CHAPTER 1

The Beginnings

> It is not because things are difficult that we do not dare, it is because we do not dare that things are difficult.
>
> *—Lucius Annaeus Seneca, ca. 10 BC in the reign of Roman Emperor Tiberius*

October 14, 1980, was a breezy and chilly fall day in New York. The NASDAQ opening bell rang as usual at 10 am. This also turned out to be an auspicious day for Genentech, a South San Francisco–based company formed in 1976 to pioneer the use of recombinant DNA technology to make therapeutic proteins in microorganisms. Genentech completed their initial public offering (IPO) on this day,[a] marking the public beginning of today's multibillion-dollar molecular biology–based biotechnology industry.[1,2,3] It was one of the most successful IPOs in history with the stock trading from the initial price of $35 to $88 per share within the first 20 minutes, settling back to $56 before the closing bell.[b]

Genentech was not the first modern biotechnology company founded. That honour belongs to Cetus, which was started five years before Genentech by Nobel Prize–winning physicist Donald Glaser, with his partners Ron Cape and Peter Farley.[4] Its initial funding came from Standard Oil to support its work on using microbial processes to produce chemical feedstocks, including propylene oxide and antibiotic intermediates. The company did not embrace recombinant DNA technology until after the founding of Genentech. Cetus raised $108 million in their 1981 IPO, the largest to

[a] The IPO was managed by Blyth Eastman Paine Webber and Hambrecht and Quist[4] (H&Q) a boutique Bay Area investment bank formed by Bill Hambrecht and George Quist in San Francisco. H&Q was later acquired by Chase Manhattan Bank, who then merged with J.P. Morgan to become JPMorgan Chase.

[b] It is remarkable that the stock never traded below the $35 initial offering price right up to when Genentech was acquired in 1999 by Hoffman-La Roche.[5]

that date and three times bigger than that of Genentech, giving Cetus a market capitalization of $500 million.[5]

From an investor perspective, the Genentech and Cetus IPOs marked the beginning of the modern biotech industry.[c]

WHEN DID IT REALLY BEGIN?

This question is difficult to answer. It is clear that the origins of the modern biotechnology industry can be traced further back well before Genentech and Cetus. Perhaps it began with the solving of the structure of DNA in 1953 by James Watson and Francis Crick,[6] for which they were awarded the 1962 Nobel Prize in Physiology or Medicine. Or maybe its origins lie in the discovery of messenger RNA (mRNA) by Crick and Sydney Brenner and others, the deciphering of the genetic code by Marshall Nirenberg, and the understanding of the concept of a gene as a unit of inheritance—work dominated primarily by phage genetics. These fundamental discoveries, although critical to biotechnology, are not really its beginning. They rather mark the beginning of molecular biology, as so elegantly described in Horace Judson's book *The Eighth Day of Creation*.[7]

Biotechnology purists will argue that it was the discovery of various enzymes to modify DNA including restriction enzymes (proteins that can cut DNA at specific locations) and understanding the problem of antibiotic resistance in bacteria that together led to powerful new tools that in turn defined the first biotech companies. The problem of antibiotic resistance in bacteria was well known in the 1960s and 1970s, but the mechanism of that resistance was not. It was clear that antibiotic resistance was associated with "plasmids"—large circles of DNA replicating inside the bacteria independently of the chromosomal DNA. These plasmids could be transferred between bacteria and often carried one or more of the genes that conferred antibiotic resistance on its host bacterium.

These observations, along with the ability to make specific cuts using the newly discovered restriction enzymes, provided the ability to make "recombinant" DNA plasmids quite easily (i.e., ones combining two or more different DNA sequences of interest). This novel genetic material could then be transferred (transformed) into *Escherichia coli*, and individual clones containing a single unique recombinant plasmid derived (see Box 1).

[c] It was also the start of the fairy-tale "magic porridge pot" industry, which keeps on giving generously to patients, investors, and the people who work in it.

BOX 1. GENE CLONING

Viruses like HIV use an enzyme called "reverse transcriptase" to turn their RNA-based genomes into DNA, which can then be inserted into the host cell genome and take over its usual functions in favour of the virus. Scientists take advantage of reverse transcriptase to make cDNA libraries. They extract mRNA from cells and use reverse transcriptase as the first step in making a cDNA. The cDNA-containing plasmid, which also includes the genes for antibiotic resistance, is transferred into *E. coli* and the bacterial colonies grown on antibiotic-containing plates. Clones that grow on the appropriate antibiotic containing plate contain plasmids with cDNA inserts.

Figure 1 shows the process of making a "copy" or a "complementary" DNA (cDNA) from a messenger RNA (mRNA) from a cell of some kind, inserting ("cloning") the cDNA into a circular piece of bacterial DNA (plasmid), and putting that cDNA-containing plasmid into *E. coli* where the cDNA can be "expressed" (i.e., a mRNA can be made by the bacteria from the cDNA sequence, and that mRNA can be translated into the protein specified by the sequence). For simplicity, this is shown as one step. Usually, the cDNAs were cloned and isolated and then the cDNA inserts engineered and transferred to an expression plasmid.

cDNA cloning was the most common approach used by early biotech companies to clone genes to make proteins in *E. coli*, although chemically synthesised genes and genes taken directly from the human genome were also used instead of cDNAs.

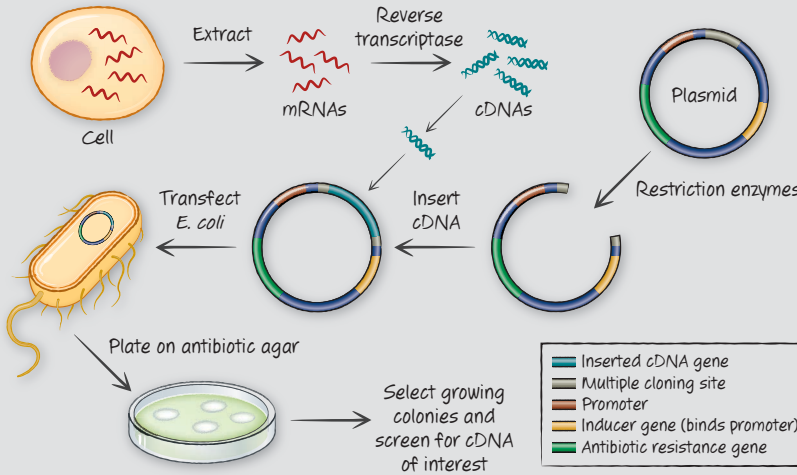

Figure 1. cDNA cloning into a basic expression plasmid.

