THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 244, No. 8, Issue of April 25, pp. 2143-2148, 1969
Printed in U.S.A.

The Enzymes of the Galactose Operon in *Escherichia coli*

IV. THE FREQUENCIES OF TRANSLATION OF THE TERMINAL CISTRONS IN THE OPERON*

(Received for publication, September 11, 1968)

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SUMMARY

The frequencies of translation of the structural genes for uridine diphosphogalactose 4-epimerase and for galactokinase have been determined in wild type *Escherichia coli* and found to be equivalent under a variety of conditions. As these structural genes are the terminal cistrons in the galactose operon, it is concluded that polarity is not an inherent characteristic of this operon.

The addition of amino acids to the growth medium doubles the growth rate, and hence the sum of all translation frequencies in the cell, but causes little, if any, change in the translation frequency of the galactose operon. This surprising result indicates that the galactose operon belongs to a subset of units of transcription the translation frequency of which is relatively insensitive to changes in the intracellular concentration of amino acids and ribosomes, and suggests that this translation frequency is strictly controlled by the transcription frequency. The model of Morse, Baker, and Yanofsky (*Proc. Nat. Acad. Sci. U. S. A.*, *60*, 1428 (1968)), in which each messenger RNA is translated once by a limited cluster of ribosomes following immediately behind the grow point for transcription, allows such a strict control. Test of this model against the existing data on the translation of the galactose operon indicates a remarkably good fit.

The three structural genes of the galactose operon are arranged as is shown in Fig. 1. Transcription, and therefore translation, proceeds from *right* to *left* in the diagram. This has been shown by the observation that the RNA transcribed from this operon can form a hybrid with the upper but not the lower of the two DNA strands indicated in the diagram (8). Such an orientation is in agreement with the general model wherein the repressor binds to the DNA of the operator (*O*) to restrict transcription near the point of its initiation.

In this model the galactose operon would constitute a single unit of transcription. This has yet to be demonstrated by measurement of the size or the cistron content of the transcribed RNA. However, the coordinate response of the synthesis of all three enzymes to the addition of inducer or to mutations in the operator region (2) is most simply explained by assuming a single point for the initiation of transcription. Consistent with this explanation is the recent evidence that nonsense (9) and other (10) mutations in gal_t exert a *cis*-dominant polar effect that decreases the ratio of the kinase to epimerase rates of formation over that observed in the wild type.

The mutational polarity generated by nonsense triplets is a general characteristic of operons and indicates a linkage among the cistrons during translation. Its orientation shows a dependence of the translation of one cistron upon the translation of cistrons nearer the operator. However, mutational polarity determined simply by the comparison of enzyme activities in mutant and wild type does not specify the relative frequencies of translation for the cistrons in the operon. Indeed, it does not even dictate that the frequency of translation for the operator-proximal member of a pair of adjacent cistrons be equal to or greater than that for the operator-distal member, since mechanisms of dependence can be imagined which allow the operator-distal member the greater frequency.[1] Conversely, the determination of the relative frequencies of translation for the cistrons in the wild type operon can be useful in restricting and suggesting possible models for the translation mechanism.

In this article we couple the structural information on UDP-galactose 4-epimerase and galactokinase presented in the previous papers of this series (7, 11, 12) with data on the rates of formation of their activities to determine the frequencies of translation of gal_e and gal_k in the wild type operon. The conclusion of these determinations is that these two cistrons are translated with the same frequency. Hence, the wild type operon exhibits no inherent polarity between the terminal cistrons, and all polar mutations in the central cistron cause the translation of the operator-distal cistron to be less frequent than the translation of the operator-proximal cistron.

* This work was supported by grants from the National Institutes of Health.

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[1] For example, it can be supposed that the operator-distal cistron has a site where ribosomes can attach and initiate translation with a higher probability than for the operator-proximal cistron, but that that site attains the required configuration only if a translating ribosome is present on the operator-proximal cistron within some specified distance.

