Understanding adaptation following exposure to radiation: What do we really know?

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Adaptive Response = Radioadaptive Response

• Non-Targeted Effects (NTE)
  • Adaptive Responses
  • Bystander Effects
  • Genomic Instability

But what is it?

Exposure to one dose of radiation, prior to exposure to a subsequent irradiation can reduce the effect compared to the second dose alone.
The Null Hypothesis...
Given a sufficient interval, sequential radiation doses will act independently.
What are the implications of dose independence?

• Previously irradiated cells will **respond** to a given radiation exposure the same as unexposed cells.

• No need to consider the ‘radiation history’ when predicting the effects of a given exposure.

  \[
  2 \text{ Gy} \\
  100 \text{ mGy} \rightarrow 2 \text{ Gy} \\
  2 \text{ Gy} \rightarrow 2 \text{ Gy}
  \]

• But this doesn’t mean the effects of any previous exposure(s) are erased, you still need to consider the totality of effects from all exposures.

• When dose responses are non-linear, continuing along a dose-response curve will be different from stopping and starting a new dose-response.

  \[
  2 \text{ Gy} + 2 \text{ Gy} \neq 4 \text{ Gy}
  \]

  Fractionation/Split-Dose effect
Fractionation/Split Dose Effects Are Explained by the Independence of Dose Effects

The Independence is Violated When the Response to a Dose is Different with Prior Irradiation
If the null hypothesis is rejected:
What kind of interactions might you see?

• Radiation might sensitise cells to a subsequent radiation dose...

• Radiation might protect cells from a subsequent radiation dose...

• Irradiation might change the response to the other dose in a way that is difficult to describe as sensitisation/protection:
  • Might change the nature of the response.
  • Might change the timing of the response.
If the null hypothesis is rejected: What kind of interactions might you see?

• Radiation might sensitise cells to a subsequent radiation dose...

• Radiation might protect cells from a subsequent radiation dose...

  The ‘Adaptive Response’ essentially describes this special case of when the null hypothesis is rejected.

• Irradiation might change the response to the other dose in a way that is difficult to describe as sensitisation/protection:
  • Might change the nature of the response.
  • Might change the timing of the response.

### ADAPTIVE RESPONSE CHARACTERIZATION

### TABLE I

Effect of 0.01- or 0.05-Gy Pretreatment of G₀ or G₁ Phase Human Lymphocytes on 1.5-Gy-Induced Chromatid and Isochromatid Breaks

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Number of chromatid and isochromatid breaks in 300 cells</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>0.01 Gy (G₀)</td>
<td>4</td>
</tr>
<tr>
<td>0.05 Gy (G₀)</td>
<td>4</td>
</tr>
<tr>
<td>1.5 Gy (48 h)</td>
<td>101</td>
</tr>
<tr>
<td>0.01 Gy (G₀) + 1.5 Gy (48 h)</td>
<td>93</td>
</tr>
<tr>
<td>0.05 Gy (G₀) + 1.5 Gy (48 h)</td>
<td>102</td>
</tr>
<tr>
<td>0.01 Gy (4 h) + 1.5 Gy (48 h)</td>
<td>78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01 Gy (8 h) + 1.5 Gy (48 h)</td>
<td>73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01 Gy (12 h) + 1.5 Gy (48 h)</td>
<td>68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01 Gy (14 h) + 1.5 Gy (48 h)</td>
<td>73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The numbers in parentheses are the times after PHA stimulation at which X rays were administered. All cultures were harvested 6 h after the 1.5-Gy exposure time.

<sup>b</sup> Significantly lower than the 1.5-Gy treatment alone; *P* < 0.05; one-tailed *t* test.
The Adaptive Response

• The word ‘Adaptive’ conveys that the effect is beneficial and active
  • Beneficial for a given endpoint? vs. Beneficial overall?
  • The first dose initiates some program in anticipation, rather than just a reduced effect due to the clearance of a sensitive sub-population.

