Perspective

The Molecule of the Year

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Science HAS SELECTED THE POLYMERASE CHAIN REACTION AS the major scientific development of 1989 and has chosen for its first "Molecule of the Year" the DNA polymerase molecule that drives the reaction. The list from which the polymerase chain reaction (PCR) was chosen included an impressive array of accomplishments in many areas of science and technology; additional kudos are therefore conferred below to 17 of the other big "stories" that made 1989 an exciting year for scientists and for followers and beneficiaries of science. Although the PCR procedure was introduced several years ago, use of the technique truly burgeoned in 1989; in much the same way, the full potentials of many of the interesting "runner-up" scientific achievements of this year are likely to be realized sometime in the years to come.

The first PCR papers were published in 1985. Since that time PCR has grown into an increasingly powerful, versatile, and useful technique. The PCR "explosion" of 1989 can be seen as the result of a combination of improvements in and optimization of the methodology, introduction of new variations on the basic PCR theme, and growing awareness by scientists of what PCR has to offer. With PCR, tiny bits of embedded, often hidden, genetic information can be amplified into large quantities of accessible, identifiable, and analyzable material. A single cell provides enough material for analysis; a single hair can be used to identify an individual.

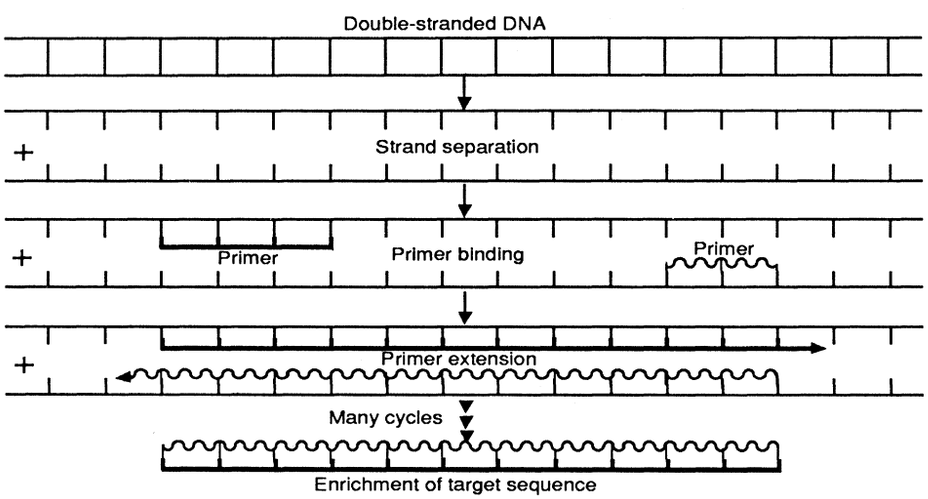
The basic PCR reaction. The starting material for PCR, the "target sequence," is a gene or segment of DNA. In a matter of hours, this target sequence can be amplified a millionfold. How this is accomplished is shown in the accompanying figure. The complementary strands of a double-stranded molecule of DNA are separated by heating. Two small pieces of synthetic DNA, each complementing a specific sequence at one end of the target sequence, serve as primers. Each primer binds to its complementary sequence. Polymerases start at each primer and copy the sequence of that strand. Within a short time, exact replicas of the target sequence have been produced. In subsequent cycles, double-stranded molecules of both the original DNA and the copies are separated; primers bind again to complementary sequences and the polymerase replicates them. At the end of many cycles, the pool is greatly enriched in the small pieces of DNA that have the target sequences, and this amplified genetic information is then available for further analysis.

Evolving PCR. Many improvements on the original PCR method have been made. One of the first was the substitution of a heat-stable enzyme for the original DNA polymerase, which was heat-labile and had to be replenished after each cycle. The stable "Taq polymerase," which comes from bacteria that live in hot springs, continues working almost indefinitely despite the heating steps. Taq polymerase improved the yield, generated more specific and longer products, and facilitated automation.

