Cellular Transforming Genes

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Summary. Cellular genes potentially capable of inducing oncogenic transformation have been identified by homology to the transforming genes of retroviruses and by the biological activity of cellular DNA's in transfection assays. DNA's of various tumors induce transformation with high efficiencies, indicating that oncogenesis can involve dominant genetic alterations resulting in activation of cellular transforming genes. The identification and characterization of cellular transforming genes and their possible involvement in naturally occurring cancers, is discussed.

The basic observation that tumor cells inherit in some stable way the transformed phenotype suggests that oncogenic transformation may be a result of genetic alterations. This is clearly the case for cells transformed by certain oncogenic viruses, where it has been demonstrated that specific viral genes are responsible for maintenance of the transformed state. However, most naturally occurring tumors, particularly in man, do not appear to be caused by viruses. The question therefore arises as to whether neoplasia can result from alterations in normal cell genes. In this article, I discuss experiments leading to the identification of cellular genes with potential oncogenic activity and the possible involvement of such genes in naturally occurring cancers.

Cellular Homologs of Retrovirus Transforming Genes

Retroviruses are a family of RNA viruses that replicate by way of a DNA provirus integrated into cellular DNA. Some members of this group, the "acute" transforming viruses, are highly oncogenic. These viruses efficiently transform cells in culture and induce neoplastic disease with short latent periods in infected animals. Approximately 20 viruses of this type, isolated from chickens, turkeys, mice, rats, cats, and monkeys, have been characterized.

The genomes of acute transforming retroviruses contain specific genes responsible for oncogenicity as well as other sequences required for virus replication (Table 1). For example, the genome of Rous sarcoma virus includes a single gene (*src*) which is responsible for cellular transformation but is not involved in virus replication (*1*). This gene encodes a 60,000-dalton phosphoprotein ($pp60^{src}$) which is a tyrosine-specific protein kinase (*2–6*). Other acute transforming retroviruses contain different transforming genes. At present, 13 distinct retrovirus transforming genes have been reported, some of which are found in multiple independent virus isolates (*7, 8*).

The transforming genes of acute transforming retroviruses are homologous to DNA sequences present in normal uninfected cells (*9*). These normal cell homologs of viral transforming genes are highly conserved in vertebrate evolution and appear to represent normal cell genes that are not linked to viral DNA (*9*). Transcription of several of these genes has been detected in normal cells and, in some cases, normal cell proteins that are closely related to proteins encoded by the homologous virus transforming genes have been identified (*9*). The cellular homologs of virus transforming genes appear to represent single-copy sequences in cellular DNA, with the exception of the *ras* genes, which constitute a multigene family (*10*).

The acute transforming retroviruses thus appear to represent recombinants in which a transforming gene, derived from a homologous gene of normal cells, has been inserted into a retrovirus genome. The transforming genes of these viruses are expressed at high levels in virus-infected cells as a consequence of their association with viral transcriptional regulatory sequences. For example, the amount of viral $pp60^{src}$ in chicken cells transformed by Rous sarcoma virus is approximately 100-fold higher than the amount of cellular $pp60^{src}$ in normal chicken cells (*11–13*). It is thus possible that transformation by these viruses is a consequence of abnormal expression of normal cell genes. Alternatively, transformation might result from structural differences between the viral and cellular proteins. In either case, the cellular homologs of retrovirus transforming genes constitute a group of normal cell genes with potential oncogenic activity.

Assay of the Biological Activity of DNA by Transfection

Investigation of the potential oncogenic activity of cellular genes is dependent on an assay for determination of the biological activity of purified cellular DNA. Transfer of biologically active DNA in bacteria was first demonstrated by Avery, MacLeod, and McCarty in 1944 (*14*) and was adapted to mammalian cells in studies of the infectivity of genomic DNA's of papovaviruses (*15*). Successful transfer of biologically active total cellular DNA was first achieved by Hill and Hillova in 1971 (*16, 17*) with the demonstration that DNA of cells transformed by Rous sarcoma virus induced transformation of recipient cultures of chicken embryo fibroblasts as a consequence of transfer of the Rous sarcoma virus genome.

