The criteria were that there be a good number of cells, good behavior on the apparatus, and at least 15 min of good sleep both before and after the behavioral session. These were informal criteria, but the data sets were chosen before any analyses were performed on them. The data were taken from animals involved in a variety of experiments, including, for example, a study of the effects of aging on rat hippocampal activity. Every data set that was analyzed is presented in this report.

12. The value of the "cross-correlation" function $\chi_{ij}(t)$, as used here, was equal to the number of pairs of spikes, one from cell $i$ and the other from cell $j$, that were separated by an interspike interval in the range $(t, t + \Delta t)$, where $\Delta t$ is the bin size. The measure of temporal asymmetry used here is, however, independent of the bin size, so long as it is small. The measure of temporal asymmetry was

$$B_{ij} = \int_{0}^{200} \chi_{ij}(t)dt - \int_{-200}^{0} \chi_{ij}(t)dt \qquad (1)$$

$B_{ij}$ measures the difference between the number of events in which a spike from cell $i$ was followed within 200 ms by a spike from cell $j$ and the number of events in which a spike from cell $j$ was followed within 200 ms by a spike from cell $i$, possibly with other spikes of either cell in between. Note that $B_{ij} = -B_{ji}$. This reversal of sign as a consequence of exchanging the cells makes the temporal bias measure quite different from a simple correlation measure of the type used by Wilson and McNaughton (*8*), which keeps the same value if the two cells are exchanged. To minimize the possibility of artifacts caused by shrinkage of spike amplitude when a cell is highly active, we used only pairs of cells recorded from different tetrodes in the analyses.

13. Our main concern in this study was to establish the reality of the phenomenon in as straightforward a way as possible. Therefore, we thought it preferable to avoid any manipulations of the data that were not absolutely necessary, such as rescaling or thresholding. It is likely that the relations reported here would be stronger if, for example, only pairs of cells with overlapping place fields were included.

14. As mentioned previously, we calculated the temporal bias in these analyses using a time window of ±200 ms. We also experimented with time windows of 50, 100, 500, and 1000 ms. Significant effects could be seen for some of the recording sessions with time windows of 100 and 500 ms, but they appeared to be less consistent. We also experimented with different time windows for the sleep and track-running sessions, but again, time windows of 200 ms for both yielded the most consistent evidence for reproduction of temporal bias. During track running, a time window of 200 ms captures the relation of most pairs of cells with overlapping place fields but yields zero for most pairs whose fields are more widely separated; during sleep, a time window of 200 ms captures relations that occur within individual sharp waves but rarely encompasses two consecutive sharp waves. Buzsáki (*5*) suggested that behavioral sequences may be compressed into the time window of individual sharp waves, and Skaggs *et al.* (*17*) have shown evidence that sequences are compressed within individual theta cycles.

15. Long-term potentiation in the rat hippocampus is thought to have a duration of days to weeks [(*2*); C. A. Barnes, *J. Comp. Physiol. Psychol.* **931**, 74 (1979)]. Why, then, would temporal bias be reproduced preferentially during sleep after behavior in a familiar environment? One possibility is that the effects are the result of the early, decremental component of LTP, known as short-term potentiation, which has a time course of 5 to 40 min [R. Malenka, *Neuron* **6**, 53 (1991); A. Colino, Y.-Y. Huang, R. C. Malenka, *J. Neurosci.* **12**, 180 (1992)]. Another theoretical possibility, which remains to be explored, is that the observed pattern of temporal bias results from a combination of long-lasting temporally asymmetric LTP with a short-lasting postbehavioral enhancement of the activity level of individual units, as observed by Pavlides and Winson (*7*). However, no such enhancement in activity was observable in our data; this discrepancy may be because Pavlides and Winson obtained their results by confining each rat inside the place field of each cell for an extended period of time.

