

Nuclear Halo from *Bradysia hygida* (Diptera:Sciaridae) Salivary Gland Polytene Cells

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ABSTRACT

*A protocol for recovered nuclear halos from insect polytene nuclei after the extraction of the nuclear proteins using LIS detergent is reported in this work. Analysis was carried out using fluorescence and confocal laser scan microscopy. The extraction of nuclear halos was possible only with nuclei-fraction isolation in hypotonic buffer without spermine and spermidine. The recovered nuclear halos from *Bradysia hygida* salivary gland polytene nuclei, contributed greatly to the study of the structure and function of these special organelles.*

Key words: Insect polytene cells; Nuclear matrix; Nuclear halo; DNA loops; Salivary gland; *Bradysia hygida*

INTRODUCTION

The extraction of interphasic nucleus with detergent and 2 M NaCl provide a residual halo picture which represents the release of the DNA loops from nuclear matrix without DNA removal by restriction nucleases or DNase digestion (Cook and Brazell, 1976). The removal of the associated DNA reveals a nuclear matrix that consists basically of the external nuclear lamina connected to the intermediary filaments of the skeleton, an internal nuclear matrix composed of 9-13-nm filaments that form a highly organized network associated with 70% of the nuclear RNA (Anderson and Roberge, 1992; Nickerson and Pennan, 1992) and the nucleolus residual structure (Bereznev et al., 1995; Pederson, 1998; Pederson, 2000). The nuclear matrix collapses when submitted to RNase A digestion, indicating that associated RNAs are essential to its maintenance (Nickerson et al., 1989). Observations using

electronic microscopy and biochemical studies of chromosomes and nuclei lacking histones supported the hypothesis that eukaryotic DNA was organized in loops associated with the chromosomal skeleton (scaffold) or the nuclear matrix (Cook and Brazell, 1976; Paulson and Laemmli, 1977). Additional analyses demonstrated that these loops, roughly ranging from 5 to 100 kb or more, were anchored in the nuclear matrix through specific sequences called Scaffold Attached Regions (SARs) or Matrix Attached Regions (MARs) (Altieri et al., 1996; Berrios et al., 1985; Phi-Van and Strätling, 1990). The nuclear matrix forms a fibrogranular structure, resulting from the extraction of a high saline concentration (2M NaCl) or the use of detergents such as LIS (3.5 lithium diiodosalicylate). Regarding these conditions, nearly 80% of the nuclear proteins are removed. However, it also depends on cell type, as well as the isolation procedure of the nuclear matrix, the internal

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network and/or the remaining nucleolar fraction, which may be absent, while the external lamina is present in all the extractions (Neri et al., 1997). A large number of proteins with enzymatic functions such as oxidoreductases, transferases, hidrolases and glycoproteins are found associated with the nuclear matrix (Altieri et al., 1996; Ferraro et al., 1994; S'iakste and S'iakste, 1994), as well as enzymes such as DNA and RNA polymerases, and DNA metilases and ligases, which are involved in the synthesis processes (processing and repairing) (Berrios et al., 1985; Stein et al., 1991; Tubo and Berezney, 1987; Tubo and Berezney, 1987a, Vemuri et al., 1993). The presence of a minimum group of constituent proteins from the nuclear matrix such as topoisomerases, histone H1 and lamina, in plant and animal cells, suggests a conserved structure (Bouliskas, 1995). Some of the internal network proteins from the nuclear matrix are specific and some changes in this protein composition have been related with tumor growth, viral infection, mitosis, cell differentiation, embryo development and sign transduction (Altieri et al., 1996; Brancolini and Schneider, 1991; Stuurman et al., 1990; Zbarskii, 1990). The characterization of specific nuclear matrix proteins has as a goal the possible use of these proteins as markers of malignant phenotypes in the early diagnosis of bladder, prostate, breast and larynx cancers, among others (Barboro et al., 1996; Donat et al., 1996; Cupo, 1991; Filatova and Zbarskii, 1993; Getzenberg et al., 1996). In *Drosophila* Kc culture cells, the identification of proteins in the chromosomal skeleton or in the nuclear matrix revealed proteins of high molecular weight (proteins Sc1 and Sc2, with 170 and 135 kDa, respectively), in addition to other smaller proteins (Lewis and Laemmli, 1982). The most abundant, Sc1, was recorded as Topoisomerase II (Earnshaw et al., 1985). In *Bombyx mori*, the abundance of DNA polymerase in the silk gland cells and the strong attachment of this enzyme to the nuclear matrix suggest that both are involved in the endoduplication process that occurs in these cells (Niranjanakumari and Gopinathan, 1991). The investigation of the nuclear matrix in insect polytene cells leads to the discovery of the polytene chromosome architecture. In addition, with the preservation of the nuclear matrix association sites, it is also an essential condition for nuclear halo recovering, and further functional analyses. The halo picture, which comprises the nuclear matrix structure with the attached loops,

