УДК 575.113/577.21

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THE PHYSIOLOGICAL SIGNIFICANCE OF NUCLEAR DNA STRUCTURAL DOMAIN DISINTEGRATION: EVIDENCE FOR NON-RANDOM DNA DOMAIN CLEAVAGE

We examined the pattern of ordered high molecular weight DNA cleavage in plant tissues showing diverse proliferative status or differentiation level, as well as that in human cultured lymphoblastoma cells during serum starvation. It was demonstrated that in quiescent or differentiated tissues there occur increased high molecular weight DNA cleavage, with the cleaved DNA domains being distinct from non-cleaved ones by the genome sequence composition. Lymphoblastoid cell culturing in serum-free medium was shown to be accompanied by progressive accumulation of high molecular weight DNA fragments which is rapidly decreased after serum addition. Transient increase in high molecular weight DNA fragmentation was found to accompany by temporal changes in C-myc sequence localization within cleaved DNA domains. The results obtained suggest that ordered disintegration of nuclear DNA into high molecular weight DNA fragments may present the specific genome reaction accompanying the physiological changes in the cells and may be presumably implicated to reprogramming of gene expression.

Introduction. Previously we showed that the treatment of agarose-embedded nuclear DNA preparations with protein denaturants resulted in appearance of two main types of nuclear DNA fragments sized about 50—100 and 250—300 kb, which proved to be unityped for various eukaryote representatives [1]. Consistent with our early data as well as those of other investigators the DNA fragment observed may be ascribed to the nuclear DNA loop domains cleaved by topoisomerase II [1—3]. Recently the similar pattern of high molecular weight (HMW) DNA fragmentation was demonstrated at the early steps of apoptosis [4—7] and upon stress influences (Solov'yan and Andreev, previous communication), thus suggesting that enhanced cleavage of nuclear DNA structural domain may be of physiological value.

In the present report we demonstrate that the pattern of ordered HMW/DNA cleavage depends on the tissue proliferative activity and (or) differentiation level, and provide evidence suggesting the non-random DNA structural domain cleavage.

Material and methods. Cell lines and culture conditions. Maize seedlings, intact plants and cultured cells of *Rauwolfia serpentina* and human lymphoblastoma cultured cells (line CEM) were used for these investigations. CEM cultured cells were routinely incubated in RPMI 1640 medium supplemented with 10 % fetal calf serum (FCS) in an atmosphere of 95 % air, 5 % CO_2 to give a final suspension of $(2—5) \cdot 10^6$ cells/ml.

Preparation of DNA samples to FIGE fractionation. Nuclei from plant tissues were prepared according to the procedure by Smith and Berezney [11] with modifications as follows. The pellet of nuclei was obtained by homogenization of tissues in cold-ice isolation buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.3 M mannitol, 0.1 % BSA (pental fraction), 4 mM 2-mercaptoethanol).

After filtration and centrifugation (1000×g for 15 min) the pellet was resuspended in the same buffer and layered on 2.2 M sucrose prepared in isolation buffer. After the next centrifugation (80.000×g

for 2 h)) the pellet of purified nuclei was washed in isolation buffer followed by addition of equal volume of 1 % low-melting agarose. The suspension of nuclei was placed in inserts of 2 mm thickness, cooled up to gelation and equal volume of 1 % sarcosyl or SDS was layered followed by incubation for 1—24 h at 55 °C.

200 μl suspension of cultured CEM cells ($2 \cdot 10^6$ cells/ml) were placed into the well of cell culture plate followed by addition of equal volume of 1 % low-melting agarose prepared on TEN-buffer (10 mM Tris-

