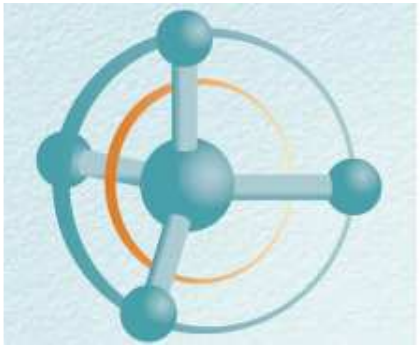

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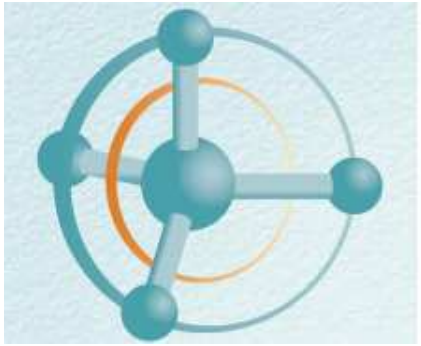
# Workshop summary

Kevin Prise and Laure Sabatier



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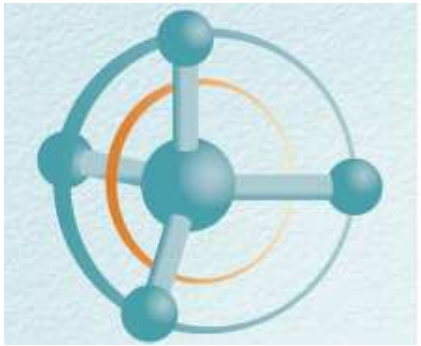
- **Biological mechanisms of action**
- Age and gender relevance for the evaluation of radiation risk
  - Wolfgang-Ulrich Müller
- Outlined that radiation risk could be discussed in terms of
  - Death
  - Malformation
  - Malignancy
  - Hereditary Disease
  - Covered two topics Age effects and gender focussing on Malignancy



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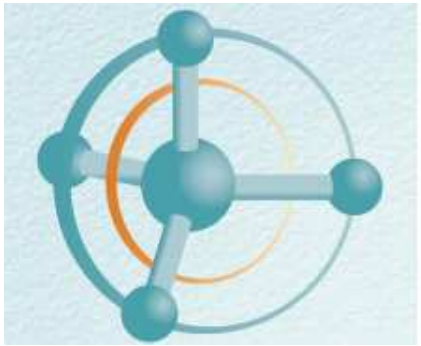
- In general radiation risk higher at younger age and declines with increasing age
  - Leukaemia – foetus and young children (covered by Wakeford)
  - Thyroid carcinoma young children (Chernobyl)
  - Basal cell carcinoma children in teens
  - Breast cancer in  girls during puberty
  - Also women under age of 30
  - High cell proliferation rates and longer life expectancy
- In older individuals ( at around 50) radiation-induced cancer risk may rise again
  - Impaired repair capacities and reduce immune functions



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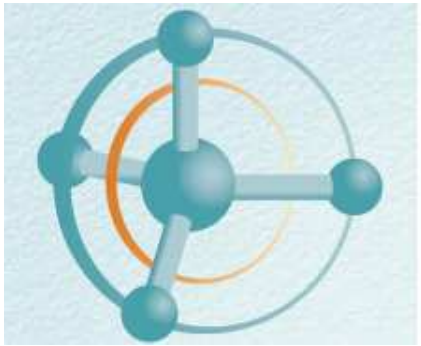
- Radiation sensitivity of the unborn child
  - Fertilisation and pre-implantation at zygote stage most sensitive (<150mSv)
  - Approx 50-60% pregnancies terminated at this early stage
  - Radiation damage can prevent implantation
  - Embryonic death during weeks 1 – 2
  - Malformations weeks 3 – 7
  - Mental retardation high weeks 8 – 15 and reduced at 16 – 25
  - All stages have increase tumour rate
  - Questions of thesholds?
  - Mouse studies, no threshold with 1 cell stage but threshold at 1Gy at 32-64 stage



