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Mutagenic Topography of the *E. coli* Chromosome

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Summary. The lactose fermenting genes in *E.coli* have been transposed to various chromosomal locations. The bacterial strains were mutagenized with different chemical mutagens and the frequency of Lac negative mutant colonies was measured as a function of lactose gene location in the chromosome. There appears to be a highly mutable location between 58-60 minutes on the *E.coli* map. This region does not appear to be correlated with the origin of DNA replication or with the terminus. The possible significance of this mutable region in the evolution of new bacterial genes is discussed.

Key words: *E.coli* - Mutagenesis - Evolution - Gene Transposition

INTRODUCTION

The present gene order in the chromosome of *E.coli* may have evolved as a consequence of natural selection or it may be the result of random processes. About five hundred genes in *E.coli,* a significant fraction of the total genetic complement, have been identified as to cellular function and map location (Taylor & Trotter, 1972). The present gene order has been determined principally by bacterial matings and generalized phage transduction using various *E.coli* strains of diverse origin. The observation that a unique genetic map can be generated from a variety of different strains of the same species suggests that the gene order of the species is not random but is the result of selection. Moreover, the gene order in *S.typhimurium,* a species of bacteria closely related to *E.coli,* is also very similar which suggests that the gene order in the enteric bacteria is a consequence of natural selection (Sanderson, 1972).

Assuming that the gene order in *E.coli* is not random, a number of basic questions can be posed. For example, do

specific *E.coli* genes function with the same efficiency irrespective of chromosomal location? Can the gene order of a specific strain be changed by enforced selection during growth in the laboratory? Is the mutation rate the same for a specific gene or gene cluster irrespective of chromosomal location or does the mutation rate vary with chromosomal location? The experiments described in this report are directed toward answering the question of mutation rate versus chromosomal location for the lactose fermenting genes in *E.coli*. The results indicate that, at least for the lactose operon, the frequency of production of mutants which are unable to ferment lactose *does* depend on the chromosomal location of the lactose genes. The other questions will be dealt with in a later report.

MATERIALS AND METHODS

Bacterial Strains. The lactose transposition Hfr strains were isolated and characterized by P.Broda. Fig.1 shows the map positions of the various Hfr strains used in this study in relation to several bacterial markers. Strain JC 411 F^- was obtained from J.Clark.

Media. LB broth (grams/liter): Bactotryptone, 10.0; Difco yeast extract, 5.0; NaCl, 10.0; glucose, 10.0 pH 7.4. Minimal M9 (grams/liter): Na_2HPO_4, 7.0; KH_2PO_4, 3.0; NH_4Cl, 1.0. After autoclaving, the following sterile solutions were added (ml/liter): 25% NaCl, 2.0; 1 *M* $MgSO_4.7\ H_2O$, 1.0; 0.01 *M* $FeCl_3$, 0.3; 0.5 *M* $CaCl_2$, 0.2; 2% thiamine, 1.0; 10% proline, 0.4. Carbon sources were added as required (Final concentrations): Glucose, 0.2%; lactose, 0.2%; casamino acids, 0.1%.

Plates. Valine plates contained M9 plus 15 grams/liter Difco agar, 0.2% glucose and 100 µg/ml valine.

Eosin methylene blue (EMB) plates were used to score Lac negative and positive colonies. These plates contained 37.5 grams/liter Difco Levine lactose agar preparation.

Mating plates (grams/liter): $(NH_4)_2SO_4$, 10.0; Na_2HPO_4, 30.0; KH_2PO_4, 15.0; NaCl, 15.0; Na_2SO_4, 0.055; Bacto-agar, 22.0. After autoclaving, the following additions were made (ml/liter): 1 *M* $MgCl_2.6\ H_2O$, 2.0; 0.5 *M* $CaCl_2$, 0.2; 0.01 *M* $FeCl_3$, 0.3; LB broth, 0.5; 0.2% thiamine, 1.0; 40% glucose, 10.0. Amino acids were added where indicated at a final concentration of 30 µg/ml.