New strategies have also been devised for flanking unknown sequences with defined primer sites. For standard PCR, the sequences at both ends of a target sequence have to be known. "Inverse" PCR provides a way of sequencing DNA outside the primer sites rather than between two primer sites. Primer molecules are synthesized with their sequences reversed. The target DNA is cut and circularized, and, when the polymerase extends the primer, it does so around the circle in the direction opposite that which would have been taken by standard PCR primers. "Anchored" PCR was developed for studying genes that encode proteins for which partial sequences are known. For anchored PCR, only one defined primer sequence is needed, not two.

The implications of inverse and anchored PCR for DNA sequencing are astounding: enormous stretches of DNA can be sequenced once a tiny bit of sequence is known. Both techniques make it possible to proceed along the DNA, continually redefining "ends" to which synthetic primers can be bound and then extended.

Applications of PCR. The basic PCR procedure has been valuable in disease diagnosis because specific DNA sequences can be amplified enormously (the needle in the haystack). One of the first uses led to improved diagnosis of a genetic disease (sickle cell anemia), because the PCR technique depended on much less clinical material than standard procedures. (Because PCR is exquisitely sensitive, unusual care is taken to avoid the amplification of contaminants.) PCR can also be used to amplify trace amounts of genetic material of infectious agents in blood, cells, water, food, and other clinical and environmental samples. PCR-based tests are especially valuable for detecting pathogens that are difficult or impossible to culture, such as the agents of Lyme disease and AIDS. For cancer diagnosis and cancer research, PCR can indicate what genes are expressed or turned off, because the messenger RNA molecules associated with such genes can be converted into complementary DNA sequences that then can be amplified.



DNA samples in trace materials (semen,

blood, hairs) found at the scene of a crime have been compared with DNA samples from crime suspects; both acquittals and convictions have resulted from such comparisons. Missing persons have also been positively identified through PCR-based comparisons. The resolution of paternity cases has been aided by comparing DNA from a child with that of the alleged father. And matches of transplant donors and recipients are facilitated with PCR. "Universal" primers are being used to determine the extent of homology in the sequences of conserved genes from different samples. Such comparisons, which help to establish evolutionary relations among organisms, can even include extinct organisms, because DNA samples extracted from mummies, bones, and other archival materials can be used.

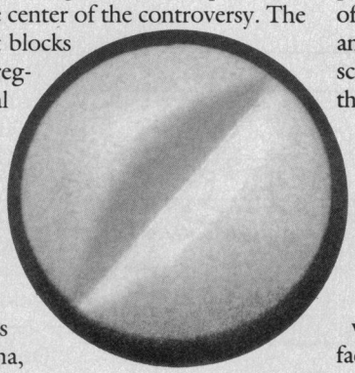
PCR may soon replace gene cloning as the amplification method of choice for gene sequencing, for which large amounts of DNA are needed. PCR is also providing new options in molecular genetics studies for adding genetic information to target materials or for altering what is already there.

The rate at which new PCR-based techniques have been developed suggests that this technology is proliferating as rapidly as its Taq polymerase molecules replicate target sequences.

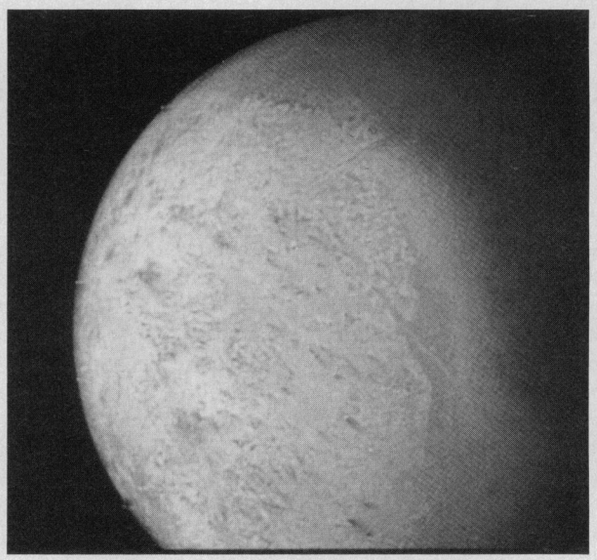
Other major scientific developments of 1989. The choice of the PCR polymerase as the Molecule of the Year was not a simple one because 1989 saw major developments in many areas of science and technology. Some of these advances are the first steps to what may develop into major discoveries but were "runners-up" because their full applicability is not yet known. Some represent steady progress, but not breakthroughs, in fields of major importance. Listed here are the scientific developments of this year that, because of their great potential, were close competitors of PCR.

