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Constitutional Hyperrecombinability and Its Consequences

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HANDLING human chromosomes is my métier. I realized that long ago; however, preparing an article for *Perspectives* led me to ask myself how this came to be. I, like others who took the premedicine curriculum in college in the 1940s, had learned about Boveri's discovery of the transmission of developmental determinants via chromosomes and of the stages of meiosis and mitosis, as well as more than a few things that were to be unlearned in due time, *e.g.*, that cells rest between mitoses and that the human has 48 chromosomes! Early on, an unusual event convinced me that the nucleus was the place to direct my attention and, specifically, that handling chromosomes experimentally could be just plain exciting: In Galveston one day in the latter part of 1951, I was surprised to see the charismatic pioneer in cell culture, Charles Pomerat, ordinarily sedate and rather slow moving, smiling and jigging happily between the benches of his research laboratory. He was waving above his head a handful of freshly printed photographs and calling out to his associates: "Chromosomes! Human chromosomes!" Those little bearers of the determinants of human development about which he was so excited that day were not tightly entwined in the cells, hopelessly inaccessible to the eye of the microscopist as they had been until then, but rather nicely separated from each other. What had happened?

The best explanation seems to be that a technical assistant erroneously had made the laboratory's stock of balanced salt solution *hypotonic*. When T. C. Hsu, Pomerat's new postdoctoral fellow, used what he thought was Tyrode's solution to rinse some cultures of human cells prior to fixing and staining them, the cells naturally had imbibed water. Those cells that by chance had been in mitosis had expanded their volume so much that their chromosomes had become sufficiently separated to permit their being counted and the observation of their individual morphology (relative lengths, centromeric constrictions). This pivotal discovery in a cell culture laboratory on the Gulf Coast of Texas in the early 1950s—that immersion of mitotic cells in a hypotonic medium will make the chromosomes suitable for microscopic analysis—was made by serendipity. *Serendipity* is a happy word that means (i) finding something of value that was not being sought (ii) by someone who has a mind suitably prepared to perceive its value (*cf.* INGRAM 2004). Pomerat, long with an interest in the mammalian nucleus and its activities during the cell cycle, and T. C. Hsu, a newcomer to mammalian cell biology but with an interest in genetics, specifically Drosophila genetics, were prepared; they perceived the importance of their observations, and fortunately they reported them (HSU and POMERAT 1953), thereby opening the modern era of human, including medical, cytogenetics.

This story is interesting historically, but why do I introduce my article by relating it? Although I had left the Pomerat laboratory before the discovery of the hyposmotic treatment was made, it did happen in the place where I first realized my calling to cell research. Just that realization seems in some unconscious way to have provided me with continuing reassurance that handling chromosomes was what I should be doing. It has always seemed *appropriate* that I should be doing so and has been a continuing source of contentment.

Preparation for a life *finding out*: As I have related elsewhere (GERMAN 2003), I was permitted to work in Pomerat's laboratory from my earliest days as a medical student (1945), and I had spent the entire summer of 1948 there learning to devise, carry out, and record laboratory experiments by using mammalian cells in culture and raising antisera to human reticulo-endothelial cells. I suppose it was because we were situated in a medical environment that Pomerat on several occasions referred to our work with cells as *wissenschaftliche Medizin*, contrasting it to *klinische Medizin*. It was my interest in and enthusiasm about cells and their culture, first awakened there, that 14 years later was to direct me into human cytogenetics, thence human genetics. The

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enthusiasm that Pomerat showed in simply handling living mammalian cells *in vitro* and his sheer pleasure in observing the beauty in their form and movement under various experimental conditions permitted me to know those same joys. Pomerat seemed happiest when showing these wonders to others. I myself have consistently made an effort to allow those working with me to develop feelings of enthusiasm and good fortune just to be working with cells, just as Pomerat first passed on his passion to me.

