Transcription of Brain Chromatin by Ribonucleic Acid Polymerases from Brain Nuclei and from *Escherichia coli*

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1. Transcription of ox brain chromatin by brain nuclear RNA polymerase II and *Escherichia coli* RNA polymerase was studied. 2. The soluble chromatin prepared from brain nuclei contained DNA, RNA, histone and non-histone proteins. Such chromatin preparations did not display any endogenous RNA polymerase activity, when assayed in the presence of concentrations of KCl as high as 0.4M. 3. The chromatin-templated activity of brain nuclear polymerase II was stimulated by KCl, with an optimum around 0.25M. 4. The template activity of brain chromatin for brain nuclear polymerase II and *E. coli* enzyme was about 20–25% of that of pure DNA. This greatly repressed template-capacity of chromatin was probably due to the acid-soluble chromosomal proteins. 5. Brain nuclear polymerase II was 3–4 times more active with dehistonized chromatin than with pure DNA as template, whereas bacterial enzyme was almost equally active with either of these two templates, reflecting the specificity of the transcriptional control mechanisms in mammalian cells.

The interphase chromosomes, in morphological terms, are collectively described as chromatin (Bonner *et al.*, 1968b). Only 5–20% of the total DNA in chromatin is available for transcription by bacterial RNA polymerase (Huang & Bonner, 1962; Allfrey *et al.*, 1963; Paul & Gilmour, 1968). This decreased template activity of the isolated chromatin for RNA synthesis *in vitro* can be restored to full capacity, the same as that obtained from an equivalent amount of pure DNA, if histone proteins are selectively removed (Marushige & Bonner, 1966; Paul & Gilmour, 1968; Murray, 1969; Smart & Bonner, 1971). These observations have implied that most of the eukaryotic genome is masked by DNA-bound macromolecules (Bonner *et al.*, 1968b; Paul *et al.*, 1970). On the other hand, physicochemical studies have shown that about 50% of the DNA in chromatin is 'naked' and not covered with proteins (Clark & Felsenfeld, 1971). Although the model of chromatin structure as proposed by these workers is different from that of Itzhaki (1971), both groups of workers have drawn the common conclusion that long sequences of DNA are available for binding large molecules like polylysine. Moreover, some evidence has been presented that the rat liver and microbial RNA polymerases bind to and transcribe different sites on the rat liver chromatin DNA (Butterworth *et al.*, 1971). These findings have led to serious doubts on the biological validity of earlier work on the transcription of chromatin by bacterial RNA polymerase (Clark & Felsenfeld, 1971; Butterworth *et al.*, 1971).

By virtue of specificity and heterogeneity in eukaryotes, not only at the cellular level but also at the genomic level, it is possible that certain distinctive characteristics of chromatin transcription

may emerge if mammalian RNA polymerase, instead of bacterial enzyme, is used. The present paper examines this possibility and deals with some of the transcriptive properties of brain chromatin.

Materials and Methods

Chemicals

Unlabelled ATP, CTP and GTP were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., or Calbiochem, San Diego, Calif., U.S.A., and [³H]UTP (specific radioactivity 18.6 Ci/mmol) was from Schwarz/Mann, Orangeburg, N.Y., U.S.A. Calf thymus DNA and yeast RNA were purchased from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Bovine serum albumin was obtained from Calbiochem.

Tissue

The cerebral cortex of ox brains freshly obtained from the slaughterhouse, processed as described elsewhere (Singh & Sung, 1972), was used.

Preparation of brain nuclear RNA polymerase

The purified nuclei were isolated in a hyperosmotic sucrose medium (2.2M-sucrose–1 mM-MgCl₂) as described by Singh & Sung (1972). The solubilization of RNA polymerase from brain cell nuclei, suspended in 0.05M-tris–HCl–0.01M-dithiothreitol–0.2M-KCl–30% (v/v) glycerol, was accomplished by sonication (Singh & Sung, 1971). The solubilized enzyme was separated, by ammonium sulphate fractionation and DEAE-cellulose chromatography

(Singh & Sung, 1972), into two DNA-dependent RNA polymerase activities, which were partly characterized and designated as RNA polymerase I and RNA polymerase II (Singh, 1972). Because of the greater instability of polymerase I, polymerase II was utilized to study the transcriptive properties of brain chromatin.

Escherichia coli DNA-dependent RNA polymerase (a DEAE-cellulose fraction) was kindly supplied by Dr. S. Su Gillam of the Biochemistry Department of this University.

RNA polymerase assay and the measurement of radioactivity

The RNA polymerase activity was measured by following the incorporation of [^3H]UTP into the trichloroacetic acid-insoluble precipitate. The conditions of enzyme assay were as described by Singh & Sung (1971), except that 0.1 mM carrier UTP was included in the assay mixture for *E. coli* RNA polymerase. Radioactivity was measured by the method of Singh & Sung (1971).

