SHORT COMMUNICATIONS

The Accumulation of 'Run-off' Ribosomes during Treatment of *Escherichia coli* with Dihydrostreptomycin *in vivo*

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Previous communications have shown that in sensitive cultures of *Escherichia coli*, treated with low concentrations of dihydrostreptomycin *in vivo*, the resulting inhibition of growth rates (Kogut, Lightbown & Isaacson, 1965; Kogut & Harris, 1969) is paralleled by increasing accumulation of 70S ribosomes (Kogut, 1969; Luzzatto, Apirion & Schlessinger, 1968, 1969; Kogut & Prizant, 1970). This has been interpreted by Luzzatto *et al.* (1968, 1969) as accumulation of special 'streptomycin monosomes' or 'stuck initiation complexes'. However, 70S monomers accumulate under many conditions which inhibit or slow down growth rates (Kohler, Ron & Davis, 1968; Kogut, 1969; Kogut, Maltby & Prizant, 1970; Kogut & Prizant, 1970) and can be characterized as 'run-off' ribosomes by their sedimentation behaviour in 2 mM-Mg²⁺ (Ron, Kohler & Davis, 1968; Hardy & Turnock, 1971). Since any 'stuck initiation complexes' resulting from action of dihydrostreptomycin *in vivo* should show resistance to 'run-off', we have examined this question by imposing run-off conditions on cultures during treatment with dihydrostreptomycin. Such run-off conditions were imposed by (a) withdrawal of energy (carbon) source and (b) addition of rifampicin which inhibits RNA polymerase and hence the synthesis of messenger RNA (Hartman, Honikel, Knusel & Nuesch, 1967; Wehrli, Nuesch, Knusel & Staehelin, 1968).

The bacterial strain, *Escherichia coli* C600, its maintenance, conditions and measurement of growth, treatment with dihydrostreptomycin, and labelling with [¹⁴C]uracil were as previously described (Kogut *et al.* 1965; Kogut & Harris, 1969; Kogut & Prizant, 1970). Harvesting and lysis were carried out according to the method of Godson & Sinsheimer (1967). Sucrose gradient centrifugation, fractionation of gradients, examination of ribosome profiles and characterization of the material in the 70S peaks, collected from gradients in 10 mM-Mg²⁺, into 'run-off' and 'stuck' 70S ribosomes were as described by Kogut & Prizant (1970). For deprivation of carbon source, cultures were grown with glucose (0.2 %, w/v) as sole source of carbon and with NH₄Cl (0.2 %, w/v) as sole source of nitrogen. They were rapidly transferred, by filtration and two washings, into warmed medium of the same composition, but lacking glucose. This procedure took about 5 to 6 min, and upon further incubation only negligible increase in *E*₅₀₀ occurred, and had ceased within 15 min. Lysates from control cultures harvested 15 min after deprivation of carbon source showed that essentially all polysomes had been converted to 70S monomers. All of these 70S particles, collected from sucrose gradients in 10 mM-Mg²⁺, then dissociated completely into 50 and 30S subunits on recentrifuging in 2 mM-Mg²⁺. A dihydrostreptomycin-treated culture, harvested when growth rate had declined by approximately 60 %, yielded a ribosome profile after sucrose gradient centrifugation in 10 mM-Mg²⁺ which showed some polysomes as well

Fig. 1. Ribosome sedimentation patterns from cultures of *Escherichia coli* c600, treated with dihydrostreptomycin and subjected to 'run-off' by treatment with rifampicin. A. Gradient profiles of lysates, prepared as described, and centrifuged in Spinco Model LSW 50 for 70 min at 50000 rev./min, 5°, through 15 to 30% (w/v) linear sucrose gradients, above a 0.5 ml 'cushion' of 50% (w/v) sucrose, all in 10 mM-tris-HCl buffer, pH 7.3, containing 10 mM Mg^{2+} and 60 mM-KCl. B. Gradient profiles of the 70S peak material harvested from the gradients in 10 mM- Mg^{2+} and recentrifuged for 2 h at 48000 rev./min, 5°, in similar sucrose gradients, but containing 2 mM- Mg^{2+} . (i) Lysate from a control culture, treated with rifampicin (20 μ g/ml) during exponential growth and harvested 20 min later when growth had ceased. (ii) Lysate from a culture treated with dihydrostreptomycin (20 μ g/ml) during exponential growth and harvested when growth rate had declined by 60%. (iii) Lysate from the same culture as (ii) but harvested after a further 20 min in the presence of rifampicin (20 μ g/ml) when growth had ceased.

