and released only 11 percent of the rat SRS-A of a control group (Table 1). Experiments with a rabbit antiserum to rat thymic lymphocytes (*12*), given intravenously in a dose of 0.2 ml 4 hours before the experiment, revealed no significant suppression of release of rat SRS-A when the absolute lymphocyte count was reduced to 20 percent of control values.

The number of hemolytic units of complement ($C'H_{50}$) of rats which had produced rat SRS-A was reduced by about 25 percent as compared to a group of control animals. The effect of the various treatments on the $C'H_{50}$ was therefore determined (Table 1). None of these procedures seriously depleted serum complement. When complement was removed from rats by means of a semipurified, nontoxic fraction of venom from cobra (*Naja haje*) (*13*), SRS-A production was markedly reduced. We cannot state whether this inhibition occurred because of dependence of the reaction on complement or because of depletion of the substrate for release of rat SRS-A; cobra venom itself releases a slow-reacting substance from tissue and serum (*14*). Investigation of the possible requirement of complement must await the establishment of a system in vitro.

Stechschulte *et al.* (*5*) successfully produced rat SRS-A in the peritoneal cavity of the rat by use of hyperimmune rat antiserums and specific antigen. In experiments with homologous antiserums in previously treated rats, we confirmed the results obtained with rabbit antiserums. Thus, in the rat, we recognize two different homologous immunoglobulins which mediate the selective release of distinctly different chemical mediators, histamine and rat SRS-A, through the participation of different cell types. One antibody, present early in immunization, sensitizes rat mast cells for the release of histamine and serotonin (*5, 15*), while the other, found in hyperimmune antiserum, prepares the rat for the release of rat SRS-A, provided that circulating leukocytes are present.

The polymorphonuclear leukocyte contains several hydrolytic enzymes which probably participate in the inflammatory process. Acid proteases and cathepsins have been suggested as possible injurious agents (*16*). A cationic protein fraction of lysosomes of polymorphonuclear leukocytes disrupts rat mast cells in vitro, releasing histamine (*17*). Kinin-forming enzyme activity also has been associated with the lysosomal fraction of polymorphonuclear leukocytes (*18*). We stress the significance of this cell in inflammation by regarding it as a prerequisite for the release of a third mediator, rat SRS-A.

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* Postdoctoral trainee supported by NIAMD training grant AM 05076-11.
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5 June 1967

Genetic Recombination in Escherichia coli: Clone Heterogeneity and the Kinetics of Segregation

Abstract. *Several recombinant types may be produced from a single* Escherichia coli *zygote when the donor parent is Hfr-Hayes and segregation is delayed; usually only one type is produced with Hfr-Cavalli. Statistical experiments confirm well-known pedigree analyses by micromanipulation and suggest that DNA synthesis is required before final genetic integration.*

Lederberg (*1*) and Anderson (*2*) using micromanipulation have made pedigree analyses of the progeny of zygotes of *Escherichia coli*; these are among the most revealing studies on genetic recombination. Lederberg found that segregation following matings with an Hfr-Cavalli (HfrC) donor strain was usually complete by the third division and that generally only one recombinant class was produced per zygote. However, with an Hfr-Hayes (HfrH) donor strain, Anderson found that segregation was much more delayed (three to more than nine divisions) and usually several recombinant classes were produced from a single zygote. Tomizawa (*3*) has used a statistical technique to study the kinetics of segregation and has confirmed the findings of Lederberg for matings with an HfrC donor parent. These studies are of particular importance in that they provide limitations on possible mechanisms for genetic integration.

Because of the differences mentioned above, the number of recombinant classes appearing in single clones (clone heterogeneity) and the kinetics of segregation for both HfrC and HfrH donor strains have been studied with a statistical method of analysis. The results confirm the observations of both Lederberg and Anderson and indicate that segregational patterns are dependent on the male strain utilized.

Donor strains were prototrophic for the markers used and were streptomycin-sensitive. For HfrH the order of marker transfer was origin, threonine, leucine, galactose, tryptophan, histidine, and streptomycin; for HfrC, the order was origin, lactose, proline, leucine, threonine, arginine, and streptomycin. The recipient strains (PA309 and PA330) could not synthesize the amino acids or ferment the sugars listed. My results are independent of the recipient strains used.

The general techniques for mating and for determination of the genetic constitution have been described (*4*). Both parental types were grown to concentrations of about 2×10^8 cells per milliliter (exponential phase), mixed in the ratio of 1 male cell per 10 female ones,

Table 1. Distribution of recombinant classes in individual primary clones (percent).

