ORIGIN OF TRANSFER RNA

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Experiments with bacteria suggest that the synthesis of transfer RNA and ribosomal RNA may be coupled in some as yet unknown way. For example, nutritional shifts cause simultaneous reduction or acceleration of the synthesis of both RNA species, while deprivation of an essential amino acid inhibits both transfer and ribosomal RNA synthesis in stringent but not in relaxed mutants. However, the ratio of transfer to ribosomal RNA is not constant at all growth rates and is higher in cultures with slow growth rates (for review see Neidhardt, 1964). In Bacillus subtilis, the DNA templates for ribosomal and transfer RNA synthesis mapped together and were not resolved by the techniques used (Dubnau et al., 1965; Oishi and Sueoka, 1965).

We have used competitive hybridization techniques to demonstrate 1) that Escherichia coli ribosomal RNA displaces hybridized transfer RNA from E. coli DNA, 2) that hybridized E. coli transfer RNA inhibits subsequent binding of ribosomal RNA to E. coli DNA. The results suggest that E. coli DNA contains nucleotide sequences which will hybridize with both E. coli transfer and ribosomal RNA and that E. coli transfer RNA may be derived from E. coli ribosomal RNA since E. coli transfer RNA and ribosomal RNA have major nucleotide sequences in common.

METHODS

The methods used for E. coli DNA and RNA preparation and for hybridization were essentially those of Gillespie and Spiegelman (1965). DNA was prepared from E. coli using sodium dodecyl sulfate, "pronase" treatment and phenol extraction to ensure removal of proteins.

In order to minimize contamination with messenger RNA (Armstrong and Boezi, 1965) radioactive RNA was prepared from stationary phase E. coli B following growth of 30ml cultures in a minimal salts medium containing either ^{3}H-orotic acid (0.2mC) or ^{32}P-orthophosphate (1mC). Nucleic acids were extracted, treated with pancreatic deoxyribonuclease (5 µg/ml) for 30 minutes at 30° and fractionated on columns of methylated serum albumin on celite to isolate the transfer and ribosomal RNA (contamination with DNA was found to be less than 0.05%).

Heat denatured DNA (90µg) was immobilized by adsorption to nitrocellulose filters. Hybridization was carried out at 65° in 1ml of 2 x SSC (0.3M sodium chloride and 0.03M tri-sodium citrate). In competitive hybridization experiments the DNA-nitrocellulose filters were hybridized first with one RNA species, washed thoroughly on both sides with 2 x SSC and then hybridized with the second RNA species. Filters carrying hybridized RNA were then washed thoroughly with 2 x SSC, ribonuclease treated, washed, dried and radioactivity measured in a Packard Tricarb liquid scintillation spectrometer.

RESULTS

The results of a series of experiments demonstrating the binding of transfer and ribosomal RNA to DNA and the effects of competition by each RNA species are presented in table I. The

TABLE I

Results of 2 successive hybridizations on a single cellulose nitrate filter containing denatured DNA.
Abbreviations: t-RNA = transfer RNA; r-RNA = ribosomal RNA.

Weight of DNA on filter	First hybridization	Second hybridization	^{3}H c.p.m.	^{32}P c.p.m.
90 µg	t-RNA 4 hr	-	157	-
90 µg	t-RNA 8 hr	-	262	-
90 µg	t-RNA 16 hr	-	258	-
-	t-RNA 4 hr	-	10	-
-	t-RNA 16 hr	-	20	-
90 µg	r-RNA 4 hr	-	-	5100
90 µg	r-RNA 8 hr	-	-	5797
90 µg	r-RNA 16 hr	-	-	6004
-	r-RNA 4 hr	-	-	99
90 µg	2 x SSC 16 hr	r-RNA 4 hr	-	5000
90 µg	t-RNA 16 hr	r-RNA 4 hr	102	2807
90 µg	t-RNA 16 hr	r-RNA 8 hr	78	3259
90 µg	t-RNA 16 hr	r-RNA 16 hr	64	3404
-	t-RNA 16 hr	r-RNA 4 hr	32	58
90 µg	t-RNA 16 hr	Yeast t-RNA 4 hr	228	-
90 µg	t-RNA 16 hr	Yeast t-RNA 8 hr	195	-
90 µg	t-RNA 16 hr	Yeast t-RNA 16 hr	140	-
90 µg	t-RNA 16 hr	2 x SSC 4 hr	246	-
90 µg	t-RNA 16 hr	2 x SSC 8 hr	205	-
90 µg	t-RNA 16 hr	2 x SSC 16 hr	167	-
90 µg	r-RNA 16 hr	t-RNA 4 hr	56	4958
90 µg	2 x SSC 16 hr	t-RNA 4 hr	157	-

