

PNRPE

PROGRAMME
NATIONAL
DE RECHERCHE
SUR LES
PERTURBATEURS
ENDOCRINIENS



Séminaire de présentation des projets de l'APR 2010

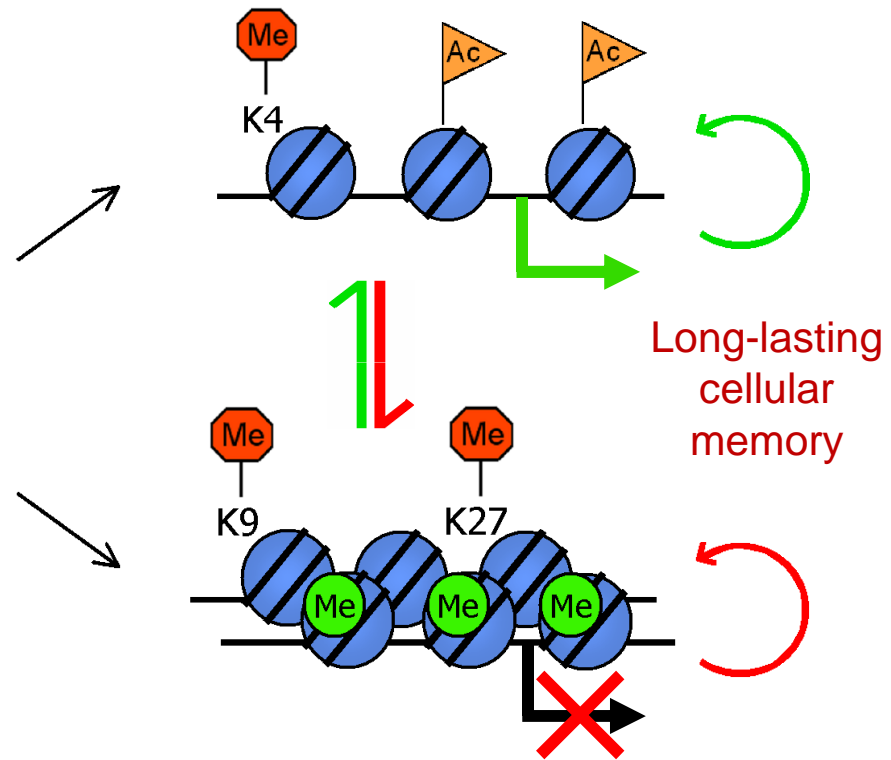
MethylED: Impact of Endocrine Disruptors on genomic DNA methylation patterns in the germline

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Jesus del Mazo, Centro de Investigaciones Biológicas, Madrid

