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Amplification of major histocompatibility complex class II gene diversity by intraexonic recombination

(ancestral polymorphism/evolution/selection)

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ABSTRACT **The roles of mutational and recombinational processes in the diversification of the exon encoding the antigen binding site in the murine major histocompatibility complex class II gene *Ab* were assessed by phylogenetic analysis of allelic nucleotide sequences. A total of 46 alleles of *Ab* exon 2 from 12 *Mus* species or subspecies and 2 *Rattus* species were sequenced after amplification by the polymerase chain reaction. Reliable allelic genealogies could not be determined by phylogenetic analyses, due to extensive homoplasy in the data set. This homoplasy results from the shuffling of polymorphisms between alleles by recombinational processes, indicating that polymorphisms in the antigen binding site encoded by *Ab* are generated by a combination of two processes. First, the accumulation of point mutations has produced highly divergent polymorphic sequence motifs in five regions of *Ab* exon 2, each encoding a portion of the binding site. Some of these motifs have persisted as polymorphisms in rodents since before the divergence of mouse and rat (>10 million years ago). The second process mediating *Ab* diversification involves the shuffling of these polymorphic sequence motifs into numerous allelic combinations by repeated intraexonic recombination. Site-specific hyperrecombinational mechanisms are not involved in this process within the exon. We postulate that these mechanisms continuously generate new *Ab* alleles with highly divergent binding sites from which alleles with advantageous antigen-binding properties are selectively maintained by some form of balancing selection.**

The class I and class II genes of the murine major histocompatibility complex (MHC) encode receptors that bind fragments of processed antigens and present them to T lymphocytes (1–4). MHC class II genes are among the most polymorphic coding loci known in vertebrates (5). Over 100 alleles of the MHC class II gene *Ab* (which encodes the β subunit of the murine A molecule) have been detected in natural mouse populations (6), and many of these alleles differ in 5–15% of their nucleotides (7–9). The molecular mechanisms responsible for the generation of this remarkable diversity are the subject of debate. Much of the controversy has centered on the relative contribution of mutation, recombination, and gene conversion in the generation of MHC diversity. MHC polymorphisms are retained in natural populations for extremely long periods (10–12). As a consequence, much of the extensive allelic diversity of MHC genes may simply reflect the accumulation of point mutations over long evolutionary periods (13–15), although the retention of ancestral polymorphisms of MHC genes does not exclude diversification by recombinational processes (10, 16, 17).

In the present study, we have assessed the contributions of recombination and point mutation to the diversification of the murine MHC class II gene *Ab*. If diversity is solely due to the accumulation of mutations over long evolutionary periods, consistent allele genealogies should be detected in different segments of the gene since the entire gene would accumulate mutations in a coordinate fashion. In contrast, recombination would shuffle polymorphic sequences among alleles, thus obscuring genealogies for the entire gene‡.

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MATERIALS AND METHODS

Mice. DNA was extracted from ethanol-preserved mouse tissues of wild-mouse-derived strains from F.B.'s laboratory in Montpellier (France) or fresh tissues from our wild-mouse-derived strains in Gainesville. The origins and characteristics of these mouse strains have been described (18, 19).

Polymerase Chain Reaction (PCR) Sequencing. Intron sequences flanking exon 2 of *Ab* were used to selectively amplify a 340-base-pair (bp) fragment of genomic DNA containing the entire exon encoding the antigen binding site (273 bp). The oligonucleotide primers were CACGGCCCGC-CGCGCTCCCGC (5′ primer) and CGGGCTGACCGCGTC-CGTCCGCAG (3′ primer). PCR amplification was performed essentially as described (20). The amplified products were purified by polyacrylamide gel electrophoresis, blunt-end-ligated into *Sma* I-cut M13, and sequenced by standard dideoxynucleotide sequencing. To eliminate potential errors introduced by PCR, two or more independent clones per sequence were analyzed.

Data Analysis. Nucleotide divergence and diversity were calculated from the aligned sequences with Nei and Jin's program SYNO (21). Phylogenetic analyses were performed using distance methods, which minimize the total nucleotide divergence on the tree (22, 23), as well as parsimony analysis, which chooses the tree requiring the fewest mutations (24, 25). Parsimony analyses were performed with the program DNAPARS in the PHYLIP package of Felsenstein (24), and unweighted pair group method analysis (UPGMA) and neighbor-joining analyses were performed with the program SEND of Nei and Jin (21).

