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Preliminary studies on the gene map of cattle

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gene map / cattle

INTRODUCTION

Gene mapping has become one of the most interesting fields in genetics research. However, data available on animal gene mapping are still very few, as compared with present knowledge of the human gene map. During the last two decades, somatic cell hybridization has proven to be one of the most useful tools for gene mapping studies both in the human and in animal species (Pontecorvo, 1975). In this report, we present our preliminary results in cattle gene mapping, obtained using this methodology.

MATERIALS AND METHODS

Somatic cell hybridization

Somatic cell hybridization was performed as described by Pontecorvo (1975). The line wg3hcl2 (a Chinese hamster cell line negative for hypoxanthine phosphoribosyltransferase (HPRT); Echard *et al*, 1984) and fibroblasts obtained from one cow were used for this purpose. Hypoxanthine–aminopterine–thymidine (HAT) culture medium was used to prevent growth of $HPRT^-$ cells. Ouabain was added to the medium at a final concentration of 10^{-7} M, to prevent growth of the unfused cattle fibroblasts.

Karyotype analysis

Karyotyping and cellular extract analysis for isozyme characterization were carried out at the same culture passage; colcemid (GIBCO) was added 3 or 3 1/2 h before harvesting, at a final concentration of 0.05 μg/ml. Cells were then removed from the culture flask, underwent a 20 min hypotonic treatment with 0.075 M KCl and were fixed twice in methanol–acetic acid (3:1), before slides were prepared.

Isozyme characterization

Cells were removed from the culture flask by trypsinization. As soon as cells started to detach, they were diluted in Hanks' balanced salt solution (HBSS). Sometimes, 10% fetal calf serum was added to HBSS to prevent trypsin degradation of enzymes. After centrifugation, cells were diluted in Shaws' buffer and subjected to three freezing-thawing cycles before being sonicated. Finally, samples were centrifuged and the supernatants (containing the enzymes) were stored in liquid nitrogen until analyzed. Electrophoresis of the samples, followed by specific staining was used to characterize the cattle isozymes expressed in the hybrid clones. The references for tank buffers and staining procedures are as follows: AK: adenylate kinase (Heuertz, 1981); PGD: phosphogluconate dehydrogenase (Heuertz, 1981); FH: fumarate hydratase (Harris and Hopkinson, 1976); MANA: α-mannosidase (Harris and Hopkinson, 1976); ACP: acid phosphatase: buffers according to Benne (1990, personal communication) and staining procedure described by Harris and Hopkinson (1976); LDHA and LDHB: lactate dehydrogenase A and B (Van Someren *et al*, 1974); PGM1, PGM2 and PGM3: phosphoglucomutase (Heuertz, 1981); NP: nucleoside phosphorylase: buffers used by Womack and Moll (1986) and staining procedure described by Ansay and Hanset (1972); PEPC: peptidase C: buffers and staining method of Van Someren *et al* (1974).

RESULTS AND DISCUSSION

We have now obtained a panel of 31 hybrid clones. So far, 14 clones have been analyzed for the following genes: AK, PGD, FH, MANA, ACP, LDHA and B, PGM1, 2 and 3, NP and PEPC (table I).

Table I. Cattle enzymes detected in the hybrid clones (hamster × cattle).

Clones	*Enzymes*											
	AK	*PGD*	*FH*	*MANA*	*ACP*	*LDHA*	*LDHB*	*PGM1*	*PGM2*	*PGM3*	*NP*	*PEPC*
4	−	−		−	−	−	+					
3-22	−	−	−	−	−	+	−					
3-15		−	−	−	−	−	−	−	−	+	+	
2-1		−	−	−	−	−	−	−			−	
2-8	−	−	+		−	−	−	−	−	−	−	
5-20	−	−	−	−		+	+	−	−	+		
2-2	−	−	−	+	−	−	−	−	−	−	−	+
3-11	−	+	−	+	−	−	−	−	−	−		
2-12		−	−	+	−	−	−	−	−	−	−	−
7-7		−	−		+	+	+	−	+	+	−	
7-1		−	−		+	+	+	−	+	+	−	
5-6		+	+		+	+	+	+	+	+	−	
FUS	−	−	−			−	−	−	−	−		−
3-17	−	−	−	+	+	−	−	−	−	−		−

Previously, PGD was found to belong to the bovine U1 syntenic group, while PGM1 was located in the U6 syntenic group (Echard *et al*, 1982; Heuertz and Hors-Cayla, 1981; Womack and Moll, 1986; Womack, 1990). FH and PEPC were not previously studied (fig 1 shows the electrophoretic enzyme pattern obtained for FH). Our data suggest that the human syntenic group including FH, PGD, PGM1 and PEPC (Van Someren *et al*, 1974) is not conserved in cattle. PGD, PGM1 and FH cannot be considered as being syntenic in light of the preliminary results shown in table I.

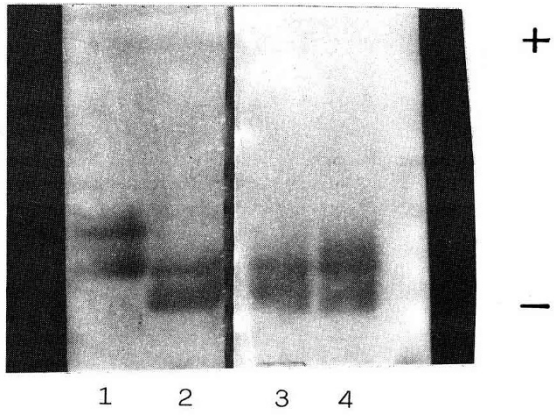

Fig 1. Cellulose acetate zymogram of FH: lane; 1: cattle; 2: hamster; 3: negative hybrid for cattle FH; 4: positive hybrid for cattle FH.

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