Transfer of biologically active eukaryotic DNA's, generally termed transfection, is usually performed by exposure of recipient cell cultures to donor DNA in the form of a calcium phosphate precipitate (*18*). In a typical assay, 10^6 recipient cells might be exposed to 20 micrograms of donor DNA. The efficiency of transformation obtained in such assays varies with the cells used as recipients. The NIH 3T3 and LtK^- mouse cell lines are commonly used recipient cells since transformation of these cells occurs by stable integration of donor DNA's with relatively high efficiencies of approximately 0.1 to 1 transformants per microgram of donor DNA (*19–22*). The LtK^- cells were derived from a mouse sarcoma and, consequently, are useful recipient cells for assays of biochemical markers but not of potential oncogenic genes. In contrast, NIH 3T3 cells are a nonneoplastic mouse cell line and have been used as recipients for most transfection assays of either viral or cellular transforming genes.

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Transforming Activity of Normal Cell DNA's

The hypothesis that normal cell genes can induce oncogenic transformation has been supported by transfection experiments in which normal cell DNA's have been found to induce transformation of NIH 3T3 cells. The transforming activity of normal cell DNA sequences has been demonstrated both by transfection of molecular clones of cellular DNA's homologous to two retrovirus transforming genes and by transfection of total genomic DNA's of normal vertebrate cells.

Ligation of molecular clones of the normal mouse gene homologous to the transforming gene of Moloney sarcoma virus (*mos*) and of the normal rat gene homologous to the transforming gene of Harvey sarcoma virus (*ras*) to viral transcriptional regulatory sequences results in activation of the transforming potential of these cellular genes (*23–25*). The transforming efficiencies of these activated cellular *mos* and *ras* genes are comparable to those of the homologous sarcoma virus DNA's. In contrast, the cellular *mos* and *ras* genes lack transforming activity when associated with their normal cellular flanking sequences (*23–25*). However, removal of the flanking sequences 5′ to the cellular *mos* gene results in activation of its transforming activity to a level that is approximately 1000-fold lower than that of the same gene after ligation to a viral transcriptional regulatory sequence (*24*). This low transforming activity is thought to represent integration of donor DNA fragments in the vicinity of active cellular transcriptional promoters, resulting in efficient expression of the donor transforming gene and cellular transformation (*24, 26, 27*). The demonstrations of transforming activity of the cellular *mos* and *ras* genes thus indicate that abnormal expression of the cellular homologs of at least two retrovirus transforming genes is sufficient for induction of oncogenic transformation.

The hypothesis that normal cells contain genes that are potentially capable of inducing transformation has been investigated in a more general way by transfection of DNA's of normal chicken embryo fibroblasts, nontransformed mouse cell lines, and normal human embryo lung fibroblasts (*28, 29*). High molecular weight DNA's (>30 kilobases) of these normal cells lacked detectable transforming activity. However, after fragmentation to sizes of 0.5 to 5 kb, normal cell DNA's induced transformation with efficiencies (~ 0.003 transformant per microgram of DNA) that were similar to those induced by subgenomic *src*-containing fragments of DNA of Rous sarcoma virus–transformed cells (*28*). DNA's of cells transformed by normal cell DNA fragments induced transformation with high efficiencies (0.1 to 1.0 transformant per microgram of DNA) in secondary transfection assays, indicating that these transformed cells contained activated transforming genes that could be efficiently transmitted by transfection (*28*).

These results suggest that potential transforming genes of normal cells can be activated as a consequence of DNA rearrangements during integration of donor DNA in transfection assays. Fragmentation of normal cell donor DNA may dissociate potential transforming genes from their normal regulatory sequences, thereby facilitating recombination with inappropriate regulatory sequences (for example, efficient transcriptional promoters), resulting in abnormal gene expression and cell transformation. Activated transforming genes can then be transmitted at high efficiencies as single transforming units in secondary transfection assays of transformed cell DNA's.

Activation of Cellular Transforming Genes in Neoplasms

The observations discussed above indicate that (i) normal cells contain genes that are potentially capable of inducing transformation and (ii) the biological activity of such genes can be detected in transfection assays. If carcinogenesis involved dominant genetic changes, affecting either the regulation or structure of such potential transforming genes, it might be possible that DNA's of some neoplasms would contain activated transforming genes and therefore induce transformation with high efficiencies upon transfection. This has been found to be the case for DNA's of a number of different types of neoplastic cells (Table 2) (*30–40*).

