ACCESSIBILITY OF METAPHASE CHROMOSOMES FROM CHINESE HAMSTER OVARY CELLS TO DNase II

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1. Introduction

The structure of metaphase chromosomes has been the subject of intensive work for many years. From electron microscopic studies with intact [1–4] or partially disaggregated [3,4] metaphase chromosomes it was concluded that also in metaphase a 100 Å filament is coiled to give a thick fiber of 200–300 Å. For the arrangement of the thick fiber in an even higher order structure different models have been proposed [4,5]. Also in metaphase chromosomes the 100 Å filament is organized in a nucleosomal structure. In digestion experiments with micrococcal nuclease no difference was observed in the spacing of the nucleosomes between interphase and metaphase [3,6,7].

Another nuclease successfully used in probing the structure of interphase chromatin is DNase II [8]. It has been shown to be sensitive to the higher order structure of chromatin leading to digestion patterns at either nucleosomal or half nucleosomal intervals. The mechanism of cleavage and structural features of chromatin affecting DNase II digestion have been thoroughly studied [9,10]. Here, we aimed to probe the structure of metaphase chromosomes with the help of DNase II and examine how exposure of chromosomes to different buffers, especially to polyamines, changes their behaviour in the digestion, since several isolation procedures for metaphase chromosomes use polyamines as stabilizing agents ([11–13], K. I., F. F., in preparation). Our results show that after exposure to polyamines there are indeed changes in the chromosomal structure as shown by differences in the DNase II digestion patterns. Metaphase chromosomes isolated in the presence of polyamines might therefore give results in subsequent structural analysis which are different from those obtained with chromosomes stabilized during preparation with divalent cations.

2. Materials and methods

Chinese hamster ovary (CHO) cells (proline requiring) were grown as monolayers in roller bottles in Dulbecco medium (supplemented with 40 mg proline/l and 10% fetal calf serum) to ~70% confluency. After renewing the culture medium and after growth in the presence of 0.06 μg colcemid/ml for 5 h the mitotic cells were selectively detached and harvested by centrifugation. The mitotic index varied from 70–90%.

The metaphase chromosomes were isolated similarly as in [14]. The mitotic cells were stored in Tris–Ca^{2+} buffer (15 mM Tris–HCl, 3 mM Ca^{2+}, pH 7.0) for 30 min at 0°C, centrifuged and after resuspension in the same buffer containing 0.1% Triton X-100 disrupted in a Dounce homogenizer. The nuclei were removed by centrifugation at 120 × *g* for 4 min. The pellet was washed under the same conditions and the metaphase chromosomes in the combined supernatants were sedimented by centrifugation (700 × *g*, 10 min). The cell debris was removed by centrifugation through 2.2 M sucrose in Tris–Ca^{2+} buffer [15]. Amounts of metaphase chromosomes are given in A_{260} units as determined after dissolving them in 0.2% sodium dodecylsulfate (SDS).

DNase II was purchased from Worthington. *Hin*dIII was a gift of R. E. Streeck from this laboratory.

3. Results and discussion

The diagram in fig.1 shows the procedure for the preparation of the metaphase chromosomes and their treatment with buffers prior to digestion with DNase II. The chromosomes were either kept at all times in the Ca^{2+}-containing buffer used for isolation or also

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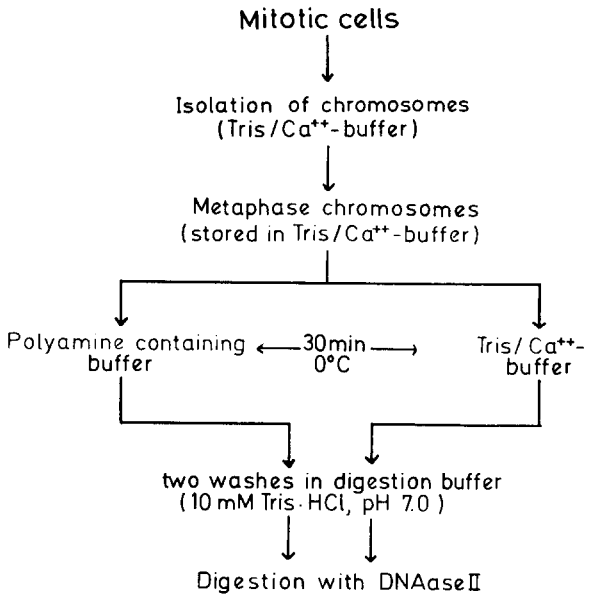

Fig.1. Schematic diagram for the buffer treatment of the metaphase chromosomes before digestion with DNase II. The buffers were Tris–Ca^{2+} buffer (15 mM Tris, 3 mM Ca^{2+}, pH 7.0) and polyamine-containing buffer similar to [16] (0.15 mM spermine, 0.5 mM spermidine, 15 mM Tris–HCl, 60 mM KCl, 15 mM NaCl, 0.2 mM EDTA and 0.2 mM ethyleneglycol [(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA)].

