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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 not 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.

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