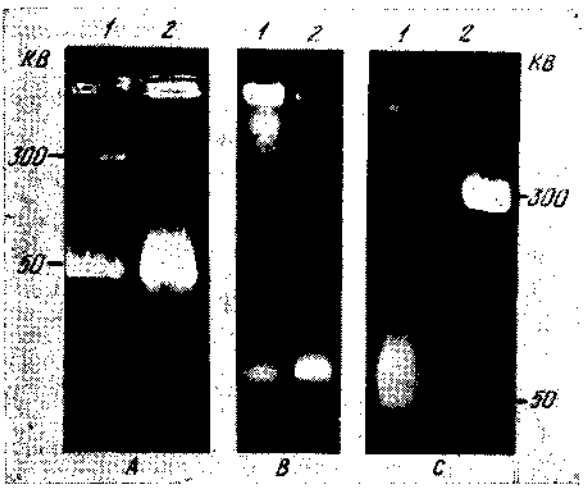

Fig. 1. The pattern of high molecular weight DNA fragmentation in plant tissues showing diverse proliferation status or differentiation level: *A* — The pattern of ordered nuclear DNA cleavage in *Zea mays* seedlings (*1* — apical root meristematic tissues; *2* — the root tissues without meristem); *B* — The pattern of ordered nuclear DNA cleavage in *Crepis capillaris* (*1*—7-days old seedlings; *2* — mesophyll leaves); *C* — The pattern of ordered nuclear DNA cleavage in *Rauwolfia serpentina* (*1* — leaves of intact plants; *2* — cultured cells)

HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl). After gelation the equal volume of lysing buffer (TEN+1 % SDS) was layered followed by incubation for 1 h at 37 °C. Agarose plugs containing the lysed nuclei or cells were used for analysis by agarose gel electrophoresis.

Gel electrophoresis. Lysed cell preparations were fractionated either by conventional or field inversion gel electrophoresis (FIGE) to detect the pattern of nuclear DNA cleavage. Conventional gel electrophoresis was carried out in 1 % agarose at 50 V for 4—5 h using 0.5×TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8—8.5). FIGE was performed in 1 % agarose at 85 V in 0.5×TBE buffer under constant pulses of electric field (24 s «forward» and 8 s «backward») allowing to monotonous resolution of DNA molecules sized up to 500 kb [12]. After electrophoresis the gel was stained with 1 μg/ml ethidium bromide for 10 min, viewed using an UV transilluminator and photographed using Mikrat 300 film.

Blot-hybridization analysis. After fractionation of agarose-embedded nuclear and cellular preparations by gel-electrophoresis DNA was transferred to nylon membrane by Southern capillary transfer technique, and hybridized with ^{32}P-labelled probes as described by Maniatis et al. [13]. After hybridization membranes were incubated twice with 2×SSC containing 0.1 % SDS at 65 °C for 30 min followed by incubation with 0.2×SSC at 65 °C for 10 min (1×SSC: 150 mM NaCl, 15 mM Na_3 citrate). Air dried membranes were wrapped in Saran Wrap and autoradiographed at —30 °C for 1—7 days.

Results and Discussion. The data presented in Fig. 1 demonstrate the pattern of ordered HMW-DNA cleavage in plant tissues showing various proliferative activities or differentiation level. As evidenced from the data in all cases under study there is a tendency to enhanced HMW-DNA fragmentation in quiescent or terminally differentiated tissues revealed as a changed proportion between the two types of DNA fragments towards 50—100 kb fragments increase. These data suggest that the pattern of HMW-DNA cleavage depends on physiological status of tissues and may be associated with the processes of cell growth and development.

Data presented in Fig. 2 show that the «cleaved» DNA domains in plant tissues seem to differ from «uncleaved» DNA by the content of some genomic sequences. Thus, notwithstanding that both types of «cleaved» DNA fragments sized 50—100 and 250—300 kb as well as «noncleaved» DNA to be left on the start share common genomic sequences, rDNA sequences in the genome of cultured plant cells are preferentially localized within the noncleaved DNA and in 250—300 kb fragments, and entirely absent within 50—100 kb fragments (Fig. 2, *B*). In the intact

