The Rabbit α-Like Globin Gene Cluster Is Polymorphic Both in the Sizes of BamHI Fragments and in the Numbers of Duplicated Sets of Genes

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The α-like globin gene cluster in rabbits contains embryonic ζ-globin genes, an adult α-globin gene, and θ-globin genes of undetermined function. The basic arrangement of genes, deduced from analysis of cloned DNA fragments, is 5'-ζ0-ζ1-α1-θ1 ζ2 ζ3 θ2 θ3 3'. However, the pattern of restriction fragments containing ζ and θ-globin genes varies among individual rabbits. Analysis of BamHI fragments of genomic DNA from 24 New Zealand white rabbits revealed eight different patterns of fragments containing ζ-globin genes. The large BamHI fragments containing genes ζ0 and ζ1 are polymorphic in length, whereas a 1.9-kb fragment containing the ζ2 gene and the 3.5-kb fragment containing the ζ3 gene do not vary in size. In contrast to this constancy in the size of the restriction fragments, the copy number of the ζ2 and ζ3 genes does vary among different rabbits. No length polymorphism was detected in the BamHI fragments containing the θ-globin genes, but again the copy number varies for restriction fragments containing the θ2 gene. The α1- and θ1-globin genes are located in a nonpolymorphic 7.2-kb BamHI fragment. The combined data from hybridization with both ζ and θ probes shows that the BamHI cleavage pattern does not vary within the region 5'-α1-θ1-ζ2-ζ3-θ2-3', but the pattern of BamHI sites within the 5'-ζ0-ζ1-3' region is highly polymorphic. Analysis of genomic blot-hybridization patterns for the progeny of parental rabbits with different α-like globin gene patterns shows that the polymorphic patterns are inherited in a Mendelian fashion. Two different haplotypes have been mapped based on the genomic blot-hybridization data. The variation in the α-like globin gene cluster in the rabbit population results both from differences in the copy number of the duplication block containing the ζ-ζ-θ gene set and from the presence or absence of polymorphic BamHI sites.

Introduction

Variation in DNA sequence detected as restriction-fragment-length polymorphisms (RFLPs) has been used in the diagnosis of genetic diseases (Kan and Dozy 1978; Orkin et al. 1978), in the construction of linkage maps (Botstein et al. 1980), and in mapping the chromosomal location of genetic disease loci (Reeders et al. 1985). RFLPs can result from base-pair substitutions that create or destroy a restriction site.
or from rearrangement of DNA segments, which alters the position of a restriction site. The latter are frequently associated with arrays of tandemly repeated sequences. The length of these arrays can vary from one allele to another, giving rise to surprisingly high heterozygosities. For example, these highly variable regions have been reported near the human insulin gene (Bell et al. 1981, 1982), within the human α-like globin gene cluster (Proudfoot et al. 1982; Goodbourn et al. 1983; Jarman et al. 1986), in human minisatellite DNA (Jeffreys et al. 1985), and in the c-Ha-Ras proto-oncogene (Capon et al. 1983). This type of polymorphism could in principle be generated by mitotic or meiotic unequal crossing-over or by DNA slippage during replication (Jeffreys et al. 1985).

