25. Photos, drawings, and lists of cut marks are provided in (*10*) and in P. Villa *et al.*, *Gallia Préhistoire*, in press.
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28. Total counts of filleting and dismembering marks on long bones from features H3, 1, 4, 6, 7, 9, and 10 are: 35, 17, 4, 8, 11, 10, and 8, respectively. See (*21*) for counting procedures.
29. Five human and seven animal crania have long sagittal marks along the midline, frontal to occipital.
30. Archeological and literary evidence indicates that Celtic tribes living in Provence at the end of the first millennium B.C. kept skull trophies in their shrines and houses. F. Benoit, in *Recent Archaeological Excavations in Europe*, R. Bruce-Mitford, Ed. (Routledge and Kegan, London, 1975), pp. 227–259; B. Cunliffe, *The Celtic World* (McGraw-Hill, New York, 1979), pp. 82–83.
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36. Amino acid analyses of two modern samples (a sheep pelvis boiled for 4 hours and a sheep humerus from a shoulder roast cooked until well-done on an open fire for 1 hour and 15 minutes) show chromatographs identical to those of modern unheated bones and to those of the archeological bones. Temperatures achieved by meat during roasting are less than 100°C [J. Child, L. Bertholle, S. Beck, *Mastering the Art of French Cooking* (Knopf, New York, 1968), p. 379].
37. Bone fragments from feature H3 have been dated by the Lyon laboratory to 3930 ± 130 B.C. (uncalibrated ^{14}C date on bone; Ly 3748).
38. Supported by grants from the Wenner Gren Foundation, the American Council of Learned Societies, and the Leakey Foundation to P.V. The Fontbrégoua excavations are funded by French Ministry of Culture grants to J. C.

Molecular Biology of the H-2 Histocompatibility Complex

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The H-2 histocompatibility complex of the mouse is a multigene family, some members of which are essential for the immune response to foreign antigens. The structure and organization of these genes have been established by molecular cloning, and their regulation and function is being defined by expression of the cloned genes.

THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) OF mammals is a multigene family whose members encode cell surface glycoproteins involved in the recognition and immune response to foreign antigens. The MHC has been conserved throughout vertebrate evolution, and the MHC's of mouse (H-2) and human (HLA) have been studied extensively. The H-2 complex, located on mouse chromosome 17, has been divided into class I and class II genes on the basis of structural and functional similarities (*1–5*).

The class I genes are located at four genetic loci defined by serologic analyses of recombinant inbred mice: H-2K, H-2D/H-2L, Qa-2,3, and Tla (Fig. 1). These genes encode heavy chains of a molecular size of approximately 45,000 (45 kD) that are noncovalently associated as heterodimers with a β_2-microglobulin (β_2m), a 12-kD polypeptide encoded by a gene on mouse chromosome 2 (*6*). The 45-kD polypeptide has three extracellular domains (here called α1, α2, and α3) anchored in the membrane by a short transmembrane segment, and a cytoplasmic peptide of some 35 amino acids (Fig. 2a).

The K, D, and L molecules are highly polymorphic (*7*), are expressed on the surface of virtually all cells, and appear to direct the recognition of virus-infected and neoplastic cells by cytotoxic T lymphocytes (CTL) (*8, 9*). The antigen-specific receptors of CTL recognize viral glycoproteins only when they are associated with these class I molecules on the cell surface. In contrast, products of the Qa-2,3 region (Qa-2,3) and the Tla region (TL) are less polymorphic and their expression is limited to certain tissues (*10–13*). The Qa-2,3 and TL molecules are not involved in associative recognition by CTL, and their function is unknown.

The class II genes are located at two genetic loci (I-A and I-E) that map between H-2K and H-2D/H-2L (Fig. 1). The I-A region contains the A_β, A_α, and E_β genes and the I-E region contains the E_α gene. These genes encode heterodimers (Ia molecules) consisting of a 35-kD α chain noncovalently associated with a 29-kD β chain (*14*). Both α and β chains consist of two extracellular domains, a transmembrane segment, and a cytoplasmic region (Fig. 2a). The Ia molecules are highly polymorphic and are expressed primarily on the surface of B lymphocytes, macrophages, dendritic cells, and certain epithelial cells. The antigen-specific receptors of helper T cells that are required for the generation of CTL and for antibody production by B cells recognize foreign antigen only when it is associated with Ia molecules (*15, 16*).

The domain organization of class I and class II molecules is reflected by the exon-intron organization of the corresponding genes. The α3 domain of class I molecules and the α2 and β2 domains of class II molecules have strong sequence homology to domains of immunoglobulin-constant regions and thus belong to the immunoglobulin supergene family (*17*).

