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# Characterization of a *Q* subregion gene in the murine major histocompatibility complex

(cell surface antigens/mutation/*H-2* complex/transfection/restriction fragment length polymorphism)

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**ABSTRACT** **We have used restriction enzyme digests, Southern blot analysis, and gene transfer experiments to identify a class I gene in the *Q* subregion of the murine major histocompatibility complex. By comparisons of class I genes from *Q* congeneic strains, five restriction fragment length polymorphisms were identified. Further studies of mutant (Qa-2$^-$) and wild-type (Qa-2$^+$) BALB/c sublines indicated that at least part of the structural or regulatory gene controlling a *Q* subregion antigen resides on a 3.7-kilobase *Xba* I DNA fragment and is absent in all tested Qa-2$^-$ strains. The spontaneously occurring Qa-2$^-$ BALB/cBy mutant appears to have an extensive deletion in this region. The identity of this gene was confirmed by gene transfer experiments as well as by the use of a single-copy probe.**

The *Tla* region of the major histocompatibility complex (MHC) of the mouse determines a series of class I molecules represented on cells of the hematopoietic system (1). It can be divided into two subregions: *Q*, determining the Qa-2, Qa-3, Qa-5, Qa-7, Qa-8, and Qa-9 antigens and *TL*, determining the Qa-1, Qa-6, and TL antigens (1–4). At least three of these antigens, Qa-1, Qa-2, and TL, are known to be present on class I molecules. Recent analyses of cosmid [(plasmid containing a *cos* site (cohesive end site)] clones, however, have indicated that many more class I genes map to these subregions (5, 6). Steinmetz *et al.* (5) have shown that as many as 36 class I genes comprise the MHC. Only five reside in the classical MHC region between *H-2K* and *H-2D*; the remaining 31 genes are determined by the *Q* and *TL* subregions.

Thus, the function and characteristics of most class I genes are unknown. These genes have not been detectable by classic serologic and biochemical approaches, possibly for several reasons. First, these genes may be pseudogenes, which do not encode a protein product. At least one of the class I genes in the *Q* subregion, *27.1* (7), has been described as a pseudogene, although recent evidence also suggests that similar genes are transcribed (8). Second, these genes may be expressed on tissues outside the hematopoietic system. Since major efforts in detecting MHC products have focused on the lymphocyte cell surface, few have examined secreted products or nonlymphoid tissues for class I gene expression. Third, these class I gene products may not be polymorphic. Since class I detection systems have concentrated on immunizations between mouse strains, any product that does not have intraspecies variation will go undetected.

Analysis of the known *Q* and *TL* genes may provide us with clues to the function of other unknown *Q* and *TL* subregion gene products, since genes in these subregions appear to be highly homologous (5). Therefore, we have been analyzing the genetic fine structure of the *Q* subregion in hopes of using this information to extend our knowledge of the MHC. In particular, we have concentrated on analysis of the *Qa-2* gene family. The Qa-2 family consists of those antigens which are coordinately expressed with the Qa-2 molecule and include Qa-2, -3, -4, -5, -6, -7, -8, and -9 (1–4). All but one of these antigens are determined by the *Q* subregion.

The Qa-2 molecule appears unique among known class I molecules. It lacks the high degree of polymorphism exhibited by other class I molecules; no structural polymorphism was detected even among *Mus* subspecies (9). Qa-2 is also highly regulated, both quantitatively and qualitatively; other genes within the MHC affect the level of *Qa-2* expression (1). In addition, other Qa-2 family antigens including Qa-3, Qa-5, and Qa-6 seem dependent on *Qa-2* expression. The function of *Qa-2* and the Qa-2 family of antigens is unknown.

Both Steinmetz *et al.* (7) and Margulies *et al.* (10) have studied some of the restriction fragment length polymorphisms (RFLP) governed by the *Q* subregion. Since several class I genes have been located in this region, it previously has not been possible to identify which of these genes is the structural gene for Qa-2 or which of them are expressed. Transfection experiments by Goodenow *et al.* (11) suggest that *Qa-2* resides on the cosmid c50.2 and not on the same cluster encoding the *Q* subregion pseudogene *27.1*. However, recent experiments by Goodenow *et al.* (12) indicate that recombination between resident L-cell genes and donor DNA could complicate the identity of putative class I genes in this type of experiment. Moreover, the results of Sharrow *et al.* (13) indicate that cross-reactivities between class I molecules also may confuse interpretations.