DNA+Histones=Chromatin

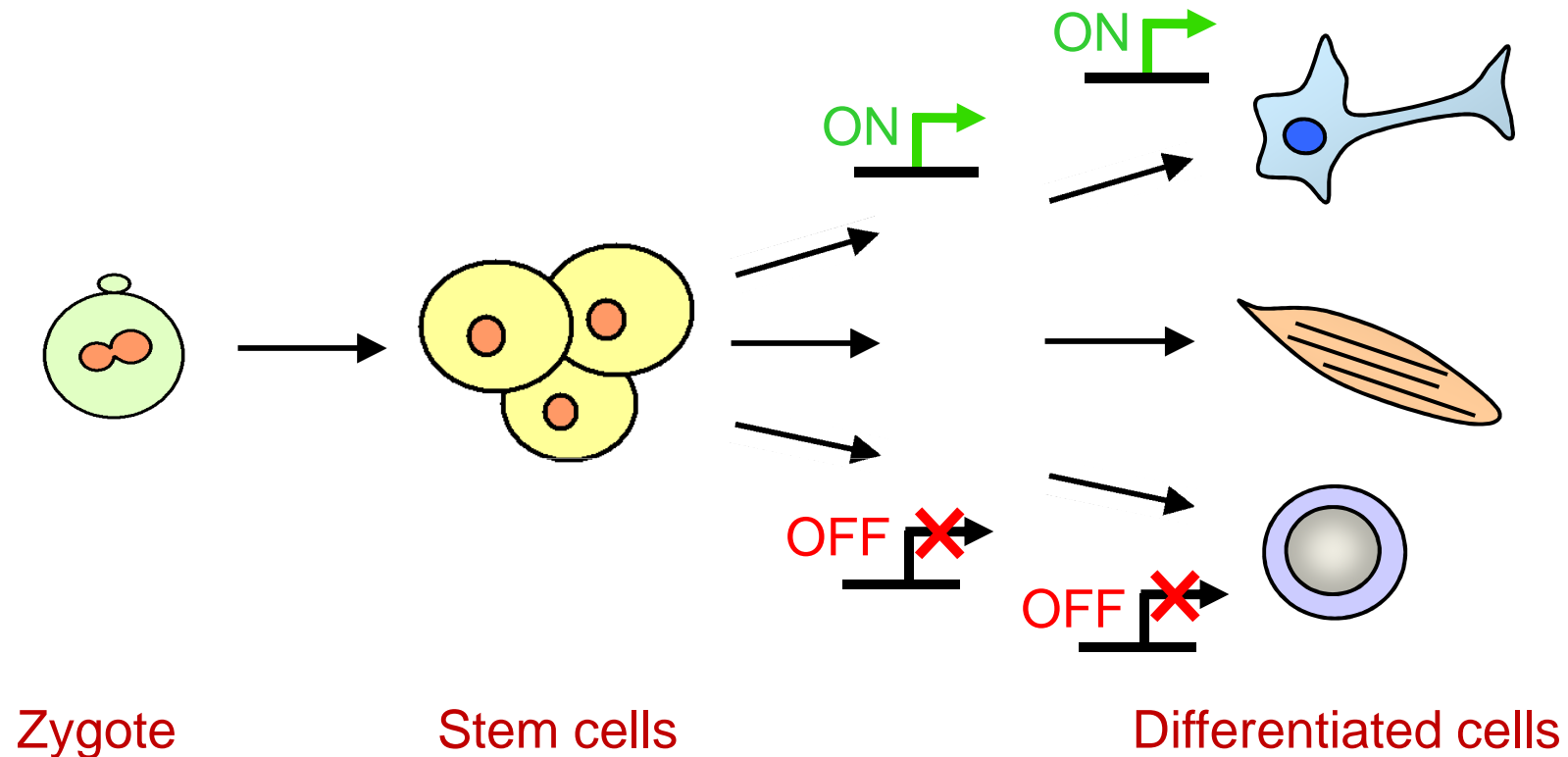
ATCGTCGAATCCGTAA
 TGCATGCCTGAAGATC
 TACGATCTAGTCGGGT
 AATCGAATCGTTTCGT



Genome

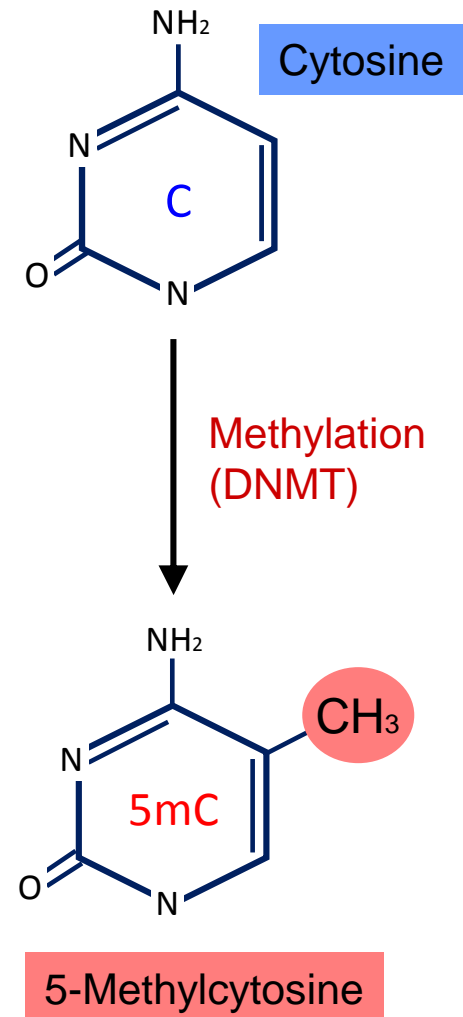
Epi-genome

