J. Mol. Biol. (1971) **61**, 275–279

L-Arabinose Operon Messenger of *Escherichia coli*

Its Inducibility and Translation Efficiency Relative to Lactose Operon Messenger

Synthesis of messenger RNA from the positively controlled L-arabinose operon is shown to be induced by the presence of arabinose in the medium. Messengers from the arabinose and lactose operons are translated with comparable efficiencies.

In *Escherichia coli* L-arabinose and a functional arabinose *C* protein are required for normal induction of the enzymes necessary for growth on arabinose (Sheppard & Englesberg, 1967). The positive control exhibited in the arabinose operon is in sharp contrast to the negative transcriptional control of the lactose operon. A repressor binding to DNA at the lactose operator prevents synthesis of messenger from the operon and induction releases the repressor allowing synthesis of the messenger (Gilbert & Müller-Hill, 1967). At present it is not known whether expression of positively controlled operons is also at the level of transcription. Also unknown is how the efficiency of translation of arabinose messenger compares with the translation efficiency of messenger from any other operon.

The purpose of the experiments presented here is twofold: first, to show that induction of the arabinose operon leads to synthesis of appreciable quantities of arabinose operon messenger and, second, to compare arabinose and lactose messenger translation efficiencies. These results show that the arabinose operon most likely is transcriptionally controlled and that the isomerase gene of its messenger is translated with nearly the same efficiency as the β-galactosidase gene on messenger from the lactose operon.

RNA–DNA hybridization was used to quantitate the arabinose specific messenger. RNA was radioactively labeled in cells whose arabinose operon was uninduced or induced. This RNA was then assayed by hybridization to DNA purified from the arabinose transducing phage λ-dara (Schleif & Greenblatt, 1970). Figure 1 shows that there is no detectable amount of arabinose specific messenger in uninduced cells, but that in induced cells arabinose specific messenger is present in amounts at least 50 times background. The same result is seen when a constitutive strain (RFS 22, C^c) (Schleif, 1969) is used instead of one induced by the addition of arabinose.

The relative translation efficiencies of messenger from the arabinose and lactose operons were determined by measuring the rates of arabinose isomerase and β-galactosidase synthesis and the amounts of operon specific messenger in a culture simultaneously induced for both operons. The rates of enzyme synthesis in monomers per unit time were calculated by assaying the enzymes and using the known turnover numbers and the relative amounts of messengers were determined by hybridization to filters containing λ*dara* or λp*lac*$_5$ DNA (Shapiro *et al.*, 1969). Both RNA's were labeled simultaneously in the same culture to avoid problems in comparing their specific activities. The hybridization measurement was made more precise by labeling

