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Genetic Fusions That Place the Lactose Genes Under Histidine Operon Control

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The genes of the *Salmonella* histidine operon (*his*) have been placed on an F′ *pro lac* plasmid using genetic methods that rely on recombinational homology provided by Tn*10* transposon insertions. The position and orientation of the transposed *his* genes permit subsequent deletion mutations to form operon fusions that put the *lac* genes under *his* operon control. Strains carrying such fusions show co-ordinate regulation of histidinol dehydrogenase and beta-galactosidase expression. While all of the operon fusions have an intact *hisD* gene, complementation testing and deletion mapping reveal that the genes downstream of *hisD* are deleted to varying extents. The beta-galactosidase produced by these operon fusions is itself a fused protein containing the amino terminus of one or another of the *his* enzymes. Two of the operon fusions having join-points in the *hisB* gene retain histidinol phosphate phosphatase activity and may produce a bifunctional protein having beta-galactosidase as well as the phosphatase activity. The methods that have been used to isolate these *his-lac* fusions should be applicable to other genetic systems.

1. Introduction

In the study of gene regulation, it has frequently been useful to fuse the control elements under investigation to well-characterized genes of an unrelated system. Such chromosome rearrangements have been called operon fusions (reviewed by Franklin, 1978); most operon fusions described in bacteria have involved the *lac* genes of *Escherichia coli* (reviewed by Bassford *et al.*, 1978). When fused to a foreign promoter, these genes provide a gratuitous indication of the level of expression of that promoter. Genetic selections have previously been devised for the isolation of mutants with either increased or decreased expression of *lac*. A *lac* fusion can thus provide a means of selecting mutants with an alteration in the regulatory mechanism under study. This paper describes the isolation and characterization of *his-lac* fusions.

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The crucial first step in obtaining fusions of the *his* and *lac* operons was to bring these unrelated genes close together on a bacterial replicon, properly positioned and oriented for the fusions to form. We have already described a means for the directed transposition of bacterial genes, relying on the genetic homology provided by insertions of the tetracycline-resistance transposon, Tn*10* (Chumley & Roth, 1980). Using this method, we integrated the *his* genes into a *lacI*::Tn*10* insertion carried by an F′ *pro lac* plasmid that already contains a deletion that fuses the *lacI* and *lacZ* genes (Muller-Hill & Kania, 1974; Beck, 1979). Subsequently, the desired operon fusions were isolated. The *his-lac* fusions are stable, and they can be used for selection of *his* regulatory mutants. This new method of forming operon fusions should be applicable to other genetic systems in any bacterium where transposable elements and F′ plasmids can be used.

2. Materials and Methods

(a) *Bacterial strains*

Table 1 lists the numerical designations and full genotypes of multiply marked strains used for this study. All strains are derived from *Salmonella typhimurium* LT2.

(b) *Media*

Vogel-Bonner E medium, NCE medium, and Difco nutrient broth were used as described (Chumley & Roth, 1980). Difco MacConkey agar medium was used (40 g/l) and contained 1% (w/v) lactose, final concentration.

(c) *Transductions and conjugations*

Generalized transductions using P22 HT105/1 *int-201* were performed as described (Chumley & Roth, 1980). F′ transfers were also performed as described (Chumley & Roth, 1980).

(d) *Enzyme assays and protein determinations*

Histidinol dehydrogenase and histidinol phosphate phosphatase were assayed in toluenized cells by the spectrophotometric methods of Martin *et al.* (1971). HDHase† was also assayed in toluenized cells using the radiochemical assay of Ciesla *et al.* (1975). [^{14}C]histidinol was a generous gift from Tadahiko Kohno. Beta-galactosidase was assayed as described by Miller (1972). In the antibody precipitation experiments, histidinol phosphate phosphatase and beta-galactosidase were assayed in crude extracts. Cells were disrupted in a French pressure cell and centrifuged at low speed to remove debris. Protein was determined using the Bio-Rad protein assay reagent (Bradford, 1976; Bio-Rad Laboratories Technical Bulletin 1051).

(e) *Antibody precipitation*

Cells grown in E medium containing histidinol were washed in TEA buffer (triethanolamine buffer (0·1 M, pH 7·5) containing 10 mM-$MgCl_2$). Crude extracts were prepared in the cold using the French press, and the protein concentration was determined by the Bio-Rad protein assay (see section (d), above). Crude extracts were serially diluted 1:2 in TEA buffer, 0·2 ml of crude extract at each dilution (1:1 to 1:128) was then mixed with 0·1 ml of undiluted antiserum, and the mixtures were incubated overnight at 4°C. Any precipitate thus formed was removed by decanting the supernatant following centrifugation at 30,000 ***g*** for 1 h. The remaining *hisB* phosphatase and beta-galactosidase activities were then assayed.

† Abbreviations used: HDHase, histidinol dehydrogenase (EC 1.1.1.23); hol, histidinol.

TABLE 1

Multiply-marked strains of Salmonella typhimurium *LT2*

Strain	Genotype
TR132	*adeC7 proA46 strA1 aza-1 gal-501 ile-405*/F'128 *pro*$^+$ *lac*$^+$
TT1770	*zee-2*::Tn*10* (*his*$^+$) *zee-1*::Tn*10*
TT2620	*hisD9652*::Tn*5*
TT2905	*hisOH2253 hisT1535*/FpZT1 *hisC-lacZ*
TT3614	*hisOE9533*/F'71–56–14 *lacI-lacZ lacI475*::Tn*10 pro*$^+$
TT3615	*hisOE9533*/F600 *lacI-lacZ lacI475*::Tn*10-his*$^+$–Tn*10 pro*$^+$
TT4801	*hisD8468 aroD5 hisT1535*$_{ts}$ *strA1 recA1*
TT4802–TT5103	*hisOE9533*/FpZT1 to FpZT302 (inclusive; *his-lac* fusion plasmid isolates: *pro*$^+$, *lacI475*::Tn*10*)
TT5104–TT5188	*hisD8468 aroD5 strA1 recA1 hisT1535*$_{ts}$/FpZT1 to FpZT279 (selected F600 *his-lac* fusions)
TT5189	*hisOH2253 aroD5 hisT1539*
TT5190	*hisOH2253* (isogenic with TT5191)
TT5191	*hisOH2253 hisT1539* (isogenic with TT5190)
TT5192–TT5210	*hisOH2253*/FpZT1 to FpZT279 (selected F600 derivatives)
TT5211–TT5228	*hisOH2253 hisT1539*/FpZT1 to FpZT279 (selected F600 derivatives)
TT5229–TT5259	*hisOH9533*/FpZT1 to FpZT279 *hisD9652*::Tn*5* (selected F600 derivatives carrying the *hisD*::Tn*5* insertion)
TT5260	*hisOH9533 hisT1535*$_{ts}$ *zej-636*::Tn*5*/F600 *lacI-lacZ lacI475*::Tn*10-his*$^+$–Tn*10 pro*$^+$
TT5261–TT5275	*hisOE9533 hisT1535*$_{ts}$ *zej-636*::Tn*5*/FpZT303 to FpZT317 (inclusive)
TT5276–TT5290	*hisD8468 aroD5 hisT1535*$_{ts}$ *strA1 recA1*/FpZT303 to FpZT317 (inclusive)
TT5291–TT5295	*hisOH2253*/FpZT303 to FpZT316 (selected F600 derivatives)
TT5296–TT5300	*hisOH2253 hisT1539*/FpZT303 to FpZT316 (selected F600 derivatives)
TT5301	*hisOE9533*/FpZT1 *hisO1242 hisC-lacZ*
TT5302	*hisOE9533*/FpZT1 *hisO*$^+$ *hisC-lacZ*

