ence **143**, 676 (1964); R. J. Cvetanovic, F. J. Duncan, W. E. Falconer, *Can. J. Chem.* **41**, 2095 (1963); W. E. Falconer and R. J. Cvetanovic, *Anal. Chem.* **34**, 1064 (1962); K. F. Wilzbach and P. Riesz, *Science* **126**, 748 (1957).

9. G. C. Pimentel, A. M. Bass, H. P. Broida, in *Formation and Trapping of Free Radicals*, A. M. Bass and H. P. Broida, Eds. (Academic Press, New York, 1960), p. 69.
10. The sticking coefficient for condensation is very sensitive to alterations in the experimental apparatus; each laboratory must determine how coefficients, measured on its apparatus, scale relative to other reported values.
11. Because the sticking coefficient for condensation is always less than unity, a measured PMI Beer's law parameter always represents a lower bound on the absolute value.
12. The volume of 0.5 μmole of gas is equivalent to about 0.01 cm^3 filled to atmospheric pressure at room temperature.
13. I thank R. V. Albarino for technical assistance.

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Streptomycin Resistance Mutation in Escherichia coli: Altered Ribosomal Protein

Abstract. *Reconstitution of 30S ribosomal particles was performed with 16S ribosomal RNA, "core" proteins, and "split" proteins from 30S particles derived from streptomycin-sensitive and streptomycin-resistant* Escherichia coli *cells in various combinations. Analysis of streptomycin sensitivity of the reconstituted particles has shown that the alteration induced by the resistance mutation resides in the core proteins, and not in the RNA or in the split proteins of the 30S particles.*

A combination of genetic and biochemical methods should be useful in the study of complex cellular organelles such as ribosomes. However, no ribosome mutation has yet been discovered where a functional alteration is correlated to a structural alteration in an obvious way (for a review, see *1*). The most extensively studied ribosomal mutation is concerned with streptomycin (Sm) resistance in *Escherichia coli*. Streptomycin inhibits polypeptide synthesis in vitro (*2*), and the site of its action is believed to be the ribosome (*3*). Thus, polypeptide synthesis of ribosomes from an Sm-resistant mutant is resistant to Sm, whereas that by ribosomes from a wild-type Sm-sensitive strain is sensitive to Sm (*2*). Furthermore, the mutational alteration which leads to Sm resistance is associated with an alteration in the 30*S* ribosomal subunit (*4*). Previous studies (*5, 6*) have shown that the alteration is in the 23*S* "core" portion of the 30*S* particle, and not in the rest of the proteins (split proteins from the 30*S* particles, designated as SP30). Although the altered component was thought to be a protein, no observable alteration had been detected in the ribosomal proteins of appropriate mutants (*1*). Thus, the possibility had not been excluded that the alteration is in the 16*S* ribosomal RNA.

We have achieved reconstitution of functionally active 30*S* ribosomal particles from free 16*S* ribosomal RNA, and the mixture of ribosomal proteins obtained from 30*S* particles, in the following way (*7*). (i) The 23*S* core particles and SP30 proteins were prepared by centrifugation of purified 30*S* particles in 5*M* CsCl (*8*). The SP30 proteins (about 40 percent of the total 30*S* ribosomal proteins) were recovered from the top of the gradient, and the 23*S* core particles were recovered from the band near the middle of the gradient. (ii) The 16*S* ribosomal RNA was prepared from 23*S* core particles by treatment with phenol. (iii) Proteins (CP30) from 23*S* core particles were prepared by treatment of the particles with an equal volume of a solution consisting of 8*M* urea and 4*M* LiCl. (iv) The 16*S* RNA, CP30 proteins, and SP30 proteins were mixed under controlled conditions and dialyzed, and the reconstituted 30*S* particles were recovered by centrifugation. Using this reconstitution system, we now show that the alteration induced by the Sm-resistance mutation resides in the CP30 proteins and not in the 16*S* ribosomal RNA.

An Sm-sensitive strain *Escherichia coli* Q13 and an Sm-resistant mutant derived from it were used (*5*). Both 30*S* and 50*S* ribosomal particles were prepared from these strains and purified (*9*); 16*S* RNA, CP30 proteins, and SP30 proteins were prepared from 30*S* particles from both the Sm-sensitive and the Sm-resistant strains. Both SP30 and CP30 protein fractions were essentially devoid of RNA (less than 2 percent), and the 16*S* RNA preparations contained almost no protein (less than 0.6 percent). The 30*S* particles were reconstituted by the mixing of 16*S* RNA, CP30 proteins, and SP30 proteins in eight different combinations (Table 1). The resultant particles were recovered by centrifugation, and their sensitivity to Sm was tested by following polyuridylic acid–directed phenylalanine incorporation (*10*) in the system containing 50*S* particles from the Sm-sensitive strain (*11, 12*).

