Fred Gilbert[1]

Chromosomes, Genes, and Cancer: A Classification of Chromosome Abnormalities in Cancer[2,3]

That single gene changes are involved in the development of cancer is clear from several lines of evidence, including the existence of familial forms of cancer and the demonstration that mutagenic agents can act as carcinogens. That chromosome abnormalities may also participate in tumor initiation and progression is suggested by the association of specific chromosome rearrangements with particular cancers. However, exactly how these karyotypic aberrations relate to the putative cancer genes is uncertain, as are the mechanisms by which single gene changes contribute to carcinogenesis.

Presented herein is a classification of chromosome abnormalities in cancer. Three classes will be described: 1) reciprocal translocations, 2) deletions or nonreciprocal rearrangements resulting in the loss of structural material at specific sites, and 3) duplications of whole chromosomes or chromosome segments. Hypotheses concerning the roles played in the development of cancer by single gene changes produced by each class will be discussed.

A discussion of the 3 classes, with definitions and examples, follows.

CLASS 1

Defined.—Reciprocal translocations, in which there is no significant loss of structural material.

Examples.—The marker rearrangements associated with particular lymphomas and leukemias, including BL and CML.

A number of leukemias and lymphomas in man and mouse have been characterized by specific chromosome rearrangements. In CML, for example, the tumor cells in approximately 90% of patients contain the Ph[1] (*1*), a small chromosome 22 in which the "missing" long arm segment is translocated to another autosome, usually a number 9 (*2, 3*). The measurement of DNA content in cells in which the 9/22 translocation is the only cytogenetic abnormality, indicates that there is no appreciable loss of DNA as a result of the rearrangement (reciprocal translocation) (*4*).

Other cancers have also been found to contain characteristic translocations in which at least one of the chromosomes contributing to the rearrangement is constant: with p = short arm and q = long arm, these include acute promyelocytic leukemia, with t(15q+;17q−) (*5*); acute myelogenous leukemia, with t(8q−;21q+) (*6*); BL, with t(8q−;14q+) (*7*), rarely t(8q+;2p−) (*8*), or t(8q+;22q−) (*9*); and non-Burkitt lymphomas, with reciprocal translocations between 14q and different autosomes (*10*).

As with the 9/22 translocations in CML, the reciprocal nature of these translocations (*11*) and the specificity of the breakpoints involved suggest that it is the activation of genes carried by the rearranged segments rather than the loss of genetic information that contributes to cell transformation. Among the genes whose activation is presumed to play such a role are the so-called "transforming sequences" or "oncogenes."

Several laboratories have now shown that the transfer of single genes (designated transforming sequences) from tumor cells to certain nontransformed cells, via DNA transfection can result in the transformation of the recipient (*12–14*). Preliminary evidence suggests, as well, that when transfecting DNA's are derived from tumors of the same differentiated cell type (e.g., human bladder cancer cells), the gene responsible for transformation in each instance is the same (*12*). Moreover, the same transforming sequence may be activated in tumors of the same cell type from different species (*15*).

The transforming sequences from different cancers are usually different (*16*). At least one exception is known, however. In human lung and colon cancer cell lines, transforming sequences that appear similar to one another have been identified (*12*). A number of transforming sequences have also been shown to be similar to the cellular homologues of retroviral *onc* genes (*c-onc* genes) (*17*).

The *c-onc* genes are normal constituents of vertebrate genomes. The coding sequences of each are similar to those of the corresponding *v-onc* genes carried by retroviruses of different species (*18*). The *v-onc* genes are responsible for cell transformation following retrovirus infection.

ABBREVIATIONS USED: ALV=avian leukosis virus(es); BL=Burkitt's lymphoma; CML=chronic myelogenous leukemia; DMS=double minutes; HSR=homogeneously staining region(s); LTR=long terminal repeat; Ph[1]=Philadelphia chromosome(s).

---

[1] Department of Pediatrics, Division of Medical Genetics, Annenberg 17-76, Mount Sinai School of Medicine, Fifth Ave. and 100 St., New York, N.Y. 10029.

[2] Received May 6, 1983.

[3] Supported by Public Health Service grants 1-R01CA-36122-01 from the National Cancer Institute and 1-R03EY-04499-01 from the National Eye Institute.

Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.

The functional significance of the *c-onc* genes in the nontransformed cell is unknown. The finding that the same transforming sequences are present in independent examples of tumors of the same differentiated cell type (*12, 14, 15*), suggests that the *c-onc* genes play a role in differentiation. Support for such a role has come from reports that the levels of transcription of individual *c-onc* genes are different during particular phases of prenatal and postnatal development in the mouse (*19*) and during liver regeneration in the rat (*20*).

That the activation of *c-onc* genes can be related to the expression of the differentiated phenotype and the presence of marker chromosome rearrangements, is suggested by the increased level of transcription of RNA specific for a single *onc* gene, *c-myc*, in certain immunoglobulin-producing lymphomas, including BL, known to contain specific chromosome translocations.

The genes responsible for the synthesis of human immunoglobulins have been assigned to chromosomes 2 (kappa light chain), 14 (heavy chain gene complex), and 22 (lambda light chain), through the analysis of somatic cell hybrids (*21, 22*). In both BL and CML, the marker translocations involve breakpoints in or near the immunoglobulin loci assigned to each chromosome- at 2p12, 14q32, and 22q11 (*23*); in the BL variants, it has also been found that cells containing t(8;22) synthesize lambda light chains and that cells containing t(8;2) synthesize kappa light chains (*23*).

