A divergent CFTR homologue: highly regulated salt transport in the euryhaline teleost *F. heteroclitus*

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[1]*Nuffield Department of Clinical Biochemistry and Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DS;* [2]*University Laboratory of Physiology, Oxford OX1 3PT, United Kingdom; and* [3]*Department of Biology, Saint Francis Xavier University, Antigonish, Nova Scotia, Canada B2G 2W5*

Singer, Thomas D., Stephen J. Tucker, William S. Marshall, and Christopher F. Higgins. A divergent CFTR homologue: highly regulated salt transport in the euryhaline teleost *F. heteroclitus. Am. J. Physiol.* 274 (*Cell Physiol.* 43): C715–C723, 1998.—The killifish, *Fundulus heteroclitus*, is a euryhaline teleost fish capable of adapting rapidly to transfer from freshwater (FW) to four times seawater (SW). To investigate osmoregulation at a molecular level, a 5.7-kilobase cDNA homologous to human cystic fibrosis transmembrane conductance regulator (hCFTR) was isolated from a gill cDNA library from SW-adapted killifish. This cDNA encodes a protein product (kfCFTR) that is 59% identical to hCFTR, the most divergent form of CFTR characterized to date. Expression of kfCFTR in *Xenopus* oocytes generated adenosine 3′,5′-cyclic monophosphate-activated, Cl^--selective currents similar to those generated by hCFTR. In SW-adapted killifish, *kfCFTR* was expressed at high levels in the gill, opercular epithelium, and intestine. After abrupt exposure of FW-adapted killifish to SW, *kfCFTR* expression in the gill increased severalfold, suggesting a role for kfCFTR in salinity adaptation. Under similar conditions, plasma Na^+ levels rose significantly after 8 h and then fell, although it is not known whether these changes are directly responsible for the changes in *kfCFTR* expression. The killifish provides a unique opportunity to understand teleost osmoregulation and the role of CFTR.

cystic fibrosis transmembrane conductance regulator; chloride channel; killifish; gill; *Xenopus* expression; osmoregulation; cystic fibrosis; *Fundulus heteroclitus*

THE EURYHALINE KILLIFISH, *Fundulus heteroclitus*, lives in tidal marshes and estuaries along the eastern coast of North America and has a remarkable capacity to adapt rapidly to changes in salinity ranging from extremely dilute [freshwater (FW); 0.1 mM NaCl] to extremely saline [4× seawater (SW); ~2.0 M NaCl] (15). This ionic adaptability makes the killifish a key model in understanding the physiology of ionoregulation (reviewed in Refs. 17, 23, 38). SW-adapted killifish, like marine teleosts, drink SW, absorb ions and water through their intestine, and secrete the excess salt through specialized mitochondria-rich "chloride" cells present in their skin and gill epithelia. However, after transfer to FW the killifish osmoregulates as a FW teleost, maintaining the same plasma NaCl levels as in SW.

The movement of Cl^- across the killifish skin and gill epithelium is believed to occur via pathways similar to those of the mammalian airway epithelium (24). Cl^- secretion across the opercular epithelium of teleosts is stimulated by adenosine 3′,5′-cyclic monophosphate (cAMP) in response to hormones such as β-adrenergic agonists, vasoactive intestinal polypeptide, glucagon, and urotensin I and is blocked by diphenylamine-2-carboxylic acid and 5-nitro-2-(3-phenylpropylamino)benzoic acid but not by 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (11, 26). A low-conductance Cl^- channel (~8.0 pS) is present in the apical membrane of primary cultures of opercular epithelium cells taken from SW-adapted killifish (26). These characteristics are similar to those of the human cystic fibrosis transmembrane conductance regulator (hCFTR), a known Cl^- channel (1, 4, 13, 30).

To investigate osmoregulation in teleosts at a molecular level, a *CFTR* homologue (designated *kfCFTR*) was cloned from the gills of SW-adapted killifish. *kfCFTR* encodes a protein that is the most divergent form of CFTR identified to date. Heterologous expression of kfCFTR in *Xenopus* oocytes demonstrated that this homologue encodes a cAMP-activated Cl^- channel. In SW-adapted killifish, *kfCFTR* was found to be most highly expressed in the gill, opercular epithelium, and intestine. Abrupt exposure of FW-adapted killifish to SW revealed increases in kfCFTR mRNA expression in the gill and increased plasma Na^+ levels, implying a role for kfCFTR in salinity adaptation. The killifish is an extremely adaptable euryhaline teleost that provides a unique opportunity to investigate the role of CFTR in an organism capable of highly regulated salt secretion.

MATERIALS AND METHODS

Tissue samples. Killifish (*F. heteroclitus*) gill tissues used to make RNA for constructing a cDNA library were collected from fish captured in esturine ponds near St. Andrews, New Brunswick, Canada, and were maintained for 1–2 wk in 100% SW (~1,000 mosM). Killifish tissue samples for Northern blot analysis were from fish captured in an estuary near Antigonish, Nova Scotia, Canada. The killifish collected from both locations were of the same genus and species and were both collected from brackish water during summer months. Fish collected for tissue distribution study were transferred directly to 100% SW and held for at least 30 days. For SW transfer experiments, fish were first transferred to 10% SW for 3 days and then transferred to FW and held for at least 30 days. In both situations water temperature was between 20 and 24°C, with a constant light-to-dark photoperiod of 15:9 h. Fish were fed twice daily a ration of tetra minimum flake food and supplemented with frozen tubifex worms.

Extraction of RNA. Total RNA used for cDNA library construction was isolated from St. Andrew's killifish gill

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tissue by lysis in guanidinium isothyocyanate followed by CsCl centrifugation (6). Poly(A)$^+$ RNA was isolated from total RNA using oligo(dT) cellulose chromatography (2). Total RNA used in Northern blot analysis was isolated separately from different tissues from Antigonish killifish using a Qiagen RNeasy kit, following the manufacturer's instructions.

Genomic library screening. A killifish genomic library, constructed by Stratagene in the vector lambda Fix II, was kindly supplied by Dr. D. Powers, Hopkins Marine Station, Stanford University, CA. Plaque lifts of this library using ICN nylon membranes were hybridized to a full-length dogfish shark CFTR (sCFTR) cDNA probe labeled with [^{32}P]dCTP by random priming (Promega Prime-a-gene) for 18 h at 42°C under low stringency in 5× SSPE [1× SSPE = (in mM) 180 NaCl, 10 sodium phosphate, and 1 EDTA, pH 7.7] containing 5× Denhardt's reagent (1× Denhardt's = 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin), 0.5% sodium dodecyl sulfate (SDS), 30% formamide, and 20 µg/ml denatured, fragmented salmon sperm DNA. The membranes were washed at a final stringency of 57°C in 2× SSC (1× SSC = 150 mM NaCl and 15 mM sodium citrate) and 0.1% SDS and were exposed to either phosphorimaging plates or autoradiographs. After screening 3×10^5 recombinants from an amplified version of this library, a positive clone was recovered. A 1,636-base pair (bp) genomic DNA fragment from this positive was cloned into a pBluescript (Stratagene) plasmid, was fully sequenced on both strands, and was found to contain a 173-bp region 91% identical to exon 22 of *hCFTR*. This 173-bp fragment was used as a probe to isolate a full-length cDNA (see below).