Mellor *et al.* (14) also have identified and sequenced a *Q* subregion gene called *Q10*. They hypothesize that this gene may be the repository for gene conversion events that cause *H-2K* region mutations. This gene is similar to one described by Cosman *et al.* (8) and codes for a liver-specific secreted product. Thus, the *Q* subregion may be important from a genetic standpoint as well as from the determination of cell surface and secreted products.

Therefore, we have used the approach of examining the *Q* subregion RFLPs in mutant vs. wild-type mice to identify the *Qa-2* family of genes. The mutants in this case are sublines of BALB/c that have lost their capacity to express detectable Qa-2 family products on the lymphocyte cell surface (15).

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Abbreviations: MHC, major histocompatibility complex; RFLP, restriction fragment length polymorphism; kb, kilobase(s).

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## MATERIALS AND METHODS

**Mice.** Mice were bred and maintained at the New York State Department of Health. The origins of the recombinant strains and the BALB/c sublines have been described (1, 15). B6.K3 and B6.K4 are new recombinants isolated from a (B6-*Tla*$^a$ × B6-*H-2*$^k$)$F_1$ × B6-*H-2*$^k$ backcross. They have the following genotypes: B6.K3 = $K^k$ $D^k$ *Qa-2*$^+$ *TL*$^+$; B6.K4 = $K^k$ $D^k$ *Qa-2*$^-$ *TL*$^+$. By serological typings, therefore, these two strains only differ by genes within the *Q* subregion.

**Isolation of High Molecular Weight DNA.** Mouse liver DNA was prepared as described by Blin and Strafford (16) with some modifications. Briefly, liver homogenates were digested in lysing buffer for 3 hr at 50°C with constant agitation. Potassium iodide was added and allowed to dissolve, and the homogenate was centrifuged at 18,000 × *g* for 15 min. The denatured protein was removed, and the DNA was precipitated with ethanol. The DNA was then redissolved overnight in 10 mM Tris chloride/1 mM EDTA, pH 8.0. The next day the DNA was treated with RNase and extracted three times with phenol, extracted twice with ether, and precipitated with ethanol.

DNA from certain of the BALB/c sublines was kindly provided by Michael Potter (National Cancer Institute, National Institutes of Health).

**Restriction Enzyme Digests and Hybridizations.** Mouse liver DNA was digested to completion with restriction enzymes (obtained from New England Biolabs, Bethesda Research Laboratories, or P-L Biochemicals) according to the suppliers' instructions. Digested samples were electrophoresed overnight on 0.8% or 1.0% agarose gels. The DNA was transferred to nitrocellulose and hybridized with a class I cDNA probe—pH-2IIa (17), pMHC-1 (18), or C1432. The probe was labeled by nick-translation with $^{32}$P-labeled deoxynucleotide (0.5–2 × $10^8$ cpm/μg). Washes were performed according to Endow (19). Filters were exposed to Kodak XAR-5 film with an intensifying screen.

**Cosmid Library.** A B6 cosmid library was prepared according to Chaplin *et al.* (20). Briefly, high molecular weight B6 liver DNA was partially digested with *Mbo* I, size-fractionated, and inserted into the *Bam*HI site of the cosmid vector pJB8; 500,000 recombinant colonies were screened with the *H-2L*$^d$ gene corresponding to most of the C2 domain. Colony hybridization and stringency conditions were according to the formamide–42°C method as described by Maniatis *et al.* (21).

**Transfection Procedure.** BW5147 cells were cotransfected by the calcium phosphate technique (22) with 15 μg of C1 cosmid DNA together with 5 μg of pMSV-neo containing the neomycin-resistance gene (*neo*), which was used as a selection marker. The cells were grown in selective medium containing 1 mg of G418 (Geneticin, GIBCO) per ml for 5–6 wk. Neomycin-resistant cells were then cloned by limiting dilution and were screened for the expression of the Qa antigens.

