Galactose-Specific Messenger Ribonucleic Acid Contents in *Escherichia coli*

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A method is described for measuring the proportion of galactose-specific mRNA (gal-mRNA) in the total RNA extracted from pulse-labelled cells of *Escherichia coli* K12, by DNA–RNA hybridization with DNA prepared from bacteriophage λ dg. RNA from wild-type *E. coli* was compared with RNA from a homogenote carrying the *gal* operon both in the chromosome and in a substituted sex-factor, and with RNA from a deletion strain that carried the galactose operon only in the exogenote. In each case the cultures were induced with fucose. Under these conditions the amount of gal-mRNA was found to be proportional to the content of galactokinase in the different cultures, and to the gene frequency. The amounts of gal-mRNA in an O^c mutant and an R^- mutant were also proportional to the observed contents of galactokinase. In cultures repressed for the enzymes of the galactose operon with thiomethylgalactoside, the content of gal-mRNA was higher than expected from the content of galactokinase. Possible explanations of this finding are discussed.

The galactose operon of *Escherichia coli* K12 consists of structural genes that specify three enzymes of galactose metabolism, together with controlling elements (Buttin, 1963b). The three enzymes (kinase, transferase and epimerase) are co-ordinately and sequentially inducible (Buttin, 1963a; Michaelis & Starlinger, 1967). The effective inducers are the substrate D-galactose and the gratuitous inducer D-fucose. Control of induction is mediated by a regulator gene situated at a remote position on the *E. coli* chromosome (Buttin, 1963b), and control is of the negative type (Saedler, Gullon & Starlinger, 1968). The galactose operon is thus closely analogous to the lactose operon, and offers the advantage that the operator and regulator loci are widely separated.

Because of its similarity to the lactose operon, regulation of the galactose operon is generally interpreted in terms of the classical Jacob–Monod mechanism. Accordingly, control of the content of the enzymes is thought to be brought about by control of the rate of mRNA synthesis, and consequently of the amount of mRNA available for transcription. We therefore expect the amount of enzyme activity in an exponentially growing culture to reflect the mRNA content. Previous direct measurements of the amounts of enzyme and mRNA in induced and non-induced cultures have not always been consistent with this expectation, however.

In the present paper we report the results of a further study of the relationship between enzyme and mRNA contents in exponentially growing cells. Enzyme contents were varied in two ways: by induction and repression, and by varying gene dosage. mRNA was measured by the method described by Bishop & Irving (1971). The results show that enzyme and mRNA contents are strongly correlated, except when the galactose enzymes are repressed with thiomethyl galactoside.

MATERIALS AND METHODS

Bacteria. The strains of *E. coli* used were as follows. Strain W3805, F⁺-gal22 (epimerase-negative); strain E1 F⁺-*histrpsr*⁺ (Frame & Bishop, 1971); strain 9-3, F⁺-*histrpsr*⁺ gal, a deletion of the entire galactose operon running from *att*^λ to *aro*-G (Frame & Bishop, 1971; see Fig. 1); strains 9-3t and 9-3h, *trp*⁺ and *his*⁺ derivatives respectively of strain 9-3; strain TMG6, F⁺-*histrpsr*⁺ Rgal, a regulator gene mutant produced from strain E1 as described by Buttin (1963b); strain CN4, F⁺-*histrpsr*⁺ O^c gal, a spontaneous operator constitutive mutant of strain E1 isolated by Miss C. Ford; strains E1 (F⁺8), E1 (FR5) and 9-3h(FR5), derivatives carrying episomes (F⁺8 or FR5) which contain the galactose operon. F⁺8 is the substituted sex-factor isolated by Hirota & Sneath (1961), and contains both the *gal* and *aro*-G loci. FR5 is a sex-factor containing *gal*, *aro*-G and genes conferring resistance to chloramphenicol (Cm), ampicillin (Pn), tetracycline (T) and streptomycin (S). This was produced at the suggestion of Dr E. Meynell, by transducing part of the

Fig. 1. Genetic map of the strains of *E. coli*, sex-factors and bacteriophage used. The distances between markers are arbitrary. The galactose operon (*gal*) is shown divided into kinase (*k*), transferase (*t*), epimerase (*e*) and operator (*o*) regions. The eight blocks of the transferase cistron were defined previously (Davison, Frame & Bishop, 1967). The left-hand end of λ dg166 defines blocks 7 and 8. The left-hand end of λ dg178 falls between *gal* and *aro-G*. Both F'8 and FR5 carry *gal* and *aro-G* and do not carry *att*⁺, but the extent of the segment of the *E. coli* chromosome is not more precisely known in either case. *E. coli* 9-3 carries *att*⁺ and lacks *aro-G* and *gal*. —, *E. coli*; ▨, F; ▩, RTF; □, λ DNA.

