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Analysis of *rpsD* Mutations in *Escherichia coli*. III:

Effects of *rpsD* Mutations on Expression of Some Ribosomal Protein Genes

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Summary. Relative rates of production and steady state levels of ribosomal proteins were determined in a temperature sensitive *rpsD* (S4) mutant of *Escherichia coli*. Some proteins (S4, S12, S13) were overproduced in the mutant at permissive temperature but steady state levels of all examined ribosomal proteins were normal. In a $rpsD^+/rpsD^+$ homodiploid strain the relative rates of production of ribosomal proteins were not affected by the increased gene dose. In a $rpsD^+/rpsD$ heterodiploid strain only wild type, but not mutant S4, was found. In such a strain S4, S7, S12 and probably S13 is overproduced. It is implied that S4 is involved in the regulation of expression of proximal genes of the two transcriptional units including the genes coding for S4 itself and S12, respectively. A degradation system for ribosomal proteins, which is rapid enough to be of regulatory significance, is demonstrated.

Introduction

Several genes which code for ribosomal proteins can be mutated and give rise to resistance to translation antibiotics as exemplified by streptomycin, spectinomycin and erythromycin. Since cells which are heterodiploid, harboring two alleles determining both sensitivity and resistance to a certain antibiotic, are phenotypically sensitive to the antibiotic (Nomura and Engbaek, 1972), the wild type allele is dominant over the mutated allele conferring the antibiotic resistance. The $rpsL^+$ gene, which codes for ribosomal protein S12, can be mutated to the *rpsL* allelic state. Such *rpsL* mutants are either resistant to streptomycin or dependent on this antibiotic for growth. Ribosomes containing the gene-product of certain *rpsL* alleles do not bind streptomycin in vitro, while ribosomes from a normal streptomycin sensitive strain do. Most ribosomes from a diploid strain which contain both an $rpsL^+$ and *rpsL* allele bind streptomycin in vitro (Chang et al., 1974). This observation suggests, that the ribosomes of the diploid strain predominantly contain the gene-product of the wild type but not the mutant allele. Why this should be so is not clear. It could either mean that wild type proteins are preferentially assembled into ribosomes compared to mutant proteins or it could mean that wild type $rpsL^+$ allele is preferentially expressed.

Mutants in *rpsL* of the streptomycin dependent type can be suppressed by a second mutation in *rpsD*, coding for ribosomal protein S4. Such S4 mutations in general give a number of effects to the cell such as the ribosomal ambiguity phenotype and temperature sensitivity for growth because of an impaired processing of 17S RNA and assembly of 30S ribosomes (Olsson and Isaksson, 1979).

Some *rpsD* mutants have a drastically altered S4 protein and in many cases a mutated and wild type S4 can be separated from each other on the same two dimensional gel electrophoresis gel slab (Kaltschmidt and Wittmann, 1970). This provides the experimental advantage that expression of a wild type and a mutated ribosomal gene in the same cell can be studied. The production of ribosomal proteins, coded for by genes in the *rpsL*- (former *strA*) region of the chromosome, is not subjected to a gene dose effect in strains which are diploid for that part of the chromosome (Geyl and Böck, 1977). This would indicate that such ribosomal protein genes, which are present in duplicate, are not fully expressed. In this paper we have studied the expression of the *rpsD* gene when it is present both as mutant and wild type allele in the same cell. Also the effect of the mutated *rpsD* gene on the expression of some other ribosomal protein genes was examined.

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Materials and Methods

Genetic Procedures

All genetic manipulations were performed as described by Miller (1972) and the selection and analysis of transductants is described elsewhere (Olsson and Isaksson, 1979). The stable mutant strains D4 and D5 were chosen for the constructions of merodiploid strains. The altered forms of S4 in these mutants are well separated from other ribosomal proteins as well as wild type S4 on two-dimensional gels (see below). D4 and D5 also represent the limits with respect to temperature sensitivity within which all mutants so far analysed are distributed. D4 is the most and D5 is the least temperature sensitive mutant.

