Cell Motility and the Cytoskeleton 47:81–107 (2000)

Review

Aneuploidy, the Somatic Mutation That Makes Cancer a Species of Its Own

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The many complex phenotypes of cancer have all been attributed to "somatic mutation." These phenotypes include anaplasia, autonomous growth, metastasis, abnormal cell morphology, DNA indices ranging from 0.5 to over 2, clonal origin but unstable and non-clonal karyotypes and phenotypes, abnormal centrosome numbers, immortality in vitro and in transplantation, spontaneous progression of malignancy, as well as the exceedingly slow kinetics from carcinogen to carcinogenesis of many months to decades. However, it has yet to be determined whether this mutation is aneuploidy, an abnormal number of chromosomes, or gene mutation. A century ago, Boveri proposed cancer is caused by aneuploidy, because it correlates with cancer and because it generates "pathological" phenotypes in sea urchins. But half a century later, when cancers were found to be non-clonal for aneuploidy, but clonal for somatic gene mutations, this hypothesis was abandoned. As a result aneuploidy is now generally viewed as a consequence, and mutated genes as a cause of cancer although, (1) many carcinogens do not mutate genes, (2) there is no functional proof that mutant genes cause cancer, and (3) mutation is fast but carcinogenesis is exceedingly slow. Intrigued by the enormous mutagenic potential of aneuploidy, we undertook biochemical and biological analyses of aneuploidy and gene mutation, which show that aneuploidy is probably the only mutation that can explain all aspects of carcinogenesis. On this basis we can now offer a coherent two-stage mechanism of carcinogenesis. In stage one, carcinogens cause aneuploidy, either by fragmenting chromosomes or by damaging the spindle apparatus. In stage two, ever new and eventually tumorigenic karyotypes evolve autocatalytically because aneuploidy destabilizes the karyotype, ie. causes genetic instability. Thus, cancer cells derive their unique and complex phenotypes from random chromosome number mutation, a process that is similar to regrouping assembly lines of a car factory and is analogous to speciation. The slow kinetics of carcinogenesis reflects the low probability of generating by random chromosome reassortments a karyotype that surpasses the viability of a normal cell, similar again to natural speciation. There is correlative

Contract grant sponsor: Abraham J. and Phyllis Katz Foundation (New York); Contract grant sponsor: Nathan Cummings Foundation (San Francisco); Contract grant sponsor: Forschungsfonds der Fakultaet for Klinische Medizin Mannheim.

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Received 10 January 2000; Accepted 28 June 2000

and functional proof of principle: (1) solid cancers are aneuploid; (2) genotoxic and non-genotoxic carcinogens cause aneuploidy; (3) the biochemical phenotypes of cells are severely altered by aneuploidy affecting the dosage of thousands of genes, but are virtually un-altered by mutations of known hypothetical oncogenes and tumor suppressor genes; (4) aneuploidy immortalizes cells; (5) non-cancerous aneuploidy generates abnormal phenotypes in all species tested, e.g., Down syndrome; (6) the degrees of aneuploidies are proportional to the degrees of abnormalities in non-cancerous and cancerous cells; (7) polyploidy also varies biological phenotypes; (8) variation of the numbers of chromosomes is the basis of speciation. Thus, aneuploidy falls within the definition of speciation, and cancer is a species of its own. The aneuploidy hypothesis offers new prospects of cancer prevention and therapy. Cell Motil. Cytoskeleton 47:81–107, 2000.

Key words: aneuploidy; cancer; gene mutation; speciation; aneuploidy-catalyzed chromosome reassortment

But one thing is certain: to understand the whole you must look at the whole
—Kacser, 1986

INTRODUCTION

For almost a century now cancer is attributed to "somatic mutation" [Tyzzer, 1916]. Indeed somatic mutation explains the clonal origin and the irreversibility of most cancers [Cairns, 1978; Pitot, 1986], as originally proposed by the biologist Boveri, ''The defect is irreparable, as the fate of cancers shows, particularly on repeated transplantation.'' [Boveri, 1914]. But it is still undecided whether the somatic mutation that causes cancer is aneuploidy, an abnormal number and balance of chromosomes, as suggested by Boveri, or whether it is gene mutation as suggested by others (see below).

The challenge is to find which kind of mutation can explain the highly complex phenotypes of cancer, such as anaplasia, autonomous growth, metastasis, DNA indices ranging from 0.5 to >2, abnormal cellular and nuclear morpholgy, abnormal centrosome structures and numbers, unstable and non-clonal karyotypes and phenotypes despite clonal origin, immortality in vitro and in transplantation, spontaneous progression of malignancy, and the exceedingly slow kinetics from carcinogen to carcinogenesis ranging from a minimum of many months to several decades, as well as the corresponding age bias of cancer (Table I) [Hansemann, 1890; Braun, 1969; Cairns, 1978; Pitot, 1986; Harris, 1995]. The term anaplasia was introduced over a century ago by the pathologist Hansemann to capture the essence of cancer, "a process carrying the cell in some entirely new direction—a direction, moreover, which is not the same in all tumors, nor even constant in the same tumor. . . . The anaplastic cell then is one in which, through some unknown agency, a progressive disorganization of the mitotic process occurs, which in turn results in the production of cells that are undifferentiated in the sense that those functions last to be acquired, most highly specialized. . . are more or less lost; but redifferentiated in the sense that the cancer cell is not at all an embryonic cell, but is a new biologic entity, differing from any cell present at any time in normal ontogenesis. But . . . this entity displays no characters absolutely and completely lacking in the mother cell. . . Its changed behavior depends on exaltation of some qualities, and depression of others, all at least potentially present in the mother cell." [transcribed by Whitman, 1919].

Here we investigate the question whether aneuploidy or gene mutation is the "unknown agency" that causes cancer, by determining how well each of the two kinds of mutations can predict and explain the complex phenotypes of cancer and the slow kinetics of carcinogenesis. Based on their different origins and ranges of action, aneuploidy and gene mutation make very different, testable predictions. For example, nature uses gene mutation for minor adjustments within a species, but reserves mutation of chromosome numbers for major, discontinuous alterations such as the generation of new species [Shapiro, 1983; Yosida, 1983; O'Brien et al., 1999]. In view of this aneuploidy appears to be a more plausible cause for the complex phenotypes of cancer than gene mutation.

Indeed, aneuploidy was originally proposed to cause cancer over 100 years ago, because it was discovered in all epithelial cancers investigated by Hansemann in 1890 [Hansemann, 1890], and because it was found to cause abnormal, "pathological" and "tumor"-like phenotypes in developing sea urchin embryos by Boveri [Boveri, 1902, 1914]. However, the aneuploidy hypothesis has gradually lost popularity for a number of different reasons:

TABLE I. Hallmarks of Cancer and Carcinogenesis

Cancer	Predicted by		
	Aneuploidy	Mutation	Ref[a]
(1) Anaplasia, autonomous growth, invasiveness, metastasis via neoantigens	Yes	No	1
(2) Abnormal cellular and nuclear morphology	Yes	No	1
(3) Abnormal growth rates	Yes	Maybe	2
(4) Abnormal metabolism and gene expression	Yes	No	2, 3
(5) Aneuploidy with DNA indices ranging from 0.5 to >2	Yes	No	4
(6) Too many and abnormal centrosomes	Yes	No	5
(7) Karyotypic or "genetic" instability	Yes	No	6
(8) Immortality in vitro and on transplantation	Yes	No	7
(9) Clonal origin	Yes	Yes	9
(10) Non-clonal karyotypes and phenotypes, including non-clonal onco- and tumor suppressor genes	Yes	No	6, 10
(11) No specific, and no transforming gene mutation	Yes	No	11
Carcinogenesis			
(1) Non-genotoxic carcinogens	Yes	No	12
(2) Non-genotoxic tumor promoters	Yes	No	13
(3) Preneoplastic aneuploidy	Yes	No	14
(4) Spontaneous progression of malignancy	Yes	No	8
(5) Latency of months to decades from carcinogen to cancer	Yes	No	15
(6) 1,000-fold age bias of cancer	Yes	No	15
(7) Suppression of malignancy by fusion with non-malignant cell, and reappearance after spontaneous chromosome loss	Yes	Maybe	16

[a] 1 [Hansemann, 1890; Hansemann, 1897; Hauser, 1903; Hauschka, 1961; Bauer, 1963; Braun, 1969; Pitot, 1986]; 2 [Boveri, 1914; Bauer, 1963; Cairns, 1978; Pitot, 1986]; 3 [Busch, 1974; Augenlicht et al., 1987; Zhang et al., 1997; Duesberg et al., 1999; Rasnick and Duesberg, 1999]; 4 [Bauer, 1963; Caspersson et al., 1963; Busch, 1974; Rasnick and Duesberg, 1999]; 5 [Brinkley and Goepfert, 1998; Lingle et al., 1998; Pihan et al., 1998; Duesberg, 1999]; 6 [Bauer, 1963; Braun, 1969; DiPaolo, 1975; Nowell, 1976; Harnden and Taylor, 1979; Pitot, 1986; Sandberg, 1990; Heim and Mitelman, 1995; Duesberg et al., 1998; Heppner and Miller, 1998; Rasnick and Duesberg, 1999]; 7 [Levan and Biesele, 1958; Saksela and Moorhead, 1963; Hayflick, 1965; Cairns, 1978; Harris, 1995]; 8 [Foulds, 1965; Braun, 1969; Wolman, 1983; Pitot, 1986]; 9 [Boveri, 1914; Cairns, 1978; Harris, 1995]; 10 [Bauer, 1963; Braun, 1969; DiPaolo, 1975; Harnden and Taylor, 1979; Albino et al., 1984; Sandberg, 1990; Heim and Mitelman, 1995; Konishi et al., 1995; Giaretti et al., 1996; Roy-Burman et al., 1997; Al-Mulla et al., 1998; Duesberg et al., 1998; Heppner and Miller, 1998; Kuwabara et al., 1998; Offner et al., 1999]; 11 [Lijinsky, 1989; Duesberg and Schwartz, 1992; Strauss, 1992; Haber and Fearon, 1998; Boland and Ricciardello, 1999; Li et al., 2000]; 12 See text [Burdette, 1955; Oshimura and Barrett, 1986; Lijinsky, 1989; Li et al., 2000]; 13 [Pitot, 1986]; 14 [Duesberg et al., 2000 and references within]; 15 [Berenblum and Shubik, 1949; Armitage and Doll, 1954; Cairns, 1978; Pitot, 1986; Li et al., 1997; Lodish et al., 1999; Duesberg et al., 2000]; 16 See text and [Pitot, 1986; Harris, 1993; Harris, 1995].

1. The first of these was certainly the lack of cancer-specific karyotypes [Rous, 1959; Bauer, 1963; Braun, 1969; DiPaolo, 1975; Nowell, 1976; Harnden and Taylor, 1979; Cram et al., 1983; Sandberg, 1990; Harris, 1995; Heim and Mitelman, 1995]. According to Rous, discoverer of Rous sarcomas virus, "Persistent search has been made, ever since Boveri's time, for chromosome alterations which might prove characteristic of the neoplastic state—all to no purpose" [Rous, 1959]. Thirty-six years later, Harris reviewed the search for cancer-specific karyotypes with the remark, "it utterly failed to identify any specific chromosomal change that might plausibly be supposed to have a direct causative role in the generation of a tumour" [Harris, 1995].
2. The second probable reason to abandon aneuploidy was the lack of conventional mechanisms for how aneuploidy is generated and how it would generate abnormal phenotypes. For example, Weinberg pointed out in an editorial in *Nature* in 1998 that, "Aneuploidy has long been speculated to be causally involved in tumorigenesis, but its importance has not been demonstrated" [Orr-Weaver and Weinberg, 1998]. Because of this widespread lack of appreciation for the mutagenic potential of aneuploidy most researchers now consider aneuploidy a consequence of cancer rather than a cause [Nowell, 1976; Harris, 1995; Heim and Mitelman, 1995; Johansson et al., 1996; Mitelman et al., 1997] or are undecided [Oenfelt, 1986; Oshimura and Barrett, 1986; Pitot, 1986; Tucker and Preston, 1996; Galitski et al., 1999; Hieter and Griffiths, 1999]. But irrespective of its mutagenic potential, the "importance" of aneuploidy in cancer could have been gleaned from the kinetics of

aneuploidization, by determining whether aneuploidy precedes cancer or is just a consequence. Indeed, several other investigators have observed preneoplastic aneuploidy earlier, but failed to interpret their data as proof for causation, probably because of the low recent currency of aneuploidy [reviewed in Duesberg et al., 2000].

3. The aneuploidy hypothesis also failed to explain the slow kinetics of carcinogenesis, a problem it shared with all other cancer hypotheses (Table I) [Bauer, 1948; Cairns, 1978].
4. Finally Boveri's premature death at 53, in 1915, proved to be yet another setback for the development of the aneuploidy hypothesis in the face of the emerging gene mutation hypothesis [Wolf, 1974; Sandberg, 1990].

As a result the aneuploidy hypothesis was eventually displaced by the gene mutation hypothesis.

