MAMMALIAN CELL GENETICS

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The DNA-mediated gene-transfer method is playing a critical role in our understanding of the genetics of animal cells, and nowhere is this more evident than in the study of cancer. This year marks the first cloning of "tumor" genes from human cells. The cloning and characterization of these genes has become a major focus of our lab. Projects on the inheritance and function of DNA methylation and the characterization of the cellular thymidine kinase (*tk*) gene continue from previous years.

Screening Human Tumor Cell Lines for Transforming Genes

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Work from two laboratories (R. Weinberg's at MIT and G. Cooper's at Sidney Farber Cancer Institute) has demonstrated the growth transformation of normal-growth-controlled mouse cells (NIH 3T3) with DNA from certain chemically transformed rodent cell lines (Shih et al., *Proc. Natl. Acad. Sci. 76:* 5714 [1979]) lines (Shih et al., *Nature 290:* 261 [1981]; Krontiris and Cooper, *Proc. Natl. Acad. Sci. 78:* 1181 [1981]). These experiments suggested the existence of dominant-acting transforming genes of cellular origin. Such genes are of obvious interest. Since, as our success with the cloning of the chicken *tk* gene demonstrated, any gene can be cloned given a means to detect its transfer, we set about to clone transforming genes of human origin.

As a first step, we screened various human tumor cell lines for the presence of dominant-acting transforming genes by using the NIH 3T3 DNA-transfer assay system. Twenty-one human cell lines provided by Jorgen Fogh (Sloan-Kettering Institute) were screened, and five were found positive: one bladder carcinoma cell line, T24; two lung carcinoma cell lines, SK-LU-1 and Calu-1; one colon carcinoma cell line, SK-CO-1; and one neuroblastoma cell line, SK-N-SH. The transforming gene or genes contained in these cells were partially characterized by the technique of "Alu blotting" (Shih et al., *Nature 290:* 261 [1981]; Perucho et al., *Cell 27:* 467 [1981]). This technique exploits the ubiquitous presence of the repeated sequence within the human genome known as the Alu family (Jelinek et al., *Proc. Natl. Acad. Sci. 77:* 1398 [1980]). The presence of these human sequences within a transformed mouse cell are readily detected by blot hybridization. The pattern one sees in transformants is characteristic for a given gene. Using this method, as well as characterization by restriction endonuclease sensitivity, we established that three distinct transforming genes were present in our five cell lines: one unique to T24, one unique to SK-N-SH, and one common to the colon and two lung carcinomas (Perucho et al., *Cell 27:* 467 [1981]). After analyzing samples exchanged with R. Weinberg's laboratory, another human colon carcinoma cell line was found also to contain this last gene. Finding a human transforming gene common to several independently derived human tumor cell lines may indicate the existence of common or overlapping pathways for carcinogenesis in humans.

We have also screened DNAs extracted from surgical specimens of human tumors and from human tumors grown in nude mice. No DNAs from human tumors have been found positive. However, DNA from one human lung tumor, maintained in nude mice, efficiently transformed NIH 3T3 cells. These cells contained the gene common to the lung and colon carcinoma cell lines. We are continuing our screening of DNAs from human tumors both to study the distribution and frequency of occurrence of the recently identified genes and to identify new sources of novel genes.

Characterization of the Transforming Gene from T24 Bladder Carcinoma Cells

M. Goldfarb, K. Shimizu, M. Wigler

Screening studies indicate that T24 cells contain a dominant-acting transforming gene. In previous years we developed a method for isolating genes from animal cells by utilizing an essentially genetic approach ("plasmid rescue") and in this way cloned the chicken *tk* gene. An improved technique ("suppressor rescue") recently developed in our laboratory also utilizes genetic selection in *E. coli,* based on the ability of a cloned tRNA suppressor gene to complement amber mutant bacteriophage. This method has been successfully applied to the isolation of the transforming gene from T24 bladder carcinoma cells (Goldfarb et al., *Nature* [In press]). A DNA sequence of less than 5.0 kb was obtained and found to be active in transforming NIH 3T3 cells. Hybridization studies indicate that these sequences are found in all NIH 3T3 cells transformed with T24 DNA and in all normal and tumor human tissue tested. No sequences were found in NIH 3T3 cells or in NIH 3T3 cells transformed with DNA from neuroblastoma or colon and lung carcinoma cells. The locus that encodes the transforming gene of T24 is highly polymorphic in the human population with respect to restriction fragment length polymorphisms. Our best work to date indicates that this gene is not grossly rearranged in T24 cells. The T24 gene encodes an mRNA of 1100 bp that has

been found expressed in every tumor cell line examined to date, but not in human placenta or in human fibroblasts cultured in vitro.

