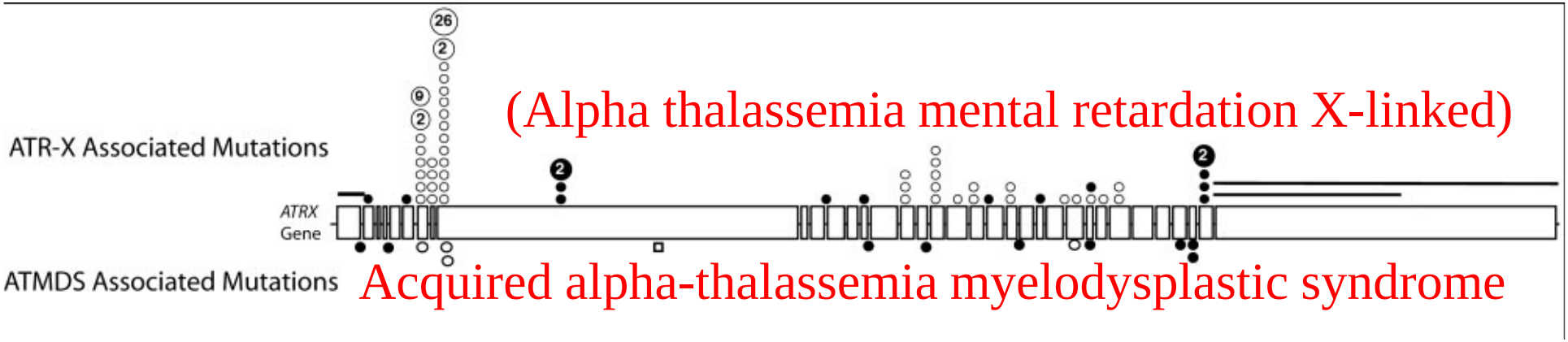
Alpha **t**halassemia mental **r**etardation **X**-linked

Acquired alpha-**t**halassemia **m**yelodysplastic **s**yndrome



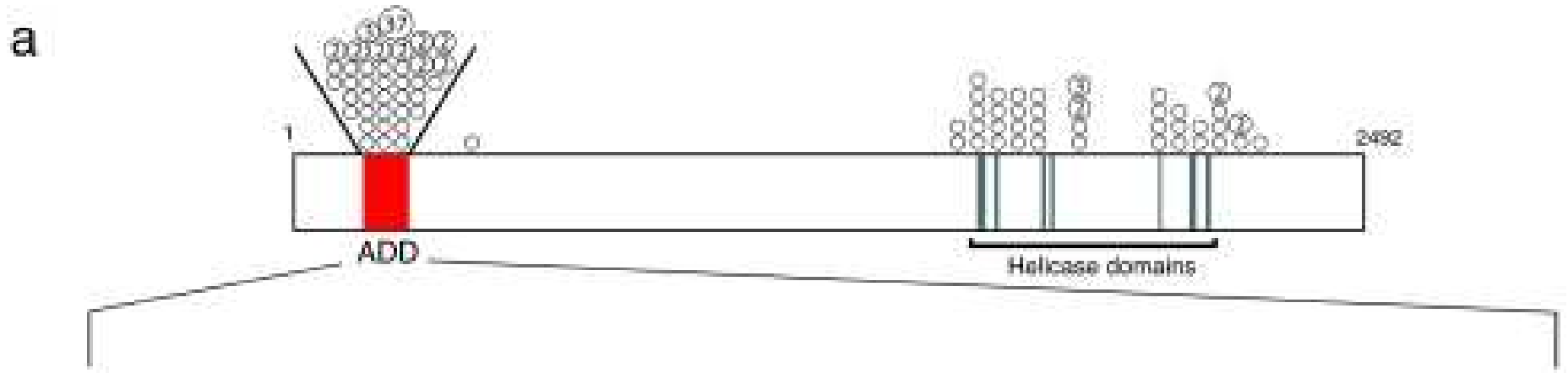
Schematic representation of the spectrum of *ATRX* mutations that have been described in boys with ATR-X syndrome with those found in ATMDS.

Mutations predicted to cause protein truncation (frameshifts and nonsense mutations) and null mutations. Amino acid changes, including in-frame insertions and deletions. Recurrent mutations in ATR-X syndrome are indicated by a number inside the circle, representing the number of families in whom the mutation has been identified.

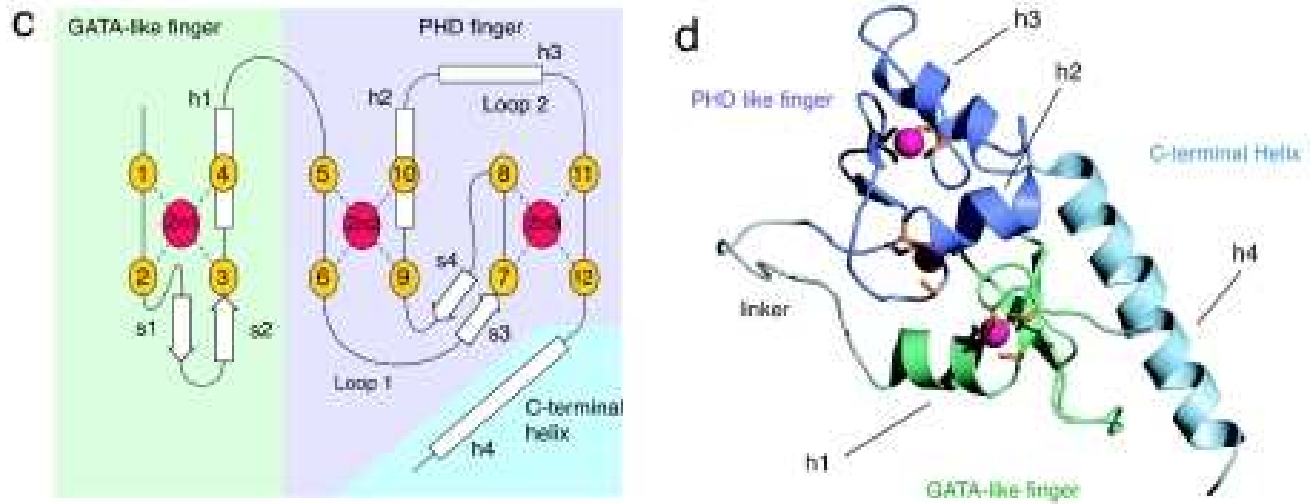
- The chromatin-associated protein ATRX was originally identified because mutations in the ATRX gene cause a severe form of syndromal X-linked mental retardation associated with α-thalassemia.

- The disease-causing mutations fall into two groups: the majority affect buried residues and hence affect the structural integrity of the ADD domain; another group affects a cluster of surface residues, and these are likely to perturb a potential protein interaction site. The effects of individual point mutations on the folding state and stability of the ADD domain correlate well with the levels of mutant ATRX protein in patients, providing insights into the molecular pathophysiology of ATR-X syndrome

ATRX mutations



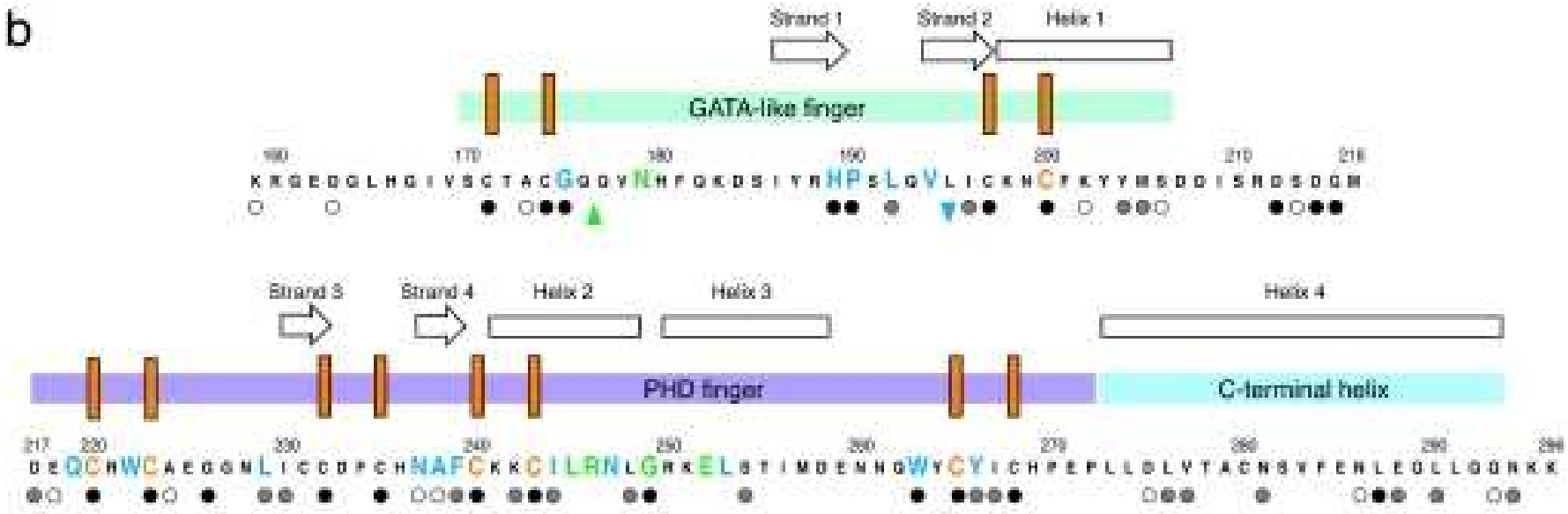
- Half of all of the disease-associated missense mutations cluster in a cysteine-rich region in the N terminus of ATRX.
  This region was named the *A*TRX-*D*NMT3-*D*NMT3L (ADD) domain, based on sequence homology with a family of DNA methyltransferases
  The positions of missense mutations are indicated with circles and the number of times (>1) the mutation has been identified in unrelated individuals is indicated within relevant circles. All of the circles drawn between the oblique lines above the bar refer to mutations within the ADD domain.



