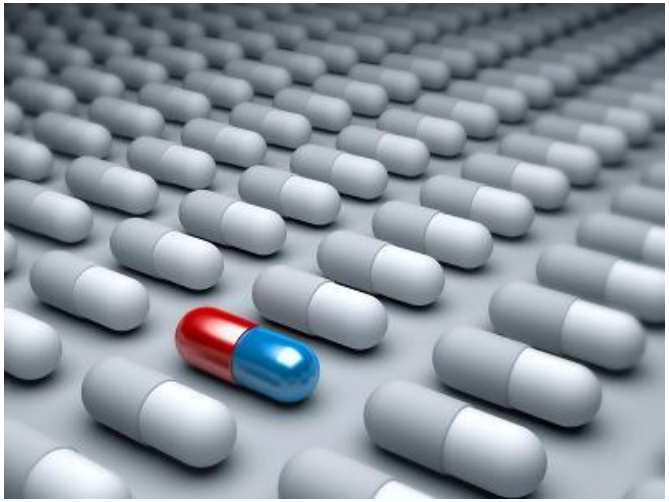

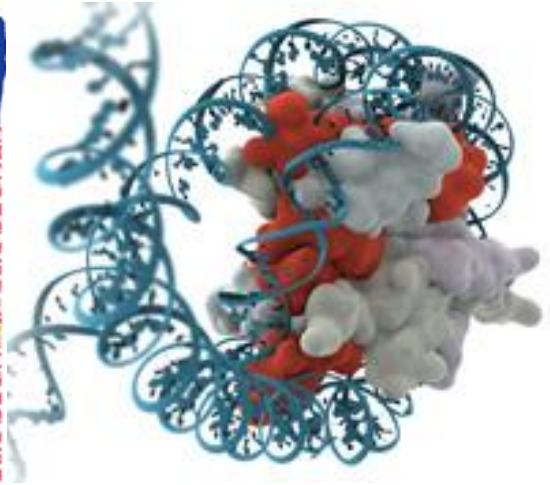

Epigenomics and Impact for Drug Safety Sciences

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NOVARTIS

Acknowledgments

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Michigan State
Jay Goodman

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Overview

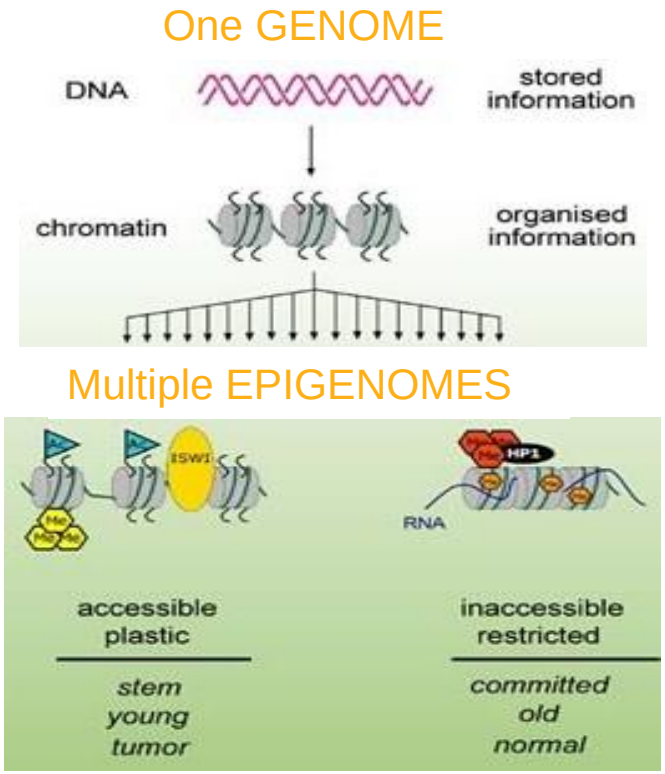
- Introduction to epigenetics and relevance to carcinogenesis
- Drug safety applications:
  - Integrated epigenomic + transcriptomic profiling in preclinical studies
  - Novel insights into early mechanisms/biomarkers of nongenotoxic carcinogenesis

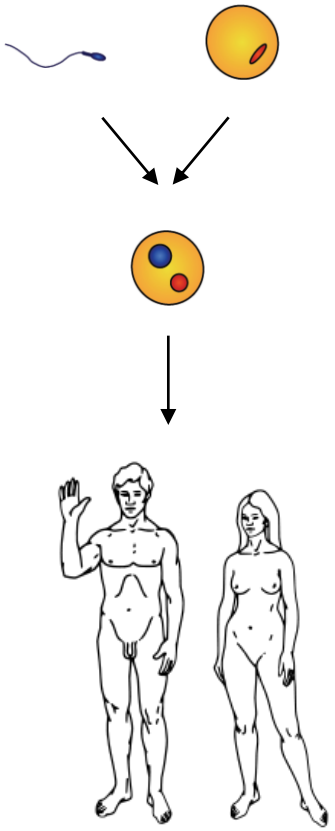
Molecular basis of epigenetic regulation

One genome, multiple epigenomes

- **Change in gene activity in the absence of a change in DNA sequence**
- Epigenetic modifications functionally organize the genome and define cell identity; they are reversible and essential to normal development and differentiation

http://www.epigenome-noe.net/WWW/aboutus/epigenetics.php





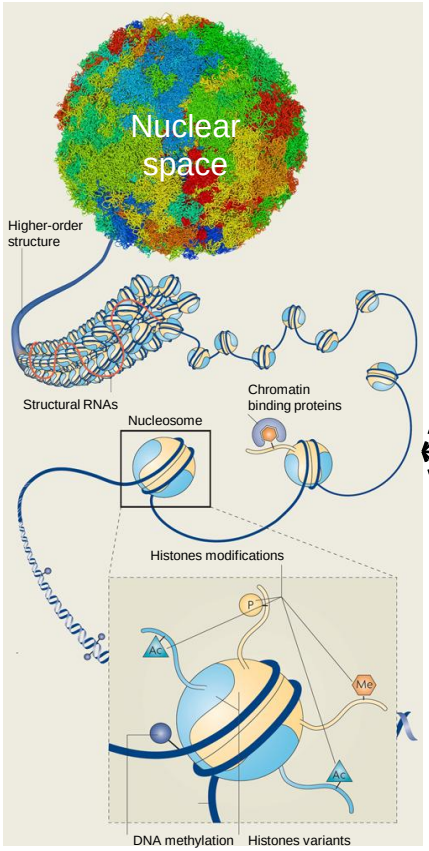
1 cell: Zygote
1 genome

- *Parental-origin specific*
- *Developmental stage specific*
- *Cell/Tissue specific*
- *Species specific*
- *Disease/drug specific*

100.000 billion cells
Multiple epigenomes

Molecular basis of epigenetic regulation

A wide spectrum of epigenetic mechanisms regulate genome function



Adapted from Probst *et al.*, *Nat Rev Mol Cell Biol* (2009)

Nuclear localization

Chromatin remodelers

Transcription factors

Non coding RNAs

Histones variants

Histones modifications

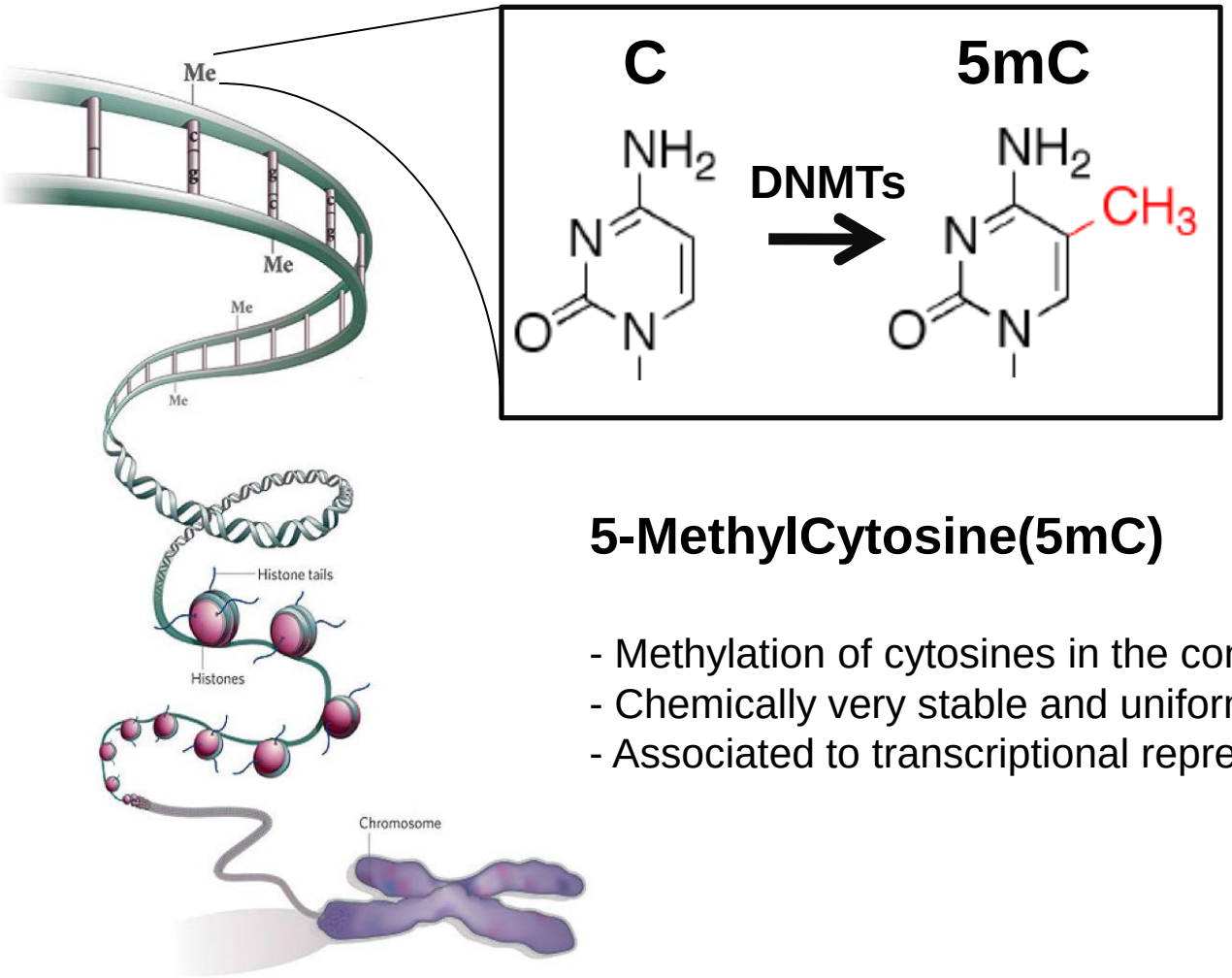
DNA methylation

- Multiple flavors → epigenetic "signatures" that specify different states of the chromatin
- Regulate genome function (transcription, replication, repair, plasticity...)
- Provide a signaling platform for long term cellular memory
- Epigenetic perturbations can lead to Human diseases
- Development of powerful detection and profiling assays

Epigenetic mechanisms are affected by several factors and processes, including development *in utero* and in childhood, environmental chemicals, **pharmaceuticals**, aging, and diet.

