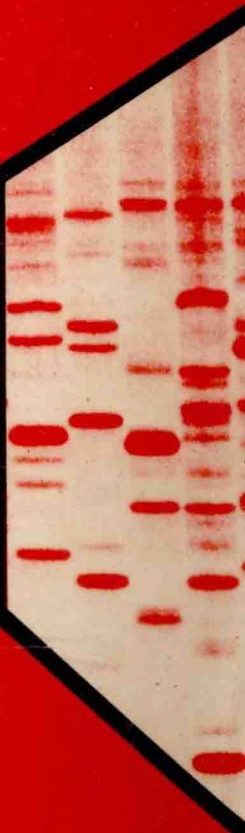
MOLECULAR BIOLOGY

A SELECTION OF PAPERS



compiled by

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MOLECULAR BIOLOGY: A SELECTION OF PAPERS

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Preface

The Journal of Molecular Biology (JMB) was founded by John Kendrew just over thirty years ago and the first issue, a modest one of less than a hundred pages, appeared in April 1959. Few present day readers can appreciate how daring this venture seemed at the time; after all, the subject had only just begun, and the number of people willing to call themselves molecular biologists in public was still quite small. But as we now all know, the subject and, for that matter, the Journal, were doomed to success from the start. Molecular biology became pervasive and now dominates areas of biological research, and, today, nearly everybody is a molecular biologist.

This book celebrates this remarkable development and marks the thirtieth anniversary of the founding of JMB by reprinting a selection of papers from the first hundred volumes. These papers span the first half of the life of JMB, up to 1975, and cover the period when most of the fundamental discoveries of molecular biology were made. During this time the first three-dimensional structures of protein were obtained by X-ray diffraction and the molecular basis of the regulation of protein function was revealed; messenger RNA was discovered, and the mechanism of protein biosynthesis and the structure of the genetic code were elucidated; DNA replication came to be understood and a new area of the structural analysis of macromolecular assemblies in viruses and cellular components was ushered in by new technical extensions of electron microscopy and X-ray diffraction. During this time the foundations were also laid for the second and more recent phase of molecular biology based on techniques of DNA cloning and sequencing developed in the mid-1970s.

The papers in this volume have been selected to illustrate some of these developments in molecular biology, and represent the twin streams of genetics and structural biochemistry that united to form the subject. The first reprint, from Volume 1 of JMB, reports the famous 'pajamo' experiment which proved that repression was dominant and that it involved the synthesis of a cytoplasmic factor. This volume also included the paper on *E. coli* ribosomes and another that showed that bacteriophages were built up of a set of structural components. Volume 3, published in 1961, contained the classic review of Jacob and Monod on genetic regulatory mechanisms which set the scene for the great developments that were to follow in this field. There was also a short note in this volume on the Theory of Mutagenesis with the essential idea that led to the use of acridine induced mutations to analyse the genetic code. The short note in Volume 4 by Champe and Benzer describes what we would now call a fusion protein, made by a genetic deletion, *r*1589. Volume 6 was marked by a second important review on "Allosteric Proteins and Cellular Control Systems" in which Monod, Changeux and Jacob first put forward a set of important ideas on regulation.

Cairns has two papers that exploit the direct use of autoradiography to visualize the chromosomes of bacteriophages and bacteria. Volume 6 contained the remarkable demonstration that the DNA of *E. coli* was one circular molecule, 1-mm long. Included are a number of important papers on electron microscopy. There is the 1963 paper by Huxley on the structure of filaments of striated muscle and a definitive paper on the structure of viruses by Klug and Finch, which appeared in 1968. Also included is the earlier 1964 paper of Klug on optical methods for image reconstruction which laid the foundations for the correct and objective analysis of negatively stained preparations.

Volume 94, 1970, contained the important paper by Unwin and Henderson on a novel and powerful method of determining molecular structure by electron microscopy.

There are many papers on the genetic code and the mechanism of protein synthesis. In 1964 Sanger and Marcker reported a transfer RNA (then called s-RNA) with formylmethione attached to it, and with this method discovered the special mechanism for chain initiation in protein synthesis. Crick's Wobble Hypothesis of codon–anticodon recognition, appeared in Volume 19, together with the experimental confirmation of the ideas by the Khorana group in the succeeding paper. Natural mutants of a tyrosine suppressor RNA are reported in Volume 47, 1970, using sequencing methods developed by Sanger and reported in a 1965 paper reprinted here. In 1971, an entire issue of JMB was devoted to the publication of Khorana's work on the total chemical synthesis of a transfer RNA, the introduction to the series is included in the collection.

