Chromosoma (1996) 104:386–392

CHROMOSOMA


# A male-specific nuclease-resistant chromatin fraction in the mealybug *Planococcus lilacinus*

**Sanjeev Khosla[1], Prameelarani Kantheti[2], Vani Brahmachari[1], H. Sharat Chandra[2]**

[1] Developmental Biology and Genetics Laboratory, Indian Institute of Science, Bangalore 560 012, India
[2] Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India

Received: 15 May 1995; in revised form: 10 August 1995/Accepted: 25 August 1995

**Abstract.** In mealybugs, chromatin condensation is related to both genomic imprinting and sex determination. The paternal chromosomal complement is condensed and genetically inactive in sons but not in daughters. During a study of chromatin organization in *Planococcus lilacinus*, digestion with micrococcal nuclease showed that 3% to 5% of the male genome is resistant to the enzyme. This Nuclease Resistant Chromatin (NRC) apparently has a nucleosomal organization. Southern hybridization of genomic DNA suggests that NRC sequences are present in both sexes and occur throughout the genome. Cloned NRC DNA is A+T-rich with stretches of adenines similar to those present in mouse α-satellite sequences. NRC DNA also contains sequence motifs that are typically associated with the nuclear matrix. Salt-fractionation experiments showed that NRC sequences are matrix associated. These observations are discussed in relation to the unusual cytological features of mealybug chromosomes, including the possible existence of multiple centres of inactivation.

## Introduction

There are two well-known examples of facultative heterochromatization of whole chromosomes. One of these is mammalian X chromosome inactivation (Lyon 1961) and the other, the inactivation of paternal chromosomes in sexually reproducing mealybugs and related coccid insects (Hughes-Schrader 1948; Brown and Chandra 1977). In the lecanoid genetic system, found in most mealybugs, there are no sex chromosomes and all embryos begin development with a diploid number of chromosomes, which is usually ten (Brown 1959; Brown and Chandra 1977). Around the blastoderm stage of development, the paternal set of chromosomes becomes heterochromatic and genetically inactive in some embryos and these embryos develop into males (Brown and Nelson-Rees 1961). There is no such heterochromatization in other embryos and these develop into females. Functional haplo-diploidy thus seems to be the basis of sex determination. Because it is always the paternal set of chromosomes that is subject to condensation and inactivation in sexually reproducing mealybugs, the mealybug genetic system also represents an example of genomic imprinting (Chandra and Brown 1975). An understanding of chromatin organization in this insect is therefore of particular interest.

Micrococcal nuclease (MNase), a $Ca^{2+}$, $Mg^{2+}$ nuclease, digests chromatin in the internucleosomal regions and accessibility of chromatin to MNase is directly related to its state of condensation (Sun et al. 1986). During a comparative study of chromatin in male and female mealybugs, a fraction of chromatin resistant to MNase was observed in males but not in females (Kantheti 1994; Kantheti and Chandra, in preparation). We report here the results of our attempts to characterize this chromatin fraction.

## Materials and methods

*Materials.* Stock cultures of a mealybug, provisionally identified as *Planococcus lilacinus*, were obtained from the Horticultural Research Station, Coorg, India. They were maintained on pumpkins at room temperature. Micrococcal nuclease was obtained from Sigma Chemical Company, St. Louis, USA. Restriction enzymes were from New England Biolabs and Amersham, England; Sequenase was from United States Biochemical, USA. Hybond N+ from Amersham, or nylon membranes from Advanced Microdevices, Ambala, India were used in Southern blotting. Other chemicals were of analytical grade.

*Preparation of nuclei.* Insects were washed briefly in a mixture of ice cold acetone and ethanol (1:1 v/v) so as to remove surface wax. They were then washed with 0.34 M sucrose in buffer A (10 mM Tris-Cl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$), homogenized in the same buffer, filtered through cheese-

*Genbank accession number:* gblU27458
*Edited by:* M.L. Pardue
*Correspondence to:* V. Brahmachari

cloth, and the filtrate centrifuged for 10 min at 4,000 rpm in a swing-out rotor. Nuclei were then purified over sucrose cushions containing 1 M sucrose in buffer A. Phenylmethylsulphonyl fluoride (PMSF) at a concentration of 0.1 mM was used in the isolation buffers when the nuclei were isolated for chromatin analysis and 1 mM EDTA was used when they were isolated for DNA. All steps were carried out at 4°C.

*Micrococcal nuclease digestion and DNA isolation.* Nuclei isolated in the presence of 0.1 mM PMSF were treated with 0.1% Triton X-100 in buffer A and washed successively with 0.34 M sucrose in buffer A and buffer B (50 mM Tris-Cl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$). Nuclei were then suspended in buffer B and treated with MNase. In most experiments, MNase was used at a concentration of 0.1 U/10 OD units of nuclei (Kantheti 1994). DNA was isolated from these digests after Proteinase K treatment, extracted with 1:1 phenol:chloroform and 23:1 chloroform:isoamyl alcohol and precipitated with ethanol. For Southern hybridization genomic DNA was extracted from nuclei isolated from gravid females. All DNA preparations were treated with RNase A (100 µg/ml) before further analysis.

