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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Control Nos.:	**90/007,542** **90/007,859**		Group Art Unit:	3991
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Patent Owner:	Genentech, Inc. and City of Hope			
For:	Merged Reexaminations of U.S. Patent No. 6,331,415 (Cabilly *et al.*)			

DECLARATION OF MICHAEL BOTCHAN UNDER 37 C.F.R. § 1.132

I, Michael Botchan, do hereby declare and state:

1. I am a citizen of the United States, and reside in Kensington, California. My C.V. is attached as Exhibit A.

2. I have been retained by Genentech and City of Hope to provide my opinion on certain issues in the patent reexamination proceedings involving U.S. Patent No. 6,331,415. I am being compensated for my time at a rate of $550 per hour.

3. I am not now affiliated with either Genentech or City of Hope. I served as an expert for Genentech in *City of Hope Nat'l Med. Center v. Genentech, Inc.*, Case No. BC215152 (Los Angeles Co. (Cal.) Super. Ct.), and provided deposition testimony in that litigation.

4. I have reviewed the following documents in the course of preparing this declaration:

   - Cabilly *et al.*, U.S. Patent No. 6,331,415 (the '415 patent)
   - Cabilly *et al.*, U.S. Patent No. 4,816,567 (the '567 patent)
   - Moore *et al.*, U.S. Patent No. 5,840,545 (the '545 patent)
   - Moore *et al.*, U.S. Patent No. 4,642,334;
   - Moore *et al.*, U.S. application no. 06/358,414 (the '414 application)
   - Boss *et al.*, U.S. Patent No. 4,816,397

- Axel *et al.*, U.S. Patent No. 4,399,216
- Rice *et al.*, *Proc. Nat'l Acad. Sci. USA* 79: 7862-65 (1982)
- Kaplan *et al.*, EP 0044722
- Builder *et al.*, U.S. Patent No. 4,511,502
- Accolla *et al.*, *Proc. Nat'l Acad. Sci. USA* 77: 563-66 (1980)
- Dallas, WO 82/03088
- Deacon *et al.*, *Biochem. Soc. Trans.* 4: 818-20 (1976)
- Valle *et al.*, *Nature* 291: 338-40 (1981)
- Valle *et al.*, *Nature* 300: 71-74 (1982)
- Ochi *et al.*, *Nature* 302: 340-42 (1981)
- Oi *et al.*, *Proc. Nat'l Acad. Sci. USA* 80: 825-29 (1983)

5. I have also reviewed the documents associated with the two reexamination proceedings, including the PTO communication dated February 16, 2007 (the Office Action).

6. I understand that patentability is to be evaluated using the perspective of a person of ordinary skill in the technical field of the invention just prior to the filing date of the patent (i.e., in this case, early April of 1983). A person of ordinary skill in the field of the '415 patent would have had a Ph.D. in molecular biology or a comparable scientific discipline and two to three years of postdoctoral experience. I believe I am well-qualified to express an opinion on what a person of ordinary skill in the art of the '415 patent would have believed or expected in early April of 1983 because I worked with many people at that time with these qualifications.

7. I understand that the '545 patent issued in 1998 from an application filed on June 5, 1995. I also understand that there were several earlier applications filed between 1982 and 1995, and that the first of these was the '414 application, which was filed in March of 1982. I understand that the question of what is described in the '414 application (the 1982 application) relative to what is described in the '545 patent is an issue in this reexamination proceeding.

8. I have been asked to explain the techniques described in the '414 application and whether there is a description of a host cell that produces two different polypeptide chains or a

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process which produces two different polypeptide chains in a single host cell in that application. I have also been asked to address what a person of ordinary skill in the art in early April of 1983 would have taken away from the information in a variety of patents and publications, and whether that information would have made the coexpression procedures in the '415 patent claims obvious at that time.

Analysis of the '414 Application and the '545 Patent

9. The '414 application describes procedures for cloning DNA that were conventional in early April of 1983. At that time, it was known that to "clone" a DNA sequence, you would:

- isolate or prepare desired DNA;
- insert the DNA into a vector;
- insert the vector into a host cell, and grow the host cell;
- isolate the copies of the DNA (within the vector) from the host cell culture (which now contains multiple progeny of the cells, and therefore multiple copies of the vector containing the desired DNA).

10. The '414 application describes cloning procedures having these steps at pages 5, line 16, to page 9, line 20.

11. The process for isolating DNA encoding the individual immunoglobulin chains is described at page 6, line 14 to page 8, line 7 of the '414 application. First, an mRNA extract is produced from a hybridoma that is making a desired antibody. This mRNA extract will contain many different mRNA "transcripts" corresponding to the messengers of the genes being expressed in the cell. Each of the mRNA transcripts is a discrete molecule containing a sequence corresponding to the amino acid sequence of a single polypeptide encoded by the DNA in the cell. The mixture of mRNA transcripts isolated from the hybridoma in the '414 application will contain mRNA transcripts produced during transcription of the immunoglobulin light chain gene, and different mRNA transcripts produced during transcription of the immunoglobulin heavy chain gene. There will be no mRNA transcripts in the extract that contain sequences from both heavy

and light chain genes, because the mRNAs for the chains are encoded by different genes expressed from separate promoters at different chromosomal positions.

12. The mRNA extract is then purified and used to prepare a cDNA library. The process as described is standard for the time, as described at page 7, line 37 to page 8, line 7. It involves using the "reverse transcriptase" enzyme that produces a complementary DNA (cDNA) molecule corresponding to each mRNA transcript in the purified mRNA extract. Again, because no mRNA transcript will contain sequences for both heavy and light chains, no individual cDNA in this cDNA library will contain heavy and light chain sequences.

13. The next step described in the application is amplification of the cDNA library. Amplification involves incorporating all of the cDNA molecules in the cDNA library into individual plasmids, and then inserting the plasmids into cells in culture by a transformation process. This procedure is specified at 8, line 12, to page 9, line 19. The procedures being described make it absolutely clear that each plasmid incorporates a single cDNA encoding a light or heavy immunoglobulin chain, and that each bacterial cell transformed will contain one plasmid.

- At page 8, lines 15-18, the application states that "the ds cDNA obtained from the reverse transcription of the mRNA" is being used. As I explained above, each discrete ds cDNA molecule in the cDNA library encodes only one immunoglobulin polypeptide sequence because it is produced from individual mRNA transcripts in the mRNA extract.
- The design of the plasmid indicates that one cDNA insert will be incorporated into each plasmid. *See* page 8, lines 20-24 ("... the vector will have a unique restriction site in one of multiple markers so that transformants may be selected by the expression of one marker and the absence of expression of the other marker"). Certainly, this is the desired outcome.

- The selection of screening techniques for bacterial clones indicates that each clone has one plasmid with one cDNA in it. The descriptions of these techniques could not be clearer in stating that each clone contains one plasmid with one cDNA encoding only one of the two immunoglobulin chains. Specifically, at page 9, lines 10-13, the '414 application states:

> The host colonies, usually bacterial, which have DNA which hybridizes to either the light or heavy chain probes are picked and then grown in culture under selective pressure.

