THE LOCUS ENCODING αA-CRYSTALLIN IS CLOSELY LINKED TO *H-2K* ON MOUSE CHROMOSOME *17*

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ABSTRACT

We have used a complementary DNA (cDNA) for mouse αA-crystallin to probe genomic DNA for restriction fragment length polymorphisms which could be used to map the αA-crystallin gene locus (*Acry-1*) in the mouse genome. Ten of 12 restriction endonucleases produced fragment polymorphism among various inbred strains of mice. A comprehensive strain survey conducted with six endonucleases resulted in the discovery of six allelic forms of *Acry-1*. Linkage analysis was conducted on DNA from three sets of recombinant inbred strains of mice and demonstrated close linkage of *Acry-1* with the major histocompatibility complex (*H-2*) on chromosome *17*. Analysis of congenic and recombinant congenic strains of mice confirmed the linkage of *Acry-1* and *H-2* and located the αA gene to the region between glyoxylase (*Glo-1*) and *H-2K*.

THE α-crystallin isolated from mammalian lens is typically composed of four types of polypeptide chains. Two of the polypeptides, αA_2 and αB_2, are the primary products of homologous genes (BERNS, STROUS and BLOEMENDAL 1972; BERNS *et al.* 1973). Deamidation of αA_2 and αB_2 produces the other α-crystallin chains, αA_1 and αB_1 (BLOEMENDAL *et al.* 1972; DELCOUR and BOUCHET 1978). In some species of rodents, a fifth α-crystallin, αA^{ins}, is found that has an insertion of 22 amino acids between positions 63 and 64 of an otherwise normal αA polypeptide (COHEN *et al.* 1978). The coding sequences of αA genes from rats and mice have recently been cloned and sequenced (MOORMAN *et al.* 1981; KING, SHINOHARA and PIATIGORSKY 1982), and the αA^{ins} polypeptide has been shown to originate from a splicing ambiguity during processing of the αA transcript (KING and PIATIGORSKY 1983).

Although much is known about the molecular features of the mouse αA-crystallin gene, its chromosomal location remains to be determined. To facilitate studies of the genetics of lens crystallins and their involvement in heritable lens defects in mice, we have identified DNA restriction fragment length polymorphisms associated with the αA gene of inbred strains of mice and utilized the variation to map the αA locus to mouse chromosome *17*, very close to the *H-2K* region of the major histocompatibility complex.

MATERIALS AND METHODS

Mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, or obtained from colonies maintained at the National Institute of Environmental Health Sciences, Research Triangle

Park, North Carolina. Additional congenic and recombinant congenic mice were provided by ALAN WHITMORE and BERNARD AMOS, Department of Microbiology and Immunology, Duke University, Durham, North Carolina. DNA was prepared from the spleens of individual mice as previously described (POPP *et al.* 1981) or was purchased from the mouse DNA resource maintained by BEN TAYLOR, The Jackson Laboratory. DNA from strain YBR/Ki was a gift from MIRIAM MEISLER, Department of Human Genetics, University of Michigan, School of Medicine, Ann Arbor, Michigan. Samples of DNA (10 μg each) were digested with 30 units of the appropriate restriction endonuclease (Bethesda Research Laboratories, Bethesda, Maryland) for 16 hr according to manufacturers' instructions and fractionated by electrophoresis in a 0.75% agarose gel submerged in 50 mM tris(hydroxymethyl)aminomethane (Tris):acetate:EDTA buffer (pH 8.6). After electrophoresis, the DNA was denatured and transferred to nitrocellulose (BA85, Schleicher and Schuell Inc., Keene, New Hampshire) or nylon (Zetabind, Bio-Rad Laboratories, Richmond, California) membranes by the procedure of SOUTHERN (1975). Filters were baked at 80° for 2 hr *in vacuo*, carefully placed in heat-sealable plastic bags (Kapok/Scotchpak, Kapok Corporation, Bloomington, Minnesota) and washed with agitation for 1 hr at 65° in 50 ml of 0.1× SSC, 0.5% sodium dodecyl sulfate (SDS). The plastic bags were opened and the wash solution replaced with 50 ml of prehybridization solution (10 mM Tris, 5× SSC, 5× Denhardt's solution, 0.1% SDS) for 3–4 hr at 42°. Filters were hybridized with αA-crystallin cDNA probe for 16 hr at 42° in 10 ml of a solution identical with the prehybridization solution but containing 50% freshly dionized formamide. The αA cDNA sequence was purified by preparative agarose electrophoresis from *Pst*I-digested pMαCr2 and radiolabeled by nick translation (RIGBY *et al.* 1977) with ^{32}P-dCTP (Amersham) to specific activities of $1–3 \times 10^8$ cpm/μg. pMαACr2 contains a nearly full-length αA cDNA cloned into the *Pst*I site of pBR322 (KING, SHINOHARA and PIATIGORSKY 1982). Radiolabeled probe was added to the hybridization solution (5 ng/ml), heat denatured at 80° for 10 min and immediately used for hybridization. After hybridization, filters were washed twice in 0.2× SSC, 0.1% SDS at 65° for 30 min prior to autoradiography for 24–48 hr using intensifying screens (Cronex, E. I. Du Pont de Nemours and Company, Wilmington, Delaware).

