

NON-HISTONE CHROMATIN PROTEINS FROM AN INSECT CELL LINE: PREFERENTIAL AFFINITY FOR SINGLE-STRAND DNA OF A HIGH MOLECULAR WEIGHT PROTEIN*

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Abstract—Nuclei and chromatin were isolated from cell line (TN-368) derived from the ovary of the cabbage looper, *Trichoplusia ni*. The chromatin proteins were examined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. The non-histone proteins extracted with 0.35 M NaCl from *T. ni* chromatin were subjected to sequential chromatography on columns containing immobilized double-strand and single-strand DNA. A protein with an apparent mol. wt of 7×10^4 was found to bind preferentially to single-strand DNA.

Key Word Index: Chromatin, single-strand DNA, *Trichoplusia ni*, non-histone

INTRODUCTION

CHROMATIN is the chromosomal material of interphase eukaryotic cells. It consists of DNA, two classes of protein (histone and non-histone), and a small amount of RNA (ELGIN and WEINTRAUB, 1975). These components have mass ratios (setting DNA = 1) of 1/0.79/1.19/0.06, respectively, in chromatin obtained from *Drosophila melanogaster* embryos (ELGIN and HOOD, 1973).

The histones have been particularly well studied. They are the major structural proteins in chromatin of all eukaryotes and are highly conserved from an evolutionary standpoint (ISENBERG, 1979). The non-histone proteins, on the other hand, are a much more heterogeneous group (ELGIN and WEINTRAUB, 1975). Except for proteins with known enzymatic activities, the biological function of no individual non-histone protein is known with certainty. Some are believed to regulate gene expression (ELGIN and WEINTRAUB, 1975; STEIN and STEIN, 1976; DASTUGUE and CREPIN, 1979). Among the non-histone proteins, particular attention has been focused in recent years on the so-called HMG (High Mobility Group) proteins. That small set of proteins was first isolated from calf thymus by GOODWIN *et al.* (1973), and homologous proteins have now been isolated from several other vertebrates. A principal defining characteristic of the HMG proteins is their solubility in 2% trichloroacetic acid (GOODWIN *et al.*, 1973).

In this paper we report isolation of chromatin from nuclei of an ovarian cell line derived from the cabbage looper moth, *Trichoplusia ni* (Hubner). We have subjected non-histone chromatin proteins to sequential chromatography on columns containing immobilized double-strand and single-strand DNA. By that approach, one major non-histone was found to exhibit preferential affinity for single-strand DNA. That result is compared to those obtained previously in similar studies of non-histone chromatin proteins from vertebrates.

MATERIALS AND METHODS

T. ni cells

Trichoplusia ni (TN-368) cells (HINK, 1972) were grown in monolayer culture in Grace's TC media (180 ml) supplemented with TC yeastolate (0.6 g)/lactalbumin hydrolysate (0.6 g)/gentamicin (10 mg) and 0.1 M *N,N*-bis(2-aminoethane sulphonic acid) (BES) as recommended by KOVAL (1978). The pH of the medium was adjusted to 6.2 with 1 M KOH before filter sterilization. Sixteen ml of foetal bovine serum (Gibco) was added just before use. Flasks had a growth area of 150 cm².

Nuclei and chromatin preparation

T. ni cells were harvested from growth medium by centrifugation at 500 *g* and 4°C for 20 min. The pellet was suspended in buffer A (0.25 M sucrose, 5 mM CaCl₂, 50 mM Tris, pH 7.5), homogenized, and collected by centrifugation at 5000 *g* and 4°C for 10 min. This step was repeated to remove growth medium. The cells were lysed by homogenization in buffer A containing 1% (w/v) Triton X-100. The nuclei were collected by centrifugation at 5000 *g* and 4°C for 10 min, washed once in buffer A, and stored frozen at -20°C.

Chromatin was prepared from fresh or frozen nuclei by homogenizing the nuclear pellet in 1 mM Tris-HCl, 1 mM EDTA (pH 7.5). The homogenate was allowed to sit on ice for 10 min before the pellet was collected by centrifugation at 5000 *g* and 4°C for 10 min. This was repeated twice to allow further swelling of the chromatin.

