IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

Application of:	)	"Production of
	)	
FU-KUEN LIN	)	Erythropoietin"
	)	
Serial No: 113,179	)	Group Art Unit 127
	)	
Filed: October 23, 1987	)	Examiner (Expected):
	)	A. Tanenholtz
(Based on S.N. 675,298,	)	
filed November 30, 1984,	)	
issued as U.S. 4,703,008	)	
on October 27, 1987)	)	

APPLICANT'S SECOND PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Consistent with the February 18, 1988 favorable Decision On Petition To Make Special and the provisions of M.P.E.P. §708.02, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please delete the entire text of page 1, lines 3-6 as amended October 23, 1987 and insert the following text in place thereof:

--This is a continuation of my co-pending U.S. Patent Application Serial No. 675,298, filed November 30, 1984 and issued as U.S. Letters Patent No. 4,703,008 on October 27, 1987, which was a continuation-in-part of my co-pending U.S. Patent Application Serial No. 561,024, filed December 13, 1983, now abandoned, and a continuation-in-part of Serial No. 582,185, filed February 21, 1984, now aban-

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doned, and a continuation-in-part of Serial No. 655,841, filed September 28, 1984.--

Page 7, line 27, "32 member" should be --32-member--.

Page 8, line 22, please delete the second occurrence of "the".

Page 11, line 3, "Expt.Hematol." should be --Exp.Hematol.--.

Page 11, line 4, "(1980:" should be --(1980);--.

Page 11, line 6, please insert a space before "1832".

Page 13, line 13, please insert --"-- after "effects".

Page 13, lines 20-21, please insert --)-- after "propagation".

Page 22, line 4, "Tables V and VI" should be --Figures 5 and 6--.

Page 22, line 22, "Table VI" should be --Figure 6--.

Page 27, line 24, "Example" should be --Examples--.

Page 32, line 35, please delete the comma (,) after "Springs".

Page 48, line 15, please delete "glutamine" and insert in place thereof --glutamic acid--.

Page 48, line 29, "Table VI" should be --Figure 6--.

Page 54, line 36, "EcoR1" should be --EcoRI--.

Page 55, line 13, "BamH1" should be --BamHI--.

Page 55, line 15, "BamH1" should be --BamHI--.

Page 61, line 25, "hemogeneous" should be --homogeneous--.

Page 88, line 36, "lablled" should be --labelled--.

Page 91, line 29, please delete "a".

Page 92, line 10, "Table VI" should be --Figure 6--.

Page 95, line 10, "membrances" should be --membranes--.

IN THE CLAIMS

Please cancel claims 61-64 without prejudice to Applicant to pursue claims of the same or similar scope in a duly-filed continuing application.

Please enter new claims 65-69.

--65. A process for the preparation of an in vivo biologically active glycosylated polypeptide comprising the steps of:

(a) growing a mammalian host cell which is capable of effecting post-translational glycosylation of polypeptides expressed therein and which is transformed or transfected with an isolated DNA sequence encoding a polypeptide having a primary structural conformation sufficiently duplicative of that of naturally occurring human erythropoietin to allow possession of the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, or the progeny thereof, under nutrient conditions suitable to allow, in sequence,

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(i) transcription within said host cell of said DNA to mRNA in the sequence of transcription reactions directed by the nucleotide sequence of said DNA;

(ii) translation within said host cell of said mRNA to a polypeptide in the sequence of translation reactions directed by the nucleotide sequence of said transcribed mRNA;

(iii) glycosylation within said host cell of said polypeptide in a pattern directed by the amino acid sequence of said translated polypeptide and sufficiently duplicative of the pattern of glycosylation of naturally occurring human erythropoietin to allow possession by the translated glycosylated polypeptide product of the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells; and

(b) isolating the glycosylated polypeptide so produced.

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66. The process according to claim 65 wherein said host cell is a CHO cell.

67. The process according to claim 65 wherein said host cell is a COS cell.

68. The process according to claim 65 wherein said DNA is cDNA.

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69. The process according to claim 65 wherein said DNA is genomic DNA.--

REMARKS

A. Amendments To The Specification

The above-requested amendments to the specification combine with those requested in Applicant's Preliminary Amendment dated October 23, 1987 to eliminate all typographical errors present in the specification as filed.

