Polymorphism and Linkage of the αA-Crystallin Gene in *t*-Haplotypes of the Mouse

Loren C. Skow,* Joseph N. Nadeau,† Jong Cheol Ahn,‡ Hee-Sup Shin,‡ Karen Artzt‡,[1] and Dorothea Bennett‡,[1]

**Department of Veterinary Anatomy and Cell Biology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, †The Jackson Laboratory, Bar Harbor, Maine 04609 and ‡Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, New York 10021*

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ABSTRACT

Restriction fragment polymorphisms were used to order the αA-crystallin locus (*Crya-1*) relative to other genes in mouse *t*-chromatin and to investigate the relatedness of α-A-crystallin sequences among different *t*-haplotypes. Analysis of DNA from *t*-recombinant mice mapped *Crya-1* to the *K* end of the *H-2* complex and within the distal inverted region characteristic of *t*-haplotypes. Hybridization with *Crya-1* cDNA revealed three distinct phenotypic groups among the 17 different *t*-haplotypes studied. A majority (9 of 17) of the *t*-haplotypes were classified into a novel group (*Crya-1^t*) characterized by restriction fragments apparently unique to *t*-chromosomes and therefore thought to contain αA-crystallin sequences descended from the original *t*-chromosome. A second group of *t*-haplotypes had restriction fragment patterns indistinguishable from those observed among many common inbred strains of mice of the *Crya-1^a* type, and a third restriction fragment pattern, observed only in the *t^{w121}* haplotype, was indistinguishable from the fragment pattern for C3H/DiSn (*Crya-1^b*) and several other inbred strains of mice. Thus, with respect to sequences around the *Crya-1* locus, different *t*-haplotypes show restriction fragment polymorphisms, some of which are comparable to those found in wild-type chromosomes and provide further evidence for genetic heterogeneity in DNA from the distal region of *t*-haplotypes.

ABOUT 25% of mice in natural populations are heterozygous for a peculiar region of genetic material called *t*-chromatin, which comprises about 14 cM of the proximal portion of chromosome *17* (BENNETT 1975, 1980; KLEIN 1975; LYON 1981; SILVER 1985), and contains at least two inversions which suppress recombination with wild-type chromosome (HERRMAN *et al.* 1986). Complete *t*-haplotypes are all defined (1) by the presence of a mutant gene, *t^{tct}*, which interacts with the Brachyury gene (*T*) to produce *T/t* heterozygous mice that are tailless, and (2) by recombination suppression over a chromosomal region of about 20 cM which includes the major histocompatibility complex (*H-2*). A large number of *t*-haplotypes have been isolated from wild mice and are thought to have originated from a common ancestral form since all *t*-haplotypes from natural populations share several distinctive characteristics. These include the presence of one (or rarely two) of several nonallelic, recessive embryo-lethal genes (ARTZT, MCCORMICK and BENNETT 1982), the preferential transmission of *t*-bearing sperm to the progeny of *t/+* heterozygous males (BENNETT, ALTON and ARTZT 1983; LYON 1984; SEITZ and BENNETT 1985; OLDS-CLARKE and PEITZ 1986), and male sterility in compound (*t^x/t^y*) heterozygotes (BENNETT and DUNN 1967 1971; LYON 1986).

Recombination suppression is abrogated in mice heterozygous for two complementing *t*-haplotypes, such that genetic recombination occurs freely along the entire length of chromosome *17* (SILVER and ARTZT 1981). The arrangement of genes in *t*-chromatin can therefore be determined by studying meiotic recombination in female mice heterozygous for complementing *t*-haplotypes. Such studies led to the discovery that the embryo-lethal genes of *t*-haplotypes are nonallelic (ARTZT, MCCORMICK and BENNETT 1982), and subsequent application of molecular techniques identified restriction fragment length polymorphisms (RFLPs) among class I and class II histocompatibility genes of different *t*-haplotypes and showed that the order of genes in the *H-2* region of *t*-chromatin is inverted relative to wild-type chromosome *17* (ARTZT, SHIN and BENNETT 1982; ROGERS and WILLISON 1983; SHIN *et al.* 1983). Recently the locus for mouse αA-crystallin (*Crya-1*) was localized by restriction fragment length polymorphisms to the mid-region of wild-type chromosome *17* just outside the centromeric boundary of the *H-2* complex (SKOW and DONNER 1985). The original gene symbol for mouse αA-crystallin (*Acry-1*) has been changed to conform to nomenclature adopted by the Human

