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Eukaryotic ternary transcription complexes. II. An approach to the determination of chromatin conformation at the site of transcription

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ABSTRACT

Digestion of rat liver nuclei by endogenous nucleases or micrococcal nuclease releases a chromatin fraction containing RNA polymerases I and II bound to DNA fragments in ternary transcription complexes. To label the DNA in these transcription complexes, the polymerases were allowed to add radioactively labelled ribonucleotides in vitro to in vivo-initiated RNA chains. During this transcription step, nucleic acids were photochemically cross-linked using 8-methoxypsoralen. Nucleic acids in transcription complexes were then sized by gel electrophoresis. Under conditions where RNA polymerases I and II were active in vitro, most of the labelled DNA was found in a series of fragments of sizes which were multiples of approximately 200 base-pairs. When polymerase I alone was active, the smallest member of this series carried the bulk of the label; when polymerase II also was active, a significant proportion of the label was carried on the dimer and higher oligomers. Proteins other than polymerase alone are shown to be responsible for the pattern of DNA fragments protected from nucleases. Therefore active RNA polymerases I and II in vivo are in close proximity to structures protecting DNA fragments, the sizes of which are similar to those found in nucleosomes. We have yet to establish that these structures are composed of histones.

INTRODUCTION

Current models of chromatin structure have developed from the discovery of the nucleosome, the repeating subunit generated by micrococcal nuclease digestion which appears to accommodate the bulk of nuclear chromatin. The repeating subunit is composed of the well-known histone octamer comprising two of each of histones H2A, H2B, H3 and H4 associated with one molecule of histone H1 and a defined length of DNA duplex, the length of which varies in size (160-240 base pairs) according to the source of the chromatin. A micrococcal nuclease limit-digest gives rise to nucleosomal cores comprising the histone octamer and 145 base pairs of DNA, regardless of the source.

A great deal of interest has centered on whether this general picture of chromatin organisation is retained in regions of transcriptional activity. Recently, Mathis et al. (2) have critically evaluated both electronmicrosco-

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pic and biochemical data which has been accumulated. For non-ribosomal sequences, the weight of evidence favours the notion that transcriptionally active chromatin is organised into repeating subunits which contain all four core histones but in a conformation less compact than bulk nucleosomes. This is indicated by the increased accessibility of active genes to nucleases such as DNase I (3), DNase II (4). In the case of micrococcal nuclease, active genes are released in 'nucleosomal-sized' fragments (5-7). Nuclease susceptibility may be due to structural modification or loss of core histones, or to interaction of non-histone proteins with the repeating subunits.

The evidence is even more tenuous with respect to the organisation of transcriptionally active ribosomal sequences. There is little evidence that these genes maintain a repeating subunit structure while being transcribed: the characteristic beaded structures are not seen in EM-spreads of active ribosomal genes whereas inactive nucleolar chromatin is indistinguishable from bulk chromatin (see e.g. ref. 8). Scheer (8) suggests that the activation of ribosomal genes is preceded by a conformational transition from a beaded to an unbeaded form. Others have detected beaded structures which may be nucleosomal in character in non-transcribed spacer regions of active genes (9, 10) and, on transcription complexes which are not fully active, Osheim et al. (11) have noticed beads between well spaced RNA polymerases. In cases where nucleases apparently give rise to repeating subunits from active ribosomal genes, doubts exist as to whether they arise from active or inactive genes in the multigene family (2).

Most of the existing experimental information, briefly summarised above, concerns transcriptionally active chromatin *in general* (i.e. complete transcription units). We have now begun to explore the organisation of active chromatin at the actual site of transcription *in particular* to establish whether transcription complexes are proximal to periodic structures within active chromatin. We have subjected rat liver nuclei to digestion with nucleases which have been shown to recognise the nucleosomal periodicity of chromatin structure and which simultaneously excise a population of transcription complexes containing RNA polymerases I and II (see accompanying paper, Sargan & Butterworth, 12). In order to identify the DNA fragments associated with the polymerases, transcription complexes have been allowed to incorporate radioactive nucleotides into RNA which, in the presence of 8-methoxypsoralen, has been photochemically cross-linked to its template (Shen & Hearst, 13). We show that RNA polymerases I and II can be excised from nuclear chromatin in close association with 'nucleosome-sized' structures. The distri-

bution of DNA fragment sizes on which labelling occurs varies when different polymerases are active, suggesting that the chromatin transcribed by them is in different organisational states during transcription.