In order to construct merodiploid derivatives of D4 and D5, they were first made rifampicin resistant and thymine auxotrophs. Spontaneous rifampicin resistant mutants were selected on plates (100 μg/ml) and ThyA-mutants were obtained by the trimethoprin selection described by Miller (1972). The strains so derived, D4B and D5B, were then mated with the *recA* strain KL16–99. Selection for Thy^+ and counter selection for rifampicin resistance gave the mutant *recA* derivatives D24 and D25. As a convenient assay for *recA* we used UV-sensitivity. The episome KLF41 was then transferred from KLF41/JC1553 to D24 and D25 by using a selection for $ArgG^+$ and rifampicin resistance. The merodiploid derivatives so obtained were called D34 and D45 respectively. Two controls were made to verify the genotype and the presence of the F′-factor in these strains. First, the diploid strains were cured. The phenotype of such cured derivatives was indistinguishable from that of their respective starting strains D24 and D25. Second, the diploid strains were tested for their ability to donate their F′-factors. For this purpose we constructed the strain DNS25, a nalidixic acid resistant and spectinomycin resistant derivative of D25. By a selection for nalidixic acid resistance and $ArgG^+$ we obtained diploid derivatives of DNS25 by using D34 or D45 as donors. The presence of the episome in the new derivatives was in every case verified by a control of their spectinomycin sensitivity and their ability to generate spectinomycin resistant haploid clones (sensitivity is dominant).

All experiments made on merodiploid strain in this report were ended with an analysis of cured haploid clones derived from the same experimental culture. The results of these analysis always verified the expected nature of the merodiploid strain, which had been used.

Preparation of Lysates and Ribosomal Components

Unlabelled ribosomes, ribosomal subunits and 30S proteins were prepared as described earlier (Kurland, 1966; Hardy et al. 1969). Samples for the analysis of ribosomal proteins on two-dimensional gels were prepared as total acid soluble cell protein extracted directly from whole cells with 66% HAc, and 0.1 M $MgCl_2$. After removal of the precipitate, formed during 1 h of incubation on ice, by centrifugation, the supernatant was dialyzed against 6M urea in 15 mM potassium phosphate buffer, pH 5,8. The supernatant proteins were occasionally precipitated in 80% acetone before the dialysis in order to reduce the sample volume.

Radioisotope Measurements. All isotope measurements were performed in a liquid scintillation counter (Beckman LS250). Gel slots obtained from 2-dimensional polyacrylamide gels (15%) were solubilized in NCS (Nuclear Chicago Tissue Solubilizer) at 50% over night and then counted in a standard PPO-POPOP-toluene solution.

Electrophoretic Fractionations. Two dimensional gel electrophoresis was performed as described by Kaltschmidt and Wittmann (1970). SDS-gel electrophoresis was performed according to the procedure of Lutter et al. (1974).

Table 1. Bacterial strains (*Escherichia coli*)

Strain	Genotype	Derivation or reference
IKA	F^-: *argG, leu, aroE, sup*	Olsson and Isaksson (1979)
59:1	F^-: *aroE*, sup^+	"
SU27	Hfr P4X: *met, rpsL, rpsD5*	"
SU34	Hfr P4X: *met, rpsL, rpsD4*	"
KL16–99	Hfr KL16: *thi, recA, relA*	B. Low and R. Takata
JC 1553	F^-: *leu, his, argG, met, lac, malA, str, xyl, mtl, recA*	A.J. Clark
01	F^-: *argG, leu, sup*	A4 in Olsson et al. (1974)
017	F^-: sup^+	Olsson and Isaksson (1979)
DL14	F^-: sup^+, *rpsD4, rpsL*	59:1 transduced with $aroE^+$ region from SU34
D14	F^-: sup^+, *rpsD4*	59:1 transduced with $aroE^+$ region from SU34
D4	F^-: *argG, leu, sup, rpsD4*	IKA transduced with $aroE^+$ region from SU34
D5	F^-: *argG, leu, sup, rpsD5*	IKA transduced with $aroE^+$ region from SU27
KLF41/JC 1553	F^-: $argG^+$, $aroE^+$, $argD^+$, $aroB^+$, mal^+/JC 1553	B. Low
D4A	F^-: *argG, leu, sup, rpsD4, rif-R*	Spontaneous rifampicin-resistance from D4
D4B	F^-: *argG, leu, sup, rpsD4, rif-R, thyA*	Spontaneous from D4A, trimethoprim-selection
D24	F^-: *argG, leu, sup, rpsD4, rif-R, recA*	KL16–99 mated with D4B
D5A	F^-: *argG, leu, sup, rpsD5, rif-R*	Spontaneous from D5
D5B	F^-: *argG, leu, sup, rpsD5, rif-R, thyA*	Spontaneous from D5A
D25	F^-: *argG, leu, sup, rpsD5, rif-R, recA*	KL16–99 mated with D5B
D34	F^-: KLF 41/D24	Episome transfer
DN25	F^-: *argG, leu, sup, rpsD5, rif-R, recA, nal-R*	Spontaneous from D25
DNS25	F^-: *argG, leu, sup, rpsD5, rif-R, recA, nal-R, spc-R*	Spontaneous from DN25
DNS45	F^-: KLF 41/DNS25	Episome transfer
D35	F^-: KLF 41/D25	Episome transfer

Results

Rate of Synthesis of Some Ribosomal Proteins in a rpsD Strain

The relative rate of synthesis of some ribosomal proteins was studied in several different related strains with normal or mutated *rpsD* (S4) or *rpsL* (S12) genes. For this purpose, cells were prelabelled with ^{14}C-lysine in early exponential phase followed by a short pulse of ^{3}H-lysine in late exponential phase immediately before harvest. Cells were then disintegrated and the total lysate was analyzed for content of radioactive ribosomal proteins in a two dimensional gel electrophoresis. The result of this analysis is shown in Table 2.

