They do not have to grow the cells in culture for several weeks to get enough to analyze, and they can usually have the results of the test as early as the 16th or 17th week of the pregnancy.

The genetic defect causing sickle cell anemia is both simple and well understood; there is a substitution of a single wrong nucleotide in the β globin gene. In contrast, the thalassemias are neither so simple nor so well understood. Rather, they constitute a complex of several anemias, of varying degrees of severity, that may be caused by any of a number of different defects in the genes coding for the hemoglobin chains. Not all of these defects have been characterized but some have been described—again largely as a result of the availability of restriction mapping techniques. Among the laboratories contributing to the progress in understanding the gene defects underlying the thalassemias are those of Kan, Arthur Bank of the College of Physicians and Surgeons of Columbia University, Bernard Forget of Yale University Medical School, and Stuart Orkin of Harvard Medical School.

For example, the α thalassemias are caused by the deletion of some or all of the four copies of the α globin gene that are carried by most persons. The α thalassemias are relatively common in Southeast Asia, where as many as 15 percent of the population of some countries are carriers. In the severest form of the disease all four genes are missing. Fetuses with this condition are stillborn because the abnormal hemoglobin they produce cannot release its oxygen to the tissues.

About 2 years ago, Kan and his colleagues devised a way to detect the most severe forms of α thalassemia. They prepare fetal DNA from fibroblasts acquired by amniocentesis, shear it into fragments, and then measure the extent to which a probe for the α globin gene binds (hybridizes) to the fetal DNA fragments. Although this hybridization procedure can determine whether the gene is missing, it is technically difficult and requires large quantities of fetal cells.

In a more recent development, reported in July of this year, Orkin, in collaboration with investigators from Harvard, Yale, and Haceteppe University in Ankara, Turkey, showed that restriction mapping could be used for the prenatal diagnosis of a rare form of thalassemia.

Orkin says the restriction method is superior to the hybridization technique for prenatal diagnosis because mapping is more sensitive. It requires only 10 to 15 percent of the DNA required by the hybridization method and is generally easier to do.

The fetus they diagnosed was at risk from a thalassemia caused by a lack of both copies of the genes for β and δ globin. (δ globin, which is structurally very similar to β globin, is produced in small quantities by normal adults.) The mapping procedure showed that the genes were present, although in reduced quantities, and the Harvard investigators concluded that the fetus was a carrier but would not be severely afflicted by the disease. This diagnosis was confirmed both by examination of fetal blood and of blood taken from the umbilical cord at the infant's birth.

Thus far, only the rare forms of thalassemia caused by gene deletions can be diagnosed by restriction mapping. Some of the more common thalassemias, including Cooley's anemia, do not involve deletion of genes. (Exact figures on the prevalence of Cooley's anemia are hard to come by. According to some estimates, a few to several percent of the populations in such Mediterranean countries as Italy and Greece may be carriers of the condition.) But the common thalassemias may also prove amenable to prenatal diagnosis by restriction mapping once the gene defects underlying them have been revealed.

Any change in DNA that results in the gain or loss of a site recognized by a restriction enzyme will alter the pattern of restriction fragments produced by digestion of the DNA by the enzyme. Thus, at least in theory, any genetic defect is detectable by restriction mapping provided the appropriate tools—restriction enzymes and gene probes with the right specificities—are available. And they may become available. Already about 80 restriction enzymes that recognize different DNA sequences have been identified and more are still being found. Moreover, as Kan's work has shown, the altered restriction site need not be within the gene of interest, although it must be closely linked to that gene.

The principal reason why the current work has centered around defects in globin genes has been the ready availability of the appropriate probes. But probes for other genes may also be produced soon. For example, Thomas Maniatis of the California Institute of Technology is currently developing a library of cloned human genes that should prove useful for preparing probes. Thus, in the future, restriction mapping may prove feasible for prenatal diagnosis of a wide range of genetic defects.—JEAN L. MARX

The 1978 Nobel Prize in Physiology or Medicine

The 1978 Nobel Prize in Physiology or Medicine was awarded to Werner Arber, 49, of the Biozentrum in Basel, Switzerland; Hamilton O. Smith, 47, of Johns Hopkins University; and to Daniel Nathans, 50, also of Johns Hopkins University. The awards recognize the development of restriction endonucleases, enzymes that can be used to study genetic organization and to manipulate DNA for "genetic engineering." Arber is credited with having first predicted the existence of the enzymes, Smith with having isolated the first such enzyme and describing its specific reaction, and Nathans with having first applied these enzymes to the study of gene organization and regulation.

