Radiation-induced bystander effects in biota- A problem for Radiation Protection?

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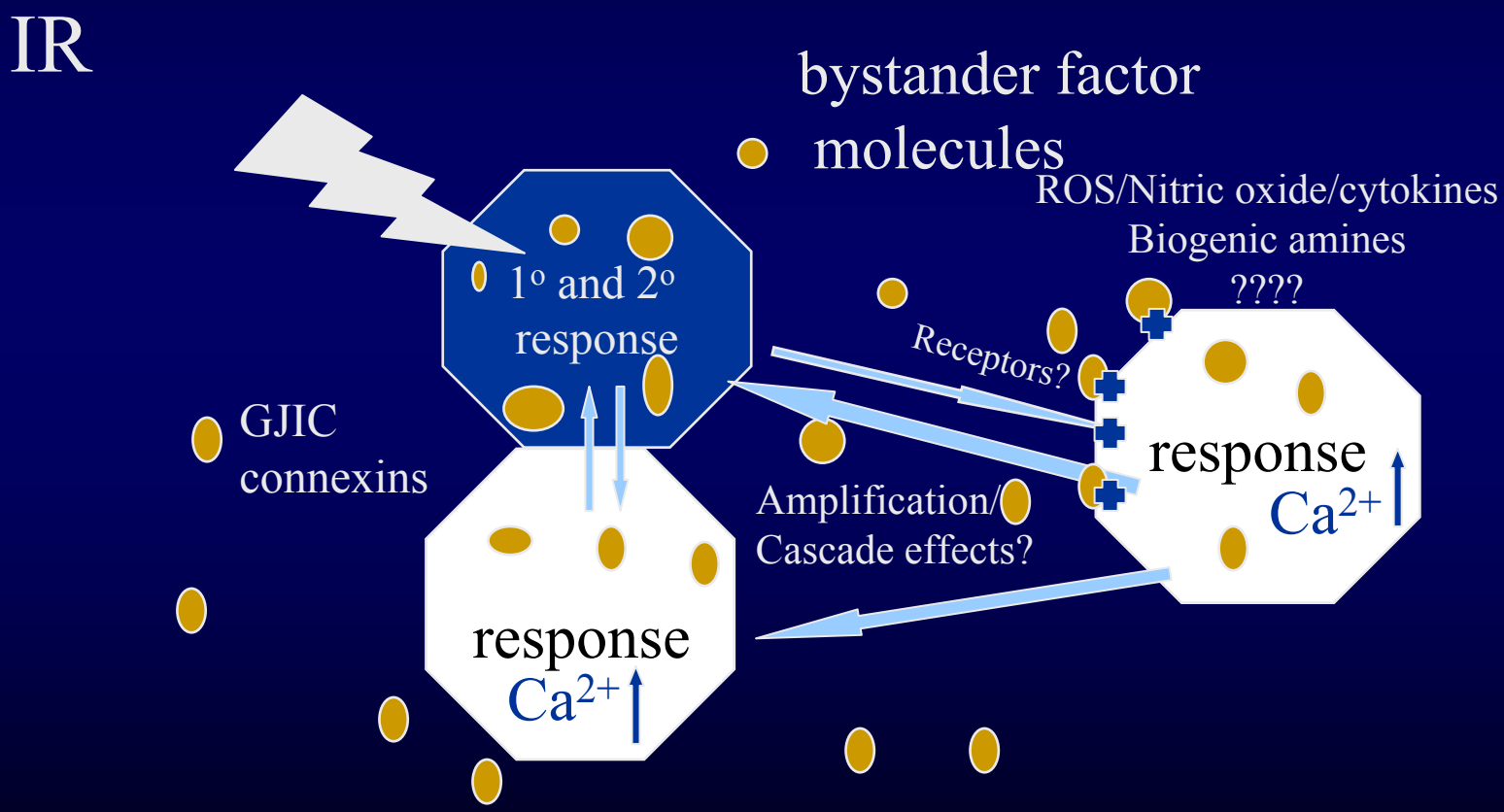
Presentation Outline

- What are radiation-induced bystander effects?
- What is special about them?
- How are they detected?
- Why/how do they happen?
- Where are they relevant?
- Can we harness them for therapeutic purposes?

The bystander effect



Detection of bystander effects

- Use targeted microbeam or high LET low fluences; detect effects in cells not targeted
- Use medium harvested from irradiated cells and look for changes in unexposed cultures receiving this medium
- Introduce unirradiated cells into co-culture with irradiated cells and measure effects
- Take blood or tissue from irradiated animals or human patients, and look for signals produced into medium/serum by cells cultured in vitro.

Bystander effects - What responses are seen to the signals?

- Apoptosis and other forms of cell death
- Genomic instability and other delayed effects
- Induction of early response proteins
- Adaptive responses
- Oxidative stress
- Proliferation
- Delayed cytogenetic effects
- Transformation

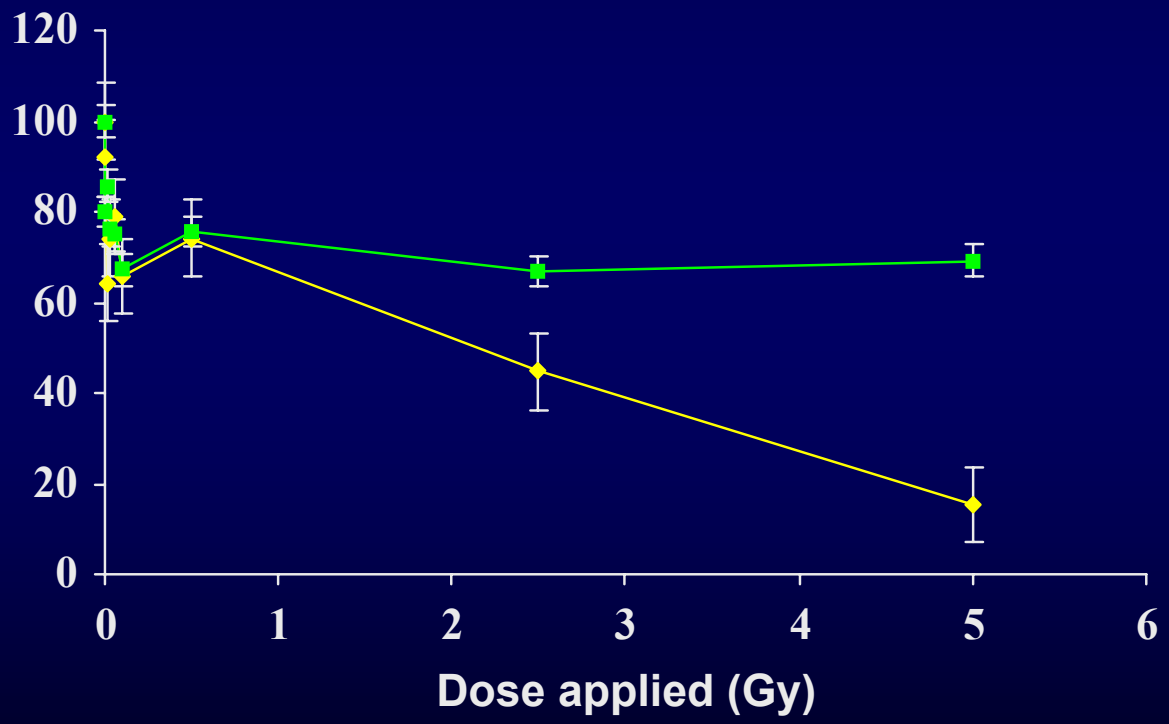
Bystander effects - How are they expressed?

- Initial mechanism similar to a stress response
- Long-term perpetuation appears to involve genomic instability type mechanisms
- Final outcome determined mainly by genetic make-up and life-style factors and not by dose.

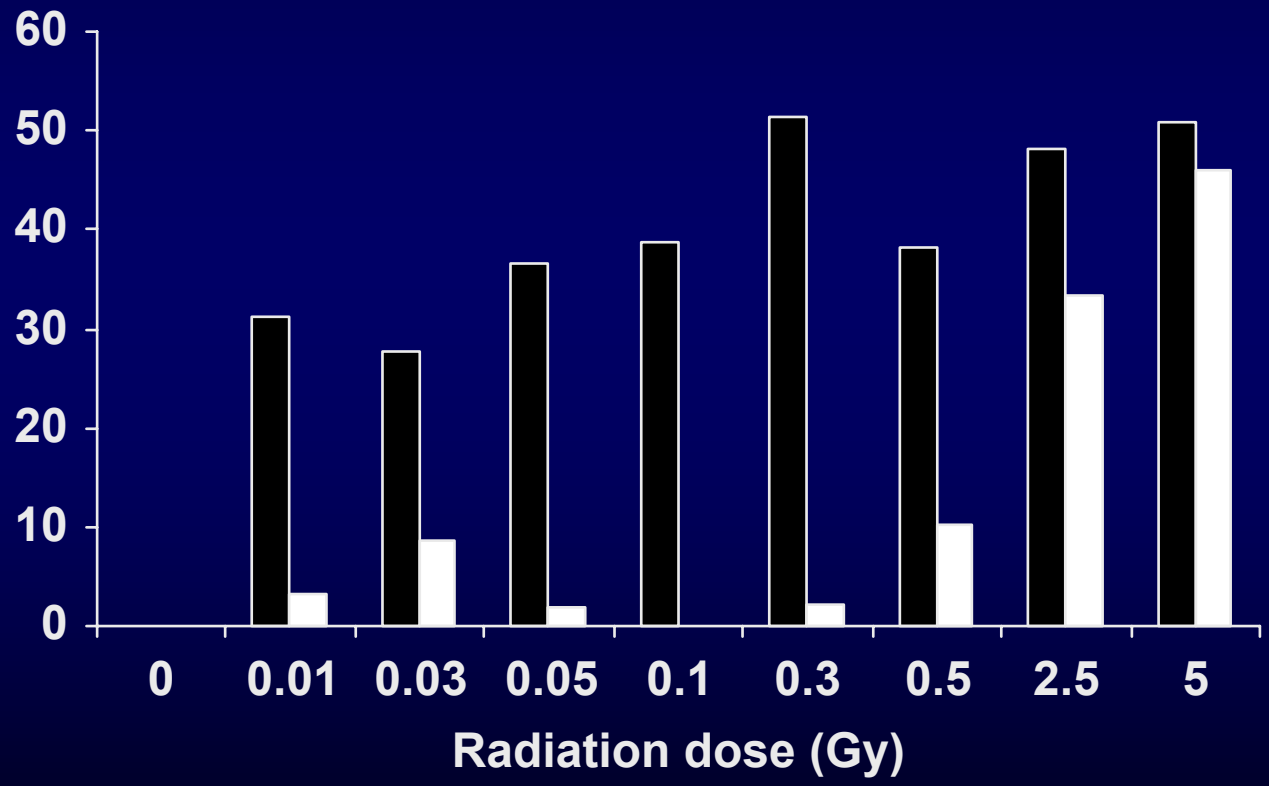
The toxic bystander effect

- Many laboratories measure cell death, chromosome damage, mutation, etc but it is entirely possible that cells which do not apparently show these effects do show other effects which are not being measured! Care is needed in interpretation of data, especially negative results.