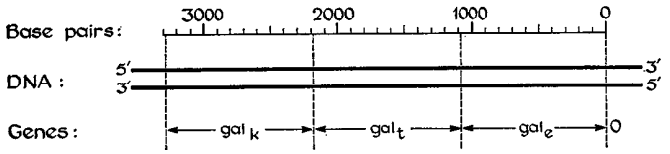

FIG. 1. The galactose operon in *Escherichia coli*. The sequence of the structural genes for galactokinase (gal_k), galactose 1-phosphate uridyl transferase (gal_t), and uridine diphosphogalactose 4-epimerase (gal_e) shown is that determined by genetic linkage (1, 2), deletion mapping (3–5), and physical mapping (4, 6). The orientation corresponds to that in the standard maps of *E. coli* and bacteriophage λdg. The number of base pairs in each gene was calculated from the number of amino acid residues in the corresponding polypeptides (see Table V of Reference 7). The position of the operator, *O*, is taken from the map position of operator-constitutive mutants (2).

We also present observations on the effect of temperature and amino acids on the frequencies of translation for gal_e and gal_k. The translation frequency has about the same temperature coefficient as does the cell growth rate. The addition of amino acids to the medium, on the other hand, doubles the growth rate without exerting much effect upon the translation frequency. These observations are considered in terms of a model recently proposed for the tryptophan operon (13), whereby translation frequencies are determined by transcription frequencies.

EXPERIMENTAL PROCEDURE

Materials

Bacteria—The wild type *E. coli* K12 used here was strain W3110 of E. Lederberg.

Media—The medium designated H is the Medium H of Kaiser and Hogness (14) supplemented with 0.04 M glycerol, and medium H + aa refers to Medium H supplemented with 0.5% Bacto-Casamino acids (Difco).

Reagents—The chemicals used in the enzyme assays and media were the same as those described previously (11, 15).

Methods

Assays—The procedures used for determining the activity of galactokinase and UDP-galactose 4-epimerase have been described previously (15), except that in the latter assay the concentration of UDP-galactose was increased to 5.0×10^{-4} M. This doubling of the substrate concentration increases the rate 1.24-fold. Hence, the unit of epimerase activity, defined as that amount of enzyme which will catalyze the formation of 1 μmole of UDP-glucose per hour under the previous conditions (15), equals the amount of enzyme which will catalyze the formation of 1.24 μmoles of UDP-glucose per hour under the conditions used here. The unit of kinase activity is the amount of enzyme that will catalyze the phosphorylation of 1 μmole of galactose per hour under the conditions used here and previously defined (15).

Protein was measured by the method of Lowry *et al.* (16), with crystalline bovine serum albumin as standard.

Growth and Vital Statistics of Cells—Cultures were grown in flasks aerated and maintained at 27° or 37° by agitation in New Brunswick Gyratory shaking water baths. Growth was followed by measuring the absorbance at 600 mμ (A_{600}) with a Zeiss PMQ II spectrophotometer with the use of a 1-cm light path. The A_{600} is related to the cell mass (dry weight) by (milligrams per ml) = $(0.3) \cdot (A_{600})$. At 37°, the average dry weight per cell for an exponentially increasing population in Medium H was determined to be 0.27 pg, whereas in Medium H + aa, it was 0.45 pg. Numbers of cells were determined as viable cells by plating on TB-agar (14) after dilution in Medium H at 0°.

RESULTS AND DISCUSSION

The following equalities can be computed from the specific activities UDP-galactose 4-epimerase (11) and galactokinase (15), and the number and molecular weight of their polypeptide subunits (7, 12).

1 unit of epimerase activity = 1.17×10^{12} epimerase polypeptides[2]
1 unit of kinase activity = 3.5×10^{12} kinase polypeptides[2]

Changes in amounts of epimerase and kinase activities can thereby be converted to changes in the amounts of the respective polypeptides, P_e and P_k. Application of these conversion factors depends upon the assumption that essentially all P_e and P_k are within the active enzymes, and upon certain assumptions regarding the extraction and measurement of enzyme activity. We consider the results of controls that relate to the validity of the assumptions regarding extraction and measurement of enzyme activity before presenting the kinetics of polypeptide synthesis.

Controls

Extraction—We have no measure of the amount of either enzyme within the cell that is independent of extraction. We have therefore estimated the quality of the extraction by comparing different methods. The results of three different methods for the extraction of epimerase and kinase activities are given in Table I. As each method gives extracts with specific activities within 9% of the average value, it seems unlikely that there is preferential extraction of either enzyme relative to the total protein or that enzyme inactivation is introduced by a particular method. Variations in the amount of total protein extracted are less important, since the calculation of the amount of enzyme per ml of culture is based on the specific activity in a given extract and a single value for the amount of protein that can be extracted per unit mass of cells (see the legend of Fig. 2, below), this value coming from the extraction of a large mass of cells by alumina grinding. Because of its convenience we have used sonic oscillation as the method of extraction in the succeeding kinetic experiments.

