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Remembering antibodies coming of age

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Fifty years ago, Norbert Hilschmann discovered that antibodies have variable immunoglobulin domains to bind antigens, and constant domains to carry out effector functions in the immune system. Just as this happened, the author of this perspective entered the field of immunology. Ten years later, the genetic basis of antibody variability was discovered by Susumu Tonegawa and his colleagues at the Basel Institute for Immunology, where the author had become a scientific member. At the same time, Georges Köhler, a former graduate student of the author's at the Basel Institute, invented with Cesar Milstein at the Laboratory of Molecular Biology in Cambridge, England, the method to produce monoclonal antibodies. The author describes here his memories connected to these three monumental, paradigm-changing discoveries, which he observed in close proximity.

Keywords: Antibodies · B cells · Biochemistry

Prologue

"Whatever you tell me, consider it published." Max Delbrück closed the page of my thesis, where a—still hypothetical, while incomplete—cloverleaf structure of serine tRNA from brewer's yeast was pictured as the summary of 3 years' of sequencing work to align 85 nucleotides [1]. "You better take it back," he added, pushing my thesis across the table. I had done this work in Hans-Georg Zachau's laboratory at the Institute of Genetics of the University of Cologne from 1961 to 1964, where Delbrück had been professor for 3 years, on leave from the California Institute of Technology (Caltech).

When Zachau learned that I had given a copy of my thesis to Delbrück in November 1964, he had asked me to tell Delbrück that the information of the structure should be treated as confidential.

Max, as we all called him, returned to Caltech in the fall of 1964, and I graduated in December of the same year. Before leaving, Max had helped me to find my first postdoctoral position at the Salk Institute in La Jolla, California, in the laboratory of Edwin (Ed) Lennox, to work on problems of antibody synthesis. In the 40s, Ed had been a member of Max's laboratory at Vanderbilt University. On the way from Germany to La Jolla, in the first days of January 1965, I learned in New York, that Robert Holley's laboratory at Cornell University had just published the first complete sequence of alanine tRNA [2]. One of the possible three-dimensional structures, proposed by Holley from the sequence, was a cloverleaf. We had lost the race to be the first.

The rest is history: In 1968 the Nobel Prize in Physiology or Medicine was awarded to Robert W. Holley, H. Gobind Khorana, and Marshall W. Nirenberg "for their interpretation of the genetic code and its function in protein synthesis."

When I arrived at the Salk Institute in the middle of January 1965, I was immediately cornered by Francis Crick and Leslie Orgel, who asked me, how much I knew of the structure of serine tRNA. "We think that a cloverleaf is the most likely structure of all tRNAs, because we have already compared Holley's alanine tRNA with partial sequences of Gobind Khorana's phenylalanine tRNA. Your sequences could tell us, whether we are right." This threw me into a dilemma: I had promised Zachau not to give out any sequence information, however, Holley had already published the first tRNA sequence, and here were virtual gods of molecular biology, requesting this—unpublished—information. I chose to tell them this information, and in doing so, in Max's way, to "publish."

For their part, Crick and Orgel promised to give all the groups working on tRNA structures the chance to publish their work as a collection of talks and articles in the upcoming Cold Spring Harbor Symposium [1].

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Figure 1. Norbert Hilschmann. © Max Planck Institute for Experimental Medicine.

Act 1

The dogma that one gene makes one polypeptide chain is abolished

I had hardly settled in Ed's laboratory at the Salk Institute, when I was allowed to participate in an Antibody Workshop at Warner Springs Ranch on the Southern Emigrant Trail in Southern California, east of La Jolla from February 8 to 11, 1965. Ed and Mel Cohn had invited some 90 immunologists to discuss antibody genetics and the problems of antibody formation with a group of world-known biochemists and molecular geneticists: Chris Anfinsen, Seymour Benzer, Francis Crick, Max Delbrück, Bill Konigsberg, Rita Levi-Montalcini, Aaron Novick, Leslie Orgel, Leo Sachs, Jon Singer, and Jim Watson.

