# Emergentism as a default: Cancer as a problem of tissue organization

ANA M SOTO* and CARLOS SONNENSCHEIN

*Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111, USA*

**Corresponding author (Fax, 617-636-3971; Email, ana.soto@tufts.edu)*

During the last fifty years the dominant stance in experimental biology has been reductionism. For the most part, research programs were based on the notion that genes were in 'the driver's seat' controlling the developmental program and determining normalcy and disease (genetic reductionism and genetic determinism). Philosophers were the first to realize that the belief that the Mendelian genes were reduced to DNA molecules was questionable. Soon after these pronouncements, experimental data confirmed their misgivings. The optimism of molecular biologists, fueled by early success in tackling relatively simple problems, has now been tempered by the difficulties found when attempting to understand complex biological problems.

Here, we analyse experimental data that illustrate the shortcomings of this sort of reductionism. We also examine the prevailing paradigm in cancer research, the somatic mutation theory (SMT), the premises of which are: (i) cancer is derived from a single somatic cell that has accumulated multiple DNA mutations; (ii) the default state of cell proliferation in metazoa is quiescence; and (iii) cancer is a disease of cell proliferation caused by mutations in genes that control proliferation and the cell cycle. We challenge the notion that cancer is a cellular problem caused by mutated genes by assessing data gathered both from within the reductionist paradigm and from an alternative view that regards carcinogenesis as a developmental process gone awry. This alternative view, explored under the name of the tissue organization field theory (TOFT), is based on premises that place cancer in a different hierarchical level of complexity from that proposed by the SMT, namely: (i) carcinogenesis represents a problem of tissue organization comparable to organogenesis, and (ii) proliferation is the default state of all cells.

We propose that the organicist view, in which the TOFT is based, is a good starting point from which to explore emergent phenomena. However, new theoretical concepts are needed in order to grapple with the apparent circular causality of complex biological phenomena in development and carcinogenesis.

[Soto A M and Sonnenschein C 2004 Emergentism as a default: Cancer as a problem of tissue organization; *J. Biosci*. **30** 103–118]

## 1. Introduction

We believe that philosophy is central to science in general and experimental biology in particular. A good number of scientists, however, are not of this persuasion. Dr Steven Weinberg, the Physics Nobelist, stated that the philosophy of science is about as useful to scientists as ornithology is to birds. Many scientists agree with this statement, and make no efforts to find theoretical underpinnings in their daily bench experience. An example of this attitude was made explicit by the developmental biologist Lewis Wolpert who, when reviewing *Science, Truth, and Democracy*, a book by the philosopher Philip Kitcher, wrote: "Once again, I have been disappointed by a philosophical analysis of the nature of science. And I am left wondering, do philosophers really have anything

**Keywords.** Cell-cell interactions; cell communication; genetic determinism; morphogenesis; neoplasms; organicism; reductionism; somatic mutation theory; supracellular phenomena; tissue organization; tissue organization field theory

Abbreviations used: APC, Adenomatous polyposis coli; EPO, erythropoietin; hDdl, human homologue of *Drosophila* discs large; HNPCC, hereditary non-polyposis colorectal cancer; Rb, retinoblastoma; SMT, somatic mutation theory of carcinogenesis; TGF-*b*, transforming growth factor-beta; TOFT, tissue organization field theory.

useful to tell scientists (Wolpert 2002)?" The inescapable fact is that, whether biologists like it or not, there are no theory-free data. As put by the philosopher D C Dennett: "There is no such thing as philosophy-free science; there is only science whose philosophical baggage is taken on board without examination (Dennett 1995a)". Ignoring the philosophical issues inherent in the practice of biology may hinder our understanding of the discipline we choose to explore.

The overwhelming majority of biologists either tacitly or explicitly adopt the ontological position that what actually exists is matter. Within this materialist stance, the dominant epistemology is reductionism. By this we mean that explanations are sought for at the lowest possible level of organization, so that biology can eventually be reduced to chemistry and physics. However, in practice, this reductive thrust goes as far down as needed to construct an explanation. For example, those studying the mechanism of action of a particular enzyme may describe their objects of study at the atomic and subatomic levels of organization. This is necessary for elucidating substrate-enzyme interactions resulting in the modification of the substrate. They are in fact working in the realm of chemistry. However, when questions are being asked about cellular activities – for example, how a particular hormone 'induces' a functional response in a particular cell type – the description of the 'signal transduction' pathway is generally made at a higher level of complexity. These researchers concentrate on describing the sequence in which proteins interact in order to transfer information from outside the cell into an observable cellular response (Morange 2002). They consider that a lower level of inquiry is unnecessary to understand how the hormone elicits cellular behaviour.

While biochemists do not strive to explain all phenomena at the lowest possible level of organization (i.e. a physical one), a great number of biologists insist that explanations should always be sought for at the gene and/or gene product level, regardless of the level of organization at which the phenomenon of interest is observed. This stance, genetic reductionism, together with its twin, genetic determinism, predicates that everything in biology may be reduced to genes because the genome is the only repository of transmissible information. In this view, genes are the only units of selection (Dawkins 1976) and development is just the unfolding of a genetic program. In sum, genes in this view are the building units of the organism, and have a privileged metaphysical status. (For an extended analysis of this subject, see Griffiths and Gray 2000.)

Although prevalent, this is not the only way in which biologists deal with philosophy of science. Evolutionary biologists have a tradition of being philosophically literate and caring about epistemological issues. Biologists concerned with the study of organismal biology in metazoa (i.e. developmental biology, physiology and cancer research) deal with epistemology in four ways:

(i) They disregard it. They think that 'data talk' and data are theory-free.
(ii) They adopt reductionism to study complex phenomena. A typical example is carcinogenesis. It is not unusual to find that, when their data in this subject contradict their hypotheses they invoke complexity and accept that something and its opposite are both true. Eventually, they hope, everything will fall into its proper place. Thus, instead of dropping one of the contradictory hypotheses they decide *a posteriori* that the *ceteris paribus* clause did not apply. No attempts are made at rejecting any hypothesis (see below, under "The Somatic Mutation Theory").
(iii) They adopt an organicist view and accept the existence of emergent phenomena. Parenthetically, organicism is also called materialistic holism (Gilbert and Sarkar 2000). These researchers choose to work at the level of organization at which the studied phenomenon is observed and venture gingerly into lower levels of organization, moving gradually through the diverse hierarchical levels of complexity, rather than jumping from phenotype to gene. Moreover, since they acknowledge emergent phenomena, their incursions into lower levels must be followed by a synthesis of how lower level phenomena bear upon upper level phenomena. This combination of analytic and synthetic approaches is called the systemic view (Bunge 2004).
(iv) They adopt an instrumentalist stance and study phenomena using heuristic models for as long as they continue to be consistent with data. They adjust their models as problems arise and care neither about the unity of science nor the reality or truth of the entities and processes their explanations postulate.

