



#### Some definitions

Words mean what I want them to mean – The Red Queen!

- Non-targeted effects:
  - Effects which cannot be ascribed to direct energy deposition in a cellular target
- Systems Biology:
  - 1. Modeling "emergent properties" of systems (bottom-up approach)
  - 3. Understanding mechanisms involved in maintaining ecosystems whether cellular i.e. tissues/organs, or organism i.e. species /population based (Top-down approach).

# The systems biology problem posed by the "new" radiobiology

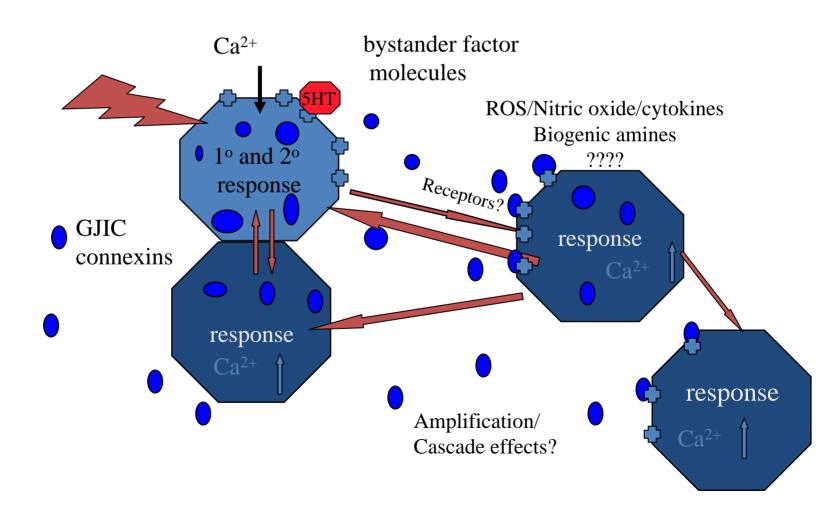
- Effects of very low radiation doses not determined by DSB's
- A mechanistic difference between high and low dose effects leading to a threshold or breakpoint
- Interactions between radiation and chemicals which can both induce delayed effects
- Cell communication related amplification or reduction of "dose/effect"
- Cellular response as the key determinant of "effect"
- Genetic background as a key determinant of "response"
- Assessment of "harm" at multiple levels of organisation
- The ability of organisms to adapt
- "Uncertainty" actually means that some individuals will benefit while others may not!

# Genomic Instability and bystander effects

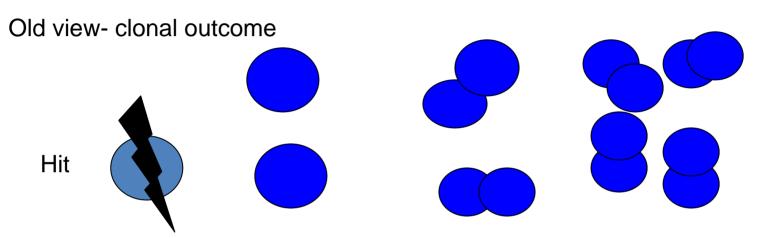
- υ Linked mechanistically
- υ Occur at very low doses (fully saturated at 5mGy acute dose)
- υ Inducible in in vivo and in a wide range of species (fish, crustaceans, molluscs as well as mammals),
- υ Perpetuated in progeny
- Detectable using many different endpoints measuring death, survival, proliferation, mutation, transformation
- υ Relevance of effects to "harm" not established