Preparation and other treatments of cerebral chromatin

Chromatin was prepared at 0–4°C by a method modified from that of Clark & Felsenfeld (1971). The purified nuclei, isolated from cerebral cortex, were gently homogenized in 'EDTA-buffer' (0.02 M-disodium EDTA, 0.08 M-NaCl and 0.005 M-tris-HCl, final pH 5.4). The nuclear homogenate was centrifuged at 9750g for 10 min and the pellet was washed twice with EDTA-buffer, followed by two additional washings with 0.02 M-tris-HCl, pH 8.0. The final gelatinous pellet was suspended in 0.02 M-tris-HCl, pH 8.0, and was sheared in a Branson Sonifier for 1.5 min at 0°C (nine bursts of 10 s each at 3 A with an interval of 20 s after every burst for cooling). The sonicated solution was centrifuged at 12100g for 30 min and the resulting supernatant constituted the final chromatin product. The chromatin, stored at 0°C, was used to study the template activity within 24 h after preparation.

Removal of histones from cerebral chromatin was done at 0°C by adding 0.25 ml of ice-cold 1 M- H_2SO_4 /ml of chromatin solution (final concentration of H_2SO_4 being 0.2 M). The solution was thoroughly mixed on a Vortex mixer, left at 0°C for 30 min with occasional mixing and then centrifuged at 27000g for 20 min. The pellet thus obtained was washed with cold water by using a glass rod and was suspended by gentle homogenization in 0.02 M-tris-HCl, pH 8.0. This material, prepared fresh, was used as the de-histone chromatin template.

The deproteinization of cerebral chromatin was based on the method of Marushige & Dixon (1969).

Chemical analysis of RNA, DNA and protein

The separation of RNA, DNA and protein into individual fractions was done by the method of Schneider (1957). A portion of the test sample was precipitated at 0°C with trichloroacetic acid (final concentration 5%, w/v), washed twice with 5% trichloroacetic acid and once with 95% (v/v) ethanol (5 ml portions each time). Insoluble precipitate was dissolved in 2 ml of 0.2 M-KOH and left overnight at 37°C. Then 0.05 ml of 70% (v/v) HClO_4 was added while the sample was pre-cooled to 0°C. The supernatant obtained by centrifugation in a clinical-model centrifuge was used for RNA determination by the orcinol reaction (Schneider, 1957). The residue was washed once with 5% trichloroacetic acid and extracted in 5% trichloroacetic acid at 90°C for 15 min. DNA in the extract was determined by the diphenylamine reaction (Schneider, 1957). The final pellet was washed once with 95% ethanol, dissolved in

Fig. 1. Effect of KCl on the chromatin-templated RNA polymerase II activity

Brain nuclear RNA polymerase II (76 μg of protein) was assayed in the presence of various concentrations of KCl with soluble chromatin (containing 60 μg of DNA) from ox brain as the template (o). Other assay conditions were as described in the Materials and Methods section. The endogenous RNA polymerase activity of the soluble chromatin (without added enzyme) was simultaneously determined (●).

dilute alkali and was used for protein determination by the method of Lowry *et al.* (1951). Yeast RNA, calf thymus DNA (in 5% trichloroacetic acid) and bovine serum albumin were employed as the standards for the determination of RNA, DNA and protein, respectively.

The determination of basic proteins (histones) was done as follows. A portion of chromatin fraction was separately treated with H_2SO_4 (0.2M) and the acid-soluble fraction was mixed with 3 vol. of 95% ethanol and left overnight at $-20^\circ C$. The precipitate obtained by centrifugation at 27000g for 20min was dissolved in dilute alkali and the content of histone proteins was determined by the method of Lowry *et al.* (1951).

Results and Discussion

The soluble chromatin prepared from ox cerebral cortex exhibited a sharp absorption maximum at 260nm, which also coincided with the peak of native calf thymus DNA. The E_{280}/E_{260} ratio in chromatin preparations was usually around 0.6. The E_{320} was practically negligible, indicating the absence of turbidity in the soluble chromatin, which contained DNA, RNA and protein in the mass ratios

1:0.09:1.92. All these features of soluble brain chromatin are compatible with those described for the chromatin preparations from other tissues (Bonner *et al.*, 1968a; Hearst & Botchan, 1970).

To determine the correct values for the transcription of brain chromatin by brain nuclear RNA polymerase II, it was essential to check the endogenous RNA polymerase activity in the soluble chromatin preparations. Fig. 1 shows that such chromatin preparations do not contain any significant amount of endogenous RNA polymerase activity, even when assayed in the presence of concentrations of KCl as high as 0.4M. The conditions of preparing chromatin from purified nuclei are very detrimental to the nuclear RNA polymerase and perhaps do not solubilize it (Singh, 1972). Fig. 1 also shows that soluble chromatin was a template for added enzyme (brain nuclear polymerase II), whose activity is further stimulated by the presence of KCl, reaching an optimum at around 0.25M.

The isolated chromatin from ox brain supported RNA synthesis in a homologous system by brain nuclear RNA polymerase II to the extent of about 20–25% of that with calf thymus DNA as template

Fig. 2. Template activity of brain chromatin

Brain nuclear RNA polymerase II (51 μg of protein) was assayed in the presence of various amounts of calf thymus DNA (●), deproteinized cerebral cortex chromatin (■) and equivalent amounts of DNA in native cerebral cortex chromatin (○), as described in the Materials and Methods section.