From 1970 onwards, papers appear that were important steps in the development of the modern approach in molecular biology. Many are technical, and it is to the credit of the Editors that they were willing to recognize new techniques and publish papers many of which exercised a seminal role in the development of the subject. Thus, there is the first report in Volume 53, the transfection calcium-treated *E. coli* by DNA, of a method which proved to be absolutely essential in the development of cloning methods using this bacterium. Restriction enzymes make their appearance; there is the first report of a recognition site by Smith in Volume 51 and we also reprint one of Nathan's papers in Volume 78 on the use of these enzymes to map a DNA, in this case, the virus SV40. Southern's paper on his method of detecting nucleic sequences by hybridization is reprinted from Volume 98, and Sanger's paper on a new method of DNA sequencing appeared in 1975. We have also included a paper by Kaiser and Lobban reprinted from Volume 78, 1973, which is an early remarkable piece of work on the beginning of DNA cloning. The important paper by Weissmann on site-directed mutagenesis, using $Q\beta$, reprinted from Volume 89, 1974, will be recognized by readers as already very much in the modern style. And, finally, we have included two papers on the analysis of protein sequences, one being the well-known computer algorithm of Needleman and Wunsch from Volume 48, 1970.

The reader should recognize that this selection is a personal one, and I must emphasize that I accept sole responsibility, not only for the papers included here but also for all the omissions. My first list would have generated a book three or four times the present size and each successive culling became more and more difficult. The papers that remained are not necessarily "The Best of JMB", although many certainly are; and in addition to papers which reflected the achievements of the subject I have included some whose value lay in the future and could not have been fully appreciated at the time. I hope too that the reader will learn something of the development of our subject. Many ideas, which today we find simple and obvious, only became established by complex and difficult argument as people struggled to extract themselves from the rigid mould of contemporary thinking, and many experiments that today are carried out with kits ordered by telephone were than heroic acts at the limit of existing technology.

Science advances at an ever-increasing rate and molecular biology is no slouch. Anything more than 2-years old is already history and anything older than 4-year lifetime of a graduate student is prehistorical. Even so, there is something we recognize as modern about all of these papers; they represent steps on the long, uncompleted road of molecular biology, leading to understanding how the nucleotide sequences of genes specify the complex functions of protein molecules, and how these interact to produce the regulated behaviour of cells.

