Nobel prize in chemistry to Berg, Gilbert and Sanger

The 1980 Nobel prize in chemistry was awarded last month to Paul Berg (Stanford University), Walter Gilbert (Harvard University) and Frederick Sanger (Cambridge University), for their work on the biochemistry of DNA. Half of the $210 000 chemistry prize was awarded to Berg for "his fundamental studies of the biochemistry of nucleic acid, with particular regard to recombinant DNA." The other half of the prize is shared between Gilbert and Sanger "for their contributions concerning the determination of base sequences in nucleic acids."

Gilbert took his PhD in theoretical physics under Abdus Salam at Cambridge. Before completing his metamorphosis into a molecular biologist in the early 1960's, he did postdoctoral work in elementary-particle theory with Julian Schwinger and then joined the Harvard physics faculty. For Sanger, this is the second Nobel prize in chemistry. In 1958 he was awarded the prize for his determination of the structure of insulin. Berg, whose Stanford group spliced together the first hybrid DNA molecule from the genetic material of different species, played a central role in calling for a moratorium and developing a set of safety guidelines for recombinant-DNA research.

Recombinant DNA. The hereditary message of a DNA molecule is embodied in the order in which four organic bases [adenine (A), thymine (T), guanine (G), cytosine (C)] are arrayed along the double helix. In general, a particular triplet sequence of these bases serves as an instruction for the insertion of a specific amino acid during protein formation, in a code that is essentially the same for all known life forms. In 1971 Berg and his Stanford colleagues David Jackson and Robert Symons succeded in splicing the 5-gene DNA molecule of the mammalian tumor virus SV40 into a DNA loop containing bacterial genes from *E. coli* and viral genes from bacteriophage lambda.

The original intention was to reinsert this hybrid genetic message into a bacterium, where it would be faithfully replicated as the bacterium multiplies. This is the essence of the recombinant-DNA method—a technology that now bids fair to become a major industry. One uses bacteria to replicate an alien gene in very large numbers—thus making available in useful quantities the gene or the protein for which it serves as a code. This has been described as advancing from the arduous techniques of vaccine production in animal tissue to the much simpler business of bacterial growth—essentially beer-brewing technology. Protein molecules previously available only in small numbers become easily obtainable in macroscopic quantities, either for chemical studies or for pharmaceutical purposes.

Berg originally planned to reinsert his hybrid DNA into *E. coli*. When the potential dangers of implanting a mammalian tumor gene in this ubiquitous intestinal bacterium were pointed out, he terminated the experiment in 1972. Two years later, when improved techniques had made such implantation relatively easy to carry out, Berg argued on behalf of a temporary moratorium and a conference to formulate safety guidelines for recombinant-DNA research.

The splicing of specific genes into chosen sites on a DNA molecule is made possible by the existence of "restriction" enzymes, and by the capacity of gel electrophoresis to separate DNA fragments by length. Restriction enzymes cleave the DNA double helix at sites where particular short sequences of bases occur. A collection of DNA fragments of different lengths produced by the restriction enzymes can be separated according to size by the distance through which they migrate in a gel subjected to an electric field. DNA fragments are spliced together by using enzymes that produce "sticky ends"—short single-strand protrusions to which "complementary" base sequences will adhere.

Base sequencing. The fact that gel electrophoresis can distinguish DNA

BERG

GILBERT

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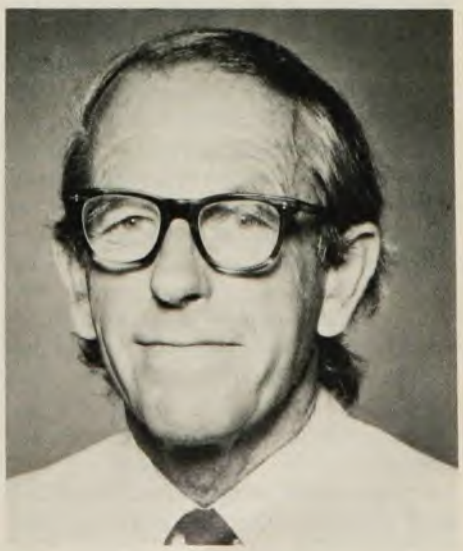

JOE WRINN

fragments differing in length by only a single base—a resolution of better than 5Å—makes possible the techniques by which Sanger and Gilbert in the mid-1970's revolutionized the determination of the sequence of bases along a DNA molecule. In the early 1970's it had taken Gilbert and his student Allan Maxam several years to work out the base sequence of a DNA strand only 20 bases long. It is now possible, by the methods for which Gilbert and Sanger won the prize, for a single person to sequence a strand of several hundred bases in a day. In 1977 Sanger's Cambridge group performed the first determination of the entire base-sequence code of a complete organism—the 5347-base genetic message of the bacterial virus $\phi X174$.

The rapid sequencing method developed by Gilbert and Maxam in 1975 employs four chemicals (not enzymes), each of which breaks DNA at the site of a particular base. When, for example, the chemical that attacks only G sites is applied to a large number of identical DNA strands that had been radioactively labelled at one end defined by a restriction-enzyme cut, the result is a collection of fragments of many different lengths—each corresponding to the distance between the labelled end and some occurrence of G further down the line. (It is essential to apply the chemical in sufficiently dilute solution that not all G sites are broken.) One can then easily read off all the G sites from an autoradiograph of the gel-electrophoretic separation of these fragments by length. Laying four such autoradigraphs side by side, each produced by one of the four chemicals, one gets a complete map of the order of the bases G,T,A and C on the original DNA strands.