• Often conceptually linked with evolutionary adaptation.
  • A trait that has evolved and is maintained by natural selection.
  • The processes which mediate the ‘protection’ exist because they have provided/continue to provide an advantage.
Detecting Adaptive Responses

\[ E(A_{d_{r1}} \rightarrow B_{d_{r2}}^{d}) < E(B_{d_{r2}}^{d}) \]

\[ E(A_{d_{r1}} \rightarrow B_{d_{r2}}^{d}) < E(A + B_{d_{r2}}^{d}) \]

\[ E(A_{d_{r1}} \rightarrow B_{d_{r2}}^{d}) < E(A_{d_{r1}}^{d+i}) + E(B_{d_{r2}}^{d}) \]
<table>
<thead>
<tr>
<th>Definition</th>
<th>Reference</th>
<th>Year</th>
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<td>Human lymphocytes exposed to low doses of endogenous radiation in the form of incorporated tritiated thymidine, [3H]dThd, become less susceptible to chromatid damage induced by a subsequent high dose of X rays</td>
<td>Shadley et al.</td>
<td>1987</td>
</tr>
</tbody>
</table>

“Human lymphocytes exposed to low doses of endogenous radiation in the form of incorporated tritiated thymidine, [3H]dThd, become less susceptible to chromatid damage induced by a subsequent high dose of X rays”
Human lymphocytes exposed to low doses of endogenous radiation in the form of incorporated tritiated thymidine, \(^{3}\text{H}\)dThd, become less susceptible to chromatid damage induced by a subsequent high dose of X rays

... a low "adapting" dose of ionizing radiation can make cells less susceptible to a second high "challenging" dose.

Wojcik et al. 1993

... exposure of lymphocytes or fibroblasts to doses as low as 0.01 Gy induces a process for the repair of chromosomal breaks which renders these cells less susceptible to chromosomal damage caused by a subsequent exposure.

Azzam et al. 1994

Lymphocytes exposed to a low dose of ionizing radiation had fewer chromatid aberrations induced by a subsequent high dose exposure, compared to lymphocytes not receiving a low dose.

Shadley et al. 1994

Preliminary irradiation of cells with low doses increases their resistance to subsequent exposure to high doses of radiation.

Filippovich et al. 1998

... a process induced by radiation whereby the consequences of a subsequent radiation exposure are modified

Mitchel et al. 1999

Very low dose of ionizing radiation also induces mechanisms whereby cell or tissue become better fit to cope with subsequent exposures of high doses

Bhattacharjee et al. 2001

... exposure to a low level of DNA stress resulting, for example, from a low dose of radiation renders cells resistant to a subsequent exposure

Sawant et al. 2001

Low doses of ionizing radiation can produce a stimulatory effect and can induce adaptive responses that reduce the harmful effects of subsequent exposure to high-dose radiation

Lee et al. 2002

... the adaptive response, induced by a low initial priming dose, reduces damage from a subsequent challenging dose.

Zhou et al. 2003

... low doses increase the ability of normal human fibroblasts, including unhit bystander cells, to repair chromosomal breaks resulting from a subsequent high-dose, acute exposure

Mitchel et al. 2004

... the phenomenon whereby a priming low-dose irradiation induces resistance to a subsequent irradiation at higher doses

Wang et al. 2004

Exposure to low doses of ionizing radiation (<10 cGy) alters gene expression profiles in cells and animal tissues but, under certain circumstances, protects cells against the damaging effects of subsequent higher-dose exposures

Coleman et al. 2005

An adaptive response is a response to an external stress, such as radiation, that is modified by prior exposure to the same or a different stress and results in a lower than expected biological response...

Day et al. 2006

... such exposure up-regulates protective mechanisms, which attenuate damage caused by normal endogenous metabolism and/or the damage caused by the radiation delivered at low dose rates or by subsequent challenge high-dose-rate exposures

de Toledo et al. 2006

... the acquisition of radiosensitivity induced by low-dose pre-irradiation both in vitro and in vivo.

Otsuka et al. 2006

... where by a small priming dose of low-LET radiation induces increased resistance to later challenging doses of radiation

Hafer et al. 2007

... low-dose induction of an adaptive response in cells and tissues that resulted in them being resistant to a high challenge dose of radiation.

Elmore et al. 2008

... the ability of cells and animals to adapt to low doses of ionizing radiation and lessen the detrimental effects of further radiation exposure or of other stressors, as well as spontaneous events ... The adaptive response to radiation is a response to low doses and dose rates

Mitchel et al. 2008

... cells become more resistant to a challenge dose of ionizing radiation if they have been pretreated with a small conditioning dose some time earlier

Pinto et al. 2010

... defined as the ability of cells or organisms exposed to a very low dose of ionizing radiation in the range of 0.1–100 mGy to confer enhanced resistance to a subsequent exposure to a much larger dose of radiation,

Grdina et al. 2013

... prior exposure to a low dose of an agent, protects against later exposure to a high dose.

