



# Are epigenetic mechanisms involved in radiation-induced bystander effects?

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The “non-targeted effects” of ionizing radiation including bystander effects and genomic instability are unique in that no classic mutagenic event occurs in the cell showing the effect. In the case of bystander effects, cells which were not in the field affected by the radiation show high levels of mutations, chromosome aberrations, and membrane signaling changes leading to what is termed “horizontal transmission” of mutations and information which may be damaging while in the case of genomic instability, generations of cells derived from an irradiated progenitor appear normal but then lethal and non-lethal mutations appear in distant progeny. This is known as “vertical transmission.” In both situations high yields of non-clonal mutations leading to distant occurrence of mutation events both in space and time. This precludes a mutator phenotype or other conventional explanation and appears to indicate a generalized form of stress-induced mutagenesis which is well documented in bacteria. This review will discuss the phenomenology of what we term “non-targeted effects,” and will consider to what extent they challenge conventional ideas in genetics and epigenetics.

**Keywords:** radiation, non-targeted effects, bystander effects, genomic instability, evolution

## INTRODUCTION

As early as 1915, a mere 20 years after Roentgen discovered x-rays, a report was published (Murphy and Norton, 1915) suggesting that ionizing radiation to one part of the body could result in effects in a distant part. This in a nutshell is “the bystander effect.” Further discoveries reviewed by the authors (Mothersill and Seymour, 2001, 2012) were published over the years but largely ignored as the field of radiobiology became dominated by target theory and the DNA paradigm. This assumed that DNA was the target for radiation damage in cells and that the damage was the direct result of energy deposition in the cells. The energy deposited, and hence the DNA damage, was directly proportional to dose, leading to complicated explanations for why the experimental evidence demonstrated a curved or linear quadratic response. Repair theory stated that departures from this linear dose–response relationship were due to DNA repair of single or double strand breaks (Hall and Giaccia, 2006).

“The Structure of Scientific Revolutions” by Kuhn (1970) describes in great detail the course of scientific change and the history of low dose radiation biology over the last 25 years fits the theory well. All the classic stages of paradigm shift are there from disbelief, to grudging acceptance, to the claim that of course the “discoveries” were obvious and in the literature since time began.

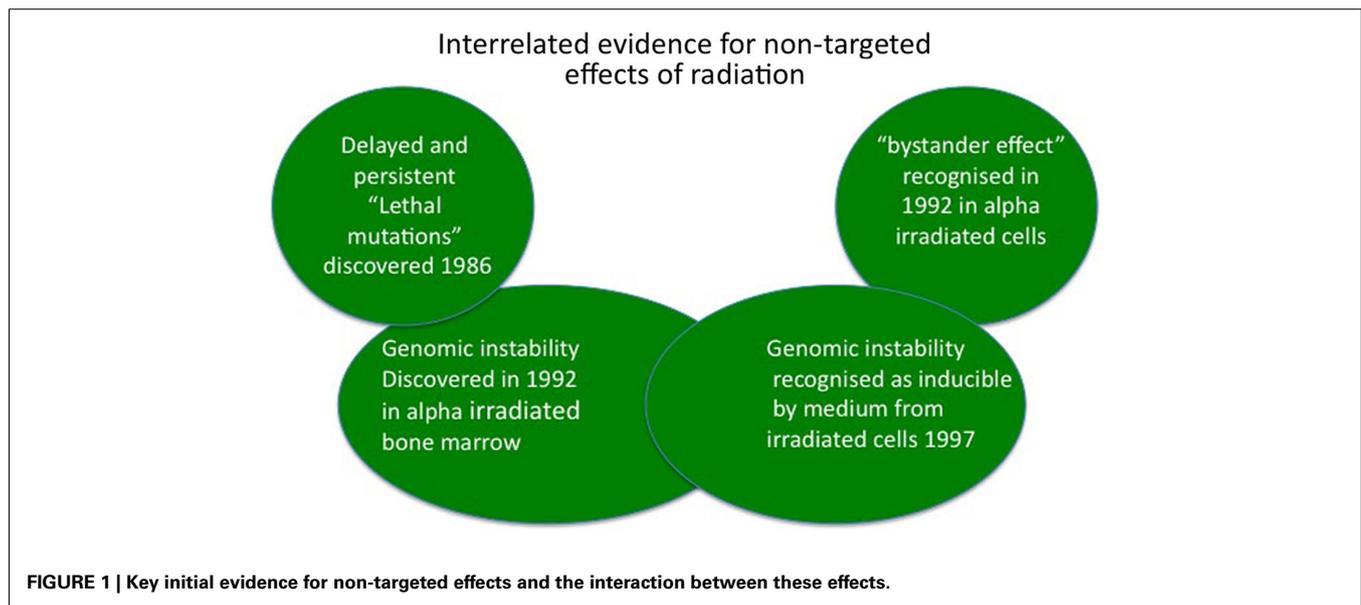
Over a 10-year period from 1986 to 1996, the dominant idea that all radiation damage resulted in hit cells from energy deposition in those cells’ DNA was finally challenged by four key lines of evidence (Figure 1). First in 1986 our group published a paper saying that *de novo* appearance of lethal mutations could occur in cells which had “recovered” from irradiation and successfully divided for several generations (Seymour et al., 1986). Second, delayed appearance of *de novo* chromosome aberrations was demonstrated