As currently mapped, the rabbit (*Oryctolagus cuniculus*) α-like globin gene cluster includes one adult α-, five embryonic ζ-, and two α-related θ-globin genes, arranged in the order 5'-ζ0-ζ1-α1-θ1-ζ2-ζ3-θ2-ζ4-3' within a 38-kb DNA segment (Cheng et al. 1986, 1987, 1988). The α-like globin gene clusters of other mammals likewise consist of linked sets of ζ-, α-, and θ-globin genes, and, with the exception of rabbits, the α-globin gene is duplicated within the cluster (reviewed in Hardison and Gelinas 1986; Hardison et al. 1987). The rabbit α1-globin gene is actively expressed during late embryonic, fetal, and adult life. The ζ-globin genes are homologous to the embryonic ζ-globin genes of other mammals, but genes ζ1, ζ2, and ζ3 in rabbits do not encode functional globin polypeptides (Cheng et al., 1988). The ζ0 gene may be functional, but the ζ4 gene, like its paralogous homologue ζ2, lacks a normal 5' end and is not functional (Cheng et al., 1988). Although θ-globin genes are found in several mammals, the θ1-globin gene in rabbits has a frameshift mutation and is missing a normal promoter for a globin gene (Cheng et al. 1986), and partial sequence determination of the θ2-globin gene indicates that it also may be nonfunctional. The θ-globin gene has also suffered inactivating mutations in the prosimian galago (Sawada and Schmid 1986), and the encoded polypeptide in horses is not a normal globin (Clegg 1987). However, specific transcripts from the human θ-globin gene have been detected in fetal erythroid tissues and in a human erythroid cell line (Leung et al. 1987; Hsu et al. 1988), indicating that the θ-globin gene may be active in higher primates. The rabbit α-like globin gene cluster probably evolved by a duplication of a large DNA fragment containing the ζ-ζ-α-θ gene set, followed by deletion of an α-globin gene to generate the ζ2-ζ3-θ2 gene set (Cheng et al. 1987). The ζ-ζ-θ gene set has apparently duplicated further, as indicated by the results of genomic blot-hybridization analyses (Cheng et al. 1987). In the present paper, some regions of this gene cluster are shown to be polymorphic by using cloned ζ and θ genes as hybridization probes. A large portion of this gene cluster—that containing 5'-α1-θ1-ζ2-ζ3-θ2-3'—shows no length polymorphism. The length polymorphisms are found only in the region containing the genes ζ0 and ζ1. Additionally, the rabbit α-like globin gene cluster is also polymorphic for the number of ζ-ζ-θ gene sets.

**Material and Methods**

**Isolation of Rabbit Genomic DNA**

Genomic DNA was isolated from peripheral white blood cells of New Zealand white rabbits by the method described by Baas et al. (1984). Typically, 5-10-ml blood samples were collected in heparinized tubes, and red cells were lysed by incubation with 3 vol solution A (155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM ethylenediaminetetraacetate [EDTA]) for 10 min on ice. The white cells were collected by centrifugation for 10 min at 160 g and resuspended in an equal volume of solution B.
FIG. 1.—The physical map of cloned rabbit α-like globin genes. The positions of the cloned genes are shown by the boxes. Boxes shaded with the same pattern represent genes descended from the same gene in an ancestral duplication unit. The J (junction) sequences found between homology blocks are indicated. The horizontal bars under the map indicate the positions of the BamHI fragments containing the ζ-globin genes. The sizes of the DNA fragments are those generated by BamHI cleavage or the distance from a BamHI site to the end of the cloned DNA.

(150 mM NaCl, 15 mM Na citrate, 20 mM EDTA, and 100 μg proteinase K/ml). The suspension was adjusted to 1% sodium dodecyl sulfate (SDS) and incubated for 2 h at 37 C. After extraction with phenol and chloroform, the DNA was precipitated by adding 1/30 vol 3 M NaOAc, pH 5.5, and 1 vol isopropanol. After a 70% ethanol wash, the precipitate was dissolved in 3 ml 10 mM tris(hydroxymethyl) aminomethane (Tris)-HCl, 10 mM EDTA, pH 7.4, and treated with RNase A (20 μg/ml) for 2 h at 37 C, followed by a 2-h digestion with proteinase K (100 μg/ml) in the presence of 0.5% SDS. After extraction with phenol and chloroform, the DNA was precipitated with isopropanol and dissolved in 10 mM Tris-HCl, 0.5 mM EDTA, pH 7.4, to a concentration of 1 mg/ml (measured by its absorbance at 260 nm). Rabbit fetus tissue was homogenized in 10 ml solution B in a Sorvall Omni-mixer (DuPont Instruments), and the DNA was isolated by the same procedure as described above.

