Table 2. Formation of double-stranded DNA of satellite virus from plus and minus single strands. S, single stranded; D, double stranded. Conditions: Satellite virus in standard saline citrate (SSC) is diluted to 1/10 containing 0.1 percent sodium lauroyl sarcosinate (SLS), heated to 100°C for 10 minutes, then cooled in ice water, and brought to 2 × SSC in concentration; then 2C DNA is added (final concentration 0.5 μg/ml). Final concentration of satellite DNA is about 1 μg/ml. Satellite DNA is heated in water bath at 67°C.

Time (min)	DNA density (g/cm³)	Appearance of band	Strandedness
0	1.729	Fuzzy	S
15	1.724	Broad	S → D
30	1.718	Sharp	D
60	1.719	Hypersharp	Aggregated D
120	1.718	Hypersharp	Aggregated D
180	1.718	Hypersharp	Aggregated D

stranded satellite DNA is obtained, whereas control native DNA (bacteriophage 2C) contained in the mixture remains double-stranded (Table 1).

(iii) It is known that the rate of renaturation of fully denatured DNA is a second-order reaction and that the reaction rate is at a maximum at $T_m - 25$°C and high ionic strengths (*13*). To study renaturation kinetics of satellite DNA, material prepared as described in (ii) at a concentration of 1 μg/ml was heated to 100°C for 10 minutes and immediately cooled in ice. Solvent concentration was then brought to twice standard saline citrate, marker 2C DNA was added, and the preparation held at 67°C. Samples were withdrawn, added to solid CsCl, and adjusted to a mean density of 1.71 g/cm³. The buoyant densities achieved at various time intervals are shown in Table 2 and Fig. 1.

Electron microscopic examination (*12*) of DNA during renaturation showed conversion of the collapsed coil characteristic of single-stranded DNA to a linear rigid form characteristic of a double-stranded helix within 15 minutes after initiating renaturation (Fig. 2). Published data on molecular weight from alkaline band sedimentation and contour length measurements [molecular species is linear and 1.5 μ long (*2*)] together with the fact that renaturation is not instantaneous (Table 2) militate against the possibility that the molecule is self-complementary with a hairpin-like reflexion. Renaturation data and the physical data presented here and in a previous publication (*2*), therefore, are consistent with the possibility of a single-stranded linear satellite genome with complementary plus and minus strands in different particles (Fig. 3).

"Renaturation" kinetics for satellite DNA proceed at an extremely high rate, indicative of two completely complementary linear molecules, and show no evidence of residual single-stranded material on completion of the double-stranded form.

Wetmur and Davidson (*13*) have shown that the second-order renaturation coefficient for DNA is $k_2 = 3.5 \times 10^5 \, L^{0.5}/N$ at $T_m - 25$°C, where N is the complexity and L the average number of nucleotides per single strand. The k_2 also increases slightly with the guanine-cytosine content of the DNA and is proportional to the reciprocal of the molecular weight. Assembly of double-stranded satellite DNA proceeds at a high rate, indicating presence of linear complementary molecules of low molecular weight with the possibility of many redundancies (low N). The band-sharpening observed during "renaturation" and the rate of decrease in buoyant density during "renaturation" are consistent with these findings (*14*). The hypersharp profile achieved (Table 2) is consistent with the possibility of an aggregated molecular species and may indicate the presence of permuted ends. The branched molecules observed in the electron microscope during renaturation are consistent with this finding. Circular molecules which might be expected to be present in a permuted population (*15*) were not observed.

There are now numerous reports in the literature on the single-stranded nature of the DNA of autonomous pico-DNA, or parvo viruses (*3, 6, 16*). Their DNA, however, remains single-stranded after extraction, indicating that the normal rules for replication of single-stranded molecules are being followed. Adeno-satellites are unique among the small DNA viruses in that they are defective. Their DNA's also are unique. Study of the replicative mechanisms leading to encapsidation of complementary plus and minus single-strands in separate particles could be important in unraveling the mystery of viral defectiveness.