The structural rearrangements that characterize CML and BL involve, as well, the movement of at least two *c-onc* genes from their positions in the normal karyotype to positions adjacent to identified immunoglobulin loci. In CML, the *onc* gene *c-abl* is moved from chromosome 9q to a position on chromosome 22q near the lambda light chain locus (*24*); in BL, the *onc* gene *c-myc* is moved from its position on chromosome 8q to a position adjacent to the heavy chain gene complex on chromosome 14q (*25*). An analogous situation exists in the mouse plasmacytomas that contain reciprocal translocations between chromosomes 12 and 15. In these tumors, the *c-myc* gene (on chromosome 15 in the mouse) is translocated close to, or within, the heavy chain gene complex on chromosome 12 (*26*).

Although the submicroscopic rearrangements responsible for the deletion and joining of variable and constant region sequences of the immunoglobulin chain genes can involve both loci on the homologous pair of chromosomes, only one is a productive rearrangement (i.e. able to direct the synthesis of an intact immunoglobulin chain). In most, if not all, instances, the marker translocations in the leukemias/lymphomas involve the nonproductively rearranged member of the chromosome pair (*27*).

Most important in terms of the control of *onc* gene transcription, however, is the finding that following translocation in both BL and the mouse plasmacytomas, the *myc* and heavy chain genes are commonly aligned head to head, 5′-end to 5′-end (*27*). In this orientation, transcription from the *myc* gene would proceed in a direction opposite to that dictated by the heavy chain gene promoter, if that promoter were active. How, then, can we explain the increase in transcription of *myc*-specific RNA found in almost all of the B-cell lymphomas of man and mouse that have been analyzed (*28*)? [An exception is described in (*29*)].

It is known that there are "hot spots" in the karyotype: regions that are susceptible to breakage when exposed to radiation or clastogenic agents (*30*). One can speculate that differentiated genes that require submicroscopic rearrangements for functional activity, such as the immunoglobulin genes, constitute another category of hot spots: that these submicroscopic rearrangements, whether productive or nonproductive (in terms of transcription), produce an instability in the chromosomes carrying the genes, predisposing to breakage and the exchange of segments within the karyotype.

The particular segments participating in such rearrangements may or may not be selected at random; the involvement of segments containing *c-onc* genes are likely to be rare events, however (in light of the frequency of these tumors in the population).

The exchange of segments between chromosomes could, theoretically, contribute to the activation of genes carried by those segments in several ways: through the movement of the gene in question (e.g. a *c-onc* gene) away from an adjacent *cis*-acting suppressor of transcription, for example, or through the movement of that gene next to an endogenous, active promoter of transcription, or, if the break occurs in the sequence of the gene itself, through an alteration in processing of the mRNA coded for by that gene. It is the latter possibility which is presumed to apply in the mouse plasmacytomas containing *myc*-specific RNA that is smaller than that found in nontransformed cells; the plasmacytoma RNA appears to be missing a sequence coded for by the 5′-terminal exon of the *c-myc* gene (*27, 29*). The explanation for *onc* gene activation in other tumors characterized by specific translocations, is unknown.

There are at least two other mechanisms not involving translocation by which activation of an endogenous *onc* gene might be achieved: point mutation and "promoter insertion".

The nucleotide sequences of the oncogenes from two human bladder cancer lines (EJ and T24) have been found to differ from that of the homologous *c-onc* gene cloned from normal tissue (*c-Ha-ras*^{1}) at a single site (*31, 32*). The base substitution (thymidine for guanosine) results in the incorporation of valine instead of glycine in the bladder oncogene-encoded protein, p21. Because the amount of *Ha-ras*-specific RNA is essentially the same in the bladder cancer lines as in normal cells (*31*), it is probable that the altered *ras* gene activity found in the cancer cells is the direct result of mutation-induced changes in one or more properties of the gene product. (One caveat must be added, however. Before a role in tumorigenesis can be postulated for any point mutation in an oncogene, it must first be established that such a change is not simply a population polymorphism.)

"Promoter insertion" refers to the integration of a sequence directing the active transcription of mRNA

next to a structural gene (*33*). The infection of certain chicken cells by retroviruses known to lack their own transforming sequences, such as ALV, is associated with the insertion of the viral LTR next to an endogenous *onc* gene, *c-myc*. The LTR includes a putative promoter of transcription; in the lymphomas that follow ALV infection, an RNA containing both leukosis virus and *c-myc* information can be detected at levels thirtyfold to one hundredfold higher than that of *c-myc*-specific RNA in normal tissues. This phenomenon suggests a direct relationship between insertion of the viral promoter and activation of the endogenous *c-onc* gene.

Though transfection of the mouse cell line NIH 3T3 by the cloned *c-Ha-ras'* gene alone is insufficient to induce transformation, transformation can be achieved following transfection by the chimeric DNA resulting from the ligation of retrovirus-derived LTR sequences to the *c-Ha-ras*[1] gene (*34*). However, when DNA from several tumor cell lines was used to transfect NIH 3T3, the transformants produced did not contain viral sequences linked to the identified oncogenes (*35, 36*). Promoter insertion is, therefore, unlikely to account for oncogene activation in most cancers.

