Polarity and Transcription in the Galactose Operon of E. coli

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SUMMARY: In wild type E. coli, gal mRNA shows the same survival time as the average mRNA, but in a strain carrying a strong polar mutation in the gal operon, the percentage of the total mRNA which is gal mRNA decreases as the length of the labeling time is increased. Most of this decrease is due to a loss of mRNA distal to the polar mutation. These results indicate that the decreased level of gal mRNA present in this strain results from an increased rate of degradation of the mRNA distal to the polar mutation. In contrast, the low level of gal mRNA found (at the restrictive temperature) in a strain with a mutation rendering protein synthesis temperature sensitive does not appear to result from degradation.

One of the common features of polar mutations in the bacterial operon (see the review by Zipser, 1969) is the reduction in the level of mRNA corresponding to regions distal to the mutation (Contesse, Naono, and Gros, 1966; Imamoto and Yanofsky, 1967). There are essentially two theories to explain this finding. The first suggests that untranslated mRNA distal to the nonsense codon is readily degraded by nucleases, a process which may functionally inactivate the mRNA coding for the next cistron (Morse and Yanofsky, 1969; Contesse, Crepin and Gros, 1970). Support for this notion comes from the finding that strains carrying the suA allele show a general relief of polarity (Beckwith, 1963; Morse and Primakoff, 1970), and appear to lack a dispensible ribonuclease (Kuwano, Schlessinger and Morse, 1971). An alternate hypothesis is that transcription is coupled to translation (Ames and Hartman, 1963; Stent, 1964; Imamoto, 1970; Imamoto, Kano, and Tani, 1970), that is, the transcription of a given cistron requires the simultaneous translation of the corresponding mRNA. This would explain the absence of mRNA and enzyme products from regions distal to the terminating codon.

These two conflicting models for polarity have been developed largely

from work with the tryptophan operon. We decided to test these models in the case of the galactose operon by examining the transcription of this operon in two situations where synthesis of galactose enzymes is blocked. In one case, a nonsense codon in the transferase cistron terminates translation of the distal kinase cistron. In the other case, a temperature-sensitive mutation in the translocation (G) factor of strain G1 (Tocchini-Valentini, Felicetti and Rinaldi, 1969) serves as a means of introducing a general block in protein synthesis.

MATERIALS AND METHODS

Strains: The bacterial strains used in this work were: 3300 (HfrH, thi^-, $lacI^-$, gal^+), supplied by Dr. A. Markovitz, Univ. of Chicago; GM113 (HfrH, thi^-, $lacI^-$, $galT^-$); G1 (HfrC, met^-, RC^{rel}, G^{ts}) (Tocchini-Valentini et al., 1969), supplied by Dr. D. Schlessinger, Washington Univ. Bacteriophage strains included λdg30, which transduces the entire gal operon, and all the E. coli chromosome between it and the λ attachment site; λdgK17 (Pfeiffer and Oellermann, 1967), (which deletes the galactokinase cistron); $\lambda pgal_8S_7$ (Echols, 1970) which carries much less "extraneous" host DNA (see Mackie and Wilson (1972) for a discussion of this problem), supplied by Dr. I. Pastan, N.I.H.; and λcI857. Phages λdg30 and λdgK17 were essential to the experiment described in Table II, as they carry equivalent amounts of the host chromosome outside the gal operon.

Hybridization: Methods for the labeling of cells with ^{3}H-uridine, the isolation of ^{3}H-RNA, and hybridization of RNA to DNA are described briefly in the legend to Table I and in more detail in Mackie and Wilson (1972). All hybridizations were performed in duplicate or triplicate, under conditions of DNA excess.

RESULTS AND DISCUSSION

Strain GM113 carries a strongly polar mutation in the transferase cistron which abolishes transferase activity, and reduces galactokinase, which is de-

TABLE I

Strain	Pulse length (min.)	Labeling of RNA (cpm/μg)	% Labeled RNA Annealed To: λpgal8S7	λDNA	Net
3300	0.5	15,100	0.167	0.033	0.134
	1.5	30,500	0.197	0.034	0.163
	3.0	54,600	0.165	0.025	0.140
GM113	0.5	28,500	0.149	0.029	0.120
	1.7	39,500	0.127	0.029	0.098
	3.0	51,800	0.098	0.019	0.077

Synthesis of Gal mRNA in a Polar Mutant galT⁻ Strain (GM113) and Parent Strain (3300).

Strains 3300 and GM113 were grown at 35° in H-1 medium containing 10 μg/ml thiamine, 0.4% glycerol, 0.2% casamino acids, and 2 mM D-fucose (to induce the gal operon). When the cultures reached a density of 7 x 10^8 cells/ml, they were labeled with 100-125 μC of ^{3}H-uridine (New England Nuclear Corp.) at a final concentration of uridine between 1.0 and 1.2 μM. Labeling was terminated by pouring the cultures into frozen buffer containing NaN_3. The RNA was isolated by the hot phenol method and its specific activity determined by counting in a scintillation counter. It was then hybridized to λ or λpgal8S7 DNA. The results are expressed as the percentage of the input counts annealed in an RNase-resistant hybrid in the presence of excess DNA.