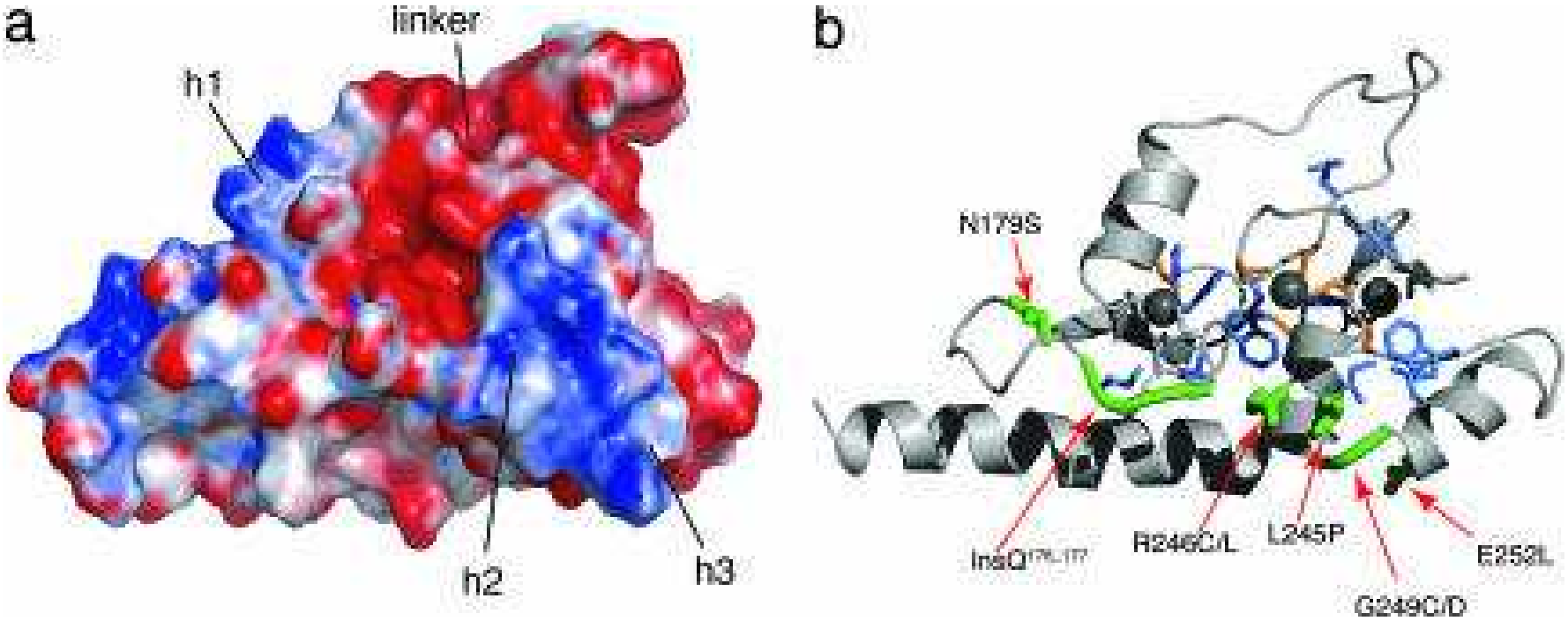
the structure of the ADD domain of ATRX consists of an N-terminal GATA-like zinc finger, a plant homeodomain finger (PHD), and a long C-terminal α-helix that pack together to form a single globular domain. The α-helix of the GATA-like finger is exposed and highly basic, suggesting a DNA-binding function for ATRX.

β-Strands are labeled s1–s4 and helices h1–h4.

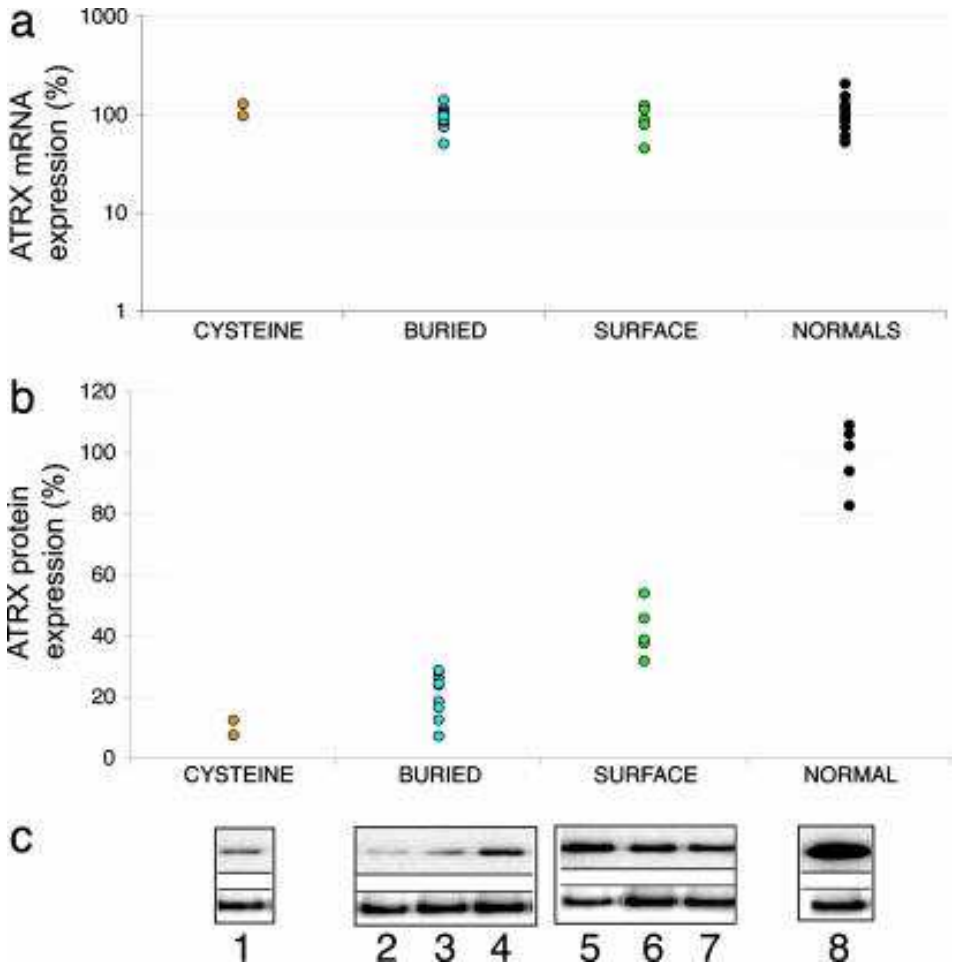
Locations of mutations and secondary structural elements in the ADD domain



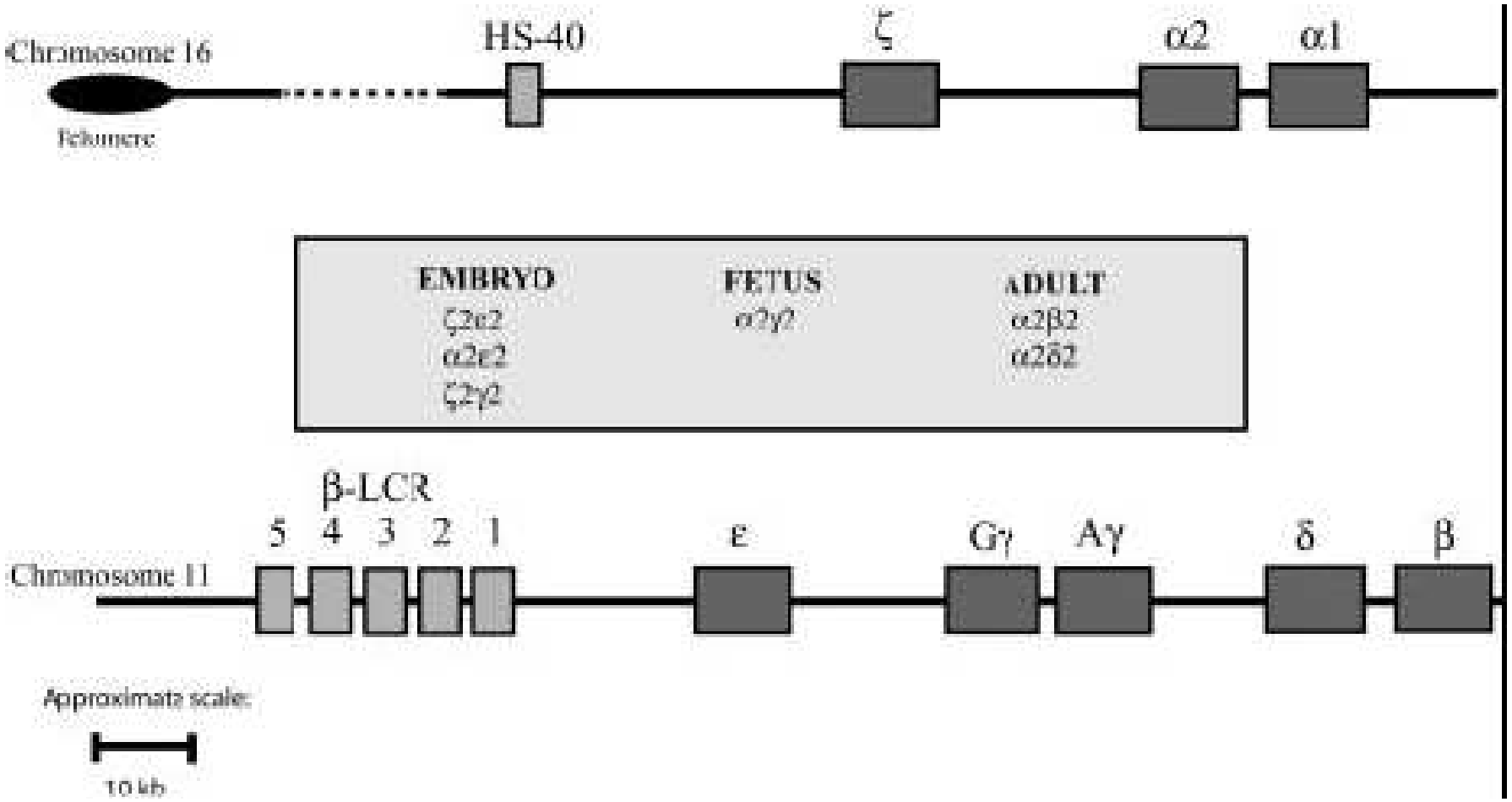
- Locations of mutations and secondary structural elements in the ADD domain. The N-terminal GATA-like zinc finger is indicated by a light green bar, the PHD finger by a mauve bar, and the C-terminal extension by a light blue bar. The conserved cysteine residues are marked as orange vertical bars.
- Missense mutations are highlighted in green (surface), blue (buried), and orange (cysteines); the insertion mutation is highlighted by an upward green arrow and the deletion by a downward blue arrow. Residues where there is homology across the whole family of ADD domain sequences (ATRX, DNMT3A, DNMT3B, and DNMT3L) are marked with filled circles (absolute conservation), gray circles (strong conservation), and open circles (weak conservation)



- Electrostatic potential and location of mutations in the structure of the ADD domain.
- (*a*) Surface electrostatic potential of the ADD domain. The helix in the GATA-like finger (h1) is solvent-exposed and basic, and the two helices within loop 2 of the PHD finger (h2 and h3) form another basic patch. The linker between the GATA-like and PHD fingers is highly acidic.
- (*b*) Ribbon structure of the ADD domain showing the locations of mutations found in patients with ATR-X syndrome. Mutations are classified as surface (green), buried (blue), or cysteine (orange) and are represented by using their side chains, except for the glycine mutation G249C/D and the glutamine insertion, which are represented by thickening the backbone. The surface mutations are individually labeled



- ATRX *in vivo* expression in EBV-transformed patient lymphocytes.
- (*a*) ATRX mRNA levels of patient mutations and normal controls as determined by quantitative RT-PCR. Patients are grouped according to the nature of their underlying mutation: cysteine mutations are orange, buried mutations are blue, and surface mutations are green. Values for normal individuals are represented by black circles. For each case, the ATRX mRNA level is expressed as the percentage of the average for 18 normal control individuals.
- (*b*) ATRX protein levels of patients and normal controls. ATRX protein levels are expressed as a percentage of the average value for seven normal control individuals.
- (*c*) Representative Western blots showing ATRX protein levels (including loading control). Lane 1 represents the ATRX protein level for a cysteine mutation, lanes 2–4 are buried mutations, lanes 5–7 are surface mutations, and lane 8 is a normal control.


