Enzymology:

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Demonstration of Phosphoryl Group Transfer Indicates That the ATP-binding Cassette (ABC) Transporter Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Exhibits Adenylate Kinase Activity*

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Background: Electrophysiological studies indicated that Cl^- channel function of cystic fibrosis transmembrane conductance regulator (CFTR) can be coupled to adenylate kinase activity (ATP+AMP ⇆ 2 ADP).
Results: CFTR catalyzes phosphoryl group transfer between a nucleotide triphosphate and a photoactivatable AMP analog.
Conclusion: CFTR exhibits adenylate kinase activity.
Significance: These data demonstrate biochemically that a membrane-bound ABC transporter can function as an adenylate kinase.

Cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane-spanning adenosine 5′-triphosphate (ATP)-binding cassette (ABC) transporter. ABC transporters and other nuclear and cytoplasmic ABC proteins have ATPase activity that is coupled to their biological function. Recent studies with CFTR and two nonmembrane-bound ABC proteins, the DNA repair enzyme Rad50 and a structural maintenance of chromosome (SMC) protein, challenge the model that the function of all ABC proteins depends solely on their associated ATPase activity. Patch clamp studies indicated that in the presence of physiologically relevant concentrations of adenosine 5′-monophosphate (AMP), CFTR Cl^- channel function is coupled to adenylate kinase activity (ATP+AMP ⇆ 2 ADP). Work with Rad50 and SMC showed that these enzymes catalyze both ATPase and adenylate kinase reactions. However, despite the supportive electrophysiological results with CFTR, there are no biochemical data demonstrating intrinsic adenylate kinase activity of a membrane-bound ABC transporter. We developed a biochemical assay for adenylate kinase activity, in which the radioactive γ-phosphate of a nucleotide triphosphate could transfer to a photoactivatable AMP analog. UV irradiation could then trap the ^{32}P on the adenylate kinase. With this assay, we discovered phosphoryl group transfer that labeled CFTR, thereby demonstrating its adenylate kinase activity. Our results also suggested that the interaction of nucleotide triphosphate with CFTR at ATP-binding site 2 is required for adenylate kinase activity. These biochemical data complement earlier biophysical studies of CFTR and indicate that the ABC transporter CFTR can function as an adenylate kinase.

Cystic fibrosis transmembrane conductance regulator (CFTR)[3] is an apical membrane anion channel that mediates chloride and bicarbonate flux across several epithelia (1). Loss of CFTR function causes the recessive genetic disease cystic fibrosis (2). CFTR is a member of the adenosine 5′-triphosphate (ATP)-binding cassette (ABC) family of proteins (3). ABC proteins are defined by two highly conserved ABC-type nucleotide-binding domains (NBDs) (4) that dimerize and form two ATP-binding sites (site 1 and site 2) (5–7). This family includes membrane-spanning ABC transporters, such as CFTR, that translocate an array of substrates across cell membranes and a variety of nuclear and cytoplasmic proteins involved in many essential biological functions, such as DNA repair and mRNA translation (3). It is well established that ABC proteins are ATPases (8, 9), *i.e.* they can hydrolyze ATP to adenosine 5′-diphosphate (ADP) and inorganic phosphate (P_i) ($ATP+H_2O \rightarrow ADP+P_i$). The conformational changes associated with ATP binding and hydrolysis are coupled to the biological function of the ABC protein (10–13); in CFTR, this is opening and closing of the channel (14–17).

