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RASA contains a polymorphic microsatellite and maps to bovine syntenic group U22 on Chromosome 7q2.4-qter

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Abstract. The bovine gene for the $p21^{ras}$ protein activator (*RASA*) includes in its 5′ untranslated region a $(TG)_n$ repeat. Analysis of this $(TG)_n$ repeat by PCR amplification of genomic DNA revealed a four-allele polymorphism. A cDNA probe was used to assign *RASA* to the region 2.4-qter of bovine Chromosome (Chr) 7 by in situ hybridization. PCR analysis of a panel of somatic hybrid lines allowed the assignment of *RASA* to the unassigned syntenic group 22 (U22) and thus localizes U22 on Chr 7.

Introduction

Systematic mapping of eukaryote genomes depends on the availability of a sufficient number of highly polymorphic marker loci, preferentially at the DNA level (Botstein et al. 1980; White et al. 1985). O'Brien (1991) discriminates between two different types of markers, type I and type II anchor loci. The type I anchor loci are evolutionarily conserved coding genes that are mapped, at least in humans and mice. These markers facilitate the construction of comparative maps. To a certain extent, comparative mapping can be useful for a directed marker-searching approach (Womack 1987). Comparative maps will also be a source of information for identifying candidate genes, after an unknown gene has been assigned to a chromosome region. The type I anchor loci represent coding genes and are often monomorphic or only slightly polymorphic. To map economic trait loci (ETLs), however, highly polymorphic loci, i.e., the type II loci, are needed for efficient linkage analysis in animal pedigrees. Variable number of tandem repeat (VNTR), specifically microsatellite loci, represent a new class of high-resolution polymorphisms that can be found in most animals, including cattle (Fries et al. 1990; Moore et al. 1991). To extend the number of useful markers in the bovine genome, we performed a database search in GenEMBL and found several sequences containing microsatellites (Fries et al. 1990). One of these is the bovine gene for the $p21^{ras}$ protein activator (*RASA*), a 125-kDa cytoplasmic protein that can accelerate the GTPase activity of $p21^{ras}$ (Lowy et al. 1991). The published bovine *RASA* cDNA (Vogel et al. 1988) includes a $(TG)_n$ microsatellite in the 5′ untranslated region. Here, we describe the analysis of the microsatellite for polymorphism and the physical mapping of *RASA* by both in situ hybridization and somatic cell genetics. Since *RASA* is an evolutionarily conserved locus that was assigned to human Chr 5 and mouse Chr 13, respectively, it can be considered as a marker with both type I and type II characteristics, extremely important for eventually anchoring the bovine linkage map with the physical map and the comparative maps of other mammals.

Materials and methods

DNA samples

DNA from animals of three different breeds (US Brown-Swiss, Holstein-Friesian, and Simmental) was extracted according to standard procedures from 10–20 ml whole blood. DNA samples from 20 unrelated animals were used in the analysis of the $(TG)_{12}$ repeat in the *RASA* gene.

PCR for visualization of the polymorphism

For amplification of the *RASA* microsatellites, an upstream 5′(CCC TTC CGC TTT AGT GCA GCC AG)3′ and a downstream 5′(GGG CCA CAG CCC AGG ATC GGG AGC)3′ primer were designed on the basis of the published sequence (Vogel et al. 1988). The PCRs

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were carried out as described in a previous publication (Fries et al. 1990) with the following modification: the 30 amplification cycles were performed with denaturation at 94°C (30 s), annealing at 65°C (30 s), and polymerization at 72°C (30 s). The amplified DNA fragments were resolved on a polyacrylamide sequencing gel (6%, 7 M urea) at a constant power of 45 W for 2 h.

Sequencing

Sequencing of PCR-amplified DNA was performed directly in low-gelling-temperature agarose (Sigma) with a modified dideoxynucleotide chain termination technique (Sanger et al. 1977; Steffen et al. 1991).

