SHORT PAPERS

SYNTHESIS OF SPECIFIC, STABILIZED MESSENGER RNA WHEN TRANSLOCATION IS BLOCKED IN *ESCHERICHIA COLI*

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IN growing bacteria, ribosomes join to nascent messenger RNA as the mRNA is formed, and move at very nearly the rate of RNA polymerase (GEIDUSCHEK and HASELKORN 1969). Anticipating this efficient relationship of transcription and translation, STENT called it "coupling" (STENT 1964). The notion of "coupling" can be extended to the possible relationship of translation and mRNA degradation; for *in vivo*, mRNA degradation has been shown to follow closely behind the last translating ribosome, and to degrade mRNA in the same sense as translation (MORIKAWA and IMAMOTO 1969; MORSE, MOSTELLER and YANOFSKY 1969). Also, in a complex assay system *in vitro* ("RNase V"), hints have been obtained that ribosome addition may be *required* to initiate degradation of a chain of mRNA (MANGIAROTTI, SCHLESSINGER and KUWANO 1971).

This paper reports the results of an attempt at a critical test *in vivo* of a possible causal relationship between mRNA translation and either its transcription or degradation.

"Coupling" models predict that when translation is blocked, mRNA transcription should also stop if transcription and translation are coupled; similarly mRNA degradation should also stop if translation and degradation are coupled.

The antibiotics fusidic acid, which specifically inhibits the G translocation factor (KINOSHITA, KAWANO and TANAKA 1968), and chloramphenicol arrest translation. In treated cultures, mRNA continues to form and tends to accumulate (SCHLESSINGER *et al.* 1969). However, these antibiotics are somewhat leaky *in vivo* and the precise degree to which mRNA was stabilized was unclear.

I have therefore turned to a quantitative comparison of the effects of antibiotics with the effect of a mutation, GI (TOCCHINI-VALENTINI and MATTOCHIA 1968), a hard temperature-sensitive lesion in the G factor. In cultures of GI at 43°C, growth and amino acid incorporation stop completely, and few, if any, polyribosomes are formed (SCHLESSINGER, PHILLIPS and CRAIG 1971). Therefore in this case translation is truly stopped.

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TABLE 1

Hybridization of pulse-labeled RNA from GI at 30° and 43°C to DNA trapped on a filter

		1/1000 RNA/DNA		1/20 RNA/DNA		% mRNA
	temperature	input	cts. hybridized	input	cts. hybridized	
Part A.	30° C	4480	1951	863,000	202,000	53%
	43° C	4830	1598	333,228	49,893	45%
		11,200	4034	375,000	63,620	47%

				counts hybridized		
	temperature	input	RNA/DNA	-rRNA	+rRNA	% mRNA
Part B.	30° C	2758	1/1000	2334	1132	48%
		1562	1/1000	806	415	51%
	43° C	3080	1/1000	1232	721	58%
		1950	1/1000	988	545	54.5%
		1901	1/1000	1055	516	49%
		1050	1/2000	950	521	55%

		1/1000 RNA/DNA		1/20 RNA/DNA		% rRNA
	temperature	input	cts. hybridized	input	cts. hybridized	
Part C.	30°	1320	745	185,000	7815	93%
	43°	620	316	62,170	5276	84%

The amount of DNA on filters was always kept at 100 to 200 μg even at low ratios to prevent a concentration effect which would decrease the efficiency of hybridization.

Part A: RNA, pulse-labeled for 1 min with ^{3}H-uracil (as in Figure 1), was hybridized over a wide range of RNA/DNA ratios. Reported here are the values obtained at a 1/1000 ratio at which all RNA species hybridize, and 1/20 ratio at which only mRNA hybridizes. The percent hybridized at 1/1000 RNA/DNA was set at 100% and the relative percent hybridized at 1/10 RNA/DNA is the percent mRNA.

Part B: RNA was hybridized to DNA in the presence and absence of a 20-fold excess of unlabeled ribosomal RNA. The percent of the counts remaining hybridized in the presence of rRNA is the percent of mRNA in the sample.