cDNA library construction/screening. To clone a full-length *kfCFTR* cDNA, an oligo(dT)-primed cDNA library was prepared using 2 µg of poly(A)$^+$ mRNA from the gill of St. Andrew's SW-adapted killifish and was cloned into the Stratagene vector lambda Zap II following the manufacturer's instructions. Plaque lifts were hybridized for 18 h at 60°C under moderate stringency in 5× SSC, 10× Denhardt's reagent, 0.5% SDS, 100 µg/ml tRNA, and 10% Dextran SO_4, using as a probe the 173-bp kfCFTR genomic fragment (see above) labeled with [^{32}P]dCTP by random priming (Promega Prime-a-gene). This probe, designated kfCFTR22, was amplified from the genomic plasmid (see above) using the primer pair

5′-GTTGGCTTGAAGTCAGTAATCG-3′ (KFC22-5)
5′-ATGGGGTCCAGGTAGGAGGA-3′ (KFC22-3)

The membranes were washed at a final stringency of 60°C in 2× SSC, 0.1% SDS and were autoradiographed. After screening 480,000 recombinants from the unamplified library, four positive clones were isolated. The cDNA inserts from these lambda clones were rescued by in vivo excision as pBluescript (Stratagene) plasmid recombinants. One of the plasmids contained a 7,250-bp insert that was fully sequenced on both strands.

Sequence analysis revealed that the insert contained the full-length *kfCFTR* cDNA sequence. However, the sequence was interrupted by two intronlike regions of 1,274 and 393 bp corresponding precisely in location to introns 18 and 20 of *hCFTR* (42). Stop signals within these putative introns would disrupt the kfCFTR coding sequence. In addition, this cDNA lacked the 126-bp region corresponding to *hCFTR* exon 6b. Investigation by reverse transcriptase-polymerase chain reaction of the same gill total RNA sample used to construct the cDNA library revealed that these introns are absent from bulk cellular kfCFTR RNA and that we had cloned a very rare variant. In addition, the region homologous to *hCFTR* exon 6b was shown to be part of the bulk of cellular RNA (data not shown). On the basis of these results, the original 7,250-bp clone was modified to "correct" these three differences: the two introns were removed and the *kfCFTR* exon 6b was replaced through a series of polymerase chain reaction amplifications, restriction digests, and ligations. After these manipulations, the final cDNA clone was fully sequenced on both strands to ensure that no mutations had been introduced during the repair process. This final cDNA (5,709 bp) contained 173 bp of the 5′-untranslated region (UTR), a 4,509-bp open reading frame encoding kfCFTR, and a 1,027-bp 3′-UTR.

DNA sequencing. Sequencing was performed using double-stranded templates with a combination of specific oligonucleotides and the T3/T7 universal primers using the Sequenase system (US Biochemical) and an ABI prism 377 auto sequencer. DNA sequences were assembled and analyzed using Wisconsin Package Genetics Computer Group software. The nucleotide and amino acid sequence data have been deposited in the GenBank/EMBL Data Bank with accession number AF000271.

Northern blot hybridization. Total RNA (10 µg), isolated from the tissues of SW-adapted killifish collected from Antigonish, was heated to 60°C for 5 min in a solution of 30% deionized formamide, 2% formaldehyde, and 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS). Glycerol-dye buffer was added, and the RNA was fractionated at 60 V for 5 h on a 1% agarose gel containing 20 mM MOPS and 6% formaldehyde. RNA size markers (GIBCO BRL) were treated in an identical manner. The RNA was transferred onto Hybond-N membrane (Amersham) by standard methods and fixed by ultraviolet cross-linking (Stratagene). The membrane was hybridized for 2 h at 65°C under high stringency in rapid-hyb buffer (Amersham; following the manufacturer's instructions) with a randomly primed (Promega Prime-a-gene), [^{32}P]dCTP-labeled 929-bp *kfCFTR* cDNA fragment amplified using the primer pair

5′-GCTTGGGCTTGGATCTTATGAC-3′ (KFC13-5)
5′-GCCAGTATGCTATCTGAGTGAGC-3′ (KFC15-3)

This 929-bp fragment was used as a probe since it corresponded to the R domain that is unique to CFTR and is unlikely to cross-hybridize with related ATP-binding cassette (ABC) transporters (16). The membrane was washed at a final stringency of 70°C in 0.1× SSC and 0.1% SDS and was autoradiographed.

Transfer of killifish from FW to SW. Killifish collected from Antigonish were held for at least 30 days in FW, and then a group of eight fish were randomly netted from a large holding tank and transferred in pairs to four 4.5-liter buckets also containing FW. Air stones maintained oxygen levels. After 24 h, the water in these buckets was replaced with 100% SW from a header tank over a period of 7 min. The transfer of fish was repeated for seven groups of eight fish, with each group exposed to SW for a set period of time (1, 3, 8, and 24 h and 2, 7, and 28 days). In addition, a group of eight fish were transferred from the FW holding tank to the 4.5-liter buckets also containing FW and sampled after 24 h. This FW control group was intended to control for possible effects of the transfer procedure. A final group of eight fish were sampled directly from the FW holding tank. After the fish had been collected, they were double pithed, and the blood was collected in capillary tubes after removal of the caudal peduncle. Blood plasma was separated by centrifugation and frozen at −20°C for later analysis of Na^+ levels by atomic absorption (Varian AA-375). Na^+ levels were compared between groups by one-way analysis of variance followed by the a posteriori sum-of-squares simultaneous test procedure. For each treat-

ment group, between four and eight fish were sampled for plasma Na^+.

Immediately after the fish blood was collected, gill arches from each of the eight fish were removed, placed into separate cryovials, and plunged into liquid nitrogen for subsequent RNA extraction. Total RNA from the gills of between one and three individuals from each treatment group was extracted and pooled. Northern blot hybridization was conducted as above, using a single 10-μg sample from each group and a randomly primed (Promega Prime-a-gene), [^{32}P]dCTP-labeled 929-bp *kfCFTR* cDNA fragment as a probe. The blot was then stripped with boiling 0.01% SDS and reprobed for loading control with a randomly primed (Promega Prime-a-gene), [^{32}P]dCTP-labeled 700-bp killifish β-actin cDNA fragment amplified using the mouse actin primer pair

5′-TGAACCCCAAGGCCAACCGT GAGAAGATGA-3′ (MACTIN-5)
5′-CCCAATCCAGACAGAGTATT TACGCTCAGG-3′ (MACTIN-3)

The hybridization signals for both *kfCFTR* and killifish *β-actin* [lower 1.5-kilobase (kb) band] were quantified by densitometric scanning of autoradiographs (BioImage densitometer, Millipore) using whole band analyzer software. Densities collected as integrated intensities for *kfCFTR* were normalized to the corresponding *β-actin* integrated intensity and were expressed relative to values from fish transferred from FW to FW.