Bystander effects at low doses in Human Keratinocytes



Direct v bystander effect



What is the signal?

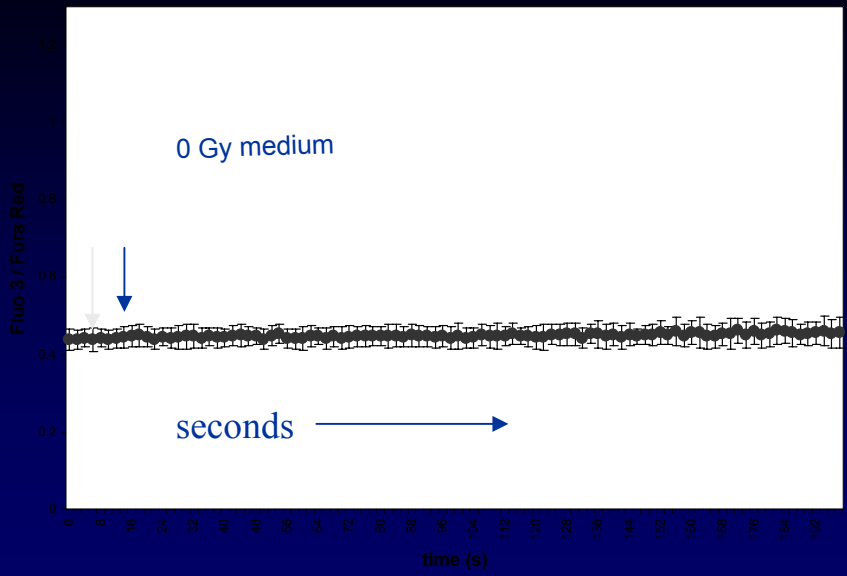
- Nature of the signal is unknown
- Destroyed by repeated freeze thaw cycles and destroyed by heating, very small size (<400 daltons).

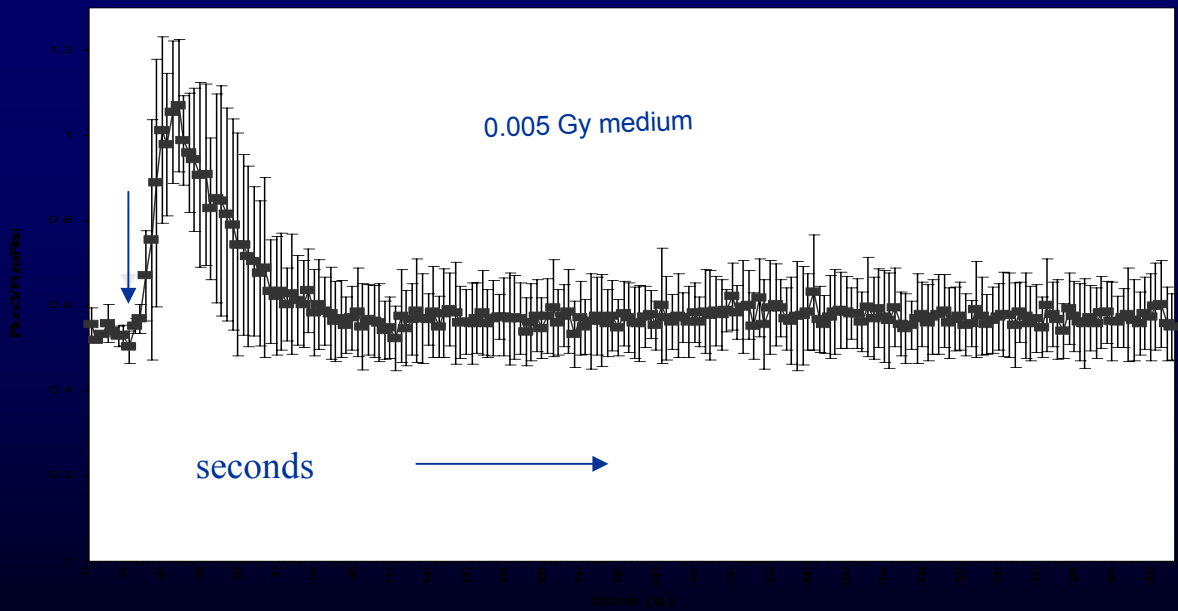
Transduction of the response

The initial cellular response to the signal

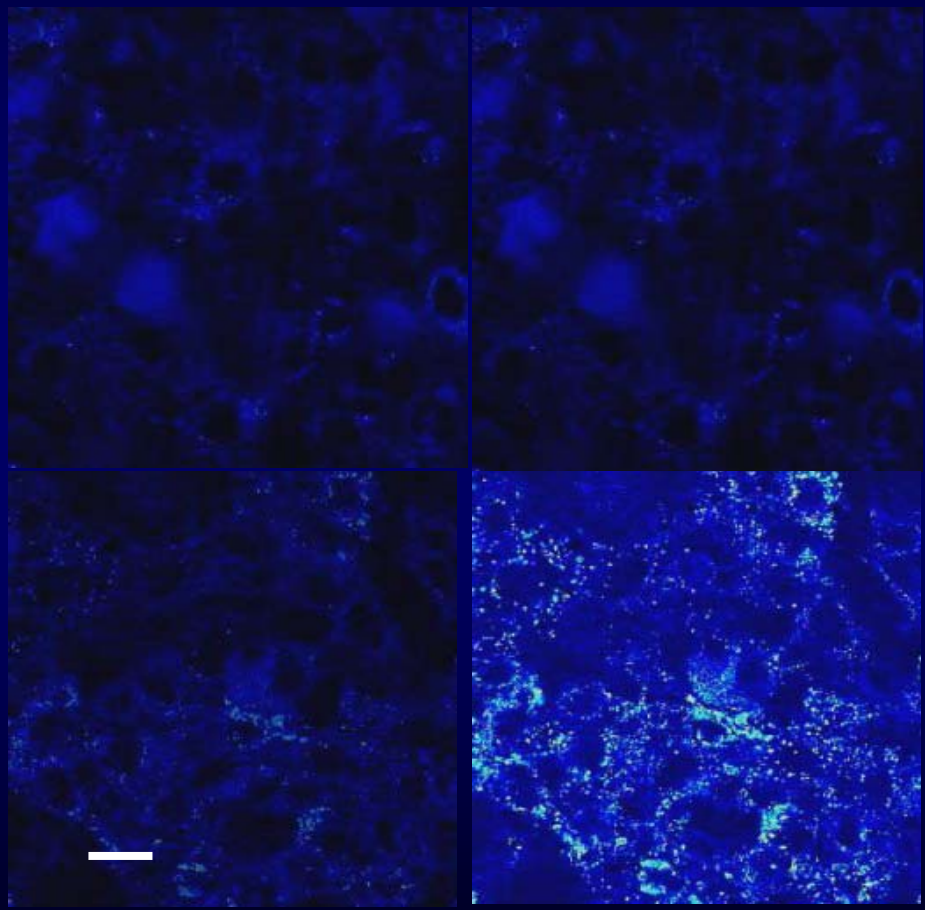
- Induction of 2 min calcium flux in 10sec
- Long-term (greater than 6hrs) induction of mitochondrial membrane potential collapse
- Long-term induction of oxy-radical production
- p53 independent

Signal after exposure to ICCM from 5mGy irradiated cells

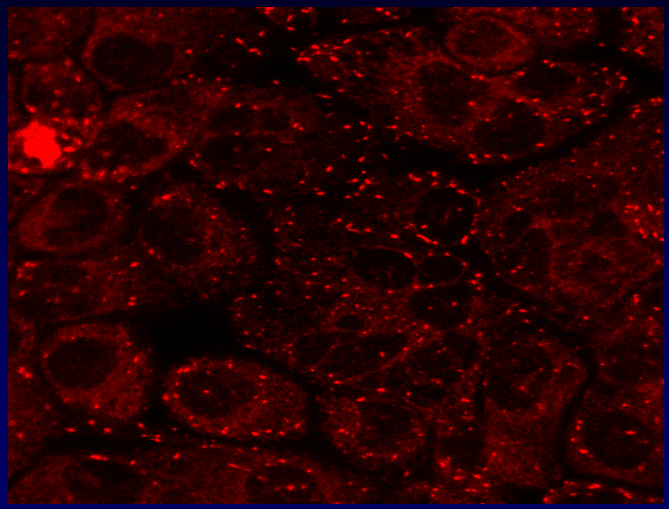


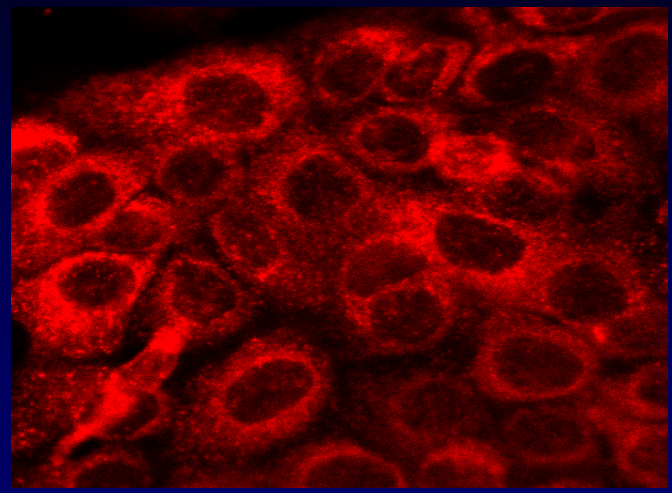


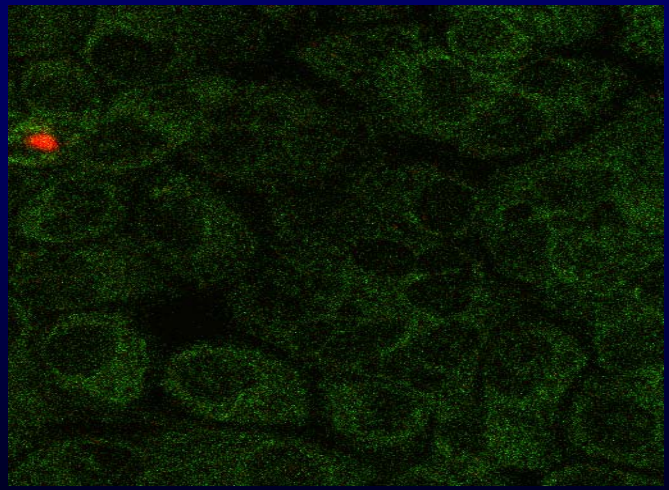
Calcium fluorescence following addition of ICCM to cells

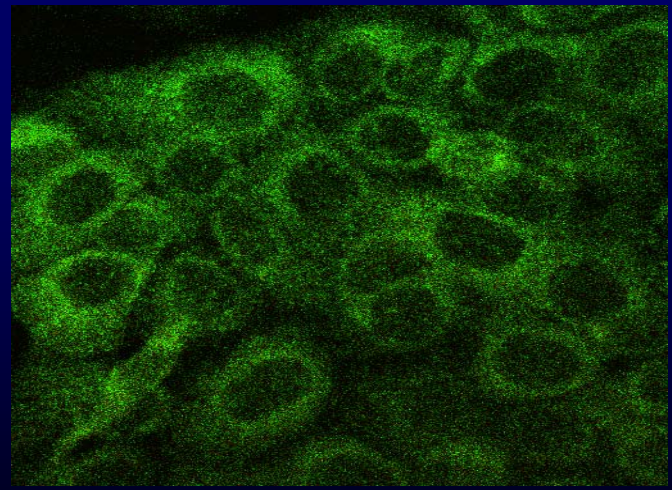


Mitochondrial membrane depolarisation









0 Gy

0.005Gy

Is the effect relevant in vivo??