*Digestion with restriction endonucleases.* Mealybug genomic DNA was incubated with restriction enzymes at a DNA to enzyme ratio of 1:5 for 8–12 h at optimum temperature for complete digestion. For partial digestion with Sau3AI the DNA to enzyme ratio was reduced to 1:1 and incubation was carried out for 1 h. Complete digestion was carried out with Sau3AI, DraI, HindIII, BamHI, HpaI and XmaI.

*DNA electrophoresis, Southern blotting and hybridization.* Agarose gel electrophoresis was performed as described by Sambrook et al. (1989). Agarose gels were stained with ethidium bromide and photographed. For sequencing, polyacrylamide gel electrophoresis was carried out as described by Sambrook et al. (1989). DNA was transferred onto nylon membranes following the protocol of Chomozyski (1992) except that denaturation and transfer were carried out in 0.4 N NaOH after treating the gel with 0.25 N HCl for 10 min. For preparing radiolabelled nuclease resistant chromatin (NRC) probes, the DNA band corresponding to NRC was cut out from a 2% low-melting agarose gel, the agarose removed by phenol/chloroform extraction and the DNA recovered by ethanol precipitation. This DNA was then nick-translated according to standard protocols (Sambrook et al. 1989). Prehybridization and hybridization were as described by Sambrook et al. (1989) in 6 × SSPE, 50% formamide at 42°C. Final wash was in 0.1 × SSPE, 0.5% SDS at 65°C. DNA was sequenced by the dideoxy method using Sequenase (USB) as specified by the supplier.

*Sizing of NRC DNA.* Gel-eluted NRC DNA was used for size estimation by pulsed-field gel electrophoresis (PFGE) on a Pharmacia-LKB Pulsaphor system. Run conditions were as follows: 1.1% agarose gel prepared in 0.5 × modified TBE buffer (50 mM Tris, 50 mM boric acid, 0.1 mM EDTA) and electrophoresis at 10 V/cm for 7 h at 9°C with a pulse time of 5 s.

*Fractionation of chromatin.* Following the method of Sanders (1978), MNase-digested nuclei were pelleted at 3,000 rpm in a swing-out rotor and the nuclear pellet was resuspended in buffer A containing 0.6 M NaCl to separate the nuclease-resistant fraction from the digested chromatin. The DNA and proteins associated with the supernatant and the pellet were analyzed on 2% agarose and 12% SDS-polyacrylamide gels respectively. Isolation and analysis of acid extractable protein was carried out according to Rao et al. (1979).

*Isolation of NRC-specific clones.* NRC DNA was purified from a 2% low-melting agarose gel by phenol/chloroform extraction and ethanol precipitation. NRC DNA was digested with Sau3AI and cloned at the BamHI site of Bluescript vector pSKII(+) (Stratagene). The Sure strain of *Escherichia coli* (Stratagene) was used for transformation. NRC-specific clones were selected by hybridizing slot blots of plasmid DNA extracted from clones with NRC DNA. The same blots were deprobed and hybridized with $^{32}P$-labelled mononucleosomal DNA. Those clones that hybridized only to NRC DNA were selected for further analysis.

*Isolation of matrix-associated DNA.* The protocol followed was that of Basler et al. (1981). Briefly, MNase-digested nuclear suspensions were chilled on ice and centrifuged at 3,000 rpm for 10 min at 4°C. The nuclear pellet was resuspended in low salt buffer (10 mM Tris-Cl, pH 7.5, 0.2 mM $MgCl_2$), incubated for 10-20 min on ice and centrifuged. The pellet obtained was then extracted with high-salt buffer (2.0 M NaCl, 10 mM Tris-Cl, pH 7.5, 0.2 mM $MgCl_2$). DNA contained in the supernatant and the pellet obtained after high-salt treatment was extracted and analyzed on a 2% agarose gel.

## Results

### *Differential sensitivity to MNase of chromatin from males and females*

Permeabilized nuclei from males and females were digested with MNase. DNA was extracted from aliquots of nuclear preparations taken at different time intervals during digestion and analysed on a 2% agarose gel as described. Chromatin extracted from females shows progressive digestion with time giving rise to a typical ladder pattern (Fig. 1A). However, when nuclei from males are treated similarly, in addition to the ladder, a chromatin fraction that remains resistant to digestion is seen as high molecular weight DNA (Fig. 1B). Even at high concentrations of MNase (10 U/10 OD units of nuclei) and in spite of longer periods of digestion (180 min), this fraction remains resistant to nuclease (data not shown, Kantheti and Chandra, in preparation). This NRC constitutes 3%–5% of the total genome as estimated by densitometric scanning of the photographic negatives.