Donor strain	Colonies analyzed	Number of recombinant classes per clone						
		1	2	3	4	5	6	7 or 8
Hayes	321	49.8	24.9	13.1	7.2	2.5	1.6	0.9
Cavalli	244	91.4	7.0	1.2		0.4		

and mated in broth at 37°C; the cultures were gently agitated for 90 minutes (in some cases 45 minutes). At the end of this period, the mating mixture was subjected to a high shear force to separate the mating pairs, diluted in warm broth (by 10 to 100 times) containing streptomycin (60 μg/ml), and incubated again. The mixtures were shaken vigorously during the incubation period, and portions were removed at intervals, diluted, blended, and plated on selective media. Cellular clumping was negligible as judged by microscopy. The primary selection was for a distal male marker (histidine for HfrH, and arginine for HfrC) and for the streptomycin-resistant female marker.

After incubation at 37°C for 36 hours, entire recombinant clones (20 or 40 in number) were removed from the selective agar, resuspended in buffer, and portions were plated on fresh plates of the same selective media. From these plates 52 individual clones were chosen at random and inoculated on additional plates, from which replica printings were made to determine the presence or absence of the four unselected markers (the genetic constitution) of the original 20 (or 40) clones. If all 52 of the secondary clones had the same genetic constitution, the primary clone was considered within the resolution of this experiment to have only one class of recombinant and was defined as homogeneous; if there were two or more classes of recombinants appearing among the 52 secondary clones, the primary clone would have had at least as many classes of recombinants and would be defined as heterogeneous.

An analysis of recombinant clones produced from samples taken from the mating mixture at the end of the mating period (no incubation) is given in Table 1. Approximately half of the primary recombinant colonies from matings involving HfrH have two or more recombinant types (an average of 2.0 types per colony) while over 90 percent of the recombinant colonies from matings involving HfrC have only one recombinant type (an average of 1.1 types per colony). These results provide only a lower limit on the number of recombinant classes present in a recombinant colony. For four unselected markers, the maximum number of classes per clone would be 16, while the maximum number detected to date is eight; a larger number would be expected with a more extended analysis. In addition, the number of classes would increase if a larger number of proximal markers were used. Furthermore, the detection of the various classes of recombinants is dependent on their frequencies of appearance in the primary colony, which is related to their relative growth rates; a recombinant class having a growth rate 20-percent lower than the fastest growing class would in general not be detected. This multiplicity of classes changes the significance of the genetic analysis usually obtained through replica printing, as classes having the auxotrophic characteristics will be masked by those having prototrophic ones, producing thereby an apparent increase in linkage between donor markers (*5*).

The frequent appearance of several recombinant classes per zygote in matings with HfrH places limitations on mechanisms which may be operative in the integration process. The simplest model for genetic recombination by way of a break and reunion mechanism is based on several assumptions: (i) both donor and recipient DNA is double-stranded and homozygous; (ii) the donor DNA does not replicate within the recipient parent before integration; (iii) integration is accomplished through double-strand interactions between the donor and recipient DNA; (iv) integration is fairly rapid (within a generation); and (v) there is no subsequent crossing-over between a recombinant chromosome and a recipient one. No multiplicity of recombinant classes is expected under such conditions. Anderson, to account for both multiple recombinant classes and delayed segregation, postulated that integration involved a copy-choice type of mechanism in which the donor DNA persisted for several generations (*2*). However, there are now indications that at least some recombinant formation in bacteria is due to break-and-reunion type mechanisms (*6*, *7*). Relaxation of assumptions (iii) or (v), or both, would allow two classes of recombinants to be formed per zygote. The strongest evidence for assumption (iv) is supplied by the Tomizawa experiment (*3*) in which only a Cavalli-type donor was used, and by radiation experiments in which a diminution in radiation effects on linkage is taken as evidence of integration (*8*); I have some evidence that with HfrH the Tomizawa experiment is much less dramatic.

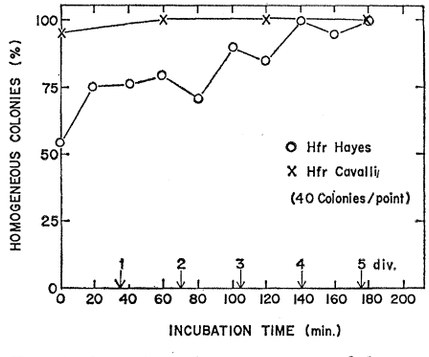

Fig. 1. Kinetics of appearance of homogeneous clones. Average division times of the recipient parent are indicated on the abscissa.

Furthermore, radiation stabilization would probably be afforded by noncovalent linkages between donor and recipient chromosomes similar to those suggested by Oppenheim and Riley (*6*). To account for the multiplicity of recombinant types found in the progeny from a single zygote and to retain a break-and-reunion mechanism for integration, it is necessary for the donor DNA to replicate within the recipient parent before final integration. Such zygotes would constitute a type of partial diploid in which a new recombinant class could be produced with each round of DNA replication as a result of integration between the two types of DNA which derive their informational content from the donor and recipient parents. For matings with HfrH in which 2.0 recombinant classes are found, on the average, per zygote, integration would take from two to three divisions, and segregation would require at least one more division.

