**Drosophila Salivary Gland Polytenic Nuclear Halo: a New Approach for Polytenic Nuclear Matrix Analysis**

Liya Regina Mikami, Celso Aparecido Polinarski, José Luis da Conceição Silva and Maria Aparecida Fernandez*

Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, 87020-900, Maringá, Paraná Brasil

Accepted March 6, 2001

**Summary**  This work presents a procedure to perform nuclear halos, nuclear matrix with the associated DNA loops, from *Drosophila* salivary gland polytenic nucleus. The purification with hypo-osmotic buffers in the absence of polyamines, as spermine and spermidine, whose function seems to be related to the chromatin compaction, was considered essential. The non-compacted polytenic chromosome in the giant nucleus could be used for protein extraction that allows the liberation of the loops. The profile of the DNA matrix and loop fractions, left after nuclear halo digestion with restriction enzyme, confirms the relationship between these fractions suitable for functional analysis. This procedure could be important to obtain a direct relationship between nuclear matrix association and transcription process in the interphase polytenic chromosomes of *Drosophila* salivary gland nucleus.

**Key words**  Salivary polytenic nucleus, *Drosophila*, Nuclear halos, Nuclear matrix.

The description of the induced “lampbrush stage” in the giant polytenic chromosomes date from many years ago (Sorsa et al. 1970). This effect was possible with urea-alkali treatment of the *Drosophila* polytenic nucleus. In this condition, masses of the coiled and folded fibrils with 15 to 50 nm were observed around a fibrillar core, due to a removal of at least most of the acidic proteins.

The giant chromosomes are highly organized in their nucleus. The position of the polytenic chromosomes within the salivary gland nucleus is not at random. They are located preferentially on the nucleus periphery with the chromocenter and intercalary heterochromatin attached to the nuclear membrane (Agard and Sedat 1983, Mathog et al. 1984). Although no correlation was observed between this attachment and transcription process (Hochstrasser and Sedat 1987a, b), the occurrence of the transitional associations remains to be studied.

The disruption of the polytenic chromosome architecture, with the preservation of the nuclear matrix association sites, is an indispensable condition for nuclear halos recovering, and further functional analysis. The halo picture, that comprises the nuclear matrix structure with the attached loops, must be suitable for restriction enzymes digestion, revealing the MARs (Nuclear Matrix Attachment Regions) or SARs (Scaffold Attachment Regions). MARs/SARs regions have been related to specific sequences located in *Drosophila* polytenic chromosome bands and interbands, but the investigations are carried out with other nuclei rather than the homogenous polytenic nuclei fraction (Mirkovich et al. 1986, Iarovaia et al. 1996, Schwartz et al. 1999).

In this paper it is reported that the hypo-osmotic buffers and the absence of polyamines, as spermine and spermidine, whose function seems to be related to the chromatin compaction (Delpire et al. 1985, Belmont et al. 1989) allow the obtainment of halos from *Drosophila* salivary gland polytenic nuclei, using the detergent LIS (lithium diiodosalicylate) for protein extraction. Figures of non-compacted polytenic chromosome nucleus before and after protein extraction and the profile

*Corresponding author, e-mail: mafernandez@uem.br
of the DNA matrix and loop fractions are shown. This procedure is important to obtain a direct relationship between nuclear association and functional events in the interphase polytene chromosomes of *Drosophila* salivary gland nucleus.

Material and methods

**Insect culture**

*Drosophila melanogaster* is maintained in culture at 22°C in 250 ml glass bottles containing alimentary diet composed of sugar cane, yeast, corn flour and agar. In its life cycle 4 different stages occur: embryo, larva, pupa and adult, and the larva presents 3 stadia: L1, L2 and L3 with the respective duration of 24, 36 and 48 h. At the end of the L3 the larvae stop feeding and migrate to the flask wall for the pupal molt and the ecdysis.

In this work L3 larvae were collected, its salivary glands were dissected under stereomicroscope and stored at −20°C in a solution containing 0.7% NaCl and 84% glycerol (1:1).

**Nuclear halo preparation**

The methodology was the same for *Bradysia hygida* salivary gland polytene nucleus (Polinarski, C. A., unpublished results). Two samples, with 100 salivary glands each, were carried out in ice, except when there were contrary indications. For nuclei purification steps, the used buffers were CWB buffer (5 mM Tris-HCl pH 7.4, 50 mM KCl, 0.5% thiodiglycol, 0.25 mM PMSF and 0.5 mM EDTA, Dijkwel and Hamlin 1988, Fernandez et al. 1997), or A buffer (60 mM KCl, 15 mM NaCl, 1 mM EDTA, 0.1 mM EGTA, 15 mM Tris-HCl pH 7.4, 0.5 mM DTE, 0.1 mM PMSF, Shermoen and Beckendorf 1982). Independently of the buffer used, the 2 samples were always purified in the same buffer, but only one of the samples received the addition of the 0.05 mM spermine and 0.125 mM spermidine. We detail here only the method with CWB buffer because the procedures and results were the same.