Kalantari et al. 2014

The radio-adaptive response is induced by a very small priming dose administered in the range of 1–200 mGy followed a short time later by a larger dose in the range of 1–10 Gy

Grdina et al. 2015

... a form of cellular response that could be induced by low doses of radiation (priming dose, D1) followed by higher dose of radiation (challenging dose, D2), the chromosome aberrations that D2 caused will be attenuated by the pretreatment of D1

Zhao et al. 2015
An adaptive response is a response to an external stress, such as radiation, that is modified by prior exposure to the same or a different stress and results in a lower than expected biological response...

... such exposure up-regulates protective mechanisms, which attenuate damage caused by normal endogenous metabolism and/or the damage caused by the radiation delivered at low dose rates or by subsequent challenge of high-dose-rate exposures.

... the acquisition of radiosensitivity induced by low-dose pre-irradiation both in vitro and in vivo.

... where a small priming dose of low-LET radiation induces increased resistance to later challenging doses of radiation.

... low-dose induction of an adaptive response in cells and tissues that resulted in them being resistant to a high challenge dose of radiation.

... the ability of cells and animals to adapt to low doses of ionizing radiation and lessen the detrimental effects of further radiation exposure or of other stressors, as well as spontaneous events... The adaptive response to radiation is a response to low doses and dose rates.

... cells become more resistant to a challenge dose of ionizing radiation if they have been pretreated with a small conditioning dose some time earlier.

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... prior exposure to a low dose of an agent, protects against later exposure to a high dose.

The radio-adaptive response is induced by a very small priming dose administered in the range of 0.01–100 mGy followed a short time later by a larger dose in the range of 1–10 Gy.

... a form of cellular response that could be induced by low doses of radiation (priming dose, D1) followed by higher dose of radiation (challenging dose, D2). The chromosome aberrations that D2 caused will be attenuated by the pretreatment of D1.
What are the ‘rules’ that define adaptive responses?

With more than 30 years of adaptive response research, it shouldn’t be difficult to answer such a question...

We need some way to integrate data from a wide variety of experimental designs, systems, endpoints.
Cell Type (Donor)
Other conditions: Cell cycle, temperature

- **250 kVp X-rays**
  - **Dose**: 50 mGy
  - **Time**: 10 seconds
  - **Endpoint**: Apoptosis

- **137Cs**
  - **Dose**: 100 mGy
  - **Time**: 1 week
  - **Endpoint**: γH2AX

- **200 kVp X-rays**
  - **Dose**: 4 Gy
  - **Time**: 2.5 minutes
### Priming Dose

<table>
<thead>
<tr>
<th>Priming Dose</th>
<th>Challenge Dose</th>
<th>Challenge Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1 mGy</td>
<td>&lt;100 mGy</td>
<td>0.5 mGy/min</td>
</tr>
<tr>
<td>0.1 mGy</td>
<td>0.4, 0.8, 1 Gy</td>
<td>8.3 mGy/min</td>
</tr>
<tr>
<td>1 mGy</td>
<td>1.5 Gy, 1.6 Gy</td>
<td>270 mGy/min</td>
</tr>
<tr>
<td>5 mGy</td>
<td>2 Gy, 3 Gy, 4 Gy</td>
<td>1000-2000 mGy/min</td>
</tr>
<tr>
<td>10 mGy</td>
<td>4980 mGy/min</td>
<td></td>
</tr>
<tr>
<td>20 mGy</td>
<td>7830 mGy/min</td>
<td></td>
</tr>
<tr>
<td>25 mGy</td>
<td>2370 mGy/min*</td>
<td></td>
</tr>
<tr>
<td>50 mGy</td>
<td>40, 44, 48, 72 h</td>
<td></td>
</tr>
<tr>
<td>75 mGy</td>
<td>50 mGy</td>
<td></td>
</tr>
<tr>
<td>100 mGy</td>
<td>75 mGy</td>
<td></td>
</tr>
<tr>
<td>200, 216, 350 mGy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500, 650, 670 mGy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1300, 1500 mGy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### γ Radiation

