The Case of the Misplaced Gene

Cancers may develop as a result of the movement of the normal cellular counterparts of the viral onc *genes to new locations*

The idea that specific chromosomal abnormalities are associated with certain kinds of cancer has been around for a long time, even though the significance of the abnormalities has been far from clear. Also long-standing is the hope that what is learned from animal cancer viruses might shed some light on human cancer, despite the fact that most human cancers are thought to be of nonviral origin.

Within the past year or so, research on the chromosome abnormalities has begun to merge with that on the viruses, to the gratification of all concerned. The aberrant chromosomes seen in cells of a human cancer, Burkitt's lymphoma, and in cells of a similar cancer of mice have turned out to contain a misplaced gene, designated *myc*, that has moved from its normal chromosomal location into the region coding for the heavier of the two protein chains of antibody molecules.

The *myc* gene was originally detected as an *onc* (for oncogenic) gene in a virus that causes a blood cell cancer in chickens. Like the *onc* genes of other animal cancer viruses, the *myc* gene has a cellular counterpart from which it is derived. The cellular gene is not itself capable of transforming cells to the malignant state, but acquires that ability when it is picked up by the virus. The current work implies that the oncogenic potential of the normal *myc* gene may also be activated by its movement within cells.

There is an additional implication. The cancers may be a side effect of a normal developmental event that has gone awry. A few years ago, investigators found that the complete genes for both the light and heavy chains of antibody molecules are assembled by joining shorter segments of DNA during the development of antibody-producing cells. This requires that the DNA be broken and then reconnected. The *myc* gene often moves into the heavy chain coding region at precisely the sites where the breaks occur. Leroy Hood of the California Institute of Technology, who was one of the pioneers of the antibody work, says of the current developments, "They tie together in a brilliant fusion research on antibodies and cancer. I certainly never expected to be studying cancer genes."

Circumstantial evidence for the possible involvement of antibody gene rearrangements in carcinogenesis began building in studies in which the chromosomal locations of the genes were mapped. For example, Carlo Croce's group at the Wistar Institute mapped the human heavy chain genes to chromosome 14. The genes for the human kappa chain, one of the two types of light chains that may be found in antibody molecules, were mapped to chromosome 2 by S. Malcolm of Queen Elizabeth College in London, Terence Rabbitts of the MRC Laboratory of Molecular Biology in Cambridge, and their colleagues. And the genes for the other type of light chain, the lambda chain, were mapped to human chromosome 22 by the Croce group and by the groups led by Philip Leder at Harvard Medical School and O. Wesley McBride at the National Cancer Institute (NCI).

> The aberrant chromosomes of the cancers turned out to contain a misplaced gene.

These results were intriguing because specific abnormalities in the same chromosomes have been associated with Burkitt's lymphoma, a cancer of the antibody-producing B cells. Lymphoma cells from about 90 percent of the patients with this cancer show aberrant chromosomes formed by an exchange of segments between chromosomes 8 and 14. In cells from about 5 percent of the patients, the exchange is between chromosomes 8 and 2. And in cells from another 5 percent, the exchange is between chromosomes 8 and 22. The antibody genes are located in the regions where the breaks occur on their respective chromosomes.

A similar picture is seen in the mouse plasmacytomas, which are also tumors of antibody-producing cells. According to George Klein of the Karolinska Institutet, plasmacytomas show two characteristic chromosomal abnormalities. Most often there is a translocation of a segment of chromosome 15 to chromosome 12, which carries the antibody heavy chain genes in the mouse. But some tumors have instead a reciprocal translocation between chromosomes 15 and 6, which carries the mouse kappa chain genes.

About a year ago, Klein suggested* that DNA rearrangements such as these translocations might lead to cancer by causing the activation of normal cellular genes. Although the identities of those genes were unknown, the most obvious candidates were the cellular counterparts of the viral *onc* genes.

The antibody gene mapping studies, taken together with those demonstrating specific chromosomal abnormalities in Burkitt's lymphoma and plasmacytoma cells, provided circumstantial evidence that antibody gene rearrangements might somehow participate in the development of the cancers. However, the mapping studies could not pinpoint the chromosomal breakpoints to the antibody genes themselves, nor could they identify the cellular genes that might be undergoing activation.

