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Major histocompatibility complex gene organization in the mole rat *Spalax ehrenbergi*: Evidence for transfer of function between class II genes

(evolution)

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ABSTRACT **A genomic DNA library prepared from the kidney of the mole rat *Spalax ehrenbergi* was screened with mouse probes representing major histocompatibility complex genes that encode α and β polypeptide chains of class II molecules (α and β genes). Restriction maps were constructed for the cross-hybridizing clones, and the class II genes borne by these clones were identified. By this procedure, five main regions containing class II genes were established. One region contained four genes and two gene fragments, the second region contained two genes, the third region contained one gene and one gene fragment, and the remaining two regions contained one gene each. Altogether, six β genes, two α genes, and three α-gene fragments were identified. Two of the genes (one α and one β) were established as belonging to the *DQ* subclass, and all other genes were found to be members of the *DP* subclass. (Subclass designations are based on the human *HLA* class II genes.) No genes belonging to the *DR* and *DO* (*DZ*) subclasses were found in the library. The absence of *DR* genes in *S. ehrenbergi* was also indicated when other experimental methods were used. At least some of the *DP* loci are polymorphic and most likely also functional. Thus, in the evolution of the mole rat, the *DR* (and probably also the *DO*) loci have been deleted and their function(s) has been taken over by the *DP* loci, which have expanded to a great extent. These findings argue for functional interchangeability of the individual subclasses of class II loci.**

The class II loci of the major histocompatibility complex (MHC) fall into four subclasses, which in the human are referred to as *HLA-DO* (*DZ*), *-DP*, *-DQ*, and *-DR* (1–3). Each subclass further consists of two types of loci, α and β, and the polypeptide chains encoded in these types combine to form $\alpha\beta$ heterodimers expressed in the plasma membrane. The number of loci in each subclass may vary depending on the species. In the human MHC (*HLA*), there are at least four *DP* (two β, two α), one DO_β, one $DZ_{\alpha 1}$, four *DQ* (two β, two α), and four *DR* (three β, one α) loci, probably arranged in this order on chromosome 6. In the mouse MHC (*H-2*), there is only one *DP* ($A_{\beta 3}$) and one *DO* ($A_{\beta 2}$) locus, but there are two DQ ($A_{\beta 1}$, A_α) and four *DR* ($E_{\beta 1}$, $E_{\beta 2}$, $E_{\alpha 1}$, $E_{\beta 3}$) loci, arranged in this order on chromosome 17 (4, 5). The mouse has thus undergone a contraction of its class II loci during evolution, although it has preserved all four subclasses of these loci. In both the human and the mouse, however, the same two subclasses, *DQ* and *DR*, are most prominent functionally. These are the subclasses that display the highest polymorphism of their β genes and that code for molecules providing the context for the recognition of foreign antigens by the T lymphocytes of the immune system (6). Unequivocal evidence for the functionality of the other two subclasses of class II loci (*DP* and *DO*) is not available; if any of these loci are functional at all, they may contribute very little to the immune response in these two species.

In this report, we describe the class II gene organization in the rodent *Spalax ehrenbergi*, a mole rat. We come to the conclusion that one of the subclasses that is functional in the human and the mouse is lacking in the mole rat, whereas one of the nonfunctional subclasses has expanded considerably to become both polymorphically and functionally the most prominent.

MATERIALS AND METHODS

Preparation of a Genomic Library. DNA was isolated from the kidneys of a single adult female mole rat, *S. ehrenbergi* subspecies $2N = 58$, captured near Zippori, Israel. The isolation was carried out following the procedure described by Steinmetz *et al.* (7). The DNA was partially digested with restriction endonuclease *Sau*3A and the fragments, ≈30–50 kilobases (kb) in length, were ligated to pNNL cosmid arms (8). The ligated DNA was then packaged into λ phages by use of a commercially available packaging kit (9). The phages were used to transform *Escherichia coli* 490A suspended in 10 mM $MgSO_4$. The bacteria were plated and screened by probe hybridization to class II genes on 22 × 22-cm GeneScreen*Plus* filters (New England Nuclear).