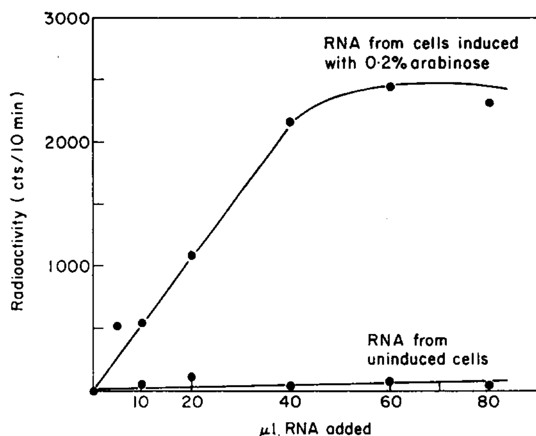

Fig. 1. Hybridization of RNA from uninduced and arabinose induced cells to DNA from phage λ*dara* immobilized on filters. Cells of K12 strain HfrH were grown with a doubling time of 60 min in 10 ml. M9–B1–0·2% glycerol to 5×10^8 per ml., and 100 μCi [^{3}H]uridine at 25 Ci/m-mole were added. After 3 min, 10 ml. of medium at -10°C containing 0·02 M-NaN_3 were added. Cells were spun down and resuspended in 2 ml. SSC (SSC is 0·15 M-NaCl, 0·015 M-Na_3 citrate), 0·5 ml. 5% sodium dodecyl sulfate, 5×10^{-3} M-EDTA were added and the cells were frozen in acetone–dry ice and thawed twice at 37°C. Nucleic acids were extracted by adding 2·5 ml. chilled distilled phenol, saturated with SSC, and mixing for 5 min at 4°C after which the phases were separated by a low speed centrifugation. The phenol phase was re-extracted with another 2·5 ml. SSC and 0·5 ml. of 2 M-KCl and 10 ml. of 95% ethanol were added to the combined aqueous phases and the solution was put at -10°C overnight. The solution was centrifuged for 30 min at 3500 *g*, and the invisible pellet resuspended in 0·5 ml. SSC. This was then dialyzed against two changes of SSC and used in the hybridizations. DNA for the hybridizations was prepared from the λ*dara* phage. The phage was purified on a CsCl step gradient followed by 2 equilibrium bandings in CsCl to separate the helper phage. After dialysis against 0·01 M-Tris·HCl (pH 7·6), 0·05 M-NaCl, 0·001 M-NaEDTA, DNA was extracted with 50% recovery by adding sodium dodecyl sulfate to 0·5% and incubating for 10 min at 65°C. KCl was added to 0·5 M and K^+–sodium dodecyl sulfate was spun out at 0°C. The DNA was extensively dialyzed into SSC. Fifty μg of denatured DNA was bound to 47 mm Millipore nitrocellulose filters by the method of Gillespie & Spiegelman (1965). For hybridizations, small circles were punched out with a sharp cork borer and marked with a ballpoint pen. The annealing was done in 13 mm × 100 mm tubes capped with a marble and covered with Saran wrap. The tubes contained 0·2 ml. of 2 × SSC, 5% dimethylsulfoxide and indicated amounts of the RNA in addition to a blank filter without DNA and filters containing λ*dara*. After annealing for 12 hr at 65 ± 0·5 deg. C, all filters were combined in a tube and given 5 1-min rinses with 5 ml. 2 × SSC. They were then resuspended in 1 ml. of 2 × SSC and one drop of 1 mg RNase/ml. (previously heated to 95°C for 10 min in water) was added for 1 hr. The filters were then given 5 more rinses, dried and counted. Non-specific binding was less than 20 cts/min in all cases.

the DNA's to be immobilized on the filters with [^{32}P]phosphate in order to allow an accurate measure both of the amount of DNA immobilized and the amount retained at the end of the annealing period.

For any single species of RNA the rate of hybrid formation is proportional to the product of the concentration of free RNA and the concentration of unhybridized homologous DNA; hence, for relatively short incubations during which the concentrations of free RNA and homologous DNA do not change appreciably, the rate of hybrid formation is linear with time. Thus, if linear hybridization kinetics are seen for two RNA's of similar molecular weights, a measurement of these rates and of the DNA on the respective filters suffices to determine the relative concentrations of the two RNA's. The size distribution of the two classes of RNA's being measured here should be very similar, since their maximum sizes are close. The sum of the subunit

molecular weights of the three
(Patrick & Lee, 1969; Lee & Ber
subunit molecular weight of β-ga
1965). (The lac RNA is hybridized
but only part of the *y* gene. The r
slightly reduces the hybridizatio
the diffusion rate of the molecule
tion are approximately linear; the
did not change significantly duri
hybridizing RNA's was not excess
relative translation efficiencies of
that an arabinose messenger co
lactose messenger, the errors inhe

The inducibility of the arabi
messenger is controlled in the ara
to the reservation that messenger
but protected from degradation i
can tentatively be assigned a role i

Measurements presented here al
are translated with very similar e
efficiencies was not necessarily ex
even within a single polycistroni
efficiencies. The coat gene of the
efficiently as the gene coding for t

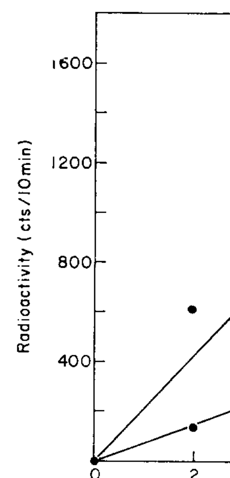

Fig. 2. Kinetics of hybridization of a
^{3}H-labeled RNA was prepared as desc
enzymes (RFS 22) and induced for lact
which had been added 5 generations be
DNA's immobilized on the hybridizatio
for the phage were grown on YT (Schlei
$1 \cdot 6 \times 10^{-2}$ M (Ames, 1966). At the time
were added to 300 ml. of cells. The filt

molecular weights of the three arabinose enzymes is 60,000 + 50,000 + 48,000 (Patrick & Lee, 1969; Lee & Bendet, 1967; Lee, Patrick & Masson, 1968), and the subunit molecular weight of β-galactosidase is 135,000 (Craven, Steers & Anfinsen, 1965). (The lac RNA is hybridized to DNA containing an intact β-galactosidase gene, but only part of the y gene. The remaining lac RNA without homologous DNA only slightly reduces the hybridization rate of an intact messenger by slightly lowering the diffusion rate of the molecule.) Figure 2 shows that the kinetics of hybrid formation are approximately linear; therefore, the amounts of RNA and homologous DNA did not change significantly during the hybridization and the size distribution of hybridizing RNA's was not excessively broad. Table 1 shows the calculation of the relative translation efficiencies of the two messengers. Although the results show that an arabinose messenger codes for 60% more monomers of enzyme than a lactose messenger, the errors inherent in this type of measurement are at least 30%.

