Chapter 4

Patenting of Micro-Organisms and Cells

"The grant or denial of patents on micro-organisms is not likely to put an end to genetic research or its attendant risks. The large amount of research that has already occurred when no researcher had sure knowledge that patent protection would be available suggests that legislative or judicial fiat as to patentability will not deter the scientific mind from probing into the unknown any more than Canute could command the tides. "

Chief Justice Warren Burger
Chakrabarty v.. Diamond

"Those companies in the private sector which are investing hundreds of millions of dollars in this new science do not accept the theory that patents are unimportant. Such a concept is particularly repugnant to patent-conscious, research-intensive pharmaceutical firms dealing in global markets with drugs which require staggering investments of time and money before ultimately yielding a commercial return. To them the patent shelter is paramount. It is quite literally their sole incentive for risk taking."

William Duffey
Patent Lawyer, Monsanto

CONTENTS

Figures

Tables

Chapter 4
Patenting of Micro-Organisms and Cells

INTRODUCTION

The development of recombinant DNA technology in the 1970s led to debate on many policy questions, one of which concerned the patenting of living matter. The purpose of this chapter is to discuss process patent protection available prior to 1980, the Supreme Court's landmark decision permitting the patenting of living matter (in this case bacteria), and several patent-related events and trends that occurred or were identified subsequent to the Supreme Court case.

PROCESS PATENT PROTECTION PRIOR TO 1980

Patents on biotechnological developments date from the early days of the United States patent system. Louis Pasteur received a patent for a process of fermenting beer. Acetic acid fermentation and other food patents date from the early 1800s, while therapeutic patents in biotechnology were issued as early as 1895. The first patent for isolating nucleic acid was issued in 1945, and the first patent for preparing ribonucleic acid by a fermentation process was issued in 1966. Until the recent advances in biotechnology, such process patent applications were examined primarily by the U.S. Patent and Trademark Office's (PTO) examining group in fermentation chemistry (18). Since March 1988, a special biotechnology examining group handles these patent applications.

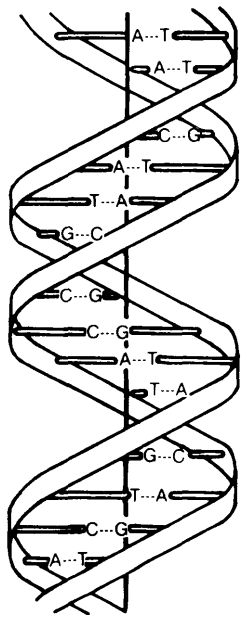
The development of recombinant DNA technology—the joining of DNA from different organisms—has resulted in greatly increased understanding of the genetic and molecular basis of life (see figure 4-l). Following the first successful directed insertion of recombinant DNA in a host micro-organism in 1973, scientific researchers began to recognize the potential for directing the cellular machinery to develop new and improved products and processes in a wide variety of industrial sectors (see figure 4-2). Many of these products were micro-organisms (microscopic living entities) or cells (the smallest component of life capable of carrying on all essential life processes). With the development of rDNA technology arose the issue of patenting the inventive results of the technology.

Prior to 1980, PTO would not grant patents for such inventions, deeming them to be "products of nature" and not statutory subject matter as defined by 35 U.S.C. 101.[1] Although patent applications were rejected if directed to living organisms per se, patent protection was granted for many compositions containing living things (e.g., sterility test devices containing living microbial spores, food yeast compositions, vaccines containing attenuated bacteria, milky spore insecticides, and various dairy products) (18). In the absence of congressional action, it took a catalytic court decision to clarify the issue of patentability of living subject matter.

THE *CHAKRABARTY* CASE

The Supreme Court's single foray into biotechnology occurred in 1980 with its ruling in the patent law case of *Diamond v. Chakrabarty (4).*

Figure 4-l-The Structure of DNA



SOURCE: Office of Technology Assessment, 1989.

[1] *Section 101. Inventions Patentable.* Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Ananda Chakrabarty, a microbiologist at the General Electric Research and Development Center in Schenectady, New York, had developed a genetically engineered (but not recombinant) bacterium capable of breaking down multiple components of crude oil. Because this property was not possessed by any naturally occurring bacteria, Chakrabarty's bacterium was thought to have significant value for the cleanup of oil spills.

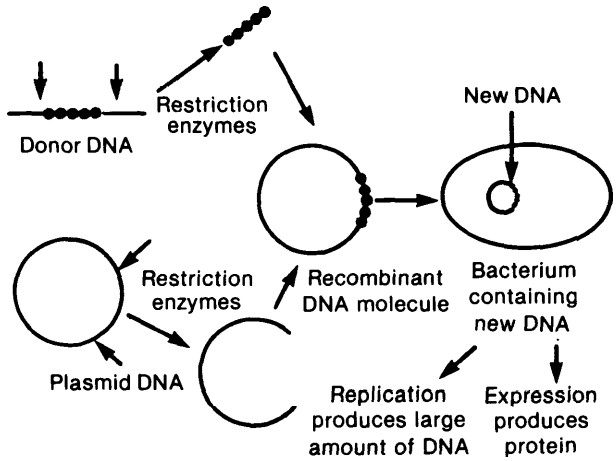
Chakrabarty filed a patent application asserting 36 claims relating to "a bacterium from the genus *Pseudomonas* containing therein at least two stable energy-generating plasmids, each of said plasmids providing a separate hydrocarbon degradative pathway." The patent claims were of three types:

- process claims for the method of producing the bacteria;
- claims for an inoculum comprised of a carrier material floating on water (e.g., straw); and
- product claims for the bacteria.

The patent examiner allowed the claims for the process and for the inoculum but rejected the claims for the bacteria on two grounds: 1) micro-organisms are "products of nature" and 2) as living things, micro-organisms are not patentable subject matter under 35 U.S.C. 101. Chakrabarty appealed the rejection of these claims to the PTO Board of Appeals. The Board reversed the examiner on the first ground, concluding that the new bacteria were not products of nature, because *Pseudomonas* bacteria containing two or more different energy-generating plasmids are not naturally occurring. The second ground of rejection—that the bacteria did not constitute statutorily protectable subject matter—was affirmed.

Chakrabarty then appealed the PTO decision to the Court of Customs and Patent Appeals, which reversed the decision (3). Judge Rich, writing for the majority in the split decision, relied upon an earlier lower court decision which held that the fact that micro-organisms are alive is without legal significance for purposes of the patent law (9).[2] The case was then appealed to the U.S. Supreme Court by the Government. **The Supreme Court, in a 5-4 ruling, held that a live, human-made micro-organism is patentable subject matter under section** 101 as a "manufacture>' or "composition of matter."

Figure 4-2-Recombinant DNA: The Technique of Recombining Genes From One Species With Those From Another



Restriction enzymes recognize sequences along the DNA and can chemically cut the DNA at those sites. This makes it possible to remove selected genes from donor DNA molecules to form the recombinant DNA. The recombinant molecule can then be inserted into a host organism and large amounts of the cloned gene, the protein that is coded for by the DNA, or both, can be produced.