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6 November 1995; accepted 7 February 1996

# An RNA Polymerase II Elongation Factor Encoded by the Human *ELL* Gene

Ali Shilatifard, William S. Lane, Kenneth W. Jackson, Ronald C. Conaway, Joan W. Conaway*

The human *ELL* gene on chromosome 19 undergoes frequent translocations with the *trithorax*-like *MLL* gene on chromosome 11 in acute myeloid leukemias. Here, *ELL* was shown to encode a previously uncharacterized elongation factor that can increase the catalytic rate of RNA polymerase II transcription by suppressing transient pausing by polymerase at multiple sites along the DNA. Functionally, ELL resembles Elongin (SIII), a transcription elongation factor regulated by the product of the von Hippel–Lindau (*VHL*) tumor suppressor gene. The discovery of a second elongation factor implicated in oncogenesis provides further support for a close connection between the regulation of transcription elongation and cell growth.

The identification of genes at breakpoints of frequently occurring chromosomal translocations has led to the discovery of cellular proteins that are involved in oncogenesis. The fact that many of these proteins are transcription factors illustrates the critical role of transcriptional deregulation in human cancer (*1*, *2*). One form of acute myeloid leukemia results from a t(11;19)(q23;p13.1) translocation between the *ELL* gene (also called *MEN*) on chromosome 19 and the *MLL* gene (also called *Htrx*, *ALL-1*, and *HRX*) on chromosome 11 (*3*). The predicted open reading frame (ORF) of the human *ELL* gene provides few clues to its role in either normal cell growth or leukemogenesis; the *ELL* gene encodes a basic, 621–amino acid protein that is ubiquitously expressed and highly conserved throughout evolution but exhibits no obvious homology to known proteins (*3*). The *MLL* gene encodes a 3968–amino acid protein; the $NH_2$-terminal regions of MLL are similar to A-T hook DNA-binding and methyltransferase-like domains, and the COOH-terminal region of MLL is similar to that encoded by the *Drosophila* gene *trithorax* with a transcriptional activation domain downstream of several contiguous zinc fingers (*4*, *5*). The putative oncogene generated by the t(11;19) translocation encodes an $NH_2$-MLL-ELL-COOH fusion protein that contains nearly the entire *ELL* ORF and the $NH_2$-terminal 1300 amino acids of MLL, including its A-T hook and methyltransferase-like domains but lacking its COOH-terminal transcriptional activation domain and zinc fingers (*3*).

Recently, we purified an RNA polymerase II transcription factor from rat liver nuclear extracts by means of the procedure outlined in Fig. 1A (*6*). During purification, this factor was assayed by its ability to stimulate the rate of accumulation of 135-nucleotide (nt) transcripts synthesized by RNA polymerase II on the T-less cassette of the oligo(dC)-tailed template pCpGR220 S/P/X (*7*, *8*). Analysis by SDS–polyacrylamide gel electrophoresis (PAGE) of fractions from the final PLRP-S reversed-phase high-performance liquid chromatography (rpHPLC) column revealed that transcriptional activity copurified with a single ~80-kD polypeptide (p80) (Fig. 1B), which stimulates transcription by RNA polymerase II in a dose-dependent manner (Fig. 1C). Results of pulse-chase experiments indicated that the factor is capable of stimulating the rate of elongation of promoter-specific transcripts synthesized by RNA polymerase II. Transcription was initiated at the adenovirus 2 major late (AdML) promoter by addition of adenosine triphosphate (ATP), guanosine triphosphate

A. Shilatifard, R. C. Conaway, J. W. Conaway, Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA.
W. S. Lane, Harvard Microchemistry Facility, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA.
K. W. Jackson, William K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190, USA.

*To whom correspondence should be addressed.