Ever since Morgan's first papers on *Drosophilia* genetics first appeared in 1910 [Morgan, 1910] gene mutation, rather than aneuploidy, was on everybody's mind as the mechanism of generating abnormal phenotypes. Moreover, Morgan and Bridges directly attacked Boveri's aneuploidy hypothesis, "At present, however, reference to such possible sources," i.e., "imperfect or irregular division of the chromosomal complex," " is too uncertain to be of great value, for there are no instances where irregularities of this kind are known to give rise to prolific growth processes. The cancer-like or tumor-like growth shown by a mutant of Drosophila . . . is caused by a sex-linked Mendelian gene. . ." [Morgan and Bridges, 1919]. The mutation hypothesis derived further support in 1927 when Muller, a former student of Morgan, had discovered that X-rays mutate genes [Muller, 1927]. Since X-rays were a previously known carcinogen, this discovery was interpreted as experimental support for the mutation hypothesis. It set off the same searches for mutagenicity of all carcinogens and for the corresponding cancer-causing mutations, that still monopolize cancer research today [Muller, 1927; Miller and Miller, 1971; Ames et al., 1973; Cairns, 1978; Pitot, 1986; Alberts et al., 1994; Harris, 1995; Lodish et al., 1999].

However, over 70 years later, proponents of the mutation hypothesis cannot as yet (1) explain the growing lists of non-genotoxic carcinogens, (2) demonstrate any cancer-specific mutations, (3) offer functional proof that cellular mutant genes cause cancer, (4) explain the complex and unstable phenotypes of cancer, (5) offer a genetic explanation for the slow kinetics of carcinogenesis based on mutations that are typically fast [Harris, 1995; Boland and Ricciardello, 1999; Li et al., 2000] (see Table I).

Intrigued by its enormous mutagenic potential, we and others have recently reconsidered aneuploidy as a cause of cancer [Li et al., 1997; Brinkley and Goepfert, 1998; Duesberg et al., 1998; Rasnick and Duesberg, 1999]. We show here biochemical and biological analyses of aneuploidy and of gene mutation, which indicate that aneuploidy is probably the only mutation that can generate the complex phenotypes of cancer. In view of this, we can now propose a coherent two-stage mechanism for all aspects of cancer and carcinogenesis. In stage one, both genotoxic and non-genotoxic carcinogens cause aneuploidy. In stage two, aneuploidy generates ever new and eventually neoplastic phenotypes autocatalytically, because aneuploidy destabilizes the karyotype.

Our analysis deals only peripherally with germline mutations that affect the cancer risk of somatic cells, as for example the mutations that lead to retinoblastoma and Bloom's syndromes, because such mutations only cause cancer indirectly [Knudson, 1985; Pitot, 1986; Duesberg et al., 1998]. Instead we focus here on the question of which somatic mutations are directly responsible for malignant transformation, i.e., either gene mutation or aneuploidy. Once this question can be answered, we expect to be in a better position to determine how germline mutations affect the cancer risk of somatic cells.

MUTATION HYPOTHESIS TAKES OVER, BUT FAILS TO ACHIEVE FUNCTIONAL PROOF

The gene mutation hypothesis, in contrast to the competing aneuploidy hypothesis, derived instant support from its conventional mechanism of phenotype alteration. Moreover the gene mutation hypothesis attracted steady attention by adopting and adapting results of the rapidly evolving fields of sexual and later molecular genetics, which offered plenty of "doable" experiments [Fujimura, 1996]. The following two examples illustrate this development.

Example 1: "Carcinogens Are Mutagens"

After his discovery that X rays, a previously known carcinogen, can mutate genes, Muller was the first to point out in 1927 that the "effect of X-rays, in occasionally producing cancer, may also be associated with their action in producing mutations" [Muller, 1927]. Soon ever more carcinogens were shown to have mutagenic function with ever more sensitive techniques [Bauer, 1928; Braun, 1969; Miller and Miller, 1971]. Even the chemically inert polycyclic aromatic hydrocarbons were found to react with DNA, although only after enzymatic oxidation [Brookes and Lawley, 1964; Cairns, 1978]. The quest for mutagenic carcinogens reached a high point with Ames' slogan, "Carcinogens are mutagens" [Ames et al., 1973].

But in the excitement over matching carcinogens with mutagenic function it was simply disregarded that many, including the most effective, carcinogens were not mutagenic in established test systems, as for example the polycyclic hydrocarbons [Berenblum and Shubik, 1949; Burdette, 1955; Ashby and Purchase, 1988]. Even Rous was ignored, "The evidence as a whole makes plain though that some carcinogens induce somatic mutations whereas others do not, that some mutagenic agents fail to be carcinogenic, and that many substances closely related chemically to agents of both sorts do neither" [Rous, 1959]. So was Lijinsky, who also acknowledged that many carcinogens are mutagenic, but warned, "that [if] chemicals, which are mutagenic cause neoplastic transformation does not mean that a mutagenic process is involved" and that "the mutagenic reaction of carcinogens might be coincidental rather than causal: alternative mechanisms of carcinogenesis should be considered" [Lijinsky, 1989].

Example 2: "Cellular Oncogenes" Like Retroviral Oncogenes

The discovery of dominant, retroviral oncogenes in the 1970s, beginning with the *src* gene of Rous sarcoma virus [Duesberg and Vogt, 1970; Martin, 1970; Lai et al., 1973], was also quickly adopted by the gene mutation hypothesis as a substitute for functional proof based on the following argument. The promoters of these oncogenes are shared with the virus, but their coding regions are derived from cellular genes by a conventional but rare process, termed transduction, which involves illegitimate recombination between viral and cellular DNAs [Duesberg, 1987; Goodrich and Duesberg, 1990; Schwartz et al., 1995]. In view of this relationship, it was proposed that the cellular relatives of the retroviral oncogenes are the long-sought cellular targets of mutation by carcinogens and that they should, therefore, be termed cellular oncogenes [Bishop, 1981, 1995]. But this proposal did not take into consideration one profound difference between the viral oncogenes and their cellular relatives, namely that the promoters of the oncogenic retroviruses are at least 1,000-stronger than those of cellular oncogenes [Duesberg and Schwartz, 1992; Duesberg, 1995; Hua et al., 1997].

In the words of Bishop, one of the original proponents of this view, "We now believe that we know 'the why for these happenings' [cancer].'' Perhaps proto-oncogenes [the cellular genes to which retroviral oncogenes are related] exemplify a genetic keyboard on which all manner of carcinogens might play. Any influence that can damage a proto-oncogene might give rise to an oncogene, even if the damage occurred without the gene ever leaving the cell, without the gene ever encountering a virus. In this view, proto-oncogenes become precursors to cancer genes within our cells, and damage to genes becomes the underpinning of all cancers—even those that are not caused by viruses. . . . An enemy has been found and we are beginning to understand its lines of attack" [Bishop, 1995]. The impact of this view was described by Harris, a prominent cell biologist, as follows, "it was a small step to conclude that mutations in proto-oncogenes within the genome might also convert them into active oncogenes that could induce transformation and thus contribute to the production of tumours. This idea met with widespread enthusiasm and at once became the focus of numerous investigations" [Harris, 1995].

Shortly after this idea was advanced, the first proof seemed to be at hand. According to two parallel studies, a point mutation in the coding region had converted a proto-oncogene of a human bladder carcinoma cell line to the functional equivalent of the *ras* oncogene of murine Harvey sarcoma virus. The basis for this claim was the ability of the proto-*ras* DNA of the human cell line to transform morphologically the mouse 3T3 cell line. This result was interpreted as the discovery of the first human cancer gene [Logan and Cairns, 1982; Reddy et al., 1982; Tabin et al., 1982]. In view of this, the human mutant *ras* DNA was called a "dominant" cellular oncogene [Cooper, 1990; Alberts et al., 1994; Harris, 1995; Lodish et al., 1995].

However, the mouse 3T3 line is not an appropriate substrate to identify a human cancer gene. The 3T3 cell is not human, is already tumorigenic [Boone and Jacobs, 1976], and is also highly aneuploid, carrying over 70 instead of the normal 40 chromosomes of mice [American Type Culture Collection, 1992; Lodish et al., 1999]. Moreover, "DNA from normal cells did [also] produce some transformed colonies . . . in NIH 3T3 cells," and "the NIH 3T3 mouse cell line . . . did [also] spontaneously generate transformed colonies" [Harris, 1995]. The unstable morphological phenotype of 3T3 cells is probably a direct consequence of the unstable karyotype typical of aneuploid cells (see Fig. 1 and Stage Two: Generation of neoplastic karyotypes). The 3T3 cell is, therefore, not even close to an authentic model for a normal, diploid human cell, which is the starting material of human cancers. Indeed, normal diploid human cells, unlike rodent cells, are exceedingly difficult to transform in culture [Rhim and Dritschilo, 1991; Harris, 1995].

Therefore, it is not surprising that subsequent work soon showed that the cancer-derived *ras* DNA that transforms 3T3 cells was unable to transform normal, diploid mouse cells and, above all, normal human cells [Duesberg and Schwartz, 1992; Duesberg, 1995; Hua et al., 1997; Lodish et al., 1999]. In fact, this disappointing result could have been anticipated if the ability of authentic retroviral *ras* genes to transform human cells had

first been investigated. Such a control experiment would have demonstrated that the cell-transforming host range of *ras* genes is limited to rodents, and does not include human cells [Li et al., 1996].

Moreover, even the ability of the mutant human *ras* DNA to transform mouse 3T3 cells proved to be an artifact of the method to introduce exogenous DNA into cells by transfection, rather than a dominant property of the cancer-derived *ras* DNA. During the transfection test, mutant *ras* DNAs are artificially recombined to large concatamers that express about 1,000-times more *ras* RNA than the human cancer cells from which the DNA was isolated. Such high levels of *ras* expression are naturally only seen in cells transformed by retroviruses with promoters that are 1,000-fold stronger than those of cellular counterparts.

By contrast, cellular *ras* RNA in human cancer cells transcribed from either normal or mutated *ras* genes with native cellular promoters is expressed so poorly, that it is practically undetectable [Duesberg and Schwartz, 1992; Duesberg, 1995; Hua et al., 1997], as for example in colon cancer cells with mutant *ras* genes [Zhang et al., 1997; Rasnick and Duesberg, 1999]. In other words the 3T3 transfection assay creates *ras* expression artifacts that are functionally similar to viral *ras* oncogenes. This transfection artifact was erroneously interpreted as evidence for functional equivalence between a point-mutated cellular gene and the authentic *ras* oncogene of Harvey sarcoma virus [Duesberg and Schwartz, 1992; Duesberg, 1995; Hua et al., 1997; Lodish et al., 1999].

Thus, there is no direct functional proof for the hypothesis that mutation of *ras* and other cellular genes, related to retroviral oncogenes, causes cancer (see below). But in view of the perceived functional precedent, mutant *ras*, the literature abounds with efforts to establish correlations between such mutations and cancer, and with the functions of artificial derivatives of these genes in animals and cultured cells [Cooper, 1990; Lodish et al., 1995; Hahn et al., 1999; Li et al., 2000]. Among these genes, mutant proto-*abl* stands out for its high correlation with chronic myeloid leukemia (CML), and for the various functions of artificial derivatives.

Human CML proceeds in two distinct phases. The first is a chronic phase lasting on average between 3 to 4 years in which undifferentiated and differentiated, functional myelocytes, granulocytes, and neutrophils are overproduced. Since the overproduced cells differentiate to functional blood cells, this phase of the disease is a clonal hyperplasia. In about 85% of CML cases these hyperplastic cells carry a clonal variant of chromosome 22, termed Philadelphia chromosome. The remaining CML cases have no Philadelphia chromosome [Nowell, 1982; Sandberg, 1990]. The second phase of CML is a terminal leukemia of several months, termed blast crisis, in which new, autonomous clones of non-differentiating myeloblasts take over that are typically aneuploid and also carry the Philadelphia chromosome. These cells are no longer functionally normal [Koeffler and Golde, 1981a,b; Sandberg, 1990].

In about 80% of CMLs with Philadelphia chromosomes, the variant chromosomes are generated by a reciprocal translocation in which a small piece of chromosome 9 is translocated to chromosome 22, and a smaller piece of 22 goes to 9 [Rowley, 1973]. Since this translocation moves the coding region of the proto-*abl* gene to a promoter region from a gene termed *bcr* on chromosome 22, and since proto-*abl* is related to the oncogene of the murine Abelson leukemia virus, the hybrid *bcr-abl* gene is now thought to be the cause of CML [Heisterkamp et al., 1985].

However, there is a conceptual problem with this hypothesis. The Abelson virus carries a dominant oncogene, termed *abl*, which causes a polyclonal leukemia in mice that is fatal within a few weeks [Weiss et al., 1985; Duesberg and Schwartz, 1992]. But the chronic phase of CML is a hyperplasia, not a terminal leukemia. Thus the *bcr-abl*-CML hypothesis postulates that a cellular mutant gene causes hyperplasia, because this gene is related to a dominant retroviral oncogene. Experimental evidence confirms and extends the discrepancy. The transcripts of *abl* genes are barely detectable or even un-detectable in CML patients by conventional hybridization with radioactive DNA probes [Gale and Canaani, 1984]. Therefore, transcripts are now typically detected by artificial amplification with the polymerase chain reaction [Bose et al., 1998]. By contrast, transcription of the oncogene of Abelson virus in leukemic mice is 100- to 1,000-fold higher than that of the mouse or human *abl* genes [Duesberg and Schwartz, 1992]. Thus, the fast, viral leukemia with highly active *abl* genes is not a model for the slow, chronic phase of human CML with inactive *bcr-abl* genes. But owing to the magic spell of the word oncogene, such discrepancies seem to be tolerated, even if the facts speak otherwise.