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rived from the distal cistron, to 15-20% of its level in the parent strain 3300. The specific activity of epimerase, the first enzyme in the operon, is identical in both strains. The data of Table I indicate that in strain GM113 there is a progressive reduction in the proportion of ^{3}H-RNA hybridizable to λpgal 8 DNA with the lengthening of the uridine labeling period. In RNA from strain GM113 labeled for 0.5 min, there is only a small reduction in the level of gal mRNA

TABLE II

Strain	% Labeled RNA Annealed To			% Galacto-kinase mRNA
	λ DNA	λdg30 DNA	λdgK17 DNA	
3300	0.029	0.212 (0.183)	0.159 (0.130)	29
GM113	0.025	0.128 (0.103)	0.123 (0.098)	5

Galactokinase mRNA in Strains 3300 and GM113.

RNA isolated from the strains indicated after labeling for 3.0 min. was hybridized to the DNAs noted in the column headings. The figures in parentheses represent the net hybridization after the background to λ DNA is subtracted. The percentage of galactokinase mRNA is calculated as follows:

$$\frac{(\%\text{ hybridized to }\lambda dg30\text{ DNA}) - (\%\text{ hybridized to }\lambda dgK17\text{ DNA})}{(\%\text{ hybridized to }\lambda dg30\text{ DNA})} \times 100$$

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relative to that found in the parent strain labeled in parallel. After pulses of 1.7 or 3.0 min., the level of gal mRNA decreases noticeably in the mutant, whereas it remains essentially constant in the parent strain. RNA from strain GM113 hybridizable to λpgal 8 DNA, does not completely disappear in long pulses, presumably due to the continued synthesis of epimerase and "non-gal" mRNAs which hybridize to the host-derived regions of λpgal 8 DNA.

The data of Table II indicate that a large part of the RNA which disappears in strain GM113 with long labeling times corresponds to mRNA from the kinase cistron. The amount of galactokinase mRNA is taken as the difference between the amount hybridizing to λdg30 DNA and that hybridizing to λdgK17 DNA; from this, the percentage of galactokinase mRNA relative to total gal mRNA can be calculated as in the legend to Table II. (What is called total gal mRNA here includes some mRNA, about 40%, which is not gal mRNA, but hybridizes to λdg30 DNA (Mackie and Wilson (1972)). The results indicate the following. After three minutes labeling there is less than one fifth as much galactokinase mRNA in the polar mutant as in the wild type cells. Second, the percentage of

labeled RNA which hybridizes to λdgK17 DNA (largely epimerase and transferase mRNAs) decreases by 40% in strain GM113 relative to strain 3300. This most likely represents the disappearance of mRNA from the transferase cistron, as the yield of epimerase activity in extracts of both strains is the same. Finally, as an internal control, the estimate of the proportion of galactokinase mRNA in strain 3300 is consistent with the knowledge that the galactokinase cistron is roughly one third the length of the entire galactose operon (Wilson and Hogness, 1969).

In a single experiment, the level of galactokinase mRNA in strain GM113 labeled for 1.7 min. was one half that in cells which had been labeled for 0.5 min., and the latter was lower than in strain 3300 labeled for 0.5 or 1.5 min. It would appear that the disappearance of galactokinase mRNA in strain GM113 is time-dependent as is the case for total gal mRNA.

The finding that the recovery of material hybridizable to λpgal 8 DNA in the polar mutant strain GM113 is only a little less than in the parent strain 3300 after a 0.5 min. pulse of uridine (Table I) implies that the entire gal operon is transcribed with equivalent efficiencies in both strains. The small decrease observed can be explained by the rapidity of the decline in gal mRNA seen by 3 min. The disappearance of gal mRNA in strain GM113 is consistent with the hypothesis that mRNA distal to a terminating codon is rendered more labile to nuclease attack.

We have made a similar analysis of transcription of the galactose operon in strain G1 at permissive and non-permissive temperatures. When cultures of this strain with a mutant G factor are shifted from 29° to 42° they cease synthesizing protein within ten minutes as illustrated in Figure 1. After this, there is at least a 90% inhibition of amino acid incorporation into protein, in agreement with the results obtained *in vitro* by Tocchini-Valentini *et al.* (1969). Table III shows that there is no concomitant inhibition of RNA synthesis since G1 is a relaxed control mutant. Table III also contains the results of assays for gal mRNA in total RNA extracted from cultures of strain G1 pulse-

