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For: Merged Reexaminations of U.S. Patent No. 6,331,415 (Cabilly *et al.*)

SECOND DECLARATION OF DR. STEVEN L. McKNIGHT UNDER 37 C.F.R. § 1.132

I, Dr. Steven L. McKnight, do hereby declare and state:

1. I am a citizen of the United States, and reside in Dallas, Texas. I previously provided a declaration in this reexamination proceeding. The circumstances of my involvement in this case remain the same as I had described them in my earlier declaration.

2. I analyzed the Final Action and the references discussed in the Final Action.[1] My opinions about the references and the Final Action are provided in this declaration.

3. The opinions I provide in this declaration are what I believe would have been the views of a person of ordinary skill in the art as of early April 1983. I believe I can accurately describe that person's perspective. In early April 1983, I was actively experimenting in the area of recombinant DNA technology, including cloning and expressing recombinant eukaryotic genes. By that time, I had worked with both bacterial and mammalian expression systems, and had extensive experience using the *Xenopus* oocyte microinjection technique.

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[1] U.S. Patent No. 4,399,216 (the Axel patent); Ochi *et al.*, *Nature* 302:340-42 (1983) (Ochi); Oi *et al.*, *Proc. Nat'l Acad. Sci. (USA)* 80:825-29 (1983) (Oi); Rice & Baltimore, *Proc. Nat'l Acad. Sci. (USA)* 79:7862-65 (1982) (Rice); Deacon & Ebringer, *Biochemical Society Transactions* 4:818-20 (1976) (Deacon); Valle et al., *Nature* 291:338-340 (1981) (Valle 1981); U.S. Patent No. 5,840,545 (Moore) ; EP004722 (Kaplan); U.S. Patent No. 4,511,502 (Builder); Accolla et al., *Proc. Nat'l Acad. Sci. (USA)* 77(1):563-566 (1980) (Accolla); PCT Patent Publication No. WO 82/03088 (Dallas); and claims 1-7 of U.S. Patent No. 4,816,567 (the '567 patent).

The Patented Invention and the State of the Art in April 1983

4. The ’415 patent requires the production of an immunoglobulin molecule or immunologically functional fragment by expression of DNA sequences encoding both heavy and light immunoglobulin chain polypeptides in a single transformed host cell. This means that all of the following things must happen:

   (i) host cells must have been successfully transformed with DNA sequences encoding the heavy and the light chain polypeptide sequences;

   (ii) the transformed host cell must independently express both sequences (*e.g.*, each DNA sequence must be accurately transcribed into an mRNA, and each mRNA must be translated into an appropriate amino acid sequence corresponding to each chain); and

   (iii) the polypeptides must be assembled into an immunoglobulin tetramer or antigen binding fragment either inside or outside of the cell.

5. None of the references cited by the Office (or any other publications of which I was aware in April 1983) describe or suggest performing an experiment comparable in complexity to what is required by the ’415 patent. In fact, I was not aware of a single paper published by April 1983 that even suggested the concept of producing more than one eukaryotic polypeptide at a time in a single recombinantly transformed host cell.

6. Where experimental results are reported in these references, the results show a significant amount of unpredictability. Experimental results would have been important to a person of ordinary skill in the art in April 1983 because many of the biological mechanisms that controlled expression of foreign DNA and assembly of proteins were not well understood at that time. As Dr. Harris observed in his article, “it is clear that not all the rules governing the expression of cloned genes have been elaborated and those rules that do exist are still largely empirical.”[2]

7. In my opinion, the publications and patents cited in the Final Action would not have led me (or any other person of ordinary skill in the art) in April 1983 to believe that what was required by the ’415 patent could be predictably achieved. Each of the cited references discloses something far less complicated than what the ’415 patent requires, and those that report results show significant unpredictability in achieving success in these simpler experiments. In addition, none of the references provide any answers to the questions that these references would have raised in the mind of a person of ordinary skill in the art in April 1983 about making an immunoglobulin molecule or fragment by producing the heavy and light chain polypeptides together in one transformed host cell.

8. Considering these scientific observations in aggregate, I believe these references would have told a person of ordinary skill in the art in April 1983 to <u>not</u> attempt to produce an immunoglobulin molecule by expressing two different DNA sequences encoding the heavy and light chains in one transformed host cell. Instead, I believe the references suggested

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[2] Harris, *Genetic Engineering* 4: 127-85, at p. 129 (1983).

taking the opposite approach, namely, to produce each chain in a separate cell culture and then (if that succeeds) attempt to assemble the immunoglobulin using these individually produced chains. Trying to produce the immunoglobulin this way would reduce some of the uncertainty by breaking the process down into more manageable steps. Only this approach would have been consistent with the prevailing mindset in April 1983 of producing only one eukaryotic polypeptide at a time in a transformed host cell.