## 2. Reductionism vs organicism

In the 1960s, it seemed that genes (hitherto considered abstract, operational entities) were finally transformed into material, specific DNA sequences (Benson 2001). Molecular biologists concluded that, at last, biology was being reduced to chemistry. However, the early optimism about reduction was proven premature. David Hull (1974) was probably the first to call attention to the difficulties in achieving this reduction. Additional arguments for the irreducibility of the Mendelian gene to the molecular gene were provided by the discoveries of the modular structure of the molecular gene and of alternative splicings of gene products. Modern genes resulted from the duplication and recombination of ancestral ones. For example, the part of DNA coding for a given protein is

made up of modular 'domains' which serve a particular biochemical function (i.e. having a given enzyme property, binding a given ion, recognizing a protein structure). The messenger RNA resulting from a particular DNA is 'spliced' (cut and pasted) before leaving the cell nucleus. Thus, one gene may produce many different RNAs (Moss 2003c). These and other theoretical considerations implied that not all biological phenomena could be meaningfully reduced to the molecular level even when adopting a materialistic stance (Rosenberg 1994). Nevertheless, the reductionistic approach prevailed. By conflating the Mendelian and the molecular gene, biologists adopt a genetic determinist worldview – genes are in the driver's seat (Moss 2003c). Development is therefore viewed as a set of 'orders' given by a 'genetic program' that unfolds seamlessly from the zygote to the viable newborn organism and beyond.

Organicists work at the periphery of the reductionistic mainstream, continuing the tradition of Developmental Mechanics. They study 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 the external world (environment) and the internal world (gene products and other chemicals synthesized by the organism); there is no causal primacy to the DNA (Griffiths and Gray 2000). As the reductionistic view became dominant in biology, the organicists still continued their studies of self-organization. Their explanations are operational – i.e. they are made in terms of how a cell (or a tissue) influences another cell's (or tissue's) behaviour. 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 purported to be reduced to the phenomenon of differential gene expression, which was thought to be similar in bacteria and in multicellular organisms. Hence the aphorism 'what's true for *Escherichia coli* is true for an elephant'. The mechanicist rhetoric of geneticists won the day in the second half of the 20th century. Embryologists became second-class experimentalists that were said to simply be engaged in doing 'phenomenology' or 'descriptive' science.

In hindsight, we consider that all experimental biologists do 'descriptive research' and provide explanatory narratives for the phenomena they study; this surely also applies to the research done by the genetic reductionists. Only at the level of chemical reactions is it possible to describe mechanisms (for example, by precisely defining the workings of enzymes or receptors). When the question explored is a biological one, we are far from building a hierarchical set of relations between laws like those in physics. Biologists would like to instead construct causal chains. However, this is often hindered by the apparent circular causality exhibited by some phenomena (Downie and Newmann 1994; van Obberghen-Schilling *et al* 1988).

A main obstacle to the success of reductionism is the historicity of the organism, i.e. evolution and ontogeny. As François Jacob (1982) noted, nature is not an engineer, but a tinker – driven by evolution, a given molecule is put to different uses. Even in the same organism, a protein may have different functions in different cells. For example, lactate dehydrogenase and crystallin are the same molecule; the former is an enzyme in muscle while the latter plays a structural role in the eye's lens. *b*-catenin is both a transcription factor and a cell-adhesion protein (Gilbert and Sarkar 2000). In addition, a signal pathway effector may lead to the induction of different gene products and therefore different differentiation programs in different cell lineages (Brisken *et al* 2002). This lack of a unique correlation between a given protein and its function was addressed by Hull (1974) as the problem of 'the many and the many'. In other words, one phenotype may result from several different molecular mechanisms, while a single molecule may be involved in other, different phenotypes. A clear example of this divergence is polyphenism, a single genotype producing different phenotypes. These cases (from single proteins with multiple activities to diverse phenotypes coexisting with a single genotype) make reduction difficult, if not impossible.

A change in the perception of biologists has been taking place during recent years regarding the success of reductionism when addressing complex phenomena. This is evidenced by a shift in strategy proposed by those who previously practiced genetic reductionism, and now preach a 'postgenomic' research program whereby computer analysis will identify patterns of gene expression, which will be used for hypothesis building (Bassett *et al* 1999; Brown and Botstein 1999). Others, being more philosophically inclined, propose a new epistemology instead of this brute force postgenomic approach. The following quotation illustrates the new thinking: "Isaac Newton might have liked the neat view of biological systems made up of dedicated components, with causal roles that can be studied in isolation, and in which particular starting conditions give rise to uniquely predictable responses. Charles Darwin, by contrast, might have felt more at home with the idea of a complex, emergent system made up of many non-identical components, with non-exclusive roles, non-exclusive relationships, several ways of producing any given output, and a great deal of slop along the way. We have been Newtonians for the past several decades in our thinking about gene action. It is time to become Darwinians (Greenspan 2001)".

Regarding the historicity of ontogeny, only in unicellular organisms that can facultatively associate into multicellu-

lar colonies can the part (one cell) exist independently from the whole (the multicellular organism). In multicellular organisms, single cells do not have an existence independent from the whole organism, so they are ontogenetically linked. This means that the usual way of thinking about organisms as made up of cells that relinquished their independence is inaccurate and/or misguided. Rather, a zygote – which is a cell resulting from the union of a female and a male gamete – divides, producing more cells, which are organized in a tri-dimensional pattern. Both association patterns and cell types change as tissues and organs are formed. From the beginning of ontogeny, each cell undergoes 'differentiation' under the influence of neighbouring cells. This reciprocity makes it difficult to establish detailed cause and effect relationships, since 'signals' are being sent from one cell to all its immediate neighbours, and vice-versa. This fact may also preclude the isolated cells from revealing their role *in situ* in the originating organism. Acknowledging these problems is not an exercise in nihilism, but a first step in trying to devise ways of studying organisms while taking into consideration the problems posed by their historicity. Hence, developing an epistemology that takes evolutionary and developmental history, elements that play a central role in biology but not in chemistry or physics, into consideration is a worthy objective.

We will next explore the shortcomings of the prevailing doctrine on cancer, which has been based on a reductionist stance whereby genes determine the neoplastic phenotype. We will critically review the data collected under the somatic mutation paradigm 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.

## 3. Research on carcinogenesis illustrates the shortcomings of the reductionistic program

The somatic mutation theory of carcinogenesis (SMT) has been the prevailing paradigm in cancer research for the last 50 years (Curtis 1965; Hahn and Weinberg 2002b). Its main premise is 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 (Weinberg 1998). A second implicit premise is that the default state of cell proliferation in multicellular organisms is quiescence (Alberts *et al* 2002). By default state we mean the state in which cells are found when they are freed from any active control (Sonnenschein and Soto 1999a). 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 (Alberts *et al* 2001; Wang *et al* 2002).

The rise of the SMT as the mainstream theory of carcinogenesis was helped by the following findings: First, a considerable number of carcinogenic chemicals were found to be mutagenic. Second, specific genes in so-called tumour viruses (named 'transforming' genes) enabled such phenomena as *in vitro* transformation and the development of tumours 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 the role of DNA mutations was brought 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 (Cooper 1983). Finally, the DNA sequences involved were identified as mutated versions of 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 (i) that the products of the mutated oncogenes were activated and (ii) that their activation led to an increased proliferation rate. Thus, oncogenes were considered 'gain of function' mutations that led the cells harbouring these mutants to enhanced proliferation. This latter concept strengthened the research program on signal transduction. These research programs resulted in an enormous contribution of knowledge in the biochemistry of cellular processes at large.

The study of familial, hereditary cancers (representing 5% of all human cancers) revealed, instead, that the DNA defects transmitted along the germ line were due to deletions in specific genes. Unlike the case of oncogenes, these deletions implied a loss of function. The first of these anti-oncogenes (also called 'tumour suppressor' genes) was the retinoblastoma (Rb) gene that was soon implicated in the regulation of cell division. Thus, mutations affecting cell cycle regulatory genes also 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 15 tumour suppressor genes have been identified. Additionally, the number of mutated genes found in human cancers challenged the known facts about the frequency of mutations. 'Mutator' genes were postulated to play a role in carcinogenesis to accommodate this 'lack of fit' (Loeb 2001).