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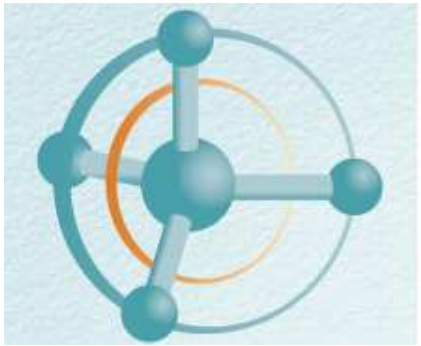
- Age and Leukaemia
- Dramatic increase in ERR at 10 years of exposure against a very low background spontaneous risk
- Leukaemia risk become significant at 10 mSv
- Doubling dose in 50 mSv
- Spontaneous frequency 5 cases per 100,000



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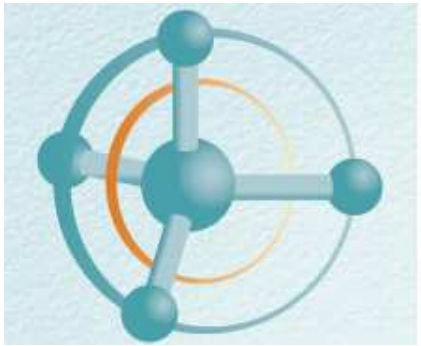
- Radiation Risk and Gender
- UNSCEAR 2000 Women ERR 0.75 per Sv and 0.3 per Sv for men
- BEIRVII ERR 1Gy
  - Males 0.33 cases and females 0.57 (1.7x)
  - Males 0.23 deaths and females 0.47 (2.0x)
- When looked at in detail problems with most datasets
- Some datasets show males more sensitive than females
- No systematic studies of gender
- Clinical studies and biological studies have not been able to provide answers so far



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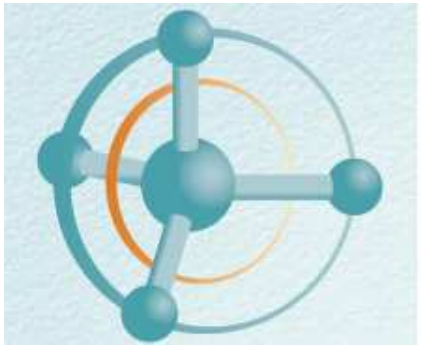
- ICRP argues for averaging over males and females
  - High uncertainties over tissue weighting factors
  - The dose limits are so low that the differences in gender ratio sensitivity does not play a significant role
  - Discrimination must be avoided
  - In the case of individual retrospective evaluations gender specific differences should be taken into account
  - One solution (Smeeters) use gender specific tissue weighting factors
  - ICRP equivalent dose is calculated for males and females then effective dose taken that is averaged
- Need systematic studies of impact of gender on radiation risk



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- **MELODI SRA -** Medium term priorities Statement 2010
- Identification and analysis of suitable epidemiological cohorts if available with archived biomaterial **to improve low dose radiation risk assessment by reducing uncertainties especially for the age and gender-dependency of radiation risk** and including those uncertainties contributed by exposure assessment. These may include cohorts exposed to internal contaminations.

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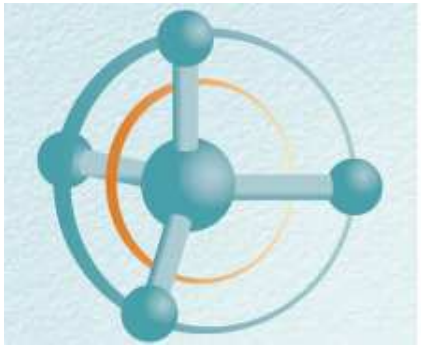
- **Biological mechanisms of action**
- The limitation of DNA double-strand break repair and checkpoint control after low radiation doses
  - Markus Lobrich