in bone marrow stem cell lineages derived from irradiated stem cells (Kadhim et al., 1992). These non-clonal aberrations could not have been present at the time of irradiation. Third, a very low dose exposure to alpha radiation resulted in more cells showing chromosome damage than could have been hit by the ionizing particles (Nagasawa and Little, 1992) and fourth, medium from irradiated cells was found to cause similar levels of clonogenic cell death and genomic instability as direct irradiation (Mothersill and Seymour, 1997; Seymour and Mothersill, 1997). Taken together, these papers started the scientific revolution establishing a new paradigm in low dose radiobiology which now is accepted by most radiation biologists but still not understood. The first two papers revealed that genetic change could occur in distant descendants of irradiated progenitor cells after multiple normal successful divisions (genomic instability) while the latter three papers established that genetic change could be induced in cells which were not affected by the mutagen (ionizing radiation) but were in receipt of signals from the irradiated cells (bystander effect). This review will highlight some of the controversies and discuss implications for mainstream biology.

## CHALLENGES TO CONVENTIONAL GENETICS POSED BY NON-TARGETED EFFECTS

### HOW CAN YOU HAVE A MUTATION WITHOUT A MUTAGEN?

Perhaps the biggest challenge posed by the discoveries was that cells not targeted by a mutagen could demonstrate heritable genetic change. Ionizing external radiation is a “clean” mutagen in that it does not leave any residue so long term effects cannot be attributed to a persistent chemical legacy. Initial theories to explain genomic instability within the existing framework centered around ideas that a mutator phenotype had been activated (Loeb, 2011) but the



