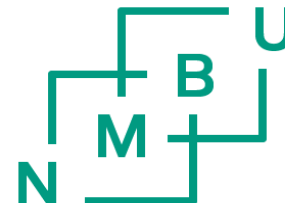




Generational effects of low dose ionizing radiation on DNA methylation in zebrafish.

Jorke Kamstra, Leonardo Martin, Leif Lindeman, Selma Hurem, Juliette Legler, Dag Brede, Deborah Oughton, Brit Salbu, Jan Lyche, Peter Aleström

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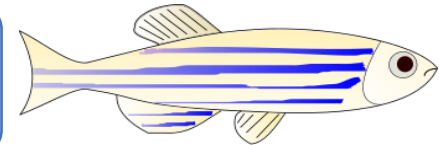
Norwegian University
of Life Sciences

Aims of the study

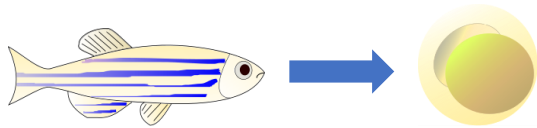
Study the effects of ionizing radiation in offspring from exposed parents.



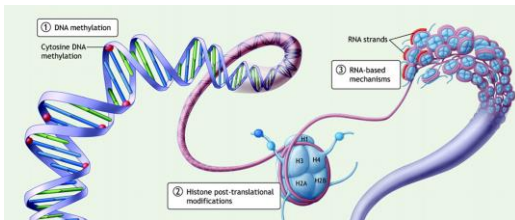
Zebrafish as a model to assess the effects of ionizing radiation over generations.



OBJECTIVES



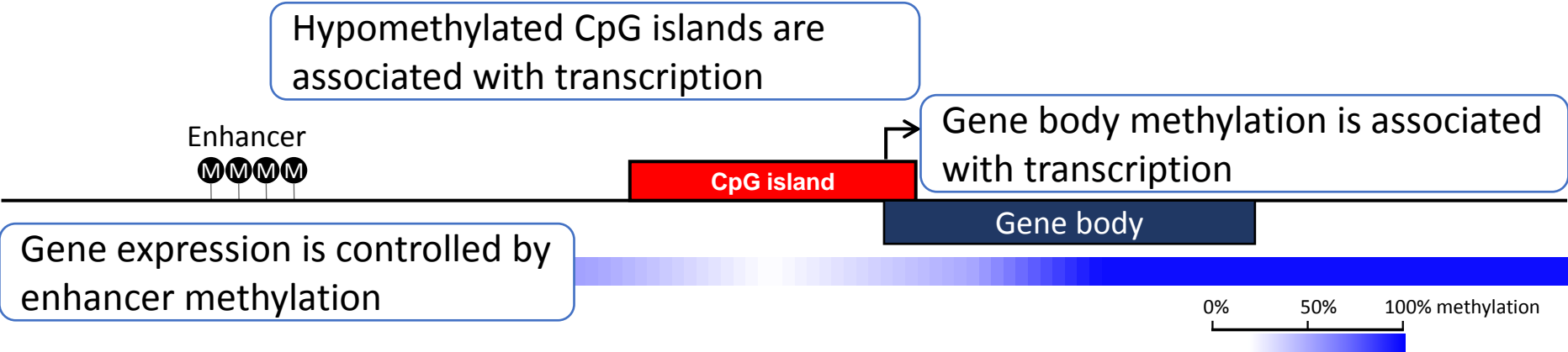
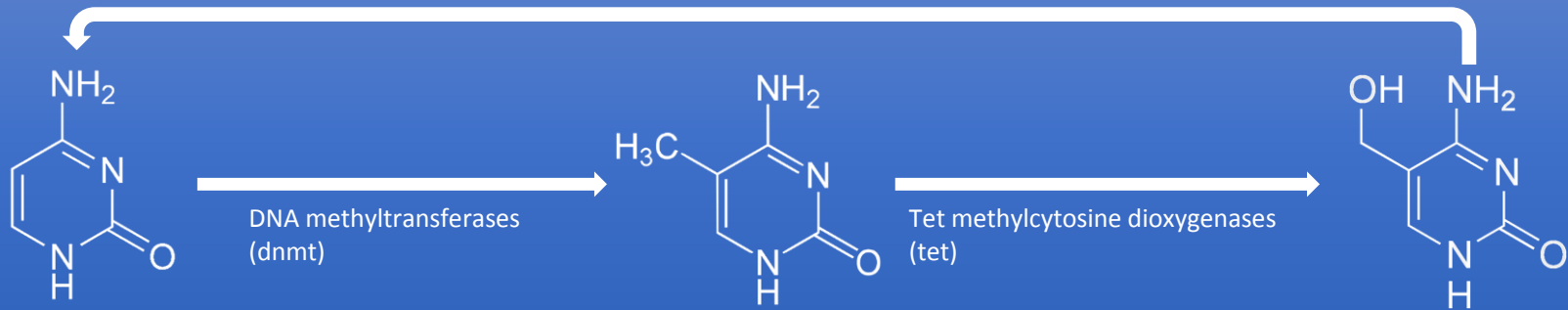
Does ionizing radiation exposure during gametogenesis leads effects in offspring of subsequent generations?