[1] Present address: Department of Zoology, University of Texas, Austin, Texas 78712.

Gene Mapping Conference 1985 for the human homolog, CRYA1 (QUAX-JEUKEN *et al.* 1985). From studies of gene order in *t*-haplotypes (SHIN, BENNETT and ARTZT 1984; PLA and CONDAMINE 1984), the mouse αA-crystallin locus is expected to be located within the distal inversion characteristic of complete *t*-haplotypes and present an opportunity to further investigate the origin and organization of *t*-chromatin. The results presented here describe the map position of *Crya-1* relative to other genes on *t*-chromosomes and identify at least three classes of *t*-haplotypes based on restriction enzyme analysis of DNA probed with αA-crystallin cDNA.

MATERIALS AND METHODS

Mice were produced in research colonies at The Jackson Laboratory, Bar Harbor, Maine, or Sloan-Kettering Institute for Cancer Research, New York, New York. The t^6-haplotype DNA was obtained from random bred mice of genotype Ttf/t^6+. All other *t*-haplotypes used in this study were maintained congenic with a C3H/DiSn genetic background. Mice with recombinant *t*-chromosomes were produced from compound female mice containing the complementing *t*-lethal haplotypes, Tt^{s6}/t^{12} or Tt^{m455}/t^{s500}, mated to C3H·*tf*(+*tf*/+*tf*) males. The origins of the $Tf^{s6}/(t^{w12})$, Tt^{m455} (t^{12}) and Tt^{s600} (t^{w5}) chromosomes have been described and tissues from these mice have been utilized in previous studies to define the map positions of several gene markers in *t*-chromosomes (ARTZT, MCCORMICK and BENNETT 1982, ARTZT, SHIN and BENNETT 1982; ARTZT 1984; SHIN, BENNETT and ARTZT 1984).

DNA was prepared from liver by the method of CAYRE *et al.* (1981). Samples of DNA (approximately 10 μg each) were digested with 50 units of the appropriate restriction endonuclease according to supplier's instructions (Bethesda Research Laboratories, Bethesda, Maryland) for 16 hr and fractionated by horizontal electrophoresis in 0.75% agarose gels submerged in 50 mM tris(hydroxymethyl)aminomethane (Tris):acetate: 2 mM EDTA buffer (pH 8.5). Following electrophoresis, the DNA was denatured and transferred to nylon membranes (Zetabind, AMF Cuno) by the procedure of SOUTHERN (1975). Membranes were baked for 90 min at 80° *in vacuo*, removed to heat-sealable plastic bags and washed with agitation for 1 hr at 65° in 50 ml of 0.1 × SSC:0.5% sodium dodecyl sulfate (SDS). A 1 × SSC solution contained 8.73 g NaCl and 4.41 g sodium citrate/liter adjusted to pH 7.0 with NaOH. The wash solution was replaced with 50 ml of prehybridization solution (10 mM Tris, 5 × SSC, 5 × Denhardt's solution, 0.1% SDS for 3–4 hr with agitation at 42°. Mouse αA-crystallin cDNA was electrophoretically purified from *Pst*I-digested pMαACr2 (KING, SHINOHARA and PIATIGORSKY 1982) and nick-translated with ^{32}P-dCTP (RIGBY *et al.* 1977) to specific activities $>10^8$ cpm/μg. Filters were hybridized with ^{32}P-labeled cDNA probe as previously described (SKOW and DONNER 1985). After hybridization, membranes were washed twice in 0.2 × SSC, 0.1% SDS at 65° for 30 min each and subjected to autoradiography for 24–48 hr on Kodak XAR film between two intensifying screens (Cronex, DuPont).

