Identification of MeCP2 target genes in a mouse model of Rett Syndrome

Hospital Sant Joan de Deu
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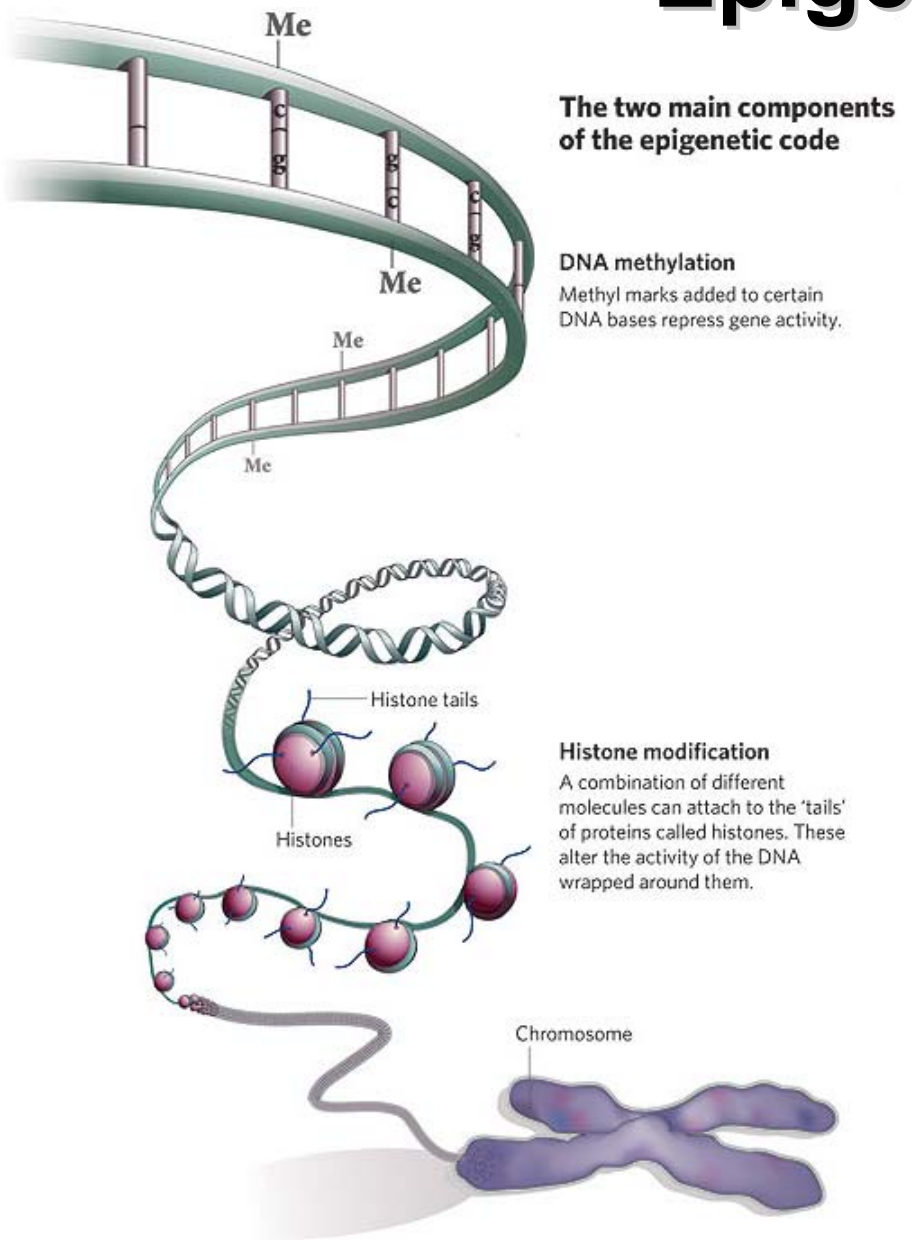
Manel Esteller
Cancer Epigenetics and Biology Program (PEBC)
Bellvitge Institute for Biomedical Research (IDIBELL)

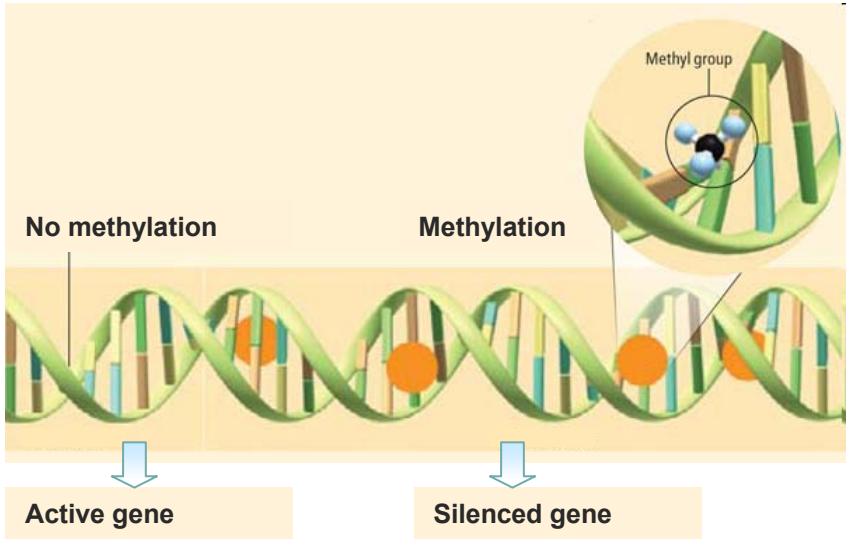
Epigenetics

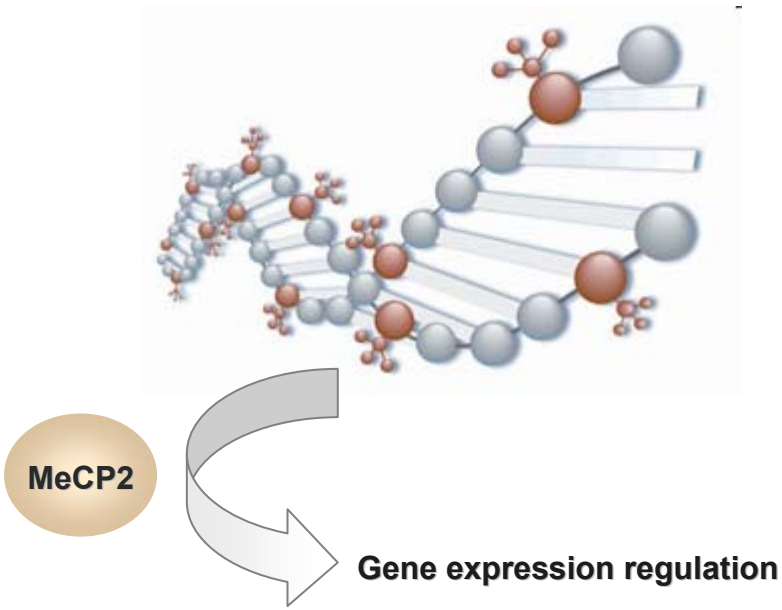


The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.





Rett syndrome

- First described in 1966 by Andreas Rett
- Second most common cause of mental retardation in women
- Frequency: 1 in 10.000-15.000
- Associated with mutations in MECP2 gene

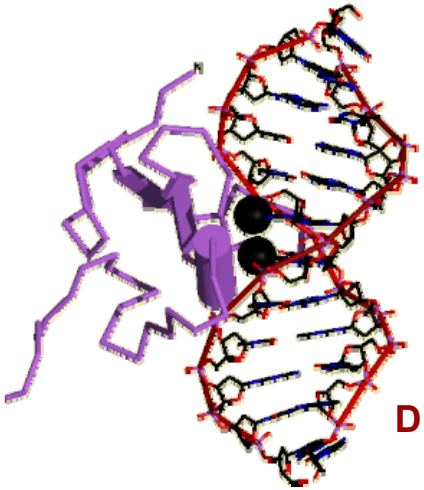
MeCP2

- Protein with affinity for methylated DNA

Two main functional domains:

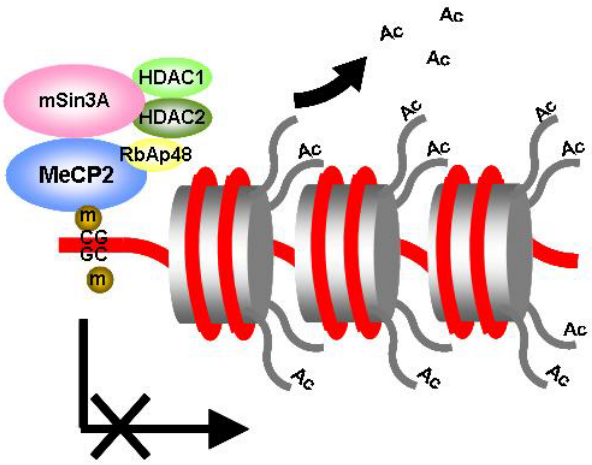
⇨MBD

⇨TRD

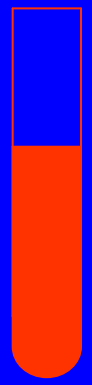


DNA CpG pairs

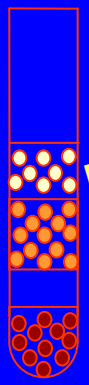
•Transcriptional regulator



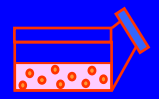
Rett Syndrome: Germline Mutations in MeCP2



