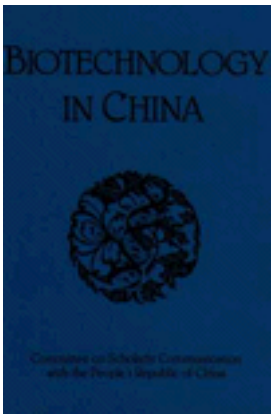

Biotechnology in China

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Committee on Scholarly Communication with the People's Republic of China, National Academy of Sciences

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NOTICE: The biotechnology program was established in 1986 as a collaborative project between the National Academy of Sciences and the Chinese Academy of Sciences to promote the exchange of information and expertise related to biotechnology research and techniques. The program was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. It was supported under Master Agreement number 86-18643 between the National Science Foundation and the National Academy of Sciences and Contract Number INT 85-06451 between the National Science Foundation and the Committee on Scholarly Communication with the People's Republic of China (CSCPRC). Program activities in China were supported by the Chinese Academy of Sciences.

Founded in 1966, CSCPRC represents American scholars in the natural and engineering sciences as well as scholars in the social sciences and humanities. In addition to administering exchange programs, it advises individuals and institutions on means of communication with their Chinese colleagues on China's international activities, and on the state of China's scientific and scholarly pursuits. CSCPRC members are scholars from a broad range of fields, including China studies. Administrative offices of the CSCPRC are located in the National Academy of Sciences, Washington, D.C.

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Preface

During a regular bi-academy meeting in 1986 in Beijing, Lu Jiaxi, then president of the Chinese Academy of Sciences (CAS), and Frank Press, president of the National Academy of Sciences (NAS), agreed upon the need and importance of a collaborative program on biotechnology. Program activities for this joint initiative were developed and finalized by members of the Committee on Scholarly Communication with the People's Republic of China (CSCPRC) Subcommittee on Biotechnology in a meeting with representatives of CAS. They agreed to a 3-year program that would include biotechnology minicourses to be held in China under the instruction of teams of American scientists, a major joint symposium in Shanghai on gene expression and gene regulation, and an assessment of Chinese biotechnology.

Four minicourses were designed to cover important current research areas: nematode molecular genetics, gene expression and amplification in yeast, immunotoxins and tumor markers, and plant molecular biology. Alexander Rich, chairman of the CSCPRC subcommittee, proposed a format which combined laboratory training with complementary, broader based lectures. Chinese students came from all parts of the country to learn the latest research methodologies and techniques, and the results were overwhelmingly successful.

The symposium was scheduled to take place at the end of May 1989. It was postponed because of the growing instability resulting from prodemocracy demonstrations taking place in China at that time.

Attendant with the substantial increase in Sino-American collaboration in the past 10 years is a need to extend American understanding of Chinese science goals, funding policies, and research infrastructure. For this reason, it was

decided the biotechnology program should include an assessment of Chinese biotechnology. CSCPRC subcommittee member Dean H. Hamer, an innovative researcher and leader of one of the minicourse delegations, and Shain-dow Kung, a professor of botany and biotechnology researcher who is known and valued for his inside track among the Chinese hierarchies, took up this challenge. They reviewed post-minicourse reports made by each American minicourse instructor, conducted a survey of published biotechnology research, both in Chinese and English, and drew on their own personal communications and previous experiences in China. Furthermore, in the fall of 1988, they traveled to China in order to make a firsthand evaluation of current biotechnology research activities and funding. They met with high-level officials in charge of science and technology policies, and they visited research institutes and biotechnology bases. *Biotechnology in China* is the result of all of these efforts: a comprehensive, evaluative, and at times, provocative documentary of Chinese biotechnology research.

It should be noted that Hamer and Kung completed their report prior to the prodemocracy demonstrations in the spring of 1989. The massacre in Tiananmen Square on June 3-4 and the government's subsequent repressive actions have inevitably altered the environment for scholarly communication with China. Regretfully, the pace of development and change in biotechnology in China and, at least in the short term, the extent and type of American participation have been significantly affected. Nonetheless, I feel strongly that the authors' findings and recommendations remain timely and noteworthy.

The CSCPRC would like to express its appreciation to the authors for making this assessment available to the American scientific community. Also, CSCPRC is especially appreciative of Alexander Rich's leadership of the subcommittee and Eric Davidson's perceptive accounts of Chinese biotechnology research. In this vein, CSCPRC would like to acknowledge all members of the CSCPRC Subcommittee on Biotechnology:

Alexander Rich (*Chairman*), Massachusetts Institute of Technology
Michael Bjorn, NeoRx Corporation
Eric Davidson, California Institute of Technology
Dean Hamer, National Institutes of Health
Robert Horvitz, Massachusetts Institute of Technology
Ernest Jaworski, Monsanto Company
Sidney Pestka, University of Medicine and Dentistry of New Jersey
Paul Williams, University of Wisconsin

CSCPRC would also like to acknowledge the capable leaders of the minicourse delegations: Michael Bjorn, Dean Hamer, Robert Horvitz, and Thomas Osborn, University of Wisconsin, for their valuable contributions to this collaborative

program. Finally, I personally would like to thank Terry Price for his indispensable assistance in managing these activities and Beryl Leach for her excellent editorial work on this report.

JANE LIU JERNOW
DIRECTOR, SCIENCE AND TECHNOLOGY PROGRAMS
CSCPRC

Acknowledgments

We gratefully acknowledge the support of the Committee on Scholarly Communication with the People's Republic of China and the National Science Foundation. We especially thank Jane Jernow and Terry Price, whose encouragement and efforts helped make our evaluation trip and report possible, and Beryl Leach, who was responsible for the editing and production of this publication. We also thank the many scientists who donated their time and effort to participate in the Chinese biotechnology literature survey: Alex Rich, James Leung, Jim Shi, Esther Cheng, Cao Xu, Lin Seyu, Bruce Paterson, and Eric Davidson (who also kindly provided Appendix D). Many of the articles reviewed in the survey were given to us by Chinese colleagues during our trip. We thank them for making these reprints available. Our evaluation of Chinese biotechnology funding would not have been possible without the valuable information provided by Cai Dalie (State Planning Commission), Xu Chengman (China National Center for Biotechnology Development), Hu Zhaosen (National Natural Science Foundation of China), and Li Zhensheng (Chinese Academy of Sciences). We thank the Chinese Academy of Sciences and the Beijing Office of CSCPRC for making arrangements in China and the many Chinese scientists who took the time to meet with us. For these many valuable contributions, we are appreciative, but we retain full responsibility for the contents of and views expressed in the following pages.

Note on Spelling of Chinese Names

At various times and places, several different systems have been used to romanize Chinese names. In the text, we use China's official *Pinyin* system, which includes retaining the Chinese order of last name first. For example, Mao Zedong's last name is Mao. We have made exceptions in the few cases where scientists are well known in the United States by the initials of their first names followed by their last names. In the references, names are rendered as they appeared in the research publications.

Contents

1

Introduction

Biotechnology refers to the manipulation of living organisms and their constituents to benefit mankind. Traditional forms of biotechnology, such as alcohol fermentation and selective livestock breeding, have existed since prehistoric times. In the 1970s, scientists developed new techniques to isolate and characterize deoxyribonucleic acid (DNA), the molecule that acts as a blueprint for the development of all living creatures. This new technology, known as recombinant DNA, or gene cloning, has allowed scientists to achieve hitherto unprecedented control over living systems. The transfer of new genetic information into living organisms provides the means to create improved crop species and livestock breeds, to produce valuable pharmaceuticals and natural products, and perhaps even to cure human genetic diseases.

China* was still in the throes of the Cultural Revolution in the 1970s, and thus, Chinese scientists had little chance to participate in the development of modern biotechnology. But in the past decade, China has chosen economic reform and development over political ideology by emphasizing the Four Modernizations of agriculture, industry, national defense, and science and technology. In just the past 5 years, Chinese leaders have made biotechnology the top priority in the high technology field. Funding for biological research has been increased more than 25-fold during this period, and new mechanisms have been introduced to allocate these monies by competitive, peer-reviewed grants. At the present time, China's investment in biotechnology, as a percentage of its gross national product, is comparable to that in many Western countries.

* Throughout this report, China refers to the People's Republic of China.

China's attitude toward the United States and other developed countries has also undergone a major shift from strict isolationism to ever increasing contact and cooperation. Since 1978, in the biological sciences alone, China has sent more than 2,000 students and researchers to the United States for advanced training. In addition, many joint research and training programs in China are currently being supported by American and other foreign academic institutions, private foundations, commercial enterprises, and government agencies. Such cooperative ventures have the potential to provide a rapid and efficient mechanism for Chinese scientists to obtain the training and technology needed to perform advanced biotechnology research.

The Committee on Scholarly Communication with the People's Republic of China (CSCPRC) has been one important conduit for exchange between American and Chinese scientists. Founded in 1966, CSCPRC is sponsored by the National Academy of Sciences (NAS), the American Council of Learned Societies, and the Social Science Research Council. In January 1987, CSCPRC's Subcommittee on Biotechnology signed a 3-year agreement with the Chinese Academy of Sciences (CAS; also known as Academia Sinica) to promote Sino-American cooperation in biotechnology. To date, CSCPRC and CAS have cosponsored three combined laboratory and lecture minicourses at the Shanghai Institute of Biochemistry and one minicourse at the Beijing Institute of Microbiology. A symposium on gene regulation and gene expression is planned to cap off this 3-year program.

The purpose of this report is to help CSCPRC, together with its sponsoring and funding organizations, to formulate strategies to continue and expand cooperation with China in biotechnology. Toward this end, the report focuses on three areas:

1. The mechanisms by which China sets priorities and funds biotechnology research.
2. The current status of China's biotechnology research. Particular emphasis is placed on areas of potential interest to American scientists.
3. The roles of various types of international cooperation programs in the development of biotechnology in China.

After a brief historical introduction in Chapter 2, Chapters 3 through 5 of the report deal with China's biotechnology policy, administration, and infrastructure. They include a summary of current research expenditures; many of these figures were made available to foreigners for the first time in 1988. The literature survey results presented in Chapter 6 and the research highlights in Chapter 7 offer the basis for a systematic analysis of the topics and quality of China's biotechnology research. Chapter 8 gives an anecdotal accounting of research at 19 institutions visited by the authors during a 1-month evaluation trip. The final two chapters discuss the role of international cooperation and areas of special interest to CSCPRC and its sponsoring organizations. The appendixes include lists of contacts for readers interested in China's biotechnology administration and research.

2

China's Long History of Biotechnology

Traditional forms of biotechnology have existed in China since its earliest history. According to legend, Shen Nong, a mythical king, introduced China to grain cultivation and crop rotation, and invented a transparent stomach covering in order to observe the effects of herbal medicines on the digestive tract. During the late Neolithic period, the Chinese were already adept at alcohol fermentation, as evidenced by the discovery of wine cups and containers from the Longshan culture and of winery ruins in Henan Province. Records from the eleventh century B.C. show that the importance of temperature and water quality to grain fermentation was understood. By the end of the Zhou Dynasty in 221 B.C., the Chinese were producing bean curd, soy sauce, and vinegar by methods still used today. The process of flax maceration by anaerobic bacteria is alluded to in a verse from the *Book of Songs,* China's earliest collection of poetry (200 B.C.), while the rotation of bean crops is described in writings from A.D. 500. As early as the sixth century, the Chinese understood that rabies could be spread by mad dogs. During the Sui Dynasty (581-618), a vaccine against smallpox was developed, and by the Ming Dynasty (1368-1644), it was widely available to the masses.

Despite this early inventiveness, China's science and technology, including biotechnology and medicine, failed to go through the explosive changes that altered Western science in the seventeenth to nineteenth centuries. As noted by Joseph Needham in his epic *Science and Civilization in China* (Cambridge: Cambridge University Press, 1961), China never underwent a scientific revolution; there are no Chinese equivalents of Locke, Newton, or Darwin. Consequently, the fundamental concept of testing hypotheses by experimentation was still

unknown in China when the door to the West was reluctantly opened to traders and missionaries during the sixteenth and seventeenth centuries.

China's defeat by the gunboats of European imperialism in the Opium War (1840-1842) ushered in the "half feudal, half colonial" period of the late nineteenth and early twentieth centuries, when China flirted with Western technology. One legacy of the period was the establishment of European-style alcohol- and yeast-based industries, including the famous German brewery at Qingdao. Many students, including Sun Yatsen, went to Japan, Europe, and the United States for training. However, except for the brief Hundred Days Reform of 1898, efforts to modernize China's science and education systems were suppressed by the government.

In 1911, the fall of the last emperor and establishment of the Republic marked a turning point in China's science policy. Under the influence of Sun Yatsen, a physician and firm believer in science, learned societies were formed, scientific journals began publication, science departments were established at several universities, and students were once again sent abroad. An important development was the founding of the Central Academy of Sciences and the Beijing Academy of Sciences, which were later combined to form CAS. China's efforts to build a scientific establishment were stymied, however, by political unrest, and were completely halted by the war with Japan (1937-1945) and by the subsequent civil war between the Nationalists and Communists.

After the Communist Party's victory in 1949, China began restructuring its scientific research and educational institutions. Following the example of the Soviet Union, basic research was assigned to CAS, applied research to various state ministries such as agriculture and public health, and education to the universities. An unfortunate consequence of this dependence on the Soviet model was the abandonment of classical genetics in favor of the more socialist, but scientifically incorrect, theories of Lysenkoism.

The government also made a major effort to attract scientists who had left the country during the war, particularly nuclear physicists and doctors, by promising them the opportunity to help build a new Chinese society. These promises were soon broken as China embarked on a series of vicious antirightist campaigns in which scientists, as members of the intellectual class, were castigated as "evil cow snakes" and "foreign devil lovers." The situation was exacerbated by the split with the Soviet Union, China's main provider of technological and scientific training in the 1950s.

This anti-intellectualism culminated in the Cultural Revolution (1966-1976), during which most scientists were sent to the countryside or factories for reeducation, and most research institutes were either closed or converted to production facilities. A rare exception was the Shanghai Institute of Biochemistry, which carried out work on the synthesis of insulin and transfer ribonucleic acid (tRNA) during this period. A direct result of the complete collapse of the

education system during the Cultural Revolution is a major generational gap in trained scientists.

Following Mao Zedong's death in 1976, the Chinese leadership was embroiled in internal power struggles and debate about the country's direction, which culminated in 1978 in the ascendancy of Deng Xiaoping and the declaration of the Four Modernizations as China's state policy. In that year, biotechnology was first mentioned as a focal point of the country's science and technology development program. During the Sixth 5-Year Plan (1981-1985), funds were allocated to support biotechnology research in the fields of agriculture, food processing, and pharmaceutical production; and in 1983, the China National Center for Biotechnology Development (CNCBD) was established to coordinate these activities. During the Seventh 5-Year Plan (1986-1990), the level and scope of biotechnology funding have been greatly increased. In March 1986, the State Council Leading Group on Science and Technology published a pivotal document, often referred to as the "8 6 3 Plan," describing China's high technology development program and making biotechnology its top priority. That same year, the National Natural Science Foundation of China (NSFC) was founded to support basic research. In 1988, the State Science and Technology Commission (SSTC) published its second white paper on science and technology, which reinforced biotechnology as China's number one priority for high technology development. These events set the stage for the current mechanisms for determining biotechnology research priorities, administration, and funding.

3

Research Priorities and Funding Mechanisms

Within China's overall goal to quadruple total industrial and agricultural output by the end of the century, the main role of biotechnology is to improve human health through advances in agriculture and medicine. Chinese leaders have frequently emphasized that they want to pursue "those scientific and technological results that can yield the best and fastest results." They have also promoted the concept that "economic construction must rely upon science and technology and the latter must cater to the needs of the former." In 1988, an informal 6-month survey was conducted of the *People's Daily*, a Chinese newspaper acknowledged as a reliable indicator of government thinking and policy priorities, in which every article and editorial concerning science and technology also gave prominent attention to China's economy. Given this ideological framework, it is not surprising that current research priorities are strongly biased toward applied rather than basic research.

At present, research priorities are reflected in two major grant programs, the High Technology Program and the Seventh 5-Year Plan, and in the four grant programs administered by NSFC. The resources and allocations of these programs are summarized in Table 1A, and the distribution of funds between various types of research institutions is described in Table 2. It should be noted that these grants cover only research expenses and minimal (typically 10 percent) overhead. Salaries are paid by the government administrative agencies (known as work organizations or *danwei*) responsible for the institutions where the research is carried out, for example, the State Education Commission (SEDC) for major universities, CAS for its research institutes, the Ministry of Agriculture, and the Ministry of Public Health. In comparison, note that salaries and overhead typically consume nearly

TABLE 1 China's Biotechnology Funding Programs

A. Research Grants

Program	Emphasis	Funds[a] (1987)	Number of Grants (1987)	Median Grant[a]
High Technology Program	Applied projects in agriculture, medicine, and protein engineering	30	100	1.0 (3-5)[b]
Seventh 5-Year Plan	Applied projects	20	108	0.2 (4)
NSFC[c]	Basic science	10	326	0.03 (3)

B. Key Laboratories

Laboratory	Emphasis	Total Funding[a] (1984-1988)
Jiangmen Single Cell Protein Biotechnology Base	Food processing	60
Shanghai Center of Biotechnology	Downstream processing (no designated product)	57
Key laboratories (11)	Research and training	55

[a]In millions of *yuan* (3.71 *yuan* = US$1).
[b]Values in parentheses are grant durations (in years).
[c]Includes only expenditures for biotechnology and closely related fields. For total biology expenditures, see Table 3.

TABLE 2 Distribution of Research Funds by Type of Research Institution (in percent)

Program	Agricultural and Universities	Medical Colleges	Academies	Other
High Technology Program	22	20	38	20
Seventh 5-Year Plan	60[a]		40	
NSFC	63[a]		37	

[a] Values are for universities and agricultural institutes and medical colleges combined.

80 percent of allocated grant funds in the United States. In short, a much higher percentage of Chinese grant funds are spent on research. The amount of funds that can be converted to hard currency is variable from grant to grant, and it is dependent on complex arrangements with the granting agency and institute administrators.

HIGH TECHNOLOGY PROGRAM AND THE CHINA NATIONAL CENTER FOR BIOTECHNOLOGY DEVELOPMENT

Funds for the High Technology Program are allocated directly by SSTC. They are administered through CNCBD, a purely administrative body that was founded in 1983 at the suggestion of a group of eight visiting Chinese-American scientists headed by Ray Wu and Shain-dow Kung. In 1986, the "8 6 3 Plan" mandated CNCBD to spend approximately 30 million *yuan*[*] per year on highly applied projects in the areas of agriculture, medicine, and protein engineering. Grant proposals are peer reviewed by a separate subcommittee in each area and approved by an 11-member panel whose members are selected directly by SSTC. Grants are awarded for 3 to 5 years and are reviewed annually. Because of the highly focused nature of this program, the individual grants are by far the largest in China, typically in the range of 500,000 to 2 million *yuan* for 4 years. In 1987, 100 grants were awarded out of a pool of 500 applications. The distribution of grants according to subject area was 40 percent for agriculture, 40 percent for medicine, and 20 percent for protein engineering. These grants were approximately equally distributed between CAS, universities, and medical and agricultural institutes.

The center is headed by Chief Engineer Liu Yonghui, who is assisted by Deputy Chief Engineer Xu Chengman. Scientific leadership is provided by Hou Yunde, chief scientist of the China National Expert Committee for Biotechnology Development and director of the Beijing Institute of Virology.

The original concept was for CNCBD to coordinate all of China's biotechnology activities and to establish research centers in Beijing, Shanghai, and Jiangmen (Guangdong Province). As it now stands, however, CNCBD is primarily responsible only for the High Technology Program. The idea of a research center in Beijing has been abandoned, the Shanghai center is still under construction, and the Jiangmen base has been left to its own devices (see Chapter 4).

Additional responsibilities include consultation and promotion activities, management of experimental animals and equipment, and supply procurement. The CNCBD is staffed by 45 people and has an annual operating budget of 300,000 *yuan*. Part of these funds are supplied by import-export companies that

[*] The official rate of exchange is 3.71 *yuan* to US$1.

CNCBD operates in Beijing, Hong Kong, and New York. Their primary export is experimental monkeys.

Although the High Technology Program grants are peer reviewed, they are, in fact, more like contracts than Western-style investigator-initiated grants. This difference stems from the fact that essentially all of the grants support projects that are preselected through a complex negotiation process involving the SSTC, CNCBD administrators, and members of the China National Expert Committee for Biotechnology Development. While most Chinese scientists feel that the High Technology Program grants are fairly reviewed, there are those who signal their reservations by quoting a Chinese proverb, "Pavilions near the water receive the most moonlight."

SEVENTH 5-YEAR PLAN (1986-1990)

In the Seventh 5-Year Plan, total investment in biotechnology and closely related fields is approximately 100 million *yuan*, or 20 million *yuan* per year. These monies are provided through CAS, SEDC, and the Ministries of Agriculture, Public Health, Medicine, and Light Industry. The Seventh 5-Year Plan solicits and funds research in the areas of basic genetic engineering, plant genetic engineering, chromosome engineering, cell engineering, enzyme engineering, downstream processing, and bioengineering products. In 1987, 108 projects were approved and supported from a pool of 150 applications. The average grant was 200,000 *yuan*, although certain key projects were funded up to 2 million *yuan*. Thus, the Seventh 5-Year Plan grants generally have been smaller than High Technology Program grants but substantially greater than NSFC grants (see below).

The Seventh 5-Year Plan grants are administered by CAS. Scientific direction is provided by Mang Keqiang, director of the CAS Expert Committee for Biotechnology and a professor at the Beijing Institute of Microbiology. Grants are reviewed by a single committee of 24 scientists and administrators. The high percentage of accepted applications would indicate a review process that is less rigorous than the one applied at CNCBD or NSFC, to the extent that it reflects primary concern about an application's conformity to goals set by the expert committee.

