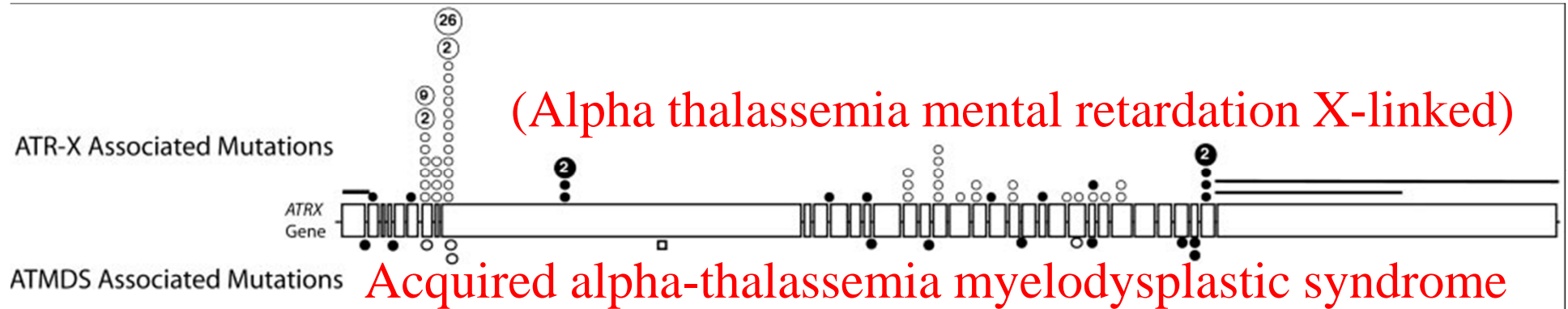


Alpha thalassemia mental retardation X-linked

Acquired alpha-thalassemia myelodysplastic syndrome



Schematic representation of the spectrum of *ATR-X* 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.

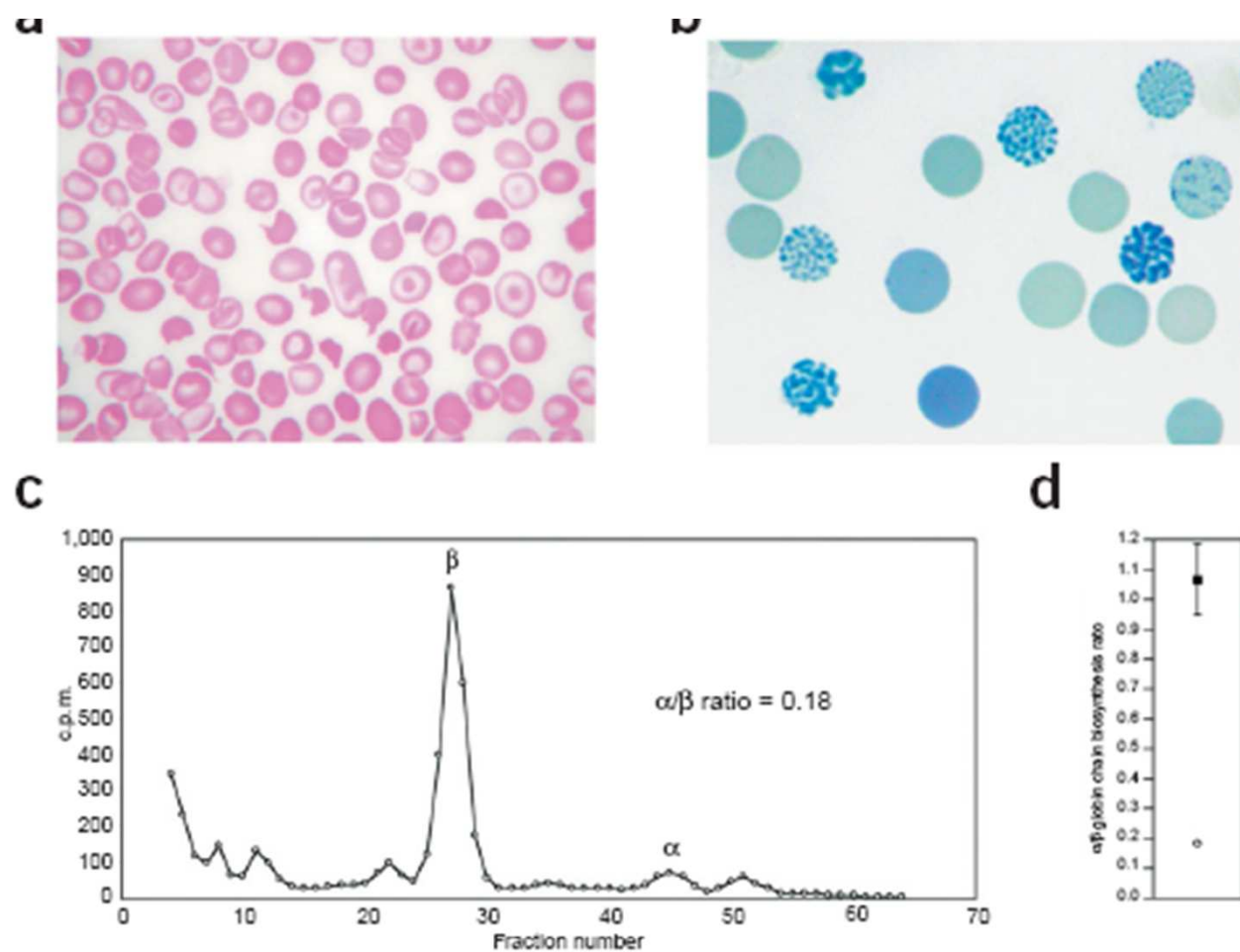


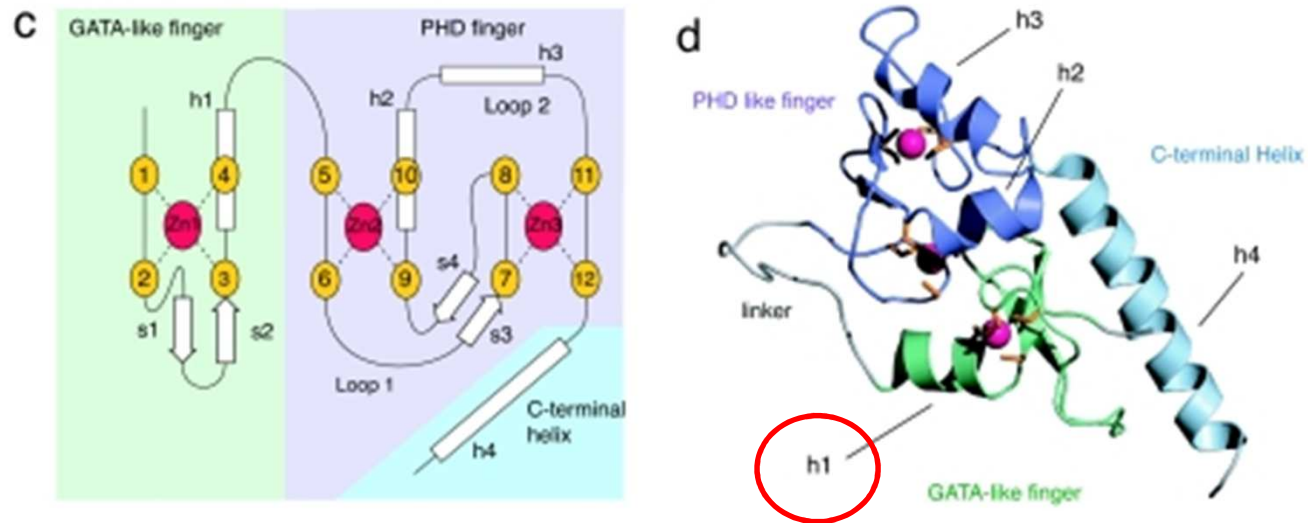
Figure 1 Hematological analysis in individuals with ATMDS. (a) Blood film from individual 1 showing hypochromia, anisocytosis, poikilocytosis and target cells. (b) Blood film of blood from individual 1 stained with brilliant cresyl blue showing the presence of cells with hemoglobin H (β_4) inclusions. (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 chain biosynthesis ratios for individual 2 (open circle) and the mean for normal controls (filled square).

