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34. R. Thiessen in (*4*); R. T. Greer, *Iowa State Univ. Energy Min. Res. Inst. Working Pap. 4* (1975), pp. 1–76. Considerable nonframboidal microglobular pyrite also is widely reported in coal. Much of this may be recrystallized framboidal pyrite.
35. The filling of a plant cell by pyrite demands far more sulfur and iron than are available in the immediately enclosing tissue. The dependence of pyrite on organic sulfur thus implies that pyrite is initially nucleated or fixed, with some growth, by sulfide generated from oxysulfur compounds, at the site of bacterial grazing, and that the initial pyrite is nourished by diffusion of sulfide of the same origin and of iron from the surrounding mass of degrading tissue. Conceivably tissue-bound nucleation may be localized by sulfidation of iron-rich structures and compounds within the cells, such as the mitochondrial flavoproteins or the ferridoxins of chloroplasts, both iron-sulfur proteins.
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48. The tin II–strong phosphoric reagent is applied to a fresh subsample for which total sulfur and its mineral forms were previously determined and organic sulfur defined by difference. The system is purged with nitrogen, heated to 250°C for 30 minutes, and the resultant H_2S is collected in zinc acetate solution, as ZnS. Reducible sulfur is determined by idiometric titration of the ZnS. The reducible sulfur is subtracted from total sulfur to define carbon-bonded sulfide (carbon-bonded sulfur of the soil literature). The reducible organic sulfur (ester sulfur of the soil literature) is defined by subtracting carbon-bonded sulfide from organic sulfur.
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53. Frenay (*24*) documents anaerobic bacterial degradation of cysteine, producing mercaptopyruvate and thiocysteine, both yielding H_2S, and of methionine, producing methyl mercaptane and dimethyl disulfide.
54. Sulfhydrolase activity of undetermined origin is known in soils [P. J. Cooper, *Soil Biol. Biochem.* **4**, 333 (1972); M. A. Tabatabai and J. M. Bremner, *Soil Sci. Soc. Am. Proc.* **34**, 225 (1974)] and from organic-rich lake mud in which it appears to be unrelated to bacterial sulfate reduction, though the released sulfate can be utilized in dissimilatory respiration [G. M. King and M. J. Klug, *Appl. Environ. Microbiol.* **39**, 950 (1980)].
55. M. B. Goldhaber and I. R. Kaplan, *Mar. Chem.* **9**, 95 (1980). R. A. Berner, T. Baldwin, and G. R. Holden, Jr. [*J. Sediment. Petrol.* **49**, 1345 (1979)] find higher persistence of FeS in low-sulfate brackish marshes than high-sulfate marine mud, thus supporting the expectation that FeS should be present and display depth relations to pyrite, if it is a necessary intermediate in low-sulfur peat.
56. Determinations on 10-g samples from P. J. Aruscavage, U.S. Geological Survey (personal communication).
57. This is opposed to conversion of a preexisting globule of FeS. The origin of framboids is obscure. Their architecture appears to demand crystallization of a prior gel or amorphous phase, as argued by Rickard (*12*), or a sequence from mackinawite to glubular greigite to pyrite, as noted by Sweeney and Kaplan (*14*). By urging direct fixation of pyrite in Everglades peat we do not preclude crystallization from amorphous or gel-like pyrite to form framboids. However, the presence of isolated, large octahedrons of pyrite, among the framboids, indicates some direct fixation of crystalline.
58. We thank R. A. Berner, E. C. T. Chao, M. Goldhaber, P. G. Hatcher, and J. R. Postgate for helpful comments, E. J. Dwornik for assistance in electron microscope observation, and D. Dwornik for photographic help.

The Chromosomal Basis of Human Neoplasia

Jorge J. Yunis

Burkitt's lymphoma, for example, the human cellular oncogene *myc* (c-*myc*), normally located in chromosome 8, appears to become activated when rearranged with either the immunoglobulin heavy chain genes of chromosome 14 or the immunoglobulin light chain genes of chromosomes 2 and 22 (*9*). Such findings together suggest that chromosomal abnormalities play an important role in human malignancy and could represent, at the molecular level, mechanisms of altered oncogene activity.

That certain chromosomal defects are consistently associated with some types of human cancer was established in the 1970's (*1*). Yet the frequency of these defects and the molecular mechanisms involved remained unknown. During the same decade, numerous data were collected on the structure and function of oncogenes of animal cancer viruses (*2*), but no definitive evidence of a viral origin of human neoplasia was presented (*3*).

In the last 2 years, the use of improved methods for culturing tumor cells and of high-banding techniques for studying human chromosomes has shown that chromosomal defects are present in most neoplasias (*4–7*). It has also been shown that active oncogenes occur in various types of human cancer and that they represent normal cellular genes of vertebrates (*8, 9*). Such genes, when taken up by certain viruses, can be used to induce neoplasia in experimental animals or to transform cells in culture (*3*).

The knowledge accumulated on chromosomal abnormalities and cellular oncogenes has just begun to merge. In

Most Cancers Have a Chromosomal Defect

The malignant cells of most neoplasias show chromosomal abnormalities and in many the defects are consistent (*4–7*). The most common of the recurring defects is either a band deletion or a reciprocal translocation between two chromo-

The author is professor in the Department of Laboratory Medicine and Pathology, University of Minnesota, Medical School, Minneapolis 55455.

somes in which one remains constant and breaks at a specific site (donor or constant chromosome) while the other may vary (receptor chromosome). In a few instances, however, a specific chromosome may occur in triplicate (trisomy).

In 1970, banding methods for the study of human metaphase chromosomes (250 and 320 band stages) were introduced (*10*) that allowed for the delineation of several chromosomal abnormalities (*1, 11*). Recently, a technique using methotrexate cell synchronization and brief exposure of cells to Colcemid was introduced for the routine study of leukemia, lymphoma, and carcinoma cells at the 400, 550, 850, and 1200 band stages (*4–7*). These techniques make it possible to visualize previously undetectable chromosomal defects and to determine specific sites that may be important in carcinogenesis (Fig. 1).

Summary. High-resolution banding techniques for the study of human chromosomes have revealed that the malignant cells of most tumors analyzed have characteristic chromosomal defects. Translocations of the same chromosome segments with precise breakpoints occur in many leukemias and lymphomas, and a specific chromosome band is deleted in several carcinomas. Trisomy, or the occurrence of a particular chromosome in triplicate, is the only abnormality observed in a few neoplasias. It is proposed that chromosomal rearrangements play a central role in human neoplasia and may exert their effects through related genomic mechanisms. Thus, a translocation could serve to place an oncogene next to an activating DNA sequence, a deletion to eliminate an oncogene repressor, and trisomy to carry extra gene dosage.

Previous methods indicated that only 50 percent of patients with acute nonlymphocytic leukemia (ANLL) and acute lymphocytic leukemia (ALL) had a chromosomal abnormality in their malignant cells, and that a consistent defect was present in a relatively small percentage of the total number of patients (*1, 11*). Using the new technologies in a study of 73 consecutive patients with ANLL and 10 with ALL, we found that most of the patients (97 and 90 percent, respectively) had a clonal chromosomal defect, with two-thirds demonstrating one of ten distinctive recurring abnormalities (Table 1) (*5*). With highly banded chromosomes and B- and T-cell markers we have also successfully studied 84 of 90 patients (93 percent) with non-Hodgkin's non-Burkitt's lymphomas. Eighty-two of the 84 patients (98 percent) showed a chromosomal abnormality, and two-thirds of them showed one of four defects (*6*). Among them, one-third had a low-grade follicular lymphoma and a translocation involving chromosomes 14 and 18 [t(14;18)], and an additional 10 percent had a high-grade B-cell lymphoma and a t(8;14) [Fig. 1, a and b]. Previous techniques had indicated that satisfactory preparations of the chromosomes could be obtained from only 60 to 70 percent of patients, and no consistent anomaly for a given histologic type of lymphoma, other than Burkitt's, was definitely established (*12*).