Electrophysiology. For oocyte expression, the *kfCFTR* and *hCFTR* open reading frames were subcloned into the oocyte expression vector (pBF) (Dr. Bernd Fakler, University of Tubingen, Germany; unpublished observations), which provides the 5′- and 3′-UTRs of the *Xenopus* β-globin genes. Capped mRNA was synthesized by in vitro transcription from the linearized cDNA. Oocyte preparation and maintenance was as previously described (14). Stage V or VI oocytes were injected with ~1 ng of kfCFTR mRNA, in a final injection volume of 50 nl. Control oocytes were injected with water. Whole cell currents were measured 24–48 h after injection using a two-electrode voltage clamp essentially as described previously (14), except that the recording solution was ND96 [in mM: 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid pH 7.5]. Stimulation of currents by cAMP was achieved by perfusion with recording solution containing 10 μM forskolin, 200 μM dibutyryl cAMP, and 100 μM 3-isobutyl-1-methylxanthine. This was originally prepared as a 1,000× stock dissolved in dimethyl sulfoxide and was diluted as required. Experiments were performed at room temperature (18–24°C).

RESULTS

The kfCFTR gene. A 5.7-kb cDNA encoding the killifish *CFTR* homologue was cloned and sequenced (see MATERIALS AND METHODS). The cDNA has a single long open reading frame encoding a protein of 1,503 amino acids. At the nucleotide level the *kfCFTR* coding sequence is 62.2% identical to *hCFTR* and 63.7% identical to dogfish shark *CFTR* (*sCFTR*). The ATG triplet assigned as the translational initiation codon was based on homology with other forms of CFTR and the similarity of the sequence surrounding this site to the consensus sequence for translation initiation in higher eukaryotes (20). This cDNA contains a 173-bp 5′-UTR, whereas the 1,027-bp 3′-UTR contains a poly(A)$^+$ addition signal sequence, although no poly(A)$^+$ tail was found.

The kfCFTR protein displays less sequence identity to hCFTR than any other CFTR homologue identified to date (Fig. 1). At the amino acid level the two proteins show 59.0% identity and 77.3% similarity. The kfCFTR homologue is similarly divergent from sCFTR, with 60.2% identity and 78.0% similarity. Figure 1 summarizes the sequence identity between seven known CFTR protein homologues. Alignment of the kfCFTR amino acid sequence with other published CFTR homologues (Fig. 2) reveals differences in the degree of conservation of different domains of the protein. Table 1 shows sequence identity between hCFTR and kfCFTR on a domain basis.

Expression of the kfCFTR gene. To study the pattern of *kfCFTR* expression, Northern analysis of total RNA isolated from different SW-adapted killifish tissues was performed using a 929-bp *kfCFTR* cDNA probe. The panel directly below the autoradiograph shows controls for RNA loading of each sample (Fig. 3). The predominant mRNA species was ~7.5 kb in size, larger than the 6.5-kb mRNA of *hCFTR* (30). A second smaller mRNA species of 5.5 kb present in gill, opercular epithelium, and posterior intestine may represent an additional cross-reactive homologue or an alternatively spliced form.

kfCFTR is expressed at high levels in the gill, opercular epithelium, and intestine of SW-adapted killifish, with moderate expression in the brain (Fig. 3). Low levels of expression, at least 20-fold less than the gill, were detected in the kidney, liver, spleen, ovary, and testes on slightly overexposed autoradiographs (data not shown). No expression was detected in either the eye or heart tissue.

kfCFTR expression and plasma Na^+ levels after abrupt transfer from FW to SW. To examine the effect of rapid transfer from FW to SW on *kfCFTR* expression,

	Human	Ovine	Bovine	Mouse	Xenopus	Shark
Ovine	90.8/95.3					
Bovine	90.1/95.2	97.7/99.1				
Mouse	78.4/89.1	77.7/88.7	77.2/88.3			
Xenopus	77.5/88.4	76.6/87.7	75.5/87.1	71.7/85.4		
Shark	72.4/85.3	70.7/84.6	70.4/84.5	65.6/81.5	69.9/84.1	
Killifish	59.0/77.3	58.6/77.0	58.0/76.7	55.5/75.0	57.5/76.2	60.2/78.0

Fig. 1. Percentage amino acid sequence identity/similarity between cystic fibrosis transmembrane conductance regulator (CFTR) homologues. These sequences were taken from Refs. 7, 22, 30, 34, 35, and 37.

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Human      1 MQRSPLEKASVVSKlFFSWTRPILrKGYRQRLELSDIYQIpSvDSADNLSEKLEREWDRELA.SKKNPKLINALRRCFFWRFMFYGIfLYLGEVTKAVQPLLLGRIIASY
Bovine     1 MQRSPLEKASVVSKvFFSWTRPILkKGYRQRLELSDIYhIsSsDSADNLSEKLEREWDRELA.SKKNPKLINALRRCFFWRFMFYGIiLYLGEVTKAVQPLLLGRIIASY
Mouse      1 MQKSPLEKASfiSKlFFSWTtPILrKGYRhhLELSDIYQapSaDSADhLSEKLEREWDREqA.SKKNPqLIhALRRCFFWRFLFYGILLYLGEVTKAVQPvLLGRIIASY
Xenopus    1 MQKtPLEKASifSqiFFSWTkPILwKGYRQrLELSDIYQIhpgDSADnLSErLEREWDREvATSKKNPKLINALkRCFFWkFLFYGILLYLGEVTKAVQPLLLGRIIASY
Dogfish    1 MQrSPiEKANaFSklFFwprPILkKGYRQKLELSDIYQIPSsDSADeLSEmLEREWDRElATSKKNPKLvNALrRCFFWrFLFYGILLYfvEfTKAVQPLcLGRIIASY
Killifish  1 MQkSPvEdANflSrfvFwWitPlLrKGftkKELEtDvYkaPSfDlADtLSErLEREWDkEvvlaKgrPKLlkALaRCFFlpFaFfGvLLYlgEasKtVQPqlsGRIIASf

Human    110 DPDNKeERSIAIYLGIGLCLLFIVRTLLLHPAIFGLHHIGMQMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFVWIAPLQVaLLMGLiWELL
Bovine   110 DPDNKVERSIAIYLGIGLCLLFIVRTLLLHPAIFGLHHIGMQMRIAMFSLIYKKTLKLSSRVLDKISIGrLVSLLSNNLNKFDEGLALAHFVWIAPLQVTLLMGLLWELL
Mouse    110 DPeNKVERSIAIYLGIGLCLLFIVRTLLLHPAIFGLHrIGMQMRtAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFiWIAPLQVTLLMGLLWDLL
Xenopus  111 DrdNehERSIAYYLAIGLCLLFVVRmLLLHPAIFGLHHIGMQMRIAMFSLIYKKTLKLSSkVLDKISTGQLVSLLSNNLNKFDEGLALAHFVWIAPLQVlLLMGLLWDLL
Dogfish  111 nakNtyERelAYYLALGLCLLFVVRtLfLHPAvFGLqHLGMQMRIALFSLIYKKiLKmSSrVLDKIdTGQLVSLLSNNLNKFDEGvAvAHFVWIAPvQVvLLMGLIWneL
Killifish 111 dpfhaaERsqgYYLALGLgLLFtaRfilLqPAiyGLhHLGMQiRIALFSLIYKKtLKlSSkVLDKIsTGQLVSLmSahLNKlDEslglAHFVWItPlQciLctGLIWeli

