IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Cabilly et al v. Boss et al	Interference No. 102,572 Examiner-in-Chief Mary F. Downey

DECLARATION OF RONALD WETZEL

1. I, Ron Wetzel, declare and state that I am a citizen of the United States residing in Phoenixville, Pennsylvania. My Curriculum Vitae is attached as Cabilly Exhibit No. 5. I am a co-inventor of the United States Patent 4,816,561 entitled "Recombinant Immunoglobulin Preparations".

2. From November 1, 1978, to April 15, 1989, I was a Senior Scientist at Genentech, Inc. (Genentech) located at 460 Pt. San Bruno Blvd., South San Francisco, California 94080. During that time I was responsible for conducting experiments, supervising others in conducting experiments, investigating methods to produce proteins by recombinant means, characterizing protein structures and protein structure/function relationships.

3. I am currently employed by SmithKline Beecham Pharmaceuticals, in King of Prussia, Pennsylvania as a Research Fellow in the Macromolecular Sciences Department.

4. In the fall of 1982, Dr. Herb Heyneker approached me to discuss developments in a research project to express immunoglobulins in E.coli. At that time I knew that such a project was going on at Genentech. I was aware that Dr. Art Riggs from the City of Hope, in Duarte, California, had spent a sabbatical period at Genentech doing initial work in the cloning of the cDNA from a hybridoma cell line producing an antibody. I also was aware that Dr. Heyneker had been working with him during that time, and that Dr. Heyneker's group was involved in the project after Dr. Riggs returned to the City of Hope.

5. Dr. Heyneker told me that his group, working with Shmuel Cabilly, a post-doctorial fellow in Dr. Art Riggs laboratory at the City of Hope, had expressed

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immunoglobulins chains directed against human carcinoembryonic antigen in E.coli. Since a major hurdle of the expression of immunoglobulin was expected to be in the folding of the protein, and since I had recently had some success in folding other recombinant proteins in my lab, it seemed to me that my group might be able to contribute to the project.

6. Jeanne Perry, a research associate in my lab, and I began work on the E.coli immunoglobulin project on or about January, 1983. I have reconstructed the course of our experiments on immunoglobulin chains given below by examining Jeanne Perry's and my notebooks from this time (Cabilly Exhibits No. 6, 8 and 9).

7. I began work on January 16, 1983, by attempting to isolate and purify the immunoglobulin heavy chain and light chain produced in two different E.coli strains from cell pastes which I received from Mike Mumford (Cabilly Exhibit No. 18, Bates Nos. 00701 and 00727). We solubilized the inclusion body material in a guanidine hydrochloride solution followed by S300 gel permeation chromatography. Jeanne Perry and I continued to try to make purified preparations of immunoglobulin chains, after converting them to their S-sulfonate derivatives. DEAE chromatography in urea was tried as a follow-up step to the S-300 column to further purify the proteins.

8. On January 25, 1983, (Cabilly Exhibit No. 6, Bates No. 0042-0044, 0047, 0050, and 0064). I began a refolding experiment using our purified preparations of light and heavy chains. However, analysis of SDS-polyacrylamide gel electrophoresis (PAGE) alerted us that by dialyzing our heavy chain into native buffer it became susceptible to proteolysis thus compromising the refolding reactions. We found a means to eliminate the protease by DEAE chromatography. We also found that it could be inhibited by PMSF. At this point we decided to continue our refolding experiments working with either denaturant-solubilized inclusion body preparations, or total lysates, without any further purification, and with PMSF added to the refolding buffers and/or to buffers used to prepare lysates.

9. On February 24, 1983, I recorded in my notebook the results from a Western blot of an SDS-PAGE gel by Jeanne Perry (Cabilly Exhibit No. 8, Bates Nos. 00223-00231). From this Western blot we noted the production of heavy and light chain protein produced in the co-transformed E.coli cells. Furthermore, we were able to estimate the level of their expression from cell paste which we received from Mike Mumford (Cabilly Exhibit No. 18, Bates No. 00730). The results of the Western blot analysis of the production levels of immunoglobulin chains in E.coli were used

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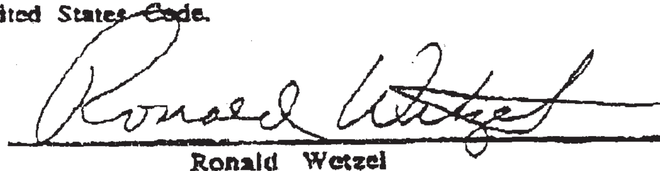
later to calculate the theoretical maximum possible yield in refolding experiments, which in turn was used to calculate % yield.

10. After a number of refolding reactions using E.coli material, we decided to use the authentic monoclonal antibody, denatured and derivatized as the S-sulfonate, as a substrate for optimization of the refolding conditions. According to these conditions a mixture of the immunoglobulin S-sulfonates were incubated with mercaptoethanol and EDTA at pH 8.5 in concentrated urea for about two hours at 37°C, then this solution was dialyzed against a nitrogen-saturated buffer consisting of 0.5 M urea in 0.1 M sodium glycinate, pH 10.8, 1mM EDTA, 5mM reduced glutathione, 0.1mM oxidized glutathione, and 10mM glycine ethyl ester at 4° C. Reactions were further dialyzed against PBS (phosphate-buffered saline) before being assayed. The result gave a titre significantly higher than that for an untreated sample (Cabilly Exhibit No. 6, Bates Nos. 0061, 0077-0081).

11. We then used the above refolding conditions on the E.coli derived material. Between March 18, 1983 and March 24, 1983, we conducted an experiment in which CEA-binding activity was generated after refolding. The results show a refolding yield of 0.76% starting from a cotransformed cellular extract, and a yield of 0.32% starting from a mixture of a heavy chain S-sulfonate and the urea-solubilized crude extract of light chain producing cells. The value of 1580 ng/ml in the cotransformed refolding reaction was significantly higher than the background levels of apparent activity obtained from controls of either heavy chain alone (441 ng/ml) or light chain alone (108 ng/ml); these latter values arise from non-specific binding to CEA in the assay. This data shows that heavy chain and light chain recombine in the refolding reaction to generate antigen binding activity (Cabilly Exhibit No. 6, Bates Nos. 0087-0088).

12. I further declare that all statements made 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.



Ronald Wetzel

Dated: Oct. 28 1991

GNE-MED-02018

GNE-GSK00003421

GNE-HGS 00082032

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