Prior to 1980, research on carcinoma was also hampered by technical limitations in the ability to gently disaggregate solid tumor cells and stimulate them to proliferate preferentially in short-term culture. With standard methods, approximately 50 percent of carcinomas could be analyzed and, even though most reported cases showed chromosomal abnormalities, no specific lesions were detected (*1*). The recent use of mild tissue disaggregating enzymes (*13*) and selective growth factors (*14*) or feeder fibroblast layers (*18, 19*) for cultured tumor cells have facilitated the discovery of different carcinomas with recurrent defects (Table 1). In a recent examination of 53 of 56 consecutive primary tumors, all 53 that were analyzed successfully had a chromosomal aberration. These tumors included disseminated neuroblastoma (5 cases), retinoblastoma (5 cases), and cancer of the cervix (8), vulva (7), lung (13), ovary (3), retina (5), kidney (3), colon (3), bladder (2), and breast (2) [see (*7*)].

Consistency of Chromosomal Lesions

Numerous reports in the 1970's, each based on a large number of patients (from 50 to 1000) (*15*) indicated the presence of a translocation involving chromosomes 9 and 22 or a variant translocation of chromosome 22 in 95 percent of adults with chronic myelogenous leukemia (CML) (*1, 11*). A translocation of chromosomes 8 and 14 or a variant form of this defect was present in 100 percent of the cases of Burkitt's lymphoma (*4, 15*); monosomy of chromosome 22 was found in 95 percent of cases of meningiomas (*16*); and translocation of chromosomes 15 and 17 in 41 percent of patients with acute nonlymphocytic leukemia of the M3 type (ANLL-M3) (*17*). Recently, with the improved techniques for the culture of solid tumors, a deletion of bands 31 to 36 of the short arm of chromosome 1 (1p31p36) was found in 11 of 14 patients with disseminated neuroblastoma (*18*), a deletion of 3p21p23 in 26 of 26 patients with small cell carcinoma of the lung (*14*), a t(6;14) in 26 of 26 cases of ovarian papillary cystadenocarcinoma (*13*), and a deletion of 13q14 in five of six cases of retinoblastoma (*19*).

We have now successfully analyzed a total of 240 cancer patients (including 24 patients with CML not previously cited). Because most (72 percent) of the neoplasias were studied at diagnosis and 98 percent of them showed a chromosomal abnormality, it became important to determine whether a high percentage of neoplasias have recurring or nonrandom aberrations. We therefore focused on specific types of leukemia, lymphoma, and carcinoma, where sufficient cases were available for study, and found a consistent defect in most of them. These defects included a t(9;22) in 24 of 24 cases of CML (*20*); a t(14;18) in 26 of 32 cases of follicular cell lymphoma (*6*); a t(15;17) in 12 of 12 cases of ANLL-M3; a +8 or a deletion of the long arm of either chromosome 5 or 7, or both, in 11 of 24 patients with ANLL-M2; an inversion of chromosome 16 in five of five patients with acute myelomonocytic leukemia (M4) with an increased number of abnormal eosinophils; a translocation involving chromosome 11 (band q23) with a chromosome 6, 9, 10, or 17 in six of eleven cases of acute myelomonocytic (M4) or acute monocytic leukemia (M5) (*5*); a t(9;22) or t(4;11) in five of seven cases of ALL-L1 and -L2; a terminal deletion of the short arm of chromosome 1 in four of five patients with disseminated neuroblastoma; a partial deletion of the short arm of chromosome 1 in four of five patients with disseminated neuroblastoma; a partial deletion of the short arm of chromosome 3 in four of four patients with small cell lung carcinoma (SCLC); and a deletion of the long arm of chromosome 13 in three of five patients with nonconstitutional retinoblastoma (*7*).

Table 1 shows approximately one-third of the disorders in which consistent chromosomal defects have been found with high resolution banding. A definition at the subband level of the breakpoints in various malignancies has also been obtained. For example, using prophasic and prometaphasic chromosomes

[1200 and 850 band stages, respectively (*4*)] from patients with CML, we have found that a chromosome 22 appears to consistently break at subband 22p11.21 and a chromosome 9 at q34.1 (Fig. 1c and Table 1). This was found not only in six CML patients but also in two ANLL and two ALL patients, all with a t(9;22) (*20*). In addition, in three patients with Burkitt's lymphoma, three with small noncleaved, non-Burkitt's lymphoma, and three with immunoblastic lymphoma, the breakpoint in chromosome 8 was at the Giemsa negative subband q24.13 and in chromosome 14 at q32.33 (Table 1) (*6*). At the level of chromosome resolution used, no evidence was found for loss or amplification of chromosomal material, and the translocations appeared to be reciprocal (*20*).

Basic Chromosomal Mechanisms in Human Malignancy

The cells of most solid tumors defined chromosomally to date show a loss of a specific band or segment (Table 1). Of particular interest is the finding that in constitutional retinoblastoma, all cells of the body have a loss of the midportion of band 13q14 (*21*) (Fig. 1d), whereas in the more common types of retinoblastoma (hereditary and sporadic), a deletion involving this same site is found only in the tumor (*19*). The absence of the small segment in the constitutional, hereditary, and sporadic forms of retinoblastoma strongly suggests an association between retinoblastoma and a specific DNA sequence in chromosome 13. Also, there appears to be an actual loss of function rather than an altered activity at this site, since in patients with constitutional retinoblastoma the lost segment varies considerably in size at both ends of 13q14 (*21*) and is associated with a 50 percent loss of esterase D activity (the loci for retinoblastoma and esterase D are linked), whereas individuals with duplication of this site show no tumor and 150 percent esterase D activity (*22*).

A similar situation exists for Wilms' tumor, since individuals with a constitutional absence of the distal half of band p13 in one chromosome 11 appear to be predisposed to the aniridia-Wilms' tumor syndrome (involving loss of the iris) (*23*) (Fig. 1e) and show a 50 percent loss of catalase activity. Duplication of band 11p13, in contrast, is associated with 150 percent enzyme activity and no tumor development (the catalase gene is located in 11p13) (*26*). Again, the missing chromosomal segment in the aniridia-Wilms' tumor syndrome may vary in length at either end of band p13 (*23*). In a few nonconstitutional cases of Wilms' tumor, a deletion of 11p13 has been found only in the tumor tissue (*24, 25*).

