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Nucleolus organizer regions and heterochromatin in the zebu (*Bos indicus* L.)

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Summary. Ag-NOR staining and a counterstain enhanced fluorescence technique (chromomycin A_3/distamycin A/DAPI-staining = CDD-method) and G-banding, respectively, have been applied to the zebu (*Bos indicus* L.) chromosomes. The nucleolus organizer regions (NORs) were found in the telomeric regions of chromosomes nos. 2, 3, 4, 11, and 28. CDD staining led to a well-defined R-banding pattern along the chromosome arms and to the visualization of centric heterochromatic bands of variable sizes.

Key words: Zebu cattle – Nucleolus organizers – Heterochromatin polymorphisms

Introduction

Nucleolus organizer regions (NORs) are defined as the areas of chromosomes that organize nucleoli and contain DNA sequences coding for ribosomal RNA production. The NORs can be specifically demonstrated in chromosomal preparations by in situ hybridization (Henderson et al. 1972), silver staining (Howell and Black 1980), and N-band methods (Matsui and Sasaki 1973).

In many species of the subfamily *Bovidae* the positions of the NORs have been determined by silver staining. Examples are taurus cattle (Di Berardino et al. 1979; Henderson and Bruere 1979; Mayr and Czaker 1981; Mayr et al. 1985) and water buffalo (Di Berardino et al. 1979).

The C-band patterns have been demonstrated in the karyotypes of several species of *Bovidae* including zebu cattle, banteng, bison, yak and water buffalo (Buckland and Evans 1978; Pathak and Kieffer 1979; Bongso and Hilmi 1982). These investigations have revealed the centromeres to be the only regions harbouring C-band material. Moreover, the centromeric regions exhibit bright fluorescence after staining with the GC-specific drug chromomycin A_3 (Jorgenson et al. 1978; Sahar and Latt 1980). Counterstain enhanced fluorescence staining by the use of the dye combination chromomycin A_3/distamycin A/DAPI ("CDD-staining," Schweizer 1980) is useful in generating a general banding pattern with good definition of intercalary and terminal R-bands as well as in defining the heterochromatin pattern.

In the present study, we applied sequential silver/CDD-staining and silver/G-band staining, respectively, for the exact localization of the nucleolus organizer regions in zebu cattle (*Bos indicus* L.). Imposing size polymorphisms of chromomycin A_3-positive bands were not only restricted to centromeric regions but were also observed to be associated with some Ag-positive NORs.

Material and methods

Five zebu cattle, *Bos indicus* L. (2 male, 3 female), were included in the present study. Blood was taken from the jugular vein. Forty-eight-hour pokeweed stimulated peripheral blood lymphocyte cultures were set up for chromosome preparations. Some of the slides were stained for R-bands and potential DA-DAPI-bands by the chromomycin A_3/distamycin A/DAPI-("CDD-")method (Schweizer 1980).

After staining, the slides were "aged" in the dark at 37 °C for 7–21 days prior to examination. By photographing with different filter combinations (e.g. Reichert filter blocks B_1 and U_1) both the DA-DAPI staining behaviour and the R-bands produced by chromomycin A_3 were documented.

Other preparations were analyzed by a silver-NOR staining method (Howell and Black 1980) and, after destaining with Farmer's solution, subjected to CDD staining or trypsin-Giemsa-banding (Wang and Fedoroff 1972) for chromosome identification.