- Evidence from fresh human, mouse, fish and prawn tissue irradiated ex vivo
- Evidence from mice irradiated in vivo to low total body doses
- Evidence from bloods taken from radiotherapy patients showing variation during therapy

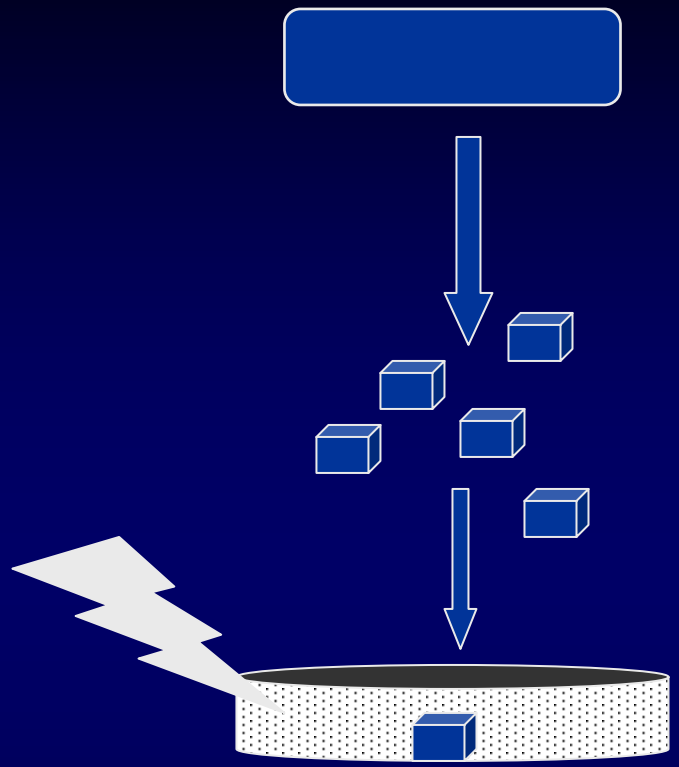
Methods for detecting signals in tissues

- Media harvest from exposed explants or whole tissues
- Detection of signals using reporter cells (clonogenic responders or autologous explants) which are exposed only to media from irradiated samples
- Endpoints include growth,apoptosis, protein expression, calcium fluxes and mitochondrial responses

Explant technique

Original tissue explant
with cells stained in situ

Measuring response *in vitro*



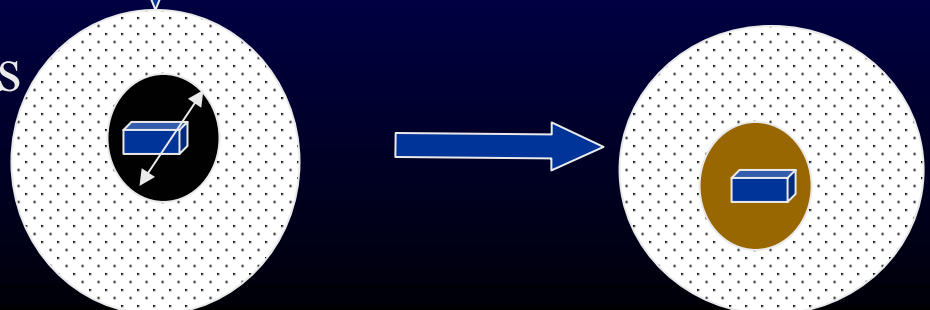
Fresh tissue

Explant pieces

Culture and irradiation of explants

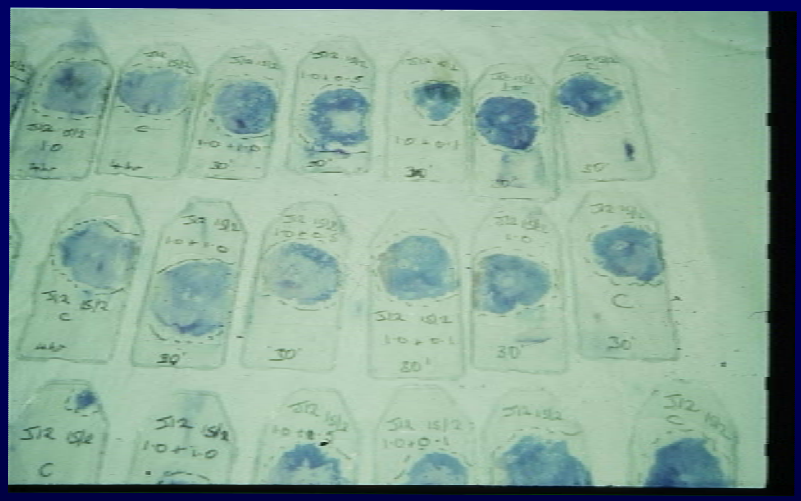
Measure Outgrowth up to 14 days

Quantify growth and response in irradiated and control cultures and harvest medium



Stain outgrowth and quantify protein expression

Explant culture technique

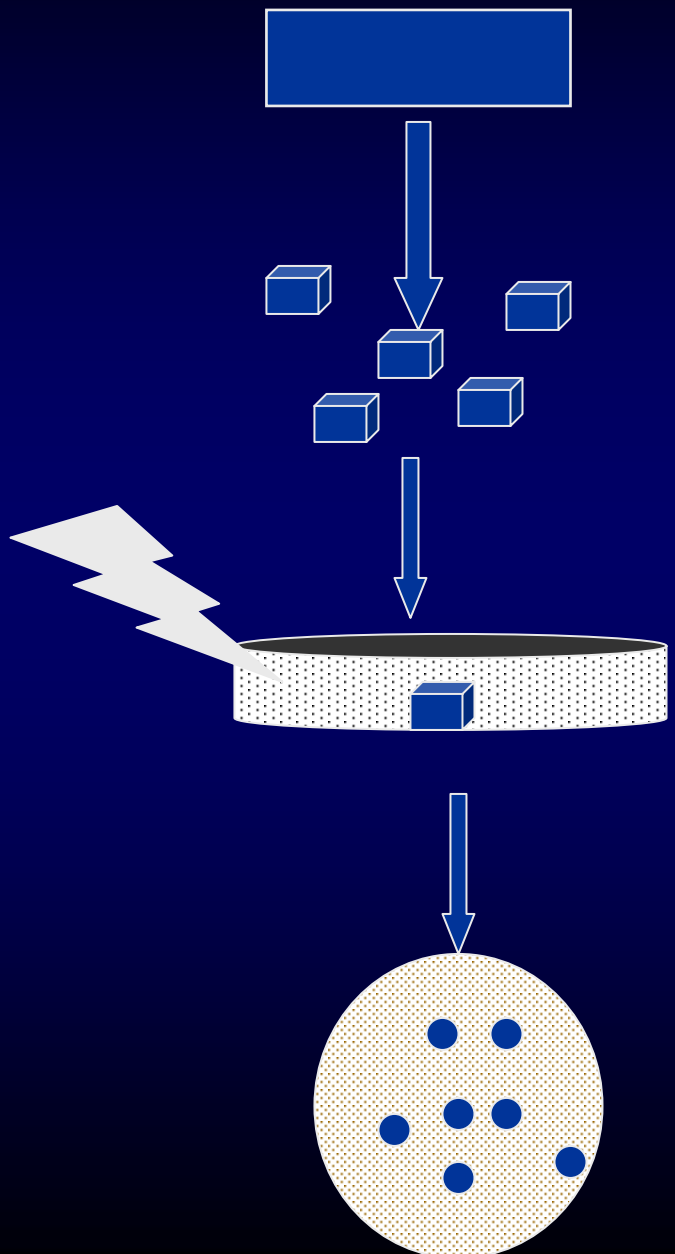


- Typical result from an explant culture experiment aimed at comparing the delayed effects of low level radiation exposure on growth and differentiation of tissue cultured in vitro

Human data

- 300 normal human urothelial samples show wide variation between subjects and three basic response catagories
- 50 samples from benign prostate where blood samples from the same patient were available show correlation between response of both tissues
- New data from nephrectomy patients show normal tissue signals following ex vivo irradiation but none from tumour cells

