27 July 1979, Volume 205, Number 4404 SCIENCE

Promotion and Limitation of Genetic Exchange

Werner Arber

Exchange of genetic material has widely been observed in practically all living organisms. This may suggest that genetic exchange must have been practiced for a long time, perhaps ever since life began. The rules followed by nature in the exchange of genetic information have been studied by geneticists. However, as long as the chemical nature of the genetic material remained unknown, genetics had to remain a rather abstract branch of the biological sciences. This began to change gradually after Avery *et al.* (*1*) had identified DNA as the carrier of genetic information. Their evidence received independent support from the work of Hershey and Chase (*2*) and it was accepted by a majority of biologists by 1953, when Watson and Crick (*3*) presented their structural model of DNA. Hence, it was clear 25 years ago that very long, filamentous macromolecules of DNA contained the genes. As is usual in fundamental research, the knowledge acquired pointed to a number of new important questions. Among them were those on the structure and function of genes, as well as those on the molecular mechanisms of exchange of genetic materials.

At that time, in the fall of 1953, I joined, more or less by chance, a small group of investigators inspired by Jean Weigle and Eduard Kellenberger. Among their main interests was the mechanism of genetic recombination. Feeling that the time was not ripe to carry out such studies on higher organisms, they had chosen to work with a bacterial virus, the now-famous bacteriophage lambda (λ). It is interesting to see today how knowledge acquired in work with phage λ later strongly influenced other research in molecular genetics. In this article I trace the origin of some discoveries made in work with λ and point to their importance for subsequent investigations. But let me first define in more general terms what I mean by genetic exchange.

Escherichia coli and other bacteria carry all their genes on a single, very long DNA molecule, except for occasional cases when bacteria have one or several additional, much shorter DNA molecules, called plasmids, endowed with the ability of autonomous replication. A bacterial strain harboring, besides its chromosome, a fertility plasmid F can, at times, donate by conjugation a copy of its F plasmid to a recipient strain. The F plasmid then establishes itself in the recipient cell as an autonomous plasmid, and it is propagated in its new environment. The donor strain has thus exchanged genetic information with the recipient strain, and in this case the genetic material transferred had not existed in the recipient strain before the conjugation. We also note that the exchanged material replicates autonomously and does not need to be integrated into the bacterial chromosome. Therefore, this process is an example of reassortment of DNA molecules.

This situation contrasts with the one encountered in the so-called general recombination. Here two individuals exchange homologous genetic information. An example for this is also seen in bacterial conjugation. With a low probability, the fertility plasmid F can integrate into the host chromosome. In conjugation, the resulting strain (the Hfr strain, for high frequency of recombination) transfers a relatively long segment of a copy of its own chromosome to the F^- recipient cell. Maintenance of the information acquired by the recipient depends on its integration into the recipient chromosome, and this integration usually follows the rules of general recombination. This means that recombination depends on the finding of homologous sections on the two interacting genomes; and homology means identity in the nucleotide sequences, with an allowance made for rare exceptions to this rule at sites of mutations, which in fact allow the geneticist to explore these phenomena. Therefore, an exconjugant recombinant genome is a hybrid that has received part of its information from one, and part from the other parent. The total information content of the hybrid is the same as that of each parent. The same rule holds true in general recombination between two bacteriophages of the same strain, and it was in work with bacteriophage λ that physical exchange between the two parental genomes was experimentally demonstrated to occur in general recombination by Kellenberger *et al.* (*4*) and Meselson and Weigle (*5*).

We know that the molecular mechanism of general recombination is quite complex and depends on a number of

The author is professor of microbiology at the Biozentrum, University of Basel, Basel, Switzerland. This article is the text of the lecture he delivered in Stockholm, Sweden, 8 December 1978 when he received the Nobel Prize in Physiology or Medicine, a prize which he shared with Hamilton O. Smith and Daniel Nathans. Minor corrections and additions have been made by the author. The article is published here with the permission of the Nobel Foundation and will also be included in the complete volume of the *Les Prix Nobel en 1978* as well as in the series *Nobel Lectures* (in English) published by the Elsevier Publishing Company, Amsterdam and New York. The lectures by Dr. Smith and Dr. Nathans will appear in subsequent issues.

0036-8075/79/0727-0361$01.25/0

specific gene products. Some of these proteins also carry out key functions in DNA replication, as well as in DNA repair.