Most mind-boggling synthesis. The correct synthesis of the compound palytoxin, which has one sextillion (10^{21}) possible isomers, was a major triumph of synthetic organic chemistry. Palytoxin has the chemical formula $C_{129}H_{223}N_3O_{54}$; it has been described as a substance that gives "new meaning to the word macromolecule." Natural palytoxin is extracted from coral and is a potent toxin that once was used for poisoning spear tips. Its only known source—coral that live in a 6 foot by 2 foot tidepool near Hana, Maui—proved difficult to discover, because local lore had it that those who collected palytoxin were cursed. The curse apparently did not apply to chemists: 8 years of strategic planning and experimental work culminated this year in the correct synthesis of this complex compound. The mammoth project has yielded many new procedures and strategies that can be applied to other difficult organic syntheses.

Most controversial. One world problem whose solution could solve many others is overpopulation. So the availability this year of RU 486, a pill that is effective at halting gestation and thereby at terminating pregnancies, has caused great excitement. RU 486 also may be one of the most controversial drugs ever developed, with politics and ethics, not efficacy, at the center of the controversy. The pill is a steroid hormone analog that blocks the action of progesterone during pregnancy. Since progesterone is essential both for establishing and sustaining a pregnancy, blockage by RU 486 causes miscarriage. In developing countries, some 200,000 women die each year from bungled abortions, and, for them, an antigestation pill would be lifesaving. (At present, however, RU 486 is available only in France and China, thus raising the spector of a black market in RU 486 sales.) The full potential of RU 486 is only hinted at by available studies: in addition to terminating pregnancies, it facilitates problem deliveries, and its steroid-like actions may be effective in the treatment of certain types of cancer, Cushing's disease (which is characterized by excess cortisone production), wound healing, and glaucoma.



Most universal appeal. The year 1989 was a banner one for space exploration. The "Planet of the Year" was clearly Neptune and the "Spacecraft of the Decade" was clearly Voyager 2. On 24 August, Voyager's Neptune flyby capped a more than 10-billion-kilometer Grand Tour of the solar system, and the final sets of dazzling images



of planets, moons, and rings (of a total of 100,000 sent altogether) and bits of data (of 5 trillion sent) were beamed back to Earth. The Voyager mission, which included two Voyager spacecraft, began in 1977 and has been an unmitigated success. Voyager 1 visited Jupiter and Saturn; Voyager 2 visited the Jupiter, Saturn, Uranus, and Neptune systems. Of the outer planets only Pluto was not in the right place at the right time for a flyby. Another space highlight of the year was the Soviet Phobos mission, which provided new information about Mars before contact with Earth was lost. And two important missions were begun: the Magellan spacecraft set out for a 1990 encounter with Venus, and the Gallileo spacecraft left for Jupiter, which it should reach in 1995.

