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Mapping of human chromosomal regions related to neoplasia: Evidence from chromosomes 1 and 17

(gene mapping/virus interaction/hematologic disorders)

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ABSTRACT **In clonal aberrations leading to an excess or partial excess of chromosome 1, trisomy for bands 1q25–1q32 was noted in the myeloid cells from all of 34 patients who had various disorders such as acute leukemia, polycythemia vera, and myelofibrosis. This was not the result of a particularly fragile site in that region of the chromosome because the break points in reciprocal translocations that involve it occurred almost exclusively in the short arm. Two consistent rearrangements that have been observed in chromosome 17 produced either duplication of the entire long arm or a translocation of the distal portion of the long arm to chromosome 15. The nonrandom chromosomal changes found in hematologic disorders can now be correlated with the gene loci on these chromosomes or chromosomal segments. Seventy-five genes related to various metabolic enzymes have been mapped; it may be significant that chromosomes carrying gene loci related to nucleic acid metabolism are more frequently involved in hematologic disorders (and other malignancies as well) than are gene loci related to intermediary or carbohydrate metabolism. Furthermore, the known virus–human chromosome associations are closely correlated with the chromosomes affected in hematologic disorders. If one of the effects of carcinogens (including viruses) is to activate genes that regulate host cell DNA synthesis, and if translocations or duplications of specific chromosomal segments produce the same effect, then either of these mechanisms might provide the affected cell with a proliferative advantage.**

The relationship of chromosomal changes to neoplasia is one of the most significant unanswered questions in cancer research. The use of new staining techniques has provided evidence that requires the reexamination of the eariler view that chromosomal changes are variable epiphenomena unrelated to the fundamental processes of malignant transformation (1). Nonrandom clonal chromosomal changes are observed in human malignancies (2, 3) when the cells are examined with quinacrine fluorescence (4) or with one of the Giemsa binding techniques (5, 6) which allow the precise identification not only of whole chromosomes but frequently of parts of chromosomes as well. Analysis of hematologic disorders, particularly the leukemias, provides most of the information on chromosomal patterns and thus on the types of nonrandom chromosomal changes that occur in malignancy (7). In addition to analyses of the nonrandom pattern of gains and losses of whole chromosomes, sufficient data are now available to indicate whether specific regions of these chromosomes are involved in the changes.

As our understanding of nonrandom patterns has increased, there has been a concurrent development of the human gene map. The use of somatic cell hybrids, complemented by linkage data from pedigree analyses, has led to the chromosomal localization of at least 75 genes coding for enzymes or for other factors involved in intermediary metabolism and in carbohydrate, protein, lipid, and nucleic acid metabolism (8). The correlation of specific chromosomal abnormalities with various known genetic loci is now possible. One can now ask what advantage these particular changes provide for the mutant cell.

Two chromosomes especially prone to structural rearrangements are chromosomes 1 and 17. In this paper, data are presented on the specific regions of each chromosome that are affected and on the genes that are located in these regions. One hypothesis suggested by this report, and by other evidence, is that the various nonrandom karyotypic changes alter the number or location, or both, of gene loci related to nucleic acid biosynthesis and that these alterations provide the mutant cell with a proliferative advantage over normal cells.

MATERIALS AND METHODS

Studies, by chromosome banding techniques in my laboratory between January 1970 and January 1977, of cells from patients with hematologic disorders provide the basis for this report. From this group, patients were selected as follows.

1. *All patients who showed any clonal abnormality affecting chromosome 1.* Three patients had an additional chromosome 1; six had structural rearrangements leading to a duplication of part of chromosome 1 (Fig. 1); six had an apparently balanced translocation affecting chromosome 1, and one of these also had a deletion of part of it. To these data I have added information from the karyotypic analysis of 30 patients with abnormalities of chromosome 1 who have been reported by others. These patients comprise the total population of which I am aware having any abnormality of chromosome 1. Except for eight patients, the data have been taken from published reports; details regarding the complete karyotype, hematologic disease, and investigator are given elsewhere (9).

2. *Patients with consistent abnormalities of chromosome 17.* These included four patients with a rearrangement involving chromosomes 15 and 17 [t(15;17)(q22;q21)] who had acute promyelocytic leukemia and six patients with an isochromosome for the long arm of chromosome 17 [i(17q)], five of whom had chronic myelogenous leukemia.