The inducibility of the arabinose-specific messenger implies that synthesis of messenger is controlled in the arabinose operon. However, this conclusion is subject to the reservation that messenger may be degraded very rapidly in uninduced cells, but protected from degradation in induced cells. Subject to this proviso, C protein can tentatively be assigned a role in controlling transcription of the arabinose operon.

Measurements presented here also show that the arabinose and lactose messengers are translated with very similar efficiencies. The near identity in the two translation efficiencies was not necessarily expected, for it has become clear that different genes even within a single polycistronic message may be translated at greatly different efficiencies. The coat gene of the RNA phage MS2 is translated at least ten times as efficiently as the gene coding for the maturation protein (Nathans, Oeschger, Polmar

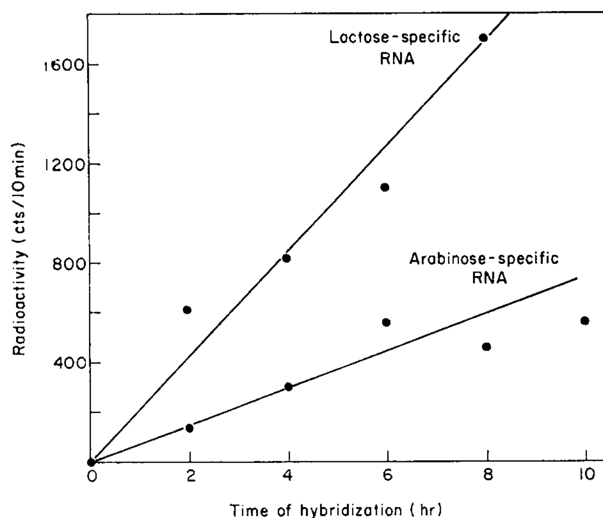

FIG. 2. Kinetics of hybridization of arabinose and lactose messages to $\lambda dara$ and $\lambda plac_5$ DNA's. ^{3}H-labeled RNA was prepared as described in Fig. 1 from a strain constitutive for arabinose enzymes (RFS 22) and induced for lactose messages by 5×10^{-4} M-isopropyl-β-D-thiogalactoside, which had been added 5 generations before the labeling. In this experiment the $\lambda dara$ and $\lambda plac_5$ DNA's immobilized on the hybridization filters were labeled with [^{32}P]phosphate. Cells lysogenic for the phage were grown on YT (Schleif, 1969) medium with phosphate concentration adjusted to $1 \cdot 6 \times 10^{-2}$ M (Ames, 1966). At the time the phages were heat induced, 0·1 mCi of [^{32}P]phosphate were added to 300 ml. of cells. The filters containing DNA were prepared as described in Fig. 1.

TABLE 1

Relative translation efficiencies of arabinose and lactose messengers

	Enzyme assay, (monomers/0·1 ml. cells[a])	λd*ara* or λp*lac* DNA bound to filter[b]	Relative rate of hybrid formation messenger mass/unit time[c]	Relative number of messengers[d]	Relative rate of enzyme synthesis/ messenger equivalent[e]	Relative translation efficiency[f]
Arabinose	$3{\cdot}02\times10^{11}$	1·00	1·00	1·00	$3{\cdot}0\times10^{11}$	$\frac{3{\cdot}0\times10^{11}}{1{\cdot}9\times10^{11}} = 1{\cdot}6$[g]
Lactose	$2{\cdot}65\times10^{11}$	2·01	2·84	1·41	$1{\cdot}9\times10^{11}$	

[a] Since both enzymes have been induced for many cell doublings, a measurement of their relative numbers is equivalent to a measurement of their relative rates of synthesis. The arabinose isomerase assay was done in duplicate at 3 concentrations as described by Schleif (1969). For calculation of the number of monomers, the specific activity of pure isomerase was used (Patrick & Lee, 1968) with a subunit molecular weight of 60,000 (Patrick & Lee, 1969) and the values were corrected for the 0·54 activity which isomerase has in my buffers, compared to its activity in Lee's buffers. The β-galactosidase assay was done in duplicate, as described by Craven *et al.* (1965), and the number of monomers was calculated from the specific activity of pure enzyme taking the subunit molecular weight to be 135,000 and the molar extinction of *O*-nitrophenol at 420 nm to be 21,300.