must be suitable for restriction enzyme digestion, revealing the MARs/SARs. These regions have been related to specific sequences located in *Drosophila* polytene chromosome bands and inter-bands, but the investigations are carried out using nuclei other than the polytene nuclei fraction (Iarovaia et al., 1996; Mirkovitch et al., 1986; Schwartz et al., 1999). In a previous paper, our laboratory reported that in hypo-osmotic buffers and during the absence of polyamines such as spermine and spermidine, it is possible to obtain halos from *Drosophila* salivary gland polytene nuclei using LIS detergent (lithium diiodosalicylate) for protein extraction (Mikami et al., 2001). Regarding the beginning of the characterization of the nuclear matrix of the *Bradysia hygida* salivary gland polytene nucleus, this trial describes the protocol for recovering nuclear halos of Sciaridae, since this salivary gland and polytene nucleus are very different from the ones observed in *Drosophila*. Along this line of investigation, this nuclear halo fraction offers the possibility of MARs mapping in the polytene nuclei during the differential replication and developmental transcription regulation process that occurs in this special interphasic nucleus.

MATERIALS AND METHODS

Bradysia hygida (Sauaia and Alves, 1968) rearing, have been previously reported (Laicine et al., 1984; da Conceição Silva and Fernandez, 2000). Each larva presents a pair of salivary glands that is divided into three regions: S1, S2 and S3. The salivary gland polytene cells present four chromosomes where the DNA and RNA puffs are developed at the end of the larval stage. DNA puffs are sites where differential replication and gene transcription regulation occur during larval development (Monesi et al., 1995; Fontes et al., 1999). The salivary glands were dissected from last instar larvae under a Zeiss stereomicroscope and stored in eppendorf tubes at 0.7% NaCl and 84% glycerol (1:1) at -20°C. To purify the polytene nucleus, the samples with one hundred dissected glands were washed twice in cold CWB buffer (5 mM Tris-HCl pH 7.4; 50 mM KCl; 0.5% thiodiglicol; 0.25 mM PMSF and 0.5 mM EDTA) and incubated for 15 minutes in 1 ml of cold CWBD buffer -CWB supplemented with 0.05% digitonin -(Dijkwel et al., 1991; Fernandez et al., 1997) in ice before being disrupted ten times in a