On the first evening, at the bar, I met Norbert Hilschmann (Fig. 1). He asked me, what I had been doing, so I told him of the cloverleaf structure of serine tRNA. I told him of the great interest of Crick and Orgel in my data, and I told him of my dilemma, that our findings had not yet been published.

Hilschmann replied, "I also have a dilemma. I do not know whether I should tell the meeting, what I have discovered. It is totally new and unexpected, but I have also not yet published it." Remembering my conversation with Max in Cologne, I replied, "Some of the most outstanding biochemists and molecular biologists are present at the meeting. Max, at least, will consider published, whatever you will tell him here. So, either say nothing, or tell them, what you found."

The prevailing spirit of the time, after the discovery of DNA and of the genetic code as common principles of inheritance of all living beings, was that molecular genetics would offer new ways to understand biology—and, hence, also the immune system. When, on day 2 of the meeting, someone described Charles "Chas" Todd's finding, that a serologically defined allotype (a) on rabbit antibodies could be detected on three different classes—IgM, IgG, and IgA [3]—Francis Crick said, "Young man, you should realize that the dogma of molecular genetics states, that one gene makes one polypeptide. So, the same allotype (a) cannot be on three different genes for antibodies (M, G, A)."

Then came February 10, 1965, the third day of the antibody workshop. After Frank Putnam, Norbert Hilschmann was the second speaker of the morning, scheduled to speak for 20 min.

Hilschmann had done his graduate work at the Max Planck Institute for Biochemistry in Munich, where he learned how to sequence allelic forms of hemoglobin in the laboratory of Gerhard Braunitzer. Hilschmann then joined the Rockefeller Institute in New York to purify Bence-Jones proteins (given to him by Henry Kunkel) by the method of countercurrent distribution that had been developed in Lyman Craig's laboratory—and thereafter to do something that nobody else knew how to do: sequence them.

"The molecular weight of this Bence-Jones protein is 27 168," Norbert Hilschmann said.

The audience, almost asleep after listening for 20 boring minutes to a detailed description of the purification of this protein [4], woke up and started reaching for their notebooks. The meeting room of the Warner Springs Ranch had only simple chairs—the notebooks had been resting on the floor.

The audience knew that Hilschmann, a biochemist, would not give the exact molecular weight, unless he knew the full sequence. "On the next slide, you see the sequence of the 212 amino acids" (Fig. 2, Bence-Jones protein Roy) [5], he said, and after a few seconds, "Slide off, please. I have purified a second Bence-Jones protein, and I have also determined the sequence of its 212 amino acids, shown in the next slide."

This all happened much too fast to see even parts of the sequences, even for Frank Putnam, one of Hilschmann's competitors, who sat in the front row, as Hilschmann, again, asked to turn off this second slide. In his talk preceeding Hilschmann's presentation, Putnam had shown tryptic peptide sequences of Bence-Jones proteins, but had been unable to align them into a sequence. Hilschmann positioned himself directly in front of Putnam: "With Doctor Putnam's permission I will now align the tryptic peptides of one of his Bence-Jones proteins" (Bence-Jones protein Ag, see [5]). The next slide, as difficult to read in detail as the two previous ones, was projected. Hilschmann kept obstructing Putnam's view of the slide. Pointing his hand over his shoulder, Hilschmann pointed to the slide behind him. "The comparison of these three sequences shows, that the carboxyterminal halves of the three Bence-Jones proteins are identical, common to all three, except for a single replacement at position 187. On the other hand, the amino terminal halves of the three proteins show a considerable amount of variation in sequences. Slide off, please."

Figure 2. At the party in the Basel Institute for Immunology on January 18, 1988, honoring Susumu Tonegawa's winning of the Nobel Prize. Also in the picture (from right to left) are Renato Dulbecco, Andreas Strasser, Fritz Melchers, and an unidentified person. © Fritz Melchers.

Hilschmann had just described constant and variable parts of immunoglobulin light chains. He ended his presentation by proposing that "individual differences in immunoglobulins might be explained by somatic crossing over between genes determining the synthesis of these proteins."