High molecular weight DNA's of these neoplastic cells induce transformation with efficiencies ranging from approximately 0.05 to 1 transformant per microgram of DNA (*30–40*). Similar efficiencies of transformation are obtained when NIH cells transformed by these tumor DNA's are used as donors of DNA in secondary transfection assays, as is expected for serial transfer of transforming genes that were activated in the original neoplasms. In several cases, identical patterns of susceptibility to digestion with different restriction endonucleases have been observed in assays of the transforming activities of DNA's of both primary neoplastic cells and transformed NIH cells, indicating that the same transforming sequences are serially passaged in primary and secondary transfection assays (*34, 36, 41*). In addition, the presence of human repetitive DNA sequences has been demonstrated in NIH cells transformed by human tumor DNA's, indicating that transformation is mediated by transfer of human DNA (*32, 33, 35–37, 39*).

The results summarized in Table 2 indicate that DNA's of different types of neoplastic cells from several different vertebrate species, including man, induce transformation of NIH 3T3 cells with high efficiencies. These neoplasms include spontaneously occurring tumors, chemically induced tumors, and virus-induced tumors that do not contain specific virus transforming genes. In addition, they include primary tumors of chicken, mouse, and human origin (*31, 34, 36*) as well as tumor-derived cell lines. Carcinogenesis in various neoplasms thus appears to involve dominant genetic alterations, either by mutations or gene rearrangements, which result in the activation of cellular transforming genes that are then detectable by transfection.

DNA's of approximately 50 percent of chemically induced and spontaneously occurring tumors, however, do not induce efficient transformation on transfection (*30, 32–39*). Carcinogenesis in these neoplasms may have involved epigenetic changes or recessive genetic alterations that would not be detectable by transfection of tumor DNA's. Alternatively, these neoplasms may contain dominant activated transforming genes which do not induce transformation of the NIH 3T3 cells used as recipients in transfection assays.

Virus-Induced Neoplasms: Possible Model for Multistep Carcinogenesis

Some of the neoplasms in which activated transforming genes have been detected by transfection were induced by infection with weakly oncogenic retroviruses that, in contrast to the acute transforming retroviruses, do not contain specific viral transforming genes (*1*). These neoplasms (Table 2) include chicken B cell lymphomas and a nephroblastoma induced by avian lymphoid leukosis virus (LLV), mouse mammary carcinomas induced by mouse mammary tumor virus (MMTV), and mouse T cell neoplasms induced by murine leukemia viruses. Unlike the acute transforming

retroviruses, these weakly oncogenic viruses do not transform cells in culture and require long latent periods before the formation of neoplasms in vivo. Oncogenesis by these viruses thus appears to proceed by indirect mechanisms that may resemble spontaneous or chemically induced oncogenesis more closely than oncogenesis by acute transforming viruses.

To determine whether the transforming genes detected by transfection of DNA's of LLV-induced B cell lymphomas and MMTV-induced mammary carcinomas were linked to viral DNA sequences, NIH cells transformed by DNA's of these neoplasms were analyzed by nucleic acid hybridization. No LLV DNA sequences were detected in six lines of NIH cells transformed by DNA's of four different LLV-induced B cell lymphomas (*31*) and no exogenous MMTV DNA sequences were detected in ten lines of NIH cells transformed by DNA's of five different MMTV-induced mammary carcinomas (*34*). Neoplasms induced by these viruses thus contain transforming genes which are not linked to viral DNA, indicating that oncogenesis involved indirect activation of cellular transforming genes at some stage of the disease process.

The long latent period and pathogenesis of neoplasms induced by these viruses suggests a multistep process that might be expected to involve more than a single transformation event. Virus-induced events might therefore be involved at an early stage of the disease, preceding activation of the cellular transforming genes detected by transfection. One possibility for such virus-induced events, proposed by McGrath and Weissman (*42*), is that proliferation of T cell lymphomas induced by murine leukemia viruses may be activated by binding of a virus encoded gene product (for example, the envelope glycoprotein) to mitogenic surface receptors. Another hypothesis, which has been termed promoter insertion, is that integration of viral DNA in the vicinity of a potential cellular transforming gene may result in abnormal gene expression as a consequence of the activity of the viral transcriptional promoter (*43*).

The latter hypothesis has been supported by studies of LLV-induced B cell lymphomas (*43*). Approximately 80 percent of such lymphomas were found to contain viral DNA sequences, including the viral transcriptional promoter, integrated in the vicinity of the cellular gene homologous to be transforming gene (*myc*) of the acute transforming virus MC-29 (*43*). Integration of these viral DNA sequences apparently resulted in increased transcription of the cellular *myc* gene, implicating activation of this gene as a direct consequence of LLV infection in lymphomagenesis (*43*).