Strain numbers and full genotypes are listed. In order to keep the list as short as possible, closely related strains have been entered as a group (i.e. TT4802 to TT5103). In such cases, the strains differ only by the F600-derived *his-lac* fusions that they contain. Many of these fusions are described in detail in the text.

(f) *Nomenclature*

We have followed the nomenclature conventions for insertion mutations suggested by Campbell *et al.* (1977) and modified by Chumley *et al.* (1979), and by Chumley & Roth (1980). In naming *his-lac* fusion derivatives of F600, we have assigned each fusion a unique plasmid identification number in accordance with the recommendations of Novick *et al.* (1976). The initials, ZT, used in naming these plasmids were assigned by E. Lederberg (personal communication).

3. Results

(a) *Construction of the F600 plasmid carrying* his *and* lac

The F600 plasmid was constructed by transposition of the *his* operon to a site within the *lacI* gene of an F' *lac* plasmid. This transposition occurs by standard recombination events between appropriately placed Tn*10* insertion elements. The transposition occurs in a generalized transduction cross, as follows.

The donor strain carries an intact *his* operon flanked by Tn*10* elements in direct

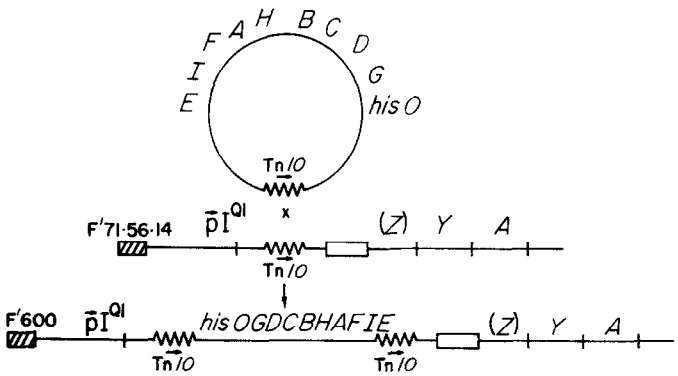

FIG. 1. Translocation of the *his* operon to the F'71–56–14 plasmid carrying *lacI475*::Tn*10*. Using Tn*10-his*-Tn*10* (strain TT1770) as a donor of His^+ in P22-mediated transduction, a circular chromosome fragment containing the *his* operon and a single Tn*10* element may form (see the text). Recombination between the Tn*10* sequence in this circle and *lacI475*::Tn*10* integrates the *his* genes into the plasmid within the *lacI* gene. The recipient *lac* region includes a *lacI* promoter-up mutation I^{Q1} and a deletion (71–56–14) fusing the *lacI* and *lacZ* genes (open box). Insertion of Tn*10-his*-Tn*10* into this *lac* region leaves the *lac* structural genes unexpressed due to lack of a promoter, since the Tn*10* insertions block all transcripts. The orientation of the integrated *his* operon is determined by the orientation of the Tn*10* insertions involved. The resulting plasmid has been designated F600. The *lacI-lacZ* fusion deletion present in the original strain is that of strain 71–56–14 described by Müller-Hill & Kania (1974) and by Brake *et al.* (1978).

repeat (TT1770; "Tn*10-his*-Tn*10*"). When this chromosomal region is transduced into a recipient, the transduced fragment can circularize using Tn*10* as a region of genetic homology. The recipient strain used (TT3614) has a large deletion of the entire *his* region: this deletion is too large to be repaired by any single transduced fragment. The recipient also carries an F′ *lac* plasmid that has a Tn*10* insertion within the *lacI* gene. Most His^+ transductants arise by circularization of the donated fragment (Tn*10-his*-Tn*10*) followed by recombination between the Tn*10* elements in *lacI* and the circular transduced fragment. This event results in an F′ plasmid that carries the entire *his* operon, flanked by Tn*10* elements, inserted in the *lacI* gene. The transposition event and the resulting F600 are diagrammed in Figure 1.

As seen in Figure 1, the F′ *lac* plasmid carries a deletion (71–56–14) that fuses the *lacI* and *lacZ* genes (Muller-Hill & Kania, 1974; Brake *et al.*, 1978). Normally this deletion places the *lacZ* gene under control of the *lacI* promoter. In F600, two Tn*10* elements and an entire *his* operon are located between the *lacZ* sequences and the *lacI* promoter. This interrupts transcription and leaves the *lac* genes unexpressed. The details of the construction of F600 and evidence that the events occur as diagrammed in Figure 1 have been presented previously (Chumley & Roth, 1980; Schmid & Roth, 1980).