MATERIALS AND METHODS

Nuclear Preparation and Digestion

Rat liver nuclei were prepared as described previously (12).

Endogenous nuclear nuclease digestion were a modification of the protocol of Sargan and Butterworth (12). Nuclear pellets were suspended usually at a density of 10^9 ml^{-1} in Buffer A (12% glycerol, 20mM Tris/HCl pH 8.0, 0.5mM DTT, 0.1mM EDTA, 5mM $MgCl_2$, 70mM KCl) on ice. They were incubated at 37°C for 30 min and the nuclei pelleted by centrifugation at 3,000 x g for 5 min. The supernatant was retained and the pellet resuspended in an equal volume of Buffer B (as Buffer A except 120mM KCl). Once again inucbation was for 30 min at 37°C. A supernatant fraction was obtained as before, and for the experiments described here the 2 supernatants were pooled.

For micrococcal nuclease digestions, nuclei at 5 x 10^8 ml^{-1} were suspended in the digestion buffer of Pauletti (14) (8% glycerol, 10mM Tris/HCl pH 8.0, 0.1mM DTT, 60mM KCl, 1.5mM NaCl, 0.15mM spermine, 0.5mM spermidine, 0.2% Triton-X-100) and preincubated at 37°C for 3 min. The digest was brought to 2mM Ca^{2+} prior to addition of 50 units ml^{-1} micrococcal nuclease (Sigma). After incubation for a further 5' at 37°C, digestion was stopped by transfer to ice and addition of EGTA to 2mM.

Transcription and Psoralen Crosslinking in the Extracts

In vitro transcription was performed in the presence of 0.4mM CTP, GTP and ATP and 0.05mM radiolabelled UTP. The latter was labelled either with ^3H at 12Ci/mMol or with ^{32}P at 16Ci/mMol (Amersham International). In experiments to study RNA polymerase I activity transcription was carried out at 80mM KCl and 100µg ml^{-1} α-amanitin (Boehringer). For RNA polymerase II activity, transcription was at 250mM KCl: at this salt concentration, polymerase I is still active but the activity of polymerase II predominates. In each case reaction mixtures were made 1mM Mn^{2+} and 5mM Mg^{2+}. For transcription in the presence of heparin, 1mg/ml heparin was added to the reaction mixture 5 min before the addition of nucleoside triphosphates at 28°C. In all cases a 1ml transcription cocktail contained 0.78ml nuclear extract.

For psoralen crosslinking, 20µl of a 5mg ml^{-1} stock of 8-methoxypsoralen (Sigma) in ethanol was added to the reaction mix at the same time as the ribonucleoside triphosphates. The mixture was transferred to 50µl thin walled glass capillaries (Gelman Hawksley no. 1651) and transcription started in the dark by bringing to 25°C for 5 min. Crosslinking was then initiated by irradiation at 366nm on the floor of a UV Products 'Chromato-Vue Cabinet' type C-70 with an intensity of 6m Watts cm^{-2} for 25 min; reducing the length of the dark phase so the reaction did not affect the fragment-labelling patterns significantly (data not shown). The contents of the capillaries was pooled and brought to 10mM EDTA. In control assays, UV-irradiation was omitted and transcription allowed to occur for 30 min in the dark.

Nuclease Digestion and Purification of Transcription Complexes

Pooled transcribed extracts were digested with 500µg ml^{-1} RNase A for 30 min at 37°C and either made 0.5% (v/v) with sarkosyl (Ciba) or prepared

for DNase 1 (Sigma) digestion by dialysis against 10mM $MgCl_2$, 100mM KCl, 10mM Tris/HCl pH 8.0 for 60'. DNase 1 digestion was for 60 min at 20µg ml^{-1} at 37°C (there are approx. 100µg ml^{-1} DNA in the extracts at the start of these digestions as measured by the Giles and Myers (15) procedure). Digestions were stopped by adding EDTA to 10mM and sarkosyl to 0.5%.

All extracts were then passed through G-50 Sephadex (using as elution buffer 10mM EDTA, 10mM Tris/HCl pH 8, 100mM KCl, 0.05% sarkosyl), and fractions eluting at the void collected. This removed a substantial proportion of residual double stranded RNA structures as well as excess ribonucleoside triphosphates. After extensive dialysis to remove the bulk of sarkosyl and to bring to 10mM Mg^{2+}, fractions were either subject to DNase 1 digestion as before or immediately proteolysed, and nucleic acids were collected by phenol /chloroform extraction and ethanol precipitation (in some cases tRNA carrier was added).