Molecular basis of epigenetic regulation

DNA methylation, a potential epigenetic biomarker



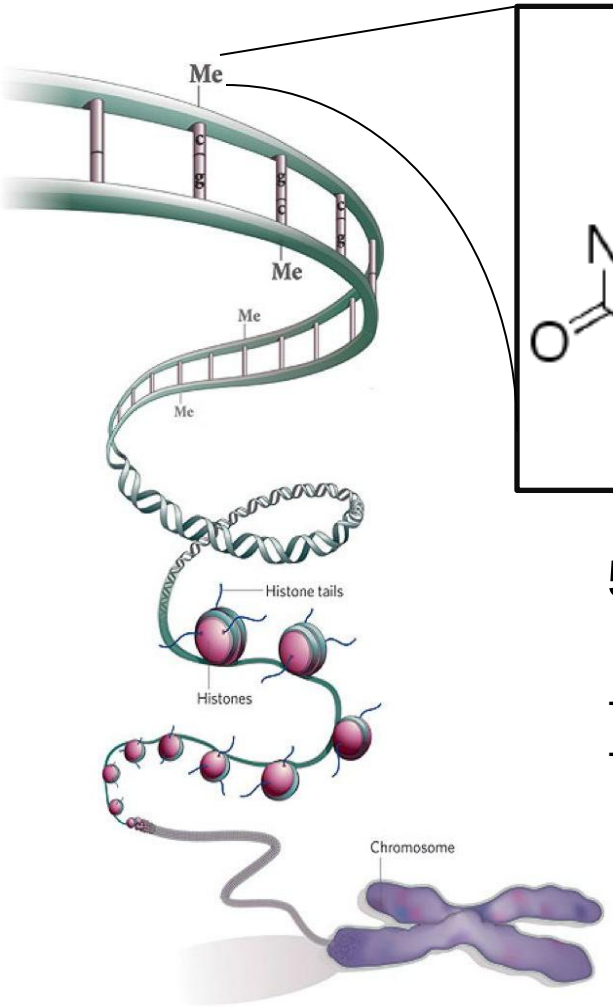
5-MethylCytosine(5mC)

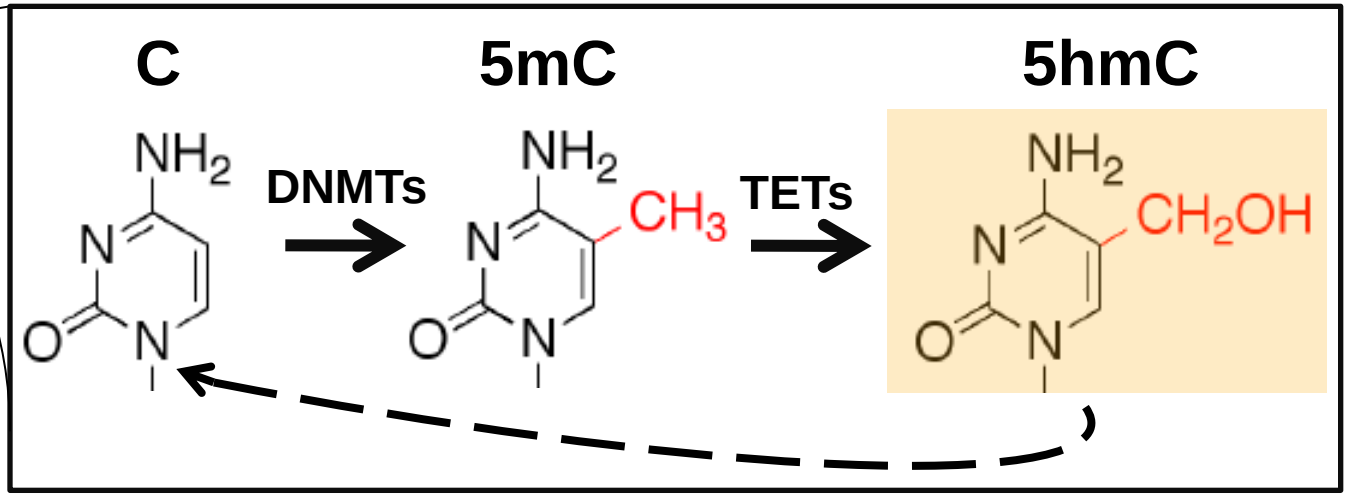
ACACGTTG
TGTGCAAC

- Methylation of cytosines in the context of CpG
- Chemically very stable and uniform in cell populations
- Associated to transcriptional repression and genome stability

Molecular basis of epigenetic regulation

DNA methylation, a potential epigenetic biomarker





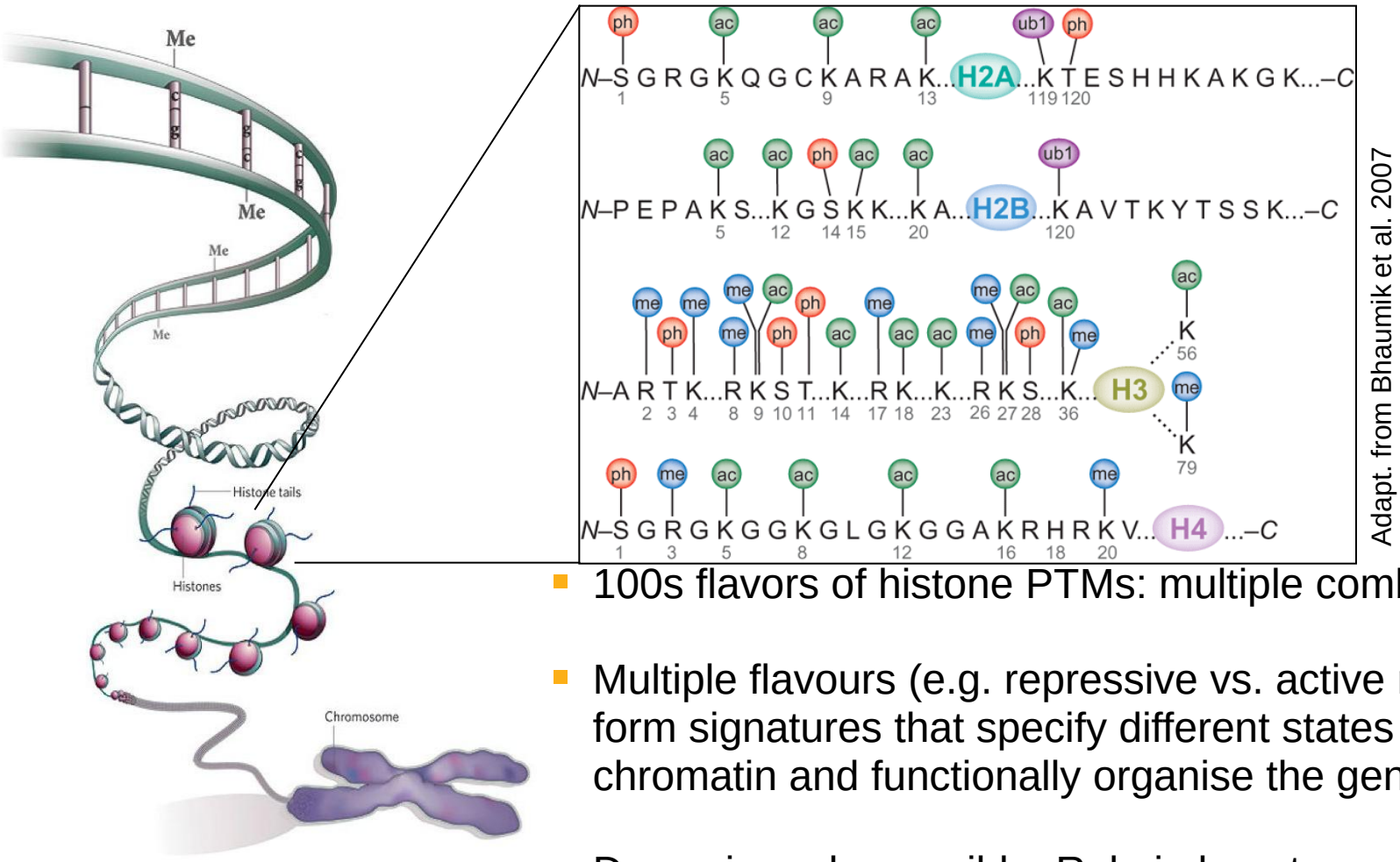
5-HydroxyMethylCytosine(5hmC)

- "Novel" DNA base – discover in mammals in 2009
- Work in ES cells suggests that 5hmC is an intermediate in DNA demethylation pathway, and is associated with expressed genes

Kriaucionis S, Heintz N. (2009). *Science*, 15;324(5929):929-30.
Tahiliani M et al. (2009). *Science*,15;324(5929):930-5.

