A Closer Look at the Genes of the MHC

Cloning the genes of the major histocompatibility complex helps to clarify both the function and the evolution of the genes

Airlie, Virginia. If any development testifies to the rapid advances that may now be achieved in molecular biology, it is the recent progress in cloning the genes of the major histocompatibility complex (MHC), a large genetic region that controls many of the activities of immune cells. Last year, a mere 18 months after researchers first gained entry to the MHC, they held the first meeting devoted entirely to the cloning work (*Science*, 23 April 1982, p. 400).

Participants had then predicted that cloning of the MHC would shed new light on the evolution of the genes and their products, the histocompatibility antigens, and facilitate analysis of the antigens' functions in mediating the many cellular interactions necessary for normal immune responses. Those predictions are now being borne out, as this year's meeting* made clear, although there was one rather surprising exception in what might be described as the case of the missing suppressor region.

Diversity is one of the hallmarks of the histocompatibility antigens. Class I includes the classic transplantation antigens that trigger rejection of organ grafts by the recipient's immune system. The same antigens are necessary for ordinary immune responses such as the recognition and killing of virus-infected cells by specific killer T cells.

Many different variants of the transplantation antigens have been identified—for example, there are 30 to 50 variants of some class I antigens. The origin of this polymorphism, as it is called, has been a puzzle.

Last year there was much discussion of the possibility that a mechanism in which segments of one gene are replaced by segments of another might contribute. Such a mechanism might be similar to gene conversion as it occurs in yeast, although other ways of achieving the replacement are also possible. This year saw the presentation of additional evidence for a gene conversion–like mechanism. As Jonathan Seidman of Harvard Medical School put it, "Gene conversion was predicted last year and it is turning out to be true."

A class I antigen consists of a large protein, which has a molecular weight of about 45,000 and varies from antigen to antigen, and a small protein called β2-microglobulin, which has a molecular weight of 12,000 and is a constant feature of the antigen molecule. Analyses first of the heavy chain molecules and more recently of the cloned genes have shown that the differences in the various genes occur not as single-point mutations but as clusters of nucleotide changes within short DNA segments. Almost all the clusters are in the gene segments that code for the two outermost domains (regions) of the protein. (A class I heavy chain contains five domains: three on the outside of the cell membrane, each including about 90 amino acid residues; one of about 25 amino acid residues that is embedded in the membrane; and a third of about 30 residues that extends into the cell cytoplasm.)

There may be more than one way of generating diversity in the MHC.

Some of the best evidence for the operation of a gene conversion–like mechanism in the class I heavy chain genes comes from analyzing a series of mutants of the K^b histocompatibility antigen of the mouse. Stanley Nathenson and his colleagues at Albert Einstein College of Medicine have identified seven different K^b mutants (in 50,000 mice) and are comparing the nucleotide sequences of the mutant heavy chain genes with that of the parent gene. They find clusters of nucleotide substitutions in the mutants that correspond to the alterations in protein structure which they had previously determined. Except for these alterations, the mutant genes are identical to the parent. For example, the mutant designated K^{bm1} has a cluster of seven nucleotide changes within a 13-base-pair segment of the gene. "This confers all the characteristics of bm1-ness on the mutant," says Daniel Schulze of the Nathenson group. Richard Flavell and his colleagues at Biogen, Inc., in Cambridge, have also compared the K^b parent and the K^{bm1} mutant and obtained the same results.

A cluster of seven changes would be unlikely to result from single random base changes. If the mutant acquired the altered sequence from another gene, as predicted, then it should be possible to detect the donor in the mouse genome. The Nathenson group did not find a potential donor in a small library of some 30 class I gene clones, but with R. Bruce Wallace of the City of Hope Research Institute in Duarte, California, they found a potential donor in the mouse line from which the mutant arose.

Moreover, the Flavell group identified two potential donors in a much larger library of some 90 class I clones. They determined the nucleotide sequence of one of them and found it to contain a 51-base-pair segment identical to the sequence of the mutant that contains the altered region. Both this and the other potential donor are located in the Tla, Qa region of the mouse MHC, which codes for antigens of unknown function that are present on cells during some stages of differentiation. The function of this region is of particular interest, incidentally, because the cloning work shows that the mouse has some 35 class I genes per haploid genome, and 30 of them are in the Tla, Qa region.