Others called attention to the incomplete explanation provided by the above approach. As summarized in a

recent review by Hahn and Weinberg (2002a): "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". To overcome these shortcomings these authors offered the alternative of searching for unifying rules governing the behaviour 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" (Hahn and Weinberg 2002a). Thus, additional research has been proposed, always within the same paradigm that conceptualizes cancer is a cellular problem.

### 3.1 *The murky notion of neoplasm*

Literally, neoplasm means new growth. For over a century, pathologists have tried to define neoplasms. Their definitions were unsatisfactory since properties attributed to 'cancer cells' were also present in normal cells. Because tumour size increases with time, researchers have considered that the underlying cause must have been either excessive or autonomous cell proliferation (Willis 1967). However, in addition to the accumulation of cells, the hallmark of neoplasms is altered tissue organization (Rowlatt 1994). To unambiguously diagnose neoplasms, pathologists examine tissue samples using light microscopes. 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*.' Consequently, researchers postulate hypothetical narratives of what may have happened in the transition from normalcy to cancer.

Different theories of carcinogenesis emerged. Some centered at the cellular level of biological organization and viewed cancer as a problem of cell proliferation (Hahn and Weinberg 2002a) or cell differentiation (Harris 1995). Others locked on the tissue level of biological organization and saw cancer as a problem akin to histogenesis (Clark 1991; Pierce *et al* 1978). Among many of the former, gross chromosome alterations and somatic mutations observed in advanced neoplasms were considered to be the causes of carcinogenesis. Others interpreted these alterations to be just epiphenomena and considered carcinogenesis as an epigenetic process (Sonnenschein and Soto 1999c). These varied theories of carcinogenesis coexisted during the 20th 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.

### 3.2 *Mainstream research reveals inconsistencies within the SMT*

From the SMT perspective, cancer is a cell-centered problem, and hence, the aim of cancer research has been 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 (i) the existence of neoplasms transmitted by the germ-line (we will address this phenomenon below) and (ii) the observation that animals exposed to mutagens often developed neoplasms.

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 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 (Newbold and Overell 1983). For example, infection with the Rous-sarcoma virus resulted

in the transformation of chicken cells, an effect attributed to the *src* oncogene (Bishop 1985). While the injection of Rous-sarcoma viruses into chickens resulted in the integration of the *src* oncogene in all tissues, tumours only developed in places where wounds were inflicted (Martins-Green *et al* 1994). In addition, the transformation of mouse fibroblasts by a single oncogene was attributed to the fact that the cells used as a model were abnormal (Hahn and Weinberg 2002a) because normal mouse fibroblasts were not transformed upon transfection with a single oncogene. At the beginning of several course corrections to come, at least two oncogenes were required (Land *et al* 1983). And, according to Hahn and Weinberg (2002a), "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". 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. This inconsistency is yet to be addressed by the proponents of the oncogene theory.

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 (Knudson 1995). Retinoblastomas appeared to represent this type of tumour. The discovery of the Rb pathway allowed for 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. It was believed that in the genesis of retinoblastomas in humans, in addition to the germ-line deletion, a second mutational event in the normal allele was sufficient to determine the neoplastic transformation of the retina (the two-hit hypothesis) (Knudson 1989). However, hemizygosity of the Rb gene in mice did not predispose 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 containing Rb-/- p107-/- cells developed tumours. Hence, additional events (mutational or not) appeared to be necessary for tumour development (Robanus-Maandag *et al* 1998). 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 (Rangarajan and Weinberg 2003).

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 (Kinzler and Vogelstein 1996). 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 (Smith *et al* 1993). 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 were 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* tumour suppressor gene discs large (*hDlg*) (Matsumine *et al* 1996) and to EB-1, a protein of unknown function (Su *et al* 1995). The central portion of APC binds *b*-catenin, a protein that has at least two functions (Rubinfeld *et al* 1993). One is related to cell-to-cell adhesion through binding to cadherin and the other plays a role in the wnt pathway and is thus involved in signal transduction. 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 neighbours, the subject of the competing tissue organization field theory (TOFT) of carcinogenesis (see below).

Other mutations, such as inactivation of p53, the 'gatekeeper of the genome' (Levine 1997), 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 cells are found in foci of proliferating cells. The problems posed by these findings led Kinzler and Vogelstein (1996) 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". 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 (Loeb 2001). The study of hereditary non-

polyposis colorectal cancer (HNPCC) that harbours mutations in mismatch repair genes provided an example of hypermutability in colorectal cancer. However, these tumours 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 (these tumours are usually diploid) 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 (Parsons *et al* 1995). 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 (Reitmair *et al* 1996). Kinzler and Vogelstein (1996) also pondered about this paradox and tentatively explained it as follows: ". . . it is possible that the dietary factors which lead to colorectal cancer are not mutagens, but rather irritants that lead to tissue regeneration. Dietary fibers may absorb these irritants, explaining part of their protective effect". This explanation brings to the forefront processes that take place at the tissue level of organization such as injury and inflammation, which are, instead, central to alternative views of carcinogenesis (see below).

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 tumour 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" (Weinstein 2002). 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.

### 3.3 *The notion of specificity, central to genetic determinism, is being challenged*

A couple of examples in the field of developmental biology point to the difficulties encountered by embracing the notion of specificity for each signal and for each pathway in determining a phenotype. The first stems from studies on mice generated through cloning by nuclear transplantation. 'Cloning' of embryos through enucleation of oocytes and transplantation with nuclei from somatic cells yields a very low percentage of embryos that develop to term. In turn, a large percentage of these animals die soon after birth of respiratory and circulatory problems and show increased placental and body weights (large offspring syndrome). These anomalies are thought to be due to the abnormal expression of imprinted genes (i.e. genes that are 'marked' or modified in a reversible way, as by methylation). A series of publications by Jaenisch *et al* (Rideout *et al* 2001) showed that many imprinted genes are misregulated in the tissues of the surviving mice, and that no pattern of abnormal gene expression correlates with large placentas or with increased body weights. They concluded that the embryos and fetuses that die before term must have had even more alterations, a point difficult to prove after the fact (i.e. they cannot be identified as the ones that will die before death). Jaenisch's group also investigated global gene expression of a set of more than 10,000 genes by microarray analysis of RNA isolated from the placentas and livers of neonatal cloned mice derived by nuclear transfer from both cultured embryonic stem cells and freshly isolated cumulus cells (somatic cells from the ovarian follicle). They show that altered gene expression involving as many as 4% of the 10,000 genes analysed by DNA microarray results in a normal phenotype at the cell, tissue and organ levels of complexity (Humpherys *et al* 2002). This demonstrates a great degree of tolerance of abnormal gene expression consistent with normal development, and hence, of the inadequacy of reductionism to understand the robustness and self regulation of the developmental process. Embryologists active during the first half of the 20th century were aware of this robustness. Waddington addressed the phenomenon he called 'canalization' (developmental stability coexisting with genetic variation) both theoretically and experimentally for three decades, starting in 1940 (Waddington 1942); however, his views for the most part were ignored by his contemporaries who were intent on placing the gene at the driver's seat (Fox-Keller 2000).