Organization of Class I Genes

The organization of class I genes of the BALB/c (H-2^d) and C57BL/10, or B10 (H-2^b), haplotypes is known in detail, and the

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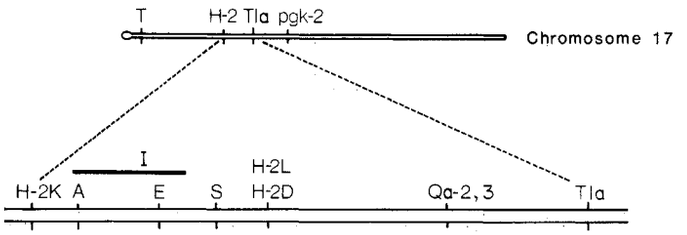

Fig. 1. Genetic map of the murine MHC defined by serologic analysis of recombinant inbred mice. The centromere of chromosome 17 is to the left. The murine MHC is 1 to 2 centimorgans (approximately 2000 to 4000 kb) in length and encompasses the region from H-2K through Tla, as shown in more detail at the bottom. The class I genes are located at the H-2K, H-2L, H-2D, Qa-2,3, and Tla loci. The class II genes are in the I region at the I-A and I-E loci. The S region includes class III genes, which are not discussed here.

two have been aligned by comparison of the restriction maps of their respective gene clusters (*18–20*) (Fig. 3).

The H-2K region. These regions are very similar in the two haplotypes and contain two genes in a head-to-tail configuration (Fig. 3a). Chromosomal walking experiments have demonstrated that the K-region cluster of B10 is linked to $A_{\beta 3}$, a class II pseudogene (*21*) (Fig. 4).

The H-2D/H-2L and Qa region. The genes in this region are arranged in head-to-tail configurations (Fig. 3b). In B10, this region is represented by 11 genes on overlapping cosmids that link H-2D^b to 10 Qa-region genes (Q1 to Q10). In BALB/c, this region is represented by 13 genes on five clusters that have recently been linked together in the order: clusters 13, 6, 2, 1, 9 (*22, 23*).

Cluster 13 is the most centromeric and contains the H-2D^d gene only. Cluster 6 cannot be aligned with any B10 cluster (*18*) and contains two class I genes between H-2D^d and H-2L^d (*23*), one of which can encode a molecule detectable by antibodies to Qa-2,3 (*24*). Cluster 2 contains the H-2L^d gene and an unidentified class I sequence. Cluster 1 contains seven genes corresponding to the Q1 to Q9 region of B10, and the single gene on cluster 9 corresponds to Q10 of B10 (*18, 25*). Thus, the genes located on BALB/c clusters 1 and 9 are most similar to the Qa region of B10 (Fig. 3b).

In B10, the Q5, Q7, and Q9 genes are similar to each other, and the DNA sequence of Q7, from exon 1 through exon 3, differs from Q9 by only a single nucleotide (*26*). Q6 and Q8 are more closely related to each other but are similar to the odd-numbered genes (*18, 26*). It has been suggested that the region from Q5 to Q9 arose from a series of gene duplications (*18*).

Gene 6 (27.1) of BALB/c (*27*) corresponds to Q7 of B10, as determined by restriction map analysis (*18*) and sequence comparison (*26*). Gene 7 of BALB/c appears to be derived from an unequal crossing-over of genes corresponding to Q8 and Q9 of B10. Analysis of BALB/cBy (*28*), a subline of BALB/cJ, shows that a similar unequal crossing-over has occurred between genes 5 and 6 (27.1) of BALB/cJ. This event correlates with the loss of Qa-2,3 expression in BALB/cBy, suggesting that the region corresponding to Q6 through Q9 of B10 is responsible for Qa-2,3 expression in the BALB/c mouse (*28*).

The Tla region. The Tla region contains the majority of class I sequences in mice (*18, 29*); however, Tla genes are the least similar to the usual H-2 class I genes, and the least conserved in mammalian evolution (*18–20, 30–34*). The greatest differences between B10 and BALB/c are also in their Tla regions, especially in terms of the number of genes (Fig. 3c).

In B10, the Tla region is on a single cluster containing 13 genes (*18*), whereas in BALB/c it is on three clusters (A, B, and 10) containing 18 genes (*19, 20*) (Fig. 3c). The T1 through T10 region of B10 is very similar to T1 through T10 of BALB/c cluster A. No B10 clusters have been isolated that correspond to BALB/c cluster B (T11 to T17) or cluster 10 (T18). However, the T11 to T17 genes of cluster B are homologous by restriction map analysis to the T1 to T6 genes of both BALB/c and B10. It appears, therefore, that part of the Tla region has been duplicated in the BALB/c haplotype (*19, 20*).