Chromosome 16
Telomere
HS-40
ζ
α2
α1
EMBRYO
ζ2ε2
α2ε2
ζ2γ2
FETUS
α2γ2
ADULT
α2β2
α2δ2
β-LCR
5 4 3 2 1
ε
Gγ
Aγ
δ
β
Chromosome 11
Approximate scale:
10 kb

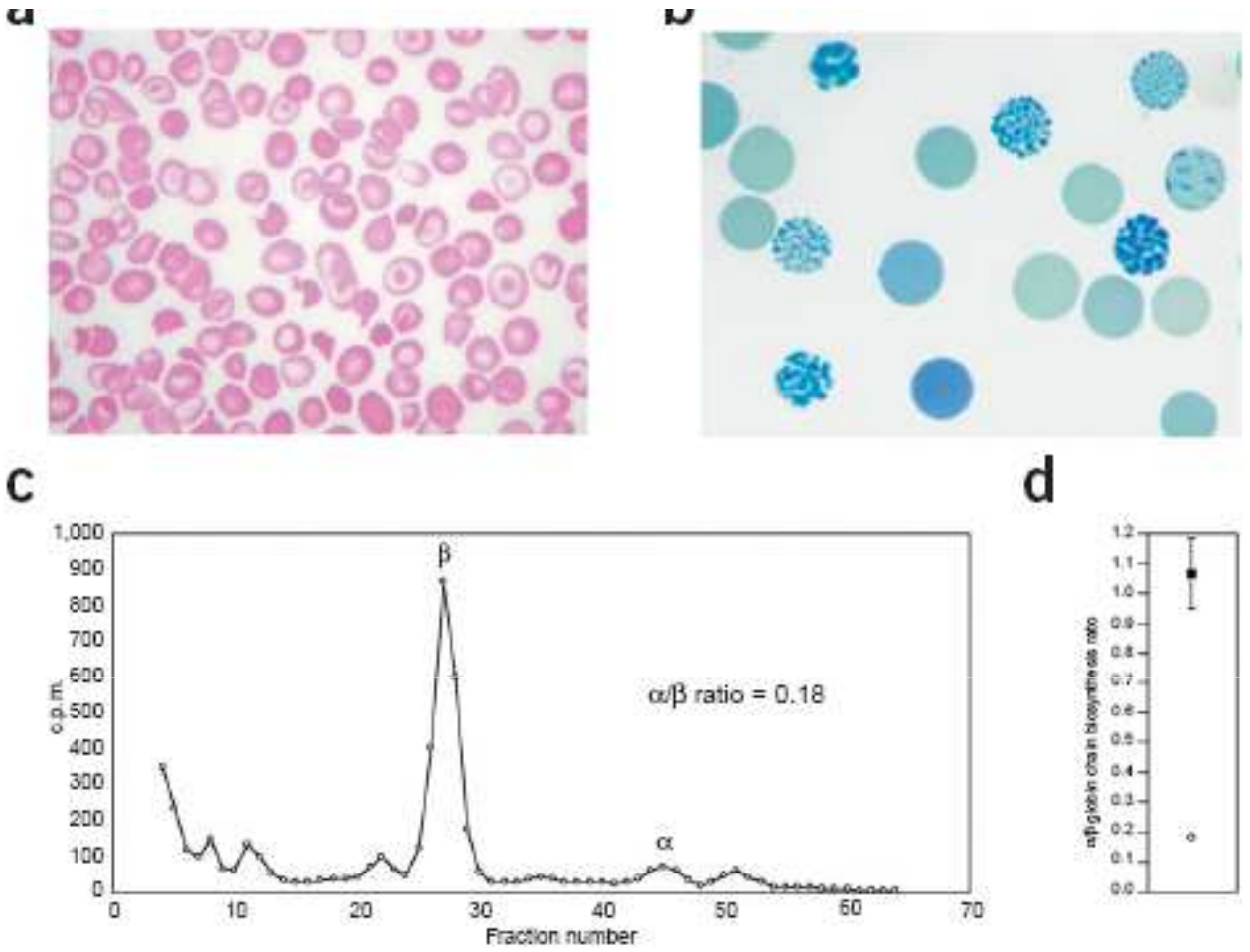
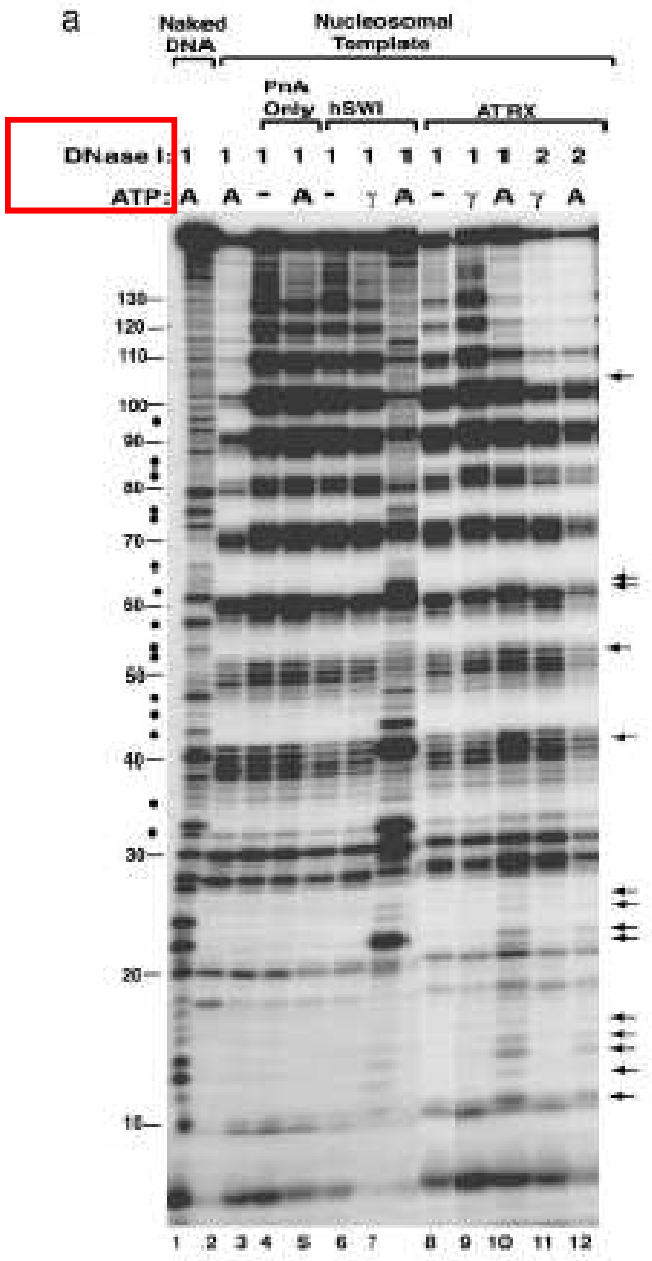


Figure 1 Hematological analysis in individuals with ATMDS. (**a**) Blood film from individual 1 showing hypochromia, anisocytosis, poikilocytosis and target cells. (**b**) Blood film of blood from individual 1 stained with brilliant cresyl blue showing the presence of cells with hemoglobin H (β_4) inclusions. (**c**) Globin chain biosynthesis plot showing marked reduction in the ratio of α/β globin synthesis in individual 2. (**d**) A comparison of the α/β globin chair biosynthesis ratios for individual 2 (open circle) and the mean for normal controls (filled square).

- ATRX is part of a multiprotein complex that uses the energy of ATP to remodel chromatin or its associated DNA in a way that affects transcriptional activity at euchromatic loci, including the -globin gene cluster.

POSIZIONMENTO NUCLEOSOMA Rimodellamento Cromatina


a
Naked DNA
Nucleosomal Template
PnA Only
hSWI
ATRX
DNase I: 1 1 1 1 1 1 1 1 1 1 2 2
ATP: A A - A - γ A - γ A γ A
130
120
110
100
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12

ATRX function may be associated with its presence at one of its nuclear locations:

Heterochromatin

ribosomal DNA repeats

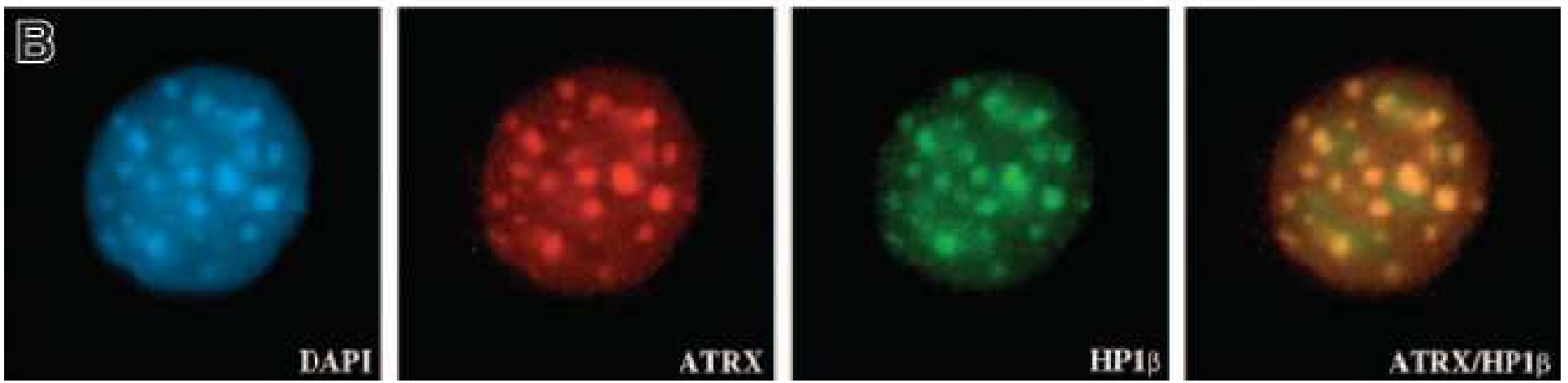
PML bodies

euchromatic sites.