The activity of the reconstituted 30*S* particles assayed in the absence of Sm was very high in every case and nearly the same as the activity of native 30*S* particles (Table 1). Also, Sm inhibited the incorporation only when the reconstituted 30*S* particles contained the CP30 proteins derived from sensitive cells, and the degree of inhibition was about the same as that found with sensitive native 30*S* particles. The origin of the SP30 proteins did not show any correlation with the Sm-sensitivity of the reconstituted particles, confirming previous results (*5, 6*). Similarly, the origin of 16*S* RNA did not affect the sensitivity of the reconstituted particles to Sm. Thus, the alteration induced by the Sm-resistance mutation resides in the CP30 proteins and not in the 16*S* ribosomal RNA.

Earlier studies have demonstrated that

Table 1. Sensitivity to streptomycin (Sm) of 30*S* ribosomal particles reconstituted from 16*S* RNA, CP30 proteins, and SP30 proteins. The origin of the components is described in Table 1. The letters r or s indicate that they are derived from Sm-resistant or Sm-sensitive cells, respectively. The reconstituted particles or control undissociated 30*S* particles (60 μg) were assayed for their activity in polyuridylic acid–directed incorporation of phenylalanine in the presence of 50 *S* particles (120 μg) from Sm-sensitive cells and, when indicated, in the presence of Sm (5×10^{-5} mole/liter). The Sm was mixed with ribosomes before the addition of other components. Incorporation was assayed as described (*9*).

Reconstituted "30*S*"			Control 30*S*	Incorporation activity (count/min)		Inhibition by Sm (%)
16*S* RNA	CP30	SP30		− Sm	+ Sm	
			s	11063	7042	36
			r	13174	12995	1.4
s	s	s		10498	6979	34
s	s	r		11904	7889	34
s	r	s		9898	9471	4.3
s	r	r		12037	11921	1.0
r	s	s		11112	7208	37
r	s	r		13098	8486	35
r	r	s		10695	10615	0.6
r	r	r		12491	12579	0 (−0.7)

streptomycin also causes misreading of the genetic message (*12, 13*). Therefore, the structure of the ribosomal site which responds to Sm should be important in influencing the specificity of the interaction between the codon in messenger RNA and the anticodon in transfer RNA. Our work shows that a protein in the CP30 protein fraction is an essential component of this structure.

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References and Notes

1. J. G. Flaks, P. S. Leboy, E. A. Birge, C. G. Kurland, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 623 (1966); D. Apirion, *J. Mol. Biol.* **30**, 255 (1967).
2. J. F. Speyer, P. Lengyel, C Basilio, *Proc. Nat. Acad. Sci. U.S.* **48**, 684 (1962); J. G. Flaks, E. C. Cox, M. L. Witting, J. R. White, *Biochem. Biophys. Res. Commun.* **7**, 390 (1962).
3. C. R. Spotts and R. T. Stanier, *Nature* **192**, 633 (1961); G. A. Jacoby and L. C. Gorini, in *Antibiotics I*, D. Gottlieb and P. D. Shaw, Eds. (Springer, New York, 1967), p. 726
4. E. C. Cox, J. R. White, J. G. Flaks, *Proc. Nat. Acad. Sci. U.S.* **51**, 703 (1964); J. E. Davies, *ibid.*, p. 659.
5. P. Traub, K. Hosokawa, M. Nomura, *J. Mol. Biol.* **19**, 215 (1966).
6. T. Staehelin and M. Meselson, *ibid.*, p. 207.
7. P. Traub and M. Nomura, *Proc. Nat. Acad. Sci. U.S.*, in press.
8. M. Meselson, M. Nomura, S. Brenner, C. Davern, D. Schlessinger, *J. Mol. Biol.* **9**, 696 (1964).
9. K. Hosokawa, R. K. Fujimura, M. Nomura, *Proc. Nat. Acad. Sci. U.S.* **55**, 198 (1966); P. Traub and M. Nomura, *J. Mol. Biol.*, in press.
10. M. Nirenberg, and J. H. Matthaei, *Proc. Nat. Acad. Sci. U.S.* **47**, 1589 (1961).
11. Although test of the misreading effect of Sm (*12*) is one way to identify the Sm-resistance, we have tested only the inhibitory effect and not the misreading effect of Sm.
12. J. E. Davies, W. Gilbert, L. Gorini, *Proc. Nat. Acad. Sci. U.S.* **51**, 883 (1964).
13. T. E. Likover and C. G Kurland, *ibid.* **58**, 2385 (1967).
14. Paper No. 1186 from the Laboratory of Genetics, University of Wisconsin. We thank Drs. C. G. Kurland and M. Susman for reading the manuscript, Miss H. Bechmann and Mrs. U. Traub for technical assistance. Supported by PHS research grant GM-15422, from the National Center for Urban and Industrial Health, and NSF grant GB-6594.