However, on the day of the "Chromosomes!" excitement, I had just dropped in for a day's visit with Pomerat. By then I had received a doctorate in medicine and was about to become a resident physician, specializing in internal medicine in the brilliant environment just created in Dallas under the dynamic internist, cardiac physiologist, and teacher, Tinsley Harrison. For me, the years 1947–1955 (age 21–29) were devoted to shaping myself into a polished internist, presumably to practice medicine in Dallas. I make this comment to emphasize that, although Pomerat had introduced me to a life of laboratory experimentation with cells, I had also become a very well-trained and self-confident physician. Yet the private practice of medicine was never to be for me; the time was soon to come when I myself could begin handling chromosomes in my own cell culture laboratory, never really to leave "the campus." My competence in medicine together with my pleasure in growing cells and experimenting with them were to make it possible for me to develop a life's work that combined clinical medicine with experimental laboratory investigation, a program of activity that already spans almost half a century.

My purpose here is only to show how one particular discovery initiated that program of investigation—the one I was invited to write about by the editors of *Perspectives*—that, like the discovery of Pomerat and Hsu, was made by serendipity. I was to find out that one of the most striking features of the genetic material, namely its stability in cell cycle after cell cycle and generation after generation, can in certain very rare human individuals be constitutionally faulty. In short, I was to come upon the first example to be identified in any species of *genetically determined genomic instability*. That discovery initiated a program that eventually would identify a mammalian nuclear protein that is essential for maintaining the fidelity of the genetic material. Furthermore, through long-term clinical contact with a small population of individuals who feature this genomic instability, the program also would identify the human state that predisposes, more than any other known, to a wide variety of types and sites of human cancer—the very rare and still little-known clinical entity called Bloom's syndrome.

Upon completion of my residency in medicine in the mid-1950s, I was surprised to receive a letter from Washington drafting me back into the military; yes, physicians still were being drafted a decade after World War II had ended. (I had seen 3 years of active duty in the U.S. Navy during the war, but not as a physician!) I immediately joined the U.S. Public Health Service, my plan, which was successful, being to obtain a position in the Clinical Center of the National Institutes of Health (NIH). There I had the pleasure, quite fortuitously, to observe through the microscope the formation of human lupus erythematosus (LE) cells and then experimentally to produce in animals the renal lesions uniquely characteristic of LE (German 1959). This, in turn, led to an invitation for me to join the New York City laboratory of the highly stimulating and creative immunologist and physician, Henry Kunkel. Kunkel and Pomerat turned out to be the two most important scientific preceptors I was ever to have. Pomerat and Kunkel had totally different approaches to science, but for me they were complementary. Pomerat was an artist, a dreamer, delighting in cells as things of beauty and perfection to be in awe of. Kunkel was driven to find new truths and to be the *first* to find them, never ceasing to "wonder if . . ." but refusing ever to deal intellectually with information other than that hard gained by either careful and confirmed clinical observation or well-planned (and confirmed) laboratory experimentation, preferably his own, and intensive and unrelenting in his search for further understanding of immunity and autoimmunity. The purpose of my appointment to the faculty of The Rockefeller Institute was to have Kunkel's associate, A. G. Bearn, and me develop a program in human genetics, which we accomplished by forming the Laboratory of Heritable Disorders in Man.

At Rockefeller (1958–1963) I worked both in that institution's small but historically highly significant hospital with Bearn's families with Wilson's disease and in the Kunkel laboratory, learning to separate human proteins by electrophoresis and to purify them, without great enthusiasm for either activity. At this time Jerome Lejeune and Raymond Turpin were reporting, orally in 1958 and in print early in 1959 (Lejeune *et al.* 1959), the finding of an extra chromosome, or trisomy, in *trois garçons mongoliens* (now Down syndrome). The observation of this devastating developmental defect present in 1 of ~650 newborns was to have major impacts on clinical medicine and the relatively new fields of human and medical genetics—and also on me. As soon as I learned of Lejeune's discovery, I wanted to begin growing human cells again and looking at their chromosomes. After I passed a little test that Bearn devised to prove me capable of handling chromosomes (described in German 2003), he agreed, and I immediately abandoned protein separation in the laboratory and—now with unbounded enthusiasm and enormous commitment of time—came into my element: cell culture and cytogenetics in a clinical setting.