Although patients with constitutional Wilms' tumor have deletion in one of the two chromosomes 11, this defect is not in itself sufficient for development of the tumor, since only 40 percent of patients with this deletion and aniridia developed

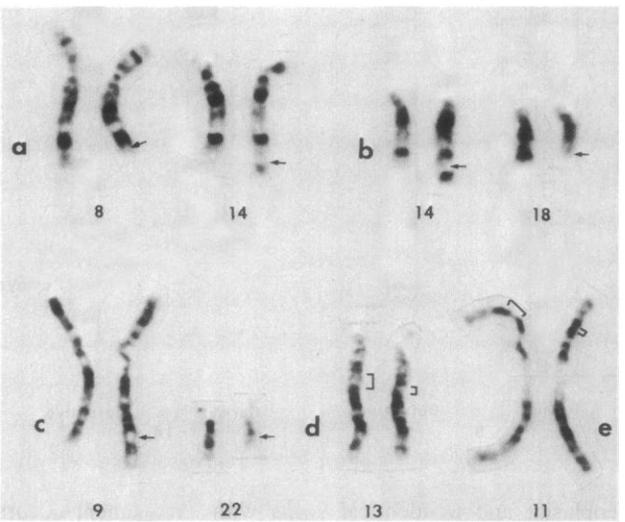

Fig. 1. Selected Giemsa banded chromosomes prepared by high-resolution technique at the 850 and 1200 band stages from patients with: (a) non-Burkitt small cell lymphoma and t(8;14); (b) follicular small cleaved cell lymphoma and t(14;18); (c) chronic myelogenous leukemia and t(9;22); (d) constitutional retinoblastoma and partial loss of band 13q14; and (e) constitutional Wilms' tumor and partial loss of band 11p13. Arrows indicate breakpoints involved in the translocations, and brackets illustrate band deletion.

Table 1. Neoplasms with a known consistent chromosomal defect. Subbands, denoted by a decimal-digit system, were defined by using high-resolution banding techniques (*4*).

Disease	Chromosome defect	Breakpoints or deletion	Reference
Leukemias			
Chronic myelogenous leukemia	t(9;22)	9q34.1 and 22q11.21	(*20, 63*)
Acute nonlymphocytic leukemia			
M1	t(9;22)	9q34.1 and 22q11.21	(*20, 64*)
M2	t(8;21)	8q22.1 and 21q22.3	(*65*)
M3	t(15;17)	15q22 and 17q11.2	(*37*)
M4*	inv 16†	p13.2 and q22	(*5*)
M4*, M5*	t(9‡;11)	9p22 and 11q23	(*5, 66*)
M1, M2, M4, M5, M6	del 5q	5q22q23	(*67*)
	del 7q	7q33q36	(*68*)
	+8		(*1*)
Chronic lymphocytic leukemia	+12		(*33*)
	t(11;14)†	11q13 and 14q32	(*33, 69*)
Acute lymphocytic leukemia			
L1-L2	t(9;22)	9q34.1 and 22q11.21	(*44*)
L2§	t(4;11)	4q21 and 11q23	(*70*)
L3	t(8;14)	8q24.13 and 14q32.33	(*45*)
Lymphomas			
Burkitt's, small noncleaved cell (non-Burkitt),* large cell immunoblastic*†	t(8;14)	8q24.13 and 14q32.33	(*6, 27*)
Follicular small cleaved,* follicular mixed,* and follicular large cell*	t(14;18)	14q32.3 and 18q21.3	(*6*)
Small cell lymphocytic*	+12		
Small cell lymphocytic, transformed to diffuse large cell*	t(11;14)†	11q13 and 14q32	(*6*)
Carcinomas			
Neuroblastoma, disseminated	del 1p	1p31p36	(*18*)
Small cell lung carcinoma	del 3p	3p14p23	(*14*)
Papillary cystadenocarcinoma of ovary	t(6;14)	6q21 and 14q24	(*13*)
Constitutional retinoblastoma*	del 13q	13q14.13	(*21*)
Retinoblastoma†	del 13q	13q14	(*19*)
Aniridia-Wilms' tumor*	del 11p	11p13	(*23*)
Wilms' tumor†	del 11p	11p13	(*24, 25*)
Benign solid tumors			
Mixed parotid gland tumor	t(3;8)†	3p25 and 8q21	(*71*)
Meningioma	−22	22	(*16*)

*Consistent chromosomal defects revealed by high-resolution banding techniques. †Few cases described. ‡Chromosomes 6, 10, 17, and 19 may serve as alternative receptor chromosomes. §Recently suspected to represent an undifferentiated form of ANLL-M4 leukemia, with the same breakpoint 11q23 as the 9;11 translocation (*70*).

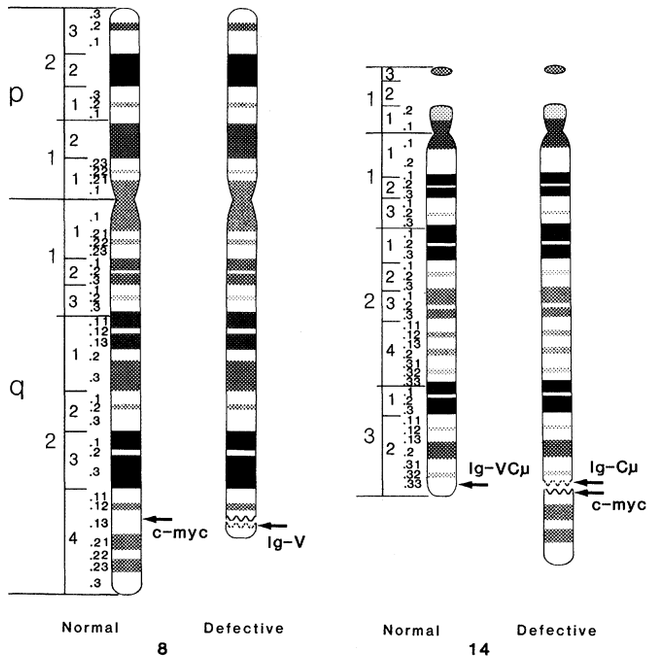

Fig. 2. Location of c-*myc* oncogene and heavy chain immunoglobulin variable (*V*) and constant μ (*C*μ) genes on normal and defective chromosome 8 and 14 in Burkitt's lymphoma, represented at the 1200 Giemsa band stage. The defective chromosome 8 loses the c-*myc* and gains V genes. The defective chromosome 14 gains c-*myc* from chromosome 8, becoming contiguous or near to Cμ. Arrows point to the normal and rearranged location of these genes. Broken ends of defective chromosomes indicate breakpoint sites.

neoplasia; and in identical twins with deletion of 11p13 and aniridia, one sibling may develop Wilms' tumor while the other may not (*23*). Such information suggests that a deletion of band p13 in one chromosome 11 strongly predisposes a patient to neoplasia but does not in itself induce the condition; and for malignancy to be initiated, a second defect may be required.

In most leukemias and lymphomas, and in some carcinomas, the malignant cells show a reciprocal translocation (Table 1). In patients with Burkitt's lymphoma, a high-grade malignancy, the malignant cells have a chromosomal translocation involving chromosomes 8 and 14 with breakpoints at subbands q24.13 and q32.3, respectively (*6, 27*) (Fig. 1a). Yet, in most patients with folicular small cleaved cell lymphoma, a low-grade malignancy, we found that the translocation usually involved chromosomes 18 and 14, with breakpoints at subbands q21.3 and q32.3, respectively (*6*) (Fig. 1b). The finding of a differing donor chromosome (8 or 18) and a common receptor chromosome (*20*) in these lymphomas implies that the donor site may determine the type of neoplasia (*6*).

The consistency of the breakpoint (band q32) in the receptor chromosome 14 in Burkitt's and non-Burkitt's lymphoma led investigators to suspect that this site confers proliferative advantage (*28*). Recent identification of the genes for immunoglobulin heavy chain on this same band (*29*) raised the possibility that the 14q+ translocation is related to the rearrangement of DNA coding for immunoglobulin heavy chain; such rearrangement occurs during normal differentiation of lymphoid cells (*30*). In support of this concept, it has been found that in Burkitt's tumor cell lines, the 14q+ chromosome retains the genes coding for the constant region of the heavy chain, whereas genes coding for all or a portion of the variable region translocate to the 8q− chromosome (Fig. 2) (*9*).

