Regulation of the *gal* Operon of *Escherichia coli* by the *capR* Gene*

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SUMMARY

We have examined in greater detail the derepression of uridine diphosphate galactose-4-epimerase synthesis in *capR* mutants of *Escherichia coli* first observed by Markovitz. (Markovitz, A. (1964) *Proc. Nat. Acad. Sci. U. S. A.* 51, 239–246). All three enzymes of the *gal* operon are derepressed from 2- to 4-fold by this mutation. This derepression may be superimposed on the conventional derepression seen when the *gal* operon is induced with fucose or by a regulatory mutation. Measurement of the level of *gal* messenger ribonucleic acid indicates that the derepression caused by the *capR* mutation probably occurs at the level of transcription since there is a coordinate increase in *gal* enzyme activity and in the level of *gal* mRNA. The effect of the *capR* mutation on the *gal* operon appears to be independent of cyclic 3',5'-monophosphate or glucose-mediated repression. Thus the product of the *capR* locus behaves as if it is an additional negative control element affecting the expression of the *gal* operon.

The *gal* operon of *Escherichia coli*, like a number of other operons, is under negative control (2-5). Mutations in either *galR* or *galO* result in the constitutive synthesis of all three enzymes of the operon. There is no firm evidence for a promoter region, but several mutants have been described with properties similar to those expected of strains carrying promoter mutations (5, 6). It has also been proposed that a positive mechanism of control involving cyclic adenosine 3', 5'-monophosphate and the cyclic AMP receptor protein (7) also acts on the *gal* operon, presumably at a promoter site (8-10).

Mutations in the *capR* locus (11, 12) result in the overproduction and excretion of a galactose-containing polysaccharide and cause an increased sensitivity to ultraviolet (13, 14) and a defect in septum formation (15, 16). Markovitz has shown that a number of enzymes involved in the synthesis of the precursors of the excreted polymer are synthesized at a higher rate in *capR* mutants. One of these enzymes, epimerase, is encoded in the *gal* operon. The others include phosphomannomutase, GDP mannose hydrolyase, GDP-L-fucose synthetase, GDP mannose pyrophosphorylase, UDP glucose dehydrogenase, and UDP glucose pyrophosphorylase (11, 17). Markovitz has postulated that the product of the *capR* locus is a repressor protein which binds to the operator region of the structural genes for each of the above enzymes. Loss of the product of the *capR* locus results in the derepression of all of the genes normally regulated by this protein.

We undertook to characterize further the effect of the product of the *capR* locus on the *gal* operon. First, we determined whether or not the entire operon responded to the product of the *capR* gene and, if so, whether this response occurred at the level of transcription or translation. In fact, all three enzymes are derepressed in *capR* mutants, with a corresponding increase in the level of *gal* mRNA. Second, we demonstrated that the effect of mutations in *capR* is for the most part independent of other regulatory phenomena in the *gal* operon such as induction and glucose-mediated repression. Finally, we have attempted to distinguish between the model of negative control suggested by Markovitz and other models involving positive mechanisms. We conclude that Markovitz's original proposal is consistent with our results.

**MATERIALS AND METHODS**

**Strains**—Bacterial strains used in this work are described in Table I. Bacteriophage λ<sub>Gal</sub> was obtained from lysates of W3350 (λ<sub>Gal</sub>) and was used to construct λG30 which retains the c1857 marker and which transduces the entire *gal* operon. Stocks of λ<sub>Gal</sub> were obtained from Dr. Ira Pastan (National Institutes of Health). This phage carries a deletion of the host chromosome between the bacterial attachment site (attB) and the *gal* operon (18).

**Growth Media**—H-1 salts consist of 11.2 g of KH<sub>2</sub>PO<sub>4</sub>, 2.0 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.0 ml of 1.8 mM FeSO<sub>4</sub>, and water to 1 liter. MgCl<sub>2</sub> to 1 mM, carbon source, and supplements were added after autoclaving. Mannitol was most often used as a carbon source, as it allowed good growth for the strains under study and did not interfere with inducer entry. Tryptone broth consists of 10 g of Difco Bacto-tryptone and 5 g of NaCl per liter.
Table I
Strains used

