

# **EXHIBIT 41**



US005441868A

**United States Patent** [19]

[11] **Patent Number:** 5,441,868

Lin

[45] **Date of Patent:** Aug. 15, 1995

[54] **PRODUCTION OF RECOMBINANT ERYTHROPOIETIN**

4,667,016 5/1987 Lai et al. .... 530/397  
 4,677,195 6/1987 Hewick et al. .... 530/397  
 (List continued on next page.)

[75] **Inventor:** Fu-Kuen Lin, Thousand Oaks, Calif.

[73] **Assignee:** Kirin-Amgen, Inc., Thousand Oaks, Calif.

[21] **Appl. No.:** 113,179

[22] **Filed:** Oct. 23, 1987

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**Related U.S. Application Data**

[63] Continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983, abandoned, and a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, and Ser. No. 655,841, Sep. 28, 1984, abandoned.

[51] **Int. Cl.<sup>6</sup>** ..... C12P 21/02; C12N 15/27  
 [52] **U.S. Cl.** ..... 435/69.4; 435/240.1;  
 435/240.2; 536/23.51; 935/13

[58] **Field of Search** ..... 435/70, 69.5, 172.3,  
 435/69.1, 69.4, 69.6, 240.2, 320.1;  
 935/50, 13; 536/27, 23.5, 23.51

[56] **References Cited**

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4,254,095	3/1981	Fisher et al. ....	424/88
4,264,731	4/1981	Shine ..... ..	435/91.41
4,273,875	6/1981	Manis ..... ..	435/253.5
4,293,652	10/1981	Cohen ..... ..	435/172.3
4,303,650	12/1981	Takezawa et al. ....	424/345
4,338,397	7/1982	Gilbert et al. ....	435/69.1
4,358,535	11/1982	Falkow et al. ....	435/5
4,377,513	3/1983	Sugimoto et al. ....	530/395
4,394,443	7/1983	Weissman et al. ....	435/6
4,397,840	8/1983	Takezawa et al. ....	530/399
4,399,216	8/1983	Axel et al. .... ..	435/6
4,411,994	10/1983	Gilbert et al. ....	435/69.7
4,442,205	4/1984	Hamer et al. .... ..	435/69.1
4,465,624	8/1984	Chiba et al. .... ..	530/395
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4,503,151	3/1985	Paddock ..... ..	435/69.1
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4,568,488	2/1986	Lee-Huang ..... ..	530/380

*Primary Examiner*—James Martinell  
*Attorney, Agent, or Firm*—Marshall, O'Toole, Gerstein, Murray & Borun

[57] **ABSTRACT**

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of procaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable procaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

5 Claims, 27 Drawing Sheets



**United States Patent** [19]

[11] **Patent Number:** 5,547,933

**Lin**

[45] **Date of Patent:** Aug. 20, 1996

[54] **PRODUCTION OF ERYTHROPOIETIN**

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 012394 10/1984 European Pat. Off. .  
 0136490 4/1985 European Pat. Off. .  
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 85/01961 5/1985 WIPO .  
 85/03079 7/1985 WIPO .  
 85/04419 10/1985 WIPO .  
 86/03520 6/1986 WIPO .

[75] **Inventor:** Fu-Kuen Lin, Thousand Oaks, Calif.

[73] **Assignee:** Kirin-Amgen, Inc., Thousand Oaks, Calif.

[21] **Appl. No.:** 487,774

[22] **Filed:** Jun. 7, 1995

**Related U.S. Application Data**

[63] Continuation of Ser. No. 202,874, Feb. 28, 1994, abandoned, which is a continuation of Ser. No. 113,178, Oct. 23, 1987, abandoned, which is a continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, abandoned, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983, abandoned.

