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Short Communication

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Structural domains of plant nuclear DNA as a constitutive component of the topoisomerase II/DNA complex

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The treatment of agarose embedded plant nuclei by strong protein denaturants was demonstrated to result in discrete self-fragmentation of intact nuclear DNA. The set of resultant DNA cleavage products involves two main types of DNA fragments sized about 50–100 kb and 300–500 kb, being of the same type in various eukaryotic representatives. The pattern of ordered DNA fragmentation has been shown to be similar both in intact nuclei and in histone-depleted ones thus suggesting that the observed DNA fragments represent preexisting DNA structural domains, corresponding to the higher levels of chromatin folding. The topoisomerase II-specific poison teniposide (VM-26) has been shown to increase the ordered DNA cleavage while the conditions stimulating the topoisomerase II-mediated reverse reaction lead to the reassociation of the cleaved DNA domains. The data presented suggest that the nuclear DNA structural domains are involved in functioning of the topoisomerase II/DNA complex, the main property of which is its ability to mediate the cleavage/ /reassociation reactions.

The loop model of chromatin organization predicts the specific attachment sites spaced along DNA for scaffolding proteins [1, 2]. Topoisomerase II has been shown to be a major component of the nuclear matrix- and chromosome scaffold fraction [3, 4], and to play an important role in chromosome structure and condensation [5–9]. Several lines of evidence show that the type II enzyme is concentrated in a number of discrete anchoring complexes, which probably form the basis of the chromatin loop domains [10–12].

In this paper we present evidences suggesting that the nuclear DNA structural domains are involved in functioning of the topoisomerase II/DNA complex, the main property of which is its ability to mediate the cleavage/reassociation reaction.

MATERIALS AND METHODS

Preparation of nuclear DNA samples and field inversion gel electrophoresis (FIGE)[1]. Rat liver, human lymphocytes, young leaves of various plant species and plant cell cultures were used for preparation of nuclear DNA. Nuclei were prepared according to Smith & Berezney [13] with some modifications. Histone-depleted nuclei were prepared by extensive treatment of the agarose embedded nuclei with 2 M NaCl. Nuclear DNA samples were fractionated by field inversion gel electrophoresis in 0.5 × TBE buffer, pH 8.0–8.3, at 10 V/cm for 20–24 h under constant pulses of electric field (24 s "forward" and 8 s "back-

[1]Abbreviations: FIGE, field inversion gel electrophoresis; TBE buffer, Tris/borate/EDTA buffer.

ward"). Lambda phage oligomers were used as relative molecular mass markers.

DNA cleavage assay. Topoisomerase II-mediated nuclear DNA cleavage was achieved by incubation of agarose-embedded nuclei in the cleavage buffer (50 mM Tris/HCl, pH 8.0; 10 mM Mg^{2+}; 50 mM NaCl; 0.1 mM EDTA) for 20 min at 30°C. In some experiments a topoisomerase II-specific poison teniposide (VM-26) at a final concentration of 50 mkM was added to the cleavage buffer. After incubation, the nuclei were treated with 1% SDS and fractionated by FIGE as described above. The DNA remaining on the start was extracted by a standard procedure involving the treatment of melted nuclei-containing agarose with 1% SDS and proteinase K; this was followed by gel-electrophoresis analysis.

RESULTS AND DISCUSSION

The data presented in Fig. 1 for *Crepis capillaris* show that fractionation of the agarose-embedded lysed nuclei samples by field inversion gel electrophoresis resulted in the appearance of discrete DNA fragments sized about 50–100 kb. The large DNA fragments of about 300–500 kb represented the limited mobility zone under the FIGE conditions used and involved DNA fragments sized up to 2000 kb (not shown). The DNA fragment set of various eukaryotic organisms (Fig. 2) visualized by FIGE appeared to be of a single type and was similar to that found in fractionated cell-, nuclei- and "nucleoid" preparations (Fig. 3). The same fragmentation of nuclear DNA from mammalian cells was also observed by other authors [14].

The similarity of nuclear DNA fragmentation pattern both in intact cells and in "nucleoids" gives essential credence to the idea that the discrete nuclear DNA cleavage appears to be independent of the presence of histones. One could speculate that the ordered fragmentation reflects a feature of nuclear DNA organization and may be due to the periodicity of DNA folding in the cell nucleus, while the resultant DNA fragments seem to represent the pre-existing structures corresponding to the higher order chromatin organization. Based on the data on structural organization of histone-depleted nuclei, it seems justified to ascribe the 50 kb DNA fragments to the DNA loop domains the average length of which varies between 40 kb and 100 kb [15, 16].

