

Molecular biology

tions for the treatment of solid cancers, which, because of low oxygen tension in the tumor mass, are generally more resistant to irradiation treatment than surrounding healthy tissue. It would, therefore, be desirable to make these tumors at least as sensitive as the more fully oxygenated surrounding tissue by the use of such drugs.

Future trends. After several years of decline and stagnation, the U.S. budget for science appeared to be on the increase. The emphasis, however, would be on applied research, with the National Science Foundation proposing to eliminate its science development grants and further reduce funds for graduate traineeships and institutional grants for science. Basic research funds would increase slightly, but the largest increases would be for mission-oriented research and development. Funds for research on cancer, and probably on diseases of the heart and lungs, would be increased sharply. Thus, human cells and their viruses would be the new *E. coli* as molecular pathology replaced molecular biology.

—Jeremy Baptist

Genetics

Molecular genetics remained the focus of intensive exploration, with ever more sophisticated techniques, and ever more specific questions being asked about cell mechanisms. The year was marked by no excitements to compare with the discovery the year before of reverse transcriptase, an enzyme responsible for the production of deoxyribonucleic acid (DNA) from a model provided by ribonucleic acid (RNA), a thoroughly unexpected operation for RNA. There was, however, growing appreciation that every seemingly simple mechanism of DNA is involved in increasingly more complex networks of relationships within the cell.

This overall complexity, rather than any profound difference in basic operation, may well have accounted for the puzzling recalcitrance of some underlying problems in the molecular biology of eukaryotic cells (those with distinct nuclei). These problems included the physical structure of the chromosome and the mechanisms of regulation of gene activity—problems that are rather well understood now for the simpler viral and bacterial systems. In responding to these challenges of higher organisms, molecular geneticists began crossing traditional boundaries that once compartmentalized biological research.

Reverse transcriptase studies. The main thrust of studies with the enzyme reverse transcriptase was the tracing of viruses newly implicated in cancer in primates and perhaps in man. Research

groups led by Sol Spiegelman at Columbia University, David Baltimore at the Massachusetts Institute of Technology (MIT), and J. Ross at the U.S. National Institutes of Health (NIH), Bethesda, Md., also used this enzyme with a sample of messenger RNA (m-RNA) as a template. The m-RNA, isolated from immature red blood cells of rabbits and other mammals, was prepared in a way believed to encode for the globin protein portion of hemoglobin. In a cell-free protein-synthesizing system—containing ribosomes, amino acids linked to transfer RNA (t-RNA) molecules, iron-containing hemin molecules, and the appropriate enzymes—this m-RNA did indeed effect the synthesis of mammalian hemoglobin. The DNA that was synthesized under the influence of the reverse transcriptase may, therefore, be equivalent to the gene for the globin, an identity that remained to be confirmed.

Apart from speculations about its use for the genetic "engineering" of tissue cells, the ability to produce quantities of a specific gene would be technically invaluable. It would be an ideal method of assaying specific m-RNAs in cells at different stages of development or in different states. It would also facilitate the analysis of the nucleotide sequence of RNA through stepwise synthesis of the corresponding DNA by the action of transcriptase. In its natural occurrence, the DNA for a specific gene accounts for less than one millionth of the DNA content in a mammalian cell, which evokes despair about attempts to isolate it directly. However, m-RNAs, which reflect the activity of specific genes, are produced differentially in various tissues, and often amplified manyfold. We can, therefore, be more optimistic about extracting specific RNAs, and then using reverse-transcriptase to produce the DNA corresponding to a specific gene.

Further studies with m-RNAs will be facilitated by the finding, by J. B. Gurdon and his colleagues at the University of Oxford, that living frog eggs can "translate" injected samples of RNA into their corresponding proteins. The eggs are a relatively simple biological system technically and retain their activity for 24 hours or longer, during which each m-RNA molecule can be translated many times. The system can recognize and encode into DNA as little as one billionth of a gram of RNA added to it.

Reverse transcriptase is inactive on pure, single-stranded RNA templates; a short segment of complementary RNA is required as a primer to which DNA can attach and assemble itself. This primer segment functions like the poly-A (adenine)-rich ends found naturally in many RNAs. In the reverse transcriptase experiments just described, the prim-

er was a synthetic poly-T (thymine), which binds to the complementary As on the RNA and helps initiate DNA synthesis.

Similar requirements had been established in 1967 by A. Kornberg's group at Stanford University for the replication of viral DNA. In later studies it was found that the RNA polymerase enzyme probably initiates replication in bacterial cells infected with the bacteriophage (virus) M13. RNA polymerase was now commonly thought of as the agent of translation, the production of m-RNA copies of DNA genes. Short segments of such copies may also be indispensable for the initiation of replication of DNA, which once begun is sustained by DNA polymerase.