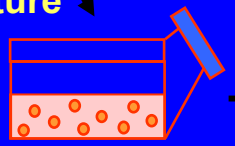
Ficoll

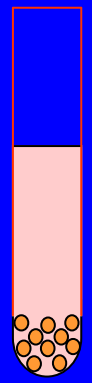


EBV Immortalization

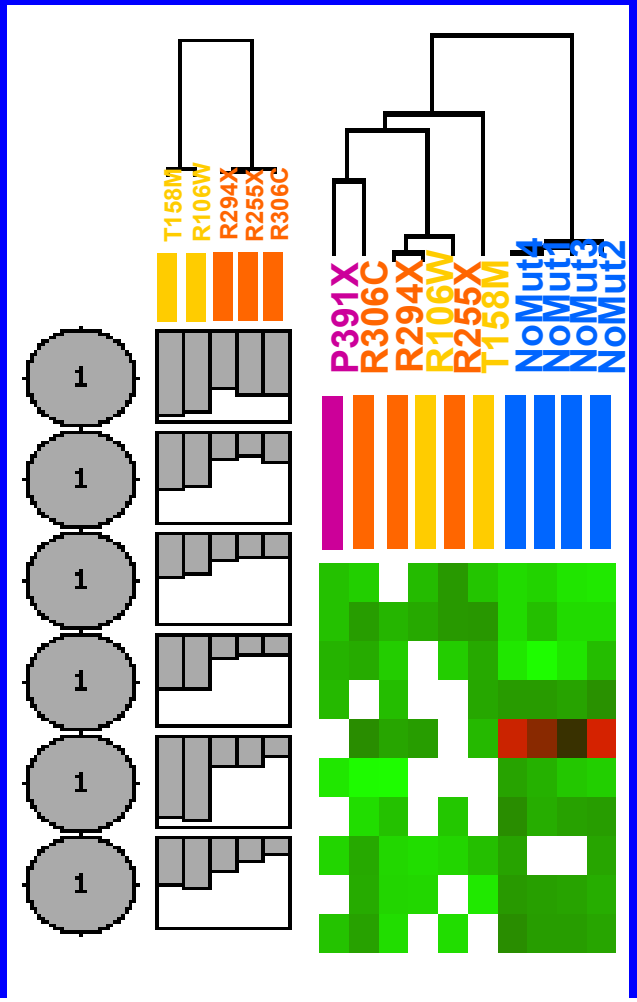


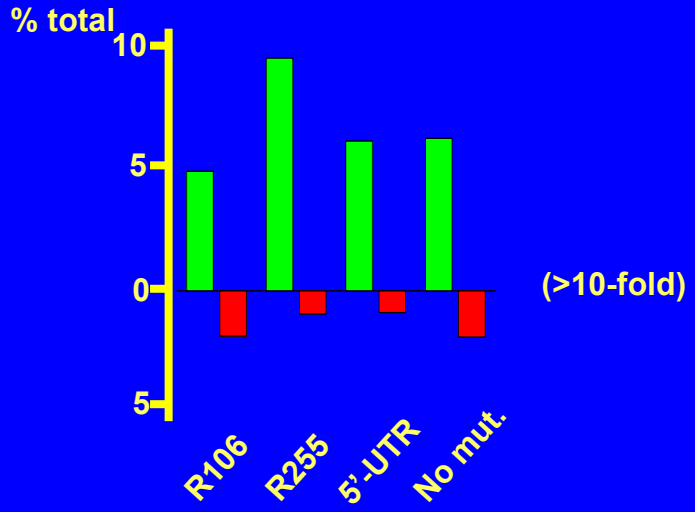
Cell culture

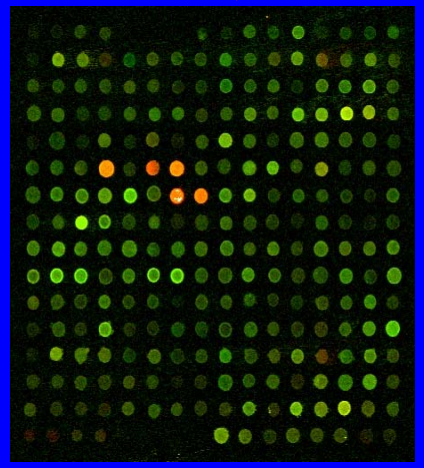




Tota l RNA





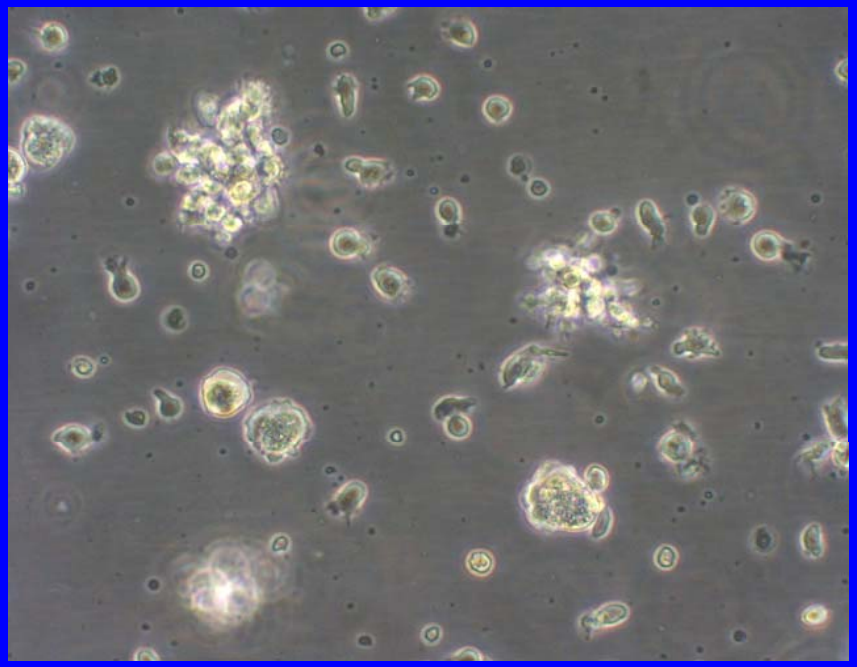


ChIP Candidate genes targeted by MeCP2

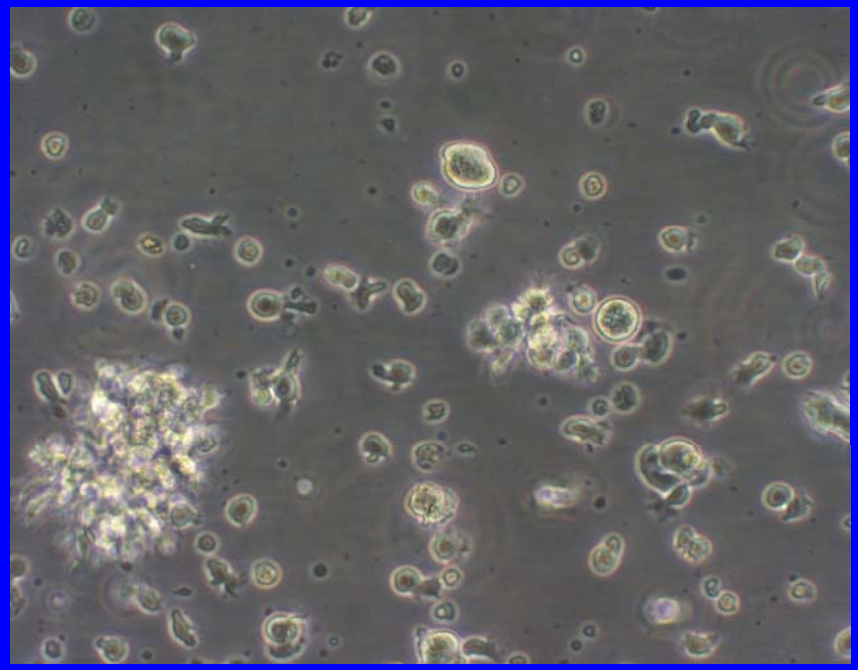
CNIO Oncochip v1.1 6500 clone

Establishment of Immortalized Lymphocyte Cell Li

Healthy Donor



Rett Patient

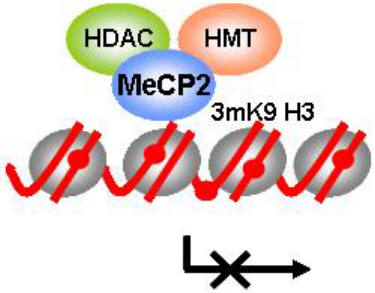


Mouse model of Rett syndrome

A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome

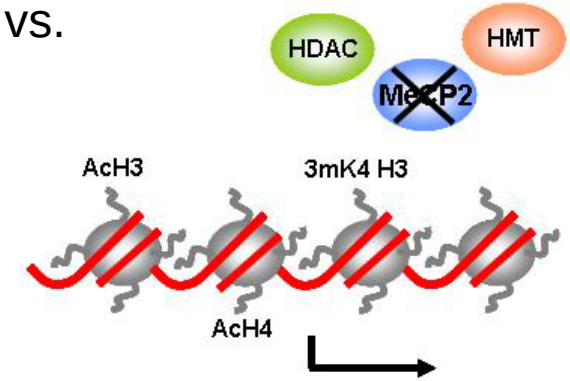
Jacky Guy[1], Brian Hendrich[1], Megan Holmes[2], Joanne E. Martin[3] & Adrian Bird[1]

VS.



Transcriptional regulation

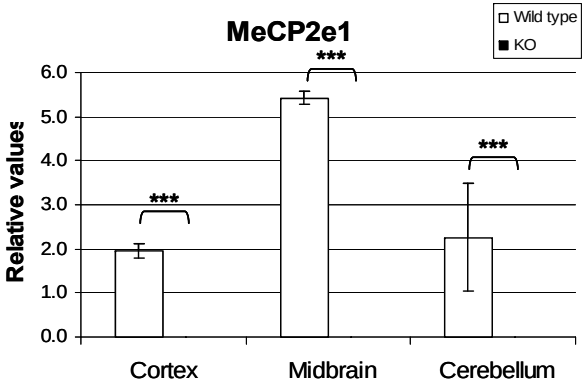
Wild-type mice (WT)

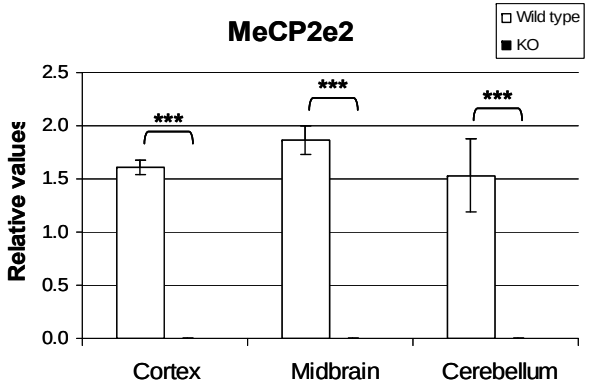


Transcriptional deregulation

Mecp2-null mice (KO)

Mecp2-null mice do not express any Mecp2 isoform





*** p-value <0.001

Mecp2-null mice

WT
Cortex
Midbrain
Cerebellum

KO
Cortex
Midbrain
Cerebellum

EXPRESSION MICROARRAY

29 up-regulated probes in KO mice (Fold >1.5)

POSSIBLE RETT SYNDROME RELATIONSHIP:
- NEURONAL GENES,
- X-CHROMOSOME,
- IMPRINTING…

20 up-regulated genes

qRT-PCR

10 consistently up-regulated genes

ChIP

3 indirectly up-regulated genes

Irak1
Prodh
Dlk1

7 up-regulated genes directly affected by Mecp2

Fkbp5 *Mllt2h*
Mobp *Eya2*
Plagl1 *S100a9*
Ddc

BISULFITE SEQUENCING

All of them with methylated CpG island

Wild type mice
Cortex
Midbrain
Cerebellum

Mecp2-null mice
Cortex
Midbrain
Cerebellum

RNA Extraction → Amplification → Reverse transcription and labeling

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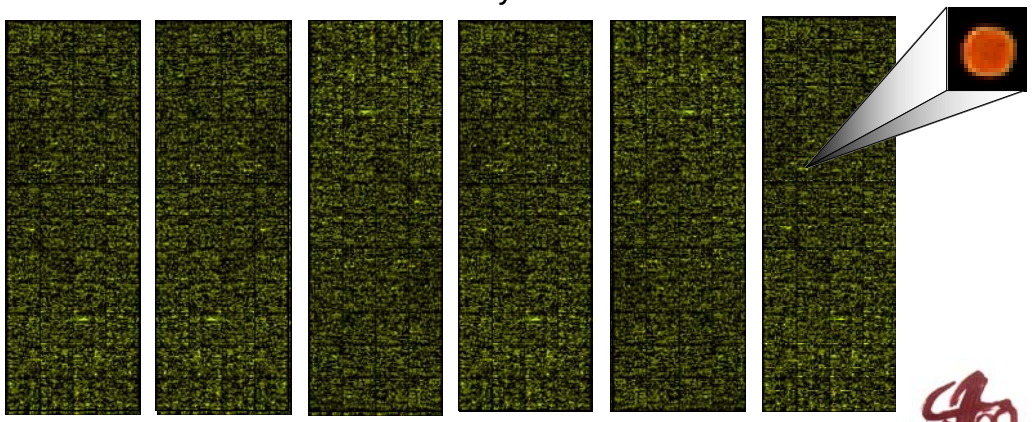
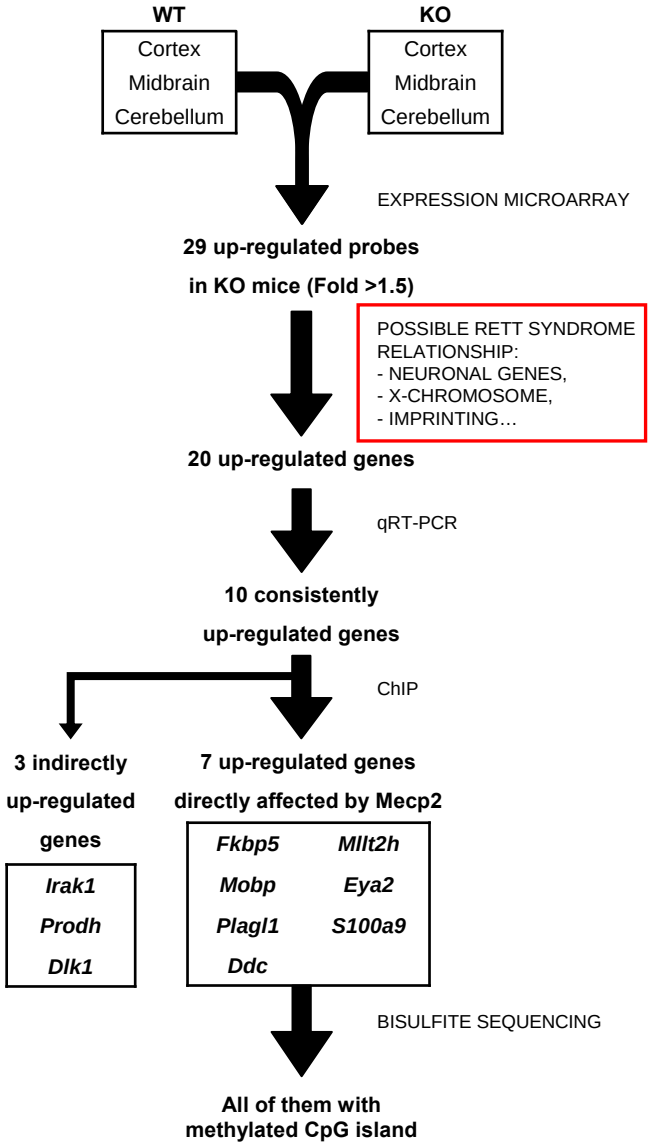
Hybridization

