PCR AMPLIFICATION AND LOCALIZATION BY FISH OF THE NGFB GENE IN CATTLE

AMPLIFICACIÓN MEDIANTE PCR Y LOCALIZACIÓN POR FISH DEL GEN NGFB DEL GANADO BOVINO

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Additional keywords

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Palabras clave adicionales

Factor beta de crecimiento del nervio.

SUMMARY

In this work, our aim was to localize precisely the bovine beta-nerve growth factor (NGFB) gene (previously assigned to bovine chromosome 3 (BTA3) by Elduque and Womack, 1992), one of the anchor *loci* listed by O'Brien *et al.* (1993).

Taking advantage of NGFB sequences previously described in various species, we have cloned a sequence representing part of the bovine gene encoding NGFB and determined its localization on BTA3q23.

RESUMEN

El objetivo de este trabajo fue localizar precisamente el gen del factor de crecimiento beta del nervio de ganado bovino (NGFB) (previamente asignado al cromosoma bovino 3 (BTA3) por Elduque y Womack, 1992) uno de los *loci* de anclaje relacionados por O'Brien *et al.* (1993).

Aprovechando las secuencias del NGFB previamente descritas en varias especies se ha clonado una parte representando la secuencia del gen bovino que codifica el NGFB y se ha determinado su localización en BTA3q23

INTRODUCTION

Comparative genomic analysis has led to the identification of conserved segments between chromosomes of man and cattle. These conserved regions have been described basically by two approaches: comparing the location of evolutionary conserved *loci* (Type I *loci*) and by heterologous chromosome painting.

Today, 314 coding genes or pseudogenes have been mapped in cattle (Eggen and Fries, 1995). Most of these loci have been assigned by syntenic analysis of bovine-rodent hybrid cells, which provide no information at the subchromosomal level. Only 72 of them have been localized precisely by *in situ* hybridization.

Recently, it has been shown that human chromosomes-specific probes can be used to paint regions of homology in cattle (Zoo-FISH). Several groups have described a comparative genome map of human and cattle (Hayes, 1995; Solinas-Toldo *et al.*, 1995; Chowdhary *et al.*, 1996) using this technique. However, the

level of definition of this approach does not exceed that of chromosomal bands and the conservation of gene order within segments of homology rest unknown.

Focus on the precise chromosome localization of anchored reference *loci* (O'Brien *et al.*, 1993) should lead to define precisely the intrachromosomal rearrangements events which have occurred in these conserved segments between species.

In this study, we report the PCR amplification, sequence and physical localization in cattle of the subunit b of

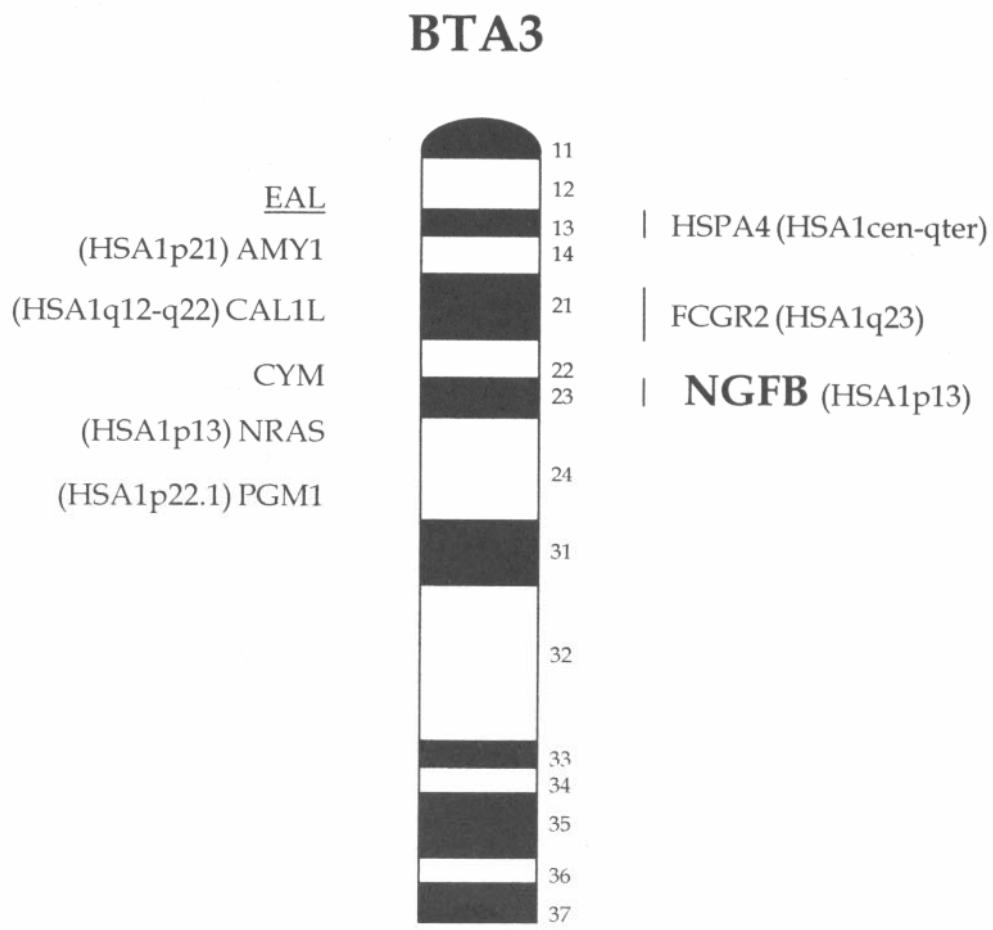

Figure 1. *A schematic diagram of BTA3 (R-banded) showing Type I* loci *already assigned to BTA3 by in situ hybridization (right), somatic cell hybrid analysis (left) or genetic analysis (left and underlined). If known, band assignments on human chromosomes are given in brackets.* (Diagrama esquemático de BTA3 (R-bandeado) mostrando los *loci* tipo I ya asignados a BTA3 mediante hibridación *in situ* (derecha), análisis de híbridos celulares somáticos (izquierda) o análisis genético (a la izquierda y subrayado). Las asignaciones de banda para los cromosomas humanos se indican entre paréntesis cuando se conocen.