### *Characterization of NRC*

It is known that sperm bundles form about 70% of the abdominal tissue in adult male mealybugs (Nur 1962). Chromatin in sperm is known to have a highly compact organisation different from that in somatic nuclei (Kierzenbaum and Tres 1975). Therefore, to investigate whether NRC is derived from the differentially organized chromatin of sperm, the head region of males, representing the somatic tissue, was dissected out from the sperm-bearing abdominal region. MNase digestion was then done separately on nuclei derived from these two regions and the DNA extracted was analysed as before. NRC is present in digests of nuclei from both the head and the abdominal regions (Fig. 2), suggesting that NRC occurs in both somatic and germline chromatin.

To investigate the nature of NRC, the association of histones with NRC DNA was examined. After MNase digestion, the nuclei were extracted with 0.6 M NaCl as described in Materials and methods. Both mononucleo-

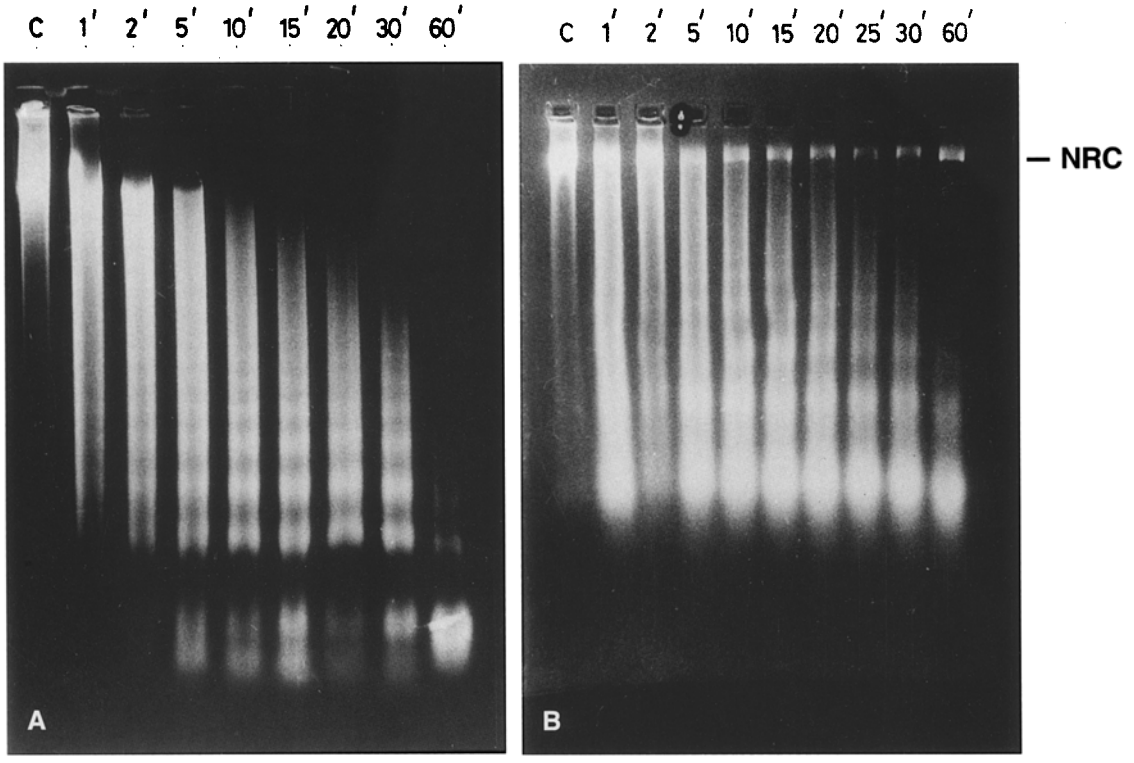


**Fig. 1A, B.** Pattern of micrococcal nuclease (MNase) digestion of nuclei from females (**A**) and males (**B**). DNA extracted from aliquots of nuclei taken at different time points during digestion with MNase was analysed on a 2% agarose gel in 0.5 × TBE. The duration of digestion is indicated above each lane in minutes. *NRC* nuclease resistant chromatin. *C* DNA from undigested nuclei