9. This mindset is shown in Dr. Harris' paper, which listed all of the published reports of production of eukaryotic proteins using recombinant DNA techniques in bacterial host cells as of March 1983. Every example, without exception, reports production of only one polypeptide at a time in a transformed host cell.

10. This mindset is also shown by the approach people had taken to produce insulin. Insulin was the only multimeric protein that had been produced using recombinant DNA techniques before April 1983. Insulin is a relatively simple multimeric[3] protein made up of two polypeptide chains linked by two inter-chain disulfide bonds (and containing one intra-chain disulfide bond). Each of the insulin polypeptides is small (*i.e.*, 21 and 30 amino acid residues). An immunoglobulin molecule is a much larger and more complicated protein than insulin. It is made up of two heterodimers and has many inter- and intra-chain disulfide bonds. Each immunoglobulin chain is also substantially larger than either chain of insulin (*i.e.*, light chains have between 210 to 220 residues while heavy chains have between 455 to 550 residues).

11. Goeddel et al.,[4] for example, reported production of insulin by expressing each insulin chain in a separate host cell culture. Then after each chain had been expressed and recovered, the two chains were combined in a test tube to form the insulin structure. The other approach that had been proposed by April 1983 was to produce a single chain insulin precursor polypeptide, isolate that polypeptide from the cell culture, cleave it in a test tube to produce the two insulin chains, and then form the insulin multimer in the test tube.[5]

12. The Moore patent also clearly reflects this one polypeptide-one host cell mindset. This patent describes a way of producing a "multimeric" antigen-binding molecule made up of short polypeptides corresponding to variable domain sequences found in heavy and light immunoglobulin chains.[6] What Moore says to do is produce each of the heavy and light chain polypeptides in <u>separate</u> host cell cultures, and then combine them in a test tube to form the rFv.[7]

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[3] A multimeric protein is a protein complex made up of more than one polypeptide subunit. The polypeptides form a stable complex through disulfide bonds and/or non-covalent interactions.

[4] *Proc. Nat'l Acad. Sci. (USA)* 76:106-110 (1979).

[5] *See, e.g.*, Harris, *supra* note 2, at p. 138; Wetzel et al., *Gene* 16:63-71 (1981); Brousseau et al., *Gene* 17:279-289 (1982).

[6] *See, e.g.*, Moore at col. 2, lns. 22-35. The Moore patent does not include any experimental results showing that a functional rFv molecule was actually made. *See also* May 18, 2007 Declaration of Steven McKnight at ¶¶ 48-54.

[7] *See, e.g.,* May 18, 2007 Declaration of Steven McKnight at ¶¶ 8-31.

13. The same approach of producing each chain of the immunoglobulin molecule in a separate host cell is what the Kaplan publication also says to do. For example, at page 10, the Kaplan publication says to produce each of the heavy and light chains in separate host cells, isolate each chain, and then attempt to produce the immunoglobulin molecule by combining the chains under mildly oxidizing conditions in a test tube (which they do not identify).[8]

14. The Cabilly '567 patent also follows this same mindset. The '567 patent claims require production of only one chimeric heavy or light immunoglobulin chain at a time in a host cell.

15. The Cabilly specification also identifies certain benefits of producing the different chains in separate host cell cultures. For example, it indicates that an "additional area of flexibility which arises from the use of recombinant techniques results from the power to produce heavy and light chains or fragments thereof in separate cultures . . . and to prevent reconstitution of the antibody or immunoglobulin aggregation until the suitable components are assembled."[9] It also explains that different types of immunoglobulin molecules can be made using separately produced heavy and light chains.[10]

16. All of these references clearly call for production of only one desired polypeptide at a time in a recombinant host cell, even if the ultimate objective might have been to produce a multimeric protein. This is the opposite of what the '415 patent requires (*i.e.*, production of two different immunoglobulin polypeptides in one host cell).

Co-Transformation of Host Cells is Not Equivalent to Co-Expression of Two DNA Sequences

17. The Axel patent describes a technique where the goal was transformation and expression of foreign DNA sequences in eukaryotic host cells. The experimental results reported in the patent show that eukaryotic host cells could be co-transformed with two different DNA sequences, but that these co-transformed host cells did not properly transcribe both DNA sequences and did not produce the desired protein.

