Hybrids of Escherichia and Salmonella

The genetic homologies of these bacteria are determined by mating and transduction.

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Baron *et al.* (*1*) and Miyake and Demerec (*2*) have reported the hybridization of *Escherichia coli* Hfr with certain strains of *Salmonella*. The Hfr strain of *E. coli* donates part of its genome to the recipient *Salmonella*.

By introducing *E. coli* genes into *Salmonella*, it is now possible to begin to observe whether there are any homologies of the genetic material in these closely related organisms. For studying the macrohomology, with respect to chromosomes or segments of chromosomes, recombination itself is available. For studying the microhomologies, the process of transduction is available.

Methods

The derivatives of *E. coli* K-12 used were strains Hfr (Cavalli) (*3*) and Hfr (Hayes) (*4*) and an F+ strain. The *S. typhimurium* strain was a derivative of LT7 (*5*), obtained from Miyake (*2*).

Suitable mutant genes were introduced into these strains for the particular purposes of the experiments. They are described below. The symbols used are as follows. For auxotrophic mutants, "meth" refers to methionine, "leu" to leucine, and "try" to tryptophan. For fermentation mutants, "gal" refers to galactose, "lac" to lactose, and "arab" to arabinose. Drug resistance to sodium azide is symbolized by "az." In addition, superscript "s" or "c" is used when necessary to indicate whether the allele in question was derived from *Salmonella* or from *E. coli*.

The techniques used for obtaining the mutant alleles and the media employed were those described by Lederberg (*5*).

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Bacterial crosses and transduction were accomplished by the routine procedures described by Lederberg *et al.* (*6*) and Zinder (*7, 8*), respectively.

Experiments

Recombinational analysis and macrohomologies. Figure 1 shows the region of the *E. coli* chromosome with which we are concerned. It is the map that was obtained when strain Hfr (Cavalli) was crossed with strain F− coli. The orientation of the chromosome is as shown, with the T6 lac region entering first. There is therefore immediately available a very potent selective marker in this region, since salmonella are by nature lactose nonfermenters and do not mutate to lactose fermentation. The meth locus in the Hfr is outside the region transmitted to the F−, and the means to select against the coli parent is thereby provided. The cross then is lac+ meth− coli by lac− meth+ salmonella when the bacteria are placed on a minimal medium containing lactose as the only carbon source. The selection is for meth+ lac+. By introducing, prior to crossing, the mutant alleles of the genes presumed to be located in this region, it is possible to look at the general linkage structure. It is significant that when the cross is set up as described, recombinant progeny appear at the lower frequency characteristic of F+ by F− matings in coli, not at the frequency of Hfr by F− matings. However, hybrids will, upon backcross to Hfr coli, give a high frequency of recombinants and even yield progeny when mated with F+ coli. It might be that only special cells of the salmonella population can mate with coli and that these are selected by the first round of mating, as has been proposed by Baron *et al.* (*1*). It is not known whether the selection would be for greater mating capacity or greater integration capacity.

Table 1 gives the data for two sets of crosses for markers in this region. Since salmonella lack the receptors for the coli bacteriophage T6, another marker is available. Almost all of the progeny obtain the coli T6 marker. This is manifest by the fact that they are killed by infection with a multiplicity of T6, but still they do not propagate this bacteriophage. One further naturally existing marker lying in this region is of some import. It is one that produces a host-controlled modification (*9*) of phage P22. Since P22 does not attach to coli strains, nothing can be said about its growth on these strains. However, when P22 is grown on the majority of the hybrids it undergoes modification such that it grows well on the hybrid (the determinant is defined here as Mp^c) but only with an efficiency of 10^{-4} on salmonella (Mp^s). The modification is nonsymmetrical, as P22 grown on Mp^s bacteria grows equally well on the two alternative bacteria. The Mp locus seems to be between T6 and lac, as some of the few progeny that did not obtain $T6^c$ did not obtain Mp^c. It should also be noted that the vast majority of the progeny selected in this way retain all of the characteristic salmonella antigens, and hence it might be assumed that the genes controlling these properties lie elsewhere in the genome. There are, however, some serological hybrids, and these are being analyzed further.

The most important point that is shown in the table is the obvious identity of the order of the genes lac, az, and arab in coli and salmonella. It might be argued that the order is imposed by the sequence in the Hfr. This, however, could not lead to multiple substitutions of genes but only to single substitutions. That these are indeed substitutions and not additions of genetic material is attested to by the facts that selection for lac^c gives frequent linked substitution of either $arab+^c$ or $arab-^c$, according to the genotypes of the parents, and that these strains give no evidence of instability.

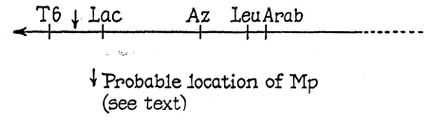

Fig. 1. Genetic map of *E. coli* Hfr (Cavalli).

Table 1. Recombination between *E. coli* and *S. typhimurium*.

