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# Inhibition of *lacZ* Gene Translation Initiation in *trp-lac* Fusion Strains

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Different levels of β-galactosidase are found in various *trp-lac* fusion strains. These levels of β-galactosidase fall within a 60-fold range. The amount of thiogalactoside transacetylase activity detected in these same strains only varies 10-fold and is found in amounts greater than those predicted from the β-galactosidase levels. The observation that the β-galactosidase and thiogalactoside transacetylase levels are not directly proportional, that the *lacZ* messenger ribonucleic acid (mRNA) levels are not proportional to the β-galactosidase activity, that, at least for the one fusion strain tested, the SuA polarity suppressor does not affect the β-galactosidase level, and that, in all but one strain, the β-galactosidase activity appears to reside in normal β-galactosidase molecules suggests that the disproportionately low production of β-galactosidase is due to a decrease in the frequency of translation initiation of *lacZ* mRNA in these strains. Several mechanisms are proposed to explain this decrease. Some possible bases for the disproportional production of β-galactosidase and thiogalactoside transacetylase are also described. The preferred explanation for these disproportional enzyme levels is that only a fraction of the full complement of ribosomes need initiate translation at *lacZ* for the functional synthesis of *lac* mRNA to occur and that once the *lac* ribonucleic acid is made a full complement of ribosomes can bind at internal translation initiation sites at *Y* and *A*.

Gene expression is dependent in part upon the process of translation. The initiation of this process is known to require an appropriate initiation sequence (or ribosome binding site) in the messenger ribonucleic acid (mRNA) containing at least an AUG codon. Experiments with several systems suggest that the existence of a ribosome binding site sequence per se is not sufficient to result in initiation of translation. Rather, the ability of this sequence to be recognized by ribosomes is affected by the structure of neighboring mRNA sequences. For instance, the ribosome binding site for the R17 replicase gene does not appear to be functional unless exposed by translation of the coat protein gene (5, 7, 12, 31), and there exist internal translation initiation sites within the *lacI* gene that only function in the presence of a preceding nonsense codon (20). Thus, it might be possible to find mutations in which the efficiency of translation initiation for a particular gene is altered due to an alteration in the structure of neighboring mRNA sequences.

Recently a large collection of *trp-lac* fusion deletions have been isolated, all of which have the same property; they fuse all of the *lac* structural genes to the tryptophan (*trp*) operon. Many of these deletions end within the *lac*-controlling element region (between *I* and *Z*) (23, 24; W. Jürgen Schrenk, Ph.D. thesis, Universität zu Köln, 1972; and D. Mitchell, W. S. Reznikoff, and J. R. Beckwith, manuscript in preparation) and therefore result in the presence of new mRNA sequences near the *lacZ* ribosome binding site. The experiments described in this paper show that, although the *lac* structural genes in these deletion strains are in all cases fused to the same transcription unit acting at the same level (the genetically derepressed *trp* operon), they manifest an apparent 60-fold range in levels of β-galactosidase production. It is proposed that most of the strains

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produce different levels of β-galactosidase because initiation of *lacZ* translation occurs at different efficiencies in the various strains. (This generalization may only apply to those strains in which the fusion deletions end within the *lac*-controlling element region. Recent studies have shown that two of the strains studied in these experiments [F101 and F104] contain *trp-lacI* deletions in combination with the *lac* promoter mutation L8 [Mitchell et al., manuscript in preparation]. In these strains the low level of *trp*-controlled *lacZ* expression may be due to two phenomena: the termination of some of the *trp*-initiated transcription at the end of the *I* gene, and inefficient initiation of *lacZ* translation on the fused mRNA that is made.)