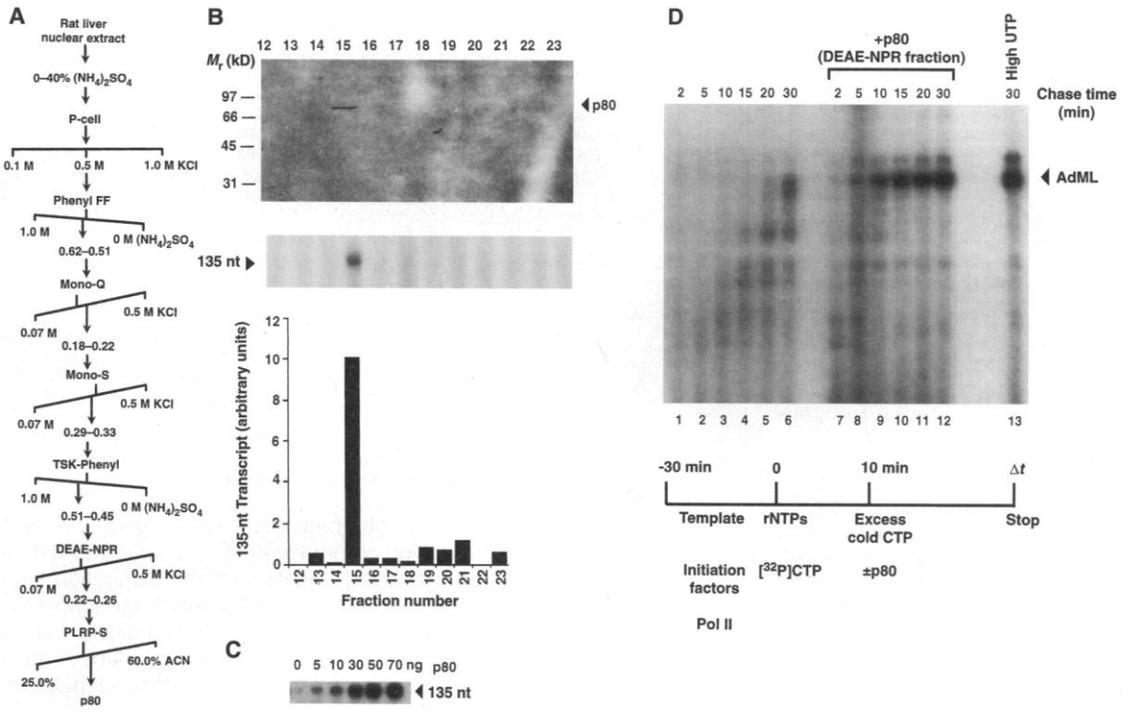


**Fig. 1.** Purification and transcription activity of rat p80. (**A**) Purification of rat p80. P-cell, phosphocellulose P-11; Phenyl FF, Phenyl Sepharose-6 Fast Flow; TSK-Phenyl, Bio-Gel TSK phenyl-5-PW. (**B**) Cochromatography of rat p80 and transcription activity. Upper panel: Aliquots of PLRP-S fractions were subjected to 10% SDS-PAGE, and proteins were visualized by silver staining. Middle panel: Aliquots of PLRP-S fractions were renatured (*21*) and assayed for their ability to stimulate synthesis of the 135-nt transcript from the T-less cassette of the oligo(dC)-tailed template pCpGR220 S/P/X (*8*). Lower panel: Synthesis of the 135-nt transcript was quantitated with a Molecular Dynamics PhosphorImager. (**C**) Renatured PLRP-S–purified rat p80 stimulates synthesis of the135-nt transcript in a dose-dependent fashion. (**D**) Effect of the p80 DEAE-NPR fraction on the kinetics of promoter-dependent transcription by RNA polymerase II. Preinitiation complexes were assembled at the AdML promoter as described (*22*) with recombinant TBP, TFIIB, TFIIE, TFIIF, TFIIH, and purified rat RNA polymerase II. Transcription was initiated by addition of 50 μM ATP, 50 μM GTP, 2 μM UTP, 10 μCi of [α-$^{32}$P]CTP (400 Ci/mmol, Amersham) (rNTPs), and 7 mM $MgCl_2$. After 10 min at 28°C, 100 μM nonradioactive CTP was added to reaction mixtures. Where indicated, 1 μl of the p80 DEAE-NPR fraction (*6*) was added to reaction mixtures, which were then incubated further for the times indicated. Transcripts were analyzed by electrophoresis through a 6% polyacrylamide, 7.0 M urea gel.

(GTP), uridine triphosphate (UTP), and [α-$^{32}$P]cytidine triphosphate (CTP) to reaction mixtures that contained RNA polymerase II, the general initiation factors TBP, TFIIB, TFIIE, TFIIF, and TFIIH, and a DNA fragment containing the AdML promoter. The short, accurately initiated transcripts synthesized during a 10-min incubation were then chased by addition of a large excess of nonradioactive CTP in the absence or presence of p80 purified through the DEAE-NPR step (6). Comparison of lanes 1 to 6 and 7 to 12 of Fig. 1D reveals that the p80 fraction stimulated the rate of appearance of full-length runoff transcripts. After p80 was digested with trypsin, the $NH_2$-terminal sequences of several tryptic peptides were determined by sequential Edman degradation (9). A database search revealed that the sequences of four p80 tryptic peptides exactly matched sequences in the human *ELL* gene (*3*) and that the sequences of three additional peptides were 71, 62, and 50% identical to human *ELL* sequences (Fig. 2).