This work began a new lexicon of terms like cloning, gene splicing, recombinant DNA, and genetic engineering.[d]

This new genetic engineering capability was species-agnostic: DNA from widely disparate organisms such as bacteria and humans could be cut and joined together in a single plasmid. These artificial constructs could then be transferred into bacteria that would make the human proteins coded for by those genes. This technology, more than any other, defined the emerging industry of modern "biotechnology." The first manuscript fully describing this technology was published by Stanley (Stan) Cohen and Herbert (Herb) Boyer in *The Proceedings of the National Academy of Sciences* in July of 1973.[8,e] The foundational patent on the technology was issued to Stanford University in 1980.[9,f]

CONCERNS OVER THE TECHNOLOGY

The artisans that were doing this work, in particular Professor Paul Berg at Stanford,[10] were very aware that this new technology had the potential to do harm through the inadvertent or deliberate cloning of genes causing cancer or antibiotic resistance into bacteria that could infect humans (e.g., the strains of *E. coli* that commonly reside in the human gut). Furthermore, these organisms can exchange genetic information with other types of bacteria, some of which are pathogenic to humans. In an unprecedented move, scientists voluntarily stopped using this technology and began to meet to discuss its potential implications and how to manage them. An initial discussion involving the principal scientists practicing genetic engineering was followed by highlighting recombinant DNA technology at the 1973 Nucleic Acids Gordon Conference, held at New Hampton, New Hampshire. Stan Cohen and Herb Boyer, the pioneers of the new cloning

[d] I must mention here the contribution made by David Baltimore and Howard Temin in the discovery of the enzyme reverse transcriptase, which turns mRNA into DNA ("cDNA") that can also then be cloned using these methods (see Box 1). They were awarded the Nobel Prize in Physiology or Medicine for this discovery with Renato Dulbecco in 1975.

[e] Curiously, the Nobel Prize committee chose to overlook the contribution of Herb Boyer and Stan Cohen.

[f] It was filed one week before the publication deadline thanks largely to the persuasive powers of Neils Reimers, who went on to run the Stanford University Tech Transfer office. Stanford University chose to license this patent nonexclusively and it brought in hundreds of millions of dollars to the university as a result.

techniques, presented some of their data at the meeting and suggested that the scientists should discuss the implications more completely. Maxine Singer and Dieter Soll were tasked with writing to the National Academy of Sciences (NAS) to ask them to set up a committee to investigate the issue and to lead communications, along with the National Institutes of Health (NIH), on the subject.[11]

A group of scientists sponsored by the NAS (called the Committee on Recombinant DNA) led by Berg, subsequently met at the Massachusetts Institute of Technology (MIT) in April 1974 to discuss concerns about the potential hazards of recombinant DNA (rDNA). A letter from the group that laid out their worries was published in *Science* in July 1974.[12] The letter called for an unprecedented temporary and voluntary pause on certain rDNA experiments, to buy further time to evaluate the risks of the technology and to avoid precipitating an "unanticipated hazardous event." Specifically, they asked scientists to defer experiments that involved inserting into bacteria genes that conferred either resistance to antibiotics or the ability to form bacterial toxins and the genes of animal viruses. They also recommended that caution be exercised before cloning genes from eukaryotic species, including humans, into bacteria.[g]

Many expressed concern that the guidance offered was way too vague, as well as to whether the temporary halt would stick. Several people questioned the risks associated with the cloning of eukaryotic DNA from *Drosophila* (the fruit fly), *Xenopus* (African clawed toad), or *Bombyx mori* (the silkworm)—experiments that had, in fact, already been done. Anticipating the uncertainties, the Committee on Recombinant DNA letter also called for NIH guidelines to be established and for a larger meeting to discuss potential hazards to be organised as soon as possible.

THE ASILOMAR CONFERENCE

The resulting meeting was the now-iconic Asilomar Conference, convened in February 1975. The conference was unusual in several ways. There were about 150 invited participants from 12 countries, including not only scientists but also reporters from the scientific and mainstream press, as well as selected representatives of church and state. It was made clear from the outset that the

[g]The genome DNA of eukaryotic organisms is contained in a nucleus, a structure that is absent in prokaryotic cells.

meeting would not consider the moral and ethical considerations of the technology, but rather the potential risks to humanity by employing it. It was clear that the scientific experts felt (probably rightly) that they understood the technology better than anyone else and were quite able to render the appropriate judgements in regulating themselves. Few believed that recombinant DNA technology was without some level of risk: the questions were what types of experiments were riskier than others, and what biological and physical containment conditions were appropriate for different types of experiments.

The Asilomar gathering resulted in a set of regulations that have stood the test of time. First, the participants agreed to change the blanket moratorium set up by the Berg letter and endorsed by the NIH to a system based on rational risk assessment. The questions of both the modification of the host organisms (mostly *E. coli*) and the cloning vectors were discussed at length. The attendees agreed that further research should focus on developing further disabled host/vector combinations that could not exist successfully outside the lab. The question of the scale at which the experiments were done was also addressed, given that physical containment was one of the key issues. Notably, the personal responsibility of the lab leaders was also stressed. In the end, the Asilomar participants established three categories of risk: low, intermediate, and high. DNAs from prokaryotes, bacteriophages, and other plasmids fell into the first group. Cloning animal virus genomes (whether DNA or RNA viruses) was considered an intermediate risk. Cloning eukaryotic DNA was classed as the highest risk, largely because very little was known about eukaryotic genes and gene expression at the time, making the actual risk very difficult to assess.