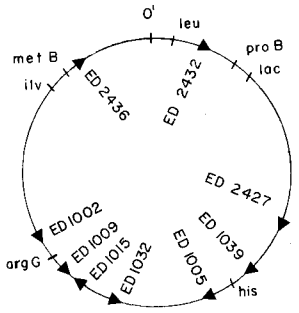

Fig.1. Map position of various Hfr *lac* transposition strains and selected *E.coli* nutritional markers

Mutagenesis. N-methyl-N'-nitro-N-nitrosoguanidine (NTG). Optimal conditions were determined according to Adelberg et al., (1965). 50% survival was obtained using 50 µg/ml NTG in 0.1 *M* citrate buffer, pH 5.5. Bacteria were incubated for 90 min at 30°C in the dark and without aeration after having been centrifuged, washed and resuspended in citrate buffer pH 5.5. After mutagenesis the bacteria were centrifuged, washed with buffer and grown overnight in M9 plus glucose and casamino acids at 30°C.

Ethylmethanesulfonate (EMS). Bacteria were treated according to the method described by Clark (1967). Bacteria were grown overnight in LB broth at 30°C without aeration. The bacteria were centrifuged and resuspended in fresh LB broth plus EMS at a final concentration of 2% and incubated for two hours at 30°C without aeration. The bacteria were centrifuged, washed twice with 5% sodium thiosulfate and grown overnight in LB broth at 30°C.

ICR-191. Optimal conditions were determined according to the procedures outlined by Miller (1972). Good mutagenesis with about 20% survival was obtained by growing cells in M9 medium plus glucose and 10 µg/ml ICR-191 for sixteen hours at 30°C in the dark. The overnight cultures were diluted and plated to give several hundred colonies per plate.

Ultraviolet (UV). The bacteria exposed to UV were obtained from cultures previously treated with NTG. This procedure raised the background level to a point where Lac^- colonies could be detected (i.e., a frequency of at least 1 per 1000). Bacteria were grown overnight in M9 medium plus glucose and casamino acids. They were diluted into 0.1 *M* $MgSO_4$ to a final concentration of 5 x 10^8 cells/ml. They were exposed to UV (254 nm, 660 erg/sq. cm) in plastic dishes with constant swirling. This exposure gave a survival of 5% and these bacteria were grown overnight in M9 medium plus glucose and casamino acids. All operations were performed in the dark to minimize UV repair due to photoreactivation.

For all the above procedures, cultures were started from single Lac^+ colonies isolated on EMB plates. After mutagenesis, the number of Lac^- colonies was determined by plating the bacteria onto EMB plates and incubating at 30° for two days. Control platings of the same culture prior to mutagenesis were also made. The background frequency of Lac^- colonies varies with each Hfr strain and a minimum of 1000 total colonies were examined. The increase in valine-resistant (Val^R) mutants before and after mutagenesis was determined by plating cells onto M9 minimal agar plates plus glucose and 100 µg/ml valine. Plates were incubated for 72 hours at 30°. Since all the Hfr strains are isogenic except for the transposition of the *lac* genes, the number of valine-resistant cells was used as a standard measure of the mutagenesis which differs from strain to strain and experiment to experiment.

Mating. Bacterial matings were performed to determine whether the Lac^- colonies arising after mutagenesis resulted from loss of the F'*lac* episome (converting the Hfr strain to an F^- strain) or from a mutation in the *lac* genes. The mating conditions were essentially those described by Clark & Margulies (1965) and Adelberg & Burns (1960). JC 411 was used as the F^- recipient strain and requires arginine, leucine, methionine and histidine for growth. Both Lac^+ and Lac^- bacteria were spread in small areas on a lawn of JC 411 on medium that will not allow growth of either parent. Recombinant bacteria can grow if the Hfr transfers the two proximal markers. Plates were incubated for several days and scored for recombinant formation.