Molecular basis of epigenetic regulation

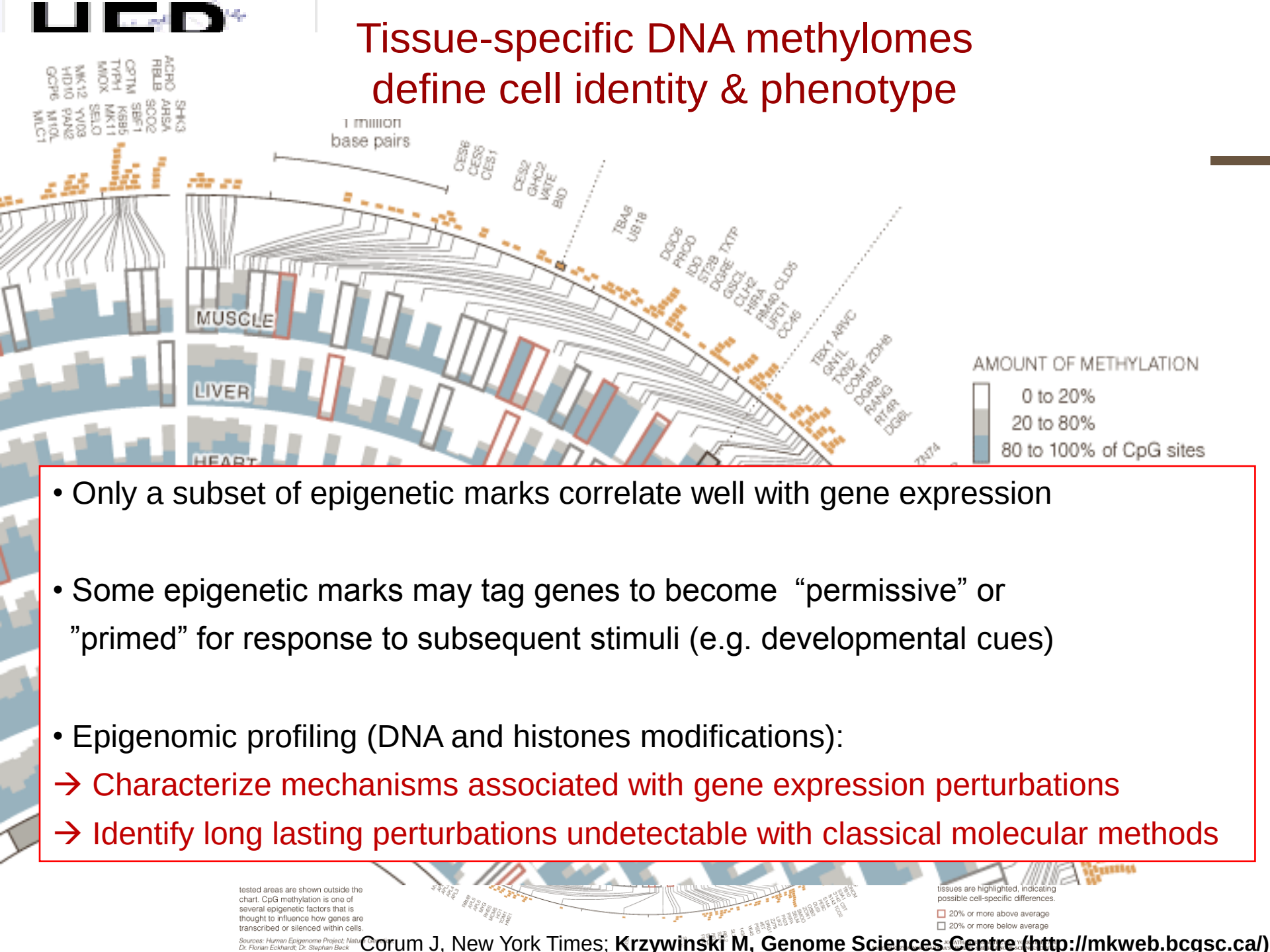
Combinations of post-translational histone modifications functionally organize the genome



Adapt. from Bhaumik et al. 2007

- 100s flavors of histone PTMs: multiple combinations
- Multiple flavours (e.g. repressive vs. active marks) form signatures that specify different states of the chromatin and functionally organise the genome.
- Dynamic and reversible, Role in long term cellular memory, Aberrant chromatin landscape in Human disease.

Tissue-specific DNA methylomes define cell identity & phenotype



- Only a subset of epigenetic marks correlate well with gene expression
- Some epigenetic marks may tag genes to become "permissive" or "primed" for response to subsequent stimuli (e.g. developmental cues)
- Epigenomic profiling (DNA and histones modifications):

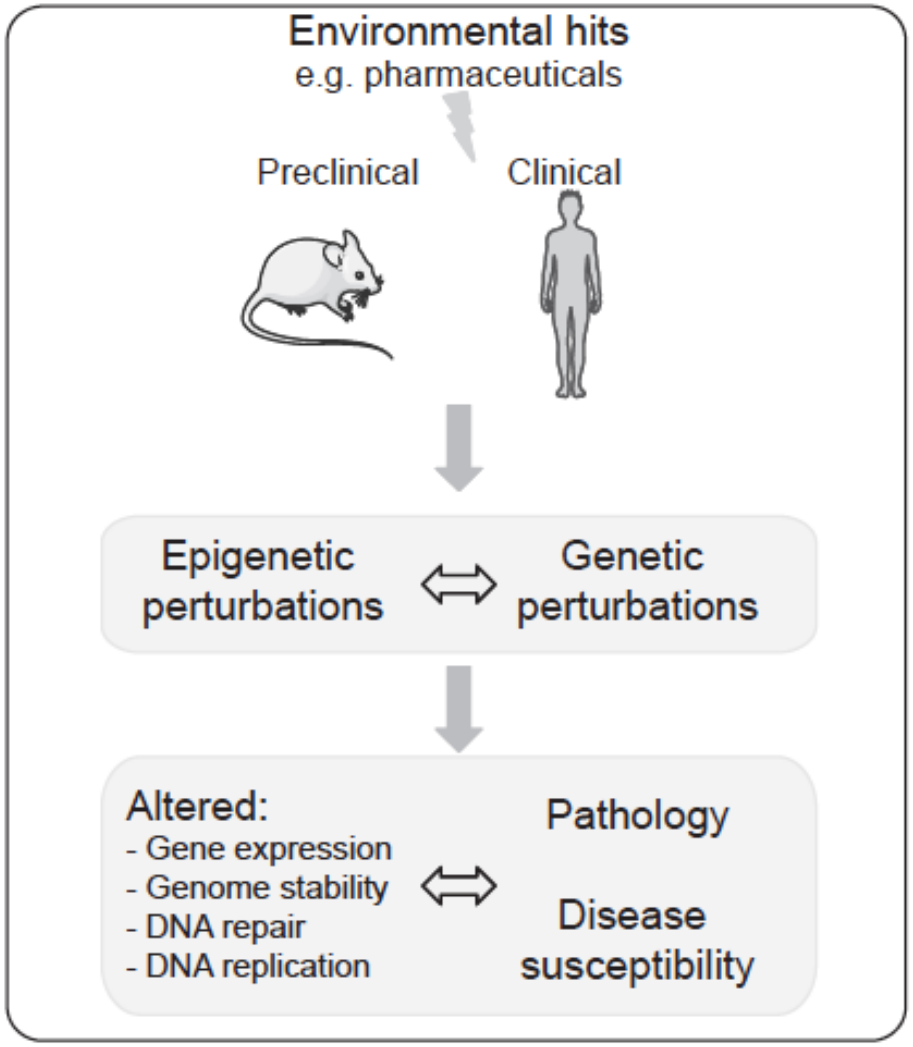
→ Characterize mechanisms associated with gene expression perturbations

→ Identify long lasting perturbations undetectable with classical molecular methods

Corum J, New York Times; **Krzywinski M, Genome Sciences Centre (http://mkweb.bcgsc.ca/)**

Environmental perturbations of (epi)genetic patterns

Impact for drug safety



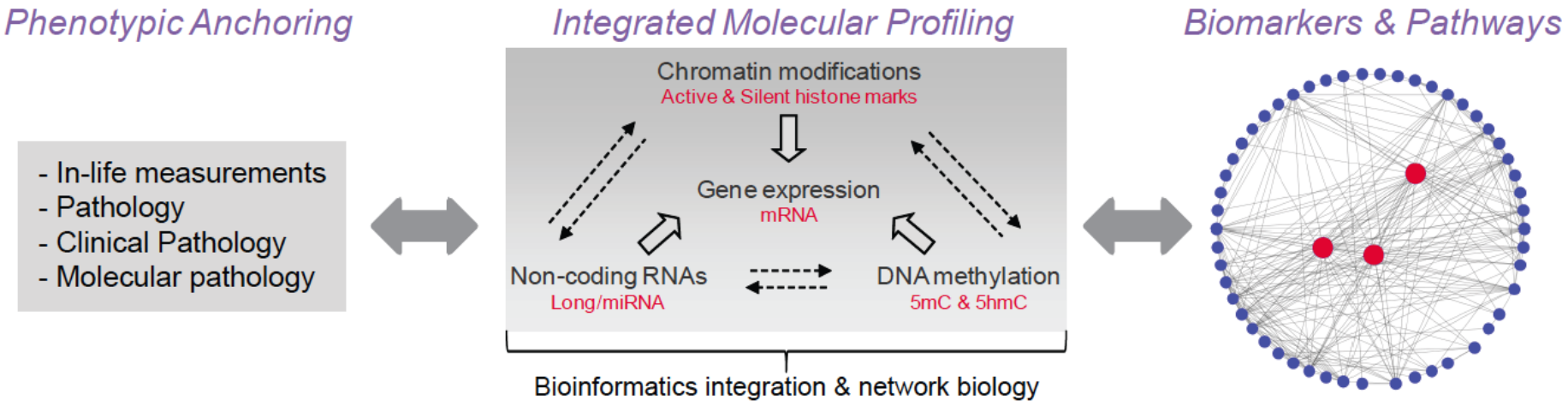
Integrated molecular profiling

Mechanistic toxicology
- Toxicity MoA / Biomarkers
- Human relevance

Predictive toxicology
- Risk management / Patient stratification
- Toxicity / Disease biomarkers

Roadmap to integrated molecular profiling in drug safety

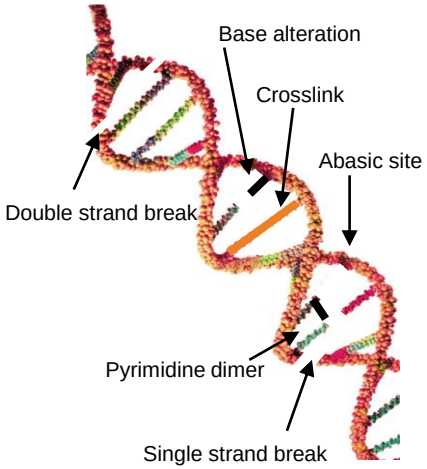
- Gain novel insight into **mechanisms** & **biomarkers** for **long-lasting** drug-induced cellular perturbations
  - Increased susceptibility to disease or toxicity (e.g. cancer)
  - Memory of prior immune stimulation or drug exposure (e.g. tolerance; chronic inflammation)
  - Aberrant developmental reprogramming & transgenerational effects (e.g. valproic acid; endocrine disruptors)



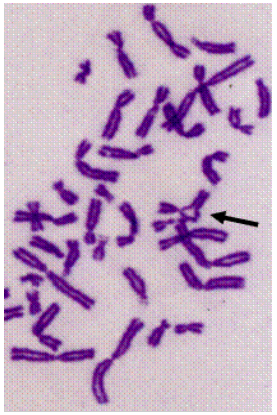
Case study: Genetic Toxicity & Carcinogenesis Testing

Pharma R&D strategy: Why focus on Non-Genotoxic Carcinogenesis (**NGC**)?