Genomic Blot Hybridization

Seventeen micrograms of rabbit genomic DNA was digested by BamHI restriction endonuclease, fractionated by electrophoresis on a 1% agarose gel, and blotted onto nylon filters (Nytran; Schleicher & Schull) by the method of Southern (1975). The filters were hybridized to nick-translated, 32P-labeled DNA probes (specific activity 1–5 × 108 cpmp/μg) by using the SDS-phosphate buffer method of Church and Gilbert (1984). Two DNA plasmids, BS+ζ3 and πBBst0.3, were nick-translated and used as hybridization probes. BS+ζ3 contains a 1.9-kb insert of the rabbit ζ-globin gene sequence extending from the upstream RsaI site to the downstream BamHI site (Cheng et al. 1988). This sequence was inserted into the Bluescript plus plasmid (Stratagene) at the HincII and BamHI sites of the polylinker. πBBst0.3 is a miniplasmid containing part of the θ1-globin gene sequence; its construction is described by Cheng et al. (1987). The DNA blotted to the nylon filter was reused after two washings in distilled, deionized H2O at 80 C for 15 min to remove the hybridized probe. The size markers included in the electrophoretic gels were DNA from recombinant lambda phage clones cut with EcoRI. The autoradiograms of the blot-hybridization results were scanned in a densitometer to quantitate the intensity of the bands.

Results
Detection of RFLPs in the Rabbit α-Like Globin Gene Cluster

A physical map of the rabbit α-like globin gene cluster is shown in figure 1. This map was determined by analysis of a set of overlapping λ clones isolated from a rabbit genomic DNA library (Cheng et al. 1986, 1987, 1988). The θ- and ζ-globin genes are
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contained within large homology blocks, consistent with the proposed duplications of long DNA segments during the evolution of this gene cluster (Cheng et al. 1987). The ends of the duplication blocks have the same sequence (Cheng et al. 1987), which is labeled "J" (junction) in figure 1. The central region of the J segment is homologous to the 3' untranslated region of the α-globin gene. Because of its sequence and location, a role for this J segment has been proposed both in the deletion of an α-globin gene and in the block duplications generating the repeating sets of genes in this cluster (Cheng et al. 1987). Five ζ-globin genes are in the cloned DNA segment, and they are contained in four different BamHI fragments (fig. 1). The sizes of these BamHI fragments are 1.9 kb (ζ2), 3.5 kb (ζ3), >2.9 kb (ζ4), and >10 kb (ζ0 and ζ1). DNA isolated from 24 New Zealand white rabbits was digested with BamHI, electrophoresed on an agarose gel, blotted onto a nylon filter, and hybridized with a labeled probe from gene ζ3. This probe will hybridize to all the ζ-globin genes.

The hybridization patterns of the BamHI fragments containing ζ genes from 11 rabbits are shown in figure 2; this set includes all the patterns found in the 24 rabbits. Eight different patterns of the ζ hybridizing bands were found, designated as groups 1 (fig. 1, lane 1), 2 (fig. 1, lanes 2 and 11), 3 (fig. 1, lane 3), 4 (fig. 1, lanes 4 and 5), 5 (fig. 1, lane 6), 6 (fig. 1, lanes 7 and 9), 7 (fig. 1, lane 8), and 8 (fig. 1, lane 10). All of the groups have a 3.5-kb fragment containing ζ3 and a 1.9-kb fragment containing ζ2. Thus, for this group of 24 rabbits, the region of the α-like globin gene cluster containing ζ2 and ζ3 is not polymorphic in length. In contrast, the presence of a variety of fragments ranging from 3 kb to 19 kb in size in different rabbits shows that other regions of the gene cluster are polymorphic. At least four different large BamHI fragments are detected with the ζ gene probe; these fragments are ~10 kb, ~12 kb, ~15 kb, and ~19 kb in length. These four fragments are large enough to contain the ζ0–ζ1 region, which is measured to be >10 kb as determined by the analysis of cloned DNA (fig. 1). The ζ1 gene was previously determined to be in one of the large BamHI fragments (Cheng et al. 1987). Thus, the variation in the genomic blot-hybridization patterns in the 10–19-kb size range (fig. 2) indicates that the ζ0–ζ1 region is polymorphic in rabbits. Smaller DNA fragments are detected by the ζ gene in some but not all rabbits. These fragments are 3.0 kb, 5.4 kb, 6.0 kb, 6.7 kb, and 7.5 kb in size (fig. 2).

In general, each rabbit has either one or two large BamHI fragments, although the rabbits in group 5 (fig. 2, lane 6) have three large BamHI fragments. Some rabbits have no polymorphic bands in the 3.0–7.5-kb range. Of the 24 rabbits examined, one has the pattern characteristic of group 1, five are in group 2, three are in group 3, two are in group 4, three are in group 5, six are in group 6, three are in group 7, and one is in group 8.