In situ hybridization

Chromosomes were prepared from peripheral blood cells of a male bovine animal as described by Fries and co-workers (1986). The probe, kindly provided by J.B. Gibbs, was a 3826-bp cDNA insert cloned in pBluescript SK (Vogel et al. 1988). The plasmid was linearized with *Sac*I and labeled by the random priming method (Feinberg and Vogelstein 1983). The incorporation of three tritiated nucleotides yielded a specific activity of 3×10^8 dpm/μg. The prehybridization identification of the chromosomes based on Q-bands by fluorescence with quinacrine (QFQ) and the in situ hybridization were described by Fries and colleagues (1991). The probe concentration was 50 ng/ml, and 50 μl of the hybridization mixture was applied per slide. After hybridization, the slides were dipped in Ilford K2 emulsion, and autoradiography was carried out for 2 weeks. The position of the silver grains was marked on the previously photographed QFQ-banded metaphase chromosomes. The identification of the chromosomes was based on the international standard [ISCNDA (1989) 1990].

Analysis of hybrid panel

Bovine leukocytes were fused with HPRT-deficient rodent CHO, E-36, and thymidine kinase-deficient ($LMTK^-$) cell lines as previously described (Womack and Moll 1986; Womack 1991). Genomic DNA was extracted from bovine, rodent, and bovine–rodent cells according to established protocols.

PCR amplification of the *RASA*-specific sequence from 100 ng bovine, CHO, E36, $LMTK^-$, or hybrid cell DNA per reaction was essentially as described above for the visualization of the microsatellite polymorphism. The reaction cycled for 1 min at 94°C, 2 min at 65°C, and 2 min at 72°C for 25 cycles. After amplification, the reaction products were electrophoresed on a 3% Nusieve, 1% Seakem Me agarose gel containing ethidium bromide.

Presence or absence of bovine-specific PCR amplification products was tested in 20 clones from the previously developed hybrid somatic cell panel. Segregation profiles of bovine-specific PCR products were compared with those of previously assigned syntenic group markers representing the 29 autosomes and the X Chr. Statistical analysis based on correlation (Chevalet and Corpet 1986) was performed.

Results

The RASA microsatellite is polymorphic

To detect polymorphism, we used PCR primers corresponding to sequences on either side of the $(TG)_n$ repeat in the 5′ untranslated region of *RASA*. Sequencing of the product amplified from bovine genomic DNA confirmed its *RASA* specificity. In total, four alleles were identified in 20 animals. A representative autoradiogram is shown in Fig. 1. The sizes of these alleles differed by two nucleotides, suggesting that each allele was generated by the gain or loss of a single TG dinucleotide. The amplified product varied between 184 and 190 nucleotides, as determined by comparison with an M13 sequencing ladder. The sequenced fragment contained 12 TG repeats and comigrated with the 186-bp fragment. The fragment size, the derived repeat number, and the frequencies of the different alleles are given in Table 1. The frequencies are not evenly distributed, and the two first alleles account for 90% of the alleles at this locus.

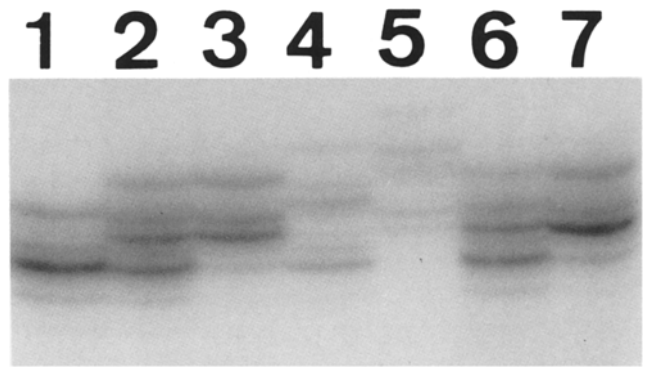

Fig. 1. Representative autoradiogram showing the four alleles identified at the *RASA* microsatellite locus. Fragments in the seven animals are as follows: **1**, 184-184; **2**, 184-186; **3**, 186-186; **4**, 184-188; **5**, 186-190; **6**, 184-186; **7**, 186-186.

RASA maps to 7q2.4-qter

The analysis of the distribution of 325 autoradiographic silver grains in 79 metaphases with preidentified chromosomes yielded a histogram as shown in Fig. 2. Of 83 grains found over Chr 7 (25% of the total grains), 73 (88%) were located above 7q2.4-qter (Fig. 3). The peak of the grain accumulation was observed over band q2.5. Fig. 4 shows a metaphase spread exhibiting silver grains on the distal portion of the two homologous Chrs 7.