as 70S particles. Recentrifugation of the latter material in 2 mM- Mg^{2+} gave a peak in the 70S region, indicating the presence of polysome-derived, or 'stuck' particles, as well as peaks at 50S and 30S. However, a portion of the same culture harvested after an additional 15 min incubation without glucose, showed complete disappearance of polysomes and accumulation of 70S particles in 10 mM- Mg^{2+} : recentrifugation of the latter particles in 2 mM- Mg^{2+} gave

peaks only in the 50 and 30s regions. Thus, imposition of 'run-off' conditions by glucose deprivation on cultures during treatment with dihydrostreptomycin *in vivo*, resulted in accumulation of 70s ribosomes which could all be characterized as 'run-off' ribosomes by their sedimentation behaviour. Under these conditions however, extracellular dihydrostreptomycin was removed along with the glucose. It could be argued, therefore, that at the time of glucose deprivation, some ribosomes, i.e. those showing as polysomes in 10 mM-Mg²⁺, were as yet unaffected by the antibiotic.

Rifampicin, by inhibiting the synthesis of messenger RNA, afforded a means of imposing 'run-off' conditions in the presence of intracellular dihydrostreptomycin. Addition of rifampicin (20 µg/ml) to exponential-phase control cultures led to complete cessation of growth within 20 min. Lysates from cultures harvested at that time showed complete disappearance of polysomes and accumulation of 70s particles which behaved as 'run-off' ribosomes on resedimentation in 2 mM-Mg²⁺. Fig. 1 shows the ribosome profiles (i) from such a control culture; (ii) from a dihydrostreptomycin-treated culture harvested when growth had declined by about 70 %; and (iii) from the same culture harvested after a further 20 min in the presence of rifampicin (20 µg/ml) at which time growth had ceased.

Our earlier study of the parallel accumulation of 70s ribosomes with increasing growth inhibition, brought about by treatment with dihydrostreptomycin *in vivo* (Kogut & Prizant, 1970), showed the presence of a small amount of 'stuck' polysomal 70s ribosomes when growth inhibition was nearly complete. This could allow the inference that the 'run-off' ribosomes were in fact those which had not interacted with the antibiotic. If that were the case, some of the ribosomes present during such *in vivo* treatment should show resistance to imposed run-off. We have shown here that this is not the case. The alternative argument that 'stuck, polysomal' 70s ribosomes, present in lysates from dihydrostreptomycin-treated cultures, were derived from polysomes which did not contain any antibiotic-affected ribosomes, agrees with the time-course of changes in ribosome patterns, relative to growth inhibition (Kogut, 1969; Kogut & Prizant, 1970). Such 'stuck' 70s particles should disappear as a result of imposed 'run-off' conditions. We have now shown that all ribosomes (excluding perhaps the free 50 and 30s subunits) present in dihydrostreptomycin-treated cultures can be converted to 'run-off' ribosomes. There does not, therefore, seem to be any evidence that dihydrostreptomycin *in vivo* 'freezes' ribosomes on polysomes, either as stuck initiation complexes (Luzzatto *et al.* 1968, 1969) or at any subsequent stage. Interference with a dissociation factor (Subramanian, Ron & Davis, 1968; Subramanian & Davis, 1970) seems also unlikely, since it would be expected to yield 'stuck 70s particles'. Other mechanisms to account for the inhibition of protein synthesis and growth, by dihydrostreptomycin (and streptomycin) *in vivo*, include inhibition of an early step in initiation. This is contra-indicated by much of the kinetic evidence (Kogut, 1969; Kogut & Harris, 1969) and by Lenette & Apirion (1970). Furthermore, this should lead to the accumulation of free 30 and 50s subunits, detectable during the early stages of growth inhibition, which again has not been observed (Kogut, 1969; Kogut & Prizant, 1970; and M. Kogut & E. Prizant, unpublished observations). We are led to the conclusion that 30s ribosomal subunits, after combining with intracellular dihydrostreptomycin while in the free state (Cox, White & Flaks, 1964; Kogut & Harris, 1969; Kogut & Prizant, 1970; Lenette & Apirion, 1970), can initiate and engage in translational movement without, however, completing normal peptide chains. They might fall off, or be released before reaching the terminator codon, and then go through one or more abortive ribosome cycles (Davis, 1971). Perhaps they mistranslate one or more aminoacid codons as terminator codons.