Photoreversal of Psoralen Crosslinks

Crosslinked nucleic acid fragments (usually 20µg) were dissolved in 100µl 10mM Tris/HCl (pH 8.0) and placed in 2 Eppendorf minicentrifuge tubes on ice. These were irradiated using a UV Products 'Mineralite' light with the filters removed at a filament to sample distance of 4 cm. Irradiation was for 60 min, after which samples were precipitated with ethanol, dissolved in 4M urea, 20% glycerol and 90mM Tris/borate 2.5mM EDTA pH 8.3 and boiled for 10 min to denature prior to electrophoresis.

Gel electrophoresis was performed as described elsewhere (12). Gels were prepared for fluorography as described by Bonner and Laskey (16). Gels for autoradiography were dried prior to exposure.

Caesium Chloride Gradient Centrifugation

Samples for caesium chloride gradient centrifugation were brought to ρ = 1.67g ml^{-1} by adding solid caesium chloride. 4.5 ml aliquots were placed in 14 ml tubes and spun at 250,000 x g for 144 hours in an MSE 8 x 14 ml angle rotor at 15°C. After centrifugation, gradients were fractionated by upward displacements and 100µl aliquots precipitated by addition to 1ml of 10% (w/v) trichloroacetic acid containing 5% (w/v) tetrasodium pyrophosphate and 50µg ml^{-1} bovine serum albumin. Precipitates were collected by filtration through Whatman GF/C filters, washed 3 times with 5% (w/v) trichloroacetic aicd/3% (w/v) tetrasodium pyrophosphate, dried and counted in toluene Butyl-PBD scintillant.

Other Materials

Except where stated otherwise, all materials were from Fisons UK Ltd. or British Drug Houses Ltd. and were analytical reagent grade.

RESULTS

The use of 8-methoxypsoralen to covalently crosslink RNA to its DNA template in transcription complexes

We have shown that endogenous nucleases excise a population of transcription complexes from rat liver nuclear chromatin (12).

These ternary complexes contain either RNA polymerase I or II in a stable association with both RNA and DNA and are capable of limited RNA synthesis *in vitro* in the presence of inhibitors of the initiation of new RNA

chains, i.e. in vivo-initiated RNA chains can be extended in vitro. By radioactively labelling this nascent RNA and crosslinking it to its DNA template, it should be possible to characterise the DNA fragments on which in vivo-assembled transcription complexes have been excised.

The psoralens (or furocoumarins) can be used to crosslink nucleic acids by virtue of their ability to intercalate between base pairs; on irradiation with long-wave UV light, mono-adducts are formed with pyrimidine bases and diadduct formation may occur if there is an adjacent pyrimidine base in the opposite strand of the duplex (17, 18). These photochemical crosslinking reactions have been used to study chromosome structure and organisation (19, 20) and it has also been shown to be possible to covalently crosslink RNA to its DNA template in ternary transcription complexes using psoralen derivatives (13).

The protocol we have used to label the DNA in in vivo-assembled transcription complexes is summarised in Fig. 1 (described in detail in Methods). The rat liver nuclear extract containing transcription complexes arising from autodigestion in the presence of Mg^{2+} as described in Methods (see Sargan and Butterworth (12)). No attempt was made to isolate or purify the complexes from the nuclear extract. Aliquots of the extract were incubated at 25°C with ribonucleoside triphosphates (one of which was highly radioactively labelled) and 100µg ml^{-1} 8-methoxypsoralen. After allowing for a short burst of RNA synthesis (Fig. 1b), the transcribing complexes were irradiated at 366nm to crosslink the nascent RNA to its DNA template (Fig. 1c). Nascent RNA was reduced to a limit size using ribonuclease A (Fig. 1d).

To demonstrate the presence of stable DNA/RNA structures, the irradiated complexes were treated with RNase A, made to 0.5% with sarkosyl and passed through Sephadex G-50 as described in Methods. Radioactivity eluting in the void volume of the column was made to 1.67 g ml^{-1} with CsCl and centrifuged to equilibrium. Analysis of this gradient showed that the bulk of the DNA banded at a density of 1.64 - 1.66 g ml^{-1} (Fig. 2). Displacement from the normal banding density of 1.700 g ml^{-1} for rat liver main-band DNA is due to the intercalation of 8-methoxypsoralen in the DNA duplex (21). Acid precipitable counts banded at approximately 1.67 - 1.68 g ml^{-1}, a displacement from the main DNA band ascribable to the covelent crosslinking to DNA of a short oligoribonucleotide. A small proportion of the total radioactivity reproducibly lies at the top of the CsCl-gradient, the significance of which is not known.