RASA belongs to syntenic group U22

The analysis of 20 hybrid cell lines by use of PCR with *RASA*-specific primers and the statistical analysis using correlation coefficients demonstrate the assignment of *RASA* to bovine syntenic group U22 (Table 2). Statistical analysis involving each of the other syntenic groups eliminates *RASA* from those groups with the

Table 1. Number of chromosomes with a specific $(TG)_n$ repeat in 20 unrelated animals.

Size	$(TG)_n$	Simmental	US Brown-Swiss	Friesian-Holstein
184	$(TG)_{10}$	5	5	0
186	$(TG)_{12}$	9	15	2
188	$(TG)_{14}$	1	1	0
190	$(TG)_{16}$	1	1	0

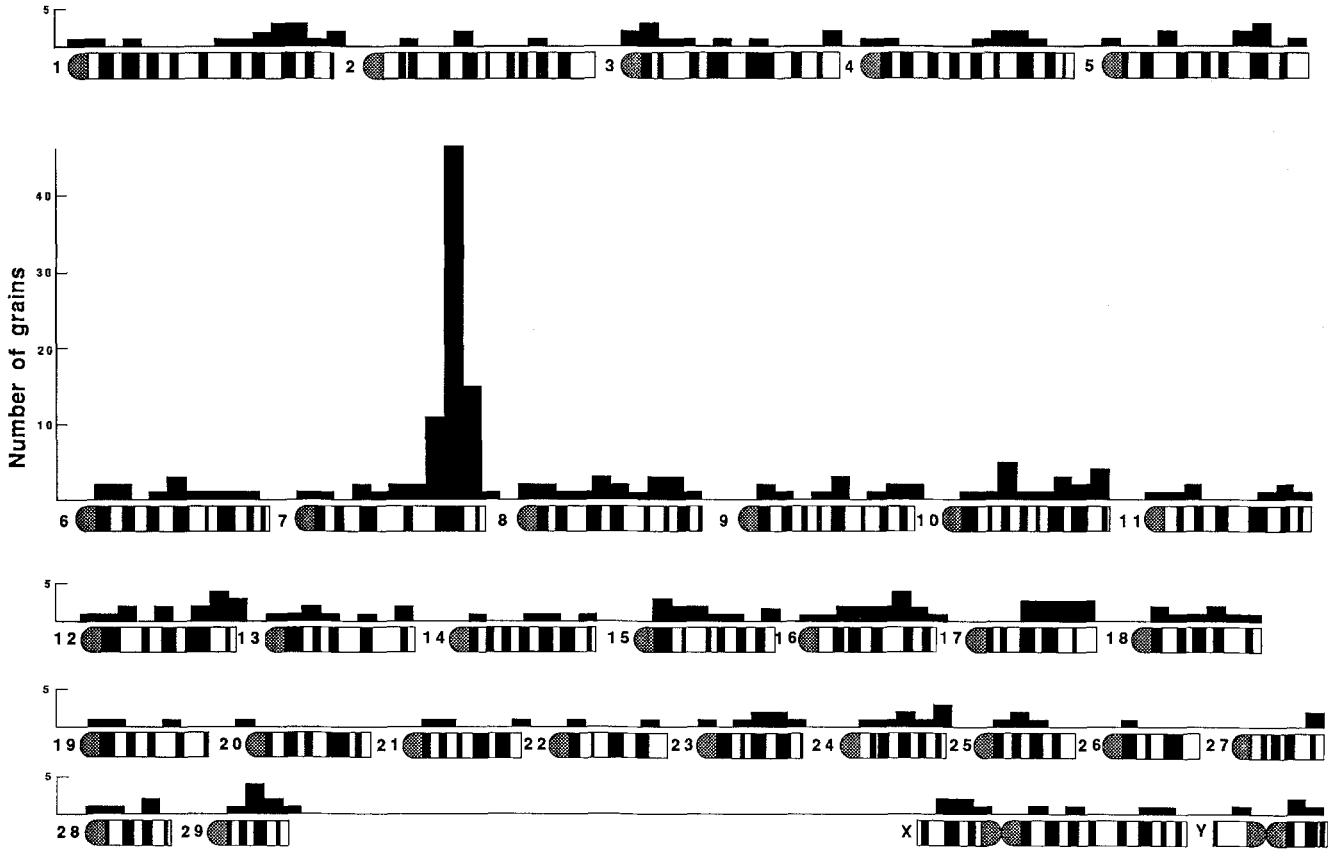

Fig. 2. Histogram showing the distribution of autoradiographic silver grains from the analysis of 79 metaphases.

exception of U19 and U26, where no decision can be reached at a high confidence level (Table 2). Consequently, all loci of syntenic group U22 can now be provisionally assigned to Chr 7.

