COORDINATE REGULATION OF THE INDIVIDUAL RIBOSOMAL RNA OPERONS IN ESCHERICHIA COLI

Edward A. Morgan and Samuel Kaplan

Department of Microbiology, 131 Burrill Hall
University of Illinois, Urbana, Illinois 61801

Received December 19,1975

SUMMARY: When Escherichia coli is growing in steady state with doubling times of 40 minutes or 20 hours, during shift up conditions, or under conditions of relaxed control of RNA synthesis, all rRNA cistrons are transcribed equally.

There are, by most estimates, approximately 6-7 copies of the ribosomal RNA (rRNA) genes in E. coli (1, 2). These genes are organized in 6-7 operons in the order 16S-23S-5S (3) and are transcribed as a single molecule which undergoes maturational cleavage (4). Four of these operons have been mapped by transductional analysis of 5S rRNA sequences which differ between E. coli K12 and B strains (5). The location of two operons identified by electron microscope heteroduplex mapping of 16S and 23S rRNA to DNA of F prime factor F14 is consistent with the location of two of the 5S rRNA genes determined by transductional analysis (6). An additional gene appears to have been located recently (7) and evidence has been presented for the general location of other cistrons (8, 9). Map position dictates that each rRNA "transcripton" be an independent operon.

We have examined whether these operons are transcribed coordinately at high and low growth rates, during shift up, and under relaxed conditions in a rel A^- strain. We have used the sequence heterogeneity within E. coli K12 5S rRNA as reported by Jarry and Rosset (10) in order to distinguish transcription of the individual operons. The relative molarity of fractional oligonucleotides in relation to each other and to those that occur in all rRNA transcripts can in principle be used to determine whether there is an equivalence in transcription of the individual rRNA operons. Published data

Copyright © 1976 by Academic Press, Inc.
All rights of reproduction in any form reserved.

(10) indicate that all cistrons are expressed approximately equally under conditions where growth is optimum or near optimum. We have sought to examine extreme physiological states in order to determine whether or not preferential transcription of individual rRNA operons exists.

METHODS

All strains were grown at 37° C with aeration. E. coli K12 strain W3110 was grown on GRCLP salts (24g Tris-HCl, 6g KCl, 0.7g Na_2SO_4, 0.2g $MgCl_2$, 0.1g Na_2HPO_4 per liter, pH 7.0) supplemented with carbon and nitrogen sources chosen to obtain the desired growth rates. Cells grown in GRCLP supplemented with 0.1% Casamino acids, 50 µg/ml tryptophan, 0.4% glucose, and 20 mM NH_4Cl had a doubling time of 40 minutes. Cells grown in GRCLP supplemented with 0.4% glycerol and 0.2% histidine had a doubling time of 20 hours. RNA was purified from exponential cultures grown for at least two doublings in the presence of [^{32}P]-orthophosphate.

Cells labeled during shift up were initially growing in GRCLP media supplemented with 0.4% glycerol, 0.2% histidine and shifted up by the simultaneous addition of [^{32}P]-orthophosphate and Casamino acids to 0.1%, tryptophan to 50 µg/ml, glucose to 0.4% and NH_4Cl to 20 mM. A lag of approximately 60 minutes is observed before growth characteristic of this media begins. During this interval the rate of RNA accumulation increases in an approximately exponential fashion until the rate characteristic of the new growth rate is achieved at nearly 60 minutes following shift up (unpublished data). In [^{32}P] labeling experiments RNA synthesis was terminated 40 minutes after shift up by addition of rifampicin to 50 µg/ml and the cells harvested by centrifugation.