- Promyelocytic leukaemia (PML) nuclear bodies (NBs) are macromolecular nuclear domains present in virtually every mammalian cell. PML nuclear bodies (PML-NBs) were functionally linked to various fundamental cellular processes, including transcriptional control, tumour suppression and apoptosis regulation.

ATRX: funzioni

- Alteration of the regional distribution of heterochromatin by the complex or of recruitment of transcription factors that alter gene expression ?



ATRX colocalizes with HP1 at heterochromatin (in murine L929 cells).

HP1

- structural protein that plays a role in heterochromatin formation, gene silencing
- three genes HP1Hsα, HP1Hsβ and HP1Hsγ.
- distinct chromosomal localization patterns
- HP1 contain a chromo domain that binds methylated **K9H3**
- HP1 dimers establish a platform in which nuclear proteins interact
- Association of HP1 with a target gene causes alterations in chromatin structure and gene silencing

Table 1 | Chromatin modifications

Mark*	Transcriptionally relevant sites†	Transcriptional role‡
DNA methylation		
Methylated cytosine (meC)	CpG islands	Repression
Histone PTMs		
Acetylated lysine (Kac)	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A, H2B	Activation
Phosphorylated serine/threonine (S/Tph)	H3 (3, 10, 28), H2A, H2B	Activation
Methylated arginine (Rme)	H3 (17, 23), H4 (3)	Activation
Methylated lysine (Kme)	H3 (4, 36, 79) H3 (9, 27), H4 (20)	Activation Repression
Ubiquitylated lysine (Kub)	H2B (123§/120¶) H2A (119¶)	Activation Repression
Sumoylated lysine (Ksu)	H2B (6/7), H2A (126)	Repression
Isomerized proline (Pisom)	H3 (30–38)	Activation/repression

*The modification on either DNA or a histone.
†Well-characterized sites with regard to genomic location for DNA methylation or residues within histones for PTMs.
‡Whether the epigenetic mark is associated with activation or repression.
§Yeast (*Saccharomyces cerevisiae*).
¶Mammals.

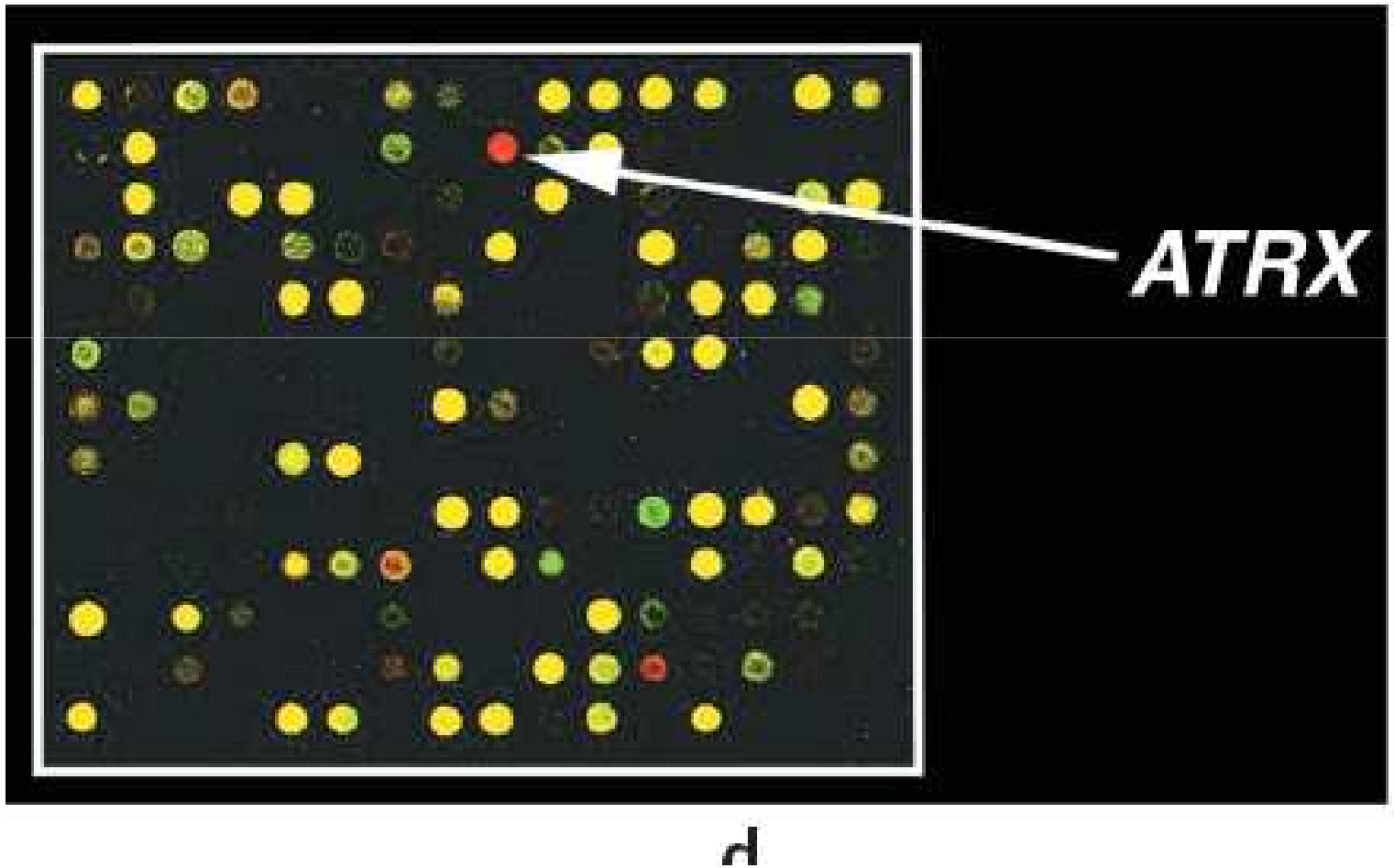
ATRX: funzioni

- Alteration of the regional distribution of heterochromatin by the complex or of recruitment of transcription factors (directly or indirectly) that alter gene expression ?

  epigenetic modifications of histone-associated DNA, leading to alterations in local chromatin conformation.

  binding to DNA of transcription factors is affected by the ATRX complex

RNA profiling in ATMD and **normal** granulocytes



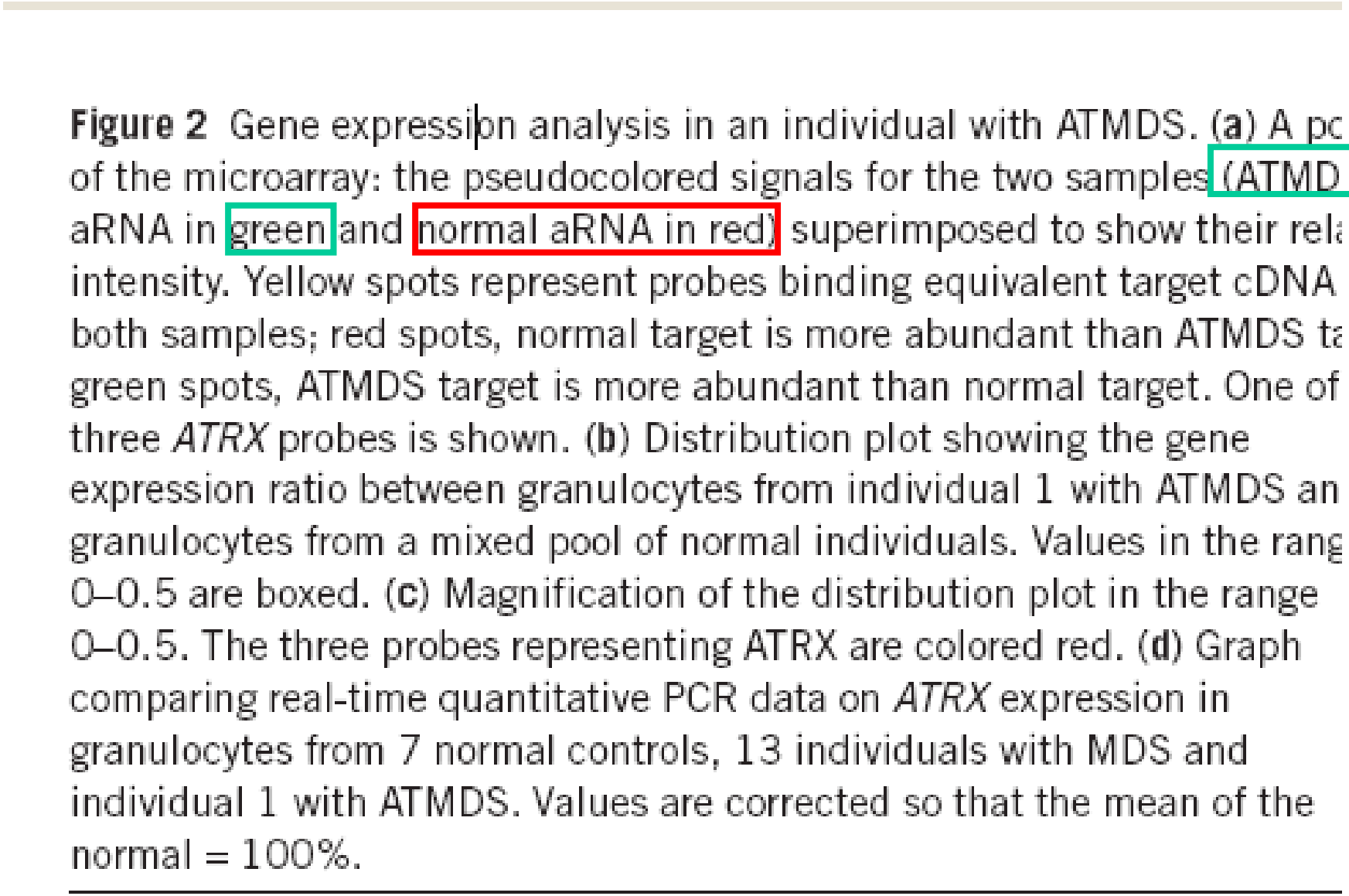
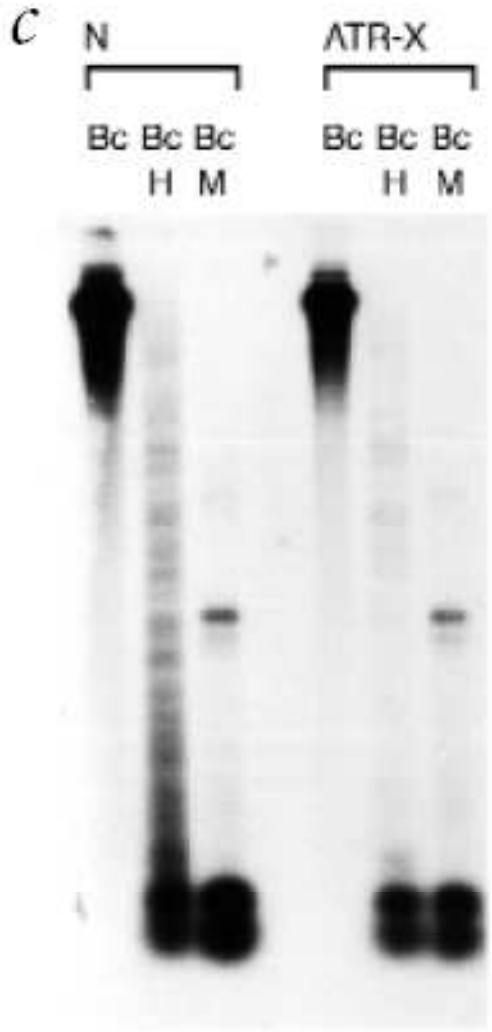