RESULTS

The locus occupied by the αA-crystallin DNA sequence is hereafter referred to as *Acry-1* in accordance with recommended guidelines for mouse gene nomenclature (LYON 1981). We also propose that a second gene symbol, *Acry-2*, be reserved for the homologous αB locus.

Restriction analysis of the region containing the αA gene revealed a large amount of variation in genomic DNA from inbred strains of mice (Figure 1). To date, 12 of 14 restriction enzymes tested have detected restriction fragment polymorphisms in DNAs from inbred strains of mice, but not all strains have been examined with each enzyme. Therefore, this report will present data on restriction polymorphisms generated by six endonucleases which have been applied to a comprehensive strain survey. These enzymes include *Bam*HI, *Bcl*I, *Bgl*II, *Hinc*II, *Hind*III and *Msp*I. A more complete analysis of the variation associated with the αA gene among inbred and wild mice is in preparation. In any single endonuclease digest only one to three fragments were produced that hybridized with αA cDNA (Figure 1). The simple restriction patterns found in this survey are in agreement with previous studies that have shown mouse αA to exist as a single copy with no closely related pseudogenes (KING and PIATIGORSKY 1983) and that αA cDNA does not hybridize to αB sequences (KING, SHINOHARA and PIATIGORSKY 1982; DODEMONT *et al.* 1981) under stringent hybridization conditions.

The restriction analysis data, summarized in Table 1, demonstrates that the

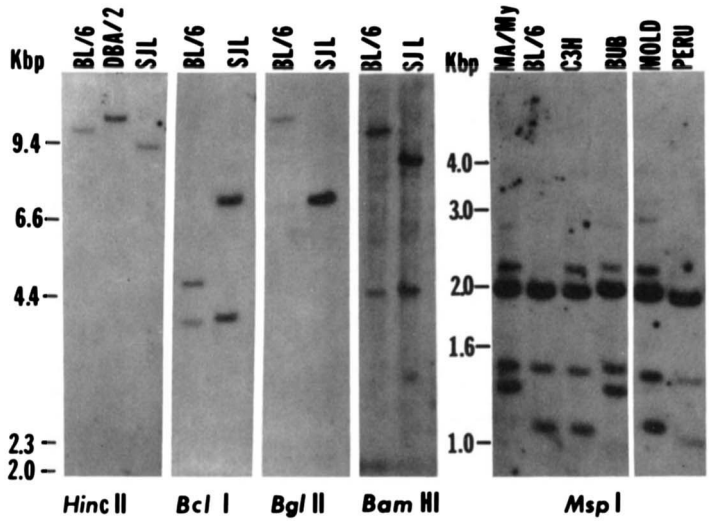

FIGURE 1.—Selected restriction fragment polymorphisms observed among DNAs from inbred strains of mice. The 2.2-kb fragment observed in some samples treated with *Msp*I is due to incomplete digestion of the DNA. Kbp = kilobase pairs.

