

DELETION MAPPING OF *zwf*, THE GENE FOR A CONSTITUTIVE  
ENZYME, GLUCOSE 6-PHOSPHATE DEHYDROGENASE IN  
*ESCHERICHIA COLI*

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ABSTRACT

Genes for three enzymes of intermediary sugar metabolism in *E. coli*, *zwf* (glucose 6-phosphate dehydrogenase, constitutive), *edd* (gluconate 6-phosphate dehydrase, inducible), and *eda* (2-keto-3-deoxygluconate 6-phosphate aldolase, differently inducible) are closely linked on the *E. coli* genetic map, the overall gene order being *man . . . old . . . eda . edd . zwf . . . cheB . . . uvrC . . . his*. One class of apparent revertants of an *eda* mutant strain contains a secondary mutation in *edd*, and some of these mutations are deletions extending into *zwf*. We have used a series of spontaneous *edd-zwf* deletions to map a series of point mutants in *zwf* and thus report the first fine structure map of a gene for a constitutive enzyme (*zwf*).

THREE genes for enzymes of central intermediary metabolism in *Escherichia coli* are at about 35 min. on the chromosome: *zwf edd* and *eda* (Figure 1). *zwf* is the gene for glucose 6-phosphate dehydrogenase, the first enzyme of the hexose monophosphate shunt (Figure 2). The locus was defined by deficiency mutations (FRAENKEL 1968) and is known to be the structural gene for the enzyme. The gene is expressed constitutively and we recently described a possible "up-promoter" mutation of it, *zwfL1*, closely linked to *zwf* (FRAENKEL and BANERJEE 1971). *Edd* (Entner-Doudoroff dehydrase) is the gene for gluconate 6-phosphate dehydrase, the first enzyme of the Entner-Doudoroff pathway (Figure 2). The enzyme is inducible, being found in high activity in cells grown on gluconate (EISENBERG and DOBROGOSZ 1967; FRAENKEL and LEVISOHN 1967). The locus was also defined by deficiency mutations (ZABLOTNY and FRAENKEL 1967) and no information is yet available on whether it is the structural gene for the enzyme. *Eda* is the gene for 2-keto-3-deoxygluconate 6-phosphate (KDGP) aldolase, the second enzyme of the Entner-Doudoroff pathway. This enzyme is also inducible, but differently from *edd*, being found in high level in cells grown on gluconic or on uronic acids (POUYSSÉGUR and STOEBER 1971; FRADKIN and FRAENKEL 1971). The locus was defined by deficiency mutations (FAIK, KORNBERG and McEVOY-BOWE 1971; FRADKIN and FRAENKEL 1971; POUYSSÉGUR 1971) and is the structural gene for the enzyme (POUYSSÉGUR and STOEBER 1972).

The main purpose of the present report is to describe a deletion analysis of

the *eda edd zwf*-cluster and the first fine structure genetic map for a constitutive enzyme of *E. coli zwf*. We also show that the overall order of genes is *man* . . . *oldD* . . . *eda. edd. zwf* . . . *cheB* . . . *uvrC* . . . *his*.

#### MATERIALS AND METHODS

*Strains and scoring:* Bacterial strains are listed in Table 1, and the positions of markers on the *E. coli* map are shown in Figure 1. Media, growth conditions, enzyme assays, and methods of conjugation and transduction were described earlier (FRAENKEL 1967; FRAENKEL and LEVISOHN 1967; FRANKLIN and FRAENKEL 1971). The gene this paper is mainly concerned with is *zwf* (glucose 6-phosphate dehydrogenase). *Zwf* mutants are only easily recognizable in strains also containing a *pgi* (phosphoglucose isomerase) mutation, since *zwf pgi*<sup>-</sup> strains are unable to grow on glucose while *zwf pgi*<sup>+</sup> strains do grow on glucose (FRAENKEL 1968). Thus, in this paper the *zwf* point mutants and deletions were selected in strains carrying a *pgi* mutation, and direct selection of intragenic recombination between *zwf* mutants could thus be done on glucose minimal medium. *Edd* (gluconate 6-phosphate dehydrase) mutants grow slowly on gluconate (using the hexose monophosphate shunt); the mutation is also recognizable on tetrazolium-gluconate plates (PEYRU and FRAENKEL 1968). *Eda* (KDGP aldolase) mutants fail to grow on glucuronate or gluconate (FRADKIN and FRAENKEL 1971), and *eda*<sup>+</sup> recombinants were selected directly on minimal medium containing glucuronate as sole carbon source; (this selection may not be done on gluconate since reversion to growth on gluconate is frequent and occurs by *edd* mutation—see text). *CheB* and *uvrC* were isolated as in ARMSTRONG and ADLER (1969). *OldD* was scored on minimal medium containing palmitic acid (OVERATH, PAULI and SCHAIER 1969). *Man*<sup>+</sup> was selected on minimal medium containing mannose (TAYLOR and TROTTER 1967).