The Guidelines

The "high, medium, low" risk designations were viewed as "interim assignments," which could be revised upward or downward in the light of future experience. The highest level of containment meant the building of labs with special containment characteristics, such as negative pressure gradients, biological containment hoods, clothing changes, and showers. Other recommendations included a focus on training, education, and reassessment.[13,14]

It was very important for the group to set a realistic set of guidelines to avoid unnecessary legislation: guidelines could be altered in the light of new knowledge, whereas legislation, once it has been set up, is very difficult to

change. The recommendations formed the basis of both the Recombinant DNA Advisory Committee (RAC) experimental assessment regulations in the United States and the GMAG (Genetic Manipulation Advisory Group) rules in the United Kingdom. Sydney Brenner, who was at the Asilomar meeting, took a very prominent role in drawing up the conclusions, which were important for the subsequent establishment of the recombinant DNA experimental guidelines in the United Kingdom.[15]

Asilomar undoubtedly helped to set the scene for the birth of the biotechnology industry on the west coast of the United States. In Cambridge, Massachusetts (MA), Harvard and MIT personnel worked hard to ensure recombinant DNA facilities adhered to NIH guidelines. This challenge was compounded by public opposition to the use of recombinant DNA technologies in Cambridge, including a moratorium on such experiments imposed in 1976. MIT faculty and their administration met with the citizens of Cambridge to help them understand recombinant DNA research, explaining how the NIH guidelines would ensure safety. By 1977, the scientific community won its case, when the City of Cambridge passed an ordinance adopting the NIH guidelines and lifting the rDNA moratorium. The competitive disadvantages of not participating in the biotech revolution—well under way on the west coast—had also become apparent.

Before introducing the biotechnology companies that were set up in those very early days and the products that they were trying to make, I need to help you get a bit more familiar with some of the cloning technology, especially as it pertains to making proteins in microorganisms like *E. coli*. An accessible (I hope) description of the cloning technologies is shown in Box 1. I will do this for the other technologies that we will explore together in upcoming chapters, as it is the best way to understand most of the companies and events. Those readers who either know about cloning technology or do not feel the need to know can skip on ahead to the description of the companies set up to exploit it.

THE FIRST COMPANIES AND THEIR PRODUCTS: BIOGEN, CETUS, GENENTECH, AND GENEX

Biogen

Biogen was set up in 1978 by Walter (Wally) Gilbert from Harvard and Phillip (Phil) Sharp from MIT. It started as a European company with

headquarters in Geneva. It later would add a facility on Binney St. in Cambridge, MA. The venture capitalists (VCs) involved initially were Ray Schaefer, who knew Phil Sharp and Wally Gilbert personally, and who was one of the founding investors in the biotechnology industry, and Daniel (Dan) Adams, the head of venture capital at Inco, who had founded and had been the CEO at several companies (Adams had previously invested in Cetus and had made the first outside equity investment in Genentech).[h]

Because of the moratorium on genetic engineering in Cambridge at that time, Biogen did not initially have its own labs. Most of the early cloning work was done in the labs of the non-Cambridge founders and collaborators. In addition to Gilbert and Sharp, the Biogen scientific founders included Charles Weissman (Zürich), Bernard Mach (Geneva), Kenneth Murray (Edinburgh), and Heinz Schaller (Heidelberg).[i]

Cetus

Cetus was founded in 1971 by Ron Cape, Peter Farley, and Don Glaser. They initially focused on industrial microbiology and the development of antibiotics and chemical feedstocks but their focus altered after the development of recombinant DNA technology. The game really changed for Cetus when they raised $108 million in their IPO following that of Genentech. It was the largest IPO to that date, raising much more than Genentech, which had gone public in October 1980.

Genentech

In mid-1975, an out-of-work wannabe venture capitalist named Robert (Bob) Swanson, who was intrigued by the revenue-driving potential of the new recombinant DNA science, met a reluctant University of California San Francisco (UCSF) professor Herb Boyer for what Boyer thought would be a 10-minute discussion. The meeting turned into a several-hour marathon at Churchill's Bar in San Francisco, where the two men sketched out a plan for a genetic engineering technology (GenEnTech) company. Swanson and

[h] All of these investments would achieve a combined value of many millions of dollars.

[i] I remember visiting the original Biogen facility in Geneva for a job interview in 1980 before joining Celltech. At that time, it was an 8000-sq-ft lab in an old watch factory with about 16 scientists.

Boyer[j] both kicked in $500 of their own money to incorporate Genentech on April 7, 1976. In addition, Swanson, who had been "let go" earlier by the venture capital firm Kleiner Perkins (KP),[k] convinced his former employers, specifically KP founding partner Thomas Perkins, to invest $100,000 for 25% of the new company at that time.

Genentech's founders were truly undertaking the task of building something entirely new. They considered their initial late-1970s competition to be limited to the companies Cetus and Biogen and later Genetics Institute (founded 1981). Amgen, also founded in 1981, was chasing projects such as chicken growth hormone and cloning the plant genes that synthesised indigo (the dye used for blue jeans), and thus were not taken very seriously as scientific or business competition at that time (Chapter 2).[l]

Genex

Genex, which was formed in 1977 in Gaithersburg, Maryland, focused on the industrial application of biotechnology for enzymes, involving structural biology and nascent computational methods for structure prediction. Genex was founded by Princeton-based venture investor Robert (Bob) Johnston, with Leslie Glick as the CEO and Kevin Ulmer the most senior scientist. Genex somehow managed to broker a deal with the Bendix Corporation, which brought considerable dollars to the research table to fund their protein engineering aspirations for industrial enzymes. One of the projects was to make phenylalanine for making the artificial sweetener aspartame. Genex invested heavily in building a production plant for aspartame in Paducah, Kentucky, in an old Seagram's drinks cannery. Bendix was taken over by Allied Corp.[m] G.D. Searle, who owned aspartame already, had

[j] Initially there was a certain amount of political sensitivity and jealousy surrounding Herb Boyer and his associations with the company, especially from other UCSF faculty, who subsequently got into their own commercial ventures. Boyer was on the Genentech Board of Directors but deliberately kept a distance between the work being done in the Genentech labs and his lab at UCSF. Nonetheless, the animosity persisted.

[k] The company has been through several naming iterations. Biotech venture capitalist Brook Byers, who worked for Asset Management (another venture firm in the Bay Area) shared a workspace with Swanson but did not invest in Genentech. Byers later joined Kleiner Perkins and the firm became the law firm–sounding Kleiner Perkins Caufield & Byers. It is now simply KP.