A coffee break had been scheduled after Hilschmann's presentation, but now this was completely forgotten. A flood of questions rained on Hilschmann and on the chairman of the session, Jon Singer. Everybody wanted to see the sequences again, to discuss possible mechanisms, by which different variable regions could be joined to the same constant region of light chains. Maybe the dogma of molecular genetics, that one gene encodes one polypeptide, was now violated. At first, Hilschmann refused, saying that the paper describing these results had not yet been completed, and hence, not yet been submitted for publication. The audience insisted to see the sequences again, and the chairman, finally, convinced Hilschmann to show them once more. Hilschmann did so as rapidly as the first time—nobody was able to contemplate how this apparently antiparadigmatic finding could be explained.

Afterwards, one of Hilschmann's most avid competitors even tried—unsuccessfully—to extract the slides of the talk from the projectionist. As the audience finally went for the coffee break, Hilschmann was cornered by several of the Nobel laureates in the audience. One said, "This was the most awful scientific presentation that I can remember. I find your refusal to show these sequences inacceptable—and I will make sure, that you never get invited to another international scientific meeting." To all of it, Seymour Benzer remarked to Ed Lennox, "Immunology has come of age. It now is a mature science—with severe fighting."

For me, my entry into the field of immunology could not have been more dramatic.

The rest is history: in 1972 Rodney Porter and Gerald Edelman were awarded the Nobel Prize in Physiology or Medicine for "for their discoveries concerning the chemical structure of antibodies."

Thus, this Nobel Prize honored the elucidation of the structure of the Fab and Fc portions (Porter) and of heavy and light chains (Edelman), but not of variable and constant region domains (Hilschmann) of antibodies.

Act 2

The dogma that all the cells of our body contain all the genes that code for all functions is abolished

In a letter to Niels Kaj Jerne (Niels, as we all called him), Director of the Basel Institute for Immunology, dated October 17, 1970, Susumu Tonegawa applied for scientific membership at the Basel Institute for Immunology:

> As you might have heard from Dr. Dulbecco,[1] I am most interested in the problems of control of gene expression and cell division in animal cells, and have been using the virus transformation system for this purpose. Antibody synthesis systems also seem to be very promising in this respect, although I have not been able to formulate the detail of specific approaches. It seems to me, as probably to many others, that an establishment of a clonal cell line, which can be induced for a specific antibody synthesis, is of utmost importance in order for this system to be susceptible to molecular biological approaches. Use of tumor RNA virus to transform the stem cells may help in establishing such a clonal line.
>
> Is it in any way possible for me to continue to use the virus transformation system? For example, one thing I have been planning to do is to isolate, using negative selection by FUdR or a plant agglutinin like Concanavalin A, SV 40 temperature sensitive mutant in the genes whose normal function is necessary for maintaining the unlimited growth of transformed cells. This information obtained by this mutant may help in understanding other growth control phenomena in animal cells, such as antigen-stimulated growth of the target cells.

[1]Renato Dulbecco, Fellow of the Salk Institute for Biological Studies in La Jolla, California, a former member of Max Delbrück's laboratory at Caltech, where he developed a plaque assay to quantitate animal viruses, for which he received the Nobel Prize for Physiology or Medicine in 1975. He became a scientific advisor to the Basel Institute for Immunology from the start of the institute in1968, until 1974 and again in 1983. Tonegawa, who had graduated in Hayashi's laboratory at the University of California at San Diego, was a postdoc in Dulbecco's laboratory. Dulbecco recommended Tonegawa to Jerne.

No question, if transformation of antibody-producing B lymphocytes would have been successful, Tonegawa would have established monoclonal antibody-producing cell lines.

However, when Tonegawa—Susumu as we called him—joined the institute in February 1971 on a 2-year contract, he—as an expert in molecular biological techniques—was drawn into discussions with many members of the institute, notably by Charles (Charlie) Steinberg, to think of ways of measuring the number of genes encoding constant and variable regions of antibody heavy and light chains in the genome. After Hilschmann's discovery of variable and constant regions of light chains this was the most eminent question, which needed to be answered, to understand the genetic basis of antibody diversity.