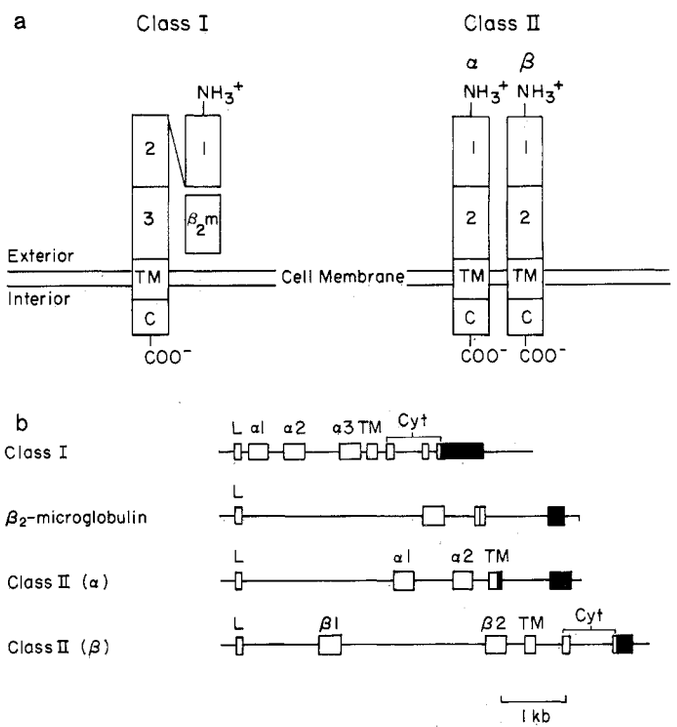

Fig. 2. (a) Schematic structure of class I and class II antigens. These antigens are depicted as consisting of protein domains labeled according to standard nomenclature. For the class I antigen, 1, 2, and 3 are external domains α1, α2, and α3; TM, transmembrane segment; C, cytoplasmic peptide; β_2m, β_2-microglobulin. Similarly for the class II antigen, 1 and 2 are external domains α1, α2 and β1, β2 of the α and β chains, respectively. (b) Exon-intron organization of class I genes, β_2-microglobulin, and the class II α and β genes. The exons encoding extracellular protein domains are numbered; those labeled L, TM, and Cyt encode the leader, transmembrane, and cytoplasmic sequences, respectively; 3′ untranslated sequences are indicated by shaded areas.

Generation of Polymorphism in Class I Genes

In the last 4 years it has become clear that intergenic exchanges of DNA between nonalleles (called gene conversion here) generate polymorphism in class I and class II genes. It was first shown that the H-2 class I mutant H-2K^{bm1} (an H-2K^b mutant) has a total of seven clustered nucleotide changes causing three amino acid substitutions (*34–39*). It was further determined that this apparently single mutagenic event had involved a short segment of DNA of between 13 and 50 nucleotides (nt), and subsequently Q10 was identified as the putative donor gene for this gene conversion event (*40*). In a second case, Geliebter *et al.* (*41*) showed that the H-2K^{bm6} mutation probably arose through sequence exchange between exons 3 of the Q4 gene and the H-2K^b gene, introducing two closely linked single-nucleotide changes. The Q4 gene sequence in this region is identical to H-2K^b except for the very two nucleotides whose introduction created the H-2K^{bm6} mutant. Recent work has

implicated gene conversion in the generation of six other mutants in the H-2K gene: H-2K^{bm3}, H-2K^{bm4}, H-2K^{bm9}, H-2K^{bm10}, H-2K^{bm11}, and H-2K^{bm23} (*42*).

The class I–related genes in the Qa and Tla regions appear to be strongly conserved in DNA sequence. This was first shown for the Q10 gene (*13*), which has fewer changes (by more than one order of magnitude) in sequence than exist between two H-2K alleles, and recently for the Q7 gene of the b haplotype, which is 99% homologous to its allele 27.1 of the d haplotype (*26*). Similarly, the T3 gene of the b haplotype and its pseudoallele T13 of the d are highly homologous (*32*). Conservation of sequence is exhibited not only by alleles but by neighboring duplicated genes as well. The Q7 and Q9 genes of the b haplotype are 99% homologous, and the homology between Q7 and Q8 is 93% (*26*).

Sequence conservation in Qa/Tla genes could mean that gene conversion involving short segments of DNA is suppressed in this region. However, we believe that short-scale exchanges of DNA may well occur, but that subsequent events may serve to erase these mutations. Gene conversion involving long DNA sequences of hundreds or thousands of nucleotides will not generate polymorphism but, to the contrary, will render genes identical. Larger scale conversion has occurred between Q7 (or Q9) and Q8—the leader sequence and first intron of Q8 is identical to Q7 over a stretch of 200 base pairs (bp). The transmembrane exon of Q8, however, shows homology in the 5′ and 3′ ends to the corresponding segments of the H-2K^b and H-2D^b genes, respectively, and thus appears to be a mosaic exon (*26*). Similarly, the T13 and the H-2L^d genes of the d haplotype have a 350-bp, 97% homologous segment in exon 4 and intron 4 (*20*).

Brégégère (*43*) has presented a mathematical model that suggests that gene conversion would generate polymorphism rather than homogeneity in the H-2 complex only if these conversions were directional, that is, if there are strong biases toward the nonpolymorphic Qa/Tla-region genes donating sequence information to the polymorphic H-2 genes. Even if gene conversions involving short segments occur in the Qa region, these will be eliminated by subsequent conversions involving large segments, and thus they impart an apparent directionality to the process. If selection is the driving force for the appearance of new polymorphic alleles at H-2K and H-2D, the absence of a role for Qa antigens in CTL recognition, coupled with the genetic mechanisms discussed above, will prevent the appearance of highly polymorphic Qa-region genes.

Expression of H-2 Class I Genes

H-2 antigens are present on virtually all adult tissues and are developmentally regulated. They are detectable during embryogenesis only after the midsomite stage on gestation day 10 (*44*). Experimental evidence suggests that interferons may play a role in activating these genes in vivo (*45*).

