

# The somatic mutation theory of cancer: growing problems with the paradigm?

Ana M. Soto\* and Carlos Sonnenschein

## Summary

The somatic mutation theory has been the prevailing paradigm in cancer research for the last 50 years. Its premises are: (1) cancer is derived from a single somatic cell that has accumulated multiple DNA mutations, (2) the default state of cell proliferation in metazoa is quiescence, and (3) cancer is a disease of cell proliferation caused by mutations in genes that control proliferation and the cell cycle. From this compelling simplicity, an increasingly complicated picture has emerged as more than 100 oncogenes and 30 tumor suppressor genes have been identified. To accommodate this complexity, additional ad hoc explanations have been postulated. After a critical review of the data gathered from this perspective, an alternative research program has been proposed. It is based on the tissue organization field theory, the premises of which are that carcinogenesis represents a problem of tissue organization, comparable to organogenesis, and that proliferation is the default state of all cells. The merits of these competing theories are evaluated herein. *BioEssays* 26:1097–1107, 2004. © 2004 Wiley Periodicals, Inc.

## Introduction

The somatic mutation theory of carcinogenesis (SMT) deals with sporadic cancers, which represent over 95% of all cancers. The SMT has been the prevailing paradigm in cancer research for the last 50 years.<sup>(1–3)</sup> Its main premise claims that cancer is derived from a single somatic cell that has accumulated multiple DNA mutations over time. This implies that cancers are monoclonal, i.e., they are all derived from a single faulty, mutated cell.<sup>(4)</sup> A second implicit premise is that in the absence of regulatory stimuli, metazoan cells in situ are proliferatively quiescent.<sup>(5)</sup> In other words, the default state of cell proliferation in multicellular organisms is quiescence (See Ref. 6, pp 1–13). A third premise of this theory considers that cancer is a disease of cell proliferation and that

cancer-causing mutations occur in genes that control cell proliferation and/or the cell cycle.<sup>(7,8)</sup>

On what bases have research programs favored the SMT as the main theory of carcinogenesis? First, a sizable number of carcinogenic chemicals were found to be mutagenic. Second, specific genes in so-called tumor viruses (called “transforming” genes) enabled such phenomena as in vitro transformation and the development of tumors at the injection site in some animal models. Next was the discovery that these transforming genes, or oncogenes, were homologous to genes present in non-infected cells. This shifted the search for exogenous genetic causes to endogenous ones and brought the role of DNA mutations back to prominence, now as cellular oncogenes, or proto-oncogenes. The major event in this unifying process was probably data showing that DNA fragments from chemically transformed cells were in turn able to transform recipient cells.<sup>(9)</sup> Finally, the DNA sequences involved were identified as mutated versions of the endogenous, “normal” cellular genes. A series of observations relating these oncogenes to growth factor receptors and to signal transduction pathways bolstered this updated version of the SMT. The implications of these findings were 1) that the products of the mutated oncogenes were activated and 2) that their activation led to increased cell proliferation.

Thus, oncogenes were considered gain-of-function mutations that led the cells harboring these mutants to enhanced proliferation. This latter concept strengthened the research program on signal transduction and, consequently, resulted in a staggering contribution of knowledge in the biochemistry of cellular processes.

Meanwhile, the study of familial, hereditary cancers (about 5% of all human cancers) revealed that the DNA defects transmitted along the germline were due to deletions in specific genes. Unlike the oncogenes, these deletions implied a loss-of-function. The first of these anti-oncogenes (later dubbed “tumor suppressor” genes) was the retinoblastoma gene (Rb), which was soon implicated in the regulation of cell division. Thus, mutations affecting cell cycle regulatory genes became a major cancer research topic.

The initial appeal of the oncogene theory was its simplicity, an assumption later challenged by the increasingly complicated picture that emerged after two decades of intensive research. To date, more than 100 oncogenes and more than

Tufts University School of Medicine, Department of Anatomy and Cellular Biology, Boston, MA

\*Correspondence to: Dr. Ana M. Soto, Tufts University School of Medicine, Department of Anatomy and Cellular Biology, 136 Harrison Ave, Boston, MA 02111. E-mail: ana.soto@tufts.edu

DOI 10.1002/bies.20087

Published online in Wiley InterScience (www.interscience.wiley.com).

30 tumor suppressor genes have been identified. As summarized in a recent review by Hahn and Weinberg: “For those who believe in the simplification and rationalization of the cancer process, the actual course of research on the molecular basis of cancer has been largely disappointing. Rather than revealing a small number of genetic and biochemical determinants operating within cancer cells, molecular analyses of human cancers have revealed a bewilderingly complex array of such factors.”<sup>(10)</sup> To overcome these shortcomings they proposed searching for unifying rules governing the behavior of cancer cells, such as, “[the abilities] to generate their own mitogenic signals, to resist exogenous growth-inhibitory signals, to evade apoptosis, to proliferate without limits (i.e., to undergo immortalization), to acquire vasculature (i.e., to undergo angiogenesis), and in more advanced cancers, to invade and metastasize.”<sup>(10)</sup> Thus, additional research has been proposed albeit within the same paradigm that cancer is a phenomenon explicable at the cellular level of biological organization.

In this article, we will critically review the data collected under the somatic mutation paradigm to explain sporadic cancers and then offer an alternative research program centered on the premises that carcinogenesis represents a problem of tissue organization and that proliferation is the default state of all cells.

### What is a neoplasm?

Literally, neoplasm means new growth. Pathologists have tried to define neoplasms for a century. Their definitions were unsatisfactory since properties attributed to “cancer cells” were also present in normal cells. Because tumor size increases with time, researchers have considered that the underlying cause must have been either excessive or autonomous cell proliferation.<sup>(11)</sup> However, in addition to the accumulation of cells, the hallmark of neoplasms is altered tissue organization.<sup>(12)</sup> Pathologists examine tissue samples using light microscopes to unambiguously diagnose neoplasms. For the most part, neoplasms retain the distinctive structures that characterize the organ of origin. Like normal organs, neoplasms also contain a parenchyma (the distinctive cell type of an organ) and a supporting tissue or stroma. For their normal development and function, tissues require a normal architecture where parenchymal and stromal constituents operate in a coordinated way through reciprocal interactions. A principal aim of cancer research is to elucidate the mechanisms by which neoplasms arise. However, as Boveri already remarked in 1914, a major problem in the study of carcinogenesis is that it is impossible to identify a neoplasm “*in statu nascendi*”.<sup>(13)</sup> Consequently, researchers postulate hypothetical narratives of what may have happened in the transition from normalcy to cancer.

Alternative theories of carcinogenesis were proposed based on competing premises. Some centered at the cellular level of biological organization and viewed cancer as a

problem of cell proliferation or cell differentiation. Others, locked on the tissue level of biological organization, saw cancer as a problem akin to histogenesis.<sup>(14)</sup> Among many of the former, gross chromosome alterations and somatic mutations observed in advanced neoplasms were considered to be the causes of carcinogenesis.<sup>(15)</sup> Others interpreted these alterations to be just epiphenomena and considered carcinogenesis as an epigenetic process<sup>(16–18)</sup> (See Ref. 6, pp 99–111). These varied theories of carcinogenesis coexisted for most of the twentieth century. The methodological emphasis on molecular biological approaches initiated in the 1950s and 60s, plus the discovery of oncogenes in the 1970s, shifted this balance toward the acceptance of the SMT as the mainstream narrative of how neoplasms develop.