Finding out: Beginning in 1959 I was able to study human chromosomes, first right in the Kunkel labora-

tory in the midst of the electrophoresis equipment, and after 1960 in my own nicely appointed tissue culture and cytogenetics research laboratory on the newly renovated seventh floor of The Hospital of The Rockefeller Institute, part of the new unit headed by Bearn. My main objective was not to find abnormal karyotypes in various clinical disorders—the enticing objective of most of the laboratories that appeared in America, Europe, and Japan shortly after publication of Lejeune's report of trisomy 21—but instead to study a certain aspect of what might be referred to as the biology of human chromosomes, namely their replication. Tritiated thymidine labeling of DNA and autoradiography to demonstrate its location in cells had just been developed, and asynchronous replication of chromosomes had been detected in both experimental animals and plants. The first project I set for myself was to find out whether an established sequence exists as the various human chromosomes replicate themselves—it does (German 1962, 1964a)—and then to find out whether the sequence varies between differentiated tissues—it does (German and Aronian 1971). I also learned that, although homologous chromosomal regions usually replicate synchronously, prominent examples of homolog asynchrony exist in some segments of autosomes. Most dramatically, the two X chromosomes in female cells replicate asynchronously, and that observation integrated nicely with Murray Barr's discovery of the sex chromatin in female nuclei (Barr and Bertram 1949) and Mary Lyon's (1961) and Liane Russell's (1963) theory of X inactivation. (Incidentally, the biological significance of replication asynchrony of autosomal homologs remains unknown.)

While carrying out the time-consuming autoradiographic study of chromosome replication, I did find time to examine the chromosome complements in a limited number of persons with developmental disorders, my discovery of the "late X" of the human female (German 1962) naturally directing me particularly toward disturbances of sexual development (*e.g.*, German 1970). One of the variously abnormal persons I had examined by the end of 1960 was a 10-year-old girl who, only because of her unusually small stature, was being followed in Reginald Archibald's clinic in the Institute's small research hospital, having been referred to him from The New York Hospital just across 68th Street. I had agreed to examine this child's karyotype at the request of a particular New York City physician, who had telephoned me. This physician, known to me at that time only as "Dr. Bloom," did let me know that he was the private dermatologist of a highly respected scientist on our faculty, Peyton Rous. (Rous knew I had begun to study human chromosomes and had told Bloom.) Immediately I made arrangements to see the child with Archibald.

In the clinic the girl's mother told me that, although born at term, Susan had weighed but 3 pounds. She then had grown at a normal rate and become normally proportioned, but had always remained exceptionally thin and much shorter than other children her age. Susan's other significant abnormal feature was an ugly, sun-sensitive, erythematous skin lesion that was limited to the butterfly area of her face. Therefore, this tiny but truly delightful child had what in those years was being referred to by our local dermatologists as "Dr. Bloom's disease." Their colleague Bloom had published an article in 1954 (Bloom 1954) in which he had described "a dwarf who presented an eruption on the face resembling lupus erythematosus," an individual born in 1933 whom he had followed from the age of 8 at the skin clinic of the New York University Post-Graduate Medical School. In 1953 two other unusually small children with similar skin lesions, and with the faces and general body characteristics resembling the child Dr. Bloom had been following, were presented by two other dermatologists at the New York Academy of Medicine's Section of Dermatology and Syphilology. Their patients were a boy from Columbia University's College of Physicians and Surgeons and a girl from The New York Hospital (the latter to become my patient Susan in a few years). Because of the impressive similarity in appearance of the three children, Dr. Bloom had correctly written that "three almost identical cases speak strongly for a syndrome entity." Publishing without coauthors (Bloom 1954), Bloom named the "new" entity *congenital telangiectatic erythema resembling lupus erythematosus in dwarfs.* (This history would seem to provide a noteworthy lesson for those seeking immortality via eponymy! In the early 1960s, articles did begin to be published in the dermatology literature referring to the entity as "Bloom's syndrome," and this is the name—with the apostrophe!—that I also prefer to use.)