| Strain  | Genotype | Source |
|---------|----------|--------|
| 3300    | HfrH; lacI; thi; galT* | Dr. A. Markovitz |
| GM91    | HfrH; lacI; thi; galR  | This lab. |
| GM130   | HfrH; lacI; thi; galO49 | This lab. |
| GM131   | HfrH; lacI; thi; galO59 | This lab. |
| GM132   | HfrH; lacI; thi; galO61 | This lab. |
| M6      | HfrH; lacI; thi; capR6; galT* | Dr. A. Markovitz |
| GM100   | HfrH; lacI; thi; capR6; galR  | This lab. |
| GM140   | HfrH; lacI; thi; capR6; galO49 | This lab. |
| GM141   | HfrH; lacI; thi; capR6; galO59 | This lab. |
| GM142   | HfrH; lacI; thi; capR6; galO61 | This lab. |
| GM30    | galT; (λgalS7, λgalS7dig30) | Dr. R. Wu |
| W3350   | galT; galK  | Dr. W. Gilbert |
| N99     | Nonpermissive for λgalS7 | Dr. I. Pastan |
| PR13    | thi, leu, thi, rrs, pro, lac, mal, xyl, mtl | Dr. W. Gilbert |

Growth of Bacteria—Bacterial strains were maintained on tryptone broth slants. For measurements of enzyme activity, fresh stationary broth cultures were diluted 1:100 into H-1 medium, grown as indicated in each table, and harvested while still in exponential phase.

Growth of Bacteriophage—Strains W3350 (λgalS7) and GM30 were grown in tryptone broth at 28° in a fermentor or in 4-liter flasks with vigorous shaking. When the cells had grown to a density of 5 x 10⁸ cells per ml, they were induced by heating for 15 min to 42° and then cooled to 37° where growth was continued until lysis occurred. Debris from the lysate was removed by centrifugation and lysed with chloroform, and the phage was recovered from the supernatant by prolonged differential centrifugation followed by centrifugation through sucrose. Ribosomal RNA was extracted from the ribosomes by the method of Bolton (20). The material was greater than 80% intact as judged by gel electrophoresis.

DNA-RNA Hybridization—In general, we followed the procedures of Spiegelman and Gillespie (24) with the following modifications. DNA was denatured with alkali, neutralized, and loaded onto 13-mm nitrocellulose filters (type B-6, Carl Schleicher and Schuell Company) at 5 or 10 μg per filter. Retention of DNA on the filter was usually over 95%. DNA was fixed to the filters by drying them under vacuum, first for 1 hour at room temperature and then for at least 2 hours at 65°.

Components of the hybridization mixture included [3H]uridine, usually from 2 to 5 μg depending on the specific activity; 30 μg of unlabeled ribosomal RNA to act as carrier and to compete against nonspecific binding; and buffer (2 x SSC, 2° neutralized phenol, 0.05% sodium dodecyl sulfate, pH 6.0) to 0.25 ml in a 2-cm vial. A filter containing the appropriate DNA was wetted with a minimal volume of buffer and added last. The vial was sealed carefully and incubated for 24 hours at 65°. At the end of this period, the filter was removed from the vial and washed with 2 x SSC (15 ml per side). The filter was incubated for 40 to 50 min at 32° with 20 μg of ribonuclease in 1.0 ml of 2 x SSC and then rewashed. The filter was dried thoroughly before counting in a toluene-based liquid scintillation fluid. The results are expressed as the percentage of input counts hybridized. In any one experiment, all filters were from the same lot.

Enzyme Assays—The assays for β-galactosidase (25), epimerase (26), kinase (26), and transferase (the two-step assay of Reference 27) were as described. One unit of activity equals 1 μmole of product formed per hour except for β-galactosidase where 1 unit equals 1 μmole of O-nitrophenol per min.

Extracts for these assays were prepared by suspending each frozen cell pellet (frozen at −20°) in 1.5 to 2.0 ml of a buffer containing 0.02 M triethanolamine acetate, pH 7.0, 1 mM EDTA, and 1 mM dithiothreitol. The suspension was transferred to a polyallomer centrifuge tube (1.5 x 7 cm), covered with paraffin, and placed in the chamber of a Raytheon sonicator (DF101) containing 30 ml of H₂O at 4°. The sonicator was operated at full power for 1 min, chilled, and operated again for 1 min more.

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Cellular debris was removed by centrifugation, and the resultant extract was assayed directly for enzyme activity. Protein concentration was determined by the method of Lowry et al. (28).

