

the rate of synthesis of RNA were due to an antibiotic-induced change in the rate of transcription of the cistrons, or to changes in the rate of initiation of new chains of RNA.

The following results were obtained.

(1) In all media it took 2.2–2.8 min for completion of 23S rRNA chains (at 37°C), indicating a fairly constant rate of elongation (about 25 nucleotides/s) even when the rates of growth and division of the organisms varied fourfold in different media. In this the results agreed with those of Bremer & Yuan (1968) and Manor, Goodman & Stent (1969), although my values for the rate of RNA chain elongation are lower and approximate to those found by Mangiarotti, Apirion, Schlessinger & Silengo (1968).

(2) On addition of chloramphenicol the rate of chain growth of 23S RNA remained constant, even though the overall rate of RNA synthesis rose by as much as 3.5-fold over that typical of cells growing normally in untreated medium. It appears that the increase in RNA synthesis in antibiotic-inhibited cultures is due almost entirely to an increase in the rate of initiation of new chains of rRNA, tRNA and mRNA being much less affected.

(3) In media supporting a lower rate of growth of bacterial cells (e.g. lactate-salts or glucose-salts) *E. coli* may have a pool of DNA-dependent RNA polymerase in excess of its requirements for steady-state conditions in the synthesis of rRNA. On the other hand in broth media all the available enzymes may be engaged in RNA synthesis, so that increases are not observed when chloramphenicol is added.

- Bremer, H. & Yuan, D. (1968). *J. molec. Biol.* **38**, 163.
 Fraenkel, D. G. & Neidhardt, F. C. (1961). *Biochim. biophys. Acta*, **53**, 96.
 Friesen, J. D. (1966). *J. molec. Biol.* **20**, 559.
 Gray, W. J. H. (1970). *Biochem. J.* **117**, 60P.
 Kurland, C. G. & Maaløe, O. (1962). *J. molec. Biol.* **4**, 193.
 Mangiarotti, G., Apirion, D., Schlessinger, D. & Silengo, L. (1968). *Biochemistry, Easton*, **7**, 456.
 Manor, H., Goodman, D. & Stent, G. D. (1969). *J. molec. Biol.* **39**, 1.

The Function of Ribosomal Thiol Groups

By JOSIE A. BEELEY. (*Department of Biochemistry and School of Dentistry, University of Glasgow, Glasgow, W.2, U.K.*)

Reports on *Escherichia coli* ribosomes suggest that thiol groups are involved in the association of the two subunits (Miyazawa & Tamaoki, 1967) and in protein biosynthesis (Traut & Haenni, 1967). In mammalian systems, thiol-blocking reagents effect partial dissociation of rat liver ribosomes (Incefy & Petermann, 1969) and inhibit binding of phenyl-

alanyl-tRNA to reticulocyte ribosomes (McAllister & Schweet, 1968).

Highly purified ribosomes from canine pancreas (Beeley, Cohen & Keller, 1968), a tissue virtually free from ribonuclease (Barnard, 1968), were incubated with 1 mM-*p*-hydroxymercuribenzoate at 37°C. Extensive dissociation to subunits occurred, the process being accompanied by the appearance of a species sedimenting between the monomer and the large subunit. This particle has been designated a 'loosened' ribosome. Further incubation resulted in complete conversion of the monoribosomes and subunits into species remaining at the top of a sucrose density gradient. A similar result was observed on incubation with ribonuclease (0.125 µg/mg of ribosomes).

By using trichloroacetic acid precipitation of 23S [¹⁴C]rRNA from *E. coli*, the exogenous ribonuclease activity was shown to be 1.4% of the added ribonuclease activity necessary to effect complete ribosomal disintegration; this was increased to 2.3% in the presence of *p*-hydroxymercuribenzoate. Hence the ribosomes contained a small amount of ribonuclease, some of which was inhibited. Sucrose-density-gradient analysis of 23S [¹⁴C]rRNA after incubation with ribosomes in the presence or absence of *p*-hydroxymercuribenzoate supported this conclusion.

However, analysis by agarose-gel electrophoresis of RNA extracted from canine pancreatic ribosomes incubated with *p*-hydroxymercuribenzoate revealed extensive degradation.

It is proposed that thiol-blocking reagents react with ribosomal protein thiol groups, these groups normally being involved in molecular interactions that maintain the integrity of the ribosome. This process gives rise to 'loosened' ribosomes. Such ribosomes appear significantly more sensitive to ribonuclease because there are fewer intermolecular interactions to maintain the integrity of the monoribosome or its subunits after limited cleavage of the RNA. One possible role of ribosomal thiol groups in ribosome structure may be in maintaining the ribosome in a compact configuration.

- Barnard, E. A. (1968). *Nature, Lond.*, **221**, 340.
 Beeley, J. A. H., Cohen, E. & Keller, P. J. (1968). *J. biol. Chem.* **243**, 1262.
 Incefy, G. S. & Petermann, M. L. (1969). *Biochemistry, Easton*, **8**, 1482.
 McAllister, H. C. & Schweet, R. S. (1968). *J. molec. Biol.* **34**, 519.
 Miyazawa, F. & Tamaoki, T. (1967). *J. molec. Biol.* **24**, 485.
 Traut, R. R. & Haenni, A. L. (1967). *Eur. J. Biochem.* **2**, 64.