Cell. Mol. Life Sci. (2012) 69:3351–3360
DOI 10.1007/s00018-012-1148-5

Cellular and Molecular Life Sciences

VISIONS AND REFLECTIONS

What mechanisms/processes underlie radiation-induced genomic instability?

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Received: 12 April 2012 / Revised: 22 August 2012 / Accepted: 23 August 2012 / Published online: 6 September 2012

Abstract Radiation-induced genomic instability is a modification of the cell genome found in the progeny of irradiated somatic and germ cells but that is not confined on the initial radiation-induced damage and may occur de novo many generations after irradiation. Genomic instability in the germ line does not follow Mendelian segregation and may have unpredictable outcomes in every succeeding generation. This phenomenon, for which there is extensive experimental data and some evidence in human populations exposed to ionising radiation, is not taken into account in health risk assessments. It poses an unknown morbidity/mortality burden. Based on experimental data derived over the last 20 years (up to January 2012) six mechanistic explanations for the phenomenon have been proposed in the peer-reviewed literature. This article compares these hypotheses with the empirical data to test their fitness to explain the phenomenon. As a conclusion, the most convincing explanation of radiation-induced genomic instability attributes it to an irreversible regulatory change in the dynamic interaction network of the cellular gene products, as a response to non-specific molecular damage, thus entailing the rejection of the machine metaphor for the cell in favour of one appropriate to a complex dissipative dynamic system, such as a whirlpool. It is concluded that in order to evaluate the likely morbidity/mortality associated with radiation-induced genomic instability, it will be necessary to study the damage to processes by radiation rather than damage to molecules.

Keywords Non-targeted effects · Genomic instability · Ionising radiation · Self-organisation · High dimensional complex system · Cell regulation · Epigenetic processes · Information · Extracellular matrix · Centrosome · DNA methylation · Chromosomal damage · Reactive oxygen species

Statement of the problem

In 1992, Kadhim et al. [1] uncovered chromosomal instability, a sub-category of a more general phenomenon known as genomic instability (GI), in the clonal descendants of explanted haemopoetic cells exposed to ionising radiation. GI in response to ionising radiation has since been observed in many contexts [2, 3] and has been found to be induced by other agents, for example, heavy metals [4]. Application of target theory[1] to GI induced by radiation indicates a target comparable in size to the cell nucleus [5], a strong indication that the phenomenon is epigenetically regulated. The theoretical framework assumed to govern radiobiology prior to 1992, based on targets of dimensions of a micron or less [6] and radiation damage to specific gene coding sequences, is unable to explain GI [7].

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[1] Due to the discrete and localised nature of energy deposition by ionising radiation, it is possible to estimate from the dose response the minimum size of the biological target (chromosome, gene, etc.) that when hit gives rise to the observed biological effect, in this case GI.

Strategy adopted in this article

The empirical features that define radiation-induced GI have accumulated over the past 20 years as the result of an extensive programme of experimental work funded by, among other organisations, the European Commission under its Framework Programme and the US Department of Energy under its Low Dose Radiation Research Programme. During that period six hypotheses for mechanisms governing GI have been published in the peer-reviewed literature. In this article we summarise the defining features of GI and evaluate each of the six hypotheses against this empirical evidence to identify the most promising candidate mechanisms/processes best able to explain the phenomenon of radiation-induced GI.