Recent studies with CFTR (18, 19) and two other ABC proteins, the DNA repair enzyme Rad50 (20) and a structural maintenance of chromosome (SMC) protein (21), challenge the model that the function of all ABC proteins depends solely on

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[3] The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; ABC, ATP-binding cassette; 2-N_3-AMP, 2-azidoadenosine 5′monophosphate; 8-N_3-AMP, 8-azidoadenosine 5′ monophosphate; AMP-NH_2, adenosine 5′-monophosphoramidate; AMPPNP, adenosine 5′-(β,γ-imido)triphosphate; Ap_5A, P^1,P^5-di(adenosine-5′) pentaphosphate; Gp_5A, P^1-(5′-guanosyl) P^5-(5′-adenosyl) pentaphosphate; Gp_5G, P^1,P^5-di(guanosine-5′) pentaphosphate; NBD, nucleotide-binding domain; SMC, structural maintenance of chromosome; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; E-64, *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane.

their associated ATPase activity. CFTR opening and closing depends on ATPase activity if ATP is the only nucleotide present (14–17). However, patch clamp studies using excised membrane patches containing CFTR indicated that in the presence of physiologically relevant concentrations of adenosine 5′-monophosphate (AMP), adenylate kinase activity is coupled to channel function (18). Adenylate kinases are enzymes that bind ATP and AMP at separate sites and catalyze the transfer of the γ-phosphoryl group of ATP onto the α-phosphate of AMP (ATP+AMP ⇆ 2 ADP) (22). The ABC proteins Rad50 (20) and SMC (21), which are not transporters, but nuclear proteins involved in DNA repair and chromosome maintenance, have been shown to catalyze both ATPase and adenylate kinase reactions. Furthermore, Bhaskara *et al.* (20) showed that a yeast strain with a Rad50 mutation that reduced its adenylate kinase but not its ATPase activity resembled a Rad50 null strain with regard to meiosis and telomere maintenance. This result suggests an important physiologic role for Rad50 adenylate kinase activity.

Lammens and Hopfner (21) solved the crystal structure of the ABC-NBD of the *Pyrococcus furiosus* SMC protein in complex with the adenylate kinase inhibitor Ap_5A, providing the first structural view of the active center of an ABC adenylate kinase. Ap_5A contains two adenosine groups connected by five phosphate groups allowing it to bind simultaneously to an ATP- and an AMP-binding site (23). The structure showed the two adenosine moieties of Ap_5A attached to two binding sites separated by ~15 Å. A Mg^{2+} ion, one adenosine, plus α-, β-, and γ-phosphates of Ap_5A bound the canonical Mg^{2+}-ATP-binding site on lobe I of the SMC NBD. The other adenosine, the "AMP" adenosine group, stacked onto the side chain of a conserved glutamine of the Q-loop at the interface of lobe I and lobe II.

A recent study measured ATPase and adenylate kinase activity of recombinant CFTR after solubilizing it from membranes using 8% (v/v) pentadecafluorooctanoic acid (24). The study failed to detect adenylate kinase activity, and the authors concluded that CFTR is an ATPase, but not an adenylate kinase. That study raised questions of whether or not a membrane-bound ABC transporter could function as an adenylate kinase. In addition, the discrepancy between the electrophysiological studies with membrane-embedded CFTR and the biochemical studies with membrane-solubilized CFTR suggested the importance of testing for adenylate kinase activity in the absence of solubilizing agents, while CFTR is present in membranes. In this study, we developed a biochemical assay that allowed us to test the hypothesis that full-length CFTR displays adenylate kinase activity while it is embedded in the membrane.

EXPERIMENTAL PROCEDURES

Materials— 8-N_3-AMP and 2-N_3-AMP, dissolved as triethylammonium salt in absolute methanol, were from Affinity Photoprobes, LLC, Lexington, KY. Immediately before use, the methanol was evaporated under a stream of argon, and the AMP analog was dissolved in a buffer of 20 mM Hepes (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$. [γ-^{32}P]GTP, dissolved in 10 mM Tricine, pH 7.6, was from PerkinElmer Life Sciences. Nonradioactive ATP, AMP, and Ap_5A were from Sigma-Aldrich. ATP was used as magnesium salt. AMP and Ap_5A were used as sodium salts. The protease inhibitors used in this study were purchased from Sigma-Aldrich.

