United States Patent [19]

Duan et al.

[11] **Patent Number:** **6,008,018**

[45] **Date of Patent:** **Dec. 28, 1999**

[54] **ELL2, A NEW MEMBER OF AN ELL FAMILY OF RNA POLYMERASE II ELONGATION FACTORS**

[75] Inventors: **D. Roxanne Duan**, Bethesda, Md.; **Ali Shilatifard**, St. Louis, Mo.; **Joan W. Conaway**; **Ronald C. Conway**, both of Oklahoma City, Okla.

[73] Assignees: **Human Genome Sciences, Inc.**, Rockville, Md.; **Oklahoma Medical Research Foundation**, Oklahoma City, Okla.

[21] Appl. No.: **09/026,343**

[22] Filed: **Feb. 19, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/038,447, Feb. 19, 1997.

[51] **Int. Cl.**[6] **C12N 15/63**; C12N 15/64; C12N 15/11; C12N 15/12

[52] **U.S. Cl.** **435/69.1**; 435/91.41; 435/243; 435/252.3; 435/254.11; 435/320.1; 435/325; 435/419; 435/455; 435/468; 536/23.1; 536/23.4; 536/23.5

[58] **Field of Search** 435/69.1, 91.4, 435/320.1, 455, 325, 91.41, 243, 252.3, 254.11, 419, 468; 536/23.5, 23.1, 23.4

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

WO 94/01548   1/1994   WIPO .

OTHER PUBLICATIONS

Shilatifard A., et al., "ELL2, a new member of an ELL family of RNA polymerase II elongation factors," *Proc. Natl. Acad. Sci. USA*, 94:3639–3643 (Apr. 1997).

Adams, M.D. et al. "Complementary DNA Sequencing: Expressed Sequence Tags and Human Genome Project," *Science 252*:1651–1656 (1991).

Adams, M.D. et al., "Sequence identification of 2,375 human brain genes," *Nature 355*:632–634 (1992).

Adams, M.D. et al., "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," *Nature 377*:3–174 (1995).

Amann, E. et al., "Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*," *Gene 69*:301–315 (1988).

Aso, T. et al., "Elongin (SIII): A Multisubunit Regulator of Elongation by RNA Polymerase II," *Science 269*:1439–1443 (1995).

Aso, T. et al., "Transcription Syndromes and the Role of RNA Polymerase II General Transcription Factors in Human Disease," *J. Clin. Invest. 97*:1561–1569 (Apr. 1996).

Bentley, D.L., "Regulation of transcriptional elongation by RNA polymerase II," *Curr. Opin. Genetics & Develop. 5*:210–216 (1995).

Duan, D.R. et al., "Inhibition of Transcription Elongation by the VHL Tumor Suppressor Protein," *Science 269*:1402–1406 (1995).

Furuse, M. et al., "Direct Association of Occludin with ZO–1 and Its Possible Involvement in the Localization of Occludin at Tight Junctions," *J. Cell Biol. 127*:1617–1626 (1994).

Furuse, M. et al., "Occludin: A Novel Integral Membrane Protein Localization at Tight Junctions," *J. Cell Biol. 123*(6):1777–1788 (1993).

Gribskov, M. and R.R. Burgess, "Sigma Factors from *E. coli, B. subtilis*, phage SP01, and phage T4 are homologous proteins," *Nucl. Acids Res. 14*:6745–6763 (1986).

Jesaitis, L.A. and D.A. Goodenough, "Molecular Characterization and Tissue Distribution of ZO–2, A Tight Junction Protein Homologous to ZO–1 and the Drosophila Discs–Large Tumor Suppressor Protein," *J. Cell Biol. 124*:949–961 (1994).

Jones, K.A. and B.M. Peterlin, "Control of RNA Initiation and Elongation at the HIV–1 Promoter," *Annu. Rev. Biochem. 63*:717–743 (1994).

Kibel, A. et al., "Binding of the von Hippel–Lindau Tumor Suppressor Protein to Elongin B and C," *Science 269*:1444–1446 (1995).

Kistner, U. et al., "SAP90, a Rat Presynaptic Protein Related to the Product of the Drosophila Tumor Suppressor Gene dlg–A," *J. Biol. Chem. 268*:4580–4583 (1993).

