EVOLUTION OF THE BACTERIAL GENOME

(bacterial DNA evolution, vertical vs. horizontal evolution)

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SUMMARY

Not all parts of bacterial genomes have evolved at the same rate. Among related genomes, some gene segments are highly conserved, some are less conserved and some are highly variable. We have studied the relatedness of selected gene segments in the genomes of a group of **E. coli** *strains and other enteric bacteria, using* **E. coli** *K12 DNA as a reference standard. We found variation in the degree of relatedness among the gene regions sampled, implying that evolutionary rates varied in different parts of enteric genomes and/or that different mechanisms of evolution have predominated in different gene regions.*

Mechanisms of evolution of the bacterial genome are discussed. These can be grouped as those mechanisms that are vertical in character, and those that are horizontal in character. Criteria are suggested that may help to identify genes whose inheritance has been primarily horizontal rather than vertical.

Arguments are given that neither internal genome rearrangement nor promiscuous exchange of genes among the chromosomes of dissimilar bacteria have played dominant roles during evolution of the bacterial genome.

INTRODUCTION

Continued progress in the application of techniques of DNA hybridization following Southern transfer, the isolation, cloning and sequencing of specific DNA fragments, as well as rapid advances in understanding the action of transposable genetic elements and the mechanisms of gene duplication and transposition, all of these advances are opening avenues of experimentation that can begin to answer questions concerning mechanisms of evolution of bacterial genomes. At present, the mechanisms that seem likely to have played roles in bringing about evolutionarily significant changes in bacterial genomes are base substitution, duplication, internal rearrangement, and genetic recombi-

nation with externally derived genetic elements. Taken togeth-
er, these kinds of molecular events could account for the gener-
ation of a multiplicity of diverse bacterial genomes from one or
a few progenitor ancestral genomes.

Some of these mechanisms, such as point mutation, duplica-
tion and internal rearrangement, are vertical in character and
are passed on to progeny in the absence of genetic exchange with
DNA of external origin. Other mechanisms involving gene trans-
fer and recombination through sexual processes or uptake of ex-
trachromosomal elements, followed by genetic exchange with the
chromosome, are horizontal in character. Studies on the extent
and type of relatedness between selected portions of evolution-
arily related bacterial genomes are beginning to provide infor-
mation on the evolutionary history of those regions and are be-
ginning to show that all parts of the genomes have not evolved
in concert, but instead different small segments have had dif-
ferent evolutionary histories. Comparative studies have been
carried out among closely related strains within a bacterial
species and among more distantly related members of a family.
Examples of such studies, with emphasis on the enteric bacteria,
are described below and are discussed in the context of distin-
guishing vertical and horizontal modes of evolutionary change.

VARIATION AMONG GENOMES OF CLOSELY RELATED BACTERIA

NUCLEOTIDE-SEQUENCE DIVERGENCE

The extent of relatedness of the total DNAs of certain
strains of *Escherichia coli* has been assessed by hybridizing total
DNAs under conditions of varied stringency (BRENNER and FALKOW
1971; BRENNER *et al.* 1972). Significant differences in the DNAs
were found. For instance, only 89% of the DNA of *E. coli* BB was
stringently highly homologous to the DNA of *E. coli* Kl2 (Uni-
versity of Washington), and the genome of *E. coli* BB appeared to
be 9% larger than that of *E. coli* Kl2. Thus significant differ-
ences have accumulated in the genomes of the *E. coli* strains that
were examined in these studies.

We have assessed the amount of variation in the nucleotide
sequences of the DNAs of a group of *E. coli* strains: 6 labora-
tory strains and 23 strains isolated from nature, the latter
generously donated from the collection of Dr. Roger Milkman (Ta-
ble 1). Variations in the nucleotide sequences of many of these
strains were seen in the electrophoretic distributions of the
cleavage products in restriction endonuclease digests of the
chromosomal DNAs (ANILIONIS and RILEY 1980; HARSHMAN and RILEY
in preparation). The profiles of the molecular-weight distri-
butions (data not shown) were closely similar for two closely
related *E. coli* Kl2 laboratory strains and for some of the Milk-
man strains that were isolated from the same fecal sample, but
the profiles were detectably different in all other cases.

To investigate the relatedness of selected small segments
of the genomes of this group of *E. coli* strains, we used the Sou-
thern   transfer technique (SOUTHERN 1975) and hybridized a set of

radioactively labeled probe DNAs to chromosomal digests. Three
of the probes were λ phage DNAs in which some of the phage genes
had been replaced with bacterial genes: λ*trp*, λ*tna*A and λ*thy*A.
Since λ phage DNA was present as vector DNA in all probes, λ
DNA was hybridized to the bacterial chromosomal digests to iden-
tify chromosomal fragments that contained regions of homology
to λ DNA. Chromosomal λ-homologs, presumed to be remnants of
inactive prophages (BERG and DRUMMOND 1978; KAISER and MURRAY
1979), were distinctly more variable than the chromosomal gene

Table 1. Sources of *E. coli* strains

Laboratory strains	Source
K12(λ)	Coli Genetic Stock Center
K12 W1485	Coli Genetic Stock Center
C	R. Calendar
W	H. Vogel
B	M. Baylor
15	A. Pardee

Strains from natural sources*	Host	Geographic region
10 A,B,C	Bison	Alberta, Canada
66 A,B,C	Human	Iowa
75 A,B,C	Human	Iowa
209 B,I	Ape	Celebes
210 D,F,J	Ape	Celebes
211 D,F,G	Sheep	New Guinea
213 E,I,K	Steer	Bali
215 A,B,C	Steer	Bali

*Kindly provided by Dr. Roger Milkman. Numbers represent fecal
 samples from different individuals. Letters represent separate
 isolates from one fecal sample; these could be, but need not
 be, members of one clone.

homologs that were used in these experiments (ANILIONIS and
RILEY in preparation).

     Fig. 1 shows that the distribution of sizes of the chromo-
somal fragments that contained λ-homologs varied from *E. coli*
strain to *E. coli* strain. Setting aside the lysogenic K12 strain
in which chromosomal λ-homologs were difficult to distinguish
from prophage end fragments, the pattern of sizes of chromosomal
Hind III fragments that contained λ-homologs differed for each of
the *E. coli* strains examined. Unique size distribution patterns
were also found in *Eco* RI chromosomal digests (data not shown).