**Immunization Procedures.** B6-*H-2*$^k$ mice were immunized with BW5147 cells transfected with the C1 cosmid according to the protocol of Shen *et al.* (23). Briefly, increasing doses of washed cultured cells were injected at 0, 4, 6, 8, 10, 12, and 14 wk. The first injection was subcutaneous, and the later injections were intraperitoneal. Doses increased from 10 × $10^6$ to 100 × $10^6$ cells per mouse. Mice were bled 1 wk after the third through seventh injection.

## RESULTS

By the use of restriction enzymes and Southern blot analyses with the class I cDNA probe pH-2IIa, we have identified several RFLPs mapping to the *Q* and *TL* subregions. With *Xba* I-digested genomic DNA, at least five RFLPs were observed (Fig. 1). These RFLPs were mapped on the basis of differences between B6.K1 (lane d) and B6.K2 (lane e), two recombinant congeneic strains that differ only in the *Q* subregion. These fragments are sized at 13.7, 11.7, 6.9, 5.2, and 3.7 kilobases (kb). Similar comparisons of C57BL/6 (B6) and B6-*Tla*$^a$, which differ only in the *TL* subregion, revealed 10 RFLP differences sized at 17.6, 9.8, 6.1, 4.7, 3.9, 2.8, 2.6, 2.3, 2.0, and 1.7 kb.

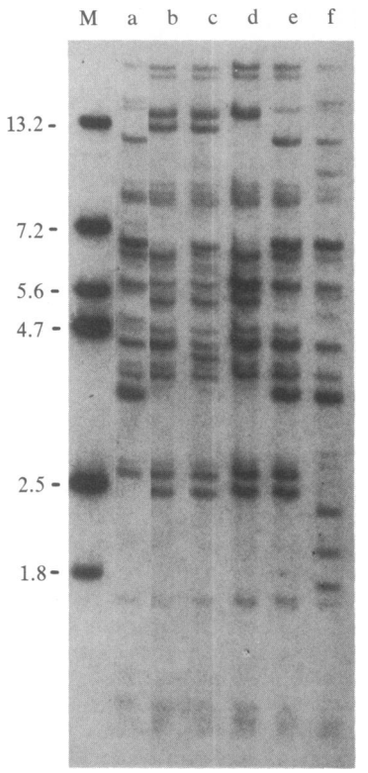


FIG. 1. *Xba* I restriction maps of genomic DNA from congeneic strains by hybridization to the pH-2IIa probe. Lanes: M, markers (sizes are shown in kb); a, B6; b, B6.AK1; c, B6-*H-2*$^k$; d, B6.K1; e, B6.K2; f, B6-*Tla*$^a$. Samples were digested to completion with *Xba* I and electrophoresed overnight on 0.8% agarose gels.

Next we compared mutant vs. wild-type strains with the pMHC-1 probe. In particular, BALB/cBy (Qa-2$^-$) was compared with BALB/cFla (Qa-2$^+$), and B6/Fla (Qa-6$^-$) was compared with B6/J (Qa-6$^+$) (Fig. 2). Only one RFLP was detected; BALB/cFla possessed a 3.7-kb fragment; BALB/cBy did not. No differences were observed between B6/J and the *Qa-6* mutant B6/Fla. This preliminary evidence suggested that an RFLP mapping to the *Q* subregion was missing in the Qa-2$^-$ family mutant BALB/cBy.

To confirm the correlation between the Qa-2$^-$ family mutation and the 3.7-kb fragment, other BALB/c sublines were examined (Table 1). These results showed that all of the Qa-2$^+$ BALB/c sublines possess the 3.7-kb fragment, whereas none of the Qa-2$^-$ BALB/c sublines did. Congeneic controls indicated that an identically sized band is missing in B6.K1 but present in B6.K2 and B6 (Fig. 2). No compensation fragment (i.e., a fragment present in the mutant Qa-2$^-$ strain but absent from Qa-2$^+$ strains) has so far been detected. Another restriction enzyme, *Bam*HI, revealed no differences between mutant and wild-type strains (data not shown).

