can be calculated from V^i independently of the particular "utility of money" function U_i.
15. A. W. Williams, V. L. Smith, J. O. Ledyard, *Simultaneous Trading in Two Competitive Markets: An Experimental Examination* (Department of Economics, Indiana University, Bloomington, 1985).
16. This result is particularly significant because of the direct evidence from choice surveys showing that most people do not treat the implicit cost of foregone opportunities on a par with explicit incurred costs [see R. Thaler, *J. Econ. Behav. Organ.* **1**, 39 (1980)]. Many other studies (see D. Coursey, J. Hovis, W. Schulze, *Q. J. Econ.*, in press) support the proposition that revealed behavior in the context of markets differs from that found in psychological choice surveys, with the former more consistent with "rational" choice theory than the latter.
17. V. L. Smith, G. L. Suchanek, A. W. Williams, "Bubbles, crashes and endogenous expectations in experimental asset markets" (working paper 86-2, Department of Economics, University of Arizona, Tucson, 1986).
18. V. Coppinger, V. Smith, J. Titus, *Econ. Inquiry* **18**, 1 (1980).
19. W. Vickrey, *J. Finance* **16**, 8 (1961).
20. J. Cox, B. Roberson, V. L. Smith, in *Research in Experimental Economics,* V. L. Smith, Ed. (JAI Press, Greenwich, CT, 1982), vol. 2, p. 1.
21. J. Cox, V. L. Smith, J. Walker, *Theory and Individual Behavior of First Price Auctions* (Department of Economics, University of Arizona, Tucson, May 1986).
22. M. Fiorina and C. Plott, *Am. Polit. Sci. Rev.* **72**, 575 (1978).
23. M. Levine and C. Plott, *Va. Law Rev.* **63**, 561 (1977).
24. C. R. Plott, "A comparative analysis of direct democracy, two candidate and three candidate elections in an experimental environment" (Social Science working paper 457, California Institute of Technology, Pasadena, 1982).
25. V. L. Smith, *J. Polit. Econ.* **85**, 1125 (1977).
26. E. Hoffman and M. Spitzer, *J. Legal Stud.* **14**, 259 (1985).
27. R. Coase, *J. Law Econ.* **3**, 1 (1960).
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Research Articles

Molecular Analysis of the Hotspot of Recombination in the Murine Major Histocompatibility Complex

JOAN A. KOBORI, ERICH STRAUSS, KARYL MINARD, LEROY HOOD

Biological and serological assays have been used to define four subregions for the I region of the major histocompatibility complex (MHC) in the order I-A, I-B, I-J, and I-E. The I-J subregion presumably encodes the I-J polypeptide of the elusive T-cell suppressor factors. Restriction enzyme site polymorphisms and DNA sequence analyses of the I region from four recombinant mouse strains were used to localize the putative I-B and I-J subregions to a 1.0-kilobase (kb) region within the E_β gene. Sequencing this region from E_β clones derived from the two mouse strains: B10.A(3R), I-J^b and B10.A(5R), I-J^k initially used to define the I-J subregion revealed that these regions are identical, hence the distinct I-J^b and I-J^k molecules cannot be encoded by this DNA. In addition, the DNA sequence data also refute the earlier mapping of the I-B subregion. Analysis of the DNA sequences of three parental and four I region recombinants reveals that the recombinant events in three of the recombinant strains occurred within a 1-kb region of DNA, supporting the proposition that a hotspot for recombination exists in the I region. The only striking feature of this hotspot is a tetramer repeat $(AGGC)_n$ that shows 80 percent homology to the minisatellite sequence which may facilitate recombination in human chromosomes.

THE I REGION OF THE MAJOR HISTOCOMPATIBILITY COMplex (MHC) of the mouse encodes two class II or Ia molecules denoted I-A and I-E (*1–4*). The class II genes encode restricting elements for helper T cells and for some cytotoxic T cells. These Ia molecules are cell-surface heterodimers, $A_\alpha A_\beta$ and $E_\alpha E_\beta$, shown to be identical to the Ir gene products that regulate immune responses to specific antigens. Differing inbred strains of mice exhibit different constellations of I region alleles; these constellations are denoted haplotypes and are indicated by superscripts; for example, a mouse of the k haplotype has A^k_α, A^k_β, E^k_α, and E^k_β genes.

Before the advent of molecular cloning of the MHC genes, the I region was considered by immunologists to include, as judged by recombinational analysis, four subregions, I-A, I-B, I-J, and I-E. These analyses were based on serological and biological (immune responsiveness) assays (Fig. 1) (*1, 2, 4*). The I-A and I-E subregions were serologically defined and encode the conventional Ia antigens. The genes for the A_α, A_β, and E_β polypeptides mapped to the I-A subregion, whereas the E_α gene mapped to the I-E subregion. The I-B subregion was defined by the regulation of immune responses to mouse immunoglobulin G_{2a} (IgG_{2a}) and lactate dehydrogenase B (LDH_B) antigens (*5, 6*). The I-J subregion was defined serologically by reagents directed against the I-J polypeptide, which is believed to be expressed as a component of secreted and membrane-bound suppressor factors of suppressor T cells (*7, 8*). Understanding the expression and function of the I-J polypeptide would provide major insights into how immune responses can be suppressed. Estimates of molecular size of the I-J polypeptide range from 20 to 25 kilodaltons (*9–11*). However, repeated attempts to purify enough I-J material for protein sequence analysis have failed.