[l] Early critics of Amgen turned out to be quite wrong.

[m] Who had absolutely no idea how to value or deal with the Genex project.

a relationship with Ajinomoto in Japan as commercial suppliers of the dipeptide, and Genex inevitably ran into financial difficulties.[n]

INSULIN: BIOGEN, GENENTECH, AND THE UNIVERSITY OF CALIFORNIA SAN FRANCISCO (UCSF)

The first highly public research undertaking at Genentech was to try to produce insulin in bacteria through genetic engineering.

The insulin hormone protein was first isolated by Drs. Frederick Banting and John Macleod at the University of Toronto over 100 years ago as a substance from dog pancreas that could reverse diabetes symptoms in dogs after injection.[o] In 1922, the first human was treated successfully with insulin isolated from bovine pancreases.[16]

Insulin was also the first protein for which the complete amino acid sequence was determined by Frederick (Fred) Sanger in Cambridge (UK) in 1955. Sanger subsequently was awarded his first Chemistry Nobel Prize in 1958 for this technological tour de force. Dorothy Hodgkin in Oxford was also awarded a Nobel Prize in Chemistry in 1964 for determining the three-dimensional structure of insulin (and other macromolecules) by X-ray crystallography.

Despite its relatively small size as proteins go, insulin is somewhat complex. It is made up of two different proteins chains, an A chain of 21 amino acids and a B chain of 30 amino acids joined by two disulphide bonds.[17] It is made by the pancreatic β cells as a single 74-amino acid precursor called pre-proinsulin that contains a 31-amino acid C peptide that is chopped out to produce the final insulin heterodimer.

The pharmaceutical company Eli Lilly became the principal provider of insulin for human use, obtaining it from either bovine or porcine sources. Even though this was a massive breakthrough for many diabetes sufferers, allergic reactions could and often did occur in patients whose immune systems saw the animal-derived insulin (with some minor variations in amino acid sequence) as foreign. In addition, it was often challenging to obtain animal pancreases in sufficient quantity to meet the demand for insulin. It was not

[n] In 1985 they fired many of the staff in Maryland and closed the Paducah plant to focus on contract research.

[o] Banting and McLeod (but not Charles Best, who was a student) were awarded the Nobel Prize in Physiology or Medicine in 1923 for this discovery.

a big jump, even in the mid-1970s, to think about producing insulin using the new recombinant DNA techniques. However, the technical challenges were prodigious.

The Groups Racing to Clone the Insulin Gene[p]

Three main groups set out to make human insulin by recombinant DNA methods. The principal players were Genentech working with Keiichi Itakura, Art Riggs, and Roberto Crea at the City of Hope Hospital in Duarte, California and Herb Boyer at UCSF. A second group at Harvard led by Wally Gilbert was sponsored by Biogen, and a third group at UCSF (not involving Boyer) led by Bill Rutter and Howard Goodman in the same biochemistry department, jumped into the race. The City of Hope/Genentech team pursued a chemical synthesis method to make the insulin genes (i.e., they synthesised the insulin gene one DNA base at a time), whereas the other two groups attempted to make an insulin-encoding cDNA from human mRNA (see Box 1).

Genentech's City of Hope collaborators had already established that a gene coding for a small 14-amino acid hormone called somatostatin could be made by chemical synthesis and cloned into the bacteria *E. coli,* which would then make small amounts of the protein.[18,q] The stage was set for doing the same with the insulin A and B chains. This approach had the added advantage of not falling under the Recombinant DNA guidelines, because the DNA was synthetic rather than obtained from the human genome or via mRNA.

The Biogen/Harvard group led by Wally Gilbert, consisting of Argiris Efstratiadis, Lydia Villa-Komaroff, and Forrest Fuller, took the cDNA cloning route following the work of recombinant DNA pioneer Tom Maniatis, who had already cloned rabbit β globin this way. They focused first on cloning the rat insulin gene from mRNA isolated from a rat

[p] This race is recounted in detail in the book *Invisible Frontiers* by Stephen Hall (referred to in the Preface).

[q] The chemical synthesis of nucleic acids had been pioneered by Har Gobind Khorana. Solid phase synthesis had been recently developed by Bruce Merrifield, who had been awarded the Nobel Prize in Chemistry in 1984. But it was not until 1982 that the first commercial DNA synthesizers became available from Applied Biosystems (the ABI 380A) and others, making the technology much more broadly accessible.

insulinoma, before attempting the human version. The Rutter–Goodman group, including postdocs Peter Seeburg and Axel Ullrich,[r] used the same general approach as the Harvard team.

Technical and other kinds of challenges were immense particularly for both cDNA-focused groups. The rat insulinoma as a source of mRNA turned out to be scarcely any better than that extracted directly from pancreas. The internecine politics in the labs at UCSF did not help either as it was clear to everyone that more than one team in the biochemistry groups at UCSF was trying to clone and produce insulin at the same time. Also, the Harvard group in 1976–1977 were forced to work remotely at Cold Spring Harbor, owing to the moratorium on recombinant DNA experiments that was in place at that time in Cambridge (MA). Recombinant DNA regulations notwithstanding, Ullrich (the UCSF Rutter–Goodman group) managed to clone most of the human pre-proinsulin cDNA into a bacterial plasmid called "pBR322," one of the preferred (from a regulatory perspective) cloning vectors.

A Trip to Porton Down

Efstratiadis and the Harvard team eventually succeeded in cloning rat insulin cDNA. But regulations required the use of a category 4 laboratory—the most secure laboratory possible—to clone the human version. They decided to approach the Ministry of Defence–operated Microbiological Research Institute (MRE) at Porton Down in Wiltshire in the United Kingdom.[19] MRE was the U.K. equivalent of Fort Detrick in Frederick, Maryland. It was directed at the time by my father Bob Harris, who was also a member of GMAG. He helped to organise the Biogen/Harvard visit, and Peter Greenaway served as their guide on the ground.[s]

As beautifully recorded in Hall's *Invisible Frontiers*, this was not a successful experience for the Biogen/Harvard team. The biological safety precautions were very onerous and commuting back and forth from Salisbury (the

[r]Seeburg and Ullrich both later joined Genentech to work on the cloning of human growth hormone.

