Acta Biochimica Polonica

Vol. 42 No. 2/1995

205–210

QUARTERLY

Short Communication

The paper was presented at the "First International Seminar on Nuclear Matrix"

Interactions of the matrix attachment region of DNA with the matrix proteins from the copper preincubated liver nuclei*

Piotr Widłak, Jacek Rogoliński and Joanna Rzeszowska-Wolny

Department of Tumor Biology, Institute of Oncology, Wybrzeże Armii Krajowej 15, 44–100 Gliwice, Poland

Key words: MAR sequences, nuclear matrix proteins, copper ions

Preincubation of rat liver nuclei with copper ions influenced the stability and protein composition of the nuclear matrices isolated by a "high salt" method. Also the specific interaction between matrix proteins and the kappa Ig matrix attachment region of DNA was affected.

Genomic DNA of eukaryotic cells is organized into loops anchored to a proteinaceous structure called nuclear matrix (nuclear scaffold). The anchorage is mediated by defined segments of DNA called the matrix (scaffold) attachment regions (MAR[1] or SAR) which interact with specific matrix proteins. MARs have been identified in many gene regions and it has been suggested that these regions might serve as a *cis* regulatory elements for transcription, replication and recombination (for review see [1]).

The specificity of interactions between MAR sequences and matrix proteins, and factors which may influence these interactions remain unelucidated. It has been established that Cu^{2+} takes part in the higher organization of chromatin *in vitro* [2]. The incubation of chicken erythroblast nuclei with Cu^{2+} ions significantly stabilized nuclear matrix, but it seems that copper did not affect properties of the nuclear skeleton-associated DNA [3]. We have attempted to examine whether copper pretreatment of nuclei affects specificity of MAR DNA binding to matrix proteins.

METHODS

Probed DNA. The MAR sequences from 5′ end of the mouse kappa immunoglobulin gene [4] cloned into pTZ19R plasmid was used for DNA binding studies. The 593-bp fragment was excised from the plasmid by *Hin*dIII and *Eco*RI. As a control, a non-MAR DNA, the whole linearized pUC19 plasmid, its 181-bp fragment excised by *Hin*dIII and *Pvu*II, or alternatively the 495-bp fragment of the rat repetitive sequence MspI8 [5], were used. The restriction fragments of probed DNA were ^{32}P-3′-end-labeled using Klenow enzyme [6]. Labeled sequences were purified by repeated electrophoretical separation and extraction from polyacrylamide gel [6].

Preparation of nuclear matrices. The nuclear matrices from liver of adult male Wistar rats

*This work was supported by Grant No. 6 P203 028 05 from the State Committee for Scientific Research and from the Foundation for Polish Science, BIMOL 76/93.

[1]Abbreviations: BSA, bovine serum albumin; M, nuclear matrix; MAR or SAR, matrix (or scaffold) attachement regions; P, pellet; S, supernatant.

were prepared by the "high-salt" method. All steps were performed at 4°C in the presence of proteinase inhibitors (phenylmethylsulfonyl fluoride, pepstatin, aprotinin and leupeptin) and 3 mM $MgCl_2$. The nuclei were purified by centrifugation in 2.2 M sucrose and washed with 1% Triton X-100. Alternatively, 10 min incubation in 5 mM $CuSO_4$ (or in a single experiment 10 mM EDTA) preceded washing with Triton. The nuclei were then treated with DNase I (10 µg/mg of protein, 60 min, 20°C) in 0.1 M NaCl. The remnant nuclei were extracted with 0.5 M NaCl followed by 2 M NaCl. The proteins of residual nuclear matrices and of total nuclei were quantitated by the Bio-Rad Protein Assay.

DNA binding assay. Binding of DNA to the nuclear matrices was assayed according to Cockerill & Garrard [4]. Nuclear matrices containing about 30 µg of proteins were suspended in 200 µl of the assay solution comprising: 50 mM NaCl, 10 mM Tris/HCl (pH 7.4), 2 mM EDTA, 0.25 M sucrose, 0.25 mg/ml BSA, different amounts of non-specific competitor (sonicated *E. coli* DNA or poly[dIdC]-poly[dIdC]) and about 10 ng of ^{32}P-end-labeled DNA fragments. After 1 h incubation at room temperature matrices were recovered by centrifugation. Both the pellet and the supernatant fractions were treated with proteinase K, extracted with phenol and precipitated. The purified DNAs were electrophoretically resolved on 1% agarose or 8% polyacrylamide gel, gels were dried and autoradiographed.