Human    220 QAsaFCGLgFLIVLALfQAGLGrMMMKYRDQRAGKIsERlVITSEMIENIQSVKAYCWEEAMEKmIENLRQTELKLTRKAAYVRYfNSSAFFFSGFFVVFLSVLPYALiK
Bovine   220 QAftFCGLaFLIVLALlQAGLGKMMMKYRDQRAGKINERvVITSEMIENIQSVKAYCWEEAMEKmIENLRQTELKLTRKAAYVRYlNSSAFFFSGFFVVFLSVLPYALlK
Mouse    220 QfSAFCGLGlLIILviFQAiLGKMMvKYRDQRAaKINERLVITSEIIdNIYSVKAYCWESAMEKmIENLREvELKmTRKAAYmRfFtSSAFFFSGFFVVFLSVLPYtvin
Xenopus  221 QaSAFCGLGFLIILsLFQArLGrMMMKYkDKRAGKINERLVITSqIIeNIQSVKAYCWEnAMEKIIEtIREtELKLTRKAAYVRYFnSSAFFFSGFFVVFLSiVPhllld
Dogfish  221 tefvFCGLGFLImLaLFQAWLGkKMMqYRDKRAGKINERLAITSEIIdNIQSVKvYCWEdAMEKIIddIRQvELKLTRKvAYcRYFsSSAFFFSGFFVVFLSVPyAfih
Killifish 221 evngFCaLasLtlLgilQAWLslKMgppRaqRAGlINrRLAlTSEIveNIhSVKaYgWEevMEtIIknIRQdEmtLTRKigslRYFySasyFFSailVivsaiVPhAlsn

Human    330 GIILRKIFTTISFCIVLRMAVTRQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTeVVMeNVTAFWEEGFgeLFEKAKqNNNNRKtSNGDdSLFFSNfsLLGTP
Bovine   330 GIILRKIFTTISFCIVLRMAVTRQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTdVVMdNVTAFWEEGFskLFEKAKeNNNNRKiSNGDNSLFFSNL.LLGTP
Mouse    330 GIvLRKIFTTISFCIVLRMsVTRQFPtAVQiWYDSfGmIrKIQDFLQKQEYKvLEYNLmTTgiiMENVTAFWEEGFGELlqKAqqsNgdRKhSsdeNnvsFShLcLVGnP
Xenopus  331 GIsLRKIFTTISFsIVLRMaVTRQFPWAVQTWYDSLGVInKIQeFLQKeEYKsLEYNLTTTEVaMENVsASWDEGIGEfFEKAKlevnggniSNeDpsaFFSNFSLHVaP
Dogfish  331 tIkLRRIFTTISYnIVLRMTVTRQFPsAIQTWYDSLGaIrKIQDFLhKdEhKtvEYNLTTkEVEMvNVtASWDEGIGELFEKvKqndserkmaNGDDGLFFSNFSLHVTP
Killifish 331 gIiLRRIFTTaSYcvVLRMTlTRQlPgsIQmWYDtLalvtKIeDFLlKeEyKvmdYsLTTvglEliNVssSWDEGIvELFEKiKrenkangqltGDDGLFFtN..LyVTP

Human    440 VLKDInFKIERGQLLAVAGSTGAGKTSLLmMIMGELEpSEGKIKHSGRISFCSQfSWIMPGTIKeNIIFGVSYDEYRYRSVIKACQLEEDISKFAEKDNiVLGEGGITLS
Bovine   439 VLKDIsFKIERGQLLAVAGSTGAGKTSLLlMIMGELEASEGKIKHSGRISFCSQySWIMPGTIKdNIIFGVSYDEYRYRSVIKACQLEEDISKFAEKDNvVLGEGGITLS
Mouse    440 VLKNINlnIEKGemLAItGSTGlGKTSLLMlIlGELEASEGiIKHSGRvSFCSQfSWIMPGTIKENIIFGVSYDEYRYkSVvKACQLqqDItKFAEqDNTVLGEGGvTLS
Xenopus  441 VLrNINFKIEKGqLLAIAGSTGaGKTSLLMMIMGELEPSaGKIKHSGRISFSPQVSWIMPGTIKENIvFGVSYDqYRYlSViKACQLEEDIsKFPEKDNTVLGEGGITLS
Dogfish  441 VLKNIsFKLEKGeLLAIAGSTGSGKSSLLMMIMGELEPSdGKIKHSGRISYSPQVpWIMPGTIKDNIiFGLSYDEYRYTSVvnACQLEEDItvFPnKDKTVLGdGGITLS
Killifish 439 VLKNInlrLEeGmmLAvAGSTGSGKSSLLMMIlGELvPSeGKIrHSGRISYSPQtSWIMPGTIrDNIlFGLtYDEfRYTSiirACQLEEDldllPeKDKTaiaeGGvTLS

Human    550 GGQRARISLARAVYKDADLYLLDSPFGYLDVLTEKEIFESCvCKLMANKTRILVTSKMEHLKKADKILILnEGSsYFYGTFSELQNlqPDFSSKLMGCDsFDQFsAERRN
Bovine   549 GGQRARISLARAVYKDADLYLLDSPFGYLDVLTEKEIFESCiCKLMANKTRILVTSKMEHLKKADKILILHEGSiYFYGTFSELQNqRPDFSSKLMGCDTFDQFTAERRN
Mouse    550 GGQRARISLARAVYKDADLYLLDSPFGYLDVFTEeqvFESCVCKLMANKTRILVTSKMEHLrKADKILILHqGtsYFYGTFSELQslRPsFSSKLMGyDTFDQFTeERRs
Xenopus  551 GGQRARISLARAVYKDADLYLLDSPFSYLDlFTEKeIFESCVCKLMANKTRILVTSKvEqLKKADKvLILHEGsCYFYGTFSELedqRPeFSShLiG...FDhFnAERRN
Dogfish  551 GGQRARISLARAlYKDADLYLLDSPFSHLDvtTEKdIFESClCKLMvNKTRILVTSKLEHLKKADKILLLHEGhCYFYGTFSELQgekPDFSSqLLGsvhFDsFSAERRN
Killifish 549 GGQRARIcLARAVYKDADLYLLDaPFtHLDiaTEKeIFdkCvCKLiasKTRvvVTSKLEHLKrADrILLLHnGdCYFYGTFSELQaqrPDFSSlLLGlgsyDniSAERRs

Human    660 SIlTETLhRFSL...EGDapVSWtETKKqSFKQ.T.......GEFGEKRKNS.ILnpINSIRKFSiVQKTpL..QMNGIEed..sDePLERRLSLVPdSEqGEaILPRis
Bovine   659 SIiTETLRRFSL...EGDtsVSWnETKKpSFKQ.T.......GEFGEKRKNS.ILsSINSIRKFSvVQKTsL..QMNGIEGa..aDaPLERRLSLVPhSEpGEgILPRSN
Mouse    660 SIlTETLRRFSv...Ddssapwsk..pKqSFrQ.T.......GEvGEKRKNS.ILNSfsSvRKiSiVQKTpL.....cIdGE..sDdlqEkRLSLVPdSEQGEAaLPRSN
Xenopus  658 SIiTETLRRCSi...DsdpsavrnEvknkSFKQ.v.......adFtEKRKSS.IiNPrkSsRKFSLmQKsqp..QMsgIeeEDmpaEqGERkLSLVPESEQGEAsLPRSN
Dogfish  661 SILTETfRRCSVssgDgaglgsysEtrkaSFKQpP.......peFnEKRKSSLIvNPitSnkKFSLvQtamS..ypqtngmEDatsEpGERhFSLiPEnElGEptkPRSN
Killifish 659 SILTETlRRvSV...DetagfrghdpnrqSFrhePfhshamgdgypEKRKpSLIlNPlaaarKFSfignsqStnnfpssaiEDgghElsdRrFSvvPEddqvEealPRSN