Sydney Brenner

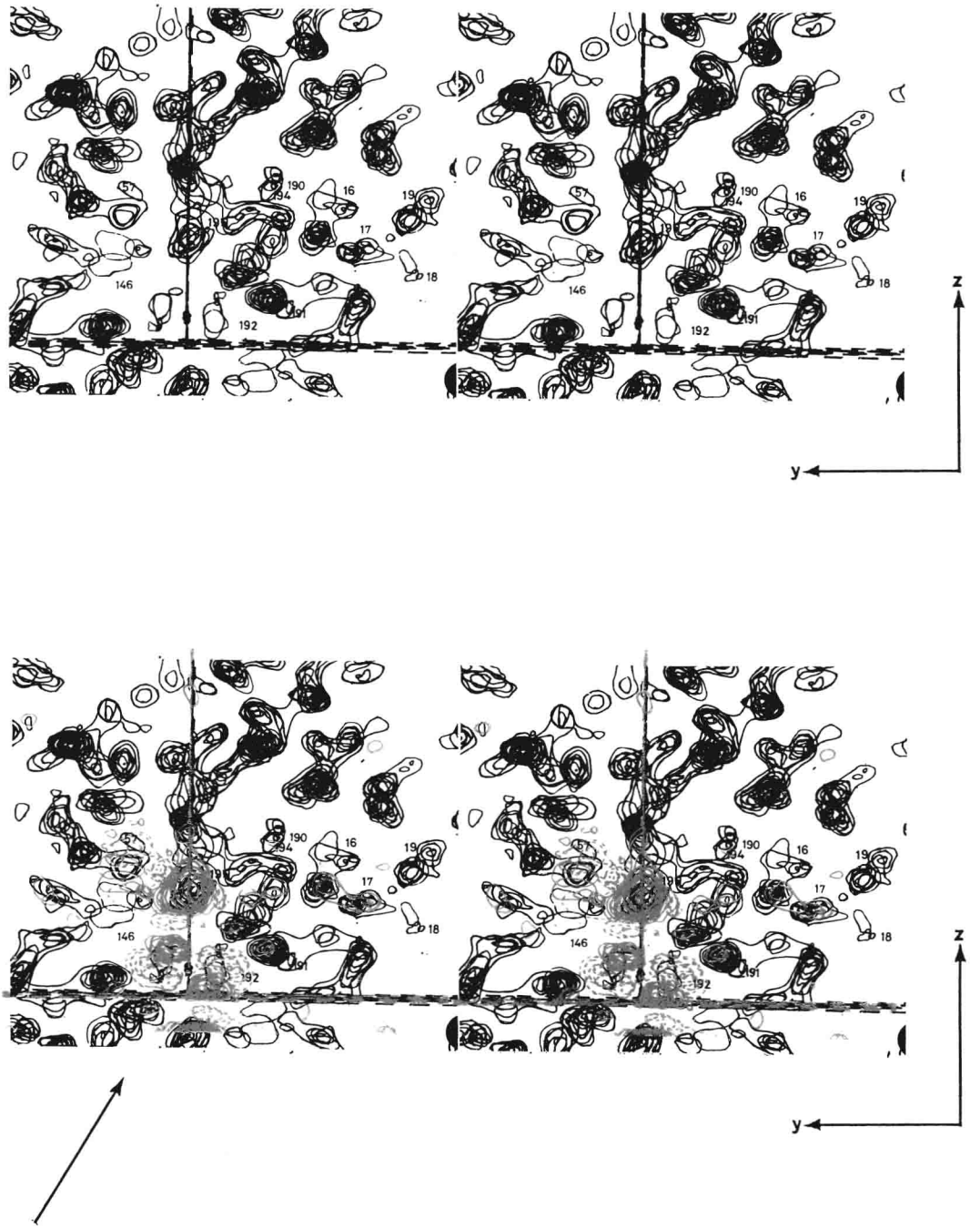

FIG. 5. Part of the electron density map in the vicinity of the active centre, including the sections $x = 17$ to 24. Most of the residues in Fig. 4 are included. In blue is shown the difference of density between the tosyl- and the native enzyme. The contours of the difference map are separated by about 0·04 el/Å^3; the zero contour is omitted, and negative contours are dashed. For clarity, in the vicinity of the sulphonyl group, five higher contours of difference density are omitted. The arrow indicates the approximate viewpoint from which Figs 4 and 6 are drawn.

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The Genetic Control and Cytoplasmic Expression of "Inducibility" in the Synthesis of β-galactosidase by *E. Coli*†

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(*Received 16 March 1959*)

A number of extremely closely linked mutations have been found to affect the synthesis of β-galactosidase in *E. coli*. Some of these (*z* mutations) are expressed by loss of the capacity to synthesize active enzyme. Others (*i* mutations) allow the enzyme to be synthesized constitutively instead of inducibly as in the wild type. The study of galactosidase synthesis in heteromerozygotes of *E. coli* indicates that the *z* and *i* mutations belong to different cistrons. Moreover the constitutive allele of the *i* cistron is recessive over the inducible allele. The kinetics of expression of the i^+ (inducible) character suggest that the *i* gene controls the synthesis of a specific substance which represses the synthesis of β-galactosidase. The constitutive state results from loss of the capacity to synthesize active repressor.

1. Introduction

Any hypothesis on the mechanism of enzyme induction implies an interpretation of the difference between "inducible" and "constitutive" systems. Conversely, since specific, one-step mutations are known, in some cases, to convert a typical inducible into a fully constitutive system, an analysis of the genetic nature and of the biochemical effects of such a mutation should lead to an interpretation of the control mechanisms involved in induction. This is the subject of the present paper.

It should be recalled that the metabolism of lactose and other β-galactosides by intact *E. coli* requires the sequential participation of two distinct factors:

(1) The galactoside-permease, responsible for allowing the entrance of galactosides into the cell.

(2) The intracellular β-galactosidase, responsible for the hydrolysis of β-galactosides.

Both the permease and the hydrolase are inducible in wild type *E. coli*. Three main types of mutations have been found to affect this sequential system:

(1) $z^+ \rightarrow z^-$: loss of the capacity to synthesize β-galactosidase;

(2) $y^+ \rightarrow y^-$: loss of the capacity to synthesize galactoside-permease;

(3) $i^+ \rightarrow i^-$: conversion from the inducible (i^+) to the constitutive (i^-) state.

The $i^+ \rightarrow i^-$ mutation always affects *both* the permease and the hydrolase. All these mutations are extremely closely linked: so far all independent occurences of each of these types have turned out to be located in the "*Lac*" region of the *E. coli* K 12 chromosome. However, the mutations appear to be *independent* since all the different phenotypes resulting from combinations of the different alleles are observed (Rickenberg, Cohen, Buttin & Monod, 1955; Cohen & Monod, 1957; Cohn, 1957).

† This work has been aided by a grant from the Jane Coffin Childs Memorial Fund.

‡ Senior Postdoctoral Fellow of the National Science Foundation (1957–58).

It should also be recalled that conjugation in *E. coli* involves the injection of a chromosome from a ♂ (Hfr) into a ♀ (F^-) cell, and results generally in the formation of an incomplete zygote (merozygote) (Wollman, Jacob & Hayes, 1956). Recombination between ♂ and ♀ chromosome segments does not take place until about 60 to 90 min after injection; moreover segregation of recombinants from heteromerozygotes occurs only after several hours, thus allowing ample time for experimentation.

In order to study the interaction of these factors, their expression in the cytoplasm and their dominance relationships, we have developed a technique which allows one to determine the kinetics of β-galactosidase synthesis in merozygotes of *E. coli*, formed by conjugation of ♂ (Hfr) and ♀ (F^-) cells carrying different alleles of the factors *z*, *y* and *i* (Pardee, Jacob & Monod, 1958). Before discussing the results obtained with this technique, we shall summarize some preliminary observations on the genetic structure of the "*Lac*" region in *E. coli* K 12.