Introduction: DNA methylation in mammals

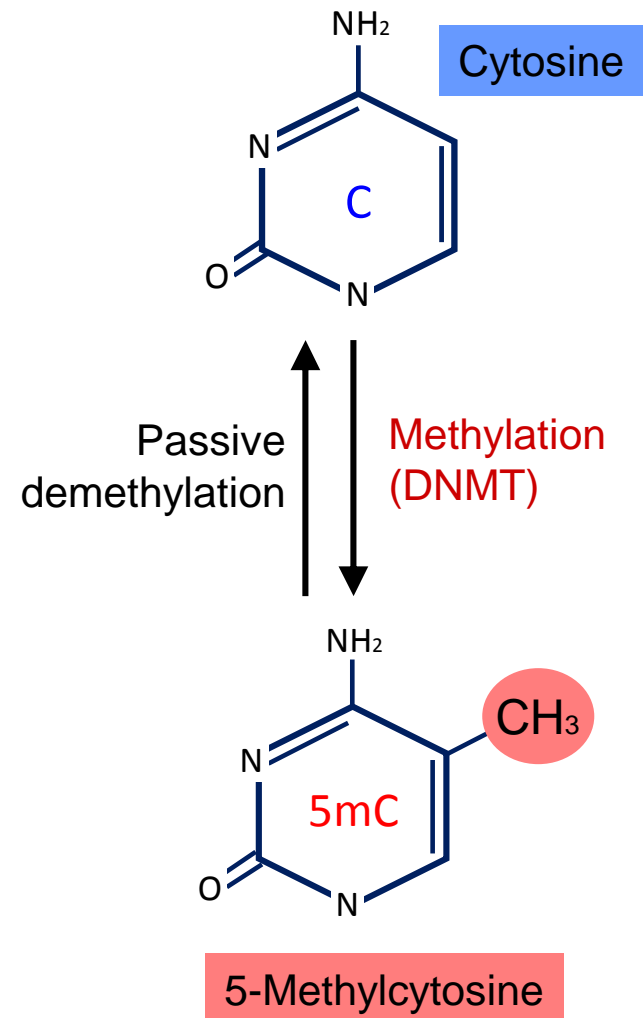


Heritable epigenetic marks create a memory of gene expression states

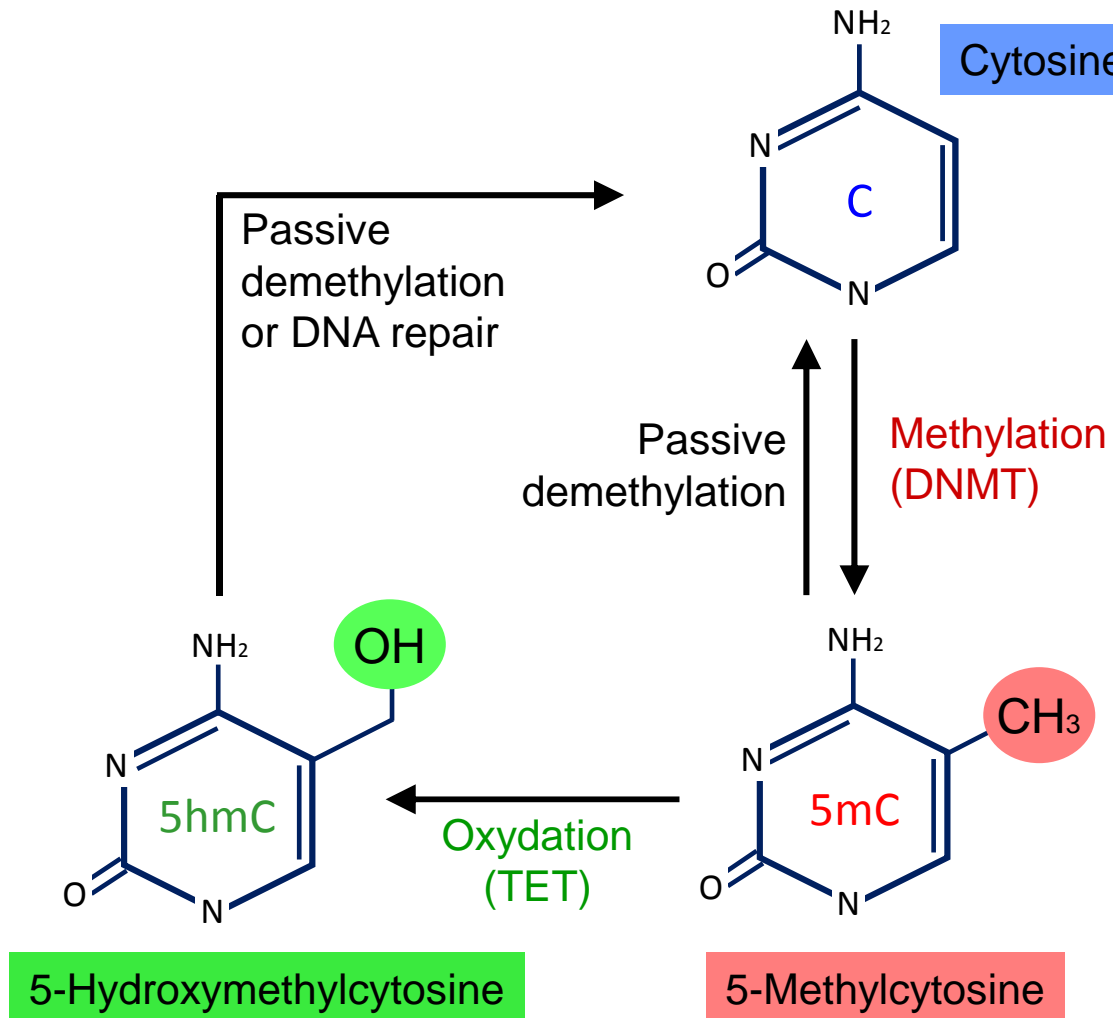
Introduction: DNA methylation in mammals