DNA damage

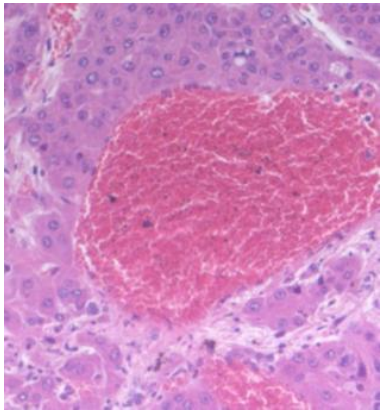


Chromosome damage
Point mutations



Carcinogenesis
Multistage process

Cancer



Genotoxicity testing

- **Required for IND**
- **Genetox battery**
- **Cost: $60K/cmpd**
- **Time: 1-3 month**

Non-genotoxic mechanisms

- Proliferation
  - Regenerative hyperplasia
  - Hormone changes
- Nuclear hormone receptor activation
- Epigenetics
  - miRNA
  - Methylation

Carcinogenicity testing

- **Required for NDA**
- **2-year bioassay**
- **Cost:$3M/cmpd**
- **Time: 3 years**

- **No sufficiently accurate or well-validated short-term assays to identify NGC**
- **Need early mechanism-based biomarkers for the design of more predictive tests & improved cancer risk assessment**

Ellinger-Ziegelbauer H *et al*. 2009. *Toxicol Lett*. 2009

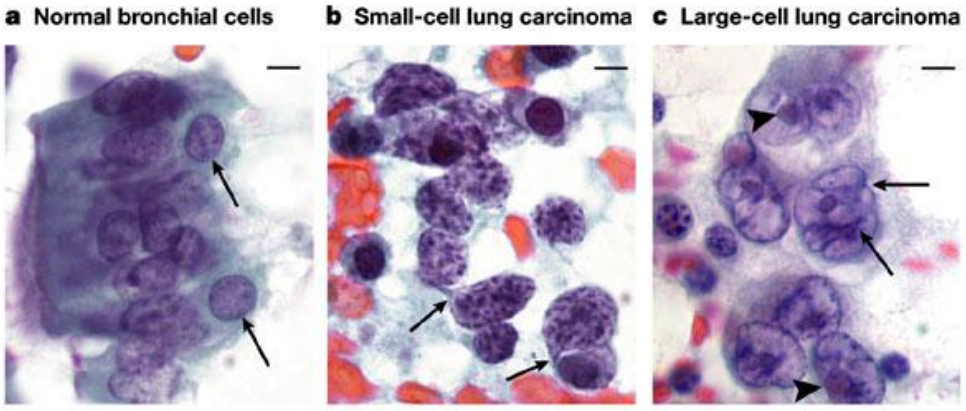
Epigenetic perturbations in tumor cells

- **DNA methylation:** promoter / gene body DNAme perturbations → oncogene (hypomethylation) and tumor supressor gene (hypermethylation) alterations + genomic instability due to global hypomethylation

- **Histone modifications:** chromatin modifier (e.g. MLL, EZH2) perturbations → altered chromatin landscape → permanent changes in gene expression

- **Non-coding RNAs:** perturbations of miRNA and lncRNA expression detected in multiple cancer types → mechanistic links to (post-)transcriptional silencing of tumor suppressor genes

- **Nuclear architecture:** strong evidence for (sub)cellular remodelling in cancer cells (e.g. changes in the structure/localization of heterochromatin, nucleoli and PML bodies)

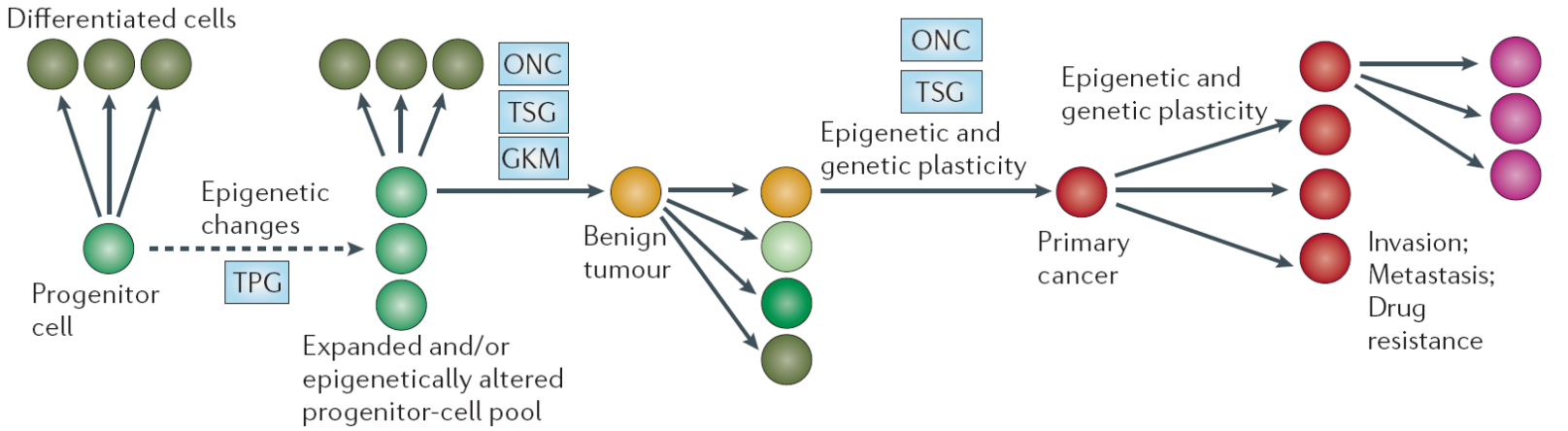


Zink et al., Nat Rev Cancer 2004

Gaining novel insights into early mechanisms/biomarkers of NGC

Early epigenetic changes may predict cancer susceptibility

Epigenetic progenitor model of cancer: Epigenetic changes may be the earliest events during NGC, their identification may help predict cancer susceptibility.

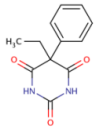


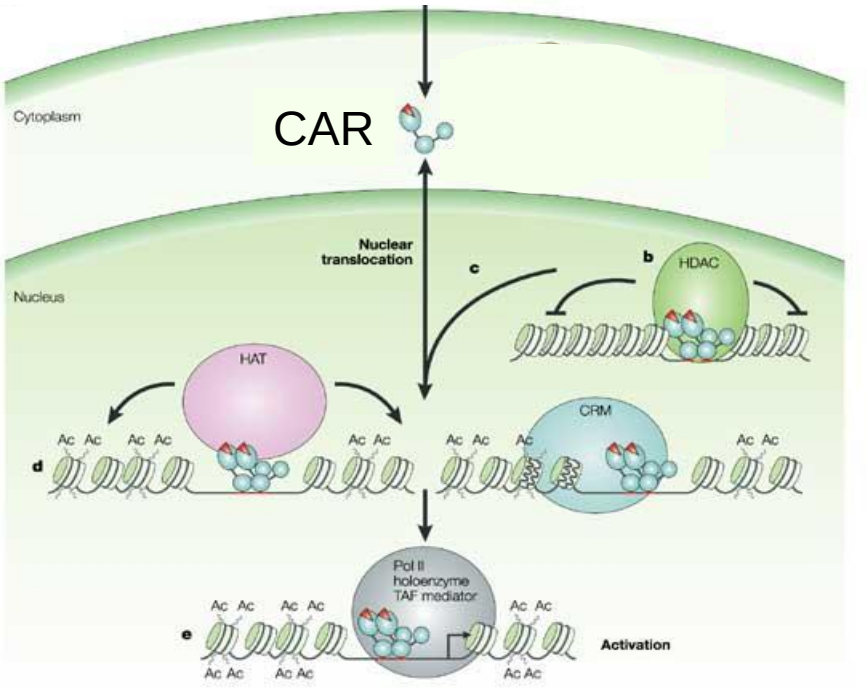
(Feinberg et al. 2006 Nat Rev Genet. 7:21-33)

Phenobarbital (PB): a well characterized rodent NGC

Investigating mechanisms of rodent NGC using integrated epigenomic profiling

Phenobarbital





Adapted from Gronemeyer et al 2004

"In B6C3F1 mice chronic PB treatment increases hepatic cancer incidences from spontaneous 29% to 100% in a 12 month *in vivo study (Becker, Cancer Res 1982)."*

2-year Rodent Bioassay

- PB induces liver tumours in rodents. No evidence for genotoxicity: Model for NGC
- Activation of the Constitutive Androstane Receptor: CAR pathway. CAR KO mice do not develop liver cancer.
- Humanised CAR mice refractory to hyperplastic effects: species specificity
- No evidence for liver cancer in humans following long-term anti-epileptic therapy