Further support for the role of the immunoglobulin genes in the translocation process comes from the fact that in variants of Burkitt's lymphoma, the end of the long arm of chromosome 8 translocates to the short arm of chromosome 2 (band p11) or the long arm of chromosome 22 (band q11), sites where the genes for the κ and λ light chains of immunoglobulin are located, respectively (*31*). Thus, it is conceivable that a donor chromosome (for example, chromosome 8 or 18) (*6*) carries a cancer gene which, when placed contiguous to the immunoglobulin genes, initiates a given type of lymphoid neoplasia. It has now been shown that the oncogene c-*myc* is located on a segment of chromosome 8, at band q24 ("Burkitt's" band) (*32*), which, in Burkitt's lymphoma cells, often becomes joined close to a switch region on chromosome 14 that carries the immunoglobulin constant region μ (Cμ) chain gene (Fig. 2) (*9*).

Even though a chromosome band deletion or a reciprocal translocation has been observed in most cytogenetically defined human neoplasias studied at diagnosis, there are two types of disorders in which a nonrandom trisomy can be found as the sole abnormality. (i) Trisomy of chromosome 12 has been observed in about one-third of patients with either chronic lymphocytic leukemia (CLL) or its lymphomatous counterpart (small lymphocytic lymphoma or SLL) (*6, 33*). (ii) Trisomy of chromosome 8 has been found in 5 to 10 percent of patients with ANLL (subtypes M1, M2, and M4 to M6) (*1, 11*) (Table 1). This raises the possibility that some malignancies are established through a mechanism of gene dosage, since in patients with a trisomy, chromosome duplication through nondisjunction could result in a double dose of an activated oncongene (*30*). Whether the role of trisomy of chromosome 8 and 12 is primary or secondary is not clear, however, because the trisomy can occur as a secondary defect in other malignancies. For example, an extra chromosome 8 is frequently seen as a secondary chromosomal defect in ANLL patients undergoing a relapse and in patients undergoing the blastic or terminal crisis of CML (*1, 11*). Also, malignant cells from follicular lymphomas are often found to have a translocation of chromosomes 14 and 18 associated with trisomy of chromosome 12 (*6*).

Relation of Oncogenes, Fragile Sites, and Carcinogens to Chromosomal Defects

Oncogenes. Fifteen oncogenes are known in the cellular genome of eukaryotes. These genes are remarkably preserved among widely separated species (*34*). The best studied is c-*myc*, an oncogene originally found in B-cell avian myelocytoma and recently assigned by hybridization in situ to band q24 of human chromosome 8 (*32*). As a result of the 8;14 translocation in Burkitt's lymphoma cells (*9*), c-*myc* transcription may increase up to 20 times over the normal level in some patients; in others, there appears to be no clear increased transcription but an altered gene product. The mechanism by which the chromosomal rearrangement triggers malignant growth is not known. However, the translocation often appears to result in a break at the 5′ side of a c-*myc* gene with a consequent loss of its 5′ exon; the immunoglobulin genes then become rearranged with the oncogene in a 5′ to 5′ configuration (*9*).

A similar translocation to that in Burkitt's lymphoma occurs in mouse and rat plasmacytomas (*30*). In the mouse, the terminal segment of chromosome 15, carrying c-*myc*, rearranges with chromosome 12 at the switch region of the heavy-chain constant-region gene (*9*). The similarity of the transposition of

genetic elements in these human and mouse B-cell lymphoid malignancies suggests that a related mechanism occurs in the two species.

In addition to c-*myc*, eight oncogenes have been found on specific human chromosomes and four on individual bands (Fig. 3) (*23, 32, 35, 36*). Because of their possible correlation with structural defects in certain malignancies, the cellular oncogenes c-*abl*, c-*mos*, c-*fes*, and c-*myb* (so named for their associations with Abelson leukemia virus, Moloney sarcoma virus, Snyder-Theilin feline sarcoma virus, and avian myeloblastosis virus, respectively) are being scrutinized. An interesting reported correlation is between c-*abl* and the translocation of chromosomes 9 and 22 in CML. C-*abl* is located on the terminal band of the long arm of chromosome 9 at band q34 (*35*), the same band involved in the breakpoint of the 9;22 translocation. This suggests the possibility that the translocation could result in activation of the oncogene (*35*). However, several laboratories have failed to show an increased transcription of c-*abl* in CML; and it is not clear that chromosome 9 is always involved in variant translocations found in 4 percent of patients with CML (*1, 11*).

An oncogene of possible relevance to ANLL is c-*mos*, mapped to band 22 of the long arm of chromosome 8 (*32*). This band is involved in one of the two breakpoints of the t(8;21) of an ANLL-M2 subgroup. A search is under way to determine if c-*mos* activation occurs in this disorder. The oncogene c-*fes* has been correlated with the 15;17 translocation that occurs in ANLL-M3. C-*fes* has been mapped to bands q24q25 of chromosome 15 (*36*), the same area where chromosome 15 was previously reported to break and rearrange with a chromosome 17 (*37*). By using high-resolution banding, however, we have found that the breakpoint actually occurs in band q22 (Table 1 and Fig. 3) (*5*).

The oncogene c-*myb* has been mapped to band q23 of the long arm of chromosome 6 (*36*), the arm that translocates to chromosome 14 in ovarian carcinoma (*13*) and that is deleted in some patients with ALL or lymphoma (*11*). Since the breakpoint in chromosome 6 in ovarian carcinoma is at q21, and at q15, q21, or q27 in lymphoid malignancies, the relation between c-*myb* and these malignancies is probably only a casual one.

Fragile sites. Although the mechanisms that trigger chromosomal translocations in cancer are unknown, it is conceivable that such rearrangements could arise without the help of specific carcinogens and be facilitated by the presence of familial or constitutional chromosome fragile sites. Expression of fragile sites is often obtained by lowering the concentration of folic acid and thymidine in the medium of cultured lymphocytes (*38*). This in turn suggests that they can be elicited when cells become deprived of DNA precursor substances.

Inherited fragile sites have been found in specific chromosome bands, including q27 of the X chromosome; q11 of chromosome 2; p13 of chromosome 9; q23 and q25 of chromosome 10; q13 of chromosomes 11 and 12; p12 and q22 of chromosome 16; p12 of chromosome 17; and p11 of chromosome 20 (see Fig. 3) (*38*). At least three fragile sites (11q13, 12q13, and 16q22) coincide with breakpoints involved in the structural rearrangements of some neoplasias (*5, 6*). We recently found a correlation between these three fragile sites and chromosomal defects in five cancer patients. Two patients with a small lymphocytic cell lymphoma showed the translocation 11;14 (q13;q32). A fragile site in band 11q13 was present in 6 percent of PHA-stimulated cultured lymphocytes from one of these patients. In a third patient, with a malignant lymphoma and a t(12;14) (q13;q32), 13 percent of the cells showed a fragile site at 12q13 (*6*). More strikingly, in two out of two patients with ANLL-M4 and an inversion of chromosome 16 (p13.2q22), a fragile site at 16q22 was found in 36 and 13 percent of the blood cells, respectively. One of these two patients had a polymorphic centromeric variant in one of the chromosomes 16, and this marker chromosome was consistently involved with a fragile site in the normal lymphocyte and with the inversion in the leukemic cells. The converse was found to be true in the second case (*5*).