amounts of radioactivity in transfer and ribosomal RNA which hybridized separately to the DNA corresponded to 0.027% and 0.27% of the DNA. These amounts agreed well with earlier observations on the binding of transfer and ribosomal RNA to

E. coli DNA (Yankofsky and Spiegelman, 1963; Giacomoni and Spiegelman, 1962; Goodman and Rich, 1962).

When hybrids formed with E. coli DNA and transfer RNA were subsequently incubated with 2 x SSC alone, the bound transfer RNA was slowly reduced after 16 hours at 65° to 57% of that originally bound. Incubation with yeast transfer RNA reduced the bound E. coli transfer RNA after 16 hours to 46% of that originally bound. A striking reduction in the amount of bound E. coli transfer RNA to 12% of that originally bound was observed after incubation of DNA - transfer RNA hybrids with E. coli ribosomal RNA for 16 hours (Fig. 1A). Yeast transfer RNA has approximately the same base composition as E. coli transfer RNA and probably some similar nucleotide sequences, since both yeast and E. coli transfer RNA's are aminoacylated to some degree by yeast or E. coli amino acid activating enzymes (Brown, 1963). Despite possible similarities, 10 μg of yeast transfer RNA was considerably less efficient than 4 μg of E. coli ribosomal RNA in displacing E. coli transfer RNA from E. coli DNA.

Hybridization of E. coli ribosomal RNA to E. coli DNA was inhibited by 44% when the DNA was first hybridized with E. coli transfer RNA for 16 hours then hybridized with ribosomal RNA for 4 hours. After a further 12 hours hybridization with ribosomal RNA, binding of the ribosomal RNA was still inhibited by 40% (Fig. 1B). Furthermore, hybridization of the transfer RNA to DNA was 70% inhibited by prior hybridization of the DNA to ribosomal RNA (see Table I). Prior incubation of filters carrying DNA for 16 hours at 65° in 2 x SSC had no effect on the subsequent degree of hybridization of transfer RNA or of ribosomal RNA.

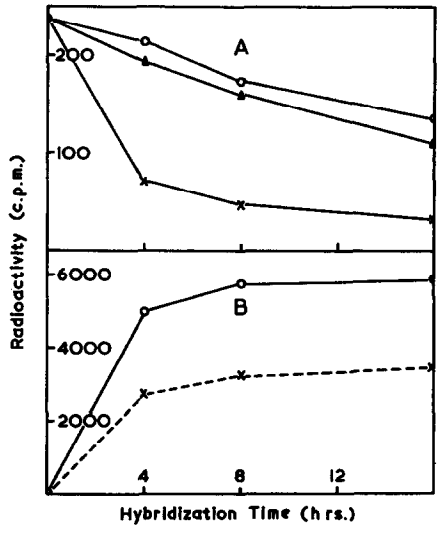

FIG. 1A. Cellulose nitrate filters containing denatured DNA (90 µg) were incubated with ^{3}H-transfer RNA (5 µg) for 16 hours at 65° in 2 x SSC (1 ml). Filters were washed thoroughly on both sides and re-incubated at 65° in 2 x SSC with or without added RNA. The filters were then washed, treated with ribonuclease, washed, and dried. ^{3}H-Radioactivity remaining hybridized to DNA in the presence of 4 µg of ^{32}P-ribosomal RNA (X——X), in the presence of 10 µg of bakers' yeast transfer RNA (Δ ——— Δ) and with no additions (0——0). Specific activity of ^{3}H-transfer RNA: 10^{4} counts/min/µg.

