ACCUMULATION OF DIFFERENT PRECURSOR RIBONUCLEOPROTEIN PARTICLES BY VARIOUS COLD SENSITIVE, ANTIBIOTIC RESISTANT MUTANTS

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SUMMARY

An analysis was carried out of precursor ribonucleoprotein particles produced by cold sensitive (subunit assembly defective) mutants from Escherichia coli which are either resistant to spectinomycin alone or to both spectinomycin and streptomyci It was found that while most spectinomycin-resistant mutants accumulated precursor particles sedimenting at 26-28S and around 30S, several streptomycin-spectinomycin double resistant mutants accumulated a 21S particle. Precursor 26-28S and 30S particles contain 17S precursor RNA which can be chased into mature RNA by a temperature shift-up. The nature of accumulation of precursor particles was discussed in relation to the scheme of biosynthesis of the 30S ribosomal subunit.

INTRODUCTION

Mutants defective in assembly of ribosomal subunits (sad mutants) have been isolated and shown to be extremely useful in studying the biosynthesis of ribosomal subunits (1,2). Such mutants show decreased growth at temperatures of 20° or below, being defective in some steps of ribosome subunit assembly. Three classes of sad mutants were found and studied by Nomura and his coworkers (3). They are as follows: (i) mutants which fail to synthesize 50S subunits and accumulate a 32S particle at 20° . One such mutant has been mapped at a locus very closely linked to spc which determines the 30S ribosomal protein S5 (3; using the nomenclature of Wittmann et al (4)). (ii) Mutants which fail to synthesize 50S subunits and accumulate a 43S particle at 20° and (iii) mutants which show drastic defects in both 50S and 30S subunit assembly and which accumulate both 32S and 21S particles at 20° . The cold-sensitive phenotype in the last class results not only in defective 50S biosynthesis but also the accumulation of 21S precursor particles of the 30S subunit (3). The apparent simultaneous defects in both 50S

and 30S subunit assembly had suggested to Nomura and his coworkers that the biosynthesis of the 30S subunit is somehow coupled with that of the 50S subunit. The accumulated 21S particle contains precursor type 16S rRNA (which sediments at 17S) which can be distinguished from mature 16S rRNA and also contains 12 (out of a total of 21) proteins (5). These proteins are all involved in the early steps of the ribosome assembly process (6). We have been interested in the ribosome assembly occurring in antibiotic resistant mutants that are cold sensitive. In this communication we report on *sad* mutants derived from *Escherichia coli* which are either resistant to spectinomycin (*spc*^{r}) alone or to both spectinomycin and streptomycin (*spc*^{r}, *str*^{r}). It was observed that while several *str*^{r}, *spc*^{r} mutants accumulate 21S as reported by Nomura and his coworkers (2,5), most *spc*^{r} mutants accumulate particles sedimenting at 26-28S and around 30S.

MATERIALS AND METHODS

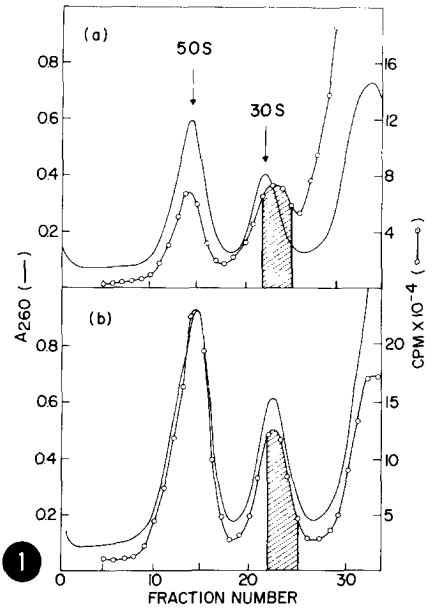
All strains used were derived from JF-600 which is a RNase I$^-$ derivative of JC-355. JC-355 is a K12, F$^-$, SM^S, SPC^S strain originating with A. J. Clark. The RNase I marker was introduced by conjugation of JC-355 with strain Q13; the latter is a K12, Hfr, SM^S, SPC^S, RNase I$^-$, polynucleotide phosphorylase$^-$ strain originating with A. Reiner and W. Gilbert (7). Both the *spc*^{r} and *spc*^{r}, *str*^{r} mutants were obtained from JF-600 by mutagenesis with nitrosoguanidine or ethylmethane sulfonate followed by selection for colonies that grew on plates containing 50 μg/ml spectinomycin or 50 μg/ml each of spectinomycin and streptomycin, respectively. These mutants were checked for cold sensitivity at 20°.