### The somatic mutation theory

#### *Internal inconsistencies and difficulties*

From the SMT perspective, neoplasia is a cell-centered problem and, thus, the aim of cancer research was to uncover how a normal cell becomes a cancer cell. When Boveri introduced the first version of the SMT in 1914, it was believed that, in order to change the phenotype of a cell, its genotype had to be changed. Boveri assumed that cellular differentiation during embryogenesis was due to the unequal segregation of genetic material during cell division, a concept that was later abandoned because of the demonstrated genomic equivalence among somatic cells in adult organisms. However, the former concept was retained within the SMT due to (1) the existence of neoplasms transmitted by the germline (we will address this phenomenon below as “inborn errors of development”) and (2) the observation that animals exposed to mutagens often developed neoplasms.

In the 1960s, genes (hitherto considered abstract, operational entities) were finally transformed into material, specific DNA sequences.<sup>(19)</sup> Molecular biologists concluded that biology was at last being reduced to chemistry. Consequently, describing chemical alterations in the genetic material became a more appealing approach to carcinogenesis than searching for messy, difficult-to-define interactions among cells and tissues.

The discovery that oncogenes were mutated versions of normal cellular genes led to the conceptualization of the cancer problem as that of gain-of-function mutations in genes that control cell proliferation and the cell cycle. Most of this research was conducted using *in vitro* models, such as primary cultures and established cell lines. Organismic phenomena were purportedly reduced to cellular phenomena. Neoplasms were reduced to a transformed cell and carcinogenesis was reduced to enhanced proliferation of cells in a dish. Verification of the tumorigenic potential of “transformed cells” was occasionally done by injecting millions of these “transformed cells” into the subcutaneous tissue of syngeneic animals and nude mice.

Soon after these one-step transformations were reported, amid much optimism that the phenomenon of carcinogenesis could at last be understood, the first critical voices noticed that carcinogenesis in animals, including humans, was a long process and, hence, something was missing in the models.<sup>(20)</sup> For example, infection with the Rous-sarcoma virus resulted in the transformation of chicken cells, an effect attributed to the *sarc* oncogene.<sup>(21)</sup> While the injection of Rous sarcoma viruses into chickens resulted in the integration of the *sarc* oncogene in all tissues, tumors only developed in places where wounds were inflicted.<sup>(22)</sup> In addition, the transformation of mouse fibroblasts by a single oncogene was attributed to the fact that the cells used as a model were not normal<sup>(10)</sup> because normal mouse fibroblasts were not transformed upon transfection with a single oncogene. At least two oncogenes were required.<sup>(23)</sup> In addition, according to Hahn and Weinberg, “attempts to transform primary human cells with combinations of oncogenes failed unless chemical or physical agents or stringent selection for rare immortalized variants was used”.<sup>(10)</sup> This was attributed to a need for multiple additional mutations. If this were the case, then the dominant, gain-of-function effect attributed to the oncogene did not fulfill the original claims.

The study of heritable cancers, however, pointed in another direction. The gene alterations found were mostly deletions and cancer was therefore inherited when the genes were rendered inactive.<sup>(24)</sup> Retinoblastomas appeared to represent this type of tumor. The discovery of the *Rb* pathway allowed an explanation of transformation by means of SV40, a DNA virus that, unlike retroviruses, did not contain oncogenes. The large SV40 antigen interfered with the activity of *Rb*. Later on, it was reported that the small t protein of SV40 was necessary to achieve a tumorigenic state. This protein disturbed the activity of protein phosphatase 2A, which acts on a multitude of substrates. Mutations in subunits of this enzyme have been associated with cancers; however, these mutations have not illuminated the role of this enzyme in carcinogenesis.<sup>(10)</sup>

It was believed that, in the genesis of retinoblastomas in humans, in addition to the germline deletion, a second mutational event in the normal allele was sufficient to determine the neoplastic transformation of the retina (the two-hit hypothesis).<sup>(25)</sup> However, hemizyosity of the *Rb* gene in mice did not predispose animals to the disease, and *Rb*-deficient retinal cells underwent apoptosis in chimeras. Only the inactivation of *Rb* and *p107* resulted in the development of retinoblastomas; yet, not all chimeric retinas in *Rb*<sup>-/-</sup> *p107*<sup>-/-</sup> mice developed tumors. Hence, additional events (mutational or not) appeared to be necessary for tumor development.<sup>(26)</sup> This and other examples of lack of fit led the supporters of the SMT to claim that mice may not be good models for human carcinogenesis after all.<sup>(27)</sup>

Among other familial cancers, colorectal cancer has probably yielded the most support for the SMT. About 15%

of these cancers occur in dominantly inherited patterns. In one of its forms, familial adenomatous polyposis, there is a deletion that, in most cases, results in a C-terminal truncated gene product in one of the two adenomatous polyposis coli (*APC*) genes. This disease results in the development of hundreds or even thousands of polyps between the second and third decade of life. However, inheritance of this mutated gene does not determine whether the carrier will always develop a cancer. For cancer to materialize, according to the SMT, other mutations have to occur. Yet, the same DNA lesion does not result in similar phenotypes. In addition, *APC* mutations are not absolutely required, since 15% of the carcinomas apparently express the full-length *APC* product. The function of the *APC* gene, which is expressed in many tissues, is unknown. Clues to the downstream effects of its inactivation were provided by the proteins that are recognized by the missing sequence in familial adenomatous polyposis. *APC* is expressed in the basolateral aspect of epithelial luminal cells. The C terminus binds to the human homolog of the *Drosophila* tumor suppressor gene discs large (*DLG*)<sup>(28)</sup> and to EB-1, a protein of unknown function.<sup>(29)</sup> The central portion of *APC* binds  $\beta$ -catenin, a protein that has at least two roles.<sup>(30)</sup> One is related to cell-cell adhesion through binding to cadherin and the other is signal transduction (*wnt* pathway). This suggests at least two ways through which *APC* inactivation may affect cellular processes connecting a cell with its surroundings. Rather than pointing directly to the control of cell cycle or cell proliferation, as expected from the tenets of the SMT, they point to the relation of the affected cell with its neighbors, the subject of the competing *tissue organization field theory* of carcinogenesis (TOFT), (see Ref 6, pp 91–143 and narrative below).

Other mutations, such as inactivation of *p53*, the “gate-keeper of the genome”,<sup>(31)</sup> are also frequently observed in colorectal carcinomas. However, patients with germline mutations of *p53* do not develop colorectal carcinomas. Mutations in *RAS* frequently appear during progression of colorectal cancer; nevertheless, *RAS* mutations in the absence of *APC* alterations do not lead to the neoplastic state. Yet these mutations are found in foci of proliferating cells. The problems posed by these findings led Kinzler and Vogelstein to ponder, “. . . it is not simply the accumulation of mutations, but rather it is also their order, that determines the propensity for neoplasia, and that only a subset of the genes which can affect cell growth can actually initiate the neoplastic process”.<sup>(32)</sup> However, these cumulative findings are not supportive of the main notion imbedded in the SMT, that is, that the genotype drives the phenotype through alterations of the ability of cells to proliferate.