*Selection of zwf point mutants:* *Zwf* point mutants were selected in DF44 (HfrH *pgi*<sup>-</sup> *str*<sup>s</sup>) as described previously for the selection in HfrC (FRAENKEL 1968), but using ultraviolet mutagenesis and intermediate growth in minimal medium with mannitol as sole carbon source. Penicillin selection was done in minimal medium with glucose and survivors were grown on mannitol plates and tested on glucose. Isolates which did not grow on glucose were generally found to be *zwf*<sup>-</sup>, by enzyme assay. Only a single *zwf*<sup>-</sup> isolate was saved from each selection (strains DF44*zwf*25 through DF44*zwf*53).

*Selection of deletions:* DF1671 (*pgi*<sup>-</sup> *eda*<sup>-</sup>) was grown from single colonies to full turbidity in broth and 0.1 ml portions of the turbid culture, as well as of 1:10 and 1:100 dilutions of it, were spread on minimal-gluconate plates containing histidine and streptomycin. The revertant frequency varied between different cultures, but was typically 10<sup>-4</sup>. Revertants were spotted to

TABLE 1  
*Bacterial strains (E. coli K-12)*

Strain	Genotype	Reference or source
AW7	F- <i>arg</i> <sup>-</sup> <i>thr</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>pro</i> <sup>-</sup> <i>his</i> <sup>-</sup> <i>thi</i> <sup>-</sup> <i>cheB</i> 590 <i>uvrC</i> <sup>-</sup>	From PARKINSON and ADLER
CA8000	HfrH <i>thi</i> <sup>-</sup> <i>str</i> <sup>s</sup>	From J. R. BECKWITH
DF10	HfrC <i>edd-1 str</i> <sup>s</sup>	PEYRU and FRAENKEL 1968
DF40	HfrC <i>pgi-2 str</i> <sup>s</sup>	FRAENKEL and LEVISOHN 1967
DF44	HfrH <i>pgi-2 str</i> <sup>s</sup>	See text
DF44 <i>zwf</i> 25, etc.	( <i>zwf</i> point mutants in DF44)	See text
DF71	HfrH <i>eda-1 str</i> <sup>s</sup>	FRADKIN and FRAENKEL 1971
DF1671	F- <i>his</i> <sup>-</sup> <i>pgi-2 eda-1 str</i> <sup>r</sup>	FRADKIN and FRAENKEL 1971
DZ1, etc.	( <i>edd-zwf</i> Δ's in DF1671)	See text
DF2000	HfrC <i>pgi-2 zwf-2 str</i> <sup>s</sup>	PEYRU and FRAENKEL 1968
K63	F- <i>his</i> <sup>-</sup> <i>oldD</i> 88 <i>man-1 str</i> <sup>r</sup>	POUYSSÉGUR 1971 (from P. OVERATH)

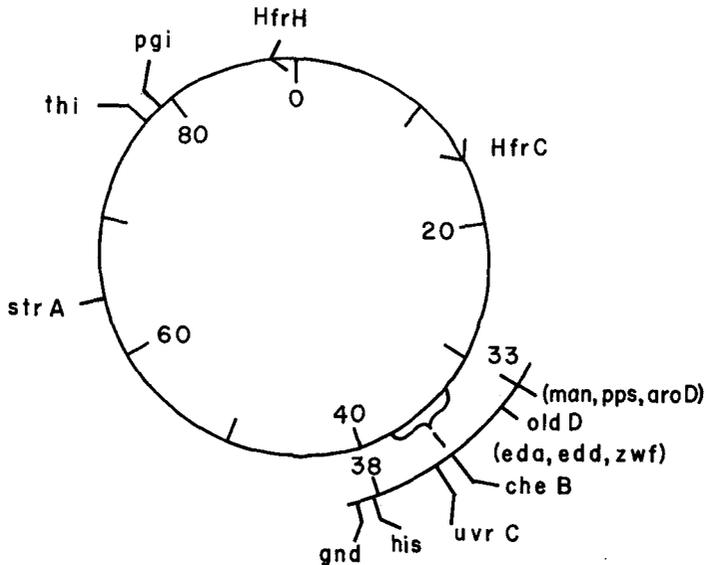


FIGURE 1.—The *E. coli* genetic map (after TAYLOR 1970), and see text.