- 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



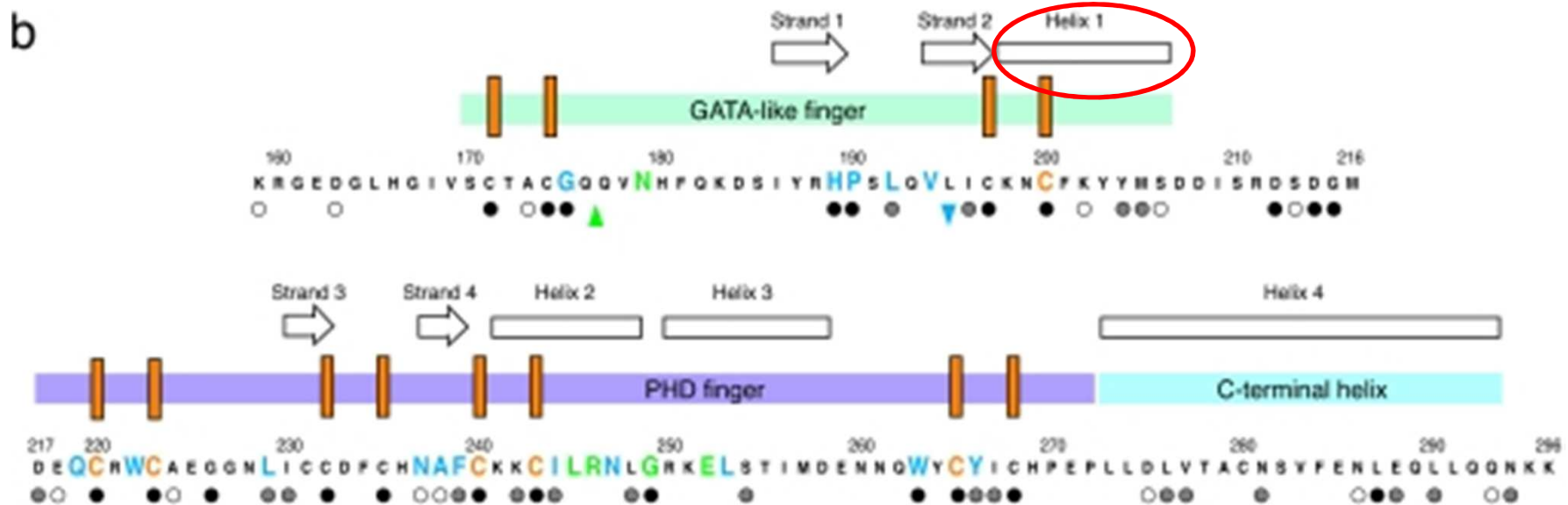
- Half of all of the disease-associated missense mutations cluster in a cysteine-rich region in the N terminus of ATRX. This region was named the *ATRX-DNMT3-DNMT3L* (**ADD**) domain, based on sequence **homology with a family of DNA methyltransferases**. The positions of missense mutations are indicated 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.



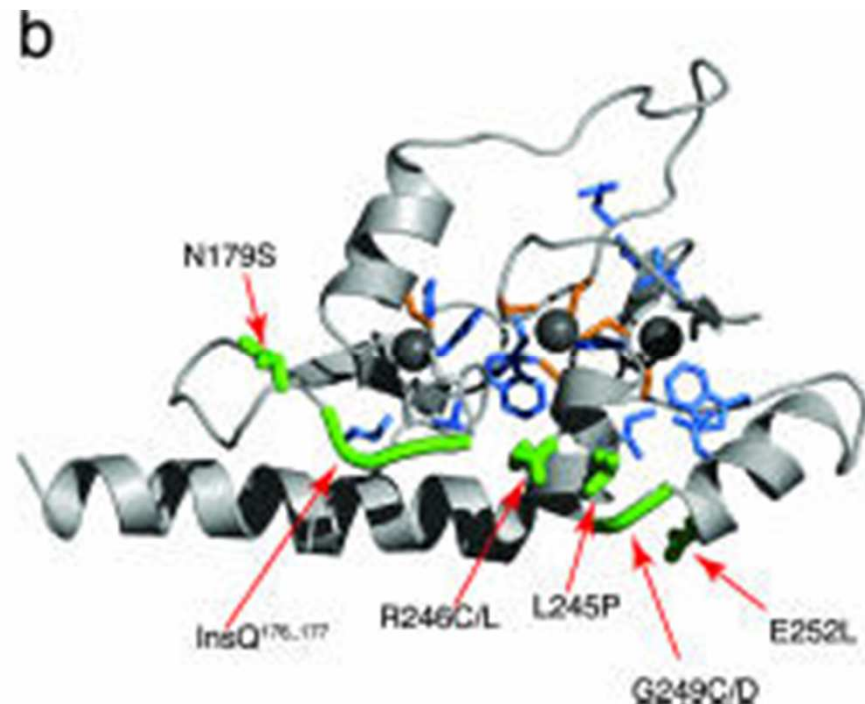
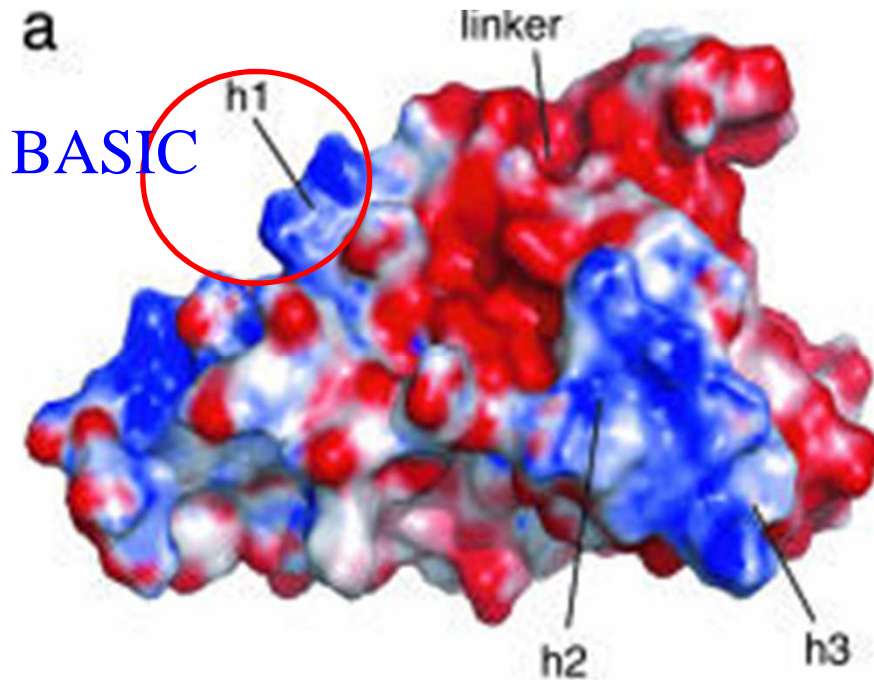
the structure of the ADD domain of ATRX consists of an N-terminal GATA-like zinc finger, a plant homeodomain finger (PHD), and a long C-terminal α -helix that pack together to form a single globular domain. The α -helix of the GATA-like finger is exposed and highly basic, suggesting a DNA-binding function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

