Journal of Chromatography, 418 (1987) 73–95
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3628

REVIEW

NON-HISTONE CHROMATIN PROTEINS THAT RECOGNIZE SPECIFIC SEQUENCES OF DNA

RAYMOND REEVES*

Department of Biochemistry/Biophysics and Program in Genetics and Cell Biology, Washington State University, Pullman, WA 99164 (U.S.A.)

and

TERRY S. ELTON

Department of Biochemistry/Biophysics, Washington State University, Pullman, WA 99164 (U.S.A.)

(First received January 29th, 1987; revised manuscript received March 11th, 1987)

CONTENTS

1. INTRODUCTION

Nuclear proteins associated with the DNA of eukaryotic cells can be conveniently divided into two major categories, histone and non-histone chromosomal (NHC) proteins. The extensively studied major histone proteins (H1, H2A, H2B, H3 and H4) are quite similar in most cells and are thought to be the primary structural components of the nucleus involved in packaging the large amounts of

0378-4347/87/$03.50

DNA characteristic of eukaryotes [1, 2]. The "core" histones (H3, H4, H2A and H2B) represent a relatively simple and highly conserved group of proteins that are essential for the structural integrity of the basic repeating subunit of chromatin, the nucleosome. In contrast to histones, the NHC proteins represent an extremely complex group of proteins comprising not only structural proteins but also numerous enzymes, polymerases, DNA- and RNA-binding proteins, genomic regulatory proteins as well as many other components of chromosomes.

While it has been demonstrated that a wide variety of well defined enzymatic activities exist among the many different species constituting the NHC proteins [3, 4], most of the NHC proteins are either poorly described or not characterized at all. In fact, most of these proteins are largely understood only in terms of their preferential association with nuclear structures or their selective extractability from isolated nuclei or purified chromatin preparations. Furthermore, these unknown proteins are frequently identified only by their reactivity with specific anti-nuclear protein antibodies or by their relative electrophoretic mobilities on polyacrylamide gels. The limited progress that has been achieved in this field may be attributed in part to NHC protein heterogeneity, relatively low abundance of individual NHC proteins, poor solubility and the lack of in vitro functional assays for most of these proteins.

These characteristics have prevented the extensive purification of many individual NHC proteins for detailed biochemical analyses and for use in elucidating their role in chromatin structure and function. Recently, however, a number of books and reviews have appeared describing many of the different methods that have been developed for the purification and characterization of certain NHC proteins [5–8]. In this review we will discuss the use of recently described new chromatographic techniques and procedures for purifying selected NHC proteins believed to play critical roles in the transcription of eukaryotic genes. We purposefully limit our coverage to purification of proteins that recognize and bind to specific sequences of DNA. This will include discussion of the isolation of the promoter-specific transcription factor Sp1 [9], topoisomerase enzymes [10], the ribosomal gene transcription factor IIIA (TFIIIA) [11, 12], the gene regulating glucocorticoid receptor (GR) proteins [13, 14] of hormone-responsive mammalian cells, and the high-mobility group protein HMG-I which binds to A-T-rich stretches of DNA [15].

The procedures employed for the purification of these NHC proteins are varied and represent an overview of the current methodologies for isolation of proteins that bind to particular regions of DNA. Many of the initial steps in purification and the experimental precautions that must be taken during such isolations are similar for most of the NHC proteins. Thus, many classical fractionation procedures such as phosphocellulose, size-exclusion and ion-exchange chromatography are common to most of the isolation procedures currently in use as is the near universal use of protease inhibitors. In addition to these older methods, many new techniques such as photoaffinity and DNA sequence-specific affinity chromatography are becoming more widely used as supplemental, or alternative, steps during these purification procedures. The advent of high-performance liquid chromatographic (HPLC) and fast protein liquid chromatographic (FPLC)

techniques have already revolutionized the methods available for the rapid purification of specific proteins and enzymes from complex mixtures. Taken together, a combination of classical and modern techniques offers considerable promise for facilitating future purification of many of the rare gene-regulatory NHC proteins that, until recently, have been inaccessible due to their extremely low concentrations in nuclei.

2. PURIFICATION OF DNA SEQUENCE-SPECIFIC BINDING NHC PROTEINS

Of the many different NHC proteins associated with chromosomes, those that recognize specific sequences of DNA are assuming special importance because, in several cases, they are known to be involved with the control of messenger RNA (mRNA) transcription (reviewed in refs. 16 and 17). Development of reliable methods for purifying these generally rare nuclear proteins for detailed biochemical and biological investigations, therefore, is of primary concern to those interested in the mechanisms that regulate gene expression in eukaryotic cells. The following discussion covers many of the chromatographic methods currently employed for purifying or enriching for such NHC proteins.

2.1. Transcription factor TFIIIA

The transcription of *Xenopus* 5S ribosomal RNA (rRNA) genes requires RNA polymerase III and a minimum of three other NHC protein factors. One of these proteins has an approximate relative molecular mass (M_r) of 37 000 and is called TFIIIA [18]. TFIIIA binds specifically to an internal region of the 5S rRNA gene [11, 19, 20] and, with the other NHC proteins, forms a stable complex that directs the accurate transcription of 5S rRNA genes [21, 22]. In addition, in immature oocytes of *Xenopus* the transcription factor is found associated with 5S rRNA in a storage particle [23]. The 5S rRNA in these abundant 7S ribonucleoprotein (RNP) particles is used later in oogenesis for the formation of the ribosomes of the mature oocyte [24].

Purified TFIIIA has been shown to specifically bind to an intragenic domain of the 5S rRNA gene that extends from nucleotide 45 to about nucleotide 95 [11], and deletion mutant mapping has further delimited the intragenic control sequence necessary for faithful initiation of transcription to a location between nucleotides 50 and 83 [19, 20]. When Miller et al. [25] analyzed both proteolytic fragments of the factor and the sequence of TFIIIA predicted from a complementary DNA (cDNA) clone coding for the protein [26], they identified nine homologous repeated metal (Zn^{2+}) binding domains ("fingers") in the protein which they suggested specifically bind to the intragenic control sequence of the 5S gene by hydrophobic interactions [25]. Recent reports of the primary structures of other sequence-specific regulatory NHC proteins, including that of the Krupple segmentation gene of *Drosophila* [27], also reveal the presence of repeated fingers closely homologous to those of TFIIIA. It thus seems likely that finger-like metal-binding domains are frequently used as structural units in sequence-specific nucleic acid-binding NHC proteins [28].

The storage of TFIIIA as 7S RNP complexes accounts for the exceptionally high levels of this NHC protein in *Xenopus* oocytes and ovaries, the starting materials for most chromatographic procedures developed to purify this transcription factor. Two principle methods have been described for purification of TFIIIA from ovaries. The first method involves gentle chromatographic fractionation of homogenates from large, mature ovaries containing a mixture of oocytes of different developmental stages [29]. The second method involves isolation of 7S RNP complexes from immature ovaries followed by DEAE-52 chromatography of the particles, separation of RNA and elution of TFIIIA under denaturing conditions [12]. The first procedure rigorously avoids denaturing conditions but is rather lengthy and results in low yields. The second, although involving protein denaturation conditions which may alter the tertiary structure of the protein and possibly some of its functional properties, has the advantage of high yield and rapidity. Nonetheless, TFIIIA proteins isolated by either of these procedures appear to be structurally and functionally similar, although it is not known if they are identical in all respects.

Before outlining the procedures commonly employed for purifying TFIIIA, a few words should be said about the complementation assay method used for identifying TFIIIA activity in various cell-free extracts. The complementation assay is based on the observation [11] that S100 (see below) extracts of unfertilized *Xenopus* eggs do not actively support in vitro 5S rRNA synthesis by RNA polymerase III. However, a combination of unfertilized egg S100 extract and an extract derived from oocytes or ovaries can direct the accurate initiation and transcription of 5S rRNA genes in vitro. The mature egg extract provides all of the other components, including RNA polymerase III, necessary for selective transcription of 5S rRNA genes [11] and is complemented by the TFIIIA protein present in the ovary or oocyte extract so that in vitro transcription can occur. This in vitro complementation assay provides a powerful system to detect functional TFIIIA during its purification.

2.1.1. TFIIIA purification method 1

The first step in this procedure is the homogenization of about 100 g of mature ovaries in a buffer (50 m*M* Tris, pH 7.9; 4°C) containing 25% glycerol and 50 m*M* potassium chloride followed by filtration of the homogenate through cheese cloth. The filtrated homogenate is centrifuged for 20 min at 3000 *g*, the yolk is removed and the supernatant is recentrifuged at 100 000 *g* at 4°C for 90 min. The supernatant, designated the S100, is aspirated slowly and can be frozen in aliquots at −80°C after assaying for TFIIIA activity [11].