RTF R1-19 (R;S,Su,K,Cm,Pn,T) from one strain of *Salmonella typhimurium* LT-2 to another, and then mobilizing the drug-resistance factors by introducing F'8 and conjugation with *E. coli* 9-3t, selecting both for drug resistance and for the ability to ferment galactose. The factor was initially very unstable, but was successfully stabilized by five cycles of conjugal transfer (from 9-3h to 9-3t or vice versa) each followed by a period of relaxation and then re-selection for drug-resistance and ability to ferment galactose.

Bacteriophage. The bacteriophages used were as follows: bacteriophage λ c₁⁸⁵⁷; bacteriophage λ dg 178, a derivative of bacteriophage λ c₁⁸⁵⁷ which carries the entire galactose operon, but does not carry the *aro-G* locus; bacteriophage λ dg 166, a derivative of wild-type bacteriophage λ that carries the epimerase cistron and a small piece of the transferase cistron, subsequently made heat-inducible by recombination with bacteriophage λ c₁⁸⁵⁷.

Growth and purification of transducing bacteriophage. Strain W3805, doubly lysogenic for the wild-type and transducing bacteriophage, was inoculated from minimal-galactose plates into minimal-galactose medium and grown overnight at 33°C with vigorous shaking. The culture was added to 12 litre of broth (1% tryptone, 0.1% yeast extract, 0.8% NaCl, 0.1% MgSO₄, 0.03% CaCl₂, 0.2% galactose) and grown with vigorous aeration to a density of about 10⁹ cells/ml (*E*₆₅₀ 0.8). The temperature was raised to 42°C, and after 20 min it was lowered again to 37°C. Aeration was continued until the cells were lysed. Chloroform was added and the culture was vigorously agitated, but not aerated. After being kept overnight at room temperature, the lysate was decanted, and transferred to the cold-room. To each litre of lysate were added 23 g of NaCl, 300 ml of 30% (w/v) polyethylene glycol 6000 and 15 ml of 40% (w/v) dextran sulphate 500. After 1–2 days at 4°C the lower phase and interphase were withdrawn and centrifuged at 10000 rev./min for 40 min in a Sorvall RC-2B centrifuge. The interphase was

recovered and dissolved in water to give a final volume of 20 ml/litre of initial lysate. One-quarter of the volume of 3 M-KCl was added and after being kept overnight at 4°C insoluble material was removed by centrifugation and discarded. The supernatant was centrifuged at 30000 rev./min in the Spinco no. 30 rotor at 4°C for 120 min to sediment the bacteriophage, and the pellet was taken up in 2 ml of 0.1 M-NaCl–7 mM-MgCl₂/litre of initial lysate and treated with 5 μ g of ribonuclease/ml and 10 μ g of deoxyribonuclease/ml for 30 min at 37°C. The bacteriophage was again sedimented by centrifuging for 45 min at 30000 rev./min in the no. 30 rotor, and the pellets were taken up in 0.1 M-NaCl–0.01 M-tris-HCl buffer, pH 7.5. Solid CsCl was added to 46% (w/w) and the solution was centrifuged for 1 h at 20000 rev./min in the Sorvall SS-34 rotor. The supernatant solution was then centrifuged (15 ml/tube overlaid with liquid paraffin) for 42 h in the Spinco no. 30 rotor at 27000 rev./min. Fractions were collected from the tubes, the transducing bacteriophages were identified and pooled fractions were dialysed against 0.1 M-NaCl–0.01 M-tris-HCl buffer, pH 7.5.

³²P-labelled wild-type bacteriophage λ was prepared by heat-inducing strain E1 (λ c₁⁸⁵⁷) grown in broth (as described above, but with glucose substituted for galactose) containing 1–2 mCi of [³²P]P_i/100 ml. The labelled bacteriophage was purified as described above, except that each overnight period at 4°C was replaced by stirring for 1 h in an ice-bath, and the final equilibrium centrifugation was carried out at 45000 rev./min in the Spinco no. 50 rotor.