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

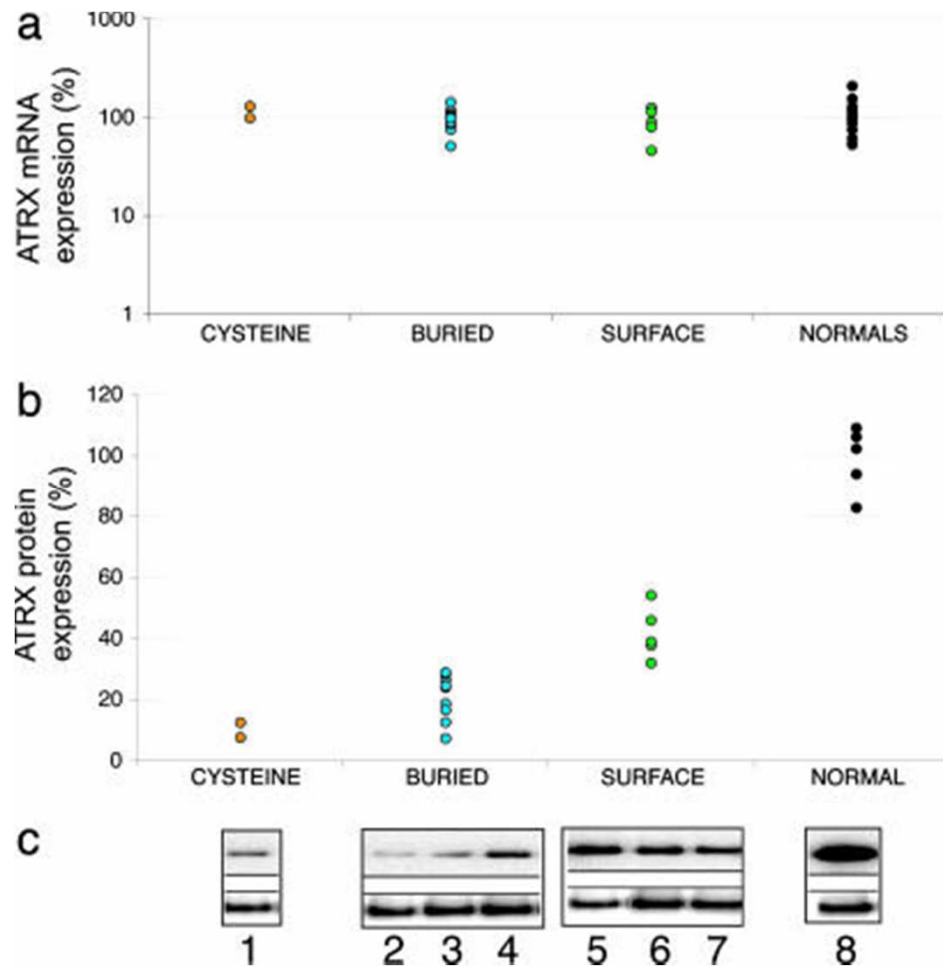
Locations of mutations and secondary structural elements in the ADD domain



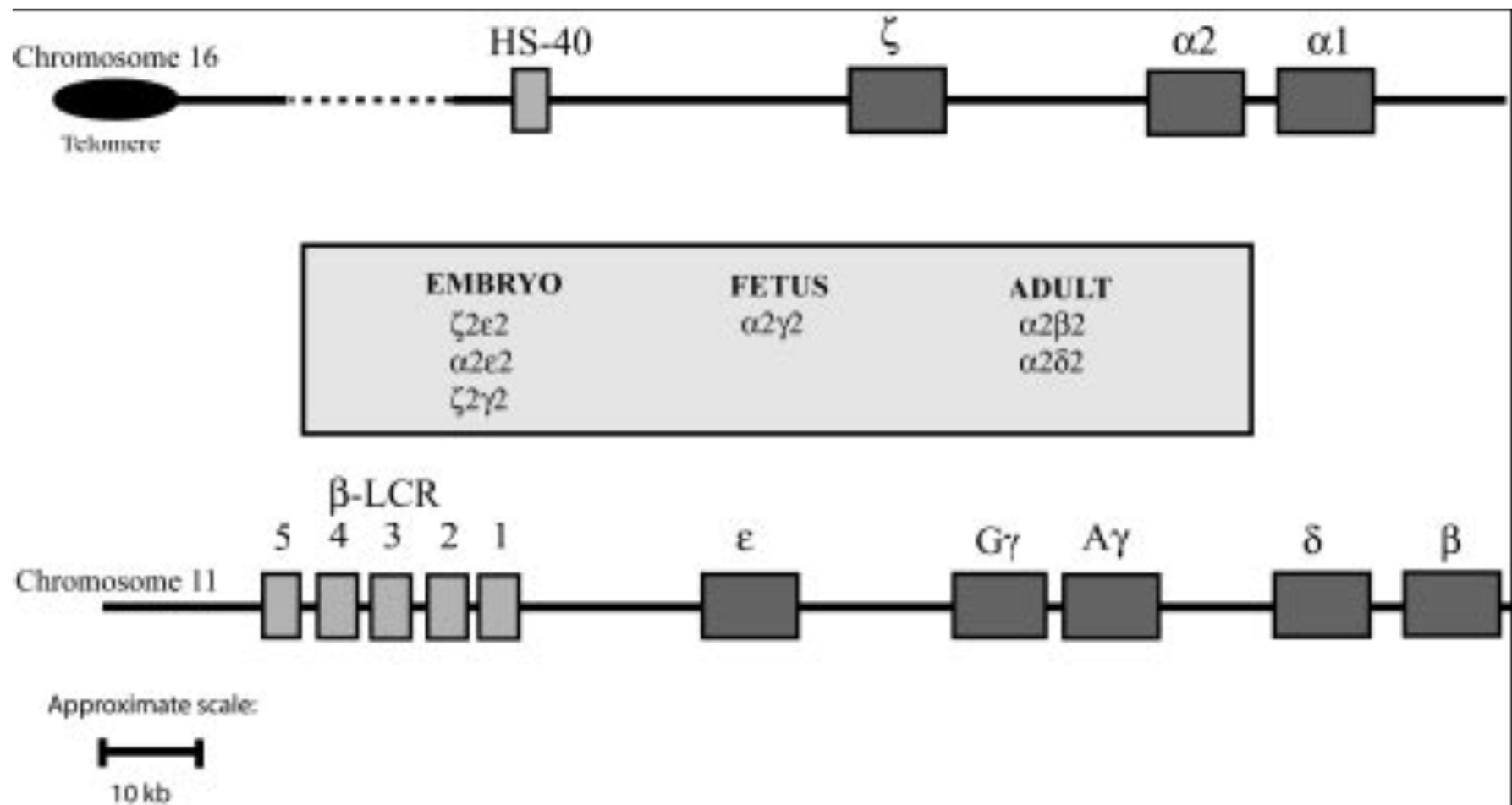
- Locations of mutations and secondary structural elements in the ADD domain. The N-terminal GATA-like zinc finger is indicated by a light green bar, the PHD finger by a mauve bar, and the C-terminal extension by a light blue bar. The conserved cysteine residues are marked as orange vertical bars.
- 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)

