CHROMOSOME MUTATION AND NEOPLASIA

JAMES GERMAN, Editor

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*The Editor and Advisors note, with sadness, the passing of two of their distinguished colleagues: Klaus Patau—born in Gelsenkirchen, Germany, 30 September 1908 and died in Madison, 30 November 1975; Curt Stern—born in Hamburg, 30 August 1902 and died near Berkeley, 23 October 1981.

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THE CHROMOSOMES SERIES

Each volume in the Chromosomes series* is devoted to cytogenetic aspects of one broad subject, or to some aspect of cytogenetics itself. The chapters within a volume review various areas of knowledge pertaining to the given subject. Each chapter is authoritative and definitive, and each is written by an eminent scientist who himself has made important experimental contributions.

Authors have been given a free hand in style of writing and in approach. However, because the review paper plays an important role in contemporary science, a major objective in the preparation of this series is that the articles be comprehensible not only to those in cytogenetics or in the specific discipline of an author but also to those working in other branches of science.

I acknowledge with gratitude my Advisors, whose valuable views aid me both in the choice of major subject areas and possible chapter topics for review and in the selection of individual contributors.

—J. G.

*The first volume in the Chromosomes series was published in 1974 by John Wiley & Sons, Inc.

INTRODUCTION TO *CHROMOSOME MUTATION AND NEOPLASIA*

This is a book about cancer, but not cancer only. The term *cancer* is predominantly for clinical use, and this book is for biologists generally. The book's title and the following comments indicate my preference for viewing cancer as just one extreme of a spectrum; the name of the entire spectrum?—*neoplasia.* This view not only places in an appropriate perspective a process of immense importance to human health—cancer—but it also introduces a valuable dimension to the study of normal mammalian cell growth by contrasting the normal with the myriad variations from normal easily observable in neoplastic cells.

This book also is about clones, specifically, the populations of cells that develop a degree of proliferative autonomy as result of chromosome mutation.* Its contents derive heavily from observations made in five rare, genetically determined human disorders (to be mentioned again below) that predispose to the emergence of such clones, including clones that qualify for the diagnosis "cancer."

Why Is the Volume About Clones?

The acceptance of the notion that most cancers are populations of cells composed of the progeny of a single cell has developed gradually. Early in this century an authority wrote in the *Encyclopaedia Britannica* that "certain cells (emphasis added), which are apparently of a normal character and have previously performed normal functions, begin to grow and multiply in an abnormal way in some part of the body" [*29*]. However, by 1914 Boveri had concluded that "typically each tumor takes its origin from one and a single cell" [*32*]. In 1937 experimental leukemia was transmitted to 5 mice (out of 97 tries!) by the micromanipulation and intravenous injection into each mouse of a single leukemic cell [*11*]; this accomplishment provided evidence that "leukemia—which has hitherto been regarded

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**Chromosome mutation* is defined as "any structural change involving the gain, loss or relocation of chromosome segments" [*28*]. Excluded are mutations that affect only one base pair in the DNA duplex. The term has been in use for at least 50 years, having been employed, for example, by Blakeslee and Davenport [*6*]. Although many chromosome mutations can be detected microscopically, some that cannot be will be demonstrable only by the techniques of molecular genetics, particularly deletions, duplications, and inversions that involve very short segments of a chromosome.

by many workers as having a multicentric origin..." might itself be a disease of single cell origin.

By the 1950s newly improved cytogenetics techniques [*21*] revealed that the cells of certain "ascites tumors" that could be propagated in rodents by the transfer of intact cells had abnormal chromosome complements that included so-called marker chromosomes, i.e., chromosomes with morphologically detectable rearrangements; such observations led to the concept that a neoplastic cell population depends on the existence of a mutant stemline of cells, the stemline cell having a characteristic abnormal chromosome constitution [*20*]. In the mid-1950s, leukemias in rodents induced experimentally by X-irradiation were shown to consist of cell lineages with marker chromosome rearrangements [*10,* and discussed in *4*]. (None of these observations indicated whether the visible genomic alterations were of etiological significance in the neoplasia or merely manifestations of it.)