Image scanning

Data analysis



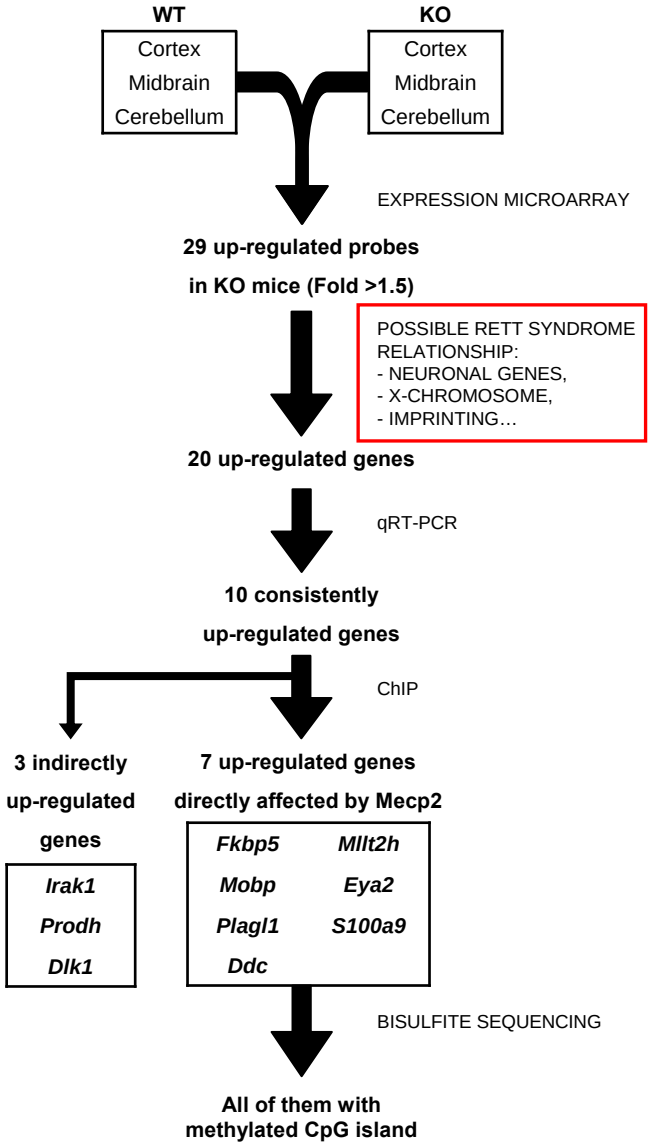
Microarray Results



UPREGULATED GENES

	Name	UniGene	GeneBank	Description	n-fold change	
►	***Fkbp5***	Mm.276405	BG087373	FK506 binding protein 5	2.76	◄
►	***Mt1***	Mm.192991	BG077818	Metallothionein 1	2.25	◄
►	***Bai1***	Mm.43133	BC037726	Brain-specific angiogenesis inhibitor 1	1.99	◄
►	***Mt2***	Mm.147226	BG063925	Metallothionein 2	1.94	◄
►	***Pcolce2***	Mm.46016	BQ561584	Procollagen C-endopeptidase enhancer 2	1.90	◄
	Pnpla2	Mm.29998	BG077619	Patatin-like phospholipase domain containing 2	1.73	
	Rpl36	Mm.379094	BG072993	Sulfatase modifying factor 1	1.72	
►	***Irak1***	Mm.38241	BG076768	Interleukin-1 receptor-associated kinase 1	1.71	◄
►	***Mobp***	Mm.40461	AK013799	Myelin-associated oligodendrocytic basic protein	1.62	◄
			BQ550269		1.58	
►	***Prodh***	Mm.28456	BQ553283	Proline dehydrogenase	1.55	◄
►	***Dlk1***	Mm.157069	BQ550065	Delta-like 1 homolog (Drosophila)	1.55	◄
	Rps15	Mm.643	BG088213	Ribosomal protein S15	1.55	
►	***Plagl1***	Mm.287857	BG085853	Pleiomorphic adenoma gene-like 1	1.54	◄
►	***Ddc***	Mm.12906	BQ554377	Dopa decarboxylase	1.52	◄
►	***Gas5***	Mm.270065	BG085421	Growth arrest specific 5	1.52	◄
►	***Mllt2h***	Mm.6949	BQ554269	AF4/FMR2 family, member 1	1.52	◄
			AW555131		1.52	
►	***Txnip***	Mm.271877	BG086605	Thioredoxin interacting protein	1.52	◄
►	***Eya2***	Mm.282719	BG069346	Eyes absent 2 homolog (Drosophila)	1.51	◄
►	***Cpm***	Mm.339332	BQ560184	Carboxypeptidase M	1.51	◄
►	***Nudt9***	Mm.241484	BQ561264	Nudix (nucleoside diphosphate linked moiety X)-type motif 9	1.51	◄
	1700084E18Rik	Mm.297949	BG070023	RIKEN cDNA 1700084E18 gene	1.50	
	1700023B02Rik	Mm.292140	BG065239	RIKEN cDNA 1700023B02 gene	1.50	
►	***Pxmp2***	Mm.21853	BG086336	Peroxisomal membrane protein 2	1.50	◄
	2010315L10Rik	Mm.41890	BG063434	RIKEN cDNA 2010315L10 gene	1.50	
	Rpl18a	Mm.379251	BG072556	Ribosomal protein L18A	1.50	
►	***Ucp2***	Mm.171378	BG087187	Uncoupling protein 2 (mitochondrial, proton carrier)	1.50	◄
►	***S100a9***	Mm.2128	BG072801	S100 calcium binding protein A9 (calgranulin B)	1.50	◄