18. The focus of the Axel patent is its technique for transforming a eukaryotic host cell with a gene encoding a selectable marker. The patent also shows that cells could be "co-transformed" with a second DNA sequence along with the marker gene. The second DNA

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[8] Kaplan refers to one dsDNA per vector, per host followed by "separately purified" light and heavy chains (page 10). Even with the minimal detail in Kaplan, it is clear that the chains should be separately purified and then assembled. Kaplan refers to "assembling of the light and heavy chains" (page 3), and, at page 10, to "combining" the purified light and heavy chains under "mildly oxidizing conditions," so it is clear to me that separate Ig chain production is all that is described and intended.

[9] '567 patent at col. 14, ln. 65 to col. 15, ln. 4.

[10] *See, e.g.*, *id.* at col. 15, lns. 44-57 (hybrid antibodies) ("Pairs of heavy and light chains . . . are prepared in four separate cultures, thus preventing premature assembly of the tetramer"); col. 16, lns. 33-54 (univalent antibodies) ("[T]he desired Fc region [is] expressed . . . . This portion is then bound using the technique of D.2 to separately produced heavy chain . . . and separately produced light chain [is] added.").

sequence was shown to "go along for the ride" and become stably integrated into the chromosomal DNA of the transformed cell, meaning that both DNA sequences would be passed on to the progeny of the "co-transformed" cell.[11]

19. The Axel patent outlines a strategy of using the co-transformation technique to produce a "desired proteinaceous material." The process described in the Axel patent uses two DNA sequences, each encoding one polypeptide.[12] One DNA sequence is the gene that encodes the selectable marker (DNA II). The other encodes the protein of interest to be produced and recovered from the cell (DNA I). This DNA I + DNA II process is designed to produce only one polypeptide that is recovered from the cell -- the marker gene protein is not recovered from the transformed cells under the Axel patent process.

20. The Axel patent reports that co-transformed cells successfully expressed the selectable marker gene (*i.e.*, DNA II) and produced "functional" marker gene protein. As a result of expressing the functional marker gene protein, the transformed cells exhibited a changed phenotype that made them resistant to a chemical that was toxic to untransformed cells.[13]

21. The Axel patent does not show production of any "functional" protein encoded by DNA I, much less a functional multimeric protein.[14] Instead, it reports experimental results showing that the two attempts to express a "DNA I" sequence (*i.e.*, a gene encoding a desired polypeptide) in a co-transformed cell both failed.[15] In both experiments, the Axel patent reports that host cells were successfully "co-transformed" with the "DNA I"

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[11] *See* Axel patent at col. 4, lns. 15-21.

[12] Axel explains that a proteinaceous material is a biopolymer formed from amino acids. *See* Axel patent at col. 4, lns. 28-29. I read this as meaning a single polypeptide (*i.e.*, a sequence of amino acid residues linked by peptide bonds) rather than a multimeric protein complex made up of different polypeptides associated through non-covalent interactions or disulfide bonds.

[13] *See*, *e.g.*, Axel patent at col. 2, lns. 16-27 (discussing Wigler et al., *Cell* 11:223-232 (1977)). This passage shows that this section discusses production of a functional "selectable phenotype" protein and not production of "functional" proteins encoded by DNA I sequences. I note this because the Office is relying on this incorrect assumption about which "functional" protein was made to justify its conclusion that Axel describes procedures for producing "functional antibodies." *See* Final Action at pp. 30-31.

[14] The Axel patent lists interferon as one of the types of proteins that could be made by its procedures. In February 1980, when the Axel patent was filed, the only interferon proteins known were monomeric proteins – meaning they only had one polypeptide chain. The Final Action (at page 30) mistakenly states that interferon is a multimeric protein.

[15] The first experiment used the rabbit ß-globin gene as DNA I. *See* Axel patent, First, Second, and Third Series of Experiments, at col. 9, ln. 59 to col. 25, ln. 68. The second experiment used human ß-globin gene as DNA I. *See id.* at Fifth Series of Experiments, at col. 30, ln. 60 to col. 42, ln. 10. The Axel patent also reports results of co-transformation experiments using a marker gene and a second model DNA sequence (*i.e.*, the pBR322 or ΦX174 bacteriophage sequence). *See id.* at col. 16, lns. 52-54 ("The stable transfer of Φ DNA sequences to mammalian cells serves as a model system for the introduction of defined genes for which no selective criteria exist."). Nothing is reported in the patent about expression of these model sequences.

sequence and the marker gene,[16] but that the polypeptide encoded by the DNA I sequence was not produced in these co-transformed host cells.[17]

22. The Axel patent also reports that in one of these experiments, abnormal transcription of the DNA I sequence was observed (*i.e.*, the rabbit β-globin DNA I sequence was not properly transcribed in the co-transformed cell).[18] The Axel inventors apparently did not investigate whether the other DNA I sequence tested (the human β-globin gene) was properly transcribed or translated, because nothing is reported about this in the patent.