**Reagents**—The following products were obtained commercially: cyclic AMP, β-fucose, β-galactose, egg white lysozyme, and reagents for the assay of the galactose enzymes (Sigma); O-nitrophenylgalactoside (Cyclo Chemical Company); radiochemicals (New England Nuclear); pancreatic ribonuclease and deoxyribonuclease (Worthington); optical grade cesium chloride (Harshaw Chemical Company); Macaloid (Boroid Division, National Lead Company, Houston, Tex.); and diethyl pyrocarbonate (K & K Chemical Company). Chloramphenicol was a gift from Dr. G. Schatz, Cornell University. All other materials were of reagent grade.

**RESULTS**

Effect of capR Mutation on gal Enzyme Synthesis—Markovitz's original work (11) indicated that epimerase activity was 4- to 5-fold higher in a mucoid (capR-) strain than in the parent (3300). The data in Table II demonstrate that all three enzymes of the galactose operon are synthesized at a higher rate in the capR strain. Growing the cells in a different medium, such as M9 (29), or using a different carbon source, such as glucose, does not substantially alter the results.

The epimerase appeared to be synthesized at a higher rate than the other two enzymes. However, the higher epimerase activity may be an artifact, resulting from the error caused by substantial blanks present in the assays for kinase and transcrase which partially obscure the basal level of activity in 3300.

Mixing experiments (data not shown) were performed to ascertain whether one of the extracts contains an inhibitor or activator not present in the other. For all three enzymes, activity was completely additive in mixed extracts. These experiments rule out the presence of a dissociable inhibitor or activator in extracts of these strains.

Effect of Interruption of Normal Regulation—It was of interest to determine whether or not the capR- strain used would show higher enzyme levels in situations where the gal enzymes are normally derepressed. Such situations arise when the growth medium contains β-fucose, a gratuitous inducer of the gal operon (2), or when the cells are in question carry mutations in the galR or galO regions. The results in Table III indicate that strains carrying a capR mutation always possess higher levels of epimerase than do the corresponding parents. Assays of kinase activity (and in the case of cells grown with fucose, transferase activity) gave results directly parallel with those shown.

**Transcription in capR- and Parent Strains**—We measured the synthesis of gal mRNA in both Strain 3300 and Strain M6 by hybridization techniques using pulse-labeled RNA. This method is valid provided that the kinetics of RNA synthesis is identical in the strains under study. Experiments demonstrating this for M6 and 3300 are presented in Fig. 1 and indicate...
Hybridization of RNA from capR (M6) and parent (3300) strains

Cells were grown as described in the legend to Table II with 1.5 mM fucose where indicated. At a density of $3.5 \times 10^6$ cells per ml, a 10-ml portion of each culture in question was exposed to 200 $\mu$Ci of [3H]uridine at a final concentration of 1.7 $\mu$M for 3.0 min. The RNA was isolated as described under "Materials and Methods." Final specific activities ranged from 43,000 cpm per $\mu$g to 54,000 cpm per $\mu$g. The hybridization assay was performed as described under "Materials and Methods." The difference in the amount of radioactivity hybridized to a DNA in the two experiments reflects the fact that a different lot of filters was used in each case.

### Table IV

| Source of RNA | Hybridization to different λ DNAs | % | % |
|---------------|----------------------------------|---|---|
| 3300          |                                  | Net | 0.000 | 0.054 | 0.049 |
| 3300 induced with fucose | 0.018 | 0.133 | 0.12 | 0.024 | 0.293 | 0.27 |
| M6           | 0.010 | 0.18 | 0.16 | 0.012 | 0.082 | 0.07 |
| M6 induced with fucose | 0.013 | 0.038 | 0.06 | 0.013 | 0.038 | 0.07 |

Comparison of level of epimerase and of gal mRNA

Enzyme assays were performed as indicated in the legend to Table II on 10.0-ml aliquots of the cultures used for pulse-labeling described in the legend to Table IV. The relative level of gal mRNA was calculated as described in the text.

| Strain       | Relative epimerase activity (observed) | Relative gal mRNA (calculated) |
|--------------|----------------------------------------|-------------------------------|
| 3300         | 1                                      | 1                             |
| 3300 induced with fucose | 13                                   | 13                            |
| M6           | 4                                      | 4                             |
| M6 induced with fucose | 20                                   | 20                            |

that the uptake of uridine from the medium, under conditions similar to those used in pulse-labeling, and its incorporation into acid-insoluble material are nearly identical in the two strains.