All irradiations were done using a Mark I $^{137}$Cs γ irradiator (J. L. Shepherd and Associates, Glendale, CA). A dose rate of 4.98 Gy/min was used for all irradiations with 1, 3 and 5 Gy. The priming exposure of 5 cGy was done with a dose rate of ~2.37 Gy/min for a total time of 0.02 min. The actual exposure for the nominal 5-cGy dose may be as much as 20% higher due to the dose delivered while the source travels into and out of the chamber. Cells were irradiated in aerobic conditions at room temperature and returned to the incubator between priming and challenge dose exposures.
<table>
<thead>
<tr>
<th>Priming Source</th>
<th>Challenge Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I seeds</td>
<td>$^{137}$Cs</td>
</tr>
<tr>
<td>$^{137}$Cs</td>
<td>$^{60}$Co</td>
</tr>
<tr>
<td>$^{60}$Co</td>
<td>160, 200, 250, 300 kVp X-rays</td>
</tr>
<tr>
<td>$^3$H</td>
<td>$^{241}$Am Alpha particles</td>
</tr>
<tr>
<td>37, 200 or 250 kVp X-rays</td>
<td>Radon</td>
</tr>
<tr>
<td>$^{241}$Am Alpha particles</td>
<td></td>
</tr>
<tr>
<td>3.37 MeV Protons</td>
<td></td>
</tr>
<tr>
<td>Endpoints</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td></td>
</tr>
<tr>
<td>Cell Survival</td>
<td></td>
</tr>
<tr>
<td>Chromatid and isochromatid breaks</td>
<td></td>
</tr>
<tr>
<td>Clonogenic Survival</td>
<td></td>
</tr>
<tr>
<td>Comet Assay</td>
<td></td>
</tr>
<tr>
<td>γH2AX</td>
<td></td>
</tr>
<tr>
<td>HPRT Mutations</td>
<td></td>
</tr>
<tr>
<td>Chromosomal Inversions</td>
<td></td>
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<tr>
<td>Micronuclei</td>
<td></td>
</tr>
<tr>
<td>Neoplastic Transformation</td>
<td></td>
</tr>
<tr>
<td>p53 Foci</td>
<td></td>
</tr>
<tr>
<td>Recovery of Peripheral Blood Cells</td>
<td></td>
</tr>
<tr>
<td>Tumour Latency/Frequency</td>
<td></td>
</tr>
</tbody>
</table>
Characterising the Adaptive Response

What are the limits of the priming dose?

1. Take all the studies where some doses showed an adaptive response while other doses didn’t.

2. Pool them all together and plot them on a graph.

Interestingly, more than half of adaptive response papers do not show any case where an adaptive response was not observed...
Adaptation vs. No Adaptation in Single Studies
Characterising the Adaptive Response

• There are plenty of studies showing evidence of the adaptive response.

• Tendency to select a single ‘optimal’ or ‘standard’ priming dose and exposure conditions.

• What we really need are studies that show both adaptive responses, no adaptive responses, and/or mal-adaptive responses that allow us to define the limits of the adaptation.

• Focus on studies/experimental designs that allow us to learn more about adaptive responses.
Dose Responses for Adaption to Low Doses of $^{60}$Co $\gamma$ Rays and $^3$H $\beta$ Particles in Normal Human Fibroblasts

E. J. Broussard, D. L. Brown, and R. E. J. Mitchell

Radiation Biology and Health Physics, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada, K0J 1J0; and Department of Biology, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

FIG. 1. Adaption induced by exposure to various doses of $^{60}$Co $\gamma$ radiation at 37°C, as measured by micronucleus frequency within binucleate cells. All cells were incubated for 3 h at 37°C after exposure to the adapting dose, and prior to irradiation with the 4-Gy (or sham) challenge dose. Control, unadapted cells were exposed to the 4-Gy challenge dose alone. Cells exposed to any of the adapting doses prior to the 4-Gy challenge dose were significantly different from the cells exposed to the 4-Gy challenge dose alone. $P < 0.05$. Mean results are reported ± standard deviation, $n = 3$. 

Normal Human Fibroblasts

[Micronuclei]
Priming doses can be effective over multiple orders of magnitude.

Transgenic pKZ1 Mouse [Chromosomal Inversions]

FIG. 2. Mean uncorrected inversion frequency (± SE) in pKZ1 mouse prostate after priming + challenge whole-body X irradiation. T: transgenic; NT: nontransgenic. 5 ≤ n ≤ 10 transgenic mice, and 2 ≤ n ≤ 5 nontransgenic mice. *Significant change in corrected inversion frequency between sham-treated and treated mice. **Significant change in corrected inversion frequency between priming + challenge and challenge only groups. P < 0.05, two-tailed Mann-Whitney U test. Corrected inversion frequencies are shown in Table 1 and were calculated by subtracting frequencies for nontransgenic mice from those for transgenic mice to correct for nonspecific staining.
Can the adapting effect of the priming dose be observed without a challenge dose...?

- It can be hard to untangle an adaptive response (where the priming dose changes the response to the challenge dose) from the addition of opposing effects at two different doses.
Priming doses alone can sometimes show effects that might simply oppose the effect of a subsequent dose.
Can the adapting effect of the priming dose be observed without a challenge dose...?