14. After each clone has been propagated in culture, the bacterial cells are lysed, and the copies of the plasmid are isolated, sequenced, and subjected to restriction mapping. The sites for specific restriction enzyme hydrolysis are mapped on the genome of the plasmid. The sequencing and restriction mapping techniques in the application indicate that individual cDNA sequences encoding the light or heavy immunoglobulin chain are being used in the process. For example, at page 9, lines 22-31, the application states:

> These analyses insure that the isolated cDNA clones completely encode the variable region and, optionally, the leader sequences for the light or heavy chain of the desired immunoglobulin. Furthermore, by having a restriction map of the variable regions and leader sequences, as well as the flanking sequences, one can determine the appropriate restriction sites for excising a DNA fragment which will allow for appropriate modification of the DNA sequence for insertion into a vector and expression of the polypeptide of interest. (emphasis added)

15. Someone who was familiar with basic molecular biology principles would know that unless special steps were taken to culture the bacterial cells under "selective pressure," those cell cultures will become uniform with respect to plasmid content within each cell. Specifically, if a bacterial cell is transformed with a plasmid that contains an antibiotic resistance gene, copies (clones) of that bacterial cell can be selectively cultivated by adding the relevant antibiotic to the cell culture (i.e., the antibiotic kills the cells that have not incorporated the plasmid). This concept of selective pressure is central to the design of genetic engineering experiments. In the case of the '414 application, the procedures employ cell culture techniques that use only a single source of selective pressure (i.e., a single antibiotic is used to exert selective pressure on transformed cells).

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16. In my opinion, a person of ordinary skill also would have known that if a bacterial cell was transformed to contain two plasmids that contain the same marker and regulatory elements, within an overnight period of growth, in the absence of appropriate selective pressure, the bacterial culture would be devoid of "double transformants." This is the consequence of several aspects of cell biology.

- First, propagation of bacterial cells is geometric (one cell divides into two, two divide into four, etc.) and the final number of cells in the culture is limited by nutrient resources and other competitive forces in the culture medium.
- Second, transformation efficiency using procedures prevalent in the early 1980's were low – approximately one in 10,000 bacterial cells would incorporate a foreign plasmid. *See* S. N. Cohen et al., *Proc. Nat'l Acad. Sci. USA* 69:2110-2114, 2112 (1972). In the absence of some strategy to increase the odds of incorporation of two different plasmids, the probability of one cell incorporating two different plasmids during a single transformation step is roughly the square of the rate of transformation with a single plasmid (i.e., one in 10^8). This means that a "double transformant," if it were produced at all, would be vastly outnumbered in the culture medium by "single" transformants.
- Finally, and very significantly, bacterial cells exhibit "plasmid incompatibility" when plasmids with the same regulatory elements but different neutral genetic elements (e.g., cDNA inserts which have no selective influence on the plasmid replication or survival) are inserted into a cell. B. Polisky, *Cell* 55:929-932, 929 (1988). This incompatibility results from the mechanisms of plasmid DNA replication as follows.
    1. Individual plasmids within a cell are chosen randomly from the pool to be replicated.
    2. The total number of plasmids within a cell are under strict copy number control, and once a copy number is achieved, repression of plasmid replication occurs.

3. Thus the progeny of any given cell will contain one or the other of the original rare "double transformants" but not both. This is because a bias is introduced toward one or the other of the plasmids in the first cell doubling (see points 1 and 2 above). This bias is amplified in each successive doubling until all copies of the other plasmid are lost to the progeny of the first transformed cell.

17. When all these factors are considered together, it would be very clear to a person skilled in this field that a bacterial cell culture, left alone, will eventually be dominated by the "most successful" bacterial clones. Given the natural forces exerted on these cells during propagation, and in the absence of multiple sources of selective pressure, a bacterial cell culture that contained one or more double transformants would, soon would contain effectively no progeny of that double transformant that maintained the two different plasmids.

18. Accordingly, I do not believe a person skilled in the field of molecular biology would have read any of the sections of the '414 application as describing transformation procedures where bacterial cells are being transformed with two different plasmids. None of the steps listed in the application indicate that two different plasmids should be inserted into a single host cell, and there is no description of any strategy for exerting selective pressure to cause a cell culture to maintain "doubly transformed" clones. There are simply no suggestions of these types of techniques or approaches anywhere in the '414 application.

19. Someone who was familiar with molecular biology techniques also would immediately recognize that the amplification steps described in the '414 application involve manipulations of individual plasmids. For example, the restriction mapping procedures described in the application are procedures where arrays of fragments of a nucleotide sequence are produced by enzymatic digestion of a particular nucleotide sequence. Restriction maps may be used to confirm the presence or absence of a particular DNA sequence (here the sequence encoding either the immunoglobulin heavy or light chain) in a particular transformed cell. Ordinarily, each map is produced from a single sequence, because of the complexity of mapping multiple sequences from a single test medium. If

these steps were being performed on mixtures of plasmids or different cDNA sequences, the application would have clearly indicated this.

20. The next step in the process is preparation of the tailored cDNA sequences encoding the variable region sequence of each immunoglobulin chain. To do this, a short oligomer is synthesized which will hybridize to a region in the light or heavy chain variable region being produced. The oligomer incorporates a "stop codon" which will terminate translation of the cDNA at the end of the variable region. The oligomer is combined with a restriction fragment encoding the light or heavy chain variable region from the amplified cDNA step (see paragraph 12 above). After the oligomer hybridizes to the restriction fragment, it is enzymatically elongated to produce a DNA strand complementary to the original source heavy or light chain sequence – except that it has the incorporated stop codon at the end of the variable region. This produces a double stranded (ds) cDNA sequence encoding the variable region and upstream flanking regions of the immunoglobulin chain sequence being manipulated.

21. This ds cDNA is referred to as a "heteroduplexed" ds cDNA in the application, meaning that the two DNA strands are not 100% complementary. The application also refers to the plasmid into which this ds cDNA is incorporated as a "hybrid" plasmid because it contains "mismatched" sequences.

22. I note that these hybridization techniques described in the '414 application depend on the use of specific reaction conditions (e.g., temperature, salt concentration, etc.) appropriate for forming each heteroduplex. Because the "melting temperature" of a heteroduplex will be sensitive to a particular mix of these conditions, it would not be possible to manipulate both a heavy chain cDNA and a light chain cDNA to introduce stop codons in the same reaction mixture.

23. As the application points out, when the resulting plasmid is incorporated into a bacterial cell, and the cell divides; one of the daughter cells will contain a ds cDNA corresponding to the original or "native" sequence (i.e., produced from the mRNA extract); and the other daughter cell will contain a ds cDNA having the sequence of the "tailored" cDNA.

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24. The clones containing the tailored sequence are then amplified. I note that at page 12, where this amplification step is described, all of the references to the tailored cDNA indicate that it encodes only one polypeptide. *See, e.g.,* page 12, lines 15-18 ("... to provide individual clones replicating the tailored sequence") (emphasis added). After amplification, the tailored cDNA is treated to introduce a start codon at the other end (the 5′ terminus) of the sequence. *See*, page 12, line 26 to page 14, line 15. Once that is completed, the tailored cDNA is incorporated into a plasmid for expression of the tailored gene and production of the desired polypeptide.

25. At this point, the application is crystal clear that individual heavy chain and light chain polypeptides are being produced in separate cell cultures. At page 14, the process for preparing an expression vector is described. As it explains, the vector (plasmid) contains the transcriptional and translational regulatory signals required for successful expression of an introduced cDNA sequence. This indicates that each vector will be instructing the cell to express only a single cDNA inserted into the vector. Even in the very general guidance provided at page 15, lines 6 to 19, the application indicates that the host cells are being engineered to produce a single polypeptide. *See, e.g.,* page 15, lines 9 to 11 ("the availability of vectors which allow for insertion of the ds cDNA sequence into the vector and expression of the variable region polypeptide"). *See also* page 16, lines 33 to 37 ("The ribosome binding site and variable-region initiation codon may be properly spaced to optimize expression of the variable region polypeptide").