(b) *Isolation of F600 derivatives that express* lac

Fusions were selected using a strain (TT3615) that has a large deletion of the chromosomal *his* region and carries F600. This strain is His^+ by virtue of the presence of F600, but Lac^-. By selecting Lac^+ revertants of such a strain, we

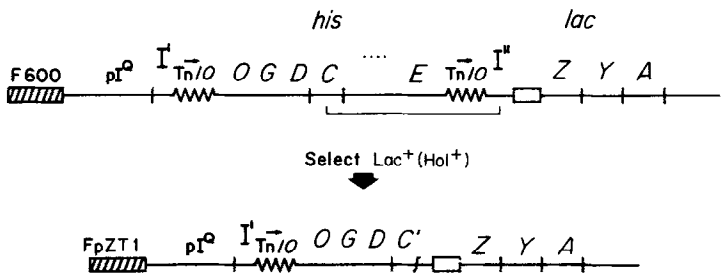

FIG. 2. Formation of a *his-lac* gene fusion on F600. A *Salmonella* strain carrying F600 is His$^+$ but Lac$^-$. By selecting Lac$^+$ (Hol$^+$) derivatives, individuals can be isolated in which a deletion fuses *hisC* to *lacZ*. Fusions to any other *his* genes downstream of *hisD* can be isolated (see the text). The resulting *his-lac* fusion plasmids still contain one Tn*10* insertion, which, for simplicity, has still been called *lac1475*::Tn*10*. It should be noted that this Tn*10* insertion is actually a hybrid, consisting of the "left" end of *lac1475*::Tn*10* and the "right" end of *zee-2*::Tn*10* (Chumley & Roth, 1980).

hoped to isolate individuals in which appropriate deletion mutations had occurred in the F-prime, resulting in *his-lac* gene fusions. Deletions of this type are diagrammed in Figure 2. All the Lac$^+$ revertants obtained were required to maintain the function of at least the *hisD* gene by selecting for utilization of the histidine intermediate, histidinol, as the only source of histidine. (Histidine auxotrophs that express the *hisD* gene can convert histidinol to histidine and thus satisfy their growth requirement; this is referred to as a Hol$^+$ phenotype.)

Eight individual isolates of strain TT3615 carrying F600 were used to inoculate separate tubes of liquid E medium. These cultures were grown to full density at 37°C with vigorous shaking, and the cells were concentrated tenfold in sterile saline. Lac$^+$ revertants were selected by plating 0·1 ml of cell suspension on an NCE lactose plate containing histidinol. After three days incubation at 37°C, Lac$^+$ colonies were picked from these plates, purified by streaking on NB plates, and then checked for growth properties by replica printing. The overall frequency of reversion to Lac$^+$ was approximately 1×10^{-7}. Among 302 revertants examined, all were Tet^R and Lac$^+$, while 250 were His$^-$ (Hol$^+$) and 52 were still His$^+$. All the revertants grew well on NCE lactose plates containing histidine. These mutants were retained as potential *his-lac* fusions (strains TT4802 to TT5103). The His$^-$ isolates were of primary interest, because it seemed likely that all of these candidates would prove to be *his-lac* fusions. The His$^+$ isolates, on the other hand, seemed more likely to include other types of Lac$^+$ revertants, such as fusions to Tn*10* genes (Beck, 1979). Also, among the His$^-$ isolates we hoped to identify *his-lac* fusions that deleted the *hisH* and *hisF* genes, which would potentially be most useful for the study and isolation of *his* regulatory mutants with increased levels of operon expression (see Discussion).

(c) *Genetic analysis of the His$^-$ defect in potential* his-lac *fusions: complementation testing and deletion mapping*

A number of His$^-$ (Hol$^+$) Lac$^+$ isolates were selected from each independent isolation group for further study. These strains were used as F′ donors in conjugation with the *hisD*$^-$ *rec*$^-$ *hisT*$^-$ Sm^R strain, TT4801, selecting Hol$^+$

TABLE 2

Complementation analysis of his-lac *fusions*

his genes functioning									Isolates per group								
G	*D*	*C*	*B*	*H*	*A*	*F*	*I*	*E*	1	2	3	4	5	6	7	8	Totals
+	+	−	−	−	−	−	−	−	0	0	0	1	0	1	0	0	2
+	+	+	−	−	−	−	−	−	1	1	1	0	0	0	0	0	3
+	+	+	+	−	−	−	−	−	1	0	1	0	0	0	0	0	2
+	+	+	+	+	+	−	−	−	2	1	1	2	1	0	2	2	11
+	+	+	+	+	+	+	−	−	0	2	2	0	0	4	2	1	11
+	+	+	+	+	+	+	+	−	2	1	1	2	3	5	2	2	18
															Total tested:		47

Results of complementation testing for 47 potential *his-lac* fusions. Functional *his* genes remaining on the plasmids are indicated by a plus (+) sign; those genes whose function has been lost are indicated by a minus (−) sign. The number of isolates of each type from each of the independent groups (groups 1 to 8) is also shown.

(HisD$^+$) streptomycin-resistant transconjugants. Consistent with expectations for transfer of an F′ *his-lac* fusion plasmid, all the Hol$^+$ transconjugants obtained proved to be Lac$^+$ and Tet^R (strains TT5104 to TT5188).

A number of these transconjugants were subsequently used as F′ donors in complementation testing with a standard set of strains carrying *his* mutations in the chromosome, as described by Johnston & Roth (1979). These tests revealed that each of the potential *his-lac* fusions had lost the function of a contiguous block of distal *his* genes. In all cases, *hisE* function was missing, with the apparent defect extending to various points between *hisE* and *hisD*. A tally of the complementation data for 47 mutants is presented in Table 2. These results agree with expectations for F′ *his-lac* fusions formed on F600 by deletions of the type shown in Figure 2.

In order to confirm the nature of their His$^-$ defect, strains containing F600 *his-lac* fusions were used as donors in P22-mediated mapping crosses. Phage lysates were prepared on 49 of the Lac$^+$ derivatives of TT3615 (chosen from among TT4802 to TT5103), selecting individuals from each of the eight independent groups. The His$^-$ recipients used in these crosses were a set of *his* operon deletions (Hartman *et al.*, 1971) whose endpoints are internal to the operon and divide it into 11 deletion intervals distal to *hisD*. The donors were also tested for their ability to recombine with five different point mutations on the *hisI* and hisE genes. In all cases, it was determined that the fusion candidates contained *his* deletions originating in one of the 11 intervals and extending through the *hisE* gene at the promoter-distal end of the operon (data not shown). A total of 46 of the mutants examined in these crosses had previously been tested for complementation as reported above. There was good agreement between the recombination and complementation results, with the exception of a few cases where recombination was not detected with point mutations in a gene that was known to be functioning on the plasmid. Such discrepancies were attributed to the relative insensitivity of