Are these effects mediated by epigenetic mechanisms?

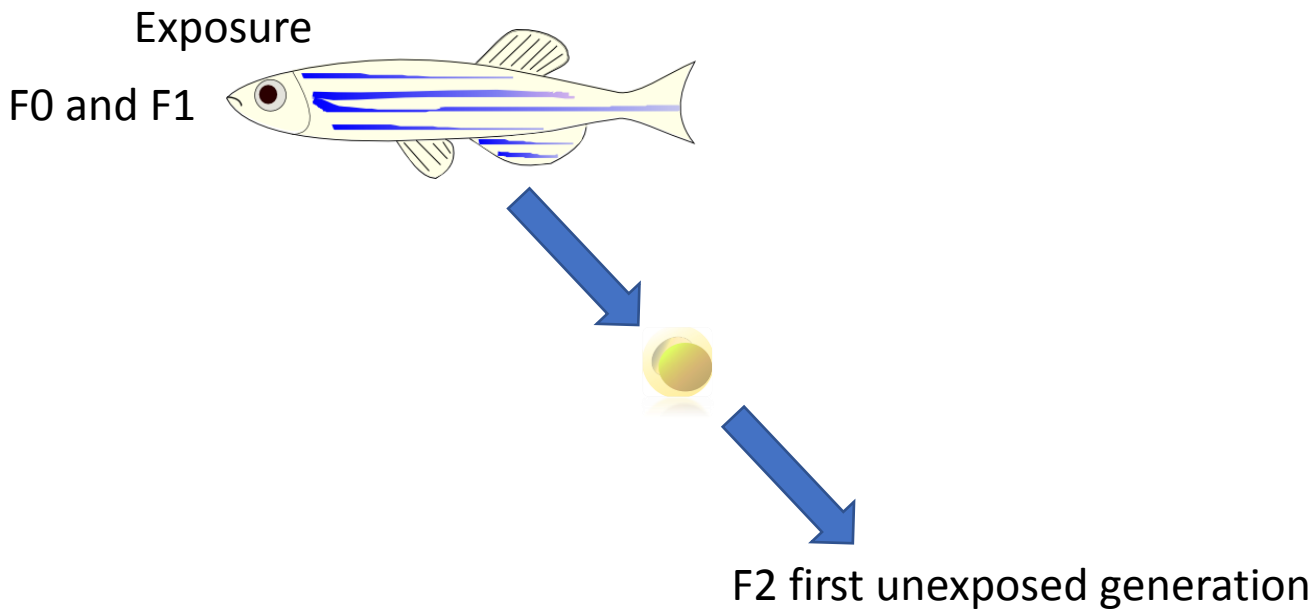
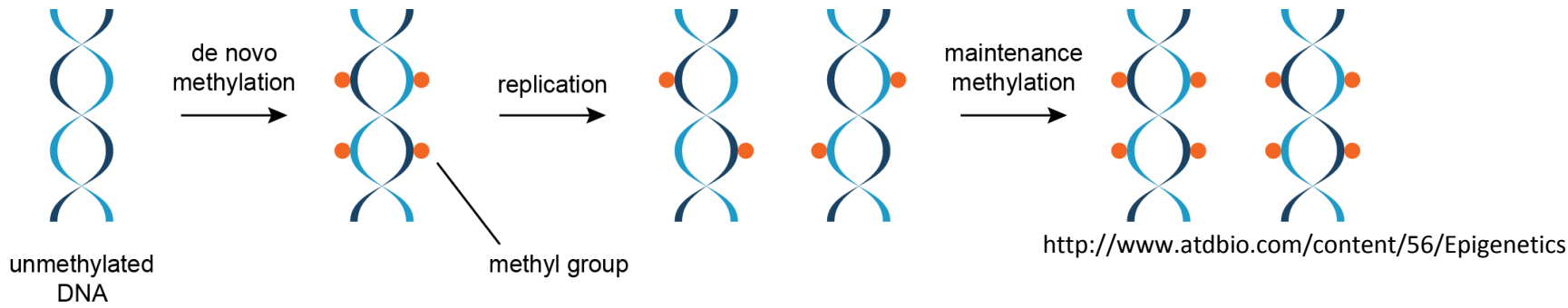
Background epigenetics