The normalized ^{3}H-cpm/^{14}C-cpm value given for every individual protein is obtained by dividing the observed ^{3}H/^{14}C value by the average ^{3}H/^{14}C value for those ribosomal proteins analyzed on the gel. The ratio so obtained is used as indicating the "relative rate" of synthesis of a certain protein during the pulse period. It can be seen that most, but not all, ribosomal proteins are close to unity in the strains investigated. The only exceptional protein in the case of the control haploid strain (01) seems to be S21. Besides this protein, *rpsD* mutant strain D14 also appears to have significantly increased values for S12 and S13 and also the mutated S4 protein itself. Strain DL14, which is a double mutant harboring the mutated *rpsD* allele of D14 plus a *rpsL* mutation, is similar to D14.

Taken together the results revealed by pulse-labelling experiments, shown in Table 2, indicate that a mutation in *rpsD* can lead to an increase of gene expression of *rpsD* and *rpsM*, both of which are located in the same operon, and possibly also *rpsL*. By using another *rpsD* mutant and a slightly different labelling procedure the ratio for *rpsL* was found to be 1.7 (see Table 2 in Olsson, 1979). This suggests that a mutation in *rpsD* also might affect expression of *rpsL*, which is located in another operon. The gene for L17, (*rplQ*) which is suggested to be in the same operon as *rpsD*, (Nomura et al., 1977) is not affected by the *rpsD* mutation.

Table 2. Relative rate of synthesis of individual ribosomal 30S proteins in wild type and mutant strains

Protein	01 *rpsD*$^{+}$, *rpsL*$^{+}$	D14 *rpsD4*, *rpsL*$^{+}$	DL14 *rpsD4*, *rpsL*
S3	1.0	0.8	1.1
S4 wt	1.0	–	–
S4 ts	–	1.4	1.4
S5/L11	0.8	0.6	0.9
S7	1.1	1.0	1.0
S8	1.0	1.0	0.9
S9	1.0	1.1	1.1
S10	1.1	1.0	1.0
S12	1.1	1.3	1.2
S13	1.0	1.7	1.5
S14	1.0	1.1	1.0
S15	1.0	1.1	1.0
S16	1.1	1.1	1.0
S18	1.0	0.6	0.8
S19	1.1	1.0	1.1
S20	1.2	1.2	1.1
S21	1.5	2.5	1.9

The indicated strains, growing exponentially at 37° in 50 ml of a M9-glucose-aminoacid medium lacking lysine, were prelabelled with 10 μCi ^{14}C-lysine. Several generations later, at a cell density of 10^{8} cells/ml and when all isotope was consumed, the cells were pulse labelled with 0.5 mCi of ^{3}H-lysine. After 1 min of pulse labelling, the cultures were harvested on frozen and crushed TMK (10 mM Tris, 10 mM $MgCl_2$, 50 mM KCl pH 7.5). The cells were rapidly collected by centrifugation and lysed and extracted with 66% HAc as described in Materials and Methods. The ribosomal proteins were isolated and identified on two-dimensional polyacrylamide gels and stained spots were punched out from the gels and analysed for radioactivity. The ratio between ^{3}H-counts (pulse label) and ^{14}C-counts (prelabel) was calculated for the identified proteins and these values were then normalized by setting the average ratio typical for each gel to 1. Actual cpm-values (^{3}H/^{14}C) were for strain 01; S4 wt (38425/2368), S7 (23058/1275), S12 (6809/381), S13 (21102/1213). Strain D14; S4 ts (13973/2166), S7 (8421/1873), S12 (1920/318), S13 (15783/2021). Strain DL14; S4 ts (2743/1842), S7 (2944/2747), S12 (625/571), S13 (3767/2422).

Analysis of Merodiploid Strains Containing Genes for Both Mutant and Wild Type Protein S4

Merodiploid strains containing both mutant and wild type genes for protein S4 are very similar to wild type strains. They grow well at otherwise non-permissive temperatures and their growth rates are only marginally affected by the mutations. If such merodiploid strains are cured of their episomes they regain temperature sensitivity. Thus the mutations studied are recessive.