2. Materials and Methods

(a) *Bacterial strains*

A ♂ (Hfr) strain (no. 4,000) of *E. coli* K 12 was used in most experiments. It was derived from strain 58,161 F^+, and was selected for early injection of the "*Lac*" marker (Jacob & Wollman, 1957). This strain is streptomycin sensitive (S^s), requires methionine for growth and carries the phage λ. A second Hfr strain (no. 3,000), isolated by Hayes (1953), was used in some experiments. This strain is S^s, requires vitamin B_1, and does not carry λ prophage. Other Hfr strains carrying mutations for galactosidase (*z*), inducibility-constitutivity (*i*), and permease (*y*) were isolated from the Hayes strain after u.v. irradiation. These markers were also put into ♀ (F^-) strains, by appropriate matings and selection of the desired recombinants.

A synthetic medium (M 63) was commonly used. It contained per liter: 13·6 g KH_2PO_4 2·0 g $(NH_4)_2SO_4$, 0·2 g $MgSO_4 . 7H_2O$, 0·5 mg $FeSO_4 . 7 H_2O$, 2·0 g glycerol, and KOH to make pH 7·0. If amino-acids were required, they were added at a concentration of 10 mg/l. of the L-form. For mating experiments, the above stock medium was adjusted to pH 6·3 and vitamin B_1 (0·5 mg/l.) was added prior to use. Aspartate (0·1 mg/ml.) was generally added at the time of mating, according to Fisher (1957).

(b) *Mating experiments*

The desired volume of fresh medium was inoculated with an overnight culture (grown in the same medium) to an initial density of approximately 2×10^7 bacteria/ml. This culture was aerated by shaking at 37°C in a water bath. Turbidity was measured from time to time; and when the density reached 1 to 2×10^8 bacteria/ml., the experiment was started. Usually small volumes of ♂ and ♀ bacteria were mixed in a large Erlenmeyer flask, with the ♀ strain in excess (e.g. 3 ml. ♂ plus 7 ml. ♀ in a 300 ml. flask). The mixed bacteria were agitated very gently so that the motion of the liquid was barely perceptible. From time to time samples were removed for enzyme assay and plating on selective media, usually lactose-B_1-streptomycin agar, for measurement of recombinants. Under these conditions, in a mating of ♂ z^+Sm^s by ♀ z^-Sm^r, up to 20 % of the ♂ population formed z^+Sm^r recombinants (as tested by selection on lactose-streptomycin agar). More often 5 to 10 % recombinants were found.

Streptomycin (Sm)† was used in many mating experiments, to block enzyme synthesis by z^+Sm^s ♂ cells. Controls showed that the synthesis of β-galactosidase was blocked in these strains immediately upon addition of 1 mg/ml. of Sm. Incorporation of ^{35}S from $^{35}SO_4^=$ as well as increase of turbidity were also suppressed by this treatment. This concentration of Sm had no effect on Sm-resistant (Sm^r) mutants. In some experiments, virulent phage (T6) was used to kill the ♂ cells, thus preventing remating.

† The following abbreviations are used in this paper:

Sm = streptomycin
IPTG = *iso*propyl-thio-β-D-galactoside
ONPG = *o*-nitrophenyl-β-D-galactoside
TMG = methyl-thio-β-D-galactoside

It should be noted that if streptomycin was added initially, it significantly reduced the number of recombinants (e.g., 75 % fewer colonies were formed on lactose-B_1-streptomycin plates after 80 min mating in the presence of 1 mg/ml. streptomycin) relative to mating in the absence of streptomycin; but the antibiotic had little effect on enzyme formation by zygotes if added at the commencement of the experiment or after the z^+ locus had been injected.

When galactosidase synthesis had to be induced in zygotes, *iso*propyl-thio-β-D-galactoside (IPTG) was used at 10^{-3}M, a concentration at which this inducer is known to be active even in the absence of permease (Rickenberg *et al.*, 1956).

(c) *Recombination studies*

The blender technique of Wollman & Jacob (1955) was used to determine the times of penetration of markers into the zygotes. It should be noted that this treatment reduces enzyme-forming capacity in zygotes by 30 to 60 %. Recombinant colonies, selected on appropriate selective media, were restreaked on the selector medium and replica plating was used to determine unselected characters. Tests for galactosidase synthesis (with or without induction) were performed on maltose-synthetic agar plates with or without IPTG, using filter paper impregnated with ONPG, according to Cohen-Bazire & Jolit (1953).

Transductions were performed with phage 363, according to Jacob (1955).