The kinetics of integration-segregation have been studied by testing the heterogeneity of recombinant clones produced from portions removed from the mating mixture at various times after the termination of mating (Fig.

1). In matings with HfrH clone homogeneity increases with incubation time from a value of about 50 percent to 100 percent in approximately 140 minutes or about four divisions of the recipient parent. In view of the fact that 90 percent of the clones produced from matings from the Cavalli strain are homogeneous with no incubation, the resolution of this type of experiment is inadequate to study the kinetics of segregation in this strain.

These two types of experiments suggest that DNA synthesis is required in the recipient parent if a multiplicity of recombinant classes per zygote greater than two is found regardless of whether recombination occurs via copy-choice, break-and-reunion, or a combination of the two. The studies reported here with HfrC indicate that generally only one recombinant type is produced per zygote, a result consistent with the earlier studies of Lederberg (*1*) and of Tomizawa (*3*). Less complete studies with other Hfr strains (AB735, Hfrl, and Ral) indicate that the segregational pattern of the Cavalli strain is typical of these strains and that the Hayes strain may be considered to have the aberrant behavior.

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9. Supported by a grant from PHS (C-6629). I thank C. Fetterolf, E. Ambush, and S. Randolph for technical assistance.

4 April 1967

Human Growth Hormone and Placental Lactogen: Structural Similarity

Abstract. *Sequential analysis of the first 17 amino acids from the amino-terminus of human placental lactogen reveals similarity to the sequence of human growth hormone, 11 of the residues being identical.*

The similar immunologic and biologic properties of human growth hormone (HGH) and placental lactogen (HPL) led us to look for structural similarities. The molecular weight of HGH is 21,500 (*1*), and the complete amino acid sequence has been reported (*2*).

A purified preparation of HPL for structural analysis was isolated from human term placentas by salt extraction, ethanol precipitation, gel filtration, isoelectric precipitation, and ion-exchange chromatography (*3*); it was homogeneous on gel filtration, disc electrophoresis, and analytical ultracentrifugation. The COOH-terminal amino acid was phenylalanine, the NH_2-terminal sequence was H-Val-Gln-, and the amino acid composition was similar to that of HGH.

The molecular weight of the hormone, as determined by ultracentrifugation, is reported to be 37,500 (*4*) and 39,000 (*3*), whereas the minimum molecular weight calculated from quantitative NH_2-terminal data is 21,500 (*3*). Ultracentrifugal studies of HPL have shown evidence of the presence of a monomer having a molecular weight of 18,600 (*4*).

These findings suggested that the HPL molecule may be chemically related to HGH. To further examine this possibility we have determined the sequence of the first 17 residues from the NH_2-terminus, using the protein sequenator of Edman and Begg (*5*). The purified HPL (7 mg) was directly subjected to degradation in the sequenator by use of the technique described for myoglobin (*5*).

Each degradation cycle produced one main amino acid component and a very faint second component. We present the sequence of the major component, with the NH_2-terminal structure of HGH (*2*) for comparison:

HPL: H-*Val-Gln*-Thr-*Val*-Pro-Leu-Ser-Arg-
HGH: H-*Phe-Pro*-Thr-*Ile*-Pro-Leu-Ser-Arg-
1 2 3 4 5 6 7 8
Leu-Phe-Asp-*His*-Ala-Met-Leu-*Gln-Ala*-
Leu-Phe-Asp-*Asn*-Ala-Met-Leu-*Arg-Ile*-
9 10 11 12 13 14 15 16 17

In this sequence of 17 amino acids from the NH_2-terminus of HPL, a total of 11 are identical with those occupying the same position in the HGH molecule. Such extensive similarity between the two hormones establishes beyond doubt a structural relation providing a basis for the well-documented immunologic and biologic similarities of HGH and HPL (*6*). Our results support the proposal that the HPL monomer consists of a single peptide chain with considerable similarity to HGH.

The minor component of each cycle had a sequence identical with that of the main component, with the exception that the NH_2-terminal sequence H-Val-Gln- was missing. The presence of such a "shortened" molecule in the preparation is interesting in view of the suggestion (*7*) that the two unidentical chains of bovine growth hormone may differ only by the absence of a small NH_2-terminal peptide.

Further sequence studies of HGH and HPL should provide structural explanations for the differences in potency of the antigenic, lactogenic, and somatotrophic properties that are shared by these molecules. The production of similar proteins by such diverse cells as the pituitary acidophils and the placental syncytiotrophoblast (*8*) doubtless reflects a biochemical need analogous to that which evokes the production of chorionic gonadotrophin by the placenta to maintain the corpus luteum of pregnancy. However, since the function of HPL has not been fully elucidated, the nature of this need remains unapparent.

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11 May 1967