Reliability of Enzyme Assays in Crude Extracts—In applying the above factors converting units of activity to numbers of poly-

[2] The term "epimerase polypeptide" refers to the sequence of 360 residues represented twice within the native enzyme molecule (12). Although our evidence indicates that this sequence is contained in a single polypeptide, we have noted (12) that the 360 residues could be distributed among two or more different polypeptides if two *ad hoc* assumptions are made: (*a*) all but one of the polypeptides contain blocked amino-terminal residues, (*b*) the different polypeptides are connected by covalent bonds. However, as all the structures require that each of the 360 residues be specified by a separate codon which must be translated twice to form one enzyme molecule, the possibility of the unlikely alternate structures does not affect the calculation of the translation frequency.

The term "kinase polypeptide" refers to the sequence of 368 residues represented only once in the galactokinase molecule, which our evidence indicates is contained in a single polypeptide (7). Arguments equivalent to those given above with respect to alternate structures and their lack of effect on the calculation of translation frequency can be made (7).

peptides, it is assumed that 1 unit of activity represents the same amount of enzyme when assayed in crude extracts as when assayed in the purified preparations used to determine the conversion factors. This can be tested by mixing the purified enzyme with a crude extract of induced cells, and comparing the sum of the activities measured before mixing to the activity in the mixture. When the number of units of either enzyme in the crude extract was normalized to 1.00, the number of units added from either purified preparation was 1.00. The number of units of each enzyme in the mixture should be 2.00 if the assumption is valid. The values found were 1.96 and 2.06 for the epimerase and kinase activities respectively; each is within experimental error of the expected value.

A structural alteration in the enzyme population, induced by purification and resulting in a change of the mean turnover number, would invalidate application of the conversion factors to crude extracts and would not be detected by the above mixing experiment. Such a possibility would, of course, be rendered most unlikely if one could account for all of the initial activity of the crude extract in the various fractions obtained during purification. Although we did not have this in mind when purifying either enzyme and hence did not take any particular effort to allow a complete accounting, we find that for each enzyme the sum of the activities in the various fractions represents at least three-quarters of the initial activity, of which 40 to 45% is in the final purified preparations (15). Considering possible trivial losses for which we lack information, this sum is not significantly different from the initial activity.

Lack of Polarity in Wild Type Operon

The experiments on the kinetics of polypeptide synthesis generally conform to the following design. Wild type *E. coli* K12 were exposed to a saturating concentration of the inducer, D-fucose, after they had adapted to the conditions of media and temperature as witnessed by strictly exponential growth during the preceding six generations. At various times after the addition of inducer, samples were removed to provide crude extracts which were assayed for protein and enzyme activity. The results of two such experiments are given in Fig. 2, where the number of polypeptides per ml of the induced culture is plotted against the increasing cell mass per ml.

