UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC.,	)	
	)	
Plaintiff,	)	
	)	Civil Action No.: 05-12237 WGY
v.	)	
	)	
	)	
F. HOFFMANN-LA ROCHE LTD., a Swiss Company, ROCHE DIAGNOSTICS GmbH, a German Company and HOFFMANN-LA ROCHE INC., a New Jersey Corporation,	)	
	)	
Defendants.	)	

DECLARATION OF HARVEY F. LODISH, Ph.D. IN SUPPORT OF AMGEN INC.'S REPLY TO DEFENDANTS' CLAIM CONSTRUCTION BRIEF

Dockets.Justia.com

1. I am a Professor of Biology and Professor of Bioengineering at the Massachusetts Institute of Technology (MIT) and a Member of the Whitehead Institute for Biomedical Research.

2. I understand that Dr. Fu-Kuen Lin of Amgen has received a number of patents in recognition of his work. In this case, I understand that Amgen has asserted the following claims in some of those patents against Roche (the "Asserted Claims"), which I have categorized as follows: Asserted Product Claims ('422 claim 1, '933 claims 3, 7-9, and 12), Asserted Process Claims ('349 claim 7, '698 claims 4-9, '868 claims 1 and 2), Asserted Method of Treatment Claims ('933 claims 11 and 14), and Asserted Equivalence Claims ('080 claims 3-4 and 6).

3. I have reviewed both Amgen's and Roche's claim constructions submitted in this case, and I have considered both sides' competing claim constructions when forming my opinions.

4. I am submitting this Declaration in support of Amgen Inc.'s Reply to Defendants' Claim Construction Brief. In particular, I will address issues relating to (1) how an ordinarily skilled artisan would interpret certain portions of the Lin patent specification, and (2) the ordinarily skilled artisan's knowledge in the relevant fields of science at the time when Dr. Lin filed his application which led to the patents-in-suit. Moreover, I provide some specific comments concerning statements made in the declaration submitted by Defendants' expert Dr. Thomas Kadesch in support of Defendants' proposed claim construction.

BACKGROUND

5. A copy of my curriculum vitae, reflecting my professional experience, affiliations, and work is attached to this report as Exhibit A. The following are some highlights of my background and experience that are most relevant to this Declaration.

6. I received an A.B. degree summa cum laude from Kenyon College in 1962, and a Ph.D. from the Rockefeller University in 1966. I was a post-doctoral Fellow at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England from 1966 to 1968. I held the positions of Assistant Professor and Associate Professor at the Massachusetts Institute of Technology (MIT) during the years 1968-71 and 1971-76, respectively. Since 1976, I have been a full Professor of Biology at MIT and since 1999 Professor of Bioengineering. In 1982, I became a Founding Member of the Whitehead Institute for Biomedical Research.

7. Since 1961, I have authored or co-authored more than 500 scientific publications, in a variety of peer- reviewed scientific journals, as detailed in Exhibit A.

8. I was elected to the National Academy of Sciences in 1987. In 2004, I was President of the American Society for Cell Biology, an international organization of more than 10,000 scientists. I have also served on a variety of external advisory boards and grant review panels. A complete list is provided in Exhibit A.

9. I have served on the Editorial Boards for many peer-reviewed scientific journals. For example, I was a member of the Board of Reviewing Editors of the journal *Science* from 1991 to 1999, and a Member of the Editorial Board of the journal *Proceedings of the National Academy of Sciences* from 1996 to 2000. Furthermore, I have reviewed hundreds of articles for publication in many different journals. When I review papers for potential publication, I must consider critically whether the work is well conceived, controlled, and performed in order to

establish whether its scientific conclusions are correct. Additionally, I consider whether the work is sufficiently described such that other workers in the field can repeat, confirm, and extend the reported findings.

10. I am also the senior author of a widely-used university-level textbook entitled *Molecular Cell Biology,* now in its fifth edition.[1] The sixth edition will be published this spring. The textbook presents a comprehensive, authoritative review of the fields of molecular and cellular biology, and is intended for advanced undergraduates and graduate students. In the course of preparing my book over the past 25 years, I have regularly studied the published scientific literature in the fields of molecular and cellular biology to determine what experimental work is new, significant, and sufficiently credible to merit reliance by the scientific community at large.

11. In the course of my career as a researcher, I have personally applied the scientific method to many different avenues of research, including cell signaling, protein synthesis, protein glycosylation, cell membranes and their formation, cell death, cell differentiation, and fat cell biology. Most relevant here, I have made significant contributions in the fields of glycoprotein synthesis and function and blood cell differentiation.

12. I have been studying glycoprotein synthesis and function in mammalian cells since about 1976. My laboratory has made several significant contributions to the understanding of the glycosylation process. Prominent examples of our work include first establishing that the addition of oligosaccharides to asparagines on glycoproteins occurs during the synthesis of the polypeptide and its translocation into the endoplasmic reticulum, and characterizing the hepatocyte asialoglycoprotein receptor, a major component of the system of clearance of

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[1] Lodish, H.F. et al., Molecular Cell Biology, 5th Edition (W.H. Freeman & Co., New York

glycoproteins from the circulation. My work on these and other topics in glycoprotein synthesis and function are reported in several publications listed in Exhibit A.

13. One example of my experience in blood cell differentiation is my work concerning the cloning and characterization of the murine erythropoietin ("EPO") receptor, the protein on the surface of red blood cell precursors that binds to EPO and that mediates the activity of EPO in cells and *in vivo* (in the body). I have been involved in substantial additional research relating to EPO and its receptor as reported in several publications listed in Exhibit A.

14. During the course of prior litigation involving the patents-in-suit between Amgen and Transkaryotic Therapies and Hoechst Marion Roussel, I reviewed in detail the patents-in-suit, portions of the prosecution histories, and related scientific publications. I testified at trial in connection with that action and prepared several expert reports.

