EXHIBIT Z

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

IN RE COLUMBIA UNIVERSITY PATENT LITIGATION	No. 04-MD-01592

REBUTTAL EXPERT REPORT OF HARVEY F. LODISH, Ph.D.
September 17, 2004

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I. Introduction

1. I submit this report in response to the expert report of Francis H. Ruddle, Ph.D., submitted on behalf of the Trustees of Columbia University in the City of New York, dated August 27, 2004 ("Ruddle Report"). My qualifications, compensation, and prior opinions are set forth in my initial expert report.

2. In connection with preparing this report, I have relied upon the materials cited in my initial expert report (listed at Tab B thereof), the Ruddle Report and its supporting materials, and the materials listed at Tab A to this report.

3. I reserve the right to supplement or amend my opinions in response to opinions expressed by Columbia's experts, or in light of any additional evidence, testimony, or other information, including any claim construction that the Court may make, that may be provided to me after the date of this report.

4. I may rely on visual aids and demonstrative exhibits that demonstrate the bases of my opinions. Examples of these visual aids and demonstrative exhibits may include excerpts from the specification, patent claims, and file histories, as well as charts, diagrams, videos, and animated or computer-generated video presentations describing the technology relevant to the asserted claims, the prior art, and my opinions.

II. The Level of Ordinary Skill

5. Professor Ruddle asserts that the "level of ordinary skill in the art was quite low as of February 1980." Ruddle Report at p. 5. However, Professor Ruddle does not appear to have considered the teachings of the prior claims in making that assessment.

6. Even without the teachings of the prior claims, Professor Ruddle understates the level of ordinary skill in the art at the time of the invention, despite noting

that a "person of ordinary skill in the art would … have had multiple years of laboratory experience in recombinant DNA technology." Ruddle Report at p. 4. Indeed, the people of ordinary skill at the time were generally working in one of a handful of sophisticated laboratories around the world under the supervision of leading scientists in the field.

III. The Axel Disclosure

A. The Specification's References to Glycosylation and Stable Incorporation

7. Professor Ruddle focuses on two aspects of claims 3, 5-14, and 16-19 of the '275 patent in comparison with the earlier claims: the recitation that the transformed cell produce a glycoprotein, and the requirement that DNA II be stably incorporated into chromosomal DNA. Professor Ruddle asserts that neither of the two would have been obvious to a person skilled in the art at the time of the invention. He even asserts that none of the claims of the earlier patents "reports on whether the cell is competent to transcribe and translate DNA I." Ruddle Report at p. 17.

8. The '275 specification itself does not provide any explicit guidance regarding either the production of glycoproteins or the stable incorporation of DNA II. Thus, I presume that the inventors, in seeking the claims of the '275 patent, assumed that a person of ordinary skill in the art would not need explicit guidance to practice the claims of the '275 patent without undue experimentation.

9. As to the production of glycoproteins, the specification states:

> Still another aspect of the present invention involves the preparation of materials normally produced within eucaryotic cells in minute amounts such as glycoproteins including interferon, which are in part protein but additionally include other chemical species such as sugars, ribonucleic acids, histones and the like. Although the method or methods by which cells synthesize complicated cellular materials such as the glycoproteins are poorly understood, it is anticipated that by using the process of the present invention it will be possible to synthesize such materials in

> commercially useful quantities. Specifically, ***it is anticipated that after inserting a gene or genes for the protein portion of a cellular material such as a glycoprotein, which includes a non-protein portion, into a eucaryotic cell of the type, which normally produces such material, the cell will not only produce the corresponding proteinaceous material but will utilize already existing cellular mechanisms to process the proteinaceous materials, if and to the extent necessary, and will also add the appropriate non-proteinaceous material to form the complete, biologically active material***. Thus, for example, the complete biologically active glycoprotein, interferon, could be prepared by first synthesizing interferon protein in the manner described and additionally permitting the cell to produce the non-proteinaceous or sugar portion of interferon and to synthesize or assemble true interferon therefrom. The interferon so prepared could then be recovered using conventional techniques.

(Col. 7, lines 31-58; emphasis added). These statements reflect the understanding of persons skilled in the art at the time of the invention. Thus, the inventors themselves considered it obvious to expect that a cell transformed with a gene that encoded a glycoprotein would produce a glycoprotein.

10. The specification communicates even less concerning the stable incorporation of either DNA I or DNA II into the chromosomal DNA of the transformed cell. The inventors apparently assumed that one of ordinary skill in the art practicing, for example, claim 54 of the '216 patent and claim 1 of the '017 patent, would generate and select transformed cells where both DNA I and DNA II were stably incorporated into chromosomal DNA.

B. The Experiments

11. None of the experiments reported in the Axel patents discloses a transformed cell where DNA I has been successfully translated into a protein or glycoprotein. None of the experiments demonstrated the production of any recombinant foreign protein encoded by DNA I by the transformed cells. There is no discussion of any structure, composition, or sequence of oligosaccharides that might be attached to any

natural or recombinant protein. Furthermore, there is no disclosure of what specific culture conditions, if any, would be required to achieve such a result.

