Session 4: Epigenetics in ecology and (eco)toxicology: science and technology

30 | Epigenetics in (Eco-)Toxicology

Juliette Legler, Institute for Environment, Health and Societies, Brunel University London

How is possible that exposure to a chemical during development affects a tissue in a way that its function is changed long after the exposure has stopped? How can chemicals 'program' an organism during development, making it more susceptible to diseases or adverse effects later in life, or even in subsequent generations? One area of molecular biology where new discoveries are made at an astonishing rate is the field of epigenetics. Epigenetics describes the array of chemical markers and switches that lie along DNA providing instructions to genes for what to do, and where and when to do it. Newfound insights in this field will help us understand how chemicals may alter basic processes in development at levels that may not produce overt toxicity. I will introduce this field, as well as recent research involving the zebrafish *Danio rerio* as a model of epigenetic (eco-) toxicology.

31 |

32 |

33 | Technologies to study epigenetic traits in humans and environmental species

Emilia Danilowicz-Luebert[1], A-C. Veillard[1], T. Ribarska[2], J. Hendrickx[1], I. Panteleeva[1], M. Laczik[1], C. Sabatel[1], D. Poncelet[1]

[1]Diagenode s.a., Belgium

[2]German Cancer Research Center, Germany

Interplay of epigenetic marks, repression of transcription can be via protein complexes, transcription factors, repressive histone marks or through DNA methylation. Both, H3K27me3 and DNA methylation are associated with transcriptional silencing and play an important role in the establishment of gene expression patterns during development or as a response to environmental stressors with an impact on human health. Application of high-throughput analyses to better understand this regulation opens new research areas. One of the robust tools is ChIP-seq, a combination of chromatin immunoprecipitation (ChIP) with genome-wide analysis using Next-Generation Sequencing. This method provides insights into transcriptional and epigenetic gene regulation and greatly assists the interpretation of events central to many biological processes, disease states as possible outcomes of changing environments. Despite its widespread use, there are considerable differences in how these steps are conducted, which affect the quality and reproducibility of the results. Furthermore, the performance of ChIP-seq is strongly dependent on the use of validated reagents such as ChIP-seq grade antibodies. Here we will share our 10 plus years' experience with ChIP-seq tools which enable reproducible and efficient results. We outline the ChIP-seq workflow and discuss the experimental challenges and different strategies depending on the protein of interest. The focus is set on quality requirements and optimization procedures that increase the reproducibility and allow comparison between experiments. Finally, the experimental challenges of ChIP-seq are exemplified with some ChIP-seq results, while providing tips for their solution. In this presentation we will also discuss methylation analysis methods such as bisulfite sequencing or methylated DNA immunoprecipitation (MeDIP-seq) assays that steadily decreasing price and increasing quality, can be automated.

34 | Towards a Generalised Method for Environmental Epigenetics: Global and Differential Genome-Wide Epigenetic Signatures in an Earthworm Exposed to a Multi-stressor Environment

Oliver Rimington[1], M. Novo[2], L. Cunha[1], P. Kille[1]

[1]Cardiff University, UK

[2]UNED, Spain

This presentation covers both the results of a physical experiment on A.gracilis, and the novel in silico methods used to generate a large scale comprehensive analysis of an organism's spatial epigenetic profile. The primary objective in an environmental epigenetics experiment is often to map a set of short-reads to a genome and produce an assessment of functional enrichment in differentially affected genes. However the methodology presented seeks to go further than this: to take advantage of the full scope and size of the huge datasets that are generated in the process of such an experiment. The methods developed generalise a form of spatial statistical enquiry such that they be applicable to the maximum possible range of attributes possessed by a 'feature' on the genome. Such a feature can include RNAseq read alignments, epigenetic annotations or read alignments, DNA motifs, variants/SNPs, and almost any other general set of discrete annotations. By placing all these genomic features in a singular analytical framework a huge variety of interesting non-random abnormalities can be discovered. The physical experiment in focus aims to understand the structure and effects of genomic methylation in an earthworm exposed to a multi-stressor environment. A reciprocal transplantation experiment was performed in which Azorian earthworms were taken from, and placed in, both active and inactive volcanic soils. Differential stressors include high CO2 content, heavy metal exposure, temperature, and soil pH. Gene expression and global methylation data was collected from all transplant groups. A draft genome has also been assembled and annotated – the analysis presented is combination of these data sources. Here we show some of the potential of these novel methods by showcasing the global statistical 'features' of A. gracilis.

35 | Environmental heat stress induces epigenetic inheritance of robustness in Artemia model

Parisa Norouzitallab[1], K. Baruah[1], M. Vandegehuchte[2], G. Van Stappen[1], L. Merga[2], K. De Schamphelaere[2], P. Sorgeloos[1], P. Bossier[1]

[1]Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium

[2]Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium

All living organisms are constantly challenged by different environmental stressors in their living environment to which they react with a battery of responses. The general consensus suggests that most of the phenotypes determining stress tolerance have a genetic basis and are subject to Darwinian natural selection and Mendelian inheritance. However, the recent notion that phenotypic traits can emerge directly from environmental experiences and can be inherited has long been debated. The recent report of non-Mendelian transgenerational epigenetic inheritance i.e. the inheritance of traits that are not determined by the DNA sequence, might make such a phenomenon plausible. In our study, by carrying out common garden experiments, we could provide clear evidences that, upon exposure to non-lethal heat shocks, a parental population of parthenogenetic Artemia (originating from one single female) experiences an increase in the levels of Hsp70 production, tolerance towards lethal heat stress, resistance against pathogenic V. campbellii and increased tolerance against zinc toxicity. Interestingly, these acquired phenotypes were transmitted to three successive generations, none of which was exposed to the parental stressor. This transgenerational inheritance of the acquired traits was associated with altered levels of acetylation on histones H3 and H4 in the heat shocked group compared to the control group, where both the parental and its successive generations were reared at standard temperature.

36 | Is inbreeding and inbreeding depression associated with epigenetic regulation? A case study in Daphnia magna

Mieke Jansen[1], P. Vergeer[2], J. Ouborg[3], L. De Meester[1]

[1]KULeuven, Belgium

[2]Universiteit Wageningen, The Netherlands

[3]Radboud University Nijmegen, The Netherlands

Inbreeding results when related individuals mate. This may, for instance, occur when populations have become small, often the case for (locally) threatened species. Inbreeding is often associated with a lowered fitness (inbreeding depression) and its occurrence has therefore important evolutionary implications. Recent work on plants has revealed that inbred lineages often show increased levels of DNA methylation, one of the key mechanisms of epigenetic regulation. Moreover, there is evidence that this epigenetic regulation may be associated with observed inbreeding depression. Here we want to test whether inbreeding is associated with increased methylation in the water flea Daphnia. Daphnia is a very convenient model system for this work because of its cyclic parthenogenetic life cycle allowing clonal lineages, its short generation time which facilitates multiple-generation studies, and the ease of culture and fitness quantification. Using Daphnia as a model system, we have the opportunity to take this line of research to a next stage. The epiGBS analysis shows a strong effect of inbreeding on GC methylation. Inbreds do not only show more methylation but also other GC sites are methylated compared to outbred individuals. This in agreement with our first hypothesis that inbreeding leads to increased levels of methylation, in analogy to the results obtained in plants. Secondly, we could observe that pollution in the surrounding of the pond could influence genome methylation of the isolated individuals.