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Sucrose gradient centrifugation

Solid sucrose and SDS were added to 2 ml of insect chromatin to give 10% (w/v) sucrose and 2.5% (w/v) SDS. Chromatin was then layered over a 9.5 ml sucrose gradient which contained 2.5% SDS. The gradient (in 1.4 × 8.8 cm cellulose nitrate tubes) was 10–40% (w/v) sucrose over 0.5 ml of 60% sucrose, 2.5% SDS. Samples were subjected to centrifugation in a Spinco SW-41 rotor at 240,000 *g* and 4°C for 6 hr (BIDNEY and REECK, 1978a). Fractions (0.7 ml) were collected by pumping 80% sucrose, 2.5% SDS into the bottom of the tubes.

Determination of protein, DNA and RNA concentrations

Protein concentrations were determined by the method of BRADFORD (1976) using Coomassie brilliant blue G-250 (Eastman). A standard curve was generated with ovalbumin (Sigma) using absorbance at 595 nm and was linear from 0 to 30 μ g per ml.

DNA concentrations were routinely estimated spectrophotometrically at 260 nm in 1% (w/v) SDS, assuming $A_{260}^{1\%} = 21$. To determine the DNA content of nuclei, a diphenylamine assay was used (BURTON, 1956).

RNA concentrations were determined using the modified orcinol assay of ALMOG and SHIREY (1978). A standard curve was generated at 500 nm using 0.50 μ g of yeast RNA (Sigma) per ml.

Electrophoresis

Polyacrylamide electrophoresis was conducted in the presence of SDS according to the procedure of LAEMMLI (1970) except at half the bisacrylamide concentration. Electrophoresis was carried out in slabs (0.2 × 14 × 14 cm) for 4 hr at 25 mA and 4°C. Gels were stained overnight in 0.25% Coomassie brilliant blue R-250 (Sigma), 50% (v/v) ethanol, 10% (v/v) acetic acid. The gels were destained in 25% ethanol, 10% acetic acid.

Samples were concentrated for electrophoresis by precipitation with 20% (w/v) TCA. Solid TCA was added to achieve the final concentration of 20%. After approx. 1 hr at 4°C, the samples were centrifuged at 5000 *g* for 5 min and the supernatant discarded. The pellet was washed twice with 5 ml of acetone to remove any remaining TCA and centrifuged at 5000 *g* after each wash. The pellet was then suspended in electrophoresis buffer: 10% (v/v) glycerol, 60 mM Tris-phosphate, pH 6.7, 1% (v/v) 2-mercaptoethanol, 1% (w/v) SDS.

Preparation of double-strand and single-strand DNA columns

Calf thymus DNA (Sigma) was purified by extraction with chloroform/isoamyl alcohol (25:1, v/v). Purified DNA was ethanol precipitated and dissolved in 100 ml of 0.9% NaCl, 10 mM Tris-HCl (pH 7.5). It was sonicated by three

10-sec bursts at a setting of 115 W using a Branson Sonifier equipped with a microtip. The sonicated DNA was dialyzed against 10 mM potassium phosphate (pH 8.5). Denatured (single-strand) DNA was obtained by placing a 50 ml portion of double-strand DNA in boiling water for 30 min, followed by rapid cooling in an ice bath.

Double-strand DNA was adsorbed to cellulose as described by ALBERTS and HERRICK (1971) and single-strand DNA was coupled to CNBr activated Sepharose 4B (Sigma) as described in ARNDT-JOVIN *et al.* (1975). The double-strand DNA column was then equilibrated to 5% (v/v) glycerol, 10 mM NaCl, 50 mM sodium acetate (pH 4.5), 1 mM ZnSO₄. Ten units of S₁ nuclease (Calbiochem) was added to the column and slowly circulated for 24 hr to remove any single-strand DNA. The column was then rinsed with 10 mM Tris-HCl (pH 7.5) and finally 2 mM NaCl, 1 mM Tris-HCl (pH 7.5). All columns were stored at 4°C in 2 M NaCl, 1 mM Tris-HCl (pH 7.5) when not in use.