As further evidence for the formation of DNA/RNA crosslinks in the

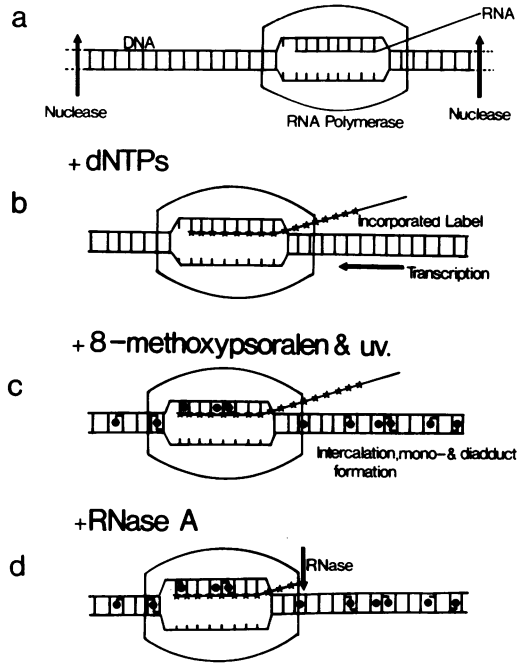

Fig. 1. Radioactive labelling of DNA in ternary transcription complexes. (a) Ternary transcription complexes are released from chromatin by nuclease digestion; (b) in vitro transcription by these complexes incorporates radioactive ribonucleotides into RNA, ~~X X X~~ ; (c) transcription is then blocked by intercalation, ● , and crosslinking of 8-methoxypsoralen to DNA and RNA through the formation of monoadducts, ◕ , and diadducts ◔ .

transcription complexes, use was made of the fact that psoralen/nucleic acid adduct formation can be reversed by irradiation with UV-light, putatively of wave-lengths between 240 and 290nm (22). Hyde and Hearst (23) reported partial breakdown of DNA/aminomethyl-trioxalen diadducts by irradiation at a longer wavelength (340nm) and they predicted that other psoralens would undergo similar reactions. Rabin and Crothers (24) showed that photo-breakdown of RNA/aminomethyl-trioxalen adducts can be driven to completion using UV-light at 250nm. Attempts were therefore made to break crosslinks by photo-irradiation. Maximal release of the labelled RNA fragment was produced by using a 'Mineralite' S-68 lamp with its filters removed (200-280nm). The irradiation flux used was 17mW cm^{-2} for 60 minutes on ice. This caused the release of approximately 70% of RNA into an RNase A-susceptible form (Fig. 3). Neither RNA nor DNA are substantially fragmented by irradiation of this intensity and duration (data not shown).

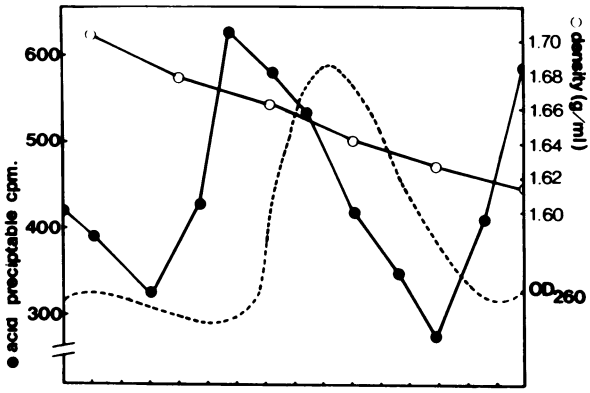

Fig. 2. Caesium chloride gradient analysis of 8-methoxypsoralen-crosslinked transcription complexes. Transcription complexes in the nuclear extracts arising from endogenous nuclease digestion were labelled by transcription, 8-methoxypsoralen crosslinked, treated with RNase A (see Fig. 1), made to 0.5% sarkosyl and chromatographed on Sephadex G-50 as described in Methods. Radioactive fractions eluting at the void were pooled and made to ρ = 1.67g ml^{-1} with CsCl (solid). After centrifugation for 144hr at 250,000 x g. fractions were monitored for optical density at 260nm (----) and acid precipitable radioactivity (●). Density measurements were made by refractometry (○).

