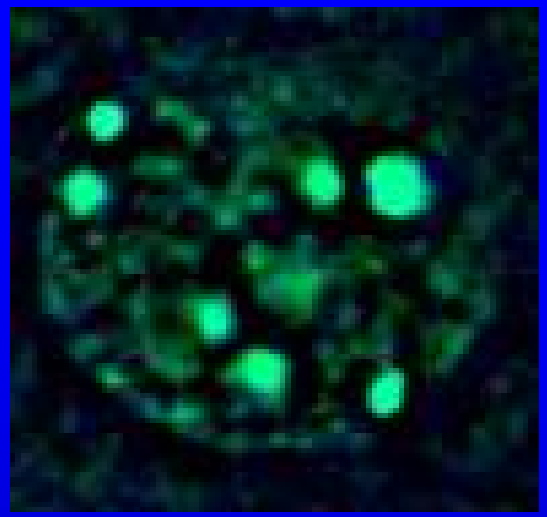
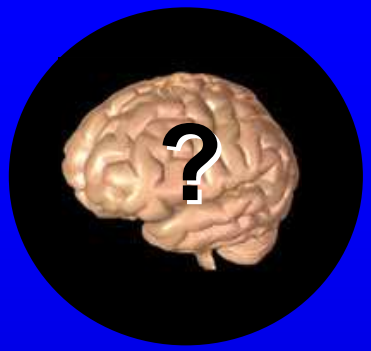
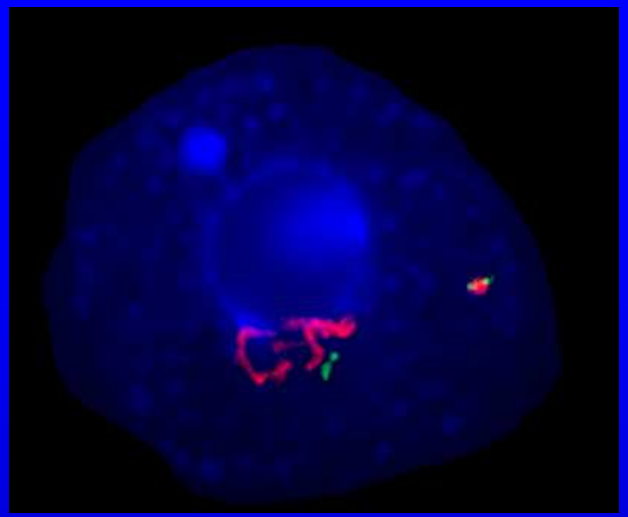


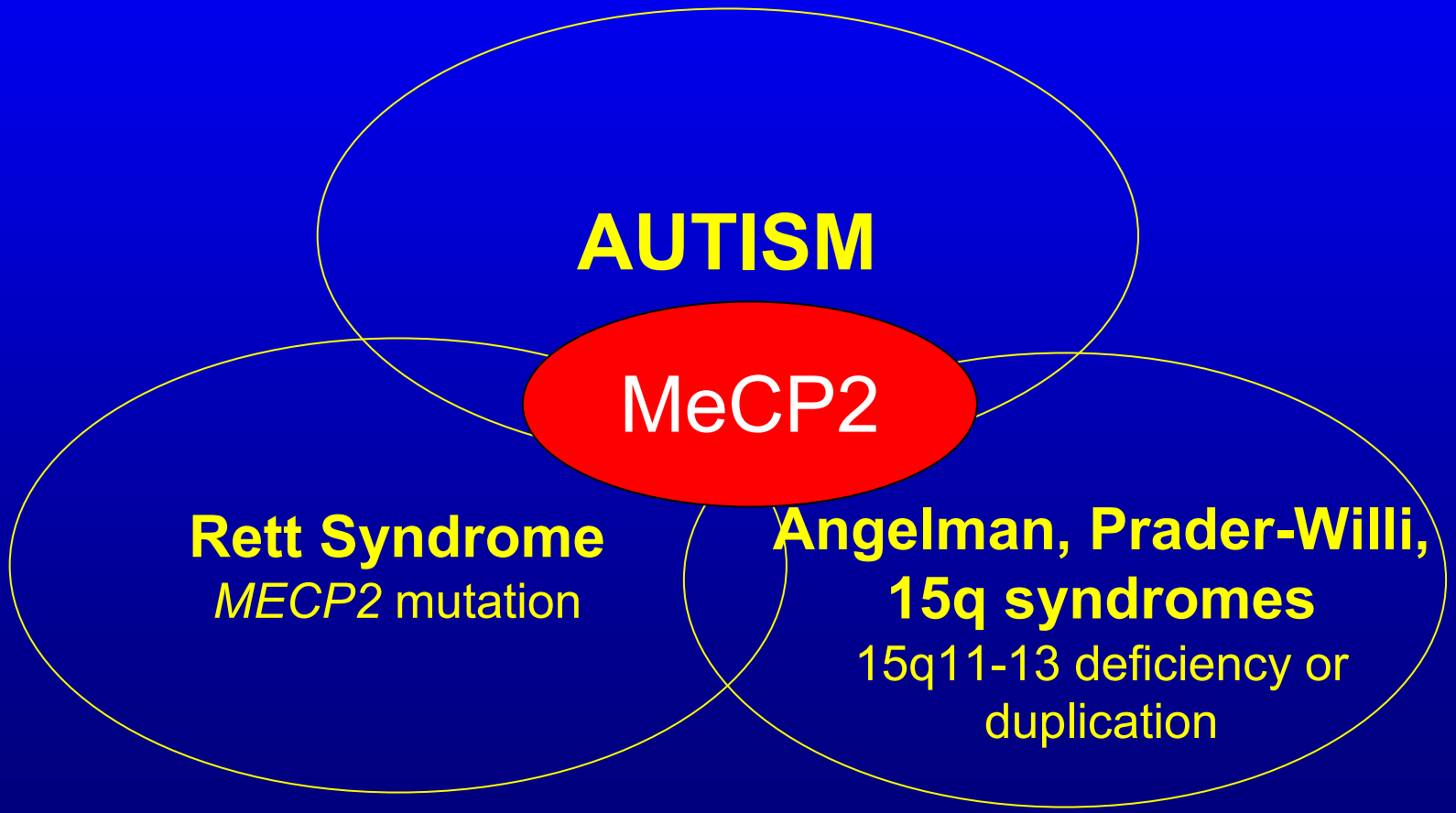




The Evolving Role of MeCP2 in Rett Syndrome and Autism

Janine LaSalle, Ph.D
Medical Microbiology and Immunology
Rowe Program in Human Genetics
UC Davis School of Medicine

Epigenetics and Autism
What's the connection?



Autism

- Complex developmental disorder that usually appears 1-3 yr
- Deficits in social interaction and communication
- Repetitive stereotyped behavior and abnormal preoccupations
- Complex genetic etiology influenced by environmental factors

Rett Syndrome (RTT)

- RTT is the only one of the five Pervasive Developmental Disorders with a known genetic cause
- X-linked dominant, ~80% *MECP2* mutation
- Neurodevelopmental regression around 6 to 18 months of age
- Mental retardation, ataxia, loss of purposeful hand movements, loss of vocalization skills, seizures

Angelman and Prader-willi syndromes

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Imprinted disorders caused by 15q11-13 deletions or deficiency

AS: Maternal 15q11-13 deletion, paternal disomy, maternal UBE3A mutation, imprinting defects

PWS: Paternal 15q11-13 deletion, maternal disomy, imprinting defects

QuickTime™ and a
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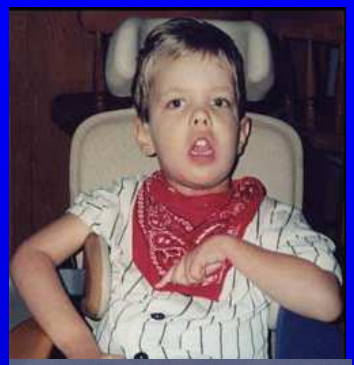
are needed to see this picture.

15q duplication syndrome isodicentric chromosome 15



- Cytogenetic abnormality of proximal 15q
  - interstitial duplication or idic 15
- Hypotonia, small stature
- Autistic features, anxiety, seizures
- Defects in sensory processing, language, and attention

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

idic 15

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

interstitial duplication

Overlap between autism, Rett and 15q11-13 syndromes

Phenotypic overlap

- stereotypic behaviors
- language impairments
- seizures (RTT, AS, 20-30% autism)
- delayed postnatal onset, regression (RTT and autism)
- mental retardation (RTT, AS, ~80% autism)
- sleep abnormalities

Evidence for genetic overlap

- *MECP2* mutations in rare cases of autism
- *MECP2* mutations in rare cases with AS phenotype
- 15q11-13 maternal duplications in 1-3% of autism
- 15q11-13 rearrangements in 3 RTT cases
- linkage to 15q11-13 loci in autism families

Evidence for epigenetic overlap between autism, RTT, and 15q

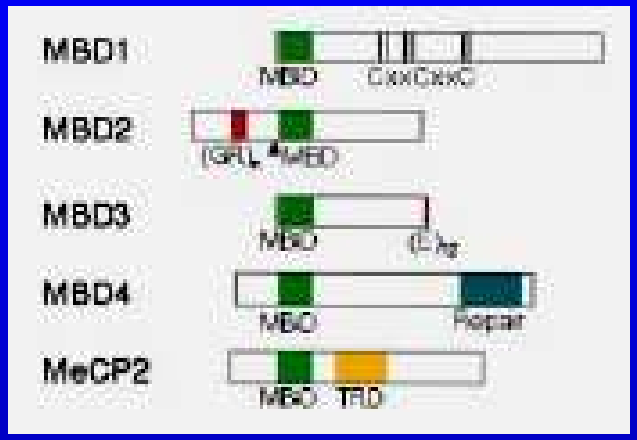
- MeCP2 expression is significantly reduced in 79% of autism post-mortem brain samples
- Methylation of the *MECP2* promoter correlates with reduced expression in male autism brain samples
- GABRB3 expression (15q11-13) is significantly reduced in 56% of autism post-mortem brain samples
- Biallelic expression levels of GABRB3 are epigenetically dysregulated in Rett and autism postmortem brain
- Homologous pairing of 15q11-13 in mature neurons is deficient in RTT, autism, and AS