Restriction enzyme site polymorphisms detected by genomic DNA blotting techniques have been used to correlate the genetic map with the molecular map of the major histocompatibility complex. By mapping the right-most boundary of the I-A subregion

J. A. Kobori is a senior research fellow, E. Strauss is an undergraduate research assistant, K. Minard is a research technician, and L. Hood is Bowles Professor in the Division of Biology, California Institute of Technology, Pasadena, CA 91125.

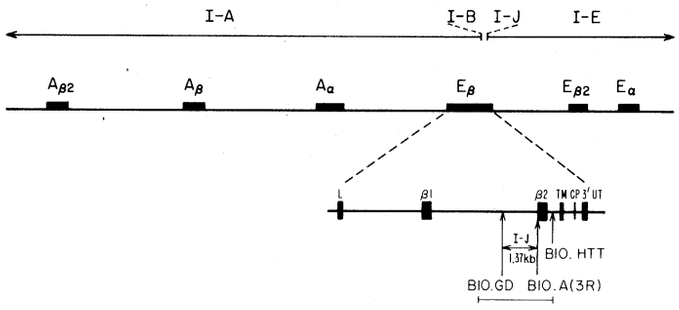


Fig. 1. Schematic representation of the I region. The I region is represented with the $A_{\beta 2}$, A_β, A_α, E_β, $E_{\beta 2}$, and E_α genes. The E_β gene region is expanded to show the exon-intron organization based on (*31*). The I-J subregion as mapped by restriction site polymorphisms is indicated. The arrows indicate the particular mouse recombinant used to define either boundary. The strain B10.HTT indicates the localization of the right-hand boundary of I-J as previously described (*13*). The horizontal bar represents the region subjected to DNA sequence analysis.

and the left-most boundary of the I-E subregion, Steinmetz *et al.* (*12*) suggested that the I-B and I-J subregions must be contained within a region of DNA no larger than 3.4 kilobases (kb). More recent restriction map data of Kobori *et al.* from isolated clones of four parental and six recombinant strains support the notion that the I-J subregion must be confined to a region of just 2 kb of DNA within the 3′ end of the class II gene E_β (*13*). In addition, 16 I region recombinants analyzed mapped to within a region of 9.8 kb or less, indicating the presence of a recombinational hotspot (*12–16*).

We now report our effort, using restriction enzyme site polymorphisms and DNA sequence analysis, to further define the putative I-J (and I-B) subregion to a 1.0-kb region that maps entirely within the second intron of the E_β gene. We have used specific oligonucleotides as primers to facilitate the rapid DNA sequence analyses of 3.1

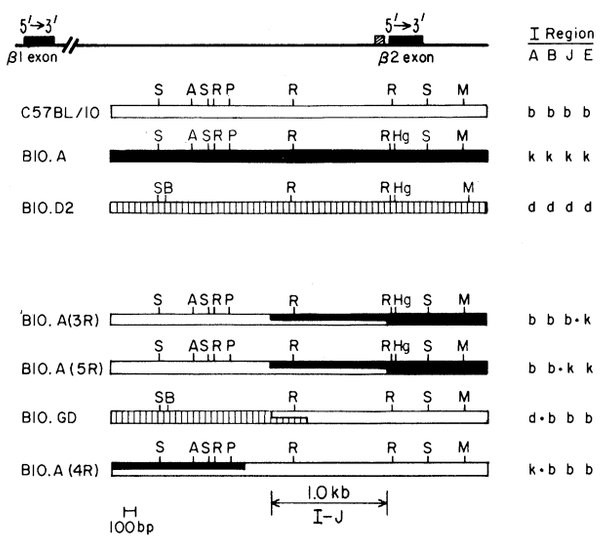


Fig. 2. Maps of partial E_β clones defining the I-J subregion. Only enzymes indicating polymorphism within the 3.1-kb region cloned into pUC8 are shown. Nonpolymorphic Bam HI sites are not shown. S, Sma I; B, Bam HI; A, Aha III; R, Rsa I; P, Apa I; Hg, Hga I; M, Mst II. The β2 exon of the E_β gene is indicated by a black box. This diagram also indicates the parental origin of I-J subregion DNA in recombinant strains as judged from DNA sequence data. The origin of DNA in the four recombinants is represented by the pattern indicated in the three parental strains. The hatched box located 5′ to the β2 exon of the E_β gene represents a repeat sequence found in all strains analyzed.

kb each of four I region recombinant and three parental chromosomes in and around a hotspot of recombination.

Mapping the right boundary of I-A and the left boundary of I-E by restriction site polymorphisms. For higher resolution mapping of the I-J subregion, we subcloned a 3.1-kb Bgl II restriction fragment from seven bacteriophage lambda clones derived from three parental and four I region recombinant strains into the Bam HI site of the plasmid, pUC8. (*13*) (Fig. 2). The polymorphism analyses allow the right-hand I-A and left-hand I-E borders to be located more accurately. The colinearity of the restriction map from one mouse strain to another permits exact alignment and comparison of sites in one strain to another strain. The right-hand border of the I-A subregion is operationally defined in the plasmid clone derived from B10.GD DNA (a d/b recombinant) because of the lack of an Apa I site indicating d haplotype DNA encodes the I-A subregion (Fig. 2). Hence, the putative I-J subregion must map to the right of this site. The left-hand border of the I-E subregion is defined in the plasmid clone derived from B10.A(3R) DNA (a b/k recombinant) by the presence of the Hga I site indicating that k haplotype DNA encodes the I-E subregion. The location of the right-hand boundary of the I-A subregion is supported by neighboring polymorphisms with the enzymes Sma I, Aha III, Rsa I, Apa I, and Fok I. The location of the left-hand boundary of the I-E subregion is supported by neighboring polymorphisms with Mst II, Hga I, and Fnu 4HI. These data narrow the amount of DNA mapping to the right of the I-A and to the left of the I-E subregions as not more than 1.37 kb. This 1.37 kb of DNA encompasses part of the intron between the β1 and β2 exons and the first 11 amino acids of the β2 exon of the E_β gene (see DNA sequence) (Fig. 1). We denote this 1.37-kb region the putative I-J subregion.