Measuring bystander response *in vitro*



Fresh tissue

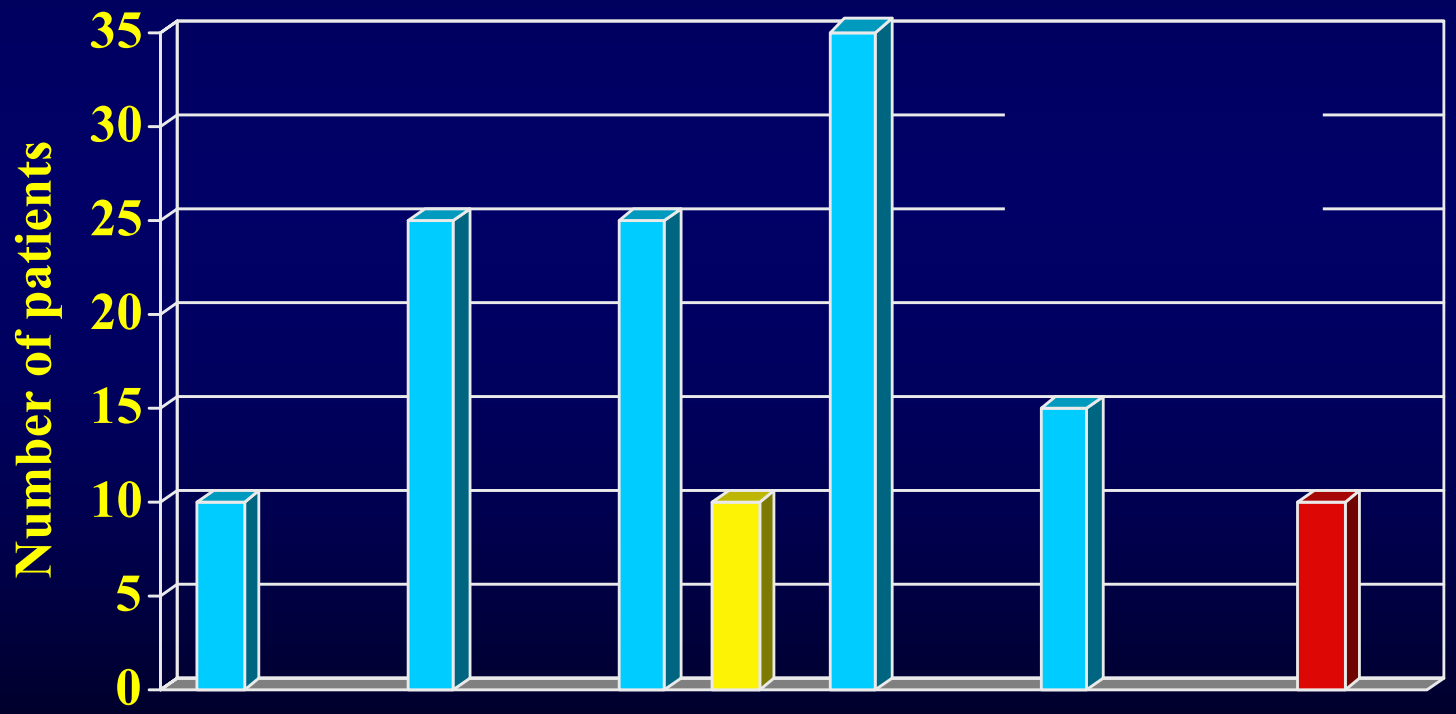
Explant pieces

Culture and irradiation of explants

Harvest culture medium

Add to unirradiated clonogenic cell line and determine SF

Individual variation in the cytotoxic properties of bystander medium

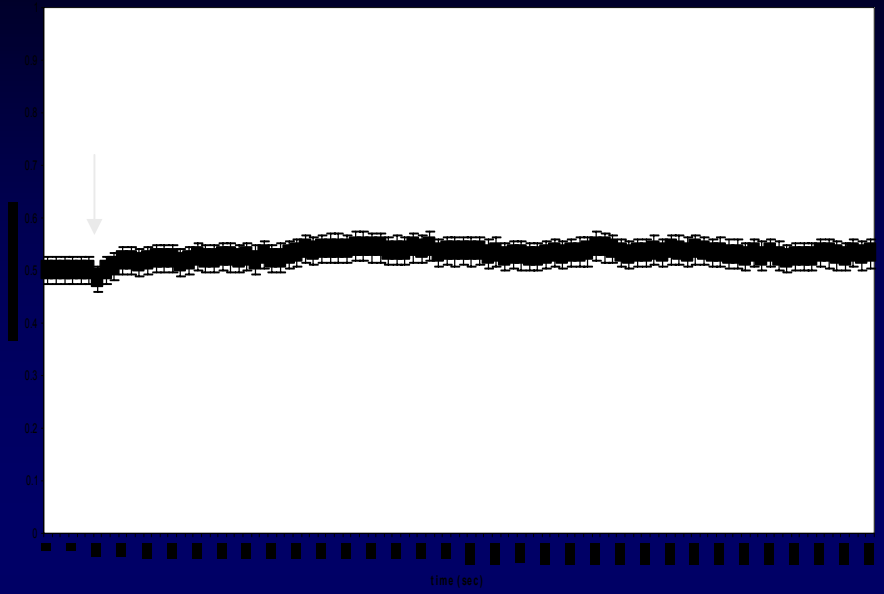


Mouse data

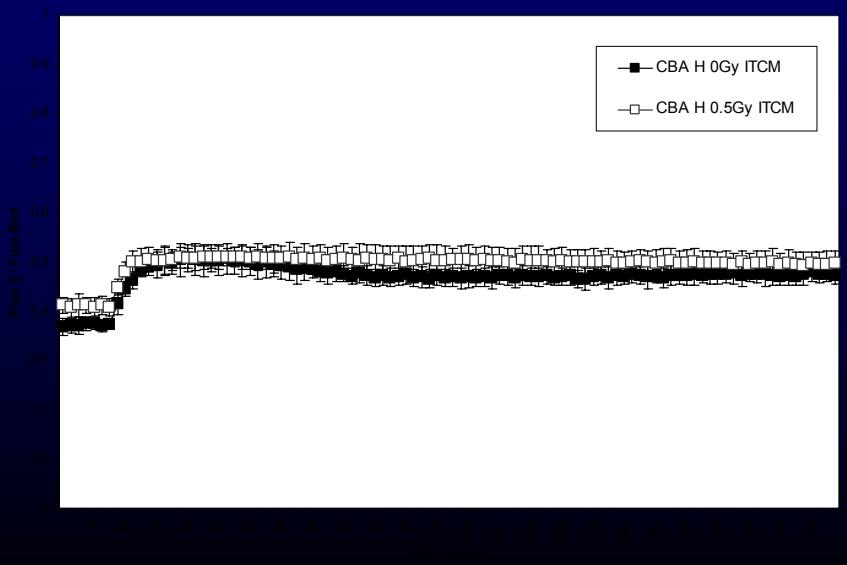
- Bladders taken from mice given 0.5 Gy TBI or irradiation to bladder explants ex vivo.
- CBA/Ca strain is radiation resistant, C57Bl/6 is radiosensitive
- Apoptotic cascade induced in cells exposed to signals from the sensitive mice only