Ravi Nagarajan
GGG student

Amber Hogart
GGG student

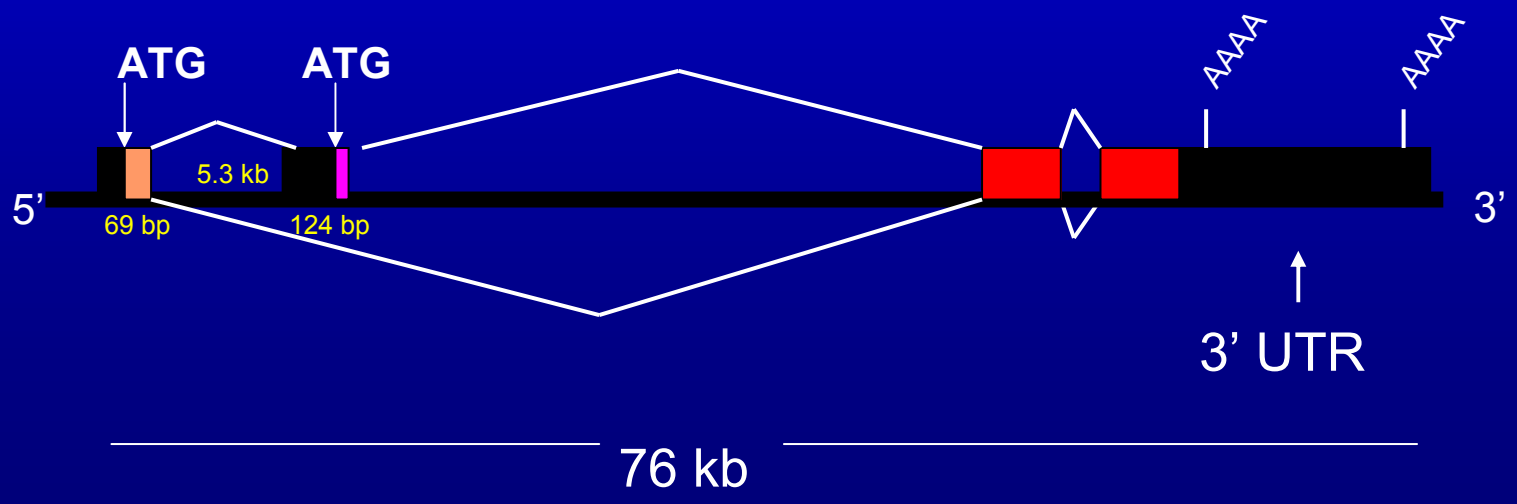
Karen Thatcher
GGG student

Methyl CpG Binding Protein 2

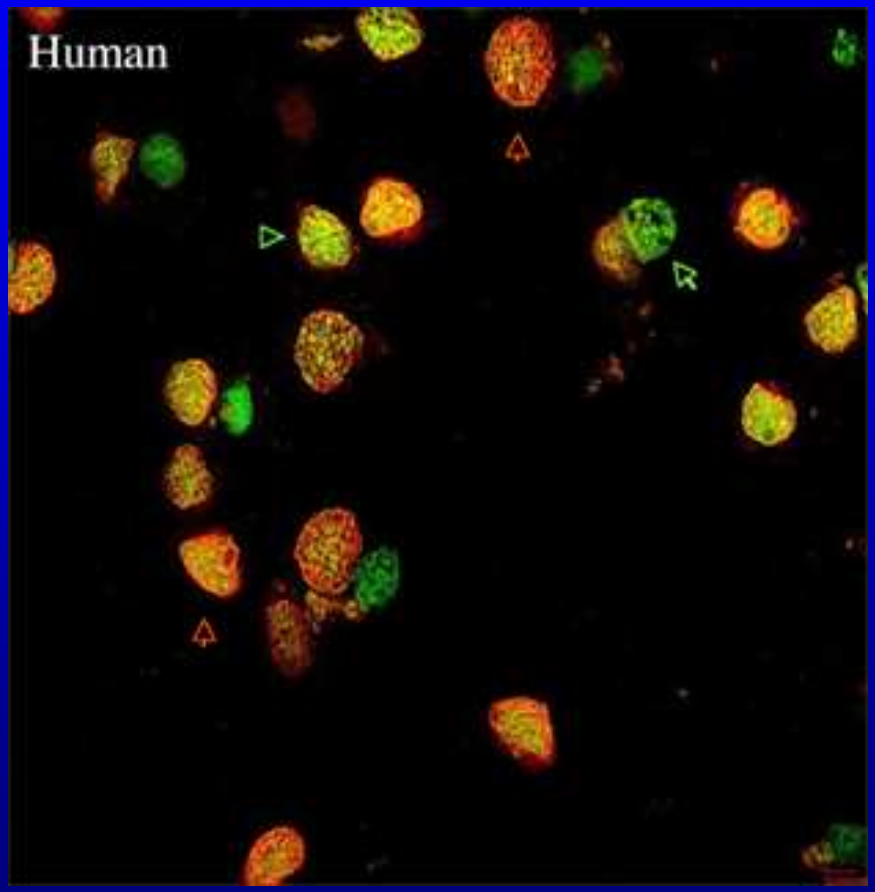


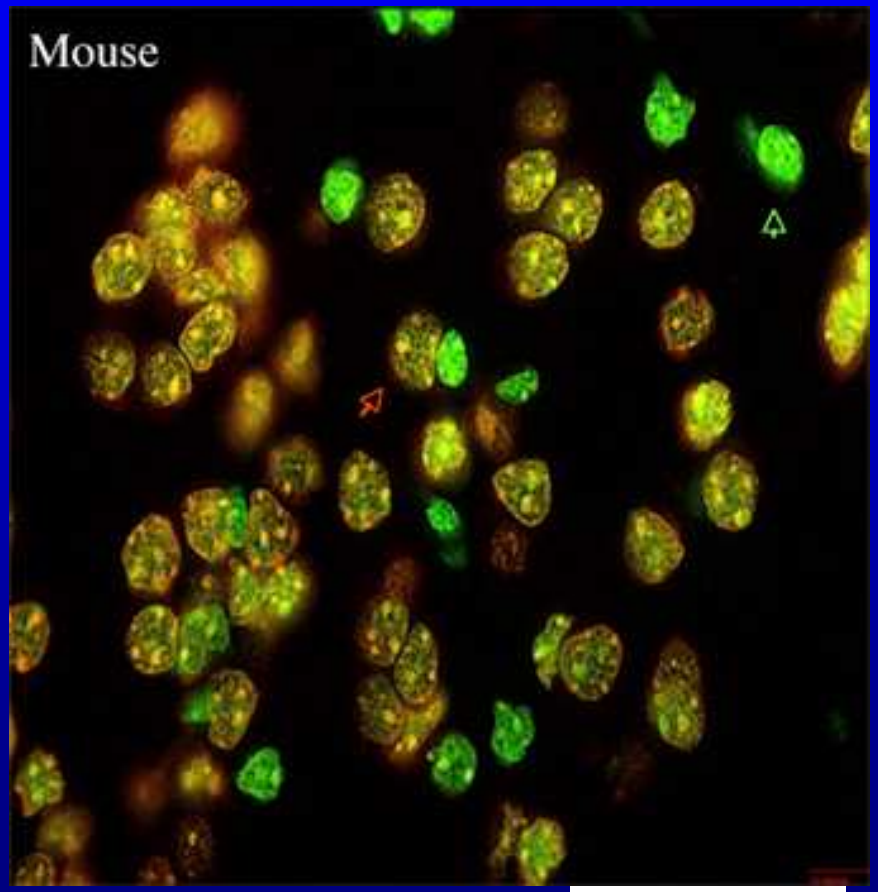
(Bird and Wolffe, *Cell* 1999)

- Binds methylated CpG dinucleotides
- Transcriptional repressor
- Xq28 (X chromosome inactivation)
- Complex developmental regulation



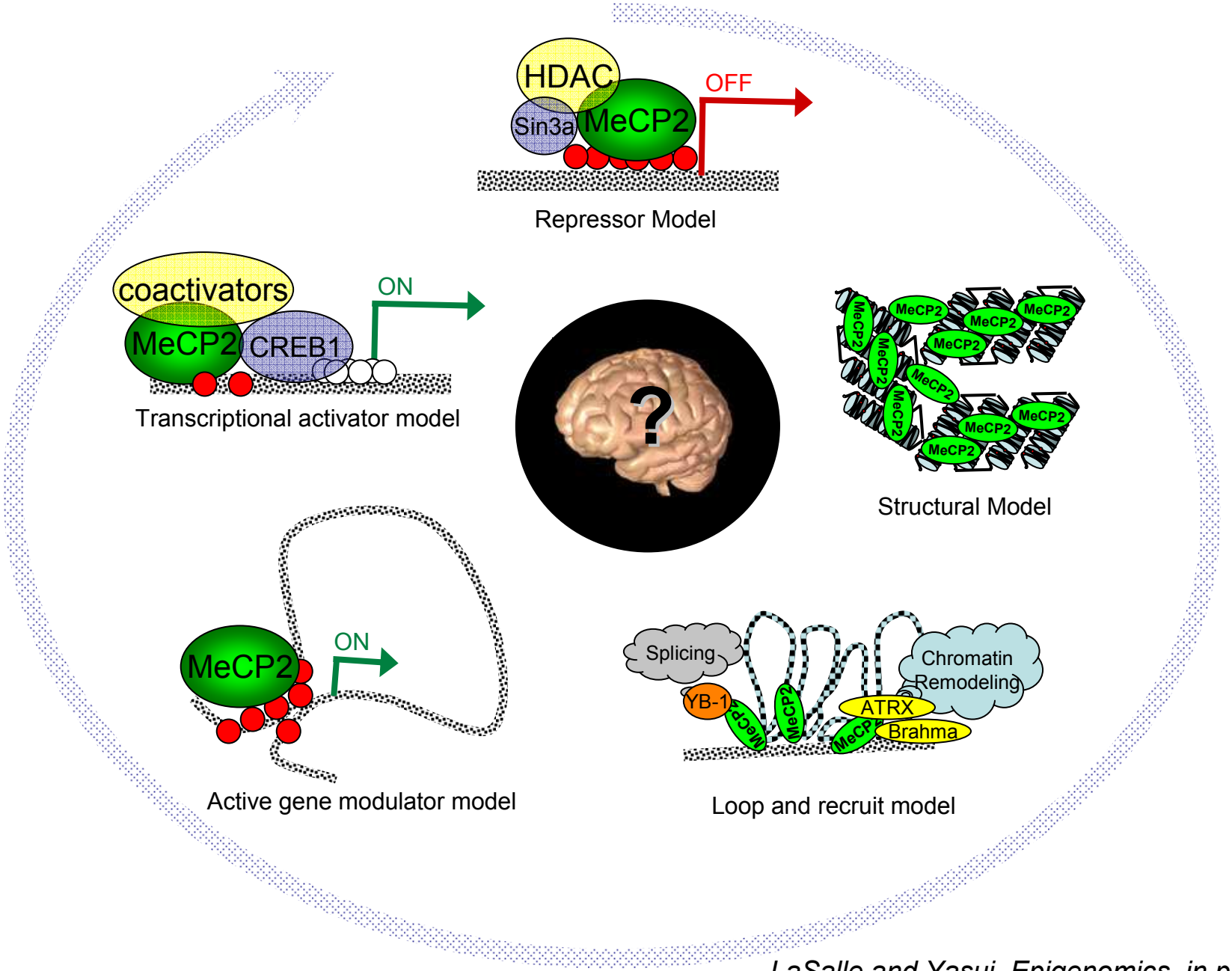
MeCP2 is a marker for mature neurons in the post-natal mammalian brain





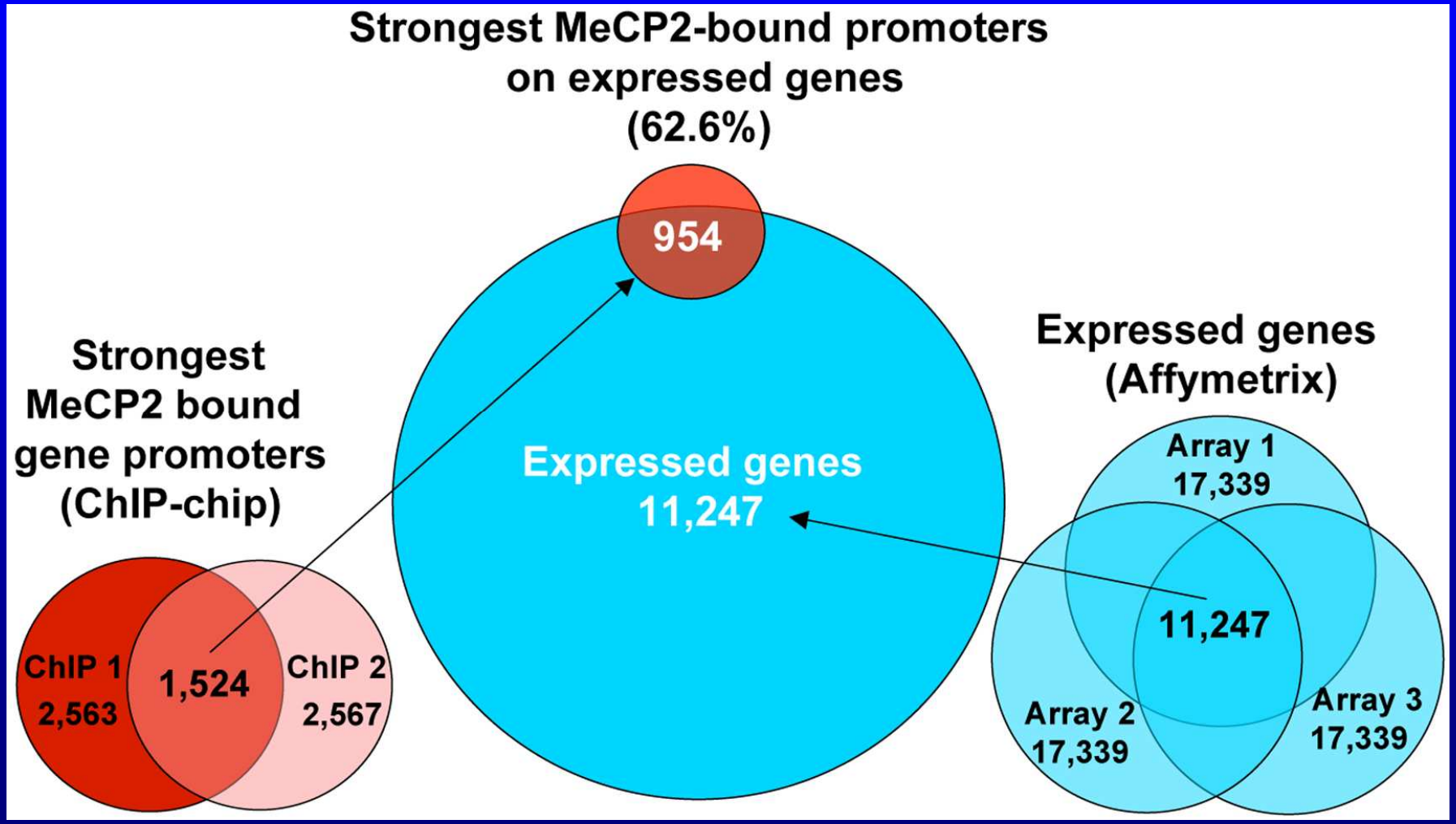
Sytox Green
Anti-MeCP2

Evolving models of MeCP2 come full circle



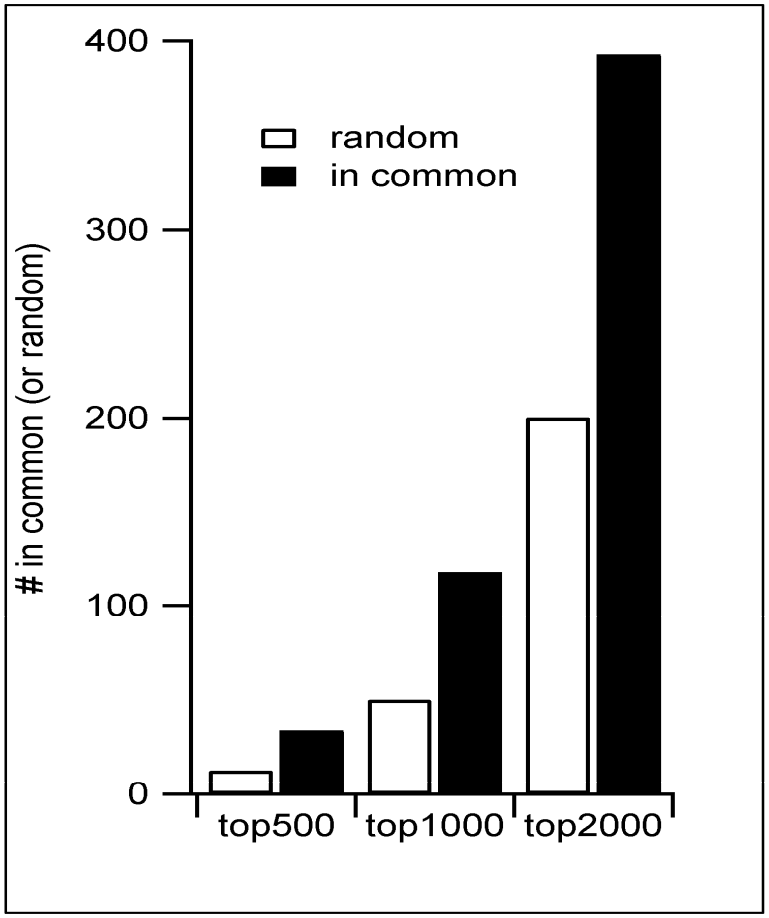
LaSalle and Yasui, Epigenomics, in press