RESULTS

Chemical analyses of nuclei and chromatin

An ovarian cell line from the cabbage looper moth, *T. ni*, was grown in monolayer cultures as outlined in Methods. Approximately 10⁹ cells were obtained every two weeks. If the cells were transferred from a seed flask as they reached confluency, approx. 60 monolayer flasks were needed during the two-week period. About 10 mg of chromatin was prepared from 10⁹ cells.

DNA, RNA and protein levels were measured in nuclei and chromatin as described in Methods. The results are presented in Table 1, along with previous analyses of chromatins from *Drosophila* tissues (ELGIN and HOOD, 1973; HELMSING and VAN EUPEN, 1973). As expected, *T. ni* chromatin had lower protein/DNA and RNA/DNA ratios than *T. ni* nuclei. The protein and RNA contents of *T. ni* chromatin were higher than those of chromatins from *D. melanogaster* tissues. This may simply be a result of the mold isolation procedure used for *T. ni* chromatin [in particular the disruption of nuclei in a solution of low ionic strength (10 mM Tris-HCl, pH 7.5)].

Gel electrophoresis of chromatin proteins

Essentially all of the protein bound to DNA in chromatin can be dissociated by the detergent SDS (SHIREY and HUANG, 1969). Figure 1, Track A, shows

Table 1. DNA, RNA and protein content* and relative mass ratios† of nuclei and chromatin from *T. ni* cells

	DNA	RNA	Protein
<i>T. ni</i> nuclei from ovary	9.9 ± 0.9 (1)	5.5 ± 2.4 (0.54 ± 0.18)	57.9 ± 2.6 (6.2 ± 0.1)
<i>T. ni</i> chromatin from ovary	(1)	(0.29 ± 0.01)	(2.8 ± 0.6)
<i>D. melanogaster</i> chromatin from			
Embryo‡	(1)	(0.06)	(2.0)
Salivary gland§	(1)	(0.04)	(2.1)
Midgut§	(1)	(0.09)	(2.2)

* Picograms per nucleus ± S.E. Averaged values of single determinations using nuclei and chromatin from three separate cultures.

† Mass ratios given in parentheses. DNA = 1.

‡ From ELGIN and HOOD (1973).

§ From HELMSING and VAN EUPEN (1972).

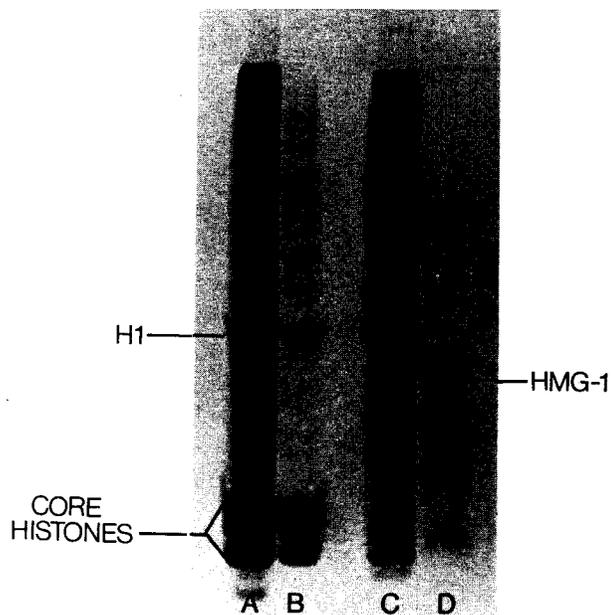


Fig. 1. Sodium dodecyl sulphate slab gel electrophoresis of *T. ni* chromatin proteins. Samples for electrophoresis were prepared as described in Methods. Track A: protein peak from 10 to 40% sucrose gradient containing 2.5% SDS. Track B: material from insect chromatin that was extracted with 0.2 M H_2SO_4 . Track C: proteins dissociated with 0.35 M NaCl. Track D: Calf thymus HMG proteins prepared according to GOODWIN *et al.* (1973). This figure was constructed by juxtaposing appropriate tracks from a single slab gel.