The functional discrepancy between the Abelson virus oncogene and the cellular *bcr-abl* gene has been confirmed unintentionally by all efforts to prove the *bcr-abl*-CML hypothesis. For example, to generate a leukemia in mice with the *bcr-abl* of human CML, Baltimore et al. had to make the gene part of an artificial Abelson virus [Daley et al., 1990], which enhanced its activity 100- to 1,000-fold compared to its activity in CML [Duesberg and Schwartz, 1992]. Likewise Era and Witte had to rely on heterologous promoters derived from cytomegalovirus and a chicken actin gene in order to find, "... Bcr-Abl being the sole genetic change needed for the establishment of the chronic phase of

CML" [Era and Witte, 2000]. However, these studies, like its antecedents with mutant *ras* genes, failed to consider that the cellular and pathogenic effects of these artificial *bcr-abl* constructs depended on 100- to 1,000-fold transcriptional activation compared to the inactive *bcr-abl* genes of human CML [Duesberg and Schwartz, 1992]. Thus, these studies confirm the lesson of the mouse Abelson virus, i.e., that a highly over-expressed *abl* gene is leukemogenic, but they say little about the function of the poorly expressed *abl* genes in the chronic phase of CML.

Moreover, since the discovery of the reciprocal translocation between chromosomes 22 and 9 in human CML [Rowley, 1973], about 20% of Philadelphia chromosomes were shown to be translocations of chromosome 22 with chromosomes that do not carry *abl* genes, i.e., with chromosomes 2, 6, 7, 11, 13, 16, 17, 19, and 21 [Nowell, 1982; Sandberg, 1990; Harris, 1995]. According to Nowell, the discoverer of the Philadelphia chromosome [Nowell and Hungerford, 1960], "These variants appear to have no significance with respect to the clinical characteristics of the disease, and so it appears that it is the displacement of the sequence of chromosome 22 that is of major importance, rather than the site to which it goes" [Nowell, 1982]. In other words, the mutation of proto-*abl* is not necessary for the generation of a Philadelphia chromosome nor for CML.

This leaves open the question whether mutation of proto-*abl* happens to be sufficient to initiate the chronic, hyperplastic phase of CML by some unknown mechanism that does not rely on high transcriptional activity. But, two facts suggest that this is not the case: (1) Transgenic mice carrying a *bcr-abl* gene in every cell of their body, even with promoters that are much stronger than those of native *bcr-abl* genes, are not born with CML. Instead, many develop a non-CML type leukemia after "long latency," because " BCR/ABL expression is not the sole cause of leukemia but rather predisposes for the cancer" [Voncken et al., 1995]. (2) CML-specific, poorly expressed *bcr-abl* transcripts have recently also been detected in up to 75% of normal humans with the polymerase chain reaction [Biernaux et al., 1995; Bose et al., 1998]. It follows that the *bcr-abl* gene is not sufficient to initiate even the chronic phase of CML.

Thus, the hypothesis that mutation of cellular genes related to retroviral oncogenes causes cancer, is unconfirmed. But, in the view of the apparent functional proof for cellular oncogenes, a plethora of mutated genes has been identified in cancer cells that are all now assumed to cause cancer either directly, as hypothetical oncogenes, or indirectly, as hypothetical tumor suppressor genes [Alberts et al., 1994; Haber and Fearon, 1998; Boland and Ricciardello, 1999; Lodish et al., 1999; Hanahan and Weinberg, 2000]. Most of these mutant genes do not even transform 3T3 cells, but they are nevertheless called "oncogenes" because they were first identified in cancer cells [Watson et al., 1987]. Indeed, in the following it is shown that to this very day it has not been possible to isolate cellular genes from any cancer that transform normal human cells to cancer cells [Li et al., 2000], "after more than 15 years of trying" [Weitzman and Yaniv, 1999].

Nevertheless, the evidence that these mutations are neither necessary nor sufficient for cancer does not exclude the possibility that these mutations, if present, play indirect roles in carcinogenesis as, for example, in clonal expansion [Cha et al., 1994] or in increasing the risk of aneuploidy (see conclusions). Indeed the transition from the chronic, preneoplastic phase of CML to the neoplastic phase, termed blast crisis, is preceded by and coincides with aneuploidy [Sadamori et al., 1983, 1985; Harris, 1995], suggesting that the Philadelphia chromosome and/or its reciprocal counterpart may increase the risk of aneuploidization.

Aneuploidy Hypothesis "Got Lost"

In the excitement over gene mutation and mutant genes, the aneuploidy hypothesis was virtually forgotten. According to an editorial in *Science* in 1999, "Over the following decades, however, [Boveri's] idea got lost, as researchers concentrated on understanding the specific gene malfunctions that lead to cancer" [Pennisi, 1999]. The idea got lost so completely that it is now no longer mentioned in the leading textbooks of biology [Watson et al., 1987; Alberts et al., 1994; Lewin, 1994; Lodish et al., 1999]. As a result scientists studying aneuploidy now compare their work to "resurrection" [Brinkley and Goepfert, 1998].

Even cytogenticists have disregarded the aneuploidy hypothesis in favor of gene mutation. For example, Nowell wrote in an influential article in *Science* in 1976, "It is certainly clear that visible alterations in chromosome structure are not essential to the initial change" [Nowell, 1976]. Twenty years later Mitelman et al. wrote, "We propose that unbalanced primary changes [aneuploidy], in fact, are secondary, the primary being submicroscopic. There are no unbalanced primary changes, only secondary imbalances masquerading as primary" [Johansson et al., 1996]. Aneuploidy, if considered at all, is now viewed as just one of several mechanisms that alter the dosage of hypothetical oncogenes or inactivate tumor suppressor genes [Orr-Weaver and Weinberg, 1998; Cahill et al., 1999]. For example Mitelman et al. state, "Obviously, the pathogenetically important outcome of cytogenetically identified gains or losses of chromosomal material may simply be ascribed to amplification or deletion of single oncogenes or tumor suppressor genes. . ." [Mitelman et al., 1997].

Gene Mutation Hypothesis Now, Popular But Unconfirmed

Despite its current popularity, the gene mutation hypothesis has failed to meet many of its own predictions (see also Table I).

1. The hypothesis predicts that carcinogens function as mutagens. But, there is a growing list of non-genotoxic carcinogens, including asbestos, Ni^{++}, hormones, butter yellow, arsenic, acrylamide, urethan, hydrazin, and polycyclic hydrocarbons [Berenblum and Shubik, 1949; Burdette, 1955; Rous, 1959; Scribner and Suess, 1978; Oshimura and Barrett, 1986; Ashby and Purchase, 1988; Lijinsky, 1989; Preussman, 1990]. Although some oxidative derivatives of the polycyclic hydrocarbons have modest mutagenic functions, the paradox remains that they are 1,000-fold better carcinogens per mutation than directly genotoxic carcinogens such as methyl-nitrosoguanidine, signaling a non-mutagenic mechanism [Scribner and Suess, 1978; Preussman, 1990].
2. The hypothesis predicts that substances that enhance malignant transformation, termed tumor promoters, are mutagenic. But tumor promoters are non-genotoxic by definition [Pitot, 1986].
3. The hypothesis predicts cancer-specific gene mutations. But no such mutations have yet been found [Vogelstein et al., 1988; Cooper, 1990; Duesberg and Schwartz, 1992; Strauss, 1992; Hollstein et al., 1994; Haber and Fearon, 1998; Little, 2000]. According to a recent commentary ("How many mutations does it take to make a tumor?"), "There are no oncogenes or tumor suppressor genes that are activated or deleted from all cancers. Even tumors of a single organ rarely have uniform genetic alterations, although tumor types from one specific organ have a tendency to share mutations" [Boland and Ricciardello, 1999]. When no specific mutations are found, other, as yet unknown, mutations are suggested to "phenocopy" the known mutations (even though there is no functional evidence) [Hanahan and Weinberg, 2000].
4. The hypothesis predicts that causative mutations are clonal, i.e., shared by all cells of a tumor. However, recent evidence shows that even known, hypothetically causative mutations are not shared by all cells of the same tumor, e.g., mutant *ras* and the hypothetical mutant tumor suppressor gene p53 [Albino et al., 1984; Shibata et al., 1993; Konishi et al., 1995; Giaretti et al., 1996; Roy-Burman et al., 1997; Al-Mulla et al., 1998; Heppner and Miller, 1998; Kuwabara et al., 1998; Offner et al., 1999]. Thus, known oncogene and tumor suppressor gene mutations are not necessary for the maintenance and probably not even for the initiation cancer, although they are present in some of its cells. (Their non-clonality is predicted by the aneuploidy hypothesis. See below, Non-clonal karyotypes, but clonal aneuploidy.) Likewise, the spontaneous loss of the presumed oncogene, mutant *ras*, does not revert the phenotype of a cancer cell back to normal [Plattner et al., 1996].
5. The mutation hypothesis predicts cancer-specific mutant genes to transform normal human or animal cells into cancer cells. But no such genes have been isolated from cancers, despite enormous efforts [Augenlicht et al., 1987; Lijinsky, 1989; Stanbridge, 1990; Thraves et al., 1991; Duesberg and Schwartz, 1992; Duesberg, 1995; Harris, 1995; Hua et al., 1997; Weitzman and Yaniv, 1999; Li et al., 2000]. On the contrary, several hypothetical mutant cancer genes, including *myc*, *ras*, and p53, have even been introduced into the germline of mice. But such transgenic mice are initially healthy and are breedable, although some appear to have a slightly higher cancer risk than other laboratory mice (see below) [Sinn et al., 1987; Hariharan et al., 1989; Donehower et al., 1992; Duesberg and Schwartz, 1992; Purdie et al., 1994; Li et al., 2000]. For example, one study of the genes said to cause colon cancer reports that, "Transgenic pedigrees that produce K-*ras*Val12 alone, p53Ala143 alone, or K-*ras*Val12 and p53Ala143 have no detectable phenotypic abnormalities" [Kim et al., 1993]. According to Harris," Experiments with transgenic animals are unanimous in their demonstration that oncogenes do not produce tumours directly, but merely establish a predisposition to tumour formation that ultimately requires other genetic changes which occur in a stochastic fashion" [Harris, 1995]. And even this predisposition may be an artifact of the ectopic position of the trans-gene in the chromosome rather than of its function.
6. Mutagenic carcinogens predict instant transformation, because carcinogen-mediated mutation is instantaneous [Muller, 1927; Brookes and Lawley, 1964]. But the latent periods between

carcinogen treatment and cancer are exceedingly long, ranging from a minimum of many months to decades [Haldane, 1933; Berenblum and Shubik, 1949; Rous, 1959; Braun, 1969; Cairns, 1978].

7. Carcinogenesis initiated by gene mutation predicts that the various phenotypes of tumor progression follow a reproducible sequence initiated by the causative mutation. But, according to "Foulds' rules," "progression occurs independently in different characters in the same tumor", and "follows one of alternative paths of development" [Foulds, 1965; Braun, 1969; Pitot, 1986].
8. Gene mutation predicts stable phenotypes. But the phenotypes of cancer cells are notoriously unstable generating phenotypic heterogeneity within tumors, which provides the basis for the notorious progression of malignancy via selection (see below) [Nowell, 1976; Duesberg et al., 1998; Heppner and Miller, 1998; Cahill et al., 1999].
9. The hypothesis that mutation converts proto-oncogenes to cancer genes is hard to reconcile with the survival of multicellular organisms in view of the spontaneous mutation rates of mammalian cells and the plethora of hypothetical oncogenes and tumor suppressor genes postulated so far [Mitelman et al., 1997; Haber and Fearon, 1998; Lodish et al., 1999; Hanahan and Weinberg, 2000]. The spontaneous, net mutation rate (after proofreading) is about 1 out of 10^9 nucleotides per mitosis [Strauss, 1992; Lewin, 1994; Li et al., 1997]. Since the DNA of human and all other mammalian species is made up of about 10^9 nucleotides [O'Brien et al., 1999], one in 10^9 cells will contain a mutation in every position of the human or mammalian genome. Considering that humans are made up out of 10^{14} cells [Cairns, 1978; Strauss, 1992], every human should contain 10^5 cancer cells even if just one dominant oncogene existed that could be activated by just one point mutation. Since there is now a plethora of such genes and "activating" mutations are found in multiple positions of the same gene [Seeburg et al., 1984], cancer should be ubiquitous. In response to this, the proponents of the mutation hypothesis now argue that it takes between 3 and 20 gene mutations to generate a human cancer cell [Lodish et al., 1999]. Hahn et al. [1999] postulate that three mutant genes "suffice" to create a human tumor cell, whereas Kinzler and Vogelstein [1996] postulate 7 mutations for colon cancer. However, this argument creates a new paradox, because in view of the above mutation rates, cancer would be practically nonexistent. For example, if 3 mutations were required only 1 in $10^{9\times3}$ or 10^{27} human cells would ever turn into a cancer cell by spontaneous mutation, and if 7 were required only one in 10^{63} would ever turn into a cancer cell. Thus only 1 in 10^{11} or in 10^{47} humans would ever develop cancer, since an average human life corresponds to about 10^{16} cells [Cairns, 1978; Duesberg and Schwartz, 1992]. In other words, cancer would never occur. In view of this paradox, the proponents of the gene mutation hypothesis have postulated that malignant transformation depends on a "mutator phenotype" [Loeb, 1991]. However, the "mutator phenotype" cannot be detected in most cancer cells [Barrett et al., 1990; Harris, 1991; Strauss, 1992; Jakubezak et al., 1996; Kinzler and Vogelstein, 1996; Duesberg et al., 1998; Heppner and Miller, 1998; Orr-Weaver and Weinberg, 1998]. Therefore, it is now claimed that the "mutator phenotype" is "transient", i.e., undetectable once a cancer cell is generated [Loeb, 1997]. But, until this "transient" mutator becomes detectable or a functional test for mutant oncogenes can be developed the mutation hypothesis is just speculation.
10. Conventional gene mutation generates diploid mutant cells. But, virtually all solid cancers are aneuploid [Sandberg, 1990; Mitelman, 1994; Mertens et al., 1997; Mitelman et al., 1997; Gebhart and Liehr, 2000] (see below, Proof of principle I:. . .).