This is what investigators have now done for both Burkitt's lymphoma and mouse plasmacytomas, showing directly that the *myc* gene moves from its normal chromosomal position to the chromosome carrying the heavy chain genes, where it may become directly connected to DNA coding for heavy chain segments. The researchers came to this conclusion by different routes.

The Croce group, in an extension of their mapping studies, showed that all or most of the DNA segments coding for the heavy chain variable regions moved from their normal position on chromosome 14 to chromosome 8 in Burkitt's lymphoma cells. In collaboration with Riccardo Dalla-Favera and Robert Gallo of NCI, Croce mapped the normal location of the cellular *myc* gene to chromosome 8. Then, he told participants in a recent symposium,† "We immediately

*G. Klein, *Nature (London)* **294**, 313 (1981).
†The symposium, "Chromosomes and Cancer: From Molecules to Man," was sponsored by the Bristol-Myers Company and organized under the aegis of the University of Chicago Cancer Research Center. It was held on 18 and 19 October at the University of Chicago.

0036-8075/82/1203-0983$01.00/0

tried to determine whether c-*myc* [the cellular *myc* gene] was on the segment translocated in Burkitt's lymphoma, and in fact we found that was the case." In about one-third of the cell lines examined, the *myc* gene is in close proximity to one of the heavy chain genes.

For three other groups, those of Hood, Leder, and Kenneth Marcu of the State University of New York at Stony Brook, the cancer gene work was a direct outgrowth of studies on antibody gene structure and rearrangement. All three groups had identified unusual DNA clones that had been prepared from mouse plasmacytoma cells. The clones carried DNA segments coding for the constant portions of the heavy chain but not for the variable regions. "The really interesting thing about our clone," Leder remarks, "is that it seemed to be bringing in something from beyond the immunoglobulin locus." The same was true of those identified in the laboratories of Hood and Marcu.

The investigators went on to look for similarly rearranged DNA in additional plasmacytoma lines—and they found it. Rearranged DNA was present in five of the six lines examined by Leder's group, in all ten lines examined by Hood's, and in 15 of the 20 plasmacytomas tested by Marcu and his colleagues.

"It turned out further that the aberrantly rearranged mouse gene has an analog in human cells, in chicken cells, and even in *Drosophila*," Leder continues. The human analog mapped to the region of chromosome 8 that is broken in the translocation seen in Burkitt's lymphoma. Using a probe supplied by Stuart Aaronson of NCI, Leder, Rebecca Taub of Harvard, and Ilan Kirsch of the National Institute of Child Health and Human Development determined that the mouse DNA clone carried the *myc* gene sequence as did human chromosome 8. Moreover, in 8 of 12 lines of Burkitt's lymphoma cells, the *myc* gene had moved into very close proximity to DNA regions coding for heavy chain constant regions.

Similarly, Marcu and his colleagues showed that the nonimmunoglobulin portion of the rearranged DNA of mouse plasmacytomas mapped to chromosome 15, the mouse analog of human chromosome 8. With Croce, they identified the *myc* gene in the rearranged DNA segments. The Hood group's results with their mouse DNA clones were comparable.

Finally, Michael Cole of St. Louis University School of Medicine looked directly for *myc* gene rearrangements in mouse plasmacytomas. He found them in six of seven cell lines. In five of them, the gene had moved into the heavy chain coding region.

In some, but not all, cell lines, the investigators find that the recombination site between the *myc* gene and the heavy chain coding segments maps to "class switch" regions—repeated sequences of DNA that mediate the DNA rearrangements necessary for antibody-producing cells to switch from one class of heavy chain to another. Leder says, "This suggests that mechanisms similar to those involved in normal antibody rearrangements might play a role in the translocation."

The possibility that the *myc* gene translocation is a consequence of normal antibody gene rearrangements is supported by observations suggesting a correlation between the type of antibody chain secreted by tumor cells and the particular chromosomal aberration in the cells. For example, Gilbert Lenoir of the International Agency for Research on Cancer in Lyon, France, and his colleagues have found this to be the case for Burkitt's lymphoma cells.

> " . . . mechanisms similar to those involved in normal antibody rearrangements might play a role in the translocation."

