GENETIC MAP OF THE LACTOSE REPRESSOR GENE (*i*) OF *ESCHERICHIA COLI*

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ABSTRACT

39 *tonB* i^- deletions were used to map 57 independently isolated *i* gene mutants. Methods selective for i^+ recombinants from various types of *i* mutants are described. i^+ recombination frequencies of 6×10^{-6} to 6×10^{-7} can be detected in the different selective systems. Twenty i^s mutations and 12 i^{-d} mutations map in distinct but different regions of the gene. i^-_{sus} mutations are scattered over the gene.

ALTHOUGH a number of *i* gene mutants have been isolated, until recently there existed no mapping system which easily enabled one to order the various *i* gene mutations. DAVIES and JACOB (1968) have exploited 3-point tests in ordering some mutations in the *i* gene. However, marker effects and high negative interference (JACOB and WOLLMAN 1961; YANOFSKY *et al.* 1967; NORKIN 1970), particularly in the case of closely linked mutations, can result in mismapping. A much less ambiguous method for mapping closely linked mutations is deletion mapping (BENZER 1959). Here, rather than comparing recombination frequencies, only a positive or negative answer is scored. Fortunately a set of deletions in the *i* gene already existed (MILLER, REZNIKOFF *et al.* 1970). These deletions were produced in strains whose *lac* genes were transposed near the *att80* site. The deletions extend from the *tonB* region into the *i* gene leaving the structural genes intact. In addition the original *lac pro* region of these strains is deleted. Therefore, the strains can be made diploid for the *lac* region by a lac^+ pro^+ episome, which can itself carry a mutation.

If such strains, carrying a point mutation and a deletion, are grown up to high density in liquid medium, recombinants, if they occur, accumulate in higher frequency than in a transduction or Hfr $\times$ F^- cross (IPPEN *et al.* 1968; MILLER *et al.* 1968).

To order the deletions, I decided to map suppressible nonsense mutations of the *i* gene which were additionally i^q. These mutations, expected to be randomly distributed over the gene, might show whether hot spots for the ends of deletions exist in the *i* gene. The i^q mutation (q = quantity, the i^q mutant produces about ten times more repressor than wild type, MÜLLER-HILL, CRAPO and GILBERT 1968) may later enable one to isolate the protein fragments and thereby deduce the exact positions of the mutations. I was also interested in determining whether

there were particular regions of the *i* gene map which code for the inducer and operator binding-sites of the repressor.

In 1964 two superrepressed (i^s) mutants of the *lac* operon were described by WILLSON *et al.* They behave as noninducible mutants whose *lac* negative phenotype is dominant over the wild-type inducible one. MÜLLER-HILL *et al.* (1968) demonstrated that i^q is dominant over i^s. Since the active form of wild-type repressor is a tetramer, these authors proposed that in an i^q/i^s heteromerozygote most of the i^s subunits occur in the mixed tetramer $i^q_3\ i^s$, and that these mixed tetramers are sensitive to the inducer. JOBE, RIGGS and BOURGEOIS (1972) have isolated additional i^s mutants. Employing *in vitro* techniques they were able to examine directly the affinity of mutated repressor for inducer and for the operator, as well as the interaction of IPTG with the different i^s repressor-operator complexes. They concluded that the majority of the i^s mutations only alter the affinity of the repressor for its ligands. Therefore, the map position of such mutants may show which regions of the *i* gene code for the inducer binding-site.

A similar type of mutation also exists for the operator binding-site. This anomalous class of i^- mutants, originally called "$i^-\ o^c$" are known now as i^{-d} (i^- dominant) mutants because of their *trans* dominance over wild type (MÜLLER-HILL *et al.* 1968). Repressor from such mutants still binds inducer but not operator (ADLER *et al.* 1972). In this paper mapping procedures for these mutants are described.

I also mapped the thermolabile mutation i^{TL} (HORIUCHI and NOVICK 1961), the temperature-sensitive synthesis mutation i^{TSS} (NOVICK, LENNOX and JACOB 1963), the repressible constitutive mutation i^{rc} (MEYERS and SADLER 1971), and *L1* (IPPEN *et al.* 1968; SCAIFE and BECKWITH 1966), a small deletion cutting into both the *i* gene and the promoter.

MATERIALS AND METHODS

Bacterial strains: Strains are alphabetically arranged (Table 1). All BMH strains were obtained from B. MÜLLER-HILL, the SBOU strains from S. BOURGEOSIS, the JD strains from J. DAVIES, the JS strains from J. R. SADLER, the JM strains from J. MILLER, and the BG strains from B. GRONENBORN. The other strains were constructed by me. The terminology of TAYLOR (1970) is used, except for the lactose operon where the terminology of BECKWITH and ZIPSER (1970) is followed. The sensitivity of a strain to antibiotics is not mentioned. All JM strains are *thi*$^-$. The F'*lac pro* episome used is *lac*+ *proAB*+ (SCAIFE 1967). The [*lac-pro*]$_\Delta$ is the deletion XIII *lac*$^-$ *proAB*$^-$ (CUZIN and JACOB 1964). The *galE* mutant *PL132,* is that used by MALAMY (1966).

Media: Dyt-broth: 1.6% Bacto tryptone (Difco), 1.0% yeast extract (Difco), 0.5% NaCl. Mineral medium (NM): 0.7% K_2HPO_4, 0.3% KH_2PO_4, 0.1% NH_4Cl, 0.08% Na_2SO_4, 0.025% $MgSO_4 \cdot 7H_2O$, 0.12% Na_3-citrate $\cdot$ $2H_2O$, 0.005% thiamine (B_1). Mineral glucose streptomycin medium (NM-gluc-Sm): NM + 0.5% glucose (gluc) (Merck), 200 μg streptomycin (Sm)/ml (Serva) (tryptophan (try) (Serva) 0.02% if needed). Mineral lactose medium (NM-lac): NM + 0.5% lactose (lac) (Merck) (try 0.02%) if needed). Mineral phenyl-β-D-galactoside Nalidixic acid medium (NM-PG): NM-gluc + 0.03% phenyl-β-D-galactoside (PG) (Sigma), 40 μg 5-bromo-4-chloro-3-indolyl-β-D-galactoside (xgal)/ml (Cyclo), (stock solution: 20 mg/ml dimethyl-formamide), 20 μg nalidixic acid/ml (Calbiochem), (stock solution: 100 mg/ml NaOH 1N). This medium is used to score i^+ recombinants among i^-'s. NM glucose xgal medium (NM-