The decisive prerequisite for discrete nuclear DNA cleavage is the treatment of nuclear preparation with protein denaturing agents (SDS or sarcosyl). No DNA fragment is released into gel when these agents are lacking. Moreover, when the nuclei are destroyed with melittin or with high concentration of NaCl or EDTA, the DNA fragments fail to appear (not shown). The need for protein denaturants as a decisive prerequisite for the DNA fragment appearance makes the phenomenon of discrete nuclear DNA fragmentation strikingly analogous to the double-strand DNA cleavage reactions mediated by topoisomerase II [17, 18]. This is further confirmed by the data presented in Fig. 4, which indicate that incubation of isolated nuclei with teniposide (VM-26) enhances the SDS-dependent DNA cleavage, resulting in both an increased amount of DNA released into the gel and in breaking down of large DNA fragments into 50 kb ones.

About 20%–80% of nuclear DNA is usually released into gel on treatment of various nuclear preparations with strong protein denatu-

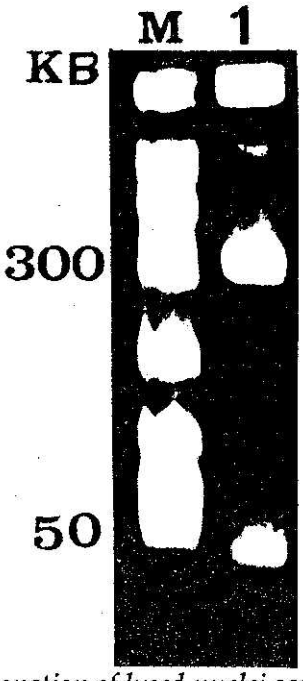


Fig. 1. Fractionation of lysed nuclei samples by gel electrophoresis. Detection of discrete DNA fragments.

Agarose embedded *Crepis capillaris* nuclei were treated with 1% sarcosyl and fractionated by field inversion gel electrophoresis. M, relative molecular mass standards: lambda phage oligomers.

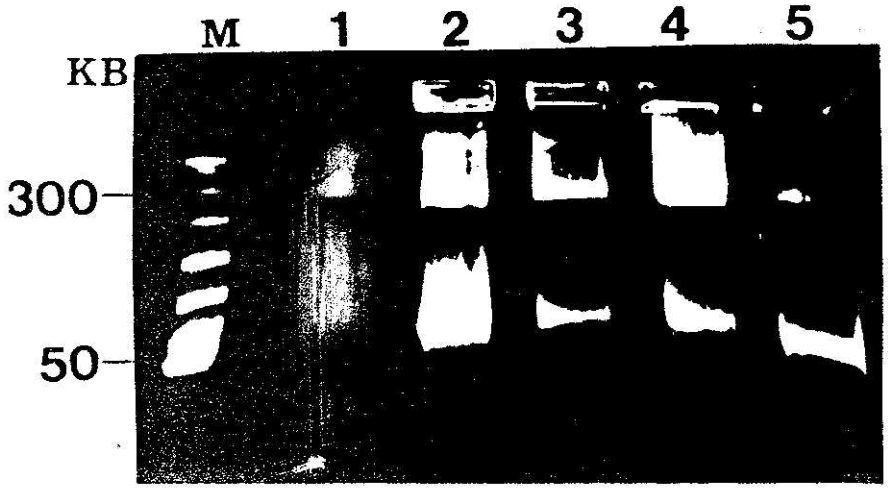


Fig. 2. The set of DNA fragments belonging to various eukaryotic organisms.
1, Rat liver; 2, 3, leaves of *Rauwolfia serpentina* and *Nicotiana tabacum*, respectively; 4, 5, cultured cells and leaves of *Crepis capillaris*, respectively; M, lambda phage oligomers.

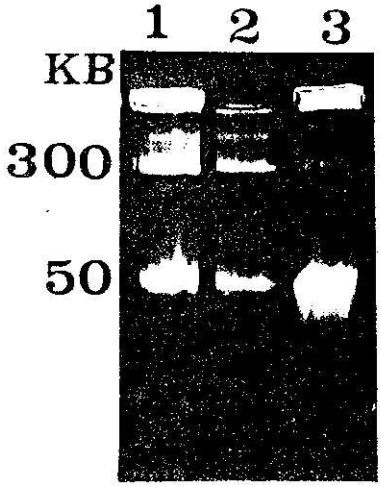
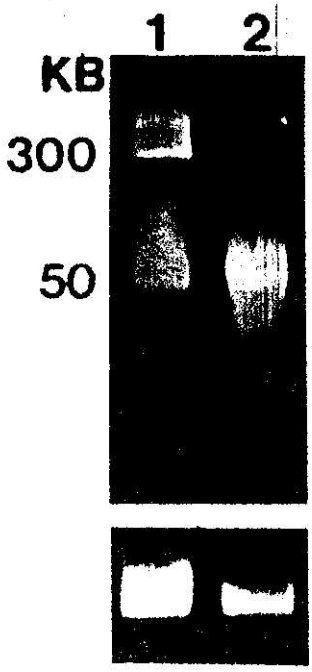


Fig. 3. The pattern of human lymphocytes nuclear DNA fragmentation in agarose embedded preparations of cells (1), nuclei (2) and nucleoid (3).