syringe with a large needle (type 21G1). The suspension was layered over 0.4 ml of 20% glycerol in cold CWBD buffer, in 15-ml conical centrifuge tubes (Corning). The polytene nuclei were recovered at 4°C for 5 min at 1,000 rpm in a Beckman GS 15R centrifuge (swing S4180 rotor) and stabilized for 25 min at 4°C in 0.4 ml of CWB without EDTA, complemented with 0.0025 mM CuSO₄. The suspension was then poured into eppendorf tubes with 1 ml of LIS buffer (10 mM of 3.5 diiodosalicylic acid; 100 mM of lithium acetate; 0.05% digitonin; 0.05 mM spermine; 0.125 mM of spermidine; 0.25 mM of PMSF and 20 mM of HEPES/KOH, pH 7.2). It was incubated for 15 min at room temperature and the nuclear halos were collected using centrifugation at 3,000 rpm for 10 min at 4°C. The pellet was washed once with cold CWBD without EDTA. 10 µl of the solution containing polytene nuclei (before LIS extraction) and nuclear halos were placed onto glass slides and stained with 1 µl propidium iodide (20 µg/ml) in anti-fading solution (2% DABCO in solution containing glycerol and PBS 1:1). The preparations were analyzed using a Carl-Zeiss Axioskop MC 100 photomicroscope with a x40, 1.3 NA planapochromat objective lens and combination filters (excitation filter 546 nm; dichroic mirror 580 nm and barrier filter 590 nm) and photographed with TEMAX (KODAK) film. Aliquots were also observed in a Confocal Laser Scan microscope (Carl-Zeiss LSM 410). Samples were observed with a x40, 1.3 NA planapochromat objective lens and a laser (543 nm). The pictures were saved as digital images. For electrophoretic analysis, the extracted nuclear halo (in a fluffy pellet form) was recovered and washed three times in buffer for enzyme restriction on the recommendation of the enzyme manufacturer. After the washes, the nuclear halo was recovered with 1 ml of restriction buffer and incubated at 37°C with 100 to 300U/ml of the restriction enzymes *EcoRI* and *XhoI* for 2 hs, adding also 50 µg/ml of RNAase A in the last 30 minutes. After cleavage, the nuclear matrix and associated DNA (matrix fraction) were recovered in a Beckman GS 15R centrifuge (swing S4180 rotor) at 4,000 rpm for 10 minutes at 4°C. The loop fraction, supernatant material, was precipitated with 0.2M NaCl and 0.7 volumes of isopropanol and stored at -20°C. The main fraction was recovered in proteinase K buffer (10 mM Tris-HCl pH 8.0; 1 mM EDTA; 0.3 M NaCl), digested using

proteinase K (500 µg/ml) addition for 18-24 hours at 30°C or 2 hours at 37°C, extracted using phenol/chloroform and precipitated as described above. The matrix and loop fractions were centrifuged at 14,000 rpm for 30 minutes at 4°C, and recovered in 30 µl of TE buffer (10 mM Tris-HCl pH 8.0; 1 mM EDTA). Electrophoresis occurred on a 0.7% agarose gel in TBE (45 mM Tris-borate, 1mM EDTA, pH 8.0) with ethidium bromide and photographed using Polaroid 667 film.

RESULTS

The nuclear halos were recovered after the extraction of the nuclear proteins using LIS. This detergent removed almost 80% of the nuclear proteins, so that a DNA halo could be observed. The recovery of nuclear halos was only possible using hypotonic buffer free of both spermine and spermidine. Fig. 1 shows different polytene nucleus and halo aspects using fluorescence and confocal microscopy. In Fig. 1a, the nucleus belonged to the control, which was purified with buffers CWB and CWBD supplemented with spermine and spermidine. No chromatin decondensation was observed in any of the nuclei of this fraction. In 1b, the polytene chromosome architecture was disorganized when the polyamines spermidine and spermine were not present in CWB and CWBD buffers. In that condition, almost all of the polytene nuclei from LIS fraction showed total chromosome decondensation, which is essential for the posterior development of the extraction protocol.

