FISH MAPPING OF ACTIVIN RECEPTOR TYPE IIA (ActRIIA) TO BOVINE CHROMOSOME 2 (2q2.2-2.4)

MAPEO MEDIANTE FISH DEL RECEPTOR DE ACTIVINA TIPO IIA (ActRIIA) PARA EL CROMOSOMA BOVINO 2 (2q2.2-2.4)

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Palabras clave adicionales

ActRIIA bovino. Localización por FISH.

SUMMARY

A λ genomic bovine DNA library was screened by using a specific bovine ActRIIA 730 bp cDNA probe. Several lambda clones containing this gene were isolated.

Such clones (including λ arms) were biotin-labelled. For purpose of Fluorescent *In Situ* Hybridization, a mix up of three different labelled clones was prepared. In this way, we tried to get a maximum lenght molecular target. Since bovine inserts in the clones were large, homologous competitor sonicated DNA was added before precipitation. To prevent any possible crosshybridization, sonicated λ DNA was also added.

After 40 hours incubation at 37°C, stringency whasing was carried out and a double cycle streptavidin-FITC and byotinilated antisptreptavidin antibodies detection procedure was performed.

The results indicate that bovine ActrIIA *locus* position is on chromosome 2 (2q2.2-2.4). This results are consistent with our previous synteny and linkage data.

For the first time in any species, an activin receptor (and more precisely ActRIIA) is mapped to its chromosomal position.

RESUMEN

Utilizando una sonda específica de 730 pares de bases de ADN copia se seleccionaron y aislaron varios clones de una genoteca bovina clonada en vector λ portadores del gen ActRIIA.

Dichos clones (incluyendo los brazos λ) fueron marcados con biotina. Una mezcla de tres de dichos clones así marcados se utilizó como sonda especifica para hibridación *in situ*. De esta forma se buscó maximizar el tamaño de región con la que la sonda hibrida. Dado el gran tamaño de los clones, se añadió

ADN homólogo sonicado como competidor para evitar el exceso de ruido de fondo. Así mismo, se utilizó ADN sonicado de fago λ para evitar toda posible hibridación cruzada.

Tras 40 horas de hibridación a 37°C, se efectuaron lavados de astringencia y un doble ciclo de detección de la señal de hibridación basado en el uso de estreptavidina-FTIC y de anticuerpos antiesptreptavidina biotinilados.

Los resultados indican que la posición del *locus* ActrIIA se sitúa en el cromosoma 2 (2q2.2-2.4). Estos resultados están en concordancia con los datos previos de sintenia y de ligamiento.

INTRODUCTION

Activin receptor type IIA role is described elsewhere in this Colloquium. Its cDNA sequence, synteny and linkage position are presented in the same contribution.

FISH localization of the ActRIIA *locuss* is decribed in the present proceeding, together with its effect on the genetic map of bovine chromosome 2 (BTA2).

MATERIAL AND METHODS

A.- CHROMOSOME SPREADS

Metaphase plates were obtained according to the method of Moorhead *et al.* (1960). G-banding was performed following Echard *et al.*(1984). After photographs, chromosomes were treated with RNAse A (100 mg/ml in 2 x SSC) for 1 hour at 37°C.

Chromosomes were denatured for 2 minutes in 70 p. cent formamide 2 x SSC (pH:7) at 70°C and immersed in an ethanol series at -20°C. Slides were then air dried, trealed with proteinase K (100 ng/ml in $CaC1_2$, Tris-HCl 20mM pH:7.4) for 7 minutes at 37°C.

Dehydration in alcohol series and air drying at room temperature were performed after RNAse A and Proteinase K treatments.

B.-MOLECULAR PROBES LABELLING, PREPARATION AND DENATURATION

Three clones carrying this gene were identified in a lambda genomic library.Cloned cattle DNA segments were not removed from the vector, so that any possible biotin label on λ could increase fluorescence intensity. To optimise FISH results, two complete lambda clones (50 ng DNA per clone) were used in each oligolabelling mixture in order to maximize possible molecular target .

Biotin-oligolabelling of lambda clones was performed according to standard procedures by using 16-bio-dUTP. Free nucleotides were removed by using a Sephadex G-50 column.

Since the cloned DNA fragments were long (20, 30 and 35 Kb) competitor homologous sonicated DNA was added in 200 fold excess prior to the precipitation of labelled DNA. A similar amount of sonicated lambda DNA was also added as a carrier and to prevent any non-specific hybridisation. The full DNA solutions (lambda clones, competitor homologous DNA and sonicated DNA) are pre annealed at 37°C for 3 hours after denaturation at 90°C (10 minutes).