The question of how many DNA mutations a single normal cell has to withstand to become a cancer cell has been a major concern, since the normal rate of mutations in somatic cells could not account for the number found in neoplasms.<sup>(33)</sup> The study of hereditary non-polyposis colorectal cancer (HNPCC)

that harbors mutations in mismatch repair genes provided an example of hypermutability in colorectal cancer. However, these tumors represent only a small percentage of colorectal cancer; 85% do not show this high mutation rate, but have instead a propensity to show aneuploidy. The absence of aneuploidy in HNPCC (cells are usually diploid in these tumors) challenges the long-held idea that these rearrangements were the consequence of excessive cell divisions. Some HNPCC patients were found to undergo elevated rates of mutations in their phenotypically normal cells, which were explained by a deficit in mismatch repair activity.<sup>(34)</sup> Remarkably, these patients do not have increased rates of cancer in tissues other than the colon. This is consistent with experiments in mice whereby targeted disruption of these genes does not result in high cancer incidence.<sup>(35)</sup>

Proponents of the SMT assume that more research along the current lines will provide data that will reconcile the present paradoxes and reveal general unifying rules. However, the search for those unifying rules appears thwarted by reports claiming that “. . . oncogenes and tumor suppressor genes are important not only for cell proliferation but also for cell fate determination (differentiation, senescence, and apoptosis), their effects often depending on the type of cell in which they are expressed. Thus, overexpression of a given oncogene can enhance growth in one cell type but inhibit growth or induce apoptosis in another”.<sup>(36)</sup> This statement about the context-dependence of oncogene activity contradicts the original concept, namely, that oncogenes are dominant gain-of-function mutants of normal genes that should cause increased cell proliferation.

### *Criticism from without: is the default state of metazoan cells proliferative quiescence?*

As noted above, the second premise adopted by those who favor the SMT is that the default state of cell proliferation in metazoa is quiescence.<sup>(5)</sup> By default state, we mean the state under which cells are found when they are freed from any active control. We consider this an implicit premise because it is seldom acknowledged. Since growth factors are invoked as the levers that putatively stimulate proliferation, quiescence implicitly becomes the default state of these cells (See Ref 6, pp 1–13).

Why should we care about the default state? We have previously addressed this issue both experimentally as well as epistemologically<sup>(37–40)</sup> (see Ref. 6, pp 1–30). From a practical point of view, it matters because in adopting the premise that the default state is quiescence, researchers become committed to favoring the notion of positive control of cell proliferation and, thus, to the search for growth factors. If, instead, researchers adopt the opposite premise, namely that the default state of cells is proliferation, they would introduce the notion of negative control of cell proliferation and would search for inhibitors. But why do we have to choose among

these postulates when dealing with carcinogenesis, or with developmental biology at large, for that matter? The default state of unicellular organisms (both prokaryotes and eukaryotes) and metaphyta is widely accepted to be proliferation. However, not much discussion has been devoted to the default state of cells in metazoa. In fact, it has been assumed all along that the default state of metazoan cells is quiescence. No explanations or data are given to support such a drastic evolutionary change.<sup>(5,41)</sup> Thus, researchers are left to choose between these exclusive postulates.

To resolve this conundrum, the choice need not be arbitrary. From an evolutionary perspective, the generation of multicellular organisms from unicellular eukaryotes involved the conservation of pre-existing levels of organization. The built-in capacity for self-replication by cells within a multicellular organism must have remained unaltered and hence, their default state conserved. The following arguments support this concept.

- (1) Multicellular organisms develop from a single cell—the zygote—that in many species initiates development outside the parental organism, and therefore proliferates without exogenous intervention of putative growth factors.
- (2) There is almost complete homology between the replication machinery of yeast and human cells, suggesting that the process remained constant throughout evolution. Unicellular organisms multiply as long as nutrients are available. With the advent of multicellularity, the coordination of the proliferative activity of each lineage making the different tissues of the organism required the emergence of negative controls that impose a quiescent state upon cells. Once these cells are freed from organismal restraints, they reacquire their default state and proliferate (see Ref. 6, pp 41–77 and 134–143).
- (3) The few studies performed to experimentally address the nature of the default state suggest that proliferation is the default state of metazoan cells.<sup>(37,40,42)</sup>

How could proliferation as the common default state have been ignored? At the beginning of the 20th century, experimentalists resorted in earnest to growing cells in culture conditions to resolve problems raised by the complexity of organisms. From an experimental perspective, evidence that the default state of unicellular organisms and metaphyta is proliferation is not hard to find, since a multitude of unicellular organisms and plant cells can be propagated in a simple nutrient mixture.

The problem posed by cells from metazoa is that, for the most part, they require a complex medium containing macromolecules. Only a few cell lines are easily propagated in defined medium. It may be argued that the difficulty found by early practitioners in getting metazoan cells to propagate in

glass flasks created the misconception that they had to be “stimulated” by adding singly, or in combination, a variety of supplements (i.e. embryo extracts or serum) to the culture medium. Under these operational circumstances, these supplements became generically known as “growth factors”. It should be mentioned that this was the operational definition of any substance that improved the propagation of bacteria as well; some pathogens absolutely required macromolecules in order to propagate. Later, the requirement of these “growth factors” for the propagation of metazoan cells was construed to mean that their default state was quiescence and that serum contained specific signals that induced cell proliferation. The term “growth factors” then acquired a narrow, regulatory meaning. The fact that, in the absence of these macromolecules, the metazoan cells were not quiescent but dead must have been overlooked.

Despite granting that this was the dawn of a novel approach to experimental biology, the patent lack of fit with evolutionary theory should have caught the attention of rigorously trained biologists (see Ref. 6, pp 14–30). Through ontological economy or by application of the parsimony principle (Ockham’s razor), no new entity should be needlessly postulated. Experimentally accumulated evidence supported the notion that cells that did not proliferate much in the intact animal organism, e.g. fibroblasts, did so soon after being transferred to a synthetic, serumless culture medium in a glass or plastic dish. The failure to permanently maintain this dominant proliferative condition may have also mislead researchers and uncommitted observers into favoring the need to add operational growth factors (usually polypeptides) to the culture medium. Recent data on the role of these putative “growth factors” supports the notion that they are either “survival” factors,<sup>(43,44)</sup> or, as in the case of hormones, that they act indirectly by neutralizing the effect of specific inhibitors.<sup>(40)</sup> The literature on genetically engineered knockout mice also shows that the so-called growth factors play important roles in cell fate, migration, and a myriad of developmental processes, but they do not specifically act on the process they were originally supposed to control, i.e., to induce quiescent ( $G_0/G_1$ ) cells to enter the cycle.

For the last two decades, our research program has been based on the premise that the default state of all cells was proliferation.<sup>(39,45,46)</sup> Recently, Henry Harris, a pioneer of somatic cell genetics, concurred with this notion.<sup>(47)</sup> Our reinterpretation of a concept so central to life is not an academic issue. Its implications on the understanding of carcinogenesis cannot be overemphasized, especially in the context of the TOFT (see below).