Figure 2 Gene expression analysis in an individual with ATMDS. (**a**) A pc of the microarray: the pseudocolored signals for the two samples (ATMD aRNA in green and normal aRNA in red) superimposed to show their rel intensity. Yellow spots represent probes binding equivalent target cDNA both samples; red spots, normal target is more abundant than ATMDS ta green spots, ATMDS target is more abundant than normal target. One of three *ATRX* probes is shown. (**b**) Distribution plot showing the gene expression ratio between granulocytes from individual 1 with ATMDS an granulocytes from a mixed pool of normal individuals. Values in the rang 0–0.5 are boxed. (**c**) Magnification of the distribution plot in the range 0–0.5. The three probes representing ATRX are colored red. (**d**) Graph comparing real-time quantitative PCR data on *ATRX* expression in granulocytes from 7 normal controls, 13 individuals with MDS and individual 1 with ATMDS. Values are corrected so that the mean of the normal = 100%.

METILAZIONE del DNA e ATRX

Paziente



Bcl I + *Hpa*II or *Msp*I
RibC7b5

Table 1 • The pattern of methylation in ATR-X syndrome[a]

Sequence	Hypomethylation	Hypermethylation	Unaffected
rDNA	+		
5SDNA			+
satellite 1 (oligo)			+
satellite 1 (DYZ2)		+	
satellite 2 (oligo)			+
satellite 3 (DYZ1)			+
α-satellite (oligo)			+
β-satellite (oligo)			+
Alu (consensus)			+
L1 (consensus)			+
TTAGGG (oligo) (telomeric)			+
Tel Bam 3.4 (subtelomeric)		(+)	
Tel Bam 11 (subtelomeric)			+
XIST[b]			+
D15S63 (PWS)[c]			+
α-globin cluster[d]			+

- **Mutations in the human methyl-CpG-binding protein gene *MECP2* cause a neurological disorder (Rett syndrome)**

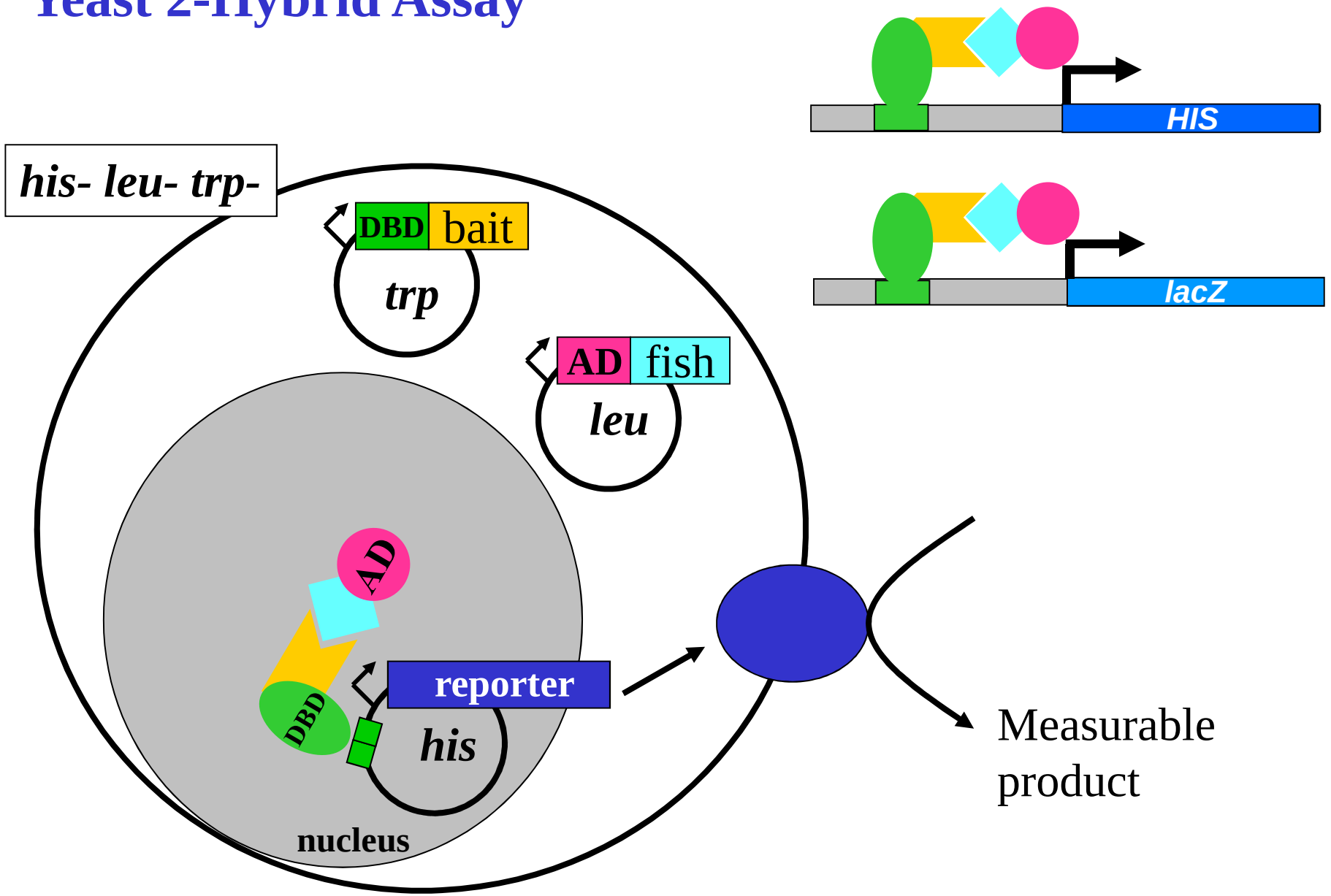
- **Interaction between chromatin proteins MECP2 and ATRX**

Kriaucionis *PNAS* 2007;104;2709-2714**.**

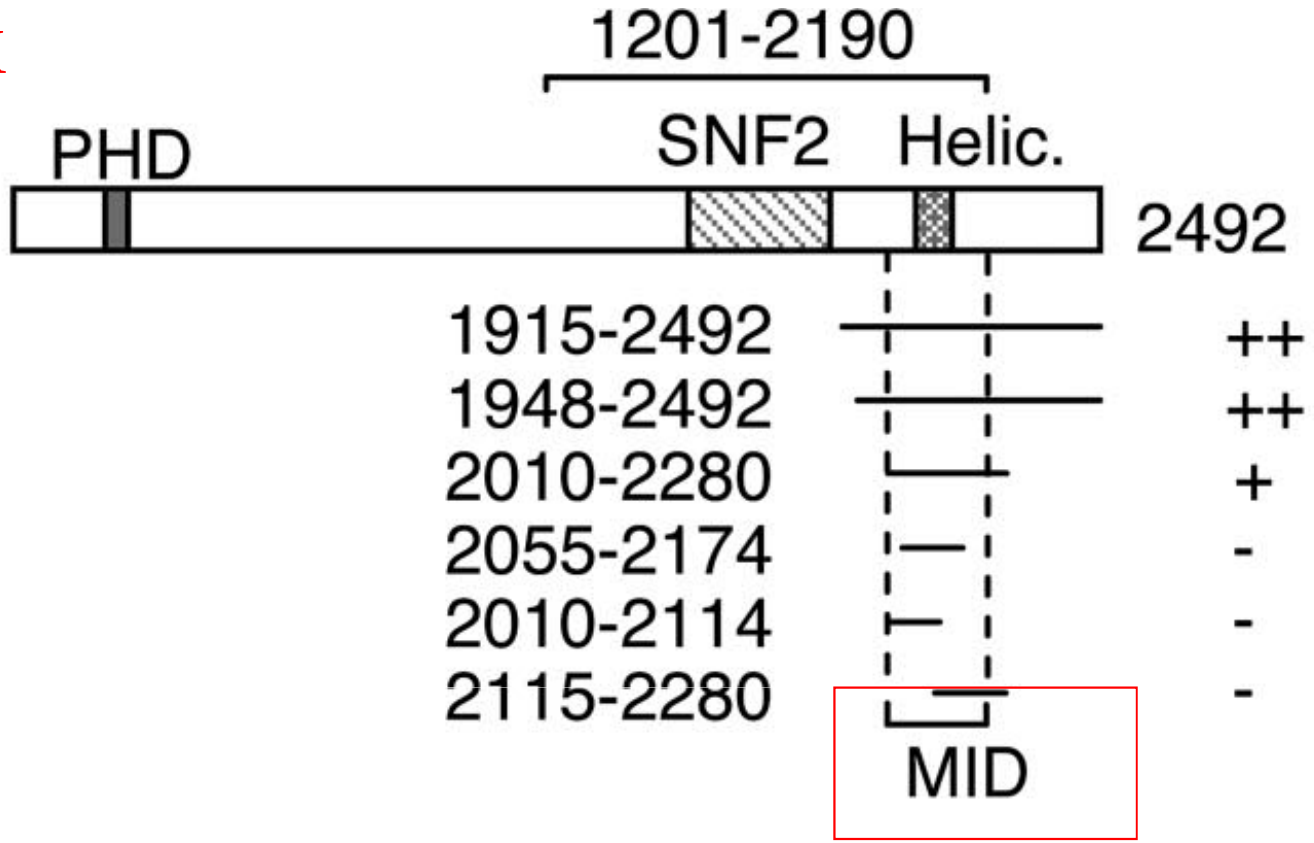
MeCP2 interacts with ATRX.