These and other discrepancies between gene mutation and cancer have been noted by several cancer researchers in the past [Burdette, 1955; Rous, 1959; Braun, 1969; Cairns, 1978; Pitot, 1986; Lijinsky, 1989; Preussman, 1990; Strauss, 1992; Harris, 1995]. For example, Berenblum and Shubik were some of the first to raise questions about gene mutation as the cause of cancer, "the theory has rested largely on the assumption that, given an irreversible change as the basis of carcinogenesis, the only known biological phenomenon to explain this would be a gene mutation. However, a closer examination of other common biological phenomena instantly reveals that this is not so" [Berenblum and Shubik, 1949]. And Rous concluded in 1959, despite a potential conflict of interest with regard to the cancer gene of his Rous sarcoma virus (see above, Introduction) [Rous, 1967] that "the somatic mutation hypothesis, after more

than half a century, remains an analogy: 'it is presumptive reasoning based on the assumption that if things have similar attributes they will have other similar attributes' " [Rous, 1959]. Rous's reservations about the hypothesis included non-genotoxic carcinogens (see above, Mutation hypotheses takes over but. . .), the slow action of carcinogens, and the inadequacy of known mutations to explain the many differences between cancer and normal cells (see below, Chromosome number variation. . .) [Rous, 1959]. But despite these and other calls, an alternative cancer hypothesis was not advanced. In the following, we present new arguments for an old alternative cancer hypothesis: aneuploidy.

MECHANISM OF HOW ANEUPLOIDY IS THOUGHT TO CAUSE CANCER

The challenge was to find an aneuploidy-cancer mechanism that explains:

1. how carcinogens could cause aneuploidy without gene mutation,
2. how aneuploidy would generate the many abnormal phenotypes of cancer cells (Table I),
3. why cancer occurs only many months to decades after exposure to, or experimental treatment with carcinogens,
4. why not all aneuploidies, e.g., Down syndrome, cause cancer,
5. why cancer-specific phenotypes are genetically unstable, unlike the phenotypes of conventional mutations (Table I),
6. how to reconcile non-clonal karyotypes and heterogeneous phenotypes with clonal cancers (Table I).

Based on comparative analyses of the biochemical and biological consequences of aneuploidy vs. gene mutation, we have recently proposed a two-stage mechanism of carcinogenesis that meets these challenges [Li et al., 1997; Duesberg et al., 1998; Rasnick and Duesberg, 1999; Li et al., 2000]. This mechanism runs as follows (Fig. 1).

Stage One: Generation of Aneuploidy

Both genotoxic and non-genotoxic chemical carcinogens are proposed to generate aneuploidy by chemically or physically altering either the chromosomes or the spindle apparatus. This has already been demonstrated by us and others [Liang and Brinkley, 1985; Oenfelt, 1986; Oshimura and Barrett, 1986; Jensen et al., 1993; Parry et al., 1996; Li et al., 1997; Matsuoka et al., 1997; Duesberg et al., 2000]. For example, the lipophilic polycyclic hydrocarbons may disrupt microtubules by binding to tubulin proteins (compare the phenol method for protein extraction), and thus induce chromosome non-disjunction [Jensen et al., 1993; Li et al., 1997; Matsuoka et al., 1997]. As originally demonstrated by Boveri [Wolf, 1974], genotoxic physical carcinogens, such as X- or α-rays, can generate aneuploidy, by fragmenting chromosomes [Muller, 1927; Bauer, 1939; Borek et al., 1977; Levy et al., 1983; Kadhim et al., 1992]. Recent evidence indicates that radiation can also cause aneuploidy by damaging the spindle apparatus (see below, Proof of principle II:. . .) [Little, 2000].

An alternative hypothesis suggests that mutation of mitosis genes causes aneuploidy. Three such mutant genes have so far been identified; two of these are thought to control centrosome replication, i.e., mutant p53 [Fukasawa et al., 1996] and an over-expressed kinase SKT15 [Zhou et al., 1998], and one is thought to be a ''mitotic checkpoint gene'' [Lengauer et al., 1997; Cahill et al., 1998]. However, the mutant p53 was found in less than 50% [Lengauer et al., 1997] and the mutated checkpoint gene in only 11% of aneuploid colon cancers [Cahill et al., 1998]. Likewise, the mutant kinase was found in only 12% of primary breast cancers whereas presumably all cancers were aneuploid because they carried "six or more [kinase] signals" [Zhou et al., 1998]. Thus, either other genes or other mechanisms must have caused aneuploidy in the majority of these cancers.

The following facts favor non-mutational mechanisms as causes of aneuploidy:

1. All cancers caused by non-genotoxic carcinogens should be diploid. But this is not observed in experimental cancers [Marquardt and Glaess, 1957; Oshimura and Barrett, 1986; Li et al., 1997; Duesberg et al., 1998].
2. Many cancers caused by genotoxic carcinogens should be diploid, because both cancer and aneuploidy are extremely rare, cellular events and thus unlikely to coincide in the same cell. Yet cancers caused by genotoxic physical and chemical carcinogens are aneuploid [Kirkland and Venitt, 1976; Borek et al., 1977; Connell, 1984; Sudilovsky and Hei, 1991; Duesberg et al., 2000]. The only possible reconciliation would be that the genotoxic carcinogens cause cancer via aneuploidy, which is what we postulate.
3. If gene mutations cause aneuploidy, equally aneuploid cells from different cancers should fall into different classes of karyotypic instability depending on the aneuploidizing mutation. But evidence from us and others shows that karyotypic instability of a cell is proportional to its degree of aneuploidy, not to its origin. The more aneuploid the cell, the more unstable is the

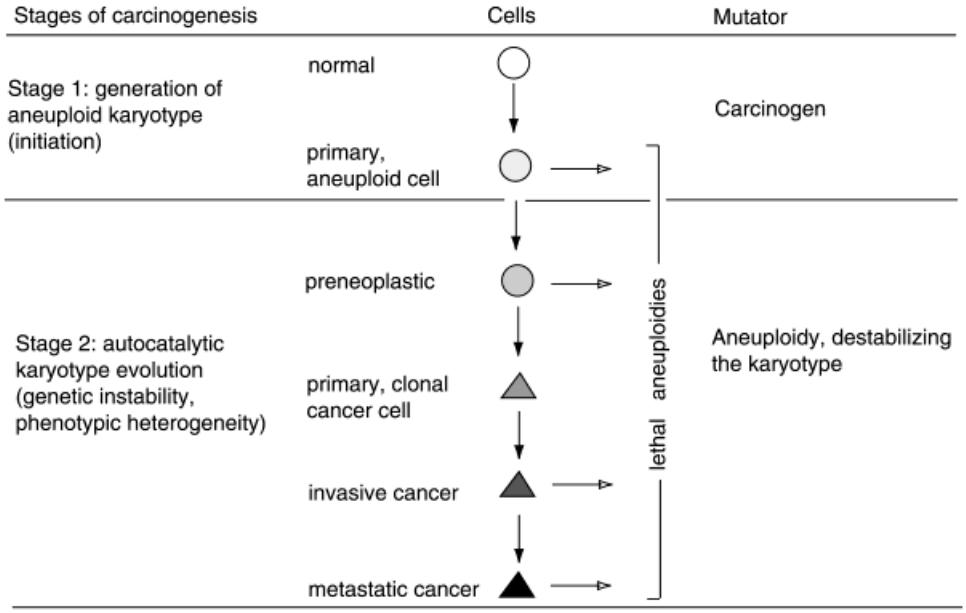


Fig. 1. A two-stage model for how carcinogens may cause cancer via aneuploidy. Stage one, a carcinogen "initiates" [Cairns, 1978] carcinogenesis by generating a random, but typically minor, ie. non-cancerous, aneuploidy. Stage two, the aneuploid cell autocatalytically generates new karyotypes including lethal, preneoplastic, and neoplastic ones. Normal and preneoplastic cells are shown as circles. Increasing degrees of aneuploidy are depicted by increasing densities of black. The primary "clonal" [Cairns, 1978] and advanced cancer cells are shown as triangles. Karyotype variation of aneuploid cells is autocatalytic because aneuploidy destabilizes the karyotype by unbalancing the dosages of spindle proteins via their chromosomal templates (see text). Autocatalytic karyotype evolution explains the non-clonal karyotypes and phenotypes of cancers, i.e., the notorious "genetic instability" of cancer cells (Table I). The inherent karyotype instability of aneuploid cells is also the basis for the spontaneous progression of malignancy, the notorious development of drug-resistance, and of the necrosis, alias apoptosis, of cancer cells by lethal aneuploidies. Karyotype evolution catalyzed by aneuploidy further explains the previously unresolved, carcinogen-independent transformation of a preneoplastic into a neoplastic cell after exceedingly long latent periods. The long latent periods from initiation to carcinogenesis would be a consequence of the low probability of generating by chance a karyotype that can out-perform normal cells.

karyotype [Lengauer et al., 1997; Duesberg et al., 1998].

4. DNA of cancers rendered aneuploid by somatic mutation should be able to convert normal diploid cells to aneuploid cells via aneuploidy genes, because such mutations are reportedly dominant [Lengauer et al., 1997]. But animals carrying mutated ''mitotic checkpoint genes,'' such as p53 [Cahill et al., 1998] in their germlines are viable (see above) and thus not aneuploid, although the cells of some of these animals are at a relatively high risk of aneuploidy (see Conclusions) [Kim et al., 1993; Purdie et al., 1994; Bouffler et al., 1995]. As yet all transfections that have generated aneuploidy in a dominant fashion have done so by artificially unbalancing the dosage of normal, un-mutated mitosis genes [Futcher and Carbon, 1986; Burke et al., 1989; Mayer and Aguilera, 1990].
5. The polycyclic aromatic hydrocarbons are inefficient and indirect mutagens, but they are outstanding chemical carcinogens and very effective aneuploidogens (see above)[Berenblum and Shubik, 1949; Cairns, 1978; Scribner and Suess, 1978; Bradley et al., 1981; Lijinsky, 1989]. For example, at micromolar concentrations aromatic hydrocarbons generate aneuploidy in 20 to 80% (!) of embryo cells and near diploid cell lines of the Chinese hamster within one or several days [Matsuoka et al., 1997; Duesberg et al., 2000]. By contrast, only a few percent of polycyclic aromatic hydrocarbons are ever converted to potentially mutagenic derivatives by animal cells [Richards and Nandi, 1978], and even the most effective, direct mutagens, such as N-nitroso compounds and ethyl-sulfonate, mutate at micromolar concentrations a given genetic locus of only 1 in 10^4 to 10^7 animal cells [Orkin and Littlefield, 1971; Terzi, 1974; Bradley et al., 1981]. In other words, the odds that a cell aneuploidized by a polycyclic hydrocarbon is also mutated in any given locus, as for example a mitosis gene, are only 10^{-4} to 10^{-7}. Thus, practically all aneuploidization by polycyclic aro-

matic hydrocarbons is due to non-mutational mechanisms.

6. If aneuploidy is caused by mutation of mitosis genes, the ratio of hypodiploid to hyperdiploid cells would be initially the same. By contrast, aneupolidy generated by physical or chemical fragmentation of chromosomes would initially generate mostly hypodiploid cells. Indeed the ratio of spontaneous aneuploidy in human cells is between 5 and 10 to 1 in favor of hypodiploidy [Galloway and Buckton, 1978]. The primary ratios may be even higher, because cells with some haploid chromosomes may be nonviable owing to otherwise recessive mutations in essential genes. It follows that most spontaneous aneuploidization is initiated by direct alteration or fragmentation of chromosomes rather than by mutation of mitosis genes.

In view of this damage to either the spindle apparatus or the integrity of chromosomes by interactions with carcinogens is considered a more likely source of aneuploidy than mutation of mitosis genes.

Stage Two: Generation of Neoplastic Karyotypes by Autocatalytic Karyotype Variation

Aneuploidy is proposed to catalyze karyotype variation and evolution, because it destabilizes the karyotype. The source of the karyotype instability is the imbalance that aneuploidy imparts on the genes of the spindle apparatus, resulting in abnormal ratios of spindle proteins, centrosomal proteins, and even abnormal numbers of centrosomes [Brinkley and Goepfert, 1998; Duesberg et al., 1998; Duesberg, 1999; Rasnick and Duesberg, 1999]. Chromosome non-disjunction via an unbalanced spindle, i.e., abnormal ratios of spindle proteins, will be more error-prone than via a balanced spindle, just like a person with uneven legs is more likely to fall than one with even legs. Thus, aneuploidy destabilzes itself, a process that has been termed "chromosome error propagation" [Holliday, 1989]. As a result, the aneuploid karyotype will vary autocatalytically (catalyzing its own variation) and evolve according to its habitat [Duesberg et al., 1998; Rasnick and Duesberg, 1999].