For many operons in *Escherichia coli*, synthesis of detectable mRNA seems to be dependent upon the concomitant translation of the mRNA into proteins (4, 11). Two general hypotheses have been advanced to explain this phenomenon. Ribonucleic acid (RNA) polymerase may require ribosomes to be actively reading the message in order to progress (8) or the mRNA which is produced in the absence of translation may be rapidly degraded by a combined endonucleolytic and exonucleolytic attack (17, 18). Whichever hypothesis is correct, it is possible that only a small fraction of the wild-type ribosome complement is sufficient to permit the successful propagation of mRNA. As will be shown in this paper, the *trp-lac* fusion strains manifest a second characteristic that suggests that this is in fact the case. β-Galactosidase and thiogalactoside transacetylase are not produced in these strains in the same proportions as in the wild-type strain. Rather, β-galactosidase is produced in reduced amounts relative to the level of thiogalactoside transacetylase (and *lacZ* and *-A* mRNA). The simplest explanation for this observation is that only a small fraction of the normal ribosome complement must initiate translation at the beginning of *lacZ* for *lac* mRNA to be successfully propagated through to *lacA* where a normal complement of ribosomes could then initiate *lacA* translation.

## MATERIALS AND METHODS

**Strains.** The *trp-lac* fusion strains all have the same genotype, except for the particular deletion ends. All of these strains are $F^-$, $Su^-$. They carry the *lac-pro A,B* deletion XIII, a *trpR*$^-$ allele, a *strA* marker, a φ80d*lac* prophage, and the *tonB* deletions shown in Fig. 1. The *lac* end of these deletions can be categorized as type 1 (those ending in *lacI* in which *lac* expression results from transcription initiation at *lacP*), type 2 (those ending in *lacI* in which *lacP* activity is reduced due to the presence of the L8 promoter mutation; in these strains most of the *lac* expression results from *trp*-initiated transcription), type 3 (those ending within the promoter), type 4 (those ending between the promoter and the operator), and type 5 (those deleting the operator but presumably ending before *Z*). The isolation and characterization of these deletions has been described previously (16, 23, 24; Schrenk, Ph.D. thesis; and Mitchell et al., manuscript in preparation).

The *trpR*$^-$ $SuA^+$ and $SuA^-$ strains were constructed by mating an Hfr Cavalli containing the *trpR*$^-$ marker with an $F^-SuA^+$, *strA*, Δ(*lac-proA,B*) XIII strain and selecting for 5-methyltryptophan-resistant, streptomycin-resistant progeny. The purified recombinants were screened for their Lac character on Lac McConkey plates. The $Lac^-$ recombinants were tested for their SuA character by introducing an F'*lac-proA,B* episome carrying the early *Z* nonsense mutation U118. Recombinants which gave rise to a melibiose-positive phenotype at 42 C after introduction of the episome were judged to be $SuA^+$. $SuA^+$ *trpR*$^-$ and $SuA^-$ *trpR*$^-$ recombinants were picked from among the $Lac^-$ recombinants. The F'W1 episome was provided to us by B. Beckett (University of Wisconsin). It was derived by crossing the W1 deletion onto an F', *trp*, *tonB*, *lac* episome isolated from EC-8 (3) by D. Schwartz and J. Beckwith (Harvard Medical School).

To look for the presence of the ω peptide production in *trp-lac* fusion strains, two F'*lac*, *proA,B* episomes were introduced into these strains by the method described previously (24). One of these episomes carried the late *lacZ* nonsense mutation X90, which is known to be an ω acceptor (27, 28), and the other episome carried the early *lac* nonsense mutation U118, which served as a negative control.

The other strains used in this study are: 3000, a wild-type *lac*$^+$ strain from the Luria collection, and X7026, an $F^-$ Δ(*lac-proA,B*) XIII strain.

**Media and chemicals.** Media and chemicals are as described elsewhere (14, 24, 26).

**Enzyme assays.** These experiments derived from independent observations made by C.A.M. and W.S.R. during the course of experiments described in reference 14 and by A.E.S. and B.M. made during the course of the experiments described in reference 26. Therefore, the exact procedures used in the assays for each experiment depended upon the laboratory where the work was performed. These are specifically referred to in each figure or table legend and are described in detail elsewhere (15, 16, 24, 25). The units of enzyme activity were in all cases normalized to the value for W1 found in Table 1 for ease of comparison.

**mRNA measurements.** The *lacZ* and *-A* mRNA determinations were performed by RNA-deoxyribonucleic acid hybridization as described by T. G. Cooper, P. A. Whitney, and B. Magasanik (manuscript submitted for publication).