In the mid-1960s certain benign tumors of the human uterus, the common leiomyomata, were shown by a biochemical method, electrophoretic protein separation, to be clones of cells; in women heterozygous (A/B) at the X-chromosome locus for glucose-6-phosphate dehydrogenase (G6PD), any one tumor was shown to be composed of cells of just one G6PD type, either A or B, rather than of a mixture of the two as were the non-tumorous myometria from which they had arisen [*12*]. (The Lyon hypothesis had been advanced early in that decade; by the Lyon effect, the G6PD locus on one of the two X chromosomes in a female (XX) cell undergoes selective inactivation, so that a cell and all its progeny express one type of the enzyme.) Subsequently, by the same approach, most human leukemias and "solid" cancers, and even the bone marrow in certain hematological disorders not generally classed as cancer but as precancerous [*9*], have been shown to be clones.

In a different type of experiment, monolayers of non-neoplastic cells proliferating in vitro that had been treated with some carcinogenic agent were found to contain foci of cells with altered growth patterns. That foci and not the whole monolayer of treated cells exhibited aberrant growth indicated that single cells had been "transformed," each giving rise to a colony that manifested features characteristic of neoplastic cells in vitro.

During the past two decades, cytogenetic evidence, viz., the presence of the identical marker chromosome(s) in each cell, has accumulated to indicate that several human leukemias and lymphomata, many solid tumors, and at least one benign type of tumor consist of clones of cells. (Subclones in the neoplastic populations have been

observed also, and these are believed to be important in the evolution and changing clinical character of the cancers [*24,25*].) Even before banding techniques came into general use in the early 1970s, a unique marker chromosome in each cell of a tumor population, with or without additional gains or losses of chromosomes or rearrangements, had identified many human cancers of diverse types as descendants of single cells, the progenitor cells themselves necessarily having either produced or inherited the unique markers in each case. (Again, such studies did not bear on the possibility that the progenitor cell itself had been a member of a population that had inherited some alteration even before a chromosome mutation occurred in it, i.e., that in some obscure way it had become what has been termed "preneoplastic" [*16*].)

Chromosome banding techniques have permitted the recognition of more marker chromosomes and the better characterization of the rearrangements. Such studies are disclosing a striking degree of specificity between the breakpoint locations in the rearrangement and the types of cancer. (It is noteworthy in this respect that in 1981 the sixth of the international workshops convened at intervals since 1973 to summarize knowledge of human-chromosome mapping made an official tabulation not made by previous groups, of specific breakpoints of human cancers [*14*].) The first example to have been recognized of specificity of a chromosome rearrangement in a neoplasm is the translocation affecting the Nos. 22 and 9 that gives rise to the marker known as the Philadelphia chromosome [*23*] in chronic granulocytic leukemia. Subsequently, other examples have been recognized, in some of the acute leukemias, Burkitt's lymphoma, and (benign) meningioma. Examples of specificity of breakpoints have been identified also in certain malignant murine lymphoid neoplasms [*16,17*]; this information, in conjunction with the extensive mapping of genes to mammalian chromosomes that has been accomplished since 1968 (when the first gene was assigned to a human autosome [*7*]), makes apparent the important fact that in the mouse, as also in the human Burkitt's lymphoma, some of the breakpoints in malignant neoplasms are at or near structural loci for immunoglobulin genes, loci active in the type tissue in which the neoplasms develop [*16,17*]. Furthermore, the normal sites of so-called cellular oncogenes have in several cases been found to coincide with the specific chromosome breakpoints, with translocation of major portions of the oncogenes to new (abnormal) positions in the genome.

