DIFFERENTIAL INHIBITION OF RNA SYNTHESIS BY ACTINOMYCIN

Hans Klenow and Sune Frederiksen

The Fibiger Laboratory
Biochemical Section
Copenhagen, Denmark

Received July 15, 1964

Actinomycin has been shown to be a potent inhibitor of the DNA-dependent RNA nucleotidyltransferase (Hurwitz and August, 1963). A large number of experiments with both bacteria and mammalian cells have, furthermore, revealed that actinomycin is a very potent inhibitor of RNA synthesis in whole cells. According to Reich et al. (1962) and to Franklin (1963) all de novo synthesis of RNA may be completely inhibited in L-cells by actinomycin. This has been taken as evidence that all RNA synthesis in these cells is DNA-dependent.

Paul and Struthers (1963) observed, however, that LS-cells (a subline of the strain L-fibroblast) contained a nuclear RNA fraction, the formation of which is resistant to inhibition with actinomycin. Muramatser et al. (1964) have likewise obtained evidence for the presence of an actinomycin D resistant nuclear RNA fraction of mammalian cells.

Coleman and Elliot (1964) have studied the effects of actinomycin on the formation of ribonuclease in B.subtilis. Their results suggested a particular low preference of the drug for combining with the ribonuclease gene. According to

Georgiev et al. (1963) the biosynthesis in the normal rat liver of nuclear RNA of the ribosomal type is more sensitive to inhibition by actinomycin D than that of RNA with a composition similar to DNA.

In the preceding publication (Frederiksen and Klenow, 1964) it was described that 3'-deATP causes a differential inhibition of RNA synthesis in Ehrlich ascites tumor cells in vitro. RNA was separated into three fractions: cytoplasmic RNA (c-RNA) which was strongly inhibited, a major nuclear fraction (n-RNA I) which was about 40 % inhibited, and a minor nuclear fraction (n-RNA II) which was not inhibited. The latter fraction amounted to only about 4 % of the total cellular RNA, and had the highest rate of labelling with ^{32}P. It remained in the interphase after treatment of the cells first with phenol at 0° followed by treatment of the interphase with lauryl sulphate and phenol at 70°.

The effect of actinomycin (5 µg/ml) on the rate of incorporation of $^{32}P_i$ into these three RNA fractions has been investigated. As may be seen from Fig. 1 the labelling of n-RNA II was not inhibited by preincubation of the cells in the presence of 5 µg actinomycin per ml. In contrast the n-RNA I and the c-RNA fractions were strongly inhibited (about 90 %) under the same conditions. This situation was not changed when both actinomycin and 3'-deATP were present in the cells. These findings show that the incorporation of $^{32}P_i$ into a particular RNA fraction (n-RNA II) is not inhibited by the presence of actinomycin D under conditions which cause a strong inhibition of the other RNA fractions. In order further to characterize the n-RNA II fraction its base ratio has been determined. According to preliminary results this ratio corresponds to an unusually high content of

guanine and cytosine.