- (*a*) Yeast two-hybrid assays identified a region of ATRX that interacts with MeCP2. The diagram depicts ATRX and its domains. Fragments 1915–2492 and 1948–2492 (amino acids numbered from the N terminus) were original clones from the library screen. Symbols indicate the strength of the MeCP2 interaction. The MeCP2 interaction domain (MID) is marked.
- (*b*) The ATRX-interaction region overlaps theMBDof MeCP2. The MeCP2 region that binds ATRX was identified by GST pulldown by using a deletion series of MeCP2-GST fusion proteins (for polyacrylamide gel of proteins, see SI Fig. 7) incubated with *in vitro*-translated [35S]-labeled ATRX fragment 1201–2190 (bracket; *a*). The input lane (*Top*) contained 25% of labeled protein used for the pull-down assay.
- (*c*) Summary of GST-pulldown showing presence or absence of an ATRX interaction leading to identification of an ATRX-interacting domain (AxID). Point mutations that inhibit ATRX binding (R133C, A140V, and R168X; see Fig. 5) are marked by arrows.
- (*d*) Native ATRX in nuclear extracts from brain is ‘‘pulled down’’ by immobilized full-length MeCP2.AMeCP2-GST fusion protein (M) or GST alone (G) were immobilized on glutathione beads and mixed with extract. Bound proteins and input (In; 10%) were separated and probed with anti-ATRX antibody on a Western blot.
- (*e*) CoIP of HA-tagged MeCP2 and GFP-fusion ATRX(NLS1201–2492) expressed transiently in mouse L cells. ‘‘Input’’ lane shows 10% of input amount. Antibodies used for coIP and blot visualization are labeled above and beside the figure, respectively

Yeast 2-Hybrid Assay



ATRX



MecP2 Interaction Domain

MeCP2 interacts with ATRX.

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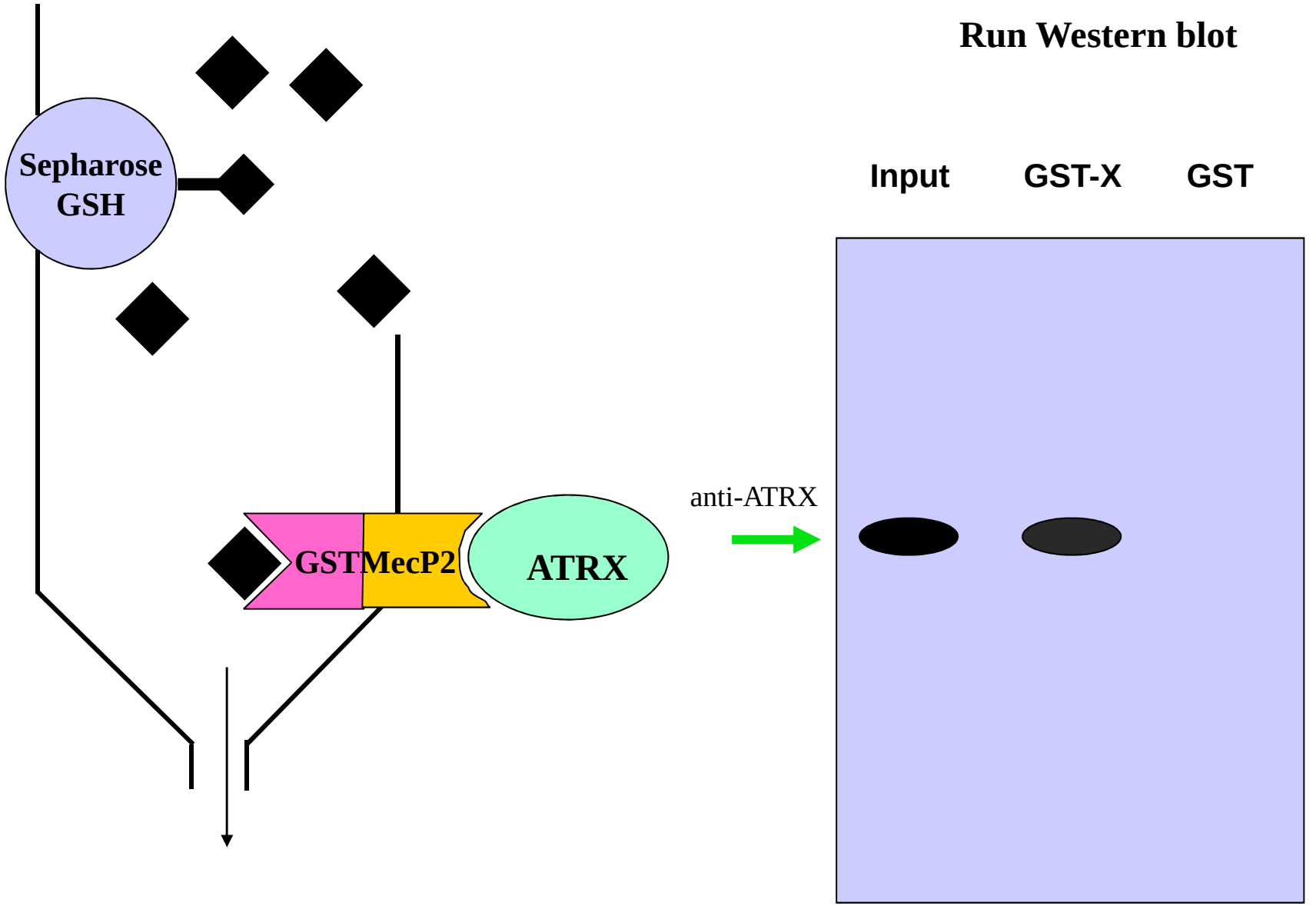
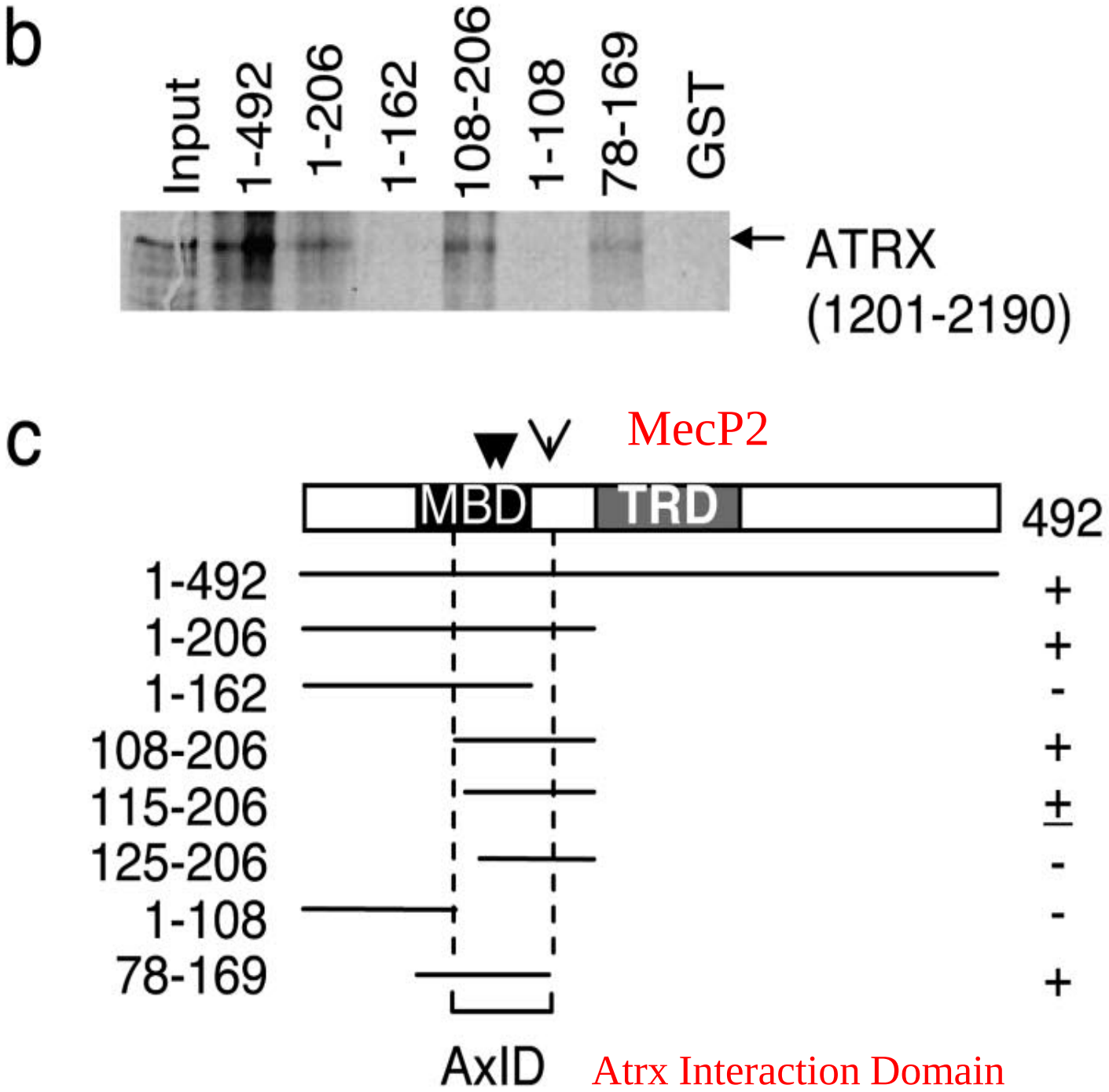
- Symbols indicate the strength of the MeCP2 interaction. The MeCP2 interaction domain (MID) is marked.