The second example relates to the validity of the 'instructive' hypothesis of differentiation. It proposes that hormones and cytokines determine a specific phenotype in target cells by inducing a lineage-specific gene-activation program. However, recent data do not support this view. For example, precursor erythroid cells, which generate red blood cells, were engineered to lack the erythropoietin (EPO) receptor. As expected, these cells failed to generate mature erythroid cells. In order to further test the specificity of the process, EPO receptor$^{-/-}$ cells were engineered to express the receptor for the reproductive hormone prolactin, which normally plays no role in the development of erythroid cells. The rationale behind this experiment was that the intracellular portions of these receptor molecules were about 20% homologous and that the signal transduction pathway for both hor-

mones contained many similar proteins (Brisken *et al* 2002). These EPO receptor$^{-/-}$ precursor erythroid cells, now bearing the prolactin receptor, were able to produce normal red blood cells in response to the hormone prolactin (Socolovsky *et al* 1998). These researchers further investigated whether it was possible to obtain similar results now using the natural target cells for prolactin, namely, the epithelial cells in the mammary gland. Prolactin receptor$^{-/-}$ mammary gland epithelial cells were placed into the mammary glands of normal mice. As expected, these cells failed to develop into alveoli when these animals underwent pregnancy, which is the natural way of exposing these cells to prolactin. To test for the specificity of the response, these prolactin receptor$^{-/-}$ mammary gland epithelial cells were made to express a fusion construct having the extracellular portion of the prolactin receptor and the intracellular portion of EPO receptor. This construct was able to restitute normal alveolar development during pregnancy when these cells were transplanted into the mammary fat pad (Brisken *et al* 2002). As mentioned above, several of the downstream signal transduction effectors in both erythroid cells and mammary epithelial cells are similar; however, the genes that are expressed after the activation of the signal transduction pathway are entirely different. In other words, these hormone-regulated pathways seem to be "generic" rather than specific. Hence, their role may be permissive rather than instructive, allowing a predetermined differentiation pattern to be expressed. The specificity of the response therefore does not reside in the hormone, its receptor, or the signal transduction pathway. Instead, it appears to be determined by an apparently unrelated 'differentiation' process. Again, we observe the lack of a unique, exclusive correlation between a given protein and its biological function. The promise that the specificity of the effect of a given hormone could be understood by the study of interactions between the receptor and the hormone, and the subsequent activation of the transduction pathway downstream, could not be fulfilled. Specificity becomes contextual, and it probably emerges during commitment to a specific fate – that is, it results from the developmental history of the target cell.

## 4. An organicist perspective of carcinogenesis

### 4.1 *Is the default state of metazoan cells proliferative quiescence?*

As noted above, the second premise adopted by those who favour the SMT is that the default state of cell proliferation in metazoa is quiescence (Alberts *et al* 2002). 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 of the SMT 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 (Sonnenschein and Soto 1999a).

Why is the default state relevant to carcinogenesis? We have previously addressed this issue both experimentally (Powell *et al* 2003; Sonnenschein *et al* 1996; Soto and Sonnenschein 1985, 1987) as well as epistemologically (Sonnenschein and Soto 1991, 1999d; Soto and Sonnenschein 1991, 1993). From a practical point of view, it matters because if we adopt the premise that the default state is quiescence, we become committed to favour the notion of positive control of cell proliferation and thus, to the search for growth factors. If we instead adopt the opposite premise, namely that the default state of cells is proliferation, we 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 (Alberts *et al* 1994, 2002). Thus, researchers are left to choose between these exclusive postulates. This decision will determine what strategy they will follow to resolve it.

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 of cells within a multicellular organism must have remained unaltered; hence, the default state must have been conserved. There is almost complete homology between the replication machinery of yeast and human cells; this suggests that the process remained constant throughout evolution. Unicellular organisms multiply as long as nutrients are available. The novelty that emerged with the advent of multicellularity has been the coordination of the proliferative activity of each lineage making the different tissues of the organism, and thus, the emergence of organismal negative controls that impose a quiescent state to cells. Once these cells become freed from organismal restraints, they return to their default state and proliferate (Sonnenschein and Soto 1999b).

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 has been 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 (e.g. embryo extracts, serum) to the culture medium. Under these operational circumstances, these supplements became generically known as 'growth factors.' It is worth mentioning that this was the operational definition of any substance that improved the propagation of bacteria as well; some pathogens absolutely require 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. In contrast, the literature on genetically engineered knockout mice shows that the so-called growth factors play important roles in cell fate, migration, and a myriad of developmental processes. However, they do not specifically act on quiescent (Go/G1) cells to induce them to enter the S phase, the process they were originally supposed to control (Sonnenschein and Soto 1999d).

In short, for the last two decades, our own research program has been based on the premise that the default state of all cells is proliferation (Soto and Sonnenschein 1985; Baron *et al* 1997; Sonnenschein *et al* 1996; Sonnenschein and Soto 1999f). Recently, Harris (2004) concurred with this notion. 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).

### 4.2 *Is cancer a disease of cell proliferation?*

Practically every book and article dealing with cancer asserts that cancer is a problem of uncontrolled cell proliferation. However, cells in neoplasms do not proliferate faster than normal cells in the same organism. In addition, the growth of neoplasms is not autonomous. For example, neoplasms of the breast and prostate usually regress following the withdrawal of their trophic hormones (estrogens and androgens, respectively). These conceptual problems led to alternative views, namely, that cancer was a problem of differentiation.

### 4.3 *A cellular approach to differentiation using somatic cell hybrids*

H Harris, a pioneer of somatic cell genetics, considered carcinogenesis to be a cellular phenomenon, whereby 'loss of function' changes in the DNA determined the cancer phenotype. He observed that cancer cells were 'normalized' by hybridization with normal cells. These data were consistent with the existence of suppressor genes and inconsistent with that of oncogenes (Harris 1995). In his view, carcinogenesis does not require acquisition of a new function, but rather the disruption of the pattern of differentiation (Steel and Harris 1989).

### 4.4 '*Normalization*' *of cancer cells*

When early embryos are transplanted into ectopic places (e.g. the kidney capsule or the peritoneal cavity) they acquire properties of malignant neoplasms called teratocarcinomas. When teratocarcinoma cells were injected into early embryos (blastocyst stage) they generated normal tissues and organs. In fact, these 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 (Stewart and Mintz 1981). Also, when nuclei from frog Lucke renal carcinoma cells were transplanted into enucleated and activated ova they developed and reached the swimming tadpole stage (DiBerardino *et al* 1986). Additionally, transplantation of tissues from these tadpoles into normal recipients generated normal tissues that were indistinguishable from those of the host (McKinnell *et al* 1993). These data contradicted 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 while the data suggested instead an epigenetic control of the expression of neoplastic phenotypes (Pierce *et al* 1978).

These experiments have demonstrated the reversibility of the neoplastic phenotype; however, they do 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 organization does carcinogenesis occur?

## 5. At what level of biological organization does carcinogenesis occur?

Ernst Mayr (1982) remarked that, for most biological phenomena, exploring levels of complexity lower than that at which the phenomenon of interest occurs usually adds little to what was learned at the original level of inquiry. For example, understanding the structure of the muscle protein myosin has not significantly helped in the under-

standing of how the heart works as a pump. In other cases, lower levels are informative, as is the case of striated muscle contraction. Hence, the exploration of the lower levels should be gradual. Only *a posteriori* will it be determined whether the lower level exploration resulted in a better explanation of the problem being addressed.

Cancer occupies multiple levels of biological organization (Sonnenschein and Soto 1999e, 2000). The preliminary diagnosis is usually made at the organismal level by a physician who examines the symptoms and signs presented by the patient. 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; this corresponds to the tissue level of biological complexity. We postulate that this is the level at which carcinogenesis takes place (see below). 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 by us as directly responsible for the development of neoplasms.