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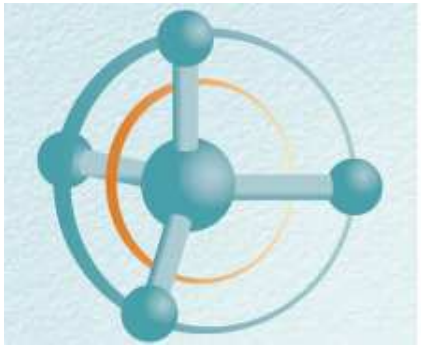
- Understanding of DNA repair pathways for dsb and role of cell cycle checkpoints
- DSB two main repair pathways
  - NHEJ predominantly G1 –"simple repair, error prone"
  - HR predominantly G2 "resection followed by homology search, error free"
  - Repair consists of "fast" and "slow" component
  - NHEJ repair in G1 and G2
  - HR represents slow component in G2



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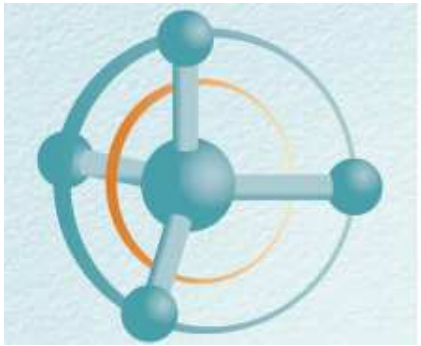
## Which lesions repaired by slow process?

- Use high LET carbon ions repaired more slowly and undergo more end resection
- etoposide clean breaks repaired quickly
- RPA/RAD51 loading on to resected DNA
- HR factors rad54 and Ctip and artemis and ATM are required for nearly all carbon ion repair.

## •Slow component in G1

- Translocations arise during G1 with slow kinetics
- Ctip initiates resection and depletion diminishes translocation formation
- Interrelationship between dsb processing and chromosome aberration formation
- Repair processes in stem cells?



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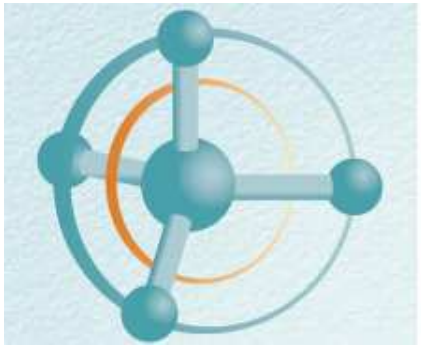
- **Cell cycle Checkpoints**
- G1/S prior to DNA replication and G2/M prior to mitosis in presence of damage to prevent transmission to daughter cells
- Can measure fraction of cells in mitosis
  - G2M checkpoint delay dose dependent
  - Cells released from checkpoint with 20 dsb present / G2 checkpoint has threshold 10-20 dsb
- Live cell imaging -follow cells irradiation before or after mitosis using GFP-53BP1
  - G2 phase cells twice number of breaks initially
  - Cells exposed in G2 higher level of residual damage than G1
  - GFP-mdc1 remains at break site during mitosis
  - More foci at end of mitosis than beginning
  - Foci distributed pairwise to daughter cells
  - HR contributes to anaphase bridge formation Chromosome breakage occurs during anaphase



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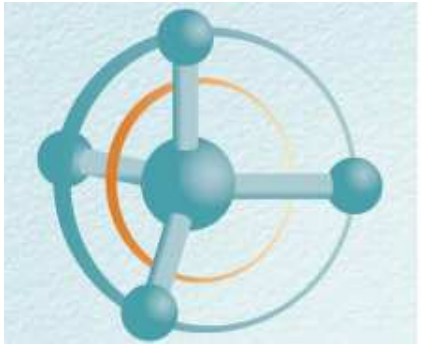
- MELODI SRA
- 2.3.1. Dose response relationship for cancer
- Furthermore, a greater understanding is needed on the relationships between oxidative stress, DNA damage complexity, chromosomal damage, translocations, DNA damage signalling, perturbed cell cycle regulation, senescence and apoptosis and how the interplay of all of these contribute to cancer induction following exposure to different radiation qualities at low doses and dose-rates. Therefore, basic cellular mechanistic studies are required.