Drug-induced perturbations of mouse liver transcriptome and epigenome

Kinetics & broad gene regulatory landscape in a 13 week PB study

Strain: B6C3F1
Compound: 0.05% (w/v) phenobarbital (PB)
Vehicle: drinking water
n=5 per group
Duration of treatment: 1, 3, 7, 14, 28, 57, 91 days

LC-MS exposure

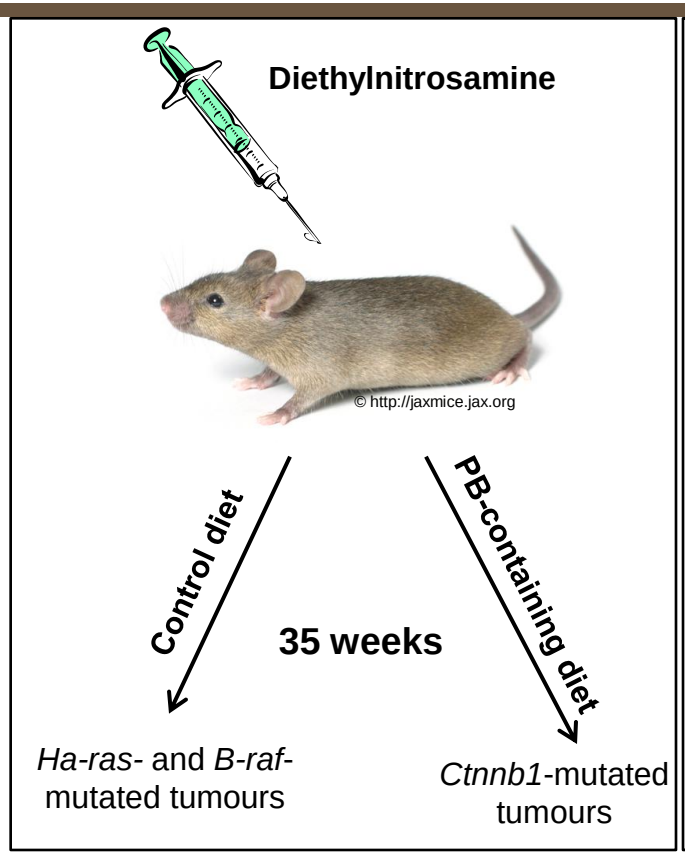
PLASMA

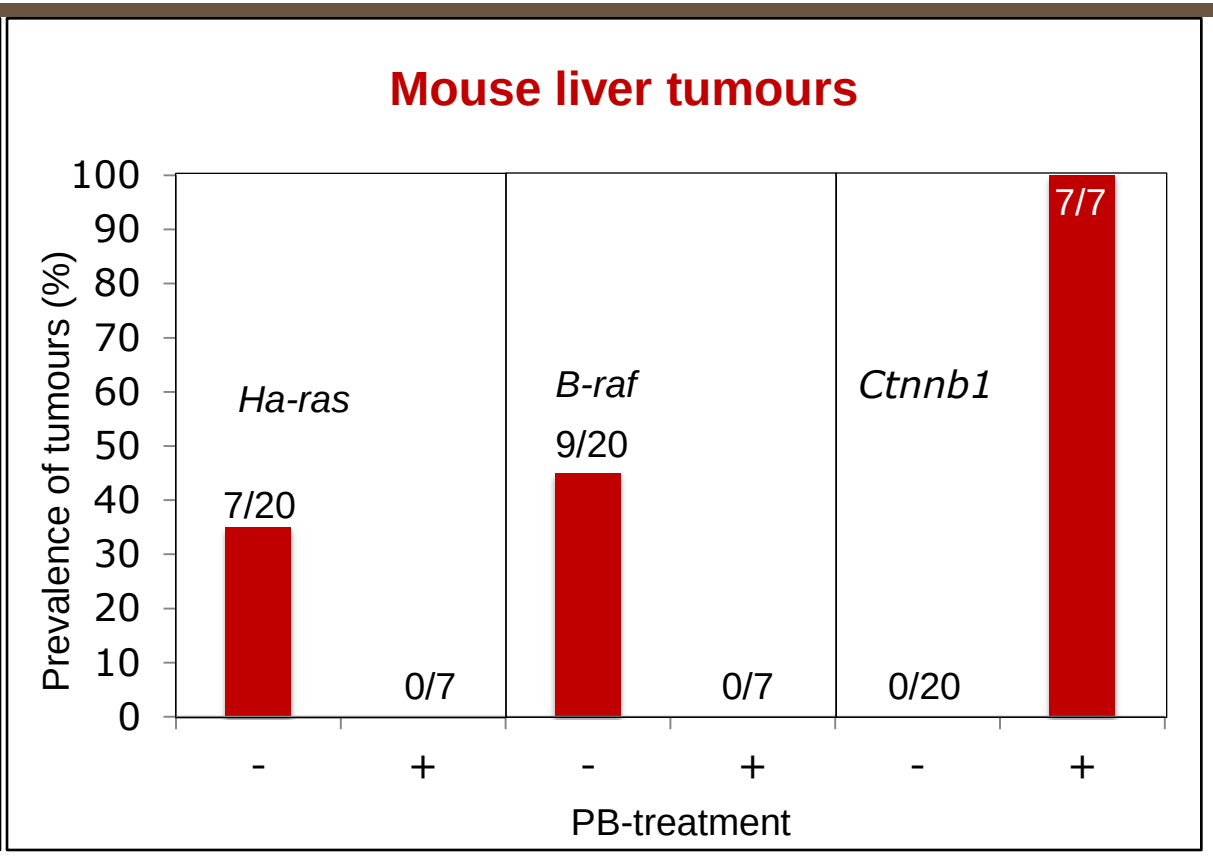
LIVER

- Gene expression
- miRNAs
- DNA methylation
- Chromatin PTMs
- Histopathology
- In situ localization

PB carcinogenesis experiment and tumour sample set

PB positively selects for tumours with activated β-Catenin

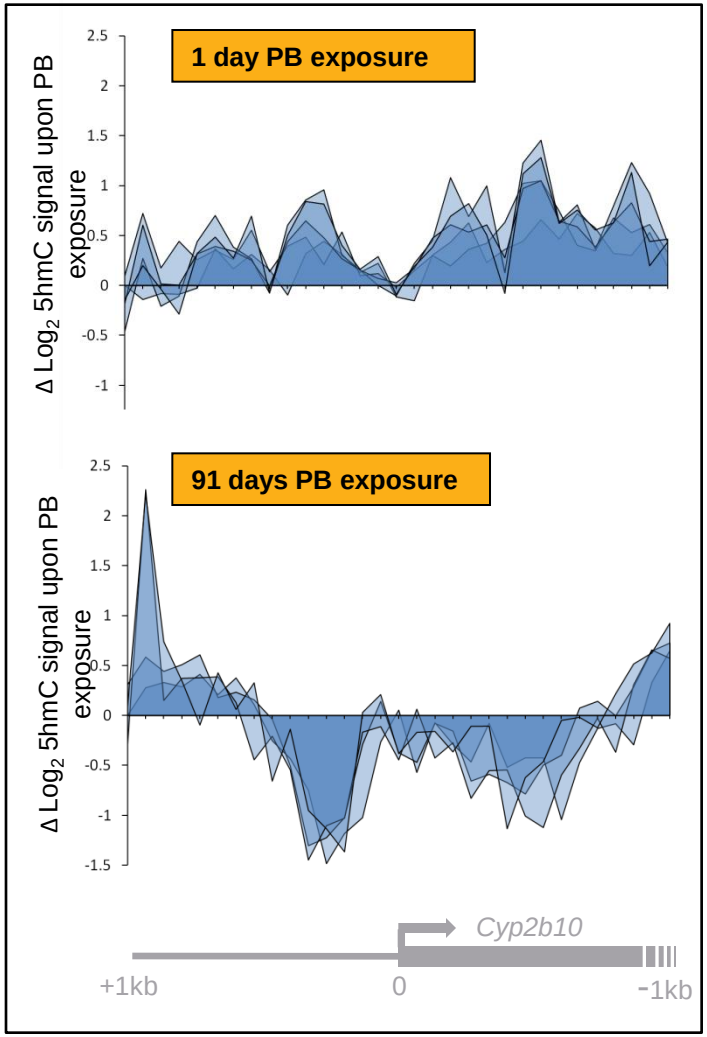


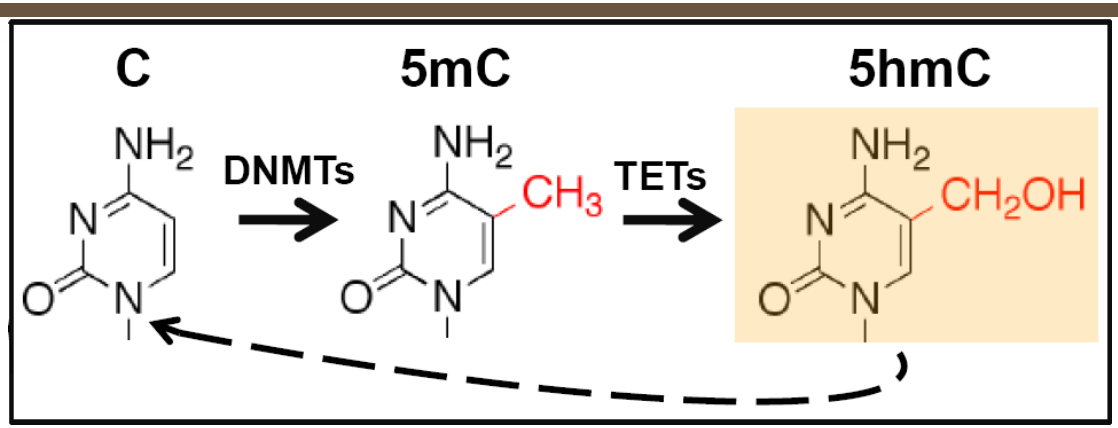


"In B6C3F1 mice chronic PB treatment increases hepatic cancer incidences from spontaneous 29% to 100% in a 12 month *in vivo study (Becker, Cancer Res 1982)."*