Thus, a rationale that favours 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 (Sonnenschein and Soto 2000). This conclusion does not imply that the gigantic effort invested in describing changes at the gene and cellular levels was fruitless. These data, frequently obtained while using human and rodent tumour 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 lack of relevance of data gathered at a lower level of organization than that at which the phenomenon to be understood takes place may be understood by the following metaphorical example: "if you want to know why traffic jams tend to happen at a certain hour every day, you will still be baffled after you have painstakenly reconstructed the steering, braking and accelerating processes of the thousands of drivers whose various trajectories have summed to create those traffic jams." This pursuit is what the philosopher Daniel C Dennett (1995b), who proudly calls himself a reductionist, called 'preposterous reductionism'.

## 6. Organicism and developmental mechanics as sources of the tissue organization field theory of carcinogenesis

Developmental mechanics, the forerunner of modern developmental biology, established the concept of 'fields of organization' or 'morphogenetic fields' (Needham 1931). These entities were defined as "a collection of cells by whose interactions a particular organ formed" (http://www.devbio.com/about.php). The morphogenetic field became the basic paradigm of embryology. In the 1930s, Needham (1936) and Waddington (1935) 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 neighbouring cells and tissues both during early development and throughout adulthood.

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 all controlled by the same regulatory gene. In fact, the morphogenetic field hypothesis was not disproved, it was just forgotten (http://www.devbio.com/about.php). 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 (De Robertis *et al* 1991). 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 tumours.

## 7. The tissue organization field theory of carcinogenesis

### 7.1 *Premises*

The TOFT is based on two main premises: (i) proliferation is the default state of all cells; and (ii) carcinogens act initially by disrupting the normal interactions that take place among cells in the stroma and parenchyma of an organ (Waddington 1935; Needham 1936; Orr 1958; Sonnenschein and Soto 2000). During embryonal and fetal development, epithelium and the subjacent stroma exert instructive influences over each other. These mor-

phogenetic fields remain operational during adulthood (Rubin 1985). 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 (Clark 1991). However, this pattern prevails in carcinomas and adenocarcinomas, which represent the substantial majority of human neoplasms. Central to this dynamic process of carcinogenesis is its reversibility (Clark 1991). The neoplastic phenotype can be experimentally reversed through cell-cell interactions as demonstrated for embryonal carcinoma cells injected into blastocysts (Illmensee and Mintz 1976), hepatocellular carcinoma cells injected into normal livers (McCullough *et al* 1998), or by modification of the extracellular matrix components (Bissell and Radisky 2001; Weaver *et al* 1997). 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 to a return to the *status quo ante* (Sonnenschein and Soto 1999e).

### 7.2 *Supporting evidence*

Ectopic expression of normal genes in transgenic mice results in neoplastic development. For example, Sternlicht *et al* (1999) observed that manipulations of the microenvironment, such as overexpression of stromelysin-1, can stimulate 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 proteinase inhibitors suppresses the carcinogenic process that ensues when the stromelysin-1 transgene is expressed (Sternlicht *et al* 1999). Interestingly, the resulting neoplasms displayed DNA losses in chromosomes 4 and 7, and those showing epithelial to mesenchymal transitions displayed DNA gains. Hence, alterations in tissue architecture can and do induce neoplasms that, like the spontaneous ones, may end up showing aneuploidy and mutations. As Prehn (1994) remarked, "it may be more correct to say that cancers beget mutations than it is to say that mutations beget cancers".

Using a theory-neutral experimental strategy, we have observed that the recombination of mammary gland stroma exposed to a carcinogen with normal mammary epithelial cells resulted in the development of carcinomas. The reverse combination did not. Carcinomas are tumours of epithelial origin; according to the SMT, the carcinogen should have caused mutations in the epithelial cells. In contrast, these observations suggest that the stroma, rather than the epithelium, is the target of the carcinogen (Maffini *et al* 2004). These results challenge the value of the SMT, while buttressing the TOFT.

### 7.3 *Sporadic versus hereditary cancers*

From our perspective, hereditary cancers (Knudson 1995) should be considered as inborn errors of development. Analogous to inborn errors of metabolism that were extensively described during the second half of the 20th century (Schaub 1991), these cancers represent syndromes that involve the appearing of uni- or multi-locular tumours at different times during development. For instance, these syndromes may appear shortly after birth as in retinoblastoma (Knudson 1993), after puberty or in early adulthood like in multiple endocrine cancers (Poisson *et al* 2003), or prior to the age of incidence for the non-familial form in breast cancers due to BRCA1 and BRCA2 gene mutations (Iau *et al* 2001), 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).

### 7.4 *Cause versus explanation in hereditary cancer*

Finding the genetic cause but not an explanation occurs frequently when studying human genetic diseases. For example, the Lesch-Nyhan syndrome is caused by a germ line mutation in the enzyme hypoxanthine guanine phosphoribosyl transferase. The boys carrying this defect are asymptomatic at birth, but show the first signs of neurological impairment at 3–4 months of age. Later on, the main signs of this fatal disease, automutilation and coreoathetosis become apparent. So far, after more of 30 years of research on this subject, there is no explanation of how a defect in an enzyme of purine metabolism leads to the neurological manifestations of the syndrome (Nyhan 2000). A comparable argument could be made regarding causation and explanation of inborn errors of development (hereditary cancers). Namely, a deletion of the *lethal giant larva-2 (lgl-2)* gene in *Drosophila* is responsible for the development of neuroblastomas in homozygote flies. This gene is expressed when the embryo is a synctytium and is never expressed in neuroblasts, the cell type that becomes cancerous when the gene is defective. As the nervous tissue develops in the mutant *Drosophila* larva, it appears less organized than in its normal coun-

terpart (Mechler *et al* 1991). 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.

## 8. Are the SMT and the TOFT two faces of the same coin?

These two theories are not compatible; they may even be incommensurable, as in Kuhn's lexicon. While one centers on 'one renegade cell,' as asserted by Weinberg (1998) and views cancer as a cell-based disease involving unregulated cell proliferation, the other focuses on a 'society of cells' and views cancer as a problem of tissue organization (Sonnenschein and Soto 1999f). 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 hemizygous for a defective APC, 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 *b*-catenin, which in turn binds to cell adhesion molecules called cadherins (Kemler 1993). APC also binds to the human homologue of *Drosophila* discs large (hDdl), which is also involved in cell-cell adhesion through septate junctions (Hough *et al* 1997). Deletions of this gene in flies result in the loosening of cell-cell contacts, abnormal morphology of the imaginal discs, and neoplastic development (Jursnich *et al* 1990).

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 *b*-catenin as a transcription factor and look at the epithelial cell nucleus (where the transcriptional machinery resides) for putative alterations in the control of cell proliferation, cell cycle and apoptosis.

In sum, genes causing inborn errors of development and cancer could be easily 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 (2003a): "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".

## 9. Conclusion: Does philosophy matter?

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 an 'anything goes' attitude where if results do not fit one hypothesis, they may fit its opposite or an *ad hoc* alternative one; in other words, nothing is rejected and everything is explained. This attitude contrasts with the objectives of science as described by Ayala (1968), namely: "(i) science seeks to organize knowledge in a systematic way, endeavouring patterns of relationship between phenomena and processes; (ii) science strives to provide explanations for the occurrence of events; and finally, (iii) science proposes explanatory hypotheses that must be testable, that is, accessible to the possibility of rejection or falsification".

As we have analysed 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 (Guerra *et al* 2003). In short, something can be anything and its opposite.