Most likely to succeed. Various technologic advances have, over the years, made possible the preparation of pure populations of highly specific antibody molecules; such antibodies bind to target substances (antigens) and have many uses in vivo and in clinical and laboratory tests. The latest advance, which was made this year, is likely to revolutionize the antibody industry: it involves antibody production by genetically engineered bacteria. A complete "library" of antibody genes from an animal can be introduced into bacteria and the binding regions of antibodies produced. The antibodies are screened for the desired specific reactivities. The appropriate gene is then amplified and antibodies churned out in quantity, and it is a comparatively easy job to screen a million molecules a day. This procedure may generate antibodies with higher affinities than those induced in animals; immunization is not necessary, and therefore difficult immunizations—for example, where the appropriate antigen is not known or is toxic—no longer present a problem. This system should be useful for producing antibodies that catalyze enzyme reactions, that bind to target antigens in vivo or in diagnostic tests, that function as biosensors, and that facilitate clinical and basic research.

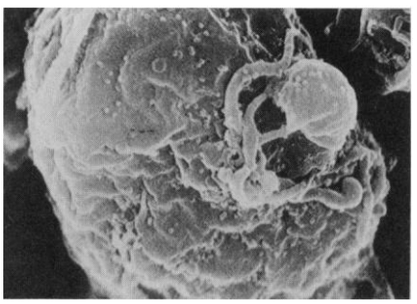
Most absorbing. The ability of scientists and engineers to depart from and improve upon what nature has to offer is not unique to the biological sciences but has also been a goal of materials science research. The 1990s have in fact been designated by the National Research Council as the "Age of Materials." Many novel materials that are lighter, stronger, or harder than known substances or have other exceptional features have been fashioned by materials scientists by combining molecules and atoms in new and different ways. An interesting example that was unveiled in 1989 was Stealth technology; the goal of this technology is to evade radar detection, and the B2 Stealth bombers absorb, rather than reflect, radar signals. Along with the advanced materials, advanced methods for fabricating them, such as molecular beam epitaxy and laser vapor deposition, came into wide use this year.

Best supporting actor. An extremely potent new immunosuppressive drug, FK506, was made available this year for experimental and clinical use. Immunosuppression is crucial to the success of tissue and organ transplants but, until now, only cyclosporin A has been widely used for suppressing the immune system. FK506 and cyclosporin A have entirely different structures; therefore, it was surprising that their actions were much the same. Each binds to an abundant (but different) protein inside target cells. Each inhibits the enzymatic activity of the target protein. The two target proteins appear to have the same enzymatic activity and to influence the folding of cellular proteins, the transduction of signals in cells, and the activation of certain lymphocytic cells. Comparisons of the actions of these two different immunosuppressors may assist in sorting out the molecular events that work together to bring about immunosuppression.

Most refreshing. September brought some long-awaited good news for individuals with cystic fibrosis (CF) and CF carriers: the CF gene was identified. There is no animal model for this disease, and the identification of the gene was an important milestone in CF research. In CF, the lungs, the pancreas, and the sweat glands all malfunction; a thick mucus clogs the lungs and this promotes opportunistic infections that destroy lung tissue; affected individuals usually die before they reach the age of 30. The identification of the CF gene is expected to have immediate payoffs in screening for carriers of the gene, in prenatal diagnosis, and for developing therapies based on improved understanding of the consequences of the genetic defect. The predicted sequence of the protein product of the CF gene has been helpful in suggesting a function for this protein in normal individuals and the nature of the aberration in affected individuals.

Most penetrating. Although the scanning tunneling microscope (STM) was developed some years ago, widespread use of this technology really began in 1989. STM provides atomic-scale information about surface topography. Images of a surface's atomic peaks and valleys are generated as a probe moves along the contours; electrons jump from the scanning probe to the surface, tunneling into "forbidden" areas, when an electric field is applied. The atomic structures and electronic properties of semiconductors, high-temperature superconductors, and biologic molecules in air and under water are now being almost routinely determined by STM and its offshoot, atomic force microscopy. A recent innovation involves the use of the sharp tip of the scanner for drawing lines on and punching holes in the surfaces of crystals; this application of the instruments for a purpose other than scanning and tunneling surfaces may make it possible to generate nanometer-size patterns on electronic devices.

