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Short Sequence-Paper

Sequence and functional expression of an amphibian water channel, FA-CHIP: a new member of the MIP family

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Abstract

A new member of the family of water channel proteins (aquaporin-CHIP) related to the major intrinsic protein (MIP) family is described. The cDNA coding for this amphibian CHIP was cloned from frog (*Rana esculenta*) urinary bladder, a model for the kidney collecting duct, using a RT-PCR cloning strategy. The encoded protein, designated FA-CHIP (frog aquaporin-CHIP), shows 77.4%, 42.4% and 35.6% identity with the three proteins now referred to as the aquaporins of the MIP family, i.e., human CHIP28, WCH-CD and γ-TIP, respectively. *Xenopus leavis* injected with FA-CHIP cRNA exhibited a marked increase of the osmotic water permeability.

Key words: Major intrinsic protein (MIP); Channel forming integral protein (CHIP28); Osmotic water permeability; Amphibian urinary bladder; RACE PCR; (*R. esculenta*)

A large family of integral membrane proteins related to major intrinsic protein of lens, MIP26, has been identified in diverse organisms [1,2]. The structure of these proteins suggests that they may be transmembrane channels, but the physiological functions of most are undefined. Three proteins of the the family, now referred to as the 'aquaporins' [3], facilitate rapid and selective movement of water across plasma membrane: (1) the aquaporin-CHIP28 initially identified and purified from human red blood cells [4] where it provides osmoregulation of volume, and also from rat kidney [5,6], where it promotes permanent reabsorption of water; (2) the WCH-CD presented as the vasopressin-activated water channel of rat kidney that permits maximal water reabsorption by renal collecting ducts [7]; (3) the γ-TIP found in the vacuolar membrane (tonoplast) of certain plants where it facilitates rapid exchange of water between the vacuole and the cytoplasm [8]. So far, the distribution of both renal water channels is not clearly defined [5,6,7,9] and their supposed sensitivity to vasopressin has been indirectly deduced from their localization. To approach this problem, we have isolated and fully sequenced a complementary DNA coding for a new aquaporin-CHIP from the frog urinary bladder, the frog aquaporin-CHIP (FA-CHIP). Indeed, the amphibian urinary bladder is an easily accessible model for water transport studies and particularly for vasopressin-stimulated water permeability in mammalian kidney.

Cloning and sequencing of the FA-CHIP cDNA. The full-length cDNA coding for FA-CHIP was obtained by a three step RT-PCR amplification on frog urinary bladder cells RNA template (Fig. 1). Frog urinary bladder RNA was used in a reverse-transcribed polymerase chain reaction (RT-PCR) with two degenerate primers corresponding to the conserved amino-acid sequences of the MIP26 family members, enclosing the tandem repeat NPA (Asn-Pro-Ala): nucleotides 214–239 (primer A) and 549–591 (primer B) of human aquaporin-CHIP28 [4]. The sequence of one PCR clone pE6 of 378-bp represented a part of a new protein of the MIP family with 80.95% amino-acid sequence identity with human aquaporin-CHIP28. To obtain a full-length clone, pE6 was used to design non-degenerate primers C, D, E and F (Fig. 1). The 5′ missing region was deduced from the clone pE222 of 453-bp obtained

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The nucleotide sequence data reported in this paper have been deposited in the GenBank Data Library under the accession number L24754.