Not all clones with a visible chromosome mutation in their genome are neoplastic. A totally new observation was made soon after modern cytogenetics techniques began to be applied to the study of

populations of human cells. Subpopulations of cells identified as clones by microscopically distinctive mutations in their chromosome complements are to be found growing among cells with normal complements, clones that by no pathologist's criteria would be classified malignant. First, some liveborn humans were found with mosaicism in multiple tissues, cells bearing a normal complement coexisting with cells bearing an abnormal one, e.g., translocation or deletion of a portion of some chromosome. That normal cells are present in such persons implies that the zygote was normal and that a chromosome mutation had occurred in a cell of the early conceptus and was transmitted thereafter by that cell's progeny. Although such major abnormal populations in mosaic individuals ordinarily are not thought of as clones, they in fact are. If their genome is unbalanced, the abnormal population may interfere with embryonic development. At other times mosaicism is detected in normally developed adults only after the clinical cytogeneticist has demonstrated that an occult abnormal clone had been responsible for the production of a gamete with an unbalanced genome; in such cases the mutant clone often comprises only a small proportion of body cells.

Clones of cells bearing chromosome rearrangements sometimes are found in cultures of fibroblasts derived from minute fragments of tissue (usually skin) taken from completely normal people [3]. Members of the clones are in the minority in such cultures, and it usually remains undetermined whether they were present in vivo or arose in vitro; however, in cell lines derived from tissue that has been X-irradiated in vivo, they may be present in abundance [8], indicating an in vivo origin at least under that unusual circumstance. Such mutant clones detected in fibroblast cultures usually have no clinical significance. Their proliferative capabilities in comparison to that of the fibroblasts with non-mutated complements have not been studied, but general observation suggests that they have no impressive growth advantage. Thus, mutation of the genome can occur in cells at various post-zygotic stages of life and give rise to non-neoplastic clones.

(I must exclude from my discourse here non-neoplastic clones in the immune system that arise in conjunction with a chromosome mutation that will permit a specific response to an antigen, because those rearrangements are not detectable microscopically. Time will tell what other non-neoplastic systems of immense interest I, for the same reason, have excluded unknowingly, for they have yet to be discovered!)

If mutant clones exist in the circulating blood from normal people, they usually are not detected. Suggesting that very small clones may exist in vivo is the occasional observation made in many laboratories of single cells that have undergone a balanced translocation affecting specific points on a No. 7 and a No. 14 chromosome (discussed in [*4*]). (It also suggests that in T lymphocytes, the cells that can be brought into metaphase by phytohemagglutinin (PHA), certain specific chromosome regions are predisposed to rearrangement. It seems reasonable to speculate that loci so identified are regions undergoing active transcription as result of cell differentiation, being the loci concerned with specialized cell products and, or, with cell cycling in that particular cell type.)

In contrast to this apparent specificity of breakpoints and to the infrequency of occurrence of clones in blood from normal populations, mutant clones with various rearrangements apparently lacking specificity with respect to the chromosomes affected have been detected with no great difficulty in the blood of members of populations that had been exposed excessively to ionizing radiation, e.g., persons who have received roentgen therapy and survivors of the atomic blasts. In these populations, such mutant clones of T lymphocytes have exhibited no malignant potential, nor any clinical effect, although the exposed human populations from which the blood samples had been taken were strongly predisposed to cancer. That the clones are detectable at all, however, and that they persist and can be demonstrated at serial samplings of the blood as long as 35 years post-irradiation [*1,2*] suggest that from their inception they enjoy a small proliferative advantage over non-mutated lymphocytes, and, in this sense, a degree of autonomy with respect to cell cycling. (Note that the peak incidence of clinical chronic granulocytic leukemia in survivors of the atomic blasts in Japan occurred in 1953 [*15*], eight years after the events occurred that gave rise directly or indirectly to formation of a Philadelphia chromosome in a cell in each of the to-be-leukemic persons. This indicates that that well-known rearrangement of the genome endows the cell and its progeny with only a slight growth advantage—but a highly significant one. It also emphasizes the dependence on the passage of time, sometimes a long time, for the clinical "surfacing" of a malignant neoplasm.)