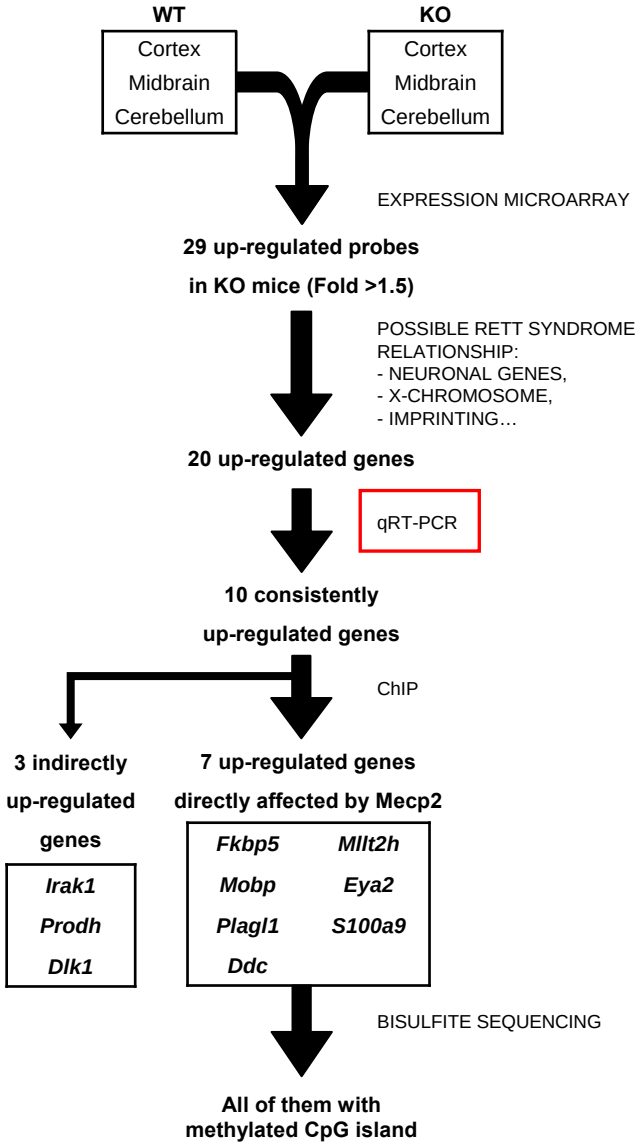
Microarray Results



DOWNREGULATED GENES

	Name	UniGene	GeneBank	Description	n-fold change	
	Sqle	Mm.296169	BG077950	Squalene epoxidase	0.67	
	Rock1	Mm.6710	BG088815	Rho-associated coiled-coil forming kinase 1	0.67	
			BG067593		0.67	
	Scd2	Mm.193096	BG066641	Stearoyl-Coenzyme A desaturase 2	0.66	
	5031439A09Rik	Mm.369129	BQ559666	RIKEN cDNA 5031439A09 gene	0.66	
	Eif4g2	Mm.185453	BG070960	Eukaryotic translation initiation factor 4, gamma 2	0.66	
►	***Calb1***	Mm.277665	BG071405	Calbindin-28K	0.66	◄
	Taf5l	Mm.291777	CK335137	TAF5-like RNA polymerase II, p300/CBP-associated factor	0.65	
	Tcea1	Mm.207263	BG081444	Transcription elongation factor A (SII) 1	0.65	
►	***Calb1***	Mm.277665	BG072229	Calbindin-28K	0.64	◄
			AW539369		0.63	
			BG087011		0.63	
►	***Fabp7***	Mm.3644	AK021271	Fatty acid binding protein 7, brain	0.63	◄
			BQ551801		0.59	
	Rhoa	Mm.757	BG081880	Ras homolog gene family, member A	0.59	
			BQ551690		0.58	
			BG076236		0.58	
►	***Sc4mol***	Mm.30119	BG083949	Sterol-C4-methyl oxidase-like	0.58	◄
		Mm.336117	BG081054	X-linked lymphocyte-regulated 3A	0.58	
			BG085378		0.57	
►	***Itm2a***	Mm.193	BG088255	Integral membrane protein 2A	0.53	◄
			AW558689		0.42	
►	***Gprasp1***	Mm.271980	AW553322	G protein-coupled receptor associated sorting protein 1	0.25	◄
►	***Mecp2***	Mm.131408	BQ553027	Methyl CpG binding protein 2	0.05	◄

qPCR to confirm microarray results



Significative differences

UPREGULATED GENES

	Cortex		Midbrain		Cerebellum	
Fkbp5	**0.0001**	***	**0.0158**	*	**0.0015**	**
Mt1	0.0535		0.1844		0.0939	
Bai1	**0.0299**	*	0.1142		0.0903	
Mt2	0.0776		**0.0191**	*	0.0778	
Pcolce2	0.1591		**0.0064**	**	0.0944	
Irak1	**0.0022**	**	**0.0087**	**	**0.0019**	**
Mobp	**0.0217**	*	**0.0378**	*	**0.0016**	**
ProDH	**0.0488**	*	0.1732		**0.0319**	*
Dlk1	**0.0017**	**	**0.0469**	*	**0.0029**	**
Plagl1	**0.0022**	**	**0.0148**	*	**0.0314**	*
Ddc	**0.0061**	**	**0.0011**	**	**0.0028**	**
Gas5	0.8500		0.5158		0.0810	
Mllt2h	**0.0063**	**	**0.0047**	**	**0.0104**	*
Txnip	0.0610		**0.0380**	*	0.2077	
Eya2	**0.0019**	**	**0.0014**	**	**0.0061**	**
CPM	0.0693		0.1563		0.0502	
Nudt9	0.2216		0.7973		0.8545	
Pxmp2	0.3373		**0.0474**	*	0.1439	
Ucp2	0.0632		0.6477		0.1687	
S100a9	**0.0256**	*	**0.0198**	*	0.0728	
Gtl2	0.5726		0.3441		0.3158	

DOWNREGULATED GENES

	Cortex		Midbrain		Cerebellum	
MeCP2e1	**0.0027**	**	**0.0010**	***	**0.0210**	*
MeCP2e2	**0.0023**	**	**0.0009**	***	**0.0119**	*
Gprasp1	**0.0323**	*	**0.0080**	**	**0.0321**	*
Itm2a	**0.0185**	*	**0.0050**	**	**0.0017**	**
Sc4mol	**0.0078**	**	**0.0459**	*	0.1178	
Fabp7	**0.0205**	*	0.0743		**0.0088**	**
Calb1	**0.0086**	**	0.3338		**0.0405**	*

* p-value < 0.05
** p-value < 0.01
*** p-value < 0.001

qPCR to confirm microarray results

WT
Cortex
Midbrain
Cerebellum

KO
Cortex
Midbrain
Cerebellum

EXPRESSION MICROARRAY

29 up-regulated probes in KO mice (Fold >1.5)

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20 up-regulated genes

qRT-PCR

10 consistently up-regulated genes

ChIP

3 indirectly up-regulated genes

Irak1
Prodh
Dlk1

7 up-regulated genes directly affected by Mecp2

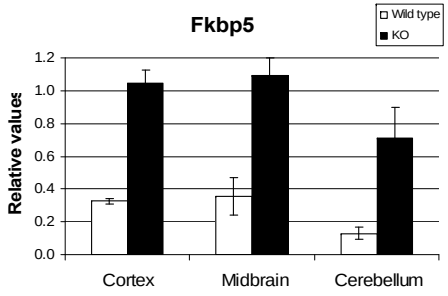
Fkbp5 *Mllt2h*
Mobp *Eya2*
Plagl1 *S100a9*
Ddc