The majority of MeCP2 bound promoters are on expressed genes



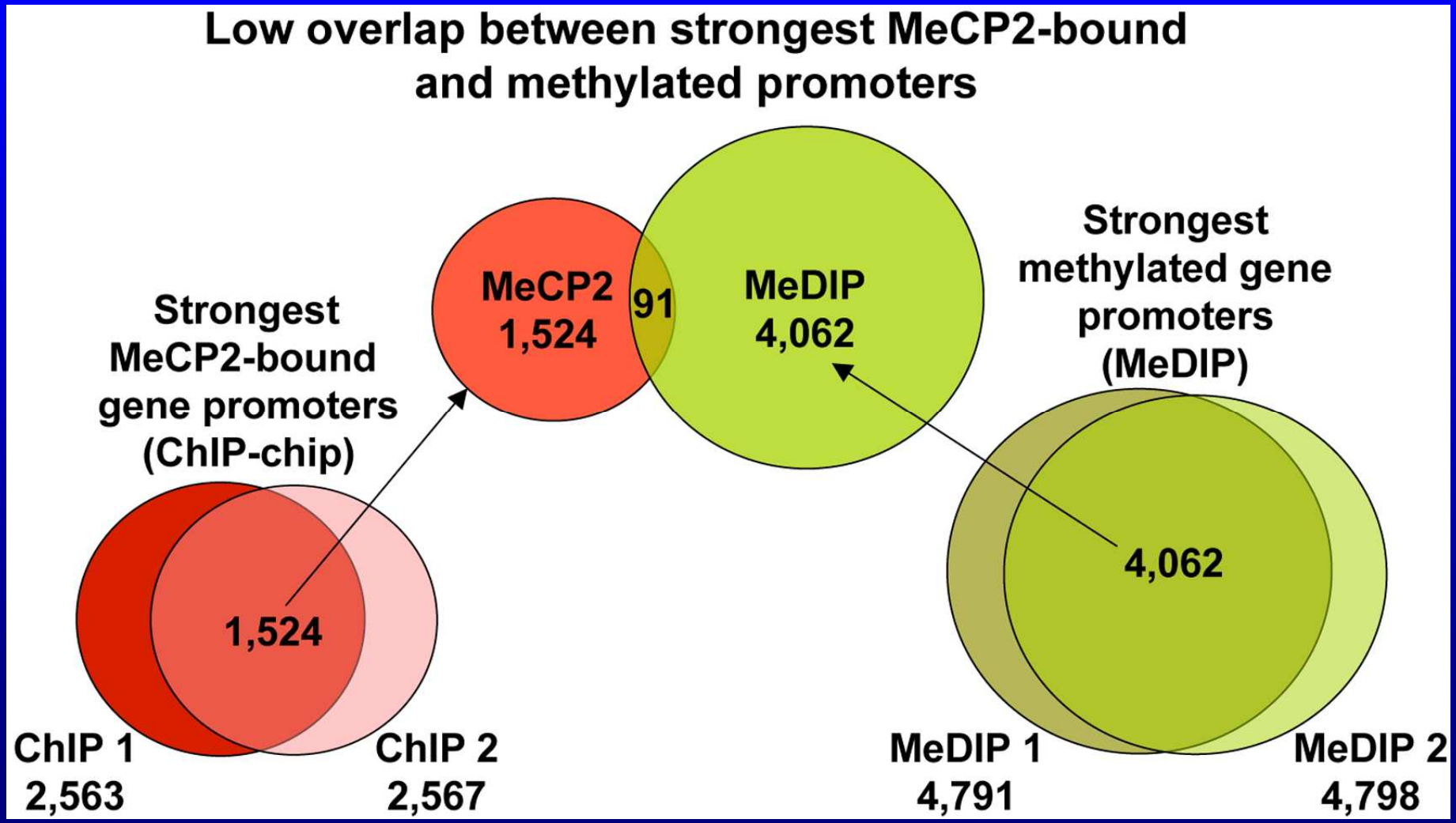
Yasui et al, PNAS, 2007

MeCP2 is bound to promoters of highly expressed genes



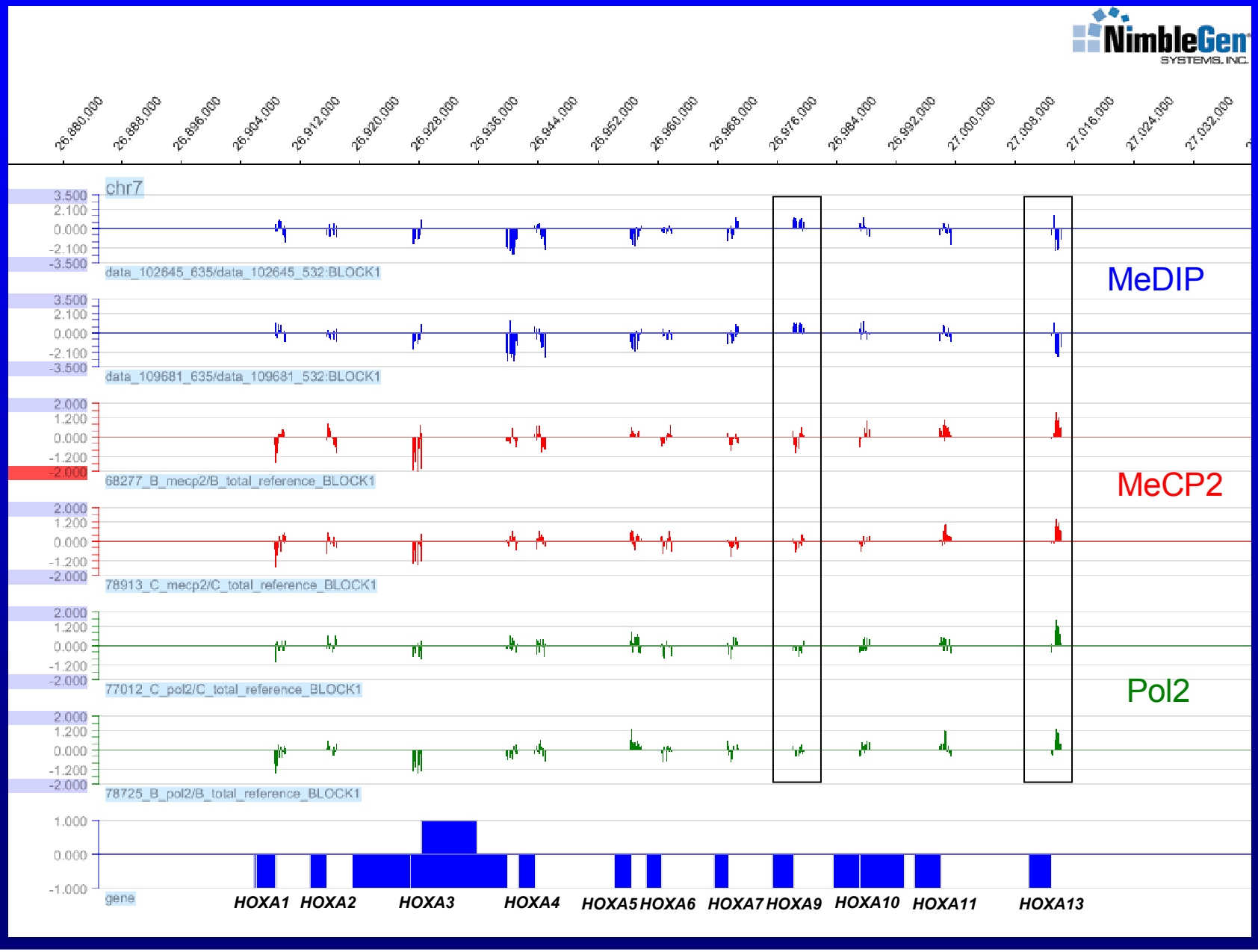
Yasui et al, PNAS, 2007

Highly methylated promoters are not bound by MeCP2



Yasui et al, PNAS, 2007

Example: MeDIP + MeCP2 and Pol2 ChIP at HOXA locus



More examples from Nimblegen promoter arrays for MeDIP, MeCP2, and Pol2

QuickTime™ and a
decompressor
are needed to see this picture.

POU5F1 (OCT4)

QuickTime™ and a
decompressor
are needed to see this picture.

GAPDH

NOL1

CHD4

Micrococcal nuclease fractionation of chromatin shows MeCP2 primarily in active fraction

QuickTime™ and a
decompressor
are needed to see this picture.

Western blot

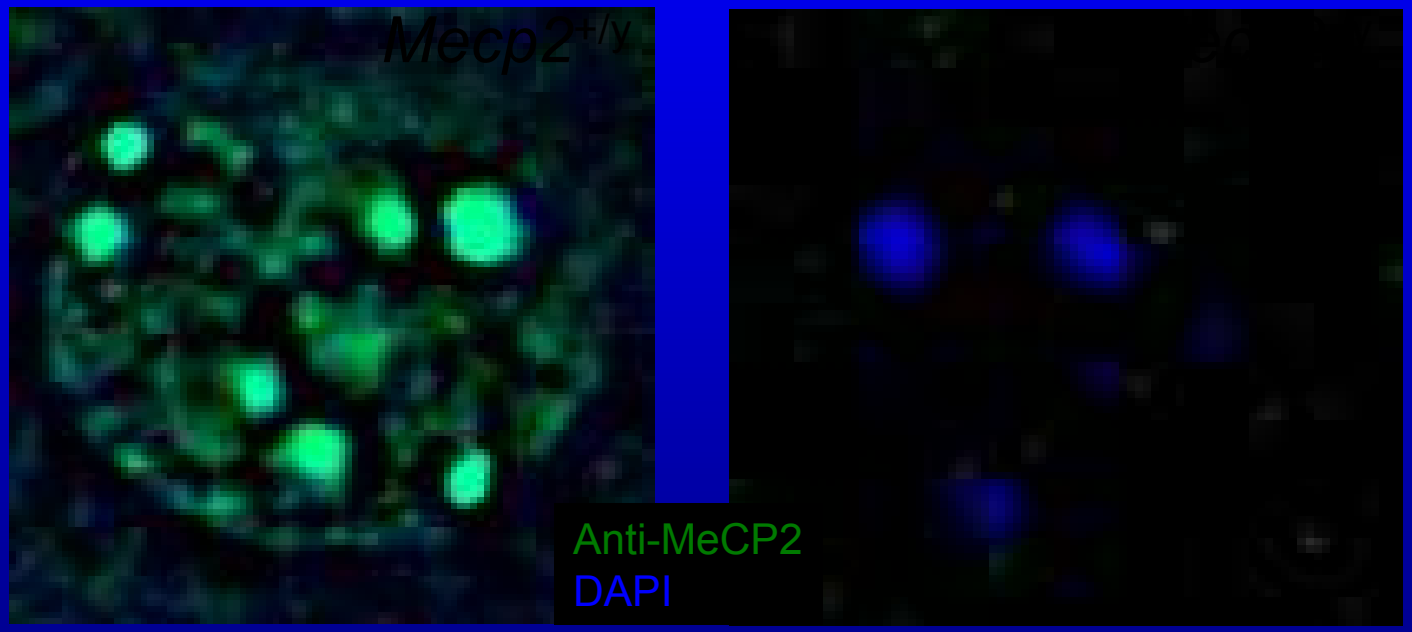
QuickTime™ and a
decompressor
are needed to see this picture.

DNA dot-blot

QuickTime™ and a
decompressor
are needed to see this picture.