I counted the chromosomes in Susan's metaphase cells—there were 46—and examined them for structural rearrangement; I could not find any. I really had not expected to find a chromosome abnormality because by questioning Bloom over the telephone (our only means of contact until that time) I had obtained enough information to make me feel fairly certain that "his" syndrome was a recessively transmitted trait: The few affected persons he knew of had unaffected parents, affecteds had been of both sexes, and in one of the cases the parents had been cousins. However, while at the microscope observing Susan's metaphase chromosomes, I became aware of something I certainly had not set out to find: Somewhat more isochromatid breaks and acentric fragments were present than in the other preparations that I had made up to that time. Breaks with sister-chromatid reunions were common, as were what appeared to be dicentric chromosomes (some of which, however, once chromosome-banding techniques became available, would be shown to have been two chromosomes with tight telomeric associations). Structurally rearranged chromosomes were increased in frequency, the breakpoints of which varied. In some meta-

phases there were curiously despiralized, faintly stained chromosomes. But what captured my interest most was a four-armed, cross-shaped, symmetrical configuration that I determined by karyotypic analysis was composed of the two homologs of a pair of chromosomes; 1–2% of Susan's cells contained these configurations, and various chromosome pairs could be involved in their formation (German 1964b). My attention focused especially on these quadriradial configurations, or "Qrs," as I began to call them, not only because they provided satisfying surprises as I moved from mitosis to mitosis but also because they reminded me of paired meiotic homologs that had undergone crossing over. Could these Qrs signify something important genetically? [By chance, I already had observed and reported "familial" Qrs in the mother and uncle of a child with translocation mongolism (German *et al.* 1962). I still do not know the significance of that early observation, nor why I seem to have been the only person to have seen Qrs, or at least to have paid them any particular attention. But here, in a child with Bloom's syndrome, I was coming on them in abundance.]

But what does it mean? I called Susan back to the clinic several times to examine her more thoroughly and to obtain additional samples of blood: the chromosome "breakage," including the Qrs, was always there. Although Bloom promptly lost interest in cytogenetics when he learned that I could find neither an extra nor a rearranged chromosome in "his" syndrome, he did help me contact the other living person of the three he had reported (Bloom 1954), as well as the few persons his fellow dermatologists had by that time recognized as affected. The bloods from one after another of them showed "breakage" along with the intriguing Qrs. I convinced myself of the constancy of this feature—the cellular phenotype—of the rare and little-known condition, and then certain questions could be asked: Does "breakage" take place spontaneously *in vivo*? More importantly, does it perhaps take place also in normal people, although not as often as in the people with Bloom's syndrome? (This was a good question because all the chromosome lesions I could so readily see in cells from persons with the syndrome I also had seen in cultured cells from other people, although much less often.) Does "breakage" have any biological significance and, if so, what is it? Does "breakage" play a role in any human disease states and, if so, in which? In my experiment notebook of the time I wondered about roles in cancer and autoimmunity. The Qrs seemed to me to represent a mechanism by which new genetic constitutions of cells could be generated, *i.e.,* genetic constitutions different from those of the host, and so I even wondered about that playing a role in cell differentiation during development. Although during the coming years I was to work on many other things, these questions seemed important enough for me to keep Bloom's disease "on the back burner," which I did. In the late 1980s, almost three decades after the "breakage" had first been detected, the subject was brought to the fore by intensive laboratory experimentation. This step, of course, was taken when it had become feasible to apply recombinant DNA technology to certain ancient questions that finally could be answered [*e.g.*, as to whether crossing over occurs in mammalian somatic cells, compare German (1964b) with Groden *et al.* (1990)].

After having set as a goal the examination of the chromosomes of every person with the condition whom I could contact—just a handful of affected persons was known then—I began to be aware of two things that later turned out to be important: (i) that the condition was genetically determined and (ii) that it predisposed to malignant neoplasia. By then, Bloom had lost all contact with his own patient, Gerald. In late 1960 I urged him to obtain follow-up information from the private physician who had referred the boy in the first place, and he wrote to that man. The physician's response came as a note scribbled at the bottom of the letter, saying that Gerald had died at age 25 of "subacute leukemia." Through this physician I later succeeded in contacting Gerald's mother, who graciously invited me to her home in Brooklyn, my purpose being to obtain clinical and genetic histories. On entering her living room I noticed and commented on a picture sitting on the radio of an infant I took to be Gerald, having seen photographs of Gerald that Bloom had obtained. "No, that was Roslyn, Gerry's older sister. She was tiny and looked *just* like Gerry." Bloom's article (1954) had stated, "No other member of the family was affected with any . . . growth abnormality." To me, Bloom's syndrome now was seeming quite certainly to be a recessive trait, and I realized a formal analysis of the condition's genetics would be in order as soon as enough *bona fide* affected families could be ascertained (German 1969a).

