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Workshop on Transgenerational and Epigenetic Mechanisms of Radiation Toxicity at Chronic Doses

St Catherine's College

University of Oxford, Oxford, UK

10th – 12th December 2014

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Date of issue of this report: 12/02/2015

Start date of project: 01/06/2013 Duration: 48 Months

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PU	Public	
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Executive Summary

The international workshop on Transgenerational and Epigenetic Mechanisms of Radiation Toxicity at Chronic Doses organised on 10-12 December 2014 at St Catherine's College (Oxford, UK) organised by COMET and STAR was intended as an integrating activity between related research fields. The meeting focused on theoretical discussions on epigenetics and on the role of epigenetics in (eco)toxicology and radioecology, including biological processes such as development, aging and neurological diseases, adaptation and the use of epigenetic endpoints as generalized or even stressor-specific biomarkers. The agenda, and this report can be downloaded form the Radioecology Exchange here: https://wiki.ceh.ac.uk/x/u4XXD; the presentations of all the speakers can be downloaded by COMET members and workshop participants from the COMET project web site here: https://wiki.ceh.ac.uk/x/foiaDQ (a policy agreed so that presenters would be willing to bring their most recent "straight from the bench data" to present.

The workshop addressed a wide spectrum of questions related to long-term and transgenerational exposure, in laboratory studies of radiation and chemical effects, molecular biology relating to epigenetic mechanisms, human and ecological risk assessment and radiological protection. World leading experts in each of the subjects discussed attended the workshop. In total there were 48 participants from 12 countries (Belgium, Canada, USA, Spain, France, Germany, Japan, Norway, Portugal, United Kingdom, Russia and Sweden). Discussion groups at the end of the plenary sessions addressed similar points on the role of epigenetics in radiobiology and ecotoxicology, but from a different perspective: one from and ecological an evolutionary biology viewpoint and the other with a focusing on mechanistic issues and systems biology.

The two discussion groups outlined key issues that unified studies across the two areas, such as how the revolution in understanding of epigenetic mechanisms has provided researchers with a wealth of new methods and tools. To date the major focus has been on DNA methylation, however, the widespread availability of methods for analysis of miRNAs and histones suggests that these should also be a focus of future studies of epigenetics mechanisms relating to radionuclide and other stressor exposures. High quality epigenetic and evolutionary biology studies will emerge when experts in radiobiology and in genetics and systems biology work together to address particular hypothesis-driven questions. Developing such partnerships is important to help move the field forward and this meeting was a clear step in this direction.

The COMET-STAR Workshop on Molecular Mechanisms of Radiation Toxicity at Chronic Low Dose Levels

In recent years, an enormous amount of work has been undertaken to assess the role of epigenetic mechanisms in development and disease. The understanding of the role of epigenetics in the determination of response to physioloBOLNlight on previously unexplained effects in the fields of ecotoxicology and radioecology, with the suggestion that epigenetic mechanisms may also be involved in responses to these stressors. The potential of epigenetics is exciting and application of methods of epigenetic analysis in ecologically relevant species has opened up the research field to include studies of trans-generational effects and their mechanisms. The links between epigenetic processes and exposure to environmental stresses, hereditary and transgenerational effects, and not the least the connection to adaptation, tolerance and resistance in exposed populations/species makes the topic one of clear relevance to environmental science and toxicology. Conversely, studies in ecotoxicology and radioecology have a lot that they can contribute to the field of epigenetics, including opportunities to test hypotheses under real-world conditions, and a wealth of organisms to study with wide-ranging physiological and ecological adaptations.

The biological effects induced by low levels of ionizing radiation are considered within COMET WP4 and STAR WP5. In COMET WP4, research actions are focused on epigenetic changes and transgenerational effects in organisms exposed to ionizing radiation. In STAR Task 5.2 had the aim to compare the modes of toxic action of alpha vs gamma irradiation including the effects that arise from long-term and potential transgenerational exposure. The international workshop on Transgenerational and Epigenetic Mechanisms of Radiation Toxicity at Chronic Doses organised that was held 10-12 December 2014 at St Catherine's College (Oxford, UK) was intended as a forum to discuss the results arising from this works within the two projects and also as a forum that would allow ecotoxicologists and radiobiologists to meet in an open forum to discuss current development in epigenetics within these two complementary fields. This meeting built on the output from the MELODI meeting 7-9 October 2014 (www.melodi2014.org/) in Barcelona on epigenetics and included a full feedback of the outcomes from that meeting. The scope of the program was focused on theoretical discussions on epigenetics and on the role of epigenetics in (eco)toxicology and radioecology, including biological processes such as development, aging and neurological diseases, adaptation and the use of epigenetic endpoints as generalized or even stressor-specific biomarkers.