In pairwise comparisons, a few of the hybrid fragments were the same or similar in size in two bacterial digests, implying that the restriction-sensitive nucleotide sequences in or near these particular λ-homologs were not different in the two chromosomal DNAs. However, most of the hybrid fragments differed in size, implying either that nucleotide-sequence changes abolished or created restriction-sensitive sites in or near λ-homologs, or that genetic rearrangement has taken place at these loci.
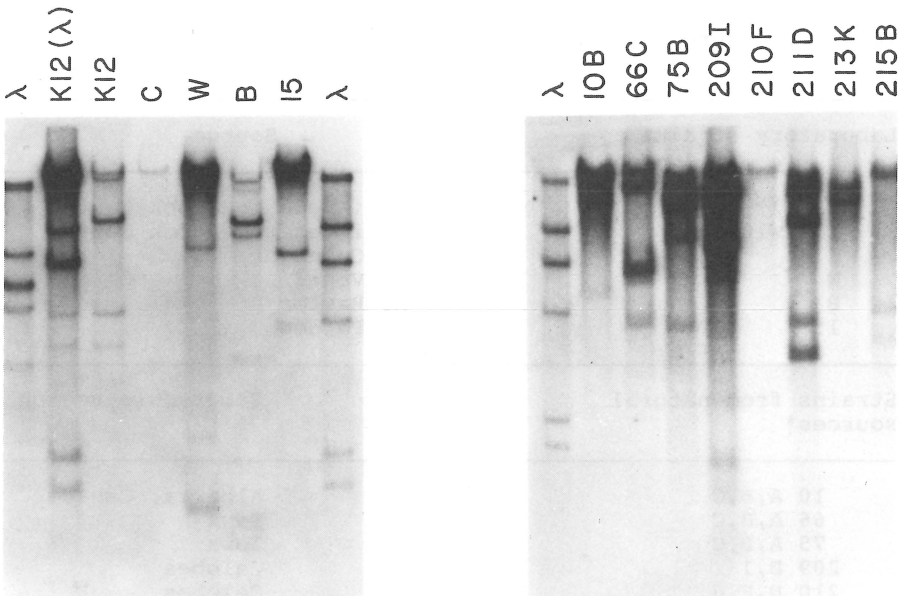

Fig. 1.   λDNA probe hybridized to *Hind* III digests of *E. coli* DNAs.   0.5 µg of bacterial DNA *Hind* III digests and 10 ng of λ DNA *Hind* III and *Eco* RI digests (used as molecular-weight markers) were subjected to agarose electrophoresis, transferred to nitro-cellulose filter paper (Southern, 1975), and hybridized with 10 ng/ml ^{32}P λ DNA ($\sim$10^8 cpm/µg) in 50% Formamide-4x SSC at 37°C. Filters were washed, dried, and exposed to X-ray film to obtain autoradiograms.

The variation observed in these λ-homolog portions of the *E. coli* genomes was substantially greater than the variation observed for structural gene regions (see below). The greater variability in and near λ-homologs might reflect a rate of base change in those parts of the bacterial genome that are not constrained by functional requirements. Presumably the DNAs of non-functional cryptic prophages are free to accept and retain all base substitutions. Alternatively, the high degree of variability might not be a consequence of purely vertical evolution, but might reflect an unusual amount of recombinational activity in these regions.

The variability of positions of restriction sites in other segments of the *E. coli* genome was examined in the same way in order to gain an estimate of the frequency of nucleotide sequence changes in or near functional bacterial genes. When λ*trp* DNA was used as probe, chromosomal fragments homologous to *trp* operon DNA were visualized in addition to the previously visualized λ-homologs. Representative results are shown in Fig. 2. Three *Hind* III *trp*-homologs were seen in *E. coli* K12 DNA, as expected from restriction analysis of a related λ*trp* phage (HOPKINS *et al.* 1976). *Trp*-homologs of the same or closely similar sizes were present in the DNA of many of the *E. coli* strains, but in some cases size variants were observed. The molecular **sizes** of the *Hind* III *trp*-homologs in 28 *E. coli* isolates are listed in Table 2.
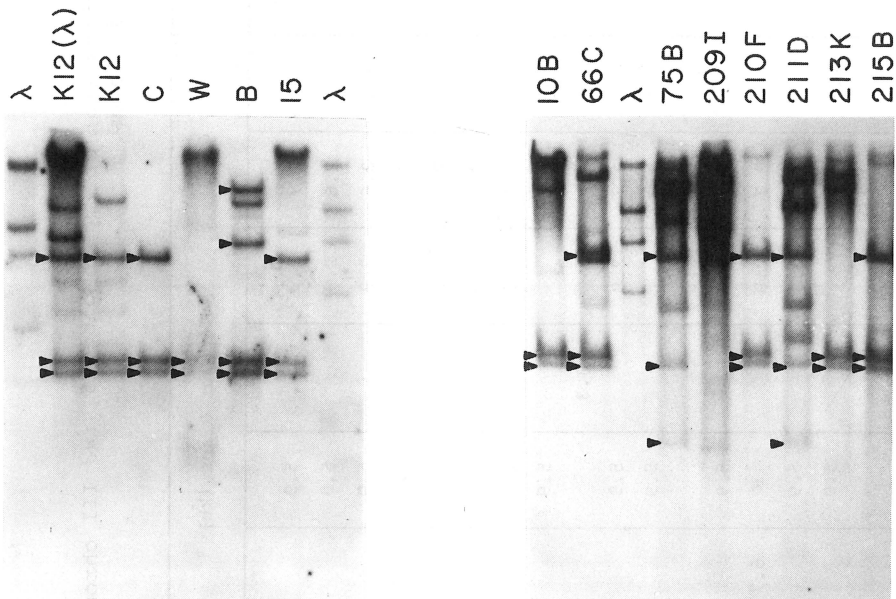

Fig. 2.   λ*trp* DNA probe hybridized to *Hind* III digests of *E. coli* DNAs.   Methods as for Fig. 1.   Pointers mark *trp*-homologs.

The *E. coli* strains having the same size patterns (within experimental error) were grouped together and assigned a letter description, A through I. In all pairwise comparisons of the sizes of the *trp*-homolog fragments of each class, the fraction of fragments having the same size ranged from 0 to 0.86. Invoking a relationship between the fraction of fragments conserved to the frequency of base substitution (UPHOLT 1977; NEI and LI 1979), and assuming that no significant genetic rearrangement has occurred at these loci, one can estimate the amount of base substitution that has taken place in and near the *trp* operon since any two of the classes of *E. coli* bacteria diverged from each other. Although estimates based on data from only one restriction enzyme can only approximate true base substitution

Table 2. Molecular sizes (kb) of *trp*-homologous *Hind* III chromosomal fragments in 28 *E. coli* isolates.

E. coli Strains	Molecular Sizes (kb)							
K12 W1485		2.7	2.9		5.9			
C		2.6	2.9		5.9			
W	1.9	2.6	2.9		5.9			
B		2.6	2.9		5.9	6.7	11.8	
15		2.6	2.9		5.9			
10 A,B,C		2.7	2.9					
66 A,B,C		2.7	2.9		5.9			
75 A,B,C	1.8	2.7			5.9			
209 I				3.3				
210 D,F		2.7	2.9		5.9			
211 D,F,G	1.8	2.7			5.9			
213 K		2.7	2.9					23
215 B,C		2.7	2.9		5.9			
210 J		2.7	2.9			6.7		
213 E,I		2.7	2.9		5.9			
215 A	1.8	2.7			5.9			
209 B								

frequencies, a preliminary picture of the relatedness of the *trp* operons in these strains was gained. Table 3 shows that two of the classes (containing one member each) were so unlike the others that no estimation of relatedness was possible using this approach. Among the rest, the chance that a given base in the *trp* region was replaced during the divergence of any two strains from a common ancestor ranged from 0.008 to 0.066.