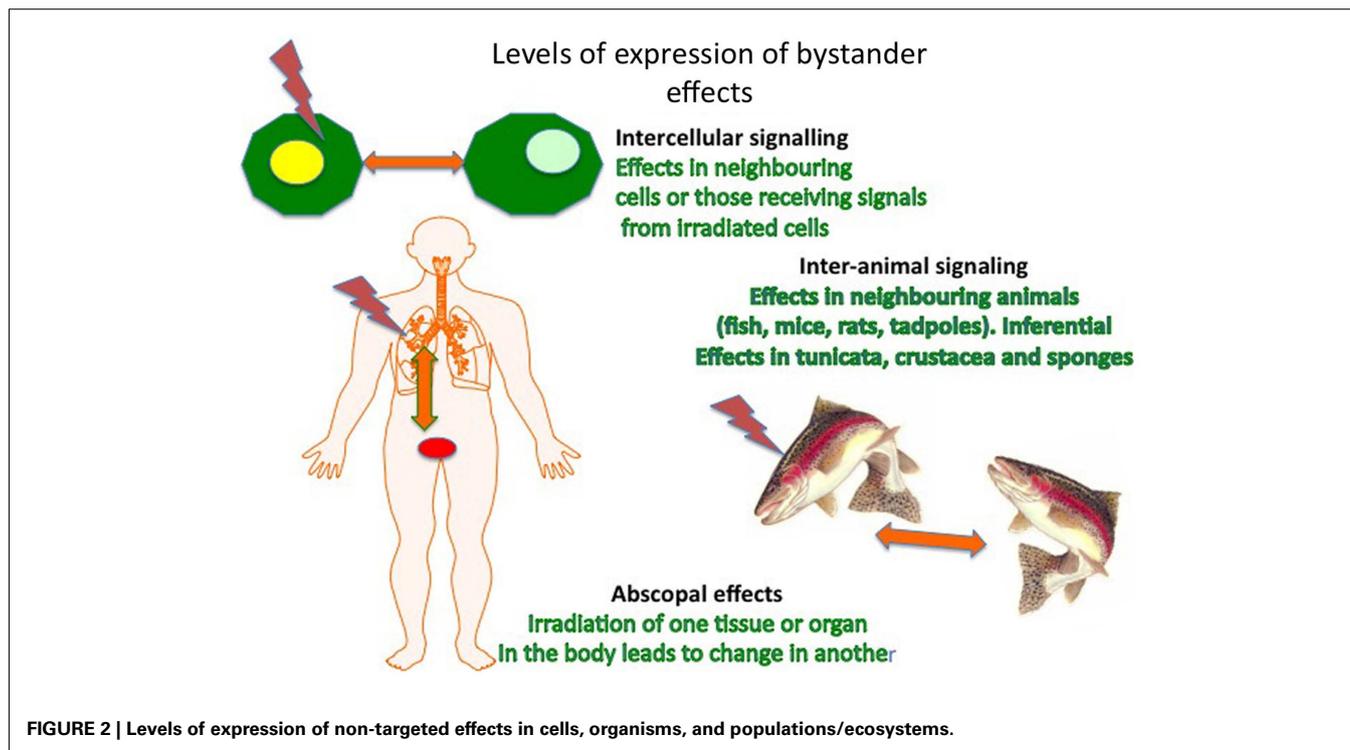
very high yields of non-clonal genetic damage inducible even by culture medium from irradiated cells made this unlikely as did the persistent nature of the effect which was neither selected out nor ultimately dominant (Seymour and Mothersill, 1988, 1997; Mendonca et al., 1989). It has been suggested that an external epigenetic driver is involved such as oxy-radicals (Hamada et al., 2011), methylation changes (Kaup et al., 2006; Tamminga and Kovalchuk, 2011), or miRNA mediated signaling (Illynskyy et al., 2009; Kovalchuk et al., 2010) but because a single exposure to radiation can turn on the process indefinitely both *in vitro* and *in vivo* (O'Reilly et al., 1994; Mothersill et al., 2000, 2010) this hypothesis requires the driver to be permanently activated both in time and in space following a single exposure to ultra-low doses of radiation. An important conceptual point here is that signal generation and response to the signal are separate processes and may not both occur in a single system (Vines et al., 2008). Signal generation appears to be a consequence of electrochemical processes much like those occurring in the nervous system. Depolarization of cell membranes, ion-fluxes, and sensitive responses to neurotransmitters and their inhibitors, characterize signal generation (Poon et al., 2007). The cellular response has been demonstrated to be an inflammatory-like response, involving cytokines, TGF beta, p53, and other proteins involved in the apoptotic or checkpoint control pathways (Hei et al., 2008). The response of the cell or organism is genetically determined with cancer prone organisms and cells having a growth response to the signal while cancer resistant systems have an apoptotic response (Mothersill et al., 2005). It is not known if the initial bystander signal generation is genetically controlled. Efforts to isolate the factor involved in transmission of information from irradiated to bystander cells has been singularly unsuccessful suggesting it is small, transient, or perhaps not chemical. Very recent data from our laboratory (Mothersill et al., *in press*) suggests a weak electromagnetic or pressure signal might play a role. This idea is highly attractive given the nature of ionizing radiation and the importance of ion-gated signaling in biology but is difficult to prove.

#### IS LAMARCKIAN GENETICS INVOLVED?

A second major challenge was posed by the discovery that these effects could be demonstrated not only in cells but also at the level of the tissue, organism, and even population (Figure 2). The tissue and organ level effects were known for some time as episcopal effects (Sgouros et al., 2007) but were ignored as an unimportant curiosity of radiotherapy. Transmission between organisms was first demonstrated by Surinov et al. (2001) in mice and has now been demonstrated in rats, several species of fish and in tadpoles, and is reviewed by the authors (Mothersill and Seymour, 2012). This has practical implications for radiation protection of ecosystems because it implies that protection of the individual might not suffice. It also has fascinating biological implications supporting Lamarckian ideas that cumulative inheritance of modified characteristics could be induced through an environmentally directed mutational shift obviating the randomness characteristic of Darwinian ideas of mutation. In the fields of stress-induced bacterial mutagenesis and plant breeding genetics there is acceptance that exposure to environmental stress can lead to persistent high rates of mutation which are adaptive *ab initio* rather than selected for. In fact inducible adaptive responses or hormetic responses are characteristic of low dose radiation exposure (Calabrese et al., 2011) and appear to be transmissible through bystander-like signaling processes even between individual animals and plants (Morgan, 2011; Mothersill and Seymour, 2012). This implies that "fitness" can spread through a population before the majority of individuals have experienced the stressor.

