Likely T Cell Receptor Gene Cloned

If further research confirms cloning of the T cell receptor gene, understanding of regulation of immune responses may follow

Immunologists appear to have taken another big step toward the characterization of the T cell receptor for antigen, according to data presented last month at the World Immunology Congress in Kyoto, Japan. Mark Davis, who until recently was at the National Institute of Allergy and Infectious Diseases (NIAID), described the cloning of a gene that has all the characteristics expected of a gene coding for a T cell receptor protein.

Earlier this year (*Science*, 29 July, p. 444), several investigators reported that they had identified proteins with the predicted characteristics of the T cell receptor. But a major goal all along has been to identify and clone the receptor genes themselves because it is now much easier to determine the nucleotide sequences of genes and then derive the amino acid sequences of the proteins they encode than it is to sequence the proteins themselves. Biochemical characterization of the T cell receptor should help clear up many of the mysteries concerning T cell recognition of antigen and help in attaining a better understanding of the myriad interactions between the cells and their targets and, consequently, of the regulation of immune responses.

Analysis of the receptor proteins themselves would have ultimately led to cloning of the genes. But Davis, who began his efforts to clone a T cell receptor gene about 3 years ago at NIAID, pioneered a different approach to the problem of identifying genes that are expressed at low levels.

Davis, who is now at Stanford University School of Medicine, and his NIAID colleague Stephen Hedrick, started by making three assumptions about the nature of the genes coding for T cell receptor proteins: that the genes would be expressed in T cells but not in the antibody-producing B cells that use immunoglobulins as their receptor for antigen; that the products of the genes would be located on the T cell membrane, the only site where they could make contact with the appropriate targets; and that the genes would be rearranged in mature T cells as compared to their organization in germ line cells.

This last assumption was based on analogy to the structures of immunoglobulin genes. Both T cell and B cell receptors appear to recognize an essentially unlimited number of foreign antigens. B cells generate the required diversity of immunoglobulins partly by assembling the peptide chains of which the molecules are formed from three or four separate pieces of DNA. These rearrangements take place as the B cells mature. Davis and Hedrick assumed that T cells would generate their receptor genes in the same way.

To obtain a probe for the T cell receptor gene, the investigators first separated the messenger RNA's (mRNA's) that were attached to ribosomes bound to the membranes of the T cell endoplasmic reticulum from the mRNA's that were attached to the free cytoplasmic ribosomes. Membrane proteins, such as those of the T cell receptor, should be synthesized on the bound ribosomes, not on the free ones. They then copied the mRNA's from the membrane-bound ribosomes into DNA (cDNA).

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To eliminate genes that are also expressed in B cells, Davis and Hedrick repeatedly hybridized the T cell cDNA's with mRNA's prepared from B cells. The double-stranded cDNA-mRNA hybrids could easily be separated from the single-stranded cDNA's that corresponded to genes that are transcribed into mRNA's only in T cells. The product of this step consisted of the cDNA's of genes for membrane-bound proteins that are expressed in T cells but not in B cells.

These cDNA's served as a probe for detecting receptor genes in a library of T cell specific cDNA clones prepared from a T cell hybridoma line by David Cohen of NIAID.

The probe picked out approximately 500 of these as potential T cell receptor genes. The NIAID workers selected about 150 of these, primarily those present in only a few copies, and reselected with the probe. This second screening step identified about 40 clones for further study, which turned out to represent sequences from 11 distinct genes. Davis and Hedrick eventually ended up with seven candidate genes.

One of these genes coded for the protein Thy-1, a membrane protein that is characteristic of mature T cells. "We thought we were pretty close at this point," Davis says. The investigators then looked to see which of the final group of cDNA's represented genes that might be rearranged in mature T cells by comparing their structures with the gene structures in liver cells, which should not be expressing any T cell receptor proteins and should thus have the genes in their unrearranged form.

They found that one of the cDNA's had been rearranged. The same gene is rearranged in all of the T cell lines that have been examined. "There was a systematic rearrangement that seems different in the gene from different cell lines," Davis says. Variations in T cell receptor genes from cell line to cell line are also expected.

Finally, Davis and Hedrick used the cDNA of the rearranged gene to screen a library of gene clones prepared from normal thymocytes by Christophe Benoist, who has recently moved from Stanford to the University of Strasbourg. Examination of three of the clones thus identified revealed that they were different at their 5′ ends and identical at their 3′ ends, a structure which suggests that their products may have features in common with the variable and constant regions of immunoglobulin chains.

The largest of the three thymocyte DNA clones, which was some 930 base pairs in length, was sequenced and checked to see what other proteins its product resembled. "The first 21 best matches were immunoglobulins," Davis says, "and 29 of the first 40 were immunoglobulins." The regions of greatest homology tended to be centered around the amino acid cysteine, which occupies similar positions in the protein encoded by the candidate T cell receptor gene and in the immunoglobulins.

Despite these similarities most investigators do not expect the T cell receptor proteins to be encoded by immunoglobulin genes. Instead, the situation regarding the T cell receptor genes may be analogous to that of the genes for the histocompatibility antigens, which are also important for interactions between immune cells.