NATIONAL NATURAL SCIENCE FOUNDATION OF CHINA

The NSFC was founded in 1986 expressly to support basic research in China. It is somewhat similar to the U.S. National Science Foundation (NSF) in that it funds, in addition to biology, research in a wide variety of disciplines such as math, physics, and geology. In 1987, the Department of Biological Sciences received 3,507 applications, of which 979 were funded following the peer review

process described below. The grants were distributed among four types of awards, as shown in Table 3, and are summarized here.

General Awards

In 1987, the standard general award was 30,000 *yuan* for 3 years, with little variation between individual grants. Universities and colleges received 63 percent of these grants. Notably, 67 percent of the awards were to investigators between 36 and 55 years of age.

Key Projects

This program, initiated in 1987, supports the ongoing projects of reputable investigators that are judged to have a high probability of success and benefit. In the first year, average funding was 40,000 *yuan* annually, or four times higher than that for the general awards, and 61 percent of the investigators were from academic institutions.

Frontiers of High Technology

This program, also initiated in 1987, is designed to support high technology fields such as biotechnology, information sciences, and aerospace sciences. Although these areas represent more applied science, this program constitutes only 8 percent of the NSFC grant budget. The average grant is only slightly higher than that for general awards.

Young Scientist Awards

This program is designed to support young scientists (under 35 years of age) who are starting their first independent projects. It is hoped that the program will entice scientists who have received their Ph.D.'s abroad to return to China. To this end, an applicant is allowed to apply for this award while he or she is living in another country. In 1987, 29 percent of the recipients had received training outside of China.

The concept for NSFC grew out of the CAS Science Foundation, which was created in 1982 in order to award CAS research grants selectively. The NSFC adopted a peer review system directly modeled on that of NSF in the United States. As China's first peer review granting system, it represented an important step in modernizing and improving funding mechanisms that has come to be widely regarded by Chinese scientists as a fair and unbiased method for allocating the scarce funds available for basic research.

TABLE 3 Biology Grants of the National Natural Science Foundation of China

			Percentage of Total	
Type of Award	Funds (1987)[a]	Median Grant[a]	Biology Funding	NSFC Awards[b]
General awards	24.5	0.03 (3)[c]	83	30.6
Key projects	2.0	0.04 (1)	7	3.4
Frontiers of high technology	2.14	0.045 (3)	7	21.9
Young scientists	0.75	0.035 (3)	3	21.9

[a] In millions of *yuan*.
[b] The remaining percentage of awards were given for non-biology-related research.
[c] Values in parentheses are grant durations (in years).

After 4 years of provisional operation, NSFC was institutionalized as a national organization administered directly by the State Council. The governing body of NSFC is an executive council consisting of 25 members appointed by the State Council. Administrative leadership is provided by Chairman Tang Aoqing, Executive Vice Chairman Hu Zhaosen, and four vice chairmen. The total 1988 budget for NSFC was 120 million *yuan*, representing a 20 percent increase from 1987 and a 300 percent incremental increase compared with average funding levels from 1982 to 1985.

The NSFC publishes an annual list of research goals that are prioritized on a sliding scale. However, most of the goals, especially in biology, are broadly stated, providing ample room for initiative by individual investigators. Grant applications are evaluated using a year-long, five-step process:

1. Approval by the sponsoring institution.
2. Preliminary evaluation by the appropriate department of NSFC. Reasons for immediate rejection include concurrent funding by another agency, lack of appropriate laboratory facilities, unqualified principal investigator, lack of progress from the previous grant period, or inappropriate subject matter.
3. Solicited peer reviews by three to seven experts in the specialty area of the application. The reviews may include recommendations for modification of the proposal. (More than 10,000 scientists helped to review grants last year.)
4. Review by a panel of approximately 10 members for each program area. (In 1987, there were 41 panels comprising 488 scientists selected by NSFC from universities [51 percent], CAS [22 percent], and other research institutions [27 percent]). The panel members evaluate the peer reviews and suggest a funding priority.
5. Approval by the executive council.

The NSFC also administers the National Science Awards, which were established in 1956 to recognize outstanding achievements by Chinese scientists. Although they were given only twice between 1965 and 1987, the awards are now being presented biannually. In 1988, there were 777 nominees, of whom 180 were selected for awards: 11 first prizes of 20,000 *yuan*, 39 second prizes of 10,000 *yuan*, 89 third prizes of 5,000 *yuan*, and 41 fourth prizes of 2,000 *yuan*, for a total expenditure of 1.13 million *yuan*.

EVOLUTION AND CONSEQUENCES OF THE NEW FUNDING MECHANISMS

The three programs just summarized represent a radical alteration in both the levels and methods of funding biological research in China. Just 5 years ago, total expenditures on biotechnology and related fields were less than 5 million *yuan* per year and were spent almost exclusively by CAS through a noncompetitive allocation system. Now expenditures are greater than 100 million *yuan* per year and are more evenly distributed among various types of research institutions through competitive grants. In the evolution of the new funding policies, three trends have become clear: the declining role of CAS, decentralization of the granting process, and strongly increased emphasis on applied research.

The declining role of CAS, the first major trend, is signalled by its decreasing direct funding. In the past, CAS was primarily responsible for all of China's basic biological research and much of the applied research as well. However, concomitant with the greatly increased funding for the three new grant programs, the State Council and State Planning Commission decided to decrease direct funding for CAS according to the following formula: for basic research, a 6 percent decrease per year over 5 years to a final level of 70 percent; and for applied research, a 20 percent decrease per year over 5 years to a final level of zero. The only area that will be increased is research on China's natural resources. At present, CAS's total expenditures on biological research are 7 million *yuan* per year, of which 1 million *yuan* is earmarked for biotechnology. When seen in comparison with the figures presented in Table 1A, this represents only 1.7 percent of total biotechnology research expenditures. After the planned 5-year cuts and inflation have taken their toll, CAS direct expenditures will account for an even smaller proportion of total biology and biotechnology funding.

In addition to these decreases in research funds, CAS is also under strong pressure to reduce the size of its administrative staff and to freeze the number of research workers. In order to achieve the latter aim, students and staff members studying abroad are counted as still occupying a CAS position, even in cases where researchers have already long overstayed their originally planned visits.

Not surprisingly, CAS administrators have bitterly opposed the new funding policies. In some cases, such as the idea of establishing a biotechnology center

under CNCBD, their arguments have been successful. In other cases, such as the formation of key laboratories at universities, their protests have been ignored. While many of the CAS arguments can simply be attributed to fear of competition, others are well founded. In particular, basic research is being short-changed both in this reorganization and by underfunding NSFC's mandate to support this type of research. As pointed out by Wang Yinglai, director emeritus of the Shanghai Institute of Biochemistry, it would have been difficult or impossible under current policies to have carried out one of China's greatest scientific achievements: the total synthesis of insulin.

A second major trend has been the effect of the new granting process on the decentralization of research units. Previously, each research organization was funded exclusively by allocations from its *danwei* which, in turn, were set by the State Planning Commission. In the past, academic institutions received virtually no funds from their *danwei* (SEDC) for research. Now each organization, be they research institutes or university-based science departments, is expected to compete openly for funds from the three major granting organizations and other sources such as provincial governments and companies. As indicated in Table 2, the big winners in this game have been the universities and colleges, which now receive roughly half of all funding. The new policies have resulted in a hitherto unprecedented degree of autonomy both for research institute administrators and for individual researchers. The one constant is that the amount of funds allocated to each major program is still determined by the State Planning Commission through its Department of Science and Technology Planning, which is headed by Cai Dalie.

The third major trend concerns the effects of the stated aim of both the High Technology Program and the Seventh 5-Year Plan to promote highly applied research that will become self-supporting within a short period of time. As elaborated in Chapters 6 and 8, this, for the most part, has involved the direct copying of Western research results with little attention to innovation, long-term development, or technology that is suited to China's unique circumstances. The only formal support mechanisms for basic research are grants from CAS, which as noted above are rapidly diminishing, and from NSFC. While NSFC controls a substantial amount of money, it is spread out among a large number of grants so that the usual grant of 30,000 *yuan* for 3 years is insufficient to equip or run a laboratory. The practical result is that only those investigators who already hold a High Technology Program or Seventh 5-Year Plan grant can effectively use their NSFC money to carry out basic research. In fact, many of China's top researchers do just that by diverting funds from goal-oriented grants to more basic research.

In many other instances, however, poor applied research by unqualified investigators is being supported at the expense of good basic research by more qualified investigators simply because it is considered to be practical in terms of addressing the pressure to generate revenue-producing results. Clearly, this trend

toward applied research is motivated by political policy considerations at higher levels. In contrast, the scientists interviewed during the evaluation trip were almost universally in favor of increased funding for basic research. Given the previous paucity of any research support, it is not surprising that many scientists have set aside this disagreement and have acquiesced to the new policies because they increase total funding.

4

New Research Centers

More than 200 research facilities and 50 different colleges and universities perform biotechnology and related research in China. During the past 5 years, the Chinese government has inaugurated two new types of research centers to promote the development and commercialization of this field. The first are the biotechnology bases at Jiangmen and Shanghai, which are meant to bring research results to the production stage. The second are the key laboratories, which provide research training for scientists from throughout China.

BIOTECHNOLOGY BASES

The Jiangmen Single Cell Protein Biotechnology Base is located about 100 kilometers from Guangzhou in Guangdong Province. At present, the base is located on 200,000 square meters of land containing—all at various stages of completion—an office building, worker housing, a 5,000-squaremeter laboratory building, a pilot plant, the single-cell protein plant, a tool factory, and a power plant. Plans are to have 400 employees when construction is completed in 1989 or 1990. The initial investment by SSTC was 60 million *yuan*, but the whole idea has fallen out of favor with Chinese administrators in the past 2 years, and the base itself will have to raise the additional 20 million *yuan* needed for completion.

The original concept for the base was to ferment molasses (a by-product of the large sugar industry in Guangdong Province) into single-cell feed protein. Because of economic reform policies, however, nearby counties are no longer required to sell their molasses to the base, resulting in a shortage of raw supplies. Instead, the

base is surviving on sales of low technology products (e.g., shrimp feed and meat tenderizer) and electricity from its power plant.

The Shanghai Center of Biotechnology is located in an industrial sector of southwest Shanghai and will eventually contain 30,000 square meters of buildings, including a guest house, laboratory building, pilot plant, library, and offices. The Shanghai center is a national project administered by CAS and supported by 57 million *yuan* from the State Planning Commission. It is hoped that the center will represent a new type of research institute in China, one which runs the gamut from laboratory research to pilot plant production. Project areas include genetic engineering and cell, enzyme, and fermentation technologies. At present, the center employs 200 people located at a biochemical factory and various CAS research institutes. They are attempting to develop processing techniques for products such as human insulin, human growth factor, and epidermal growth factor, but a definite product has not been identified as of this writing.

As shown in Table 1B, construction of the Jiangmen and Shanghai bases has consumed a sizable fraction of China's biotechnology funds. Both bases are large—probably larger than any comparable facility in the United States, even those built by major pharmaceutical and chemical companies. However, the construction quality at both sites appears to be low. Moreover, both have already or are in the process of purchasing large quantities of expensive fermentation equipment without knowing exactly what will be produced. It is unlikely that either base will produce a major product within the next 5 years, by which time much of this equipment will be outdated and will have suffered from the neglect that accompanies idleness. Over time, questionable construction standards and the lack of clear production planning will frame judgment of the utility of this investment strategy.

KEY LABORATORIES

The key laboratories represent the second new type of biotechnology center. To date, the State Planning Commission and SSTC have funded 11 such laboratories, each at an average cost of 5 million *yuan*. An additional one-half million *yuan* per year is provided by CAS to each of its laboratories for maintenance, whereas laboratories at universities and other institutes are expected to raise their own operating expenses. The laboratories are open to investigators from outside institutions and are intended to serve as national training centers. In general, these key laboratories have been established at the most advanced biotechnology research centers in China: Peking University, Fudan University, Beijing Institute of Virology, Beijing Institute of Biophysics, Shanghai Institute of Biochemistry, Shanghai Institute of Plant Physiology, and Shanghai Institute of Cell Biology.

The value of the key laboratories as training centers is dubious since, in general, visiting scientists from distant provinces are unable to apply their new knowledge after returning to their home institutions that lack adequate facilities.

On the other hand, the program has allowed some of China's best biology research centers to make great improvements in their facilities and equipment. An example is the Laboratory of Genetic Engineering at Fudan University. Rather than building a new facility, this key laboratory was integrated with existing laboratories of the university's Institute of Genetics, and the money was spent on new instrumentation. This allows visiting investigators maximum contact with well-trained university scientists and, at the same time, permits access by scientists to highly sophisticated laboratory instruments.

5

Infrastructure

INSTRUMENTATION

The most obvious sign of the improvement in biotechnology funding in China is the tremendous increase in the number of modern, imported instruments. Of the 19 research institutes visited during the evaluation trip, virtually every one had at least some of the following items: DNA synthesizers, amino acid analyzers, protein sequencers, high-performance liquid chromatographs, liquid scintillation counters, ultracentrifuges, computerized fermentors, ultraviolet spectrophotometers, electron microscopes, tissue culture incubators, and laser densitometers. At certain centers, particularly those financed by World Bank education and equipment loans, there were more instruments than available space.

In general, these instruments are well maintained. Most Chinese scientists are more familiar with how an instrument actually works than are their American counterparts, and are able to strip parts from an abandoned or outmoded instrument to repair a new one. Furthermore, the Chinese are paying close attention to the ability of foreign suppliers to provide prompt, reliable maintenance service. The clear impression was that American suppliers are quickly being replaced by Japanese and West German competitors.

Most laboratories are also well equipped with small instruments such as water baths, gel electrophoresis devices, shakers, vortex mixers, and bacterial incubators.

Many of these items are produced in China by using Western designs. These locally manufactured instruments are inexpensive and easily repaired.

PROCUREMENT SYSTEM AND SUPPLIES

In contrast to the system for instrument purchases, the system for procuring chemicals, disposable laboratory supplies, and other supplies is poor. Many of the items essential for biotechnology research still must be purchased from foreign sources; this includes most restriction and DNA modification enzymes, radioisotopes, specialty chemicals, and plastic ware. These items, which must be purchased with hard currency, can only be ordered once a year. This makes it extremely difficult to do experiments efficiently because researchers are often unable to follow up on interesting leads because of a lack of reagents.

The stated rationale for this system is that all orders requiring foreign exchange should be consolidated in order to increase efficiency. (Many of the scientists interviewed during the evaluation trip complained that the real reason was middle-level bureaucrats who see the control of hard currency as a way to gain power.) Recognizing the negative effect of this rationale on the advancement of research, a certain number of institutes have recently introduced more efficient systems that allow perishable supplies, such as radioisotopes, to be ordered on a more regular basis. In contrast to the bureaucratic efficiencies being pursued, these changes will demonstrate the real value of making the procurement system responsive to the research community's needs and goals.

China is gradually trying to improve its own production of biotechnology supplies. Most simple chemicals (e.g., sodium chloride and sodium phosphate) are produced in China and appear to be of reasonably high quality. A fairly wide spectrum of more specialized biochemicals (e.g., adenosine triphosphate and deoxyribonucleotides) are available from biochemical factories such as the one associated with the Shanghai Institute of Biochemistry; the quality of these reagents is more variable, and several scientists complained of poor quality control. A notable recent development is the formation of the Sino-American Biotechnology Company, a joint venture between Promega Corporation, SSTC, and the Henan provincial government. Under the supervision of an American director and with the assistance of several scientists who have trained in the United States, this new company's production facility produces several of the most widely used restriction enzymes. In addition, they import, store, and offer for sale a number of enzymes and other reagents from the Promega catalog. Although these items are priced at American levels, an important advantage is that they can be purchased with *yuan* rather than hard currency. The headquarters

of the Sino-American Biotechnology Company is inconveniently located in Luoyang in Henan Province, but more convenient branch offices have recently opened in Beijing and Shanghai.

INTELLECTUAL PROPERTY RIGHTS

China's newly instituted patent system provides for the granting of patents on inventions, utility models, and designs. Patents are granted for 15 years and may be licensed to third parties for a negotiated fee. Infringers, in principle, are subject to compensation for damages and criminal liability; such fines can be levied either by the Patent Office or the People's Court. Similar to the European system, patents must be filed prior to publication of research results. The Chinese patent system strongly emphasizes the application and spreading of patents as well as the protection of the patent right. Accordingly, if a patentee has not worked on an invention within 3 years without any justified reason, the Patent Office has the right to issue a compulsory license to a third party. Such compulsory licensing provisions are found in the patent systems of many developing countries.

China has acceded to the Paris Convention for the Protection of Industrial Property, thereby allowing nationals from countries that are party to the convention (including the United States) to obtain patent rights in China. Foreigners are required to use a patent agency designated by the State Council; these include agents in Beijing, Shanghai, and Hong Kong. A foreign party may use an invention through a subsidiary in China, a Chinese-foreign joint venture, or a licensee. The Chinese patent law includes specific restrictions against the government's expropriation of foreign-owned patents. However, if progress toward production is not demonstrated within 3 years, the right to issue a compulsory license to a Chinese entity is in effect.

The actual effectiveness of the new Chinese patent system is weakened by two difficulties. First, the law specifically excludes inventions in the following areas: food, beverages, and flavorings; pharmaceutical products and substances obtained by means of a chemical process; and animal and plant varieties. In principle, these exceptions cover virtually every possible product of biotechnology. The situation is somewhat alleviated by the fact that it is possible to patent the processes, including microbiological processes, leading to such products. For example, a company could patent the process used to produce a new form of tissue plasminogen activator by a genetically engineered microbe but could not patent the drug or the microbe itself. In the United States, such process patents are generally considered inferior to product patents, especially in the pharmaceutical field. A report from the Chinese Patent Office justifies the exception of chemicals and pharmaceuticals by stating that they are "important raw materials and . . . necessities for safe-guarding the health of the people and the increase of livestock which relate to the national economy" and "should not be granted patent until we have had enough experience."

A second difficulty is enforcement. Given the short period that the new patent system has been in effect, together with the general disinclination of Chinese parties to litigate, it is not surprising that foreign investors are dubious about the real worth of a Chinese patent. As an anecdotal example, an American pharmaceutical firm established a joint venture factory in China and obtained a patent on their method for antibiotic production. Within a year, a virtually identical factory, producing the same product by the same method, was opened in an adjacent county. It is alleged to have taken at least half of the American company's market share. When it was explained that the people's need for antibiotics took precedence over profit making, the American company decided not to press the case.

China has no copyright law. Foreign journals are routinely copied at a central facility in Beijing and then distributed to libraries at research institutes and universities. Computer software is also unprotected by law.

In sum, China's new patent law provides an important first step in protecting the intellectual property rights of Chinese scientists. However, substantial improvements in the content and enforcement of the law are required to afford the level of protection needed to assure potential foreign collaborators and investors.

6

Biotechnology Literature Survey

The following section summarizes the analysis of 160 articles published in Chinese scientific journals. Conducting a literature survey to gain insights into the content and quality of China's biotechnology research was justified for several reasons. First, essentially all research performed in China is published in Chinese journals, in part because scientists receive a bonus for each article. Second, it was hoped that the survey would provide access to research performed at institutes in remote locations that were not visited during the evaluation trip. Third, it was possible to have outside experts review articles on scientific topics with which the authors were not familiar. All articles were read by at least one and, in many cases, by two or more expert reviewers. As all of the reviewers had some familiarity with science in China, the assessments of research originality and accuracy are reasonably uniform for different disciplines.

SOURCES

The journals and number of articles reviewed in the survey are listed in Table 4. The selection of articles was from three major sources.

Chinese Journal of Biotechnology. This is a specialty journal that publishes scientific articles and reviews on applied biotechnology and closely related basic research. Because of the relevance of this journal to this report, every research article published between Volume 1-1 (1985) and Volume 3-4 (1987) was surveyed.

Scientia Sinica. This journal, which is published by CAS in English, is generally considered to be China's most prestigious scientific journal. Similar to

the *Proceedings of the National Academy of Sciences* in the United States, it publishes articles considered to be of broad interest in several areas of the natural sciences, physical sciences, and mathematics. The survey included every article published between January 1987 and February 1988 relevant to either basic or applied biotechnology.

TABLE 4 Sources for Literature Survey

Journal	Language	Number of Papers Reviewed
Chinese Journal of Biotechnology	Chinese	71
Scientia Sinica	English	41
Acta Genetica Sinica	Chinese	10
Acta Biochimica et Biophysica	Chinese	10
Kexue Tongbao (Science Reports)	English	5
Journal of Xiamen University	Chinese	5
Acta Microbiologica Sinica	Chinese	4
Acta Biologicae Experimentalis Sinica	Chinese	4
Acta Physiologica Sinica	Chinese	3
Acta Zoologica Sinica	Chinese	2
Journal of Fudan University	Chinese	2
Acta Anthropological Sinica	Chinese	1
Journal of China University of Science and Technology	Chinese	1
Acta Scientiarium Naturalium Universitatis Pekinensis	Chinese	1
Total		160

Chinese Science Abstracts (Life Sciences). This publication contains English translations of abstracts of all articles published in 61 Chinese journals on the life sciences. All abstracts relating to biotechnology were surveyed for the period 1986 to 1987, and relevant articles were selected and obtained from the National Library of Medicine or the Library of Congress.

Journals in China, as elsewhere, vary in prestige, quality, and the rigor of the peer review process. Except for the arbitrary inclusion of all articles from the *Chinese Journal of Biotechnology*, the survey was strongly biased toward the most interesting articles in what are considered to be the best journals in China. In addition, a serious endeavor was made to include articles on basic research, in particular in biochemistry (e.g., protein structure) and genetics (e.g., gene regulation). Besides the final 160 articles that were thoroughly reviewed and included in the survey, about 200 additional manuscripts were examined briefly. While including these articles in the survey might have better met standards for

statistical analysis, it seems unlikely that it would have affected the qualitative conclusions.