The salivary glands samples were washed twice in cold CWB buffer and homogenized in 1 ml of cold buffer supplemented with 0.05% digitonin, CWBD buffer, by 3 passages in large needle syringe type 21G1. The homogenate was carefully layered over 1 ml of 20% glycerol in cold CWBD buffer, and centrifuged at 2,000 rpm in Beckman (swing S4180 rotor) centrifuge for 10 min at 4°C. The supernatant was removed by aspiration and the nucleus fraction recovered in 200 µl of CWBD buffer. The nuclei were stabilized with 0.0025 mM CuSO₄ at 0°C during 20 min, and 10 percent of this sample was taken for nuclei microscopy analysis. The nuclei fractions were then injected in 1 ml of the LIS buffer (10 mM LIS, 100 mM lithium acetate, 0.05% digitin, 0.05 mM spermine, 0.125 mM spermidine, 0.25 mM PMSF, 20 mM Hepes/KOH pH 7.2) and incubated for 5 min at room temperature. The extracted nuclear halo, in a fluffy pellet form, was recovered and washed three times in buffer for enzyme restriction recommended by the enzyme manufactory, and a sample for nuclear halo microscopy analysis was taken. After the washes, the nuclear halo was recovered in 1 ml of restriction buffer and incubated at 37°C with 100 to 300 U/ml of the restriction enzyme EcoRI during 2 h, with the addition of 50 µg/ml of RNase A in the last 30 min. After the cleavage, nuclear matrix and associated DNA (matrix fraction) were recovered by centrifugation at 4,000 rpm during 10 min at 4°C. The loop fraction, supernatant material, was precipitated with 0.2 M 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) and digested with Proteinase K (500 µg/ml) addition, during 18–24 h at 30°C or 2 h at 37°C, extracted with phenol/chloroform and precipitated as described above. The matrix and loop fractions were centrifuged at 14,000 rpm during 30 min at 4°C, and recovered in 30µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).
Fluorescence microscopy analysis

10 μl of the nuclei (before LIS extraction) and nuclear halo fractions, of both extractions, with and without spermine and spermidine, were mixed with 1 μl fluorescence dye propidium iodide or DAPI (1 μg/ml) in antifading solution (2% DABCO in solution containing glycerol and PBS 1:1). The preparations were analyzed in the appropriated fluorescence filter using the photomicroscope Axioscop Zeiss and photographed in Temax (KODAK) films.

Electrophoresis analysis of the matrix and loop enzyme restriction digested fractions

0.7% agarose gels in TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) with ethidium bromide were run with the matrix and loop enzyme restriction digested fractions, and photographed with Polaroid film.

Results and discussion

In order to obtain the nuclear halos, nuclear matrix with the associated DNA loops, of Drosophila salivary gland polytene nucleus, a new experimental approach was standardized with hypotonic buffers without polyamines addition. Several experiments were carried out in both CWB (Dijkwel and Hamlin 1988, Fernandez et al. 1997) and A buffer (Shermoen and Beckendorf 1982) with the same results. No important differences were found with one or another buffer for polytene nucleus isolation and the disruption of the chromosomes compaction. The hypotonic composition of both CWB and A buffers take advantages when the disruption of the compacted chromatin is necessary. It has been determined that a decondensation of the chromatin and a fragmentation of the granular material in the nucleolus are observed when hypo-osmotic conditions are applied for purification of the cells and nucleus (Delpire et al. 1985). Our results appoint that a hypotonic buffer have an important role in the polytene chromosome decondensation. Another point was the polyamines absence. When the polyamines spermidine and spermine were present in CWB buffer, almost all of the polytene nuclei purified did not show total chromosome decondensation (Fig. 1A). The action of the polyamines over the stabilization of the chromosome condensation was previously reported (Belmont et al. 1989), and here these compounds block the disruption of the chromosome structure and the nuclear halo recovered. In the Fig. 1B a polytene nucleus purified with CWB buffer without spermine and spermidine is shown. It is a notable picture, showing a total decondensation of the chromosomes, and the nucleus looks like a common interphase nucleus. Only this kind of the nucleus can be submitted to the LIS extraction (Fig. 1C). When the nuclei were purified with polyamines and submitted to LIS extraction, almost all the nuclei were fragmented. This effect is

![Image](https://example.com/image1.png)

Fig. 1. Drosophila polytene nucleus and nuclear halo. Salivary gland giant nuclei were purified in CWBD buffer with (A) and whitout (B) polyamines spermidine and spermine. The nuclear halo (C) was recovered after LIS extraction. Propidium iodide stain. Bar=5 μm.
devoid a large portion of the chromatin, in compacted form, that are released out of the nucleus by the purification steps and LIS extraction. The recovery of the nuclear halo in these conditions is difficult.

The electrophoretic profile of DNA associated to nuclear matrix and released from the loop after digestion with restriction enzyme can be observed in the Fig. 2. 50–75% of the DNA was found in the loop fraction. This distribution between matrix and loop fractions is also observed in other systems (Dijkwel and Hamlin 1988, Fernandez et al. 1997). Another typical feature is that the matrix fraction shows DNA fragments with higher molecular weight than the observed in loop fraction. These fractions can be used in experimental investigations to determine MARs/SARs sites. In our lab, matrix and loop fractions from salivary gland polytene nucleus of Drosophila transgenic lines (Monesi et al. 1998) and Bradysia hygida (Sauaia and Alves 1968, Laicine et al. 1984, da Conceição Silva and Fernandez 2000) are used for MARs/SARs analysis of an amplified BhC4-1 gene promoter segment. The results indicate that a functional relationship between the association sites and the transcriptional process in the salivary gland polytene nucleus are observed (Mikami, L. R., unpublished results).

In conclusion, our work describes, for the first time, an approach suitable for the polytene nuclear matrix analysis, opening the opportunity to a direct relationship between nuclear organization and function in these special organelles.

Acknowledgments

We thank Valmir Peron and Jimes R. da S. Santos for their dedicated technical assistance, and Vanessa Pinatto Gaspar for revising the manuscript. The authors are indebted to Dr. Nadia Monesi and Dr. Maria Luiza Paço-Larson from Laboratório de Biologia Celular do Departamento de Morfologia da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Brasil, due to important discussions and support. Mikami L. R. received a master fellowship from Conselho Nacional de Desenvolvimento Tecnológico-CNPq. This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq and The Third World Academy of Sciences-TWAS.
References