A useful model for in vitro differentiation studies is the F9 embryonal carcinoma (F9 EC) line, which expresses no detectable H-2 antigens on the cell surface until it is induced chemically. Regulatory sequences necessary for the expression of the H-2K gene in F9 EC cells most likely interact with a *trans*-acting factor (*46*). Expression of the H-2K^b gene upon differentiation in F9 EC cells has also been correlated with an increase in the level of DNA methylation of this gene (*47*). Blocking DNA methylation sites resulted in reduced expression of H-2K^b in differentiated F9 EC cells, in contrast to many examples where transcriptional activity is increased (*48*).

Understanding H-2 gene expression in tumorigenesis may clarify its role in cell-mediated immunity of tumor rejection (*49*). A

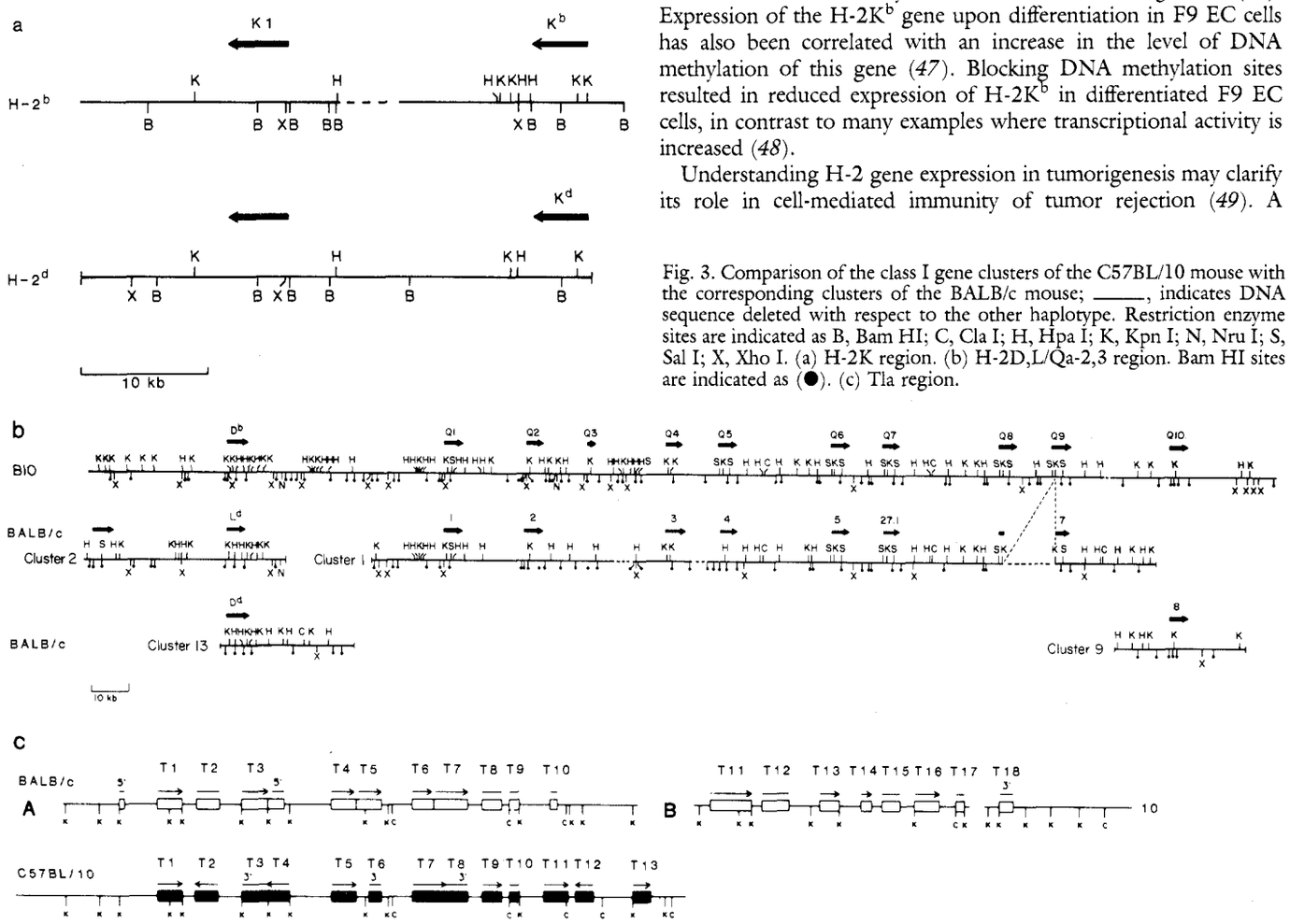

Fig. 3. Comparison of the class I gene clusters of the C57BL/10 mouse with the corresponding clusters of the BALB/c mouse; ——, indicates DNA sequence deleted with respect to the other haplotype. Restriction enzyme sites are indicated as B, Bam HI; C, Cla I; H, Hpa I; K, Kpn I; N, Nru I; S, Sal I; X, Xho I. (a) H-2K region. (b) H-2D,L/Qa-2,3 region. Bam HI sites are indicated as (●). (c) Tla region.

number of studies have described a significant decrease or a total loss of H-2 antigens on the surface of metastatic tumor cells. However, increased expression of class I genes or induction of previously nonexpressed class I genes has been observed in other cases. Mutation or recombination of class I genes also appears to occur in some tumor cells, as suggested by the finding of novel class I gene products on the cell surface or by isolation of unique clones of class I genes from these cell lines.