23. The results reported in the Axel patent clearly show that "co-transformation" of cells was not being equated with successful "co-expression" of two foreign DNA sequences. Instead, these results show that successfully co-transformed cells did not properly transcribe DNA I to produce correct mRNA, and did not produce any DNA I polypeptide.

24. In April 1983, I would not have read the Axel patent as describing – or even suggesting – production of three different proteins (*i.e.*, two different proteins of interest plus a marker protein) in a single transformed host cell. That does not match the DNA I + DNA II process described in the patent, and ignores the experimental results showing unsuccessful expression of each DNA I sequence actually tested.

25. The Axel patent certainly does not explain how to make three different proteins in one host cell. For example, it does not contain any kind of plan for transforming a host cell with three different DNA sequences. It also does not contain any suggestions for improving the odds of successful transformation or expression of the three genes (as it does for experiments using just two genes).[19]

26. A person of ordinary skill in the art would not have read the Axel patent as specifically suggesting production of two different antibody polypeptides in a single transformed host cell. This is because if that person followed the DNA I + DNA II scheme outlined in the patent, and used the procedure described for obtaining the DNA I sequence (*i.e.*, restriction endonuclease digestion of chromosomal DNA), he or she would have obtained a DNA I sequence that encoded only one antibody polypeptide. The restriction endonuclease

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[16] *See id.* at col. 13, ln. 56 to col. 14, ln. 23; *see also id.* at col. 15, lns. 12-22; col. 17, lns. 43-46; col. 22, lns. 39-41.

[17] *See, e.g.*, *id.* at col. 21, lns. 37-57 ("Attempts to detect this protein [the rabbit ß-globin encoded by DNA I] in cell lysates using a purified anti-rabbit ß-globin antibody have thus far been unsuccessful.").

[18] *See id.* at col. 20, lns. 13-15 ("Taken together, these results indicate that although the intervening sequences expressed in transformed mouse fibroblast are removed from the RNA transcripts precisely, the 5' termini of the cytoplasmic transcripts observed do not contain about 48±5 nucleotides present in mature 9S RNA of rabbit erythroblasts."); *see also id.* at col. 19, ln. 7 to col. 20, ln. 67 (reporting the nature of aberrant (*i.e.*, incorrect) transcription of the rabbit ß-globin DNA I sequence).

[19] *See id.* at col. 5, lns. 29-50; col.6, lns. 47-53; col. 7, lns. 3-26. These techniques of using excess ratios of copies of the DNA I sequence to the DNA II sequence or gene amplification of linked DNA I all result in cells that will have identical copies of the same DNA I sequence in the cell.

technique digests chromosomal DNA to recover the desired sequence. Because the antibody genes are located on different chromosomes, digestion will produce a DNA sequence that has only one antibody gene, not both.

27. A person of ordinary skill also would not have read the Axel patent as saying that the heavy and light chains should be produced in one transformed host cell if that person wanted to use its procedures to try to make an antibody molecule. Instead, that person would have understood that the way to approach producing an antibody (or any other multimeric protein) using the Axel methodology would have been to produce only one antibody polypeptide at a time using a host cell co-transformed with a marker gene and a DNA I sequence encoding the desired antibody polypeptide (*i.e.*, produce one co-transformed host cell with a DNA I encoding the antibody heavy chain, and a different co-transformed host cell with a DNA I encoding the antibody light chain). If each chain were successfully produced and isolated from the separate host cell cultures, then the next step would have been to try to combine the chains in a test tube to form the immunoglobulin tetramer or binding fragment. This is the only approach that is consistent with the DNA I + DNA II scheme outlined in the Axel patent, and with the recommendations in the Moore and Kaplan references.

28. I note that the Office has pointed out that the Axel patent identifies potential benefits of producing eukaryotic proteins in eukaryotic cells. These benefits include that the proteins may be glycosylated or subjected to other types of post-translational chemical modification. These properties of eukaryotic cells were known before April 1983. I do not believe a person of ordinary skill in the art would have read these comments as suggesting that different antibody polypeptides should be produced in a single transformed host cell.