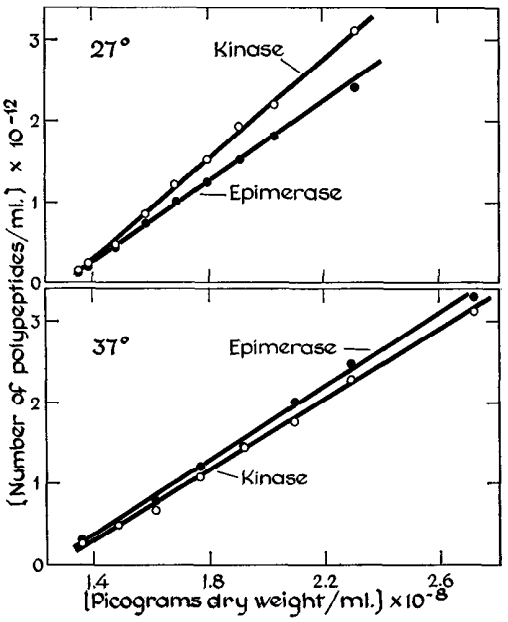

FIG. 2. Differential rate of synthesis for epimerase and kinase polypeptides. The general design of the experiment is given in the text. D-Fucose, as a 1.0 M solution, was added to yield a final concentration of 1.0×10^{-3} M at a cell concentration of 1.35 pg per ml (27°) or 1.30 pg per ml (37°). At the indicated cell concentrations, 5- to 10-ml samples were pipetted into chilled tubes containing 0.01 the sample volume of 1.0 M sodium azide and then centrifuged at 1–2°; the cells were resuspended and subjected to sonic oscillation as is described in the legend to Table I. The extracts were assayed for protein and for epimerase and kinase activities. The units of enzyme per ml of culture were calculated from the specific activities in the extract, with the use of the fact that 1 ml of culture with $A_{600} = 1.0$ yields 0.15 mg of extractable protein. Units of enzyme were converted to numbers of polypeptides as described in the text.

TABLE I

Extraction of galactokinase and UDP-galactose 4-epimerase

Strain W3110 was grown in 1 liter of Medium H + aa at 37° in the presence of 1.0×10^{-3} M D-fucose for five generations of exponential growth; the 6×10^8 cells per ml were then harvested by centrifugation. Two small samples equivalent to 25 ml of culture were resuspended in 2 ml of 5.0×10^{-3} M mercaptoacetic acid-1.0×10^{-2} M potassium phosphate buffer, pH 6.9. One of these was treated for 1.0 min in a Mullard Sonicator. Egg white lysozyme and EDTA were added to the other to obtain a final concentration of 5 μg per ml and 0.01 M, respectively, and the mixture was then incubated for 20 min at 30°. Cells (1.1 g, wet wt) were ground with 2.5 g of alumina, and this mixture was extracted with 30 ml of the solution used for sonic treatment. All three extracts were centrifuged at 14,000 × *g* for 20 min (0–5°), and the supernatants were assayed for protein and for kinase and epimerase activities.

Extraction procedure	Specific activities	
	Galactokinase	UDP-galactose 4-epimerase
	units/mg	*units/mg*
Sonic oscillation	7.5	19.3
Lysozyme	7.4	17.3
Alumina grinding	8.5	20.4
Average	7.8	18.9

The slope of the curve generated by such a plot indicates the number of polypeptides formed per unit increase in cell mass, and is termed the differential rate of synthesis (17). The straight lines observed in Fig. 2 indicate that the cells are in the induced steady state, typified by a constant differential rate of synthesis which is at least 16-fold greater than that for the uninduced steady state (see Table II, Footnote *a*). The differential rates in the minimal Medium H determined from the data in Fig. 2 are given in Table II along with a similar set of rates determined in minimal medium supplemented with amino acids (Medium H + aa).

In either medium, whether at 27° or 37°, the differential rate for P_e synthesis is essentially the same as that for P_k synthesis. The rate for P_e is always within 20% of that for P_k, and, on the average, is within 10%. This equivalence also characterizes the uninduced steady state, since the increase in differential rate due to the addition of inducer is the same (±10%) for each polypeptide. It should be noted, however, that significant derepression

TABLE II

Translation frequencies

Conditions of growth			Differential rates of induced synthesis[a]			No. of P_e or P_k synthesized per cell per generation	Translations per genome per min
Medium	Temperature	Generation time[b]	P_e	P_k	Mean of P_e and P_k		
		min	(*polypeptides/pg*) $\times 10^{-4}$				
H	37°	80	2.2	2.2	2.2	5900	43
H	27	220	2.4	3.0	2.7		
H + aa	37	40	1.2	1.3	1.25	5600	50
H + aa	27	97	1.1	1.0	1.05		

[a] These induced rates are 16-, 31-, and 18-fold greater than the uninduced rates for the conditions, Medium H at 37°, H at 27°, and H + aa at 27°, respectively. Except for the conditions of H + aa at 37°, the differential rates were computed from the straight lines of plots such as those shown in Fig. 2. The differential rates in H + aa at 37° were calculated from the specific activities of the sonically treated extract given in Table I; the number of polypeptides per pg, dry weight, of cells after five generations of induction should be 97% of the differential rate of induced synthesis.

[b] The generation time is defined here as the time necessary for the exponentially growing culture to double its size.

exists even in the uninduced state (defined here as the absence of external inducer), and is presumably due to the presence of internal inducers (18). It is therefore possible that more complete repression could reveal a lack of coordinate translation of gal_e and gal_k similar to that observed for the terminal cistrons in the repressed tryptophan operon of either *E. coli* (19) or *Salmonella typhimurium* (20), which is presumed to be due to a low efficiency site for the initiation of transcription located between the terminal cistrons.