- In most cases, the priming dose alone induces a small ‘increase’ in the endpoint (i.e. same direction as challenge, not opposing) or shows no significant change.

- Sometimes the ‘protection’ is much larger than the background, such that an offset of spontaneous damage is not a good explanation.
Low Dose Radiation-Induced Adaptive Survival Response in Mouse Spleen T-lymphocytes in vivo

NAOKI YOSHIDA, HAJIME IMADA, NAOKI KUNUGITA AND TOSHIYUKI NORIMURA

Fig. 2. Relationship between the adaptive survival response and the priming dose given. The interval between priming and the challenge exposure is 7 hours. Challenge dose: 3 Gy. Sample size: 5 per point. The 0.05- and 0.1-Gy points differ significantly from the 0 Gy point; p<0.05, one-tailed t test.
Effective priming doses may have a narrow dose window.
Do higher doses not induce adaptive responses, or are they just harder to see against co-induced damage?

![Graph showing contribution of responses to first dose on net yield of damage.](image)
Mutagenic radioadaptation in a human lymphoblastoid cell line

Fumio Yatagai, Yukihiro Umebayashi, Masamitsu Homma, Kaoru Sugasawa, Yuko Takayama, Fumio Hanaoka

1 Advanced Development and Support Center, The Institute of Physical and Chemical Research (RIKEN), Saitama 351-0198, Japan
2 Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo 158-8501, Japan
3 Graduate Program, Frontier in Biosciences, Osaka University, Osaka 565-0871, Japan

Received 6 April 2007; revised 15 August 2007; accepted 22 August 2007
Available online 1 September 2007

Table 1a

<table>
<thead>
<tr>
<th>Time interval (h)</th>
<th>0</th>
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<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
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</thead>
<tbody>
<tr>
<td>TK MF ($\times 10^{-6}$)</td>
<td>19.8</td>
<td>18.1</td>
<td>14.4</td>
<td>13.5</td>
<td>17.8</td>
<td>19.7</td>
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Table 1b

<table>
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<tr>
<th>Priming X-ray dose (cGy)</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
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<tbody>
<tr>
<td>TK MF ($\times 10^{-6}$)</td>
<td>13.3</td>
<td>15.8</td>
<td>4.5</td>
<td>6.3</td>
</tr>
</tbody>
</table>

The interval time and priming dose can both have effects on adaptation.
Effective priming doses can vary in their effective interval times.

Lethal mouse irradiation [Mouse Survival]

Two types of X-ray-induced radioresistance in mice: Presence of 4 dose ranges with distinct biological effects

Morio Yonezawa a,*, Jun Misonoh b, Yasushi Hosokawa a

a Division of Radiation Biology, Research Center of Radiotopes, Research Institute for Advanced Science and Technology, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 593, Japan
b Radiation Safety Group, Nuclear Energy System Department, Konoe Research Laboratory, Central Research Institute of Electric Power Industry, Iwato-ko, Komae, Tokyo 201, Japan

Fig. 4. Effects of the priming dose and the time interval on the acquired radioresistance in mice. ○, significant radioresistance ($p < 0.05$), ×, insignificant ($p > 0.05$).
Adaptive response and split-dose effect of radiation on the survival of mice

ASHU BHAN TIKU and R K KALE*

Free Radical Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Table 2. Effect of conditioning doses of gamma rays on the 30 day survival of mice challenged by lethal dose of 8 Gy.

<table>
<thead>
<tr>
<th>Conditioning dose I (Gy)</th>
<th>Inter treatment time (h)</th>
<th>Challenging/conditioning dose II (Gy)</th>
<th>Inter treatment time (h)</th>
<th>Challenging dose (Gy)</th>
<th>No. of mice</th>
<th>No. of mice that survived till day 90th</th>
<th>Survival (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>0.25</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>24</td>
<td>10</td>
<td>41.66</td>
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<tr>
<td>0.5</td>
<td>6</td>
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<td>8</td>
<td>19</td>
<td>17</td>
<td>89.47*</td>
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<td>0.25</td>
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<td>8</td>
<td>31</td>
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<td>67.74</td>
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<td>0.5</td>
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<td>0</td>
<td>8</td>
<td>18</td>
<td>14</td>
<td>77.77**</td>
</tr>
<tr>
<td>0.25</td>
<td>6</td>
<td>4</td>
<td>24</td>
<td>4</td>
<td>24</td>
<td>16</td>
<td>66.66</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>4</td>
<td>24</td>
<td>4</td>
<td>20</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>0.25</td>
<td>6</td>
<td>0.25</td>
<td>24</td>
<td>8</td>
<td>20</td>
<td>16</td>
<td>80*</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>0.5</td>
<td>24</td>
<td>8</td>
<td>24</td>
<td>6</td>
<td>25</td>
</tr>
</tbody>
</table>