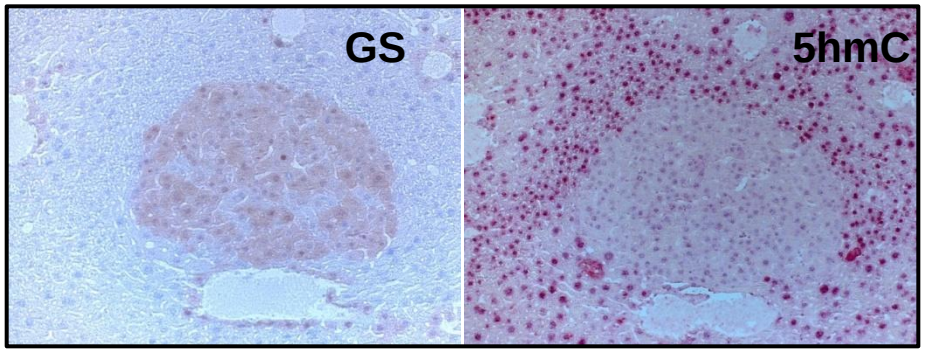
Dynamic changes in 5-hydroxymethylcytosine in liver induced by NGC

5-hmC is decreased in mouse liver tumours





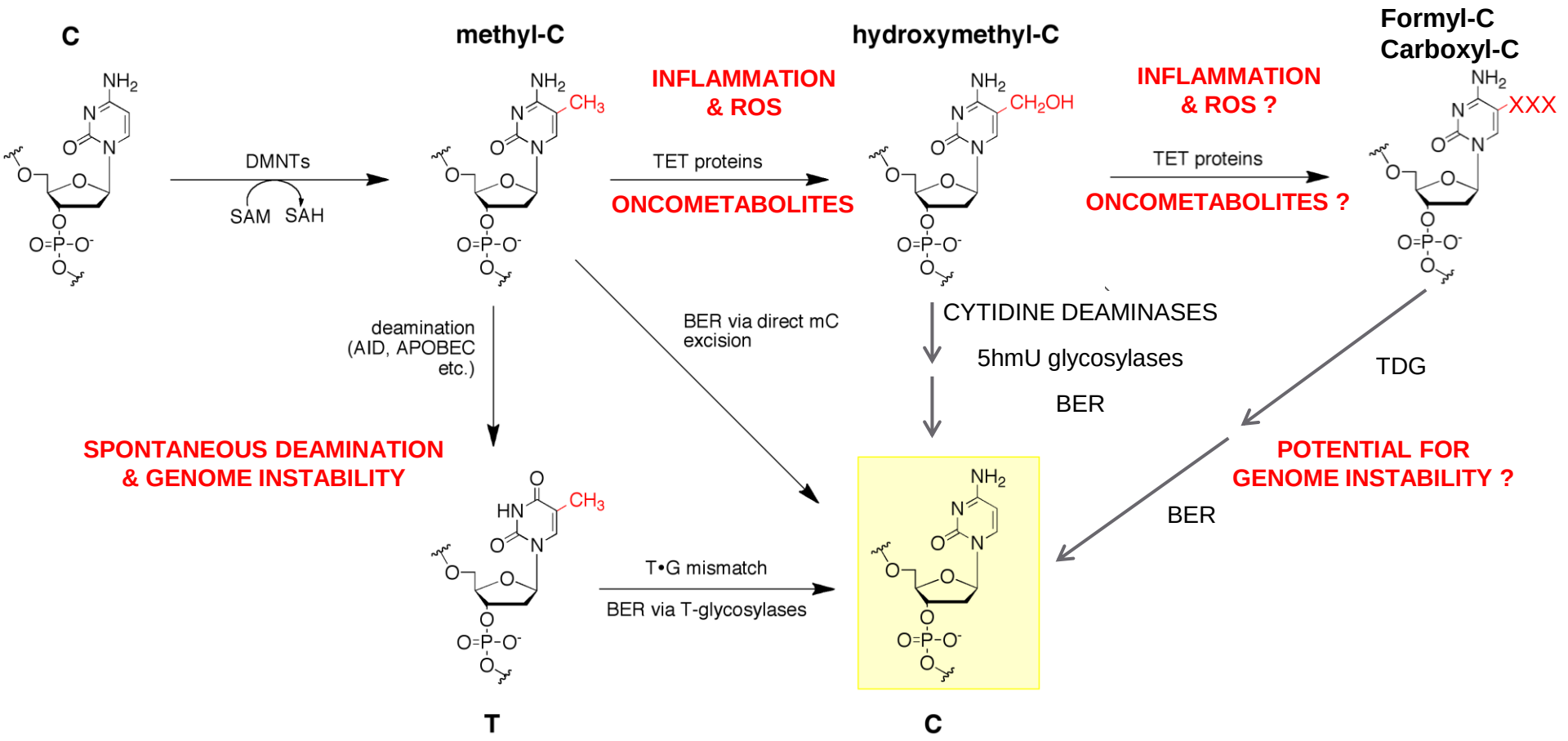
C3H mouse liver tumour (*Ctnnb1*-mutated): DEN injection + 35 weeks PB treatment



Unpublished data:
John Thomson and Richard Meehan, Univ. Edinburgh
Elif Unterberger and Michael Schwarz, Univ. Tübingen

Changes in DNA methylation potentially relevant for carcinogenesis

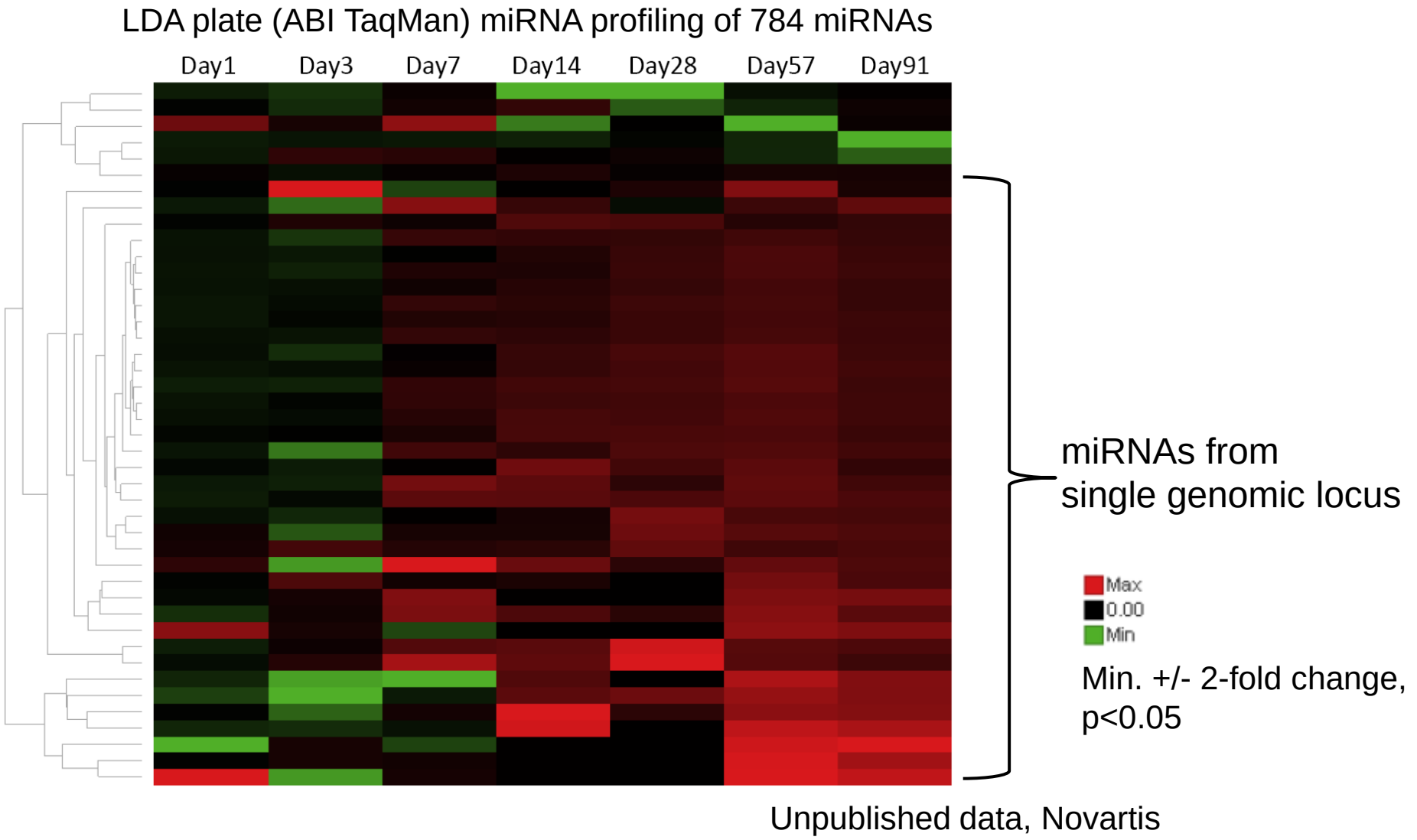
Interplay between Epigenetics and Genetics; 6th DNA base and beyond



Adapted with permission from Liang Wu, Santiago Uribe-Lewis, Adele Murrell, Cancer Research UK, Cambridge

Drug-induced perturbations of non-coding RNAs (ncRNAs)