15. For my analysis and testimony, I am being compensated at my consulting rate of $800 per hour.

SCIENTIFIC TUTORIAL

16. During the *Amgen v. Hoechst Marion Roussel* matter, I prepared a brief slide presentation concerning the expression of the EPO glycoprotein. The presentation is attached as Exhibit B.

17. During the same matter, I also prepared graphics depicting Example 10 from Dr. Lin's patents. This presentation is attached as Exhibit C.

18. Moreover, I incorporate by reference the text and figures from my textbook with respect to these general tutorial concepts relating to proteins, protein expression, hormones, cell structure, DNA, mRNA, transcription, translation, post-translation modifications, glycosylation,

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(2004)).

oligosaccharides, erythropoietin ("EPO"), and EPO isoforms, which can be found at pp. 1-145, 361-71, 447-470, 533-41, 578-87, 605-07, 673-76, 904-05 of the 5th Edition.

19. If called upon, I may provide oral testimony at a hearing or at trial consistent with the attached slides and the cited pages of my textbook.

THE ERYTHROPOIETIN HORMONE AND ITS CELL-SURFACE RECEPTOR

20. EPO is a glycoprotein hormone in the body that regulates production of red blood cells. Hormones are signaling molecules produced in the body that act on target cells distant from their site of synthesis. Protein hormones typically circulate in the blood and induce specific responses only in certain target cells by specifically activating receptors present on the target cells.

21. EPO belongs to a family of related protein hormones called cytokines. Cytokines are small, secreted proteins (generally around 160 amino acids) that activate cell-surface receptors on specific cells with the appropriate receptor. Activation of the receptor then triggers the cells to differentiate, proliferate, and/or change their metabolism. Prolactin, interferons, interleukins, growth hormone, and G-CSF are other cytokines. For example, during pregnancy, prolactin induces epithelial cells in the mammary gland to differentiate into cells that can produce milk proteins. G-CSF stimulates white blood cell production by inducing certain progenitor cells to differentiate into white blood cells.

22. EPO stimulates erythrocyte (red blood cell) production through a process called erythropoiesis. Red blood cells transport oxygen throughout the body. Hemoglobin, the major protein inside of red blood cells, binds to oxygen in the lungs and carries bound oxygen through the bloodstream to the tissues where it is required. A drop in blood oxygen level indicates a

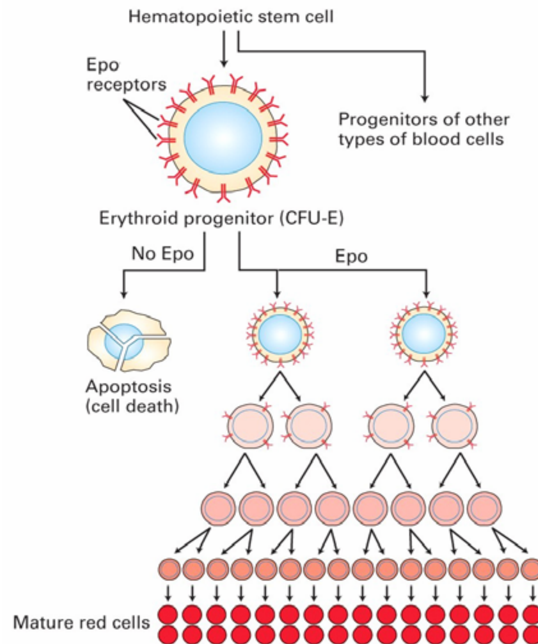
sub-optimal level of erythrocytes in the blood. When blood oxygen levels are low or in decline, certain specialized kidney cells are induced to make more EPO and secrete it into the blood. EPO, in turn, will travel though the bloodstream and bind to the specific EPO receptor on certain progenitor cells in the bone marrow (called burst forming units erythroid – BFU-E – and colony forming units erythroid – CFU-E) thereby inducing them to proliferate and differentiate into red blood cells.

23. In healthy humans, the amount of EPO in circulation in the bloodstream is exquisitely regulated to produce just the required numbers of red cells. Maintaining proper levels of red blood cell production is essential to maintaining appropriate oxygen levels in tissues throughout the body. Inadequate red blood cell production can lead to anemia and death. Levels of red cells that are too high, a state called polycythemia, can lead to blood clots and death.

24. Once secreted from the kidney cells, the EPO circulates through the blood stream on its way to the target erythroid progenitor cells in the bone marrow. While circulating through the blood stream, EPO is exposed to attack by proteases and other components found in the blood that can degrade the EPO molecule. EPO in the blood stream is also subject to clearance from the body via the kidney or degradation in certain liver cells. EPO is also internalized and degraded when bound to the EPO receptor on its target cells. Which of these (or other) mechanisms represents the dominant route of EPO clearance in the body is unknown.[2] It is clear, however, that the EPO glycoprotein has evolved over time to have a structure that not only protects it from degradation and clearance before it discharges its function, but also allows it to specifically bind only to the specific EPO receptors on the surface of erythroid progenitor cells.

[2] Gross, A. and Lodish, H., "Cellular Trafficking and Degradation of Erythropoietin and Novel Erythropoiesis Stimulating (NESP)" J. Biol. Chem. 281:2024-32 ("How Epo is cleared from the circulation and degraded in the body is not understood.").

25. When EPO binds to the EPO receptors on the target erythroid progenitor cells, it initiates a signal transduction process that ultimately causes the progenitor cells to survive, proliferate and differentiate into mature red blood cells. Figure 14-7 from my textbook, *Molecular Cell Biology*, depicts this process:



26. A glycoprotein hormone like EPO cannot function effectively in the body unless it possesses the appropriate three-dimensional structure (also known as "conformation") required to survive its passage through the bloodstream to the target erythroid progenitor cells in the bone marrow, and then bind and activate the EPO receptor on those cells. The amino acid sequence of a protein, and, in addition, post-translational modifications (such as glycosylation) determine the three-dimensional structure of a protein like EPO. The amino acid sequence (or primary structure) dictates the secondary structure (localized folding of parts of the polypeptide chain), and tertiary structure (long-range folding of the polypeptide), as well as the sites of post-translational modification of the protein. However, as discussed below, the particular post-translational modifications (such as oligosaccharide structures) that are imparted to the

polypeptide are determined by the host cell. Based upon a protein's sequence of amino acid residues, many noncovalent bonds between the amino acids and with their surrounding medium will further determine the protein's conformation. These include ionic interactions that are based on electrical charge, hydrogen bonds, and hydrophobic interactions. Additionally, in many secreted proteins such as EPO, covalent bonds between two cysteine residues, termed disulfide bonds, are important for stabilizing the protein's conformation. EPO has two such disulfide bonds in its normal conformation.