Radiation-induced genomic instability defined

In their original experiment [1], Kadhim et al. exposed explanted bone marrow cells to mono-energetic alpha particles such that, on average, each cell was transited by about one alpha particle. After irradiation, surviving cells were grown singly into clones and subjected to karyotypic analysis. Several clones were found to contain both normal cells and cells with various kinds of chromosomal damage. The expectation, on the basis of the then prevailing dogma, was that a minority of clones would contain cells with chromosome aberrations but that all such cells would bear the same aberration and that the majority of clones would be normal. This expectation is based on the assumption that after the first cell division following irradiation, if a damaged cell could divide, the damage would be replicated in all further viable progeny. The rationalisation of the Kadhim et al. result was that the early divisions in the clone gave rise to apparently normal cells but after some number of cell divisions chromosomal aberrations appeared de novo. Thus, GI is characterised by late damage markers in the progeny of the irradiated cells at a high frequency in relation to the dose delivered, indicating that the "target" for the effect is large [5]. This latter point precludes a genetic origin for GI. GI has also been observed for up to 24 months post irradiation both in vivo in mice subjected to whole-body irradiation with X-rays and neutrons and in bone marrow irradiated in vitro and transplanted into ablated mice [8]. More recently, irradiated *Caenorhabditis elegans* have exhited GI several generations after irradiation [9]. Other studies have shown GI appearing in the progeny of males irradiated at the spermatogonial cell stage of spermatogenesis, for example in the case of the reversion of the pink eye mutation in mice [10], mini-satellite mutation in humans [11–14] and extended sequence tandem repeat (ESTR) mutations in mice [15–17].

Several other endpoints exhibit the characteristics of radiation-induced GI, for example, mutations at the *hprt* locus in cells irradiated in vitro, which can be sub-categorised as "late" or "delayed" and due to point mutations [18], or "early" or directly inflicted mutations that are predominantly deletions [19, 20]. Furthermore, GI can be induced with a number of agents other than radiation, including heavy metals [4], chemical mutagens [21, 22], bacterial infection [23] and incubation with heat-killed bacteria [24]. GI has also been observed in yeast [25] and in fish cells [26]. It is, therefore, reasonable to conclude that GI is a generic response of cells to ionising radiation and other agents.

Thus, evidence accumulated since 1992 indicates that GI is:

- a universal radiation-induced effect in vitro and in vivo, which may persist in the absence of molecular damage;
- heritable both at somatic cell division and trans-generationally and is essentially the same process for both categories of cell although the manifestations may differ;
- characterised by a mutator phenotype, i.e. an increased spontaneous mutation rate, leading to increases in DNA mutations and molecular/chromosomal damage;
- contingent on the properties of the affected cell in terms of the form (i.e., chromosomal damage, mutation, etc.) in which GI is manifested;
- characterised by a target size approximating to that of the cell nucleus, therefore, being much larger than a gene coding sequence.

The six hypotheses that have been proposed to explain the mechanistic basis for radiation-induced GI up to January 2012 are evaluated below:

Hypothesis 1 GI is a consequence of tissue dysregulation with the extra-cellular matrix (ECM) serving as the long-term damage "memory" and as the primary regulator of cellular function.

Explanation

Signalling between cells through the microenvironment is hypothesised to be the cause of GI and to provide support for its persistence [27] ultimately leading to the appearance of cancer in the affected tissues. This hypothesis is based on earlier ideas that give a major regulatory role to the ECM [28, 29]. In accordance with the stem cell niche theory and experimental data, the disruption of communication between cells in the tissue or with the ECM removes the limiting factors for cell proliferation and malignant transformation [30–32]. Cells growing in tissues tend to be more quiescent than single cells or those cultured in vitro

[33], and this is attributed to inhibition of proliferation by the tissue. Thus, alterations in stromal signalling resulting from the presence of GI cells can be expected to have major effects on cancer progression in stromal tissues [34]. In the context of this hypothesis radiation can then be seen as a non-specific stress factor that modifies signalling between cells. For example, in vivo studies showed changes in the ECM of the irradiated murine mammary gland as well as the activation of the transforming growth factor β (TGF β) [35]. This signalling molecule regulates cell differentiation, apoptosis, cell growth, and chemotaxis [36, 37]. TGF β is postulated to be an important molecule for mediating the effects of radiation exposure of the ECM [38] and is suggested as a central mediator of abnormal extra-cellular signalling resulting from radiation damage. The authors recognise that tissue function is greater than the sum of its parts and they envisage radiation-induced GI, closely related to carcinogenesis, as a "two-compartment" problem, encompassing genomic sequence damage and damage to cellular communication [39]. They further assume that the cellular phenotype is dictated and regulated by the tissue microenvironment [40].