Control slides indicated no evidence of lambda vector hybridisation to bovine chromosomes.

C.- HYBRIDISATION CONDITIONS

The hybridisation medium contained deionised formamide 50 p. cent (V/V),

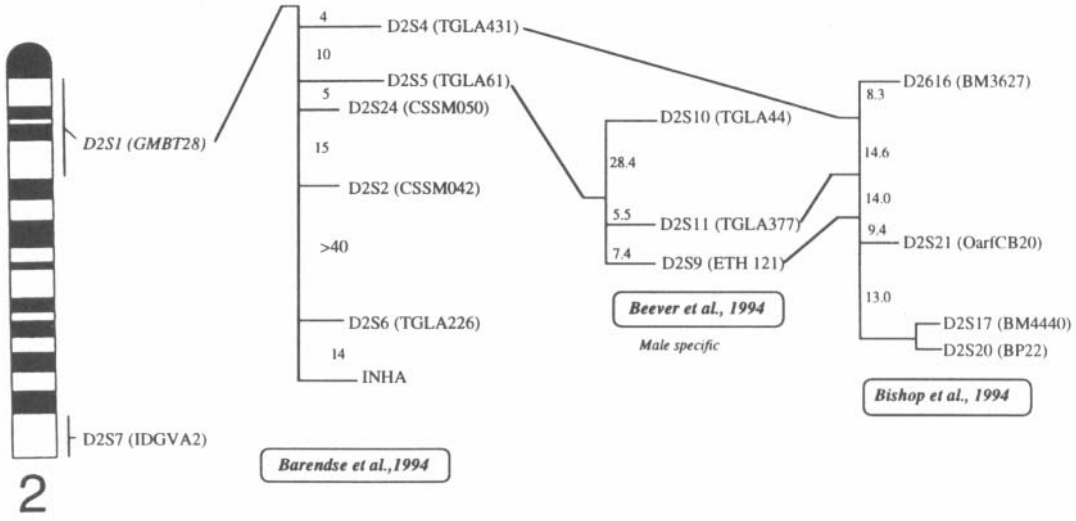

Figure 1a. *Status of the gene map of bovine chromosome 2 prior to the localisation of ActRIIA and its intronic microsatellite UCD2 (adapted and summarised from Eggen and Fries, 1995).* (Estado del mapa génico del cromosoma bovino 2 previo a la localización de ActRIIA y su satélite intrónico UCD2(adaptado y resumido de Eggen and Fries, 1995)).

dextran 10 p. cent (W/V) in 2 x SSC pH 6.8. Hybridisation was performed for 40 hours at 37°C.

D.- STRINGENCY WASHING

After hybridisation, slides are washed for 3 minutes in 50 p. cent formamide 2 x SSC (pH 7) at 45°C. This wash was repeated twice prior to S washes in 2 x SSC (2 minutes at 45°C). A final wash in BN buffer (pH 8) was also performed. BN is composed of $NaHC0_3$ 0.1 M, Nonidet P40 (Sigma) 0.05 p. cent V/V and sodium azide 0.002 p. cent W/V.

E.- HYBRIDISATION SIGNAL DETECTION

Biotin-labelled probes are detected by avidine FITC. To intensify the fluorescence, a second detection procedure using anti-avidine biotin-conjugated antibodies followed by avidine FITC was performed. In order to obtain a more intense signal, a third amplification was performed. The intensity of the signal was indeed increased, however, background level were also increased.

Slides are Propidium Iodide (PI) stained before observation according to Pinkel *et al.* (1986). A Leitz Aristoplan equipped with a fluorescent lamp and a double excitation filter G/R was used. 400 ASA photographic slides were taken for further signal analysis.