The relation between the transforming genes detected by transfection of LLV-induced B cell lymphoma DNA's and the activated *myc* gene of these neoplasms was further investigated by analysis of *myc* sequences in the lymphomas used as DNA donors in transfection experiments and in NIH cells transformed by these lymphoma DNA's. In confirmation of the results of Hayward *et al.* (*43*), it was found that the LLV-induced B cell lymphomas used as donors of DNA in transfection assays contained LLV DNA sequences integrated in the vicinity of the cellular *myc* gene (*44*). However, chicken *myc* sequences were not transferred to NIH cells transformed by these lymphoma DNA's (*44*). These results indicate that LLV-induced B cell lymphomas contain at least two transforming genes: (i) a cellular *myc* gene activat-

Table 1. Transforming genes of acute transforming retroviruses.

Gene	Prototype virus	Isolation source
src	Rous sarcoma virus	Chicken
*fps**	Fujinami sarcoma virus	Chicken
*fes**	Snyder-Theilin feline sarcoma virus	Cat
yes	Y73 sarcoma virus	Chicken
fms	McDonough feline sarcoma virus	Cat
mos	Moloney sarcoma virus	Mouse
ras†	Harvey sarcoma virus	Rat, mouse
sis	Simian sarcoma virus	Woolly monkey
myc	Myelocytomatosis virus strain MC29	Chicken
erb	Avian erythroblastosis virus	Chicken
myb	Avian myeloblastosis virus	Chicken
abl	Abelson leukemia virus	Mouse
rel	Reticuloendotheliosis virus strain T	Turkey
ros	UR2 sarcoma virus	Chicken

*The genes designated *fps* and *fes* are homologous genes of viruses isolated from different species (*55*). †The genes designated *ras* constitute a multigene family (*10*).

Table 2. Transforming genes activated in neoplastic cells.

Cell type	Species	Mode of induction	Positive neoplasms (No.)	References
Transformed fibroblasts	Mouse	Chemical	5	(*30*)
Bladder carcinoma	Human	Spontaneous	3	(*32, 33, 37, 39*)
	Mouse	Chemical	1	(*33*)
	Rabbit	Chemical	1	(*33*)
Lung carcinoma	Human	Spontaneous	4	(*37, 39, 40*)
	Mouse	Spontaneous	1	(*33*)
Mammary carcinoma	Human	Spontaneous	1	(*34*)
	Mouse	Chemical or virus	6	(*34*)
Colon carcinoma	Human	Spontaneous	2	(*35, 37*)
Promyelocytic leukemia	Human	Spontaneous	1	(*35*)
Nephroblastoma	Chicken	Virus	1	(*31*)
Neuroblastoma	Human	Spontaneous	1	(*37*)
	Rat	Chemical	3	(*33*)
Glioblastoma	Mouse	Chemical	1	(*33*)
Pre-B lymphocyte neoplasm	Human	Spontaneous	4	(*36*)
B cell lymphoma	Chicken	Virus	6	(*31*)
	Human	Spontaneous	6	(*36*)
	Mouse	Spontaneous	3	(*36*)
Plasmacytoma/myeloma	Human	Spontaneous	2	(*36*)
	Mouse	Chemical	2	(*36*)
T cell lymphoma	Human	Spontaneous	1	(*36*)
	Mouse	Spontaneous, chemical, radiation, or virus	6	(*36*)
Mature T helper cell neoplasm	Human	Spontaneous	1	(*36*)
	Mouse	Virus	1	(*36*)
Sarcoma	Human	Spontaneous	1	(*39*)
	Mouse	Chemical	1	(*38*)

ed by integration of viral DNA sequences and (ii) a distinct cellular gene that is not linked to viral DNA and that efficiently induces transformation of NIH 3T3 cells.

Activation of these two genes may correspond to events involved at different stages of the neoplastic process. The earliest event detected in the course of LLV-induced lymphomagenesis is the formation of multiple preneoplastic follicles within the bursas of most birds (*45*). The majority of these preneoplastic follicles regress, but a small fraction appear to progress to the neoplastic state (*45*). Thus, one might consider the possibility that viral activation of the cellular *myc* gene is an early event in lymphomagenesis which results in preneoplastic follicle proliferation. Progression to neoplasia might then involve further genetic alterations resulting in the activation of the cellular transforming genes detected by transfection.