The cells used in these analyses were obtained directly from bone marrow specimens or from 24- or 48-hr cultures of immature bone marrow-derived (myeloid) cells circulating in the peripheral blood (10). The abnormalities observed were confined to the bone marrow cells. Karyotypes obtained from other tissues, usually mitogen-stimulated lymphocytes, were normal. The chromosomal changes discussed here represent somatic mutations in otherwise chromosomally normal persons.

The identification of chromosomes and band numbers used in this report is based on the nomenclature adopted at the Paris Conference (11). A + or − sign before a number indicates a gain or loss, respectively, of a whole chromosome. The letters "p" and "q" refer to the short and long arms of the chromosome, respectively; "ter" refers to terminal or end of chromosome; "i" and "del" are "isochromosome" and "deletion." Translocations are identified by "t" followed by the chromosomes in-

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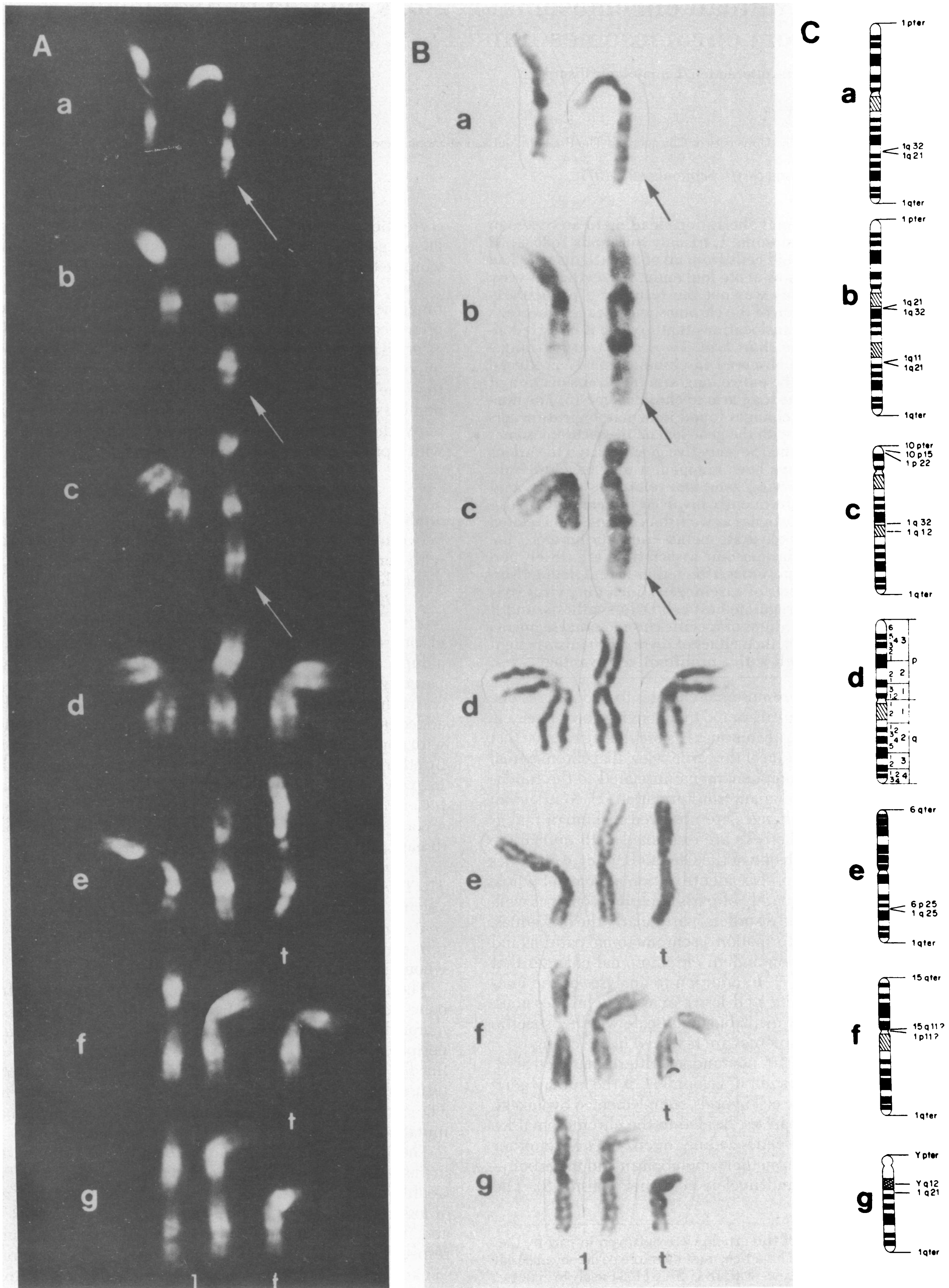