To investigate the possibility that the human *ELL* gene encodes an RNA polymerase II transcription factor similar to rat p80, we subcloned the *ELL* complementary DNA (cDNA) into a bacteriophage M13 expression vector under the control of the T7 RNA polymerase promoter and expressed it in *Escherichia coli* with an $NH_2$-terminal histidine (His) tag (*10*). The recombinant ELL protein was purified to homogeneity from guanidine-solubilized inclusion bodies (*10*) and was assayed for transcriptional activity (*8*). The *ELL* cDNA encoded an ~80-kD polypeptide with an electrophoretic mobility that was indistinguishable from that of purified rat p80 (Fig. 3A). Like rat p80, the recombinant ELL protein could stimulate the rate of accumulation of 135-nt transcripts synthesized by RNA polymerase II on the T-less cassette of the oligo(dC)-tailed template pCpGR220 S/P/X (Fig. 3B). In contrast, an identically prepared protein fraction from bacterial cells expressing an irrelevant protein had no detectable effect on transcription (Fig. 3B). In control experiments, ELL-stimulated transcription was sensitive to α-amanitin (Fig. 3B, lane 10) and was dependent on added RNA polymerase II (*11*).

The results of pulse-chase experiments indicate that the ELL protein is an RNA polymerase II elongation factor that can function either during promoter-independent transcription on an oligo(dC)-tailed template or during promoter-specific transcription in the presence of the general initiation factors. In one experiment (Fig. 3C), transcription was initiated by addition of RNA polymerase II to reaction mixtures that contained oligo(dC)-tailed template pCpGR220 S/P/X, ATP, GTP, and [α-$^{32}$P]CTP but did not contain UTP. The resulting 135-nt transcripts were then chased, in the absence or presence of ELL protein, by addition of UTP and a large excess of nonradioactive CTP. At the conclusion of the reaction, transcripts synthesized in the presence of ELL protein were substantially

```
Rat p80      1 DWPGYSEGDQQLLK 14
                ||||||||||||||
Human ELL  272 DWPGYSEGDQQLLK 285

Rat p80      1 RLIAEYDQRQLQAWP 15
                |||||||||||||||
Human ELL  607 RLIAEYDQRQLQAWP 621

Rat p80      1 DKER 4
                ||||
Human ELL  457 DKER 460

Rat p80      1 aeLIIrLQk 9
                |||||||||
Human ELL  223 AELLLRLQK 231

Rat p80      1 DTLDSLLQQVASVNPK 16
                |.||:|||||.:.:|
Human ELL  240 DALDGLLQQVANMSAK 255

Rat p80      1 RSQQTDFIDPLASK 14
                |  |..||||||||.|
Human ELL  324 RLQPPDFIDPLANK 337

Rat p80      1 LPLLTDFPQAEQPAsd 16
                ||||||  :|:..|  :.
Human ELL  427 LPLLTDCAQPSRPHGS 442
```

**Fig. 2.** Sequence similarity of tryptic fragments of rat p80 and predicted amino acid sequence of human ELL protein. Capital letters indicate the highest probability sequence. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