RESULTS

PCR Amplification and Sequencing of Rodent *Ab* Alleles. The origins and properties of the rodent strains analyzed are presented in Table 1. Alleles were designated according to the conventions recently proposed by Klein *et al.* (28). The phylogeny of the 12 *Mus* and 2 *Rattus* species in this collection has been extensively studied (29).

A 340-bp fragment containing *Ab* exon 2 was amplified from genomic DNAs by using PCR primers homologous to

Abbreviations: MHC, major histocompatibility complex; PCR, polymerase chain reaction; UPGMA, unweighted pair group method analysis.

‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M57794–M57839).

Table 1. Origins of *Ab* alleles analyzed

Species	*Ab* allele(s)	Geographic origin(s)
Mus musculus		
Laboratory	*b, d, f, k, p, u, q, r, s, nod*	Unknown
domesticus	$MudoAb^{1}$–$MudoAb^{15}$	United States, Morocco, Yugoslavia, Egypt, Israel, Italy
musculus	$MumuAb^{1}$–$MumuAb^{6}$	Bulgaria, Czechoslovakia, Denmark, Yugoslavia
castaneus	$MucaAb^{1}$–$MucaAb^{2}$	Thailand
molossinus	$MumoAb^{1}$	Japan
Mus spretus	$MuspAb^{1}$–$MuspAb^{3}$	Spain, Tunisia
Mus spicilegus	$MusiAb^{1}$–$MusiAb^{6}$	Bulgaria, Yugoslavia
Mus spretoides	$MustAb^{1}$	Bulgaria
Mus cookii	$MucoAb^{1}$	Thailand
Mus cervicolor	$MuceAb^{1}$	Thailand
Mus caroli	$MucrAb^{1}$, $MucrAb^{2}$	Thailand
Mus platythrix	$MuplAb^{1}$	India
Rattus norvegicus	$RT\text{-}1^{u}$, $RT\text{-}1^{b}$	Laboratory strain
Rattus rattus	$RaraAb^{1}$–$RaraAb^{3}$	United States

Published sequences are from the indicated references: *d* (8); *b* (7); *f*, *k*, *s*, and *u* (9); *nod* (26); $RT\text{-}1^{u}$ (11); $RT\text{-}1^{b}$ (27).

evolutionarily conserved intron sequences. These primers amplified exon 2 from all *Mus* and *Rattus* alleles tested. A total of 46 *Ab* alleles were amplified, cloned into M13mp19, and sequenced. Representative nucleotide sequences are presented in Fig. 1, and all of the sequences have been submitted to GenBank.

***Ab* Exon 2 Is Highly Diversified.** These sequences were combined with 10 previously published sequences from laboratory mouse and rat strains (7–9, 11, 26, 27) to produce a panel of 56 sequences of *Ab* exon 2. Analysis of these 56 sequences revealed 52 different alleles (4 pairs of identical alleles were found in independent samples from the *M. musculus* complex).

The majority of the polymorphisms in exon 2 occur in five specific regions, termed polymorphic segments (Fig. 1). As illustrated in Fig. 2, each of these segments encodes a specific