Cancer was turning up in several persons with Bloom's syndrome. In the summer of 1963, my patient Susan, now 13, abruptly became pale, feverish, and weak and was dead 6 weeks later of acute leukemia (Sawitsky *et al.* 1966). I recalled then the scribbled note about Bloom's own patient also having developed a leukemia, but there was no published documentation of that; I had to know. Gerald's mother, now known to me personally, supplied me information that, after a tortuous search, led me to his bone marrow slides (they having been laid supposedly to eternal rest in a dusty file cabinet in a private Brooklyn hospital), permitting confirmation of the diagnosis—acute myelogenous leukemia.

A few years later the New York hematologist Arthur Sawitsky, who had cared for my patient Susan when she developed leukemia, told me that he had heard a paper read at a hematology meeting about a strikingly diminutive young woman in Mexico City who had died at 23 of acute leukemia. David Bloom and I flew there at once and visited her parents, an elderly Ashkenazi couple, cousins named Abraham and Sarah, who had been born

near Bialystok in Poland and emigrated to Mexico. With David's help as translator using Russian, Polish, and Yiddish (David himself having grown up in Warsaw, immigrating to New York City in 1920), we obtained clinical information about their daughter and examined photographs of her; we both became convinced she had had "the syndrome." I was becoming more and more interested in the possibility that persons with this rare form of growth deficiency were cancer prone. Obviously, however, I needed to find and follow the clinical courses of many more affected persons. (There were many more "Bloom's tours" to come, some fortunately and pleasantly with David to Appalachia, Philadelphia, Boston, Toronto, Paris, and Jerusalem. My tours without him included England, Germany, France, Switzerland, The Netherlands, Belgium, Italy, Israel, and Japan, as well as Canada, Mexico, and many parts of the United States. David and I remained close and in fact were to have seen a patient together at a local hospital the day after he died, in 1985 at the age of 93.)

The course ahead seemed clear. I was prepared as a physician to undertake a long-term, prospective study of people with the trait to determine their clinical courses and, in particular, to carry out a surveillance program for neoplasia. This also would permit me to determine the syndrome's genetics. (The study is still in effect over four decades later.) I, of course, needed some affected people to follow, so in lectures at scientific meetings and in published papers, (i) I tried to make Bloom's syndrome better known as a genetic/clinical entity and (ii) I announced that I would like to know of any persons diagnosed anywhere in the world. Since at that time I was one of the relatively small set of persons working seriously with human chromosomes, I had made friends with many other cytogeneticists throughout the world or at least was known to them. I realized that it was a cytogeneticist who would be the final arbiter as to whether a person suspected by a clinician or geneticist of having Bloom's syndrome really was affected: Was there increased breakage? Were there Qrs? I worked at this with persistence, and the cytogeneticists, as well as clinical geneticists and practicing physicians, contacted me about cases they diagnosed. I believe I was able to ascertain and to follow the course of almost every case diagnosed from 1954 (when Bloom's article appeared) through 1990—169 individuals in 139 families—at which time I published announcements in several journals that I arbitrarily was discontinuing the accessioning of affected persons into what had by then become known as the Bloom's Syndrome Registry (German *et al.* 1977). Regardless of those announcements, several cases each year continued to be referred, so that finally the Registry holds extensive clinical, genetic, and experimental laboratory information in the files maintained on 238 affected persons. Accessions really have been discontinued now, as of April 15, 2003, a date known to those who now are obliged to practice Washingtonian medicine as "according to HIPAA" (Cole and Fleisher 2003). Two important pieces of information that the study still was to show were: (i) the enormous *degree* to which affected persons are predisposed to cancer and (ii) that the predisposition was to the *generality* of human cancer, not just to leukemia as suggested at the outset nor, as in many of the genetic disorders that predispose to cancer, predominantly to only one type or only one anatomical site (German 1993, a review article).