The identification of two genes that appear to be involved in LLV-induced lymphomagenesis may be an example of progressive alterations that appear to occur during carcinogenesis in a variety of neoplasms. If this is the case, transformation of NIH 3T3 cells by tumor DNA's may detect only some of these alterations. In this regard, it should be noted that NIH 3T3 cells are transformed by DNA of MC-29–infected cells only with a very low efficiency of approximately 0.005 transformant per microgram of DNA (*46*). It is likely that, since NIH 3T3 cells are a permanent heteroploid cell line, they may have already undergone some of the alterations that would be required for transformation of normal cells. If this is the case, transfection of NIH 3T3 cells may identify only a subset of cellular transforming genes involved in stages of carcinogenesis that are required for final oncogenic transformation of these cells. The role of the transforming genes identified by transfection of NIH 3T3 cells in the possibly more complex pathways of normal cell oncogenesis remains to be established.

Specificity of Transforming Genes Activated in Neoplasms

The transforming genes detected by transfection of neoplastic cell DNA's have been characterized by (i) analysis of the sensitivity of the transforming activities of DNA's to cleavage with different restriction endonucleases and (ii) analysis of repetitive human DNA sequences linked to transforming genes of human neoplasms. The results of such characterizations indicate that different transforming genes are activated in different types of tumors but that the same transforming gene is repeatedly activated in independent tumors of the same differentiated cell type.

Restriction endonucleases cleave DNA at specific recognition sites, usually consisting of four to six nucleotides, which are distributed in cellular DNA's. Consequently, digestion of a biologically active DNA with a particular restriction endonuclease would be expected to inactivate transforming activity only if those sequences necessary for transformation contained a recognition site for that specific restriction endonuclease. Digestion of a series of transforming DNA's with a series of different restriction endonucleases therefore allows a comparison of the restriction sites contained within the transforming sequences activated in different neoplasms.

Data from experiments of this type with DNA's of chemically transformed mouse fibroblasts (*41*), human and mouse mammary carcinomas (*34*), and human and mouse lymphoid neoplasms (*36*) are summarized in Table 3. It is apparent that the transforming activities of DNA's from related types of transformed cells display similar patterns of susceptibility to cleavage with different restriction endonucleases. Thus, specific patterns of restriction endonuclease sensitivity were observed for the transforming activities of DNA's of four chemically transformed lines of mouse fibroblasts (*41*), a human mammary carcinoma cell line, and six mouse mammary carcinomas induced by either viral or chemical carcinogens (*34*), four human pre-B cell neoplasms (*36*), three mouse and two human B cell lymphomas (*36*), two human and two mouse mature B cell neoplasms (plasmacytomas and myelomas) (*36*), one human and six mouse T cell lymphomas (*36*), and human and mouse mature T helper cell neoplasms (*36*). In the case of T cell lymphomas, digestion with Xho I inactivated the transforming activities of DNA's of neoplasms of BALB/c and C57 mice but did not affect the transforming activities of DNA's of neoplasms of AKR mice or of a human neoplasm (*36*). This Xho I site may, therefore, represent a strain-specific polymorphism in a common T lymphoma transforming gene.

The above results indicate that specific transforming genes are activated in neoplasms of the same differentiated cell type, whether neoplasia was spontaneously occurring or induced by viral or chemical carcinogens. In addition, closely related transforming genes appear to be activated in some neoplasms of both human and mouse origin. However, different transforming genes are activated in neoplasms of different types of differentiated cells. Furthermore, different transforming genes appear to be activated in neoplasms corresponding to distinct stages of B and T lymphocyte development.

Data obtained from analysis of repetitive DNA sequences linked to the transforming DNA's of some human neoplasms is also consistent with these conclusions. Different repetitive DNA sequences are linked to the transforming genes of a human bladder carcinoma, a human colon carcinoma, a human promyelocytic leukemia, and a human neuroblastoma, indicating that different transforming genes are activated in these four different human neoplasms (*35, 37*). However, a common transforming gene appears to be activated in independent human lung and colon carcinomas (*37*).

It thus appears that different cellular transforming genes are activated in most different types of neoplasms, but that specific transforming genes are activated in multiple neoplasms of specific differentiated cell types. This relation between differentiation phenotype and neoplasm transforming gene suggests that transformation may result from alterations of genes that are normally involved in differentiation-specific control of cell proliferation. In addition, these observations suggest that the number of tumor-specific transforming genes detectable by transfection is limited and may not exceed 50 to 100.