CpG DNA Methylation



Background epigenetics

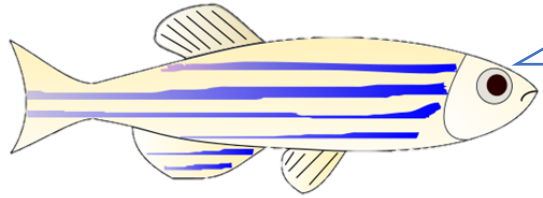
Epigenetic inheritance



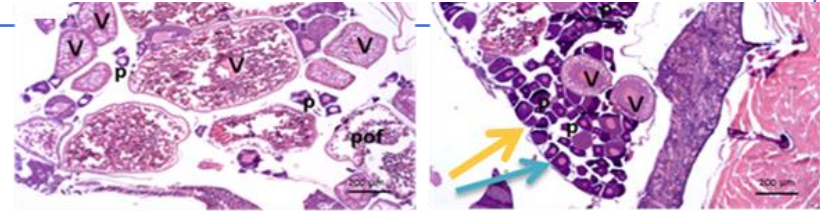
Ionizing radiation in zebrafish (Hurem et al. In prep)



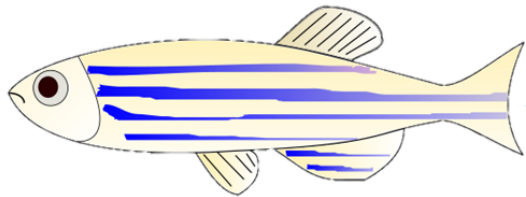
Adults exposed to 8.7 mGy/h, 27 days, inbreeding



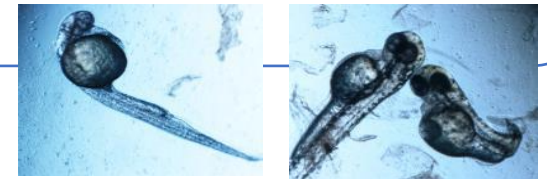
- Irradiated oocytes have a large number of pre-vitellogenic follicles.



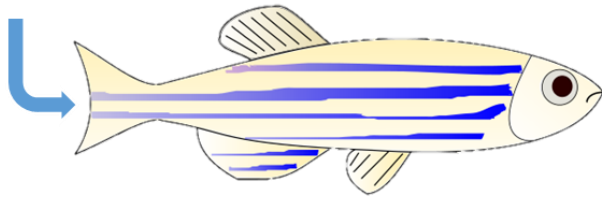
F1



- Early hatching, increased mortality, increased deformities (changes in eye morphology).
- Increased lipid peroxidation and DNA Damage one year after parental irradiation.