Fig. 3. Template activity of brain dehistonized chromatin for *E. coli* RNA polymerase

E. coli RNA polymerase (22.5 μg of protein) was assayed in the presence of calf thymus (●) and equivalent amounts of DNA in dehistonized chromatin (■) and native chromatin (○).

Fig. 4. Template activity of brain dehistonized chromatin for brain nuclear RNA polymerase II

Brain nuclear RNA polymerase II (51 μg of protein) was assayed in the presence of calf thymus DNA (●) and equivalent amounts of DNA in dehistonized chromatin (■) and native chromatin (○).

(Fig. 2). Moreover, the synthesis of RNA by polymerase II on deproteinized chromatin (brain DNA) was of an equal magnitude to that obtained with calf thymus DNA, indicating that the source of DNA used as a reference for comparison does not matter. These results indicate that most of the genome in nerve cells is somehow repressed, reinforcing the support for the 'masking' hypothesis of gene transcription in the cells of higher organisms (Bonner *et al.*, 1968b; Paul *et al.*, 1970).

A group of DNA-bound basic proteins, the histones, have long been suggested as the repressors of gene expression (Stedman & Stedman, 1950). The repressor function of histones is well exemplified by the fact that the selective removal of histones raises the template capacity of the isolated chromatin to the same value as that obtained with pure DNA as template for bacterial RNA polymerase (Marushige

Fig. 5. Relative rates of transcription of chromatin, dehistonized chromatin and calf thymus DNA by brain nuclear DNA polymerase II

The enzyme (76 μg of protein) was assayed in the presence of chromatin (60 μg of DNA, ○) or dehistonized chromatin (60 μg of DNA, ■) or calf thymus DNA (60 μg, ●) as template.

& Bonner, 1966; Paul & Gilmour, 1968; Murray, 1969; Smart & Bonner, 1971). In accord with these reports, we found that the dehistonized chromatin from brain was active as a template for *E. coli* RNA polymerase to the same extent as pure DNA (Fig. 3). However, when similarly prepared dehistonized chromatin from brain was utilized as template for brain nuclear RNA polymerase II, the resulting enzyme activity was about 3–4 times greater than that obtained with pure DNA (Fig. 4). Further, the rate of transcription of dehistonized chromatin by brain polymerase II (Fig. 5), but not by *E. coli* enzyme (Fig. 6), was about three times greater than that of an equivalent amount of pure DNA as template. These observations imply that an activating mechanism of DNA transcription exists in the genome of brain cells which is detectable by using an homologous enzyme (i.e. brain nuclear RNA polymerase II) but not by using an heterologous enzyme (i.e. *E. coli* RNA polymerase).

Fig. 6. Relative rates of transcription of chromatin, dehistonized chromatin and calf thymus DNA by *E. coli* RNA polymerase

The enzyme (22.5 μ g of protein) was assayed in the presence of chromatin (60 μ g of DNA, ○) or dehistonized chromatin (60 μ g of DNA, ■) or calf thymus DNA (60 μ g, ●) as template.

As RNA polymerase II from brain nuclei prefers heat-denatured DNA rather than native DNA (Singh, 1972), it may be argued that the treatment of chromatin with acid might have caused the denaturation of chromatin DNA and that is why dehistonized chromatin is the preferred template to pure DNA for this enzyme. However, the treatment of chromatin with 0.2M- H_2SO_4 at 0°C (the conditions utilized in the experiments described in the present paper) does not cause the denaturation of DNA in chromatin (Bonner *et al.*, 1968a; Murray, 1969). This kind of argument may also be excluded, on the basis of the finding that *E. coli* RNA polymerase utilizes dehistonized chromatin as a template as well as pure DNA. Such a result would not be expected if the DNA in dehistonized chromatin had undergone denaturation, because with denatured DNA as template the *E. coli* enzyme is not very active (Chamberlin & Berg, 1962; Hurwitz *et al.*, 1962; Furth & Loh, 1964). However, 'nicks' in DNA template (produced by limited digestion with deoxyribonuclease) stimulate transcription by bacterial RNA polymerase (Vogt, 1969) and it may, therefore, be questioned whether the enhanced transcription of dehistonized chromatin from brain by RNA polymerase II from brain nuclei results at least in part from the action of a contaminat-

ing nuclease. This possibility is difficult to eliminate, but it appears rather unlikely.

At present, it is premature to envisage a mechanism for the activated transcription of dehistonized chromatin by homologous RNA polymerase, but two comments are worth making: (i) polymerases of different origins might be highly selective in their action, perhaps in the step of chain initiation, and (ii) the structure of the dehistonized chromatin may enforce the specificity with homologous RNA polymerase but not with heterologous enzyme. Therefore, it is possible that the transcriptional control mechanisms which operate at the level of RNA polymerase may be highly specific in mammalian cells.

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