12. None of the experiments expressly addresses the stable incorporation of amplified DNA I or DNA II into the chromosomal DNA of the transformed cell. Furthermore, none of the experiments explicitly discusses how to generate transformed cells that contain stably incorporated DNA I without the concomitant stable incorporation of DNA II or vice versa.

IV. Glycosylation Claims of the Axel Patents

A. Claims of the '216 and the '017 Patents

13. **Transcription and translation.** Professor Ruddle declares that none of the claims in the '216 patent "reports on" whether the cell is "competent to transcribe and translate DNA I" or whether "the transcription and translation would be proper." Ruddle Report at p. 17. Similarly, he asserts that "Claims 1 – 4 (of the '017 patent) do not report on whether the cell is competent to transcribe and translate DNA I." Ruddle Report at p. 18. Claim 5 of the '017 patent claims production of proteinaceous material using the cell of claim 1. Prof. Ruddle asserts that even this claim "does not report on whether DNA I is accurately transcribed and translated." *Id.*

14. One of skill in the art would find the claims of the '275 patent that explicitly recite the production of proteins obvious in view of the prior art. As I emphasized in my initial expert report, at the time the original Axel patents were filed, a great deal was known concerning regulatory sequences that could cause transcription of a foreign DNA. Lodish Report at ¶72. For example, it was known by February 25, 1980 that one could cause the globin gene, the ovalbumin gene, or another foreign gene encoding a protein to be translated and/or transcribed in a cultured mammalian cell by

including the appropriate regulatory sequences, such as a promoter, that would instruct the RNA synthesis machinery present in the cultured cell to copy the foreign DNA into RNA. Mulligan, R.C, *et al.,* "Synthesis of Rabbit β-globin in Cultured Monkey Kidney Cells Following Infection with a SV40 β-globin Recombinant Genome," *Nature* 277:108-114 (1979); Hamer, D. H. and Leder, P., "Expression of the Chromosomal Mouse β^{maj}-globin Gene Cloned in SV40," *Nature* 281:35-40 (1979); Mantei, N., *et al.*, "Rabbit ß-globin mRNA Production in Mouse L Cells Transformed with Cloned Rabbit Beta-globin Chromosomal DNA," *Nature* 281(5726):40-46 (1979); Mantei, N., *et al.,* "Synthesis of Rabbit ß-Globin-Specific RNA in Mouse L Cells and Yeast Transformed with Cloned Rabbit Chromosomal ß-Globin DNA," in *Eucaryotic Gene Regulation* (Axel *et al.* eds.), Academic Press, Inc., pp. 477-499 (1979); Lai, E., *et al.,* "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Nat'l. Acad. Sci. U.S.A.* 77(1):244-248 (1980).

15. These results attest to the fact that the processes of DNA transcription, RNA splicing, and messenger RNA translation function in substantially the same way in all mammalian cells. An investigator of ordinary skill in the art at the time of the filing of the original application would have a reasonable expectation of producing desired foreign vertebrate proteins, including glycoproteins, in a variety of types of cultured mammalian cells including CHO cells.

16. **Glycoproteins.** Claim 3 of the '017 patent teaches that the transformed CHO cell may comprise a DNA I that encodes one of several types of specific glycoproteins – interferons, clotting factors, and antibodies. Claims 56, 59, and 60 of the '216 patent also teach that the transformed eucaryotic cell may comprise a DNA I

molecule that encodes these same glycoproteins. As discussed in this report and in paragraphs 139-142 of my initial report, a person of ordinary skill in the art would have a reasonable expectation that eucaryotic cells would perform post-translational modification of these glycoproteins when they were encoded by the DNA I recited in these claims. The claims of the '275 patent that recite glycoproteins generally are obvious in view of the recital of particular glycoproteins in the earlier claims.

B. The '275 Patent's Glycoprotein Claims

17. A glycoprotein is simply a protein that has at least one sugar residue attached to it. Watson, J.D., *Molecular Biology of the Gene*, 3rd Ed., W. A. Benjamin, Inc., Menlo Park (1976); Spiro, R.G., "Glycoproteins," in *Advances in Protein Chemistry* Vol. 27:349-467 (Anfinsen, C.B. *et al.*, ed. 1973). Professor Ruddle reads limitations into claims 4, 15, and 16-19 of the '275 patent that, simply put, do not exist. None of these claims requires any specific type, composition, or sequence of sugars attached to any amino acid on a protein. None of these claims requires that the glycoprotein be functional or therapeutically useful following administration to humans or animals. None of these claims requires that the protein be glycosylated in the same manner as in the donor species or that the cells reproduce any specific pattern of glycosylation. None requires any particular post-translational modification. All that is required is that the stated protein be a glycoprotein, *i.e.*, that it have at least one sugar attached to it.