27. Human EPO has a primary structure of 165 amino acids linked to one another in a unique and specific order. The amino acid sequence for human EPO is depicted at positions +1 through +165 in Figure 6 of Amgen's Patents. EPO's two disulfide bonds (bridges) link the sulfur atom on the cysteine residue at position 7 with the sulfur atom on the cysteine residue at position 161, and the sulfur atom on cysteine residue 29 with the sulfur atom on cysteine residue 33 in the polypeptide sequence. EPO has a secondary and tertiary structure (four long conserved alpha helices folded together in a particular arrangement) that is very similar to that of other cytokines. This structural homology suggests that cytokines evolved from a common ancestral protein.

28. Long after Lin's inventions, and as a direct result of having the EPO DNA and protein products his inventions made possible, the three-dimensional structure of a human EPO analog was determined using x-ray crystallography and nuclear magnetic resonance ("NMR"). (Syed et al., "Efficiency of signaling through cytokine receptors depends critically on receptor orientation." *Nature* 395:511-516 (1998); Cheetham et al., "NMR structure of human erythropoietin and a comparison with its receptor bound conformation," *Nat. Struct. Biol.* 5:861-66).

AN ORDINARILY SKILLED ARTISAN'S UNDERSTANDING OF "HUMAN ERYTHROPOIETIN"

29. I agree with the interpretation of the term "human erythropoietin" in Dr. Lin's patent specification to mean a protein having the amino acid sequence of human EPO, such as the amino acid sequence of EPO isolated from human urine. I do not believe that "human erythropoietin" requires that the EPO be glycosylated, or have the same structure as would be manufactured by mammalian cells as of 1984.[3] These additional requirements are not supported by the analysis of Lin's patent specification or the knowledge in the art as of 1984.

30. First, I do not agree that the term "human erythropoietin" necessarily requires that the human erythropoietin protein be glycosylated. First, Lin's patent specification clearly states the "polypeptides of the invention," in other words, erythropoietin, may be "non-glycosylated": "Depending upon the host employed, ***polypeptides of the invention*** may be glycosylated with mammalian or other eucaryotic carbohydrates or ***may be non-glycosylated***."[4] The various examples of the Lin patent further confirm that Lin contemplated non-glycosylated human erythropoietin. For example, Examples 11 and 12 of the patent are directed, among other things, at the expression of human erythropoietin (comprising the exact 165 amino acid sequence of human EPO set forth in Lin's Figure 6) in bacterial cells of the species *Escherichia coli*.[5] It was well-known before 1983 that prokaryotic cells like *E. coli* are incapable of adding carbohydrates to proteins.[6] Thus, the Lin patent specification makes clear that human erythropoietin can be

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[3] *See* Defendants' Opening Memorandum in Support of Their Proposed Claim Construction at 1 (Docket No. 311).

[4] Amgen Brief, Appendix B, '933 Patent at 10:28-33 (Docket No. 312) (emphasis added).

[5] *Id.* at columns 29-32.

[6] *See, e.g.*, Lodish, H.F., "Post-translational modification of proteins," *Enzyme Microb Technol.*, 3(3):177-188 (1981) (attached as Exhibit D).

glycosylated or unglycosylated. The addition of carbohydrates to the 165-amino-acid polypeptide backbone via the formation of covalent bonds at the point of attachment creates a ***glycosylated*** erythropoietin polypeptide. But both glycosylated forms and the unglycosylated form are human erythropoietin polypeptides. Specifically, in an N-glycosylated protein, the amide $-NH_2$ of asparagine is chemically modified in that a hydrogen atom is replaced by a sugar, but nonetheless the amino acid's identity as asparagine is unchanged. Likewise, the identity of the polypeptide — here, human erythropoietin — is also unchanged by the addition of an oligosaccharide.

31. Second, I do not agree that the Lin patent specification or the knowledge in the art as of 1984 would require that "human erythropoietin" have a "structure that would be produced in mammalian cells as of the invention date." Again, Lin's patent examples directed towards production of human erythropoietin from non-mammalian cells make it plain that Roche's interpretation is incorrect. In addition to *E. coli* expression, Examples 11 and 12 also disclose production of human erythropoietin in the baker's yeast, *Saccharomyces cerevisiae*. It was well known in 1983 that while *S. cerevisiae* cells are capable of glycosylation, the particular glycans that they impart to polypeptides are considerably different than those imparted by mammalian cells.[7] I illustrated precisely this phenomenon in a review article I published in 1981:

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[7] *See id.*

[9] Amgen Brief, Appendix B at 10:28-33 (Docket No. 312) (emphasis added).