Part C: RNA was pulse labeled for 1 min; then 200 μg/ml rifampicin was added and the culture allowed to shake for 30 min before harvesting. The RNA was extracted and hybridized as in Part A.

In all cases pulse labeling of GI at 43°C was carried out one hour after transfer to the higher temperature.

mRNA is formed in the absence of translocation: GI at 43°C continues to form pulse-labeled RNA at a rate comparable to that in growing cultures (± 20%), for at least 1 hr after transfer from 30°C. How much of this RNA is messenger RNA? Because the result is of critical importance, two methods of hybridization analysis were used: (1) DNA:RNA hybridization titration curves were carried out according to the design of KENNELL (1968) and (2) RNA samples were hybridized in the presence and absence of a 20-fold excess of cold rRNA. Typical

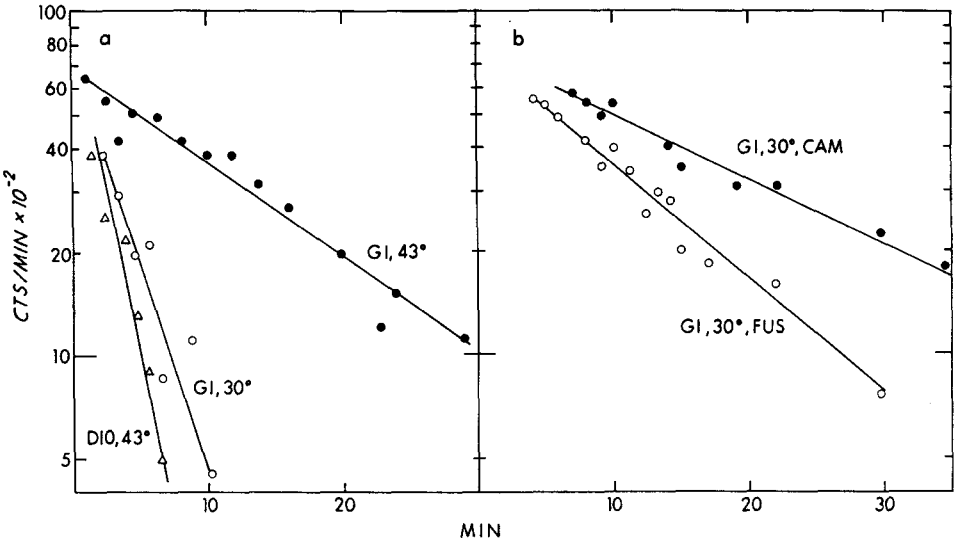

FIGURE 1.—Breakdown of pulse-labeled RNA in D10 or G1 treated with rifampicin in the presence or absence of chloramphenicol and fusidic acid. 4 ml cultures at 3×10^8 cells/ml at 30°C or 43°C where indicated were pulse-labeled 1 min with 3 μC/ml ^{3}H-uracil (27 C/μmole). Then 200 μg/ml rifampicin was added to block further initiation of RNA. The further incorporation of label and subsequent breakdown of unstable RNA was followed by measuring acid-insoluble c.p.m. on portions of culture. The total amount of RNA that broke down in 60 min was about 6000 c.p.m. The c.p.m. of that unstable RNA remaining at each time is plotted.

Frame a: O———O, G1 30°; △———△, D10 43°; ●———●, G1 43°. Pulse-labeling of G1 at 43° was carried out one hour after the transfer to the higher temperature. D10 is an RNase 1-derivative of a wild-type Hfr Hayes strain obtained from the laboratory of R. GESTELAND.

Frame b: breakdown of pulse-labeled RNA in cells pretreated with chloramphenicol or fusidic acid for 30 min. ●———●, G1, 30° with 200 μg/ml CAM; O———O, G1, 30° with 300 μg/ml fusidic acid.

results (Table 1) show that RNA pulse-labeled at either 30° or 43°C is about 50 and 55% mRNA, as determined by both techniques. Therefore, GI at 43°C continues to make mRNA at a nearly normal rate.

mRNA is stabilized in the absence of translocation: Arrest of translocation—for example, by chloramphenicol—protects preformed mRNA against degradation (LEVINTHAL *et al.* 1963; MANGIAROTTI and SCHLESSINGER 1966; FLESSEL 1968; MORSE 1971). But this result can be trivial, resulting from the protection of mRNA in polyribosomes from nucleases. However, new mRNA chains initiated after translocation are also protected. This protection is shown by the data in Figure 1. RNA was pulse-labeled in growing cells or in cells in which translocation is blocked; rifampicin was then added to block further initiation of mRNA synthesis, and the decay of pulse-labeled mRNA was followed; about half the RNA was unstable.