# The bystander effect-a bridge between levels

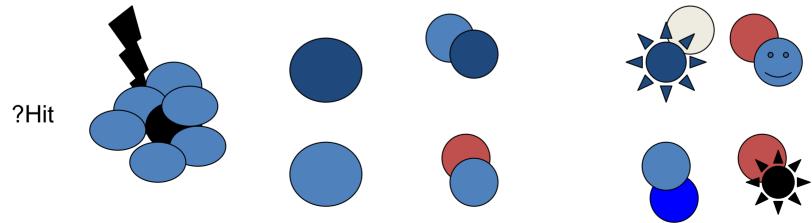
Ionizing radiation



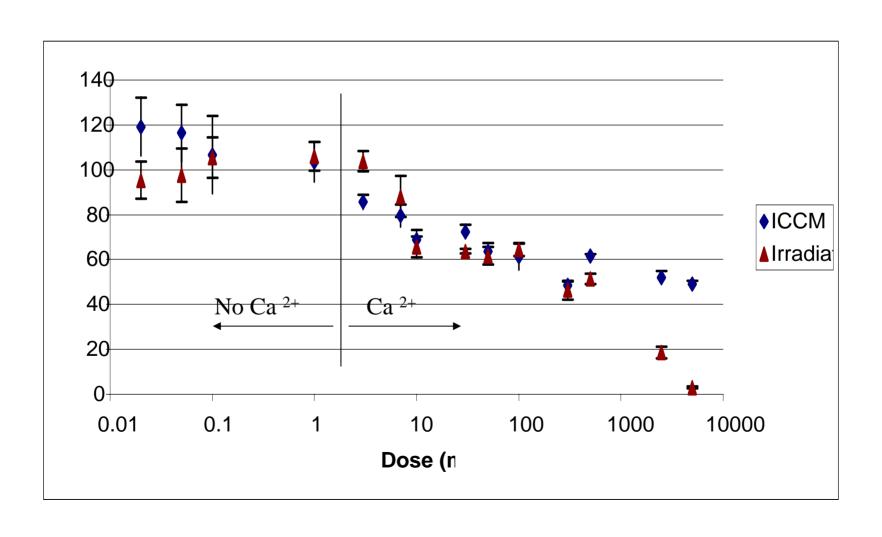
## The link between bystander effects and delayed death/instability



New view-non-clonal, population-determined outcome



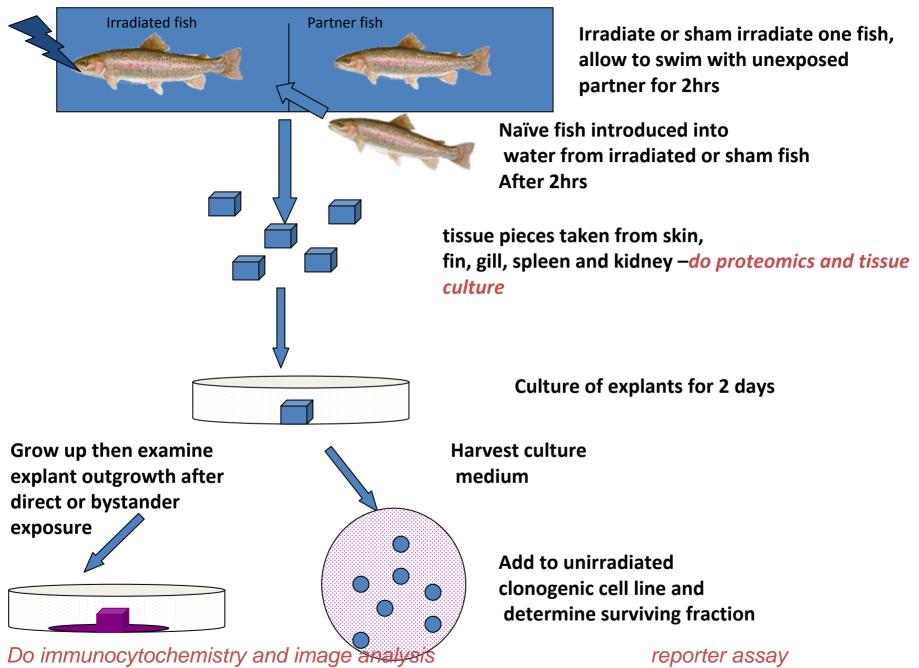
### Bystander and direct dose survival curves over six orders of magnitude <sup>60</sup> Co with calcium data



# Explant model for mechanistic studies

- Ex-vivo system
- All tissues tried to date from humans, rodents, fish, frogs, molluscs and prawns have yielded viable growing cells capable of producing and responding to bystander signals, expressing relevant proteins after irradiation and showing apoptosis and necrosis.
  Differentiation in 2D can also be seen.