29. The Office also indicates that the abstract of the Axel patent makes references to using multiple copies of genes. These comments are not suggesting that different proteins of interest should be produced in one host cell. The more detailed explanation in the body of the patent[20] explains that by including multiple copies of the DNA I sequence relative to the DNA II sequence, or by using gene amplification of DNA I linked to DNA II, one can generate a host cell with multiple copies of the DNA I sequence. If the Axel patent had intended "multiple genes" to mean co-transformation and co-expression of three (or more) different DNA sequences, the patent would have clearly said so and would have explained how to accomplish that goal. For example, the patent could have said to prepare a "DNA III" sequence and use it to "co-transform" the host cell with the DNA I and DNA II sequences. Since it does not, I do not believe the Axel patent is saying to do this.

<u>The Ochi, Oi, and Rice Publications Illustrate the Unpredictability in the Field as of April 1983</u>

30. The uncertainty reported in the Axel patent in achieving successful expression of even one mammalian protein of interest is also seen in the Rice, Ochi, and Oi papers. Each of these papers describes efforts to express one immunoglobulin light chain gene in a lymphoid cell.

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[20] *See, e.g.*, *id.* at col. 6, ln. 44 to col. 7, ln. 26.

31. The Ochi paper provides a good illustration of the unpredictability reported in these transformation experiments. The Ochi researchers selected a mutant of an otherwise functional hybridoma cell line.[21] The mutant had lost the ability to express its endogenous light chain gene, but continued to produce its heavy chain.[22] The Ochi researchers then isolated the light chain gene from the functional parent hybridoma line, and transformed the mutant cell line with that gene. In other words, they put the same light chain gene back into the mutant cell line that had lost the ability to express that gene.

32. Despite the simple design of this experiment, Ochi reports that 8 of the 14 successfully transformed B-cell lines failed to regain any antibody production.[23] Of the remaining 6 successfully transformed cell lines, only one produced antibodies at levels comparable to the parent line. In other words, the Ochi researchers report that they failed to achieve success 13 out of 14 times in the simplest type of lymphoid cell experiment they could design.

33. The Ochi researchers report the same types of problems that the Axel patent reports: they were able to successfully transform host cells with the gene encoding their protein of interest – an immunoglobulin light chain – but most of the transformed cell lines did not successfully express that gene.[24] Rice and Oi also report unsuccessful expression in successfully transformed lymphoid cells.[25]

34. Each of these papers shows that successful transformation and expression of even one foreign immunoglobulin gene in a lymphoid host cell could not be reasonably expected in April 1983. I do not believe these references can be read as suggesting that something even more challenging – expressing two different foreign immunoglobulin genes in one transformed cell – would have been something that could be predictably achieved at that time.

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21 *See* Ochi at p. 340, col. 2 ("As recipient cells, we used the mutant cell line igk-14 which was derived from Sp603 and does not produce the κ_{TNP} chain.").

22 *See id.* ("As shown in Fig. 3, the κ_{TNP} gene is apparently deleted from the igk-14 cell line. Because the igk-14 cells still produce the TNP-specific p heavy chain, it would be expected that the expression of the κ_{TNP} gene in these cells would restore the production of TNP-specific IgM.").

23 *See id.* at p. 341 (Table 1).

24 Ochi reports that each of the 14 cell lines was successfully "transformed" with the foreign light chain gene. *See id.* p. 341, col. 1, middle paragraph. It then reports that 10 of these transformed cell lines produced virtually no protein, and all but one of the remaining "successfully transformed" lines produced protein at significantly lower levels than the parental line. *See id.* at p. 341 (Table 1); pp. 341-342. The Ochi paper also points out that many of the successfully transformed B-cell lines did not successfully express the introduced light chain gene.

25 The Rice paper reports that several of the transformed cell lines showed aberrant transcription of the introduced light chain gene. *See* Rice at p. 7864, col. 1. The Oi paper also reports problems with transcription. *See* Oi at p. 827 ("Twelve independently transformed Y3 and seven BW5147 cell lines did not produce detectable amounts of the S107 light chain, as judged by immunoprecipitation and gel analysis. XGRPT analyses verified that these cells were, indeed, transformants.").