The information of the Workshop on Molecular Mechanisms of Radiation Toxicity at Chronic Low Dose Levels (agenda, and minutes of the discussion sessions (this deliverable) can be accessed from the Radioecology Exchange here: https://wiki.ceh.ac.uk/x/u4XXD. The presentations can be accessed by COMET members and workshop attendees from the COMET project web site here: https://wiki.ceh.ac.uk/x/foiaDQ. The overall aim of the meeting was, as stated, to bring together scientists involved in epigenetic studies, environmental and/or laboratory studies of radiation effects, allied human and ecological fields, and radiation protection specialists.

Within this context, the workshop was designed to feed research priorities for the ALLIANCE roadmap, as initiated recently in the ALLIANCE working groups. The workshop sought to bring

together biological scientists studying transgenerational and epigenetic effects during chronic and long-term chemical and radiological exposures in the laboratory and field to:

- gain a greater understanding of the epigenetic changes in organisms exposed to ionizing radiations and of their relevance for key biological functions and transgenerational effects
- discuss current methods for epigenetic studies applicable across disciplines
- discuss current methods for data integration in systems biology
- agree future research priorities and identify promising approaches within two working groups (WGs)

Within the work programme, the main topics of the meeting were covered within four main topics each overseen by a chair from within the STAR / COMET projects as detailed below:

- workshop aims and the MELODI Roadmap (Chair N. Horemans, SCK•CEN)
- introducing the role of epigenetics in biology and toxicology (Chair D. Spurgeon, CEH)
- epigenetics in ecotoxicology (Chair: D. Spurgeon, CEH)
- epigenetics and transgenerational effects in radioecology (Chair: C. Adam-Guillermin, IRSN)

Additionally, discussion sessions were included to allow delegates to exchange ideas and identify major topics for future development within and outside COMET and STAR. During the organization of the Workshop, a priority was to given to ensuring that have as much time as possible for these open discussions, taking advantage that many of the experts in the field of low dose effects met at the event in the presence of experts in field such as epigenetics and systems biology. To build on the presence of these complementary experts, two discussion sessions were included in the programme:

1. Epigenetics and transgenerational effects. Chaired by Karel De Schamphelaere (Ghent University, Belgium)
2. Epigenetics and systems biology. Chaired by Peter Aleström (CERAD/NMBU, Norway)

The outcomes of these Discussion sessions are presented below.

Discussion session on Epigenetics and transgenerational effects:

In order to structure the discussion, a list of questions was distributed to the participants in this working group as detailed below:

1. Which of the epigenetic mechanisms is most important to study and what techniques should be used and how?
2. What can other scientific domains teach us?
3. What kind of designs and approaches are needed to analyse transgenerational effects?
4. What endpoints should be measured, including existing and known mutation endpoints?

5. What are the easy "wins" (e.g. meta-analysis)?
6. What dose rate to focus on? Should we focus on dose or on dose rate?
7. Is this field just interesting or also important (from a management perspective) and what knowledge is needed and what skills do we need to nurture to deliver what is needed?

Based on the very interesting and useful discussions that took place, there was agreement on the following conclusions:

1. All epigenetic mechanisms are important topics of study (DNA methylation, histone modification, miRNAs…). It is recommended to use whole genome studies in order to avoid missing important features. However, the cost of these techniques, which may run into the €100s per sample, can be prohibitive. It is possible when resources are limited to use reduced representation methods or targeted approaches, but these raise the potential issue that important modifications driving a given effect may be missed. Relevant analyses can be focused on key samples or samples may be frozen until further analyses can be undertaken.