Differential splicing of messenger RNA's (mRNA's) could be an additional mechanism for generating novel class I genes. This has been shown to occur at the 5′ end of the H-2K^d gene (*50*) and in the 3′ region of the H-2K^b (*51, 52*) and H-2D^d (*53, 54*) genes. This phenomenon has also been observed in some Qa/Tla-region genes (see below).

Expression of Non-H-2 Class I Genes

Qa-2,3 and TL antigens differ from H-2 antigens in tissue distribution, with their expression limited to cells of hematopoietic origin (*11*). In addition, they do not serve as restriction elements for CTL, possibly because of their lack of polymorphism. In contrast to H-2 antigens, Qa-2,3 and TL undergo antigenic modulation (*55, 56*) when subjected to treatment with antibodies to Qa-2,3 (*57*) or TL (*55, 56*). This phenomenon appears to be general for all Qa/Tla-region gene products expressed on the cell surface and involves the total loss of their surface expression. Antigenic modulation appears to be the reason that TL-positive syngeneic leukemia cells escape destruction in TL-negative immunized recipient mouse strains.

Expression of Qa-region genes. The Qa-2,3 determinant was first identified by Flaherty (*11*). Originally thought to be a single molecular species (*58*), the Qa-2,3 epitope was shown subsequently to exist on several Qa-region gene products. Biochemical analysis of Qa-2,3 molecules on the surface of cloned CTL cell lines by Sherman *et al.* (*59*) first demonstrated variation in both molecular size and isoelectric point (*p*I). Additional evidence for the expression of multiple Qa-region genes was provided by analysis of L cells transfected with the ten genes of the C57BL/10 Qa region (*28*). Of these, none was expressed on the surface and only gene products from Q6, Q7, Q8, and Q9 were immunoprecipitated from cell lysates by antiserum to Qa-2,3. Variations in molecular size and *p*I were also detected, revealing the close molecular relationship of Q6 and Q8 and of Q7 and Q9. Whether a correlation exists between the variability in the Qa-region glycoproteins detected on the surface of CTL and the Qa-region glycoproteins expressed in L cells has not been determined.

Other approaches to the study of Qa-region gene expression have involved the transfection into L cells of hybrid BALB/c genes, constructed by exchanging the 5′ (L, α1, α2) and 3′ (α3, transmembrane segment, cytoplasmic peptide) segments of Qa-region genes with those of H-2K, H-2D, or H-2L genes (*60, 61*). These studies showed that the Qa-2,3 serologic determinants are associated with the α1 or α2 protein domains, or both, but the hybrid molecules were expressed on the surface of L cells only when the 3′ segment was derived from H-2K, H-2D, or H-2L. These results suggest that the 3′ segment of Qa genes may be important in the tissue-specific expression of Qa-2,3 molecules.

We have analyzed a complementary DNA (cDNA) library from the 2.1.1 cloned CTL line that expresses only one form of Qa-2,3 on the cell surface (*59, 62*). A cDNA clone has been identified as Q7 from B10, and, consistent with both the 27.1 (*27*) and Q7 (*26*) genomic sequences, it has an in-frame termination codon at the end of the exon encoding the transmembrane domain (*63*). Further studies will be required to determine whether the protein expressed on the surface of this CTL line is encoded by Q7.

It has been shown that the Q10 gene (*13*) is expressed specifically in mouse liver (*12, 64*). This gene is nonpolymorphic, and was predicted to encode a secreted protein based on the presence of an in-frame termination codon in the middle of exon 5 (encoding the transmembrane domain). Subsequent studies revealed the presence of a class I–related antigen in mouse serum (*64*). Q10-transfected L cells (*65*) were then shown to secrete an antigen reactive with an antiserum to a Q10-specific peptide (*66*). A nonpolymorphic secreted class I molecule may have a role in the induction of immunological tolerance (*64*).

A study of class I genes expressed in DBA/2 mouse liver has revealed eight distinct mRNA's (*67*). In addition to H-2K, H-2D, and H-2L, cDNA's corresponding to the Q10 gene and Q7/27.1 gene were identified. An alternatively spliced Q10 gene transcript was found that lacks the third exon. Another of the cDNA clones is highly related to the BALB/c Tla-region gene T6 but is clearly not identical. This study has established that genes from all three class I subregions are transcribed in DBA/2 mouse liver.

Expression of Tla-region genes. The TL antigens were originally discovered by Old *et al.* (*68*). A large number of mouse strains now have been typed serologically as TL$^+$ or TL$^-$ on the basis of expression of TL in normal thymocytes (*69*). An unusual feature of the expression of TL antigens is that certain TL$^-$ mouse strains (that is, C57BL/6, Tla^b) do express TL antigens on leukemic cells. The mechanism of this induced expression in leukemic cells has yet to be determined.