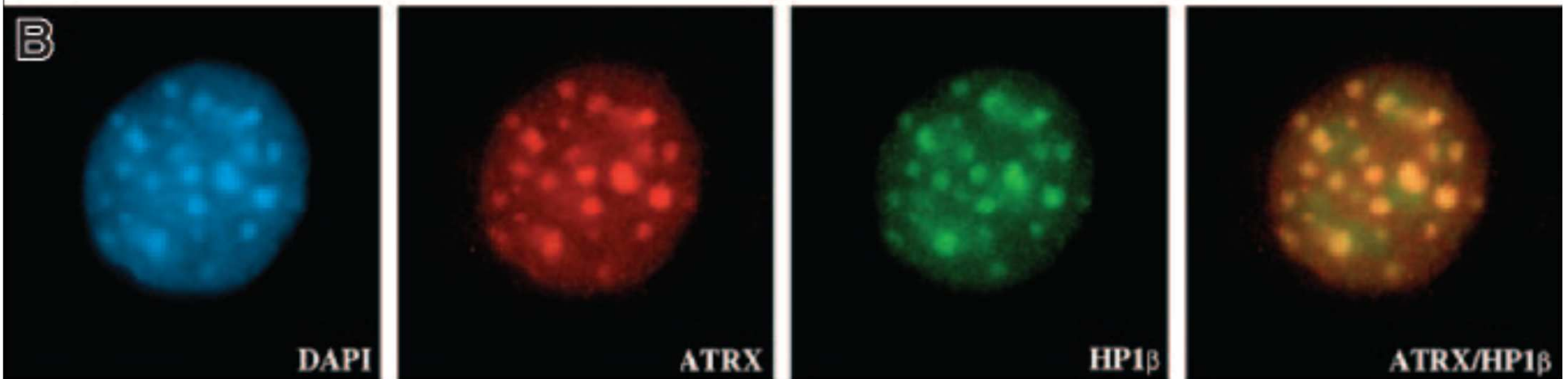
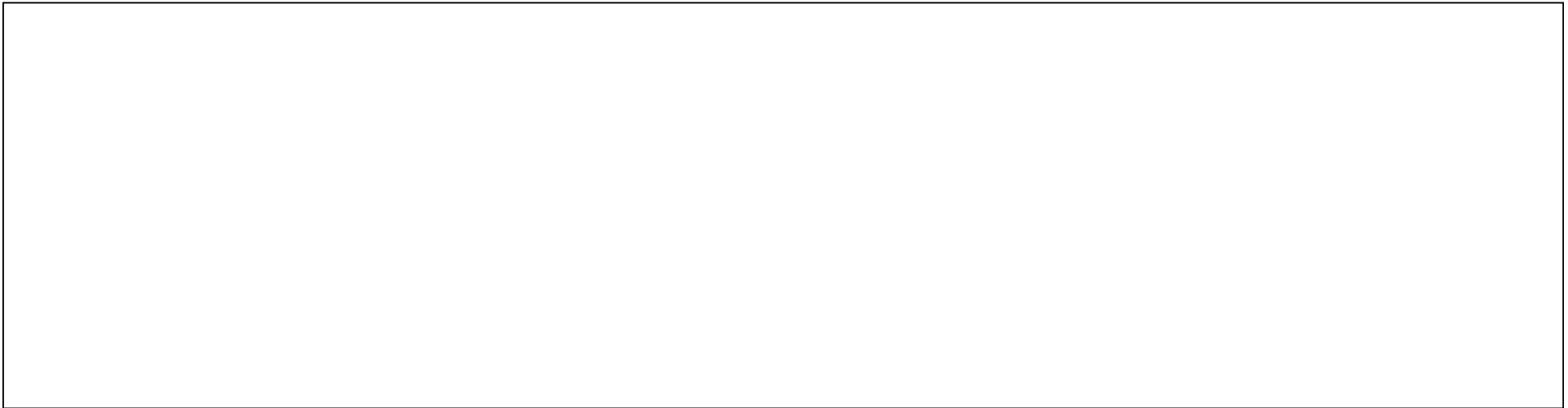


- Electrostatic potential and location of mutations in the structure of the ADD domain.
- (a) Surface electrostatic potential of the ADD domain. The helix in the GATA-like finger (h1) is solvent-exposed and basic, and the two helices within loop 2 of the PHD finger (h2 and h3) form another basic patch. 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.





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

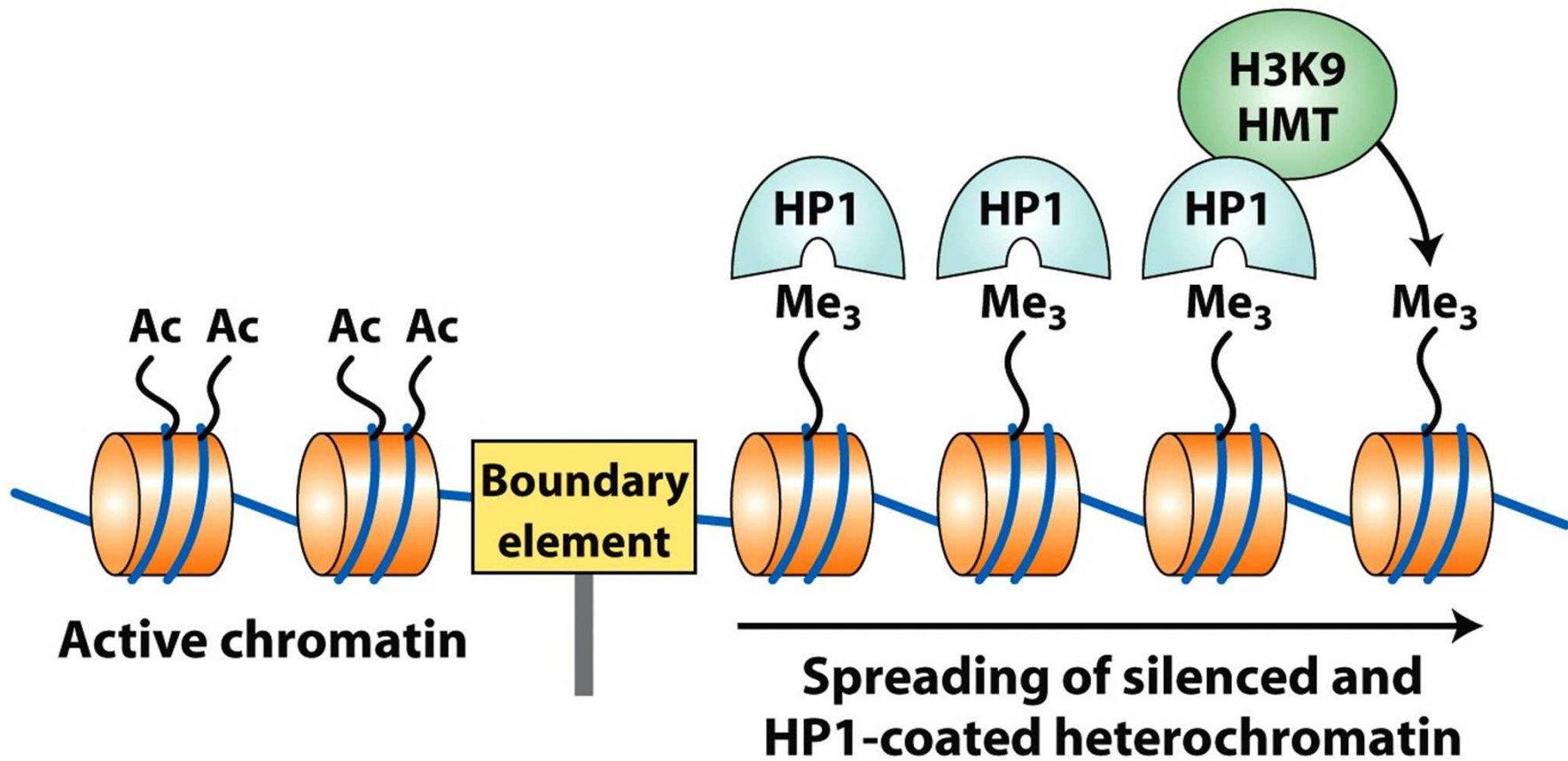


Figure 6-34b
Molecular Cell Biology, Sixth Edition
 © 2008 W. H. Freeman and Company