26. If there were any doubt that the procedures described in the '414 application are designed to produce one polypeptide in each host cell, that doubt is erased by the explanation of the procedures for isolation and purification of the expression product, and preparation of the rFv. For example, at page 15, lines 27-30, the application states that the polypeptides made by this procedure "are prepared as a homogeneous composition containing identical sequences and chain lengths." If each cell were producing a mixture of heavy and light chain variable region polypeptides, the composition would not be "a homogeneous composition containing identical sequences."

27. Then, the application outlines the procedure for assembling the rFv. In very clear terms, the application indicates that each transformed host cell will produce one of the two

variable region polypeptides, and then, after each is isolated, the polypeptides will be combined outside of the cell to form the rFv. As the application explains at page 16, lines 24 to 29:

> The resulting construct is then introduced into an appropriate host to provide expression of the heavy or light polypeptide members of the rFv and the polypeptides isolated. The heavy and light polypeptide members of the rFv are then combined in an appropriate medium to form the rFv.

28. This one protein-one host cell concept is reiterated throughout the section of the application describing polypeptide isolation procedures. *See*, for example, page 17, line 35, to page 18, line 7:

> Where the light or heavy chain is not secreted, the transformed microorganisms containing the appropriate ds cDNA for either light or heavy chains are grown in liquid cultures and cleared lysates prepared. ... The bound variable regions are eluted from the column with an appropriate denaturing solvent. The eluates from each of the heavy and light chain isolations are pooled, followed by treatment to renature the polypeptides to form L-rFv and H-rFv respectively.

29. Again, this makes it absolutely clear that each variable region polypeptide is produced in a separate host cell.

30. The application contains an example that illustrates use of the general procedure outlined earlier. *See* Example 1, starting at page 19, line 10 and continuing to page 42, line 13. I note that several details in this example clearly demonstrate that only one variable region polypeptide will be produced in each host cell.

31. At pages 40-41, the example describes a procedure where each tailored cDNA is incorporated into a separate plasmid. As the application states at page 41, lines 6 to 15, "the 'tailored' pGM1 is isolated, partially restricted with PstI and the DNA sequences coding for the light and heavy chain variable regions prepared above inserted individually into the tailored site to provide two plasmids having DNA sequences coding for the light (pGM1L) and heavy (pGM1H) chains .... The resulting plasmids are used to transform *E. coli* HB101 and clones having the light and heavy variable region sequences in the desired orientation identified by restriction mapping and purified."

32. Reading this, a person familiar with basic molecular biology techniques would immediately see several points that would erase any doubt about the procedures being described.

- First, because separate plasmids are being produced, the "genetic constructs" used for expression will contain only one cDNA encoding either the light or heavy chain variable region polypeptide, not both.
- Second, because the same plasmid (pGM1) having the same regulatory elements and antibiotic resistance gene is used to prepare the two plasmids (i.e., the pGM1H plasmid containing the heavy chain sequence, and the pGM1L plasmid containing the light chain sequence), the two plasmids are clearly not being incorporated into the same host cell. The PstI site used in the pGM1 plasmids lies within the ampicillin resistance gene. *See* F. Bolivar et al., *Gene* 2:95-113, 95 (1977). Inserting a cDNA at this site will render the ampicillin resistance gene non-functional. Host cells transformed with this plasmid thus will be resistant to tetracycline, but not ampicillin. Transforming a single cell culture with both plasmids would make little sense in this process as it is described. One cannot use tetracycline to select "double transformants" using the methods described because of the plasmid incompatability mechanism discussed above. Furthermore, using only tetracycline, it would not have been possible to differentiate the host cells that had been transformed with the first plasmid, the second plasmid, or both. Thus, any reading of the '414 application reveals a strategy where only a single plasmid is to be propagated in an individual clone.
- Third, as I explained above, the few bacterial clones in the culture that might have incorporated two different plasmids would quickly be outnumbered by clones containing only one of the plasmids. The culture would then become uniform and not contain any of these "double transformants" – especially since no selective pressure for double transformants could be exerted by the scheme that has been described.

- Finally, the use of restriction mapping as described to confirm successful transformation indicates to me that each host cell is being transformed with, and will contain, only one plasmid containing one cDNA encoding a single polypeptide sequence.

33. The example also follows the procedures outlined earlier in the application for isolating the individual light chain polypeptides and combining them *in vitro* to form the rFv. Specifically, at page 41, lines 29-35, the application refers to plural column extracts ("the supernatants are passed over the immunoabsorbant columns"), and at page 52, lines 1-3, it refers to the process of mixing these separately prepared eluates to form the rFv ("the renatured heavy and light chains of the rFv are further purified by combining the eluates containing the rFv components").

34. As I indicated earlier, I do not believe a person familiar with basic molecular biology techniques and concepts in early April of 1983 could have read these various sections of the application and in any way conclude the application is describing a procedure for coexpression of heavy and light chain variable region sequences in a single transformed host cell.

35. The PTO indicates that it interprets portions of the application as stating that individual bacterial clones containing DNA encoding both the heavy and the light immunoglobulin chains will be produced. For example, in the Office Action at page 20, the PTO states that there is a disclosure of "a 'host cell' transformed with a single genetic construct ... or two separate constructs ... comprising DNA encoding variable light and heavy chains." The PTO indicates specific passages in the '545 patent support this reading. I do not agree.

36. As I explained earlier, the procedures outlined in the application will not produce any single cDNA that contains heavy and light chain sequences. The cDNA being amplified during the initial steps is produced via reverse transcription of an mRNA extract. None of the mRNA transcripts will contain sequences corresponding to both immunoglobulin chains because the immunoglobulin chains are encoded by separate genes. Consequently, none of the cDNA molecules will contain heavy and light chain

sequences. Similarly, the procedures for producing the tailored cDNA make it clear that the cDNA will not contain heavy and light chain sequences. Also, the application contains no description of a procedure where two different cDNA sequences are incorporated into a single genetic construct (e.g., a single plasmid). Instead, both in the general description and in the example, each cDNA is incorporated into a separate plasmid. Thus, there is no description in the '414 application of a procedure for producing two different polypeptides in a single transformed host cell. I could find nothing that supports the PTO's view that the '414 application describes a host cell that contains a single genetic construct or two separate constructs containing cDNAs encoding variable light and heavy chains (pages 20-21 of the Office Action). The sections of the patent the PTO identifies certainly do not describe what the PTO states.

- Col. 5, lines 32-35, is simply indicating that many vector choices were available for amplification and expression of cDNA sequences. This is the opening sentence of a section of the patent that is explaining a process where cDNAs in a library are amplified. The "host cells" made during this process each incorporate a single plasmid, into which has been inserted a single cDNA from the cDNA library. None of these cells will contain a single plasmid with two cDNA inserts, or two plasmids each with a different cDNA.
- Col. 7, lines 39-50, is describing the steps where the "hybrid" plasmid containing the "mismatched" DNA strands is being replicated. The mismatched double stranded sequence separates during division of the transformed cell, and forms two sets of double stranded cDNA, one with the native sequence, and one with the tailored sequence. Each new plasmid is contained in one daughter cell; this is basic cell biology. As I explained above (see paragraph 31), the sections preceding this passage clearly indicate that a single cDNA encoding either the light or the heavy chain variable region is used to prepare the "mismatched" double-stranded cDNA. There is no indication here that two different cDNAs are to be inserted into a single host cell for amplification.