35. Dr. Baltimore's declaration in the reexamination proceeding recognizes this uncertainty. He states in his declaration that "if the two chains were expressed" in the same suitably transformed mammalian cell, he believes that the cell would properly fold two chains and assemble them into a functional antibody. He avoids saying two important things in his declaration. First, he does not say that producing a transformed mammalian cell that successfully transcribed foreign heavy and foreign light chain genes and also properly produced the foreign heavy and foreign light chain polypeptides would have been predictable. Second, he does not say that a person of ordinary skill would have been motivated by his paper or anything else in the literature at the time of the invention to try to produce an immunoglobulin by independently expressing foreign heavy and foreign light chain genes in a single transformed cell. I believe Dr. Baltimore did not say anything about the prospects or idea of making his hypothetical co-transformed cell because even he would have not considered this predictable based on what was known in April 1983.

36. I also find it important that the Rice, Ochi, and Oi papers describe experiments done by the preeminent researchers in the field of immunoglobulin gene expression in April 1983. Each was also published in a leading scientific journal. Despite this, none of the papers even suggests the idea of transforming a cell line with more than one immunoglobulin gene. If these authors believed their work was showing people how to make a recombinant antibody by expressing two different foreign immunoglobulin genes in one host cell, they would have said something about this somewhere in their papers. Of the three, none accomplished it, tried it, or even suggested it.

37. The Rice, Ochi, and Oi papers, considered with the Axel patent, reinforce my belief that if someone wanted to try to produce an immunoglobulin molecule using recombinant DNA techniques in April 1983, that person would have tried to produce each of the two chains in separate host cells, rather than trying to produce both chains in one host cell. That is the same message delivered by the Kaplan and Moore references, and is consistent with the one polypeptide-one host cell mindset that existed in April 1983.

Dallas Focuses On an Unrelated Goal and Would Not Have Influenced the Beliefs or Expectations of a Person of Ordinary Skill in the Art

38. All of the references I have discussed above tell me that if a person of ordinary skill in the art wanted to try to produce an immunoglobulin molecule using recombinant DNA techniques in April 1983, that person would not have tried to do this by producing both immunoglobulin chains in one transformed host cell. The Axel, Rice, Ochi, and Oi references make this clear in the context of eukaryotic host cells, and the Kaplan and Moore references explicitly say to produce only one immunoglobulin chain at a time in a single prokaryotic host cell. The experimental results reported in these references would have given a person of ordinary skill no basis to predict or expect that it would be possible to achieve what is required by the '415 patent.

39. The Dallas publication would not have changed the clear message I see in these references. This is because the Dallas publication is describing a simple experiment where an *E. coli* cell was transformed with two different *E. coli* genes, and the expression product of these *E. coli* genes was not isolated or recovered. The Dallas publication simply would not have

provided any relevant guidance or insights into how to successfully produce and recover multiple eukaryotic proteins from a single host cell using recombinant DNA techniques in April 1983.

40. One reason for my conclusion is the very different goal of the Dallas experiments – production of a whole cell bacterial vaccine. In these experiments, there was no need to isolate, purify, or even evaluate the proteins encoded by the *E. coli* genes. There also was no need to attempt to assemble the isolated bacterial proteins into a more complicated multimeric structure. Instead, the Dallas researchers just had to get an *E. coli* cell to express one or two of the *E. coli* genes for the experiments to be considered a success.[26] The desired product of the Dallas researchers was the transformed *E. coli* cell, not an isolated functional protein.

41. Another reason for my conclusion is that the Dallas experiments involved transforming and expressing *E. coli* genes in an *E. coli* cell. These experiments would not have presented technical challenges comparable to expressing foreign DNA sequences encoding eukaryotic proteins in *E. coli* cells in April 1983.[27] By April 1983, it was known that *E. coli* cells could easily incorporate and express *E. coli* genes from other *E. coli* cells or bacteriophages.[28] The mechanisms that enabled this to happen in *E. coli* are unique to these types of prokaryotic cells. For example, it was known that bacterial genes often included specific sequences that enabled them to be readily taken up by the bacterial cells.[29] Eukaryotic DNA does not have these bacterial "uptake" sequences, and there were no analogous systems known to exist in April 1983 for eukaryotic genes to be selectively uptaken by eukaryotic cells.

42. It is also important to note that the bacterial proteins expressed in the Dallas experiments are not "foreign" to the bacterial cell. Instead, they are proteins that normally are destined for display on the cell surface of the *E. coli.* By contrast, when foreign eukaryotic proteins like immunoglobulin polypeptides are produced in a bacterial cell, they usually form inclusion bodies – solid aggregates of the polypeptides. The inclusion body is believed to be *E. coli's* defense mechanism against the damaging effects of the foreign protein in its

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[26] The Dallas publication reported success on the basis of positive agglutination tests, which show binding of antisera to transformed cells. *See, e.g.,* Dallas at p. 6, lns. 32-34; p. 8, lns. 21-24.