Most scrutinized disease. AIDS continues to be elusive, but this year some headway may have been made both in treatment and in vaccine development. The search for effective drugs for treating AIDS patients has intensified recently as the efficacy of AZT, the only currently approved AIDS drug, has begun to wane. AZT often works well for many months; but after about 6 months, AIDS viruses begin showing reduced susceptibility to the drug, and by 18 months the drug's clinical value is sharply reduced. In early summer, the drug ddI passed muster in preliminary clinical trials designed to determine its toxicity. A group of AIDS patients, some of whom could not tolerate AZT, tolerated this purine analog for a period of 42 weeks and had increased energy levels and some improved immune functioning. This month the possibility was raised that protective immunity against AIDS might be induced with a vaccine: in a monkey model, vaccination against a simian immunodeficiency virus prevented AIDS-like disease when the macaques were later challenged with lethal viruses. The study raised the possibility that protection might be achieved even if infection was not entirely prevented.



Most fundamental. Physicists are no longer losing sleep trying to generate enough Zs. Z^0 bosons are the vectors of the weak nuclear force. The standard model of high-energy physics posits that all elementary particles are members of distinct but parallel families. Physicists have long thought that the number of such families is three, but, in theory, more families are possible. This year at the Stanford Linear Accelerator Center, the CERN European Center for Particle Physics, and the Fermi National Accelerator Laboratory, Z^0 bosons were for the first time produced in quantities that were sufficient for analyzing particle masses and lifetimes. The results from CERN and Stanford have narrowed the family number to three, thus bolstering the validity of the standard model as well as the validity of the Big Bang theory for the origin of matter.

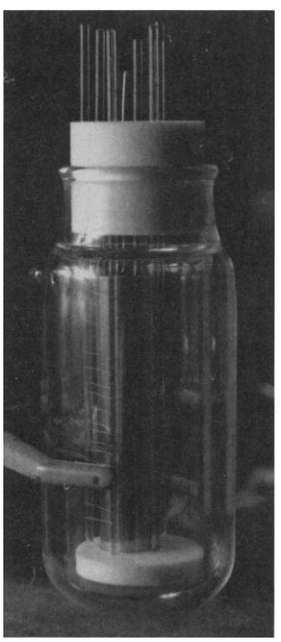
Most heated discussion. The condition of the global environment, both present and future, was one of the biggest scientific topics last year, and still at issue this year are many of the same questions. Has global warming from greenhouse gases been detected, when might larger changes occur, and what should be done about the situation? New measurements of the depletion of ozone in the atmosphere and the expansion of the ozone hole suggest that ozone losses are occurring outside the hole, although the specific mechanisms of this type of loss are unclear. Clouds continue to be of interest because of their role in the earth's energy budget and because of the part played by polar stratospheric clouds in chemical reactions that lead to ozone depletion. Global climate models, which are undergoing steady improvement and have been used more and more, should figure into the future environment-related decisions and actions of the international community.

Most productive. In June, a genetically engineered kidney hormone was approved for use by the U.S. Food and Drug Administration. This could turn out to be one of the biotechnology industry's big commercial medical successes. Erythropoietin, known by the trade name Epogen, stimulates the production of red blood cells. In normal individuals, the natural hormone is instrumental in replenishing the billion or more red blood cells that turn over each day; in patients with kidney failure (of whom there are 95,000 in the United States), there is a tremendous red blood cell deficit. These patients typically receive regular post-dialysis transfusions, because dialysis leaves them weak and anemic. However, with injections of Epogen, red blood cell production resumes, the patients are ener-

gized, and the need for transfusions is obviated. (Patients with AIDS and certain cancers might also benefit from the boost to red blood cell production provided by Epogen.) If, like Epogen, other blood products can be made commercially by genetic engineering procedures, reliance on transfusions might be lessened and the incidence of transfusion-associated cases of AIDS and hepatitis could be lowered. Already in the commercial pipeline are other blood products like factor VIII.