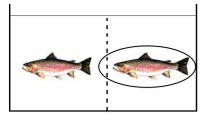
#### Measuring bystander response to radiation in vivo



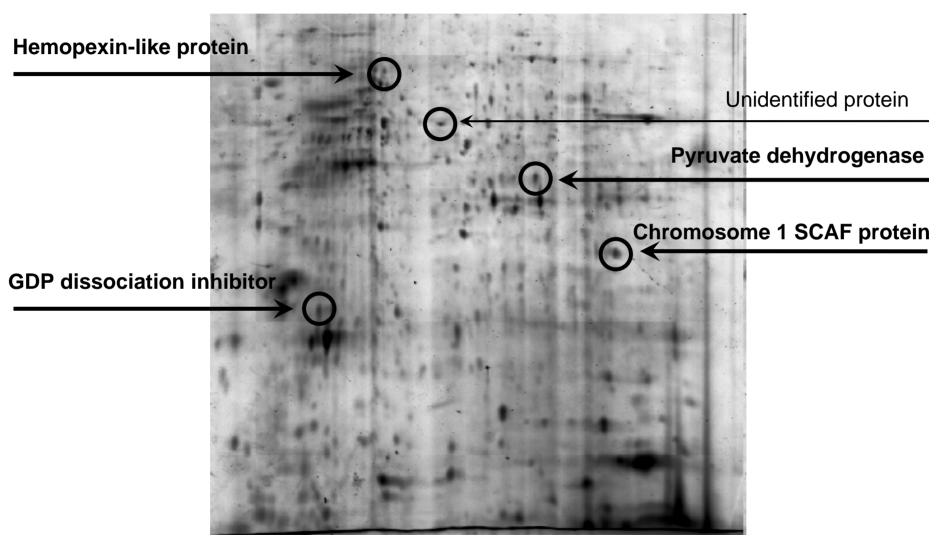


### Highlights of Fish bystander studies

- Bystander effect induced in 3 species of fish exposed to irradiated fish/or their water – ie evolutionary conserved mechanism.
- Attenuation of signal only seen after fish removed for 6hrs from water and live fish continue to emit signal for over 12hrs – ie stable water soluble signal
- Chronically exposed Medaka confer an adaptive response on reported cells- ie chronic radiation effect different to acute effect.
- Multiple stressors appear to have sub-additive effects suggests a saturable or antagonistic mechanism
- Bystander proteome and direct irradiation proteome very different very important for understanding potential risk outcomes
- Effect can be demonstrated in trout as early as the eyed egg stage and is still there in retested adults one year on – persistent effect once induced
- Serotonin involved in vivo and in vitro in fish and mammalian cells conserved mechanism