Mendelian Inheritance of the Polymorphisms

The different banding patterns detected in the Southern blot-hybridization analysis (fig. 2) could have two different sources. One source of variation is the fact that the α-like globin gene cluster could have a distinctive array of alleles for each gene on a given chromosome; such an array of alleles is called a haplotype. These haplotypes can associate randomly in heterozygotes to give a second source of variability in the genomic blot-hybridization patterns. To examine the contribution of allelic variation to the diverse patterns of α-like globin genes, we mated a male and female rabbit with
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different ζ-globin gene hybridization patterns, and the genomic DNA of nine offspring was analyzed by Southern blot hybridization. The BamHI fragments containing ζ genes from each individual rabbit are shown in figure 3A. The buck (male parent) shows three bands hybridizing to the ζ probe; they are 12 kb, 3.5 kb, and 1.9 kb in length. The 12-kb fragment probably contains the ζ0 and ζ1 genes, as discussed above. This pattern for the buck is characteristic of group 2 rabbits (fig. 2, lanes 2 and 11). The doe contains two additional bands, of 19 kb and 5.4 kb; this pattern is characteristic of group 1 rabbits (fig. 2, lane 1). The fetuses from the litter show only the fraternal or maternal pattern (fig. 3A). These results indicate that the buck was homozygous for one haplotype, which can be called haplotype A, and that the doe was heterozygous for haplotype A and another haplotype, which can be called B. The combination of the 19-kb and the 5.4-kb bands is a distinctive characteristic of haplotype B.

This mating produced three homozygotes (F4, F8, and F9; fig. 3A) and six heterozygotes (F1–F3 and F5–F7; fig. 3A) in the litter, whereas a Mendelian pattern of inheritance would predict equal numbers of homozygotes and heterozygotes among the progeny. The closest approach to equal numbers, given a litter of nine, would generate a 4:5 ratio between homozygotes and heterozygotes. The observed ratio of 3:6 is close to the theoretical prediction, and the deviation from the prediction is likely the result of the small sample size. The data indicate that the RFLPs in these two rabbits represent two different haplotypes that are inherited in a Mendelian fashion.

Variation in Copy Number of the ζ-ζ-θ Duplication Block

The intensities of the hybridization signals to the ζ- and θ-globin gene fragments vary in a consistent manner among the haplotypes, and one can estimate the numbers of these genes from these differences in signal intensity. The average intensity of the hybridization signal for the 3.5-kb ζ3 fragment in the heterozygous rabbits is approximately 1.5-fold the intensity observed in the homozygous rabbits (fig. 3A), and a similar relationship is seen for the 1.9-kb ζ2 fragments (fig. 3A). The difference in hybridization intensity most likely results from a difference in the copy number of each fragment. This possibility was examined further by hybridizing the DNA samples with a θ gene probe (fig. 3B). The fragments containing θ genes in these rabbits are not polymorphic in length. The two bands correspond to the 7.2-kb fragment containing the α1 and θ1 genes (fig. 3B, band θ1) and the 5.0-kb fragment containing the θ2 gene (fig. 3B, band θ2-n). The data in figure 3B show that the hybridization signals for θ1 are of equivalent intensities for both the homozygous (AA) and heterozygous (AB) rabbits but that the signal for θ2 is twice as strong in the heterozygotes as in the homozygotes. The equivalent signal for gene θ1 observed for all rabbits shows that an equal amount of DNA was present in each lane and that these comparisons between individuals are meaningful. Thus, the rabbits with a strong signal for θ2 also have strong signals for ζ2 and ζ3. These are the genes in the duplicated ζ2-ζ3-θ2 gene set.
and the data in figure 3 indicate that the heterozygotes have a larger number of copies of this gene set. The additional copies of the θ-globin genes are specified as θ2-n in figure 3B because the exact number of duplicated ζ-ζ-θ gene sets is difficult to determine from these data. The 7.2-kb BamHI fragment that hybridizes to the θ gene probe also hybridizes to an α-globin cDNA probe (Cheng et al. 1987). In confirmation of the results in figure 3B, no variation in the size of BamHI fragments hybridizing to the α gene probe was observed in this group of 24 rabbits (data not shown).