Results

In the investigated zebus, the silver stained NORs appeared at the telomeric regions of five chromosomes. The concerned chromosomes were nos. 2, 3, 4, 11, and 28 (Figs. 1 and 2). In some animals, no Ag-NORs were

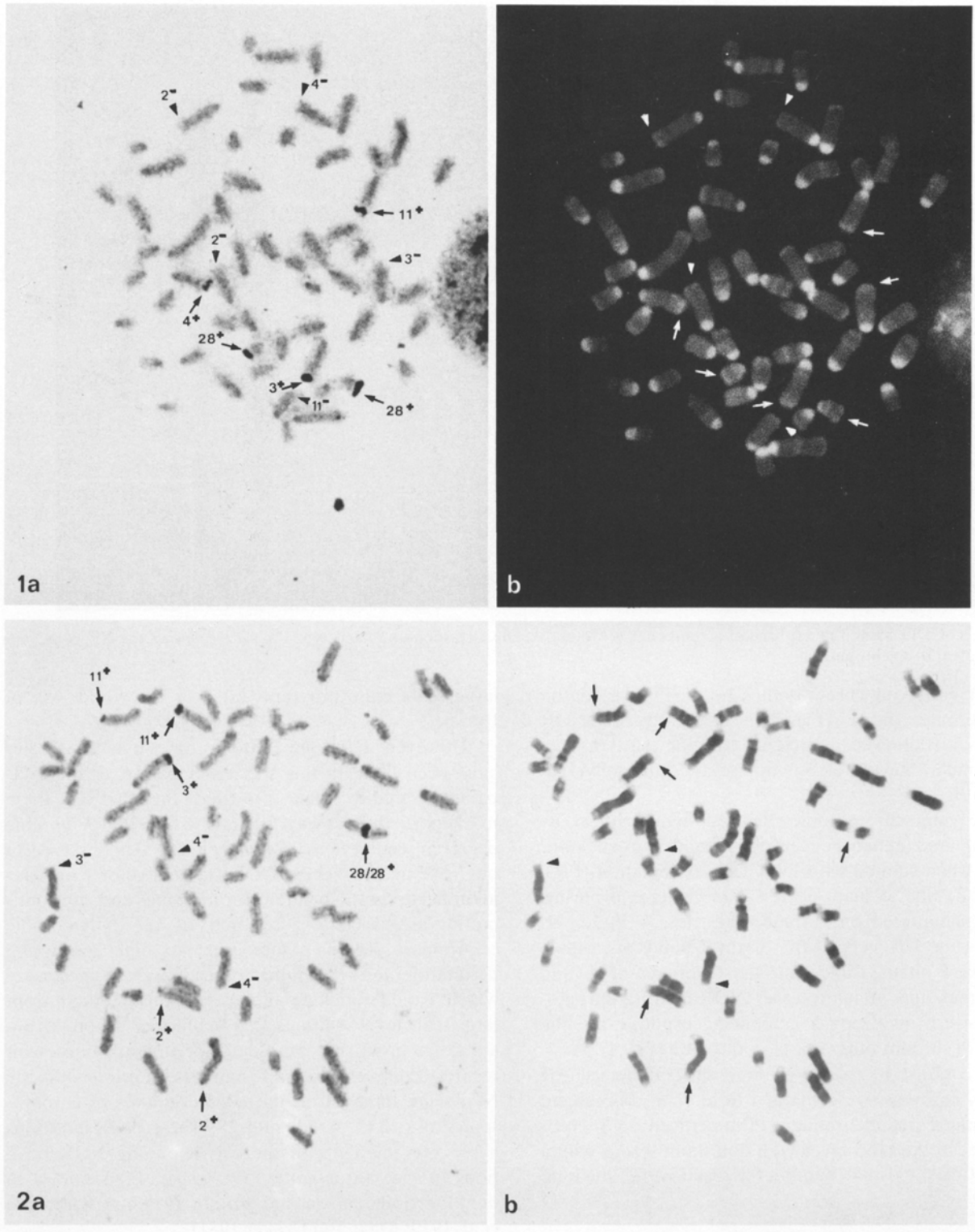

Fig. 1a, b. Sequentially silver-CDD stained metaphase of a zebu bull. Note the absence of NORs on both chromosomes no. 2. **a** Silver staining. Arrows (+) indicate silver NORs, arrowheads (–) indicate telomeres where NORs are absent. **b** CDD staining