Susumu has been quoted by Niels for saying that "if you want to study genes, you must study DNA." However, when Susumu joined the BII in 1971, neither cloning nor sequencing of DNA had yet been invented.

Thus, he employed the knowledge of Günther von Ehrenstein's laboratory at the Max Planck Institute for Medical Research in Göttingen, Germany, and convinced two of his collaborators there, Shaim Dube and Bernd Weimann, to radioactively label and purify full-length or 3'-light-chain-encoding mRNA, i.e. mRNA encoding sequences of the variable (v) and constant (c) region, or only those of the constant (c) region, from a collection of Michael Potter's plasmocytomas. The P32-labeled mRNAs were hybridized in solution with DNA to determine the number of genes in the genome. From these studies the authors "concluded that the number of germ line genes is too small to account for the observed diversity of antibody molecules" [6, 7]. The numbers of hybridizeable genes for v- and c-regions were all similar to the numbers for genes encoding hemoglobin, not thousands of v-genes, as one would have expected, if the diversity of v-regions was inherited in the germ line. Hence, these results pointed to somatic generation of v-region diversity, by somatic mutation—as Niels had postulated in his programmatic review article at the beginning of the Basel Institute for Immunology [8].

Susumu had done these experiments with a technician, Rita Schuller, with the help of the RNA specialists in Göttingen, and with the continuous input of ideas from Charlie Steinberg. His working schedules were extreme: he would come to the institute at noon, and continue to work until 2 or 3 in the morning—interrupted only by his wife, who would bring him a Japanese dinner at midnight. No wonder that he saw the diligently prepared sucrose gradients in the refrigerator of the lab next door, where Theo Staehelin and Max Schreier worked on translation factors guiding protein synthesis, as a temptation to use them for his own—so urgent—experiments. He also drew the critique of the radiation safety department of Roche, the company that fully financed the BII, for his excessive and often sloppy use of P32. Niels had already decided, in the spring of 1973, not to prolong Susumu's appointment at the institute, but kept him employed for one more year, with the expectation that his application for a visum to the United States would eventually be granted.

This all changed—and Susumu's contract was prolonged—when Nobumichi Hozumi and Susumu discovered "that the VK and CK genes, which are some distance away from each other in the embryo cells, are joined to form a contiguous polynucleotide stretch during differentiation of lymphocyes. Such joining occurs in both of the homologous chromosomes" [9]. Today, we recognize, how lucky they were, that Michael Potter's plasmocytoma MOPC 321 had rearranged both alleles of the κ light chain locus. Susumu was also lucky, that cloning and sequencing of DNA became doable—and to be able to do it with lambda light chain genes at the right time at the right place. The 1250 bp long intron between v- and c-regions of a v-c-rearranged light chain gene was found and published in 1977 [10]. Christine Brack brought her expertise in electron microscopic R-loop mapping to Susumu's laboratory—and discovered J-regions [11], soon confirmed by sequencing of germline (embryo) and v-c-rearranged somatic forms of the light chain genes [12]. Susumu's spectacular work secured him invitations to the most attractive scientific meetings, and Jim Watson made sure that he came to the Cold Spring Harbor Symposia of Quantitative Biology in 1977, 1978, 1981, and 1989. Many excellent scientists joined Susumu's laboratory in Basel to expand the work on the structure of immunoglobulin genes. I remember Niels sitting in his office, looking at the genomic sequence containing the five Jk-segments of the mouse kL-chain locus [13]: "Fritz, do you think we need this? Five J-regions, and one of them is even a pseudogene!" His theory had proposed that one, or a few genes encoded one, or a few preexisting, original antibodies, which could be somatically mutated, and thereafter be selected by antigen to develop better antibodies. Multiple J-regions (and later D-regions) encoding pieces of V-region genes, which recombine somatically to form V-domains of antibodies with highly variable complementarity region three antigen-binding sites, was a mutational mechanism simply not envisaged by bacterial genetics.