In the second step, ammonium sulfate (0.3 g per 120 ml of S100) is used to precipitate (30 min at 4°C) an enriched protein fraction containing TFIIIA activity which can be pelleted by centrifugation at 100 000 *g* for 1 h at 4°C. The pellet is resuspended in buffer [20 m*M* N-(2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH 7.5] containing 20% glycerol, 50 m*M* ammonium sulfate and 5 m*M* magnesium chloride (among other components) and at this stage can also be stored at −80°C, if desired.

The next two steps involve sequential anion-exchange chromatography of the

ammonium sulfate fractions containing TFIIIA activity (from step 2) on DEAE-cellulose and DEAE-Sephadex A-25. These steps not only lead to a substantial enrichment in TFIIIA specific activity in the eluting peak fractions but also remove residual nucleic acids and RNA polymerase III which would otherwise interfere in subsequent steps of purification [11]. The peak fraction from the DEAE-Sephadex A-25 column is dialyzed for 5 h against a buffer solution containing 25% glycerol and 50 m*M* potassium chloride as the primary constituents.

The final two chromatographic purification steps involve the sequential absorption and elution of the enriched ion-exchange fractions on columns of phosphocellulose (P-11) and BioRex-70 (BR-70). In both cases, the protein fractions are absorbed to the columns in buffers containing relatively low concentrations of ammonium sulfate (100 and 50 m*M*, respectively) and then subsequently eluted from the columns by linear gradients of increasing ammonium sulfate concentrations (95–700 and 50–500 m*M* ammonium sulfate, respectively). After dialysis against a buffered solution containing 25% glycerol, the purified TFIIIA preparation can be frozen in aliquots at −80°C, where the activity is stable for several months [11].

After the final chromatographic step, the TFIIIA preparation is judged to be greater than 95% pure by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). About 500 μg of purified protein are routinely obtained from about 100 g of starting ovary material [11].

2.1.2. TFIIIA purification method 2

Purification of TFIIIA from immature ovaries begins by the isolation of 7S RNP particles by centrifugation of ovary homogenates on 15–30% sucrose gradients. Ovaries from young *Xenopus* are homogenized in a buffer (50 m*M* Tris, pH 7.5, 4°C) containing 150 m*M* sodium chloride, the homogenate is centrifuged for 15 min at 20 000 *g* and the supernatant collected. This yolk-free supernatant is layered on top of 15–30% sucrose gradients, and the gradients are centrifuged in a Beckman VTi50 rotor for 6 h at 50 000 rpm. The gradients are fractionated and the 7S RNP particles located by their absorbance at 260 nm. Under these conditions of centrifugation, the 7S particles form a prominent peak approximately half way down the gradient [12].

The final step of purification is separation of TFIIIA from the other components of the 7S RNP particles by anion-exchange chromatography on a column of DEAE-cellulose. The preparation of 7S particles is adjusted to 5 m*M* EDTA and loaded onto the DEAE-cellulose in low salt [10 m*M* Tris, pH 7.5, 0.25 m*M* dithiothreitol (DTT)] and, after washing, eluted from the column with a buffered solution containing 7 *M* urea and 2.5 m*M* DTT. The protein-containing fractions are then pooled and dialyzed against a buffer containing 20% glycerol and 50 m*M* potassium chloride and frozen in aliquots at −80°C.

The TFIIIA protein isolated by this second method is greater than 90% pure as judged by SDS-PAGE and is functionally similar in complementation assays to the protein isolated by procedure 1 from mature oocytes. Additionally, the second method is not only more rapid than the first but is also more efficient in

terms of yield for a given amount of starting material. A 10-g amount of tissue yields 700–1000 μg of purified protein [12].

The two methods described for TFIIIA purification may not be of general applicability for isolating other types of DNA sequence-specific binding proteins unless they happen, fortuitously, to be present in exceedingly large amounts within cells. The special situation offered by the developmentally regulated *Xenopus* 5S ribosomal genes and accumulation of TFIIIA in oocytes represents an unusual occurrence in eukaryotic cells. In most cells the NHC proteins that specifically recognize nucleic acid sequences are typically present in low amounts in nuclei, and thus other purification methods must be used to isolate these proteins.

2.2. Promoter-specific transcription factor Sp1

A more typical eukaryotic NHC protein that recognizes DNA sequence and is present in low concentrations within nuclei (approximately 0.001% of nuclear proteins) is transcription factor Sp1 recently isolated by Briggs et al. [9]. Transcription factor Sp1 is a sequence-specific, DNA-binding protein originally isolated from HeLa (human) cells that enhances transcription of mRNA by RNA polymerase II, 10- to 50-fold from a select group of viral and cellular promoters [30–32]. The promoter sequence recognized by Sp1 is an asymmetric GC-rich decanucleotide (the "GC-box") with the consensus sequence (5′-G/TGGGCGGPuPuPy); the affinity of binding is determined by the match of a given sequence to the consensus [16, 30, 31]. Recently two Sp1 proteins of greater than 95% purity (representing a gain in specific activity of greater than 30 000-fold during the purification) have been isolated from HeLa cells [30, 31]. The two proteins, one with an M_r of 105 000 and the other of M_r 95 000, have been demonstrated to have both specific-sequence binding capability and the ability to stimulate transcriptional activity of gene promoters containing the consensus recognition sequence [9].

The steps of purification of Sp1 from HeLa cells as described by Briggs et al. [9] are summarized in Table 1. The assay for Sp1 binding activity in different fractions was by deoxyribonuclease (DNase) I protection experiments (footprinting) with a fragment of SV40 viral DNA that contains multiple copies of the GC-box recognition site acting as the substrate for Sp1 binding. The stepwise isolation and chromatographic procedures followed in the purification of Sp1 are potentially useful for the isolation of most types of NHC proteins that bind to specific sequences of DNA and are thus worthwhile discussing in some detail.

The initial processing step involved the isolation of nuclei from HeLa cells by hypotonic Dounce homogenization and low-speed centrifugation: the soluble cytoplasmic fraction was discarded. The nuclear pellet was extracted with buffer containing 0.42 *M* potassium chloride (a salt concentration typically used for the extraction of NHC proteins [6, 7]) and the crude nuclear extract was adjusted to 53% ammonium sulfate. The precipitated proteins, containing Sp1, were collected by centrifugation, resolubilized and separated by sequential steps of column chromatography as outlined in Table 1.

TABLE 1

PURIFICATION OF Sp1

On the basis that there is 100% recovery of Sp1 at the Sephacryl S300 step; accurate determination of binding activity in crude nuclear extract cannot be made. Taken from ref. 9 with modifications.

Procedure	Cumulative yield (%)	Total protein (mg)	Activity		Purification factor
			Total (U)	Specific (U/mg)	
HeLa cells (60 g)		3000			
Nuclear extract	100	600	10 000	17	5
Sephacryl S300	100	40	10 000	250	15
DEAE Sepharose	75	30	7500	250	1.0
Heparin agarose	40	8	4000	500	2
FPLC Mono S	18	1.5	1800	1200	2.4
DNA affinity	10	0.01	1000	100 000	83

The first step employed was Sephacryl S300 gel permeation chromatography (using a buffer containing 0.1 *M* potassium chloride for elution) which gave a symmetrical and highly reproducible peak of Sp1 binding activity with an apparent native M_r of approximately $500 \cdot 10^3$ [9]. This chromatography step gave about a five-fold purification of Sp1 binding activity, but the peak fraction was heavily contaminated with nucleic acids, phosphotases, polymerases and other proteins. Many of these contaminants were subsequently removed by chromatography on the anion-exchange resin DEAE-Sepharose CL-6B under conditions in which Sp1 was not retained on the column. The proteins that flowed through the DEAE-Sepharose column were directly applied to a heparin-agarose column in a buffer containing 0.1 *M* potassium chloride and eluted in steps with the buffer containing either 0.2, 0.3 or 1 *M* potassium chloride. The Sp1 activity eluted in the 0.3 *M* potassium chloride step fraction. This highly active Sp1 fraction was further purified on an HPLC cation-exchange column (FPLC Mono S; Pharmacia) using a linear 0.06–0.4 *M* potassium chloride gradient for elution. At this stage of purification, the peak fraction of Sp1 activity from the FPLC column represented about a 500-fold increase in specific activity relative to the whole cell extract (Table 1), and Sp1 constituted about 1–2% of the protein in the preparation. Efforts to further purify Sp1 by conventional chromatographic procedures, including FPLC Mono P, FPLC Sepharose 6, Biorex-70 (Bio-Rad), various hydrophobic resins and hydroxyapatite, were apparently not successful due to low efficiencies of recovery of the protein [9].