Evaluation

This is one of three hypotheses that invoke a regulatory origin of GI. Regulation entails information: in the case of genetic regulation, which is the prevailing dogma, mutational damage to regulatory gene coding sequences is assumed to result in regulatory changes in the affected cells. Genetic regulatory mechanisms are ruled out in the case of GI on target size grounds. Hypothesis 1 however proposes that regulation from the ECM is able to "over-rule" genetic regulatory processes. What is lacking in this proposal is the origin of the information that is deployed by the ECM in this regulatory role. The ECM is as much the product of the genotype as any cell in the organism and as such cannot overrule its progenitor without acquiring the necessary information from an independent source. To be tenable this hypothesis needs to identify the source of the implied information.

Hypothesis 2 GI results from damage to centrosomes leading to delayed effects such as spindle multi-polarity followed by aneuploidy and chromosomal instability.

Explanation

The authors propose a leading role for centrosome aberrations and deregulation in the triggering and inheritance of chromosomal instability based on in vitro studies on normal human mammary epithelial cells [41]. This form of GI apparently has a threshold of about 10 mGy. The centrosome is an organelle responsible for organising the microtubule cytoskeleton of animal cells [42]. Centrosome amplification, e.g., the presence of more than two centrosomes in a dividing cell, is associated with aneuploidy and cell death in many cancers because of spindle multi-polarity and is the cause of an unequal distribution of chromosomes between daughter cells [43]. The authors show that centrosome deregulation occurs in the first generation of radiation-treated cells and has detrimental effects, including GI, in subsequent cell progeny by unidentified mechanisms.

Evaluation

The centrosome is much smaller (sub-micron) than the cell nucleus and thus cannot itself present a big enough target to be responsible for GI. This organelle is not essential as microtubules are able to self-organise [44]: ablation of centrosomes from transformed [45] and untransformed [46] cells does not prevent cell division and centrosomes self-organise in the daughter cells. No mechanism by which radiation, or any other environmental agent, can result indirectly in the deregulation of the centrosome is advanced and in the absence of that this hypothesis cannot explain GI.

Hypothesis 3 The induction of inflammation in tissue by radiation results in raised levels of ROS, leading to the GI state, and these are sustained as a result of the inheritance of damaged mitochondrial DNA.

Explanation

An association between free-radical mediated processes and GI has been proposed and extensively studied (reviewed in [47, 48]). Triggered by ionising radiation, oxidative stress and inflammation in tissues are tightly bound to the production of reactive oxygen (ROS) and nitrogen (RNS) species by the affected cells. Moreover, persistent inflammation is associated with an increase in DNA mutations and the induction of cancer [47, 49]. In vitro studies of haematopoietic and CHO cells showed that DNA damage, cell membrane damage and apoptosis or necrosis were due to the increase of ROS in cell cultures [50–53]. Mitochondria, which produce high concentrations of ROS as a part of normal metabolic processes, are proposed as the origin of chronic oxidative stress also associated with unbalanced respiration processes, reduced activity of superoxide dismutase, or mutations in succinate dehydrogenase genes [54–57]. An attempt to measure chromosomal instability and detect evidence for the bystander effect[2] in vivo at doses up to 500 mGy failed

[2] The bystander effect occurs when a cell that does not directly receive ionising events responds, by becoming unstable, to ionising events occurring in neighbouring cells as a result of some signalling process.

[58]. The most recent publication on this hypothesis [59], based on the study of gamma-irradiated mice fed on a diet containing an anti-inflammatory drug, has demonstrated a reduction in chromosomal instability in bone marrow cells together with a marked reduction in markers of inflammation. The authors conclude that this finding supports the hypothesis that the chromosomal instability observed in that system in vivo is not an intrinsic property of cells but a consequence of inflammatory processes.

