

Regional Assignment of the Rat Alpha-Feto Protein Gene (Afp) to Chromosome 14p21-p22

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ABSTRACT

The gene encoding alpha fetoprotein (locus symbol Afp) was assigned to rat chromosome 14 at band p21-p22 using fluorescence *in situ* hybridization method. The present result suggests that there is a conserved syntenic group between human 4q11-q13, mouse 5F-G, and rat 14p21-p22.

Keywords: AFP, Rat, Gene mapping, chromosome 14

INTRODUCTION

The comparative gene mapping is proving of considerable value in either studies on human hereditary disease or in our understanding of the organization of mammalian genome during the evolution. Because the rat (*Rattus norvegicus*) is used extensively as an experimental animal in several fields of biological and medical research, the assignment of genetic markers on rat chromosomes is highly desirable [1-3]. In the past few years, considerable progress has been made in the rat gene mapping [2]. However, more refined mapping is necessary to define evolutionary breakpoints between conserved chromosomal segments.

Although the gene encoding rat alpha-fetoprotein (locus symbol Afp) was mapped on rat chromosome 14 [4-8] its regional localization is unknown. This report describes sub-localization of the Afp locus on the rat chromosome 14 using fluorescence *in situ* hybridization (FISH) method.

MATERIALS AND METHODS

Chromosome preparations were obtained from primary skin cultures of a male Wistar rat. The full length of the rat AFP cDNA [9] was labeled as a probe by nick translation kit with biotin-16-dUTP (Boehringer Mannheim). The labeled-probe DNA was denatured in 100% formamide (Boehringer Mannheim) at 70°C for 5 min. FISH method was performed as described previously [10] with minor modification. In brief, chromosomes were denatured

in 70% formamide/2 × SSC (1 × SSC = 150 mM NaCl, 15 mM Sodium citrate pH 7.0) at 70°C for 2 min followed by dehydration in ethanol series, prior to applying probe mixture. The slides were incubated with denatured DNA probe (500 ng/slide) in a humidified box at 37°C for 16-18 h. The hybridization mixture consisted of 50% formamide, 4 × SSC, 10% dextran sulfate, and 1% bovine serum albumin. After hybridization, the slides were washed as described previously [10]. Hybridization signals were detected with fluorescein isothiocyanate-conjugated avidin (avidin FITC) (Vector Laboratories) and amplified by incubation with biotinylated anti-avidin D antibodies (Vector Laboratories) and avidin-FITC. Chromosomes counter-stained with propidium iodide, and metaphases with hybridization signals were photographed. In order to precise localization of specific signals, the preparations were destained, then chromosomes were Q-banded with quinacrine mustard and Hoechst 33258 double staining [11] and photographed. The chromosome numbering system of rat by Satoh *et al.* [12] were adopted.

RESULTS AND DISCUSION

Analysis of 60 rat metaphases showed that 41 (about 70%) of metaphases had a twin-spot signal on one of the homologous chromosome 14 at region p21-p22, indicating that the gene encoding rat AFP located on chromosome 14p21-p22 and confirming the previous assignments [4-8]. The comparative gene mapping demonstrated that there is an evolutionary conserved segment between human chromosome 4q11-q13, mouse chromosome 5F-G, and rat chromosome 14

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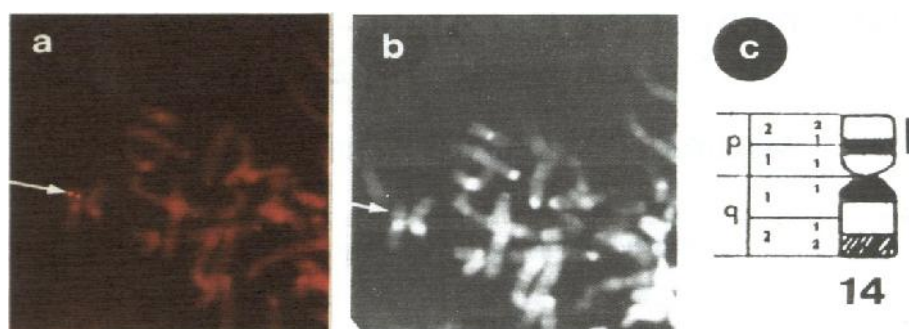


Fig. 1. Chromosomal localization of the rat Afp by FISH. (a) Partial rat metaphase after FISH with rat AFP cDNA hybridization. (b) Same spread as in (a) after Q-band staining. Arrows indicate the positions of hybridization signals. (c) Black bar on ideogram shows the respective mapped site.

[1, 2]. The gene encoding AFP is belonging to this conserved syntenic group [2, 3, 6]. Because the regional assignment of gene is not made on the rat chromosome 14 the conserved syntenic segment between rat chromosome 14 and the chromosomes of other two species is not defined [1, 2]. The present study showed that the Afp located on rat chromosome 14p21-p22. Therefore, the region p21-p22 of rat chromosome 14 is homologous to human chromosome 4q11-q13 and mouse chromosome 5F-G.

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