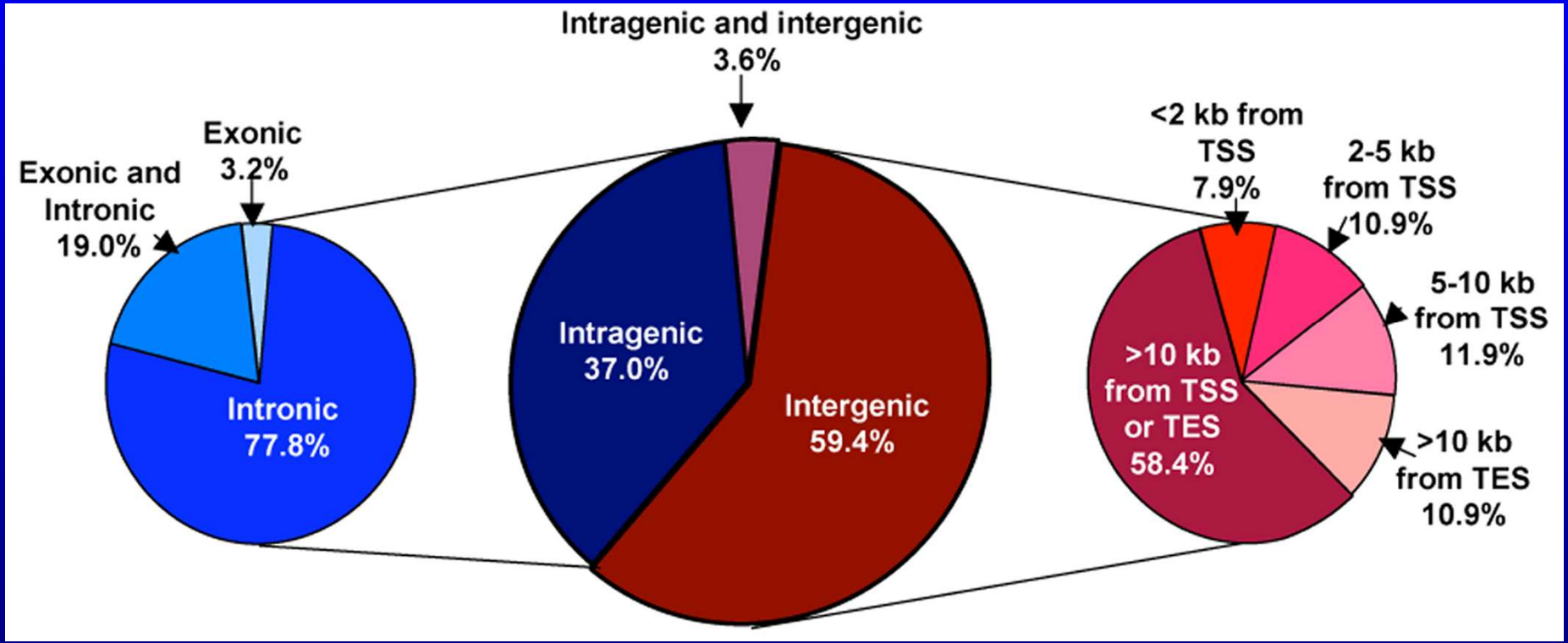
Mike Gonzales, unpublished

MeCP2 localizes to both inert nuclear heterochromatin as well as non-heterochromatic regions



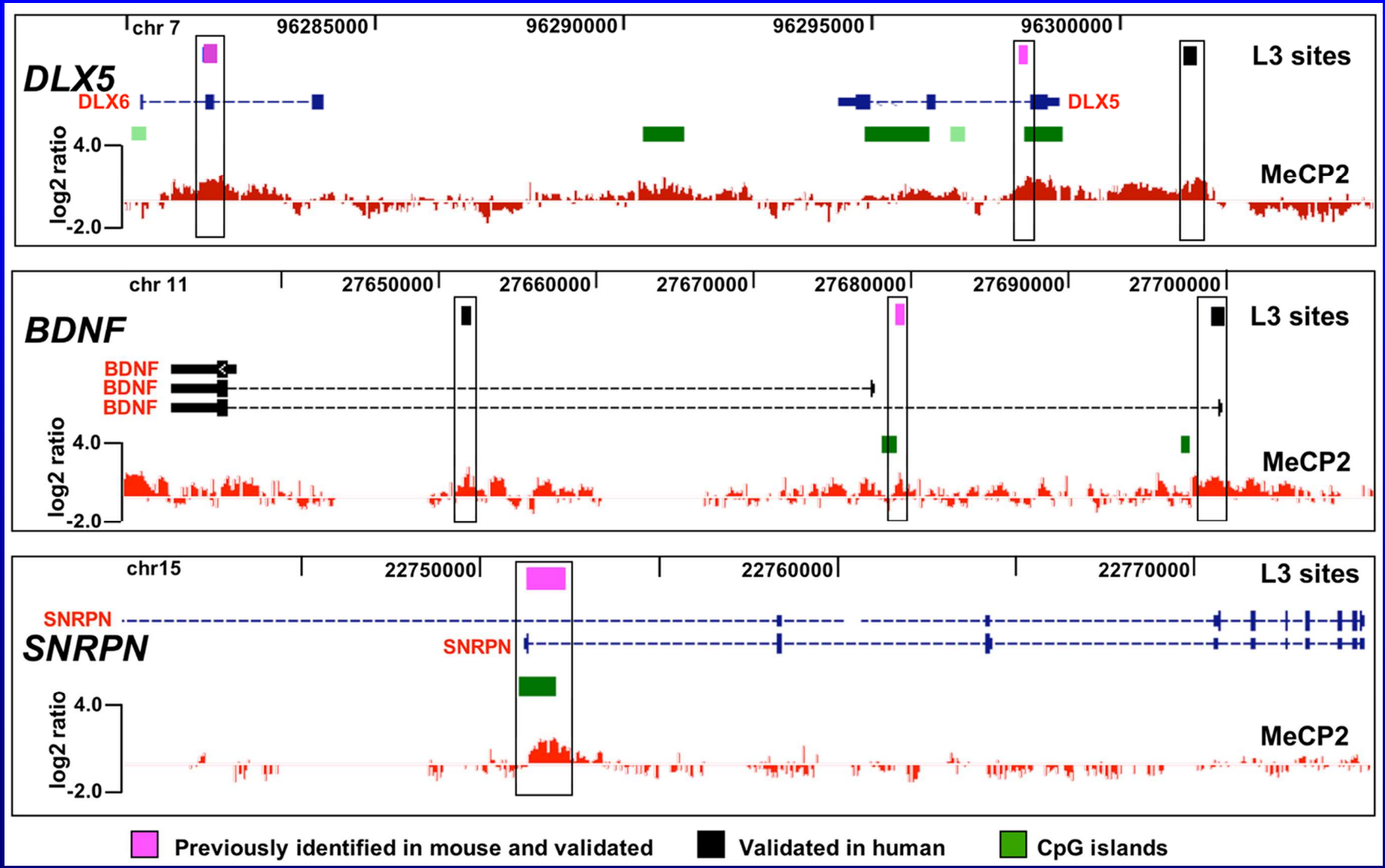
Sub-nuclear localization patterns of MeCP2 are consistent with dual functions in repression and activation

The majority of MeCP2 binding sites are intergenic or intronic



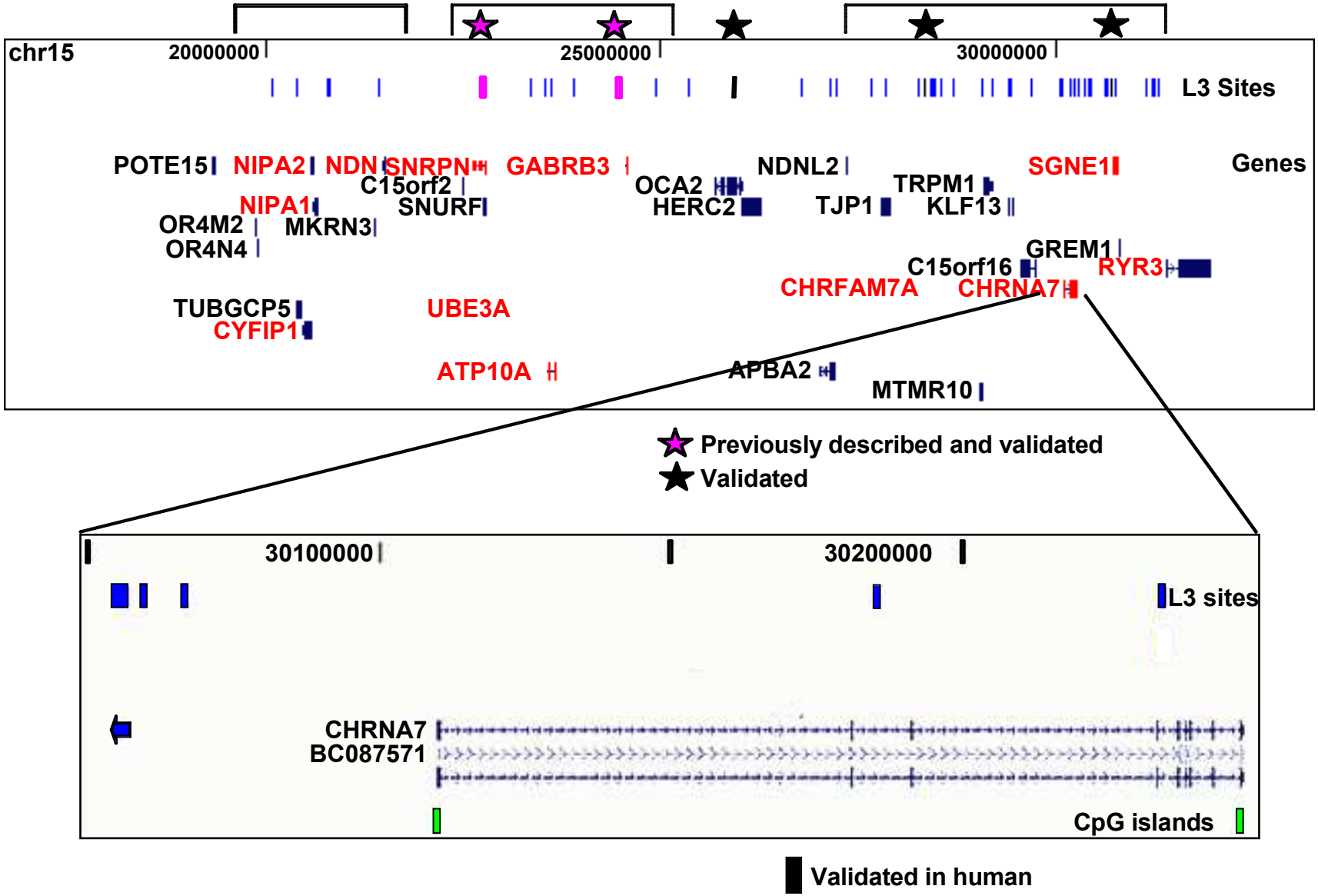
Yasui et al, PNAS, 2007

ChIP-chip analysis of established MeCP2 target genes



Yasui et al, PNAS, 2007

MeCP2 ChIP-chip analysis of imprinted region 15q11-13



Yasui et al, PNAS, 2007

MeCP2 target genes at closer examination

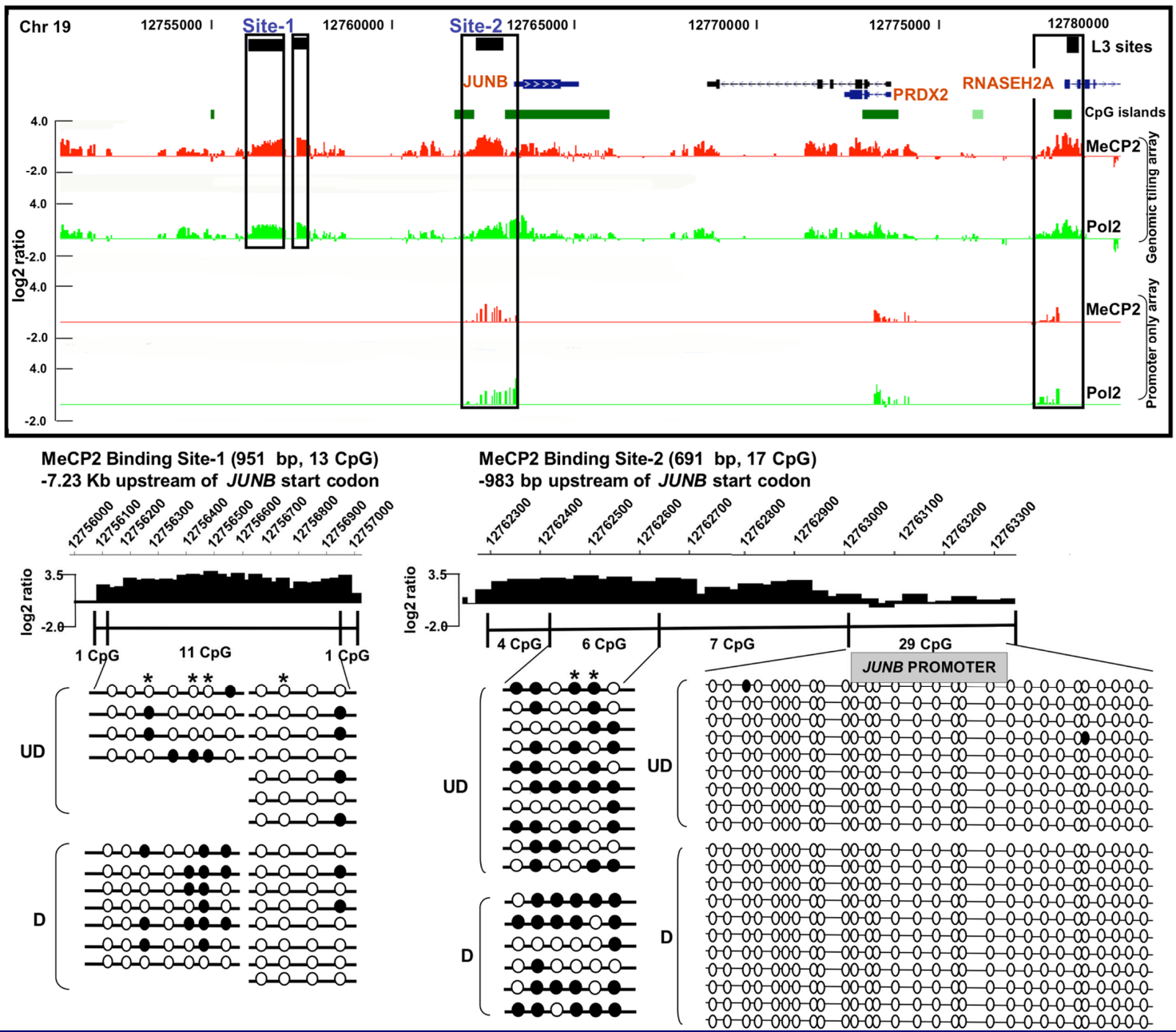
Active genes that MeCP2 represses through intergenic sites

