MAMMALIAN CELL GENETICS

M. WIGLER, D. KURTZ, *C. Fraser, M. Hallaran, D. Hanahan, D. Levy, L. Lipsich, C. Nicodemus, C. Perucho, M. Perucho*

Certain cultured cell lines can readily be transformed by exogenous DNA when given as a calcium phosphate coprecipitate. Although stable transformants, detected by biochemical selection, can arise from exposure of 10^6 cells to as little as 50 pg of a purified gene, such efficient gene transfer requires the use of vast excesses of carrier DNA. Cells that incorporate a selectable marker, such as thymidine kinase, are also likely to incorporate significant amounts of carrier DNA, a process we have called cotransformation. By adding well-defined DNA sequences to the carrier, it is possible to construct cell lines containing virtually any defined DNA sequence. This method is a potentially powerful tool in the study of animal cell genetics and gene expression. In our laboratory we are attempting to increase our understanding of this tool. To exploit this understanding in specific ways, we are (1) attempting to determine the physical state of transforming elements in the transformed host, (2) designing more general animal cell vectors, (3) studying the expression of transforming elements, (4) collaborating with the Tumor Virology Section to engineer new mutant-specific host cells that will be useful in the development and analysis of new viral mutants, and (5) utilizing the method of plasmid rescue to isolate cellular genes coding for selectable markers.

Transfer and amplification of an altered dhfr gene

Successful transfer of the cellular genes coding for thymidine kinase (tk) and adenine phosphoribosyl transferase (aprt) has been demonstrated using total genomic DNA from vertebrate species as donor. Isoelectric focusing of enzymatic activity in transformants has shown this activity to be donor-derived. These findings suggest that it may now be possible to transfer any cellular gene that codes for a selectable marker. More recently, we have demonstrated the transfer to mouse cells of a mutant gene from hamster cells (A29), which codes for a dihydrofolate reductase (dhfr) with altered binding affinity of methotrexate (Mtx). This gene confers resistance to high concentrations of Mtx. Murine recipients were shown to contain the hamster dhfr gene by blot hybridization, using as probe a cDNA clone of dhfr mRNA (kindly provided by R. Schimke, Stanford University). When the Mtx concentration was raised slowly from 0.1 μg/ml to 10 μg/ml, the hamster dhfr gene resident in surviving mouse cells was shown to undergo a commensurate increase in copy number (amplification). Moreover, if genomic A29 DNA is ligated to a defined genetic element prior to transformation and the transformants are subsequently amplified, the ligated genetic marker is also often amplified. In this way we have constructed mouse cells containing up to 50 copies of a yeast suppressor tRNA gene (see below).

Cloning the HSV-1 tk gene

Herpes simplex virus type 1 (HSV-1) codes for a thymidine kinase activity that can complement tk^- animal cells. We have cloned the HSV-1 tk gene as a 3.5-kb fragment inserted into the *Bam*I site of the prokaryotic plasmid pBR322. This prokaryotic/eukaryotic chimeric molecule (ptk-2) transforms tk^- mutant mouse cells (Ltk^-) with the same efficiency as the HSV tk gene purified from viral DNA. When ptk-2 is cotransformed into Ltk^- $aprt^-$ cells in a nonselective manner (using a cellular aprt gene as the selectable marker), eight of nine cotransformants express tk, suggesting that the cloned HSV-1 tk gene carries its own promoter.

Plasmid rescue

It is possible to cotransform animal cells with the prokaryotic antibiotic resistance plasmid pBR322. These bacterial sequences are found integrated into the high-molecular-weight nuclear DNA of the host. The integrated pBR322 sequences often include a contiguous origin of plasmid replication and an antibiotic resistance factor. In such cases, we have demonstrated that the pBR322 sequences can act as a transducing element from the animal host to *Escherichia coli*. Host DNA is digested with restriction endonucleases and the resulting restriction fragments are then ligated with T4 DNA ligase under cyclization conditions. Those cyclized molecules containing pBR322 and its flanking host sequences can be used to transform *E. coli* to antibiotic resistance. We refer to this process as plasmid rescue.

Cointegration of cotransforming DNA

Cotransforming elements become genetically linked in the transformed host. This has been demonstrated in a variety of ways. We have used prokaryotic sequences such as pBR322 and ϕX174 RF DNA as the cotransforming markers and ptk-2 as the selectable marker. tk^- revertants can be selected from tk^+ transformants. Revertants that have deleted the tk gene will also often delete the cotransforming markers as well. In addition, when the tk gene resident in a transformed host is transferred to tk^- recipient cells by chromo-

some-mediated transfer, cotransforming markers are also cotransferred. Finally, when cells are transformed with A29 dhfr and cotransformed with pBR322 derivatives, amplification of the dhfr gene will often result in amplification of pBR322 sequences. We used the method of plasmid rescue to demonstrate that the flanking sequences of cotransforming pBR322 derive not from the endogenous host sequences but rather from carrier sequences. These observations have led us to hypothesize that transforming elements do not integrate directly into the chromosomes of the host cell but, rather, are first ligated to carrier DNA into a single unit, which we have called the pekelasome. We do not know at present if pekelasomes are sometimes or usually stably integrated into host chromosomes. Thus, the picture that emerges of transformation in animal cells differs markedly from that in yeast or bacteria.