The following media were used: Hershey's tris (0.1 M tris, 1 mM $MgCl_2$, 0.01 mM $FeCl_3$, 0.1 mM $CaCl_2$, 1 mM KH_2PO_4 and 0.32 mM Na_2SO_4, adjusted to a final pH of 7.4) supplemented with 20 μg/ml of each of 20 amino acids, 0.3% glucose and 2 μg/ml thiamine; tryptose phosphate medium (obtained from Difco and used according to manufacturer's directions). Labeling of cells was carried out either with carrier-free ^{32}P or [^{14}C]uracil (obtained from New England Nuclear and Amersham/Searle Corp., respectively). Carrier cells (Q13, purchased from

General Biochemical Inc.) were added to sample of radioactive cells before cell disruption with a French press in a buffer containing 0.01 M tris-HCl pH 7.8, 0.05 M KCl and 0.1 mM $MgCl_2$. After centrifuging at 17,000 rpm for 30 min., extracts were layered directly on 5-20% sucrose gradients prepared in the same buffer and centrifuged overnight at 17,000 rpm in a Spinco SW 25.2 rotor. The fractions were collected after passage through a Gilford spectrophotometer equipped with a flow-cell device. About 30 fractions from each gradient were collected, 75 µl portions from each fraction pipetted into a scintillation vial containing toluene based cocktail (5 g PPO, 0.3 g POPOP per liter of toluene) with 10% BBS-3 (obtained from Beckman Instrument Co.), and counted in a scintillation counter. In some experiments, 75 µl fractions were also diluted with 2 ml of buffer and filtered through millipore filters followed by washing 3 times with 5 ml of the same buffer. The filters were dried and counted in the above scintillation cocktail without BBS-3. Such a washing procedure was sometimes necessary to identify the presence of the 21S particle which was occasionally obscured by the presence of large amounts of precursor RNAs (unpublished observations). RNA electrophoresis was carried out according to the procedure of Bishop *et al* (8).

RESULTS AND DISCUSSION

We have screened a total of 17 *E. coli* spc^r mutants which are cold sensitive. Of these, 4 have been found to accumulate particles sedimenting at 26-28S, 3 have been found to accumulate 40-43S particles and the rest showed disproportional distribution of 50S and 30S peaks (instead of the usual 1 to 1 ratio). None of these strains accumulate a 21S particle. Since the isolation of the 26-28S particle has not been previously reported from cold sensitive mutants, one of the four strains which produces 26-28S particles (JF-810) has been extensively studied. Fig. 1a shows the accumulation of the 26-28S particle at 20°, and its subsequent chase into 30S subunits when the temperature was shifted to 37° (Fig. 1b). Analysis of the fractions in Fig. 1a by Millipore filtration showed no 21S particle was present. RNA sedimentation analysis (shaded area from Fig. 1) was carried out and



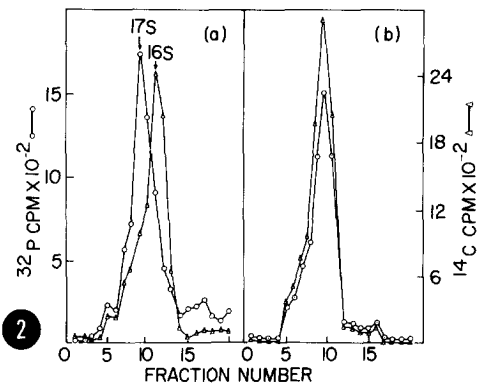


Fig. 1 Accumulation of 26-28S precursor particle by JF-810 at 20° and its chase into mature 30S after a temperature shift-up to 37° . Strain JF-810 was grown at 37° in 30 ml Hershey's tris medium supplemented with 20 µg/ml of all amino acids. When the A_{420} reading of the culture reached 0.5, cells were chilled and transferred to 20° . After 30 min. at 20° , 0.4 mc of carrier-free ^{32}P was added and further incubated for 2 hrs. Cells were chilled to 0° and divided into two equal parts and centrifuged. One part was stored whereas the other part was resuspended in the same medium and further incubated at 37° for 2/3 of the generation time at 37° . Extracts were prepared from both cultures after the addition of 75 mg of carrier Q13 cells and layered on 5-20% sucrose gradients in a Spinco SW 25.2 rotor as described in Materials and Methods. a) Extract from culture grown at 20°, b) extract from culture after a temperature shift-up from 20° to 37°.