RESULTS

The *lac* transposition strains used in this study and their growth characteristics are shown in Table 1. These strains were isolated by forced integration of an F'*lac* episome into the bacterial chromosome at various locations. This particular episome carries a mutation which prevents autonomous replication of the episome at 40°C. Thus, if these bacteria are plated on lactose plates and incubated at 40°C the only cells able to grow and produce colonies are those in which the episome has become integrated into the host chromosome. In addition, the chromosome is deleted for the *lac* genes so that there is no preferred site of integration. The Hfr strains that result from such a selection have been mapped

Table 1. Characteristics of Hfr strains

Strain	Map Position (Minutes)	Generation Time (Min)[a] 0.4% lactose, 37°C	Stability of Lac$^+$ [b]	Spontaneous Lac$^-$ [c] Frequency x 10^{-3}
ED 2432	5	80	unstable	35
ED 2427	30	95	very unstable	336
ED 1039	35	90	stable	4
ED 1005	40	95	stable	4
ED 1032	50	95	unstable	36
ED 1015	58	95	very unstable	107
ED 1003	58	-	stable	2
ED 1044	58	80	stable	2
ED 1049	58	95	stable	5
ED 1052	58	150	stable	5
ED 1009	60	90	stable	9
ED 1010	60	-	unstable	29
ED 1012	60	95	stable	6
ED 1023	60	190	unstable	34
ED 1002	65	105	unstable	12
ED 1007	65	95	stable	4
ED 1008	65	190	stable	2
ED 1029	65	95	stable	4
ED 2436	80	70	very unstable	412

[a] Generation times for all strains in LB broth at 37°C are 35 minutes.

[b] Strains were maintained on EMB agar by periodic transfer of a single Lac$^+$ colony.
Stable: Lac$^-$ colonies appearing at a frequency of 1-10/thousand
Unstable: Lac$^-$ colonies appearing at a frequency of 10-100/thousand
Very unstable: Lac$^-$ colonies appearing at a frequency greater than 100/thousand

[c] Values represent an average of several experiments with a total of 10^4 colonies counted.

and characterized by Masters & Broda (1971) and Broda et al. (1972).

The details of this selection for *lac* transposition strains have been discussed previously by Beckwith et al. (1966). Their results showed that the expression and regulation of the *lac* genes in *E.coli* are normal irrespective of chromosomal location or DNA strand orientation. Table 1 shows that all of the Hfr strains used in this study also are able to grow on lactose although there are some differences in generation time. More interesting is the marked difference in the frequency of appearance of Lac^- bacteria. The spontaneous frequency of Lac^- colonies varies by more than two orders of magnitude. The question is whether these Lac^- colonies result from mutations or from loss of the F' episome which carries the *lac* genes. To answer this, a number of spontaneous and mutagenized Lac^+ and Lac^- colonies of each strain were picked and mated to an appropriate female strain to determine the Hfr character of each strain. Table 2 shows the results of these experiments. All of the spontaneously appearing Lac^- colonies are still the original Hfr strains showing that the F'*lac* episome is still integrated. Even after mutagenesis the majority of the Lac^- colonies are still Hfr showing that the integrity of the chromosome is maintained and that the Lac^- colonies derive from a chromosomal alteration in the *lac* segment of the DNA which does not affect the F factor. It is interesting to note that of the two very unstable strains, one (ED 2427) is located near the terminus of DNA replication and the other (ED 2436) is located close to the origin of vegetative DNA replication. These preliminary results suggest that certain regions of the *E.coli* chromosome may be particularly mutable and that genes located in these regions or other regions may be genetically unstable. To further test these ideas, the susceptibility of the *lac* genes in various chromosomal locations to different mutagens was tested. Each Hfr strain was mutagenized as described in Methods. The frequency of Lac^- bacteria in the population was determined before and after mutagenesis. As a control the overall mutagenesis in each experiment was measured by determining the increase in valine resistant colonies. Fig.2 shows the results of one series of experiments using NTG. These data show that there are two regions of the *E.coli* chromosome that are particularly sensitive to NTG mutagenesis - one region located at about sixty minutes on the *E.coli* map and another less sensitive region located at about thirty-five minutes on the map. This experiment was repeated three times yielding the same results each time. Both the absolute increase in Lac^- colonies (closed circles) and the