Discussion

The localization of *RASA* on bovine chromosome region 7q2.4-qter and the demonstration of its synteny with a marker for syntenic group 22 now allow the provisional assignment of 15 additional loci to Chr 7 (R. Fries, unpublished data). Two of these loci are polymorphic, *D7S1* and *D7S2* (formerly *DU22S1* and *DU22S2;* Georges et al. 1991). *RASA,* containing a polymorphic microsatellite, adds to the coverage of bovine Chr 7 with polymorphic markers. Genetic mapping involving the three loci will provide information about their spacing. The *D7S1* and *D7S2* loci, being polymorphic (heterozygosity: 58% and 68% respectively) but anonymous, are examples of type II loci. *RASA,* representing a conserved locus and exhibiting a heterozygosity of 55% and a PIC of 0.42, combines the characteristics of both types of loci. Since the *RASA* polymorphism is visualized by PCR, it represents an STMS (sequence-tagged microsatellite site; Beckmann and Soller 1990).

Synteny of gene loci is highly conserved among mammalian species (O'Brien and Marshall Graves, 1991). More than 200 loci mapped in human, mouse,

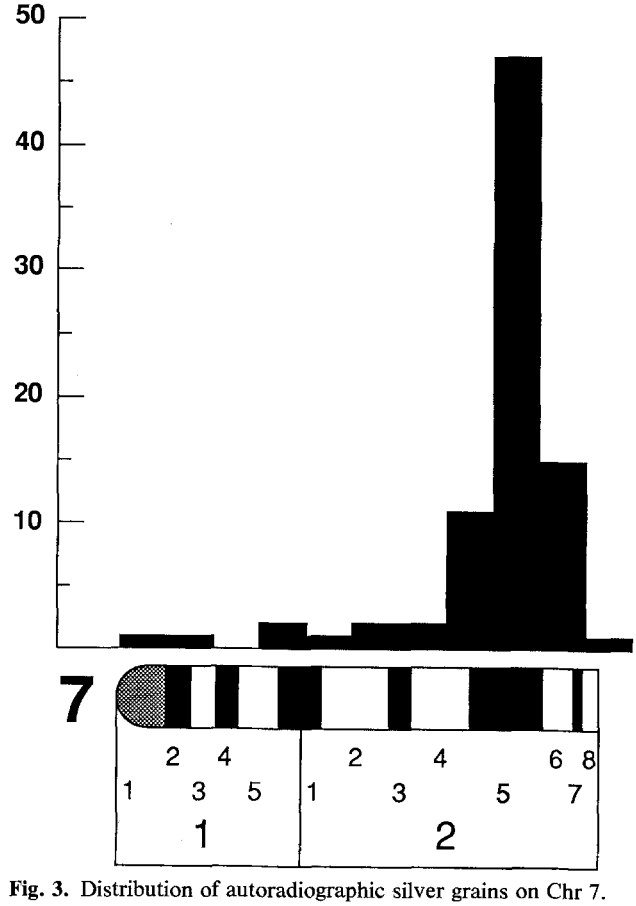

Fig. 3. Distribution of autoradiographic silver grains on Chr 7.