Table IV shows the results of hybridizing pulse-labeled RNA from strains M6 and 3300 (with or without fucose) to different DNAs. Table V shows the relative epimerase levels of these same cultures. Although there is an increase in hybridizable RNA in M6 relative to 3300, this increase is much less than the increase in gal enzyme activity. Moreover, although fucose induces the gal enzymes 10- to 13-fold, the increase in gal mRNA synthesis is only 2.4-fold in the culture of endogenous DNA is used in the assay and 4-fold when λgd40 DNA is used. λgd40 carries the entire gal operon, but it lacks a segment of E. coli chromosomal material carried in λgd40. This strongly suggests that other RNA species, in addition to gal mRNA, are hybridizing to both types of DNA and obscuring the increase in gal mRNA.

### Analysis of Hybridization Data

We have analyzed our data making the assumption that, upon induction with fucose, gal mRNA and gal enzyme levels are directly proportional. For this discussion, we define the following quantities using the enzyme ratios measured in fucose induced cells (Table V).

\[
\begin{align*}
    x & = \% \text{ hybridizable RNA from 3300 that is not gal mRNA} \\
    x' & = \% \text{ hybridizable RNA from M6 that is not gal mRNA} \\
    y & = \% \text{ hybridizable RNA from 3300 that is gal mRNA} \\
    z & = \% \text{ hybridizable RNA from M6 that is gal mRNA} \\
    z' & = \% \text{ hybridizable RNA from M6 grown with fucose that is not gal mRNA} \\
    y' & = \% \text{ hybridizable RNA from 3300 grown with fucose that is not gal mRNA} \\
    13y & = \% \text{ hybridizable RNA from 3300 grown with fucose that is gal mRNA} \\
    13z & = \% \text{ hybridizable RNA from M6 grown with fucose that is gal mRNA} \\
\end{align*}
\]

Applying these symbols to the data from Table IV for hybridization to λgd40 DNA, we can set up the following equations.

\[
\begin{align*}
    x + y + z & = 0.12 \\
    x + 13y & = 0.27 \\
    x' + z' & = 0.16 \\
    x' + 13z' & = 0.36
\end{align*}
\]

Solving these equations yields the following solutions.

\[
\begin{align*}
    x = 0.11 & \quad x' = 0.11 \\
    y = 0.013 & \quad z = 0.03 \\
    13y = 0.16 & \quad 13z = 0.25
\end{align*}
\]

These results are striking confirmation of our original assumptions. First, one would expect $x = x'$, since these represent the mRNA which is not gal mRNA and which should not respond to fucose. This is the mRNA from the genes which lie between the phage attachment site on the host chromosome (attg) and the gal operon. The amount of this message ought to be the same in both strains, and our calculations show it is.

Second, the derepression of gal mRNA in strain M6 relative to 3300 is now virtually identical with the extent of derepression of epimerase which is the best measure of all the gal enzymes (Table V). These data indicate that the product of the capR gene affects transcription, that is, it normally represses transcription some $4 \times$ fold. The data further imply that if there is translational control in the gal operon it is not affected by the capR product.

Third, if the results obtained from using λgd40 DNA in the hybridization assay are substituted into the equations developed previously, we obtain the following solutions.

\[
\begin{align*}
    x = 0.037 & \quad x' = 0.047 \\
    y = 0.013 & \quad z = 0.05 \\
    13y = 0.17 & \quad 13z = 0.23
\end{align*}
\]

That is, identical results are derived for the percentage of gal mRNA in the mutant and parent strains using different DNAs in the hybridization assay. In the case of λgd40l DNA, only the values of $x$ and $x'$ are reduced as expected since this phase is derived from a strain carrying a deletion between the phage attachment site and the gal operon (18). It is interesting to note that even with this deletion, the amount of RNA other than gal mRNA which hybridizes to this DNA is still twice as great as the amount of gal mRNA in uninduced cells. Thus it would appear that λgd40l phage carries significant amounts of DNA derived from the host chromosome in addition to the gal operon. Finally, our values for both the basal and induced levels of gal mRNA in strain 3300 are very close to those obtained by Miller et al. (90).
which was further supplemented with cyclic AMP to 5 mM. Growth was continued until the cells were harvested at 3 × 10^8 cells per ml. The cell pellets were stored at −20°C until assayed as described previously.

