ALLELE CONTRIBUTION TO Mecp2 EXPRESSION IN MOUSE BRAIN DEVELOPMENT: AN *ESCAPING* MATTER?

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POSTER #53

BACKGROUND

WHAT

- In humans, alterations in dosage of the X-linked-MECP2 gene are associated with neurological disorders with **a sex-biased susceptibility**
- In mice, **delay in the inactivation kinetics** of Mecp2 in normal embryos has been reported [1] and some evidence suggests that Mecp2 may be a **facultative escapee from XCI**, in a lineage specific manner (i.e., trophoblast giant cells & neural progenitors) [2, 3]
- The Xi-linked allele of Mecp2 is **embedded in TAD-like subdomains** [4]

OPEN QUESTIONS

- Allelic expression of Mecp2 in presence of rXCI has been limited to transcripts (nascent RNA FISH or SNPs-based RNA-seq) >> **WHAT ABOUT PROTEINS?**
- The silencing pattern of Mecp2 over tissues and developmental stages is still unexplored >> **HOW TO PERFORME *in vivo* LONGITUDINAL STUDIES?**
- Mecp2 biallelic cells may exert a neuroprotective effect [6] >> **ARE THERE ANY BIOMARKERS TO IDENTIFY THEM IN THE BRAIN?**

METHODS

HOW?

- Generation of a new dual color Mecp2-reporter transgenic mouse (*under a physiological regulation of XCI*)
- Qualitative and quantitative FACS analyses of the allelic expression of Mecp2 at the protein level and single cell resolution (*during mouse neurogenesis*)
- Imaging of fresh brain biopsies (*unfixed samples*)
- Cell sorting
- RNA extraction from sorted cells and qRT-PCR

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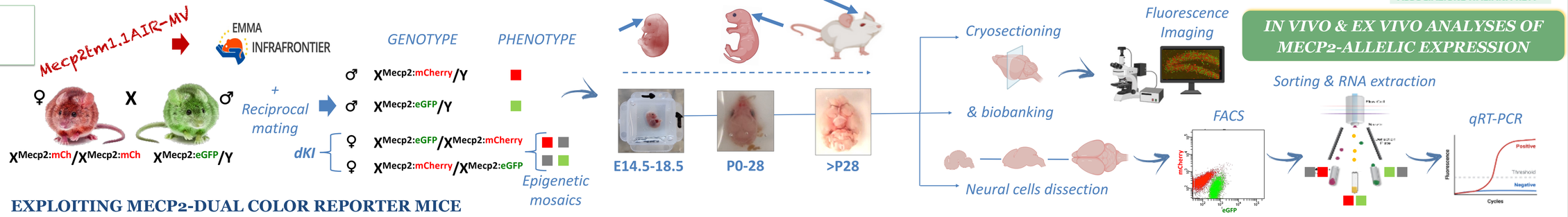
WORKFLOW

Gap of Knowledge
How extensive is Mecp2 escaping from the XCI in the mouse BRAIN?



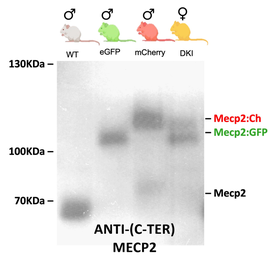
EXPLOITING MECP2-DUAL COLOR REPORTER MICE

Mecp2:mCherry (Mecp2tm1.1AIR-MV) mice generated at the IGB-CNR and crossed with Mecp2:eGFP mice (Mecp2tm3.1Bird/J [5], kindly provided by dr. Jim Selfridge) produce dKI females. Here, both Mecp2 alleles are tagged with different fluorophores, thus allowing to distinguish their active or inactive state at the cellular level, under physiological XCI conditions. Both Mecp2 transgenes are expressed under the endogenous regulative sequences and obey to XCI. 3'UTR and splicing isoforms are all preserved. Males are used as controls.