F2



F3



Whole genome bisulfite sequencing and mRNA sequencing



Whole genome bisulfite sequencing

- Analysis of genome wide DNA methylation
- > 100 million reads (150 bp paired end)
- Differentially methylated regions by logistic regression

mRNA sequencing

- Differentially expressed genes by Deseq2



Multiplex Amplicon Bisulfite PCR (BisPCR2)

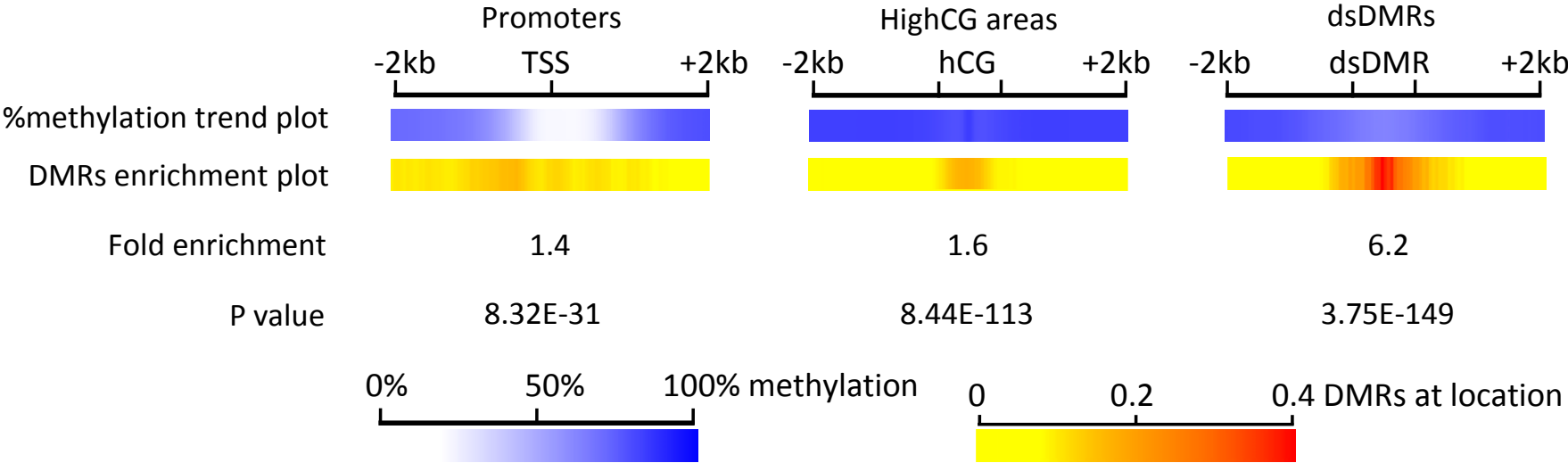
- Analysis of specific loci for DNA methylation
- Measurement of 19 loci in 46 DNA samples
- Validation of WGBS results
- Analysis of F2 and F3 generations



DNA methylation – Global analysis

Differentially methylated regions:

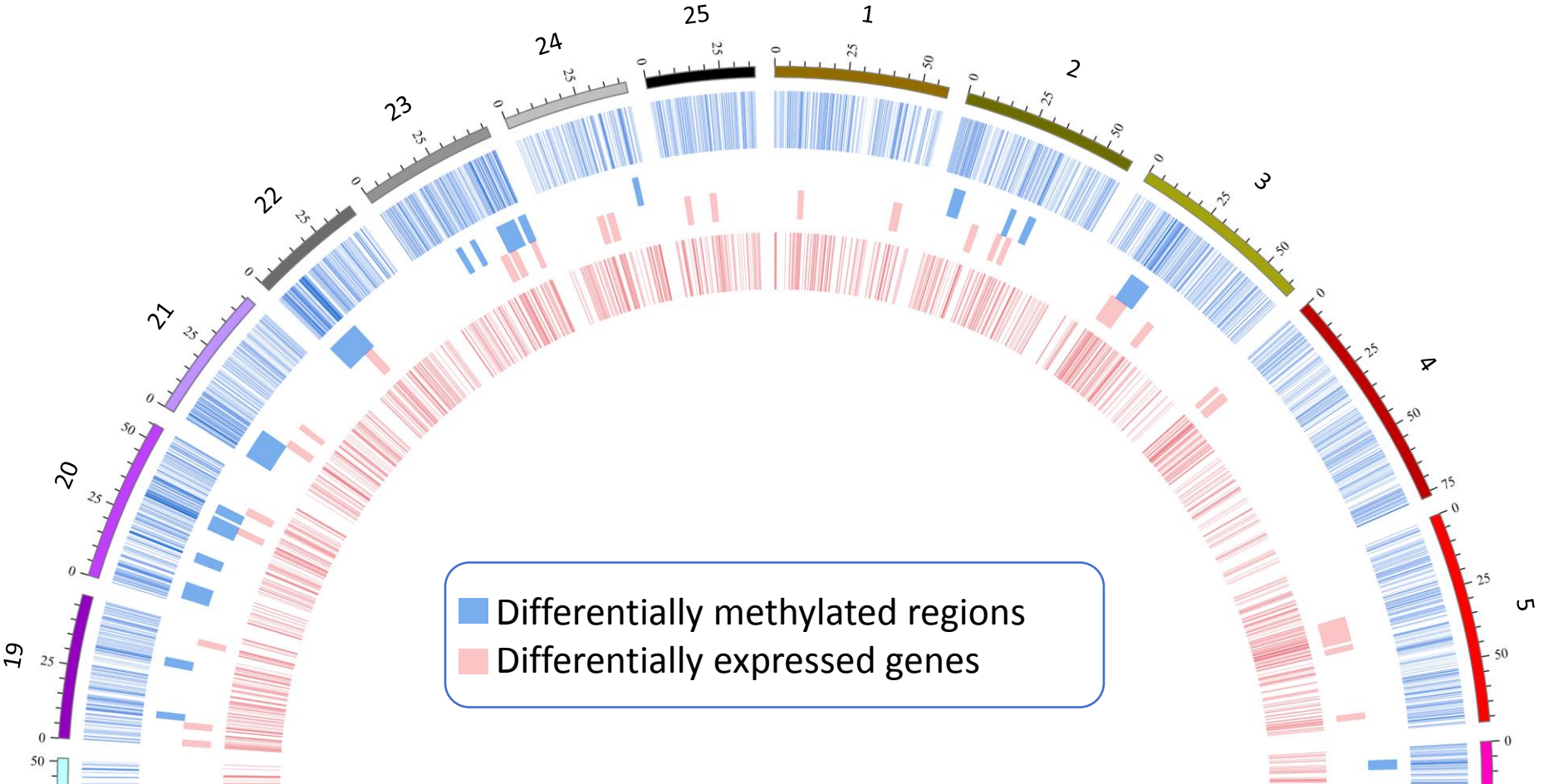
- Located at transcriptional start sites
- High content CG areas
- Developmental DMRs (dsDMRs)