TABLE 5 Geographical Distribution of Articles Cited

City or Province	Number of Articles
Shanghai	68
Beijing	59
Xiamen	5
Wuhan	4
Hangzhou	3
Tianjin	3
Hefei	3
Guangzhou	2
Chengdu	1
Jiangsu Province	1
Jilin	1
Quanzhou	1
Shijiazhuang	1
Wuxi	1
Dalian	1
Fuzhou	1
Harbin	1
Hunan Province	1
Nanjing	1
Suzhou	1
Xianyang	1
Total	160

GEOGRAPHICAL DISTRIBUTION

Despite a desire to have the survey compensate for the necessity of confining the evaluation trip to major cities in eastern China, it did not, in the end, reveal much information about the research being conducted in remote areas. As shown in Table 5, the majority (80 percent) of the articles were from institutes in Shanghai and Beijing, reflecting the concentration of research centers in these two cities. The relative paucity of articles from other geographical areas suggests that the increased funding of CAS institutes and universities in provincial areas has not yet had a substantial effect on their scientific productivity.

TECHNIQUES

The use of various techniques reported in the articles surveyed is summarized in Table 6. Several interesting points emerge. First, techniques for cloning and analyzing DNA molecules were noted in more than half of the articles, which

TABLE 6 Techniques Used in the Articles Surveyed

Technique[a]	Number of Citations	Percentage of Citations[b]
DNA analysis		
DNA cloning	33	21
Restriction enzyme analysis	37	23
Southern blot	8	5
DNA sequencing	9	6
Oligonucleotide synthesis	3	2
		57
RNA analysis		
cDNA cloning	1	1
Northern blot	2	1
RNA sequencing	1	1
In vitro transcription, translation	2	1
		4
Protein analysis		
High-resolution separation: HPLC, PAGE	10	6
Affinity purification	2	1
Protein sequencing	4	3
Peptide synthesis	2	1
X-ray crystallography, NMR	5	3
		14
Immunological methods		
Monoclonal and polyclonal antibody production	8	5
Assays: RIA, ELISA	6	4
		9
Animal techniques		
Cell culture and hybrid cell lines	7	4
Gene transfer into cultured cells	6	4
Gene transfer into animals	4	3
		11
Plant and microbial techniques		
Cell and tissue culture	18	11
Plant regeneration	10	6
Protoplast fusion	10	6
		23
Bioprocessing		
Controlled fermentation	12	8
Immobilized enzymes and cells	10	6
		14
Other		
Electron microscopy, x-ray microanalysis	4	3
Computer modeling	3	2
		5

[a]Abbreviations: cDNA, complementary DNA; HPLC, high-performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; NMR nuclear magnetic resonance; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay.
[b]Rounded to the nearest integer. Note that these percentages add up to greater than 100 because some articles described the use of more than one technique.

indicates their widespread use. In contrast, techniques for analyzing RNA molecules were strikingly underrepresented. Thus, although there is good capability for isolating genes, there is weakness in understanding how they are expressed and regulated. Second, the use of sophisticated techniques for protein analysis was surprisingly well represented. Third, as might be expected from the emphasis on agriculture in China, there was widespread use of plant tissue culture and regeneration techniques. In general, this analysis suggests that the various techniques composing modern biotechnology have become widely available in China.

EXPERIMENTAL ORGANISMS

The organisms used in each article were classified under two categories: (1) The "organism studied" refers to the species whose biology is being investigated, and (2) the "organism used" refers to the species actually used in the experiments. For example, for an article describing the production of human interferon in *Escherichia coli*, the organism studied is human and the organism used is *E. coli*.

A summary of the data on experimental organisms is presented in Table 7. The most important organism used was *E. coli*, the routine host for DNA-cloning experiments. Also popular were the yeast *Saccharomyces cerevisiae*, used for protein expression work, and economically important strains of antibiotic-producing fungi. The organisms studied reflected the emphasis of China's biotechnology research. There was a relatively strong emphasis on agricultural research, whereas animal research was predominantly directed toward humans. An interesting sidelight was the substantial number of articles on species of special interest to China, namely, fish (mostly species used for aquaculture), the silkworm, the panda bear, and the Peking duck.

A noteworthy point of this analysis was the complete lack of research using certain species widely used in Western countries. In particular, there were no articles on *Drosophila melanogaster* or *Caenorhabditis elegans*, the two most useful organisms for studying animal genetics and developmental biology. There was only one article (of low quality) on *Xenopus* species, and none on sea urchins, despite the wealth of embryological data on these organisms.

RESEARCH TOPICS AND GOALS

Because of the multidisciplinary nature of biotechnology research, it was not always simple to assign a single research topic to any given article. Taking this into account, each article was categorized according to two systems: scientific discipline (Table 8) and research goal (Table 9). For instance, an article on computer modeling of the hepatitis B surface antigen protein would be classified as "biochemistry, protein structure" under scientific discipline and "applied, human vaccine" under research goal.

TABLE 7 Experimental Organisms

Organism	Organism Studied[a,b]		Organism Used[a]	
	Number of Citations	Percentage of Citations[c]	Number of Citations	Percentage of Citations[c]
Bacteria				
E. coli	9	6	47	30
Bacillus	9	6	5	3
Streptomyces	6	4	4	3
Other	5	3	4	3
		19		39
Fungi				
S. cerevisiae	5	3	16	10
Antibiotic producers	14	7	14	9
Other	4	3	4	3
		13		22
Plants				
Rice	8	5	5	3
Maize	6	4	4	3
Tobacco	9	6	7	4
Fruits	6	4	6	4
Other	4	3	4	3
		22		17
Animals				
Human	16	10	5	3
Other mammals and rodents	6	4	3	2
Birds (mostly duck)	5	3	2	1
Amphibians and fish	9	6	8	5
Silkworm	5	2	2	1
		5		12
Viruses				
Hepatitis B	9	6	4	3
Other animal	4	3	2	1
Insect	5	3	9	6
Plant	8	5	5	3
		17		13

[a]See text for definitions.
[b]In 10 technique-related articles, no "organism studied" was assigned.
[c]Rounded to the nearest integer.

As expected, the predominant scientific disciplines represented in the articles were molecular biology and genetics, mostly because of the large number of articles on gene cloning. Next most popular was biochemistry, with a concentration of articles on protein structure. The fields of microbiology, virology, botany, and immunology were all reasonably well represented. (Note that the apparent paucity of articles on virology is because most of them were assigned to other disciplines; see Table 7.) The weakest areas noted were developmental and cell

TABLE 8 Scientific Discipline

Discipline	Number of Citations	Percentage of Citations[a]
Molecular biology and genetics		
Gene cloning and expression	20	13
Gene regulation	11	7
Population genetics	5	3
Gene mapping	4	3
Mutation and recombination	6	4
Other	3	2
		32
Biochemistry		
Protein structure	14	9
Enzymology	5	3
Structure and metabolism of natural products	5	3
Immobilized catalysts	11	7
Other	3	7
		29
Microbiology[b]		
Strain isolation and characterization	11	7
Fermentation	9	6
		13
Virology[b]		
Structure	5	3
Detection and prevention	3	2
		5
Botany[b]		
Plant propagation	16	10
Physiology and anatomy	5	3
		13
Immunology		
Diagnostic reagents	10	6
Other	2	1
		7
Other		
Developmental biology	3	2
Cell biology	4	3
Neurobiology	5	3
	160	8

[a] Rounded to the nearest integer. Each citation was assigned to a single discipline.
[b] Other than molecular biology and genetic citations.

TABLE 9 Research Goal

Goal	Number of Citations	Percentage of Citations[a]
Basic		
Genetics	15	9
Biochemistry	18	11
Other	17	11
		31
Applied		
Foodstuffs and natural products	21	13
Antibiotics	14	9
Therapeutic proteins	11	7
Diagnostics	11	7
Improved animal species	6	4
Improved plant species	14	9
Human vaccines	10	6
Animal vaccines	9	6
Plant vaccines and herbicides	6	4
Other	8	5
	160	70

[a] Rounded to the nearest integer.

biology (seven articles for both fields combined) and protein-nucleic acid interactions (no articles).

The research goals of the articles surveyed are summarized in Table 9. Applied research was favored over basic research by a more than two-to-one margin. The most important applied goal was the production of foodstuffs and other natural products, with antibiotic fermentation and the improvement of agricultural crops close behind. The most important conclusion from this analysis is that a close correlation exists between research funding priorities and the research that is actually performed.

EVALUATION OF RESEARCH

Reviewers were asked to evaluate the scientific originality and accuracy of each article according to the following criteria:

Originality

A. *Very original.* Describes a new phenomenon or technique or yields significant information on an important problem.

B. *Original.* Describes new results in an area of basic or practical significance.

C. *Somewhat derivative.* Repeats previous research but uses a different organism or a slightly different approach.
D. *Very derivative.* An exact copy of already published research.

Accuracy

A. *Very thorough.* Proves the point conclusively.
B. *Good.* The main point is not in doubt.
C. *Weak.* Insufficient data.
D. *Unacceptable.* Poor experimental design or interpretation—would not be published in a U.S. journal.

A summary of these evaluations, broken down by type as basic or applied research, is provided in Table 10.

In terms of originality, basic research articles far outstripped applied research articles. For example, 22 percent of the basic research articles were considered to be "very original" as compared with only 3 percent of the applied research articles. More striking was the fact that 81 percent of the applied research articles "repeat previous research," either completely or with only minor changes in methodology or experimental material. Many of these articles described either the isolation of genes already cloned in the West or the production of previously described materials. For example, more than half of the applied genetics articles described the cloning of genes already sequenced and published in international journals. Because virtually all such journals have the policy that published clones must be made available to all who ask for them, this represents an especially fruitless expenditure of time and resources. One Chinese colleague quipped, "Many of the experiments are like Xerox copies, only made with brush and ink."

TABLE 10 Evaluation of Research

Type of Research	Number of Citations per Category[a]			
	Originality			
	A	B	C	D
Basic (50 articles)	11	18	17	4
Applied (110 articles)	3	17	46	44
	Accuracy			
	A	B	C	D
Basic (50 articles)	10	21	16	3
Applied (110 articles)	31	47	23	9

[a] See text for definitions of the letters under the headings of originality and accuracy.

The evaluations of scientific accuracy showed a greater equality between basic and applied research. More than half of both types of articles were considered to "prove the point." Those that failed this test did so more often because of a lack of completeness rather than genuine faults in experimental design or interpretation. Several reviewers commented on the tendency to split a single body of work into several articles.

7

Research Highlights

During the course of the literature survey, it became apparent that certain research in China has progressed to an international level. The following section describes the most promising of these projects in both basic and applied biotechnology. This material is elaborated on, and in some cases overlaps the reports on individual research institutes, in Chapter 8.

X-RAY CRYSTALLOGRAPHY

X-ray crystallography is the method of choice for determining the precise three-dimensional structure of many biologically important molecules. X-ray structure determination techniques have evolved in two major directions: direct methods, for small molecules; and indirect methods, involving multiple derivative sets of diffraction data, for macromolecules such as proteins. China possesses a high level of expertise in both of these state-of-the-art technologies.

At the Beijing Institute of Physics, Fan Haifu and colleagues have been working on probability phasing methods to determine the crystal structures of increasingly large biological molecules. They were among the first to develop and successfully apply *ab initio* random-start phasing techniques. The advantage of such methods is that phasing can be determined without the need for multiple measurements at different wavelengths on heavy atom derivatives. Recently, Fan's group has demonstrated the accuracy of their methodology by redetermining the structure of avian pancreatic peptide from one-wavelength, anomalous-scattering x-ray data at 2 Å resolution. Ultimately, this methodology may make it

possible to determine the structure of a variety of peptides and proteins by direct means. This would have broad and important implications for protein engineering.

Groups at the Beijing Institute of Biophysics and the Fujian Institute of Research on the Structure of Matter have used more traditional diffraction methods to solve the structures of several interesting molecules such as insulin and trichosanthin. A comparison of native and despentapeptide insulin at 1.5 Å resolution revealed important differences in the position of the B-chain aminoterminal region. The positions of 84 water oxygen atoms and some hydrogen bonds have also been determined. These observations provide important clues to the design of new insulin molecules with altered receptor-activation and multimerization properties. The structural determination of trichosanthin, an abortifacient from a traditional Chinese medicine, revealed it to be a two-domain protein. The three-dimensional structure revealed striking homology to the ricin A-chain, a potent toxin, immediately suggesting the mechanism of action. It was recently reported that trichosanthin selectively kills cells infected with the human immunodeficiency virus, which causes the acquired immune deficiency syndrome (AIDS). Such findings put this research in an interesting new light.

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CONTROL OF PLANT VIRUSES

Plant viruses cause great harm to China's agriculture. Scientists at the Beijing Institute of Microbiology have been using molecular biological techniques to fight two of the most important menaces, cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV).

CMV can infect more than 775 plant species, including green peppers, tomatoes, and tobacco (but, despite the name of the virus, not Chinese cucumbers). Previously, there were no means to prevent viral infection since the aphid vectors are difficult to control and no CMV-resistant host genes have been discovered. In

1976, an American group found that certain strains of CMV carry a small RNA, termed satellite RNA, in addition to the four major genomic species. Satellite RNA, which is completely dependent on its helper virus for replication, bears no nucleotide sequence homology to CMV but can interfere with its replication and ability to cause disease symptoms.

Tien Po's group at the institute has been studying various strategies to use satellite RNA as a biological control agent for CMV. They prepared two attenuated strains of CMV by coinfecting plants with genomic RNA from Chinese tomato or green pepper together with satellite RNA from a laboratory strain. Greenhouse experiments showed that inoculation of green pepper plants with the attenuated green pepper strain reduced infection by virulent CMV by up to 75 percent over a 10- to 20-day time frame. Plants inoculated less than 10 or more than 30 days prior to challenge were less well protected. An obvious risk to this approach is that the attenuated strain might actually be virulent in other species, thus greatly reducing the practicability of field applications. However, infection of 38 different plant species produced no obvious disease symptoms except for slight mosaic symptoms in *Cucurbita pepo*. In addition, the attenuated CMV strain did not accelerate infection by other plant viruses or pathogens. Indeed, infection of tobacco appears to actually inhibit the growth of certain fungi such as *Phytophthora infestans* and *Cladosporium fulrum*. It is presumed, but not proven, that this fortuitous resistance is caused by an interferon-like response to the infection. Additionally, in some cases, it is claimed that infection with the attenuated strains can accelerate plant growth and increase fruit yields.

Between 1981 and 1985, the attenuated viruses were field tested on green pepper and tomato plants in several localities throughout China. A 5-year test on green pepper plants in Beijing, Handan, and Yantai gave 70 to 80 percent decreases in disease indexes and 20 to 55 percent increases in fruit yields. A 3-year test on tomato plants in Taiyuan gave a 31 to 40 percent reduction in disease and a 31 to 45 percent increase in fruit yield. Since 1986, these control agents have been applied to much larger areas constituting about 3 percent of the fields around some large cities (e.g., Beijing and Shanghai) and up to 80 percent of the fields around certain small cities (e.g., Handan and Anda).

While the attenuated virus approach has had success, it would obviously be preferable to develop plants that are permanently resistant to viral infection, and, consequently, bypass the necessity for seasonal inoculations. In 1986, Baulcombe showed that a dimer insert of satellite RNA introduced into tobacco on a tumor-inducing (Ti) plasmid vector could be expressed into biologically active RNA. Following up on this lead, Tien's group obtained and sequenced their own satellite cDNA clone, inserted a monomer into the Ti plasmid, and obtained transgenic tobacco. Most of the transformed plants produce large amounts of satellite RNA and are 10-fold more resistant to CMV infection than are control or nonsatellite RNA-producing plants. The transgenic plants grow almost as well as normal plants both in the greenhouse and in the field; the influence on yield and quality is now being tested. An interesting difference from Baulcombe's results is

that these plants are resistant in inoculated as well as systemically infected leaves. This might reflect a difference between the replication of the monomer satellite cDNA insert used by Tien as compared with the dimer insert used by Baulcombe.

Mang Keqiang's group is using a related, but distinct approach to combat TMV. It has long been known that inoculation of tobacco with a mild strain of virus confers resistance to later superinfection with a more virulent strain, a phenomenon known as cross-protection. In 1986, Abel and colleagues showed that such cross-protection could be conferred by the expression of a single protein, the TMV coat protein, in transgenic plants. Following this lead, Mang's group cloned the coat protein gene from the common Chinese strain of TMV and confirmed its identity by DNA sequencing and comparison with the vulgar strain sequence already published by Western scientists. They then fused the coat protein gene to a strong promoter sequence, mobilized the fusion gene into the Ti plasmid, and obtained transgenic tobacco plants. Such plants have been shown to produce the coat gene RNA and protein. Although they are still susceptible to TMV infection, symptoms are delayed by 1 to 2 months, which is a substantial advantage under field conditions.

The basic mechanism(s) by which expression of satellite RNA or coat protein blocks viral infection is still unknown. Possibilities include competition for replication or packaging factors, blockage of receptors, or a more general response to infection and stress. This is an interesting problem that could form the basis for a useful Sino-American collaborative research project. American scientists may also be interested to know that field testing of genetically altered organisms is possible in China. Given the large area of cultivated land and the variety of soil and climate conditions in China, this might provide the basis for joint ventures between American agricultural biotechnology companies and their Chinese counterparts.

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TOWARD A HEPATITIS B VIRUS VACCINE

Hepatitis B virus (HBV), the causative agent of serum hepatitis, is a serious health problem in China, as it is throughout much of Asia. It is estimated that there are 100 million antibody-positive individuals in China, many of whom are at

high risk for chronic liver disease and hepatocarcinoma. A notable characteristic of HBV is that it can be transmitted before or at birth from infected mother to baby. A vaccine, derived from the serum of HBV carriers, has been available in the West and in China for several years. However, this vaccine is very expensive (more than $100 in the United States), thus prohibiting the mass inoculations needed to eradicate the disease. Moreover, no matter how well the vaccine material is purified, there is always the fear that it might be contaminated with unknown viruses. Thus, there is a strong incentive to use biotechnology to produce a safe and inexpensive HBV vaccine. The groups of Li Zaiping, at the Shanghai Institute of Biochemistry, and of Hou Yunde and C. M. Chu, both at the Beijing Institute of Virology, have been particularly active in this field.

China is pursuing two strategies to develop such a vaccine. The first, already in use in the United States and Europe, is to employ hepatitis B surface antigen (HBsAg) as a subunit vaccine. Toward this end, the strain of HBV most prevalent in China was cloned and characterized by complete DNA sequencing. The HBsAg-coding sequences were then expressed by using several different eukaryotic vector systems: yeast plasmids with high-efficiency promoters; bovine papilloma virus vectors that can stably replicate in mouse cells; dihydrofolate reductase (DHFR) vectors that can be amplified in Chinese hamster ovary (CHO) cells; and vaccinia virus. In agreement with previous findings of Western scientists, HBsAg was shown to be appropriately glycosylated, assembled into particles, and secreted into the medium of the cultured mammalian cells. The most efficient systems have been transferred to the Biological Products Factory at Changchun (Jilin Province) for medium-scale production. Phase one human trials on two of these products have given encouraging results: no obvious side reactions and a high level of antigenicity. The plan for 1989 is to carry out phase two trials of the two Chinese products that will compare them with a vaccine produced in Shenzhen using foreign recombinant DNA technology and with the Chinese serum vaccine. After determining which preparation gives the best protection against HBV infection, large-scale production will be started at Changchun.

Attempts to develop second-generation HBsAg subunit vaccines are also under way. One strategy is to produce a mixture of the short form of HBsAg together with a longer form containing the pre-S2 region. The rationale is that both forms are found in serum particles and that the longer variant may contain a binding site for albumin; theoretically, this might increase antigenicity, but clinical trials will be required to test the actual efficacy of this approach. A second idea is to produce synthetic peptide antigens. Although this research has been publicized in the Chinese press, it seems unlikely to be practical in view of the many studies showing that a much smaller fraction of the human population will respond to a single epitope than to the mixed epitopes of complete HBsAg.

While the efficacy of HBsAg subunit vaccines is now established, there remain serious difficulties in using this approach for the massive inoculations needed to eradicate HBV in China: the high cost, the necessity for multiple injections, and the requirement for careful storage of the vaccines. With these problems in mind,

the Beijing Institute of Virology is attempting to develop a more practical vaccine based on live vaccinia virus recombinants. Toward this end, the HBsAg gene was linked to a strong viral promoter and then inserted into the Tian Tan strain of vaccinia virus; this is a vaccine strain that has been used extensively for the eradication of smallpox in China. The recombinant viruses have been propagated in primary cells and shown to be antigenic in experimental animals. (For further details and comparison with American research, see Chapter 8.) It was originally planned that testing of this material on humans would begin in 1988, but apparently there was some controversy because the recombinant virus was originally plaqued on an established human cell line, and therefore, might have "picked up" some "tumor material." Fortunately, material that has been grown only on primary cells will soon be available for testing. This vaccine has many potential advantages: low cost, stability of the vaccine at room temperature, and single inoculation with an air gun. Ultimately, it may be possible to make multivalent vaccinia viruses that include antigens for hepatitis A virus and herpesvirus.

There are three noteworthy points about China's efforts to develop new HBV vaccines. First, there has been real cooperation between basic science units (CAS and the Chinese Academy of Preventive Medicine) and a downstream production facility. Second, China is making its own comparison of various forms of the HBV vaccine rather than simply accepting Western findings; this is critical in view of the different target groups (children and potential mothers in China versus doctors, dentists, and sexually active male homosexuals in the United States). Lastly, China appears ready to respond to the urgency of its HBV problem with a novel approach—the vaccinia virus recombinants—which probably will not be clinically tested in the West.

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PLANT CELL AND TISSUE CULTURE

The in vitro culture of plant cells and tissues has numerous applications in agricultural biotechnology: the regeneration of plants from cells with desirable traits introduced by conventional genetic selections or recombinant DNA methods; rapid propagation of valuable flowering and fruit-bearing plants; eradication of certain persistent viral diseases; and generation of haploid species with useful properties. Although the principles of plant cell and tissue culture are straightforward, the actual application can involve testing hundreds or thousands of variations in culture conditions.