Telling others: Being in New York City in the early days of human genetics, and Idlewild (now JFK) Airport being the main port of entry for Europeans, we at Rockefeller were fortunate to have visits from many geneticists, including cytogeneticists. When I would ask the human or medical cytogeneticists whether they had ever observed spontaneous "chromosome breakage" in cultures, and then put some Bloom's syndrome chromosomes under my laboratory microscope, they would say that they had not, and, to my disappointment, they showed little or no interest in the fact that I had. (Their interest in those years was mainly in the amazing array of imbalances being detected in the chromosomal complements of humans and in the developmental consequences. Those observations of course also were exciting to me.) One of our visitors, Patricia Jacobs, who had by far the vastest experience of any human cytogeneticist at that time (if not even now) in examining human karyotypes, did tell me that she had observed Qrs, but only on very rare occasions and never more than one in the many cells examined from any one person. Others had never come on a Qr. The first time I was to mention my observation of "breakage" and Qr formation in public was at a meeting of the American Society of Human Genetics in New York City in 1963, not as a formal presentation but in an open discussion period that followed several papers on human cytogenetics presented by others. It was in a small room. I went to a blackboard and described having found an increased amount of chromosome "breakage" and rearrangement in Bloom's syndrome (a clinical condition that no one present had ever heard of, of course). Again, little interest. In fact, the session's chairman, Klaus Patau, older and better established as a geneticist than most of us then working in human cytogenetics, and formidable in his Germanic, authoritarian *présence*, commented in an avuncular but not particularly endearing way that in his own laboratory such "artifacts" had on occasion been seen. He explained to me that my problem quite simply was to identify the toxins responsible in my tissue culture medium or culture vessels! A series of almost a dozen publications on chromosome breakage in Bloom's syndrome eventually did emanate from the Patau laboratory over the decade beginning in 1976 (Kuhn 1976; Kuhn and Therman 1986)! In due time Klaus and I actually did become friends.

Shortly after 1963 another distinguished cytogeneticist, Charles Ford, then working at Harwell, paid us a

visit. He was a person who, like me, derived real pleasure in just handling chromosomes, and I decided to discuss my finding of "breakage" with him. I invited him to have lunch with me at a small French restaurant; the paper tablecloth served as my blackboard. Charles showed sincere interest in my observations and my interpretation of Qrs as cytological evidence that crossing over can take place in mammalian somatic cells. Early in 1964 I sent a short article off to Science, reporting and interpreting the Qrs (German 1964b, including an acknowledgment of Ford), and in this and in an even shorter article there the following year (German *et al.* 1965) I presented the following information and ideas:

1. Qrs are found in cultured human cells, but they are rare except in cells from persons with Bloom's syndrome, where they are relatively common, being increased along with other evidence of excessive genomic instability, namely chromatid breaks and gaps and structurally rearranged chromosomes.
2. The fact that the symmetrical interchange figures involved various homologous pairs of chromosomes indicated necessarily that "a degree of pairing or association of at least portions of homologous chromosomes" can take place in somatic cells—"a form of homolog pairing."
3. The symmetrical Qr of the Bloom's syndrome cells—affecting the two homologs of a pair, with centromeres in opposite arms of the four-armed figure and with opposite arms of the same lengths—was interpreted as cytological evidence that "somatic crossing-over may occur in mammalian cells." [Proof of this would await molecular technology in the Ph.D. thesis of Joanna Groden 26 years later (Groden *et al.* 1990)]. That somatic crossing over occurs *in vivo* at a greatly increased rate in Bloom's syndrome also was to be demonstrated much later (Rosin and German 1985). Also, much later it was shown by others (Cavenee *et al.* 1983) to occur in certain neoplastic lineages and sometimes to be an important event in their generation.
4. Somatic crossing over is "one cellular mechanism by which mammalian cells *in vitro* may develop a genome which differs from that of . . . other cells in the culture." The cytological evidence for it, *i.e.*, the Qrs, suggested "the feasibility of detecting a recombination of genes *in vitro* in mammalian cell systems of certain types, pertinent to the theoretical possibility of replacing experimental breeding by genetical analysis of somatic cells in culture." [This was an idea then being championed by Guido Pontecorvo (Pontecorvo 1962).] Somatic crossing over also was suggested to be responsible for autoimmunity: "a clone of cells could develop which (i) lacks certain genes (and antigenes) [*sic*] present in the other cells of the host, yet (ii) contains no genes not already present. . . . Somatic crossing over provides a means for producing, by loss of genes, lymphoid cells with a genetic complement different from that of the host."
5. Evidence based on observation of only 13 families manifesting "Bloom's syndrome" supported the hypothesis of autosomal recessive inheritance of that newly described condition. Because in cells from other normal and variously abnormal persons Qrs and complex rearrangements such as "triradials, asymmetrical quadriradials, and dicentrics" were "extremely infrequent . . . except for those from one patient, . . . a child with Fanconi's anemia, another genetically determined disorder," it seemed "plausible to assume, for the present, that it is associated with the genetic abnormality," *i.e.*, that Qrs and complex rearrangements are constant features of Bloom's syndrome. It was apparent that "an increased tendency to chromosomal breakage may be part of a *genetically* [my emphasis here] determined disorder."
6. My maintenance of clinical contact with the persons with Bloom's syndrome, ascertained throughout the world after 1954, even by 1965 had suggested that Bloom's syndrome is a state predisposing to cancer, leukemia having developed in 2 of the first 19 persons diagnosed (one being Susan) and carcinoma in a third. In 1964 I suggested that crossing over, if it occurs *in vivo*, could explain "antigen loss in neoplasms" (a feature of some cancers, which by then was already known to occur). By 1965, the chromosomal breakage was considered "possibly related to the apparent increase in frequency of malignant neoplasia in persons with this syndrome."

What is the significance of "chromosome breakage" in a genetically determined, highly cancer-predisposing human disorder? This finding, along with the demonstration by Peter Nowell and David Hungerford in 1960 that the structurally rearranged "Philadelphia chromosome" is a mutation found consistently in chronic granulocytic (myelogenous) leukemia (Nowell and Hungerford 1960), constituted the first strong pieces of evidence in support of the theory of Theodor Boveri that chromosome mutation causes cancer (Boveri 1914). [A valuable "review" in English of Boveri's book was published by Ulrich Wolf (Wolf 1974).] And, of course, Boveri was absolutely right.

In 1972, by which time I had applied the term "chromosome-breakage syndromes" to the several recessively transmitted conditions that along with Bloom's syndrome feature microscopic evidence of excessive mutation (German 1969b), I could write a major article establishing my conviction that Bloom's syndrome was proclaiming, loudly, that chromosome mutation can be responsible for the conversion of a normal cell to a neoplastic one and that the excessive number of the type of chromosome mutations that arise spontaneously in Bloom's syndrome provides the background from

which excessive numbers of neoplasms could/would arise (German 1972). Nevertheless, a viral basis for human cancer was the prevailing idea during the 1960s and 1970s, and few leading tumor biologists paid attention to this evidence nor to me on my soapbox trying to get the message across. In an editorial, John Cairns was one of the first of those not himself working with chromosomes to perceive and emphasize the etiological importance of genetic transpositions (which would include *microscopically visible* chromosome mutations) in the origin of neoplasia, and his was a powerful article (Cairns 1981). The virologists prevailed until abnormal activation of specific cellular loci—proto-oncogenes, growth (tumor) suppressors—was demonstrated in neoplasia (Hayward *et al.* 1981; Shilo and Weinberg 1981) and until chromosome-translocation breakpoints were shown capable of mutating those loci and deregulating them or creating neo-fusion genes, information that necessarily had awaited the advent of the age of molecular genetics and the integration of molecular genetics into human cytogenetics. For me, 1981 was the turning point, when scientific evidence overwhelmingly supported the mutational origin of human cancer.

