Induction of Helical Arrays of Ribosomes by Vinblastine Sulfate in Escherichia coli

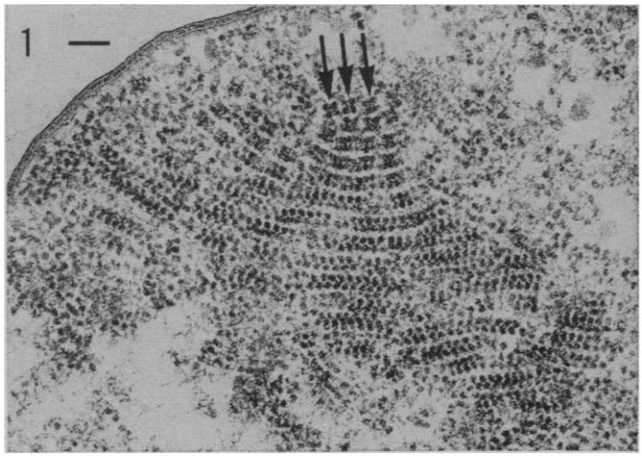
Abstract. *Physiological concentrations of vinblastine sulfate elicited ribosomal helices in large numbers in growing cultures of the osmotically sensitive mutant* sud 24 *and, after treatment with ethylenediaminetetraacetate, also in the K-12 strain. The helices were usually seen at the division plane and were often connected to the membrane. This method of induction of ribosomal helices offers a unique system for studying in vivo structure and function of the translational apparatus in relation to other cell components.*

Biochemical methods of analysis have provided information about the composition and function of ribosomes. However, the spatial structure of ribosomes, still hypothetical, may be better understood through x-ray diffraction analysis of suitable crystals or by optical diffraction of electron micrographs of in vivo structures. Ribosomal helices, believed to be polyribosomes, have been demonstrated in animal (*1*), plant (*2*), and bacterial cells (*3*). Crystals of ribonucleoprotein have been found in cysts of *Entamoeba invadens* and occasionally occur in vegetative cells of *Myxococcus xanthus* (*4*). Previously, we reported the presence of helical "supracoils" in the cytoplasm of *Escherichia coli* K-12 (*5*) and have proposed that these structures may be polyribosomes. Below we shall refer to these structures as ribosomal helices. Since these structures seem to occur naturally in a small proportion of growing cells, a method was required which would not only enhance their frequency of occurrence but also improve the discernment of structural details in electron micrographs.

We have developed such a method by which ribosomal helices are induced in *E. coli,* through exposure of a growing population to vinblastine sulfate, an oncolytic alkaloid from *Vinca rosea* Linn. (*6*). This substance was chosen since it causes hypertrophy of granular endoplasmic reticulum (*7*) and arrests dividing mammalian cells in the metaphase, perhaps through the crystallization of the spindle fibers (*8*). It was thought that a similar effect could be achieved aiming at "freezing" polyribosomes and, as a consequence, retarding or blocking cell division.

Escherichia coli sud 24, an osmotically sensitive mutant (*9*), was cultured in medium C containing 20 percent sucrose with continuous shaking at 30°C. Cells, from the logarithmic phase of growth, were harvested by centrifugation and resuspended in fresh medium containing $5.4 \times 10^{-4}M$ or $5.4 \times 10^{-5}M$ freshly reconstituted vinblastine sulfate. (Once reconstituted, vinblastine sulfate appears to deteriorate within 1 month when stored at 4°C.) Samples were harvested over an equal volume of crushed, frozen 20 percent sucrose (*10*) at consecutive time intervals up to 4 hours. After centrifugation, cells were washed twice with cold (4°C) Kellenberger buffer, *p*H 6.0, containing 20 percent sucrose and fixed with 1 percent glutaraldehyde in the same buffer for 30 minutes in an ice bath. After washing five times with cold Kellenberger sucrose-buffer, the specimen was processed according to the Ryter-Kellenberger method (*11*). Epon 812 was used for embedding. Thin sections, double stained with uranyl acetate and lead citrate, were examined in the RCA EMU-3G electron microscope at 100 kv. Growth rates were measured during exposure to vinblastine sulfate by the method of optical density measurement in a Bausch and Lomb Spectronic 20.