Calcium ratios in contol and 0.5Gy TBI CBA/Ca and C57BL/6 mice

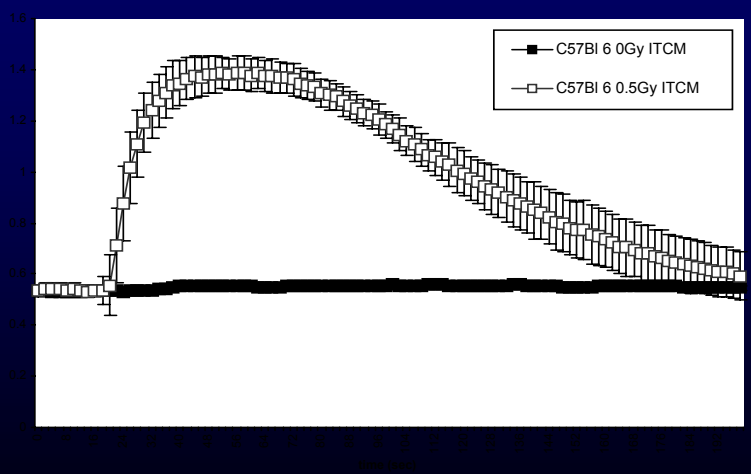
Medium only



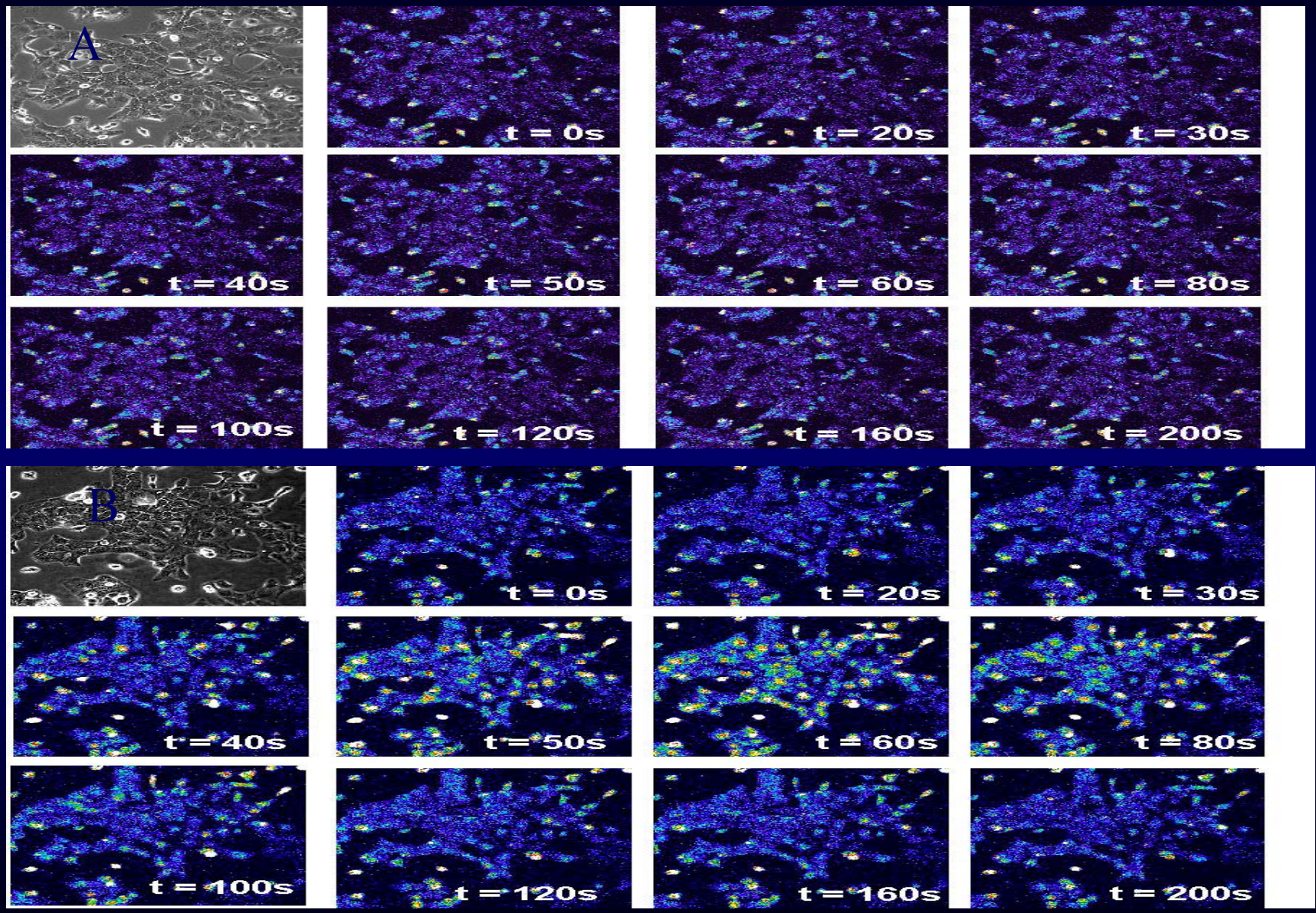
CBA/Ca



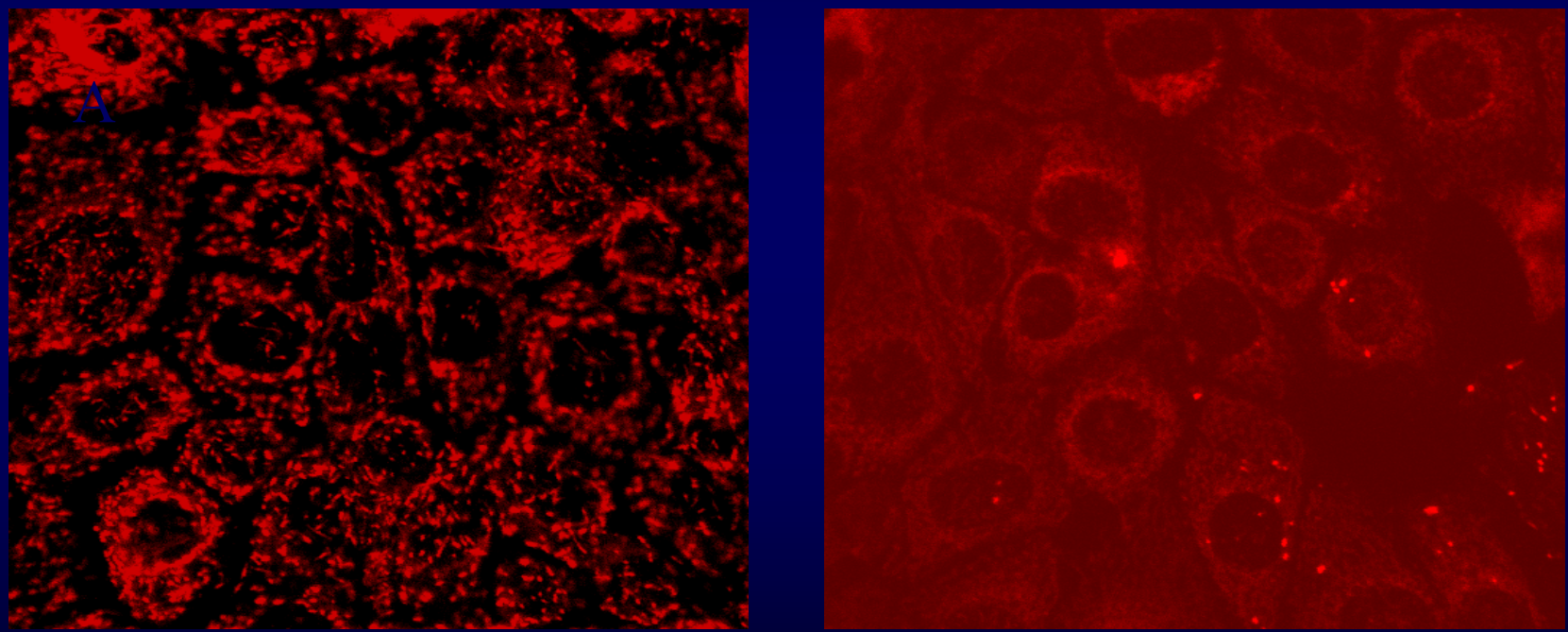
C57BL/6



Real time calcium flux for Control and CBA/Ca mice (A) and C57BL 6 0.5Gy TBI (B)



Mitochondrial membrane potential decrease in C57BL/6 0.5Gy TBI



Significance of bystander effects

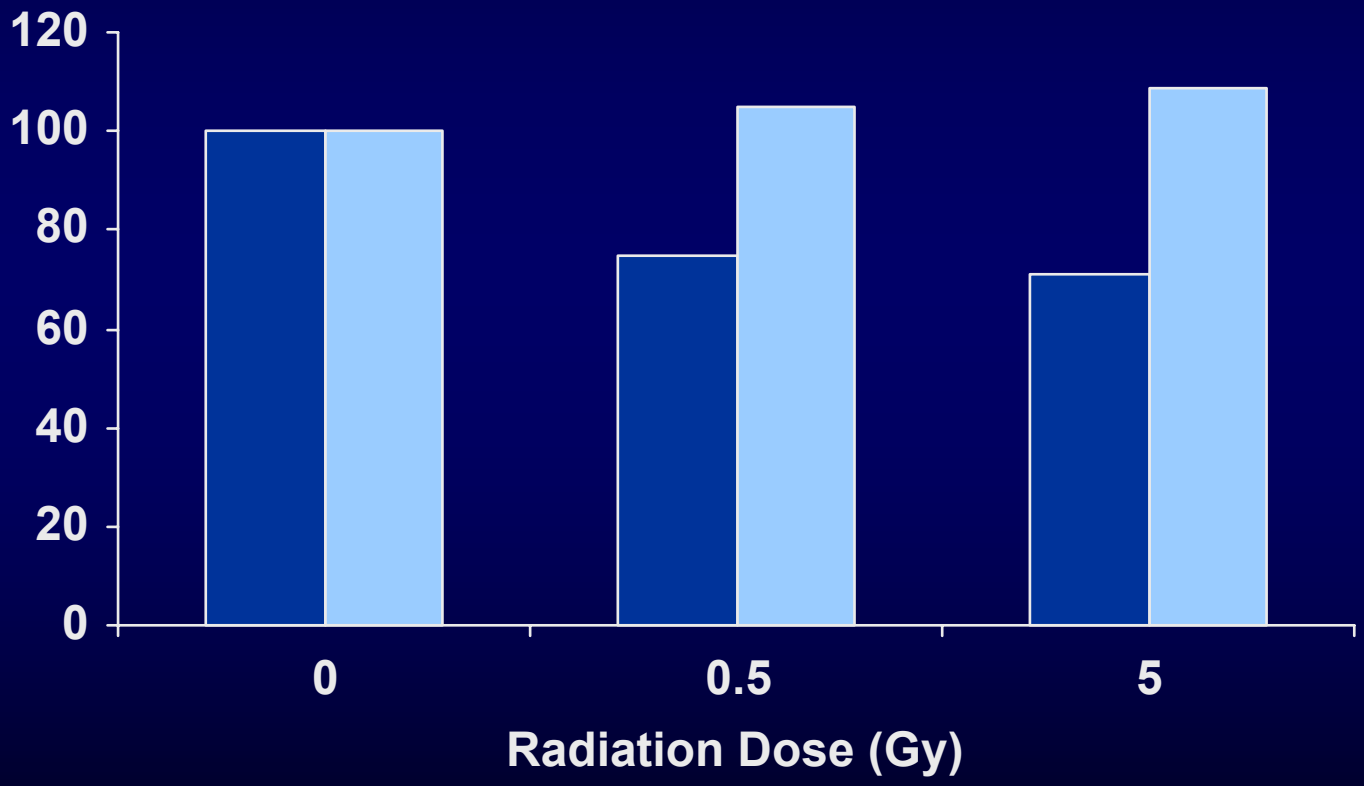
- Therapy
- Carcinogenesis
- Protection of biota
- Production of novel biological compounds

Models, techniques and mechanisms

Radiotherapy: Key questions

- What is the signal and can we inhibit it or harness it?
- What mechanisms control signal production and response?
- What is the basis of the genetic relationship?
- How can bystander effects be modulated?

Tumour and associated normal bystander effect



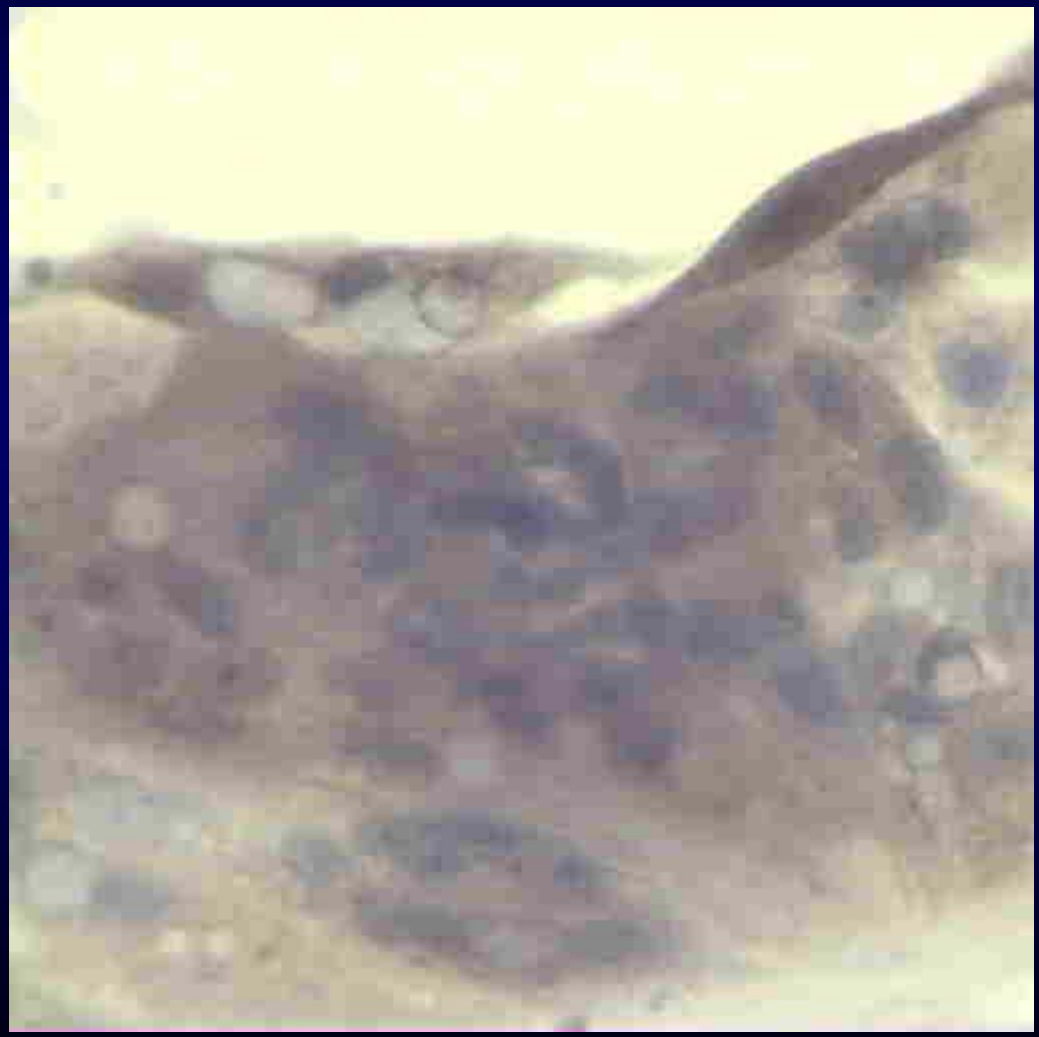
Prevention of bystander effects using *l*-deprenyl

- Signal production unchanged
- Recipients induce bcl-2
- Using explant technique, bladder tumours can be shown to express high levels of bcl-2 in response to irradiation (2Gy)
- Normal explants have lower induction but tumour derived bystander medium or Normal +l-deprenyl leads to greater expression