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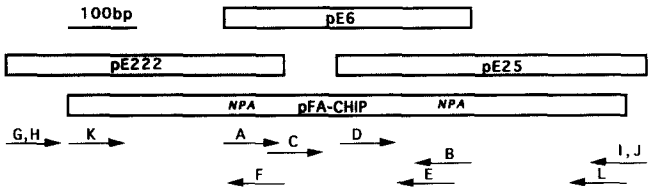

Fig. 1. Diagram representing the organization of FA-CHIP RT-PCR clones obtained by a three step RT-PCR strategy from frog bladder cells. In step I, pE6 (378 bp) was obtained by RT-PCR (30 cycles; 96°C, 30 s; 50°C, 30 s and 72°C, 45 s.) with degenerate sense primer A (5'-GG(A/C/G/T)GC(A/C/G/T)CA(C/T)(G/C)T(A/C/G/T)AA(T/C)CC(A/C/G/T)GC(A/C/G/T)GT(A/C/G/T)AC-3') which corresponds to nucleotides 214 to 239 of human CHIP28 (G-A-H-L-N-P-A-V-T) and antisense primer B (5'-AAAGGA(C/G)CG(A/G)GCAGGGTT(C/A)AT(G/C)CC(A/T)(C/G)(C/A)-(A/G)CCAGT-3') which corresponds to nucleotides 549 to 591 of human CHIP28 (T-G-C-G-I-N-P-A-R-S-F). In step II, pE222 (453-bp) was obtained by using in succession: (1) sense primer G (5'-GGCCACGCGTCGACTAGTAC$(G)_{16}$-3') and antisense primer E (5'-GTTCATTCCACATCCAGTGTAGTC-3') which corresponds to nucleotides 661 to 684 of FA-CHIP (D-Y-T-G-C-G-M-N), 35 cycles at 94°C, 45 s; 54°C, 30 s and 72°C, 2 min.; (2) sense primer H (5'-GGCCACGCGTCGACTAGTAC-3') and antisense primer F (5'-GAGGCACTGGGCGATTATGTA-3') which corresponds to nucleotides 397 to 417 of FA-CHIP (Y-I-I-A-Q-C-L), 30 cycles at 94°C, 45 s; 57°C, 30 s and 72°C, 2 min. In step III, pE25 was obtained using in succession: (1) sense primer C (5'-GCGGTCACGCTCGGGTGTCTTCTTAG-3') which corresponds to nucleotides 340 to 365 of FA-CHIP (A-V-T-L-G-C-L-L-S) and antisense primer I (5'-CCGCATGCGGCCGCAGATCTAGATATCGA$(T)_{16}$(A/C/G)-(A/C/G/T)-3'),25 cycles at 94°C, 1 min; 50°C, 1 min and 72°C, 3 min; (2) sense primer D (5'-CCGGGAGTCTCTGCAGGA CAAGGA-3') corresponding to nucleotides 499 to 522 of FA-CHIP (P-G-V-S-A-G-Q-G) and antisense primer J (5'-GGCCGCAGA TCTAGATATCG-3'), 25 cycles at 94°C, 30 s; 54°C, 1 min and 72°C, 3 min. Sense primer K (5'-GCGAGATCTAATGGCGAGCGAA TTCAAGAAGA-3') corresponding to nucleotide 96 to 118 of FA-CHIP (M-A-S-E-F-K-K-K) and antisense primer L (5'-GCCACT AGTTTGACTCAATCTCTATTTTGG-3') corresponding to nucleotide 907 to 928 of FA-CHIP (P-K- tag) were deduced from pE222 and pE25 and were used to obtain the full-length cDNA coding for FA-CHIP, pFA-CHIP (850-bp) 30 cycles at 94°C, 30 s; 52°C, 30 s and 72°C, 1 min.