Human    754 vistGPTlqarRRQSVLNLMT.hSVNQGQnIHRKTtaSTR.KvSLAPQAnL.t.ElDIYSRRLSQeTGLEISEEINEEDLkeClFDDmEsIPAVTTWNTYLRYITVHKSL
Bovine   753 avnsGPTFlGgRRQSVLNLMTgsSVNQGQSIHRKTaTSTR.KmSLAPQASL.a.EiDIYSRRLSQDTGLEISEEINEEDLrdCFFDDVEnIPAVTTWNTYLRYITVHKSL
Mouse    749 miaTGPTFpGRRRQSVLdLMTfTpnSgsSNlqR.TrTSiR.KiSLvPQiSL.n.EvDvYSRRLSQDStLnItEEINEEDLKECFLDDVikIPPVTTWNTYLRYfTlHKgL
Xenopus  754 flnTGPTFQGRRRQSVLnLMTrTSiSQGSNafatRnaSVR.KMSvnSysNs.SfdlDIYnRRLSQDSLiEvSEEINEEDLKECFLDDtdsQsPTTTWNTYLRflTaHKNf
Dogfish  762 ifkseLpFQahRRQSVLALMThsStS..pNkihaRrSaVR.KMSmlSQtNfASsEiDIYSRRLSeDgsFEISEEINEEDLKECFADeeEiQnvTTTWSTYLRYVTNRNL
Killifish 766 lyhhvLqhlngRRQSVLAfiT...naQGqerreqmqSsfRkKlSitpQcNlAS.ElDIYaRRLSkDsvFdISEEvdEEDmeECFADdrEdifeTTsWSTYLRYVsTNRsL

Human    860 iFVLIWCLViFLaEVAASLVVLwLlgntplQ...DKGNS.......ThSrN..NSYAVIITSTSSYYvFYIYVGVADTLLAmGfFRGLPLVHTLITVSKiLHHKMLhSVL
Bovine   860 mFVLIWCLVVFLVEVAASLVVLcLfpkiffQ...DKGNS.......TKSaN..NSYAVIITSTSSYYIFYIYVGVADTLLALGLFRGLPLVHTLITVSKtLHHKMLqSVL
Mouse    855 llVLIWCvlVFLVEVAASLfVLWLlKnNpvn...sgNNg.......TKisN..sSYvVIITSTSFYYIFYIYVGVADTLLALsLFRGLPLVHTLITaSKiLHrKMLHSIL
Xenopus  862 iFiLvfCLVIPfVEVAASsAwLWIIKrNApainmtsNen.......vsevS..dtlSVIvThTSFYYvFYIYVGVADSLLALGifRGLPLVHTLItVSKvLHKKMLHaIL
Dogfish  869 vFVLIlCLVIFlaEVAASlAGLWIIsglAintGsqtNdt.......stdlShlsvfSkfitngShYYIFYIYVGlADSfLALGViRGLPLVHTLvTVSKdLHKQMLHSVL
Killifish 872 iyVLIfiLivFaiEVAgcviGiflItseiwrdGanpNspnyideqhpnasStpvhlavivTptSaYYiIiYIffahsDSiLALGVfRGLPLVHTLlTVSKrLHeQMLsSVL

Human    958 QAPMSTLNTLKaGGILNRFSKDIAiLDDLLPLTIFDFiQLLLIVIGAiaVVaVLQPYIFVATVPVIVAFIMLRAYFLQTSQQLKQLESEGRSPIFTHLVTSLKGLWTLRA
Bovine   958 QAPMSTLNTLKtGGILNRFSKDIAvLDDLLPLTIFDFvQLLLIVIGAvvVVSVLQPYIFLATVPVIaAFILLRAYFLHTSQQLKQLESEGRSPIFTHLVTSLKGLWTLRA
Mouse    953 HAPMSTiskLKAGGILNRFSKDIAILDDfLPLTIFDFiQLvfIVIGAIiVVSaLQPYIFLATVPglVvFILLRAYFLHTaQQLKQLESEGRSPIFTHLVTSLKGLWTLRA
Xenopus  963 HAPMSTFNtMrAGRILNRFSKDTAILDDiLPLsIFDltQLVLIVIGAItVVSlLePYIFLATVPViVaFILLRsYFLHTSQQLKQLESkARSPIFaHLITSLKGLWTLRA
Dogfish  972 qgPMtaFNkMKAGRILNRFiKDTAIIDDMLPLtvFDfvQLiLIVvGAIcVVSvLqPYtlLAAIPVAVIFImLRaYFLRTSQQLKQLESEARSPIFSHLITSLrGLWTvRA
Killifish 982 raPMsvlNtMKtGRImNRFtKDmAvIDDMLPLvlFDliQLtLlVIGcIftVSimrPYifisAIPlAVIFvvmRkYFLRTgQQLKQLEaEARSPIFSHLIiSLkGLWTiRA

Human   1068 fgRQPYFETLFHKALNLHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISILTTGEGEGRVGIILTLAMNIMsTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTE.GKPt
Bovine  1068 ldRQPYFETLFHKALNLHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISILTTGEGEGRVGIILTLAMNIMgTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTEdGKPn
Mouse   1063 FrRQtYFETLFHKALNLHTANWFmYLaTLRWFQMRIdMIFVlFFIvVTFISILTTGEGEGtaGIILTLAMNIMnTLQWAVNSSIDtDSLMRSVSRVFKFIDiqTEEsmyt
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Dogfish 1082 FGRQsYFETLFHKALNLHTANWFLYLSTLRWFQMRIDivFVLFFIAVtFIAIAThdvGEGqVGIILTLAMNItsTLQWAVNsSIDVDGLMRSVSRVFKyIDiPpEgsetk
Killifish 1092 FGRQtYFETLFHKALNtHTAtWFhYLSTLRWFlfRcDviFVLFFtAaaFIAvgTnqdrpGeiGIIvaLAMlIlgTfQWAiitSInVDGLMRSVdRVFKfIDlasEetlqg

Human   1177 kStkPyKngQlSKVMIIENsHVKKDDIWPSGGQMTVKDLTAKYTeGGNAILENISFSISpGQRVGLLGRTGSGKSTLLSaFLRLLNTeGEIQIDGVSWDSITLQQWRKAF
Bovine  1178 nSfrPsKdsQpSKVMIIENqHVKKDDIWPSGGQMTVKDLTAKYTDGGNAILENISFSISsGQRVGLLGRTGSGKSTLLlAFLRLLNTKGEIQIDGVSWDSITLQQWRKAF
Mouse   1173 qiiKElprEgsSdVLvIkNeHVKKsDIWPSGGeMvVKDLTvKYmDdGNAVLENISFSISPGQRVGLLGRTGSGKSTLLSAFLRmLNiKGDIeIDGVSWnSvTLQeWRKAF
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Dogfish 1192 Nrhna...nnpSdVLVIENkhLtKe..WPSGGQMmVnNLTAKYTsdGRAVLqDlSFSVnaGQRVGLIGRTGaGKSTLLSALLRLLNTEGEIQIDGiSWnSvsLQKWRKAF
Killifish 1202 ks.....ggkggpdLVInN..LsthsyWPnrGQleVqgLTvKYTeaGRAVLnDiSFSVdgGQsVGLLGRTGsGKSTLLSALLgLnyTEGEmfIDGvSssSmpLQaWRKAF

