Centromeric loss in translocations of centric fusion type in cattle and water buffalo

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Robertsonian translocations in Podolian and Romagna cattle (Bos taurus L.) and Asiatic river buffaloes (Bubalus bubalis L.) were studied with C, G + C, and R-banding techniques. The investigation demonstrated: (a) presence of one block of constitutive heterochromatin in the q-arm of the cattle translocation chromosome; (b) chromosomes identified as 1 and 25 according to the system used - but presumptively 1 and 29 as earlier described - being involved in the cattle centric fusions, with the centromere region of chromosome 25(29) being lost and that of chromosome 1 being retained; (c) no constitutive heterochromatin in the q-chromosome arm of the bi-armed chromosomes 1 and 5 in the water buffaloes (with retention of the constitutive heterochromatin in their p-arms) and partial losses of centromeric heterochromatin in both arms of chromosomes 2, 3 and 4; (d) presence of a C-positive segment in the telomere region of the p-arm of chromosome 4 in the water buffalo; (e) no bi-armed chromosome common for the two species.

Karyotype evolution is discussed and the urgency of an appropriate nomenclature system for cattle and buffalo chromosomes is briefly stressed.

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Robertsonian or centric fusion translocation is known to be the most common mechanism in karyotype evolution of mammals (Hsu 1979). The family Bovidae includes several species demonstrating variable diploid chromosome numbers but having similar fundamental numbers (NFs), which, with the exception of a few cases, vary between 58 and 62. The karyotypes contain variable numbers of centric fusions, which have changed the diploid number but not the NF (WURSTER and BENIRSCHKE 1968). These rearrangements of a basic karyotype consisting of one-armed chromosomes have later been confirmed by studies using banding techniques in various species of Bovidae (EVANS et al. 1973; BUCKLAND and EVANS 1978a; BUNCH and NADLER 1980; DI BERARDINO and IANNUZZI 1981, 1984).

In cattle (2n=60, NF=62), several centric fusion translocations involving different chromosome pairs have been found so far (POPESCU 1977a). Only one of them, however, the translocation involving chromosomes 1 and 29 (GUSTAVSSON and ROCKBORN 1964), seems to be extensively distributed although with different incidences in the breeds studied. The observations of the deleterious effects on fertility induced by this translocation (GUSTAVSSON 1969) and the attention these observations attracted initiated work in cytogenetics of domestic animals. Hitherto the 1/29 translocation has been found in approximately 40 breeds of domestic cattle, including the Podolian and the Romagna breeds of Italy (for nomenclature see MASON 1969) as well as breeds of Bos indicus (POPESCU 1984). The distribution and the effects of the 1/29 were described by, among others, GUSTAVSSON (1979). The banding techniques applied so far on mitotic chromosomes of animals carrying this translocation revealed one block of constitutive heterochromatin (HC) and identified the chromosomes involved in the fusion as 1 and 29 (POPESCU and BOSCHER 1974; POPESCU 1975; GUSTAVSSON and HAGELTORN 1976; GUSTAVSSON et al. 1976; GUSTAVSSON 1979; DI BERARDINO et al. 1979; MASUDA et al. 1980; CIUPERCESCU et al. 1984).

In the Asiatic river buffalo (Bubalus bubalis, 2n = 50) five bi-armed chromosome pairs correspond
to ten one-armed pairs of the cattle karyotype (Di Berardino et al. 1981; Di Berardino and Ianuzzi 1984), and one can assume that they originated from centric fusion translocations. Also these bi-armed autosomes of the buffaloes demonstrate single HC blocks.

The application of the RBA-technique (Dutril-Laux et al. 1973), in addition to fluorescent G + C-banding (Ianuzzi et al. 1985), on chromosomes of late and early prometaphase stages (Di Berardino et al. 1985) to-day allow better characterization of the chromosomes. We therefore found it to be of interest, by application of the karyotype systems described by Di Berardino and Ianuzzi (1982, 1984) and Di Berardino et al. (1985) to characterize the centric fusion translocation found in cattle as well as the normal bi-armed chromosomes of water buffalo and their relationship to the cattle karyotype.

Material and methods

Six male cattle, four of the Podolian breed and two of the Romagna breed, heterozygous for a centric fusion, and four river buffaloes (two males and two females) reared in Southern Italy were used for the study.