[s]This was an interesting connection for me. I was not only quite familiar with Porton Down in the late seventies, but I also worked at SAIC-Frederick in 2006 and became familiar with Fort Detrick. The MRE midsummer garden parties were legendary. Although quite formal in a military way, the booze flowed liberally from each corner of the marquee that was set up. I remember them well and they were not to be missed.

nearest large city), was far from ideal. The team failed to meet their objective, succeeding only in recloning rat rather than human insulin cDNA.[t] In the end Biogen gave up on its efforts to clone the cDNA for human insulin. In the meantime, the Rutter–Goodman UCSF team, although they learned a lot about the molecular biology of insulin gene expression and protein formation, fell short of finishing first to clone it.

The chemical synthesis approach of Genentech won both the cloning and expression race.[20] A subsequent collaboration with Eli Lilly, who needed to remain relevant in the insulin market in this new rDNA age, led to the successful commercialisation of recombinant human insulin. Following rapid clinical trials, Humulin was approved in late 1982, only six years after Genentech was founded. Humulin is still sold by Eli Lilly alongside a faster acting version called Humalog, with combined 2020 sales of ~$4 billion.

GROWTH HORMONE: GENENTECH AND UCSF

Another obvious target for the early recombinant DNA companies was human growth hormone (hGH). The bovine version (like insulin), available for the treatment of pituitary dwarfism, was obtained by extraction and purification from cadaver-derived human pituitary glands. The Swedish company Kabi Vitrum was the only provider of growth hormone made this way.

Although the protein is relatively small (around 200 amino acids), the gene was still too big for the chemical synthesis approach used for cloning somatostatin or insulin. But its size was in the range for cDNA cloning. Peter Seeburg, who did his PhD research with phage geneticist Heinz Schaller in Heidelberg (a Biogen founder), came to Herb Boyer's lab at UCSF and ended up working with John Baxter on the cloning of rat growth hormone, from a rat tumour that overexpressed the hormone. The small team pursued the project in stealth mode, reporting the cloning and the comparative sequence of rat and human growth hormone in December 1977 in *Nature*.[21] The paper also described the pro-growth hormone precursor of 216 amino acids and showed how it was matured to the natural hormone of 190 amino acids after secretion. It should have been simple to express the cDNA in *E. coli*, but making a construct that expressed active protein in bacteria turned out to be less straightforward than expected. A paper describing the synthesis of

[t] Presumably from some contamination in their reagents.

the hormone in *E. coli* eventually was eventually published (also in *Nature*), but the amounts made were small.[22]

As Chairman of the UCSF Biochemistry department, Bill Rutter wasted no time protecting the intellectual property (IP) for the growth hormone cloning project. He also established a collaboration on growth hormone with Eli Lilly.

In September 1978, after the successful cloning and expression of human insulin, Genentech also decided to pursue human growth hormone and were very well aware of what was transpiring at UCSF. Genentech teamed up with Kabi Vitrum (the Swedish pharmaceutical growth hormone provider). After cloning most of the human growth hormone cDNA from mRNA, Genentech decided to use its chemical synthesis capabilities to make an hGH expression construct that would express mRNA in *E. coli* from a hybrid DNA molecule, consisting of chemically synthesised DNA coding for the first 23 amino acids of the protein, with the remaining 24–191 amino acids coming from cDNA. This was not only an elegant means to avoid having to look for a cDNA that expressed the whole protein, but it was also a smart move from an IP perspective, because the DNA was now semisynthetic and not derived solely from mRNA. This construct was also engineered for successful transcription and translation in *E. coli*.[23]

The ambitious and talented young scientists Peter Seeburg and Axel Ullrich (mentioned above) and John Shine (also at UCSF) decided to join Genentech, not only because they knew people there quite well, but also because they were all tired of the UCSF academic politics and the hierarchical infighting. They were also much encouraged by the attitude of the Genentech scientists and management in treating each other as colleagues and equals while providing the opportunity to publish their important results in the scientific literature.[u]

Their transition to Genentech, however, was fraught with intrigue. Contrary to commercial practice but consistent with academic practice, the investigators took their growth hormone clones with them to Genentech after a nighttime visit to the UCSF lab to retrieve them. The disputed origin of the clones resulted in a protracted lawsuit between UCSF and Genentech, settled by the latter paying the university $200 million in 1999.[24]

[u] Promotion of publication was quite unusual for both big and small companies at the time and remains so in some places still today.

From a commercial perspective the cloning of growth hormone was important for the upcoming planned Genentech IPO, as it led to a product called Protropin that underwent successful clinical trials demonstrating efficacy in pituitary dwarfism and people of short stature. Protropin, FDA-approved and launched in 1985, was a commercial success. The fact that, in early 1985, Creutzfeldt–Jakob disease—the human equivalent of mad cow disease (bovine spongiform encephalopathy)—had been seen in some patients treated with pituitary-derived human growth hormone certainly helped. Recombinant hGH (despite some allergic reactions in some patients) was safer than the natural material derived from bovine pituitaries, which was subsequently banned.

Following its separate collaboration with UCSF, Eli Lilly launched their own version of recombinant hGH (Humatrope) in 1987. Unlike the Genentech product, the Eli Lilly version was missing the amino acid methionine at the beginning of the protein, perhaps making the Eli Lilly product less antigenic. Despite that difference, Genentech did very well in the market competing with Eli Lilly, an established, traditional pharmaceutical company. A big and likely unwelcome surprise for the "big player," a result that has been repeated many times since then by the biotech industry.[v]

Going Public

The day of the Genentech IPO was as exciting for its scientists as it was for investors (Plate 3). All watched the ticker with bated breath. According to Mike Ross (the 10th employee at Genentech and now a managing partner at SV Health Investors), the day was filled with naïve conversation about liquidity and fortunes.[w]

There were many reactions to the IPO. The investors and VCs (e.g., Lubrizol, which owned 25% of Genentech at this point) were pleased with the liquidity, as it represented an opportunity for a real return and real cash. Others felt it was just a step along the way to building a completely new type of drug company. Neither view was wrong.