The risk of autocatalytic karyotype variation would be proportional to the degree of aneuploidy, i.e., the more the balance of mitosis proteins is biased the more unstable is the karyotype [Lengauer et al., 1997; Duesberg et al., 1998; Miazaki et al., 1999; Furuya et al., 2000]. This process would generate lethal, preneoplastic, and eventually neoplastic karyotypes (Fig. 1) [Li et al., 1997; Duesberg et al., 1998; Duesberg, 1999; Rasnick and Duesberg, 1999]. The preneoplastic karyotypes would include aneuploid cells that are "immortal," i.e., cell lines with unlimited growth potential like cancer cells, but that are not necessarily tumorigenic (Table I, see Aneuploidy ''immortalizes'') [Levan and Biesele, 1958; Saksela and Moorhead, 1963; Hayflick, 1965; Cairns, 1978; Cram et al., 1983; Harris, 1995; Trott et al., 1995; Rasnick, 2000].

EXPLANATIONS AND PREDICTIONS MADE BY THE ANEUPLOIDY-CANCER HYPOTHESIS

Our hypothesis offers testable explanations for each of the following eleven characteristics of cancer and carcinogenesis (see also Table I).

Non-Genotoxic Carcinogenes

The aneuploidy hypothesis exactly predicts the growing lists of non-genotoxic carcinogens that are incompatible with cancer by gene mutation (see above, Gene mutation hypothesis, now popular but unconfirmed, item 1).

Preneoplastic and Non-Neoplastic Aneuploidy

Our hypothesis predicts preneoplastic aneuploidy (Fig. 1). We have recently confirmed this prediction by demonstrating that "aneuploidy precedes and segregates with carcinogenesis" [Duesberg et al., 2000]. Indeed, several other investigators have observed preneoplastic aneuploidy earlier, but failed to interpret their data as evidence for causation, probably because of the low recent currency of aneuploidy [Rubin et al., 1992; Giaretti, 1994; Furuya et al., 2000; Duesberg et al., 2000; and references within]. Instead, most other researchers currently suggest that aneuploidy is a consequence of cancer (see Introduction).

According to our mechanism, neoplastic aneuploidy differs from non-neoplastic aneuploidy quantitatively and qualitatively, i.e., we postulate an as yet poorly defined threshold for neoplastic aneuploidy (see below, Fig. 2) [Duesberg et al., 1998, 2000; Rasnick and Duesberg, 1999]. Non-neoplastic aneuploidies typically involve the loss and less frequently the gain of only one or a few chromosomes [Harnden et al., 1976; Galloway and Buckton, 1978]. For example, Nowell points out, "Usually, the karyotypic alterations in these non-neoplastic clones are relatively minor, involving balanced translocations or loss of a sex chromosome" [Nowell, 1982].

Cancer-Specific Phenotypes

According to the proposed mechanism (Fig. 1), aneuploidy generates abnormal phenotypes, including the complex, cancer-specific phenotypes such as anaplasia, autonomous growth, and metastasis described previously (Table I), by unbalancing the dosages of thousands of regulatory and structural genes. The effect of aneu-

ploidy on the phenotypes of cells would be analogous to that of randomizing assembly lines of an automobile factory on cars, i.e., cars with abnormal ratios of normal (rather than mutated) wheels, bodies, and engines (see below, Fig. 2). It is acknowledged that this analogy is a simplification that assumes biochemical assembly lines to be colinear with chromosomes, which is often not the case [Epstein, 1986].

By contrast, the range of altering phenotypes by mutation of individual genes in vivo is much more limited than by mutating their numbers. It would be equivalent to mutating individual workers in an assembly line, who typically work at only a small fraction of their capacity (see below, Biochemical phenotypes are controlled. . .) [Kacser and Burns, 1981; Cornish-Bowden, 1995; Rasnick and Duesberg, 1999]. In an assembly line, the output of both activated and inactivated workers would be buffered by un-mutated workers working upstream and downstream and by redundant capacity. Even null mutations are buffered by a second unmutated allele.

Long Latent Periods From Carcinogen to Carcinogenesis

The exceedingly long latent periods from the initial aneuploidization to cancer reflect the low probability of evolving by chance a karyotype that surpasses the viability of a normal, diploid cell. In view of this, Boveri proposed in 1914 that the odds of generating a cell that is more viable than a normal cell, by random karyotype variation is as low as winning in the "lottery" [Boveri, 1914].

Even the statistical odds for generating the kinds of aneuploidy that are commonly seen in cancer cells by random chromosome non-disjunctions are low. For example, to generate a cell with more than three copies of a given chromosome requires at least two consecutive non-disjunctions affecting that particular chromosome. Since the odds of a given chromosome even of a highly aneuploid cell to undergo non-disjunction are only about 2% [Lengauer et al., 1997; Duesberg et al., 1998], on average 50 mitoses are necessary to generate a cell with four or more copies of a given chromosome. It is for this reason that experimental cancers appear on average no sooner than 6 months after treatment with a carcinogen. By contrast, cancer appears within less than a month after inoculation of one or more authentic cancer cells [Haldane, 1933; Bauer, 1948; Pitot, 1986; Harris, 1995; Duesberg et al., 2000].

Spontaneous Progression of Malignancy

Autocatalyzed karyotype evolution and selection of variants based on aggressiveness also predicts the spontaneous progression of malignancy from docile cancers in situ to invasive and metastatic variants (Table I) [Foulds, 1965; Braun, 1969; Wolman, 1983; Pitot, 1986; Sandberg, 1990].

Age Bias of Cancer

The exceedingly slow kinetics from a spontaneous or carcinogen-initiated aneuploidy to a neoplastic one via autocatalytic karyotype evolution, and the non-heritability of aneuploidy [Muller, 1927; Hook, 1985; Hassold, 1986] also explain the 1,000-fold age bias of cancer (Table I) [Armitage and Doll, 1954; Cairns, 1978; Lodish et al., 1999]. Since aneuploidy is not heritable, because the product would either be non-viable or it would be a new species of its own (see below, Chromosome number variation as. . .), it must be acquired somatically. (Rare congenital aneuploidies, such as Down syndrome (see below, Aneuploidy causing biologically abnormal. . .), are acquired during meiosis [Sandberg, 1990].) Such somatically acquired aneuploidy would then take many years to evolve into a neoplastic one.

By contrast, the gene mutation hypothesis tries to explain the age bias with the hypothesis that multiple mutations have to occur in the same cell (see above, Gene mutation hypothesis. . . item 9.) [Armitage and Doll, 1954; Cairns, 1978; Lodish et al., 1999]. However, in this case cancer should occur in newborns who have inherited an incomplete set of oncogenic mutations, once a final mutation has occurred somatically [Li et al., 1997]. But this is not observed.

Genetic Instability and Phenotypic Heterogeneity

The notorious "genetic instability" of cancer cells (Table I) and the resulting phenotypic heterogeneity would all simply reflect the inherent karyotype instability of aneuploid cells [Duesberg et al., 1998; Rasnick and Duesberg, 1999]. Examples are the spontaneous progression of malignancy from cancers in situ to invasive and metastatic cancers (see below, Table I) [Pitot, 1986; Heppner and Miller, 1998] and likewise, the appearance of lethal karyotypes, owing to the loss of all copies of a chromosome, termed necrosis or recently also apoptosis [Bauer, 1948; Pitot, 1986] (Fig. 1).

Mutation of Cancer Cells to Drug-Resistance and Multidrug-Resistance at Paradoxically High Rates

The rapid generation of drug-resistant cancer cells during chemotherapy has been a challenge to both clinicians and geneticists since the 1960s [Skipper, 1965; Siminovitch, 1976; Harris, 1995]. Numerous efforts to reconcile the rapid generation of drug-resistance among aneuploid cancer cells with conventional gene mutation have failed in view of the paradoxically high rates of mutation. For example, at least one in 10^6 human leukemic cells in vivo is resistant to amethopterin [Skipper, 1965]. Likewise, drug-resistant variants of cancer cells

and aneuploid cell lines appear in vitro at frequencies of 10^{-3} to 10^{-6} [Gartler and Pious, 1966; Breslow and Goldsby, 1969; Coffino and Scharff, 1971]. By contrast, the estimated frequencies with which diploid somatic cells would lose both alleles of recessive drug-resistance genes by spontaneous gene mutation are in the order of 10^{-12} to 10^{-14}, based on a haploid human mutation rate of about 10^{-6} to 10^{-7} [Gartler and Pious, 1996; Breslow and Goldsby, 1969; Vogel and Motulsky, 1986; Harris, 1995]. Indeed, only a few cancer cells have been found to have higher than normal gene mutation rates (see above, Gene mutation hypothesis, popular but unconfirmed, item 9).

However, the paradox can be resolved by the unique ability of aneuploid cells to vary phenotypes by chromosome reassortments instead of gene mutation. Since phenotype alterations by chromosome reassortment is catalyzed by aneuploidy, it occurs at high rates, proportional to the degree of aneuploidy, in aneuploid cells [Lengauer et al., 1997; Duesberg et al., 1998]. By contrast, normal diploid cells lack the ability of phenotype alteration by chromosome reassortment because chromosome non-disjunction in a cell with a spindle apparatus that is balanced by the normal, species-defining karyotype is extremely rare [Harnden et al., 1976; Galloway and Buckton, 1978]. Moreover, it would be a long way from a random primary aneuploidy to one that encodes a drug-resistant phenotype, about as long as from a primary aneuploidy to a cancer cell (Fig. 1). Thus this mechanism of phenotype alteration is unique for aneuploid cells, and explains the notorious, high mutation rates of aneuploid cancer cells and aneuploid cells in culture, ie. the above described genetic instability [Siminovitch, 1976; Pitot, 1986; Harris, 1995].

The hypothesis also predicts multidrug resistance of cancer cells as a consequence of the multigene reassortments that are necessarily associated with chromosome reassortments. Multidrug resistance is observed, ''When cultured cells are exposed to . . . a chemotherapeutic drug, individual clones can be selected that express . . . resistance to multiple drugs that may be structurally and functionally unrelated. Such cross-resistance occurs frequently in cultured cell lines and is termed the multidrug resistance (MDR) phenotype. The MDR phenotype is also encountered in the clinical setting where many human cancers are refractory to multi-agent chemotherapy.'' [Schoenlein, 1993]. By contrast, multidrug resistance is incompatible with conventional gene mutation of one or even a few genes that is selected by only one specific drug.

Independent Progression of Characters, or Foulds' Rules

According to Foulds, the various cancer-specific characters that accumulate in tumor progression (Table I) [Pitot, 1986], are independently, rather than sequentially acquired [Foulds, 1965; Braun, 1969; Pitot, 1986]. This is exactly what is predicted by random karyotype variation and selection (Fig. 1).

Non-Clonal Karyotypes But Clonal Aneuploidy

According to the proposed mechanism, cancers are clonal for aneuploidy (above a threshold), but not for a particular karyotype. The aneuploidy above a threshold is clonal because it causes the cancer. The specific karyotypes of individual cells of a clonal tumor are non-clonal because of variations among neoplastic karyotypes and because neoplastic aneuploidy is masked by non-neoplastic noise generated because aneuploidy is inherently unstable (see above, Stage two: generation of neoplastic karyotypes. . .).

This also explains the recently discovered non-clonality of various hypothetical oncogenes and tumor-suppressor genes [Albino et al., 1984; Konishi et al., 1995; Giaretti et al., 1996; Roy-Burman et al., 1997; Al-Mulla et al., 1998; Heppner and Miller, 1998; Kuwabara et al., 1998; Offner et al., 1999], which is paradoxical in view of the mutation hypothesis (see above, Gene mutation hypothesis. . .). These mutations would have pre-existed in one chromosome of a diploid prospective cancer cell [Fialkow, 1979; Shibata et al., 1993], and would have been lost in some descendent cancer cells as a result of karyotype shuffling.

"Non-Random" Karyotypes

Most cancer researchers have abandoned the aneuploidy hypothesis because no cancer-specific aneuploidy could be found (see Introduction) [Rous, 1959; Bauer, 1963; Braun, 1969; DiPaolo, 1975; Nowell, 1976; Harnden and Taylor, 1979; Cram et al., 1983; Sandberg, 1990; Harris, 1995; Heim and Mitelman, 1995].

Nevertheless recent cytogenetic studies have succeeded to identify some specificity after all [Sandberg, 1990; Gebhart and Liehr, 2000], i.e., "non-random" karyotypes [Heim and Mitelman, 1995]. These can be reconciled with the aneuploidy hypothesis if one considers that cancer results from dedifferentiation of many sorts of differentiated cells by random karyotype variation. In the light of this, one can see that those chromosomes that are involved in the specific differentiation of a prospective cancer cell must be "non-randomly" unbalanced in order to convert it to a cancer cell. Indeed, most cancers retain sufficient differentiation-specific markers to identify their tissue origin, despite aneuploidy [Hauschka, 1961; Braun, 1969; Pitot, 1986].

