Journal of Cellular Biochemistry 34:163–168 (1987)

Crosslinking of DNA to Nuclear Lamina Proteins by UV Irradiation In Vivo

Z. Galcheva-Gargova and G.N. Dessev

Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

We have been able to demonstrate that a fraction of DNA becomes crosslinked to nuclear lamina shells isolated from Ehrlich ascites tumour cells irradiated with UV light. Terminal labeling of short DNA fragments covalently attached to proteins reveals that DNA has become crosslinked to all three lamins and to a protein comigrating with vimentin.

Key words: nuclear lamina, UV crosslinking, DNA

A number of experimental data suggest that there is a close physical association between chromatin and the nuclear lamina (NL) in the interphase nucleus. For example, electron microscopic observations have shown chromatin fibers attached to the NL [1,2]. High molecular weight DNA cosediments with isolated NL shells and can be seen directly associated with them [3,4]. Specificity of the interaction between chromatin and the NL is suggested by the location of condensed centromere chromatin blocks containing satellite DNA at the nuclear periphery [5–7], although NL can apparently assemble around chromatin containing phage DNA [8]. In any case, chromatin provides nucleation sites for the postmitotic NL assembly [8,9]. All these data suggest that the NL may be important in the structural organization of chromatin.

A study of DNA sequences specifically associated with the NL requires a method for isolation of NL structures free of intranuclear material and associated with relatively long DNA fragments. So far, in most studies on DNA bound to nuclear skeletal structures, the latter have consisted of both NL and intranuclear matrix material [10]. Interpretation of the results of such experiments have proved difficult for the following reasons. In vitro reconstitution studies have shown that the lamins are strong DNA (and particularly single stranded DNA) binding proteins [11–13]. It is not clear how this property is related to the situation in the living cell, since we have been unable to obtain evidence for the existence of "tight" complexes between DNA and NL in vivo [4]. However, the high in vitro affinity of the lamins

G.N. Dessev's present address is Northwestern University Medical School, Department of Cell Biology and Anatomy, 303 East Chicago Avenue, Chicago, Ill 60611.

Received November 4, 1986; revised and accepted January 14, 1987.

to DNA may lead to formation of artifactual complexes in the course of cell fractionation [4]. Recently, we have developed a method for isolating NL from Ehrlich ascites tumour (EAT) cells as intact shells [14] under conditions that minimize the in vitro artifacts. We have shown that DNA appears loosely attached to these shells and undergoes a size-dependent release from them upon fragmentation [4]. However, under these conditions DNA may slide relative to its in vivo attachment sites, which would result in a randomization of the sequences found in the NL fraction.

To avoid these difficulties and to address the problem of DNA sequence specificity of attachment to the NL we have used UV irradiation of whole EAT cells to crosslink DNA to the NL proteins. Our results indicate that a small fraction of DNA copurifies with NL structures isolated from irradiated, but not from control, cells, and that all three lamins become crosslinked to DNA. We also observe formation of covalent complexes between DNA and a protein that comigrates with vimentin.

MATERIALS AND MEHODS

EAT cells were propagated in mice and labeled in suspension, as previously described [14]. For UV irradiation, the cells were suspended in the medium used for labeling at a density of 5.10^6 per ml. Twenty-milliliter aliquots were placed in 90-mm petri dishes and irradiated at room temperature for different time periods using a 15-W bactericidal lamp (Medicor, Hungary; emission at 254 nm) fixed 4 cm above the dish in a box lined with aluminum foil.

The proportion of DNA crosslinked to proteins was estimated using partitioning in the water/phenol system [15]. Chromatin samples were dissociated by boiling in 1% SDS, 1 M NaCl for 3 min. After cooling, an equal phenol/chloroform/isoamyl alcohol (1:1:0.05) was added, the samples were shaken for 15 min at room temperature, and centrifuged in an Eppendorf microfuge. The organic phase with interphase was extracted twice with the same solution. The amount of acid-precipitable [^{3}H] DNA was estimated in the aqueous phase and in the phenol phase plus interphase.