Evaluation

As this hypothesis invokes a tissue response, inflammation, which requires the active participation of mitochondria to sustain it, it cannot account for the transmission of GI trans-generationally after paternal irradiation or, in the context of the latest statement of the hypothesis [59], GI in in vitro experiments. As noted by Morgan [2] in respect of inflammatory signals, "While secreted factors may explain radiation-induced genomic instability, bystander effects, death-inducing effects and clastogenic factors, it is difficult to imagine a scenario whereby a secreted factor could influence the reported trans-generational effects. It is unlikely that the radiation directly damages the expanded simple tandem repeats themselves or that the negligible cytoplasmic component of the mature sperm could carry a secreted factor or other radiation-induced species into the egg during fertilization". Thus, acceptance that radiation-induced GI is the same phenomenon in both somatic and germ cells effectively rules out oxidative stress as the cause of GI. Perhaps the strongest evidence that they are the same phenomenon comes from the early work of Schiestl et al. [10] on the induced reversion of the pink eye mutation in mice, where it is clear that reversion can take place both before and after fusion of the irradiated sperm with the egg. This result also sheds light on the claim that GI is not an intrinsic property of cells [59]. It is clear that what is transmitted by the irradiated father is the *propensity* for the mutation to revert, and it is equally clear that what was transmitted from the irradiated cell in the Kadhim et al. [1] experiment was the *propensity* to form delayed chromosomal aberrations. The chromosomal damage, in vitro and in vivo, is one material (molecular) manifestation, one that was chosen for the experiment, of an underlying process. The conclusion that being able to manipulate (by interfering with the inflammatory process) that molecular endpoint necessarily signifies that an underlying GI process has been manipulated is based on a category error, namely, confusing the material cause with the efficient cause.

The authors claim in their most recent results using the in vivo mouse model [58, 59] that the chromosomal instability observed is not a result of GI but of a tissue response to radiation involving inflammation; however, they need now to explain their original results using mouse bone marrow cells in vitro [1], where inflammation is highly unlikely to be involved.

Hypothesis 4 Telomere damage by radiation unmasks large fractions of genetic damage accumulated during the history of the cell because of the loss of heterozygosity and chromosomal imbalance.

Explanation

This approach to understanding the origins of radiation-induced GI is based on telomere damage as a causal event. Damage to telomeres leads to chromosomal instability and eventually cancer [60]. Telomeres are the end-capping DNA–protein complexes on chromosomes, protecting them from degradation and fusion [61]. Damaged telomeres are problematic regions for repair by non-homologous end joining or homologous recombination pathways, and their maintenance by telomerases is limited in humans to normal germ cells. However, it has also been observed in some leukocytes, bone marrow cells, embryonic cells and abnormal somatic cells, as a stage in carcinogenesis [62] and in stem cells [63]. Telomere loss causes a series of breakage/fusion/bridge (bfb) cycles resulting in DNA amplification and large deletions [62]. It is proposed that such radiation-induced damage of telomeres can be the causal event for unmasking primary recessive genomic damage accumulated in previous cell generations and the cell's own history through chromosome imbalance or loss of heterozygosity [60].

Evaluation

Telomeres in mammalian cells are composed of repeat sequences totalling 2–50 kb at the ends of chromosomes [64] and are therefore far too small to satisfy the target size criterion for the induction of GI. Telomere-based mechanisms can also only explain chromosomal instability but not other GI manifestations such as sequence mutations or the transgenerational effects. This hypothesis should take into consideration the situation in which the telomerase activity is present in the cell (see explanation above). Such activity indicates that part of the telomere damage would be eliminated because of the telomerase-associated concatenation of the telomeric sequences. The overall b/f/b outcome must, therefore, be dependent on the ratio between the speed of the b/f/b cycle build-up and the telomerase turnover frequency and concentration.