The question remains as to whether activation of an oncogene is, by itself, sufficient for transformation (i.e., are single oncogenes "dominant" genes for cancer?) (*12–14*). Against the dominant gene hypothesis is the observation that the only cell lines that have been transformed following transfection are certain permanently dividing, aneuploid lines such as NIH 3T3. It can be argued that these cells already carry one or more preneoplastic gene changes and that the transfected oncogene represents the final step in a multistep process. It has yet to be demonstrated that the integration of a single exogenous oncogene into the genome of a completely normal cell will directly result in the expression of the malignant phenotype.

Moreover, the identified transforming sequences isolated from cancer cell lines from two different species were clearly different from the *onc* genes, which were activated in these lines. In chicken lymphomas, for example, though *myc*-specific RNA was found to be synthesized at high levels (*33*), the transforming sequence, as defined by the transfection-induced transformation of NIH 3T3, was not *myc* (*36*). [The nucleotide sequence of the transforming gene, now designated *B-lym*, appears to specify a protein that is homologous to part of another normal constituent of the chicken genome, transferrin (*37*).]

Also, in a human leukemia line known to contain high levels of a *c-abl*-specific RNA (SMS-SB), the transforming sequence was shown to be different from *c-abl* (*38*). Both the chicken lymphomas and SMS-SB, therefore, contain at least two activated genes, each of which is presumed to be capable of modifying the normal pattern of cell growth and division.

In summary, reciprocal translocations in cancer cells are postulated to produce the activation of specific genes, now termed oncogenes. Oncogenes are likely to be genes whose expression is normally restricted to particular stages of development; once activated, however, they are expressed constitutively and act to alter the normal pattern of cell growth and division. Whereas an increase in activity of specific genes can, in theory, be produced by point mutations in structural genes, by gene multiplication, and by alterations in mRNA processing or by an induction of mRNA transcription, it is likely to be the induction or alteration of specific mRNA that accounts for oncogene activation in cancers characterized by specific reciprocal translocations.

CLASS 2

Defined.—Deletions or nonreciprocal rearrangements resulting in the loss of structural material at specific sites.

Examples.—Deletions in chromosomes 13q and 11p in retinoblastoma and Wilms' tumor, respectively.

Three solid tumors of children—retinoblastoma, Wilms' tumor, and neuroblastoma, occur in familial and sporadic forms (*39, 40*). In familial cases, predisposition to development of the cancer is transmitted as a dominant trait from parent to child. The inherited gene is, however, not sufficient for carcinogenesis because not everyone who receives it develops the cancer and not every susceptible cell carrying it becomes transformed. To explain these observations, Knudson proposed the "two-hit" hypothesis (*40*), which states that in certain cancers, at least two gene changes are required for expression of the malignant phenotype. One can inherit the predisposing germ line gene ("first hit"), but only after (at least) a second gene change ("second hit") has occurred in a susceptible cell (e.g., a neuroblast) will that cell become transformed. (In sporadic cases, the necessary "two hits" are assumed to occur as chance events in the same somatic target cell.)

Consistent chromosome abnormalities have now been identified in tumor cell karyotypes from each of these cancers, both from individuals whose constitutional karyotypes are normal and in association with constitutional deletion syndromes [i.e., the Wilms' tumor–aniridia syndrome (*41*) and the 13q deletion syndrome (*42*)]. In each instance, the chromosome rearrangement (deletion, translocation) results in the loss of structural material from a specific site or segment in the genome, in Wilms' tumor, at 11p13 (*43*); in retinoblastoma, at 13q14 (*44*); and in neuroblastoma, in chromosome 1, distal to 1p31 (*45*).

Not every example of each tumor type contains the deleted marker chromosome; in neuroblastoma, for instance, 1p deletions and or rearrangements were present in only 11 of 14 tumors in one series (*45*) (Gilbert F: Unpublished observations). That the same gene may, however, be involved in the development of all cases of at least one of these tumors is indicated by family studies linking a gene for retinoblastoma in chromosomally normal individuals to another gene (that for esterase D) at 13q14, the site previously identified in the constitutional deletion cases (*46*). If the loss of activity of specific genes is a prerequisite for transformation in particular cell types (e.g., genes at 1p, 11p, 13q in neuroblastoma,

Wilms' tumor, and retinoblastoma, respectively), this finding suggests that such losses can be achieved through submicroscopic changes (such as point mutations), as well as by visible chromosome rearrangements.

How might the single gene changes resulting from class two abnormalities predispose to carcinogenesis? The association of particular deletions with cancers of specific differentiated cell types suggests that contained within the deleted segments are genes whose normal responsibility is the control of differentiated function. Included in this category are genes modifying cell growth and division, inasmuch as full expression of the differentiated phenotype in certain cell types can be related to the cessation of cell division (*47*).

A decrease in activity of such a differentiation-associated gene could presumably be achieved by deletion of the structural locus or by point mutation; an increase in transcriptional activity would likely result from the deletion of a *cis*-acting suppressor. Among the genes whose expression might play a role in cellular differentiation, as noted previously, are the *c-onc* genes; among these is *c-Ha-ras*^{1} (*18*), which has been mapped to the site of the deletion in patients with Wilms' tumor-aniridia syndrome, 11p13 (*48*). Because each band in the normal human karyotype contains millions of base pairs of DNA, it is not clear whether the 11p deletion in Wilms' tumors results in the loss of all, part, or none of the *c-Ha-ras*^{1} gene or of a suppressor of onc gene activity.