RESULTS

Previous mapping experiments in wild-type mice utilized recombinant inbred and congenic inbred strains of mice to assign the αA-crystallin locus to chromosome 17, about 1 cM centromeric from *H-2K* (SKOW and DONNER 1985). To provide independent confirmation of the close linkage of *Crya-1* to the *H-2* complex in a segregating population, we utilized restriction fragment length polymorphisms to follow the inheritance of a class II gene, *Eβ*, and *Crya-1* among 17 F_2 progeny of (BALB/c × C57BL/6)F_1 mice. The *Eβ* polymorphism was produced by *Bam*HI (BALB = 5.2 kb and B6 = 9.4 kb) and the *Crya-1* polymorphism by *Hinc*II (BALB = 12.8 kb and B6 = 10.8 kb). No recombinants between *Eβ* and *Crya-1* were observed among the F_2 progeny (data not shown), supporting the close linkage of these loci.

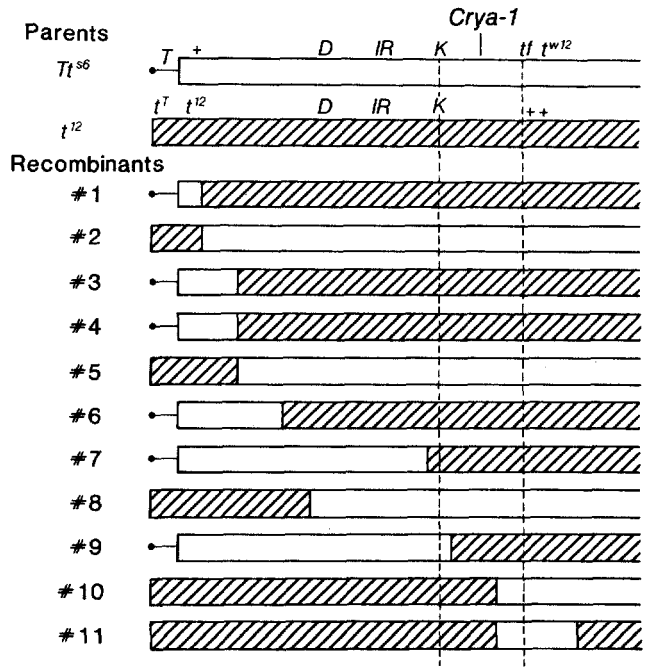

FIGURE 1.—Diagrammatic constitution of parental chromosomes and localization of breakpoints in recombinant chromosomes. Chromosomes are represented with centromeric end to the left and loci are noted above the respective parental chromosomes in linear order but not to scale. By typing parental forms of αA-crystallin RFLPs among recombinant chromosomes of known breakpoints, we can assign the *Crya-1* to the region between *H-2* and *tf*.

To confirm that the relative orientation of *H-2*, *Crya-1*, and *tf* in *t*-haplotypes was the same as in wild type chromosomes, we analyzed a set of 11 recombinants obtained from $Tt^{s6}tf/t^{12}$ females. Figure 1 gives a diagrammatic representation of the two parental chromosomes and the recombinants, showing their breakpoints. Restriction enzyme analysis of the parental *Crya-1* sequences with *Taq*I demonstrated no differences in the fragment patterns for Tt^{s6} and C3H whereas the t^{12} haplotype has a *Taq*I fragment slightly larger than that found in C3H DNA (Figure 2). Analysis of *Taq*I restriction fragments in recombinants 1–8 show that the *Crya-1* locus must be to the right of the break point in recombinant 7 which was defined as having an intra-*H-2* crossover between the *I* and *K* regions. Recombinants 9, 10 and 11 all have breakpoints between *K* and *tf* and demonstrate that the