In this atmosphere, 'tissue-based' cancer research is also being blended into the oncogene theory. Namely, data showing that extracellular matrix and tissue architecture can normalize the behaviour of cancer cells (Weaver *et al* 2002) 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" (Jacks and Weinberg 2002). Hence, for these committed supporters of the SMT, the problem of how extracellular matrix controls cell phenotypes be-

comes 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. 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 18th century homunculus that determined morphogenesis in the embryo morphed into a genetic program at the middle of the 20th century. Instead, the modern view about epigenesis is 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 (2003b): "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". This organicist perspective acknowledges the existence of emergent phenomena.

Philosophers debate whether emergence is a real phenomenon, or just an epiphenomenon (Kim 1999). A main concern of philosophers about emergentism is 'downward' causation. As Kim (1999) states about synchronic emergence: ". . . apart from any recondite metaphysical principle that might be involved, one cannot escape the uneasy feeling that there is something circular and incoherent about this variety of downward causation". In a general and perhaps trivial sense, molecules mediate these high-level phenomena. However, there are many interactions that occur simultaneously to maintain the structure of a tissue; hence, it is practically impossible to sort out cause and effect in a way that would precisely reveal whether emergents have true causal agency. Therefore, researchers take for granted that emergent phenomena exist and adopt an organicist stance, or alternatively, assume a reductionist stance hoping that a neat, linear causal chain will eventually be identified.

The genetic reductionist claim that morphogenesis is controlled by the genetic patterning of the body plan through a gene-induction cascade is now being modified to include mechanical forces. The unidirectional flow from genes to shape is being modified to include cell movements that cause 'physical stress' in neighbouring cells inducing specific gene expression (Farge 2003). This causal chain, from a molecular event to physical stress inducing the next molecular event appears as an emergent (i.e. an increased number of cells moving) acting as a downward cause. Of course, one may argue that this is not a truly downward cause because an operator could apply compression and generate the same outcome. However the fact is that the only way the embryo has to exercise compression is by creating a tri-dimensional structure that can generate this force.

Perhaps the problem centers in the literal way in which cells are taken as 'low level' parts of the higher level 'tissue.' Historicity is of the essence here. A tissue results from a long series of interactions during which cells move around in relation to one another and change in the process. By the time the tissue is formed, the 'parts' that we identify in them are no longer the parts that interacted in their formation. The cellular components present now did not pre-exist the tissue itself – they are interacting now in a particular way that is reciprocal. When we artificially separate the components of the tissue, for instance the cells forming epithelium and its subjacent stroma, the cells cease to perform the functions they executed when together in their proper tri-dimensional arrangement. However, when recombined, they form a tissue similar to the one from which they originated. Parenthetically, this is also an oversimplification; as previously stated, neither the parenchyma nor the stroma exists in isolation from one other.

Recombining stroma and parenchyma (usually epithelium) from different organs has provided some hints about the inductive role of the stroma over the epithelium, as well as some indications that the epithelium possesses some degree of cellular identity that is not influenced by the stroma. As researchers tend to think that the important part of the tissue is the parenchyma, a rigorous search for subtle reciprocal changes in the stroma is needed. Interestingly, the basement membrane that is interposed between the epithelium and the stroma is made 'in collaboration' by the stroma and epithelial cells. Notably, when epithelial cells are placed in a plastic culture dish, they form a flat layer, quite different from the epithelium of origin. If instead, they are placed in a similar dish coated with basement membrane proteins they associate and recover the original tri-dimensional architecture of the epithelium of origin. They attach to each other forming either sheets or ducts. Moreover, the individual cell shapes also change, as does the intracellular placement of their organelles. How can causation be studied here? Is the tissue causing the formation of a basement membrane? And then, is the basement membrane 'causing' the normal architecture of the epithelium – and thus the tissue? This looks like circular causation.

Limb development also offers an example of circular causation. The skeletal structures of the limb are initiated

by the expansion of precartilage condensations within the mesenchyma. Proteins of the transforming growth factor-beta (TGF-*b*) family regulate this process (Downie and Newmann 1994). This process is mediated by TGF-*b* through a positive autoregulatory pathway (van Obberghen-Schilling *et al* 1988), namely, cells in these precartilage condensations secrete TGF-*b*, and TGF-*b* in turn makes them secrete more of it.

Tooth development offers other examples of quasi 'circular causation'. The mandibular epithelium 'causes' the underlying mesenchyme to condense (i.e. it appears more cell-dense at the microscope). If the epithelium is recombined with other kinds of mesenchyme at this stage of development, it induces the generation of tooth structures. Soon thereafter this 'inductive' potential is lost. If the condensed mesenchyme from underneath the mandibular epithelium is now recombined with other epithelia, the recombinant generates tooth structures. Moreover, the gene products involved in early tooth development also are implied in mammary gland development and in hair follicle formation. Hence, in order to understand how form is generated it may be more productive to concentrate on the higher-level phenomena (cell movement, cell proliferation, cell death) than exclusively in patterns of gene expression.

In sum, we think that at best, in biology several levels of explanation are necessary. Development and cancer will not be reduced to complex series of protein interactions, but rather a multilevel explanation will be required. In some instances molecules will do the explanatory job, in others physical forces, but at the core they will remain a problem of tri-dimensional tissue organization. To pretend that technological innovations will enable the understanding of these complex phenomena may just be wishful thinking. We instead need a novel way of thinking about these problems that takes all levels of biological organization into consideration.

## Acknowledgements

We are grateful to Drs Michel Morange and Jean-Jacques Kupiec (both at Centre Cavailles, Ecole Normale Superieure, Paris, France), Pierre Sonigo (Institut Cochin, Paris, France), Stuart Newman, Evelyn Fox-Keller and Vidyanand Nanjundiah. 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) and the Pelling conference. We are also grateful to Cheryl Schaeberle, Janine Calabro and April Flynn for their technical and editorial assistance. An earlier version of this analysis was presented at the Conference on Reduction and Emergence organized by Max Kistler at the Ecole Normale Superieure in Paris on 12–15 November 2003, and published as a commentary in *BioEssays* (September Issue, 2004).

## References

Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson J D 1994 *Molecular biology of the cell* (New York: Garland Publishing Inc.) p. 891

Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P 2001 *Molecular biology of the cell* (New York: Garland Publishing Inc.) pp 1313–1362

Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P 2002 *Molecular biology of the cell* (New York: Garland Publishing Inc.) p. 1015

Ayala F J 1968 Biology as an autonomous science; *Am. Sci.* **56** 207–221

Baron U, Gossen M and Bujard H 1997 Tetracycline-controlled transcription in eukaryotes: novel transactivators with graded transactivation potential; *Nucleic Acids Res.* **25** 2723–2729

Bassett D E Jr, Eisen M B and Boguski M S 1999 Gene expression informatics – It's all in your mine; *Nature Genet.* **21** 51–55

Benson K 2001 T H Morgan's resistance to the chromosome theory; *Nat. Rev. Genet.* **2** 469–474

Bishop J M 1985 Viral oncogenes; *Cell* **42** 23–38

Bissell M J and Radisky D 2001 Putting tumours in context; *Nat. Rev. Cancer* **1** 46–54

Brisken C, Socolovsky M, Lodish H F and Weinberg R 2002 The signaling domain of the erythropoietin receptor rescues prolactin receptor-mutant mammary epithelium; *Proc. Natl. Acad. Sci. USA* **99** 14241–14245

Brown P O and Botstein D 1999 Exploring the new world of the genome with DNA microarrays; *Nature Genet.* **21** 33–37