The B10.GD recombinant clone between d and b haplotypes is the only one in which both sides of the recombination event can be identified by restriction site polymorphism analysis. The DNA on the left-hand side, up to the location of the Apa I site present in the k or b haplotypes, is definitely of d origin. The DNA on the right-hand side, up to the location of the Hga I site present in the d or k haplotypes but absent in the b haplotype, is definitely of b origin. Hence, our data suggest for the B10.GD recombinant that the leader and β1 exons are of d origin and the β2 exon, transmembrane and cytoplasmic domains are of b origin. The B10.GD mouse is known to express a hybrid E_β polypeptide consistent with this result (*17, 18*).

DNA sequence analysis of the putative I-J subregion. We decided to sequence 3.1 kb of DNA in and around the putative I-J (I-B) subregion. These data would allow us to determine whether there was an intact I-J (I-B) gene present in the second E_β intron, or whether alternative RNA splicing patterns could create an I-J gene. Moreover, the sequence of the putative I-J subregion in the B10.A(3R) and B10.A(5R) strains initially used to define the $I\text{-}J^b$ and $I\text{-}J^k$ alleles should allow us to determine whether sequence differences are localized to this region.

We aligned 3.1 kb of sequence from three parental and four I region recombinant mouse strains. Comparing the nucleotide sequence of the parental clones to the recombinant clones, the left-hand boundary of the I-E subregion and the right-hand boundary of the I-A subregion can be localized (Figs. 2 and 3). The sequence analysis allows us to narrow the putative I-J subregion to 1.0 kb of DNA entirely contained within the intron separating the β1 and β2 exons of the E_β gene. All nucleotides present in the recombinants can be directly aligned with the parental DNA sequences. No base changes or deletions of unknown origin are present. Both B10.A(3R) and B10.A(5R) are identical for the 3.1-kb region sequenced. This result rules out the possibility that their I-J phenotypic difference maps here (see below).

Our DNA sequence analysis provides an explanation for the serological recombinational analyses mapping the class II gene E_β into the I-A subregion. The first major protein-coding domain, the β1 exon, maps at the DNA level into the I-A subregion. This polymorphic exon expresses the determinants detected by the serological reagents used for the recombinational mapping studies (*19*). The second major protein-coding domain, the β2 exon, is highly conserved and maps into the I-E subregion. All I region recombinant mouse strains examined so far should have hybrid E_β molecules as has been reported for the B10.GD mouse strain (*17, 18*).

The above observations allow us to eliminate several models for the I-J gene. (i) A stretch of 1 kb of DNA can encode about 333 amino acid residues. However, there are no significant open reading frames on either DNA strand for any of the six reading frames examined. Hence the putative I-J subregion cannot completely encode a 25,000-dalton polypeptide. (ii) Multiple short open reading frames are found in the putative I-J subregion with suitable donor and acceptor signals for RNA splicing. An analysis of the introns of the A_β and E_α genes reveals open reading frames of similar length. One open reading frame of 387 nucleotides extends through the β2 exon. However, Kronenberg *et al.* (*20*) were unable to detect in suppressor T cells any messenger RNA species homologous to the putative I-J subregion or the surrounding area when they used DNA probes spanning 250 kb throughout the I region. There is the possibility, however, that the I-J polypeptide is encoded by a very low abundance messenger RNA. Second, the I-J polymorphism cannot be encoded in the putative I-J subregion. The putative I-J subregion is identical in B10.A(3R) and B10.A(5R) mice, which encode the $I\text{-}J^b$ and $I\text{-}J^k$ polypeptides, respectively. These data formally eliminate the possibility that allelic forms of the I-J polypeptide are encoded in the putative I-J subregion. RNA splicing differences appear unlikely as an explanation for I-J polymorphisms because of the identity of the putative I-J subregion in B10.A(3R) and B10.A(5R) mice (the β2 exons are also identical) and the data of Kronenberg *et al.* (*20*) discussed above.

Several other models to explain the I-J paradox have been proposed. First, data of Hayes *et al.* (*21*) suggest that two genes are required for $I\text{-}J^k$ expression—one mapping in the MHC and one mapping on chromosome 4, denoted J_t. Two recent reports do not strongly support this study (*22, 23*). However, the discrepancies may be attributed to the complexity of different assays used in the different laboratories to detect I-J expression. Second, a MHC gene may regulate the expression of the I-J molecule. The I-J molecule may be part of a T-cell receptor that must recognize antigen in the context of an appropriate MHC molecule such as E_β. Suppressor T cells may be selected during ontogeny for expressing the I-J molecule that results in their ability to recognize E_β on appropriate immune cells (*24, 25*). Thus, the genes for the I-J suppressor molecules might appear to map to the MHC (or E_β) even though their structural genes map elsewhere. Ikezawa reported I-J positive suppressor factors that react with conventional Ia monoclonal antibodies, suggesting that a modified E_β molecule shares I-J determinants (*26*). One major problem with these molecules is the existence of mouse strains that cannot express I-E molecules but do express I-J molecules (*4*). Reports by Uracz *et al.* (*27*) and Sumida *et al.* (*28*) suggest that expression of I-J determinants is adaptively acquired and determined by the MHC environment. The available data do not support the possibility that the I-J gene may be derived partially from E_β coding sequences, novel gene structure within the E_β gene, or an alternative splicing mechanism of the E_β gene.