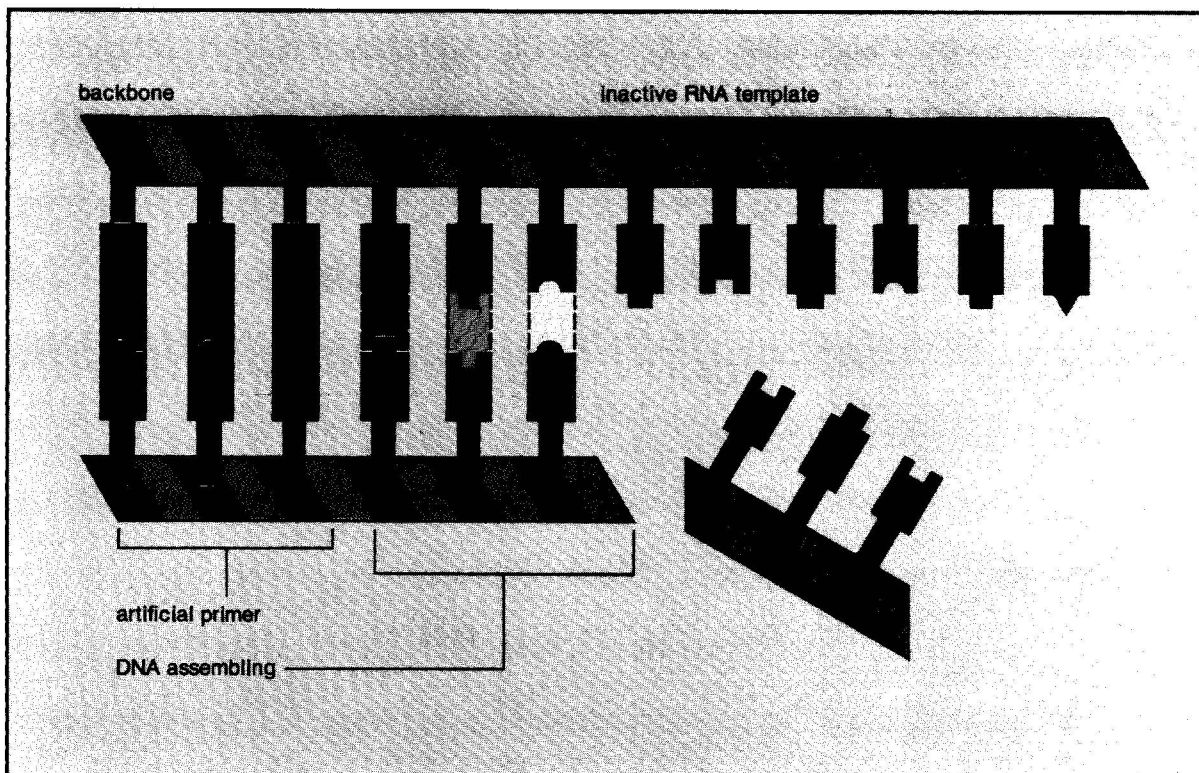
Ligase and the swivelling enzyme. Ligases, enzymes that repair nicks in one strand of a duplex of DNA, were now a routine part of the repertoire of molecular genetics. V. Sgaramella, H. H. van de Sande, and H. G. Khorana (working at the University of Wisconsin) reported that ligase associated with the bacterial virus T4, besides showing the nick-sealing activity of other ligases, can join free ends of two separate DNA duplexes. The application of this tool to biologically active genes derived

from various types of cells was under active study in several laboratories.

Many DNA molecules are found in closed circles, and much thought has been given to the way a double helix might be wrapped into simple circles without excessive twists. When DNA circles were proven to be pure DNA lacking any protein linkers, wrapping was thought to be due to a combined action of nicking enzymes and sealing enzymes. J. C. Wang at the University of California at Berkeley and J. J. Champou and R. Dulbecco at the Salk Institute, San Diego, Calif., have now described a protein (swivelling enzyme) that appears to untwist supercoiled DNA molecules. The reaction appeared to occur at one turn at a time, rather than the simultaneous relief afforded by a nicking enzyme. The molecular mechanism of the swivelling enzyme would be difficult to determine unless intermediate stages could be captured.

Solving DNA puzzles. The molecular requirements for DNA recombination were illuminated by studies on a protein coded by T4 and called simply the product of gene 34 of that virus. Because an enormous number of gene-34-protein molecules are produced during viral growth, some struc-

In the test tube the enzyme reverse transcriptase requires a primer to initiate DNA production from an RNA model, or template. The primer is poly-T (dT), a synthetic of the nucleic acid thymine. When this piece of artificial RNA bonds with its complementary nucleic acid, adenine (A), on the template, the RNA becomes active. DNA then begins assembling with the aid of the enzyme.