**Heat inactivation of β-galactosidase.** The cells of the control strain $R^-$X8618 and of two or more experimental strains were harvested by centrifugation at 4 C from late-log-phase cultures grown in LB broth. The cell pellets were resuspended in one-tenth the original volume in Z buffer and were sonically dis-

rupted by using the microtip attachment of the sonifier cell disruptor (Heat Systems-Ultrasonics, Inc.). These crude extracts were clarified by centrifugation at 10,000 rpm for 15 min in an IEC centrifuge model HR-1 with an 856 rotor. The supernatants were then assayed for β-galactosidase and were diluted with a similar extract of X7026 so that they had the same approximate specific activity. A 0.5-ml volume of these diluted extracts was then pipetted into 2 ml of prewarmed Z buffer and heated at 60 C. At the indicated time points (Fig. 3), 0.2 ml of the heated extract was removed and pipetted into 0.8 ml of ice-cold Z buffer. These diluted samples were then assayed for β-galactosidase. Since the water bath temperature might vary between and during experiments, a wild-type control was always included, and samples for corresponding time points from different extracts were removed within 1 min of each other.

TABLE 1. *β-Galactosidase and thiogalactoside transacetylase activities in trp-lac fusion strains*[a]

| Strain | *trp* control of *lac* expression | β-Galactosidase (β-Gz) | Thiogalactoside transacetylase (TA) | β-Gz/TA |
|---|---|---|---|---|
| R⁻X8618 | No | 5775 | 9.95 | 580.4 |
| X7713 | Yes | 177 | 3.10 | 57.1 |
| W1 | Yes | 519 | 10.85 | 47.8 |
| W200 | Yes | 135 | 5.10 | 26.5 |
| W205 | Yes | 6805 | 9.25 | 735.7 |
| W209 | Yes | 2655 | 12.65 | 209.9 |
| W211 | Yes | 3290 | 15.70 | 209.6 |
| W227 | Yes | 204 | 5.45 | 37.4 |
| F23a | Yes | 561 | 9.15 | 61.3 |
| F36a | Yes | 114 | 1.80 | 63.3 |
| F101 | Yes | 146 | 2.05 | 71.2 |
| F104 | Yes | 95 | 2.45 | 38.8 |

[a] All assays were performed in duplicate. The exact protocols and definitions of unit activities are as described by Michels and Zipser (15). R⁻X8618 is a control *trp-lacI* deletion strain in which *lac* operon expression is under the control of the *lac* promoter.

## RESULTS

**β-Galactosidase and thiogalactoside transacetylase activities in trp-lac fusion strains.** Starting with a strain in which the *lac* operon is transposed to a site near the *trp* operon (Fig. 1), it has been possible to isolate *tonB* fusion deletions that fuse the *lac* structural genes *Z*, *Y*, and *A* to the *trp* operon so that the *lac* genes are under the control of the *trp* regulatory elements. Most of these deletions appear to end within the *lac*-controlling elements (Fig. 1) (16, 23, 24; Shrenk, Ph.D. thesis; and Mitchell et al., manuscript in preparation).

Two striking characteristics of these strains are revealed by the data in Tables 1 and 2, and Fig. 2. First, the various *trp-lac* fusion strains produce dramatically different levels of β-galactosidase. For instance, the amount of β-galactosidase found in F36a is more than 60-fold lower than that found in W205. Second, the β-galactosidase production in these strains is not proportional to the thiogalactoside transacetylase, *lacZ* mRNA, and *lacA* mRNA production if compared to the ratios found in the control strains R⁻X8618 and 3000. (The twofold difference in the Z enzyme-A enzyme ratio for 3000 as compared to R⁻X8618 is presumably due to differences in the genetic background of the two strains, and this difference has been noticed before when comparing 3000 to strains similar to R⁻X8618 [26].) Rather, with the exception of W205, less β-galactosidase is found than would be predicted. As will be discussed subsequently, these two properties of the *trp-lac* fusion strains are probably closely related; however, this paper will first present observations which suggest a possible explanation for the differing levels of β-galactosidase in the various strains and then will discuss the observed disproportionate production of the two enzymes.