To confirm that this gene is the one controlling *Qa* expression, a B6 cosmid library was examined. Three cosmids were found that contained a 3.7-kb *Xba* I fragment that hybridized to the pMHC-1 probe. At least two of these cosmids contain extensive overlapping sequences; the third has not been fully analyzed. The cosmid c50.2 described by Goodenow *et al.* in 1982 also was tested and found not to contain a 3.7-kb *Xba* I fragment. On restriction enzyme analyses with

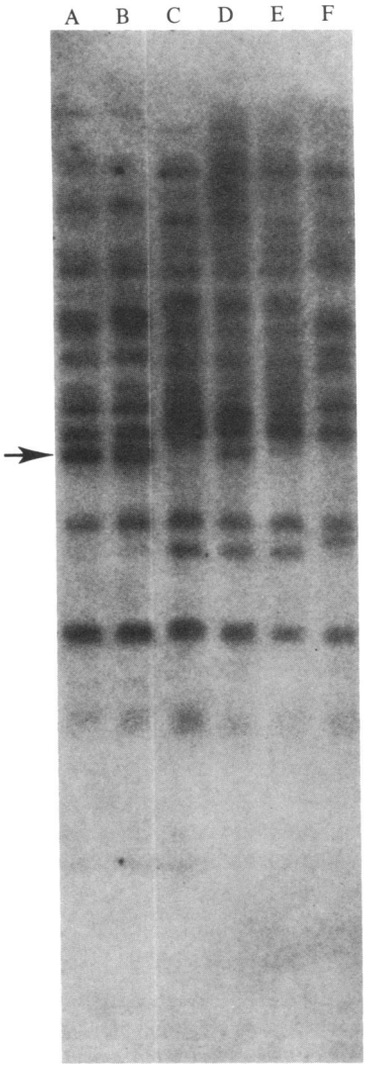


FIG. 2. *Xba* I restriction maps of genomic DNA from mutant strains by hybridization with the pMHC-1 probe. Lanes: A, B6/J; B, B6/Fla; C, BALB/cBy; D, BALB/cFla; E, BALB/cKh-*H-2*$^{dm2}$; F, B6.K1. The arrow indicates the 3.7-kb fragment.

six enzymes, c50.2 appeared dissimilar from the B6 cosmids containing the 3.7-kb *Xba* fragments (i.e., there were very few restriction fragments in common).

One of these cosmids, C1, was chosen for further study. BW5147 cells, transfected with this cosmid, were analyzed for the expression of Qa antigens. Two approaches were used. First, the transfected cells were tested in the direct cytotoxicity test with a variety of Qa antibodies. Second, the transfected cells were used to immunize B6-*H-2*$^k$ mice, and the resulting antisera were analyzed for anti-Qa activity. The results of the first approach were ambiguous, probably because leukemia cells often lose their Qa antigen expression upon passage *in vitro* (unpublished results). The second approach, however, led to some fruitful observations. Antisera from immunizations involving these transfected cells showed *Qa-2* family activity (Table 2). Its activity on B6.K3 and not on B6.K4 indicates its *Q* subregion specificity, while its activity on all *H-2*$^b$ strains shows its cross-reactivity with *H-2K*$^b$ and/or *H-2D*$^b$. This activity correlated strikingly with the activity of a monoclonal antibody, 20-8-4, described by Sharrow *et al.* (13).