An alteration in activity of these differentiation-associated genes, or their expression at an inappropriate time in development, could affect the preprogrammed pattern of cell division, which is characteristic of each differentiated lineage (*49*). Such an alteration could lead to the continued proliferation of certain cell populations and might produce the so-called "intermediate" lesions which have been described as preceding cancer in a number of genetic disorders. Examples of intermediate lesions include nodular renal blastema (which may precede Wilms' tumor) and neuroblastoma-in situ; other benign lesions which may also become malignant over time, include the intestinal polyposes in familial polyposis syndromes and neurofibromas in neurofibromatosis patients. Presumably, it is the accumulation in individual dividing cells of these lesions of the gene changes responsible for malignant transformation, that produces the cancer.

One can also speculate that the effect specified by certain "first hits" is a self-limited one, allowing the somatic target cell (e.g. a neuroblast) to go through a finite number of divisions before it differentiates or dies. Indeed, maturation to a benign, more differentiated tumor, ganglioneuroma, has been reported in cases of neuroblastoma (*50*), and spontaneous regression of tumor has been documented in both neuroblastoma and retinoblastoma (*51*). Though restricting the number of divisions a cell could go through would reduce the statistical likelihood of any "second hit", it would never be zero; this fact could account for the coexistence of neuroblastoma and ganglioneuroma in the same pedigree.

The loss of specific chromosomes or chromosome segments has been associated with only a few other cancers or conditions predisposing to cancer. These include meningioma (monosomy 22 or 22q−) (*52*), small cell carcinoma of the lung (3p−) (*53*), and the preleukemias (particularly monosomy 7 and 5, or 5q−) (*54*). In the preleukemias, the chromosome change may precede frank cancer by months to years, again indicating that the loss of single copies of particular genes (the effect of deletion or monosomy) is, by itself, insufficient for malignant transformation.

If it is correct that the gene changes produced by individual class 2 abnormalities cannot induce cell transformation by themselves, one would predict that the homologous normal gene from a nontransformed cell would be capable of correcting the alteration in gene activity resulting from a class 2 rearrangement (a form of complementation). The fusion, then, of a nontransformed cell with a tumor cell in which transformation was the result of multiple gene changes produced by class 2 abnormalities should initially lead to the suppression of the malignant phenotype in the hybrids that are generated. Over time, as chromosomes randomly segregate from the hybrids, the loss of complementing normal gene activity could result in the reexpression of tumorigenicity. Evidence supporting the hypothesis that complementation leading to the reversible suppression of the malignant phenotype can occur in cell hybrids is summarized in (*55*).

CLASS 3

Defined.—Duplication of whole chromosomes or chromosome segments.

Examples.—Trisomies for chromosomes 8 and 9 and for 1q and 17q.

"Clonal evolution" (*56*) refers to the stepwise selection over time of mutant subpopulations derived from a common precursor cell. In CML, for example, when patients enter the terminal acute phase of their illness, chromosome abnormalities, in addition to the postulate class 1 change, the Ph^1, are evident in 80% of cases (*11*). The most common abnormalities are an extra chromosome 8, an extra Ph^1, a duplication of 17q (either as an iso(17q) or as an extra No. 17), and an extra chromosome 19. The change in karyotype in such patients is a grave prognostic sign, with death usually occurring within 3 months (*57*).

Other nonrandom chromosome changes reported in the evolution of lymphoproliferative and myeloproliferative disorders, either preceding or following tumor initiation, include trisomies for numbers 1q, 17 (or 17q), 8, and 9 (*58, 59*). Extra chromosomes 1q and 17q have also been found in solid tumors, including neuroblastomas (*59*); (Gilbert F: In preparation).

The fact that particular class 3 abnormalities are not limited to any one tumor type suggests that the genes involved are not cell type specific. The fact that they may precede or follow the development of cancer by months to years (in polycythemia vera, for example, which predisposes to the development of acute leuke-

mias), is consistent with the hypothesis that they need not be directly responsible for carcinogenesis.

Also consistent with this hypothesis is the fact that constitutional trisomies for chomosomes 8, (parts of) 9, 13, and 18 are not associated with an increased frequency of cancer. Trisomy 21 (Down's syndrome) would appear to be an exception, in that both an increased frequency of acute leukemias and an unusual hematologic disorder have been reported in a significant fraction of these patients (*60, 61*). The neonatal disorder results from the proliferation of primitive myeloid elements and resembles a leukemoid reaction. It usually regresses spontaneously. In individuals mosaic for trisomy 21, the leukemoid reaction involves cells containing the additional chromosome (*62*). Regression of the disorder in these individuals is paralleled by a decrease in the number of marrow cells containing the extra number 21.

Trisomy 21 is by itself, therefore, insufficient for carcinogenesis. The increase in acute leukemias seen in Down's patients suggests that the extra chromosome 21 alters the proliferative capacity of a particular cell population, and it is in these cells that the additional gene change(s) required for transformation occur.

Two novel chromosome abnormalities have also been associated with the multiplication of single genes in cells. These abnormalities, HSR and DMS, share unusual staining properties (*63*) and have been reported in many human and experimental animal tumors (*64*). Evidence has been presented that indicates that DMS can be derived from the breakdown of HSR (*65*).