glucose-minimal plates (with histidine and streptomycin), and apparent glucose-negatives were purified and retested. Almost all such isolates proved, upon assay, to now lack both glucose 6-phosphate dehydrogenase and gluconate 6-phosphate dehydrase; they also still were *eda*-negative (failed to grow on glucuronate). A few of the isolates were selected on tetrazolium gluconate plates, since the parental strain DF1671 (*eda*<sup>-</sup>) is inhibited by gluconate in rich medium, while the *edd*<sup>-</sup> derivatives are not (FRADKIN and FRAENKEL 1971). The deletion strains DZ1-DZ61, thus, are (*F*<sup>-</sup> *pgi*<sup>-</sup> *eda*<sup>-</sup> *edd-zwf*Δ *his*<sup>-</sup> *str*<sup>r</sup>). Most were obtained in independent selections and no mutagen was used.

**Deletion mapping:** Each of the *zwf* point mutants (DF44*zwf*25, etc.) was crossed with the deletion strains (DF1671, DZ1, etc.) by the following technique. The strains were inoculated from single colonies and grown to full turbidity in broth containing gluconate (the donors without aeration and recipients with aeration (CURTISS *et al.*, 1969); the donors were then subcultured 1:10, and the recipients 1:40, and grown 2.5 hr. Matings were done using 95 cm aluminum plates containing an array of 20 wells of 1 cm diameter. Each well was inoculated with 0.25 ml fresh gluconate broth, 0.025 ml of the donor, and 0.02 ml of the recipient culture. A typical plate contained 16 different crosses (e.g. 16 different *zwf* point mutants with the same deletion strain); one well contained the recipient only. Before addition of the recipient strain the array of donors alone was also spotted to the selective plates, as another control. The mating mixtures were incubated at 37°C for 4 hr, and spotted, using a metal replicator, to plates selective for *zwf*<sup>+</sup> recombinants (glucose-minimal with streptomycin), and *his*<sup>+</sup> recombinants (glycerol-minimal with streptomycin). The plates were scored after 48 hr at 37°C. All crosses gave confluent spots on *his*<sup>+</sup>-selective plates. On the plates selective for *zwf*<sup>+</sup> recombination, when recombination occurred small discrete colonies were visible on the spot; their number was estimated by inspection with a dissecting microscope, and ranged from ten to hundreds (confluence). Non-recombining crosses gave zero colonies, or occasionally, one or two revertants. In a cross giving *zwf*<sup>+</sup> recombinants, the number of recombinants was roughly proportional to the distance between the point mutant and the deletion, as finally derived; although recorded, these values were not used in constructing the map, which was made from the “+” and “-” tallies alone. Any cross giving *zwf*<sup>+</sup> recombinants also gave *zwf*<sup>+</sup> *his*<sup>+</sup> recombinants, but usually fewer. Any crosses giving exceptional results (e.g. some, but too few colonies to be scored as “+”) were

repeated, as were crosses where reversion of *pgi* or *zwf* occurred in one of the parents. (*Pgi* reversion was evident on inspection, since such colonies were much larger than *zwf*<sup>+</sup> *pgi*<sup>-</sup> colonies.) In some of the crosses the scoring was certified by enzyme assay of some recombinants.

