The Interleukin-2 Receptor Gene Is Cloned

Cloning of the interleukin-2 receptor gene should help researchers understand both the normal and abnormal responses of T cells

Persistence, it seems, pays off. The secrets of the T cell, once a major source of frustration for immunologists, are now being resolved at an accelerating pace. Researchers have taken two major steps toward understanding the activation of T cells, which play a central role in bringing about immune responses. The long elusive T-cell receptor for antigen is finally in hand (see box, p. 1065). And three groups of investigators have now cloned DNA's that correspond to the gene coding for the receptor for the lymphokine interleukin-2.

Whereas the receptor for antigen is part of the first stage of T-cell activation, in which certain T cells are specifically primed to begin working, the interleukin-2 receptor is part of the common pathway that they must then follow to mount an effective immune attack. Interleukin-2, by binding to the receptors that appear on the antigen-stimulated cells, causes the cells to proliferate, thus greatly enlarging the active population. "Expression of the interleukin-2 receptor is pivotal to whether the T-cell response succeeds or fails," notes Warner Greene of the National Cancer Institute (NCI).

Not only will cloning of the interleukin-2 receptor gene greatly facilitate the study of normal T-cell responses, but it may also aid in the understanding of diseases in which T-cell proliferation is abnormal. In particular, it may help to understand adult T-cell leukemia, which is caused by human T-cell leukemia virus I (HTLV-I). The leukemic cells carry greatly increased numbers of the interleukin-2 receptor, which may contribute to their uncontrolled division. Moreover, another member of the HTLV family, HTLV-III, causes AIDS (acquired immune deficiency syndrome), which is characterized by T-cell death rather than proliferation, and it will be interesting to determine whether this virus has any effect on production of the receptor.

The three groups that cloned DNA's corresponding to the interleukin-2 receptor gene all used similar approaches and obtained comparable results. They first isolated the receptor by precipitating it with a monoclonal antibody. Greene, Warren Leonard, and their NCI colleagues and also Tasuku Honjo and his colleagues at Kyoto University in Japan* used the same monoclonal antibody, which was prepared by Takashi Uchiyama of the Kyoto group when he was working in Thomas Waldmann's NCI laboratory. The third group, from Immunex Corporation in Seattle, which has not yet published their data, isolated the receptor with their own monoclonal antibody.

The investigators then determined the sequence of amino acids on the amino terminus of the receptor protein, constructed DNA probes corresponding to that sequence, and used the probes to pick out the cDNA's (DNA's copied from the messenger RNA's of cells that make the receptor) with the complementary structure.

The investigators have determined the nucleotide sequence of the cloned cDNA's and from it deduced the amino acid sequence of the receptor protein.

> "Expression of the interleukin-2 receptor is pivotal to whether the T-cell response succeeds or fails."

The protein consists of 251 amino acids and has a molecular weight of about 28,500, but this is brought up to 55,000 by the addition of sugars and sulfate groups, among other things. Neither the receptor gene nor the protein shares any significant homologies with other known normal genes or oncogenes.

The structural organization of the receptor protein is somewhat unusual. The largest portion, consisting of some 220 amino acids on the amino terminus, is on the outer portion of the cell where it can bind interleukin-2. Then there is a very hydrophobic segment of 19 amino acids that is embedded in the membrane. The surprising feature is the small size of the region that projects into the cell cytoplasm. It consists of only 13 amino acids on the carboxyl end of the protein.

The intracytoplasmic regions of receptors for other growth factors, including epidermal growth factor, are much larger and are kinases, enzymes that attach phosphate groups to cellular proteins, usually to tyrosine residues in the case of growth factor receptors. This activity is probably important for transmitting the growth factor signals to the cell interior. But as Greene points out, "The intracytoplasmic domain of the interleukin-2 receptor is probably too small for an enzymatic function. It is very different from all the other growth factor receptors that are tyrosine kinases." How this receptor transmits its signals to the cell interior remains a puzzle.

Expression of the gene also displays some interesting features. Although there appears to be only one copy of the gene per haploid cell, both the NCI and Kyoto workers find two major size classes of messenger RNA (mRNA) transcripts. These are 1500 and 3500 base pairs long, and both appear capable of producing a functional receptor protein. The gene contains at least two sites at which transcription may be terminated, and the use of alternative sites apparently gives rise to the two size classes of mRNA. Whether the RNA's have different functions is currently unclear.

In addition, Leonard, Greene, and their colleagues have noted that both major mRNA groups contain a subclass of transcripts that have lost a segment of 216 nucleotides from within the protein coding sequence. Because the missing segment is bordered by sequences that typically mark the boundaries of regions to be cut from mRNA molecules, the investigators propose that it has been spliced out of the messengers. Somewhat surprisingly they find that only the unspliced version can produce a functional receptor. Ordinarily, the spliced messenger produces the active protein.

Studying the control of interleukin-2 receptor synthesis is one of the major goals of the investigators. In contrast to the situation with most receptor systems, the formation both of interleukin-2 and its receptor must be induced. The receptor appears on the surfaces of T cells only after they have been specifically activated by antigen binding. This presumably helps to ensure that only the activated cells proliferate in response to the growth factor. Interleukin-2 itself induces an increase in the receptor number. Eventually, however, the number of receptors declines. The decrease, which may contribute to the normal termination of the immune response, results from a decrease in transcription of the receptor gene, according to Greene and

*W. J. Leonard *et al.*, *Nature (London)* **311**, 626 (1984); T. Nikaido *et al.*, *ibid.*, p. 631.

