

# Variability: The common factor linking low dose-induced genomic instability, adaptation and bystander effects

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## Abstract

The characteristics of low dose radiation-induced genomic instability, adaptive responses, and bystander effects were compared in order to probe possible underlying mechanisms, and develop models for predicting response to *in vivo* low dose radiation exposures. While there are some features that are common to all three (e.g., absence of a true dose–response, the multiple endpoints affected by each), other characteristics appear to distinguish one from the other (e.g., TP53 involvement, LET response, influence of DNA repair). Each of the responses is also highly variable; not all cell and tissue models show the same response and there is much interindividual variation in response. Most of these studies have employed *in vitro* cell culture or tissue explant models, and understanding underlying mechanisms and the biological significance of these low dose–responses will require study of tissue-specific *in vivo* endpoints. The *in vitro* studies strongly suggest that modeling low dose radiation effects will be a complex process, and will likely require separate study of each of these low dose phenomena. Knowledge of instability responses, for example, may not aid in predicting other low dose effects in the same tissue.

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**Keywords:** Adaptation; Genomic instability; Bystander effects; Ionizing radiation; Low dose

Bystander, adaptation, and instability  
Sat down to chat about their variability.  
They talked in gray phenomenology  
And even probed molecularly  
About their lives intertwined, so intimately  
Are they part of some intelligent design  
Or of some evolutionary outline  
A complex model of act and reaction  
Or simply some quantum uncertain contraption

## 1. Introduction

For many decades the primary model for radiation-induced effects was based on the notion of hits and

targets. Radiation produced damage (hits) in critical structures (targets). If the damage was repaired, the cell returned to a normal physiological state. If the damage failed to be repaired, the result was cell death. And if the damage was misrepaired, the result could be cell death or mutation, with mutation potentially leading to cell dysfunction and transformation. The primary target for all these effects was considered to be DNA. In the early 1980s there began to appear in the literature first a trickle and then a torrent of articles suggesting that not all effects of radiation result from directly targeted effects of radiation on DNA. Exposure of cytoplasm alone can lead to toxicity, mutation, and cell transformation. New mutations can develop long after the initial exposure, and some effects of radiation can be observed in neighboring unexposed cells. The interest in these nontargeted effects has grown in part because of the realization that they likely predominate over targeted

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Table 1  
Characteristics of instability, adaptation and bystander effects

	Instability	Adaptation	Bystander
Endpoints affected	Mutations, delayed lethality	Mutations, lethality, cell transformation, carcinogenesis, <i>in utero</i> development	Mutations, lethality, sister chromatid exchanges, cell transformation, differentiation
Dose–response	Linear, no true dose–response	No true dose–response	No true dose–response
Sensitivity	>5 cGy	0.5–20 cGy	>1 cGy
Cycling cells	Not required for induction	No induction in G0 cells	Not required for either induction or response
Lifespan	Variable, can last many generations	~3 cell generations	Variable, can last many generations
Let dependence	RBE > 1 for induction	Only low LET induced	RBE > 1 for induction
DNA repair involvement	Influences induction	Mixed reports	No obvious effect on induction Affects response
ROS involvement	Important for induction and persistence	Involved in induction	Involved in induction of response
TP53 role	Mixed reports on TP53 role in induced instability	Intact TP53 required	May mediate response TP53 pathways involved in response
Expression	Variable	Variable	Not required for induction Variable

effects of radiation at low dose exposures and therefore they may help define risks from low dose radiation exposures. There is also growing evidence for nontargeted effects underlying some of the late responses of patients to therapeutic radiation exposures [1,2].

A number of investigators have looked for links between some of these low dose effects (see, for example [3,4]). The present review focuses on common features of low dose-induced genomic instability, adaptive response, and bystander effects. This review was not designed to be exhaustive. Rather, the aim was to compare and contrast different characteristics in order to probe possible underlying mechanisms and thereby aid in the development of models for predicting *in vivo* response to low dose radiation exposures. Table 1 contains a summary of the key features compared in this review. The primary feature common to all three endpoints is their variability; responses differ widely depending on the model system studied.

## 2. Genomic instability

Radiation-induced genomic instability is observed as elevated mutation rates seen over many cell cycles after irradiation (reviewed in [4–6]). Both gene and chromosome instability have been reported as has the phenomenon of delayed lethality. The type of instability observed varies depending on the test system being studied. Initially, linear dose–responses were reported. Most reports, however, find no true dose–response; the instability phenotype is either on or off. Instability can

be observed following doses as low as 5 cGy [7]. The response can be observed after exposure of either cycling or noncycling cells, although in order to detect instability, one usually requires cycling cells. It has been shown that instability has a variable lifespan that can last many generations.

It is also observed after *in utero* irradiation [8]. High LET radiation is generally considered to be more effective in inducing instability and defective DNA repair is associated with increased susceptibility to the induction of instability [9–12]. Reactive oxygen (ROS) production is associated with unstable cells and may induce and/or perpetuate instability. While it is well established that the functional loss of TP53 is associated with spontaneous genome instability [7], the role of TP53 in radiation-induced instability remains unclear. Some studies suggest that TP53 loss leads to increased sensitivity to instability induction while others report reduced sensitivity to instability induction in TP53-deficient cells [7,13–16]. The ability to induce instability is also variable. Not all cells in an exposed population develop instability and not all cell types are susceptible to instability induction (see, for example [8,17]).

