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Brief report

Chromosomal localization of conglutinin (*CGN1*) gene to river buffalo by sequential RBH-banding and FISH

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The human-cattle comparative gene map reveals numerous conserved syntenies (WOMACK and MOLL 1986; THREADGILL et al. 1990; ZHANG and WOMACK 1992). In light of this syntenic conservation, gene mapping efforts in cattle benefit greatly from the rapid development of the human map. Likewise, gene mapping studies in other bovids are advanced through the development of the cattle gene map, especially when considering the high degree of chromosome band conservation among bovids (BUCKLAND and EVANS 1978; GALLAGHER and WOMACK 1992; HEDIGER et al. 1991; IANNUZZI et al. 1990b; HAYES et al. 1991).

We have begun to use the cattle gene map in conjunction with chromosome band homologies to rapidly develop the river buffalo (*Bubalus bubalis* L., 2n = 50) physical gene map. Presently, only five loci have been mapped to four different river buffalo chromosomes using in situ hybridization (IANNUZZI et al. 1993a,b,c; HASSANANE et al. 1993), but since these loci marked four cattle syntenic groups, i.e., U3, U4, U18, and U20, and were assigned to homologous chromosomes of cattle and river buffalo, the entire syntenic groups, consisting of approximately 78 loci (FRIES et al. 1993), are tentatively assigned to river buffalo chromosomes.

Conglutinin (*CGN1*) belongs to the vertebrate C-type (calcium-dependent) lectin family, and recently it has been placed in a subfamily of lectins called collectins because of the presence of a collagen-like domain (MALHOTRA et al. 1992). Recently, *CGN1* gene was assigned to cattle syntenic group U29 through analysis of cattle-rodent hybrid cells, and sublocalized to cattle (*Bos taurus* L.) chromosome 28q18 band by fluoresence in situ hybridization (FISH) of a genomic clone containing all of the *CGN1* (GALLAGHER et al. 1993). This gene assignment was the first marker for the syntenic group U29 in cattle, and it is supposed that this entire syntenic group resides on BTA 28 (GALLAGHER et al. 1993). In the present paper, we sublocalize the *CGN1* gene to a river buffalo chromosome by FISH as part of our continued effort to extend the river buffalo physical map and to test chromosome band homologies between cattle and river buffalo.

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Materials and methods

Cell culture, chromosome preparation, FISH, and probe detection were carried out as previously reported (IANNUZZI et al. 1993a).

For R-banding, slides were stained for 10 min with Hoechst 33258 (50 μg/ml in distilled water), washed in tap and distilled water and mounted in phosphate buffer (pH = 7.0) under a sealed cover slip. Slides were observed using a fluorescent Aristoplan Leitz microscope (combination filter *A*). The best RBH-H (R-banding by late incorporation of BrdU and Hoechst 33258 followed by Hoechst fluorochrome staining) banded early- and prometaphase preparations were photographed with Kodak 2415 film. The slides were prepared for FISH by removing the cover slip, immersing in ethanol, rinsing in tap and distilled water, and air drying.