#### RESULTS AND DISCUSSION

1. *The overall order of the genes:* *Zwf* and *edd* were known to be closely linked (97%) in transduction with phage P1, and to lie between the cluster of genes at 33 min (which includes *aroD pps* and *man*) and the *his* operon at 38 min (PEYRU and FRAENKEL 1968). *Eda* and *zwf* were shown to be closely linked (98%) in conjugation (FRADKIN and FRAENKEL 1971). *Eda* and *edd* are closely linked (95%) in transduction (FAIK, KORNBERG and MCEVOY-BOWE 1971; POUYSSEGUR 1971), and *eda* cotransduced (16%) with *oldD*, the overall gene order being *man . . . oldD . . . (eda edd) . . . his*, with the genes in parentheses probably being in the order given (POUYSSEGUR 1971). We also have found cotransduction of *eda* with *edd* and with *zwf*. Thus, with DF1671 (*pgi*<sup>-</sup> *eda*<sup>-</sup>) as recipient, transduction of *eda*<sup>+</sup> (ability to grow on glucuronic acid) from DF10 (*edd*<sup>-</sup>) gave 92% linkage (126/137 recombinants inherited *edd*<sup>-</sup> from the donor). When the donor was DF2000 (*zwf*<sup>-</sup>) with the same recipient there was 95% linkage with *zwf* (105/110 recombinants inherited *zwf*<sup>-</sup> from the donor). We have also confirmed, by conjugation of DF71 (HfrH *eda*<sup>-</sup>) with K63 (F<sup>-</sup> *man*<sup>-</sup> *oldD*<sup>-</sup> *his*<sup>-</sup>) the gene order *man . . . oldD . . . eda . . . his*. *Eda edd* and *zwf* are a closely linked cluster and we will show their relative order in section 3.

ARMSTRONG and ADLER (1969) ordered several genes counterclockwise to *his* on the *E. coli* chromosome (*aroD . . . cheB . . . cheA . . . mot . . . uvrC . . . his*). We now show that the *eda edd zwf*-cluster lies slightly counterclockwise from *cheB*. Thus, with DF1671 (*eda*<sup>-</sup>) as recipient, when the donor phage was grown on strain AW7 (*cheB*<sup>-</sup> *uvrC*<sup>-</sup>), with selection of *eda*<sup>+</sup> there was 17% linkage with *cheB* (32 *cheB*<sup>-</sup> out of 192 recombinants), and none of these recombinants had inherited *uvrC*<sup>-</sup>. (*CheB* and *uvrC* were 57% linked in ARMSTRONG and ADLER's experiments (1969).) In order to look for very low cotransduction of *eda* and *uvrC* we repeated the transduction, but screened *eda*<sup>+</sup> transductants for inheritance of *uvrC*<sup>-</sup> by directly patching the recombinants to tryptone-yeast extract plates for the ultraviolet sensitivity test (instead of first purifying them on the selective media): likely isolates were purified and retested. In this way, we found that 1 out of 750 *eda*<sup>+</sup> transductants had inherited *uvrC*<sup>-</sup>; this strain was also *cheB*<sup>-</sup>. The overall order of genes is, therefore, (*pps man*) . . . *oldD* . . . (*eda edd zwf*) . . . *cheB. cheA . . . uvrC . . . his*.

2. *Edd deletions:* KDGP aldolase is on the pathway of glucuronic acid degradation (Figure 2), and, thus, *eda* mutants are unable to grow on this compound. They also do not grow on gluconic acid, a surprising result, since the hexose monosulfate shunt is still present for the metabolism of gluconate 6-phosphate, and *edd* mutants do grow on gluconate using the shunt. The inability of *eda* mutants to grow on gluconate is related to their accumulation of KDGP, which is toxic to growth, and it has been shown that reversion of an *eda* mutant to the ability to grow on gluconate, without recovery of ability to grow on glucuronate,