(d) *β-galactosidase assay*

For this enzyme assay, 1 ml. aliquots of culture were pipeted into tubes containing 1 drop of toluene. The tubes were shaken vigorously and were incubated for 30 min at 37°C. They were then brought to 28°C; 0·2 ml. of a solution of M/75 *o*-nitrophenyl-β-D-galactoside in M/4 sodium phosphate (pH 7·0) was added, and the tubes were incubated a measured time, until the desired intensity of color had developed. The reaction was halted by addition of 0·5 ml. of 1 M-Na_2CO_3, and the optical density was measured at 420 mμ with the Beckman spectrophotometer. A correction for turbidity could be made by multiplying the optical density at 550 mμ by 1·65 and subtracting this value from the density at 420 mμ. One unit of enzyme is defined as producing 1 mμ-mole *o*-nitrophenol/minute at 28°C, pH 7·0. The units of enzyme in the sample can be calculated from the fact that 1 mμ-mole/ml. *o*-nitrophenol has an optical density of 0·0075 under the above conditions (using 10 mm light-path).

(e) *Chemicals*

o-nitrophenyl-β-D-galactoside (ONPG), methyl-thio-β-D-galactoside (TMG) and *iso*propyl-thio-β-D-galactoside (IPTG) were synthesized at the Institut Pasteur by Dr. D. Türk. Other chemicals were commercial products.

3. Genetic Structure of the " Lac " Region

Figure 1 presents the structure of the "*Lac*" region, as it can be sketched from the data available at present. This complex locus, as established long ago by Lederberg (1947) and confirmed by the blender experiments of Wollman & Jacob (1955), lies at about equal distances from the classical markers *TL* and *Gal*. The closest known markers are *Proline* (left) and *Adenine* (or *T6*) (right). As shown in the map, the several (about 10) occurrences of the y^- mutation all lie together probably at the left of the segment, while the different z^- mutations and the i^- mutant are packed together at the other end. No attempt has been made to establish the order of individual y^- mutations. The order of the z^- mutations relative to each other and to the i^- marker is unambiguously established, as shown, except for the z_U^- mutation, whose position is largely undetermined. Several independent occurrences of the i^- mutation have been isolated. They all appear to be closely linked to the i_3^- marker, but they have not been mapped, for lack of adequate methods of selection i^+ recombinants. The evidence for this structure is briefly as follows:

(1) The frequency of recombination between *z* and *y* mutations is very low:

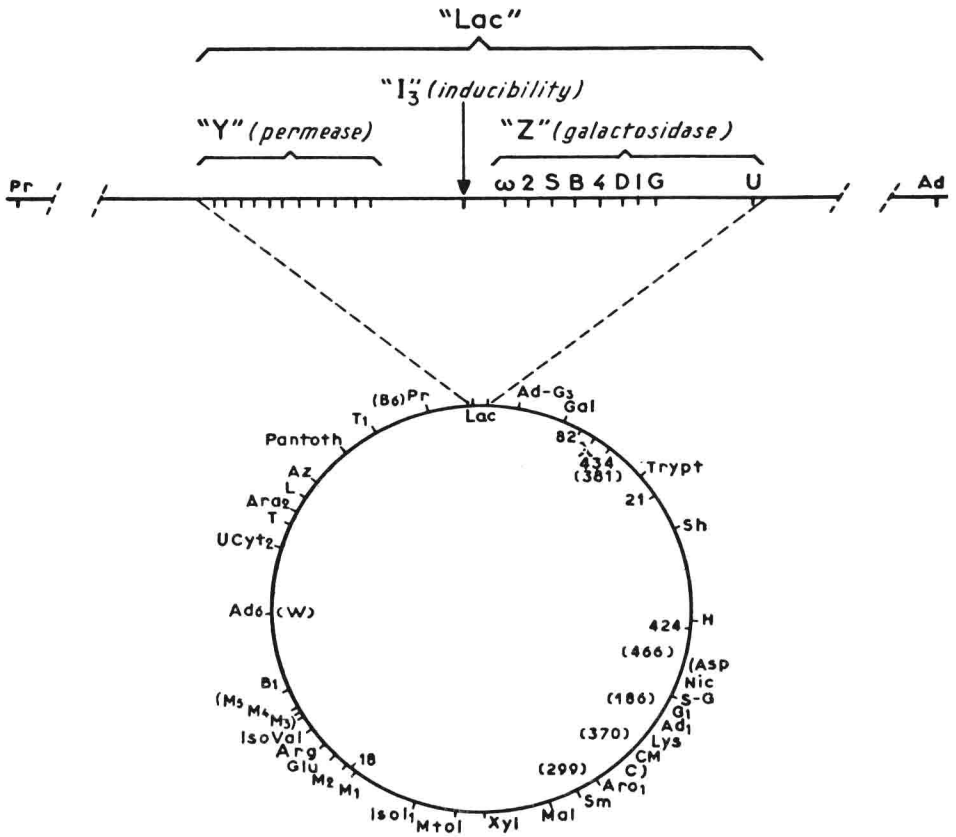


FIG. 1. Fine structure of the "*Lac*" segment.

The "*Lac*" segment is shown enlarged and positioned with respect to the rest of the *E. coli* K 12 linkage group for which the circular model (Jacob & Wollman, 1958) has been adopted.

roughly 1/100th of the frequency of recombination between *TL* and *Gal*. The frequency of recombination between individual *z* markers is about one order of magnitude lower.