Table 3. Inferred frequency of base substitution in *trp* regions of 9 classes* of 28 *E. coli* isolates

	B	C	D	E	F	G	H	I
A	.022	.033	.008	.018	.022	.022	$\geqslant$.25	$\geqslant$.25
B		.066	.008	.04	.066	.066	$\geqslant$.25	$\geqslant$.25
C			.032	.008	.022	.022	$\geqslant$.25	$\geqslant$.25
D				.022	.032	.032	$\geqslant$.25	$\geqslant$.25
E					.032	.04	$\geqslant$.25	$\geqslant$.25
F						.022	$\geqslant$.25	$\geqslant$.25
G							$\geqslant$.25	$\geqslant$.25
H								$\geqslant$.25

*Members of classes are as follows:

A - K12 W1485, C, 15, 66 ABC,
    210 DF, 215 BC, 213 EI
B - 75 ABC, 211 DFG, 215 A
C - 210 J
D - W
E - B
F - 213 K
G - 10 ABC
H - 209I
I - 209B

Variability in the vicinity of the *tna*A and *thy*A genes was assessed in the same way (data not shown). These regions showed a degree of variation that was similar to that found for the *trp* genes, less than that found for λ-homologs. It seems likely that functional constraints have acted to reject some of the base substitutions that arose in the *trp*, *tna* and *thy* regions, all presumed to be functional portions of the bacterial genomes.

One can ask whether any of the 28 *E. coli* isolates examined

in these ways are more closely related to one another than they are to other strains. With the exception of clonally related strains, each strain differed from the others in the molecular-weight distributions of total chromosomal restriction digests and in the patterns of sizes of restriction fragments bearing homology to λ genes, and many of the strains were different with respect to the positions of restriction-sensitive sites near the *trp* operon. However there were some strains that could not be distinguished from one another with respect to the restriction sites that lay in or near the *trp*, *thy*A and *tna*A genes. This group contained *E. coli* laboratory strains K12 and C and isolates from a human, an ape and two steers, whereas other laboratory strains and other human, ape and steer isolates fell in other groups. No exception-free correlations could be made between these groupings and the origin of the strains, either in terms of the host animal or geographic region. Thus, the genomes of a random collection of *E. coli* strains differ in readily detectable ways from one another, reflecting past, and presumably ongoing, processes of divergence.

We note with interest that the laboratory K12 strain is a member of the majority group within the set we examined, and by this measure appears not to be an aberrant *E. coli* strain, but rather seems to have at least as much claim to stand as a representative of the species as any other strain included in this study.

Generalizing, these experiments show that approaches of this kind offer opportunities to assess relatively easily the extent of relatedness of the DNAs of closely similar bacteria in the absence of base-sequence information. Only extremely closely related strains exhibit molecular-weight distributions of chromosomal restriction fragments that are indistinguishable. The extent of relatedness of corresponding small segments of the genome can be gauged using the Southern transfer and hybridization technique. Some parts of the bacterial genome, among which are the λ-homolog regions discussed above, are highly variable and are suitable to use as probes in comparative studies of very closely related genomes. Other parts, such as *trp*, *thy* and *tna* segments discussed above, are less variable and are appropriate for comparative studies of less closely related strains. Using a chosen genetic segment as probe and a roster of restriction enzymes to digest chromosomal DNAs, then if the genetic regions being studied differ only by base substitution, the fraction of restriction fragments whose size is conserved gives information on the frequency of base substitution that has occurred in the region during divergence of the DNAs (for applications in eucaryotic systems, see UPHOLT and DAWID 1977; BROWN, GEORGE and WILSON 1979). Thus, since bacterial genomes are heterogeneous with respect to variability, appropriate portions of the genome can be selected for the comparative study of bacteria over a range of relatedness.

DUPLICATIONS

Another source of variation among the genomes of closely related bacteria is provided by duplication of a gene or group

of genes. Information on tandem duplication in bacteria was reviewed recently (ANDERSON and ROTH 1977). Duplications are formed and, in the absence of selection, are also lost at high frequencies in bacterial populations. Well-studied duplications range from the size of a gene, as in the case of the *argE* gene in *E. coli* (CHARLIER *et al.* 1979), to around 35% of the genome, as in the case of a particularly large duplication in *Salmonella typhimurium* (STRAUS and HOFMANN 1975).

A set of duplications that has been analysed in detail in *E. coli* appear to have been formed by errors of replication involving redundant *rrn* loci (HILL, GRAFSTROM and HILLMAN 1978) (see Fig. 3 for schematic diagrams). Involvement of duplicate *rrn* loci has been substantiated by the isolation, as segregation products, of DNA circles that were found to have contour lengths corresponding to the physical distances between relevant *rrn* genes. Selection for duplication irrespective of genetic position of end-points in *S. typhimurium* yielded a collection of duplications, some of which appeared to be *rrn*-mediated, (ROTH 1978). Involvement of *rrn* loci has been demonstrated by LEHNER and HILL (1980). Circles of predicted contour length have been isolated from certain of these mutants and the circles were shown to form R loops with ribosomal RNA.

Some gene duplications apparently have become stabilized during evolution of the bacterial genome. Seven *rrn* loci are currently maintained in the genome of *E. coli* Kl2 which, although not identical, are highly similar. Duplicate genes *tuf*A and *tuf*B are present in *E. coli* Kl2 and *S. typhimurium* genomes, but other bacteria contain only one copy. *E. coli* Kl2 bacteria, but not *E. coli* B or W, carry very similar genes *arg*I and *arg*F. On the basis of DNA heteroduplex studies and NH_2-terminal amino acid sequences, the duplicate genes *arg*I and *arg*F genes are presumed to have a common ancestry (KIKUCHI and GORINI 1975; GIGOT *et al.* 1978).

Although there is no definitive proof in bacteria, it is generally supposed that duplication of genes has provided an avenue for evolution of new functions by alteration of one of the gene copies toward a new function. One pair of genes that might *a priori* seem a likely candidate for such an evolutionary history, *trp*B and *tna*A, do not, however, show similarity in immunological specificity of either the native or denatured proteins (CHAFFOTTE, ZAKIN and GOLDBERG 1980), nor homology of base sequence as judged by failure to form DNA-DNA hybrids even under relaxed conditions (unpublished observations). The adjacent genes *pyr*B and *arg*I seem to be more likely candidates for this type of evolutionary history. These genes encode aspartate transcarbamylase and ornithine transcarbamylase, respectively. The NH_2-terminal amino acid sequences of the two enzymes bear close resemblances, having more than 35% homology in the sequenced segment (GIGOT *et al.* 1977).