18. Professor Ruddle nowhere asserts that one of ordinary skill in the art would have expected *no* glycosylation to occur in a eucaryotic cell, including a CHO cell, transformed with a gene encoding a glycoprotein. His opinions appear to be premised on improperly reading specific structures, compositions, and sequences of glycosylation into the claims.

19. In claim 16 of the '275 patent, DNA I corresponds to a "gene encoding a glycoprotein of interest." Col. 42, lines 10-18. The specification does not define the phrase "glycoprotein of interest." The phrase does not appear anywhere in the specification and is only used in claims 16-19 of the '275 patent. I understand the phrase to mean simply a glycoprotein that one is interested in for any purpose, including basic research.

V. Post-Translational Modifications

20. Even though none of the claims of the '275 patent requires that the protein be "properly" modified following translation, the state of the art was sufficiently advanced as of February 1980 that a person of ordinary skill in the art would have expected that a eucaryotic cell would perform post-translational modifications to a protein produced according to the claims of the original Axel patents.

A. Signal Sequences

21. Professor Ruddle suggests (at p. 10) that a person of ordinary skill in the art "would have been very concerned whether the host cell possessed the appropriate mechanism to process the foreign polypeptide's signaling structures." He further asserts that "[u]nfortunately there was not a lot known about how differences in the signaling structures affected what a cell thought it was supposed to do." Ruddle Report at pp. 9-10.

22. I am puzzled by these comments in view of the state of the art at the time of the filing of the initial application in February 1980. As I discussed in my prior report at ¶73, signal sequences comprising ~20 amino acids are found at the beginning of all proteins secreted by mammalian cells (and, in fact, by all eucaryotic cells). These sequences differ slightly from one secreted protein to another, but all of them direct the protein, as it is being made on the ribosome, into the endoplasmic reticulum. It was also

well known at this time that the initial steps in production of certain membrane proteins were identical to those for secreted proteins; such membrane proteins also have signal sequences comprising ~20 amino acids that direct the newly made protein into the endoplasmic reticulum. For both secreted and membrane proteins, cellular enzymes cleave the signal sequence from the growing polypeptide. All this was well-known before February 1980. Devillers-Thiery, A., *et al.*, "Homology in Amino-terminal Sequence of Precursors to Pancreatic Secretory Proteins," *Proc. Nat'l. Acad. Sci. U.S.A.* 72(12):5016-5020 (1975); Rothman, J.E. and Lodish, H.F., "Synchronized Transmembrane Insertion and Glycosylation of a Nascent Membrane Protein," *Nature* 269: 775-780 (1977); Rothman, J.E., *et al.*, "Glycosylation of a Membrane Protein is Restricted to the Growing Polypeptide Chain but is not Necessary for Insertion as a Transmembrane Protein," *Cell* 15:1447-1454 (1978); Lingappa, V.R., *et al.*, "A Signal Sequence for the Insertion of a Transmembrane Glycoprotein: Similarities to the Signals of Secretory Proteins in Primary Structure and Function," *J. Biol. Chem.* 253:8667-8670 (1978); Katz, F.N. and Lodish, H.F. "Transmembrane Biogenesis of the Vesicular Stomatitis Virus Glycoprotein," *J. Cell Biol.* 80: 416-426 (1979).

23. There is no mention of signal sequences either in the common specification of the Axel patents or in the claims of the earlier Axel patents. However, claim 5 of the '017 patent explicitly teaches the production and recovery of proteinaceous materials, a term that includes secreted proteins, by transformed CHO cells. The term "proteinaceous material" must include secreted proteins because claim 3 of the '017 patent, which depends from claim 1, recites that the proteinaceous material may be one of a number of specified secreted proteins, including interferon, insulin, a growth hormone,

a clotting factor, an antibody, or an enzyme. On reading these claims, one skilled in the art at the time of the invention would have expected that the necessary signal sequences were present and that these would function in transformed CHO cells.

B. The Biosynthesis of Glycoproteins

24. Professor Ruddle questions what was known about the general mechanisms of protein glycosylation at the time of filing of the original Axel patents. In fact, a great deal was known about protein glycosylation at the time. Nevertheless, scientists would not have needed to understand all aspects of glycosylation to have a reasonable expectation of success in attempting to practice the claims of the '275 patent upon following the teachings of any one claim of the earlier Axel patents.

25. As of February 25, 1980, scientists were aware that the same basic processes of protein glycosylation occurred in all eucaryotic cells. A nascent glycoprotein may undergo *N*-linked or *O*-linked glycosylation, or both. *N*-linked glycosylation is by far the most widespread and important type of protein glycosylation in mammals. All eucaryotic cells, including yeasts, plants, and animals, produce the same carbohydrate precursor of *N*-linked oligosaccharides.

26. As discussed in paragraph 80 of my initial report, the gene determines the amino acid sequence of a protein, and it is the amino acid sequence that dictates the glycosylation pattern of a glycoprotein. For example, asparagine residues in the tripeptide sequences Asn-X-Ser and Asn-X-Thr (where X is any amino acid except proline) acquire a carbohydrate chain. Marshall, R., "Some Observations on Why Many Proteins are Glycosylated," *Biochemical Society Transactions* 7(4):800-805 (1979).