[27] The Dallas publication reports uncertainty about these very simple bacterial transformation experiments. For example, it reports that bacterial cells transformed with two different plasmids (each with a different bacterial gene) were not stable. *See id.* at p. 10, lns. 3-7. I read this as meaning that the transformed bacterial host cells did not stably retain both plasmids. The Dallas publication also reports reduced levels of expression where two different bacterial genes were placed in one plasmid relative to levels of expression when only one gene was put into the bacterial cell. *See id*. at p. 8, lns. 21-24.

[28] To me, the Dallas experiments describe a process that is no different than what happens when an *E. coli* host is infected by bacteriophage lambda, which can insert 50 or more *E. coli* genes into an infected *E. coli* cell and cause that cell to express those genes. *See, e.g.,* Echols & Murialdo, *Microbiol. Rev.* 42(3):577-591 (1978).

[29] *See, e.g.,* Graves et al., *J. Bacteriol.* 152:1071-77 (1982).

cytoplasm. The *E. coli* walls off the proteins in a solid, improperly folded mass to ensure that the proteins are not biologically active within the cell. This phenomenon does not occur when endogenous bacterial proteins like those encoded by the *E. coli* genes used in the Dallas experiments are expressed in the bacterial cell. Recognizing this, I would have expected that the transformed *E. coli* cells in Dallas could express the *E. coli* genes that had made it into the cells, and that the expression products of these *E. coli* genes would not have caused any problems for these transformed *E. coli* cells.

43. The Dallas publication and the other references cited by the Office do not discuss how to produce a multimeric protein structure using the expression products of a co-transformed host cell. This is important to achieving the '415 patented invention, and there is simply no guidance in any of these publications about doing this.

44. These are some of the reasons why I do not believe a person of ordinary skill in the art in April 1983 would have considered the Dallas publication to even be relevant to production of eukaryotic proteins in a bacterial host cell.

45. I note the *Harris* review article does not cite any papers by the Dallas group or mention this type of experiment. The Harris paper was an exhaustive review of the technology available to express eukaryotic genes in prokaryotic cells around the time of the '415 patent invention. The fact that the paper does not even mention the Dallas work confirms my belief that a person of ordinary skill in the art would not have found anything useful or insightful in the Dallas publication about how to produce multiple foreign eukaryotic genes in a single bacterial cell.

46. A person of ordinary skill in the art would have had no reason to even attempt production of heavy and light immunoglobulin chains in single bacterial host cell. Even if that person were able to co-express both chains in a bacterial cell, there would have been no apparent benefit of doing this.

47. I say this for two reasons. First, my experience at the time would have led me to believe that if the immunoglobulin chains had been formed, they would have immediately precipitated into inclusion bodies. Second, the reducing environment of the bacterial cell would have prevented disulfide bond formation, which was known to be important for the correct folding of the heavy and light chains, and formation of the tetrameric antibody structure.[30] Because a person of ordinary skill would have anticipated having to solubilize and "untangle" the aggregated and misfolded chains from the inclusion bodies and try to refold them in a test tube anyway, that person would not have perceived any advantage to producing the two chains in one host cell. This is because bacterial cells do not have any of the special attributes or capabilities of the "professional" B-cells that enables those cells to facilitate immunoglobulin polypeptide folding and assembly of the immunoglobulin tetramer (*e.g.*, specialized organelles, chaperone proteins, and proper reduction/oxidation

[30] *See* Harris, *supra* note 2, at p. 173.

environment). In other words, the person of ordinary skill would not have seen any reason to produce both polypeptide chains in one host cell.[31]

48. I believe a person of ordinary skill would have simply avoided all these problems and uncertainties by producing the heavy and light immunoglobulin chains in separate bacterial host cell cultures. This is what each of the Moore and Kaplan references recommends doing and what the '567 Cabilly patent claims call for. The Dallas publication would not have altered my conclusions from these other references because it had a very different goal and because it provides no guidance at all about producing and recovering foreign eukaryotic proteins from transformed host cells.

The Deacon and Valle 1981 Publications Would Not Alter Expectations

49. I do not believe the mRNA microinjection experiments with frog (*Xenopus*) oocytes would have changed the expectations of a person of ordinary skill about trying to produce heavy and light immunoglobulin chains in a single transformed host cell in April 1983.