TABLE 1

Bacterial strains

BG1001, -02, -03, -04, -05, -07, -11, -12, -13, -14:
[*lac–pro*]Δ *thi*⁻ *strA*ʳ *ϕ80dlac* $i^{-d}_{(\text{No. BG1 to BG14})}$ *o*+ *z*+ *y*+ *a*+
BG1, -2, -3, -4, -5, -7, -11, -12, -13, -14:
[*lac–pro*]Δ *nalA*ʳ/F′*lac* $i^{-d}_{(\text{No. BG1 to BG14})}$ *o*+ *z*+ *y*+ *a*+
BMH34: i^{s}_{34} *o*+ *z*+ *y*+ *a*+
BMH68: i^{s}_{68} *o*+ *z*+ *y*+ *a*+/F′*lac* i^{s}_{68} *o*+ *z*+ *y*+
BMH157: *lac*+/F′*lac* i^{s}_{y18} *o*+ *z*+ *y*+ *a*+ (i^{s}_{y18} marker: WILLSON *et al.* 1964)
BMH236: i^{s}_{236} *o*+ *z*+ *y*+ *a*+ *strA*ʳ/F′*lac* i^{s}_{236} *o*+ *z*+ *y*+ *a*+
BMH240: i^{s}_{240} *o*+ *z*+ *y*+ *a*+ *strA*ʳ/F′*lac* i^{s}_{240} *o*+ *z*+ *y*+ *a*+
BMH270: i^{s}_{270} *o*+ *z*+ *y*+ *a*+ (duplication for *lac*+, see RESULTS)
BMH271: i^{s}_{271} *o*+ *z*+ *y*+ *a*+
BMH272: i^{s}_{272} *o*+ *z*+ *y*+ *a*+ (duplication for *lac*+, see RESULTS)
BMH274: i^{s}_{274} *o*+ *z*+ *y*+ *a*+
BMH275: i^{s}_{275} *o*+ *z*⁻/F′i^{s}_{275} *o*+ *z*⁻
BMH277: i^{s}_{277} *o*+ *z*+ *y*+ *a*+ (duplication for *lac*+, see RESULTS)
BMH380: i^{TL} *o*+ *z*+ *y*+ *a*+ *strA*ʳ (alias E103, HORIUCHI and NOVICK 1961)
BMH395: i^{TSS} *o*+ *z*+ *y*+ *a*+ *thi*⁻ (i^{TSS} marker: SADLER and NOVICK 1965)
BMH425: [*lac–pro*]Δ *thi*⁻/F′*lac*+ *pro*+
BMH596: Wild type
BMH606: [*lac–pro*]Δ *thi*⁻ $\phi 80c_{I857}t_{68}dlac$ i^{sq} *o*+ z^{-}_{U118} *y*+ *a*+ (originates from J. H. MILLER's M965, pers. communication; z^{-}_{U118} marker: JACOB and MONOD 1961)
BMH611: [*lac–pro*]Δ/F′*lac* i^{q} *o*+ z^{-}_{U118} *y*+ *a*+ *pro*+ (originates from J. H. MILLER's M965)
JD24ep: *thi*⁻, *leu*⁻, *purE*⁻, *proC*⁻, *lac* i^{-d} *o*+ *z*+ *y*+ *a*+, *strA*ʳ
JD522ep: *thi*⁻, *leu*⁻, *purE*⁻, *proC*⁻, *galE*⁻, *lac* i^{-d} *o*+ *z*+ *y*+ *a*+, *strA*ʳ
JM446, -449, -535, -550, -790, -912, -914, -917, -921, -923, -932, -935, -936:
[*lac–pro*]Δ/F′*lac* $i^{q,-}_{\text{ochre(No. o446 to o936)}}$ p_{L8} *o*+ *z*+ *y*+ *a*+ (PLATT *et al.* 1972)
JM95, -100, -136, -258, -315, -568, -907, -913, -919, -959, -960:
[*lac–pro*]Δ/F′*lac* $i^{q,-}_{\text{amber(No. a95 to a960)}}$ p_{L8} *o*+ *z*+ *y*+ *a*+ *pro*+ (PLATT *et al.* 1972)
JMx7700r: [*lac–pro*]Δ,*recA*⁻ *strA*ʳ *ϕ80dlac i*+ *o*+ *z*+ *y*+ *a*+
JM7733r: [*lac–pro*]Δ,*recA*⁻ *galE*⁻$_{PL132}$, *strA*ʳ (grows mucoid at temperatures below 41°C)
JMx8508: [*lac–pro*]Δ *strA*ʳ *ϕ80dlac* [*trp*⁻*tonB*⁻ *lac a*⁻ *y*⁻ *z*⁻ *o*⁻ *i*⁻]Δ (MILLER *et al.* 1968)
JMx8601, -03, -04, -07, -08, -15, -16, -21, -22, -23, -26, -29, -33, -36, -40, -41, -42, -43, -47:
[*lac-pro*]Δ *strA*ʳ *ϕ80dlac* [*trp*⁻ *tonB*⁻ *lac*+ *i*⁻]Δ (MILLER, REZNIKOFF *et al.* 1970)
JMx8606, -10, -11, -12, -13, -17, -18, -24, -25, -27, -28, -30, -32, -35, -38, -43, -46, -60, -61:
[*lac–pro*]Δ *str*ʳ *ϕ80dlac* [*trp*⁻ *tonB*⁻ *lac*+ *i*⁻]Δ (MILLER, REZNIKOFF *et al.* 1970)
JM101: [*lac–pro*]Δ/F′*lac* i^{s}_{694} *o*+ *z*+ *y*+ *a*+ *pro*+ (i^{s}_{694} marker: WILLSON *et al.* 1964)
JSD113: *lac* i^{rc} *o*+ *z*+ *y*+ *a*+ (MEYERS and SADLER 1971)
MPD113: i^{rc} from JSD113 on F′*lac*+*pro*+ in MP29
MPN1: i^{s} from SBN2 on F′*lac*+*pro*+ in MP29
MPTL: i^{TL} on F′*lac*+*pro*+ in MP29
MPTSS: i^{TSS} on F′*lac*+*pro*+ in MP29
MPy18: i^{s}_{y18} on F′*lac*+*pro*+ in MP29
MP2A: i^{s} from SB2A on F′*lac*+*pro*+ in MP29
MP14A: i^{s} from SB14A on F′*lac*+*pro*+ in MP29
MP10: i^{s} from BMH270 in BMH596
MP11: i^{s} from BMH272 in BMH596
MP12: i^{s} from BMH277 in BMH596
MP16z: i^{s} from SB16z on F′*lac*+*pro*+ in MP29
MP24: i^{-d} from JD24 on F′*lac*+*pro*+ in MP29
MP27: [*lac–pro*]Δ *thi*⁻ *strA*ʳ