HEATHER D. MAYOR
KATSUTAKA TORIKAI
JOSEPH L. MELNICK
Baylor College of Medicine, Houston, Texas 77025
MANLEY MANDEL
University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston 77025

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Altered Ribosomal Protein in Streptomycin-Dependent Escherichia coli

Abstract. *We have compared the 30S ribosomal proteins of strains of* Escherichia coli *sensitive to and dependent on streptomycin and identified a single protein that is functionally altered in the ribosomes dependent on streptomycin. This protein (30S-15) is the same protein that is functionally altered in ribosomes resistant to streptomycin.*

The functional analysis of genetically altered ribosomal proteins has been made possible by the development of procedures for the purification of individual ribosomal proteins (*1, 2*) and for reconstituting functional 30*S* ribosomal particles from 16*S* RNA and the 30*S* proteins (*3*). Consequently, genetic

alterations affecting the ribosome can be localized readily in specific ribosomal components. The rapid elaboration of a genetic map for all of the ribosomal components is impeded by the scarcity of suitable mutant phenotypes. One of the more effective procedures for selecting bacteria with genetically altered ribosomes has been to choose mutants with an altered response to antibiotics (*4*). In this way the two ribosomal proteins responsible for the streptomycin-resistant (SmR) phenotype and the spectinomycin-resistant (SpcR) phenotype in *Escherichia coli* have been identified (*5, 6*). We now report the identification of the 30*S* ribosomal protein which is altered in a streptomycin-dependent (SmD) strain of *E. coli*.

When appropriate ionic conditions are maintained in vitro, it is possible to show that ribosomes from SmD bacteria require streptomycin (Sm) for maximum polyphenylalanine synthesis primed by polyuridylic acid and that this SmD polypeptide synthesis is a property of the 30*S* ribosomal subunit (*7*). Therefore, the altered component in this strain must be either the 16*S* ribosomal RNA or one of the 30*S* proteins. This altered component, like that of SmR ribosomes (*5*), is a protein (Table 1). If 30*S* particles are reconstituted with protein from SmD ribosomes, the reconstituted particles are slightly stimulated by Sm, and this effect is independent of the source of the ribosomal RNA. In contrast, Sm causes strong inhibition of polypeptide synthesis if the reconstituted 30*S* particles contain wild-type protein.

Unfortunately, we have never been able to obtain a marked stimulation of polypeptide synthesis with reconstituted ribosomes under any conditions. This failure is evident even when ribosomes are reconstituted from protein and RNA of native ribosomes that are stimulated to function in vitro three times faster in the presence of Sm than in its absence. We do not understand this, and we are forced to rely on the difference between slight stimulation and strong inhibition of polypeptide synthesis in vitro to distinguish the SmD from the Sm-sensitive (SmS) phenotype of reconstituted ribosomes.

To determine which of the 30*S* proteins is altered in the SmD ribosomes we assumed that the degree of SmS character of the reconstituted ribosomes would reflect the relative amount of the altered protein in the reconstitution mixture. Therefore, by adding to the reconstitution mixture an excess of the normal (SmS) proteins, as well as unfractionated 30*S* protein from SmD particles, we should be able to obtain ribosomes which have an SmS phenotype. Such experiments were successful and led to the same kind of "dilution" experiments with individual proteins or groups of proteins from the SmS ribosomes. The only protein from SmS ribosomes capable of converting the SmD ribosomes to the SmS phenotype is one which we designate 30*S*-15 (*2*) (Table 2).

More convincing evidence that 30*S*-15 is the altered ribosomal protein of SmD ribosomes is provided in Fig. 1. This nominal protein was purified from SmS and SmD ribosomes. When the SmS protein (30*S*-15 SmS) is added in increasing amounts to a reconstitution mixture containing unfractionated SmD protein, the resulting 30*S* particles show a progressive degree of SmS phenotype. The reciprocal experiment also yields the expected results.

Table 1. Incorporation of ^{14}C-phenylalanine directed by polyuridylic acid (poly U) by reconstituted 30*S* ribosomes (0.67 optical density units measured at 260 nm). Preparation of the ribosomes, RNA, and protein have been described (*2, 11*). Protein solutions were prepared by the lithium chloride-urea method, although protein extracted with acetic acid is equally active. The reconstitution system, modified from Traub and Nomura (*3*), consisted of 6 O.D. units (260 nm) of 16*S* RNA, 0.18 mg of total protein, and 0.04*M* 2-mercaptoethanol in a final volume of 1.37 ml with an ionic strength of 0.43. Reconstituted 30*S* ribosomes were assayed (*7*) with the following changes: 22 m*M* tris-(hydroxymethyl)aminomethane, *p*H 8.5; 0.2 m*M* all amino acids except phenylalanine; 0.04 m*M* ^{14}C-phenylalanine (10 μc/μmole); 7 O.D. units (260 nm) of tRNA; 25 μg of poly U; and 1.33 O.D. units (260 nm) of SmS 50*S* ribosomes; incubation time was 50 minutes. A background of 90 count/min was subtracted from all incorporations. Recovery of activity for the experiments shown varies from 20 to 50 percent, which can be accounted for by the fact that the strains used (PL1 SmS and a spontaneously arising SmD mutation from it) are not ribonuclease free.