Chinese scientists have achieved several notable accomplishments both in culturing new species and in applying this technology commercially. Chinese scientists were among the first to succeed in the use of anther culture to breed haploid wheat and sugar cane varieties, many of which are now in widespread field use. For examples of the painstaking research used to optimize culture conditions, see the references below by Ouyang Junwen and colleagues. Meristem culture has been used to obtain plants free of two important viral pests, the potato degeneration virus and gladiolus mosaic virus. Tissue culture is routinely used in several Guangdong institutes for the rapid propagation of various fruiting and flowering plants such as grape, orchid, gladiolus, chrysanthemum, African violet, narcissus, and fuchsia. An important advantage of this technology, which the Chinese (and the Japanese) are just beginning to exploit, is that valuable flower varieties can be exported without the usual plant pest control restrictions. In the field of plant regeneration, Chinese scientists have succeeded in obtaining maize embryonic calli from pollen and protoplasts, soybean from protoplasts, and rice (*Indica* strain) from embryos and protoplasts.

Many Western scientists are now working on the isolation of plant genes of economic importance. Collaboration with Chinese scientists could provide an important step in transferring such genes to useful plant species.

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8

Current Research at Selected Institutes

The following section describes current biotechnology-related research at 19 institutions that were visited during a 1-month evaluation trip to China. The purposes of this somewhat anecdotal recounting are threefold: to support, in a concrete way, the general conclusions of Chapter 3 on how research funding is prioritized and allocated; to elaborate on research trends and quality examined in Chapters 6 and 7; and to provide a road map for American scientists interested in cooperation and collaboration with Chinese colleagues. A list of contacts at these institutions is included in Appendix C.

BEIJING

Beijing Agricultural University

Beijing Agricultural University, formed in 1952 from the agricultural departments of Beijing and Qinghua Universities, is China's largest agricultural university with a staff of 1,050, including 100 professors, 235 associate professors, and 350 lecturers. The large campus on the outskirts of Beijing includes an experimental farm, and the university also operates experimental stations in Hubei and Harbin. Of the 21 departments, five actively pursue research involving biotechnology. The amount and type of equipment in the research laboratories have recently been improved by using funds from a World Bank loan. The university operates a national key laboratory for agricultural biology and plans to establish an open laboratory for agricultural biotechnology in the near future.

The generation of transgenic plants is of interest to several groups at the

university. Yun Longfei's group has cloned a soybean 7S storage protein gene and shown, in agreement with results of other groups, that it is expressed in a tissue-specific fashion in Ti plasmid-generated transgenic tomato, potato, and tobacco plants. They are also attempting to generate CMV-resistant plants by using the strategy employed at the Beijing Institute of Microbiology. Attempts to clone plant photosynthetic and high nutritional value protein genes, by using probes from other groups, are still at the genomic library construction stage.

Work on transgenic animals at the Beijing Agricultural University focuses on the use of growth hormone and growth hormone-releasing factor genes to improve the growth characteristics of pigs and rabbits. Although the relevant genes have already been cloned by Western biotechnology companies, they are trying to get their own genes and are still in the process of making genomic libraries.

Chinese Academy of Agricultural Sciences

The Chinese Academy of Agricultural Sciences was established in 1957 to coordinate agricultural research activities at the national level. Currently, it has 33 research institutes, a graduate school, an agricultural library, an agriculture and technology publishing company, a center for computer sciences, and the Center for Biotechnology. There are a total of 10,575 staff members, including 5,063 scientists, 812 administrative personnel, and 4,700 support staff.

The Center for Biotechnology was established in 1986 with a mission that includes research and training. According to the original plan, the center will have 40 new positions. At the present time, there are three major laboratories: molecular biology and genetic engineering, plant cell technology, and tumors and monoclonal antibodies. Research subjects carried out at the center include vaccine and genetic engineering, plant genetic engineering, biological control and viral genetic engineering, protoplast culture and fusion, and plant virus and monoclonal antibody studies.

The Molecular Biology and Genetic Engineering Laboratory is led by Y.L. Fang, director of the center. Currently, there are about 20 people: one associate professor, two instructors, one postdoctoral fellow, three Ph.D. students, eight M.S. students, and five research associates. This laboratory is well funded with approximately 400,000 *yuan* from the Chinese government and $20,000 from the Rockefeller Foundation.

They are carrying out four major projects. In the isolation and identification of plant genes project, the genes that have been cloned are leghemoglobin and 11S seed storage protein from soybean. In the nutritional improvement of crop species project, they are at the stage of constructing genomic libraries from alfalfa. In the insect-resistant plants project, they are transferring plants with *Bacillus thuringiensis* toxin gene by using β-glucuronidase as the reporter gene. The isolation of toxin genes from insects project has just been started.

The Plant Cell Technology Laboratory is led by S.C. Chia, who is currently

taking sabbatical leave in Singapore. He has a group of nearly 10 people consisting of one associate professor, two research associates, and several M.S. students. This laboratory has been credited with making contributions in fusing cucumber protoplasts and subsequently regenerating them into plants. It receives good support at 200,000 *yuan* per year.

The Tumor and Monoclonal Antibody Laboratory conducts research on potato viruses. It is the newest among the three laboratories. Currently, there are about 10 people receiving about 200,000 *yuan* per year for research.

Institute of Biophysics (CAS)

The Beijing Institute of Biophysics, founded in 1959, currently houses some 800 workers, including 400 scientists. The institute is divided into 12 departments that include, in biotechnology-related fields, bioengineering, enzymology, x-ray crystallography, protein engineering, and cell biology. An interview conducted with Lei Kejian concentrated on scientific topics rather than research support. However, it was obvious from the sophisticated instrumentation in the laboratories that the institute is well funded.

The research highlight at the institute is their internationally recognized work on structure-function relationships in insulin. These studies involve high-resolution x-ray crystallography to determine the precise structure of the molecule, chemical and enzymatic modifications to determine the roles of various residues in molecule function, and genetic engineering to produce novel derivatives. The structure of native insulin (Zn2 hexamer form) has now been completed at a 1.2 Å resolution, the highest yet reported. At this resolution, it is possible to visualize three hydrogen bonds and to detect asymmetry at two disulfide bonds. The structure of chemically prepared despentapeptide insulin, which lacks five residues and has lost 8 percent of its biological activity, has been solved at a 1.5 Å resolution and appears identical to the native form except for the position of the carboxy-terminal residue. Narrowing in on the active site, attempts are now under way to solve the structure of deshexapeptide insulin, which lacks one additional residue and has lost all biological and receptor-binding activities. Structure-function relationships of insulin are also being studied by a comparative approach, and the structure of a fish liver insulin has been solved at a 2.8 Å resolution.

Building on this basic research, the protein engineering group is attempting to engineer long-acting forms of insulin. Insulin is stored and secreted as a Zncoordinated hexamer, whereas the active form is either a monomer or dimer (a point of dispute among the Beijing Institute of Biophysics and other groups). Therefore, if the association constant for the hexamer could be increased, administered insulin might have a longer action time. Using computer graphics, it was shown that there is an empty area between adjacent dimers in the hexamer structure, and that a valine and a glutamic acid residue reside on opposing forces of this region. Molecular orbital calculations suggested that changing the valine

to arginine, a basic amino acid, would allow formation of a salt bridge and substantially increase the association constant. This change has now been achieved by site-directed mutagenesis, and mass production in *E. coli* is under way. Another useful alteration, which has been achieved by chemical methods, is the deletion of phenylalanine residue B1. This produces an insulin which is still 100 percent biologically active but lacks immunological reactivity, an important step in treating diabetics that mount an immune response to administered insulin. The same change is now being made by genetic engineering methods to allow large-scale production.

Two organizational aspects of the insulin work are noteworthy. First, although the bulk of funding is from High Technology Program grants, it is clear that there is a major nonapplied component to this work and that the researchers' hearts are really in basic research. Second, the work has proceeded in close collaboration with groups at the Beijing Institute of Physics, the Shanghai Institute of Biochemistry, and Peking University. Such collaborative research, especially between CAS and non-CAS institutes, is unusual in China; the success of the insulin work illuminates its importance.

The Beijing Institute of Biophysics is pursuing research on the structure of several other interesting proteins and small molecules. The structure of trichosanthin, a component of a traditional Chinese medicine used as an abortifacient, has been solved at 2.6 Å (R = 0.29), and data for the 1.8 Å map have been collected. The three-dimensional structure shows an even closer resemblance to the ricin A-chain, a related ribosome inhibitory protein, than does the primary structure. In collaboration with the visual sciences group at the institute, a lightsensing protein has been purified to homogeneity, and crystallization attempts are under way. The structures of several small molecules, such as a natural antimalarial drug, have also been solved.

Institute of Developmental Biology (CAS)*

When the Beijing Institute of Developmental Biology was established in 1980, it was the realization of the vision of T.C. Tung, who, during his years as a highly respected senior embryologist at the Beijing Institute of Zoology, had inspired the idea of creating a separate institute of embryology or developmental biology. Although Tung died in the late 1970s, his idea was adopted and promoted by Niu Mann-Chiang, a Chinese-American biologist who had worked in Tung's laboratory when he started splitting his time between China and his own laboratory at Temple University. Niu capitalized on his association with Tung, and Tung's relationships with government leaders including Deng Xiaoping, to orchestrate the founding of the institute.

* This section has been revised from the earlier edition. For a statement by Dr. Niu regarding this section, see Appendix E.

The institute houses 60 scientists, including 13 senior researchers and 15 research associates. Although it does not have a formal Ph.D. program, there are several doctoral students in Niu's laboratory. Several of the institute's students and scientists have studied abroad, where they have shown themselves to good advantage. The institute is divided into five research groups that are working on 21 individual projects. Current support from the High Technology Program, the Seventh 5-Year Plan, and NSFC is 1.8 million *yuan* per year, plus an undisclosed amount from CAS specifically for Niu's laboratory.

The institute occupies a unique position among China's biology research organizations. Under Niu's leadership, the institute obtained $650,000 from the Rockefeller Foundation, $550,000 from the United Nations Fund for Population Activities, and several million *yuan* from the Chinese government in order to build and equip its research facility. The institute's laboratories are the most modern, well equipped, and best maintained of those the authors visited in China. Clearly, Niu has been influential in the establishment of this institute, a role that is reflected in his position and the amount of funding the institute has been able to attract.

However, the following overview of Niu's research casts doubt on the extent to which his enquiries can be counted as contributions to the international scientific community. The focus of Niu's research is on the role of RNA in biological systems and during development. In 1975, he claimed that the fungus *Neurospora crassa* could be stably, genetically transformed by RNA.[*] This is a remarkable claim since it is universally believed that in *Neurospora*, as in all other organisms, it is DNA rather than RNA that serves as the genetic material. Moreover, despite intense work on the genetic transformation of *Neurospora*, this observation has never been reproduced in the published literature. Because transformation experiments on *Neurospora* are easy to perform, it is likely that these experiments have in fact been repeated but without positive results.

Niu has also claimed that RNA can be stably inherited in higher organisms and can cause the derepression of normally silent genes. For example, in 1977 he reported that mouse uterus injected with RNA from rats or chicken synthesized both mouse and the donor species albumin in uterine epithelial cells, suggesting that the mouse genes were depressed by the heterologous RNA. However, it is difficult to interpret these experiments because the method used to detect albumin synthesis was not sufficiently documented. Moreover, similar experiments using cloned, purified genes and well established gene transfer methods have failed to reveal any derepression of the endogenous genes.

Starting in 1981, Niu described experiments in which goldfish developing from eggs microinjected with rabbit globin mRNA were purported to express rabbit globin, as determined by an immunological assay. The results were again

[*] Mishra, N.C., Niu, M.C, and Tatum, E.L., *Proceedings of the National Academy of Sciences USA* (1975): 642-645.

ambiguous because the globin was not purified or adequately characterized. Subsequently, successful gene transfer has been reported in fish, but these experiments all used DNA rather than RNA as the nucleic acid and purified genes rather than a crude mixture of species. Judging from the rate of transformation with purified genes, it is difficult to believe the results claimed by Niu using total, unfractionated RNA containing thousands of different messages.

The most spectacular of Niu's claims is the ability of RNA to redirect the formation of whole organs in intact animals. Specifically, he claims to have created goldfish that have balancer appendages or tail shapes derived from newts or carp, and that these new traits can be inherited. Such fish, if they existed, would be of intense interest to many biologists. However, when the authors asked to see these animals, Niu claimed that they are all kept at another location even though the Institute of Developmental Biology has extensive fish-raising facilities on its own campus. If and when these fish are made available to other investigators, it should be straightforward to check them for the presence of newt or carp genetic information using modern methods. Only when these tests have been conducted will it be possible to determine whether the fish represent a real phenomenon. Appendix D, provided by Eric Davidson, provides a further analysis of the experimental and theoretical problems raised by Niu's work.

The laboratory of Yan Shaoyi, Director of the Institute of Developmental Biology, studies nuclear-cytoplasmic interactions in fish. His group has shown that it is possible to recover fertile adult fish from enucleated eggs injected with the diploid nucleus from early embryonic cells. In agreement with work by other laboratories on amphibians, they observe that the ability to recover viable progeny declines with the developmental stage of the donor cells. They have also made hybrids between different species of fish and claim that it is possible to obtain viable progeny from crosses between different genera and some different subfamilies, but not between different families. While most of the hybrid progeny appear to be identical to the nucleus donor species, they also make the surprising claim that some offspring display morphological traits similar to those of the recipient species or intermediate between those of the donor and recipient. Even more remarkably, they claim that the recipient traits can be passed on to offspring and that the penetration of the recipient characteristics can be increased by serial transplantations. One example, which has been reported widely in the Chinese press, is the cross between nuclei of the common carp *Cyprinus carpio* L. and enucleated eggs of the crucian carp *Carassius auratus* L. It is claimed that this hybrid has a higher growth rate, higher protein content, and lower fat content than the common carp and that it can be bred stably. Yan attempts to explain these results by a theory in which "silent genes" in the donor nucleus undergo activation and rearrangement under the influence of the recipient cytoplasm. However, a major flaw of these experiments is the lack of distinct markers for the donor nucleus. Without such markers, there is no way to be sure whether the "hybrid" fish are really derived from the nucleus of the donor or from residual material of

the recipient. There are some simple biochemical and molecular biological experiments that could clearly resolve this question, but Yan shows no inclination to perform these analyses. Another serious flaw is the lack of quantitative analysis and controls to show that these fish are not simply mutants induced by the various experimental manipulations. Until these controls are performed, Yan's results must be viewed with skepticism.

Xiao Shuxi studies DNA polymerase in Erlich ascites cells. She claims to have discovered a new polymerase with a different pH optimum and size than any of the polymerases in normal cells and hopes to use it as a marker for cancer cells. However, the experiments are impossible to interpret because of a complete lack of controls. Moreover, since the Erlich cell polymerase has not actually been purified, the idea of making diagnostic reagents is premature.

The laboratory of Lu Deyu works on the genetic manipulation of mammalian embryos. They have spent several years developing an electroporation method to perform nuclear transplantation in rabbits. The idea is to test Niu's cytoplasmic activation of the nucleus theory in a mammal, but so far no progeny animals have been recovered. They are also working on the production of transgenic farm animals by the more conventional DNA injection method and claim to have obtained one cow which expresses HBsAg.

Visits to the laboratories of two associate professors, Wu Naihu and Wu Zhengan, demonstrated that the Institute of Developmental Biology does conduct some research at an international level. Wu Naihu studies the rice chloroplast genome. He is continuing his work, started in Ray Wu's laboratory in the United States, on the structure and regulated expression of various chloroplast genes such as *psbA* and *psbB*. He has also made the interesting observation that in certain crosses between different rice species, the F-1 plants display a new chloroplast DNA restriction band. This is quite surprising, since the chloroplast genome is known to be maternally inherited.

Wu Zhengan studies highly repeated DNA sequences in amphibians and mammals. He has made the interesting observation that an extra C band in one chromosome of a Chinese ground squirrel is due to the transposition of a satellite DNA. He is also studying a series of satellite DNAs from Chinese newts and has shown that one form is specific to Asian species. Wu's work demonstrates the use of modern molecular techniques to study the basic biology of uniquely Chinese species. He was proud to note that his recent work, which has been published in an international journal, was conducted exclusively in China.

In summary, the quality of the research conducted at the Beijing Institute of Developmental Biology is highly variable. The work in several of the laboratories, particularly that of Niu, is so poorly controlled and documented that the results will continue to be questioned in China and abroad. Continued support for such research will not advance developmental biology in China, sets a poor example for other Chinese scientists, and casts doubts on the efficacy of peer review

procedures. On the other hand, the institute does have some laboratories, such as those of Wu Naihu and Wu Zhengan, that are performing creditable research on worthwhile topics. Given China's general weakness in developmental biology (see Chapter 6), it is urgent that this institute focus its considerable resources on supporting the best possible research.

Institute of Genetics (CAS)

The Beijing Institute of Genetics employs 470 workers, including 80 senior scientists, 180 middle-level scientists, and 40 graduate students. There are 40 research groups working in the areas of molecular genetics, gene and chromosome engineering, plant breeding and tissue culture, animal developmental genetics, and human and medical genetics. Approximately 70 percent of the research is on plants. Funding is obtained through CAS, NSFC, the Seventh 5-Year Plan, the National Key Project Program, and the High Technology Program. Approximately half the research is classified as basic, half as applied. Located in the new Datun Road complex, it maintains an experimental farm and animal facility, as well as a laboratory building.

Plant regeneration is a key research area and a point of pride for the Beijing Institute of Genetics. Ouyang Junwen's laboratory was the first to achieve anther culture of wheat and has continued to work on this project for the past 10 years. By systematically varying the medium and temperatures used for anther cultivation and callus induction, they have achieved up to 10 percent plant propagation efficiencies. They have also shown that the efficiency of callus formation and the percentage of albino plantlets, a serious problem in anther culture, are both complex multigenic traits. A focus of current research is the use of anther culture to obtain mutant plants resistant to diseases, such as the wheat scab fungus, *Fusarium* species, or to herbicides such as atrazine. Two scab-resistant lines have been isolated and are currently being field tested. Attempts are also in progress to obtain wheat with a high lysine content, by selection with a lysine analog, but the investigator did not seem convinced of the physiological soundness of this approach. The laboratory is also beginning gene transfer attempts, using marker genes such as kanamycin resistance, but there was no clear focus on what useful genes might be employed in the future. In testimony to the somewhat unique position of this laboratory in the anther culture field, Monsanto Company has contracted with Ouyang to culture and provide seeds from their germplasm. The laboratory is supported by 140,000 *yuan* for 5 years from the Seventh 5-Year Plan and High Technology Program grants and $60,000 from Monsanto.

Li Xianghu's laboratory concentrates on the culturing, fusion, and DNA transformation of plant protoplasts. Over the past 10 years, this group has successfully regenerated plants from rice, tobacco, petunia, and others and has had initially encouraging results with wheat—a species so far resistant to this technique. They recently made front-page headlines in the Chinese press by

transferring an α-interferon gene into tobacco. The transgenic plants produce low levels of interferon (1,000 units per gram of leaf tissue) and are currently being tested for resistance to TMV. Curiously, Li's group has not done the control experiment of determining whether direct interferon treatment protects against this virus. Zhen Zhu, who recently obtained his Ph.D. from the University of Tennessee and who holds a Rockefeller Foundation fellowship to return to the United States for 3 months each year, is studying the more basic problem of the relationship between methylation and gene expression in plants. Another very interesting basic project is an attempt to create new plant species by protoplast fusion. This group is handsomely supported by 700,000 *yuan* for 5 years from the High Technology Program and the Seventh 5-Year Plan.

Li Liangcai's group also works on DNA transformation of plants using a two-step protoplast preparation method worked out in his laboratory. They have transferred two marker genes, those for kanamycin resistance and β-glucuronidase, in both stable and transient transfection systems. The main emphasis is on methodology rather than potential applications. The laboratory is supported by 150,000 *yuan* for 5 years from the Seventh 5-Year Plan and High Technology Program and by $15,000 from the Rockefeller Foundation.

From the above review, it is clear that the Beijing Institute of Genetics conducts research in several areas of modern biotechnology and gene manipulation. Therefore, it was surprising to note that few—if any—of the senior scientists have a background in classical genetics, and that the institute does little research in the traditional areas that provide the foundation for modern molecular biology.

Institute of Microbiology (CAS)

The Beijing Institute of Microbiology houses 580 workers, including 236 middle- and senior-level scientists and 20 Ph.D. students. There are eight divisions: mycology, bacteriology, virology, genetics, physiology, ecology, natural resources, and a fermentation facility. The institute is supported by 6 million *yuan* per year, of which 60 percent is derived from various project grants. The mycology and bacteriology divisions, which are concerned mostly with systematics, have recently been designated as a national key laboratory with two postdoctoral positions.

Plant virology is a highlight of the institute's biotechnology research. Tien Po's laboratory studies CMV, which infects several important crops such as tomatoes, green peppers, and tobacco. During propagation, small satellite RNAs that depend on the main genome for their replicative functions occasionally occur. Tien's laboratory and other laboratories showed that these satellite RNAs can interfere with the replication and disease-causing properties of superinfecting viruses. Therefore, they prepared a mixture of CMV and excess satellite particles and showed, in greenhouse experiments, that it protected plants against CMV superinfection. This "plant vaccine" can be administered either by individual inoculation of plants or, in more recent experiments, by a convenient spray gun

technique. Subsequently, large amounts of the material have been prepared in the institute's fermentation facility and have been used on tomato and green pepper plants in several parts of China. Tien claims that infections, in some cases, have been cut by 50 percent and yields increased by 30 percent. Current research focuses on incorporating the interfering gene sequence into the tobacco genome to provide permanent protection.