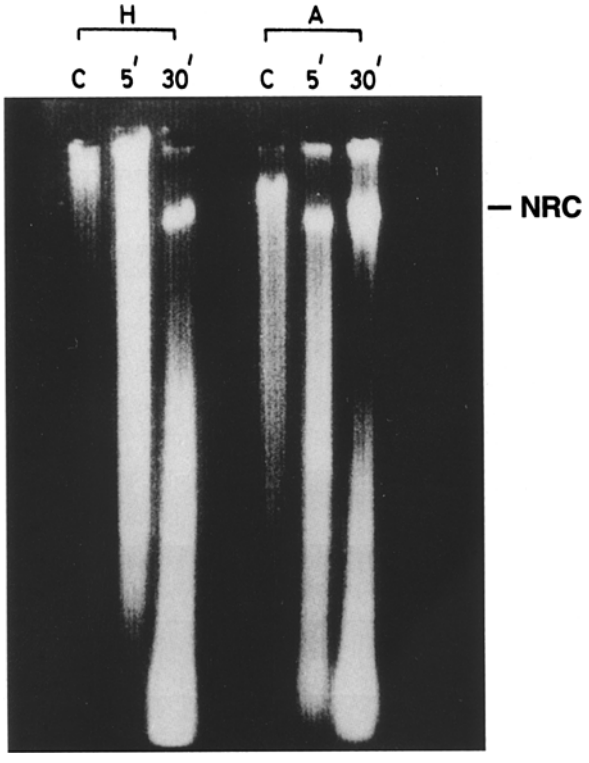


**Fig. 2.** MNase sensitivity of nuclei from the head region (*H*) and abdomen (*A*). *C* DNA from undigested nuclei. MNase digestion was carried out for 5 and 30 min as indicated

somes and oligonucleosomes are solubilized whereas NRC remains in the insoluble pellet (Fig. 3A). An analysis of this pellet fraction by SDS-polyacrylamide gel electrophoresis shows that a complete complement of core histones and H1 is present in this fraction (Fig. 3B). Since the 0.6 M NaCl pellet is virtually free of mononucleosomal DNA (Fig. 3A), this would suggest that NRC has a nucleosomal structure. However, the possibility that a subset of DNA sequences within NRC may be nonnucleosomal cannot be ruled out at this stage.

The distribution of NRC DNA sequences within chromatin was studied by Southern hybridization. DNA extracted from MNase-digested chromatin of males and females was fractionated on a 2% agarose gel (Fig. 4A), transferred to a nylon membrane and probed with $^{32}$P-labelled NRC DNA (Fig. 4B). These results indicate that in nuclei derived from males NRC DNA hybridizes to the MNase-resistant fraction and not to mononucleosomal DNA; it also hybridizes to DNA of females, thus ruling out the possibility that NRC contains only male-specific DNA sequences. In females, NRC sequences hybridize predominantly to DNA derived from oligonucleosomes, suggesting that in females also NRC is relatively nuclease resistant.

DNA derived from the NRC fraction when analysed on a 1% agarose gel is seen to consist of fragments of various sizes, suggesting that it is heterogeneous in this respect. To estimate the size range of NRC DNA, PFGE was performed under conditions recommended for the

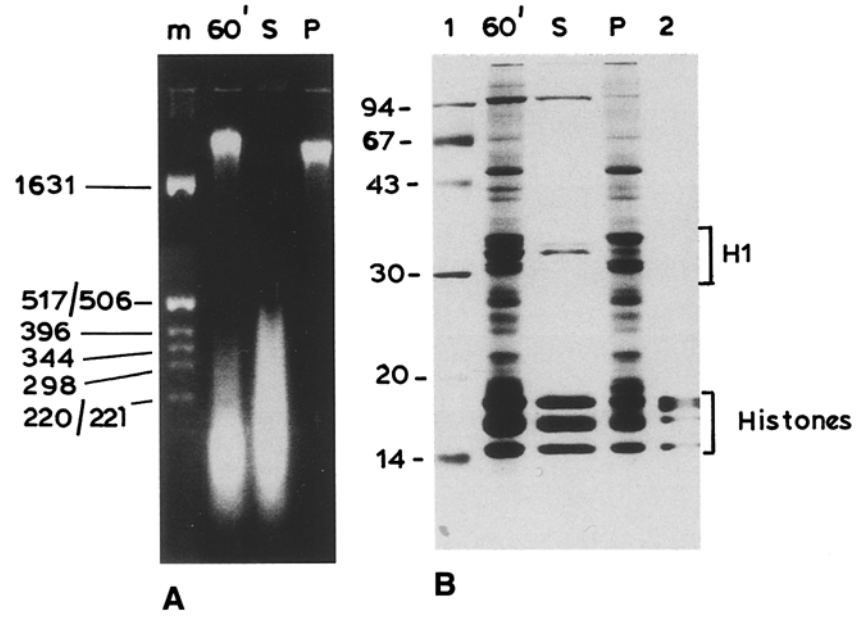


**Fig. 3A, B.** Detection of histones in NRC. MNase-digested nuclei of males were fractionated with 0.6 M NaCl. **A** DNA from various fractions was analysed on a 2% agarose gel. *m* HinfI digest of pBR322 DNA as marker (in base pairs), *60′* DNA from nuclei digested for 60 min with MNase, *S* supernatant of salt-fractionated 60 min digest, *P* pellet of salt-fractionated 60 min digest. **B** Acid-extracted proteins from the fractions corresponding to 60′, S and P, analysed by electrophoresis on a 12% SDS-polyacrylamide gel. *1* protein molecular weight marker (in kDa), *2* chicken erythrocyte core histones as marker

