

Aneuploidy versus gene mutation as cause of cancer

Peter Duesberg^{*,‡}, Reinhard Stindl^{*}, Ruhong Li^{*}, Ruediger Hehlmann[†] and David Rasnick^{*}

^{*}Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA 94720, USA

[†]III Medizinische Klinik Mannheim of the University of Heidelberg at Mannheim, Wiesbadener Str. 7-11, 68305 Mannheim, Germany

The mutagenic ranges of aneuploidy, an abnormal number of chromosomes, and gene mutation are analyzed for their abilities to cause the dominant phenotypes of cancer. In the cell, activating gene mutations are buffered because virtually all gene products are kinetically linked into biochemical assembly lines and thus functionally controlled by upstream and downstream enzymes working at their native rates. Inactivating mutations are also buffered, because (i) they are oversupplied with substrate from unmutated upstream enzymes, (ii) are functionally complemented by a second un-mutated allele, and (iii) because in the cell all enzymes work far below saturation. Therefore, gene mutations are typically recessive and thus unable to generate dominant phenotypes. The argument, that all hypothetically carcinogenic gene mutations are exceptional dominants, is hard to reconcile with their failure to transform cells *in vitro* and in transgenic animals. By contrast, numerical variations of chromosomes, encoding complete biochemical assembly lines, inevitably generate dominant phenotypes, consider the chromosomes that determine sex or Down syndrome. Thus aneuploidy above an as yet poorly defined threshold emerges as the only plausible mutation to cause the dominant phenotypes of cancer. The aneuploidy hypothesis also explains the exceedingly long latent periods, years to decades, between carcinogen and carcinogenesis. Since aneuploidy destabilizes mitosis by unbalancing mitosis proteins, it catalyzes karyotype evolution that eventually generates carcinogenic karyotypes. Three predictions of the hypothesis have been confirmed experimentally, (i) that human cancer cells, reportedly generated by 'three defined genetic elements', are aneuploid, (ii) that an 'immortal' liver cell line, reportedly safe for human transplantation, is aneuploid and thus preneoplastic, (iii) that the high mutation rates of cancer cells to drug and multidrug-resistance are due to chromosome reassortments.

LIKE many others, our article attempts to identify the cause of cancer. Since any valid theory of cancer must be able to explain all relevant facts, we begin our quest with a survey of the many cancer-specific phenotypes as well as the peculiar kinetics of carcinogenesis. The long list of cancer-specific phenotypes shown in Table 1 includes auto-

nomous growth, metastasis, dedifferentiation, irreversibility, immortality, genetic instability, abnormal DNA indices ranging from 0.5 to over 2, abnormal centrosome numbers, and many others. The Table also lists the peculiar properties of carcinogenesis including the exceedingly long latent periods from an 'initiating' carcinogen¹ to carcinogenesis. Indeed it may take years, even decades and many cell generations for cancer to appear, long after the initiated cell and the initiating carcinogen such as X-rays or tobacco smoke have disappeared (Table 1).

In view of some of these facts, it has been proposed a century ago that cancer is caused by 'somatic mutation'²⁻⁶. The mutation hypothesis correctly predicts that cancer is irreversible, and that cancer cells are immortal in culture

Table 1. Hallmarks of cancer and carcinogenesis*

	Predicted by	
	Aneuploidy	Mutation
Cancer		
Anaplasia, autonomous growth, invasiveness, metastasis via neoantigens	Yes	No
Abnormal cellular and nuclear morphology	Yes	No
Abnormal growth rates	Yes	Maybe
Abnormal metabolism and gene expression	Yes	No
Aneuploidy with DNA indices ranging from 0.5 to > 2	Yes	No
Too many and abnormal centrosomes	Yes	No
Irreversible	Yes	Yes
Karyotypic or 'genetic' instability	Yes	No
Immortality <i>in vitro</i> and on transplantation	Yes	No
Spontaneous progression of malignancy	Yes	No
Clonal origin	Yes	Yes
Non-clonal karyotypes and phenotypes, including non-clonal onco- and tumor-suppressor genes	Yes	No
No specific, and no transforming gene mutation	Yes	No
Carcinogenesis		
Non-genotoxic carcinogens	Yes	No
Non-genotoxic tumor promoters	Yes	No
Prenoplastic aneuploidy	Yes	No
Latency of months to decades from carcinogen to cancer	Yes	No
1000-fold age bias of cancer	Yes	No
Suppression of malignancy by fusion with non-malignant cell, and reappearance after spontaneous chromosome loss	Yes	Maybe

*This table is a modification of the one published previously²². Because of editorial limitations for references, the reader is referred for specific references for each item of this Table to Duesberg and Rasnick²², and for additional references to Cairns¹, Pitot⁶⁵, Bauer⁷⁰, Harris²³, and Li *et al.*⁹.

[‡]For correspondence. (e-mail: duesberg@uclink4.berkeley.edu)

or transplantation, which has been first confirmed by historic observations and was subsequently proved experimentally by transplantation *in vivo* and *in vitro*²⁻⁵. The hypothesis also predicts that cancer is typically clonal, i.e. derived from a single mutated cell, as originally proposed by Boveri⁷. This has also since been confirmed based on both somatic and germinal genetic markers^{1,8}.

But despite a century of cancer research, it is still unclear whether the somatic mutation that causes cancer is one that alters the normal number of chromosomes, i.e. causes aneuploidy, or one that alters specific genes^{9,10}. Originally, aneuploidy was proposed to be that mutation, after it was first discovered in cancer cells in 1890 (refs 7, 11). But after the discovery in 1927 that X-rays, a previously known carcinogen, were mutagenic, gene mutation became the most popular cause of cancer to this day^{1,3,12}.

Surprisingly, in view of the enormous efforts in cancer research, it has not been possible in almost a century to prove the gene mutation hypothesis, nor has it been possible to disprove the aneuploidy hypothesis. Yet, the two kinds of mutation have very different ranges and thus make very different, testable predictions. In the following we have first analyzed the mutagenic ranges of the two kinds of mutation to determine which is best qualified to cause cancer, and then we have analyzed how well each hypothesis predicts cancer (Table 1).

Effects of gene- and chromosome-number mutations

Range of gene mutation

Any assessment of the effects of mutations of singular genes in the cell must take into consideration that virtually all gene products within the cell are kinetically linked into biochemical assembly lines^{13,14}.

One consequence of the kinetic linkage of most intracellular functions is that activating mutations are unlikely to generate dominant new phenotypes because they are very effectively buffered by un-mutated, upstream and downstream enzymes from within their biochemical assembly line working at their native rates¹⁵. Even if the activity of a hypothetically 'dominant' oncogene¹⁶ were increased 10-fold, the phenotype of the corresponding assembly line, or signal cascade^{16,17}, would remain unchanged. Just like a single over-productive assembly line worker cannot increase the output of an assembly line in a car factory. Thus activating gene mutations are unlikely to generate dominant phenotypes in the cell.

Loss of function due to inactivating mutations is also very effectively buffered, particularly in eukaryotic cells, at three different levels^{13-15,18,19}: (i) Inactivating mutations are buffered, because in the cell all enzymes work far below saturation; (ii) Un-mutated upstream enzymes

from within the respective biochemical assembly line or cascade compensate in part for loss of function by an oversupply of substrate; (iii) Even null mutations are buffered in eukaryotic cells by functional compensation from the second un-mutated allele.