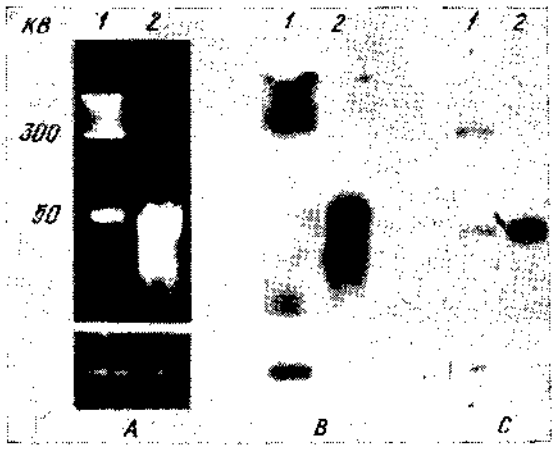

Fig. 2. Non-random high molecular weight DNA cleavage in plant tissues. Agarose embedded nuclear preparations of *R. serpentina* cultured cells *(1)* and intact plant leaves *(2)* were treated with SDS and fractionated by FIGE. Agarose plugs containing noncleaved DNA left on the start were melted and DNA was isolated by standard procedure. *A* — The pattern of nuclear DNA cleavage; *B* — the hybridization of cleaved DNA domains (top panels) and isolated noncleaved DNA with ^{32}P-labelled rDNA; *C* — The same hybridization with ^{32}P-labelled total DNA

plant genome, however, the above sequences disappear from the «noncleaved» DNA and are localized within fragments of 50—100 kb (Fig. 2, *B*).

As evidenced from the data presented in Fig. 3, the incubation of human lymphoblastoma cultured cells (line CEM) in serum free medium

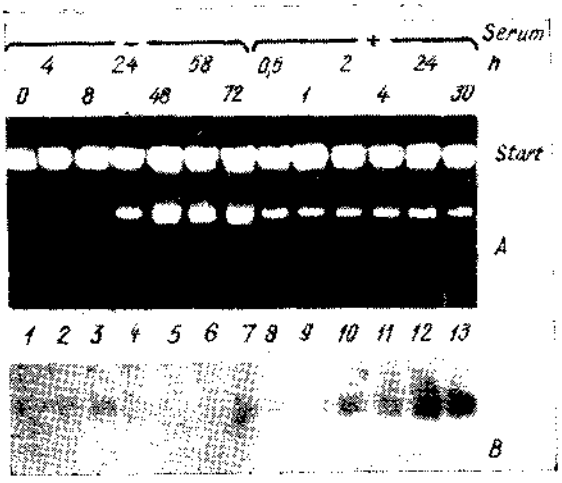

Fig. 3. Temporal re-localization of *c-myc* sequences within cleaved DNA domains in cultured CEM cells during serum starvation. Cells were incubated in serum free medium (—) for the time (h) indicated at the top of figure. After 24 h of incubation serum was added followed by incubation in serum supplemented medium (+) for the time indicated in the figure. Following incubation cells were embedded into agarose, treated with SDS and fractionated by conventional gel-electrophoresis: *A* — The time course of nuclear DNA cleavage; *B* — The hybridization of cleaved DNA with ^{32}P-labelled *c-myc* sequences

is accompanied by the progressive formation of HMW-DNA fragments to be rapidly declining following serum addition. Besides, transient enhancement of HMW-DNA cleavage during serum starvation is accompanied by the temporal changes in *c-myc* sequence localization between «cleaved» and «noncleaved» DNA. Thus, with serum present and during the early steps of serum starvation *c-myc* sequences were detected within the «cleaved» DNA domains despite that the relative proportion of these domains appeared to be negligible. As the starvation proceeded the proportion of «cleaved» domains rose, however, *c-myc* sequences proved undetectable in these domains. Finally, upon serum addition the partial

reduction in «cleaved» DNA took place with the sequences tested being re-localized within «cleaved» domains.

The data presented so far show that the formation of HMW-DNA fragments depends on the physiological status of the tissues, occurs in cells subjected to the stress challenge, and seems to be of non-random occurrence. This allows us to interpret the ordered disintegration of nuclear DNA as the specific genomic reaction reflecting physiological changes in the cells.