A related and possibly larger class of constitutional breaks and gaps has been found in the general population. Chromosomes 1(q44), 2(q23), 3(p14), 6(q26), 7(q31), 9(q13), and 13(q34) are among those affected (*38*). Two deletions possibly related to chemical carcinogens have been reported to have a breakpoint in a similar location to constitutional fragile sites. Band 3p14 has been described as a breakpoint in the deletion 3(p14p23) of small cell carcinoma of the lung, a carcinoma associated with heavy smoking (Fig. 3) (*14*), and band 7q31 appeared to be a constitutional fragile site involved in a terminal deletion 7q in five patients with ANLL and a history of exposure to radiation, alkylating agents, or pesticides (*5*).

These findings are of interest since they suggest that fragile sites may act as predisposition factors for certain chromosomal rearrangements in human neoplasia and that some of them may represent oncogenic sites. In addition, it is possible that certain genes of differentiated cells require submicroscopic rearrangements for normal functional activity, as shown for immunoglobulin genes (*30*). Such physiologic changes in gene structure could provide a mechanism whereby unique chromosome sites become inherently susceptible to rearrangement in specific cell types.

Carcinogens. In the last few years a different avenue of research has pointed to a possible relation between carcinogens and chromosomal defects and certain neoplasias involving deletion or trisomy. The most detailed studies relate to the finding of consistent defects of chromosomes 5, 7, and 8 in both de novo and secondary ANLL among patients with a history of heavy exposure to mutagens (*39*). Among patients with secondary ANLL, consistent chromosomal changes were found in the bone marrow cells of patients who developed ANLL approximately 5 years after treatment with radiation or cytotoxic drugs for a previous unrelated malignancy (*40*). These changes involved primarily (in 39 of 43 patients) the loss of the entire chromosome or loss of part of the long arm of chromosome 5 or 7 (-5 or $5q-$, -7 or $7q-$).

Two other recent studies on de novo ANLL (*41*) have provided information relevant to the search for karyotypic changes in leukemic cells that might indicate previous exposure to strong mutagenic agents. (i) Of 256 adults (over 50 years of age) with de novo ANLL, 133 had a chromosomal defect and 64 of these showed loss of chromosome 7 or 5 or a trisomy of chromosome 8. In contrast, of 50 children with ANLL, 25 had an abnormal karyotype but only five had abnormalities of chromosomes 5, 7, or 8 (*41*). (ii) When the same chromosome aberrations were correlated with the patient's occupation, 25 of 68 adult patients (37 percent) exposed to pesticides, chemical solvents, or petroleum products showed loss of chromosomes 5 or 7 or trisomy of chromosome 8, whereas only 20 of 188 nonexposed patients (11 percent) showed these defects. These data taken together suggest that deletions and trisomy occur more often in adults than in children with ANLL and that such changes may provide a marker for ANLL that has been induced by exposure to potent mutagenic agents.

The most direct evidence of a relation between mutagenic agents and neoplasia

comes from tumors induced in experimental animals. In the rat, sarcomas induced by Rous sarcoma virus often show a trisomy of chromosome 7, while cells from sarcomas, carcinomas, and leukemias induced by dimethylbenz[*a*]-anthracene have a complete or partial trisomy of chromosome 2. There is also evidence that different etiological agents can produce the same defect; for example, trisomy of chromosome 15 occurs in mice with T-cell leukemia induced by Moloney virus, x-irradiation, or various chemical carcinogens (*42*).

Since there appears to be an association between consistent chromosomal defects in cancer and certain carcinogens and oncogenes, it should become possible to develop models in which the same chromosome lesions that occur in vivo can be induced or inhibited in vitro by manipulation of the molecular steps involved in the process. Such models might reveal the role of certain chromosomal rearrangements in the initial stages of some neoplasias.

Chromosomal defects often are not unique to one type of neoplasia. Our understanding of the role of chromosomal defects in human neoplasia has developed in three stages. In the first stage, from 1956 to 1970, chromosomal abnormalities were thought to represent