JUNB
ID1

Active genes that MeCP2 activates through intronic enhancers

EGR2
GABRB3

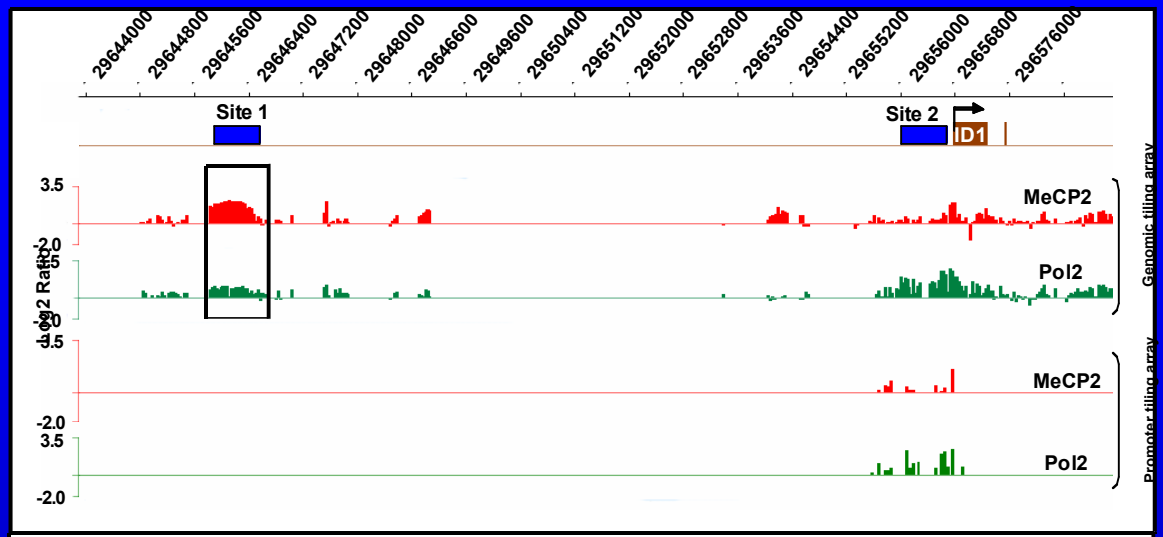
ChIP-chip analysis of 19p13.2 and *JUNB*

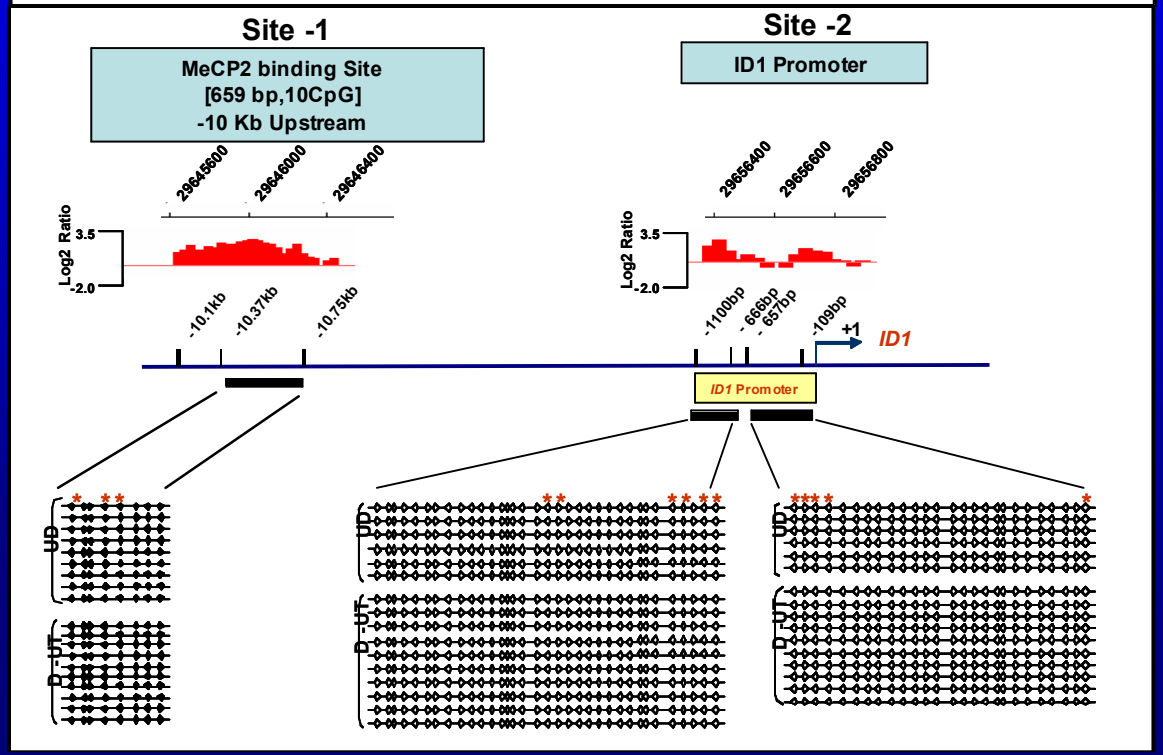


Yasui et al, PNAS, 2007

Methylation of upstream binding site for *ID1* with unmethylated promoter

QuickTime™ and a
decompressor
are needed to see this picture.





ID1 is upregulated in *Mecp2*$^{-/y}$ brain
Peddada et al, 2006

DNA methylation patterns do not change with differentiation

S. Peddada, unpublished

Reciprocal regulation of *MECP2* and *EGR2* intronic enhancer

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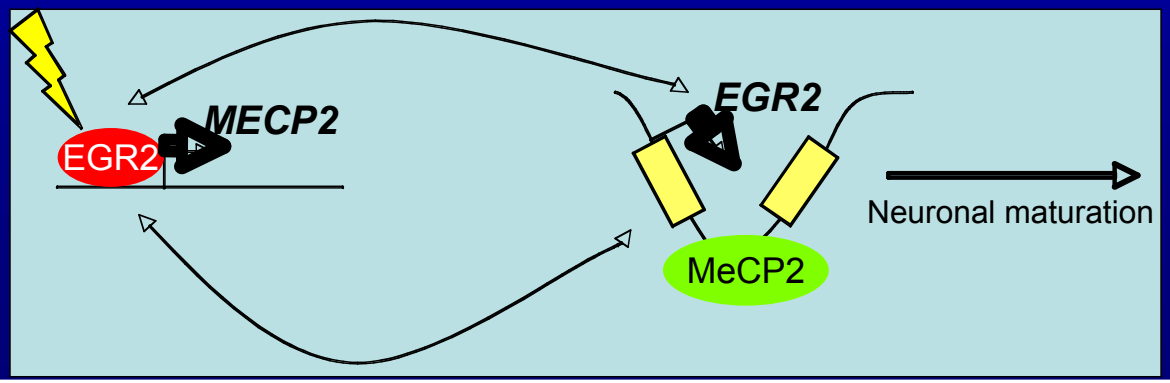
decompressor
are needed to see this picture.

QuickTime™ and a
decompressor
are needed to see this picture.



Swanberg et al, 2009

15q11-13 $GABA_A$ receptor subunit *GABRB3* has MeCP2 in intronic enhancer for homologous pairing and biallelic expression

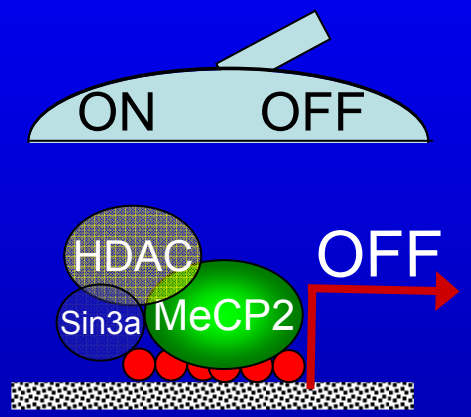
QuickTime™ and a
decompressor
are needed to see this picture.

MeCP2 intronic binding predicted to act as a pairing factor for optimal maternal expression

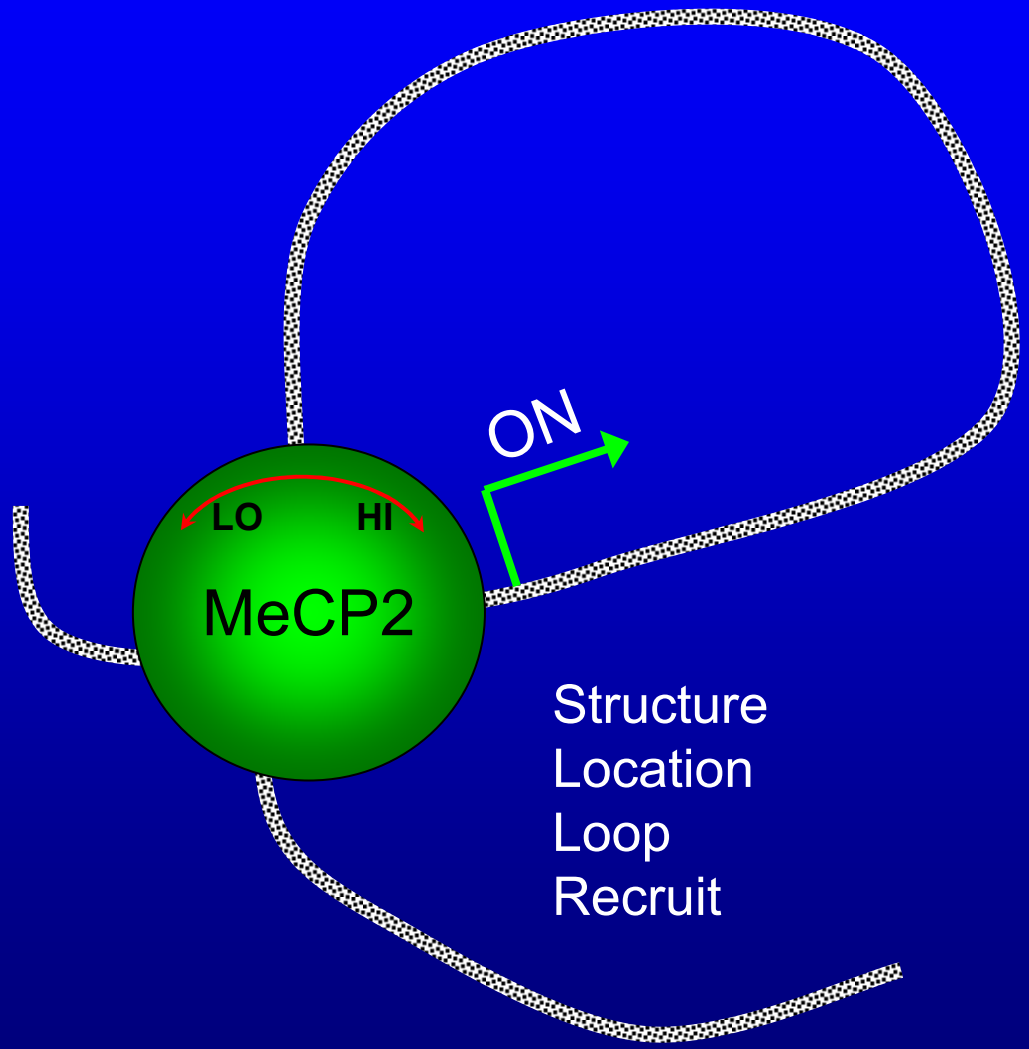
QuickTime™ and a
decompressor
are needed to see this picture.

Hogart et al, HMG, 2007

“Dimmer switch” model of MeCP2 as long range modulator of gene expression



Textbook enzymatic model as “off” switch



Structure
Location
Loop
Recruit

Modulator rather than silencer

Future goals for improved understanding of MeCP2

- Characterize and develop reagents for investigating all MeCP2 post-translational modifications
- Characterize MeCP2 alternative isoforms, particularly MeCP2e1 in brain
- Genome-wide ChIP-seq in primary neurons
- Correlate MeCP2 binding sites with DNA methylation, histone marks, and gene expression genome-wide

Imprinting regulates mammalian snoRNA-encoding chromatin decondensation and neuronal nucleolar size

