Mapping of the interferon gamma (IFNG) gene in river and swamp buffaloes by in situ hybridization

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Interferons (IFNs) are a family of proteins characterized by a potent ability to provide virus-resistant state in their target cells, inhibit cell proliferation and modulate immune response (STEWART et al. 1980). Of the three major types of interferons, i.e., IFN-α (IFNA), IFN-β (IFNB), and IFN-γ (IFNG), the latter is considered as an important immunoregulatory agent (SONNENFELD et al. 1978), with a 10–100-fold antiproliferative effect on transformed cells, as compared to the other two types (RUBIN and GUPTA 1980; BLALOCK et al. 1980). In the Bovidae family, IFNG has been studied in detail only in cattle. The bovine IFNG gene has been cloned and sequenced (CERRETTI et al. 1986), and recently localized to the q22–q24 bands of chromosome 5 (CHAUDHARY et al. 1993).

Comparative chromosomal studies conducted in the Bovidae indicate that cattle, sheep, goat, and buffalo (both river and swamp types) chromosomes share marked similarities in banding patterns (ISCNDA 1989; DE HONDT et al. 1989; IANNUZZI et al. 1990). A number of gene localizations in the former two have demonstrated that banding similarities reflect homologies at the genetic level (HEDIGER et al. 1990, 1991; CHOWDHARY et al. 1991). Although it has been proposed that these genetic homologies extend also to the goat and buffalo chromosomes, there is very little evidence to support the concept. The present study was therefore undertaken to verify possible correlation between banding and genetic homologies in buffaloes and other related Bovidae species, through localization of the IFNG gene in river and swamp buffaloes.

Material and methods

A 700 bp bovine IFNG cDNA probe, isolated from a bovine lymphnode cDNA library (CERRETTI et al. 1986) was used in the present study. The probe was amplified by the polymerase chain reaction (PCR), using primers specific for the PGEM vector in which the probe DNA had been cloned. Tritium (3H) labelling of the probe was carried out by the random priming method modified by LIN et al. (1985). The specific activity of the probe was \(2.1 \times 10^8\) dpm/μg DNA.

Standard methods were employed for obtaining metaphase chromosome preparations from peripheral blood lymphocytes collected from two male and two female Egyptian river buffaloes, and two male and one female Indonesian swamp buffaloes. In situ hybridization was carried out as described earlier (MAKINEN et al. 1989; CHOWDHARY et al. 1989b). After autoradiography, the preparations were stained with 5% Giemsa. Metaphase spreads with silver grains on the chromosomes were photographed, and the chromosomes were later iden-
An idiogrammatic representation of the distribution of silver grains on G-banded river buffalo chromosomes. The bovine IFNG cDNA specifically hybridized to the 4q23-q26 bands.

Results

River buffaloes

One hundred metaphase spreads from one hybridization experiment were analysed for silver grain distribution on the chromosomes (Fig. 1). A representative metaphase, Giemsa-stained after autoradiography and subsequently G-banded, is presented in Fig. 2a and b, respectively. A total of 196 grains were scored, of which 64 (33 %) were located on the q arm of chromosome 4 (P < 0.001). Analysis of grains on chromosome 4 showed that, of the total grains on this chromosome, 45 (71 %) were located within the q23-q26 bands, making this region the most likely location of the IFNG gene in river buffalo.

Swamp buffaloes

A total of 100 metaphase cells from one in situ hybridization experiment were analysed for silver grain distribution on the chromosomes. In 35 % of the metaphase cells analysed, the specificity of the signal could be detected on the middle part of the p arm of chromosome 1. This chromosome can be unambiguously identified, as it is the largest sub-metacentric chromosome in the swamp buffalo karyotype. Totally, 195 grains were scored from all metaphases, of which 43 (22 %) were present on chromosome 1 (P < 0.001). The background signal was low and uniformly distributed. Hence the grain distribution only on chromosome 1 is presented (Fig. 3). On this chromosome, 98 % of the grains were present on the p arm, mainly clustering (77 %) on the middle part, making this region the most probable location of the IFNG gene in swamp buffaloes. In the absence of any standard nomenclature for G-banded swamp buffalo chromosomes, we have adopted the band designations available from river buffalo chromosomes. Thus, the IFNG gene can be assigned to the lp24-p26 bands, in swamp buffaloes. Six partial metaphase
Fig. 2a and b. a A Giemsa stained metaphase cell showing (arrow) specific hybridization site of the IFNG cDNA on chromosome 4q in river buffaloes; b the same metaphase subsequently G-banded.