Table 2. Percentage of Lac$^-$ Colonies which are Hfr

Strain	Spontaneous	Mutagenized		
		NTG	EMS	ICR-191
ED 2432	100	-	100	100
ED 2427	100	-	100	100
ED 1039	100	100	100	100
ED 1005	100	100	100	100
ED 1032	100	100	75	100
ED 1015	100	60-90	100	75
ED 1003	100	50-90	-	-
ED 1044	100	33	-	-
ED 1049	100	55	-	-
ED 1052	100	50	-	-
ED 1009	100	90-100	100	100
ED 1010	100	100	-	-
ED 1012	100	50-90	-	-
ED 1023	100	100	-	-
ED 1002	100	100	100	100
ED 1007	100	75	-	-
ED 1008	100	65	-	-
ED 1029	100	65	-	-
ED 2436	100	-	100	100

Matings were carried out as described in Methods. Plates were incubated for a maximum of five days at 37°C. In most cases, sixteen colonies of Lac$^-$ and Lac$^+$ phenotype were tested. For all strains Lac$^+$ colonies from both spontaneous and mutagenized cultures were all Hfr.

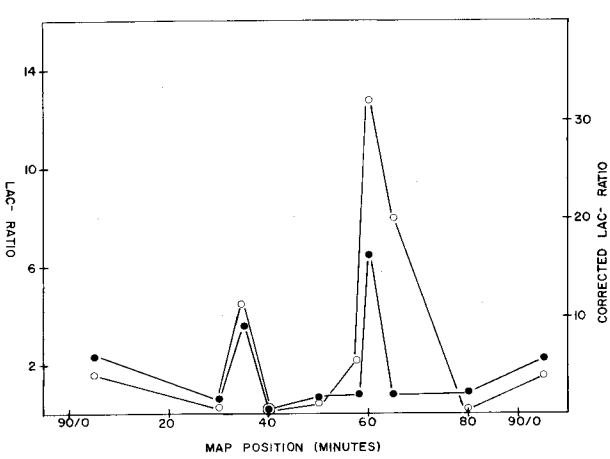

Fig.2. NTG mutagenesis of various *E.coli* Hfr *lac* transposition strains. Each strain was mutagenized as described in Methods. ●—●, frequency of Lac$^-$ colonies (mutagenized/spontaneous). o—o, corrected frequency of Lac$^-$ colonies (Lac$^-$ mutagenized/Lac$^-$ spontaneous divided by Val^R mutagenized/Val^R spontaneous)

corrected increase (open circles), which takes into account strain and experimental differences in the degree of mutagenesis, give the same result.

To test the generality of this result three other mutagens were tested. Figs.3, 4 and 5 show the results for EMS, ICR-191 and ultraviolet irradiation in that order. EMS produces a pronounced increase in Lac^- colonies at 58-60 minutes on the map (Fig.3) as does ICR-191 (Fig.4). Even though the mode of action of these mutagens is known to be very different, they all appear to react most effectively with a particular region of the DNA. Fig.5 shows that UV is not very mutagenic and does not show any specific increase as do the other mutagens.

Since only a single bacterial strain was tested at the different map locations it seemed possible that the Lac^- colony increase observed at 58-60 minutes on the map is a property of the particular Hfr strain tested. Since a number of Hfr strains in this region of the map have been isolated independently, NTG mutagenesis was performed on each of these strains. Fig.6 shows that the increase in frequency of Lac^- colonies does depend on the chromosomal location of the *lac* genes and is not a function of the Hfr strain employed. For comparison, the original NTG curve from Fig.2 is replotted in Fig.6. Independent Hfr isolates are not available for every map position shown but the observation that all Hfr strains located at 58-60 minutes on the genetic map exhibit increased frequency of Lac^- colonies suggests that this region is indeed a chromosomal "hot spot". (Benzer, 1961).