The functional significance of HSR and DMS has been established for only a few cell lines; in each instance, the abnormal segments have been shown to contain multiple copies of (as few as) a single gene, usually one conferring resistance to specific drugs (*65, 66*). This finding has led to the hypothesis that all HSR and DMS are sites of gene amplification (*63*). In most cell lines the particular genes that have been amplified are unknown. Because most of the cell lines containing HSR or DMS were from tumors from untreated patients, it is reasonable to predict that amplification encompasses a number of genes other than those conferring drug resistance.

Recently, the human promyelocytic leukemia line HL-60 has been shown to contain multiple copies of the *onc* gene *c-myc* (*67, 68*). This line also contains DMS, and though it has yet to be proved, it is likely that the DMS carry the multiple copies of *c-myc*. Two other cell lines containing HSR–DMS have also been found to contain multiple copies of particular *c-onc* genes; the mouse adrenocortical tumor line Y1, containing amplified *c-Ki-ras* (*69*), and the human colon carcinoma line COLO 320, containing amplified *c-myc* (*70*). In the latter 2 lines it has been demonstrated (by in situ hybridization) that the HSR–DMS contain the amplified *c-onc* gene sequences.

One can conclude that class 3 abnormalities alter the division of both normal and malignant cells through a dosage effect. [A possible association between tumor progression and alterations in gene dosage resulting from abnormalities in chromosome constitution had earlier been proposed by Ohno (*71*) and Hsu (*72*).] The fact that class 3 abnormalities tend to accumulate as a tumor spreads and becomes more refractory to therapy suggests that the duplication–amplification of particular genes confers a selective growth advantage on cells in which such changes occur.

CONCLUSION

Since the first proposal, by Boveri in 1914 (*73*), of a possible role for chromosome abnormalities in the development of cancer, the precise definition of that role has remained an important, though elusive, goal of cancer biology. The classification I discuss is both an organization of the nonrandom karyotypic changes that have been associated with cancer and an attempt to define the possible genetic consequences of such changes.

Individual cancers, in this classification, are presumed to be the product of a multistep process; to result from the accumulation of single gene changes, each of which alters the proliferative capacity of the involved cell. [Possible mechanisms by which single gene changes might contribute to carcinogenesis have also been discussed in several recent reviews (*74–77*)].

The first gene change, which can result from chromosome abnormalities of class 1, 2, or 3, as well as from submicroscopic changes (including point mutations), is postulated to produce the limited expansion of a particular cell population. Only after one or more additional gene changes have occurred in the same cell, however, will that cell be transformed.

Virus infections may also play a role in the sequence of changes responsible for carcinogenesis. Epstein-Barr virus infection, for example, has been associated with certain human B-cell lymphomas containing a class 1 change—an 8;14 translocation (*78*). One can hypothesize that infection induces the division of certain susceptible cells, and it is in these dividing cells that the accumulation of changes required for cell transformation, among which may be the gene change resulting from the 8;14 translocation, occurs.

The gene changes produced by chromosome rearrangements of class 2, as discussed, are likely to be cell type specific. Those produced by class 3 may be cell type related (as with trisomy 21 and the neonatal leukemoid reaction), but they are more commonly cell type independent. Class 1 changes are cell type associated in that they can result from breaks at sites involved in the expression of specific differentiated functions.

Because an increase in gene transcription, or the loss of gene activity, can also be produced by submicroscopic changes, it is not necessary that every tumor contain visible chromosome abnormalities. This is consistent with the observation that up to 50% of acute leukemias appear to have normal karyotypes (*11*). It is also possible that this reflects the limitations of previous banding techniques; Yunis et al. (*79*) have recently indicated that with high-resolution banding, one can identify chromosome rearrangements in every acute leukemia.

Because more than one gene is likely to be involved in the control of differentiation or proliferation in any one

cell type, the pattern of gene changes responsible for carcinogenesis in individual cancers may differ (even within a single type of cancer). Because gene changes and chromosome rearrangements can develop spontaneously following transformation, either or both may differ in serial tumor samples obtained from the same patient at different points in his illness. It is also possible that more than one class of chromosome change may result from a single event; the nonrandom occurrence of iso(17q) in hematopoietic disorders, for example, may reflect both an increase in dosage of genes on 17q (class 3 change) and the loss of genes on 17p (class 2 change).

Reports of specific transforming sequences in only 2 out of several human bladder cancers (*12*) and in 1 out of 3 human neuroblastomas (*12, 13*) would also be consistent with the development of a class 1 change as either one of the primary events in tumorigenesis or as a secondary event following tumorigenesis. The fact that chromosome abnormalities of all 3 classes, especially class 3, tend to accumulate as cells continue to divide, suggests that the gene changes produced following transformation can contribute to tumor progression; that is, to the capacity of a tumor to invade locally, to metastasize, and, ultimately, to kill the host.

That abnormalities of both class 1 and class 3 can produce changes in a single category of normal genes, the *c-onc* genes, has been demonstrated in tumors containing specific translocations, such as CML or BL, and in those containing HSR–DMS, such as HL-60 and COLO 320; that abnormalities of class 2 may likewise affect the same category of genes is suggested by the position of the specific deletion in Wilms' tumor–aniridia patients. This raises the possibility that alterations in a limited number of gene families may be responsible for tumorigenesis and tumor progression in all cancers.