Bunge M 2004 *Emergence and convergence* (Toronto: University of Toronto Press) pp 40–52

Clark W H 1991 Tumour progression and the nature of cancer; *Br. J. Cancer* **64** 631–644

Cooper G M 1983 Transforming genes of neoplasms; *Prog. Nucleic Acid Res. Mol. Biol.* **29** 273–277

Curtis H J 1965 Formal discussion of: Somatic mutations and carcinogenesis; *Cancer Res.* **25** 1305–1308

Dawkins R 1976 *The selfish gene* (Oxford: Oxford University Press)

De Robertis E A, Morita E M and Cho K W Y 1991 Gradient fields and homeobox genes; *Development* **112** 669–678

Dennett D C 1995a *Darwin's dangerous idea* (New York: Simon and Schuster) p. 21

Dennett D C 1995b *Darwin's dangerous idea* (New York: Simon and Schuster)

DiBerardino M A, Orr N H and McKinnell R G 1986 Feeding tadpoles cloned from Rana erythrocyte nuclei; *Proc. Natl. Acad. Sci. USA* **83** 8231–8234

Downie S A and Newmann S A 1994 Morphogenetic differences between fore and hind limb precartilage mesenchyme: Relation to mechanisms of skeletal pattern formation; *Dev. Biol.* **162** 195–208

Farge E 2003 Mechanical induction of twist in the *Drosophila* foregut/stomodeal primordium; *Curr. Biol.* **13** 1365–1377

Fox-Keller E 2000 *The century of the gene* (Cambridge: Harvard University Press) pp 73–132

Gilbert S F and Sarkar S 2000 Embracing complexity: Organicism for the 21st century; *Dev. Dynamics* **219** 1–9

Greenspan R J 2001 The flexible genome; *Nat. Rev. Genet.* **2** 383–387

Griffiths P E and Gray R D 2000 Darwinism and developmental systems; in *Cycles of contingency: Developmental systems and evolution* (eds) S Oyama, P E Griffiths and R D Gray (Cambridge: MIT Press) pp 195–218

Guerra C, Mijimolle N, Dhawahir A, Dubus P, Barradas M, Serrano M, Campuzano V and Barbacid M 2003 Tumor induction by an endogenous *K-ras* oncogene is highly dependent on cellular context; *Cancer Cell.* **4** 111–120

Hahn W C and Weinberg R A 2002a Mechanisms of disease: Rules for making human tumor cells; *New Engl. J. Med.* **347** 1593–1603

Hahn W C and Weinberg R A 2002b Modelling the molecular circuitry of cancer; *Nat. Rev. Cancer* **2** 331–342

Harris H 1995 *The cells of the body: A history of somatic cell genetics* (New York: Cold Spring Harbor Laboratory Press) pp 211–247

Harris H 2004 Tumor suppression: putting on the breaks; *Nature* (*London*) **427** 201

Hough C D, Woods D F, Park S and Bryant P J 1997 Organizing a functional junctional complex requires specific domains of the *Drosophila* MAGUK Discs large; *Genes Dev.* **11** 3242–3253

Hull D 1974 *The philosophy of biological science* (Englewood Clifts NJ: Prentice Hall) pp 8–44

Humpherys D, Eggan K, Akutsu H, Friedman A, Hochedlinger K, Yanagimachi R, Lander E S, Golub T R and Jaenisch R 2002 Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei; *Proc. Natl. Acad. Sci. USA* **99** 12889–12894

Iau P T, Macmillian R D and Blamey R W 2001 Germ line mutations associated with breast cancer susceptibility; *Eur. J. Cancer* **37** 300–321

Illmensee K and Mintz B 1976 Totipotency and normal differentiation of single teratocarcinoma cell cloned by injection into blastocysts; *Proc. Natl. Acad. Sci. USA* **73** 549–553

Jacks T and Weinberg R A 2002 Taking the study of cancer cell survival to a new dimension; *Cell* **111** 923–925

Jacob F 1982 *The possible and the actual* (Seattle: University of Washington Press)

Jursnich V A, Fraser S E, Held L I Jr, Ryerse J and Bryant P J 1990 Defective gap-junctional communication associated with imaginal disc overgrowth and degneration caused by mutations of the dco gene in *Drosophila*; *Dev. Biol.* **140** 413–429

Kemler R 1993 From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion; *Trends Genet.* **9** 317–321

Kim J 1999 Making Sense of Emergence; *Philos. Stud.* **95** 3–36

Kinzler K W and Vogelstein B 1996 Lessons from hereditary colorectal cancer; *Cell* **87** 159–170

Knudson A G Jr 1989 Hereditary cancers disclose a class of cancer genes; *Cancer* **63** 1888–1891

Knudson A G Jr 1993 Pediatric molecular oncology: Past as prologue to the future; *Cancer* **71** 3320–3324

Knudson A G Jr 1995 Mutation and cancer: a personal odyssey; *Adv. Cancer Res.* **67** 1–23

Land H, Parada L F and Weinberg R A 1983 Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes; *Nature* (*London*) **304** 596–602

Levine A J 1997 p53, the cellular gatekeeper for growth and division; *Cell* **88** 323–331

Loeb L A 2001 A mutator phenotype in cancer; *Cancer Res.* **61** 3230–3239

Maffini M V, Soto A M, Calabro J M, Ucci A A and Sonnenschein C 2004 The stroma as a crucial target in rat mammary gland carcinogenesis; *J. Cell Sci.* **117** 1495–1502

Martins-Green M, Boudreau N and Bissell M J 1994 Inflammation is responsible for the development of wound-induced tumors in chickens infected with Rous Sarcoma virus; *Cancer Res.* **54** 4334–4341

Matsumine A *et al* 1996 Binding of APC to the human homolog of the *Drosophila* discs large tumour suppressor protein; *Science* **272** 1020–1023

Mayr E 1982 *The growth of biological thought: Diversity, evolution and inheritance* (Cambridge: Belknap Press) pp 1–146

McCullough K, Coleman W B, Ricketts S L, Wilson A W, Smita G J and Grisham J W 1998 Plasticity of the neoplastic phenotype *in vivo* is regulated by epigenetic factors; *Proc. Natl. Acad. Sci. USA* **95** 15333–15338

McKinnell R G *et al* 1993 Genomic plasticity of the Lucke renal carcinoma: a review; *Int. J. Dev. Biol.* **37** 213–219

Mechler B M *et al* 1991 *Drosophila* as a model system for molecular analysis of tumorogenesis; *Environ. Health Perspect.* **93** 63–71

Morange M 2002 The gene: Between holism and generalism; in *Promises and limits of reductionism in the biomedical sciences* (eds) D L Hull and M H V Van Regenmortel (Weinheim: John Wiley) pp 179–190

Moss L 2003a *What genes can't do* (Cambridge: MIT Press) p. 129

Moss L 2003b *What genes can't do* (Cambridge: MIT Press) p. 186

Moss L 2003c *What genes can't do* (Cambridge: MIT Press)

Needham J 1931 *Chemical embryology* (Cambridge: Cambridge University Press)

Needham J 1936 New advances in chemistry and biology of organized growth; *Proc. R. Soc. B.* **29** 1577–1626

Newbold R F and Overell R W 1983 Fibroblast immortality is a prerequisite for transformation by EJ c-HA-ras oncogene; *Nature* (*London*) **304** 648–651

Nyhan W L 2000 Dopamine function in Lesch-Nyhan disease; *Environ. Health Perspect.* (*Suppl. 3*) **108** 409–411

Orr J W 1958 The mechanism of chemical carcinogenesis; *Br. Med. Bull.* **14** 99–101