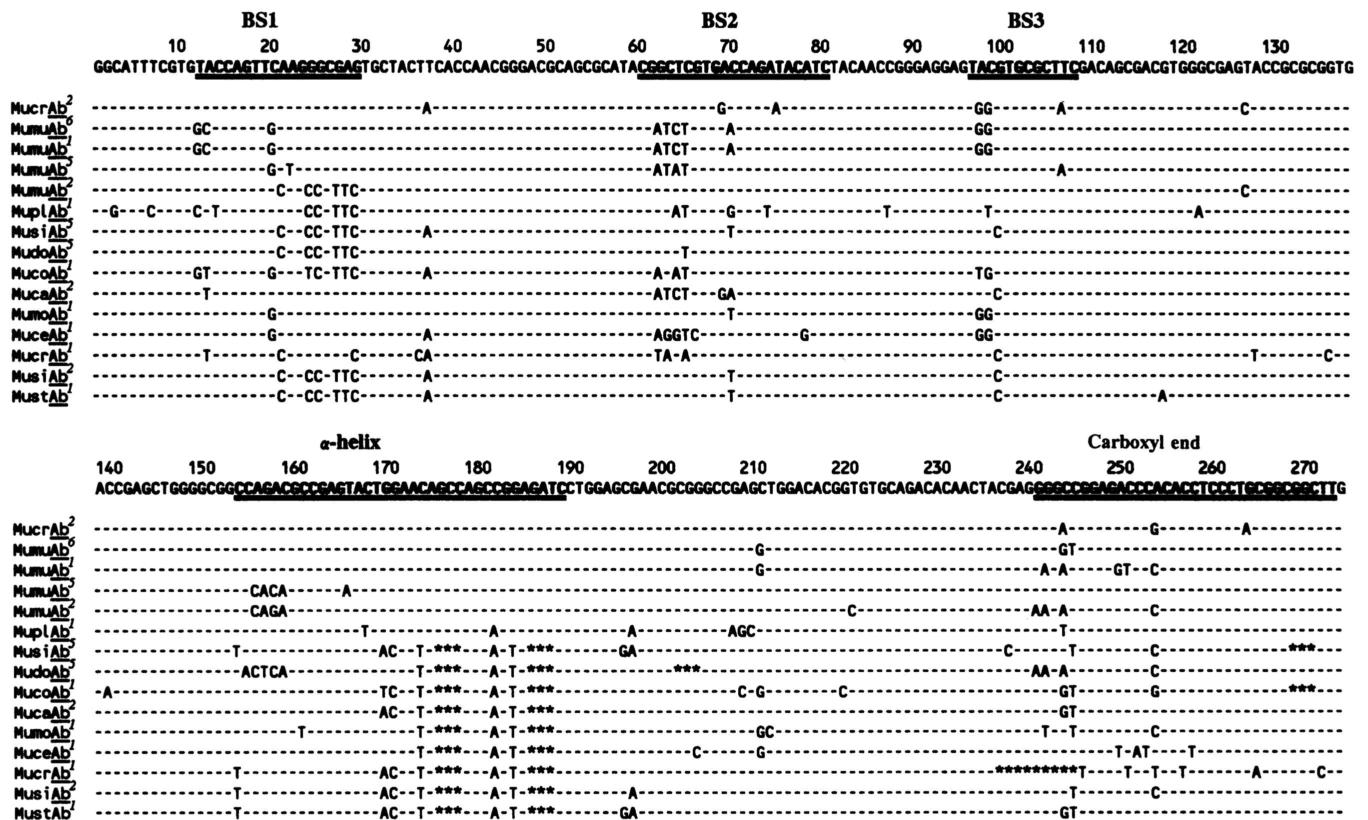

FIG. 1. Nucleotide sequences of the *Ab* exon 2 of 15 representative alleles showing the five highly polymorphic segments and the sequence motifs (bold and underlined). The precise borders of the sequence motifs are as follows: BS1, 12–29; BS2, 60–80; BS3, 96–107; α-helix, 152–188; and carboxyl end, 240–272. Only different nucleotides are shown in the figure and identical nucleotides are indicated by dashes. Asterisk indicates base deletion. The alignment between the alleles with the two-codon deletion (at nucleotide positions 175–177 and 185–187) and alleles without the deletion is different from the conventional alignment (previously designated as 65 and 67 codon deletion). This alignment represents an improvement of two nucleotides to the previous one. The first codon deletion (at positions 175–177) is the last two bases of codon 63 and the first base of codon 64. The second codon deletion is the last base of codon 66 and the first two bases of codon 67.

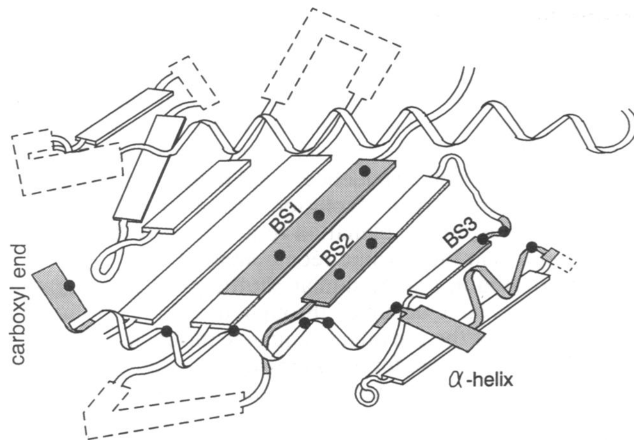

FIG. 2. Hypothetical three-dimensional model of MHC class II molecules developed by Brown *et al.* (3). Stippled areas indicate the positions of the sequence motifs of the A_β chain identified in Fig. 1. Filled circles identify contact amino acids to antigens as defined by Brown *et al.* (3).

element of the hypothetical antigen binding site of the A molecule (3). The first three segments (BS1, BS2, and BS3) encode three β-pleated-sheet strands that form the floor of the antigen binding groove. The fourth encodes amino acids 56–67 of the α-helix and the fifth encodes the carboxyl end.