FIG. 1. *(Legend appears at bottom of the next page.)*

volved in the first set of brackets; the chromosome bands in which the breaks occurred are shown in the second brackets.

RESULTS

Abnormalities of Chromosome 1. Ten patients studied in my laboratory had abnormalities of chromosome 1 that altered the amount of genetic material of this chromosome in the bone marrow cells; in nine, the aberration led to duplication of part or all of the chromosome (Fig. 1). Information was obtained from other investigators on 26 patients who had an abnormal amount of chromosome 1 in clones of bone marrow cells; in 25 of these there was an excess. The detailed karyotypic and clinical data are reported elsewhere (9). In all of these patients, the specific aberration was present in 50–100% of the myeloid cells. The presence of the same abnormality in the cells of these patients confirms their clonal origin—i.e., their origin from a single mutant cell. These patients had various hematologic abnormalities: polycythemia vera in 11; myelofibrosis in 6; acute nonlymphocytic leukemia in 8; and various other disorders in the rest.

One question to be asked is whether some regions are invariably or preferentially present in the trisomic state. Are the sites of chromosomal breaks random or clustered? Are the break points different from those seen in other clonal rearrangements not leading to trisomy or from those in inborn chromosomal abnoramlities? The region that was trisomic in each patient is represented by a vertical line in Fig. 2. All partial trisomies involved the long arm. Despite the fact that these patients had various hematologic disorders, *bands 1q25–1q32 were trisomic in every one of the 34 patients*, and bands 1q21–1q25 and 1q32 to the end of the long arm (qter) were trisomic in 30 and 28 patients, respectively. The break points on chromosome 1 are limited to bands p22–q42, with clusters at p22, the centromere, q12, q21, q25, and q32.

Two patients had deletions of chromosome 1, involving loss of 1q32–qter in one and loss of 1p32–pter in the second (9).

In addition to these 36 patients, 13 had balanced reciprocal rearrangements (9). With one exception, the break points were located in the short arm of chromosome 1, specifically in bands p22, p34, and p36. The break points found in constitutional abnormalities (translocations, inversions, deletions, etc.) are distributed along the entire length of the chromosome, although there is some evidence of clustering at bands q32, p32, p36, q21, and q12 in order of frequency (9).

Abnormalities of Chromosome 17. Four patients with acute promyelocytic leukemia showed the same structural rearrangement involving chromosomes 17 and 15 at bands q21 and q22, respectively (12). This finding has been confirmed by others in two patients (13, 14).

Five patients with chronic myelogenous leukemia in the blast phase and one patient with an undifferentiated myeloproliferative disorder had an isochromosome for the long arm of chromosome 17, i(17q), usually accompanied by other changes such as an extra chromosome 8. The i(17q) has also been reported in 41 other patients with chronic myelogenous leukemia (3, 7) and in 7 patients with acute leukemia (15).

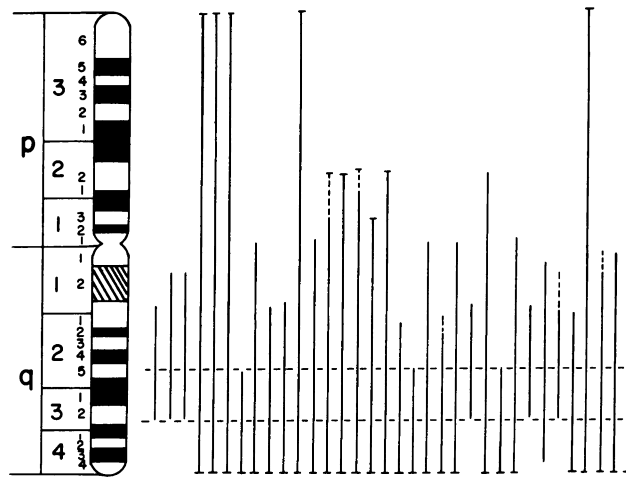

FIG. 2. Diagram of abnormalities illustrated in Fig. 1. Each vertical line represents the trisomic region in each patient; dashed vertical lines indicate break points. Patients *a–d* are represented by lines 1–4 and *e–g*, by lines 7–9. The dashed horizontal lines enclosed the segment that is trisomic in every one of the 34 patients. The detailed karyotypes and hematologic disorders are listed in ref. 9.