**Production of β-galactosidase.** The follow-

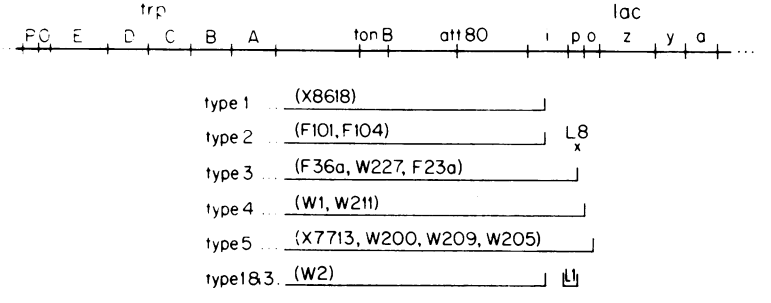


FIG. 1. *Deletions fusing the trp and lac operons. The isolation and characterization of the deletions pictured are described in detail elsewhere (13, 23, 24; Schrenk, Ph.D. thesis; and Mitchell et al., manuscript in preparation). The type 2 deletions are all present in combination with the promoter point mutation L8 indicated by an X.*

ing three models could be envisaged to explain the differing levels of β-galactosidase in the *trp-lac* fusion strains. (i) *Z* gene expression in some strains might be reduced due to a polar effect generated by a nonsense codon possibly located in the residual *lacP-O* region. (ii) The β-galactosidase activity in some strains might reside in abnormal trp-β-galactosidase hybrid protein molecules which are only partially active. (iii) There may be an inhibition to varying degrees in different strains of *lacZ* mRNA translation, probably at the level of translation initiation.

The observations described in Tables 1 and 2 and in Fig. 2 seem to rule out a polar effect on *lacZ* expression as being the primary reason for the reduced levels of β-galactosidase. This is because polar effects resulting from nonsense codons are known to have proportional effects on distal gene expression and on detectable mRNA synthesis (1, 4, 11, 19, 30). An experiment which further examines the possibility mentioned above is described in Table 3. The effect of the polarity suppressor, SuA (2), on the synthesis of β-galactosidase coded for by one *trp-lac* fusion was tested by introducing an F′ episome carrying the W1 *trp-lac* fusion into $SuA^+$ and $SuA^-$ strains that were otherwise isogenic and measuring the amount of β-galactosidase produced. The level of β-galactosidase activity was not affected by the presence of the SuA suppressor (Table 3).

A *trp-lac* fusion strain might produce an abnormal hybrid trp-β-galactosidase protein if the fusion deletion began in a *trp* structural gene and ended in phase within the *lac*-controlling element region or within the sequence

TABLE 2. *Lac enzyme and mRNA production in trp-lac fusion strains*[a]

| Strain | *trp* control of *lac* expression | *Z* enzyme[b] | *Z* mRNA | *A* enzyme[c] | *A* mRNA | *Z* enzyme/ *Z* mRNA | *A* enzyme/ *A* mRNA | *Z* enzyme/ *A* enzyme | *Z* RNA/ *A* RNA |
|---|---|---|---|---|---|---|---|---|---|
| 3000 | No | 3,147 | 7,860 | 12.15 | 3,340 | 0.40 | $3.69 \times 10^{-3}$ | 259.0 | 2.3 |
| W1 | Yes | 519 | 5,810 | 10.85 | 2,480 | 0.089 | $4.38 \times 10^{-3}$ | 47.8 | 2.3 |
| W2 | Yes | 236 | 3,210 | 3.41 | 2,010 | 0.074 | $1.70 \times 10^{-3}$ | 69.2 | 1.6 |
| X7713 | Yes | 100 | 1,510 | 2.06 | 930 | 0.066 | $2.06 \times 10^{-3}$ | 48.5 | 1.6 |

[a] The assays were performed as follows: β-galactosidase as in Silverstone et al. (25) and thiogalactoside transacetylase as in Miller et al. (16). The enzyme activities were normalized to the values for W1 found in Table 1. The *lac* mRNA was isolated from cells which had been exposed to 5 mM IPTG for 20 min and then labeled for 4 min with [$^3$H]uracil, and the hybridization assays were performed as described (Cooper et al., manuscript submitted for publication), with activity shown as counts per minute of hybridized RNA.