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- Col. 10, lines 1-5, is describing the process of inserting start and stop codons at either end of the coding sequence for the variable region polypeptide. This is not describing a host cell transformed with a single genetic construct containing cDNA encoding light and heavy chain variable region polypeptides, or two plasmids that encode, respectively, light and heavy chain variable region polypeptides.

- Col. 23, lines 35-45, is describing an example corresponding to the "hybrid plasmid" replication step outlined at col. 7, lines 39-50. This section concerns cDNA cloning and amplification, not protein expression. As I explained above (see paragraph 19), the process of generating "tailored" sequences involves insertion of a plasmid containing a mismatched double-stranded cDNA sequence into a host cell. When the cell replicates, it produces daughter cells that contain double stranded copies of the two mismatched cDNA sequences. In addition, it is absolutely clear that each of the tailored cDNA sequences is inserted into a separate plasmid, and only one plasmid is incorporated into each transformed cell. Each of these cDNA inserts (and thus each plasmid) encodes only one of the two immunoglobulin polypeptides. There is no description at this point of the patent of a host cell with one plasmid that has incorporated two different cDNA inserts, or two plasmids each with one insert.

- Col. 24, lines 50-60, outlines the procedure which produces the two plasmids, pGM1L and pGM1H, each containing, respectively a cDNA encoding the light chain variable region polypeptide, and the heavy chain variable region polypeptide. As I explained earlier, the host cells transformed with these plasmids will each contain one plasmid, not two.

- Col. 11, lines 5-12, is describing the attributes of rFV binding proteins (i.e., that they are "polypeptide duplexes of the variable region of a light and heavy chains of immunoglobulins, retaining the specificity of immunoglobulins"). It also identifies the benefits of these molecules (e.g., smaller size, less immunogenic than complete immunoglobulins). There is nothing in this section of the patent that discusses host cells, genetic constructs, or coexpression concepts.

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37. I also could find nothing in the sections of the disclosure identified at page 20 of the Office that describes "a method of making an immunologically functional fragment ... comprising independently expressing in a host cell variable heavy and light chain domains ... lacking constant regions."

- Col. 1, lines 33-42, is generally describing the characteristics of an immunoglobulin. There is no discussion in this part of the patent relating to expression of recombinant DNA or host cells.
- Col. 3, lines 59-63, is identifying how to obtain desired antibodies through conventional immunization and hybridoma production techniques. There is nothing in this section concerning expression of recombinant DNA or host cells.
- Col. 17, lines 4-8, has a description of the amino acid and nucleic acid sequences of a light chain variable region. This passage does not discuss host cells, genetic constructs, or methods of expressing polypeptides.

38. Instead, based on my careful review of the entire '414 application (which I understand to be the part of the '545 patent that was filed with the PTO in March of 1982), I could find nothing that suggests "coexpression" of heavy and light immunoglobulin chains in a single transformed host cell. Instead, in my opinion, the processes being described very clearly outline a procedure where individual heavy or light chain variable region polypeptides are being separately produced in different cell cultures, and then combined outside the cells to form the rFv.

39. I also could find nothing in the '414 application that could be reasonably interpreted by someone with basic training in molecular biology techniques as describing a coexpression protocol. Specifically, I found nothing that would be consistent with known molecular biology principles that would indicate that:

- a single cDNA, either "native" or "tailored," encodes both heavy and light chain sequences;

- a single genetic construct incorporating two distinct cDNA molecules encoding light and heavy chain polypeptides or variable region polypeptides is produced; or a single host cell has been transformed with and will retain two different plasmids.

40. Taking all of the points above into account, I could find no description in the '414 application of a host cell that meets the requirements of claim 1 of the '545 patent (i.e., that contains and expresses cDNA sequences encoding two different immunoglobulin chain variable region polypeptides, as the PTO suggests), or claim 2 of the patent (i.e., a process which calls for expression in one transformed host cell of two different cDNAs encoding light and heavy variable region polypeptides, via use of a plasmid that has the two cDNA sequences in it, as the PTO suggests).

Discussion of the Findings of Obviousness of the Coexpression Procedures in the '415 Patent

41. I have been asked to review the statements of the PTO in the Office Action relating to the question of "obviousness" of the co-transformation procedures outlined in the '415 patent claims. To do this, I have reviewed the Office Action, the expert declarations of Drs. Harris, Rice and Colman, the previous communications from the PTO and the patent owners, and the literature being discussed in these communications.

42. The PTO outlines several different theories why it believes the coexpression processes claimed in the '415 patent would have been obvious to a person of ordinary skill in the art in early April of 1983 from the information in the '545 patent. *See* pages 12 to 15 of the Office Action. As I explained above, I did not find any description in the 1982 version of the '545 patent (i.e., the '414 application) of a procedure where light and heavy chain polypeptides are produced in a single host cell. There also is no description in that application of a host cell that has been transformed with two different plasmids, each containing a cDNA encoding one of immunoglobulin chains, or a host cell transformed with a single plasmid that contains two different cDNA inserts. Instead, the process described in the application calls for independent production in separate host cell cultures of the light and heavy variable region polypeptides, followed by assembly of those separately produced chains *in vitro* to form an rFv.

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43. This makes it clear to me that the PTO's conclusion that the '415 processes would have been obvious from the '545 patent is incorrect. I also do not agree that these processes would have been obvious to a person of skill in the art who reviewed the '545 patent disclosure with the Axel patent and the Accolla publication, as the PTO states. Neither of those documents discusses recombinant expression of heavy and light chains in a single host cell. There is no connection I can see between these different publications that would have made the coexpression processes in the '415 patent obvious in early April of 1983.

44. Another PTO theory (Office Action, pages 15-25) is that the '567 patent claims, which define a process of producing either a chimeric light chain or a chimeric heavy chain in a host cell, when considered with the '545 patent disclosure, would have made the coexpression procedures of the '415 patent claims obvious to a person of ordinary skill in early April of 1983. The PTO is again reading the '545 patent disclosure as teaching something that it does not, namely, a method which produces within a single host cell both the light and the heavy chain variable region polypeptides. Since the PTO is not correctly reading the '545 patent disclosure, I do not believe its obviousness conclusion is sound. I also do not believe that the additional information in the Axel and Accolla publications would change the opinion of a person of ordinary skill in early April of 1983, because neither of these documents describes a procedure for coexpressing heavy and light chain polypeptides in a transformed host cell.

45. Beginning at page 26 of the Office Action, the PTO outlines a more complex set of theories why it believes the coexpression process defined in the '415 patent claims would have been obvious. I understand that all of these theories are grounded on an initial view that the '567 patent claims define a process for producing either a chimeric heavy chain or a chimeric light chain in a host cell. The remaining references are then used by the PTO to support its opinion that a person of ordinary skill, in early April of 1983, would have been encouraged to express both heavy and light chains in one host cell. The reasons that the PTO identifies are set out at pages 29 to 34 of the Office Action. I understand that the PTO also has identified a number of publications to support its opinion that several aspects of the process of producing heavy and light

immunoglobulin chains in one host cell would have been predictable to a person of ordinary skill in the art in early April of 1983. These are outlined at pages 35 to 36 of the Office Action.