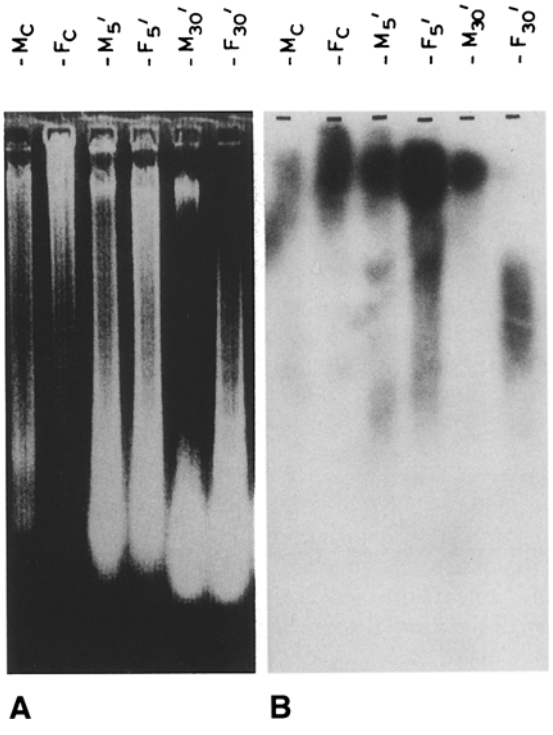


**Fig. 4A, B.** Hybridization of NRC DNA to MNase digests. DNA from MNase-digested nuclei from females (*F*) and males (*M*) was run on a 2% agarose gel and hybridized with $^{32}P$ labelled NRC DNA as probe. **A** Ethidium bromide stained gel; **B** autoradiogram. *F5′*, *F30′*, *M5′*, *M30′* indicate DNA of nuclei derived from females and males respectively, digested for 5 and 30 min with MNase. $M_c$ and $F_c$ are DNA samples from undigested nuclei from males and females, respectively

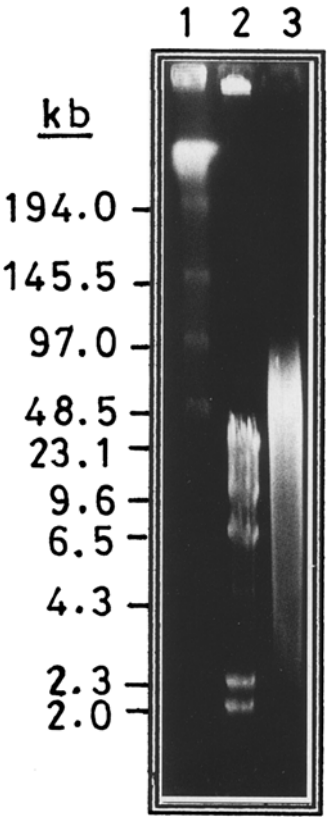


**Fig. 5.** Analysis of NRC DNA by pulsed-field gel electrophoresis. PFGE was carried out in a 1.1% agarose gel in 0.5 × modified TBE at 10 V/cm for 7 h at 9°C. The pulse time was 5 s. *1* Phage λ DNA concatamers, *2* phage λ DNA digested with HindIII, *3* NRC DNA

separation of DNA fragments of up to 100 kb (Instruction Manual for LKB 2015 Pulsophor system). As shown in Fig. 5, the size range of NRC DNA is approximately 2 to 100 kb. The distribution of NRC DNA sequences within the genome was studied by Southern analysis of genomic DNA digested with different restriction endonucleases and probed with $^{32}P$-labelled NRC DNA. Hybridization of NRC is observed all over the genomic digests (Fig. 6B), indicating that the composition of DNA sequences in NRC is heterogeneous. In Sau3AI partial digests, NRC DNA hybridizes more intensely to particular DNA sequences as indicated by arrows (Fig. 6B, lane 1). Even on complete digestion with Sau3AI (Fig. 6B, lane 6) an unequal hybridization inten-