Although gene duplication as a source of genetic diversity is widely accepted as an evolutionary mechanism, one is not able at present to assess the relative importance over evolutionary time of duplications as compared to single base substitutions.

INTERNAL REARRANGEMENTS

A potential source of variation in bacterial genomes is
rearrangement of segments of DNA such as inversion or trans-
position (STARLINGER 1977). Such events could, in theory, bring
about changes in regulation or even gene fusions at junction
points. Some large-scale rearrangement seems to have occurred
in nature: *E. coli* and *S. typhimurium* are inverted with respect to
each other over a 10-map-unit length (CASSEL, PASCAL and
CHIPPAUX 1973). A very few transposition strains of *E. coli* K12
have been isolated (JACOB and WOLLMAN 1971; DeWITT and ADELBERG
1962).

In *Bacillus subtilis*, a particular mutant that is defective
in the *trp*E gene was found on analysis to have undergone exten-
sive chromosomal rearrangements involving a transposition and
inversion of large segments of the genome (ANAGNOSTOPOULOS 1976).
Trp$^+$ merodiploid derivatives of this mutant contain very large
duplications as well. Some of the massively rearranged mero-
diploid strains are genetically stable, others are not
(TROWSDALE and ANAGNOSTOPOULOS 1976). Insight into factors
governing tolerance or intolerance of chromosome rearrangements
may emerge from further study of this interesting system.

One might expect inversions to occur with ease in bacte-
rial genomes between any two duplicate loci of opposite orienta-
tion. For instance, pairs of identical insertion sequences of
opposite orientation seem to offer such an opportunity. Even
more extensive homology is offered by pairs of *rrn* loci. Poten-
tial genetic activities of the duplicate *rrn* loci are diagrammed
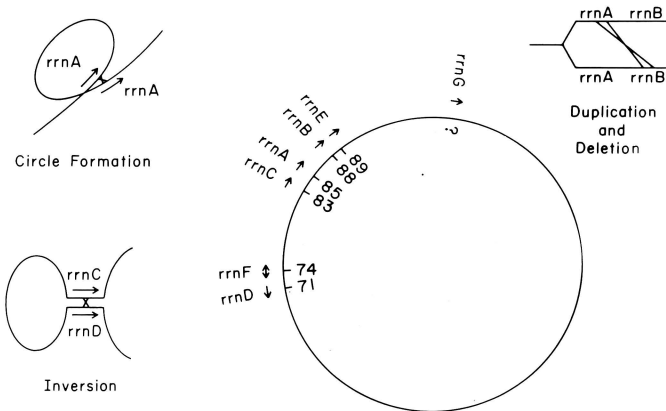in Fig. 3. Inversions between oppositely oriented *rrn* loci,

Fig. 3. Ribosomal RNA loci as agents of chromosome rearrange-
ment.

duplications between *rrn* loci of the same orientation and trans-
position of segregated circles all seem diagrammatically possi-
ble. Yet in the ordinary course of interrupted-mating mapping
studies using *E. coli* K12 Hfr strains, one does not observe re-
arrangements of the genome at detectable frequencies.

Small-scale rearrangements may be important regulatory
devices, as in the inversion that appears to govern phase varia-
tion in *S. typhimurium* (ZEIG *et al.* 1977), and may also be impor-
tant sources of genetic diversity within genes (ORNSTON and YEH
1979). Rearrangements and inversions that extend over small
distances appear to be common in the *E. coli* and *S. typhimurium* ge-
nomes (RILEY and ANILIONIS 1978). However, since large-scale,
stable genetic rearrangements have not been observed frequently
in bacterial populations, it seems possible that massive inter-
nal rearrangement has not played a prominent role in nature in
the evolution of bacterial genomes.

VARIATION AMONG GENOMES OF RELATED BACTERIAL GENERA

HOMOLOGIES BETWEEN ENTEROBACTERIAL DNAs

The extent to which the genomes of different bacterial
taxa are related to one another has been assessed by determin-
ing pairwise the extent of hybridization between the total DNAs
of the bacteria under various conditions of annealing that re-
quire the base sequences either to be well-matched or, over a
range, partially matched (e.g. BRENNER 1973; BRENNER, FANNING
and STEIGERWALT 1974; COLWELL *et al.* 1974). These studies have
shown that the DNAs of enteric bacteria are heterogeneous with
respect to degree of homology. For instance, between *E. coli*
and *Serratia marcescens* DNAs, there is a small fraction, 3%, that
is highly homologous, a larger fraction, 24%, that is about 14%
divergent, and a remainder that is poorly homologous and does
not form duplexes under any of the conditions employed (BRENNER
1973). Thus it appears that different portions of the enteric
genomes have diverged from one another to different extents, or
have evolved separately and later combined by horizontal trans-
fer.

We have visualized some of the "core" sequence homologies
that are highly conserved among more distantly related members
of the family Enterobacteriaceae. When either *E. coli* or *S.
typhimurium* total chromosomal DNAs were used as probes to hybri-
dize with an *Eco*RI digest of *S. marcescens* chromosomal DNA under
conditions that require hybrid base pairs to be well-matched,
only a few hybrid bands were seen on the autoradiograms and
these hybrid bands in most cases appeared to be identical for
the two probe DNAs (Fig. 4). Comparable results were obtained
when *E.. coli* and *S. typhimurium* DNAs were hybridized with *P. morganii*
DNA. Such shared sequences must be those that are highly con-
served among enteric bacteria, such as ribosomal RNA (WOESE
et al. 1975). Using ribosomal RNA as a probe, the majority of
the "core" sequences that had been visualized could be identi-
fied as chromosomal fragments containing rRNA sequences (Fig. 4,
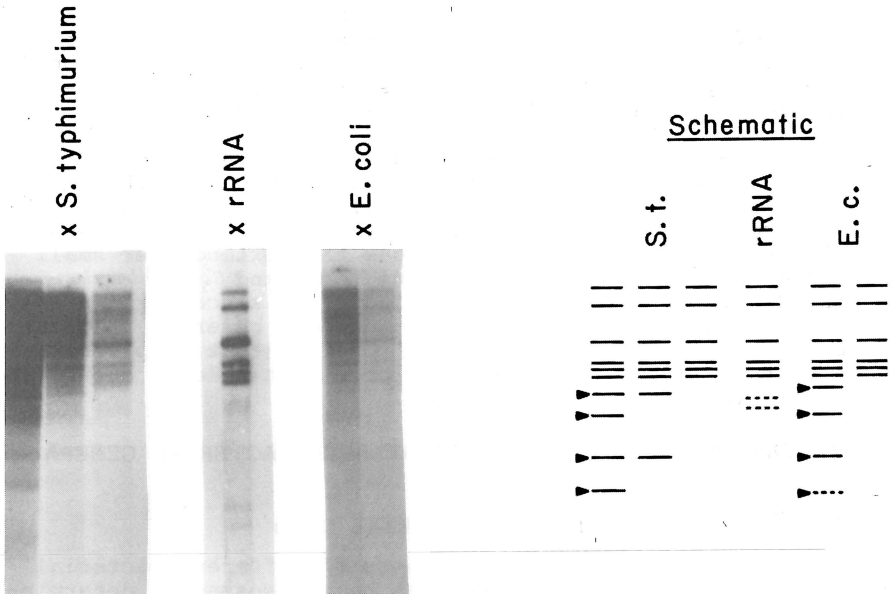ANILIONIS and RILEY, in preparation). The remaining non-rRNA