Why Either Hyper-Triploid or Near-Diploid Karyotypes Are Common in Cancers

The modal chromosome numbers of most common cancers is either hyper-triploid or near diploid [Sandberg,

1990; Rubin et al., 1992; Giaretti, 1994; Lengauer et al., 1997; Cahill et al., 1998; Ghadimi et al., 2000]. This reflects two competing mechanisms of achieving neoplastic properties via aneuploidy: One of these would be to maximize the adaptability of a neoplastic cell to different histogenetic habitats, i.e., the ability to metastasize, by regrouping chromosomes, but at the same time to minimize the risk of losing a vital chromosome. This is best accomplished with the most unstable karyotype, which is the furthest away from diploid and tetra-ploid [Rasnick and Duesberg, 1999], and with the highest number of redundant chromosomes to replace losses, i.e., with a hyper-triploid karyotype. If a vital function of a chromosome is lost, another could take over, or the karyotype could be regrouped to form a new cancer species. The other mechanism would be to retain vital chromosomes via the proven stability of the normal karyotype and to introduce sufficient variation for carcinogenicity by a minor, near-diploid aneuploidy.

Transient Suppression of Malignancy by Cell Fusion

Based on the same kind of experiments, malignancy has been described as either dominant by some or as recessive by others. Originally Barski and Cornefert found that hybrids of malignant and non-malignant mouse cells were malignant [Barski and Cornefert, 1962]. Indeed, the existence of thousands of immortal hybridoma cell lines, which are artificial hybrids of specific normal immune cells with immortal cancer cells, confirm this observation [American Type Culture Collection, 1992; Lewin, 1994].

By contrast, Harris and his collaborators found that some hybrids formed between malignant and normal mouse cells were initially non-malignant, but regained malignancy spontaneously once they "had lost a substantial number of chromosomes relative to what was to be expected from the sum of the two parental chromosome sets" [Harris, 1995]. It was on this basis that recessive tumor suppressor chromosomes and later suppressor genes were postulated, the physical or functional loss of which would cause cancer (Table I) [Harris, 1993].

However, the hypothesis that the cancer phenotype is recessive failed to explain the hybrids that were malignant from the beginning, despite fusion with non-malignant cells [Barski and Cornefert, 1962; Pitot, 1986]. Moreover, despite enormous efforts, no specific suppressor chromosomes, or suppressor genes were found (see above, Gene mutation hypothesis. . .item 3) [Pitot, 1986; Harris, 1993]. Even Harris confirms the defective correlations, "Mutations and deletions in the p53 [tumor suppressor] gene were found to be extremely common in human malignancies; they were found in 40% of mammary carcinomas and in 30% of colorectal carcinomas" [Harris, 1995]. Others have reported that the hypothetical suppressor genes lack the expected phenotype, "Transgenic pedigrees that produce . . . [mutant] p53Ala143 alone, or K-*ras*Val12 and p53Ala143 have no detectable phenotypic abnormalities." [Kim et al., 1993].

But both seemingly contradictory results of cell fusion experiments can be readily reconciled by the aneuploidy hypothesis: Fusion with a non-malignant cell can, but may not, unbalance the neoplastic aneuploidy of a malignant cell. If the neoplastic karyotype is lost as a result of a non-cancerous chromosome combination, a malignant karyotype is likely to re-emerge sooner or later owing to the inherent instability of the aneuploid karyotype, as for example by the loss of chromosomes "relative to what was expected from the sum the two parental chromosome sets." Indeed, Harris points out that the tumors produced by such hybrids appeared "after a very long lag period compared with that given by the [unfused malignant] Ehrlich cells" [Harris, 1993].

Proof of principle that aneuploidy can cause cancer is provided in the following sections.

PROOF OF PRINCIPLE I: CORRELATIVE EVIDENCE FOR ANEUPLOIDY AS CAUSE OF CANCER

Since Hansemann first described ''asymmetric mitoses'' in cancer cells in 1890 [Hansemann, 1890], aneuploidy has been observed in virtually all of the over 5,000 solid human cancers that have been analyzed [Sandberg, 1990; Harris, 1995; Mertens et al., 1997]. The correlations between aneuploidy and solid cancers are so tight that neither one of the two text books of cancer cytogenetics, i.e., Heim and Mitelman's *Cancer Cytogenetics* [Heim and Mitelman, 1995] and Sandberg's *The Chromosomes in Human Cancer and Leukemia* [Sandberg, 1990], lists confirmed examples of solid cancers that are diploid, or euploid. In view of this, Oshimura and Barrett commented that "a better correlation with cell transformation is observed with induction of aneuploidy than of point mutations" [Oshimura and Barrett, 1986]. And the cytogeneticist Atkin asked in 1990, "Are human cancers ever diploid?" [Atkin and Baker, 1990]. The tight correlations have since been confirmed and extended by comparative genomic hybridization, a technique that is particularly sensitive to segmental aneuploidy. According to a recent survey, all "of over 2,400 human solid tumors" analyzed by this technique were aneuploid with regard to either segments of or complete complements of chromosomes [Gebhart and Liehr, 2000].

Nevertheless, there are sporadic reports about "diploid tumors," as for example the recent one by Ghadimi et al. that describes three "diploid colorectal cancer cell

lines" [Ghadimi et al., 2000]. However, Ghadimi et al. also report that "DNA copy number changes were present in all cancer cell lines", i.e., segmental aneuploidy. Moreover, both Ghadimi et al. and the commercial supplier of the lines, the American Type Culture Collection (ATCC), report the following additional evidence for aneuploidy: According to Ghadimi et al., the line SW48 has one extra chromosome and three marker chromosomes. And ATTC reports that SW48 is trisomic for chromosome 7 and has two marker chromosomes of unknown origin. The chromosome distribution of the line ranges from 38 to 50 with a modal chromosome number of 47. Ghadimi et al. report that the line DLD 1 has 3 marker chromosomes, and the ATCC reports a chromosome of unknown origin instead of the normal chromosome 2, and that the chromosome distribution of DLD 1 ranges from 40 to 51. The HCT 116 line contains three marker chromosomes according to Ghadimi et al., and according to the ATTC its chromosome distribution ranges from 43 to 47 with a modal number of 45.

By contrast, the chromosome number distribution of normal diploid cells is narrowly censored around the species-specific chromosome number [Hauschka, 1961; White, 1978]. It follows that none of the reportedly "diploid tumor" cells is truly diploid.

The only apparent exceptions are the diploid tumors caused by the dominant oncogenes of retroviruses [Mitelman, 1974; Duesberg, 1987]. However, retroviral oncogenes can generate functional aneuploidy in transformed cells by increasing the expression of thousands of genes and simultaneously by decreasing the expression of others (see below) [Groudine and Weintraub, 1980].

Thus, aneuploidy meets the first of Koch's postulates, i.e., a perfect correlation, as a cause of cancer.

PROOF OF PRINCIPLE II: FUNCTIONAL EVIDENCE FOR ANEUPLOIDY AS CAUSE OF CANCER

In the following, we describe biochemical and biological evidence that provides functional proof of principle that aneuploidy may cause cancer.

Carcinogens Cause Aneuploidy

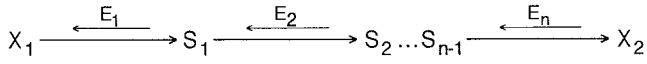
Once more, Boveri was probably the first to point out that carcinogens function by causing aneuploidy, "If I survey reports about the etiology of carcinoma and the many suggestions of physical and chemical insults, and if I consider on the other hand that pressure, shaking, narcotics, and abnormal temperatures are precisely the agents with whose help we may produce multipolar mitoses in young eggs, then it appears possible to me that we have before us the entire causal sequence of certain tumors" [Boveri, 1902]. In 1914, Boveri supplemented his list of aneuploidogens with carcinogenic potential by X rays, radium, quinine, paraffin, chloralhydrate, morphine, nicotine "and probaly many others" [Boveri, 1914]. The search for aneuploidogenicity or "aneugenicity" of carcinogens was only continued over 70 years later by a few cancer researchers, for example Oshimura and Barrett [Oshimura and Barrett, 1986].

However, most recent evidence for aneugenicity of carcinogens was collected not by cancer researchers, but by other biologists investigating the causes of congenital aneuploidy-diseases, infertility, aging, and aneugenicity of environmental and industrial chemicals and of radiation. Their data collectively show that probably all chemical carcinogens, both genotoxic and non-genotoxic ones, can function as aneugens or physically altering either the chromosomes or the spindle apparatus (see, above, Stage one: generation of aneuploidy) [Natarajan et al., 1984; Liang and Brinkley, 1985; Cimino et al., 1986; Galloway and Ivett, 1986; Jensen and Thilly, 1986; Oenfelt, 1986; Parry and Sors, 1993; Parry et al., 1996; Aardema et al., 1998; Duesberg et al., 2000].

Beginning with the demonstration that X-rays eliminate chromosomes from Ascaris embryos by Boveri in 1909 [Wolf, 1974], and from Drosophila by Mavor in 1921 [Mavor, 1921], X-, α-, and UV radiation have been found to cause aneuploidy in animal and human cells [Bauer, 1939; Borek et al., 1977; Borek, 1982; Levy et al., 1983; Kadhim et al., 1992; Harris, 1995; Trott et al., 1995]. Even Muller, who first proposed that X-rays cause cancer by gene mutation, pointed out in his 1927 article, that the "truly mutational" effects of X-rays are "not to be confused with the well known effects of X-rays upon the distribution of chromosomes, expressed by non-disjunction, non-inherited crossover modifications, etc." [Muller, 1927]. But like most other geneticists and cancer researchers since Morgan, Muller disregarded aneuploidy as a cause of cancer (see above, Mutation hypothesis takes over. . .).

In addition to causing aneuploidy by fragmenting chromosomes, radiation may also cause aneuploidy by targeting the spindle apparatus. This view is directly supported by recent evidence for "extranuclear targets" of cellular mutation including the loss of chromosomes by α-radiation [Wu et al., 1999]. According to Little the yield of cellular mutations is significantly higher than expected per "alpha-particle traversals per nucleus" [Nagasawa and Little, 1999], and "irradiation targeted to the cytoplasm yields a significant increase in the frequency of mutations" [Little, 2000].

Thus both chemical and physical carcinogens can function as aneugens.



Scheme 1.

Biochemical Phenotypes Are Controlled by the Dosage of Cellular Genes

Normal Diploid Cells. The comprehensive biochemical phenotype of a cell is determined by the action and interaction of all of its active genes, i.e., the biochemical flux [Kacser and Burns, 1981; Fell, 1997]. Since the production of gene products is, in a first approximation, proportional to gene dose [Oshimura and Barrett, 1986; Leitch and Bennett, 1997; Hieter and Griffiths, 1999; Matzke et al., 1999; Rasnick and Duesberg, 1999], the biochemical flux of normal cells can be roughly determined from the species-specific pool of genes.

As originally proposed by Kacser and Burns all active genes of a cell have an approximately equal share of the biochemical flux of the cell, because they are all kinetically connected within and even between the distinct biochemical assembly lines of a cell [Kacser and Burns, 1981; Fell, 1997]. Thus, the cell can be viewed as one large assembly line, just like a car factory can be seen as one large assembly line that combines the outputs of numerous component assembly lines that are required for the production of normal cars. At steady state, the biochemical phenotype of a cell that is generated by *n* enzymatic steps can thus be described by Scheme 1 [Rasnick and Duesberg, 1999].

In this scheme X_1 is the "source" (of nutrients) and X_2 is the resulting comprehensive phenotype or "sink", and E_i is the enzyme concentration for the *i*th step in the cellular assembly line [Kacser and Burns, 1981]. Using the fact that at steady state each intermediate flux is equal to the overall flux of a connected system, equation 1 was derived for the overall steady state flux, F, for the production of X_2 according to Scheme 1.

$$F = \frac{X_1 - \dfrac{X_2}{K_1K_2 \ldots K_n}}{\dfrac{K_{m_1}}{V_1} + \dfrac{K_{m_2}}{V_2K_1} + \cdots + \dfrac{K_{m_n}}{V_nK_1K_2 \ldots K_{n-1}}} \quad (1)$$

The K values are equilibrium constants, the K_m values are Michaelis constants, and the V values are maximum rates [Rasnick and Duesberg, 1999].