The cytoplasmic domain of Tla-region genes is encoded by exon 6 only, in contrast to that of H-2 class I genes, which is encoded by exons 6, 7, and 8 (Fig. 2). The nucleic acid homology of Tla genes to H-2 class I genes (H-2K, H-2D, H-2L) is only 60 to 70% in exons 1 to 3 but greater than 90% in exon 4. Comparison of three Tla genes from BALB/c with two other genomic clones from B6 (*29, 31*) reveals differences in the length of the cytoplasmic domain. The overall homology of exon 6 (40 to 50%) is lower than that observed among class I genes.

Northern analysis with Tla region–specific probes has also revealed differences with H-2 antigens. The mature H-2K, H-2D, and H-2L mRNA's of all haplotypes are 1.6 kilobases (kb), whereas Tla transcripts show marked heterogeneity, with sizes ranging from 1.3 to 3.5 kb. Chen *et al.* (*31*) provided a possible explanation by showing length variation in the 3′ untranslated region of Tla cDNA clones.

Tla-region probes from TL$^+$ strains do not hybridize to thymus poly(A)$^+$ mRNA from TL$^-$ strains of mice (*31*), but do detect mRNA from leukemic cells derived from TL$^-$ mouse strains. It is likely, therefore, that the Tla-region genes expressed in leukemic cells and in thymocytes are the same or are at least closely related.

Functional Domains of Class I Antigens

The H-2 class I antigens, K, D, and L, appear to direct recognition of virus-infected cells by CTL. A major question about the molecular basis of CTL recognition is whether a single T-cell receptor recognizes both class I and viral antigen, or whether two receptors recognize each independently. While this is still unknown, two related questions have been resolved. First, regions of the class I antigen that interact with the T-cell receptor have been defined. Second, postulated roles of the cytoplasmic domain in either recognition or the lytic mechanism, or both, have been tested and excluded.

To identify regions of the class I antigen involved in CTL recognition, we and others constructed hybrid genes by exchanging exons between different pairs of the H-2K, H-2D, and H-2L genes

(*70–76*). These class I genes were expressed in mouse fibroblasts, and recognition of the hybrid class I antigens by CTL and monoclonal antibodies (mAb) specific for K, D, or L was investigated. The pattern of CTL and mAb recognition obtained shows that the majority of CTL and mAb recognition sites are located on the domains α1 and α2 (Fig. 2a). Furthermore, these recognition sites are minimally influenced by interaction of domains α1 and α2 with polymorphic regions of domain α3. In contrast, interaction between domains α1 and α2 appears to alter these recognition sites (*73–75*). We have further shown that a hybrid K^b/D^b molecule, consisting of domain α1 from K^b and the rest of the molecule from D^b, expresses new antigenic determinants that are recognized by alloreactive CTL (*77*). The alteration of CTL recognition sites by interaction between domains α1 and α2 suggests that recognition sites may be formed by amino acids from both domains or that the conformation of amino acids at a recognition site may be altered by interactions between domains α1 and α2, or that both obtain. These two features may allow the conformation of CTL recognition sites on H-2 class I antigens to be sensitive to alteration by interaction of either domain α1 or α2 with viral antigens.

The absence of a role for the cytoplasmic domain of class I antigens (Fig. 2a) in CTL recognition and lysis was shown by the construction of truncated H-$2L^d$ genes in which all or parts of exons 6, 7, and 8, encoding the cytoplasmic domain, were deleted (*78, 79*). These genes were expressed in mouse fibroblasts, and the truncated L^d antigens were recognized by L^d-allospecific and L^d-restricted virus-specific CTL. The levels of lysis of transformants expressing the native and the truncated H-$2L^d$ antigens were similar, with the exception of the combination of one virus with one of the three truncated L^d antigens (*79*). However, the three truncated L^d antigens retain six amino acids, encoded by exon 5, that immediately follow the hydrophobic membrane-spanning segment on the carboxyl terminal side of the native L^d antigen. Therefore, it is possible that these six amino acids of the cytoplasmic domain are required for CTL recognition and lysis.

Organization of Class II Genes

Figure 4 shows the DNA organization of the I region of the C57BL/10 mouse (*21, 80, 81*). The genes $A_{\beta 1}$, A_α, $E_{\beta 1}$, and E_α of the I-A and I-E subregions encode the α and β polypeptide chains of Ia molecules. Class II protein and gene structure are shown in Fig. 2, a and b, respectively. Sequence analysis of $A_{\beta 2}$ and $E_{\beta 2}$ and detection of mRNA suggest that these also may be functional genes (*82–84*).

$E_{\beta 3}$ and $A_{\beta 3}$ are two additional β-related sequences (*5*). $E_{\beta 3}$ maps to the MHC class III S region. $A_{\beta 3}$, a pseudogene ($\psi A_{\beta 3}$) in the two haplotypes examined, is 75 kb telomeric to the H-2K gene (*21*) and is about 160 kb from the $A_{\beta 2}$ gene (*81*).

Function of Class II Molecules

Helper T cells recognize foreign antigens only in the context of an Ia molecule. Functional studies have revealed that structural differences between allelic class II molecules determine the initiation and level of the immune response (*15, 16*). Several groups have suggested that there is a specific interaction of antigen with Ia (*85–88*). However, the location and number of sites on the class II molecule that are involved in the interaction with antigen and T-cell receptor are unknown.