Pattern of DNA fragment sizes which bear transcription complexes

Only a minute proportion of the DNA released from nuclei by endogenous nuclease-digestion of chromatin will carry transcription complexes (12). We have used the psoralen crosslinking of labelled RNA to DNA in transcription complexes to see whether the DNA fragments on which the ternary complexes have been excised exhibit any characteristic nuclease-cutting periodicities.

After radioactive labelling using the crosslinking protocol described above (Fig. 1) and limit digestion with RNase A, the nucleic acids were purified and analysed by electrophoresis on 1.2% agarose gels (Fig. 4) or 12% polyacrylamide gels (Fig. 5). Both the endogenous nuclear nuclease(s) and micrococcal nuclease released nucleic acid fragments which labelled as ladders of sizes resembling the nucleosomal periodicity (i.e. multiples of approx. 200 base pairs). The majority of the label was, in all cases, carried by the lower members of the series. However, the number of fragments labelled varied with individual preparations using the endogenous nuclear nuclease activity and with the intensity of the micrococcal nuclease digestion conditions employed. The pattern differed according to which RNA polymerase or combination of polymerases was active under the transcription conditions used to label the excised transcription complexes. Thus, when RNA polymerase I alone

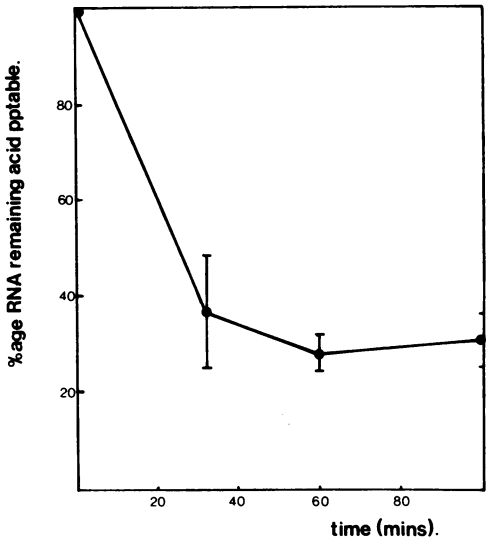

Fig. 3. Psoralen crosslinks between RNA and DNA in transcription complexes can be broken by short-wave UV-irradiation. Transcription complexes in the nuclear extracts arising from endogenous nuclease digestion were labelled according to the protocol in Fig. 1. After RNase digestion (Fig. 1d), the labelled complexes were irradiated with short-wave UV-light (see text) for the times shown, boiled for 5 min, redigested with 200µg ml^{-1} RNase A for 60 min and acid precipitated. Control incubations on ice without UV-irradiation showed no increase in susceptibility to RNase A.

was active (in the presence of α-amanitin to suppress polymerase II), most of the label was on the smallest 'monosomal'-like fragment, with smaller amounts on higher oligomers. When both polymerases I and II were active (polymerase II predominating), the monomer was labelled but an increased proportion of the label was associated with the dimer and higher oligomers.

It is important to exclude the possibility that any fragment labelling was due to initiation of RNA synthesis by RNA polymerases on released DNA fragments not associated with the polymerases in vivo. Preincubation of the nuclear digests at 25°C for 5 min with 1 mg ml^{-1} heparin prior to the in vitro transcription phase of the labelling protocol will totally eliminate any initiation events (25). Such treatment left the transcription complexes intact and functional and the pattern of labelled DNA fragments remained substantially unchanged (compare lanes 1 and 2 with lanes 3 and 4 of Fig. 5).

When the major transcriptionally labelled monomer and dimer fragments derived from the endogenous nuclear digests were sized by electrophoresis on 12% polyacrylamide gels (e.g. Fig. 5), they showed average sizes (at the cen-