B. Reference To Related Applications

The present application is a continuation of U.S. Patent Application Serial No. 675,298, filed November 30, 1984 (as a continuation-in-part of Serial No. 655,841, filed September 28, 1984, and Serial No. 582, 185, filed February 21, 1984, now abandoned, and Serial No. 561,024, filed December 13, 1983, now abandoned). Application Serial No. 675,298 issued on October 27, 1987 as U.S. Letters Patent No. 4,703,008 (hereinafter "the '008 Patent"), with claims relating to novel erythropoietin DNA sequences. To facilitate consideration of the claims presented herein, Applicant attaches as Exhibit "A" hereto a copy of columns 39 to 42 of the '008 Patent which includes the 31 issued claims.

C. The Presently Claimed Subject Matter

Without prejudice to Applicant's right to pursue such further process claims as are supported by the disclosures of the present specification, Applicant has

submitted new claims 65-69 herein to that aspect of his invention which relates to novel methods for the production in mammalian host cells of human erythropoietin glycoprotein -- the first article of human manufacture ever to possess both the amino acid sequence and glycosylation needed for in vivo erythropoietin biological activity. As set out in detail hereafter, the practice in late 1983 of processes herein claimed is believed to constitute one of the first instances (if not the first instance) of the recombinant production of an in vivo biologically active obligate human glycoprotein, an event which has in turn allowed, for the first time, the provision of a human erythropoietin product in quantities necessary for human hematopoietic therapy.

Briefly summarized, independent claim 65 relates to a novel series of process steps wherein a mammalian host cell[1] capable of glycosylating the expressed polypeptides is first transformed or transfected with a DNA sequence[2] encoding a specifically delineated polypeptide, i.e., one having sufficient amino acid sequence homology to natural human erythropoietin to allow it to qualify, amino acid sequence-wise, for potential in vivo biological activity. (The DNA reagent employed in the transformation/transfection process is itself the novel and unobvious subject matter of '008 Patent claim 7 and the resulting host cells are as recited in claim 24 of the Patent.) The claim 65 process calls for host cell growth in culture under conditions

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1 Including such non-human, non-kidney cells as COS and CHO cells as specified in claims 66 and 67.

2 Including, e.g., cDNA and genomic DNA as specified in claims 68 and 69.

wherein transcription, translation and glycosylation processing occurs. More particularly, the claim calls for mRNA transcript formation according to the per se unique directions provided by the recited DNA sequence. (Illustratively, the formation of a full length coding region transcript of erythropoietin cDNA ordinarily involves no less than 582 specifically ordered nucleotide additions for the formation of the mRNA polymer.) Also delineated by claim 65 is performance of a specific sequence of translational events giving rise to polypeptide formation. (Again, a minimum of 193 specific alignments of tRNA's to the mRNA and 192 peptide bond formations are involved to link, in order, the amino acids constituting the full length primary structural conformation.) Further required by claim 65 is the glycosylation processing of the translated polypeptide at sites directed by the order of amino acids of the translated polypeptide so that the resulting product, upon isolation, will have the pattern of glycosylation which is also required for in vivo biological activity.

D. The Examiner's Position Regarding Method Method Claims During '008 Patent Prosecution

In the course of prosecution of Application Serial No. 675,298 leading up to issuance of the '008 Patent, certain erythropoietin production method claims (later withdrawn without prejudice) were objected to by Examiner Tanenholtz (one of three Examiners handling the application) on the following grounds:

> Claims 69-72 are rejected under 35 U.S.C. 103 as being unpatentable over Talmadge et al and who disclose the basic process of recombinantly expressing and isolating polypeptides as claimed herein. Even where it is considered that one more of the starting

> materials is novel, the application of an old process to such materials to produce the expected result would still be obvious within the meaning of 35 U.S.C. 103; In re Durden, supra; In re Larsen, 141 U.S.P.Q. 730 (1964). Whether or not a product produced by the claimed process possesses any unique or unexpected properties is not material to the question of whether or not the process itself would have been obvious." (Emphasis added)

Applicant respectfully submits that the above statements of the Examiner construing the decision of the Durden case were legally erroneous and that application of such constructions to the presently claimed subject matter would also constitute error.