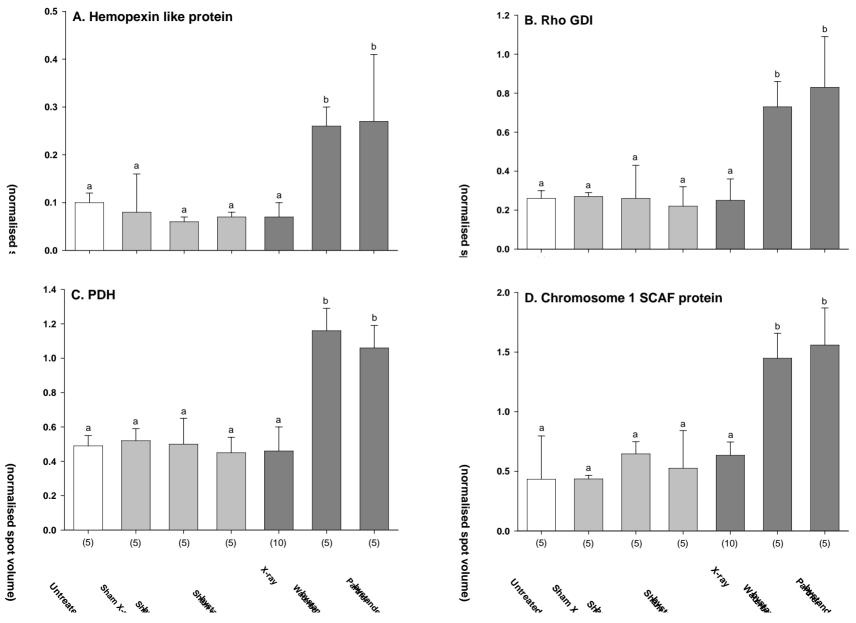


#### **BYSTANDER PROTEIN IDENTITIES**

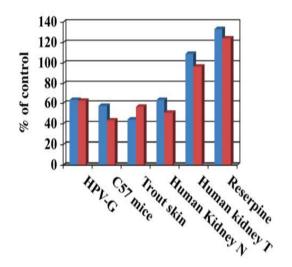


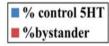


#### PROTEOMIC RESPONSES TO THE BYSTANDER EFFECT



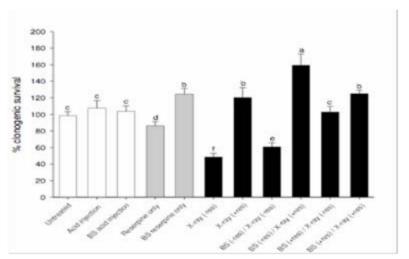
# Serotonin important in vivo and in vitro





Serotonin bound by irradiated cells In vitro, leading to Calcium pulse.

Reserpine inhibits bystander effect in vitro and in vivo

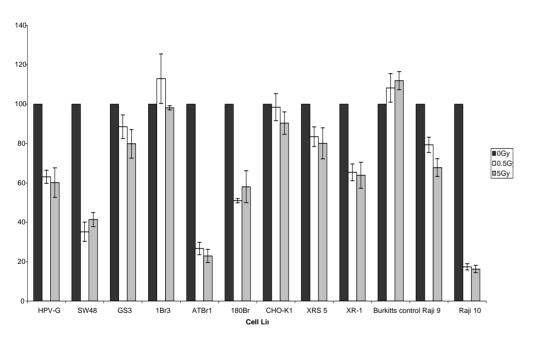


Sham treatment	% clonogenic survival
Sham X-ray (-reserpine injection)	103.8 ± 8.1*. <sup>2.†</sup>
Sham X-ray (+reserpine injection)	111.7 ± 8.6 <sup>‡</sup>
Bystander (-reserpine) / sham X-ray (-reserpine)	102.0 ± 4.8***
Bystander (-reserpine) / sham X-ray (+reserpine)	137.0 ± 6.8*. <sup>‡</sup>
Bystander (+reserpine) / sham X-ray (-reserpine)	93.5 ± 3.61
Bystander (+reserpine) / sham X-ray (+reserpine)	$130.6 \pm 4.3^{\ddagger}$

- \* significantly different to equivalent X-ray treatment
- ‡ significantly different to untreated and reserpine injected fish.
- † significantly different to reservine injection only induced bystander effect.

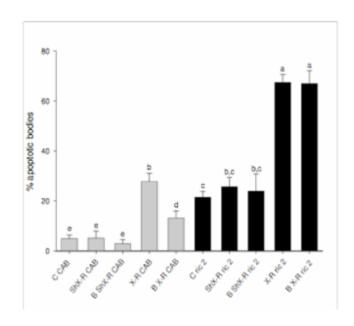
# DNA repair is important in vivo and in vitro

Reduced reproductive survival in vitro



DNA repair deficient cell lines and transgenic medaka both produce highly toxic bystander signals after low dose irradiation

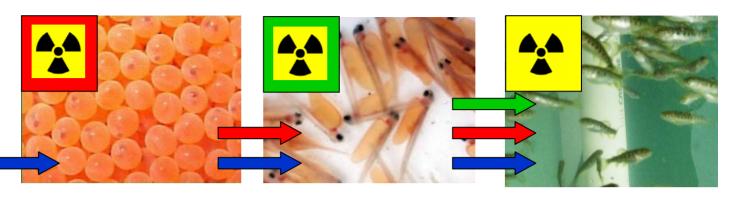
#### Increased apoptosis in vivo





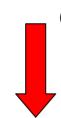
#### LEGACY OF EARLY LIFE STAGE IRRADIATION