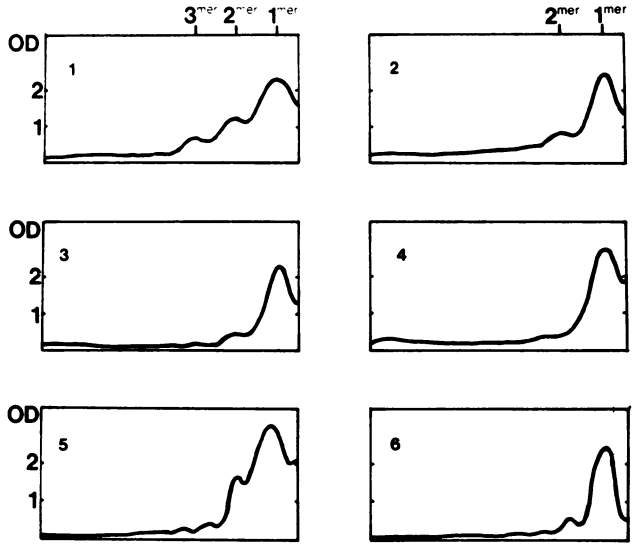

Fig. 4. The pattern of transcription complex-labelled chromatin fragments analysed by agarose gel electrophoresis. Transcription complexes in nuclear extracts derived from endogenous nuclear nuclease or micrococcal nuclease digestion were labelled in vitro by transcription and 8-methoxypsoralen cross-linking as shown in Fig. 1. Nucleic acids were purified and subjected to electrophoresis on 1.2% agarose gels and subsequent autoradiography. Autoradiograms were scanned by densitometry at 480nm (N.B. the relationship between optical density of the autoradiogram and number of counts on the gel is not linear). Lane 1: micrococcal nuclease-cut chromatin fragments transcribed at 200mM KCl and crosslinked; lane 2: micrococcal nuclease-cut chromatin fragments transcribed at 80mM KCl in the presence of 100µg ml^{-1} α-amanitin; lane 3: endogenous nuclease-cut chromatin fragments transcribed and crosslinked as in lane 1; lane 4: endogenous nuclease-cut chromatin fragments transcribed and cross-linked as in lane 2; lane 5: endogenous nuclease-cut chromatin fragments transcribed and crosslinked at 200mM KCl in the presence of 1mg ml^{-1} heparin; lane 6: endogenous nuclease-cut chromatin fragments transcribed and crosslinked at 80mM KCl in 100µg ml^{-1} α-amanitin and 1mg ml^{-1} heparin.

tre of the band) of 205 ± 11 base-pairs and 370 ± 16 base-pairs respectively (5 determinations). These figures were independent of the class of RNA polymerase active during the labelling. The equivalent figures for the unlabelled 'bulk' DNA fragments in the extract were 173 ± 9 and 364 ± 11 base-pairs. Most of this difference may be due to the presence of the crosslinked RNA tags on the labelled material (see below, the description of Fig. 6, lane 4). Some RNase-A resistant material less than 145 base-pairs in length is also evident (Fig. 5) and is discussed below.

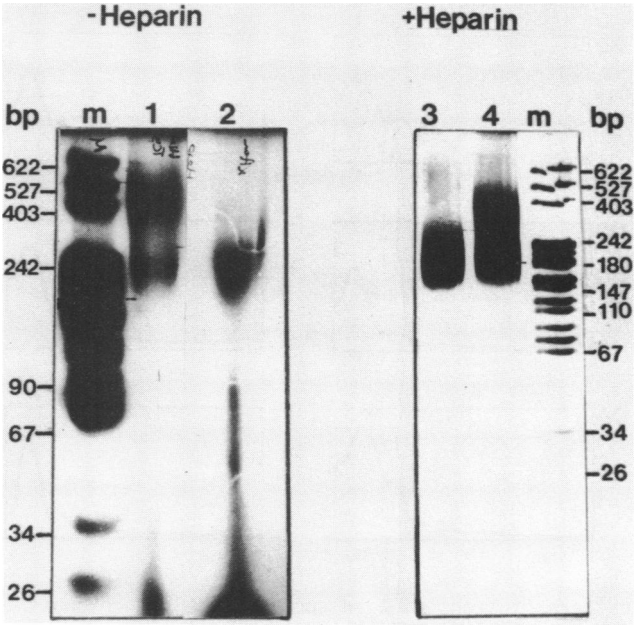

Fig. 5. The effect of heparin on the labelling pattern of chromatin fragments analysed on polyacrylamide gels. Chromatin fragments in the nuclear extracts derived from endogenous nuclease digestion were labelled by in vitro transcription and crosslinking as shown in Fig. 1. Purified nucleic acids were analysed on non-denaturing 12% polyacrylamide gels and autoradiography. Lane M: DNA markers (Hpa II digest of pBR 322, end-labelled by reverse transcription); lane 1: transcription in 200mM KCl, - α-amanitin, - heparin; lane 2: transcription in 80mM KCl, + 100 g ml^{-1} α-amanitin, - heparin; lane 3: as for lane 2 + 1mg ml^{-1} heparin; lane 4: as for lane 1 + 1mg ml^{-1} heparin.