Human   1287 GVIPQKVFIFSGTFRKNLDPYeQWSDQEIWKVADEVGLRsVIEQFPGKLDFVLVDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPvTYQIIRRTLKQAFAdCT
Bovine  1288 GVIPQKVFIFSGTFRKNLDPYGQWSDQEIWKVADEVGLRaVIEQFPGKLDFVLVDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPITYQIIRRTLKQAFAnCT
Mouse   1283 GVItQKVFIFSGTFRqNLDPnGKWkDEEIWKVADEVGLKsVIEQFPGQLnFtLVDGGyVLSHGHKQLMCLARSVLSKAKIILLDEPSAHLDPITYQvIRRvLKQAFAgCT
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Killifish 1305 GVvPQKVFiltGTFRmNLDPYgrySDdElWrVaEEVGLKSvIEQFPDKLdFeLkDGGsvLSNGHKQLMCLARSILSKArILLLDEPssyLDPiTlQvlRKTLKqsFSgCT

Human   1397 VILcEHRIEAMLECQqFlVIEENKVRQYDSIQklLnErSLFRQAISPsDRvKLfP..HRNSSkckSkpq...IAALKEETEEEVQdTrL 1480
Bovine  1398 VILsEHRIEAMLECQRFfVIEENKVRQYDSIQrmLSEKSLFRQAISPaDRlKLlP..HRNSSrqrSRsn...IAALKEETEEEVQETkL 1481
Mouse   1393 VILcEHRIEAMLdCQRFLVIEEsnVwQYDSlQaLLSEKSiFqQAISsSekmrffq..gRhSSKhKpRtQ...ItALKEETEEEVQETRL 1476
Xenopus 1400 VILSEHRlEAMLECQRFLVIEdntVrQYDSiQKLvnEKSfFKQAISHSDRlKLFplHRRNSSKRKsRPQ...ISALQEETEEEVQdTRL 1485
Dogfish 1407 VILSEHRVEALLECQRFLVIEgcSVKQfDalQKLltEaSlFKQvfgHlDRaKLFtaHRRNSSKRKtRPk...ISALQEEAEEEdlQeTRL 1492
Killifish 1415 VILSEHKVEpLLECQsFLmIEksSVKsyDsiQKLmnEmShlKQaispaDRlhLFptphRlnSikrpqPqttkISsLpEAEEdeiQdTRL 1503
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Fig. 2. Alignment of the deduced amino acid sequences of CFTR from 6 species. Amino acids invariant between all species are highlighted in bold. Residues identical in adjacent sequences are in capital letters, and others are in lower case. The 12 membrane-spanning regions (TM1-TM12) are boxed, the 2 nucleotide-binding domains are double underlined, and the R domain is highlighted with a dashed line. Putative glycosylation sites between TM7 and TM8 are underlined in each of the 6 sequences. These sequences were taken from Refs. 7, 22, 30, 34, and 37.

Table 1. *Percentage identity and similarity of individual domains of killifish CFTR compared with equivalent domains of human CFTR*

	%Identity	%Similarity
First transmembrane domain	60.5	79.8
First nucleotide-binding domain	71.1	86.8
R domain	50.2	72.0
Second transmembrane domain	58.0	76.4
Second nucleotide-binding domain	72.6	88.1
Total	59.0	77.3

CFTR, cystic fibrosis transmembrane conductance regulator.

FW-adapted killifish were exposed to SW and sampled after 1, 3, 8, and 24 h and 2, 7, and 28 days. Expression of *kfCFTR* in the gills increased 8 h after transfer to SW (Fig. 4). Control fish transferred from FW to FW showed levels of *kfCFTR* expression similar to the levels from fish sampled directly from the FW holding tank, demonstrating that expression changes were not due to stress of handling. Levels of *kfCFTR* expression were quantitated from the Northern blot as the ratio of kfCFTR to β-actin mRNA. A ninefold increase in *kfCFTR* expression was seen 24 h after transfer to SW, which decreased to a threefold higher level by 28 days compared with fish transferred from FW to FW.

Plasma Na^+ levels were also measured in these fish to verify that the response of the killifish used to study *kfCFTR* expression was as expected. Plasma Na^+ levels reached a peak after 8 h of exposure to SW (226.3 ± 4.9 mM) and were significantly higher than any other group ($P < 0.05$; Fig. 5). Fish transferred from FW to FW also showed a significant increase in plasma Na^+ levels (188.7 ± 5.0 mM) compared with fish sampled directly from the FW holding tank (164.5 ± 2.8 mM; $P < 0.05$).

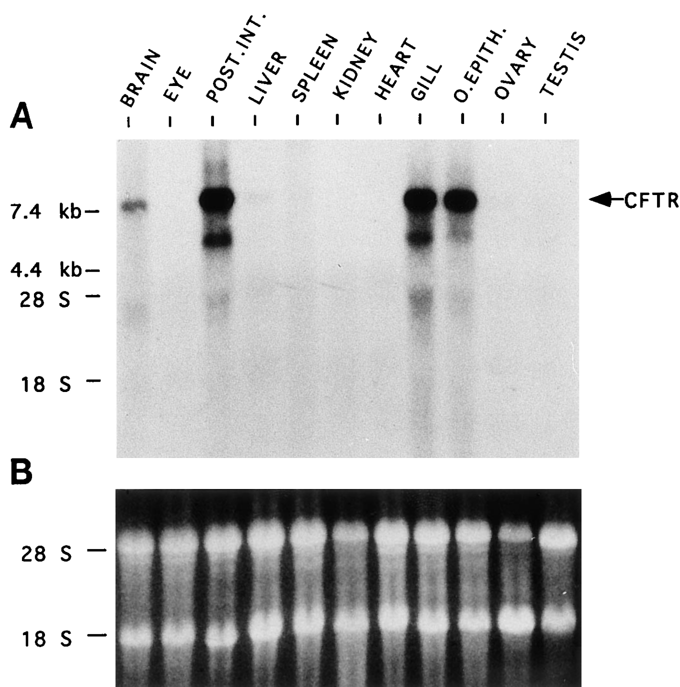

Fig. 3. Tissue-specific expression of kfCFTR mRNA. *A*: Northern blot of total RNA isolated from different seawater (SW)-adapted killifish tissues probed with a 929-base pair (bp) *kfCFTR* cDNA fragment. Lanes were loaded with 10 µg of total RNA from each tissue. *B*: controls for RNA loading of each sample. Positions of 28S and 18S ribosomal RNA molecules are indicated. Post int, posterior intestine; O epith, opercular epithelium.