Equation 1 can be simplified. Since all terms in the numerator of 1 are constants, they can be combined into a single constant term C_n, which represents the environmental and constitutive parameters for the specific system or phenotype being considered. Furthermore, since $V_i = E_i k_{cat(i)}$, all the V_i terms are proportional to their respective enzyme concentrations. Each fraction in the denominator of 1, then, can be replaced by the composite e_i terms, all of which are proportional to enzyme concentration. The e_i terms represent the functions of the *n* gene products contributing to the flux. These modifications result in the simple equation 2 that gives the overall metabolic output or flux for a normal cell composed of *n* individual functions or genes.

$$F = \frac{C_n}{\dfrac{1}{e_1} + \dfrac{1}{e_2} + \cdots + \dfrac{1}{e_n}} \quad (2)$$

Equation 2 can be rearranged to 3, which shows that the reciprocal of the cellular phenotype F multiplied by a constant is the linear combination of the reciprocals of all *n* elemental phenotypes e_i that comprise a cell.

$$\frac{C_n}{F} = \frac{1}{e_1} + \frac{1}{e_2} + \cdots + \frac{1}{e_n} \quad (3)$$

For a system as complex as a diploid cell, the number of gene products necessary to determine its phenotype, *n*, is on the order of tens of thousands. For systems this complex, the $1/e_i$ terms make only small individual contributions and can be approximated by replacing them with $1/\hat{e}$ the mean of all the $1/e_i$ terms. Making this substitution in 3 gives 4, which can be used to describe the phenotype of a normal, diploid cell, F_d, for a given environment.

$$\frac{C_n}{F_d} = \left(\frac{1}{\hat{e}} + \frac{1}{\hat{e}} + \cdots + \frac{1}{\hat{e}}\right)_n = \frac{n}{\hat{e}} \quad (4)$$

Aneuploid Cells. The effects of aneuploidy on the collective biochemical phenotype of a cell can be quantitated, if we determine how the flux of a normal cell is altered in proportion to the dosages of the aneuploid genes [Matzke et al., 1999; Rasnick and Duesberg, 1999]. Therefore, we have recently modified equation 4 to calculate the effects of aneuploidy on the phenotypes of eukaryotic cells, which increases or decreases substantial fractions of the genes, but not all genes, of a cell [Rasnick and Duesberg, 1999]. If only a subset of the n cellular genes is involved, the fluxes in 4 can be partitioned into those that are affected by aneuploidy (m) and those that are not (n-m) to give 5.

$$\frac{C_n}{F_a} = \frac{n-m}{\hat{e}} + \frac{m}{\pi\hat{e}} \quad (5)$$

F_a is the phenotype of a eukaryotic cell resulting from aneuploidy. The number of genes experiencing a change in dosage due to aneuploidy is m. The variable π is the ploidy factor, reflecting the change in the number of gene

copies for m. For example, $\pi = 1.5$ for trisomy of m genes. The difference n-m is the number of genes not experiencing aneuploidy. The relative effect of aneuploidy compared to normal diploid cells can be obtained by dividing 5 by 4 to give 6.

$$\frac{F_d}{F_a} = \frac{n - m + \frac{m}{\pi}}{n} = 1 - \frac{m}{n} + \frac{m}{n\pi} \qquad (6)$$

To further simplify 6, we set the normal, diploid phenotype $F_d = 1$, and replace the quotient m/n with f, which is just the fraction of the cell's gene products experiencing changes in dosage due to aneuploidy relative to the normal cell. These modifications give the dimensionless equation 7, the fundamental equation of the analysis of phenotypes, where F_a is now the relative flux.

$$\frac{1}{F_a} = 1 - \phi + \frac{\phi}{\pi} \qquad (7)$$

The 1- ϕ term represents the fraction of unaffected gene products. The composite term ϕ/π is the fraction, ϕ, of gene products undergoing a π-fold change in expression.

The relation of the biochemical phenotype to the DNA index of aneuploid cells can be estimated by assuming that the production of gene products is proportional to gene dose (see above). Thus, the DNA index equals $1\text{-}\phi + \phi\pi$ [Rasnick and Duesberg, 1999]. The product $\phi\pi$ is a measure of the increase or decrease in the gene products themselves.

A graphical representation of **7**, where the normal diploid phenotype, F_d, is perturbed by varying the ploidy factor π and the genome fraction ϕ to produce an ensemble of aneuploid phenotypes F_a is shown in Figure 2. The variable ϕ defines the shape of the curve as well as the limiting metabolic flux at the plateau for a genome fraction $\phi < 1$. The ploidy factor π determines the specific values of F_a within the limits set by ϕ. It is acknowledged that all ploidy increments are quantal, i.e., additions or deletions of whole, or segments of, chromosomes and thus generate steps rather than a continuous curve. However, since any subset of chromosomes may be aneuploid in a given cell the resulting π values are practically continuous curves.

Figure 2 also shows that for negative aneuploidy, $\pi < 1$, there is a decline in F_a, indicating a loss of function compared to the normal phenotype, and for $\pi > 1$, there is a gain. The slopes are steeper for $\pi < 1$ than for $\pi > 1$, which is consistent with a loss of gene dose being more deleterious than a gain as has been shown for both Drosophila and humans [Lindsley et al., 1972; Sandler and Hecht, 1973]. The shaded areas of both positive and negative F_as indicate aneuploidies that are thought to be past our hypothetical threshold for cancer (see above).

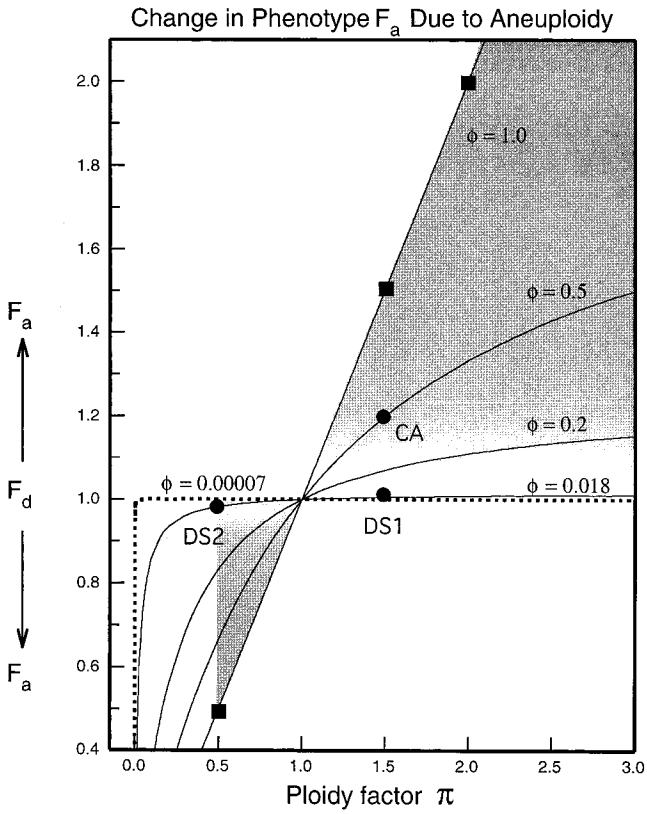


Fig. 2. The consequences of aneuploidy (*closed circles*), polyploidy (*closed squares*), and gene mutation (*dotted line*) on the biochemical phenotype of eukaryotic cells. $F_d = 1$, is the phenotype of a normal, diploid cell (see Equation 4, text). The phenotypes of polyploid cells with integral multimers of the normal chromosome set of a species were obtained by multiplying ê of diploid cells in Equation 4 by 0.5, 1. 5, and 2, respectively (see text). Their phenotypes (F) fall on a straight line with haploids at $\pi = 0.5$, diploids at $\pi = 1$, triploids at $\pi = 1.5$ and tetraploids with $\pi = 2$ differing by equal increments of 0.5 F units. An ensemble of aneuploid phenotypes, F_a, was produced by varying the ploidy factor, π, and the fraction of the normal chromosome set, ϕ, according to Equation 7 (see text). $F_a > 1$ represents positive aneuploidy, corresponding to gain-of-flux relative to the diploid cell, and $F_a < 1$ represents negative aneuploidy, corresponding to loss of biochemical flux. Specific examples of aneuploid phenotypes are Down syndrome with trisomy ($\pi = 1.5$) of chromosome 21 and $\phi = 0.018$ and an $F_a = 1.006$ (DS1), or with monosomy ($\pi = 0.5$) of chromosome 21 and an $F_a = 0.98$ (DS2) (see text). Another example is a typical, near triploid colon cancer (CA) with an average of 69 chromosomes, corresponding to $\phi = 0.5$ and $\pi = 1.5$, and an $F_a = 1.2$ according to Equation 7. The effect on the phenotype of increasing or decreasing the functional dosage of seven genes, within kinetically linked assembly lines, by gene mutations from $\pi = 0$ to 3 is shown by a *dotted line*. The same number of mutant genes is thought to cause colon cancer (see text). It can be seen that the biochemical phenotype described by the dotted line nearly coincides with that of the normal diploid cell and thus is unlikely to generate cancer. The *shaded area* represents phenotypes above the hypothetical threshold for cancer described in the text.

The positions of two specific examples of human aneuploidies, i.e., trisomy or monosomy of chromosome 21 or Down syndrome [Sandler and Hecht, 1973] and a typical, pseudotriploid colon cancer with 69 chromosomes [Sandberg, 1990] are identified in Figure 2. Since chromosome 21 represents about 1.8% of the haploid human genome ($\phi = 0.018$), trisomy ($\pi = 1.5$) only changes the phenotype from $F_d = 1$ to 1.006 (DS1 in Fig. 2) and monosomy changes it to $F_d = 0.98$ (DS2 in Fig. 2). Both F values lie below our hypothetical threshold for cancer (without shaded area of Fig. 2). But the pseudo-triploid colon cancer with 69 chromosomes ($\phi = 0.5$, $\pi = 1.5$) would generate a flux or phenotype of about $F_a = 1.2$, and would thus readily surpass our hypothetical threshold for a cancer causing aneuploidy (i.e., CA, within shaded area of Fig. 2).

Polyploid Cells. Equation 4 can also be used to describe the phenotypes of polyploidization, i.e., all integral multimers of the complete haploid chromosome set of a cell. Since the production of gene products is, in a first approximation, proportional to gene dose [Leitch and Bennett, 1997; Hieter and Griffiths, 1999; Matzke et al., 1999; Rasnick and Duesberg, 1999], haploidization of a diploid cell will half the dose of the ê gene products. Thus, by setting the flux of a normal, diploid cell $F_d = 1$, that of a haploid counterpart comes out as $F = 0.5$, which corresponds to the biochemically rather inert gametes (see squares in Fig. 2) [Hieter and Griffiths, 1999]. According to the same equation, the F values, and thus the biochemical activities, of polyploid cells are increased in proportion to their degrees of polyploidization (see the straight line with squares in Fig. 2). For example, the F value of tetraploid liver cells would be 2, that of 8-ploid and 16-ploid heart muscle cells would be 4 and 8, respectively, and that of 16-ploid and 64-ploid megakaryocytes would be 8 and 32, respectively [Hieter and Griffiths, 1999].

Diploid Cells With Gene Mutations. Equation 7 can also be used to investigate directly the effect of gene mutation on the biochemical flux, i.e., the probability of generating abnormal phenotypes by gene mutations including those proposed to cause cancer. Because virtually all enzymes and functions of cells are integrated into kinetically linked biochemical assembly lines, and work in vivo at only a small fraction of their capacity [Kacser and Burns, 1981], rare positive or activating mutations of enzymes or of hypothetical oncogenes are very effectively buffered in vivo via supplies and demands of un-mutated upstream and downstream enzymes. For example, transfecting 10 to 50 copies of each of the five enzymes of the tryptophan pathway into yeast increases the yield of tryptophan no more than 2–30% [Cornish-Bowden, 1995]. This can be shown by entering the corresponding number of gene mutations into Equation 7.

Figure 2 graphically demonstrates the effects of mutating the dosage of 7 genes, as is postulated for colon carcinogenesis via oncogenes [Kinzler and Vogelstein, 1996], from null to a functional dosage of 6 ($\pi = 3$) by a dotted line. This would correspond to a threefold gene "activation" by mutations. As can be seen in Figure 2, this line almost coincides with the phenotype $F_d = 1$ of a normal diploid cell. Based on Equation 7, the effect on the cellular phenotype of changing the dosage of any seven kinetically linked genes by mutation, the same number of mutations that is thought to cause colon cancer [Kinzler and Vogelstein, 1996], is negligible because only 7 out of about 100,000 human genes [O'Brien et al., 1999] are altered, i.e., $\phi = 0.00007$. In other words, altering the dosage and function of limited numbers of functionally connected genes through mutation, compared with the biochemical consequences of aneuploidy of a sizeable fraction of the genome, is comparable in magnitude to the difference between chemical and nuclear combustion.

However, it may be argued that the mutant genes that cause cancer are "dominant" [Alberts et al., 1994], i.e., independent of others and highly pleiotropic, affecting the function of many others. Possibly some genes that govern differentiation play such roles [Fell, 1997; Bailey, 2000]. But it is unlikely that the currently known, hypothetical cancer genes are dominant, because they do not transform normal diploid cells in culture nor in transgenic animals, which carry these genes in their germ line (see above).

The only known exceptions to date are the dominant genes of viruses that transform or kill cells, without delay and with single hit kinetics, owing to truly dominant viral promoters that increase the functional π values of these genes up to about 1,000 (see above, Proof of principle I:. . .) [Duesberg and Schwartz, 1992; Duesberg, 1995; Hua et al., 1997]. This is exactly the reason why biotechnologists always use viral promoters in synthetic vectors designed to maximize gene expression.

Aneuploidy Causing Biologically Abnormal, Non-Cancerous Phenotypes

Boveri was probably the first to provide proof of principle that experimental aneuploidy generates abnormal phenotypes, e.g., in developing embryos [Boveri, 1902]. And after originally rejecting Boveri's proposal that aneuploidy causes intersexual phenotypes (see Introduction) [Morgan and Bridges, 1919], Morgan et al. [1925] and Harris [1995] later confirmed Boveri's proposal in *Drosophila* (see Harris [1995]).