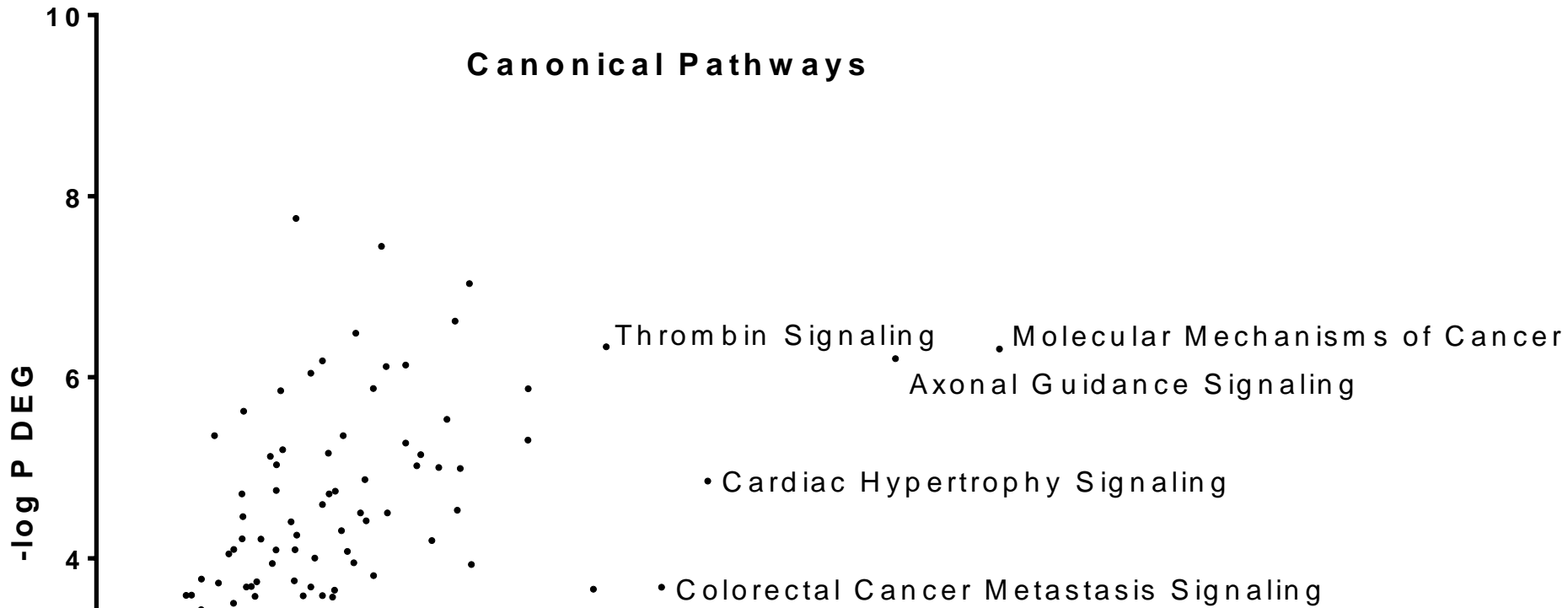
DNA methylation and mRNA seq

Non random distribution of differentially methylated regions and differentially expressed genes

Epigenetic control regions



Ingenuity pathway analysis



Similar pathways affected by both DNA methylation and RNA seq analysis

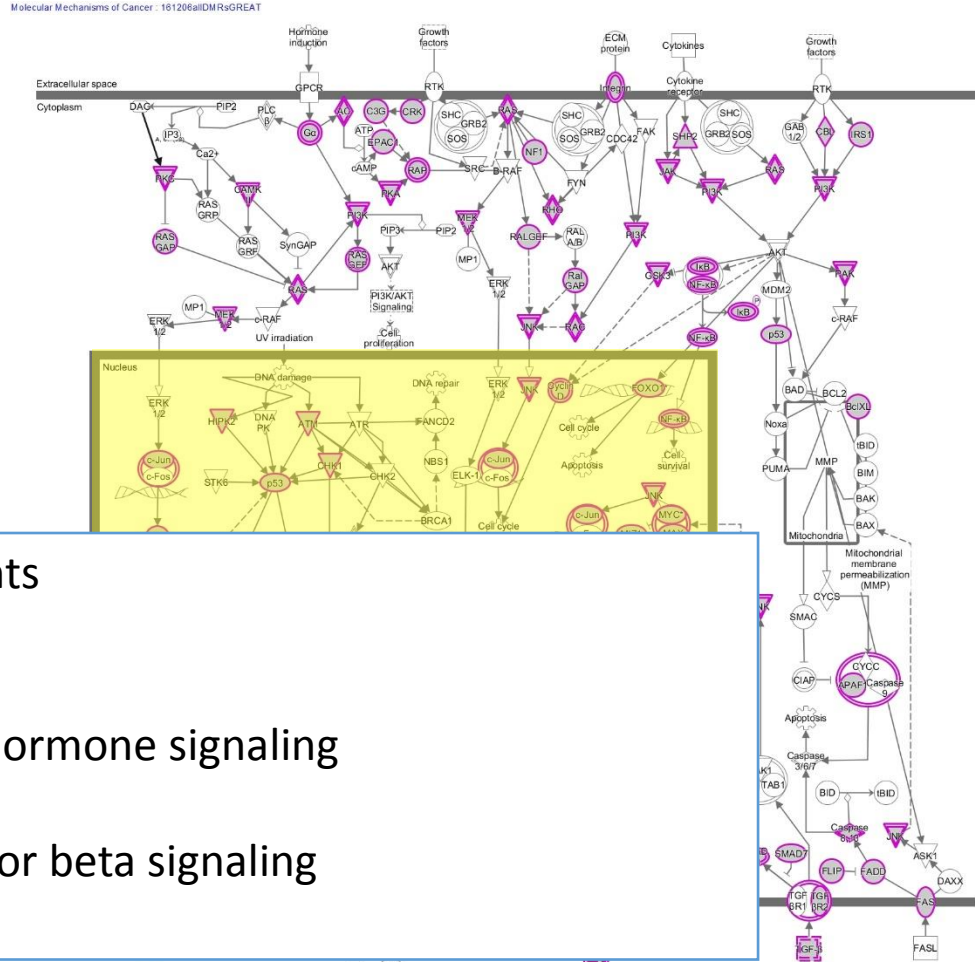
- With low overlap of similarly regulated genes

