First Portrait of an Oncogene Product

The ras *protein structure, the first to be determined for an oncogene product, provides clues to how the protein might regulate cell activities*

A research article in this issue of *Science* marks a new milestone in the quest to understand the action of the cancer-causing oncogenes. On page 888, Sung-Hou Kim of the University of California, Berkeley, Susumu Nishimura of the National Cancer Research Institute in Tokyo, Eiko Ohtsuka of Hokkaido University in Sapporo, and their colleagues report the three-dimensional structure of a protein encoded by a member of the *ras* oncogene family. "This is the first oncogene protein for which the structure has been determined," Kim says. "It provides a structural basis for understanding the biological function of *ras*."

The *ras* genes are the most common oncogenes isolated from cancer cells. Active *ras* genes have been found in 40% of the human colon cancers removed during surgery and also in several other types of malignant cells. But studies of the *ras* genes may do more than contribute to a better understanding of cancer development. All oncogenes, including those of the *ras* family, are mutated forms of cellular genes that regulate growth and development. The hope is that a better understanding of oncogene action will aid in unlocking the mysteries, not just of cancer, but of normal cell regulation as well.

In the current article, the Kim group describes the three-dimensional structure assumed by the first 171 amino acids of a normal *ras* gene, the human counterpart of an oncogene originally detected in the Harvey sarcoma virus. The researchers used recombinant DNA technology to make the protein that they analyzed in bacterial cells.

The recombinant protein has the same biochemical activity as the natural *ras* protein, Kim notes, even though it was made without the last 18 amino acids of the normal protein. This segment was omitted because it is very flexible and might have interfered with the crystallization of the protein. The 171–amino acid segment that was analyzed contains the active sites that perform the known biochemical functions of the molecule, however.

Determination of the amino acid sequences of the *ras* proteins had previously shown that they are structurally related to the G proteins, which are thus called because they bind guanosine triphosphate (GTP). The G proteins, and apparently the *ras* proteins as well, transmit hormonal and growth factor signals from the cell membrane to the interior of the cell where the changes elicited by the stimuli are brought about.

The G proteins, including the normal *ras* proteins, are also enzymes that split off the third phosphate of GTP, thus producing guanosine diphosphate (GDP), which remains bound to the G protein, and inorganic phosphate. This conversion of GTP to GDP may serve to inactivate the proteins temporarily so that they do not continue to transmit signals after the original stimulus has ceased.

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The crystallographic analysis is helping to identify the amino acid residues that participate in the binding and splitting of GTP by the *ras* protein. Overall the molecule has an almost round shape. The protein chain is folded so that it contains four α helices, a β sheet consisting of six strands, and nine loops that connect the helices and β strands.

The GDP bound to the *ras* protein is visible in the crystal structure. The amino acids of loop 1 are in contact with the GDP phosphates and are therefore prime candidates to be part of the active site that splits the GTP. This agrees with what is already known about *ras* protein action. "The information that we are getting from these [structural] studies fits very well with what we know from molecular and biochemical studies," says Mariano Barbacid of the National Cancer Institute's Cancer Research Facility in Frederick, Maryland, who has been studying the *ras* gene activities for many years.

Mutations that change only one of a limited number of critical amino acids are sufficient to convert a normal *ras* gene to an active oncogene. As a consequence of these activating mutations, the proteins produced by the genes lose their ability to split GTP. The supposition is that this effectively locks them in the "on" position, so that they continue sending stimulatory signals to the cell interior under circumstances in which they should be shut off.

The x-ray structure shows that all of the critical amino acids are contained within loops 1, 4, and 7. As mentioned, the loop 1 amino acids are in contact with the GDP phosphates. Those of loop 7 contact the guanine. Changes in the amino acids of the two loops might therefore affect the ability of a *ras* protein to bind or split GTP. Although loop 4 is not itself in contact with the GDP, it is in direct contact with loop 1, which might well be distorted and inactivated as a result of alterations in the amino acids of loop 4.

The three-dimensional structure of the human *ras* protein resembles that proposed for the *ras* products a few years ago by Frances Jurnak of the University of California, Riverside, and, independently by a group of investigators including Frank McCormick of the Cetus Corporation in Emeryville, California, and Brian Clark of the Aarhus University in Denmark. These investigators had determined the three-dimensional structure of a bacterial GDP-binding protein that is related to the *ras* proteins, and from this they deduced what the *ras* proteins themselves might look like.

Kim and his colleagues are now moving ahead to determine the crystal structures of the proteins encoded by two activated *ras* oncogenes. Preliminary results indicate little difference between the structures of the mutant proteins and that of the normal molecule. This indicates, Barbacid says, "that the mutations affect the catalytic site, but do not affect the structure."

Small structural differences at the catalytic site may not have been picked up in this early comparison, however. The importance of the *ras* gene proteins, both in normal hormonal signaling and in cancer development, ensures that interest in the structures will continue. ■ JEAN L. MARX