#### *A cellular approach to differentiation: somatic cell hybrids*

Harris considered carcinogenesis to be a cellular phenomenon, whereby loss-of-function changes in the DNA deter-

mined the cancer phenotype. He observed that the behavior of cancer cells was “normalized” by hybridization with normal cells; this resulted in the lack of tumor formation when the hybrid cells were injected subcutaneously into nude mice. These data are consistent with the existence of suppressor genes and inconsistent with that of oncogenes. In his own words, “As things now stand, it appears that the key cellular events determining malignancy are heritable losses of function, and, in particular, loss of the ability to complete specific patterns of differentiation. This may well be true not only for genetic lesions involving tumor suppressor genes, where the evidence is in some cases compelling, but also for mutated oncogenes. The two great peaks that somatic cell geneticists have long been attempting to scale, cancer and differentiation, seem to have merged into one”.<sup>(48)</sup> In order to explore the mechanisms underlying the suppression of the neoplastic phenotype in hybrids between normal and neoplastic cells, Harris transfected neoplastic cells with cDNAs expressing proteins such as keratins, which are markers of terminal differentiation of keratinocytes, in order to force cells to differentiate and thus behave normally. This particular strategy did not produce the anticipated results.<sup>(49)</sup> Harris also disrupted the pattern of fibronectin expression by the introduction of antisense fibronectin constructs into non-tumorigenic hybrid cells. The “malignant” phenotype re-appeared in the cells in which the antisense construct resulted in reduction of fibronectin synthesis.<sup>(50)</sup> Hence, in his view, carcinogenesis does not require acquisition of a new function, but rather the disruption of the pattern of cellular differentiation.<sup>(47)</sup>

#### *“Normalization” of cancer cells in an organismal, tri-dimensional context*

When early embryos were transplanted into ectopic places (e.g. the kidney capsule or the peritoneal cavity), they behaved like malignant neoplasms called teratocarcinomas. Conversely, teratocarcinoma cells injected into early embryos (blastocyst stage) generated normal tissues and organs. In fact, those cancer cells became gametes (oocytes and sperm cells), which in turn generated normal progeny. Thus, embryonal cells produced neoplasms when misplaced in adult tissues and reverted to normalcy when placed into an early embryo.<sup>(51)</sup> In addition, when nuclei from Lucke’s frog renal carcinoma cells were transplanted into enucleated and activated ova, they developed and reached the swimming tadpole stage.<sup>(52)</sup> Additionally, transplantation of tissues from these tadpoles into normal recipients generated normal tissues that were indistinguishable from those of the host.<sup>(53)</sup> These data challenged the view that cancer was caused by DNA mutations, since the neoplastic phenotype could be normalized at a frequency much higher than was needed to revert a DNA mutation back to the wild type. Hence, the dictum “once a cancer cell, always a cancer cell” was invalidated and

the data instead suggested an epigenetic control of the expression of neoplastic phenotypes.<sup>(17)</sup>

Although these experiments clearly showed the reversibility of the neoplastic phenotype, and hence are inconsistent with the SMT, they did not address the issue of how neoplasms arise. In this regard, the relevant question that needs to be asked is: at what level of biological complexity does carcinogenesis occur?

### **Carcinogenesis and biological organization**

Cancer occupies multiple levels of biological organization<sup>(46)</sup> (see Ref 6, pp 91–143). Within this perspective, determining at which of these levels carcinogenesis occurs is controversial. To illustrate this concept, we introduce the image of a metaphorical bookshelf. In this bookshelf, each separate volume would deal with and represent a different level of complexity. Each volume would be lying side by side with others addressing “higher” or “lower” levels. The information contained in each volume may have only a limited relatedness, or none at all, with that presented in another volume of the collection. A historic example will be representative of this type of relationship at the population level of organization. Over a half-century ago, epidemiologists and public health officials were able to design and promote effective preventive campaigns for a good number of cancers. Specifically, reducing tobacco consumption in order to lower lung cancer incidence did not require that those epidemiologists and public health professionals of yesteryears knew much about DNA replication, gene expression, signal transduction pathways, or epithelium-stroma interactions. A comparable case could apply to the design of a vaccination campaign against the hepatitis B virus to reduce the incidence of hepatocellular carcinomas, or to the eradication of schistosomiasis to diminish the number of bladder adenocarcinoma cases.

Returning to our metaphorical bookshelf, another volume should be dedicated to cancer at the organismal level, the equivalent to cancer disease management. This is the level at which a patient interacts with his/her physician. These protagonists exchange information about the symptoms and signs of the cancer syndrome. After the initial contact with the patient, the clinician makes a preliminary diagnosis of the disease through physical examination and by reading the results from a battery of tests that he/she has ordered. Later on, if the diagnosis is confirmed, an interactive managerial relationship is established between the patient and his/her treatment group.

However, another physician, the pathologist, is the one who makes the final, definitive diagnosis when he/she “reads” a biopsy of the suspected neoplastic tissue through an uncomplicated light microscope. Thus, by this objective criterion, a separate volume in our metaphorical cancer bookshelf should be dedicated to carcinogenesis at the level where it is identified, i.e. at the tissue level of biological complexity. We

postulate that this is the level at which carcinogenesis takes place (see below).

By extending the metaphorical argument of the cancer bookshelf, we conclude that a volume dealing with cancer at the subcellular level of organization should, at best, be moved to a library shelf where generic subcellular and biochemical topics are placed, or at worst, be considered apocryphal. We hasten to add that the effects of carcinogens on subcellular structures and organelles (including genomic mutations), while variably deleterious to each and every cell in the host, are not viewed as directly responsible for the development of neoplasias.

Thus, a rationale that favors discarding the SMT is predicated on the grounds that its niche is at the subcellular level of biological complexity, a level that appears as irrelevant to carcinogenesis.<sup>(46)</sup> This conclusion does not imply that the gigantic effort invested in describing changes at the gene level (gene mutations, methylation patterns, gene expression, etc.) and/or that of the cellular organelles (endoplasmic reticulum, Golgi apparatus, mitochondria, etc.) was fruitless. These data, frequently obtained while using human and rodent tumor cells in culture conditions, have significantly increased our understanding of normal intracellular processes. We posit, however, that these features are not unique or specific to the cancer state, that they are instead part of the flexible set of phenotypic variations with which cells are normally endowed. Hence, it would be understandable that they have fallen short of providing an explanation for carcinogenesis. To the contrary, an examination of a biopsy by a competent specialist would be enough to discriminate between a normal histoarchitectural pattern and that of a neoplasm.

### **The tissue organization field theory of carcinogenesis**

#### *Organicism and developmental mechanics as sources of TOFT*

Reconstructing the history of concepts that led to the TOFT is beyond the scope of this review. Suffice it to say that at the end of the 19<sup>th</sup> century, Boll proposed that cancer resulted from inductive interactions between tissues, Cohnheim suggested that cancer originated in embryonic residues, and Ribbert postulated that epithelial cells do not possess special proliferative powers, but that their proliferation results from being freed from the restrictions imposed by normal tissue organization.<sup>(54,55)</sup> Yet, the introduction of the morphogenetic field concept was a central event in the genesis of the TOFT (see Ref. 6, pp 91–143). “Fields of organization” or “morphogenetic fields”<sup>(56)</sup> were defined as “a collection of cells by whose interactions a particular organ formed”.<sup>(57)</sup> The morphogenetic field became the basic paradigm of embryology. In the 1930s, Needham<sup>(58)</sup> and Waddington<sup>(59)</sup> speculated that neoplastic development resulted from alterations of the normal

interactions that occur in those morphogenetic fields. In other words, carcinogens, as teratogens (i.e. agents that interfere with normal embryonic development), would disrupt the normal dynamic interaction of neighboring cells and tissues both during early development and throughout adulthood. This concept was updated by Rubin 50 years later.<sup>(60)</sup>

Organicism has provided the philosophical bases for the study of embryology's modern beginnings.<sup>(61)</sup> Biologists of the organicist persuasion ask questions about self-organization, cell–cell interactions, tissue–tissue interactions, and organogenesis. They posit that the organism is the zygote that organizes itself into a newborn and beyond. By virtue of being an open system, the organism utilizes resources from both the external (environment) and the internal (gene products and other chemicals synthesized by the organism) worlds. As the reductionistic and genetic determinist view became dominant in biology, the organicists continued their studies of self-organization. Their explanations are operational and are made in terms of cell–cell and tissue–tissue interactions. In contrast, reductionist explanations are made in terms construed as material entities such as genes and their products. From this perspective, histogenesis and organogenesis were supposed to be reduced to the phenomenon of differential gene expression, which was thought to be similar in bacteria and in multicellular organisms. As stated by Jacques Monod “what's true for *E. coli* is true for an elephant”.<sup>(62)</sup> For a long period, the mechanistic rhetoric of geneticists won the day.