Analysis of the protein composition of ribosomes from these diploid strains reveal that there is no mutant protein S4 incorporated into their ribosomes. This is so independently of the temperature at which the cells are grown (not shown). Even if total acid soluble proteins, extracted directly from whole cells, are analysed, no mutant S4 can be seen (Fig. 1). SU34 and SU27 are two haploid strains containing two different mutant alleles of *rpsD*. D34 and D35 are the corresponding merodiploid derivatives containing both mutant and wild type alleles of *rpsD*.

It is immediately clear from the gels illustrated in Fig. 1 that none of the merodiploid strains contains a detectable amount of its mutated S4 protein.

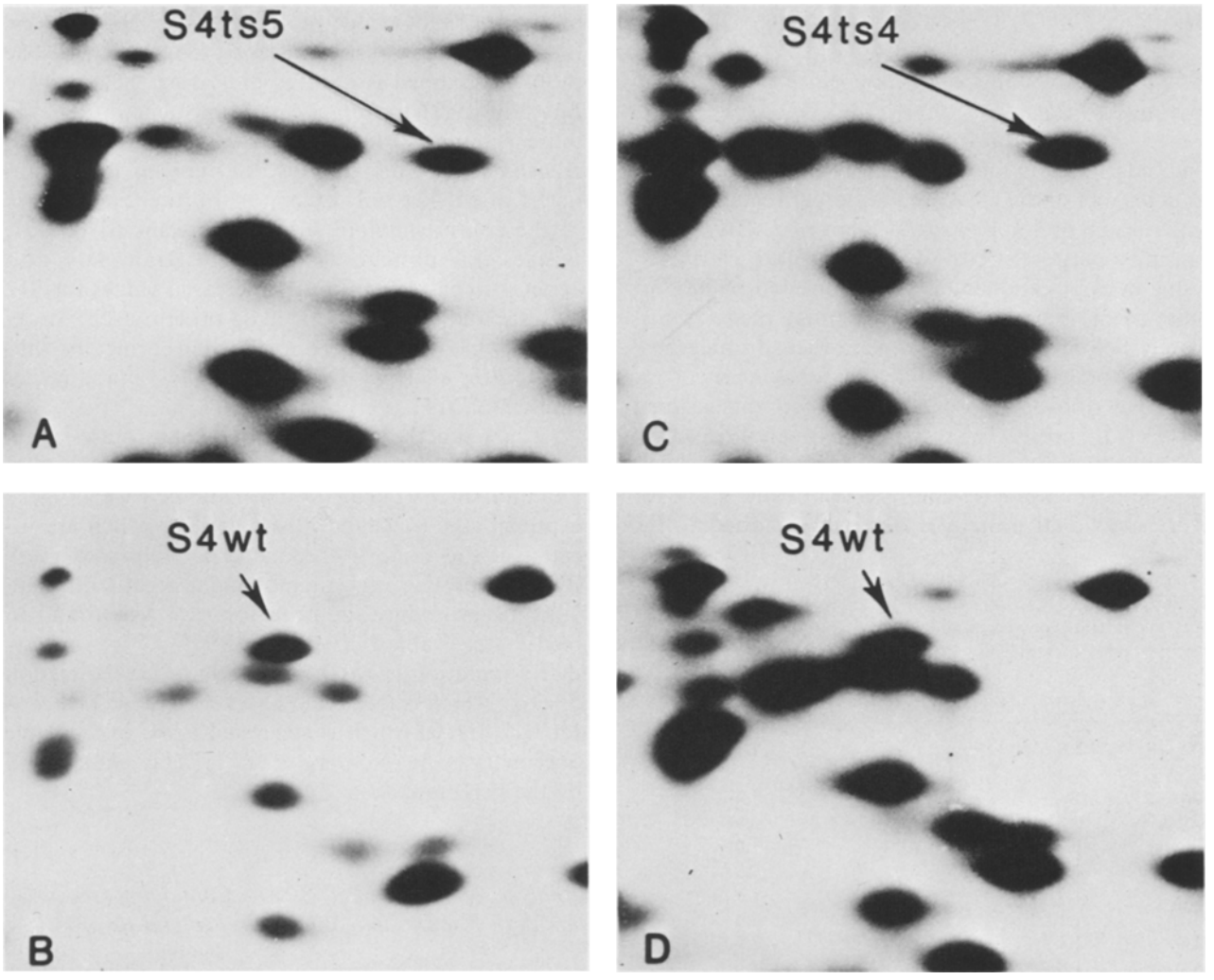

Fig. 1 A–D. Electrophoretic analysis of ribosomal proteins in haploid and heterodiploid mutant derivatives. Acid soluble proteins were extracted directly from whole cells and separated on two-dimensional poly-acrylamide gels as described in Materials and Methods. The position of protein S4 is indicated by an arrow in each case. **A** SU27, **B** D35, **C** SU34 and **D** D34