#### ARE THERE IMPLICATIONS FOR ESTABLISHED VIEWS OF CANCER INITIATION?

A further challenge presented by bystander effects in particular but also by genomic instability was that conventional theories of radiation carcinogenesis were based on the idea of initiation by an ionizing track of a mutation in a cell which ultimately gave rise to a cancer. This clonal origin of radiogenic cancer was used to



calculate risk of cancer induction by relating risk to number of double strand breaks induced by the dose received. Such thinking is still the basis of radiation epidemiology and is the fundamental assumption of the linear non-threshold (LNT) hypothesis which is the basis of radiation protection. Horizontal and vertical transmission of damage undermine the direct association of a DNA “hit” with development of a cancer and support instead the idea that radiation induces a field change which allows unstable cells to proliferate. Interestingly, a major factor underlying the shift away from LNT is the recent concern to establish a new radiation protection system for non-human species (Bréchnac et al., 2011). Here of course, concern is to protect populations not individuals and cancer is not an issue. The conceptual framework needed to develop new endpoints and biomarkers indicating population level damage at the macro level in biota may also provide insights into possible dose–response relationships and markers for risk from non-targeted effects at the micro level in both cells and tissues.

#### **DO NON-TARGETED EFFECTS CHALLENGE THE VIEW THAT EVOLUTION INVOLVES CONTINUAL IMPROVEMENT?**

Current thinking in radiobiology and radiation protection is divided as to whether non-targeted effects are “good” or “bad.” On the one hand, genetic damage such as chromosome aberrations and micronucleus formation can be seen and quantified. Stress effects and induction of DNA repair occur which must indicate damage. However on the other hand protective effects such as induction of adaptive responses and hormesis also occur – so do these effects mean radiation is more or less dangerous than previously thought? There is no clear answer to this question which continually pitches pro- and anti-nuclear

activists against each other but it is important to make a number of points and to list endpoints which seem common to both targeted and non-targeted effects (summarized in the **Box 1** below).

An important conclusion is that much depends on the level of organization at which “goodness” or “badness” is being assessed. To quote Lamarck “Since each part must necessarily change and cease to exist to make way for the formation of another, each part has an interest which is contrary to that of the whole; and if it reasons, it finds that the whole is badly made. In reality, however, this whole is perfect, and completely fulfills the purpose for which it is destined” (Lamarck, 1963). Implicit in this quotation is the idea that “good” or “bad” can only be answered in terms of the “whole” and that at other levels change equals improvement. Is this true? Many hypothesize (including these authors earlier in this review) that non-targeted effects are drivers of evolutionary change allowing adaptation to environmental change to occur quickly and efficiently. “Mistakes” would lead to the death of the “whole.” Darwinian arguments would suggest that bystander factors provide greater rates of random mutation, and hence provide material for natural selection to occur.

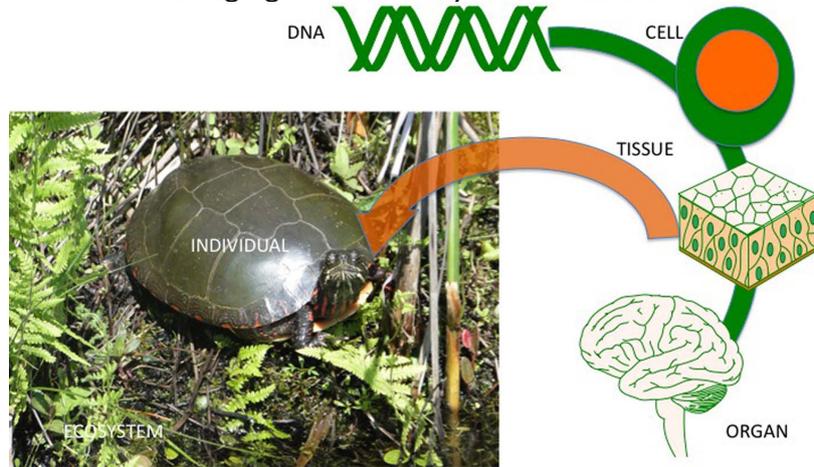
The idea of a driver may suggest cause, reason, direction, and improvement, which are central ideas in human philosophy. However it is neither necessary nor possible to comment effectively on this. Just as ionizing radiation tracks in cells are random, their consequences may also be random? If this is true it introduces an uncertainty into radiation biology, which means that radiation is unlike chemical carcinogens and mutagens, which have definable chemical pathways of action and where cause and effect can be plausibly linked. With radiation, electrical perturbation