Peripheral blood was cultured for about 72 hours at 38.5°C in 7 ml of McCoy's 5A modified medium (Gibco), supplemented with autologous plasma (20%) and Poxeweed mitogen (1%). For each animal, cultures were set up and treated as follows: (A) conventional cultures (without addition of any base analogue, for application of the CBG and CBA techniques; (B) addition of 20 μg/ml of the base analogue 5-bromodeoxyuridine (BrdU) to the cultures 6 hours before harvest for application of the RBA-technique; (C) treatment of cultures for simultaneous demonstration of fluorescent G and C-bands according to the procedure reported by Ianuzzi et al. (1985). The latter technique is now standardized: 24 hours before harvest, 20 μg/ml of BrdU and 0.5 μg/ml of methotrexate (MTX) are added to block the cell cycle in the S-phase to allow early BrdU incorporation. After 17 hours, the cells are washed in physiological solution (Puck's saline) to eliminate BrdU and MTX, and allowed to recover for 5.5 - 6 hours in the same medium containing thymidine (10 μg/ml). According to the proposal of ISCN (1978), this combined technique can in the following be designated GBA+CBA. Colcemid in a concentration of 0.01 and 0.03 μg/ml medium for the cattle and buffalo chromosomes, respectively, was added 1.5 and 1 hours to cultures A and B-C, respectively. The cell suspension was treated with a hypotonic solution (0.075M KCl) for 20 min and fixed three times in 3:1 methanol-acetic acid with the first fixation overnight.

C-bands were obtained in bright field as well as in fluorescence, the former (CBG) according to the technique described by Sumner (1972), the latter by staining for 30 min with acridine orange. For the last stainings, designated CFA and CBA, respectively, slides previously treated with the method of Sumner (1972) as well as slides obtained by the procedure "C" (see above) were used after treatment in 2 × SSC for 30 min at 60°C.

Thirty metaphases were analysed from each male cattle and each one of two buffaloes (male and female). Acridine orange staining for GBA+CBA and RBA-bands was performed as reported for RBA-banding by Di Berardino and Ianuzzi (1982). Ten complete karyotypes were examined in each animal.

A Leitz epifluorescent microscope was used for the chromosome analyses. The photomicrographs were taken with Kodak TPN 2415 film and printed on Kodabrome paper no. 2 and 3. The cattle chromosomes were arranged according to the RBA-banded karyotype (Di Berardino and Ianuzzi 1982; Di Berardino et al. 1985), which is a 'translation' based on direct comparison with the standard G-banded karyotype (Proceedings of the First...
Fig. 2. Simultaneous demonstration of GBA and CBA-bands in the chromosomes of a lymphocyte of a bull heterozygous for the 1/25-translocation.

International Conference for the Standardization of Banded Karyotypes of Domestic Animals 1980. The chromosomes of the water buffaloes were arranged according to the RBA-karyotype reported by Di Berardino and Iannuzzi (1984), which is also based on direct comparison with the cattle standard karyotype.

Results

Cattle

*CBG and CBA-bands.* In all cells studied, only one block of HC located in the proximal region of the q-arm of the translocation chromosome was observed (Fig. 1).

*GBA+CBA and RBA-bands.* By detailed analysis the prometaphase chromosomes (Fig. 2) involved in the centric fusion were classified as 1 and 25 (Fig. 3) with the HC block of chromosome 25 being lost and that of chromosome 1 being retained. Chromosome 1 demonstrated a size similar to the long arm of the translocation, but chromosome 25 was longer than the corresponding arm because of the loss of chromosome material, a fact which was confirmed in detailed studies of metaphases stained according to the GBA+CBA (Fig. 4) and RBA (Fig. 5) techniques. The HC block of each centric fusion chromosome showed an apparent division into two segments in comparison with the normal chromosome 1, where this region appeared smaller and only rarely divided (Fig. 4).

Fig. 3. The karyotype of the prometaphase demonstrated in Fig. 2. Notice the C-band polymorphism in chromosome pairs 11, 18, 19, 26, 27, and 29.

River buffalo

*CFA-bands.* There was a remarkable difference in HC size between the bi-armed pairs (Fig. 6) and other chromosomes, including the sex chromosomes. The X had a proximal HC segment in addition to the centromeric region and all the Y was fluorescing bright. There was also an evident size variability of HC between different one-armed chromosomes.

