INHIBITION OF DNA POLYMERASE BY HISTONES[1]

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Previous work in this laboratory on the time-course of synthesis of DNA, RNA, and histones in cell nuclei of regenerating liver (Holbrook, et al., 1962), (Evans, et al., 1962) and on the effects of added histones upon incorporation of labeled precursors into nucleic acids of Ehrlich ascites carcinoma cells (Irvin, et al., 1963) has led us to suggest that histones may play a role in the regulation of nucleic acid synthesis in cells preparing for mitosis. According to this hypothesis, the rising ratio of histones to DNA, which results from the fact that histone synthesis commences before DNA synthesis: (a) inhibits RNA synthesis and, directly or indirectly, stimulates DNA synthesis at intermediate values of the ratio; and (b) inhibits synthesis of both nucleic acids at the high ratios (approaching 2:1) which occur immediately preceding mitosis. Huang and Bonner (1962), Allfrey, et al. (1963), Barr and Butler (1963) have reported that histones may play an important role in regulation of RNA synthesis through inhibition of the DNA-dependent RNA polymerase.

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The present communication is concerned with a study of the effects of histones upon the activity of a DNA polymerase system from regenerating liver.

Methods. The effect of histones on DNA synthesis was measured by use of an enzyme preparation from regenerating rat liver (Behki and Schneider, 1963). Two types of experiments were conducted: Type 1, an assay for the effect of histones on thymidine kinase and deoxyribonucleotide kinases. Type 2, an assay for the effect of histones on DNA polymerase. Each of these assays involved two successive incubations or stages which were performed by mixing 0.45 ml of enzyme and 0.45 ml of incubation medium[3] containing H^3-thymidine (Behki and Schneider, 1963). To this mixture was added either 0.05 ml aqueous calf thymus histone solution in the type 1 kinase assay, or 0.05 ml water in the type 2 DNA polymerase assay. The mixture was incubated 30 minutes at 38° to generate deoxyribonucleoside triphosphates. The reaction was stopped by heating 1 minute at 100° followed by cooling on ice. The precipitated protein was removed by centrifugation. The supernatant (0.45 ml) was mixed with 0.1 ml calf thymus DNA primer solution (1 mg DNA/ml 0.001 M NaCl). To this mixture was added either 0.05 ml water in the type 1 kinase assay or 0.05 ml histone solution in the type 2 DNA polymerase assay; 0.40 ml of the enzyme was added and the mixture incubated 30 minutes at 38° to synthesize DNA. The reaction was stopped by adding 0.2 ml 6 N NaOH. The DNA was then isolated from the mixture (Orlov and Orlova, 1962),

[3] 0.45 ml of incubation medium contained 100 mμmoles each of 5'-deoxycytidylic acid, 5'-deoxyadenylic acid, 5'-deoxyguanylic acid; 10 μmoles adenosine triphosphate, 12 μmoles potassium 3-phosphoglycerate, 10 μmoles $MgCl_2$, 80 μmoles tris(hydroxymethyl)aminomethane buffer, pH 8.0, 120 mμmoles (methyl-H^3)thymidine, 10 mc/mmole.

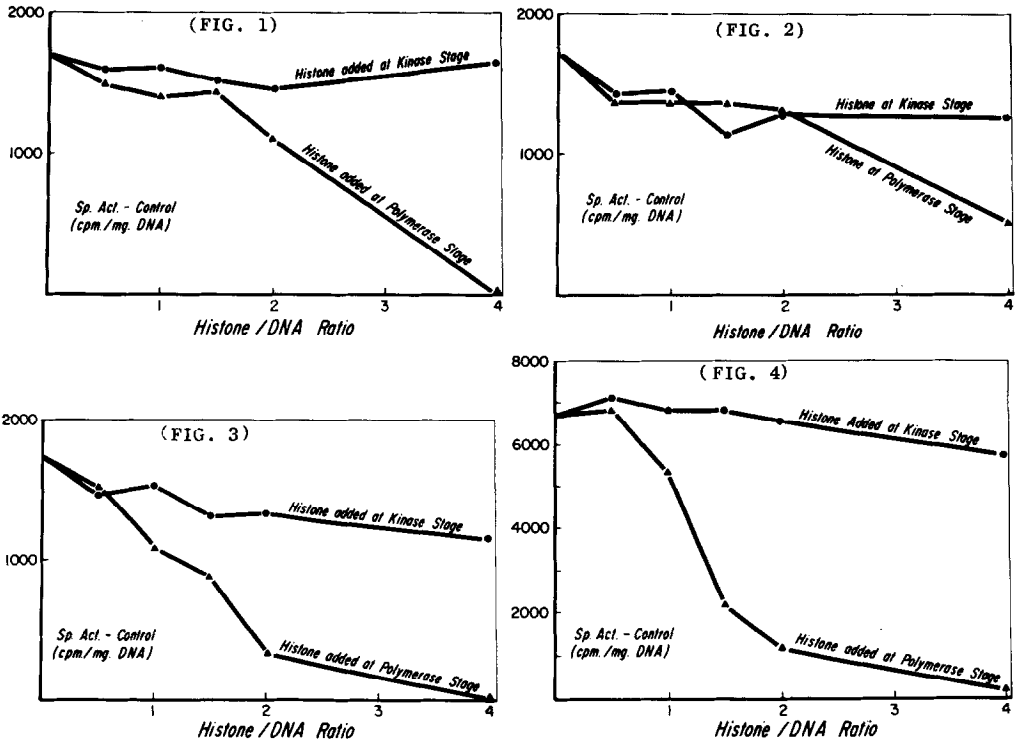
washed once with 40-50° alcohol:ether mixture (3:1) to remove lipid, twice with 5% trichloroacetic acid (TCA) to remove water soluble salts, and twice with ethanol to remove the TCA. The DNA was dissolved in 0.05 N NaOH. Samples were taken for DNA analysis (Burton, 1956) and for the determination of radioactivity in a Packard Tricarb Liquid Scintillation Spectrometer using Bray's liquid scintillation mixture (Bray, 1960). The specific activity of the DNA in cpm/mg was calculated.