The studies of bacteriophage lysogeny in the 1950's ripened the concept that other mechanisms of molecular exchange between DNA molecules must exist. When bacteriophage λ infects a bacterial host cell it can either reproduce vegetatively to yield a progeny of phage particles or it can lysogenize the host cell. In the latter situation, the infected host survives, and it will accept the λ genome as a part of its own chromosome. This is similar to what we have discussed as reassortment of DNA molecules. However, the λ prophage, as the λ genome carried in a lysogenic cell is called, does not replicate autonomously and its maintenance depends on its integration into the host chromosome, which usually occurs at a site close to the genes determining galactose (gal) fermentation [see Wollmann (*6*), Lederberg and Lederberg (*7*), and Jacob (*8*)]. Lysogenic bacteria can be induced to phage production and in this process the prophage gets excised again from the host chromosome. Morse *et al.* (*9*) observed that phage lysates obtained by such induction of λ-lysogenic gal$^+$ bacteria were able to render gal$^-$ bacteria gal$^+$. This phenomenon is called specialized phage-mediated transduction. The authors mentioned that some of the gal$^+$ transductants obtained did not produce plaque-forming phage upon induction, although these bacteria were immune to superinfection with λ, a property usually displayed by λ-lysogenic bacteria. At that time, I studied λ prophage mutants with defects in genes controlling the cycle of vegetative phage reproduction. Therefore, gal$^+$ transductants as just described were a welcome addition to my materials to be studied.

Let me now show what I still consider a simple, straightforward experiment published in my Ph.D. thesis exactly 20 years ago (*10*). A phage λ lysate transducing the gal$^+$ characters at high frequency (HFT lysate) was used to infect a gal$^-$ bacterial strain at various multiplicities of infection of phage particles per cell, and the surviving bacteria were tested for their gal and lysogenicity characters (Fig. 1).

As was expected, the overall probability that an infected cell might become lysogenic remained constant in the range of multiplicities of infection below 1; that is, the number of normal lysogenic bacteria linearly dropped with decreasing amounts of phage added (Fig. 1, curve 3). This curve is exactly paralleled by the one (curve 1) representing gal$^+$ transductants found to be immune to superinfection with λ, but which produced no plaque-forming phage upon induction. In contrast, the number of gal$^+$ transductants which both were λ immune and produced plaque-forming phage upon induction (curve 2) is proportional to the square of the multiplicity of infection (*10, 11*).

The interpretation given to these observations was that the HFT lysate used was a mixed population of λ phage particles: (i) normal λ phages and (ii) λgal transducing phages which were defective in their capacity to reproduce serially and thus to form plaques, but which were still able to lysogenize even after single infection although they did so with reduced probability. This interpretation found support in a number of additional experiments, which I shall not discuss now. In summary, by 1958 it was shown that in the excision of λ prophage from the bacterial chromosome errors could sometimes produce aberrant phage genomes having acquired a segment from the host genome and having deleted from the λ genome a segment carrying essential genes for phage reproduction. A molecular model to explain both precise λ excision and the illegitimate formation of λgal was drawn by Campbell (*12*), who had also brought very important experimental contributions to this field. The analysis of a large number of independently produced λgal genomes made it clear that recombination within DNA molecules, and by extrapolation also between DNA molecules, occurs sometimes at more or less randomly chosen sites, and not on the basis of extended regions of homology. Obviously, the likelihood for such recombinants to be viable is relatively small, and nature seems to limit their production to a level several orders of magnitude below the level of general recombination. In the course of evolution, however, this kind of illegitimate recombination may be of great importance.