2. Radiobiologists, ecotoxicologists and radioecologists should work together without any barriers, as there is a lot to share between these communities as seen during the workshop. A working group exists at SETAC on Evolutionary & Transgenerational issues (EVOGENERATE), chaired by Karel De Schamphelaere, which is open to participation and can act as a forum to continue discussion arising from the outcome of this meeting.

3. Phenotype anchoring and top-down approaches must be used. Different radiosensitivities should be studied by choosing organisms of different phylogeny. The choice of gamma dose rates should allow easy comparison across species. Ambitious projects are needed to go further and deeper in this topic. Combinations of strong phenotypic analysis and detailed epigenetic analyses are needed to establish the role of each mechanism in transgenerational effects.

4. Other endpoints should not be forgotten including those presented within the meeting relating to macrophenotypes (growth, development, reproduction etc.) and effects on genome architecture. These are of vital relevance when considering the nature of transgenerational effects. The power of these studies comes when strong phenotypic observation relating to any transgenerational effects are linked to detailed studies of the underlying mechanisms.

5. It was proposed to use a modelling approach to determine relevant dose rates, based on the data currently available.

6. From a management perspective, epigenetics is important but we need to know what protection goals are of most importance (human vs ecosystem) and the context to focus on (chronic vs accidental releases).

7. The presentation relating to potential transgenerational effects including identification of the sensitisation of subsequent generations following parental exposure are clearly of high importance for risk assessment. Currently uncertainty factor used in current risk assessment

programs do not include any consideration of such effects even though the magnitude of such effects in model species suggest that they may be significant (e.g. leading to fold change difference in the sensitivity of the progeny of exposure parents compared to the previously unexposed parent generation).

Discussion group on Epigenetics and systems biology

To structure the discussion the questions detailed above were distributed to the participants to provide a starting point for the discussion.

1. Which of the epigenetic mechanisms is the most important to study and what techniques should be used and how?
2. What can other scientific domains teach us?
3. Do we have models for DNA methylation in invertebrates, plants and vertebrates? How do these models differ?
4. Can we adopt the use of modelling / mathematical analyses / integrating approach building on the methods that have already been developed in system biology?
5. What should be the balance between genome-wide studies and targeted work and are their common hallmarks for ecotoxicological assessment in group?
6. Is this field just interesting or also important (from a management perspective) and what knowledge and skills do we need to nurture?

Some of these questions were similar for both discussion groups to get a view on them from both perspectives. The discussion was chaired by Peter Aleström whom first gave some introductory slides to focus the discussion. This was followed by a very fruitful discussion that led to the following consensus answers or ideas on the questions posed:

1. Methylation is the most studied epigenetic endpoint and hence the best known. Histone modifications are more difficult to study, but might be very important regulators of the eventual effect of methylation changes. The range of ncRNAs on the other hand have not often been studied, but in many ways are easier to investigate than histone modifications and might, therefore, be a good target for future research. It was generally agreed that it is dependent on organism being studied (e.g. *C. elegans* does not have methylation) and the interplay between different epigenetic mechanisms might be most important to study. This is challenging, but could be beneficial.

2. A number of scientific domains were mentioned as being of potential importance for the development of some aspect of epigenetic research
    - Bioinformatics is essential (in house or outsourced)
    - Upcoming technologies like Crispr Cas9-genome editing will enable studies in non-model organisms
    - Evolutionary ecotoxicology: e.g. looking at how a receptor evolved in different organisms over time can lead to insight on drug/pesticide development

The importance of individual endpoints or mechanistic models like DEBtox and other toxicokinetic/toxicodynamic models was stressed. The use of these models should enable a more complete picture to be generated of phenotypes that can be related to epigenetic changes.

3. There is a big difference between reference organisms in radioecology and the major test organisms under in ecotoxicology, although for effect studies radioecology often adopts the same species (e.g. zebrafish, *Lemna*, earthworms, *C. elegans*, *Daphnia*) as are used in ecotoxicology.
    - For radioecology reference organisms have been set from an exposure point of view. These are often not even a specific organism but a group (like grasses). However, they have ecological relevance.
    - Within ecotoxicology reference organisms show some overlap with the molecular model organisms and there are some ecotoxicological species (such as *Daphnia* sp.) that are emerging and models. The species are characterised by:

        Sequenced and/or annotated genome

        Knock-Out libraries are often available

        Easy to handle in the laboratory plus existence of guidelines for ecotox tests

    - Both have their pros and cons: for example *Arabidopsis thaliana* is a superb molecular model organism but is not radiosensitive, while *C. elegans* is a good model for radioecological effect studies but not for work on DNA methylation.