46. I believe each of these reasons is based on an inaccurate reading of the particular document being discussed. I also believe that the PTO is extrapolating the results reported in these documents in a way that a person of skill in the art in early April of 1983 would not have. For example, I do not believe a person of ordinary skill in the art in early April of 1983 would have made the assumptions that the PTO has made in its reading of these documents, or would have had the degree of confidence that seems to be reflected in the PTO's statements about likelihood of successfully coexpressing heavy and light chains in a single transformed host cell.

47. Finally, I note that the PTO refers to the '545 patent as providing an additional motivation to produce heavy and light immunoglobulin polypeptides in one host cell. Again, I believe this is based on the PTO's incorrect analysis of the contents of the '545 patent.

The Axel Patent Would Not Have Rendered Coexpression of Heavy and Light Chains Obvious

48. The Axel patent describes procedures for "co-transforming" a eukaryotic cell with a foreign "DNA I" (which codes for proteinaceous material which is not associated with a selectable phenotype) and "DNA II" (which codes for a protein that confers a selectable phenotype not expressed by the eukaryotic cell). The polypeptide encoded by DNA II is not recovered from the host cell, but introduces a new phenotype into the transformed cell that allows it to be selected or identified.

49. The PTO indicates that the Axel patent discloses and claims the expression of antibodies in mammalian hosts "as intact (assembled) antibodies." *See* Office Action, pages 29-30. Moreover, the PTO indicates that because the Axel patent "clearly encompasses one *or more* genes which encode one or more proteins" and "an antibody [] necessarily possesses both light and heavy immunoglobulin chains" the Axel patent suggests a procedure where immunoglobulin heavy and light chains are coexpressed in one

transformed host cell. See Office Action, page 51. I believe the PTO is not accurately reading the Axel patent.

50. It is very clear to me that the Axel patent is describing procedures where only a single protein of interest is being produced. The scheme describes co-transformation using only two DNAs. Only one of the two DNAs encodes the protein of interest (i.e., DNA I). The other DNA (DNA II) introduces a selectable phenotype into the cell. DNA II must encode a gene that imparts a selectable phenotype into the cell – if the cells do not incorporate and express this DNA, the cells cannot be differentiated from cells that have not been transformed. *See* for example, col. 4, lines 61-66.

51. Even more telling is the absence of any discussion in the Axel patent of strategies or techniques for producing multiple polypeptides in a single transformed host cell. Given when the Axel patent was written (February of 1980), a person of skill in the art would find it hard to believe that producing two different polypeptides of interest in a single host cell would be so trivial that even the idea of doing so would not have been mentioned.

52. There also is no mention of a strategy in the Axel patent for incorporating into "DNA I" multiple DNA sequences encoding different polypeptides. This stands in stark contrast to sections in the Axel patent which discuss other general expression strategies. For example, the Axel patent has sections which discuss ways of increasing the odds of successful transformation and expression of foreign DNA sequences. See, e.g., col. 4, lines 61-66 and col. 7, lines 3-9, which discuss whether DNA I and DNA II should be "unlinked" or "linked," and column 5, lines 45-50, which discusses varying the ratio of copies of DNA I to DNA II to maximize production of the protein of interest.

53. If the Axel patent had intended DNA I to include two different coding sequences (e.g., encoding heavy chain and light chain immunoglobulin genes), I would have expected it at least to have mentioned the nature of the genetic construct that should be used. Similarly, if the Axel patent had contemplated that its procedure could be extended by transforming a host cell with a third DNA sequence (DNA III), it would at least have mentioned this clearly somewhere in the patent. Neither point is even mentioned in the Axel patent.

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54. All of the examples in the Axel patent use the two DNA system, with DNA I encoding only one polypeptide. *See* col. 9, line 57 through col 42, line 31. This is consistent with describing procedures for producing only a single protein of interest.

55. In the Office Action (Page 13, lines 8-19), the PTO indicates that the Axel patent must be describing production of heavy and light chains of an antibody in one host cell because it lists "antibodies" as one of the proteins that can be produced using the procedure. I disagree. The Axel patent simply lists "antibodies" as one of many types of proteins that can be produced. The way that the patent mentions this does not suggest anything about coexpressing the two antibody chains in one host cell or producing an intact, assembled antibody. In my opinion, a person skilled in this field would read this passing reference to "antibodies" as simply indicating that antibody polypeptides (i.e., heavy or light chains) can be produced by the Axel procedure.

56. The PTO suggests that a person of ordinary skill in the art would consider expression of a gene encoding a protein of interest along with a "marker gene" to be equivalent to the concurrent production in a single transformed host cell of two or more constituent polypeptides of a multimeric protein, such as an antibody. For example, page 50 of the Office Action states that "the patentee's arguments fail to address the *combined* teaching by Axel of coexpressing two separate proteins (an exogenous gene of interest and an exogenous reporter gene) in a single eukaryotic (mammalian cell) in the context of producing an antibody with the Cabilly I patent teaching of the separate expression of heavy and light chains." I do not believe a person of ordinary skill in the art would have held this opinion, particularly in early April of 1983.

57. Under the Office's rationale, there would be no selectable marker expressed in the transformed host cell. This is because DNA I would encode the light chain and DNA II would encode the heavy chain. In this scheme, there would be no mechanism to select cell populations that have effectively taken up and expressed the exogenous DNA.

58. As Axel explains, the expression product of the marker gene, unlike a protein of interest, must change the phenotype of the cell (e.g., to make it resistant to a chemical agent). The phenotype also must be maintained during the culturing process to allow the desired

selective pressure to have effect (i.e., to cause only the cells with the marker gene to propagate).

59. The PTO states at pages 29-30 of the Office Action that the Axel patent abstract; column 5, lines 3-7 and 24-28; and patent claims 1, 7, 22-24, 28, and 29 show expression of antibodies in mammalian host cells as "intact (assembled)" proteins. I have reviewed these sections of the Axel patent and found no reference to "intact" or "assembled" antibodies. I also could find no references to "intact" or "assembled" antibodies anywhere else in the Axel patent.

60. The PTO also refers, at page 51 of the Office Action, to the abstract of the Axel patent as disclosing "DNA which includes a gene or genes coding for desired proteinaceous materials." In my opinion, the PTO is not reading the abstract accurately. Instead, I believe a person skilled in the art would understand that is referring to the idea of inserting multiple copies of the same gene encoding a single polypeptide of interest, as explained in the Axel patent at column 6, lines 44-66.

61. I read the Axel declaration dated August 26, 1999, that is attached to the Office Action. It is clear to me that Dr. Axel attributes the guidance needed to extend the procedures in the Axel patent to make a recombinant antibody or antibody fragment through coexpression to the information in the '415 patent. *See* Axel Declaration, paragraph 7.

62. In view of all of these points, I do not agree with the PTO's conclusions that a person of ordinary skill would have considered the information in the Axel patent to provide any motivation to modify the procedures for individual chimeric chain production specified in the '567 patent. Given the uncertain nature of the procedures governing expression of foreign DNA in host cells and the lack of information in Axel about procedures or techniques focused on production of multiple proteins of interest, I believe a person of ordinary skill would have not have reached the conclusions that the PTO states.