Hypothesis 5 Chromatin marking (DNA methylation and histone acetylation) and changes in non-coding RNA (ncRNA) profiles lead to altered protein expression patterns and GI both in vivo and in vitro.

Explanation

Many examples of exposure to ionising radiation associated with the modification of methylation or acetylation patterns of chromatin marking and of ncRNA profiles, are cited in extensive reviews of the connections between epigenetic changes and non-targeted effects, including GI, observed in somatic and germ cells [65, 66]. For example, the disruption of two genes involved in methylation in cultured embryonic stem cells eliminated the transmission of GI at mitosis [67]. Ilnytskyy and Kovalchuk [66] acknowledge that the mechanism of the global DNA methylation changes in irradiated tissue is unknown and a similar situation exists for the transcription of regulatory ncRNA elements.

In addition to DNA methylation and regulatory RNAs, other markers of non-mendelian inheritance of GI, namely mini-satellite mutations in humans [11–14] and ESTR mutations in mice [15–17], have been observed. The small size of the repeat sequences that are mutated, the frequency of mutation observed and the persistence of these effects are taken to indicate an epigenetic mechanism that identifies with the phenomenon of GI (reviewed in [68]). The clearest illustrations of such epigenetic effects are the experimentally determined mutation rates of ESTR in mice. Paternal exposure of mice to another mutagen, ethylnitrosourea (ENU), which causes DNA base alkylation rather than strand breakage, also results in increased ESTR mutations of the offspring [21]. The authors believe that the evidence clearly points to some sort of epigenetic chromatin-contingent trans-generational inheritance of GI in the case of spermatogonia [68].

The level of expression of several genes, for example, mitogen-activated protein kinases, has been shown to be altered in the progeny of irradiated mice over four generations in a way that exhibits substantial variation between individuals [69, 70]. The authors postulated that they are observing GI associated with de novo methylation changes. Studies of the progeny of irradiated mice (paternal and combined paternal/maternal exposure) showed DNA methylation loss in several organs of the offspring as well as changes in DNA methyltransferase methyl-binding protein MeCP2 levels [71, 72].

Evaluation

This is the second of the three hypotheses invoking a regulatory origin of GI. There is clear evidence that chromatin and DNA marking and ncRNAs are all involved in regulation of cellular function [73]. The question of from where the information to determine the DNA methylation, histone modification, and ncRNA expression patterns derives is so far unanswered. Methylation of DNA in the germ cell undergoes extensive, although not full, removal and reinstatement of the marking pattern before and after the formation of the zygote [74–76]. No source of information has been advanced by the hypothesis developers to explain how the correct reinstatement is achieved. Thus, the information source that determines the correct reprogramming is not known, and until it is, it is not possible to say whether such marking is causal or consequential. Furthermore, in a wider context, Huang argues [77] that marking has neither the locus specificity nor the stability, a point confirmed experimentally by Deal et al. [78], to be the primary cell regulatory mechanism. The initiation step of transcription resulting in the introduction of a gene product into the cell has been intensively studied and has been found to depend on several seemingly loosely related factors in addition to chromatin and DNA marking, including spatial location in the nucleus, state of chromatin in the coding sequence, position of nucleosomes in relation to transcription initiation sites, and the expression of transcription factors [79–81]. Finally, marking controls transcription but it is clear that post-transcriptional processes play an important role in regulation (see for example [82, 83]).

Hypothesis 6 Changes in the organisation and stability of the gene product dynamic interaction network in response to non-specific molecular damage are proposed to explain GI and its trans-generational inheritance.