Genes apparently do not need to be near each other to exchange segments. "The donor gene is a long way away—at least a centimorgan away—from the K locus," Flavell says. (A centimorgan is a measure of relative genetic distance; in the mouse it represents about 2000 kilobases.)

There may be more than one way of generating diversity in the MHC. Leroy Hood of the California Institute of Technology presented evidence that the number of gene copies may vary from animal to animal. Gene loss or gain may result from unequal crossing-over if the corresponding genes on the two chromosomes

*"The Second Meeting on Cloning of the H-2 and HLA Regions," held on 17 to 19 April in Airlie, Virginia, was organized by Stanley Nathenson and Jack Strominger and sponsored by the Naval Medical Research and Development Command and the Office of Naval Research.

of a homologous pair are not lined up exactly right when the chromosomes exchange segments during meiosis. Hood says, "It seems quite likely that not only does one have gene conversion but also unequal crossing-over with expansion and contraction of gene numbers."

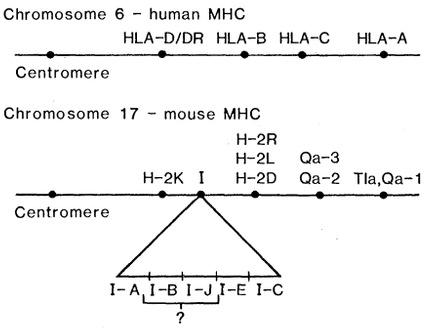
For technical reasons, class II antigens are harder to study than class I, although that situation is changing rapidly. The past year has seen an explosion in the number of clones of class II genes that have been isolated and sequenced, thus greatly facilitating analysis of the structures of the proteins themselves.

Class II antigens, which regulate such immune responses as antibody production by B cells, consist of three protein chains. In some of the antigens, the α chain, which has a molecular weight of 33,000, varies from molecule to molecule. In others this chain appears constant, at least at the level of protein structure. However, the β chain, which has a molecular weight of 28,000, is polymorphic in all the class II antigens. The third chain, the γ chain, is invariant.

The α and β chains, despite their size differences, have remarkably similar overall structures. Each has four domains, two on the outside of the cell, a transmembrane region, and a cytoplasmic region. Moreover, the patterns of nucleotide changes are similar in the chains that vary. "For class II β, as for DCα, there is a region which is very variable and one that doesn't vary much at all," says Per Peterson of the University of Uppsala, Sweden. Most of the differences occur in the first external domain, whereas the second external domain is highly conserved and has a structure similar to an antibody domain. These structural features are found in the class II antigens of both mouse and human.

The full extent of polymorphism in the class II genes is not yet known. However, Hugh McDevitt of Stanford University School of Medicine points out, "There is a lot of polymorphism, but it doesn't appear to be as great as in class I." Some class II genes may have 10 to 15 variants, compared to the 30 to 50 for some of the class I genes.

Gene conversion may help to generate the polymorphism of some class II genes, at least in the human. Charles Auffray, Jack Strominger, and their colleagues at Harvard University compared the sequences of two alleles (variants of the same gene) of a human α chain gene and found that the products specified by these alleles differed by 24 amino acids, 20 of which are in the first external domain. "A simple accumulation of point mutations is unlikely to explain that many changes clustered in one region," Strominger says. The Strominger group found another class II α gene that has nearly all the altered sequences found in one of the two alleles and might be a donor for gene conversion.



The MHC's of mice and men

In the human, the genes for the class I (transplantation) antigens map to the HLA-A, -B, and -C regions of the MHC. The HLA-D/DR region contains the genes of the class II antigens. In the mouse the class II genes are in the region designated I, which lies between the class I regions. The I region had been subdivided as shown, but the cloning work has failed to find the I-B and I-J regions in that position. Their locations are currently unknown. Although the Qa and Tla regions carry most of the class I genes of the mouse, the functions of these genes are unknown.

For the mouse α chains, the situation may be different, according to McDevitt and Christophe Benoist, also of Stanford. They, too, find clusters of mutations in the first external domain, but have not detected any donor sequences. Whereas the human MHC carries four or five different α chain genes per haploid genome, the mouse MHC apparently carries only two and has less potential for gene conversion.