SOURCE: Office of Technology Assessment, 1989.

How did the Court reach its conclusion? Because the case involved statutory construction, i.e., the meaning of the language of the statute and the intent of the legislature in enacting the statute, the Court conducted an analysis of the language and legislative history of section 101. In so doing, the Court reached the following conclusions:

- In looking at the **plain meaning of the statutory language,** words are to be interpreted as taking their ordinary, contemporary, common meaning. In addition, courts should not read into the patent laws limitations and conditions which the legislature has not expressed (23). Therefore, the terms "manufacture" and "composition of matter" must be interpreted in accordance with their dictionary definitions. Because both terms are expansive in their meaning, and are modified in the statutory language by the expansive term "any," Congress plainly contemplated that the patent laws

[2] Although the Supreme Court *decided* to hear both the *Bergy* and *Chakrabarty* cases, Bergy withdrew his claim so only the *Chakrabarty* case was argued.

U.S. patent 3,813,316, issued to Ananda M. Chakrabarty.

United States Patent Office

3,813,316
Patented May 28, 1974

3,813,316

MICROORGANISMS HAVING MULTIPLE COMPATIBLE DEGRADATIVE ENERGY-GENERATING PLASMIDS AND PREPARATION THEREOF

Ananda M. Chakrabarty, Latham, N.Y., assignor to General Electric Company

Filed June 7, 1972, Ser. No. 260,488
Int. Cl. C12b 1/00
U.S. Cl. 195—28 R 18 Claims

ABSTRACT OF THE DISCLOSURE

Unique microorganisms have been developed by the application of genetic engineering techniques. These microorganisms contain at least two stable (compatible) energy-generating plasmids, these plasmids specifying separate degradative pathways. The techniques for preparing such multi-plasmid strains from bacteria of the genus Pseudomonas are described. Living cultures of two strains of Pseudomonas (*P. aeruginosa* [NRRL B-5472] and *P. putida* [NRRL B-5473]) have been deposited with the United States Department of Agriculture, Agricultural Research Service, Northern Marketing and Nutrient Research Division, Peoria, Ill. The *P. aeruginosa* NRRL B-5472 was derived from *Pseudomonas aeruginosa* strain 1c by the genetic transfer thereto, and containment therein, of camphor, octane, salicylate and naphthalene degradative pathways in the form of plasmids. The *P. putida* NRRL B-5473 was derived from *Pseudomonas putida* strain PpGl by genetic transfer thereto, and containment therein, of camphor, salicylate and naphthalene degradative pathways and drug resistance factor RP-1, all in the form of plasmids.

BACKGROUND OF THE INVENTION

The terminology of microbial genetics is sufficiently complicated that certain definitions will be particularly useful in the understanding of this invention:

Extrachromosomal element.—A hereditary unit that is physically separate from the chromosome of the cell; the terms "extrachromosomal element" and "plasmid" are synonymous; when physically separated from the chromosome, some plasmids can be transmitted at high frequency to other cells, the transfer being without associated chromosomal transfer;

Episome.—A class of plasmids that can exist in a state of integration into the chromosome of their host cell or as an autonomous, independently replicating, cytoplasmic inclusion;

Transmissible plasmid.—A plasmid that carries genetic determinants for its own intercell transfer via conjugation;

DNA.—Deoxyribonucleic acid;

Bacteriophage.—A particle composed of a piece of DNA encoded and contained within a protein head portion and having a tail and tail fibers composed of protein;

Transducing phage.—A bacteriophage that carries fragments of bacterial chromosomal DNA and transfers this DNA on subsequent infection of another bacterium;

Conjugation.—The process by which a bacterium establishes cellular contact with another bacterium and the transfer of genetic material occurs;

Curing.—The process by which selective plasmids can be eliminated from the microorganism;

Curing agent.—A chemical material or a physical treatment that enhances curing;

Genome.—A combination of genes in some given sequence;

Degradative pathway.—A sequence of enzymatic reactions (e.g. 5 to 10 enzymes are produced by the microbe) converting the primary substrate to some simple common

metabolite, a normal food substance for microorganisms;

(Sole carbon source)$^-$.—Indicative of a mutant incapable of growing on the given sole carbon source;

(Plasmid)^{del}.—Indicative of cells from which the given plasmid has been completely driven out by curing or in which no portion of the plasmid ever existed;

(Plasmid)$^-$.—Indicative of cells lacking in the given plasmid; or cells harboring a non-functional derivative of the given plasmid;

(Amino-acid)$^-$.—Indicative of a strain that cannot manufacture the given amino acid;

(Vitamin)$^-$.—Indicative of a strain that cannot manufacture the given vitamin and

(Plamid)$^+$.—Indicates that the cells contain the given plasmid.

Plasmids are believed to consist of double-stranded DNA molecules. The genetic organization of a plasmid is believed to include at least one replication site and a maintenance site for attachment thereof to a structural component of the host cell. Generally, plasmids are not essential for cell viability.

Much work has been done supporting the existence, functions and genetic organization of plasmids. As is reported in the review by Richard P. Novick "Extrachromosomal Inheritance in Bacteria" (Bacteriological Reviews, June 1969, pp. 210–263, [1969]) on page 229, "DNA corresponding to a number of different plasmids has been isolated by various methods from plasmid-positive cells, characterized physicochemically and in some cases examined in the electron microscope."

There is no recognition in the Novick review of the existence of energy-generating plasmids specifying degradative pathways. As reported on page 237 of the Novick review, of the known (non energy-generating) plasmids "Combinations of four or five different plasmids in a cell seem to be stable."

Plasmids may be compatible (i.e. they can reside stably in the same host cell) or incompatible (i.e. they are unable to reside stably in a single cell). Among the known plasmids, for example, are sex factor plasmids and drug-resistance plasmids.

Also, as stated on page 240 of the Novick review, "Cells provide specific maintenance systems or sites for plasmids. It is thought that attachment of such sites is required for replication and for segregation of replicas. Each plasmid is matched to a particular maintenance site . . . ". Once a plasimd enters a given cell, if there is no maintenance site available, because of prior occupancy by another plasmid, these plasmids will be incompatible.

The biodegradation of aromatic hydrocarbons such as phenol, cresols and salicylate has been studied rather extensively with emphasis on the biochemistry of these processes, notably enzyme characterization, nature of intermediates involved and the regulatory aspects of the enzymic actions. The genetic basis of such biodegradation, on the other hand, has not been as thoroughly studied because of the lack of suitable transducing phages and other genetic tools.

The work of Chakrabarty and Gunsalus (Genetics, *68*, No. 1, page S10 [1971]) has showed that the genes governing the synthesis of the enzymes responsible for the degradation of camphor constitute a plasmid. Similarily, this work has shown the plasmid nature of the octane-degradative pathway. However, attempts by the authors to provide a microorganism with both CAM and OCT plasmids were unsuccessful, these plasmids being incompatible.