with the RACE (rapid amplification of cDNA ends) PCR procedure [10], using oligonucleotide E to prime the first strand cDNA synthesis. Terminal deoxynucleotidyl transferase (TdT) was used to attach homopolymeric tails to the 3' ends of the cDNA. The tailed cDNA was then amplified by PCR using primers E and G (Fig. 1), which annealed to the homopolymeric tail, and then reamplified using a nested primer F, which annealed 3' to E and primer H (Fig. 1). Twelve clones were sequenced, and the largest one (pE222) was found to contain a 453-bp insert with 96-bp in the 5'-untranslated region. The 3' missing region was sequenced from the clone pE25 (465-bp): the first-strand cDNA synthesis was performed using frog urinary bladder RNA as template which was reverse transcribed using antisense primer I, a lock-docking oligo$(dT)_{16}$ primer which is able to lock in at the beginning of polyadenylation rather than at random points along a potentially lengthy poly(A) tail, to avoid a population of varying length products. The first strand cDNA pool was treated by RNAse H and amplified with sense primer C and antisense primer I to enrich the template mixture. An aliquot (1%) was used for PCR amplification employing primers D and J, which resulted in a cDNA fragment of 465 bp (pE25) corresponding to a part of pE6 and the 3' end missing sequence of FA-CHIP cDNA with a 3'-untranslated region of 30-bp. The frog urinary bladder PCR products (pE6, pE222, pE25) were subcloned into pBluescript vector and sequenced.

To obtain the full FA-CHIP clone, primers L and K (Fig. 1), deduced from the nucleotide sequences of pE222 and pE25, were used for RT-PCR on frog urinary bladder RNA. The RT-PCR products of the expected size (850 bp) coding for FA-CHIP were purified and directly cloned into the *Bgl*II and *Spe*I sites of pT7TS, a pGEM4ZT-derived Bluescript vector carrying 5' and 3' untranslated sequences of a β-globin gene of *Xenopus* (kindly provided by P. Krieg, Austin, TX). This vector has a sense strand transcription driven by the T7 promoter and provides high mRNA stability and translation efficiency in *Xenopus* oocytes [11]. Three plasmids were completely sequenced on both strands. Two additional primers were synthesized to sequence the full FA-CHIP cDNA. Only one plasmid did not show any mistake induced by the TAQ polymerase (pFA-CHIP). The sequences of pE222, pE25, pE6 and pFA-CHIP partially overlap and together

