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The Effects of Chloramphenicol on the Synthesis of Bacterial Ribonucleic Acids

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It has been known for some time that amino acids play an important role in regulating the rate of biosynthesis of bacterial RNA (Pardee & Prestidge, 1956; Gros & Gros, 1958; Aronson & Spiegelman, 1961a,b). High concentrations of chloramphenicol (up to 0.2 g/l) inhibit protein synthesis severely and spare the requirement for amino acids as stimulants of RNA synthesis (Aronson & Spiegelman, 1961a; Kurland & Maaløe, 1962). Further, when bacteria are grown in an enriched medium containing amino acids the overall rate of RNA synthesis is virtually unaffected by adding chloramphenicol, whereas it is almost doubled when the antibiotic is added to cultures growing in glucose-salts medium (Fraenkel & Neidhardt, 1961; Kurland & Maaløe, 1962).

Using the technique of DNA-RNA hybridization (Gillespie & Spiegelman, 1965; Pigott & Midgley, 1968), I have studied the antibiotic-induced changes in the rate of mRNA synthesis in *Escherichia coli* M.R.E. 600 growing in broth, glucose-salts or sodium lactate-salts media at 37°C. On comparing the kinetics and relative rates of incorporation of [5-³H]uracil into mRNA, rRNA and tRNA, after addition of chloramphenicol, with those holding for all states of steady exponential growth (Gray & Midgley, 1969), I found the following.

(1) mRNA is stabilized in the presence of chloramphenicol and accumulates as about 6% of the total cellular RNA (Midgley, 1963; Friesen, 1966). Though also RNA turnover can be demonstrated in antibiotic-inhibited cells, and the pools of nucleotides are of normal size, the kinetics of labelling all RNA fractions gives incorporation curves that extrapolate to the origin.

(2) Whereas in exponentially growing cells in any medium and at all temperatures exogenous uracil enters mRNA and stable RNA pools in a 1:2 (weight/unit time) ratio, only 14% of the RNA formed in the presence of chloramphenicol has the hybridization characteristics of mRNA. Thus the synthesis of mRNA in bacteria is not rigidly coordinated with that of rRNA + tRNA.

(3) The kinetics of labelling mRNA in broth cultures suggests that, when chloramphenicol is

added, about half the mRNA present eventually decays, and the remainder persists throughout chloramphenicol inhibition.

(4) It appears that, in antibiotic-inhibited cultures, new steady states of synthesis of the various RNA fractions are set up that are unlike those found in normally growing cultures. The question whether these changes are brought about by alterations in the rate of RNA transcription by DNA-dependent RNA polymerase or in the rates of initiation of new chains is considered by Midgley (1970).

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The Rate of Transcription of 23S Ribosomal Ribonucleic Acid in Normal and Chloramphenicol-Inhibited Bacteria

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Gray (1970) has shown that, when exponentially growing and dividing cultures of *Escherichia coli* M.R.E. 600 are inhibited by the addition of chloramphenicol, a rapid change takes place in the relative rates of production of various RNA species. Further, when cultures growing in simple defined media such as lactate-salts or glucose-salts are incubated with the antibiotic, the overall rate of RNA synthesis is rapidly increased (Fraenkel & Neidhardt, 1961; Kurland & Maaløe, 1962). In broth media, where amino acids are in plentiful supply, there is virtually no change in the rate of RNA synthesis in the presence of chloramphenicol, although mRNA is stabilized and accumulates (Friesen, 1966).

I have studied the kinetics of synthesis of the 23S rRNA of *E. coli* at 37°C in each of three media, in the presence or the absence of 0.2 g of chloramphenicol/l. My object was to discover if the alterations in

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.

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The Function of Ribosomal Thiol Groups

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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.

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