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The Rice Publication Does Not Make Obvious Coexpression of Exogenous Heavy and Light Chains in a Single Host Cell

63. The *PNAS* paper by Rice and Baltimore reports that a rearranged light chain gene could be introduced into and expressed in a lymphoid cell line (i.e., the 81A-2 line, a mutant murine lymphoid cell line that synthesizes only its endogenous heavy chain, and has lost its kappa constant region genes). *See* Rice, page 7862, left column. The details of this experiment, and the observations of the authors of the paper, reflect the high degree of unpredictability that existed in this field at the time.

64. The PTO states that this paper demonstrates the successful expression in a single host cell of light and heavy immunoglobulin chains, and the successful assembly of the chains into an immunoglobulin tetramer. *See* Office Action, page 30. The PTO interpretation of the experimental results and findings in the Rice paper are inaccurate for several reasons.

65. Importantly, the experiments in Rice are not focused on making proteins. Instead, they are focused on understanding the processes in lymphocytes that affect expression of immunoglobulin genes. Given this focus of the paper, a person of ordinary skill in the art would not attach much, if any, significance to the report in Rice of the possible association of the exogenous light chain gene expression product with the endogenous heavy chain polypeptide.

66. In fact, the Rice paper raises more questions than it answers concerning the transcriptional regulation of the exogenous light chain gene. The PTO has failed to appreciate this uncertainty in the paper. The PTO has apparently overlooked the observations in the paper in which the authors question why they saw successful expression of the exogenous light chain gene in their experimental cell line. For example, the paper observes that the light chain gene they introduced contained uncharacterized control elements which the authors suggest were playing a role in directing expression of the introduced gene. *See* page 7865, lines 9-11. They also suggest that expression of the exogenous light chain gene could have been influenced by the continued expression of the endogenous heavy chain gene in the cell line they used. *See* page 7865, lines 26-37.

67. The experimental result reported in Rice is that a mature lymphoid cell line expressing its endogenous heavy chain gene but having lost its endogenous κ light chain constant region genes could be made to express an exogenous light chain gene. In my opinion, a person skilled in this field would not have extrapolated this limited experimental result to other experimental models, particularly the expression of exogenous light and exogenous heavy genes in a lymphoid cell or other types of cell lines. To do so would require the person to assume that the control mechanisms that made expression of the light chain gene possible in Rice's experimental system, as well as what was not known about those mechanisms at the time, were unimportant. This assumption would run counter to what is actually reported in the paper, and the fact that Drs. Rice and Baltimore pointed out that these were issues that required further research.

68. The PTO also is improperly equating expression of an exogenous gene in a lymphoid cell (the introduced light chain gene) with the continued expression by the cell of one of its endogenous genes (the endogenous heavy chain gene). Particularly for a cell of lymphoid origin, the developmental state of the cell profoundly affects its ability to express various cell type-specific genes. M. C. Howard, *CRC Critical Reviews in Immunology* 3(3):181-208, 190 (1982). For example, by April of 1983, it was known that the expression of immunoglobulin genes in a B cell occurs only in specific steps as the cells differentiate and mature. The factors that regulate the expression of immunoglobulin genes were, as the Rice paper notes, poorly understood in 1983. Based on this state of knowledge, a person of skill in this field would not have assumed that expression of exogenous heavy and light chain genes could be successfully introduced into and expressed in a lymphocyte.

69. In my opinion, the PTO's views are inconsistent with the views of a person of ordinary skill in the art in early April of 1983. In fact, I believe they are inconsistent with the views of the authors of the Rice paper. For example, the authors observed that uncharacterized or novel mechanisms in the transformants regulated the expression of the light chain gene they introduced. The logical implication of this observation is that a person of ordinary skill in the art would have concluded that those regulatory mechanisms should be deciphered and understood before attempting to extend the

experimental model (e.g., by attempting to express another exogenous immunoglobulin gene).

70. In addition, I believe the reported association of the exogenous light chain gene expression product with the endogenous heavy chain gene in the Rice paper would not have created broader expectations about immunoglobulin assembly, as the PTO states. In this respect, I disagree with the PTO's interpretation of Dr. Baltimore's declaration at page 53 of the Office Action. Dr. Baltimore states his expectation that "if two [immunoglobulin] chains were expressed [in the same mammalian cell], they would form a functional antibody." What Dr. Baltimore carefully avoids stating is whether he believed at the time it would have been possible to successfully transform and express exogenous heavy and light chain genes in a mammalian cell line. Thus, I conclude that Dr. Baltimore's observations are of limited relevance to understanding what a person of ordinary skill would have expected regarding the prospects for co-transforming a host cell with heavy chain and light chain immunoglobulin genes and causing it to produce assembled antibody proteins.

71. For example, in the time frame of the Rice paper, there was extensive literature documenting the complexity of the expression of immunoglobulin genes and the assembly and secretion of immunoglobulins in lymphocytes. Much of the literature related to work done in hybridomas and other stable lymphocyte cell lines. This work would have led a molecular biologist to conclude that how and when heavy and light chain genes were expressed in the cell would affect the assembly of an immunoglobulin tetramer, as well as the viability of the cell. *See, for example,* M. Wabl et al., *Proc. Nat'l Acad. Sci. USA* 79:6976-6978 (1982); I.G. Hass & M. Wabl, *Nature* 306:387-9 (1983); L. Hendershot et al., *J. Cell. Biol.* 104:761-7 (1987); G. Kohler, *Proc. Nat'l Acad. Sci. USA* 77(4):2197-2199 (1980); C. D. Wilde & C. Milstein, *Eur. J. Immunol.* 10:462-7 (1980).

72. In view of all of these points, I do not believe the Rice paper would have led a person of ordinary skill in the art to assume that the individual chain expression procedures of the '567 patent claims could be extended with any degree of confidence. Instead, the Rice paper demonstrated that there was a substantial amount of unpredictability associated with expression of immunoglobulin genes. The Rice publication does not resolve any of

this unpredictability. Instead, its authors emphasize that the factors governing expression of immunoglobulin genes in lymphocytes were not known or characterized, but were subjects requiring additional research.

The Kaplan Reference Does Not Suggest Coexpression

73. The PTO states at pages 30 of the Office Action that the Kaplan publication "teaches that a variety of host cells (e.g. bacteria and yeast) and plasmids (particularly pBR322) may be used to express recombinant heavy and light chains." Moreover at page 55 of the Office Action the PTO suggests that the Kaplan publication provides a "roadmap" for recombinantly making antibodies through coexpression of the heavy and light chains in one host cell.

74. Expression of heavy and light immunoglobulin chains in a single bacterial or yeast host cell is not described in Kaplan.

75. Pages 9 and 10 of Kaplan outline, in extremely general terms, a procedure for expressing immunoglobulin heavy or light chain cDNAs produced using mRNA isolated from human hybridomas. The use of mRNA as the starting point of the process reveals that the procedures in Kaplan, like the procedures in the '545 patent, will not involve production of cDNAs containing both heavy and light chain sequences. As I noted above, the heavy and light chain genes are separate and will be transcribed separately in the cell.

76. There is no suggestion in Kaplan to produce a genetic construct that contains both heavy and light chain cDNAs. Instead, the procedure described in Kaplan follows a conventional model of one protein per host cell. There also is nothing in Kaplan to outline a procedure in which host cell cultures would be transformed with, and would maintain clones having multiple plasmids. And, critically, the Kaplan procedure, like the '545 patent process, calls for assembly of the immunoglobulin *in vitro* after the heavy and light chains have been produced. *See* page 10, lines 27-33.