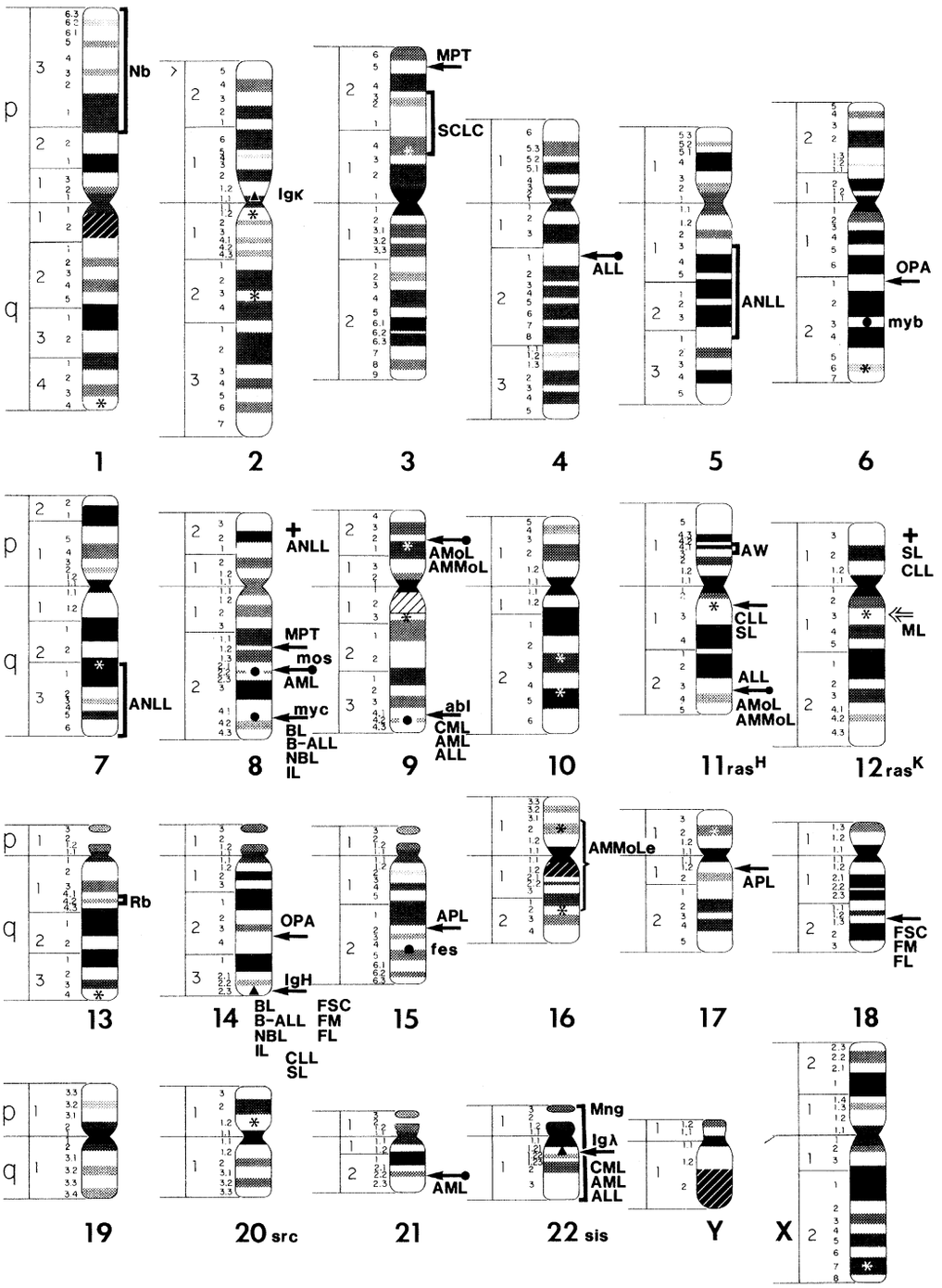

Fig. 3. Human chromosome map of oncogenes (dots), fragile sites (asterisks), immunoglobulin genes (triangles), and consistent chromosome defects in human neoplasia. Deletions are represented with a bracket, inversion with a brace, trisomy with a plus sign, and reciprocal translocations with solid arrows. The karyotype represents Giemsa bands at the 400 band stage, according to the international nomenclature (*4*). Beginning with chromosome 1, abbreviations denote the following: *Nb*, neuroblastoma; *Igκ*, kappa light chain immunoglobulin genes; MPT, mixed parotid gland tumor with t(3;8); *SCLC*, small cell lung cancer; *ALL*, acute "lymphocytic" leukemia with t(4;11); *ANLL*, acute nonlymphocytic leukemia; *OPA*, ovarian papillary adenocarcinoma with t(6;14); *mos*, Moloney sarcoma oncogene; *AML*, acute myelogenous leukemia with t(8;21); *myc*, myelocytoma oncogene; *BL*, *B-ALL*, *NBL*, and *IL*, Burkitt's lymphoma, B-cell type ALL, small noncleaved non-Burkitt's lymphoma, and immunoblastic lymphoma, respectively, with t(8;14); *AMoL* and *AMMoL*, acute monocytic and acute myelomonocytic leukemia with t(9;11); *abl*, Abelson oncogene; *CML*, chronic myelogenous leukemia with t(9;22); *ML* and broken arrows, not well defined malignant lymphoma associated with a t(12;14); *AW*, aniridia 'Wilms' tumor syndrome; *CLL*, *SL*, chronic lymphocytic leukemia and small lymphocytic lymphoma, respectively; *ras*^{H}, *ras* Harvey oncogene identified at 11p; *ras*^{K}, Kirsten sarcoma oncogene identified on chromosome 12; *Rb*, retinoblastoma; *IgH*, heavy-chain immunoglobulin genes; *fes*, Snyder-Theilin feline sarcoma oncogene; *AMMoLe*, acute myelomonocytic leukemia with increased eosinophils and inversion 16; *FSC*, *FM*, *FL*, follicular small cleaved cell, follicular mixed, and follicular large cell lymphomas, respectively, with t(14;18); *src*, Rous sarcoma virus oncogene; *Mng*, meningioma; *sis*, Simian sarcoma oncogene; *Igλ*, immunoglobulin light lambda chain genes. Igκ and Igλ are involved in the Burkitt's lymphoma variant with t(2;8) or t(8;22), respectively. Heritable fragile sites (asterisks) are found in Xq27, 2q11, 9p21, 10q23, 10q25, 11q13, 12q13, 16p124, 16q22, 17p12, and 20p11. Constitutional fragile sites occur in 1q44, 2q23, 3p14, 6q26, 7q31, 9q13, and 13q34.

an epiphenomenon of neoplasia. This was before the advent of banding techniques, and only one cancer (CML) with a consistent chromosomal defect (the Philadelphia chromosome) had been found (*43*). In 1970, when the first banding technique was introduced (*10*), we entered a second stage. During this stage, which lasted to the present, over 30 disorders were shown to have a consistent clonal chromosomal anomaly (Table 1) (*4*). This led to the assumption that each lesion was specific for certain neoplasias. I believe that we are now entering a third stage in which a given chromosomal abnormality will often be found to be associated with a variety of malignancies. As an example, among the best studied neoplasias (leukemias and lymphomas) most subgroups have been found not to have a unique defect (Table 2).

The best known of shared defects is the translocation 9;22, which is found in 90 percent of all cases of CML, 25 percent of adults and 10 percent of children with ALL, and 3 percent of adults with ANLL (Table 1). Although the same stem cell is believed to be affected in these three disorders (*44*), there may be additional mechanisms that affect the differentiation of the cell lineage and result in the different hematologic pictures of CML, ALL, and ANLL.

Another shared chromosomal lesion is that of the translocation 8;14, first thought to be characteristic of Burkitt's lymphoma and later also found to be typical of ALL of the B lymphocyte type (B-ALL) (*45*). Recently, we have observed the same characteristic translocation in small noncleaved non-Burkitt's lymphomas and a subgroup of immunoblastic lymphomas (*6*). Although there is some question as to whether Burkitt and small noncleaved non-Burkitt's lymphomas are different disorders (*6*), their clinical differences suggest the presence of a factor or factors other than the translocation 8;14. Also, even though B-ALL represents the leukemic counterpart of Burkitt's lymphoma, the fact that one disease is a peripheral disorder and the other is not (*4*) suggests the existence of a controlling step (or steps) that determines whether the tumor develops as a leukemia or lymphoma. The finding of a rearrangement between c-*myc* and Cμ in humans with Burkitt's lymphoma and the translocation 8;14 (q21;q32), and in mice with plasmacytoma and the translocation 12;15, again suggests the presence of factors determining the type of malignant process after a critical genomic event has occurred.

A third example of a shared chromosomal defect is the translocation 14;18 (q32.3;q21.3), which we recently found in three related follicular B-cell lymphomas (follicular small cleaved, follicular mixed, and follicular large-cell lymphomas). These three lymphomas make up one-third of all non-Hodgkin's lymphomas (*6*) and involve the same receptor chromosome and breakpoint (14q32.3) as in Burkitt's lymphoma.

An interesting possible counterpart to the importance of band 14q32 in lymphoid malignancies may have now been found in acute leukemias with monocytic differentiation, involving band 11q23. A translocation t(4;11)(q21;q23) results in a highly undifferentiated acute myelomonocytic leukemia previously diagnosed as ALL; a t(9;11)(p22;q23) is most often associated with poorly differentiated monocytic leukemia; and a translocation of a chromosome 6, 10, 17, or 19 to a chromosome 11 (band q23) often results in a myelomonocytic leukemia (*5*). These findings suggest the existence in band 11q23 of a gene important in myelomonocytic differentiation, equivalent to the heavy chain immunoglobulin genes of band 14q32, active during B-lymphoid cell differentiation.

Secondary chromosomal defects in tumor development. In addition to the defects present early in the development of malignancy, there may be other recurrent chromosomal defects or "permissive" sites that potentiate a malignant process. This idea gains credence from the finding that nonrandom chromosomal changes occur during the progression of neoplasia that are believed to confer proliferative advantage to the tumor cell. Probably the best known example is the acquisition of an extra Philadelphia (Ph^1) chromosome, and either an extra chromosome 8 or an extra 17q (as an isochromosome 17q or extra chromosome 17) in most patients with Ph^1 positive CML (80 percent), when they enter the blastic or terminal phase of the disease (*1, 11*). Recently, we found that most patients with follicular small cleaved cell lymphoma have a 14;18 translocation (*6*). If this is the only demonstrable chromosomal defect, the patient's prognosis is much better than if there are other associated defects such as deletions involving chromosomes 6 or 17, or trisomies of chromosomes 3, 7 or 12 (*6*).

Other chromosomal abnormalities that appear to confer proliferative advantage include the deletion 6q, which is frequently found in patients with ALL and patients with Hodgkin's and non-Hodgkin's lymphomas (*1, 11, 12*); and the duplication q25q32 of chromosome 1 that occurs in a wide range of disorders such as carcinoma of the breast, cervix, and colon, and melanoma, myeloma, non-Hodgkin's lymphoma, preleukemia, and ANLL (*46*).