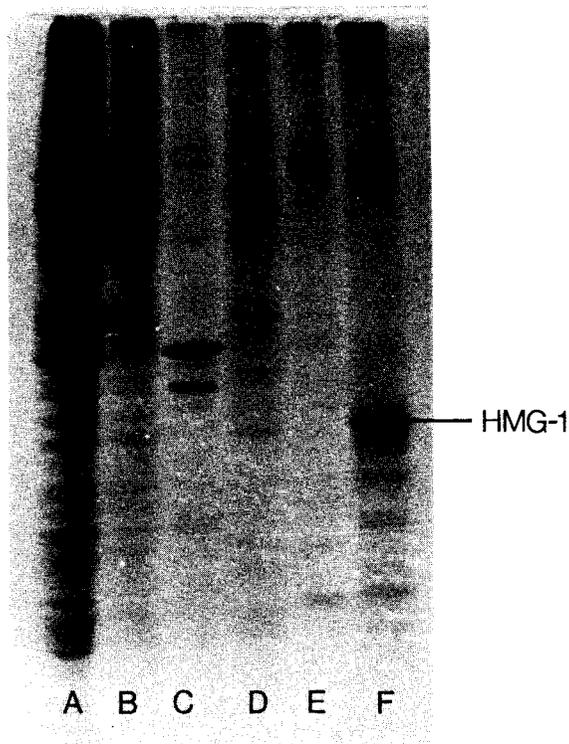


Fig. 2. Sodium dodecyl sulphate slab gel electrophoresis of *T. ni* chromatin proteins. Samples from sequential DNA chromatography of proteins dissociated from *T. ni* cell chromatin were prepared as described in Methods. Track A: proteins from chromatin dissociated by 0.35 M NaCl. Track B: material that did not bind to double-strand DNA cellulose equilibrated to 0.2 M NaCl, 1 mM Tris-HCl (pH 7.5). Track C: material bound to double-strand DNA cellulose that was subsequently eluted with 2 M NaCl. Track D: material that did not bind to single-strand DNA Sepharose. Track E: material that bound to single-strand DNA. Track F: calf thymus HMG protein preparation.

the proteins dissociated from *T. ni* chromatin by 2.5% SDS. By analogy with chromatins from other animals, including other insects (ELGIN and HOOD, 1973; HELMSING and VAN EUPEN, 1973), the most intense bands are expected to be histones. Extraction of chromatin with 0.2 M sulphuric acid yielded the histones, which are shown in Track B. These are similar electrophoretically to histones obtained from other tissue sources. No attempt was made to identify the individual core histones. Track C shows protein dissociated with 0.35 M NaCl. That salt concentration has been used to extract the high mobility group (HMG) proteins from mammalian chromatins (GOODWIN *et al.*, 1973). As can be seen by comparison to a calf thymus HMG protein preparation (Track D), there is no prominent protein in the 0.35 M NaCl extract of *T. ni* chromatin with the electrophoretic mobility of HMG-1.

Search for non-histone proteins with preferential affinity for single-strand DNA

Sequential chromatography of protein samples on double-strand and single-strand DNA columns was introduced by HERRICK and ALBERTS (1976) as a means of screening for proteins with preferential affinity for single-strand DNA. Such proteins are identified by their being retained by the single-strand DNA column after having failed to bind to the double-strand DNA column. We have successfully applied the technique to non-histone chromatin protein samples from cultured rat hepatoma cells (BIDNEY and REECK, 1978b) and from chicken erythrocytes and calf thymus (ISACKSON *et al.*, 1979).

Chromatin from insect cells, prepared as outlined in Methods, was extracted with 0.35 M NaCl (Fig. 2, Track A). Following centrifugation at 25,000 *g* and 4°C for 30 min, the extract was diluted to 0.2 M NaCl with 1 mM Tris-HCl, 1 mM EDTA (pH 7.5). The extract was then applied to double-strand DNA cellulose equilibrated to 0.2 M NaCl, 1 mM Tris-HCl (pH 7.5). The void volume material from double-strand DNA (Track B) was applied to single-strand DNA Sepharose equilibrated to the same ionic strength. Proteins that bound to single-strand DNA were eluted with 2 M NaCl/1 mM Tris-HCl (pH 7.5).