It is for this reason that inactivation of genes is unlikely to generate dominant phenotypes, except perhaps homozygous null mutations which are not a probable basis for cancer.

It follows that in cells, particularly in eukaryotic cells, the effects of both activating and inactivating gene mutations are almost completely buffered, and thus unable to generate dominant new phenotypes. In other words, virtually all gene mutation is recessive¹⁴, and thus not a likely basis to explain the plethora of dominant, cancer-specific phenotypes, that are never found in diploid, non-cancerous cells (Table 1). According to Cairns, 'one of the problems is that most mutations lead to loss of functions, rather than creation of new function'¹. Other researchers have also pointed out that gene mutations do not typically generate the multiplicity of new phenotypes and functions that are characteristic of cancer (Table 1)^{6,20-22}.

It may be argued, however, that all cancer-specific gene mutations are exceptional dominants because they are somehow biochemically independent. However, this argument is hard to reconcile with the failure of mutant genes from cancer cells to transform diploid cells *in vitro* and in transgenic animals (see below)^{22,23}.

Range of chromosome number mutation

In contrast to gene mutation, there is apparently no buffering against mutation by chromosome number mutation. For example, normal chromosome number variation dominantly determines whether an organism is male or female by the presence or absence of a Y chromosome, and determines a species by a phylogenetically fixed number of chromosomes²⁴.

All abnormal chromosome number variations also generate dominant phenotypes. Indeed Boveri was probably the first to provide proof of principle that chromosome number mutation generates abnormal phenotypes, e.g. in developing sea urchin embryos²⁵. 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 humans²⁶. Since then several other human birth defects have been attributed to congenital aneuploidy^{27,28}. More recently, aneuploidy has been confirmed experimentally as a dominant mutator, that is independent of gene mutation, in other eukaryotes including *Drosophila*²⁹, yeast³⁰, plants³¹, and mice³². Yeast mutations conferring dominant 'growth advantages' have recently all been attributed to specific, aneuploid chromosomes with DNA microarrays³³.

It follows that chromosome number mutation, but not gene mutation, is a probable cause of the many dominant phenotypes of cancer cells (Table 1).

Gene mutation–cancer hypothesis

The most direct test of the merits of a hypothesis is to examine how well it can predict and explain the observation it is meant to solve. Therefore, we have applied this test to the gene mutation hypothesis of cancer, although gene mutation is not a probable cause of the dominant phenotypes of cancer.

Predictions

The mutation hypothesis makes the following predictions:

1. *Carcinogens mutate cellular genes.* But, there is a growing list of non-mutagenic, alias non-genotoxic, carcinogens such as asbestos, Ni²⁺, butter yellow, urethan, mineral oil, hydrazin and many others³⁴ (Table 1).
2. *Cancer-specific gene mutations, i.e. oncogenes and tumor suppressor genes*³⁵. But, enormous efforts in the last 20 years have failed to demonstrate any cancer-specific mutations^{9,22,36} (Table 1).
3. *Cancer genes that ‘dominantly’ transform*^{16,37} *normal diploid cells to tumorigenic cells in vitro, and cause polyclonal cancers in transgenic animals.* But, despite popular claims^{38–40}, there is as yet no functional proof that any cellular mutant genes cause cancer^{9,10,22,23,41}.
4. *Cancer genes that dominantly increase the expression of thousands of genes, and simultaneously decrease the expression of thousands of other genes, as is the case in cancer cells*^{42,43} (Table 1). Such genes should be able to generate abnormal phenotypes *de novo*, even if a given combination of hypothetical cancer genes were insufficient to cause malignant transformation. But, fertile, transgenic mice carrying one or several hypothetical cancer genes are commercially propagated for generations without displaying abnormal, cancer-specific phenotypes (except perhaps an increased risk of clonal cancers)^{22,23,41}.
5. *Transformation coincident with mutation of prospective cancer genes, because the consequences of mutation become manifest within one or a few generations after a cell has reacted with a mutagen.* But, transformation follows reaction with carcinogens only after exceedingly long latent periods of years to decades¹ (Table 1).
6. *Cancer phenotypes are as stable as those of conventional mutations.* But, most cancer phenotypes are notoriously unstable. This phenomenon is termed the ‘genetic instability’ of cancer cells (Table 1).
7. *Cancer cells are diploid with specific gene mutations.* But, probably all solid (non-viral) cancers are aneuploid (see Table 1).

Thus the gene mutation hypothesis cannot predict or explain many of the most critical aspects of cancer,

neither is gene mutation a probable cause of the dominant phenotypes of cancer. Nevertheless, this hypothesis is currently favored by most cancer researchers, virtually to the exclusion of all other hypotheses^{12,16,35,37,44}.

Are oncogenic retroviruses the exception to the rule of no-dominant-cancer genes?

Ever since the discovery of dominant retroviral oncogenes in the seventies⁴⁵, viral oncogenes are perceived as functionally proven models for dominant cellular oncogenes^{23,44,46}. This perception is based entirely on the close sequence relationship between the coding regions of the viral genes with cellular genes. But in the meantime not a single cellular gene with dominant transforming function has been isolated from cancers^{22,23} (see above). This raises the question, ‘Are retroviral oncogenes the exception to the rule of no-dominant-cancer genes’? All things considered, the answer is, ‘probably not’.

The following has been considered in arriving at this answer:

1. Retroviral oncogenes are indeed instant and dominant transforming genes, meeting #2 to #7 of the above predictions of a cancer gene^{9,47}.
2. Retroviral oncogenes have very limited host ranges, limited to a few related species⁴⁸, and limited within a species to only some cell lineages, as for example sarcomas or leukemias⁴⁹.
3. The dominant transforming function of retroviral oncogenes depends entirely on the highly active viral promoters, that are not found in any hypothetical cellular oncogenes²².

In view of this, a retroviral oncogene can be seen as an autonomous mini-chromosome producing a dominant transforming protein. This mini-chromosome is biochemically quasi-independent because it only depends on a relatively small percentage of the large cellular supplies of amino acids and nucleotides, but not on specific precursor proteins or enzymes, to make a transforming protein. The resulting oncogenic effects would be analogous to those of a peptide hormone generating, at least initially, ‘a continuing viral hyperplasia of an extreme type’^{50,50a} made up of diploid cells^{47,50}. Indeed retroviral transformation is initially reversible as demonstrated by temperature-sensitive mutants⁵¹.

However, retroviral transformation also confers a high risk of subsequent aneuploidization, generating irreversible cancer phenotypes^{23,52,53}. Thus retroviral oncogenes are not an exception to the rule of no-dominant-cancer genes.

Aneuploidy–cancer-hypothesis

Intrigued by its enormous mutagenic potential we and others have recently reconsidered aneuploidy as a cause

of cancer^{22,54–56}. This idea derived further strong support from exact correlations between aneuploidy and solid cancer (Table 1). Apparently aneuploidy is so common in solid cancer, that it is an open question now whether there is such a thing as a (non-viral) solid cancer that is diploid^{10,22,47,57,58}.