After the extraction of the nuclear proteins using LIS, the fluorescence microscopy analyses supply the visualization of a halo picture called nuclear halo (Fig. 1c). Analyses of nuclear halo optic sections in a LSCM (Fig. 1d-f) showed a uniform picture at different depths (2-µm intervals), due to a total decondensation of the chromosomes after extraction using LIS. The existence of the DNA halo, observed in both fluorescence and confocal microscopes, suggested the presence of a structure, the nuclear matrix, which was possibly constituted by the arrangement of the nuclear lamina, filaments and associated proteins. The different plans supplied by confocal microscopy provided the images that confirmed the total chromatin decondensation, where images with a repetitive

pattern in different focal plans did not allow the visualization of the three-dimensional structuring of the nuclei. The electrophoretic profile of DNA associated with the nuclear matrix and released from the loop after digestion using restriction enzymes can be observed in Fig. 2. Almost 75% of the DNA was found in the loop fraction. This distribution between matrix and loop fractions was

also observed in other systems (Fernandez et al., 1997). Another typical feature was that the matrix fraction showed DNA fragments with higher molecular weight than the ones observed in the loop fraction. These fractions could be used in experimental investigations to determine MARs/SARs sites.

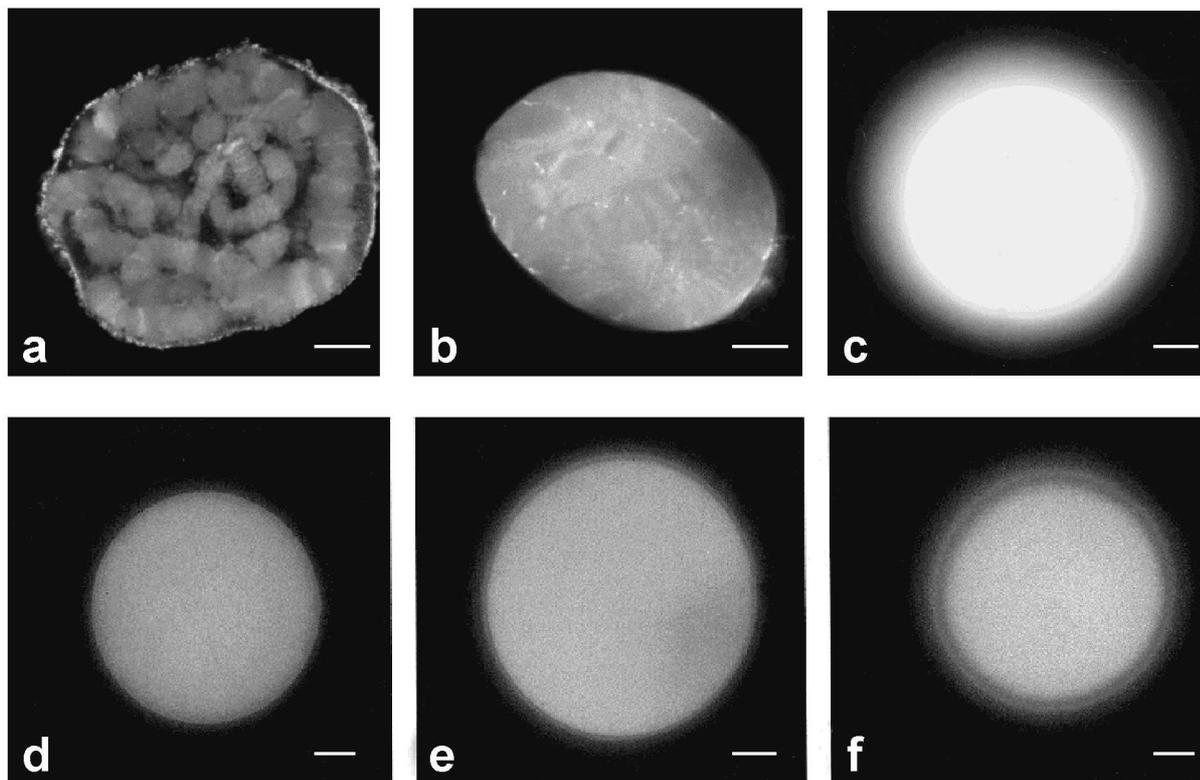


Figure 1 - *Bradysia hygida* salivary gland polytene nucleus and nuclear halos.

