The Physical State of Deoxyribonucleoprotein and its Ability to Direct Ribonucleic Acid Synthesis *in vitro*

By TANYA A. HOARE and E. W. JOHNS

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London S.W.3, U.K.

(Received 10 August 1970)

The procedures for measuring the ability of DNA to act as a template for RNA synthesis *in vitro* are now well established (Chamberlin & Berg, 1962; Nakamoto, Fox & Weiss, 1964; Burgess, 1969). These methods are often applied to deoxyribonucleoprotein, or chromatin, which, unlike DNA, is insoluble under the normal assay conditions (Sonnenberg & Zubay, 1965; Butler & Chipperfield, 1967). It has been suggested that the physical state of the chromatin may be important for transcription *in vivo* (Johns, 1969), and Sonnenberg & Zubay (1965) have demonstrated that RNA synthesis *in vitro* can be increased by sonication of the deoxyribonucleoprotein used as a template. This procedure may cause breaks in the deoxyribonucleoprotein structure, and Butler & Chipperfield (1967) suggested that the effects observed may have resulted from a loss of histone.

It is therefore necessary to investigate more fully the manner in which the ability of deoxyribonucleoprotein to direct transcription can be increased by altering its physical state under conditions giving minimum deviation from conditions *in vivo*. It is important also to standardize the conditions for

measuring deoxyribonucleoprotein template ability and to attempt to relate these to the situation *in vivo*. We have attempted to do this by measuring the template ability of deoxyribonucleoprotein in various states of subdivision, brought about by precipitation from solutions of different concentrations by 0.14M-sodium chloride. As a control against artificially increasing the template ability of the deoxyribonucleoprotein, the completely repressed chromatin from chicken erythrocytes was similarly treated.

When deoxyribonucleoprotein is precipitated from a concentrated solution (1.5mg/ml) by a final concentration of 0.14M-sodium chloride, it is recovered as a dense fibrous material; if a very dilute solution of deoxyribonucleoprotein (0.15mg/ml) is precipitated in a similar manner a fine precipitate is obtained consisting of micro-fibres. This enabled a series of nucleoproteins (containing the same amount of DNA) to be isolated in physical states varying from dense fibrous clumps to finely dispersed micro-fibres. Calf thymus deoxyribonucleoprotein and chicken erythrocyte deoxyribonucleoprotein were made as described previously (Johns, 1964; Johns & Diggle, 1969) and gels were produced by dissolving them in water. The DNA content was determined by the method of Dische (1930). The gels were then diluted to give various concentrations as shown in Fig. 1. Different volumes of these diluted gels were taken for precipitation by a final concentration of 0.14M-sodium chloride in order to give the same final quantity of precipitated deoxyribonucleoprotein. The assay for template ability and the preparation of RNA polymerase have been described previously (Johns & Hoare, 1970).

Fig. 1 clearly shows that with calf thymus deoxyribonucleoprotein the template ability increases considerably with the state of subdivision. This does not occur with chicken erythrocyte deoxyribonucleoprotein under the same conditions, and indicates that the deoxyribonucleoprotein is not being artificially activated by the procedures used.

The physical state of the deoxyribonucleoprotein is clearly of importance when its template ability *in*

Volume of deoxyribonucleoprotein solution from which deoxyribonucleoprotein containing 500 μ g of DNA was precipitated (ml)

Fig. 1. Effect of the state of subdivision of calf thymus deoxyribonucleoprotein (O) and chicken erythrocyte deoxyribonucleoprotein (Δ) on the incorporation of [¹⁴C]AMP into RNA *in vitro*.

vitro is being measured, and this should be taken into account in experiments designed to investigate factors affecting transcription. That chicken erythrocyte deoxyribonucleoprotein remains inactive under identical conditions indicates that it is a completely repressed chromatin (Harris, 1965); this complete repression of RNA synthesis is probably due to the histone fraction F2C, which is unique to nucleated erythrocytes (Edwards & Hnilica, 1968) and which replaces most of histone F1 (Dick & Johns, 1969).

This work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research. We thank the Medical Research Council for a scholarship to T. A. H.

Burgess, R. R. (1969). *J. biol. Chem.* **244**, 6160.

Butler, J. A. V. & Chipperfield, A. R. (1967). *Nature, Lond.*, **215**, 1188.

Chamberlin, M. & Berg, P. (1962). *Proc. natn. Acad. Sci. U.S.A.* **48**, 81.

Dick, C. & Johns, E. W. (1969). *Biochim. biophys. Acta*, **175**, 414.

Dische, Z. (1930). *Mikrochemie*, **9**, 4.

Edwards, L. J. & Hnilica, L. S. (1968). *Experientia*, **24**, 228.

Harris, H. (1965). *Nature, Lond.*, **206**, 583.

Johns, E. W. (1964). *Biochem. J.* **92**, 55.

Johns, E. W. (1969). In *Ciba Found. Symp.: Homeostatic Regulators*, p. 128. Ed. by Wolstenholme, G. E. W. & Knight, J. London: J. and A. Churchill Ltd.

Johns, E. W. & Diggie, J. H. (1969). *Eur. J. Biochem.* **11**, 495.

Johns, E. W. & Hoare, T. A. (1970). *Nature, Lond.*, **226**, 650.

Nakamoto, T., Fox, C. F. & Weiss, S. B. (1964). *J. biol. Chem.* **238**, 167.

Sonnenberg, B. P. & Zubay, G. (1965). *Proc. natn. Acad. Sci. U.S.A.* **54**, 415.