The mice used to obtain DNA for all studies presented in this article were provided by laboratories doing immunological studies of B10.A(3R) and B10.A(5R) strains. The exact mice used were not subjected to typing immediately before isolation of the DNA. Because of the lack of differences identified by restriction site polymorphisms or sequence analysis in the 3.1-kb region examined, finer restriction mapping would not distinguish the B10.A(3R) clone from the B10.A(5R) clone. We handled these samples as carefully as possible and feel it is highly unlikely that the samples were mixed up.

The sequence analysis included a total of 2.1 kb more than the 1.0-kb region designated as the putative I-J subregion and the recombination region of B10.A(3R) and B10.A(5R). We cannot rule out the possibility that a small gene conversion event or other genetic alteration outside this region not detectable by the extensive restriction site analysis (*12, 13*) could account for the observed I-J phenotypic differences. The suggestion has been made that perhaps differences in the β1 exons of the E_β genes could be responsible for I-J. However, the β1 exon does not map into the region that putatively controls the I-J phenotype. Moreover, the probability that a second independent event, a gene conversion, occurred in the I region simultaneously with a recombination event at a nearby location does not seem very likely.

Our study definitively rules out the possibility that an I-J gene product is encoded in the 3.1-kb region of sequenced DNA. This result therefore does not identify the location of the I-J gene nor does it explain why the I-J gene appears to map to this region. The biology of the I-J gene product is controversial, and we believe that the resolution of the I-J paradox requires biochemical studies of the I-J molecule and the eventual molecular cloning of its structural gene.

The I-B subregion as initially mapped is incorrect. The I-B subregion was originally defined with two recombinants, $H\text{-}2^{i5}$ [B10.A(5R)] and $H\text{-}2^{h4}$ [B10.A(4R)] derived from an $H\text{-}2^a/H\text{-}2^b$ heterozygote. The $H\text{-}2^a$ haplotype (A^k B^{LR} J^k E^k) is a low responder (LR) to both IgG myeloma protein and LDH_B while $H\text{-}2^b$ (A^b B^{HR} J^b E^b) and the two recombinants are high responders (HR) (*5, 6*). Baxevanis *et al.* have demonstrated that the requirement of an I-B subregion to explain immune response differences to LDH_B can be explained by an interaction between the I-A and I-E subregion gene products (*29*). They demonstrated the conversion of a nonresponder strain to a responder by blocking I-E molecules with antibody. They proposed the removal of the I-B subregion from the H-2 map.

Our DNA sequence analysis indicates that both k (low responder) and b (high responder) haplotypes are identical for the 1.0 kb of DNA that includes the recombination region of B10.GD, $I\text{-}A^d$ $I\text{-}B^b$, and B10.A(5R), $I\text{-}B^b$ $I\text{-}J^k$. This region must encompass the I-B subregion. We have ruled out the possibility that a distinct immune response gene can map in this region and therefore we conclude that the initial mapping of the I-B subregion is incorrect. These data do not provide an explanation for the biological observations related to the I-B subregion. A recent report discusses and reevaluates the immunological and molecular data concerning the I-B subregion (*30*).

Prior to the advent of molecular cloning, the best genetic description of the I region involved recombinational analysis by the classical assays of immunogenetics—serology and immune responsiveness. This generated various maps that evolved to the current picture of four subregions. At present, the use of relatively crude recombinational analysis to describe the I region is no longer warranted. Its use led to a concept of regions which is not tenable, as was shown later by artifacts in the biological or serological assays. It also led to the idea of the E_β gene being split between the I-A and I-E subregion. Therefore, we suggest that, in the future, the I region should be defined in terms of class II genes and their locations.

