J. Membrane Biol. 151, 139–148 (1996)

The Journal of
Membrane
Biology

CFTR Channels Expressed in CHO Cells Do Not Have Detectable ATP Conductance

R. Grygorczyk, J.A. Tabcharani, J.W. Hanrahan
Department of Physiology, McGill University, 3655 Drummond Street, Montréal, Québec, Canada H3G 1Y6

Received: 8 November 1995/Revised: 23 January 1996

Abstract. The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated, ATP-dependent chloride channel which may have additional functions. Recent reports that CFTR mediates substantial electrodiffusion of ATP from epithelial cells have led to the proposal that CFTR regulates other ion channels through an autocrine mechanism involving ATP. The aim of this study was to determine the ATP conductance of wild-type CFTR channels stably expressed in Chinese hamster ovary cells using patch clamp techniques. In the cell-attached configuration with 100 mM Mg · ATP or Tris · ATP solution in the pipette and 140 mM NaCl in the bath, exposing cells to forskolin caused the activation of a low-conductance channel having kinetics resembling those of CFTR. Single channel currents were negative at the resting membrane potential (V_m), consistent with net diffusion of Cl from the cell into the pipette. The transitions decreased in amplitude, but did not reverse direction, as V_m was clamped at increasingly positive potentials to enhance the driving force for inward ATP flow (>+80 mV). In excised patches, single channel currents did not reverse under essentially biionic conditions (Cl_{in}/ATP_{out} or ATP_{in}/Cl_{out}), although PKA-activated currents were clearly visible in the same patches at voltages where they would be carried by chloride ions. Moreover, with NaCl solution in the bath and a mixture of ATP and Cl in the pipette, the single channel *I/V* curve reversed at the predicted equilibrium potential for chloride. CFTR channel currents disappeared when patches were exposed to symmetrical ATP solutions and were restored by reexposure to Cl solution. Finally, in the whole-cell configuration with NaCl in the bath and 100 mM MgATP or TrisATP in the pipette, cAMP-stimulated cells had time-independent, outwardly rectifying currents consistent with CFTR selectivity for external Cl over internal ATP. Whole-cell currents reversed near $V_m = -55$ mV under these conditions, however the whole cell resistance measured at −100 mV was comparable to that of the gigaohm seal between the plasma membrane and glass pipette (7 GΩ). We conclude that CFTR does not mediate detectable electrodiffusion of ATP.

Key words: CFTR — Cystic fibrosis — Chloride channel — ATP conductance — Secretion

Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a nonrectifying, low-conductance chloride channel which mediates cAMP-regulated Cl permeability in epithelial and some nonepithelial cells [15]. Expression studies have suggested a loose correlation between the severity of disease symptoms observed in patients with cystic fibrosis (CF) and the amount of CFTR Cl channel function remaining for particular mutants. Thus, mutations that cause mislocalization (ΔF508; [11]) or inhibit responsiveness to secretagogues (G551D; [13]) are usually associated with severe disease symptoms, whereas mutations that only partially reduce open probability (R117H; [26, 5]), single channel conductance (R347P/H; [25, 26]), or targeting to the plasma membrane (A445E; [25]) correlate with milder disease symptoms [31]. This relationship between disease severity and residual channel activity is consistent with the main role of CFTR being that of a cAMP-regulated Cl channel [3, 16] but does not exclude other functions, which might also contribute to the variable severity of disease symptoms.

Recently, it has been reported that the CFTR channel conducts ATP at rates that can be measured electrophysiologically [22, 24]. ATP is an important extracellular agonist for many cells, and purinergic (P_{2U}) recep-

Correspondence to: R. Grygorczyk

tors having high affinity for ATP are expressed on airway epithelia [20]. It has been proposed that ATP diffusion out of epithelial cells is mediated by CFTR channels, is stimulated by cAMP, and serves as an autocrine activator of CFTR itself [9], outwardly rectifying anion channels [22, 24], and perhaps other transporters present in the apical membrane [22, 1]. ATP is known to stimulate secretion by airway epithelia, thus ATP efflux through CFTR could potentially be involved in the normal regulation of transepithelial transport. The P_{2U} purinergic receptor agonist UTP is presently in clinical trails as a potential treatment for CF [17], thus regulation by extracellular ATP may have therapeutic implications.

This paper investigates the ATP conductance of wild-type CFTR channels stably expressed in Chinese hamster ovary cells. Several patch clamp configurations, protocols, and salts of ATP have been used to evaluate its permeation through CFTR. Contrary to previous reports, we could find no evidence for electrodiffusion of ATP through the CFTR chloride channel. These results have been presented in preliminary form [14].

Materials and Methods

Cells

Chinese hamster ovary cells expressing wild-type CFTR [10, 27] were plated at low density on glass coverslips and cultured at 37°C in 5% CO_2 for 2–4 days before being used in patch clamp experiments. Cultures were maintained in αMEM supplemented with 8% FBS, 20–100 μM methotrexate, penicillin (100 U/ml) and streptomycin(100 μg/ml). Media constituents were from GIBCO (Burlington, Ont.) except methotrexate (D. Bull Lab., Mulgrave, Australia).

Solutions

Cells were transferred to a recording chamber that initially contained (in mM): 140 NaCl, 5 KCl, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, 10 TES, pH 7.4 (Soln. A). To measure control Cl currents through single CFTR channels under cell-attached conditions, the pipette solution contained (in mM): 140 NMDG · Cl, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 30 sucrose, 10 TES, pH 7.4 (Soln. B). ATP permeation in the inward direction was examined using a pipette solution containing (in mM): 100 MgATP or Tris-ATP, 10 TES, pH 7.4 (Soln. C or D, respectively). To measure outward ATP flow in the inside-out configuration the pipette was filled with 150 NaCl, 2 $CaCl_2$, 10 TES, pH 7.4 (Soln. E) and the cytoplasmic side was exposed to 161 Na_2ATP, 10 TES, pH 7.4 (Soln. F) or 80.5 Na_2ATP, 77 NaCl, 10 TES, pH 7.4 (Soln. G). To measure the ATP conductance of single cells, whole-cell currents were recorded after breaking into the cell with the pipette containing Soln. C or D.

Channel activity was stimulated during cell-attached and whole-cell experiments by including 10 μM forskolin and, in some experiments, 400 μM 8-BrcAMP in the bath solution. Forskolin, 8-BrcAMP, and sodium, Tris and magnesium salts of adenosine triphosphate were all from Sigma Chemical (St. Louis, MO). The cytoplasmic side of inside-out patches was exposed to 1 mM MgATP and purified catalytic subunit of cyclic-AMP dependent protein kinase (PKA; 180.5 nM) to maintain CFTR channel activity. Type II bovine cardiac PKA was obtained from Promega or from the laboratory of Dr. M.P. Walsh (*ref.* 27 for description). Experiments were carried out at room temperature (~21°C).