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[v] I suspect it has something to do with the passion, commitment, and teamwork of small start-up companies.

[w] There was an earlier partial liquidity event for Genentech stockholders as some external people from Hollywood bought some Genentech stock from the staff before they went public.

The first building that Genentech leased was a 5000-sq-ft former dialysis center at 460 Point San Bruno Boulevard in South San Francisco. Over time other buildings on the street, now called "DNA Way," were taken over by Genentech. In Building 3, for example, there was a manufacturing suite where the first lots of recombinant t-PA (tissue plasminogen activator)[x] were made in 10,000-L fermenters in suspension Chinese hamster ovary cells using the dihydrofolate reductase (DHFR) amplification system. It was a replica of the plant that Boehringer Ingelheim, the Genentech partner in Europe for tPA, had built.

Like many companies after an IPO, Genentech did "follow on" rounds to raise more money. They were also one of the first companies to make use of Research and Development Limited Partnerships (RDLPs). This was a concept for off-balance sheet financing catalysed for use in biotech, having been used in the oil and gas industry before by Stephen Evans-Freke, as a tax shelter strategy. RDLPs were a way for the company to raise additional money from investors who invested specifically in the project that the partnership covered, in return for profits from any products that resulted from the partnership. Genentech raised money this way to finance the cloning of interferon-γ and tumour necrosis factor. RDLPs were also used by Centocor, Amgen, and Genzyme. They were subsequently disallowed via new accounting rules as a means of off-balance sheet financing for biotech companies.

Being the first "real" biotech company, Genentech worked on many cloning projects before anyone else, but they did not win all the cloning races.[y] Strange as it may seem, Genentech abandoned their erythropoietin (EPO) project (see Chapter 2) even though the scientists wanted to do it, based on what turned out to be a less than enthusiastic (and erroneous) marketing assessment from their commercial team.[z]

The Genentech Culture

The HoHos were a defining feature of the Genentech culture, regular parties held every week where all staff got together. I happened to be invited to one of these in 1980, where all the management team, including Bob Swanson,

[x] We will talk later about *t-PA*, a gene that my lab was involved in cloning at Celltech, in Chapter 2.

[y] They usually won the ones I happened to be working on at Celltech, so they were not only my bête noire but also the people I looked up to as being some of the best in the business.

[z] Not the first or last time has such a thing happened in either biotech or pharma.

wore pink tutus. It was an event where I felt unusually out of place as the "reserved" Englishman, at least for a while. Eventually, these company-sponsored events grew ever larger—persisting through the 1990 Roche acquisition—requiring considerable space and organising. Many early biotech companies had equivalent weekly functions for staff at which people tended to let their hair down.[aa]

Practical jokes were also popular. One true story concerned Mark Matteucci, one of the first chemists at Genentech originally from the Marv Carruthers lab in Boulder. Mark had bought a small pink car. He was very proud of it. One time when he was out of town the car was removed from its parking space, hidden, and replaced by a similar pink car that had been crushed at the local breaker's yard. You can imagine Mark's surprise when he returned to pick up his car after the trip to drive it home to find a pink cube with no wheels in its place. Such high jinks defined the "work hard–play hard" Genentech culture, which is missing from many start-up companies now.[bb]

Not everything at Genentech was magical. There were so-called "shitstorms," and many key mistakes made. The first volunteers treated with growth hormone, for example, got symptoms (chills, etc.) from endotoxin contamination of the product. It took a year to figure out why and to correct it.[cc]

INTERFERON: BIOGEN, GENENTECH, AND CETUS

Cloning Interferon

Interferon was discovered by Alick Isaacs and Jean Lindenmann in the United Kingdom in 1957 as a substance that reduced (interfered with) the replication of influenza virus in eggs and other viruses in plaque assays. By the late 1970s, Wellcome Research Laboratories were making lymphoblastoid interferon from large cell cultures. Today, several different types of interferons (and eight interferon genes) are known, called interferon α, β,

[aa] Some of the behaviours would simply not be tolerated now (think the culture of the 1980s depicted in many movies like *Wall Street*[25]).

[bb] Sometimes for misguided political correctness.

[cc] Mike Ross, a protein chemist who looked after protein production from the very early days of Genentech and a colleague of mine at SV Health Investors, helped to sort out the endotoxin contamination problem.

γ, and λ, with several subtypes (especially for interferon-α), with different modes of action, and with different cellular receptors. These interferons are made by different types of cells but were originally defined as fibroblast interferon or leukocyte (white blood cell) interferon. The interferon genes were one of the first set of mammalian genes (or rather cDNAs) to be cloned and expressed in *E. coli* in anticipation that the recombinant protein could be a highly useful antiviral or anticancer drug.

Before joining Biogen, the Weissman lab had been trying to clone mouse interferon in a collaboration with Peter Lengyel, whom Weissman knew from Severo Ochoa's lab at New York University where they were postdocs together. Their approach was to clone cDNA from mRNA from mouse cells that were known to make interferon and use "hybrid selection" to screen for the *E. coli* clones containing the cDNA (see Box 2). When Weissman teamed up with Biogen, the members of the lab turned their attention to cloning human interferon by the same methods.

At Genentech, David Goeddel was also cloning human interferon in a collaboration with Roche and Sidney Pestka at the Roche Institute of Molecular Biology in Nutley, New Jersey. They had some protein sequence

BOX 2. FINDING INTERFERON CLONES

Hybrid Selection

In hybrid selection the plasmid DNA from the clones is immobilised in arrays on filters and mRNA from mouse cells hybridised to them. If a clone contains interferon cDNA, then the mRNA should hybridise to it. Recognition of the appropriate mRNA is done by washing off the mRNA from colonies on the arrays and translating it in *Xenopus* oocytes to look for any interferon protein activity (by antiviral assay) that had been translated from the mRNA. This would mark the appropriate "interferon cDNA-containing" clone. This is a very time-consuming and difficult assay.