- a) optical section of polytene nucleus with the chromatin condensed in chromosomes;
 - b) optical section of the polytene nucleus showing the decondensation of the chromosomes;
 - c-f) nuclear halos from salivary gland polytene nucleus (only picture c was taken in a fluorescence microscope (Carl-Zeiss Axioskop);
 - d-f) 2- μ m optical sections of the Z axis interval were taken in a Confocal Laser Scan microscope (Carl-Zeiss LSM 410).
- All the nuclei or nuclear halos were stained with propidium iodide in anti-fading solution. Bar represents 10 μ m.

DISCUSSION

The possibility that polytene nucleus extraction results in a nuclear halo depends on two basic conditions: the CWB hypotonic buffer conditions, which are important for chromatin decondensation, as described for isolated nuclei in other systems (Delpire et al., 1985); polytene nuclei buffer extraction must be free of spermine

and spermidine. Although the effects of these polyamines on the chromatin structure are not completely known, their presence is important in the structuring of the chromosomes (Belmont et al., 1989). The recovery of the nuclear halos of the *B. hygida* salivary gland polytene nucleus indicated the existence of a substructure such as the nuclear matrix in these special nuclei. Nuclear halo visualization using fluorescence and confocal

microscopy suggested that the loops were associated with an internal structure that should participate actively in processes such as an additional replication and transcription that occurred in polytene nuclei. In our laboratory, matrix and loop fractions from *B. hygida* salivary gland polytene nuclei were used for MARs/SARs analyses of an amplified *BhC4-1* gene promoter

segment. The results suggested a functional relationship between the association sites and the transcription, as well as the replication process in the salivary gland polytene nucleus (Mikami, 2000). This protocol is highly reproducible if the total decondensation of the nuclear DNA is achieved.

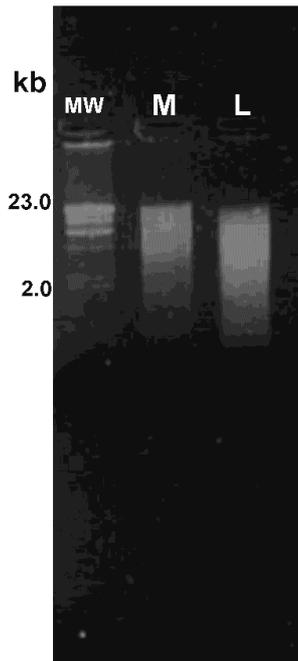


Figure 2 - Nuclear matrix and DNA loop fractions from a *Bradysia hygida* salivary gland polytene nucleus. The recovered nuclear halos in CWBD were submitted to *EcoRI* and *XhoI* digestion and the DNA associated with the nuclear matrix (M) separated from liberates DNA (L). The 0.7% agarose gel was running with 2 μ l of ethidium bromide (1 mg/ml). MW - molecular weight marker: *HindIII*-digested lambda DNA.

This is possible in hypotonic buffer (CWBD). For *D. melanogaster* salivary gland polytene nuclei, the disruption of the salivary glands and chromosome decondensation were very rapid; but for *B. hygida*, a 15-minute incubation before syringe disruption was necessary. After that, it was imperative not to relax the loops too much with extensive time in LIS buffer incubation. For *D. melanogaster* preparations LIS incubation was carried out for 5 minutes (Mikami et al., 2001) but the same procedure took 15 minutes in *B. hygida* polytene nuclei fractions. The salivary gland tissue complexity (Laicine et al., 1984) and the larger DNA quantity (~ 3 ng/nucleus) in the *B. hygida*

polytene nucleus could explain these singularities in each protocol.