Class II Probes. The $H\text{-}2A_\beta$ probe was a genomic *Eco*RI fragment 5.6 kb long and containing the entire gene (4). The $H\text{-}2A_\alpha$ probe was a cDNA *Pst* I fragment 1 kb long and containing the entire coding sequence (10). The *Spalax* MHC probes were designated a–e. Probe a was a genomic *Kpn* I–*Hin*dIII fragment, 5.2 kb in length and isolated from the cosmid 1.8, that contained almost the entire Q_β gene (see Fig. 2). Probe b was a genomic *Bam*HI fragment, 750 base pairs (bp) in length and isolated from the cosmid clone 9.6, that contained the 3′ end of the $P_{\beta 2}$ gene. The *Spalax* MHC probe c was a 1.25-kb genomic *Taq* I–*Hin*dIII fragment isolated from the cosmid clone 9.6 and containing the 3′ end of the $P_{\alpha 2}$ gene. Probe d was a genomic *Xho* I–*Bam*HI fragment, 4 kb in length and isolated from the cosmid 9.6, that contained most of the $P_{\alpha 1}$ gene. Finally, *Spalax* MHC probe e was a genomic *Pst* I–*Eco*RI fragment, 600 bp in length and isolated from the cosmid clone 7.7, containing the 5′ end of the Q_α gene. The *Spalax* MHC fragments were selected for the

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Abbreviation: MHC, major histocompatibility complex.

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absence of repetitive sequences by hybridization against the labeled, total genomic DNA. They were subcloned in plasmid pUC8 (34). All other class II probes are depicted in Fig. 1.

Southern Blot Hybridizations. These were carried out according to Southern (11) in 46% (vol/vol) formamide/1× Denhardt's solution/20 mM $Na_4P_2O_7$/5× SSC/10% dextran sulfate for 14 hr at 42°C. Filters were washed twice in 2× SSC and at least twice in 0.1× SSC at 65°C. (1× SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7; 1× Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin.)

RESULTS

Isolation and Mapping of Cosmid Clones Containing Class II Genes. The cosmid library prepared from a single mole rat contained approximately 600,000 clones. Screening of the library with mouse A_α, E_α, A_β, and E_β probes (Fig. 1) revealed the presence of a total of 15 hybridizing clones—10 clones hybridized with both A_α and A_β probes, 3 only with the A_α probe, and 2 only with the A_β probe. All clones were then digested by several restriction endonucleases, either singly or in combinations of two enzymes, and their restriction maps were constructed (Fig. 2). Three clones, 6.9, 7.7, and 1.11, apparently contained cloning artifacts (vector fragments of unidentified length were present in the insert), which made the construction of their restriction maps difficult. For all other clones, restriction maps could be constructed, and for some of the clones overlapping regions could be identified. It was possible to arrange the clones into five clusters (Fig. 2). Cluster 1 is composed of six partially overlapping cosmid clones encompassing ≈90 kb of contiguous DNA sequence. One additional clone, 8.7, showed a striking homology with part of cluster 1, but its restriction map was nonetheless different. Forty-one restriction sites were shared, two sites were lost, and six new sites appeared in this clone. The clone probably represents an allelic chromosomal region of the original heterozygote. "Cluster" 3 is composed of two clones, but we cannot exclude the possibility that these clones are in fact allelic. The remaining "clusters" are each composed of a single clone. Altogether we have been able to identify some 260 kb of DNA as belonging to the *Spalax* MHC class II region.