Patch Clamp Recording

Standard methods were used to record single-channel and whole-cell currents. Pipettes were prepared using a conventional puller (PP-83, Narishige Instruments, Tokyo) and had resistances of 4–6 MΩ when filled with Soln. A. Electrical connection to the Ag/AgCl electrode in the pipette was made through an agar bridge containing Soln. A when the pipette contained ATP (Soln. C or D). The bath was grounded through an agar bridge containing Soln A. Liquid junction potentials at the 150 mM NaCl agar bridge were measured against a flowing saturated KCl electrode (19) and were −12 mV for 100 mM MgATP and 100 mM Tris · ATP, −21 mV for 161 mM Na_2ATP and −7.5 mV for the mixture of 77 mM NaCl and 80.5 mM Na_2ATP. All voltages shown in the figures were corrected for these junction potentials. Clamp commands were generated by a computer and the resulting single channel currents were amplified (Axopatch 1C, Axon Instruments, Foster City, CA) and recorded on videocassette tape by a pulse-coded modulation-type recording adapter (DR384, Neurodata Instruments, NY). In whole-cell experiments, membrane capacitance was measured and then canceled using the internal circuitry of the amplifier. Whole-cell currents were low-pass filtered at 1 KHz and sampled on-line at 2 KHz using a TL-1 interface (Axon Instruments). Single channel recordings were filtered at 50–150 Hz (4–8 pole Bessel), digitized at 0.25–1 KHz, and stored on the computer's hard disk. V_p refers to the command voltage applied to the pipette interior with reference to the bath. In whole cell experiments $V_p = V_m$, the membrane potential. In the excised, inside-out patch configuration $-V_p = V_m$, and in cell-attached patches $-V_p$ is the applied shift in membrane potential. Reversal potentials were estimated by interpolation after fitting polynomial functions to the *I/V* curves. Slope conductance was determined by linear regression over the voltage ranges specified in the Results section.

Results

When CHO cells stably expressing CFTR were pre-stimulated with 10 μM forskolin, a low-conductance channel with slow gating and outward rectification was observed (Fig. 1*B*; 145 mEq/L Cl, bath Soln. A, pipette Soln. B). These channels were not present in control CHO cells that had been transfected with vector alone ([27], *data not shown*). The current-voltage relationship reversed near 0 mV, consistent with a passive distribution of Cl across the membrane during forskolin stimulation. The single channel conductance was 6 pS at positive membrane potentials (between +20 and +60 mV) and approximately 2 pS at the corresponding negative potentials. Identical results were obtained in three experiments during which the large, impermeant cation N-methyl-D-glucamine was replaced by sodium. These electrophysiological properties are consistent with those of the CFTR Cl channel as shown in previous expression [6, 27], mutagenesis [2,29], and reconstitution [4] studies. The properties of recombinant CFTR channels are indistinguishable from those of a low-conductance Cl channel found on cells from pancreas, colon and lung;

tissues which are usually affected in CF (*see review* [15]).

CFTR channels were recorded in the cell-attached configuration with pipette solution containing 100 mM MgATP to assess their permeability to external ATP (Fig. 1). Large inward single channel currents were observed at 0 mV pipette potential under these conditions after correction for the liquid junction potential, as would be expected for a net flow of chloride ions from the cell to the pipette. Transitions became smaller as the membrane potential was made increasingly positive, however the current did not reverse at potentials up to +90 mV. Single channel currents compatible with outward flow of Cl ions were clearly observed in the patches but there was no evidence for inward electrodiffusion of ATP.

To examine ATP conductance under more defined ionic conditions, patches were excised in the inside-out configuration with 161 mM Na_2ATP in the pipette and 154 mM/L NaCl in the bath (Fig. 2). The bath solution also contained PKA and 1 mM ATP to maintain channel activity. Large inward currents indicative of anion diffusion from bath to pipette were observed at 0 mV applied potential. Channels recorded under these essentially biionic conditions had slow gating that is characteristic of CFTR (Fig. 2*A*). The amplitude of these currents varied linearly with voltage between −20 and +70 mV and became too small to resolve at potentials greater than +70 mV. Positive currents were not observed at voltages exceeding $V_m = +120$ mV, suggesting a lack of ATP permeation from the extracellular side. For comparison, the *I/V* curve was linear and the conductance was 7 pS when the pipette and bath solutions contained 154 mM/L NaCl, as reported previously [29].

To obtain a reversal potential that could be compared more definitively with the one predicted for a Cl^- selective electrode, *I/V* relationships were determined with a mixture of 77 NaCl and 80.5 mM Na_2ATP in the pipette (extracellular), and 154 mEq/L Cl in the bath (intracellular). The *I/V* relationship was less steep under these conditions and reversed at +17 ± 1 mV ($n = 4$; Fig. 2*B*), close to the value calculated from the Goldman-Hodgkin-Katz equation (17.9 mV) by assuming ATP im-