TABLE 1

Restriction fragments (in kilobases) *observed among the DNAs of six* Acry-1 *alleles of mice*

Restriction endonuclease	No. of fragments	*Acry-1*^{a}	*Acry-1*^{b}	*Acry-1*^{c}	*Acry-1*^{d}	*Acry-1*^{e}	*Acry-1*^{f}
*Hinc*II	1	12.0	10.5	10.5	10.5	10.5	9.0
*Hind*III	2	13.0	13.0	13.0	13.0	8.5	10.5
		6.0	6.0	5.9	5.9	5.9	6.0
*Bam*HI	3	9.5	9.5	9.8	9.8	12.5	8.0
		3.8	3.8	3.6	3.6	3.6	3.8
		1.0	1.0	1.0	1.0	1.0	1.0
*Bgl*II	1 or 2	13.0	13.0	10.3	11.0	11.0	8.0
				2.7	2.7	2.7	
*Bcl*I	1 or 2	5.2	5.4	5.4	5.4	5.4	7.5
		4.4	4.4				4.4
*Msp*I	3	2.0	2.0	2.0	2.0	2.0	2.0
		1.4	1.4	1.4	1.4	1.4	1.4
		1.3	1.1	1.2	1.2	1.2	1.1

mouse αA gene is contained within a polymorphic stretch of DNA that can be resolved into at least six distinct restriction fragment patterns. It is likely that much of the restriction fragment variation detected with the αA cDNA lies outside the boundaries of the regulatory and structural sequences of the αA

TABLE 2

Distribution of Acry-1 *alleles among inbred strains of mice*

Acry-1^a	*Acry-1*^{b}	*Acry-1*^{c}	*Acry-1*^{d}	*Acry-1*^{e}	*Acry-1*^{f}
AU/Ss (*q*)	A/J (*a*)	PL/J (*u*)	MOLD/Rk –	CASO/Rk –	SJL/J (*s*)
BALB/cJ (*d*)	AKR/J (*k*)				
BUB/BnJ (*q*)	CBA/J (*k*)				
DBA/2J (*d*)	CE/J (*k*)				
MA/My (*k*)	C3H/HeJ (*k*)				
NZB/BINJ (*d*)	C57BL/6J (*b*)				
RIII/J (*r*)	C57BL/10Sn (*b*)				
SEC/1ReJ (*d*)	C57BR/cd (*k*)				
SWR/J (*q*)	C57L/J (*b*)				
YBR/Ki (*d*)	C58/J (*k*)				
129/J (*b*)	SM/J (*v*)				

Classification of alleles is based on restriction fragment polymorphisms generated by *Hinc*II, *Hin*dIII, *Bgl*II, *Bcl*I, *Bam*HI and *Msp*I. *H-2* haplotypes are given in parentheses. Note the strong association between *H-2* haplotype (given in parenthesis) and *Acry-1*. In only two strains (MA/My and 129/J) are the *H-2* haplotypes not predictive of *Acry-1* alleles.

gene proper. Nonetheless, we have attributed the various restriction fragment phenotypes to alleles of *Acry-1* so as to conform with standard rules of mouse gene nomenclature, pending clarification regarding the designation of restriction fragment polymorphisms in mice. A complete understanding of the basis for the restriction fragment polymorphisms detected with the αA cDNA probe must await the cloning and analysis of genomic sequences from each of the allelic forms of *Acry-1*. However, two conclusions are apparent from an examination of the data in Table 1. First, base substitution mutations either cannot account for much of the detected polymorphism or most of the variation occurs in flanking sequences where a gain or loss of a restriction site is not detectable with the αA cDNA probe. Of the 23 polymorphisms listed in Table 1, only the *Bgl*II restriction fragment patterns for alleles *Acry-1*^{a,b} *vs.* *Acry-1*^{c,d,e} can be explained by a base substitution event which results in the gain or loss of a restriction site. An alternate and seemingly more plausible explanation for the observed high level of variation is that the region of the genome containing the αA gene has undergone a series of sequence rearrangements, by insertions, deletions or gene conversions to produce alleles which can be defined by multiple, independent restriction endonuclease sites.