Relation Between Transforming Genes of Neoplasms and of Retroviruses

The relation between the transforming genes detected by transfection of tumor DNA's and the transforming genes of acute transforming retroviruses has been investigated with the use of molecular clones of *src, myc, fes, ras, erb, mos, myb*, and *sis* as probes for hybridization to restriction endonuclease–digested DNA's of transformed NIH cells. These experiments indicated that the transforming genes detected by transfection of DNA's of a human mammary carcinoma, human pre-B cell lymphoma, human myelomas, human T cell lymphomas and a human mature helper T cell neoplasm were not homologous to these retroviral transforming genes. However, the transforming genes detected by transfection of human bladder and lung carcinoma DNA's were homologous, respectively,

Table 3. Restriction endonuclease analysis of transforming sequences activated in neoplastic cells. N.D., not determined.

Cell type	Species	Mode of induction	Independent neoplasms (No.)	Transforming activity after digestion*					
				Bam H1	Eco R1	Hind III	Pvu II	Sac I	Xho I
Transformed fibroblasts	Mouse	Chemical	4	+	−	−	N.D.	N.D.	+
Mammary carcinomas	Human, mouse	Spontaneous, chemical, or virus	7	+	+	+	−	−	+
Pre B cell neoplasms	Human	Spontaneous	4	−	+	+	N.D.	N.D.	−
B cell lymphomas	Human, mouse	Spontaneous	5	−	+	+	N.D.	N.D.	+
Mature B cell neoplasms (myelomas/plasmacytomas)	Human, mouse	Spontaneous or chemical	4	+	+	+	+	−	+
T cell lymphomas	Human, mouse	Spontaneous, chemical, radiation, or virus	7	+	−	+	N.D.	N.D.	+/−
Mature T helper neoplasms	Human, mouse	Spontaneous or virus	2	+	+	−	N.D.	N.D.	−

*Results are summarized from references (*34*), (*36*), and (*41*). Plus signs indicate transforming activity unaffected by digestion. Minus signs indicate transforming activity inactivated by digestion.

to the *ras* genes of Harvey (ras^H) and Kirsten (ras^K) sarcoma viruses (*40*). The relation of the bladder carcinoma transforming gene to ras^H was also observed in two other laboratories (*47, 48*).

These results indicate that the transforming genes activated in human bladder and lung carcinomas are cellular homologs of two different members of the *ras* gene family. Since a common transforming gene is activated in human lung and colon carcinomas (*37*), the colon carcinoma transforming gene is also implicated as a cellular homolog of ras^K. The involvement of two different cellular *ras* genes in three different types of human carcinomas further suggests the possibility that members of the *ras* gene family may be involved in a variety of human neoplasms of epithelial origin.

The activation of cellular *ras* genes in these human carcinomas provides the first direct link between the transforming genes of retroviruses and human neoplastic disease. Further studies of these genes and their gene products may significantly contribute to understanding the etiology of human neoplasms.

Molecular Analysis of Neoplasm Transforming Genes

The organization of *ras* genes in bladder and lung carcinomas and in normal cells has been investigated by analysis of restriction endonuclease–digested cellular DNA's with cloned probes of viral ras^H and ras^K sequences (*40*) and of biologically active ras^H transforming genes isolated from human bladder carcinomas (*39, 49, 50*). The results of these analyses indicate that the activated transforming genes of human bladder and lung carcinomas are not distinguishable in their general organization from homologous genes of normal cells. Activation of the transforming activity of these genes in neoplasms thus does not appear to be a consequence of either DNA amplification or gross DNA rearrangements but may instead result from point mutations or minor DNA rearrangements that have not been detected in these experiments.

Molecular clones of normal cell ras^H genes efficiently induce transformation when activated by ligation to viral transcriptional promoter sequences, an indication that abnormal expression of these genes is sufficient to induce transformation (*25*). The level of expression of the *ras* gene product in bladder and lung carcinoma cell lines, and in NIH cells transformed by these carcinoma DNA's, is two- to fourfold higher than in NIH 3T3 cells (*40, 51*). It is possible that this increase in *ras* gene product may be sufficient to account for transformation. However, it is also possible that the transforming activity of the neoplasm *ras* genes may be a consequence of structural mutations.