BISULFITE SEQUENCING

All of them with methylated CpG island

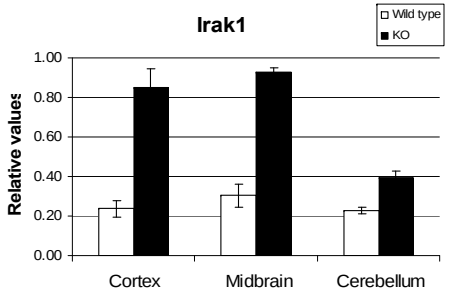
Expression levels

More than 2-fold upregulation



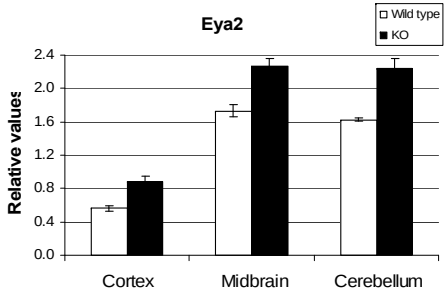
Fkbp5

Around 2-fold upregulation



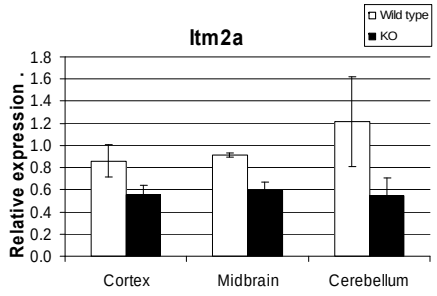
Irak1 **Ddc**
Dlk1 **Mllt2h**

Around 30% upregulation



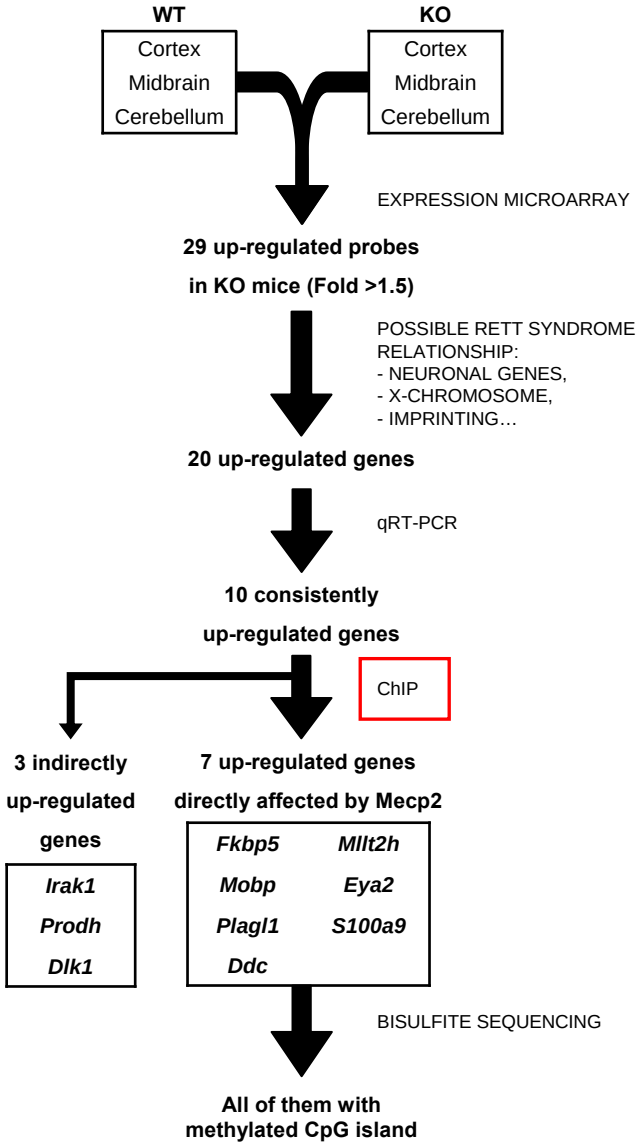
Mobp **Eya2**
ProDH **S100a9**
Plagl1

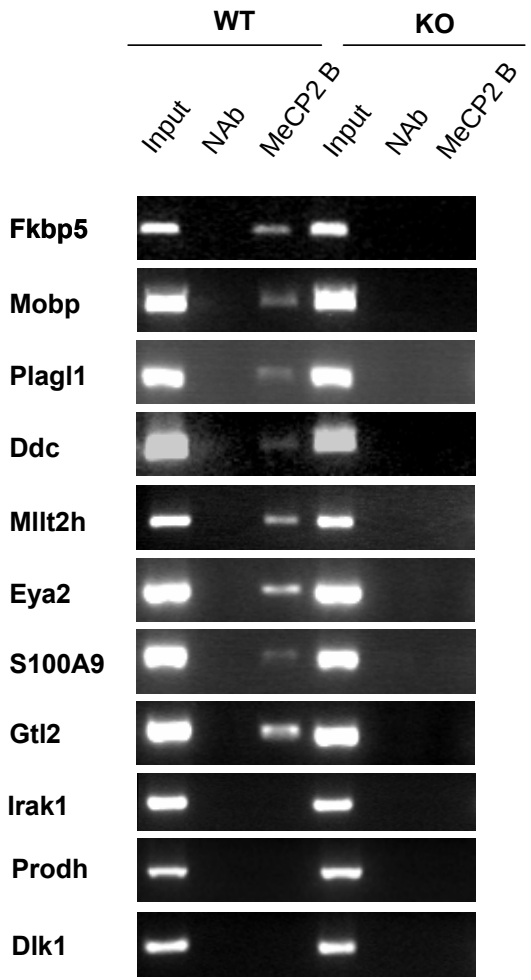
Around 30% downregulation



Gprasp1 **Fabp7**
Itm2a **Calb1**
Sc4mol

ChIP: protein-DNA interactions





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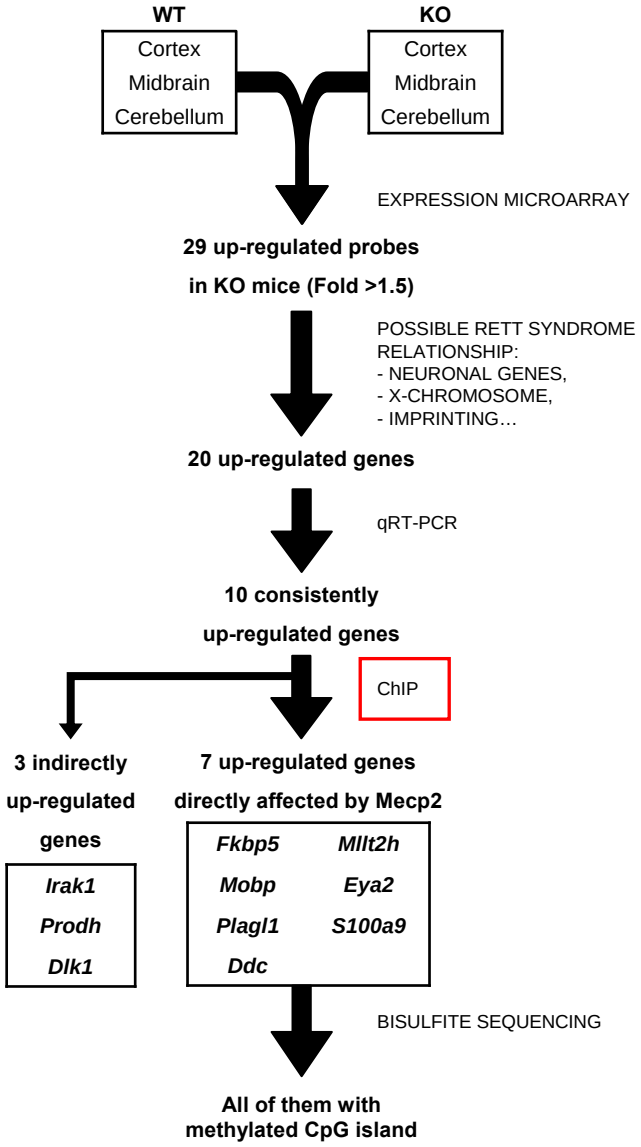
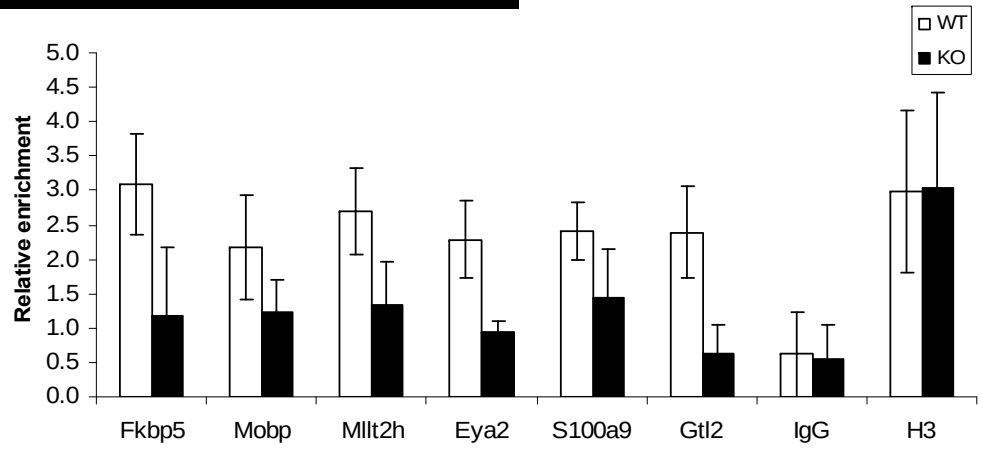
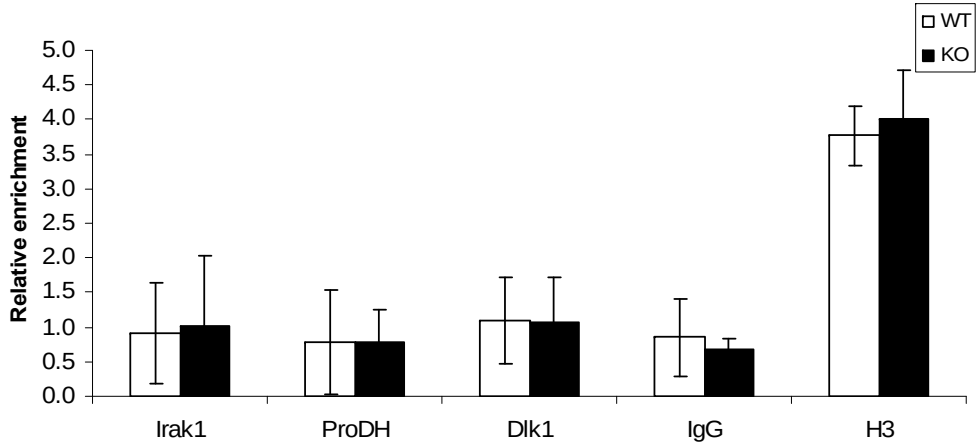