Another example of consistent variation in the intensity of the hybridization
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signals is seen in the large fragments that hybridize to the ζ gene probe. The average hybridization intensity of the 12-kb fragments in the homozygous AA rabbits is twice the intensity observed in the heterozygous AB rabbits (fig. 3A, ζ1 band). The heterozygous rabbits have a second large fragment of 19 kb that hybridizes to the ζ probe, and the sum of the intensities for the 12-kb plus 19-kb bands in the heterozygotes is about the same as the intensity of the 12-kb band in the homozygotes (fig. 3A). This indicates that the ζ0-ζ1 region is contained on a 12-kb fragment in haplotype A whereas it is contained on a 19-kb fragment in haplotype B. Thus, the homozygotes for haplotype A would have two copies of the 12-kb fragment, and heterozygotes for haplotypes A and B would have one copy each of the 12-kb and 19-kb fragments.

Maps of Two Haplotypes in the α-Like Globin Gene Cluster

An interpretation of the blot-hybridization data is summarized in the gene maps for two haplotypes of the rabbit α-like globin gene family (fig. 4). The haplotypes differ in the copy number of the ζ-ζ0 duplication blocks and in the length of restriction fragments containing the ζ0 and ζ1 genes. Group 2 rabbits (fig. 2, lanes 2 and 11) are homozygous for haplotype A, which contains seven α-like globin genes. Group 1 rabbits (fig. 2, lane 1) are heterozygous for haplotypes A and B. Haplotype B contains a total of 13 α-like globin genes within four duplication blocks. The 5.4-kb BamHI fragment is proposed to contain the ζ4 and ζ5 genes located 3' to the ζ2-ζ3-ζ2 duplication block. This postulate is consistent with the observation that the ζ4 gene is in the cloned gene cluster is located in a BamHI fragment that must be >2.9 kb long in the rabbit genome (fig. 1). Also, a fusion, by the loss of a BamHI site, of the 1.9-kb fragment containing ζ2 with the 3.5-kb fragment containing ζ3 would generate a 5.4-kb fragment. The gene maps of these two haplotypes are consistent with the blot-hybridization patterns and band intensities described above. In particular, both the ζ3 3.5-kb fragment and the ζ2 1.9-kb fragment are present twice in the AA homozygotes and three times in the AB heterozygotes. This would generate the 1.5-fold increase in band intensity observed in the heterozygotes. The θ2 5.0-kb band is present twice in the AA homozygotes and four times in the AB heterozygotes, thus accounting for the twofold increase in signal intensity in the heterozygotes. The θ1 7.2-kb band is present twice in both cases, and it gives the same hybridization intensity in each lane of figure 3B.

The pattern of fragments hybridizing to the ζ gene probe shown in figure 2 can be analyzed as a group of haplotypes by making the simplifying assumptions that each of the large BamHI fragments contains the ζ0-ζ1 region and that the 3.0-kb, 5.4-kb, 6.0-kb, 6.7-kb, and 7.5-kb fragments contain genes derived from duplication of the ζ-ζ-θ gene set. Only group 2 rabbits contain a single large ζ0-ζ1 fragment (and are therefore homozygous), so the other patterns containing more than one large BamHI fragment probably reflect heterozygosity. The seven heterozygous patterns could contain as many as 14 different haplotypes, and evidence for at least eight haplotypes in addition to haplotype A can be deduced from the data for heterozygotes in figure 2. Thus, in this analysis of 24 rabbits, nine different haplotypes can be detected. Many of these additional haplotypes are simple extensions or recombinations of haplotypes A and B diagrammed in figure 4. For example, the pattern for group 3 (fig. 2, lane 3) can be described as a heterozygote of haplotype B and another haplotype C, which is like haplotype A except that it extends into the ζ4-ζ5 region for 6.0 kb. Groups 4, 5, and 8 (fig. 2, lanes 4-6 and 10) appear to have a haplotype D, which would result
Haplotype A

\[ S_0 \ S_1 \ \alpha_1 \ \theta_1 \ S_2 \ S_3 \ \theta_2 \]

12 Kb
7.2 Kb
1.8 Kb 3.6 Kb
5.0 Kb

Haplotype B

\[ S_0 \ S_1 \ \alpha_1 \ \theta_1 \ S_2 \ S_3 \ \theta_2 \ S_4 \ S_5 \ \theta_3 \ S_6 \ S_7 \ \theta_4 \]

16 Kb
7.2 Kb
1.8 Kb 3.6 Kb
5.0 Kb
5.4 Kb
5.0 Kb
5.0 Kb 3.8 Kb
5.0 Kb
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from a recombination between haplotypes A and B to produce characteristic BamHI fragments of 19 kb, 3.5 kb, and 1.9 kb.