**Box 1 | Comparison of endpoints of damage or change in directly irradiated cells, bystander cells, and progeny of directly irradiated cells.**

The data in the table summarize 25 years of research by numerous authors are comprehensively reviewed in Morgan (2003a,b), Lorimore et al. (2003), Hei et al. (2008), Mothersill and Seymour (2012), and many other reviews.

Endpoint	Directly irradiated cells	Radiation-induced bystander cells	Progeny of directly irradiated or bystander cells
Death	Reproductive death, apoptosis	Apoptosis and other forms of cell death	Delayed reproductive death, apoptosis
Protein induction	Induction of repair and checkpoint proteins	Induction of early response proteins	Persistent over-expression of stress proteins in progeny
Reactive oxygen species	Generation of free radicals	Oxidative stress	Persistent oxidative stress
Growth stimulation	Adaptive response	Proliferation and adaptive response	Adaptive response
Non-clonal persistent mutations	Chromosomal aberrations	Genomic instability, lethal mutations	Genomic instability in progeny and lethal mutations
Micronucleus (MN) assay	Increased MN	Cytogenetic effects and increased MN	Cytogenetic effects and increased MN
Carcinogenesis	Transformed foci	Transformed foci	Transformation and cancer <i>in vivo</i>
Mitochondrial function	Aberrant	Aberrant	Aberrant
P53 function	Critical	Critical to response outcome	Critical to response outcome
Genotype dependent?	Yes	Yes	Yes

**Hierarchical levels in biological systems: A bridging role of the bystander effect?**



**FIGURE 3 | Hierarchical levels in biological systems indicating a coordinating role for stress-induced bystander effects.**

may lead to random and unpredictable consequences, which are intrinsically chaotic and can only be assessed (if at all) at levels of organization much greater than those at which the events occur (Figure 3). The argument against it being true is the reproducibility of effects, which would suggest that the response to random ionizations becomes coordinated suggesting an emergent property of the system similar to quorum sensing in bacteria.

**WIDER IMPLICATIONS OF NON-TARGETED EFFECTS IN GENETICS AND EPIGENETICS**

Bystander effects and genomic instability have revolutionized low dose radiobiology, leading to questions about radiation risk

and carcinogenesis and suggesting new targets for cancer therapy (Mothersill and Seymour, 2006; Prise and O’Sullivan, 2009). Have they wider implications in biology? Certainly the mechanisms involved suggest a key role for electromagnetic radiation in cell signaling and that this signaling is key to coordinating responses at higher levels of organization – providing a communication system which is highly responsive to environmental conditions. The involvement of neural-like processes such as depolarization of membranes, involvement of neurotransmitter binding to ion-gated channel receptors and ion-fluxes across membranes (Lyng et al., 2000, 2006; Poon et al., 2007; Saroya et al., 2009) is exciting and may provide a mechanism for such

alternative medicine therapies as Reiki and acupuncture, which employ the concept of a “biofield” in living organisms, coordinating health and well being. At the genetic level, the phenomena challenge gene-centric paradigms and suggest the existence of mechanisms even more epigenetic than those such as histone acetylation, mRNAs, or mitochondrial inheritance which are causing so much excitement at present (Calvanese et al., 2012). Non-targeted effects especially if mediated by an electromagnetic information transfer system may provide some very interesting science.

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## CONCLUSION

This review discusses the key phenomena, which are challenging our understanding of radiation action in living systems. The controversies and implications are then considered. Finally the implications for biology in its widest sense are considered.

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