4. Systems biology includes proteomic and metabolomics to be integrated with the genomics, transcriptomic epigenetics.
    - Proteomics and genomics are the letters in the book, but metabolomics make the story, with epigenetics being the parts of the story taken across to the next generation.
    - For proteomics: it was said that at the moment the technology does not seem to have sufficiently high resolution. Studies of the functions of specific proteins may be very important and can be used to unravel important post-translational protein modifications such as phosphorylation on physiological functions.
    - Metabolites on the other hand are relatively easy to measure and can be cheap (after initial capital outlay), but they need a good model of the biological system to be available that is not the case for all potential species of interest to gain the greatest impact from metabolomic studies.

5. Further the discussion turned to the advantages and disadvantages of genome wide vs. targeted research: this will depends on the following.
    - The questions you want to answer from the specific analyses.
    - The skills and resources you have (e.g. in-house bioinformatics or not, budget for next generation sequencing etc.).
    - Genome-wide research should be a hypothesis generator whereas targeted research will follow from that basis.

- Meta-analysis of existing data seems difficult, as data often are not in same format. Therefore despite the importance meta-analysis is not often done. It, could, however, identify and characterise general mechanisms that cross species borders and could identify topics for targeted research.

6. What will be gained from such studies?
    - This research could generate biomarker fingerprints for exposure or effect.
    - It will bring evidence (or not) for the existence of long-term effects across generations. If a site is contaminated this knowledge might be important to define clean up measurements and to see if they are sufficient.
    - Knowledge on long-term effects can indicate key species that are more vulnerable to the stressor in the future or to an alternative stressor.

Summary conclusions and actions arising from the Workshop and Discussion groups

The two discussion groups were complementary in the scope and addressed similar points on the role of epigenetics in radiobiology and ecotoxicology from different perspective: one from an ecological and evolutionary biology view and the other from a mechanistic direction. The transgenerational effects of stressor that were presented and discussed emphasize that the epigenetic and evolutionary effects of chemicals and radioisotopes are not just of academic interest. Instead they can have real influences on the impacts that these stressors may have on the environment. Transgenerational mechanisms can result in effects that occur at concentrations ten times lower than those that have the same effect on the previously unexposed parent generation. The studies of the epigenome indicate important roles of major epigenetic factors in the longevity of these effects over generations.

The revolution in understanding of epigenetic mechanisms that has occurred in medical research over the last decade has provided environmental toxicologists with access to a wealth of new methods and tools to understand these epigenetic effects. To date the major focus has been on DNA methylation, however, the widespread availability of methods for analysis of miRNAs and histones suggests that these should also be a focus of study – indeed it is entirely possible that these will prove to be more important mechanisms. High quality epigenetic and evolutionary biology studies will emerge when experts in radiobiology and in genetics and systems biology work together to address particular hypothesis-driven questions. This will mean that all aspects of work, exposure and dosimetry, and epigenetics and mechanistic toxicology use appropriate tools within an integrated study. Both Discussion Groups agreed that forming such partnerships is important to help move the field forward. Key areas that emerged from these discussion include suggestion of work in the following:

- Fundamental studies of genome-wide methylation patterns across species from different taxa to assess the different roles of DNA methylation in gene regulation, including expression levels and alternative splicing.
- Further work on the role of DNA methylation as a first case study of the role of epigenetic mechanisms in species responses to radionuclide (and chemical) exposure.

- Studies to assess the specific role of DNA methylation as a potential biomarker of exposure, including the potential for cytosine modifications to act as a "memory" of exposure for individual subject to pulsed exposures.
- Studies using non-coding RNAs in radiological and ecotoxicological species including assessment of the link between the changing non-coding RNA complement and gene expression.
- Assessing comparative radio-sensitivities for different organisms of different phylogeny to allow assessment of the role of physiological traits including the epigenome in sensitivity.
- For risk management, to know what protection goals are of most importance (human vs ecosystem) and in which contexts (chronic vs accidental releases) and to understand how information that may be gained from epigenetic studies can support decision making within these different assessments.