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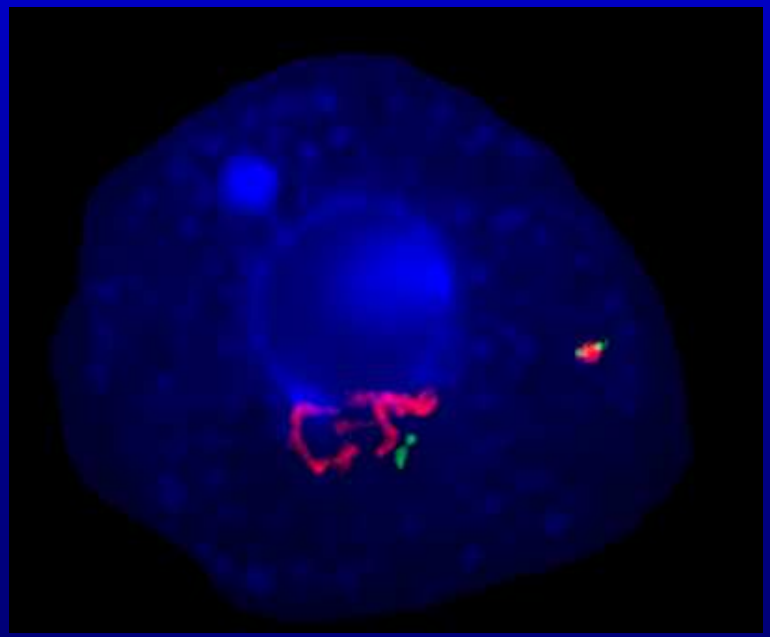
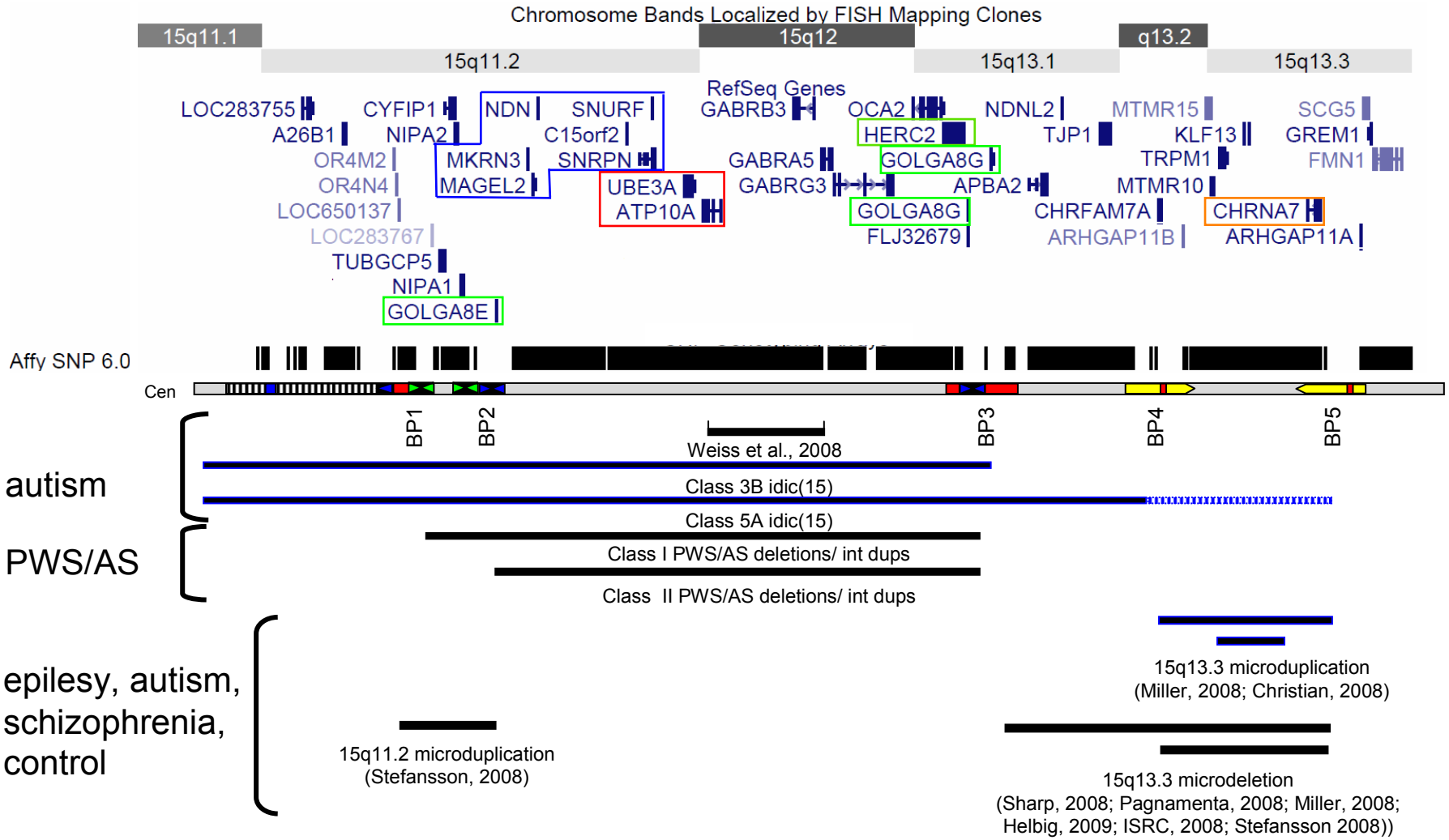
Received June 10, 2009; Revised July 24, 2009; Accepted August 3, 2009



Genomic map of the 15q11-13 deletion/duplication disorders



Imprinted and neuronal regulation of 15q11-13

Paternally Expressed

expressed in neurons

MKRN3 MAGEL2 NDN PWS-ICR SNRPN HBII-13 HBII-85 HBII-52 UBE3A-AS

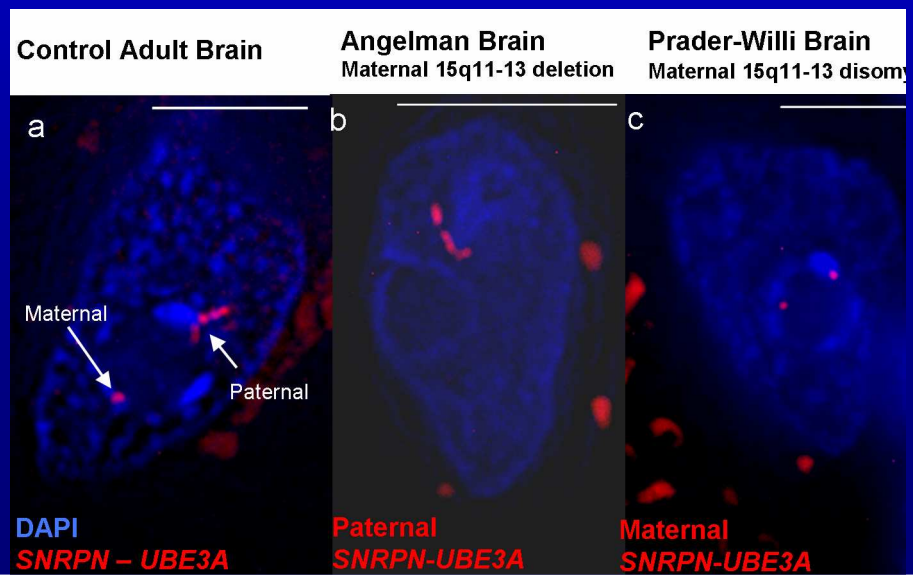
CEN TEL

Maternally Expressed

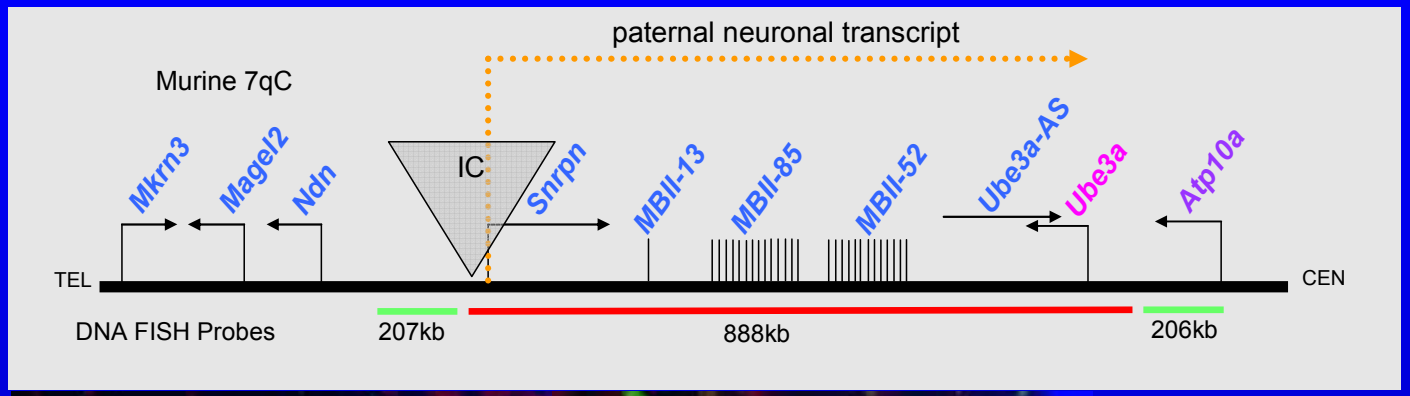
AS-ICR UBE3A ATP10A

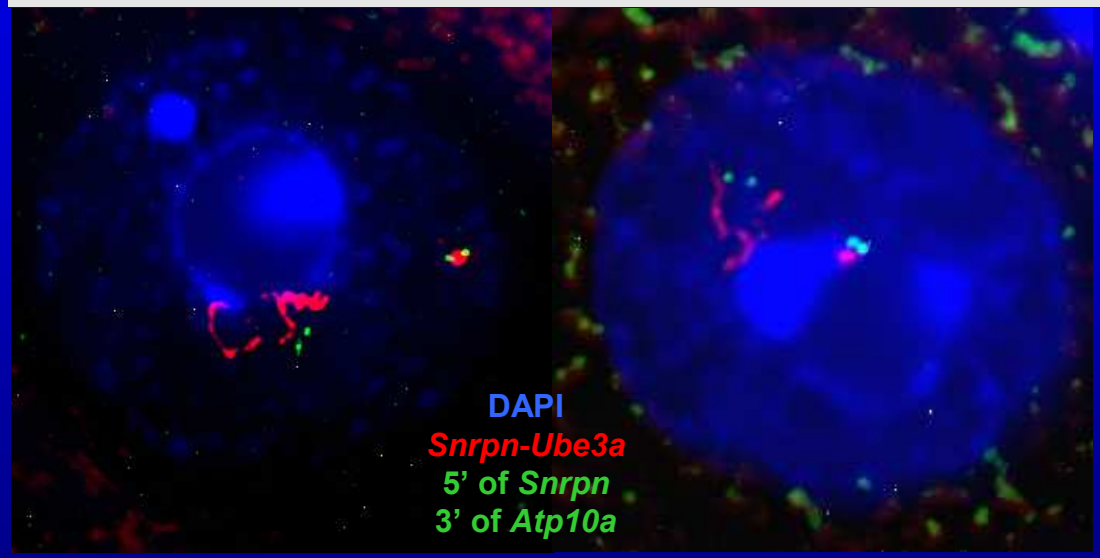
DNA FISH Probes

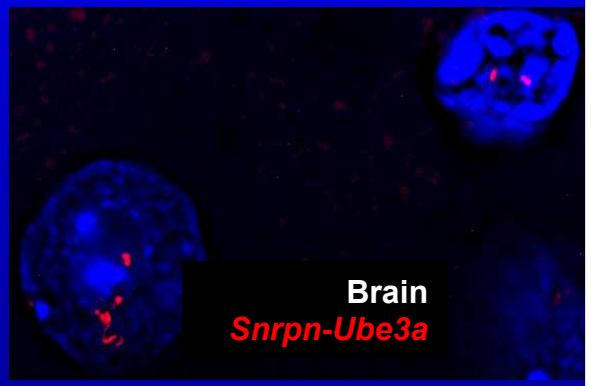
175kb 170kb 161kb

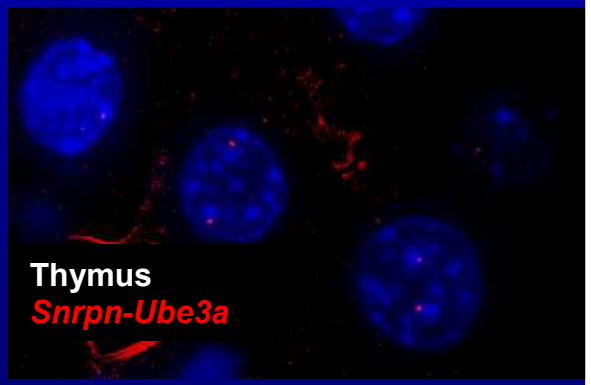


Chromatin decondensation of *Snrpn* to *Ube3a* is neuron-specific





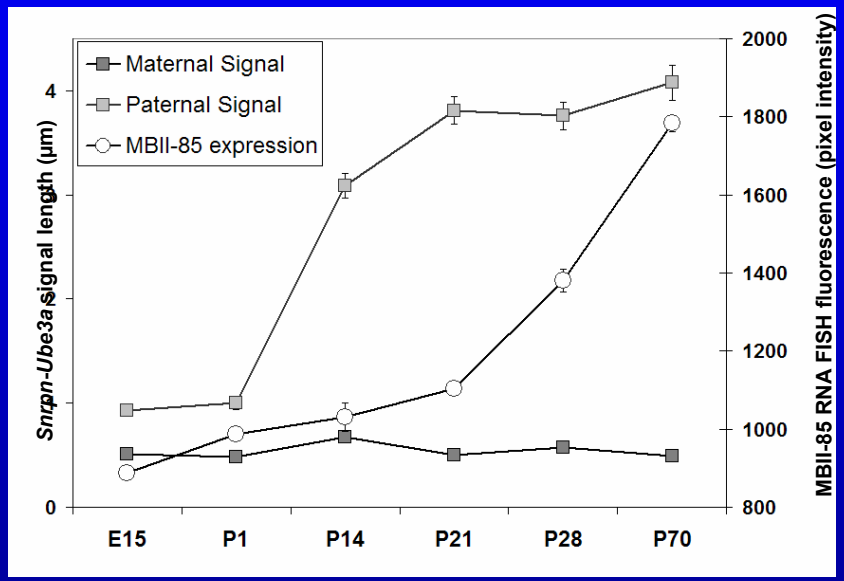




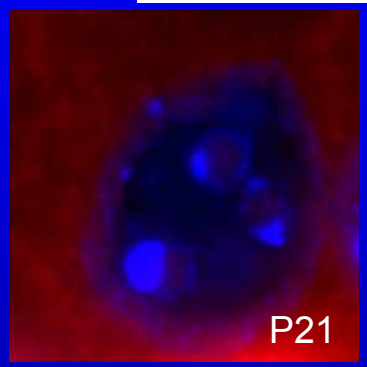
Paternal signal ~5.1µm (average)
266.4µm/5.1µm = 52.2:1 packing ratio
1.3x more compact then a 30nm fiber