*Significantly different from irradiated control P < 0.01.
**Significantly different from irradiated control P < 0.05.
Characterization of the Adaptive Response to Ionizing Radiation Induced by Low Doses of X Rays to Human Lymphocytes

JEFFERY D. SHADLEY, VEENA AFZAL, AND SHELDON WOLFF

Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, California 94143


The degree of adaptation can vary with interval time (not binary on/off)

Human Lymphocytes [Chromatid & Isochromatid Breaks]

Fig. 1. Time course of expression of the adaptive response. The number of chromatid and isochromatid breaks per cell ±SEM for each time interval between 0.01- and 1.5-Gy doses is shown. Squares and circles represent two separate experiments. The 4-, 5-, and 6-h points are significantly different from the 0-h point (1.5 Gy alone); $P < 0.05$, one-tailed $t$ test.
Adaptation could be induced in waves.

Human Tumour Lines [Apoptosis]
Some adaptation can be similarly induced across a range of conditions.
Cells can be kept in an adapted state.

Low Doses of Very Low-Dose-Rate Low-LET Radiation Suppress Radiation-Induced Neoplastic Transformation In Vitro and Induce an Adaptive Response

E. Elmore, X-Y. Lao, R. Kapadia, E. Giedzinski, C. Limoli and J. L. Redpath

Department of Radiation Oncology and Busch Family Comprehensive Cancer Center, University of California Irvine, Irvine, California 92697

FIG. 4. Adaptive response as assessed by response to a high challenge dose (3 Gy). Experiment 5 is for cells that had accumulated a dose of 216 mGy over a period of 102 days at an average dose rate of 2.12 mGy/day. Error bars are ±SE. VLDR, very low-dose-rate radiation.
Adaptive Responses in the Progeny of Irradiated Cells

• What does it mean when the challenge dose is received by the daughter cells or distant progeny of irradiated cells?

• Priming doses which prevent bystander effects...?

• Adaptation of cells via a bystander effect...?
Adaptive Response and the Bystander Effect Induced by Radiation in C3H 10T½ Cells in Culture

Satin G. Sawant,*1 Gerhard Rands-Pehrson,* Noelle F. Metting*2 and Eric J. Hall*3

* Center for Radiological Research, Columbia University, New York, New York; and * Pacific Northwest National Laboratory, Richland, Washington

A priming dose can protect from Bystander Effects.

Mouse C3H 10T½ fibroblasts
[Cell Survival]

FIG. 1. The adaptive response and the bystander effect for cell survival in C3H 10T½ cells. The dotted line shows the percentage of cells that would be expected to survive when 10% of the cells are exposed to various numbers of α particles calculated from the survival curve for all cells irradiated. The squares show survival for various numbers of α particles, from 1 to 12, traversing 10% of the cell population. The extent to which this falls below the dotted line is an indication of the magnitude of the bystander effect. The circles show survival for cells exposed to 2 cGy of γ rays, 6 h before exposure to various numbers of α particles traversing 10% of the population. The extent to which the circles are above the squares reflects the adaptive response.
Variability of the adaptive response to ionizing radiations in humans *

Alba Bosi and Gregorio Olivieri

Dipartimento di Genetica e Biologia Molecolare, Università di Roma 'La Sapienza', Rome (Italy)

(Received 22 June 1988)
(Revision received 26 September 1988)
(Accepted 5 October 1988)

Individuals do not show a consistent adaptive response.

Fig. 1. The effect of pretreatments with \(^{3} \text{H}\)dThd (0.01 \(\mu\)Ci/ml; spec. act. 78.8 Ci/mmmole), from 32 h of culture until fixation, or 0.01 Gy of X-rays at 32 h of culture, on the frequency of chromatid aberrations induced in human lymphocytes of different donors. (———) 0.75 Gy; (○) \(^{3} \text{H}\)dThd + 0.75 Gy; (△) 0.01 Gy + 0.75 Gy.
Individuals do not always show a repeatable adaptive response.
Low-Dose Irradiation Alters the Transcript Profiles of Human Lymphoblastoid Cells Including Genes Associated with Cytogenetic Radioadaptive Response

Matthew A. Coleman, a Eric Yin, a Leif E. Peterson, a David Nelson, a Karen Sorensen, a James D. Tackett a and Andrew J. Wyrobek a

a Biology & Biotechnology Research Program, Lawrence Livermore, National Laboratory, Livermore, California 94551; and

Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

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$k$ value ± SE

Adaptation

Cell Line

GM15036 GM15510 GM15268
Is There an Adaptive Response in Spleen Lymphocytes of C57Bl/6 Mice as Assessed by Chromosomal Aberrations?