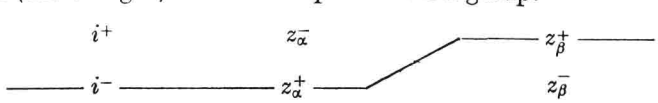
(2) When y^+z^+ recombinants are selected (by growth on lactose-agar) in crosses of the type:

$$y^+i^-z^- \times y^-i^+z^+$$

the i^+ marker remains associated with z^+ 85 % of the time.

(3) The frequency of cotransduction of *i* with *z* (selecting for z^+ alone) is very high (> 90 %), while the frequency for *i* and *y* is also high, although definitely lower (about 70 %). (These data are somewhat ambiguous, because of the heterogeneity of the clones resulting from a transduction.)

(4) The selection of z^+ recombinants in crosses involving different z^- mutants, and *i* as unselected marker, invariably results in about 90 % of the progeny being either i^- or i^+, depending on the particular z^- mutants used. Assuming this result to be due to the position (left or right) of *i* with respect to the *z* group:



a linear order can be established, without contradictions, for the eight markers shown. This however leaves an ambiguity as to whether *i* lies *between* the *y* and the *z* groups, or outside.

Let us emphasize that this sketch of the *Lac* region is preliminary and very incomplete, and that the results concerning the relationships of certain markers are not understood. For instance, the z_U marker recombines rather freely with all the other mutants shown (both *y* and *z*) yet, by cotransduction tests, it is closely linked to *i* (25 % cotransduction). It should also be mentioned that certain of the z^- mutants (z_ω^- ; z_2^-; z_G^-) have apparently lost the capacity to synthesize *both* the galactosidase *and* the permease. Yet these mutations do not seem to be deletions. We shall not attempt, here, to interpret this finding, since we shall center our attention on the interaction between the *i* marker and the *z* region.†

A question which should now be considered is whether we may regard the *z* region as possessing the specific structural information concerning the galactosidase molecule. The fact that so far all the independent mutations resulting in loss of the capacity to synthesize galactosidase were located in this region might not constitute sufficient evidence‡. However, it has been found by Perrin, Bussard & Monod (1959, in preparation) that several of the z^- mutants synthesize, instead of active galactosidase, an antigenically identical, or closely allied, protein. Moreover several of these mutant proteins are different from one another by antigenic and other tests. These findings appear to prove that the *z* region indeed corresponds to the "structural" genetic unit for β-galactosidase.

4. β-Galactosidase Synthesis by Heteromerozygotes

(a) *Preliminary experiments*

The feasibility and significance of experiments on the expression and interaction of the *z*, *y* and *i* factors depended primarily on whether *E. coli* merozygotes are physiologically able to synthesize significant amounts of enzyme very soon after mating. It was equally important to determine whether the mating involved any cytoplasmic mixing. These questions were investigated in a series of preliminary experiments.

Since the physical separation of *E. coli* zygotes from unmated or exconjugant parent cells cannot be achieved at present, test conditions must be set up, such that the zygotes only, but not the parents, can synthesize the enzyme. This is obtained when the following mating:

$$\text{♂ } z^+y^+i^+Sm^s \times \text{♀ } z_s\,y^+i^+Sm^r \qquad \text{(A)}$$

is performed in the presence of inducer (IPTG) and of 1 mg/ml. of streptomycin. The ♀ lack the z^+ factor; the ♂ are inhibited by streptomycin (cf. Methods); the zygotes are not, because they inherit their cytoplasm from the ♀ cells (see below

† Interaction of *i* with the *y* region is of course equally interesting, but since determinations of activity are much less sensitive with the galactoside-permease than with the galactosidase, we have used the latter almost exclusively.

‡ In addition to the mutants shown on Fig. 1, 20 other galactosidase-negative mutants, as yet unmapped, have been found to belong to the same segment by contransduction tests. None was found outside. Lederberg *et al.* (1951), however, have isolated some lactose-"non-fermenting" mutants (as tested on EMB-lactose agar) which are located at other points on the *E. coli* chromosome. In our hands, one of these mutants (Lac_3^-) formed normal amounts of both galactosidase and galactoside-permease (although it did form white colonies on EMB-lactose). Another one (Lac_7^-) formed reduced, but significant, amounts of both. A third (Lac_8^-) which is a galactosidase-negative, appears to belong to the "*Lac*" segment, by cotransduction tests.

pages 170 and 171), and because the type of ♂ used transfers the Sm^s gene to only a very small percentage of the cells. Under these conditions, enzyme is formed in the mated population with a time course and in amounts showing that the synthesis can be due only to zygotes having received the z^+ factor. Figure 2 shows the

