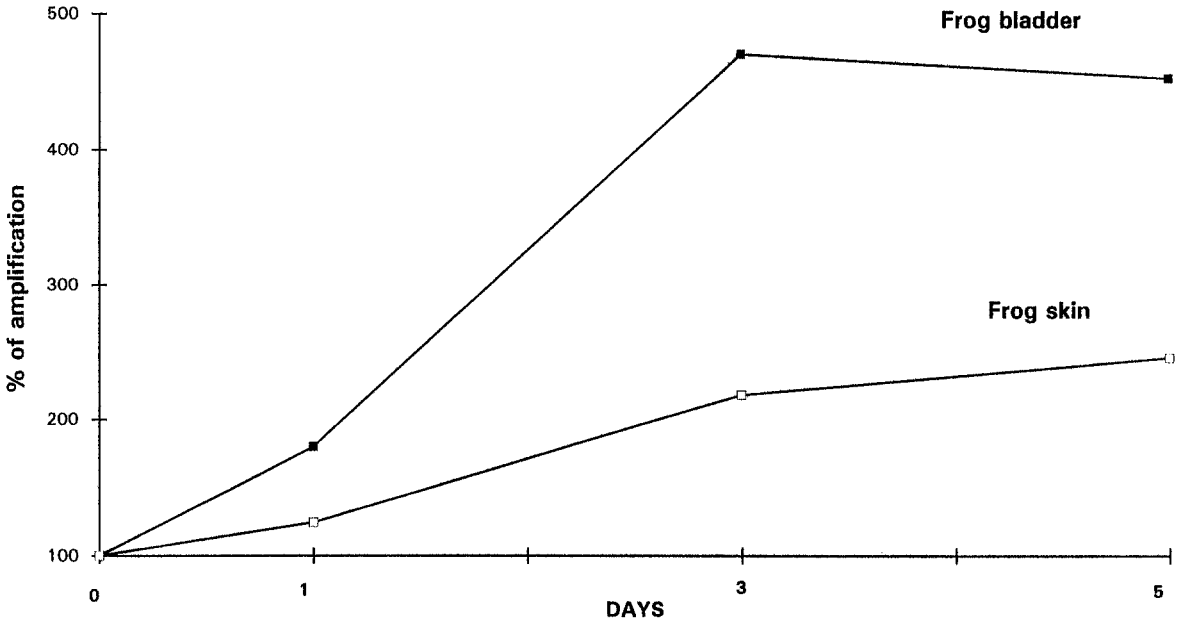


Fig. 4. Study of the percent amplification of PCR products as a function of salt-adaptation times. The graph shows that the percentage of the PCR products increases at 1, 3 and 5 days from the PCR products obtained from tap water animals used as controls (100%).

Corman, & Ripoche, 1991). However, several studies have shown that the expression of CHIP28 is not affected by dehydration of the animals (Deen et al., 1992; Nielsen et al., 1993*c*) while, Smith et al. (1993) showed a relationship between CHIP28 detection in kidney epithelial cells and the beginning of the urine concentration process of the newborn rat.

The FA-CHIP protein may be a CHIP28-like protein, specific for amphibia, permanently present in basolateral membranes with its own regulation of the FA-CHIP mRNA level expression. This mRNA level expression induction could be closely related to the amphibia medium conditions and could be absent in mammals. These data confirm that related but distinct water channel proteins exist in mammals, plants, amphibia, and probably other organisms, with different physiological functions.

References

Abrami, L., Simon, M., Rousselet, G., Berthonaud, V., Buhler, J.M., Ripoche, P. 1994. Sequence and functional expression of an amphibian water channel, FA-CHIP: a new member of the MIP family. *Biochim. Biophys. Acta* **1192:**147–151

Agre, P., Preston, G.M., Smith B.L., Jung, J.S., Raina, S., Moon, C., Guggino, W.B., Nielsen, S. 1993. Aquaporin CHIP: the archetypal molecular water channel. *Am. J. Physiol.* **265:**F463–F476

Bentley, P.J. 1964. Physiological properties of the isolated frog bladder in hyperosmotic solutions. *Comp. Biochem. Physiol.* **12:**233–239

Brown, D., Grosso, A., De Sousa, R.C. 1990. Membrane architecture and water transport in epithelia cell membranes. *In:* Membrane transport and information storage. Alan R. Liss, Inc. editor. pp. 103–132

Brown, D., Verbavatz, J.M., Valenti, G., Lui, B., Sabolić, I. 1993. Localization of the CHIP28 water channel in reabsortive segments of the rat male reproductive tract. *Eur. J. Cell Biol.* **61:**264–273

