sheet they would quickly move apart again. So it is difficult to see how gravitational clustering alone could have produced the kind of structure seen in the Local Supercluster, says Tully.

Testing these ideas in more distant superclusters is more difficult, he says. Outside our own neighborhood, individual galaxies cannot be located accurately enough in the line of sight to know whether they lie on the front side or the back side of their supercluster. Moreover, the dim galaxies, which actually outnumber the bright ones, are undetectable at great distances. It is only in the local supercluster that the census can be relatively complete.

None of the models of large-scale structure is without its problems in any case, notes Tully. Most important, none of them can explain where the initial density fluctuations came from. So in an ultimate sense, no one really knows why the Local Supercluster exists. But at least, he says, we are learning how to formulate the questions that address the problem.—M. MITCHELL WALDROP

Gene Transfer Yields Cancer Clues

Some cancer cells carry genes that transform cultured cells. Researchers are beginning to isolate and clone the transforming genes

Using gene transfer techniques, investigators have recently shown that cultured cancer cells derived from human and animal tumors often carry transforming genes that cause normal cells to acquire cancerous characteristics. The experiments provide direct support for what everyone has thought all along, namely that gene changes contribute to the development of many cancers. But they do more than that. For the first time, researchers are gaining the ability to isolate, clone, and study in detail transforming genes from cancers that have arisen spontaneously or been induced by chemicals.

Substantial progress has already been made in identifying the transforming genes carried by many of the viruses that cause cancers in animals. Studies of these genes, which are called *onc* (for oncogenic) genes, are providing much information about the biochemical basis of viral transformation, and possibly about transformation in general. Nevertheless, the applicability of the viral results to the problem of human cancer remains to be proven.

As Robert Weinberg of the Massachusetts Institute of Technology (MIT) points out, "Hopes of finding viral agents that cause human cancers have largely been frustrated." Even though viruses have been implicated as the cause of some, mostly rare, forms of cancer, Weinberg continues, "In general, it is likely to be the case that the cancers common in this country are not going to have a viral etiology. If it is not a viral agent, then what kinds of changes in the cell are causing cancer?"

The evidence suggesting that they are gene changes includes demonstrations by Bruce Ames of the University of California at Berkeley and others that radiation and chemicals that are carcinogenic are usually mutagenic, effecting alterations in DNA. In the past, investigators were not able to identify the affected genes because they lacked probes that could pick them out from among the many tens of thousands present in a mammalian cell.

With the normal road to gene isolation blocked, investigators, including Weinberg and Geoffrey Cooper of the Sidney Farber Cancer Institute and Harvard Medical School, turned in the late 1970's to "transfection" methods, gene transfer techniques that have developed rapidly in recent years (*Science*, 19 December 1980, p. 1334). As long as the transferred gene confers some detectable new property on the recipient cells, the methods provide an assay for its presence that can be used in lieu of a more conventional probe. Acquisition of a transforming gene, for example, should alter the growth pattern and shape of the recipient cells in a characteristic fashion.

In an early series of experiments, Chiaho Shih of MIT and Weinberg transferred DNA prepared from each of 15 different lines of mouse cells that had been transformed with chemical carcinogens to mouse cells (fibroblasts) of the NIH3T3 line. The results suggested that some of the lines carried a transmissible transforming gene. Shih, Weinberg, and their collaborators found that DNA from five of them, all transformed by 3-methylcholanthrene, caused the recipient cells to be transformed at a frequency 10 times higher than the frequency of transformation by DNA from normal cells. Weinberg says, "The DNA from transformed cells functioned differently from the DNA of normal cells. It carried transforming sequences."

The transforming trait appeared to be carried on a single fragment of DNA. "The behavior of the DNA suggested that the transforming activity was located in a single discrete segment," Weinberg explains. "It was incompatible with a series of genes scattered through the genome acting together to create this phenotype." Even in the best cases, the efficiency of gene transfer is low, only about one in 100,000 cells successfully acquiring a new gene. Since the probability of transferring one gene is low, it is mathematically unlikely that two or more unlinked genes will be transferred.

