Sheep gene mapping by somatic cell hybridization

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INTRODUCTION

Twenty-five hamster × sheep hybrid cell lines were previously obtained (Saïdi-Mehtar *et al*, 1981b). Analysis of these hybrids has already enabled the identification of 5 syntenic groups and 10 independent markers (Saïdi-Mehtar *et al*, 1981a, b, 1987; Millot *et al*, 1981; Imam-Ghali *et al*, 1987). In this paper, we report results obtained with 3 other enzyme markers: aconitase 1 (ACO1), inosine triphosphatase (ITPA) and glyceraldehyde-3-phosphate dehydrogenase (GAPD); and 6 DNA markers: ornithine *trans*carbamylase (OTC), color vision red pigment (RCB), *raf* oncogene (ARAF1) and β-gene locus DR of human lymphocyte antigen region (HLA-DRβ).

ENZYME MARKERS

ACO1, ITPA and GAPD were studied by cellulose acetate electrophoresis using modified versions of the methods described by Womack and Moll (1986). The cytoplasmic (ACO1) and mitochondrial (ACO2) forms of aconitase were identified by successive freezing and thawing of cells followed by electrophoresis of the supernatants. ACO1 was obtained first and corresponded to the most anodal band. A different electrophoretic migration between sheep and hamster was only observed for ACO1. Ovine ITPA migrated more anodally than hamster ITPA. Ovine GAPD is a trimeric enzyme: positive hybrids for sheep GAPD showed 2 intermediate bands (fig 1). Comparison between ACO1, ITPA, GAPD and the other 21 markers previously studied in these hybrids showed 2 syntenic groups.

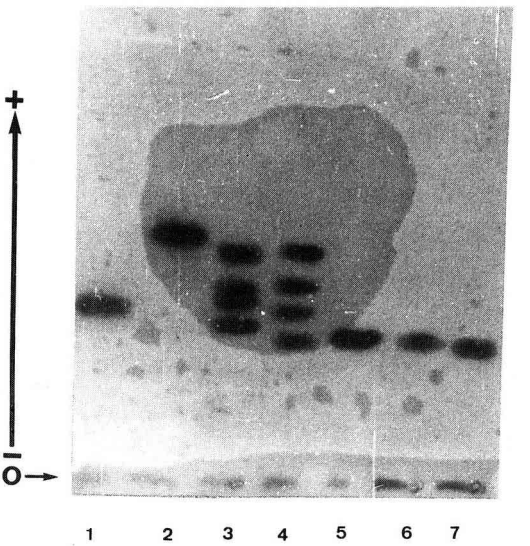

Fig 1. Zymogram of glyceraldehyde-3-phosphate dehydrogenase in Chinese hamster (1), sheep fibroblasts (2), hamster × sheep hybrids (3–7). The hybrids in lanes 3 and 4 are positive for ovine GAPD and show 2 intermediate bands, the hybrids in lanes 5, 6 and 7 are negative.

ITPA–adenosine deaminase (ADA)

This synteny is conserved in man (Hopkinson *et al*, 1976), hamster (Stalling *et al*, 1982), mouse (Siciliano *et al*, 1984), cat (Berman *et al*, 1986), cattle (Womack and Moll, 1986), dog and mink (Human Gene Mapping 9, 1987).

GAPD and lactate dehydrogenase B (LDHB)–peptidase B (PEPB)–triose-phosphate isomerase (TPI)

This group is syntenic only in man (Bootsma and Ruddle, 1978; Ruddle and Meera Khan, 1976), cattle (Womack and Moll, 1986) and mink (Human Gene Mapping 10, 1989). This synteny is not conserved in mouse, hamster and rabbit, where an independent segregation is observed between GAPD–TPI–LDHB and PEPB (Human Gene Mapping 10, 1989).

Like ADA, ITPA is on U15. Like LDHB-PEPB-TPI, GAPD is assigned to chromosome 3. ACO1 segregates independently from the other 23 markers and is assigned to U2 (Human Gene Mapping 10, 1989).

DNA MARKERS

DNA was extracted from the parental hamster cell line, ovine lymphocytes and from 23 hamster × sheep hybrid lines; 3 hybrid lines were grown concomitantly in hypoxanthine–aminopterine–thymidine (HAT) medium and in 6-thioguanine medium. This DNA was digested with *Eco*RI and analyzed with the Southern

blotting technique using the 6 following DNA probes: OTC, isolated by Horwich *et al* (1984); color vision red pigment, isolated by Nathans *et al* (1986); one human autosomal probe: HLA-DRβ, isolated by Wiman *et al* (1982); two rat X-linked probes: brain myelin proteolipoprotein (PLP), isolated by Dautigny *et al* (1985); synapsin 1, isolated by Kilimann and De Gennaro (1985); and one mouse X-linked probe ARAF1 oncogene, isolated by Huebner *et al* (1986).

Analysis of hybrids showed: 1) the localization on the sheep X chromosome of sequences homologous to the following genes: OTC, color vision red pigment, ARAF1 oncogene, brain myelin PLP and synapsin 1 (presence of molecular signals in hybrids grown in HAT medium and absence of signals in the same hybrids grown in 6-thioguanine medium) (fig 2); 2) the absence of a positive correlation between the serological signal obtained with anti-ovine histocompatibility complex (OLA) sera (Millot *et al*, 1981) and the molecular signal obtained with the HLA-DRβ probe.

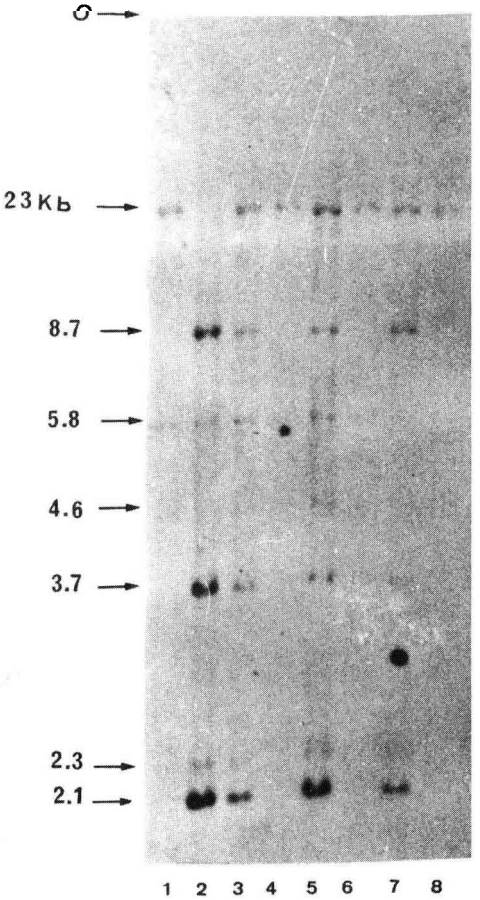

Fig 2. Hybridization of radioactive human OTC cDNA probe to *Eco*RI fragments from DNA of the Chinese hamster cell line (1); ovine lymphocytes (2); hamster × sheep hybrid lines grown in HAT medium (3, 5, 7); hamster × sheep hybrid lines grown in 6-thioguanine medium (4, 6, 8).

In this study, we showed two new syntenies for the sheep: GAPD–LDHB–PEPB–TPI on chromosome 3 and ITPA–ADA on U15. The assignment to the X chromosome of 5 genes, known to be X-linked in several other species, confirms the concept of the conservation of the X chromosomal genome in mammalian vertebrates.

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