Differential Chromosomal Staining in *Bos taurus* (Cattle)

P.K. Seth¹ and W.P. Kunze²

Pathologisches Institut der Universität, 53 Bonn-Venusberg, West Germany

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The differential staining property of the constitutive heterochromatin is of lately being emphasized (Arrighi and Hsu 1971, Weisblum and de Haseth 1972, Hsu *et al.* 1972, Yunis and Yasmineh 1971). While it is known from the *in situ* hybridization techniques that repetitive DNA and constitutive heterochromatin in *Mus musculus* is localized in the centromeric regions of the chromosomes (Pardue and Gall 1970, Jones 1970), the distribution of the corresponding DNA fractions and of heterochromatin in the similarly telocentric chromosomes of *Bos taurus* is unclear and thereby raises some fundamental questions (Schnedl 1972, Seth and Gropp 1973). Therefore, the staining of the corresponding centromeric regions in cattle chromosomes with a benzimidazol derivative "Hoechst 33258", which is known to visualize the heterochromatic segments in certain other species, viz. mouse and the hedgehog (Hilwig and Gropp 1972), vis-a-vis the staining with the regular C- and G- banding techniques seems to be of particular interest.

Material and methods

Chromosome preparations: Metaphase chromosome spreads were obtained from fibroblasts of fetal tissue (perichondrium and testis) of four fetuses (2 males, 2 females) of *Bos taurus*.

Fluorescence staining, microscopy and photography: Chromosome spreads were stained with the benzimidazol derivative "Hoechst 33258" as described elsewhere (Seth and Gropp 1973). A Zeiss Universal Fluorescence microscope with an exciter filter KP 500 and the barrier filter combination 65/50/44 was used. Metaphase chromosomes were photographed with a Neofluor 63/1: 25 oil objective on an Ilford FP 4 film.

C- and G- staining: Slides were stained for the constitutive heterochromatin and Giemsa banding patterns following the known techniques of Arrighi and Hsu (1971), Sumner *et al.* (1971), Schnedl (1971 a, b, c) and Yunis *et al.* (1971).

Results

Fluorochrome staining with "Hoechst 33258" benzimidazol derivative: The centromeric regions of the autosomes in *Bos taurus* do not fluoresce at all (Fig. 1). One arm of the small metacentric Y chromosome exhibits bright fluorescence sug-

¹ Alexander von Humboldt Fellow. Address: Department of Anthropology, University of Delhi, Delhi-7, India.

² Present address: Institute für Pathologie, Medizinische Hochschule, 24 Lübeck, West Germany.

gestive of the Q-banding (Fig. 2a) as shown by Hansen (1971, 1972) and Schnedl (1972). The X-chromosome, however, shows bright fluorescence at a large pericentric segment of both arms (Figs. 1, 2a). While the centromeric areas of the cattle chromosomes remain unstained, the fluorochrome "Hoechst 33258" reveals along the chromatids a banding pattern of weak fluorescence (Figs. 1, 2a). They facilitate identification of the individual cattle chromosomes.

C- and G-staining: Similarly, no darkly stained centromeric regions in the cattle chromosomes are revealed following the techniques of Arrighi and Hsu (1971)¹, Schnedl (1971) and Sumner *et al.* (1971) (Fig. 2b). If, however, a different pan-

Fig. 1. Metaphase chromosomes (fibroblast culture) of *Bos taurus* ♀ stained with fluorochrome benzimidazol derivative "Hoechst 33258". Note the non-fluorescing centromeric regions of the autosomes. The arrows indicate the brightly fluorescing centromeric areas of the X-chromosomes.

creatic RNase² is used, the centromeric regions are darkly stained (Fig. 2c) comparable to the results obtained with these methods in the mouse chromosomes. Again, using the strong denaturation and reassociation procedures of Yunis *et al.* (1971), all the autosomes and the sex chromosomes show distinctly dark stained centromeric regions (Fig. 3). An additional dark stained segment is observed immediately below the stained centromeric region in the X-chromosome (Figs. 2c, 3).

¹ Using pancreatic RNase as supplied by Serva (West Germany) following National Formular XII.

² Böhringer's pancreatic RNase (West Germany).