The human MHC also seems to carry more functional β chain genes, at least six compared to the two functional genes found so far in the mouse. "Either the mouse people have missed something, which is unlikely," Strominger says, "or man has undergone gene expansion in this region."

Several groups reported at Airlie that they had cloned the gene for the γ chain of class II antigens. This gene and its product have some unusual features. The transmembrane region of the protein is near the amino terminal. In the other proteins of the class I and class II antigens the transmembrane region is near the carboxyl terminal. Moreover, the γ chain gene is not located in the MHC but is at some other unknown site.

Histocompatibility antigens mediate recognition and interaction between cells, whether killer T cells and their targets or immune cells that cooperate to regulate immune responses. Researchers have been using transfection experiments, in which the cloned genes are transferred into cultured cells of a different histocompatibility type, to study the functions of the class I molecules. They have shown that the products of transferred class I genes are made in the recipient cells, integrated into the cell membrane, and recognized by immune cells of the corresponding histocompatibility type. In other words, the genes and their products work normally.

Results of gene transfer experiments that were presented last year by Robert Goodenow of the Caltech group suggested that an incomplete gene of a class I heavy chain might combine with the DNA of the corresponding endogenous gene to form a complete gene. Since then the original observation has been confirmed and extended, according to Hood, who presented the new findings at Airlie.

The Caltech workers made a series of truncated L^d genes and transferred them separately into cultured mouse cells, which carry a different L allele. Apparently complete L^d antigen subsequently appeared on the surfaces of the transfected cells. "I can say that in every case where the third exon was present we were able to generate putative hybrid [antigen] molecules," Hood explains. "We generated molecules incredibly similar to L^d molecules." (The third exon of the gene had to be present in the truncated gene because this coding sequence specifies the region of the histocompatibility antigen that reacts with the specific antibodies used for detection.)

The Caltech workers repeated the transfer experiments with cloned truncated K and D genes. "We always generated products similar to the original," Hood says. Each truncated gene appeared to incorporate into the genome in such a way that it formed a complete gene of the right type. For reasons that are not yet clear, however, this type of site-specific recombination was not seen by Seidman and David Margulies of the National Institute of Child Health and Human Development (NICHD), although they used conditions similar to those of the Caltech experiments. They did obtain recombination, resulting in the formation of a complete hybrid gene, when they transferred together the left-hand portion of one class I gene and the right-hand portion of a second.

Whether such specific recombination is a peculiar property of class I histocompatibility genes or a more general property of the members of multigene families or even of unique single genes is current-

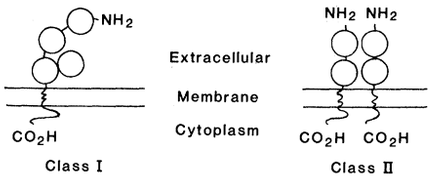
ly not known. It could be very beneficial to gene transfer experiments if a general effect. A transferred gene segment might then be used to repair a defective endogenous gene, permitting it to be expressed normally, which is a must for successful gene therapy.

Specific recombination could also be a complicating factor in interpretation of transfection results if, for example, even intact genes should undergo recombination with endogenous histocompatibility genes, although this may not be likely. Very high concentrations of cloned genes—about ten times higher than are normally used in transfection experiments—had to be used to obtain the recombination seen in the Caltech experiments.

By producing defined alterations in class I genes before transferring them into cultured cells, investigators can determine which portions of the antigens are needed for recognition by attacking immune cells and for other aspects of antigen function. Seidman and his collaborators at Harvard and NICHD transfected cultured cells with hybrid genes consisting of some L^d gene segments and others contributed by the D^d gene. They found that T cells recognize primarily the outer two domains of the heavy chain. In analogous experiments with hybrid human class I genes, Bertrand Jordan and his colleagues at the Centre d'Immunologie de Marseille-Luminy obtained similar results. The importance of these two domains for cellular recognition is not unexpected because they are the sites of most of the differences seen in the antigens. The third domain—the part of the molecule that binds to β2-microglobulin—is highly conserved among the class I heavy chain genes.

Deletion of the gene segments that code for the intracytoplasmic portion of the class I heavy chain has little effect on its function. Seidman says, "In most assays, this truncated protein works normally and can't be distinguished from the normal protein." Because the cytoplasmic domain is in contact with internal cell structures, there have been suggestions that it might be involved in signaling the information about the recognition event to the cell interior, but that is apparently not the case.