Inspection of the allelic sequence variations in each polymorphic segment reveals the presence of recurrent sequence motifs. For example, in positions 22–30 in BS1, the sequence motifs CAGCCCTTC and AAGGGCGAG are each found in several alleles (Fig. 1). Only a few (from 4 to 11) highly divergent sequence motifs are prevalent at each polymorphic segment. Representative examples of motifs in the α-helix and carboxyl-end polymorphic segments are presented in Fig. 3.

Genealogies of *Ab* Exon 2 Alleles Are Not Reliable. Genealogies of the 52 *Ab* exon 2 alleles in our data set were constructed using both distance methods (UPGMA and neighbor-joining) and parsimony methods. Fig. 4 presents the genealogy produced by the UPGMA method. As expected, alleles in separate species are commonly more closely related than alleles in the same species, consistent with their retention as ancestral polymorphisms. However, several aspects of the analysis indicate that this tree is unreliable. First, the predicted branching pattern of alleles varies depending upon the phylogenetic method used (data not shown). Second, all *Ab* exon 2 alleles form two groups on the basis of the presence or absence of a two-codon deletion polymorphism at nucleotide positions 175–177 and 185–187 (Fig. 1). Double codon deletions are rare and represent reliable phylogenetic markers that should distinguish two distinct lineages of *Ab* exon 2. However, as indicated by the dots in Fig. 4, alleles having the two codon deletions do not group together. Consequently, if this were the true phylogeny, then the two codon deletions would have evolved on numerous occasions in separate *Ab* lineages.

	α-helix		Carboxyl end	Intron
Cons	CAGCCAGCCGGAGATC	.	GGGCCGGAGACCCAC	2
MumuA[2]	----------------		AA-A---------C-	?
p	----------------		-A-A-----GT--C-	1
MudoAb[2]	----------------		-A-A-----GT--C-	1
MumuAb[1]	----------------		-A-A-----GT--C-	1
RanoAb[3]	-------A--------		-A-A-----GT--C-	#
q	----------------		---GT----------	1
MudoAb[8]	----------------		---GT----------	1
MudoAb[4]	----------------		---------------	1
MuplAb[1]	---------A------		---T-----------	1
b	----------------		---------------	2
MumuAb[5]	----------------		---------------	2
MudoAb[10]	T-***---A-T-***-		AA-A---------C-	2
MuspAb[2]	T-***---A-T-***-		AA-A---------C-	2
k	T-***---A-T-***-		AA-A---------C-	3
nod	T-***---A-T-***-		-A-A-----GT--C-	1
MumuAb[4]	T-***---A-T-***-		-A-A-----GT--C-	2
u	T-***---A-T-***-		-A-A-----GT--C-	3
MucaAb[2]	T-***---A-T-***-		---GT----------	1
MustAb[1]	T-***---A-T-***-		---GT----------	1
s	T-***---A-T-***-		---GT----------	2
MumuAb[3]	T-***---A-T-***-		---GT----------	2
r	T-***---A-T-***-		---------------	1
MusiAb[6]	T-***---A-T-***-		---------------	1
MudoAb[12]	T-***---A-T-***-		---------------	2
MuspAb[1]	T-***---A-T-***-		---------------	2

FIG. 3. Nucleotide sequences of the α-helix and carboxyl-end segments of some representative alleles. The retroposon lineage origin of intron 2 from each allele is given in the last column. Alleles marked with "?" are from heterozygote individual for intron 2 and intron 2 data are not available for alleles marked with "#". Restriction fragment length polymorphism data were from McConnell *et al.* (10) and C. C. Lu, Y. Ye, J.X.S., and E.K.W. (unpublished work).