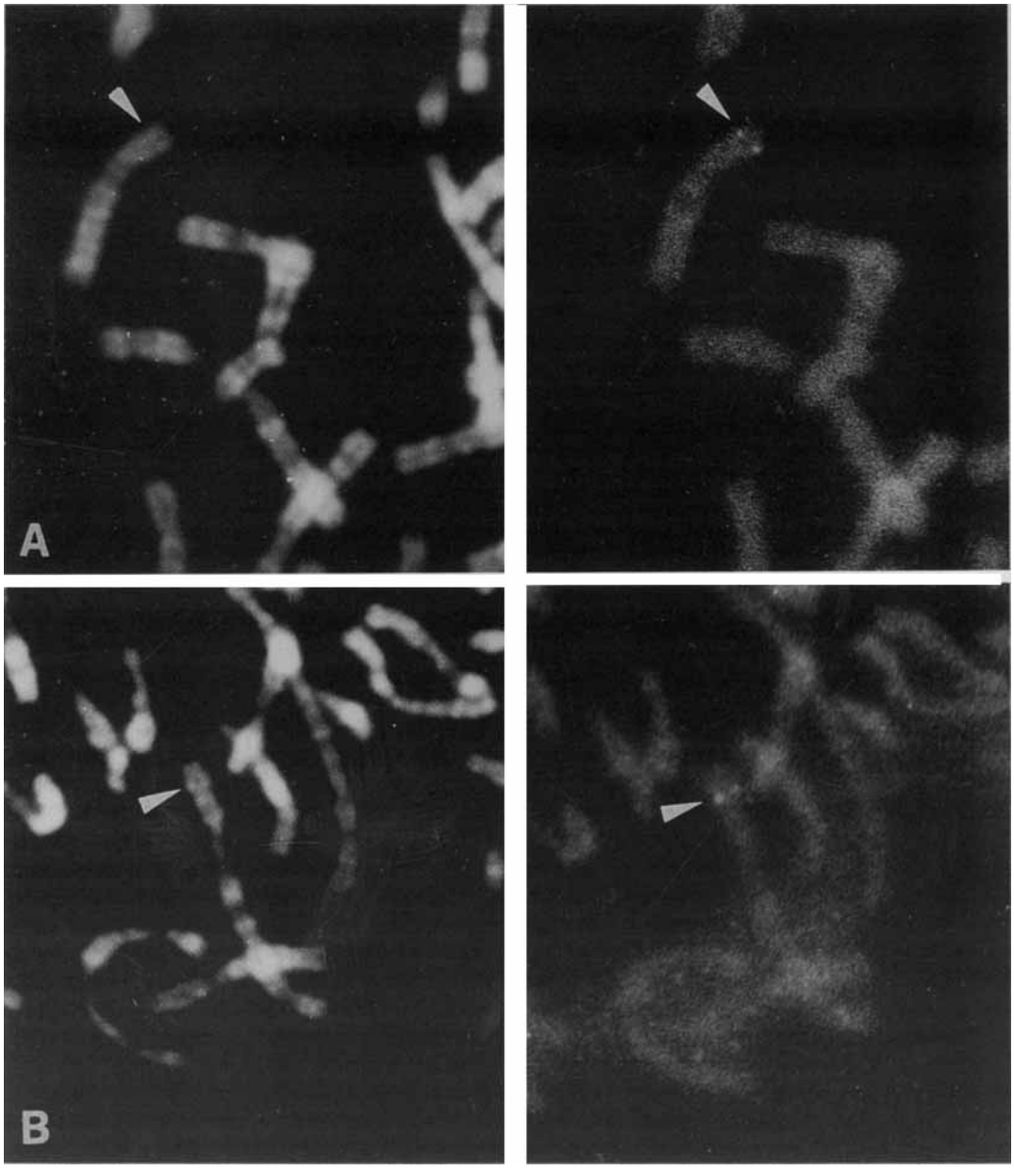

Fig. 1A and B. *CGN1* FISH results. Partial early-(**A**) and prometaphase-(**B**) cells sequentially treated for RBH-banding (left) and FISH (right). Arrows mark the site of *CGN1* probe hybridization signals on chromosome 4p16 band.

A 35 kb genomic clone containing the entire conglutinin gene (cosmid clone #16-2; LIOU et al. 1994) was used as a probe in the present study and hybridized in the presence of cattle genomic DNA enriched for the highly repeated fraction to suppress repetitive sequences of the probe. Probe hybridization signals were photographed with Kodak Tri-X Pan film (D-11 as developer, dil. 1:1). After detection of the hybridized probe, some slides were counterstained with propidium iodide so as to allow precise localization of probe signals relative to positive and negative R-bands. The single detections in metaphase and prometaphase cells were analysed according to the chromosome band