Because transfection experiments can sometimes lead to incorrect interpretations (12), a single-copy probe was made to this 3.7-kb *Xba* I fragment. The 3.7-kb *Xba* I fragment was excised from the C1 cosmid and cloned into the plasmid pUC13. Because this fragment was found to contain repetitive sequences and was not useful for Southern blot analyses, further subclones were made. A number of subclones were used as probes in the Southern blot analyses of genomic DNA. One such subclone, C1432, was particularly useful in identifying the mutant difference (Fig. 3). Under stringent conditions, it only recognized one *Xba* I fragment, the one originally identified as being deficient in the BALB/cBy strain. In addition, the insert from this subclone did not appreciably cross-hybridize to the class I gene in c50.2 (data not shown). Under less stringent washes (60°C instead of 65°C), a weakly hybridizing high molecular weight fragment could also be seen. No compensation band was evident in the BALB/cBy strain under either stringent or nonstringent washes. Moreover, the hybridizing gene sequence in B6 and B6.K2 appears very similar to that in BALB/c since cross-hybridization was specific for this fragment. At least one other restriction enzyme, *Pvu* II, revealed this deletion. Again, no compensation band was evident.

Table 1. Strain distribution of 3.7-kb *Xba* I fragment containing a class I gene recognized by either pH-2IIa or pMHC-1

| | Strains* | |
|---|---|---|
| | With 3.7-kb fragment | Without 3.7-kb fragment |
| B6 congeneic strains | B6/Fla†, B6/J†, B6.K2‡, B6-*T1a*$^a$ | B6.K1†, B6-*H-2*$^k$ B6.AK1 |
| BALB/c congeneic strains | BALB/cFla†, BALB/cAR, BALB/cJ, BALB/cORNL, BALB/c-PC1$^-$, BALB/cWt | BALB/cKh, BALB/cKh-*H-2*$^{dm2}$†, BALB/cBy† |
| Others | A†, A-*T1a*$^b$, DBA/2, MOLI/E†, CAST/Ei† | P/J, C3H/HeJ† A.CA |

*All tested strains with the 3.7-kb fragment were Qa-2$^+$, while all tested strains lacking the 3.7-kb fragment were Qa-2$^-$.
†Strains also tested with the C1432 probe. In each case, the genotype was confirmed.

## DISCUSSION

On the basis of genetic mapping studies and comparisons of mutant vs. wild-type strains, we have identified a DNA fragment that maps to the *Q* subregion and appears missing in all tested Qa-2$^-$ strains and mutants. Gene transfer studies indicated that this fragment is responsible for a Qa-2 family antigen that is very similar to that described by Sharrow *et al.* (13) and Oudshoorn-Snoek *et al.* (24). The use of a single-copy probe prepared from this fragment confirmed its identity and its deficiency in the spontaneously occurring Qa-2$^-$ family mutant, BALB/cBy.

Several points are worth noting about this fragment and its gene product. First, the strain distribution of our prepared antisera against transfected cells did not completely correlate with the presence of *Qa-2*. This antiserum was cross-reactive with *H-2*$^b$ molecule(s). One possible explanation for this cross-reactivity is that presentation on the *H-2*$^k$ genotype rather than on *H-2*$^b$ (used for most Qa-2 immunizations) altered Qa-2 antigenicity. Evidence so far does not support this observation since recently performed H-2$^k$ immunizations (B6-*H-2*$^k$ anti-B6.K3) have never produced such activity (unpublished results). Alternatively, one can postulate that this gene is not the structural gene for Qa-2 but rather a neighboring one. This hypothesis is also difficult to reconcile in light of the complete correlation between the presence of the 3.7-kb *Xba* I fragment and *Qa-2* expression even when *Mus* subspecies and mutants are examined (Table 2). No other fragment besides this 3.7-kb *Xba* I fragment appears changed in the Qa-2$^-$ BALB/cBy strain, although further