From a reductionistic perspective, tissues became collections of independent cells and explanations of carcinogenesis were sought primarily at the cellular, subcellular and molecular levels of organization. To explain differentiation and epigenesis, the morphogenetic field was overcome by the operon, a group of genes controlled by the same regulatory gene. In fact, the morphogenetic field hypothesis was not disproved, it was just forgotten.<sup>(57)</sup> Only when morphogen gradients were visualized toward the end of the 1990s did developmental biology resuscitate this old concept so central to its previous success.<sup>(63)</sup> Morphogens are diffusible substances that “determine” the differentiation that cells “perceiving” this information will undergo (<http://www.books.md/M/dic/morphogen.php>).

As briefly noted above, despite the dominance of the reductionistic program, a few research groups studied the expression of the neoplastic phenotype in a developmental context such as in teratocarcinomas and Lucke's tumors, while others addressed the role of tri-dimensional organization and extracellular matrix.<sup>(64)</sup>

### *Premises and supporting evidence*

The TOFT is based on two main premises: (1) that carcinogens act initially by disrupting the normal interactions that take place among cells in the stroma and parenchyma of an organ,<sup>(46,58,59,65)</sup> and (2) that proliferation is the default state

of all cells (see Ref 6, pp 1–30). During embryonal and fetal development, epithelium and the subjacent stroma exert instructive influences over each other. These morphogenetic fields remain operational during adulthood.<sup>(60)</sup> The disruption of these interactions by carcinogens results in a lessening of the cells' ability to “read” their positional and historical background. This, in turn, allows the epithelial cells to exercise their constitutive property to proliferate (hyperplasia). Next, the tissue organizational pattern would become disrupted (dysplasia) or would even adopt a different tissue type (metaplasia). The pattern of progression to carcinoma *in situ* may not always exactly follow this sequence.<sup>(66)</sup> However, this pattern prevails in carcinomas and adenocarcinomas, which represent the substantial majority of human neoplasms.

Central to this dynamic process is its reversibility.<sup>(66)</sup> The neoplastic phenotype can be experimentally reversed through cell–cell interactions as demonstrated by embryonal carcinoma cells injected into blastocysts,<sup>(67)</sup> hepatocellular carcinoma cells injected into normal livers,<sup>(68)</sup> or modification of the extracellular matrix components.<sup>(69,70)</sup> Hence, the cancer phenotype is an adaptive, emergent phenomenon occurring at the tissue level of organization and is susceptible to being normalized. Of course, if the irritative action of the carcinogen persists, or if the histoarchitecture has been severely compromised, eventually a full neoplastic state evolves, thus diminishing the chances of returning to the status quo ante (see Ref. 6, pp 91–143).

Using a theory-neutral experimental strategy, we recently collected data on rat mammary carcinogenesis. We observed that the recombination of stroma exposed to a carcinogen with normal epithelial cells resulted in neoplasms. The reverse combination did not. This observation suggests that the stroma, rather than the epithelium, is the target of the carcinogen.<sup>(71)</sup> These results challenge the validity of the SMT, while buttressing the TOFT.

### *Sporadic versus hereditary cancers*

From our perspective, hereditary cancers<sup>(24)</sup> should be considered as *inborn errors of development*. Analogous to inborn errors of metabolism that were extensively described during the second half of the twentieth century,<sup>(72)</sup> these cancers represent syndromes that involve the appearing of uni or multilocular tumors at different times during development. For instance, these syndromes may appear shortly after birth as in retinoblastoma,<sup>(73)</sup> after puberty or in early adulthood like in multiple endocrine cancers,<sup>(74)</sup> or prior to the age of incidence for the non-familial form in breast cancers due to BRCA1 and BRCA2 gene mutations,<sup>(75)</sup> and in colorectal cancers due to APC mutations. The distinction between sporadic and hereditary cancers is intended to separate two sets of tumors that have a distinct etiology (epigenetic versus genetic, respectively) but share a common pathogenesis (tissue architecture disruption).

### Are there ways to reconcile the SMT and the TOFT?

These two theories are not compatible in principle. While one centers on “one renegade cell”, as asserted by R.A. Weinberg<sup>(4)</sup> and views cancer as a cell-based disease involving unregulated cell proliferation, the other focuses on a “society of cells”<sup>(6)</sup> and views cancer as a problem of tissue organization. However, this does not mean that the data gathered from experiments based on the SMT cannot be interpreted in the light and context of the TOFT. The polyps in patients who are hemizygous for a defective *APC* and the displasias appearing prior to neoplasia in retinoblastoma and in the lethal giant larva mutant in *Drosophila* are all tissue organization alterations. In the case of inactivated *APC*, one may even hint at the mechanisms that may be involved, since, as mentioned above, *APC* binds to  $\beta$ -catenin, which in turn binds to cell adhesion molecules called cadherins.<sup>(76)</sup> *APC* also binds to the human homologue of *Drosophila discs large (hDdl)*, which is also involved in cell–cell adhesion through septate junctions.<sup>(77)</sup> Deletions of this gene in flies result in the loosening of cell–cell contacts, abnormal morphology of the imaginal discs, and neoplastic development.<sup>(78)</sup>

Altered communication among cells is at the core of the TOFT. From this perspective, one would study how specific alterations in *APC*, catenins, cadherins and *hDdl* affect the development of the intestinal crypt and give rise to polyps. Instead, the SMT-based research effort centers on the role of  $\beta$ -catenin as a transcription factor and looks at the epithelial cell nucleus (the transcriptional machinery) for putative alterations in the control of cell proliferation, cell cycle and apoptosis. It is thus theoretically conceivable that spontaneous gene mutations causing altered cell–cell communication may lead to carcinogenesis; the biological effects of these mutations, however, would only become apparent at the tissue level of organization.

A different problem is revealed by the study of the *lethal giant larva-2 (lgl-2)* gene in *Drosophila*. A deletion in this gene is responsible for the development of neuroblastomas in homozygote flies. This gene is expressed when the embryo is a syncytium and is never expressed in the cell type that becomes cancerous when the gene is defective, i.e., neuroblasts. As the nervous tissue develops in the mutant *Drosophila* larva, it appears less organized than in its normal counterpart.<sup>(79)</sup> Thus, the gene deletion somehow affects tissue organization several steps downstream after it failed to be expressed much earlier. Hence, even finding the mutated gene and showing its causal role in carcinogenesis has fallen short of explaining the cancer phenotype.

