Deoxyribonucleoproteins and the Tissue-Specific Restriction of the Deoxyribonucleic Acid in Chromatin

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Although histones appear necessary for the restriction of DNA (Bonner et al. 1968), their lack of tissue and species specificity point to the necessary presence of other factors in chromatin that are required for the highly organ-specific genetic restriction of the DNA. The present paper analyses the type of restriction of the DNA in native chromatin by analysing the type of restriction in hybrid chromatins composed of portions of the dissociated products of the chromatins from two different organs.

Techniques for the isolation of chromatin, the RNA synthesis in vitro, the isolation of RNA formed in vitro from chromatin template and the DNA–RNA hybridization have been reported elsewhere (Spelsberg & Hnilica, 1970a). Briefly, isolated rat (male Sprague–Dawley) liver and thymus nuclei (Blobel & Potter, 1966) were excised three times with 80 mM-NaCl–20 mM-EDTA, pH 6.3, then once with 0.3 M-NaCl and twice with 0.01 × SSC† with a Teflon homogenizer. Each extraction was followed by centrifugation at 4000 × g, for 20 min. The RNA synthesis in vitro was carried out for 4 h. In each reaction 200 μg of DNA or 600–1000 μg of chromatin DNA was used as a template with 400–500 units of RNA polymerase from Micrococcus luteus (Nakamoto, Fox & Weiss, 1964). Each reaction mixture contained 400 μmol of tris–HCl buffer, pH 8.0, 25 μmol of MgCl₂, 10 μmol of MnCl₂, 30 μmol of 2-mercaptoethanol, 4 μmol each of GTP, ATP and CTP, 2.35 μmol of [³H]UTP (50 μCi/μmol; from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A.), 10 μmol of spermidine phosphate and 50 μg of bentonite. The reactions were incubated at room temperature. After the reaction mixture was made 0.3 M with respect to KCl, incubated for 20 min and centrifuged at 20000 × g for 10 min. This procedure releases 85–95% of the newly synthesized RNA with a little or no release of the endogenous chromatin RNA. The DNA–RNA hybridization method was essentially that of Gillespie & Spiegelman (1965). The annealing was carried out in 48% formamide–4 × SSC, pH 7.0, at 37°C for 16 h. Competition experiments involved incubating the DNA on membrane filters with the competing RNA, followed by washing and ribonuclease treatment and finally incubation with the labelled RNA.

The hybrid chromatins were formed as follows. The chromatin was homogenized by hand in 2.0 M-NaCl–50 mM-sodium acetate, pH 6.0. The lower pH (6.0) was used in our experiments to prevent proteolysis and to more thoroughly extract all of the histones while leaving most of the non-histone proteins associated with the DNA (see Spelsberg & Hnilica, 1970b). The dissociated chromatin was centrifuged at 90000 × g, for 36 h to sediment the DNA with associated non-histone proteins. The supernatants of histones along with RNA and about 5% of the non-histone proteins were carefully decanted from the pellets, precipitated with 70% (NH₄)₂SO₄ treatment, re suspended in 2.0 M-NaCl–5.0 M-urea–10 mM-sodium acetate, pH 6.0, and used as solvents to resuspend the DNA–protein pellets of the other tissue. Reconstitution of the solubilized histone fraction of one organ to the DNA with acidic proteins of another organ was accomplished by gradual dialysis of this mixture from 2.0 M-NaCl–5.0 M-urea–50 mM-sodium acetate, pH 6.0, to 0.4 M-NaCl–5.0 M-urea–40 mM-sodium acetate, pH 6.0, over a 16 h period. This was followed by a 6 h dialysis against 50 mM-sodium acetate buffer, pH 6.0, to remove urea. The chromatin in solution was sedimented by centrifugation at 30000 × g for 10 min and subjected to the chromatin purification procedure in 80 mM-NaCl–20 mM-EDTA, pH 6.3. The native as well as reconstituted chromatin, resuspended in 0.01 × SSC, was used as template for the RNA synthesis in vitro.

As reported by several authors, isolated chromatin exhibits organ-specific restriction of its DNA similar to that of native tissues (Huang & Bonner, 1965; Bonner et al. 1968; Paul & Gilmour, 1968;
protein in the hybrid chromatins was determined by the source of the DNA–non-histone protein used in the reconstitutions.

Unlabelled RNA, formed from the hybrid chromatin or native chromatin templates, was made to compete against labelled RNA formed from native rat liver chromatin template. In Fig. 1 the organ-specific restriction of DNA in the reconstituted hybrid chromatins appears to be determined by the DNA–non-histone proteins pelleted by centrifugation. Consequently the non-histone proteins or some other components associated with the DNA and not the histone-containing supernatants determined the pattern of restriction of the DNA in chromatin.

Experiments utilizing DNA–RNA hybridization in eucaryotes have certain limitations. If the RNA synthesized in nuclei of higher organisms were strictly heterogeneous, with no repetitive or closely similar nucleotide sequences, the large number of RNA species to be hybridized would make it impossible to obtain conclusive information in a reasonable length of time (Smith et al., 1969). Since a large portion of DNA in higher organisms was found to exist in a large number of either identical or highly similar copies (Britten & Kohne, 1968), these repetitive sequences allow a rather quick annealing of RNA to DNA. The redundant DNA sequences represent the major, if not total, fraction of the cell DNA involved in the hybrid formation in these experiments.

Previous evidence has indicated that either a special class of RNA (Huang & Bonner, 1965; Bonner et al., 1968; Bekhor et al., 1969) or the acidic proteins (Paul & Gilmour, 1968; Gilmour & Paul, 1969) of chromatin determine the pattern of restriction of the DNA. Our evidence here suggests that the total histone complement, along with some non-histone protein and RNA of chromatin extracted by 2.0 M NaCl at pH 6.0, are not involved in the tissue-specific restriction of the DNA, but do participate in the general restriction of the DNA. The non-histone proteins and possibly RNA, remaining with the DNA after the salt extraction, specify this organ-specific restriction.

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Fig. 1. Competitive hybridization of unlabelled RNA formed in vitro from the following templates: •, native rat liver chromatin; ○, hybrid chromatin composed of histones from rat liver and DNA with associated non-histone proteins from rat thymus chromatin; △, native rat thymus chromatin; A, native rat liver chromatin; ▲, hybrid chromatin composed of histones from rat thymus and the DNA with associated non-histone proteins from rat liver chromatin. The reconstitution was performed by dialysis at pH 6.0. The unlabelled RNA was made to compete against 3H-labelled RNA formed in vitro from native rat liver chromatin template. The 100% hybridization represents 410 c.p.m. with 4 µg of DNA/filter.