DISCUSSION

The evidence presented here indicates that certain regions of chromosomes 1 and 17 appear to be selectively affected in myeloid cells with clonal aberrations of these chromosomes. One specific region of the long arm of chromosome 1 (q25–q32) was present in a trisomic state in *every one of 34 patients* whose marrow cells were trisomic for some portion of chromosome 1. This consistent aberration in the long arm is not the result of a particularly fragile site in that region of the chromosome, because the break points in reciprocal translocations were almost exclusively in the short arm. The two consistent rearrangements of chromosome 17 produced either duplication of the entire long arm or a translocation of the distal portion of the long arm to chromosome 15. Other common karyotypic aberrations seen in hematologic disorders are summarized in Table 1. Trisomy for chromosome 8 is the aberration seen most frequently, and preliminary evidence indicates that the critical region is in the long arm (16, 17). Two consistent translocations of chromosome 8—namely, t(8;21) in acute nonlymphocytic leukemia (18) and t(8;14) in Burkitt lymphoma (19, 20)—also involve the long arm.

Because several different nonrandom chromosomal aberrations have been identified in patients with hematologic disorders, it is apparent that no single gene locus provides the mutant cells with a proliferative advantage. One can then ask whether the different aberrations such as trisomy 1q or i(17q) have a certain class of gene loci in common. To provide a proliferative advantage, the mutation must occur in a cell that is capable of continued division, because all present evidence,

FIG. 1. (*on preceding page*). No. 1 chromosomes from seven patients (*a–g*): (*A*) stained with quinacrine mustard and photographed with ultraviolet heterochromatin; (*B*) same chromosomes stained with conventional Giemsa (*d, e, f*) or after NaOH treatment to reveal constitutive heterochromatin (*a, b, d, g*); (*C*) diagram, according to Paris nomenclature, of the rearrangement involving chromosome 1. (*a*) Myelofibrosis; the abnormality results in the duplication of 1q21 to 1q32. There is only one block of constitutive heterochromatin, at the centromere. (*b*) Histiocytic lymphoma; there is an inversion of the duplicated segment 1q11 to 1q32 between two block of constitutive heterochromatin. (*c*) Plasma cell leukemia; the duplicated segment of 1q11 to 1q32 is between two blocks of constitutive heterochromatin. (*d*) Acute myelogenous leukemia; an extra intact chromosome 1 is present. Diagram shows complete numbering system for a normal chromosome 1. (*e*) Myelofibrosis; a translocation (t) between the end of the short arm of chromosome 6 (p25) and chromosome 1 (q25). (Patients *e, f,* and *g* also had two normal chromosome 1's.) (*f*) Acute myelocytic leukemia following polycythemia vera; the entire long arm of one chromosome 1 is translocated to the entire long arm of chromosome 15 (t). (*g*) Polycythemia vera; part of the long arm of chromosome 1(q21) has been translocated to the end of the Y chromosome (t).

Table 1. Summary of chromosome abnormalities and gene loci

Disease*	Chromosome 1	5	7	8	9	14	15	17	19	20	21	22
CML					t							t
AL				+				q+	+			+ph[1]
ANLL	+	q−	−	t/+				+			t/+−	
APL							t	t				
PV	q+			+	+					q−		
MMM	q+			+	+							
Ref. an.		q−	−	+						q−		
Lymphoma				t		t		+				
Nucleic acid gene loci†												
	AK2 UMPK GUK1-2 U-CK§ 5sRNA	adeB‡			AK1 AK3	NP rRNA	rRNA	TK		ADA ITP	GAPS§ rRNA	rRNA

* CML, chronic myelogenous leukemia; AL, acute phase of CML; ANNL, acute nonlymphocytic leukemia; APL, acute promyelocytic leukemia; PV, polycythemia vera; MMM, myelofibrosis; Ref. an., refractory anemia.