Ectopic expression of normal genes in transgenic mice results in neoplastic development as observed by Sternlicht et al,<sup>(80)</sup> who reported that manipulations of the microenvironment, such as overexpression of stromelysin 1, may result in carcinogenesis. This matrix metalloproteinase would alter

cell–cell and cell–extracellular matrix interactions. These alterations, in turn, would promote the neoplastic transformation of the mammary gland. Moreover, administration of protease inhibitors suppressed the carcinogenic process that ensued when the stromelysin-1 transgene was expressed.<sup>(80)</sup> Interestingly, the resulting neoplasms displayed DNA losses in chromosomes 4 and 7, and those showing epithelial–mesenchymal transitions displayed DNA gains. Hence, alterations in tissue architecture can and do induce neoplasms, and those neoplasms, like the sporadic ones, may end up showing aneuploidy. As Prehn remarked, “. . . it may be more correct to say that cancers beget mutations than it is to say that mutations beget cancers”.<sup>(16)</sup>

In sum, genes causing inborn errors of development and cancer could easily be incorporated into the TOFT, but the questions asked about the role of these genes would be different from those formulated by the SMT. While the former looks at cell interactions in a tissue-based, developmental context, the latter looks at the cell as a quasi-autonomous entity, governed from the inside by its genes. As put by L. Moss: “To heirs of nineteenth century holism (‘organicism’- is the materialist, contemporary version of holism-author’s note), autonomy was understood in terms of ‘totipotency’, the possession by the cell of the potential of the whole. The autonomy of the cell understood this way is then the precondition for either normal or aberrant growth and a prior guarantee of neither. What determines which way it will go, normal or aberrant, is not its internal features but the subsequent history of its interactions” (see Ref 81, p 129).

### Back to the beginning: a historical and philosophical perspective

For four centuries, choices between competing postulates, hypothesis testing and falsification have been central to the long, successful tradition of science. Only after a rigorous weeding-out process is a synthesis possible. Through this synthesis, contradictions are resolved and both spurious “facts” and wrong premises are recognized and dismissed. A misguided, premature synthesis may lead to a confusing state of affairs where, if results do not fit one hypothesis, they may fit its opposite; in other words, nothing is rejected and everything is explained by the piling up of ad hoc explanations. This attitude contrasts with the objectives of science as described by Ayala, namely: “(1) science seeks to organize knowledge in a systematic way, endeavoring patterns of relationship between phenomena and processes; (2) science strives to provide explanations for the occurrence of events; and finally, (3) science proposes explanatory hypotheses that must be testable, that is, accessible to the possibility of rejection or falsification”.<sup>(82)</sup>

When assessing the state of the art in carcinogenesis at the beginning of the 21st century, we are reminded of a similar evaluation done in 1926 about the state of the art in

embryology. H.S. Jennings recalled that embryologists often did similar experiments and arrived at different conclusions. “All the conflicting reports were correct. The situation was that of the Gilbertian comic opera chorus, ‘For you are right, and I am right, and he is right and all is right’”.<sup>(83)</sup> Maienschein’s historical analysis shows that, at the root, those were issues of epistemology; researchers were disagreeing not only about the biological phenomenon, but also about how it should be studied.<sup>(84)</sup> A rigorous epistemologic foundation helps guide experimental design, the gathering of data, and the interpretation for or against a given hypothesis.<sup>(85–87)</sup> Hence, it is not disruptive, but actually productive, that alternative views coexist before the body of evidence gathered allows for a synthesis and/or the rejection of the wrong concepts and hypotheses.

As we have analyzed above, the emergence of conflicting data within the SMT did not result in the rejection of premises and hypotheses. For example, an oncogene could be “dominant” and express a gain of function with respect to the non-mutated homologue, and its biological effect could be contextual at the same time. That is, a mutation that should have produced uncontrolled cell proliferation resulted in cell death or arrest of cell proliferation. Again, ad hoc explanations were proposed to resolve conflicting evidence, leading to a situation whereby any possible conclusion is valid because no alternative concept is ever disproved and abandoned. The lack of fit is attributed to the unfathomable complexity of nature/biology.<sup>(88)</sup> In short, something can be anything and its opposite.

In this atmosphere, an attempt to blend “tissue-based” cancer research into the oncogene theory has been proposed. Namely, data showing that extracellular matrix and tissue architecture can normalize the behavior of cancer cells<sup>(89)</sup> are re-interpreted by adherents to the SMT as important steps towards understanding the mechanisms that determine how “...cancer genes perturb the biological interactions of individual cells with their immediate surroundings”.<sup>(90)</sup> Hence, for these committed supporters of the SMT, the problem of how extracellular matrix controls cell phenotypes becomes at best a quest to unravel how oncogenes affect interactions between mutated and normal cells.

The philosopher L. Moss has put forward the argument that most of the problems inherent to the SMT are due to the amalgamation of the Mendelian gene (as used in transmission genetics to trace the inheritance of a given character) with the molecular gene (a DNA sequence) and to the adoption of a preformationistic view in the long and still ongoing debate between epigeneticists and preformationists (see Ref. 81, pp 183–198). Indeed a substantial literature, both biological and epistemological, clearly shows that the Mendelian gene was not reduced to the DNA “gene” and that the relationship between the two is rendered ambiguous because of splicing (one gene–many possible transcripts) as well as by the

classical properties of pleiotropism (one gene–diverse effects) and polyphenism (one genotype–multiple phenotypes).

Regarding the preformationism/epigenesis argument in embryology, the 18<sup>th</sup> century homunculus that determined morphogenesis in the embryo morphed into a genetic program in the middle of the 20<sup>th</sup> century. The modern view about epigenesis is instead that the embryo constructs itself, using not only the proteins and RNA coded in the genome, but all sorts of environmental resources. According to Moss: “The critical decisions made at the nodal points of organismic development and organismic life are not made by a prewritten script, program, or master plan but rather are made on the spot by an ad hoc committee” (see Ref. 81, p.186).

### Conclusions

During the last decades, developmental biology benefited from many conceptual and methodological advances involving the role of interactions among extracellular matrix, cells and tissues in morphogenesis. The application of the morphogenetic field concept to cancer research has revealed that the neoplastic phenotype can be reversed when cells from a neoplasm are placed in a normal environment.<sup>(6,46,67–69,81)</sup> The normal interaction among tissues during development may be disrupted by a variety of physical, chemical and biological agents resulting in malformations. Similarly, disruption of these same interactions during adulthood may result in neoplasia.

From a methodological standpoint, those favoring the premises of the SMT adopted an in vitro, two-dimensional approach involving a single cell type to study carcinogenesis. Instead, the TOFT favors adopting the methods and strategies used by developmental biologists to study histogenesis and organogenesis, including the use of tissue recombination in animals and, when warranted, a three-dimensional model where different, interacting cell types in culture evolve into a series of changes that mimic what happens in the complex environment of tissues in situ.

From an epistemological viewpoint, the TOFT removes the gene from the driver’s seat (genetic determinism), and brings the organism and its ability to self-organize as the conceptual focus (organicism). This parallels the position of Smithers who, over four decades ago, compared the merits of cytologism and an organismal view of carcinogenesis.<sup>(14)</sup>

Historically, replacing an old paradigm with a new one is a drawn-out enterprise.<sup>(91)</sup> Science, being a creation of the human intellect, becomes subject to the vagaries of social activities where the participants have interests that transcend the objective value of the competing paradigms.<sup>(92)</sup> In the modern era, awareness of these vagaries on the part of governmental and private funding agencies may accelerate and productively stir these changes for the benefit of both the public at large and that of the research community. In the meantime, much will be accomplished when cancer research

rejoins the long and successful tradition of discarding premises and rejecting hypotheses.

We are grateful to Drs. Michel Morange and Jean-Jacques Kupiec (both at Centre Cavailles, Ecole Normale Supérieure, Paris, France) and Pierre Sonigo (Institut Cochin, Paris, France). This commentary is based on discussions we have had with them during our sabbatical stage in Paris (AMS at the Centre Cavailles, CS at the Institut Cochin). We are grateful to Cheryl Schaeberle, Janine Calabro and April Flynn for their technical and editorial assistance. This work was supported by the Bradshaw Foundation (Geneva, Switzerland) and by the NIH (grants ES 08314, CA 55574 and CA13410).