Southwestern blot analysis. Total nuclear proteins (200 µg) and the nuclear matrix proteins (100 µg per slot) were fractionated in 10 % polyacrylamide/SDS gel [6]. The separated proteins were electrophoretically transferred onto nitrocellulose membrane (Hybond-C extra) in 25 mM Tris, 190 mM glycine and 20% methanol. Filter-bound proteins were renatured by incubation for 6 h at 20°C in 50 mM Tris (pH 7.4), 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol and 2.5% BSA in the hybridization oven. After washing with the binding buffer (the same composition as above except that 0.25% BSA was added) filters were incubated for 6 h at 20°C in 6 ml of the binding buffer supplemented with different amounts of sonicated *E. coli* DNA and about 50 ng of ^{32}P-end-labeled DNA probe. Then filters were washed twice with the binding buffer (without DNA), dried and autoradiographed.

RESULTS

We have studied binding of the established mouse kappa Ig MAR DNA and its subfragments (Fig. 1) to rat liver nuclear matrices obtained by the standard "high salt" method in the presence of magnesium ions (M/Mg^{2+}), or from nuclei preincubated with copper ions (M/Cu^{2+}) or EDTA (M/EDTA). The electrophoretical patterns of the 593-bp fragment of kappa MAR (and its subfragments) either bound to matrices (P) or present as unbound fractions in supernatant (S) are compared (Fig. 2). The 593-bp *Hin*dIII/*Eco*RI fragment of MAR DNA revealed the highest binding ability to copper pretreated matrices. In the presence of unlabeled *E. coli* competitor DNA the *Hin*dIII/*Eco*RI fragment interacted with proteins of M/Cu^{2+} matrices much more effectively than the control plasmid DNA. On the other hand, in the presence of competitor DNA similar binding of MAR and control DNA to M/EDTA matrices was observed (Fig. 2A). Binding of the two kappa MAR subfragments to M/Mg^{2+} and M/Cu^{2+} matrices is illustrated in Fig. 2B and 2C. Binding of the longer *Hin*dIII/*Ava*II (or *Hin*dIII/*Hin*fI) subfragment to the M/Mg^{2+} matrices was more effective as compared to binding of the shorter *Ava*II/*Eco*RI (or *Hin*fI/*Eco*RI) subfragment. The binding efficiency towards the M/Cu^{2+} matrices of both longer and shorter subfragments were similar.

Using the Southwestern analysis, in which binding of labeled DNA fragment to electrophoretically resolved proteins takes place, we tried to establish which proteins may be en-

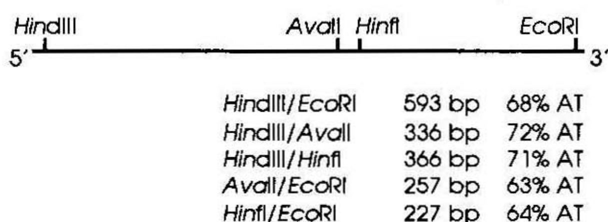

Fig. 1. Fragments of the MAR sequence of the mouse kappa immunoglobulin gene used for DNA binding studies.
The length of the fragments and AT-pair content in each fragment is shown.