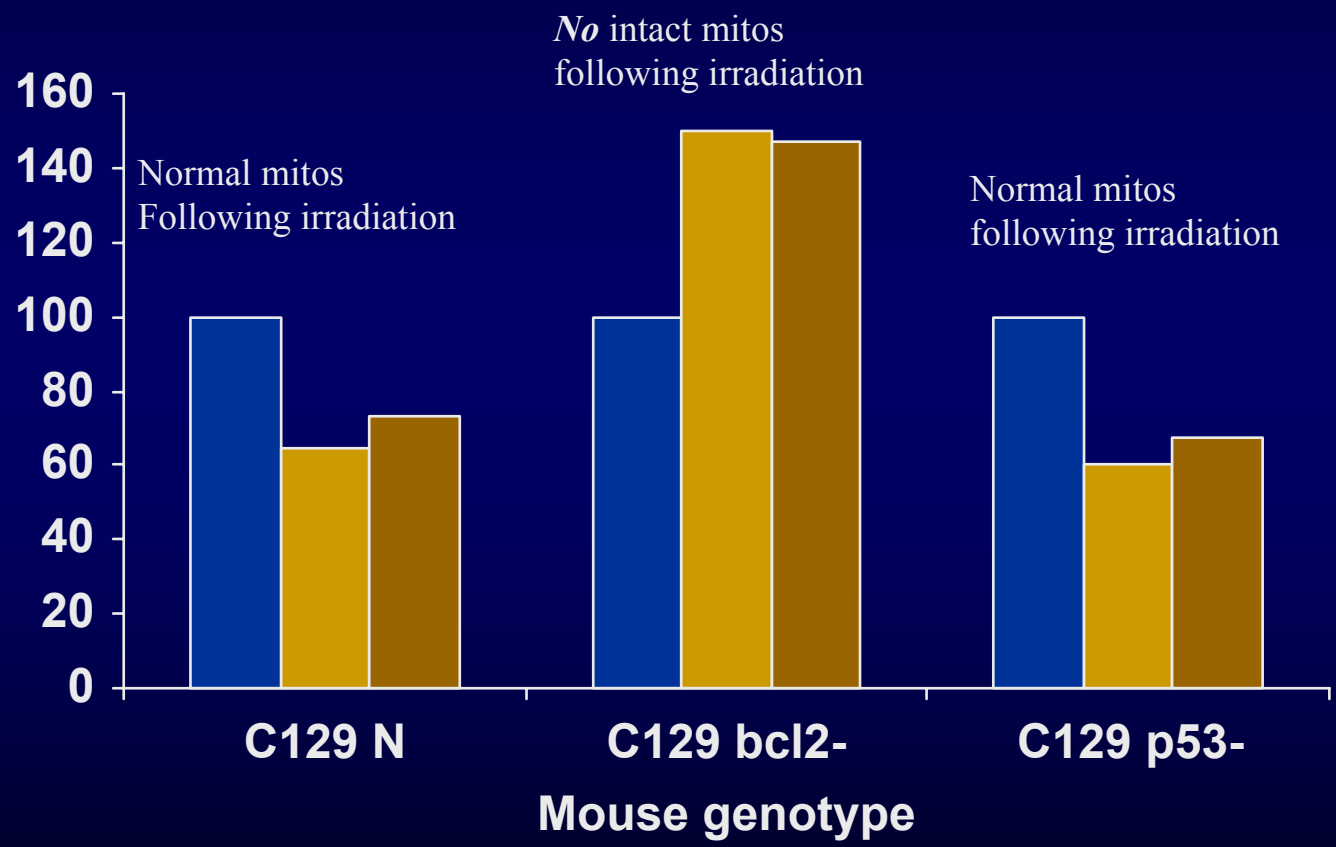
Effect of L-deprenyl on bcl 2 expression in explant cultures

Treatment	**%bcl 2 + Normal**	**% bcl 2 + Tumour**
control	0	100
9nM L-deprenyl	100	100
5Gy	49±13.2	100
5Gy+ 9nM L-deprenyl	100	100

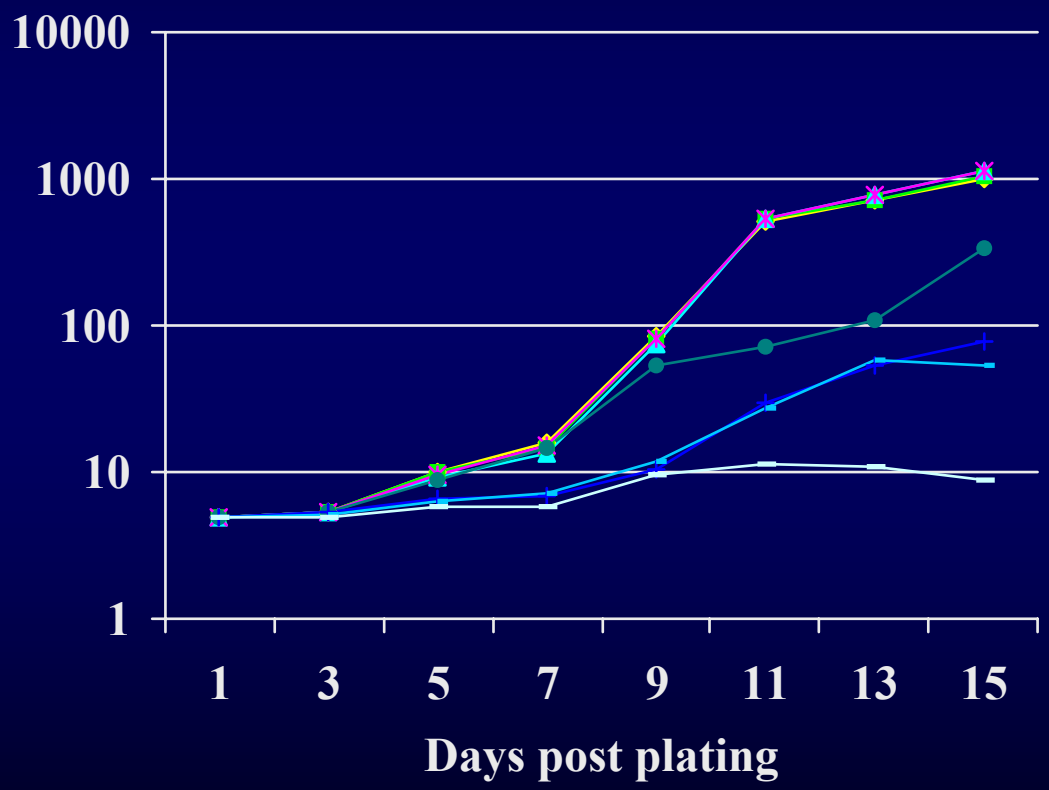
Bcl 2 expression and lack of cellular damage in bladder culture treated with ICCM +9nM Deprenyl



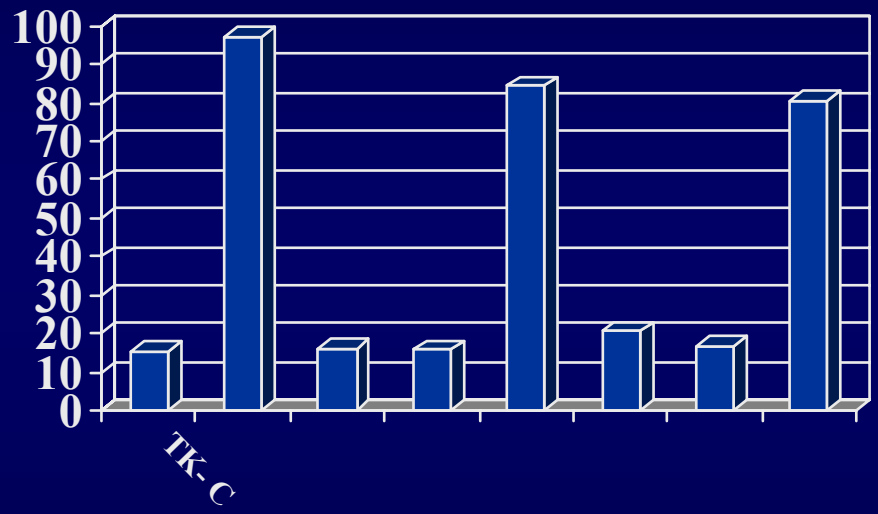
Bystander effects in Knockout mice



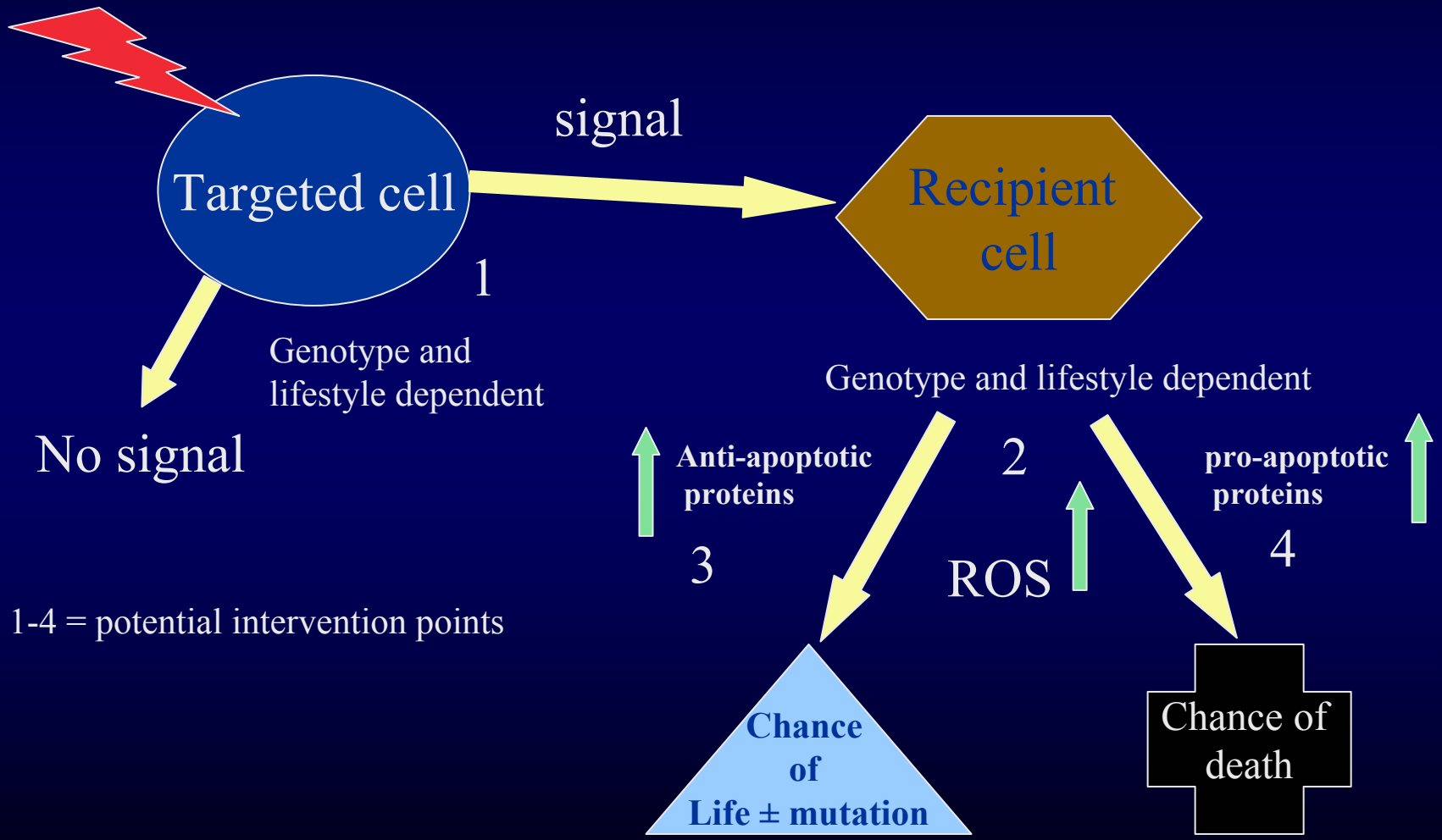
Growth curves for TK- and Raji cells post 0.5Gy or ICCM