Escherichia coli artificial, transmissible plasmids (one per cell) have been made, each containing a degradative pathway. These plasimds, not naturally occurring, are F'*lac* and F'*gal*, wherein the lactose- and galactose-

would be given wide scope. Federal courts should not read into patent laws limitations and conditions which the legislature has not expressed.

- The legislative history of the patent statute also supports a broad construction. Congress originally adopted Jefferson's view that "ingenuity should receive a liberal encouragement." Jefferson's original subject matter statutory language remained virtually intact through five rewrites of the patent statute spanning 187 years. Indeed, committee reports accompanying the most recent patent act revision "inform us that Congress intended statutory subject matter to include anything under the sun made by man."
- **Laws of nature, physical phenomena, and abstract ideas are not patentable. New** minerals discovered in the earth, a new plant found in the wild, Einstein's celebrated law of $E=mc^2$, and Newton's law of gravity were all cited by the Court as "manifestations of. . . nature, free to all men and reserved exclusively to none." Unlike such manifestations, Chakrabarty's micro-organism was a product of human ingenuity having a distinct name, character, and use.
- **The passage of the 1930 Plant Patent Act** (PPA) (affording patent protection for certain asexually reproduced plants) **and the 1970 Plant Variety Protection Act** (PVPA) (providing protection for certain sexually reproduced plants) does not evidence congressional understanding that the terms "manufacture" or "composition of matter" do not include living things.
- **The fact that genetic technology was unforeseen** when Congress enacted Section 101 does not require the conclusion that micro-organisms cannot qualify as patentable subject matter until Congress expressly authorizes such protection.
- **Arguments against patentability based on potential hazards** that may be generated by genetic research should be addressed to the Congress and the Executive for regulation or control, not to the Judiciary.

The dissenting opinion opposed the patentability of living things and concluded that PPA and PVPA evidenced Congress* understanding, at least from 1930, that living things were not patentable subject matter. The dissenters reasoned that if living things were patentable, then "the plants included in the scope of the 1930 [PPA] and 1970 [PVPA] Acts could have been patented without new legislation." Because Congress thought it had to legislate in order to make agricultural "human-made inventions patentable" in 1930, and because bacteria were specifically excluded from coverage in the PVPA, the dissenters reasoned that "Congress plainly legislated in the belief that Section 101 does not encompass living organisms."

Although *Chakrabarty* held that a live, human-made micro-organism was patentable, the specific issue of whether plants and animals are patentable was not addressed. The *Chakrabarty* decision did, however, provide the judicial framework for PTO to later determine that plants and animals were patentable subject matter under the U.S. Code (see chs. 5 and 6). Many observers agree that the *Chakrabarty* decision provided great economic stimulus to patenting of micro-organisms and cells, which in turn provided stimulus to the growth of the biotechnology industry in the 1980s. One patent examiner notes, however, that even without *Chakrabarty,* some aspects of patenting of recombinant DNA technology probably would not have been adversely affected since plasmids, phage, and viruses are not living and thus would have been ultimately embraced as patentable subject matter (18).

POST-CHAKRABARTY EVENTS AND TRENDS

Federal Patent Policy

In addition to the *Chakrabarty* decision, revisions in Federal patent policy encouraged increased patenting of living organisms and related processes. Prior to 1980, no single patent policy existed for government-supported research, despite the Federal Government's preeminence in biotechnology-related research funding. Instead, each Federal agency developed its own rules, resulting in 26 different patent policies. Under this system, only about 4 percent of some 30,000 government-owned patents were licensed. Furthermore, the government policy of granting nonexclusive licenses discouraged private investment, since a company lacking an exclusive license was unlikely to pay the cost of

developing, producing, and marketing a product. Thus, potentially valuable research remained unexploited.

To resolve this problem, Congress passed the Patent and Trademark Amendments of 1980 (Public Law 96-517) as amended in 1984 (Public Law 98-260) to promote efforts to develop a uniform patent policy that would encourage cooperative relationships and to commercialize government-funded inventions. From 1980 through 1984 patent applications by universities and hospitals for inventions containing human biological increased more than 300 percent as compared to the previous 5-year period (20).

The policies adopted by Congress in 1980 and 1984, which gave statutory preference to small businesses and nonprofit organizations, were extended to larger businesses (with some exceptions) in 1983 (12). The Technology Transfer Act of 1986 (Public Law 99-502) granted Federal authority to form consortia with private concerns. Executive Order 12591, issued in 1987, further encouraged technology transfer programs, including the transfer of patent rights to government grantees. In combination with the *Chakrabarty* decision, these actions helped spur patent activity.

Patents and the Commercialization of Biotechnology

The *Chakrabarty* decision helped to precipitate increased research and development, assuring the commercialization of biotechnology in the United States. The commercialization of biotechnology was the focus of an earlier report in OTA's New *Development in Biotechnology* assessment series (21). In that report, OTA noted that **patent protection of biotechnology products is a major unresolved issue that presents a potential barrier to commercialization.**

Patents are very important to commercial entities. For an emerging biotechnology company, patents can help attract venture capital, collaborative arrangements, and new research and development leads. Investors watch biotechnology patent developments and sometimes react quickly to news. The initial public offering of stock by Genentech in 1980 set a Wall Street record for the fastest price per share increase ($35 to $89 in 20 minutes); the initial public offering by Cetus in 1980 set a record for the largest amount of money raised in an initial public offering ($1 15 million) (19). In September 1986, Genentech's stock dropped 10.5 points following the news that Hoffmann-La Roche had sued it for infringing a patent for human growth hormone. Genentech's stock rose the previous year when it sued Burroughs-Wellcome (PLC) in Great Britain for allegedly infringing a British patent on tissue plasminogen activator (21).

By 1987, 403 American companies dedicated to biotechnology and 70 established corporations with significant investments in biotechnology yielded an estimated 35,900 jobs, including 18,600 scientists and engineers. Combined, U.S. industry is spending $1.5 billion to ***$2.0*** billion annually in biotechnology research and development. On average, dedicated biotechnology companies—those entrepreneurial ventures started specifically to commercialize innovations in biotechnology—have filed fewer biotechnology patent applications than larger, diversified firms that use biotechniques—1.5 v. 10 applications, respectively, in 1986. This is likely due to a greater institutional capacity to file multiple patents in the larger, more diversified companies (21).

Patent Activity Following Chakrabarty

Although *Chakrabarty* addressed the subject matter patentability of a human-made micro-organism, i.e., a patent on the end **product,** many patent law developments involve the use of such micro-organisms and cells in **processes** that could be patented. Data compiled by PTO within the first 3 years of the *Chakrabarty* decision focused on six areas of U.S. patent activity relating to micro-organisms and cells (22). The six areas present a cross-section of the types of patents issued in this field.