The '205 base-pair' labelled fragment is not protected by RNA polymerase alone

It is necessary to ascertain that the '205 base-pair' nucleic acid fragment, labelled in the transcripiton complex did not arise through the coincidental protection of DNA from nucleases by the RNA polymerase alone. This was achieved by comparing the effect of DNase I digestion on transcriptionally labelled and crosslinked extracts before and after protein-stripping by sarkosyl: sarkosyl will dissociate the histone and most of the non-histone protein from nucleo-protein complexes, leaving transcription complexes intact and functional (26).

In these experiments, the pattern of nucleic acid labelling by the transcription/crosslinking technique was displayed on 18% polyacrylamide gels un-

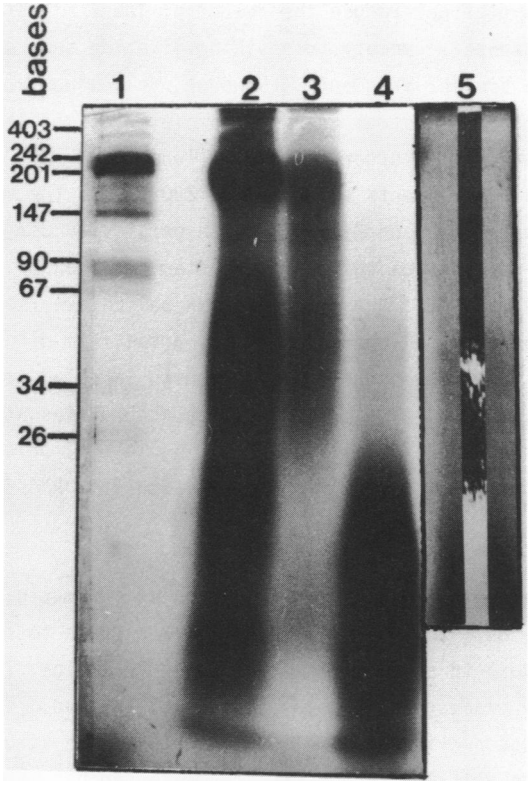

Fig. 6. The effect of DNase I digestion on labelled transcription complexes before and after the removal of protein by sarkosyl. Transcription complexes in the nuclear extracts from endogenous nuclease digests were labelled by *in vitro* transcription as before at 200mM KCl (Fig. 1), and subsequently digested with DNase I at 20μg ml^{-1} for 1hr at 10mM $MgCl_2$ either before or after treatment with sarkosyl at 0.5% to remove proteins (see text). Nucleic acids were purified, denatured and analysed on 18% polyacrylamide gels containing 7M urea. *lane 1*: DNA markers (Hpa II digest of pTP8, labelled by reverse transcription), *lane 2*: before DNase I-digestion (25,000 count min^{-1}); *lane 3*: DNase I-digested prior to sarkosyl treatment (18,000 counts min^{-1}); *lane 4*: DNase I-digested after sarkosyl treatment (25,000 counts min^{-1}); *lane 5*: image analysis of lane 4: inset over lane 4 is a photograph of the screen of a 'Quantimet 720' image analyser with the cursor set to show the disappearance of the band centered at 200 bases with the retention of that centered at 44 bases after nuclease digestion of sarkosyl-treated material.

der denaturing conditions (7M urea) to resolve both the '205 base-pair' and smaller labelled fragments (Fig. 6). The control lane (lane 2) shows the prominent '205 base-pair' fragment and a smear of fragments between 60 and 12 bases, the heaviest labelling being in the regions of 14, 26 and 45 bases. DNase I digestion of these fragments in the nuclear extract prior to sarkosyl

treatment (lane 3) does not reduce the amount of label migrating in the region of the '205 base-pair' moiety to anything like the same extent as it reduces label in the smaller fragments. However, following protein-stripping with sarkosyl, DNase I digestion causes a complete loss of label in the range of the '205 base-pair' and larger fragments (lane 4).

Of the labelled fragments smaller than 200 bases, the most interesting appears to be that centered on 44 bases which persists under DNase I-digestion conditions regardless of whether the system has been pretreated with sarkosyl: this may represent DNA masked by RNA polymerase (see Discussion below). Other smaller labelled fragments may arise from digestion barriers in DNA structures which are protein-free to which labelled transcript has been cross-linked, whereas that material which accumulates at less than 26 bases after extensive DNase I-digestion (Fig. 6, lane 4) of the sarkosyl-treated fraction in probably labelled RNA released from RNA/DNA complexes.