To obtain further purification of Sp1 past that offered by FPLC Mono S chromatography, Kadonaga and Tjian [30] found it necessary to use sequence-specific DNA bioaffinity chromatography in which the Sp1-enriched Mono S fraction was applied to a DNA affinity column prepared by covalently linking tandemly ligated synthetic oligonucleotide fragments containing high-affinity Sp1 recognition sequences (5′-GGGGCGGGGC-3′) to Sepharose CL-2B. An important aspect of this bioaffinity chromatography step was the addition of an appropriate amount of non-specific competitor DNA, such as sonicated calf thymus DNA or

synthetic poly d(I-C), prior to application of the Mono S protein fraction to the DNA bioaffinity column. This DNA bioaffinity step provided a further purification of Sp1, up to 60- to 100-fold, with the cumulative gain in specific activity throughout the purification being approximately 30 000-fold or greater (Table 1), and with the Sp1 binding proteins being greater than 95% pure [9]. Analysis of purified Sp1 by SDS gel electrophoresis indicated that specific DNA binding activity was present in two prominent polypeptide species, one of M_r 105 000 and the other of M_r 95 000. Both of these polypeptides could be individually eluted from SDS gels, renatured in vitro, and shown to retain specific GC-box binding to fragments of SV40 DNA. The relationship of the two Sp1 polypeptides to each other, e.g., whether the larger species is a modified form of the smaller one or whether the M_r 95 000 moiety represents a proteolytic fragment of the larger species, is unknown at the present time [9].

The strategy employed by Briggs et al. [9] for the purification of Sp1 should be generally applicable to the purification of other rare NHC proteins that bind specific sequences when different DNA resins of known sequence are used as the last step in the purification scheme. For example, a similar purification procedure employing plasmid DNA containing multiple copies of a specific recognition sequence absorbed to cellulose as the bioaffinity resin has recently been used by Rosenfeld and Kelly [33] to purify Nuclear Factor I, a specific binding protein that enhances initiation of adenovirus DNA replication in vitro, from nuclear extracts of HeLa cells. Other examples of sequence-specific proteins that have been isolated by such techniques are also known [9].

2.3. Topoisomerases

DNA topoisomerases are enzymes that control and modify the topological states of DNA. Two classes of topoisomerases, type I (Topo-I) and II (Topo-II), are known to exist in both prokaryotes and eukaryotes [34, 35]. These enzymes are able to alter the topology of DNA by transient protein-linked cleavages of either one (type I enzymes) or both (type II enzymes) DNA strands [34, 35]. Among the reactions catalyzed by these enzymes are relaxation–supercoiling, knotting–unknotting and catenation–decatenation of DNA.

The sites of topoisomerase-mediated cleavage are non-randomly distributed in DNA fragments suggesting that the reaction is sequence dependent. Recently a tentative consensus sequence (5′-GAN-A/T-A-C/T-ATTNATNNG-3′, where N denotes no preferred nucleotide) for *Drosophila* Topo-II cleavage sites was reported [36] supporting the view that Topo-II is a sequence-specific DNA-binding protein.

The alteration of the topological state of DNA in bacteria has been shown to affect replication, repair, recombination and transcription [34, 35]. In contrast, the role(s) that topoisomerases play in modifying DNA topology in eukaryotes is not as yet understood. Nevertheless, topoisomerases have been implicated in facilitating replication [37, 38], transcription [39], recombination [40], chromosome disjunction [41–43], and the establishment, maintenance and organization of chromosomal loops or "domains" [44–47].

Clearly, before the in vivo functions of eukaryotic topoisomerases can be properly determined, their in vitro activities must be well characterized. The recent purification of these enzymes from several eukaryotic species [10, 48–51] has made such characterizations possible.

Purification results have shown that both species of topoisomerases are enzymes with monomer subunits of M_r 90–135·10^3 [10, 50] and M_r 150–180·10^3 [10, 48, 49] for Topo-I and Topo-II enzymes, respectively. Sedimentation and gel permeation data suggest that the type I enzymes are monomeric [10] and type II enzymes are dimeric [10, 49].

One major problem that has plagued investigators in the purification of topoisomerase enzymes has been degradation by proteolysis. Recently, Goto et al. [10] have described a purification scheme for yeast DNA topoisomerases in which such proteolysis is minimized. First, two protease inhibitors, phenylmethylsulfonyl fluoride (PMSF) and sodium bisulfite, were included in their initial lysis buffer. Second, PMSF (1 m*M*) was present in all of the buffers used throughout subsequent purification steps. Third, a final concentration of 0.7% Polymin P was used to precipitate the nucleic acids and associated proteins directly after cell lysis in order to enhance the stability of the enzymes. Fourth, both Topo-I and -II were found to be relatively stable after chromatography on phosphocellulose. Finally, and perhaps most importantly, the initial steps were carried out as rapidly as possible at 4 °C and, in particular, Goto et al. [10] chose to use a fast batch phosphocellulose adsorption method as the first chromatographic purification step to minimize enzyme degradation.

To follow the purification of Topo-I and -II during isolation, Goto et al. [10] employed two different enzyme assays. Topo-I purification was followed by a simple relaxation assay which measures the ability of the enzyme to relax supercoiled plasmid DNA. Removal of supercoils was monitored by the decrease in mobilities of relaxed, closed circular plasmid DNAs relative to more supercoiled species during electrophoresis in agarose gels. Topo-II activity was followed through the purification steps by measuring the ability of different preparations to unknot P4 phage DNA in the presence of ATP [10].

Fig. 1 shows a flow chart that schematically outlines the early steps of Topo-I and -II purification as employed by Goto et al. [10]. Initially, yeast cells were homogenized in a purification buffer (TEG) which contained 50 m*M* Tris–HCl (pH 7.4), 1 m*M* Na_3EDTA, 10% glycerol, 1 m*M* PMSF, 1 m*M* sodium bisulfite, 1 m*M* 2-mercaptoethanol and 20 m*M* potassium chloride. The homogenate was centrifuged and the supernatant (fraction I) was used in Polymin P fractionations.

Fraction I was made 0.7% in Polymin P to precipitate DNA and Topo-II. Topo-II was extracted from the resulting precipitate with 0.8 *M* potassium chloride and the extract (fraction IIb) further purified by two sequential ammonium sulfate fractionations. The second (65%) ammonium sulfate precipitate, which is highly enriched for Topo-II activity, was dissolved in TEG buffer (fraction IIIb) and subjected to batch phosphocellulose chromatography.

The Polymin P supernatant (fraction IIa), which consists of Topo-I and many other contaminating proteins, was purified further by ammonium sulfate fractionation. A precipitated fraction from 80% ammonium sulfate was dissolved in

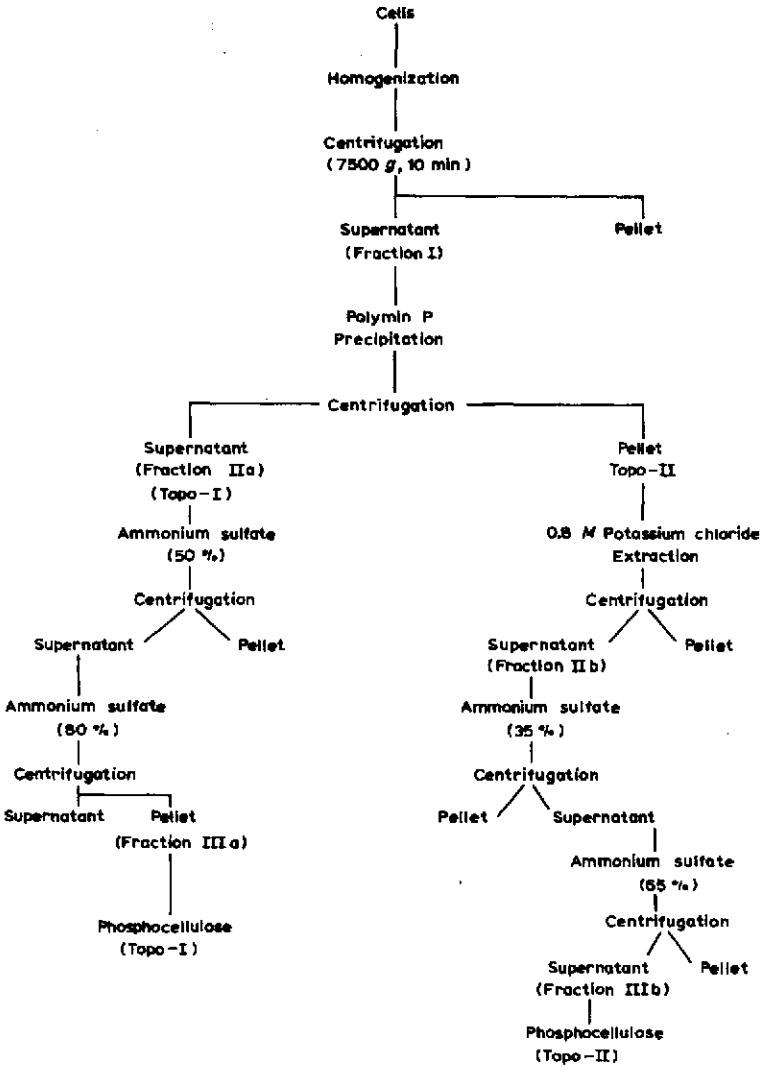

Fig. 1. Schematic outline of the procedures for fractionating yeast homogenates prior to chromatographic purification of topoisomerase enzymes [10].