Discussion

The present study localizes the IFNG gene to the q23–q26 bands of chromosome 4 in river buffaloes, and to the p24–p26 bands of chromosome 1 in swamp buffaloes, thus providing the first mapping data for these chromosomes in each buffalo type.

A number of studies have demonstrated a high degree of banding homology between chromosomes of different species belonging to the family Bovidae (HAGELTORN and GUSTAVSSON 1974; BUNCH et al. 1976; ISCNDA 1989). In recent years, this homology has been confirmed by gene localizations in, e.g., cattle, sheep and goat (HEDIGER et al. 1990, 1991; CHOWDHARY et al. 1991). Banding comparisons have also been carried out between the chromosomes of river and swamp buffaloes (BONGSO and HILMI 1982; IANNUZZI and DI BERARDINO 1985; CHOWDHARY et al. 1989a). These studies clearly indicate that the difference in chromosome number between the two types (river 2n = 50 and swamp 2n = 48) most probably arose from a tandem fusion between chromosomes 4 and 9 of the river buffalo karyotype, resulting in a big submetacentric chromosome, considered as chromosome 1 in swamp buffaloes. Comparison between river buffalo and cattle chromosomes indicates that chromosome 4 in river buffalo is the result of a Robertsonian
Fig. 4. Six partial metaphase cells showing specific hybridization site (arrows) on chromosome 1p in swamp buffaloes.

translocation between chromosomes 5 and 28 in cattle (De Hondt et al. 1989; Iannuzzi et al. 1990), while chromosome 9 in river buffaloes corresponds to cattle chromosome 7.

Recently, using a bovine cDNA probe the IFNG gene was mapped to 5q25–q27 in cattle (Chaudhary et al. 1993). The same probe was used in the present study to localize the gene in river and swamp buffaloes. The probe also hybridized to those chromosomes in the two species which, on the basis of banding patterns, resemble cattle chromosome 5. Moreover, the peak of the signal was also observed on the corresponding bands, further supporting the contention that banding homologies between different species within the Bovidae, reflect genetic similarities. However, it must be kept in mind that, if a conserved synteny is detected for these chromosomes, the physical order of the genes will not necessarily be the same in all the species, since minor (or sometimes even substantial) rearrangements within the chromosomes, can be expected in the course of evolution.

It is known that chromosomes 3q, 5, 4q, and 1p in sheep, goat, river buffaloes and swamp buffaloes, respectively, resemble chromosome 5 in cattle in banding patterns. Evidence of homology between chromosome 1p in sheep and bovine chromosome 5, at the molecular level, is provided by the fact that all the three loci, viz. keratin type II, lactatedehydrogenase B (LDHB) and peptidase B (PEPB), mapped to chromosome 3 in sheep (Jones et al. 1985), have also been mapped to chromosome 5 in cattle. A recent review (Fries 1992) shows that almost 30 genes have now been assigned to bovine chromosome 5. Based on the evidence provided in the present study, it can be expected that these genes may also be found on the q arm of chromosome 4 in river buffaloes, and the p arm of chromosome 1 in swamp buffaloes. It would therefore be interesting to map some of these genes in both types of buffaloes, mainly in context of comparative gene mapping studies.

Acknowledgements. — We wish to thank Dr Rudy Setiabudi and Dr Ahmed Farid for kindly providing the buffalo blood samples. The study was financed by the Swedish Council for Forestry and Agricultural Research.

References


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