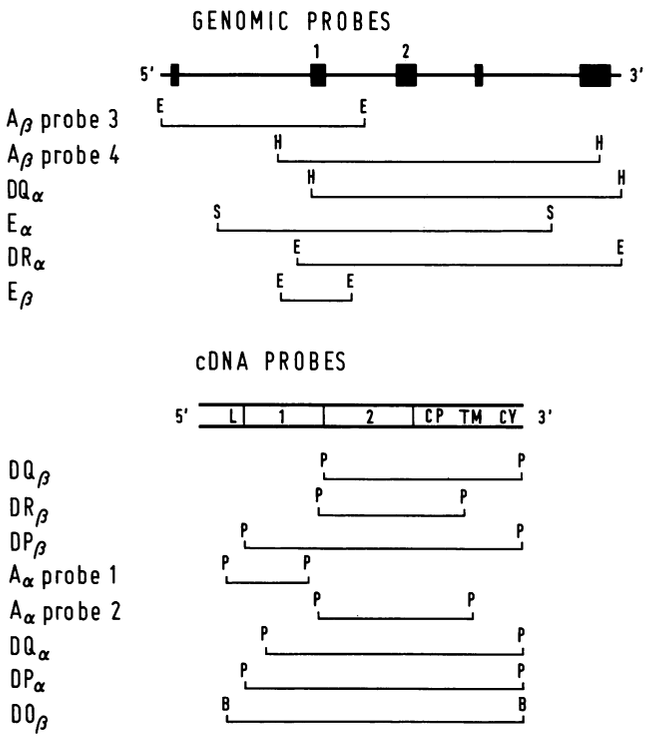

FIG. 1. Probes used for the identification of *Spalax* class II genes. Probes 1–4 were used for orienting the genes. Probe sizes were as follows: A_β probe 3, 2.5 kb (ref. 4); A_β probe 4, 3.1 kb (ref. 4); DQ_α (*DQA* in standard human-gene nomenclature) genomic, 2.4 kb (ref. 12); E_α, 3.4 kb (ref. 4); DR_α (*DRA*) 3.2 kb (ref. 13); E_β, 2 kb (ref. 4); DQ_β (*DQB*), 850 bp (ref. 14); DR_β (*DRB*), 800 bp (ref. 15); DP_β (*DPB*), 880 bp (ref. 16); $A_{\alpha 1}$, 400 bp (ref. 10); $A_{\alpha 2}$, 600 bp (ref. 10); DQ_α (*DQA*), cDNA, 780 bp (ref. 17); DP_α (*DPA*), 1044 bp (ref. 12); DO_β (*DZB*), 1.3 kb (ref. 18). Restriction endonuclease sites: E, *Eco*RI; H, *Hin*dIII; S, *Sac* I; P, *Pst* I; B, *Bam*HI. Exons 1 and 2 (black boxes in genomic map) encode domains 1 and 2 shown in the cDNA map. Other regions: L, leader; CP, connecting peptide; TM, transmembrane region; CY, cytoplasmic region.

Mapping of Class II Genes and the Determination of Their Orientation. As a further step in the characterization of the isolated cosmid clones, we digested them, singly or doubly, with appropriate restriction endonucleases and blotted the resulting fragments on nitrocellulose (Southern) filters. We then hybridized the fragments, under medium-stringency conditions sequentially, with both A_α probes (1 and 2 in Fig. 1) and both A_β probes (3 and 4 in Fig. 1), whereby one probe in each pair encompassed the 5′ and the other the 3′ region of the *H-2A* gene. Using this approach, we have been able to identify 12 separate DNA segments hybridizing with at least one of the probes used. Six of the segments hybridized with β probes, five only with α probes, and one segment, which was derived from clone 1.10, hybridized with both α and β probes. The singly hybridizing segments (either α or β but not both) apparently represent individual class II α or β genes of the *Spalax* MHC. The doubly hybridizing segment (both α and β) could represent two very closely spaced α and β gene fragments that are not separated from each other by the restriction enzymes used. The orientation of the α and β genes was determined by the hybridization of a given gene with either the 5′- or the 3′-specific probe (Fig. 2).

Identification of Class II Gene Subclasses. To determine the homology of the identified *Spalax* MHC class II genes, the gene-containing cosmid clones were digested with *Bst*EII, and the fragments were blotted on nitrocellulose filters and then sequentially hybridized with all human and mouse probes available to us, as well as with *Spalax* class II probes that we had isolated in the meantime. Each hybridization pattern was scored for the number of fragments and for their intensity (an example of the pattern obtained is shown in Fig. 3). The intensity was scored on an arbitrary scale from 0 to 4, where 0 indicates no hybridization under conditions in which the control DNA hybridizes well, and 4 indicates as strong a hybridization with a given gene as with the control DNA. (The control DNA was derived from the same species as the probe.) On the basis of the data thus obtained, we were able to divide the *Spalax* class II genes into two subclasses, which we designate *Smh-P* and *Smh-Q* (Fig. 4). The genes in the *P* subclass display a homology with human *HLA-DP* genes; the two genes in the *Q* subclass show a homology with the human *DQ* genes. In the *P* subclass, two types of β genes could be identified: those that hybridize strongly with *DP* and weakly with *DQ* probes ($P_\beta A$ loci) and those that hybridize almost equally well with *DP* and *DQ* probes (the $P_\beta B$ loci). We include the $P_\beta B$ loci in the *P* subclass because sequence comparisons (R. Schöpfer, F.F., E.N., and J.K., unpublished data) indicate a somewhat stronger homology of these genes with human *DP* than with *DQ* loci.