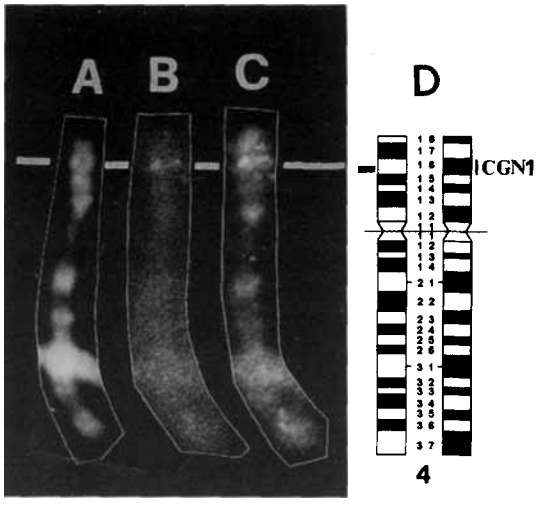

Fig. 2. A single river buffalo chromosome 4 (the same of Fig. 1B), sequentially treated for RBH-banding (A), FISH (B) and propidium iodide counterstaining (C), and positioned to the left of its G- and R-banded idiogram (D). Note the hybridization signals on chromosome 4p16 band.

nomenclature described by IANNUZZI et al. (1990a).

Results

Fig. 1 shows the sequential RBH-banding and FISH of cosmid clone #16-2 in river buffalo early- and prometaphase cells. Hybridization signals of the probe assign the *CGN1* gene to river buffalo chromosome 4p16, idiogrammatically represented in Fig. 2.

A total of 37 early- and prometaphase cells sequentially treated for RBH-banding and FISH were analyzed. Hybridization signals were observed in 15 (40 %) spreads, of which 5 showed hybridization signals in both chromatids of each homologue, 7 in both chromatids of one chromosome, and 3 in one chromatid of one chromosome.

Discussion

Since the bovine *CGN1* gene was previously localized to BTA 28 (GALLAGHER et al. 1993), we expected to find hybridization signals on the homologous river buffalo chromosome 4p (IANNUZZI et al. 1990b). As shown in Fig. 1, hybridization was to 4p, but our sublocalization to a chromosome band in river buffalo differs from that proposed by GALLAGHER et al. (1993) for cattle. In cattle, the *CGN1* gene was assigned to BTA 28q18 band (G-positive), while in the present study we sublocalize the *CGN1* gene to river buffalo chromosome 4p16 band (R-positive) (Fig. 2). River buffalo 4p16 is believed to be homologous to BTA 28q17 band (ISCNDA 1989). The difference in chromosomal localization of the *CGN1* gene in the two species might be real or might simply be a result of problems (limits) in resolution. If the difference is real, then the chromosomal location of the *CGN1* gene in river buffalo is slightly more centromeric in comparison with cattle.

Despite this discrepancy, our assignment of the *CGN1* gene to river buffalo 4p confirms the homology between BTA 28 and river buffalo 4p (IANNUZZI et al. 1990b), which until now was based exclusively on partial band similarities. River buffalo 4p differs in banding pattern from BTA 28 due to loss of constitutive heterochromatin (IANNUZZI et al. 1987) and a pericentric G-positive band (IANNUZZI et al. 1990b) during the centric fusion translocation which formed the biarmed river buffalo chromosome 4 [rob(5;28)] (IANNUZZI et al. 1990b). Since high resolution G- and R-banding techniques have been applied to river buffalo chromosomes (IANNUZZI et al. 1990a,b), chromosome 4p might prove useful in resolving nomenclature ambiguities related to BTA 28, as recently pointed out by GALLAGHER et al. (1993).

Because the *CGN1* gene has been placed in cattle syntenic group U29 (GALLAGHER et al. 1993), the localization of *CGN1* to river buffalo 4p allows us to tentatively assign this entire syntenic group (*HK1*, *PLAU*, *RBP3*, *SAP2*, and *CGN1*; WOMACK et al. 1993) to river buffalo 4p. Furthermore, since we have previously assigned the lysozyme gene cluster (a marker for cattle syntenic group U3) to river buffalo 4q (IANNUZZI et al. 1993c), molecular markers are now assigned to both arms of this chromosome.

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