77. The roadmap that I see in the Kaplan publication calls for individual production of the heavy and light chain sequences in separate host cell cultures. I do not agree with the

PTO that the Kaplan publication describes, much less suggests a coexpression procedure. I do not believe a person of ordinary skill in the art would have been motivated by Kaplan to change the procedures outlined in the '567 patent claims where individual heavy or light chimeric immunoglobulin chains are to be produced in separate host cells. Instead, I believe Kaplan simply reiterates what the '567 patent claims call for – individual expression of the heavy and light chain DNAs in separate host cells.

The Dallas Publication Would Not Make Obvious Coexpression of Heavy and Light Immunoglobulin Chains in a Single Host Cell

78. The Office Action indicates that the Dallas publication describes methods for expressing two different genes (in addition to a selectable marker gene) in a single host cell. *See* pages 30-31, and page 56. The PTO apparently believes Dallas has outlined a broadly applicable procedure for coexpressing different proteins in a single host cell. I do not agree with the PTO's use of Dallas, because I believe that a person of ordinary skill in 1983 would not have considered the Dallas application relevant to the problem of making large eukaryotic proteins, such as immunoglobulins, much less to the problem of producing an antibody through coexpression of two complex eukaryotic proteins in one host cell.

79. The Dallas publication is describing a process for making a new bacterial vaccine. It involves expressing in a bacterial cell one or two bacterial genes. The bacterial genes encode small bacterial proteins, which apparently will be resident on the cell surface of the transformed bacterial cell. This transformed bacterial cell is injected into a farm animal to trigger an immune response to the bacterial antigens encoded by the inserted bacterial genes.

80. Dallas, read literally, is not focused on expressing proteins that are meant to be isolated from the cell. The entire focus of the Dallas publication is on production of vaccines. I believe this would influence how a person of ordinary skill would interpret the significance of the experiments described in Dallas. Specifically, I believe this focus in Dallas would not lead one to believe that the techniques being described would be

broadly applicable to procedures for recombinantly producing and isolating desired proteins from a transformed bacterial cell.

81. The experiments described in Dallas are simple. They involve inserting bacterial genes into a bacterial cell. In addition, the bacterial antigens (K88(ac), K99, and TL-B) encoded by the genes described in Dallas are small, simple proteins, and much different from large complex immunoglobulin proteins.

82. The differences in the complexity of the proteins being made and in the techniques for expressing the inserted DNA, coupled with the fact that Dallas does not call for recovery of its bacterial gene expression products, in my opinion, would have led a person of ordinary skill to not even consider Dallas relevant to the procedures outlined in the '415 patent. Simply put, that person, considering these differences, would not have considered Dallas relevant to production of heavy and light chain proteins in a single co-transformed host cell.

83. I also do not agree with the comments of the PTO at page 56, where they state that "the Dallas reference provides support for the Axel reference teaching of eukaryotic expression of both a light and heavy antibody chains along with the reporter gene for producing an antibody." The Dallas publication, in my opinion, provides no guidance for extending the simple procedures described in it to expression systems in eukaryotic host cells described in Axel and in Rice.

Expectations Set by the Xenopus Oocyte Publications

84. The PTO refers to publications involving experiments where *Xenopus* oocytes are injected with mRNA extracts isolated from antibody-producing cells. *See* Office Action, page 35 and 62. The PTO indicates that, in its view, these experiments would have led a person of ordinary skill in the art to expect that heavy and light immunoglobulin chains, once present in a eukaryotic cell, would assemble and form functional antibodies. In particular, the PTO states:

> Although the above-discussed Deacon and 1981 Valle references utilize m-RNA as compared to the use of vector DNA in the Cabilly 1 claims for encoding the corresponding light and heavy immunoglobulin chains, once

> the m-RNA or vector DNA is expressed, the ability of the two chains to assemble into an immunoglobulin does not depend on the genetic material used for such expression. Accordingly, the difference between using vector DNA and mRNA for host transformation is not substantive.

85. The PTO also states that it considers *Xenopus* oocytes to be "host cells" and that injection of mRNA into a *Xenopus* oocyte to be "host transformation," analogous to incorporation of foreign DNA into a host cell. Office Action, page 62. The PTO's observations are scientifically incorrect and logically inconsistent.

86. A *Xenopus* oocyte would not have been considered to be a "host cell" within the meaning of the '415 patent by a person of ordinary skill in the art in April of 1983. A host cell is transformed by incorporating foreign DNA in a way that will ensure that the progeny of the host cell will contain a copy of the foreign DNA. Host cells also make copies of themselves by cell division. Because both the original host cell and the progeny host cells contain the introduced DNA sequence, they become useful, either for amplification or expression of the introduced DNA sequence. A *Xenopus* oocyte cannot make copies of itself. It either becomes an embryo (if it is fertilized), or it dies. In addition, after the *Xenopus* oocyte translates the injected mRNA, the mRNA is degraded and no longer exists within the cell. As such, injecting mRNA into the cytoplasm of a cell is not equivalent in any sense to incorporating DNA into a host cell.

87. *Xenopus* oocytes also were known to be unique cells. They have an unusual ability to tolerate the microinjection of large quantities of foreign mRNA. The *Xenopus* oocyte is a storehouse of "pre-transcribed" mRNA and protein translation machinery to be used (after fertilization) to synthesize polypeptides for the developing frog embryo. Their large stores of translational factors, coupled with their unique ability to store endogenous mRNA made these *Xenopus* oocytes an ideal experimental platform for translation experiments. J. B. Gurdon & D. A. Melton, *Ann. Rev. Genet.* 15:189-218, 192 (1981).

88. The mRNA found in the cytoplasm of cells that proliferate is limited to mRNA that is actively being translated. In such cells – for example, differentiated host cells – mRNA is present in the cytoplasm only transiently, and only during the active translation of the message into protein. *See, e.g.,* Ross, *Microb. Rev.* 59(3): 423-450 (1995). This cellular environment is not at all analogous to the environment of the *Xenopus* oocyte, where

large amounts of mRNA are stored in the cytoplasm, translation of the stored mRNA has been suppressed, and components of the protein translation machinery (e.g., ribosomes) are abundant.

89. Microinjection experiments in *Xenopus* oocytes do not result in a cell that replicates the cellular environment of a host cell that is translating an mRNA transcribed from integrated DNA. In the microinjection experiment, a very high local concentration of mRNA is injected into a compartment of the oocyte that is prepared to receive and translate the mRNA. In a cell producing protein after transcription of a gene, a very different pathway and a very different cellular environment is observed.

90. The ability of a transformed host cell to incorporate "genetic information" in a way that allows such information to be passed on to its progeny is central to its role as a protein production system. DNA that is introduced into the cell must replicate along with the cell's DNA so that the information is stably maintained as the cell proliferates. The introduced genetic information must also remain accessible to the machinery of the cell and capable of being expressed. This relates not only to transcribing the DNA into mRNA in the nucleus of the cell, but also transporting the mRNA into the cytoplasm for translation at levels that do not impair the viability of the cell.

91. Cells process endogenous RNA transcripts using specialized mechanisms. These mechanisms process and deliver mRNA to the proper location in the cell, in proper amounts, and with the correct timing to enable the cell to use its protein translational machinery to convert the mRNA into a polypeptide. The mRNA microinjection process entirely bypasses this process. The experiments reported in Deacon and Valle 1981, which use a poly(A+) mRNA extract from a lymphocyte, "import" the result of successfully expressing the endogenous immunoglobulin genes in the lymphoid cell. As such, a person of ordinary skill in the art would not consider the microinjection of mRNA into a *Xenopus* oocyte as the equivalent of a process of transforming a host cell with DNA encoding a polypeptide sequence of interest.