Isolation of chromatin and NL was carried out as previously described [14]. Non-equilibrium metrizamide (MA) gradients [4] were prepared in 12-ml tubes for the SW 40 Beckman rotor. One milliliter of 55% metrizamide was overlayered with a 4-ml linear 20-40% MA gradient and 4.5 ml 15% sucrose. All layers contained 2 M NaCl, 10 mM Tris·Cl, 1 mM EDTA (pH 7.5), 0.1% bovine serum albumin, and 0.1 mM phenylmethylsulfonyl fluoride. Two-milliliter samples of chromatin digested with 6 units DNAse II per A_{260} plus 20 μg RNAse per ml and dissociated in 2 M NaCl [4] were layered on the gradient and centrifuged for 60 min at 10,000 rmp. Fractions (0.5-ml) were collected from the meniscus.

DNA fragments covalently linked to proteins were labeled at the 5′ terminus with T4 polynucleotide kinase in 75 mM Tris. Cl, pH 7.6, 10 mM $MgCl_2$ in the presence of 5–10 μCi [$\gamma-^{32}$P] ATP (1,000 Ci/mmol). After incubation for 30 min at 37°C, the reaction was stopped by addition of EDTA to 20 mM.

RESULTS AND DISCUSSION

EAT cells were labeled with [^{3}H] thymidine and irradiated with UV light for different periods of time, as described in Materials and Methods. To monitor the formation of covalent bonds between DNA and protein, dissociated chromatin sam-

ples were subjected to partitioning between two phases in the phenol/water system [15]. Figure 1 shows that in the case of unirradiated cells about 95% of DNA is found in the aqueous phase. Upon UV irradiation, increasing amounts of DNA become associated with the organic phase, reaching a plateau at about 30% of the input after 30 min of irradiation. Similar results were obtained with the NL fraction isolated after partial digestion of DNA (Fig. 1).

To separate DNA crosslinked to the NL we used nonequilibrium MA gradients, prepared as described above. These gradients set a density barrier to free DNA (buoyant density 1.15 g/cm^3) or to single DNA molecules attached to single proteins. Only those DNA fragments associated with large protein structures such as NL will sediment until reaching the equilibrium density of free protein (about 1.30 g/cm^3) [4,16]. Figure 2 shows that in both control and irradiated samples, about 4% of the input protein has sedimented to the bottom of the gradient. This material has been previously characterized by electron microscopy and biochemical techniques as representing purified NL shells [4,14]. Its protein composition analysed by SDS polyacrylamide gel electrophoresis [17] is shown in Figure 3, lane 1.

In the case of unirradiated controls (Fig. 2A) virtually no DNA cosediments with NL, in agreement with our previous results [4]. In irradiated samples (Fig. 2B, about 2% of the input DNA is found at the bottom, suggesting that it has become crosslinked to the NL proteins.

To identify the NL proteins crosslinked to DNA, the material was recovered from the bottom of the gradient by low speed centrifugation, after dilution with 10 mM Tris·Cl, 1 mM EDTA (TE), pH 7.0, resuspended in TE, pH 7.0, and digested exhaustively with DNAse II (50 units enzyme/A_{260} initial O.D. for 30 min at 25°C). In this step, most of the DNA is hydrolysed, leaving only short fragments with 5′ OH ends covalently attached to NL proteins, suitable for terminal labeling with T4 polynucleotide kinase. The material was then washed several times with the kinase buffer, resuspended in the same buffer to a volume of 50 μl, heated at 80°C for 10 min to inactivate the protein kinases present in the NL preparations (unpublished

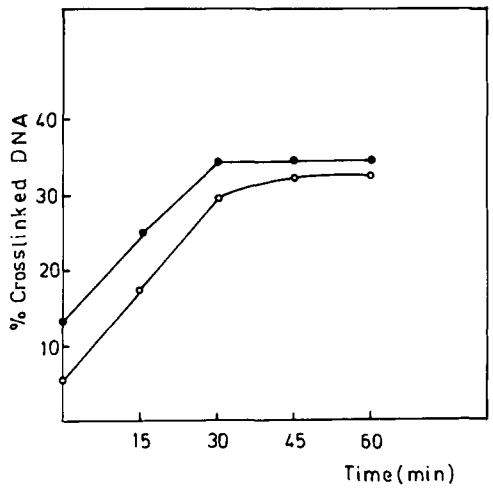

Fig. 1. DNA in chromatin (○) or NL (●) samples (percentage of the input) associated with the phenol phase plus interphase as a function of UV irradiation time.