Isolating the Transforming Genes from Lung and Colon Carcinoma Cells

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We have adopted a different strategy to clone the common transforming genes of lung and colon carcinoma cells. From the number and sizes of gene-associated restriction fragments hybridizing with human repetitive DNA, we have estimated this gene to be in excess of 30 kb. Cloning it by purely genetic means would therefore be very difficult. We have used an alternative method based on screening genomic libraries constructed in phage λ from mouse cells transformed by these human genes and using human repetitive DNA as our hybridization probe. We obtained one phage with a 12-kb human DNA insert. This insert contained sequences that are present in all NIH 3T3 cells transformed with the common lung/colon gene but not in NIH 3T3 cells transformed with other human genes. Using the "left most" and "right most" parts of the insert, we are attempting to clone additional parts of the gene by "chromosome walking." We have not yet detected a transcript of this gene in transformed mouse cells.

Characterization of the Cellular *tk* Gene

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Efforts to understand the control of the cellular *tk* gene are continuing. Jesse Kwoh has now precisely mapped the more than 80 linker insertion mutants of the cloned chicken *tk* gene and correlated these with their transformation efficiencies. Results of combined physical/genetic mapping and nuclease-S1 heteroduplex mapping suggest that the 3′ noncoding region is encompassed in an 800-base unspliced region of transcript and that the 5′ coding region consists of at least three exons (the largest no greater than 400 bases) totaling about 1000 bases. John Lewis (Molecular Genetics Section) has shown that expression of *tk* is cell-cycle-regulated in mouse cells transformed with the 3.0-kb clone of chicken *tk*, pchtk-5. This suggests that (1) the control sequences for cell-cycle regulation of *tk* expression are contained on that 3.0-kb fragment and (2) that the regulatory mechanisms have been conserved from chicken to mouse. In related work, Kenji Shimizu is isolating the human *tk* gene and John Lewis is isolating the Chinese hamster *tk* gene. Both are utilizing the technique of suppressor rescue. These genes will be useful in identifying and studying the regulatory control sequences. The isolation of the human *tk* gene will further enable us to study mutational events in the various disorders involving DNA repair that afflict humans.

The Inheritance and Function of DNA Methylation in Mammalian Cells

D. Levy, M. Wigler

In previous years we had begun work on the study of DNA methylation in mammalian cells. These studies involved methylating cloned genes in vitro with *Hpa*II methylase, a bacterial enzyme that modifies DNA at a subset of sites (CCGG) that are normally found methylated in the genomic DNA of vertebrates. We demonstrated two things: first, that methylated genes do not transform as well as the unmethylated controls; and second, that DNA methylation patterns are heritable. The first point confirmed the findings of others that methylation was roughly associated with gene activity, and the second point suggested, as had been hypothesized, that methylation might play a role in somatic inheritance. Current work has centered about these two points. First, we have established that the reduced transformation efficiency of methylated *tk* genes is likely to be the result of an effect on the expression of those genes, since methylation does not affect the efficiency of their transfer. Second, we have established that methylation of the coding regions of the herpes simplex virus (HSV) *tk* gene is sufficient to inhibit transformation, and methylation of the 5′ untranscribed region is without effect. Third, treatment with azacytidine, a cytidine analog that inhibits DNA methylation, can induce reactivation of unexpressed methylated *tk* genes. Fourth, the inheritance of methylation can be very faithful, approaching greater than 99% per cell generation in rat fibroblast cells. Our previous estimate was 95% per generation on the basis of studies in mouse L cells.

Publications

Goldfarb, M., K. Shimizu, M. Perucho, and M. Wigler. 1982. Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. (Submitted.)

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