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RESUMO

Observações à microscopia eletrônica e estudos bioquímicos de cromossomos e núcleos sem histonas tem suportado a hipótese que o DNA de eucariotos é organizado em alças associadas com o esqueleto cromossômico ou à matriz nuclear. A observação da matriz nuclear sem a remoção do DNA, através da digestão com enzimas de restrição, apresenta uma figura em halo que representa a liberação das alças de DNA. Um protocolo para a obtenção de halos nucleares de núcleos politênicos de insetos, através da extração de proteínas usando o detergente LIS, é reportado nesse trabalho. Foram realizadas análises utilizando-se microscopia de fluorescência e microscopia de varredura confocal a laser. A extração de halos nucleares foi possível somente com o isolamento da fração nuclear em tampão sem espermina e espermidina. A obtenção de halos nucleares de núcleos politênicos de glândula salivar de *Bradysia hygida* contribuiu significativamente para o estudo da estrutura e função dessas organelas tão especiais.

REFERENCES

- Altieri, F.; Maras, B.; Ferraro, A. and Turano, C. (1996), Purification of a 60-kDa protein from chicken liver associated with the internal nuclear matrix and closely related to carboxylesterases. *Eur. J. Biochem.*, **236**, 806-813.
- Anderson, H. J. and Roberge, M. (1992), DNA Topoisomerase II: A review of its involvement in chromosome structure, DNA replication, transcription and mitosis. *Cell Biol. Int. Rep.*, **16**, 717-724.
- Barboro, P.; Alberti, I.; Sanna, P.; Parodi, S.; Balbi, C.; Allera, C. and Patrone, E. (1996), Changes in the cytoskeletal and nuclear matrix proteins in rat hepatocyte neoplastic nodules in their relation to the process of transformation. *Exp. Cell Res.*, **225**, 2315-2332.
- Belmont, A. S.; Braunfeld, M. B.; Sedat, J. W. and Agard, D. A. (1989), Large-scale chromatin structural domains within mitotic and interphase chromosomes in vivo and in vitro. *Chromosoma*, **98**, 129-143.
- Berezney, R.; Mortillaro, M. J.; Ma, H.; Wei, X. and Samarabandu, J. (1995), The nuclear matrix: a structural milieu for genomic function. *Int Rev Cytol*, **162A**, 1-65.
- Berrios, M.; Osheroff, N. and Fisher, P. A. (1985), In situ localization of DNA Topoisomerase II, a major polypeptide component of the Drosophila nuclear matrix fraction. *Proc. Natl. Acad. Sci. USA*, **82**, 4142-4150.
- Bouliskas, T. (1995), Chromatin domains and prediction of MAR sequences. *Int. Rev. Cytol*, **162A**, 279-388.
- Brancolini, C. and Schneider, C. (1991), Change in the expression of a nuclear matrix-associated protein is correlated with cellular transformation. *Proc. Natl. Acad. Sci. USA*, **88**, 6936-6940.
- Cook, P. R. and Brazell, I. A. (1976), Characterization of nuclear structures containing superhelical DNA. *J. Cell Sci.*, **22**, 303-324.
- Cupo, J. F. (1991), Electrophoretic analysis of nuclear matrix proteins and the potential clinical applications. *J. Chromatography*, **569**, 389-406.
- da Conceição Silva, J. L. and Fernandez, M. A. (2000), Feulgen stain in heterogeneous egg samples from non-synchronized *Bradysia hygida* (Diptera: Sciaridae) laboratory culture. *Cytologia*, **65**, 167-171.
- Delpire, E.; Duchêne, C.; Goessens, G. and Gilles, R. (1985), Effects of osmotic shocks on the ultrastructure of different tissues and cell types. *Exper Cell Research*, **160**, 106-116.
- Dijkwel, P. A.; Vaughn, J. P. and Hamlin, J. (1991), Mapping of replication initiation sites in mammalian genomes by two-dimensional gel analysis: stabilization and enrichment of replication intermediates by isolation on the nuclear matrix. *Mol. Cell Biol.*, **11**, 3850-3859.
- Donat, T. L.; Sakr, W.; Lehr, J. E. and Pienta, K. J. (1996), Unique nuclear matrix protein alterations in head and neck squamous cell carcinomas: intermediate biomarker candidates. *Otolaryngol Head Neck Surg*, **114**, 387-393.
- Earnshaw, W. C.; Halligan, B. Cooke, C. A. and Heck, M. M. S. and Liu, L. F. (1985), Topoisomerase II is a structural component of mitotic chromosome scaffolds. *J. Cell Biol.*, **100**, 1706-1715.
- Fernandez, M. A.; Baron, B.; Prigent, M.; Toledo F.; Buttin, G. and Debatisse, M. (1997), Matrix attachment regions and transcriptions units in a polygenic mammalian locus overlapping two isochores. *J. Cell Biochem.*, **67**, 541-551.
- Ferraro, A.; Eufemi, M.; Altieri, F.; Cervoni, L. and Turano, C. (1994), Glycoproteins of the nuclear matrix from chicken liver cells. *Cell Biol. Inter.*, **18**, 655-661.
- Filatova, L. S. and Zbarskii, I. B. (1993), The immunochemical demonstration of high-molecular proteins in the nuclear matrix of tumor cells. *Bull. Esp. Biol. Med.*, **116**, 418-421.