Expression of CFTR in HeLa Cells and Preparation of Membranes—Wild-type and mutant CFTR were transiently expressed in HeLa cells using a vaccinia virus/T7 hybrid expression system (25). Cell membranes were prepared following methods described by Travis *et al.* (26) in the presence of a proteinase inhibitor mixture of 125 μg/ml benzamidine, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 100 μg/ml Pefabloc, and 7 μg/ml *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane (E-64). A high-speed membrane pellet (70,000 × *g*, 40 min, 4 °C) was resuspended in 20 mM Hepes (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$, 2 μg/ml leupeptin, 100 μg/ml Pefabloc, and 7 μg/ml E-64.

CFTR Adenylate Kinase Assay—Membranes containing either 30 μg of protein (from cells expressing wild-type CFTR) or 90 μg of protein (from cells expressing S1248F CFTR) were incubated gently shaking with nonradioactive 8- or 2-N_3-AMP (at concentrations given in the figure legends), radioactive [γ-^{32}P]GTP (30 μCi, 6000 Ci/mmol), 20 mM Hepes (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$, and 1 mM Tricine (pH 7.6) for 5 min at 37 °C in a total volume of 30 μl followed by UV irradiation for 30 s (302 nm, 8-watt lamp) at a distance of 5 cm. Immediately after exposure to UV light, first 20 μl of Stop buffer (25 mM dithiothreitol, 4% SDS, 20 mM Hepes (pH 7.5), 50 mM NaCl, 125 μg/ml benzamidine, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 100 μg/ml Pefabloc, 7 μg/ml E-64) and then 875 μl of 1% Triton X-100 in 20 mM Hepes (pH 7.5), 50 mM NaCl, 125 μg/ml benzamidine, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 100 μg/ml Pefabloc, and 7 μg/ml E-64 were added. Samples were stored at −80 °C overnight and thawed on ice before adding CFTR antibodies for immunoprecipitation. CFTR was immunoprecipitated by adding monoclonal CFTR antibodies to its regulatory (R) domain (13-1, 0.2 μg/sample) (R&D Systems, Inc., Minneapolis, MN) (27) and NBD2 (M3A7, 1 μg/sample) (EMD Millipore, Billerica, MA) (28). Immunocomplexes were fractionated on 6% SDS-polyacrylamide gels. After electrophoresis, either the gels were dried or the fractionated proteins were transferred onto a PVDF membrane (Immobilon®-FL transfer membrane, EMD Millipore) for Western blotting. The dried gels or the PVDF membranes were then subjected to digital autoradiography using a FLA-7000 imaging system (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Western Blotting—PVDF membranes blocked in 0.01% casein were incubated for 2 h with the monoclonal anti-human CFTR antibody indicated for each experiment in the figures, diluted 1:1,000 in TTBS buffer (137 mM NaCl, 2.7 mM KCl, 25 mM Tris-Cl (pH 8.0), 0.05% Tween 20). Membranes were washed twice in TTBS buffer and then incubated for 1 h with donkey anti-mouse IRDye (0.1 μg/ml, in TTBS plus 0.01% casein, 0.01% SDS) (LI-COR Biosciences, Lincoln, NE) as secondary antibody. Immunoreactive proteins were visualized with the Odyssey Infrared Imaging System (LI-COR Biosciences).

Data Presentation and Statistics—Data are presented as means ± S.E. p values <0.05 were considered statistically sig-