Chevalier, J., Bourguet, J., Hugon, J.S. 1974. Membrane associated particles: distribution in frog urinary bladder epithelium at rest and after oxytocin treatment. *Cell Tissue Res.* **152:**129–140

Chomczynski, P., Sacchi, N. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:**156–159

Deen, P.M.T., Dempster, J.A., Wieringa, B., Van Os, C.H. 1992. Isolation of a cDNA for rat CHIP28 water channel: high mRNA expression in kidney cortex and inner medulla. *Biochem. Biophys. Res. Commun.* **188:**1267–1273

Fushimi, K., Uchida, S., Hara, Y., Hirata, Y., Marumo, F., Sasaki, S. 1993. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* **361:**549–552

Gorin, M.B., Yancey, S.B., Cline, J., Revel, J.P., Horwitz, J. 1984. The major intrinsic protein (MIP) of the bovine lens fiber membrane: characterization and structure based on cDNA cloning. *Cell* **39:**49–59

Hasegawa, H., Lian, S.C., Finkbeiner, W.E., Verkman, A.S. 1994. Extrarenal tissue distribution of CHIP28 water channels by in situ hybridization and antibody staining. *Am. J. Physiol.* **266:**C893–903

Katz, U., Ben-Sasson, Y. 1984. A possible role of the kidney and urinary bladder in urea conservation of *Bufo viridis* under high salt acclimation. *J. Exp. Biol.* **109:**373–377

Ma, T., Frigeri, A., Skach, W., Verkman, A.S. 1993. Cloning of a novel rat kidney cDNA homologous to CHIP28 and WCH-CD water channel. *Biochem. Biophys. Res. Commun.* **197:**654–659

Maurel, C., Reizer, J., Schroeder, J.I., Chrispeels, M.J. 1993. The vacuolar membrane protein γ-TIP creates water specific channels in *Xenopus* oocytes. *The EMBO Journal* **12:**2241–2247

Moon, C., Preston, G.M., Griffin, C.A., Wang Jabs, E., Agre, P. 1993. The human Aquaporin-CHIP gene. *J. Bio. Chem.* **268:**15772–15778

Nielsen, S., Digiovanni, S.R., Christensen, E.I., Knepper, M.A., Harris,

H.W. 1993a. Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. *Proc. Natl. Acad. Sci. USA* **90:**11663–11667

Nielsen, S., Smith, B.L., Christensen, E.I., Agre, P. 1993b. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. *Proc. Natl. Acad. Sci. USA* **90:**7275–7279

Nielsen, S., Smith, B.L., Christensen, E.I., Knepper, M.A., Agre, P. 1993c. CHIP28 water channels are localized in constitutively water-permeable segments of the nephron. *J. Cell Biol.* **120:**371–383

Preston, G.M., Agre, P. 1991. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: Member of an ancient channel family. *Proc. Natl. Acad. Sci. USA* **88:**11110–11114

Preston, G.M., Carroll, T.P., Guggino, W.B., Agre, P. 1992. Appearance of water channel in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science* **256:**385–387

Ripoche, P., Parisi, M., Bourguet, J. 1969. Mechanism of the antidiuretic hormone-like action of hypertonic media on the frog urinary bladder. *Biochim. Biophys. Acta* **193:**231–234

Smith, B.L., Baumgarten, R., Nielsen, S., Raben, D., Zeidel, M.L., Agre, P. 1993. Concurrent expression of erythroid and renal aquaporin CHIP and appearance of water channel activity in perinatal rats. *J. Clin. Invest.* **92:**2035–2041

Van Der Goot, F., Corman, B., Ripoche, P. 1991. Evidence for permanent water channels in the basolateral membrane of an ADH-sensitive epithelium. *J. Membrane Biol.* **120:**59–65

Verbavatz, J.M., Frigeri, A., Gobin, R., Ripoche, P., Bourguet, J. 1992. Effects of salt acclimation on water and urea permeabilities across the frog bladder: relationship with intramembrane particle aggregates. *Comp. Biochem. Physiol.* **4:**827–833

Wade, J.B. 1980. Hormonal modulation of epithelium structure. *In:* Current topics in membranes and transport. F. Bronner, editor. pp. 123–147. Academic Press, New York

Zhang, R., Skach, W., Hasegawa, H., Van Hoek, A.N., Verkman, A.S. 1993. Cloning, functional analysis and cell localization of a kidney proximal tubule water transporter homologous to CHIP28. *J. Cell Biol.* **120:**359–369