Mang Keqiang, together with young colleagues Qing Xiaofong and Yang Maozhou, work on TMV, a close relative of CMV that also infects tomatoes and tobacco. Building on work performed in the United States, they are attempting to prevent TMV infection by persistent expression of the coat protein of the virus in transgenic plants. The idea is that the coat protein, which normally forms the viral envelope, will bind to cell surface receptors and therefore compete with virus particles for a binding site. This group cloned a Chinese strain of TMV, showed that it has a sequence very similar to that of a previously characterized American strain, and introduced the coat protein gene into tobacco by Ti plasmid transformation. The resulting transgenic plants express coat protein, and although they are still susceptible to TMV infection, the symptoms are delayed by 1 to 2 months under field conditions. Mang's laboratory has also carried out comparative studies of various isolates of CMV, TMV, barley stripe mosaic virus, wheat mosaic virus, cereal mosaic virus, and a gladiolus virus by using DNA sequencing, RNA sequencing, and antibody techniques.

The Beijing Institute of Microbiology also has a long history in industrial fermentation. Between 1972 and 1978, they developed a simple two-step fermentation technique for the production of vitamin C, and in 1985, this process was sold to Hoffmann-La Roche, Incorporated. They are currently working on the production of enzyme inhibitors to increase the efficiency of various β-lactam antibiotics against resistant strains of bacteria, and on a kit for blood cholesterol analysis by using two enzymes produced by fermentation. Other fermentation studies involve protoplast fusion of useful penicillin producers and characterization of a new virus in *Aspergillus niger*.

Institute of Virology (CAPM)

The Institute of Virology, which is part of the Chinese Academy of Preventive Medicine (CAPM; previously Chinese Academy of Basic Medical Sciences), is the major research center for animal virology in China. Although a substantial proportion of the institute's research is in classical medical virology, there are four projects involving molecular biology: bio-engineered vaccines; basic studies of vaccinia virus replication; production of interferon, interleukin-2, and other lymphokines; and protein engineering. This institute, with 12 High Technology Program grants and 18 Seventh 5-Year Plan grants, is one of the two most richly supported research institutes in China. They are currently erecting a national key laboratory funded by 5 million *yuan* from the State Planning Commission.

Although the physical facilities appear dilapidated and are located in a *hutong*, or alley, district far from other institutes in Beijing, a relatively high proportion of scientists and students were seen to be actively performing experiments during several visits.

Groups under the supervision of the institute's director Hou Yunde and former director C.M. Chu are involved in several biotechnology projects. One major area of interest is the use of vaccinia virus as a vector for vaccine production. They have constructed a new vector with two important differences from the vectors used by Bernard Moss's laboratory in the United States. First, they use the hemagglutinin gene rather than the thymidine kinase gene as an insertion site because hemagglutinin mutants can be grown on normal cell lines, whereas the thymidine kinase mutants must be propagated on a special cell line. Moreover, the virus yield is higher. Second, the Chinese vector is based on the Tian Tan strain, which has been widely used for smallpox vaccination in China. The construction of the new vector required a substantial amount of basic research on vaccinia virus gene expression, including the sequencing of 30,000 base pairs of the vaccinia virus genome. An unexpected result of this research was the discovery that the vaccine virus hemagglutinin gene is a member of the immunoglobulin gene superfamily. Their results suggest that hemagglutinin binds to lymphocytes in a manner analogous to that of the CD2 surface protein with T lymphocytes. Much of the basic research has been published in Western journals.

Development of a vaccine against HBV is another priority of the institute. Closely following work in the West, institute scientists cloned and sequenced the surface antigen gene of a Chinese HBV strain, inserted it into a DHFR vector, and introduced it into CHO cells. After amplification by methotrexate selection, the cells were found to secrete 5 to 7.5 mgl of HBsAg per liter that was appropriately glycosylated and assembled into subviral particles. It is possible to culture the cells, and collect the media every 2 days, for up to 120 days. In animal tests and phase one human clinical trials, the genetically engineered vaccine is four times more potent as an antigen than is the current human plasma vaccine. The vaccine, which meets World Health Organization standards, is currently produced and purified in a small laboratory at the institute. The production process is similar to one developed by Genentech in the United States.

Production and genetic engineering of interferon is a long-standing project. Using standard *E. coli* methods, they have produced interferon which appears to be clinically useful against chronic cervical condylomas caused by human papillomaviruses. They have also produced hybrids between interferon and tumor necrosis factor, which they hope will target the interferon to tumor cells, and between interferon and the pre-S region of HBsAg, which they hope will direct it to liver cells. These experiments also address the basic question of which parts of the interferon molecule are responsible for its antiviral activity.

The Institute of Virology's work on viral replication is one of China's few examples of basic research that has garnered notice internationally. At the same

time, its work on vaccine development shows a sensitivity to practical needs as well as to the necessity of tailoring Western inventions to China's needs. In summary, this institute shows that it is possible to integrate basic and applied research in the context of China's new scientific policies.

Institute of Zoology (CAS)

The Beijing Institute of Zoology is a multidisciplinary research center with an emphasis on animal reproduction and classical fields such as taxonomy and ecology. Biotechnology-related research is carried out in the Laboratory of Genetic Engineering, which is supported by a Seventh 5-Year Plan grant, under the supervision of Shen Xiaozhou.

The genetic engineering group focuses on the biology and applications of growth hormones. Using techniques similar to those developed at Genentech and Integrated Genetics Corporations, they have generated a CHO cell line carrying a metallothionein-human growth hormone fusion gene and linked DHFR gene. After methotrexate amplification, the cell line produces and secretes high levels of human growth hormone (100 μg/106 cells/day) which will be used for the treatment of hereditary dwarfism. This group is also trying to create "superfish" by microinjection of a mouse metallothionein-bovine growth hormone fusion gene into freshly fertilized eggs of the common carp. Of 100 injected eggs that developed into fish, 30 contained the foreign gene, as shown by appropriately controlled Southern blots. Of these, 10 displayed rapid growth compared with control fish, and three of these that survived past 1 month are now being bred to test for germline inheritance. The creation of transgenic cows and sheep is also under way. In order to create appropriate vectors, student Yang Weimin has performed interesting and competent basic research on the organization of the metallothionein gene cluster in the cow.

Peking University

Peking University is China's most famous institution of higher learning both inside and outside the country. Many of China's leaders, including Mao Zedong, have at least passed through, if not been graduated from, this prestigious and historic university.

The Department of Biology at Peking University is staffed by a large faculty of 20 full professors, 40 associate professors, and more than 100 teaching assistants and lecturers. The rotating chair of the department is currently held by Gu Xiaocheng, an articulate spokesman for the role of universities in China's research program. The biology department has several subdivisions: botany (including plant physiology, plant genetics, and plant cell biology), zoology (including insect physiology and ecology), cell biology, genetics, biophysics, microbiology, biochemistry (the largest division), and a special teaching group to coordinate and teach courses for the 640 undergraduates in the department. There are 150 M.S.

students and 50 Ph.D. students. Undergraduates are admitted on the basis of a national test whose results are curved to fit the requirement for geographical quotas. The department also provides training for 90 premedical students from the Beijing Union Medical College. Of the 12 students who received Ph.D.'s in 1987, five stayed on as instructors and the rest either went abroad or took faculty positions at other universities.

The department is richly supported by some 6 million to 7 million *yuan* in research grants held in 1988. This represents a 150 percent increase over funding levels in 1987 and a more than 1,000 percent increase over levels in 1983. Of the current grants held, 11 are from the High Technology Program, 5 are from the Seventh 5-Year Plan, and 55 are from NSFC. Nearly 90 percent of the senior faculty hold research grants. The State Education Commission provided the construction costs for the department's new laboratory facility and supplies 300,000 *yuan* per year for teaching costs, but it provides no direct research support. The rather lavish equipment for the new laboratories was purchased with a loan from the World Bank. In addition, the Department of Biology houses a national open laboratory on protein engineering and has received funds for five national postdoctoral fellow positions. The department's prominence is evidenced by their winning more Natural Science Awards than any other department in China and by having an NSFC approval rating of 50 percent compared with 30 percent nationally.

It is making an active effort to attract returning students who have obtained their Ph.D.'s or other advanced training abroad. The most prominent example is Chen Zhangliang, who was a top student at Washington University, where he studied with Roger Beachy; he could have obtained a permanent position in the United States. Peking University offered Chen several inducements to return, including an associate professor position, a large laboratory, and, perhaps most importantly, the chance to go abroad on an annual basis. Chen is currently supported by a 1.5 million *yuan* grant from the High Technology Program and a $30,000 grant from the Rockefeller Foundation. Chen's research focuses on the genetic engineering of resistance to viral diseases in plants, especially rice.

Several other research projects at Peking University relate directly or indirectly to biotechnology. Zhang Longxiang's group focuses on comparative protein structure analyses and has determined the sequences of lactate dehydrogenase from the panda bear and C-reactive protein from a Chinese clam. Dr. Li's group is attempting to use somatic seeds to study somaclonal variation in plants and to propagate Chinese medicinal herbs. The protein structure groups of Gu Xiaocheng and colleagues are studying structure-function relationships of insulin, trypsin, and urokinase with an eye to eventual protein engineering.

Although the department is one of the most dynamic in China, it also suffers from a high degree of inbreeding and from a top-heavy staff structure (features somewhat common in Chinese academic institutions). Whether the department's research accomplishments live up to its prestige and high funding priority or not will depend critically on the continuing recruitment of top-notch young scientists.

SHANGHAI

Fudan University

Biotechnology research at Fudan University is predominately carried out at the Institute of Genetics, an academic department of the School of Life Sciences that performs research and graduate education but is not responsible for undergraduate students. The leader of the university's Institute of Genetics and of the School of Life Sciences is C.C. Tan, one of China's most highly regarded scientists and educators. Tan, who was a student of Thomas H. Morgan, received his Ph.D. from the California Institute of Technology in 1936 and returned to China in 1937. He was one of the discoverers of transposable elements in ladybugs; this discovery, together with a similar finding in corn, has allowed one of the basic frameworks for the development of genetic engineering to be formed. In China, Tan and his students continued their work on genetics, despite the complete disregard of this subject when Chinese life sciences were guided by Lysenkoism. Perhaps his greatest contribution was his role in influencing Chairman Mao to turn China from Lysenkoism. Agricultural production in China increased enormously because of his contribution in preserving classical genetics. Tan has received many awards and honorary degrees and is a foreign associate of the U.S. National Academy of Sciences.

Research at the university's Institute of Genetics is carried out by 120 staff members and 93 graduate students. Total research support is 8 million *yuan*, making this, on a per capita basis, the most prosperous of all such research centers in China. These funds are derived primarily from 6.2 million *yuan* in High Technology Program grants, 960,000 *yuan* in Seventh 5-Year Plan grants, and 156,000 *yuan* in NSFC grants. The institute has also actively sought research support from abroad and receives approximately $100,000 from the Rockefeller Foundation and $70,000 from Interferon Sciences. In addition, the Fudan Foundation, an educational and lobbying organization based in Washington, D.C., has raised over $5 million and aims at raising another $10 million in order to establish an American studies center and the Thomas H. Morgan Science Center at Fudan University. It is hoped that the Morgan center, which is still in the initial planning stages, will provide an opportunity for Chinese scientists and students to cooperate with American scientists in a high-quality educational and research program.

The section on microbial molecular genetics performs both basic and applied research on bacteria. The section chief, Sheng Zujia, received his Ph.D. from Columbia University and has a long and distinguished career in microbial genetics in China. Research support, mostly from the Seventh 5-Year Plan, amounts to 350,000 *yuan*. This section's basic research focuses on DNA replication in *E. coli*. Strains carrying a mutation in the *dnaA* gene, which codes for a replicase, are incapable of growing. This defe ct can be overcome by integration of a new

replication origin from a plasmid or F-factor. Mao Yumin, an instructor who recently received his Ph.D. in this section, has made the surprising discovery that such integrative suppression is dependent on *recA*, a gene involved in DNA recombination. The effect of *recA* is highly dependent upon the integration site of the new origin in a manner that suggests that the recombinase allows DNA replication to proceed past a termination site. Control experiments show that this effect is dependent on the recombination activity of *recA*, not on SOS repair, and that it is independent of the type of replication origin. Current research focuses on proving the terminator bypass model by direct measurements of DNA synthesis and accumulation.

The second major focus of the section on microbial genetics is the molecular biology of thermophilic bacteria, in particular, *Bacillus stearothermophilus*, which is capable of normal growth at 55°C. As might be expected, many of the enzymes from such thermophilic organisms are very stable at high temperatures, a useful property for industrial fermentation and biocatalysis processes. The gene for a thermostable α-amylase, potentially useful for direct fermentations from starch, has been cloned, partially characterized, and expressed in several vector-host systems. The cloning and preliminary characterization of heat-stable glucose phosphate isomerase, DNA polymerase, proteases, and lipases are also under way. At the same time, this group is carrying out more basic work on gene expression and genetic exchange in *B. stearothermophilus* in order to improve host-vector systems and facilitate genetic analyses. Promoter DNA sequences, which are required for high-level gene expression, have been cloned both from total DNA and from the glucophosphate isomerase gene. Vectors have been constructed using naturally occurring cryptic *B. stearothermophilus* plasmids and mutated, thermostable antibiotic resistance genes from *E. coli* . Two thermostable restriction enzymes, which may be important for genetic exchange, have been purified and characterized in terms of cleavage specificity. During the course of vector construction it was noted that a plasmid that expressed a foreign gene with high efficiency had picked up an extra piece of DNA from the chromosome. Subsequent studies showed that the extra DNA is one member of a family of transposons, a class of DNA element that is capable of excision and integration into the chromosome and extrachromosomal replicons. This transposon, which has now been marked with an antibiotic resistance gene, is already proving to be a highly useful genetic tool. Clearly, one goal of the *B. stearothermophilus* work, and the reason for its 300,000-*yuan* funding from the High Technology Program, is to produce thermostable enzymes for industry. But along the way there is the possibility of doing some very interesting basic work on protein structure-function relationships, the regulation of transcription, and mechanisms of genetic exchange in this unusual class of organism.

The section of human and medical genetics consists of three groups under the leadership of Xue Jinglun, Zhao Shouyuan, and Chai Jianhua. Research support totals 2.7 million *yuan*, mostly from High Technology Program grants. The group

of Xue Jinglun, who trained at Roswell Park Memorial Institute in Buffalo, New York, focuses on human gene therapy with the ultimate aim of curing inherited diseases by inserting a normal gene into the somatic cells of an affected individual. As a model system, they are studying hemophilia B, a blood-clotting disease caused by a mutation in the X-chromosome-linked gene for factor IX. They have transfected the factor IX gene, obtained from a group in England, into CHO cells and shown that it is expressed at the protein level. They have also, in collaboration with the Shanghai Second Medical University, established skin fibroblast cell lines from patients with hemophilia A and B. While good progress has been made, the ultimate success of this ambitious project will require many additional advances in delivery systems and understanding of factor IX gene regulation.

The group of Zhao Shouyuan, who has been a visiting scientist at Yale University, concentrates on various problems relating to human cancer. Stomach cancer is much more prevalent in China than in other parts of the world. Zhao's group showed that stomach cancer cells express the Ha-*ras* oncogene and that DNA from these cells can neoplastically transform normal cells. However, they did not prove that Ha-*ras* is the active oncogene by DNA cloning; this was accomplished at another institute in Beijing. A second project is to characterize the regulation of and to overproduce lymphokines and cytokines, such as interleukin-2 and tumor necrosis factor, that may be useful in tumor therapy. Together with Li Changben, who trained for 2 years at the University of Maryland and 2 years at Yale, they cloned and sequenced the human interleukin-2 gene that had previously been described by Genentech. As a basic research topic, they are attempting to characterize the regulatory DNA sequences that control lymphocyte-specific expression of this gene by deletion analyses. In more applied work, they have successfully produced interleukin-2 in both bacteria and mammalian cells by genetic engineering. However, major improvements in yield and purification will be required to prepare sufficient amounts for human testing.

The group of Chai Jianhua, who recently returned from 4 years at the European Molecular Biology Laboratory and Max Planck Institute in West Germany, is just starting an ambitious project to prepare a restriction and genetic map of the entire human X-chromosome. Their mapping will start at two known X-chromosome loci, those for hemophilia A and muscular dystrophy, for which probes have been obtained from other groups. Long-range restriction maps will be determined by an ingenuous "jumping clone" method whereby overlapping segments of DNA can be ordered without detailed characterization of the intervening sequences. The construction of the "jumping" and "linking" clone libraries from DNA of a multi-X-chromosome cell line is under way. The methodologies being developed in Chai's laboratory are potentially applicable to any chromosomal or organelle DNA.

The institute's plant genetics section is divided into two groups working on the interrelated topics of cellular and molecular biology. The section is supported by 830,000 *yuan* from the Chinese government and $180,000 from the Rockefeller

Foundation and Interferon Sciences. The cellular biology group, headed by Ge Koulin, focuses on methods for plant regeneration and gene transfer. They were among the first to succeed in protoplast regeneration of *Phaseolus* species, the common green bean that is an important food crop in China, and together with the sponsor, Interferon Sciences, have applied for a U.S. patent on this process. In common with many other groups in China and abroad, they have also regenerated rice from protoplasts of the *Japonica* but not the *Indica* strain. They are now in the process of DNA transformation of *Phaseolus* and other species with marker genes such as those for kanamycin resistance. Eventually, they hope to use this technique to improve food crops, but they have not yet decided the exact genes that they will use.

The molecular biology group, under Wang Xunming, studies the structure and expression of plant chromosomal and organellar genes. In the past, they compared chloroplast tRNA genes of *Vicia fabia* and *Brassica napus* and showed that they have evolved at different rates. They also detected differences in the mitochondrial DNA of sterile male and maintainer rice strains, but the molecular basis of such variation was not determined. A current project is to obtain tissue-specific promoters to be used eventually for transgenic plant construction.

The institute's genetic engineering section comprises three research groups led by Wang Qisong, Li Yuyang, and Zheng Zhaoxin. The general aim of the section, which is strongly supported by 3.3 million *yuan* in High Technology Program grants, is to produce useful proteins by recombinant DNA techniques. The group of Wang Qisong, who trained in Canada, focuses on methods for the synthesis and overexpression of genes encoding peptides. Using an automated DNA synthesizer, they have synthesized and assembled over 20 different genes including those for calcitonin gene-related peptide, which is potentially useful for control of hypertension; atrial natriuretic factor, which is also involved in blood pressure control; a foot-and-mouth virus coat protein peptide (a potential vaccine); and α-interferon and interleukin-2 (potential anticancer drugs). Most of these genes have now been expressed either in yeast or bacteria. A second project is to develop new methods for protein engineering through multiple, simultaneous point mutagenesis. This approach is being used to make novel hybrids between two drugs useful against myocardial infarction, namely, tissue plasminogen activator and urokinase. Finally, Wang has invented a clever new method for synthesizing mRNA from a synthetic DNA template. This RNA can be translated in vitro, thus providing a rapid assay method for genetically engineered enzymes and hormones. While many institutes in China now have DNA synthesizers, few, if any, are used as frequently and productively as they are in this laboratory.

Li Yuyang's group is trying to use yeast to express various useful proteins such as HBsAg, atrial natriuretic factor, and calcitonin gene-related peptide. They employ strategies previously worked out in Western biotechnology companies, i.e., high-copy-number vectors, strong promoter sequences, and secretion signal leader sequences. A more novel strategy, which will be tested by a returning

postdoctoral scholar, will be the use of the naturally high secreter strain *Kluyveromyces lactis*.

Zheng Zhaoxin and associates work on genetic engineering in bacteria. They are particularly interested in the use of *Corynebacterium glutamicum* , an organism that is widely used for amino acid production and that possesses several advantages over *E. coli* for fermentation purposes, i.e., fast growth, safety, lack of proteases, and good secretion. They have constructed a *Corynebacterium* vector and established a transformation system; attempts to ferment a useful product are now in progress. This work is sufficiently novel to have attracted the attention of several European groups. A second project is to produce the calcitonin generelated peptide hormone which, in a collaboration with Peking Medical University, has been shown to reduce blood pressure within 90 minutes of administration. A major new effort revolves around *Schistosoma japonicum*, a liver parasite that is carried by rice paddy snails and affects three million people in China. Following Australian scientists' work on the Filipino parasite strain, two major antigen genes have been cloned and characterized. Overproduction of these proteins, and of corresponding monoclonal antibodies, should allow development of a reliable diagnosis method.

Institute of Biochemistry (CAS)

The Shanghai Institute of Biochemistry, established in 1958, is one of China's best known research centers. It was one of the only research institutes to remain active throughout the Cultural Revolution, during which important work on the synthesis of insulin and alanine tRNA was performed, and in the past 20 years, it has been at the forefront of bringing molecular biology to China.

The recent history of the institute provides insight into the effects of China's new scientific policies on how research is conducted and supported at a major center. In 1984, CAS provided for all research costs, whereas now it is responsible for only about 10 percent. At that time, the institute was organized into several large divisions, each with about 40 people, and the pathway for funding was from institute director to division head to individual researcher. Now the institute has been reorganized into 37 much smaller groups, each responsible for obtaining its own funding. While the new system allows greater autonomy for individual scientists, it lacks the cohesiveness that made major projects such as the synthesis of insulin possible. Previously, the institute took great pride in its emphasis on basic research, but because of the new funding priorities, this stance has been abandoned.

The decentralization and diversification of the institute's administration is evidenced by the opening, in 1988, of the Shanghai Molecular Biology Laboratory as a center for scientific training and international cooperation. Although this laboratory has its own director and publishes its own annual report, it is housed in

the same building as the Shanghai Institute of Biochemistry and numbers among its members Lin Qishui, director of the institute. Plans are also afloat for two new national laboratories focusing on eukaryotic gene regulation and neuropeptides. One clear benefit of the new policies is increased attention to international cooperation. In 1987, the institute hosted over 300 foreign scientists and conducted numerous symposia, workshops, and minicourses (including two organized jointly by CSCPRC and CAS).

The institute has a staff of 646, including 396 research and technical workers. It is located in the CAS Yueyang Road complex, in the old French quarter, adjacent to the Shanghai Institutes of Cell Biology, Physiology, and Materia Medica. Laboratories are located in a large, old, but well-equipped main building and a new wing devoted to molecular genetics.