As an additional control the frequency of $Lac^+ \rightarrow Lac^-$ mutant colony formation was measured in an independent strain of *E.coli* carrying the *lac* genes in the normal position (AB 257) and also in the parent strain from which all of the Hfr strains in this study were derived (ED 903). This parent strain is deleted for the *lac* genes on the chromosome and carries the *lac* genes on the F sex factor which means that each bacterium has, on the average, 2-3 *lac* genes since there are 2-3 F sex factors per bacterium. NTG mutagenesis of these strains gave frequencies of Lac^- mutants in the range of the base line shown in Fig.2. Therefore, when the *lac* genes are in the wild-type chromosomal location or on an episome, they are not unusually mutable underscoring the high mutability of the "hot spot" located at 58-60 minutes.

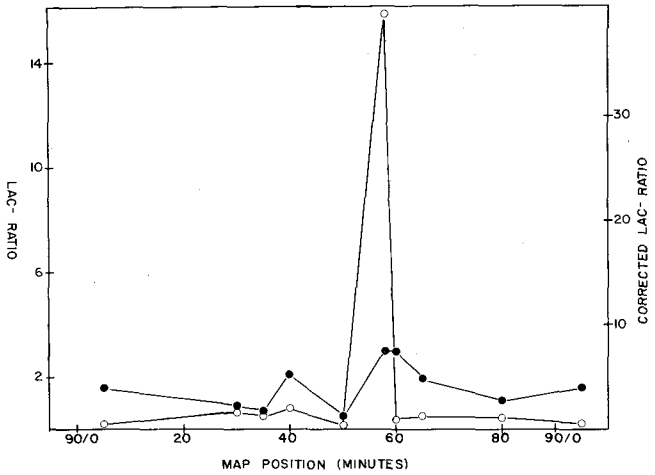

Fig.3. EMS mutagenesis of various *E.coli* Hfr *lac* transposition strains. Each strain was mutagenized as described in Methods. The plot is the same as Fig.2

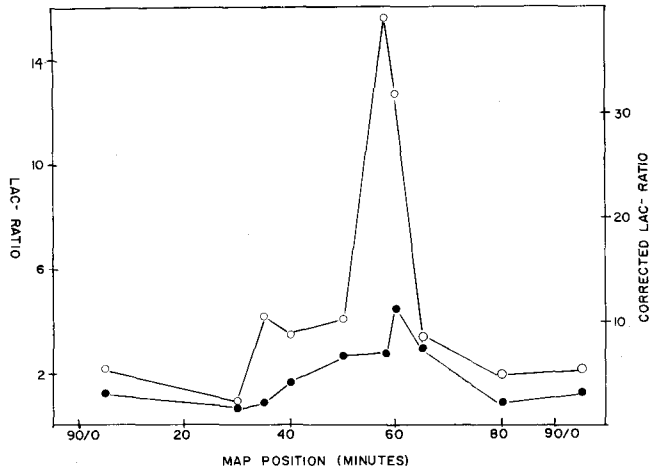

Fig.4. ICR-191 mutagenesis of various *E.coli* Hfr *lac* transposition strains. Each strain was mutagenized as described in Methods. The plot is the same as Fig.2