TEG buffer (fraction IIIa) and, likewise, subjected to batch phosphocellulose chromatography. Topoisomerase activities were eluted from the phosphocellulose resins packaged into columns using linear gradients of potassium chloride, and fractions containing either type I (fraction IVa) or type II (fraction IVb) activity were recovered and pooled.

Fraction IVa, the eluate from the phosphocellulose column which contained Topo-I activity, was further fractionated by carboxymethyl-Sepharose chromatography using a linear potassium chloride gradient and again the eluted peak fractions of enzyme activity were pooled (fraction Va). Fraction Va was finally

TABLE 2

PURIFICATION OF TOPOISOMERASE II ENZYME ACTIVITY

One unit of topoisomerase II activity is defined as the amount of enzyme that converts 50% of knotted P4 phage DNA into nicked linear form without knots in 30 min under standard conditions. N.D. = not determined. Taken from Goto et al. [10] with modifications.

Cumulative yield (%)	Fraction	Total protein (mg)	Activity		Purification factor* (%)
			Total ($U \times 10^6$)	Specific ($U/mg \times 10^3$)	
100	I: crude homogenate	70 500	48	0.68	1
N.D.	IIb: 0.8% potassium chloride fraction	17 800	N.D.	N.D.	N.D.
N.D.	IIIb: 35-65% ammonium sulfate	7300	N.D.	N.D.	N.D.
83	IVb: phosphocellulose	840	40	48	78
45	Vb: heparin-agarose	99	22	222	330
17	VIb: double-stranded DNA cellulose	8.5	8.5	1000	1470
7	VIIb: aminopentyl-agarose	0.47	3.3	7000	10 300

*Crude lysate taken as 100%.

chromatographed on a column of double-stranded DNA (dsDNA) cellulose, eluted with a linear potassium chloride gradient, and the peak fractions of activity (fraction VIa) were pooled and concentrated.

During the chromatographic purification of Topo-I activity, two steps accounted for the greatest increases in purification of the enzyme. The first step involved elution of fraction Va from the carboxymethyl-Sepharose column resulting in a cumulative 740-fold increase in purity and an increase in specific activity of the enzyme to 4800 U/mg of protein. However, the second (and most substantial) increase in purity resulted from the chromatographic separation of fraction VIa on the dsDNA cellulose column which resulted in a cumulative 3100-fold increase in purification and a resulting preparation of Topo-I enzyme activity of $20 \cdot 10^6$ U/mg of protein [10]. This preparation of Topo-I appeared to be greater than 90% pure.

In contrast to Topo-I purification, the eluate from the phosphocellulose column that contained Topo-II activity (fraction IVb) was fractionated on a heparin-agarose column using a linear potassium chloride gradient. Eluted fractions containing Topo-II activity were pooled (fraction Vb) and, as before, subjected to dsDNA cellulose chromatography giving a further enriched preparation, fraction VIb. A final chromatographic separation on a column of aminopentyl-agarose gives a purified fraction, VIIb, a preparation of Topo-II that was greater than 90% pure. A summary of the steps of purification of Topo-II activity is given in Table 2.

After the final chromatographic steps, the preparations of both Topo-I and Topo-II enzymes approached homogeneity as judged by SDS-PAGE. The subunit sizes were estimated to be M_r 90 000 for Topo-I and M_r 150 000 for Topo-II of yeast [10].

The purification of Topo-I and -II has allowed detailed physical and enzymatic

characterization of these proteins. Furthermore, a number of useful antibodies against these proteins have been prepared. Recently, Goto and Wang [51], using specific anti-Topo-II antibodies, have isolated several molecular cDNA clones that contain the structural coding regions for yeast Topo-II. Because of these recent advances, much more should be learned in the near future about the in vivo function of eukaryotic topoisomerases.

2.4. Glucocorticoid receptor proteins

Steroid hormone-responsive genes are good model systems to study transcriptional regulation of mammalian genes. Several investigations have shown that one of the first steps in the activation of transcription in cells responding to treatment with glucocorticoids is binding of these hormones to specific receptor molecules in the target cells [52]. Binding of hormone with the glucocorticoid receptor (GR) results in a marked increase in affinity of the resulting hormone–protein complex for binding to specific sequences of DNA associated with cellular genes induced by the hormone [53, 54].

The GR–hormone complex binding regions (termed response elements) [55] commonly reside in multiple copies within or near regulated genes and function as receptor-dependent transcriptional "enhancers". That is, a glucocorticoid response element to which GR–hormone complexes bind is not itself a promoter; rather, it stimulates the activity of promoters to which it is linked without stringent constraints upon its position or orientation relative to the promoter.

Elucidation of many of the steps in such steroid gene regulation has come from investigations of the effects of glucocorticoids on stimulation of mouse mammary tumor virus (MMTV) gene transcription, both in vivo and in vitro (reviewed in ref. 55). In particular, it has been demonstrated that glucocorticoid stimulation of MMTV transcription requires binding of the GR–hormone complex to several specific nucleotide enhancer sequences located in the viral long terminal repeat (LTR) near the promoter [56]. Nuclease footprinting experiments have shown that a common consensus sequence for binding of the GR–hormone complex in the viral LTR enhancer region appears to be the hexanucleotide 5′-TGTTCT-3′ [56].

Steroid receptor proteins have been isolated and purified from a number of different sources including the glucocorticoid receptors of rat [13, 14] and mouse [57], the avian progesterone receptor [58, 59], and the human [60] and avian estrogen receptors, among others [52]. In addition, a number of useful monoclonal antibodies have been produced against GRs from several species [61, 62] which have been used both for purifying the protein and for detecting and quantifying the protein in crude extracts [52]. Furthermore, molecular cDNA clones for the coding sequence of GRs have recently been isolated from a number of sources including rat [63], mouse (reported in ref. 64) and human [65], as have the cDNAs for the estrogen receptors of humans [66, 67] and chickens.

From these and other studies, it appears that the intact GR in a variety of tissues and species is a protein of approximately 94 000 M_r [14] that can be divided into a number of functional domains including a ligand-binding region, a

DNA-binding domain and a region that selectively interacts with the enzymatic apparatus for transcription [52, 64]. In this regard, it is of considerable interest that the viral oncogene (v-erb-A) appears to be very similar to the domains of the glucocorticoid and estrogen receptors that bind both hormones and DNA [67, 68], suggesting that all of these receptors share an ancient progenitor.

Most of the purification methods available for isolating GR proteins rely on the high-affinity binding of the receptor with radiolabeled steroid ligands (such as dexamethasone or triamcinolone acetonide) which allows selective labeling and biochemical characterization of receptors in whole cells and in crude extracts, as well as in purified preparations [13, 14]. In recent years photoaffinity labeling methods (with radiolabeled ligands such as dexamethasone 21-mesylate or tamoxifen aziridine) that yield covalent hormone–receptor complexes [69–71] have been developed; this important approach is now being widely utilized for the purification of GR proteins.

Preparation of GR protein (to a purity of up to 95%) from rat liver cytosol has been reported by Wrange and co-workers [13, 14] and serves as a general model for similar receptor isolations from other sources. The purification scheme involved sequential chromatography of cytosol extracts on phosphocellulose, DNA-cellulose twice, DEAE-Sepharose and Sephadex G-200. Between the two chromatographic steps on DNA-cellulose, the receptor was heat-"activated" (or transformed). The final receptor preparation was concentrated by hydroxyapatite chromatography and analyzed by SDS-PAGE to determine purity and by centrifugation on sucrose density gradients to determine sedimentation coefficients of the hormone–receptor complex [14].

The following description is a more detailed account of the receptor purification scheme of Wrange and co-workers [13, 14]. Fifteen young rats (eight to nine weeks old) were adrenalectomized three to six days before killing by cervical dislocation. The livers were perfused in situ via the inferior vena cava with ice-cold phosphate-buffered saline (PBS) containing 10% glycerol and DTT as a reducing agent, removed, and finely minced together in the same cold PBS solution. After homogenization of the fragments in a glass homogenizer, the homogenate from fifteen livers was centrifuged directly at 80 000 *g* for 70 min in a Beckman Type 35 rotor with the temperature being kept as close as possible to 0°C to limit proteolysis. After centrifugation, the clear supernatant cytosol fraction was carefully removed from the tube and incubated together with 100 n*M* of [^{3}H]triamcinolone acetonide at 0°C for 60 min. In the subsequent chromatographic steps, the purification of the hormone–receptor complex was followed by monitoring bound radioactivity in a liquid scintillation counter [13].