In all cases the unstable RNA decayed exponentially. However, the half-life of mRNA in growing GI cells was 2.5 min, while the half-life in cultures of GI at 43°C was 12 min. The half-life of mRNA in control ts$^-$ cells at 43°C was

1.5 min. The half-life of mRNA in cultures of GI treated with fusidic acid and chloramphenicol was 9 min and 18.5 min, respectively (Figure 1). The same results with antibiotics were obtained with a wild-type K12 3000 strain (data not shown).

To determine what type of RNA was stable in GI, RNA was extracted 30 min after the addition of rifampicin and tested by DNA:RNA hybridization. For RNA pulse-labeled at 30°C or 43°C, 93% or 84% of the remaining RNA, respectively, was rRNA (Table 1, part C). Therefore, at 43°C, as in growing cells, mRNA is unstable and rRNA is stable.

It is important to note that in some other strains of *E. coli* including GPI, a strain with low adenyl cyclase levels (OHNISHI, unpublished results), much less stabilization of new mRNA in the presence of antibiotics was seen, suggesting that the protection of mRNA may be indirect. Nevertheless, in all the cases reported here, there is a normal coupling of translocation and degradation of mRNA. The case of GI is especially extreme; since few or no polyribosomes are formed, most or all of each chain of mRNA must be exposed to scavenger nuclease action, and yet survives for a considerable time.

Only some mRNA species are made in absence of translocation: One continuation of this line of experimentation is its extension to specific mRNA species. Since detailed analyses have already been done of the mRNA's specific for the *lac* (VARMUS and PERLMAN 1971) and *trp* operons (IMAMOTO and KANO 1971), one can quote from their results that those two mRNA species are very strongly under-represented in the mRNA made in absence of translocation.

D. MORSE (personal communication) has observed a similar fall in *trp* operon expression in GI cells at 42°C. After 1 hr at 42°C, as described here, the labeling of total RNA was unaffected (± 20%). However, the percent of pulse-labeled RNA which was *trp* mRNA, determined in a series of hybridization trials, dropped from 0.5% to 0.03%. In an isogenic non-GI strain, there was a drop to an average value of about 0.3%.

Thus, as in the case of degradation of mRNA, transcription of some mRNA species is formally coupled to translocation. Since the two operons in question, *lac* and *trp*, share no known mechanism of regulation of mRNA synthesis, an unknown mechanism of regulation, or several, may be operative here. The regulation may not be at the site of repressor action; for example, a full complement of mRNAs may start to be transcribed, but transcription may proceed only to a certain point. Some mRNAs might be completely transcribed, while others, particularly from polycistronic messages would be prematurely interrupted.

Possible models: The study by IMAMOTO and KANO (1971) is complementary to this one, for they have used a mutant (KANG 1970) that cannot initiate protein chains at high temperature. They find results comparable to those with strain GI, and come to similar conclusions. Combining the two sets of data, one can infer that neither initiation of protein chains nor ribosome translocation is required for synthesis of some species of mRNA, but that synthesis of other mRNAs requires not only initiation but actual ribosome movement. Whether this is a direct

effect of translation, or an effect through some other agent (charged transfer RNA? ribosomal proteins?) is unclear.

Stabilization of new mRNA in the absence of translocation may also be indirect. There may be a 5′-exonuclease (MORSE, MOSTELLER and YANOFSKY 1969) on or behind the last translating ribosome, and the ribosome must then move along the mRNA to permit degradation to proceed. Some evidence for this notion is given by experiments with the *lac* operon (VARMUS and PERLMAN 1971); treatment with puromycin *in vivo*, which would promote release of blocked ribosomes from mRNA, accelerated the decay of the mRNA.

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