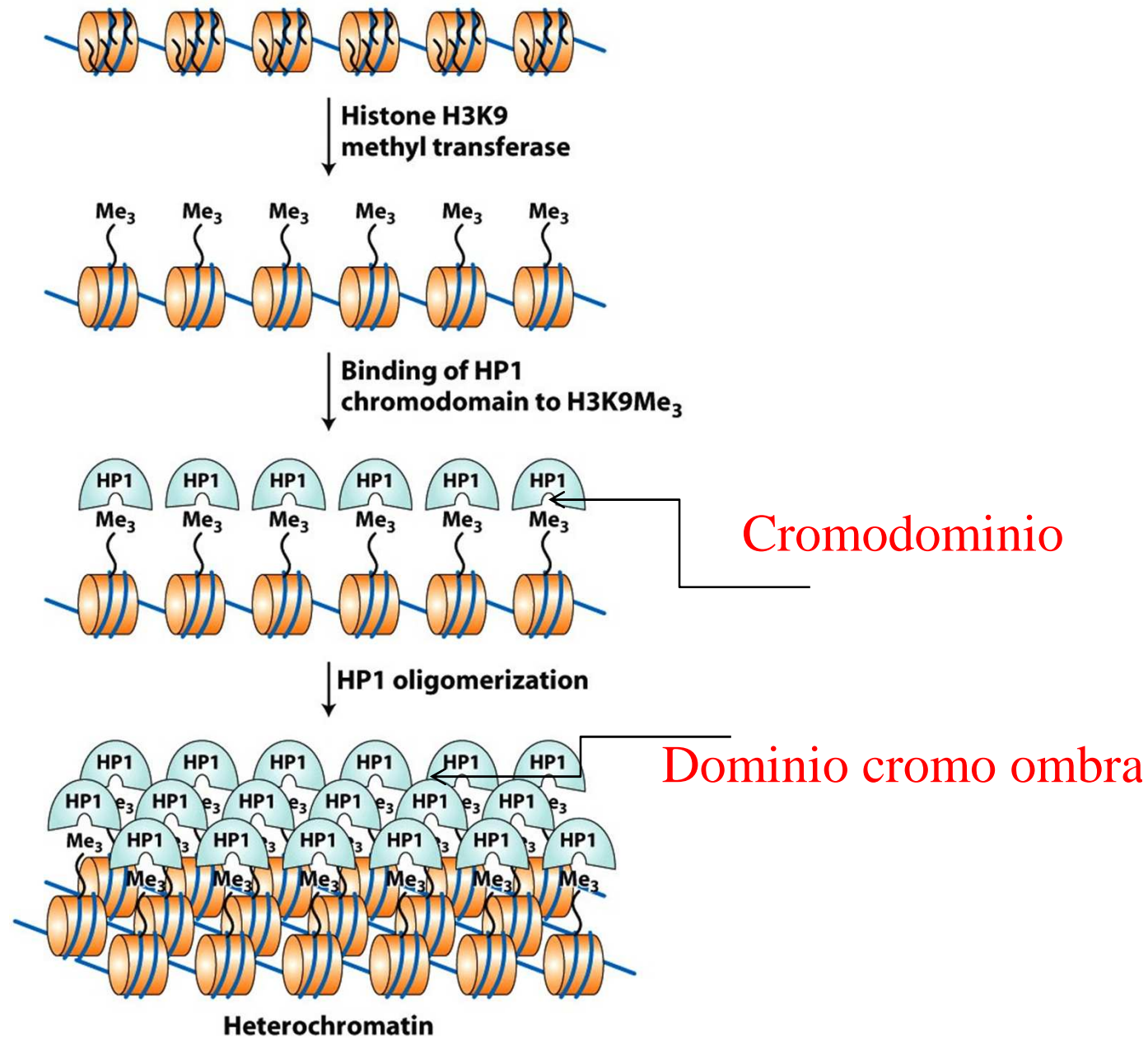


Figure 6-34a
Molecular Cell Biology, Sixth Edition
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HP1

- structural protein that plays a role in heterochromatin formation, gene silencing
- three genes HP1Hs α , HP1Hs β and HP1Hs γ .
- 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.

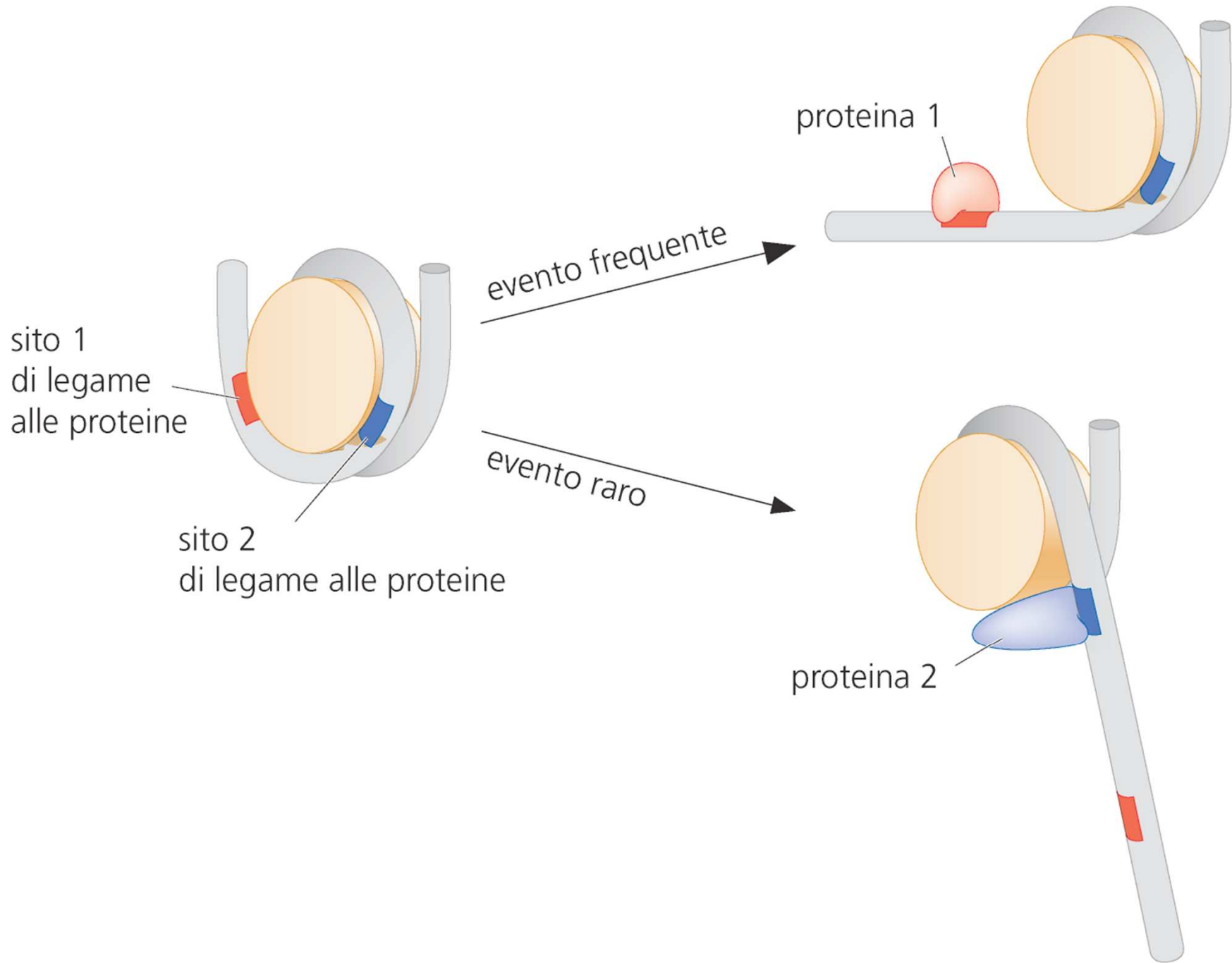
§Yeast (*Saccharomyces cerevisiae*).