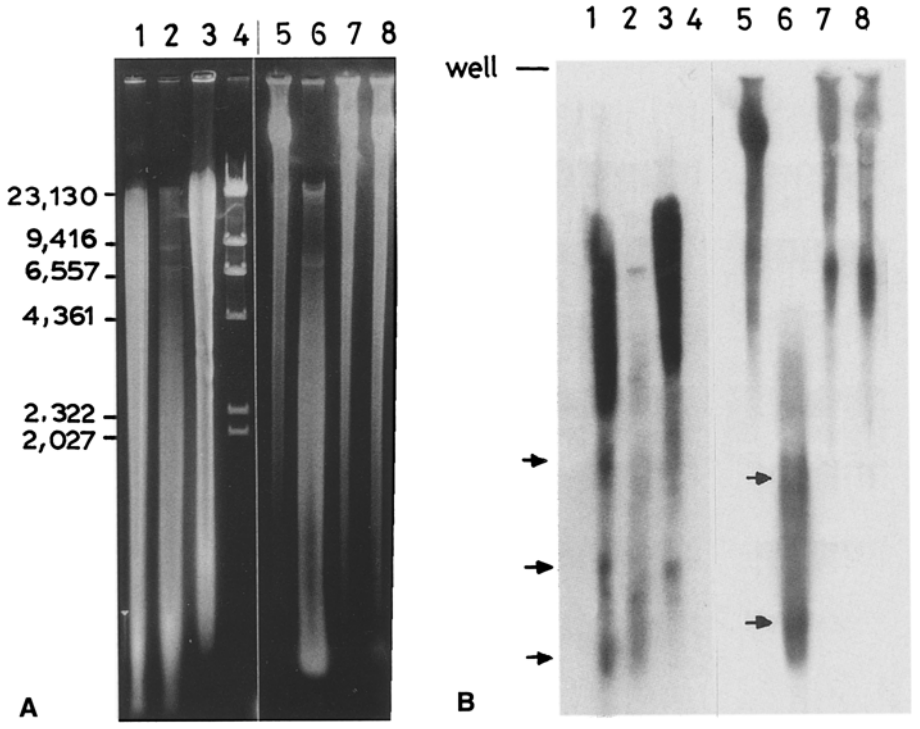


**Fig. 6A, B.** Distribution of NRC DNA sequences within genomic DNA. Mealybug genomic DNA digested with different restriction enzymes, and separated on a 1% agarose gel was Southern blotted and probed with $^{32}$P-labelled NRC DNA. **A** Ethidium bromide stained gel, **B** autoradiogram. Lanes 1–3 and 5–8 contain mealybug genomic DNA digested with: 1, Sau3AI (partial); 2, DraI; 3, HindIII; 5, BamHI; 6, Sau3AI (complete); 7, HpaI; 8, XmaI. Lane 4, phage λ DNA digested with HindIII

**Table 1.** Percentage A+T content of NRC-specific clones

| Clone | %A+T |
|---|---|
| NRC 1 | 40.2 |
| NRC 6 | 65.9 |
| NRC 7 | 67.3 |
| NRC 8 | 67.3 |
| NRC 9 | 46.0 |
| NRC10 | 59.6 |
| NRC11 | 67.8 |
| NRC13 | 71.4 |
| NRC14 | 64.3 |
| NRC20 | 58.0 |
| NRC36 | 59.6 |
| NRC42 | 58.6 |
| NRC46 | 66.8 |
| NRC50 | 50.5 |
| NRC51 | 69.3 |

sity in certain regions is seen along with background hybridization (indicated by arrows). This suggests that NRC DNA probably contains certain sequences that are present in multiple copies. The pattern of hybridization with the DraI digest suggests the presence of two classes of sequences in NRC DNA, one that has few sites for a rare cutter like DraI, and another that is probably A+T rich and frequently cut by DraI. This pattern of hybridization is reproducible.

To characterize NRC further, the Sau3AI fragments of NRC DNA were cloned into the BamHI site of a Bluescript plasmid vector. Of the 28 NRC-specific clones obtained, 15 have been sequenced. The base composition of these clones is shown in Table 1. Except for

```
GATCTCAATA GATGTGTGAA AGTACAGGAA AATTGGTGCT
TTTGATTCAT ACAGAAATAT GAAATAAAAT AATAAAACAC
CCGGAAAGCT GAATAGCTGG AAAttttcGA AATTTTTAAT
TTTCTACAAG GGTGTTTATC TGTTGTTGTT GAATTGGAAT
TGACTACCCC CTAACTACCA CCTGATC
```

**Fig. 7.** Nucleotide sequence of clone NRC7 (Accession no. gb|U27458). (GAAA) sequences are in **bold**, corresponding complementary sequences (TTTC) are in **lowercase**; **underlining** indicates the matrix-associated sequences (MAR)

three clones all others have an A+T content higher than 50%, in the range of 58–71%. A homology search using the BLASTn and FASTA programmes was done with the nucleotide database available on the public domain of the Internet. None of the clones showed significant similarity to any of the known sequences. Two clones, NRC7 and 8, which were selected independently, had exactly the same insert size (187 bp) and sequence (Fig. 7, Accession no. gb|U27458). However, a polypurine motif (GAAAA), similar to that observed in centromeric alphoid sequences (Radic et al. 1987), was identified by visual comparison. Similarly, an A+T-rich motif that is believed to be involved in matrix association (Cockerill and Garrad 1986) was detected in several NRC clones. Figure 7 illustrates these features for clone NRC7.

After digestion of permeabilized nuclei with MNase, extraction with 2 M NaCl solubilizes all DNA sequences other than those that are matrix associated (Basler et al. 1981). Similar fractionation of chromatin of the male mealybug leaves NRC sequences in the pellet, suggesting that these sequences are matrix associated (Fig. 8). These putative matrix-associated sequences range in size from 2 to 100 kb (data not shown).