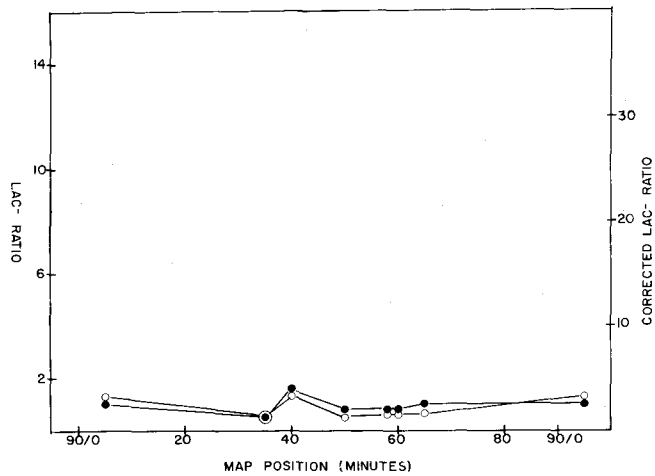

Fig.5. Ultraviolet mutagenesis of various *E.coli* Hfr *lac* transposition strains. Each strain was mutagenized as described in Methods. The plot is the same as Fig.2

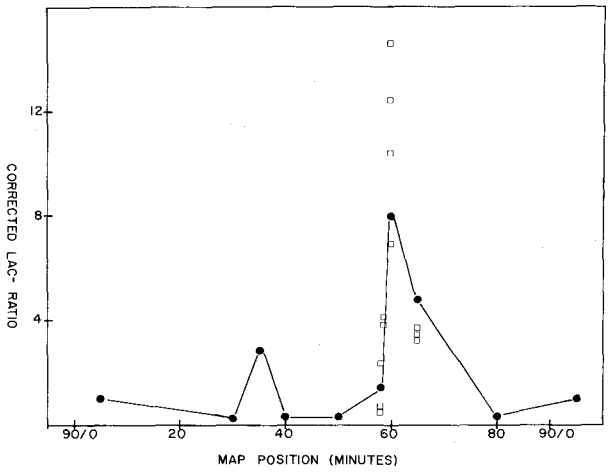

Fig.6. NTG mutagenesis of independently isolated Hfr *lac* transposition strains located in "hot spot" region. The curve represented by closed circles (●—●) is replotted from Fig.2. The open squares (□) represent the frequency of Lac^- colonies (Lac^- mutagenized/Lac^- spontaneous divided by Val^R mutagenized/Val^R spontaneous) for independent Hfr isolates in that map position

DISCUSSION

The ultimate origin of genetic change and diversity is mutation operated on by selection. Because of the ease with which bacteria can be grown under a variety of selective conditions, they provide excellent material for studying the mechanisms underlying evolutionary change and adaptation at the biochemical level. Horowitz (1965) has proposed a scheme of "retrograde evolution" whereby new enzymes arise which allow the organism to utilize preformed organic molecules from the immediate environment. The evolution of different metabolic pathways in bacteria and their regulation has been summarized in a review by Hegeman and Rosenberg (1972).

In this study we have attempted to answer the question "Are genes located in a particular region of the *E.coli* chromosome more susceptible to mutation than if the same genes are located in other regions of the chromosome?"All the data presented indicate that the answer is affirmative, although we are aware that alternative explanations are possible. When the *lac* genes are inserted into the *E.coli* chromosome at 58-60 minutes on the genetic map the frequency with which Lac^- colonies are produced is at least ten fold higher than the frequency at any other chromosomal location (see Figs.2, 3 and 4). This increase in Lac^- colonies is observed for each of the three chemical mutagens tested even though the molecular mode of action of these mutagens is very different (Miller, 1972). Thus, it seems reasonable to conclude that there is some unique structural property of the *E.coli* chromosome in this region that makes it particularly susceptible to