The tentative arrangement of loci shown in Fig. 4 is based on analogies with maps of the mouse and human class II loci. Among the identified *Spalax* genes, none could be found that would hybridize more strongly with the DR_β probe than with the other probes. Furthermore, on Southern blots *Spalax*

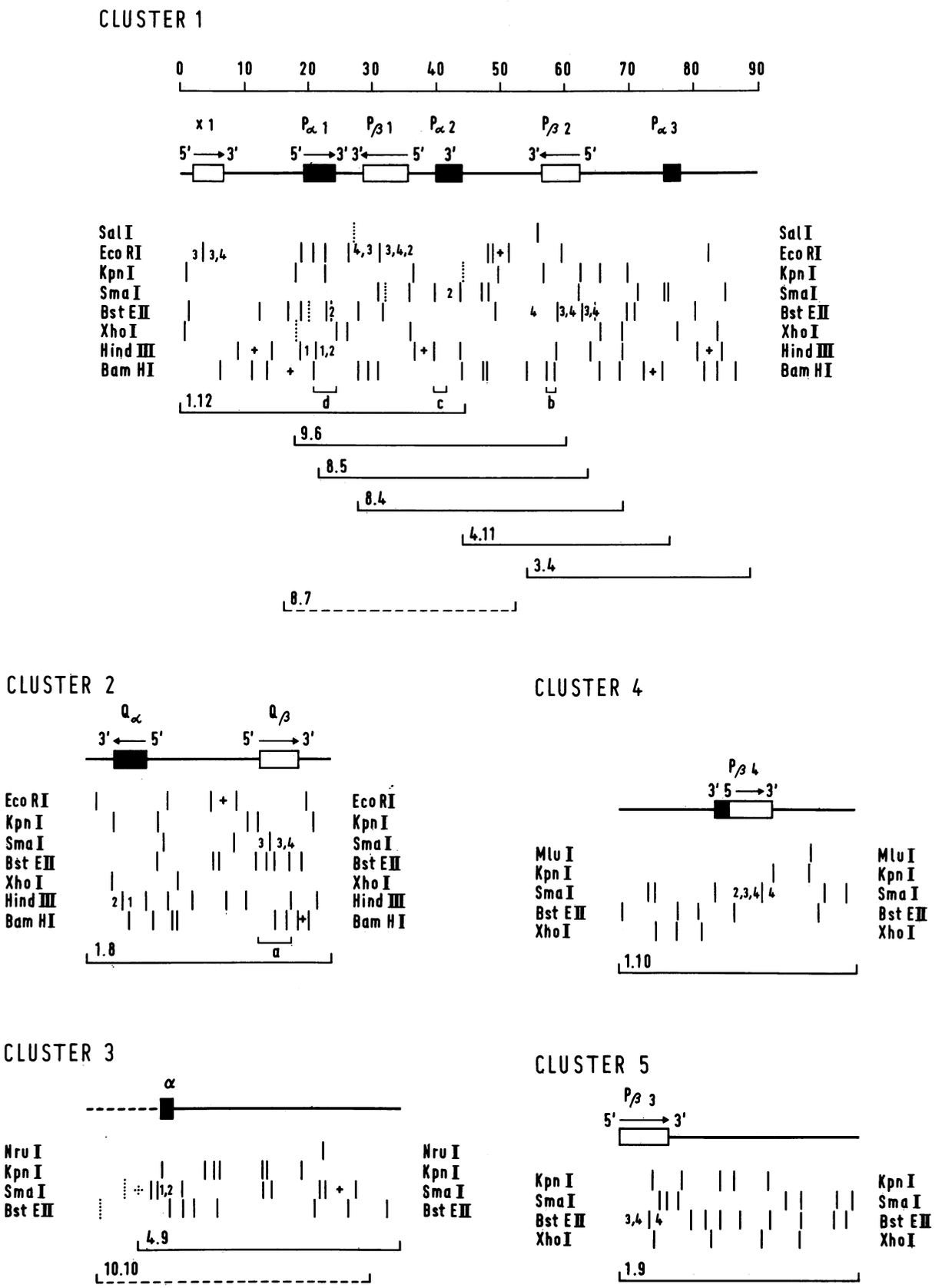

FIG. 2. Restriction maps of cosmid clones defining five "clusters" of *Spalax* class II genes. Scale at top indicates length in kb. α genes are indicated by filled boxes, and β genes by open boxes. The vertical dotted lines indicate restriction sites present in presumed allelic forms of the genes. Orientation of genes is indicated by arrows. Small numbers indicate probes (see Fig. 1) hybridizing with given fragments; large numbers designate individual clones. *Spalax* MHC probes were isolated from fragments a, b, c, and d. *P* and *Q* are *Spalax* class II genes; *X* is a gene whose assignment to the *DP* subclass is tentative.