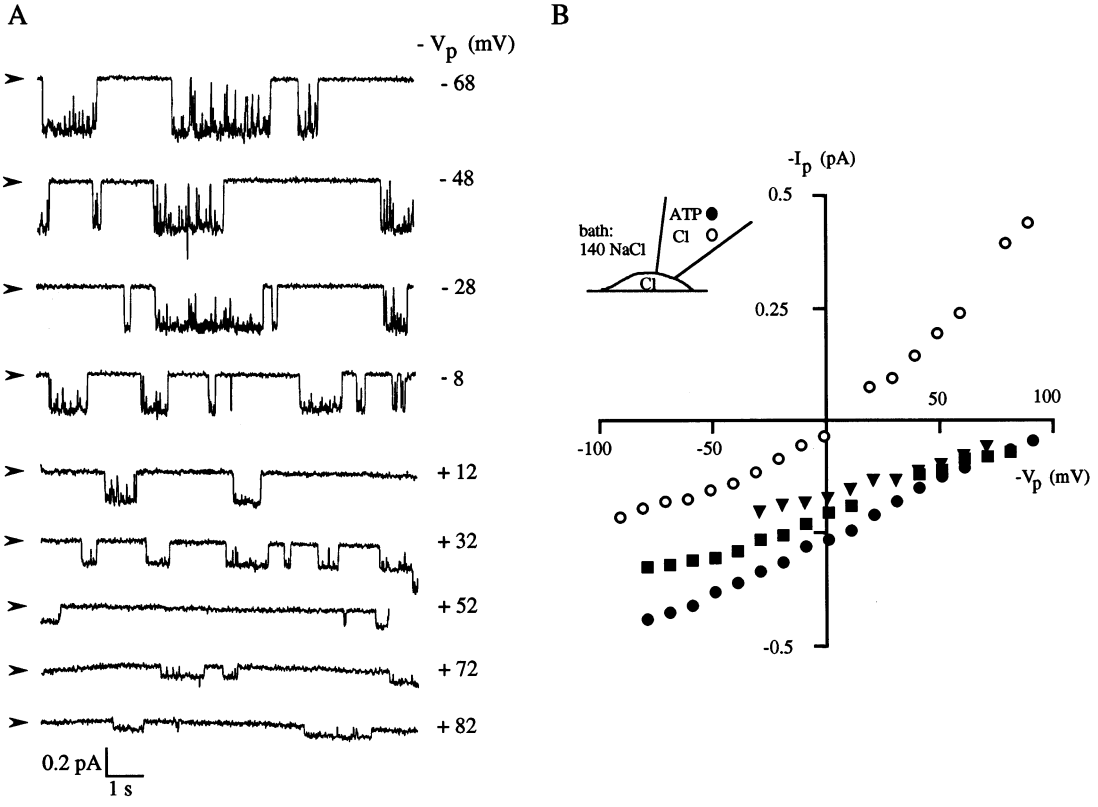

Fig. 1. Permeability of CFTR channels to external ATP in the cell-attached configuration. (*A*) Representative traces obtained with 100 mM MgATP solution in the pipette and 140 mM NaCl in the bath. Arrows indicate the baseline level (channel closed). Experiment R5904#1. (*B*) Current-voltage relationships determined with Cl or ATP solution in the pipette and NaCl solution in the bath. Open circles indicate *I/V* with 140 mM NMDG-Gl in the pipette; Experiment R5O04#6, representative of four cell-attached patches. Filled symbols indicate data from three patches with 100 mM MgATP in the pipette; (●) Experiment R5904#1, (■) Experiment R5830#2, (▼) Experiment R5830#4, representative of five patches. Inset shows a schematic of the patch pipette and intact CHO cell during cell-attached recording with pipette solution containing (○) 140 mM NMDG · Cl or (●) 100 mM MgATP.

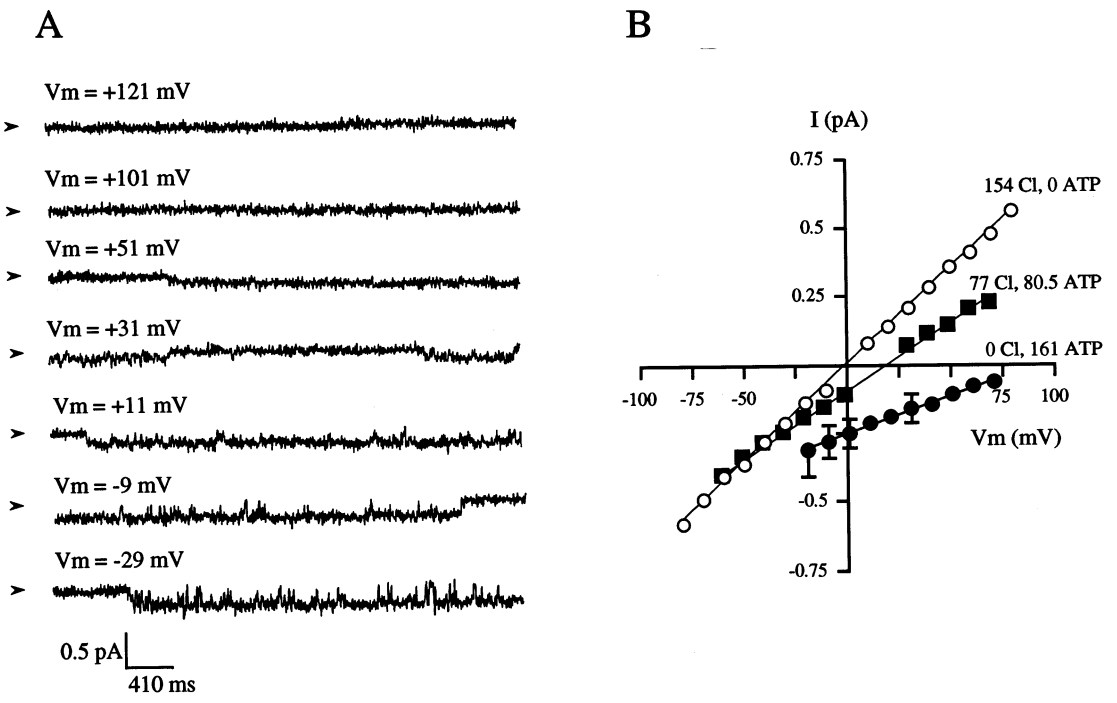

Fig. 2. Permeability of CFTR to external ATP using excised, inside-out patches. (*A*) Recordings obtained with 161 Na_2ATP in the pipette and bath solution containing 150 mM NaCl and 2 mM $MgCl_2$ ($n = 5$). (*B*) Current-voltage relationships obtained with (○) symmetrical 154 mEq/L Cl solutions bathing each side of the membrane, (●) same conditions as in (*A*), and (■) 154 mEq/L in the bath and pipette solution containing 77 mM Cl and 80.5 mM MgATP ($n = 4$). A reversal potential could not be measured with pure ATP solution in the pipette however with the ATP+Cl mixture in the pipette the *I/V* curve reversed at 17.0 mV, very close to the value predicted using the Nernst equation, assuming negligible ATP permeability, finite Na conductance, and similar activity coefficients for Cl on both sides of the membrane (*see text*). Error bars, if not shown, are smaller than the symbols.

permeability ($P_{ATP}/P_{Cl} = 0$), $P_{Na}/P_{Cl} = 0.08$ (obtained previously from dilution potential experiments [27], and similar Cl activities in NaCl and the Na_2ATP + NaCl mixture. The close agreement between the predicted values and observed reversal potentials provides further evidence that CFTR has negligible ATP conductance.

We considered the possibility that ATP permeation is asymmetric and occurs only in the outward (i.e., proposed physiological) direction. The conductance for outward ATP flow was studied by replacing bath NaCl in Soln. A (mM) with 161 mM Na_2ATP. The pipette solution contained 154 mM NaCl. Figure 3 shows representative traces (Fig. 3*A*) and the mean single channel current-voltage relationship (Fig. 3*B*) under these conditions. Single channel currents did not reverse direction at the most negative potentials tested (<−120 mV), well beyond the reversal potential of −22 mV reported previously under similar conditions [24]. This indicates that if ATP does permeate through the CFTR pore, the ATP:Cl permeability ratio is several orders of magnitude lower than has been reported.