No differences between treated and untreated cells were seen in the early hours of growth. At 3 hours, numerous ribosomal helices were observed in



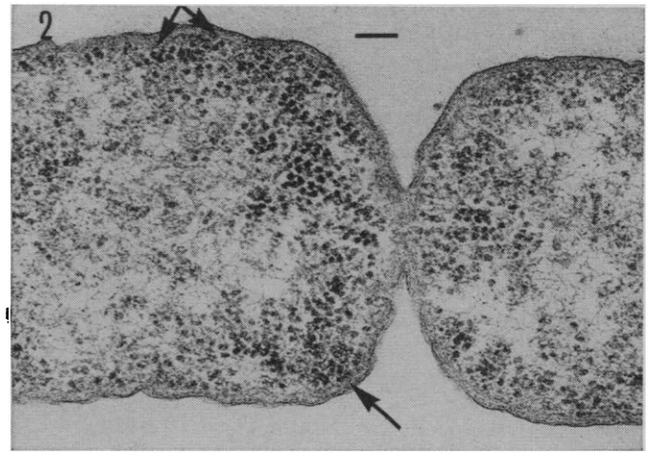


Fig. 1. Ribosomal helices at the cell pole of *E. coli sud 24.* Arrows, "cross sections" of the helices. Bar = 0.1 μ.

Fig. 2. Ribosomal helices at the division plane of cells of *E. coli sud 24*. Single arrow, attachment to the membrane. Double arrows, ribosomal helix oriented parallel to the membrane. Bar = 0.1 μ.

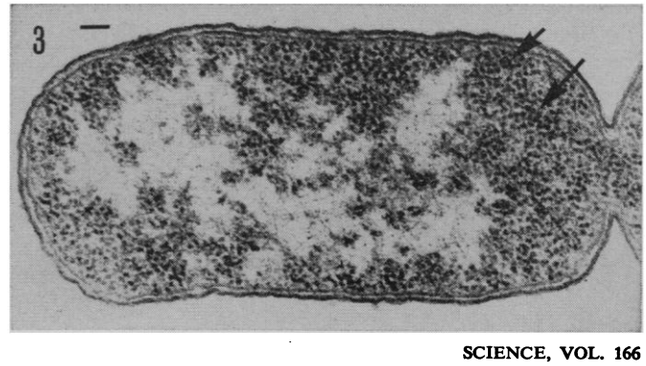


Fig. 3. Noninduced cell (control) in division. Note "pairing" of ribosomes near division plane (arrows). Bar = 0.1 μ.

about 70 to 80 percent of the population exposed to vinblastine sulfate at both concentrations, whereas none or only a few were observed in the control culture. As seen in Fig. 1, the structures are distinct. Both longitudinal and what appear to be cross sections can be observed. Depending on the sectioning plane, these structures often seemed to be composed of alternating units, about 160 Å in diameter, suggesting a helical configuration. A single helix is about 600 Å in width but varies in length. The pitch is on the average 180 Å. According to these data the structures are similar, if not identical, to those naturally occurring in *E. coli*. In the assumed cross sections, the helices appear to contain four closely packed units. The majority of cells in which ribosomal helices were observed were dividing, and the structures were perpendicular to the plane of division or occasionally oriented parallel to the membrane (Fig. 2). Frequently they appeared to be attached directly to the membrane. No distinct ribosomal helices were seen in control cells (Fig. 3), but "pairing" of ribosomes was often noticed near the division plane. The vinblastine sulfate–treated specimen contained many more dividing cells than the control specimen, suggesting either a partial arrest of division or an increased degree of synchrony in the culture. Growth curves indicated that vinblastine sulfate reduces the growth rate of *E. coli sud 24*.

We also subjected strain K-12 to the same vinblastine sulfate treatment as that of *sud 24*, but omitted sucrose from the medium and fixation reagents. Neither increase in the number of helices nor a decrease in growth rate was observed. We concluded that the difference in response to vinblastine sulfate of K-12 and *sud 24* may be a matter of permeability. Since *sud 24* is an osmotically fragile mutant, this strain might allow the passage of vinblastine sulfate into the cell, whereas K-12 may be impermeable to the alkaloid. In order to test this theory, we applied the method of Leive (*12*), of increasing cell permeability by removal of lipopolysaccharides from the cell wall through a brief exposure to ethylenediaminetetraacetate. Thus treated, cells were transferred to fresh medium C with and without vinblastine sulfate ($5.4 \times 10^{-4} M$) and incubated as stated previously. Samples for electron microscopy were taken at various time intervals and processed as above.

After 2 hours of growth, vinblastine sulfate induced a great number of ribosomal helices in K-12 cells similar to the *sud 24* cultures. We concluded that, normally, *E. coli* is impermeable to vinblastine sulfate, but alteration of the permeability barriers either by ethylenediaminetetraacetate or mutation permits the uptake of vinblastine sulfate.