The initial two chromatographic steps involved the sequential elution of the isotopically labeled hormone–receptor complex in the cytosol extract first from a phosphocellulose and then from a DNA-cellulose column. At this stage of purification, the hormone–receptor complex had not yet been "activated" (or transformed) and therefore did not bind to either the phosphocellulose or the DNA-cellulose column with high affinity. The receptor complex eluted in the flow-through volume from both columns [13]. These two chromatographic steps, however, did remove many proteins, proteases and other contaminants from the

receptor complex preparation. The hormone–receptor complex was then activated to a high DNA binding affinity state by incubation of the flow-through volume from the first DNA-cellulose column at 25°C for 30 min.

After heat activation, the cytosol extract was chromatographed on a second DNA-cellulose column at a low flow-rate (9.5 ml/cm^2/h) to allow the receptor complex to bind to the matrix DNA. The hormone–receptor complex was eluted from the second DNA-cellulose column in one of two ways. In the original method [13], the receptor complex was affinity-eluted from the column with pyridoxal 5′-phosphate resulting in a purification of approximately 6700-fold and a purity of about 74%. However, the affinity elution of the receptor complex with pyridoxyl 5′-phosphate was later shown to block the specific binding of the GR to DNA [14]. Therefore, in later modifications of this procedure the receptor complex was eluted from the DNA-cellulose column with 25 m*M* magnesium chloride, giving a hormone–receptor preparation of lower purity (about 63%) but one retaining the ability to specifically bind to DNA [14]. The 25 m*M* magnesium chloride-eluted GR complex was applied directly on a DEAE-Sepharose column and rapidly eluted with a linear salt gradient resulting in a significant purification (8470-fold) [13]. The recovery in the DEAE-Sepharose step was approximately 66%, and the purity ranged from about 80% [14] to about 95% [13].

Analysis by SDS-PAGE indicated that the major GR protein species (80% of the protein) isolated by this method [13] had an M_r of about 94 000. Two other minor proteins, one of M_r 79 000 (constituting about 9% of the total protein) and the other of M_r 72 000 (representing about 11% of the protein), were also present in these receptor preparations [14]. Photoaffinity labeling with [^{3}H]triamcinolone acetonide of the three protein components in the preparation indicated that only the 94 000 M_r proteins bound labeled hormone, thus indicating that the larger species was most likely the true receptor protein and that the smaller one probably represented a proteolytic cleavage product [14].

These purified GR preparations have been extensively utilized to produce monoclonal antibodies against the receptor protein for use as analytical reagents. They have also been used in experiments demonstrating the binding of the receptor complexes to specific enhancer sequences of steroid hormone responsive genes and, more recently, have been used for amino acid sequence determinations in the molecular cloning of the cDNAs coding for the receptor proteins.

2.5. High-mobility group protein HMG-I

The high-mobility group (HMG) non-histone chromatin proteins, so named because of their rapid migration during acid/urea PAGE, were first characterized by Goodwin and Johns [72]. Detailed biochemical analyses conducted on these proteins have established that at least four major HMG protein groups can be isolated from mammalian cells. These can be further divided into two categories: the high-molecular-mass proteins, HMG-1 and HMG-2 (approximate M_r 26 000), and the low-molecular-mass proteins, HMG-14 and HMG-17 (approximate M_r 10 000–12 000) [73].

Recently, Lund et al. [74] isolated a new HMG-like protein (HMG-I) with

an approximate M_r of 10 000 from proliferating human (HeLa S3) cells. The amino acid composition of HMG-I was similar to, but still distinct from, the known amino acid compositions of calf thymus HMG-14 and HMG-17 proteins [74]. Very recently it has been shown that HMG-I is also present in proliferating African green monkey cells [75], Ehrlich ascites mouse cells [76], rat fibroblast cells [77], Friend erythroleukemic cells [78] and proliferating rat thymus tissue [77]. In contrast to these results, HMG-I is present in very low concentrations (if at all) in non-dividing tissues such as rat liver [77] and calf or pig thymus [15]. These results suggest that HMG-I may be involved with a proliferative function of mammalian cells.

By way of their composition and primary structure (which includes regions of both highly acidic and highly basic amino acid residues) all of the HMG proteins bind to isolated DNA [79–81], to histones [82–84] and, in some cases, to nucleosomes [85–89]. In addition, HMG-1, -2, -14 and -17 bind preferentially to single-stranded as compared to double-stranded DNA [90, 91]. HMG-1 and -2 also demonstrate, in vitro, some degree of specificity of binding to DNA with various non-B-form conformations [92–96], but do not seem to display any sequence specificity in such binding.

In contrast to the DNA binding characteristics of HMG-1, -2, -14 and -17, the newly discovered HMG-I (α-protein) is an A-T sequence-specific dsDNA binding protein [95,102]. Furthermore, unlike other well characterized sequence-specific DNA binding proteins, such as bacterial repressors, Sp1 and TFIIIA, HMG-I makes extensive contacts with the minor (rather than the major) groove of B-form DNA [95].

Recently, Strauss and Varshavsky [75] have shown that HMG-I (α-protein) can be purified to apparent homogeneity by a combination of three separate chromatographic steps (phosphocellulose, DNA-Sephacryl and hydroxyapatite). Although this purification procedure is adequate, it is definitely very time-consuming. Furthermore, this multiple-step purification method decreases the yield of purified HMG-I from the starting material. By comparison, Elton and Reeves [78] have developed an extremely rapid (25 min), one-step reversed-phase HPLC (RP-HPLC) purification procedure for the preparation of homogeneous HMG-I protein. Since HPLC is becoming a common technique in the isolation of proteins, as evidenced by the purification of functional HMG-I (see below), we expect its wide-spread future use in the isolation of other DNA sequence-specific binding proteins.

The RP-HPLC purification procedure for isolation of functional HMG-I as described by Elton and Reeves [78] starts with the isolation of nuclei from exponentially growing murine Friend erythroleukemia cells and extraction of total NHC proteins from these with 0.35 *M* sodium chloride. Highly enriched HMG protein samples were obtained from this crude salt extract by selective acid fractionations using varying percentages of trichloroacetic acid (TCA) to isolate the proteins (i.e., HMG proteins are soluble in 2% TCA but insoluble in 25% TCA) [78]. Enriched HMG protein samples were injected onto a Vydac C_4 RP-HPLC column and eluted with a linear trifluoroacetic acid–acetonitrile gradient [78].

From the typical chromatographic elution profile shown in Fig. 2A, taken from

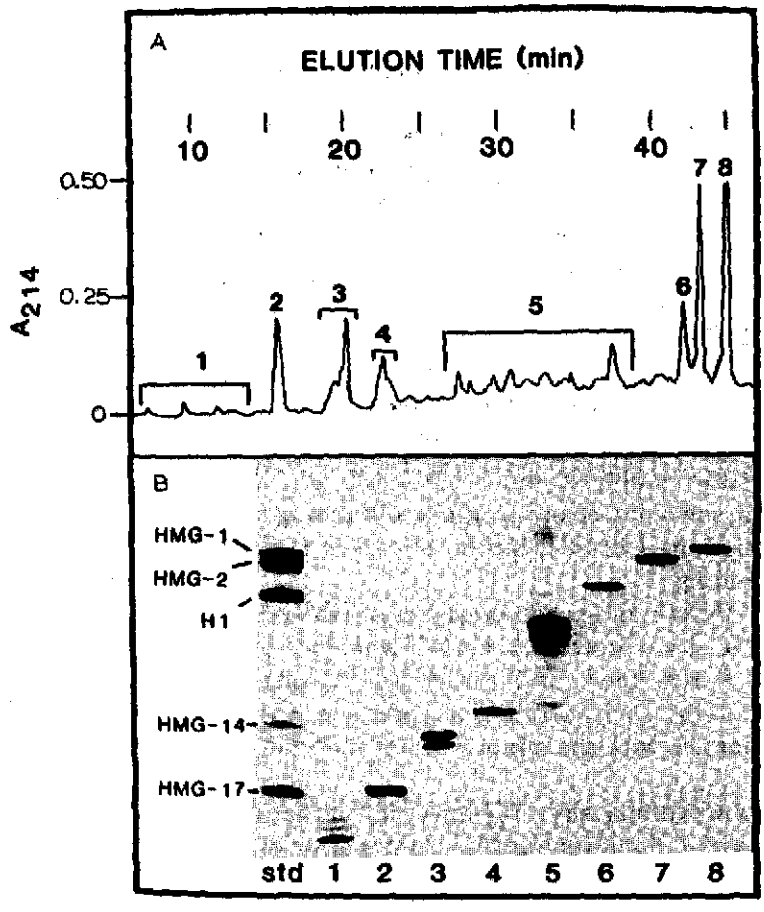

Fig. 2. Ion-pair reversed-phase HPLC analysis of Friend erythroleukemic cell HMG proteins [15]. (A) Enriched HMG protein samples (approximately 100–200 μg) were isolated from Friend cell nuclei, dissolved in 0.1% (w/v) aqueous trifluoroacetic acid (TFA), injected onto the reversed-phase HPLC column and eluted with a 15–50% linear gradient of acetonitrile–0.1% TFA at a flow-rate of 1.0 ml/min. Absorbance was monitored at 214 nm, 0.5 a.u.f.s. The column effluent from profile A was collected, lyophilized and electrophoresed. (B) Acid/urea polyacrylamide slab gel (15%); std: HMG-1, -2, -14 and -17 calf thymus protein standards. Lanes 1–8 correspond to the protein peaks labeled 1–8 in A.