Mutation/Genetic Engineering

Patents in this emerging area within biotechnology refer to laboratory processes for producing a stable, inheritable change in the genotype of an animal, a plant, or a micro-organism. This can be accomplished by artificially inducing a structural change in a gene or through the incorporation of genetic material from an outside source (e.g., a

chemically synthesized or modified gene). Patents in this area include methods of modifying plasmids by chemical or biochemical processes.

Probably the best known patent in this area is Patent 4,237,224, covering the process for producing biologically functioning molecular chimeras. Soon after the *Chakrabarty* decision, Stanley N. Cohen and Herbert Boyer (at Stanford University and the University of California at San Francisco, respectively) patented a process for inserting foreign genetic material into a bacterial plasmid, a technique widely used in recombinant DNA research. The Cohen-Boyer patent was assigned to their universities, who split royalty payment income received from those wishing to use the patented process. By 1987, the Cohen-Boyer patent was Stanford's top earning patent ($1.7 million annually), surpassing the former leader, a 1971 patent on the FM synthesizer chip used in music synthesizers (2),

Enzymes Per Se

An enzyme is a protein that acts as a catalyst, speeding the rate at which a biochemical reaction proceeds, but not altering its direction or nature. An important tool in biotechnology, patents in this area have included products (enzymes per se and enzyme compositions) and processes for preparing, separating, purifying, and treating enzymes.

Immobilized Enzymes

Immobilization of an enzyme occurs when the enzyme or microbe is bonded to a carrier or entrapped within a carrier. The carrier material physically confines the enzyme or microbe, making them more stable when exposed to changes in reaction conditions. Binding often makes the enzyme insoluble, offering additional economic advantages. Examples of such bonded or entrapped enzymes include enzymes chemically or physically bonded to a water-soluble matrix, enzymes contained within a polymer or gel, and enzymes absorbed in resin.

Tissue and Cell Culture

Tissue and cell culture refers to the propagation of cells removed from organisms in a laboratory environment that has strict sterility, temperature, and nutrient requirements. Techniques in this area are of extreme importance to the medical sciences for the production of vaccines, pharmaceuticals, and antibodies. Patents in the area include those covering processes, apparatus and nutrient media that permit the growth and maintenance of cell lines, as well as cell lines per se.

Starch Hydrolysates

Hydrolysis is a chemical process of decomposition involving splitting of a chemical bond and addition of the elements of water. Patents in this area include those covering processes for synthesizing monosaccharides by the action of an enzyme or micro-organism. An example of such a process is the hydrolysis of starch to sugar.

Amino Acids

Amino acids are the building blocks of proteins. Each different protein is made up of a specific sequence of amino acids—which number some 20 molecules—with the unique sequence coded for by DNA. Patents in the area include processes for preparing alpha or beta amino acids and salts by a biological transformation of matter.

Emerging Patent Litigation

Early patents in the biotechnology field have resulted in the emergence of patent litigation. Factors leading to litigation include the presence of pioneer inventions, high value-added products, major investments, and personality factors. Where litigation is avoided, mitigating factors can include economic considerations and the ability of parties to enter into licensing or cross-licensing arrangements (1 1). Courts are being asked to determine whether patent holders have met the requisite requirements of novelty, usefulness, and nonobviousness. In addition, issues relating to the scope of claims, infringement, and enforcement of patents have occurred.

Uncertainty over patent protection is likely to be costly and will undoubtedly influence the research and development strategy of many companies. Eighty-five percent of large companies responding to an OTA survey indicated that they expect to pursue trade secret protection for biotechnology

The **patent awarded to Stanley Cohen and Herbert Boyer in 1980. This patent has since become Stanford University's top earning patent ($1.7 million annually).**

United States Patent [19]

Cohen et al.

[11] ***4,237,224***

[45] **Dec. 2, 1980**

[54] PROCESS FOR PRODUCING BIOLOGICALLY FUNCTIONAL MOLECULAR CHIMERAS

[75] Inventors: **Stanley N. Cohen, Portola Valley;** Herbert W. Boyer, Mill Valley, both of Calif.

[73] **Assignee:** Board of Trustees of the Leland **Stanford Jr. University, Stanford, Calif.**

[21] Appl. No.: 1,021

[22] Filed: Jan. 4, 1979

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 959,288, Nov. 9, 1978, which is a continuation-in-part of Ser. No. 687,430, May 17, 1976, abandoned, which is a continuation-in part of Ser. No. 520,691, Nov. 4, 1974.

[51] **Int. Cl.**³ **C12P 21/W**

[52] U.S. Cl. ..... **435/68; 435/172; 435/231; 435/183; 435/317; 435/849; 435/820; 435/91; 435/207; 260/1 12.5 S; 260/27R; 435/212**

[58] Field of Search ..... **195/1, 28 N, 28 R, 112, 195/78, 79; 435/68, 172, 231, 183**

[56] References Cited

U.S. PATENT DOCUMENTS

3,813,316 5/1974 **Chakrabarty** ..... 195/28 R

OTHER PUBLICATIONS

Morrow et al., Proc. Nat. Acad. Sci. USA, vol. 69, pp. *3365-3369, Nov. 1972.*

Morrow et al., Proc. Nat. Acad. Sci. USA, vol. 71, pp. 1743-1747, May 1974.

Hershfield et al., Proc. Nat. Acad. Sci. USA, vol. 71, pp. 3455 et seq. (1974).

Jackson et al., Proc. Nat. Acad. Sci. USA, vol. 69, pp. *2904-2909,* Oct. 1972.

Mertz et al., Proc. Nat. Acad. Sci. USA, vol. 69, pp. ***3370-3374, Nov. 1972.***

Cohen, et al., Proc. Nat. Acad. Sci. USA, vol. 70, pp. 1293-1297, May 1973.

Cohen et al., Proc. Nat. Acad. Sci. USA, vol. 70, pp. 3240-3244, Nov. 1973.

Chang et al., Proc. Nat. Acad. Sci, USA, vol. 71, pp. 1030-1034, **Apr. 1974.**

Ullrich et al., Science vol. 196, pp. 1313-1319, Jun. 1977.

Singer et al., Science vol. 181, p. 1114 (1973).

Itakura et al., Science vol. 198, pp. 1056-1063 Dec. 1977.

Komaroff et al., Proc. Nat. Acad. Sci. USA, vol. 75, pp. 3727-3731, Aug. 1978.

Chemical and Engineering News, p. 4, May 30, 1977.

Chemical and Engineering News, p. 6, Sep. 11, 1978.