### Note added in proof

Almost three decades after B. Mintz's group showed the "normalization" of teratocarcinoma cells implanted into mouse blastocysts, Hochedlinger et al (Reprogramming of a melanoma genome by nuclear transplantation, Hochedlinger K., Blelloch R., Brennan C., Yamada Y., Kim M., Chin L. and Jaenisch R. 2004 *Genes & Development*, 18:1875–1885) now demonstrate the pluripotentiality of the nuclei of mouse melanoma cells. In their elegant experimental approach, these authors extend further the notion of the potential reversibility of the cancer phenotype under the influence of a normal multicellular environment.

### References

1. Curtis HJ. 1965. Formal discussion of: Somatic mutations and carcinogenesis. *Cancer Res* 25:1305–1308.
2. Hahn WC, Weinberg RA. 2002. Modelling the molecular circuitry of cancer. *Nat Rev Cancer* 2:331–342.
3. Frank SA, Nowak MA. 2004. Problems of somatic mutation and cancer. *Bioessays* 26:291–299.
4. Weinberg RA. 1998. One renegade cell: how cancer begins. New York: Basic Books.
5. Alberts B, Johnson A, Lewis JG, Raff M, Roberts K, Walter P. 2002. *Molecular Biology of the Cell*. New York, NY: Garland Publishing Inc. p 1015
6. Sonnenschein C, Soto AM. 1999. *The Society of Cells: Cancer and Control of Cell Proliferation*. New York: Springer Verlag.
7. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2001. *Molecular Biology of the Cell*. New York, NY: Garland Publishing Inc. p 1313–1362
8. Wang T-L, Rago C, Silliman N, Ptak J, Markowitz S et al. 2002. Prevalence of somatic alterations in the colorectal cancer cell genome. *Proc Nat Acad Sci USA* 99:3076–3080.
9. Cooper GM. 1983. Transforming genes of neoplasms. *Progress in Nucleic Acid Research & Molecular Biology* 29:273–277.
10. Hahn WC, Weinberg RA. 2002. Mechanisms of disease: Rules for making human tumor cells. *New Engl J Med* 347:1593–1603.
11. Willis RA. 1967. *Pathology of Tumors*. London: Butterworths.
12. Rowlatt C. 1994. Some consequences of defining the neoplasm as focal self-perpetuating tissue disorganization. In: Iversen OH, editor. *New Frontiers in Cancer Causation*. Washington, DC: Taylor & Francis. p 45–58.
13. Boveri T. 1929. *The Origin of Malignant Tumors*. Baltimore, MD: Williams & Wilkins. p 115
14. Smithers DW. 1962. Cancer: an attack of cytologism. *Lancet* 493–499.
15. Nowell PC, Hungerford DA. 1960. Chromosome studies on normal and leukemic lymphocytes. *J Nat Cancer Inst* 25:85–109.
16. Prehn RT. 1994. Cancers beget mutations *versus* mutations beget cancers. *Cancer Res* 54:5296–5300.
17. Pierce GB, Shikes R, Fink LM. 1978. *Cancer: A Problem of Developmental Biology*. Englewood Cliffs, NJ: Prentice-Hall.
18. Harris H. 1988. The analysis of malignancy by cell fusion: the position in 1988. *Cancer Res* 48:3302.
19. Benson KTH. 2001. Morgan's resistance to the chromosome theory. *Nat Rev Genet* 2:469–474.
20. Newbold RF, Overell RW. 1983. Fibroblast immortality is a prerequisite for transformation by EJ c-HA-ras oncogene. *Nature* 304:648–651.
21. Bishop JM. 1985. Viral oncogenes. *Cell* 42:23–38.
22. Martins-Green M, Boudreau N, Bissell MJ. 1994. Inflammation is responsible for the development of wound-induced tumors in chickens infected with Rous Sarcoma virus. *Cancer Res* 54:4334–4341.
23. Land H, Parada LF, Weinberg RA. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 304:596–602.
24. Knudson AG Jr. 1995. Mutation and cancer: a personal odyssey. *Adv Cancer Res* 67:1–23.
25. Knudson AG Jr. 1989. Hereditary cancers disclose a class of cancer genes. *Cancer* 63:1888–1891.
26. Robanus-Maandag E, Dekker M, van der Valk M, Carrozza M-L, Jeanny J-C, et al. 1998. p107 is a suppressor of retinoblastoma development in pRb-deficient mice. *Genes Dev* 12:1599–1609.
27. Rangarajan A, Weinberg RA. 2003. Comparative biology of mouse versus human cells: modeling human cancer in mice. *Nat Rev Cancer* 3:952–959.
28. Matsumine A, Ogai A, Senda T, Okumura N, Satoh K, et al. 1996. Binding of APC to the human homolog of the drosophila discs large tumor suppressor protein. *Science* 272:1020–1023.
29. Su LK, Burrell M, Hill DE, Gyuris J, Brent R. 1995. APC binds to the novel protein EB1. *Cancer Res* 55:2972–2977.
30. Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH. 1993. Association of the APC gene product with beta-catenin. *Science* 262:1731–1734.
31. Levine AJ. 1997. p53, the cellular gatekeeper for growth and division. *Cell* 88:323–331.
32. Kinzler KW, Vogelstein B. 1996. Lessons from hereditary colorectal cancer. *Cell* 87:159–170.
33. Loeb LA. 2001. A mutator phenotype in cancer. *Cancer Res* 61:3230–3239.
34. Parsons R, Li GM, Longley M, Modrich P, Liu B, et al. 1995. Mismatch repair deficiency in phenotypically normal human cells. *Science* 268:738–740.
35. Reitmair AH, Cai J-C, Bjerknes M, Redston M, Cheng H, et al. 1996. MSH2 deficiency contributes to accelerated APC-mediated intestinal tumorigenesis. *Cancer Res* 56:2922–2926.
36. Weinstein IB. 2002. Cancer. Addiction to oncogenes—the Achilles heel of cancer. *Science* 297:63–64.
37. Soto AM, Sonnenschein C. 1985. The role of estrogens on the proliferation of human breast tumor cells (MCF-7). *J Steroid Biochem* 23:87–94.
38. Sonnenschein C, Soto AM. 1980. But are estrogens per se growth-promoting hormones? *J Nat Cancer Inst* 64:211–215.
39. Soto AM, Sonnenschein C. 1993. Regulation of cell proliferation: is the ultimate control positive or negative? In: Iversen OH, editor. *New Frontiers in Cancer Causation*. Washington, DC: Taylor & Francis. p 109–123.
40. Sonnenschein C, Soto AM, Michaelson CL. 1996. Human serum albumin shares the properties of estradiol-like, the inhibitor of the proliferation of estrogen-target cells. *J Steroid Biochem Molec Biol* 59:147–154.
41. Alberts B, Bray D, Lewis JG, Raff M, Roberts K, et al. 1994. *Molecular Biology of the Cell*. New York, NY: Garland Publishing Inc. p 891.
42. Yusuf I, Fruman DA. 2003. Regulation of quiescence in lymphocytes. *Trends in Immunology* 24:380–386.
43. Kelly LL, Green WF, Hicks GG, Bondurant MC, Koury MJ, Ruley HE. 1994. Apoptosis in erythroid progenitors deprived of erythropoietin occurs during the G<sub>1</sub> and S phases of the cell cycle without growth arrest or stabilization of wild-type p53. *Mol Cell Biol* 14:4183–4192.
44. Barrandon Y, Green H. 1987. Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor  $\alpha$  and epidermal growth factor. *Cell* 50:1131–1137.