DNA was prepared as required from suitable mixtures of labelled and unlabelled bacteriophage by extracting three times with water-saturated phenol. The final aqueous phase was dialysed against 10 mM-NaCl–10 mM-tris-HCl buffer, pH 7.5. The specific radioactivity of the DNA was measured by carefully measuring its extinction at 260 nm and its radioactivity when applied to membrane filters of the type used for DNA–RNA hybridization experiments. The extinction of a 1 mg/ml solution was taken to be 20. Less than 1% of labelled wild-type bacteriophage was added to unlabelled transducing bacteriophage preparations.

Growth of bacteria. Cells were grown in 400 ml batches in a rotary shaker at 37°C in glycerol–casamino acids (casein hydrolysate) medium containing 5 mM-D-fucose or 2.5 mM-thiomethyl galactoside in some cases. The cells were pulse-labelled at a cell density of about 10⁹/ml (*E*₆₅₀ 0.8) by adding 50 μ Ci of [¹⁴C]uracil/400 ml of culture, and harvested after precisely 1% of the doubling time by pouring each 400 ml of culture on to 160 ml of crushed frozen buffer [10 mM-tris-HCl (pH 7.5 at 0°C)–10 mM-MgCl₂–10 mM-Na₂N₃]. The cells were collected by centrifugation and taken up in 10 mM-Na₂N₃–5 mM-EDTA–0.1 M-NaCl–20 mM-sodium acetate buffer, pH 5. At this point the preparation could be stored at –20°C.

RNA was prepared from freshly harvested or frozen and thawed cells as described by Bishop & Robertson (1969).

DNA–RNA hybridization. The method of Gillespie & Spiegelman (1965) was used, with the minor modifications described in the preceding paper. Annealing was for 40 h at 65°C in 0.3 M-NaCl–30 mM-sodium citrate buffer, pH 7.

Assay of galactokinase. During growth in glycerol–casamino acids medium (see above) a 40 ml portion was

removed when the E_{650} of the culture was 0.6, and chilled in ice. After 30 min the E_{650} was again measured. The cells were collected by centrifugation, washed with 40 ml of 0.1M-sodium phosphate buffer, pH 7.2, recentrifuged and resuspended in 4 ml of phosphate buffer containing 5 mM-GSH. A 0.25 ml portion of the suspension was mixed with 0.75 ml of a solution containing 1 mg of lysozyme/ml, 5 mM-tris-acetate buffer, pH 8, 0.25 mM-EDTA, 1 mM-GSH and incubated for 5 min at 37°C. Lysis was checked by microscopic examination. The assay was carried out by mixing three 0.025 ml samples of lysate with 0.025 ml of a solution containing 0.05 μ mol of [14 C]-galactose (specific radioactivity 1 Ci/mol), 0.1 μ mol of ATP, 0.0832 μ mol of $MgCl_2$, 0.2 μ mol of NaF and 2.5 μ mol of tris-HCl buffer, pH 7.5 at 37°C. After 0, 5 and 10 min at 37°C the reaction in one tube was stopped by adding 0.95 ml of ice-cold water and placing the tube in an ice bath. Samples (0.1 ml) were spotted on 2.3 cm diameter DEAE-paper discs (Whatman DE81) and the discs were washed four times in water to remove galactose. The discs, which retain galactose 1-phosphate quantitatively, were dried and assayed in a liquid-scintillation counter. Enzyme activities were normalized by dividing by the extinction of the chilled culture, and by the activity/extinction ratio for a culture of *E. coli* strain E1 grown, harvested, and assayed in parallel.

Calculation of the proportion of galactose mRNA. The principle of the method is described by Bishop & Irving (1971). After annealing various amounts of total cellular RNA with a constant amount of DNA a plot is constructed of H/c against H , where H is the ratio of input RNA to DNA recovered (DNA recovered being the only estimate available of the DNA input) and c is the radioactivity in galactose mRNA hybridized/ μ g of DNA recovered. A straight line is fitted to the plot and values of c_s and K are obtained from the slope and intercept respectively according to the relationship:

$$H/c = H/c_s + K \quad (1)$$

In the present case we are faced with the difficulty that mRNA other than gal-mRNA hybridizes with bacteriophage λ dg DNA (Guha, Tabaczynski & Szybalski, 1968). We dealt with this in the following way. The polypeptide chains specified by the kinase, transferase and epimerase cistrons are approximately equal in size [kinase, 38 000 daltons (Wilson & Hogness, 1964); transferase, 40 000 daltons (Saito, Ozutsumi & Kurahashi, 1967); epimerase, 40 000 daltons (Wilson & Hogness, 1966)]. The transducing bacteriophage λ dg 166 carries only the epimerase cistron and a portion, probably very small, of the transferase cistron. We assume that the gal-mRNA that hybridizes with λ dg 166 DNA is one-third of the total, ignoring the small amount of transferase RNA and any RNA that may be transcribed from the controlling elements of the operon. Secondly, we assume that the difference between hybridization to λ dg 178 DNA and λ dg 166 DNA represents two-thirds of all gal-mRNA, ignoring any RNA that may hybridize with λ dg 178 DNA to the left of the *gal* operon (see Fig. 1). Thus the radioactivity in gal-mRNA hybridizing at any given value of H is taken to be $3(c_{178} - c_{166})/2$, where c_{178} and c_{166} are the amounts of radioactivity in mRNA hybridized to λ dg 178 and λ dg 166 DNA respectively at a given value of H . In practice, it was impossible to arrange that the amounts

of the two types of DNA were precisely the same, owing to variable retention by the membrane filters. Values of $(c_{178} - c_{166})$ were therefore obtained by fitting straight lines to plots of H/c_{178} against H and H/c_{166} against H , reading off values for several values of H , and calculating $(c_{178} - c_{166})$ from these values.

The specific radioactivity of gal-mRNA (s , c.p.m./ μ g) was calculated from c_s according to eqn. 5 of the previous paper (Bishop & Irving, 1971), taking $M\lambda = 30 \times 10^6$. The value of $Mgal$ was calculated to be 1.02×10^6 by multiplying the sum of the molecular weights of the three polypeptide chains by 8.5 (the ratio of the molecular weight of an average trinucleotide, less one molecule of water, to the molecular weight of an average *E. coli* amino acid, less one molecule of water):

$$s = 1.75c_s M\lambda / Mgal = 53c_s \quad (2)$$

Then the proportion of the total RNA which is gal-mRNA is:

$$\alpha = 1/K's = 0.019/K'c_s \quad (3)$$

and the proportion of the total radioactivity contained in gal-mRNA is:

$$\beta = 1/K'S \quad (4)$$

where S is the overall specific radioactivity of the total RNA.

RESULTS

Control experiments. (a) Time-course of hybridization. Fig. 2 shows the result of a time-course experiment with λ dg178 DNA and total pulse-labelled RNA from fucose-induced cells of the homogenote E1 (F'8). The amount of radioactivity becoming hybridized increased at least up to 40 h, which was adopted as the standard annealing time.

(b) Effect of varying the amount of DNA. The results for rRNA (Bishop & Irving, 1970) show that under the conditions used for these experiments, the specific hybridization (RNA hybridized/ μ g of DNA) is more directly related to the RNA/DNA ratio than to the RNA concentration. Fig. 3 shows that this is true also for the hybridization of *E. coli* pulse-labelled RNA with λ dg178 DNA. In Fig. 3(a) the hybridization (c , c.p.m./ μ g of DNA) is plotted against the RNA/DNA ratio (H). The curves generated by using 1.2 and 4.4 μ g of DNA per sample are virtually the same. In contrast, when c is plotted against the RNA concentration, the two curves are quite different (Fig. 3b).

(c) Hybridization to DNA of wild-type λ and hybridization of RNA from a deletion strain. RNA extracted from pulse-labelled cells of strain E1 (F'8) was compared with RNA from strain 9-3t, which carries a deletion from *att* ^{λ} to beyond *aro*-G. Both strains were grown in the presence of D-fucose. The RNA was annealed with DNA from wild-type λ and from λ dg178. When RNA from strain E1 (F'8) was used, about 20 times more radioactivity

hybridized to λ dg178 DNA than to wild-type DNA (Fig. 4). Thus at least 95% of the homology between pulse-labelled RNA and λ dg178 DNA is due to sequences not present in the wild-type. Very little hybridization was observed between RNA from the deletion strain and either type of DNA. Thus, when hybridization is observed, it is due to transcription from the region deleted, i.e. from *att*^A to beyond *aro*-G. This conclusion was tested further by hybridization-competition experiments between labelled E1 (F'8) RNA and unlabelled 9-3t RNA. In agreement with the results of the saturation experiments, very little competition was observed.