Finally, one small and heterogeneous, cancer-prone human population is known in which mutant clones of cells can be detected in various tissues relatively easily even without excessive exposure to exogenous clastogens. These are the persons with one of the exceedingly rare genetic disorders that, for our purposes here, may be

grouped as "the chromosome-breakage syndromes," because, although the syndromes are dissimilar clinically, cells from affected persons present evidence that their genomes are unusually mutable, either spontaneously or following some environmental insult, or both [*19,26,27*]. Thus, in Fanconi's anemia, mutant clones have been found in fresh bone marrow aspirates and in circulating blood lymphocytes; in ataxia-telangiectasia, in circulating lymphocytes, and here a specificity as to the chromosomes affected has been detected, chromosomes Nos. 7, 14, or both predominating in the rearrangements; and in Werner's syndrome, in skin fibroblasts and B lymphocytes proliferating in vitro. Sometimes a very few cells will constitute the only evidence of the presence of a mutant clone—but, note that the finding of just 1–2% of T lymphocytes with the same rearrangement in a sample drawn from the blood of an adult reflects the existence at the moment of sampling of millions of members of the clone circulating in his blood, and many more stationed in lymphoid tissues elsewhere. In other cases the entire population of PHA-responding cells circulating in the blood will display the same mutated genome, and even then clinical evidence of leukemia may be lacking. In people with one of these syndromes, frank cancers will develop more often than expected in the general population, and marker-chromosome rearrangements have been found in most of the few cancers that have become available for cytogenetic study (e.g., Fig. 1), similar in type to those we have become familiar with in the benign clones. As cells and cell lineages from persons with these rare genetic constitutions are scrutinized, difficulty is encountered in deciding whether a clone proliferating excessively has graduated from benign to malignant status. In fact, the concept neoplasia itself becomes hazy here, and we begin to discern the spectrum of disturbed growth to which I referred at the outset. "Few if any areas of biology exist with a greater potential for elucidation of the step or steps taken as cells transform from 'normal' to 'neoplastic' and from 'benign' to 'malignant' " [*26*]. Since the early 1960s, the strikingly increased cancer occurrence in persons with genetically determined chromosome instability has been known. This has been a steady signal to many students of human cancer that pointed to a crucial role—or roles—for chromosome mutation in the etiology and, or, progression of neoplasia. It is for this reason that this particular volume on cancer—on neoplasia and on clones—takes as its point of departure the chromosome-breakage syndromes.