A number of questions remain to be answered about the gene cloned by Davis and his colleagues. Although it has the expected characteristics of a gene coding for a T cell receptor protein, there is as yet no evidence regarding its function or that of its product. Nevertheless, it should soon be possible to transfer the cloned gene into cells, see whether the product is expressed on the membrane as it should be, and determine whether the product can alter antigenic recognition by T cells and whether antibody to the product can block T cell function.

Also important are the questions about the number and organization of the sequences in the genome that carry information that may be used to produce functional T cell receptor genes and whether the gene cloned by the Davis group can be used as a probe to identify related sequences. But if further work confirms that the cloned gene is the first example of a T cell receptor gene, as it appears to be, it may provide the key to unlocking the secrets of T cell recognition of antigen.—**JEAN L. MARX**

Scheme to Foil Software Pirates

Three Israeli scientists have proposed a new scheme to protect computer programs from illegal copying. If adopted, their method could eliminate a major headache in the computer software industry and one that has driven some manufacturers out of business. The scheme was devised by Adi Shamir, a mathematician at the Weizmann Institute of Science, who is known for his innovative work in cryptography, and his two students Amos Fiat and Yossi Tulpan. A manuscript describing their idea is just now being circulated in the scientific community. "It is a very clever idea," says Ronald Rivest of the Massachusetts Institute of Technology.

Shamir proposes that software manufacturers modify their computer programs to create special sections that contain weak bits—meaning bits that are sometimes read as a 0 and sometimes as a 1. These special areas will serve in his scheme as "the software equivalent of a coin-operated machine," he says. Most important, personal computer owners will not be able to duplicate the weak bits on their own machines and the programs will not run without them.

The problem facing the software industry is that computer programs on floppy disks can easily be copied. These programs often are quite expensive; business programs frequently cost hundreds of dollars and even game programs typically cost about $35. Blank disks, however, are cheap, costing only a few dollars. Thus many people who buy computer programs copy them and distribute them to their friends. In the end, Shamir points out, "this practice penalizes other users by forcing them to pay more for legally obtained software."

A few years ago, the manufacturers thought they could solve the piracy problem by writing the program on the disk in nonstandard ways—writing in blocks distributed in a spiral pattern along the disk or writing between the grooves of the disk, for example, so that the disk could not be copied. But a few enterprising entrepreneurs quickly began selling special programs, with names like Locksmith and Nibbles Away, that unscrambled this copy protection scheme. It was legal to sell such programs because users of computer software are entitled to make backup copies for their own use.

Shamir thinks his method, based on a statistical approach to error analysis, may "be the ideal solution: high security, low cost, no hardware modifications, and complete transparency to the user. The new scheme even solves the harder problem of controlling the number of times rented software can be used." Shamir has been talking to software manufacturers on an informal basis. "All of them like the idea," he says.

Shamir and his colleagues describe their scheme as adding "coupons" to software so that each time the program is run the coupon is reduced in value. The user cannot produce copies of the coupons and cannot modify them. As a result, Shamir says, "A home computer owner will be able to buy a new video game or a cheap diskette which can be used 100 times, and if he likes the game he can buy more expensive 500, 1000, or unlimited use diskettes."

The "coupons" are produced by software manufacturers who modify their disk drives so that they write some weak bits on the diskette. Weak bits sometimes occur by chance when disks are produced but they are corrected by rerecording the data or by error-correcting codes. Shamir proposes, however, that hundreds of weak bits be intentionally written on certain tracks or sectors of the diskette which are explicitly chosen by the computer programmer and hidden within the program.

When a consumer uses such a program, his computer is instructed to check for weak bits by reading over the coupon section several times. If weak bits are there, they will show up sometimes as a 0 and sometimes as a 1. The computer checks to see that there is no consistency in the way the coupon is read. Every time the program runs, it is instructed to destroy one of the weak bits by changing it to an unambiguous 0 or 1. Eventually, there will be few weak bits left and the program will no longer run unless a new coupon is purchased.

Consumers could also purchase programs that will run indefinitely. This would be done by instructing the home computer never to alter the coupon, a method known as write-protecting.

The program also can be copied onto backup disks or moved to a faster and larger hard disk. The original disk then would be used for license verification. The Israeli scientists note that this license has the advantage that it is not affected by wear and tear. "A few more errors in some of the coupons are unlikely to change the validity of the license," they say. The program can also monitor how many coupons are left so that users can know well in advance that they need to replace their disk.

The reason the coupon scheme should work, Shamir points out, is that disk drives on home computers are designed to work reliably. They are incapable of introducing weak bits. If a consumer tries to copy the coupons, he will get a copy containing only normal bits, meaning unambiguous 0's and 1's that are put in more or less arbitrarily by the computer whenever a weak bit is encountered.

Of course, no protection scheme can ever be completely secure. In the case of Shamir's system, some hardware experts will undoubtedly be able to modify their own disk drives to copy weak bits. But most software pirates are unlikely to want to fool around with the hardware in their delicate and expensive disk drives, Shamir contends. "The real problem is not to create a fool-proof system but to make sure that for most users it makes more economic sense to rent or buy the software than to try to steal it. In this billion dollar industry, even a partial reduction in software misuse will have enormous economic impact," he says.

—**GINA KOLATA**