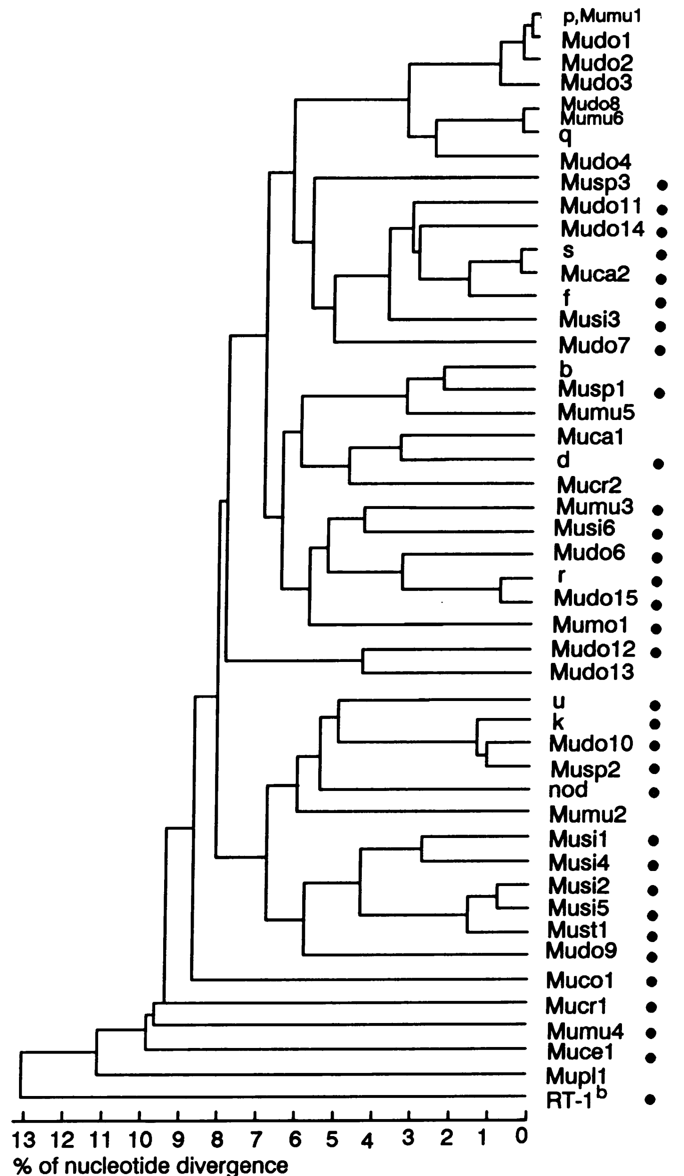

FIG. 4. Phylogenetic tree of *Ab* exon 2 alleles constructed with UPGMA. Alleles marked with a dot have the α-helix double codon deletion.

The failure to detect a solid phylogeny is not due to insufficient phylogenetic information, because there are 82 informative sites (nucleotide positions that exhibit at least

two character states represented by two or more alleles in the data set and distinguish between alternative branching orders of alleles) out of the 273 nucleotides in exon 2. This amount of diversity is sufficient to develop a robust phylogeny. The main problem with *Ab* exon 2 is the presence of extensive homoplasy (including reverse, parallel, and convergent mutations) among the alleles. The most parsimonious network for all 52 alleles requires 356 base substitutions at 82 informative sites, as compared with a theoretical minimum of 135 substitutions [this was calculated based on the assumption that each variant nucleotide state was generated by a single mutational event—i.e., theoretical minimum = Σ(number of states at each site -1)]. The 221 additional substitutions are due to the repeated introduction of identical substitutions in separate lineages in the parsimony network.

Intraexonic Recombination During Exon 2 Diversification. The extensive homoplasy may reflect a high frequency of reverse, parallel, and convergent mutations or may indicate that recombinational processes have played a role in the divergence of *Ab* exon 2. If recombinational events have been common during the divergence of *Ab* exon 2, then the allelic lineages of this exon should not coincide with those predicted for other segments of *Ab*. This can be assessed by comparing the predicted phylogenies of exon 2 and intron 2. Intron 2 alleles can be divided into three distinct lineages according to the presence or absence of a retroposon insertion (10). These insertional events provide solid phylogenetic information about the evolutionary history of *Ab* intron 2. As illustrated in Fig. 3, *Ab* exon 2 alleles with and without the double codon deletion are present in alleles with and without retroposon insertions in intron 2. Although this result might be interpreted as indicating that the double codon deletion has evolved independently in separate *Ab* lineages, such an event is highly unlikely. Also, such convergent evolution cannot readily explain the presence of identical silent mutations in the deleted motifs (nucleotide position 173) of different retroposon lineages.