Primary Examiner—**Alvin E. Tanenholtz**

Attorney, Agent, or **Firm--Bertram 1. Rowland**

[57] ABSTRACT

Method and compositions are provided for replication and expression of exogenous genes in microorganisms. Plasmids or virus DNA are cleaved to provide linear DNA having ligatable termini to which is inserted a **gene having complementary termini, to provide a biologically functional replicon with a desired phenotypical property. The replicon is inserted into a microorganism cell by transformation. Isolation of the transformants provides cells for replication and expression of the DNA molecules present in the modified plasmid. The method provides a convenient and efficient way** to **introduce genetic capability into microorganisms for the production of nucleic acids and proteins, such as medically or commercially useful enzymes, which may have direct usefulness, or may find expression in the production of drugs, such as hormones, antibiotics, or the like, fixation of nitrogen, fermentation, utilization of specific feedstocks, or the like.**

14 Claims, No Drawings

SOURCE: Office of Technology Licensing, Stanford University

Photo credit: *Robyn Nishimi*

Human cells in culture

lines in addition to patent protection, although patent protection is more desirable for many companies (21). When intellectual property rights are unclear, valuable resources are invested in expensive and time-consuming litigation.

Infringement

The patenting of micro-organisms and cells and related processes have, in several early cases, involved issues resulting from questions of patent infringement. Patent infringement issues arise mainly in three contexts: literal infringement, infringement through the doctrine of equivalents, or noninfringement through exceptions from infringement.

Literal infringement occurs whenever a person without authority makes, uses, or sells any product or process that is covered by the patent claims within the United States during the term of the patent (35 U.S.C. 271(a)). This is the most common form of infringement litigation. In addition to literal, or statutory infringement, the Supreme Court has established the rule that in order to prevent an infringer from stealing the benefit of a patented invention, a patentee may proceed against the producer of a product or process if it performs substantially the same function in substantially the same way to obtain the same result (7). This is the rule of the **doctrine of equivalents.** The rule applies in instances where the accused product or process in question does not constitute literal infringement, yet remains an "equivalent" of the patented invention.

In one case, a court found that certain "antibody fragments" do the same thing in essentially the same way as previously patented whole antibodies and, therefore, infringe the patent *'either literally or by the doctrine of equivalents" (8). From this case it seems possible that the doctrine of equivalents is applicable to other areas of biotechnology as well.

In biotechnology, the most relevant exemption from patent infringement is the **experimental use exception,** a court-created doctrine which holds that an experiment with a patented invention for the sole purpose of gratifying true scientific inquiry or philosophical curiosity does not attack the right of a patentee, and thus does not constitute infringement.

In 1984, the Court of Appeals for the Federal Circuit decided that "the limited use of a patented drug for testing and investigation strictly related to Food and Drug Administration (FDA) drug approval requirements during the . . . term of the patent" did not fall within the experimental use exemption, and thus constituted infringement (15). Roche, the plaintiff, held a patent on the brand name prescription sleeping drug "Dalmane." Bolar, a generic drug manufacturer, began taking steps near the end of the term of Roche's patent. to gain FDA approval of a generic drug equivalent. Bolar's actions (bioequivalency tests) were conducted pursuant to the requirements of the Federal Food, Drug, and Cosmetic Act (FDCA) (21 U.S.C. 301-392), which govern actions required for FDA drug approval.

Roche argued that Bolar's *use* of the patented drug constituted infringement. Bolar argued that FDCA requirements created a conflict with the patent infringement statute; because FDCA increased the time necessary for FDA drug approval, and because the patent code did not allow for FDA-mandated testing until the end of the patent term, the patentees "gain for themselves . . . a de *facto* monopoly of upwards of two years" by preventing the testing of a generic drug until the patent expires. Although admitting that it used Dalmane, Bolar claimed that the use was "experimental." The court found that Bolar's use did not fall within the narrow confines of the experimental use doctrine, and thus infringed Roche's patent.

In the wake of *Roche,* Congress amended the patent code (Public Law 98-417) to allow a statutory exemption with respect to human drug products which in part overruled the court decision. Thus, it is "not. . . an act of infringement to make, use, or sell a patented invention . . . solely for uses reasonably related to the development and submission of information under a Federal law which regulated the manufacture, use, or sale of drugs" (35 U.S.C. 271 (e)(l)).

Where the testing of a patented drug is found to be not solely **for purposes of meeting FDA approval requirements, however, the testing will still be found to constitute infringement.** A **1987 case** tested the limits of this provision. In *Scripps Clinic and Research Foundation v. Genentech (16), the* plaintiff, owner of a patent for blood-clotting factor VIII:C, brought an infringement suit against Genentech, which defended by arguing that all its uses of the factor VIII:C, though not solely for purposes related to FDA testing, bore some reasonable relationship to such purposes and hence fit the new 271 (e) exception. The court disagreed with Genentech, finding that actions taken by the company (e.g., preparation of a European patent and the development of an agreement to commercially market Factor VIII:C) constituted more than was permitted by statute, which creates an exception **solely** for the development and submission of information required by a Federal law.

It remains unclear how other courts will interpret exemption from infringement issues raised by the application of various fact patterns to Section 271 (c)(l). A strict interpretation of the statute could result in slower development of generic copies of previously patented organisms. A looser interpretation could result in infringers taking advantage, early in a patent's term, of the amendment in circumstances where it was not intended to operate (10).

Scope of Protection

A significant issue presented by several cases involves the scope of protection for naturally occurring proteins as opposed to those that have been genetically engineered. Although a protein found in nature is not patentable, purified compositions of the protein may be patented.

An example of this involves current development of tissue plasminogen activator (tPA), a genetically engineered protein drug that helps to dissolve blood clots in patients who have suffered heart attacks. Genentech, Inc. received FDA approval in 1987 to market its form of tPA. During the first 5 months following government approval, sales totaled $100 million (1). Subsequently, Genentech received exclusive license to a patent claiming broad protection for the way tPA acts on blood clots (U.S. 4,752,603) (6). Nonetheless, other companies also filed patent applications for their forms of tPA, based on small changes in the molecular structure of the drug.

The scope of protection (i.e., whether patent protection will be on the fundamental characteristics and uses of an organism or product, or on the slight

Pho credi ona Hea

as ed w th m be at a be ge e ca
g p oduce

modifications of the organism or product) is an issue that will determine the degree of exclusivity that patent holders will enjoy. The Patent Office and the courts have a long history of experience in dealing with questions of claim scope (17), but comparatively little experience in applying this law to biotechnology inventions. Until court decisions resolve emerging issues, neither a patentee nor the patentee's competitors can be entirely clear on the limits of patent claim enforcement (5).

Already, patent battles are being fought over Interleukin-2, tissue plasminogen activator, human growth hormone, hybridoma technology, alpha interferon, factor VIII, and use of dual monoclinal antibody sandwich immunoassay in diagnostic test kits. Companies receiving basic product patents are in court enforcing their rights against infringement or defending the patent grant in opposition or revocation proceedings. It is likely that patent litigation in biotechnology will increase given the complex web of partially overlapping patent claims, the high-value products, the problem of prior publication, and the fact that many companies are chasing the same products (21).

U.S. Patent and Trademark Office Activity

The majority of biotechnology patent applications involving micro-organisms and cells fit into one of two classes established by PTO for examination purposes. **Class 935 is a comprehensive cross--reference collection of patents and other technical documents relating to genetic engineering technology. Within the Class 935 are various subclasses (see table 4-l). Micro-organisms per se that are not provided for in other classes are listed in **Class** 435, Chemistry: Molecular Biology and Microbiology (see table 4-2).