Discussion

Analysis of restriction-enzyme-site polymorphism within the rabbit α-like globin gene cluster has revealed eight different BamHI digest patterns in 24 New Zealand white rabbits. The differences include both length polymorphisms in the restriction fragments and variation in the number of copies of the duplicated ζ-θ-0 three-gene set. This gene cluster can be divided into regions with or without length polymorphism. The central portion of this gene cluster is not polymorphic; this region includes the 7.2-kb BamHI fragment containing the α1 and θ1 genes, the 1.9-kb BamHI fragment containing the ε2 gene, the 3.5-kb BamHI fragment containing the ε3 gene, and the 5.0-kb BamHI fragment containing the θ2 gene. DNA sequence analysis of the genes within the nonpolymorphic region 5'-α1-θ1-ε2-ε3-θ2-3' has shown that both the θ1 and θ2 genes have lost the normal 5' promoter sequences (Cheng et al. 1986; J.-F. Cheng and R. Hardison, unpublished data), that the ε2 gene contains no exon 1 sequences, and that the ε3 gene encodes a polypeptide containing amino acid replacements at positions important for globin function (Cheng et al. 1988). Thus, the α1-globin gene is the only functional gene in this nonpolymorphic region.

The restriction fragments with length polymorphisms include those containing the ζ0-ζ1 region (large BamHI fragments 12 kb and 19 kb in size) and the 5.4-kb BamHI fragments that probably include ζ4 and ζ5. The large 10-kb and 15-kb BamHI fragments are also large enough to include the ζ0-ζ1 region. The observation that, in general, each rabbit examined has either one or two fragments in the 10-19 kb range suggests that the 10-kb, 12-kb, 15-kb, and 19-kb fragments each contain the ζ0 and ζ1 genes but that they are in separate haplotypes. However, this question cannot be resolved definitively until probes have been identified that are specific for ζ0 and ζ1 and that will not cross-hybridize with ζ2 and ζ3. Other BamHI fragments, ranging from 3.0 kb to 7.5 kb in size, may also include ζ4 and ζ5. These fragments are not present in all the rabbits examined, which indicates that the small size fragments are not essential for the production of sufficient amounts of the ζ-globin polypeptide.

The length polymorphisms can be generated by two different mechanisms, either by the creation or elimination of a restriction site by nucleotide substitutions or by rearrangement of DNA segments (deletions, insertions, or inversions). In one case, the polymorphic 5.4-kb fragment in haplotype B apparently results from the loss of a BamHI cleavage site, possibly by a nucleotide substitution. The length polymorphisms in the large BamHI fragments containing ζ0 and ζ1 in haplotypes A and B could be generated by either mechanism, although the large number of variants suggests that they may be generated by differences in copy numbers of repeated sequences. For example, Bell et al. (1982) showed that variation in the copy number of a 14-bp tandemly repeated sequence generates a length polymorphism in the 5'-flanking region of the human insulin gene. The human α-like globin gene cluster contains three hypervariable regions located within the ζ1 intron (Proudfoot et al. 1982), between the ζ genes (Goodbourn et al. 1983), and downstream from the α1-globin gene (Jarman et al. 1986). Again, variation in the copy number of a tandemly repeated sequence is responsible for these length polymorphisms in the human α-like globin gene cluster. It is possible that the region containing the rabbit ζ0 and ζ1 genes also includes a repeating DNA segment variable in copy number that could account for the length polymorphism in the large BamHI fragments.
The results from blot-hybridization studies show that the copy number of the \( \zeta-\zeta-\theta \) duplication block varies from one to three or more in different haplotypes. The copy-number polymorphisms are common in the rabbit population, which indicates that these differences in \( \zeta-\zeta-\theta \) copy numbers have little effect on phenotype. In fact, the additional copies of the \( \zeta \) and \( \theta \) genes may not be functional, and the observed variation in copy number supports the conclusion, based on quantitative sequence comparisons (Cheng et al. 1988), that the genes in the \( \zeta-\zeta-\theta \) block were rendered nonfunctional before the duplication of \( \zeta-\zeta-\theta \) occurred. The variation in the copy number of the \( \zeta-\zeta-\theta \) gene set could result from the unequal crossing-over between misaligned gene sets. However, it is curious that only one region of the \( \alpha \)-like globin gene cluster seems to be involved in these unequal crossovers at a high frequency. In this regard, it is interesting that the J sequences discovered at the junctions of the duplication blocks (Cheng et al. 1987) flank the \( \zeta-\zeta-\theta \) gene region (fig. 1). The J sequences have been proposed as hot spots for recombination (Cheng et al. 1987), and they may contribute to a high frequency of recombination involving the \( \zeta-\zeta-\theta \) gene set. Alternatively, the explanation for the high frequency of copy-number variants need not rely on a high frequency of recombination in the \( \zeta-\zeta-\theta \) gene region. Rather, the recombination products from the unequal crossing-over may be more frequently fixed in the population because the variation in copy number of the \( \zeta-\zeta-\theta \) genes has little physiological effect on the rabbit (i.e., it is selectively neutral).

