Stimulation of Hen Erythrocyte Chromatin by Extracts of Rat Liver or Uterus

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Hen erythrocyte nuclei, and certain other nuclei, when introduced into HeLa cells of *Xenopus* eggs are often induced to synthesize RNA or DNA. Before synthesis begins the nuclei swell (Harris, 1967; Gurdon & Woodland, 1968). We describe here related effects in isolated hen erythrocyte nuclei.

Nuclei were isolated from washed hen erythrocytes by lysis in 1.5 mM-MgCl₂ followed by homogenization in a medium containing the neutral detergent BRIJ 35. After centrifuging through 2.1 M-sucrose-1.5 mM-MgCl₂ they were suspended in 0.25 M-sucrose-1.5 mM-MgCl₂. Liver (10 g.) from virgin female Wistar rats was homogenized to a final volume of 20 ml. in 0.25 M-sucrose-1.5 mM-MgCl₂ and the supernatant retained from a final centrifugation at 100 000g. Defatted uteri from twelve 23-day-old female Wistar rats were minced and homogenized in 2.5 ml. of 0.25 M-sucrose-1.5 mM-MgCl₂ and the supernatant was retained after centrifuging. *Escherichia coli* RNA polymerase was isolated by the method of Chamberlin & Berg (1962). RNA synthesis was assayed as described by Leake, Mayne & Barry (1968).

Hen erythrocyte nuclei synthesized little or no RNA with or without added RNA polymerase. But after preincubation for 30 min. at 37° in the liver supernatant the nuclei acted as template for added *E. coli* polymerase. The nuclei swelled to two to three times their original diameter in the liver supernatant. If this swelling was prevented by increasing the MgCl₂ concentration to 4 mM the nuclei would not subsequently act as template. Although cytoplasmic extracts of hen erythrocytes can be prepared that cause erythrocyte nuclei to swell, the nuclei do not acquire template activity. Erythrocyte nuclei when preincubated in uterine supernatant from control rats swelled, but only acquired low template activity/μg. of DNA. If the supernatant was from rats that had received oestradiol-17β (5 μg.) 4 hr. previously, the nuclei again swelled and they acquired greater template activity. In extracts of uteri from rats that had received oestradiol-17β (5 μg.) every 4 hr. for 24 hr., the erythrocyte nuclei did not swell and they did not subsequently act as template to *E. coli* polymerase. But if the nuclei were induced to swell in this supernatant by adding ATP (2 mM) to bind Mg²⁺, their subsequent template activity was greater than ever.

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Physical Studies on Partially Dissociated Nucleohistone

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Calf thymus deoxyribonucleohistone dissociated progressively into DNA and histone in the presence of NaCl between 0.5 and 2.0 M. The two components were separated by gel filtration on agarose beads and the viscosity, sedimentation and thermal-denaturation behaviour of the depleted nucleohistone was examined.

On removing up to 30% of the histone in 0.5-0.7 M-NaCl the intrinsic viscosity of the depleted nucleohistone remained similar to that of the native material. In 1.0 M-NaCl a further 50% of the histone was dissociated and the intrinsic viscosity increased considerably. In 2.0 M-NaCl the remainder of the histone was removed and a further increase in intrinsic viscosity was observed, giving a value close to that of DNA under similar conditions.

The sedimentation coefficient of the nucleohistone fraction decreased progressively as histone was removed. After allowance for the decrease in molecular weight caused by the removal of the histone the sedimentation properties showed an increase in the equivalent hydrodynamic volume of the particle that closely paralleled the increases in viscosity. These changes are interpreted in terms of a supercoiled model for native nucleohistone (Pardon, Wilkins & Richards, 1967) and the contributions that the various histone fractions make to this structure.

The thermal-denaturation curves of partially depleted nucleohistone showed that the 'melting' temperature decreased as histone was removed. The results will be discussed in terms of the arrangement of the various histone fractions on the DNA.

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