LOCATIONS OF 18S AND 28S RIBOSOMAL GENES ON THE CHROMOSOMES OF THE INDIAN MUNTJAC

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Cytologists have long associated the achromatic secondary constrictions of chromosomes with nucleoli and termed these achromatic regions the nucleolus organizers. More recently, applications of the in situ nucleic acid hybridization techniques have positively identified the locations of the 18S and 28S ribosomal cistrons (rDNA) in metaphase chromosomes of some animal species (4, 7-9) and the 5S genes in some others (10, 14).

The results of in situ hybridization experiments largely confirmed the classic cytologic concept, i.e., the nucleolus organizers are indeed the chromosomal locations for rDNA. In this report we attempt to identify the locations of rDNA in the chromosomes of the Indian muntjac Muntiacus muntjak because this species has some unique cytological features. It has the lowest diploid numbers among mammals (96, 67), and every chromosome is morphologically distinct (15). There are conspicuous secondary constrictions on the X and Y chromosomes. We also employ the various chromosome-banding techniques to determine the staining properties of the nucleolus organizer regions.

MATERIALS AND METHODS

The cell line used in the present study was originally initiated from a subcutaneous tissue of a male specimen by Wurster and Benirschke (15). In the Houston laboratory it was designated as TCH-1626 and has been continuously cultivated since 1970. Harvest of cells was made by a 2-h Colcemid treatment and a 10-min hypotonic solution treatment. For in situ hybridization experiments, squashed preparations on chrome alum-gelatin-coated slides were used, and for banding experiments air-dried slides were prepared. Heterochromatin (C band) staining followed the procedure of Stefos and Arrighi (13); the Giemsa banding (G band), the trypsin procedure of Seabright (11); and the quinacrine mustard fluorescence (Q band), the procedure of Caspersson et al. (2).

Tritiated ribosomal RNA (rRNA) was obtained from cells of a Xenopus laevis kidney cell line grown for 7 days in medium containing [3H]uridine (100 μCi/ml, 40.4 Ci/mM). The cells were lysed at 4°C in 0.1 M NaAc, pH 5.0 + 0.5% sodium dodecyl sulfate, and protein was extracted with cold phenol. The aqueous phase was brought to 0.1 M NaCl and the nucleic acids were precipitated by the addition of 2 vol of 95% ethanol. The precipitate was dissolved in 0.05 M 2-(N-morpholine)-ethane-sulfonic acid, 0.002 M MgAc, pH 7.0 and treated with DNase I (50 μg/ml) at 4°C for 15 min. After a second ethanol precipitation, the RNA was dissolved in 1 mM EDTA, 0.1 M NaCl, 0.02 M NaAc, pH 5.0 and centrifuged on a 5-40% (wt/vol) sucrose gradient at 15°C. The combined 18S and 28S RNA peaks from the gradient had a specific activity of 10^6 cpm/μg as determined by scintillation counting of known amounts of RNA on nitrocellulose filters (efficiency of counting about 20%).

In situ hybridization was carried out by the method of Gall and Pardue (5) except that the treatment with HCl was omitted. The hybridization mixture contained 10 μg/ml of [3H]18S and 28S ribosomal RNA from X. laevis and 50 μg/ml nonradioactive ribosomal RNA.
Figure 1 Karyotype of male muntjac (2n = 7), conventional staining. A telocentric autosome is translocated onto the X chromosome to become its long arm but the homolog remains free in male individuals. This homolog is termed \( Y_1 \) in this paper. The real Y chromosome (\( Y_2 \)) is a small submetacentric. A deep secondary constriction (nucleolus organizer) is present in the long arm of the X and in the equivalent area of \( Y_1 \).

Figure 2 Constitutive heterochromatin (C band). No demonstrable C band on either side of the nucleolus organizers.

Figures 3 and 4 In situ hybrids showing locations of ribosomal cistrons. In addition to the anticipated locations on X long arm and \( Y_1 \), two minor sites near the distal ends of the long arm of chromosome 1 show heavy label.