Fig. 2. Time-course of hybridization. The ¹⁴C-labelled RNA was extracted from a fucose-induced culture of E1 (F'8). ³²P-labelled λ dg178 DNA was prepared as described. The samples contained an average of 4.6 μ g of DNA (2.3 μ g/filter) and the RNA/DNA ratio was 96.

Measurement of galactose-specific mRNA. Fig. 5 shows the results of an experiment in which pulse-labelled RNA from a fucose-induced culture of strain E1 (F'8) was annealed in parallel with λ dg178 and λ dg166 DNA. Fig. 5(a) shows the hybridization observed as a function of RNA/DNA ratio (*H*). Fig. 5(b) shows the hybridization of gal-mRNA deduced from the data as described above, and Fig. 5(c) shows the reciprocal plot for gal-mRNA. This plot gives K' 1.08, c_s 42.6, from which *s* is

Fig. 4. Hybridization of RNA from a fucose-induced culture of E1 (F'8) and from the deletion strain 9-3h with DNA from λ dg178 and wild-type λ . $\times$, E1 (F'8) RNA $\times$ λ dg178 DNA; $\blacksquare$, E1 (F'8) RNA $\times$ λ DNA; $\circ$, 9-3h RNA $\times$ λ DNA.

Fig. 3. Effect of DNA concentration on DNA-RNA hybridization. The samples contained an average of 1.2 ($\bullet$) and 4.4 ($\circ$) μ g of DNA (0.6 and 2.2 μ g per filter). The annealing time was 40 h. (a) Hybridization (*c*) plotted against DNA/RNA ratio (*H*). (b) Hybridization plotted against RNA concentration (*h*).

Fig. 5. Calculation of the hybridization of gal-mRNA. (a) Hybridization of RNA extracted from a fucose-induced culture of E1 (F'8) with λdg178 (○) and λdg166 (●) DNA. (b) Hybridization of gal-mRNA deduced from 5(a). (c) Reciprocal plot of the hybridization of gal-mRNA.

Table 1. *Variation of gal-mRNA with gene dosage and enzyme content*

All cultures were induced with D-fucose and pulse-labelled with [¹⁴C]uracil. The number of gene copies is given relative to wild-type assuming that in glycerol-casamino acids (generation time about 50 min) there is one replicating fork per chromosome on average, and two episomes per fork (Frame & Bishop, 1971). With one exception the amount of galactokinase was measured in a sample of the culture removed just before the pulse of uracil, and compared with a parallel sample of strain E1. In the exceptional case (*) the content of galactokinase commonly found in strains of E1 (F'8) was used.

Strain	Genotype	Gene copies	Kinase	Kinase	$10^5 \times \alpha$	$10^5 \alpha / \text{Kinase}$
				Gene copies		
E1	<i>gal</i> ⁺	(1)	(1)	(1)	23	23
E1 (F'8)	<i>gal</i> ⁺ / <i>Fgal</i> ⁺	2.4	2.4*	1*	41.3	17
E1 (FR5)	<i>gal</i> ⁺ / <i>Fgal</i> ⁺	2.4	2.32	0.97	49.5	21
9-3h (FR5)	Δgal / <i>Fgal</i> ⁺	1.4	1.05	0.75	17.7	17
		1.4	1.31	0.94	24.8	19
					Average	19

Table 2. *Variation of gal-mRNA content with enzyme induction and repression*

Cultures were grown in glycerol-casamino acids medium and induced with D-fucose or repressed with thiomethyl galactoside. In most cases enzyme activities were measured on samples removed from the cultures just before pulse-labelling and compared with parallel samples of strain E1. In some cases (*) the enzyme activity characteristic of the strain and growth conditions was adopted. —, Indicates neither induced nor repressed, i.e. with glycerol alone.

Strain	Genotype	State of culture	Kinase	$10^5 \times \alpha$	$10^5 \alpha / \text{Kinase}$
E1	gal ⁺	Induced	(1)	23	23
CN 4	gal ⁺ 0 ^c	—	1.73	37.6	22
TMG 6	gal ⁺ R ⁻	—	1.13	27.8	24
E1 (F'8)	gal ⁺ /Fgal ⁺	Induced	2.4*	41.3	17*
		Repressed	0.12*	6.2	52*
9-3h (FR5)	$\Delta gal/Fgal^+$	Induced	1.31	24.8	19
		—	0.15	3.9	26
		Repressed	0.08	3.2	40

Table 3. *Specific radioactivities of total and gal-mRNA*

The specific radioactivity of the total RNA was calculated directly. That of the gal-mRNA was obtained by using eqn. 2. [The overall specific radioactivities of the E1 (F⁺8) preparations shown in Tables 1 and 2 are not available.]