Explanation

This hypothesis, based on the independent attractor model, treats the cell as a thermodynamically open, complex (high-dimensional) dynamic system in which self-organisation plays a dominant regulatory role [84, 85]. Many subcellular components, microtubules, for example, are generally accepted as being "self-organised" [86], and self-organisation in simpler physical systems is a well-understood phenomenon based on free energy readily available in thermodynamically open systems [87]. In this model phenotype is represented by a dynamic attractor state that is stable within limits. The earliest life forms must have been self-organised, and there is no event in the calendar of evolution that would mark a transition to something akin to a Turing machine [88]. Under this hypothesis it is proposed that the modern cell is essentially regulated by the interactions between gene products that conform to certain rules that constitute a second and independent source of information in addition to the DNA base sequence [85]. The importance of the DNA base sequence to the maintenance of the phenotype is in ensuring that after cell division the correct gene products can be transcribed in the progeny. This entails a battery of

damage detection and repair processes, the functioning of which is subject to stress from environmental sources and if overloaded can lead to an attractor/phenotypic transition. Cells belonging to stably replicating species have attractors that are more stable in this respect (termed home attractors [5]) but it is postulated that systems of the degree of complexity of the cell have numerous attractors that evolution has not exploited [88]. Thus genomic instability is seen as a response to stress entailing a transition from a home to a variant attractor as empirically demonstrated in vitro [89] and in vivo [9].

Evaluation

This hypothesis can account for all of the main empirical features of GI. Most notably, the implied target size is large because although the transition to instability is (in the case of radiation-induced GI) most probably the result of damage to the DNA, there is no specificity as to localisation of the damage, as is implied in target theory, since it is not the damage per se that leads to the effect but the stress on processes that respond to it. Thus, in target theory terms the whole genome is the target or, put another way, the effect is a generic response of the system at the genome level. This also means that the model is not specific to radiation: any environmentally sourced stress on those processes involved in cell regulation can, in principle, lead to a transition from the home to a variant attractor and thus GI. The model is applicable to any cell of a stably replicating species and in metazoa to both somatic and germ cells. Neither does the model imply a restriction on how the presence of GI is manifested at the molecular level.

Conclusions

Hypotheses 1, 5 and 6 have in common the idea that GI is a consequence of the dysregulation of the cell, but in three different contexts. Fundamental to the concept of regulation is information and if radiation is to disrupt a regulatory process then the source of the information that it modifies needs to be identified. At the present state of knowledge only hypothesis 6 gives a clear idea of the possible source. Hypotheses 1 and 5 are therefore not explanatory until such time as a viable source of information can be identified. Despite the explanatory superiority of hypothesis 6, it has its weakness in the difficulties of the experimental setups that might be used to confirm it. It is also not associated with specific material/molecular markers because of its nature. Hypotheses 2 and 4 are ruled out on the grounds that the targets of radiation action, centrosomes and telomeres are too small to account for the high sensitivity of the induction of GI by radiation. Hypothesis 3, GI as a result of sustained increased levels of ROS, has in effect been withdrawn in the two most recent reports [58, 59]. It could not have accounted for GI as a trans-generational phenomenon and would attribute the sustained chromosomal instability observed in mouse bone marrow to a tissue level phenomenon, inflammation, rather than GI.

That the evidence points overwhelmingly to a regulatory origin of GI should not be surprising. That the damage that characterises GI appears late and is sometimes different in character to that directly inflicted by radiation [18] is an indication that the cause of GI lies not in the material but in the efficient cause, i.e., it is a result of the "process" underlying molecular change rather than the molecular change itself. Another compelling example can be found in the studies of mutations in ESTRs after paternal irradiation [17]. One of the elevated markers of instability in the somatic cells of the offspring was mutation at the *hprt* locus on the X-chromosome and, therefore, inherited not from the irradiated father but from the unirradiated mother.