Introduction: DNA methylation in mammals

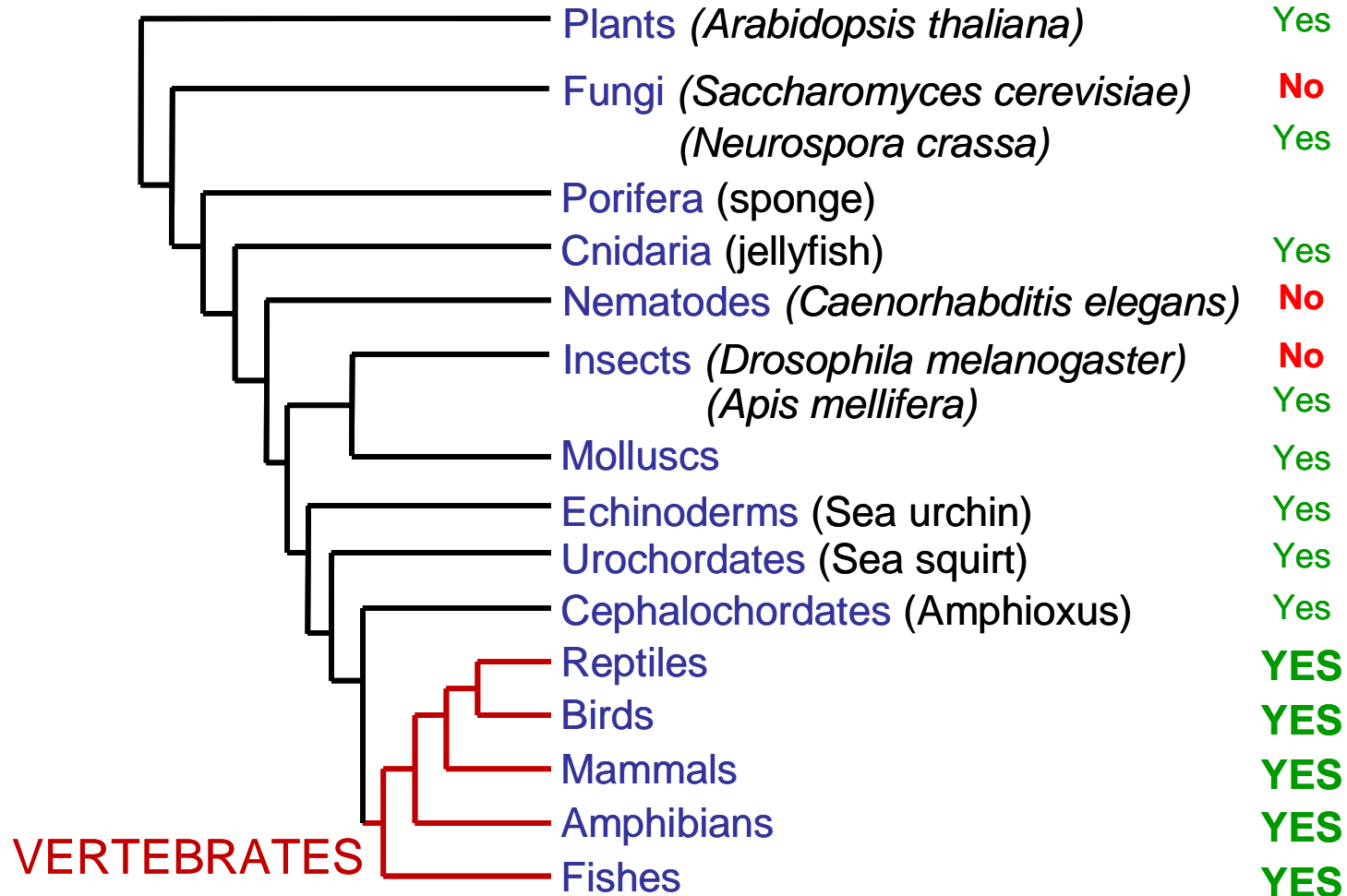


Introduction: DNA methylation in mammals





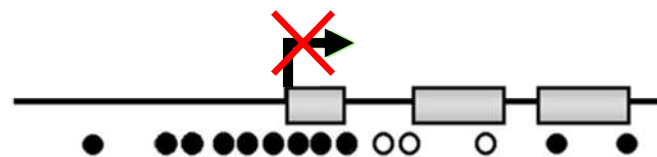
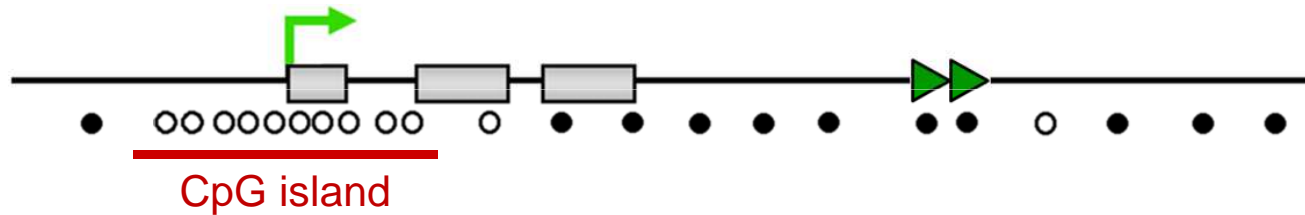
Introduction: DNA methylation in mammals