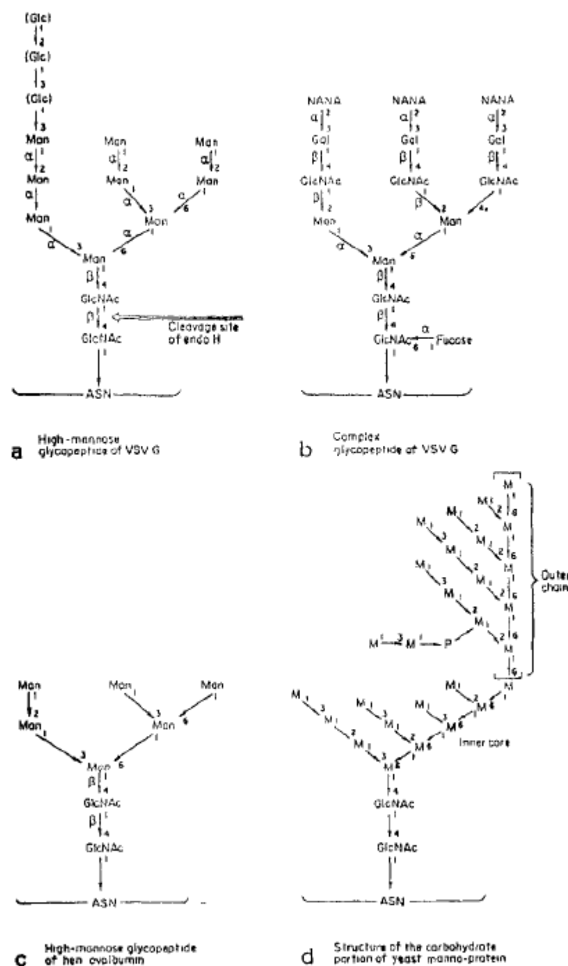

Figure 10 Structures of asparagine-linked oligosaccharides. (a) Structure of the two high mannose carbohydrate chains found on the microsomal form of VSV G protein (drawn from refs 76 and 15); including the site of cleavage by endoglycosidase H. (b) Structure of the asparagine-linked oligosaccharide found on the VSV glycoprotein.[57] (c) Structure of one of the related high-mannose glycopeptides of hen ovalbumin.[80] (d) Structure of the carbohydrate portion of yeast mannoprotein[99]

As shown in Figure 10 of my review article, the N-linked oligosaccharides of vertebrate cells (panels a-c) are significantly different than those found in yeast proteins (panel d).

32. In addition to differences in glycosylation state, the patent specification also contemplates human erythropoietin with other differences in structure as compared to the "structure that would be produced in mammalian cells as of the invention date." In particular, the patent specification states that human erythropoietin can include a methionine residue linked to the amino-terminus of human erythropoietin by an amide bond: "Polypeptides of the

invention may also include an initial methionine amino acid residue (at position -1)."[9] In the context of Example 11, the patent specification further states: "FIGS. 10 through 15 and 7 illustrate the design and assembly of a manufactured gene encoding a human EPO translation product lacking any leader or presequence but including an initial methionine residue at position -1." This passage in particular makes plain that according to Lin (as well as the common understanding at the time), that even when an additional molecule, here methionine, is added to the polypeptide sequence of human EPO, it is still a "human EPO." Again, like glycosylation, if a bond to a hydrogen atom from the nitrogen atom in the amino group of Ala_{+1} is replaced by an amide bond to a methionine, this does not change the identity of that amino acid as alanine, nor does it change the identity of the polypeptide as human erythropoietin.

33. Example 11 describes construction of a synthetic gene for human erythropoietin that has been optimized for production in *E. coli* by, among other things, the replacement of the codons for the normal 23 amino acid signal sequence[10] found in the native EPO gene with a methionine codon. Upon expression in *E. coli*, this would result in the synthesis of a human erythropoietin with an addition methionine amino acid at position –1. I set forth below a comparison of the chemical structures between the human EPO produced by mammalian cells (such as the CHO cells of Lin's Example 10) and the human EPO produced by *E. coli* cells as described in Examples 11 and 12, which has an additional amide bond between Ala+1 and Met-1 which is not present in human EPO produced by mammalian cells:

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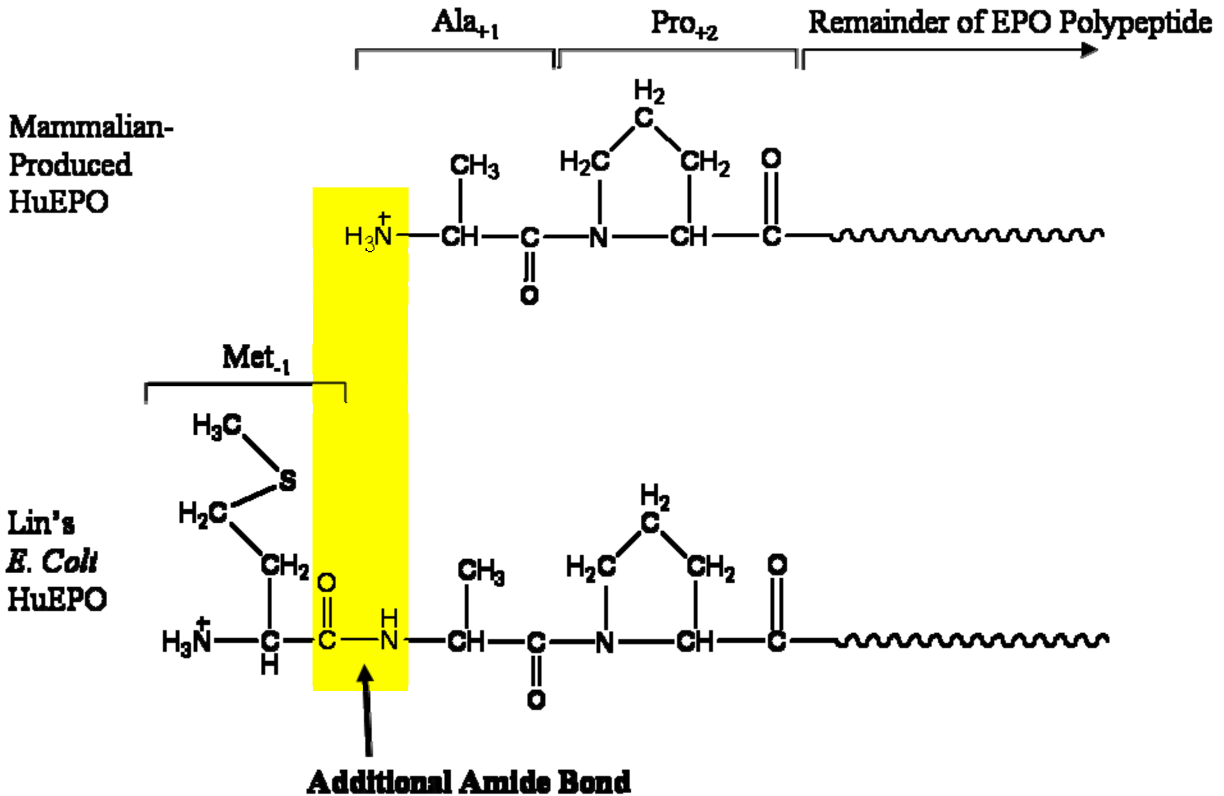
[10] In mammalian cells, the signal sequence is cleaved from the nascent erythropoietin polypeptide in the endoplasmic reticulum. Prokaryotes like *E. coli* often lack the ability to cleave off a signal sequence from a mammalian protein.