Patent activity in both areas has increased dramatically during the past 10 years, both as a function of application filing date (that date when the application is filed) and patent grant date (the date of those patents which issued) (see figures 4-3 and 4-4). In both classes, the majority of patentees are Americans, and the vast majority of patents are owned by U.S. corporations (see table 4-3).

A recent survey of genetic engineering patents confirms the dominance of U.S. inventors in the area of biotechnology patents as related to pharmaceuticals and health care (14). The Pharmaceutical Manufacturers Association found that of 1,476 biotechnology patents issued by PTO in 1987, some 206 used techniques of the "new biotechnology." Fully 80 percent of these patents (159) were of U.S. origin, as opposed to only 20 percent (40) from foreign sources. Within the United States, corporations accounted for 45 percent of the patents (89), nearly *21/2* times as many patents as U.S. universities.

One negative trend from the increase of patent applications is the inability of PTO to process biotechnology applications in a timely manner. The number of biotechnology patent applications has severely challenged the process and examination capabilities of PTO. In March 1988, PTO reorganized its biotechnology effort into a separate patent examining group. As of July 1988, 5,850 biotechnology applications had not yet been acted on. **Currently, it is approximately 15.5 months, on average, before examination of a biotechnology application is initiated, and an average of 27 months before the examination process is completed by grant of the patentor abandonment of the patent application (24).** Turnover among patent examiners, lured to the private sector by higher pay, is cited as a significant reason for the delay in reviewing patents (21).

SUMMARY

Prior to 1980, the U.S. Patent and Trademark Office did not grant patents on living organisms per se, deeming such organisms to be outside the scope of statutory subject matter. This policy was reversed by the Supreme Court's landmark decision in *Diamond v. Chakrabarty* in 1980, a case involving a genetically engineered bacterium capable of breaking down multiple components of crude oil. The *Chakrabarty* decision, in concert with revisions to Federal patent policy, led to increased numbers of patent applications for living micro-organisms and cells, as well as related processes. The majority of such patents are filed by U.S. inventors and owned by U.S. corporations. Patent activity is one measure of the increased commercialization of biotechnology during the 1980s. One predictable and costly result has been the emergence of patent infringement litigation, as patent holders and alleged infringers attempt to define the scope of biotechnology patent

Table 4-l--Class 935, Genetic Engineering

1 Obtaining the desired gene; DNA, RNA per se and the modification thereof other than vector modification
2 DNA—RNA hybrid
3 RNA
4 mRNA
5 2-100 nucleotides in length, e.g., t-RNA, etc.
6 DNA, e.g., regulatory sequences, etc.
7 Homopolymeric, e.g., poly d(A) sequence, etc.
8 12-75 nucleotides in length, e.g., primers, etc.
9 Structural gene sequence
10 Modified structural gene, e.g., nonnaturally occurring sequence, etc.
11 Polypeptide
12 Antigenic material
13 Hormone, e.g., human growth factor, insulin, etc.
14 Enzyme
15 Antibody
16 Methods of producing DNA or RNA other than by expression vectors, e.g., culture of cells high in DNA, etc.
17 Cell free production
18 cDNA synthesis
19 Isolation or purification of DNA or RNA
20 RNA
21 mRNA
22 Vectors and methods of modifying vectors
23 Inserting a gene into a vector to form a recombinant vector, i.e., cleavage and ligation
24 Vector utilized, e.g., episomes, etc.
25 Plant virus
26 Cosmid
27 Plasmid
28 Yeast
29 Prokaryotic
30 Plant
31 Bacteriophage
32 Animal Virus, e.g., SV40, etc.
33 Methods of enhancing or diminishing expression
34 Eukaryotic cell
35 Plant cell
36 Transcription
37 Yeast Cell
38 Prokaryotic cell
39 Transcription
40 Operon selection
41 Promoter, e.g., portable promoters, etc.
42 Gene dosage modification, e.g., copy number amplification, etc.
43 Inducible, e.g., temperature inducible, etc.
44 Translation
45 Ribosome binding site
46 Initiation
47 Fused protein or peptide
48 Signal peptide, e.g., secretion, etc.
49 Post translational modification
50 Glycosylation
51 Peptide bond cleavage
52 Methods of introducing a gene into a host cell, e.g., transformation or transfection, etc.
53 Microinjection
54 Microencapsulation, e.g., liposome vesicle etc.
55 Using vector, e.g., plasmid, etc.
56 Plasmid
57 Virus
58 Phage, e.g., phage lambda, etc.
59 Method of use of genetically engineered cells, e.g., oil spill cleanup, etc.
60 To produce an identified chemical product e.g., amino acid, etc.
61 Yield optimization
62 Control of genetic diseases or defects by use of added gene
63 Use in animal husbandry
64 Use in agriculture
65 Vaccine production
66 Cells containing a vector and/or exogenous gene per se; propagation thereof; other membrane encapsulated DNA, e.g., protoplasts, etc.
67 Plant cells
68 Fungal cells
69 Yeast cells
70 Animal cell
71 Human cell
72 Bacteria
73 Escherichia
74 Bacillus
75 Streptomyces
76 Assay related to genetic engineering
77 Methods of analysis of nucleic acids
78 Including hybridization
79 Methods of selection of recombinant gene containing vector; materials therefore, e.g., replica plating, etc.
80 Gene library manipulations
81 Antigen-antibody
82 Enzyme activity
83 Host suicide
84 Selection medium
85 Genetic engineering apparatus
86 Analytical, e.g., for autoradiography, etc.
87 Automated
88 Synthesis, e.g., peptide or gene synthesizers, etc.
89 Hybrid or fused cell technology, e.g., hybridoma, etc.
90 Method of selection of the desired cell
91 Of plant cells, e.g., protoplasts, etc.
92 Using positive selection technique
93 Method of production of hybrid or fused cells, e.g., chromosome or genome transfer techniques, etc.
94 of plant cells
95 Fused or hybrid cell per se
96 Interspecies fusion
97 Fungi, e.g., yeasts, etc.
98 Plant cells
99 Human cells
100 B lymphocyte
101 T lymphocyte
102 Animal cell
103 Murine cell, e.g., mouse cell, etc.
104 B lymphocyte
105 T lymphocyte
106 Method of use of the fused or hybrid cell or the product thereof
107 In vivo use of product
108 In vitro, e.g., cell cultivation
109 Production of non-antibody product
110 For use as testing material
111 Miscellaneous

SOURCE: U.S. Patent and Trademark Office, 1988.

