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Ribosomal Protein Conferring Sensitivity to the Antibiotic Spectinomycin in Escherichia coli

Abstract. *Reconstitution of 30S ribosomal particles from 16S ribosomal RNA and total proteins, or from core proteins and split proteins obtained from the ribosomes of strains of* Escherichia coli *sensitive to and resistant to spectinomycin, shows that the split protein fraction determines the response of polypeptide synthesis in virto to spectinomycin. Reconstitution of active particles in the presence of isolated split proteins allowed the identification of the single split protein responsible for spectinomycin sensitivity.*

The aminoglycoside antibiotic spectinomycin is a strong inhibitor of protein synthesis in whole cells and in extracts, and it has been demonstrated that sensitivity and resistance to this drug are properties of the 30*S* ribosomal subunit (*1*). The genetic locus conferring resistance to spectinomycin is located close to the locus for streptomycin resistance on the *Escherichia coli* linkage map; the results of mapping by conjugation (*1*), transduction (*1–3*), and complementation studies in diploids (*4*, *5*) strongly suggest that these two determinants are related to changes in two separate proteins of the 30*S* subunit.

Active 30*S* ribosomal particles have been reconstituted from split proteins and core particles (*6*), and more fundamentally from ribosomal RNA and ribosomal proteins (*7*). These ribosomal proteins have been separated and characterized (*8*, *9*). We now report identification of the ribosomal component that confers sensitivity to spectinomycin in a manner identical to the identification of the protein which confers sensitivity (*10*, *11*) and dependence (*12*) to streptomycin.

Ribosomal subunits were prepared by zonal centrifugation of extracts from a strain of *Escherichia coli* MRE600 sensitive to spectinomycin and a mutant resistant to spectinomycin derived from this strain by spontaneous mutation. Split proteins and core particles were prepared according to Meselson *et al.* (*13*). Total 30*S* ribosomal protein was obtained by lithium chloride–urea extraction of 30*S* subunits, and purified single ribosomal proteins were obtained by phosphocellulose chromatography (*8*). Reconstitution of 30*S* ribosome subunits from 16*S* ribosomal RNA and total proteins has been described by Traub and Nomura (*7*). The integrity of all reconstituted particles was confirmed by sucrose density-gradient centrifugation. Because spectinomycin shows considerable variation in its inhibitory effects against polypeptide synthesis directed by synthetic polynucleotides, we used protein synthesis directed by RNA from bacteriophage R17 or f2 as assays for the properties of 30*S* subunits.

The results of reconstitution of 30*S* subunits from 16*S* ribosomal RNA and total core and split proteins (Table 1) show that a split protein is involved in sensitivity to spectinomycin. This distinguishes the protein determinant of spectinomycin sensitivity from that of streptomycin sensitivity, because the latter is a core protein (*10*).

The results of reconstitution of 30*S* subunits from 16*S* ribosomal RNA, total proteins, and single split proteins (Table 2) show that a single protein (P_4) [formerly called B_2, see (*8*)] purified from the split protein fraction, determines sensitivity to spectinomycin in 30*S* ribosomal subunits.

In similar experiments, protein P_4, purified directly from total 30*S* ribosomal proteins (*11*), was used and the same conclusion was obtained. Previous experiments have shown that the pro-

Table 1. Sensitivity to spectinomycin of 30*S* ribosomal particles reconstituted from 16*S* RNA (optical density at 260 nm, 25 units), total proteins (TP) (750 μg), core proteins (CP30), and split proteins (SP30) (a threefold excess). The letters S or R indicate that they are derived from spectinomycin-sensitive or spectinomycin-resistant cells, respectively. The reconstituted particles or control undissociated 30*S* ribosomal particles (60 μg) were assayed for their activity in f2-bacteriophage RNA-directed incorporation of valine in the presence of 50*S* particles (120 μg) from spectinomycin-sensitive cells, and when indicated, in the presence of spectinomycin (+Spc) (6×10^{-6} mole/liter). Sensitivity of native and reconstituted particles to spectinomycin ranged between 65 and 80 percent inhibition. Samples were counted for at least 1000 counts, and experiments were repeated at least two times with independent preparations.

Reconstituted 30*S*				Control 30*S*	Incorporation (count/min)		Inhibition by Spc (%)
16*S* RNA	TP	CP	SP		Control Spc	+Spc	
				S	159	51	66
				R	280	224	20
S	S				314	112	65
S	R				169	117	30
S	R	S			255	185	27
S	R	R			249	184	26
S	R		S		269	76	72
S	R		R		186	162	15

Table 2. Sensitivity to spectinomycin (Spc) of 30*S* ribosomal particles reconstituted from 16*S* RNA (optical density at 260 nm, 25 units), total proteins (TP) (750 μg), and single split proteins (threefold excess) (*8*). Symbols and procedure are as described in the legend of Table 1; P_1, P_4, and P_6 correspond to split proteins A_1, B_2, and B_3, as described previously (*8*).