Leonard. The unusual splicing pattern also raises the possibility that receptor number may be partially controlled by regulating splicing of the mRNA's.

Of special interest are the interleukin-2 receptors of cells that have been made leukemic by HTLV-I. These receptors are present in high numbers, some five to ten times more than appear on normal, activated T cells. They may also show qualitative differences. In some leukemic cell lines they are slightly smaller than normal, although this difference is in the carbohydrate and sulfate residues, not in the protein portion of the molecule. However, until receptor genes of normal and HTLV-I–transformed cells are compared, slight differences in amino acid sequence can not be ruled out. All three groups originally cloned the gene from transformed cell lines. Work on the normal gene is under way.

Current work suggests that HTLV-I may transform cells by producing a protein that increases gene transcription. For example, the uncontrolled proliferation of the cells might result if genes that regulate cell division are abnormally switched on. One obvious candidate for this is the interleukin-2 receptor gene. The cloning work means that this hypothesis can now be readily tested.

—JEAN L. MARX

More on the T-Cell Receptor

The remarkable progress in characterizing the T-cell receptor for antigen continues. Two groups, one from Mark Davis's laboratory at Stanford University School of Medicine and the other including Susumu Tonegawa, Herman Eisen, and their colleagues at the Massachusetts Institute of Technology, have just reported the cloning of what they carefully call "a third type of T-cell receptor gene"(*1*). However, there can be very little doubt that the investigators have in fact cloned genes for the α-chain of the T-cell receptor, which, as other investigators have shown, consists of two protein chains. Davis, in collaboration with Stephen Hedrick of the University of California at San Diego, and, independently, Tak Mak, Yusuke Yanagi, and their colleagues at the Ontario Cancer Institute in Toronto, cloned the first β-chain genes about a year ago. With the α-chain gene now in hand, immunologists are getting their first look at both T-cell receptor proteins.

The new clones displace another cloned gene that the MIT workers had proposed earlier this summer as a candidate α-chain gene. This gene has many of the predicted characteristics of a T-cell receptor gene. It is expressed only in T cells, is structurally related to the β-chain and immunoglobulin genes, and, like these genes, rearranges to produce the active form as T cells mature. Further work has made it extremely unlikely that this is an α-chain gene, however. In particular, the protein it encodes lacks sites for attaching the *N*-linked sugar residues that are commonly found on receptor proteins and have now been shown by various investigators to be present on α-chains from several sources. Moreover, John Kappler and Philippa Marrack of the National Jewish Hospital in Denver and their colleagues have determined portions of the amino acid sequence of a human α-chain and find little resemblance to the protein encoded by the earlier MIT clone (*2*).

The evidence that the new clones represent α-chain genes appears unassailable. They meet all the criteria listed above and their proteins have appropriate glycosylation sites. The structural organization of their products resembles those of the β-chain and antibody proteins. The β-chain, like the heavier of the two chains of which antibodies are composed, consists of four separately encoded regions, which have been designated as the V (for variable), D (diversity), J (joining), and C (constant) regions. The current work shows that the α-chain consists at least of V, J, and C regions. It may also contain a D region, although this is not yet certain. Moreover, the clone prepared by the Stanford group from helper T cells, and the one prepared by the MIT group from cytotoxic T cells, contain the same C-region coding sequence. Their V regions are different, which is to be expected because the cells of origin recognize different antigens.

In addition, an α-chain peptide with 17 amino acids that was sequenced by the Kappler-Marrack group is almost identical to corresponding segments of the proteins encoded by the clones of the Stanford and MIT groups. Finally, Davis and his colleagues note that comparison of their murine α-chain sequence with that of a human α-chain studied in Jack Strominger's laboratory at Harvard University suggests that the two are species variants of the same molecule (*3*).

The function of the gene represented by the earlier MIT clone remains an intriguing puzzle. The gene has a number of interesting features. According to Tonegawa, it varies slightly in structure from one T-cell line to another, which is one of the predicted characteristics of a gene coding for a receptor, such as the T-cell receptor, that must recognize many different antigens. The T-cell receptor recognizes antigens only in conjunction with appropriate histocompatibility molecules. For many years there has been a debate over whether there are two separate receptors on the T cell, one for antigen and one for the histocompatibility molecule, or a single receptor that recognizes both together. The evidence generally favors the single receptor hypothesis but, Tonegawa suggests, the variability of the mystery gene raises the possibility that it might encode a second receptor. More work will be required to resolve this issue.

Meanwhile, Davis and his Stanford colleague Phillip Patten have evidence suggesting that the β-chain may have sites for recognizing both the antigen and histocompatibility molecule (*4*). They have compared the amino acid sequences of three recently determined β-chain variable regions with the four that were already known and find that β-chains appear to have seven especially variable subregions, in contrast to the three hypervariable regions found in antibody chains, which are part of the antigen-binding site. The corresponding segments of the β-chain may also bind antigen, the Stanford work suggests, whereas the remaining hypervariable regions may be involved in contacts with the histocompatibility molecule—provided, of course, that one receptor recognizes both.—JEAN L. MARX

Additional Reading

1. Y-H. Chien *et al.*, *Nature (London)* **312**, 31 (1984); H. Saito *et al.*, *ibid.*, p. 36.
2. C. H. Hannum *et al.*, *ibid.*, p. 65.
3. N. Jones *et al.*, *Science*, in press.
4. P. Patten *et al.*, *Nature (London)* **312**, 40 (1984).