PB induces expression of the miRNAs from a genomic cluster

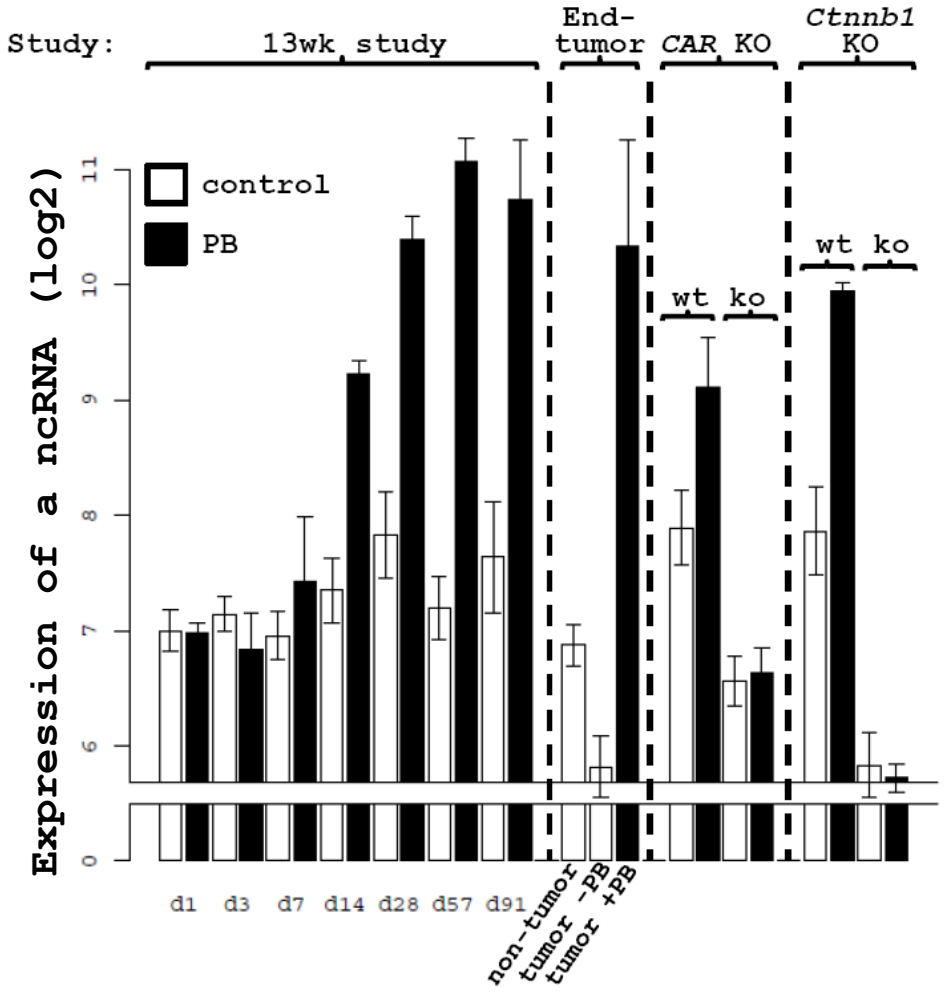


Unpublished data, Novartis

Drug-induced perturbations of non-coding RNAs (ncRNAs)

ncRNAs are overexpressed in NGC tumours and induction by PB is CAR & Ctnnb1 dependent

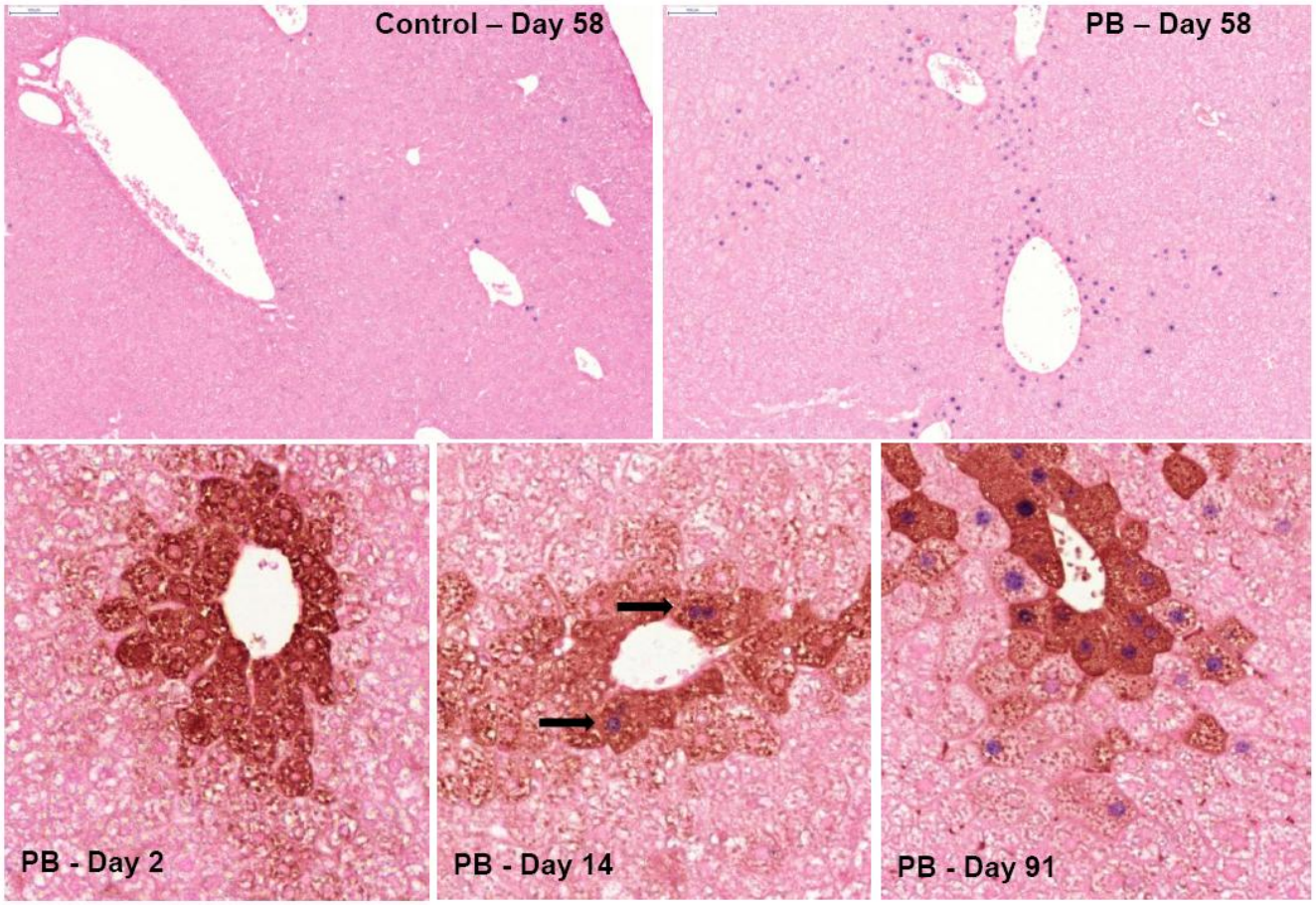
Tumour promotion by PB is *CAR* and *Ctnnb1* dependent (Yamamoto at al. 2004, Rignall et al. 2010)



Drug-induced perturbations of non-coding RNAs (ncRNAs)

ncRNA expression is induced in GS-positive hypertrophic hepatocytes

- PB-mediated liver tumour promotion may involve transcriptional activation of *Glutamine Synthetase* (*GS*) by activated β-catenin
- GS positive clones is a hallmark of tumour promotion in *Ctnnb1* mutated tumours



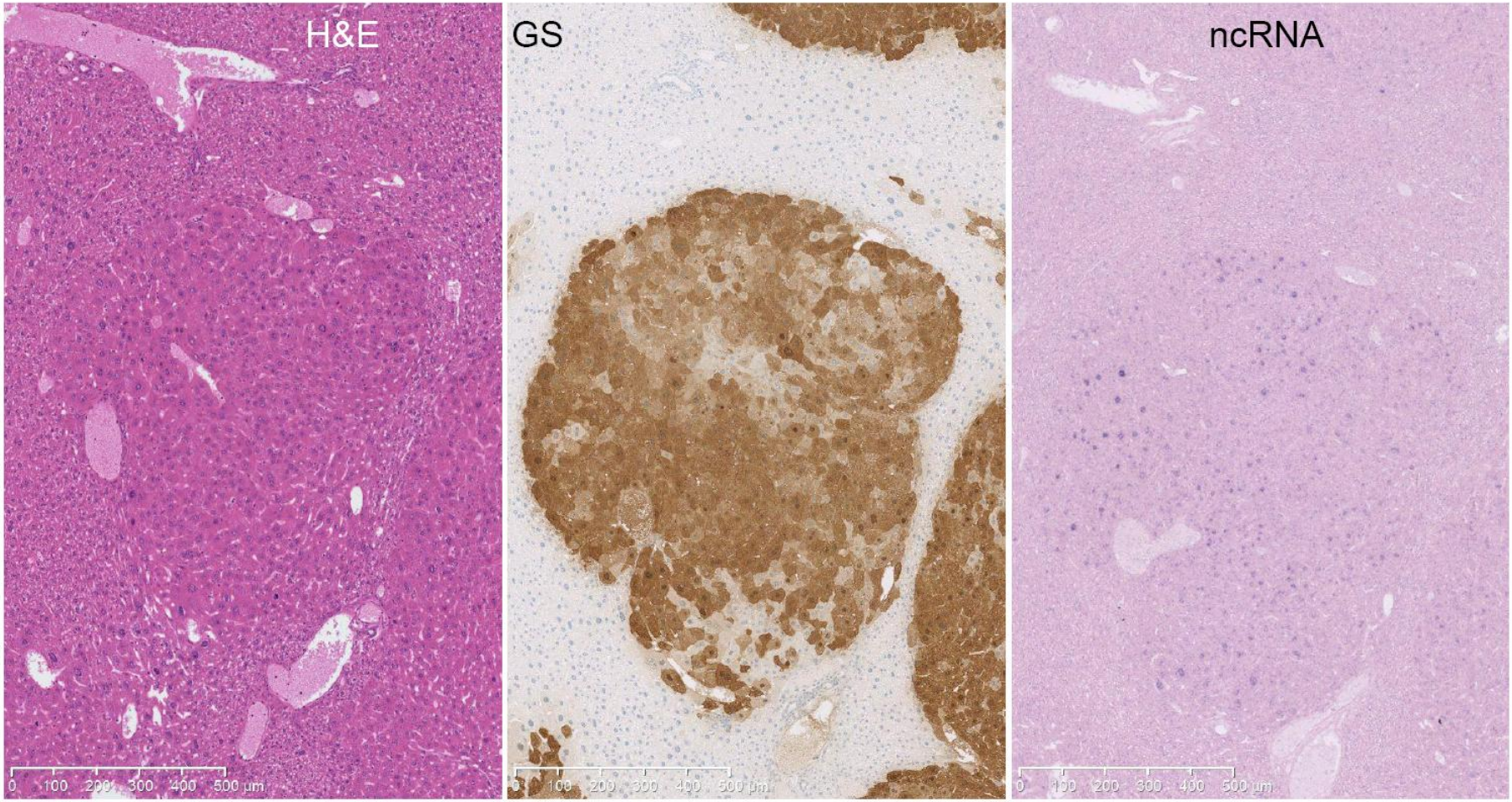
ncRNA

ncRNA
GS

Drug-induced perturbations of non-coding RNAs (ncRNAs)