TABLE 1—Continued

MP29: [*lac-pro*]Δ *thi*⁻ *nalA*ʳ
MP30: as JMx7733r but *nalA*ʳ
MP43: i^s from SB43 on F′*lac*+*pro*+ in MP29
MP44: i^s from SB44 on F′*lac*+*pro*+ in MP29
MP68: i^s from BMH68 on F′*lac*+*pro*+ of BMH425
MP236: i^s from BMH236 on F′*lac*+*pro*+ in MP29
MP240: i^s from BMH240 on F′*lac*+*pro*+ in MP29
MP258: as BMH212 but i^-_{a258}
MP270: i^s from BMH270 on F′*lac*+*pro*+ in MP29
MP271: i^s from BMH271 on F′*lac*+*pro*+ in MP29
MP272: i^s from BMH272 on F′*lac*+*pro*+ in MP29
MP274: i^s from BMH274 on F′*lac*+*pro*+ in MP29
MP275: i^s from BMH275 on F′*lac*⁻*pro*+ in MP29
MP277: i^s from BMH277 in BMH425
MP315: as BMH212 but i^-_{a315}
MP445: as BMH212 but i^-_{o446}
MP449: as BMH212 but i^-_{o449}
MP522: i^{-d} from JD522ep on F′*lac*+*pro*+ in MP29
MP790: as BMH212 but i^-_{a790}
MP907: as BMH212 but i^-_{a907}
MP913: as BMH212 but i^-_{a913}
MP932: as BMH212 but i^-_{o932}
MP935: as BMH212 but i^-_{o935}
MP959: as BMH212 but i^-_{a959}
MP960: as BMH212 but i^-_{a960}
SBOU-N1, -N2, -2A, -14A, -16z, -43, -44, -45: independently isolated i^s mutants (14A and 16z came out of the same culture) in C600: *thi*⁻ *thr*⁻ *leu*⁻ *strA*ʳ (JOBE *et al.* 1972)

gluc-xgal): NM-gluc + 40 μg xgal/ml. On this medium i^+ or *lac*⁻ colonies are white. NM glucose xgal isopropyl-thio-β-D-galactoside medium (NM-gluc-xgal-IPTG): NM-gluc-xgal + 5×10^{-4} M isopropyl-thio-β-D-galactoside (IPTG) (Sigma). On this medium i^+ colonies are blue. NM glucose Isopropyl-thio-β-D-galactoside medium (NM-gluc-IPTG): NM-gluc + 5×10^{-4} M isopropyl-thio-β-D-galactoside (IPTG) (Sigma). For solid media bacto agar (Difco), is added to 1.5% final concentration. MacConkey -plates: 51.5 g MacConkey agar No. 3 (Oxoid)/liter H_2O. On this medium *lac*⁺'s show up as red colonies, whereas *lac*⁻'s are white. Tc-lac-plates: 0.15% Beef extract (Difco), 0.3% yeast extract, 0.6% bacto peptone (Difco), 1.5% bacto agar, 50 μg 2,3,5-triphenyltetrazolium chloride/ml (Merck); after autoclaving sterile lactose solution (Merck) is added, 0.2% final concentration. *lac*⁺ colonies are white on this medium whereas *lac*⁻'s are red.

Mating procedures: Overnight cultures of the recipient and the donor, grown in dyt, were diluted 1 : 10 (0.1 ml : 1 ml) in the same medium and incubated for 1 hr at 37°C (in 20 ml test tubes) on a culture roller (New Brunswick). One ml of both cultures was mixed and incubated at 37°C without shaking for 30 min. The mating mixture was then plated on a suitable selection medium.

For high mating efficiencies an overnight culture of the donor, grown in dyt (maximal incubation time: 12 hr at 30°C) was diluted 1 : 10 in the same medium and incubated at 37°C for 1.5 hr on a culture roller. The recipient was diluted 1 : 5 and incubated for the same time. Two ml of the donor plus 2 ml of the recipient plus 4 ml dyt were mixed in a 100 ml Erlenmeyer flask and incubated at 37°C with slight shaking (30 rpm) for 30 min. Under these conditions 10^8 diploids/ml were obtained. In some cases, as indicated later, the mating mixture was incubated for another 2 hr with strong shaking (200 rpm). During this time the culture grew to saturation.

Strains constructions: For mapping, all *i* gene mutations were crossed on an F'*lac*+ *pro*+ episome. The procedures used are described below. To test for the episome, the strains were streaked out against MP27 or MP29 (Table 1) on NM-gluc-Sm or -Nal plates.

i^s *mutants:* Strains carrying the mutation on a normal *lac* episome were cured with acridine orange (HIROTA 1960) assuming that 0.3% of the bacteria had become homozygous for the i^s mutation before having lost the episome. A suitable dilution of the cured culture was spread on Tc-lac-plates. The dark red colonies—about 1 among 300—were tested for the absence of the episome (the strains were tested for sensitivity to phage fr (ZINDER 1965)). The F'*lac*+ *pro*+ episome from BMH425 was then transferred into strains which were *lac*− and had lost the normal *lac* episome. These strains were mated with MP29 ([*lac*−*pro*−]Δ $nalA^r$). Appropriate dilutions of the mating mixture were plated onto NM-gluc-xgal-IPTG-Nal media. xgal, a substrate but not an inducer of β-galactosidase at the concentration used, is colorless. When it is hydrolyzed, two indolyl moities associate spontaneously to produce the insoluble colored dye, indigo (DAVIES and JACOB 1968). The IPTG at the concentration used (5×10^{-4} M) induces only *i*+'s, which show up as deep blue colonies on this medium. The white or light blue colonies (dependent on the i^s mutation) growing on these plates—1 to 5 among 300 blue colonies—had the i^s mutation on the F'*lac pro*+ episome transferred to the recipient MP29.