RESULTS

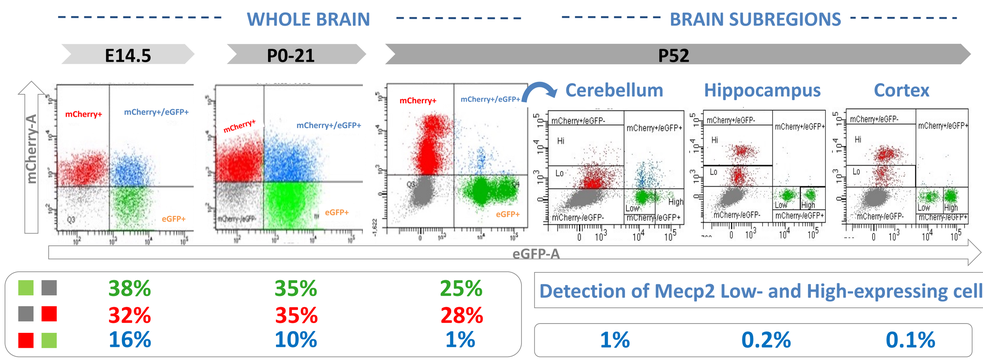
FACS and CONFOCAL MICROSCOPY DETECT RARE MECP2-BIALLELIC CELLS WITHIN THE MOSAIC

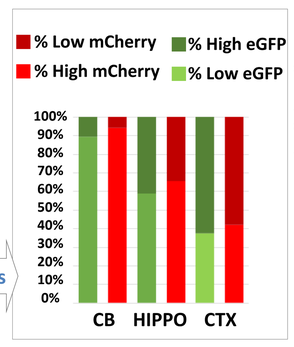
Recombinant Mecp2:reporter proteins retain the expression levels and the nuclear localization of the endogenous one.

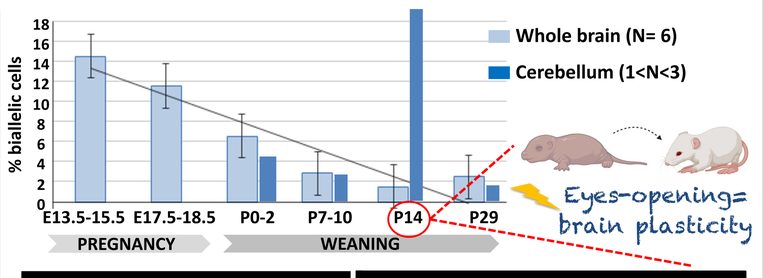


Heterozygous dKI females are an epigenetic mosaic of cells that express alternatively one recombinant protein, whereas in hemizygous males all cells are mCherry+ or eGFP+.

However, fluorescence enables phenotyping to the single cell resolution and reveals FOR THE FIRST TIME **WHERE** and **WHEN** Mecp2 PROTEIN is expressed bi-allelically



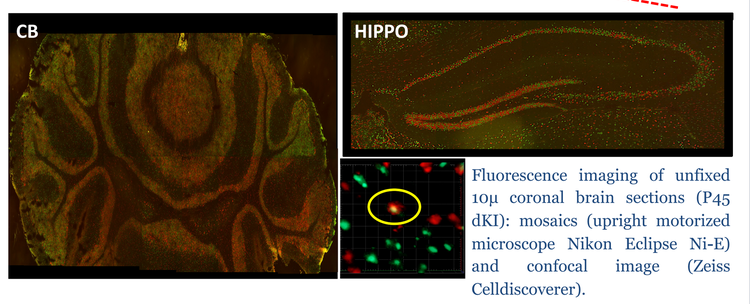




1. QUANTITATIVE & QUALITATIVE ANALYSES ALONG BRAIN DEVELOPMENT

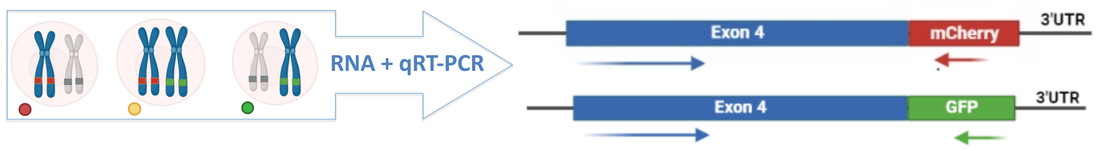
2. IN VIVO IMAGING IN ADULT BRAIN SUBREGIONS