Strain	State of culture	Specific radioactivity of total RNA (<i>S</i>) (c.p.m./ μ g)	Specific radioactivity of gal-mRNA (<i>s</i>) (c.p.m./ μ g)	<i>s/S</i> ratio
E1	Induced	693	21 360	30.8
E1 (FR5)	Induced	654	21 360	32.6
CN4	—	338	12 640	37.4
TMG6	—	507	17 070	33.6
9-3h (FR5)	Induced	533	30 500	57.2
	Induced	344	19 860	57.8
9-3h (FR5)	—	256	18 640	73
	Repressed	277	26 140	94

Table 4. *Proportion of total radioactive label contained in gal-mRNA*

Values of β were calculated by using eqn. 4. Values of α are taken from Tables 1 and 2.

Strain	State of culture	β (%)	β (normalized)	α (normalized)
E1	Induced	0.5	(1)	(1)
E1 (FR5)	Induced	1.2	2.4	2.2
CN4	—	1.0	2.0	1.6
TMG6	—	0.7	1.4	1.2
9-3h (FR5)	Induced	0.6	1.2	0.8
	Induced	0.5	1.0	1.1
9-3h (FR5)	—	0.13	0.26	0.17
	Repressed	0.15	0.30	0.14

found to be 2260 c.p.m./ μ g and $\alpha = 41.3 \times 10^{-4}$; i.e. in this case the gal-mRNA is estimated to make up 0.04% of the total cellular RNA.

(a) Variation of gal-mRNA with gene dosage. The results of a number of experiments are shown in Table 1. As previously reported, the galactokinase content in fucose-induced cells closely parallels the calculated gene dosage (Frame & Bishop, 1971). The values of α for the different genotypes are compared in Table 1 with the galactokinase contents observed in the same cultures. The ratios in the last column are relatively constant, showing that as gene dosage is varied in fucose-induced cultures the same proportional change occurs in both the mRNA and enzyme contents. The closeness of agreement here, where agreement is fully expected, is a partial justification of the method used to measure the mRNA content. Our estimate is that in fucose-induced wild-type cells, in the exponential phase of growth, galactose-specific mRNA makes up about 0.02% of the total mRNA.

(b) Variation of gal-mRNA with enzyme induction and repression. The contents of mRNA found in O⁺ and R⁻ strains were higher than that found

in the wild-type (Table 2). The increases, however, were exactly proportional to the higher enzyme contents in these strains. The mRNA contents found in repressed cultures were less than those in corresponding induced cultures, but the reduction was less than the fall in the galactokinase content. The results suggest that each gal-mRNA molecule is translated 2-3 times less often in the repressed culture. There is an indication that in the non-induced culture mRNA may also be less frequently translated than in fucose-induced cells. However, we consider the difference observed (Table 2, line 7) too small for confident interpretation at this time.

(c) Specific radioactivities of total RNA and gal-mRNA. The specific radioactivities of the total cellular RNA and specific radioactivities calculated for gal-mRNA are shown in Table 3. The highest gal-mRNA specific radioactivities are about half that expected (55×10^3 c.p.m./ μ g) if the [¹⁴C]uracil had been incorporated directly without dilution by endogenous uracil, and if all the pre-existing mRNA had been degraded during the pulse.

Because of variation in the specific radioactivity of the [¹⁴C]uracil and the difficulty of controlling precisely the timing of the pulse, the rate of cooling

of the pulsed culture, and other features of the labelling procedure, variations in the absolute specific radioactivities of the different RNA preparations are probably not meaningful. (An exception is the total RNA from strain CN 4, which in repeated tests consistently had a specific radioactivity close to half that of RNA from a parallel culture of strain E1. The reason for this is unknown.) The ratio of specific radioactivities, s/S (Table 3), being internally controlled, provides a more valid basis for comparison. Two main effects are seen. (1) Strain 9-3h, with a deletion from *att*^A to *aro*-G, consistently shows a higher ratio (s/S) than the other strains. All of these strains, it should be noted, were derived from strain E1. The reason for this effect is not clear, but it may be related to the slightly longer generation time (10% longer) of strain 9-3h. (2) The ratio (s/S) is greater for the non-induced and repressed cultures of strain 9-3h (FR5) than for the induced culture. Since these cultures grow at the same rate, and the inducer and repressor are not metabolized, this suggests that gal-mRNA becomes labelled more rapidly in the induced culture, relative to the other species of RNA. The most direct explanation of this is that the lifetime of gal-mRNA is longer in the induced culture, so that newly synthesized gal-mRNA is diluted to a greater extent than it is in the non-induced and repressed cultures.