E. Remarks In Support Of Patentability of the Claims

Applicant respectfully submits that the subject matter of claims 65-69 is clearly patentable and that no proper basis exists for rejection of the claims under 35 U.S.C. §103. In support of this position, Applicant provides the following series of remarks relating to: (1) the characteristics of human erythropoietin as an "obligate glycoprotein"; (2) the distinctness of the patentability issues herein from issues addressed in the decision in In re Durden; (3) the lack of relevance to patentability of prior art cited during prosecution of the '008 Patent; and (4) the lack of relevance to patentability of prior art recently ascertained and relating generally to recombinant production of glycoproteins.

1. The Characteristics of Erythropoietin As an Obligate Glycoprotein

"State of the art" knowledge with respect to erythropoietin at the time of the present invention revealed to the skilled worker that the in vivo biological activity

of this human glycoprotein was dependent not only on its specific amino acid sequence conformation, but also its array of glycosylation. Thus, Goldwasser et al., J.Biol.Chem., 249(18), 4302-4306 (1974) [Exhibit "B" hereto"] report at page 4302 that:

> Erythropoietin, a glycoprotein that induces normal erythrocyte development, has 16 to 18 sialic acid residues per mole. Desialation results in complete loss of biological activity when it is assayed in vivo. When the assay is done in vitro asialoerythropoietin has full activity, or when assayed at low levels of hormones is about three times more active than the native hormone. The loss of activity can be explained by the hepatic removal of asialo-glycoproteins from the circulation.

Goldwasser, Fed.Proc., 34(13), 2285-2292 (1975) [Exhibit "C" hereto] further reports at page 2288 that:

> The role of sialic acid in epo activity appeared for a time to be paradoxical since asialo epo has no activity when assayed by in vivo methods, but had full, or increased, activity when assayed [in vitro] by a marrow cell culture method. The explanation of this apparent paradox was derived from the studies of Morell et al. who showed that, in general, asialo glycoproteins were cleared from the circulation at a much greater rate than the native form. This rapid clearance was effected by hepatic cells that specifically interacted with the newly terminal galactose residues exposed by desialation. The liver system was shown to be a saturatable one, so that other asialoglycoproteins could occupy all of the binding sites and permit a nearly normal clearance rate for a particular test asialo glycoprotein. These authors also showed that, if the terminal galactose residues of asialo glycoproteins were oxidized or removed, the modified asialo glycoproteins would remain in the circulation for a nearly normal period.
>
> Lukowsky and Painter have shown that partially desialated epo, when oxidized with galactose oxidase, had its in vivo biological activity restored. In this laboratory, we have shown that completely desialated epo similarly treated had about 45% of the original in vivo activity. These data

> suggest that galactose is the penultimate carbohydrate residue but is not required for biological activity. In addition, asialo-orosomucoid and the tetrasaccharide stachyose could act as competitors in assay animals, permitting about 30% of the original (native) activity to be found in asialo epo. Lactose, on the other hand, does not act as a competitor, so the minimal size of oligosaccharide required must be either three or four.
>
> We have also found that asialo epo is more susceptible to tryptic digestion and heat inactivation than is the native form. The asialo hormone has about three times more activity, when assayed in vitro at the lower end of the dose-response curve (1-4 milliunits), than the native form. This may be due to greater ease of binding of asialo epo to the epo-responsive cell receptors when the negative charge on the hormone is reduced by desialation. (Citations omitted)

The skilled worker at the time of the present invention would thus have understood that if preparations of in vivo biologically active human erythropoietin were to be provided in therapeutic quantities by recombinant means, a method would have to be devised whereby (a) an appropriate array of glycosylation including sialic acid terminal residues and, possibly, penultimate galactose residues would be provided on (b) a polypeptide with requisite amino acid sequence homology to erythropoietin. Unlike other human glycoproteins such as the interferons and Interleukin-2, human erythropoietin was conspicuously known to be an obligate glycoprotein and no hope at all existed for isolating in vivo active material from recombinant host cells unless, at a minimum, both the issues of required polypeptide sequence and of required glycosylation could be successfully attended to.