34. I note that Dr. Kadesch addresses the structure of amino acids and the amide bonds in polypeptides in his declaration at paragraphs 19-22. I must make a few corrections and clarifications with respect to Dr. Kadesch's descriptions. First, Dr. Kadesch incorrectly represents the terminal amino group of amino acids as the structure $-NH_2$ and the terminal carboxylate group as COOH. In fact, the terminal amino group is comprised of a mixture of the structures $-NH_2$ and $-NH_3^+$, where the terminal nitrogen atom of the polypeptide chain is covalently linked to either two or three hydrogen atoms. In a solution of amino acids (or the EPO protein), these two structures are present in equilibrium, meaning that the forms can freely exchange by the addition or subtraction of a proton (a hydrogen atom with a positive charge, "H^+") from the medium. The average proportion of the $-NH_2$ and $-NH_3^+$ species is dependant on the pH of the medium in which the EPO is suspended. The same equilibrium situation exists for the carboxylate group on amino acids, which are a mixture of COOH and COO^-. Contrary to Dr.

Kadesch's depiction, under physiological conditions (pH of about 7), the amino acids predominantly exist in a form with $-NH_3^+$ as the amino group and COO^- as the carboxylate group.

35. This equilibrium phenomenon is well-illustrated in Lubert Stryer's 1981 Biochemistry textbook (excerpted in Kadesch's Exh. 3), however, Dr. Kadesch does not include these illustrations and descriptions (the entire Chapter 2 from the Stryer textbook is attached to this declaration as Exhibit E). For example, Stryer's Figure 2-6 illustrates the ionization states of a free amino group of an amino acid,[11] and a carboxylate group on the other end of the amino acid:[12]

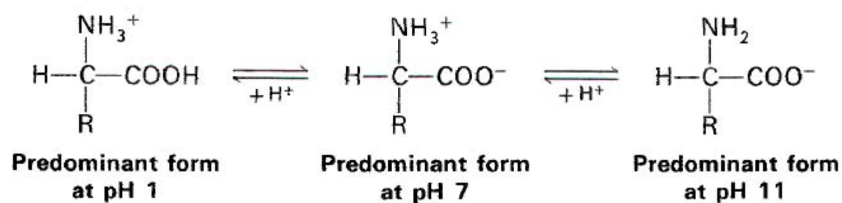


Figure 2-6
Ionization states of an amino acid as a function of pH.

36. More importantly, Dr. Kadesch's depiction of the chemical reaction by which an amide bond is formed also omits the third hydrogen and the positive charge found on a free amino group at physiological pH, as shown in Stryer's Figure 2-17:

[11] The structure of the amino group on a single amino acid is equivalent to the free amino group on the amino-terminus of a polypeptide.

[12] The structure of the carboxylate group on a single amino acid is equivalent to the free carboxylate group on the carboxy-terminus of a polypeptide.

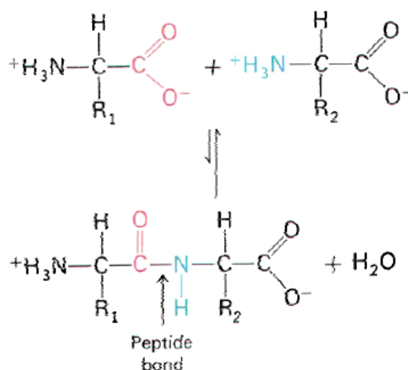


Figure 2-17
Formation of a peptide bond.

Compare Stryer's figures to Dr. Kadesch's figure at page 7 of his report. Dr. Kadesch improperly depicts the amino group in the uncharged, NH_2 state, and the carboxylate group in its uncharged COOH state, when in fact, as shown by Stryer, under the physiological conditions that this reaction occurs in cells, the amino group is predominantly in the charged, $-NH_3^+$ state, and the carboxylate group is predominantly in the charged COO^- state. In any event, it is abundantly clear that both the $-NH_2$ or $-NH_3^+$ species of EPO are both "human erythropoietin."

HOW ERYTHROPOIETIN IS GLYCOSYLATED IN VERTEBRATE AND MAMMALIAN CELLS

37. Human EPO found in the body is a glycoprotein. A "glycoprotein" is a protein to which one or more sugar residues (carbohydrates) have been added. A carbohydrate is a covalently bonded combination of carbon and water in a one-to-one ratio $(CH_2O)_n$, where n equals 3, 4, 5, 6, or 7.[13] In 1983, as well as today, this was the commonly understood meaning of the term glycoprotein. The following quotations from the contemporaneous literature

[13] Molecular Cell Biology, 5th Ed. at 41.

establish that the term "glycoprotein" was well known in the art in 1983, and had a very consistent usage:

- A "glycoprotein" is a "[p]olypeptide to which sugar residues are attached." Watson, J.D., MOLECULAR BIOLOGY OF THE GENE, 3rd Ed., (W. A. Benjamin, Inc., Menlo Park, 1976).