Since aneuploidy alters the dosage of thousands of structural and regulatory gene products because it multiplies or divides complete biochemical assembly lines, it offers a plausible explanation for the many dominant phenotypes of cancer cells. Indeed a multiplicity of dominant new phenotypes would be expected, if a set of chromosomes carrying assembly lines for structural and regulatory proteins were changed randomly, as in aneuploid cancer cells. This hypothesis would exactly explain the cancer-specific DNA indices, expression profiles of thousands of genes^{42,43}, neoantigens, autonomous growth, nuclear morphology (Table 1) – alterations that are not observed in conventional gene mutation. The results would be analogous to randomly altering assembly lines of a car factory, i.e. chaotic products carrying for example 5 wheels, two engines, no brakes – which would be the equivalent of an aneuploid cancer cell made up of abnormal ratios of un-mutated components. The hypothesis that a cancer cell is made up by an abnormal combination of entirely normal genes and proteins also explains the consistent failures of the gene mutation hypothesis to find a cancer-specific mutations or proteins.

Indeed, aneuploidy was originally proposed to cause cancer over a century ago by Hansemann and Boveri^{7,11} (see above). But the aneuploidy hypothesis has since been abandoned, in favor of gene mutation, for a number of reasons:

1. The first of these was certainly the lack of cancer-specific karyotypes²². According to Rous⁵⁹, discoverer of Rous sarcomas virus in 1959, ‘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’⁵⁹. Thirty-six years later, Harris²³ 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 tumor’²³.
2. The second probable reason to abandon aneuploidy was the lack of theories for how aneuploidy 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’⁶⁰. 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^{23,57,61,62} or are undecided^{63–68}. However, the aneuploidy-is-a-consequence-hypothesis collides with past and recent evidence that ‘aneuploidy precedes

and segregates with chemical (and spontaneous) carcinogenesis’⁶⁹.

3. Prior to our recasting the aneuploidy hypothesis as a cause of cancer (see below), the hypothesis has failed to explain the slow kinetics of carcinogenesis – a problem it shared with all other cancer hypotheses (Table 1)^{1,70,71}.
4. Aneuploidy also occurs in non-cancerous cells, as for example, trisomy #21 in Down’s syndrome, losses or gains of one or a few of the smaller chromosomes in non-cancerous cells of aging animals and humans, and minor or major aneuploidies in preneoplastic lesions^{22,69,72}.
5. Sporadic claims, as yet unconfirmed by current technology, of solid cancers that are diploid^{22,23,57,58,69}. The most recent of these claims, again unconfirmed, has just been registered by Weinberg¹⁰.

In view of this, the challenge was to rethink the aneuploidy hypothesis in an effort to find explanations for:

1. How to reconcile non-clonal karyotypes and heterogeneous phenotypes with clonal cancers (Table 1).
2. How aneuploidy would generate the many abnormal phenotypes of cancer cells (Table 1).
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’s syndrome, cause cancer.
5. How carcinogens could cause aneuploidy without gene mutation.
6. Why cancer-specific phenotypes are genetically unstable, unlike the phenotypes of conventional mutations (Table 1).

A two-stage mechanism of carcinogenesis that meets these challenges, runs as follows (Figure 1).

Mechanism of carcinogenesis via aneuploidy:

(a) Stage one, generation of aneuploidy

Both mutagenic and non-mutagenic, alias genotoxic, chemical carcinogens are proposed to generate aneuploidy by chemically or physically altering one or more of the chromosomes or of the many proteins of the spindle apparatus^{22,54}. 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^{54,73,74}. As originally demonstrated by Boveri⁷, genotoxic physical carcinogens, such as X-rays or α -rays, can generate aneuploidy, by fragmenting chromosomes²². Recent evidence indicates that radiation can also cause aneuploidy by damaging the spindle apparatus⁷⁶.

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 (ref. 77) and an over-expressed kinase SKT15 (ref. 78), and one is thought to be a mitotic checkpoint gene^{79,80}. However, the mutant p53 was found in less than 50% (ref. 79) and the mutated checkpoint gene in only 11% of aneuploid colon cancers⁸⁰. 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'⁷⁸. 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. If aneuploidy were the result of mutation, all cancers caused by non-mutagenic, alias non-genotoxic, carcinogens should be diploid. But this is not observed in experimental cancers²².
2. Many cancers caused by mutagenic carcinogens should be diploid, because aneuploidy and particularly cancer are very rare consequences of mutation, and thus unlikely to coincide in the same cell. Yet, cancers caused by

genotoxic physical and chemical carcinogens are always aneuploid^{69,81-84}. The only possible reconciliation would be that the genotoxic carcinogens cause cancer via aneuploidy which is what we postulate.

3. Mutated 'checkpoint genes' should cause aneuploidy. But transgenic animals carrying mutated p53 in their germlines are fertile (see above) and thus not aneuploid, although the cells of some of these animals are at a relatively high risk of aneuploidy (see below)^{23,85-87}.

4. The polycyclic aromatic hydrocarbons are inefficient and only indirect mutagens, but they are outstanding chemical carcinogens and very effective aneuploidogens^{1,71,88-90}. 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^{69,74}. By contrast, only a few per cent of polycyclic aromatic hydrocarbons are ever converted to potentially mutagenic derivatives by animal cells⁹¹, and even the most effective, direct mutagens, such as N-nitroso compounds and ethyl-sulfonate, mutate at 50% lethal (micromolar) concentrations in a given genetic locus of only 1 in 10^4 to 10^7 animal cells^{90,92,93}. 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 aromatic hydrocarbons is due to non-mutational mechanisms.

5. If aneuploidy is caused by mutation of mitosis genes, the ratio of hypodiploid to hyperdiploid cells would be initially the same. By contrast, aneuploidy generated by physical or chemical fragmentation of chromosomes would initially generate mostly hypodiploid cells. Indeed spontaneous aneuploidy in human cells is between 5 and 10 to 1 in favor of hypodiploidy⁹⁴. The primary ratios may be even higher, because cells with some haploid chromosomes may be non-viable owing to otherwise recessive mutations in essential genes. It follows that most spontaneous aneuploidization is initiated by direct alteration of the spindle or fragmentation of chromosomes rather than by mutation of mitosis genes.

In view of this, direct interference of carcinogens with the many components of the spindle apparatus and with chromosome structure is considered a more likely source of aneuploidy than gene mutation.