Fig. 4. *S. marcescens* chromosomal DNA *Eco*RI digest hybridized with
three probes: *E. coli* K12 chromosomal DNA, *S. typhimurium* LT2
chromosomal DNA and *E. coli* K12 ribosomal RNA. Methods as for
Fig. 1. Pointers mark non-ribosomal-RNA conserved hybrid bands.

conserved sequences have yet to be identified.

    Information is accumulating on homology relationships in
the less highly conserved majority fraction of enteric genomes.
Homology relationships among the *trp* operons of a group of bac-
terial genomes has been assessed by hybridizing φ80 DNA contain-
ing *trp* operon DNA to the *trp* mRNAs of several bacteria (DENNEY
and YANOFSKY 1972). To learn more about the relationships of
specific segments of the *E. coli* K12 genome to the corresponding
segments of other enterobacterial genomes, we hybridized λ probe
DNAs that carried *trp*, *tna*A, *thy*A and *lac*Z genes to the digests of
the chromosomal DNAs of nine enteric bacteria (RILEY and
ANILIONIS 1980). In these experiments, frequencies of base sub-
stitution in restriction-sensitive sites could not be estimated
by comparing sizes of hybrid fragments since only few instances
of conservation of size of hybrid restriction fragments were
seen among the enteric genomes. Instead, the amount of DNA that
was highly homologous to probe DNA was estimated. The hybridi-
zations were carried out under stringent conditions that only
allowed duplex formation between the small probe DNA fragments
and the corresponding sequences in the chromosomal restriction
fragments when these duplexes had Tm's ranging from 0 to 8°C
below that of *E. coli* DNA duplexes. Densitometric tracings of
autoradiograms permitted an estimate of the sum of the segments
of DNA within chromosomal restriction fragments that were well-
matched with probe DNA. Thus, comparisons of relative homolo-
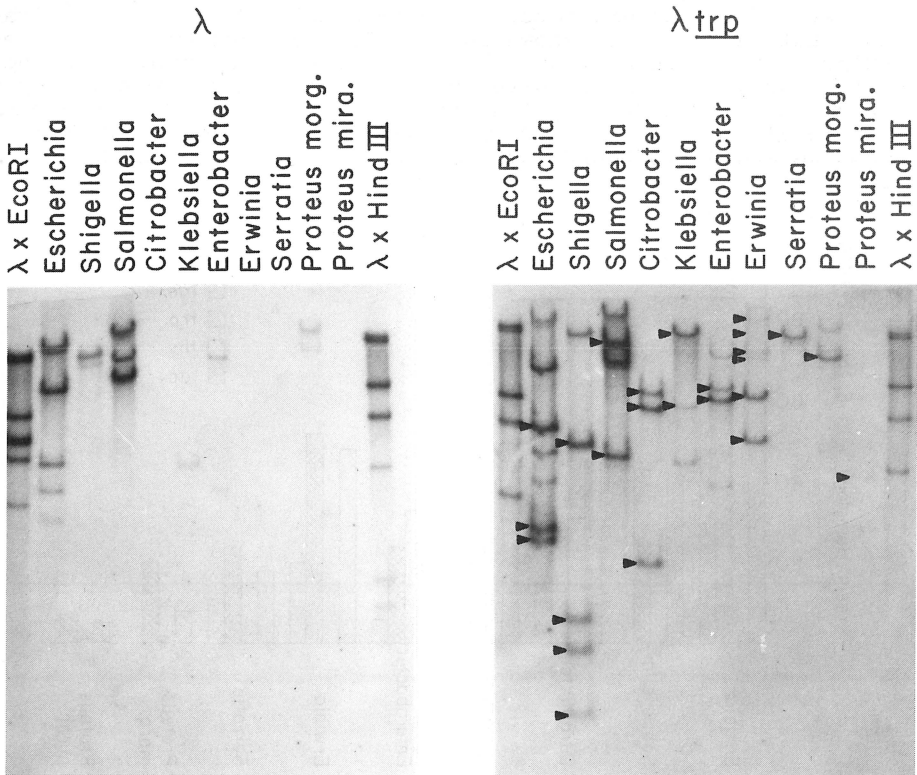gies in each enteric genome to the probe DNAs were possible.

Fig. 5. λ and λ*trp* DNA probes hybridized to *Hind* III digests of enterobacterial DNAs. Methods as for Fig. 1. Pointers mark *trp* -homologs.

When phage λ DNA was used as probe, hybridization patterns showed that not all enteric bacterial DNAs contained λ-homologs (Fig. 5a), and that among those that did hybridize, the amount of λ-homologous DNA in the chromosomes did not correspond either to commonly accepted taxonomic groupings or to the relative homologies of the total DNAs. Expressed as relative to *E. coli* K12 W1485 (λ⁻) set at 1.0, *S. typhimurium* registered 0.7, *Shigella dysenteriae* 0.26, *Proteus morganii* 0.10, *Enterobacter aerogenes* 0.07, *Klebsiella aerogenes* 0.01, whereas no homologs were detected in *Citrobacter freundii*, *Erwinia amylovora*, *Serratia marcescens* or *P. mirabilis*. The presence or absence or the amount of λ-homologous DNA does not seem related to the extent to which these bacterial genomes have diverged from one another during evolution. The number and extent of cryptic lambdoid prophages in bacterial genomes can reasonably be thought of as arising from more than one origin and as a result of multiple events involving genetic exchange and lateral transmission, and thus could be independent of vertically transmitted evolutionary change.

Amounts of well-matched homologs to the *E. coli trp, thy*A, *tna*A and *lac*Z regions were determined for both *Hind* III and *Eco*RI digests of the enterobacterial chromosomal DNAs. A representative autoradiogram is shown in Fig. 5b and a summary of the results is given in Fig. 6. The *trp, tna*A and *thy*A portions of the enterobacterial genomes have about the same relative homology to *E. coli trp, tna*A and *thy*A DNA as the genomes as a whole have to *E. coli* total DNA (cf. BRENNER *et al.*1972; BRENNER 1973; BRENNER, FANNING and STEIGERWALT 1975; COLWELL *et al.* 1974; BRENNER *et al.* 1978).