When Niels retired as director of the institute in 1980 (upon which I took his place), Susumu had already accepted a professorship at the Massachusetts Institute of Technology (MIT). I asked Susumu, what would make him stay in Basel. His answer shocked me. "If you are prepared to give me one-third of the Basel Institute, budget and space, and if you separate my third from the rest, which you can direct, I will consider it." To me, that meant the end of the nonhierarchical organization of scientists at the Basel Institute, which had been so attractive and successful for so many scientists for so many years. I asked the chairman of the scientific council of the board of directors of the institute, Manfred Eigen, and director at the Max Planck Institute for Biophysical Chemistry in Göttingen, for his opinion. "In the Max Planck Society, such a demand by a scientist who might get the Nobel Prize, would be considered reasonable, and would be granted," he replied. Convinced that I would not be able to continue as director of the institute under such changed conditions, I met Fritz Gerber, chairman of the board of directors of the institute, and chairman of the board of Roche, the company that owned and financed the institute, for lunch. "You are the director. You decide what is best for the institute," he said. And so I did.

The rest is history: Susumu Tonegawa joined MIT in 1981 and received the Nobel Prize for Physiology or Medicine in 1987 "for

his discovery of the genetic principle for generation of antibody diversity" (Fig. 2).

Act 3

The dream of making an immortal plasma cell that can produce a ton of a single antibody with a desired specificity comes true

On March 2, 1981, soon after I had become director of the Basel Institute for Immunology, I received a letter from Werner Henle, virologist at the Children's Hospital of Philadelphia: "I have a question to which I believe you will have the answer. Among the nominations for this year's General Motors Sloan Prize is, not surprisingly, Cesar Milstein but not Georges Köhler, despite the fact that he was senior author on what appears to be the key publications deemed worthy of the prize [14]. Was Köhler merely assigned by Milstein to these studies or did he contribute pertinent ideas to the success of the work?"

This was not the only request for clarification: Lennart Philipson, then director of the EMBO laboratory in Heidelberg and a member of the board of scientific advisors of the Basel Institute, suggested that I ask Georges himself, by then a permanent scientific member of the institute, to write down, how he remembered his work in Cesar Milstein's laboratory.

In his "detailed personal account to the discovery of monoclonal antibodies by one of the inventors—Georges Köhler" he wrote on June 29, 1981 [15]:

> My thesis work, done at the Basel Institute under the supervision of F. Melchers, was dealing with antibody repertoire and diversity in the mouse using the enzyme β-galactosidase as a model antigen. To explain the vast repertoire (10^7 different antibodies in the mouse), somatic mutation, and subsequent selection of lymphocytes making altered antibodies was the idea favored by many members of the institute, particularly by N. Jerne at that time its director.
>
> To measure mutation rates of antibody genes and to study the impact of mutations on the antibody binding site seemed to me a logical continuation of my work.[2] I joined Cesar.
>
> Milstein's group because he and his collaborators had already described variants of the MOPC-21 cell line[3] which differed in the electrophoretic mobility of the Ig secreted which, however, had no known antigenic specificity. Unfortunately MOPC-315, the only cell line which was available at that time (1974) secreting IgA anti-dinitrophenyl antibody could not easily be grown in vitro culture, so this project was abandoned ...
>
> I introduced the 8-azaguanine resistance into X63, a subline of MOPC-21 which was well adapted to tissue culture growth. X63-Ag8 was then fused to P1-BU1 (BUdR resistance was introduced by R. Cotton). The results were interesting in two ways: coexpression of heavy and light chains of both parental lines was observed and the frequency of hybrids was much higher than expected ...
>
> C. Milstein asked me several times to start a screening program to find an antigen which fitted the IgG1 (k) of MOPC-21. I refused to do this as I was not sure if I would find it within a reasonable time.
>
> The idea to generate myself lines secreting antibodies with known specificity and to solve this problem by cell fusion is certainly understandable from the above. Laying in bed unable to sleep I had the idea of fusing the myeloma lines and normal antibody producing spleen cells. The excitement wouldn't let me sleep for a long time that night. Next morning I told my wife (no reaction, it wasn't the first all solving idea!) and then C. Milstein. I presented the idea as a crazy possibility to solve the problem of having no tissue culture line secreting antibody with known specificity in the lab. I was terribly uncertain if it could work out and it was Cesar's willingness to discuss seriously at any time any crazy idea which has made the idea survive. We discussed the odds against such an experiment: low fusion frequencies were reported for normal lymphocytes and were also found in the myeloma fusion experiment of R. Cotton, but my own experience had not been so bad.
>
> The frequency of B lymphocytes secreting antigen-specific antibodies is low (the figures we thought of were 1/1000), so we calculated that if we were lucky enough to obtain ten hybrids per fusion, we had to do 100 fusions to get one specific hybridoma. This meant that I had to do about one fusion every third day to have a chance to get such a hybridoma during my post doc time. This was quite depressing. But we decided that it might be worthwile to pursue the idea as a side project.
>
> We chose to immunize the mice with sheep erythrocytes (SRC), which were known to be potent immunogens and for which easy techniques were described to identify antibodies against. Although I came from the Basel Institute for Immunology whose former director, Niels Jerne, was the inventor (together with A. Nordin) of the plaque assay detecting single anti-sheep erythrocyte antibody producing cells, I had to learn the technique in Cambridge very much to the amazement of Cesar.[4]