[51] **Int. Cl.<sup>5</sup>** ..... A61K 38/18; C12P 21/02

[52] **U.S. Cl.** ..... 514/8; 435/686; 530/388.7; 530/397; 530/835

[58] **Field of Search** ..... 435/69.1, 69.4, 435/69.6, 240.2, 320.1, 172.3, 13; 530/350, 380, 397, 834, 835, 23.5, 23.51, 388.7; 514/8

[56] **References Cited**

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4,254,095	3/1981	Fisher et al.	424/88
4,264,731	4/1981	Shine	435/91.41
4,273,875	6/1981	Manis	435/320.1
4,293,652	10/1981	Cohen	435/172.3
4,303,650	12/1981	Takezawa et al.	530/397
4,338,397	7/1982	Gilbert et al.	435/69.1
4,358,535	11/1982	Falkow et al.	435/5
4,377,513	3/1983	Sugimoto et al.	530/397
4,394,443	7/1983	Weissman et al.	435/6
4,397,840	8/1933	Takezawa et al.	424/99
4,399,216	8/1983	Axel et al.	435/6
4,411,994	10/1983	Gilbert et al.	435/69.7
4,442,205	4/1984	Hamer et al.	435/69.1
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4,667,016	5/1987	Lai et al.	530/397
4,677,195	6/1987	Hewick et al.	530/397
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4,703,008	10/1987	Lin	435/240.2
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4,757,006	7/1988	Toole et al.	435/69.6

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0093619	11/1983	European Pat. Off. .
0116446	8/1984	European Pat. Off. .
0117058	8/1984	European Pat. Off. .

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Abraham et al., "Nucleotide Sequence of a Bovine Clone Encoding the Angiogenic Protein, Basic Fibroblast Growth Factor," *Science*, 233, 545-548 (Aug. 1, 1986) [F].  
 Adamson, "The Polycythemia: Diagnosis and Treatment," *Hosp. Practice*, 18(12), 49-57 (Dec. 1983) [A].  
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(List continued on next page.)

**Primary Examiner**—James Martinell  
**Attorney, Agent, or Firm**—Marshall, O'Toole, Gerstein, Murray & Borun

[57] **ABSTRACT**

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of prokaryotic or eukaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable prokaryotic or eukaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

**14 Claims, 27 Drawing Sheets**



US005621080A

**United States Patent** [19]

[11] **Patent Number:** **5,621,080**

**Lin**

[45] **Date of Patent:** **\*Apr. 15, 1997**

[54] **PRODUCTION OF ERYTHROPOIETIN**

[75] **Inventor:** Fu-Kuen Lin, Thousand Oaks, Calif.

[73] **Assignee:** Kirin-Amgen, Inc., Thousand Oaks, Calif.

[\*] **Notice:** The portion of the term of this patent subsequent to Aug. 20, 2013, has been disclaimed.

[21] **Appl. No.:** 468,556

[22] **Filed:** Jun. 6, 1995

**Related U.S. Application Data**

[63] Continuation of Ser. No. 202,874, Feb. 28, 1994, abandoned, which is a continuation of Ser. No. 113,178, Oct. 23, 1987, abandoned, which is a continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, abandoned, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983, abandoned.

[51] **Int. Cl.<sup>6</sup>** ..... C07K 14/505; A61K 38/00

[52] **U.S. Cl.** ..... 530/350; 530/380; 530/399

[58] **Field of Search** ..... 435/240.2, 172.3, 435/320.1, 69.4, 240.1, 69.1, 69.6; 536/23.5, 23.51; 530/350, 380, 399; 514/12

[56] **References Cited**

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 Antonsson et al., "Posttranslational Modifications of Fibro-modulin," *J. Biol. Chem.*, 266(25), 16859-16861 (1991).

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*Primary Examiner*—James Martinell  
*Attorney, Agent, or Firm*—Marshall, O'Toole, Gerstein, Murray & Borun

[57] **ABSTRACT**

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of procaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable procaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

7 Claims, 27 Drawing Sheets



US005618698A

**United States Patent** [19]

[11] **Patent Number:** **5,618,698**

**Lin**

[45] **Date of Patent:** **\*Apr. 8, 1997**

[54] **PRODUCTION OF ERYTHROPOIETIN**

[75] **Inventor:** **Fu-Kuen Lin**, Thousand Oaks, Calif.

[73] **Assignee:** **Kirin-Amgen, Inc.**, Thousand Oaks, Calif.

[\*] **Notice:** The portion of the term of this patent subsequent to Aug. 15, 2012, has been disclaimed.