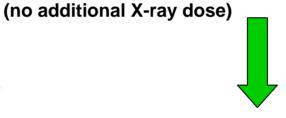




1. Legacy effect at each subsequent early life stage









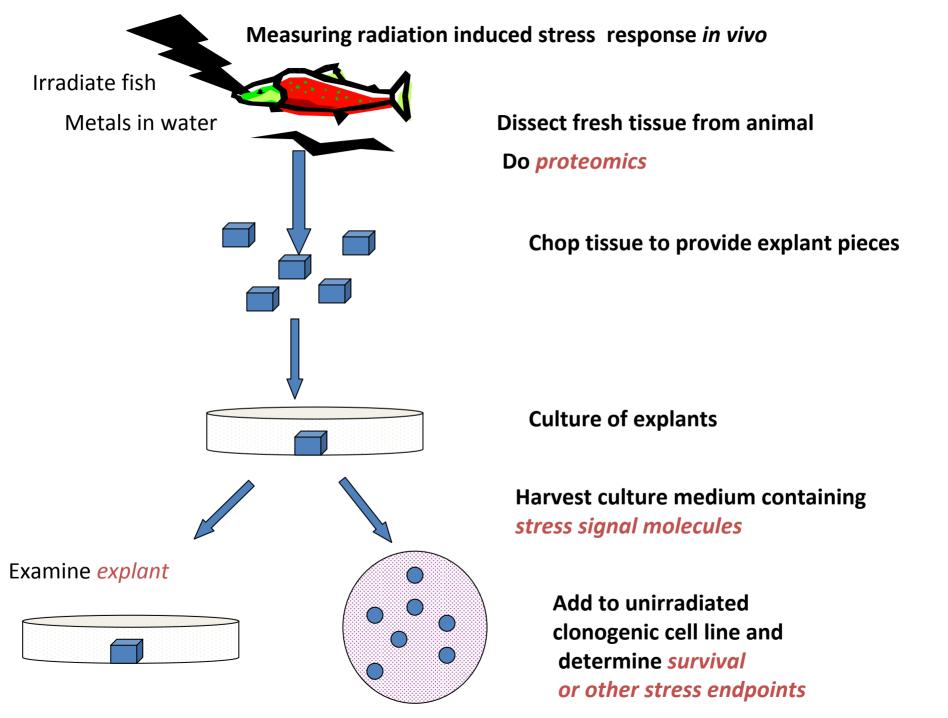
2. Legacy effect after eggs, larvae and first feeders allowed to grow for 1 year (with / without an additional 0.5 Gy X-ray dose)



• From eyed egg onwards rainbow trout are affected by a 0.5 Gy X-ray dose and an X-ray induced bystander effect.

- Exposure of the egg, larvae and first feeding stages results in a legacy of these effects which extends to 1 year old fish.
  - Nature of these responses (pro- / anti- apoptotic) are dependent on when the radiation dose was administered.

• We propose these results have implications for the radiological protection of the aquatic environment.



# Multiple stressor fish experiments (Norwegian collaborations)

- Summary conclusions
  - Stress (bystander) signals are produced in vivo by salmonids in response to acute or chronic low doses of radiation (4-75mGy)
  - Al, Cu and Cd all show complex effects when combined with low doses of radiation (4-75mGy)
  - Tissue specific differences are seen with gills being more sensitive than skin

#### Summary mechanistic findings

- Serotonin is bound by irradiated cells and leads to a calcium pulse characteristic of type 3 ion-gated receptors
- TGFb and p53 are involved in response of signal recipients but not in production of the signal
- DNA repair deficient cells and live fish have big apoptotic bystander effects
- The "memory" effect in fish lasts at least a year and is inducible even in adults which were irradiated at the egg stage eggs
- In our in vivo model protective proteins are induced in bystander fish and carcinogenic proteins in directly exposed fish
- A gamma dose threshold of 2-3mGy has been demonstrated in vivo and in vitro
- Neutrons induce direct cell killing in vivo and in vitro but no bystander effects can be demonstrated for doses up to 300mGy even if the associated gamma dose exceeds the gamma threshold of 2-3mGy

### Justification of the fish communication model - 1

- This is a model system for looking at bystander effects isolated from direct effects in vivo – we are not looking at fish social life!
- It is a unique system where the *production* of a signal in vivo in an irradiated animal can be studied separately from the *response* in vivo to that signal.
- Bystander response as distinct from signal production can be studied in vivo in a whole totally unexposed animal, without the complication of body scatter, circulating blood or stress hormones associated with whole body experiments.

# Justification continued – lots of justification!!!!!

- In terms of evolution, it shows conservation of the mechanism and bystander pathway across species and suggests a very primitive origin in vertebrates since teleost fish split from the main vertebrate line early in vertebrate evolution.
- 5. Because the signal is secreted into water in this model, and is stable in the water for at least six hrs, it is possible to analyze the water and get information about the chemistry and stability of the signals without the complicating factors of medium and serum components.
- We are basically using a whole organism instead of a cell culture as a "reporter" for our signal.