In the single channel studies described above CFTR channels were exposed to both Cl and ATP. This would also be true in vivo, however other laboratories [21, 22] have described currents under symmetrical ATP conditions, and it is conceivable that Cl may have blocked ATP permeation in the experiments described above. To test this possibility, the cytoplasmic side of excised, inside-out membrane patches was alternately exposed to high-Cl bath solution or to a continuous stream of Tris · ATP, with Tris · ATP solution in the pipette. The continuous trace in Fig. 4 is an example of one such experiment. Negative single channel currents carried by Cl flowing from bath to pipette were observed at $V_m = -18$ mV after excision. When the pipette tip was moved into the stream of Tris · ATP, the change in liquid junction potential at the interface between ATP and NaCl solutions (−12 mV) caused the baseline current to shift by ~−0.5 pA. More importantly, the amplitude of single channel currents declined to zero despite the increased driving force for outward anion movement (−18 → −30 mV). Transitions were not observed over a range of 60 mV during the next 90 sec of ATP exposure. CFTR channel currents reappeared within 400 millisec after the pipette tip was moved from the ATP stream back to the NaCl solution.

Taken together these data suggest there is little if any ATP electrodiffusion through single CFTR channels. The whole cell patch configuration is potentially a more sensitive assay of ATP conductance since it sums con-

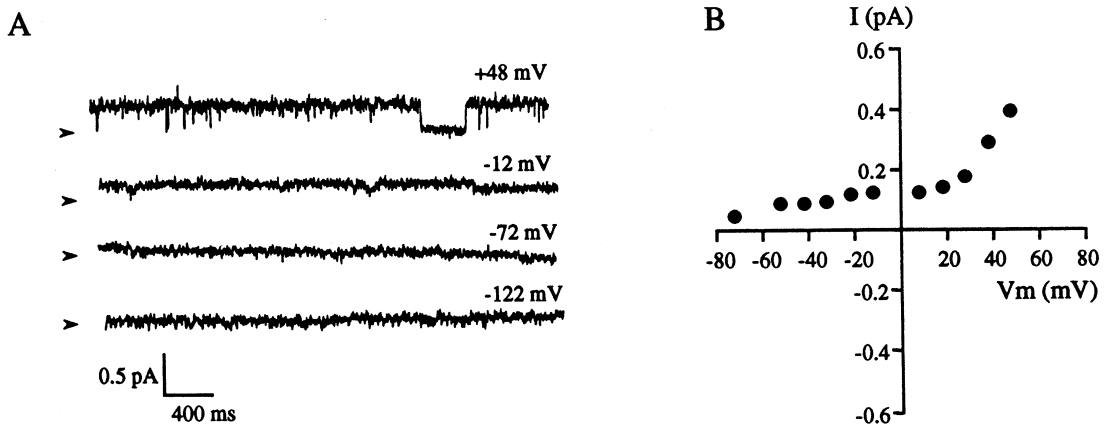

Fig. 3. Permeability of CFTR to ATP on the cytoplasmic side of excised patches. (*A*) Single channel currents recorded at several potentials with Cl in the pipette and ATP in the bath. (*B*) Mean current-voltage relationship determined under the same conditions as in (*A; n* = 3). Currents became too small to resolve at potentials more negative than −72 mV, but they did not reverse at large negative voltages (*ca.* −122 mV). Standard errors are smaller than the symbols, which represent 3 patches at each potential.

tributions from all the CFTR channels present in the cell. Based on comparisons of the whole cell and single channel *I/V* curves at +50 mV and assuming a value for open probability of ~0.4 [27] we estimate there are 300–500 functional CFTR channels per cell.

Currents immediately before and after breaking into the cells with 100 mM MgATP in the pipette and 150 mM Cl in the bath are shown in Fig. 5. As expected, the macroscopic currents measured in the cell-attached configuration were small and independent of time (Fig. 5*A*), and inward single channel events were observed at all applied potentials over the range of −112 and +88 mV, consistent with CFTR-mediated Cl efflux into the pipette containing ATP solution (*see also* Fig. 1). The time course of macroscopic currents measured at +48 mV and −72 mV are plotted before (Fig. 5*B*; open symbols), and after (closed symbols), applying excess suction to break into the cell. Whole cell currents at V_m = +48 mV were largest immediately after entering this configuration and relaxed within 2–3 min to steady-state values that were about 60% their initial amplitude. Importantly, the steady-state currents were much larger at +48 mV, where they could be carried by inward Cl flow than at −72 mV, where the only intracellular anion available to carry current was ATP. The whole-cell current at −72 mV declined rapidly to the same level as in the cell-attached configuration, suggesting that most negative whole cell current flowed through the seal resistance between cell membrane and glass pipette. Figure 5*C* shows the macroscopic currents recorded during pulses of −112 to +88 mV. The whole cell currents were strongly rectified in the outward direction and showed time- and voltage-independent gating. Figure 5*D* shows a typical current-voltage relationship for macroscopic currents before and after breaking into the whole cell configuration. The *I/V* relationship determined for the leak current in cell-attached recordings when CFTR channels were closed was nearly linear. The slope conductance of the uncorrected whole cell *I/V* between −80 and −112 mV after breaking into the cell was ~140 pS (~7 GΩ), which is in the range of typical gigaohm seals in these experiments. More significantly, when the leak current (measured on-cell) was subtracted from the whole cell current, the ''difference'' current was strongly rectified and did not cross the abscissa within −120 mV. This leak-subtracted current reflects inward diffusion of chloride ions. Any inward difference current that might appear at more hyperpolarized voltages could reflect inward flow of sodium and/or outward flow of ATP.

Discussion

The goal of this study was to characterize ATP conductance in the CFTR chloride channel using transfected CHO cells and patch clamp techniques. However, ATP conductance was not detected in cell-attached recordings with external (pipette) ATP, excised patch recordings with ATP bathing the extracellular, intracellular or both sides of the membrane, or in whole-cell recordings with magnesium or Tris salts of ATP on the cytoplasmic side. We conclude that the CFTR channel does not mediate detectable electrodiffusion of ATP.