Sequence comparisons between class II genes from different haplotypes have revealed extensive polymorphisms associated with

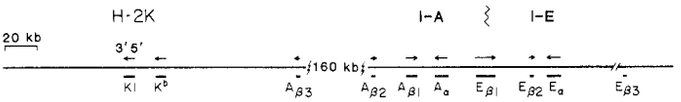

Fig. 4. The I region of the B10 mouse gene cluster is divided into two subregions, I-A, which encodes $A_{\beta 2}$, $A_{\beta 1}$ and the 5′ part of the $E_{\beta 1}$ gene, and I-E, which encodes the 3′ part of the $E_{\beta 1}$ gene, the E_α and the $E_{\beta 2}$ gene. A hotspot of recombination maps in the large intron the E_β gene (*81*). An additional β-related sequence $\psi A_{\beta 3}$ is located between the $A_{\beta 2}$ gene and the class I H-2K region. Analysis of the I region of other haplotypes reveals a similar arrangement (*80, 81*).

A_α, $E_{\beta 1}$, and $A_{\beta 1}$ loci, whereas only minor variations have been found among E_α alleles. The allelic variations are restricted to three specific areas of hypervariability in the amino-terminal domain (*14, 89*).

To investigate the structure-function relations of class II molecules, several approaches have been taken, including cotransfection of α- and β-chain genes, construction of hybrid molecules, analysis of a class II mutant mouse strain, site-directed mutagenesis, and immunoselection of class II mutants.

Cotransfection of α- and β-chain genes of various haplotypes has revealed that most T cells respond to antigen only in the context of the α-β pair to which they were originally restricted. These studies (*90*) provide evidence for conformational or combinatorial epitopes that form as a result of particular interactions between α and β chains. Changes in Ia structure, either by the construction of hybrid molecules or by mutations, may affect the conformation of the individual polypeptide chains or α-β pairing. Thus, these approaches may localize regions important for function but not necessarily the actual sites for interaction.

Hybrid class II molecules were constructed by exchanging sequences that encode the β1 domain of one haplotype with those of another haplotype (*91, 92*). Transfection of the hybrid β chains with the appropriate α chain demonstrated that the β1 domain contains sequences required for reactivity with monoclonal antibodies and for restricted recognition by T cells.

Analysis of A_β^{bm12}, a naturally occurring class II mutant, has shown that its difference from wild-type A_β^b is due to a cluster of 3 nt in the β1 domain, resulting in three amino acid substitutions (*93*). The DNA sequence of the E_β^b gene could have been the donor for a gene conversion event that created the bm12 mutation (*94, 95*). This change in structure correlates with a change in function. Mice of the b haplotype are nonresponders to sheep insulin, whereas A_β^{bm12} mutant mice do respond. Recognition of this antigen in the context of A_β^{bm12} (*96*) clearly maps residues critical for T-cell response to sheep insulin.

To analyze further the importance of individual amino acids in the function of class II molecules, several groups have utilized the technique of site-directed mutagenesis. In addition, the class II genes of immunoselected mutants that show functional differences (*97*) have been sequenced. These studies (*90*) indicate that multiple distinct regions of class II molecules can be involved in recognition by different T cells. Furthermore, changes both in widely separated and closely spaced regions of the linear sequence of an Ia molecule can have major effects on T-cell recognition.

In addition to the interaction of Ia with antigen-specific class II–restricted T-cell receptors, Ia molecules also interact with accessory molecules on the T-cell surface. The L3T4 molecule is expressed on the surface of class II–restricted T cells (*98, 99*), and is thought to contribute to the overall avidity of T-cell interactions by binding to nonpolymorphic determinants on the class II molecule. Studies with a hybrid MHC gene having a polymorphic class II β1 domain and the class I α3, transmembrane, and cytoplasmic domains of H-$2D^d$

Table 1. Comparison of promoter regions of class II genes. Numbers refer to distance in nucleotides (nt) 5′ of CAP site. Dashed lines represent identity with consensus sequence.

Gene	$^{-124}$ 14-nt region $^{-80}$	~20-nt spacer	10-nt region
α and β chain consensus sequence	CCYAGNRACNGATG	Spacer	CTGATTGGYY
E_α	--T--CA-- A----	TGTCAGTCTNGAAACATTTTT	--------TT
E_β	A-T--CA--T----	ATGCTGGACTCCTTTGATG	--------CT
A_β	--C--AG--A----	ACAGACTTCANGTCCAATG	--------TT

(*100*), indicate that the conserved β2 domain of the class II molecule is not the site for L3T4 binding.

Expression of Class II Genes

The expression of Ia antigens is restricted primarily to B cells, macrophages (including Kupffer cells of the liver and Langerhans' cells of the skin), dendritic cells, and to thymic epithelium (*101*). Low levels of Ia expression have also been observed in other tissues (*102–105*).

Precursor B cells are Ia-negative, but mature B cells and plasma cells constitutively express Ia (*106*). The levels of Ia can be augmented by various treatments (*107–110*), but not by γ interferon (IFN-γ). In contrast, resting macrophages do not express Ia, but it can be induced by a phagocytic stimulus or by treatment with IFN-γ (*111*). The molecular mechanism of Ia induction has not been fully elucidated, but it is known that IFN-γ enhances levels of Ia mRNA in macrophages (*112*).