Fig. 2a, b. Sequentially silver-trypsin-Giemsa stained metaphase of a zebu bull. Note the absence of NORs on both chromosomes no. 4. **a** Silver staining. Arrows (+) indicate silver NORs, arrowheads (–) indicate telomeres where NORs are absent. **b** Trysin-Giemsa staining

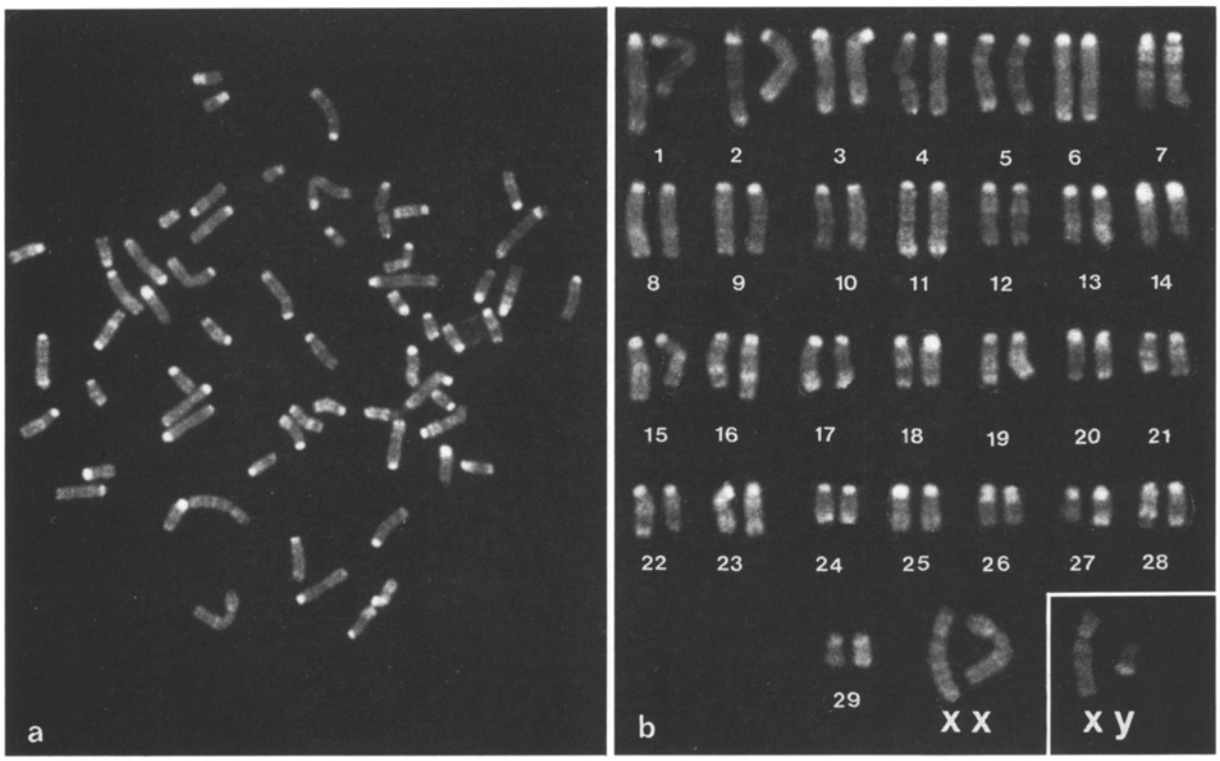

Fig. 3a, b. CDD stained metaphase of a zebu cow. **a** Metaphase. **b** Corresponding karyotype. The insert shows the XY gonosome complement of a zebu bull

present either on chromosomes no. 2 (Fig. 1a) or on chromosomes no. 4 (Fig. 2a). Generally, Ag-NOR expression followed individual specific patterns accompanied, nevertheless, by some intraindividual variability.