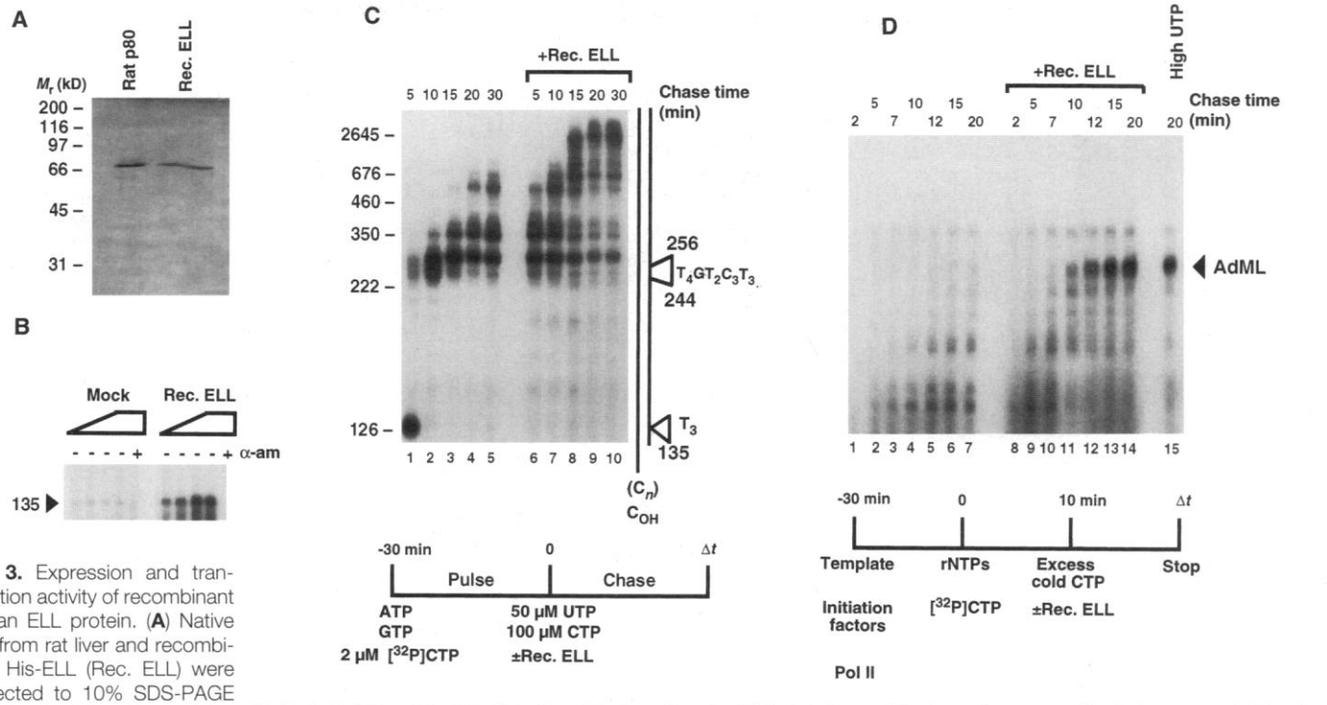


**Fig. 3.** Expression and transcription activity of recombinant human ELL protein. (**A**) Native p80 from rat liver and recombinant His-ELL (Rec. ELL) were subjected to 10% SDS-PAGE and visualized by silver staining. (**B**) SDS-PAGE–purified His-ELL (Rec. ELL) (*10*) or an identically prepared protein fraction (mock) prepared from *E. coli* infected with an M13mpET vector carrying the gene for Elongin B (*22*), which is transcriptionally inactive in the absence of Elongin A and C, were assayed (*8*) for their ability to stimulate synthesis of the 135-nt transcript from the T-less cassette of the oligo(dC)-tailed template pCpGR220 S/P/X (*7*). Reaction mixtures in lanes 1 to 5 contained 1, 3, 5, 7, and 7 μl, respectively, of the renatured mock protein fraction, and reaction mixtures in lanes 6 to 10 contained 1, 3, 5, 7, and 7 μl, respectively, of the renatured recombinant ELL fraction. Where indicated, reaction mixtures contained α-amanitin (α-am; 1 μg/ml). (**C**) Effect of ELL protein on the kinetics of promoter-independent transcription by RNA polymerase II. Oligo(dC)-tailed template assays were carried out essentially as described (*8*) with the modifications diagrammed in the lower panel. Reaction mixtures in lanes 6 to 10 contained 5 μl of renatured, nickel agarose–purified His-ELL protein (*10*) (Rec. ELL). (**D**) Effect of ELL protein on the kinetics of promoter-dependent transcription by RNA polymerase II. Preinitiation complexes were assembled at the AdML promoter as described (*22*) with recombinant TBP, TFIIB, TFIIE, TFIIF, TFIIH, and purified rat RNA polymerase II. Transcription was initiated by addition of 50 μM ATP, 50 μM GTP, 2 μM UTP, 10 μCi of [α-$^{32}$P]CTP (400 Ci/mmol, Amersham), and 7 mM $MgCl_2$. After 10 min at 28°C, 100 μM nonradioactive CTP was added to reaction mixtures. Where indicated, 5 μl of renatured His-ELL protein (*10*) was added to reaction mixtures, which were then incubated further for the times indicated. Transcripts were analyzed by electrophoresis through a 6% polyacrylamide, 7.0 M urea gel.