(b) Stage two, generation of neoplastic karyotypes by autocatalytic karyotype variation

Aneuploidy is proposed to initiate autocatalytic 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 balance-sensitive spindle apparatus, resulting in abnormal ratios of spindle proteins, centrosomal proteins, and even

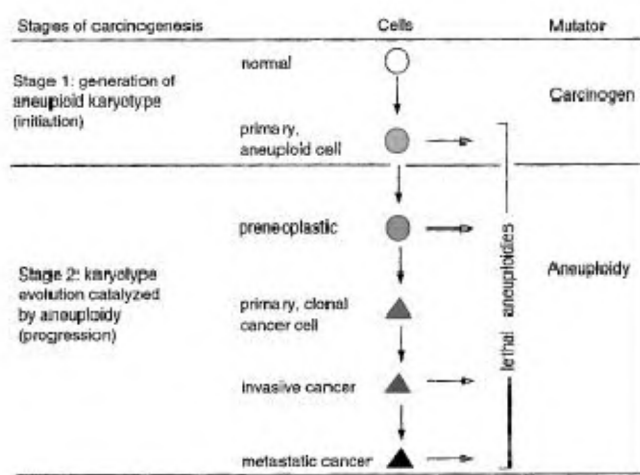


Figure 1. A two-stage model to show how carcinogens may cause cancer via aneuploidy. Stage one, a carcinogen 'initiates'¹ carcinogenesis by generating a random, but typically minor, i.e. non-cancerous, aneuploidy. Stage two, the aneuploid cell generates new karyotypes including lethal, preneoplastic and neoplastic ones autocatalytically, i.e. catalyzed by aneuploidy. Aneuploidy catalyzes chromosome reassortment because it unbalances balance-sensitive mitosis proteins, even centrosome numbers, via the dosage of the corresponding chromosomal templates. Normal and preneoplastic cells are shown as circles. Increasing degrees of aneuploidy are depicted by increasing densities of black. The primary 'clonal'¹ and advanced cancer cells are shown as triangles. The inherent karyotype instability of aneuploid cells explains the non-clonal karyotypes and phenotypes of cancers, i.e. the notorious 'genetic instability' of cancer cells (Table 1). Autocatalytic karyotype evolution is also the common 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. The low probability of generating by autocatalytic karyotype evolution a phenotype, that out-performs normal cells in their habitat, explains the exceedingly long latent periods of carcinogenesis. Further, it explains the previously unresolved, carcinogen-independence from 'initiation'¹ of carcinogenesis by a carcinogen to carcinogenesis occurring long after the initiating carcinogen has disappeared.

abnormal numbers of centrosomes^{55,56,95-97}. 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.

For example, we have shown recently that the risk of gaining or losing a given chromosome per mitosis in a highly aneuploid cancer cell is about 2%, which corresponds to a 46% risk for a highly aneuploid human cell to gain or lose one chromosome per mitosis^{79,95,97}. The more aneuploid the karyotype, the more unstable it will be^{56,95,98}. By contrast, the risk of normal diploid cells of gaining or losing a single chromosome during mitosis is very low, ranging from 0% in human embryos⁹⁹ and adolescents⁹⁴ to 0.4% in adults, which included finding trisomic chromosomes in 0.2% (ref. 72).

Once initiated by a primary, spontaneous or carcinogen-mediated aneuploidy, this process of aneuploidy-catalyzed chromosome reassortment would generate, via discrete chromosomal units, lethal, preneoplastic and eventually neoplastic karyotypes (Figure 1)⁹⁷. The majority of aneuploid cells would be less viable than their normal predecessors or even lethal, analogous to conventional gene mutation (Figure 1).

The low probability to generate by random chromosome reassortments a cell that outgrows a normal differentiated cell in its environment would explain the long latent periods from initiation of aneuploidy to the generation of a cancer cell. The process would be analogous to the evolution of a new species via new karyotypes. Nevertheless the evolution of a new, phylogenetic species would be much more complex, and thus much rarer than the evolution of a cancer cell, because a cancer cell is an entirely parasitic species, whereas a phylogenetic species must be autonomous²².

Thus the aneuploidy hypothesis provides a rational explanation for the long latent periods from initiation of carcinogenesis by a carcinogen to a cancer cell many years later. Indeed Boveri predicted that the probability of generating a cancer cell by randomizing chromosomes would be as low as 'winning in a lottery'⁷.

(c) What is the threshold of aneuploidy for cancer?

It remains to be explained why not all aneuploidies cause cancer (see above). In view of this we have proposed that there must be an as yet poorly defined threshold above which a cancer cell is generated^{22,56}. There is both statistical and logical support for this hypothesis.

Indeed the majority of malignant cancers are highly aneuploid, with modal chromosome numbers between trisomic and tetrasomic suggesting a high threshold of aneuploidy for cancer. But some cancers are near-diploid, suggesting instead a low threshold of aneuploidy^{23,58,100,101}. These near-diploid cancers typically have a 'better prog-

nosis', i.e. are less abnormal, less malignant and better treatable than their highly aneuploid counterparts.

A consistent explanation suggests that liberation of the many buffering controls of normal eukaryotic cells is best achieved by the most dominant mutation that is possible, i.e. near triploid aneuploidy. But sufficient liberation can apparently also be achieved by certain near-diploid aneuploidies that are sufficient to neutralize just the differentiated state of cell, while preserving a maximum of the robustness and viability of the normal karyotype. Further work is required to identify the predicted cancer-specific karyotypes, that are typically masked by chromosome variations that are not essential for oncogenicity.

Proof of principle

The acid test of any hypothesis is its ability to explain observations and predict experimental results. Here we have subjected the aneuploidy hypothesis to three such experimental tests.

Human cancer cells, reportedly caused by 'three defined genetic elements', are predicted to be aneuploid

A high profile publication by Weinberg *et al.* recently reported that three genes 'suffice to convert normal human cells to tumorigenic cells'⁴⁰. However, the study did not investigate the karyotype of the resulting tumorigenic cells, presumably because tumorigenicity was exclusively attributed to the mutant genes.

Since this claim is not compatible with the aneuploidy hypothesis, we have analyzed the karyotypes of the two published tumorigenic cell lines, termed HA1 ER and BJ ELR, originally by direct microscopy⁹, and here again by fluorescent microscopy after hybridization with chromosome-specific colored probes (multicolor FISH). Our new results have confirmed and extended that both lines are highly aneuploid, containing even more aneuploid cells than estimated previously, e.g. HA1 ER 90% and BJ ELR 100%. The chromosome numbers of individual cells of HA1 ER ranged from 45 to 86; and those of BJ ELR from 45 to 91. A representative karyotype of each of the two cell lines, with 89 (BJ ELR) and 83 (HA1 ER) chromosomes is shown in Figure 2. In addition to numerical aneuploidy, BJ ELR included five rearranged chromosomes, and HA1 ER included four rearranged chromosomes (Figure 2).

Thus our hypothesis correctly predicted the aneuploidy of the tumorigenic cells and suggests that aneuploidy, rather than the three transfected genes, were the cause of transformation (see Li *et al.*, for further reasons in support of this suggestion⁹). Indeed a subsequent study by Weinberg *et al.*, again generating human cancer cells with