27. 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, a defined chain of 14 sugars is transferred *en bloc* to an asparagine residue on a protein while it is being fabricated on a ribosome. This occurs in the cellular subcompartment called the rough endoplasmic reticulum and is catalyzed by enzymes present in all eucaryotic cells. The protein then moves to a different cellular subcompartment, the Golgi complex, where these sugar chains undergo extensive modifications in reactions catalyzed by other sets of enzymes. Knipe, D.M., *et al.*, "Localization of Two Cellular Forms of the Vesicular Stomatitis Virus Glycoprotein," *J. Virol.* 21:1121-1139 (1977); Wirth, D.F., *et al.*, "How a Single Sindbis Virus mRNA Directs the Synthesis of One Soluble Protein and Two Integral Membrane Glycoproteins," *Cell* 10:253-263 (1977); Katz, F.N., *et al.*, "Membrane Assembly *In Vitro*: Synthesis, Glycosylation and Asymmetric Insertion of a Transmembrane Protein," *Proc. Nat'l. Acad. Sci. USA* 74:3278-3282 (1977); Katz, F.N., *et al.*, "Membrane Assembly: Synthesis and Intracellular Processing of the Vesicular Stomatitis Virus Glycoprotein," *J. Supramolec. Struct.* 7: 353-370 (1977); Rothman, J.E. and Lodish, H.F., "Synchronized Transmembrane Insertion and Glycosylation of a Nascent Membrane Protein," *Nature* 269:775-780 (1977); Rothman, J.E., *et al.*, "Glycosylation of a Membrane Protein is Restricted to the Growing Polypeptide Chain but is not Necessary for Insertion as a Transmembrane Protein," *Cell* 15:1447-1454 (1978); Lingappa, V.R., *et al.*, "A Signal Sequence for the Insertion of a Transmembrane Glycoprotein: Similarities to the Signals of Secretory Proteins in Primary Structure and Function," *J. Biol. Chem.* 253:8667-8670 (1978).

28. These processes occur in substantially the same manner in all eucaryotic cells, and this was generally understood at the time the original Axel patents were filed.

Thus there was and is a reasonable expectation that expression of a foreign gene encoding a glycoprotein with *N*-linked oligosaccharides in any eucaryotic cell will result in attachment of sugars to that protein.

29. Professor Ruddle's remark that "it was only after February 1980 that scientific journals began publishing papers establishing that a host cell has properly glycosylated a foreign polypeptide" is immaterial. Ruddle Report at p. 16. As discussed above in paragraph 17, none of the claims of the '275 patent recites a particular or "proper" pattern of glycosylation or requires that the glycoprotein be functional or therapeutically useful. Furthermore, Professor Ruddle's observation does not contradict my opinion that a person skilled in the art at the time of the invention would have found it obvious to use CHO cells as host cells, and would have expected the transformed CHO cells to attach sugars to the nascent protein.

C. Intermediates in Synthesis and Processing of Glycoproteins Are Also Glycoproteins.

30. As discussed above in paragraph 17, a glycoprotein is simply a protein that has at least one sugar attached to it. Thus, all of the cellular intermediates in the generation of the ultimate glycoprotein – which in the case of secreted proteins is the entity secreted from the cell – are themselves glycoproteins since they contain at least one attached sugar, although they may not all be functional or therapeutically useful.

31. As I discussed in my initial report (paragraphs 77 – 82) and reviewed in more detail below, the mode of synthesis of *N*-linked oligosaccharides by cells involves many intermediate steps. Similarly, *O*-glycosylation, the process of adding sugar chains to the hydroxyl group of serine and threonine residues of a protein, involves many

cellular enzymes and many intermediate forms of the sugar chain(s) that are attached to any protein. All of these intermediates are glycoproteins.

32. Regardless of their functionality and therapeutic benefit, all such intermediates in *N*- and *O*-glycosylation would be encompassed by the claims of the '275 patent, including claim 19, since a cell that produces a glycoprotein of interest would necessarily "comprise" these glycoproteins that are intermediates in generation of the final glycoprotein. This is because claim 19 of the '275 patent embraces functional, nonfunctional, therapeutically useful, and therapeutically useless glycoproteins.