The simplest conclusion from these results is that the terminal cistrons are translated with equal frequencies in the wild type and, consequently, that the galactose operon exhibits no inherent polarity. The corollary to this conclusion is that all polar mutations in gal_t cause gal_k to be translated less frequently than gal_e.

It should be noted that since the epimerase cistron must be translated twice to produce one enzyme molecule (12) whereas only one translation of the kinase cistron is required per galactokinase molecule (7), the rate of formation of galactokinase is twice that of UDP-galactose 4-epimerase.

The galactose operon is like the tryptophan operon in *E. coli* in regard to the equal frequencies of translation of the terminal cistrons. Although occupying 6700 base pairs (twice that for the galactose operon, Fig. 1) and containing five structural genes, the terminal cistrons of the tryptophan operon are translated with the same frequency when the operon is in the derepressed wild type state (13, 19). This is certainly not the case for the lactose operon in *E. coli*, which in most other regards is like the galactose operon. It includes three structural genes contained in a segment of 5400 base pairs, of which the 3700 closest to the operator (*z* cistron) specify the β-galactosidase polypeptide; the remainder are about equally divided between the *y* and *a* genes which specify the M and acetylase polypeptides.[3] Brown, Brown, and Zabin (24) and Zabin (25) have observed that in the derepressed wild type state, the *z* cistron is translated about 5-fold more frequently than is the *a* cistron located at the opposite terminus; apparently the *y* cistron is translated with the same frequency as is the *a*.[4] The status of the inherent polarity among the nine genes of the histidine operon in *S. typhimurium* is uncertain. It may exhibit a polarity having the same orientation as that in the lactose operon since it appears that the first two genes (counting from the operator) are translated more frequently than the third and sixth, which are translated with equal frequency.[5]

Hence there is a general rule by which all these operons abide, whether they are wild type or whether they contain polar mutations. Each translation of a given cistron is accompanied by at least one translation of every cistron nearer the 5′ terminus of the polycistronic RNA chain, translations which abort because of nonsense triplets being included in the count. The absence of significant exceptions to this rule strongly suggests that these operons follow a common mode of translation in which their RNA is sequentially translated starting at the 5′ end, a model to which we shall return.

Nonrepressive Factors Which Could Influence Enzyme Formation

Post-translational Reactions—Were post-translational reactions rate-limiting, polypeptide precursors could accumulate in sufficient amounts to make the values given in Table II but a small fraction of the total P_e or P_k translated. This possibility is made unlikely by the recent observations of Michaelis and Starlinger (27) on the kinetics of the transition from uninduced to induced steady states.

They observed intervals for completion of the epimerase, transferase, and kinase transitions which occupied 0.010, 0.019, and 0.026 generations after the addition of inducer, respectively (generation time, 120 min). This chronological sequence equals the genetic sequence (Fig. 1, starting from the operator), and is consistent with the sequential translation given above. The transition intervals are, however, inconsistent with post-translational rate-limiting reactions which allow accumulations of polypeptide precursors in amounts comparable to or larger than the respective enzymes. The rate of such reactions would be expected to be proportional either to the concentration of precursor or to the square of that concentration, depending upon whether the enzyme contains one polypeptide chain (galactokinase) or two (UDP-galactose 4-epimerase). In either case, the transition intervals are so short that in order to increase the precursor concentration sufficiently to account for the at least 16-fold increase in rate of enzyme formation, the translation frequency during the interval would have to be at least an order of magnitude greater than that necessary to maintain the induced steady state (this calculation proceeds from an uninduced state satisfying the condition that the amount of precursor is equal to or larger than the amount of enzyme). As we have likewise observed such short intervals for the epimerase and kinase transitions under the conditions given in Table II, we assume that post-translational reactions are not rate-limiting here.

Temperature—The differential rates given in Table II exhibit little change in either medium when the temperature is changed by 10°. This insensitivity to temperature indicates that the reactions limiting translation of the galactose operon have about

[3] The gene sizes are calculated from the molecular weights of the β-galactosidase (21, 22), M (23), and acetylase (24) polypeptides assuming an average residue weight of 110 daltons and three base pairs per residue.

[4] Eugene Kennedy, personal communication.