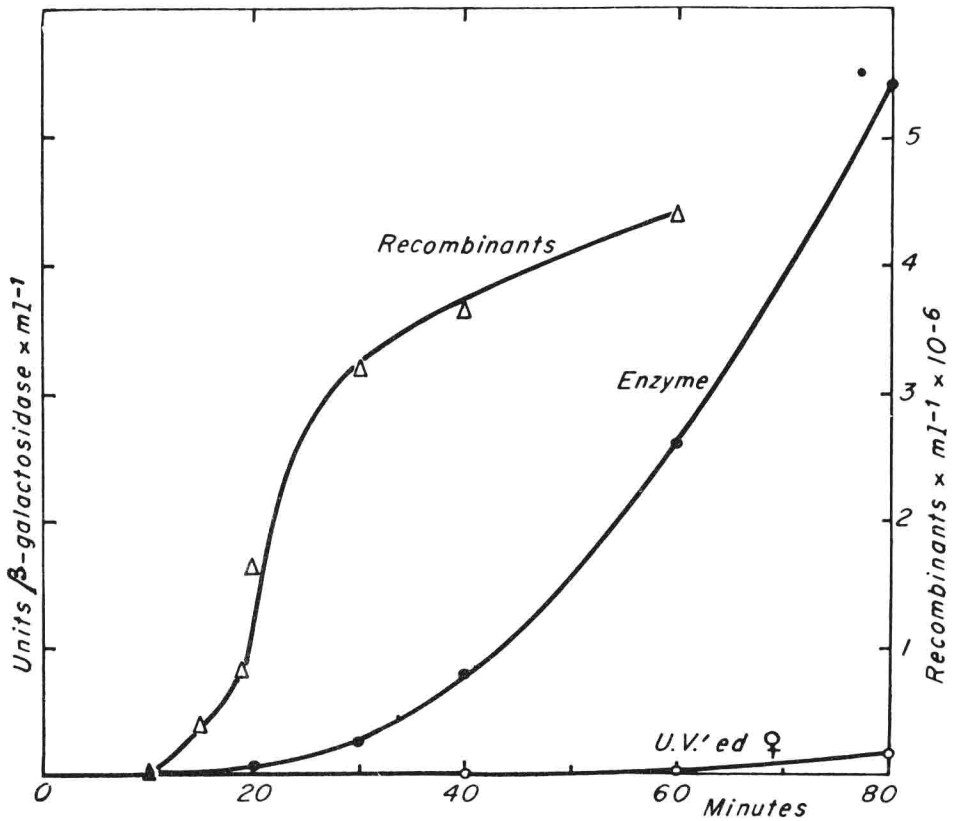


FIG. 2. Enzyme formation and appearance of recombinants in mating A.
Mating in presence of streptomycin (1 mg/ml.) and IPTG (10^{-3}M). A control with u.v.-treated ♀ cells (0·01 % survival) is shown. Recombinants (z^+Sm^r) selected by plating on Sm-lactose agar after blending (separate experiment with the same ♂ culture).

kinetics of galactosidase accumulation, compared with the appearance of z^+Sm^r recombinants, determined on aliquots of the same population (cf. Methods). The latter curve corresponds, as shown by Wollman & Jacob (1955), to the distribution of times of penetration of z^+ genes in the zygote population. It will be remarked that enzyme synthesis commences just within a few minutes after the first z^+ genes enter into zygotes. Assuming that the number of zygotes having received a z^+ gene is 4 to 5 times the number of recovered z^+Sm^r recombinants, and taking into account the fact that normal cells are on the average trinucleate (i.e., have three z^+ genes), the rate of enzyme synthesis per injected z^+ appears nearly normal.

This rapid expression of the z^+ factor poses the problem whether cytoplasmic constituents are injected from the ♂ into the zygote. This already appeared unlikely from the previous observations of Jacob & Wollman (1956). We reasoned that if there occurred any significant cytoplasmic mixing, such a mixing should allow the

♂ cells to feed the ♀ cells with any small metabolites which the ♂ had and the ♀ lacked. This condition is obtained in the following mating:

$$♂\ z^+Sm^s\ maltose^+ \times ♀\ z^-Sm^r\ maltose^-$$

if it is performed in presence of maltose as sole carbon source, using a ♂ which virtually does not inject the *maltose*$^+$ gene. It results in a very strong inhibition of enzyme synthesis (and recombinant formation) showing that the ♂ cannot effectively

TABLE 1

Enzyme formation in nutritionally deficient zygotes

Deficiency	Rate of enzyme formation †			Mean % inhibition of recombinant formation
	Control	Deficient	Mean % inhibition	
Carbon source ‡	1·6	0·4	73	75
	0·66	0·20		
Arginine §	0·28	0·02	96	65
	0·36	0·01		

† Units of enzyme × hr^{-1}.

‡ ♂ $z^+Sm^s\ maltose^+$ × ♀ $z^-Sm^r\ maltose^-$ mated in presence of inducer and Sm, with glycerol plus maltose (control) or maltose as sole carbon source.