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REFERENCES

- COX, E. C., WHITE, J. R. & FLAKS, J. G. (1964). Streptomycin action and the ribosome. *Proceedings of the National Academy of Sciences of the United States of America* **51**, 703–709.
- DAVIS, B. D. (1971). Role of subunits in the ribosome cycle. *Nature, London* **231**, 153–157.
- GODSON, N. & SINSHEIMER, R. L. (1967). Lysis of *Escherichia coli* with a neutral detergent. *Biochimica et biophysica acta* **149**, 476–488.
- HARDY, S. J. S. & TURNOCK, G. (1971). Stabilization of 70s ribosomes by speridine. *Nature New Biology, London* **229**, 17–19.
- HARTMANN, G., HONIKEL, K. O., KNUSEL, F. & NUESCH, J. (1967). The specific inhibition of the DNA-directed RNA synthesis by rifamycin. *Biochimica et biophysica acta* **145**, 843–844.
- KOGUT, M. (1969). Effects of antibiotics on bacterial lysis and intracellular ribosomes. *Journal of General Microbiology* **57**, xxv–xxvi.
- KOGUT, M. & HARRIS, M. (1969). Effects of streptomycin in bacterial cultures growing at different rates; interaction with bacterial ribosomes *in vivo*. *European Journal of Biochemistry* **9**, 42–49.
- KOGUT, M., LIGHTBOWN, J. W. & ISAACSON, P. (1965). Effects of dihydrostreptomycin treatment on the growth of *Escherichia coli* after removal of extracellular antibiotic. *Journal of General Microbiology* **39**, 165–183.
- KOGUT, M., MALTBY, E. N. & PRIZANT, E. (1970). Effects of dihydrostreptomycin on bacterial ribosomes *in vivo*. *Biochemical Journal* **119**, 121–123.
- KOGUT, M. & PRIZANT, E. (1970). Effects of dihydrostreptomycin treatment *in vivo* on the ribosome cycle in *Escherichia coli*. *FEBS Letters* **12**, 17–20.
- KOHLER, R., RON, E. & DAVIS, B. D. (1968). Significance of the free 70s ribosomes in *Escherichia coli* extracts. *Journal of Molecular Biology* **36**, 71–82.
- LENNETTE, E. T. & APIRION, D. (1970). The level of F met-tRNA on ribosomes from streptomycin treated cells. *Biochemical and Biophysical Research Communications* **41**, 804–811.
- LUZZATTO, L., APIRION, D. & SCHLESSINGER, D. (1968). Mechanism of action of streptomycin in *Escherichia coli*: interruption of the ribosome cycle at the initiation of protein synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **60**, 873–880.
- LUZZATTO, L., APIRION, D. & SCHLESSINGER, D. (1969). Polyribosome depletion and blockage of the ribosome cycle by streptomycin in *Escherichia coli*. *Journal of Molecular Biology* **42**, 315–335.
- RON, E., KOHLER, R. & DAVIS, B. D. (1968). Magnesium ion dependence of free and polysomal ribosomes from *Escherichia coli*. *Journal of Molecular Biology* **36**, 83–89.
- SUBRAMANIAN, A. R. & DAVIS, B. D. (1970). Activity of initiation factor F₃ in dissociating *Escherichia coli* ribosomes. *Nature, London* **228**, 1273–1275.
- SUBRAMANIAN, A. R., RON, E. & DAVIS, B. D. (1968). A factor required for ribosome dissociation in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **61**, 761–767.
- WEHRLI, W., NUESCH, J., KNUSEL, F. & STAEHELIN, M. (1968). Action of rifamycins on RNA polymerase. *Biochimica et biophysica acta* **157**, 215–217.