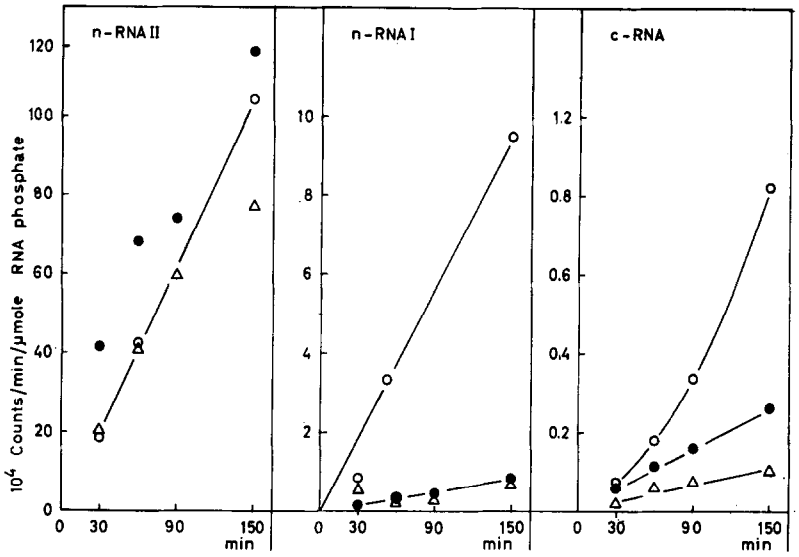

Fig. 1. The effect of actinomycin and actinomycin plus 3'-deoxyadenosine on the incorporation of $^{32}P_i$ into different RNA fractions of Ehrlich ascites cells *in vitro*. Each vessel contained per ml: Ehrlich ascites tumor cells, 85 mg (wet weight); ascites fluid, 415 µl; Robinson's medium (Robinson, 1949) containing glucose (5.6×10^{-3} M), 500 µl; folic acid, 20 mµmoles, and $^{32}P_i$, 16 µC. Additions: o - o none; ● - ● actinomycin D (5 µg); $^1\Delta$-Δ actinomycin D (5 µg plus 3'-deoxyadenosine (0.3 µmole). Reaction mixtures were incubated with shaking for 30 min at 37° in the presence of inhibitors. $^{32}P_i$ was then added (0 min) and aliquots withdrawn at different time intervals. The cells were washed with 0.15 M NaCl, and suspensions of washed cells were treated with 1 vol. phenol at 0° for about 10 min. After centrifugation the aqueous phase containing cytoplasmic RNA was made 10 mM with respect to $MgCl_2$, and treated with 2 vol. 96 % ethanol. The RNA precipitate was dissolved and reprecipitated essentially as described by Holland (1963). The interphase was washed several times with phenol-phosphate buffer mixtures, and finally treated with sodium lauryl sulphate and phenol at 70°. After centrifugation of the cooled mixture, n-RNA I was obtained from the aqueous phase by precipitation with $MgCl_2$ and 2 vol. ethanol. The n-RNA II fraction was obtained together with protein as a precipitate from the remaining interphase after addition of about 10 vol. 66 % ethanol. Specific activities of RNA were determined as previously described (Klenow, 1963).

Actinomycin D has been found to be attached specifically to the guanine base of DNA (Goldberg *et al.*, 1963; Kahan *et al.*, 1963), and the inhibiting effect of the drug

on both the DNA-dependent RNA nucleotidyltransferase and the cellular RNA synthesis is assumed to be due to this fact. The finding that the n-RNA II fraction has a very high content of guanine and cytosine would suggest that it is formed on a template with a similar high content of these two bases. It is remarkable, therefore, that in whole cells the formation of especially this RNA fraction is insensitive to actinomycin D in contrast to the formation of the rest of the RNA. It is equally remarkable that another potent inhibitor of the DNA-dependent RNA nucleotidyltransferase i.e. 3'-deATP neither alone nor together with actinomycin D has any inhibiting effect on incorporation of ^{32}P into the n-RNA II fraction. It is difficult to explain these results by assuming an intracellular compartmentation which would prevent the access of the two inhibitors to the center for the incorporation of ^{32}P into n-RNA II. Such a mechanism should exclude both 3'-deATP formed in the cells from 3'-deoxyadenosine and actinomycin D from the place of the incorporation, and at the same time permit the access of the four ribonucleotide triphosphates.

If the incorporation of ^{32}P into RNA as studied here reflects a synthesis of RNA the available data seem to favour the following hypothesis: The formation of n-RNA II in Ehrlich cells in vitro in the presence of actinomycin D and/or 3'-deATP is catalyzed by an enzyme different from the DNA-dependent RNA nucleotidyltransferase. The activity of the responsible enzyme might be dependent on the presence of RNA which may possibly be of viral origin.

REFERENCES

Coleman, G. and Elliot, W.H., Nature, 202, 1083 (1964).

Franklin, R.M., Biochim. Biophys. Acta, 72, 555 (1963).

Frederiksen, S. and Klenow, H., Biochem. Biophys. Res. Comm., this issue (1964).

Georgiev, G.P., Nature, 200, 1291 (1963).

Goldberg, I.H., Reich, E. and Rabinowitz, M., Nature, 199, 44 (1963).

Holland, J.J., Proc. Natl.Acad. Sci. U.S., 50, 436 (1963).

Hurwitz, J., August, J.T., in J.N. Davidson and W.E. Cohn: Progress in Nucleic Acid Research, Vol. I, Academic Press Inc., New York, 1963, p. 59.

Kahan, E., Kahan, F.M. and Hurwitz, J., J. Biol. Chem., 238, 2491 (1963).

Klenow, H., Biochim. Biophys. Acta, 76, 354 (1963).

Muramatser, M., Hodnett, J.L., Adams, H.R. and Busch, H., Proc. Am. Ass. Cancer Res., 5, 47 (1964).

Paul, J. and Struters, M.G., Biochim. Biophys. Res. Comm., 11, 135 (1963).

Reich, E., Franklin, R.M., Shatkin, A.J. and Tatum, E.L., Proc. Natl. Acad. Sci., 48, 1238 (1962).

Robinson, J.R., Biochem. J., 45, 68 (1949).