The development and application of the restriction endonucleases is a prime example of how scientific progress can be achieved through interlaboratory communication, with a concomitant "explosion" of results and developments simultaneously in many institutions throughout the world.

The story began in the early 1950's with the description of host-controlled variation among bacterial viruses (bacteriophages), probably most systematically presented by Salvador E. Luria of MIT and by Giuseppe Bertani and Jean J. Weigle. For example, when the bacteriophage lambda (Table 1), is isolated after growth on a particular strain of bacteria and replated on the same strain, it forms plaques (infects) with a relatively high efficiency. However, when these phage particles infect a different strain, only a few of them survive to form plaques. But the progeny of these survivors are adapted to the new strain in that they now plate with high efficiency on this strain. Thus, for example, when grown on *Escherichia coli* B, lambda is

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modified so that it becomes able to grow well on strain B; concomitantly its growth on *E. coli* K becomes restricted.

By 1958 Arber had received a doctorate from the University of Geneva for work done with Eduard Kellenberger and Weigle; he then worked in 1958 and 1959 in the United States with Bertani, Gunther Stent, Joshua Lederberg, and Luria. Upon his return to Geneva, Arber, with Daisy Dussoix, a graduate student in his laboratory, embarked on a study of the molecular basis of host-controlled variation.

In 1962, Dussoix and Arber reported their experiments showing that the host-controlled "modification" involved changes in the DNA of the phage and that "restriction" was accompanied by degradation of the DNA. Although the DNA breakdown was efficient in preventing phage infection, it was limited in that certain phage genes were still expressed in abortively infected bacteria.

Arber next reported in 1965 that methionine was required for the host-specific modification of DNA, and in 1966 William B. Wood—working in Arber's laboratory—showed genetically that the abilities to modify or restrict DNA were properties of the host and were applicable to bacterial DNA that was foreign to the cell as well as to virus DNA's.

Based on these accumulated observations, a model evolved in Arber's laboratory to explain restriction and modification (Fig. 1). According to the model, DNA would contain specific sites (sequences of nucleotides) that are recognized and cleaved by restriction endonucleases with a specificity characteristic of a particular bacterial strain. In order to protect its own DNA, the bacterium would have a methylase that recognizes the same sequence specificity; it would methylate such sequences, thus conferring a "modification" that protects them from the restriction endonuclease. The model was soon confirmed by (i) the isolation by Urs Kuhnlein and Arber of phage mutants that had become inert to restriction and modification by mutation at recognition sites; (ii) the direct correlation of DNA methylation with host-controlled modification that was observed in studies of the phage mutants by Kuhnlein, Arber, and John Smith of the Medical Research Council at Cambridge; (iii) the isolation of the *E. coli* B modification DNA methylase by Kuhnlein, Linn, and Arber; and, most notably, (iv) the isolation of restriction DNA endonucleases.

Table 1. Relative plating efficiencies of phage lambda on *Escherichia coli* strains B and K. Phages λ · B and λ · K were isolated after growth on strains B and K, respectively.

Phage	B	K
λ · B	1	4×10^{-4}
λ · K	10^{-4}	1

The recognition that site-specific DNA endonucleases might exist set off a burst of excitement among nucleic acid biochemists because of their appreciation that such enzymes would be invaluable for sequencing DNA as well as for studying its genetic organization. In addition, such enzymes would perhaps open up the possibility of manipulating DNA structures in the test tube.

In Japan, in 1966, the first demonstration of restriction activity in vitro appeared. Toshiya Takano, Tsutomu Watanabe, and Toshio Fukasawa observed an activity controlled by the R factor, N-3, which could specifically inactivate the infectivity of phage lambda DNA. The R factors are episomal elements of bacteria that are generally known for their ability to confer resistance to antibiotics on their host; however, in some instances they had also been observed to introduce a modification and restriction system into bacteria. Unfortunately, the assay system used by the Japanese workers did not suffice for the purification of this activity away from other nonspecific nucleases and the enzyme could not be exploited.