Implicit in hypothesis 6 is a departure from the normally accepted machine metaphor for cells as well as, and more importantly, a move away from attributing a central role in regulation to genomic DNA, as indicated by empirical evidence. Now that genome-wide sequencing is a routine procedure and hypotheses linking diseases to specific base sequence changes in DNA can be tested, there is mounting evidence in favour of the latter shift. For example, there is the problem of the "missing heritability" in complex diseases [90, 91]; the clustering of disease in families is mostly not reflected in the modification of the DNA sequence. The other side of that coin is apparent in the observation that specific disease phenotypes seem to entail mutations in many different genes [92]. A study over 20,000 generations of the adaptation of a bacterial population to nutritional stress showed little or no correlation with the mutations acquired [93] with about 50 % of the ultimately acquired adaptive fitness gained within the first 1,000 generations and the acquisition of just two mutations. An in vitro reconstitution of a circadian oscillation, largely independent of temperature, was achieved with three proteins extracted and purified from cyanobacteria and incubated with ATP [94]. Subsequent studies showed transcription-independent circadian rhythm in a eukaryote [83] and in human red blood cells [95]. In an attempt to simulate the metabolic system of *Mycoplasma pneumoniae,* a bacterium with a reduced genome, by constructing the complete metabolic map, Yus et al. [82] found that the organism was capable of functions for which it did not have transcription factors, indicating post-transcriptional processes alone can modify phenotype. Finally, in an attempt to study the adaptive process in a bacterium challenged with a totally novel stress, one for which no pathway would exist, Kashiwagi et al. [96] introduced into

E. coli a plasmid containing genes, along with reporters, able to compensate for two nutrients required by the bacterium. When introduced to a medium deficient in one or the other nutrient, the engineered bacteria initially reduced the metabolic rate, but after an hour or so resumed growth expressing the gene that compensated for the nutrient deficiency. Thus, the evidence that runs counter to the prevailing dogma that the regulation of cells derives primarily from DNA is mounting, and hypothesis 6, which sees cell regulation as an epigenetic phenomenon resulting from the self-organisation of gene products, can account for these phenomena.

The vision

The study of biological effects of radiation commenced quite quickly after the discovery in 1895 of X-rays by Roentgen: an early form of target theory was proposed by Crowther [97] in 1924 and geneticists and physicists such as Timoféeff-Ressovsky and Delbrück [98] were studying X-ray-induced mutations in fruit flies in 1935. At the time of the discovery of the structure of DNA in 1953, there was deep concern about the population genetic consequences of radioactive fallout from atmospheric weapons testing, particularly in terms of the recessive disease. The concept, therefore, that radiation effects could be understood in terms of material changes to the coding sequence of DNA (mutations) had considerable traction and indeed single locus hereditary effects can be explained in that way. The extension of these ideas to somatic disease, particularly cancer, was therefore a natural progression.

However, the focus on material causation to explain radiation effects allowed little incentive to explore the role of process, i.e., efficient causation. Radiobiology was not exceptional in this respect; the modern synthesis viewed evolution as entirely due to mutations in the genomic DNA and it is only recently that this dogma has been challenged vigorously [99], and the still dominant metaphor for the cell is the machine. A consequence has been an almost complete failure to realise that radiation effects could also result from damage to processes taking place in the cell as well as to DNA or other material components of the cell. After all machines most commonly fail as a result of component failure. In the context of complex systems we can understand, for example, how a petrol price hike could cause a shortage of cheese on supermarket shelves because fuel distribution depots have been blockaded in protest and consequent fuel shortages result in un-stocked supermarkets. Complex systems fail because of stress on their normal and relied upon processes. The need, dictated by natural selection, for cells to repair all damage before cell division creates a vulnerability to environmental agents such as radiation but at the same time provides an opportunity to escape a stressful environment into a better adapted form though probing variant attractors [88].

Thus, it might be argued that the uncovering of GI in 1992 was the precursor to a change of course for biology from being primarily causally based on *material* to a much greater appreciation of the importance of *process*; to basing cell biology on a more relevant metaphor, for example, a whirlpool rather than a machine.

Acknowledgments A.K. was supported by a postdoctoral fellowship for training in cancer research of the International Agency for Research on Cancer, Lyon, France. This work is a part of the European Commission's FP7 project Agenda for Research on Chernobyl Health (ARCH). The authors acknowledge helpful discussion with Dillwyn Williams.

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