Kunkel, T.A., "Rapid and efficient site–specific mutagenesis without phenotypic selection," *Proc. Natl. Acad. Sci. USA 82*:488–492 (1985).

Lee, S. et al., "Nuclear/cytoplasmic localization of the von Hippel–Lindau tumor suppressor gene product is determined by cell density," *Proc. Natl. Acad. Sci. USA 93*:1770–1775 (Mar. 1996).

Lue, R.A. et al., "Cloning and characterization of hdlg: The human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1," *Proc. Natl. Acad. Sci. USA 91*:9818–9822 (1994).

Marshall, N.F. and D.H. Price, "Purification of P–TEFb, a Transcription Factor Required for the Transition into Productive Elongation," *J. Biol. Chem. 270*:12335–12338 (1995).

Marshall, N.F. et al., "Control of RNA Polymerase II Elongation Potential by a Novel Carboxyl–terminal Domain Kinase," *J. Biol. Chem. 271*:27176–27183 (Oct. 1996).

Mitani, K. et al., "Cloning of Several Species of MLL/MEN Chimeric cDNAs in Myeloid Leukemia With t(11;19)(q23;p13.1) Translocation," *Blood 85*:2017–2024 (1995).

(List continued on next page.)

Primary Examiner—John S. Brusca

Attorney, Agent, or Firm—Sterne, Kressler, Goldstein & Fox, p.l.l.c.

[57] **ABSTRACT**

ELL2 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing ELL2 polypeptides and polynucleotides in the design of protocols for the treatment of neoplastic disorders, among others and diagnostic assays for such conditions.

96 Claims, 7 Drawing Sheets

OTHER PUBLICATIONS

Price, D.H. et al., "Dynamic Interaction between a Drosophila Transcription Factor and RNA Polymerase II," *Mol. Cell. Biol. 9*:1465–1475 (1989).

Reines, D., "Nascent RNA Cleavage by Transcription Elongation Complexes," In: *Transcription: Mechanisms and Regulation*, Conaway, R.C. and J.W. Conaway, eds., Raven Press, Ltd., New York, NY, pp. 263–278 (1994).

Reines, D. et al., "The RNA polymerase II general elongation factors," *Trends in Biochemical Sci. (TiBS) 21*:351–355 (Sep. 1996).

Rice, G.A. et al., "Footprinting analysis of mammalian RNA polymerase II along its transcript: An alternative view of transcription elongation," *Proc. Natl. Acad. Sci. USA 88*:4245–4249 (1991).

Ruff, P. et al., "Molecular identification of a major palmitoylated erythrocyte membrane protein containing the src homology 3 motif," *Proc. Natl. Sci. USA 88*:6595–6599 (1991).

Shilatifard, A. et al., "An RNA Polymerase II Elongation Factor Encoded by the Human ELL Gene," *Science 271*:1873–1876 (Mar. 1996).

Stark, M.J.R., "Multicopy expression vectors carrying the lac repressor gene for regulated high–level expression of genes in *Escherichia coli*," *Gene 51*:255–267 (1987).

Tan, S. et al., "A Bacteriophage Vector Suitable for Site–Directed Mutagenesis and High–Level Expression of Multisubunit Proteins in *E.coli*," *BioTechniques 16*:824–828 (1994).

Thirman, M.J. et al., "Cloning of ELL, a gene that fuses to MLL in a t(11;19)(q23;p13.1) in acute myeloid leukemia," *Proc. Natl. Acad. Sci. USA 91*:12110–12114 (1994).

Willott, E. et al., "The tight junction protein ZO–1 is homologous to the Drosphila discs–large tumor suppressor protein of a septate junctions," *Proc. Natl. Acad. Sci. USA 90*:7834–7838 (1993).

Woods, D.F. and P.J. Bryant, "The Discs–Large Tumor Suppressor Gene of Drosophila Encodes a Guanylate Kinase Homolog Localized at Septate Junctions," *Cell 66*:451–464 (1991).

Yu, H. et al., "Structural Basis for the Binding of Proline–Rich Peptides to SH3 Domains," *Cell 76*:933–945 (1994).