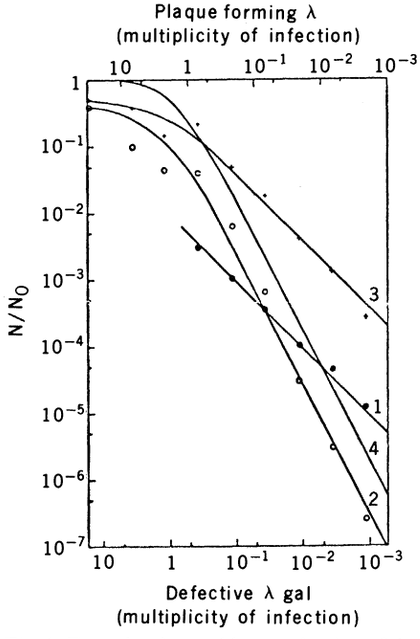


Fig. 1. Transduction and lysogenization of the *galT galK* strain W3350 of *E. coli* K12 by an HFT (high frequency of transduction) lysate. The HFT lysate used was a phage stock composed of 4.4×10^{10} plaque-forming λ phage particles and an estimated (from electron microscopical counts and physiological experiments) 2.3×10^{10} λgal phage particles, which were concluded from this and additional experiments to be defective in vegetative growth and partially affected in lysogenization. Portions of the host bacteria were treated at various multiplicities of infection with the HFT lysate and then spread on EMB galactose indicator plates containing antiserum to λ. Colonies grown after incubation were tested by replication for immunity to λ and for the ability to produce plaque-forming λ upon induction. N_0, the number of recipient bacteria in the infection mixture; N, the number of recipient bacteria from the infection mixture found to be (curve 1) gal$^+$, λ immune, not producing plaque-forming λ, hence carrying a λgal prophage; (curve 2) gal$^+$, λ immune, producing plaque-forming λ, hence being doubly lysogenic for λ and λgal; (curve 3) gal$^-$, λ immune, producing plaque-forming λ, hence carrying a λ prophage; (curve 4) the calculated fraction of bacteria simultaneously infected with at least one λ and one λgal. [From Arber (*10*); courtesy of the *Archives des Sciences (Genève)*]

In the meantime, molecular geneticists have learned to isolate in vivo derivatives of λ able to transduce practically any desired segment of the host chromosome, and this work has greatly facilitated detailed structural and functional studies of several selected *E. coli* genes. In addition, these studies pointed the way to more recently undertaken approaches to produce in vitro recombinational hybrids between λ (or other vector DNA molecules) and DNA fragments from any chosen origin.

The studies of λ lysogeny and of the defective nature of λgal, which could be complemented by helper phage infection, also influenced work on animal and plant viruses. The knowledge acquired with λ was taken as a model and this turned out to be extremely fruitful. We indeed know today that many situations similar to that of λ exist, where viruses are found integrated into the host chromosome and where viruses or fragments thereof can be shown to be defective but able to be activated by superinfection with exogenous helper viruses.

Let me now return to the process of integration and precise excision of the λ prophage. As has been already stated, this site-specific recombination was explained in a model devised by Campbell (*12*). A long and careful study on this system culminated a few years ago by its demonstration in vitro. Some of the few enzymes needed for the process are contributed by the λ phage itself and others by the host bacteria (*13*). We also know the nucleotide sequences at which the interaction between the λ genome and the bacterial chromosome occurs (*14*). These show homology over a stretch of 15 nucleotide pairs, but it has been shown that this length is not sufficient for efficient integration. Rather, the considerably longer, nonhomologous flanking segments have additional, key roles in the interaction. This is the system of site-specific recombination on which our knowledge is the most advanced.

The demonstration of recombinational events occurring independently of extended nucleotide homology, be they site-specific or at random, brought up the question on possible limitations set by nature to such exchange, which might be considered rather undesirable for the life of a cell.

Before two DNA molecules of different origin can interact with each other directly, they must be brought into proximity, into the same compartment. Nature has certainly set up a number of mechanical barriers, such as membranes, to limit free diffusion of genetic materials. We also know that a number of mechanisms exist that precisely allow the transfer of DNA from one cell to another, and that sometimes this exchange occurs between cells that are not directly related. May I recall that some bacterial conjugation plasmids have a relatively wide host range, and so do some bacteriophage strains able to transduce segments of the host chromosome, either by the already described specialized transduction, or also by the mechanism of general transduction, in which upon maturation the phage wrongly packages a segment of the host chromosome instead of its own phage genome. However, it is also clear that the host range is always limited by the need for specific cell surface interactions, and this seems to hold also for the penetration of free DNA into bacterial cells in the process known as transformation. In contract, bypass mechanisms have been demonstrated, for example, that a phage genome is transferable by bacterial conjugation (*15*) or that a conjugation plasmid is transduced by bacteriophage (*16*).