Protocols were designed so that the presence of CFTR channels could be verified with chloride-containing solutions at some point during each experiment. The channels were not observed in the absence of PKA stimulation or in untransfected cells. Depending on the conditions (i.e., cell-attached or excised patches), the single channel conductance was 5–7 pS at voltages where currents could be carried by chloride ions as reported previously for CFTR [15]. Identical results were

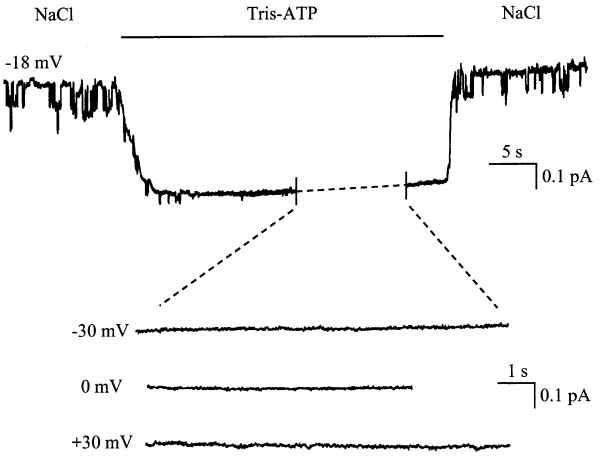

Fig. 4. Effect of symmetrical ATP solutions on single CFTR channels. Traces were recorded with 100 mM TrisATP in the pipette and 150 mEq/L Cl in the bath. As indicated by the horizontal bar, the pipette tip was moved from the chloride-containing bath solution into the opening of a perfusion line through which 100 mM TrisATP solution was flowing. The expanded traces show that CFTR channel currents were abolished in symmetrical ATP when examined at three different potentials. Channel currents were restored instantly when the tip was shifted back to Cl-containing bath solution. Experiment R5O20#2. Representative of 5 trials with two patches.

obtained when N-methyl-D-glucamine, Tris, magnesium or sodium were used as the cation. Some outward rectification was observed in the cell attached configuration with high-Cl solutions in the pipette as reported previously for the human colonic cell line T_{84} expressing endogenous CFTR [3] and CFTR-expressing CHO cells [27] and is probably caused by Goldman-type rectification induced by low intracellular Cl concentration (~40 mEq/L in most cells *vs.* 150 mEq/L Cl in the pipette solution), and attenuation by the low-pass filter of intrinsic channel flickering and voltage-dependent fast block by large intracellular anions. Flickering decreased and the single channel *I/V* relationship became linear when patches were excised into symmetrical high-Cl solutions as described previously [27], and the channels opened in long bursts lasting from hundreds of milliseconds to several seconds, which is typical for CFTR channel gating [12].

Positive and negative single channel currents were recorded with chloride in the pipette and the bath solutions. When the pipette contained ATP, negative currents were greatly increased and positive currents (carried by an influx of anions) were not observed. The extrapolated reversal potential was shifted by $\geqslant$+100 mV, although this would be an underestimate of the reversal if the *I/V* relationship approached the *x* axis asymptotically. To avoid this uncertainty, the reversal potential was measured using a mixture of 80.5 mM Na_2ATP and 77 mM NaCl (Soln. G) in the pipette and a high-Cl solution in the bath. The value obtained under these conditions was 17.0 mV, very close to the reversal potential expected for a perfectly Cl-selective electrode having no ATP permeability (17.9 mV). This agreement within the range of experimental error indicates that the CFTR channel has little, if any, conductance for inward ATP flow. Electrodiffusion of ATP in the outward direction was not observed with ATP on the cytoplasmic side and Cl extracellularly (i.e., in the pipette solution). The amplitudes of single channel events under these conditions declined as the membrane potential was hyperpolarized and were eventually obscured by the baseline noise but they did not reverse despite voltages exceeding −120 mV. The concentration of each major ATP species in our solutions was estimated using a program that corrects association constants for temperature and pH [8]. The starting values used for log Keq (at 0.1 M ionic strength) were: 6.51 $HATP^{3-}$, 4.06 H_2ATP^{2-}, 1.1 $NaATP^{3-}$, 4.06 $MgATP^{2-}$, 1.6 Mg_2ATP, 4.55 $MgHATP^{-}$, 3.26 MgH_2ATP. The following concentrations were obtained for significant ATP species (mM): Soln. C, 5.83 ATP^{4-}, 0.87 $HATP^{3-}$, 88.14 $MgATP^{2-}$, 0.16 $MgHATP^{1-}$, 4.98 Mg_2ATP; Soln. F, 52.83 ATP^{4-}, 4.68 $HATP^{3-}$, 103.47 $NaATP^{3-}$, Soln. G, 22.24 ATP^{4-}, 2.07 $HATP^{3-}$, 56.17 $NaATP^{3-}$. Thus most ATP would be in a divalent anion form in high magnesium solutions, and trivalent anion form with high sodium. Finally, we examined single CFTR channels in symmetrical ATP using a perfusion system that allowed alternating exposure of the cytoplasmic side to ATP or Cl solutions. CFTR channel currents disappeared within one second following exposure to symmetrical ATP and were instantly restored by returning the pipette to chloride-containing solution.

These results differ from those reported recently by other groups. Using mouse mammary cells stably expressing human CFTR, Reisin et al. [22] observed single channel events when patches were bathed symmetrically with Tris or Mg salts of ATP (100 mM). Their currents were activated by PKA, sensitive to diphenylamine-2-carboxylate, and had a linear current-voltage relationship indicating a conductance of 4.8 pS. Interestingly, the *I/V* relationship became outwardly rectifying and the conductance for inward ATP flow increased to 24.8 pS with external ATP and internal Cl, although the conductance for outward Cl permeation remained ~5 pS. The reason for the different results obtained in their study are unclear and may be methodological. When recording with high ATP in the pipette, Reisin et al. filled the tip with ATP solution and backfilled the pipette with a chloride-containing solution which sometimes contained a dye as a visual check for mixing. The adequacy of this method for assessing Cl contamination of the ATP solution near the membrane is uncertain, therefore we used NaCl agar bridges in the bath and pipette.