In more recent experiments, DNA's from a variety of cell lines derived from human cancers have been found to transform NIH3T3 cells. Weinberg's group found this to be the case for lines derived from colon and bladder carcinoma cells and from promyelocytic leukemia cells. Cooper and Theodore Krontiris of the Sidney Farber Cancer Institute obtained transformation with DNA's from two lines of bladder carcinoma cells. In collaboration with Mary-Ann Lane, who is also at Sidney Farber, Cooper transformed NIH3T3 cells with DNA from a line of mammary carcinoma cells, as well as with DNA's from a number of kinds of malignant human lymphocytes. And Michael Wigler and Manuel Perucho of the Cold Spring Harbor Laboratory obtained similar results with DNA's from two lines of lung carcinoma cells and one line each of bladder, colon carcinoma, and neuroblastoma cells.

Explaining transformation might have been simplified if all these cell types turned out to have the same transforming gene. That did not happen, although cancers of a particular cell type may be traceable to the activation of a specific transforming gene. According to Weinberg, "The hypothesis, which is becoming increasingly validated, is that each given type of tissue will have a characteristic activated oncogene." Investiga-

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tors have generally found that cancerous cells of the same type have the same, or very similar, transforming genes. Conversely, with one current exception, they have found different transforming genes in different types of cancerous cells.

Ben-Zion Shilo of MIT and Weinberg have evidence that four lines of the 3-methylcholanthrene-transformed mouse cells, which were independently produced in different laboratories, have the same transforming gene. They determined this by comparing the sensitivities of the four DNA's to digestion with five restriction enzymes, each of which cuts DNA at a specific site. The treated DNA's from all four lines behaved in the same way when they were subsequently transferred to NIH3T3 cells. They lost the ability to transform the recipients when treated with two of the enzymes, but not when exposed to the other three.

Similarly, Cooper and Lane found that the transforming gene of the human mammary carcinoma was at least very similar to those carried by six mouse mammary carcinomas, five of which were virally caused and the sixth induced by a chemical. The same transforming gene can apparently be activated in comparable cells of different species and by different treatments. Recently, Cooper and Lane extended these findings to several kinds of malignant lymphocytes. Cooper says, "Specific transforming genes may be activated in neoplasms of specific differentiated cell types."

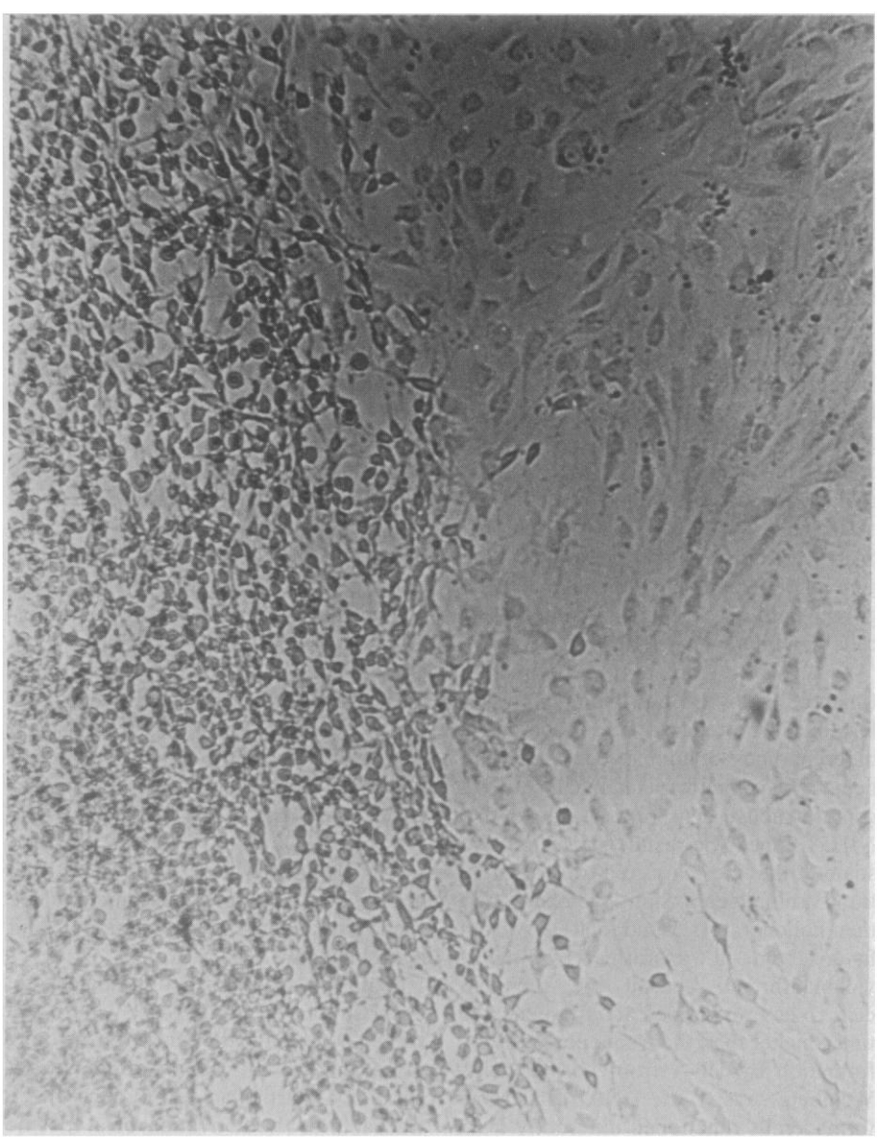
Dissimilar transforming genes were found in the human colon, bladder, and leukemia cell lines studied by the MIT workers. They compared these DNA's on the basis of the patterns of their *Alu* sequences, repeated segments of DNA scattered throughout the human genome, but not found in the mouse genome. The *Alu* patterns of the transforming DNA's from the three lines were different.