Second, the distribution of restriction fragment polymorphisms among the alleles of *Acry-1* suggest an evolutionary relatedness among two groups of alleles. *Acry-1*^{a} and *Acry-1*^{b} may represent a related pair of alleles distinguished only by polymorphisms in *Hinc*II or *Msp*I digests. Similarly, the restriction patterns of *Acry-1*^{c}, *Acry-1*^{d} and *Acry-1*^{e} share several features including the additional *Bgl*II restriction site and the absence of a 4.4-kb *Bcl*I fragment. The *Acry-1*^{f} allele appears to be peculiarly unique with respect to its restriction fragment patterns.

The distribution of *Acry-1* alleles among inbred strains is presented in Table 2. Most of the standard inbred strains fall into one of two allelic groups,

Acry-1^a or *Acry-1*^{b}. Each of the remaining alleles was observed only in single inbred strains; *Acry-1*^{c}(PL/J), *Acry-1*^{d}(MOLD/Rk), *Acry-1*^{e}(CASO/Rk) and *Acry-1*^{f}(SJL/J).

Comparison of the strain distribution pattern of *Acry-1* alleles with other gene markers in the mouse revealed a strong association with haplotypes of the *H-2* complex (Table 2). The *H-2* complex contains genes of the mouse major histocompatibility complex and has been mapped to the midregion of chromosome *17* (KLEIN 1981). All strains of mice of the *H-2*^{d}, *H-2*^{q} or *H-2*^{r} haplotypes carried the *Acry-1*^{a} allele. With two exceptions, MA/My and 129/J, all strains of *H-2*^{b} or *H-2*^{k} haplotypes had the *Acry-1*^{b} allele, as did strains of the *H-2*^{a} (an *H-2*$^{k/d}$ recombinant) or *H-2*^{v} haplotypes. *Acry-1*^{c} and *Acry-1*^{f} were found only in combination with *H-2*^{u} or *H-2*^{s}, respectively. The *Acry-1*^{d} and *Acry-1*^{e} alleles were observed only in strains derived from *M. musculus molossinus* (MOLD/Rk) or *M. musculus castaneus* (CASO/Rk), respectively, and may be restricted to the Asian subspecies of *M. musculus*. CASO/Rk and MOLD/Rk have not been typed for *H-2* specificites, therefore, it is not possible to determine whether these strains also have unique *H-2* haplotypes. *H-2* haplotypes have been determined for two related strains, MOLC-(*H-2*^{k}) and CASA-(*H-2*^{w17}) (ARDEN and KLEIN 1982). The *H-2*^{w17} haplotype is unique and suggests that the *Acry-1*^{e} allele may also be uniquely associated with a single *H-2* haplotype.

The strong association observed for *Acry-1* alleles and *H-2* haplotypes suggests that the *Acry-1* locus is closely linked to *H-2* on chromosome *17*. To more precisely identify the chromosomal location of *Acry-1*, we analyzed *Hin*cII-digested DNA from three sets of recombinant inbred (RI) strains derived from progenitor inbred strains homozygous for either the *Acry-1*^{a} or *Acry-1*^{b} alleles. A total of 55 RI strains were typed for *Acry-1*. A comparison of the strain distribution pattern of *Acry-1* alleles with other gene markers (BEN TAYLOR, personal communication; MANN, ELLIOT and HOHMAN 1984; D'EUSTACHIO *et al.* 1984) revealed a strong concordancy with loci on chromosome *17*, especially *H-2* (Table 3). The recombination distance (expressed as percent recombination in a single meiosis) for the interval *Acry-1—H-2* was calculated as 1.05 ± 0.6. This estimate is based on the predicted relationship between the probability of fixing a recombinant genotype (R) in an RI strain and the probability of recombination in a single meiosis (r), such that $R = 4r/1 + 6r$ (HALDANE and WADDINGTON 1931). An excellent summary of the use of RI strains in linkage analysis has been presented by BAILEY (1981).