† AK1,2,3, adenylate kinases; ADA, adenosine deaminase; adeB, FGAR amidotransferase; GAPS, phosphoribosyl glycinamide synthetase; GUK 1, 2, guanylate kinases; ITP, inosine triphosphatase; NP, nucleoside phosphorylase; TK, thymidine kinase; U-CK, uridine-cytidine kinase; UMPK, uridine monophosphate kinase.

‡ On chromosome 4 or 5.

§ Provisional assignment.

both cytogenetic and enzymologic (21–23), supports the single-cell origin of these mutant clones. The defect may lead to an increase in the proportion of cells committed to the "stem" cell compartment compared with those having differentiated functions (24). Alternatively, the defect may allow a mature, supposedly end-stage cell to continue to divide, which would not be possible in a similar, chromosomally normal cell.

Only gross chromosomal changes are considered here, but subtle changes at the genetic level that do not produce detectable chromosomal lesions must also be involved in neoplastic transformation. Because the average chromosome band contains 5×10^6 base pairs, deletions or duplications of one-third of a band, or 2×10^6 nucleotide pairs, would be difficult to detect with current methods.

The Human Gene Map. Of the approximately 75 enzymes that have been located in the gene map (8), only those related to carbohydrate and nucleic acid metabolism are sufficiently numerous to allow a useful correlation between chromosomes that are abnormal in hematologic disorders and chromosomes carrying genes related to these two cell functions. The 42 genes involved in intermediary or carbohydrate metabolism are located in 18 chromosomes, 9 of which (chromosomes 1, 5, 7, 8, 9, 15, 17, 19, and 22) are abnormal in these patients. The 16 genes related to nucleic acid metabolism are located on 10 chromosomes, 7 of which (chromosomes 1, 5, 9, 14, 17, 20, and 21) have been found to be abnormal in patients with hematologic disorders. One other chromosome, the X, which carries the gene for hypoxanthine phosphoribosyltransferase, occasionally is abnormal in acute nonlymphocytic leukemia. Of the remaining uninvolved chromosomes (namely, chromosomes 10 and 16), the gene for adenosine kinase is on the former and the genes for adenine phosphoribosyltransferase and mitochondrial thymidine kinase are on the latter. Thus, 12 of the 16 genes are located on the seven affected chromosomes, compared with 4 genes on the three less involved chromosomes. Not included in these previous categories are the genes for 5S RNA which is on 1q and the genes for ribosomal RNA which are located in the satellite region of the five acrocentric chromosomes. The genes related to nucleic acid metabolism, and the chromosomes carrying them, are summarized in Table 1.

It should be recognized that, although these correlations provide evidence in support of the hypothesis that chromosomal aberrations lead to changes in gene loci related to nucleic acid metabolism, the data are relatively sparse and the associations are therefore quite tenuous. If the hypothesis is correct, a significant number of gene loci that are unmapped at present, related to nucleic acid metabolism, will be found to be located on the affected chromosomes.

An increased number of autosomal gene loci (i.e., trisomy) should result in a 50% increase in gene product, because recent evidence in man indicates that, in trisomy, all three gene loci are functional (25, 26). This fact has led to the localization of a number of genes; for example, purine nucleoside phosphorylase is assigned to bands 14q11–14q21 (25) and adenylate kinase 1 and the closely linked ABO blood group locus, to 9q34 (26). By analogy, then, trisomy for a part or all of a chromosome in a malignant cell may result in an increase in gene product. Balanced reciprocal translocations, on the other hand, may affect enzyme levels in several ways; for example, the regulation of gene loci adjacent to the translocation site may be altered with either an increase or a decrease in gene product.

Gene loci on chromosome 1 that are related to nucleic acid metabolism are adenylate kinase 1 and uridine monophosphate kinase on the short arm, guanylate kinases 1 and 2 in bands q31–qter, and uridine-cytidine kinase in bands 1q42 to qter. Three genes are present in 17q (band 17q21–22)—namely, thymidine kinase, galactokinase, and a site of interaction with adenovirus 12. In patients with an i(17q), these genes are present in triplicate whereas, in patients with the 15;17 rearrangement, the break site in chromosome 17 appears to be in band q21, with translocation of this band to chromosome 15.