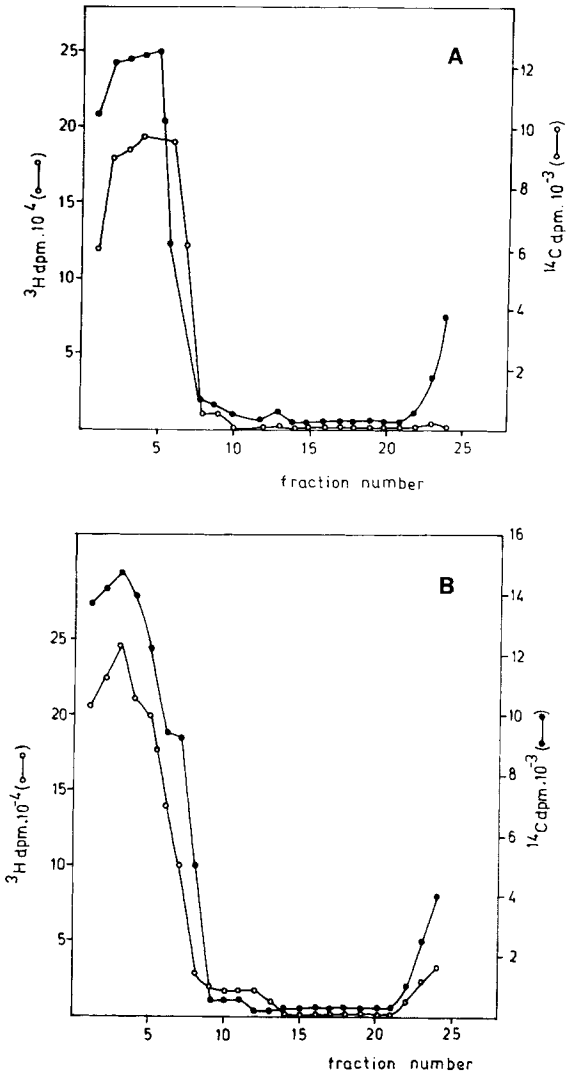

Fig. 2. Distribution of [^{3}H]DNA (○) and [^{14}C] protein (●) in nonequilibrium metrizamide gradients. **A,** Control cells: **B,** cells irradiated with UV light for 30 min. Sedimentation from left to right.

data), cooled to 37°C, and terminally labeled as described above. The material was washed several times with TE, dissolved in SDS sample buffer by boiling for 3 min, and fractionated in SDS containing 10% polyacrylamide gels. The gels were dried and autoradiographed.

The electrophoretic analysis (Fig. 3, lane 2) shows the presence of four fractions in the UV-irradiated samples, which have become radiolabeled due to covalently attached, short, terminally labeled DNA fragments. Protein-bound RNA fragments with 5′ phosphate ends generated by pancreatic RNAse, if present in our preparations, may have become labeled by a T4 kinase catalysed 5′ phosphate exchange driven by excess ADP [18]. However, the efficiency of this reaction under our conditions should have been very low, and we consider it unlikely to account for our results.

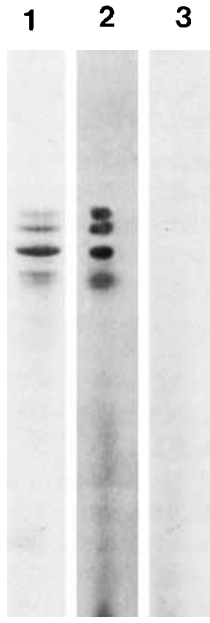

Fig. 3. Electrophoresis of NL proteins crosslinked to DNA in 10%, SDS-containing polyacrylamide gels. **Lane 1,** total NL proteins (Coomassie-blue-stained gel): **Lane 2,** covalent complexes of NL proteins and terminally labeled short DNA fragments from UV irradiated cells (autoradiography): **Lane 3,** unirradiated control (autoradiography).