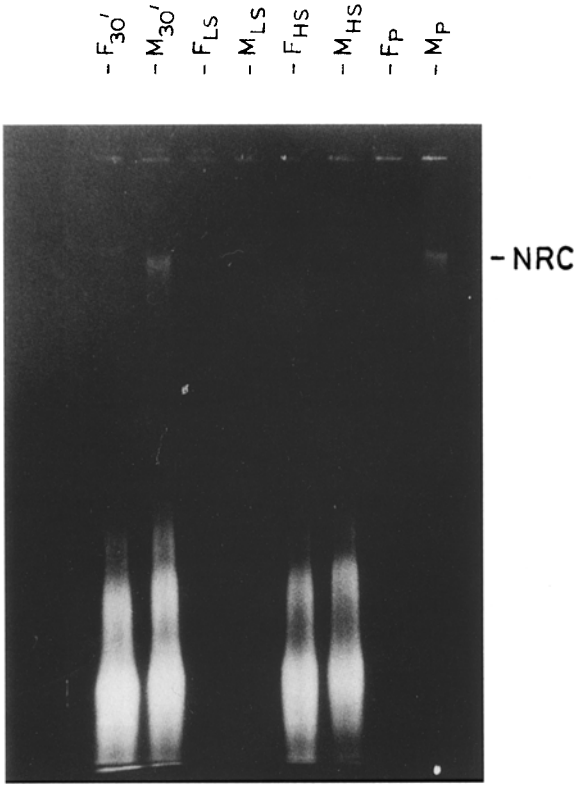


**Fig. 8.** Association of NRC DNA with the nuclear matrix. MNase-digested nuclei of males and females were extracted with high salt, and DNA from soluble and pellet fractions was analysed on a 2% agarose gel. $F_{30'}$ is DNA from nuclei of females digested with MNase for 30 min; $M_{30'}$ is DNA from nuclei of males digested with MNase for 30 min; $F_{LS}$, $F_{HS}$ and $F_P$ are low-salt soluble, high-salt soluble and pellet fractions, respectively, from nuclei of females digested with MNase. $M_{LS}$, $M_{HS}$ and $M_P$ are low-salt soluble, high-salt soluble and pellet fractions, respectively, from nuclei of males digested with MNase

## Discussion

Chromosomal condensation seems to function as a point of divergence between the developmental pathways of males and females in mealybugs. Condensation of the paternal chromosomal set is correlated with maleness (Hughes-Schrader 1948; Brown 1966; Chandra 1963b). In mealybugs and other coccids, radiation-induced chromosomal fragments are not lost during mitosis, but perpetuate themselves as stable entities in nuclei of both sexes, demonstrating that the centromere is diffuse and that freshly broken chromosomal ends can form telomeres (Brown and Nelson-Rees 1961; Chandra 1963a). Moreover, when broken chromosomes are transmitted by fathers to their sons, each chromosomal fragment undergoes heterochromatization (Brown and Nelson-Rees 1961; Chandra 1963a), suggesting that there are multiple centres of inactivation. This is in contrast to the facultative condensation of the X chromosome in mammalian females in which a single centre is thought to control inactivation (Cattanach 1975; Rastan 1983; Brown et al. 1991). The inactive X chromosome in mammalian females shows a typical chromatin organization as assessed by MNase sensitivity, but transcriptional factors do not bind to its condensed domains (Pfeifer and Riggs 1991). As far as we are aware, a sex-specific chromatin fraction such as that observed in *P. lilacinus* (Kantheti 1994; Kantheti and Chandra, in preparation) has not been observed in other systems. Resistance of this chromatin to MNase may be a consequence of its compact organization, or its association with the matrix, or both. Since NRC DNA hybridizes predominantly to oligonucleosomal DNA in females also, it appears to be organized in a partially MNase-resistant manner even in the active or potentially active female chromatin. This may mean NRC sequences are matrix associated even in females, or that a subset of NRC sequences are centromeric in origin. The centromeric regions of *Saccharomyces cerevisiae* are organized into nucleosomes, with a spacing, including the linker, of 200-300 bp (Saunders et al. 1990; Bloom 1993). In mammals also, centromeric DNA is organized into nucleosomes and centromeric proteins such as CENP-A are involved in this organization (Bloom 1993). In mealybugs, although both males and females have diffuse centromeres, NRC is observed only in the males, thus suggesting a connection between NRC and the condensed state of paternal chromosomes in the males rather than with the centromeres.

NRC DNA sequences appear to be heterogeneous in size and dispersed within the genome. In male mealybugs, large NRC DNA sequences, approximately 2–100 kb in size, are matrix associated. This is in contrast to other systems where matrix-associated regions are much smaller, ranging in size from 300-500 bp (Basler et al. 1981). Several clones derived from NRC contain the A+T-rich motif implicated in nuclear matrix-association (Cockerill and Garrad 1986). Matrix association may help confer nuclease resistance to NRC in male mealybugs.