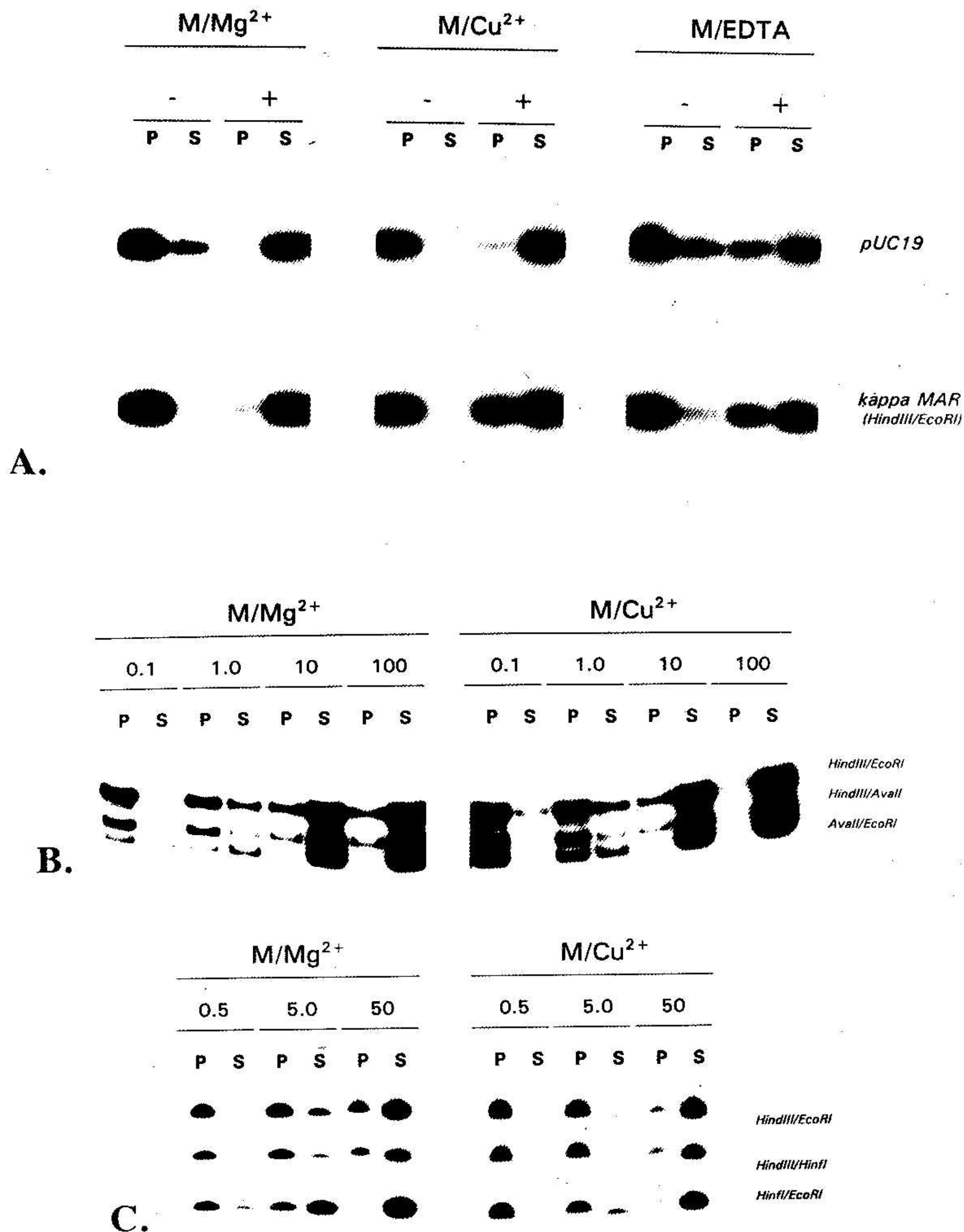

Fig. 2. Electrophoretic pattern of probed DNA incubated with proteins of different matrix preparations in bound (P, pellet) and unbound (S, supernatant) fractions.

A. Binding of *Hind*III/*Eco*RI MAR fragment to the standard "high salt" matrices (M/Mg^{2+}), to the copper stabilized matrices (M/Cu^{2+}) and to the matrices from EDTA washed nuclei (M/EDTA). Complexes were formed without competitor (–) and in the presence of 200 μg/ml of sonicated *E. coli* DNA (+). B. Binding of *Hind*III/*Eco*RI, *Hind*III/*Ava*II and *Ava*II/*Eco*RI MAR fragments to the M/Mg^{2+} and M/Cu^{2+} matrices. Complexes were formed in the presence of increasing amounts (0.1, 1.0, 10, 100 μg/ml) of poly[dIdC]-poly[dIdC]. C. Binding of *Hind*III/*Eco*RI, *Hind*III/*Hin*fI and *Hin*fI/*Eco*RI MAR fragments to the M/Mg^{2+} and M/Cu^{2+} matrices. Complexes were formed in the presence of increasing amounts (0.5, 5.0, 50 μg/ml) of poly[dIdC]-poly[dIdC].