Bcl_2 positive cells post exposure of TK- and Raji 10 cells to 0.5Gy or ICCM



Possible model for expression of bystander effects in humans of relevance to therapy

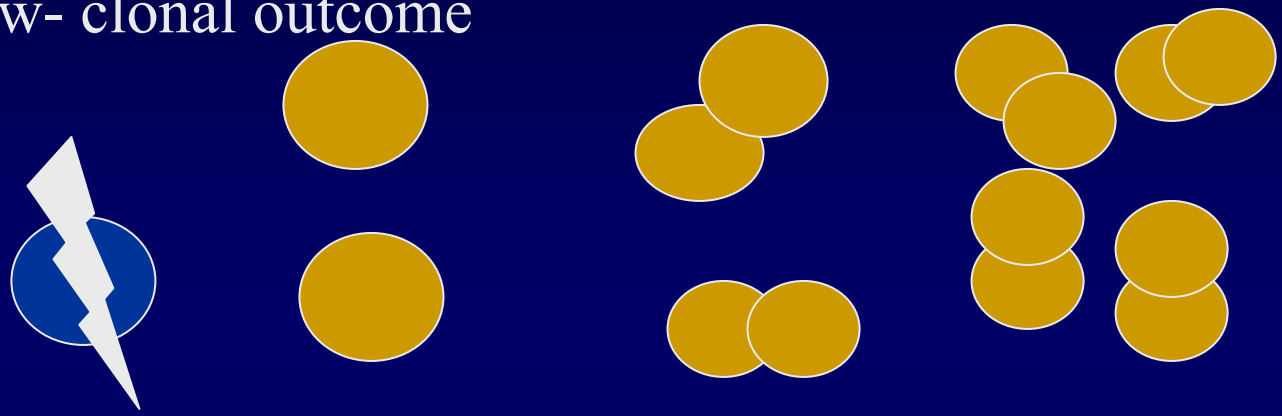


Carcinogenesis and the link between genomic instability and bystander effects

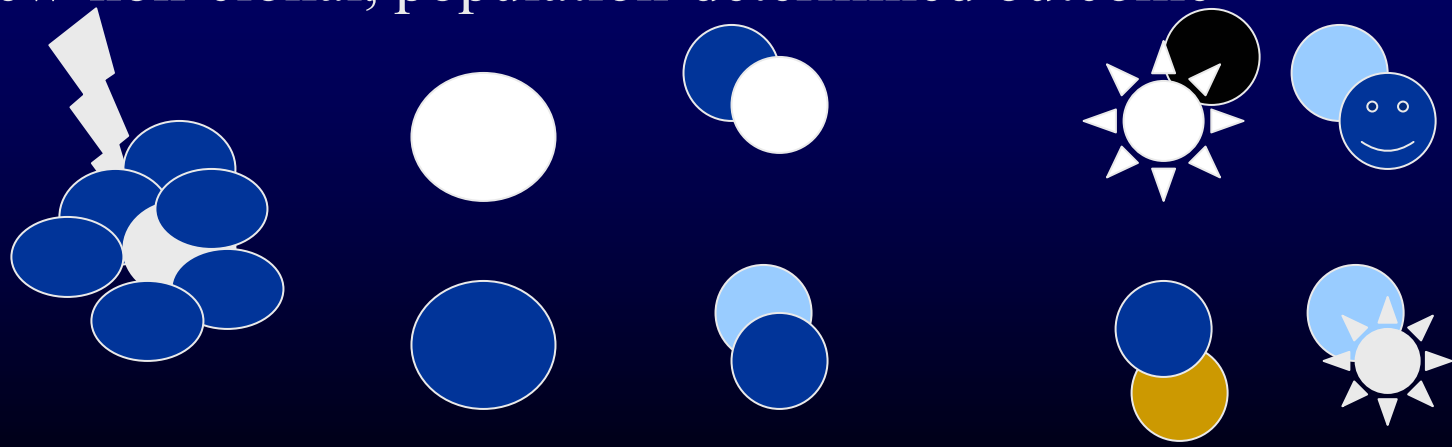
- Linked mechanistically
- Occur at very low doses (fully saturated at 5mGy acute dose)
- Inducible in vivo and in a wide range of species (fish, crustaceans, molluscs and sponges as well as mammals)
- Linked to innate immunity (self-non-self recognition) in tunicates and probably in other species
- Perpetuated in progeny
- Detectable using many different endpoints measuring death, survival, proliferation, mutation, transformation
- Relevance of effects to “harm” not established

The link between bystander effects and genomic instability

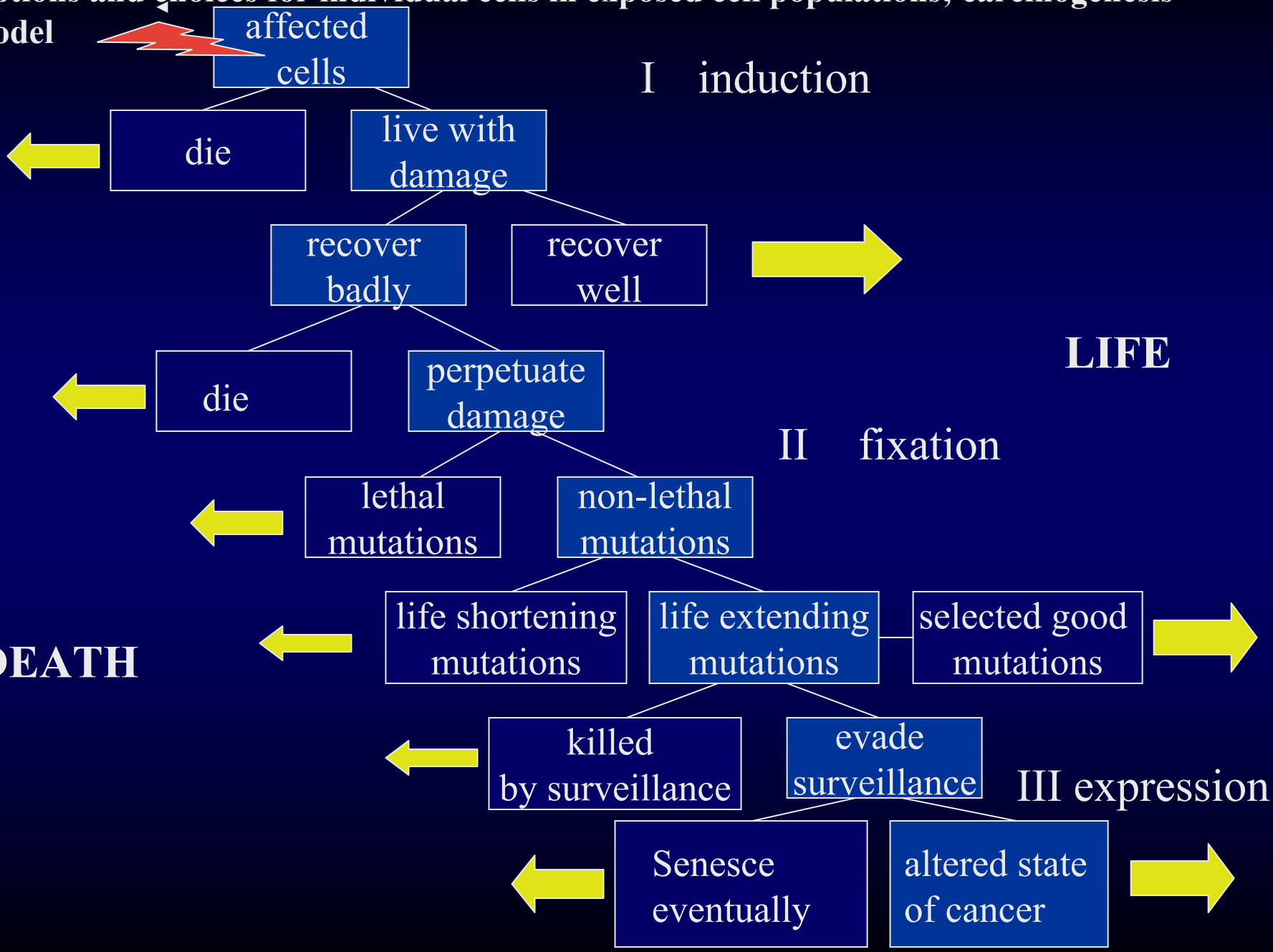
Old view- clonal outcome



New view-non-clonal, population-determined outcome



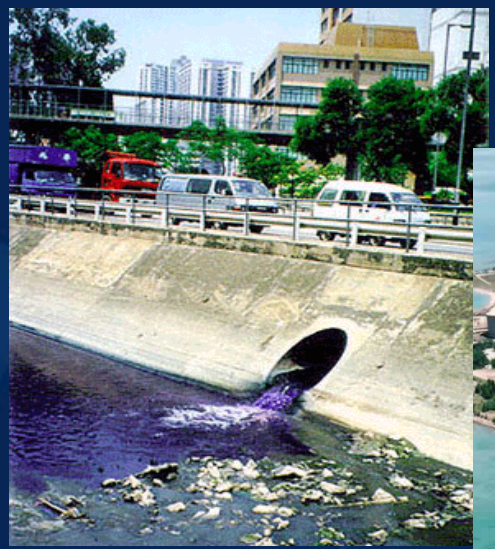
Options and choices for individual cells in exposed cell populations; carcinogenesis model

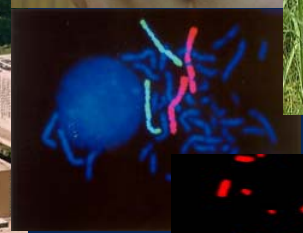


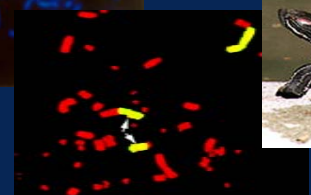
What do bystander effects do to radiation protection?