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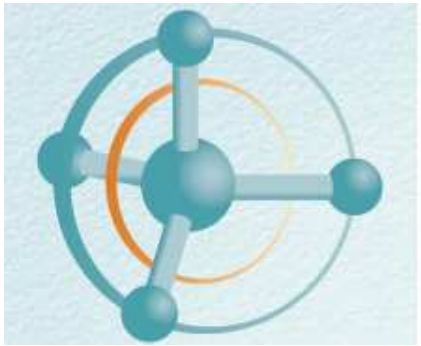
- **Biological mechanisms of action**
- Biomimetic models of radiation-induced radical stress and biomarker discovery
  - Chryssostosmos Chatgillialoglu

- Outlined the importance of the biological consequences of free radicals
  - ROS/RNS in physiological concentrations play a role in regulating the activation of transcription factors, cell proliferation and apoptosis.
  - ROS/RNS play a critical role in eliminating viral and microbial infections and thus function as an efficient cellular defense mechanism.
  - The overproduction of ROS/RNS has been linked with the etiology of various diseases and the inflammatory response.



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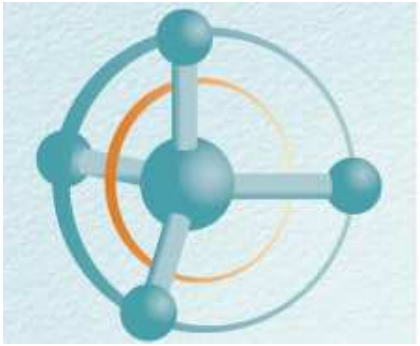
- Use of biomimetic approaches where experiments conducted in simple environment but relevant to cellular conditions
- Three examples described from work on lipidomics, genomics and proteomics focussing on radical stress induced changes
  - Cis-trans isomerisation of unsaturated fatty acids with the formation of trans-lipids in liposomes
  - Radical generation in different positions of sugar residues and purine bases
  - Tandem damage of lipids and sulfur-containing proteins by post-translational modification of amino acid sequences containing cysteine and methionine
- This approach is leading to the discovery of new biomarkers and molecular libraries for the evaluation of biological effects



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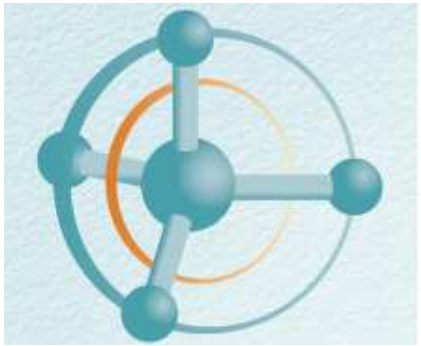
- **Targets of low dose radiation: physical and biological issues:**
- Early events relevant for biological damage
  - Andrea Ottolenghi



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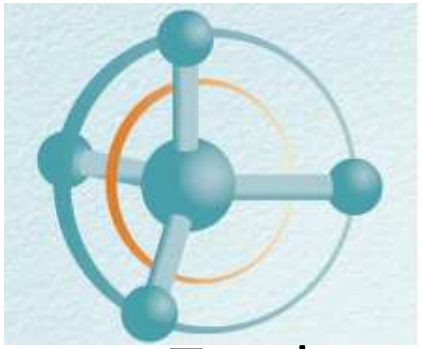
- Many cell components involved in cancer, but all not necessarily initial targets
- Correlated damages does not imply cause-effect relationship
- Need multiscale systems biology approach
- Radiation response needs to be analysed as a perturbation of a complex system
- Radiation Quality and dose-rate can be important tools for testing hypothesis