A biologically active molecular clone of the transforming gene detected by transfection of chicken B cell lymphoma DNA has also been isolated (*52*). This transforming sequence is homologous to a family of genes that are present in both avian and mammalian, including human, DNA's; this sequence is not homologous to any of the known retroviral transforming genes. As in the case of *ras* genes activated in bladder and lung carcinomas, the organization of sequences homologous to the chicken B-cell lymphoma transforming gene is similar in DNA's of normal and neoplastic cells. Homologous polyadenylated RNA's are also detected, at roughly comparable levels, in both B cell lymphomas and normal B lymphocytes. Nucleotide sequencing suggests that this gene encodes a small polypeptide of approximately 7000 daltons.

Further analysis of these cloned transforming genes should elucidate the molecular events involved in transforming gene activation and may contribute to understanding the mechanisms of transforming gene function.

Proteins Associated with Cellular Transforming Genes

Another approach to elucidation of the function of cellular transforming genes is analysis of proteins associated with these genes. The use of serum from tumor-bearing mice in immunoprecipitation assays has allowed the detection of proteins associated with the transforming genes of mammary carcinomas (*53*) and neuroblastomas (*54*). In addition, association of a tumor-specific transplantation antigen with the transforming gene of a chemically induced mouse sarcoma has been detected (*38*).

The protein associated with the mammary carcinoma transforming gene is a glycoprotein of 86,000 daltons that is present in NIH cells transformed by human mammary carcinoma DNA in either primary or secondary transfection assays (*53*). This protein is not present in other transformed NIH cells but is immunoprecipitated by serum from mice bearing tumors induced by NIH cells transformed by mouse mammary carcinoma DNA's and by serum from mice bearing primary mammary carcinomas (*53*). These results confirm the conclusion that related genes are activated in human and mouse mammary carcinomas

and indicate that this 86,000-dalton glycoprotein is specifically associated with the transforming genes of these neoplasms.

The neuroblastoma transforming gene–associated protein is a phosphoprotein of 185,000 daltons (*54*). It was detected in cells transformed by DNA's of three independent neuroblastomas and one glioma, as well as in the original tumors. These results thus provide further evidence for activation of a specific transforming sequence in these neoplasms.

At present, two alternative possibilities could account for the association of these antigens with cellular transforming genes. They might be directly encoded by transforming genes expressed in the primary neoplasms and transferred to NIH cells by transfection. Alternatively, expression of these antigens might be induced as a secondary consequence of expression of the neoplasm transforming genes. In either case, further studies of proteins specifically associated with cellular transforming genes may contribute to biochemical analysis of the pathways of oncogenesis induced by these genes.

Summary and Perspectives

A number of normal cellular genes with potential oncogenic activity have been identified. In at least some cases, it appears that abnormal expression of these genes is sufficient for induction of oncogenic transformation. Analysis of the transforming activity of tumor DNA's further indicates that carcinogenesis can involve dominant genetic alterations resulting in activation of cellular transforming genes. Analysis of the transforming genes activated in different neoplasms indicates that the same genes are activated in independent neoplasms of the same cell type, suggesting that specific pathways of oncogenesis may be involved in specific types of neoplastic disease. Furthermore, the finding that specific transforming genes are activated in neoplasms corresponding to discrete stages of lymphocyte differentiation suggests that the transforming genes activated in neoplasms are closely related to the state of normal differentiation exhibited by the neoplastic cells.

The transforming genes of human bladder and lung carcinomas have been identified as cellular homologs of the *ras* genes of Harvey and Kirsten sarcoma viruses and molecular cloning of the cellular transforming genes activated in some neoplasms has been accomplished. Analysis of these isolated transforming genes should elucidate the molecular events involved in transforming gene activation during oncogenesis. Studies of cloned transforming genes and associated proteins may also lead to understanding of the mechanisms by which these genes induce transformation.

The normal role of cellular genes with potential oncogenic activity is not known. The specificity of transforming genes activated in neoplasms of particular types of differentiated cells suggests that these genes may be involved in differentiation-specific control of cell proliferation. The use of cloned transforming genes as probes to study gene expression in normal cells may elucidate their normal functions.

The cellular transforming genes identified by transfection apparently induce oncogenic transformation of NIH 3T3 cells as a single-step event. Since naturally occurring oncogenesis appears to be a multistep process, it is likely that activation of these genes is only one of several events involved in carcinogenesis. Studies of the expression and biological activity of these genes at different stages of naturally occurring oncogenesis will be required to determine the steps of oncogenesis in which they are involved.

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