Occasionally, different cancers may share a common transforming gene, however. When Wigler and Perucho looked at the restriction enzyme sensitivities or *Alu* sequence patterns of the transforming DNA's of their five cell lines, they found the neuroblastoma and bladder carcinoma lines had different transforming genes, as expected. Also as expected, the two lines of lung carcinoma cells had the same transforming gene. This was different from those of the bladder carcinoma and neuroblastoma cells but the same as the transforming gene of the colon carcinoma cell line.

As things now stand, it appears that cancers of a given cell type have the same activated transforming gene, but different cancers need not have different transforming genes, although they usually do. More work will be required to verify these generalizations and to determine how many transforming genes there are. For the moment, Wigler expresses caution: "I think the rule will have a lot of exceptions. It is premature to say that there is one gene per tissue."

Because investigators are just beginning to work on the biochemical nature of the transforming genes and their products, there is currently little information about how they cause transformation. But there is evidence that a gene does not have to be abnormal, in the sense of directing the synthesis of a faulty product, to cause cancer. Instead, control of gene expression may be defective, leading to synthesis of the product in excessive amounts or at the wrong time. Many investigators think that the transforming genes may be turned on as the result of gene rearrangements that bring them under the influence of new control elements.

Transfection experiments by the Cooper group support the hypothesis that even normal cells contain latent transforming genes that may be exposed when their usual environment is disrupted. When Cooper transferred high-molecular-weight DNA from normal cells to NIH3T3 recipients, there was no detectable transformation. But if the DNA was first bombarded with high-frequency sound waves to break it into smaller pieces, the transformation frequency was increased to a low, but detectable,



Cells transformed by tumor cell DNA

Mouse cells were exposed to DNA prepared from a line of tumor cells transformed by the chemical carcinogen 3-methylcholanthrene. Two weeks later, a dense focus of transformed cells could be seen on the left. The monolayer of untransformed cells is to the right. [Source: Chiaho Shih and Robert Weinberg, Massachusetts Institute of Technology]

level. If a second round of transfection was carried out, this time with DNA from cells transformed by the sonicated DNA, the result was a high frequency of transformation similar to that produced by transfection with DNA from cells infected by a transforming virus known to carry an *onc* gene.

Cooper says, "Gene rearrangements could result in the activation of transforming genes." For example, the potential transforming gene might be stripped of a regulatory DNA sequence that normally keeps the gene turned off and then be integrated into recipient cell DNA near a promoter site that turns it on. This would be a relatively rare event during the first transfer of sonicated DNA, but, once it happened, a second DNA transfer could transform with a higher frequency.

Recent studies of transforming viruses, principally those having RNA as their genetic material, have also indicated that faulty control of normal cellular genes may underlie transformation. The *onc* genes carried by many of these viruses were found to be homologous, perhaps exactly, to genes found in the cells they infect. Apparently the viruses acquired the ability to transform when they picked up the cellular gene homolog, which then came under the influence of viral regulatory sequences that caused the gene products to be made in large amounts.