genetic change. It should be noted, however, that in all of the experiments reported, we are measuring the appearance of a new phenotype. Thus, there is the possibility that we are not measuring an increased *mutation rate* in this region but only an increased *frequency* of appearance of mutants. This increased mutant frequency could result from any number of factors which might affect the survival of mutant phenotypes. Even though this possibility cannot be definitively excluded, it seems reasonable to conclude that we are actually observing a differential mutagenesis of the lactose genes as a function of chromosomal location. This conclusion is also supported by the data in Fig.6 which shows that all the independently isolated Hfr strains that map at 58 minutes show an increased frequency of Lac$^-$ mutants. The observation that virtually all of the Lac$^-$ mutants which arise are still Hfr (Table 2) also suggests that we are measuring mutagenesis and not preferential loss of the F' factor in certain Hfr strains or regions of the chromosome. One other bit of evidence that suggests that the *E.coli* chromosome may be genetically unique in this region is the recent isolation of a new β-galactosidose gene *(ebg)* (Campbell et al., 1973; Hall & Hartl, 1974; Warren, 1972). This new lactose fermenting activity has been mapped at 59 minutes. This new activity is the result of many mutations accumulated during prolonged selection. Thus, it is particularly interesting that this newly evolved activity which requires numerous mutations should be located in the mutable region that we have delineated. It may be that in this mutable region of the *E.coli* chromosome new genes may evolve more easily.

It should be noted that this site is some distance from the origin of vegetative replication. This origin has been located by different techniques at 60-65 minutes (Masters & Broda, 1971), 74 minutes (Bird et al., 1972), and 75 minutes (Hohlfeld & Vielmetter, 1973). There is more general agreement by these same workers that the replication terminus is located in the region of 25-30 minutes. Of all the Hfr strains listed in Table 1, three are categorized as being very unstable. One of these, strain ED 1015 is located in the region defined as being exceptionally mutable by the experiments in this report (58-60 minutes) and of the other two, ED 2427 is located near the terminus and ED 2436 is located near the origin. While no conclusion can be drawn from these three Hfr strains, the existence of such unstable strains may suggest that certain regions of the *E.coli* chromosome are genetically less stable than other regions.

In regard to the non-random organization of the chromosome, there may be a selective advantage to having the genes

which govern indispensible activities such as ribosome proteins, amino acid biosynthetic pathways, etc., located in the genetically most stable regions of the chromosome. Conversely, mutable regions may be the location for newly evolved or more rapidly evolving genes.

REFERENCES

1. Adelberg,E.A., Burns,S.N. (1960). J.Bacteriol.79, 321
2. Adelberg,E.A., Mandel,M., Chen,G.C.C. (1965). Biochem.Biophys.Res. Comm.18, 788
3. Beckwith,J.R., Signer,E.R., Epstein,W. (1966). Cold Spr.Har.Symp. Quant.Biol.31, 393
4. Benzer,S. (1961). Proc.Nat.Acad.Sci. USA 47, 403
5. Bird,R.E., Louarn,J., Martuscelli,J., Caro,L. (1972). J.Mol.Biol.70, 579
6. Broda,P., Meacock,P., Achtman,M. (1972). Molec.Gen.Genet.116, 336
7. Campbell,J.H., Lengyel,J.A., Langridge,J. (1973). Proc.Nat.Acad. USA 70, 1841
8. Clark,A.J. (1967). J.Cell.Physiol.70, 165
9. Clark,A.J., Margulies,A.D. (1965). Proc.Nat.Acad.Sci. USA 53, 451
10. Hall,B., Hartl,D.L. (1974). Genetics 76, 391
11. Hegeman,G.D., Rosenberg,S.L. (1972). Ann.Rev.Micro.24, 504
12. Hohlfeld,R., Vielmetter,W. (1973). Nature 242, 130
13. Horowitz,N.H., Bryson,V., Vogel,H.J., Eds.(1965). Evolving genes and proteins. New York: Academic Press
14. Masters,M., Broda,P. (1971). Nature 232, 137
15. Miller,J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbor Laboratory
16. Sanderson,K. (1972). Bact.Rev.36, 558
17. Taylor,A.L., Trotter,C.D. (1972). Bact.Rev.36, 504
18. Warren,R.A.J. (1972). Can.J.Microbiol.18, 1439

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