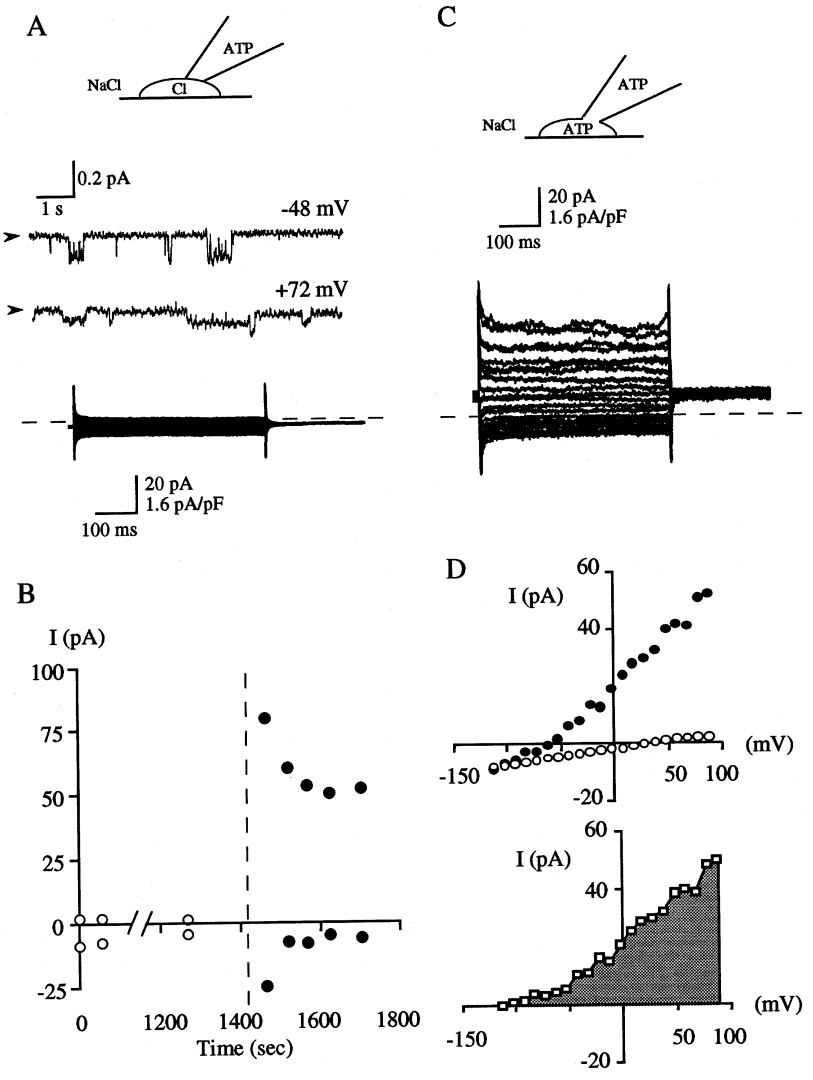

Fig. 5. Recording CFTR currents before and after establishment of the whole cell configuration with 100 mM ATP solution in the pipette and 140 mM NaCl in the bath. The cell was prestimulated with 10 μM forskolin. (*A*) (lower part) Macroscopic currents recorded in the cell-attached configuration during a series of 500 msec voltage pulses of −112 to +88 mV in 10 mV increments. The cell was held at −12 mV between the pulses and the dashed line indicates zero current. (upper part) The single channel currents recorded at $-V_p$ = −48 and +72 mV under these conditions shown at 100-fold higher gain. Arrow heads indicate baseline current level when channels are closed. (*B*) Time course of macroscopic currents recorded at V_p = −72 and +48 mV; (○) before, and (●) after applying a pulse of suction to break into the cell. Note that the currents measured at −72 mV increased briefly and then relaxed back to the same level, presumably due to washout of cytoplasmic Cl by ATP solution from the pipette. (*C*) Steady-state whole cell currents about 4 min after entering the whole cell configuration, recorded in response to a series of voltage pulses as described in (*A*). ATP and NaCl solutions were present in the pipette and bath, respectively. Note the strong outward rectification consistent with selectivity for Cl influx *vs.* ATP efflux. (*D*) (Top) Current voltage relationships obtained (○) before, and (●) after breaking into the whole cell configuration showing convergence of the negative currents. (Bottom) The leak-corrected difference current flowing through the cell membrane with chloride solution in the bath and ATP solution in the cell. Experiment R5830#3; representative of three experiments with MgATP in the pipette.

A study of transfected airway epithelial cells also reported that CFTR has a conductance of 5 pS when bathed with symmetrical ATP solutions, although the *I/V* relationship in asymmetrical (Cl_o/ATP_i) solutions was very different from that described by Reisin et al. in that it was not strongly rectifying and indicated Cl and ATP conductances of 9 and 6 pS, respectively [24]. Schweibert et al. observed a reversal potential of −22 mV with external Cl and internal ATP solutions [24], indicating moderate selectivity for Cl over ATP whereas we found no current reversal. It is difficult to reconcile these data if CFTR was indeed the channel being recorded in both studies. Subtle difference in experimental conditions may have contributed to the disparities, however these

probably not due to the use of CHO rather than airway cells because ATP conductance is also not observed in the CFTR channels which are expressed endogenously on the human airway cell line Calu 3 (C. Haws and J.J. Wine, *personal communication*).

In mammary [22] and airway epithelial cells [24], the channel observed in symmetrical ATP solutions was activated by PKA and blocked by diphenylamine-2-carboxylate (DPC). A recent study of isolated nuclei and plasma membranes from transfected CHO cells also demonstrated a CFTR-like channel in patches bathed with symmetrical 100 mM ATP [21]. The channel was activated by PKA, had a linear *I/V* relationship and conductance of 4.6 pS in symmetrical ATP solutions, and was DIDS insensitive [21]. Since all three studies reporting the ATP conductance of CFTR used symmetrical solutions, we considered the possibility that our negative results with chloride on one side of the membrane might be due to block by Cl ions. Experiments were carried out with Tris · ATP solution in the pipette and Cl or ATP solution on the cytoplasmic side of excised patches. A fine stream of ATP solution enabled rapid and repeated switching between cytoplasmic Cl and ATP solutions while continuously recording CFTR channel currents. Moving the pipette tip into the ATP stream rapidly abolished the CFTR channel currents, which reappeared immediately upon returning the pipette to the Cl-containing bath solution. These effects were almost instantaneous and therefore unlikely to be mediated by alterations in phosphorylation. For comparison, rundown of CFTR activity after PKA removal takes about 100 sec when patches from CHO cells are studied at room temperature [5].

The whole-cell recordings further strengthen the conclusion that CFTR conducts little if any ATP. CFTR channel activity was observed on forskolin-stimulated cells before breaking into the whole cell configuration. Inward single channel currents carried by Cl efflux from cell to pipette containing ATP were superimposed on a baseline current that presumably flowed through the shunt between glass pipette and cell membrane. The seal resistance was typically greater than 5 gigaohms and was comparable to the resistance in the whole cell configuration at strongly negative pipette potentials with intracellular Tris · ATP or Mg · ATP. Inward whole cell current was not observed at −120 mV after subtracting the leak current which had been measured in the cell-attached configuration, therefore much of the negative current in the whole cell configuration was probably carried by sodium influx and ATP efflux through the seal. The finite permeability ratio $P_{Na}/P_{Cl} = 0.08$ suggests there might be some sodium flux through CFTR channels at very large negative potentials.