Appendix A: Agenda of the meeting

The meeting took place at St Catherine's College, Oxford University.

The agenda of the meeting and the list of invited and project speakers is as set-out below

Day 1 - Wednesday 10th December			
12.30	1.15	**Arrival**	
		Introducing the aims of the meeting and the MELODI Roadmap	
1.15	1.30	Introduction to the meeting. Oxford, topics, working groups and aims	Dave Spurgeon
1.30	2.00	ALLIANCE and a Roadmap for Challenge 2:epigenetics and transgenerational effects	Hildegarde Vandenhove
2.00	2.30	Report on the MELODI Barcelona meeting on epigenetics	Simon Bouffler
		Epigenetics in biology and toxicology (Chair: D. Spurgeon)	
2.30	3.00	Epigenetic Marking of the Zebrafish Developmental Program	Peter Alestrom
3.00	3.30	***Tea Break***	
3.30	4.00	Epigenetics and systems biology in endocrine disruption	Eduardo Santos
4.00	4.30	Epigenetics of the model organism *E12* global and single-base resolution DNA methylation and its response to natural and chemical stressors	Karel De Schamphelaere
4.30	5.00	Like father like son – transgenerational effects of paternal exposure to mutagens	Yuri Dubrova
		Day ends	
6.30		***Dinner in St Catherine's College Main Hall***	

Day 2- Thursday 11th December			
8.00	9.00	**Breakfast in College**	
		Epigenetics and transgen effects in ecotoxicology (Chair: D. Spurgeon)	
9.00	9.30	Multigenerational effects and epigenetic control in ecotoxicology of invertebrates	Dick Roelofs
9.30	10.00	Genomes, epigenome and transcriptome: Analysis and integration for system biology in a soil sentinel	Peter Kille
10.00	10.30	Discussion on common transgenerational and epigenetics research themes in ecotoxicology	All in plenary
10.30	11.00	***Tea D42Break***	
		Epigenetics and transgen effects in radioecology (Chair: C. Adam-Guillermin)	
11.00	11.30	Transgenerational effects of radium and gamma exposure in fish and mammals	Carmel Mothersill
11.30	12.00	DNA alterations and reprotoxic effects of gamma radiation over 3 generations of *Daphnia magna*	Floran Parisot
12.00	12.30	Linking DNA damages and transgenerational effects of radionuclides in invertebrates	Frédéric Alonzo
12.45	1.30	***Lunch***	
		Epigenetics and transgen effects in radioecology (Chair: C. Adam-Guillermin)	
1.30	2.15	Study of epigenetic changes induced by ionizing radiations in non human organisms : approach adopted within COMET-WP4 and first results	Christelle Adam-Guillermin
2.00	2.45	Transgenerational non-targeted effects of parental exposure to ionizing radiation in *Daphnia*	Elena I. Sarapultseva
2.30	3.15	Epigenetics of low dose radiation effects in eukaryotes	Olga Kovalchuk
3.00	3.45	***Tea Break***	
		Epigenetics and transgen effects in radioecology (Chair: C. Adam-Guillermin)	
3.45	4.15	Origin and inheritance of spontaneous and induced epigenetic variants: lessons from *Arabidopsis thaliana*	Claude Becker
4.15	4.45	Interactions between genetic and epigenetic effects	Munira Kadhim
4.45	5.15	Discussion on common transgenerational and epigenetics research themes in radioecology	All in plenary
		Day ends	
7.00		***Dinner in the pub***	

Day 3 - Friday 12rd December			
8.00	9.00	**Breakfast in College**	
9.00	11.00	Breakout session. Epigenetics in radio-(eco)toxicology. Prioritising research questions. WG1: Transgenerational and epigenetics effects WG2: Epigenetic integration within system biology	2 Groups
11.00	11.20	***Tea Break***	
11.20	12.15	Breakout group priority lists	G1, G2
12.15	1.00	Consolidation of priority options	All
		Meeting ends	
		Head for Home	

Appendix B: Delegates list

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