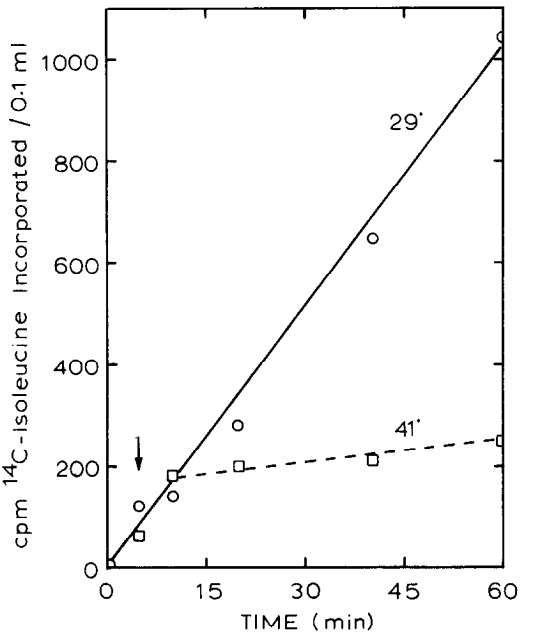

Figure 1

Protein Synthesis in Strain G1 with a Temperature-Sensitive G Factor.

Strain G1 was grown at 29° in H-1 salts supplemented with 0.4% galactose, 19 amino acids at 50 μg/ml and isoleucine at 10 μg/ml. At a cell density of 4 x 10^8 cells/ml, ^{14}C-isoleucine (New England Nuclear Corp.) was added to 0.05 μC/ml. Five minutes later, the culture was divided into two lots of 5 ml, one of which was transferred to 41°, the other kept at 29°, as indicated by the arrow in the figure. At the times indicated, 0.1 ml aliquots were removed from the cultures and pipetted into 5% trichloroacetic acid. The samples were boiled, collected by filtration, and counted by liquid scintillation. The results are expressed as acid-insoluble cpm/0.1 ml of culture.

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labeled for different times, starting 15 minutes after a shift in temperature. It is clear that at 42° there is much less RNA hybridizable to λpgal 8 DNA, even with the shortest pulse, 0.5 min., and furthermore, there is no decrease with a longer pulse. In the control culture, the level of gal mRNA remains constant, but lower than in strain 3300 grown under similar conditions. This may reflect real differences between the two strains, as strain G1 grows slowly on galactose. Labeling strain G1 at 42° also increases the level of background hybridization to λ DNA quite reproducibly, perhaps due to the generation by degradation of radioactive oligonucleotides which can hybridize to non-homolo-

TABLE III

Labeling Temperature (°C)	Pulse Length (min.)	Labeling of RNA (cpm/μg)	% Labeled RNA Annealed to λpgal8S7 DNA	λ DNA	Net
29	0.5	10,500	0.136	0.032	0.104
29	3.0	29,000	0.109	0.017	0.092
42	0.5	9,800	0.075	0.049	0.026
42	3.0	26,000	0.070	0.043	0.027

Effect of Temperature on the Synthesis of gal mRNA in Strain G1 with a Temperature-Sensitive G Factor.

A culture of strain G1 was grown at 29° in H-1 medium supplemented with 0.4% galactose and all twenty amino acids at 50 μg/ml each. At a density of 5×10^8 cells/ml, half the culture was transferred to 42°. After 15 minutes, 5 ml aliquots of the cultures at both temperatures were labeled with 200 μC of ^{3}H-uridine for 0.5 min., or with 100 μC for 3.0 min., in both cases at a uridine concentration of 1.8 μM. The isolated RNA was hybridized to λ or λpgal 8 DNA as in Table I.

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gous DNAs such as λ DNA. A similar situation occurs with RNA extracted from cells poisoned with puromycin (G. Mackie, unpublished).

Our results for the polar mutant, strain GM113, are consistent with the theory that untranslated mRNA distal to a chain-terminating mutation is rapidly degraded. The data obtained with strain G1, however, do not lend themselves to unambiguous interpretation. The apparent absence of gal mRNA after relatively short pulses may be a consequence of extremely rapid degradation of nascent messenger which is essentially unprotected in the absence of translation. Morse (1971) and Morse and Guertin (1971) have proposed a rapid degradation to explain their results on trp mRNA. But the degradation would have to

be much more rapid than that seen in Table I so it seems very unlikely that it accounts for the absence of gal mRNA seen here.

Since total RNA labeling has been maintained under these conditions, it is necessary to find an explanation for inhibition of synthesis of certain specific RNAs. Two alternate explanations are conceivable. First, the absence of gal mRNA in strain G1 at high temperature may be due to the onset of catabolic repression induced by the cessation of protein synthesis. Normally, the gal operon is relatively insensitive to catabolic repression (Mackie, 1972), but the unusual metabolic state of strain G1 at 42° might alter this. Secondly, these results might be taken as support for the hypothesis that transcription is more tightly coupled to translation in some operons. Similar findings have been obtained in a study of transcription of the tryptophan operon in a relaxed control strain blocked for protein synthesis (Imamoto and Kano, 1971). Attempts to reconcile this idea with the contrary conclusion drawn from the experiments with strain GM113 have led us to consider the following. It is entirely possible that the effects on transcription of a polar mutation and of blocking translation at the beginning of the operon (as occurs when protein synthesis is inhibited overall) are quite different. In the former case, untranslated messenger may simply be degraded, whereas in the latter, it may inhibit subsequent rounds of transcription by virtue of its proximity to the promoter.

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