Cross No. 1*					Cross No. 2*			
E. coli / *S. typh.*	lac+ / lac−	az^s / az^r	arab+ / arab−	meth− / meth+	Lac+ / Lac−	az^s / az^r	arab− / arab+	meth− / meth+
	Az^r		arab+	1	Az^r		arab−	0
	Az^r		arab−	112	Az^r		arab+	27
	Az^s		arab+	39	Az^s		arab−	13
	Az^s		arab−	18	Az^s		arab+	6

* Selection for meth+ lac+.

The possibility remains that what is selected in the primary cross is a salmonella which has changed the natural order of its genes in such a way as to be colinear with that of the coli Hfr. The low probability of this occurrence could then account for the low frequency of the occurrence recombinants. Further clarification of these points will become available as the genetic map of salmonella is determined independently in salmonella-by-salmonella crosses and salmonella-by-coli crosses.

Transduction between coli genes and salmonella genes. Through the system described above, the appropriate alleles with which to study interspecific transduction were introduced into hybrids. P22 was grown on these hybrids, and the transduction efficiency of a number of alleles in this region was studied. Careful attention was paid to the source of the allele, to whether the phage was or was not modified with respect to the recipient, and to whether the recipient, in turn, would or would not modify the phage. Let me repeat that phage grown on salmonella (Mp^s) grows equally well on the hybrids but, after growth on the usual hybrid (Mp^c), grows poorly on salmonella. Wherever possible, the attempt has been made to analyze transduction independently of phage-growth characteristics.

Table 2 contains the data which were obtained. They are expressed in terms of the number of transductions per infective phage particle. The minimum frequencies are, of course, determined by the spontaneous mutability of the alleles being studied.

The following points can be developed by analyzing the data in the table.

1) Because of the intrinsic stability of the lac− nature of Salmonella, it may be stated with a very high degree of confidence that the $lac+^c$ is not transduced. However, this very stability may imply that the nature of the change necessary to cause salmonella to ferment lactose is beyond the capacity of the restricted nature of transduction (*8*).

2) With regard to the arabinose alleles, where all of the combinations are available, it may be noted that coli genes do not transduce salmonella genes and salmonella genes do not transduce coli genes, or do so only at a frequency of 1/20 that of the normal. However, coli genes do transduce coli genes and salmonella genes transduce salmonella genes.

3) Although leucine requirement was not a marker used directly in the crosses, when the hybrid has an arab coli gene, the transduction of leu^s is restricted. Abrabinose fermentation and leucine requirement are closely linked genes and are linked in transduction.

4) With all of the combinations, the marker $try\pm^s$ was affected in transduction efficiency only when the phage was also restricted in its growth. It lies outside the region under study, and therefore all experiments were $try+^s$ on $try-^s$.

Table 2. Transduction of *E. coli* and *S. typhimurium* genes.

Bacterial strain	No. of transductions per infective phage particle				
	Bacteriophage				
	$Lac+^c$ $arab+^s$ Mp^c	$Arab+^s$ Mp^s		$Lac+^c$ $arab+^c$ Mp^c	$Lac+^c$ $arab+^c$ Mp^s
		$lac+^c$	$lac-^s$		
$Lac-^s$ Mp^s	$<10^{-10}$	$<10^{-10}$*		$<10^{-10}$	$<10^{-10}$*
$Arab-^s$ Mp^c	$\frac{4.1}{10^8}$*	$\frac{6.8}{10^8}$*	$\frac{5}{10^8}$*	$<\frac{3}{10^9}$*	$<\frac{3}{10^9}$*
$Arab-^s$ Mp^s	$<\frac{2}{10^9}$	$\frac{7.2}{10^8}$*	$\frac{6.4}{10^8}$*	$<\frac{3}{10^9}$	$<\frac{2}{10^9}$*
$Arab-^{c1}$ Mp^c	$<\frac{2}{10^9}$*	$<\frac{2}{10^9}$*	$<\frac{2}{10^9}$*	$\frac{4.2}{10^8}$*	$\frac{5.6}{10^8}$*
$Arab-^{c2}$ Mp^c	$<\frac{2}{10^9}$*	$<\frac{2}{10^9}$*	$<\frac{2}{10^7}$*	$\frac{1.2}{10^8}$*	$\frac{3.2}{10^8}$*
$Leu-^s$ Mp^s	$<10^{-9}$	$\frac{3.2}{10^7}$*	$\frac{1.3}{10^7}$*	$<10^{-9}$	$<10^{-9}$*
$Gal-^s$ Mp^s	$<10^{-9}$	$\frac{1}{10^7}$*		$\frac{5}{10^8}$	
$Try-^s$ Mp^s	$<10^{-9}$	$\frac{9}{10^7}$*	$\frac{6.8}{10^7}$*	$<10^{-9}$	$\frac{5.3}{10^7}$*

* Bacteriophage grows with an efficiency of about 100 percent.