Figure 5 An in situ hybrid similar to Figs. 3 and 4. A tetraploid metaphase.

Figure 6 Quinacrine mustard fluorescence. Note that all rDNA regions are negative. \( Y_2 \) has practically no fluorescence.

Figure 7 Trypsin-induced G banding. Note that all rDNA regions are G-band negative.
from Escherichia coli in a buffer of 0.6 M NaCl, 4 x 10^{-3} M Tris, pH 7.0. The hybridization was carried out for 14 h at 65°C.

RESULTS

The diploid number of the male muntjac is 7 (Fig. 1). There are two pairs of autosomes: one pair of large submetacentrics and one pair of large subtelocentrics. The X chromosome is submetacentric. A long "neck" region is found to connect the short arm (the real X) and the long arm (a translocated autosome). A conspicuous achromatic region is located in the proximal third of the long arm of the X. The homolog of the translocated autosome (customarily termed the Y1 chromosome) is telocentric with a secondary constriction at the location equivalent to the one on the long arm of the X. The real Y chromosome (Y~) is a small submetacentric.

This species has very little constitutive heterochromatin as measured by C-band staining (3). Each chromosome has a small piece at its centromeric region except the X where the entire neck region is C-band positive. The secondary constrictions are not bordered by any visible C band (Fig. 2).

The in situ hybrids made with rRNA showed unequivocally four grain clusters: one at the secondary constriction of the long arm of the X, one at the secondary constriction of Y1, and one near the distal end of the long arm of each member of the largest pair of autosomes (Figs. 3–5). This pattern was found to be consistent in more than 100 metaphases observed. In a large proportion of metaphases the grain clusters over Y1 appear to be heavier than those over the X.

With the information on the locations of ribosomal cistrons, it becomes evident that these chromosomal segments are invariably Q negative and G negative (Figs. 6 and 7, respectively).

DISCUSSION

Although in situ hybridization experiments have shown a good correlation between sites of rDNA and secondary constrictions on metaphase chromosomes, the results presented here show that the correlation is not absolute. The cluster of rDNA on chromosome 1 of the muntjac shares the G-banding and Q-banding characteristics of the other rDNA regions in the complement but it does not bear a secondary constriction on the metaphase chromosome. We assume that all sites of rDNA are functioning in these cells because interphase nuclei frequently have more than two nucleoli and because the end of the long arm of chromosome 1 does exhibit a secondary constriction in prophase. (This was observed after realizing the existence of a nucleolus organizer from in situ hybrids.) If our assumption is correct, the lack of a secondary constriction at metaphase does not necessarily signify that the rDNA cluster on chromosome 1 is inactive. Probably different nucleolus organizers contain different numbers of ribosomal cistrons, and those in chromosome 1 may have a smaller number than those in the sex chromosomes, thus accounting for the early disappearance of the secondary constrictions in metaphase.

We have used [3H]rRNA from the toad X. laevis for these experiments because Xenopus cell lines tolerate very high levels of [3H]uridine and will produce in vivo-labeled [3H]rRNA of exceptionally high specific radioactivity. Several studies show that some regions of 18S and 28S ribosomal RNA have been strongly conserved during evolution in eucaryotes (1, 6, 12). The Xenopus rRNA which hybridizes to human DNA is completely competed by human rRNA (4). The previous biochemical evidence and our in situ experiments show sufficient sequence homology between the ribosomal cistrons of amphibia and mammals to warrant the use of amphibian rRNA in mapping chromosomal sites of rDNA in mammals.

SUMMARY

The locations of genes coding for 18S and 28S ribosomal RNA have been mapped on metaphase chromosomes of the Indian muntjac M. muntjak by in situ hybridization with [3H]rRNA from the toad X. laevis. The results show that, in the muntjac, rDNA clusters are associated with the prominent secondary constrictions on the X and the Y1 chromosomes. In addition a cluster of rDNA is found near the tip of one arm on the longest pair of autosomes. The autosomal cluster of rDNAs usually does not express as a secondary constriction at metaphase.

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