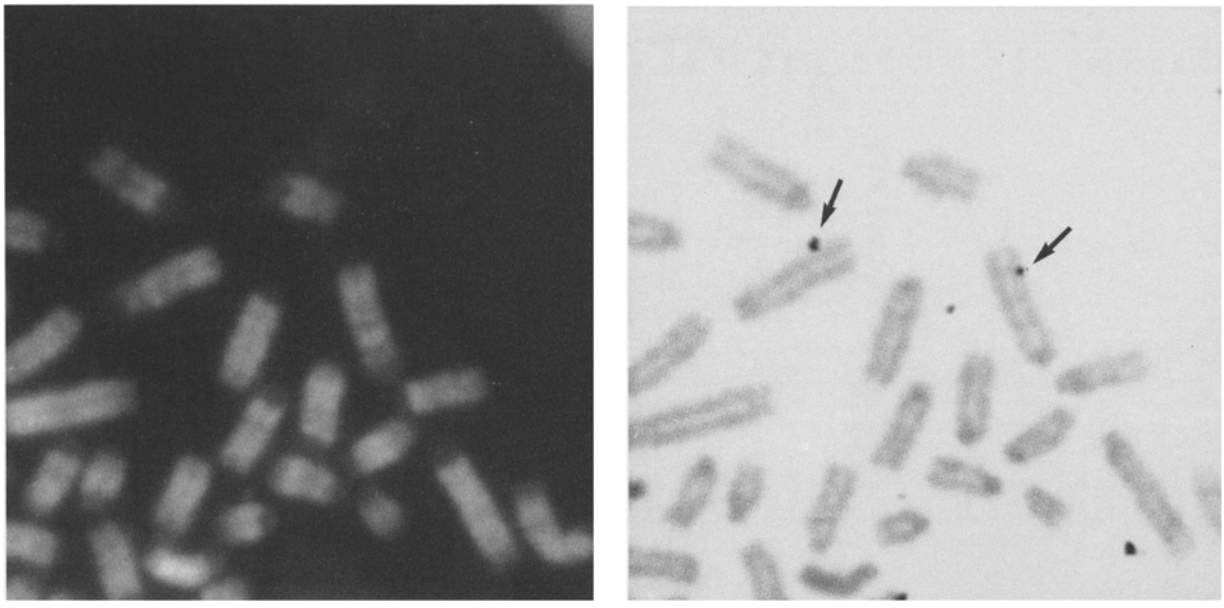

Fig. 4. Partial metaphase spread illustrating specific labeling of both Chr 7. **Left:** QFQ-banding (before hybridization). **Right:** Giemsa staining (after hybridization). The **arrows** indicate specific labeling.

and cattle define 49 mouse–human evolutionary linkage disruptions, but only 35 bovine–human disruptions (Womack et al. 1991). Two loci from the long arm of human Chr 5 are now mapped in cattle and mice, *RASA* and *HMGCR* (3-hydroxy-3-methylglutaryl coenzyme A gene). In mice, both loci are on Chr 13 (Hsieh et al. 1989; Sundaresan et al. 1989). In cattle, however, *HMGCR* is on Chr 10 (Womack et al. 1991) and *RASA*, as shown in the present study, on Chr 7. Thus a linkage disruption is present when one compares the human and the bovine genome, but not between the human and murine genome, indicating a deviation from the tendency of higher degree of conservation between the human and bovine than between the human and the murine genomes. Considering this finding, it is important to use extrapolation of synteny from the human to the bovine genome cautiously, and its species-specific confirmation is recommended in each instance.

Table 2. Concordancy, statistical analysis, and assignment of *RASA* to bovine syntenic group U22.

	RASA	
Syntenic group	Φ	Concordancy
U1	−.24	45
U2	−.39	32
U3	−.13	55
U4	−.38	45
U5	.24	55
U6	.55	80
U7	.35	65
U8	.24	55
U9	−.22	63
U10	.52	70
U11	−.13	55
U12	−.18	50
U13	−.18	50
U14	.29	63
U15	.35	65
U16	−.07	60
U17	−.18	50
U18	−.19	65
U19	.63	85
U20	.20	70
U21	.42	60
U22	**1.0**	**100**
U23	.24	55
U24	.29	60
U25[a]	.00	33
U26	.63	85
U27	.06	60
U28	.24	55
U29	.34	65
X	−.52	30

Φ = correlation coefficient; concordancy = percent concordancy.
[a] Limited number of clones were tested for syntenic group U25. Each locus was tested against each syntenic group with statistically significant values for syntenic in bold, asyntenic groups in plain text, and underlined values representing undetermined relationships between the tested locus and that syntenic group (error rate Q = 0.025, and probability for correct decision P = 0.91).

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