The Molecular Basis of Codominant Expression of the Histocompatibility-2 Genetic Region
(transplantation antigens/membrane glycoproteins)

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ABSTRACT H-2 alloantigens in an intact, undegraded form can be solubilized by the nonionic detergent NP-40 and isolated by indirect immunoprecipitation and electrophoresis in sodium dodecyl sulfate on polyacrylamide gels. With the use of an immunological precipitation method, the question about the number of cellular H-2 gene products in heterozygous cells has been partially resolved. At least four separable gene products were detected in cells heterozygous at the H-2 genetic region—one for each of the H-2D genes of the two parental haplotypes, and one for each of the H-2K genes of the two parental haplotypes. This is in contrast to the two H-2 gene products reported previously for cells homozygous at the H-2 region. The findings establish that the two parental H-2 haplotypes on homologous chromosomes in heterozygous cells are ultimately expressed as different glycoprotein molecules.

The murine H-2 alloantigens are cell-membrane glycoproteins that carry some of the antigenic determinants involved in tissue-graft rejection. A complex and markedly polymorphic genetic region in Linkage Group IX controls the molecular expression of these antigens (see refs. 1–3). The genetic polymorphism is well documented by the finding of more than 30 different H-2 haplotypes in inbred laboratory mouse strains and in wild mice (4). Each haplotype determines the cellular expression of a unique profile of H-2 antigenic specificities.

The complexity of the H-2 genetic region is clearly demonstrated by the fact that genes apparently not involved in histocompatibility [e.g., Ss (5) and Ir-i (6)] separate H-2 determinants into two parts. According to the simplest genetic hypothesis, these can be termed the H-2D and H-2K genes (7, 8).

A full understanding of the genetic and evolutionary aspects, and the cellular regulation and biosynthesis of the H-2 system of alloantigens can only come from an understanding of the molecular properties of the gene products themselves. Studies on the chemistry of the H-2 alloantigens have demonstrated that they are glycoproteins (9, 10), whose antigenic sites are probably determined by their primary amino-acid sequence (11, 12). Recent studies (13, 14) with alloantigens solubilized by the detergent Nonidet P-40 (NP-40) have further shown that at least two different H-2 glycoproteins, each the product of either the H-2D or H-2K gene, can be isolated from the cells of mouse strains homozygous at the H-2 region.

Strong immunological evidence exists that antigen expression in F1 heterozygous cells is codominant, i.e., the H-2 haplotypes of both parental chromosomes are expressed (1, 3, 16). By the same methods that allowed us to demonstrate separate H-2D and H-2K gene products in cells homozygous for both H-2 genes, it should be possible to examine the mechanism of the cellular expression of the H-2 genes located on separate chromosomes in the F1 heterozygote.

The present paper presents the results of such studies on the H-2 gene products in cells heterozygous for both the H-2D and H-2K genes, and provides information on the expression of codominance at the molecular level.

MATERIALS AND METHODS

Mouse Strains. Inbred mice were C57BL/10, B10.D2, B10.A, and (B10.A x B10) F1; they were obtained from The Jackson Laboratory, Bar Harbor, Me.

Radiolabeling and Solubilization of Antigen. Short-term cell cultures were used to label spleen cells with tritiated fucose. The methods will be described in detail elsewhere (Cullen, S. E., Nathenson, S. G. & Lundquist, M., in preparation). Briefly, spleens were collected from about 4–6 mice and teased apart, and the cell suspension was strained through cotton gauze into a conical 12-ml tube. The cells were then incubated at 37° under 5% CO2 in RPMI 1640 medium (GIBCO) supplemented with 10% fetal-calf serum and 50 μCi of [3H]fucose (New England Nuclear Corp.)/ml for about 4 hr. The cellular incorporation of [3H]fucose was around 1–3%. Cells were then centrifugated, washed twice in a saline buffer [0.15 M NaCl–0.01 M Tris–HCl (pH 7.4)]–1.5 mM MgCl2], and treated with 0.5% NP-40 (Nonidet P-40, Shell Chemical Co.) as described (13).