The \( \beta \)-like globin gene clusters of ruminants have also evolved by block duplications of sets of genes (Townes et al. 1984; Schimenti and Duncan 1985). In sheep, two common haplotypes have been observed, and these differ in the number of duplication blocks present (Garner and Lingrel 1988). In both lagomorphs and artiodactyls, the \( \alpha \)- or \( \beta \)-globin gene clusters are expanding by block duplications and possibly are contracting by deletions of these same sets of genes. One of the results of these block duplications is the accumulation of many pseudogenes, and, as shown in the present paper, the number of pseudogenes varies among individual rabbits, depending on their haplotypes. In the artiodactyls, however, at least one gene in the duplicated set has remained active, and in fact the \( \beta \)-globin gene has become expressed at different times of development after the block duplications (Townes et al. 1984). The functional status of all the \( \zeta \) and \( \theta \)-globin genes in the larger haplotypes of rabbits has not been established, and it is possible that a similar switch in the development regulation of some duplicated genes may have occurred in rabbits. However, for the A haplotype, the only candidates for active genes are \( \alpha_1, \zeta_0 \), and possibly \( \theta_2 \), which again indicates that the additional genes are not needed for survival.

Polymorphism is observed at a high frequency in the \( \alpha \)-like globin gene cluster of rabbits. In 24 rabbits, eight different patterns representing at least nine haplotypes were found. Despite this variability, only one adult \( \alpha \)-globin gene has been detected in each haplotype. The presence of only one active \( \alpha \)-globin gene is unique to rabbits (Cheng et al. 1986), and the data in the present paper show that it is the common condition in New Zealand white rabbits; it is not restricted to a few rare individuals. Much polymorphism has also been observed in the human \( \alpha \)-like globin gene cluster, resulting from the presence of hypervariable regions (Goodbourn et al. 1983; Jarman et al. 1986), sequence changes at restriction sites (Higgs et al. 1986), and deletions that are prevalent in some populations (Nicholls et al. 1987). No RFLP has been observed in the 7.2 kb surrounding the \( \beta_1 \)-globin gene of rabbits (Jeffreys and Flavell 1977), but, in the 3' flank of this gene, a length polymorphism has been discovered that results from the insertion or deletion of a 2.7-kb segment of DNA (Masina et al.
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1984). Two different length polymorphisms were detected in this region in a group of 25 New Zealand white rabbits. This observation is similar to those presented here for the α-like globin gene cluster in that the central part of the gene cluster shows no detectable polymorphism but the flanking regions vary in length. However, only two different patterns from the β-like globin gene cluster were detected in 25 rabbits, whereas eight different patterns from the α-like globin gene cluster were detected in 24 rabbits. Several lines of evidence suggest that the α-like globin gene clusters of mammals show more variability than do the β-like globin gene clusters (Hardison and Gelinas 1986). If, in fact, polymorphisms are more frequent in the α-like globin gene cluster, it will be most informative to determine the basis for that higher frequency.

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