[b] β-Galactosidase.

[c] Thiogalactoside transacetylase.

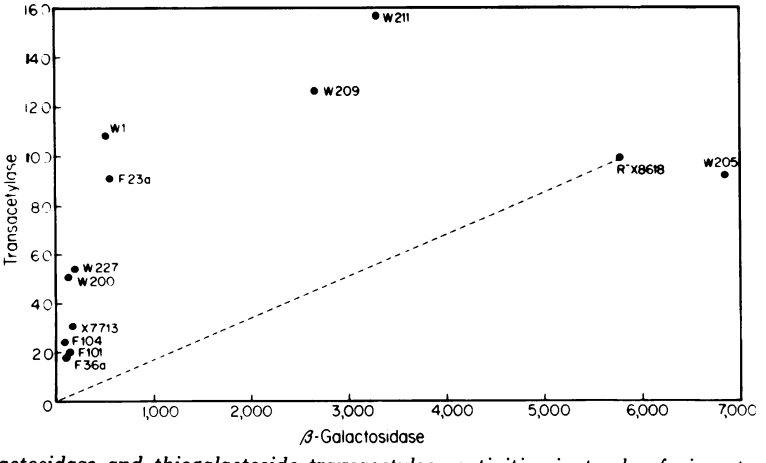


FIG. 2. *β-Galactosidase and thiogalactoside transacetylase activities in trp-lac fusion strains. The data presented in Table 1 are plotted with the dashed line indicating the expected enzyme activity values for proportional Z and A expression.*

TABLE 3. *Effect of the SuA suppressor on β-galactosidase production by the W1 trp-lac fusion*[a]

| Strain | β-Galactosidase |
|---|---|
| F'W1/SuA$^+$ | 519 |
| F'W1/SuA$^-$ | 507 |

[a] An F' episome carrying the W1 *trp-lac* fusion was introduced into SuA$^+$ and SuA$^-$ strains, and the β-galactosidase activity produced by the merozygotes was measured by using the techniques described by Reznikoff et al. (24). The enzyme activities were normalized to the values for W1 found in Table 1.

corresponding to the amino terminus of β-galactosidase. To test whether abnormal β-galactosidase is made in any of the fusion strains, the thermostability of the β-galactosidase produced in these strains when incubated at identical specific activities in Z buffer at 60 C was examined. Representative inactivation curves for the control strain R$^-$X8618 and for the fusions, W1 and W209, are shown in Fig 3. All of the strains tested (W1, W2, W200, W205, W211, W227, F23a, F36a, F101, and F104) except for one (W209) produced β-galactosidase that was indistinguishable from that of the control strain by this test. The β-galactosidase of W209 from three independent cultures has been tested. In all cases it was approximately 2.7 times more thermolabile than the wild-type enzyme. These results are consistent with the preliminary results of sodium dodecyl sulfate (SDS)-acrylimide gel analysis of the crude extracts from fusion strains to be reported subsequently. Extracts of all of the fusion strains except for W209 (and possibly W200) manifest a single band at the position expected for β-galactosidase in an amount approximately proportional to the β-galactosidase activity found in the strain. Therefore, with the possible exception of W209, it can be concluded that the decreased levels of β-galactosidase activity do not result from structural alterations in the enzyme itself but from a decreased rate of formation of the enzyme.