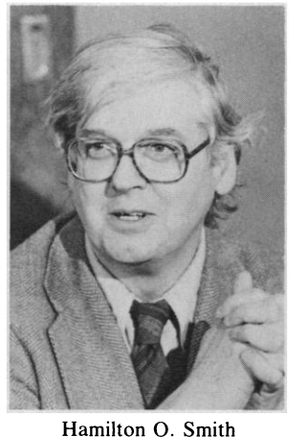
In 1968, Linn and Arber in Geneva reported the partial purification and characterization of the restriction endonucleases controlled by *E. coli* B and by phage P1, while Matthew Meselson and Robert Yuan similarly purified and characterized the enzyme from *E. coli* K. These enzymes were subsequently shown to belong to a peculiar class—now called type I—of restriction endonucleases that recognize specific unmodified DNA sequences, but cleave the DNA at relatively random locations that are far removed from the recognition site. This was disappointing because it meant that the type I restriction enzymes were unsuitable for studying and manipulating gene structure.

Two years later, however, Hamilton O. Smith and his co-workers K. W. Wilcox and Thomas J. Kelley reported the purification and characterization of the first type II, or "specific" restriction endonuclease. Smith had received an M.D. at Hopkins and, after an internship and residency, decided to do research in microbial genetics—first working at the University of Michigan from 1962 to 1967, then as a faculty member in the Microbiology Department at Johns Hopkins. In 1969, while studying the uptake of DNA from the *Salmonella* phage P22 into cells of *Hemophilus influenzae*, strain Rd, Smith and his co-workers made the chance observation that the phage DNA was extensively degraded. The degradative activity was subsequently observed in extracts from the bacteria, and was shown to be a restriction endonuclease by virtue of its inability to act on DNA from the extract source, *H. influenzae*. The restriction enzyme activity, subsequently called Hind II, was purified of contaminating activities and was shown to recognize the set of sequences

5′...pGpTpPy ↓ pPuPApCp...3′
3′...pCpApPup ↑ PypTpGp...5′

where Py and Pu represent any pyrimidine or purine residue, respectively, and the arrows indicate the cleavage sites of the individual strands. The pattern of a twofold rotational symmetry (both strands have the same sequence when read with the same polarity) has sub-


Werner Arber


Hamilton O. Smith


Daniel Nathans

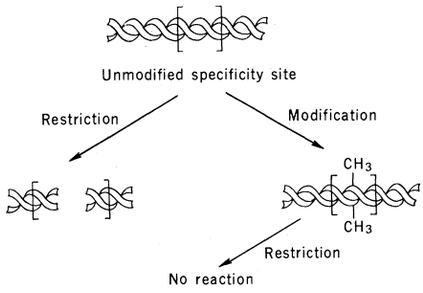


Fig. 1. A model for host-controlled restriction and modification based on early in vivo observations. [Modified from S. Linn *et al.*, in *The Biochemistry of Adenosylmethionine*, F. Salvatore *et al.*, Eds. (Columbia University Press, New York, 1977)]

sequently been found to occur for virtually all of the sequences recognized by type II enzymes.

The characterization of several R factor restriction endonucleases at the University of California, San Francisco, by Herbert Boyer and co-workers (including Howard Goodman, Daisy Dussoix, Robert Yoshimori, and Joe Hedgepath) rapidly followed. Those investigators purified the restriction enzyme Eco RI, which was originally thought to be controlled by an R factor but was subsequently shown to be controlled by a different kind of plasmid. They showed that Eco R1 recognized the sequence

$$5'...pG \downarrow pApApTpTpCp...3'$$
$$3'...pCpTpTpApA \uparrow pGp...5'$$

and cleaved it in the staggered manner shown. Likewise, the Eco RII, controlled by the R factor, R-15, was purified and shown to recognize the set of two sequences

$$5'... \downarrow pCpCp(\overset{A}{\underset{T}{}})pGpGp...3'$$
$$3'...pGpGp(\overset{T}{\underset{A}{}})pCpCp \uparrow ...5'$$

making the staggered breaks shown. As originally noted by Janet E. Mertz and Ronald W. Davis at Stanford, the staggered nature of the breaks and their ability to be rejoined at low temperature have important implications for the use of these enzymes as reagents. For example, since all DNA treated with Eco RI will have the same termini, "recombination" in the test tube becomes a relatively simple task (Fig. 2).