Sanger's sequencing technique is similar in spirit and efficiency. An enzyme is used to copy strands of DNA cleaved at known sites. But mixed in with the nucleotide triphosphates that provide the bases for the copy are analogs—fakes that closely resemble the four bases. When, for example, copying takes place in the presence of a small amount of dideoxythymidine, some of the sites where a T should have been copied, will get a counterfeit analog of T that brings the copying process to an abrupt halt. Each of the four base analogs can then produce an autoradiographic map just as is done with the four chemical cleavers of Gilbert and Maxam. The sequencing techniques of Gilbert and Sanger are currently used interchangeably in hundreds of laboratories throughout the world. —BMS

Gel collapse is phase transition

An infinitesimal change in temperature or fluid composition can, under the right conditions, abruptly collapse a gel to 1/500th of its original volume. This appears to be a phase transition in the classic thermodynamic sense. A gel is a crosslinked polymer network that holds a fluid in its interstices—for example, a jellied consommé. Considerable theoretical and experimental work has been done since the 1940's on the sol–gel transition, the phase transition from the liquid to the gel state. But prior to the recent work of Toyoichi Tanaka and his colleagues at MIT,[1,2] it was not known that a phase transition could occur within the gel state itself.

This newly discovered phenomenon is a transition between two gel phases, differing only in the degree of swelling—by as much as two orders of magnitude. It suggests that gels might provide useful mechanochemical engines or artifical muscle fibers. The study of these phase transitions may also shed light on pathological changes of gels in the human body, for example retinal detachment, caused by a sudden shrinkage of the vitreous humor in the eyeball.

Critical opalescence. The first hint of such a phase transition came in 1977, when Tanaka was studying the temperature dependence of thermal density fluctuations in gels by means of laser light scattered by the gel. As he lowered the temperature of the polyacrylamide gel under study, the clear gel developed what appeared to be "critical opalescence"—a sudden clouding, with a strong increase in the intensity of the scattered light. Correlation studies of the noise pattern of the scattered light showed that the characteristic relaxation time of the fluctuations of polymer concentration was diverging to infinity. This is analagous to what one sees when supercritical steam is cooled to its "critical point," the point on the phase diagram where the distinction between the liquid and vapor phases first appears. Above this critical point (647 K at 217 atmospheres) the discontinuous density difference between steam and water vanishes.

The observation of critical phenomena such as critical opalescence thus implies the onset of discontinuous phase transitions. Tanaka told us it was not at first obvious that he had seen a critical phenomenon. But when he added acetone to the gel fluid to raise the temperature at which opalescence occurred, he discovered the discontinuous volume changes that signaled the phase transition lying below the critical temperature.

The acrylamide gels studied in Tanaka's laboratory consist of long polyacrylamide chains cross-linked by bisacrylamide monomers. It turns out that the thermodynamic behavior of these gels depends crucially on their degree of ionization. On the unadulterated polyacrilamide network, every second carbon has an aminocarbonyl ($CONH_2$) side chain. If one treats the gel for an extended period with a basic solution, some of the side chains are hydrolized to carboxyl (COOH) groups. The degree of conversion depends on the hydrolysis time, reaching a maximum of about 25% carboxyl groups after 60 days. After the hydrolyzing base is washed away and the gel is saturated with a water–acetone mixture, the carboxyl groups liberate hydrogen ions into the gel fluid, leaving fixed negative charges on the polymer network.

When the temperature of such swollen gels is gradually lowered (or equivalently, when the acetone concentration is gradually increased), a discontinuous volume change is seen only in the strongly ionized gels. That is to say, the abrupt shrinkage that signals a phase transition is observed only in gels that had been hydrolized for at least six days. Tanaka and his MIT colleagues David Fillmore, Shao-Tang Sun, Izumi Nishio, Gerald Swislow and Arati Shah have recently reported[2] that a maximally ionized acrylamide gel (hydrolyzed for 60 days) will shrink suddenly by a factor of about 350 when it is cooled through its transition temperature (25 °C in a 57% acetone solution). Gels hydrolized for lesser times will shrink by smaller factors, and at somewhat higher transition temperatures. Finally, for gels hydrolized less than six days, the discontinuous volume change is no longer seen; one has merely an inflection point on a continuous curve of volume against temperature.

Flory–Huggins theory. Tanaka told us that these phase transitions and their dependence on the ionization of the gel can be well understood in terms of the mean-field theory developed for gels by Paul Flory and Maurice Huggins in the 1940's. The first of the mean-field theories was introduced by van der Waals in 1873. All such theories approximate the difficult many-body interactions of fluids, ferromagnets and the like by considering only the interaction of any particle with the averaged effect of all other particles in the system.

With a conventional choice of gel parameters, the isotherms of osmotic pressure against gel volume derived from the Flory–Huggins theory turn out to be monotonic; as the gel expands at fixed temperature, its osmotic pressure (its tendency to suck up fluid) falls.