It may be needed to recognize some viruses, however. Seidman and his colleagues found that killer cells could not recognize vesicular stomatitis virus in conjunction with a histocompatibility antigen that lacked the cytoplasmic component, although they, and Martha Zuniga of the Caltech group, found that other viruses could still be recognized.



Histocompatibility antigens

A class I antigen consists of a heavy, variable chain and a light chain, β2-microglobulin, which binds to the heavy chain domain nearest to the cell membrane. A class II antigen consists of three chains. Two of these, designated α and β, have similar overall structures, even though the molecular weight of the α chain is about 5000 higher than that of the β chain. They are arranged in the membrane as shown. The way in which the third, invariant chain fits into the molecular is currently unknown.

Finally, investigators are using transfection experiments to study the regulation of class I gene expression, both at the level of transcription of the gene into messenger RNA, the first step in protein synthesis, and at later stages. Interferon has been shown by several groups, including those of Jordan and of Sherman Weissman at Yale University School of Medicine, to increase the transcription of class I genes. Enhanced expression of class I antigens on cell surfaces, which aids in the recognition and killing of viral infected cells, is part of interferon's antiviral action.

The amount of class I antigen actually expressed on the cell surface does not depend just on how much heavy chain messenger is made, however. Availability of β2-microglobulin may limit cell surface expression, according to Seidman and John Weis, who is also at Harvard.

Like the class I genes, the class II genes can be transferred into cultured mouse cells, where their products are made and expressed on the cell surface. For example, Bernard Mach and his colleagues at the University of Geneva, Switzerland, found that α human class II antigen could be expressed on mouse cells provided both the α and β chain genes were transferred together. Somewhat surprisingly, the γ chain gene was not required for such expression, even though the γ chain is thought to be a component of class II antigens.

Functional studies of class II antigens cannot easily be carried out in L cells, which are fibroblasts. The class II antigens mediate the activities and interactions of immune cells, including B cells and macrophages. Several groups are now trying to introduce class II genes into appropriate effector cells.

One disappointing and surprising finding reported at the Airlie meeting was the inability to find the gene or genes coding for the factors that suppress antibody production by B cells. These had been mapped to the I-J region of the mouse MHC.

Last year, Michael Steinmetz, who was then working in Hood's laboratory, reported that he had identified a probe that extended into the I-J region. The hope was that the suppressor gene could be cloned, permitting many uncertainties about the nature and function of the suppressor molecules to be cleared up. But, as Steinmetz, who is now at the Basel Institute of Immunology, and his Caltech collaborators told the Airlie participants, the putative suppressor region has now been thoroughly examined by restriction mapping and cannot contain a suppressor gene. The I-J and also the controversial I-B site had been mapped to a position between the I-A and I-E sites in the class II region of the mouse MHC. However, Steinmetz showed that there were at most 3.4 kb of DNA between the I-A and I-E sites. Moreover, that region contains segments of a gene coding for one of the mouse β chains, which is designated $I\text{-}E_\beta$. The investigators have eliminated the possibility that some modified product of this gene serves as the suppressor molecule.

That leaves the conclusion that the suppressor gene was erroneously mapped, although the work has not ruled out the possibility that the I-J site somehow regulates the formation of suppressor, which could give it the appearance in genetic mapping studies of encoding suppressor itself.

Steinmetz has found a great deal of polymorphism in the region extending from the K region to the I-E gene, and also in the D region of the mouse MHC, which indicates that these areas are undergoing genetic rearrangements. The regions between these are more conserved in structure. But there is a "hotspot" for recombination just at the $I\text{-}E_\beta$ gene, which could have affected the accuracy of the genetic mapping studies that assigned the suppressor gene to the I-J region. For now, the location of the suppressor region remains unknown.

Cloning of histocompatibility genes for both class I and class II antigens has now become routine. With a growing repertoire of the genes available for gene transfer studies, Mach suggests that it may be time to turn the research over to cellular immunologists, who have the expertise to do the more detailed analysis of immune cell interactions that are needed to unlock the mysteries of how the histocompatibility antigens work.

—JEAN L. MARX