The zebu chromosomes showed high contrast R-banding and enhanced centric C-bands on all autosomes when stained with the CDD combination (Figs. 1b and 3). Spectacular centric C-band heteromorphisms were encountered on chromosomes nos. 4, 9, 16, 17, and 18 (Fig. 3b). Very CMA_3-bright R-bands of variable sizes were often visible near the telomeres of several autosomes and in many cases NOR-bearing chromosomes were involved. A convincing example are the NORs of chromosomes no. 11 in the zebu (Fig. 1).

In contrast to the autosomes, the submetacentric zebu X chromosome contained little or no fluorescent centromeric heterochromatin. The acrocentric Y chromosome of the zebu revealed a dull fluorescence with a bright CMA_3-positive band in the distal half of the long arm.

Discussion

Zebu cattle (*Bos indicus* L.) belong to the most important species in animal breeding and human food supply. It is well established in many different pure breed stocks and holds a prominent position in several large scale cross programs all over the world, except Europe.

However, very few chromosome marker studies have been done in this species. Recently, detailed G-banding studies have provided the first of these markers in the zebu (Krutzler et al. 1986). A notable degree of centromeric polymorphisms was observed in our investigated zebus. CDD-staining allows an easy identification of R-banded chromosomes and, concomitantly, an excellent resolution of the polymorphic centromeric regions. Moreoever, no time consuming destaining and restaining procedures are necessary which would preclude the use of this method from large scale investigations. This fact makes this procedure a further promising candidate for investigations with regard to zebu chromosome markers. In zebu cattle, the NORs are localized at the telomeric ends of chromosomes nos. 2, 3, 4, 11, and 28. These NOR positions agree very highly with the corresponding NOR positions in the taurus cattle (*Bos taurus* L.) reported in literature (Henderson and Bruere 1979; Di Berardino et al. 1979; Mayr and Czaker 1981).

Moreover, an individual characteristic distribution of silver-NORs was observed in our zebus. The metaphase of a zebu bull in Fig. 1 is a good example of this individual specific expression of silver-NORs. Both silver-NORs on chromosome 28 are expressed, whereas, on chromosomes 3, 4, 11, only one homologue is silver-

NOR positive and silver-NORs are completely lacking on both chromosomes no. 2. On the other hand, another bull (Fig. 2) does not possess silver-NORs on both chromosomes no. 4.

A comparable individual characteristic silver-NOR distribution has also been suggested in man (Goodpasture et al. 1976; Varley 1977) and domestic pig (Mayr and Schleger 1981; Stefanova 1983).

Moreover, the silver-NOR distribution has been found to be heritable in a simple Mendelian fashion in man (Varley 1977; Mikelsaar 1977; Markovic et al. 1978) and in domestic pig (Christensen 1980). Their proposed underlying silver-NOR genotypes were ++ (positive homozygous), +− (heterozygous and −− (negative homozygous) for the individual NOR chromosomes.

The absence of silver-NORs on certain chromosomes may be due to the fact that transcriptionally active rDNA is either lacking or only present in very small and therefore cytologically undetectable amounts. Unequal crossing-over and amplification events are regarded as probable reasons for these NOR variations (for review see Babu and Verma 1985).

In the future, systematic NOR studies will be of interest in the detection of NOR polymorphisms of the zebu. Moreover, the NOR expression patterns in different cross-products, i.e. zebu to taurus cattle, mithun, gaur, or yak, will be informative in terms of nucleolar suppression. Our preliminary data in these four cross systems clearly demonstrate that in each of them NORs of both species are active in stimulated peripheral blood lymphocytes (own unpublished results). Thus, the existance of complete nucleolar suppression can be definitely excluded in these interspecific crosses. This fact may be explained by the close relationship between these bovine species which is convincingly manifested in the crossability between them, sometimes even connected with a full reproductive capacity of their crossbreeds.

Therefore, centromeric and NOR markers should be a hopeful implement in the instrumentary of fundamental and applied genetics.

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