The classification proposed provides a framework for future discussions of the significance of chromosome abnormalities in cancer; the hypotheses generated concerning the roles played by the single gene changes produced by each of the 3 defined classes of chromosome abnormalities in cancer can be tested with the use of current techniques of somatic cell genetics and molecular biology. Comparisons of the chromosome complements of individual cancers with clinical course and outcome should also make it possible to determine whether particular chromosome abnormalities are of value in defining prognosis and therapeutic responsiveness.

NOTE ADDED IN PROOF

The product of the oncogene *v-sis* has recently been shown to be at least partially homologous to an identified cellular growth factor, platelet-derived growth factor; this is the first association between an oncogene and a protein with a known physiological function in normal cells (*80, 81*).

Several laboratories have also demonstrated that the interaction of at least two transforming agents is required for the transformation of primary diploid cells from a number of species. In embryonic rat fibroblasts and baby rat kidney cells, for example, the transfection of cloned DNA from at least two different oncogenes (of viral or cellular origin) is necessary for transformation (*82, 83*), and in hamster fibroblasts, DNA from a cloned oncogene would produce transformation only in cells already "immortalized" (capable of continuous growth) by exposure to chemical carcinogens (*84*). These observations are consistent with the hypothesis discussed herein that tumorigenesis is a multistep process.

REFERENCES

(*1*) Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960; 132:1497.

(*2*) Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 1977; 243:290–293.

(*3*) Yunis JJ. Specific fine chromosomal defects in cancer. Hum Pathol 1981; 12:503–515.

(*4*) Mayall BH, Carrano AV, Moore DH II, Rowley JD. Quantitation by DNA-based cytophotometry of the 9q+/22q− chromosomal translocation associated with chronic myelogenous leukemia. Cancer Res 1977; 37:3590–3593.

(*5*) Rowley JD, Golomb H, Vardiman J, Fukuhara S, Dougherty C, Potter D. Further evidence for a non-random chromosomal abnormality in acute promyelocytic leukemia. Int J Cancer 1977; 20:869–872.

(*6*) Rowley JD. Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet (Paris) 1973; 16:109–112.

(*7*) Manolov G, Manolova Y. Marker band in one chromosome from Burkitt's lymphomas. Nature 1972; 237:33–34.

(*8*) Berger R, Bernheim A, Weh H-J, et al. A new translocation in Burkitt's tumor cells. Hum Genet 1979; 53:111–112.

(*9*) Van den Berghe H, Parloir C, Gosseye S, Englebienne V, Cornu G, Soka G. Variant translocation in Burkitt's lymphoma. Cancer Genet Cytogenet 1979; 1:9–14.

(*10*) Fukuhara S, Rowley JD, Variakojis D, Golomb H. Chromosome abnormalities in poorly differentiated lymphocytic leukemias. Cancer Res 1979; 39:3119–3128.

(*11*) Rowley JD. Chromosome abnormalities in cancer. Cancer Genet Cytogenet 1980; 2:175–198.

(*12*) Perucho M, Goldfarb M, Shimizu K, Lama C, Fogh J, Wigler M. Human tumor derived cell lines contain common and different transforming genes. Cell 1981; 27:467–476.

(*13*) Krontiris TG, Cooper GM. Transforming activity of human tumor DNAs. Proc Natl Acad Sci USA 1981; 78:1181–1184.

(*14*) Shih C, Padhy L, Murray M, Weinberg R. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. Nature 1981; 290:261–264.

(*15*) Lane M, Sainten A, Cooper G. Activation of related transforming genes in mouse and human mammary carcinomas. Proc Natl Acad Sci USA 1981; 78:5185–5189.

(*16*) Murray MJ, Shilo B, Shih C, Cowing D, Hsu H, Weinberg R. Three different human tumor cell lines contain different oncogenes. Cell 1981; 25:355–361.

(*17*) Der CJ, Krontiris TG, Cooper G. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. Proc Natl Acad Sci USA 1982; 79:3637–3640.

(*18*) Coffin JM, Varmus H, Bishop J, et al. Proposal for naming host cell-derived inserts in retrovirus genomes. J Virol 1981; 40:953–957.

(*19*) Muller R, Slamon DJ, Tremblay JM, Cline MJ, Verma IM. Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. Nature 1982; 299:640–644.

(*20*) Goyette M, Petropoulos DJ, Shank PR, Fausto N. Expression of a cellular oncogene during liver regeneration. Science 1983; 219:510–512.

(*21*) Malcolm S, Barton P, Murphy C, Ferguson-Smith MA, Ben-

TLEY DL, RABBITTS TH. Localization of human immunoglobulin κ light chain variable region genes to the short arm of chromosome 2 by in situ hybridization. Proc Natl Acad Sci USA 1982; 79:4956–4961.

(22) ERIKSON J, MARTINIS J, CROCE CM. Assignment of the genes for human lambda immunoglobulin chains to chromosome 22. Nature 1981; 294:173–175.

(23) LENOIR GM, PREUD'HOMME JL, BERNHEIM A, BERGER R. Correlation between immunoglobulin light chain expression and variant translocation in Burkitt's lymphoma. Nature 1982; 298:474–476.

(24) DE KLEIN A, GEURTS VAN KESSEL A, GROSVELD G, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelogenous leukaemia. Nature 1982; 300:765–767.