When technical advances permitted, my group mapped the Bloom's syndrome locus (German *et al.* 1994). Then, by harnessing Bloom's syndrome's own tendency to homologous recombination in somatic cells, utilizing particularly the information we had obtained by then that recombination within the Bloom's syndrome gene itself can occur—intragenic recombination (Ellis *et al.* 1995a)—we isolated the gene (Ellis *et al.* 1995b). The protein encoded by that gene, *BLM*, turned out to be a recQ helicase, a subclass of DNA-RNA helicases, the prototype of which had been discovered in bacteria in 1984 by the Nakayamas while working in Phil Hanawalt's laboratory (Nakayama 2002). ("Q" is for Kyushu, the home of the Nakayamas!) When recQ helicases are mutated in various experimental organisms, the result is defective control of recombination. At present, the DNA transactions in which the Bloom's syndrome proteins play roles are being identified, and their mechanisms of action are being defined. Yeast, Drosophila, and mammalian genetics are being employed in sophisticated and elegant ways by molecular cell biologists and students of DNA repair.

Much of the Bloom's syndrome story has been told: The protein whose function is missing from Bloom's syndrome cells is essential for the normal maintenance of genomic stability. BLM protein in as-yet-unknown ways appears to suppress recombination between juxtaposed homologous/near-homologous segments of DNA. When BLM protein is lacking from human somatic cells, they become hyperrecombinable, and the activated hyperrecombinational mechanism apparently is error prone, leading to the accrual of a great burden of mutations throughout the genome (Vijayalaxmi *et al.* 1983; Langlois *et al.* 1989). The hypermutability of Bloom's syndrome cells, including their hyperrecombinability at both homologous and near-homologous regions, would seem to explain the enormously increased number of neoplasms that arise in the various proliferating tissues of affected persons. (Abnormally small size, the presenting and most constant clinical feature of the syndrome and the reason Susan was a patient in our clinic, remains unexplained!)

Why study rare Bloom's syndrome? The study of Bloom's syndrome provides insight into and understanding of an attribute of normal cells that is of singular importance, namely their ability to maintain their genetic material unchanged over many generations. The information yielded by the study of this very rare condition can be viewed as but another beautiful affirmation of the wisdom of William Harvey. During the last year of his life, he responded to a physician in Holland who had written him about a patient with a very unusual clinical condition: "Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her working apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature, by the careful investigation of cases of rarer forms of disease. For it has been found in almost all things, that what they contain of useful or applicable, is hardly perceived unless we are deprived of them, or they become deranged in some way" (Harvey 1989). Harvey wrote those words almost three-and-a-half centuries ago, but I had not read them until I was many years into the surveillance program. This intuition doubtless had driven my entire study of the very rare Bloom's syndrome. To gain some understanding of the "secret mystery" of how genomic *stability* is maintained, I seized the opportunity that I alone seemed to have and with tenacity studied a rare variation from normal in which there is genomic *instability*.

Notes:

1. As I was preparing my article for *Perspectives*, a little arithmetic I scribbled at the edge of the paper surprised me: it is 90 years since Boveri published his theory that chromosome mutation is the cause of cancer (Boveri 1914) and exactly 50 years since Bloom published his article describing the "new" syndrome that now bears his name (Bloom 1954). *Tempus omnia revelat.*
2. Also, as I wrote, it surprised me how many of those with whom I had interacted with great pleasure or who had influenced me have gone on. *Tempus fugit.* The following are their birth and death years: Murray Barr (1908–1995), Theodor Boveri (1862–1915), Charles Ford (1912–1999), Tinsley Harrison (1900–1978), William Harvey (1578–1657), David Hungerford (1927–1993), T. C. Hsu (1917–2003), Henry Kunkel (1916–1983), Jerome Lejeune (1926–1994), Klaus Patau (1908–1975), Charles Pomerat (1905–

1951), Guido Pontecorvo (1907–1999), Peyton Rous (1879–1970), and Raymond Turpin (1895–1988). *Requiescat in pace.*

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