Table 4-2-Class 435, Chemistry: Molecular Biology and Microbiology

This class provides for the following subject matter when *not provided for elsewhere:*

A. A process of using a micro-organism or enzyme to synthesize a chemical product.
B. A process of treating a material with a micro-organism or enzyme to separate, liberate, or purify a preexisting substance.
C. An **in vitro process of measuring and testing** in which:
   (1) A micro-organism or enzyme is used to determine the presence or identity of a compound or composition in a sample.
   (2) A micro-organism is identified by propagation.
   (3) An enzyme is identified by its catalytic activity.
   (4) The presence of micro-organisms is detected.
   (5) A live micro-organism is used in an antigen antibody test as an antigen.
D. A process of propagating a micro-organism.
E. A process in which the genetic structure of a micro-organism or extrachromosomal genetic structure is altered.
F. A process of organ or tissue maintenance.
G. A process of mashing or malting.
H. Apparatus claimed or solely disclosed as for A-G.
I. Micro-organisms *per* se or the subcellular parts thereof.
J. Enzymes, immobilized enzymes or enzyme containing compositions not otherwise provided for and the processes for purifying enzymes or forming immobilized enzymes.
K. Compositions claimed or solely disclosed as for the propagation of micro-organisms or for measuring and testing processes in C above.

SOURCE: U.S. Patent and Trademark Office, 1988

Table 4-3-Patents: Applications and Ownership, by Class

	Class	
	935	435
Percent of applications, US inventor	77	59
Percent of applications, foreign inventor	23	41
Percent of patents, corporate owned	91	88
Percent of patents, government owned	4	4

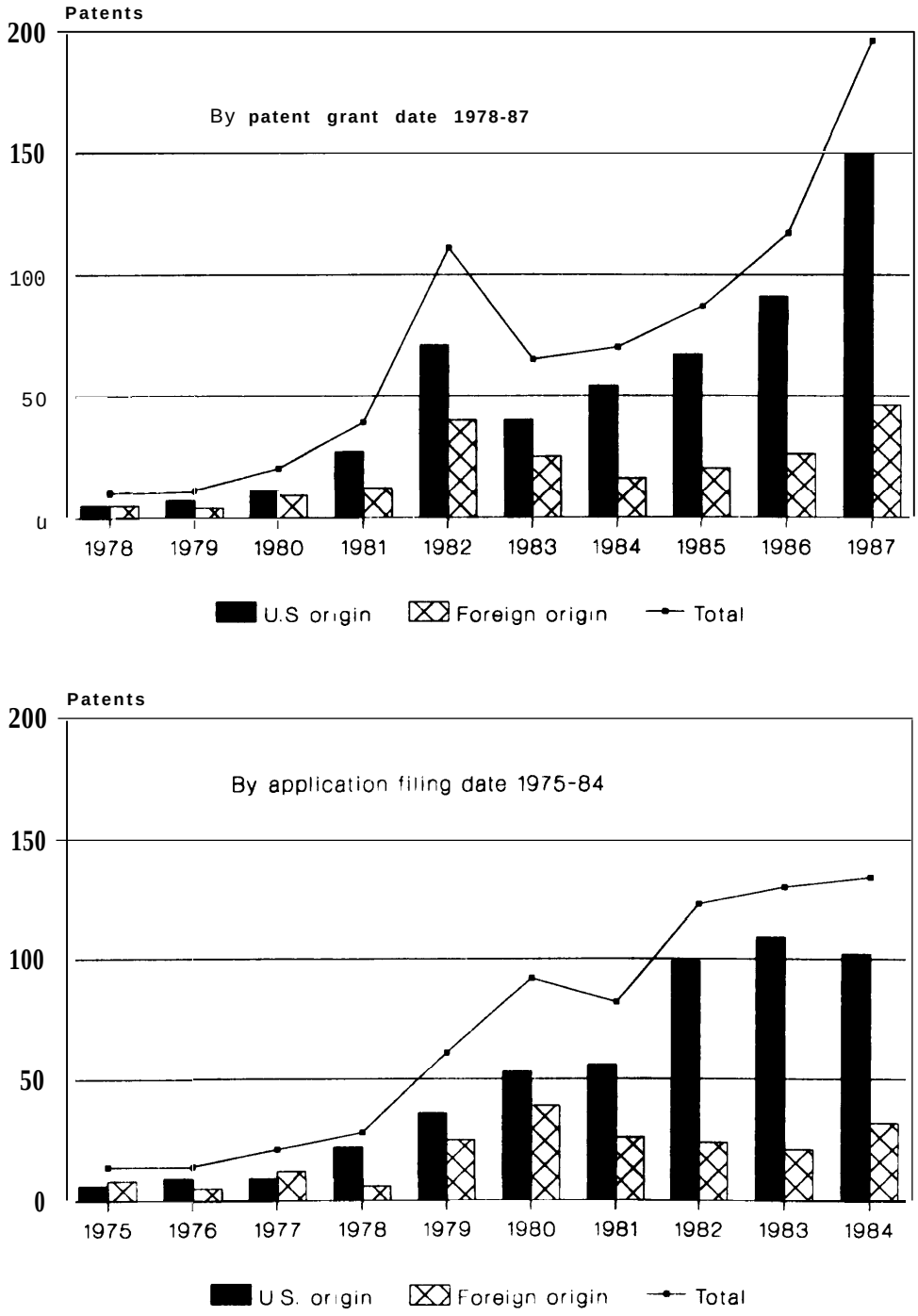
SOURCE: U.S. Patent and Trademark Office, 1988.

protection. It is unclear at this time what the result of such litigation will be. One negative result of increased numbers of biotechnology patent applications is PTO's inability to examine such applications in a timely manner.