GST pull-down assay




b
Input
1-492
1-206
1-162
108-206
1-108
78-169
GST
ATRX
(1201-2190)
c
MecP2
MBD
TRD
492
1-492
1-206
1-162
108-206
115-206
125-206
1-108
78-169
+
+
-
+
±
-
-
+
AxID
Atrx Interaction Domain

MeCP2 interacts with ATRX.

- (*b*) The ATRX-interaction region overlaps theMBDof MeCP2. The MeCP2 region that binds ATRX was identified by GST pulldown by using a deletion series of MeCP2-GST fusion proteins incubated with *in vitro*-translated [35S]-labeled ATRX fragment 1201–2190 (bracket; *a*). The input lane (*Top*) contained 25% of labeled protein used for the pull-down assay.
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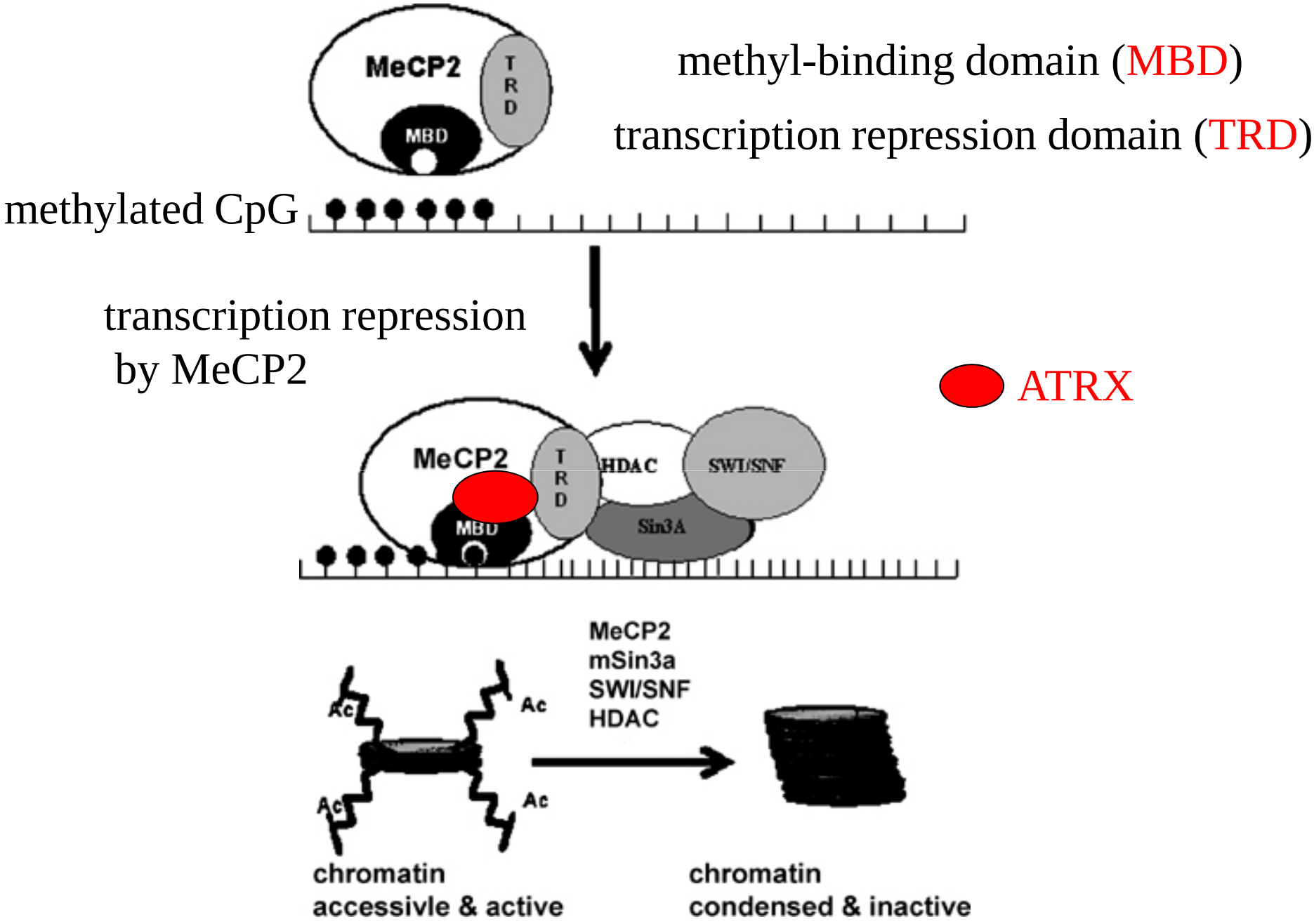
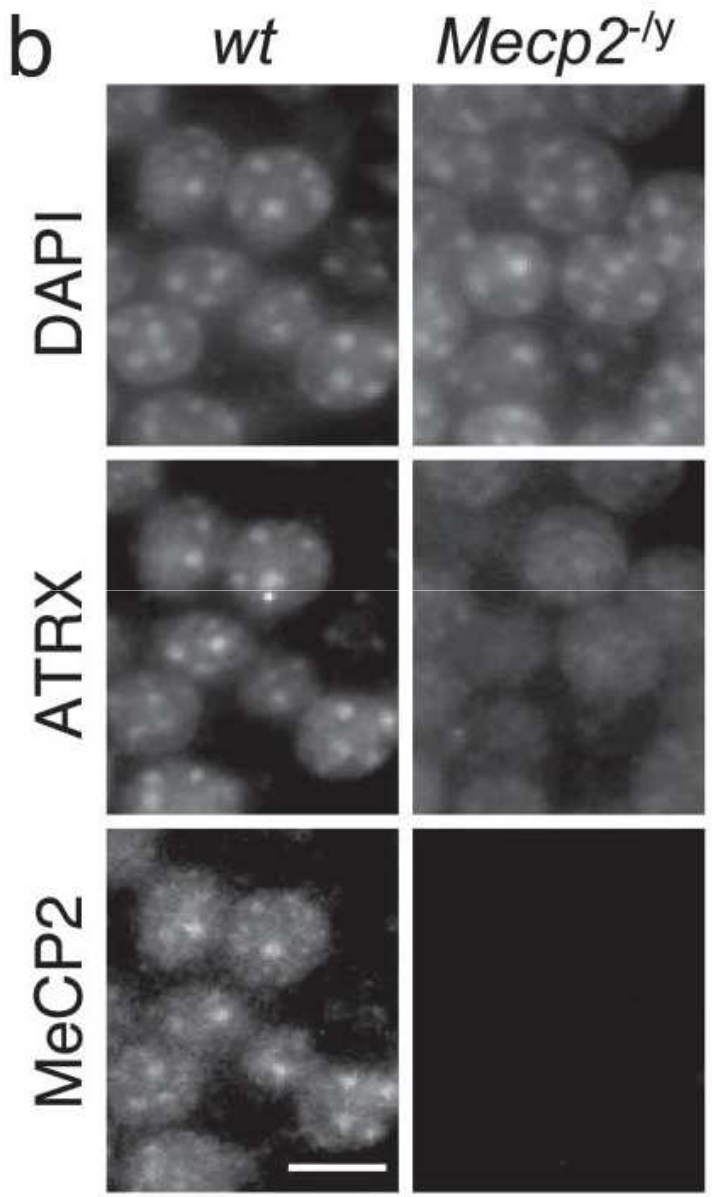

MeCP2
TRD
MBD
methyl-binding domain (MBD)
transcription repression domain (TRD)
methylated CpG
transcription repression
by MeCP2
ATRX
MeCP2
TRD
HDAC
SWI/SNF
MBD
Sin3A
MeCP2
mSin3a
SWI/SNF
HDAC
Ac
Ac
Ac
Ac
chromatin
accessivle & active
chromatin
condensed & inactive

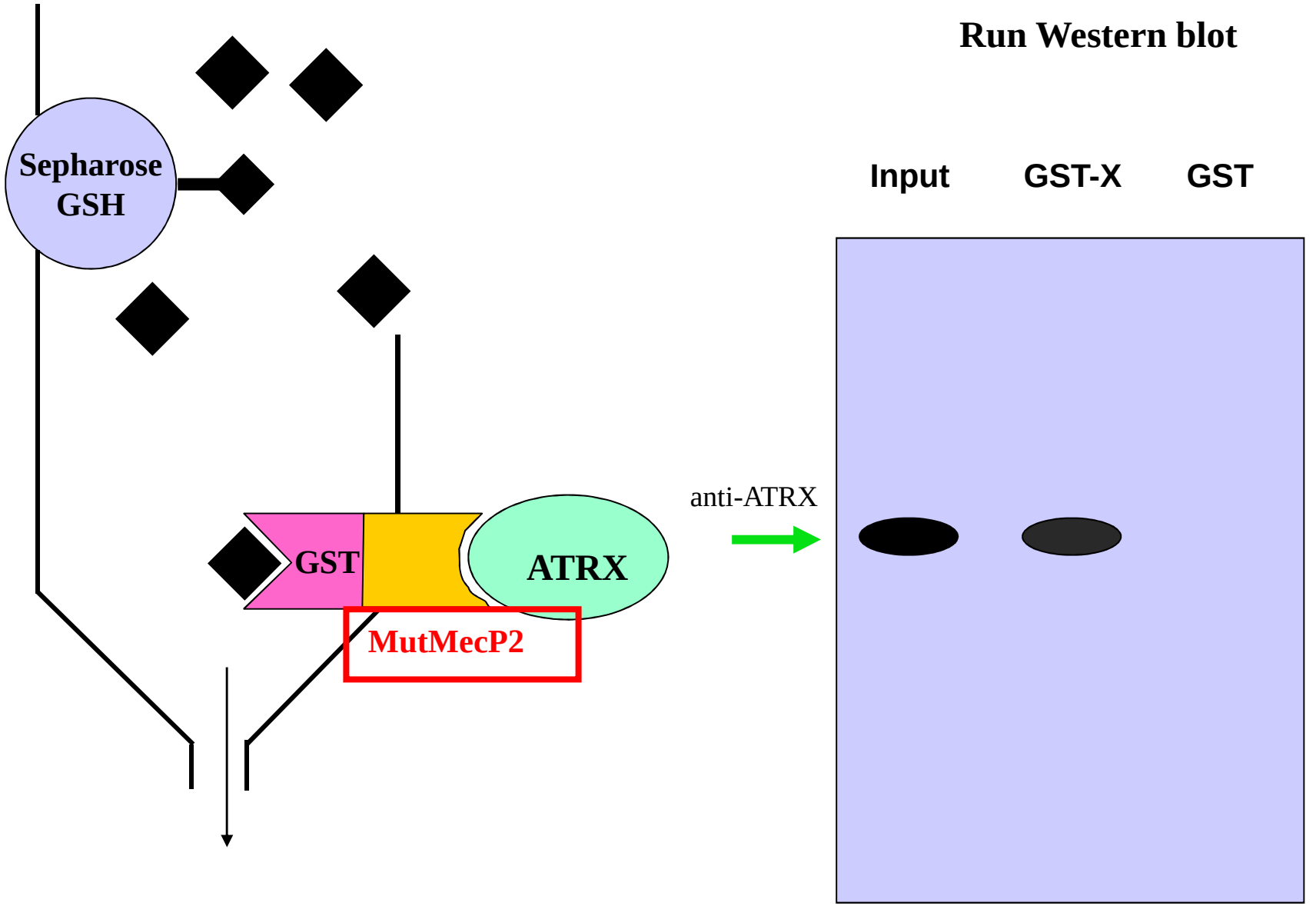
ATRX localization in neurons of *Mecp2*-null mice