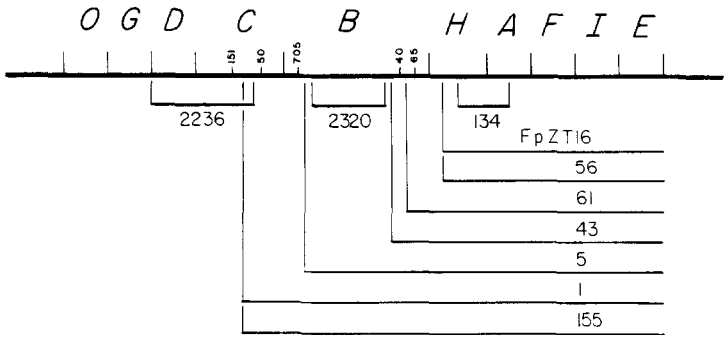

FIG. 3. Deletion map of 7 *his-lac* fusions. P22 phage lysates grown on strains with the genotype, *his-9533*/F'*his-lac* fusion (TT4805, TT4816, TT4843, TT4856, TT4861, TT4891, TT4956) were used as donors in deletion mapping crosses with various *his*$^-$ deletion and point mutations. The Figure shows only a few of the *his*$^-$ mutations used. The bottom 7 horizontal lines indicate the extent of the *his* deletions in the *his-lac* fusions. FpZT43 and FpZT61 retain *hisB* phosphatase expression, while FpZT5 has lost the phosphatase.

these deletion mapping crosses. Several of the fusions with deletion endpoints in *hisC*, *hisB* and *hisH* were subsequently mapped with greater precision, as reported below.

(d) *A fused* hisB-lacZ *protein with both histidinol phosphate phosphatase and beta-galactosidase activity*

Fusions of the *lac* operon derived from F600 would be expected to produce a chimeric beta-galactosidase enzyme that includes the amino terminus of some other gene product (Brake *et al.*, 1978; Bassford *et al.*, 1978; Beck 1979). In order to test this expectation regarding the *his-lac* fusions, we examined fusions with deletion endpoints in the *hisB* gene. The *hisB* gene determines the structure of a bifunctional enzyme; coding sequences for the histidinol phosphate phosphatase domain reside in the promoter-proximal portion of the gene, while sequences for the indole glycerol phosphate dehydratase domain reside in the distal portion (Houston, 1973). We therefore expect *his-lac* fusions that inactivate *hisB* dehydratase, but retain phosphatase activity, to produce a single protein having both activities.

As shown in Table 2, three independent potential *his-lac* fusions appeared to have join-points in the *hisB* gene. Along with two fusion candidates deleted for *hisC* through *hisE* and two other candidates deleted for *hisH* through *hisE*, these potential *hisB* fusions were mapped more precisely. Figure 3 indicates the extent of the *his* deletions in these seven isolates. The deletions in FpZT61 and FpZT43 fail to recombine with only a few of the most distal *hisB* point mutations, while the deletion in FpZT5 extends much further toward the promoter-proximal end of the gene.

Based on these results, we suspected that strains carrying FpZT61 and FpZT43 might retain *hisB* phosphatase activity. Enzyme assays reported in Table 3 bore out this possibility. The phosphatase produced by the truncated *hisB* genes on these two plasmids apparently has only slightly reduced specific activity by

TABLE 3

Enzyme activity in hisB-lacZ *fusions*

Strain	Relevant genotype	*hisB* specific activity
hisO1242	*hisB*$^+$	54·9
hisBH22	*hisBΔ*	0
TT5218	FpZT125 *hisB*$^+$; *hisE-lacZ*	45·2
TT5226	FpZT43 *hisB-lacZ*	33·5
TT5228	FpZT61 *hisB-lacZ*	23·4
TT5211	FpZT5 *hisB-lacZ*	0

Histidinol phosphate phosphatase activity remaining in various *his-lac* fusion strains. Enzyme activities are reported as increase in ΔO.D.$_{820}$/20 min per mg protein in crude extracts that had been dialyzed against 0·1 M-triethanolamine buffer (pH 7·5) containing 10 mM-$MgCl_2$ (Martin *et al.*, 1971). Strain *hisO1242* carries a *his* regulatory mutation (attenuator deletion). Strains TT5211 to 5228 carry a deletion of the chromosomal *hisB* gene and have high levels of episomal operon expression due to the presence of a *hisT* regulatory mutation in the chromosome.

comparison to the wild-type protein. Enzyme levels are high in the fusion strains due to the presence of a *hisT* regulatory mutation in strains assayed.

To determine whether the *hisB* phosphatase produced by FpZT43 was associated with beta-galactosidase, we used rabbit antiserum prepared against partially purified *hisB* enzyme (a gift from B. Cooper Kohno and T. Kohno, personal communication) and precipitated *hisB* phosphatase from crude extracts. We also assayed the effects of antibody treatment on beta-galactosidase in the same crude extracts. Table 4 shows the results of a representative experiment. A number of experiments were done, as described in the legend to Table 4, and the results clearly indicate that antibody to *hisB* enzyme cross-reacts with beta-galactosidase produced by the presumptive *hisB-lacZ* gene fusion. Several possibilities could account for the failure of anti-*hisB* antiserum to completely inactivate beta-galactosidase in crude extracts of the fusion strain, including partial proteolysis of the fused protein or residual activity of beta-galactosidase in the antigen–antibody complex. It should be noted that the enzyme activity levels of the fused protein from TT5226 are lower in Table 4 (assayed after incubation overnight at 4°C) than in Table 3 (assayed immediately after cell disruption). This may be due to instability of the fused protein.

(e) *HDHase and beta-galactosidase from* his-lac *fusions increase co-ordinately in a* hisT$^-$ *genetic background*

Mutants defective in the *hisT*-encoded enzyme activity, tRNA pseudouridylase, show elevated *his* operon expression (Chang *et al.*, 1971 : Cortese *et al.*, 1974). For a fused operon with *lac* under *his* control, the levels of both *his* and *lac* enzymes would

TABLE 4

Immune precipitation of fused hisB-lacZ *protein*

Strain	Genotype	Protein (mg/ml)	*hisB* activity Before Ab	*hisB* activity After Ab	*hisB* activity % Lost	β-Gal activity Before Ab	β-Gal activity After Ab	β-Gal activity % Lost
TT5226	his*Δ*9533/ FpZT43 *hisB-lacZ*	3·8	6·6	0	100	960	342	65
TR132	*hisB*$^+$/ F′*lacZ*$^+$	4·0	17·0	1·5	91	34,500	37,625	0

Cross-reaction between antibody to *hisB* enzyme and *his-lac* fusion beta-galactosidase. Strains were grown in E medium containing histidinol. Extracts were made and material cross-reacting with antibody to *hisB* enzyme was removed (see Materials and Methods). The remaining material was assayed for *hisB* phosphatase and beta-galactosidase activities. Enzyme activities for *hisB* are reported as ΔO.D.$_{820}$/20 min per mg of crude extract. Activity of beta-galactosidase is ΔO.D.$_{420}$/min per mg protein. The effects of antibody treatment on extracts of TT5226 and TR132 were compared for dilutions of crude extract that contained equivalent amounts of total cell protein. The experiments reported in the Table were performed simultaneously. Antiserum was a generous gift from Beth Cooper Kohno and Tadahiko Kohno.