[2] I remember discussions with Georges in Basel before his departure to Cambridge, on how "classic" it would be to develop a Luria–Delbrück-type fluctuation assay [20–22] with antibody-secreting plasmocytomas to measure the frequencies of mutations in antibody variable region genes, which would change the binding to the antigen. However, we could not think of an antigen-specific plaque assay like the one developed by Nordin and Jerne [17] for SRC-specific antibody-secreting cells, since neither Michael Potter nor Mel Cohn had ever been able to develop a plasmocytoma secreting SRC-specific antibody by immunizations with SRC, and since the hapten-specific plasmocytomas, which they did find, neither had been developed to tissue culture lines, nor appeared the SRC-plaque assay easily adaptable to a hapten-specific assay. In retrospect, it is amusing that Georges never went on to determine rates of mutations, once he had made his SRC-specific hybridomas.

[3] At the Antibody Workshop in Warner Springs Michael Potter agreed to give the laboratories of Ed Lennox and Mel Cohn 10 of his mineral oil-induced plasmocytomas (MOPC), which could be maintained, as transformed, apparently clonal plasma cell lines by transplantation in BALB/c mice. They were renamed P (for Potter) 1 to 10. P3, originally MOPC 21, was adapted, as the first mouse plasmocytoma line, to tissue culture by Leo Sachs, on sabbatical leave from Israel, and Kengo Horibata, as the IgG-secreting cell line P3K[16]. In Cesar Milstein's laboratory at the Molecular Biology Laboratory in Cambridge, England, Georges Köhler introduced resistance to azaguanine into P3K, renaming it P3-X63-Ag8. He later also developed the SP2/0 cell line, which he had selected for loss of synthesis of the heavy and light chains of the original P3-X63-Ag14, which, according to Georges' own words "was ... derived from a hybridoma between Balb/c and X63-Ag8... and was the first and for about 1 year the only Ig negative line which could be used for fusion."

[4] Yes, it was amazing, considering the fact that Jan Andersson and myself were using the plaque assay each day in the same laboratory [18].

. . . .the experiment was performed the following way: spleens of three mice immunized with SRC one month and four days before sacrifice were removed. This time was used because we thought this to be the time of maximum secondary response as indicated by the number of anti-SRC plaque-forming B cells of the spleen. Using Sendai virus, 10^8 spleen cells were fused to 10^7 and 10^6 cells of each of the three HAT sensitive mouse myeloma lines we had (X63-Ag8, NSI-Ag4/1, and P1-BU1). These 6 fusions were placed in separate tissue culture bottles. This I did to have independent fusions in case the fusion was variable or myeloma line dependent and to see if by chance we had more than just a few hybrids!