Unfractionated histones were prepared from calf thymus glands (McAllister, et al., 1963); arginine-rich and lysine-rich histone fractions were prepared from the unfractionated histones (Ui, 1957); and a very lysine-rich histone fraction was prepared directly from calf thymus glands (Johns and Butler, 1962). These fractions are electrophoretically heterogeneous (McAllister, et al., 1963). The sodium salt of highly polymerized calf thymus DNA, type 1, was purchased from Sigma Chemical Company and used as DNA primer.

Results. In all the experiments, the concentration of DNA was held constant while varying concentrations of histones were added at the two stages of incubation. The figures present data for the effects of histone fractions on DNA synthesis in terms of varying histone:DNA ratios.

When unfractionated histones were added to the DNA polymerase assay (experiment type 2) (Figure 1), only a slight inhibition of DNA synthesis (approximately 15%) was observed at histone/DNA ratios of 1.5 or less. 35% inhibition of DNA synthesis occurred at a ratio of 2.0, and 100% inhibition occurred at a ratio of 4.0. Arginine-rich histones added to the DNA polymerase assay caused only a slight inhibition of DNA synthesis (approximately 20%) at histone/DNA ratios of 2.0 or less and only 68% inhibition at a ratio of 4.0 (Figure 2). Lysine-rich histones were more

inhibitory in the DNA polymerase assay and caused 12%, 35%, 47%, 80% and 100% inhibition at ratios of 0.5, 1.0, 1.5, 2.0, and 4.0, respectively (Figure 3). Very lysine-rich histones inhibited DNA synthesis slightly more than the lysine-rich histones at the higher ratios (Figure 4).



Figures 1, 2, 3, and 4: The effect of histone fractions on DNA synthesis. Each point in the figures is the average of two determinations. The specific activity of the isolated DNA was corrected for small non-specific radioactive contamination by subtracting c.p.m. for a control sample in which DNA primer was present but DNA synthesis was prevented by addition of NaOH to the enzyme before incubation.

When various fractions of histones were added to kinase assay (experiment type 1), only a small, or no, inhibition of DNA synthesis was observed (Figures 1, 2, 3 and 4). The small, apparent inhibitions may result from incomplete removal of histones by the 1 minute heat

treatment between the first and second incubation leaving trace amounts of active histones in the supernatant during the DNA polymerase incubation. Therefore, these small, apparent effects of histone on the kinase assay possibly can be interpreted as residual effects on the polymerase rather than on the kinases.

Discussion. Huang and Bonner (1962) have suggested that histones may control RNA synthesis by inhibiting RNA polymerase. They have shown, in vitro, that at a histone/DNA ratio of 1.0, RNA synthesis is completely inhibited in a system isolated from pea seedling nuclei. The data presented in this report show that at this ratio, in vitro, an arginine-rich histone fraction causes only a 21% inhibition of DNA synthesis, and a lysine-rich histone fraction inhibits DNA synthesis only about 35%. At a histone/DNA ratio of 2.0, however, DNA synthesis is still affected only moderately by the arginine-rich histone fraction, but the lysine-rich histone fraction inhibits DNA synthesis by 80%. These data lend some support to the previous experiments in vivo (Irvin, et al., 1963) from the results of which it was proposed that DNA synthesis is stopped when high histone/DNA ratios are attained in nuclei preparing for mitosis while RNA synthesis declines at lower ratios. Preliminary work (unpublished observations of E. L. Cooler, R. F. Fisher, and J. L. Irvin) on regenerating liver indicates that a histone/DNA ratio of about 2 is reached shortly before mitosis. The fact that the lysine-rich fraction is a stronger inhibitor of DNA polymerase than the arginine-rich fraction suggests the possibility that the lysine-rich fraction is particularly involved in cessation of DNA synthesis prior to mitosis.

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