The gene order that minimizes the number of recombinations among the RI strains would locate *Acry-1* to the centromeric side of *H-2* but distal from the α-globin pseudogene, *Hba-a4*. The calculated recombination value of 1.05% between *Acry-1* and *H-2* is much less than the 6.6% for *Hba-a4—H-2* (D'EUSTACHIO *et al.* 1984) and suggests that *Acry-1* is located nearer to *H-2* than *Hba-a4*.

To further define the linkage relationships between *H-2* and *Acry-1*, DNA was prepared from congenic and recombinant congenic strains of mice in which the *H-2* donor and recipient strains differed for alleles at *Acry-1*. If *Acry-*

TABLE 3

Strain distribution patterns for Acry-1 *and other loci on chromosome* 17

	RI Strains	Loci					RI Strains	Loci				
		Hba-a4	*Acry-1*		*H-2*	*Upg*		*Hba-a4*	*Acry-1*		*H-2*	*Upg*
1	CXB-D	C	C		C	C	AKXD-27		A			
	CXB-E	B	B		B	B	AKXD-28		A		A	
	CXB-G	C	C	X	B	B	AKR/J		A		A	
	CXB-H	C	C		C	C	DBA/2J		D		D	
	CXB-I	B	B		B	B	3 BXD-1	D	D		D	D
	CKB-J	B	B		B	B	BXD-2	B	B		B	X D
	CKB-K	B	B		B	B	BXD-6	D	D		D	D
	BALB/c	C	C		C	C	BXD-8	B	B		B	B
	C57BL/6	B	B		B	B	BXD-9	B	D		D	X B
							BXD-11	B X	D		D	D
2	AKXD-1		A		A		BXD-12	D	D		D	X B
	AKXD-2		D		D		BXD-13	B	B		B	X D
	AKXD-3		A		A		BXD-14		B		B	B
	AKXD-6		A				BXD-15	B	B		B	B
	AKXD-7		A		A		BXD-16	B X	D		D	D
	AKXD-8		D		D		BXD-18	D	D		D	D
	AKXD-9		D		D		BXD-19	B	B		B	B
	AKXD-10		A		A		BXD-20	B				
	AKXD-11		A				BXD-21	D	D		D	X B
	AKXD-12		D		D		BXD-22	D	D		D	X B
	AKXD-13		D		D		BXD-23	B	B		B	B
	AKXD-14		D		D		BXD-24	D X	B	X	D	D
	AKXD-15		A		A		BXD-25	D	D		D	D
	AKXD-16		D		D		BXD-27	B			D	D
	AKXD-17		A		A		BXD-28	D	D		D	D
	AKXD-18		D		D		BXD-29	B	B		B	B
	AKXD-20		D		D		BXD-30	D	D		D	D
	AKXD-21		A				BXD-31	D	D		D	D
	AKXD-22		D		D		BXD-32	D	D		D	D
	AKXD-23		D		D							
	AKXD-24		D		D		C57BL/6	B	B		B	B
	AKXD-25		A		A		DBA/2J	D	D		D	D
	AKXD-26		A									
							Recombinants/total RIS		4/29	2/50	6/31	

Recombination (r) frequency ± SD for the interval *Acry-1*—*H-2* = 1.06 ± 0.83.

Alleles are designated by strain of origin and recombinations are marked with "X". Recombination frequency was calculated by a rearrangement (BAILEY 1981) of HALDANE and WADDINGTON'S (1931) equation.