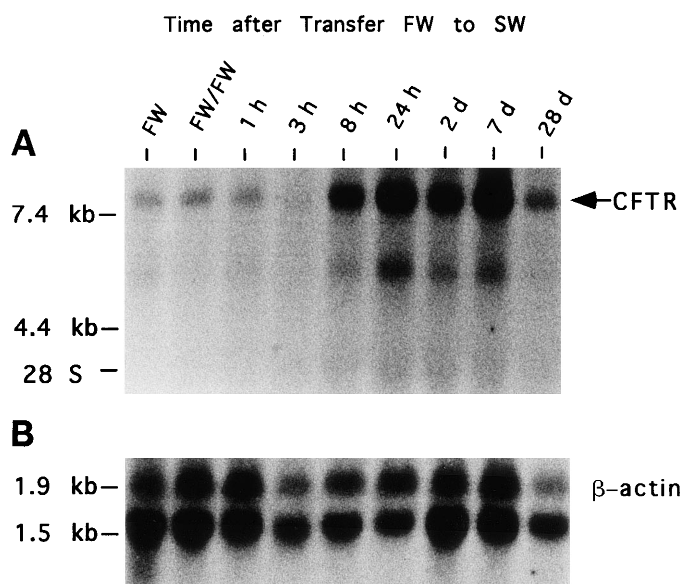

Fig. 4. Effect of rapid transfer from freshwater (FW) to SW on *kfCFTR* expression. *A*: Northern blot of 10 µg of total RNA isolated from gill tissue of FW-adapted killifish exposed to SW for indicated period of time, probed with a 929-bp *kfCFTR* cDNA fragment. *B*: same blot reprobed with a 700-bp killifish β-actin cDNA fragment as a control for RNA loading of each sample. FW/FW, FW to FW control fish; d, days.

kfCFTR functions as a cAMP-activated Cl^- channel. To assess whether the *kfCFTR* cDNA encodes a cAMP-activated Cl^- channel, it was expressed in *Xenopus* oocytes. Using the two-electrode voltage-clamp configuration, currents were measured at +30 mV, since at these potentials no background currents were seen in mock/uninjected oocytes. Basal currents were observed in oocytes injected with kfCFTR mRNA (3.88 ± 0.15 µA; $n = 8$) compared with water-injected oocytes (0.55 ± 0.15 µA; $n = 8$; Fig. 6). These basal currents were of a magnitude similar to those observed in oocytes injected with an equivalent amount of hCFTR mRNA (4.16 ± 0.65 µA; $n = 6$). In kfCFTR-expressing oocytes these basal currents were increased significantly by cAMP stimulation (16.55 ± 3.95 µA; $n = 8$; Fig. 6), equivalent to currents generated by expression of hCFTR on cAMP stimulation (18.73 ± 3.15 mA; $n = 6$).

Figure 7*A* shows a representative current trace from an oocyte expressing kfCFTR before stimulation, 10 min after addition of cAMP agonist, and 15 min after removal of agonist. The stimulated kfCFTR currents exhibited a linear current-voltage (*I-V*) relationship with time- and voltage-independent characteristics (Fig. 7*B*). The effects of cAMP-dependent activation could be reversed by removal of the agonist from the bath.

To assess the ion selectivity of kfCFTR, the bath Cl^- was replaced by the impermeant anion gluconate (96 mM NaCl was substituted with 96 mM sodium gluconate) after activation. This caused a 37.2 ± 2.8 mV

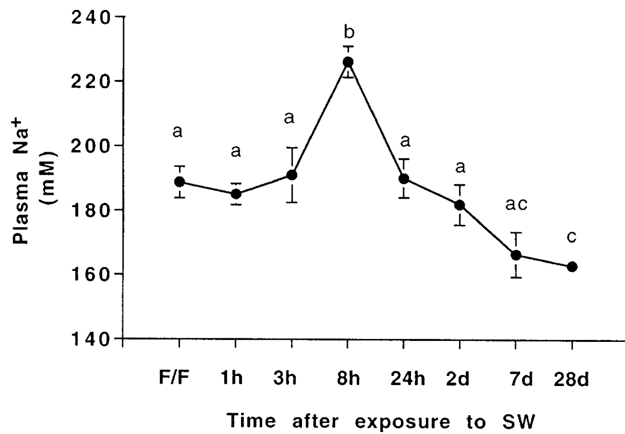

Fig. 5. Effect of rapid transfer from FW to SW on plasma Na^+ levels. Plasma Na^+ concentration of FW-adapted killifish exposed to SW for indicated period of time. Data points without a common letter are significantly different at $P < 0.05$. Each point represents mean ± SE of data obtained from 4–8 fish. Note: 28-day error bar is within symbol.

($n = 4$) shift in the reversal potential, from −33.8 ± 1.8 mV (NaCl) to 4.6 ± 1.9 mV (sodium gluconate), indicating a permeability (P) ratio of $P_{Cl}/P_{gluconate} > 5.8$. Figure 8 shows a representative *I-V* relationship from an agonist-stimulated oocyte before and after gluconate substitution.

DISCUSSION

To study osmoregulation in the killifish, a homologue of the *hCFTR* gene was characterized. The deduced amino acid sequence of kfCFTR is the most divergent form of CFTR identified to date, only 59.0% identical to hCFTR and considerably more divergent than the dogfish shark sequence that is 72.4% identical to hCFTR (22). Despite this relatively low level of sequence conservation, function has been conserved. Heterologous expression of kfCFTR in *Xenopus* oocytes generated a cAMP-regulated Cl^--selective conductance with properties similar to those of hCFTR (3): a linear *I-V* relationship that is time and voltage independent and stimulated by cAMP.

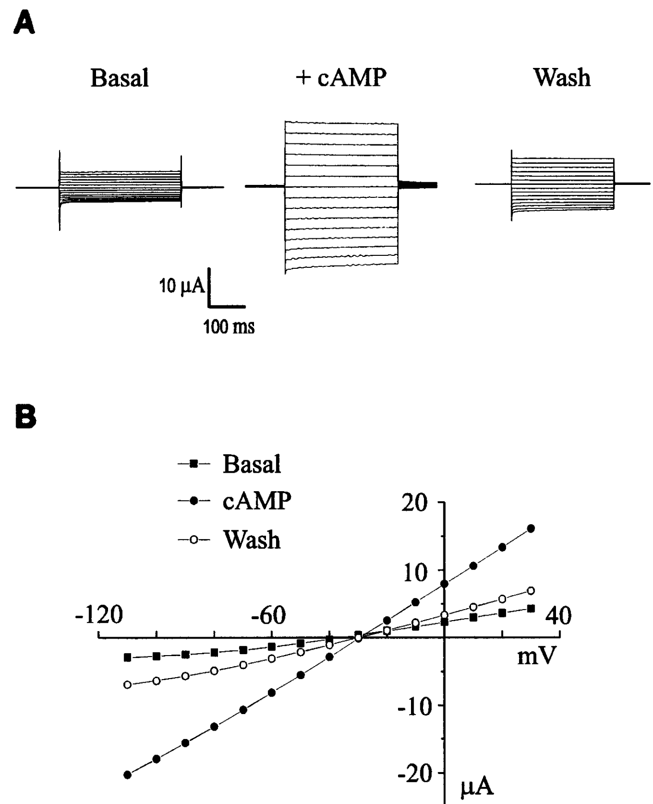

Fig. 7. Current-voltage relationship of kfCFTR channel activity. *A*: typical current trace recorded from an oocyte expressing kfCFTR before (basal) and 10 min after addition of a cAMP agonist cocktail. Currents recorded 10 min after removal of agonist are also shown (Wash). Currents were elicited by a series of voltage steps from −110 to +30 mV in 10-mV steps from a holding potential of −30 mV. *B*: corresponding current-voltage relationship before, during, and after cAMP stimulation.