[b] Relative amounts of DNA as determined from the [^{32}P]phosphate in the DNA. The amount of λd*ara* DNA on the filters is taken to be 1·0.

[c] Calculated from Fig. 2. Cts/min hybridized/unit time taking the rate for *ara* messenger to be 1·00. The relative masses of the 2 messenger RNA's found after prolonged induction will equal the relative amounts of radioactivity in the 2 messenger RNA's following the 3-min labeling if their rates of decay are equal. The half-lives for decay of the 2 messengers were shown to be equal, both $1{\cdot}75 \pm 0{\cdot}25$ minutes by sensitizing cells with EDTA (Leive, 1965), growing 10 min, inducing both operons for 1 min, adding rifampicin to 50 μg/ml. and measuring the kinetics of residual enzyme synthesis.

[d] The relative masses of each messenger present, equal the rates of hybrid formation divided by the relative amounts of homologous DNA on the filters. To convert these numbers to relative *numbers* of messenger molecules, the masses must be divided by their molecular weights, which are here taken to be equal.

[e] Equals the number of monomers/0·1 ml./relative number of messengers.

[f] Equals the relative rate of isomerase synthesis/isomerase messenger divided by the relative rate of galactosidase synthesis/galactosidase messenger.

[g] Discussion of errors. The least precise datum for the calculations is the rate of hybrid formation, as shown in Fig. 2, where it is apparent that the precision is approximately 30%. The most likely source of systematic errors appears to be in my measurement of enzyme activities or the literature values of the specific activity or the subunit molecular weights of the enzymes; however, it seems unlikely that any of these are in error by more than 20%.

& Eggen, 1969) and Hendrix (1970) has shown that in ultraviolet irradiated cells infected with λ there is as large as a 100-fold difference in the translation efficiency of several of the late genes coded for by the same messenger.

The method used here to compare the arabinose and lactose messenger translation efficiencies is general and can be used for any genes present on transducing phages for which an absolute rate of synthesis can be measured. Once one absolute measurement of the translation efficiency of an operon has been made, i.e. the number of ribosomes that translate a messenger and its average lifetime, or equivalently, the rate of movement and the average spacing between ribosomes on a messenger, then measurements of the type presented here can be used to calculate the values for any other suitable operon.

This work is publication no. 794 from the Graduate Department of Biochemistry, Brandeis University. It was supported in part by a research grant from the National Institutes of Health (no. GM-18,277) and by U.S. Public Health Service Career Development Award (no. K4-GM-38,797).

Graduate Department of Biochemistry
Brandeis University
Waltham, Mass. 02154, U.S.A.

ROBERT F. SCHLEIF

Received 9 March 1971, and in revised form 15 July 1971

REFERENCES

Ames, B. (1966). In *Methods in Enzymology*, ed. by E. Neufeld and V. Ginsburg, vol. 8, p. 116. New York: Academic Press.
Craven, G., Steers, E. & Anfinsen, C. (1965). *J. Biol. Chem.* **240**, 2468.
Gilbert, W. & Müller-Hill, B. (1967). *Proc. Nat. Acad. Sci., Wash.* **58**, 2415.
Gillespie, B. & Spiegelman, S. (1965). *J. Mol. Biol.* **12**, 829.
Hendrix, R. (1970). Ph.D. thesis, Harvard University.
Lee, N. & Bendet, I. (1967). *J. Biol. Chem.* **242**, 2043.
Lee, N., Patrick, J. & Masson, M. (1968). *J. Biol. Chem.* **243**, 4700.
Leive, L. (1965). *J. Mol. Biol.* **13**, 862.
Nathans, D., Oeschger, M., Polmar, S. & Eggen, K. (1969). *J. Mol. Biol.* **39**, 279.
Patrick, J. & Lee, N. (1968). *J. Biol. Chem.* **243**, 4312.
Patrick, J. & Lee, N. (1969). *J. Biol. Chem.* **244**, 4277.
Schleif, R. (1969). *J. Mol. Biol.* **46**, 185.
Schleif, R. & Greenblatt, J. (1970). *Cold Spr. Harb. Symp. Quant. Biol.* **35**, 369.
Shapiro, J., MacHattie, L., Eron, L., Ihler, G., Ippen, K. & Beckwith, J. (1969). *Nature*, **224**, 768.
Sheppard, D. & Engelsberg, E. (1967). *J. Mol. Biol.* **25**, 443.