- Dissociate
  - Dose from effect
  - Effect from harm
  - Harm from risk
- Enables the concept of a “zone of uncertainty” where outcome can be assessed relative to the context in which the dose is delivered

The complexity of the radiation protection problem

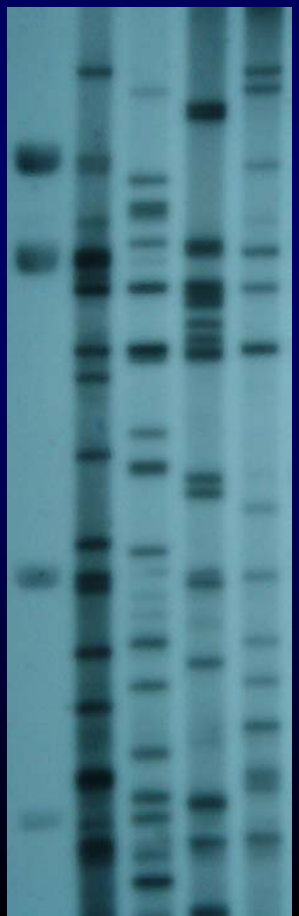






Challenges in Interpreting Comparisons Among Natural Populations:

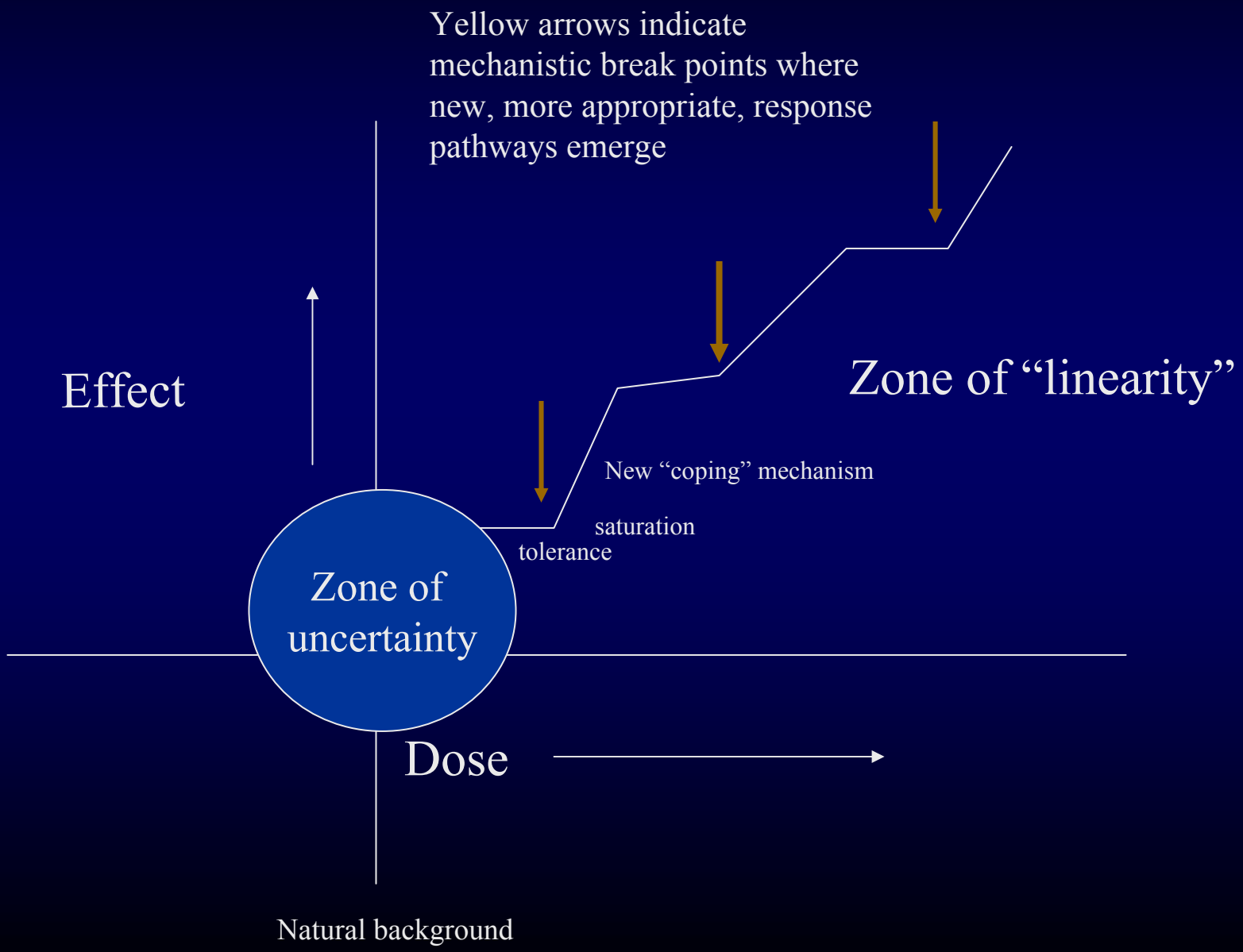
Underlying Genetics



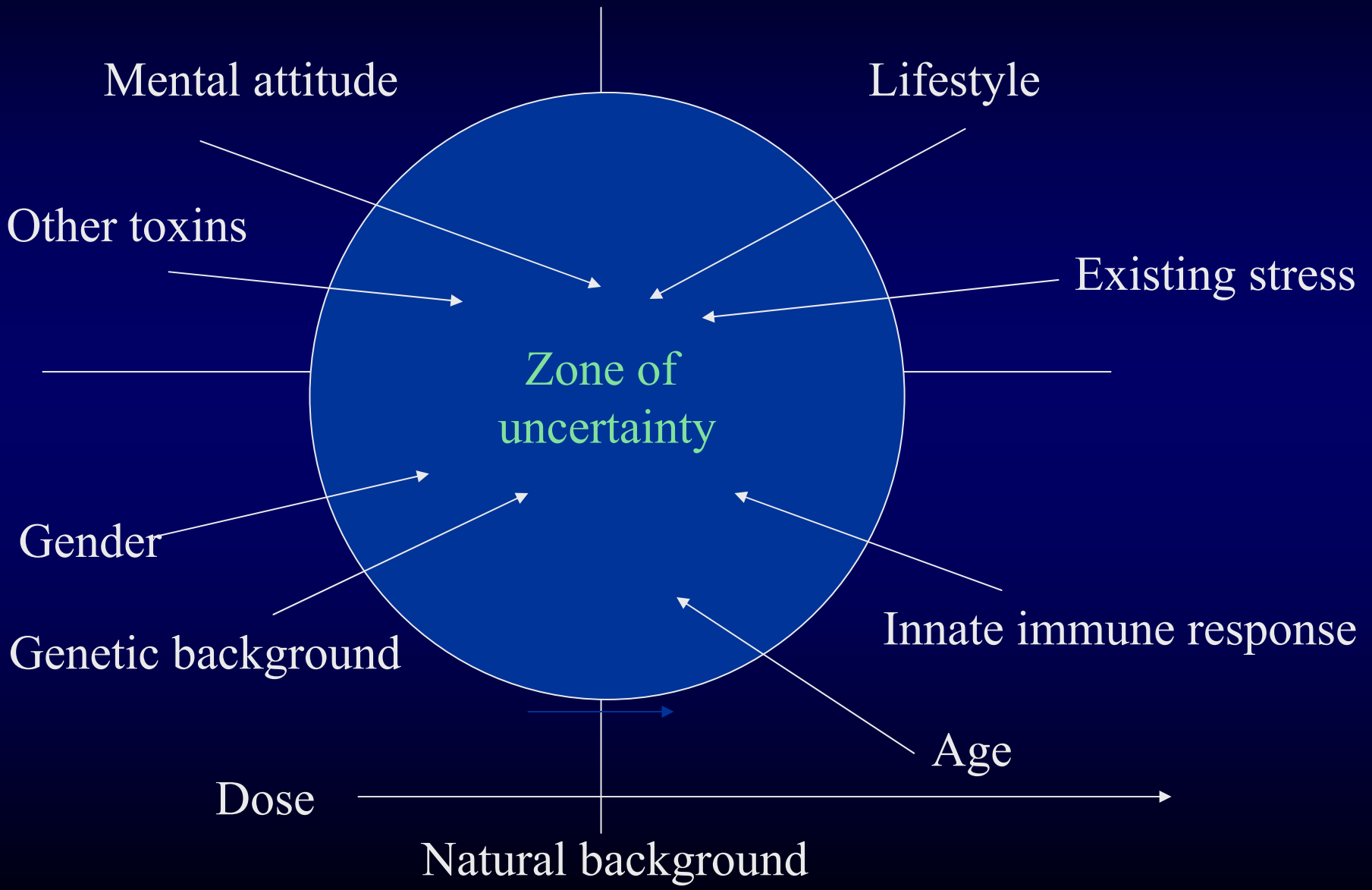
Isolating Route of Exposure

Life History / Behaviour Differences

Proposed dose response relationship for radiation-induced effects



Factors influencing outcome in the zone of uncertainty



Outcome possibilities in the zone of uncertainty

- Dose related cancer induction
- Adaptation/induced response
- Negation of the damage
- Hormesis
- No effect

ALL POSSIBILITIES ARE DEPENDENT ON SIGNALS RECEIVED NOT DOSE

Bystander effects and responses - Which response predominates?

- Which effect predominates depends on factors *independent of dose* (genetic and environmental)
- Death responses or life responses are major choices, but the *consequences* of these choices need to be assessed at several levels of organisation (QUORUM SENSING TYPE MECHANISM???)
- Radiation dose in terms of the amount of damage caused in the system is relevant to the *determination of consequences*

Potential in biotechnology

- Can we harness bystander signal molecules as new natural therapeutics?
- Can we enable sustainable production by applying radiation stress to cultures or fragments of tissues of rare organisms, then use reporter cells to carry on production?
- Can we understand the genetic basis of these effects and produce genetically engineered production systems?

Driving hypothesis for novel therapeutic applications

- Bystander effects represent a homeostatic stress response, and control growth at a cellular level. Thus they might be produced in species which are sessile and where defense at the colony boundary is an issue.
- Application of low dose radiation stress should enhance production of bystander factors in susceptible species. This is long-term!
- Preliminary evidence suggests that bystander signals can induce signal production in unrelated cells - potential for sustainable production?

Future directions

- Test ability of harvested medium from target tissues to induce effects in unrelated reporter cells
- Mix/match tissues and cell lines to optimize properties of harvested medium
- Test whether medium from stressed cells contains novel or potent signals
- Try to identify signal molecule(s)

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