Gene Dose Effect in a Merodiploid Strain with Both Mutant and Normal rpsD Alleles

It has already been shown by Geyl and Böck (1977) that the ribosomal proteins in a strain, which is partially diploid for genes mapping in the *rpsL* region of the chromosome, are not overproduced as a result of an increased gene dose. We have, however, shown here that a mutation in *rpsD* does have an effect on the production of several ribosomal proteins. The level of stable ribosomal proteins in a *rpsD* strain carrying the F′-factor KLF41, which contains many ribosomal protein genes including $rpsD^+$, was therefore compared with the level of the same proteins in a haploid strain with the same *rpsD* mutation. For this purpose the two strains were labelled separately with ^{14}C-lysine and ^{3}H-lysine respectively, whereafter they were chased with an excess of cold lysine. The analysis of the mixed lysate is shown in Table 3. In addition, the results from a control experiment where a haploid *rpsD* strain was mixed with a haploid $rpsD^+$ strain, is shown. Although many genes are present in duplicate in the merodiploid strain, the relative steady state amount of different ribosomal proteins is close to unity. In the case of protein S4 however, the wild type form is predominantly found in the merodiploid. Thus, with the exception of S4 itself, our data suggest that there is no gene dose effect on the level of ribosomal proteins of the *rpsL* region, even in a merodiploid strain with a $rpsD^+/rpsD$ arrangement.

Table 3. Relative steady-state levels of ribosomal proteins

Protein	Strains D14/017 $^3H/^{14}C$	Strains D34/D24 $^{14}C/^3H$	Number of genes in D34
L1	1.0	1.1	1
L10	1.1	1.0	1
L27	1.0	1.0	1
S15	1.1	1.0	1
S18	0.8	0.9	1
S20	1.1	0.6	1
S4 wt	0.3	3.0	1
S4 ts	3.9	0.2	1
S3	0.9	1.1	2
S7	0.8	1.0	2
S8	1.1	0.9	2
S10	1.1	0.9	2
S12	1.0	1.0	2
S13	1.2	1.0	2
S14	1.0	1.0	2
S19	1.0	1.0	2

Strains D14 (*rpsD4*), 017 (*rpsD*$^+$), D24 (*rpsD4*) and D34 (*rpsD*$^+$/*rpsD4*), growing exponentially at 37° in 50 ml of M9-glucose-aminoacid medium lacking lysine, were labelled for 25 min with 0.5 mCi ^{3}H-lysine (D14, D24) or 2 μCi ^{14}C-lysine (D34, 017). After 5 min of chase with non-radioactive lysine, cultures were harvested and mixed as indicated in the table. The composition of the radioactivity of the individual ribosomal proteins was derived as described in the legend to Table 2. Mutant and normal S4 are not found in the same gel spot and the diploid strain D34 only accumulates normal S4. The radioactivity of both forms of S4 therefore is related to the background radioactivity of the other isotope in the respective spot.

Rate of Synthesis of Ribosomal Proteins in a Merodiploid rpsD$^+$*/rpsD Strain*

The rate of production of some ribosomal proteins was studied in three merodiploid strains carrying the F'-factor KLF41. One of the strains is *rpsD*$^+$ in the chromosome and the other two harbor a mutated *rpsD* allele. The three strains were prelabelled with ^{14}C-lysine and then pulselabelled with ^{3}H-lysine. Total cell extracts were analyzed in a two-dimensional gel electrophoresis, which separates normal and mutated S4 from each other. The result is shown in Table 4. In the case of the *rpsD*$^+$/*rpsD*$^+$ control strain, the rate of production of all proteins, except S21, is close to unity. Several experiments using two independent hetero-diploid strains, which both have a *rpsD* allele in the chromosome, demonstrate that several proteins (S4, S12, S13, S21 and in one strain S7) are overproduced. The high ratio for S21, which is rather variable, is probably due to turnover of this protein, made during the prelabelling period. As a result the ratio, which is based on this internal control, increases.