DISCUSSION

This work seeks to probe the organisation of chromatin at the actual site of transcription. The ability of certain nucleases to recognise repeating subunit structure in chromatin has been used to excise, from nuclear chromatin, ternary transcription complexes formed *in vivo*. The nature of these complexes must provide some insight into chromatin structure at the focal point of gene activity. The evidence presented has shown that transcription complexes containing either RNA polymerases I or II are located in rat liver nuclei *in vivo* in chromatin structure with periodicities recognisable by the nuclease systems we have used. The repeating subunits cannot be related with certainty to the nucleosome-periodicity defined by the cutting patterns of these nucleases although the DNA fragment sizes bearing transcription complexes are highly emotive of the nucleosomal organisation of chromatin.

For transcription complexes formed on non-ribosomal sequences (i.e. those containing RNA polymerase II), the pattern of labelled fragments mirrors the nuclease susceptibility of some very actively-transcribed genes: for example, the ovalbumin gene in induced chick oviduct (6, 7) and viral thymidine kinase genes (27, 26) are selectively reduced primarily to mono- and di-nucleosomal fragments by mild nuclease digestion. However, some recent work has shown that for the two major 'heat-shock' protein loci of *Drosophila melanogaster*, the active gene does not have a regular distribution of nucleosomes (28, 29). The 'heat-shock' genes are luxury genes transcribed at a very

high rate when active and may therefore represent a special case.

The labelling patterns of DNA fragments derived from transcription complexes on ribosomal genes (complexes containing RNA polymerase I, see ref. 30) provides firm evidence that the site of ribosomal RNA synthesis in rat liver chromatin also has discrete structure. In this case, there is a predominance of a '205 base-pair' labelled DNA fragment.

The fact that the pattern of DNA fragment-labelling is different when either RNA polymerase I alone or RNA polymerases I and II are both active suggests that the two enzymes must be active on chromatin templates having different structures in vivo. Our data nicely complements and extends that of Hatayama et al. (31) who have shown that RNA polymerase II copurifies with mono- and di-nucleosomal-sized DNA fragments after micrococcal nuclease digestion of rat liver nuclei.

This approach of labelling in vitro the DNA of transcription complexes formed in vivo obviates the problems inherent in (i) the potential instability of chromatin structure in the low ionic strength steps of the electron-microscopic analysis; (ii) distinguishing between the data arising from active and/or inactive genes in a multigene family (see Mathis et al. ref. 2) and (iii) attempts to reconstitute in vitro functional chromatin.

It is certain that nuclease protection of the transcription complexes is not due solely to the RNA polymerase component. The digestion barrier in the region of 44 base-pairs which persists following protein-stripping by sarkosyl is most probably due to bound polymerase. Chandler and Gralla (32) have previously reported that a eucaryotic RNA polymerase II protects 40 base-pairs of DNA from DNase I digestion or 34 base-pairs from micrococcal nuclease digestion. E. coli RNA polymerase has also been shown to protect a very similar amount of DNA from these nucleases (see Siebenlist et al., ref. 33).

While we cannot be sure that nuclease-protection of the transcription complexes arises from the normal components of the nucleosome per se, it has been shown in in vitro transcription studies that the E. coli RNA polymerase can come into fairly close proximity with isolated nucleosomes (34) and SV-40 minichromosomes (35) and no local sliding or dissociation of nucleosomes was detected. Furthermore, Hodo et al. (36) and Gould et al. (37) have indicated that RNA polymerases may be able to transcribe across nucleosomes. However, transcription may be from free DNA because Lilley et al. (38) have suggested that the core protein of nucleosomes and free DNA may exist in equilibrium with assembled nucleosomes in the dilute solution conditions

encountered in the in vitro assay. Many of these experiments have been carried out using isolated 'bulk' nucleosomes or nucleosomes reconstituted in vitro from histones and DNA alone and therefore may not have properties of 'active' chromatin (e.g. DNase I sensitivity). The use of E. coli RNA polymerase, an enzyme not adapted to encountering nucleosomes in vivo, or over-purified homologous polymerases which have probably lost initiation and/or elongation factors (see e.g. ref. 39) conspire to make definitive interpretations difficult.

The nature of the chromatin components which confer nuclease protection to the transcription complexes are now being examined. Assuming that no gross organisational changes occur during the excision of transcription complexes from isolated nuclei, the interactions between the components of the transcription complexes are the interactions which were formed in vivo. Preliminary data suggest that the endogenous nuclease extracts in which these complexes are found are enriched for transcribed DNA (Sargan and Butterworth (40) and manuscript in preparation).

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