33. Many intermediates in the modification of the sugar chains attached to glycoproteins had been identified by 1980, and mutant CHO and other cells had been isolated in which several of these processing events did not occur, giving scientists knowledge of specific cellular mechanisms of glycosylation. Furthermore, many of the enzymes that catalyze these modifications had already been identified. Beyer, T.A., *et al.*, "Biosynthesis of Mammalian Glycoproteins," *J. Biol. Chem.* 254(24):12531-12541 (1979); Kornfeld, R. *et al.*, "Comparative Aspects of Glycoprotein Structure," in *Annual Review of Biochemistry* Vol. 45, 217-237 (Snell *et al.* eds. 1976); Gottlieb, C., *et al.*, "Deficient Uridine Diphosphate-*N*-acetylglucosamine: Glycoprotein *N*-Acetylglucosaminyltransferase Activity in a Clone of Chinese Hamster Ovary Cells with Altered Surface Glycoproteins," *J. Biol. Chem.* 250(9):3303-3309 (1975); Gottlieb, C. *et al.*, "Isolation of a Cloned of Chinese Hamster Ovary Cells Deficient in Plant Lectin-Binding Sites," *Proc. Nat'l. Acad. Sci. U.S.A.* 71(4):1078-1082 (1974). Thus the mechanism of protein glycosylation in mammalian cells, and CHO cells in particular, was not so poorly understood as Professor Ruddle suggests.

34. In fact, investigators used CHO cells to study the mechanisms of glycosylation prior to February 25, 1980. For instance, Stuart Kornfeld's laboratory infected CHO cells with vesicular stomatitis virus and other viruses in order to characterize the glycoprotein biosynthesis pathway. Schlesinger, S., *et al.*, "Growth of Enveloped RNA Viruses in a Line of Chinese Hamster Ovary Cells with Deficient *N*-acetylglucosaminyltransferase activity," *J. Virol.* 17(1):239-46 (1975); Tabas, I., *et al.*, "Processing of High Mannose Oligosaccharides to Form Complex Type Oligosaccharides on the Newly Synthesized Polypeptides of the Vesicular Stomatitis Virus G Protein and the IgG Heavy Chain," *J. Biol. Chem.* 253:716-722 (1978); Li, E., and Kornfeld, S., "Biosynthesis of Lipid-linked Oligosaccharides. Isolation and Structure of a Second Lipid-linked Oligosaccharide in Chinese Hamster Ovary Cells," *J. Biol. Chem.* 254(8):2754-8 (1979).

D. Heterogeneity of Carbohydrate Chains Attached to Proteins

35. Professor Ruddle suggests (at p. 14) that, as of 1980, a researcher "would not have considered there to be a reasonable likelihood of success that a given host cell would be able to reproduce a glycosylation pattern for a foreign glycoprotein." As already discussed above in paragraph 17, none of the claims of the '275 patent requires that the recombinant glycoprotein possess any specific "glycosylation pattern." Indeed, as I discuss in more detail below, the carbohydrate chains that are attached to any one type of protein made in any one type of cell are heterogeneous. Even today, a researcher would not expect a host cell to reproducibly generate recombinant proteins with identical sugar structures. Thus, the problem Professor Ruddle identifies is illusory.

36. As discussed above in paragraph 26, genes encode proteins with defined sequences of amino acids. However, the sugar chains that become attached to the same

residues of a particular protein made will vary. This heterogeneity occurs because the cellular machinery that adds and removes sugars from proteins sometimes will not modify all of the proteins of a particular type being produced. In part this occurs because certain enzymes or certain sugars or other molecules needed for these reactions may be present in limiting amounts. This statement applies both to glycoproteins naturally produced in the body and to recombinant glycoproteins produced in cultured cells such as CHO cells.

37. There are two broad types of heterogeneity of the carbohydrate chains that are attached to proteins. First, for any given protein, a particular amino acid may or may not have a carbohydrate chain attached to it. In some cases this difference has little or no effect on the function of the protein. An example of heterogeneity having no effect on function, well known before January 1980, is ribonucleases A and B. These are similar RNA-degrading enzymes that are produced and secreted by the human pancreas that digest RNA in foods. Ribonucleases A and B contain the identical sequence of amino acids and are encoded by the same gene. Ribonuclease B is also identical in protein conformation to ribonuclease A. The only difference is that in ribonuclease B, asparagine residue 34, has attached to it an oligosaccharide chain that contains mannose and *N*-acetylglucosamine residues; the corresponding asparagine in ribonuclease A has no attached sugars. Both proteins are functional, thus establishing that the presence or absence of a sugar chain on a particular amino acid of a protein may have little or no effect. Plummer, Jr., T. and Hirs, C., "The Isolation of Ribonuclease B, a Glycoprotein, from Bovine Pancreatic Juice," *J. Biol. Chem.* 238:1396–1401 (1963); Plummer, Jr., T.,

"Glycoproteins of Bovine Pancreatic Juice. Isolation of Ribonucleases C and D," *J. Biol. Chem.* 243:5961–5988 (1968).