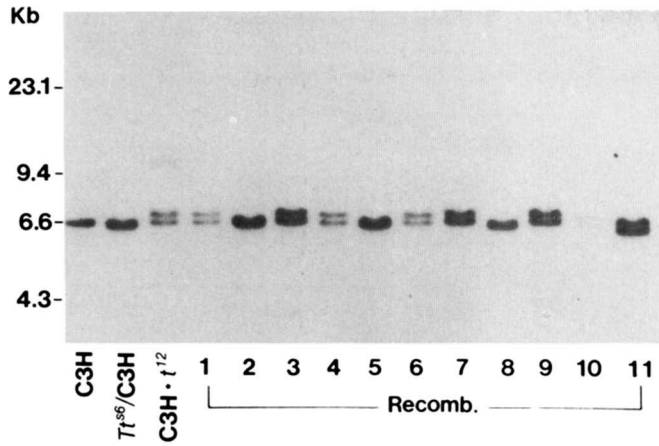

FIGURE 2.—Representative *Taq*I restriction fragment patterns for *Crya-1* from mice with *t*-haplotypes in parental (C3H, $Tt^{s6}tf$/C3H, and C3H t^{12}) or recombinant chromosome arrangements. Recombinant chromosomes are *trans* to C3H/DiSn.

Crya-1 locus must lie between the *K* region and *tf*, in the same, albeit inverted, order as in wild type. To map *Crya-1* more precisely, we analyzed an additional 40 recombinants obtained from females carrying Tt^{s6} and t^{12} or t^{w5} that were known to have breakpoints between *K* and *tf* (t^{w5} and t^{12} have the same *Taq*I restriction fragment pattern for *Crya-1*). Of the total of 43 recombinants tested, 51% (22 of 43) had breakpoints between *H-2K* and *Crya-1*. Previous studies (SHIN *et al.* 1982) have estimated the map distance between *H-2K* and *tf* in *t*-haplotypes at 5.9 cM, thus *Crya-1* is located about 3.0 cM equidistant from *H-2K* and *tf*.

The search for restriction fragment length polymorphisms among the *t*-haplotypes was conducted using eight different restriction endonucleases selected for efficiency in detecting RFLPs in mammalian DNA (WIJSMAN 1984). These included *Bam*HI, *Bcl*I, *Bgl*I, *Eco*RI, *Hin*dIII, *Hin*cII, *Pst*I, and *Taq*I. Each restriction endonuclease revealed variation among the 17 *t*-haplotypes examined. Most endonucleases (*Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Pst*I, and *Taq*I) produced two classes of restriction fragment patterns but two enzymes (*Hin*cII and *Bcl*I) gave three classes of restriction patterns. All the *t*-haplotypes studied could be classified into one of three categories based on CRYA-1 phenotypes. Representative patterns for each category are shown in Figure 3 and estimated restriction fragment sizes are presented in Table 1. The majority of the *t*-haplotypes (t^{w1}, t^{w2}, t^{w8}, t^{12}, t^{w32}, t^{w71}, t^{w73}, t^{w75}, and $t^{WPA\text{-}1}$) were assigned to a genotypic class, designated *Crya-1*^{t} that is unique with respect to the αA-crystallin restriction fragment patterns observed so far among inbred strains of mice and non-*t*-chromosome-bearing wild mice (SKOW and DONNER 1985; E. K. WAKELAND and L. C. SKOW, unpublished data). The restriction fragment patterns of the next most abundant class (haplotypes t^0, t^{w5}, t^6, t^{w12}, $t^{w12}tf$, t^{w120} and $t^{LUB\text{-}1}$) were indistinguishable from the *Crya-1*^{a} type commonly found among inbred strains of mice. The third type of restriction fragment patterns was observed only in the t^{w121} haplotype and was indistinguishable from the *Crya-1*^{b} pattern, also common among standard inbred strains of mice. The origins of t^{W120} and t^{W121} were described by ARTZT *et al.* 1985.