Most original. RNA molecules, which once were thought to be the middlemen in the process by which proteins were made from DNA, have come into their own. RNA can cut, splice, and assemble RNA sequences without help from any other types of molecules. It is now clear that in RNA-protein complexes, the RNA segments do the catalytic work while some of their partner proteins serve mainly in structural capacities. Thus it may eventually be possible to block gene expression inside cells or interfere with virus infections through the use of catalytic RNA molecules. It is also becoming clear how transfer RNA molecules, which take amino acids to the ribosome where they are added to growing protein chains, get properly "charged" by synthetase enzymes. The discovery that RNA can be self-sufficient lends support to the hypothesis that the most primitive biotic world was an RNA-based world; it was into this world that proteins came only later to specialize in some of the activities that RNA could already do.

Most tantalizing. If one science news event most excited the imaginations of the scientific community, the public, and the press it was certainly the claim in March of the achievement of cold nuclear fusion in a jar. The prospect that a plentiful and cheap energy supply might become available for an energy-hungry world engendered great interest and excitement. As soon as the claim was made, laboratories all over the world scrambled to repeat the experiment, apparently to no avail. Yet some still believe something intriguing is going on in those jars, although what it is continues to elude explanation. Investigations of cold fusion continue but at lower energy levels.



Most earthshaking. For some time, structural engineers in California have been devising ways to shore up structures against earthquakes, and a test of their work came this year. On 17 October 1989, an earthquake with a mainshock magnitude close to 7 rocked the San Francisco Bay Area. In the city's hard-hit Marina district, artificial fill amplified the waves of the earthquake, and many buildings were destroyed. Elsewhere the extent of damage varied, and altogether fewer than 100 people were killed. The experience pointed up the importance of reinforcing older structures and of attending closely to new building design and city planning (on what types of soil should buildings be built?). These are currently the best defensive actions that can be taken against earthquakes, because consistently accurate short-term predictions of earthquakes are still a long way off. Earth scientists have been gaining new insights into dynamic features of the mantle and core through seismologic and experimental studies (in particular, through use of the diamond anvil cell) and theoretical considerations. New microanalytic techniques have increased understanding of reaction mechanisms and processes in the earth, have improved dating accuracy, and should soon provide data on isotopic compositions of individual mineral grains.

Wide World

Most antithetical pair. There is increasing understanding of the types of genes that contribute to the development of cancer and how they do it. A fruitful new approach for cancer therapy may someday involve intervention in the expression or loss of expression of cancer-associated genes. Two functionally opposite types of genes, both active in normal cells, play a part in tumor development. Oncogenes are cellular genes that affect normal growth, development, and the transduction of signals in cells; disruption of the normal functioning of oncogenes (for example, when they are picked up by tumor viruses or acted on by carcinogens) leads to abnormal growth of cells and the development of cancer. Other genes, the tumor suppressor genes, act in a very different manner. They normally block tumor development and drive cells toward normality, but, when specific mutations accumulate in a cell or when tumor suppressor gene functions are lost, cancers can arise. The balance struck between oncogenes and tumor suppressor genes appears key to whether cell behavior will be normal or aberrant.

Most thought-provoking. Can a machine be taught to think like a human? And, if the workings of the machine can be delineated, will the workings of the human mind also become clear? These twin questions are at the center of neural network, or connectionism, research, a fiercely competitive interdisciplinary field (combining electrical engineering, neurophysiology, physics, behavioral psychology, and others) that has gained new momentum in the past 2 years. (For example, the United States is reported to have close to 3000 researchers already working in this area, Japan has several hundred, and the European Economic Community has 1000.) Learning algorithms are being designed, circuits are being wired up like neurons in the brain (each usually with many inputs but only one output), and machines are learning such skills as how to pronounce previously unencountered English words and how to calculate the curvature of an image from its shading patterns. The commercial potential of robots that can write, speak, and in other ways interact with their environments appears to be limitless; what insights such machines will provide about how thinking occurs remains to be seen.