The issue of how the *myc* gene translocation might cause Burkitt's lymphoma and mouse plasmacytomas is still far from settled. From the start, suspicion had fallen heavily on *myc* gene involvement in the lymphomas because of results already obtained with avian leukosis virus (ALV). This virus causes a variety of cancers in chickens, including B cell lymphomas, but does not carry an *onc* gene. According to results obtained by William Hayward of Rockefeller University and Susan Astrin of the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, and by J. Michael Bishop and Harold Varmus of the University of California at San Francisco, about 80 percent of the lymphomas induced by ALV have a viral control sequence that has become integrated near the cellular *myc* gene. The tumors also show increased production of the *myc* gene product, and the assumption is that integration of the viral control sequence activates the cellular gene. It was these results, incidentally, that prompted several of the investigators to look first for *myc* involvement in the mouse and human lymphomas.

In any event, work with both ALV and MC29, the virus that carries *myc* as its own *onc* gene, suggests that it is increased expression of the gene that underlies the malignant transformation of susceptible cells. If the Burkitt's lymphoma and mouse plasmacytoma cells followed the viral precedents, researchers postulated, the cancers might have been caused by increased expression of the translocated *myc* gene due to the influence of regulatory sequences that ordinarily promote the expression of heavy chain genes.

Not all tumor cells have the translocated *myc* gene immediately attached to heavy chain sequences, however. So if its activation does contribute to development of the lymphomas, the activation does not depend absolutely on connection with the antibody chain genes. Croce argues, "What is important is the translocation, whether or not the *myc* gene is rearranged with antibody chain genes. It is still very close to those genes, even when it is not directly attached."

Also, the investigators find that the translocated *myc* gene and heavy chain coding regions, when they are joined, are connected head-to-head, or 5′ end to 5′ end. This means that transcription of the *myc* gene into messenger RNA would have to proceed in the opposite direction to that of the heavy chain segment. Nevertheless, there is clear agreement that the translocated *myc* gene is transcribed in tumor cells. Where agreement fails is over the question of whether it is increased expression of the gene, as predicted by the viral work, or production of an altered gene product that contributes to development of the cancers. There is a recent precedent for the latter from work on a transforming gene isolated from human bladder carcinoma cells (*Science*, 12 November, p. 667).

Cole, for one, favors the altered gene alternative. He does not find any significant differences in the amounts of *myc* RNA transcripts produced by normal B cells and by tumor cells having the rearranged gene. But he does find a new messenger RNA in the tumor cells which is 0.4 kilobase (kb) shorter than the normal 2.5-kb *myc* messenger. This suggests that a segment of the *myc* gene may have been lost as a result of the translocation. A 0.4-kb segment could code for about

16,000 daltons of protein. If all the lost segment came from coding regions, the *myc* gene product would be substantially shortened.

According to Cole, the viral and rearranged tumor *myc* genes contain the same two coding regions. Since viral coding regions total only about 1.6 kb, considerably shorter than the normal *myc* messenger RNA, he suggests that a *myc* gene segment was also lost when it was picked up by the virus. Cole maintains, "Increased *myc* gene expression may be relevant to transformation in some way, but there is clear evidence for an altered gene. I think that it's the disruption of the gene itself that is important."

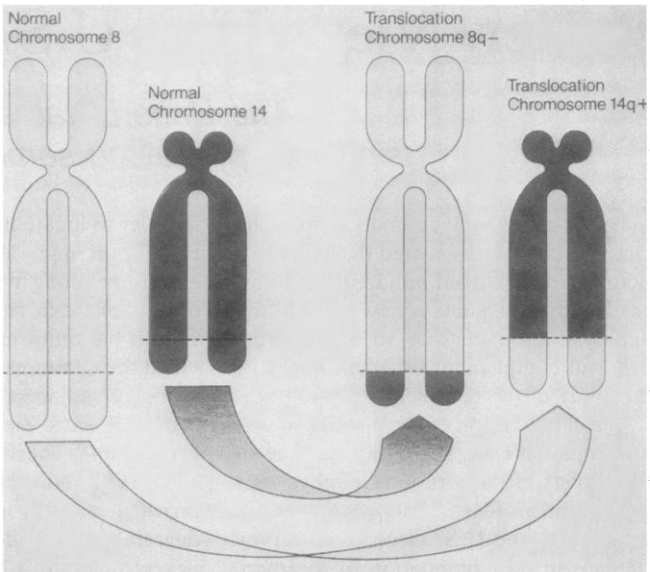
Croce takes the other view. He strongly favors the idea that there is increased *myc* gene expression and that this is involved in transformation. "We find a dramatic increase of *myc*-specific RNA, about 10- to 20-fold more in tumor cells than in normal cells," he asserts. The messenger RNA's appear to be of normal size in Burkitt's lymphoma cells, but Croce and Marcu find that mouse plasmacytoma cells contain transcripts that are about 0.5 kb shorter than normal, a decrease that is similar to the 0.4-kb difference observed by Cole.