In the chromosome arm 4p there was a HC segment localized at the telomere (Fig. 6B-D). The buffalo bi-armed chromosomes compared to corresponding cattle chromosomes. A comparison of the bi-armed chromosomes 1 to 5 of the river buffalo karyotype with their presumptive homologues in the cattle karyotype by the GBA + CBA and RBA-technique is demonstrated in Fig. 7. From this comparison it is possible to confirm that the bi-armed buffalo chromosomes originated by centric fusions of chromosomes corresponding to 1 and 29, 2 and 22, 8 and 19, 5 and 28 and 16 and 25 of the cattle karyotype. Furthermore, in comparison with the corresponding chromosomes of cattle, an
extensive reduction of HC in all the bi-armed pairs was noticed. There was also a loss of the entire centromeric regions in chromosome arms 1q and 5q and retention of the centromeric regions in their p-arms. Amounts of HC appeared to be lost from the centromere regions of both arms for chromosomes 2, 3 and 4. Although there was a HC segment at the telomere of the chromosome arm 4p in the water buffalo, a high degree of G- and R-band homologies between the two species could be observed in all chromosomes. High resolution RBA- (left chromosome of Fig. 8A) and GBA + CBA-banding patterns (right chromosome of Fig. 8A) of the translocation in cattle were compared with the chromosome arms 1q and 5p of the buffalo (Fig. 8B-C). It should be noted that the large block of HC is absent in the buffalo chromosome arm 1q but present in the long arm of the cattle translocation. Instead, there is a HC block in the chromosome arm 5p of the buffalo which has no correspondence in the short arm of the cattle translocation.
Fig. 5 a-c. The six smallest chromosome pairs of cattle, including pair 25, here involved in the 1/25-translocation, cut out from three metaphases of one animal (not the same as in Fig. 4) and stained according to the RBA technique. Notice the large size of chromosome 25 compared to the corresponding arm 25 in the translocation.

Discussion

Morphology and cytological origin of the cattle centric fusion translocation

Previous studies revealed one block of HC in the 1/29 Robertsonian translocation (Gustavsson 1979; Di Berardino et al. 1979; Masuda et al. 1980; Ciupercescu et al. 1984) localized in the q-arm (Popescu 1973, 1975; Popescu and Boscher 1974) and two blocks in other centric fusions involving variable chromosomal pairs (Eldridge 1974; Popescu 1977b; Di Berardino et al. 1979; Papp and Kovacs 1980; Masuda et al. 1980; Ciupercescu et al. 1984). For this reason, the 1/29 translocation has been considered to have an ancient origin while the others are thought to have originated more recently. The CBG and CBA-banding patterns observed in the six cattle studied in the present paper demonstrated one block of HC localized in the q-arm. This was confirmed also by GBA + CBA and RBA-bands which enabled us to identify the chromosomes as 1 and 25 according to the chromosome
Fig. 6 A-D. CFA-banding patterns of male river buffalo chromosomes. (A) The sex chromosomes are indicated as well as the bi-armed chromosome arm 4p (large arrow) and the centromeres of the bi-armed chromosomes (small arrows). (B-D) Cut out pictures demonstrating the terminal C-positive segment of 4p, which shows clear heteromorphism within the pair (D).

arrangement system used. From these observations it is possible to verify the conservation of the centromeric region in chromosome 1 and loss in chromosome 25. This event has reduced the length of the p-arm. The C-banding patterns thus agree with the patterns of the 1/29 translocation earlier described in cattle and since the latter translocation occurs with a high incidence in Podolian and Romagna cattle (Di Berardino et al. 1980b; Succi et al. 1980) it is reasonable to assume that the translocation described here is the so-called 1/29 earlier observed. A loss of a proximal segment in chromosome 29 preceding the translocation into 1/29 has also recently been revealed in synaptosomal complex analysis by electron microscopy (Switoski et al., in prep). So until proven otherwise, we adhere to the conclusion that the translocation here identified as 1/25 in fact is the well-known 1/29.

More work is necessary in order to establish whether the larger size and the apparent division of the HC block in the translocated chromosome, compared to the homologous chromosome 1, is due to the acquisition of the HC lost from chromosome 25 or not, especially in relation to the extensive polymorphism of HC between and within cattle karyotypes (Di Berardino et al. 1980a).