**Disproportionate production of β-galactosidase and thiogalactoside transacetylase.** A possible basis for the observed disproportionate β-galactosidase and thiogalactoside transacetylase activities in the fusion strains might be that the β-galactosidase activity observed underestimates the actual amount of *Z* gene translation occurring in these strains. One could assume that two types of *Z* gene translation are occurring. For instance, strains which produce approximately normal levels of thiogalactoside transacetylase but low levels of β-galactosidase might contain in-phase deletions so that they produce both normal β-galactosidase and a hybrid inactive trp-β-galactosidase protein. The total translation of *Z* might be normal, although the amount of normal, enzymatically active β-galactosidase would be reduced. If this model were correct, a fused protein containing the β-galactosidase ω peptide fragment should be made in these strains. This might be detected by introducing an episome coding for an ω acceptor into the fusion strains and looking for increased β-galactosidase activity (the total activity would correspond to normal β-galactosidase plus ω donor plus ω acceptor hybrid molecules). Partial diploids were constructed by introducing either an F' which carries the X90 ω-accepting mutation or a control F' carrying the U118 mutation into several of the fusion strains. These partial diploids were assayed for β-galactosidase activity. No increase in β-galactosidase activity was detected in the merozygotes carrying the F'X90 (Table 4). This result

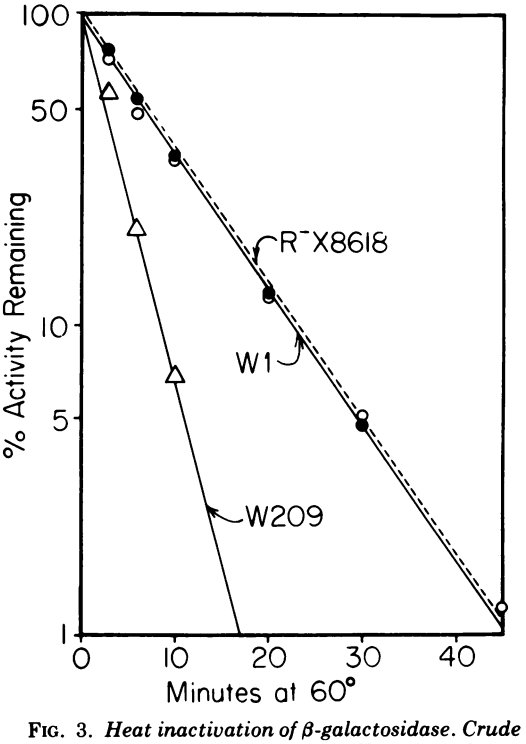


FIG. 3. *Heat inactivation of β-galactosidase. Crude extracts of the control strain (R$^-$X8618) and two experimental strains (W1 and W209) were diluted in a crude extract of a lac$^-$ strain (X7026) to the same specific activity. These extracts were diluted into prewarmed Z buffer, and samples were removed at the indicated times and assayed for β-galactosidase as described in Reznikoff et al. (24).*

TABLE 4. *β-Galactosidase ω peptide production by trp-lac fusions*

| Strain | F' *lacZ*⁻ X90 | F' *lacZ*⁻ U118 |
|---|---|---|
| W209 | 847 | 846 |
| W1 | 376 | 519 |
| W227 | 316 | 288 |
| W200 | 255 | 302 |
| F104 | 186 | 160 |

[a] An F' *lac* episome coding for the X90 ω-accepting fragment or a control F' *lacZ*⁻ U118 episome was introduced into various *trp-lac* fusion strains, and the β-galactosidase activity produced by the merozygotes was measured by using techniques described before (24), except that the cells were grown by using 0.4% glycerol as the carbon source. The activities were normalized to values for W1 found in Table 1.

does not rule out the above model, since the maximum level of β-galactosidase activity resulting from such complementation is only 25% of the wild-type level (or approximately 600 units in this experiment) and frequently is considerably lower (27, 28) and since an unstable ω peptide might be made in these strains and not be detected by our assay. But other considerations to be presented in the Discussion suggest that this model is not correct.