Elton and Reeves [78], it is seen that an enriched Friend cell HMG protein sample is fractionated into several major and numerous minor peaks by RP-HPLC. To identify the protein(s) present in each of the elution peaks, samples were collected and subjected to PAGE (Fig. 2B) and amino acid analysis [78]. Based upon those results Elton and Reeves [78] concluded that fraction 4 (Fig. 2A, Fig. 2B, lane 4) is composed of homogeneous HMG-I. Furthermore, these workers demonstrated that RP-HPLC is useful for obtaining HMG-1, -2, -14 and -17 in highly purified form in a single chromatographic step in less than 50 min (Fig. 2B, lanes 2, 3, 7 and 8).

For some proteins the utility of an RP-HPLC purification is of questionable

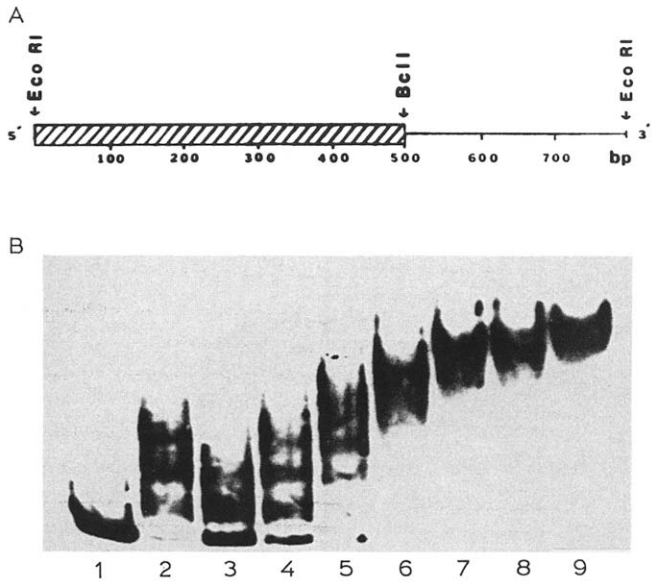

Fig. 3. (A) Restriction enzyme map of the 791-bp cDNA clone of the bovine interleukin-2 (bIL-2) gene, showing the open reading frame for the protein coding region (hatched box) and the 3'-non-translated tail region. (B) Electrophoretic mobility shift assay of a 295-bp Bcl I-Eco RI restriction fragment. End-labeled 295-bp 3'-untranslated tail of bIL-2 (0.5 ng of DNA, 10 000 cpm per binding reaction) incubated with a fixed amount of competitor DNA (2 μg of poly I-C per reaction) and increasing amounts of reversed-phase HPLC-purified HMG-I before electrophoresis in a low-ionic-strength 4% polyacrylamide gel. Lane 1, free DNA fragment; lane 2, 10 ng of HMG-I in the absence of competitor DNA; lanes 3–9 contain 5, 10, 20, 30, 40, 50 and 60 ng of HMG-I, respectively.

value since the secondary and tertiary structures of proteins are not preserved during chromatography carried out under denaturing conditions [97–100]. Therefore, Elton and Reeves [78] investigated whether the HMG-I preparation purified by their denaturing RP-HPLC method retained its function of specifically binding to A-T-rich sequences of DNA as does the protein isolated under non-denaturing conditions [95]. For these determinations these workers used both DNA fragment electrophoretic mobility shift assays [75] and DNase I footprinting assays [101] to investigate the DNA-binding activity of RP-HPLC-purified HMG-I.

The DNA fragment electrophoretic mobility shift assay is based on the principle that there is a difference in relative mobility of nucleic acid–protein complexes compared to free nucleic acids during electrophoresis in non-denaturing polyacrylamide gels. To perform such assays Elton et al. [102] incubated a radiolabeled 295-bp Bcl I/Eco RI DNA restriction fragment (Fig. 3A) rich in A-T sequences (which comprised the entire 3'-untranslated tail region of the bovine interleukin-2 (bIL-2) cDNA sequence [103]) with RP-HPLC-purified HMG-I. After a short incubation period (10 min) the sample was applied to a low-ionic-strength non-denaturing polyacrylamide gel and electrophoresed for a period of time (2–3 h) that gave reasonable separation of DNA–protein complexes and free DNA. The gels were then processed for autoradiography and the resulting

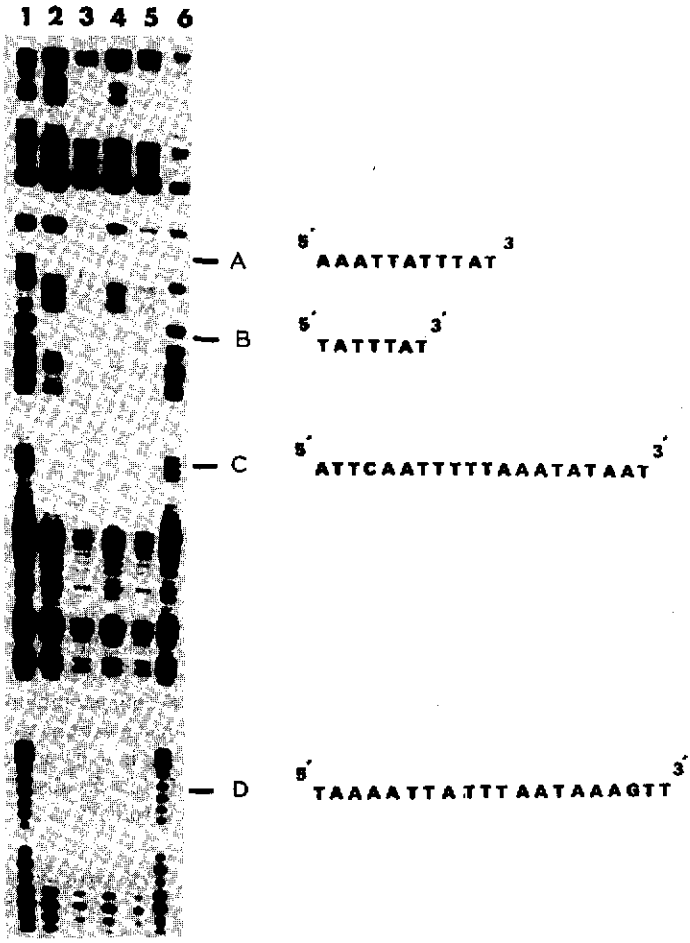

Fig. 4. DNase I footprint analyses of reversed-phase HPLC-purified HMG-I binding sites in the 3'-untranslated region of bIL-2 cDNA [102]. Lanes 1 and 6 show control DNase I digestion patterns in the absence of HMG-I. Lanes 2–5 show the DNase digestion patterns in the presence of increasing amounts of HMG-I (25, 50, 75 and 100 ng). Four protected sites within the 3'-untranslated tail region of bIL-2 are denoted A, B, C and D. The DNA sequences protected by HMG-I (shown at the right) were deduced by Sanger dideoxy sequencing.

radiolabeled electrophoretic band patterns are shown in Fig. 3B. Lanes 3–9 show that with increasing amounts of added HMG-I, slower migrating radiolabeled bands containing specific DNA–HMG-I protein complexes begin to appear. These results suggested that although HMG-I had been isolated under denaturing conditions it still retained its ability to form specific DNA–protein complexes [102].

To localize the specific HMG-I binding sites on the bIL-2 cDNA, DNase I footprinting experiments were performed. Elton and co-workers [102,105] radiolabeled the 5'-end of a 295-bp DNA fragment corresponding to the 3'-untranslated tail region of the bIL-2 cDNA clone with ^{32}P and then partially digested the fragment with DNase I in the presence or absence of purified HMG-I protein. Fig. 4, lanes 1 and 6, shows the DNase I digestion pattern obtained in

the absence of protein. Lanes 2–5 show the DNase I digestion patterns obtained when increasing amounts of HMG-I were bound to the DNA prior to nuclease addition. It is obvious from these results that HMG-I protected at least four (labeled A–D) regions of DNA in the 3′-untranslated tail region of bIL-2 from cleavage by DNase I. By performing Sanger dideoxy sequencing reactions [104] in parallel with the DNase I footprinting experiments, Elton and co-workers [102,105] were able to determine the DNA sequences to which the HMG-I bound in regions A–D (Fig. 4). Their sequencing results indicated that regions A–D all consisted of long stretches of A-T-rich DNA. Thus, they concluded that RP-HPLC-purified HMG-I, although it had been denatured, still retained its native ability to specifically bind to A-T-rich stretches of DNA in vitro.