NCBI Entrez, GenBank Report, Accession No. Z20670, from MRC Human Genome Mapping Project Resource Centre (1995), with Revision History.

NCBI Entrez, GenBank Report, Accession No. T89063, from Hillier, L. et al. (1995), with Revision History.

NCBI Entrez, GenBank Report, Accession No. R12663, from Hillier, L. et al. (1995), with Revision History.

NCBI Entrez, GenBank Report, Accession No. R16400, from Hillier, L. et al. (1995), with Revision History.

NCBI Entrez, GenBank Report, Accession No. N39822, from Hillier, L. et al. (Jan. 1996), with Revision History.

NCBI Entrez, GenBank Report, Accession No. W92650, from Hillier, L. et al. (Nov. 1996), with Revision History.

NCBI Entrez, GenBank Report, Accession No. W94585, from Hillier, L. et al. (Nov. 1996), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA191245, from Hillier, L. et al. (Jan. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA414900, from Marra, M. et al. (May 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA370180, from Adams, M.D. et al. (Apr. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA370048, from Adams, M.D. et al. (Apr. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA375277, from Adams, M.D. et al. (Apr. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA009921, from Hillier, L. et al. (May 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA545429, from Marra, M. et al. (Aug. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA252607, from NCI–CGAP (Aug. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA243384, from NCI–CGAP (Aug. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA524290, from NCI–CGAP (Aug. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA655966, from Marra, M. et al. (Nov. 1997), with Revision History.

NCBI Entrez, GenBank Report, Accession No. AA743118, from NCI–CGAP (Jan. 1998), with Revision History.

NCBI Entrez, GenBank Report, Accession No. W13766, from Marra, M. et al. (Oct. 1997), with Revision History.

```
    CAGTGGCGGCGGGTGCAGAAGCCCAAGCAGCGCGGCCGCAGTGGAGGCTAGAGCCGGAGC
  1 ---------+---------+---------+---------+---------+---------+   60

    GGCGGCGGCGGCGGCACCCCGGGGGAGGTTTAAGATGGCGGCGGGGGGGGACAGGGGGCCTG
 61 ---------+---------+---------+---------+---------+---------+   120
                                        M  A  A  G  G  T  G  G  L

    CGGGAGGAGCAGCGCTATGGGCTGTCGTGCGGACGGCTGGGGCAGGACAACATCACCGTA
121 ---------+---------+---------+---------+---------+---------+   180
     R  E  E  Q  R  Y  G  L  S  C  G  R  L  G  Q  D  N  I  T  V

    CTGCATGTGAAGCTCACCGAGACGGCGATCCGGGCGCTCGAGACTTACCAGAGCCACAAG
181 ---------+---------+---------+---------+---------+---------+   240
     L  H  V  K  L  T  E  T  A  I  R  A  L  E  T  Y  Q  S  H  K

    AATTTAATTCCTTTTCGACCTTCAATCCAGTTCCAAGGACTCCACGGGCTTGTCAAAATT
241 ---------+---------+---------+---------+---------+---------+   300
     N  L  I  P  F  R  P  S  I  Q  F  Q  G  L  H  G  L  V  K  I

    CCCAAAAATGATCCCCTCAATGAAGTTCATAACTTTAACTTTTATTTGTCAAATGTGGGC
301 ---------+---------+---------+---------+---------+---------+   360
     P  K  N  D  P  L  N  E  V  H  N  F  N  F  Y  L  S  N  V  G

    AAAGACAACCCTCAGGGCAGCTTTGACTGCATCCAGCAAACATTCTCCAGCTCTGGAGCC
361 ---------+---------+---------+---------+---------+---------+   420
     K  D  N  P  Q  G  S  F  D  C  I  Q  Q  T  F  S  S  S  G  A

    TCCCAGCTCAATTGCCTGGGATTTATACAAGATAAAATTACAGTGTGTGCAACAAACGAC
421 ---------+---------+---------+---------+---------+---------+   480
     S  Q  L  N  C  L  G  F  I  Q  D  K  I  T  V  C  A  T  N  D

    TCGTATCAGATGACACGAGAAAGAATGACCCAGGCAGAGGAGGAATCCCGCAACCGAAGC
481 ---------+---------+---------+---------+---------+---------+   540
     S  Y  Q  M  T  R  E  R  M  T  Q  A  E  E  E  S  R  N  R  S

    ACAAAAGTTATCAAACCCGGTGGACCATATGTAGGGAAAAGAGTGCAAATTCGGAAAGCA
541 ---------+---------+---------+---------+---------+---------+   600
     T  K  V  I  K  P  G  G  P  Y  V  G  K  R  V  Q  I  R  K  A

    CCTCAAGCTGTTTCAGATACAGTTCCTGAGAGGAAAAGGTCAACCCCCATGAACCCTGCA
601 ---------+---------+---------+---------+---------+---------+   660
     P  Q  A  V  S  D  T  V  P  E  R  K  R  S  T  P  M  N  P  A

    AATACAATTCGAAAGACACATAGCAGCAGCACCATCTCTCAGAGGCCATACAGGGACAGG
661 ---------+---------+---------+---------+---------+---------+   720
     N  T  I  R  K  T  H  S  S  S  T  I  S  Q  R  P  Y  R  D  R

    GTGATTCACTTACTGGCCCTGAAGGCCTACAAGAAACCGGAGCTACTTGCTAGACTCCAG
721 ---------+---------+---------+---------+---------+---------+   780
     V  I  H  L  L  A  L  K  A  Y  K  K  P  E  L  L  A  R  L  Q
```