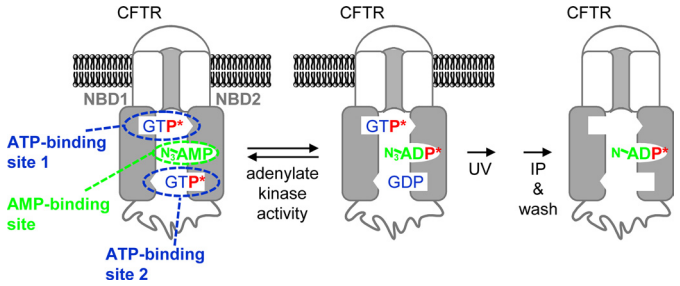

FIGURE 1. **Model of CFTR labeling through phosphoryl group transfer between [γ-^{32}P]GTP and N_3-AMP followed by UV-mediated cross-linking of the resulting N_3-[β-^{32}P]ADP and solubilization and immunoprecipitation (*IP*) of CFTR.** P^* indicates a radioactive phosphoryl group containing ^{32}P. In each NBD, the *open rectangle* represents the Walker A motif, and the *open triangle* represents the signature motif. The binding site for AMP is not known.

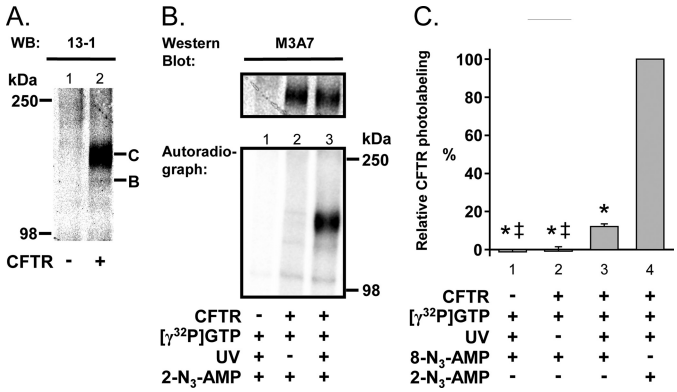

FIGURE 2. **Membrane-inserted CFTR catalyzes phosphotransfer from [γ-^{32}P]GTP to N_3-AMP.** *A*, Western blot (*WB*) probed with antibody 13-1. *Letters* label highly (*C*) and core glycosylated (*B*) CFTR. Each lane represents 30 μg of membrane protein. *B*, autoradiograph and Western blot (probed with antibody M3A7) of the same gel. Experiments were performed as illustrated in Fig. 1. Experimental conditions are indicated below the lanes. N_3-AMP concentration was 65 μM. Comparing the autoradiograph and Western blot corroborated that the labeled band was CFTR. *C*, CFTR photolabeling with 8-N_3-AMP and 2-N_3-AMP. N_3-AMP concentration was 65 μM. To compare the results from different autoradiographs, data were normalized to CFTR radioactivity under conditions indicated below bar 4. *Asterisks* indicate $p \leq 0.001$ when compared with bar 4, and *double daggers* indicate $p \leq 0.001$ when compared with bar 3 (one-way analysis of variance followed by the Holm-Sidak method for multiple comparisons, $n = 3$).

nificant. SigmaStat software (SPSS Inc., Chicago, IL) was used for statistical analysis.

RESULTS

To test the hypothesis that full-length CFTR displays adenylate kinase activity, we developed the strategy illustrated in Fig. 1. CFTR has two ATP-binding sites formed at the NBD head-to-tail dimer interface. As in other ABC proteins, the three ATP phosphates are bound to the phosphate-binding loop or Walker A motif (29) of one NBD and the ABC signature motif of the other NBD. In ATP-binding site 1, this is the Walker A motif of NBD1 and the signature motif of NBD2. In ATP-binding site 2, it is the Walker A motif of NBD2 and the signature motif of NBD1. Previous patch clamp studies indicated the existence of a separate binding site for AMP (18). These studies together with structural studies on other ABC protein NBDs provided three lines of evidence indicating that the AMP-binding site is distinct from the two ATP-binding sites. 1) AMP induced positive cooperativity for ATP in the relationship between ATP concentration and CFTR current (18). This finding indicates that in the presence of AMP, two ATP molecules interact simultaneously with CFTR. Therefore, AMP must interact with a different site. 2) ATP- and AMP-binding sites showed different nucleotide base specificities; the ATP-binding sites accept both ATP and GTP (guanosine 5′-triphosphate) (18, 26, 30, 31). In contrast, the AMP-binding site showed high specificity for the adenine base; *i.e.* GMP (guanosine 5′-monophosphate) did not mimic the effects of AMP on current. This discrimination was also revealed by the effect of agents that interact with both an ATP-binding site and an AMP-binding site to inhibit adenylate kinase activity; Ap_5A and Gp_5A inhibited CFTR Cl^- current, whereas Gp_5G did not (18). 3) In Ap_5A bound to an adenylate kinase, the ribose oxygens are ~16 Å apart (23). Crystal structures of other ABC protein NBDs in the dimeric state with bound ATP showed the ribose oxygens of the two ATPs 34–37 Å apart (5, 6). Therefore, a similar dimeric structure in CFTR would not permit Ap_5A binding simultaneously to the two ATP-binding sites. The recently solved crystal structure of the ABC-NBD of an SMC protein in complex with Ap_5A confirmed a binding site for one Ap_5A adenosine that is distinct from the two ATP-binding sites (21).