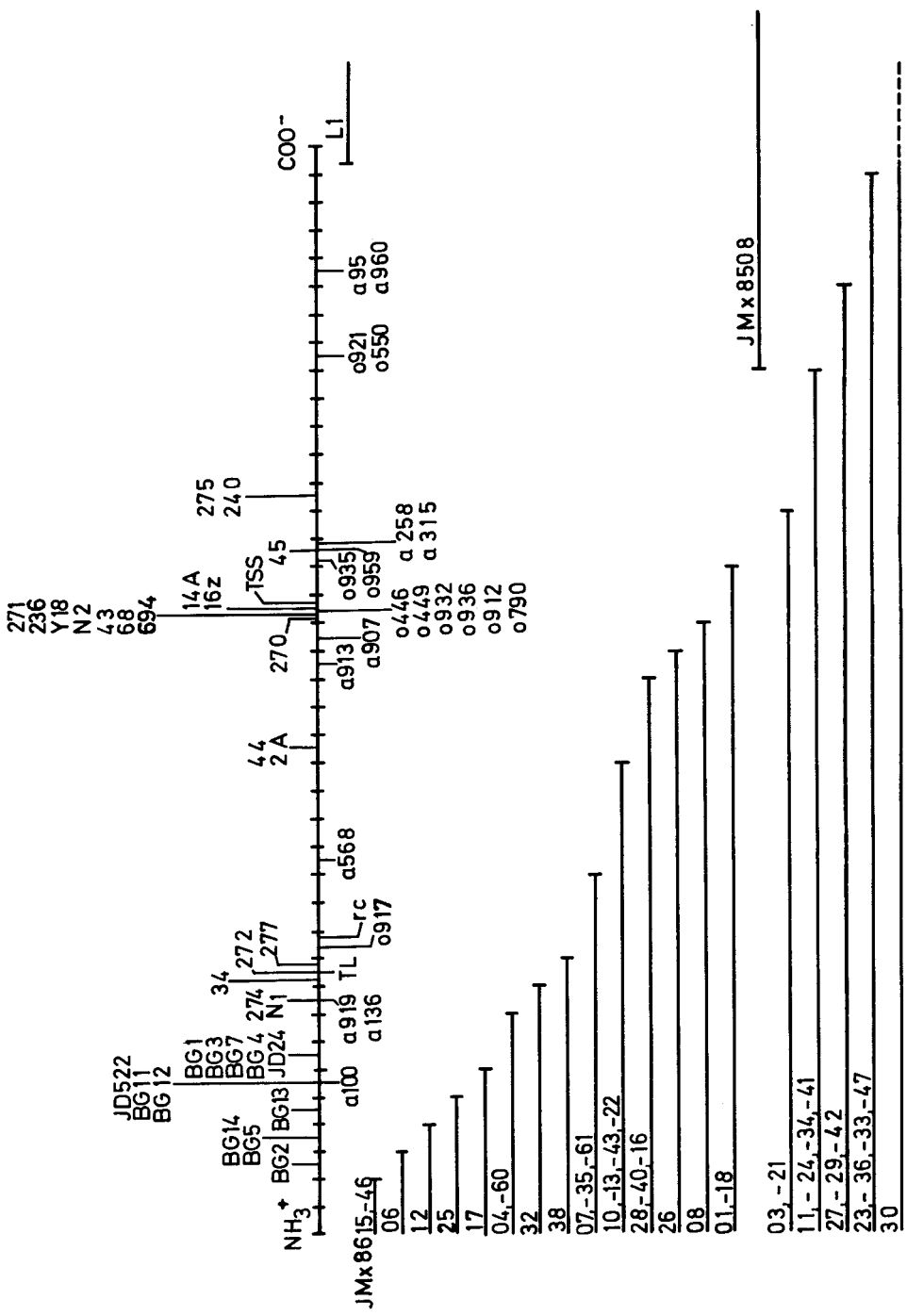
i−, i^{-d}, i^{rc} *mutants:* The procedures used were the same as for the i^s mutants, but the xgal indicator plates did not contain IPTG. The blue colonies which grew on these plates were due to the mutation on the F'*lac*+ *pro*+ episome after transfer to strain MP29.

Thermosensitive i− *mutants:* The same methods were used as before, but the xgal plates were incubated at 42°C.

Mapping: The different *i* mutants were tested for recombination with various *i* deletions. Therefore diploids of the type F'*lac-i*^{mutated}*pro*+/[*lac-pro*]Δ partial *i* deletion had to be constructed first. To do this, 1 ml of an exponentially growing donor culture (carrying the mutation to be mapped on an F'*lac*+*pro*+) episome) was mixed with the F− *i*− deletion strain. The mating mixture was incubated for 30 min at 37°C without shaking. One drop of the mixture was spread with a glass rod to give single colonies on a NM-gluc-Sm plate, which was incubated for about 36 hr at 37°C.

i^s *mutants:* A diploid colony was grown up overnight at 30°C in dyt (final titer 5 to 6×10^9). One tenth ml of 10^{-1} and 10^{-3} dilutions were spread on NM-lac-Sm-tryptophan agar (final tryptophan (try) concentration 0.02%), and 0.1 ml of the 10^{-7} dilution on NM-gluc-try-Sm agar. After two days the lac plates were screened for *lac*+ recombinants. Since the i^s mutation reverts to *lac*+ (*i*− or o^c) at an appreciable rate, a number of potential *lac*+ recombinants were tested on NM-gluc-xgal-try-Sm plates to insure that they were not constitutive revertants (REZNIKOFF *et al.* 1969). Constitutive revertants show up as deep blue colonies on this medium. When no recombinants were found, the procedure was repeated twice and each time 50 of the *lac*+ colonies were tested. The number of the colonies on the minimal gluc plates was counted to determine the titer of the diploids. To measure the sensitivity of the recombination techniques used, a diploid which had been previously shown to give no recombinants was mixed in different ratios with a diploid which carried an F' *lac*+*i*+*pro*+ episome. The screening for *lac*+ colonies was done as before.

i− *and* i^{-d} *mutants:* For selecting *i*+ recombinants, the diploid strains were mated with MP30 (F− [*lac-pro*−]Δ, *galE*−, *recA*−, $nalA^r$). One tenth ml of the 10^{-1} dilution of this mating mixture was spread on NM-gluc-PG-xgal-Nal agar and 0.1 ml of the 10^{-6} dilution on NM-gluc-Nal medium. The constitutive episomes produce β-galactosidase, hydrolyzing the non-inducing phenyl-β-D-galactosidase (PG), and yielding galactose. The galactose kills the strain since it is *galE*− (FUKASAWA and NIKAIDO 1961). Inducible recombinants have no β-galactosidase and therefore survive (DAVIES and JACOB 1968). The xgal enables one to distinguish between *i*+ recombinants and galactose resistant mutants which show up as deep blue colonies on this medium. *i*+'s are white and do not differ from *lac*− mutants, whereas $i^{q,+}$ strains are slightly blue. The white, or


JD522
BG11
BG12
BG14
BG5
NH3+
BG1
BG3
BG7
BG4
JD24
BG2
BG13
34
274
N1
272
277
TL
rc
a100
a919
a136
a568
o917
JMx8615-46
06
12
25
17
04,-60
32
38
07,-35,-61
10,-13,-43,-22
28,-40,-16
26
08
01,-18
03,-21
11,-24,-34,-41
27,-29,-42
23,-36,-33,-47
30
44
2A
270
271
236
Y18
N2
43
68
694
14A
16z
TSS
45
275
240
COO-
L1
a913
a907
o446
o449
o932
o936
o912
o790
o935
o959
a258
a315
o921
o550
a95
a960
JMx8508

the light blue colonies if i^q was used, were streaked on xgal plates with and without IPTG to determine whether they were inducible for *lac*. To get optimal transfer of episome from the diploid strain into MP30, ([*lac-pro*]Δ, *galE*⁻, *recA*, *nalA*ʳ) the following procedures were used. Diploid colonies were grown up in dyt overnight at 30°C. A very small drop of the culture was diluted in 2 ml dyt broth and incubated for another 12 hr. This subculturing was repeated once more. Then the high efficiency mating method described above was used. Synthesis of β-galactosidase escapes from repression in cells which received an *i*+ episome (PARDEE, JACOB and MONOD 1959) and induced *i*+ cells are killed on the selection medium. Therefore the mating mixture was incubated for two more hours with strong shaking before plating in order to dilute out the β-galactosidase. To verify that all of the recipients which received an *i*+ or i^{q+} episome grew on the selection medium, diploids containing an F′*lac*+*i*+ —or F′*lac*+i^{q+}*pro*+ episome were constructed and mated as described above with MP30. Suitable dilutions of the mating mixture were plated on the selective medium as well as on NM-gluc-Nal agar. To measure the sensitivity of the recombination techniques used here, a diploid, which had been previously shown to give no recombinants, was mixed in different ratios with a diploid carrying an F′*lac*+i^{q+}*pro*+ episome. The strains were mixed just before the mating with MP30 was started. Screening for *i*+'s was done as before. The two diploids were also mated individually with MP30. Suitable dilutions were spread on NM-gluc-Nal agar, to test that both strains had the same donor qualities.