These results indicate that intragenic recombinational events between intron 2 and exon 2 must have occurred during the evolutionary divergence of *Ab*, but do not establish that recombinational events have occurred *within* exon 2. Evidence favoring intraexonic recombination during the diversification of exon 2 is presented in Fig. 3. The *Ab* alleles were organized into two lineages according to the presence or absence of the two-codon deletion in their α-helix segment. Four distinct sequence motifs in the carboxyl-end polymorphic segment are found in alleles with either deleted or undeleted motifs in the α-helix segment. Although these blocks of polymorphic sequence could have been repeatedly generated via convergent evolution during the divergence of these *Ab* alleles, this explanation requires many more mutational steps than intraexonic recombination.

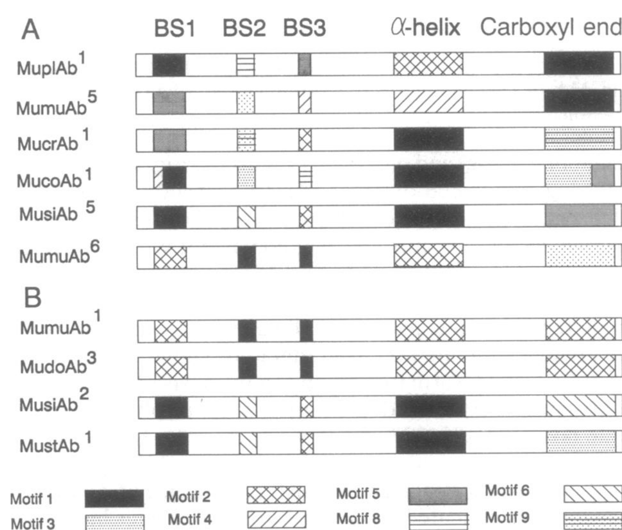

FIG. 5. Schematic diagram of *Ab* exon 2 polymorphisms for some representative alleles. Sequence motifs are represented by different fill patterns. (*A*) Alleles sharing only one to three subdomains, illustrating shuffling of motifs by intraexonic recombination. These alleles are derived from distantly related species. (*B*) Alleles sharing four to five identical subdomains. They are derived from the same species or closely related species.

As illustrated in Fig. 5, similar shufflings of the polymorphic segments have occurred throughout exon 2. Numerous combinations of the prevalent sequence motifs in each polymorphic segment were found among the *Ab* alleles in our data set. For example, *MucrAb[1]*, *MucoAb[1]* and *MusiAb[5]* all have sequence motif 1 of the α-helix segment but have sequence motifs 9, 3, and 5, respectively, for the carboxyl end (Fig. 5*A*). Similar random associations are found for all motifs, suggesting that shuffling of polymorphic segments to generate novel alleles has been a primary evolutionary mechanism for *Ab* exon 2 diversification. Many alleles in the data set shared only one or two polymorphic segments throughout exon 2, indicating that several intraexonic recombinational events have accumulated during their evolutionary divergence. Such alleles generally differ in >10% of their nucleotides in exon 2. This shuffling process has resulted in the formation of an array of alleles with highly divergent binding sites.

A hyperrecombinational mechanism probably is not responsible for these intraexonic recombinational events. As illustrated in Fig. 5*B*, several alleles with identical or similar combinations of polymorphic sequence motifs throughout exon 2 were found within species or between closely related species or subspecies. Analysis of the entire data set revealed that 18 of the 52 alleles could be organized into six groups in the mouse on the basis of sharing four or more contiguous polymorphic sequence motifs. These groups are identical to the closely related clusters defined by UPGMA analysis in Fig. 4, indicating that specific combinations of sequence motifs from all five polymorphic segments (i.e., intact exon 2 alleles) are stable over relatively short evolutionary times (<1–2 million years). These results suggest that intraexonic recombinational events are relatively infrequent and that their prevalence in the diversification of exon 2 results from their accumulation over evolutionary time spans.