This highly divergent kfCFTR homologue highlights those amino acids that are conserved in all forms of CFTR. The first and second nucleotide-binding domains (NBDs) are the most highly conserved domains, and the site of the most frequent mutation in humans, ΔF508, is retained. The Walker A and B motifs located in each NBD of kfCFTR, hallmarks of ATP binding proteins, are identical to the same motifs in hCFTR except for four conservative substitutions: two located

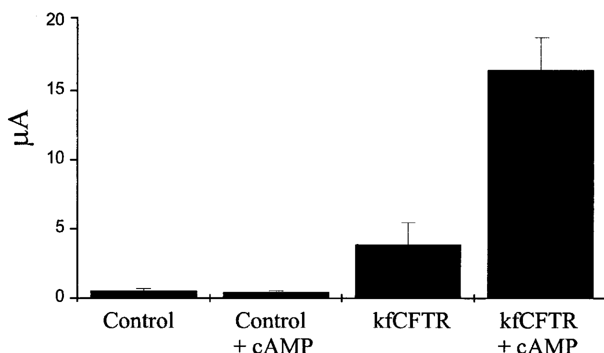

Fig. 6. Generation of cAMP-activated currents by kfCFTR. Histogram showing currents recorded from control (water-injected) or kfCFTR cRNA-injected oocytes, both before and 10 min after exposure to a cAMP agonist cocktail. Currents were measured at +30 mV (holding potential −30 mV) and expressed as means ± SE ($n = 8$).

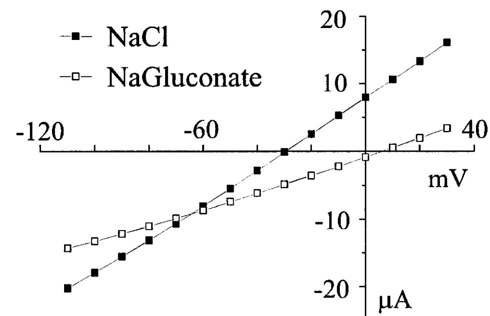

Fig. 8. Anion selectivity of kfCFTR-generated currents. Representative current-voltage relationship from an oocyte expressing kfCFTR recorded in Cl^--containing or gluconate-containing extracellular medium. In both cases currents were recorded in the presence of the cAMP agonist.

in Walker A from NBD1 and two located in Walker B in NBD2. The presence of an R domain demonstrates that kfCFTR is a true CFTR homologue, distinct from other members of the ABC superfamily of transporters (16). The R domain is the least highly conserved domain, although it retains eight consensus protein kinase A (PKA) phosphorylation sites, similar to nine for the R domain from both hCFTR (30) and sCFTR (22). This is consistent with the model in which the R domain regulates channel activation through phosphorylation by cAMP-dependent protein kinases. In the putative transmembrane domains (TMDs), predicted membrane-spanning segments TM6 and TM12, which are believed to line the channel pore (5, 27), show the highest identity with those of hCFTR. The four cytoplasmic loops (CL) linking the TMs on the cytoplasmic side of the membrane also show high levels of conservation. CL1 and CL2 are thought to be involved in determining the open probability and conductive states of the channel (39, 40), whereas CL3 and CL4 may regulate channel opening and closing (31, 32). The extracellular loop between TM7 and TM8 contains a single *N*-linked glycosylation site (N-X-S/T) in kfCFTR, compared with two sites for all other known CFTR homologues.

CFTR-like anion conductances have previously been measured in primary cultures of opercular epithelium from SW-adapted killifish using patch-clamp techniques (26). The functional and expression data obtained here strongly suggest that kfCFTR is responsible for these currents. Interestingly, the Cl^- conductance in the opercular epithelium has been suggested to be regulated differently from CFTR in mammalian airway epithelium (25, 26). First, killifish opercular epithelium shows an α_2-adrenergic-mediated downregulation of Cl^- transport via intracellular Ca^{2+} (25). Second, the opercular epithelium transports Cl^- at a very high rate even when unstimulated (26). These novel features of regulation were not observed from the kfCFTR expressed in *Xenopus* oocytes and may be due to interactions of kfCFTR with endogenous regulatory elements present in killifish opercular epithelium that are likely absent from *Xenopus* oocytes. The kfCFTR homologue characterized here will allow for closer examination of such functional differences in relation to structure, with the use of the appropriate expression systems.

The high expression of kfCFTR in the gill and opercular epithelium of SW-adapted killifish is consistent with the finding that these tissues contain an abundance of specialized Cl^--secreting cells (18). Using the vibrating probe technique, Foskett and Scheffy (12) demonstrated that, in SW-adapted teleosts, Cl^- movement was localized to these cells. The gills and opercular epithelium that contain these specialized cells are the tissues responsible for maintaining blood NaCl levels significantly lower than those of the surrounding environment in SW-adapted killifish. The current model for ion transport by chloride cells in SW-adapted teleosts is similar to that for mammalian airway epithelium (24). The driving force for Cl^- secretion is the Na^+ gradient established by the Na^+-K^+-ATPase located in the basolateral membrane (19), and a basolateral Na^+-K^+-$2Cl^-$ cotransporter generates increased intracellular Cl^- concentrations (9). The gills and opercular epithelium act much like specialized salt-secreting tissues such as the shark rectal gland (29) and the duck salt gland (10), where CFTR is expressed at high levels and is thought to be the primary Cl^- channel. Significantly, similar chloride cells in amphibian skin express an anion conductance similar to CFTR (33), and these cells have been compared with a subpopulation of cells in the human submucosal glands where CFTR mRNA and protein expression is localized (8). The abundance of these cells in the killifish gill and opercular epithelium make it an ideal model for studying the role of CFTR and the chloride cell.

In addition to the expected high level of *kfCFTR* expression in the gill and opercular epithelium, *kfCFTR* was also expressed at high levels in the posterior intestine. Unlike the gill and the opercular epithelium, the intestine of marine teleosts is mainly NaCl absorptive (21). This contrasts with the human intestine where *CFTR* is expressed at high levels in the secretory crypts of the small intestine and distal colon (36). It will be interesting to determine whether the intestinal form of *kfCFTR* plays an absorptive rather than secretory role. Interestingly, in the human sweat duct *CFTR* serves an absorptive role (28).

The killifish provides a unique opportunity to examine the role of CFTR in an organism capable of highly regulated salt transport. *kfCFTR* showed a rapid, ninefold increase in expression 24 h after FW-adapted killifish were exposed to SW followed by a drop in expression such that, after 28 days of SW exposure, they were only threefold higher than fish transferred from FW to FW. Also associated with the abrupt exposure to SW, plasma Na^+ levels increased significantly from 188 to 226 mM in just 8 h and subsequently fell. A similar transient 65 mosM increase in plasma osmolarity was observed after 10–15 h in killifish transferred from FW to SW (41). It is not yet known whether plasma salt levels provide a direct signal for increased *kfCFTR* gene expression.

The increases in *kfCFTR* expression strongly suggest a role in SW adaptation and osmoregulation. The mechanism by which *kfCFTR* gene expression increases during rapid SW adaptation is not known and could be a result of transcriptional activation or a reduction in mRNA turnover. Further investigation of the role of kfCFTR and its regulation at both the gene and protein level during rapid adaptation to changes in salinity will not only lead to an understanding of the molecular mechanisms of teleost osmoregulation but may also increase our understanding of hCFTR function and dysfunction.

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