ANDRZEJ WOJCIK, KAREN BONK, AND CHRISTIAN STREFFER

Institut für Medizinische Strahlenbiologie, Klinikum Essen, Hufelandstr. 55, D-4300 Essen 1, Germany
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Medium change

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0.1 + 1.5 Gy | 44 |
16 | Control | 1 |
0.1 Gy | 1 |
1.5 Gy | 56 |
0.1 + 1.5 Gy | 46 |
20 | Control | 2 |
0.1 Gy | 2 |
1.5 Gy | 55 |
0.1 + 1.5 Gy | 66 |
17 | Control | 1 |
0.1 Gy | 0 |
1.5 Gy | 65 |
0.1 + 1.5 Gy | 58 |
17 | Control | 1 |
0.1 Gy | 2 |
1.5 Gy | 55 |
0.1 + 1.5 Gy | 69 |
21 | Control | 5 |
0.1 Gy | 0 |
1.5 Gy | 53 |
0.1 + 1.5 Gy | 64 |
18 | Control | 2 |
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1.5 Gy | 60 |
0.1 + 1.5 Gy | 49 |
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0.1 Gy | 0 |
1.5 Gy | 60 |
0.1 + 1.5 Gy | 66 |
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0.1 + 1.5 Gy | 38 |
18 | Control | 1 |
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0.1 + 1.5 Gy | 67 |
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1.5 Gy | 55 |
0.1 + 1.5 Gy | 44 |
18 | Control | 0 |
0.1 Gy | 3 |
1.5 Gy | 50 |
0.1 + 1.5 Gy | 50 |
18 | Control | 0 |
0.1 Gy | 3 |
1.5 Gy | 65 |
Radioadaptive response: Efficient repair of radiation-induced DNA damage in adapted cells

Takaji Ikushima *, Hisako Aritomi, Jun Morisita

Biology Division, Kyoto University of Education, 1 Fukakusa-Fujinomori, Fushimi-ku, Kyoto 612, Japan

Adapted cells still show the same initial DNA damage response.

CHO V79 Cells [Comet Assay]

Fig. 2. Induction of DNA double-strand breaks by $\gamma$-rays in adapted and non-adapted cells. Immediately after challenging doses, cells were subjected to single cell gel electrophoresis as described in the Materials and method section. Each point represents the mean of 50 cells from each sample. The range bars indicate standard deviations.
Fig. 4. Repair kinetics of DNA double-strand breaks induced by 1.5 Gy of γ-rays in adapted and non-adapted cells. After irradiation, repair was allowed by incubating the cells at 37°C. The analysis of DNA strand breaks was done as described in Section 2: Materials and methods. Each point represents the mean of 50 cells from each sample.

Fig. 3. Repair kinetics of DNA double-strand breaks induced by 5 Gy of γ-rays in adapted and non-adapted cells. After irradiation, repair was allowed by incubating the cells at 37°C. The analysis of DNA strand breaks was done as described in the Section 2: Materials and methods. Each point represents the mean of 50 cells from each sample.

But they may show faster repair.
Activation of Antioxidative Enzymes Induced by Low-Dose-Rate Whole-Body γ Irradiation: Adaptive Response in Terms of Initial DNA Damage

Kensuke Otsuka,† Takao Koana, Hiroshi Tauchi* and Kazuo Sakai†

* Low Dose Radiation Research Center, Central Research Institute of Electric Power Industry, Tokyo, Japan; and † Department of Environmental Sciences, Faculty of Science, Ibaraki University, Ibaraki, Japan

Or, adapted cells may show reduced initial DNA damage.

Mouse spleen cells [Comet Assay]

**FIG. 1.** Increase in tail moment in mouse spleen cells estimated by the comet assay. The tail moment was estimated immediately after irradiation with the challenge dose. Diamonds: mice irradiated only with a challenge dose. Squares: mice pretreated 0.5 Gy of low-dose-rate radiation before the challenge dose (*P < 0.05). Triangles: spleen cells irradiated on ice in vitro. The broken lines show the regression lines for low-dose-rate irradiated and control groups. Based on ANCOVA, the slopes of the lines are not significantly different.
Low-dose Radiation Attenuates Chemical Mutagenesis In Vivo

-Cross Adaptation-

Shizuko KAKINUMA, Kazumi YAMAUCHI, Yoshiko AMASAKI,
Mayumi NISHIMURA and Yoshiya SHIMADA

Radiation may induce adaptation against chemical-induced mutations.