longer than transcripts synthesized in its absence; this finding indicated that the ELL protein stimulates elongation by RNA polymerase II during promoter-independent transcription in the absence of initiation factors. In another pulse-chase experiment (Fig. 3D), promoter-specific transcription reactions were performed as in Fig. 1D. Comparison of the kinetics of accumulation of full-length runoff transcripts synthesized from the AdML promoter revealed that the ELL protein also stimulated the rate of elongation of promoter-specific transcripts synthesized by RNA polymerase II in the presence of the general initiation factors.

Molecular analysis of a large number of chromosomal abnormalities in human cancers has revealed that the *MLL* gene is a recurring target for translocations in a variety of phenotypically distinct leukemias. To date, genes encoding six MLL translocation partners in addition to ELL have been cloned: *AF4* in t(4;11)(q21;q23) (*5*, *12*), *ENL* in t(11;19)(q23;p13.3) (*4*), *AF9* in t(9;11)(p22;q23) (*13*), *AF6* in t(6;11)(q27;q23) (*14*), *AF1p* in t(1;11)(p32;q23) (*15*), and *AFX* in t(X;11)(q13;q23) (*16*). A common feature of these translocations is that their breakpoints result in the creation of a putative oncogene encoding nearly the entire translocation partner fused to the $NH_2$-terminal ~1400 amino acids of MLL, including the A-T hook and methyltransferase-like domains but lacking the COOH-terminal transcriptional activation and zinc finger domains (*2*). The observation that these translocations occur within the same region of the *MLL* gene but are associated with leukemias exhibiting different clinical features (*2*) suggests that the partner gene plays a critical role in establishing the leukemic phenotype. However, despite the availability of cloned cDNAs encoding the MLL protein and its translocation partners, neither the roles of these proteins in oncogenesis nor their functions in normal cell growth have been established. Our identification of the product of the *ELL* gene as an RNA polymerase II elongation factor may help to establish such a role for one of these proteins. By virtue of its ability to regulate the activity of the RNA polymerase II elongation complex, the ELL protein joins a small but growing family (*17*) of biochemically defined elongation factors including SII, TFIIF, P-TEFb (*18*), and Elongin (SIII), which was recently found to be a target for regulation by the product of the *VHL* tumor suppressor gene (*19*). The discovery of a function for the ELL protein should expedite investigation of its role in both acute myeloid leukemia and normal cell growth.