Morphology of the river buffalo bi-armed chromosomes and their relationship to the cattle chromosomes

Previous studies in the river buffalo demonstrated the origin of bi-armed chromosome pairs by centric fusion of chromosomes corresponding to 1 and 29, 2 and 22, 8 and 19, 5 and 28, and 16 and 25 of the cattle karyotype (Di Berardino et al. 1981; Di Berardino and Ianuzzi 1981, 1984). The bi-armed chromosomes demonstrated no or very small amounts of HC (Gupta and Ray-Chaudhuri 1978; Cribiu and Obeidah 1978). By using a more resolute banding technique the present paper confirms previous identification of homologies to the cattle chromosomes and demonstrates that the fusions were accompanied by HC losses in relation to the acrocentric chromosomes of their cattle homologues. In spite of the fact that only a few animals were investigated and that there is extensive polymorphism of HC between and within the karyotypes of the species concerned, it is possible to show the retention of the centromere regions in the p arms of chromosomes 1 and 5 and centromere region loss in their q-arms, while in chromosomes 2, 3 and 4 there was an evident HC loss from both arms. In the African buffalo (Syncerus caffer) a uniform C-banding pattern has been reported (Buckland and Evans 1978b) while in the Asiatic buffalo (Bubalus bubalis) the centromeric region appears variable in size both in swamp (Di Berardino and Ianuzzi 1981) and in river buffalo (Fig. 6). The C-banding patterns of the sex chromosomes of the river buffalo showing a large heterochromatic proximal block of HC in the X chromosome in addition to the centromeric HC and a completely heterochromatic Y chromosome are in agreement with the CBG-patterns of the sex chromosomes found in swamp buffalo (Ianuzzi and Di Berardino 1985). It is possible that parts of the HC were lost by transfer of centric fragments to other chromosomes now showing conspicuous amounts of C-positive material. However, as is well known, there are also other mechanisms of acquisition of extra chromatin, such as unequal crossing-over (Smith 1976) and saltatory replications of repeated DNA sequences (Britten and Kohne 1969; Britten and Davidson 1971).
Fig. 7. Comparison of the five biarmed chromosomes in the water buffalo with corresponding cattle chromosomes by application of the GBA+CBA and RBA techniques. The buffalo chromosomes, GBA+CBA-banded to the left (A) and RBA-banded to the right (B), were arranged in the middle, and their corresponding cattle chromosomes, to the left and right according to the banding techniques used. For the detailed comparison, see the text. Notice the high degree of correspondence between the G-positive and R-negative regions and vice versa.

It is interesting to notice that, in the 1/29 and the 16/25 centric fusions of buffalo, the centromere of the chromosome arm 1q (corresponding to 1 of cattle) was lost and the centromere of chromosome arm 5p (corresponding to 25 of cattle) was retained (Fig. 8B-C), whereas in the translocation of cattle the centromere of chromosome 1 was retained and that of chromosome 25 was lost. Another important observation was the C-positive segment localized at the telomere of the chromosome arm 4p of buffalo presumably corresponding to the cattle chromosome 28, which does not show the same banding patterns. This new observation deserves a more accurate cytogenetic investigation not only because of the limited number of animals examined but also because of the fact that the telomere of this chromosome is known to carry Ag-NORs (Di Berardino et al. 1981) and have been found to be fused, by tandem fusion, with the centromeric region of chromosome 9 in the river buffalo karyotype to give the large chromosome 1 of the swamp buffalo (Di Berardino and Iannuzzi 1981).

Karyotype evolution

Robertsonian translocation is, in the sense of the original paper (Robertson 1916), the consequence of a fusion between two acrocentric chromosomes after breakages close to the centromeres, one in the short arm of one chromosome and the other in the long arm of the other chromosome. The result is the formation of a bi-armed stable chromosome and a centric fragment which is eliminated after a few cell divisions. White (1973) suggested other mechanisms involving breakages either in both short arms, with formation of a dicentric chromosome, or within the two centromeres and their ensuing fusion into one. The present study is in agreement with the first and classical concept, especially for the fusion of 1 and 25 (the presump-
Reading system (Proceedings of the First International Conference for the Standardization of Banded Karyotypes of Domestic Animals 1980), to reinvestigate chromosome aberrations such as the 1/29 translocation, and to find out correspondences between chromosomes stained according to the two different staining techniques. Unfortunately, chromosomes can not be dually stained with the two techniques so the only way to find out homologies, as we see it, is to make use of chromosomally aberrant karyotypes (trisomies, centric fusions etc.), and karyotypes of related species containing centric fusion translocations. This is an important task to do before cattle and buffalo cytogenetics can be further developed.

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**Nomenclature of cattle and water buffalo chromosomes**

It is now highly urgent to establish a nomenclature system for cattle and buffalo chromosomes based on both G and R-banding patterns. In this work, high resolution techniques should be used. Since the R-banding by some workers to-day is considered superior to G-banding for identification of the chromosomes it is important to reconsider the
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