FIG.1A

```
     AAAGATGGTGTCAATCAAAAAGACAAGAACTCCCTGGGAGCAATTCTGCAACAGGTAGCC
781  ---------+---------+---------+---------+---------+---------+  840
     K  D  G  V  N  Q  K  D  K  N  S  L  G  A  I  L  Q  Q  V  A

     AATCTGAATTCTAAGGACCTCTCATATACCTTAAAGGATTATGTTTTTAAAGAGCTTCAA
841  ---------+---------+---------+---------+---------+---------+  900
     N  L  N  S  K  D  L  S  Y  T  L  K  D  Y  V  F  K  E  L  Q

     AGAGACTGGCCTGGATACAGTGAAATAGACAGACGGTCATTGGAGTCAGTGCTCTCTAGA
901  ---------+---------+---------+---------+---------+---------+  960
     R  D  W  P  G  Y  S  E  I  D  R  R  S  L  E  S  V  L  S  R

     AAACTAAATCCGTCTCAGAATGCTACAGGCACCAGCCGTTCAGAATCTCCTGTATGTTCT
961  ---------+---------+---------+---------+---------+---------+  1020
     K  L  N  P  S  Q  N  A  T  G  T  S  R  S  E  S  P  V  C  S

     AGTAGAGATGCTGTATCTTCTCCTCAGAAACGGCTTTTGGATTCAGAGTTTATTGATCCT
1021 ---------+---------+---------+---------+---------+---------+  1080
     S  R  D  A  V  S  S  P  Q  K  R  L  L  D  S  E  F  I  D  P

     TTAATGAATAAAAAAGCCCGAATATCTCACCTGACGAACAGAGTACCACCAACACTAAAT
1081 ---------+---------+---------+---------+---------+---------+  1140
     L  M  N  K  K  A  R  I  S  H  L  T  N  R  V  P  P  T  L  N

     GGTCATTTGAATCCCACCAGTGAAAAATCGGCTGCAGGCCTCCCACTGCCCCCTGCGGCT
1141 ---------+---------+---------+---------+---------+---------+  1200
     G  H  L  N  P  T  S  E  K  S  A  A  G  L  P  L  P  P  A  A

     GCTGCCATCCCCACCCCTCCACCGCTGCCTTCAACCTATCTGCCCATCTCACATCCTCCT
1201 ---------+---------+---------+---------+---------+---------+  1260
     A  A  I  P  T  P  P  P  L  P  S  T  Y  L  P  I  S  H  P  P

     CAGATTGTAAATTCTAACTCCAACTCCCCTAGCACTCCAGAAGGCCGGGGGGACTCAAGAC
1261 ---------+---------+---------+---------+---------+---------+  1320
     Q  I  V  N  S  N  S  N  S  P  S  T  P  E  G  R  G  T  Q  D

     CTACCTGTTGACAGTTTTAGTCAAAACGATAGTATCTATGAGGACCAGCAAGACAAATAT
1321 ---------+---------+---------+---------+---------+---------+  1380
     L  P  V  D  S  F  S  Q  N  D  S  I  Y  E  D  Q  Q  D  K  Y

     ACCTCTAGGACTTCTCTGGAAACCTTACCCCCTGGTTCCGTTCTACTAAAGTGTCCAAAG
1381 ---------+---------+---------+---------+---------+---------+  1440
     T  S  R  T  S  L  E  T  L  P  P  G  S  V  L  L  K  C  P  K

     CCTATGGAAGAAAACCATTCAATGTCTCACAAAAAGTCCAAAAAGAAGTCTAAAAAACAT
1441 ---------+---------+---------+---------+---------+---------+  1500
     P  M  E  E  N  H  S  M  S  H  K  K  S  K  K  K  S  K  K  H

     AAGGAAAAGGACCAAATAAAAAAGCACGACATTGAGACTATTGAGGAAAAGGAGGAAGAT
1501 ---------+---------+---------+---------+---------+---------+  1560
     K  E  K  D  Q  I  K  K  H  D  I  E  T  I  E  E  K  E  E  D

     CTTAAGAGAGAAGAGGAAATTGCCAAGCTAAATAACTCCAGTCCAAATTCCAGTGGAGGA
1561 ---------+---------+---------+---------+---------+---------+  1620
```