TABLE 5

Co-ordinate regulation of his *and* lac

his-lac fusion plasmid	Isolation group	HDHase activity In *histT*$^+$	HDHase activity In *histT*$^-$	D.R.†	β-Gal activity In *hisT*$^+$	β-Gal activity In *hisT*$^-$	D.R.†
FpZT5 *hisB-lacZ*	1	0·95	10·0	10·5	311	1641	5·3
FpZT16 *hisH-lacZ*	1	0·65	12·2	18·8	46	518	11·2
FpZT43 *hisB-lacZ*	2	1·8	8·9	4·9	470	3675	7·8
FpZT56 *hisH-lacZ*	3	0·96	9·4	9·8	54	733	13·6
FpZT59 *hisF-lacZ*	3	2·3	15·3	6·7	182	1151	6·3
FpZT1 *hisC-lacZ*	4	1·7	8·9	5·3	16	239	14·6
FpZT125 *hisE-lacZ*	6	1·8	7·3	4·1	358	1546	4·3
FpZT155 *hisC-lacZ*	6	1·6	12·0	7·5	158	814	5·2
FpZT197 *hisF-lacZ*	7	2·2	16·7	7·7	38	334	8·8
FpZT198 *hisF-lacZ*	7	1·6	23·9	15·4	65	488	7·5
FpZT218 *hisI-lacZ*	8	2·7	15·2	5·6	214	1296	6·1

Effects of *hisT1539* on expression of histidinol dehydrogenase and beta-galactosidase in various *his-lac* fusions. HDHase units are ΔO.D.$_{590}$/20 min per O.D.$_{650}$ unit of cells: beta-galactosidase levels are ΔO.D.$_{420}$/min per O.D.$_{650}$ unit of cells. Cultures were grown in liquid E medium containing 1 mM-histidinol in order to select for maintenance of the *his-lac* fusion plasmids. Growth in 1 mM-histidinol at 37°C results in only slight derepression of these strains, as evidenced by the effects of the *hisT1539* mutation (also F. Chumley, unpublished results). The *hisT*$^+$ isolates were chosen from among TT5192 to TT5210, while the *hisT1539* isolates were chosen from among TT5211 to TT5228.

† D.R. indicates the derepression ratio, or the relative increase in enzyme activity due to the *hisT* mutation. Enzymes were assayed spectrophotometrically in toluenized cells (see Materials and Methods).

be expected to increase in the absence of *hisT* function. In order to test this expectation, the presumptive F′ *his-lac* fusion plasmids were transferred into isogenic strains that differ only by the mutation, *hisT1539* (TT5190, TT5191). Transconjugants from these matings were purified, grown in liquid E medium supplemented with 1mM-histidinol, and then the levels of *hisD* enzyme and beta-galactosidase were assayed. The results of these assays for 11 fusion candidates from seven independent groups are presented in Table 5; the data demonstrate that the *hisT1539* mutation causes a co-ordinate increase in HDHase and beta-galactosidase levels. The assays also show a wide range in the levels of beta-galactosidase produced by the various fusions. We attribute this to differences in the specific activities of the fused beta-galactosidase proteins.

(f) *A* hisD::*Tn5 insertion is polar on* lac

When the kanamycin resistance (Kan^R) transposon, Tn*5*, inserts into an operon, it exerts strong polar effects on genes downstream from the site of the insertion (Berg *et al.*, 1978,1980). In order to confirm that *lac* transcription is dependent on the *his* promoter in the potential *his-lac* fusions, a *hisD*::Tn*5* insertion was introduced by P22 transduction, and any polar effects on *lac* were scored by observing the ability of the transductants to grow on lactose plates. A transducing lysate was prepared on strain TT2620, which carries the polar insertion, *hisD9652*::Tn*5* (D. Stetler, unpublished results). The recipients in the transductions are indicated in Table 6. Each of the recipients contains the chromosomal *his* deletion, *his-9533*, which is so large that it cannot be spanned by a single P22-transduced fragment. The recipients also harbor one of the potential F′ *his-lac* fusion plasmids derived from F600. Kan^R transductants were selected on NB kanamycin plates, picked to NB kanamycin master plates, and replica printed to analyze growth requirements. The results are presented in Table 6.

From every cross, there was a relatively large class of Kan^R transductants that remained Hol^+ (and Lac^+). These transductants result primarily from transposition of Tn*5*, and the magnitude of the class is enhanced relative to legitimate ($hisD^-$) transductants due to the restricted genetic homology available for recombination (Biek & Roth, 1980). Among the Hol^- transductants from each cross, most showed a diminished ability to use lactose as carbon source, indicating that the *lac* genes in the recipient strains do in fact depend on the primary *his* promoter for expression. However, from almost every cross there were a few Hol^- transductants that remained Lac^+. These transductants could owe their phenotype to a reversal of the polarity properties of the Tn*5* insertion during the course of transduction. Some *hisG*::Tn*5* insertions (all with the same chromosomal orientation) are known to be non-polar due to the activity of a Tn*5*-associated promoter (D. Stetler, S. Ciampi & D. Biek, unpublished results). Insertion mutants with Tn*5* in the opposite orientation invariably cause a complete polar block. Rolf Menzel has observed similar orientation-dependent Tn*5* promoter activity in the *put* genes of *S. typhimurium* (personal communication). Berg *et al.* (1980) have reported a promoter activity associated with the ends of Tn*5*, but they have not observed the orientation dependence.