ncRNA expression is increased in Glutamine Synthetase (GS) positive tumours

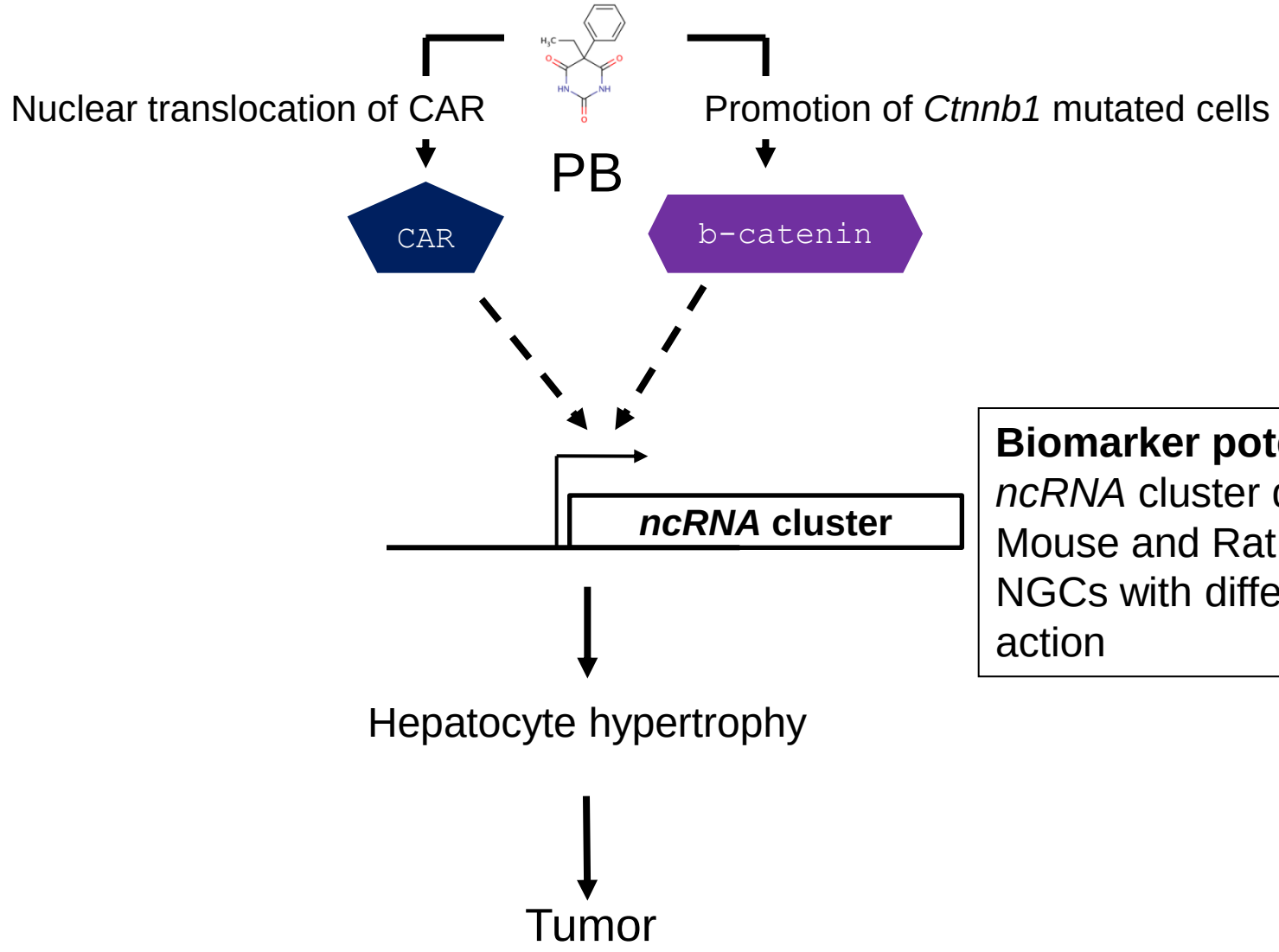
C3H mouse liver tumor: DEN injection + 35 weeks PB



Unpublished data: Elif Unterberger and Michael Schwarz, Univ. Tübingen

Drug-induced perturbations of non-coding RNAs (ncRNAs)

Model for ncRNA cluster regulation and role in tumor promotion



Biomarker potential

ncRNA cluster disregulated in Mouse and Rat by several NGCs with different modes of action

Power of consortium approach (IMI MARCAR)

Towards mechanistic understanding and biomarker consolidation of NGC

Consolidation of early NGC biomarkers:

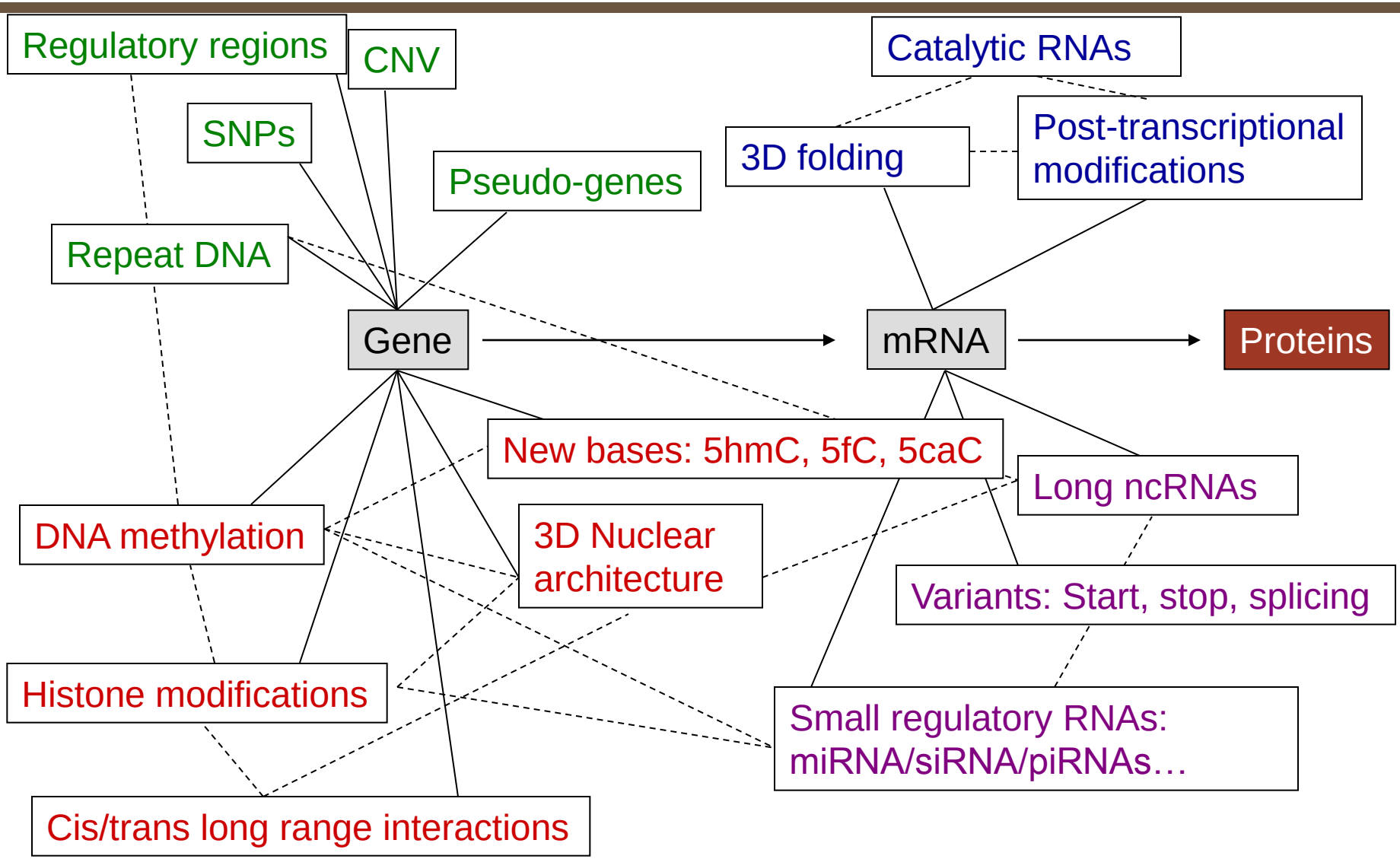
- **On epigenetic, transcriptional and genetic level**
- **Across a wide panel of drugs (NGC, GC, non carcinogens)**
- **Across different species/strains (Mouse, Rat)**
- **Via mechanistic studies (humanized/KO CAR/PXR etc)**
- **Across different stages (short-term/long-term treatment, tumours)**
- **Via localization studies**

marcar
towards novel biomarkers
for cancer risk assessment

- **Identification of reliable early biomarkers for predicting later tumour formation→ improved scientific basis for cancer risk assessment and regulatory decision-making in preclinical development of new compounds**
- **Classification / Stratification of NGC-promoted and spontaneous tumours**

2012 – Integrated Molecular View

Multiple levels of regulation = Multiple new safety biomarkers?



Tool or assay

SWOT analysis: Name of the assay

Strengths

-Provides a comprehensive view of modifications/changes that may proceed events that lead to phenotypic change/toxicity

-Rich biomarker resource

-Potential for assessment of species-specific responses in vivo and in vitro

Weaknesses

-Very large and complex datasets, need for customized bioinformatic tools

-Huge number of changes observed - many possible mechanisms/outcomes

-High probability for nonspecific or irrelevant responses

Opportunities

-Biomarkers for use in acute ot chronic toxicity studies

-Early prediction of genotoxic risk, inform or replace 2-year bioassay

-Drug-induced vs. non-drug-induced tumors

Threats

- Tremendous complexity with possiblity of compond-specific effects rather than class-effects amenable to generalized biomarkers generation

- Translation of findings from preclinical models/results to human

Acknowledgments

NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH

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