Although the in vivo function of HMG-I is not yet known, recent reports have suggested that A-T-rich DNA sequences may be sites of attachment of genes to the nuclear scaffold or matrix [47]. Our laboratory has also shown that the bIL-2 cDNA 3′-untranslated region is associated with the nuclear matrix [106]. It is possible that this nuclear scaffold binding might be mediated by HMG-I since there is some evidence that HMG-I may be a component of the nuclear matrix [95, 102]. However, we have also noted [106] that HMG-I binding site D (Fig. 4) contains at least two Topo-II consensus cleavage sites (see Section 2.3) [44–47] which, likewise, might be involved with such nuclear matrix binding.

Another possibly important observation made by Elton and co-workers [78,102,105] is that while HMG-I specifically bound to A-T-rich sequences in the 3′-untranslated tail region of bIL-2, it did not bind to similar A-T-rich sequences present in the protein coding region of the bIL-2 cDNA, indicating that the RP-HPLC-purified HMG-I still retained the ability to make subtle distinction between different regions of DNA in vitro. These results are similar to those obtained by Udvardy et al. [107] concerning Topo-II cleavage sites. These workers found that Topo-II cleavage sites were typically found in non-transcribed spacer segments close to the 5′- and 3′-boundaries of genes and few, if any, cleavage sites were found in the coding sequences of genes. Taken together, the results suggest that HMG-I, like Topo-II, may play a structural role in the establishment, maintenance and/or regulation of chromatin loops or domains inside the nucleus. Clearly, investigation of the in vivo function(s) of HMG-I will be greatly facilited by the ability to rapidly isolate homogeneous preparations of the protein by RP-HPLC.

3. ACKNOWLEDGEMENTS

We wish to thank Drs. Ken Johnson and Rob Kay for helpful discussions and criticisms during the preparation of this manuscript and for reviewing the final product. We also thank Dr. Kotty Postle for her patience in allowing us to use her word processor and laser printer. This work was supported by NSF Grant No. DCB-8602622.

4. SUMMARY

Numerous chromatographic procedures have recently been developed to purify to near homogeneity various eukaryotic non-histone chromatin proteins that recognize and bind to specific sequences of DNA. In this brief review we have discussed a number of these different methods that we feel are important and probably represent the starting points for much of the future research work in this area. We view this coverage as being only an introduction, however, and strongly recommend that the reader consult the original papers for details of methods and protocols. We, nevertheless, hope that the information presented here will be of some assistance to those researchers and students who wish to become acquainted with the latest developments in this rapidly advancing field of chromatography.

Although it is evident from what has been presented that the purification of each DNA sequence-specific non-histone chromatin protein initially starts with common or classical isolation and fractionation procedures, the final (and often crucial) steps of enrichment and purification often involve distinctive or unique procedures for each individual protein of interest. In many cases these final steps involve new techniques such as DNA sequence-specific bioaffinity and photoaffinity chromatography which not only ensure the isolation of specific protein species from complex mixtures but also result in a tremendous enrichment for nuclear proteins that are often present in the nucleus in extremely low concentrations. Furthermore, the entire process of protein purification has been remarkedly facilitated with the advent of high-performance liquid chromatographic and fast protein liquid chromatographic techniques which now allow for the very rapid separation and purification of proteins in a matter of minutes from mixtures that in the recent past would have required hours or days to purify. Thus, separation and purification techniques are now available that set the stage for the rapid isolation of rare, DNA sequence-specific, NHC nuclear proteins from almost any cellular source. It is therefore reasonable to anticipate that in the near future there will be major advances made in our understanding of the specific nuclear proteins that regulate gene expression in eukaryotic cells.

REFERENCES

1 P.J.G. Butler, CRC Crit. Rev. Biochem., 15 (1983) 57.
2 R. Reeves, Biochim. Biophys. Acta, 782 (1984) 343.
3 I.L. Cartwright, M. Keene, G. Howard, A. Abmayr, G. Fleischmann, K. Lowenhaupt and S.C.R. Elgin, CRC Crit. Rev. Biochem., 13 (1982) 1.
4 I. Bekhor (Editor), Progress in Nonhistone Protein Research, Vol. 1, CRC Press, Boca Raton, FL, 1984.
5 I. Bekhor (Editor), Progress in Nonhistone Protein Research, Vol. 2, CRC Press, Boca Raton, FL, 1984.
6 L.S. Hnilica (Editor), Chromosomal Nonhistone Proteins, Vol. 1, CRC Press, Boca Raton, FL, 1983.
7 L.S. Hnilica (Editor), Chromosomal Nonhistone Proteins, Vol. 2, CRC Press, Boca Raton, FL, 1983.

8 L.S. Hnilica (Editor), Chromosomal Nonhistone Proteins, Vol. 3, CRC Press, Boca Raton, FL, 1983.
9 M. Briggs, J. Kadonaga, S. Bell and R. Tjian, Science, 234 (1986) 47.
10 T. Goto, P. Laipis and J.C. Wang, J. Biol. Chem., 259 (1984) 10422.
11 D.R. Engelke, S.-Y. Ng, B.S. Shastry and R.G Roeder, Cell, 19 (1980) 717.
12 J.D. Dignam, P.l. Martin, B. Shastry and R.G. Roeder, Methods. Enzymol., 101 (1983) 582.
13 O. Wrange, J. Carlstedt-Duke and J.-A. Gustafsson, J. Biol. Chem., 254 (1979) 9284.
14 O.Wrange, S. Okret, M. Radojcic, J. Carlstedt-Duke and J-A. Gustafsson, J. Biol. Chem., 259 (1984) 4534.
15 T.S. Elton and R. Reeves, Anal. Biochem., 144 (1985) 403.
16 W.S. Dynan and R. Tjian, Nature, 316 (1985) 774.
17 S. McKnight and R. Tjian, Cell, 46 (1986) 795.
18 A.B. Lassar, P.L. Martin and R.G. Roeder, Science, 222 (1983) 740.
19 D.F. Bogenhagen, S. Sakonju and D.D. Brown, Cell, 19 (1980) 27.
20 S. Sakonju, D.D. Brown, D.R. Engelke, S.-Y. Ng, B.S. Shastry and R.G. Roeder, Cell, 23 (1981) 665.
21 D.D. Brown, Cell, 37 (1984) 359.
22 J.J. Bieker, P. Martin and R.G. Roeder, Cell, 40 (1985) 119.
23 B. Honda and R.G. Roeder, Cell, 23 (1980) 119.
24 M. Wegnez and H. Denis, Biochimie, 55 (1973) 1129.
25 J. Miller, A.D. McLachlan and A. Klug, EMBO J., 4 (1985) 1609.
26 A.M. Ginsberg, B.O. King and R.G. Roeder, Cell, 39 (1984) 479.
27 U.B. Rosenberg, C. Schroder, A. Preiss, A. Kienlin, S. Cote, I. Riede and H. Jackle, Nature, 319 (1986) 336.
28 J. Berg, Science, 232 (1986) 485.
29 H.R.B. Pelham and D.D. Brown, Proc. Natl. Acad. Sci. U.S.A., 77 (1980) 4170.
30 J. Kadonaga and R. Tjian, Proc. Natl. Acad. Sci. U.S.A., 83 (1986) 5889.
31 J. Ladpmaga, K. Jones and R. Tjian, Trends Biochem. Sci., 11 (1986) 20.
32 K. Jones, J. Kadonaga, P. Luciw and R. Tjian, Science, 232 (1986) 755.
33 P.J. Rosenfeld and T.J. Kelly, J. Biol. Chem., 261 (1986) 1398.
34 L.F. Liu, CRC Crit. Rev. Biochem., 54 (1983) 1.
35 J.C. Wang, Ann. Rev. Biochem., 54 (1985) 665.
36 M. Sander and T. Hsieh, Nucleic Acids Res., 13 (1985) 1054.
37 J.M. Kaguri and A. Kornberg, Cell, 38 (1984) 511.
38 M.M.S. Heck and W.C. Earnshaw, J. Cell Biol., 103 (1986) 2569.
39 A.N. Luchnik, V. Bakayev, I. Zbarsky and G.P. Georgiev, EMBO J., 1 (1982) 1353.
40 B. Singh and R.S. Gupta, Cancer Res., 43 (1983) 577.
41 S. DiNardo, K. Voelkel and R. Sternglanz, Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 266.
42 A.W. Murray, N. Schultes and J. Szostak, Cell, 45 (1986) 529.
43 T. Uemura and M. Yanagida, EMBO J., 5 (1986) 1003.
44 W.C. Earnshaw and M. Heck, J. Cell Biol., 100 (1985) 1716.
45 W.C. Earnshaw, B. Halligan, C. Cooke, M. Heck and L. Liu, J. Cell Biol., 100 (1985) 1706.
46 M. Berrios, N. Osheroff and P. Fisher, Proc. Natl. Acad. Sci. U.S.A., 82 (1985) 4142.
47 S. Gasser and U.K. Laemmli, EMBO J., 5 (1986) 511.
48 E.R. Shelton, N. Osheroff and D.L. Brutlag, J. Biol. Chem., 258 (1983) 9530.
49 B.D.Halligan, K.A. Edwards and L.F. Liu, J. Biol. Chem., 260 (1985) 2475.
50 A. Andah, K. Kshii, K. Nagasawa and N. Seno, in I. Bekhor (Editor), Chromosomal Nonhistone Proteins, Vol. 2, CRC Press, Boca Raton, FL, 1983, p. 151.
51 T. Goto and J.C. Wang, J. Biol. Chem., 257 (1982) 5866.
52 H. Eriksson and J.-A. Gustafsson (Editors), Steroid Hormone Receptors: Structure and Function, Elsevier/North Holland, Amsterdam, 1983.
53 J.N. Anderson, in R.F. Goldberger and K.R. Yamamoto (Editors), Biological Regulation and Development, Vol. 3B, Plenum Press, New York, 1984, p. 169.
54 D.D. Moore, A.R. Marks, D. Buckley, G. Kapler, F. Payvar and H.M. Goodman, Proc. Natl. Acad. Sci. U.S.A., 82 (1985) 699.