After HAT selection vigorous growth was observed in both X63 bottles. Some growth was observed in both NSI bottles (which later were lost due to contamination), and revertants were seen in both P1 bottles, as judged by appropriate controls. I considered this already as the major outcome of the experiment: it was possible to make spleen hybrids and one could obtain hybrids even by using 1/10 the number of X63 myeloma cells. I was so sure that we couldn't have specific hybridomas[5] in these bottles that I waited for 7 weeks until I finally decided that it couldn't harm to check their specificity against SRC. The plaque experiment was set up the following day: 10^6 cells of each of the X63 bottles were mixed with SRC and complement and plated in agarose (direct plaques indicative for IgM anti SRC secreting cells) and similarly 2 plates were set up containing, in addition, a rabbit anti mouse Ig developing serum (indirect plaques indicative for IgG antiSRC secreting cells).

The development of the plaques takes about 2–3 h and I went home for dinner. I convinced my wife that it would be much less boring to go together to the institute (around 9.30 p.m.) to score a negative result. The first plate I looked at was from the 10^7 bottle, direct plaques: and there they were; I looked in the microscope: no doubt about the plaques; myeloma alike looking cells were in the center of a lysed area of SRC. I shouted, embraced my wife and jumped around in the little tissue culture room in the basement of the MRC. After calming down I still had three plates to look at. The indirect plaques of the 10^7 bottle, no indication of increased plaque numbers—the direct plaques from the 10^6 bottle, no plaques—the indirect plaques of the 10^6 bottle, and to my utmost surprise there were plaques again. It was clear then, that the experiment had been extremely successful. We were able to clone out the two hybrids responsible for the plaque formation (after an awful long time of fear to lose the cultures by contamination or to lose the activity by overgrowth of other hybrid cells in the bottles).

The next thing was to reproduce the result and find out the frequency of specific hybridomas and we found with fusion 3 that it was reproducible and that instead of the expected frequency of 0.1% of anti-SRC-specific hybridomas we found 10%.

After having repeated the fusion also using trinitrophenyl hapten on different carrier molecules, thus showing its feasibility with other than SRC antigens, we were sure that we had discovered a general way to produce monoclonal antibodies against all antigens.

In retrospect, it is the completely unexpected approximately 100× enrichment of specific hybridomas, which made the hybridomas such a successful technique all over the world in such short a time.[6] I think that in 1976 when I left Cesar Milstein we both were seeing only the tip of the iceberg of the impact monoclonal antibodies would have in science.

This is to my best knowledge the account of the discovery of hybridomas. I believe that I was the driving force in it, but it is also true to say that I would not have thought about this problem in any other laboratory than Cesar Milstein's and I would not have been encouraged to do the experiment by anyone else but Cesar Milstein.

Without any corrections, and with the permission of Georges, I sent his account in October 1981 to Peter Reichard, Medical Nobel Institute, Karolinska Institute, Stockholm, and to Erling Norrby, Lennart Philipson and Renato Dulbecco. Lennart Philipson also searched the files of EMBO for Georges' application in 1975 to prolong his long-term EMBO Fellowship, with which he had joined Cesar Milstein's laboratory in 1974.

On the suggestion of the scientific advisory board of the Basel Institute and, again, with the permission of Georges, I also sent the account to Nicholas Wade at Science. In February 1982 his article "Hybridomas: The Making of a Revolution" appeared [20]. Wade had met and consulted several times with Georges to intensify and clarify the information, which Georges had given him in his original account. In his article Wade discusses the problem of who deserves the Nobel Prize. His concluding remarks are so wonderfully idealistic that I want to repeat them here:

Maybe those with prizes to award would manage to make a more significant contribution to science if they sought primarily to honor a discovery, not the discoverers per se. If the prize committees were to publish a scholarly account of how the discovery came to be made, those cited in the account would receive due credit, and the public would better understand how often an important discovery stands at the apex of a rich and diverse set of findings, contributed by many different researchers over a long period of time.

It is commonly assumed among immunologists that the invention of the hybridoma technique will eventually be the subject of a Nobel Prize. But no number of prizes can add to the distinction of so notable a discovery.

Nicholas Wade's proposal, how to better honor outstanding discoveries and inventions, would certainly also have been a wonderful alternative to the awarding of the other Nobel Prizes, which I have touched upon here.