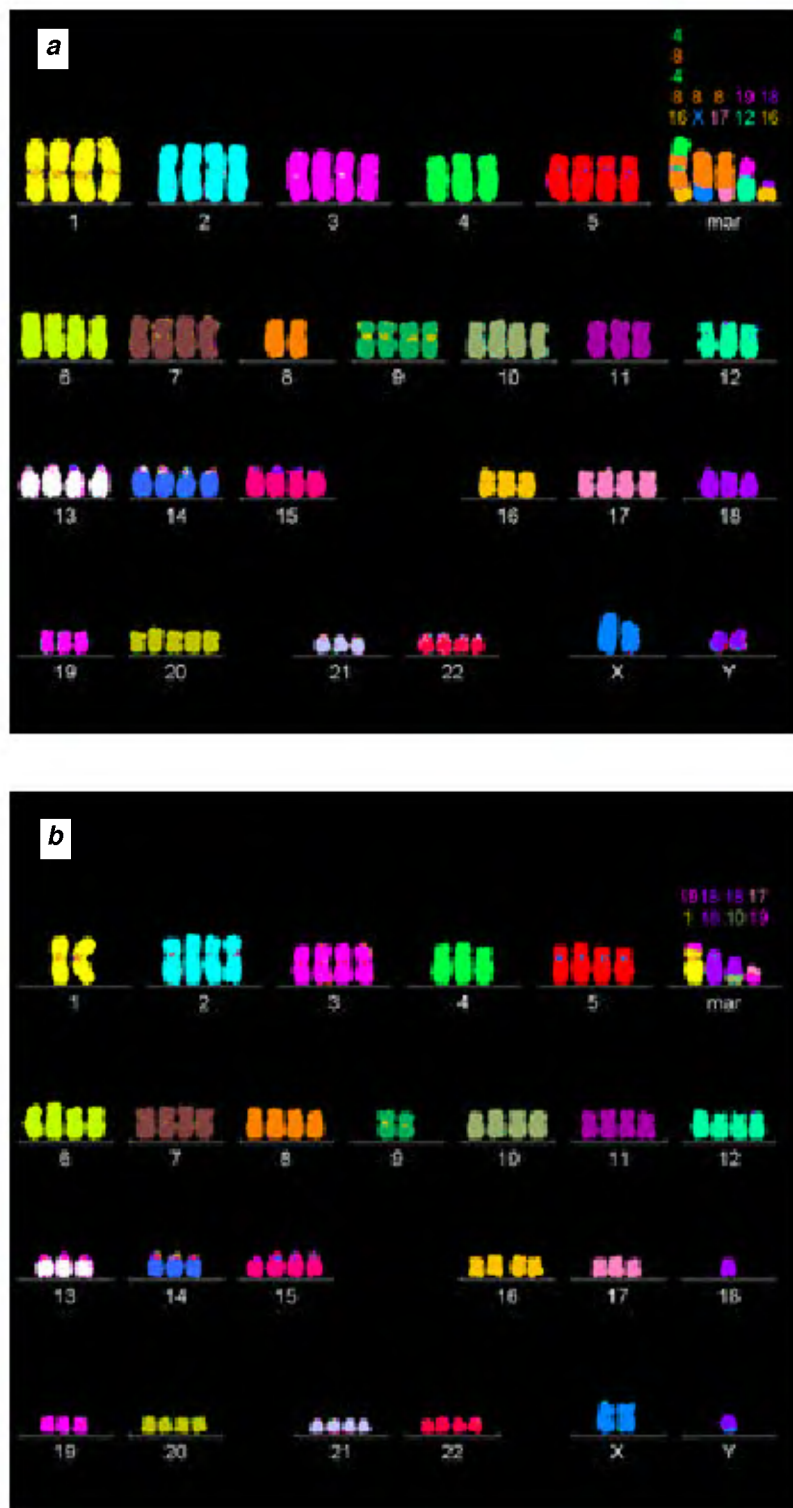


Figure 2. Typical karyotypes of cells from two human tumorigenic cell lines, termed BJ ELR (*a*) and HA1 ER (*b*), reportedly transformed by two ‘oncogenes’ (SV40 T-antigen and H-ras) and one ‘immortalization’ gene (human telomerase). The chromosomes were identified by hybridization *in situ* with chromosome-specific DNA probes carrying multiple fluorescent dye markers, i.e. the mFISH method (multicolor Fluorescence In Situ Hybridization). For this purpose chromosomes were prepared from actively growing cells as described by Fonatsch¹⁰⁷ and kept on a microscope slide at room temperature for 1–3 days prior to hybridization for multicolor FISH analysis. mFISH analysis was performed on an Ikaros4/Isis4 workstation from MetaSystems (www.metasystems.de) connected to a Zeiss Axioplan 2 imaging motorized microscope. The 24Xyte probe cocktail containing chromosome specific probes labeled by FITC, Spectrum Orange, Texas Red, Biotin and DEAC, was obtained from MetaSystems. The probes were denatured, and hybridized on denatured target slides for 3 days as recommended by the supplier. Visualization for biotin-labelled DNA was carried out with Cy5-avidin (B-tect) also according to the 24Xyte-protocol from MetaSystems. (*a*), Karyotype of a typical BJELR fibroblast-derived cell showing 89 chromosomes including 5 rearranged chromosomes. The apparent chromosomal origin of these chromosomes is indicated in the figure. (*b*), Karyotype of a typical HA1ER kidney-derived cell showing 83 chromosomes including 4 rearranged chromosomes.

the same set of three defined genes, has now reported that every transformed cell is aneuploid¹⁰².

An immortalized liver cell line, reportedly safe for human transplantation, is predicted to be aneuploid and thus preneoplastic

According to the aneuploidy hypothesis, cancer-specific phenotypes are acquired via discrete chromosome reassortments (Figure 1). One of these would generate immortality, i.e. the ability of indefinite propagation that is a hallmark of cancer (Table 1). As originally described by Foulds, each of these phenotypes can be acquired independently of the others¹⁰³. It would follow then that immortal cell lines must be aneuploid, having acquired immortality via a chromosome reassortment, and would therefore be preneoplastic if not neoplastic. Indeed most cell lines appear to be tumorigenic if tested under appropriate conditions¹⁰⁴.

By contrast, a recent publication by Fox *et al.* has proposed that an immortal human liver cell line is safe for transplantation to humans, because it appeared not to be tumorigenic in immuno-deficient mice¹⁰⁵. But, in view of

the above our hypothesis would predict that the immortality of the liver cell line is due to aneuploidy and that this aneuploidy would render the cell preneoplastic, and thus unsafe for human transplantation. We have analyzed here the karyotype of the liver cell line, kindly provided by Fox *et al.*, whose karyotype had not been investigated previously by fluorescence microscopy as described above (Stindl and Duesberg, unpublished). It was found that the liver cell line was indeed 100% aneuploid with chromosome numbers ranging from 59 to 63. A characteristic karyotype, including five rearranged chromosomes, is shown in Figure 3.

It follows that our hypothesis correctly predicted that the immortal liver cell line was aneuploid, and thus has neoplastic potential.

High mutation rates of cancer cells to drug and multidrug-resistance via chromosome reassortments that are catalysed by aneuploidy

Ever since the introduction of chemotherapy about 60 years ago, it has been observed that cancer cells mutate to drug and even multidrug-resistance at paradoxically high