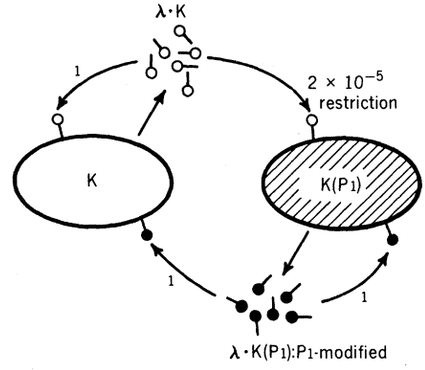
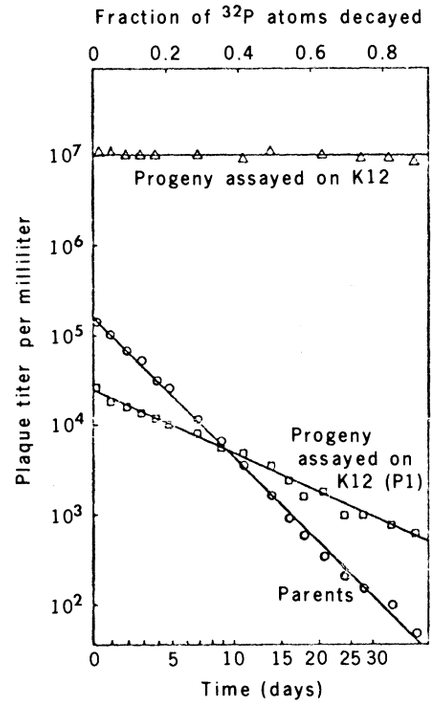


Fig. 2 (above). P1-specific restriction and modification as detected by growth of bacteriophage λ. Numbers give the efficiency of plating of phage variants λ · K and λ · K(Pl) on the hosts K and K(P1) as indicated by the arrows. Fig. 3 (right). Joint transfer of parental DNA and parental P1-specific modification of heavily ^{32}P-labeled λ · K(Pl) into the phage progeny produced after infection at a multiplicity of 0.006 phage per cell of the nonradioactively labeled host strain K. [From Arber and Dussoix (*22*); courtesy of the *Journal of Molecular Biology*]



Sometimes the host range of a bacterial virus can become extended due to a mutation in one of the phage genes. In contrast to this situation, host-controlled variation (or modification, as it is now generally called) of bacteriophage, first described in the early 1950's by Luria and Human (*17*), Bertani and Weigle (*18*), Anderson and Felix (*19*), and Ralston and Krueger (*20*), presented the puzzling situation that, when grown on different host strains, a virus could adapt to propagate on a new host without this ability being maintained upon "backgrowth" on the old host. Hence the adaptation could not find its explanation by a mutation in the phage genome.

I became interested in these phenomena in 1960 and I decided to look at the mechanisms of host-controlled modification of bacteriophage λ. The two host strains of my choice were a pair of *E. coli* strains, K12 (shortly called K), and its P1-lysogenic derivative K(P1). A few years before, Lederberg (*21*) had shown that the P1 prophage determines a system of host-controlled modification. Restriction of λ · K (phage grown on K) by K(P1) bacteria is quite strong: λ · K forms plaques on K(P1) host bacteria with an efficiency of only 2×10^{-5} (Fig. 2). In contrast, phage adapted to K(P1) grows with full efficiency on both K and K(P1). However, as is characteristic for host-controlled modification, when λ · K(P1) serves as inoculum for the growth of a multicycle stock of λ on strain K, the resulting phage behaves exactly as the original λ · K. I wanted to know how fast this readaptation occurs. Therefore, I grew λ · K(P1) on strain K for just one lytic cycle, taking care to inactivate all nonadsorbed λ with antiserum to λ, which was then removed by washing. The result was striking. The one-cycle progeny grew on the restrictive host K(P1) with an efficiency of between 3×10^{-3} and 10^{-2} instead of 2×10^{-5}. Since the burst size per singly infected host cell ranged between 100 and 200 λ particles, this result suggested that about one progeny phage per cell had "inherited" the parental P1-specific modification (*22, 23*). We were convinced that this was transferred from the infecting parental phage particle. But was it a diffusible internal phage protein or was it perhaps carried on the parental DNA molecule?