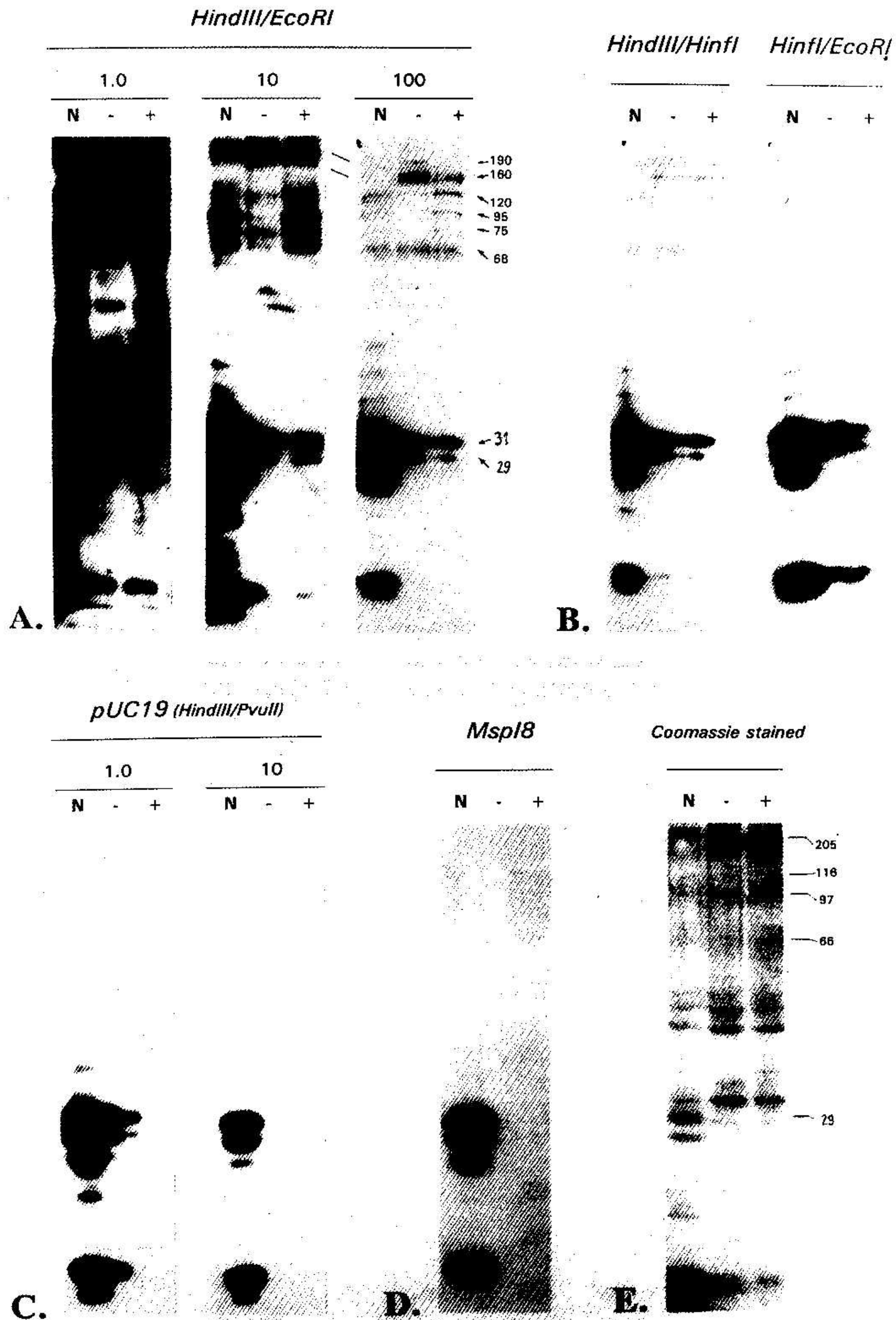

Fig. 3. Southwestern blot analysis of total nuclear proteins (N), proteins of the non-stabilized matrices M/Mg^{2+} (–) and proteins of copper stabilized matrices M/Cu^{2+} (+) interacting with kappa MAR fragments and non-MAR DNAs.

A. Proteins complexed with *Hin*dIII/*Eco*RI fragment of MAR in the presence of increasing amounts (1.0, 10, 100 μg/ml) of sonicated *E. coli* DNA. The molecular size (in kDa) of the matrix proteins which specifically interacted with MAR is marked. B. Proteins complexed with *Hin*dIII/*Hin*fI and *Hin*fI/*Eco*RI fragments of MAR in the presence of 10 μg/ml of sonicated *E. coli* DNA. C. Proteins complexed with *Hin*dIII/*Pvu*II fragment of pUC19 plasmid DNA in the presence of sonicated *E. coli* DNA (1.0 and 10 μg/ml). D. Proteins complexed with repetitive sequence MspI8 in the presence of 1.0 μg/ml of poly[dIdC]-poly[dIdC]. E. The Coomassie stained electrophoretic pattern of total nuclear and the matrix proteins. The position of molecular size markers (in kDa) is indicated.

gaged in specific MAR-nuclear matrix interactions. Figure 3 shows the binding of the MAR fragments to the specific rat liver nuclear proteins. Several matrix proteins with the molecular mass of about 29, 31, 68, 75, 95, 120, 160 and 190 kDa specifically bound the 593-bp *Hin*dIII/*Eco*RI MAR fragment. At least three protein bands of 75, 95 and 120 kDa from the M/Cu^{2+} matrices bound more effectively radioactive MAR fragments as compared to the M/Mg^{2+} matrices (Fig. 3A). The longer *Hin*dIII/*Hin*fI MAR subfragment bound to the same proteins, but with a lower affinity than the 593-bp fragment of kappa MAR. The shorter *Hin*fI/*Eco*RI subfragment bound only to the matrix proteins of about 31, 29 and about 20 kDa (Fig. 3B). We found that control non-MAR DNA (*Hin*dIII/*Pvu*II subfragment of pUC19 and MspI8 repetitive sequence) bound mainly to histones but not to the matrix-specific proteins (Fig. 3C and 3D).