Table I. *Comparison of nucleotide and derived amino acid sequences of part (687 bp) of the NGFB gene among species. Percentage of similarity was calculated with the BESTFIT program (from GCG).* (Comparación entre especies de las secuencias de los nucleótidos y aminoácidos derivados de parte (687bp) del gen NGFB. El porcentaje de semejanza fue calculado con el programa BESTFIT (de GCG)).

Amino acid sequences	Nucleotide sequences				
	Bovine	Pig	Human	Mouse	Chicken
Bovine	-	92.3	87.3	83.4	74.1
Pig	95.2	-	88.8	82.2	72.7
Human	93.5	95.2	-	85.3	74.1
Mouse	88.6	89.94	89.5	-	71.4
Chicken	77.9	76.7	77.3	74.2	-

the nerve growth factor gene (NGFB), one of the anchor *loci* proposed by O'Brien *et al.* (1993). Nerve growth factor is a polypeptide involved in the regulation of growth, differentiation and maintenance of sympathetic and embryonic sensory neurons. Its biological activity resides in the b subunit.

MATERIAL AND METHODS

Primers were defined from previously described NGFB nucleotides sequences: the upper primer in position 1 of the sequence for partial pig NGFB gene (LahbibMansais *et al.*, 1994); lower primer in position 348 of the sequence for partial bovine NGF mRNA (369 bp), corresponding to the mature NGFB mRNA (Meier *et al.*, 1 986).

The PCR was carried out in 10 µl reactions with 100 ng of bovine genomic DNA, with 1.5 mM $MgCl_2$. Samples were preheated for 5 min. at 94°C and them subjected to 30 cycles at 94°C for 30 s.; 60°C for 30 s.; 72°C for 30s.; in a Cetus 9600 thermocycler, using a Promega PCR kit.

The amplified product was prepared for ligation into vector pUC18 by using a Sure-Clone ligation kit (Pharmacia), and transformed into DH5cc competent cells (BRL). Positive clones were sequenced using a Applied Biosystems 373 automatic sequencer.

Bovine NGFB sequence was compared with NGFB sequences from other species (X52599: human NGFB mRNA; M17298: mouse NGF gene, exon 4; X04003: chicken NGF gene, exon 3'; L31898: partial pig NGFB gene, and M26809: partial bovine NGF mRNA) using the Program Manual for the Wisconsin Package, Version 8, September 1994 of the Genetics Computer Group (575 Science Drive, Madison, Wisconsin, USA 53711).

NGFB PCR products of genomic DNA from parents of the International Bovine Reference Family Panel (IBRP) were tested with the following restriction enzymes: BamHI, BstXI, EcoRI, HaeIII, HindIII, NdeII, PaeI, PstI, SacI, SalI, TaqI and XbaI.

R-banded chromosome spreads

preparation, *in situ* hybridization conditions and detection were carried out as described by Hayes *et al.* (1996). Labelled NGFB probe was prepared by PCR amplification of the plasmid containing the bovine NGFB insert, in the presence of biotin 11-dUTP, using the universal an reverse primers (Richard *et al.*, 1994).

RESULTS AND DISCUSSION

PCR amplification with the selected primers on bovine genomic DNA yielded a PCR product of the expected size. The nucleotide sequence between the two primers was determined in its entirety (687 bp). Sequence comparison revealed that this sequence contains, as expected, the bovine mature NGFB mRNA sequence (369 bp) described by Meier *et al.*, (1986), plus 318 nucleotides corresponding probably to a part of the bovine pro-NGFB precursor.

The NGFB sequences from human, mouse, pig, chicken and cattle (see material and methods) and their deduced NGFB amino acid sequences were compared witheach other and the results are shown in **table I**. These sequences was found highly homologous among the studied species. The highest homology was obtained between cattle and pig.

NGFB amplification products of genomic DNA from parents of the IBRP families were digested with several restriction enzymes (see material and methods) in order to search polymorphism for genetic analysis purposes. Unfortunately, none of the restriction endonucleases revealed any polymorphism.

The bovine NGFB gene was localized in the bovine genome by fluorescent in situ hybridization using the bovine NGFB insert as probe. A total of 30 metaphases spreads were examined: three presented double signals and 27 a single signal on one chromosome 3. The small size of this probe (687 bp) explains the low number of spreads with more than one signal. However, background was very low and permitted unambiguous interpretation of the results.

The NGFB gene is located on bovine chromosome 3 band q23. This result confirm and precise the localization of NGFB on BTA3 (assigned previously by somatic cell hybrid analysis by Elduque and Womack, 1992) and agree with the update comparative mapping data **(figure 1)** between human and bovine that show a correspondarlce between bovine chromosome 3 and part of human chromosome 1.

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