Another interesting phenomenon is the finding of homogeneous staining regions (HSR's) and acentric chromosomal

Table 2. Leukemias and lymphomas with consistent chromosomal defects.

Chromosome defect	Disease
	Unique chromosomal defects
t(4;11)(q21;q23)	Acute "lymphocytic" leukemia, L2*
t(8;21)(q22.1;q22.3)	Acute myelogenous leukemia, M2
t(15;17)(q22;q11.2)	Acute promyelocytic leukemia, M3
inv(16)(p13.2q22)	Acute myelomonocytic leukemia, M4
	Shared consistent chromosomal defects
del(5)(q22q23)	Acute nonlymphocytic leukemia, subtypes M1, M2, M4, M5, M6
del(7)(q33q36)	Acute nonlymphocytic leukemia, subtypes M1, M2, M4, M5, M6
+8	Acute nonlymphocytic leukemia, subtypes M1, M2, M4, M5, M6
t(8;14)(q24.1;q32.3)	Burkitt's lymphoma
	Acute lymphocytic leukemia—L3
	Small noncleaved non-Burkitt's lymphoma
	Immunoblastic lymphoma†
t(9;11)(p22;q23)	Acute monocytic leukemia
	Acute myelomonocytic leukemia
t(9;22)(q34.1;q11.2)	Chronic myelogenous leukemia
	Acute myelogenous leukemia—M1
	Acute lymphocytic leukemia—L1, L2
t(11;14)(q13;q32)†	Chronic lymphocytic leukemia
	Small cell lymphocytic lymphoma, transformed to diffuse large cell lymphoma
+12	Chronic lymphocytic lymphoma
	Small cell lymphocytic lymphoma
t(14;18)(q32.3;q21.3)	Follicular small cleaved cell lymphoma
	Follicular mixed cell lymphoma
	Follicular large cell lymphoma

*Recently suspected to represent an undifferentiated M4 acute nonlymphocytic leukemia. †Few cases described.

fragments called double minutes (DM's) in solid tumors (*47*). This chromosomal material appears to represent sites of gene amplification (*47*). However, little is known of their molecular effect on tumor cell aggressiveness (*48*). In our laboratory we have found HSR's in three of eight carcinomas of the cervix, two of three of the colon, and two of five cases of disseminated neuroblastoma (*7*). None of the patients had received treatment, indicating that HSR's may not be related to radiation or cytotoxic drug treatment.

We have also studied leukemias (83 cases), at diagnosis or after 1 to 2 months of treatment, and lymphomas (80 cases), at diagnosis or after 1 to 15 years of treatment, and found no indication of HSR's or DM's (*20*). The absence of these aberrations may therefore be related to the peripheral nature of lymphomyeloproliferative neoplasias and the fact that more differentiated neoplastic cells are continuously replaced in these diseases so that the amplified regions, if produced, are not retained.

That distinctive secondary chromosomal changes occur during the clinical course of some malignancies points out the need for sequential studies of the neoplastic process. The common involvement of chromosomal duplication or gene amplification in tumor development indicates that extra gene dosage may be an important secondary stage in carcinoma. Since these lesions have a tendency to accumulate as the carcinoma spreads or becomes refractory to treatment, they suggest that such secondary gene changes contribute to the capacity of the tumor to invade, metastasize, and kill the host. Identification of the nature and function of these sequences could facilitate the development of rational approaches to the control of neoplastic growth.

Chromosomes, Carcinogenesis, and New Avenues of Research

The malignant cells of most cancers in humans have a chromosomal defect. Since the defects are clonal in nature and are present throughout the disease process, it is reasonable to suggest that a rearrangement of genetic material represents a common step in the pathway to the development of malignancy (*4, 49*). Such a pathway could involve at least three different types of chromosome-gene alterations. First, a genomic rearrangement may set a stem cell toward a malignant path. Second, controlling steps of cell proliferation and differentiation may be altered in such a way as to commit a stem cell toward one or another related type of malignancy. Third, some nonrandom secondary chromosomal defects seen during the evolution of the neoplastic process, may enhance tumor aggressiveness.

In neoplasias with a recurring chromosomal translocation (for example, leukemias and lymphomas), the rearrangement may activate an oncogene, such as c-*myc* in Burkitt's lymphoma (*9*). The association between oncogene activation and lymphoid neoplasias might be confirmed by studying Cμ rearrangement in lymphoid malignancies with a 14q+ marker and a donor chromosome other than No. 8. The 14q+ abnormality occurs in about 65 percent of all non-Hodgkin's lymphomas and in some patients with ALL and CLL (Table 1) (*50*). If Cμ rearrangement with a nonimmunoglobulin sequence could be demonstrated, a second step would be to find transcriptionally active rearrangements in these malignancies and to work backward to isolate previously unknown oncogenes. One such oncogene is thought to occur in band 18q21.3 and to be responsible for the low-grade follicular lymphomas with a t(14;18) (q32.3;q21.3); another in band 11q13 is thought to participate in the development of a subgroup of CLL and small lymphocytic lymphomas (*6*).

An experimental model that might be used to decipher the type and nature of additional steps in carcinogenesis is the chicken B-cell lymphoma that is induced by the lymphoid leukosis virus (LLV). As in most cancers (*49*), the experimentally induced tumor has a long latent period after LLV infection, and, in this case, involves the integration of a viral promotor, in the vicinity of c-*myc*, which results in an increased transcription of this oncogene (*51*). By means of transfection assays (*52*) it was found that an unrelated transferrin-like transforming gene is also activated in the tumor cells (*53*). Since, at the beginning of LLV-induced lymphomagenesis, multiple preneoplastic follicles appear and a small fraction of these progress to frank lymphoma, it is possible that two different activated genes are related to the two cellular events. Whether c-*myc* or the transfection gene is responsible for the preneoplastic lesion or the irreversibility of the neoplastic process is not known. The use of transcription assays for these two genes during the two stages of lymphomagenesis may help elucidate their role.

That cells with the same chromosomal defect can differentiate into one or another type of related tumor might be explained on the basis of the different transforming genes that are activated in human pre-B-cell, B-cell (Burkitt), and mature B-cell (myeloma) malignancies (*52, 54*). These genes might control differentiation-specific cell proliferation, and by cloning such genes and using them as probes it might be possible to determine why Burkitt's and some immunoblastic lymphomas share the same translocation 8;14. A related approach might be used in studies of the 9;22 translocation in malignant cells from the related leukemias CML, ANLL, and ALL. Other molecular approaches will have to be used, however, to explain why a patient with t(9;22) positive CML enters into an "acute" or terminal crisis commonly accompanied by the presence of secondary chromosome defects that often involve extra gene dosage.

It has been postulated that in neoplasias with a consistent band deletion, loss of a DNA sequence that normally represses a certain class of oncogene represents the main event leading to neoplasia (*55, 56*). In both the constitutional and sporadic nonconstitutional forms of retinoblastoma and Wilms' tumor, there is a loss of DNA segment closely linked to the genes for esterase D and catalase, respectively. Such loss predisposes an individual to neoplasia but may not by itself produce a tumor (*56*). For malignancy to be initiated, the allelic site may have to be affected by a second deletion or mutation. Such a process was postulated to have occurred in a patient with constitutional retinoblastoma. This patient showed 50 percent esterase D activity and "normal" chromosomes 13 in the blood cells (submicroscopic deletion), while the tumor showed no esterase D activity and a loss of one chromosome (allelic loss of esterase D and retinoblastoma loci) (*57*). Another example of a possible homozygous deletion was reported in the tumor cells of a patient with sporadic Wilms' tumor; these cells showed partial deletion of the short arm of the two chromosomes 11 (*25*).

