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DNA FRAGMENTS OF 300 BASE PAIRS RELEASED FROM METAPHASE CHROMOSOMES BY DIGESTION WITH DEOXYRIBONUCLEASE I

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SUMMARY: DNase I digestion of metaphase chromosomes, that have been extensively digested with Hae III, further released chromosomal DNA and proteins; 3.3% and 10.8% of the chromosomal DNA and proteins, respectively, remained insoluble. However, digestion of chromosomes first with DNase I followed by Hae III caused most of the proteins to remain in the insoluble fraction. DNase I released DNA fragments of 300 base pairs long which were not released by Hae III digestion. These DNA fragments may be protected by protein components from further fragmentation by DNase I.

Electron microscopic observations of Paulson and Laemmli (1) have described a core-like scaffolding structure of metaphase chromosomes after treatment of chromosomes with agents that released histones from the DNA. The chromosome scaffold is not sensitive to DNase and is essentially composed of nonhistone proteins (2), from which loops of DNA at least 30-90 kbp long appear to emanate (1). These findings prompted many investigators to examine the scaffold DNA (3-6); however, less attention has paid to the DNA or protein components released into the soluble fraction following various treatments, such as nuclease digestion, of metaphase chromosomes. We describe here the differential susceptibilities of chromosomes to a serial digestion with Hae III and DNase I and the appearence of DNA fragments of 300 bp long after digestion of chromosomes with DNase I.

MATERIALS AND METHODS

Chinese hamster V79 cells were labeled with 0.05 μCi/ml [^{14}C]thymidine (53.2 mCi/mmol; New England Nuclear) and 0.5 μCi/ml [^{3}H]leucine (52 Ci/mmol;

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Abbreviations: bp, base pairs; PIPES, piperazine-N,N'-bis(2-ethane-sulfonic acid); NP-40, Nonidet P40; EDTA, ethylenediaminetetra-acetic acid disodium salt; PMSF, phenylmethylsulfonyl fluoride.

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Amersham) for 15 h. To the cultures 0.06 μg/ml colcemid was added 6 h before harvesting the mitotic cells by shaking the monolayers (7). The mitotic cells were homogenized in 1 M hexylene glycol (2-methyl-2,4-pentanediol), 0.5 mM $CaCl_2$, and 0.1 mM PIPES buffer at pH 6.8 and centrifuged at 3,000 rpm for 10 min (8). Chromosomes were purified by centrifugation at 250g for 15 min through a step sucrose gradient consisting of a mixture of equal volumes of the homogenizing buffer containing 0.1% NP-40 and 1.2 M sucrose, overlaid by a chromosome suspension in 0.25 M sucrose and 0.05% NP-40. Purified chromosomes were resuspended at 1 mg/ml in 10 mM Tris-HCl, pH 7.5, 7 mM $MgCl_2$, 60 mM NaCl, and 7 mM 2-mercaptoethanol for digestion with 1,000 units/ml Hae III or in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, and 1 mM $CaCl_2$ (9) for digestion with 1,000 units/ml DNase I (Cooper Biomedial) at 37°C for 16 h. Nuclease digestions were terminated by adding EDTA to a final concentration of 5 mM followed by centrifugation. Radioactivity in soluble and insoluble fractions was determined in ACS II (Amersham). DNA in soluble or residual insoluble fractions was ethanol-precipitated or extracted with chloroform-isoamyl alcohol (24:1), respectively, to load on 2% agarose gels. After gel electrophoresis gels were stained with ethidium bromide. Gel filtration was carried out with use of Sephacryl S-200 (Pharmacia) column (1 x 27 cm) equilibrated with 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, and 1 mM PMSF.