RESULTS AND DISCUSSION

From pictures analysis, it can be

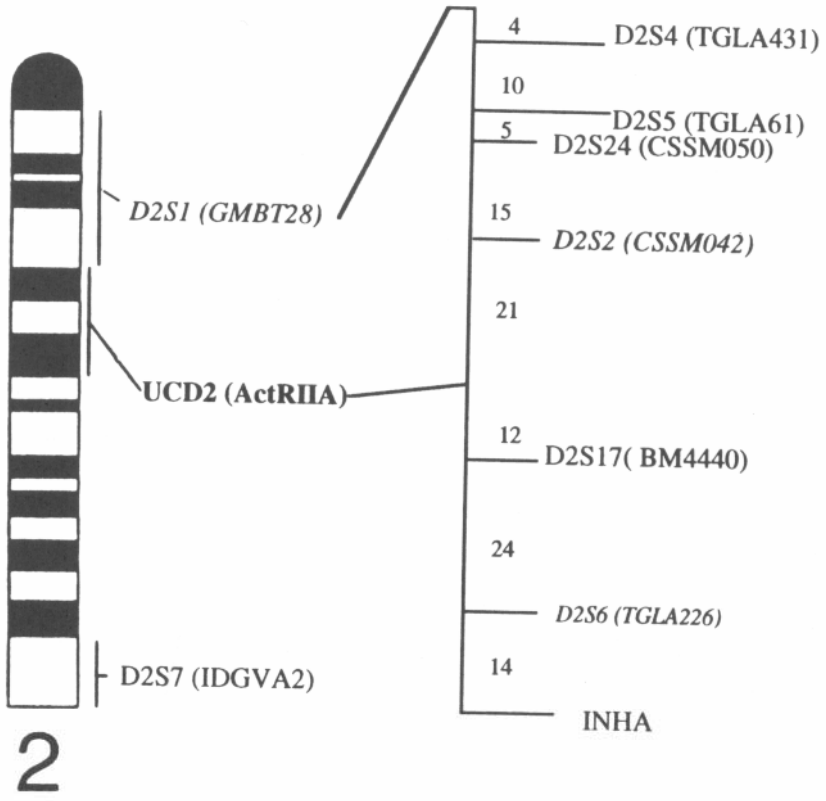

Figure 1b. *Proposed summarised in situ and linkage map for bovine chromosome 2 after the incorporation of the ActRIIA* locus. *As it can be observed, a third anchored* locus *is now available in order to obtain a more precise linkage map.* (Mapa propuesto resumido, *in situ* y de ligamiento, para el cromosoma bovino 2 después de la incorporación del *locus* ActRIIA; Como puede observarse un tercer *locus* anclado está ahora disponible para obtener un mapa de ligamiento más preciso).

concluded that ActRIIA maps to chromosome 2, on the 2q2.2-2.4 position. This result is consistent with previous linkage and somatic genetics data Moreover, it contributes to a more precise genetic map of bovine chromosome 2.

As a linkage map including type II markers was available for this chromosome, a close linkage (lods= 35.87; rec. frac.: 0.12) could then be observed between an intronic microsatellite in ActRIIA locus (UCD2) and BM4440 (Bishop *et al.*, 1994). Close linkage to ILSTS98 and URB42 was also detected. Of course, the existence and availability of the reference families (ILRDA, CSIRO and BovMap) plays a key role at this stage of research.

Finally, FISH has provided new consistent data on the chromosomal location of the *locus*. This result is not only verification but a real information

complement, since it contributes to a more exact knowledge on the position of ActRIIA and other markers previously assigned to this chromosome. Consequently, a recalculation and verification of recombination fractions among the loci assumed to be within a short distance to ActRIIA is now possible.

Eggen and Fries (1995) offer one of the most recent bovine gene map recompilation. For chromosome 2, only two *In Situ* anchored *loci* were available: GMBT28 on 2ql2-q21 and IDVGA2 on 2q45. Different papers (Barendse *et al.*, 1994; Beever *et al.*, 1994; Bishop *et al.*, 1994) have presented data on this chromosome map. However, linkage data in these reports assume GMBT28 as the only anchored *In Situ* reference. IDVGA2, on the telomere, remains unlinked to the remainder of the *loci*.

Figure 1a presents a summarised linkage map of chromosome 2 (adapted from Eggen and Fries, 1995), showing the linkage positions of BM4440, CSSM042, TGLA226 as determined prior to the mapping of ActRIIA by linkage and FISH studies. As can be seen, CSSM042 and TGLA226 were presented purely as loci distant by more than 40 cM from each other. BM4440 (mapped by Bishop *et al.*, 1994) was supposed to be equidistant from centromere and telomere. However, TGLA61 was its only indirect linkage reference to CSSM042.

As shown in our first contibution to this Colloquium ActRIIa has shown a close linkage (12 cM) to BM4440, which in turn is linked to TGLA226 (24 cM). Linkage is also shown between UCD2 and CSSM042 (0.21 cM).

FISH information is now concordant. Even if more accurate data on linkage distances are still required, a correct alignment for the mentioned type II markers should be as shown in **figure 1b.** As can be observed, the accumulated recombination fractions between CSSM042 and TGLA226 are clearly over proposed (40cM).

As a general conclusion ActRIIa (and its microsatellite UCD2) is now the third anchored locus on bovine chromosome 2 at position 2q2.2-2.4. This should be of great help for purpose of completing the gene map of this bovine chromosome.

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