Crosses of i^s *mutants against one another:* Diploids carrying one i^s mutation in the chromosome and another one in the F′*lac*+*pro*+ episome were constructed and mated with MP29 using the high efficiency mating procedure. One tenth ml of a 10^{-1} and a 10^{-2} dilution were spread on NM-lac-try-Nal agar and 0.1 ml of a 10^{-5} dilution on NM-gluc-try-Nal plates. Screening for *i*+ recombinants was done as described above.

RESULTS

Mapping of i^s *mutants:* A deletion map resulting from crosses of 20 independently isolated i^s with 39 deletion strains is shown in Figure 1. The i^s mutations are listed by number at the top, above the line indicating the genetic map. The extent of deletions is indicated below the map. Recombination data are presented in Table 2 and elsewhere (PFAHL 1971). In positive tests, the frequencies of recombination varied from 10^{-3} to 7×10^{-7} (Table 2). Control experiments indicate that recombination frequencies as low as 7×10^{-7} can be detected. One deletion (JMx8508) extends over *lac a y z o p* into the *i* gene. The other deletions extend from the *tonB* region into the *i* gene leaving *lac p o z y a* intact. Nineteen of these are *trp*⁻ (MILLER, REZNIKOFF *et al.* 1970). In these deletions transcription of the *i* gene starts from the *tonB* end (MILLER *et al.* 1968; KUMAR and SZYBALSKI 1969), allowing designation of the ends of the *i* gene responsible for the amino- and carboxyl-terminal ends of the repressor protein (Figure 1). The map is drawn to a scale that assumes the ends of deletions are spaced regularly along the length of the *i* gene, crudely approximating a random distribution.

FIGURE 1.—Map of the *i* gene obtained by crossing 57 independently isolated *i* gene mutants with 39 *tonB*⁻ *i*⁻ deletions. Deletion JMx8508 (MILLER *et al.* 1968) extends from the carboxy-terminal end into the *i* gene. The map is drawn to a scale that assumes the ends of deletions are spaced regularly along the length of the *i* gene, crudely approximating a random distribution. The i^s mutations are listed by number at the top, above the line indicating the genetic map. i^-_{sus} mutations are listed by a (amber) and o (ochre) numbers below the line, i^{-d} mutants by BG and JD numbers above the line. The extent of deletions is indicated below the line. The order of different mutations in the same regions can not be determined. Part of these deletions have been used by PLATT *et al.* 1972, to map 51 nonsense mutations.

TABLE 2

Frequency of recombination per mating of i^s *mutants with* tonB i^- *deletions*

Deletion	MPN1	MP272	MP44	MP270	MPy18	MP14A	MP45	MP240
JMx8615	$1,5 \times 10^{-3}$		$1,7 \times 10^{-3}$	$5,0 \times 10^{-4}$				$1,2 \times 10^{-3}$
JMx8616	$1,1 \times 10^{-3}$			$2,5 \times 10^{-3}$				
JMx8606	$8,5 \times 10^{-4}$							
JMx8612	$4,6 \times 10^{-4}$							
JMx8525	$1,0 \times 10^{-3}$							
JMx8517	$2,0 \times 10^{-4}$							
JMx8560	$2,5 \times 10^{-5}$	$5,0 \times 10^{-5}$						
JMx8504	$2,2 \times 10^{-6}$	$2,0 \times 10^{-6}$	$1,4 \times 10^{-3}$	$1,0 \times 10^{-4}$	$3,3 \times 10^{-3}$	$5,0 \times 10^{-4}$		$2,3 \times 10^{-3}$
JMx8532	0	$7,0 \times 10^{-7}$		$1,5 \times 10^{-3}$				
JMx8638	0	0	$2,0 \times 10^{-4}$	$1,5 \times 10^{-3}$				
JMx8607	0	0	$2,1 \times 10^{-4}$	$1,3 \times 10^{-3}$	$2,5 \times 10^{-3}$	$2,1 \times 10^{-4}$		$1,7 \times 10^{-3}$
JMx8635	0	0	$8,0 \times 10^{-4}$	$2,0 \times 10^{-3}$				
JMx8561	0	0	$2,5 \times 10^{-5}$					
JMx8610	0	0	$9,0 \times 10^{-7}$	$1,0 \times 10^{-3}$				
JMx8513	0	0	$2,5 \times 10^{-6}$	$7,0 \times 10^{-4}$				
JMx8643	0	0	$3,3 \times 10^{-5}$	$7,0 \times 10^{-4}$				
JMx8622	0	0	$6,4 \times 10^{-5}$	$7,5 \times 10^{-4}$	$1,0 \times 10^{-4}$	$3,3 \times 10^{-4}$	$1,8 \times 10^{-3}$	$3,6 \times 10^{-4}$
JMx8628			0	$2,5 \times 10^{-4}$	$5,0 \times 10^{-5}$	$3,5 \times 10^{-5}$		
JMx8540			0	$6,8 \times 10^{-4}$	$1,6 \times 10^{-4}$	$6,2 \times 10^{-4}$		$7,0 \times 10^{-4}$
JMx8616			0	$1,3 \times 10^{-4}$	$1,2 \times 10^{-4}$	$2,3 \times 10^{-4}$		$7,8 \times 10^{-4}$
JMx8525			0	$2,5 \times 10^{-4}$	$4,8 \times 10^{-5}$	$5,5 \times 10^{-5}$	$2,5 \times 10^{-4}$	$2,7 \times 10^{-3}$

TABLE 2—Continued

Deletion	MPN1	MP272	MP44	MP270	MPy18	MP14A	MP45	MP240
JMx8608			0	$2{,}1 \times 10^{-5}$	$4{,}4 \times 10^{-5}$	$1{,}1 \times 10^{-5}$		$3{,}6 \times 10^{-4}$
JMx8601	0		0	0	0	0	$1{,}4 \times 10^{-4}$	$6{,}3 \times 10^{-4}$
JMx8618	0		0	0	0	0	$2{,}0 \times 10^{-5}$	$1{,}0 \times 10^{-4}$
JMx8603			0	0	0	0	0	$1{,}4 \times 10^{-6}$
JMx8621			0	0	0	0	0	$1{,}8 \times 10^{-6}$
JMx8611				0				0
JMx8624				0				0
JMx8634				0			0	0
JMx8641				0			0	0
JMx8508							$3{,}0 \times 10^{-4}$	$2{,}0 \times 10^{-4}$
JMx8627				0	0	0	0	0
JMx8629			0	0			0	0
JMx8642			0	0	0	0	0	0
JMx8623				0			0	0
JMx8636			0	0			0	0
JMx8633			0	0			0	0
JMx8647			0	0	0	0	0	0
JMx8630				0			0	0