45. Baron U, Gossen M, Bujard H. 1997. Tetracycline-controlled transcription in eukaryotes: novel transactivators with graded transactivation potential. *Nucleic Acids Res* 25:2723–2729.
46. Sonnenschein C, Soto AM. 2000. The somatic mutation theory of carcinogenesis: Why it should be dropped and replaced. *Mol Carcinog* 29:1–7.
47. Harris H. 2004. Tumor suppression: putting on the breaks. *Nature* 427:201.
48. Harris H. 1995. *The Cells of the Body: A History of Somatic Cell Genetics*. Plainview, NY: Cold Spring Harbor Laboratory Press. p 234.
49. Harris H, Rawlins J, Sharps J. 1996. A different approach to tumour suppression. *J Cell Sci* 109:2189–2197.
50. Steel DM, Harris H. 1989. The effect of antisense RNA to fibronectin on the malignancy of hybrids between melanoma cells and normal fibroblasts. *J Cell Sci* 93:515–524.
51. Stewart TA, Mintz B. 1981. Successful generations of mice produced from an established culture line of euploid teratocarcinoma cells. *Proc Nat Acad Sci USA* 78:6314–6318.
52. DiBerardino MA, Orr NH, McKinnell RG. 1986. Feeding tadpoles cloned from *Rana erythrocyte* nuclei. *Proc Nat Acad Sci USA* 83:8231–8234.
53. McKinnell RG, Lust JM, Sauerbier W, Rollins-Smith LA, Williams JW 3, et al. 1993. Genomic plasticity of the Lucke renal carcinoma: a review. *Int J Dev Biol* 37:213–219.
54. Triolo VA. 1964. Nineteenth century foundations of cancer research origins of experimental research. *Cancer Res* 24:4–27.
55. Triolo VA. 1965. Nineteenth century foundations of cancer research advances in tumor pathology, nomenclature, and theories of oncogenesis. *Cancer Res* 25:76–98.
56. Needham J. 1931. *Chemical Embryology*. Cambridge: Cambridge University Press.
57. Gilbert SF. 2003. The rediscovery of morphogenetic fields. <http://www.devbio.com/article.php?id=188&search=morphogenetic%20field>. May 13, 2003.
58. Needham J. 1936. New advances in chemistry and biology of organized growth. *Proc Roy Soc B* 29:1577–1626.
59. Waddington CH. 1935. Cancer and the theory of organizers. *Nature* 135:606–608.
60. Rubin H. 1985. Cancer as a dynamic developmental disorder. *Cancer Res* 45:2935–2942.
61. Gilbert SF, Sarkar S. 2000. Embracing complexity: Organicism for the 21st century. *Developmental Dynamics* 219:1–9.
62. Judson HF. 1995. *The Eighth Day of Creation*. Toronto, ON: Penguin Books. p 613.
63. De Robertis EA, Morita EM, Cho KWY. 1991. Gradient fields and homeobox genes. *Development* 112:669–678.
64. Bissell MJ, Barcellos-Hoff MH. 1987. The influence of extracellular matrix on gene expression: is structure the message? *J Cell Sci* 8:327–343.
65. Orr JW. 1958. The mechanism of chemical carcinogenesis. *Br Med Bull* 14:99–101.
66. Clark WH. 1991. Tumour progression and the nature of cancer. *Br J Cancer* 64:631–644.
67. Illmensee K, Mintz B. 1976. Totipotency and normal differentiation of single teratocarcinoma cell cloned by injection into blastocysts. *Proc Nat Acad Sci USA* 73:549–553.
68. McCullough K, Coleman W, Ricketts S, Wilson J, Smith G, Grisham JW. 1998. Plasticity of the neoplastic phenotype in vivo is regulated by epigenetic factors. *Proc Nat Acad Sci USA* 95:15333–15338.
69. Bissell MJ, Radisky D. 2001. Putting tumours in context. *Nat Rev Cancer* 1:46–54.
70. Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, et al. 1997. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo integrin blocking antibody. *J Cell Biol* 137:231–245.
71. Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C. 2004. Rat mammary gland chemical carcinogenesis: the stroma as a crucial target. *J Cell Sci* 117:1495–1502.
72. Schaub J. 1991. *Inborn Errors of Metabolism*. Philadelphia: Lippincott, Williams & Wilkins.
73. Knudson AG Jr. 1993. Pediatric molecular oncology: Past as prologue to the future. *Cancer* 71:3320–3324.
74. Poisson A, Zablewska B, Gaudray P. 2003. Menin interacting proteins as clues toward the understanding of multiple endocrine neoplasia type 1. *Cancer Lett* 189:1–10.
75. Iau PT, Macmillan RD, Blamey RW. 2001. Germ line mutations associated with breast cancer susceptibility. *Eur J Cancer* 37:300–321.
76. Kemler R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends in Genetics* 9:317–321.
77. Hough CD, Woods DF, Park S, Bryant PJ. 1997. Organizing a functional junctional complex requires specific domains of the *Drosophila* MAGUK Discs large. *Genes Dev* 11:3242–3253.
78. Jursnich VA, Fraser SE, Held LI, Ryerse J, Bryant PJ. 1990. Defective gap-junctional communication associated with imaginal disc overgrowth and degeneration caused by mutations of the *dco* gene in *Drosophila*. *Dev Biol* 140:413–429.
79. Mechler BM, Strand D, Kalmes A, Merz R, Schmidt M, Torok I. 1991. *Drosophila* as a model system for molecular analysis of tumorigenesis. *Environ Health Perspect* 93:63–71.
80. Sternlicht MD, Lochter A, Sympon CJ, Huey B, Rougier J-P. 1999. The stromal proteinase MMP3/Stromelysin-1 promotes mammary carcinogenesis. *Cell* 98:137–146.
81. Moss L. 2003. *What Genes Can't Do*. Cambridge, MA: MIT Press.
82. Ayala FJ. 1968. Biology as an autonomous science. *Am Sci* 56:207–221.
83. Jennings HS. 1926. Biology and experimentation. *Science* 64:97–105.
84. Creath R, Maienschein J. 2000. Competing epistemologies and developmental biology. In: Creath R, Maienschein J, editors. *Biology and Epistemology*, Cambridge Studies in Philosophy and Biology. Cambridge, U.K: Cambridge University Press. p 122–137.
85. Isom HS, Wigdahl B, Howett MK. 1996. Molecular pathology of human oncogenic viruses. In: Sirica AE, editor. *Cellular and Molecular Pathogenesis*. Philadelphia, PA: Lippincott-Raven. p 341–387.
86. Bard J. 2000. Popper's philosophy of science: a practical tool for the working biologist. *Bioessays* 22:205.
87. de Gray ADNJ. 2000. Biologists abandon Popper at their peril. *Bioessays* 22:206.
88. Guerra C, Mijimolle N, Dhawahir A, Dubus P, Barradas M, et al. 2003. Tumor induction by an endogenous *K-ras* oncogene is highly dependent on cellular context. *Cancer Cell* 4:111–120.
89. Weaver VM, Lelievre S, Lakins JN, Chrenek MA, Jones JC. 2002. beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell* 2:205–216.
90. Jacks T, Weinberg RA. 2002. Taking the study of cancer cell survival to a new dimension. *Cell* 111:923–925.
91. Kuhn TS. 1962. *The structure of scientific revolutions*. Chicago: University of Chicago Press.
92. Fujimura J. 1996. *Crafting Science*. Cambridge: Harvard University Press.