Table 4. Relative rate of synthesis of individual ribosomal proteins in different strains, partially diploid for some of the ribosomal proteins

Protein	KLF 41/JC1553 (*rpsD*$^+$/*rpsD*$^+$)	D34 (*rpsD*$^+$/*rpsD4*)			DNS 45 *rpsD*$^+$/*rpsD5*
		exp 1	exp 2	exp 3	
S3	1.1	1.0	1.1	1.1	1.1
S4 wt	1.0	0.9	1.1	1.0	1.1
S4 ts	—	2.2	2.3	1.4	2.1
S5/L11	0.8	0.6	0.7	0.9	0.8
S7	1.0	1.7	1.7	1.2	1.1
S8	0.9	0.9	0.9	0.9	0.8
S9	1.0	1.2	1.2	1.2	0.9
S10	0.9	0.9	0.9	1.0	1.0
S12	1.2	2.1	2.1	2.0	1.7
S13	1.0	1.4	1.2	1.4	1.3
S14	1.1	1.1	0.9	1.1	0.9
S15	1.0	1.0	0.9	1.1	1.0
S16	1.0	1.0	0.9	1.1	1.1
S17	1.0	—	—	1.0	1.0
S18	1.0	—	—	1.0	1.0
S19	0.9	0.9	1.0	1.0	1.0
S20	1.2	1.2	1.0	0.9	1.0
S21	3.4	1.4	1.2	1.7	2.5
L17	0.8	1.1	1.0	1.0	0.9

The indicated strains were treated essentially as described in the legend to Table 1. At the moment of harvest the labelled cells were mixed with an equal amount of unlabelled haploid mutant carrier cells (DL14), which are necessary for identification of the mutant S4 spot in the electrophoretic analysis. No stable mutant S4 is produced in the heterodiploid derivatives. This means that the ratio between ^{3}H-counts from the pulselabelling and ^{14}C-counts from the prelabelling calculated for the mutant form of protein S4, in contrast to the values calculated for all other proteins, is based on pure background levels of ^{14}C-counts. Actual cpm-values ($^3H/^{14}C$) were for strain KLF41/JC1553; S4wt (1344/1912), S4ts (=background 180/223), S7 (859/1171), S12 (386/438), S13 (910/1216). Strain D34 exp. 1; S4wt (7441/1594), S4ts (1715/155), S7 (9238/1126), S12 (1999/189), S13 (9219/1258). Strain D34 exp. 2; S4wt (12083/1305), S4ts (2870/140), S7 (13348/956), S12 (3214/183), S13 (14850/1433). Strain D34 exp. 3; S4wt (24772/2382), S4ts (4147/302), S7 (19256/1563), S12 (10620/508), S13 (26248/1837). Strain DNS45; S4wt (964/1304), S4ts (891/637), S7 (1098/1508), S12 (524/469), S13 (1491/1750).

As indicated by the high ratios mutated S4 is produced at a seemingly high rate. This elevated ratio, however, is mainly due to the fact that the spot with mutated S4 does not contain any significant amount of radioactive prelabelled S4. This spot contains primarily ^{14}C-material, originating from the prelabelling period, as background radioactivity. Still, the ratio, which clearly is above one, indicates that also the *rpsD* allele is expressed in the merodiploid strain. Because of the extreme lability of mutant S4, this value most probably reflects a certain underestimation of the expression of the *rpsD* gene. From other experiments (not shown), where pulse-labelled

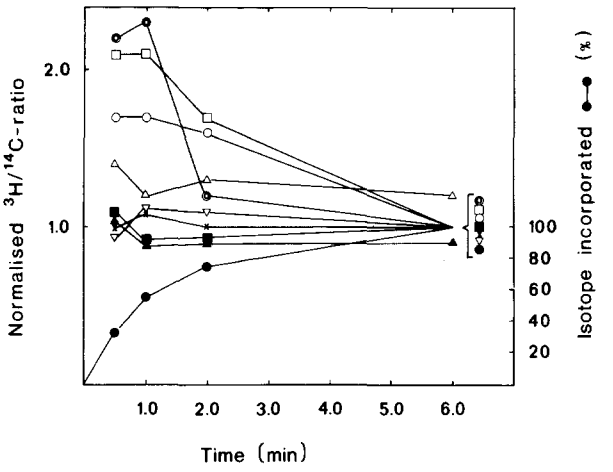

Fig. 2. Turn-over of individual ribosomal proteins during pulselabelling of strain D34. D34, growing exponentially at 37° in 200 ml of a M9-glucose aminoacid-medium lacking lysine, was prelabelled with 25 μCi of ^{14}C-lysine. Several generations later, when all isotope was consumed and the cell density had reached 10^8 cell/ml, the culture was supplied with 2 mCi ^3H-lysine. After 30, 60, 120 and 360 s, 50 ml samples were withdrawn and harvested on frozen and crushed TMK-buffer (10 mM tris, 10 mM $MgCl_2$ and 50 mM KCl pH 7.5). The composition of radioactivity of individual ribosomal proteins was derived as described in the legend to Table 3. Some representative proteins are shown; S3 (×), S4wt (▽), S4ts (◎), S7 (○), S12 (□), S13 (△), S14 (■) and S15 (▲).

haploid *rpsD* cells were mixed with diploid prelabelled *rpsD*$^+$/*rpsD* cells, the normalized ratio, which also in this case reflects an increase over background radioactivity in the mutant S4 gel spot, was found to be about 5. By taking this value as an estimate for rate of synthesis of S4 in a haploid *rpsD* strain, it can be estimated that the *rpsD* gene in a *rpsD*$^+$/*rpsD* strain is expressed to 30%, as a lower limit, compared to the *rpsD*$^+$ allele.