Three of the radioactive fractions (Mr 75,70 and 64 kilodaltons) comigrated with the three lamins. A somewhat diffuse radioactive band is also present, in the region of 56–58 kilodaltons. In NL preparations isolated in the presence of EDTA [14] this region, which shows reactivity towards a polyclonal antibody directed against vimentin, is occupied by two closely migrating polypeptides (Fig. 3, lane 1), whose peptide maps are similar, but not identical to that of purified vimentin (unpublished data). Further studies are needed to establish the nature of these two polypeptides.

No radioactive bands were present in control, unirradiated samples (Fig. 3, lane 3). Digestion with proteinase K abolished all four radioactive bands (not shown).

Our results indicate that DNA is within a crosslinking distance of the three main protein constituents of the NL of EAT cells (lamins A, B, and C) and of a putative intermediate filament protein. This finding is in agreement with the results of other investigators, who have observed formation of lamin-DNA [19] and cytokeratin-DNA [20] covalent complexes using crosslinking with potassium chromate and a different methodology.

It is known that NL is depolymerised during mitosis and the lamins become soluble [21]. The possibility that the covalent complexes observed result from contact between DNA and soluble mitotic lamins is unlikely since our experiments involve isolation of NL structures from interphase nuclei. Moreover, so far, the lamin proteins have not been found as components of the mitotic chromosomes.

Recent results suggest that during mitosis some intermediate filaments remain attached to the chromosomes [22]. This may result in formation of covalent bonds between DNA and intermediate filament proteins upon UV irradiation. Our approach,

involving isolation of interphase NL structures, makes this explanation of our results unlikely as well as the possibility that the labeling results from crosslinking between intermediate filaments and mitochondrial DNA.

Finally, our experiments provide an approach for isolation and characterization of the DNA sequences associated with the NL in vivo.

REFERENCES

1. Bouvier D, Hubert J, Bouteille M: J Ultrastruct Res 73:288, 1980.
2. Puvion-Dutilleul F, Puvion E: J Cell Sci 42:305, 1980.
3. Hancock R, Hughes ME: Biol Cell 44:201, 1982.
4. Krachmarov Ch, Iovcheva C, Hancock R, Dessev G: J Cell Biochem 31:59, 1986.
5. Jones KW: Nature 226:912, 1970.
6. Pardue ML, Gall JD: Science 168:1356, 1970.
7. Rae PMM.
8. Forbes DJ, Kirschner MW, Newport JW: Cell 34:13, 1985.
9. Burke B, Gerace L: Cell 44:639, 1986.
10. Mirkovitch J, Mirault ME, Laemmli UK: Cell 39:223, 1984.
11. Lebkowski J, Laemmli UK: J Mol Biol 156:325, 1982.
12. Dessev G: Biol Cell 45:333, 1982.
13. Hancock R: Biol Cell 46:105, 1982.
14. Krachmarov Ch, Tasheva B, Markov D, Hancock R, Dessev G: J Cell Biochem 30:351, 1986.
15. Van Eekelen CAG, Van Venrooij WJ: J Cell Biol 88:554, 1981.
16. Rickwood D, Birnie G: FEBS Lett 50:102, 1975.
17. Laemmli UK: Nature 227:680, 1970.
18. Maniatis T, Fritsch EF, Sambrook J: "Molecular Cloning." Cold Spring Harbor Laboratory, 1982.
19. Wedrychowski A, Steven Ward W, Schmidt WN, Hnilica, LS: J Biol Chem 260:7150, 1985.
20. Ward WS, Schmidt WN, Schmidt CA, Hnilica LS: Proc Natl Acad Sci USA 81:419, 1984.
21. Gerace L, Blobel G: Cell 19:227, 1980.
22. Jones JCR, Goldman AE, Yang HY, Goldman RD: J Cell Biol 100:93, 1985.