ATRX localization is disrupted in neurons of *Mecp2*-null mice

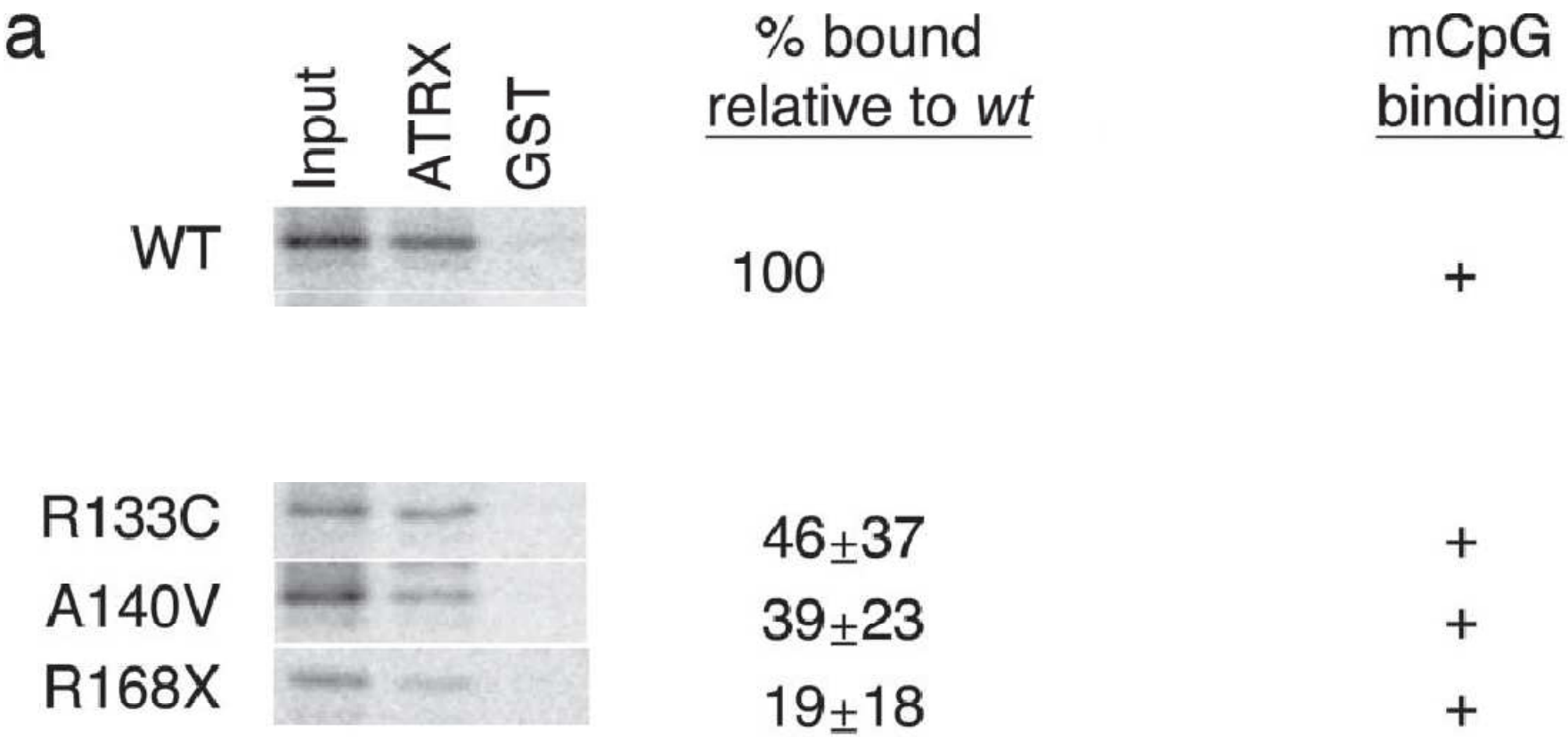


Mutations in human MeCP2 cause mental retardation

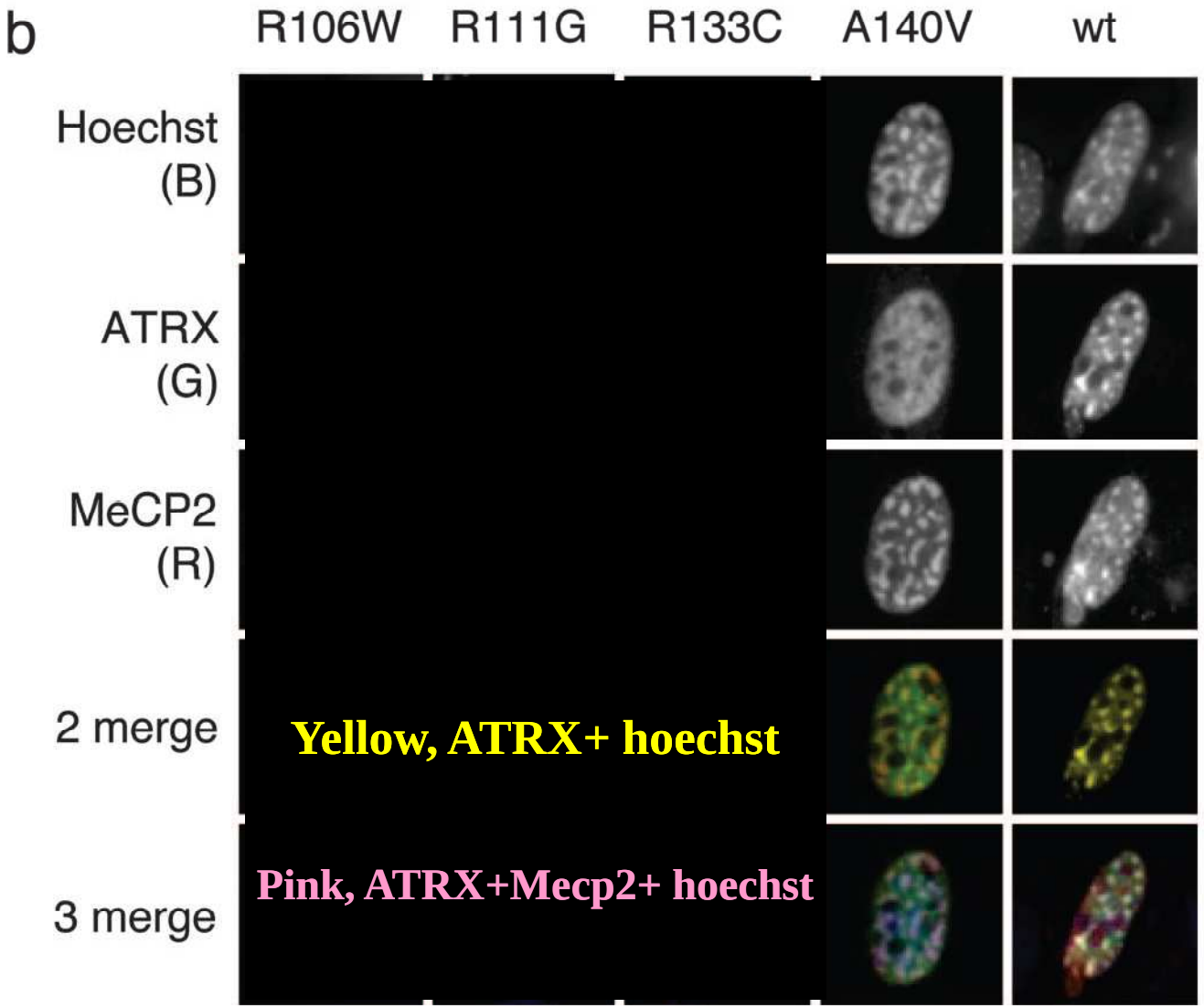
GST pull-down assay



Mutations in human in the MBD of human MeCP2 that cause mental retardation disturb the MeCP2–ATRX interaction without affecting methyl-CpG binding.



MeCP2 mutant A140V target heterochromatic foci but cannot direct ATRX to heterochromatin



In the merge
Hoechst blue
ATRX green
MeCP2 red

disruption
of the MeCP2–ATRX interaction
leads to pathological changes that
contribute to mental retardation.

Lavori recenti esemplificativi della ricerca su ATRX-Titoli

1. Distinct Factors Control Histone Variant H3.3 Localization at Specific Genomic Regions. Cell. 2010 Mar 5;140(5):678-691..
2. Abnormalities of cell packing density and dendritic complexity in the MeCP2 A140V mouse model of Rett syndrome/X-linked mental retardation. BMC Neurosci. 2010 Feb 17;11(1):19.
3. ATRX partners with cohesin and MeCP2 and contributes to developmental silencing of imprinted genes in the brain. Dev Cell. 2010 Feb 16;18(2):191-202.
4. Chromatin modifiers, cognitive disorders, and imprinted genes. Dev Cell. 2010 Feb 16;18(2):169-70.
5. PROTEIN COMPLEX OF DROSOPHILA ATRX/XNP AND HP1A IS REQUIRED FOR THE FORMATION OF PERICENTRIC BETA-HETEROCHROMATIN IN VIVO. J Biol Chem. 2010 Feb 13
6. ATRX interacts with H3.3 in maintaining telomere structural integrity in pluripotent embryonic stem cells. Genome Res. 2010 Mar;20(3):351-60.