CHAPTER 4 REFERENCES

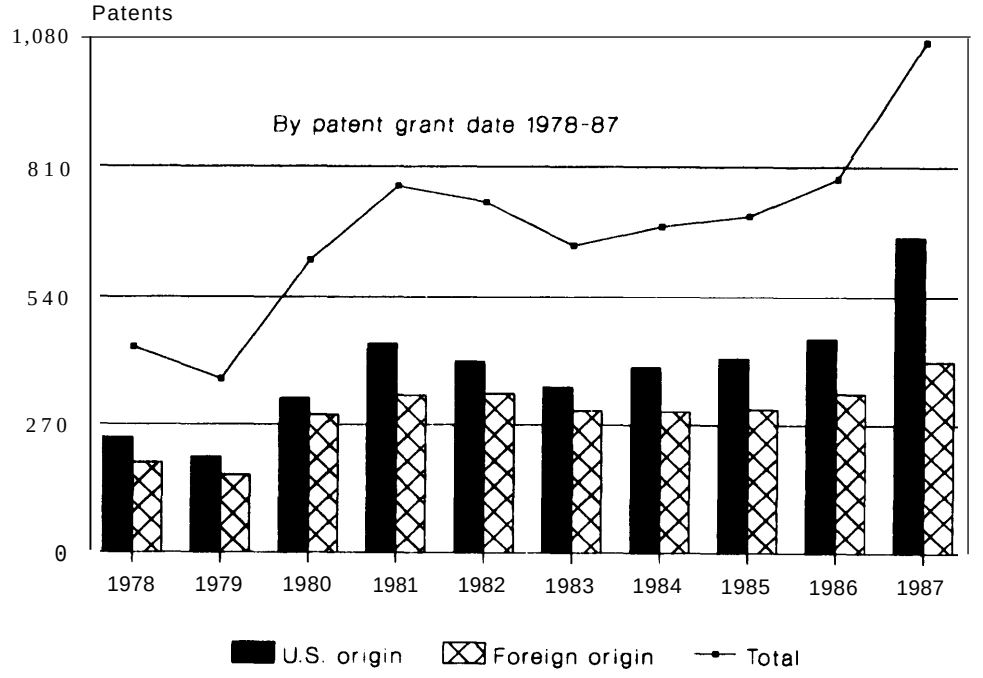
1. **Andrews,** E. L., "The Complex **Legal** Issues in TPA Fight," New *York Times,* July 2, 1988.
2. **Barinaga,** M, "Stanford Still on Top," *Nature 332* **(6161):192,** Mar. 17, 1988.
3. *Chakrabarty v. Diamond, 571* **F.2d** 40 (C. C.P.A. 1978).
4. *Diamond v. Chakrabarty, 477 U.S. 303 (1980).*
5. Duffey, **W.,** "The Marvelous Gifts of **Biotech**: Will They Be Nourished or Stifled by Our International Patent Laws?" Symposium on the Protection of **Biotechnological** Inventions, World Intellectual Property Organization and Cornell University, Ithaca, NY, June 1987.
6. **Ezzell,** C., "Genentech Gets Patent Protection for Tissue **Plasminogen** Activator,'' *Nature,* **333(6176):790,** 1988.
7. *Graver Tank & Manufacturing Co., Inc. v. Linde Air Products Co., 339* U.S. 605 (1949).
8. *Hybritech v. Monoclinal Antibodies, Inc., 802* **F.2d** 1367 (Fed. Cir. 1986).
9. *In re Bergy,* 563 **F.2d** 1031, 195 USPQ 344 (1977).
10. **Janicke,** P. M., attorney, Arnold, Durkee & White, Houston, TX, personal communication, Aug. 24, 1988.
11. Kamy, G., attorney, **Dickstein,** Shapiro & **Morin,** Vienna, VA, personal communication, Aug. 26, 1988.
12. Memorandum dated Feb. 18, 1983, from the President to the Heads of the Executive Departments and Agencies on Government Patent Policy, 19 Weekly **Comp. Pres.** Dec. 252.
13. **Nelkin,** D., *Science as Intellectual Property* **(New** York, NY; Macmillan, 1984).
14. Pharmaceutical Manufacturers Association, "update: Biotechnology Products in Development,*' Juiy 1988.
15. *Roche Products Inc. v. Bolar Pharmaceutical Co., 733* **F.2d** 858 (Fed. **Cir.** 1984).
16. *Scripps Clinic and Research Foundation v. Genentech, 666* **F.Supp** 1379 **(N.D. Cal.** 1987).
17. Sibley, K. D., "**Factual** Inquiries in Deciding the Question of Enablement," **70** J. Pat. Off. Soc. 115 (1988).
18. **Tanenholtz,** Alvin E., "Genetic **Engineering—** Technological Trends From the Perspective of a U.S. **PTO** Examiner," *Fourth Annual Biotechnology Law Institute* (Clifton, NJ: Prentice Hall Law & Business, 1988).
19. U.S. Congress, Office of Technology Assessment, *Commercial Biotechnology: An International Analysis, OTA-BA-218* **(Elmsford,** NY: Pergamon Press, January 1984).
20. U.S. Congress, Office of Technology Assessment, New *Developments in Biotechnology: Ownership of Human Tissue and Cells—Special Report,* OTA-BA-337 (Washington, DC: U.S. Government Printing Office, March 1987).

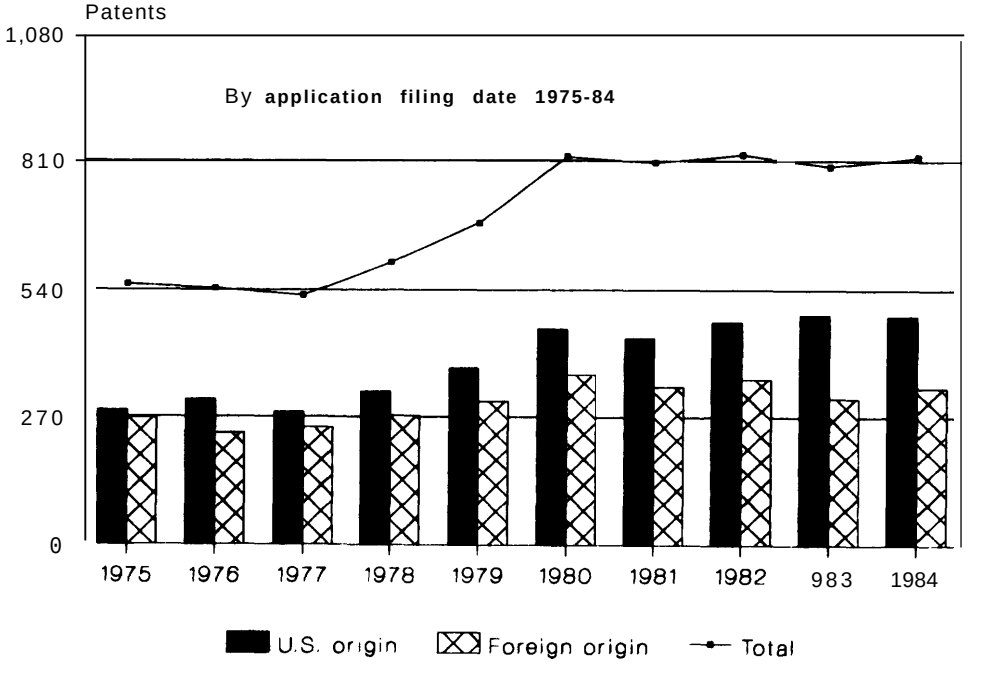
Figure 4-3-Patent Activity Class 935, Genetic Engineering



SOURCE. U S Patant and Trademark Office, 1989.

Figure 4-4-Patent Activity Class 435, Chemistry: Molecular Biology and Microbiology





SOURCE: U.S Patent and Trademark (Mice. 1989.

21. U.S. Congress, Office of Technology Assessment, *New Developments in Biotechnology: U.S. lnvestment--Special Report, OTA-BA-360* (Springfield, VA: National Technical Information Service, July 1988).
22. U.S. Department of Commerce, Patent and Trademark Office, Office of Technology Assessment and Forecast, *Patent Prqfiles-biotechnology: 1982 Update* (Washington, DC, September 1983).
23. *United States v. Dublilier Condenser Corp., 289* U.S. *178 (1933).*
24. Van Horn, C., Deputy Solicitor, U.S. Patent and Trademark Office, Washington, DC, personal communication, September 1988.