- "Much of the current interest in carbohydrates is focused on such substances as glycoproteins and glycolipids, complex carbohydrates in which sugars are linked respectively to proteins and lipids. Sharon, N. "Carbohydrates" SCIENTIFIC AMERICAN 243:90-116 (1980) at 90.

- "Only within the last two decades has it been realized that glycoproteins — that is, proteins with covalently bound sugars — are ubiquitous in nature and are found in all living organisms, with the possible exception of bacteria." Sharon, N. and Lis, H. "Glycoproteins: research booming on long-ignored, ubiquitous compounds" C & EN 21-44 at 21 (1981).

- "A very large number of proteins, subsequent to the intracellular formation by the largely understood processes of transcription and translation of information in the genome, undergo glycosylation at one or more points along the polypeptide chain. The products formed are called glycoproteins, and these have a wide range of diverse physical, chemical and biological properties depending upon the nature of the sugars present in the carbohydrate prosthetic groups, upon the general distribution of the carbohydrate moieties along the polypeptide chain and on the amount of carbohydrate in the macromolecule." Marshall, R.D., "Some Observations on Why Many Proteins are Glycosylated" Biochem. Soc. Trans. 7:800-805 (1979).

- "Glycoproteins (*Chem.*) Glucoproteins." "Glucoproteins (*Chem.*) Compounds formed by a protein with a substance containing a carbohydrate group other than a nucleic acid, e.g. mucin." CHAMBERS TECHNICAL DICTIONARY, 3rd Ed., (The Macmillan Co. New York 1961).

- "Important extracellular structural materials (such as cellulose) are composed of simple polysaccharides, and smaller but more complex, nonrepeating sequence of sugar molecules are often covalently linked to proteins in *glycoproteins* and to lipids in *glycolipids.*" Alberts *et al.*, MOLECULAR BIOLOGY OF THE CELL, (Garland Publishing Inc. New York 1983) at 51.

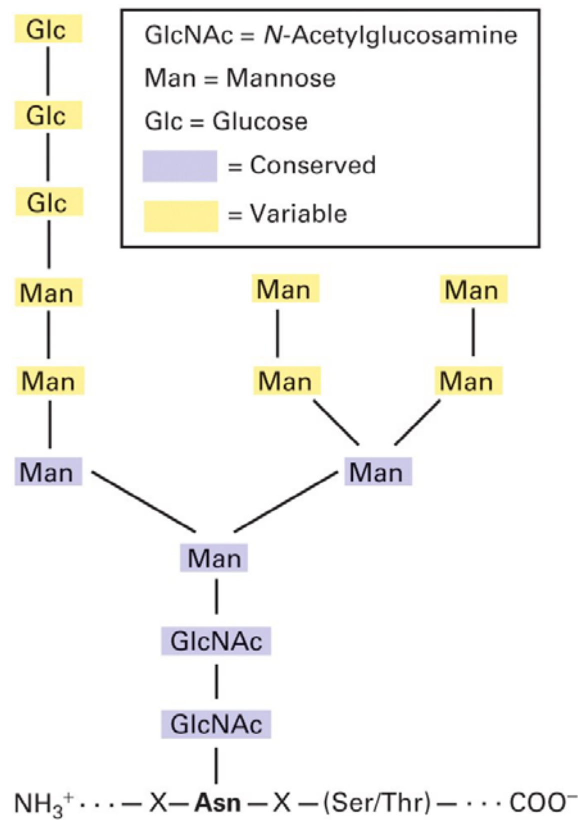
- My own 1986 textbook provided the following definitions: "Glycoproteins are Composed of Proteins Covalently Bound to Sugars. Many important membrane proteins and serum proteins contain carbohydrate chains; these are termed *glycoproteins.*" Darnell *et al.*, MOLECULAR CELL BIOLOGY (Scientific American Books 1986) at 97. "Oligosaccharide chains are often found attached to proteins or lipids, in which cases the modified molecules are called *glycoproteins* or *glycolipids.*" Id. at 94.

38. Glycosylation occurs in many eukaryotic cells, including vertebrates and specifically mammals. Different phylogenetic groups, however, impart different specific oligosaccharide structures to proteins. In the following paragraphs, I will describe in general the process by which carbohydrates are added to proteins. While my description is focused on glycosylation processes in mammalian cells, these processes are conserved to some extent across all eukaryotic organisms. A much more detailed overview of the cellular processes for building oligosaccharide chains of glycoproteins is set forth in my textbook, *Molecular Cell Biology*, Fifth Edition at pp. 673-75, which I incorporate by reference.

39. There are two principal classes of oligosaccharides (chains of sugar residues) on glycoproteins: *N*- and *O*-linked oligosaccharides. *N*-linked and *O*-linked oligosaccharides are added to the polypeptide backbone of proteins by somewhat different mechanisms. As I discuss below, *N*-linked (or "asparagine-linked") oligosaccharides are joined to polypeptides concurrent with their synthesis, then later modified, whereas *O*-linked oligosaccharides may be added to completed polypeptides.

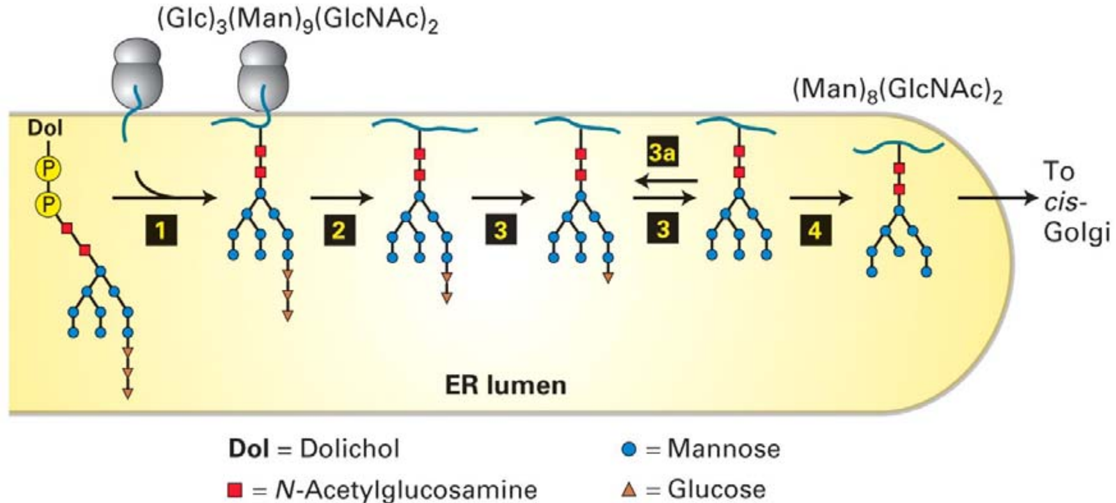
40. In brief, *N*-glycosylation occurs in mammalian cells as follows. First, a 14-sugar precursor oligosaccharide is fabricated by a set of cellular enzymes. The precursor is covalently linked to an asparagine amino acid in the nascent polypeptide chain as it is being synthesized.