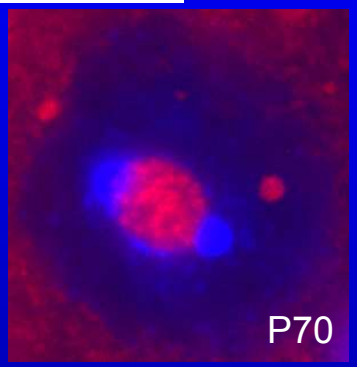
Maternal signal ~1.1µm (average)
266.4µm/1.1µm = 242.2:1 packing ratio
6x more compact then a 30nm fiber

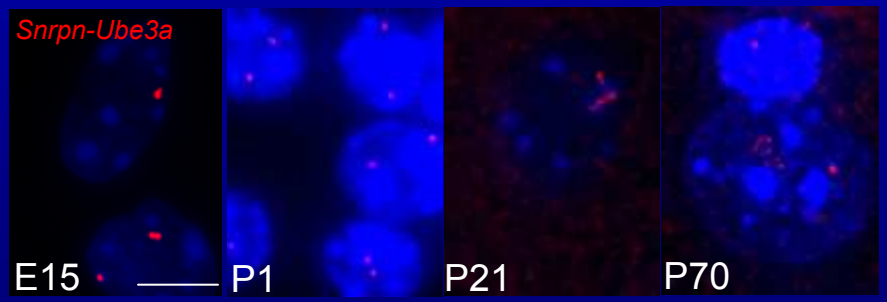
Paternal *Snrpn-Ube3a* allele decondensation is developmentally regulated and correlates to nucleolar enlargement during neuronal maturation

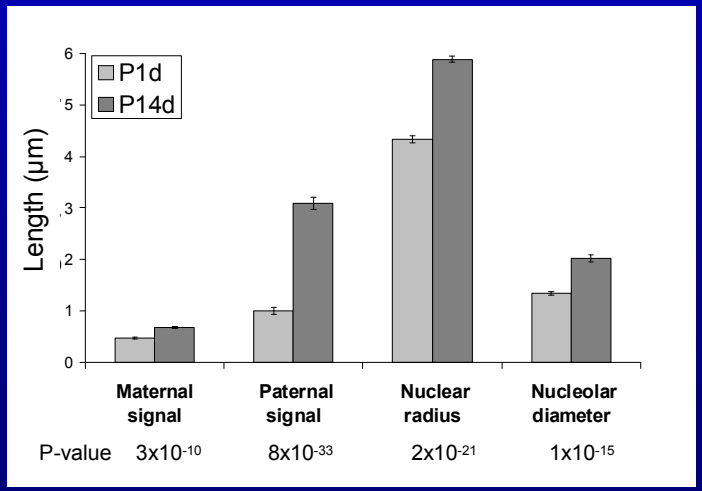


MBII-85 RNA FISH





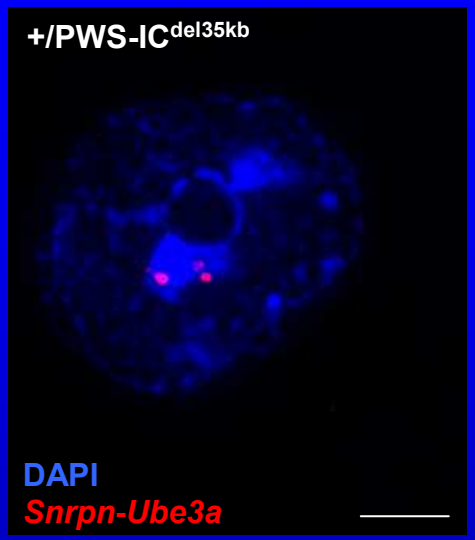


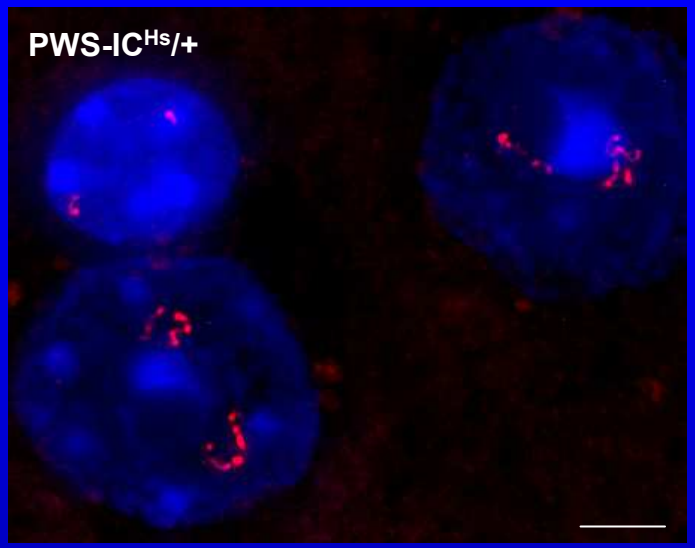


Transcription is required for paternal *Snrpn-Ube3a* chromatin decondensation

Mouse model 1
Paternal IC deletion
PWS model

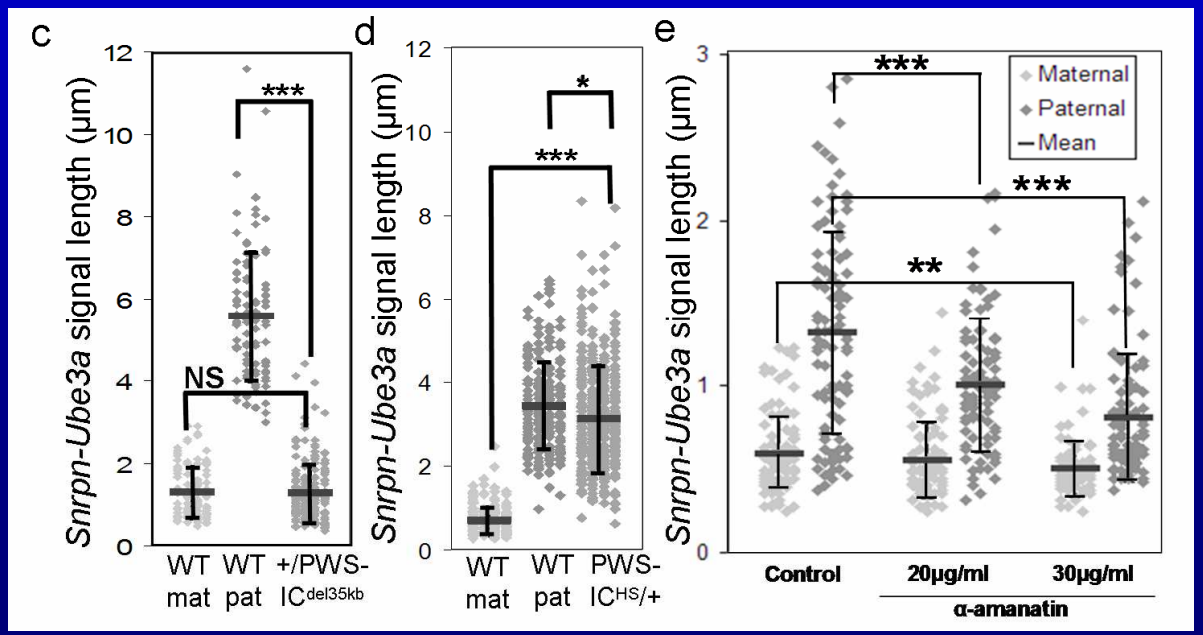
Chamberlain et al, 2004, HMG





Mouse model 2
Human IC swap
No maternal imprint

Johnstone et al, 2005, HMG



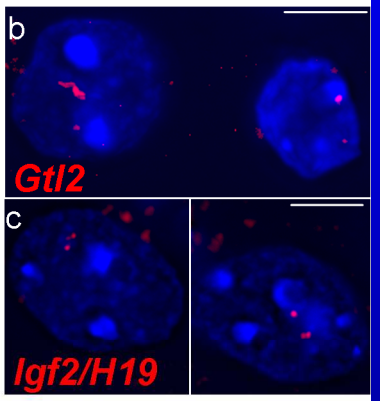
Transcriptional inhibition in mouse primary neurons

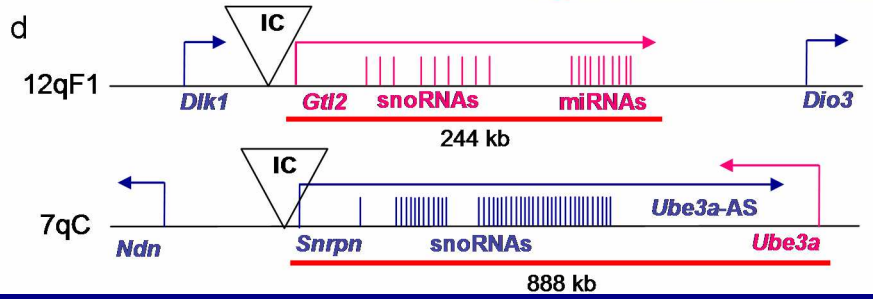
Neuron-specific chromatin decondensation is uniquely observed at imprinted snoRNA clusters and impacts nucleolar size

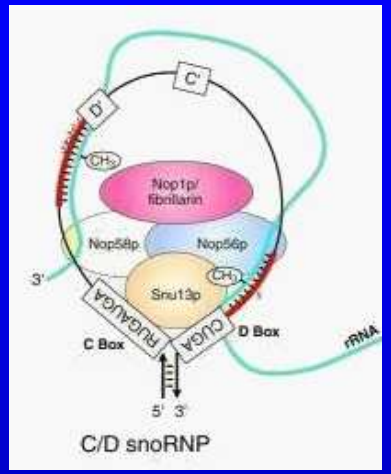
Neuronal FISH with probes from imprinted, ncRNA, and highly transcribed loci

a

Gene Locus	Imprinted	sno RNA	nc RNA	Decondensed chromatin
Snrpn-Ube3a	Yes	Yes	Yes	**Yes**
Igf2/H19	Yes	No	No	No
Dlx5/6	Yes	No	No	No
Igf2r	Yes	No	Yes	No
Ndn-Mkrn3	Yes	No	No	No
Htr2c	No	Yes	Yes	No
Tsix	No	No	Yes	No
Dmd	No	No	No	No
Gtl2	Yes	Yes	Yes	**Yes**