Fig. 2 Polyacrylamide gel electrophoresis profiles of JF-810 ribosomal RNAs. The fractions from the shaded areas in Fig. 1 were pooled and concentrated by ethanol (14). 0.5 A_{260} units of samples containing 12,000 cpm were mixed with 30,000 cpm of [^{14}C]uracil labeled Q13 30S subunit (grown at 37°). Electrophoresis and slicing of gels were carried out according to the procedure of Bishop et al (8).

is shown in Fig. 2. It was found that the 26-28S particle contains predominately 17S precursor rRNA and very small amounts of both 16S and 23S (which sediments at fractions 4 and 5 in Fig. 2a). The 17S RNA present in the 26-28S particle can be chased to mature 16S rRNA by a temperature shift from 20 to 37° (Fig. 2b). Since our present purpose was mainly to study the biosynthesis of the 30S subunit, only the shaded area was pooled and analyzed. In other experiments we have observed the

presence of a 32S particle (which contains precursor 23S RNA) to the left of the shaded area. The electrophoresis procedure can only separate RNAs from the 30S regions into three peaks, namely 23S, 17S and 16S. The 26-28S particle has also been observed in an RC^{rel} strain after starvation for an essential amino acid (9), and by treatment of cells with low concentrations of chloramphenicol (10). Although not presented in Fig. 1, the accumulation of the 50S particle at 20^{0} was diminished with very little accumulation found after 1 hr. at 20^{o} rather than 2 hrs. The 26-28S peak, on the other hand, showed only little change during this period.

Since the bulk of the $\underline{spc^r}$ mutants that we have studied accumulated particles sedimenting at around 30S rather than at 26-28S, we have also analyzed RNA compositions of these strains. Most of these strains contained both 17S and 16S RNA with little 23S RNA. The appearance of the 23S RNA would indicate the presence of a 32S particle-- a presumed intermediate in the biosynthesis of the 50S ribosomal subunit. Since the 30S particle obtained at 20^{o} with most cold sensitive, $\underline{spc^r}$ mutants showed the presence of 17S RNA in addition to the mature 16S rRNA, it would suggest that the conversion of the precursor 30S particle(s) which presumably contains 17S RNA to the mature 30S subunit (which contains 16S RNA) is the rate-limiting step in these strains. Furthermore, it would also indicate that the trimming of 17S RNA to 16S rRNA is a very late event during the biosynthesis of the 30S subunit. Very recently, Lindahl (11) also observed the presence of the precursor 30S particle. It remains to be established whether or not his precursor 30S particle is the same as ours. Nor do we know the similarity between our precursor 30S particle and the native 30S subunit which has been reported earlier by Green & Hall (12).

Nomura and his coworkers have also reported the accumulation of a 21S particle in certain $\underline{E.\ coli}$ $\underline{spc^r}$ strains (5). On closer examination we noticed that the $\underline{spc^r}$ that they studied contains an additional mutation to streptomycin resistance ($\underline{str^r}$). Consequently, we have screened 12 $\underline{str^r}$, $\underline{spc^r}$ double mutants which are also cold sensitive. Of these, 2 have been shown to accumulate 21S particles, thus confirming the finding of Nomura and his coworkers. The accumulation of the 21S

particle was partially dependent on the growth conditions with cells growing in a rich medium favoring its accumulation. From the above, the sequence of biosynthesis of the 30S subunit can be represented as follows:

$$17S \longrightarrow 21S \longrightarrow 26\text{-}28S \longrightarrow p\ 30S \longrightarrow 30S$$

The accumulation of the 21S precursor particle by *str*^{r}, *spc*^{r} but not by *spc*^{r} mutants is particularly interesting because it throws some light on the nature of the accumulation of precursor particles. Since S12 has been shown to be absent in the 21S particle (5), it would appear that the accumulation of the 21S particle is most likely due to the failure of incorporation of the mutated *str* gene product (S12) at nonpermissive temperature. If this is the case, then the sequence of addition of S12 in at least some of the strains during the biosynthesis of the 30S ribosomal subunit would lie between the 21S and 26-28S particle stages, with the addition of the *spc* gene product (S5) occurring after the 26-28S particle stage. In this regard, we already have evidence for the lowered incorporation of *str*^{r} gene product in the presence of its *str*^{s} counterpart during an *in vitro* reconstitution of the 30S particle (13).

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