Fig. 6.   Relative homologies of *tna*A, *trp, thy*A and *lac*Z gene segments to enterobacterial DNAs.

In contrast, the *lac*Z-homologous portions do not always show this parallel relationship. Leaving to one side the absence of *lac*Z homology, as one might expect, in the Lac⁻ bacteria *S. typhimurium, P. mirabilis* and *P. morganii,* the amount of *lac*Z-homology in *E. aerogenes* and *K. aerogenes* chromosomes falls well below that expected on the basis of homology relationships between the total DNAs. Such out-of-step homology relationships may indicate that while the *trp, tna,* and *thy* regions evolved generally in concert with the genomes as a whole, the *lac*Z gene may have had a different evolutionary history, perhaps having undergone lateral transmission in the relatively recent evolutionary past. Enteric bacteria may have derived β-galactosidase genes from more than one source, some having *E. coli*-type *lac*Z genes, others such as *K. aerogenes* and *E. aerogenes* having a β-galactosidase gene of another type.

HORIZONTAL VS. VERTICAL EVOLUTION

For any particular genetic segment, in order to use data from present-day living bacteria to distinguish those genes that participated mostly in vertical or in horizontal evolutionary events in the past, more than one criterion will need to be applied. A set of criteria can be suggested as a beginning in making such distinctions. One might expect that laterally transmitted gene regions could be found residing in transmissible genetic elements in some bacterial strains at the present time, caught in transit, as it were. Further, laterally transmitted genes that are not essential to life might be expected to be present in the genomes of some related strains, absent in others. One might also expect that a genetic segment that has undergone horizontal transfer in the relatively recent evolutionary past would exhibit homology relationships that are not congruent with the homology relationships of the genomes as a whole. Analysis of chromosomal DNA at the junction between a laterally transmitted segment and the resident, vertically transmitted DNA might be expected to show a sharp demarcation in terms of homology relationships to the corresponding regions of related genomes, and might show a sharp demarcation with respect to AT-GC content as well. Examples of genes that show some of these features are given below.

Returning to *lac* as an example, it is suggestive of horizontal transmission that the genetic capacity for lactose fermentation is plasmid-borne in several bacteria. (See REANNY 1976 for general discussion.) As an example, recently a plasmid was isolated from *Yersinia enterocolitica* that contained a 5.6 kb *lac* region that was highly homologous with *E. coli* DNA (CORNELIS, GHOSAL and SAEDLER 1977). Some, if not all, *Klebsiella* strains are only weakly Lac⁺ unless they carry a plasmid containing a coli-like *lac* region (REEVE and BRAITHWAITE 1973).

As mentioned above, hybridization experiments indicate that *E..coli lacZ* homologs are not present in three out of eight enterobacterial DNAs tested, consistent with acquisition of *lac* genes by some, but not all, enteric bacteria. Also relevant to the history of the *lac* region is another approach that can help identify genetic regions that may be present in some genera but absent in others: a comparison of well-developed genetic maps. When the genetic maps of *E. coli* and *S. typhimurium* were subjected to a point-by-point comparison, each of these genomes was found to contain 12 to 13 genetic segments not possessed by the other (RILEY and ANILIONIS 1978). If absence of mapped genes reflects absence of DNA, then these extra genetic segments or "loops" could represent physical additions to one genome or deletions from the other. Many of the loops carry genes that are believed to be present in *E. coli* but not in *S. typhimurium*, such as *lac* and *argF*, or active in *S. typhimurium* but not in *E. coli*, such as *tct* (citrate transport) and *inlB* (inositol catabolism). Thus it seems quite plausible that the loops represent segments of DNA that have been gained or lost from the genomes of *S. typhimurium* and *E. coli* since the genomes of these organisms began to diverge from each other. A diagram illustrating the positions and extents of the postulated loops is given in Fig. 7 with
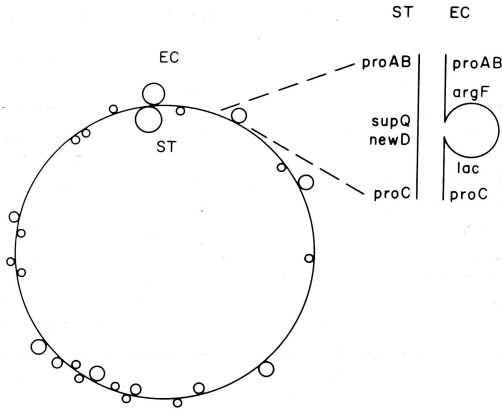
Fig. 7. Postulated additions or deletions to *E. coli* and *S. typhi-murium* genomes. (Not to scale.) See RILEY and ANILIONIS (1978).

emphasis on the *lac* region.

        Besides the genes on *E. coli* and *S. typhimurium* "loops", there are other genetic differences deriving from either presence or absence of genetic determinants. The *nif* cluster of nitrogen-fixation genes are present in *Klebsiella* species, but appear to be absent in non-nitrogen-fixing enteric bacteria. The *nif* genes can be transmitted in the laboratory to *E. coli* by transduction, becoming incorporated at the genetic location in *E. coli* corresponding to the location in *K. pneumoniae* (CANNON, DIXON and POSTGATE 1974). Such incorporation of the *nif* genes may take place in nature. An *E. coli*-like organism that is capable of nitrogen fixation has been isolated (NEILSON 1979).

        There are other examples: the two adjacent clusters of genes concerned with ribitol and arabitol catabolism, *rtl* and *dal*, are present in *K. aerogenes* and in *E. coli* C, but are absent in *E. coli* K12. These genes can also be transmitted to *E. coli* K12, taking up their characteristic genetic location in the chromosome (REINER 1975; RIGBY, GETHING and HARTLEY 1976). Such genes seem likely candidates for involvement of horizontal transmission in their evolutionary past.

        Heterogeneity in homology relationships may also be clues to intervention of genetic recombination in the past. Anomalously high or low homologies of particular genes or sections of genes when compared to homology relationships of the genomes as a whole may signal horizontal transmission. As described above, we have found that in some enteric bacteria the *lacZ* gene exhibits homology relationships that are anomalous with respect to

other genes and to the genomes as a whole, consistent with a horizontal mode of evolution.

However, caution in asserting anomaly must be exercised. Anomalously high conservation of a genetic segment relative to the rest of the genome may signify a high degree of conservation, perhaps related to particularly severe functional constraints, rather than horizontal exchange. Also, anomalously large variation of a particular gene or gene product with respect to other characteristics may not relate to a history of horizontal transmission. The amino acid sequences of some bacterial cytochrome c proteins are at variance with taxonomic relationships that have been based on gross cell morphology and metabolism, leading to a proposal that the genes for cytochrome c have undergone lateral transmission (AMBLER *et al*. 1979; AMBLER, MEYER and KAMEN 1979). However, it has been pointed out that the cytochrome c data is entirely congruent with ribosomal-RNA-sequence relationships and follows groupings of the organisms that are made on the basis of configuration of photosynthetic membranes and modes of cell division (DICKERSON 1980; WOESE *et al*. 1980). Thus the cytochrome c relationships appear to be anomalous in one context but are not in another.