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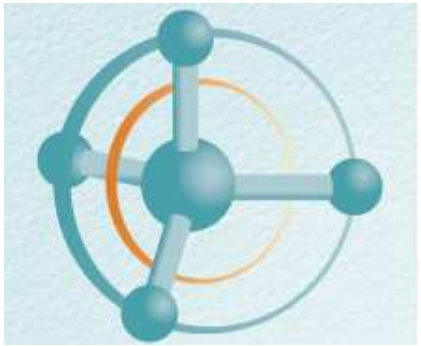
- Track structure is both spatial and temporal
  - Underpinned by cross-sections
  - Also need to consider nuclear interactions
  - Mixed fields complex to analyse
  - Impacts on validity of weighting factors
  - Challenges with internal emitters due to heterogeneity
- Chemistry
  - OH yields dependent on electron track energy
  - Shorter mean distance between radicals
  - Higher recombination probability
  - Lower relative yield of indirect radical mediated effects
  - Lower impact of oxygen



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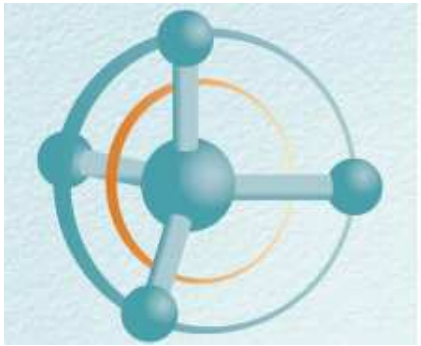
- Track structure models start from DNA perspective but only 1% of nucleus
- LET has limitations particle mass important and fluence
- Track structure models predict yields of fragments not measured experimentally
- Need to develop new models for foci formation dynamics
- Different cell targets need to be considered alongside DNA?
  - Nuclear envelope, centrioles, cytoskeleton, ER, Golgi, Mitochondria, Ribosomes, plasma membrane



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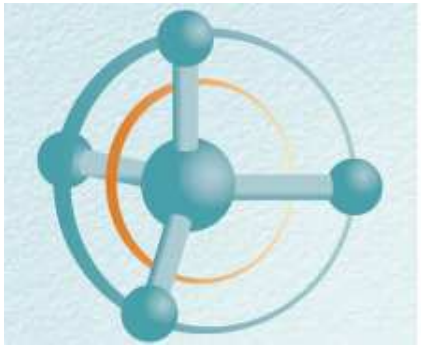
- **Targets of low dose radiation: physical and biological issues:**
- Dose rate effects: spatial and temporal damage distribution
  - Peter O'Neill



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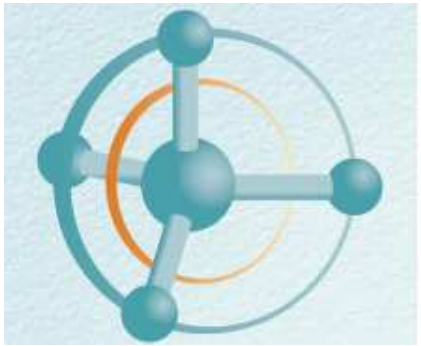
- Relationship between radiation quality and biological effectiveness – maintenance of genomic stability – radiation on top of a background of endogenous damage
- Key aspects –
  - clustered damage defined at 2 or more lesions with 1-2 turns of the DNA
  - Complex dsb -
- Timescales, early events, chemistry important, all dose-rate independent
- DNA damage
  - 30% low LET clustered, clustered damage:DSB 4:1, rest adding to increased oxidative burden
  - 90% high LET clustered