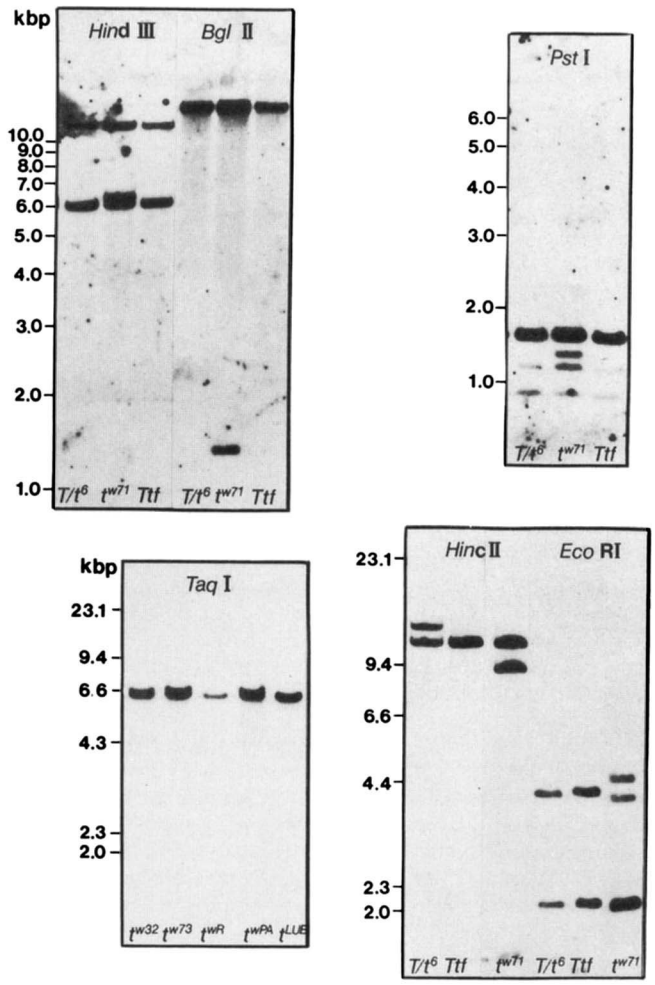

FIGURE 3.—Selected αA-crystallin restriction fragment patterns observed among DNAs of representative *t*-haplotypes. Endonucleases are listed at the top of each panel of fragments. The source of *t*-chromosome in each sample is identified by lane. All samples contain fragments from the C3H background. The *T tf* haplotype is from C3H·HeSn-*T tf*/*t tf*.

It is apparent from these data that members of the same complementation group (*i.e.*, t^{w1} and t^{w12}) do not always possess the same form of *Crya-1*. Similar discordancies were noted for class I and class II histocompatibility genes by ARTZT *et al.* (1985) who examined the 17 haplotypes used in this study serologically for class I antigenic determinants and by restriction fragment polymorphisms for class II gene sequences. A comparison of our data with that of ARTZT *et al.* (1985) also shows divergence among *t*-haplotypes with respect to *H-2* genes and *Crya-1*. For example, the t^0 and t^{WPA} haplotypes were scored as identical on the basis of restriction enzyme polymorphism for *H-2* class I and class II genes, but contain different *Crya-1*

TABLE 1

αA-Crystallin restriction fragments (kb) observed among the DNAs of mice of 17 different *t*-haplotypes

Restriction endonuclease	*Crya-1*^{t}	*Crya-1*^{a}	*Crya-1*^{b}
*Bam*HI	7.8	9.5	9.5
		3.8	3.8
	1.0	1.0	1.0
*Bcl*I	5.4	5.4	5.2
	5.0	4.4	4.4
*Bgl*II		13.0	13.0
	12.5		
	1.2		
*Eco*RI	4.5	4.3	4.3
	2.2	2.2	2.2
*Hin*dIII	13.0	13.0	13.0
	6.4		
		6.0	6.0
*Hin*cII		12.0	10.5
	8.5		
	1.9		
*Pst*I	1.8	1.8	1.8
	1.6		
		1.4	1.4
*Taq*I	6.8		
		6.6	6.6

patterns. Similarly t^6, which is serologically identical to t^{w1} and t^{w71}, contains *Crya-1*^{a}, whereas the other two haplotypes have *Crya-1*^{t}.