#### Yet more!!!!!

- Our in vitro findings concerning the role of serotonin and the importance of DNA repair capacity, are both confirmed in the fish model which validates it as a useful and meaningful system.
- We can look for health effects and transgenerational effects in short lived zebrafish much more conveniently than using mice or humans.
- A huge range of knockout zebrafish are available making mechanistic studies feasible.

#### **Evolution of mechanisms**

- Primitive mechanisms which evolved for other purposes can be harnessed eg bystander signalling, extremophiles
- Polymorphisms in enzymes can be selected for leading to population drift
- Genomic instability can result in increased diversity available for selection
- Conservation of signaling mechanisms across species

VALUABLE MATERIAL FOR EVOLUTIONARY BIOLOGISTS

### Eco – systems biology

- Bridging hierarchical levels
- Modeling emergent properties

- Modeling "demergent" properties i.e. if you know the big picture how can you predict what bio–indicators are relevant at lower hierarchical levels!
- In relation to risk what hierarchical level do we "protect"

# Ecosystems embody systems biology

#### Key issues are

Structure and function of the system (generally if structure is preserved then function is assumed to be preserved)

- Sentinel components (species, lead cells etc) which control outcomes
- Redundancy in the system
- Emergent properties of the system
- Non-linear or chaotic elements

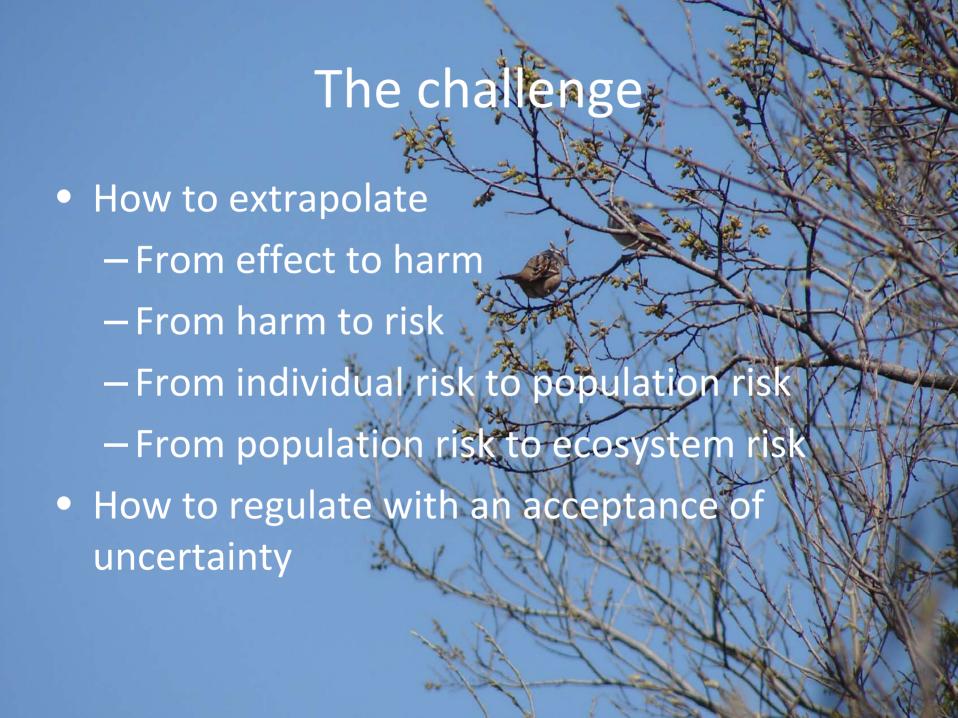
### **Evolutionary implications**

- In a stable situation an organism must establish and defend it's position
- In an unpredictable situation complexity theory requires the organism to be based on "the edge of chaos" providing just enough structure to allow it to capture the best opportunities



### Original phenotype





### Take home messages

- All bystander effects are not the same
- All bystander effects are not harmful

- The assay you use to reveal an effect is an important determinant of the effect – ie read out is important
- No one has yet demonstrated a clear detrimental health effect of exposure to bystander signals in vivo
- No one has yet identified the soluble factor(s) responsible for inducing bystander responses