Introduction: DNA methylation in mammals

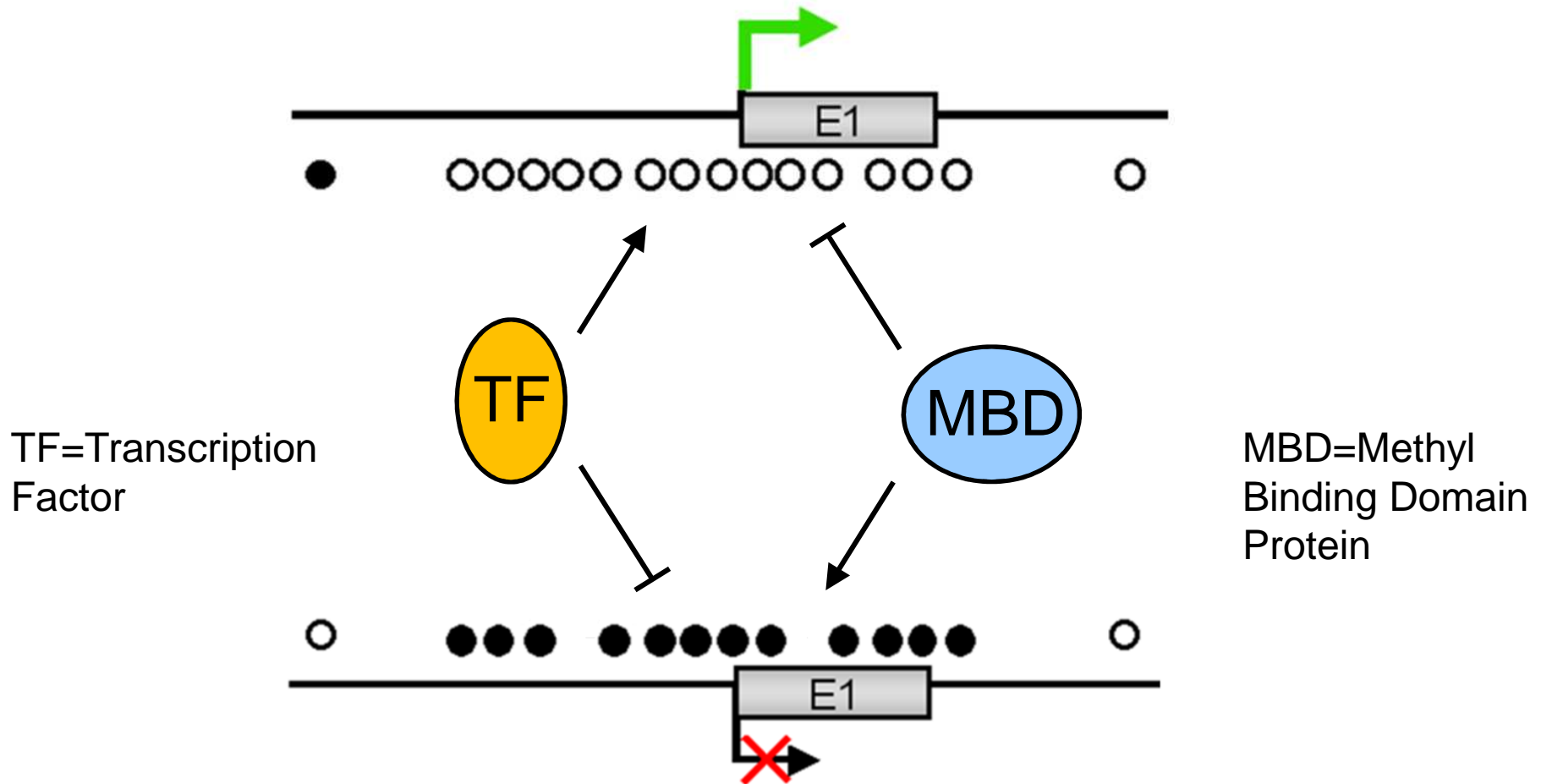


- Methylation occurs on cytosines of CpG dinucleotides, which are unevenly distributed in mammalian genomes.

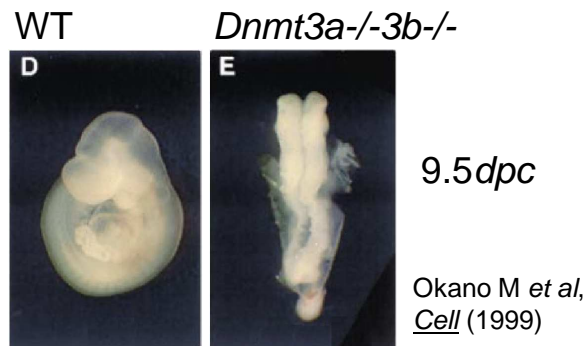
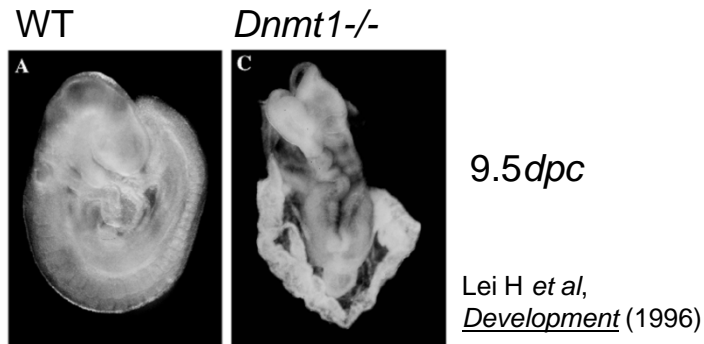


- 2-5% methylated CpG islands in normal cells.