(25) NEEL BG, JHANWAR SC, CHAGANTI RS, HAYWARD W. Two human *c-onc* genes are located on the long arm of chromosome 8. Proc Natl Acad Sci USA 1982; 79:7842–7846.

(26) CALAME K, KIM S, LALLEY P, HILL R, DAVIS M, HOOD L. Molecular cloning of translocations involving chromosomes 15 and the immunoglobulin C_a gene from chromosome 12 in two murine plasmacytomas. Proc Natl Acad Sci USA 1981; 79:6994–6998.

(27) DALLA FAVERA R, MARTINOTTI S, GALLO RC, ERIKSON J, CROCE CM. Translocation and rearrangement of the *c-myc* oncogene locus in human undifferentiated B-cell lymphomas. Science 1983; 219:963–967.

(28) ERIKSON J, AR-RUSHIDI A, DRWINGA HL, NOWELL PC, CROCE CM. Transcriptional activation of the translocated *c-myc* oncogene in Burkitt's lymphoma. Proc Natl Acad Sci USA 1983; 80:820–824.

(29) SHEN-ONG G, KEATH E, PICCOLI S, COLE M. Novel *myc* oncogene RNA from abortive immunoglobulin gene recombination in mouse plasmacytomas. Cell 1982; 31:443–452.

(30) MEYNE J, LOCKHART LH, ARRIGHI FE. Nonrandom distribution of chromosomal aberrations induced by three chemicals. Mutat Res 1979; 63:201–209.

(31) TABIN CH, BRADLEY SM, BARGMAN CE, et al. Mechanism of activation by a human oncogene. Nature 1982; 300:143–149.

(32) REDDY EP, REYNOLDS RK, SANTOS E, BARBACID M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature 1982; 300:149–152.

(33) HAYWARD WS, NEEL B, ASTRIN S. Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukosis. Nature 1981; 290:475–480.

(34) CHANG E, FURTH M, SCOLNICK E, LOWY D. Tumourigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus. Nature 1982; 297:479–483.

(35) COOPER G, NEIMAN P. Two distinct candidate transforming genes of lymphoid leukosis virus-induced neoplasms. Nature 1981; 292:857–858.

(36) LANE M, SAINTEN A, COOPER G. Stage specific transforming genes of human and mouse B- and T-lymphocyte neoplasms. Cell 1982; 28:873–880.

(37) GOUBIN G, GOLDMAN DS, LUCE J, NEIMAN PE, COOPER GM. Molecular cloning and nucleotide seqeunce of a transforming gene detected by transfection of chicken B-cell lymphoid DNA. Nature 1983; 302:114–119.

(38) OZANNE B, WHEELER T, ZACK J, SMITH G, DALE B. Transforming gene of a human leukaemia cell is unrelated to the expressed tumour virus-related gene of the cell. Nature 1982; 299:744–747.

(39) KNUDSON AG, STRONG LC. Mutation and cancer: Neuroblastoma and pheochromocytoma. Am J Hum Genet 1972; 24:514–532.

(40) KNUDSON AG. Genetics and the etiology of childhood cancer. Pediatr Res 1976; 10:513–517.

(41) FRAUMENI JF JR, GLASS AG. Wilms' tumor and congenital aniridia. JAMA 1968; 206:825–828.

(42) KNUDSON AG, MEADOWS AT, NICHOLS WW, HILL R. Chromosomal deletion and retinoblastoma. N Engl J Med 1976; 295:1120–1123.

(43) RICCARDI VM, SUJANSKY E, SMITH AC, FRANCKE U. Chromosomal imbalance in the aniridia-Wilms' tumor association. Pediatrics 1978; 61:604–610.

(44) YUNIS JJ, RAMSAY N. Retinoblastoma and subband deletion of chromosome 13. Am J Dis Child 1978; 132:161–163.

(45) GILBERT F, BALABAN G, MOORHEAD P, BIANCHI D, SCHLESINGER H. Abnormalities of chromosome 1p in human neuroblastoma tumors and cell lines. Cancer Genet Cytogenet 1982; 7:33–42.

(46) SPARKES RS, MURPHREE AL, LINGUE RW, et al. Gene for hereditary retinoblastoma assigned to human chromosome 13 by linkage to esterase D. Science 1983; 210:971–973.

(47) GILBERT F. Control of expression of differentiated functions in neuroblastoma cell hybrids. J Natl Cancer Inst 1976; 57:667–673.

(48) DE MARTINVILLE B, GIACALONE J, SHIH C, WEINBERG RA, FRANCKE U. Oncogene from human EJ bladder carcinoma is located on the short arm of chromosome 11. Science 1983; 219:498–501.

(49) HOLTZER H. Quantal cell cycle, lineages and stem cells. In: Lord B, Potten C, Cole R, eds. Stem cells and tissue homeostasis. Cambridge: Cambridge Univ Press, 1978:1–29.

(50) CUSHING H, WOHLBACH B. The transformation of a malignant paravertebral sympathicoblastoma into a benign ganglioneuroma. Am J Pathol 1927; 3:203–215.

(51) EVERSON TC, COLE WH. Spontaneous regression of cancer. Philadelphia: Saunders, 1966.

(52) ZANG KD, SINGER H. Chromosomal constitution of meningiomas. Nature 1967; 216:84–85.