Discussion

In the laboratory mouse (NMRI strain), the centromeric regions containing highly repetitive DNA sequences (Pardue and Gall 1970, Jones 1970, Arrighi and Hsu 1971, Schnedl 1971c, Yunis *et al.* 1971) are darkly stained following the regular denaturation-renaturation staining procedures. Likewise, similar regions in man are variably stained (Jones and Corneo 1971). Though highly repetitive DNA fractions have been isolated in the cattle (Polli *et al.* 1965, Frenster *et al.* 1963, Gartler and Burt 1964, Britten and Kohne 1968, Britten and Davidson 1969), the distribution along the chromosome vis-a-vis the constitutive heterochromatin is not clearly known. The centromeric regions of the cattle chromosomes exhibit a variable staining property (Figs. 1-3) if staining procedures are used which visualize the constitutive heterochromatin in some other species. In cattle, there is evidence that during the DNA replication period, the centromeric regions and the Y-chromosome as a whole start their replication later than all other regions of the chromosomes (Frenster *et al.* 1963, Gartler and Burt 1964, Schnedl 1972), thereby indicating that these regions are heterochromatic in na-

Fig. 2. Metaphase chromosomes of *Bos taurus* ♂. a. stained with Hoechst fluorochrome, showing the brightly fluorescing arm of the Y-chromosome and the brightly fluorescing centromeric region in the X-chromosome. b. using the C-staining methods of Arrighi and Hsu (1971) and Serva's pancreatic RNase, the chromosomes display unstained centromeric regions. c. using Böhlinger's pancreatic RNase and the staining technique as in (b), the centromeric regions are darkly stained.

ture. The centromeric regions of the metaphase chromosomes are non-fluorescing with the fluorochrome "Hoechst 33258", display negative C-banding following the regular staining method of Arrighi and Hsu (1971)¹, and also negative G-bandings using the techniques of Schnedl (1971) and Sumner *et al.* (1971). These observations, though supporting the findings of Schnedl (1972) should, however, be discussed in view of further results obtained in the present study (Figs. 2c, 3) using a different pancreatic RNase² but the same staining technique of Arrighi and Hsu (1971), and of the observations of Yunis and Yasmineh (1971). These latter authors report that there are large amounts of pericentromeric heterochromatin in calves. The-

Fig. 3. Metaphase chromosomes (fibroblast culture) of a male *Bos taurus* displaying the darkly stained centromeric regions using the staining methods of Yunis *et al.* (1971).

refore, the interpretation of Schnedl (1972) that the highly condensed DNA localized at the centromeric regions of all autosomes and the short arm of the Y-chromosome in cattle is really removed during the incubation period in a buffer solution is contradictory. The foregoing observations, however, suggest the heterogeneous molecular organization of the constitutive heterochromatin present in *Bos taurus*.

The bright fluorescence in one arm of the Y-chromosome (Fig. 2a) on staining

¹ See footnote 1 on page 374

² See footnote 2 on page 374

with the fluorochrome "Hoechst 33258" is in conformity with the observations of Hansen (1971, 1972) but is in contrast with the corresponding weak quinacrine fluorescence reported by Schendl (1972).

The findings in *Bos taurus* substantiate the identical failure of the type of constitutive heterochromatin present in the Algerian hedgehog to fluoresce with "33258 Hoechst" (Hilwig and Gropp 1972), whereas it is stained following the heat denaturation methods of Yunis *et al.* (1971) and remains unstained following the regular staining techniques of Arrighi and Hsu (1971) (Gropp and Natarajan 1972). This differential behaviour of the centromeric heterochromatin, supposedly possessing similar DNA replication properties, in mouse, cattle and the Algerian hedgehog, suggests that the constitutive heterochromatin is heterogeneous and thus, has to be subdivided into different types corresponding to the different staining patterns. In other words, these varying properties of the staining of the constitutive heterochromatin is due to its compositional heterogeneity or its complex structure.

Abstract

The centromeric regions of the cattle chromosomes exhibit a variable staining property, if staining procedures, which visualize the constitutive heterochromatin in some other species, are used. These regions are non-fluorescing with the fluorochrome "Hoechst 33258" (a benzimidazol derivative) and remain unstained following the staining methods for C- and G-banding. However, using the heat denaturation-renaturation procedures, the centromeric regions are darkly stained. This differential behaviour of the centromeric heterochromatin in *Bos taurus* suggests that the constitutive heterochromatin is heterogeneous in its staining intensities and thus, its variable staining properties may be due to its complex structure or its compositional heterogeneity.

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