Pathways found can be related to phenotype or known ionizing radiation effects:

- DNA damage response
- Cancer
- Brain development

Ingenuity pathway analysis

DNA methylation follows function:
Molecular mechanisms of cancer

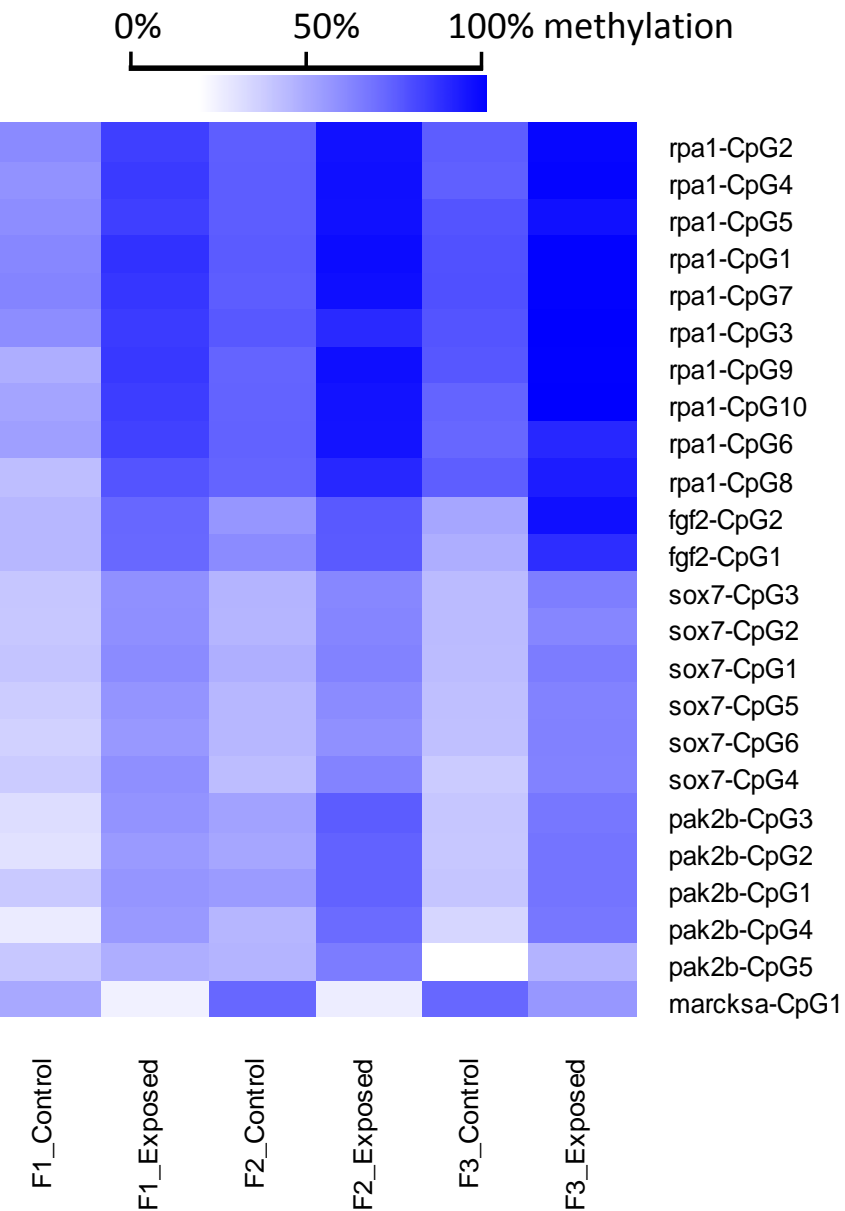
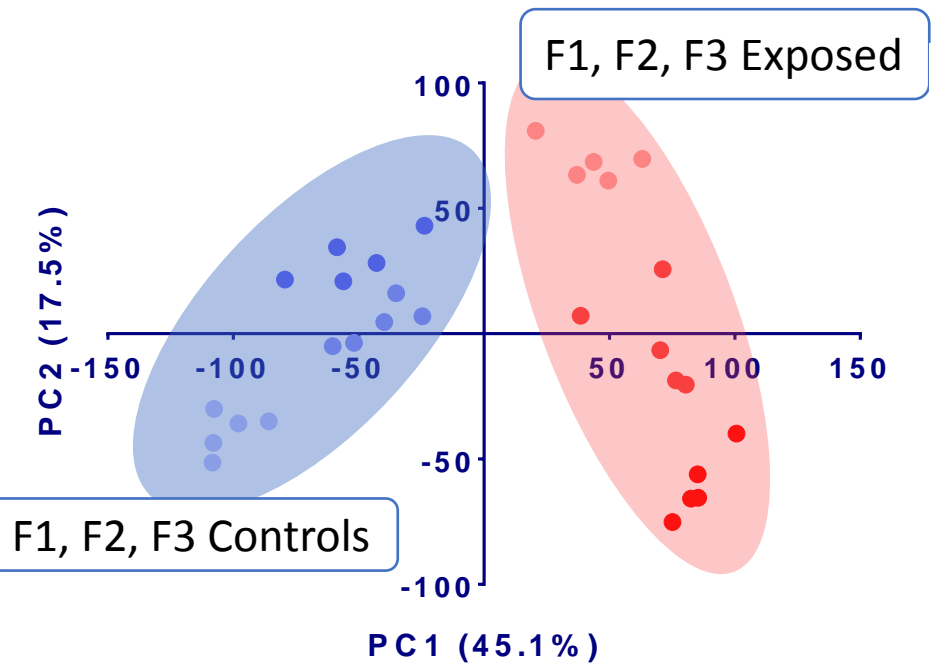


Also potential novel endpoints

For instance:

- Gonadotropin releasing hormone signaling
- Axonal guidance
- Transforming growth factor beta signaling

Transgenerational effects




- Clear separation of control and ancestral ex
- Five loci exhibited persistent effects at each

RESEARCH

Open Access



Differential DNA methylation at conserved non-genic elements and evidence for transgenerational inheritance following developmental exposure to **mono(2-ethylhexyl) phthalate** and **5-azacytidine** in zebrafish

Jorke H. Kamstra¹, Liana Bastos Sales², Peter Aleström¹ and Juliette Legler^{2,3*} 

Discussion and Outlook

Functional role of DNA methylation

- Differential gene expression and methylation have limited overlap...
- ...but, pathways and phenotypes can be linked to effects of DNA methylation

Transgenerational effects on DNA methylation

- Strong indication that DNA methylation changes are persistent over generations



Next:

Use zebrafish to explore causality and disease related effects

- Investigate the 'functional epigenomics'
- Combine with ncRNAs and histone marks
- De novo methylation analyses (CRISPR-dnmt and CRISPR-tet)
- 4C (interaction of DMRs with other parts of DNA)

DMRs as epigenetic markers following environmental stress

- High throughput analysis of found DMRs with environmental contaminants

Acknowledgements



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Peter Aleström

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