Nonrandom clonal changes occur in other malignancies, and they frequently involve the same chromosomes that are aberrant in hematologic disorders (3).

The Role of Viruses. A number of agents may be associated with an increase in chromosome breaks; some of these agents might produce specific effects correlated with the chromosomal abnormalities described here. McDougall (27) has studied the effect of adenovirus 12 on cultured human cells and has identified four sites on chromosome 1 (bands 1p36, 1p32, 1q12, and 1q42), and one on chromosome 17 that are particularly vul-

nerable to the action of this virus. The fragile site on chromosome 17 is in band 17q21–22, which also is the locus for thymidine kinase (28). Induction of host cell thymidine kinase and a high frequency of breaks in 17q21 are early functions of the virus (29), as is the synthesis of a tumor antigen that may have a role in the control of DNA synthesis. Thus, the virus may act to induce enzymes in the host cell that are related to nucleic acid metabolism and that may be essential for virus replication. As suggested before, an increased enzyme level may be one mechanism that provides the cell with a growth advantage. In regard to chromosome 1, the vulnerable sites in adenovirus 12-infected cells are 1p36, 1p32, 1q12, 1q42, which are in the region of gene loci related to nucleic acid metabolism (J. K. McDougall and V. Lewis, personal communication). With another tumor virus, simian virus 40, chromosomes 7 (30) and 17 (31) are needed to maintain the transformed state.

The chromosome lesions induced by virus infection might be indicators of gene loci that are important in cell regulation. If one of the effects of carcinogens (including viruses) were to activate genes that regulate host cell DNA synthesis, and if this activation could be maintained, these cells would have a proliferative advantage without the need for viral gene persistence and could appear to be chromosomally normal. This mechanism would explain the failure, with rare exceptions, to isolate C type virus from patients with human leukemia (32). In other neoplastic cells, an imbalance in regulation may result from altered transcription of these genes in translocated, trisomic, or monosomic chromosomal regions. This imbalance could act directly on metabolic pathways, indirectly through an effect on membrane receptors, or by some unknown other mechanism.

The evidence from hematologic disorders, and from other malignancies as well, suggests that only certain chromosomes carry genes that confer a proliferative advantage upon the mutant clone. Although the data in this paper were obtained primarily from aberrations of myeloid cells, McCaw *et al.* (33) have commented on the frequency with which chromosome 14 is involved in proliferative diseases of lymphoid origin. In their discussion, they noted that chromosomal rearrangements occurring in band 14q12 may provide a selective advantage to lymphocytes. This advantage might be related to an increased rate of cell division, prolonged cell life-span, or relaxation of proliferative controls. The end of the long arm of chromosome 14 (14q32) is frequently the site of translocations from other chromosomes, notably chromosome 8 in Burkitt lymphoma (19, 20). Some nonrandom chromosomal changes seen in various neoplasias may confer a selective advantage on any cell type. In some differentiated cells, on the other hand, specific gene changes may provide an advantage to a particular stage or may favor differentiation along a particular pathway.

In the past, a number of investigators (34–37) have considered the possibility that malignancy represents a balance phenomenon between genes related to the expression or suppression of malignancy. In some *in vitro* systems, transformation was reversible and was related to the presence or absence of specific chromosomal segments (36, 37). In the present context, a chromosomal change that promotes the expression of malignancy in such systems may be one that changes the level of some enzymes related to nucleic acid metabolism, either through a change in location or through duplication of gene loci.

Nonrandom chromosomal changes—particularly consistent, specific translocations—now seem clearly to be an important component in the proliferative advantage of the mutant cell in neoplasia. The challenge to the cell biologist is to decipher the meaning of these changes.

Conversations with Dr. James McDougall stimulated me to view the chromosomal changes in leukemia as reflecting changes in nucleic acid metabolism or changes in the cell cycle. Dr. Daniel Roth, Section of Hematology, has classified the known gene loci as to their biochemical function in the cell. Many of my colleagues at the University of Chicago have provided critical comments on the concepts presented in this paper. The Franklin McLean Memorial Research Institute is operated by the University of Chicago for the U.S. Department of Energy under Contract E4-76-C-02-0069.

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