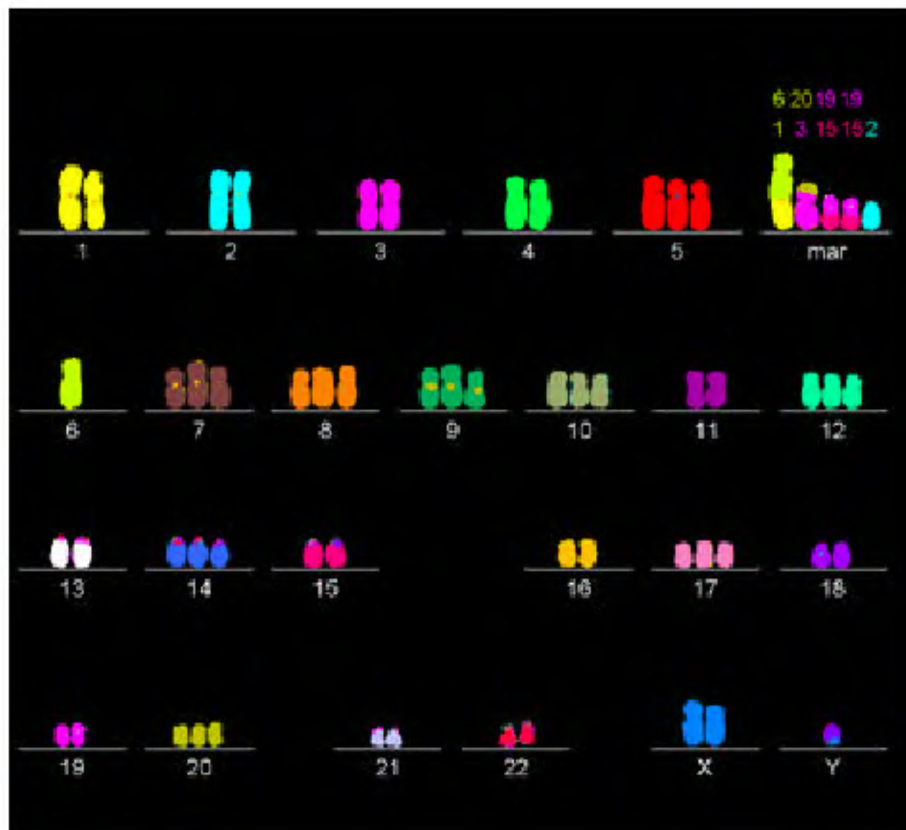


Figure 3. The karyotype of a typical cell from an immortal, human liver cell line, termed NKNT-3, reportedly safe for human transplantation. Preparation and staining of the chromosomes by the mFISH method was as described for Figure 2. The cell shown here contains 60 chromosomes including 5 rearranged chromosomes.

Table 2. Frequency of drug-resistant colonies among cultures of highly aneuploid and diploid Chinese hamster cells exposed to toxic drugs[§]

Drug	Cells	Time until resistant colonies (days)	Colonies per 2 to 4 × 10 ⁶ cells
Puromycin (1 to 20 µg/10 cm dish)	CHE*	–	0 (3 expts)
	B 644**	12, 18, 12	13, > 80, > 50***
	D 313	–, 11, 21	0, 3, 20
	M 853	14, 18	11, > 100*
	SpoT 1	17, 10	2, > 200*
	SpoT 2	10	> 200*
Cytosine arabinoside (0.25 to 10 µg/10 cm dish)	CHE	–	0 (3 expts)
	B 644	14, 15	40, > 200
	D 313	–, 15	0, 3
	M 853	–, 15	0, > 100
	SpoT 1	–, 10	0, > 50
Colcemid (0.1 µg/10 cm dish) (0.2 µg/10 cm dish)	CHE	–	0, 0
	B 644	26, 40	1, > 200
	D 313	–, 26	0, 3
	M 853	26, 40	9, > 100
	CHE	–	0, 0
	B-01col**	16, 9	10/7, 2
	M-01col	16, 9	28/75, 27
Methotrexate (1.25/ 2.5 µg/10 cm dish)	CHE	–	0
	B 644	11	82/17
	D 313	9	135
	M 853	16	91
	SpoT 1	35	164
	SpoT 2	35	68

[§]This table is a modification of precursor published previously by us⁹⁷.

*CHE, diploid Chinese hamster embryo cells.

**B 644, D 313 and M 854 are highly aneuploid, clonal lines of benzpyrene, dimethylbenzanthracene and methylcholanthrene-transformed Chinese hamster cells, B-01col and M-01col are B 644 and M 854 cells resistant to 0.1 µg colcemid, and Spot 1 and SpoT 2 are highly aneuploid spontaneously-transformed Chinese hamster cells.

***Experiments in which in addition to large colonies of cells, multiple small ones survived drug treatment, typically observed when selection was initiated at the lowest concentration listed in the table.

Table 3. Multidrug resistance profiles of Chinese hamster cells originally selected for resistance to one specific drug

Cells resistant to a given drug in µg per 10 cm petri dish	Cross-resistance to unselected drugs in%**
B 654* – puromycin 20	50% at 5 µg puromycin + 5 µg ara-C
M 853 – puromycin 20	20% at 5 µg puromycin + 5 µg ara-C
D 313 – puromycin 20	5% at 10 µg puromycin + 5 µg ara-C
B 654 – ara-C 5	75% at 5 µg ara-C + 5 µg puromycin
D 313 – ara-C 5	50% at 5 µg ara-C + 1 µg puromycin
M 853 – ara-C 5	30% at 5 µg ara-C + 5 µg puromycin
	50% at 5 µg ara-C + 1 µg puromycin
	50% at 5 µg ara-C + 5 µg puromycin
B 654 – ara-C 5	75% at 5 µg ara-C + 0.1 µg colchicine
B 654 – ara-C 5 + puromycin 5	< 5% at 5 µg ara-C + 0.2 µg colchicine
	50% at 5 µg ara-C, 5 µg puromycin + 0.1 µg colchicine
D 313 – puromycin 20	< 5% at 5 µg ara-C, 5 µg puromycin + 0.2 µg colchicine
	90% at 5 µg puromycin + 0.1 µg colchicine
	75% at 5 µg puromycin + 0.2 µg colchicine

*See text, Duesberg *et al.*⁹⁷, or Table 2 for description of cells.

**Cross-resistance of cells, with resistance to previously selected drugs, to further drugs was determined from the percentage of confluence a culture had reached by the time a parallel culture in the absence of further drugs was confluent.

rates, 10^{-3} to 10^{-6} , compared to the extremely low mutation rates of normal diploid cells of about 10^{-12} (refs 97, 106). The aneuploidy–cancer hypothesis suggests that the high mutation rates of cancer cells may reflect chromosome reassortments (Figure 1).

To test this prediction we have compared directly the mutation rates to drug and multidrug resistance of highly aneuploid and normal diploid Chinese hamster cells⁹⁷. As shown in Table 2, all aneuploid cells mutated to drug-resistance at rates ranging from 10^{-4} to 10^{-6} , whereas under the same conditions none of the diploid cells had become drug-resistant. Moreover, the aneuploid drug-resistant cells were also resistant to various other unrelated drugs, e.g. puromycin-resistant cells proved to be 50% resistant to cytosine arabinoside and colcemid (Table 3)⁹⁷. Multidrug-resistance can also be explained by chromosome reassortments, because the ‘genetic unit’ exchanged is a chromosome which carries thousands of genes, and probably many independent biochemical assembly lines including some that generate unselected markers such as multidrug resistance.

Conclusions

In order to distinguish between aneuploidy and gene mutation as hypothetical causes of cancer, we have analyzed the mutagenic ranges of chromosome number- and gene-mutation for their abilities to generate the many dominant phenotypes of cancer cells. This analysis has revealed that only chromosome number mutation has the potential to generate the dominant phenotypes of cancer cells. By contrast, virtually all gene mutations, and specifically those claimed to cause cancer, are recessive, and thus unable to cause the dominant phenotypes of cancer cells.