FIG. 1B. Filters containing denatured DNA (90 µg) were incubated with ^{32}P-ribosomal RNA (4 µg) at 65° in 2 x SSC. ^{32}P-radioactivity hybridized to DNA (0——0). ^{32}P-Radioactivity hybridized to DNA which had previously hybridized to ^{3}H-transfer RNA (X - - - X). Specific activity of ^{32}P-ribosomal RNA: 2.6×10^{4} counts/min/µg.

DISCUSSION

Midgley (1963) has investigated the synthesis of E. coli transfer RNA. He has shown that there is a kinetic delay of about one minute in the radioactive labelling of transfer RNA compared with other RNA components and that the delay is eliminated in the presence of chloramphenicol. His conclusions concerning transfer RNA synthesis are open to new interpretation in view of recent

evidence showing 1) that ribosomal RNA undergoes turnover in the presence of chloramphenicol (Dubin and Ekort, 1965), 2) that ribosomal precursor RNA functions as messenger for ribosomal protein synthesis (Roberts, 1965; Nakada, 1965), 3) that a substantial increase in functional transfer RNA occurs during prolonged growth of E. coli in the presence of chloramphenicol (Ezekiel and Valulis, 1965).

Our experiments suggest that E. coli transfer RNA may be formed by partial degradation of ribosomal RNA or ribosomal precursor RNA. We propose the following model to explain the mechanism and effects of transfer RNA synthesis in E. coli.

1. 16S and 23S E. coli ribosomal RNA are transcribed from a number of sites (about 12 to 20) comprising about 0.27% to 0.4% of the DNA (see above results and Roberts, 1965).

2. Nascent ribosomal RNA may either be translated into protein, degraded, or incorporated into ribosomes or ribosomal proteins. Translation of nascent ribosomal RNA occurs to give ribosomal proteins required for ribosome maturation (Nakada, 1965). Incorporation of ribosomal precursor RNA into ribosomes is essentially irreversible under normal growth conditions.

3. Ribosomal precursor RNA not rapidly incorporated into ribosomes is partially degraded to relatively stable 4S nucleotide sequences which are transfer RNA precursors. These subsequently receive a -CCA terminal sequence and are able to accept amino acids. Modifications of the transfer RNA (e.g. methylation, reduction etc.) may occur before or after its formation from ribosomal RNA.

4. One 16S ribosomal RNA component gives rise to two transfer RNA molecules and one 23S ribosomal RNA gives four transfer RNA molecules, since 0.02% of the DNA hybridizes with transfer RNA and 0.27% hybridizes with ribosomal RNA.

Since 16S and 23S ribosomal RNA could produce two and four ribosomal proteins respectively (Roberts, 1965), transfer RNA precursor segments may signify the start of each cistron specifying a separate ribosomal protein. Currently available data on the molecular weights of ribosomal RNA and ribosomal proteins are not sufficiently accurate to calculate whether or not precursor transfer RNA sequences in ribosomal RNA are translated into ribosomal protein. If the average molecular weight of ribosomal proteins is 25,000, then it is possible that the precursor transfer RNA sequences would not be translated.

The existence of such a precursor to transfer RNA could explain the kinetic delay observed by Midgley (1963) in incorporation of radioactive precursor into transfer RNA during transfer RNA synthesis. Chloramphenicol, by inhibiting ribosomal protein synthesis would prevent translation of the ribosomal precursor RNA. The RNA would then become susceptible to degradation to transfer RNA immediately rather than after translation. Greater degradation of ribosomal RNA would thus give rise to a higher proportion of transfer RNA (Ezekiel and Valulis, 1965). The increase in the ratio of transfer RNA to total RNA observed in E. coli grown at slow growth rates (Rosset et al., 1964) may result from reduced protein synthesis. This may in turn slow ribosome synthesis causing a greater proportion of the nascent ribosomal RNA to be degraded to transfer RNA.

If nucleotide sequences in ribosomal RNA give rise to transfer RNA and occur at the beginning of cistrons specifying individual ribosomal proteins, this may have wider implications in the regulation of ribosomal and other protein synthesis. In those cases where modified ribosomes have been suggested (Anderson et al., 1965), or where new ribosomal proteins have been detected

(Leboy et al., 1964; Reid et al., 1965) new transfer RNA species may also occur. The existence of new transfer RNAs as well as modified ribosomes might explain many of the phenomena associated with these systems such as suppression (Bremmer et al., 1965; Anderson et al., 1965).

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