## DISCUSSION

The first unusual feature of the *trp-lac* fusion strains examined in this paper is the difference in the levels of β-galactosidase made in the various strains. The evidence indicates that these differing levels of β-galactosidase are not due to polarity generated by a nonsense codon preceding *lacZ*. The strains did not have similarly depressed levels of transacetylase activity; the levels of *lacZ* mRNA were not lowered to the same degree as the β-galactosidase activity; and the polarity suppressor SuA did not alter the level of β-galactosidase generated by at least one fusion. The fact that the level of *lacZ* mRNA was greatly in excess of that expected from the amount of β-galactosidase activity found also indicates that the lowered levels of β-galactosidase do not result from defects in transcription. Another possibility, which has not been completely ruled out yet, is that these strains produce abnormal β-galactosidase proteins (probably fused to *trp* proteins). However, the heat-inactivation studies suggest that, with the exception of W209, all of the strains produced normal β-galactosidase. Furthermore, a recent sequence analysis of the *lac* operator region indicates that there is an in-phase nonsense codon (UGA) within the operator (W. Gilbert, N. Maizels, and A. Maxam, Cold Spring Harbor Symp. Quant. Biol., in press), which would suggest that no such partially active fused protein could be made in strains W1, W2, W211, W227, F23a, F36a, F101, and F104, all of which contain deletions ending before *lacO* (see Fig. 1). Additional evidence that normal β-galactosidase is produced in these strains has come from preliminary SDS-acrylimide gel analyses of crude extracts of these fusion strains.

What other phenomenon could explain the varying amounts of β-galactosidase found in these fusion strains? We propose that the amount of β-galactosidase found in these strains is a measure of the frequency of initiation of translation at the start of *lacZ*. One could hypothesize three different means by which this initiation might be depressed in these strains. (i) The deletions may have removed some nucleotides that normally code for part of the *lacZ* ribosome binding site. Presumably this site includes the AUG codon at the start of *lacZ* and perhaps 15 or 20 nucleotides on either side. This possibility cannot explain the lowered β-galactosidase production in the type 2, 3, and 4 strains, since these strains contain deletions ending before *lacO* (24; Schrenk, Ph.D. thesis; and Mitchell et al., manuscript in preparation) and therefore must end at least 35 nucleotides away from the *lacZ* AUG (6).

(ii) In several (but probably not all) of these strains, the fusion deletion extends from a *trp* structural gene to the *lac*-controlling elements. It would be expected that there might be ribosomes and associated translation machinery proceeding through from the *trp* structural gene across that section of the *lac* mRNA usually reserved for ribosome binding. This read-through translation might therefore compete with the correct binding of new ribosomes. Such a possibility has been postulated by Platt et al. (20) to explain the necessity for a chain termination codon prior to an initiation codon in order for initiation to occur. There are two strains in which the fusion deletions apparently do not end in a *trp* structural gene (W205 and F36a) but rather after *trpA* (Mitchell et al., manuscript in preparation). Presumably read-through translation cannot occur, and yet the β-galactosidase level in F36a is very low. Also, this model does not take into consideration the existence of two nonsense codons within the operator region (Gilbert et al., in press). One of these, which has been mentioned before, is an in-phase UGA codon which would terminate translation 33 nucleotides prior to the *lacZ* AUG. The second nonsense codon is a UAA in another reading frame, 25 nucleotides prior to

the *lacZ* AUG. One would therefore expect that *trp* translation would read through the *lacZ* ribosome binding site only in strains in which the fusion deletion extends into *O* beyond the two nonsense codons or in which the deletion ends so that the third reading frame is utilized. The deletions which end in *O* can be detected (23; Schrenk, Ph.D. thesis; and Mitchell et al., manuscript in preparation), but there is no way of knowing yet which reading frames are represented by the various deletions. In conclusion, it is possible that in some strains (those whose deletions end in *O* or in the third reading frame) this is the mechanism by which *lacZ* translation initiation is inhibited, although it is very unlikely in the specific case of F36a.

(iii) A general feature of all of these fusions is that abnormal RNA sequences have been added onto *lac* mRNA close to the *lacZ* ribosome binding site. It is possible that these sequences alter the configuration of the *lacZ* ribosome binding site or make it structurally inaccessible, thereby reducing the affinity of ribosomes for the site. That such a phenomenon can occur is known from the studies on R17 RNA translation. The RNA sequences close to the R17 replicase gene ribosome binding site dramatically affect its accessibility to incoming ribosomes (5, 7, 12, 31). This mechanism may well explain the depressed level of *lacZ* translation in some of the fusion strains. It may be possible to test this model directly. *trp-lac* fusion mRNA should bind ribosomes to the *lacZ* ribosome binding site at a lower efficiency than normal *lac* mRNA, but the sequence of the site should be the same.