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   1 CAGAGCTCTGCTCAGTCTTTTGCAAGGAAGAACTTTTAAAAAATTTAACAAAGTTAGAAA  60
  61 AACTTTCTTTTTTATTGTCACTTAAAAAAAAAAAA ATG GCG AGC GAA TTC AAG  114
   1                                    M   A   S   E   F   K    6
 115 AAG AAA GCT TTC TGG AGG GCG GTC ATA GCG GAG TTT TTA GCC ATG  159
   7 K   K   A   F   W   R   A   V   I   A   E   F   L   A   M   21
 160 ATC CTC TTC GTT TTT ATT AGT ATT GGT GCC GCT TTG GGG TTC AAC  204
  22 I   L   F   V   F   I   S   I   G   A   A   L   G   F   N   36
 205 TTC CCC ATC GAA GAG AAG GCT AAT CAG ACT GTT GGC CGG TCG CAA  249
  37 F   P   I   E   E   K   A   N   Q   T   V   G   R   S   Q   51
 250 GAC ATC GTA AAG GTG TCT TTG GCT TTT GGG ATA TCG ATC GCC ACC  294
  52 D   I   V   K   V   S   L   A   F   G   I   S   I   A   T   66
 295 ATG GCT CAG AGC GTG GGC CAC GTC AGC GGG GCT CAC CTG AAC CCA  339
  67 M   A   Q   S   V   G   H   V   S   G   A   H   L   N   P   81
 340 GCG GTC ACG CTC GGG TGT CTT CTT AGC TGC CAA ATC AGC ATC CTG  384
  82 A   V   T   L   G   C   L   L   S   C   Q   I   S   I   L   96
 385 AAA GCC GTC ATG TAC ATA ATC GCC CAG TGC CTC GGC GCC GTG GTC  429
  97 K   A   V   M   Y   I   I   A   Q   C   L   G   A   V   V  111
 430 GCC ACC GCC ATA CTC TCC GGT ATC ACC TCT GGC CTT GAA AAC AAT  474
 112 A   T   A   I   L   S   G   I   T   S   G   L   E   N   N  126
 475 AGC CTG GGG CTC AAT GGG CTC AGT CCG GGA GTC TCT GCA GGA CAA  519
 127 S   L   G   L   N   G   L   S   P   G   V   S   A   G   Q  141
 520 GGA TTG GGA GTG GAG ATC CTC GTT ACT TTC CAG CTG GTC CTT TGT  564
 142 G   L   G   V   E   I   L   V   T   F   Q   L   V   L   C  156
 565 GTT GTG GCA GTC ACA GAC CGA AGG CGG CAT GAC GTT TCT GGA TCT  609
 157 V   V   A   V   T   D   R   R   R   H   D   V   S   G   S  171
 610 GTT CCT CTC GCC ATT GGA CTC TCT GTT GCC TTA GGG CAT TTG ATT  654
 172 V   P   L   A   I   G   L   S   V   A   L   G   H   L   I  186
 655 GCG ATC GAC TAC ACT GGA TGT GGA ATG AAC CCA GCC AGG TCT TTT  699
 187 A   I   D   Y   T   G   C   G   M   N   P   A   R   S   F  201
 700 GGC TCT GCG GTA CTA ACT AAA AAC TTT ACA TAT CAT TGG ATC TTT  744
 202 G   S   A   V   L   T   K   N   F   T   Y   H   W   I   F  216
 745 TGG GTT GGT CCA ATG ATC GGT GGT GCT GCA GCC GCT ATT ATA TAC  789
 217 W   V   G   P   M   I   G   G   A   A   A   A   I   I   Y  231
 790 GAC TTC ATC TTA GCA CCA AGA ACC AGC GAT TTA ACA GAC CGC ATG  834
 232 D   F   I   L   A   P   R   T   S   D   L   T   D   R   M  246
 835 AAG GTG TGG ACC AAC GGG CAA GTG GAG GAG TAT GAA TTA GAC GGA  879
 247 K   V   W   T   N   G   Q   V   E   E   Y   E   L   D   G  261
 880 GAT GAC AAC ACT AGG GTC GAA ATG AAA CCA AAA TAG AGATTGAGTCAA 927
 262 D   D   N   T   R   V   E   M   K   P   K   *                273
 928 AAAAAAAAAAAAAAA                                             942
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Fig. 2. Nucleotide and deduced amino-acid of FA-CHIP, derived from pE222, pE25, pE6 and 3 pFA-CHIP clones. The cysteines and the *NPA* tandem repeats are underlined.

contain the sequence of the entire 272 amino-acid FA-CHIP protein, plus 96-bp and 30-bp at the 5′ and 3′ termini, respectively (Fig. 2). pFA-CHIP begins at nucleotide 96, with respect to the start of transcription, and contains an open reading frame of 816-bp with a stop codon at +913 and 16-bp at the 3′-end. Multiple stop codons are present in the other two reading frames. Several lines of evidence indicate that the deduced amino-acid sequence of FA-CHIP is characteristic of a new member of the MIP26 family. The predicted molecular size of the protein (M_r = 28 900) is nearly identical to the MIP26 members. The homologies between members of the MIP channel family are described in Fig. 3. The amino-acid sequence of FA-CHIP coding region shows 47.4% identity with the ancestral member frog MIP26 [12] and 78.8%, 77.4%, 42.4%, 35.6% identity with the rat aquaporin-CHIP28 [5], the human aquaporin-CHIP28 [4], the rat WCH-CD [7] and the γ-TIP [8], respectively. Computer analysis of the deduced amino-acid sequence demonstrated six strong hydrophobic regions that most likely correspond to bilayer spanning domains, and five connecting loops. Whereas the human aquaporin-CHIP28 protein contains four cysteines, five cysteines can be found in the sequence of the FA-CHIP. These cysteines are known to be potential target sites for the water flux inhibition by mercurial compounds. Furthermore, the Cys-189, which is necessary for the functional activity of aquaporin-CHIP28 [13] and WCH-CD [7], is present in the FA-CHIP, two amino-acids downstream of the first NPA repeat. Thus, the sequence of FA-CHIP is closely related to the sequence of the water channels of the MIP family.