[5] M. N. Margolies, M. J. Voll, J. C. Loper, and R. G. Martin quoted by Voll (26).

the same temperature coefficient as the reactions limiting the growth rate in the 27–37° range. This result is to be compared to the observations that both cell mass and the differential rate of total RNA synthesis appear to be invariant with changes of temperature in this range (p. 75 of Reference 28).

Amino Acids—Addition of amino acids to the medium causes about a 2-fold increase in the growth rate (2.0-fold at 37° and 2.3-fold at 27°; Table II), which is accompanied by about a 2-fold decrease in the mean differential rate of polypeptide formation (1.8-fold at 37° and 2.6-fold at 27°; Table II). This indicates that for a given bacterial mass, the frequency of translation of the galactose operon per min is relatively unchanged by the addition of amino acids.

We should like to know if the frequency of translation per genome per min is likewise unchanged, as it should be since the cell mass per genome is known to be relatively insensitive to changes in growth rate (p. 74 of Reference 28). The differential rates expressed in Table II as the number of polypeptides translated per pg of new cell mass can be converted to the number of polypeptides translated per cell per generation simply by multiplying by the cell dry weight, which at 37° is 0.27 pg in Medium H and 0.45 pg in Medium H + aa (see "Methods"). The result, given in Table II, indicates that the larger weight of the cells formed at the more rapid growth rate compensates for the lower proportion of enzyme so that the number of polypeptides per cell is about the same in the two media. However, the amount of DNA and, hence, the average number of genomes per cell increases with increased cell mass. We have not measured the average number of genomes per cell under these two growth conditions, but rather have taken the values given by Maaløe and Kjeldgaard (p. 74 of Reference 28) for the same growth rates, namely 1.7 and 2.8 for Media H and H + aa at 37°, respectively. When the number of polypeptides per cell per generation is divided by the genomes per cell and by the generation time, the desired frequencies of translation per genome per min are obtained (Table II). Clearly these frequencies are insensitive to addition of amino acids relative to the growth rate and, hence, relative to the sum of all translation frequencies (the percentage of dry weight which is protein is little, if at all, dependent on growth rate (p. 70 of Reference 28)).

We discuss the theoretical implication of this surprising result below. A practical extrapolation of the result is that decreasing the growth rate of induced cultures should increase the specific activity of the galactose enzymes in the crude extracts, making such extracts preferable as a starting material for purification of these enzymes. This effect is observed for the differential rates, given as polypeptides per pg in Table II, when shifting from Medium H + aa to Medium H. It has also been observed by us (p. 2479 of Reference 11) and by others (29) as an increase in specific activity when the growth rate decreases late in the growth phase on media essentially the same as Medium H + aa.

Application of Model of Morse, Baker, and Yanofsky (13) to Galactose Operon

The addition of amino acids to the growth medium not only increases their intracellular concentration many fold (30), but also results in an increase of the intracellular concentration of ribosomes that is almost as great as the increase in growth rate (pp. 82 and 90 of Reference 28). The translation frequency of the galactose operon is evidently saturated with respect to the intracellular concentration of ribosomes and amino acids, even in the minimal medium. Under the conditions of our experiments, some other factor apparently limits the translation frequency of this operon. As the growth rate and presumably the sum of all translation frequencies are doubled by the amino acid addition, the limiting factor would appear to be at least partially specific to the galactose operon, *i.e.* specific to some subset of cistrons which includes the galactose operon. This suggests that the frequency of transcription of this operon exerts a strict control on the frequency of translation that is not influenced by the changes in the intracellular level of amino acids and ribosomes applied here.

A model which allows such a dependence of the frequency of translation on the frequency of transcription has been proposed by Morse, Baker, and Yanofsky (13) to account for the results of their elegant experiments on the temporal relationship between translation and transcription of the tryptophan operon. We terminate this article by comparing some of the characteristics of the galactose operon to their model.

Model (13)—The messenger RNA is translated as it is transcribed by a cluster of 100 ribosomes located immediately behind the RNA polymerase and occupying 2000 ± 700 nucleotide residues of the polycistronic RNA chain. The cluster yields 100 polypeptides from each cistron, starting from the 5′ end of the RNA, and only one cluster is formed per RNA chain. Hence, 100 polypeptides are formed from each cistron per transcription event. Transcription is initiated periodically, the period for the derepressed tryptophan operon at 37° being 2½ to 3 min in minimal medium and 2 to 2½ min in enriched medium (31). The rate of transcription along the operon is 1000 and 1200 nucleotide residues per min in the two media at 37°, the slower rate being in the minimal medium (31). The maximum number of transcriptions that can occur simultaneously on a single tryptophan operon of 6700 base pairs is therefore three, spaced 2700 ± 300 base pairs apart.