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equivalent to that of buffer A containing 0.1 M KCl and was applied to a 1-liter phosphocellulose column (P11; Whatman, Maidstone, UK). The column was eluted stepwise at one packed column volume per hour with buffer A containing 0.5 M KCl. Fractions (200 ml) were collected, and active fractions were pooled, adjusted to 1 M $(NH_4)_2SO_4$ by addition of an equal volume of buffer A containing 2 M $(NH_4)_2SO_4$, and applied to a 100 ml Phenyl Sepharose-6 Fast Flow (low sub) column (Pharmacia) equilibrated in buffer D [40 mM Hepes-NaOH (pH 7.9), 0.5 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol] containing 1 M $(NH_4)_2SO_4$. The column was eluted at 10 ml/min with a 1-liter linear gradient from 1 to 0 M $(NH_4)_2SO_4$ in buffer D. Fractions (40 ml) were collected, and the active fractions, which eluted with ~0.6 M $(NH_4)_2SO_4$, were pooled and dialyzed against buffer B [40 mM tris-HCl (pH 7.9), 0.5 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol] to a conductivity equivalent to that of buffer B containing 0.07 M KCl. The dialysate was centrifuged at 20,000*g* for 30 min and applied to a 10-ml Mono-Q column (Pharmacia) equilibrated with buffer B containing 0.07 M KCl. The column was eluted at 1 ml/min with a 100-ml linear gradient from 0.07 to 0.5 M KCl in buffer B. Fractions (4 ml) were collected, and the active fractions, which eluted at ~0.2 M KCl, were pooled, dialyzed against buffer A to a conductivity equivalent to buffer A containing 0.07 M KCl, and applied to a 1-ml Mono-S column (Pharmacia) equilibrated in the same buffer. The column was eluted at 1 ml/min with a 10-ml linear gradient from 0.07 to 0.5 M KCl in buffer A. Fractions (1 ml) were collected, and the active fractions, which eluted with ~0.3 M KCl, were adjusted to 1 M $(NH_4)_2SO_4$ by addition of an equal volume of buffer A containing 2 M $(NH_4)_2SO_4$, centrifuged at 20,000*g* for 30 min, and applied to a Bio-Gel TSK phenyl-5-PW column (7.5 mm by 75 mm; Bio-Rad) equilibrated with buffer D containing 1 M $(NH_4)_2SO_4$. The column was eluted at 1 ml/min with a 30-ml linear gradient from 1.0 M to 0 M $(NH_4)_2SO_4$ in buffer D. Fractions (1 ml) were collected, and the active fractions, which eluted with ~0.5 M $(NH_4)_2SO_4$, were pooled, dialyzed against buffer B to a conductivity equivalent to that of buffer B containing 0.07 M KCl, and applied to a TSK DEAE-NPR (35 mm by 4.6 mm; Toso-Haas, Montgomeryville, PA) equilibrated in the same buffer. The column was eluted at 0.3 ml/min with a linear gradient from 0.07 to 0.5 M KCl in buffer B. Fractions (0.1 ml) were collected, and the active fractions, which eluted with ~0.2 M KCl, were pooled, diluted with an equal volume of 4 M guanidine hydrochloride, 4 M urea, 7.5% acetonitrile, 0.15% trifluoroacetic acid (TFA), and 0.2% Zwittergent ZC-8 (Calbiochem, La Jolla, CA), and applied at 0.1 ml/min to a PLRP-S (1 mm by 50 mm; pore size 1000 Å, particle size 8 μm) rpHPLC column (Michrom BioResources, Auburn, CA) preequilibrated at 40°C in 2% eluant B (90% acetonitrile, 0.09% TFA) and 98% eluant A (2% acetonitrile, 0.1% TFA). The column was developed at 0.1 ml/min with a 2-min linear gradient from 2 to 25% eluant B, followed by a 23-min linear gradient from 25 to 60% eluant B.