Frequencies of recombination for eight of the 20 i^s mutants which were crossed with 38 *tonB* i^- deletions and deletion JMx8508, which extends from *tonB* over *lac a y z o p* into the *i* gene (MILLER *et al.* 1968). $i+$ recombinants were scored as indicated in MATERIAL AND METHODS. No recombination (0) means that if $i+$ recombinants occur at all, the frequency of recombination is lower than 7×10^{-7}. Deletions and i^s mutations are ordered as in Figure 1. Frequencies of recombination for the other 12 i^s mutants are published elsewhere PFAHL 1971).

TABLE 3

Frequency of i+ *recombinants of crosses of* i^s *mutants*

	274	N1	34	272	277			
274	0	0	$1{,}5 \times 10^{-5}$		0			
N1	0	0						
34	$1{,}5 \times 10^{-5}$	$2{,}7 \times 10^{-5}$	0					
272			$1{,}2 \times 10^{-5}$	0	$3{,}8 \times 10^{-4}$			
277	$5{,}7 \times 10^{-4}$		$1{,}0 \times 10^{-5}$		0			
	14A	236	y18	N2	43	68	694	270
236		0	0					
y18		0	0	0				
N2	$1{,}0 \times 10^{-5}$	0	0	0				
43	$3{,}0 \times 10^{-5}$	0	0	0		0		
68	$2{,}1 \times 10^{-5}$	0	0	0				$5{,}6 \times 10^{-5}$
694	$3{,}1 \times 10^{-5}$	0	0	0		0		$8{,}6 \times 10^{-5}$
270	$6{,}0 \times 10^{-6}$	$6{,}4 \times 10^{-5}$	$6{,}3 \times 10^{-5}$	$1{,}1 \times 10^{-5}$	$1{,}8 \times 10^{-5}$	$1{,}8 \times 10^{-5}$		0
14A	0							
271		0	0	0	0	0		$4{,}3 \times 10^{-5}$

i^s mutations mapping in the same deletion region were crossed with each other. No recombinants (0) means that if $i+$ recombinants occur at all, the frequency of recombination is lower than 7×10^{-7}. This table shows only the numbers of the i^s mutations used in these crosses. Procedures are described in MATERIALS AND METHODS.

Mutations mapping in the same deletion region were crossed with each other. As a control experiment, each mutant was crossed with itself. Three strains (BMH270, –272, and –277) unexpectedly segregated red colonies (i^+) on control MacConkey plates; the three strains probably carry a second, wild-type copy of the *lac* genes. Therefore, the i^s mutations were transferred from the corresponding F′*lac*$^+$*pro*$^+$ episome to wild type. These new strains were numbered MP10, –11, and –12.

Some i^s mutants recombined with each other (frequency of about 10^{-5}) whereas others failed to do so. For example, BMH34, –272, and –277 are recombinationally different (Table 3). The sites of mutation within each deletion region are placed in arbitrary order on the map in Figure 1.

Repressed and induced levels of β-galactosidase for the 20 i^s mutants are shown in Table 4. Four of the 20 mutants are highly induced at the IPTG concentration used (10^{-3} M). Therefore matings using these four weak i^s mutations on the episome could not be done. "Escape" of β-galactosidase and permease from repression takes place when a *lac* episome is transferred into an i^- strain. This "escape" results in an accumulation of inducer in the cell. The inducer concentration is high enough to induce weak i^s mutants so that in these cases the *lac* system is continuously induced (NOVICK and WEINER 1957; COHN and HORIBATA 1959). Strong i^s mutants are not induced. Two of the weak i^s mutations (2A, 44) map in deletion region 18 and two (14A, 16z) map in deletion region 23. These latter

TABLE 4

β-Galactosidase levels of i^s *mutants*

Strain	β-galactosidase level uninduced	β-galactosidase level induced (IPTG 10^{-3} M)
BMH425 (wild type)	0.5	300.0
MPN1	0.3	1.3
MP274	0.8	0.8
MP34	0.9	0.9
MP272	1.8	2.1
MP277	1.3	1.0
MP2A	0.7	268.0
MP44	1.0	240.0
MP270	0.6	0.7
JM101	2.1	2.6
MP68	1.1	1.5
MP43	1.0	1.0
MPN2	1.7	2.3
MPy18	0.5	1.2
MP236	0.6	0.9
MP271	0.1	0.6
MP16z	0.9	258.0
MP14A	0.6	280.0
MP45	0.7	1.5
MP240	0.8	0.6
MP275	0.1	0.6

β-galactosidase was assayed according to PARDEE *et al.* 1959 and units are the same as in MILLER *et al.* 1968. For these assays all mutations were in a [*lac–pro*]Δ strain on an F′*lac pro*+ episome.