We predicted that when membranes containing CFTR are incubated with radioactive [γ-^{32}P]GTP and nonradioactive, photoactivatable azido (N_3)-AMP, CFTR adenylate kinase activity would catalyze transfer of the radioactive γ-phosphate of [γ-^{32}P]GTP onto N_3-AMP, forming radioactive N_3-[β-^{32}P]ADP. Subsequent exposure to UV light would mediate cross-linking of N_3-[β-^{32}P]ADP to the CFTR protein. The N_3-group absorbs UV light, which results in photolysis and formation of a reactive intermediate that reacts with nearby amino acid residues to become covalently attached (32, 33). Thus, CFTR would become radioactively labeled. We chose to use [γ-^{32}P]GTP rather than [γ-^{32}P]ATP because in preliminary experiments, we found that incubating native membranes containing CFTR with [γ-^{32}P]ATP at 37 °C resulted in some radioactive labeling of CFTR even in the absence of UV light. This result suggested that the radioactive phosphate group was incorporated into CFTR in a different way than via a cross-linked N_3-[β-^{32}P]ADP, *e.g.* perhaps by direct phosphorylation. In contrast to ATP, GTP is not a substrate of the major protein kinases known to phosphorylate CFTR in the cell membrane (34–37).

We expressed CFTR in HeLa cells using a double vaccinia virus/T7 RNA polymerase system (25) and collected cell membranes. Western blotting confirmed the presence of CFTR (Fig. 2*A*). The majority of CFTR migrated as the highly glycosylated band C (27, 38). No CFTR could be detected in membranes from HeLa cells not infected with the recombinant vaccinia virus encoding CFTR.

To test for adenylate kinase activity, we incubated membranes containing CFTR with [γ-^{32}P]GTP and nonradioactive N_3-AMP followed by UV irradiation (Fig. 1). This procedure radioactively labeled CFTR (Fig. 2*B*, *lane 3*), indicating phosphoryl group transfer activity between [γ-^{32}P]GTP and N_3-AMP, *i.e.* adenylate kinase activity.

Labeling was greater when we used azido-AMP with the N_3-group substituted at the C-2 position of the adenine ring

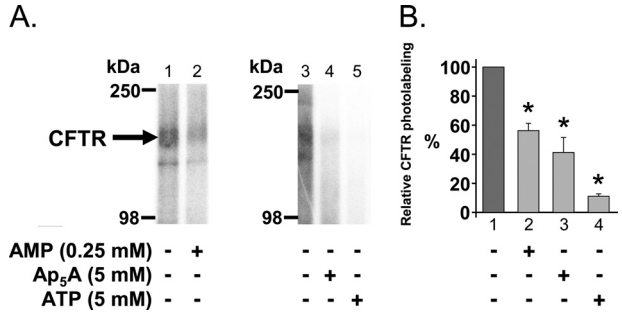

FIGURE 3. **Excess nonradioactive AMP, ATP, and Ap_5A reduce photolabeling.** *A*, autoradiographs from two different experiments. Experiments were performed as in Fig. 1. Concentration of 2-N_3-AMP was 50 μM. *B*, summary data. Amount of radioactivity incorporated into CFTR was normalized to CFTR radioactivity under conditions indicated below bar 1. *Asterisks* indicate $p \leq 0.001$ when compared with bar 1 (one-way repeated measures analysis of variance followed by the Holm-Sidak method for multiple comparisons, $n = 4–6$).