The discovery that an extra chromosome 21 is the cause of Down's syndrome, was the first demonstration

that aneuploidy can cause abnormal, non-cancerous phenotypes in humans [Lejeune et al., 1959]. Since then several other human birth defects have been attributed to congenital aneuploidy [Sandler and Hecht, 1973; Dellarco et al., 1985; Epstein, 1986; Sandberg, 1990]. More recently, aneuploidy has been confirmed experimentally as a dominant mutator that is independent of gene mutation in other eukaryotes including Drosophila [Lindsley et al., 1972], yeast [Burke et al., 1989; Mayer and Aguilera, 1990; Hartwell, 1992], and plants [Matzke et al., 1999].

Aneuploidy "Immortalizes."

Immortality in vitro or on continuous propagation in experimental animals is one of the hallmarks of cancer (Table I) [Boveri, 1914; Tyzzer, 1916; Pitot, 1986; Lewin, 1994]. Since all normal diploid cells have a finite life span, in vitro immortalization has become one of the most reliable markers of malignant transformation in vitro [Levan and Biesele, 1958; Saksela and Moorhead, 1963; Hayflick, 1965; Trott et al., 1995]. On this basis, and on the grounds that aneuploidy coincides with immortalization, aneuploidy has been proposed to be the cause of immortalization [Levan and Biesele, 1958; Saksela and Moorhead, 1963; Hayflick, 1965]. In the words of Hayflick, "escape from the inevitability of aging by normal cells in vivo and diploid cell strains in vitro is only possible when such cells acquire, respectively, properties of transplantable tumors or heteroploid [aneuploid] cell lines." Surprisingly, in view of their general disregard for aneuploidy in cancer, even modern textbooks link immortalization with aneuploidy [Lewin, 1994; Lodish et al., 1995]. According to Harris, "no permanent cell line with a strictly euploid chromosome constitution has yet been established." And while keeping the door open for gene mutation, Harris concludes that immortalization is "achieved by mechanisms whose visible manifestation is aneuploidy." [Harris, 1995]. Thus aneuploidy is necessary, if not sufficient to negate one of the most fundamental phenotypes of somatic cells: mortality.

Indeed, if immortality could be achieved by gene mutation, we would all be immortal 3 billion years into evolution! In view of this, immortality must be the consequence of karyotype variations steadily compensating for otherwise lethal gene mutations, i.e., must be due to aneuploidy. Obviously, such a mechanism is incompatible with the existence of a stable phylogenetic species, but is the hallmark of the genetically unstable cell lines and cancers (see below).

Phenotypic Abnormalities Proportional to Degree of Aneuploidy

A dose-response relationship is a direct argument for causation. This argument has first been made for the role of aneuploidy in cancer by Winge in 1930, who observed minor aneuploidies in preneoplastic lesions of tar-painted mice and major aneuploidies in cancers [Winge, 1930]. A progression of minor aneuploidies in preneoplastic lesions to major aneuploidies in cancer cells has since been confirmed by others (see above) [Conti et al., 1986; Rubin et al., 1992; Harris, 1995; Duesberg et al., 2000], and has been found to continue with the progession of malignancy [Wolman, 1983; Sandberg, 1990].

Polyploidy Causes Distinct Biological Phenotypes

The phenotypic consequences of polyploidy are yet another example of the power of chromosome number mutation. In this case, nature uses balanced chromosome number variations to increase the biochemical output of cells within a species (Fig. 2) [Hieter and Griffiths, 1999; Matzke et al., 1999]. For example, crop plants derive their highly increased output compared to diploid wild type species to various degrees of polyploidization [Leitch and Bennett, 1997; Matzke et al., 1999]. Likewise, up to 32-ploidy is the basis of the high biochemical output of normal human liver cells, heart cells and megakaryocytes [Hieter and Griffiths, 1999; Matzke et al., 1999], just like a car factory increases its output of (normal) cars by balanced increases of its assembly lines. Thus, phenotype variation by polyploidy lends further functional support to the mutagenic potential of aneuploidy.

Chromosome Number Variation as the Mechanism of Speciation

Chromosome number variation is the basis of nature's most definitive and far-ranging mutation, i.e., speciation. Because a species is defined by a specific number of chromosomes and the gene sequences within [Matthey, 1951; White, 1978; Shapiro, 1983; Yosida, 1983; O'Brien et al., 1999], and not necessarily by a species-specific gene pool [O'Brien et al., 1999], aneuploidy falls within the definition of speciation. By contrast, the number and even the function of genes is not necessarily changed in speciation. For example, among mammalian species the specific number of chromosomes and the sequences of genes within are definitive, whereas the gene pools of all mammals are basically conserved [O'Brien et al., 1999].

It follows that aneuploid cells, above all cancer cells, are by definition species of their own that differ from their diploid predecessors in both the number of chromosomes and the dosage of thousands of genes. Since there are no new genes, and no cancer-specific mutant genes, and no new chromosomes (except occasional hybrid or marker chromosomes) in cancer cells, their specific properties are due primarily to their species-specific gene dosage. However, as a species of their own

aneuploid cancer cells differ from diploid species in that they are parasitic, i.e., unable to function independently. Moreover, because of the inherent instability of aneuploid karyotypes cancer cells are unlikely to retain acquired properties long enough to evolve phylogenetic autonomy.

The view that cancer cells are a species of their own is completely compatible with Hansemann's theory of anaplasia (Table I), which postulates that cancer results from an alteration of the cell's species, "eine Artenveraenderung der Zellen" [Hansemann, 1897]. According to Hansemann, this alteration is not dedifferentiation or transdifferentiation of a normal cell to a cancer cell, "but the cells change their character in every regard morphologically and physiologically to a new species" [Hansemann, 1897]. The pathologist Hauser, a contemporary of Hansemann, described cancer cells as a "new cell-race" [Hauser, 1903; Bauer, 1963; Braun, 1969]. Hauser used this term to account for the multiplicity of characters that set apart cancer cells from normal counterparts. The new species-analogy also confirms the suspicion of the geneticist Whitman, who tried to reconcile cancer with gene mutation in 1919, "The trouble is, indeed, not that the changes observed in cancer cells prove too little, but that they seem rather to prove too much" [Whitman, 1919]. In 1932, the geneticist Haldane reached the same conclusion, "The idea that a chromosomal injury [gene mutation in this context] may alter the character of the descendents of the injured cell is certainly well founded. But known alterations in character which can be referred to chromosomal changes are of a much less fundamental kind than would be required to explain malignancy in tumours. Coordinated growth is just as characteristic, in spite of the chromosomal differences which are presumably associated, for instance, with the different limitations of growth in dwarf and in large pea plants, or the absence of definite limitations of growth in various species of fish. Even when a chromosomal mutation is inconsistent with the ultimate survival of the developing organism growth is still coordinated" [Haldane, 1932].

After World War II, Hauschka equivocated between attributing "the pathological differentiations of oncogeny" either to "differential gene activation" or to "more drastic reorganizing of the somatic karyotype in a mutation-selection sequence analogous to phylogeny" [Hauschka, 1961]. For this latter possibility, Hauschka relies on Julian Huxley's definition of autonomous growths as "equivalent to new biological species" [Huxley, 1956]. According to Huxley, "Once the neoplastic process has crossed the threshold of autonomy, the resultant tumour can be logically regarded as a new biological species, with its own specific type of self-replication and with the capacity for further evolution by the incorporation of suitable mutations. From the angle of biological classification, all tumors, whether of plants or animals, could then be regarded as constituting a special organic phylum or major taxonomic group, with the following characteristics: (1) universal parasitism, but with the parasite always originating from its host; (2) some loss of supracellular organization; (3) lack of limit to proliferation; (4) (a) in most cases each individual tumor is the equivalent of a biological species . . . and each species becomes extinct on the death of its host; (b) . . . in tumors maintained artifically . . . a certain amount of evolutionary divergence may occur in substrains." [Huxley, 1956]. Then, in 1959, Rous confirmed Haldane's view that the gap between cancer cells and their normal predecessors is too big to be explained by known gene mutations, "The cells of the most fatal human cancers are far removed from the normal in character, and almost no growths fill the gap between, much less a graded series of them, such as one might expect were they the outcome of random somatic mutations." [Rous, 1959]. In other words, Rous even pointed out missing links, an evolutionary hallmark regarding the relationships of different species.

The probable answer to the question of missing links in phylogeny and oncogeny is that both are based on the common mechanism of chromosome number variation, which involves coordinate changes of thousands of genes. The concept that aneuploidy defines a species also explains why mutations that cause cancer are "somatic" rather than germinal. Aneuploidy is not heritable because the product would either be non-viable [Muller, 1927; Hook, 1985; Hassold, 1986] or it would be a new species of its own.

By contrast, gene mutations, particularly those that are postulated to cause cancer, can be inherited by transgenic animals [Sinn et al., 1987; Hariharan et al., 1989; Donehower et al., 1992; Kim et al., 1993; Purdie et al., 1994], or congenitally in humans [Knudson, 1985; Haber and Fearon, 1998] without causing cancer, although they may increase the cancer risk.

In sum, there is both correlative and functional proof of principle that aneuploidy is a probable cause of cancer: (1) solid cancers are aneuploid; (2) carcinogens cause aneuploidy; (3) the biochemical phenotypes of cells are severely altered by aneuploidy affecting the dosage of thousands of genes, but virtually un-altered by a few gene mutations such as oncogenes; (4) aneuploidy immortalizes cells; (5) non-cancerous aneuploidy generates abnormal phenotypes in all species tested, e.g., Down syndrome; (6) the degree of non-cancerous and cancerous aneuploidies are proportional to the degrees of abnormality; (7) polyploidy generates very distinct biological phenotypes; (8) variation of the number of chromosomes is the basis of speciation. Thus aneuploidy falls

within the definition of speciation, and cancer is a species of its own.

CONCLUSIONS

By identifying aneuploidy as an autonomous mutator, and by discovering a new, coherent two-stage mechanism from carcinogen to carcinogenesis, we have demonstrated that aneuploidy may be an independent and possibly sufficient cause of cancer.

As a final test we have compared the aneuploidy hypothesis with the mutation hypothesis for their abilities to explain and predict the complex phenotypes of cancer, and the slow kinetics of carcinogenesis mentioned in the text and listed in Table I. It can be seen in Table I that the aneuploidy hypothesis meets all criteria of this test, whereas the mutation hypothesis, in its present form, fails to explain many aspects of cancer. Thus, there is ample proof of principle that aneuploidy is a gene mutation-independent and far-ranging mutation of eukaryotic cells and therefore a plausible cause of cancer.

Further work is needed to determine the role of gene mutation in cancer. Since some transgenic animals carrying mutant genes appear to have a higher than normal cancer risk, mutated hypothetical oncogenes and tumor suppressor genes may play indirect roles in carcinogenesis. We propose that this role is aneuploidization because the cells of animals with such mutations in their germlines have an abnormally high risk of aneuploidy [Bouffler et al., 1995; Fukasawa et al., 1996], and because the tumors that appear in such animals are aneuploid [Hanahan, 1988; Sandgren et al., 1989; Purdie et al., 1994]. Moreover the tumors that appear in humans with heritable cancer disposition genes, as for example the retinoblastoma and Bloom syndrome genes, are also aneuploid [German, 1974; Benedict et al., 1983; Evans, 1985; Duesberg and Schwartz, 1992; Hamel et al., 1993]. Indeed, some of these genes, such as p53, have already been suggested to generate aneuploidization [Bouffler et al., 1995; Fukasawa et al., 1996; Cahill et al., 1999]. Likewise, the genes altered in the generation of the Philadelphia chromosome may increase the risk of aneuploidization that precedes and coincides with the subsequent blast crisis of CML.

If confirmed, the aneuploidy-cancer hypothesis promises to be relevant to (1) cancer prevention and treatment, by leading to the identification and removal of substances from food and drugs that cause aneuploidy, (2) the distinction between benign and preneoplastic lesions based an aneuploidy, and (3) treatment options of cancers based on the degree of aneuploidy.

ACKNOWLEDGMENTS

We thank Bill Brinkley for inviting this analysis, Steven Beckendorf, Ruhong Li, Reinhard Stindl, Richard Strohman (University of California, Berkeley), Andreas Hochhaus and Ruediger Hehlmann (III Medizinische Klinik of the University of Heidelberg at Mannheim), and Agneta Oenfelt (Stockholm University) for critical comments regarding the manuscript, and the journalist Tom Bethell from the *American Spectator* for original questions and references and for engaging proponents of alternative cancer hypotheses. We are especially grateful to Robert Leppo (philanthropist, San Francisco), a foundation that prefers to be anonymous, the Abraham J. and Phyllis Katz Foundation (New York), the Nathan Cummings Foundation (San Francisco), the Forschungsfonds der Fakultaet for Klinische Medizin Mannheim made available to Ruediger Hehlmann, III Medizinische Klinik Mannheim, Germany, and additional donors including Carol J. Wilhelmy (San Mateo), Anthony Robbins Research International (San Diego), Friedel Hafner (Mannheim), and other private sources for support. P.D. is a recipient of a guest-professorship from the Mildred Scheel Stiftung of the Deutsche Krebshilfe, at the III Medizinische Klinik of the university of Heidelberg at Mannheim. The article is dedicated to the memory of Berta Stegmann, a non-scientist (mother-in-law of P.D.) who followed all the politics and much of the science of this article, so much that she never thought she would live to see it published. But she almost did.

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