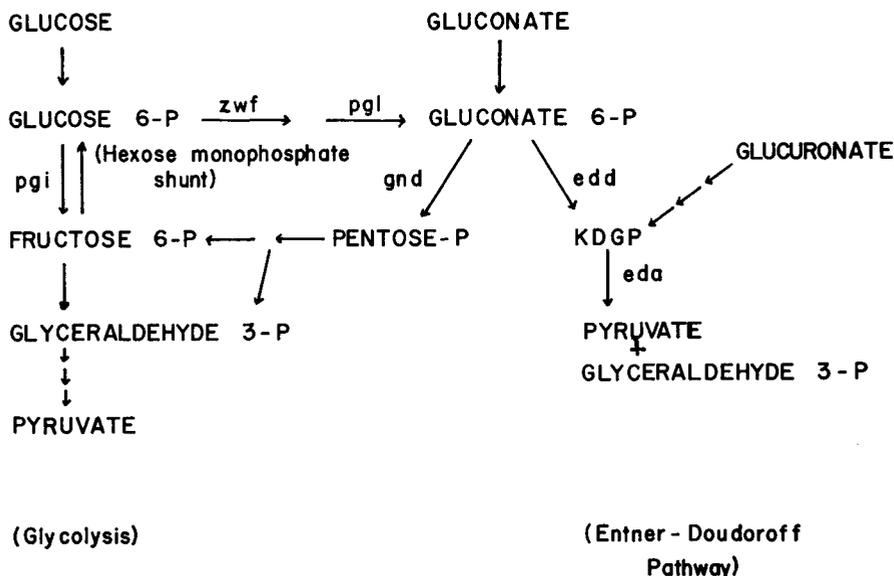


FIGURE 2.—Pathways of sugar metabolism in *E. coli*. Gene symbols are *pgi* (phosphoglucose isomerase), *zwf* (glucose 6-phosphate dehydrogenase), *pgl* (6-phosphogluconolactonase), *gnd* (gluconate 6-phosphate dehydrogenase), *edd* (gluconate 6-phosphate dehydrase), and *eda* (2-keto-3-deoxygluconate 6-phosphate (KDGP) aldolase).

can occur by *edd* mutation (POUYSSÉGUR and STOEBER 1971; FRADKIN and FRAENKEL 1971). Such reversion is very frequent. Thus, typically, a culture of strain DF1671 (*pgi<sup>-</sup> eda<sup>-</sup>*) after growth in broth (not containing gluconate) contains about  $10^{-4}$  gluconate-positive revertants, while the frequency of glucuronate positive (*eda<sup>+</sup>*) revertants is *ca.*  $10^{-8}$ . All revertants of the frequent type have been found, by enzyme assay, to lack both the aldolase and the dehydrase (i.e. to be *eda<sup>-</sup> edd<sup>-</sup>*). (There seems to be strong selective pressure for loss of the dehydrase (*edd*) in the aldolase mutant (*eda*) even without gluconate in the culture medium; this suggests that some KDGP may be formed from gluconate 6-phosphate by the uninduced basal level of the dehydrase.)

A system generating spontaneous *edd* mutations might be useful for *zwf* genetics because some spontaneous mutations are deletions, and *edd* and *zwf* are very closely linked. Thus, if no essential genes intervened, and deletion of the *zwf* gene were tolerable, then some of the *edd* mutations might delete *zwf*. This was directly examined in strain DF1671, which carries a mutation in phosphoglucose isomerase (*pgi*) as well as in *eda*. Growth on glucose of this strain is restricted to the hexose monophosphate shunt, so any *zwf<sup>-</sup>* derivative would not grow on glucose at all. We selected (MATERIALS AND METHODS) gluconate revertants of DF1671 and screened them for growth on glucose; typically (with wide variation between different cultures) 1 in 200 revertants was glucose negative and was found, by assay, to lack both gluconate 6-phosphate dehydrase and glucose 6-phosphate dehydrogenase. We thus collected about 60 derivatives of DF1671 carrying (presumed) *edd-zwf* deletions (strains DZ1 through DZ61).

3. *Extent of deletions and gene order*: The overall gene order determined above was *oldD* . . . (*eda edd zwf*) . . . *cheB* (with genes in parentheses unordered). The deletion strains were all assayed for chemotaxis and none were *che*<sup>-</sup>; thus, none of the deletions extends into *cheB*. In addition, the cotransduction frequency of *cheB* and *eda*, cited above as 12% in the cross P1 (AW7 (*cheB*<sup>-</sup>)) × DF1671 (*eda*<sup>-</sup>) was not markedly different in the deletion strains (e.g. 13 *che*<sup>-</sup>/103 with DF1671DZ20 and 13 *che*<sup>-</sup>/113 with DF1671DZ1). There are probably many genes between *zwf* and *cheB* and some may be essential.