A hotspot for recombination in the I region. In order to

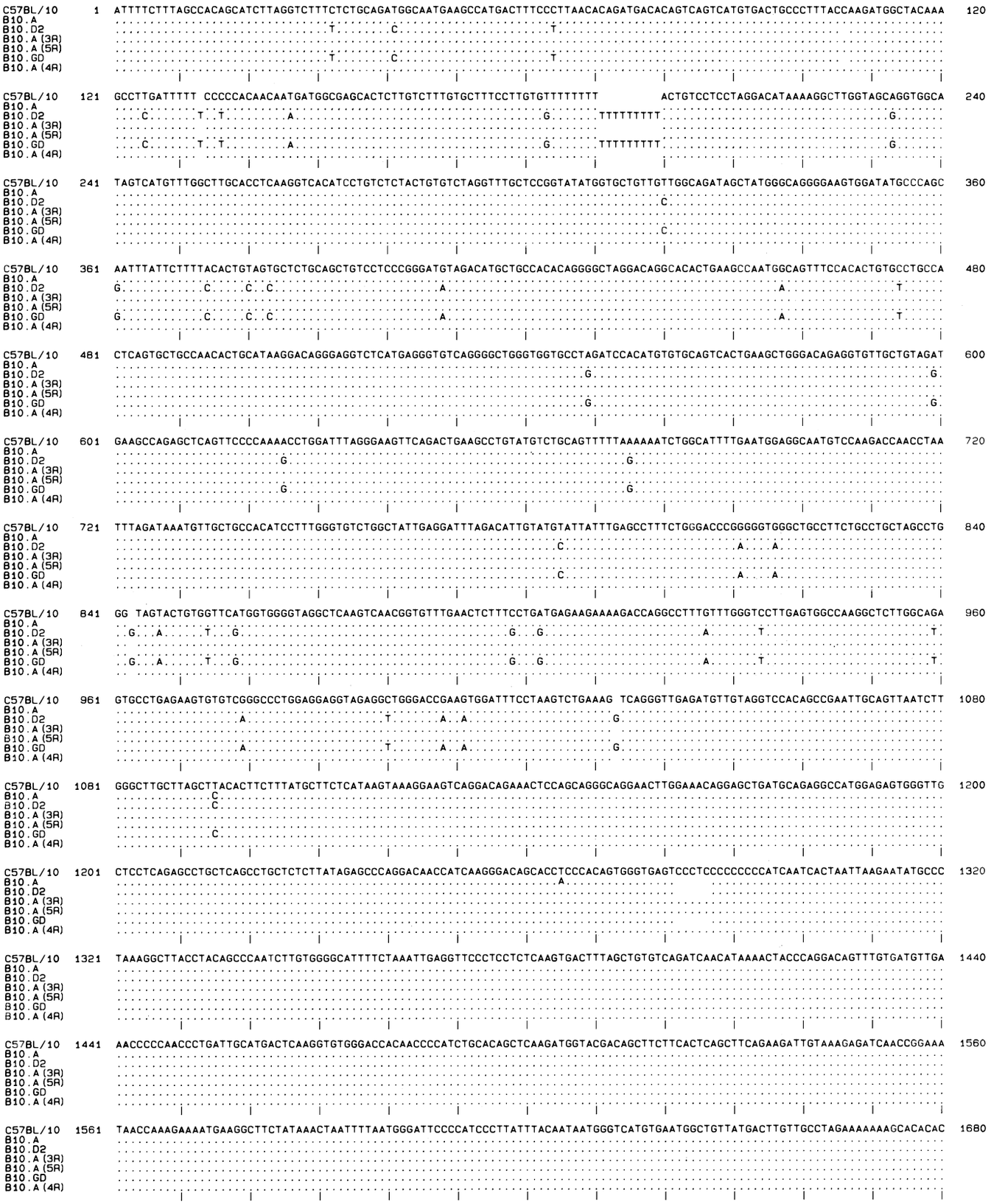
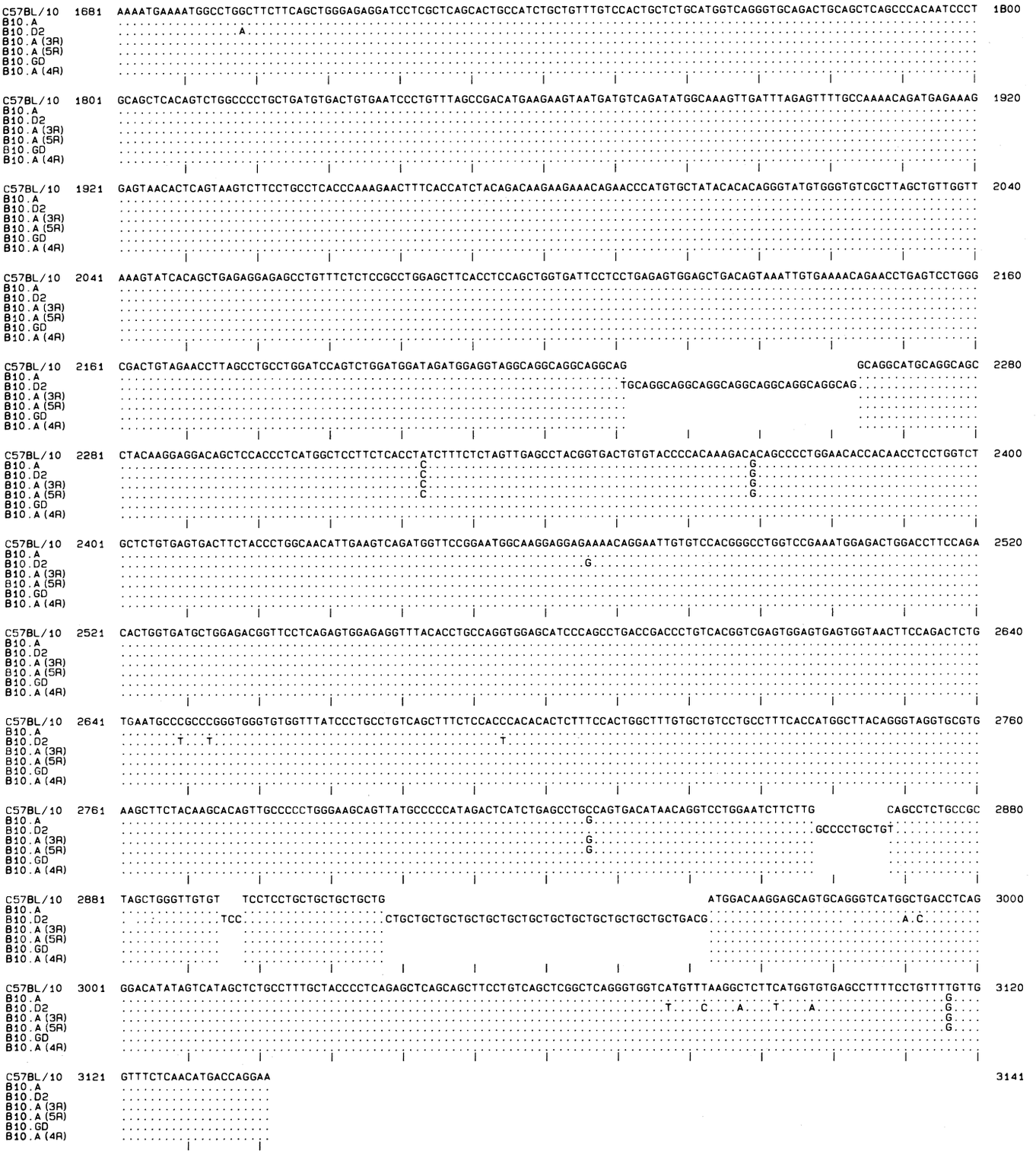