Relaxed rRNA was prepared from E. coli K12 strain LMUR (rel A$^-$, RNase I$^-$, lys$^-$, met$^-$, ura$^-$) growing in the low phosphate media of Maaløe and Hanawalt (11) supplemented with 0.4% glucose, 30 µg/ml uracil, and 50 µg/ml methionine and lysine. Starvation for methionine was induced following a one minute centrifugal collection, followed by a one minute centrifugal wash in ice-cold media without methionine, and resuspension in pre-warmed media without methionine. RNA accumulation resumes within 5 minutes and continues an additional 55 minutes with a doubling of the RNA content with little increase in the optical density or protein content of the culture (unpublished data). [^{32}P]-orthophosphate was added 10 minutes after starvation for methionine was induced. Labeling was terminated by pouring the culture over 0.4 volumes of frozen, crushed M9 salts containing 0.4% glucose, 30 µg/ml uracil, and 50 µg/ml lysine, centrifuged one minute and resuspended in an equal volume of pre-warmed M9 salts media with methionine. Cells were aerated an additional 60 minutes and harvested by centrifugation. Growth resumes approximately 25 minutes after relieving methionine starvation. 5S rRNA was isolated from 50S ribosomal subunits and was free of detectable contaminating RNA as determined by acrylamide gel electrophoresis. [^{32}P] -5S rRNA was mixed with [^{3}H]-uracil labeled 5S rRNA purified from strain LMUR grown on 20 ml of M9 media with 0.4% glucose, 15 µg/ml unlabeled uracil plus 2 MC[^{3}H]-uracil, 50 µg/ml methionine and lysine and 1% Casamino acids (doubling time 40 minutes). The RNA was digested with RNase T1 and fingerprinted as described (12). Details of purification are similar to those which will be published elsewhere (Morgan and Kaplan, in preparation).

RESULTS

The relative yield of individual oligonucleotides derived from 5S rRNA

produced under the various physiological conditions described in METHODS are presented in Table 1.

The use of a dual label technique minimizes the possibility of error in comparing the relative molarities of individual oligonucleotides produced from

Molarities of RNase T1
derived oligonucleotides of 5S rRNA
synthesized under different conditions-relative to a standard RNA

oligonucleotide	Molarity in *E. coli*	20 hr doubling time	40' doubling time	shift up	relaxed RNA
CG	5	.86	.91	1.0	.90
UG	4	1.0	1.0	1.1	1.1
CCG*	3.5	1.0	.93	.91	.91
CAG	0.5	.82	.81	1.1	.95
UAG	4	1.2	1.0	.99	1.2
AUG*	1	1.1	1.1	1.2	1.2
CCAG*	1	.93	1.0	.87	.92
CCUG	1	1.6	1.3	1.3	1.2
UCUG	.15	.74	1.2	1.4	1.1
AAACG	1	1.1	1.2	.89	.91
AACUG	1	1.1	1.1	1.3	1.4
AACUCAG	1	1.1	1.0	1.3	1.3
ACCCCUAG	1	1.0	1.1	1.0	1.1
UCCCACCUG	1	1.2	1.3	1.3	1.2
UCUCCCCAUG	0.8-0.9	.80	.87	1.1	.97
UCUCCUCAUG	0.1-0.2	.85	.74	1.3	1.3

Table 1. The molarities of ^{32}P labeled oligonucleotides derived from 5S rRNA produced under various conditions relative to a [^{3}H]-uracil labeled oligonucleotide from a 5S rRNA standard is compared to determine equivalancy of expression of individual rRNA operons. The $^{32}P/^{3}H$ ratios of individual oligonucleotides were multiplied by a correction for the pyrimidine/phosphate ratio (uracil labels uracil and cytosine equally) and divided by an average of similarly corrected values for CCG, AUG and CCAG. The approximate equal synthesis of rRNA from all cistrons under all conditions is indicated by values near 1.0 for oligonucleotides present in all, a few, or one 5S rRNA cistrons. Molarities of oligonucleotides present in *E. coli* are those determined by Jarry & Rosset (1).

5S rRNA labeled under various conditions. Recovery of oligonucleotides is in general consistent with the results presented by Jarry and Rosset (1). We find that oligonucleotide CCAUG is produced in variable amounts in our hands within a given RNA preparation and depending on the digestion conditions. This may be due to spurious production due to non-ideal enzyme cleavage of other sequences present in 5S rRNA which contain CCAUG. In any case, despite fluctuations in the relative molarity of the oligonucleotide the $^{32}P/^{3}H$ ratio relative to other spots remains constant, indicating that the fluctuation is not due to any variation in the levels of expression of individual rRNA cistrons.

It was necessary to chase the 5S rRNA produced under relaxed conditions into ribosomes in order to obtain 5S rRNA of the necessary purity. The procedure used should eliminate uptake of significant [^{32}P] into the cells after the chase, but turnover of pre-existing unpackaged 5S rRNA, with incorporation into new 5S rRNA is possible. However, since a substantial proportion of the rRNA produced under relaxed conditions is incorporated into ribosomes after release of starvation (13) and since we are unable to detect any significant indication of preferential synthesis in the RNA isolated, we conclude that even if significant label is incorporated into new RNA after the release of starvation, an equal synthesis of 5S rRNA has occurred from all cistrons under relaxed conditions.