Differential Hybridisation

Here the amount of radioactive cDNA from uninduced cells bound to clones is compared to that from similar radioactive cDNA made from "induced" cells. Induced cells were made by treatment with double-stranded oligonucleotides or virus nucleic acids, which were known to "induce" interferon synthesis (and increasing mRNA levels). Clones containing interferon cDNA would "light up" with the radioactive probe from induced cells but not the control. This was also a very time-consuming activity involving the picking of many thousands of recombinant *E. coli* clones to find the right ones.

but not enough to make appropriate short DNA "probes" to find their clones. Instead, they used differential hybridisation with radioactive cDNA made from cells that had been virus-infected to induce interferon synthesis (Box 2). Pestka was using the same sort of expression screen that the Weissman lab was using. When some protein sequence finally emerged, interferon-specific clones were found. In early 1979, when Biogen was running out of money, the pharmaceutical company Schering-Plough stepped in to rescue the work by buying 16% of the company (for $8 million), as well as to secure the rights to it (including the IP).

Science by Press Release

After filing the patent application around Christmas 1979, Biogen announced in a press release early the next January that the Weissman lab had succeeded in cloning the human α-interferon gene. According to reporter Nicholas Wade at *Science*, January 16, 1980, was "the date on which molecular biology became big business."[26] The interferon cloning news was also presented at a press conference at the Boston Park Plaza hotel by Weissman and Gilbert. *Time* magazine also ran a story about interferon as a potential anticancer agent.[27] It was called "science by press release" at the time, a practice generally derided by the industry but commonplace nevertheless.[dd] Schering-Plough stock went up on the news, although the intent was to promote Biogen and its cloning capabilities more than to enrich Schering-Plough shareholders.

Fibroblast (β-) interferon was cloned shortly afterwards by Tadasugu (Tada) Taniguchi in Japan, who had been a postdoc in the Weissman lab. Publications followed shortly thereafter in *Nature* and *Science* describing the cloning of both α1- and α2-interferon, β-interferon, and their amino acid sequence comparisons.[28] Both the Biogen and Genentech groups produced their interferons in *E. coli* using promoter-based plasmids (see Box 1).

Unusually, Genentech and David Goeddel came off second in the race to clone both α- and β-interferons, but they did succeed in filing their own patent applications. Patrick (Pat) Gray at Genentech cloned interferon-γ in 1981 ahead of anyone else. Interferon-γ, known as Actimmune, was approved by the FDA to treat chronic granulomatous disease (CGD) in children in 1990. InterMune subsequently acquired this drug from Genentech.

[dd] It still is common practice, and it is still derided by the hard-core science community. As indeed it should be.

Cetus was also working on cloning interferon(s) at the same time using by and large the same methods in a collaboration with Triton Biosciences. It subsequently cloned and developed β-interferon, which became (via Chiron) the Schering AG product Betaferon for treating multiple sclerosis.

The Commercial Potential

Genentech had their Roche connections and Biogen was working with Schering-Plough. The companies entered into cross-licensing deals for the respective patent applications, owing to the apparent size of the potential market for interferon as a drug. The cloning of α- and β-interferons was important for different reasons for both Biogen and Genentech. The milestone was a key contributor to Genentech's IPO in October 1980 (Plate 2), but the commercial success of α-interferon as a product was modest at best, despite the high hopes for it being an antiviral and anticancer drug for multiple indications. Both the Schering (Intron A, Biogen) and Roche (Roferon, Genentech) versions were approved in 1986 for hairy cell leukemia, a very uncommon blood cancer.

β-interferon (1a) had a very different outcome commercially. Schering AG–developed β-interferon, and Betaferon got approval before the Biogen product. It is still unclear precisely how β-interferon mediates its effect, but interferons generally affect the activity of the immune system, and β-interferon is known to affect regulatory T cells (T regs). Clinical studies in multiple sclerosis (MS) showed that β-interferon reduced the number of relapses in patients with RRMS (relapsing, remitting MS) and reduced inflammation. Avonex, the injectable Biogen product, was approved in 1996[29] with parallel orphan drug status given to the Schering AG product.

The Avonex Pen, a specialised prefilled syringe with a small and covered needle, now provides patients with the exact dose they need and is intended for easier self-administration. The Avonex Pen was approved in Europe and Canada in 2011 and in the United States in 2012. Avonex, which made Biogen commercially, is still used today as a very successful and important medicine for adults with MS, including clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS), and active secondary progressive MS (SPMS). Tysabri was their second MS drug. Tysabri is a monoclonal antibody that blocks a cell surface protein on T cells to prevent them from migrating into the brain. It is extremely effective in combating the disease but has a serious side effect as it can reactivate John Cunningham (JC) virus

in patients who have the latent virus in their brains and cause a neurological infection (progressive multifocal leukoencephalopathy [PML]) that is sometimes fatal. These two drugs were and still are a mainstay of the Biogen MS franchise.

In 1982, Biogen moved its headquarters from Geneva to Binney Street in Cambridge (MA). The company went public in 1983, selling 2.5 million shares at $23/share in their IPO. In July 1987, Biogen sold its Geneva research facility to Glaxo to reduce expenses. It became the Glaxo Institute of Molecular Biology (GIMB).[ee]

CETUS PRODUCTS

Frank McCormick and I were at the University of Birmingham in the United Kingdom at the same time. I was the class of '68 while Frank was class of '69. We both read biochemistry, and crossed paths frequently. When I went to the State University of New York (SUNY) at Stony Brook in 1977 to work with Eckard Wimmer on polio virus, I ran into Frank again as he was doing postdoctoral work there with Seymour Cohen on polyamines in herpesviruses, after completing his PhD in Cambridge (UK) with Alison Newton. We even worked in the same building. We have been friends ever since I helped him to go back to the United Kingdom for a job at the Imperial Cancer Research Fund (ICRF) labs in London (see Box 3).

The ICRF lab at that time was heavily into understanding the function of a protein called p53, with many groups competing internally rather than collaborating. Peter Tegtmeyer at SUNY Stony Brook (coincidentally) had discovered p53 in SV40 transformed cell lines, but no one knew what the protein did. Many people made antibodies to p53 and to SV40 T antigen (a virus protein that transforms cells) and found out that p53 and SV40 Tag

[ee] As I was at Glaxo looking after Biotechnology, I remember the Institute very well and visited it frequently. Jonathan Knowles, who went on to run Roche R&D, was the GIMB Director. As part of the sale, Glaxo also obtained marketing rights to both the cytokines IL2 and GM-CSF (which you will learn more about later on in the book). Most of the more than 100 employees at Biogen Geneva, who had a great deal of molecular biology and genetics expertise, became part of Glaxo. The Institute moved to one research building in the late 1980s. After the Glaxo Wellcome merger in 1994, despite the talent that was there, Glaxo off-loaded the whole facility to Serono. This was a short-sighted decision to save money to increase focus on drug discovery, completely missing the point that diversity of research activities and thinking leads to much better innovation.