Polyacrylamide/SDS gel electrophoretic analysis of a sequential chromatography experiment with *T. ni* non-histone chromatin proteins is shown in Fig. 2. The material that was retained by the single-strand DNA column (after having failed to be retained by the double-strand DNA column) consisted of a single major polypeptide (Track E). The protein had a mobility on polyacrylamide/SDS gels close to that of bovine serum albumin ($M_r = 6.7 \times 10^4$). Protein that adsorbed to the double-strand DNA cellulose column is separated in Track C. The predominant protein is very probably histone H1, since the analogous protein from other sources is also retained by immobilized double-strand DNA at 0.2 M NaCl (ISACKSON *et al.*, 1979). Track C also contains a faint band with mobility similar to that of the major band in Track E. Thus it is within the realm of possibility that some of the major protein retained by the single-strand DNA column was also retained by the double-strand DNA column.

DISCUSSION

Many laboratories are now studying non-histone chromatin proteins from vertebrates. It should be quite useful to investigate proteins from distantly related animal species (such as insects) to determine the extent to which structures and functions of those proteins have changed over a wide range of evolution. Cultured *T. ni* ovary cells appear to be an attractive system in which to study chromatin proteins. Even with monolayer cultures, it is possible to obtain enough chromatin at sufficiently frequent intervals to allow many types of experiments to be conducted.

In this paper we have reported an initial characterization of nuclei, chromatin, and chromatin proteins from *T. ni* cells. As expected from studies of fruit fly chromatins, the *T. ni* chromatin appears to contain the standard complement of histones and many non-histone chromatin proteins. We also carried out a search for non-histone proteins from *T. ni* chromatin that bind preferentially to single-strand DNA. In earlier studies of chromatins from rat, cow and chicken (BIDNEY and REECK 1978b; ISACKSON *et al.*, 1979), a constant picture has emerged: the predominant non-histone chromatin proteins that possess preferential affinity for single-strand DNA are the higher mol. wt members of the HMG proteins. Those proteins have apparent mol. wts of $2.5-2.6 \times 10^4$. A very different result was obtained in the current study on *T. ni* chromatin proteins: a single predominant protein with approx. a three-fold larger apparent mol. wt (7×10^4) was found to bind preferentially to single-strand DNA. We conclude that *T. ni* chromatin does contain a prominent, high mol. wt single-strand DNA binding protein but does not contain a protein that is sufficiently abundant to have been detected in our experiments and that is similar in all of its properties to the high mol. wt HMG proteins of vertebrates.

The different results obtained from the application of sequential DNA affinity chromatography to extracts of chromatin from the insect cell line, on the one hand, and from vertebrate tissues, on the other, could have been due to any of several causes. It could be, for example, that a protein similar to HMG-1 occurs in insect chromatin but it is not extracted with 0.35 M NaCl. Alternatively, a homologue of HMG may occur in the 0.35 M NaCl extract of *T. ni* chromatin, but it may not exhibit preferential affinity for single-strand DNA. These and other possibilities can be evaluated only after further study.

The physiological function of the single-strand DNA binding protein from *T. ni* chromatin is, of course, completely unknown at this time. Proteins that bind preferentially to single-strand DNA have been shown to be involved in DNA replication in prokaryotic organisms, perhaps by destabilizing the DNA double helix (FREY and ALBERTS, 1970). The possibility that high mol. wt HMG proteins serve such a role in vertebrates has been raised previously (BIDNEY and REECK, 1978b) and a similar consideration should be given to the *T. ni* protein described in this report. Regardless of the outcome of studies of the *T. ni* protein's function, the results presented here demonstrate a distinct difference in non-histone proteins in *T. ni* chromatin and in vertebrate chromatins and illustrate the potential usefulness of studying non-histone chromatin proteins in insects.

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