RESULTS

Following extensive digestion of chromosomes with Hae III, the chromosomes were then successively digested with DNase I (2,000 units/ml) at 37°C for increasing periods of time. The relative amount of both [^{14}C]DNA and [^{3}H]protein in the insoluble fraction decreased rapidly during digestion with DNase I, and the release of DNA and protein was completed 4 h after incubation (Fig. 1). The initial digestion with Hae III solubilized about 20% of either DNA or proteins from the chromosomes, and successive digestions with DNase I for

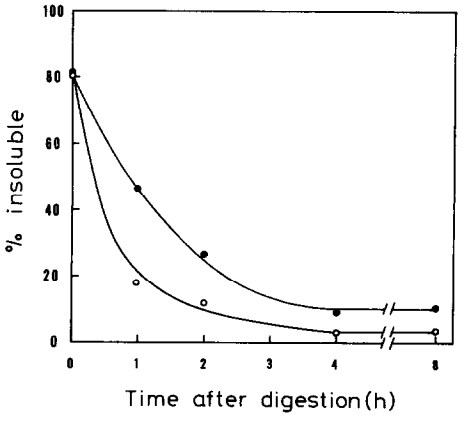

Fig. 1: Decrease in the relative amount of insoluble [^{14}C]DNA (-o-) and [^{3}H]-protein (-●-) during the second cycle of DNase I digestion of chromosomes that had been previously digested with Hae III.

TABLE 1

Relative amount of radioactivity solubilized and remaining after nuclease digestion of metaphase chromosomes

Nuclease digestion	% Solubilized		% Remaining
Fraction	1st digestion	2nd digestion	
Hae III-DNase I			
[^{14}C]DNA	19.9	76.8	3.3
[^{3}H]protein	18.9	70.3	10.8
DNase I-Hae III			
[^{14}C]DNA	94.4	4.1	1.5
[^{3}H]protein	18.5	7.7	73.8

8 h solubilized most of the remaining DNA and proteins, leaving 3.3% of the DNA and 10.8% of the proteins in the insoluble fraction (Table 1).

Conversely, when chromosomes were digested first with DNase I, most of the DNA was solubilized, whereas the amount of proteins solubilized was comparable to that with Hae III treatment (Fig. 2). Digestion of the chromosomes with Hae III following DNase I treatment decreased the insoluble DNA and protein fractions by 4% and 8%, respectively. The relative amount of DNA remaining insoluble was comparable to that after digestion with Hae III followed by

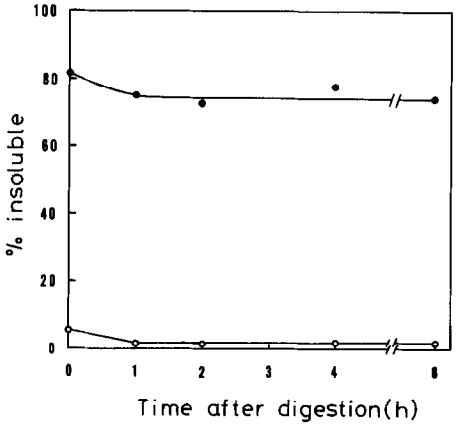

Fig. 2: Decrease in the relative amount of insoluble [^{14}C]DNA (-o-) and [^{3}H]-protein (-●-) during the second cycle of Hae III digestion of chromosomes that had been previously digested with DNase I.

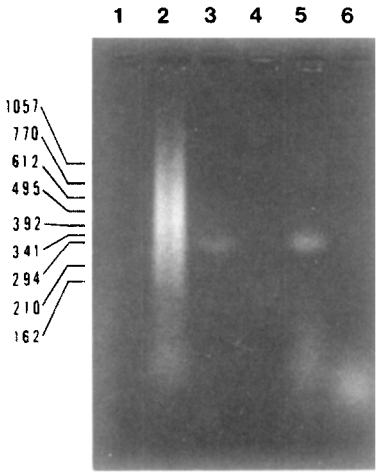

Fig. 3: Agarose gel electrophoresis of DNA fragments solubilized or remaining insoluble after digestion of chromosomes with Hae III or DNase I. 1: Hae III soluble fraction; 2: Hae III insoluble fraction; 3: Hae III insoluble-DNase I soluble fraction; 4: Hae III insoluble-DNase I insoluble fraction; 5: DNase I soluble fraction; 6: DNase I insoluble fraction.

DNase I; however, more than 70% of the total chromosomal proteins remained in the insoluble fraction after digestion with DNase I followed by Hae III treatment (Table 1).