¶Mammals.

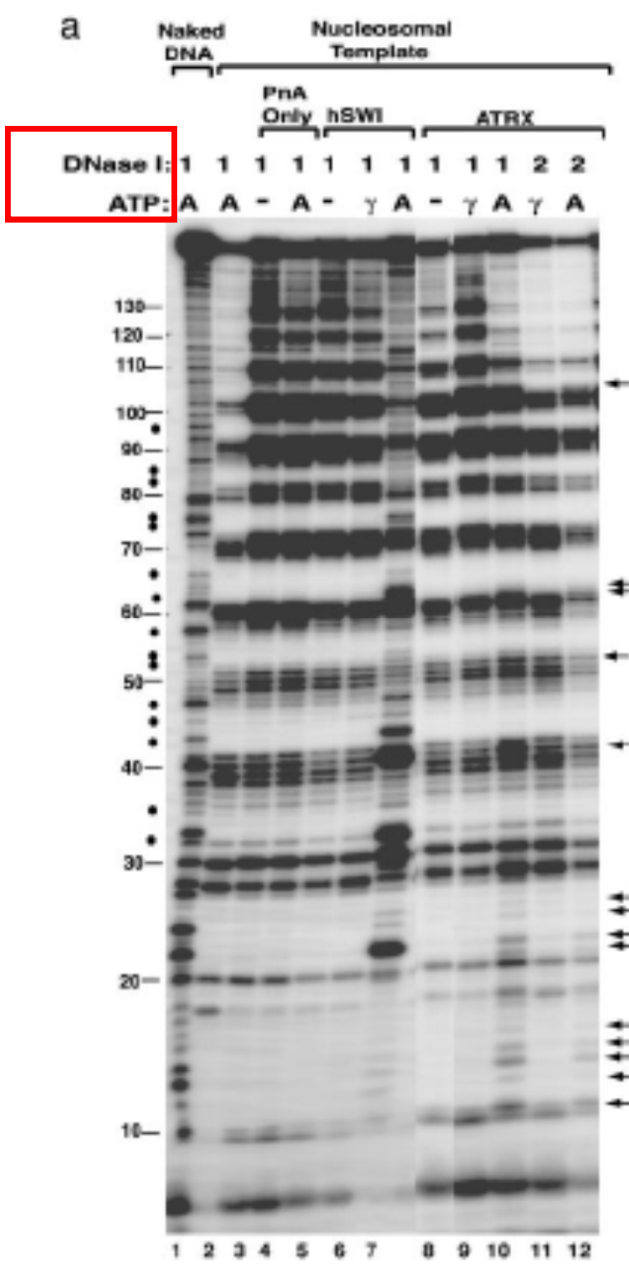
ATRX: funzioni

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

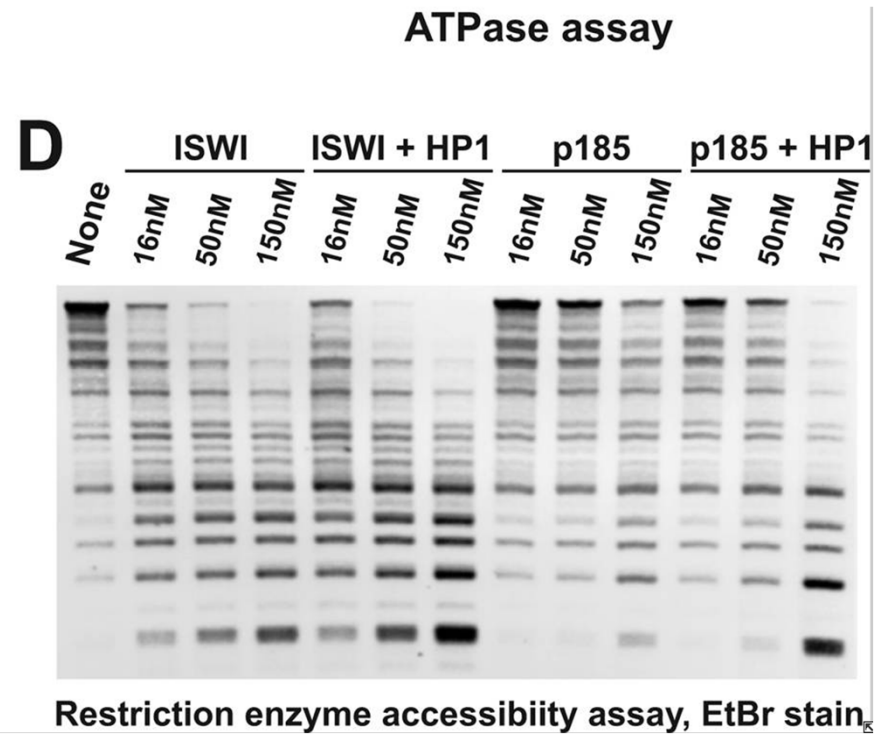
POSIZIONAMENTO
NUCLEOSOMA
Rimodellamento
Cromatina



a



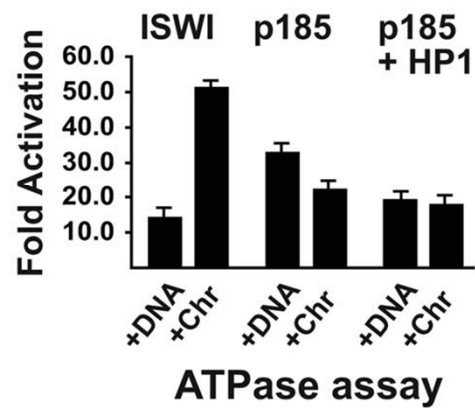
nucleosomal array remodeling activity of ATRX