1 was closely linked to *H-2*, then the donor *Acry-1* allele might "hitchhike" with the selected *H-2* haplotype and the *H-2* congenic strains would carry *Acry-1* alleles different from the inbred recipient strains. *H-2* congenic and recombinant congenic strains are particularly useful for mapping loci closely linked to *H-2* due to the precise mapping of many loci within and outside the *H-2* region and the identification of markers that delineate the extent of donor DNA in the congenic genomes (KLEIN *et al.* 1982; RINCHIK and AMOS 1983). Analysis of *Hinc*II-digested DNA from congenic strains revealed that all strains that possessed the donor *H-2K* allele also had the donor *Acry-1* allele. Similar analysis of DNA from recombinant congenic strains confirmed the close linkage of *Acry-1* and *H-2K* and permitted the unambiguous location of *Acry-1* to the region between glyoxylase (*Glo-1*) and *H-2K* (Figure 2). Particularly informative are the two recombinant congenic strains B6.TC2 and B6.TC3 which have undergone recombination between *Glo-1* and *H-2K* (RINCHIK and AMOS 1983). In both strains, *Glo-1* is of the BALB/c donor type, and *H-2K* is from the C57BL/6J recipient. Both B6.TC2 and B6.TC3 are homozygous for the C57BL/6 (*Acry-1*^{b}) allele, placing *Acry-1* between *Glo-1* and *H-2K* and suggesting that *Acry-1* may be more closely linked to *H-2K* than to *Glo-1*.

DISCUSSION

αA-crystallin is among the more highly conserved proteins (WILSON, CARLSON and WHITE 1977; KRAMPS, DE MAN and DE JONG 1975; DE JONG 1981), evolving at a rate of 1%/17 million yr. Among rodents, the αA-crystallins of rats and mice are identical (KING, SHINOHARA and PIATIGORSKY 1982) even though these species diverged about 10 million yr ago (KOHNE 1970). The highly conserved nature of the αA-coding sequences is consistent with our hypothesis that most of the restriction fragment length variation observed among strains of mice is located in sequences flanking the αA gene.

The results of the linkage analyses presented here clearly establish that the mouse αA-crystallin gene locus is on chromosome *17*, very closely linked to the *H-2K* region of the major histocompatibility complex. The chromosomal location and the presence of several alleles among inbred strains of mice make *Acry-1* a useful genetic marker for the centromeric end of the *H-2* complex. Furthermore, the αA gene locus is expected to be closely linked to the major histocompatibility complex in other organisms, including humans. Comparative gene-mapping experiments have demonstrated that the *Glo-1* ↔ *MHC* linkage group has been widely conserved among mammals (O'BRIEN, SEUANEZ and WOMACK 1985).

The proximity of *Acry-1* to *H-2K* as evidenced by linkage analysis and the allelic associations observed among inbred strains of different lineages leads to the prediction that strong linkage disequilibrium may exist between these loci in natural populations. If such disequilibrium does, in fact, occur, then *Acry-1* could be included as one of the class IV loci in the *H-2* complex, since it is not readily apparent that αA-crystallin possesses any properties characteristic of proteins encoded by classical *H-2* genes. Most molecules encoded by the *H-2* loci participate in the immune response and either mediate lymphocyte rec-