Fig. 1. Experimental design for gpt mutation analysis of thymic DNA from mice treated with X-rays, ENU or a combination of the two. Mice were exposed to X-rays weekly for 4 weeks. ENU was administered at a concentration of 200 ppm in drinking water.

Fig. 2. Mutant frequency analysis of gpt recovered from thymus DNA from control, irradiated (0.2 Gy × 4), ENU-treated, and irradiated/ENU-treated mice. *P < 0.05, significantly different from control. **P < 0.05, significantly different from ENU. Bars represent mean ± S.D.
FIG. 4. Influence of adapting treatments on leukemia latency. Panel A: Survival of mice that developed myeloid leukemia after a chronic irradiation (1.0 Gy, 0.5 Gy/h) with (■) or without (○) a prior 0.1-Gy (0.5 Gy/h) exposure compared to survival of unirradiated control animals that did not develop AML (+). Panel B: Survival of mice that developed
did not develop AML (+). Panel B: Survival of mice that developed myeloid leukemia after a chronic 1.0-Gy irradiation (0.5 Gy/h) with (■) or without (○) prior whole-body hyperthermia (40.5°C, 60 min) compared to unirradiated control animals that did not develop AML (+).
A priming dose can delay but not prevent radiation-induced cancers.

p53+/- mice
[Malignant Tumours]
Adaptive Response

Exposure to one dose of radiation prior to exposure to a subsequent irradiation can reduce the effect compared to the second dose alone.
So, what do we really know...?

- Radiation is clearly capable of inducing mechanisms that can counteract subsequent radiation-induced damage, but is also able to sensitise cells to further exposures.
- Adaptation is highly variable between individuals, even between inbred mice, suggesting that it is highly dependent on the state of the system at the time of irradiation(s).
- Cells appear receptive or refractory to adaptation depending on the cell cycle, stimulation etc.
- There is no universal priming dose ‘window’ or generic interval which can be generalised between experimental systems.
So, what do we really know…?

• The complex matrix of interacting variables means that when non-responders are identified, they may still be capable of adaptation, but may have different time/dose/dose-rate windows.

• Non-responders may already be pre-adapted, if the same adaptive responses can be induced by other stressors.

• The variety of agents which can inhibit adaptive responses suggests that radiation conditioning perturbs homeostasis rather than triggering a specific, dedicated pathway.

• Cross-adaptation experiments suggest that radiation conditioning may compete with other counteracting and sensitising stimuli.
What do we still **not** know...?

- What is the net effect on cancer from the disparate responses for the relevant lower-level endpoints?
  - What does it mean if there is adaptation for apoptosis but not DNA damage for a given exposure scenario?

- How does the complex nature of human radiation exposures over a lifetime affect the responses to rare higher doses?
  - Diagnostic radiology and radiotherapy.
  - What is the impact of varying rates of natural background radiation?

- Almost all experiments use challenge doses that are not relevant for the general population. What is the effect of sequential/concurrent radiation exposures for doses in the occupational, medical diagnostics range?

- What is the impact of publication bias?
What does the adaptive response mean for regulation?

- Regulations and safety standards cannot be personalised for millions of complex and unique irradiation histories (even if we could gather the dosimetry in the first place).
- Radiation ‘conditioning’ or even a mimetic could reasonably be expected to cause sensitisation in some people, even if a widely applicable dosing regime was shown to be useful in the majority of people.
- Yet, the mere existence of adaptive responses to radiation demonstrate that simple extrapolations or biophysical arguments are unlikely to accurately describe the complexity of low dose responses.
- **Do adaptive responses simply add to risk uncertainty?**
Strategies for Future Research

• Inclusion of common endpoints.
  • ‘Plus one’ approach: e.g. your own endpoint plus micronucleus assay
  • Gives information about how different endpoints react under the same conditions, and helps to build a collaborative dataset.

• Adoption of common models.
  • Which cells/animals/tissues would best represent human tissues at risk...

• ‘How low can you go?’
  • Choice of priming and challenge doses that fit into the most relevant human exposure scenarios.
  • Replicating realistic exposure patterns:
    • Scanning, planning and radiotherapy
    • Daily occupational patterns
Acknowledgements

• Professor Pamela Sykes
  • Flinders University

• Dr Shizuko Kakinuma, Dr Yoshiya Shimada
  • National Institute of Quantum and Radiological Science and Technology, Japan

• The authors of all the papers cited.