The group of Li Zaiping, who is well known in the West for his pioneering work on bringing molecular biology to China and promoting international cooperation, works on various basic and practical aspects of eukaryotic gene regulation and transcription. They have isolated and sequenced the 5.8S ribosomal RNA gene of the silkworm, *Attacus ricini*, and shown that the chromosomal copy contains a DNase-hypersensitive site under active transcription conditions. Interestingly, this site corresponds to a nuclease S1-sensitive site in the cloned, supercoiled DNA, perhaps indicating an underlying irregularity in the structure of the DNA. More recently, Li and colleagues have been studying the transcription of the HBV genome. They are using antisense RNA and antibodies to determine whether the "X-gene" is an autoregulator and are also searching for *trans*-acting factors encoded by the nuclear genome. A more applied project relating to HBV is the construction of vaccinia virus recombinants to be used as vaccines. This is similar to work in the United States and at the CAPM Institute of Virology. A vaccinia virus recombinant expressing HBsAg has been constructed and turned over to the Beijing Institute of Biological Products for scale-up. Recombinants expressing the pre-S1 and pre-S2 antigens, which contain binding sites for serum albumin, have also been constructed and will be compared with the shorter S-antigen for effectiveness. Yeast and *E. coli* have been used to express S- and X-fusion proteins as potential immunological screening reagents. In addition, fusion genes between the surface antigens of hepatitis B virus and hepatitis A virus are being constructed with the idea of making a multivalent hepatitis vaccine.

Li's group is also pursuing several cancer-related projects. Several tumor growth factor fusion peptides have been expressed and shown, in agreement with results from the United States, to have increased antiviral activity. In collaboration with Japanese scientists, a study of antioncogenes has been initiated. Several different classes of those genes, each capable of suppressing transformation by a different set of oncogenes, have been cloned. The detailed characterization of these genes could yield important insights into the biochemical pathways of neoplastic transformation.

One of the newer and most active staff members at the Shanghai Institute of Biochemistry is Hong Guafan, whose research focuses on the molecular biology of nitrogen fixation. Nodule formation is an essential prerequisite for symbiotic nitrogen fixation. In collaboration with workers at the John Innes Institute in England, Hong has identified a series of genes expressed by *Rhizobium leguminosarum* in peas. One of these, the *nodD* gene, was sequenced and expressed in *E. coli*, and it was shown that this protein specifically binds to the *nod* cluster intragenic region. While the initial phases of this work were done in England, students in Hong's lab in Shanghai are now actively pursuing the question of how certain organic compounds switch *nodD* from a repressor to an activator. In research initiated in Shanghai, Hong's group has also started to characterize the *nod* genes of fast-growing *Rhizobium* strains isolated from nearby fields. Following up on work started in Fred Sanger's laboratory at the Medical Research Council in Cambridge, England, Hong has also continued to improve DNA-sequencing methods by the use of a heat-stable DNA polymerase which can read through hairpin structures in the DNA at high temperatures. Several U.S. biotechnology reagent companies have expressed interest in this method.

In order to entice Hong to return to Shanghai, CAS offered several incentives: a full professorship, an independent research group, a good-sized laboratory, research support, and the opportunity to go abroad annually to perform collaborative research. These were wise investments because Hong's group is certainly one of the strongest in China. Regular collaboration with Western scientists has been especially important, particularly during the early years when Hong was still setting up his laboratory and training students. Now workers in this laboratory have the opportunity, rare in China, to do original research on a current topic rather than simply repeat experiments done in the West. In a sense, the John Innes Institute provided the critical mass to establish an active research group in China.

Structure-function relationships of insulin have been a major focus of the Shanghai Institute of Biochemistry since 1965. Using modified synthetic methods, several derivatives of insulin have been prepared and evaluated for biological activity and receptor binding. The results show that Phe26 plays a critical role in the structure of the hormone. The insulin A-chain has been expressed in *E. coli*, and several site-directed mutants are being worked up. In addition, various short peptide segments of insulin have been chemically synthesized and then tested for biological activities or the ability to compete for insulin inhibitors (i.e., inhibitor-binding sites). The conformation of these fragments has been probed by nuclear magnetic resonance methods, including nuclear Overhauser effect, with spinlattice relaxation time measurements. Many of the insulin experiments at the Shanghai Institute of Biochemistry overlap with those at the Beijing Institute of Biophysics; perhaps more rapid progress could be made by combining the Shanghai institute's expertise in synthesis and nuclear magnetic resonance methods with the Beijing institute's expertise in crystallography.

Institute of Cell Biology (CAS)

The Shanghai Institute of Cell Biology, founded in 1950 as the Institute of Experimental Biology, employs 370 personnel, including 50 senior researchers, 60 research associates, and 50 senior technicians. The institute carries out biotechnology research in several areas such as chromosome and chromatin biology, production of immunotoxins, and vaccine development using transgenic animals. This institute was one of the first to develop monoclonal antibody technology in China, and many of the basic and applied research projects use this methodology. The institute is also responsible for maintaining China's mammalian cell line bank.

The chromosome biology group, in collaboration with colleagues at the Beijing Institute of Biophysics, is attempting to develop chromosome-like vectors for plants, especially rice. Using a novel antibody approach, they are trying to clone chromosomal DNA fragments containing centromere and telomere sequences. First, they prepared a panel of monoclonal antibodies directed against nuclear structures. Second, they determined the specificity of the antibodies by immunostaining nuclei; they also tested the function of these structures by inhibition studies. Lastly, in their current work, they try to pull out specific DNA fragments associated with these antigens. While the ultimate aim of the work is applied, it also involves a healthy dose of basic research on the structure and function of important chromosome components.

A group under the leadership of L.C. Sze is attempting to use whole animals as bioreactors to produce valuable proteins such as HBsAg. In collaboration with Jiangsu Province Agricultural College, they injected a metallothionein-HBsAg hybrid gene into rabbit embryos and obtained 20 percent transgenic animals of which 10 percent produced detectable surface antigen in the blood. This work, supported by 1 million *yuan* from the High Technology Program and 1 million *yuan* from the Seventh 5-Year Plan, is being turned over to a biological products institute for further development.

Consistent with the institute's traditional emphasis on immunology, several groups are using antibodies to detect and eventually fight cancer. An immunological kit for detecting α-fetoprotein, an early indicator for liver cancer, has been developed and marketed. Several monoclonal antibodies against hepatoma cell surface antigens have been isolated, and antibody-ricin immunotoxins have been produced in collaboration with the Shanghai Institute of Biochemistry. While these appear somewhat specific in vitro, their potential applications in vivo will require much further testing. Several members of the Shanghai Institute of Cell Biology attended the joint CSCPRC-CAS minicourse on immunotoxins in 1988, which, hopefully, will help them to design the appropriate experiments.

Institute of Materia Medica (CAS)

The Shanghai Institute of Materia Medica, founded in 1932, searches for new, physiologically active compounds and studies their structure-function relationships.

The institute has over 400 staff, including 64 at senior levels. While members of the institute's staff are best known for their research on traditional Chinese medicines, they have recently begun to use the techniques of genetic engineering and molecular biology to produce and characterize new drugs.

Antibiotic production and improvement is the main focus of a group led by Yang Shenti. They cloned, and overproduced in *E. coli*, penicillin G-acylase for use in the semisynthetic production of penicillin. The immobilized enzyme is now used commercially by several pharmaceutical companies in China. Attempts to improve the enzyme by genetic engineering are under way, but in the absence of basic information about the structure of the enzyme, it is unclear what residues to change. This group has also developed a microbial technique for producing threonine, a precursor for synthesis of the moxalactam series of antibiotics. In related research, this group is carrying out structural determinations on certain β-lactamase inhibitors which could potentially allow the use of penicillin and other β-lactams against normally resistant bacteria.

A second project combining classical and modern approaches is a search for new neuropeptides from such sources as amphibian skin and gut, human brain, and certain tumor cells. Several new peptides have been isolated, sequenced, synthesized, and tested for biological activities, including analgesic effects. Attempts to produce some of these peptides by genetic engineering are just beginning.

Several projects at the Shanghai Institute of Materia Medica concern the mechanism of action of anticancer drugs derived from Chinese medicinal herbs such as *Camptotheca acuminata*, a traditional Chinese treatment for leukemia. It was found that hydroxycamptothecin, the active ingredient, inhibits the expression of the *ras* and *myc* oncogenes while having little effect on *fos* or *erb* oncogenes. However, it is far from clear that the effect is either specific or the primary mode of action. The institute also produces the active components of several potential anticancer drugs by various methods including plant tissue culture; some of these are being tested in clinical trials in the United States in collaboration with the National Cancer Institute. Eventually, it is hoped that mutant cell lines that overproduce these drugs will be isolated.

Institute of Plant Physiology (CAS)

The Shanghai Institute of Plant Physiology is one of the best known institutes in CAS and has made many significant contributions in the field of plant physiology. Therefore, it was selected to house one of among a few plant molecular biology key laboratories. Designated as the Plant Biotechnology Laboratory, it was established in 1986 and occupies 2,500 square meters of space in the institute's new building. The Plant Biotechnology Laboratory was planned to have the capacity to house 60 scientists: 20 from Shanghai and the rest from elsewhere around the nation. Currently, there are several research groups, although the number can vary as the number and interests of visiting scientists fluctuate.

The disease resistance and genetic engineering group, like other groups working

on genetic engineering for plant disease resistance, focuses on plant viruses. However, this group is attempting to use a different coat protein gene from TMV and a different experimental strategy than the other groups. The results are not yet available for evaluation. However, they anticipate that deletion of the bases coding for polymerase can suppress the synthesis of the TMV coat protein subgenomic RNA. If this is successful, TMV infection can be prevented instead of delayed. In addition, this group also initiated a project to attack diseases caused by fungi. They plan to isolate the components from a rice cultivar sensitive to *Pyricularia oryzae*. Currently, they have isolated a protein from *Gastrodia elata* that may contribute to the resistance of such fungal disease. The leader of this group is C. Wang, who joined the institute very recently. He has received substantial financial support, but the lack of trained and experienced workers is proving to be a major obstacle.

The primary goal of the regulation of gene expression group, led by the well-known professor, M.M. Hong, is to study the organization, expression, and regulation of the waxy gene from rice. In rice (*Oryza sativa*), the waxy gene is located on the number one chromosome. The starch granule-bound uridine diphosphate-glucose starch transferase is the catalyst for the synthesis of α-amylase in the pollen grain and endosperm. Since *α*-amylase is synthesized only in these tissues, the expression of the waxy gene is tissue and developmental stage specific. Therefore, this group is working on the cloning, isolation, and sequence of this gene. It is intended to study the coding as well as the regulatory regions of this gene. It is hoped that the *cis*-acting elements as well as the *trans*-acting factors can be identified. This is a very important step in the attempt to improve the quality of rice. At the same time, this group is also interested in studying the cause of differences in *α*-amylase content between *Japonica* and *Indica* strains of rice. This may be related to the regulation of expression of the waxy gene. Furthermore, the lack of α-amylase in some cultivars of rice may reflect the lack of expression of the waxy gene. This group has constructed four rice genomic libraries since 1987. Using the waxy gene probe provided by Nina Fedoroff at Johns Hopkins University, they have proceeded expeditiously in identifying the waxy genes.

The cell and tissue culture group reflects the institute's traditional strength in this area. Currently, there are four areas of investigation under the leadership of C.H. Xu, the deputy director of the institute:

1. *In vitro clonal propagation:* Using in vitro techniques, Xu's group has regenerated over 70 plant species. Many of them are important crop species including rice, wheat, maize, sorghum, soybean, cabbage, and rape. It should be noted that the orchid derived from the in vitro clonal method flowers in a few months compared with 5 years for orchids derived from seedlings.
2. *Protoplast fusion and cell hybridization:* They have obtained over 10 plant

species derived from fused protoplasts. Among them are some important crop species such as rice, soybean, and tobacco.

3. *Mutation induction:* They have focused on the selection of mutants with a high concentration of essential amino acids or with new stress resistances. Tobacco plants which can tolerate 2 percent sodium chloride and soybean cells resistant to glyphosate (the active ingredient in Roundup, an herbicide made by Monsanto) have been selected and regenerated into plants.
4. *Cell transformation:* Using Ti plasmid and reporter genes, they have obtained a series of transformed plants, but no novel contributions are forthcoming.

The cotton disease resistance group, which is led by G.Y. Zhou of the Shanghai Institute of Biochemistry, will be administratively, if not physically, transferred to be part of this open laboratory. For many years, Zhou's group has worked on a technique for the introduction of foreign DNA from disease-resistant cotton species into disease-sensitive cotton species. They take advantage of the pollen tube pathway and inject foreign DNA into the embryonic sac to transform the zygote. This approach has encountered technological difficulties as well as collegial criticisms. In collaboration with the Jiangsu Province Institute of Industrial Crops, they have obtained some cotton hybrids expressing disease-resistant traits. In addition, there is a group working on *B. thuringiensis* toxin and a group working on storage proteins.

GUANGZHOU

Guangdong Agricultural Academy of Sciences

In 1987, the Center for Agricultural Biotechnology was established in the Guangdong Agricultural Academy of Sciences. It is mainly an organization that coordinates existing activities loosely related to the broad definition of biotechnology. Currently, there are 54 staff members, 34 of whom are scientists at different levels; 1 senior, 6 associate, and 13 assistant staff scientists; and 14 technicians. According to their plan, the center will have four divisions: gene, cell, enzyme, and fermentation biotechnology. To do this, additional scientists have to be recruited, which will not be easy.

Most research activities at the present time are centered around tissue culture work for the purpose of propagation, e.g., banana and pineapple. Surprisingly, great effort has been directed to the breeding of seedless watermelon, which was successfully achieved almost 30 years ago in Taiwan.

Obviously, this center is in its infancy, and it will be a long time before it can move into molecular aspects of agricultural research. However, this center is an excellent resource for improving rice crops: they are studying a unique strain of

black rice that is claimed to be more nutritious than the ordinary strain and to possess other values.

South China Agricultural University

South China Agricultural University attained its university status in 1984. Currently, there are 10 academic departments including most of the classical agricultural disciplines such as agronomy, horticulture, animal husbandry, veterinary medicine, agricultural engineering, and forestry. Among the 820 faculty members, there are 240 professors and associate professors, 480 instructors, and 100 teaching assistants. The undergraduate population is 3,000. The university also offers graduate degrees at both M.S. and Ph.D. levels in highly selected areas. In addition, there are 36 foreign students, mostly from other developing countries.

The current administration is interested in promoting and strengthening research activities within the university. Under the present organization, there are 20 research units, one of which is genetic engineering. The others include crop genetics and postharvest physiology. The university has a very large collection of germplasm, including over 7,000 cultivars of rice.

Currently, there are six faculty members in the research unit of genetic engineering: two associate professors, one senior research staff, and three research associates who focus on two areas. In animal sciences, the emphasis is on growth hormone and antibacterial peptides. In the plant sciences, rice and rape diseases are the major interest. This unit is currently supported by grants from the Seventh 5-Year Plan, NSFC, and donations from various sources in Hong Kong (HK$500,000; HK$7.8 = US$1).

South China Institute of Botany (CAS)

The South China Institute of Botany, established in 1929, was originally known as the Institute of Agriculture and Forestry and was affiliated with Sun Yatsen University. It was reorganized in 1954 and transferred to CAS, where it was expanded from one taxonomy department to include other botanical disciplines. Currently, it has six departments and a botanic garden and is staffed with 568 workers at different levels. Among them are 334 scientists or technicians, 10 professors, 65 associate professors, 3 senior scientists, 10 senior engineers, and 25 M.S. graduate students. The six departments and their respective focuses are listed below:

1. *Taxonomy:* classification and phylogenetic studies of many plant families aided by chemotaxonomy, cytology, anatomy, and polynology are actively carried out.
2. *Phytomorphology:* includes anatomical studies on weed structure, embryology, and pollen morphology.

3. *Phytochemistry:* surveys the chemical compounds extracted from a large number of plants for useful or valuable substances such as the effective antitumor compounds Harringtonine and Homo-Harringtonine.
4. *Ecology:* major studies include ecosystem, pollution ecology, and the use of artificial plant association for the regeneration of Chinese plant species.
5. *Physiology:* investigates stress adaptation, seed and postharvest physiology, and tissue culture and protoplast fusion.
6. *Genetics:* the primary emphasis of this department is on biotechnology to study heterasis and cytoplasmic male sterility. This department invested heavily in rice breeding for high-quality, high-yield, and disease resistant crops.

The institute's biotechnology program is carried out at the interdepartmental level. The scientists involved are primarily from three units: genetics, physiology, and botanic garden. This biotechnology group is moderately supported by grants from the High Technology Program, Seventh 5-Year Plan, and NSFC at about 200,000 *yuan* per year, plus $10,000 from the Rockefeller Foundation to support research on rice. Like so many other groups in China, they started in the early 1960s with cell and tissue culture methods. In fact, most activities related to biotechnology are centered around the production of plantlets by tissue cultivars for commercial purposes. The micropropagation group is large and consists of over 40 people, including two professors and six associate professors. In the protoplast fusion groups, they have produced eggplant and potato hybrids that had already been achieved in the West. Obviously, there is need for more molecular biologists in this program. One scientist, Huang Yuwen, just returned from abroad. She is in the process of setting up a molecular biology laboratory to work on rice cytoplasmic male sterility. But progress is extremely slow, a frustration faced by almost everyone who returns from overseas.

Zhongshan (Sun Yatsen) University

Zhongshan University was established in 1952 by combining certain academic departments in basic and social sciences from two well-established universities (Ling Nan and Sun Yatsen). Currently, it has over 30 academic departments and institutes, including basic science departments in biology, chemistry, physics, mathematics, computer sciences, electronics, mechanics, geography, and geology. The student population has just reached the 9,000 mark. In addition, there are over 100 graduate students, 10 percent of whom are Ph.D. students. The faculty-to-student ratio at Zhongshan University is roughly 1:7; there are about 120 professors, 350 associate professors, 700 instructors, and 200 teaching assistants.

In 1986, the Center for Biotechnology was established at Zhongshan University by organizing into a separate administrative unit existing faculty members whose research interests were related to biotechnology. It was initially funded by 1.2 million *yuan* from SEDC and 200,000 *yuan* from Guangdong Province. The

director of the center is Li Baojian, a plant molecular biologist who has recently returned from Cornell University.

The plant genetic engineering group is the largest in the center. There are two professors, seven associate professors, five instructors, and 24 research associates at the M.S. level being supported by 120,000 *yuan* from the High Technology Program and the Seventh 5-Year Plan. Since this group has been actively working on rice, a $30,000 grant from the Rockefeller Foundation has proved to be extremely helpful. The research subjects of this group are rather diffuse and include gene transformation, cell fusion, artificial seed, and micropropagation. Formation of a highly focused program should be the top priority. One area of considerable interest is the attempt to locate and to clone the gene that is believed to play an important role in the wide compatibility of rice cultivars. It was good news to learn that they were successful in developing artificial seeds, but disappointing to learn that they failed to keep them.

Another notable group in the center is the microbial genetic engineering section. It is staffed by two full professors, three instructors, three research associates, and seven graduate students. This group's support is primarily provided by the Seventh 5-Year Plan, NSFC, and Guangdong Province at 120,000 *yuan* annually. Its main focus is on the construction of vectors from *Bacillus* species, cloning of industrially useful genes, and the isolation of heavy metal resistance plasmids. One unique project, which is just under way, deserves special mention. They have isolated a plasmid from bacteria found in the gut of an insect that grows on rice. This plasmid may have the potential to be used as a biological control agent because it kills some pathogens when it is transferred into *E. coli*.

This center is a very well-coordinated, multidisciplinary organization that includes biologists, chemists, physicists, and engineers. The petrochemical engineering group is very active, and they use immobilized enzymes for scaled up production. This is a new and energetic group that has the potential to advance rapidly.

TIANJIN

Nankai University

Nankai University, located in the major port city of Tianjin, is one of China's national key universities. Biotechnology research is carried out in the Department of Biology and the Institute of Molecular Biology. The Department of Biology, founded in 1971, is staffed by 170 people, including nine full professors and 42 associate professors. There are 450 undergraduate students, 66 M.S. candidates and four Ph.D. students. Total research funding is approximately 550,000 *yuan* per year, mostly from Seventh 5-Year Plan grants. SEDC pays staff salaries and 20,000 *yuan* for each Ph.D. student's research costs but provides no direct research grants. The Department of Biology is divided into seven groups focusing

on molecular biology, biochemistry, microbiology, genetics, enzymology, plant physiology, and marine biology. The department publishes approximately 30 articles a year, all in Chinese.

The university's Institute of Molecular Biology was founded in 1983 to provide a center for advanced biotechnology research at Nankai. There are 50 staff members, including nine full professors, together with 75 M.S. students and 21 Ph.D. students. The institute is supported by 400,000 *yuan* per year from three High Technology Program grants, nine Seventh 5-Year Plan grants, and several awards from industry and from Tianjin City. Fifteen of the institute's Ph.D. graduates have been sent abroad for postdoctoral training, and all of them apparently have returned. The institute is housed in its own well-equipped 4,500-squaremeter building.

Much of the biotechnology research focuses on microbiology with potential industrial and agricultural applications. A gene for heat-stable *α*-amylase has been cloned from a thermophilic bacterium. A naphthalene-degrading plasmid from *Pseudomonas aeruginosa* has been transferred to *E. coli* . Toxins from *Bacillus sphericus* (specific for mosquitoes) and *B. thuringiensis* (specific for cabbage worms) have been characterized, and attempts at gene cloning and transfer are under way.

While lacking the vitality of Beijing or Fudan Universities, it is still regarded as one of China's best educational institutions and attracts top-notch students. (It has also built a strong reputation in mathematical sciences, largely through the efforts of the famous Chinese-American mathematician, S.S. Chern.) The fact that biological research has gone from essentially no support to nearly 1 million *yuan* per year, all within 5 years, speaks to the influence of the new granting policies throughout China's educational system. However, judging from the several projects reviewed during a brief visit, it does not appear that competition for this funding has lent any special urgency or inventiveness to the research process itself.