Once the precursor is joined to the nascent polypeptide it has the following structure, as set forth in Fig. 16-16 of my textbook:



41. Work in my own laboratory, in part done in collaboration with Professors Gunter Blobel of the Rockefeller University and David Baltimore of MIT, demonstrated that, in *N*-linked glycosylation, the 14 sugar precursor is transferred en bloc to an asparagine residue on a protein while it is being fabricated on a ribosome. Rothman, J.E., and Lodish, H.F., "Synchronised transmembrane insertion and glycosylation of a nascent membrane protein," *Nature* 269:775-80 (1977). This occurs in the cellular subcompartment called the rough endoplasmic reticulum and is catalyzed by enzymes present in all eucaryotic cells. Only asparagine residues in the tripeptide sequences Asn-X-Ser and Asn-X-Thr (where X is any amino acid except proline) in polypeptides that are transiting into the endoplasmic reticulum are

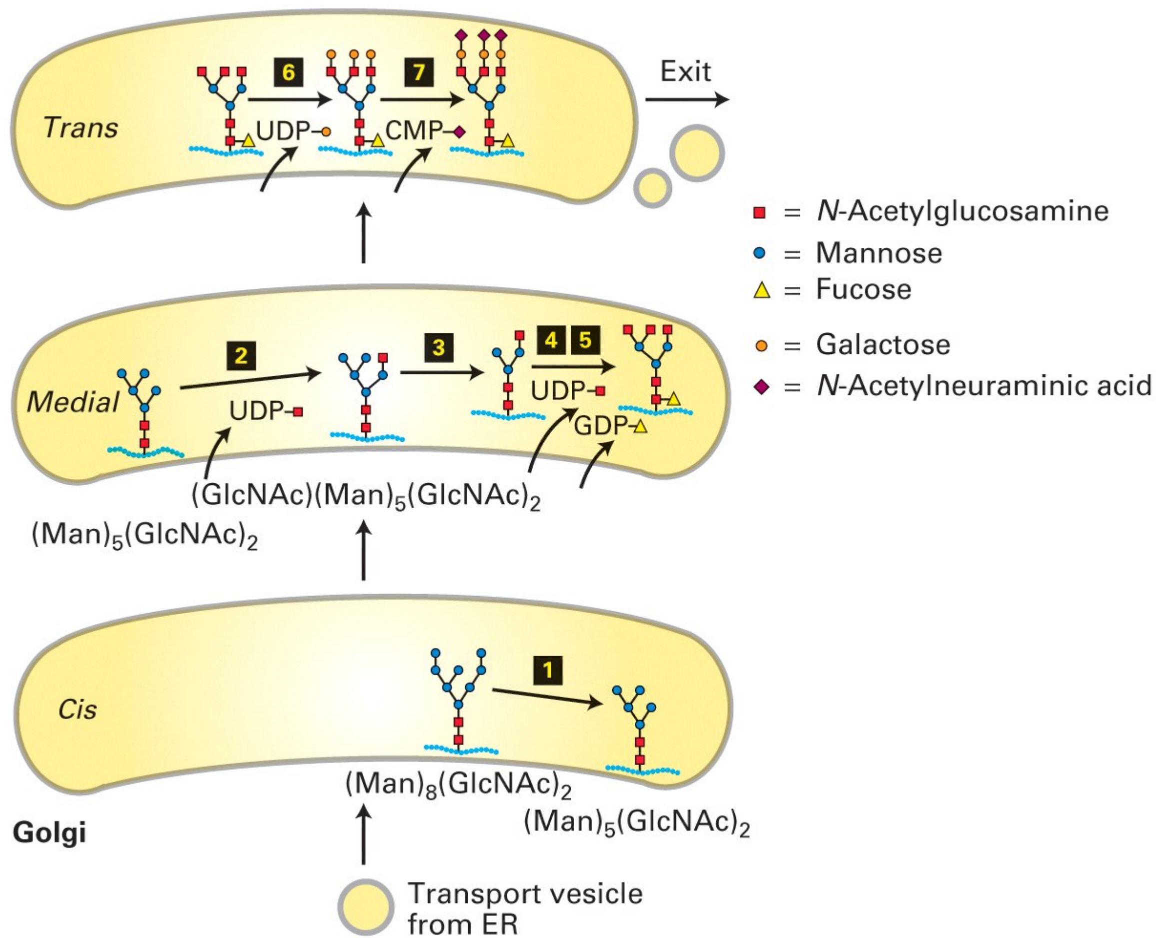
substrates for this transferase and thus acquire a carbohydrate chain. I illustrated this process in step 1 of Figure 16-18 of my textbook:



42. I must note that Dr. Kadesch's report contains a grievous error concerning the initial synthesis steps of glycosylation. In paragraph 14, Dr. Kadesch erroneously states that "Within the Golgi apparatus, proteins destined for secretion are modified so that certain specific amino acids in the protein are linked to carbohydrates or sugars made by the cell." In fact, as illustrated just above, the linkage of oligosaccharides to asparagine residues occurs in the rough endoplasmic reticulum, not the Golgi apparatus, but Dr. Kadesch's report contains no mention of the rough endoplasmic reticulum and its central role in glycosylation.