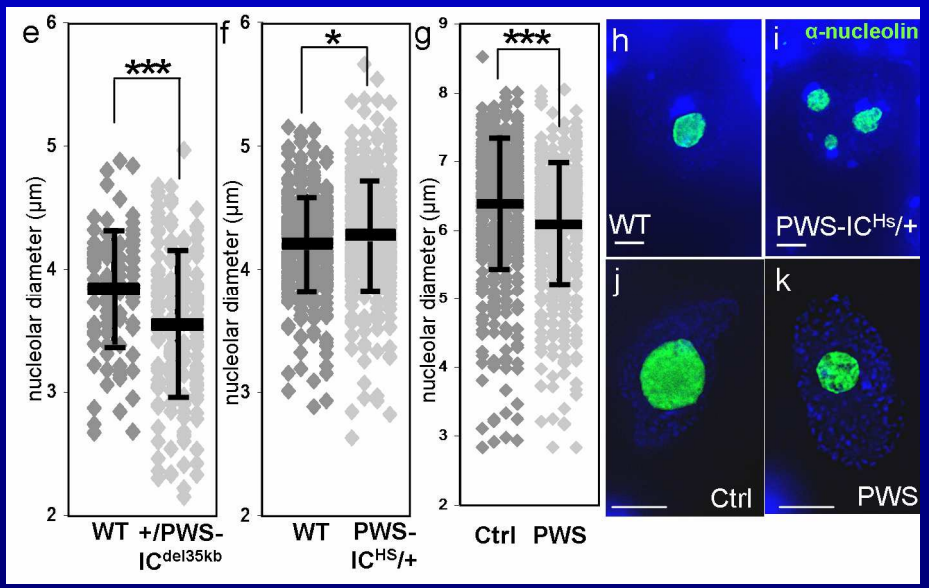




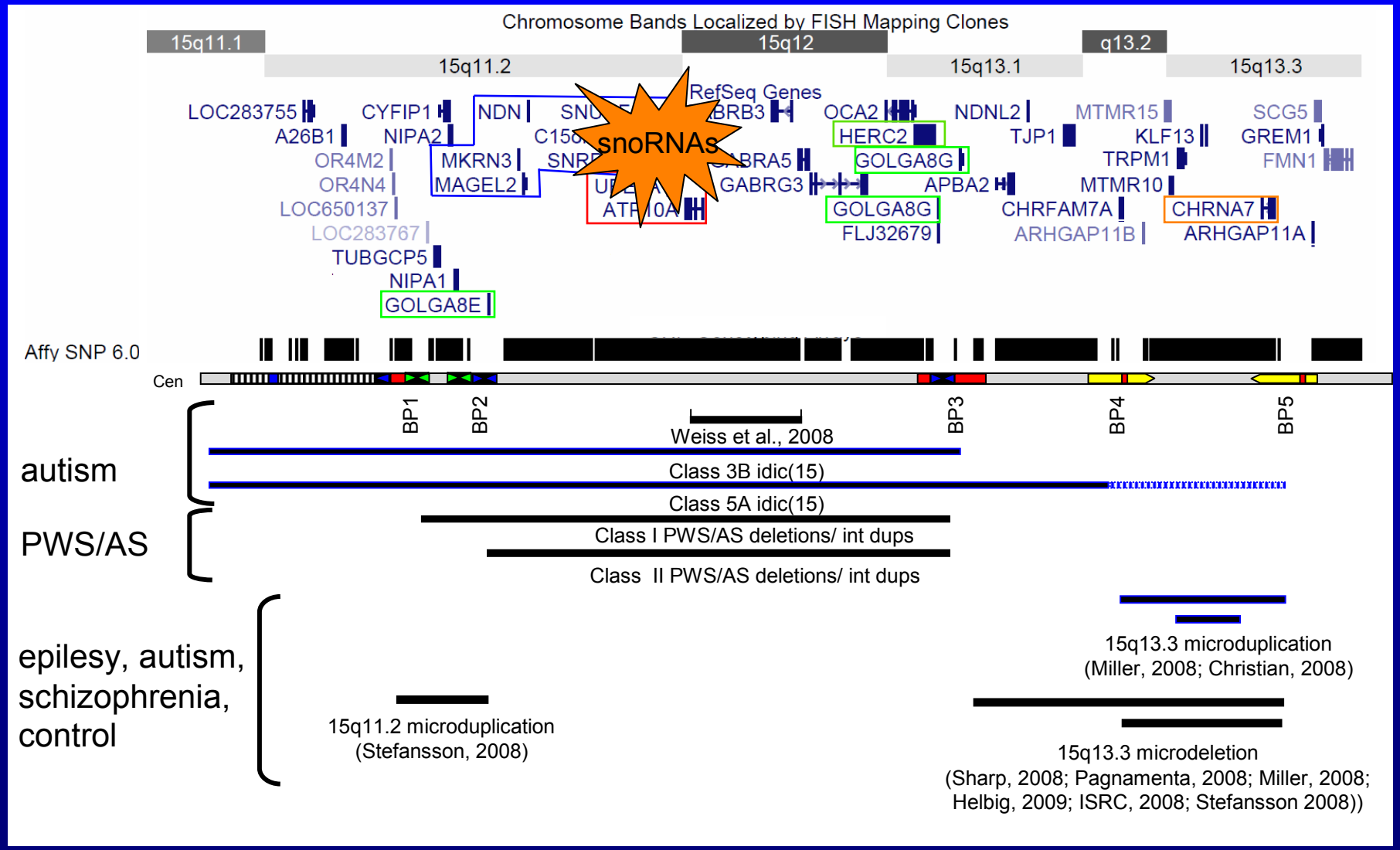


2'-0-methylation of rRNA

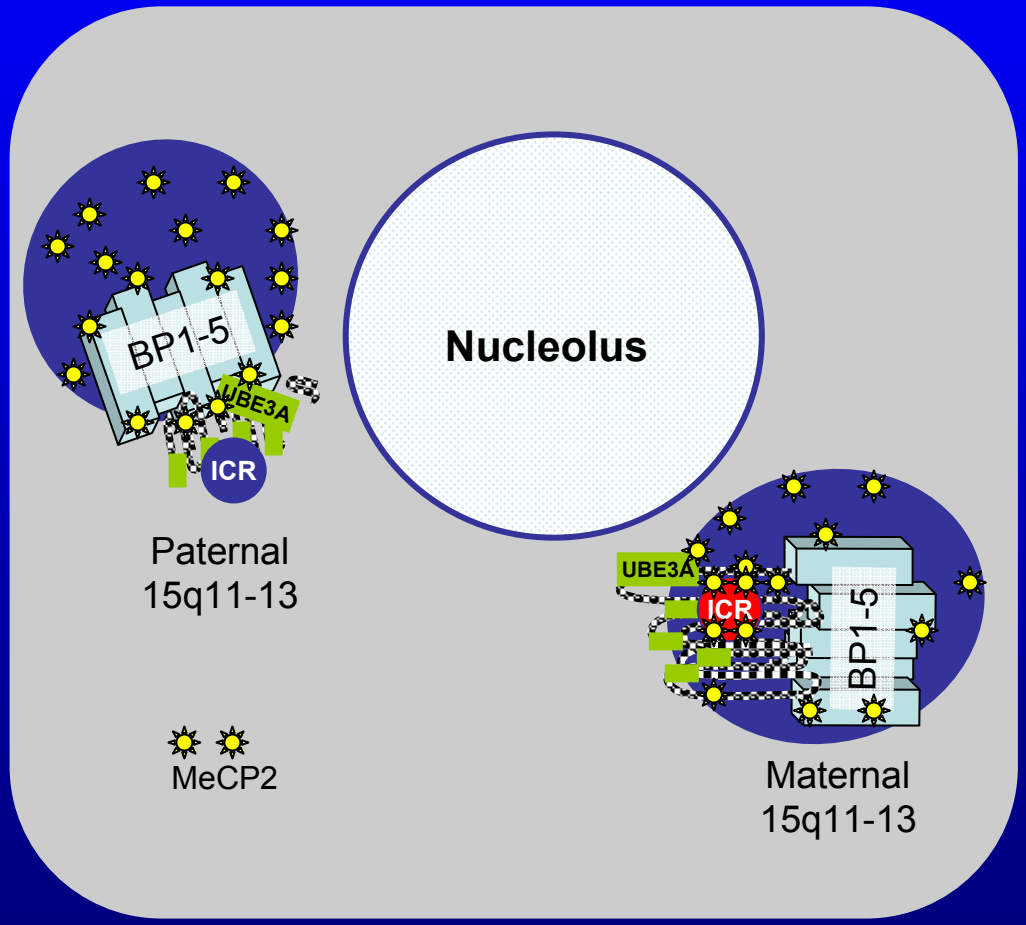
Nucleolar measurements of Purkinje neurons



Multiple copy number variants in multiple neurodevelopmental disorders map to 15q11-13

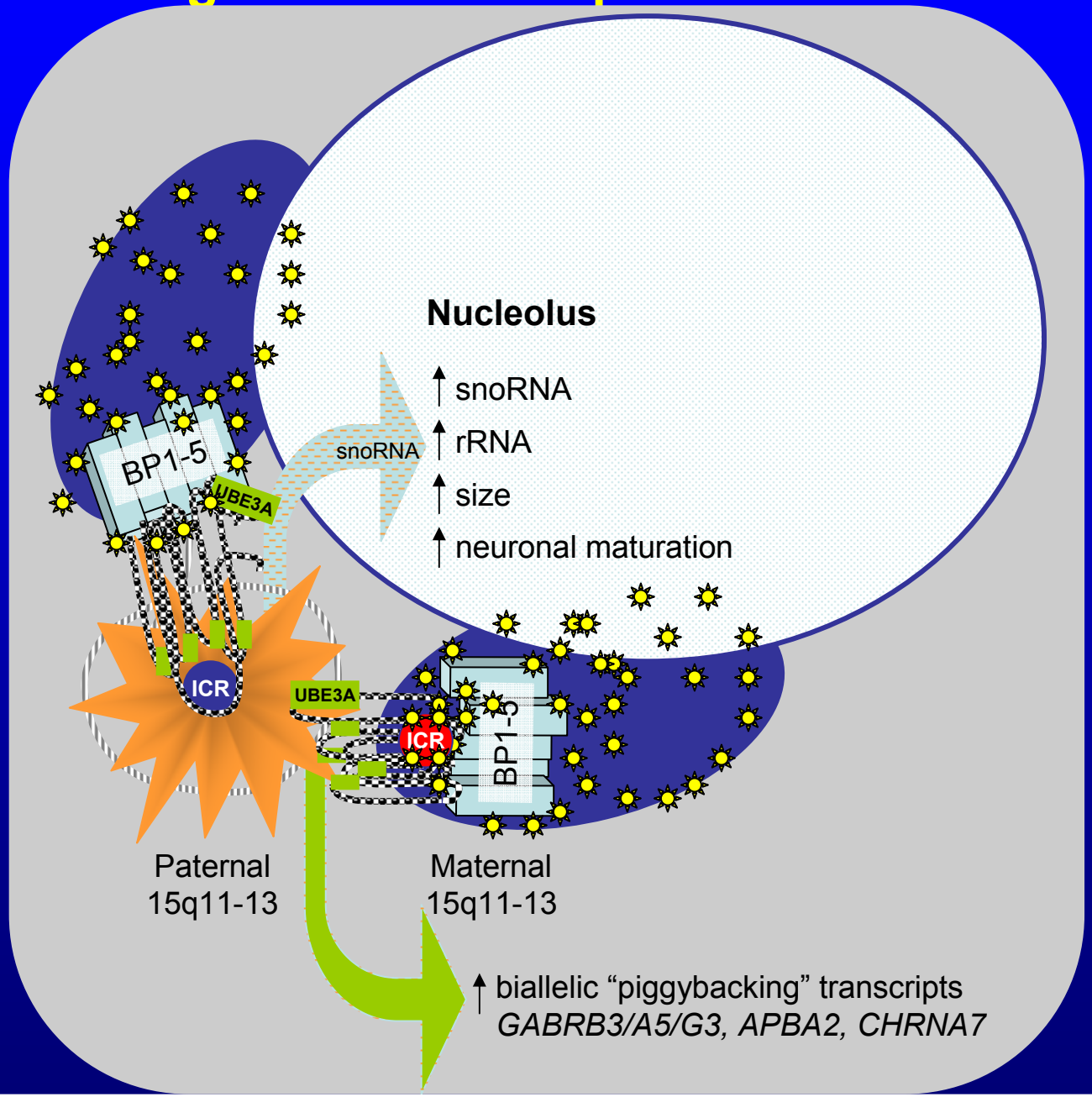


15q11-13 model for multiple genetic and epigenetic etiologies of autism spectrum disorders

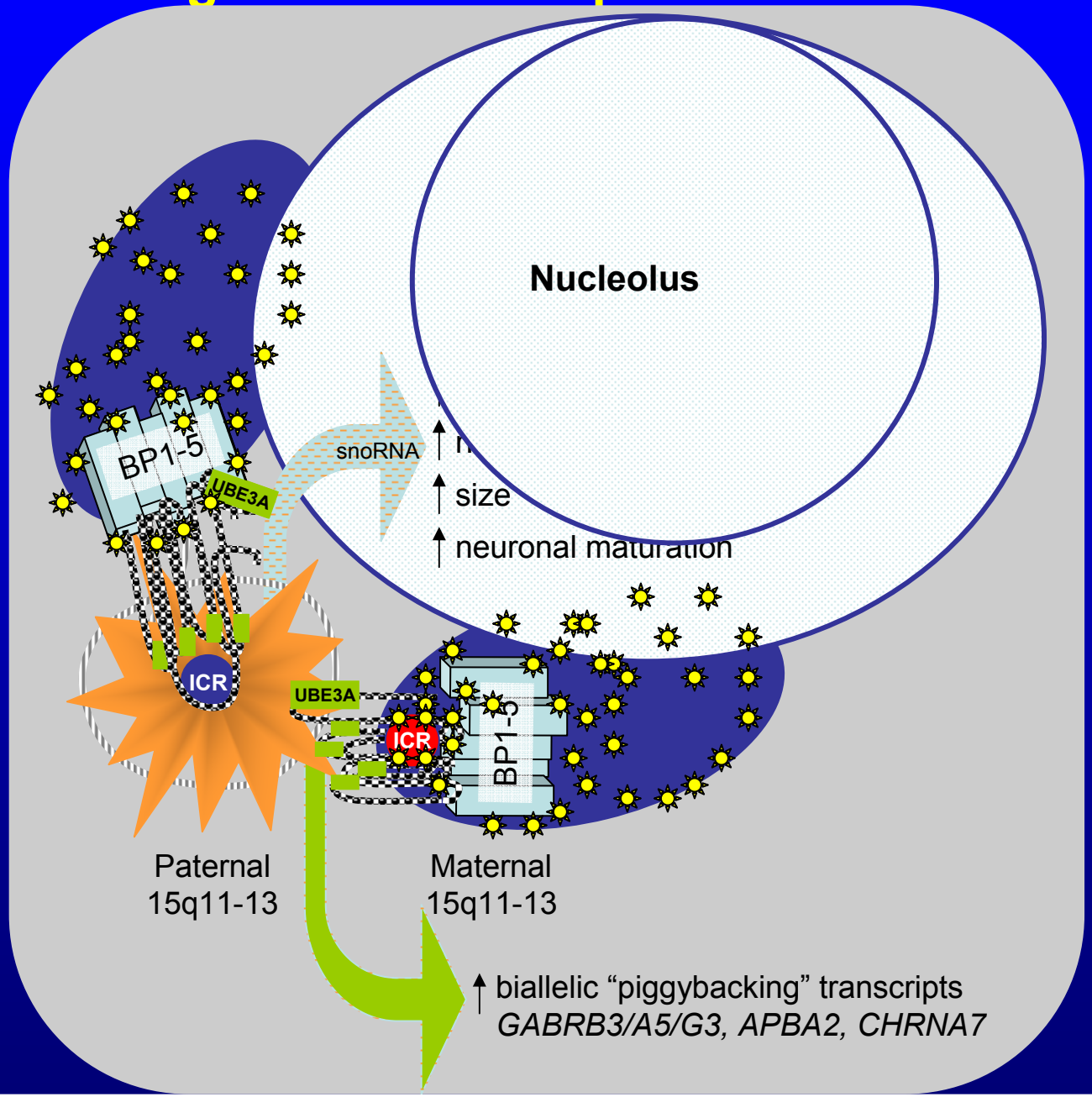


Neuronal maturation

15q11-13 model for multiple genetic and epigenetic etiologies of autism spectrum disorders



15q11-13 model for multiple genetic and epigenetic etiologies of autism spectrum disorders



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