9

Cooperation with the United States

Since 1978, Chinese scientific leaders and administrators have increasingly focused on cooperation with developed countries, particularly the United States, as a means to raise China's modest level of research and development in biotechnology. Contacts within developed countries occur through a variety of channels, including government agencies, private foundations, educational institutions, and commercial concerns. Major activities include information and technology transfer, support of Chinese research through grants and contracts, joint research projects, joint commercial ventures, training programs in China, and perhaps most important, study abroad. In China, cooperative activities are coordinated by active international cooperation departments within CAS, NSFC, SSTC, SEDC, and the Ministries of Agriculture and of Public Health. The sections below, while not all-inclusive, indicate the range of Sino-American cooperative activities.

GOVERNMENT-SUPPORTED PROGRAMS

The CSCPRC, sponsored by NAS and with financial support from NSF, and CAS have organized a joint 3-year program (1987-1989) of minicourses and a symposium aimed at introducing Chinese scientists to the new frontiers of basic biological and biotechnology research. To date, three combined laboratory and lecture minicourses have been held at the Shanghai Institute of Biochemistry and one at the Beijing Institute of Microbiology. The first, organized by Robert Horvitz (Massachusetts Institute of Technology), focused on the genetics and molecular biology of *Caenorhabditis elegans*. During the past decade, this

simple nematode has proven to be an exceptionally useful experimental organism for fundamental studies of early development, behavior, and neurobiology. The main purpose of the course was to expose young Chinese scientists to the genetic manipulations that make this organism well suited for answering basic biological questions. The course was the first introduction to this important organism for most of the attending Chinese students and researchers. The second course, organized by Dean H. Hamer (National Institutes of Health [NIH]), focused on gene cloning and expression in yeast and mammalian cells. The laboratory portion of the workshop offered instruction in several techniques, such as cDNA cloning and site-directed mutagenesis, which have wide applicability in many areas of biotechnology. Perhaps more importantly, the lecture portion focused on the most recent advances in understanding the regulation of eukaryotic gene transcription during environmental adaptation and development. The third course, organized by Mike Bjorn (NeoRx Corporation), focused on the more applied topic of immunotoxins. Laboratory exercises demonstrated state-of-the-art methods for antibody and toxin purification, chemical coupling, and immunotoxin delivery. Lectures ranged from introductory material on basic immunology to the most recent results of animal and clinical trials. The fourth course, organized by Thomas Osborn (University of Wisconsin), was on plant molecular genetics and was held at the Beijing Institute of Microbiology in May 1989.

The above minicourses lasted for 2 to 3 weeks and were taught by up to five visiting instructors, including university professors and assistant professors, NIH scientists, industrial scientists, postdoctoral fellows, and in one case, a Chinese scientist studying in the United States. Laboratory exercises were limited to between 10 and 25 people, whereas lectures were attended by larger numbers of interested Chinese scientists. Laboratory space and large equipment were provided by the Chinese, while reagents and most laboratory supplies were brought in from the United States.

In general, the Chinese participants were enthusiastic, eager to learn, and hardworking. They were excellent in the laboratory, picking up new techniques with ease, and in most cases, they had a good working knowledge of modern experimental methods. In contrast, the students' basic knowledge, particularly in genetics, was not always so strong. In some cases, they could do a Southern blot but were unable to predict the outcome of a single Mendelian cross. Others could transform years cells with high efficiency, but were incapable of distinguishing between a *cis*- and a *trans*-acting mutation. Thus, while the students knew many of the facts of modern molecular biology, they had less sense of how to design experiments to test these hypotheses or develop new ones. Perhaps the main benefit of the minicourses, in particular the lecture portions, was in emphasizing the importance of the experimental method and broad training in science. This message appeared to be warmly accepted by the participating scientists.

An original aim of the joint CSCPRC-CAS program was to establish a continuing series of minicourses, similar to those at Cold Spring Harbor Laboratory in Cold

Spring Harbor, New York, in which Chinese scientists would gradually take over the teaching responsibilities. The Chinese support this idea, and the Shanghai Institute of Biochemistry has set aside space for a dedicated training laboratory. However, at the present early stage of this cooperative program, it is clear that additional American participation and funding will be required to achieve this aim.

The CSCPRC also has administered the Visiting Scholar Exchange Program. This program has provided funding for short (1- to 3-month) visits of American scholars to China and of Chinese scholars to the United States. The main focus in the sciences has been on the establishment of collaborative research projects, especially in areas in which work in China and/or with Chinese scientists can make a unique contribution. The program has been especially useful in allowing American-trained Chinese scientists to continue their contacts with the West and update their training. The program has been supporting 15 American and 10 Chinese scholars each year, typically including two biologists. This has been an important program that bridges the gap between graduate study abroad and visits by senior scientists and administrators. It is regrettable that this program will be terminated in 1990.

The NIH, which is the main supporter of biological research in the United States, engages in several cooperative activities with China. While the general agreements between NIH and CAS, and NIH and CAPM, are largely inoperative, contract research programs are supported within several NIH institutes. The National Cancer Institute is engaged in epidemiological studies of throat and stomach cancer in China, clinical trials of traditional herbal medicines, and an analysis of the relationship between vitamins and cancer. The National Institute of Allergy and Infectious Diseases supports research on hepatitis and several parasitic diseases, while the National Heart, Lung, and Blood Institute is involved in a study on the relationship between diet and stroke. Although it is not widely known, investigator-initiated NIH grants are open to scientists of all nationalities on a competitive basis; the National Institute of Allergy and Infectious Diseases expects to fund at least one independent research project in China in 1989. The NIH, through the Fogarty International Center, is also an important training center for Chinese scientists. There are currently 125 Chinese scientists at the main NIH campus.

PRIVATE FOUNDATIONS AND ACADEMIC INSTITUTIONS

The Rockefeller Foundation, which has long historical ties with China, is the most visible of the private American foundations supporting research and training of Chinese scientists. It provides direct research grants in two major areas: population control (including basic studies of reproduction) and agriculture (particularly improvement of rice strains). Between 1979 and 1988, the

foundation's medical sciences department expended $2.1 million on 34 research grants, of which 10 dealt with basic studies of the molecular and cellular biology of reproduction, e.g., studies of plasminogen activators in early embryos and cloning of sperm surface protein genes. In addition to research grants, the Rockefeller Foundation provides Biotechnology Career Fellowships to young scientists (many of whom have trained abroad) in China and other developing countries.

These Rockefeller Foundation programs have had a major impact on Chinese biotechnology. While average grants are small, typically $30,000, they are very effective because they are provided in U.S. dollars, which can be spent outside China without the usual encumbrances of the procurement system. In addition, the fellowship program allows investigators to work abroad on a regular basis, providing a key incentive for students to return to China. Furthermore, virtually every high-caliber scientist that was met during the 1-month evaluation trip had been helped, one way or another, by the Rockefeller Foundation. Finally, most of the foundation-supported research projects, except those at the Beijing Institute of Developmental Biology, are clearly above average.

Several other private foundations support Sino-American biotechnology cooperation, mostly through small grants for travel and study abroad. Recently, the Fudan Foundation has unveiled preliminary plans to establish the Thomas H. Morgan Science Center at Fudan University's Institute of Genetics at a projected cost of several million dollars (see Chapter 8).

Many Chinese educational and research institutes have established formal or informal ties with American universities. For example, Fudan University has ties with Harvard, Princeton, and Yale Universities and with the University of Maryland, while Nanjing University has an association with Johns Hopkins University. In certain instances, attempts have been made to establish joint research projects such as the breeding of improved pig strains (Beijing Institute of Developmental Biology with North Carolina State University) and the development of anti-liver cancer immunotoxins (Shanghai Institute of Cell Biology with Stanford University). However, such ties lag behind those established by other countries, and notably, no American university conducts a regular research or training program in China such as the Max Planck Institute's program at the Shanghai Institute of Cell Biology. The major contribution of American universities lies in training Chinese students abroad, a topic discussed below.

STUDENTS ABROAD: HOW MANY WILL RETURN?

According to a recent study by Leo Orleans, *Chinese Students in America: Policies, Issues and Numbers* (Washington, D.C.: National Academy Press, 1988), some 56,000 Chinese students and scholars visited the United States between 1979 and 1987. About 60 percent of these were officially sponsored by the

Chinese government (J-1 visa status), while 40 percent were students privately supported by friends or family (F-1 visa status). At the beginning of 1988, it was estimated that there were 36,000 visitors from China in the United States: 21,000 studying or working with J-1 visas, 7,000 holding F-1 student visas, and 8,000 who managed to remain in the United States either with or without a different type of visa. Based on figures from 1985, 17 percent of the Chinese visitors were supported by the Chinese government; 9 percent by personal funds; and 64 percent by U.S. universities, foundations, corporations, and government programs. Between 1979 and 1985, there was a severe decline in the proportion of students and scholars supported by the Chinese government (from 54 percent in 1979 to 17 percent in 1985) and a corresponding increase in support from American sources, particularly universities (from 18 to 57 percent). Total expenditures by American sources in 1985 were in excess of $80 million, and it is likely that today the figure is greater than $100 million. Although no statistics are available on the number of Chinese visitors working specifically on biotechnology, it is estimated that 11 to 17 percent are involved in the combined fields of life sciences, health sciences, and agriculture and that 7 percent of undergraduate plus graduate students are studying biology or biochemistry. From these figures, together with causal observations of graduate student populations at several American universities and medical schools, it can be estimated that about 1,000 to 3,000 Chinese students and scientists are currently training in biotechnology and related fields in the United States.

Clearly, this sizable pool of students and scientists abroad presents China with an important opportunity to accelerate technological development. But how many of the students will actually return? And will those that do return be able to use their new training? Although breakdowns by field of study are not available, overall figures compiled by Orleans on the return of students and scholars to China between 1978 and 1988 are revealing. It is estimated that within this period approximately 12,500 J-1 visa holders and 7,000 F-1 visa holders returned to China. These 19,500 returnees represent approximately one-third of the total sent to the United States, including the majority of all officially sponsored students and scholars. Moreover, of the 36,000 visitors currently in the United States, only 20 percent have overstayed their originally planned visits. Since more than half of all students and scholars that have been sent to the United States are still here, it is too early to predict what the overall return rate will be; but judging from the first few years, it will undoubtedly be high enough to have a significant impact on China's science and technology development.

Despite these encouraging return rates, Chinese leaders have recently introduced several new measures to try and increase the fraction of students and scholars that return to China. These include the following steps:

1. Students are no longer officially sponsored for undergraduate study, only for graduate study and postdoctoral research.

2. Students who have obtained their undergraduate or masters degree in China must remain in the country for 2 to 3 years before going abroad for a Ph.D. degree.
3. Visas for spouses and children are no longer routinely granted. (representatives of the Chinese Embassy in Washington, D.C. deny changing this policy, but numerous Chinese colleagues assert that it has been changed.)
4. Work units are being held increasingly responsible for selecting and ensuring the return of students and scholars abroad. Even when a student or scholar has clearly emigrated, he or she is still counted as occupying a work position in China. Financial penalties are levied on the family and/or guarantor of students or scholars that fail to return within the allotted time. Since these penalties are decided by each work or administrative unit, they may vary considerably. One example cited to us was 100 *yuan* per month (about the average salary for a Chinese scientist) for the first 6 months; after that, 400 *yuan* per month.
5. Several Chinese universities and research institutes are trying to establish programs in which graduate students do their course work in China and go abroad only for their dissertation work (typically, 2 years).

While the intent of these measures is clear, their ultimate effectiveness remains to be tested. The general impression is that there has been a tangible alteration in the mood of Chinese students in the United States since the "antibourgeois democracy" movement of late 1986 and early 1987. Students who fully intended to return to China are now taking a "wait and see" attitude; recipients of an undergraduate degree are staying on for graduate studies; and Ph.D. recipients are staying on for postdoctoral work. These "lingerers" are waiting for three assurances: (1) that China will not return to an outright anti-intellectual campaign of the sort that has occurred so regularly since 1949, (2) that once they return they will have the opportunity to go abroad again to continue high-level training or collaborative research, and (3) that they will be provided with an appropriate environment and sufficient funding to continue doing science in China. Measures that help to assure students and scholars abroad on these points will undoubtedly have a positive impact.

The most commonly discussed solution to the brain-drain problem, namely, the improvement of working and living conditions in China, is unfortunately the most difficult to achieve. To their credit, certain Chinese universities and research institutes have offered considerable incentives for scientists to return from abroad. These include immediate promotion to professor, ample laboratory space, permission to go abroad on a regular basis, and virtually guaranteed research support. In addition, while scientists are abroad they can apply for the relatively small research grants provided by NSFC. By offering such incentives, a small number of universities (e.g., Peking University) and institutes (e.g., Shanghai Institute of Biochemistry and Beijing Institute of Virology) have managed to attract truly top-notch researchers trained abroad. The increased use of such incentives could have a major impact on China's biotechnology development.

COMMERCIAL ENTERPRISES

Given China's preoccupation with applied research, it is not surprising that special emphasis and hopes are placed on ties with U.S. corporations. Four types of commercial activities are under way or planned in China: (1) sponsored (contract) research, (2) joint ventures, (3) wholly owned subsidiaries, and (4) technology transfer. Although the details of many of these commercial agreements are not publicly available, a few examples will suffice to indicate the range of ongoing activities. One of the most active U.S. companies in China is the Monsanto Company, which currently supports over 50 research projects at various Chinese institutions. Most of these are contract research programs that take advantage of the special skills of Chinese scientists, e.g., plant tissue culture methods. In the realm of joint ventures, the formation of the Sino-American Biotechnology Company by Promega Corporation has been especially useful in giving Chinese scientists access to biotechnology reagents (see Chapter 5). Several major U.S. pharmaceutical firms operate joint venture factories in China. Other projects at various stages of negotiation include the genetic engineering of soybeans, HBV vaccines, and cancer detection kits.

Despite these encouraging signs, and despite the fact that a large number of U.S. biotechnology, chemical, and pharmaceutical firms have made overtures to China, the overall level of Sino-American cooperation in commercial biotechnology remains low. The main complaints on the American side are the weakness of the Chinese patent system (see Chapter 5) and the fact that many Chinese have tried to obtain new technology for free, often by playing one company against another and by offering preferential market entry. On the other hand, the number of marketable, profitable products available from biotechnology is still low. Perhaps the Chinese are wise to save their money, and at the same time build their own technological expertise, until the potential of biotechnology becomes a reality.

10

Conclusions and Recommendations

In the past 10 years, and particularly since 1986, China has made great progress in developing the capacity for biotechnology research, which will lead eventually to its application and commercialization. Funding has been increased to an absolute level greater than that in any other developing country and to a relative level (adjusted for gross national product) that compares favorably with those in many developed countries. A competitive granting system that includes peer review has been established to allocate these funds. New laboratories have been built and equipped with modern instruments. A substantial number of research groups routinely use sophisticated technologies ranging from DNA cloning to x-ray crystallography. Large numbers of scientists have been sent abroad for advanced training, and many of them have returned to China. Scientific productivity, as judged by the number of articles and journals published and the number of scientists actually engaged in research, is on the rise.

Despite these advances, China's level of biotechnology research and development remains far below that in developed countries. In basic research, Chinese scientists operate at an international level in only a few scattered areas. Genetics, first undermined by Lysenkoism, then stalled—as was most science—by the Cultural Revolution, is now too often bypassed in favor of applied genetics like gene cloning. The literature survey revealed a paucity of developmental biology papers, which indicates a bias against using biotechnology to advance understanding of the basic knowledge of genetic expression and regulation that governs organisms' development. In applied research, the majority of projects are derivative or outright imitations of Western investigations. For example,

more than one-half of the applied genetics research articles surveyed described the cloning of genes already sequenced and published in international journals. The Chinese government's insistence on funding research of such low originality has led to a large and lamentable waste of rare resources. How, then, can China ensure that recent improvements in biotechnology funding and support ultimately translate into improved research and technology? Although most of the suggestions below are neither novel nor surprising, they are sufficiently important and useful to bear mentioning.

- Policymakers should recognize that the precepts for innovative and productive molecular biology applications are dependent on a sound understanding of and training in the sciences of genetics and molecular biology. Educational and institutional reforms should be supported to bolster training in the basic sciences of biotechnology.
- A better balance between basic and applied research should be sought. The best basic projects should be supported by large grants; conversely, bad or derivative projects should not be funded solely because they are applied. This could be accomplished by setting aside a certain percentage of High Technology Program or Seventh 5-Year Plan funds for basic research or by giving NSFC a separate budget for large basic grants.
- Administrators should realize that support of biotechnology represents a long-term investment and should not expect research units to become self-supporting. Even in the United States, where more than $3 billion is spent annually on biotechnology, few biotechnology companies operate at a profit. Early withdrawal of support will result in wasted time and money.
- Downstream processing facilities should be subject to accountability and progress assessment as conditions for financial support. Large capital expenditure plans should be based on definite product identification. Budget allocations to facilities lacking these considerations would be better spent on the continued support and enlargement of key laboratories at centers that have a demonstrated capacity for high-level research.
- The procurement system needs to be reformed to ensure that scientists have a flexible and reliable supply of the materials necessary to pursue research efficiently. In this context, giving scientists direct control over their grant funds should be considered.
- Continued international cooperation at the governmental, academic, and commercial levels should be encouraged.
- The development of joint and Chinese ventures to produce biotechnology reagents should be continued and expanded.
- The protection of intellectual property rights should be improved to encourage foreign investment.
- Safeguards against scientific fraud should be instituted.
- Chinese scientists should take greater advantage of the accessibility of most

materials published in international journals, particularly DNA clones and cell lines. This would prevent much duplication of effort.

- The offering of special inducements to students and scientists returning from abroad should be continued and expanded.
- Certain measures concerning the return of students and scientists are counterproductive and should be eliminated. These include the restriction against leaving China for 2 to 3 years after completing a B.S. or M.S. degree, a period of the greatest creativity and productivity for many young scientists; the attempt to limit postdoctoral fellowships abroad to 1 year, a period too short to accomplish much work of significance; and joint Ph.D. programs (which involve coursework in China and limited subsequent research abroad) that allow students to learn techniques without understanding their scientific bases.

What are the pros and cons of the United States providing further support for biotechnology in China? On the positive side, China has a rich flora and fauna and a long history of traditional medicines that may eventually provide useful resources for American biologists and biotechnology entrepreneurs. China also represents a potentially huge market for biotechnology products, produced either with licensed technology or through joint ventures. But perhaps the most important consideration is also the simplest: China has a population of over one billion, nearly one-fifth of the global population, and it is a developing country. For these reasons, China is an important test case for the successful application of biotechnology to meeting economic development goals and basic human needs in developing countries. In this crucial way, Chinese biotechnology goals can and should diverge from those of developed countries.

On the negative side is the unfortunate conclusion of this report, namely, that the Chinese government has opted to attempt a direct replication of Western biotechnology rather than to support the basic, innovative research that is essential to tailor biotechnology to China's needs as a developing country. Because of the lack of emphasis on basic research, China is at risk of developing a cadre of highly trained, technically competent scientists who understand the mechanics of biotechnology, but not the underlying science or the road ahead. Unless corrected, this deficiency will ensure that Chinese biotechnology will remain an expensive but nonproductive activity—a poor recompense for the great effort and expenditures of the past decade. The root cause of this problem is government policy, in particular, the insistence that science generate earnings, and, moreover, do it in short order. In the past few years, many Americans have been lulled into thinking that China is becoming progressively less ideological and political. In fact, today's idea of "serving the economy" is no less rigorously pursued than was the idea of "serving the people" during the antirightist campaigns and the Cultural Revolution.

Within this context, how can the United States help to improve Chinese biotechnology and related research to the ultimate benefit of both China and

itself? Below are listed several ideas for both government-sponsored and individual activities.

- Funds should be sought to continue combined laboratory and lecture minicourses such as the ones cosponsored by CSCPRC and CAS. They represent a unique opportunity to introduce Chinese scientists to both the technologies and philosophy of modern biological science, particularly in areas that are weak in China, such as gene regulation and developmental biology. It is essential that they be held on a continuous basis at a dedicated facility; only then will it be possible to achieve the goal of full Chinese participation in teaching these courses.
- A low-cost but potentially productive activity that the CSCPRC should consider would be sponsorship at a U.S. facility in Beijing of a lecture series on the frontiers of biology. Periodically, (one to four times a year) prominent American and Chinese scientists would lecture on the most recent advances in basic and applied biology. A major aim of this program would be to foster contacts and interactions among Chinese scientists who, at present, rarely go outside their own workplace and who often fail to inform "outsiders" of visits by scientists from abroad. American participants could be drawn from the pool of visiting U.S. scientists who pass through Beijing, thereby avoiding any outlay for travel funds. Chinese lecturers could be nominated by Chinese universities, CAS, or various ministries and state commissions.
- Appropriate forums should be promoted for science policy discussions among visiting scientists, their Chinese counterparts, and Chinese government and Party officials. Discussions should expand understanding of the factors that determine a sound and productive science research infrastructure and administration. Special efforts should be made to engage senior policy planners in such forums as a way to outline and emphasize ways that government policies help or impede scientific progress.
- Additional research on the biological and agricultural resources of China should be conducted. While this report uncovers only a few exciting developments in Chinese biotechnology, it should be remembered that the findings center on modern research technologies that are almost exclusively imported from the West. Inspection of China's more traditional biotechnology areas would be useful, e.g., aquaculture, drought- and cold-resistant fruit varieties, high-nutrition rice varieties, fermentation of farm by-products, and testing of traditional medicines for unexpected activities, e.g., against the AIDS virus.

This is a critical period for the development of biotechnology, biology, and all science and technology in China. Some important administrators are now taking the view that enough money has already been spent on biotechnology and that research centers and programs should "sink or swim" on their own. But others take the more farsighted view that China should continue to fund and improve its

research capacity, especially in the basic sciences, so that when biotechnology finally does realize its potential, China will be well placed to reap its benefits. Regardless of which policy is adopted, American scientists—individually or through governmental, academic, and commercial groups—will have many opportunities to collaborate with their Chinese colleagues to advance biotechnology in China and worldwide.