Model and Galactose Operon—In the steady state, the frequency of translation per genome per min will equal the number of ribosomes per cluster divided by the period between transcriptions. With a cluster of 100 ribosomes, this translation frequency for the tryptophan operon is 33 to 40 in minimal medium, and 40 to 50 in enriched medium. These values are in good agreement with the two values given in Table II for the galactose operon in Media H and H + aa. Although this agreement indicates that the translation frequencies of the two operons are the same, in the terms of the model, it specifies neither the size of the cluster nor the transcription period for the galactose operon.

We can obtain an estimate of these quantities from the data of Michaelis and Starlinger (27). As Morse, Baker, and Yanofsky (13) have pointed out, there is a strong suggestion in the data of Michaelis and Starlinger that the increase in enzyme activity in the first few minutes after the addition of inducer obeys a step function. This is the prediction of the model provided that the translation of a given cistron is completed by the last ribosome in the first cluster well before the first ribosome in the second cluster has translated that cistron. The period between steps will represent the transcription period, and the amplitude of the step will reflect the size of the cluster.

The period of the steps discernible from their data (27) is about 2 min, while the amplitude represents an increase in enzyme equal to about one-fourth the uninduced level. The growth at 37° was in a minimal medium similar to medium H (the generation time of 120 min is longer because succinate rather than glycerol was used as the carbon source). Under these conditions we can expect the number of polypeptides of a given type per un-

induced cell to be about one-sixteenth the value in the induced cell (Table II), or roughly 400. The step amplitude therefore represents about 100 polypeptides per cell. With an estimated 1.6 genomes per cell under these conditions (p. 74 of Reference 28), each step yields about 60 polypeptides per genome. Hence we arrive at the estimates of a cluster of 60 ribosomes and a transcription period of 2 min for cells grown at 37° in succinate-minimal medium. These values provide a translation frequency of 30 translations per genome per min.

While it is clear that more accurate values for the period and amplitude exhibited by the galactose operon should be obtained by preventing successive rounds of transcription, the technique used so effectively for the tryptophan operon (13, 31), we have presented these first approximations because of their remarkable agreement with both the data in Table II and that for the tryptophan operon. Furthermore, we note that a minimum rate for the transcription of the galactose operon can be calculated from its size (Fig. 1) and the 3-min interval observed by Michaelis and Starlinger (27) between addition of inducer and the first increase of kinase activity. The resulting value is 1100 nucleotide residues per min, in agreement with the rate of transcription for the tryptophan operon at 37° (31). The characteristic of the model which places the first ribosome of the cluster in close proximity to the RNA polymerase makes this minimal rate a good approximation to the true rate, provided that the time required to initiate transcription after addition of inducer is small compared to the above interval. This provision appears to be satisfied, as the intervals between addition of inducer and the rise in epimerase and transferase activities are about 1 and 2 min, respectively (27). This rate and the estimated 2-min period between successive transcriptions indicate that a given galactose operon would undergo two simultaneous transcriptions spaced about 2200 base pairs apart.

In sum, the existing data on the kinetics of induction of the galactose operon conform closely to the model. Accepting it as our best working hypothesis, what can we say about the insensitivity of the translation frequency to the 2-fold increase in concentration of the ribosomes resulting from enrichment of the medium? We infer from the above data on the tryptophan operon that the transcription frequency is only slightly (20%), if at all, increased by such enrichment. Since the translation frequency is also only slightly increased by enrichment (16%; see the last column of Table II), and is the product of the transcription frequency and the cluster size, we can conclude that the cluster size is not influenced by the 2-fold change in ribosome concentration. The mechanism determining cluster size has yet to be specified, but the restraint imposed by the preceding conclusion makes unlikely mechanisms which depend upon a simple competition between ribosome attachment and nucleolytic degradation at the 5′ end of the RNA molecule.[6] The restraint would be satisfied, however, by a counting mechanism in which the attachment of each ribosome results in the loss of a fixed, small number of nucleotide residues from the 5′ end of the RNA molecule, making this end unrecognizable to further ribosomes after the 100th attachment. Whatever the case, it is clear that further testing of the model depends largely upon specification of the mechanisms that determine both the cluster size and the transcription frequency.