The present results do not support the hypothesis that CFTR is a multifunctional channel which mediates electrodiffusion of both Cl and ATP, but they do not exclude mechanisms in which ATP is translocated at a much lower rate than reported previously, or in electroneutral fashion. Evidence against other transport mechanisms comes from a luciferase luminometry study in which ATP effluxes were similar in control and CFTR-transfected fibroblasts and were unaffected by the cAMP agonists forskolin and isobutylmethylxanthine [30]. The CFTR-independent ATP leakage from 3T3 fibroblasts is itself an interesting and unexplained result. We occasionally observed a channel in CHO cells which could mediate this ATP efflux; it resembled voltage-dependent anion channels (VDAC) in having unitary conductance (150 pS), numerous substates, and voltage-dependent inactivation at positive potentials [7, 17, 23]. However dilution potential experiments with 100 mM MgATP on one side and 20 mM MgATP on the other side revealed little discrimination between magnesium and ATP. Whether this channel accounts for ATP leakage from CHO and 3T3 cells and mediates ATP efflux under physiological conditions remains to be determined. Constitutive ATP leakage from T_{84} and primary cultures of airway cells was lower than from fibroblasts and was not stimulated by cAMP [30], nevertheless its effect on epithelial secretion in vivo might be significant when confined to a thin layer of airway surface liquid.

The permeability of CFTR to a large number of anions has been studied previously and is restricted to polyatomic anions having diameters $\leqslant$5.5 Å [15, 18]. Figure 6 shows space-filling models for a few representative anions that have high (Cl, NO_3), low (acetate) or negligible (gluconate) permeability through the CFTR pore. For comparison, two views of gluconate and ATP are shown next to an aperture indicating an upper estimate for the effective pore diameter. Although interaction with magnesium might alter the shape of ATP somewhat it seems unlikely to reduce the diameter of ATP sufficiently to pass through the pore by electrodiffusion even if fully dehydrated.

The single channel and whole cell data in this paper indicate that CFTR does not have measurable ATP conductance that could be resolved using the patch clamp technique. The results obtained in this study are difficult to reconcile with those reported previously by others [21, 22, 24]. It seems unlikely, though possible, that CFTR function would differ qualitatively among various cell lines, and regardless, the same cell line was used in one previous study. In the unphysiological environment of high (*ca*100 mM) ATP solutions, it is possible that transitions might be attributed to CFTR when in fact it is some other channel. In this regard it is interesting that we occasionally observed unitary ATP currents, although their conductance (150 pS), kinetics (voltage-dependent), selectivity (weak) were distinct from CFTR. It is conceivable that such channels might have substates re-

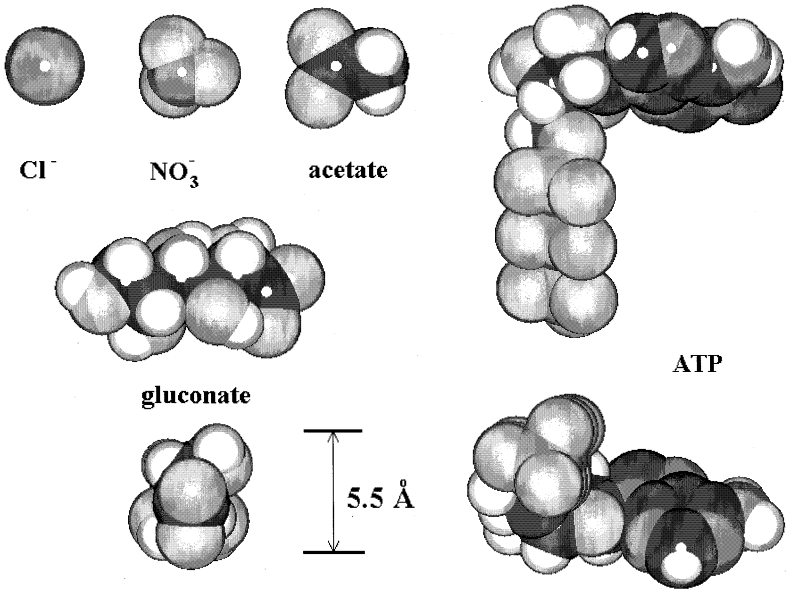

Fig. 6. Space-filling models showing the relative sizes of representative anions that permeate well (Cl and NO_3), have low permeability (acetate) or not measurably permeant (gluconate). Two views of gluconate and ATP are shown for comparison with an upper estimate for the limiting pore diameter of CFTR.

sembling CFTR under other conditions. Finally, immature CFTR channels could have different permeability properties which might explain the ATP currents recorded on isolated nucleii [21]. Further studies are needed to establish the role of ATP as an autocrine regulator of epithelial secretion.

We thank X.-B. Chang and J. Riordan, Mayo Clinic Scottsdale for providing the CHO cell line, S. Brooks and K. Storey for their program and useful discussion, and S.-X. Zheng and J. Liao for technical assistance. This work was supported by the Canadian Cystic Fibrosis Foundation, Medical Research Council (Canada), and the National Institutes of Health (National Institute of Diabetes and Digestive and Kidney Diseases).