43. Once this sugar chain becomes chemically linked to an asparagine residue, it undergoes extensive chemical modifications. Certain sugars are removed and others may be added, reactions catalyzed by cellular enzymes. The protein then moves to a different cellular subcompartment, the Golgi complex, where these sugar chains undergo further extensive modifications in reactions catalyzed by other sets of enzymes. I illustrated the modifications

made in the endoplasmic reticulum in Figure 16-18 (shown above, steps 2, 3, and 4), and the modifications made in the Golgi apparatus in Figure 17-3 of my textbook:[14]



44. As I stated above, different types of cells contain different sets of such carbohydrate modifying enzymes. Yeast cells, for example, add the same 14-sugar oligosaccharide precursor to asparagine residues in proteins as do mammalian cells, but these oligosaccharide chains are subsequently modified very differently than in mammalian cells.

[14] The general process of glycosylation as set forth in my textbook was known in the early eighties. *See* Figure 3, Kornfeld R. and Kornfeld S., "Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem. 54:631-64 (1985), which is very similar to my two textbook images.

Even different proteins secreted from the same cell, and the same protein secreted from different cells in the same mammalian organism can have different oligosaccharide structures.

45. The EPO polypeptide contains four potential glycosylation sites – three N-linked glycosylation sites and one O-linked glycosylation site. These carbohydrate chains comprise many different structures and contain different sugars that can be attached to each other in a variety of different ways. For example, at each of the N-linked glycosylation sites, the oligosaccharide can have two branches ("bi-antennary"), three branches ("tri-antennary"), or four branches ("tetra-antennary").

46. For EPO, as for most other glycoproteins, there is considerable heterogeneity in the carbohydrate structures at any given glycosylation site. The heterogeneity takes multiple forms: for example, the number of branches (or "antennae") in each chain, which as noted above can be 2, 3, or 4, and the number and nature of the carbohydrate residues that make up the branches. In another form of heterogeneity, a negatively charged carbohydrate residue, sialic acid[15] may or may not be present at the end of each branch. The maximum total number of sialic acid residues that may be present on the carbohydrate chains on each EPO molecule is 14 (*i.e.*, one sialic acid attached to the end of each of the four branches of each of the three N-linked chains (a total of 12), and one sialic acid residue attached to each of the two branches of the single O-linked chain (a total of two). There are also additional chemical modifications that can be made to the monosaccharide subunits of oligosaccharides, such as sulfation.

47. The different forms of EPO having different overall carbohydrate structures are called "isoforms" or "glycoforms." EPO produced by a single cell typically consists of a heterogeneous mixture of different isoforms, which all share the same amino acid backbone, but

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[15] Sialic acid is a generic name for a large family of sugars. In humans most of the sialic acid

differ in their glycosylation patterns. Because each EPO isoform can have different numbers of sialic acid residues (from 0 to 14) and sulfates they also differ in electric charge.

48. Isoforms of EPO that are more highly sialylated (i.e., those that have more tri- and tetra-antennary structures in which most of the branches end in sialic acid) exhibit a longer half-life in the body, lower binding affinity to the EPO receptor, and greater biological activity *in vivo* than do isoforms of EPO that are less sialylated.

49. Despite the fact that these isoforms of EPO are structurally distinct molecules with different molecular weights, different binding affinities to the EPO receptor, different half-lives in the body, and different degrees of biological activity, scientists in the fields of molecular biology and biochemistry today (as well as in 1983-84) consider each of these different molecules to be human erythropoietin because each shares the same polypeptide "backbone" comprised of the same amino acid sequence, contains the same two disulfide bonds, folds into the same three-dimensional shape, and has the same functional interaction with the erythropoietin receptor.

OTHER COMMENTS ON DR. KADESCH'S DECLARATION

50. I have found a number of other statements in Dr. Kadesch's report that are inaccurate or misleading. While by no means an exhaustive critique, I note three significant errors in Dr. Kadesch's report.

51. In paragraph number 15 of Dr. Kadesch's report, he incorrectly states that "[e]very cell within a single organism has the same DNA." While most cells within any particular organism have the same DNA, there are significant exceptions. For example, in

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attached to oligosaccharides is N-acetylneuraminic acid.

immune cells of mammals, the genes for immunoglobulins (antibodies) are rearranged into many different structures, which differ from cell to cell within the immune system and from all the other cells of the organism.

52. In describing the process of "protein synthesis" (see paragraphs 24-34), Dr. Kadesch omits the important step of mRNA splicing. Like almost all mammalian mRNAs, the EPO mRNA is produced as a precursor comprised of both exons and introns, and then is spliced into its final form in the nucleus before it is transported to the cytoplasm and translated by ribosomes on the rough endoplasmic reticulum.

53. In paragraph number 28 of Dr. Kadesch's report, he incorrectly states that serine is encoded by four codons. In fact, serine is encoded by six codons.[16]

FURTHER TESTIMONY

54. If requested by the Court, I may provide oral testimony at a hearing or at trial consistent with the statements made in this Declaration.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Executed this 19th day of March, 2007 at Cambridge, Massachusetts.

/s/ Harvey F. Lodish, Ph.D.

HARVEY F. LODISH, PH.D.

[16] Molecular Cell Biology, 5th Edition at 120.

Index of Exhibits to Lodish Expert Report

Exhibit A	Curriculum Vitae
Exhibit B	Trial Exhibit A, 97-10814-WGY
Exhibit C	Graphics Depicting Example 10 from Amgen's Patents
Exhibit D	Lodish, H.F., "Post-translational modification of proteins," *Enzyme Microb Technol.*, 3(3):177-188 (1981)
Exhibit E	L. Stryer, Biochemistry 2nd Ed. (W.H. Freeman & Co., New York (1981)), Chapter 2