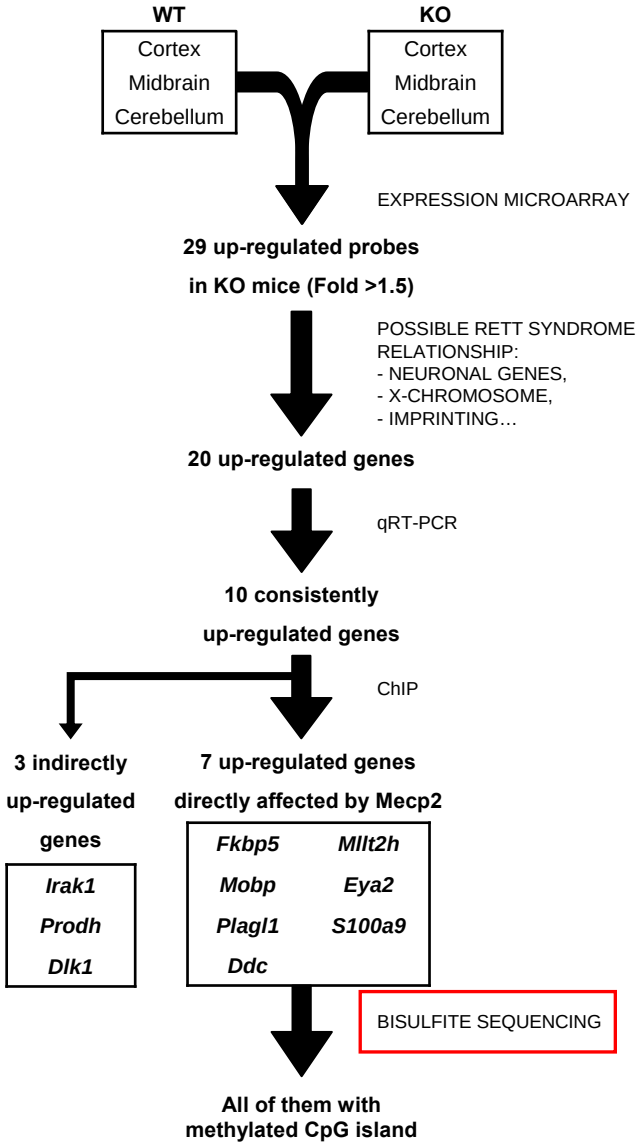
Genes directly bound by MeCP2

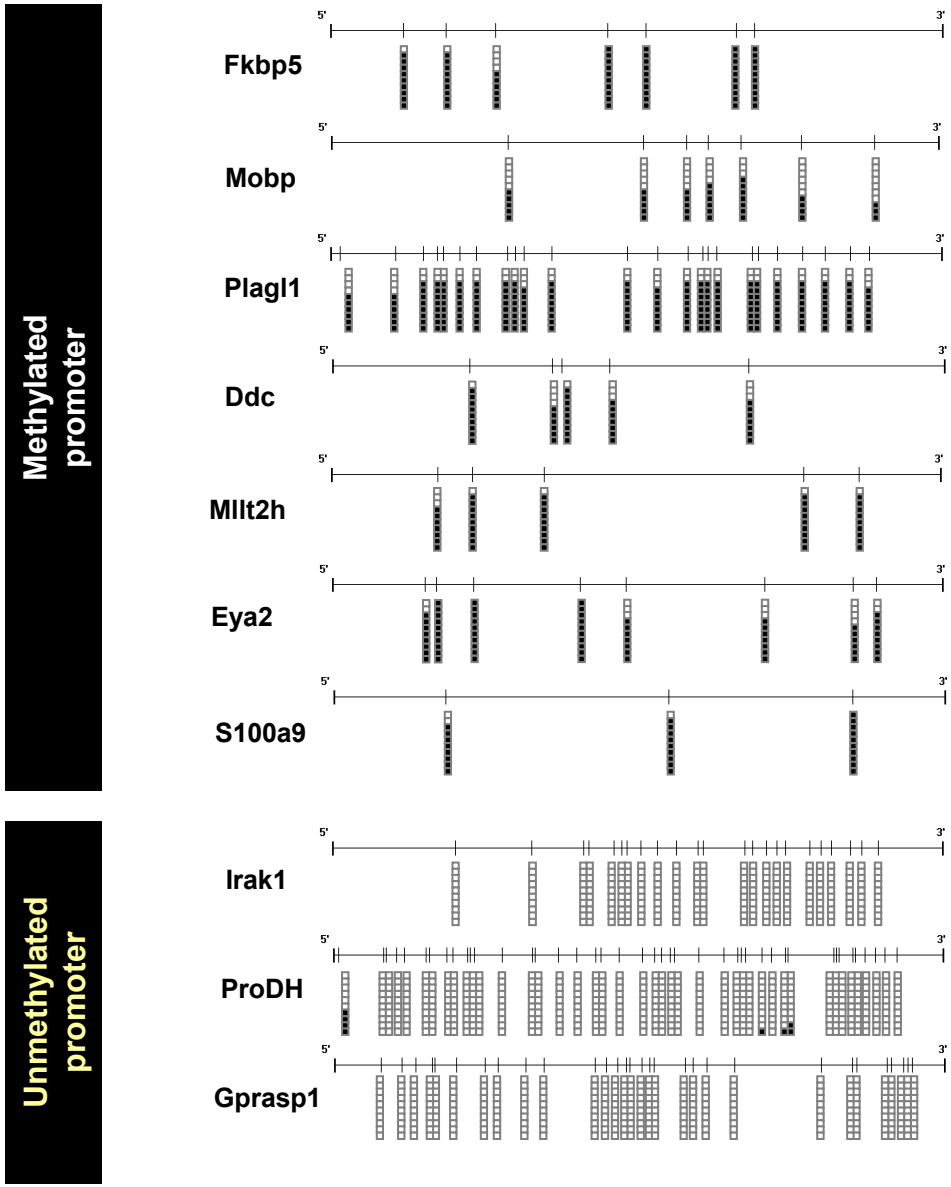


Genes not bound by MeCP2



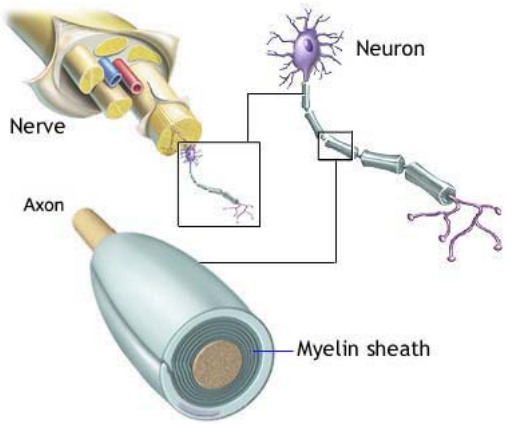
Bisulfite sequencing: Methylation





Mobp

Myelin-associated oligodendrocytic basic protein



Family of abundant CNS myelin proteins

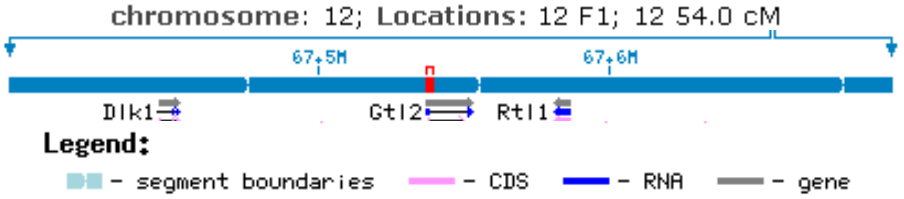
Localizes to major dense line of compact myelin

Dlk1 – Gtl2

Human Molecular Genetics, 2006, Vol. 15, No. 6 **821–830**
doi:10.1093/hmg/ddl001
Advance Access published on January 26, 2006

Imprinted *DLK1* is a putative tumor suppressor gene and inactivated by epimutation at the region upstream of *GTL2* in human renal cell carcinoma

Takahiro Kawakami[1,2,*], Tokuhiro Chano[2,3], Kahori Minami[2], Hidetoshi Okabe[2], Yusaku Okada[1] and Keisei Okamoto[1]