The second striking feature of these *trp-lac* fusion strains is the obvious disproportionate production of β-galactosidase and thiogalactoside transacetylase. A similar observation has been made in another system. Voll (29) and Rechler et al. (21, 22) have described a frame-shift mutation located late in the *D* gene of the *his* operon. This mutation reduces expression of the immediate next gene, *hisC*, 98 to 99% but reduces the expression of subsequent genes only 90%. The reduction in *hisC* expression and the disproportionate polar effect observed was not altered by introducing a nonsense codon immediately prior to the frame-shift mutation in *hisD* and therefore was not due to read-through translation competing for normal initiation. The protein produced by the immediate next gene was normal in all respects.

The experiments described in Table 2 indicate that the disproportionate production of β-galactosidase and thiogalactoside transacetylase does not seem to be reflected in the amounts of *lacZ* mRNA and *lacA* mRNA found in these strains. Rather, these experiments suggest that it is specifically due to decreased amounts of β-galactosidase. Some theories which would explain these apparent decreased levels of β-galactosidase relative to thiogalactoside transacetylase and *lacZ* mRNA rely on the notion that the β-galactoside activity found in these strains is not an accurate indicator of the level of *Z* gene translation. For instance, both normal β-galactosidase and fused proteins or restart fragments might be synthesized. Such fused proteins or restart fragments might be detectable as ω peptides, which we were unable to find. Again, the presence of the two nonsense codons in the operator suggests that only the few strains that contain deletions extending into *lacO* could synthesize such fused proteins and only strains with deletions either ending in the operator or in the third possible reading frame could give rise to restart fragments. Furthermore, in F36a the deletion ends after *trpA*. Therefore, this strain probably does not have any of the required read-through translation. These theories also cannot explain the similar observation made in the *his* system.

One possible explanation for the disproportionate production of β-galactosidase and thiogalactoside transacetylase can be derived from the following considerations. Let us assume that there is some minimum number of ribosome binding events (translation initiation events) necessary at the start of any given cistron to ensure propagation (or protection) of mRNA down to the next cistron. Any number below this minimum will result in a polar effect on the expression of the subsequent cistrons. This could either be due to a combined endonucleolytic-exonucleolytic degradation of the mRNA or to the fact that the RNA polymerase does not progress in the absence of an adequate supply of ribosomes (8, 17, 18). Presumably ribosomes can bind at the start of internal cistrons as long as mRNA is present. This assumption is supported by the fact that it has been possible to isolate *lacO-Z* deletions which remove the *lacZ* translation initiation site yet still permit expression of the *lacY* gene (6). Thus, as long as some minimum number of ribosomes translates the *Z* gene, the whole *lac* message will be produced and available for normal translation initiation at *Y* and *A*.

Although the data presented in this paper do not allow an exact determination of this minimum number of *lacZ* ribosome binding events, they do suggest that this number is considerably lower than the normal complement of ribosomes. This is indicated by the observation that

strains W1 and F23a have low levels of β-galactosidase but near normal levels of thiogalactoside transacetylase and *lacZ* and *-A* mRNA.

The question as to how many ribosomes are necessary for successful propagation of mRNA has not been resolved by work previously reported. Yanofsky and Ito (30) have found that, for the case of suppressed nonsense mutations in the *trp* operon, the relief of polarity is approximately proportional to the level of suppression. However, earlier work by Newton et al. (19) using the *lac* system did not find such a simple relationship. There have been other studies examining the production of mRNA, either in temperature-sensitive translation-defective mutants or in the presence of antibiotics inhibiting translation (see 9, 10, 13). The data in these papers are insufficient to determine whether detectable *trp* or *lac* mRNA is dependent upon a minimum number of ribosomes or is directly proportional to the number of ribosomes present.

If the model presented in this paper is correct, one would predict that a mutation in the *lacZ* ribosome binding site should have the following phenotype. It should reduce *lacZ* expression but it should not affect *lacY* or *-A* expression unless the level of fully induced β-galactosidase made by the mutant is less than that found in W1. In other words, the effects should not be proportionate.

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