Introduction: DNA methylation in mammals

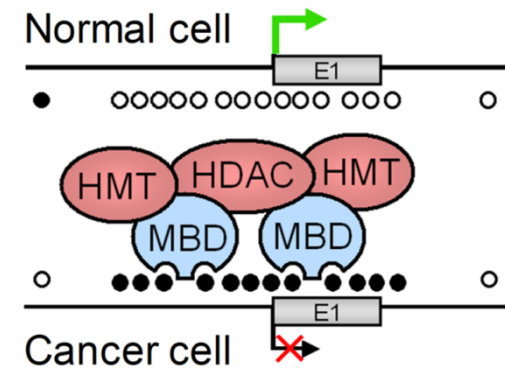


a) Essential for mammalian development



b) Perturbed in many pathologies

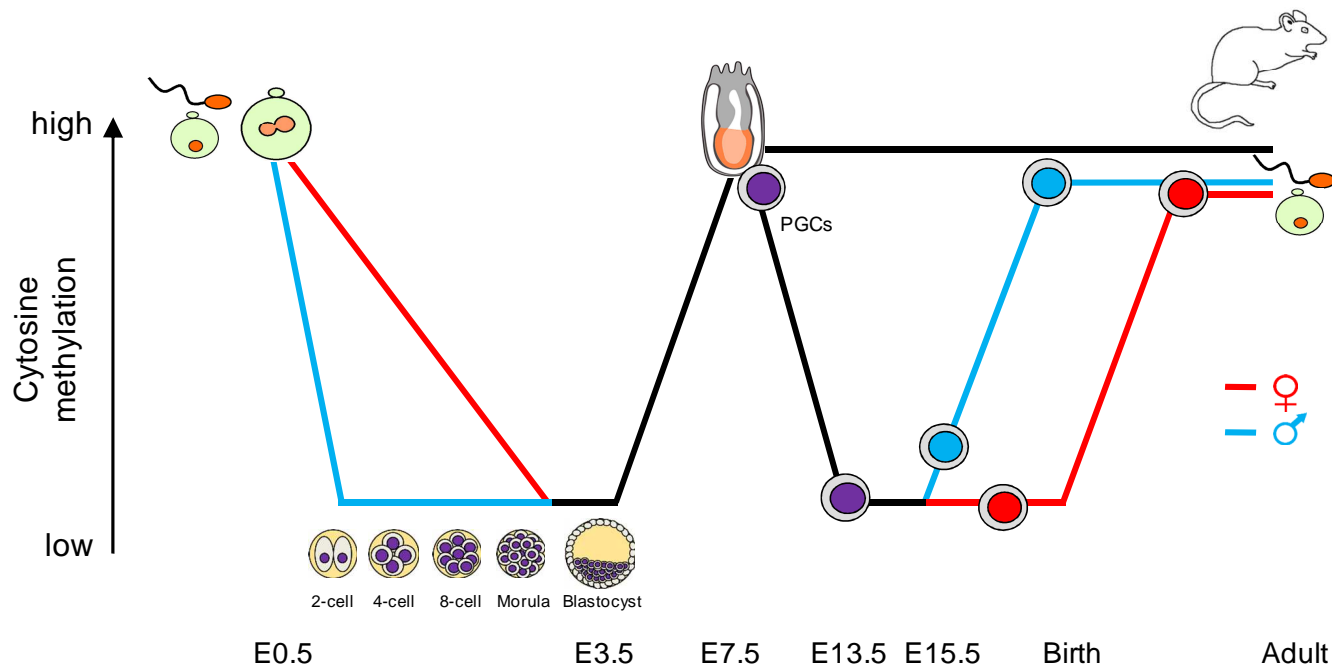
- Growth and neuro-developmental syndromes
 - ICF syndrome
 - Beckwith-Wiedemann syndrome
 - Prader-Willi/Angelman's syndrome
- Auto-immune and metabolic diseases
- All types of cancer



- Diagnostic markers
- Therapeutic targets

Objective: Test the impact of Endocrine Disruptors on DNA methylation patterns in the germline

- Primordial germ cells undergo massive DNA methylation reprogramming and are therefore susceptible to epigenetic alterations





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- Primordial germ cells undergo massive DNA methylation reprogramming and are therefore susceptible to epigenetic alterations
- Changes in DNA methylation in rodents after *in utero* maternal exposure to chemicals, diet, alcohol

Ho SM et al., *Cancer Res* 2006
Dolinoy DC et al., *PNAS* 2007

Zama AM et al., *Endocrinology* 2009
Bromer JG et al., *Endocrinology* 2009

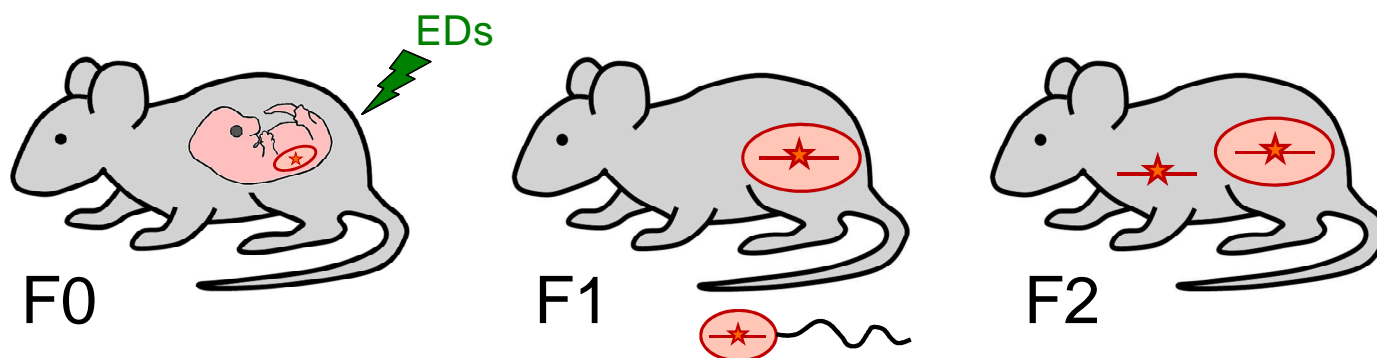
Waterland R et al., *Mol Cell Biol* 2003
Kaminen-Ahola N et al., *PLoS Genet* 2010

- Multigenerational non-mendelian effects suggest possible transmission of altered DNA methylation through generations