References

1. Al-Awqati, Q. 1995. Regulation of ion channels by ABC transporters that secrete ATP. *Science* **269:**805–806
2. Anderson, M.P., Gregory, R.J., Thompson, S., Souza, D.W., Paul, S., Mulligan, R.C., Smith, A.E., Welsh, M.J. 1991. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science* **253:**202–205
3. Anderson, M.P., Rich, D.P., Gregory, R.J., Smith, A.E., Welsh, M.J. 1991. Generation of cAMP-activated chloride currents by expression of CFTR. *Science* **251:**679–682
4. Bear, C.E., Li, C., Kartner, N., Bridges, R.J., Jensen, T.J., Ramjeesingh, M. Riordan, J.R. 1992. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* **68:**809–818
5. Becq, F., Jensen, T.J., Chang, X-B., Savoia, A., Rommens, J.M., Tsui, L-C., Buchwald, M., Riordan, J.R., Hanrahan, J.W. 1994. Phosphatase inhibitors activate normal and defective CFTR chloride channels. *Proc. Natl. Acad. Sci. USA* **91:**9160–9164
6. Berger, H.A., Anderson, M.P., Gregory, R.J., Thompson, S., Howard, P.W., Maurer, R. A., Mulligan, R., Smith, R.A., Welsh, M.J. 1991. Identification and regulation of the cystic fibrosis transmembrnae conductance regulator-generated chloride channel. *J. Clin. Invest.* **88:**1422–1431
7. Blatz, A.L. Magleby, K.L. 1983. Single voltage-dependent chloride-selective channels of large conductance in cultured rat muscle. *Biophys. J.* **43:**237–241
8. Brooks, S.P.J. Storey, K.B. 1992. Bound and determined: A computer programme for making buffers of defined ion concentrations. *Anal. Biochem.* **201:**119–126
9. Cantiello, H.F., Prat, A.G., Reisin, I.L., Ercole, L.B., Abraham, E.H., Amara, J.F., Gregory R.J., Ausiello, D.A. 1994. External ATP and its analogs activate the cystic fibrosis transmembrane conductance regulator by a cyclic AMP-independent mechanism. *J. Biol. Chem.* **269:**11224–11232
10. Chang, X-B., Tabcharani, J.A., Hou, Y-X., Jensen, T.J., Kartner, N., Alon, N., Hanrahan, J.W., Riordan, J.R. 1993. Protein kinase A (PKA) still activates CFTR chloride channel after mutagenesis of all ten PKA consensus phosphorylation sites. *J. Biol. Chem.* **268:**11304–11311
11. Cheng, S.H., Gregory, R.J., Marshall, J., Paul S., Souza, D.W., White, G.A., O'Riordan, C.R., Smith, A.E. 1990. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* **63:**827–834
12. Dalemans, W., Barbry P., Champigny, G., Jallat, S., Dott, K., Dreyer, D., Crystal, R.G., Pavirani, A., Lecocq, J-P., Lazdunski, M. 1991. Altered chloride ion channel kinetics associated with the deltaF508 cystic fibrosis mutation. *Nature* **354:**526–528
13. Drumm, M.L., Wilkinson, D.J., Smit, L.S., Worrell, R.T., Strong, T.V., Frizzell, R.A., Dawson, D.C., Collins, F.S. 1991. Chloride conductance expressed by ΔF508 and other mutant CFTRs in *Xenopus* oocytes. *Science* **254:**1797–1799.

14. Grygorczyk, R., Tabcharani J.A., Hanrahan, J.W. 1996. ATP conductance of the CFTR chloride channel. *Biophys. J.* **70:**A71 (*Abstr.*)
15. Hanrahan, J.W., Tabcharani, J.A., Grygorczyk, R. 1993. Patch clamp studies of apical membrane chloride channels. In: Cystic Fibrosis—Current Topics: Volume 1. J.A. Dodge, D.J.H. Brock, and J.H. Widdicombe, editors. pp. 931–137. John Wiley & Sons, Chichester
16. Kartner, N., Hanrahan, J.W., Jensen, T.J., Naismith, A.L., Sun, S., Ackerley, C.A., Reyes, E.F., Tsui, L-C., Rommens, J.M., Bear, C.E., Riordan, J.R. 1991. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. *Cell* **64:**681–691
17. Knowles, M.R., Olivier K., Noone, P., Boucher, R.C. 1995. Pharmacologic modulation of salt and water in the airway epithelium in cystic fibrosis. *Am. J. Resp. Crit. Care Med.* **151:**S65–S69
18. Linsdell, P., Rommens, J.M., Hou, Y-X., Chang, X-B., Tsui, L-C., Riordan, J.R., Hanrahan, J.W. 1996. Mutation of the narrow region of the CFTR channel pore. *Biophys. J.* **70:***A*72 (*Abstr.*)
19. Neher, E. 1992. Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol.* **207:**123–131
20. Parr, C.E., Sullivan, D.M., Paradiso, A.M., Lazarowski, E.R., Burch, L.H., Olsen, J.C., Erb, L., Weisman, G.A., Boucher, R.C., Turner, J.T. 1994. Cloning and expression of a human P_{2U} nucleotide receptor, a target for cystic fibrosis pharmacotherapy. *Proc. Natl. Acad. Sci. USA* **91:**3275–3279
21. Pasyk, E.A. Foskett, J.K. 1995. CFTR functions as an ATP channel in the endoplasmic reticulum of mammalian cells. *Ped. Pulmonol. suppl.* **12:**187 (*Abstr.*)
22. Reisin, I.L., Prat, A.G., Abraham, E.H., Amara, J.F., Gregory, R.J., Ausiello, D.A., Cantiello, H.F. 1994. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J. Biol. Chem.* **269:**20584–20591
23. Schlichter, L.C., Grygorczyk, R., Pahapill, P.A., Grygorczyk, C. 1990. A large, multiple-conductance chloride channel in normal human T lymphocytes. *Pfluegers Arch.* **416:**413–421
24. Schwiebert, E.M., Egan, M.E., Hwang, T-H., Fulmer, S.B., Allen, S.A., Cutting, G.R., Guggino, W.B. 1995. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* **81:**1063–1073
25. Sheppard, D.N., Ostedgaard, L.S., Winter, M.C., Welsh, M.J. 1995. Mechanism of dysfunction of two nucleotide binding domain mutations in cystic fibrosis transmembrane conductance regulator that are associated with pancreatic sufficiency. *EMBO J.* **14:**876–883
26. Sheppard, D.N., Rich, D.P., Ostedgaard, L.S., Gregory, R.J., Smith, A.E., Welsh, M.J. 1993. Mutations in CFTR associated with mild-disease form Cl^- channels with altered pore properties. *Nature* **362:**160–164
27. Tabcharani, J.A., Chang, X-B., Riordan, J.R., Hanrahan, J.W. 1991. Phosphorylation-regulated Cl^- channel in CHO cells stably expressing the cystic fibrosis gene. *Nature* **352:**628–631
28. Tabcharani, J.A., Low, W., Elie, D., Hanrahan, J.W. 1990. Low-conductance chloride channel activated by cAMP in the epithelial cell line T_{84}. *FEBS Lett.* **270:**157–164
29. Tabcharani, J.A., Rommens, J.M., Hou, Y-X., Chang, X-B., Tsui, L-C., Riordan, J.R., Hanrahan, J.W. 1993. Multi-ion pore behaviour in the CFTR chloride channel. *Nature* **366:**79–82
30. Takahashi, T., Matsushita, K., Welsh, M.J., Stokes, J.B. 1994. Effect of cAMP on intracellular and extracellular ATP content of Cl^--secreting epithelia and 3T3 fibroblasts. *J. Biol. Chem.* **269:**17853–17857
31. Tsui, L-C. 1992. The spectrum of cystic fibrosis mutations. *Trends Genet.* **8:**392–398