Recombinational Events in Exon 2 Are Not Site-Specific. Although recombination between polymorphic segments illustrates the prevalence of such events in *Ab* diversification, this is not meant to imply that recombinational breakpoints only occur at specific sites in exon 2. These events occur throughout the exon. This is illustrated by the distribution of a silent mutation (A or T) at position 37 adjacent to the BS1 polymorphic segment. A and T are both present in alleles with motif 1 (C–CC–TTC) and with other motifs (Fig. 1). This distribution is best explained by postulating that recombinational breakpoints have occurred on both sides of position 37 during the evolution of the exon, resulting in the shuffling of this silent polymorphism among alleles with different motifs in BS1. Many other examples of "wandering" single nucleotide polymorphisms can be found throughout the exon (e.g., positions 20, 106, 126, and 210).

DISCUSSION

This study presents an extensive collection of nucleotide sequences of MHC class II alleles in the genus *Mus*. All of the alleles sequenced contained open reading frames and appeared to be functional genes, consistent with previous serologic studies that have demonstrated that all mice express MHC class II A molecules (30, 31). The observed

distribution of diversity within the exon confirms the previous finding of hypervariable regions within this exon (9) and defines five separate polymorphic segments that encode specific structural elements postulated to form the sides and floor of the antigen binding groove of MHC class II molecules.

Our analyses indicate that recombinational processes have played a major role in the diversification of the antigen binding site of the *Ab* gene product. Although the polymorphic segments that encode portions of the binding site have diversified predominantly by the accumulation of point mutations, the entire antigen binding site of *Ab* does not evolve as a single entity in this manner, due to repeated intraexonic recombinations. Much of the diversity of *Ab* alleles results from the shuffling of these polymorphic segments into a variety of combinations. The exact molecular mechanism involved in the shuffling process is unclear (i.e., micro gene conversion versus homologous recombination). Our results suggest that intraexonic recombination may not occur at an extraordinary rate during the divergence of *Ab*, since exon 2 lineages are stable within species and occasionally span closely related species. Also, the breakpoints are not site-specific within the exon, suggesting that a sequence-specific segmental exchange mechanism is not involved. However, it is not known whether such events occur in other regions of MHC genes and the sizes of the recombinational intervals have not been determined.

Sequence motifs can be traced throughout the genus *Mus* and at least occasionally into *Rattus*. These findings are consistent with the results of Figueroa *et al.* (11), who reported that lineages of *Ab* exon 2 arose prior to the divergence of *Rattus* and *Mus*. Our results indicate that this is true for short segments of exon 2, such as the double deletion polymorphism in the α-helix segment that was assayed by Figueroa *et al.* (11), but not for the entire exon. The evolutionary conservation of these short sequences may reflect structural constraints imposed on *Ab* genes by the necessity of encoding a functional class II molecule or may represent selective maintenance due to an intrinsic ability to contribute to the binding of specific antigenic peptides.

The accumulation of recombinant alleles during the diversification of *Ab* suggests that MHC diversity is driven by specialized selective mechanisms. Intraexonic recombination is an efficient mechanism for the generation of new *Ab* alleles with highly divergent antigen binding sites. However, such recombinations would be predicted to be rare occurrences, and therefore the prevalence of recombinant *Ab* alleles would be surprising in the absence of some type of specialized selection. We have suggested (32, 33) that a special type of selection, which we term "divergent allele advantage," may be responsible for the specific maintenance of MHC alleles with highly divergent binding sites. This mode of selection, together with overdominant selection (34, 35) or possibly frequency-dependent selection (36, 37) could account for the observed patterns of *Ab* diversification.