Conclusions

These two bacteria, *Salmonella typhimurium* and *Escherichia coli,* have been considered, and not without reason, sufficiently different to be worthy of separate generic designation. It is somewhat surprising to find that the gene orders in these species are sufficiently similar to allow for genetic recombination to occur. Thus we find in the same place, in the same order in coli and in salmonella genes affecting the receptors for a bacteriophage (T6), resistance to a drug (sodium azide), fermentation of lactose and arabinose, and synthesis of leucine. In both organisms the genes for galactose fermentation and tryptophan synthesis lie in other areas. Unless some artifact is imposed by the fact that there is a selection of recombinant types, it would seem that the gross features of the salmonella chromosome are the same as those of the coli chromosome. That this is no artifact is indicated by some preliminary evidence from salmonella-by-salmonella crosses, which indicate a similar order of these genes.

However, if, as we suppose, transduction mirrors the finer structure of the genetic material, it is apparent that here sufficient evolutionary diversity has occurred to restrict transduction even for those genes which seem to be common to both organisms. Apparently

there have been accumulated many mutations which are relatively innocuous, in the physiological sense, but which cause sufficient diversity of genetic structure to prevent the necessary pairing over short distances prior to recombination. It may therefore be expected that individual differences relative to the "interspecific" transduction efficiency of different genes may exist, some genes being more efficiently transduced than others.

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Science in the News

United States Satellite Launched into Orbit around Sun

The United States has placed a 94.8-pound satellite, Pioneer V, in orbit around the sun between Earth and Venus (see diagram). The spherical, 26-inch aluminum payload, which was launched by a three-stage rocket on 11 March, will take 311 days to complete a circuit of the sun.

The vehicle carries instruments for five experiments, the most significant device being a 150-watt transmitter designed to permit communications between Earth and the payload at distances of up to 50 million miles. The transmitter is believed to be the most powerful ever flown in deep space—approximately 30 times more powerful than any other United States experimental space transmitter developed to date.

The launching is the third in a series of "paddle-wheel" flights. The National Aeronautics and Space Administration contracted for the series in November 1958 with the Air Force Ballistic Missile Division (Air Research and Development Command). In turn, AFBMD subcontracted with Space Technology Laboratories, Inc., of Los Angeles, for over-all system integration and payload packaging. In all, some 50 subcontractors, including universities and industrial firms, have had a part in the series.

The new satellite, which was propelled at more than 24,869 miles an hour at third-stage burnout, is designed to describe a 506-million-mile path around the sun at an average speed of about 70,000 miles an hour.

This probe differs from past successful sun-orbiting probes—the Soviet Union's Lunik I (2 January 1959) and the United States' Pioneer IV (3 March 1959)—in that it is inside the earth's orbit. Lunik I and Pioneer IV are in orbits between those of Earth and Mars.

To get it into an orbit between Earth and Venus, Pioneer V was launched in the morning. As the rocket neared escape velocity, it followed the curve and directional spin of the earth. When it escaped, the vehicle was swept into a sun orbit by the sun's gravitational force; it is moving around the sun in the same direction as the rest of the planets.

Significance of the Transmitter

There are several reasons for sending up the powerful transmitter. One objective is to demonstrate the feasibility of long-range space communications. Another involves a new method of measuring astronomical distances.

To date, distances within the universe have been computed from basic laws of physics governing bodies in motion, with positions plotted against seemingly stable distant stars. To astronomers, the basic unit of measurement is the AU or astronomical unit—the mean distance between Earth and Sun, or approximately 93 million miles.

Most scientists agree that this measurement is accurate to only plus or minus 50,000 miles. It is important to future space missions to have more precise values. Successful long-range communication with this payload should provide a more accurate measure. The transmitter and associated electronic equipment, batteries, and the solar cells for power supply make up more than half the probe's total weight.

Other Instrumentation

A high-energy radiation counter, developed by the University of Chicago, measures high-energy or "hard" radiation, particularly from the sun.

An ionization chamber and a Geiger-Müller tube, together weighing about 2 pounds, are measuring the total radiation flux encountered. They are particularly sensitive to medium-energy radiation. These instruments were supplied by the University of Minnesota.

A 1-pound micrometeorite counter, developed by the Air Force Cambridge Research Center, is measuring the number and momentum of meteoric dust particles striking the probe.

A 1-pound search-coil magnetometer, developed by Space Technology Laboratories, is designed to determine the strength and direction of magnetic fields in space.

An 8-ounce photoelectric cell called an "aspect indicator," also developed by STL, will trigger a specific electrical impulse when it "looks" directly at the sun. These "fixes" on the sun should make the information obtained from the magnetometers and radiation counters more meaningful.

In addition to the instruments listed, Pioneer V contains a number of amplifiers, "logic" units which transform various instrument-sensing actions into transmittable signals, and a command compartment capable of initiating some ten payload functions. Five tiny thermistors are recording temperatures, two on the paddles and three within the payload.

Radio Contact

The probe carries one 5-watt ultra-high-frequency transmitter which, on