RNA used	Protein used	^{14}C-phenylalanine incorporation (count/min)		Stimulation (+Sm/−Sm)
		−Sm	+Sm (3 μg/ml)	
SmS	SmS	2044	1165	0.57
SmS	SmD	1158	1466	1.26
SmD	SmS	1619	870	0.54
SmD	SmD	721	941	1.30

Table 2. Synthesis of ^{14}C-phenylalanine directed by polyuridylic acid by 30*S* ribosomes reconstituted with artificial protein mixtures. Each mixture contained the normal amount of SmD-derived total protein plus the indicated amount of SmS-derived protein purified and assayed according to the methods of Hardy *et al.* (*2*). All other conditions are the same as in Table 1.

Addition of protein		^{14}C-phenylalanine incorporation (count/min)		Stimulation (+Sm/−Sm)
Sensitive protein	Amount (μg per 100 μg of total dependent protein)	−Sm	+Sm (3 μg/ml)	
None		970	1185	1.22
1 through 12a	167	1779	2103	1.18
12b, 14	24	1512	1726	1.14
13, 15b	20	786	981	1.25
15	5.5	1571	1162	0.74
16	20	1076	1193	1.11

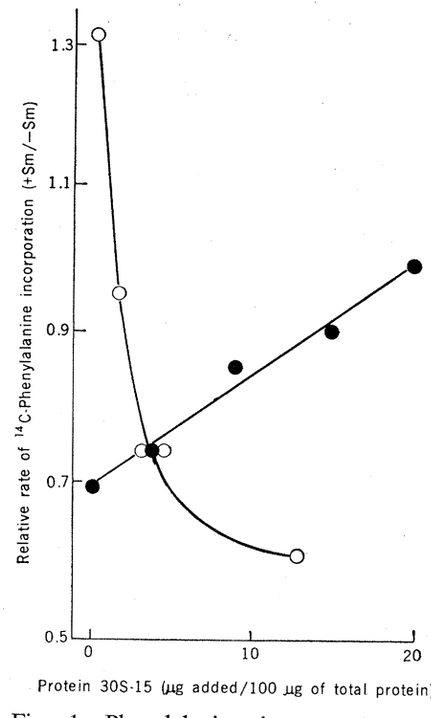

Fig. 1. Phenylalanine incorporation by 30*S* ribosomes reconstituted with the normal amount of total protein plus increasing amounts of protein 30*S*-15 purified on cellulose phosphate columns (*2*). The conditions for reconstitution and assay are given in Table 1. The open circles represent the dilution of SmD-derived total protein with protein 30*S*-15 SmS, and the closed circles represent the dilution of SmS-derived total protein with 30*S*-15 SmD.

The disc-electrophoretic patterns of 30*S*-15 from both sources reveal one component, and the material is homogeneous in sedimentation equilibrium experiments. Experiments in which total SmS and SmD proteins are combined in different ratios in the reconstitution mixture yield a population of ribosomes with intermediate phenotypes. The variation in degree of SmS character of these particles parallels the relative amount of SmS protein in a manner which is consistent with there being one protein per 30*S* particle that determines the Sm phenotype of the ribosomes. Preliminary experiments by Craven (*8*) which are designed to determine the nature of the chemical alteration of 30*S*-15 SmD have confirmed the conclusion that our active samples are at least 90 percent chemically homogeneous.