Pretreatment of nuclei with copper increased the amount of proteins in the residual matrix fraction: M/Cu^{2+} matrices contained about 20% of total nuclear proteins while the amount of proteins in M/Mg^{2+} matrices was only about 10%. However, the nuclear matrices from either the copper treated or non-treated nuclei revealed a similar protein pattern, except for two protein bands in the range of 35–40 kDa which were specific for the M/Cu^{2+} matrices (Fig. 3E).

DISCUSSION

Copper ions are a factor affecting chromatin structure. Pretreatment of nuclei with Cu^{2+} was introduced to "stabilize" the scaffold structure against lithium diiodosalicylate (LIS) extraction [7]. It was found that the LIS-extracted nuclear halo's non-stabilized with copper lost their DNA-binding activities [8]. However, the mechanism of copper action remains unclear. We found that the interactions between the defined MAR sequence and the matrix proteins were significantly enhanced when nuclei were treated with Cu^{2+} before DNase digestion and salt extraction. On the other hand, on comparing the standard matrix preparations one can see that the copper pretreated matrices bound with increased efficiency to the MAR subfragment with a relatively low AT-pair content (high AT content is one of the characteristic features of MAR DNAs).

Many proteins have been suggested to constitute the nuclear skeleton, but only few of them were reported to interact specifically with MARs. The specific interactions of MAR sequences were found with ARBP, chicken nuclear matrix protein of 95 kDa. The counterparts of ARBP from other eukaryotic cells are of 70–110 kDa [9]. The other MAR-binding matrix proteins are rat liver lamin B_1 [10] and rat brain SP120 protein of 120 kDa [11]. The mouse liver nuclear actin, histone H1 and HMG proteins were also reported to bind MAR DNA [12]. Also purified yeast topoisomerase II preferentially bound MAR-containing DNA [13]. We believe that the MAR-binding matrix proteins from rat liver (according to their molecular size) correspond to the previously described proteins. We found that binding of the MAR DNA to some proteins of the Cu^{2+} pretreated matrices (most probably corresponding to the ARBP and SP120 proteins) was stronger than to the appropriate protein bands of standard, non-stabilized matrices. It seems likely that copper pretreatment enables these proteins to become "matrix" (i.e. by preventing them from salt extraction) or that copper ions stabilized some of their structural features essential for DNA binding.

We would like to express our thanks to Prof. S.V. Razin for the kind gift of pTZ19R plasmid.

REFERENCES

1. Gasser, S.M., Amati, B.B., Cardenas, M.E. & Hofmann, J.F.X. (1989) *Int. Rev. Cytol.* **119**, 57–96.
2. Lewis, C.D. & Laemmli, U.K. (1982) *Cell* **29**, 171–181.
3. Rzeszowska-Wolny, J., Razin, S., Puvion, E., Moreau, J. & Scherrer, K. (1988) *Biol. Cell.* **64**, 13–22.
4. Cockerill, P.N. & Garrard, W.T. (1986) *Cell* **44**, 273–282.
5. Rzeszowska-Wolny, J. & Rogoliński, J. (1994) *Acta Biochim. Polon.* **41**, 459–466.
6. Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: a laboratory manual.* Cold Spring Harbor Lab. Press, New York.
7. Mirkovitch, J., Mirault, M.E. & Laemmli, U.K. (1984) *Cell* **39**, 223–232.

8. Tsutsui, K., Tsutsui, K. & Muller, M.T. (1988) *J. Biol. Chem.* **263**, 7235–7241.
9. von Kries, J.P., Buhrmester, H. & Stratling, W.H. (1991) *Cell* **64**, 123–135.
10. Luderus, M.E.E., deGraaf, A., Mattia, E., den Blaauwen, J.L., Grande, M.A., deJong, L. & van Driel, R. (1992) *Cell* **70**, 949–959.
11. Tsutsui, K., Tsutsui, K., Okada, S., Watarai, S., Seki, S., Yasuda, T. & Shohmori, T. (1993) *J. Biol. Chem.* **268**, 12886–12894.
12. Ivanchenko, M. & Avramova, Z. (1992) *J. Cell. Biochem.* **50**, 190–200.
13. Adachi, Y., Kas, E. & Laemmli, U.K. (1989) *EMBO J.* **8**, 3997–4006.