38. The second type of glycosylation heterogeneity concerns differences in the composition, sequence, and structure of the sugar chain that is attached to a particular amino acid of a protein. Kornfeld, R., *et al.*, "Comparative Aspects of Glycoprotein Structure," in *Annual Review of Biochemistry*, Vol. 45, 217-237 (Snell *et al.* eds. 1976). Even when a cell attaches a chain of sugars to a particular amino acid, the structure of the attached oligosaccharide may vary. For example, ribonucleases may have one of at least three different kinds of oligosaccharide chains attached to the asparagine side chain at position 34 of the protein. Baynes, J. W., *et al.*, "Effect of Glycosylation on the *In Vivo* Circulating Half-life of Ribonuclease," *J. Biol. Chem.* 251(19):6016-6024 (1976); Plummer, Jr., T. and Hirs, C., "The Isolation of Ribonuclease B, a Glycoprotein, from Bovine Pancreatic Juice," *J. Biol. Chem.* 238:1396–1401 (1963); Plummer, Jr., T., "Glycoproteins of Bovine Pancreatic Juice. Isolation of Ribonucleases C and D," *J. Biol. Chem.* 243:5961–5988 (1968); Clamp, J.R., *et al.*, "Heterogeneity of Glycopeptides from a Homogeneous Immunoglobulin," *Biochem. J.*, 100:35c-36c (1966).

39. Erythropoietin (Epo) provides another example of this second type of heterogeneity. The *N*-linked carbohydrate chains attached at positions 24, 38, and 83 are heterogeneous with respect to sugar composition and structure. Sasaki, H., *et al.*, "Carbohydrate Structures of Erythropoietin Expressed in Chinese Hamster Ovary Cells by a Human Erythropoietin cDNA," *J. Biol. Chem.* 262:12059–12076 (1987); Sasaki, H., *et al.*, "Site Specific Glycosylation of Recombinant Human Erythropoietin," *Biochemistry* 27:8618–8626 (1988). As a consequence, different molecules of Epo will

have different numbers of attached sialic acid residues. Egrie, J. and Browne, J., "Development and Characterization of Novel Erythropoiesis Stimulating Protein (NESP)," *Nephrol. Dial. Transplant* 16 [suppl]:3–13 (2001); Takeuchi, M., *et al.*, "Relationship Between Sugar Chain Structure and Biological Activity of Recombinant Human Erythropoietin Produced in Chinese Hamster Ovary Cells," *Proc. Nat'l. Acad. Sci. U.S.A.* 86:7819– 822 (1989). Thus, heterogeneity of protein glycosylation occurs naturally; in most if not all glycoproteins, no single structure, composition, or sequence of sugar chains is found attached to any one amino acid.

40. Further, the glycosylation of recombinant human Epo produced by CHO cells differs from normal human urinary Epo. Sasaki, H., *et al.*, "Carbohydrate Structures of Erythropoietin Expressed in Chinese Hamster Ovary Cells by a Human Erythropoietin cDNA," *J. Biol. Chem.* 262:12059–12076 (1987); Takeuchi, M., *et al.*, "Comparative Study of the Asparagine-linked Sugar Chains of Human Erythropoietins Purified from Urine and the Culture Medium of Recombinant Chinese Hamster Ovary Cells," *J. Biol. Chem.* 263(8):3657-3663 (1988). The differences in carbohydrate chains attached to recombinant and normal Epo are exploited in the forensic determination of whether or not an individual has received recombinant human Epo. This is the basis of the urine analyses for Epo "doping" used in the recent Olympic games. Lasne, F. and de Ceaurriz, J., "Recombinant Erythropoietin in Urine," *Nature* 405:635 (2000).

41. Human beta interferon (IFN-β) provides yet another example of heterogeneity in the carbohydrate chains that are attached to a glycoprotein. Mammalian cells transformed with the IFN-β gene will produce glycosylated recombinant IFN-β. However, the particular distribution of the attached carbohydrates is variable depending

on the host cell used. While the oligosaccharide structure of the IFN-β produced in CHO cells is similar to that of the native protein, the glycosylation pattern of IFN-β produced in mouse epithelial cells and human lung adenocarcinoma cells contains structurally different oligosaccharide chains. Kagawa, Y., *et al.*, "Comparative Study of the Asparagine-linked Sugar Chains of Natural Human Interferon-β1 Produced by Three Different Mammalian Cells," *J. Biol. Chem.* 263(33):17508-15515 (1988). Indeed, even within a single host cell type, such as the CHO cell, the precise distribution of oligosaccharides may vary. *Id.*; Conradt, H.S., *et al.*, "Structure of the Carbohydrate Moiety of Human Interferon-β Secreted by a Recombinant Chinese Hamster Ovary Cell Line," *J. Biol. Chem.* 262(30):14600-14605 (1987). Nonetheless, all of these IFN-β molecules are glycoproteins.

42. Thus, Professor Ruddle has greatly oversimplified the issues in implying (at p. 14) that there is one "proper" type of glycosylation for any particular recombinant protein. In contrast to what Prof. Ruddle suggests, CHO cells do not "reproduce a glycosylation pattern for a foreign glycoprotein," as shown by the examples above. Further, there is nothing in the claims of the '275 patent that requires any particular structure or composition or sequence of sugar chains attached to a protein. Whether an oligosaccharide is attached to a particular amino acid or modified in one way or another by a particular cell, the resultant proteins are glycoproteins and thus fall within the glycoprotein claims of the '275 patent.