The concept that carcinomas can be initiated by loss of genetic function as a result of a deletion or mutation is also supported by the complete suppression of malignancy that occurs when stable human cell hybrids are produced by fusion of a cancerous and a normal cell (*58*). Further credence for this concept comes from the fact that some 40 types of solid tumors are known to have a familial counterpart (*59*). These include some forms of cancers of the breast and colon (10 percent of the total incidence of these neoplasias) and childhood can-

cers, such as retinoblastoma and Wilms' tumor (where the hereditary fraction may be as high as 40 percent) (*56*).

An effort should now be made to identify the DNA sequences of the deleted segments by comparing DNA clones from normal and partially deleted chromosome pairs. This might be possible in instances where the missing DNA is less than 1×10^6 base pairs long and the sequence in question (for example, the retinoblastoma site in 13q14) is closely linked to a known gene (for example, esterase D), as in the case of the submicroscopic deletion in chromosome 13 discussed above, in which we could not find a defect using chromosomes at the 2000 band stage (*60*). From there it might be possible to transcribe the DNA and discover the postulated regulatory nature of the missing sequence (*56*).

Studies in the past 2 years have indicated that certain *ras* transforming genes are activated in carcinomas of epithelial origin. In particular, the *ras* gene of Harvey (ras^H) sarcoma virus is active in lung and bladder cancers and the *ras* gene of Kirsten (ras^K) sarcoma virus is active in colon cancer (*52, 61*). At present, little is known about the chromosomal profile of these neoplasias, except that small cell lung cancer (SCLC) cells show a consistent deletion of the short arm of chromosome 3. This deletion appears to be important in SCLC since we have not found it using high-resolution banding techniques in six patients with squamous cell carcinoma of the lung, three with adenocarcinoma of the lung, and two with squamous cell carcinoma of the bladder (*7*).

Since ras^H has been mapped to the short arm of chromosome 11 (*23*), SCLC consistently shows a del 3p, and ras^H transforming activity is found in various histologic types of lung tumors (*52, 61*), it is possible that the *ras* oncogenes play a noninitiating role in neoplasia (*62*).

Few studies are available of the detailed structure of chromosomes from a large number of solid tumors, and it is important that such studies now be conducted at diagnosis on all types of carcinoma. Given the frequency and consistency of chromosomal defects already found, and the recent advances in tumor cell culture techniques and chromosome resolution (*7, 60*), it is likely that additional consistent chromosomal abnormalities will be identified in carcinomas before their function is defined. Such studies could lead the way to molecular experimentation and might indicate which solid tumors have a related origin.

The general finding of shared chromosomal defects among certain leukemias and lymphomas implies that primary chromosomal defects in neoplasias affect stem cells and that the number of sequences initiating the common human neoplasias may be small. This is plausible since there may not be more than 200 truly different common histologic types of malignant tumors in man, and an initiating chromosomal defect may often be shared by two to five neoplasias (Table 2). These findings are of practical significance since 15 oncogenes have already been identified (*34*). This should make it somewhat easier to decipher the riddle of neoplasia, as now may be possible in Burkitt's lymphoma.

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RESEARCH ARTICLE

Structure of a Mouse Submaxillary Messenger RNA Encoding Epidermal Growth Factor and Seven Related Proteins

James Scott, Mickey Urdea, Margarita Quiroga
Ray Sanchez-Pescador, Noel Fong, Mark Selby
William J. Rutter, Graeme I. Bell

Abstract. *The structure of the messenger RNA (mRNA) encoding the precursor to mouse submaxillary epidermal growth factor (EGF) was determined from the sequence of a set of overlapping complementary DNA's (cDNA). The mRNA is unexpectedly large, about 4750 nucleotide bases, and predicts the sequence of preproEGF, a protein of 1217 amino acids (133,000 molecular weight). The EGF moiety (53 amino acids) is flanked by polypeptide segments of 976 and 188 amino acids at its amino and carboyxl termini, respectively. The amino terminal segment of the precursor contains seven peptides with sequences that are similar but not identical to EGF.*

Epidermal growth factor (EGF) is a 53 amino acid protein that has been isolated from the submaxillary gland of the male mouse and from human urine (*1*). It stimulates the proliferation and differentiation of cells of ectodermal and mesodermal origin. In addition, EGF, which is presumably identical to the hormone urogastrone, is a potent inhibitor of HCl release from the intestinal mucosa. As EGF exerts a number of effects on prenatal and neonatal tissue growth including accelerated maturation of the lung, precocious eye-opening, and incisor eruption and is found in elevated levels in milk, it may play a role in early development. Moreover, since EGF receptors are present in various adult tissues, EGF is presumably involved in the control of growth and function of cells throughout life.

Interestingly, EGF stimulates phosphorylation of its own receptor by a receptor-associated tyrosine-specific protein kinase which may be related to those encoded by the transforming genes of some retroviruses (*2*). Thus, the control of cell proliferation by EGF and retroviruses may share common features.

EGF is synthesized in the tubular cells of the submaxillary gland of the mouse, in the acinar cells of the human submaxillary gland, and in the human duodenal glands (*3*). Although the primary translation product of EGF messenger RNA (mRNA) has not been identified, EGF is probably generated by proteolytic processing of a larger molecule, as forms with molecular weights of about 9,000, and 28,000 and 30,000 have been reported in the mouse submaxillary gland and human urine, respectively (*4*).

We report here the nucleotide sequence of the mRNA encoding mouse submaxillary gland preproEGF. The mRNA which is at least 4750 bases encodes an EGF precursor of 1217 amino acids. The sequence of the precursor contains EGF and seven peptides that possess structural similarity to EGF.

EGF-specific clones were isolated from a male mouse submaxillary complementary DNA (cDNA) library (*5*) by hybridization with ^{32}P-labeled synthetic oligodeoxynucleotide probes made as four pools of 64-fold degenerate 20-base oligonucleotides according to the nucleotide sequence predicted from the amino acid sequence of EGF(17–23) (*6*). Eleven of 5000 colonies hybridized with the probes of pool 4; one colony, pmegf10, contained a plasmid with an insert of about 1700 base pairs (bp). This insert contained a continuous opening reading frame which included the sequence of mouse EGF. Hybridization of ^{32}P-labeled pmegf10 (*7*) to male and female mouse submaxillary RNA indicated that the mRNA encoding EGF is at least ten times more abundant in the male gland, as expected (*1*), and is approximately 4800 bases. Since the insert in pmegf10 was not a complete copy of the mRNA, overlapping clones were identified by screening the original 5000 and 7500 additional colonies with terminal restriction fragments prepared from the insert in pmegf10.

This strategy was repeated with other restriction fragments to identify all colonies that contained a portion of the mRNA

James Scott, Mark Selby, and William J. Rutter are in the Department of Biochemistry and Biophysics, University of California, San Francisco 94143. Mickey Urdea, Margarita Quiroga, Ray Sanchez-Pescador, Noel Fong, James Scott, and Graeme I. Bell (to whom requests for reprints should be sent) are on the staff of Chiron Corporation, 4560 Horton Street, Emeryville, California 94608. The present address for James Scott is Molecular Medicine Group, MRC, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, United Kingdom.