Antisera. The following antisera were used: (a) A/J anti-Meth A (H-2b anti-H-2b) was a control serum that detected no specificities on H-2b or H-2k cells; (b) (B10.D2 x A/Sn)F1, anti-B10.A (2R) ((H-2b x H-2k)F1, anti-H-2b) was a serum detecting specificity 2; (c) (B10 x AKR.M)F1, anti-B10.A ((H-2b x H-2k)F1, anti-H-2b) was a serum detecting specificity 4; (d) (B10.D2 x C3H.NB)F1, anti LP.RIII ((H-2b x H-2k)F1, anti-H-2b) was a serum detecting specificity 11 and possibly 25; (e) (B10.D2 x A/J)F1, anti B10.A (5R) ((H-2b x H-2k)F1, anti-H-2b) was a serum detecting specificity 33. Serum 1 was

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prepared by S. G. Nathenson (9) and sera 2 and 3 by M. Cherry and G. D. Snell, as described in (15). Sera 4 and 5 were obtained from the Transplantation Immunology Branch, NIAID, NIH, Bethesda, Md.

**Indirect Precipitin Assay and Gel Electrophoresis.** The indirect immunoprecipitin assay for radiolabeled antigen and the electrophoresis in sodium dodecyl sulfate on polyacrylamide gels were performed as described (13).

**RESULTS**

Indirect immunoprecipitation was used (13) to detect specific radiolabeled H-2 antigen in splenic cell extracts solubilized by NP-40. Four monospecific antisera were used to detect the H-2 determinants on the H-2K or H-2D gene products of both haplotypes. Each specific or control serum was reacted with an aliquot of antigen extract that was solubilized with NP-40; the antibody-antigen complex thus formed was removed, and the resultant supernatant was then retested systematically with the specific sera against other determinants and with the control serum. If two specificities were removed by prior precipitation with a monospecific serum, it could be presumed that both specificities were on the same macromolecule. Conversely, any specificity not removed by the first treatment must have been on a molecule separate from that bearing the first specificity.

For the experiments reported here, we used cells from (B10.A x B10)F1 mice that are H-2$^a$/H-2$^b$ heterozygous. Different sera detecting the four major or "private" (4) specificities of the gene products were used. For the H-2$^a$ haplotype, we measured specificity H-2A to detect the H-2D$^b$ gene products and the H-2.11 to detect the H-2K$^b$ gene product. For the H-2$^b$ haplotype, we measured specificity H-2.33 to assess the H-2K$^b$ gene product, and specificity H-2.2 to detect the H-2D$^b$ gene product.

The [3H]fucose-labeled antigen preparation that was solubilized with NP-40 was divided into five portions (A–E). The first portion, A, was reacted with a control antiserum that could detect none of the specificities known to exist in this heterozygous cell type. B was reacted with a predetermined excess of H-2.2 serum; C with an excess of H-2.4 serum; D with an excess of H-2.11 serum, and E with an excess of H-2.33 serum. Goat anti-mouse gammaglobulin was added in a proportion previously determined to result in equivalence for the precipitation reaction. The precipitates formed in each of these five reaction mixtures were removed. The precipitates in reactions B, C, D, and E contained about 0.5% of the total soluble radioactivity and were about 5–10 times more radioactive than the control precipitate, A.

In the next phase of the experiment, the supernatant solutions from A, B, C, D, and E were each subdivided into five portions and retested for the presence of radioactive material reactive with the five antisera. The results of this second set of alloantigen–antibody precipitin assays are presented in Fig. 1, which shows the polyacrylamide gel patterns obtained from these precipitates.

In the supernatant preparation that was previously treated with control serum, no peak of alloantigen was present when it was examined again with the control serum. However, antigen peaks were clearly present when the supernatant was examined with an anti-2, anti-4, anti-11, or anti-33 serum. When

![Fig. 1](image-url)