TABLE 6

Distribution of Hol and Lac phenotypes among Kan^R transductants

Recipient *his-lac* fusion plasmid	Number of transductants with phenotype			Total examined
	Hol$^+$ Lac$^+$	Hol$^-$ Lac$^+$	Hol$^-$ Lac$^-$†	
FpZT1 *hisC-lacZ*	11	1	12(−)	24
PpZT155 *hisC-lacZ*	10	0	14(−)	24
FpZT5 *hisB-lacZ*	9	7	7(±)	24‡
FpZT43 *hisB-lacZ*	8	1	15(±)	24
FpZT61 *hisB-lacZ*	3	2	19(±)	24
FpZT16 *hisH-lacZ*	11	8	31(−)	50
FpZT56 *hisH-lacZ*	3	1	20(−)	24
FpZT34 *hisF-lacZ*	7	2	15(±)	24
FpZT59 *hisF-lacZ*	3	7	14(±)	24
FpZT197 *hisF-lacZ*	6	0	18(−)	24
FpZT198 *hisF-lacZ*	3	1	20(−)	24
FpZT30 *hisI-lacZ*	5	0	19(−)	24
FpZT161 *hisI-lacZ*	2	0	8(±)	10
FpZT218 *hisI-lacZ*	10	0	13(±)	23
FpZT9 *hisE-lacZ*	2	0	22(±)	24
FpZT125 *hisE-lacZ*	4	1	19(±)	24
FpZT167 *hisE-lacZ*	0	2	19(±)	21
FpZT279 *hisE-lacZ*	3	1	16(±)	20

Polar effects of a *hisD*::Tn*5* insertion on *lac* expression. P22 phage grown on TT2620 (*hisD9652*::Tn*5*) were used to transduce Kan^R to strains carrying F′-*his-lac* fusion plasmids. The recipients all had the genotype, *his-9533*/FpZT*his-lac* fusion (selected from among TT4802 to TT5103).

† In different fusion strains, the polar *hisD*::Tn*5* insertion results in different degrees of reduced *lac* expression; (−) indicates no growth on NCE lactose plates, (+ =) indicates very weak growth, and (+ −) indicates weak growth.

‡ Includes one transductant that had acquired a new (uncharacterized) auxotrophy, presumably due to Tn*5* transposition.

The *his* operon contains two low-level internal promoters (Atkins & Loper, 1970: Ely & Ciesla, 1974). We believe the effects of these promoters, between the *hisC* and *hisB* genes and within *hisF* (M. Schmid, personal communication), are reflected in the *lac* phenotypes of the Hol$^-$ transductants shown in Table 6. It seems likely that in cases where (1) the fused *his-lac* gene lies downstream from one of these promoters and (2) the fused protein is sufficiently active, a weak Lac$^+$ phenotype is observed even when no *lac* transcription originates from the primary *his* promoter.

(g) hisO1242 *causes HDHase and beta-galactosidase levels to increase*

The regulatory mutation, *hisO1242*, is a *cis*-dominant attenuator deletion that causes increased levels of *his* operon expression (Johnston *et al.*, 1980). This mutation has been introduced into one of the presumptive *his-lac* fusions, resulting in elevated expression of both histidinol dehydrogenase and beta-galactosidase.

Strain TT5238 has the genotype, *his-9533*/FpZT1 *hisD9652*::Tn*5* (*hisC-lacZ*). P22 phage grown on *hisO1242* was used in transducing *hisD*$^+$ to the plasmid-borne *his* region in TT5238, selecting for growth on E plates containing 1mM-histidinol. After three days of incubation at 37°C, Hol$^+$ transductants were picked to

TABLE 7

Enzyme levels in a hisO1242 his-lac *fusion*

Strain	Genotype	Enzyme activity HDHase	Enzyme activity β-Gal
TT5301	FpZT1 *hisO1242* *hisC-lacZ*	40·1	766
TT5302	FpZT1 *hisO*$^+$ *hisC-lacZ*	5·4	33

Effects of *hisO1242* on expression of *hisD* and *hisC-lacZ* in FpZT1. An isogenic pair of *hisO*$^+$/*hisO1242* recombinants was constructed as described in the text. Cultures were grown in NB containing tetracycline (25 μg/ml). Histidinol dehydrogenase and beta-galactosidase were assayed in toluenized cells (using the radiological HDHase assay: see Materials and Methods). Enzyme activities are expressed as cts/min × 10^{-6} per min per A_{650} unit or as beta-galactosidase specific activity units (Miller, 1972, pp. 352–355).

MacConkey lactose plates. Following overnight incubation, two classes of patches could be distinguished on these plates: among 50 transductants examined, 23 had the same pale pink appearance as strain TT4891 (FpZT1 *hisO*$^+$ *hisD*$^+$ *hisC-lacZ*), while 27 transductants had a very intense red appearance. It seemed likely that the pink transductants were *hisO*$^+$ recombinants, while the bright red transductants contained *hisO1242*. The Hol$^+$ transductants were replica printed to check their growth properties; all were Kan^S Tet^R His$^-$ and Lac$^+$. One bright red transductant (TT5301) and one pale pink transductant (TT5302) were purified and retained. The F′ plasmid in TT5301 has been transferred into several other genetic backgrounds selecting *hisD*$^+$, always resulting in transconjugants that are bright red on MacConkey lactose plates. When P22 phage was grown on TT5301 and used to transduce a His$^+$ phenotype into recipient *hisG46*, 95% of the transductants formed very rough colonies on E plates, while 5% formed normal smooth colonies. Very rough colony morphology is typical of His$^+$ strains carrying the *hisO1242* mutation (Roth & Hartman, 1965).

Table 7 shows the results of enzyme assays performed on strains TT5301 and TT5302. The data indicate that *hisO1242* causes elevated expression of both *hisD* and (*hisC-lacZ*). However, the increase in beta-galactosidase levels appears to be about threefold greater than the increase in HDHase. This could be due to mass-action effects on the assembly or stability of the fused beta-galactosidase protein, a phenomenon that has been argued in the case of other *lac* fusions (Casadaban, 1976).

(h) *Isolation of* his-lac *fusions that require high levels of* his *expression for a Lac*$^+$ *phenotype*

One major goal of this work was to obtain *his-lac* fusions that could subsequently be used in the isolation of regulatory mutants with increased *his* operon expression, basing selective schemes on *lac* phenotypes. However, strains carrying any of the

fusions described above are Lac$^+$ even in the presence of excess histidine. They also grow well enough on minimal histidine plates containing the lactose analog, phenylgalactoside ("phi-gal", Miller, 1972, pp. 146–152), that it is not possible to select for mutants with improved ability to use even this poor carbon source. We therefore devised a scheme for the isolation of *his-lac* fusions that requires high levels of *his* operon expression for a fully Lac$^+$ phenotype. To do this, fusions (Lac$^+$ revertants of strain TT5260) were selected in a strain expressing the *his* operon at a high level. Potential fusions were then screened for those that became Lac$^-$ when basal levels of *his* expression were restored.