The altered protein of Sm resistant (SmR) ribosomes also corresponds to 30*S*-15. When a sample of our 30*S*-15 SmS was used by Ozaki *et al.* (*5*), it was found that the SmR phenotype of reconstituted ribosomes could be converted to the SmS phenotype if excess 30*S*-15 SmS is present in the reconstitution mixture. Therefore, mutational events at a single genetic locus (*9*) convert SmS ribosomes to the SmR or SmD phenotype by affecting a single protein. However, we are not certain that this genetic locus is the primary structure determinant of 30*S*-15. The only genetic locus which is known to determine the primary structure of a ribosomal protein is that determining the structure of 30*S*-8, the K character (*10*). It is likely that the alterations of 30*S*-15 which give rise to the different Sm phenotypes are alterations in the primary structure of this ribosomal protein.

E. A. BIRGE
C. G. KURLAND
Department of Zoology, University of Wisconsin, Madison 53706

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Lesions of Central Norepinephrine Terminals with 6-OH-Dopamine: Biochemistry and Fine Structure

Abstract. *Intracisternal injections of 6-hydroxydopamine produce rapid and long-lasting depletion of brain catecholamines without effects on serotonin concentrations. Depletion of norepinephrine is greatest in areas containing only nerve terminals and axons and least in areas containing monoamine cell bodies. The norepinephrine loss is accompanied by electron microscopic evidence of nerve terminal degeneration and decreased turnover. Dopamine loss is less marked and is not accompanied by degeneration or alteration of turnover rate.*

A classical approach for elucidating the function of a given neuronal population is to study the changes following their biochemical inactivation or anatomical lesioning. For the serotonin-containing nerve cells of the central nervous system this approach is typified by either electrolytic lesioning of the raphe nuclei (*1*) or inhibition of serotonin synthesis with *p*-chlorophenylalanine (*2*). Provided its accuracy and selectivity can be anatomically established, the lesioning approach has the inherent advantage that the nerve terminals undergo permanent orthograde degeneration. Both the lesioning and the biochemical approaches appear to be impractical for analysis of catecholamine nerve terminals, since the cell bodies of these monoaminergic neurons are diffusely placed in the brain stem reticular formation (*3*) and the inhibitors of norepinephrine synthesis also inhibit dopamine formation (*4*). Moreover, it is impossible to block norepinephrine biosynthesis selectively in the central nervous system without causing peripheral autonomic inhibition (*5*). The norepinephrine congener 6-hydroxydopamine (6-HDM) provides reversible chemical sympathectomy of certain portions of the peripheral sympathetic nervous system (*6, 7*) associated with ultrastructural toxic degeneration of the axons and nerve terminals (*8*). When administered intracisternally, 6-HDM will produce long-lasting depletion of brain norepinephrine levels and inhibition of uptake mechanisms (*9*). We now report that 6-HDM administered intracisternally produces selective effects on norepinephrine-containing nerve terminals accompanied by fine structural evidence of degeneration.

Sprague-Dawley male rats (NIH and Zivic Miller, Pittsburgh, strains), 150 to 300 g, were given 6-HDM by either intraventricular or intracisternal injections according to various dose schedules. In the most extensive series, rats lightly anesthetized with ether were given 200 μg of 6-HDM (in 50 μl of 0.85 percent NaCl containing 0.001 percent $Na_2S_2O_5$) followed at 72 and 96 hours by supplemental intracisternal injections of 50 μg each. Temporal assays of whole-brain monoamine levels were performed by modifying previously reported techniques (*10–15*) and were compared with control animals receiving only intracisternal injections of $Na_2S_2O_5$ in NaCl diluent.

Brains were frozen immediately after removal and stored frozen until analysis. Homogenization of tissues and their preparation for Dowex columns (*10*) was as previously described (*11*). The effluent and the eluate of a successive rinse with 4 ml of 0.1*M* Na acetate buffer, *p*H 4.5, with 0.1 percent disodium ethylenediamine tetraacetate (EDTA) were collected for tyrosine analysis (*12*). A rinse with 10 ml of 0.1*M* Na acetate buffer, *p*H 6, with 0.1 percent disodium EDTA followed. A successive wash with 5 ml of 0.4*N* HCl, containing 0.5 mg of $Na_2S_2O_5$ per milliliter, was discarded, but the following 10-ml wash with the same solution was collected for the norepinephrine analysis (sample A). The columns were rinsed with 4 ml of 4*N* HCl with 0.5 mg of $Na_2S_2O_5$ per milliliter, which was collected for dopamine analysis (sample B), and then rinsed with H_2O (2 ml). Finally, the serotonin was eluted with 4 ml of