BOX 3. FRIENDS GOING TO JFK

Frank went back to the ICRF on a fellowship in February of 1978. As a good mate, I told him I would take him to JFK Airport in my rather beat-up old pale blue Plymouth Valiant (called Elizabeth after the Queen). Elizabeth had more than 175,000 miles on her clock (odometer) and cost me the princely sum of $250. The engine in this car had such a gap between the piston rings and the cylinders that if you really tightened the oil cap too much the dipstick would blow out. It was basically a wreck—but it ran for a while. It turned out on that very day I was taking Frank to JFK the petrol tank began to leak. I had no time to fix it then, but it was still drivable.

I picked him up at his apartment on that fateful day and found out that it was not just him going to the airport but his partner at the time (Judy) and their three dogs (Toulouse, Oggie, and Crackers). We got everyone in. There were tears and shouts and a growing smell of petrol. The dogs would be in quarantine in England for six months. As we got nearer to the airport, I asked Frank where the British Airways freight terminal was. "Over there somewhere" was the reply, as the deadline for checking in the dogs got ever closer. We circled the airport for 20 minutes and found it with about five minutes to spare.

formed a complex. At ICRF, Frank made an antibody to p53. With that antibody in hand, Frank returned to the States to work with Bob Tjian, who had pure T antigen at University of California Berkeley, to investigate the relationship between p53 and T antigen further. Rick Myers, who now runs the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama, was a graduate student there at the time. Rick will appear again a bit later in the book.

Starting at Cetus

Frank went to Cetus in late 1980 after Genentech had gone public and Cetus was rebooted to work on recombinant interferons. Cetus had an impressive scientific advisory board (SAB) consisting of Stan Cohen, Francis Crick, Joshua Lederberg, Ham Smith, Tom Merigan (an interferon clinician), and Andrew Schally. Frank developed there a patented cell system for expressing recombinant proteins based on amplifying constructs containing the dihydrofolate reductase gene. Biogen infringed this patent and was sued by Cetus. Subsequently others also developed such amplifiable expression vectors, including Genentech and Genetics Institute (GI). After a chance meeting with the late Chris Marshall (who was at the Cancer Research Campaign

Labs in the United Kingdom) at a meeting in Spetsai in Greece, Frank got interested in the *ras* oncogene, and he developed a K-*ras* project at Cetus.

Cetus was an extraordinary place to do science and, of course, the company was not immune from the rather typical early eighties Bay Area culture of "partying." Cetus partied heavily and a visit there in early 1980 confirmed it for me. Apart from the great science and parties, one of the highlights I remember from that trip was Frank and I going to the movies to watch the film *The Long Good Friday* starring Helen Mirren, which had just been released. I think surreptitiously we both were hoping that Ms. Mirren (now of course Dame Helen Mirren) would disrobe as she had done before in previous movies.[ff]

Kary Mullis, who was awarded the 1993 Nobel Prize in Chemistry for discovering the polymerase chain reaction (PCR) and about whom many stories[gg] have been written, was at Cetus at the time Frank was there. He was using DNA polymerase and oligonucleotide primer extension to look for mutations in the β-globin gene, and for codon 12 and 61 mutations in K-*ras* (mutations that were oncogenic in tissue culture).

Kary Mullis realised that a newly synthesised DNA strand could be primed again with a complementary oligonucleotide and both strands could be amplified. He could never really get the concept to work very well, owing to contamination of the lab with β-globin DNA. Once a clean room was found and an able technician started to run the reactions, it worked. Heat was used to separate the complementary strands so another priming round could take place. Originally, DNA polymerase (which is heat-sensitive) would be added between each amplification step. It was Kary who realised that thermostable polymerases (like *Taq* polymerase from a thermostable bacterium) would enable continuous rounds of replication to be done. These efforts led to PCR. The protocols were developed at Cetus long before it was finally published in a landmark paper.[30] Kary was not even the first author, although he was on a later publication.[31,hh]

Neither IL2 (Proleukin) nor β-interferon was doing well enough at Cetus for it to continue as an independent company. It was taken over by Chiron for $360 million in 1991, and the PCR technology licensed to Roche

[ff] We were disappointed on that score, but it is still one of my favourite movies.

[gg] Some of them undoubtedly true.

[hh] A paper from the Khorana lab in 1971 in the *Journal of Molecular Biology* preempted the idea of PCR, but it was never taken up.[32,33]

at the same time. Wall Street perceived the deal very negatively despite the combined company having more than 20 potential products in clinical trials.

Biogen, Cetus, Genentech, and Genex were the major players in the new recombinant DNA technology in the late 1970s. Other companies existed or were formed, most notably those providing critical reagents and tools to the rapidly developing recombinant DNA technologists in industry and in academia. Collaborative Research (from whom I bought oligo-dT cellulose to purify mRNA via the poly(A) tail) formed a subsidiary called Collaborative Genetics. Bethesda Research Laboratories and New England Biolabs were also set up primarily to sell pure restriction enzymes and other important enzymes, such as DNA ligase and terminal transferase, needed to practice the art of recombinant DNA.[34,ii]

In early 1980 before any of the four major players had gone public, they had a *combined* valuation of ~$500 million, all based on perception and promise. Cetus had a valuation of $250 million and was trying to raise $55 million. Genentech had a valuation of more than $100 million, and Biogen also had a valuation of ~$100 million. Genex was valued at $75 million. These days single companies at that stage can easily have a valuation of $500 million, based entirely on promise and perception and nothing anywhere near the clinic. It was those valuations, however, that persuaded others to jump on the biotech bandwagon and build the industry in the United States, the United Kingdom, and other parts of the world.

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