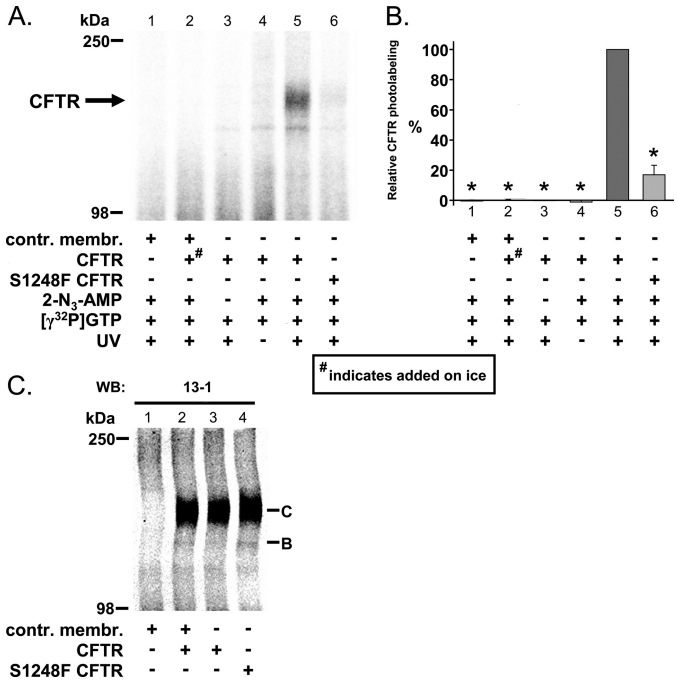

FIGURE 4. **CFTR has intrinsic adenylate kinase activity.** *A*, autoradiograph of immunoprecipitated CFTR fractionated on a 6% SDS-polyacrylamide gel. Experiments were performed as illustrated in Fig. 1. Membranes containing 30 μg of protein from CFTR-expressing HeLa cells (*lanes 3–5*) or control membranes (*contr. membr.*) containing 30 μg of protein from HeLa cells not expressing recombinant CFTR (*lane 1*) were used. In *lane 6*, membranes containing 90 μg of protein from S1248F CFTR-expressing HeLa cells were employed. Membranes were incubated together with 50 μM 2-N_3-AMP and 30 μCi of [γ-^{32}P]GTP (6000 Ci/mmol) for 5 min at 37 °C followed by UV irradiation for 30 s (302 nm, 8-watt lamp) at a distance of 5 cm as described under "Experimental Procedures." The sample of *lane 4* was not UV-irradiated. In *lane 2*, 30 μg of membranes from HeLa cells not expressing recombinant CFTR (control membranes) were incubated with 50 μM 2-N_3-AMP and 30 μCi of [γ-^{32}P]GTP (6000 Ci/mmol) for 5 min at 37 °C. Then 30 μg of membranes containing CFTR were added on ice before UV irradiation (30 s, 302 nm, 8-watt lamp). In all cases, CFTR was then solubilized and immunoprecipitated as described under "Experimental Procedures." *B*, summary data. Radioactivity incorporated into CFTR was normalized to radioactivity for conditions indicated below bar 5. *Asterisks* indicate $p = 0.029$ when compared with bar 5 (Mann-Whitney rank sum test, $n = 4$). No significant differences were detected between bars 1–4 and 6 (Kruskal-Wallis one-way analysis of variance on ranks, $n = 4$). *C*, Western blot probed with CFTR antibody 13-1. 30 μg (control membranes and membranes with wild-type CFTR, *lanes 1–3*) and 90 μg (membranes with S1248F CFTR, *lane 4*) of protein were used.

