Restriction Fragment Length Polymorphism in the 3' Flanking Region of the Rabbit $\beta_1$-Globin Gene

Piero Masina, 1 Andrea Rando, 1 and Sergio Cocozza 2

Received 6 Feb. 1984—Final 16 May 1984

By Southern blot analysis, a restriction fragment length polymorphism in the 3' flanking region of the rabbit $\beta_1$-globin gene was detected. Two alleles, characterized by 9.7- and 12.4-kb BamHI fragments and by 15.3- and 18.0-kb HindIII fragments, have been detected in a small population of White New Zealand rabbits. The long allele is the most frequent (about 70%). The simultaneous changes in the restriction patterns of the two endonucleases and the constant distance between BamHI and HindIII sites in short and long fragments suggest the possibility that the two alleles arise from a rearrangement phenomenon involving a DNA segment 2.7 kb long. In addition, the presence of the two alleles in individuals genetically unrelated to the White New Zealand breed suggests that this polymorphism is widespread.

KEY WORDS: rabbit; $\beta_1$-globin gene; restriction fragment length polymorphism; Southern blot hybridization.

INTRODUCTION

Genetic polymorphism is a well-known phenomenon which, until recently, has been identified exclusively in gene products. In 1975, Southern introduced a new method for the analysis of genomic DNA consisting of the identification of structural genes, in fragments produced by restriction endonuclease digestion, by hybridization with specific radioactive probes. This method has...
been used principally to study the organization of many eukaryotic genes, including the rabbit \(\beta\)-globin cluster (Jeffreys and Flavell, 1977a, b; Lacy et al., 1979). During the study of human globin genes by the Southern method, genetic polymorphism of restriction endonuclease sites in the \(\beta\)-globin cluster was detected (Kan and Dozy, 1978; Jeffreys, 1979; Geever et al., 1981). These genetic markers can be utilized for antenatal diagnosis of hemoglobinopathies (for review see Little, 1981).

Furthermore, Botstein et al. (1980) have initiated a project for detecting DNA polymorphism with the aim of constructing a detailed human linkage map. For this purpose, probes do not necessarily have to be associated with any specific genes. Any unique or low-copy sequence will suffice as long as it hybridizes with some part of one of the DNA fragments formed by restriction endonuclease digestion (Wyman and White, 1980).

Thus, the Southern method is a very powerful tool to reveal a new and virtually unlimited source of genetic polymorphisms: restriction fragment length polymorphisms (RFLPs).

A comprehensive review on RFLP utilization in human genetics is presented by Shows et al. (1982). The possible applications of RFLPs to genetic improvement in animal breeding have recently been discussed by Soller and Beckman (1982).

As a first step toward the identification of RFLPs in animals of economic interest, we have analyzed a region of the rabbit genome comprising the \(\beta_1\)-globin gene. A polymorphic region on the 3' side of this gene is described in the present report.

**MATERIALS AND METHODS**

*Preparation of Rabbit DNA.* Individual blood samples (20 ml), using EDTA as anticoagulant, were collected from rabbits varying in age from 3 months to some years. The subjects studied included 78 individuals of the White New Zealand breed and 5 of undefined genetic type. DNA was prepared from leukocytes according to Wyman and White (1980), except that removal of RNA was accomplished by incubation with bovine pancreatic ribonuclease A (100 \(\mu\)g/ml) and *Aspergillus oryzae* ribonuclease \(T_1\) (5 U/ml) for 3 hr at 37°C, instead of by equilibrium sedimentation in CsCl/ethidium bromide gradients.

*Restriction Endonuclease Digestion, Electrophoresis, and Transfer of DNA.* EcoRI, \(Pvu\)II, \(Kpn\)I, \(Pst\)I, \(Bam\)HI, and \(Hind\)III endonucleases were obtained from Boehringer Mannheim and Amersham England and used according to suppliers' directions. To ensure complete digestion, samples of genomic DNA were incubated with twofold more enzyme than required to