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tural rather than catalytic role for the protein had been suspected. Bruce M. Alberts and Linda Frey at Princeton University isolated the protein and showed that it binds to single-stranded DNA in such a way as to minimize self-associations and to encourage the unwinding of duplexes, in effect stretching out sequences to enable their exposure to complementary ones. The addition of gene-34-protein, therefore, facilitates the resynthesis of DNA that has been melted with the strands separated. The protein may be a prime mover in the mechanism of genetic recombination. More recently Y. Hotta and H. Stern at the University of California at San Diego found that this protein, designated by them "meiotic protein," is almost always found in cells in meiosis (reduction division).

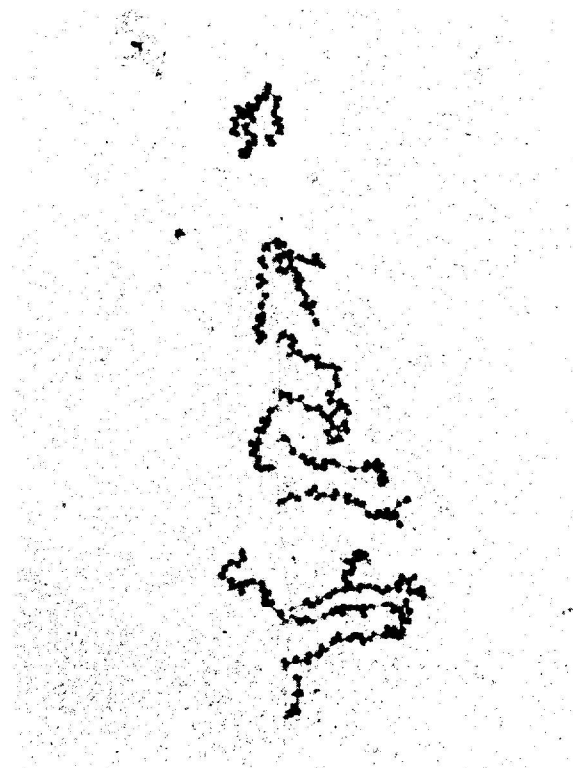
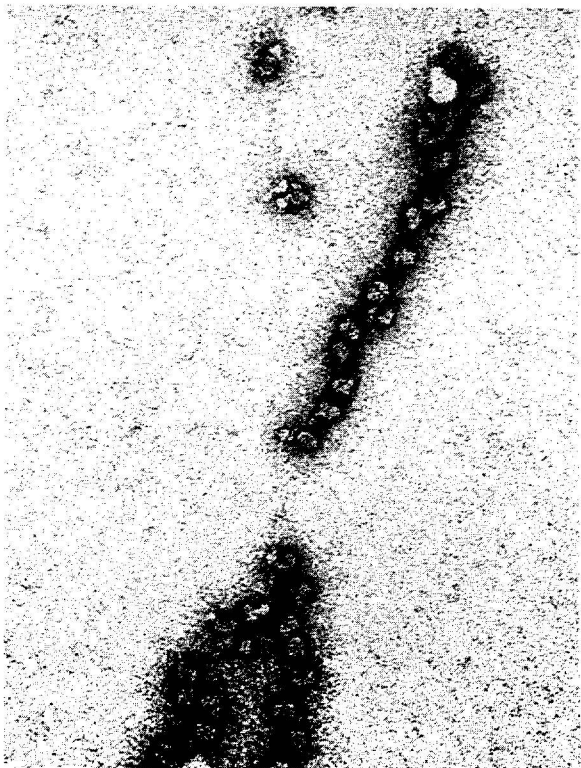
Of growing interest was the work by L. S. Lerman at Vanderbilt University, Nashville, Tenn., on another puzzle of DNA—how the extended fibers of the double helix of a phage particle in an infected cell can be condensed and packaged into the tiny space of the phage head. Similar problems of DNA compression in the sperm head and in chromosomes in higher forms had been explained (correctly?) by invoking complexes with basic pro-

teins that neutralize the multiple negative charges of the DNA phosphates and allow tight compaction. Lerman found a remarkable collapse of the extended structure of DNA solutions—but no chemical binding—in the presence of neutral water-soluble polymers. The phage head contains the compact DNA until the phage infects a new host and the DNA springs back into expanded form.

Cell fusion techniques. Parallel to the deepening of biochemical probes into basic genetic functions was the explosive development of somatic cell genetics. The underlying theme was the progressive domestication of tissue cells in culture. This consisted primarily of the accumulation of cell lines and techniques to display precise features of chromosome content or of enzymatic function—in all events revealing the potentialities and the regulation of the genetic information in the cell. The most striking advance was the fusion of cells, in many cases facilitated by the use of an enzyme associated with inactivated virus particles.