We have also detected some of the heterogeneous sequences of *E. coli* 5S RNA in *P. mirabilis* containing two *E. coli* 5S rRNA genes on F' factor F14 (unpublished data). These sequences are not present in *P. mirabilis* 5S rRNA. Our data agree well with the relevant heterogeneity and map positions determined by Jarry and Rosset (1). Both *E. coli* 5S rRNA genes appear to be expressed equally in *P. mirabilis* at high and low growth rates. *P. mirabilis* also contains heterogeneous sequences and stoichiometry of synthesis of individual *P. mirabilis* 5S genes to each other and to *E. coli* 5S rRNA genes appears to be maintained at different growth rates (unpublished data).

DISCUSSION

Several alternative possibilities exist in order to explain the existence of multiple rRNA operons in E. coli: 1) to provide for a high transcriptional rate; 2) multiple operons may be involved somehow in the regulation of rRNA, with rRNA accumulation regulated by shutting off transcription from individual operons non-coordinately; 3) the different operons may exist to give rise to functionally heterogeneous ribosomes. Multiple operons, which contain appreciable non-rRNA sequences (4, 6), may contain RNA products somehow involved in cellular regulation in a way which is not yet clear. In this respect it is interesting that "spacer" regions of the rRNA operon are at least sometimes non-homologous (14) and that two different tRNAs have been found to be co-transcribed with rRNA, at least one of which is necessary for cell growth and dictates that transcription of this operon is mandatory.

We feel that rRNA transcription is non-preferential for most and probably all operons under the conditions studied here, as relative molarities of all fractional oligonucleotides remains nearly constant. Symmetrical distribution and clustering of rRNA operons around the proposed origin of replication probably reduces changes in relative gene dosage of the rRNA operons at different chromosomal replication rates. Coordinate regulation occurs at the widely different rates of rRNA accumulation dictated by growth rate. No preferential transcription occurs during shift up, conditions in which an exponential increase in the rate of RNA accumulation occurs. These kinetics of synthesis are consistent with a gradual release of repression or a total derepression and autocatalytic transcription or translation of ribosomal protein messenger RNA. These possibilities are difficult to distinguish as at least some ribosomal proteins and RNA polymerase components are coordinately controlled (15). Coordinate control of individual operons also occurs when coordinate control of protein synthesis and rRNA accumulation is altered by the Rel A^- defect. Although the mechanism of regulation of rRNA accumulation has not been clarified, this data indicates that the regulation of rRNA is un

likely to be complicated by non-coordinate regulation of individual rRNA operons.

Acknowledgment

This research was supported by N.I.H. grant HD-03521. E.M. was supported by N.I.H. pre-doctoral training grant GM 00510. We would like to acknowledge C.R. Woese and E. Reichmann for use of space and equipment.

References

1. Jarry, B., and Rosset, R. (1971). Mol. Gen. Genet., 113, 43-50.
2. Pace, B., and Pace, N.R. (1971). J. Bact., 105, 142-149.
3. Doolittle, W.F., and Pace, N.R. (1970). Nature, 228, 125-129.
4. Ginsburg, D., and Steitz, J.A. (1975). J. Biol. Chem., 250, 5647-5654.
5. Jarry, B., and Rosset, R. (1973). Molec. Gen. Genet., 126, 29-35.
6. Deonier, R.C., Ohtsubo, E., Lee, H.J., Davidson, N. (1974). J. Molec. Biol., 89, 619-629.
7. Jaskunas, R.S., Lindahl, L., Nomura, M., and Burgess, R.R. (1975). Nature, 257, 458-462.
8. Unger, M., Birnbaum, L.S., Kaplan, S., and Pfister, A. (1972). Molec. Gen. Genet., 119, 377-380.
9. Gorelic, L. (1970). Molec. Gen. Genet., 106, 323-327.
10. Jarry, B. and Rosset, R. (1971). Molec. Gen. Genet., 113, 43-50.
11. Maaløe, O. and Hanawalt, P. (1961). J. Molec. Biol., 3, 144-155.
12. Sanger, F. and Browlee, G.G. (1967). Methods in Enzymology, 12, 361-381.
13. Nakada, D., Anderson, I.A.C., and Mayasanik, B. (1964). J. Molec. Biol. 9, 472-488.
14. Lund', E., Dahlberg, J.E., Lindahl, L., Jaskunas, S.R., Dennis, P.P., and Nomura, M. In Press.
15. Jaskunas, S.R., Burgess, R.R., Lindahl, L., and Nomura, M. In Press.