**Fig. 1.** Analysis of antigen extracts solubilized with NP-40 by immunoprecipitation. This figure shows the dodecyl sulfate–polyacrylamide gel patterns of precipitates from the reactions of test antisera (shown on the left of the figure) with the supernatant fraction remaining after reaction with the pretreatment antisera (shown across the top of the figure) and removal of the resultant precipitate. The cpm of [3H]fucose-labeled antigen is plotted along the ordinate of each graph. In each case, completeness of the treatment is shown by the lack of a specific $H-2$ peak when the sera are tested for antigen putatively removed by the antiserum treatment. The presence of three gene products beside the one removed in the treatment is demonstrated by finding specific antigen peaks when the remaining specificities are tested (see text for discussion).
the supernatants from the antigen preparations that had been previously treated with sera against specificities 2, 4, 11, or 33 were examined, no antigen peak was detectable with the serum originally used for prior treatment. This showed that the first precipitation was complete. However, antigen peaks were present for the other three specificities tested. For example, removal of specificity 2 caused loss of reactivity with anti-2 antisera, but not with anti-4, 11, or 33 antisera.

The results of these experiments in Fig. 1 thus show that at least four separate gene products of the H-2 region are detectable in NP-40 extracts of heterozygous H-2*/H-2b cells. Each product carried the antigensite characterizing the major or "private" specificity of either the H-2K or H-2D allele of each of the two haplotypes, but not both (see Fig. 2).

**DISCUSSION**

It has been fairly well established from several different lines of evidence (1, 3, 16) that cells heterozygous at the H-2 region express the antigenic determinants of the chromosomes of both parental haplotypes—a property termed codominance (1, 15). The explanation of the molecular nature of this cellular expression was the subject of the studies described in this paper. Essentially one of two possible molecular arrangements might explain the findings of codominance of the H-2 antigen system; (a) two species of separate glycoprotein molecules might exist, each carrying the antigenic site (s) determined by each of the parental haplotypes, or (b) a single species of molecules might carry the antigenic sites determined by both parental haplotypes, i.e., a so-called hybrid molecule.

A study (17) on the H-2 alloantigens from an F1, heterozygous animal has suggested the existence of a hybrid molecule carrying the antigenic sites of determinants from both parental H-2 genes. However, the conclusion was based on the elution behavior by molecular sieve chromatography of solubilized antigen that had been reacted with antibody against specificities of one of the parental genotypes. The interpretation was most likely in error because of the existence of antigen fragments of different sizes produced by the proteolytic solubilization procedure, a complication that was unknown at the time of the experiments.

A recent development in our laboratory (13) has allowed us to examine the cell membrane-free H-2 alloantigens in a condition that we feel is their intact or undegraded state, i.e., without prior breakage of a covalent bond. The detergent NP-40 was used for solubilization, for a short (0.5-hr) period at 4°C to prevent action of autolytic enzymes; a very high yield (of the order of 80-100% of antigen available on the cell surface) was obtained. An indirect immunoprecipitation, followed by polyacrylamide gel electrophoresis of the specific precipitate in sodium dodecyl sulfate, allows isolation and identification of the alloantigen as a single peak.

Studies performed by these methods (14) showed that two different antigen molecules could be isolated from mouse cells homozygous at the H-2 region. These antigen-bearing molecules carried the site of a major or "private" specificity of the H-2K or H-2D specificity profile for that particular H-2 haplotype. In addition, these molecules were found to also carry several minor or "public" specificities whose genetic determinants also mapped in association with the H-2D or H-2K genes. The cell products were, thus, multispecific. This information supported strongly the two-gene hypothesis recently presented as the most likely genetic model for the H-2 region (4, 5). However, due to the limited number of specificities examined, the possibility of more than two genes and, hence, of more than two cellular gene products could not be entirely eliminated.

The studies described in the present paper used immunologic precipitation techniques to examine the H-2 gene products of cells heterozygous at the H-2 region, specifically H-2*/H-2b F1 hybrids. At least four separate gene products were detected by our methods. Two of these cellular products were reactive for the H-2K and H-2D gene specificities 33 and 2, respectively, and two were reactive for the H-2K* and H-2D* gene specificities 11 and 4. Of course, additional gene products, if present, could have gone undetected due to limitations of the methods and the sera used.
However, our findings confirm that in heterozygous cells, as well as in homozygous cells, the H-2K and H-2D genes are expressed as different glycoprotein molecules, and further demonstrate that codominance is the result of simultaneous expression of genes from both parental chromosomes as separable cell membrane glycoproteins, and not as hybrid molecules containing antigenic sites determined by both parental genes.

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