This system can be used profitably for at least two different types of studies. First, the ribosomal helices may be studied for formation, structure, and function. Since the helical arrangement of polysomes appears to be a phenomenon pertaining to eucaryotic as well as to procaryotic cells, information in regard to these structures and their relationship to DNA and cell membranes would aid in defining the concepts which exist about the function of ribosomes in protein synthesis. Furthermore, it may be possible to gain information on the structure of ribosomes, as it exists in vivo. This approach permits the observation of unaltered cytoplasmic units which are generally distorted by the methods used for their isolation. Although we have not tried other genera or *E. coli* strains, it seems probable that this method could be adapted to a number of different organisms. Secondly, this method could be used to investigate the mode of action of vinblastine sulfate on cells, generally an easier procedure in procaryotic cells than in the structurally complicated eucaryotic cell systems.

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References and Notes

1. O. Behnke, *Exp. Cell Res.* **30**, 597 (1963); C. H. Waddington and M. M. Perry, *ibid.*, p. 5 → E. Shelton and E. L. Kuff, *J. Mol. Biol.* **22**, 23 (196 → P. Weiss and N. B. Grover, *Proc. Nat. Acad. Sci. U.S.* **59**, 763 (1968). → P. Echlin, *J. Cell Biol.* **24**, 150 (1965); V. I. Bruskov and N. A. Kiselev, *J. Mol. Biol.* **38**, 443 (196 → W. A. Jensen, *J. Cell Biol.* **36**, 403 (1968); F. B. P. Wooding, *J. Ultrastruct. Res.* **24**, 157 (1968).
3. C. H. Domermuth, M. H. Nielsen, E. A. Freundt, A. Birch-Andersen, *J. Bacteriol.* **88**, 727 (1964); J. Maniloff, H. J. Morrovitz, R. J. Barrnett, *ibid.* **90**, 193 (196 → H. Slayter, Y. Kiho, C. E. Hall, A. Rich, *J. Cell Biol.* **37**, 583 (1968); F. M. Schwartz and N. D. Zinder, *Virology* **34**, 352 (1968).
4. D. C. Barker. *Z. Zellforsch. Mikroskop. Anat.* **58**, 641 (196 → R. S. Morgan, H. S. Slayter, D. L. Weller, *J. Cell Biol.* **36**, 45 (1968); H. Voelz, *J. Ultrastruct. Res.* **25**, 29 (1968).
5. E. W. Kingsbury and H. Voelz, *J. Bacteriol.* **95**, 1478 (1968).
6. I. S. Johnson, H. F. Wright, G. H. Svoboda, J. Vlantis, *Cancer Res.* **20**, 1016 (1960). → A. Krishan, D. Hsu, P. Hutchins, *J. Cell Biol.* **39**, 211 (1968); J. H. Cutts, *Cancer Res.* **21**, 168 (1961).
8. K. G. Bensch and S. E. Malawista, *Nature* **218**, 1176 (1968).
9. G. Mangiarotti, D. Apirion, D. Schlessinger, *Science* **153**, 892 (1966).
10. G. Mangiarotti and D. Schlessinger, *J. Mol. Biol.* **20**, 123 (1966).
11. R. Ryter and E. Kellenberger, *Z. Naturforsch.* **13b**, 597 (1958).
12. L. Leive, *Biophys. Biochem. Res. Commun.* **21**, 290 (1965).
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Evoked Potentials: Modifications by Classical Conditioning

Abstract. *Visual evoked potentials to a positive discriminative stimulus change systematically during sensory conditioning and extinction. Changes due to conditioning are manifested in the increased amplitude of the late component of the evoked response. This effect is attenuated during extinction and reappears after reconditioning.*

Many changes in brain evoked potentials obtained during conditioning have been demonstrated in animals (*1*). Comparisons of wave forms obtained during "correct" and "incorrect" behavioral responses suggest that differences occur primarily in the late components. Inspection of simultaneously computed wave forms suggests a covariation between late components and the poentials evoked in mesencephalic reticular formation and centralis lateralis (*2*). During the conditioning procedure, changes in the significance of the conditioned stimulus are accompanied by modifications of the later activity of the evoked potentials.

We now report the results of a study in which we explored the relation between classical conditioning and the late components of the visual evoked potentials (VEP) in humans. All data were derived from monopolar scalp recordings of 16 college students. The active electrode was located on the midline 2.5 cm above the inion; the combination of the two ear lobes formed the reference electrode. Evoked potentials were recorded by means of a Grass Model 7 P5A wide-band AC EEG amplifier, whose low-frequency cutoff filter was set at 0.15 hz. The driver amplifier high-frequency cutoff filter was set at 75 hz, and the gain was set at 50 hz. Amplifier time-constant setting was 0.1. The averaged evoked potentials were computed with the Mnemotron (CAT 1000) and written out on a Moseley (7590 CMR) XY plotter.