(d) Proportion of the total radioactivity contained in gal-mRNA. Values of β (eqn. 4) are shown in Table 4. The absolute wild-type (induced) value of β is rather higher than values reported by Hill & Echols (1966) and Guha *et al.* (1968). This may be due in part to differences in the growth conditions and labelling of the cells, and partly to our use of an extrapolation method. The ratio of induced to non-induced 9-3h (FR5) gal-mRNA is close to the ratios previously reported. Our ratio is 4. Hill & Echols (1966) who made no allowance for the hybridization of non-galactose-specific mRNA to λ dg DNA found a ratio of 3.4, and Guha *et al.* (1968) who measured hybridization to the separated strands of λ dg DNA found a ratio of 5. Since Guha *et al.* (1968) did not use an extrapolation method, they would tend to underestimate the non-induced content more seriously than the induced.

The normalized values for β are very similar to those for α except in the case of the non-induced and repressed cultures (Table 4). This is clearly related to the exceptionally high specific radioactivity of the gal-mRNA in these cases. These differences emphasize the importance of measuring the actual amount of mRNA rather than the radioactivity it contains.

For the fully-induced wild-type culture $\beta = 0.005$ and $\alpha = 0.00023$, i.e. there is a 21-fold difference in

the absolute values. The difference is due to the lability of mRNA relative to the stable types. If in our short labelling period incorporation into stable and unstable RNA is approximately equal (Mangiarotti & Schlessinger, 1967; Salser, Janin & Levinthal, 1968), gal-mRNA in the induced culture accounts for $(2 \times \beta)$ or 1% of the radioactivity in unstable RNA. If we assume that the rate of labelling of gal-mRNA is typical of all unstable RNA, we can calculate that unstable RNA makes up about (100×0.00023) or about 2% of the total bacterial RNA. This is in good agreement with previous more direct estimates (Lieve, 1965; Mangiarotti & Schlessinger, 1967; Salser *et al.* 1968; Mueller & Bremer, 1968).

DISCUSSION

It is not possible to set limits to the accuracy of the estimates we have made, because of the number of assumptions involved. The main support for the method is the close correlation observed between gal-mRNA content and enzyme activity when gene-dosage is varied. However, this corroborates the relative contents rather than the absolute determinations. The relative contents are set by the products $K'c$, (see eqn. 3) for the different mRNA preparations, and the normalized values of α (Table 4) which depend only on $K'c$, are independent of the value assumed for M_{gal} and the adjustment made for hybridization efficiency.

However, the number of gal-mRNA molecules per gene copy may be calculated from the data, and as pointed out before this calculation is independent of M_{gal} . We use eqn. (8) of the preceding paper:

$$m = \alpha QMb/oM_{gal} = 31000\alpha$$

taking the RNA/DNA ratio of cells with a generation time of 50 min (Q) to be 11.5 (Manor, Goodman & Stent, 1969), the amount of DNA per genome (Mb) to be 2700×10^6 daltons, and the average number of genes per genome equivalent (c) to equal 1. For a fucose-induced wild-type cell (Table 1) we take a value of $\alpha = 2 \times 10^{-4}$ and $m = 6$ copies per gene. This, of course, is the steady-state content of gal-mRNA equivalents. If all mRNA molecules are in the process of simultaneous synthesis and degradation, the number of different molecules being concurrently synthesized will be greater than 6. From Table 2 we can calculate that a non-induced or repressed wild-type culture will contain just under 1 gal-mRNA equivalent per gene, and an O⁺ mutant 10 copies. These values are still subject to the adjustment made for hybridization efficiency (1.75) and would have to be modified if this should prove to be incorrect. If the average cell contains 1.4 genome equivalents of DNA, the

average number of gal-mRNA equivalents per cell is 8.4 and 1.2 for the induced and non-induced wild-type, and 14 for the O^c.