In summary, we observe that 3%–5% of the genome of *P. lilacinus* is organized as highly compact chromatin in males. These NRC sequences appear to be associated with the nuclear matrix and are present in the genomes of both males and females. The diffuse nature of the centromere and the ability to form new telomeres are features common to both sexes but nuclease-resistant chromatin is present only in males. The possibility that DNA sequences present in NRC may, in conjunction with other factors serve as centres of initiation of facultative heterochromatization merits further investigation.

*Acknowledgements.* This work was supported by grants from the Council of Scientific and Industrial Research (CSIR), India. Partial support from the Jawaharlal Nehru Centre for Advanced Scientific Research is also acknowledged. The assistance of Mr. C. Rajanna and Mr. K. Kuppuswamy in maintenance and supply of mealybugs is gratefully acknowledged. The Molecular Biophysics Unit and Centre for Genetic Engineering of this institute provided generous access to their laboratory facilities.

## References

Basler J, Hastie ND, Pietras D, Matsui S, Sandberg AA, Berezney R (1981) Hybridization of nuclear matrix attached deoxyribonucleic acid fragments. Biochemistry 20: 6921–6929

Bloom K (1993) The centromere frontier: kinetochore components, microtubule-based motility and the CEN-value paradox. Cell 73: 621–623

Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, Willard HF (1991) A gene from the region of the human X-inactivation centre is expressed exclusively from the inactive X chromosome. Nature 349: 38–44

Brown SW (1959) Lecanoid chromosome behaviour in three more families of the Coccoidea (Homoptera). Chromosoma 10: 278–300

Brown SW (1966) Heterochromatin. Science 151: 417–425

Brown SW, Chandra HS (1977) Chromosome imprinting and the differential regulation of homologous chromosomes. In: Goldstein L, Prescott DM (eds) Cell biology: a comprehensive treatise, vol I. Academic Press, New York, pp 109–189

Brown SW, Nelson-Rees WA (1961) Radiation analysis of a lecanoid genetic system. Genetics 46: 983–1007

Cattanach BM (1975) Control of chromosome inactivation. Annu Rev Genet 9: 1–18

Chandra HS (1963a) Cytogenetic studies following high dosage paternal irradiation in the mealybug *Planococcus citri*. I. Cytology of $X_1$ embryos. Chromosoma 14: 310–329

Chandra HS (1963b) Cytogenetic studies following high dosage paternal irradiation in the mealybug *Planococcus citri*. II. Cytology of $X_1$females and the problem of lecanoid sex determination. Chromosoma 14: 330–346

Chandra HS, Brown SW (1975) Chromosome imprinting and the mammalian X chromosome. Nature 253: 165–168

Chomozyski P (1992) One hour downward alkaline capillary transfer for blotting of DNA and RNA. Anal Biochem 201: 134–139

Cockerill PN, Garrad WT (1986) Chromosomal loop anchorage of the kappa immunoglobin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 44: 273–282

Hughes-Schrader S (1948) Cytology of coccids (Coccoidea-Homoptera). Adv Genet 2: 127–203

Kantheti P (1994) Studies on a female specific cDNA clone, and chromatin organization, in a mealybug, *Planococcus lilacinus*. Ph D Thesis, Indian Institute of Science, Bangalore, India

Kierzenbaum AL, Tres LL (1975) Structural and transcriptional features of the mouse spermatid genome. J Cell Biol 65: 258–270

Lyon MF (1961) Gene action in the X chromosome of mouse (*Mus musculus* L.) Nature 190: 372–373

Nur U (1962) Sperm, sperm bundles and fertilization in a mealybug *Pseudococcus obscurus* Essig (Homoptera-Coccoidea). J Morphol 111: 173–199

Pfeifer GP, Riggs AD (1991) Chromatin differences between active and inactive X chromosomes revealed by genomic footprinting of permeabilized cells using DNase I and ligation-mediated PCR. Genes Dev 5: 1102–1113

Radic MZ, Lundgren K, Hamkalo BA (1987) Curvature of mouse satellite DNA and condensation of heterochromatin. Cell 50: 1101–1108

Rao MRS, Prasad VR, Padmanabhan G, Ganguly J (1979) Isolation and characterization of binding proteins for retinol from the cytosol, nucleosol and chromatin of the oviduct magnum of laying hens. Biochem J 183: 501–506

Rastan S (1983) Non-random X-chromosome inactivation in X-autosome translocations: location of inactivation centre. J Embryol Exp Morphol 78: 1–22

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sanders MM (1978) Fractionation of nucleosomes by salt elution from Micrococcal nuclease-digested nuclei. J Cell Biol 79: 97–109

Saunders MJ, Yeh E, Grunstein M, Bloom K (1990) Nucleosome depletion alters the chromatin structure of *Saccharomyces cerevisiae* centromeres. Mol Cell Biol 10: 5721–5727

Sun YL, Xu YZ, Bellard M, Chambon P (1986) Digestion of the chicken β-globin chromatin with micrococcal nuclease reveals the presence of an altered nucleosomal array characterized by a typical ladder of DNA fragments. EMBO J 5: 293–300