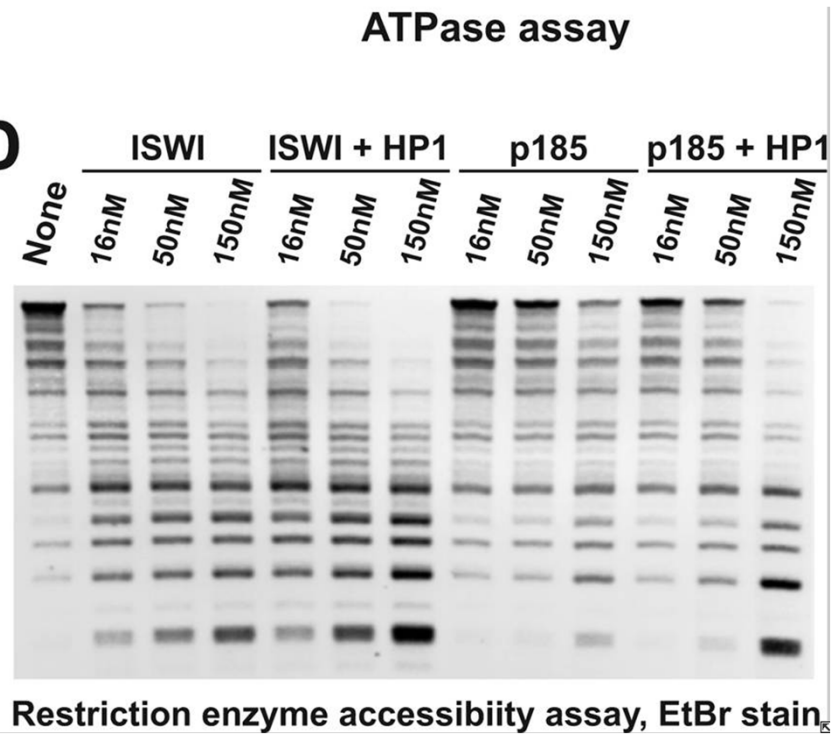
ATRXp185

nucleosomal array remodeling activity of ATRX

C



D



ATRXp185

ATRX: funzioni

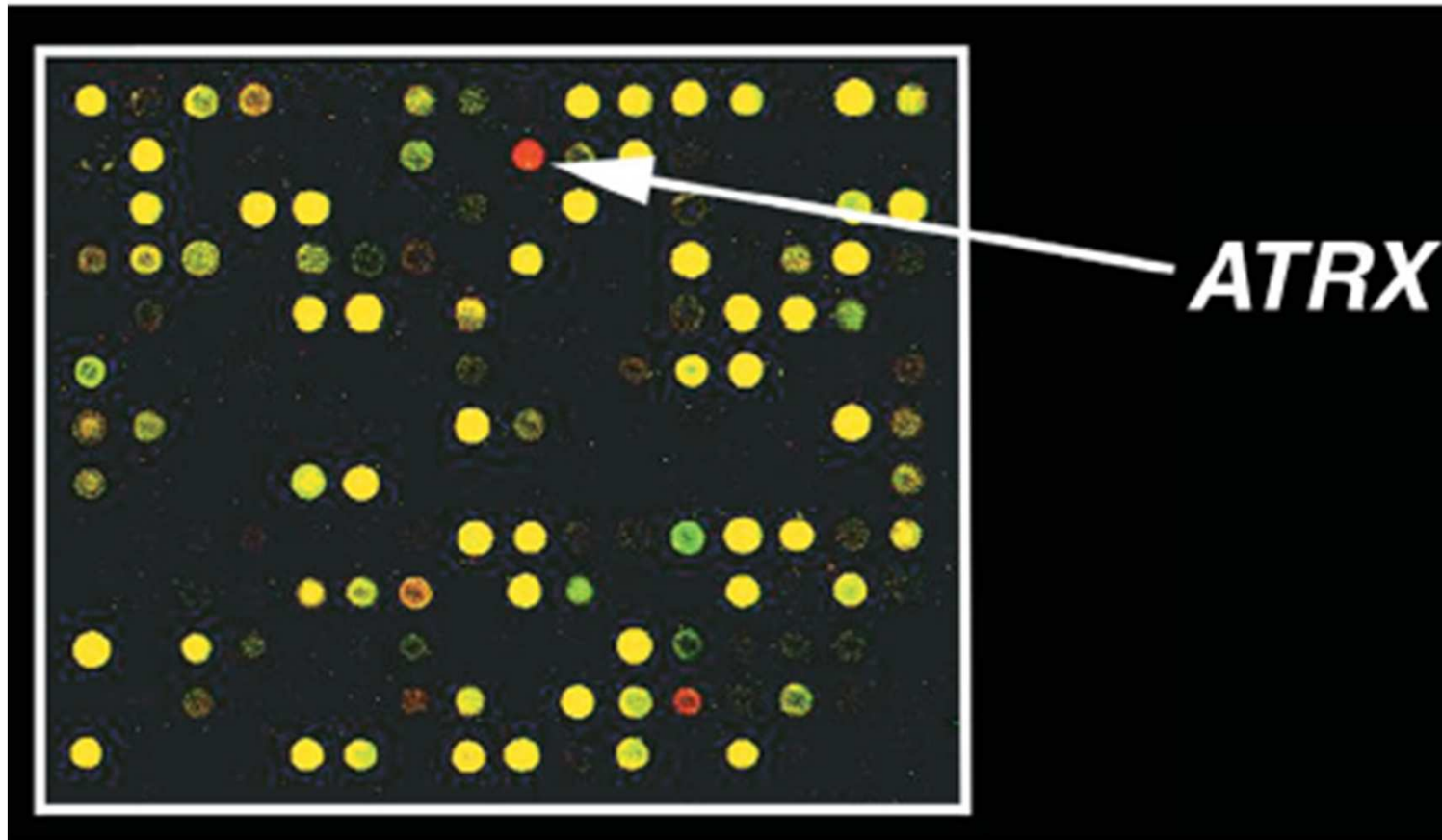
- Alteration of the regional distribution of heterochromatin by the complex or of recruitment of transcription factors (directly or indirectly) that alter gene expression ?

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

- 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 γ -globin gene cluster.

RNA profiling in **ATMD** and **normal** granulocytes



d

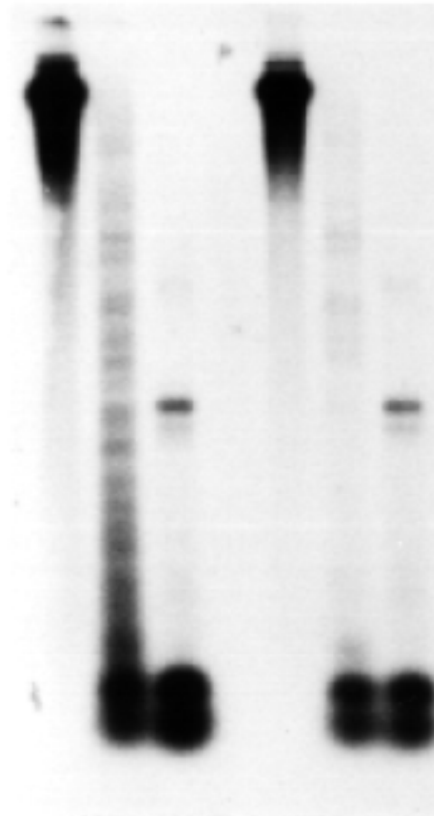
Figure 2 Gene expression analysis in an individual with ATMDS. (a) A portion of the microarray: the pseudocolored signals for the two samples (ATMDS aRNA in green and normal aRNA in red) superimposed to show their relative intensity. Yellow spots represent probes binding equivalent target cDNA in both samples; red spots, normal target is more abundant than ATMDS target; 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 and granulocytes from a mixed pool of normal individuals. Values in the range 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

C

N			ATR-X		
┌──────────┐			┌──────────┐		
Bc	Bc	Bc	Bc	Bc	Bc
	H	M		H	M



BclI + *HpaII* or *MspI*
RibC7b5

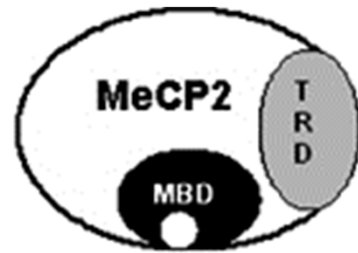
Table 1 • The pattern of methylation in ATR-X syndrome^a

