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Note

The gene map of the rabbit III. α and β casein gene synteny

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Summary

We are developing the gene map of the rabbit using DNA probes which allow us to investigate non-expressed genes. We have characterized an α and β casein gene synteny and have found that neither the α and β casein genes nor the whey acidic protein gene have a syntenic relationship with the other chromosome markers already investigated in our laboratory. Moreover, with the β casein probe we have detected a simple allelic Eco RI restriction-site polymorphism.

Key words : Rabbit, gene mapping, α and β casein genes, whey acidic protein gene, DNA polymorphism.

Résumé

Carte génique du lapin. III. Synténie entre les gènes des caséines α et β

Nous développons la carte génique du lapin en utilisant des sondes d'ADN cloné qui, par hybridation moléculaire, permettent de caractériser les gènes indépendamment de leur expression. Dans le cadre de cette étude, nous avons mis en évidence une synténie entre les gènes de la caséine α et de la caséine β. Aucune relation de synténie n'a été observée entre les gènes des caséines α et β, ou le gène de la protéine acide du lactosérum et les autres marqueurs chromosomiques déjà étudiés au laboratoire. Les résultats obtenus avec la sonde caséine β montrent un polymorphisme au locus de ce gène sous la dépendance d'un couple d'allèles.

Mots clés : Lapin, carte génique, gènes des caséines α et β, gène de la protéine acide du lactosérum, polymorphisme de l'ADN.

I. Introduction

Assignment of at least one marker per chromosome is one of the objectives of gene mapping. By molecular hybridization of nucleic acid, one can analyse non-expressed genes and thus get access to a large number of valuable chromosome markers.

The present results demonstrate that the α and β casein genes are syntenic in *New Zealand* rabbits. Taking into account the previous results obtained with DNA probes or enzyme markers, we find that α and β casein genes and the whey acidic protein gene are not syntenic with any of the 20 other chromosome markers already investigated in our laboratory.

II. Materials and methods

A. Biological material

The rabbit × Chinese hamster somatic cell hybrids used in this study have been previously described (ECHARD *et al.*, 1981). Among 3 independent series of hybrid clones, 26 clones were chosen on the basis of their growth ability and low chromosome number.

B. Molecular hybridization

Purification of high molecular weight cellular DNA, total digestion with endonuclease Eco RI, agarose gel electrophoresis, transfer onto nitrocellulose filters, nick-translation of the probes and DNA/DNA hybridization were performed as previously described (GELLIN *et al.*, 1983). The probes used : α casein, β casein, and whey acidic protein (WAP), were from a lactating rabbit mammary gland cDNA library (SUARD *et al.*, 1982).

C. Synteny analysis

Synteny analysis was performed between the 3 new markers and the markers already studied in our laboratory : enzymatic markers (ECHARD *et al.*, 1982) and uteroglobin gene (GELLIN *et al.*, 1983).

III. Results and discussion

A. Syntenic studies

Eco RI digested DNA from rabbit or hamster cells and rabbit × hamster hybrid clones, was hybridized with α casein, β casein and WAP radioactive probes. In our experimental conditions, no cross-hybridization was observed between α and β casein probes, nor between the 2 probes and the hamster DNA. Hybridization of the α casein probe with rabbit DNA from cells or hybrid clones gave 3 bands at 9.2 kilobases (kb), 5.8 kb and 4 kb (fig. 1) ; the β casein probe gave 3 different band patterns : 11 kb, 8.6 kb and 11 kb + 8.6 kb (fig. 1 and fig. 2). The β casein polymorphism observed in the hybrid clones reflects the polymorphism of the parental rabbit cells as will be discussed below. The WAP probe gave bands of different size in hamster and rabbit DNA. It is

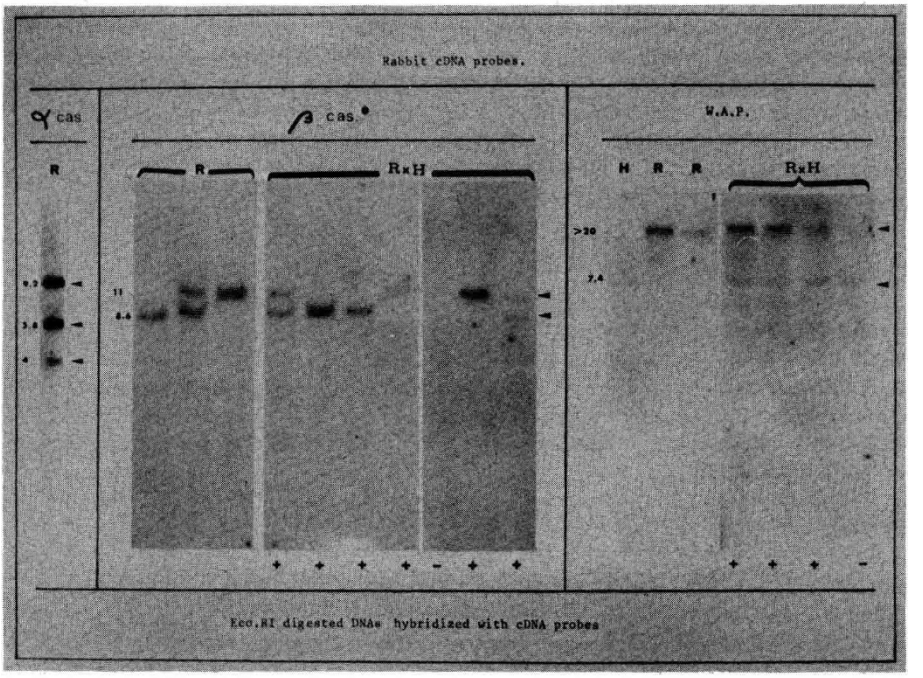

FIG. 1

Eco RI digested DNA hybridized with the various cDNA probes.