[6] The recent evidence of Morse, Mosteller, Baker, and Yanofsky (to appear in *Nature*, personal communication) that degradation of the messenger RNA of the tryptophan operon is initiated at the 5′ end, rather than at the 3′ end as originally proposed (13), allows this supposition.

REFERENCES

1. Adler, J., and Kaiser, A. D., *Virology*, **19**, 117 (1963).
2. Buttin, G., *J. Mol. Biol.*, **7**, 164, 183 (1963).
3. Adler, J., and Templeton, B., *J. Mol. Biol.*, **7**, 710 (1963).
4. Kayajanian, G., and Campbell, A., *Virology*, **30**, 482 (1966).
5. Pfeifer, D., and Oellermann, R., *Mol. Gen. Genet.*, **99**, 248 (1967).
6. Hogness, D. S., *J. Gen. Physiol.*, **49**, 29 (1966).
7. Wilson, D. B., and Hogness, D. S., *J. Biol. Chem.*, **244**, 2137 (1969).
8. Guha, A., Tabaczynski, M., and Szybalski, W., *J. Mol. Biol.*, **35**, 207 (1968).
9. Jordan, E., and Saedler, H., *Mol. Gen. Genet.*, **100**, 283 (1967).
10. Jordan, E., Saedler, H., and Starlinger, P., *Mol. Gen. Genet.*, **100**, 296 (1967).
11. Wilson, D. B., and Hogness, D. S., *J. Biol. Chem.*, **239**, 2469 (1964).
12. Wilson, D. B., and Hogness, D. S., *J. Biol. Chem.*, **244**, 2132 (1969).
13. Morse, D. E., Baker, R. F., and Yanofsky, C., *Proc. Nat. Acad. Sci. U. S. A.*, **60**, 1428 (1968).
14. Kaiser, A. D., and Hogness, D. S., *J. Mol. Biol.*, **2**, 392 (1960).
15. Wilson, D. B., and Hogness, D. S., in E. F. Neufeld and V. Ginsberg (Editors), *Methods in enzymology, Vol. 8*, Academic Press, New York, 1966, p. 229.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
17. Monod, J., Pappenheimer, A. M., Jr., and Cohen-Bazire, G., *Biochim. Biophys. Acta*, **9**, 648 (1952).
18. Wu, H. C., and Kalckar, H. M., *Proc. Nat. Acad. Sci. U. S. A.*, **55**, 622 (1966).
19. Morse, D. E., and Yanofsky, C., *J. Mol. Biol.*, in press.
20. Bauerle, R. H., and Margolin, P., *Proc. Nat. Acad. Sci. U. S. A.*, **56**, 111 (1966).
21. Steers, E., Jr., Craven, G. R., Anfinsen, C. B., and Bethune, J. L., *J. Biol. Chem.*, **240**, 2478 (1965).
22. Brown, J. L., Koorajian, S., Katze, J., and Zabin, I., *J. Biol. Chem.*, **241**, 2826 (1966).
23. Jones, T. H. D., and Kennedy, E. P., *Fed. Proc.*, **27**, 644 (1968).
24. Brown, J. L., Brown, D. M., and Zabin, I., *J. Biol. Chem.*, **242**, 4254 (1967).
25. Zabin, I., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 431 (1963).
26. Voll, M. J., *J. Mol. Biol.*, **30**, 122 (1967).
27. Michaelis, G., and Starlinger, P., *Mol. Gen. Genet.*, **100**, 210 (1967).
28. Maaløe, O., and Kjelgaard, N. O., *Control of macro-molecular synthesis*, W. A. Benjamin, New York, 1966.
29. Jordan, E., Saedler, H., Lengeler, J., and Starlinger, P., *Mol. Gen. Genet.*, **100**, 203 (1967).
30. Holden, J. T., in J. T. Holden (Editor), *Amino acid pools*, American Elsevier Publishing Company Inc., New York, 1962, pp. 73, 566.
31. Baker, R. F., and Yanofsky, C., *Proc. Nat. Acad. Sci. U. S. A.*, **60**, 313 (1968).