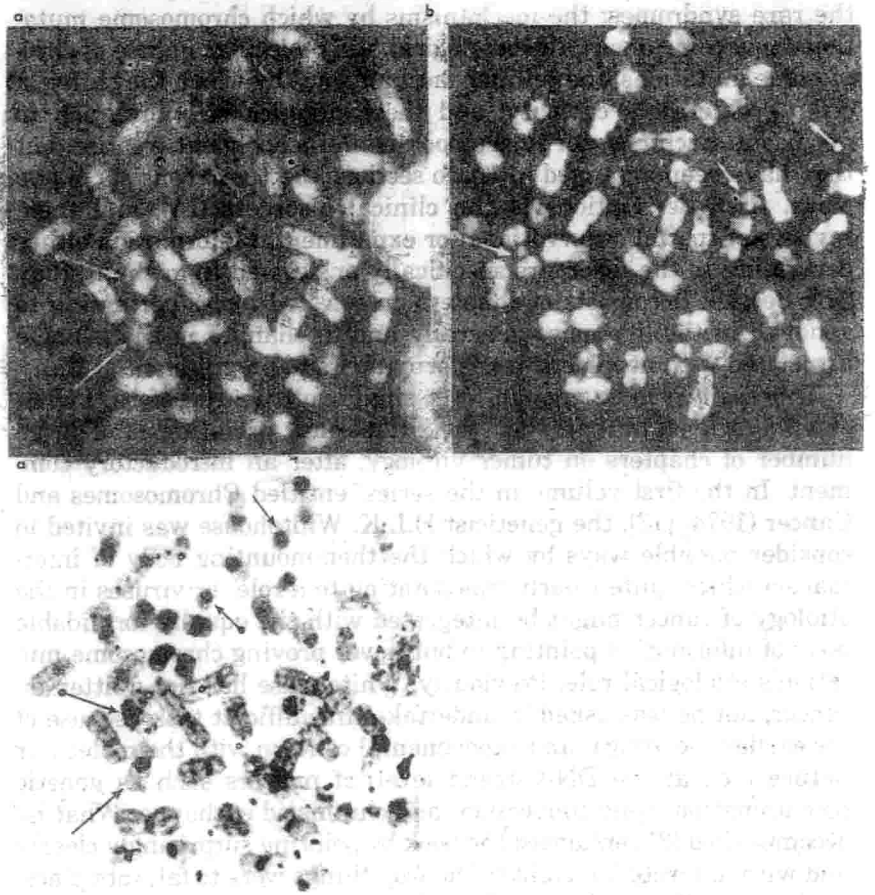

Fig. 1. Two metaphases from the leukemic clone populating the bone marrow of a young man with Bloom's syndrome and acute lymphocytic leukemia. Upper panels, Q-banding; lower panel, C-banding of the cell at upper left. Longer arrows indicate chromosome Nos. 9, the shorter ones the Nos. 20. The arrows marked with terminal dots point to structurally aberrant chromosomes, either an isochromosome for the long arm of No. 9—iso(9q)— or deletion of the long arm of No. 20—20q−. (Unpublished observation, with R.S.K. Chaganti.)

The Matters Covered in the Volume—and Some Not Covered

Among the several important matters addressed by the distinguished contributors to the volume are the following: the aberrant responses made to DNA-damaging agents by cells of persons with the rare syndromes; the mechanisms by which chromosome mutations may come about; the cytological and molecular nature of chromosome mutations; the cellular mechanism(s) by which they affect a cell's proliferative capacity; and their significance in relation to neoplasia. Each chapter in the book is an independent treatise, but they have been arranged into two sections. Section I consists of two parts, first, descriptions of the clinical disorders (IA), and then, reviews of special observational or experimental information either pertaining to the disorders specifically or derived from their study (IB); Section II consists of chapters on selected topics pertaining to genomic instability more generally and to changes in the genome associated with neoplastic transformation and progression.

This seemed to be enough for a single volume; therefore, I shall explain below the basis for the intentional exclusion of a large number of chapters on tumor virology, after an introductory comment. In the first volume in the series, entitled Chromosomes and Cancer (1974) [*13*], the geneticist H.L.K. Whitehouse was invited to consider possible ways by which the then-mounting body of information which quite clearly was pointing to a role for viruses in the etiology of cancer might be integrated with the equally formidable body of information pointing to but never proving chromosome mutation's etiological role. Previously, Whitehouse had not written on cancer, but he was asked to undertake this difficult task because of his earlier theoretical and experimental concern with the molecular nature (i.e., at the DNA-strand level) of matters such as genetic recombination, gene conversion, and chromatid exchange. What he accomplished [*31*] enhanced the book by pointing surprisingly clearly and with accurate foresight to the way things were to fall into place, as they rapidly are doing today, almost a decade after he wrote. In his "open-replicon hypothesis of carcinogenesis" he observed that host-DNA replication was needed for viral integration to take place, and that many viruses contain genes that can initiate such replication in the host. He proposed that because viral integration into a genome is advantageous to both the parasite and the host, selection will have occurred for genes in the host that will hold in check the DNA synthesis otherwise induced by the integrated virus and thereby permit survival of the host cell. Thereafter, the occasional loss from a cell of the activity of such suppressor loci, as by their mutation or deletion, could disrupt the balanced system that had