## 3. Radioadaptive response

The adaptive response to radiation was first described in 1984 by Olivieri et al. [18], who reported that peripheral blood lymphocytes cultured in [H-3]-thymidine showed a reduced frequency of chromosome aberrations following a challenge with an acute, higher dose

of X-ray. The phenomenon was subsequently studied by many different laboratories in a variety of test systems [4,8,19]. The usual protocol is to prime cells with a low dose of radiation and then follow 4 or more hours later with a challenge to a much higher dose (50–200 cGy). Adaptive responses have been reported for cell killing, transformation, mutagenesis and carcinogenesis. There is no true dose–response; adaptive responses are either off or on. Adaptation is most efficiently induced by doses of 0.5–20 cGy. The response usually requires cycling cells; at least it has been shown that low dose treatments will not increase resistance in unstimulated lymphocytes [20].

As with induced instability, detecting an adaptive response requires cycling cells. The adaptive response has been reported to last for about three generations following low dose induction [20]. It can be induced with *in utero* exposures, but its induction is dependent on the stage at which the fetus is irradiated, and the long term consequences of induction can include postnatal mortality and other detrimental effects [8]. High LET radiation will not induce the response, but adapted cells can repair high LET-induced chromosome damage [21]. The role of DNA repair is unclear as some have reported that the response is reduced or absent in repair-deficient cell lines while others see no effect of repair background on response [22,23]. There is no clear evidence for adaptive responses involving detoxification of ROS, although ROS may induce the response. TP53 has been implicated as a key element in the response [23,24]. One of the difficulties in studying the radioadaptive phenomenon is that it is highly variable. Not all protocols yield similar results. Even within a single laboratory, the magnitude and occurrence of the adaptive response has been shown to vary between donors and even within a single donor over time (see, for example [24]).

#### 4. Bystander effects

Bystander effects are defined as alterations in unexposed cells caused by nearby cell exposures [4,25–28]. In some experimental systems, bystander signals can be transmitted through the growth medium while in others gap junctions seem to be required. These modes of transfer are not mutually exclusive. Observed bystander effects include increased levels of gene mutations, sister chromatid exchanges, micronuclei, chromosome aberrations, premature cell differentiation, cell killing, and cell transformation. Like adaptation and induced instability, there is no true dose–response; bystander induction is either off or on. Bystander effects have been observed with doses as low as 1 cGy [29]. Cells need not be in

cycle to produce or respond to bystander mediators. High LET radiations appear to be more effective in inducing bystander mediators. Studies with repair-deficient cell lines suggest that a reduced ability to repair DNA damage will lead to greater responses, depending on the endpoint studied. However, repair-deficient cells show similar ability to induce bystander mediators as repair-proficient cells. ROS have been implicated as potential inducers and mediators of the effect. The role of TP53 in bystander effects is not clear. Bystander effects are associated with changes in TP53 activity, but it is not yet established whether a functional TP53 is necessary to induce or respond to bystander mediators. Like instability and adaptation, bystander effects can be variable. Some cells can be induced to release bystander mediators, while others have the capacity to respond to these factors. There also appear to be tissue-specific differences in bystander responses and not all cells within a tissue will show evidence of exposure to bystander mediators [25].

#### 5. Summary and conclusions

At first glance, there appear to be a number of features common to genomic instability, adaptive response, and bystander effects; the endpoints affected are similar, absence of a true dose–response has been reported for all three, and the minimum dose required to induce all three responses is in the same range. The similarities seem to end there. Induction of an adaptive response usually requires cycling cells, has a limited lifespan, is only seen following low LET exposure, and has a clear TP53 involvement. Instability and bystander effects can be induced in either cycling or noncycling cells, can last for many cell generations, and show an RBE of greater than 1. The role of TP53 is not fully established for induced instability, while it is established that bystander effects can be induced in TP53-deficient cells. DNA repair deficiencies influence cell sensitivity to instability induction but not bystander induction, although the expression of instability or bystander effects is influenced by repair characteristics. Based on these observations, one would have to conclude that genomic instability, adaptive response, and bystander effects represent distinct processes that may overlap in some of their mechanisms. For modelers, differences in the characteristics of low dose-induced phenomena will complicate modeling effects. Separate models may be required for each response as knowledge of the characteristics of one low dose–response may not be useful in predicting other low dose effects in the same tissue.

Perhaps the most difficult aspect of studying genomic instability, adaptive response, and bystander effects is that all three responses are highly variable. What is observed depends on the model system being used to study these low dose effects. Variation is not unexpected. Radiation exposure initiates a complex series of events in cells and tissues, many of which seem at odds with each other. Radiation can stimulate both pro- and anti-apoptotic responses [30–33]. It can lead to cell cycle arrest or growth stimulation [27,33–35]. Presumably, the nature of the response reflects in part dose- and tissue-specific mechanisms that ensure a balanced response to stress. Barcellos-Hoff [36] has maintained that modeling radiation effects will require a systems biology approach that considers multicellular and multigenerational responses. Clearly understanding mechanisms and modeling low dose effects must also consider the nature of the tissue being studied. Even within a single model system, however, there are variations in induction of low dose-responses; not all cells in a single model system respond to low dose exposures in the same way all the time. This variability has frustrated efforts to understand the molecular basis of each phenomenon. On the other hand, this variability suggests that homeostasis is controlled by a complex of different factors. No doubt these controls have evolved to provide greater flexibility in response to endogenous and exogenous factors.

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