genomic DNA failed to hybridize with mouse E_α and E_β probes (Fig. 5). We conclude, therefore, that *DR*-like genes are not present in this *Spalax* library. Similarly, no genes hybridizing with the human *DO* probe could be found.

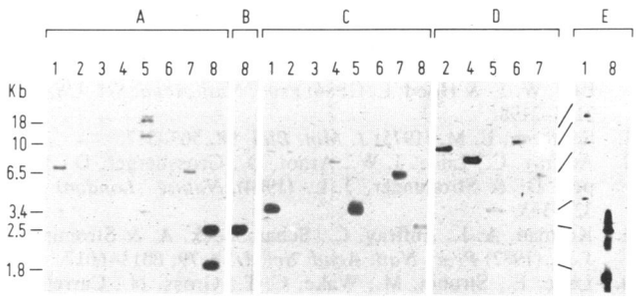

FIG. 3. Southern blots of *Spalax* MHC genomic clones hybridized with class II human, mouse, and mole rat β-gene probes. The probes used were *H-2A*$_\beta$ (blot A; *HLA-DQ*$_\beta$ gave a similar pattern), *H-2E*$_\beta$ (blot B; *HLA-DR*$_\beta$ gave a similar pattern), *HLA-DP*$_\beta$ (blot C), *Spalax P*$_{\beta 2}$ (blot D), and Spalax Q$_\beta$ (blot E). Lane numbers indicate genomic clones: 1, 8.5; 2, 4.11; 3, 4.9; 4, 3.4; 5, 1.12; 6, 1.10; 7, 1.9; 8, 1.8. Positions of size markers (kb) are indicated by lines.

DISCUSSION

The most interesting result of the present study is that the mole rat *Spalax* lacks at least one and maybe even two subclasses of the class II MHC loci. The evidence for the absence of *DR*-like loci in the mole rat is threefold. First, the results demonstrate that no *DR*-like genes could be found in a genomic library, even when it was screened under relaxed hybridization conditions with either human *DR* probes or with mouse *E* probes. One can, of course, argue that the *DR*-like genes of the mole rat diverged from the corresponding human and mouse genes to such an extent that they are no longer recognized by the probes used. Although this argument cannot be dismissed completely, it is counteracted by the fact that the *DP*-like and *DQ*-like genes could be identified in the same library without any great difficulty. Second, Southern blot analysis of total genomic DNA using mouse E_β and E_α probes failed to reveal any specific hybridizing bands (Fig. 5; see also ref. 19). Third, *Spalax* lymphocytes fail to react with all the E_α-specific antibodies tested (20). These antibodies detect a determinant that is evolutionarily highly conserved in that it has been found in all the mammals tested thus far (21–23) and even in birds, amphibians, and fish (24). Antibodies specific for this determinant could be produced only because some mice carry a deletion that prevents the expression of the E_α gene (25–27). Taken together, these three observations suggest very strongly that the *DR*-like (*E*-like) genes have been deleted in the evolutionary line leading to *S. ehrenbergi*.

The conclusion concerning the absence of the *DO*-like genes in *Spalax* rests solely on the failure of the *DO* probe to

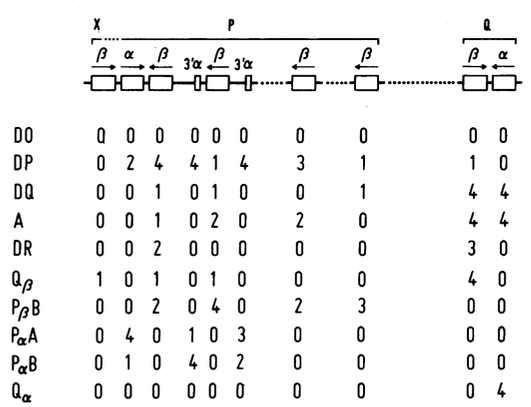

FIG. 4. Tentative organization of class II genes in the MHC of the mole rat. Increasing strength of hybridization of *Spalax* MHC genes with the individual class II probes is indicated by numbers from 0 to 4.