One portion of the culture was retained as a control, and the other was supplemented with cyclic AMP to 5 mM. Growth was continued until the cells were chilled, harvested at 3 × 10^8 cells per ml and 0.4% D-mannitol as carbon source. At a density of 1 × 10^8 cells per ml, the cultures were each divided into two lots, one of which was further supplemented with cyclic AMP to 5 mM. Growth was continued until the cells were harvested at 3 × 10^8 cells per ml. The cell pellets were stored at −20°C until assayed as described previously.

Effect of cyclic AMP on levels of epimerase and β-galactosidase in strains 3300 and M6

| Strain | Addition | Epimerase | β-Galactosidase |
|--------|----------|-----------|----------------|
| 3300   | None     | 0.67      | 4430           |
| 3300   | 5 mM cyclic AMP | 0.71      | 5060           |
| M6     | None     | 3.8       | 4220           |
| M6     | 5 mM cyclic AMP | 2.5       | 6080           |

Effect of Cyclic AMP—There is ample evidence to suggest that cyclic AMP is a positive effector in the process of transcription (7, 31, 32), and that the lowering of the intracellular concentration of cyclic AMP is the likely cause of the repression of inducible enzyme synthesis during catabolite repression (31, 33–35). It is conceivable that concentrations of cyclic AMP might be increased by the capR mutation in strain M6, thereby giving rise to the derepressed rate of transcription of the gal operon in M6.

The data of Table VI indicate that cyclic AMP added to the growth medium does not mimic the effect of a mutation in the capR gene on the synthesis of the gal enzymes. Rather, the specific activity of epimerase in strain 3300 is unaltered by growth for 14 generations in the presence of 5 mM cyclic AMP. Surprisingly, in M6 the specific activity of epimerase reproducibly falls roughly 30% under the same growth conditions. In contrast, in both strains cyclic AMP stimulates β-galactosidase synthesis by 20%. As a further test of the relation between positively regulated phenomena and mucoidy, epimerase and β-galactosidase were assayed in extracts of capR− (M6) and parent (3300) strains grown under conditions of (permanent) catabolic repression. In this case, a medium containing a poor nitrogen source was used to accentuate the repression caused by glucose (35). A comparison of the data of Table VII with those of Table VI indicates that whereas the epimerase in both strains is not subject to catabolic repression under the conditions of the experiment, i.e. at 30°C, β-galactosidase is strongly repressed at this temperature. (It is apparent that cyclic AMP does not reverse the repression of β-galactosidase synthesis in M6 to the same extent as in 3300. The decreased response of β-galactosidase to cyclic AMP in M6 suggests that M6 may be less permeable to cyclic AMP than is its parent.) Thus, under conditions of catabolic repression at 30°C, there is no repression of the gal operon in the parent strain nor any reversal of the derepression of the gal operon caused by the capR mutation.

**TABLE VI**

| Strain | Addition | Epimerase | β-Galactosidase |
|--------|----------|-----------|----------------|
| 3300   | None     | 0.67      | 4430           |
| 3300   | 5 mM cyclic AMP | 0.71      | 5060           |
| M6     | None     | 3.8       | 4220           |
| M6     | 5 mM cyclic AMP | 2.5       | 6080           |

**TABLE VII**

| Catabolic repression in capR− (M6) and parent (3300) strains |
|------------------------------------------------------------|
| Cells were grown to stationary phase in H-1 salts containing 10 μg per ml of thiamine and 0.4% glucose. These cultures were used to inoculate a medium composed of H-1 salts (without ammonium sulfate), 10 μg per ml of thiamine, 1 mM MgSO₄, 0.5% glutamic acid, 5% tryptone broth, and 0.2% glucose. These cultures were aerated at 30°C to a density of 1 × 10⁸ cells per ml. The cell pellets were stored at −20°C until assayed as described previously. |