The high ratios found for S12, S13 and apparently also for S4 and in one strain S7 suggest that in a *rpsD*$^+$/*rpsD* strain there is an overexpression of a few, but not all, of the ribosomal genes that are present in duplicate copies.

Degradation of Overproduced Ribosomal Proteins

The influence of the length of the pulse-labelling period on the incorporation of ^3H-lysine into ribosomal proteins was investigated. The merodiploid strain D34, with the *rpsD*$^+$ plus *rpsD* alleles, was used for this experiment. The strain was pregrown on ^{14}C-lysine and pulselabelled with ^3H-lysine for an increasing length of time and the apparent relative rate of synthesis was determined. The result is shown in Fig. 2. It can be seen, that some of the proteins (mutant S4, S7 and S12) are found at higher than average values immediately after the addition of the ^3H-lysine used for the pulse-labelling. At the end of the pulse, when the ^3H-lysine is consumed, the higher values obtained for these proteins is lower and approaches that of the other proteins. It thus appears that, in this strain, the excess of some ribosomal proteins which is overproduced is very rapidly degraded. The precise half-life for the overproduced protein is difficult to estimate but appears to be in the order of a couple of minutes.

Discussion

Measurements on relative rates of production of some ribosomal proteins in an *rpsD* strain show that some of these proteins have an increased rate of production. These proteins are S4, S12 and S13. When an F'-factor with an *rpsD*$^+$ allele is introduced into the same strain, the phenotypic characteristics of the *rpsD* mutant like temperature sensitivity and slow growth rate disappear. The *rpsD* allele thus appears to be recessive to the wild type allele. The disappearance of temperature sensitivity is most likely explained by the fact that the ribosomes of the heterodiploid strain contain only wild type S4 and apparently nothing of the mutant protein. Quite unexpextedly, the heterodiploid strain still produced proteins S4, S12 and probably S13 and also S7 at a significantly increased rate, although the production of the mutant form of S4 is difficult to measure. Short term pulse experiments reveal a varying but consistent production of this protein. The reason for the variation in determinations of mutant S4 in the heterodiploid strain is probably because of an extremely rapid degradation of this protein.

The complementation of the *rpsD*$^+$ allele thus only is obtained for the temperature sensitive property of the mutated allele, but not for the changed rate of production of some ribosomal proteins, which also is a characteristic feature of the haploid mutant. Although some ribosomal proteins are produced at a considerably increased rate in the *rpsD* mutant or *rpsD*$^+$/*rpsD* heterodiploid strains, the steady state level of these proteins is close to unity. The reason for this is most probably that there is a rapid degradation of such overproduced protein. In fact the turnover rate for those proteins is comparable to mRNA turnover. It is known (Olsson and Isaksson, 1979) that in an *rpsD* ts-mutant, all 30S proteins, except S4, accumulate together with 17S RNA in the cell at non permissive temperature. Such proteins, which are not assembled into ribosomes, are degraded. In that case, however, the half lives of the proteins were comparable to that of 17S RNA, i.e. about 20 min.

The failure of mutant S4 to accumulate at the non permissive temperature was suggested to be due to inability of mutant S4 to bind to 17S RNA, thus being protected from turnover.

Altogether, these data suggest that proteins, which are overproduced, are rapidly degraded unless 30S precursor particles are accumulating at the same time. Binding to such particles, even in the absence of complete ribosome maturation, would efficiently protect ribosomal proteins from this rapid degradation. Such mechanism would contribute to the matching of the pool of free ribosomal protein to the availability of ribosomal RNA. The presence of such a mechanism, which has been suggested before (Dennis, 1974) could be part of the explanation to why the pool of free ribosomal proteins is found to be very small in the cell (Marvaldi et al., 1974; Ulbrich and Nierhaus, 1975).

Mutants in *rspsL*, which give the Str-D phenotype, always counteract the abnormally high translational ambiguity which is characteristic of most *rpsD* mutants (Biswas and Gorini, 1972). Introduction of such an *rpsL* allele into an *rpsD* strain does however not significantly change the main pattern of increased rate of production of a few ribosomal proteins (Table 1), although the double mutant shows a decreased translational ambiguity (not shown). Since the observed overproduction of some ribosomal proteins is not correlated with the degree of translational ambiguity of the cell, the changed production pattern in *rpsD* strains is not likely to be a general reflection of the production of faulty proteins, which are synthesized due to the characteristic increased translational ambiguity in such strains.