No need for invasive techniques of perfusion and to fix/stain specimens



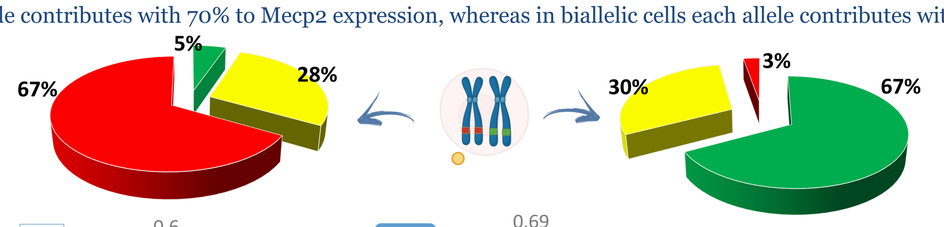
Fluorescence imaging of unfixed 10µ coronal brain sections (P45 dKI): mosaics (upright motorized microscope Nikon Eclipse Ni-E) and confocal image (Zeiss Celldiscoverer).

3. MOLECULAR INSPECTION OF SORTED CELLS



EXPRESSION ANALYSIS IN SORTED CELLS

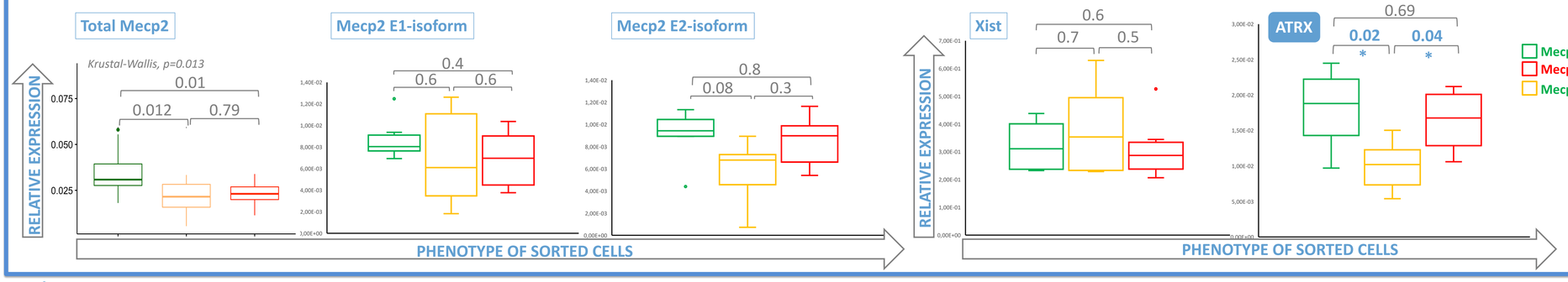
In monoallelic cells the active allele contributes with 70% to Mecp2 expression, whereas in biallelic cells each allele contributes with 30%. Thus, in biallelic cells a **dampening** of Mecp2 transcription occurs.



Neither Mecp2 nor Xist expression are useful to distinguish mono from biallelic cells.

Instead, ATRX transcript levels are significantly lower in biallelic cells (Wilcox test).

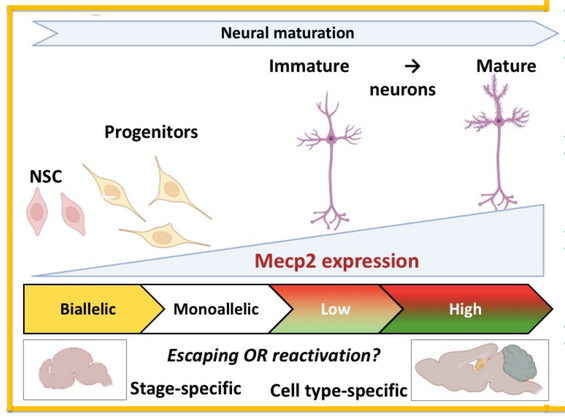
Could ATRX be used as a biomarker in WT mice?



CONCLUSIONS

Mecp2 biallelism:

- is spatially and developmentally regulated
- is prevalent upon embryonic development
- in post-natal life, persists in the cerebellum and hippocampus
- is associated with allelic dampening of Mecp2 transcription
- precedes the appearance of Mecp2 low- and high-expressing cells
- is associated with ATRX downregulation



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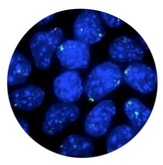

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IN MEMORY OF MAURIZIO D'ESPOSITO, MICHELE D'URSO and ALL GIRLS WITH RETT SYNDROME WHO LOST THEIR LIVES