Anway MD et al., *Science* 2005

Stouder C et al., *Reproduction* 2010

Cropley JE et al., *PNAS* 2006



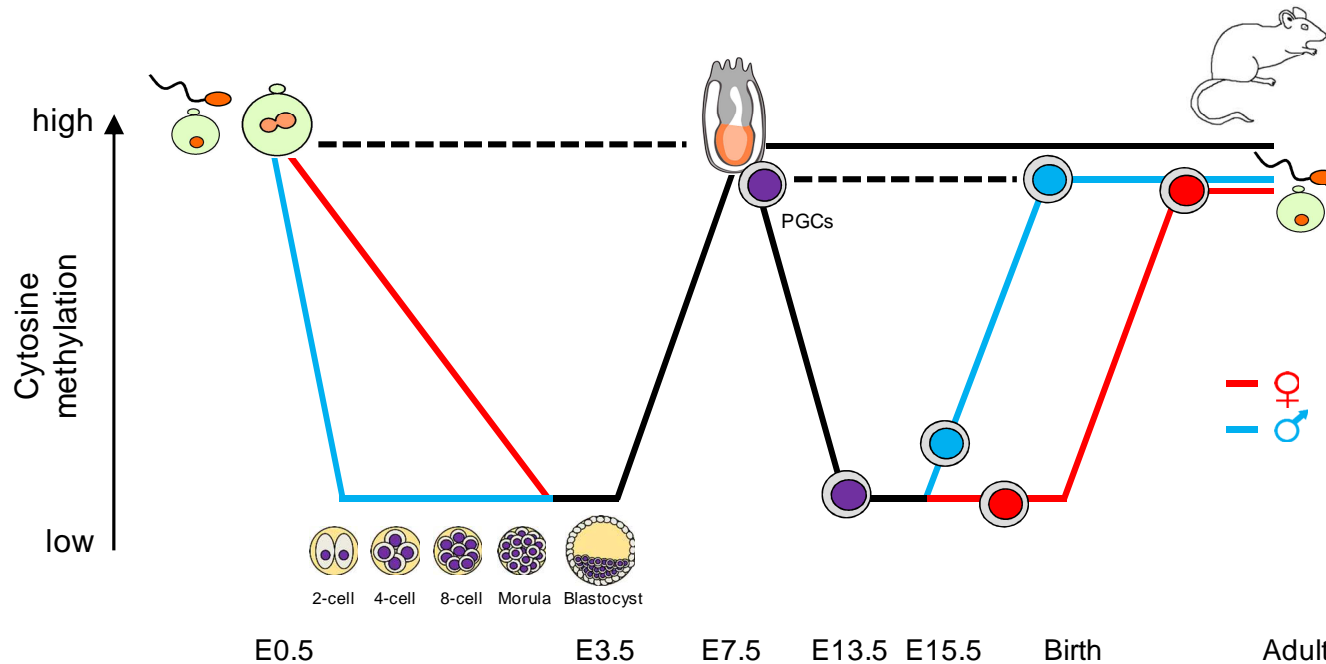


Objective: Test the impact of Endocrine Disruptors on DNA methylation patterns in the germline

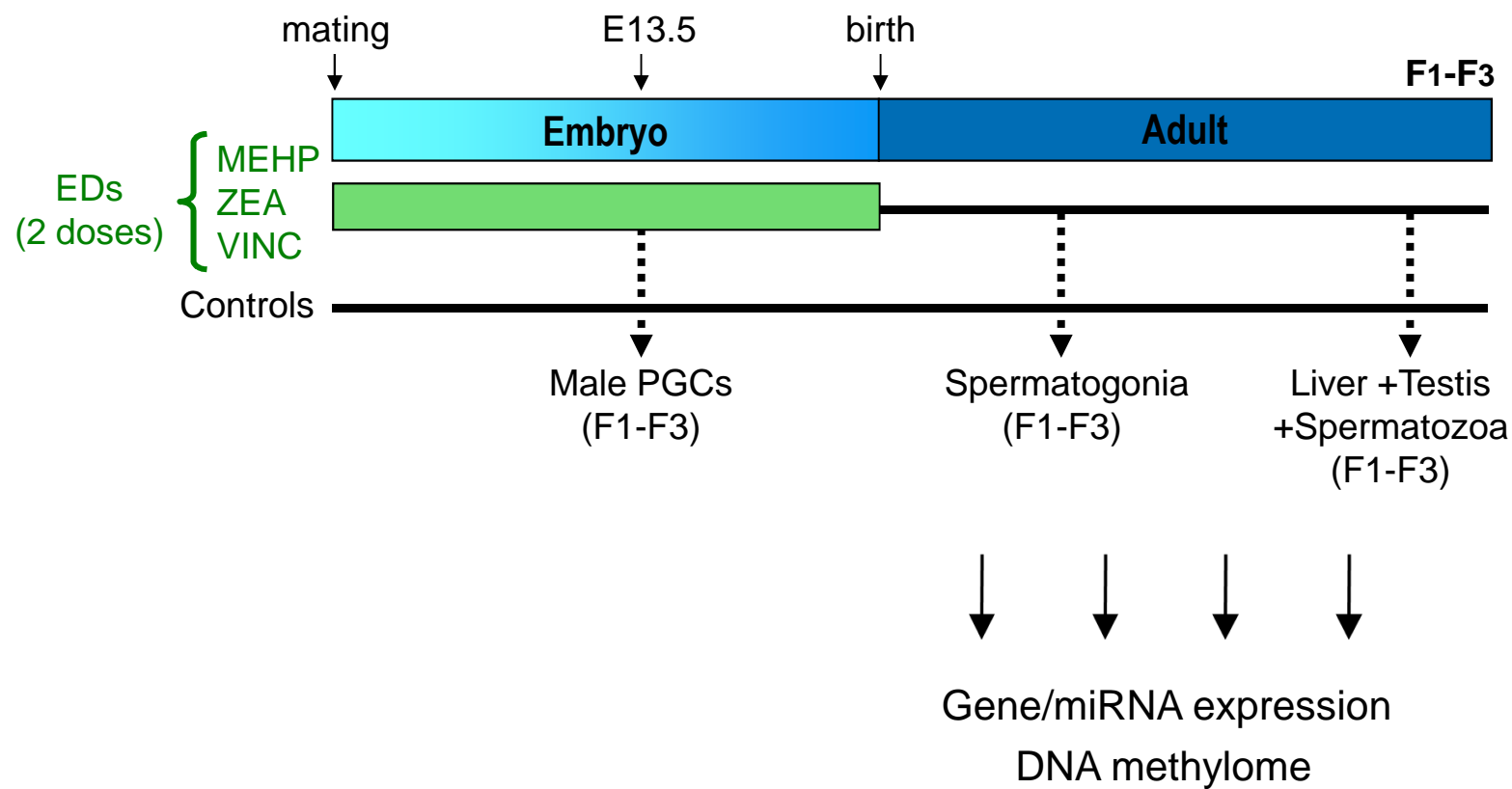
- To evaluate the potential effect of environmental factors on epigenetic states, it is first necessary to describe the « normal » epigenetic landscape
- Our results indicate that a small fraction of the genome escapes methylation reprogramming during development, which suggests possible transmission of epimutations through generations