The proteins which potentially can be overproduced in a strain, containing a *rpsD* mutation, are S4, S7, S12 and S13. Due to the rapid turnover of overproduced proteins and other technical problems, it is sometimes difficult to judge with absolute confidence in a single experiment which proteins show a "unit" relative rate of production and which are significantly altered. Our conviction that production of the indicated four proteins shows a different response to the *rpsD* mutation, compared to other proteins is, however, supported by the results of experiments using several strains in several experiments even though the technical details of the labelling protocols were varied to some extent. The genes for these proteins (*rpsD, G, L,* and *M,* respectively) are suggested to be in two transcriptional units. One of these contains *rpsM, rpsK* (S11), *rpsD, rpoA* (RNA polymerase alfa) and *rplQ* (L17) suggested in that order. The other contains *rpsL, rpsG, fus* (elongation factor G) and *tufA* (elongation factor TuA) in the indicated order (Nomura et al., 1977). The rate of production of L17 is normal in the *rpsD* mutant studied here. The expression of *rpoA, fus, tuf* and *rpsK* in this strain is not yet known. It thus appears, that the *rpsD* mutation can effect the expression of proximal genes in the operon coding for S4 itself, as well as the first two structural genes of the *rpsL* transcription unit. Also the data seem to indicate that the overproduction is most pronounced for S4 and S13 in the *rpsD* haploid strain whereas S12 is most affected in the $rpsD^+/rpsD$ heterodiploid strain. Overproduction of protein S7, which is in the same operon as S12, is only seen in a heterodiploid strain carrying a certain *rpsD* allele.

Some general features are known about the regulation of ribosomal proteins in E. coli. The structural genes are found in several operons (for a review, see Nomura et al., 1977). The synthesis of ribosomal proteins (Dennis and Nomura, 1974, 1975) as well as EFG and EFTu (Furano and Wittel, 1976) is under stringent control and ppGpp has been shown to inhibit DNA dependent synthesis of ribosomal proteins in a direct fashion in vitro (Lindahl et al., 1976). Results obtained from in vivo experiments do, however, indicate that control of ribosome biosynthesis during cellular growth is more complicated. The basal level of (p)ppGpp at normal growth is very low compared to the level, which is induced upon amino acid starvation (Lazzarini et al., 1971; Friesen et al., 1975). Furthermore, the regulation of rRNA and ribosomal protein mRNA is not identical when the effect of changing growth rate of the cell is investigated (Gausing, 1977) although both classes of macromolecules apparently are under stringent control (Stent and Brenner, 1961; Kurland and Maløe, 1961, 1962; Dennis and Nomura, 1975). Arguments have been presented both in favour of some kind of negative (Dennis and Nomura, 1975) as well as a positive autogenous control of ribosomal protein genes (Gausing, 1974). Clearly, the control mechanisms for expression of ribosomal protein genes are complicated and far from understood.

In agreement with Geyl and Böck (1977), we found that production of most ribosomal proteins in a $rpsD^+/rpsD^+$ homodiploid strain is not subjected to a gene dose effect; it produces ribosomal proteins at the same rate as a haploid strain. Even in experiments with very short pulses there was no sign of overproduction followed by rapid degradation in the merodiploid strain. A possible explanation of this result could be that expression of the duplicated genes is under positive control of the limiting product(s) of some other genes(s), which are not duplicated as a result of introduction of the F'-factor. However, such a control is not necessarily operating on all ribosomal protein genes since production of a few riboso-

mal proteins S20, S21, L21, which map outside the *rpsD-rpsL* region of the chromosome, is subjected to a gene dose effect (Geyl and Böck, 1977; Takata, 1978).

The results presented here show that a mutation in *rpsD* is associated with an increased expression of a few ribosomal protein genes, including *rpsD* itself. This correlation suggests that normal expression of these genes is dependent on wild type S4. In *rpsD*$^+$/*rpsD* heterodiploid cells both mutant and wild type S4 appear to be produced, but probably due to the lability and low affinity of the mutant protein for 16S or 17S RNA, ribosomes will contain almost exclusively wild type S4. Still, in such a strain, the overexpression of *rpsL* (S12) is even higher and now also can include the next gene *rpsD* (S7) in the same operon. This means that normal expression of the indicated genes is not conferred by the normal steady state level of wild type S4 in mature ribosomes. The results instead suggest that the observed expression is directly or indirectly due to the presence of mutant S4 itself.

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