Fig. 3. DNA sequence of the I-J subregion and flanking area. The three parental sequences derived from C57BL/10, B10.A, and B10.D2 are aligned and shown above the four recombinant sequences derived from B10.A(3R), B10.A(5R), B10.GD, and B10.A(4R). A dot indicates identity with the nucleotide presented above it. Deletion clones for sequence analysis were constructed with the use of Bal 31 nuclease (*45*). Specific oligonucleotide primers based on the sequence obtained from B10.A(3R) deletion clones were used to obtain the sequence of the other clones (*46*). All sequences were obtained from M13mp10 or M13mp11 clones (*47*). The 16- or 20-nucleotide primers were synthesized in the Caltech Biology Microchemical



Facility on an Applied Biosystems 380A DNA synthesizer. All sequences presented were obtained on both strands from overlapping clones or overlapping sequencing gel runs (or both). The following nucleotide positions correspond to polymorphic restriction sites: 549 (Bam HI), 675 (Aha III), 811 (Sma I), 847 (Rsa I), 979 (Apa I), 2369 (Hga I), and 2653 (Sma I). The left-hand boundary of the I-J subregion is designated nucleotide 1287 and the right-hand boundary is designated nucleotide 2322. The cross-over occurred between nucleotide 1287 and 2322 (inclusive) in the strains B10.A(3R) and B10.A(5R), between nucleotide 1287 and 1697 in the strain B10.GD, and to the left of nucleotide 1094 in the strain B10.A(4R). The β2 exon of the E_β gene as delineated by Saito *et al.* (*31*) is from nucleotides 2335 to 2616. The locations of nonoverlapping sequences exhibiting at least 80 percent homology to the human minisatellite repeat sequence are as follows (listing the first base): 335, 505, 1149, 2214, 2226, and 2454. The strain B10.D2 contains two additional homologous sequences at nucleotide locations 2238 and 2250 in the I-J subregion repeat region showing 80 percent homology and a change to 70 percent homology at positions 2214 and 2226.

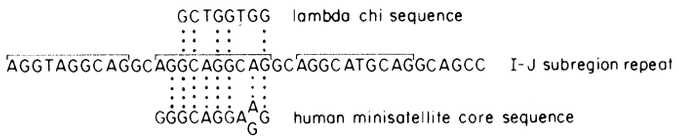


Fig. 4. Alignment of the I-J subregion repeat sequence to the lambda chi sequence and the human minisatellite core repeat sequence. The lambda chi sequence (*32, 33*) is aligned with the I-J subregion repeat found in the b or k haplotypes. The human minisatellite core sequence (*34*) gives a maximum homology of eight out of ten bases to the I-J subregion repeat. There are three nonoverlapping core-like sequences, or eight overlapping core-like sequences.

determine locations and special DNA sequence characteristics of the recombinational hotspot, if any, we analyzed the recombination regions of four I region recombinant chromosomes and compared their sequences with those from the appropriate parental chromosomes. Three of the four recombinants analyzed recombined within a 1-kb region of DNA within the intron between the β1 and β2 exon of the E_β gene (Fig. 2). Both B10.A(3R) and B10.A(5R) recombined within a 1.0-kb region and B10.GD recombined within a 410-bp region. The precise location of the recombination event in the strain B10.A(4R) cannot be identified because of a lack of polymorphism between the b and k haplotypes. The detailed analysis of I region recombinants supports the observation that recombination within the I region is highly localized in the E_β gene. These observations raise a question as to whether any DNA sequences correlate with this hotspot of recombination.

DNA sequence analysis revealed the presence of an imperfect four-base repeat sequence $(AGGC)_n$ found in all seven mouse strains examined. It is located approximately 50 bp 5′ to the β2 exon of the E_β gene (Fig. 2). This sequence has also been observed by Saito *et al.* (*31*) in the E^d_β gene sequence. However, lacking our extensive sequence analysis in this region, they could not assign any possible significance to this repeat. This repeat sequence is the only notable feature of the 3.1 kb of putative I-J subregion sequence. This sequence is repeated 18 times in the d haplotype (B10.D2) and 10 times in the b and k haplotypes (C57BL/10 and B10.A). It shows that only five bases out of eight are homologous to the lambda phage chi sequence, which promotes homologous recombination in *Escherichia coli* (Fig. 4) (*32, 33*). But the recombination hotspot repeat sequence shows three nonoverlapping sequences that are 70 or 80 percent homologous to the human minisatellite core sequence reported by Jeffreys *et al.* (Fig. 4) (*34*). This core sequence has been postulated to provide a recombination signal promoting formation of minisatellites. There is a relative clustering of minisatellite core-like sequences in the DNA repeat region of the recombinational hotspot of the I-J subregion in the E_β gene as compared to the corresponding region of the A_β gene. Hence, it is attractive to speculate that this repeat sequence in mouse may facilitate recombination. This hypothesis requires experimental test. However, there is no evidence to suggest that recombination in other parts of the I region is specifically inhibited but rather may be enhanced in the E_β gene region.