Examples of heterogeneity in homology relationships are accumulating. Using the Southern transfer technique and hybridizing various chromosomal digests with small, genetically defined probe DNAs, RUVKUN and AUSUBEL (1980) found that at least part of the base sequences of the three structural genes for the nitrogenase enzyme complex are highly conserved in the genomes of a wide spectrum of nitrogen-fixing bacteria, whereas other genes of the *nif* gene cluster are not so conserved. Are the nitrogenase genes highly conserved because of functional constraints, or have they been widely transmitted horizontally in evolutionarily recent times? Homology relationships for other genes in this group of bacteria have not to date been determined, so that intragenomic comparisons are not yet possible.

In the same vein, the *tuf* gene (protein elongation factor) of *E. coli* shows internal heterogeneity in homology relationships. Hybridization of parts of *tuf* gene mRNA to various chromosomal DNA digests has shown that the COOH-terminal end of the *tuf* gene is highly homologous to the DNA of bacteria as distant from *E. coli* as *Chromatium* whereas the middle of the gene and the NH2-terminal fragment are less homologous (FILER and FURANO 1980). Is the COOH-terminal sequence highly conserved, evolving at a different rate from the rest of the gene, or has it been altered by horizontal transmission?

Heteroduplex analysis of the *hut* genes (histidine utilization) of *S. typhimurium* and *K. aerogenes* under progressively denaturing conditions has shown that all parts of this operon have not diverged uniformly (BLUMENBERG and MAGASANIK 1979). The four structural genes of the two organisms are more closely related than are the two promoters or the repressor gene. Again, one cannot yet distinguish between conservation and genetic exchange.

The ultimate detail for comparative analysis of homologous genes is, of course, provided by base sequence determinations. Sequences of parts of the *trp* operon, and the *lpp* region have been determined in related enterobacteria. Nucleotide sequences of the *trp* operon promoter, operator and leader regions and the NH$_2$-terminal end of the *trp*E gene have been determined for *E. coli*, *S. typhimurium* and *S. marcescens* (LEE *et al.* 1978, MIOZZARI and YANOFSKY 1978, and references therein). Several sequences up to 13 nucleotides in length in the promoter, operator and leader regions are conserved among the three bacterial DNAs. These conserved sequences are interpreted as having functional impor- tance (MIOZZARI and YANOFSKY 1978). The AT content of the se- quenced region of the beginning of the *trp* operon of *S. marcescens* (47%) is significantly higher than for total *S. marcescens* DNA (39%). The range of AT-GC content within individual bacterial genomes is narrow as judged by the distribution of chromosomal DNA shear fragments in CsCl density equilibrium gradients. A- nalysis of band widths showed that AT-GC contents are distri- buted with standard deviations no greater than 0.03 (ROLFE and MESELSON 1959). Thus the fraction of *S. marcescens* DNA (with av- erage AT content of 39%) that would be expected to have an AT content of 47% is very small. Segments of DNA that have anoma- lous AT-GC contents might be thought of as having been acquired from another genome by horizontal transfer. However, there is no evidence for insertion of a piece of unusual DNA at the be- ginning of the *trp* operon of *S. marcescens* within the portion that has been sequenced. No major discontinuities in AT-GC content are apparent in this region, nor discontinuities in the amount of homology to the corresponding *E. coli* or *S. typhimurium* DNAs.

The sequences of the *trp*A genes of both *E. coli* and *S. typhi- murium* have also been determined (NICHOLS and YANOFSKY 1979). Again, no discontinuities with respect to AT content or relative homologies within the gene were seen, and the AT-GC contents were not unexpected in relation to the genomes as a whole. Se- quences of the *trp*G-*trp*D junction in *S. marcescens* (separate genes) and in *E. coli* (fused genes) have also been determined (MIOZZARI and YANOFSKY 1979). These sequences seem to be significantly more homologous to each other than are the genomes as a whole, raising the possibility of lateral movement, although again no boundaries suggestive of genetic recombination were seen within the sequenced region.

Nucleotide sequences of the *lpp* (lipoprotein) region of the *E. coli* and *S. marcescens* genomes have been determined (NAKAMURA and INOUYE 1980). These sequences show a remarkable amount of heterogeneity both with respect to relative homologies of gene segments and with respect to AT content (Fig. 8). Highly homo- logous segments are interspersed with poorly homologous seg- ments. Borders between the segments are sharp. The sequenced part of a gene for an unknown polypeptide at the left end of the sequenced region shows high homology, those base substitutions that are seen having occurred exclusively at third positions of codons. These third-position changes are such that the AT con- tents of the unknown gene (*E. coli* 45%; *S. marcescens* 35%) are not far in each case from the AT content of the respective genomes as a whole (*E. coli* 49%; *S. marcescens* 39%), each falling within the
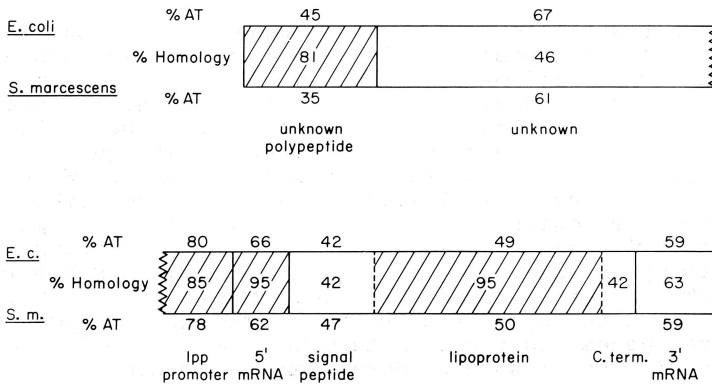
Fig. 8. A comparison of the *lpp* region of *E. coli* and *S. marcescens*. Data of NAKAMURA and INOUYE (1980).

range of AT contents that is present in the respective chromosomal DNAs. With sharp demarcation, the adjacent genetic region lying to the right (again of unknown function) has very poor relative homology. Sharply delineated, the *lpp* region that follows further on the right is generally highly homologous between the two DNAs. However, there are two clearly defined subsections of the *lpp* gene that have poor homology: (a) the coding region for the signal peptide and (b) the COOH-terminal end of the lipoprotein. The rest of the *lpp* region, including the promoter region and the non-translated mRNA sequences, are highly homologous. The AT contents of the non-translated parts of the *E. coli lpp* gene are unusually high (80%, 66% and 59%, see Fig.8). Even more extraordinary, the AT contents of all parts of the *S. marcescens lpp* gene are nearly identical to the corresponding *E. coli* values and lie far from the average AT content of the *S. marcescens* genome as a whole.