We have also found that deletion from *edd* into *zwf* does not usually delete the entire *eda* gene itself. The direct way to determine this would be to measure the frequency of *eda*<sup>-</sup> to *eda*<sup>+</sup> (reversion to growth on glucuronate) in the *eda*<sup>-</sup> *edd-zwf*Δ strains. Unfortunately, the reversion frequency in the parental strain DF1671 (*eda*<sup>-</sup>) is so low (*ca.*10<sup>-8</sup>) as to make comparison with the frequency in the deletion strains difficult. Instead, we have transduced *eda*<sup>+</sup> from an *eda*<sup>+</sup> *edd*<sup>+</sup> *zwf*<sup>+</sup> strain (AW7) into some of the deletion strains (*eda*<sup>-</sup> *edd-zwf*Δ) and scored recombinants for inheritance of *zwf*; any *zwf*<sup>-</sup> recombinants must have inherited *zwf*<sup>-</sup> (the deletion) from the recipient strain, and there would necessarily have been a crossover between the deletion and the *eda*<sup>+</sup> of the donor. In several such crosses *zwf*<sup>-</sup> recombinants were found (DZ15, 3 *zwf*<sup>-</sup>/100; DZ22, 9 *zwf*<sup>-</sup>/500; DZ46, 11 *zwf*<sup>-</sup>/500). Thus, at least in these strains the deletion did not cover the original *eda*<sup>-</sup> mutation, and therefore, *eda* cannot be between *edd* and *zwf*. (We also note that in one strain, DZ1, we found no (0/2500) *zwf*<sup>-</sup> recombinants in such a cross, so it is possible that this deletion covers the original *eda* point mutation.)

The relative order of *eda edd* and *zwf* could also be determined. Because of the results cited immediately above, the four possible orders, with respect to *oldD* on the left and *cheB* on the right, are *eda.edd.zwf*, *eda.zwf.edd*, *edd.zwf.eda*, and *zwf.edd.eda*. POUYSSEUR's (1971) transduction results fitted best the order *oldD..eda.edd*, which would leave only the first two possibilities. In fact, of the four, only the first is in accord with the result of the following experiment. We show below that some of the deletions recombine with *zwf* point mutations. If such recombination occurs by a single crossover between them, with the donor *eda*<sup>+</sup>, *edd*<sup>+</sup> *zwf*<sup>-</sup> *his*<sup>+</sup> and recipient (e.g. DZ15) *eda*<sup>-</sup> *edd-zwf*Δ *his*<sup>-</sup> with the order as given, *zwf*<sup>+</sup> recombinants would usually be *eda*<sup>+</sup> and *his*<sup>-</sup>; none of the other orders would give this as majority class. In fact, of the first 46 glucose recombinants collected from three crosses with DZ15 (the males carried the point mutations *zwf*-27 *zwf*-33 and *zwf*-38), all grew on glucuronate (were *eda*<sup>+</sup>) and 39/46 were *his*<sup>-</sup>. The gene order of the cluster is thus *oldD..eda.edd.zwf...cheB*.

4. *Selection of zwf point mutants*: The known *zwf* point mutants were obtained as derivatives of strain DF40 (HfrC *pgi*<sup>-</sup> *zwf*<sup>+</sup>) completely unable to grow on glucose (FRAENKEL 1967). Since *zwf* is a late marker in HfrC and considerably distal to the streptomycin sensitivity locus most conveniently used for counter-selection in matings, we have selected the new *zwf* point mutants in HfrH. CA8000 (HfrH *thi*<sup>-</sup>) was transduced to thiamine independence with phage P1



it and none ending to the right. Thus, there seems to be only a single locus in *E. coli* for glucose 6-phosphate dehydrogenase and mutations may be mapped linearly on it.

*E. coli* glucose 6-phosphate dehydrogenase contains only one size of polypeptide chain (52,000 daltons, BANERJEE and FRAENKEL 1972), and there is no present reason to suppose that the *zwf* gene is more than one cistron; direct proof of this point is not yet available. The point mutants have not been ordered within each group. We also note that although most are known to revert (unpublished results), it is possible that some of the "point mutants" are actually deletions extending to the right.

One type of error in the map of Figure 3 would have been caused by failure to observe recombination even though a particular point mutation was not covered by a deletion. There is as yet no independent direct estimate of distance in this system (e.g. positioning of mutations in the protein). We have measured frequency of intragenic recombination with respect to frequency of recombination with an outside marker for some of the pairs. In the cross *zwf*47 × DZ15 (which are separated by most of the gene), *zwf*<sup>+</sup> *his*<sup>+</sup> recombinants, occurred at about 0.1% the frequency of *his*<sup>+</sup> recombinants.

We show elsewhere (FRAENKEL and PAROLA 1972) that the "up-promoter" mutations (e.g. *zwf*L1, FRAENKEL and BANERJEE 1971) lie in group (1) of the map.

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