If the I-J subregion repeat serves to promote recombination in the I region and is the reason why the recombination event in I region recombinants analyzed map to the E_β gene, then this sequence might not be located elsewhere in the I region. A 40-nucleotide fragment of the repeat sequence was used as a hybridization probe against restriction-digested cosmid DNA clones derived from BALB/c (*12*). DNA blots of individual cosmid clones spanning approximately 170 kb of the I region were probed with this repeat sequence. These cosmids include the genes A_β, A_α, E_β, $E_{\beta 2}$, and E_α. Only the three cosmids containing E_β sequences hybridized to the oligonucleotide. These data demonstrate that this repeat sequence does not occur elsewhere in the I region and is, accordingly, consistent with its postulated role in facilitation of recombination. Genomic blot analysis with the repeat region as probe indicates a few major hybridizing bands, including one that corresponds to the recombinational hotspot, and a high background of weak hybridization.

Recombinational hotspots—mechanisms and examples. The recombinational hotspot in the E_β gene may arise from DNA sequence characteristics in this region, such as the repeat sequence, or from special features of chromatin structure which may promote chromosome pairing and recombination. The existence of the hotspot indicates a possibility that there is a selective pressure for hybrid E_β genes. Perhaps recombination in other locations would produce αβ heterodimer combinations that are less effective in functioning as MHC restricting elements. The observation of certain mixed-haplotype αβ dimers ($A^k_\alpha A^d_\beta$) that are unable to associate and the observation that some mixed-isotype dimers ($E^d_\alpha A^d_\beta$) can form and function as restricting elements suggest that structural constraints dictate the permissive association of class II α and β chains (*35, 36*). The location of the recombinant hotspot would then prevent the generation of nonpermissive A_α and A_β alleles (*37, 38*). In addition, the conserved E_α chain can associate with both E_β and A_β, and this too is maintained in the I region recombinants analyzed. Two recombinants have recently been mapped to two different locations 5′ to the A_β gene and thus do not affect the α and β associations (*39*). A more general effect of recombination in the I region is to provide a mechanism for generating diversity by joining the proximal end of the MHC of one haplotype to the distal end of the MHC of another haplotype.

Recent analyses of recombinants between wild mice (*Mus musculus molossinus* and *M. m. castaneus*) and laboratory mice permit the identification of recombinational hotspots between the K and I region loci of the MHC (*39, 40*). Another study suggests that recombinants between the p and k haplotypes map to an additional recombinational hotspot in the E_α gene region (*41*). This result is somewhat surprising because 16 out of 16 I region recombinants map to the E_β gene region. These latter recombinants are derived from the b, d, k, and s haplotypes. The current available data suggest that the location of recombinational hotspots may be dependent on the specific parental DNA.

Studies of the human β-globin locus and human insulin locus also indicate hotspots of recombination (*42–44*). Each of these hotspots also contains tandem repeat sequences, unrelated to those we have described above, which may promote unequal crossing-over. Our data demonstrate that the recombination frequency per kilobase of DNA is not random, which, indeed, may be due to the presence of hotspots of recombination scattered throughout the genome.

REFERENCES AND NOTES

1. J. Klein, *Biology of the Mouse Histocompatibility-2 Complex* (Springer-Verlag, New York, 1975).
2. _____, F. Figueroa, Z. A. Nagy, *Annu. Rev. Immunol.* **1**, 119 (1983).
3. L. Hood, M. Steinmetz, B. Malissen, *ibid.*, p. 529.
4. L. Mengle-Gaw and H. O. McDevitt, *ibid.* **3**, 367 (1985).
5. R. Lieberman, W. E. Paul, W. Humphrey, J. H. Stimpfling, *J. Exp. Med.* **136**, 1231 (1972).
6. I. Melchers, K. Rajewsky, D. C. Shreffler, *Eur. J. Immunol.* **3**, 754 (1973).
7. D. B. Murphy, L. A. Herzenberg, K. Okumura, L. A. Herzenberg, H. O. McDevitt, *J. Exp. Med.* **144**, 699 (1976).
8. D. B. Murphy, M. C. Horowitz, R. J. Homer, P. M. Flood, *Immunol. Rev.* **83**, 79 (1985).
9. M. Taniguchi *et al.*, *Nature (London)* **298**, 172 (1982).
10. K. J. Wieder, B. A. Araneo, J. A. Kapp, D. R. Webb, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3599 (1982).
11. Y. Kumagai, K. Okumura, T. Tada, *Mol. Immunol.* **21**, 545 (1984).
12. M. Steinmetz *et al.*, *Nature (London)* **300**, 35 (1982).
13. J. A. Kobori, A. Winoto, J. McNicholas, L. Hood, *J. Mol. Cell. Immunol.* **1**, 125 (1984).