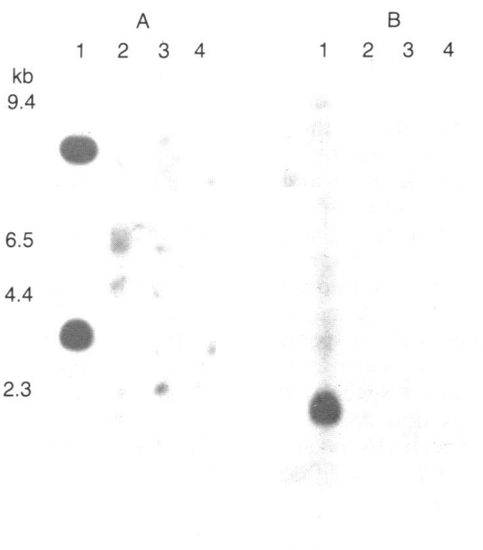

FIG. 5. Southern blot analysis of genomic DNA from *S. ehrenbergi* and mouse. *Eco*RI restriction fragments were hybridized with E_α (*A*) and E_β (*B*) class II probes. Lane 1: C57BL/10 mouse. Lanes 2–4: three independent *Spalax* individuals belonging to different chromosomal races ($2N$ = 58, 52, and 54, respectively).

hybridize with the class II genes in the genomic library. It must, therefore, be regarded as tentative. On the other hand, even in the human and particularly in the mouse, the *DO*-like genes are underrepresented compared to genes in the other subclasses (1–5). It is, therefore, conceivable that in most mammals, the *DO*-like genes are in a regression phase, their function (if they ever had one) being taken over by other subclasses of class II loci.

The total absence of *DR*-like (and probably also *DO*-like) genes in a species suggests that the subclasses of class II loci are functionally interchangeable. In *Spalax*, the function of the *DR*-like genes has apparently been taken over by the *P* genes. The *Spalax P* cluster has been expanded in comparison to the mouse and even to the human genes. Because at least some of the *Spalax P* loci are polymorphic (data not shown), they are probably functional. In *Spalax*, therefore, the *P* cluster may be the main functional set of class II loci. The ability of one subclass of genes to substitute for another subclass is also indicated by the existence of inbred mouse strains (28) and wild mice (29) that, because of a deletion in the promoter region (27), do not express any E molecules. There is no evidence that these mice are handicapped in any way. In these animals, the functional demands on the class II loci are apparently met by only two genes, A_β and A_α. We have provided evidence that the *E*-null mutation has been present in the mouse population for at least the last 2 million years (30). Although the persistence of this mutation might be artificially maintained by its association with the strong segregation distorters of the *t* complex (31), its presence in non-*t* wild mice indicates that it has no drastic adverse effect on the mouse population.

The class II gene complex may therefore be unusual because it contains genes that diverged at least 200 million years ago (30) but remain functionally equivalent to each other and because different taxa can call upon different genes within this complex to carry out the function of the whole set. This interchangeability of the individual subclasses is undoubtedly possible because of the uniqueness of the class II function. The class II molecules are needed to provide the context of recognition for soluble foreign antigens by the T lymphocytes (6). In other words, the T-cell receptor must recognize a small part of the class II molecule together with a small part of the antigen molecule. Apparently, this small

region can be present on any one of the several class II molecules, regardless of the gene subclass encoding these molecules. All the class II subclasses have retained the same general organization of their genes and their molecules; they have diverged only in their sequence. The retention of the overall organization would seem to be all that is required to keep the class II genes and molecules functional. Which of the subclasses a given species uses for context of recognition may depend largely on the polymorphism of these genes. This polymorphism, in turn, may vary among species depending on the species' evolutionary history. We have argued elsewhere (30, 32) that a major part of the MHC polymorphism is older than a given species. If so, the degree of polymorphism at a particular class II locus may depend on the polymorphism a species inherited from its ancestors at the time of speciation. As the mode in which a species arises may vary from species to species (33), different subclasses of class II genes may have a different opportunity to become functional.

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