Strain TT5260 contains the *hisT* temperature-sensitive mutation, *hisT1535ts*. Strains carrying this mutation express the *his* operon at normal basal levels at 30°C, but at 42°C the *hisT* defect results in high levels of *his* expression (Chang *et al.*, 1971). Strain TT5260 also contains F600. We reasoned that if we isolated Lac$^+$ Hol$^+$ revertants of TT5260 at 42°C, some of those might be Lac$^-$ (or only weakly Lac$^+$) at 30°C, owing to repression of the *his* operon at the lower temperature. Presumably, such a complex phenotype could result from a fused beta-galacotsidase protein with very low specific activity or with very strong concentration dependence for assembly or stability. Of course, simply a cold-sensitive fused protein would also result in the desired phenotype.

Six independent isolates of TT5260 were grown in minimal E medium, the cells were concentrated tenfold in sterile saline, and the suspensions were spread on NCE lactose histidinol or on NCE phi-gal histidinol plates. Lac$^+$ or Phi-gal$^+$ revertants were picked from these plates following incubation at 42°C for three days or six days, respectively. A total of 17 Lac$^+$ revertants was recovered from the six independent isolation groups, at an overall frequency of $0{\cdot}5 \times 10^{-9}$. Thirty Phi-gal$^+$ revertants were recovered from five groups, at a frequency of 1×10^{-9}. Reversion to Lac$^+$ or Phi-gal$^+$ at 42°C is about 100-fold less frequent than at 37°C, probably because almost all the fused beta-galactosidase proteins that can be formed are temperature-sensitive (Muller-Hill & Kania, 1974: Beck, 1979; F. Chumley, unpublished results). All the Lac$^+$ or Phi-gal$^+$ revertants were purified at 42°C and checked for growth properties; among the 47 isolates, 38 were His$^-$ and nine were His$^+$. Fifteen of the isolates (representing five independent groups and including one His$^+$ isolate) seemed to grow much better using lactose or phi-gal at 42°C than at 30°C. To check for transmissibility, the F′ plasmids from these 15 candidates were transferred into the *recA*$^-$ strain TT4801, selecting Hol$^+$ Sm^R transconjugants at 37°C. From this genetic background, the plasmids were analyzed for His$^-$ defects by complementation testing, as described above. Some of the results of this analysis are reported in Table 8.

All 15 of the candidate plasmids were transferred from the TT4801 genetic background into the isogenic pair of strains TT5190 and TT5191, which differ only by the mutation *hisT1539*. Hol$^+$ transconjugants from these matings were then examined for possible effects of the *hisT* mutation on the ability to utilize lactose or phi-gal at 37°C. In the case of strains carrying plasmids listed in Table 8, *hisT1539* isolates grew significantly better than *hisT*$^+$ isolates on NCE lactose histidine or NCE phi-gal histidine plates. When *hisB* phosphatase and beta-galactosidase activities were assayed in these *hisT*$^+$/*hisT*$^-$ pairs, the *hisT*$^-$ mutation was

TABLE 8

Properties of his-lac *fusions*

his-lac fusion plasmid	Growth† in *hisT*⁺ Lac	Growth† in *hisT*⁺ Phi-Gal	Growth† in *hisT*⁻ Lac	Growth† in *hisT*⁻ Phi-Gal	Enzyme levels *hisB* *hisT*⁺	Enzyme levels *hisB* *hisT*⁻	Enzyme levels *hisB* D.R.‡	Enzyme levels β-Gal *hisT*⁺	Enzyme levels β-Gal *hisT*⁻	Enzyme levels β-Gal D.R.‡
FpZT303 *hisF-lacZ*	1	0	5	5	3·5	32	9·2	31	316	10·2
FpZT305 *hisF-lacZ*	3	1	4	1	3·4	25	7·3	0	244	∞
FpZT308 *hisH-lacZ*	3	1	4	5	2·8	30	10·9	31	389	12·5
FpZT315 *hisF-lacZ*	5	1	5	5	3·0	23	7·7	57	354	6·2
FpZT316 *hisA-lacZ*	2	0	5	5	1·6	22	13·5	0	240	∞

Properties of F′*his-lac* fusions isolated at 42°C in a *hisTts* genetic background. For each plasmid, the gene fusion indicated was determined by complementation testing, as described in the text.

† Ability to grow on replica plates using lactose or phenylgalactoside (phi-gal) as carbon source. 0 indicates no growth; 5 indicates best growth possible; 1 to 4 indicate intermediate ranges in growth response. Enzyme levels were measured in toluenized cells (histidinol phosphate phosphatase, the product of the *hisB* gene, and beta-galactosidase; see Materials and Methods). Activities are presented as change in $O.D._{820}$/20 min per $O.D._{650}$ unit of cells (*hisB*) or as beta-galactosidase specific activity units (Miller, 1972, pp. 352–355). Enzyme levels were assayed in strains that contained either a wild-type *hisT* gene (TT5291–TT5292) or a mutated *hisT* gene (TT5296–TT5300).

‡ The columns headed D.R. (derepression ratio) indicate the relative increase in gene expression in strains containing the *hisT* defect.

observed to cause high levels of both enzymes (Table 8). Two of the *hisT*⁺ isolates showed beta-galactosidase levels that were not above background. Apparently the repressed level of *his* expression is not sufficient for expression of *lacZ* in these fusions. The plasmids mentioned in Table 8 have been useful for the isolation of *his* regulatory mutants (Chumley & Roth, unpublished results).

4. Discussion

In this paper, we have described the isolation and characterization of operon fusions that place the *lac* genes under *his* operon control. Expression of the *lac* genes in these fusions is dependent on transcription that originates at the *his* promoter: a Tn*5* insertion in the *hisD* gene is also polar on *lac*. Known regulatory mutations that cause high levels of *his* expression increase *lac* expression in these fusions as well. The fusions that are described have been formed on a modified F′ *pro lac* plasmid, permitting easy transfer from one genetic background to another.

The beta-galactosidase produced by *lac* fusions formed in this manner is a fused protein, containing in our example the amino-terminal portion of any one of the *his* enzymes encoded by genes downstream of *hisD*. Two isolates apparently fuse active *hisB* gene product to beta-galactosidase. Under identical conditions of operon expression, independently isolated fusions differ in measurable levels of beta-galactosidase. We attribute this to differences in the specific activity of the various fused proteins. Given a large number of such fusions, this variability makes it possible to choose one with a Lac phenotype that is uniquely suitable for the

desired genetic application. Various *his-lac* fusions that are described have been useful for genetic studies of the *his* operon, as well as for the selection of mutants with altered *his* regulatory phenotypes.