Analysis of $A_{\beta 2}$ has revealed mRNA in spleen cells and B-cell tumors but not in macrophages or peritoneal exudate cells (PEC), even after IFN-γ treatment (*82*). Therefore, Ia expression is regulated in a gene-specific manner and may have B cell–specific and macrophage-specific regulatory sequences.

Molecular Approaches to the Regulation of Class II Expression

Three different approaches may help define *cis*-acting elements involved in the regulation of class II gene expression.

1) Nucleotide sequence comparisons of the promoter regions of class II genes have revealed common upstream sequences. Two regions of 10 and 14 nt, separated by about 20 nt, are found to be conserved when promoter sequences of class II α and β genes are compared (Table 1). The sequence of this 20-nt spacer (located about 90 nt upstream of the start of transcription) seems to be specific for α- and β-chain genes (*113*).

2) Transfection of class II genes into cell lines has proved to be of value in determining regulatory elements. Folsom *et al.* (*114*) transferred the E^b_β gene containing about 4 kb of 5′ flanking sequences into a macrophage cell line and showed it to be inducible by IFN-γ. Gillies *et al.* (*115*) demonstrated that a tissue-specific enhancer was located approximately 600 bp from the initiation of transcription of E^b_β. This enhancer was functional in a B-lymphoma cell line, but not in L-cell fibroblasts or in an Ia^- plasma cell tumor. Further studies are necessary to define potential control elements in more detail.

3) We (*116*) and others (*117, 118*) have introduced a class II E_α gene (d or k haplotype) into mice that do not express their endogenous E^b_α gene. In mice of b haplotype the I-E antigen does not appear on the cell surface even though the E^b_β protein is present in the cytoplasm (*119*). In two cases (*116, 117*), transfer of a functional E^d_α gene resulted in constitutive tissue-specific expression in spleen and inducible expression in macrophages. In contrast, Yamamura *et al.* (*118*) found abnormal constitutive expression of the transgene in macrophages. In all cases the transgene product was found on the cell surface, was functional in mixed lymphocyte reactions, and was able to act as a restriction element for antigen presentation to helper T cells.

Initially, we transferred the E_α gene with about 35 kb of 5′ flanking DNA. In this case, transmission of the transgene to progeny occurred only with female transgenic mice; all males either were infertile or did not transmit the gene (*116*). Table 2 summarizes the expression pattern and transmission of the E_α transgene in four different animals. Expression was tissue-specific except in one case where the transgene was expressed in all tissues tested. Abnormal expression could have been the result of the high number of gene copies or the result of integration of the transferred gene into a chromatin domain that is actively transcribed in all cell types.

Le Meur *et al.* (*117*) demonstrated that a DNA segment carrying the E^k_α gene and only 2 kb of 5′ flanking sequence was sufficient to

Table 2. Expression of the H-2 E^d_α gene in transgenic mice.

Mouse no.*	Gene copies†	E^d_α/A_β mRNA level‡								Macrophage induction	Transmission frequency§
		Spleen	Thymus	Heart	Kidney	Liver	Muscle	Brain	Bone marrow		
24-3	4	+/+	+/+	−/−	B/B	B/B	B/B	−/−	B/B	Yes	0/22
25-4	19	++/+	+/+	+/−	+/B	+/B	ND	ND	ND	ND	Infertile
27-2	2	+/+	+/+	−/−	B/B	B/B	B/B	−/−	B/B	Yes	6/11
27-3	2	+/+	+/+	−/−	B/B	B/B	B/B	−/−	B/B	Yes	0/17
Control	0	−/+	−/+	−/−	−/B	−/B	−/B	−/−	−/B	ND	

*These are the founder mice that integrated the E^d_α gene. †Gene copies per cell as determined by dot hybridization. ‡The numerator denotes levels of mRNA for the injected E^d_α gene; the denominator denotes levels for the endogenous class II A_β gene. Levels were determined by hybridization intensity after Northern analysis. Symbols: ++ indicates ten times the intensity of normal class II mRNA for spleen or thymus; + indicates intensity similar to normal class II mRNA for spleen or thymus; B indicates barely detectable levels; − indicates undetectable levels; ND, not determined. §Expressed as a ratio of number of offspring with the E^d_α gene to the total number of offspring examined.

confer the typical class II tissue specific expression patterns. We have introduced a functional E^d_α gene carrying only 1.4 kb of 5′ flanking DNA sequences into the germline of H-2^b mice. We find expression in thymus, in spleen, and in PEC induced by IFN-γ. The expression in spleen is found in macrophages or dendritic cells, or both, but not in B cells (*120*). Deletion of 5′ flanking DNA sequences has apparently inactivated or eliminated sequences required for expression of the transgene in B cells but not in macrophages or dendritic cells. Surprisingly, we have separated the regulatory sequences sufficient for expression of Ia in the thymus and macrophages from those required for Ia expression in B cells. Further insights will come from experiments to elucidate the regulatory sequences involved and potential *trans*-acting factors.

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