Expression of FA-CHIP in Xenopus oocytes. FA-

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FA-CHIP     :                    masEfkkkaF WRAViAEFLA miLFVFisiG
human CHIP28 :                   masEfkkklF WRAVvAEFLA TtLFVFisiG
rat CHIP28  :                    masEikkklF WRAVvAEFLA mtLFVFisiG
rat WCH-CD  :                     mwElrsiaF sRAVlAEFLA TlLFVFfglG
Gamma-TIP   : mpirniaig rpdEatrpda lkAalAEFis TliFVvagsG
Frog MIP    :                     mwEfrsfsF WRAVfAEFfg TmfyVFfglG

FA-CHIP  : aALgFn-fpi eekanqtvgr sqdiVkvsLA FGisIATmaQ
hu.CHIP28 : sALgFk-ypv gn--nqtav- -qdnVkvsLA FGLsIATlaQ
ratCHIP28 : sALgFn-ypl er--nqtlv- -qdnVkvsLA FGLsIATlaQ
ratWCH-CD : sALqw----- --------ass ppsvlqiavA FGLgIgilvQ
Gamma-TIP : SgmaFnklte ngat-----t psglVaaavA hafglfvavs
Frog MIP  : asLkw----- --------aag panvlviaLA FGLvlATmvQ

FA-CHIP  : SvGHvSGAHl NPAVTlGcLl sCQISiLkAv mYIIAQcLGA
hu.CHIP28 : SvGHiSGAHl NPAVTlGlLl sCQISifRAl mYIIAQcvGA
ratCHIP28 : SvGHiSGAHl NPAVTlGlLl sCQISiLRAv mYIIAQcvGA
ratWCH-CD : alGHvSGAHi NPAVTvacLv gChvSfLRAa fYvaAQlLGA
Gamma-TIP : vganiSGgHv NPAVTfGafi ggnItlLRgi lYwIAQlLGs
Frog MIP  : SiGHvSGAHi NPAVTfafLi gsQmSlfRAi fYIaAQlLGA

FA-CHIP  : VVAtAILsGI TsgLennsLG lNgLspGVsa GQglgvEIlv
hu.CHIP28 : iVAtAILsGI TssLtgnsLG rNdLadGVns GQglgiEIig
ratCHIP28 : iVAsAILsGI TssLlensLG rNdLarGVns GQglgiEIig
ratWCH-CD : VagaAILheI TpveirgdLa vNaLannata GQavtvElfl
Gamma-TIP : VVAclILkfa TggLavpafG ---LsaGVgv lnafvfEIvm
Frog MIP  : VagaAvLyGv TpaairgnLa lNtLhpGVsl GQattvEIfl

FA-CHIP  : TfQLVLCVvA vTDrRRhDvs GSvp-LAIGl SVaLGHLiaI
hu.CHIP28 : TlQLVLCVlA TTDrRRrDlg GSap-LAIGl SVaLGHLlaI
ratCHIP28 : TlQLVLCVlA TTDrRRrDlg GSap-LAIGl SVaLGHLlaI
ratWCH-CD : TmQLVLCifA sTDeRRgDnl GSpa-LsIGf SVtLGHLlgI
Gamma-TIP : TfGLVytVyA Taidpkngsl GtiapiAIGf iVganiLagg
Frog MIP  : TlQfVLCifA TyDeRRngrl GSvs-LAIGf SltLGHLfgl

FA-CHIP  : dYTGCgMNPA RSFGsAVlTk NFtyHWiFWV GPmIGGAaAa
hu.CHIP28 : dYTGCgiNPA RSFGsAViTh NFsNHWiFWV GPfIGGAlAv
ratCHIP28 : dYTGCgiNPA RSFGsAVlTr NFsNHWiFWV GPfIGsAlAv
ratWCH-CD : yfTGCsMNPA RSlapAVvTg kFddHWvFWi GPlvGaiigs
Gamma-TIP : afsGasMNPA vaFGpAVvsw twtNHWvyWa GPlvGGgiAg
Frog MIP  : yYTGasMNPA RSFapAVlTr NFtNHWvyWV GPiIGGAlgg