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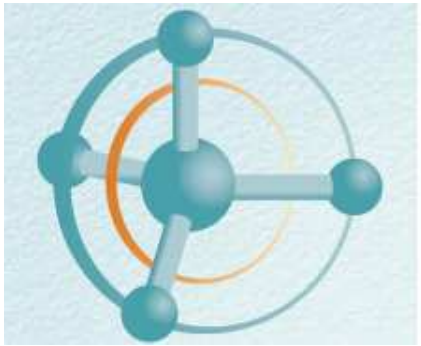
- Processing of non-dsb clusters is impaired
  - Repair occurs sequentially, SSB/AP then base lesion limiting production of dsb
  - Presence of nearby lesions extends lifetime of ssb
  - Depends on types of lesions, interlesion distances and orientation
- May not activate checkpoint and lead to stalled replication forks – mutations – cancer
- Fragments – double ended dsb, replication forks – single ended dsb
- Tracks of radiation from high LET remain for long times due to complexity, small fragments independently repaired
- At low doses, variable number of tracks per cell (different dose-rates)- increased yield of non-dsb clusters relative to complex dsb



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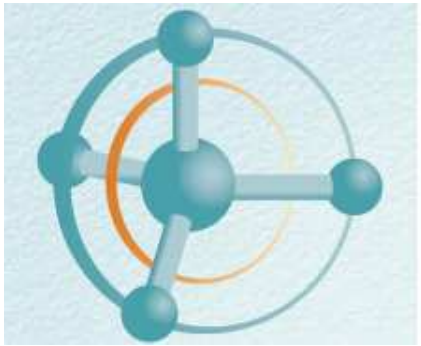
- **Targets of low dose radiation: physical and biological issues:**
- DNA damage response, senescence and cancer
  - George Garinis



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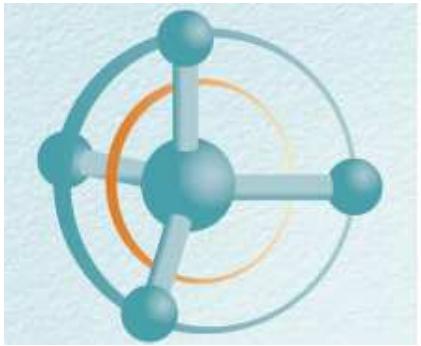
- Focussed on Nucleotide Excision Repair pathway (NER)
- Well characterised repair of UV damage via two interlinked pathways
  - Global pathway
  - Transcription coupled repair
  - Underpinning Human diseases XP, CS, TTD
  - Good mouse models for study



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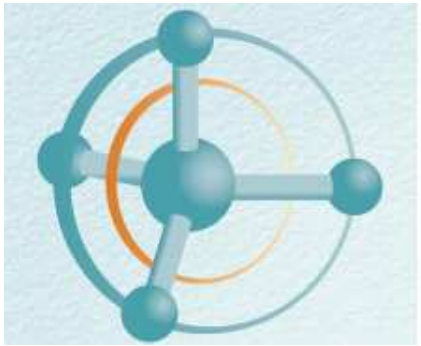
- $CSB^{m/m}$ $XPA^{-/-}$ mutant mouse
  - liver hepatocytes smaller
  - Retinal cell degeneration
- Gene array
  - Upregulation of antioxidant defence
  - Down regulation of catabolic metabolism (Gylcolysis krebs etc)
  - Down regulation of GH/IGF1 growth axis
- Response is systemic
- Mice store glycogen and fat
- Phenotype similar to naturally aged mouse
- Metabolism (GH) boosted in early life at expense of DNA damage, the maintenance at reduced GH levels and prevent DNA damage
- ERCC1 in NER also plays a role in HR after ionising radiation
- RPA involved in BER
- **Models of ageing and their relationship to stress responses**



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- Summary of this morning discussion session
- Usefulness of dose, equivalent dose/$W_R$, LET for predicting effects
- Which is the target of radiation at low doses
- Problems of distinguishing endogenous from low dose DNA damage
- Possibility of activation of repair systems after a set amount of DNA damage
- Suggestions for future research
  - More fundamental research on repair systems
  - Specific molecular epidemiology research

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## How robust is the system of radiation protection and risk assessment?

The current system of radiation protection makes judgements in several important areas: the four blue boxes indicate judgements that fall directly within the system of protection against the low dose radiation effects as recommended by ICRP, whereas the boxes on the right identify issues that are, at present, included only to a minor degree.