The presence of sharp boundaries with respect to relative homology and AT content, and the anomolously high AT content of most of the *S. marcescens lpp* region suggest, as NAKAMURA and INOUYE (1980) have pointed out, that the *lpp* region of *S. marcescens* may have an evolutionary history different from the rest of the genome. The *S. marcescens lpp* gene may have been acquired by horizontal transmission from a source whose base sequence and AT content is like that of the *E. coli lpp* region.

Returning to the suggestion that one might apply more than one criterion in order to identify gene regions that might have undergone horizontal exchange during evolution, one can ask whether enough information is now available for any one gene region to allow application of all of the criteria in any one case, and whether any presently characterized gene region seems on this basis to be likely to have a horizontal evolutionary

history.

With respect to presence of a gene in some, but absence
in other related genomes, applicable only to dispensable or
duplicate genes, all of the gene regions dealt with in this
essay that are dispensable are apparently absent in related
genomes: *lac*, λ-homologs, *hut*, *rtl*, *dal*, *nif* and the duplicate
copy of the *tuf* gene meet this description. The question is not
applicable to the indispensable *trp* and *lpp* genes. With respect
to residence in natural plasmids or other transmissible agents,
among the gene regions we are considering, only *lac*, phage λ
genes and *nif* genes have been found in nature in transmissible
forms, to my knowledge. With respect to anomalous homology
and/or AT content as compared to the rest of the genome, hy-
bridization data suggests that *lac*, λ-homologs, nitrogenase
structural genes and the COOH-terminal end of the *tuf* gene are
anomalous in this respect. Heterogeneity in homology is also
seen within the *hut* operon. Far more precise information from
base-sequence data reveals striking anomalies with sharp bound-
aries around and within the *lpp* gene and to a lesser extent at
the *trp*G–*trp*D junction as well.

From this information, no proof emerges, but the *lac*, λ-
homolog and *lpp* regions appear to be prime candidates for having
at least some horizontal component in their evolutionary histo-
ries. More information will be needed on these and other genes.
Another bit of evidence that would be consistent with a hori-
zontal history would be the existence, within a group of relat-
ed genomes, of more than one type of gene for a single physio-
logical function. Such a situation would be compatible with
independent multiple origins of genes for that function. A
hint of such a situation presents itself in the case of *lac*.
The chromosomal *lac* genes of *K. aerogenes* and *E. aerogenes* that show
relatively poor homology to the *E. coli lac*Z gene might have been
acquired from a different source than the ancestor of the *E. coli*
lac genes. Determination of relative homologies in enteric ge-
nomes to the *K. aerogenes* gene for β-galactosidase might provide
information on this point.

CONSTRAINTS ON GENE MOVEMENT

Having discussed ways by which we might hope to distin-
guish vertically evolved from horizontally acquired genes, we
might ask what fraction of bacterial genes are expected to have
evolved by these two modes. What relative importance have lat-
eral modes had in the course of the evolution of bacterial ge-
nomes? Self-replicating extra-chromosomal elements such as
plasmids are found widely distributed in bacteria, and many of
them are self-transmissible. Active genetic elements such as
transposons and insertion sequences undergo illegitimate recom-
bination and are able to potentiate the exchange of genes be-
tween taxonomically distant genomes. Horizontal transfer of
genes is seen by some as an important element in bacterial evo-
lution (REANNY, 1976), and this may well be the case for cyto-
plasmic genes. But widespread, unbridled chromosomal integra-
tion of genetic elements derived from distantly related bacteria

would, if carried to an extreme, randomize all participating genomes, eliminating the identities of the many kinds of bacteria that presently exist. Therefore, some moderating counter-force(s) must act to prevent homogenizing of bacterial chromosomes.

Conservative force(s) must also act to prevent internal scrambling of the gene order in individuals within populations of a particular species (STARLINGER, 1977; RILEY and ANILIONIS 1978). Within limits of detection, all members of a normal population of *E. coli* Hfr donors are found to transmit genes in the same order during conjugation. Even among related genera, genetic maps show that overall arrangement of genes is grossly similar. The maps of *E. coli* (BACHMANN LOW and TAYLOR 1976), *S. typhimurium* (SANDERSON and HARTMAN 1978), and *K. pneumoniae* (MATSUMOTO and TAZAKI 1971) share the same general order of genes around the circular map with the exception of the one large inversion mentioned earlier. There are similarities in the less well-developed maps of *Streptomyces coelicolor, S. rimosus* and *Nocardia mediterrani* (FRIEND and HOPWOOD 1971; SCHOPF 1970; HOPWOOD *et al.* 1973), in the maps of two *Bacillus* species: *B. subtilis* and *B. lichenoformis* (ROGOLSKY 1970), and in the maps of *Pseudomonas aeruginosa* strains PAO and PAT, which have quite separate geographical origins (HOLLOWAY, KRISHNAPILLAR and MORGAN 1979).

Thus, even though opportunities exist for internal rearrangement of bacterial genomes, for instance *via* recombination between duplicate genes (as discussed earlier), the same global gene order is apparently maintained among members of groups of related bacteria. Why is it that internal recombination between, for instance, *E. coli rrn* loci, observed to take place under selective conditions in the laboratory, has not generated many genetically rearranged strains of *E. coli*? Is there a functional advantage to particular gene orders? Some of the rearranged *trp* merodiploid strains of *B. subtilis* studied by TROWSDALE and ANAGNOSTOPOULOS (1976) were found to be unstable, whilst others were stable. Are there some types of chromosomal rearrangements that are permitted and compatible with normal cell function while other rearrangements are not permitted, violating perhaps some spatial arrangement of genes that is necessary for optimal function? No functional advantage to present gene order has yet been demonstrated. Recently, Dr. Charles Hill (personal communication) has selected *E. coli* strains that have undergone transposition of segments of DNA which are bracketed by *rrn*B and *rrn*E to locations at *rrn*C in one case and *rrn*D in two other cases. No growth disadvantage has yet been observed in these internally rearranged strains.

There is no obvious explanation at present for the observed conservation of global gene order within groups of related bacteria. This conservative tendency might be related to the counter-force that seems to have prevented complete mixing of the genomes of taxonomically distant bacteria by horizontal modes of evolution.

CONCLUSION

Studies on mechanisms of evolution of the bacterial genome have only begun. Techniques and experimental approaches lie at hand for advancing our understanding of this process. Further comparison of nucleotide sequences of isofunctional genes from related genomes by Southern   transfer and hybridization serves as a beginning, but ultimately base-sequence analysis will bring the information needed to distinguish modes of inheritance and evolutionary histories. Further study of bacteria that have undergone chromosomal rearrangement with an eye to defining any barriers that may reject some types of rearrangement may help elucidate one of the conservative evolutionary forces that restrains unbridled mixing and scrambling of bacterial genomes.

ACKNOWLEDGMENTS

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