DISCUSSION

This study estimated the linkage distance to be 3.0 ± 0.17 cM from αA-crystallin to *H-2K* in *t*-chromatin, or about three times greater than the 1.06 ± 0.83 map distance for the same loci in wild-type chromosome *17* as estimated from analysis of recombinant inbred and congenic strains of mice (SKOW and DONNER 1985). This discrepancy may simply represent variation due to sampling ($\chi^2 = 2.8$, $0.10 > P > 0.05$) but other possibilities should be considered. The frequency of genetic recombination is often higher in female than male mice for a given stretch of DNA (ROBINSON 1972) and this effect may be accentuated on different genetic backgrounds (SKOW 1981). Of necessity, linkage data for *t*-chromosomes are derived from females (*t*-heterozygous males are sterile) and may exceed the standard map distances that have been derived, at least in part, from male meioses. Also, variable map distances may be attributable to structural differences among *t*-haplotypes. CONDAMINE, GUENET and JACOB (1983) noted that map distances also vary greatly between genes in complete *t*-haplotypes and proposed that different *t*-haplotypes contain different lengths of *t*-chromatin such that recombination frequencies for genes of any two complementing haplotypes will be influenced by the proportional lengths of *t*-chromatin in the two haplotypes.

The identification of three classes of αA-crystallin restriction fragment phenotypes among *t*-haplotypes was a surprising observation given the apparent uniqueness and remarkable genetic similarity of *t*-haplotypes at other loci. In general, *t*-haplotypes demonstrate strong linkage disequilibrium for several distinctive serological and allozymic variants. No polymorphisms have been found among *t*-haplotypes for any enzyme gene markers that map within the *t*-complex (KLEIN, SIPOS and FIGUEROA 1984; NIZETIC, FIGUEROA and KLEIN 1984; NADEAU 1986) nor for several *t*-chromatin-specific testicular proteins identified by two-dimensional gel electrophoresis (SILVER *et al.* 1983). Likewise, analysis of *t*-chromosome DNA with random genomic DNA probes (ROHME *et al.* 1984; FOX *et al.* 1985) failed to detect significant variation among different *t*-haplotypes and analysis of restriction fragment polymorphisms identified with cDNA probes to *H-2* class I genes (SHIN *et al.* 1982; SILVER 1985) suggested that all *t*-haplotypes were very similar to one another and quite different from wild-type chromosomes. It should be noted in this context, however, that the genes recognized by the *H-2* class I cDNA probe are predominantly *Qa* and *Tl* genes which are relatively nonpolymorphic even in wild mice (WINOTO, STEINMETZ and HOOD 1983; WEISS *et al.* 1984). There is evidence of substantial sequence variation in and around the class II genes in *t*-haplotypes (ARTZT *et al.* 1985; GOLUBIC *et al.* 1984) and detailed comparative studies of the *H-2K* region of two haplotypes (t^{12} and t^{w5}) by high resolution restriction enzyme mapping has shown them to be very different; in less than 200 kb of DNA compared, there were 15 differences involving insertion/deletion changes and 14 restriction site differences out of 183 restriction sites compared (UEHARA *et al.* 1987). Seen in this perspective, the levels of variation for *Crya-1* that we find among *t*-haplotypes are not so surprising after all. Furthermore, many of the size differences responsible for the polymorphisms we observed are in the range of the small insertions found by UEHARA *et al.* to be so prevalent as a source of variation between t^{12} and t^{w5}.

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