E. Genetic Causes for Differences in Oligosaccharide Structures

43. As noted above, modifications to the oligosaccharide chains that are initially attached to a protein involve many intermediate steps and many cellular enzymes. One way the experimentalist can dissect such a type of complex cellular

process is to isolate mutant cells that are specifically blocked in their ability to carry out one of these steps. By understanding the nature of the block that a particular mutation induces in a cell pathway, scientists can infer the normal function of the gene in which the mutation occurred.

44. As Professor Ruddle notes in his expert report, researchers before 1980 had published descriptions of mutant cells in which the oligosaccharide chains attached to cell proteins had a slightly different structure and composition than those in non-mutant cells. Ruddle Report at pp. 11-12. These studies were part of a broad effort to elucidate each of the steps and each of the cellular enzymes involved in modifications of oligosaccharide chains attached to glycoproteins. There was no intent to use these mutant CHO (and other) cell lines for production of recombinant glycoproteins. One skilled in the art at the time the initial Axel patents were filed would know that it was inadvisable to use these mutant CHO cells for production of recombinant glycoproteins because he or she would know that these would result in production of glycoproteins that bore altered oligosaccharide chains. I note, however, that because all of the recombinant glycoproteins produced in mutant cells, such as those cited in Prof. Ruddle's report, would still have attached carbohydrate chains, they would still be glycoproteins and would still be encompassed by the claims of the '275 patent.

F. Effects of Culturing Conditions on Glycoprotein Production

45. In his expert report, Professor Ruddle states that the conditions of culture affect "the ability of a cell to perform a particular function" and questions whether this function "would be retained, modified, or lost in the course of continuous culture." Ruddle Report at p. 12 (*citation omitted*). Though not explicitly stated, I gather that this comment is meant to imply that culture conditions can somehow affect protein

glycosylation. It was widely known at the time the original Axel patent application was filed that culture conditions can affect many aspects of cell growth and metabolism. *See*, *e.g.*, Ceccarini, C., *et al.*, "Induction and Reversal of Contact Inhibition by pH Modification," *Nature New Biology* 233(43):271-273 (1971); Hsie, A., *et al.*, "Morphological Transformation of Chinese Hamster Ovary Cells by Dibutyryl Adenosine cyclic 3':5'-monophosphate and Testosterone," *Proc. Nat'l. Acad. Sci. U.S.A.* 68(2):358-361 (1971); Dulbecco, R., "Topoinhibition and Serum Requirement of Transformed and Untransformed Cells," *Nature* 227(5260):802-806 (1970); Holley, R.W., *et al.*, "'Contact inhibition' of Cell Division of 3T3 Cells," *Proc. Nat'l. Acad. Sci. U.S.A.* 60(1):300-304 (1968). One skilled in the art at the time the initial Axel application was filed would know to use appropriate conditions for culturing the cells. In addition, this is taught by claim 5 of the '017 patent, among others, which teaches a method of obtaining and recovering a protein (which would include a glycoprotein) by culturing transformed CHO cells under suitable conditions. This is discussed in my initial report at ¶142.

46. However, even if the transformed cells expressing a recombinant glycoprotein were cultured under sub-optimal conditions, it would be expected that the cell would still attach at least some sugar residues to the protein. Thus the desired protein would still be a glycoprotein. If culture conditions were so sub-optimal that the desired protein was not glycosylated, it is likely that the nonglycosylated protein would be misfolded and not secreted. One of ordinary skill in the art would know not to use such culture conditions for glycoprotein production.

G. Effects on Glycosylation Caused by Expression of Amplified Numbers of a Gene Encoding a Glycoprotein

47. On pages 20 and 21 of his expert report Prof. Ruddle refers to "squelching"; *i.e.*, he raises the possibility that "the volume of foreign polypeptides encoded by all of the amplified copies.... would overwhelm the processing apparatus of the cell and prevent the host cell from producing the glycoprotein of interest." Prof. Ruddle's observations on this score are irrelevant to the claims of the '275 patent.

48. The theoretical possibility that squelching might occur would not have deterred an experimenter from employing any particular line of cultured cells, such as CHO cells, as a host for recombinant DNA transformation and protein expression.

49. None of the claims of the '275 patent specifies that the transformed cells produce any particular amount of a glycoprotein nor, as I emphasized above, do the claims require that the resultant recombinant protein have attached oligosaccharide chains of any particular structure, composition, or sequence. Even if squelching occurred and less than optimal amounts of a particular recombinant glycoprotein were made, the glycoproteins that would be produced would still fall within the claims of the '275 patent.

VI. Stable Incorporation

A. Professor Ruddle's Assertions Concerning Gene Amplification, Stability, and Linkage

50. Professor Ruddle states that "claims 3, 5–14, and 16-19 of the '275 patent require that amplified DNA I and amplified DNA II are both stably incorporated into the chromosomal DNA of the host cell," while none of the claims of the original Axel patents expressly recites the stable incorporation of DNA II into chromosomal DNA. Ruddle Report at p. 21. He further states that "stable incorporation of DNA II would not have

been obvious in light of any of the claims of the Axel patents." Ruddle Report at pp. 21-22.