At present, more than 80 restriction enzymes have been purified and characterized, thanks in large part to the screening of a large number of bacterial strains for specific endonucleases by Richard J. Roberts at Cold Spring Harbor. The application of these enzymes to the characterization and engineering of DNA molecules over the past 7 years has virtually revolutionized molecular biology and molecular genetics.

The first applications of a restriction endonuclease to DNA characterization were reported in 1971–1972 by Daniel Nathans and his graduate student, Kathleen Danna at Johns Hopkins. Nathans received an M.D. from Washington University, St. Louis. In 1959, after his residency, he embarked on a career in biochemical research at the Rockefeller Institute; he then joined the faculty in the Department of Microbiology at Hopkins and is now director.

Having direct access to Smith's newly discovered Hind II restriction endonuclease, Danna and Nathans found that the double-stranded circular DNA genome of simian virus 40 (SV40) could be cleaved into 11 separable specific fragments. This work was followed in 1972 by an elegant series of experiments based on a paper from 1961 in which H. Dintzis showed that the direction of synthesis of the hemoglobin molecule was amino- to carboxyl-terminus. Danna and Nathans first deduced the physical order of their 11 SV40 DNA "restriction fragments" by analyzing for neighboring fragments which were contained in larger, incompletely broken pieces. Then, by pulse-labeling the DNA during its replication, treating the labeled DNA with the restriction endonuclease, and finally separating the various fragments, they could monitor the appearance of radioactivity in the individual fragments and hence deduce the order in which they were replicated. In this way, they showed that DNA replication begins at a unique origin and proceeds bidirectionally around the circle, terminating roughly 180° from the initiation point.

During the following year Nathans and co-workers went on to isolate SV40 messenger RNA (mRNA) at various stages of infection by the virus and to test the ability of the RNA to hybridize to the various DNA "restriction fragments" by virtue of their complementary nucleotide sequences. In this way, a map of the transcription sites (that is, the origin and direction of each mRNA transcript during viral infection) was obtained. In total, these pioneering experiments of Nathans and co-workers set the stage for a virtual barrage of DNA restriction maps, transcript maps, and nucleotide sequences of isolated restriction fragments.

A more controversial development has been the application of restriction enzymes to the isolation of particular genes and the construction of recombinant molecules consisting of DNA from two different sources. Many of these experiments involve the combination of eukaryotic DNA with bacterial or viral DNA in a single molecule, which is then cloned by growing multiple copies of the recombinant DNA molecules in bacterial or other host cells. Some scientists and members of the public expressed fears that such experiments could lead to the production of new pathogens. The resulting discussions led the National Institutes of Health to adopt a set of guidelines specifying the conditions under which research with recombinant DNA could be conducted.

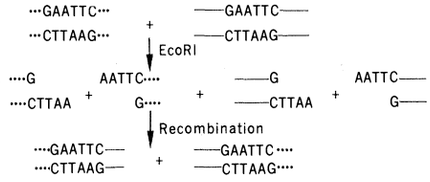


Fig. 2. Diagram showing how two different double-stranded DNA molecules can be cut by a restriction enzyme (designated Eco RI) and then recombined to form a hybrid or recombinant DNA molecule. Because the enzyme cuts the two strands of the DNA's in the staggered manner shown, it generates fragments with "sticky ends" that can adhere to one another according to normal base-pairing mechanisms. If a fragment from one DNA molecule (represented by the dotted lines) pairs up with a fragment from another molecule (represented by the solid lines), a recombinant DNA molecule can form.

Although no hazards have become apparent, recombinant DNA research has already had important practical consequences for the analysis of the organization of eukaryotic DNA's and also for the construction of bacterial "factories" for producing large amounts of previously scarce enzymes and hormones, including human insulin. The technological developments leading to these advances, many of which took place in Paul Berg's laboratory at Stanford, in Kenneth and Noreen Murray's laboratory in Edinburgh, as well as in Boyer's and in Goodman's laboratories at the University of California, San Francisco, and in Stanley Cohen's laboratory at Stanford, are certainly not to be overlooked in terms of their important consequences for future directions in biomedical research. Thus, the basic research pioneered by Arber, Smith, and Nathans, is being translated with unusual rapidity into applications that could hardly have been visualized a decade ago.

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