14. A. B. Begovich and P. P. Jones, *Immunogenetics* **22**, 523 (1985).
15. B. K. Saha and S. E. Cullen, *J. Immunol.* **136**, 1112 (1986).
16. H. C. Passmore, J. A. Kobori, E. J. Zimmerer, D. G. Spinella, L. Hood, in preparation.
17. P. P. Jones, *J. Exp. Med.* **152**, 1453 (1980).
18. M. L. Plunkett, J. E. Coligan, C. S. David, J. H. Freed, *ibid.* **155**, 937 (1982).
19. V. Folsom, D. Gay, S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1678 (1985).
20. M. Kronenberg *et al.*, *ibid.* **80**, 5704 (1983).
21. C. E. Hayes *et al.*, *Science* **223**, 559 (1984).
22. C. Waltenbaugh, L. Sun, H.-Y. Lei, *Eur. J. Immunol.* **15**, 922 (1986).
23. P. M. Flood and D. B. Murphy, *J. Mol. Cell. Immunol.* **2**, 95 (1985).
24. T. Tada *et al.*, *Ann. N.Y. Acad. Sci.* **418**, 189 (1983).
25. J. W. Schrader, *Scand. J. Immunol.* **10**, 387 (1979).
26. Z. Ikezawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6637 (1983).
27. W. Uracz, Y. Asano, R. Abe, T. Tada, *Nature (London)* **316**, 741 (1985).
28. T. Sumida *et al.*, *ibid.*, p. 738.
29. C. N. Baxevanis, Z. A. Nagy, J. Klein, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3809 (1981).
30. E. Simpson *et al.*, *Immunogenetics* **23**, 302 (1986).
31. H. Saito, R. A. Maki, L. K. Clayton, S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5520 (1983).
32. R. E. Malone, D. K. Chattoraj, D. H. Faulds, M. M. Stahl, F. W. Stahl, *J. Mol. Biol.* **121**, 473 (1978).
33. G. R. Smith, S. M. Kunes, D. W. Schultz, A. Taylor, K. L. Triman, *Cell* **24**, 429 (1981).
34. A. J. Jeffreys, V. Wilson, S. L. Thein, *Nature (London)* **314**, 67 (1985).
35. R. N. Germain and H. Quill, *ibid.* **320**, 72 (1986).
36. B. Malissen, N. Shastri, M. Pierres, L. Hood, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3958 (1986).
37. R. N. Germain, D. M. Bentley, H. Quill, *Cell* **43**, 233 (1985).
38. G. C. Schlauder, M. P. Bell, B. N. Beck, A. Nilson, D. J. McKean, *J. Immunol.* **135**, 1945 (1985).
39. M. Steinmetz, D. Stephan, K. F. Lindahl, *Cell* **44**, 895 (1986).
40. T. Shiroishi, T. Sagai, K. Moriwaki, *Nature (London)* **300**, 370 (1982).
41. W. P. Lafuse, N. Berg, S. Savarirayan, C. S. David, *J. Exp. Med.* **163**, 1518 (1986).
42. D. Treco, B. Thomas, N. Arnheim, *Mol. Cell. Biol.* **5**, 2029 (1985).
43. G. I. Bell, M. J. Selby, W. J. Rutter, *Nature (London)* **295**, 31 (1982).
44. R. V. Lebo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4808 (1983).
45. J. A. Kobori and A. Kornberg, *J. Biol. Chem.* **257**, 13757 (1982).
46. E. Strauss, J. Kobori, G. Siu, L. Hood, *Anal. Biochem.* **154**, 353 (1986).
47. J. Messing, *Methods Enzymol.* **101**, 20 (1983).
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In Vivo Half-Life of a Protein Is a Function of Its Amino-Terminal Residue

ANDREAS BACHMAIR, DANIEL FINLEY, ALEXANDER VARSHAVSKY

When a chimeric gene encoding a ubiquitin-β-galactosidase fusion protein is expressed in the yeast *Saccharomyces cerevisiae*, ubiquitin is cleaved off the nascent fusion protein, yielding a deubiquitinated β-galactosidase (βgal). With one exception, this cleavage takes place regardless of the nature of the amino acid residue of βgal at the ubiquitin-βgal junction, thereby making it possible to expose different residues at the amino-termini of the otherwise identical βgal proteins. The βgal proteins thus designed have strikingly different half-lives in vivo, from more than 20 hours to less than 3 minutes, depending on the nature of the amino acid at the amino-terminus of βgal. The set of individual amino acids can thus be ordered with respect to the half-lives that they confer on βgal when present at its amino-terminus (the "N-end rule"). The currently known amino-terminal residues in long-lived, noncompartmentalized intracellular proteins from both prokaryotes and eukaryotes belong exclusively to the stabilizing class as predicted by the N-end rule. The function of the previously described posttranslational addition of single amino acids to protein amino-termini may also be accounted for by the N-end rule. Thus the recognition of an amino-terminal residue in a protein may mediate both the metabolic stability of the protein and the potential for regulation of its stability.

IN BOTH BACTERIAL AND EUKARYOTIC CELLS, RELATIVELY long-lived proteins, whose half-lives are close to or exceed the cell generation time, coexist with proteins whose half-lives can be less than 1 percent of the cell generation time. Rates of intracellular protein degradation are a function of the cell's physiological state, and appear to be controlled differentially for individual proteins (*1*). In particular, damaged and otherwise abnormal proteins are metabolically unstable in vivo (*1*). Although the specific functions of selective protein degradation are in most cases still unknown, it is clear that many regulatory proteins are extremely short-lived in vivo (*1, 2*). Metabolic instability of such proteins allows for rapid adjustment of their intracellular concentrations through regulated changes in rates of their synthesis or degradation. The few instances in which the metabolic instability of an intracellular protein has been shown to be essential for its function include the cII protein of bacteriophage λ and the HO endonuclease of the yeast *Saccharomyces cerevisiae* (*3*).

Most of the selective turnover of intracellular proteins under normal metabolic conditions is adenosine triphosphate (ATP)–dependent and (in eukaryotes) nonlysosomal (*1, 4*). Recent biochemical and genetic evidence indicates that, in eukaryotes, covalent conjugation of ubiquitin to short-lived intracellular proteins is essential for their selective degradation (*1, 5*).

By analogy with the signal sequences that confer on a protein the

The authors are in the Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.