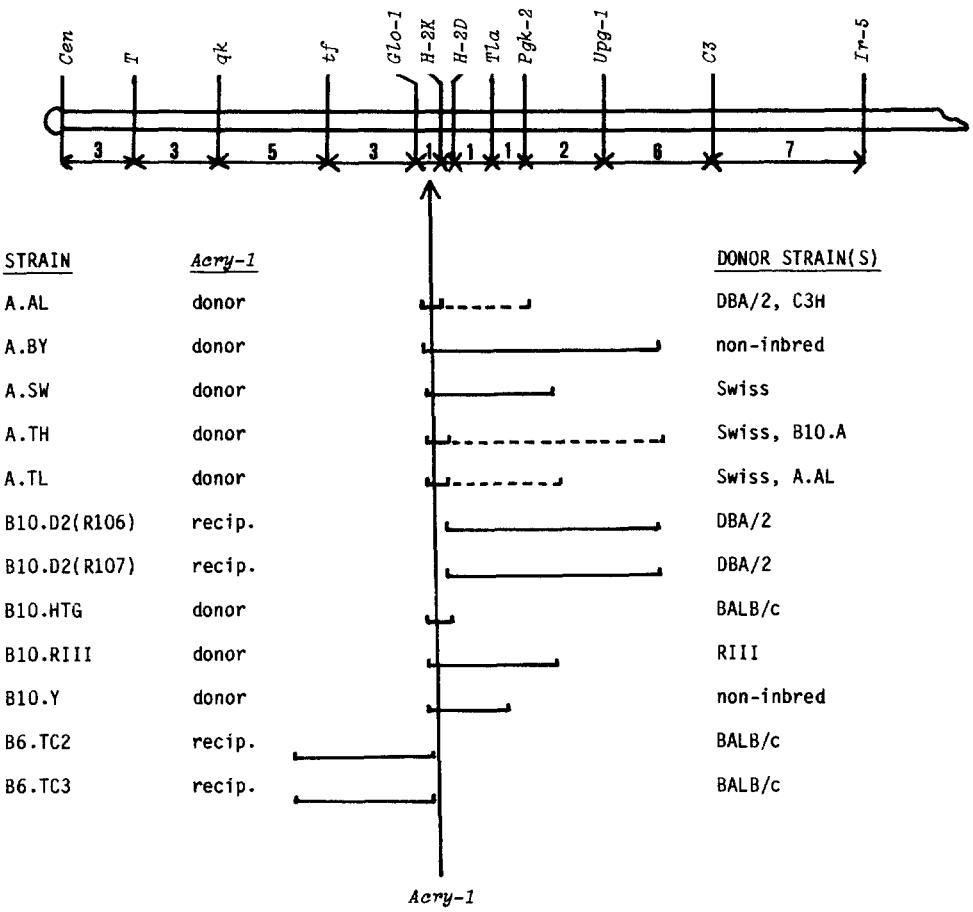

FIGURE 2.—The position of *Acry-1* on mouse chromosome *17* as determined by analysis of restriction fragment polymorphisms among congenic and recombinant congenic strains of mice. The minimal length of donor(s) chromosome present in each strain is represented by solid or solid/dotted lines as described by KLEIN *et al.* (1982). Gene markers and map distances (cM) for loci on chromosome *17* are presented at the top of the figure. By correlating the presence of donor or recipient (recip.) *Acry-1* alleles with the minimal segment of donor DNA in the congenic or recombinant congenic strains, it is possible to locate *Acry-1* to the region between *Glo-1* and *H-2K*.

ognition of foreign antigens (class I and class II loci) or demonstrate enzymatic activity related to the complement system (class III loci). Class IV loci comprise a miscellaneous assemblage of genes that share a single characteristic, that is, close linkage to *H-2*. As a class IV locus, *Acry-1* would represent the first such gene to be mapped to the *H-2K* end of the complex.

It is important to note recent evidence that suggests a more diversified function for genes of the *H-2* complex. ARTZT (1984) and SHIN, BENNETT and ARTZT (1984) demonstrated that *H-2* is included within a larger segment of chromosome *17* which is operationally defined as the *T/t* complex (BENNETT 1975). Chromosomes that bear the *t* complex can be isolated from wild pop-

ulations of mice and shown to contain several genes, intermingled with *H-2* loci, which are embryo-lethal when homozygous. The presence of developmentally important genes in the *t* complex has led to the generalized hypothesis that many genes on chromosome *17* function during embryogenesis by specifying cell surface components that direct embryonic cells through the early stages of differentiation. In this context, it may be relevant to note that α-crystallin expression is initiated early in embryonic development, intimately associated with the induction of the lens placode.

Based on linkage data, it seems unlikely that any of the described mutations affecting lens development (ano- or microphthalmia) or function (cataract) have occurred at the *Acry-1* locus. The only described locus on chromosome *17* that affects ocular function is *rds* (retinal degeneration slow), encoding a defect in the retinal epithelial layer (VAN NIE, IVANYI and DEMANT 1978; DEMANT *et al.* 1979). The lenses of *rds/rds* mice appear normal and the map position of *rds*, five units distal from *H-2*, is different from *Acry-1*, demonstrating that these two loci are physically and functionally distinct.

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