50. As I explained above, the Axel, Rice, Ochi, and Oi references each showed there was significant uncertainty in the expression of foreign DNA sequences in transformed eukaryotic host cells. These references specifically identify unpredictability associated with transforming cells with foreign DNA, getting transformed cells to properly transcribe the DNA into mRNA, and translating the RNA into protein. The Axel patent, in particular, demonstrated that while one could stably incorporate a DNA I sequence into a host cell, this could not be equated with successful transcription of that DNA I sequence, much less successful production of the protein encoded by DNA I.

51. The oocyte experiments of Valle and Deacon do not even address the issue of gene expression. Instead of trying to express foreign DNA sequences encoding immunoglobulin proteins, these researchers simply used the oocyte to translate fully mature and functional immunoglobulin messenger RNA.[32] The oocyte experiments bypass all of the problems and questions about expression of foreign DNA sequences by using mRNA extracts from B-cells that have successfully transcribed their immunoglobulin genes.

52. The Valle and Deacon experiments also use impure mRNA fractions isolated from the B-cells. These extracts would have contained many mRNA transcripts other than those encoding the light and heavy immunoglobulin chains. These other mRNA transcripts could very well have encoded proteins that played an essential role in assembling functional immunoglobulin molecules in B-cells. In essence, the use of these impure

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[31] Vassalli et al., *J. Mol. Biol*. 56(1):1-19 (1971) (showing that disulfide-bonded H_2L_2 units takes place at the level of the rough endoplasmic reticulum); Wabl et al., *Proc. Nat'l Acad. Sci. (USA)* 79:6976-6978 (1982).

[32] I understand that an attorney in Europe said that the oocyte experiments had relevance to the coexpression of heavy and light chain DNA sequences in a host cell. This is scientifically incorrect. In my opinion, a person of ordinary skill in the art would not have concluded that one could substitute DNA for Valle's mRNA and expect to get successful expression, assembly and secretion of a functional antibody.

mRNA fractions could have re-created at least some of the environment found in the successful antibody-producing B-cell that enabled it to translate and process immunoglobulin polypeptides. For example, by April 1983, scientists had predicted that a protein called BiP played this role in the B-cell.[33] Injecting the impure mRNA fractions into the oocyte could have resulted in production of BiP, which could have been why the immunoglobulin chains were assembled into antibodies.

53. I also do not believe a person of ordinary skill in the art would have characterized a microinjected *Xenopus* oocyte as a "transformed host cell."[34] Transformed host cells result from stable incorporation of foreign DNA. Successfully transformed host cells must pass the foreign DNA onto their progeny. In the experimental setting described in the Deacon and Valle 1981 papers, the *Xenopus* oocytes do not produce any progeny and they do not integrate any foreign DNA into their genome.

54. I believe a person of ordinary skill would have viewed the differences between using vector DNA and microinjection of mRNA fractions as being substantive and significant in April 1983. This is because expression of DNA and translation of mRNA are linked events in a transformed host cell – translation depends on successful transcription of the foreign DNA sequence. As such, the beliefs that person would have had about the predictability of achieving the entire process of the '415 patent would have been based on that person's expectations about achieving all of steps involved in expression of the two DNA sequences (*i.e.*, transformation, correct transcription of the DNA, and successful translation of mRNA), not just assembly of the immunoglobulin.

Conclusion

55. I do not believe the combination of the '567 patent claims with Axel, Rice, Kaplan, Builder, Accolla, Dallas, Ochi, Oi, Deacon, Valle 1981, and Moore would have made obvious the inventions defined by the '415 patent claims in April 1983. Instead, I believe these references together provide a clear message to produce only one immunoglobulin polypeptide at a time in a transformed host cell. Following a one polypeptide-one cell strategy would have helped the person of ordinary skill in the art avoid the multiple problems and uncertainties reported in these publications and known at the time of the invention.

56. Neither the cited references, nor anything else of which I was aware as of April 1983, would have led a person of ordinary skill in the art to have any basis for expecting that he or she could successfully achieve what is required by the '415 patent -- producing an immunoglobulin molecule or fragment by co-expressing DNA sequences encoding heavy and light immunoglobulin chains in a single host cell.

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[33] *See* Wabl et al., *supra* note 31; Valle 1981 at p. 339, col. 2 (speculating on possible presence of non-stoichiometric amounts of putative helper protein).

[34] *See, e.g.,* May 18, 2007 Declaration of Steven McKnight, ¶ 105.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent subject to this reexamination proceeding.

SSMcK

Dr. Steven McKnight

June 3, 2008

Date