After cells from different species have been fused, clones of hybrid cells can be found that may contain a sum of the parents' chromosomes, many of which are unfortunately unstable in such com-

Transcription, the copying of a DNA sequence into messenger RNA, and translation of that information into protein can be seen occurring simultaneously in the bacterium Escherichia coli. Special electron microscope techniques developed at Oak Ridge (Tenn.) National Laboratory captured RNA linked by spherical ribosomes attached to strands of DNA magnified 306,000 times (left) and 72,000 times (right).



Courtesy, Dr. Barbara A. Hamkalo and Dr. O. L. Miller, Jr., Oak Ridge National Laboratory

binations. In the fusion of a mouse cell and a human cell, the human chromosomes are particularly unstable. Such instability is a mixed curse: it interferes with the observation of controlled variations from the norm, but it also produces a rich variety of cell types, with different sets of chromosomes whose effects can then be measured.

The pathologist Henry Harris at Oxford, who introduced virus-mediated fusion for genetic analysis, pursued it with particular vigor to study the genetic basis of malignancy. The results vary considerably depending on the particular cell lines under study; however, overall malignancy is attenuated, or even extinguished, when cancer cells are augmented with normal chromosomes. These observations were the first steps to a formal genetic analysis of the chromosomal basis of malignancy. They would advance much further as techniques were developed for identifying specific chromosomes and for the construction of cells with a normal complement of chromosomes of known origin.

Cell fusion techniques also offered a unique opportunity to study the possible genetic role of particles in the cytoplasm of cells. The occurrence of DNA in mitochondria and other particles suggested that these may be more than mere passive reflectors of the information in chromosomal DNA. In several experiments it appeared that the human chromosomal contribution was overridden by the mouse contribution, arguing for negative control as a common cellular mechanism. Negative control was also inferred from studies on biochemical attributes, including regulatory functions. The conclusions of all such studies indicated: (1) that unexpressed functions in a cell derive from negative control (that is, the active inhibition of certain genes, an inhibition that also regenerates new chromosomes); (2) that these functions can be revived upon extraction of the controlling factors; and (3) that the controlling factors are stably associated with certain chromosomes.

The nature of the stable activation of the controlling chromosome was the central challenge to developmental genetics. One hypothesis had some support from studies of X-chromosomes: stable changes of a chromosome are often deletions of some part of the chromosome. The activation of a controlling chromosome would then result from removing a small regulating unit from it, unleashing the negative control over other specific gene functions. In other cases, such deletions are already known to unleash positive functions.

While these studies were still at an exploratory stage, cell fusion was a well-established method for mapping genetic mutations on various human chromosomes, greatly accelerating work that

formerly required the painstaking collection of pedigrees often insufficient or inappropriate to allow firm conclusions. In the course of gene mapping F. H. Ruddle's group at Yale noted the first evidence of a translocation (breakage and new union) between a mouse and a human chromosome, which may reflect the actions of a two-strand-repairing ligase, as noted earlier.

Testicular feminization. During the year testicular feminization (Tfm), a fascinating rare genetic disease, was critically reviewed. It is a disturbance whereby an XY embryo, normally male, is switched into a female pattern of development, but is sterile. A similar condition in the mouse is firmly linked to the X chromosome. S. Ohno (City of Hope National Medical Center, Duarte, Calif.) and his colleagues offered an explanation of the defect: the body cells of the Tfm type appear to be unable to respond to testosterone for lack of a primary protein receptor for it. The Tfm "female" thus differs from normal females as well as from males, both of whose tissues do respond to male hormones.

—Joshua Lederberg

Obituaries

The following persons, all of whom died between July 1, 1971, and June 30, 1972, were noted for distinguished accomplishments in one or more scientific endeavors. Biographies of those whose names are preceded by an asterisk (*) appear in *Encyclopaedia Britannica*.

***Adams, Roger** (1889—July 6, 1971). U.S. organic chemist and recipient of the National Medal of Science, Adams was head of the University of Illinois department of chemistry for 28 years. He received an A.B. degree (1909), an A.M. (1910), and a Ph.D. (1912) from Harvard University, and then studied at the Kaiser Wilhelm Institute in Berlin before returning to Harvard as an instructor. In 1916 he went to the University of Illinois as an assistant professor, served as head of the department of chemistry and chemical engineering (1926–54), and became research professor emeritus in 1957. Adams and his students explored the many methods of organic synthesis, determined the structures of synthetic and natural products, and discovered a platinum oxide catalyst for further research in organic chemistry and biochemistry. He investigated (1947) the structure of marijuana, isolating and identifying cannabidiol, and then synthesized a drug that was 70 times stronger.

Among the many honors bestowed upon Adams were the Davy Medal of the Royal Society (London, 1945), the Priestley Medal of the American Chem-