The data reported recently [4—7] demonstrated that the process of programmed cell death is accompanied by the formation of HMW-DNA fragments sized 30—50 kb or more, which precedes apoptosis-specific internucleosomal DNA fragmentation. In previous communication we showed that enhanced HMW-DNA cleavage occurs in apoptotic cells and in those subjected to stress challenges (Solov'yan and Andreev, Biopolymers and Cell, previous communication).

In this study we showed that ordered cleavage of nuclear DNA into high molecular weight fragments naturally occurs in normal tissues with the pattern of fragmentation to be correlating with the physiological status of the cells. In addition, we showed that enhanced HMW-DNA cleavage accompanies the physiological changes in cells during serum starvation and may be of transient nature. These observations suggest that ordered disintegration of nuclear DNA seems to accompany the various cell programmes, including apoptosis, stress response, and may be implicated to the processes of cell growth and development.

Our interpretation of ordered HMW-DNA cleavage is based on previously obtained data showing that nuclear DNA structural domains are involved in functioning topoisomerase II/DNA complex with its ability to mediate the cleavage/religation equilibrium reactions [14]. In terms of functioning DNA/topoisomerase complex the ordered disintegration of nuclear DNA may be interpreted as DNA structural domain transition from noncleavable to cleavable state mediated by topoisomerase II.

Our results indicate that the «cleaved» nuclear DNA domains may differ from «uncleaved» DNA by content of some genomic sequences. Moreover, we showed that the transient formation of HMW-DNA fragments during serum starvation is accompanied by the temporal changes in localization of *c-myc* sequences between «cleaved» and «noncleaved» DNAs. These data may imply that during various physiological events there may occur differential cleavage of DNA domains. In other words in response to the particular type of challenge not sporadic but the specific set of DNA structural domains may be involved in turnover between «cleaved» and «noncleaved» state. In this connection the question arises: what is implication for presumable differential cleavage of nuclear DNA domains?

C-myc sequences as is well documented represent the family of so-called immediately early response genes capable for reprogramming their expression under the effect of a wide variety of influences including serum starvation (for references see [8]). One can not exclude, therefore, that transient changes in localization of these sequences within «cleaved» domains may reflect the cell genome response to serum deficiency, associated with reprogramming of *c-myc* gene expression.

To summarize above stated one may conclude that the changes in the integrity of nuclear DNA detectable as an altered pattern of ordered HMW-DNA cleavage are of physiological value and may present specific cell reaction associated presumably with reprogramming of genome expression.

This study was supported in part by Grant of Fund «Fundamental Research» of State Committee of Science and Technology, project 5.3/140.

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ФІЗІОЛОГІЧНЕ ЗНАЧЕННЯ ДЕЗІНТЕГРАЦІЇ СТРУКТУРОВИХ ДОМЕНІВ ЯДЕРНОЇ ДНК: СВІДЧЕННЯ НЕВИПАДКОВОГО РОЗЩЕПЛЕННЯ ДОМЕНІВ ДНК

Резюме

В роботі досліджували характер крупноблочної фрагментації у тканинах рослин з різним проліферативним статусом і ступенем диференціювання, а також у культурі лімфобластоїдних клітин людини у відсутності сироватки. Показано, що для тканин у стані спокою або диференційованих тканин характерно посилене формування великих фрагментів. При цьому розщеплені домени ядерної ДНК відрізняються від нерозщеплених за складом геномних послідовностей. Культивування лімфобластоїдних клітин у середовищі без сироватки супроводжується прогресивним зростанням крупноблочної фрагментації, яка швидко зменшується в залежності від додавання сироватки. Крім того, транзитне посилення крупноблочної фрагментації викликає зміну локалізації послідовностей у розщеплених доменах.

Отримані результати вказують на те, що модифікація нативності доменів ядерної ДНК, яка проявляється у різному характері крупноблочної фрагментації, може являти собою специфічну геномну реакцію, що супроводжується фізіологічними змінами, які відбуваються у клітинах, і мати відношення до перепрограмування геномної експресії.

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Inst. of Mol. Biol. and Genet.
Nat. Acad. of Sci. of Ukraine, Kiev

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