Additional support for the activation of transforming genes by viral regulatory elements comes from studies of avian leukosis virus (ALV), which causes several kinds of cancer in birds even though it does not carry its own oncogene. According to William Hayward of Rockefeller University and Susan Astrin of the Institute for Cancer Research in Fox Chase, near Philadelphia, this virus transforms by turning on the *myc* oncogene in infected cells.

After ALV enters the cell, its RNA genome, like those of the other RNA transforming viruses, is copied into DNA and this copy (the provirus) is then integrated into the cellular DNA. An integrated provirus is flanked by two identical DNA segments, called long terminal repeats (LTR's), which contain the signals for turning on viral genes. The *myc* gene is apparently activated when the LTR segment of ALV is integrated nearby.

George Vande Woude and his colleagues at the National Cancer Institute also have evidence that LTR's can activate a transforming gene. They cloned another cellular oncogene, this one designated *mos*. In a transfection assay, they found that cells were not transformed by the *mos* gene itself, but were transformed if a viral LTR was first attached to the gene.

The growing body of evidence that transformation may be caused by the activation of otherwise dormant cellular genes, possibly as a result of gene rearrangements, does not rule out a role for altered products resulting from structural gene changes. There may be more than one path to transformation.

Cloning the various transforming genes and comparing their structures to those of their inactive cellular counterparts should help to determine whether they have been altered in transformed cells. Cloning is now under way in several laboratories. For example, Wigler's group has cloned the transforming gene of a bladder carcinoma line. The gene appears to be of human origin as human placental DNA carries a homologous DNA segment. Restriction analyses of the two DNA's suggest that the transforming gene has not undergone any major rearrangements compared to its placental counterpart, but rearrangements cannot be ruled out at this time.

There is also the intriguing possibility that two or more gene changes, which may affect either control or product or both, must interact to effect transformation. Most investigators agree that development of the cancerous state requires several steps.

Cooper and Paul Neiman of the Fred Hutchinson Cancer Research Center in Seattle suggest that two genes may participate in the transformation of cells by ALV. They found that they could transform NIH3T3 cells by transfecting them with DNA from the cells of tumors induced by the virus. The transforming DNA turned out to contain no viral DNA, however—not even any LTR sequences. Nevertheless, the tumor cells themselves contained LTR sequences integrated near the cellular *myc* gene. Cooper and Neiman hypothesize that activation of the *myc* gene might precede and contribute to the activation of the gene detected by transfection.

The transformation of NIH3T3 cells by the acquisition of a single gene would seem to be inconsistent with the presumed multistep nature of transformation. But Weinberg points out, "These cells are not normal fibroblasts. They are established in culture and immortal, and could have undergone predisposing alterations to make them susceptible to the oncogenic effects of a transferred gene."

The cells were not transformed by DNA from the majority of tumor cell lines tested, however. Shih and Weinberg did not get transformation with DNA from 10 of 15 chemically transformed lines of mouse cells. Wigler and his colleagues did not see it with DNA from 16 of the 21 lines of human tumor cell lines that they have studied. And, in one series of experiments, Cooper and Krontiris failed to observe it with DNA from 24 of 26 such lines.

Perhaps the NIH3T3 cells simply were not capable of being transformed by the transforming genes of these lines, possibly because they had not undergone the right predisposing alterations. Alternatively, the transforming genes might have been recessive and not detectable in a transfection assay. And, of course, there is also the possibility that the cells did not contain transforming genes at all.

Nevertheless, at least ten distinct transforming genes have been identified in tumor cell lines. More may be discovered, especially if it turns out that most of the hundred or so types of cancer estimated to exist are caused by the activation of different transforming genes. Moreover, results so far suggest that the transforming genes detected by transfection experiments are, for the most part, different from the dozen *onc* genes identified in transforming viruses.

Cancer researchers who once lamented the dearth of clues to the causes of transformation now have almost an embarrassment of riches with which to work. As members of both groups of genes are cloned, their products identified, and their activities compared, progress in understanding transformation should be rapid. "Transfection," says Vande Woude, "is the wave of the future."—JEAN L. MARX

Cancer researchers who once lamented the dearth of clues to the causes of transformation now have almost an embarrassment of riches with which to work.