- Fontes, A. M.; Conacci, M. E.; Monesi, N.; de Almeida, J. C. and Paçó-Larson, M. L. (1999), The DNA puff *BhB10-1* gene encodes a glycine-rich protein secreted by the late larvae salivary glands of *Bradysia hygida*. *Gene*, **231**, 67-75.
- Gerdes, M. G.; Carter, K. C.; Moen, P. T. and Lawrence, J. B. (1994), Dynamic changes in the higher-level chromatin organization of specific sequences revealed by in situ hybridization to nuclear halos. *J. Cell Biol.*, **126**, 289-304.
- Getzenberg, R. H.; Konety, B. R.; Oeler, T. A.; Quigley, M. M.; Hakam, A.; Becich, M. J. and Bahnsen, R. R. (1996), Bladder cancer-associated nuclear matrix proteins. *Cancer Res.*, **56**, 1690-1694.
- Iarovaia, O.; Hancock, R.; Lagarkova, M.; Miassod, R. and Razin, V. S. (1996), Mapping of genomic DNA loop organization in a 500-kilobase region of the *Drosophila* X chromosome by topoisomerase II-mediated DNA loop excision protocol. *Mol. Cell Biol.*, **16**, 302-308.
- Laicine, E. M.; Alves, M. A. R.; de Almeida, J. C.; Rizzo, E.; Albernaz, W. C. and Sauaia, H. (1984), Development of DNA puffs and patterns of polypeptide synthesis in the salivary glands of *Bradysia hygida*. *Chromosoma*, **89**, 280-284.
- Lewis, C. D. and Laemmli, U. K. (1982), Higher order metaphase chromosome structure: evidence for metalloprotein interactions. *Cell*, **29**, 171-181.
- Mikami, L. R. (2000), Associação à matriz nuclear do segmento à montante do gene *BhC4-1* em núcleo politênico de *Bradysia hygida* é dependente da atividade de transcrição na região. Máster thesis, Universidade Estadual de Maringá, Paraná, Brasil.
- Mikami, L. R.; Polinarski, C. A. and Fernandez, M. A. (2001), *Drosophila* salivary gland polytene nuclear halo: a new approach for polytene nuclear matrix analysis. *Cytologia*, **66**, 177-181
- Mirkovitch, J.; Spierer, P. and Laemmli, U. K. (1986), Genes and loops in 320,000 base-pairs of the *Drosophila melanogaster* chromosome. *J. Mol. Biol.*, **190**, 225-258.
- Monesi, N.; Fernandez, M. A.; Fontes, A. M.; Basso Jr., L. R.; Nakanishi, Y.; Baron, B.; Buttin, G. and Paçó-Larson, M. L. (1995), Molecular characterization of a 18 kb segment of DNA puff C4 de *B. hygida* (Diptera: Sciaridae). *Chromosoma*, **103**, 715-724.
- Neri, L. M.; Fackelmayer, F. O.; Zweyer, M.; Kohwi-Shigematsu, T. and Martelli, A. M. (1997), Subnuclear localization of S/Mar-binding proteins is differently affected by in vitro stabilization with heat or Cu²⁺. *Chromosoma*, **106**, 81-93.
- Nickerson, J. A.; Krochmalnic, G.; Wan, K. M., and Pennan, S. (1989), Chromatin architecture and nuclear RNA. *Proc. Natl. Acad. Sci. USA*, **86**, 177-181.