**Discussion**

The model presented by Markovitz (11, 12) is consistent with the data presented in this paper. The derepression of the gal enzymes and of gal mRNA caused by a mutation in the capR gene in strain M6 appears to be similar to that caused by a mutation in the galR gene. Since we have not measured the kinetics of gal mRNA synthesis, only its level, we cannot say whether the capR product effects the synthesis or the degradation of gal mRNA; however, it seems probable that it is increasing the rate of synthesis by analogy with other regulating systems. The derepression caused by the presence of the capR6 allele occurs even in induced cells, showing that the capR product acts independently of the galR product. The magnitude of the derepression of the gal enzymes by the capR allele is greater in uninduced cells than in induced cells (Table III). However the level of gal enzymes and gal mRNA in induced capR− cells is very close to the sum of the levels in induced capR+ and uninduced capR− cells. This is the expected result if the control by the capR gene was completely independent of the control by the galR gene. Another explanation is that E. coli contains two sets of gal genes, the normal set in the gal operon and another set under the control of the capR locus. This possibility has been eliminated by the isolation of a transferase-negative derivative of strain M6. This strain lacks transferase when it is grown under conditions in which the mucoid phenotype is expressed as well as when it is induced for gal enzyme synthesis. Furthermore, transferase activity is readily isolated which have transferase activity under both conditions, indicating that a mutation in a single locus is responsible for the loss of both the mucoid- and the fucoid-induced transferase activities in the mutant strain.

Markovitz’s proposal that a single repressor-like molecule, the product of the capR gene, is capable of regulating the activity of a number of diverse genes is not without precedent. It simply demands that all of the genes so regulated share a common regulatory site, analogous to an operator. The enzymes of arginine biosynthesis (36, 37) exemplify such a case. What is novel in this model is that the gal operon must respond to two different repressors, the products of the galR and capR genes. Consequently, the gal operon must possess two regions capable of recognizing regulatory proteins, the well known galO region and another site which we will designate galQ. It is this latter region which all genes under the regulation of the capR gene would share.

Evidence from investigations in vivo suggests that the gal operon is less sensitive to the presence of cyclic AMP than is...
the lactose operon. First, the enzymes of the gal operon are completely insensitive to catabolic repression (Table VII). Secondly, a mutant deficient in adenylate cyclase (38) still grows on galactose at rates comparable to glucose. Yet studies of the in vitro transcription of gal mRNA show a complete requirement for cyclic AMP and the cyclic AMP binding protein (39). One explanation for this paradox could be that the gal promoter site has a high affinity for the cyclic AMP-cyclic AMP binding protein complex so that maximal transcription could occur in vivo even at the low concentrations of cyclic AMP present in glucose-repressed cells and in an adenyl cyclase mutant.

Only recently has it been possible to show that the increase in enzyme activity following the induction of the lactose operon is accompanied by a proportional increase in mRNA synthesis from that operon (40). As mRNA from other genes can represent a substantial background. In the case of RNA from uninduced cells of strain 3300, 85 to 90% of the total RNA hybridizing to λgal DNA is probably RNA other than gal mRNA. Only by using phages whose DNA contains a minimum of extraneous material, by performing calculations such as we have, or by using competition hybridization can one arrive at a better estimate of the fraction of the total RNA which is the species of interest. We did not use competition hybridization because we had neither a source of pure gal mRNA, nor a gal deletion mutant. The other approach would be to use RNA from a strain carrying a deletion of the gal operon for competition. However such RNA would either contain some gal mRNA if the deletion were not complete or lack other RNA's if the deletion extended outside the gal operon and so would not solve the problem of precisely defining the level of gal mRNA. Our approach was to use the varied levels of gal mRNA in different strains and make the assumption that gal mRNA and enzyme levels increased proportionally upon induction in M6 and 3300. We have three independent checks which apply to both the data and this assumption. They are (a) that the non-gal hybridizing counts are the same in the two strains, (b) that the amount of non-gal counts is larger with λdg than with λgal DNA, and (c) that the level of mRNA in M6 is 4 times higher than in 3300, which is exactly the ratio of the enzyme levels for the two strains.

Other workers (41) have suggested that translation may be required for gal mRNA and enzyme levels increased proportionally upon induction in M6 and 3300. We have three independent checks which apply to both the data and this assumption. They are (a) that the non-gal hybridizing counts are the same in the two strains, (b) that the amount of non-gal counts is larger with λdg than with λgal DNA, and (c) that the level of mRNA in M6 is 4 times higher than in 3300, which is exactly the ratio of the enzyme levels for the two strains.

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