§ ♂ $z^+Sm^s\ Arg^+$ × ♀ $z^-Sm^r\ Arg^-$ mated in presence of inducer and Sm with and without arginine (10 μg/ml.).

feed the ♀. An even stronger effect is observed when the ♀ requires arginine, the ♂ not, and mating takes place in absence of arginine (again on condition that the Ar^+ gene is not injected by the ♂) (Table 1). These observations indicate that even small molecules do not readily pass from the ♂ into the ♀ cell during conjugation.†

It therefore appears that cytoplasmic fusion or mixing does not occur to an extent which might allow cross-feeding. That the contribution of the ♂ is exclusively genetic, and does not involve cytoplasmic constituents of a nature, or in amounts, significant for our purposes, is however only proved by the results of the opposite matings, which we shall consider in the next section.

(b) *Expression and interaction of the alleles of the z and i factors*

We should first consider which of the alleles of the *z* factors are dominant, and whether they all belong to a single cistron. Experiments of the type described above (mating A) were performed with each of the eight z^- mutants, used as ♀ cells, receiving a z^+ from the ♂. Enzyme was synthesized to similar extents in all cases, showing that the z^- mutants in question were all recessive. Each of the mutants was also mated (as ♂) to a z^- ♀. No enzyme was synthesized by any of these double recessive heterozygotes where the mutations were in the *trans* position

† However such leakage may occur when the concentration of a compound is exceptionally high in the ♂. This happens when a ♂ with the constitution $z^-i^-y^+$ is used in the presence of lactose. The constitutive permease then may concentrate lactose up to 20 % of the cells' dry-weight (Cohen & Monod, 1957). Adequate tests have shown that this lactose does flow from the ♂ into a permease-less ♀ during conjugation.

$$\frac{z_\alpha^+ z_\beta^-}{z_\alpha^- z_\beta^+}$$

showing that all the (tested) z^- mutants belong to the same cistron as defined by Benzer (1957).

The next and most critical problem is whether the z and i factors also belong to the same unit of function (gene or cistron) or not. Let us recall that cells with the constitution z^+i^+ synthesize enzyme in presence of inducer only, while z^+i^- cells synthesize enzyme without induction, and z^-i^+ or z^-i^- cells do not synthesize enzyme under any condition. The extremely close linkage of z and i mutations suggests that they may belong to the same unit. If this were so, they would not be able to interact through the cytoplasm, but could act together only when in *cis* position within the same genetic unit. The heterozygote, z^+i^+/z^-i^- would then be expected not to synthesize galactosidase constitutively.

In order to test this expectation, the following mating:

$$\sigma\, z^+i^+ \times \varphi\, z_2^-i_3^- \qquad \text{(B)}$$

was performed *in absence of inducer*. The σ cannot synthesize enzyme, because they are i^+. The φ cannot because they are z^-. The zygotes however do synthesize enzyme (Fig. 3): during the first hour following mating the synthesis is, if anything, even more rapid and vigorous than when both parents are i^+ and inducer is used, as in mating (A).

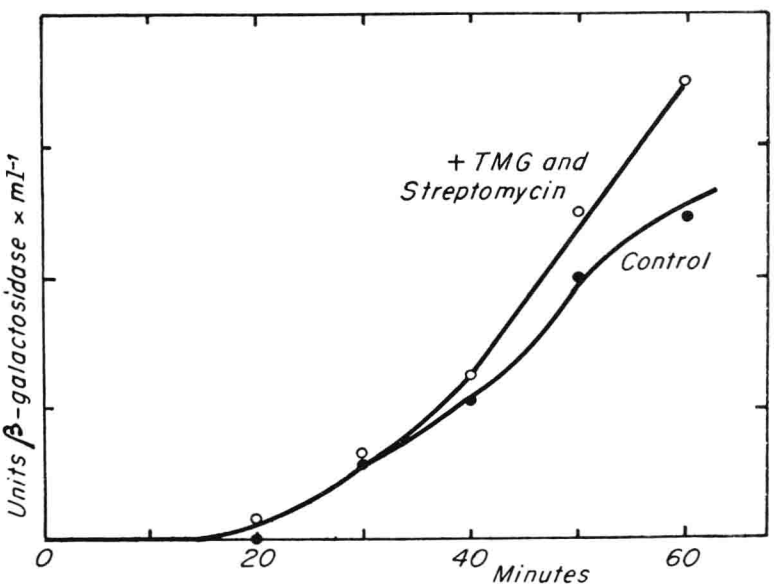


FIG. 3. Enzyme formation during first hour in mating B.
Mating under usual conditions. To an aliquot streptomycin (0·8 mg/ml.) was added at 20 minutes, and TMG at 25 minutes, to allow comparison of synthesis with and without inducer.

Such a mating therefore allows immediate and complete interaction of the z^+ from the σ with the i^- from the φ. The possibility that the interaction depends upon actual recombination yielding z^+i^- in *cis* configuration is excluded because: (a) the synthesis begins virtually immediately after injection whereas genetic recombination is known (Jacob & Wollman, 1958) not to occur until 60 to 90 min after injection; (b) the factors z and i are so closely linked that recombination is an exceedingly rare