Appendixes

A

Abbreviations

CAPM	Chinese Academy of Preventive Medicine
CAS	Chinese Academy of Sciences (Academia Sinica)
CHO	Chinese hamster ovary (cell line)
CMV	cucumber mosaic virus
CNCBD	China National Center for Biotechnology Development
CSCPRC	Committee on Scholarly Communication with the People's Republic of China
DHFR	dihydrofolate reductase
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
NAS	National Academy of Sciences (U.S.A.)
NSF	National Science Foundation (U.S.A.)
NSFC	National Natural Science Foundation of China
SEDC	State Education Commission
SSTC	State Science and Technology Commission
Ti plasmid	tumor-inducing plasmid
TMV	tobacco mosaic virus

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D

An Analysis of Niu Menchang's Research on Transformation by RNA

Eric H. Davidson

It has been claimed by Niu and his associates that amphibian and teleost eggs can be transformed heritably with injected egg poly(A) RNA.[27-32] Because of the potentially great practical, experimental, and theoretical importance of embryo germ line transformation, it is worth examining these claims carefully. In the following paragraphs, a brief review of the published reports of Niu et al. is presented. Unfortunately, this review leads to the conclusion that these claims of RNA-mediated embryo transformation are incorrect.

To obtain germ line transformation, the introduced sequences must, of course, be incorporated into the DNA genome. It could be argued that retroviral reverse transcriptase is present in eggs (though there is absolutely no evidence of this), and could copy the injected maternal poly(A) RNA into DNA that could then be incorporated into the genome. Were this likely to occur, however, it would follow that there should also be a high frequency of reverse transcription and reinsertion of the endogenous maternal poly(A) RNA. Current evidence, on the contrary, proves that this type of event is very rare, and that it occurs only on an evolutionary time scale. Several pseudogenes have recently been found[33-36] that indeed could have appeared in the germ line by the route of reverse transcription and insertion. The distinguishing feature of such genes is that they have the structure of mature

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NOTE: This analysis is an excerpt from a paper, "The Maternal RNA of Amphibian and Echinoderm Eggs," which was submitted for publication in *Scientia Sinica* in 1982. It and an earlier Letter to the Editors were never published.

messages rather than of genes. That is, they lack the intervening sequences possessed by most genes but they have terminal poly(A) sequences that in functional genes are not coded in the genomic DNA. Direct sequence analysis often displays sequence changes in the pseudogenes that have required millions of years to accumulate. This, plus the facts that only a few if any such pseudogenes are ever found for any given true gene and that most genes are single-copy sequences, shows that incorporation into germ line DNA of sequences copies from mRNA is an exceedingly infrequent event. It is consequently impossible to believe a priori that RNA injected into an egg could be efficiently reverse transcribed and incorporated in the genome. Otherwise, the genome would contain enormous numbers of copies of genes without intervening sequences, while, of course, exactly the reverse is true. In addition, the traits claimed to have been transformed by Niu and his associates are in general morphological, and their developmental creation almost certainly requires the participation of many genes working together. To transform such traits would necessitate incorporation of all the requisite genes in intact form, plus their control systems, which is even more unlikely.

Biological principles aside, the reports of Niu et al. are experimentally unconvincing. Specific examples of probable artifacts, misinterpretations, or inadequate procedures include the following:

1. The mRNA utilized by Tung and Niu[27-29] was extracted by an unreliable method, utilizing a material called "Sigmacell." This is merely an unpurified cellulose, some batches of which trap some poly(A) RNAs. There is, in fact, no evidence that the RNA extracted was polyadenylated, or that it was mRNA. The cell-free translation studies reported by Niu et al.[30] were done with RNAs prepared by a better procedure, and these experiments are not relevant to the claim that the RNA in the original reports was mRNA. Furthermore, the gel electrophoresis patterns shown for the alleged mRNA in the 1977[29] and 1973[27] papers are impossible for real poly(A) mRNA, which, of course, does not band tightly in one place as illustrated because it is extremely heterodisperse in size.
2. The same transformation results were reported to result from DNA and mRNA injection.[27] I am certain the DNA results are an error or at best not the result of nucleic acid injection. Whichever is the explanation, it no doubt pertains to the mRNA results as well because it is even more unlikely that there are two different explanations for the same unlikely observation. The data shown state the DNA was injected at "100 OD/ml," indicating that the DNA was concentrated to about 4,000 μg/ml. Unfortunately, DNA cannot be concentrated to this extent unless it is broken to very small pieces, a few hundred nucleotides long (or is supercoiled, which is irrelevant for this case). DNA a few hundred nucleotides long, of course, cannot transform anything since a single gene is usually at least a few kilobases (kb) and sometimes as much as 20 or 50 kb in length. The proposition that a whole organ-level structure can be transferred heritably by small pieces of DNA is particularly difficult to believe.

3. In the fish transformation experiments it was claimed that a heterospecific tail phenotype could be induced by mRNA injection. However, the fact that this "single tail" phenotype occurs at a significant frequency, even though breeders "have always discarded those with single fins," may be significant. Tung and Niu[27] make the key assumption that single tail must be a "genotypic character." However, no evidence is presented that single tail is in fact a simple genetic character. In fact, Tung and Niu[28] report ratios of single tail to double tail in the single parent offspring of the alleged transformed fish that cannot easily be interpreted in terms of a Mendelian character. I would guess that single tail is a minor developmental abnormality, perhaps an easily induced phenocopy of a complex genetic mutation. Many such abnormalities are known in developmental biology. I suspect this physiological lesion has nothing whatsoever to do with the introduced nucleic acids and is the result of handling, the injection procedure, or some other experimental artifact (see below).
4. Tung and Niu[31] also claimed nucleic acid-mediated transfer of an urodele amphibian embryonic trait; the balancer A version of this experiment was recently performed by Shi et al.,[37] who showed that, indeed, injection of urodele DNA into an anuran (frog) egg produces an extra "balancer" (i.e., protrusion on the chin of the embryo which may or may not be a real balancer), but so does injection of the DNA of this anuran into its own egg. Therefore, this case is clearly just what I suspect the "single tail" result is, viz., a developmental lesion that can occur naturally, and is simply a frequent experimental artifact stimulated by the operation. It might be noted that this is not the only example of such artifacts. For example, Scharf and Gerhart[38] have recently shown that the appearance of extra heads and nerve chords in amphibian eggs can be stimulated merely by turning the eggs over. Previous workers (e.g., see Curtis[39]) had claimed that transfer of cytoplasmic cortical material produced such secondary axiations, but to carry out the experimental transfers the eggs had to be turned on their sides. Scharf and Gerhart[38] showed that in fact the material transferred had nothing whatsoever to do with the result of the operation.
5. Niu and Tung[32] also claimed that injection of carp mRNA into goldfish eggs produces adult liver isozymes similar to those occurring in carp × goldfish hybrids produced by artificial insemination. The evidence is a series of lactate dehydrogenase (LDH) starch gels. These gels are unconvincing since they are severely overloaded or have been run with such poor resolution that one band cannot be distinguished from another. (For contrast, observe the precise appearance of the LDH isozyme gels in Wright and Subtelny[40]). The key observation purports to show an additional faint band, but the appearance of this band suggests an artifact caused by a salt front in the gel. There is no likely way that a variant could be present at 1/100 the concentration of all the other variants anyway, since all the liver cells must have the same genes, as they all descend from the same allegedly transformed egg.
6. Niu et al.[30] claimed that poly(A) RNA of carp eggs can be translated to produce albumin. The procedures in this paper for extraction of RNA are

acceptable, but everything depends on the specificity of the albumin antibody. No evidence on this point is presented whatsoever. In any case, the claim is irrelevant to the nucleic acid transformation claimed in the earlier papers because the RNA is prepared differently and because the presence of the albumin message in the egg, even if true, has no particular significance. Albumin or some other cross-reactive protein could be used in the early embryo and be coded by a maternal message like so many other proteins.

In another RNA transformation study, Niu et al.[41] claimed that soybean protein can be induced in rice by injection of soy seedling mRNA into rice ovaries. The RNA was extracted from seedlings from which the cotyledons had been removed. However, J. Bonner (personal communication) has shown that soy globulin is confined to the cotyledons. Therefore, Niu et al.[41] injected mRNA from the part of the plant that contains no soy globulin mRNA. Thus, it is impossible that the injected RNA caused the rice cells to synthesize soy globulin.

It can be concluded that there is no experimental support for the contention that maternal poly(A) RNA can be utilized for transformation of eggs, nor, on theoretical grounds, should this be possible. It is obvious that the correct route to development of a germ line transformation system is introduction of the genetic material, that is, DNA, not RNA. This is clear from successes already obtained with tissue culture cells,[42] *Dictyostelium*,[43] and *Drosophila* embryos.2526 Embryo DNA transformation will provide a decisive approach to many problems in the molecular biology of early development, including the role of maternal poly(A) RNA.

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E

Statement by Dr. Niu

Observation on the Evaluation of Dr. Niu's Research at Ide Published in "Biotechnology in China" by Drs. Hamer and Kung

There are several points in Drs. Hamer and Kung's evaluation pages upon which I would like to comment:

1. Based on the national competition for research grant, IDB got 1.8 million *yuan* from NSFC for 3 years and not for one year.
2. About the working hypothesis (my theory): The stored mRNA in the presence of reverse transcriptase (RT) is transcribed into cDNA which is then inserted into the genome. This cDNA would act as an agent for the differential gene activation during embryogensis. Drs. Hamer and Kung wrote "This theory directly contradicts the basic laws of Mendelian inheritance." As a developmental biologist, I appreciate fully what T.H. Morgen wrote in his book entitled *Embryology and Genetics* that "during cleavage heterogeneous cytoplasm modifies nuclear function." The question often being asked is what is the component (agent) of the heterogeneous cytoplasm? In the seventies we proposed that the egg mRNA transcript (cDNA) was the agent. Now in the later half of the eighties we have provided evidence to support that proposal. We do not see how it contradicts Mendel's laws. As to the transmission of the induced simple tail of goldfish to the next generation, we have reported on the phenomenon.
3. Hamer and Kung stated that the rabbit globin mRNA used in our experiment was impure. This was wrong and it strongly indicates that they did not read the

paper. In the abstract of the article we thanked Prof. Jerry Lingrel for the gift of rabbit globin mRNA (it was the best available at the time, 1978-1988). In order to show that red blood cells (RBC) from the rabbit globin mRNA injected fish carry the properties of rabbit RBC, we chose to detect rabbit globin from the fish's RBC by immuno-precipitation (highly specific) and by the tissue specific lactate dehydrogenase (LDH). The electrophoretic pattern of the liver homogenates from the injected fish was of the hybrid type (i.e., intermediate between goldfish and rabbit). Therefore, for our purpose, there was no need to do globin chemistry as demanded by Hamer and Kung.

4. Their comments about the "green fish" were totally invented. No such comments were made. Drs. Hamer and Kung labelled me as a poor professor without funding, unable to attract students or post-doctors. In view of my retirement, Drs. Hamer and Kung should know university rules governing retired professors. As a matter of fact, Temple University treats me very well, as evidenced by not closing my lab. As to my publications, Drs. Hamer and Kung should get a list of my publications in Chinese and Western journals, get to know the content and evaluate those papers published while working in IDB (1982-1990) and not only those from Institute of Zoology (1973-1981). Drs. Hamer and Kung did not know that the late Prof. Tung suggested to me that he would like to see my research done in China and published in China. In the past few years, however, Academia Sinica encourages people to publish abroad. This is the reason why I began to get papers published in Western Journals in the past few years. The evaluation was supposed to be on papers published by members of IDB. Drs. Hamer and Kung chose to comment only papers published in the period of 1973-1981. Their error was in not evaluating the work done at the IDB during the period 1982-1990; however, Drs. Hamer and Kung did write a sentence about Dr. Niu's work on cytoplasmic DNA synthesis and the discovery of an independent reverse transcriptase in the mid-1980's.

Dr. Niu does express great appreciation to Dr. Hans Ris, a well-known biologist, for his letter of support and criticism therein of the Drs. Hamer and Kung's false statements.

Drs. Hamer and Kung wrongfully drew the conclusion that Dr. Niu's group controls the institute's resources, positions, and power. Therefore, the research conducted by this group is open to question.

Dr. Niu's scientific and professional response to these irresponsible statements was an offer already made for the impartial selection of a highly respected, qualified member of the world scientific community to come to the Institute at Beijing. All expenses would be paid and the scientist would have the permission of the IDB to monitor and see the results of the research conducted by Dr. Niu and to write an independent report of his findings. The offer by Dr. Niu was refused. Obviously, the writers are apprehensive of finding a failure to corroborate their report.

Response to Prof. Davidson's Comments on M.C. Niu's Scientific Research in Academia Sinica, Beijing, China

Prof. Davidson subscribes to the dogma that transformation in developing systems is based on DNA and never on RNA. He also accepts the oncogenic effect of certain viral RNA based on the presence of the reverse transcriptase (RT for short) in the virus. In eggs, he argues, RT has not been demonstrated. Prof. Davidson, in 1989-1990, was not familiar with the work of Niu at IDB after 1982 and presents an excerpt from his manuscript written in 1981.

There are seven points in Prof. Davidson's analysis of Niu's work in collaboration with the late Prof. T.C. Tung at the Institute of Zoology in Beijing. I shall answer them point by point.

1. The method of our isolation of mRNA is unreliable because of the use of "Sigmacell" type 38 cellulose.

   Answer: New methodology in research develops year after year. By 1972 there were 3 new techniques in the literature for the isolation of mRNA: (1) Absorption to millipore (see Lee et al., PNAS, 68:1331, 1971 and Rosenfeld et al., B. B. Res. Commun., 47:787, 1972), (2) mRNA hybridization to the oligo dT cellulose (Aviv et al., PNAS, 69:1406, 1972), and (3) isolation of eukaryotic mRNA on cellulose and its translation *in vitro* (Schultz et al., B. B. Res. Commun., 16:377, 1972). The method of using "Sigmacell" cellulose for mRNA isolation is simple and easy. The mRNA isolated with this technique was shown to be pure and could prime the translation of a specific protein. The reason we chose "Sigmacell" cellulose was mainly economical. It goes without saying that in Philadelphia we used "sigmacell" cellulose first and then purified the mRNA further by oligo dT cellulose. The mRNA activity was tested by *in vitro* synthesis of a specific protein.

2. The mRNA and DNA mediated tail transformation from the double 2-lobed tail to the forked shaped simple goldfish tail is questionable. (Correction: 100 o.d./ml DNA is misprint of 10 o.d./ml)

   Answer: The claim of nucleic acid-mediated genetic change was sensational in early 1970. Many biologists were skeptical because they claimed that the strain of the goldfish we used was not genetically pure. Another criticism was that we did not use any controls. However we had used fish liver mRNA, rRNA, and tumor mRNA as controls and eggs injected with these mRNAs developed into fish without changed tails. They did not accept that mRNA could produce genetic change. This is to say that the scientific community demands that we repeat the experiments. We decided to use mRNA from a different species. When egg mRNA from a carp, *Cyprinus carpio* L. was injected into goldfish eggs of a pure strain maintained by inbreeding at the Institute of Zoology in Beijing, the tail was changed but with slightly reduced frequency. (Literature says that cloned rabbit globin gene (Ca. 600 b.p.) and many other genes with less than 1,000 b.p. can mediate genetic change.)

3. The transformation of the mRNA and DNA induced goldfish tail to next generation.

Answer: Published observation (Matui, J. Imp. Fisheries Institute, 30:1, 1934) and our own data show that the tail of the offspring from the cross of goldfish and crucian carp or carp is simple and never double 2-lobed. The double 2-lobed tail is recessive. The injection of carp mRNA into goldfish eggs resulted in the alteration of the tail fin, while injection of goldfish egg mRNA into carp eggs provided no change of tail fin. These data show the specificity of the carp egg mRNA in goldfish tail change. The mating of the simple tailed goldfish obtained from the injected eggs produced offspring with simple forked and double 2-lobed tails, thus showing the transmission of the induced simple tail to the next generation. This refutes Prof. Davidson's assumption that the simple tail in goldfish is a minor developmental abnormality. The strain of goldfish we used is genetically pure. Their mating produced double fancy tailed offspring.

4. The newt DNA induced development of the balancer in goldfish larvae.

Answer: Balancers are transitional organs found in the California newt, *Taricha torosa*. In newt larvae, they emerge 5-6 days after fertilization and resorb ca. 25 days later. They consist of a pair of bar-like organs on the site post-lateral to the mouth. In sections they consist of epidermis and connective tissue. The cells at the top are secretory in nature. When *Taricha torosa* DNA was injected into goldfish eggs, approximately 1 percent of the larvae developed one balancer on the right or left side. If DNA from the Chinese newt, *Cynops orientalis*, was injected into goldfish eggs, 6 percent of the larvae developed balancer. Over 90 percent of the injected eggs developed normally.

Shi et al. (Scientia Sinica, 24:402-406, 1980) repeated this experiment using DNA isolated from frog and Chinese newt. Over 90 percent of their injected eggs developed abnormally. Among the abnormal tadpoles, 0.9×10^{-3} of the frog DNA and 0.3×10^{-2} percent of the newt DNA-injected developed lateral protuberance on the operculum. Without considering the definition of the newt's balancer, they named the protuberance a balancer. Their experiments have nothing to do with induction of a balancer. Induction of a balancer is not due to the experimental manipulation as shown by the fact that injection of goldfish DNA or mRNA never produced a balancer-like structure.

5. The use of the electophoretic pattern of lactate dehydrogenase (LDH) to detect organ (liver) specificity.

Answer: Liver is one of the few organs possessing LDH-c which migrates toward the cathode. The liver from the hybrid of goldfish and carp is characterized by the presence of 2 bands in between goldfish's 2 bands. Carp liver mRNA injected goldfish were found to have 1 faint band, thus showing the effect of mRNA on the faint band formation. Prof. Davidson considered it unconvincing because the resolution of the bands in the photographs was poor. However, some photographs in the same paper shows the LDH bands from the rat liver mRNA injected fish sharp and clear.

6. Indirect demonstration of the presence organ (tissue and cell) forming mRNA in the stored mRNA of the egg.

   Answer: The egg mRNA used in the 1973 experiment has to be tested (as demanded by Prof. Davidson in his writings) for its ability of protein translation. Successful experiments were done with the egg mRNA primed synthesis of albumin and globin. This was the first report suggesting that albumin and globin messages were present in the eggs. Here is what Prof. Davidson wrote, "The presence of albumin or globin message in the egg, *even if true*, has no particular significance." On the contrary, we believe that if it were true, we would have demonstrated the mRNA nature of the "morphogen" in the egg. The role of the injected rabbit globin mRNA as a "morphogen" was shown by the appearance of rabbit globin in the red blood cells of the goldfish.

7. Transfer of information from soybean mRNA to rice.

   Answer: It was reported that soybean protein could be induced in rice by injection of soy seedling mRNA into rice ovaries. Professor Davidson insists that soy globulin is confined to the cotyledons. The mRNA we used was from seedlings with cotyledon removed. Therefore, he claims that the seeds from the injected rice could not possibly contain soy globulin. We have tested the seedling mRNA primed synthesis of soy globulin and the result was positive.

Abstract of M.C. Niu's Scientific Research in Academia Sinica, Beijing, China

Carp (*Cyprino carpio* L.) and crucian (*Carassius auretus* L.) are two species belonging to two genera of the same family, Cyprinidae. They possess a simple forked tail. Goldfish is a mutant of the crucian with double 2-lobed tail. The mating of goldfish and carp or crucian produces offspring with a simple forked tail (i.e., simple tail is dominant and the double tail is recessive).

mRNA was prepared from crucian eggs and DNA from liver. Both were injected into goldfish eggs. They develop into fish with simple tails (1). This effect of the egg-mRNA was unexpected but was confirmed repeatedly in the following two years by careful analysis of the fish developed from goldfish eggs injected with carp egg mRNA (2). It should be noted here that the goldfish egg-mRNA injected carp or crucian eggs did not change the simple forked tail into a double fancy type (3). The injection of rat liver-mRNA and rabbit globin-mRNA into goldfish eggs resulted in the development of goldfish with a liver that had some rat liver property (4) and red blood cells with rabbit RBC properties (5). The effect of foreign DNA injected into eggs was also repeated by the injection of California newt (*Taricha torosa* L.)-DNA into goldfish eggs. Approximately 1 percent of the injected fish developed a newt larval organ, the balancer. This balancer was characterized by three criteria: (1) position, (2) histological structures and the time of appearance and resorption (6,7).

The goldfish with a simple forked tail induced by nucleic acids were mated among themselves. About one-third to one-quarter of the offspring possess the simple forked tail, thus showing the transmission of the character, induced simple forked tail, to the next generation (8).

These findings lead us to explore the nature of the effect of the cytoplasm on the nucleus in *Xenopus*. White *Xenopus* is a mutant of the wild type (black). The pigment development from composite eggs (white nucleus in black cytoplasm) is close to the wild type. After metamorphosis, the pigment is confined to the dorsocaudad region (9).

Subsequently, we studied the mechanism by which egg-mRNA affect development. We found that following injection of mRNA, DNA synthesis occurred in the egg cytoplasm. We then showed that the egg cytoplasm contained a reverse transcriptase (10). This suggests that this reverse transcriptase catalyzes the transcription of the mRNA into DNA. We then showed that H3-thymidine labeled DNA injected into the egg cytoplasm is transferred to the egg chromosomes (11). Based on these data we suggested that cDNAs transcribed from the cytoplasmic mRNAs are transferred into egg chromosomes and maintained in the chromosomes during development. Our experiments showed that DNA extracted from the fish developed from eggs injected with rabbit reticulocyte mRNA contains the complimentary sequence of the rabbit globin cloned cDNA (11). However, this rabbit globin DNA, though present in other tissues of the fish, is expressed only in the red blood cells.

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