That the second possibility is the correct explanation became clear in the following experiment, which for historical reasons I would like to show. It had been demonstrated by Hershey *et al.* (*24*) and Stent and Fuerst (*25*) in the 1950's that DNA carrying radioisotopes loses its viability as a function of the radioactive decay. Therefore, phage heavily loaded in its DNA with ^{32}P undergoes suicide upon storage. To my knowledge, the experiment shown in Fig. 3 is the only important application of this rather special technique, which did allow us to trace parental DNA molecules in the course of replication at a time before density labeling methods had made their proof. A

stock of heavily ^{32}P-labeled λ · K(P1) was prepared and carefully purified. One portion was immediately used for one cycle of growth on nonradioactive K bacteria, and the phage progeny was stored and assayed from day to day. Another portion of the parental phage was directly stored and assayed from day to day. It is seen in Fig. 3 that the viability of this parental phage disappeared exponentially as a function of the ^{32}P decay. In contrast, the bulk of the progeny phage grown on nonradioactive cells for one cycle was perfectly stable as revealed by assay on K. However, those phages in the one-cycle progeny able to grow on K(P1) were inactivated, and their inactivation was about half as rapid as that of the parental phage (*22*). These results indicated that P1-specific modification carried by the parental λ · K(P1) phage is transferred together with a parental DNA strand into the population of progeny phage particles. It must be noted that fragmentation of parental λ DNA molecules can occur by recombination with progeny λ DNA molecules in the course of the intracellular phage growth, but it affects at most half of the parental input. Such recombinants with less than semiconserved parental DNA would not grow on K(P1), and their slow inactivation due to ^{32}P decay would not be detectable when assayed on K, since such phage particles represent a small minority of the progeny population.

About the same time Grete Kellenberger, who worked in the same laboratory, and Daisy Dussoix, a Ph.D. student, studied the breakdown of DNA from irradiated phage λ upon infection of normal host bacteria. We wondered if the mechanisms of P1-specific restriction and of inactivation of phage caused by irradiation had anything in common. For this reason the fate of λ DNA in restrictive host bacteria was investigated, and we could demonstrate that in the infection of K(P1) bacteria with λ · K phage an important fraction of the phage DNA was rapidly degraded (*26*). No DNA breakdown was seen in the λ · K-infected K bacteria.

The implication of these early findings, that host-controlled modification affected DNA, although the phenomenon could not be explained as a mutation, found additional support rapidly. We also realized that the phenomenon does not directly depend on phage λ, which was used in the studies, and that it affects any other DNA in the same way as λ DNA is affected, for example, bacterial DNA in conjugation (*23*). Hence restriction and modification (R-M) systems can be looked upon as serving as defense mechanisms against the uptake of foreign DNA and restriction to be brought by nucleolytic activity.

It took us some time to find out how bacteria can protect their own DNA against their restriction nucleases. They do so by postreplicative nucleotide methylation at the sites serving the R-M systems for specificity recognition (*27–29*).

Interestingly, the R-M systems *Eco* P1, *Eco* K, and *Eco* B with which the fundamental genetic and physiological experiments were carried out do not cleave the DNA precisely at the sites used for recognition (*30*). This points to rather complex molecular mechanisms by which these enzymes act. Careful investigations [see (*31–33*)] have already revealed important aspects of them so that these systems can serve as models in investigations of other nucleic acid protein interactions, particularly those showing regional rather than site specificity. I think, in particular, of some as yet poorly understood recombination phenomena pointing to regionally increased probability of interchange.

Other restriction enzymes, as is now well known, do cleave unmodified DNA at the recognition site, which is specific for each particular R-M system. My colleagues Hamilton Smith (*34*) and Daniel Nathans (*35*) discuss in their Nobel laureate presentations aspects of the mechanisms of these enzymes and of their application to studies of structure and function of DNA. Let me therefore just mention what is relevant with regard to in vivo genetic exchange. Since restriction enzymes have been widely used in recombination of DNA molecules in vitro, it is of interest that these enzymes can also trigger recombination in vivo (*36*). Hence, as in other biological activities with ability to catalyze antagonistic reactions, the restriction enzymes in question can both inhibit genetic exchange as well as promote it to some degree. This recombination does not depend on major nucleotide homologies on the interacting DNA molecules, but only on the existence of recognition sites for the enzymes determined by four to six nucleotide pairs in general.

The discovery of still another type of genetic exchange not based on nucleotide homology also has its roots in work with phage λ. Peter Starlinger (*37*) was among those fascinated by the explanation of how λgal phages were formed. In the early 1960's he and his collaborators had extended the knowledge on the randomness of the illegitimate recombination by genetically determining the end points of the bacterial material picked up by λgal. This formed a part of their studies on the structure and function of the galactose operon of *E. coli*. In the course of their work they encountered unorthodox mutations with strongly polar effects and several other unexpected properties. Further investigations of the nature of these mutations, as well as of similar mutations isolated in other laboratories, finally revealed the existence of what are now known as insertion sequences or IS elements (*37, 38*).