55 K. Yamomoto, Ann. Rev. Genet., 19 (1985) 209.
56 C. Scheidereit, S. Geisse, H.M. Westphal and M Beato, Nature, 304 (1983) 749.
57 P. Grandics, A. Miller, T. Schmidt, D. Mittman and G. Litwack, J. Biol. Chem., 259 (1984) 3173.
58 T.C. Spelsberg, B. Grosse, B. Littlefield, H. Toyoda and R. Seelke, Biochemistry, 23 (1984) 5103.
59 J.-M. Renoir, J. Mester, T. Buchou, M.-G. Catelli, P. Tuohima, N. Binar, I. Joab, C. Radanyi and E.-T. Baulieu, Biochem. J., 217 (1984) 685.
60 U. Gehring and A. Hotz, Biochemistry, 22 (1983) 4013.
61 S. Okret, A-C. Wikstrom, O. Wrange, B. Anderson and J-A. Gustafsson, Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 1609.
62 B. Gametchu and R. Harrison, Endocrinology, 114 (1984) 274.
63 R. Miesfeld, S. Okret, A.-C. Wikstrom, O. Wrange, J.-A. Gustafsson and K. Yamamoto, Nature, 312 (1984) 779.
64 J.M. Bishop, Nature, 321 (1986) 112.
65 S.M. Hollenberg, C. Weinberger, E.S. Ong, G. Cerelli, A. Oro, R. Lebo, E.B. Thompson, G. Rosenfeld and R.M. Evans, Nature, 318 (1985) 635.
66 G.L. Greene, P. Gilna, M. Waterfield, A. Baker, Y. Hort and J. Shine, Science, 231 (1986) 1150.
67 S. Green, P. Walter, V. Kumar, A. Krust, J.-M. Bornert, P. Argos and P. Chambon, Nature, 320 (1986) 134.
68 C. Weinberger, S.M. Hollenberg, M.G. Rosenfeld and R.M. Evans, Nature, 318 (1985) 670.
69 M. Biernbaumer, W.T. Schrader and B.W. O'Malley, J. Biol. Chem., 255 (1983) 1637.
70 H. Eisen, R. Schleenbaker and S.S. Simons, J. Biol. Chem., 256 (1981) 12920.
71 J.A. Katzenellenbogen, K. Carlson, D. Heiman, D.W. Robertson, L. Wei and B.S. Katzenellenbogen, J. Biol. Chem., 258 (1983) 3487.
72 G.H. Goodwin and E.W. Johns, Eur. J. Biochem., 40 (1973) 215.
73 R.H. Nicolas, in G.H. Goodwin and E.W. Johns (Editor), The HMG Chromosomal Proteins, Academic Press, New York, 1982, pp. 41-68.
74 T. Lund, J. Holtlund, M. Fredrickson and S.G. Laland, FEBS Lett., 152 (1983) 163.
75 F. Strauss and A. Varshavsky, Cell, 37 (1984) 889.
76 T. Lund, J. Hotlund and S.G. Laland, FEBS Lett., 180 (1980) 275.
77 G.H. Goodwin, P.N. Cockerill, S. Kellam and C.A. Wright, Eur. J. Biochem., 149 (1985) 47.
78 T.S. Elton and R. Reeves, Anal. Biochem,. 157 (1986) 53.
79 B. Abercrombie, G.C. Kneale, C. Crane-Robinson, E.M. Bradbury, G.H. Goodwin and E.W. Johns, Eur. J. Biochem., 84 (1978) 173.
80 P.D. Cary, D. King, C. Crane-Robinson, E.M. Bradbury, A. Rabbani, G.H. Goodwin and E.W. Johns, Eur. J. Biochem., 112 (1980) 577.
81 K. Javaherian and S. Amini, Biochim. Biophys. Acta, 478 (1977) 295.
82 S.H. Yu and T.G. Spring, Biochim. Biophys. Acta, 492 (1977) 20.
83 P.D. Cary, K. Shooter, G.H. Goodwin, E.W. Johns, J. Olayemi, P. Hartman and E.M. Bradbury, Biochem. J., 183 (1979) 657.
84 J. Bernues, E. Querol, P. Martinez, A. Barris, E. Espel and J. Lloberas, J. Biol. Chem., 258 (1983) 11020.
85 C.G. Mathew, G.H. Goodwin and E.W. Johns, Nucleic Acids Res., 6 (1979) 167.
86 C.S.M. Tohourdin, N. Neihart, I. Isenberg and M. Bustin, Biochemistry, 20 (1981) 910.
87 V.V. Bakayev, T. Bakayeva, V. Schmatchenko and G.P. Georgiev, Eur. J. Biochem., 91 (1978) 291.
89 P. Yau, B. Imai, A. Thorne, G.H. Goodwin and E.M. Bradbury, Nucleic Acids Res., 11 (1983) 2651.
89 G. Sandeen, W. Wood and G. Felsenfeld, Nucleic Acids Res., 10 (1980) 5695.
90 P.J. Isackson, J. Fishback, D. Bidney and G.R. Reeck, J. Biol. Chem., 254 (1979) 5569.
91 P.J. Isackson and G.R. Reeck, Nucleic Acids Res., 9 (1981) 3779.
92 H. Hamada and M. Bustin, Biochemistry, 24 (1985) 1428.
93 P.N. Cockerill and G.H. Goodwin, Biochem. Biophys. Res. Commun., 112 (1983) 547.

94 M. Bustin and N. Soares, Biochem. Biophys. Res. Commun., 133 (1985) 633.
95 M.J. Salomon, F. Strauss and A. Varshavsky, Proc. Natl. Acad. Sci. U.S.A., 83 (1986) 1276.
96 F. Strauss and A. Varshavsky, Cell, 37 (1984) 889.
97 L. Benedek, S. Dong and B.L. Karger, J. Chromatogr., 317 (1984) 227.
98 R.H. Ingraham, S. Lau, A. Taneja and R. Hodges, J. Chromatogr., 327 (1985) 77.
99 S.Y.M. Lau, A.K. Taneja and R.S. Hodges, J. Chromatogr., 317 (1984) 129.
100 J. Luiken, R. van der Zee and G. Welling, J. Chromatogr., 284 (1984) 482.
101 G. Pruijn, W. Van Driel and P. Van Der Vliet, Nature, 322 (1986) 656.
102 T.S. Elton, M.S. Nissen and R. Reeves, Biochem. Biophys. Res. Commun., 143 (1987) 260.
103 R. Reeves, G. Spies, M. Nissen, C. Buck, A. Weinberg, P. Barr, N. Magnuson and J. Magnuson, Proc. Natl. Acad. Sci. U.S.A., 83 (1986) 3228.
104 F. Sanger, S. Nicklen and A. Coulson, Proc. Natl. Acad. Sci. U.S.A., 74 (1977) 5463.
105 R. Reeves, T.S. Elton, M. Nissen, D. Lehn and K. Johnson, Proc. Natl. Acad. Sci. U.S.A., (1987) in press.
106 R. Reeves, unpublished results.
107 A. Udvardy, P. Schedl, M. Sander and T.-S. Hsieh, Cell, 40 (1985) 933.