FIG.1B

```
         L  K  R  E  E  E  I  A  K  L  N  N  S  S  P  N  S  S  G  G

        GTTAAAGAGGATTGCACTGCCTCCATGGAACCTTCAGCAATTGAACTCCCAGATTATTTG
1621    ---------+---------+---------+---------+---------+---------+    1680
         V  K  E  D  C  T  A  S  M  E  P  S  A  I  E  L  P  D  Y  L

        ATAAAATATATCGCTATCGTCTCCTATGAGCAACGCCAGAATTATAAGGATGACTTCAAT
1681    ---------+---------+---------+---------+---------+---------+    1740
         I  K  Y  I  A  I  V  S  Y  E  Q  R  Q  N  Y  K  D  D  F  N

        GCAGAGTATGATGAGTACAGAGCTTTGCATGCCAGGATGGAGACTGTAGCTAGAAGATTT
1741    ---------+---------+---------+---------+---------+---------+    1800
         A  E  Y  D  E  Y  R  A  L  H  A  R  M  E  T  V  A  R  R  F

        ATCAAACTAGATGCACAAAGAAAGCGCCTTTCTCCAGGCTCAAAAGAGTATCAGAATGTT
1801    ---------+---------+---------+---------+---------+---------+    1860
         I  K  L  D  A  Q  R  K  R  L  S  P  G  S  K  E  Y  Q  N  V

        CATGAAGAAGTCTTACAAGAATATCAGAAGATAAAGCAGTCTAGTCCCAATTACCATGAA
1861    ---------+---------+---------+---------+---------+---------+    1920
         H  E  E  V  L  Q  E  Y  Q  K  I  K  Q  S  S  P  N  Y  H  E

        GAAAAATACAGATGTGAATATCTTCATAACAAGCTGGCTCACATCAAAAGGCTAATAGGT
1921    ---------+---------+---------+---------+---------+---------+    1980
         E  K  Y  R  C  E  Y  L  H  N  K  L  A  H  I  K  R  L  I  G

        GAATTTGACCAACAGCAAGCAGAGTCATGGTCCTAGAACTCTGCTTGGACCAGAAGATGT
1981    ---------+---------+---------+---------+---------+---------+    2040
         E  F  D  Q  Q  Q  A  E  S  W  S  *

        GAATAAACTTAAGCTTATTTATTTAAAATTCCAAATGAGTTGCTCTAGATTCTAAAAAGG
2041    ---------+---------+---------+---------+---------+---------+    2100

        TGAAACTTTGGCTGTTGAAAGTTTCAGTATTAGTAAACT
2101    ---------+---------+---------+---------       2139
```

FIG.1C

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.