It would be premature to list general properties of IS elements and related structural entities. It is clear, however, that these elements of the size of about 600 to more than 2000 nucleotide pairs can be found in *E. coli* and in other bacteria in one or more copies carried at a number of different chromosomal sites. Such an IS element can show up spontaneously at a site not previously occupied by it. This event is called transposition, although it remains unclear whether the element really jumps from one location on the chromosome to another, or whether a resident IS element prepares a new copy in view of its integration at a new site. However, it is clear that IS elements generally can indeed be excised, either precisely or imprecisely, from a given site. Another often encountered property of IS elements is their ability to form deletions starting at one of the ends of the IS element and extending to a perhaps randomly chosen site at a distance sometimes of several genes. All these events are rare, indeed, but occur at measurable frequencies of perhaps 10^{-4} to 10^{-8} per cell division, depending on the system studied. They must be enzymatically determined, and it is likely that the rate-limiting factors are usually repressed. Presumably for this reason, no enzymological studies in vitro of these mechanisms have yet been successful. It is still a guess whether one or several of the genes determining the activities of IS elements are located on the IS element itself, which is also supposed to have specific sites involved in the events of transposition, excision, and deletion formation. Some IS elements have been shown to contain regulatory signals for gene expression.

Most of the IS elements described so far were chance isolates, and it remains largely unknown how many different IS elements are carried, for example, in *E. coli* K12, nor does one yet have good ideas on the host range of particular IS elements. We have started to look for an-

swers to these questions with the use of a large plasmid, the bacteriophage P1 prophage, to trap transposing IS elements inside the *E. coli* cell. Interestingly, an important proportion of spontaneous P1 prophage mutations affected in the functions of vegetative phage growth is explained by the incorporation of IS elements, which must originate from the host chromosome. On the other hand, several IS elements were also found by chance carried in genomes of P1 derivatives not affected in their functions of vegetative growth. Preliminary studies indicate that many of the IS elements isolated are independent of each other. This should allow us to establish a library of transposable IS elements isolated from host strains of bacteriophage P1. Hybridization studies with these IS elements and DNA from various origin is then expected to shed light on the question of the host range of particular IS elements (*39*).

IS elements have also been shown to mediate the exchange of more extended DNA segments. Transposons are DNA segments flanked by identical IS elements or at least repeated sequences. One of the important features of a transposon is that it can insert as a unit into another chromosome. For example, an r-determinant element 23 kilobase pairs long, carrying the genes for resistance to several antibiotics and originally identified as a constituent of R plasmids, has been shown to transpose into phage P1, from this into the *E. coli* chromosome, and from the chromosome into another bacteriophage genome (*39*). This clearly shows that under natural conditions relatively long DNA segments can translocate onto a transferable vector DNA molecule, such as a viral genome or a conjugative plasmid. And the same element at some later time in another host cell can transpose into a cellular chromosome. In principle, there is no limit set for genes to be picked up at one time or another on a transposable element, since the elements flanking a transposon can also transpose independently and thus by chance give rise to the formation of new transposons.

IS elements and related repeated sequences also give rise to cointegration of two DNA molecules, as well as to the dissociation of a single DNA molecule into two. Chromosomal integration and excision of F and R plasmids is just one example. Finally a few additional mechanisms contributing to genetic rearrangement and diversity should also be mentioned: gene inversion, gene amplification, and the formation of short partial duplications. These mechanisms seem also to be driven by IS elements flanking the genes involved.

I have tried to show that the deeper we penetrate as we study genetic exchange the more we discover a multitude of mechanisms either acting as promoters of exchange or acting to set limits to it, and some do both. On purpose I have discussed only prokaryotes and did so largely by taking examples from *E. coli* and its phages and plasmids.

I am aware and puzzled by the roles that site-specific exchanges may play in the ontogeny of higher organisms and at the level of the RNA in gene expression.

I gave some thought on the possible reasons why *E. coli* bacteria might have set up such a multitude of systems involved in the genetic exchange which for some reasons must be vital for them. I must confess that I did not find out why, besides trivial answers such as "serving in repair processes" or "evolutionary driving forces" for the promoting activities, and "species isolation" or "genetic stability" for the activities keeping genetic exchange within limits. More intensive research is needed for us to understand the apparent complexity of nature. But one important notion already obtained might be good to keep in mind: In spite of possessing a multitude of natural mechanisms to promote exchange between genetic materials of unrelated origin, *E. coli* and other living organisms have succeeded in achieving a relatively high overall stability in their genetic makeup.