Parsons R *et al* 1995 Mismatch repair deficiency in phenotypically normal human cells; *Science* **268** 738–740

Pierce G B, Shikes R and Fink L M 1978 *Cancer: A problem of developmental biology* (Englewoods Cliffs: Prentice-Hall)

Poisson A, Zablewska B and Gaudray P 2003 Menin interacting proteins as clues toward the understanding of multiple endocrine neoplasia type 1; *Cancer Lett.* **189** 1–10

Powell C E *et al* 2003 Plasma membrane-resident albumin binding protein associated with the proliferation of MCF7 serum-sensitive cells; *Steroids* **68** 487–496

Prehn R T 1994 Cancers beget mutations *versus* mutations beget cancers; *Cancer Res.* **54** 5296–5300

Rangarajan A and Weinberg R A 2003 Comparative biology of mouse versus human cells: modelling human cancer in mice; *Nat. Rev. Cancer* **3** 952–959

Reitmair A H, Cai J C, Bjerknes M, Redston M, Cheng C, Pind M T, Hay K, Mitri S, Bapat B V, Mak T W and Gallinger S 1996 MSH2 deficiency contributes to accelerated APC-mediated intestinal tumorigenesis; *Cancer Res.* **56** 2922–2926

Rideout W M, Eggan K and Jaenisch R 2001 Nuclear cloning and epigenetic reprogramming of the genome; *Science* **293** 1093–1098

Robanus-Maandag E, Dekker M, van der Valk M, Carrozza M L, Jeanny J C, Dannenberg J H, Berns A and te Riele H

1998 p107 is a suppressor of retinoblastoma development in pRb-deficient mice; *Genes Dev*. **12** 1599–1609

Rosenberg A 1994 *Instrumental biology, or, The disunity of science* (Chicago: University of Chicago Press)

Rowlatt C 1994 Some consequences of defining the neoplasm as focal self-perpetuating tissue disorganization; in *New frontiers in cancer causation* (ed.) O H Iversen (Washington DC: Taylor and Francis) pp 45–58

Rubin H 1985 Cancer as a dynamic developmental disorder; *Cancer Res*. **45** 2935–2942

Rubinfeld B *et al* 1993 Association of the APC gene product with beta-catenin; *Science* **262** 1731–1734

Schaub J 1991 *Inborn errors of metabolism* (Philadelphia: Lippincott, Williams and Wilkins)

Smith K J, Johnson K A, Bryan T M, Hill D E, Markowitz S, Willson J K V, Paraskeva C, Peterson G M, Hamilton S R, Vogelstein B and Kinzler K W 1993 The APC gene product in normal and tumor cells; *Proc. Natl. Acad. Sci. USA* **90** 2846–2850

Socolovsky M, Fallon A E J and Lodish H F 1998 The prolactin receptor rescues EpoR$^{-/-}$ erythroid progenitors and replaces EpoR in a synergistic interaction with c-kit; *Blood* **92** 1491–1496

Sonnenschein C and Soto A M 1991 Cell proliferation in metazoans: negative control mechanisms; *Cancer Treat. Res*. **53** 171–194

Sonnenschein C and Soto A M 1999a Cell proliferation: the background and the premises; in *The society of cells: Cancer and control of cell proliferation* (New York: Springer Verlag) pp 1–13

Sonnenschein C and Soto A M 1999b Epilogue Moving toward the integration of cell proliferation, carcinogenesis and neoplasia into biology; in *The society of cells: Cancer and control of cell proliferation* (New York: Springer Verlag) pp 134–143

Sonnenschein C and Soto A M 1999c The enormous complexity of cancer; in *The society of cells: Cancer and control of cell proliferation* (New York: Springer Verlag) pp 99–111

Sonnenschein C and Soto A M 1999d *The society of cells: Cancer and control of cell proliferation* (New York: Springer Verlag) pp 41–59

Sonnenschein C and Soto A M 1999e *The society of cells: Cancer and control of cell proliferation* (New York: Springer Verlag) pp 91–143

Sonnenschein C and Soto A M 1999f *The society of cells: Cancer and control of cell proliferation* (New York: Springer Verlag)

Sonnenschein C and Soto A M 2000 The somatic mutation theory of carcinogenesis: Why it should be dropped and replaced; *Mol. Carcinog*. **29** 1–7

Sonnenschein C, Soto A M and Michaelson C L 1996 Human serum albumin shares the properties of estrocolyone-I, the inhibitor of the proliferation of estrogen-target cells; *J. Steroid Biochem. Mol. Biol*. **59** 147–154

Soto A M and Sonnenschein C 1985 The role of estrogens on the proliferation of human breast tumor cells (MCF-7); *J. Steroid Biochem*. **23** 87–94

Soto A M and Sonnenschein C 1987 Cell proliferation of estrogen-sensitive cells: the case for negative control; *Endocrinol. Rev*. **8** 44–52

Soto A M and Sonnenschein C 1991 Regulation of cell proliferation: the negative control perspective; *Ann. NY Acad. Sci*. **628** 412–418

Soto A M and Sonnenschein C 1993 Regulation of cell proliferation: is the ultimate control positive or negative?; in *New frontiers in cancer causation* (ed.) O H Iversen (Washington DC: Taylor and Francis) pp 109–123

Steel D M and 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

Sternlicht M D, Lochter A, Sympson C J, Huey B, Rougier J-P, Gray J W, Pinkel D, Bissell M J and Werb Z 1999 The stromal proteinase MMP3/Stromelysin-1 promotes mammary carcinogenesis; *Cell* **98** 137–146

Stewart T A and Mintz B 1981 Successful generations of mice produced from an established culture line of euploid teratocarcinoma cells; *Proc. Natl. Acad. Sci. USA* **78** 6314–6318

Su L K, Burrell M, Hill D E, Gyuris J, Brent R, Wiltshire R, Trent J, Vogelstein B and Kinzler K W 1995 APC binds to the novel protein EB1; *Cancer Res*. **55** 2972–2977

van Obberghen-Schilling E, Roche N S, Flanders K C, Sporn M B and Roberts A B 1988 Transforming growth factor beta-1 positively regulates its own expression in normal and transformed cells; *J. Biol. Chem*. **263** 7741–7746

Waddington C H 1935 Cancer and the theory of organizers; *Nature* (*London*) **135** 606–608

Waddington C H 1942 Canalization of development and its inheritance of acquired characters; *Nature* (*London*) **150** 563–565

Wang T-L, Rogo C, Silliman N, Ptak J, Markowitz S, Willson J K V, Parmigiani G, Kinzler K W, Vogelstein B and Velculescu V E 2002 Prevalence of somatic alterations in the colorectal cancer cell genome; *Proc. Natl. Acad. Sci. USA* **99** 3076–3080

Weaver V M, Lelievre S, Lakins J N, Chrenek M A, Jones J C, Gianeotti F, Werb Z and Bissell M J 2002 *b*4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium; *Cancer Cell*. **2** 205–216

Weaver V M, Petersen O W, Wang F, Larabell C A, Briand P, Damsky C and Bissell M J 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

Weinberg R A 1998 *One renegade cell: how cancer begins* (New York: Basic Books)

Weinstein I B 2002 Cancer. Addiction to oncogenes – the Achilles heal of cancer; *Science* **297** 63–64

Willis R A 1967 *Pathology of tumors* (London: Butterworths)

Wolpert L 2002 Unpersuasive thoughts and unhelpful ideals; *Science* **295** 633

*e*Publication: 27 September 2004