The Hood group also find both increased production of *myc* messenger RNA and new transcripts in mouse tumor cells with the rearranged gene. "I think it could be either, or possibly both," Hood replied when asked which is important for transformation.

Sorting out this situation will require isolating the normal and translocated *myc* genes to compare their structures and transforming capabilities. It should also be possible to construct mutant genes or hybrids in which comparable segments of the two are interchanged to see whether particular gene regions are important for transformation. This approach worked for the bladder transforming gene, for example.

Currently there is a major hindrance to these studies, however. A suitable system for assaying the transforming activity of the *myc* genes is still not available. According to George Vande Woude of NCI, the cloned *myc* gene from chicken cells does not transform the mouse fibroblasts that are commonly used to assay transforming activity. Hood says, "There may be a real limitation on the *myc* gene story, the lack of a biological assay for transformation."

Another complicating factor is the likelihood that cancer development requires several steps, of which a specific gene translocation may be just one.



Chromosome translocation in Burkitt's lymphoma. Chromosomes 8 and 14 exchange portions of their long arms (designated q) to form the abnormal chromosomes 8q− and 14q+. [Source: University of Chicago Cancer Research Center/Bristol-Myers Company]

Geoffrey Cooper and Paul Neiman of the Sidney Farber Cancer Center have identified in cells transformed by ALV a gene that is capable of transforming mouse fibroblasts. This transforming gene is not the *myc* gene. In the normal cells that give rise to the lymphoma, *myc* gene activation may be an early step, followed by activation of this other transforming gene. The number of steps in this possible progression is not known.

One thing that is certain about the current work on *myc* gene translocation is that it will engender a great deal of additional research. An obvious next step is to look for *myc* gene movement in the 2;8 and 8;22 translocations of Burkitt's lymphomas to see whether the gene has moved into the light chain regions.

Moreover, the linkage of specific chromosomal abnormalities and particular cancers is not restricted to Burkitt's lymphoma. Participants in the Chicago meeting emphasized that there has been a shift from thinking that such abnormalities are rare to thinking that they are very common. As Jorge Yunis of the University of Minnesota School of Medicine said in Chicago, "Our data suggest that 96 percent of all cancers have a chromosome defect and that in 56 percent of them the defect is recurrent."

In general, cancers of the blood cells involve translocations of chromosome segments, similar to those seen in Burkitt's lymphoma. For example, the Philadelphia chromosome, which was discovered more than 20 years ago in patients with chronic myelogenous leukemia, was apparently formed by a loss of a segment of chromosome 22. However, about 10 years ago, Janet Rowley of the University of Chicago School of Medicine showed that the "lost" segment was actually translocated to other chromosomes, most often chromosome 9. Rowley has also shown that acute myeloblastic leukemia is associated with a translocation of a segment of chromosome 21 to chromosome 8 and that cancer cells from patients with acute promyelocytic leukemia show a translocation from chromosome 17 to chromosome 15.

When the cellular analogs of the viral *onc* genes are mapped to their human chromosomal locations, they are most often found on the same chromosomes that have been linked to specific cancers. Rowley said she knows of about ten such genes that have been mapped in various laboratories. If additional aberrations can be shown to involve translocations of the cellular *onc* genes or alterations in the environments near them, it would buttress the hypothesis that activation of these genes plays some part in carcinogenesis, at least for cancers affecting blood cells.

The situation may be different for tumors of solid tissues. These, too, have been linked to specific chromosomal abnormalities, but, as pointed out in Chicago by Uta Francke of Yale University School of Medicine, the aberrations seen in solid tumors usually involve deletions, rather than translocations, of chromosomal segments. The deletions associated with a given cancer may vary widely in length, although they generally have a small segment in common. The marked length variations make it unlikely that the abnormalities feature a specific change in the immediate vicinity of a given gene that might cause its activation. Still, it is a good bet that researchers will be looking for the presence of the cellular counterparts of *onc* genes in the regions affected by the deletions.

—JEAN L. MARX