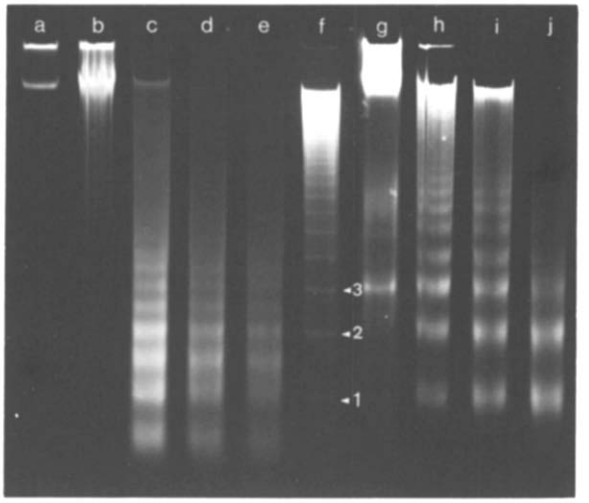

Fig.2. Digestion with DNase II leads to different DNA repeats depending on the pretreatment of the chromosomes with buffers. 0.45 A_{260} units metaphase chromosomes pretreated with polyamine-containing buffer (lanes a–e) were incubated in 45 μl buffer (10 mM Tris (pH 7.0)) at 37°C with 150 units DNase II for (b) 5 min, (c) 10 min, (d) 20 min, (e) 40 min or (a) 40 min without enzyme. Chromosome samples (0.45 A_{260} units each) pretreated in Tris–Ca^{2+} buffer (lanes g–j) were incubated with 20 units of DNase II in 45 μl digestion buffer (as above) for (h) 2 min, (i) 5 min, (j) 10 min or (g) 20 min without enzyme. (f) 0.08 A_{260} units African green monkey DNA, partially digested with *Hin*dIII (the numbers 1, 2 and 3 designate the positions of the monomer (172 basepairs), dimer (344), and trimer (516) of α-satellite DNA repeat, respectively [17]). The incubations were terminated, proteinase K added as in [18] and the DNA applied to a 2% agarose gel (Tris-phosphate buffer system as in [19] but without SDS). All samples were run on the same gel.

briefly incubated at 0°C in a polyamine-containing buffer. In order to eliminate ionic effects of the buffers on the nuclease the chromosomes in both samples were washed twice in salt-free digestion buffer and incubated under identical conditions with DNase II.

The cleavage patterns are shown in fig.2. After treatment of the chromosomes with polyamine-containing buffer, digestion with DNase II (lanes b–e) results in a series of DNA fragments with a half nucleosomal periodicity of ~90 basepairs. Chromosomes treated only with Ca^{2+}-containing buffer yield a cleavage pattern with a nucleosomal periodicity of ~177 basepairs after digestion under otherwise identical conditions. If the chromosomes pretreated with polyamine containing buffer were washed once and incubated in digestion buffer in the presence of 1–3 mM EDTA with DNase II the rate of cleavage was increased, but still a pattern with a half-nucleosomal repeat appeared. If the chromosomes were washed with even higher concentrations of EDTA (up to 20 mM) they disaggregated partially and could no longer be suspended evenly.

Chromosomes exposed to polyamines appear more dense on inspection by phase contrast microscopy and, electron microscopic studies revealed that they have a smaller diameter than those kept in Tris–Ca^{2+} buffer only [13]. This raises the question of whether this correlates with a decreased accessibility of the DNA to the nuclease. Comparison of the kinetics of digestion showed that this is the case. As judged from the DNA size distribution on the gel, ~7-times more DNase II is necessary to digest the DNA in polyamine-treated chromosomes to the same degree as those treated in Tris–Ca^{2+} buffer only.

The most remarkable feature of the structure of metaphase chromosomes is the higher compaction of the DNA compared to interphase. In order to test the influence of this higher compaction on the DNase II digestion, the rate of DNA cleavage in CHO-nuclei and metaphase chromosomes was compared after both

had been exposed to either Tris–Ca^{2+} buffer or to polyamines (not shown). Again, the samples were washed twice in digestion buffer prior to digestion. About 4-times more DNase II was necessary to digest metaphase chromosomes treated in Tris–Ca^{2+} buffer compared to nuclei treated also in this buffer. The same difference in the rate of DNA cleavage was observed, if metaphase chromosomes and nuclei exposed to polyamines were compared.

The lower accessibility of the DNA in metaphase chromosomes to DNase II in comparison to nuclei most likely reflects the higher compaction of the DNA in metaphase, since in both buffer systems used the same difference in the rate of cleavage was found. The difference in the accessibility of the DNA in chromosomes treated with Tris–Ca^{2+} buffer or exposed to polyamines cannot be attributed to an inhibition of the nuclease by polyamines, since it has been shown [20] that free DNA is degraded at the same rate in the presence or absence of polyamines. The lower rate of digestion of chromosomes exposed to polyamines is indicative of a condensed state of the chromatin. This is borne out also in the fact that the DNase II digestion patterns change from a nucleosomal to a half-nucleosomal periodicity upon exposure of the chromosomes to spermine buffer. Spermine and spermidine are known to bind more tightly to chromatin than Ca^{2+} and compact its chromatin structure in a way that can be detected by digestion with DNase II [9,10]. We show here that analogous structural differences exist in metaphase chromosome preparations and that DNase II is sensitive to these differences.

The exact mode of action of polyamines is unknown. It is clear however that they do induce structural changes in chromosomes as detected here by the use of DNase II. One should therefore be cautios when comparing results obtained with spermine-stabilized chromosomes to those found with chromosomes isolated in the presence of divalent cations.

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