"Fritz, you should be prepared, that a Nobel Prize may be given to one of your scientific members." A friend had called on Friday, October 12, 1984 from Stockholm. "I am not entitled to tell you this, and it may also not happen, when this year's Nobel Prizes for Physiology or Medicine are announced on the coming Monday before noon, because the committee will only then decide between two choices." First, I called the president of Roche, hoping that he would find the time for a possible press conference. Together with Mr. Gwinner, in charge of press relations at Roche, we assembled information on Georges Köhler,—who I guessed could be

[5]Georges uses here the term for his fused hybrid cells coined by Len Herzenberg.

[6]Georges mentions knowing that normal, mostly Go-resting cells did not fuse well. It was their unpredictable luck that activated, G1-S-M-cycling cells would fuse at least 100-fold better [19].

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dr georges j. f. koehler
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the nobel assembly of karolinska institute has today decided to award the nobel prize in physiology or medicine for 1984 jointly to yourself and cesar milstein and niels k. jerne for the discover of ''the principle for production of monoclonal

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antibodies'' and for theories concerning ''the specificity in development and control of the immune system stockholm, october 15, 1984
jerzy einhorn, chairman
the nobel assembly
david ottoson, chairman
the nobel committee
jan lindsten, professor
secretary general

col 15, 1984

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Figure 3. Telegram from the Nobel Assembly of the Karolinska Institute to Georges Köhler, on 15 October, 1984, informing Dr. Köhler that he had been awarded the Nobel Prize for Physiology or Medicine. The 1984 Prize was awarded jointly to Drs. Köhler, Cesar Milstein, and Niels K. Jerne for "the discover(y) of the principle for production of monoclonal antibodies" and for "theories concerning the specificity in development and control of the immune system." Scan of telegram kindly provided by the author.

the winner. Then, my wife Ursula and I invited Georges and his wife for dinner at our house on Sunday night. I did not mention the phone call from Stockholm. At the end of the dinner, I casually asked him, whether he would be in the institute on Monday morning, pretending that I had to discuss details of his moving to Freiburg in Breisgau, nearby in Germany, where he had already accepted an offer to become director of the Max Planck Institute for Immunobiology.

Thus, the next morning, Georges was in Basel, not in Freiburg, when—shortly after 11 in the morning—he received a telegram from the chairman of the Nobel Committee, that he had been awarded the Nobel Prize. (Fig. 3). It was no surprise that he shared the prize with Cesar Milstein (Fig. 4), since Georges had done the work leading to the prize in Cesar's laboratory at the Medical Research Council in Cambridge, England.

The third winner of the prize was a wonderful surprise: Niels. We were lucky again: Niels was in the institute. (Or was it possible that the candidates had also been warned?) Within minutes, the staff of the institute came to Georges' laboratory to congratulate him and Niels, and to drink with them, totally illegally, the champagne, which I had stored overnight in the cold room of Georges' laboratory (Fig. 5).

Since Niels had recently retired recently as institute director, we remained well-prepared for the invasion by the press. Within minutes TV and radio stations, and journalists from a wealth of

Figure 4. Cesar Milstein, Rodney Porter, and Ed Lennox preparing for a walk in 1982. © Fritz Melchers.

Figure 5. Georges Köhler with Nils Kaj Jerne on 15 October, 1984 at noon in George's laboratory at the Basel Institute for immunology—1 h after the Nobel Prizes were announced. Matthias Wabl is in the background. © Fritz Melchers.

newspapers, began to call and to come to the institute. At 2 o'clock in the afternoon, the president of Roche, the two Basel-based new Nobel Laureates and a surprise guest, Nobel Laureate Linus Pauling (who happened to be in town), met the press. Even the stock market responded favorably: shares of Roche jumped by 10%.

This event would not be the last impact of monoclonal antibodies on Roche's financial well-being: the current multibillion dollar profits resulting from the development and sales of several monoclonal antibodies with anti-inflammatory and anticancer activities are still only one tip of the amazing antibody iceberg.

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Abbreviations: **SRC**: sheep erythrocytes

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