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1. Babbitt, B. P., Allen, P. M., Matsueda, G., Haber, E. & Unanue, E. R. (1985) *Nature (London)* **317**, 359–361.
2. Borkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–512.
3. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Borkman, P. J. & Wiley, D. C. (1988) *Nature (London)* **332**, 845–850.
4. Buus, S., Sette, A. & Grey, H. M. (1987) *Immunol. Rev.* **98**, 115–141.
5. Klein, J. & Figueroa, F. (1986) *Crit. Rev. Immunol.* **6**, 295–386.
6. Wakeland, E. K., Tarnuzzer, R. W., Lu, C. C., Potts, W., McIndoe, R. A., Talbot, W. S. & McConnell, T. J. (1987) in *H-2 Antigens, Genes, Molecules, and Function*, ed. David, C. S. (Plenum, New York), 139–153.
7. Larhammar, D., Hämmerling, U., Denaro, M., Lund, T., Flavell, R. A., Rask, L. & Peterson, P. A. (1983) *Cell* **34**, 179–188.
8. Malissen, M., Hunkapiller, T. & Hood, L. (1983) *Science* **221**, 750–754.
9. Estess, P., Begovich, A. B., Koo, M., Jones, P. P. & McDevitt, H. O. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3594–3598.
10. McConnell, T. J., Talbot, W. S., McIndoe, R. A. & Wakeland, E. K. (1988) *Nature (London)* **332**, 651–654.
11. Figueroa, F., Gunther, E. & Klein, J. (1988) *Nature (London)* **335**, 265–267.
12. Lawlor, D. A., Ward, F. E., Ennis, P. D., Jackson, A. P. & Parham, P. (1988) *Nature (London)* **335**, 268–271.
13. Gyllensten, U. B. & Erlich, H. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9986–9990.
14. Klein, J. & Takahata, N. (1990) *Immunol. Rev.* **113**, 5–26.
15. Klein, J., Kasahara, M., Gutknecht, J. & Figueroa, F. (1990) in *Chemical Immunology*, eds. Kimishige, I., Lachmann, P. J., Lerner, R. & Waksman, B. H. (Karger, Basel), pp. 35–50.
16. McConnell, T. J., Darby, B. & Wakeland, E. K. (1986) *J. Immunol.* **136**, 3076–3084.
17. Parham, P., Lawlor, D. A., Lomen, C. E. & Ennis, P. D. (1989) *J. Immunol.* **142**, 3937–3946.
18. Bonhomme, F. (1986) in *Current Topics in Immunology*, eds. Potter, M., Nadeau, J. H. & Cancro, M. P. (Springer/Verlag, New York), Vol. 127, pp. 19–34.
19. Wakeland, E. K., Price LaFace, M., Henson, V. & Peck, A. B. (1987) *Immunogenetics* **26**, 115–119.
20. Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1986) *Nature (London)* **324**, 163–166.
21. Nei, M. & Jin, L. (1989) *Mol. Biol. Evol.* **6**(3), 290–300.
22. Sokal, R. R. & Sneath, P. H. A. (1963) *Principles of Numerical Taxonomy* (Freeman, New York).
23. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4**(4), 406–425.
24. Felsenstein, J. (1989) PHYLIP 3.2 Manual (Univ. Calif. Press, Berkeley).
25. Felsenstein, J. (1982) *Quart. Rev. Biol.* **57**, 379–404.
26. Acha Orbea, H. & McDevitt, H. O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2435–2439.
27. Eccles, S. J. & McMaster, W. R. (1985) *Immunogenetics* **22**, 653–663.
28. Klein, J., Bontrop, R. E., Dawkins, R. L., Erlich, H. A., Gyllensten, U. B., Heise, E. R., Jones, P. P., Parham, P., Wakeland, E. K. & Watkins, D. I. (1990) *Immunogenetics* **31**, 217–219.
29. She, J. X., Bonhomme, F., Boursot, P., Thaler, L. & Catzeflis, F. (1990) *Biol. J. Linn. Soc.* **41**, 141–161.
30. Wakeland, E. K. & Klein, J. (1979) *Immunogenetics* **8**, 27–39.
31. Gotze, D., Nadeau, J., Wakeland, E. K., Berry, R. J., Bonhomme, F., Egorov, I. K., Horth, J. P., Hoogstraal, H., Vives, J., Winking, H. & Klein, J. (1980) *J. Immunol.* **124**, 2675–2681.
32. Wakeland, E. K., Boehme, S. & She, J. X. (1990) *Immunol. Rev.* **113**, 207–226.
33. Wakeland, E. K., Boehme, S., She, J. X., Lu, C. C., McIndoe, R. A., Cheng, I., Ye, Y. & Potts, W. (1989) *Immunol. Res.* **9**, 123–131.
34. Doherty, P. C. & Zinkernagel, R. M. (1975) *Nature (London)* **256**, 50–52.
35. Hughes, A. L. & Nei, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 958–962.
36. Bodmer, W. F. (1972) *Nature (London)* **237**, 139–145.
37. Potts, W. K. & Wakeland, E. K. (1990) *Trends Ecol. Evol.* **5**, 181–187.