After extensive digestion of chromosomes with Hae III or DNase I, DNA released into the soluble fraction and DNA remaining in the insoluble fraction were loaded onto agarose gels. Fig. 3 shows that Hae III releases no detectable DNA fragments to the soluble fraction and DNA fragments of heterogeneous size remain in the insoluble fraction (lanes 1 and 2). Contrary to Hae III treatment, homogeneous DNA fragments of 300 bp were released by DNase I digestion (lane 5). Long DNA fragments were also released by the second cycle of digestion with DNase I of chromosomes which have been previously digested with Hae III (lane 3). The apparent molecular size of the latter DNA fragments was 270 bp.

To examine whether the 300 bp DNA fragments released by DNase I were protected from further fragmentation, the soluble fraction obtained by centrifugation after DNase I digestion, which have been labeled with [^{14}C]thymidine and [^{3}H]leucine, was filtered through a Sephacryl S-200 column. Fig. 4 shows that the [^{14}C]thymidine-labeled fractions, eluted between rabbit muscle aldol-

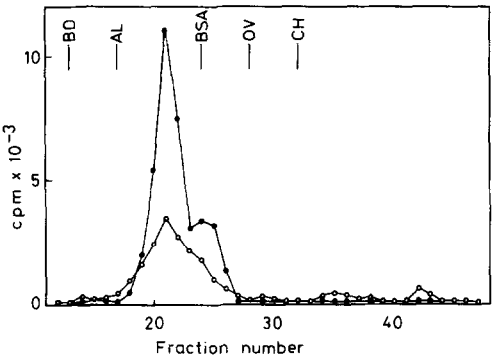

Fig. 4: Gel filtration on Sephacryl S-200 column of soluble fraction obtained by digestion with DNase I of chromosomes that have been labeled with [^{14}C]-thymidine (-●-) and [^{3}H]leucine (-o-). Locations of blue dextran 2,000 (BD), rabbit muscle aldolase (AL), bovine serum albumin (BSA), ovalbumin (OV), and chymotrypsinogen A (CH) are indicated in the figure.

ase (158 kDa) and bovine serum albumin (67 kDa), were also labeled with [^{3}H]-leucine. The content of DNA and protein in the peak fraction was 16.8 µg/ml and 55.0 µg/ml, respectively.

DISCUSSION

The present results demonstrate that digestion of metaphase chromosomes with DNase I following Hae III treatment released further chromosomal proteins and DNA into the soluble fraction (Fig. 1), whereas digestion with Hae III following DNase I digestion failed to solubilize further proteins, with more than 70% of the total proteins remaining in the insoluble fraction (Fig. 2). This difference in susceptibility of chromosomal proteins to DNase I before and after Hae III digestion may be due to an alteration in the chromosome organization by the initial digestion with Hae III so that DNase I was able to solubilize further chromosomal proteins together with DNA. Alternatively, the initial DNase I digestion makes chromosomal proteins resistant to further release by Hae III. While chromosomes are not dehistonized here, the residual chromosomes after DNase I digestion seem to be similar to the chromosome scaffold which is not sensitive to DNase I and is essentially composed of nonhistone proteins (2). The possibility that solubilization of most of the chromosomal DNA by DNase I makes the residual chromosomal proteins to be resistant to further solubilization by Hae III can not eliminated. If this

possibility is correct, DNA fragments of 300 bp found here may retain the chromosome structure from the excess aggregation of chromosomal proteins.

Micrococcal nuclease digestion of metaphase chromosomes gave 140 bp DNA fragments (4), suggesting the existance of nucleosome structures in metaphase chromosomes (10-14). Long DNA fragments, such as those found here, have been detected for yeast, HeLa and chicken erythrocyte nuclei following digestion with DNase I, and it was suggested the regions occurred in the chromatin in which nucleosome cores were closely packed (140-160 bp x n) (15). The present results suggest the possible occurrence in metaphase chromosomes of closely packed structures, involving two nucleosomes, which are protected by protein from further fragmentation by DNase I. The apparent molecular weight for the protein component accompanying the DNA fragments was estimated to be approximately 59 kDa by measurement of the content of DNA and protein in the peak fraction shown in Fig. 4; however, we have not determined the size precisely as yet.

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