The aneuploidy hypothesis also provides a plausible explanation for the exceedingly long latent periods from carcinogen treatment to carcinogenesis. According to this hypothesis, a carcinogen initiates carcinogenesis by a preneoplastic aneuploidy. This aneuploidy destabilizes mitosis because it unbalances via the corresponding chromosomes the many dosage-sensitive mitosis proteins, even centrosome numbers. As a result, aneuploidy initiates an autocatalytic karyotype evolution that generates ever new chromosomal variants including rare neoplastic aneuploidy, similar to the evolution of phylogenetic species. By contrast, the mutation hypothesis predicts instant transformation which is never observed (except with oncogenic retroviruses).

Several experimental predictions of the aneuploidy hypothesis have been confirmed experimentally, i.e. aneuploidy in cancer cells reportedly transformed solely by three mutant genes, aneuploidy and thus tumorigenic potential in a cell line reportedly safe for human transplantation, and high mutation rates of cancer cells due to aneuploidy-catalyzed chromosome reassortments. In view

of this we conclude that aneuploidy above a certain as yet poorly defined threshold is sufficient to cause cancer. The hypothesis can be refuted by identifying malignant but euploid cancers, or by generating euploid cancers with cancer-derived mutant genes.

1. Cairns, J., *Cancer: Science and Society*, W. H. Freeman and Company, San Francisco, 1978.
2. Tyzzer, E. E., *J. Cancer Res.*, 1916, **1**, 125–155.
3. Muller, H. J., *Science*, 1927, **66**, 84–87.
4. Bauer, K. H., *Mutationstheorie der Geschwulstentstehung*, Springer, Berlin, 1928.
5. Strong, L. C., *Br. J. Cancer*, 1949, **3**, 97–108.
6. Whitman, R. C., *J. Cancer Res.*, 1919, **4**, 181–202.
7. Boveri, T., *Zur Frage der Entstehung maligner Tumoren*, Gustav Fischer Verlag, Jena, Germany, 1914.
8. Heim, S., Mandahl, N. and Mitelman, F., *Cancer Res.*, 1988, **48**, 5911–5916.
9. Li, R., Sonik, A., Stindl, R., Rasnick, D. and Duesberg, P., *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 3236–3241.
10. Webb, T., *J. Natl. Cancer Inst.*, 2001, **93**, 92–94.
11. Hanseemann, D., *Virchows Arch. Pathol. Anat.*, 1890, **119**, 299–326.
12. Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D. and Darnell, J., *Molecular Cell Biology*, W. H. Freeman and Co., New York and Basingstoke UK, 1999.
13. Kacser, H. and Burns, J. A., *Genetics*, 1981, **97**, 639–666.
14. Fell, D., *Understanding the Control of Metabolism*, Portland Press, London, 1997.
15. Cornish-Bowden, A., in *Biotechnology* (eds Rehm, H.-J. and Reed, G.), VCH, Weinheim, New York, Basel, 1995, vol. 9, pp. 121–136.
16. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J. D., *Molecular Biology of the Cell*, Garland Publishing, Inc., New York, 1994.
17. Hanahan, D. and Weinberg, R. A., *Cell*, 2000, **100**, 57–70.
18. Heinrich, R. and Rapoport, T. A., *Acta Biol. Med. German.*, 1973, **31**, 479–494.
19. Hartman, J. L., Garvik, B. and Hartwell, L., *Science*, 2001, **291**, 1001–1004.
20. Haldane, J. S., in *Growth* (ed. Haldane, J. S., Oliver & Boyd, Edinburgh), pp. 121–131.
21. Rubin, H., *Science*, 1982, **219**, 1170–1171.
22. Duesberg, P. and Rasnick, D., *Cell Motil Cytoskeleton*, 2000, **47**, 81–107.
23. Harris, H., *The Cells of the Body; A History of Somatic Cell Genetics*, Cold Spring Harbor Lab Press, Plainview, NY, 1995.
24. O'Brien, S. et al., *Science*, 1999, **286**, 458–481.
25. Boveri, T., in *Foundations of Experimental Embryology* (eds Willier, B. H. and Oppenheimer, J. M.), Prentice-Hall, Englewood Cliffs, NJ, 1964, pp. 74–97.
26. Lejeune, J., Turpin, R. and Gautier, M., *Ann. Genet.*, 1959, **2**, 41–49.
27. Sandler, L. and Hecht, F., *Am. J. Hum. Genet.*, 1973, **25**, 332–339.
28. Epstein, C., *The Consequences of Chromosome Imbalance: Principles, Mechanisms, and Models*, Cambridge University Press, Cambridge, 1986.
29. Lindsley, D. L. et al., *Genetics*, 1972, **71**, 157–184.
30. Hartwell, L., *Cell*, 1992, **71**, 543–546.
31. Matzke, M. A., Mittelsten-Scheid, O. and Matzke, A. J. M., *Bio-Essays*, 1999, **21**, 761–767.
32. Liu, P., Zhang, H., McLellan, A., Vogel, H. and Bradley, A., *Genetics*, 1998, **150**, 1155–1168.
33. Hughes, T. R. et al., *Nature Genet.*, 2000, **25**, 333–337.
34. International Agency for Research on Cancer, *IARC Monogr.*, 1974, **7**, 125–146.