References and Notes

1. O. T. Avery, C. M. MacLeod, M. McCarthy, *J. Exp. Med.* **79**, 137 (1944).
2. A. D. Hershey and M. Chase, *J. Gen. Physiol.* **36**, 39 (1962).
3. J. D. Watson and F. H. C. Crick, *Nature (London)* **171**, 737 (1953).
4. G. Kellenberger, M. L. Zichichi, J. J. Weigle, *Proc. Natl. Acad. Sci. U.S.A.* **47**, 869 (1961).
5. M. Meselson and J. J. Weigle, *ibid.*, p. 857.
6. E. L. Wollman, *Ann. Inst. Pasteur Paris* **84**, 281 (1953).
7. E. M. Lederberg and J. Lederberg, *Genetics* **38**, 51 (1953).
8. F. Jacob, *Virology* **1**, 207 (1955).
9. M. L. Morse, E. M. Lederberg, J. Lederberg, *Genetics* **41**, 142 (1956); *ibid.*, p. 758.
10. W. Arber, *Arch. Sci.* **11**, 259 (1958).
11. _____, G. Kellenberger, J. J. Weigle, *Schweiz. Z. Allg. Pathol. Bakteriol.* **20**, 659 (1957).
12. A. M. Campbell, *Adv. Genet.* **11**, 101 (1962).
13. H. A. Nash, *Curr. Top. Microbiol. Immunol.* **78**, 171 (1977).
14. A. Landy and W. Ross, *Science* **197**, 1147 (1977).
15. F. Jacob and E. L. Wollman, *Ann. Inst. Pasteur Paris* **91**, 486 (1956).
16. W. Arber, *Virology* **11**, 273 (1960).
17. S. E. Luria and M. L. Human, *J. Bacteriol.* **64**, 557 (1952).
18. G. Bertani and J. J. Weigle, *ibid.* **65**, 113 (1953).
19. E. S. Anderson and A. Felix, *Nature (London)* **170**, 492 (1952).
20. D. J. Ralston and A. P. Krueger, *Proc. Soc. Exp. Biol. Med.* **80**, 217 (1952).
21. S. Lederberg, *Virology* **3**, 496 (1957).
22. W. Arber and D. Dussoix, *J. Mol. Biol.* **5**, 18 (1962).
23. W. Arber, *Pathol. Microbiol.* **25**, 668 (1962).
24. A. D. Hershey, M. D. Kamen, J. W. Kennedy, H. Gest, *J. Gen. Physiol.* **34**, 305 (1951).
25. G. S. Stent and C. R. Fuerst, *ibid.* **38**, 441 (1955).
26. D. Dussoix and W. Arber, *J. Mol. Biol.* **5**, 37 (1962).
27. W. Arber, *ibid.* **11**, 247 (1965).
28. J. D. Smith, W. Arber, U. Kuhnlein, *ibid.* **63**, 1 (1972).
29. U. Kuhnlein and W. Arber, *ibid.*, p. 9.
30. K. Horiuchi and N. D. Zinder, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3220 (1972).
31. W. Arber, R. Yuan, T. A. Bickle, "Post-synthetic modification of macromolecules," in *Proceedings of the 9th FEBS Meeting, Budapest 1974*, F. Antoni and A. Farago, Eds. (1975), vol. 34, p. 3.
32. R. Yuan, T. A. Bickle, W. Ebbers, C. Brack, *Nature (London)* **256**, 556 (1975).
33. T. A. Bickle, C. Brack, R. Yuan, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3099 (1978).
34. H. O. Smith, *Science*, in press.
35. D. Nathans, *ibid.*, in press.
36. S. Chang and S. N. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4811 (1977).
37. P. Starlinger and H. Saedler, *Curr. Top. Microbiol. Immunol.* **75**, 111 (1976).
38. A. I. Bukhari, J. A. Shapiro, S. L. Adhya, *DNA Insertion Elements, Plasmids, and Episomes* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977).
39. W. Arber, S. Iida, H. Jütte, P. Caspers, J. Meyer, Ch. Hänni, *Cold Spring Harbor Symp. Quant. Biol.*, in press.

40. In the course of the past 25 years I was very fortunate to benefit from a large number of highly qualified collaborators and I received additional stimulation from even more colleagues. An important part of my research found continued support by the Swiss National Science Foundation.