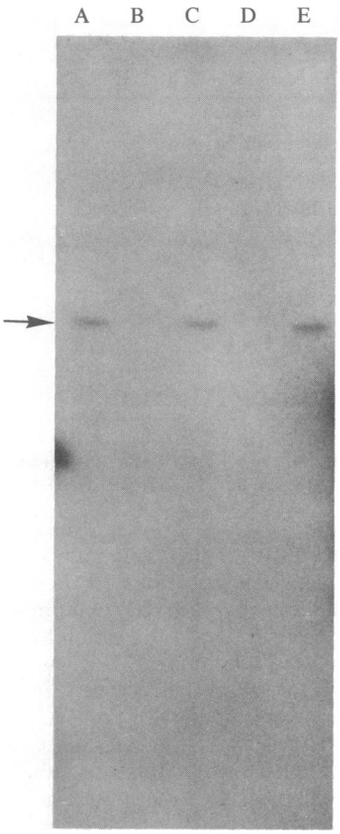


FIG. 3. *Xba* I restriction map of genomic DNA from congeneic and mutant strains by hybridization with the C1432 probe. C1432 contains a 0.5-kb *Pst* I–*Sst* I insert of the 3.7-kb fragment of the C1 cosmid. Lanes: A, B6/Fla; B, B6.K1; C, B6.K2; D, BALB/cBy; E, BALB/cFla.

experiments will be needed to confirm this point.

Our single-copy probe experiments and Southern blot analyses using less-specific cDNA class I probes indicate that the BALB/cBy mutants may have lost an extensive stretch of *Q* subregion DNA. No compensation band was detectable with any of the specific or more general probes used under either stringent or relaxed hybridization conditions. This observation is noteworthy in the light of the different types of polymorphism observed within the MHC. Whereas the *H-2D* and *H-2K* genes have over 200 alleles, each of which determines a protein with a different amino acid sequence, the only polymorphism observed in the *Q* subregion is the presence vs. absence of the various Qa-2 family antigens. Thus, the generation of diversity in this latter subregion may be operating by deletion mechanisms rather than by postulated MHC gene conversion events (14).

Table 2. Activity of B6-*H-2*$^k$ anti-BW5147 cells transfected with the C1 cosmid

| Strain | Genotype | | | | Results of cytotoxicity testing* | | |
|---|---|---|---|---|---|---|---|
| | *H-2K* | *H-2D* | *Qa-2* | *TL* | B6-*H-2*$^k$ anti-BW5147 (C1)† | D3.262‡ | 20-8-4§ |
| B6 | *b* | *b* | + | − | + | + | + |
| B6-*Tla*$^a$ | *b* | *b* | + | + | + | + | + |
| B6-*H-2*$^k$ | *k* | *k* | − | − | − | − | − |
| B6.K1 | *b* | *b* | − | − | + | − | + |
| B6.K2 | *b* | *b* | + | − | + | + | + |
| B6.K3 | *k* | *k* | + | + | + | + | + |
| B6.K4 | *k* | *k* | − | + | − | − | − |

*Lymph node cells were tested in a direct complement-dependent cytotoxicity test as described (2). +, >40% cytotoxicity above controls; −, <5% above controls.

†This antiserum is a result of multiple immunizations of B6-*H-2*$^k$ with BW5147 cells transfected with the C1 cosmid.

‡D3.262 is a monoclonal antibody against Qa-2 (22).

§The monoclonal antibody, 20-8-4, has been described by Sharrow *et al.* (13).

It is also noteworthy that the Qa-2$^+$ *Mus* subspecies MOLI/Ei and CAST/Ei possess an identically-sized 3.7-kb *Xba* I band, hybridizable with the single-copy probe C1432. On the basis of mitochondrial RFLPs, Yonekawa *et al.* (25) have estimated that the time of divergence between these two strains and inbred laboratory strains is between 2.5 and 1.1 million years. Thus, even though this gene sequence appears highly conserved throughout *Mus* evolution, its presence is not necessary for survival. Again, these results are in sharp contrast to those obtained for the *H-2D* and *H-2K* genes. Here, these genes have extensive polymorphisms yet seem to have an important survival function in immune regulation.

One possible hypothesis that could explain these apparent discrepancies is that *Q* subregion genes operate by different mechanisms from those in the *H-2K* and *H-2D* subregions. They may be important for survival, but one *Q* subregion gene can substitute for another—behavior similar to that of *H-2* genes in associative recognition. However, *Q* subregion gene mutations cannot be tolerated by the organism. This situation is true for certain other multigene families such as hemoglobin, where the persistence of fetal hemoglobin production can compensate for extensive deletions in the adult hemoglobin δ- and β-chain genes (26). Yet, certain mutations in these same hemoglobin genes can cause severe anemia. In this respect, it will be very interesting to compare the actual DNA sequences of these 3.7-kb *Xba* I fragments in evolutionary diverse *Mus* subspecies.