FA-CHIP  : iIYDFILaPR tsdLtdRmkV wtngqveEye ldgdd-nt-r
hu.CHIP28 : LIYDFILaPR ssdLtdRvkV wtsgqveEyd ldaddins-r
ratCHIP28 : LIYDFILaPR ssdftdRmkV wtsgqveEyd ldaddins-r
ratWCH-CD : LlYnylLfPs aksLqeRlaV lkglepdtdw eerevrrrqs
Gamma-TIP : LIYevffint theqlpttdy
Frog MIP  : LvYDFILfPR mrgLseRlsi lkgarpaEpe gqqea-tgep

FA-CHIP  : VEmkpk
hu.CHIP28 : VEmkpk
ratCHIP28 : VEmkpk
ratWCH-CD : VElhspqslp rgska
Gamma-TIP :
Frog MIP  : iEl-ktqsl
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Fig. 3. Amino-acid sequence comparison between FA-CHIP, human CHIP28 [4], rat kidney CHIP28 [5], WCH-CD [7], γ-TIP [8] and frog MIP [12]. At each aligned position, amino acid residues identical in four or more of the six forms are printed in capital letters.

CHIP-mediated water channel activity was demonstrated with the *Xenopus laevis* oocyte expression system. In vitro transcribed cRNAs encoding FA-CHIP with the 5′ and 3′ untranslated regions of a β-globin gene from *Xenopus laevis* were injected into defoliculated oocytes. Two days after the injection, the oocytes were transferred to hypoosmotic conditions and initial changes in the oocyte volume were measured at 22°C, using videomicroscopy. Control oocytes that were either uninjected or injected with water instead of cRNA swelled slowly, whereas FA-CHIP cRNA injected oocytes swelled rapidly and increased their volume by up to 35% in 300 s. The osmotic water permeability coefficient (P_f) of FA-CHIP cRNA injected oocytes was 6–8-times greater than P_f of water injected oocytes (Fig. 4). Moreover, 95% of the oocytes injected with FA-CHIP transcripts ruptured within 5 min after the transfer into the hypotonic solution, whereas none of the water injected oocytes ruptured, even after 30 min. Fig. 4 shows that over a set of six independent experiments, uninjected oocytes and water injected oocytes displayed similar osmotic water permeability values

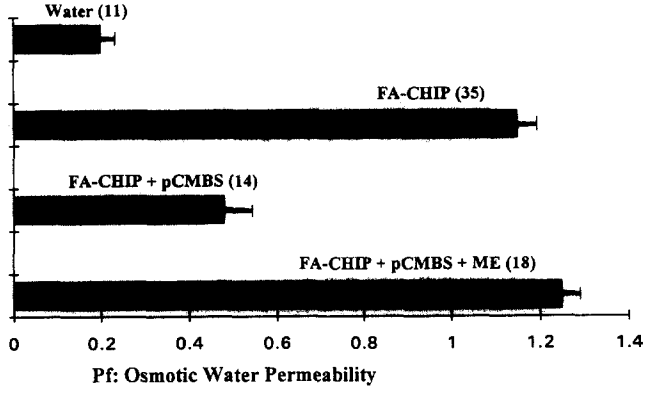

Fig. 4. Osmotic water permeability of oocytes expressing FA-CHIP at 22°C, 48 h after injection. Osmotic water permeability of oocytes was measured by a swelling assay. Oocytes were transferred from Barth's solution at 200 mosmol/kg H_2O to the same solution diluted to 40 mosmol/kg H_2O with distilled water. Change in oocyte volume was measured at 1-s intervals by a quantitative imaging method: oocytes were viewed by transmitted light on a phase-contrast microscope and imaged in a camera connected to an image-processing system. Temperature control was maintained by a circulating water bath. Osmotic water permeability P_f was determined from the initial slope of the time-course of V/V_0 ($d(V/V_0)/dt$), the initial oocyte volume V_0, the initial oocyte surface area S and the molar volume of water ($V_w = 18$ cm^3 per mol) by using the relation:

$$P_f = V_0[d(V/V_0)/dt]/[S \cdot V_w(Osm_{out} - Osm_{in})]$$

Measurements on oocytes injected with FA-CHIP cRNA stopped at the time of the oocyte rupture. Osmotic swelling was determined without pretreatment (FA-CHIP) or after 1 h in 2 mM pCMBS, followed by swelling in 5 × diluted buffer (FA-CHIP + pCMBS). Other oocytes were pretreated for 1 h in 2 mM pCMBS followed by 15 min in 5 mM β-mercaptoethanol (FA-CHIP + pCMBS + ME). Data are expressed as the means ± S.E. of data from the indicated number of oocytes isolated from eight different *Xenopus laevis* (unit: 10^{-2} cm/s.

($P_f = (0.17 \pm 0.02)10^{-2}$ cm/s). In contrast, FA-CHIP cRNA injection elicited increase in P_f over the control values ($P_f = (1.15 \pm 0.05)10^{-2}$ cm/s). Osmotic water permeability in FA-CHIP cRNA injected oocytes was reduced by 60% by a 1 h incubation in 2 mM *p*-chloromercuribenzene sulfonate (pCMBS) ($P_f = (0.48 \pm 0.07)10^{-2}$ cm/s) and this inhibition was reversed by subsequent incubation in the reducing agent β-mercaptoethanol 5 mM ($P_f = (1.25 \pm 0.04)10^{-2}$ cm/s) (Fig. 4). The appearance of high osmotic water permeability together with the inhibition by mercurial reagents which can be reversed by reducing agents, are all characteristics of channel-mediated water permeability and support the conclusion that the expressed protein in the FA-CHIP cRNA injected oocytes is a frog water channel.

Concluding remarks. In the present work, a new member of the MIP family was isolated from frog urinary bladder using RT-PCR strategy: the frog aquaporin-CHIP. The comparison of the deduced amino-acids sequences (Fig. 3) indicates that the FA-CHIP protein sequence is more similar to the human water channel CHIP28 than to the frog MIP26, which probably conducts ions as the bovine MIP26 which, once reconstituted into planar bilayers, presents a cationic conductance [1]. The transport function of the FA-CHIP protein was identified using functional expression in *Xenopus laevis* oocytes. The data presented here provide evidence that the expression of FA-CHIP increases the osmotic water permeability of the oocyte plasma membrane to an extent comparable with human CHIP28 [11] expression. These observations suggest that overall sequence similarity between the various MIP family members may reflect functional specialization rather than evolutionary divergence. In the kidney, the presence of water channels was demonstrated in both proximal tubules and collecting ducts. The FA-CHIP protein sequence shows a high degree of identity and similarity to the rat kidney aquaporin-CHIP28, proposed to be the major constitutively activated water channel of renal proximal tubules and thin descending limbs of Henle's loop [6,9]. Furthermore, FA-CHIP shares a lower identity with the cDNA encoding rat WCH-CD, the probable vasopressin-regulated water channel of renal collecting ducts [7]. In contrast, the presence of a CHIP protein in frog urinary bladder, the kidney collecting duct model, could be in favor of a vasopressin-regulated water channel. As a matter of fact, the presence of two different water channels was described [14] in the epithelial cells of frog urinary bladder, the vasopressin sensitive being present in apical membrane and the constitutive in basolateral membrane. Only the localization of FA-CHIP will allow to determine if this frog water channel, isolated from urinary bladder, is the apical or basolateral one.

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