Borgel J et al, *Nat Genet* (2010)

Guibert S et al, *Genome Res* (2012)



CD1 mice (outbreed)



RRBS (Reduced Representation Bisulfite Sequencing)

ACTCGGTACGC

↓ Sodium Bisulfite

ATTCGGTATGT

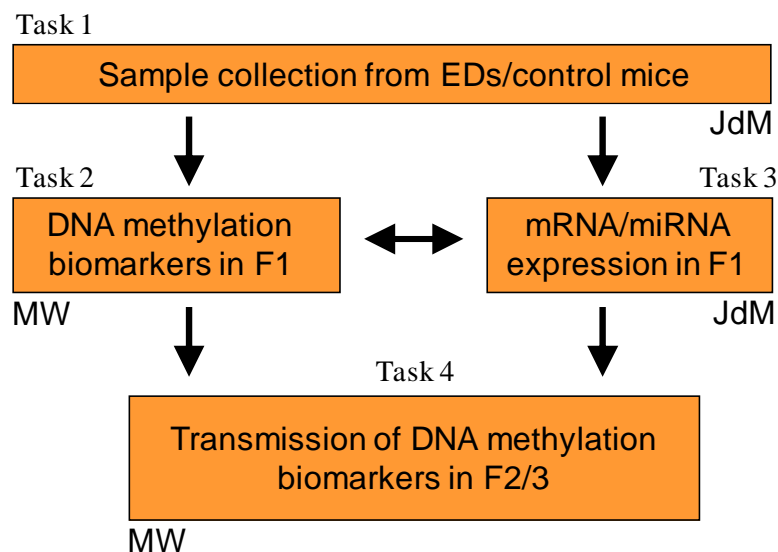


Illumina
Hi-Seq 2000



Quantitative
methylation information
for 1,200,000 CpGs

Expected results and deliverables



Tasks	Deliverables	months					
		6	12	18	24	30	36
Task 1	Isolation of E13.5 PGCs from mice of the F1-F3 generations.	█					
	Isolation of spermatogonia, testis and spermatozoa from mice of the F1-F3 generations.	█					
	Isolation of somatic cells (liver) from mice of the F1-F3 generations.	█					
Task 2	RRBS/MeDIP-Seq profiles in spermatozoa from control and exposed F1 mice		█	█	█		
	Comparative analysis of altered DNA methylation in relation to ED compound and dosage			█	█	█	
	Validation of DNA methylation biomarkers in PGCs, spermatogonia, spermatozoa and somatic cells			█	█	█	
Task 3	Transcriptome expression profiles in PGCs, spermatogonia, and testis from control and exposed F1 mice		█	█	█		
	miRNA expression profiles in PGCs, spermatogonia, spermatozoa and testis from control and exposed F1 mice		█	█	█		
	Selection and validation of gene expression biomarkers			█	█	█	
Task 4	Comparative analysis between DNA methylation and gene expression abnormalities			█	█	█	
	Analysis of DNA methylation biomarkers in germ cells and somatic cells of unexposed F2/F3 individuals				█	█	█
	Analysis of altered mRNA/miRNA expression at candidate biomarkers in unexposed F2/F3 individuals				█	█	█

- Understand epigenetic mechanisms of action of EDs
- Relate epigenetic effects to doses and compound ID
- Identify novel DNA methylation biomarkers related to ED exposure



Acknowledgements

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