(53) WHANG-PENG J, KAO-SHAW CS, LEE EC, et al. Specific chromosome defect associated with human small cell lung cancer: Deletion 3p (14–23). Science 1982; 215:181–182.

(54) NOWELL PC. Cytogenetics of preleukemia. Cancer Genet Cytogenet 1982; 5:265–278.

(55) STANBRIDGE EJ, DER CJ, DOERSON CH, et al. Human cell hybrids: Analysis of transformation and tumorigenicity. Science 1982; 215:252–259.

(56) NOWELL PC. The clonal evolution of tumor cell populations. Science 1976; 194:23–28.

(57) WHANG-PENG J, CANNELOS GP, CARBONE PP, TJIO HH. Clinical implications of cytogenetic variants in chronic myelogenous leukemia. Blood 1968; 32:755–766.

(58) TESTA JR. Cytogenetic patterns in polycythemia vera. Cancer Genet Cytogenet 1980; 1:207–215.

(59) SANDBERG AA. The chromosomes in human cancer and leukemia. New York: Elsevier, 1980.

(60) KANEKO Y, ROWLEY JD, VARIAKOJIS D, CHILCOTE R, MOOHR J, PATEL D. Chromosome abnormalities in Down syndrome patients with acute leukemia. Blood 1981; 58:459–470.

(61) ROSS JD, MALONEY WC, DESFORGES JF. Ineffective regulation of granulopoiesis masquerading as congenital leukemia in a mongoloid child. J Pediatr 1963; 63:1–10.

(62) BRODEUR G, DAHL G, WILLIAMS D, TIPTON R, KALWINSKY D. Transient leukemoid reaction and trisomy 21 mosaicism in a phenotypically normal newborn. Blood 1980; 55:691–693.

(63) BIEDLER JL, SPENGLER BA. Metaphase chromosome anomaly: Association with drug resistance and cell-specific products. Science 1976; 191:185–187.

(64) GILBERT F, BALABAN G, BREG WR, GALLIE B, REID T, NICHOLS W. Homogeneously staining region in a retinoblastoma cell line: Relevance to tumor initiation and progression. JNCI 1981; 67:301–306.

(65) COWELL JK. Double minutes and homogeneously staining regions: Gene amplification in mammalian cells. Annu Rev Genet 1982; 16:21–60.

(66) SCHIMKE R. Gene amplification. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1982.

(67) COLLINS SH, GROUDINE M. Amplification of endogenous *myc*-related DNA sequences in a human myeloid leukaemia cell line. Nature 1982; 298:679–681.

(68) DALLA FAVERA R, WONG-STAAL F, GALLO RC. *Onc* gene amplification in promyelocytic leukemia cell line HL-60 and primary leukemic cells of the same patient. Nature 1982; 299:61–63.

(69) SCHWAB M, ALITALO K, VARMUS HE, BISHOP JM, GEORGE D. A

cellular oncogene (*c-Ki-ras*) is amplified, overexpressed, and located within karyotypic abnormalities in mouse adrenocortical tumour cells. Nature 1983; 303:497–501.

(*70*) ALITALO K, SCHWAB M, LIN CC, VARMUS HE, BISHOP JM. Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (*c-myc*) in malignant neuroendocrine cells from a human colon carcinoma. Proc Natl Acad Sci USA 1983; 80:1707–1711.

(*71*) OHNO S. Genetic implications of karyological instability in malignant somatic cells. Physiol Rev 1971; 51:496–526.

(*72*) HSU TC. Chromosomal evolution in cell populations. Int Rev Cytol 1974; 12:69–161.

(*73*) BOVERI T. Zur Frage der Enstehung Maligner Tumoren. Jena: Fischer, 1914.

(*74*) CAIRNS J. The origin of human cancers. Nature 1981; 289:353–357.

(*75*) KLEIN G. The role of gene dosage and genetic transposition in carcinogenesis. Nature 1981; 294:313–318.

(*76*) COMINGS DE. A general theory of carcinogenesis. Proc Natl Acad Sci USA 1973; 70:3324–3328.

(*77*) MOOLGAVKAR SH, KNUDSON AG JR. Mutation and cancer: A model for human carcinogenesis. JNCI 1981; 66:1037–1052.

(*78*) KLEIN G. Lymphoma development in mice and humans. Proc Natl Acad Sci USA 1979; 76:2442–2446.

(*79*) YUNIS JJ, BLOOMFIELD CD, ENSRUD K. All patients with acute nonlymphocytic leukemias may have a chromosomal defect. N Engl J Med 1981; 305:135–139.

(*80*) WATERFIELD MD, SCRACE GT, WHITTLE N, et al. Platelet-derived growth factor is structurally related to the putative transforming protein $p28^{sis}$ of simian sarcoma virus. Nature 1983; 304:35–39.

(*81*) DOOLITTLE RF, HUNKATILLER MW, HOOD LE, et al. Simian sarcoma virus *onc* gene, *v-sis*, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 1983; 221:275–277.

(*82*) LAND H, PARADA LF, WEINBERG RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 1983; 304:596–602.

(*83*) RULEY HE. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature 1983; 304:602–606.

(*84*) NEWBOLD RF, OVERELL RW. Fibroblast immortality is a prerequisite for transformation by EJ *c-Ha-ras* oncogene. Nature 1983; 304:648–651.