51. I reiterate that, as I pointed out in my first Expert Report, it would have been obvious to one skilled in the art at the time of filing of the original Axel patents to use only cells in which DNA II was stably incorporated. Lodish Report ¶¶133-138.

52. Both Prof. Ruddle and I pointed out in our initial reports that it was known that in some cells, the amplified genes could be located on extrachromosomal elements termed "double minute chromosomes." Ruddle Report at pp. 23-24; Lodish Report at ¶133. It was also known that during cell division these genes would not be equally distributed among the daughter cells and thus that some cells would lose these amplified genes. In cells that stably incorporated the DNA into the chromosome, on the other hand, the amplified genes were divided equally between the two daughter cells during cell division. Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Nat'l. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979).

53. A person of ordinary skill in the art at the time of the invention would have known that the selection pressure recited in claim 54 of the '216 patent ("culturing the transformed eucaryotic cells in the presence of successively elevated concentrations of an agent permitting survival or identification of eucaryotic cells which have acquired multiple copies of said amplifiable gene") would permit the isolation of cells that have stably integrated the foreign DNA into their genome. Thus, it would be obvious to one of ordinary skill in the art to use only those cells in which the amplified DNAs are stably inserted into chromosomal DNA.

54. Professor Ruddle further asserts that the mechanism of gene amplification was unknown in 1980 and that many questions about the mechanism of DNA amplification remain even today. Ruddle Report at pp. 22-23. There is nothing in the claims or specification of the Axel patent that requires genes in cells to become amplified by any particular mechanism. Nor does an investigator need to know the cellular mechanism by which these DNAs become amplified in order to practice the claims.

55. Professor Ruddle comments that in 1980, one researcher raised the possibility that "[g]ene amplification might have occurred as a consequence of reverse transcription of mRNA into DNA." Ruddle Report, p. 27. However, the original Axel claims teach that this is not the case. As noted earlier in this report, the '216, '665, and '017 patents teach that selection for cells with amplified DNA II encoding a selectable marker can result in cells with amplified DNA I.

56. Were DNA II amplified by reverse transcription, there would be no reasonable likelihood that cells selected for amplified DNA II would also contain amplified DNA I. Regardless of whether DNAs I and II are linked or unlinked in the cell, the mRNA encoded by DNA II would be a separate molecule from the mRNA encoded by DNA I. As a consequence, were DNA II to be amplified by reverse transcription of a mRNA encoded by DNA II, this amplified DNA would not contain any sequences from DNA I. In other words, amplification of DNA II would not result in amplification of DNA I. Professor Ruddle's assertion that "it would be highly likely that DNA I would not be linked to DNA II after amplification," is irrelevant, since the claims of the original Axel patents teach co-amplification of DNA I and DNA II.

VII. Chinese Hamster Ovary Cells.

57. Professor Ruddle states that "if a person of ordinary skill wanted to attempt to practice claim 19, and have a reasonable probability of success, he or she would not have wanted to use a cell type that had not been proven consistently effective in the system – such as a CHO cell." Ruddle Report at p. 29. However, claims 1 – 5 of the '017 patent teach the use of recombinant CHO cells for production of recombinant proteins, including recombinant glycoproteins. Accordingly, these claims would teach one skilled in the art to use CHO cells to produce recombinant proteins. Furthermore, given the teachings of the claims from the '216, '017, and '665 patents that any eucaryotic cell may be used to practice the inventions claimed, it would have been obvious to a person of ordinary skill to utilize CHO cells if he or she wanted to attempt to practice the claims of the '275 patent. *See* Srinivasan, P.R., *et al.*, "Transfer of the Dihydrofolate Reductase Gene into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," in *Introduction of Macromolecules into Viable Mammalian Cells* 27-45 (Baserga, R. *et al.*, eds. 1980); Lewis, W.H., *et al.*, "Parameters Governing the Transfer of Genes for Thymidine Kinase and Dihydrofolate Reductase into Mouse Cells Using Metaphase Chromosomes or DNA," Som. Cell Genet. 6(3):333-347 (1980).

VIII. General Applicability of the Teachings of the '216 and '665 Patents

58. I find Professor Ruddle's statement (at p. 29) that "a person of ordinary skill in the art would thus have had serious doubts about the general applicability of the transformation systems disclosed in the '216 and '665 patents" puzzling. The claims and the teachings of the prior patents are very general and are applicable to many types of cultured cells. Basic cellular processes such as amplification, replication, transcription, and splicing function in substantially the same way in all mammalian cells, as do the

processes of translation and, as discussed above, protein glycosylation. The processes of transformation and coamplification of foreign DNAs have proven useful in many types of cultured cells besides CHO cells.

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