92. In my opinion, a person of ordinary skill in the art would consider these distinctions between *Xenopus* oocytes and transformed host cells, and between transformation with

DNA and microinjection with mRNA, to be very substantial. Given what was known in early April 1983 about the interrelationship between the timing and levels of expression of immunoglobulin genes and the ability of lymphocytes to successfully produce immunoglobulins, a person of ordinary skill would not dismiss these differences as not being "substantive." In addition, I believe the PTO fails to appreciate the significant difference between "using vector DNA" for "host transformation" and microinjecting mRNA – which does not result in "host transformation" of the *Xenopus* oocyte. *See* Office Action at page 36.

93. I also disagree with the PTO that any person skilled in the art, after reading the '415 patent disclosure, would conclude that a *Xenopus* oocyte would be considered, by any stretch of imagination, to be a "host cell" as that concept is used in the patent. As I explained above, a host cell must be able to incorporate introduced DNA in a stable manner that allows the DNA to replicate, and pass it on to its progeny. A *Xenopus* oocyte cannot carry out any of these functions. It is simply not a transformed host cell.

94. A person of ordinary skill in the art would immediately recognize the significant differences between experiments involving microinjection of mRNA into *Xenopus* oocytes, and expression of foreign DNA in a genetically transformed host cell. Accordingly, I do not agree that such a person would extrapolate the results from Deacon and Valle 1981 as the PTO has at pages 35 to 36 of the Office Action.

Expectations Set by the Ochi and Oi Publications

95. The PTO states that Ochi provides "further motivation" to transform a single host cell with genes encoding both the light and the heavy chain of an immunoglobulin. It observes that Ochi "restored specific antibody production by cloning light immunoglobulin chain into a cell line endogenously producing heave immunoglobulin chain." Office Action at page 35. Based on this information, the PTO concludes that Ochi "provides further motivation to perform the Cabilly 1 patented steps in a single host cell for producing a chimeric heavy and light chain … with a reasonable expectation of success."

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96. I believe the PTO's conclusion is based on a misunderstanding of the work being described in the Ochi reference. The abstract of the article (page 340) explains that:

> The technique of modifying cloned genes *in vitro* and transferring the modified genes to cells in culture provides a tool for identifying the structural features required for gene expression. To analyze immunoglobulin genes in this manner, however, it is first necessary to use, as recipients, cells that normally permit immunoglobulin production.

97. Thus, as the abstract of the Ochi paper states, the experiments reported in Ochi were performed to study the expression of immunoglobulin genes. It is important that the experiments were designed to examine the expression of a *single* introduced immunoglobulin gene – in this case, the introduced κ light chain gene. This is because an attempt to introduce two exogenous immunoglobulin genes into a single host cell would have introduced many uncontrolled variables into the experiments. Not least among those variables would be the possibility mentioned by Drs. Rice and Baltimore in their *PNAS* paper, that expression of the light chain gene might be directed or influenced by regulatory elements associated with the heavy chain gene. See Rice at page 7865. Demonstrating that the expression of *one* missing gene could be restored was what made the system described by Ochi *et al.* useful for studying "the structural features required for gene expression."

98. Ochi *et al.* noted that the levels of expression of the introduced κ gene that were observed varied considerably from experiment to experiment. They state (page 341, left column):

> It is interesting that the transformant R31L4 makes more κ_{TNP} chain than does T3L2, although T3L2 has more copies of the κ_{TNP} gene. Furthermore, R31L4 makes about 10-fold less κ_{TNP} chain per gene copy than does the wild-type hybridoma, although it should be pointed out that we do not know if all copies of the κ_{TNP} gene function equally efficiently. This variability in gene expression raises the question of whether all the regulatory elements of the normal κ_{TNP} gene are present or functioning in the cloned fragment.

99. It is clear to me from this discussion that much experimentation remained before the transformant system described in the Ochi paper might be used to obtain predictable expression of even a single heterologous light chain gene, let alone introduced heavy *and* light chain genes. Indeed, the authors stated that further research was underway to resolve some of the questions raised by their initial experiments.

100. The authors of the Ochi paper note the necessity of performing their experiments in "cells that normally permit immunoglobulin production." The igk-14 host cell that they used provided an ideal vehicle for the experiments they described, since it appeared to be identical to the parental Sp603 hybridoma except for its failure to produce a κ light chain product. Ochi does not describe any "analogous" cell lacking the capacity to produce both a heavy chain and a light chain, but otherwise identical to a closely related antibody-producing cell.

101. The *PNAS* paper by Oi *et al.* describes experiments that are similar to those described in Ochi. I note that the PTO considers that they are effectively equivalent for assessing obviousness with respect to the methods claimed in the '415 patent. *See* Office Action at pages 3-4.

102. Oi observed expression of an introduced immunoglobulin κ gene in two lymphoid cell types, a myeloma line and a hybridoma line. However, Oi reported that the κ chain expression product was only secreted in the hybridoma, which was poised to synthesize – and in fact was synthesizing – an endogenous IgG antibody. The authors also report that two other lymphoid cell lines, a myeloma and a thymoma, failed to express an exogenous κ light chain gene. In my opinion, the information and analysis in Oi does nothing to resolve the basic questions or lessen the unpredictability conveyed by the Ochi paper.

103. I believe the PTO makes a fundamental mistake when it assumes that the results described in the Ochi paper relating to the restoration of a single immunoglobulin light chain could be extrapolated to a further experiment to "restore" the expression of both a heavy and a light chain in a cell type that expresses neither gene. It compounds that mistake by discounting the experimental evidence, as well as the authors' analysis, of the variability and unpredictability associated with the expression of a single exogenous light chain gene as described in the Ochi paper. I do not agree that a scientist of ordinary skill working in this field in April 1983 would have read Ochi to "motivate" any of the experiments that the PTO concludes are obvious. I also do not agree that the experiments described in Ochi provide any "reasonable expectation of success" for expressing two exogenous immunoglobulin genes in the same host cell, as the PTO suggests.

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Conclusions

104. I have considered all of the evidence cited by the PTO together. In particular, I have taken account of the Axel, Rice, Kaplan, Builder, Accolla, Dallas, Deacon, Valle 1981, Ochi, and Moore references. The PTO concludes that in view of all of these references, it would have been "obvious" to make chimeric immunoglobulin heavy and light chains, each as in the claims of the '567 patent, in a single host cell, using the methods delineated in the claims of the '415 patent. The PTO further states that these references would have given someone of ordinary skill in the art a reason to construct a coexpression system, and would have provided a "reasonable expectation of success" that such a system would work. I disagree.

105. I do not see any way that a practicing scientist in the field of protein expression in early April of 1983 would have merged the disparate aspects of antibody technology and molecular biology reflected in the various references that the PTO cites to bridge the gap between the claims of the '567 patent and the claims of the '415 patent.

106. The reasoning that the PTO uses to arrive at its conclusion depends on many assumptions about what a person of ordinary skill would have understood from these references, and how such a person might have thought that the results described in the papers could be extended. As I explain in detail above, many of the PTO's assumptions are based on misreadings of what the references actually describe.

* * * * * * * * * * *

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.

May 20, 2007
Date

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-Howard, CRC CRIT. REV. IMMUNOL. 3(3): 181-208 (1982)

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