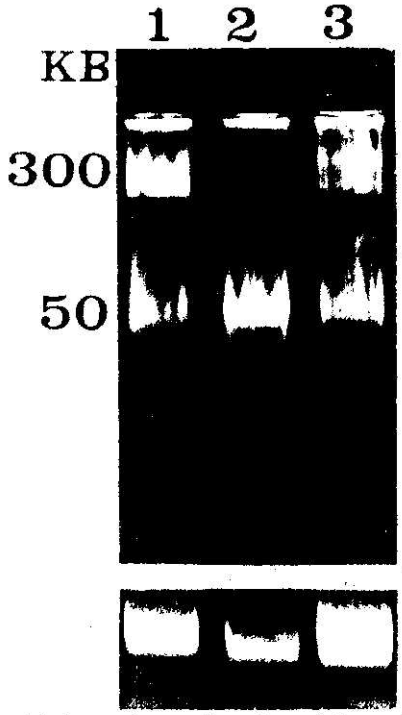


Fig. 5. NaCl-dependent religation of cleaved nuclear DNA.
Agarose embedded maize seedling nuclei prepared in the presence of Mg^{2+}, were incubated in cleavage buffer for 20 min at 0°C (1), or 30°C (2, 3), followed by NaCl addition to a final concentration of 250 mM (3). After incubation the nuclei were treated with 1% SDS and fractionated by FIGE. Top panel, DNA recovered from nuclei; bottom panel, DNA remaining on the start.

Fig. 4. Effect of teniposide (VM-26) on the ordered nuclear DNA fragmentation.
Agarose embedded maize seedling nuclei prepared in the presence of Mg^{2+}, were incubated for 20 min at 30°C in cleavage buffer without (1) and with 50 mkM teniposide (2). Following incubation the nuclear samples were treated with SDS and fractionated by FIGE. Top panel, DNA recovered from nuclei; bottom panel, DNA remaining on the start.

rants (not shown). The incomplete release of nuclear DNA into gel during fractionation of lysed nuclei preparations by FIGE suggests that the DNA that has remained on the start might represent the uncleaved DNA due to the cleavage/religation equilibrium mediated in the cell nuclei by topoisomerase II. The data presented in Fig. 5 show that the treatment of nuclei with a moderate concentration of NaCl resulted in reassociation of the cleaved DNA fragments.

Thus, the results presented so far allow to consider the nuclear DNA domains as a constituent component of the topoisomerase II/DNA complex which is able to mediate the cleavage/reassociation equilibrium reactions.

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REFERENCES

1. Marsden, M. & Laemmli, U.K. (1979) *Cell* **17,** 849–858.
2. Mirkovitch, J., Mirault, M.-E. & Laemmli, U.K. (1984) *Cell* **39,** 223–232.
3. Earnshaw, W.C. & Heck, M.M.S. (1985) *J. Cell. Biol.* **100,** 1716–1725.
4. Berrios, M., Osheroff, N. & Fisher, P.A. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82,** 4142–4146.
5. Cockerill, P.N. & Garrard, W.T. (1986) *Cell* **44,** 273–283.
6. Gasser, S.M. & Laemmli, U.K. (1986) *EMBO J.* **5,** 511–518.
7. Earnshaw, W.C., Halligan, B., Cooke, L.A., Heck, M.M.S. & Liu, L.F. (1985) *J. Cell. Biol.* **100,** 1706–1715.
8. Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. & Yanagida, M. (1987) *Cell* **50,** 917–925.
9. Newport, J. (1987) *Cell* **48,** 205–217.
10. Sperry, A.O., Blasquez, V.C. & Garrard, W.T. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86,** 5497–5501.
11. Adachi, Y., Kas, E. & Laemmli, U.K. (1989) *EMBO J.* **8,** 3997–4006.
12. Zhang, L.H., Mui, S.C., Todt, J.C. & Strauss, P.K. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83,** 5871–5874.
13. Smith, H.S. & Berezney, R. (1982) *Biochemistry* **21,** 6752–6761.
14 Filipski, J., Leblanc, J., Youdale, T., Sikorska, M. & Walker, P.R. (1990) *EMBO J.* **9,** 1319–1327.
15. Laemmli, U.K., Cheng, S.M., Adolph, K.W., Paulson, J.F., Brown, J.A. & Baumbach, W.R. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 351–360.
16. Ward, W.S., Partin, A.W. & Coffey, D.S. (1989) *Chromosoma* **98,** 153–159.
17. Osheroff, N. (1989) *Pharmacol. Ther.* **41,** 223–241.
18. Liu, L.F. (1989) *Annu. Rev. Biochem.* **58,** 351–375.