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Selection for Higher Fitness in Populations of *Drosophila pseudoobscura*

Abstract. *Four laboratory populations, each consisting of flies homozygous for a wild second chromosome, were maintained in population cages. Three of them were initially characterized by low fecundity of the females; the fourth was a control. After 15 months, in all three populations with low fecundity this character was much improved; two of them have reached the control level. The changes observed were presumably due to mutation and selection under highly competitive conditions.*

Natural populations of *Drosophila* carry concealed recessive genes which, when homozygous, decrease the fecundity of the flies. Females of *D. pseudoobscura* homozygous for wild second chromosomes lay approximately 19 percent fewer eggs ($\bar{X} = 26.0 \pm 0.7$ per day per female) than females heterozygous for the same chromosomes ($\bar{X} = 32.2 \pm 0.8$ per day per female) (*1*). If the survival from egg to adult is taken into account, the difference between the homozygotes and the heterozygotes becomes even greater, reaching a value of about 48 percent (*2*).

Among 211 wild second chromosomes analyzed for the fecundity of the homozygous females in spring 1966 (*1*), three conferring to their carriers the lowest egg-laying capacity and one conferring a high capacity (used as a control) have been chosen. Four experimental populations, each homozygous for one of these wild second chromosomes were maintained at 25°C in laboratory population cages, with an initial number of founders between 800 and 1000. For all four populations, chromosomes other than the second came from a laboratory tester stock carrying the markers Delta/Bare.

Over a period of 15 months, five samples of eggs were taken in the cages. The survival from egg to adult and the fecundity were measured simultaneously in the four populations. The eggs collected in the cages were distributed in ten groups of about 50 each, and each group was allowed to develop in a separate culture under uncrowded conditions. The percentage of emerging adults was the measure of survival from egg to adult. From these emerging adults, 30 impregnated females were distributed in ten vials with three females per vial. Fecundity was measured by counting daily the eggs laid in each vial from the 6th to the 15th day after emergence. Ten replications were thus available per sample for each measure. The determinations of the two parameters made in May 1966 were, however, made with three replications. The mean average number of eggs laid per female per day, the percentage of survival from egg to adult, and the standard errors for both measures are given in Table 1.

The fecundity of the control females fluctuated throughout the experimental period, owing to uncontrolled environmental variations in the cultures. With the performance of the control population taken as a standard, the fecundity of the experimental populations increased gradually and rather dramatically. The initial fecundity of the females in the three homozygous strains (Pinon 9, Borrego 71, and Borrego 41) was 3.2 and 8.6 times lower than that in the control strain (Borrego 3). During the period of 15 months (corresponding to about 15 generations under the experimental conditions), the fecundity increased in two populations to the degree found in the control population. The difference between the third population, Borrego 41, and the control diminished to about 15 percent. The survival from egg to adult was also improved in the populations in which it was low (Borrego 3, 41, and 71) and remained approximately constant in the Pinon 9 population where it was initially high. At the end of the experiment, the four populations were very much alike and approached the means obtained for random heterozygous com-

Table 1. Average number of eggs deposited per female per day, and percentage of survival from egg to adult, with their standard errors, in the experimental populations.

Date	Measurement	Borrego 3	Pinon 9	Borrego 71	Borrego 41
May 1966	Fecundity	43.0	13.5	5.0	12.9
May 1966	Survival	17.0	71.7	37.3	52.8
August 1966	Fecundity	31.9 ± 0.7	16.5 ± 1.3	16.9 ± 1.5	13.6 ± 3.7
August 1966	Survival	47.0 ± 3.5	74.0 ± 4.2	59.1 ± 3.8	46.1 ± 3.6
January 1967	Fecundity	39.4 ± 2.1	32.2 ± 2.1	24.2 ± 2.6	30.1 ± 2.2
January 1967	Survival	68.0 ± 4.7	77.0 ± 3.9	77.1 ± 3.5	67.1 ± 4.4
April 1967	Fecundity	41.1 ± 3.3	30.1 ± 1.7	33.9 ± 3.3	30.7 ± 1.8
April 1967	Survival	64.2 ± 5.4	63.9 ± 5.9	68.0 ± 4.9	81.5 ± 3.1
July 1967	Fecundity	27.2 ± 2.5	27.1 ± 2.6	30.8 ± 3.0	23.4 ± 2.0
July 1967	Survival	68.9 ± 3.6	74.3 ± 4.3	82.8 ± 4.1	83.7 ± 3.2
August 1967	Fecundity	33.5 ± 2.0	35.2 ± 3.1	33.6 ± 3.4	28.1 ± 3.5
August 1967	Survival	76.6 ± 5.0	76.3 ± 8.9	79.4 ± 4.8	66.3 ± 6.5