- Nickerson, J. A. and Pennan, S. (1992), Localization of nuclear matrix core filament proteins. *Cell Biol. Int. Rep.*, **16**, 811-826.
- Niranjanakumari, S. and Gopinathan, K. P. (1991), Characterisation of the DNA-polymerase-alpha-primase complex from the silk glands of *Bombyx mori*. *Europe J. Biochem.*, **201**, 431-438.
- Paulson, J. R. and Laemmli, U. K. (1977), The structure of histone-depleted metaphase chromosomes. *Cell*, **12**, 817-828.
- Pederson, T. (1998), Thinking about a nuclear matrix. *J. Mol. Biol.*, **277**, 147-159.
- Pederson, T. (2000), Half a century of "The Nuclear Matrix". *Mol. Biol. of the Cell*, **11**, 799-805.
- Phi-Van, L. and Strätling, W. H. (1990), Association of DNA with nuclear matrix. *Prog. Mol. Subcell Biol.*, **11**, 1-11.
- S'iakste, N. I. and S'iakste, T. G. (1994), Enzymatic activity of the nuclear matrix. *Biokhimiia*, **59**, 1663-1674.
- Sauaia, H. and Alves, M. A. R. (1968), A description of a new species of *Bradysia* (Diptera, Sciaridae). *Pap. Avul. Zool.*, **22**, 85-88.
- Schwartz, Y. B.; Ioidinkova, E. S.; Demakov, S. A.; Razin, S. V. and Zhimulev, I. F. (1999), Interbands of *Drosophila melanogaster* polytene chromosomes contain matrix association regions. *J Cell Biochem*, **72**, 368-372.
- Stein, G. S.; Lian, J. B.; Dworetzky, S. I.; Owen, T. A.; Bortell, R.; Bidwell, J. P. and Van Wijnen, A. J. (1991), Regulation of transcription-factor activity during growth and differentiation: involvement of the nuclear matrix in concentration and localization of promoter binding proteins. *J. Cell. Biochem.*, **47**, 300-305.
- Stuurman, N.; Meijne, A. M. L.; Van Der Pol, A. J.; DE Jong, L.; Van Driel, R. and Van Renswoude, J. (1990), The nuclear matrix from cells of different origin. *J. Biol. Chem.*, **265**, 5460-5465.
- Tube, R. A. and Berezney, R. (1987), Identification of 100 and 150 S DNA polymerase α -primase megacomplexes solubilized from the nuclear matrix of regenerating rat liver. *J. Biol. Chem.*, **262**, 5857-5865.
- Tube, R. A. and Berezney, R. (1987), Nuclear matrix-bound DNA primase: elucidation of an RNA priming system in nuclear matrix isolated from regenerating rat liver. *J. Biol. Chem.*, **262**, 6637-6642.
- Vemuri, M. C.; Raju, N. N. and Malhotra, S. K. (1993), Recent advances in nuclear matrix function. *Cytobios*, **76**, 117-128.
- Zbarskii, I. B. (1990), Skeletal structures of the cell nucleus in the normal state and pathology. *Vest. Akad. Med. Nauk SSSR*, **2**, 8-10.

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