Autoradiograms of rabbit (R), hamster (H) or rabbit × hamster (R × H) DNAs digested with Eco RI, showing the different patterns obtained after hybridization with α casein, β casein or WAP probes. Numbers represent the size (in kilobases) of the positive bands.
(*) see fig. 2 for polymorphic phenotypes with the β casein probe.
(+) positive rabbit hybrid clones.
(−) negative rabbit hybrid clones.

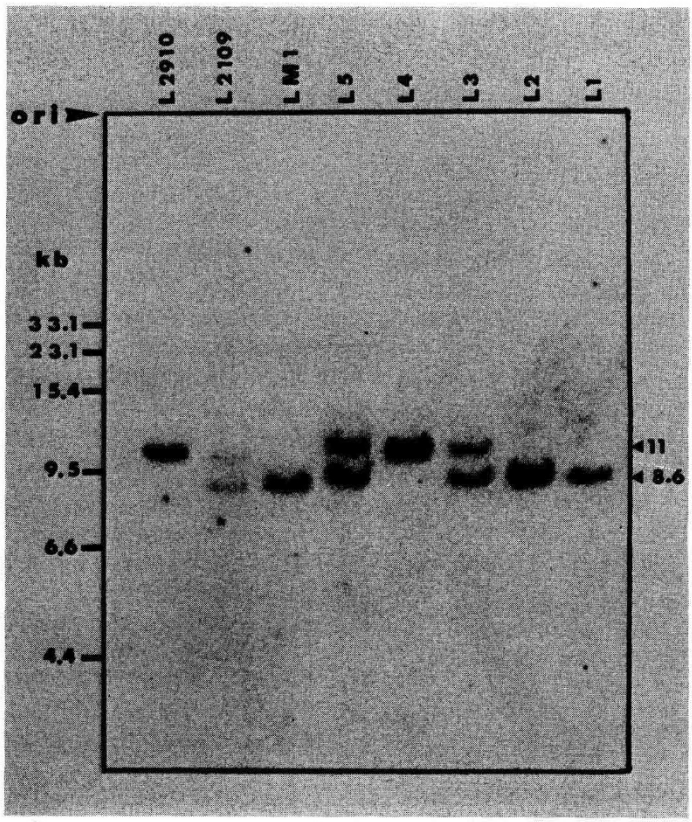

FIG. 2

Polymorphism of Eco RI digested DNA hybridized with cDNA β casein probe.

Autoradiogram of DNA sequences containing the β casein gene detected in DNA digested with Eco RI in different rabbits : L2910 ; L2109 ; LM1 ; L5 ; L4 ; L3 ; L2 ; L1. The detected polymorphism shows 3 hybridizing patterns : a fragment at 11 Kb ; a fragment at 8.6 Kb or both fragments. Numbers on the left represent the positions of Sal I and Hind III λ DNA fragments (in kilobases) used as size standard.

therefore always possible to determine if the rabbit fragment is present or not in hybrid clones (fig. 1).

Table 1 shows the correlation analysis of the results obtained by hybridization of the 3 cDNA probes with the somatic hybrid cellular DNAs.

TABLE 1

(+) somatic hybrid DNA displaying positive autoradiographic signals.
(−) somatic hybrid DNA not displaying autoradiographic signals.

β cas. \ α cas.	+	−
+	14	2
−	0	10

26

α cas. \ WAP	+	−
+	11	3
−	5	7

26

β cas. \ WAP	+	−
+	12	4
−	4	6

26

Among 26 hybrid clones tested for α and β casein genes, the same 14 were positive and the same 10 negative for both casein probes. This shows that these 2 genes are syntenic. Such casein gene synteny was already described in cattle by linkage analysis (GROSCLAUDE, 1979) and in mice by molecular hybridization (GUPTA *et al.*, 1982). No synteny was found between casein genes and the WAP gene.

By computer analysis (CORPET *et al.*, in preparation) of the present results and those previously obtained in our laboratory, we could not find any syntenic relationship between the other rabbit chromosome markers and the α casein, β casein or WAP genes.

We have now 4 syntenic groups (α and β casein genes ; ACP.2-LDH.A and uteroglobin gene ; LDH.B-TPI and GAPDH ; G6PD-PGK-HPRT and GLA) plus 11 independent asyntenic markers to mark 14 autosomal chromosomes and the X chromosome among the 22 chromosome pairs of the rabbit. Nine linkage groups and 3 more syntenic groups have been described by FOX (in *Genetic Maps*, 1984).

B. Detection of an Eco RI restriction-site polymorphism with the β casein probe

Using the β casein probe we observed 3 hybridizing patterns depending on the rabbit DNA tested : either one fragment at 11 kb (rabbits L2910 and L4), one fragment at 8.6 kb (rabbits LM1, L2 and L1) or both fragments 11 kb + 8.6 kb (rabbits L2109, L5 and L3) (fig. 2). Among the 16 hybrid clones having kept at least one rabbit β casein gene, we observed the same pattern in the cell hybrids as in the rabbit parental cells : i.e. an 11 kb fragment for 5 hybrid clones obtained from a rabbit displaying the 11 kb fragment and an 8.6 kb fragment for 7 hybrid clones derived from a rabbit displaying the 8.6 kb fragment. Among the 4 hybrid clones derived from a rabbit having both fragments, 3 hybrid clones presented both fragments and the fourth showed only the 11 kb fragment. In this last case, the homologous chromosome bearing the 8.6 kb fragment may have been lost. It therefore appears that a simple allelic Eco RI restriction-site polymorphism does exist. Further characterization of this polymorphism is in progress.

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