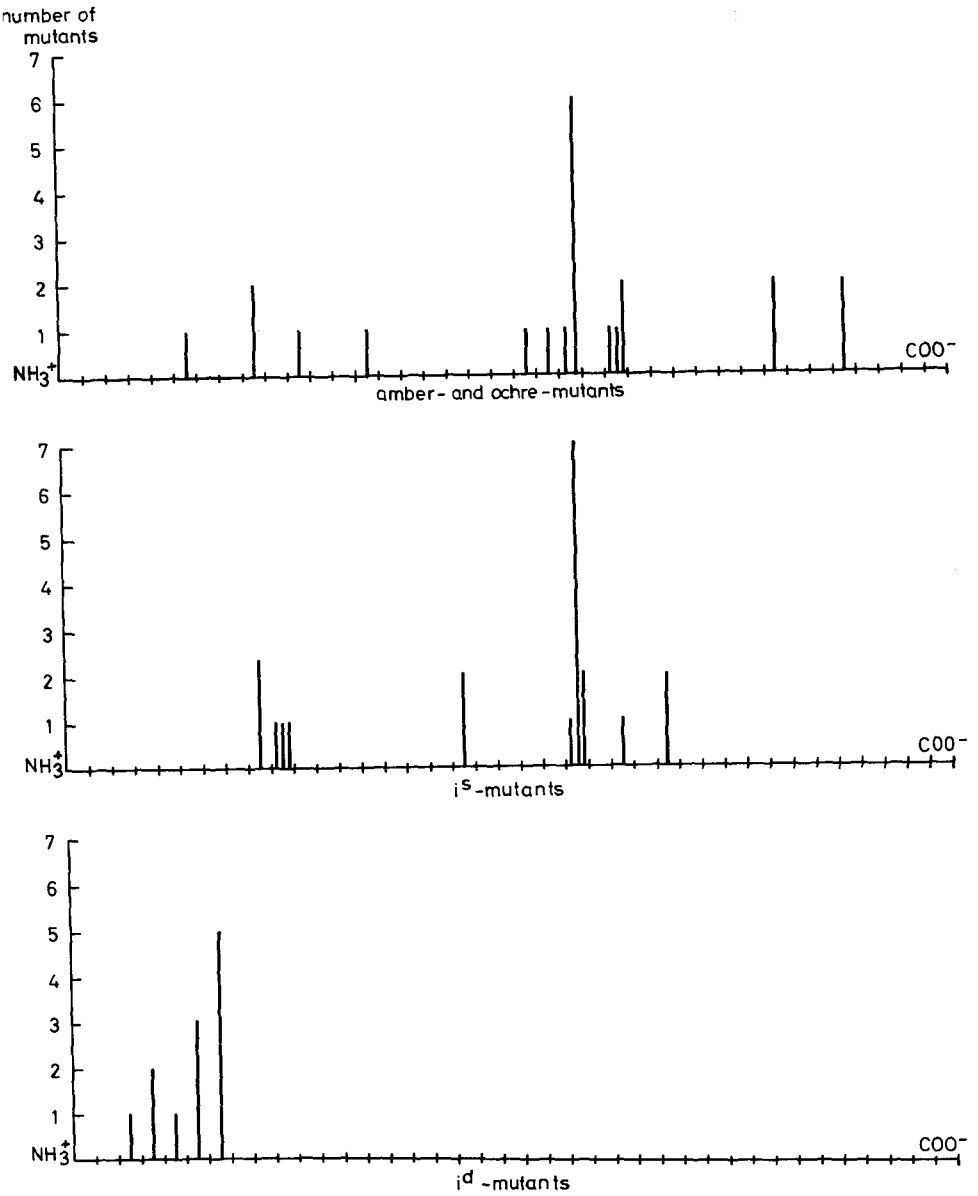


FIGURE 2.—To get a better view of which regions of the *i* gene certain mutations tend to map in, three coordinate systems are given in one figure, one for i^s's, one for i^{-d}'s, and one for i^-_{sus} mutations. The number of mutations is indicated by the ordinate and the abscissa gives the relative position of the mutation on the gene. Mutations mapping in the same region were crossed with each other. Recombinationally different mutations are drawn as separate columns. The sites of mutation within each deletion region are placed in arbitrary order. Only some of the i^{-d} mutations were crossed with each other (see RESULTS).

two mutants were isolated from one culture and are probably identical. They are shown in Figure 1 as separate from eight other mutations in region 23. Of these eight mutations, 270 recombines with the other seven which, however, fail to yield i^+ recombinants in crosses with each other (Table 3).

Mapping of i^- *mutants:* It is likely that suppressable i^- (i^-_{sus}) mutants are distributed statistically over the *i* gene. Therefore a map of them should allow one to determine whether the deletions used end only at certain preferred points in the *i* gene or whether they are distributed more evenly over the gene. 21 i^-_{sus} (i^-_o (ochre) or i^-_a (amber)) mutants obtained from J. MILLER (PLATT *et al.* 1972) were crossed against the 39 i^- deletions.

The sensitivity of the mapping system varies from 6×10^{-6} for i^+ to 6×10^{-7} for $i^{q,+}$ recombinants. The increased sensitivity for $i^{q,+}$'s is due to the fact that the "escape" from repression of the *lac* enzymes is decreased tenfold by the i^q mutation (MÜLLER-HILL *et al.* 1968; MÜLLER-HILL 1971). Ninety percent of the i^+ recombinant cells still contain high concentrations of β-galactosidase when they are plated on the selective medium. The β-galactosidase hydrolyses the PG yielding galactose, which kills the cells, since they are *galE*$^-$. Control experiments showed that 100% of the $i^{q,+}$'s survived on the selective medium, so that a tenfold lower recombination frequency could be detected (the lowest frequency of recombination detected was 9.0×10^{-7} (Table 5)).

The 21 i^-_{sus} mutants map at a minimum of 12 different points in the *i* gene (a (amber) and o (ochre) numbers below the line in Figure 1). No contradicting results concerning the endpoints of the deletions were obtained from the mapping of i^s and i^-_{sus} mutants. Seven i^-_o's map in the 23rd region, where a great number of i^s's also maps. Another but weaker "clustering" is found in the 25th region. When crossed against one another the seven i^-_o mutations in region 23 were shown to be identical. In region 25, on the other hand, it could be shown that there exist at least three different points for i^-_{sus}'s.

Mapping of the i^{TL}, i^{TSS}, i^{rc}, L1, *and 12* i^{-d} *mutants*: To map these mutants it was not necessary to cross them against all deletions, since the deletions already had been ordered. The mating results show (Figure 1) that mutant i^{TL} maps in the tenth, i^{rc} in the eleventh, and i^{TSS} in the 23rd deletion region. For these mutants the sensitivity of the system was such that recombinants could be detected at a frequency of about 6×10^{-6}, since they were not i^q. *L1*, an i^q derivative, failed to recombine only with JMx8630.

All i^{-d}'s except JD24 and -522, carried in addition the i^q mutation. The isolation and characterization of these i^{-d}'s, BG1 —BG14, will be published elsewhere (PFAHL and GRONENBORN in preparation). All twelve map within the first seven units of the *i* gene, that is near the "aminoterminal end" of the gene (BG and JD numbers above the line in Figure 1). When some of them (in region four: BG14 and BG5, in region seven: BG1, BG3, BG4, and BG7) which map in the same region were crossed against one another, no recombinants were found.

DISCUSSION

Mapping system: I have described a mapping system in which most types of

TABLE 5

Frequency of recombination per mating of i^-_{sus} *mutants with* tonB i^- *deletions*