Plagl1

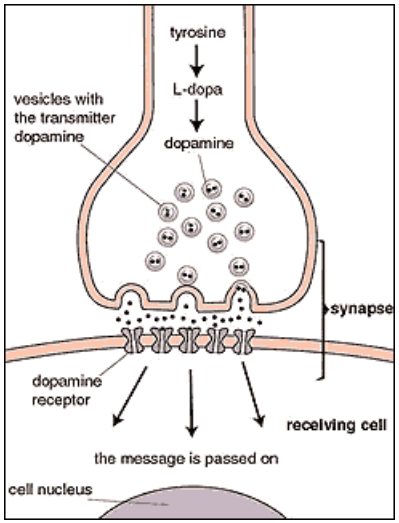
zinc finger protein regulator of apoptosis and cell cycle arrest (Zac1)

Developmental Cell *11*, 711–722, November, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2006.09.003

Zac1 Regulates an Imprinted Gene Network Critically Involved in the Control of Embryonic Growth

Annie Varrault,[1] Charlotte Gueydan,[1] Annie Delalbre,[1] Anja Bellmann,[1] Souheir Houssami,[1] Cindy Aknin,[1,2] Dany Severac,[1,2] Laetitia Chotard,[1] Malik Kahli,[1] Anne Le Digarcher,[1] Paul Pavlidis,[3,4] and Laurent Journot[1,2,*]

Ddc



DOPA decarboxylase

L-Dopa
⇩
Dopamine

L-5-HTP
⇩
Serotonine

Mllt2h

Suggested transcriptional factor

Specifically expressed in Purkinje cell (Af4)

The Journal of Neuroscience, March 1, 2003 • 23(5):1631–1637 • 1631

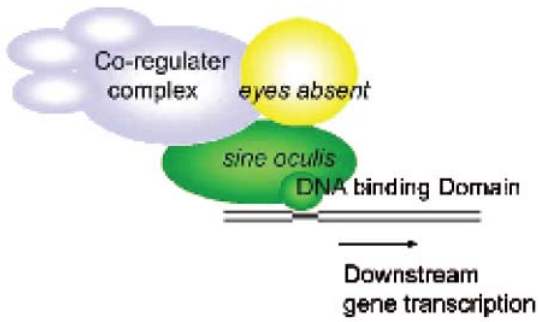
A Mutation in *Af4* Is Predicted to Cause Cerebellar Ataxia and Cataracts in the Robotic Mouse

Adrian M. Isaacs,[1*] Peter L. Oliver,[1*] Emma L. Jones,[1] Alexander Jeans,[1] Allyson Potter,[1] Berit H. Hovik,[1] Patrick M. Nolan,[2] Lucie Vizor,[2] Peter Glenister,[2] A. Katharina Simon,[3] Ian C. Gray,[4] Nigel K. Spurr,[6] Steve D. M. Brown,[2] A. Jackie Hunter,[5] and Kay E. Davies[1]

Eya2

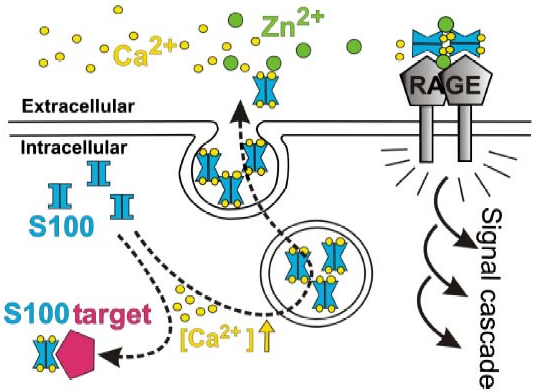
Transcriptional coactivator

Expressed during development of several organs



S100a9

S100 calcium binding protein A9 (calgranulin B)



Marenholz I, Heizmann CW and Fritz G. (2004) Biochem Biophys Res Commun. 322:1111-22

Human diseases associated with S100 proteins

Protein	Disease association
S100A1	Cardiomyopathies
S100A2, S100A3, S100A4, S100A5, S100A6, S100A10, S100P, S100B	Cancer
S100A7, S100A7L1/A15	Psoriasis
S100A8, S100A9, S100A12	Inflammatory disorders
S100B	Neurodegeneration

Summary

Mecp2-null mice — wild-type littermates

MICROARRAY

29 upregulated genes (Fold>1.5)

POSSIBLE RETT SYNDROME RELATIONSHIP

20 selected genes

qRT-PCR

10 consistently upregulated genes

ChIP

7 genes directly regulated by Mecp2

3 genes indirectly regulated by Mecp2

BISULFITE SEQUENCING

Methylated promoter: Mecp2 binding site

Unmethylated promoter

Plagl1	Ddc	Mobp	Mllt2h	Eya2	S100A9	Irak1 ProDH Dlk1
IMPRINTING	NEUROTRANSM. METABOLISM	MIELIN COMPONENT	NEURONAL REGULATOR?	INFLAMATORY RESPONSE	TRANSCRIPTIONAL COACTIVATOR	

Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice

Daniela Tropea[a,1], Emanuela Giacometti[b,1], Nathan R. Wilson[a,1], Caroline Beard[b], Cortina McCurry[a], Dong Dong Fu[b], Ruth Flannery[b], Rudolf Jaenisch[b,c,2], and Mriganka Sur[a,2]

[a]Picower Institute for Learning and Memory and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; [b]Whitehead Institute for Biomedical Research, Cambridge, MA 02142; and [c]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Rudolf Jaenisch, December 11, 2008 (sent for review November 9, 2008)

Rett Syndrome (RTT) is a severe form of X-linked mental retardation caused by mutations in the gene coding for methyl CpG-binding protein 2 (MECP2). Mice deficient in MeCP2 have a range of physiological and neurological abnormalities that mimic the human syndrome. Here we show that systemic treatment of MeCP2 mutant mice with an active peptide fragment of Insulin-like Growth Factor 1 (IGF-1) extends the life span of the mice, improves locomotor function, ameliorates breathing patterns, and reduces irregularity in heart rate. In addition, treatment with IGF-1 peptide increases brain weight of the mutant mice. Multiple measurements support the hypothesis that RTT results from a deficit in synaptic maturation in the brain: MeCP2 mutant mice have sparse dendritic spines and reduced PSD-95 in motor cortex pyramidal neurons, reduced synaptic amplitude in the same neurons, and protracted cortical plasticity in vivo. Treatment with IGF-1 peptide partially restores spine density and synaptic amplitude, increases PSD-95, and stabilizes cortical plasticity to wild-type levels. Our results thus strongly suggest IGF-1 as a candidate for pharmacological treatment of RTT and potentially of other CNS disorders caused by delayed synapse maturation.

IGF-1 | plasticity | autism | brain | synapse

phenotype (18). Furthermore, reduced BDNF expression in the brainstem correlates with respiratory dysfunction in MeCP2 mutant mice, and enhancing BDNF expression ameliorates respiratory symptoms (19). Unfortunately, the therapeutic utility of BDNF is hampered by its poor efficiency at crossing the blood–brain barrier. Nevertheless, a therapeutic intervention in humans might thus arise from identifying an agent similarly capable of stimulating synaptic maturation.

A second pleiotrophic growth factor with promise in CNS therapy is Insulin-like Growth Factor 1 (IGF-1). Like BDNF, IGF-1 is widely expressed in the CNS during normal development (20), strongly promotes neuronal cell survival and synaptic maturation (20, 21), and facilitates the maturation of functional plasticity in the developing cortex (22). While BDNF stimulates synaptic strengthening via a pathway involving PI3K/pAkt/PSD-95 (23) and MAPK signaling (17), IGF-1 stimulates the same pathways (22, 24) and has been shown to elevate excitatory postsynaptic currents significantly (25). The biological action of IGF-1 is also regulated by the binding of IGF binding proteins (IGFBP1–6), which may be of significance to RTT and other disorders. IGFBP3, for example, has a binding site for the MeCP2 protein (26), and MeCP2 null mice and RTT patients express

Epigenetics Laboratory at Epigenetics and Cancer Biology Programme

Group Leader: Manel Esteller, M.D., Ph.D.

Rocio G. Urdinguio, Bs.Sci.
Agustín Fernández, Ph.D.
Pilar López-Nieva, Ph.D.
Maria Berdasco, Ph.D.
Hiroaki Taniguchi, Ph.D.
Filipe V. Jacinto, Bs.Sci
Amaia Lujambio, Bs.Sci
Sonia M. Almeida, Bs.Sci.
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