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9. Approximately 50 pmol of PLRP-S–purified rat p80 (*6*) was reduced, *S*-carboxyamidomethylated, digested with trypsin, and fractionated by microbore HPLC. Optimal peptides were determined by differential absorbance of ultraviolet light and matrix-assisted laser desorption mass spectrometry (Lasermat; Finnigan-MAT, San Jose, CA). The peptides were then sequenced by automated Edman degradation [W. S. Lane, A. Galat, M. W. Harding, S. L. Schreiber, *J. Protein Chem.* **10**, 151 (1991)].
10. The human ELL protein was overexpressed in *E. coli* with an M13mpET bacteriophage vector (*20*). The construct for expression of histidine (His)–tagged human ELL was prepared by insertion of a polymerase chain reaction (PCR)–generated 1.8-kb fragment containing the *ELL* ORF into the Sal I and Bam HI sites of M13mpET, which contains the complete pET T7 transcription-translation regions as well as sequences encoding the His tag. The initial *ELL* ORF PCR product encoded a protein with a C494Y mutation relative to the published ELL sequence (*3*). This mutation was corrected by site-directed mutagenesis with the use of the Bio-Rad Muta-Gen kit; the transcription activities of the mutant and corrected proteins were indistinguishable. For preparation of recombinant ELL, a 500-ml culture of *E. coli* strain JM109(DE3) was grown to an absorbance (at 600 nm) of 0.3 with gentle shaking in Luria broth medium containing 2.5 mM $MgCl_2$ at 37°C. Cells were infected with M13mpET carrying the *ELL* ORF at a multiplicity of infection of 20. After 3.5 hours at 37°C, cells were equilibrated at 30°C, induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and incubated at 30°C for an additional 12 hours. Cells were harvested by centrifugation at 2000*g* for 15 min at 4°C. Inclusion bodies were solubilized by resuspension in 5 ml of ice-cold 50 mM tris-HCl (pH 8.0) containing 6 M guanidine-HCl, and recombinant His-ELL protein was purified by nickel chromatography on ProBond resin (*20, 21*). For preparation of recombinant ELL protein for transcription assays, 500 ng of His-ELL was renatured essentially as described (*21*). For preparation of SDS-PAGE–purified ELL protein, ~1 μg of His-ELL was subjected to 10% SDS-PAGE. The portion of the gel containing the intact ELL protein was eluted with a Model 422 electroeluter (Bio-Rad). Electroelution was carried out at 10 mA for 4 hours in 25 mM tris base, 192 mM glycine, and 0.1% SDS according to the manufacturer's instructions. The electroeluted His-ELL was ethanol-precipitated and resuspended in 100 μl of 6 M guanidine-HCl, 25 mM tris-HCl (pH 7.9), 300 mM imidazole, and 0.5 mM phenylmethylsulfonyl fluoride. For assays, 20 μl of resuspended His-ELL was diluted with 40 μl of 40 mM Hepes-NaOH (pH 7.9), 0.1 M KCl, 50 μM $ZnSO_4$, 1 mM DTT, and 10% (v/v) glycerol. After 90 min on ice, the protein was dialyzed for 3 hours at 4°C against the same buffer without DTT.
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23. We thank R. Robinson of the Harvard Microchemistry Facility for peptide isolation. Supported by NIH grant GM41628 and by funds provided to the Oklahoma Medical Research Foundation by the H. A. and Mary K. Chapman Charitable Trust (A.S., R.C.C., and J.W.C.) and by the William K. Warren Foundation (K.W.J.). A.S. is supported by a postdoctoral fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

4 January 1996; accepted 23 February 1996


# Failure of the Cystic Fibrosis Transmembrane Conductance Regulator to Conduct ATP


M. M. Reddy, P. M. Quinton, C. Haws, J. J. Wine, R. Grygorczyk, J. A. Tabcharani, J. W. Hanrahan, K. L. Gunderson, R. R. Kopito*



**The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride ion channel regulated by protein kinase A and adenosine triphosphate (ATP). Loss of CFTR-mediated chloride ion conductance from the apical plasma membrane of epithelial cells is a primary physiological lesion in cystic fibrosis. CFTR has also been suggested to function as an ATP channel, although the size of the ATP anion is much larger than the estimated size of the CFTR pore. ATP was not conducted through CFTR in intact organs, polarized human lung cell lines, stably transfected mammalian cell lines, or planar lipid bilayers reconstituted with CFTR protein. These findings suggest that ATP permeation through the CFTR is unlikely to contribute to the normal function of CFTR or to the pathogenesis of cystic fibrosis.**


The cloning of the CFTR gene (*1, 2*) and the demonstration that it encodes a low-conductance protein kinase A (PKA)–regulated $Cl^-$ channel (*3–5*) confirmed that the loss of epithelial plasma membrane $Cl^-$ conductance is the principal ionic mechanism underlying the pathogenesis of cystic fibrosis (CF) (*6, 7*). The precise mechanism by which this loss of $Cl^-$ conductance is linked to the complex and varied features of the CF phenotype remains obscure. CFTR has been reported to regulate other ion channels (*8, 9*) and mediate permeability of cell membranes to ATP (*10*). Both CFTR (*10*) and its distant relative P-glycoprotein (*11*) have been reported to form ATP-permeant channels with single-channel conductance of ~5 pS. However, we found that CFTR had no detectable ATP conductance in four different systems, including native sweat duct and reconstituted bilayers.

CFTR is abundantly expressed in human sweat duct (*12*), where it appears to constitute the sole pathway for $Cl^-$ absorption (*13*). When the CFTR channel is activated by addition of adenosine 3′,5′-monophos-