Expression of rat α_{2u} globulin gene in rat hepatoma cells

The α_{2u} globulin is a protein with a molecular weight of 20,000 that is synthesized in the liver of mature male rats, secreted into their serum, and excreted in their urine. The hepatic synthesis of this protein is under complex hormonal control involving the participation of the sex hormones, glucocorticoids, thyroid hormone, and pituitary growth hormone. The cDNA for this protein has been cloned into pBR322, and this clone is being used to screen a genomic rat library. Several library isolates have been obtained that show nucleotide homology to the probe, but they have not yet been mapped. At the same time, a rat hepatoma cell line has been obtained that synthesizes α_{2u}, and its hormonal responses have been characterized. Using this cell line and DNA-mediated transformation, it may be possible to localize genomic sequences responsible for hormonal control by site-specific mutagenesis of the gene and insertion into an expressing cell.

Expression of transformed genetic elements in host cells

Work in progress in our laboratory and in others indicates that the phenotypic pattern of expression of transformed elements in genetically transformed cells is very complex. In collaboration with M. Botchan and D. Lane of the Tumor Virology Section, we have used the method of cotransformation to examine the expression of SV40 T antigen in mouse cells transformed nonselectively with the early region of SV40 DNA. Some transformants express T antigen constitutively. With other transformants, subclones arise spontaneously, at high frequency, and do not express T antigen. These subclones, in turn, can spontaneously give rise to cells that express T antigen. Despite this varied phenotypic expression, the genotypic content of these cells remains constant as determined by Southern blotting. Several transformants were obtained that contain multiple copies of the entire SV40 early region but express no detectable T antigen. This perhaps represents an example of either *cis-* or *trans*-acting coordinate regulation.

Construction of mutant-specific host cells

Much effort in viral genetics centers around the isolation of conditional lethal mutants. For animal cells, temperature sensitivity is the most commonly used conditional selection, whereas host range is used much less frequently. In collaboration with T. Grodzicker and D. Klessig of the Tumor Virology Section, we have constructed a number of human cell lines that have stably incorporated various regions of the adenoviral genome. These cells can now complement adenovirus mutations that map at those loci. The use of these cells should greatly facilitate the development and analysis of new adenoviral mutants. In addition, in collaboration with J. Broach, now at the State University of New York, Stony Brook, we have constructed mouse cell lines with greater than 50 copies of a specific yeast suppressor tRNA gene. We are preparing to examine this mouse cell line for the proper expression of these genes. Suppressor animal cells provide another avenue to the development of mutant-specific host cells.

Isolation of cellular genes coding for selectable markers by the method of plasmid rescue

We have previously demonstrated that cellular genes coding for selectable markers can be transferred to appropriate recipient cells using total genomic DNA as donor. We have coupled this observation with the method of plasmid rescue to devise a means for the cloning of the cellular gene coding for thymidine kinase on bacterial plasmids. To this end we ligate restriction-endonuclease-cleaved, total genomic DNA to bacterial plasmid pBR322 prior to transformation of tk^- animal cells. DNA from transformed animal cells is then used to transform *E. coli* to ampicillin resistance. The chicken tk gene is now the first vertebrate gene isolated by purely genetic means. It also represents the first "household" gene cloned. Our isolation scheme can be applied equally as well to any dominant-acting gene coding for a selectable marker. Included in this category are various "putative" genes that code for the malignant phenotype, as well as a variety of biochemically well-defined genetic loci.

Publications

Hanahan, D., D. Lane, L. Lipsich, M. Wigler, and M. Botchan. Expression and rescue of an SV40/

pBR322 chimera in transformed mouse cells. (In preparation)

Perucho, M., D. Hanahan, and M. Wigler. Cointegration of cotransforming elements in animal cells. (In preparation)

Perucho, M., L. Lipsich, M. Wigler, and D. Hanahan. Isolation of the chicken thymidine kinase gene. (In preparation)

Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin. 1979. DNA-mediated transfer of the adenine phosphoribosyl transferase locus into mammalian cells. *Proc. Natl. Acad. Sci.* **76:**1373.

Wigler, M., M. Perucho, D. Kurtz, S. Dana, A. Pellicer, R. Axel, and S. Silverstein. Transformation of mammalian cells with an amplifiable dominant acting gene. (In preparation)

Wigler, M., R. Sweet, G.-K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel. 1979. Transformation of mammalian cells with genes from procaryotes and eucaryotes. *Cell* **16:**777.

Wold, B., M. Wigler, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel. 1979. Introduction and expression of a rabbit β-globin gene in mouse fibroblasts. *Proc. Natl. Acad. Sci.* **76:**5684.