Our results show a lack of agreement between enzyme and mRNA contents in the case of the repressed culture, and possibly also the non-induced culture. This raises the question of whether there is a separate translational control mechanism. First, however, we must take account of the fact that when the proportion of gal-mRNA is very small, the possibility of obtaining an erroneous result increases. Failure to discount a small amount of hybridization due to RNA other than gal-mRNA might not significantly affect the calculations for the induced culture, but might be enough to increase the apparent gal-mRNA content in the repressed culture by the factor of 2-3 observed. This is a distinct possibility in the case of E1 (F8) but is much less likely in the case of 9-3h (FR5). In the latter case nearly all of the hybridization observed is attributed by our method to gal-mRNA, presumably because the remaining homology between the wild-type *E. coli* RNA and λ dg DNA is deleted both from the chromosome and from the hybrid sex-factor. Our calculated ratio of 3:1 for the gal DNA in λ dg 178 and λ dg 166 is directly supported by the results of Guha *et al.* (1968). Even if it is not strictly correct, it is very unlikely to be inaccurate by a factor of two.

The lower utilization of the gal-mRNA in the repressed cultures may be due to translational control. However, the existence of a second special mechanism to bring about a two- to three-fold reduction in the enzyme activity when transcriptional control has already brought about a seven- to eight-fold reduction in the content of mRNA seems at first sight unlikely. It is reasonable, therefore, to try to interpret the effect as an incidental result of transcriptional control. The work of Ito & Imamoto (1968) and of Morse, Baker & Yanofsky (1968) suggests that a package of ribosomes is entrained behind the polymerase molecule during the simultaneous translation and transcription of the *trp* operon. Synthesis of the mRNA molecule at the 3'-end of the growing chain is thought to be accompanied by degradation from the 5'-end, following the entrained ribosomes. The results we have obtained may be interpreted formally in terms of this model by supposing that in the repressed culture the number of entrained ribosomes is twofold less than in the induced

culture. This could be brought about by an earlier onset of RNA degradation from the 5'-end of the mRNA molecule, and we have shown that more rapid degradation of gal-mRNA may occur in the repressed culture. In terms of transcriptional control we expect the principal difference between induced and repressed cultures to be in the length of time during which repressor is associated with the operator region. To complete the formal model we must suppose that mRNA degradation is facilitated by the attachment of repressor to the operator.

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REFERENCES

- Bishop, J. O. & Irving, M. I. (1971). *Biochem. J.* **121**, 105.
 Bishop, J. O. & Robertson, F. W. (1969). *Biochem. J.* **115**, 353.
 Buttin, G. (1963a). *J. molec. Biol.* **7**, 164.
 Buttin, G. (1963b). *J. molec. Biol.* **7**, 183.
 Davison, J., Frame, R. & Bishop, J. O. (1967). *Genet. Res., Camb.*, **10**, 107.
 Frame, R. & Bishop, J. O. (1971). *Biochem. J.* **121**, 93.
 Gillespie, D. & Spiegelman, S. (1965). *J. molec. Biol.* **12**, 829.
 Guha, A., Tabaczynski & Szybalski, W. (1968). *J. molec. Biol.* **35**, 207.
 Hill, C. W. & Echols, H. (1966). *J. molec. Biol.* **19**, 38.
 Hirota, Y. & Sneath, P. H. A. (1961). *Jap. J. Genet.* **36**, 308.
 Ito, J. & Imamoto, F. (1968). *Nature, Lond.*, **220**, 441.
 Lieve, L. (1965). *J. molec. Biol.* **13**, 862.
 Mangiarotti, G. & Schlessinger, D. (1967). *J. molec. Biol.* **29**, 395.
 Manor, H., Goodman, D. & Stent, G. S. (1969). *J. molec. Biol.* **39**, 1.
 Michaelis, G. & Starlinger, P. (1967). *Molec. gen. Genet.* **100**, 210.
 Morse, D. E., Baker, R. F. & Yanofsky, C. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 1428.
 Mueller, K. & Bremer, H. (1968). *J. molec. Biol.* **38**, 329.
 Saedler, H., Gullon, A. & Starlinger, P. (1968). *Molec. gen. Genet.* **102**, 79.
 Saito, S., Ozutsumi, M. & Kurahashi, K. (1967). *J. biol. Chem.* **242**, 2362.
 Salser, W., Janin, J. & Levinthal, C. (1968). *J. molec. Biol.* **31**, 237.
 Wilson, D. B. & Hogness, O. S. (1964). *J. biol. Chem.* **230**, 2469.
 Wilson, D. B. & Hogness, D. S. (1966). In *Methods in Enzymology*, vol. 8, p. 229. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.