[21] **Appl. No.:** **468,381**

[22] **Filed:** **Jun. 6, 1995**

**Related U.S. Application Data**

[63] Continuation of Ser. No. 113,179, Oct. 23, 1987, Pat. No. 5,441,868, which is a continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983.

[51] **Int. Cl.<sup>6</sup>** ..... **C12P 71/02; C12N 15/27**  
 [52] **U.S. Cl.** ..... **435/69.4; 435/69.6; 435/325; 536/23.51**

[58] **Field of Search** ..... **435/240.2, 177.3, 435/320.1, 69.4, 240.1, 69.1, 69.6; 536/23.5, 23.51**

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4,293,652	10/1981	Cohen	435/172.3
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*Primary Examiner*—James Martinell  
*Attorney, Agent, or Firm*—Marshall, O'Toole, Gerstein, Murray & Borun

[57] **ABSTRACT**

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**9 Claims, 27 Drawing Sheets**





US005756349A

**United States Patent** [19]  
**Lin**

[11] **Patent Number:** 5,756,349  
[45] **Date of Patent:** May 26, 1998

- [54] **PRODUCTION OF ERYTHROPOIETIN**
- [75] **Inventor:** Fu-Kuen Lin, Thousand Oaks, Calif.
- [73] **Assignee:** Amgen Inc., Thousand Oaks, Calif.
- [21] **Appl. No.:** 468,369
- [22] **Filed:** Jun. 6, 1995

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- Baciu et al., "Erythropoietin Interaction with the Mature Red Cell Membrane." *Ann. N.Y. Acad. Sci.*, 414, 66-72 (1983).
- Baron et al., "Antibodies against the Chemically Synthesized Genome-Linked Protein of Poliovirus React with Native Virus-Specific Proteins." *Cell*, 28, 395-404 (Feb. 1982).

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*Primary Examiner*—James Martinell  
*Attorney, Agent, or Firm*—Marshall, O'Toole, Gerstein, Murray & Borun

[57] **ABSTRACT**

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of prokaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable prokaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viralborne cDNA or genomic DNA "library".

7 Claims, 27 Drawing Sheets

**Related U.S. Application Data**

- [63] Continuation of Ser. No. 113,179, Oct. 23, 1987, Pat. No. 5,441,868, which is a continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983, abandoned, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, abandoned.

- [51] **Int. Cl.<sup>6</sup>** ..... C12N 15/00
- [52] **U.S. Cl.** ..... 435/325; 435/358; 435/365
- [58] **Field of Search** ..... 435/69.1, 69.4, 435/240.2, 240.22, 325, 358, 365

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**United States Patent** [19]  
**Lin**

[11] **Patent Number:** 5,955,422  
[45] **Date of Patent:** \*Sep. 21, 1999

[54] **PRODUCTION OF ERTHROPOIETIN**

[75] **Inventor:** Fu-Kuen Lin, Thousand Oaks, Calif.

[73] **Assignee:** Kirin-Amgen, Inc., Thousand Oaks, Calif.

[\*] **Notice:** This patent is subject to a terminal disclaimer.

[21] **Appl. No.:** 08/100,197

[22] **Filed:** Aug. 2, 1993

**Related U.S. Application Data**

[63] Continuation of application No. 07/957,073, Oct. 6, 1992, abandoned, which is a continuation of application No. 07/609,744, Nov. 6, 1990, abandoned, which is a continuation of application No. 07/113,179, Oct. 23, 1987, Pat. No. 5,441,868, which is a continuation of application No. 06/675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of application No. 06/655,841, Sep. 28, 1984, abandoned, which is a continuation-in-part of application No. 06/582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of application No. 06/561,024, Dec. 13, 1983, abandoned.

[51] **Int. Cl.<sup>6</sup>** ..... A61K 38/16

[52] **U.S. Cl.** ..... 514/8; 514/12; 530/351; 530/363; 530/395; 530/350

[58] **Field of Search** ..... 424/439; 435/69.1, 435/69.2, 69.3, 69.6, 71.1, 71.2, 172.1, 172.3; 436/8; 514/8, 778, 970, 12; 530/351, 361, 395, 350

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2 Claims, 27 Drawing Sheets