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1. Flaherty, L. (1981) in *Role of the Major Histocompatibility Complex in Immunobiology*, ed. Dorf, M. (Garland, New York), pp. 35–57.
2. Flaherty, L., Karl, M. & Reinisch, C. L. (1982) *Immunogenetics* **16**, 329–337.
3. Sandrin, M. S., Hogarth, P. M. & Mckenzie, I. F. C. (1983) *J. Immunol.* **131**, 546–547.
4. Sutton, V. R., Hogarth, P. M. & Mckenzie, I. F. C. (1983) *J. Immunol.* **131**, 1363–1367.
5. Steinmetz, M., Winoto, A., Minard, K. & Hood, L. (1982) *Cell* **28**, 489–498.
6. Mellor, A. L., Golden, L., Weiss, E., Bullman, H., Hurst, J., Simpson, E., James, R. F. L., Townsend, A. R. M., Taylor, P. M., Schmidt, W., Ferluga, J., Leben, L., Santamaria, M., Atfield, G., Festenstein, H. & Flavell, R. A. (1982) *Nature (London)* **298**, 529–534.
7. Steinmetz, M., Moore, K. W., Frelinger, J. G., Sher, B. T., Shen, F.-W., Boyse, E. A. & Hood, L. (1981) *Cell* **25**, 683–692.
8. Cosman, D., Kress, M., Khoury, G. & Jay, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4947–4951.
9. Michaelson, J., Flaherty, L., Hutchinson, B. & Yudkowitz, H. (1982) *Immunogenetics* **16**, 363–366.
10. Margulies, D., Evans, G., Flaherty, L. & Seidman, J. (1982) *Nature (London)* **295**, 168–170.
11. Goodenow, R. S., McMillan, M., Nicolson, M., Sher, B. T., Eakle, K., Davidson, N. & Hood, L. (1982) *Nature (London)* **300**, 231–237.

12. Goodenow, R. S., Stroynowski, I., McMillan, M., Nicolson, M., Eakle, K., Sher, B. T., Davidson, N. & Hood, L. (1983) *Nature (London)* **301,** 388–394.
13. Sharrow, S. O., Flaherty, L. & Sachs, D. H. (1984) *J. Exp. Med.* **159,** 21–40.
14. Mellor, A. L., Weiss, E. H., Kress, M., Jay, G. & Flavell, R. A. (1984) *Cell* **36,** 139–144.
15. Flaherty, L. (1978) in *Origins of Inbred Strains*, ed. Morse, H. (Academic, New York), pp. 409–422.
16. Blin, N. & Stafford, D. (1976) *Nucleic Acids Res.* **3,** 2302–2308.
17. Steinmetz, M., Frelinger, J. G., Fisher, D., Hunkapiller, T., Pereira, D., Weissman, S. M., Uehara, H., Nathenson, S. & Hood, L. (1981) *Cell* **24,** 125–134.
18. Evans, G. A., Margulies, D. H., Camerini-Otero, R. D., Ozato, K. & Seidman, J. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1994–1998.
19. Endow, S. A. (1982) *Genetics* **100,** 375–385.
20. Chaplin, D. D., Woods, D. E., Whitehead, A. S., Goldberger, G., Colten, H. R. & Seidman, J. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6947–6951.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 326–328.
22. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.
23. Shen, F.-W., Boyse, E. A. & Cantor, H. (1975) *Immunogenetics* **2,** 591–595.
24. Oudshoorn-Snoek, M., Mellor, A. L., Flavell, R. A. & Demant, P. (1984) *Immunogenetics* **19,** 461–474.
25. Yonekawa, H., Moriwaki, K., Gotoh, O., Watanabe, J., Hayashi, J., Miyashita, N., Pertras, M. L. & Tagashira, Y. (1980) *Jpn. J. Genet.* **55,** 289–296.
26. Maniatis, T., Fritsch, E. F. & Lauer, J. (1980) *Annu. Rev. Genet.* **14,** 145–178.