(2-N_3-AMP) *versus* at the C-8 position (8-N_3-AMP) (Fig. 2*C*). There are at least two explanations for this difference. The photoactivated reaction of the N_3-group with nearby amino acid residues (32, 33) might be sterically favored if the N_3-group is substituted at the C-2 position *versus* the C-8 position of the adenine ring. Alternatively, the affinity of 8-N_3-AMP for the AMP-binding site might be less than that of 2-N_3-AMP due to conformational differences between these two analogs. Studies in other adenylate kinases support this possibility. The torsional angle between the base and the ribose of AMP can be *syn* or *anti* (39). At physiologic pH, AMP preferentially adopts the *anti* conformation (40). An N_3-group at the adenine C-8 position likely shifts the conformation of the nucleotide toward a *syn* angle, whereas substitution at the C-2 position allows for an *anti* conformation (41). NMR studies with rabbit muscle adenylate kinase (42) and the crystal structure of *Escherichia coli* adenylate kinase in complex with AMPPNP and AMP show AMP bound in an *anti* conformation (43). Moreover, in chicken muscle adenylate kinase, when compared with 8-N_3-AMP, 2-N_3-AMP supports greater phosphoryl group transfer activity, and the AMP-binding site is preferentially photolabeled with 2-N_3- *versus* 8-N_3-nucleotide analogs (44). Our findings suggest a similar requirement for AMP in CFTR. Consequently, 2-N_3-AMP was employed in all subsequent experiments.

We did several studies to test whether radioactive labeling was due to CFTR adenylate kinase activity. 1) We predicted that if labeling required phosphoryl group transfer activity between [γ-^{32}P]GTP and N_3-AMP (adenylate kinase activity) as illustrated in Fig. 1, it should decrease in the presence of the adenylate kinase inhibitor Ap_5A. Labeling should also decrease if nonradioactive ATP was added to compete with [γ-^{32}P]GTP or if AMP was added to compete with N_3-AMP. Experimental testing showed that excess ATP, AMP, and Ap_5A indeed reduced labeling (Fig. 3). 2) If radioactive labeling depended on UV light-induced cross-linking of N_3-[β-^{32}P]ADP to CFTR after phosphoryl group transfer, labeling should not occur in the absence of either N_3-AMP or UV irradiation. Experimental testing confirmed that both were true (Figs. 2*B* and 4*A*). Thus, ^{32}P was not incorporated into CFTR by means other than the cross-linked N_3-nucleotide, *e.g.* not by a protein kinase present in the membrane preparation. 3) We considered the possibility that an adenylate kinase other than CFTR might form N_3-[β-^{32}P]ADP, which could then bind and label CFTR during UV irradiation. To test this possibility, we incubated nontransfected HeLa cell membranes with [γ-^{32}P]GTP and N_3-AMP at 37 °C. We then added membranes containing CFTR on ice and irradiated with UV light. Lack of radioactive CFTR (Fig. 4*A*, *lane 2*) suggests that it was adenylate kinase activity intrinsic to CFTR rather than another adenylate kinase that generated the N_3-[β-^{32}P]ADP that labeled CFTR.