35. Futreal, P. A., Kasprzyk, A., Birney, E., Mullikin, J. C., Wooster, R. and Stratton, M. R., *Nature*, 2001, **409**, 850–852.
36. Boland, C. R. and Ricciardello, L., *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 14675–14677.
37. Lewin, B., *Genes V*, Oxford University Press, Oxford, 1994.
38. Tabin, C. J. *et al.*, *Nature*, 1982, **300**, 143–149.
39. Reddy, E. P., Reynolds, R. K., Santos, E. and Barbacid, M., *Nature*, 1982, **300**, 149–152.
40. Hahn, W. C., Counter, C. M., Lundberg, A. S., Beijersbergen, R. L., Brooks, M. W. and Weinberg, R. A., *Nature*, 1999, **400**, 464–468.
41. Duesberg, P. H. and Schwartz, J. R., *Prog. Nucleic Acid. Res. Mol. Biol.*, 1992, **43**, 135–204.
42. DeRisi, J. *et al.*, *Nature Genet.*, 1996, **14**, 457–460.
43. Zhang, L. *et al.*, *Science*, 1997, **276**, 1268–1272.
44. Haber, D. A. and Fearon, E. R., *Lancet*, 1998, **351**, SIII–SIII8.
45. Duesberg, P. H., *Cold Spring Harbor Symp. Quant. Biol.*, 1980, **44**, 13–29.
46. Bishop, J. M., *Genes and Dev.*, 1995, **9**, 1300–1315.
47. Duesberg, P., Rasnick, D., Li, R., Winters, L., Rausch, C. and Hehlmann, R., *Anticancer Res.*, 1999, **19**, 4887–4906.
48. Li, R., Zhou, R.-P. and Duesberg, P., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 7522–7527.
49. Tooze, J., *The Molecular Biology of Tumor Viruses*, Cold Spring Harbor, New York, 1973.
50. Hauschka, T. S., *Cancer Res.*, 1961, **21**, 957–981.
- 50a. Bryan, W. R., *J. Natl. Cancer Inst.*, 1960, **24**, 221–251.
51. Martin, G. S., *Nature*, 1970, **227**, 1021–1023.
52. Mitelman, F., Mark, J., Levan, G. and Levan, A., *Science*, 1972, **176**, 1340–1341.
53. Mitelman, F., in *Chromosomes and Cancer* (ed. German, J.), John Wiley and Sons, New York, 1974, pp. 675–693.
54. Li, R., Yerganian, G., Duesberg, P., Kraemer, A., Willer, A., Rausch, C. and Hehlmann, R., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 14506–14511.
55. Brinkley, B. R. and Goepfert, T. M., *Cell. Motil. Cytoskeleton*, 1998, **41**, 281–288.
56. Rasnick, D. and Duesberg, P., *Biochem. J.*, 1999, **340**, 621–630.
57. Heim, S. and Mitelman, F., *Cancer Cytogenetics*, Wiley-Liss, New York, 1995.
58. Sandberg, A. A., *The Chromosomes in Human Cancer and Leukemia*, Elsevier Science Publishing, New York, 1990.
59. Rous, P., *Nature*, 1959, **183**, 1357–1361.
60. Orr-Weaver, T. L. and Weinberg, R. A., *Nature*, 1998, **392**, 223–224.
61. Johansson, B., Mertens, F. and Mitelman, F., *Genes Chromosome Cancer*, 1996, **16**, 155–163.
62. Mitelman, F., Mertens, F. and Johansson, B., *Nature Genet. (Supplement)*, 1997, **15**, S417–S474.
63. Oenfelt, A., *Mutat. Res.*, 1986, **168**, 249–300.
64. Oshimura, M. and Barrett, J. C., *Environ. Mutagen.*, 1986, **8**, 129–159.
65. Pitot, H. C., *Fundamentals of Oncology*, Marcel Dekker, New York, 1986.
66. Tucker, J. D. and Preston, R. J., *Mutat. Res.*, 1996, **365**, 147–159.
67. Hieter, P. and Griffiths, T., *Science*, 1999, **285**, 210–211.
68. Galitski, T., Saldanha, A. J., Styles, C. A., Lander, E. S. and Fink, G. R., *Science*, 1999, **285**, 251–254.
69. Duesberg, P. *et al.*, *Cancer Genet. Cytogenet.*, 2000, **119**, 83–93.
70. Bauer, H. K., *Das Krebsproblem*, Springer, Berlin, 1948.
71. Berenblum, I. and Shubik, P., *Br. J. Cancer*, 1949, **3**, 109–118.
72. Petersson, H. and Mitelman, F., *Hereditas*, 1985, **102**, 33–38.
73. Jensen, K. G., Oenfelt, A., Poulsen, H. E., Doehmer, J. and Loft, S., *Carcinogenesis*, 1993, **14**, 2115–2118.
74. Matsuoka, A., Ozaki, M., Takeshita, K., Sakamoto, H., Glatt, H., Hayashi, M. and Sofuni, T., *Mutagenesis*, 1997, **12**, 365–372.
75. Wolf, U., in *Chromosomes and Cancer* (ed. German, J.), John Wiley, New York, 1974, pp. 3–20.
76. Little, J. B., *Carcinogenesis*, 2000, **21**, 397–404.
77. Fukasawa, K., Choi, T., Kuriyama, R., Rulong, S. and Vande Woude, G. F., *Science*, 1996, **271**, 1744–1747.
78. Zhou, H. *et al.*, *Nature Genet.*, 1998, **20**, 189–193.
79. Lengauer, C., Kinzler, K. W. and Vogelstein, B., *Nature*, 1997, **386**, 623–627.
80. Cahill, D. P. *et al.*, *Nature*, 1998, **392**, 300–303.
81. Sudilovsky, O. and Hei, T. K., *Cancer Lett.*, 1991, **56**, 131–135.
82. Connell, J. R., *Br. J. Cancer*, 1984, **50**, 167–177.
83. Kirkland, D. J. and Venitt, S., *Br. J. Cancer*, 1976, **34**, 145–152.
84. Borek, C., Pain, C. and Mason, H., *Nature*, 1977, **266**, 452–454.
85. Bouffler, S. D., Kemp, C. J., Balmain, A. and Cox, R., *Cancer Res.*, 1995, **55**, 3883–3889.
86. Purdie, C. A. *et al.*, *Oncogene*, 1994, **9**, 603–609.
87. Kim, S. H., Roth, K. A., Moser, A. R. and Gordon, J. I., *J. Cell. Biol.*, 1993, **123**, 877–893.
88. Scribner, J. D. and Suess, R., *Int. Rev. Exp. Pathol.*, 1978, **18**, 137–187.
89. Lijinsky, W., *Env. Mol. Mutagenesis*, 1989, **14**, 78–84.
90. Bradley, M. O., Bhuyan, B., Francis, M. C., Langenbach, R., Peterson, A. and Huberman, E., *Mutat. Res.*, 1981, **87**, 81–142.
91. Richards, J. and Nandi, S., *J. Natl. Cancer Inst.*, 1978, **61**, 773–777.
92. Orkin, S. H. and Littlefield, J. W., *Exp. Cell Res.*, 1971, **69**, 174–180.
93. Terzi, M., *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 5027–5031.
94. Galloway, S. M. and Buckton, K. E., *Cytogenet. Cell. Genet.*, 1978, **20**, 78–96.
95. Duesberg, P., Rausch, C., Rasnick, D. and Hehlmann, R., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 13692–13697.
96. Duesberg, P., *Science*, 1999, **284**, 2091–2092.
97. Duesberg, P., Stindl, R. and Hehlmann, R., *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 14295–14300.
98. Furuya, T. *et al.*, *Clin. Cancer Res.*, 2000, **6**, 2815–2820.
99. Harnden, D. G., Benn, P. A., Oxford, J. M., Taylor, A. M. R. and Webb, T. P., *Somatic Cell Genet.*, 1976, **2**, 55–62.
100. Atkin, N. B., in *Chromosomes and Cancer* (ed. German, J.), John Wiley and Sons, New York, 1974, pp. 375–422.
101. Bocking, A. and Chatelain, R., *Anal. Quant. Cytol. Histol.*, 1989, **11**, 177–186.
102. Elenbaas, B. *et al.*, *Genes. Dev.*, 2001, **15**, 50–65.
103. Foulds, L., *Cancer Res.*, 1965, **25**, 1339–1347.
104. McAllister, R. M. and Corriell, L. L., *Cancer Res.*, 1959, **19**, 1040–1044.
105. Kobayashi, N. *et al.*, *Science*, 2000, **287**, 1258–1262.
106. Vogel, F. and Motulsky, A. G., *Human Genetics: Problems and Approaches*, Springer Verlag, Berlin, 1986.
107. Fonatsch, C., Schaadt, M., Kirchner, H. and Diehl, V., *Int. J. Cancer*, 1980, **26**, 749–756.

ACKNOWLEDGEMENTS. We thank Robert Leppo (philanthropist, San Francisco), an American foundation that prefers to be anonymous, the Abraham J. and Phyllis Katz Foundation (New York), other private sources, and the Forschungsfonds der Fakultät für Klinische Medizin Mannheim for support. Robert Leppo is specially thanked for the Zeiss Axioplan imaging microscope from Metasystems for fluorescence microscopy. R.S. thanks Robert Leppo for a research fellowship, and P.D. thanks the Mildred Scheel Stiftung of the Deutsche Krebshilfe for a guest professorship at the III Medizinische Klinik of the University of Heidelberg at Mannheim covering 18 months.