	a100	a919	o917	a913	a907	o449	a315	a960
JMx8615	$7{,}8 \times 10^{-4}$	$1{,}2 \times 10^{-3}$				$8{,}5 \times 10^{-4}$		
JMx8646	$5{,}5 \times 10^{-4}$	$7{,}4 \times 10^{-4}$	$3{,}3 \times 10^{-4}$	$1{,}0 \times 10^{-3}$				
JMx8606	$1{,}4 \times 10^{-4}$	$2{,}5 \times 10^{-4}$						
JMx8612	$2{,}4 \times 10^{-4}$	$3{,}2 \times 10^{-4}$	$3{,}3 \times 10^{-4}$					
JMx8625	$1{,}2 \times 10^{-5}$	$4{,}1 \times 10^{-4}$	$3{,}2 \times 10^{-4}$					
JMx8617	0	$1{,}2 \times 10^{-4}$	$2{,}2 \times 10^{-4}$					
JMx8660	0	$4{,}0 \times 10^{-6}$	$1{,}2 \times 10^{-4}$					
JMx8604	0	$1{,}0 \times 10^{-5}$	$4{,}0 \times 10^{-5}$	$5{,}0 \times 10^{-4}$				
JMx8632	0	0	$5{,}0 \times 10^{-5}$	$2{,}8 \times 10^{-4}$				
JMx8638		0	$7{,}0 \times 10^{-6}$	$2{,}0 \times 10^{-4}$				
JMx8607	0	0	0	$1{,}0 \times 10^{-4}$		$2{,}2 \times 10^{-4}$		
JMx8635			0	$1{,}8 \times 10^{-4}$				
JMx8661			0	$2{,}9 \times 10^{-4}$	$1{,}6 \times 10^{-3}$	$4{,}2 \times 10^{-4}$		
JMx8610			0	$1{,}0 \times 10^{-4}$	$2{,}0 \times 10^{-4}$			
JMx8613			0	$2{,}5 \times 10^{-4}$	$2{,}8 \times 10^{-4}$			
JMx8643			0	$2{,}2 \times 10^{-5}$	$3{,}2 \times 10^{-5}$			
JMx8628			0	$2{,}8 \times 10^{-5}$	$9{,}4 \times 10^{-5}$	$2{,}6 \times 10^{-4}$		
JMx8640	0	0	0	$1{,}0 \times 10^{-4}$	$5{,}0 \times 10^{-5}$	$8{,}5 \times 10^{-5}$	$4{,}2 \times 10^{-4}$	
JMx8616			0	$2{,}3 \times 10^{-5}$	$4{,}2 \times 10^{-5}$		$2{,}3 \times 10^{-4}$	
JMx8622			0	$2{,}0 \times 10^{-5}$	$1{,}0 \times 10^{-5}$			

TABLE 5—Continued

	a100	a919	o917	a913	a907	o449	a315	a390
JMx8626				0	$5{,}0 \times 10^{-6}$	$1{,}5 \times 10^{-4}$	$7{,}2 \times 10^{-5}$	
JMx8608			0	0	0	$2{,}3 \times 10^{-5}$	$5{,}0 \times 10^{-5}$	
JMx8601	0	0	0	0	0	0	$1{,}7 \times 10^{-5}$	
JMx8618						0	$2{,}9 \times 10^{-5}$	
JMx8603						0	0	
JMx8621						0	0	
JMx8611						0	0	$1{,}0 \times 10^{-4}$
JMx8624							0	$7{,}8 \times 10^{-5}$
JMx8634							0	$7{,}3 \times 10^{-5}$
JMx8641							0	$9{,}0 \times 10^{-7}$
JMx8508							$1{,}4 \times 10^{-3}$	0
JMx8629			0	0	0	0	0	$1{,}0 \times 10^{-6}$
JMx8627								$8{,}0 \times 10^{-6}$
JMx8642								$1{,}5 \times 10^{-6}$
JMx8623								0
JMx8636								0
JMx8633								0
JMx8647				0	0	0	0	0
JMx8630								0

Frequencies of recombination for eight of the 21 i^-_{sus} mutants which were crossed with 38 *tonB* i^- deletions and deletion JMx8508. i^+ recombinants were scored as indicated in MATERIALS AND METHODS. No recombination (0) means that if i^+ recombinants occur at all, the frequency of recombination is lower than 7×10^{-7}. Deletions and i^-_{sus} mutations are ordered as in Figure 1. Frequencies of recombination for the other 13 i^-_{sus} mutants are published elsewhere: (PFAHL 1971).

i gene mutants known so far can be tested for recombination with a set of i^- deletions. Methods were developed, which allow one to pick up very low frequencies of i^+ recombinants from crosses involving most types of mutants. I divided the *i* gene into 39 regions in 18 of which I mapped one or several mutations. Among 57 mutants 31 could be shown to be different (Figure 1). A "hot-spot" is found in region 23 for two different types of mutants. In this case a single triplet might, by a special mutation, result in an i^- mutant and by any other change in an i^s mutant. A weak proof for this hypothesis is the fact that an i^s revertant from JM446 was found (personal communication by J. H. MILLER).

Mutations were found to map in only 18 out of 39 regions. This is probably not due to a large proportion of the deletions being identical, since out of the 57 mutants I mapped, only some, the 21 i^-_{sus} mutants, may be considered to be distributed randomly over the gene. The results indicated that only 12 of these 21 i^-_{sus} mutants were different. These 12 map in ten different regions, whereas ten different i^s mutants map in only six different regions. A greater number of i^-_{sus}'s would be desirable in order to determine the endpoints of those deletions which I was not able to order. Figure 2 gives a summary of the mapping data for the three main groups (i^{-d}, i^s, and i^-_{sus}) of mutants I examined. Work in progress (PFAHL and GRONENBORN) has confirmed that mutants of the first two groups occur only in some regions of the gene.

i^s *mutants*: Of the 20 i^s's I mapped, 16 were strong i^s mutants. These map at a minimum of eight different points in two main regions, one near the aminoterminal end, the other throughout the middle of the gene. Provided all these mutants have the characteristics so far described for i^s mutants: decreased affinity to the inducer, normal affinity to the operator, and recessivity to i^q (still able to form oligomers), then one might suppose that an i^s mutant carries a defect which alters only the inducer binding site of the repressor. However JOBE *et al.* (1972) have shown that on the basis of in vitro behavior at least two main types of i^s's exist. One, the major group, has an altered affinity for inducer, while mutations in the other group synthesize a repressor which in addition has altered allosteric and or operator-binding properties. Mutants of the first group map in unit 23, implying that this region codes for at least one essential part of the inducer binding site. Only the mutants, SBOUN1 and SBOU45 were previously known to be in the second group. They map in the eighth and 25th unit. Two other mutants, BMH272 and -277, which map near SBOUN1, were sent to S. BOURGEOIS for *in vivo* investigation. BMH272 behaves similar to SBOUN1 while BMH277 has only an altered affinity for the operator (JOBE, RIGGS and BOURGEOIS 1972). These results suggest that at least a large proportion of the i^s mutants which map near the aminoterminal end of the *i* gene synthesizes a repressor which is "frozen" in the operator-binding conformation. I suggest that in regions nine and ten amino acids are coded which are important for allosteric properties of the repressor and allow it to assume either the inducer or the operator-binding conformation.

i^{-d} *mutants*: In this paper I described the mapping of twelve i^{-d} mutants. A more detailed investigation of i^{-d} mutants will be published elsewhere (PFAHL and GRONENBORN in preparation). Characteristically i^{-d} mutants lack operator

binding but retain the capacity to bind inducer and to form oligomers. Together with their map position the properties of i^{-d} mutants have led to a model of repressor-operator interaction (ADLER *et al.* 1972).

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