The *his-lac* fusions have been used to isolate new *his* regulatory mutants, either (1) by screening colonies on lactose indicator plates or (2) by selecting for mutants with improved ability to grow on the lactose analog, phenylgalactoside (Chumley & Roth, unpublished results). Significantly, *his* regulatory mutants can be isolated in this way in the presence of excess histidine and in the absence of the *hisH* and *hisF* genes, whose products are toxic when overproduced (Murray & Hartman, 1972). Such regulatory mutants are selected independently of the effects of any histidine analog. Thus they could include classes of regulatory mutants not revealed by previous selection methods.

Potential His^+ *his-lac* fusions were not further characterized, although a number of candidates have been retained. The fusion method described here requires that *lacZ* coding sequences be fused to the proximal portion of some other gene. A His^+ *his-lac* fusion would require the formation of a compound *hisE-lacZ* gene with residual *hisE* activity, or else fusion of *lacZ* to a functional translation initiator within the *his* transcription unit, but distal to all known genes.

The observed beta-galactosidase activities of the *his-lac* fusions described here suggest that the *his* promoter may be of the same relative strength as the *lac* promoter. The basal (maximally repressed) levels of transcription from the *his* promoter can generate a beta-galactosidase level in the fusion strains that is 20% of the level seen for a fully induced lac^+ strain. In a $hisT^-$ background, some of the fusions show nearly as much beta-galactosidase activity as a fully induced F′ *lac* strain. In a strain that carries a deletion of the *hisO* attenuator (Johnston *et al.*, 1980), transcription from the *his* promoter may yield levels of fused beta-galactosidase activity that are three to five times those found for the fully induced lac^+ operon. These considerations provide a minimal estimate of transcription, since the fused beta-galactosidase may have a lower specific activity than normal enzyme. A comparison of the published purification data for HDHase and beta-galactosidase indicates that these estimates of the relative strengths of the *his* and *lac* promoters may be accurate, if one assumes that translation and turnover proceed at roughly the same rates for the two proteins (F. Chumley, calculations not shown).

The fusions that have been described were formed on an F′ plasmid, relying on methods using Tn*10* genetic homology (Chumley & Roth, 1980). It should be possible to extend these methods to other bacterial systems, wherever transposons and F′ plasmids can be used.

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REFERENCES

Atkins, J. F. & Loper, J. C. (1970). *Proc. Nat. Acad. Sci., U.S.A.* **65**, 925–932.

Bassford, P., Beckwith, J., Berman, M., Brickman, E., Casadaban, M., Guarente, L., Saint-Girons, I., Sarthy, A., Schwartz, M., Shuman, H. & Silhavy, T. (1978). In *The Operon* (Miller, J. H. & Reznikoff, W. S., eds), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Beck, C. F. (1979). *Proc. Nat. Acad. Sci., U.S.A.* **76**, 2376–2380.
Berg, D. E., Jorgensen, R. & Davies, J. (1978). In *Microbiology–1978* (Schlessinger, D., ed.), American Society for Microbiology, Washington.
Berg, D. E., Weiss, A. & Crossland, L. (1980). *J. Bacteriol.* **142**, 439–446.
Biek, D. & Roth, J. R. (1980). *Proc. Nat. Acad. Sci., U.S.A.* **77**, 6047–6051.
Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.
Brake, A. J., Fowler, A. V., Zabin, I., Kania, J. & Muller-Hill, B. (1978). *Proc. Nat. Acad. Sci., U.S.A.* **75**, 4824–4827.
Campbell, A., Berg, D., Lederberg, E., Starlinger, P., Botstein, D., Novick, R. & Szybalski, W. (1977). In *DNA Insertion Elements, Plasmids, and Episomes* (Bukhari, A. I., Shapiro, J. A. & Adhya, S. L., eds), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Casadaban, M. (1976). *J. Mol. Biol.* **104**, 541–555.
Chang, G. W., Roth, J. R. & Ames, B. N. (1971). *J. Bacteriol.* **108**, 410–414.
Chumley, F. G. & Roth, J. R. (1980). *Genetics*, **94**, 1–14.
Chumley, F., Menzel, R. & Roth, J. R. (1979). *Genetics*, **91**, 639–655.
Ciesla, Z., Salvatore, F., Broach, J. R., Artz, S. W. & Ames, B. N. (1975). *Anal. Biochem.* **62**, 44–55.
Cortese, R., Kammen, H. O., Spengler, S. J. & Ames, B. N. (1974). *J. Biol. Chem.* **249**, 1103–1108.
Ely, B. & Ciesla, Z. (1974). *J. Bacteriol.* **120**, 984–986.
Franklin, N. C. (1978). *Annu. Rev. Genet.* **12**, 193–221.
Hartmann, P. E., Hartman, Z., Stahl, R. C. & Ames, B. N. (1971). *Advan. Genet.* **16**, 1–34.
Houston, L. L. (1973). *J. Bacteriol.* **113**, 82–87.
Johnston, H. M. & Roth, J. R. (1979). *Genetics*, **92**, 1–15.
Johnston, H. M., Barnes, W., Chumley, F. G., Bossi, L. & Roth, J. R. (1980). *Proc. Nat. Acad. Sci., U.S.A.* **77**, 508–512.
Martin, R. G., Berberich, M. A., Ames, B. N., Davis, W. W., Goldberger, R. F. & Yourno, J. D. (1971). In *Methods in Enzymology* (Tabor, H. & Tabor, C. W., eds), pp. 1–44, Academic Press, New York and London.
Miller, J. H. (1972). In *Experiments in Molecular Genetics*, pp. 352–355, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Müller-Hill, B. & Kania, J. (1974). *Nature (London)*, **249**, 561–563.
Murray, M. L. & Hartman, P. E. (1972). *Can. J. Microbiol.* **18**, 671–681.
Novick, R. P., Clowes, R. C., Cohen, S. N., Curtiss, R., Datta, N. & Falkow, S. (1976). *Bacteriol. Rev.* **40**, 168–189.
Roth, J. R. & Hartman, P. E. (1965). *Virology*, **27**, 297–307.
Schmid, M. & Roth, J. (1980). *Genetics*, **94**, 15–29.