To further test that the observed adenylate kinase activity was intrinsic to CFTR, we asked whether it could be eliminated by a CFTR mutation. We chose a phenylalanine substitution for serine at position 1248 (S1248F) in the phosphate-binding loop of ATP-binding site 2. A previous study showed that this mutation abolished nucleotide interaction with ATP-binding site 2 (45). However, this mutation did not interfere with intracellular processing of CFTR to the highly glycosylated form migrating

as band C (Fig. 4*C*). When we incubated membranes containing S1248F CFTR with [γ-^{32}P]GTP and nonradioactive N_3-AMP, followed by UV irradiation, we found very little labeling (Fig. 4*A*, *lane 6*). Western blotting confirmed that the mutant CFTR was present in an amount similar to that of wild-type CFTR (Fig. 4*C*). We could not assess the effect of the homologous mutation in ATP-binding site 1 (A462F mutation) on adenylate kinase activity because that mutation affected intracellular CFTR processing to an extent that we were unable to detect the mutant CFTR protein in our membrane preparations by Western blot.

DISCUSSION

In this study, we developed a biochemical assay that radioactively labeled CFTR as a consequence of phosphoryl group transfer activity between GTP and a photoactivatable AMP analog (adenylate kinase activity). Our results show that when N_3-AMP was added, membrane-bound CFTR displayed adenylate kinase activity. The findings further suggest that the interaction of nucleotide triphosphate with CFTR at ATP-binding site 2 is required for CFTR adenylate kinase activity.

Our data complement previous electrophysiological data indicating that CFTR channel activity is coupled to adenylate kinase activity. Patch clamp studies with excised inside-out membrane patches containing CFTR showed that the adenylate kinase inhibitor Ap_5A inhibited CFTR current. Inhibition was attenuated by increasing the ATP concentration or by adding AMP, suggesting a similar mechanism of inhibition as seen in other adenylate kinases, *i.e.* binding to an ATP site and an AMP site. Furthermore, AMP noncompetitively altered the response of current to different ATP concentrations. However, an AMP analog that cannot act as a phosphoryl group acceptor, AMP-NH_2, did not mimic the effects of AMP. Instead, AMP-NH_2 inhibited current partially and noncompetitively with ATP by reducing the channel opening rate. The addition of AMP reversed AMP-NH_2 inhibition (18). Patch clamp studies also suggested that physiological intracellular AMP concentrations could support the adenylate kinase activity (18). Our biochemical results are consistent with that interpretation.

A previous study failed to detect adenylate kinase activity after solubilization of recombinant CFTR from membranes (24). Because we used membrane-bound CFTR that had not been detergent-solubilized, the difference in results emphasizes the importance of the native, membrane-embedded conformation for CFTR adenylate kinase activity.

Substituting a phenylalanine into the phosphate-binding loop of NBD2 (the S1248F mutation) interfered with labeling. Possible explanations include disruption of adenylate kinase activity by the mutation and interference with UV light-dependent photolabeling. Previous observations support the interpretation that the S1248F mutation disrupted adenylate kinase activity. 1) A study characterizing the gating characteristics and the interaction of ATP with S1248F CFTR found that this mutation interfered with the interaction of nucleotides at ATP-binding site 2. It did not abolish photolabeling of ATP-binding site 1 (45). 2) Patch clamp studies showed that mutations K1250A and D1370N, located within conserved motifs of ATP-binding site 2, abolished the effects of Ap_5A and AMP on CFTR current. The homologous mutations in ATP-binding site 1, however, did not (18). Our findings plus those previous observations suggest that the interaction of nucleotide triphosphate with CFTR at ATP-binding site 2 is required for adenylate kinase activity. It may be that the phosphoryl group donor nucleotide triphosphate interacts with ATP-binding site 2, the same site at which ATP is hydrolyzed in the absence of AMP (45, 46). Alternatively, the mutation may also interfere with the interaction of AMP with CFTR. Of note, mutations within the ATP-binding site of other adenylate kinases also affect the interaction with AMP (23).

These biochemical data complement earlier biophysical studies of CFTR and further establish that CFTR can function as an adenylate kinase when embedded in the membrane. The approach employed in this study may also be useful to test whether cystic fibrosis-associated mutations interfere with adenylate kinase activity and whether the effects of such mutations can be counteracted by small molecules. The methods used here may also allow investigations into whether other ABC transporters have intrinsic adenylate kinase activity.

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