Reconstituted 30*S*			Control 30*S*	Incorporation activity (count/min)		Inhibition by Spc (%)
16*S* RNA	TP	Single SP		Control Spc	+Spc	
			S	159	51	66
			R	280	224	20
S	S			314	112	65
S	R			169	117	30
S	R	P_1 (A_1)		225	142	37
S	R	P_4 (B_2)		338	107	70
S	R	P_6 (B_3)		479	337	30

tein determined by the streptomycin-resistant locus is P_{10} and is different from the so-called K-protein (*14*), which is P_5 according to our nomenclature (*11*). Thus, the protein determined by the spectinomycin locus is different from that determined by the streptomycin locus or from the K-protein.

The function of the spectinomycin protein (P_4) has been studied previously (*8*). Omission of this protein from a reconstituted 30*S* subunit caused only a partial decrease in incorporation activity, assayed with both synthetic and natural messenger RNA (mRNA). However, omission of the streptomycin protein (P_{10}) during reconstitution gives a particle that shows a drastic decrease in incorporation activity when assayed with natural mRNA, but only a weak decrease with a synthetic mRNA (*11*). Thus, the functions of these two proteins (P_4 and P_{10}) appear to be different.

However, the function of the P_4 protein was studied with a partial reconstitution system and the possibility of contamination of "P_4-deficient particles" by P_4 could not be excluded (*8*). The reconstitution system now available, which allows 30*S* subunit formation from 16*S* RNA and each of the purified ribosomal protein components, should give more definite information as to the functional role of the protein controlled by the spectinomycin locus.

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Memory-Blocking Agents: Effects on Olfactory Discrimination in Homing Salmon

Abstract. *Homing salmon were injected intracranially with puromycin, actinomycin D, or cycloheximide. From 4 to 7 hours after such treatment these agents markedly inhibited olfactory bulbar discrimination between home water and other natural waters, including spawning sites for other groups of salmon. At longer intervals after treatment there was a partial restoration of olfactory memory-based discrimination. The dosages of the inhibitors used could be shown to have depressed incorporation of H^3-leucine into protein by 78 percent or of H^3-uridine into RNA by 41 percent in the salmon brains 4 hours after intracranial injection. These findings suggest that acute blockage of RNA synthesis or protein synthesis can interfere with long-term olfactory memory in anadromous salmon, at least as this function can be analyzed by electrophysiological methods. This implies that long-term olfactory memory depends upon continued metabolism of RNA and continued protein synthesis.*

A rapidly growing literature (*1*) indicates a relation between memory and cellular-genetic and protein-synthetic activity in the central nervous system. Much of the research in this field has utilized the goldfish (*2*) and, more specifically, relates DNA-dependent RNA synthesis [but not DNA synthesis (*3*)] to establishment of long-term memory. Actinomycin D and puromycin block establishment of long-term memory (*4*); short-term memory apparently is not dependent on this mechanism (*2*). Although there seems to be agreement that RNA synthesis is part of a memory-establishing mechanism, there is no information concerning the character of the residual memory process. Transfer of learned behavior in goldfish and rats through preparations of brain RNA (*1, 5*) would suggest persistence of long-lived RNA or de-

Table 1. Effects of puromycin, actinomycin D, or cycloheximide on electroencephalographic (EEG) olfactory discrimination in Chinook salmon that had homed to the University of Washington College of Fisheries pond. The mean evoked response is expressed as arbitrary values obtained by planimetric measurement of the integrated olfactory bulbar response. For further explanation see Fig. 1. Soos refers to Soos Creek; U.W. to the University of Washington College of Fisheries.

Treatment	Dosage	No. of fish	Integrated bulbar EEG response to test water: Mean evoked response to pond water*	Mean ratio of responses × 100 (%): Soos/U.W. fisheries	Mean ratio of responses × 100 (%): Skykomish/U.W. fisheries
	4 to 7 hours after intracranial injection				
Saline	0.05 ml	12	1.44 ± 0.06	49.8 ± 3.9	82.1 ± 4.8
Puromycin HCl	500 μg	8	0.95 ± .13†	94.8 ± 7.9‡	115.4 ± 8.2§
	1000 μg	2	.58 ± .02‡	110.3 ± 0.5‡	110.0 ± 0.7
Actinomycin D	100 μg	9	.99 ± .11‖	84.0 ± 4.3‡	121.3 ± 7.6‡
	200 μg	4	1.03 ± .17	87.0 ± 4.8	120.0 ± 11.8‖
Cycloheximide	1000 μg	5	1.07 ± .09§	95.4 ± 3.6‡	105.6 ± 7.8†
	9 to 28 hours after intracranial injection				
Saline	0.05 ml	4	1.28 ± 0.09	45.8 ± 8.5	74.5 ± 8.5
Puromycin HCl	500 μg	4	1.18 ± .15	68.8 ± 9.7	78.0 ± 8.6
Actinomycin D	100 μg	8	1.13 ± .08	74.1 ± 8.3	86.1 ± 4.1
Cycloheximide	1000 μg	4	0.92 ± .09	70.7 ± 2.1†	96.0 ± 6.0

* University of Washington fisheries pond. † $P < .025$. ‡ $P < .001$. § $P < .005$. ‖ $P < .01$.