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

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

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

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

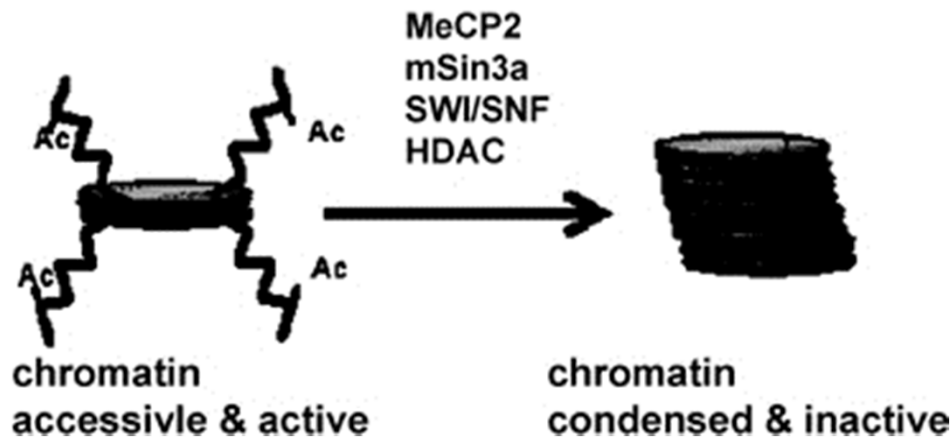
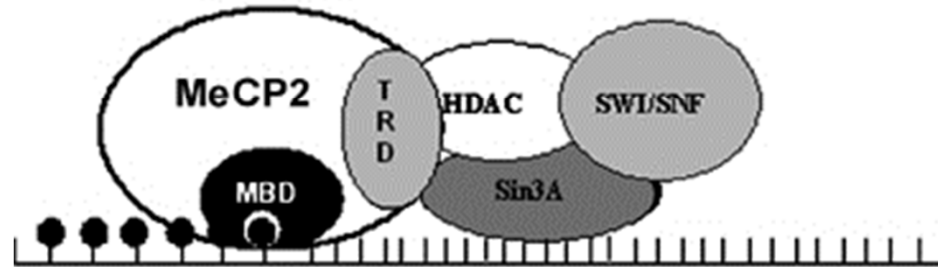


methyl-binding domain (**MBD**)

transcription repression domain (**TRD**)

methylated CpG 

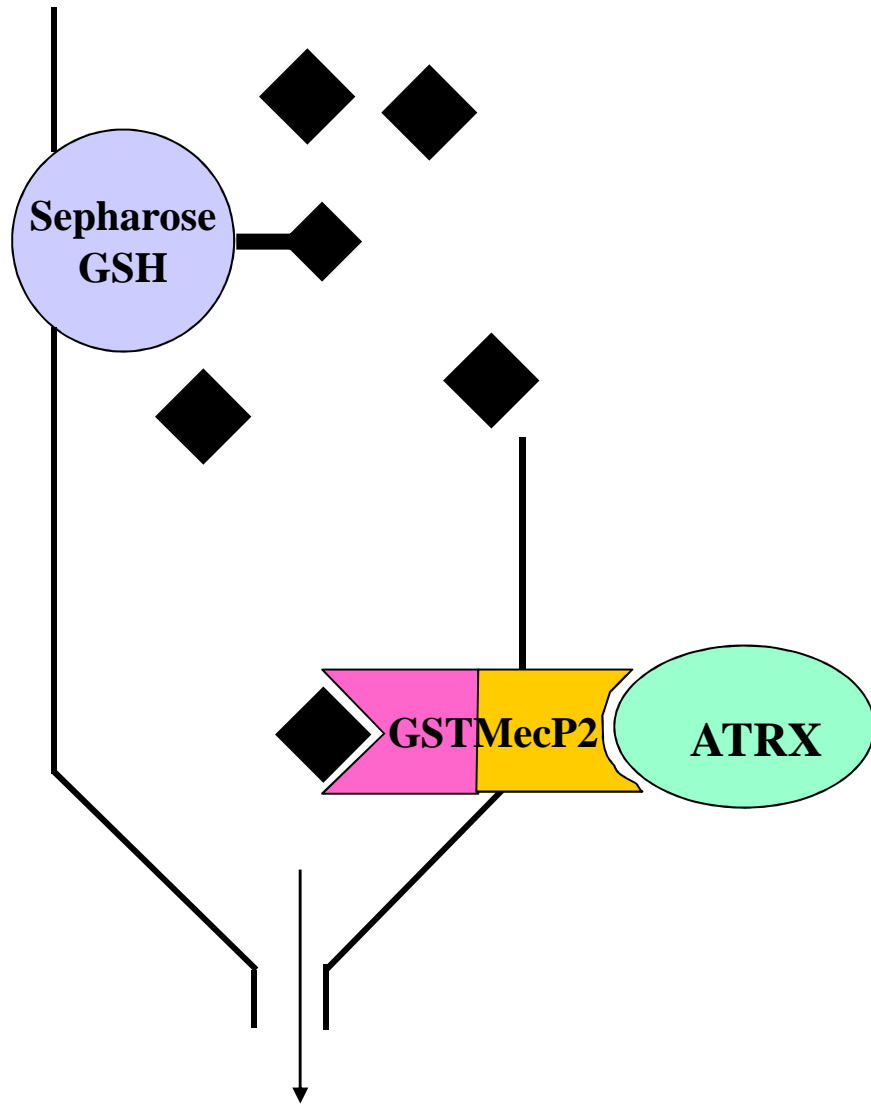
transcription repression
by MeCP2



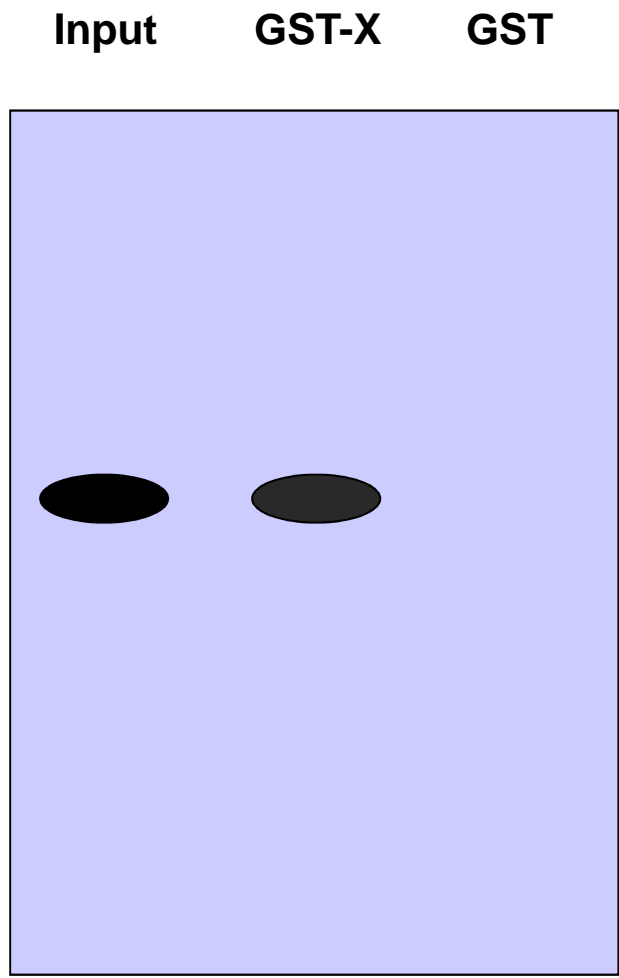
MeCP2 interacts with ATRX.

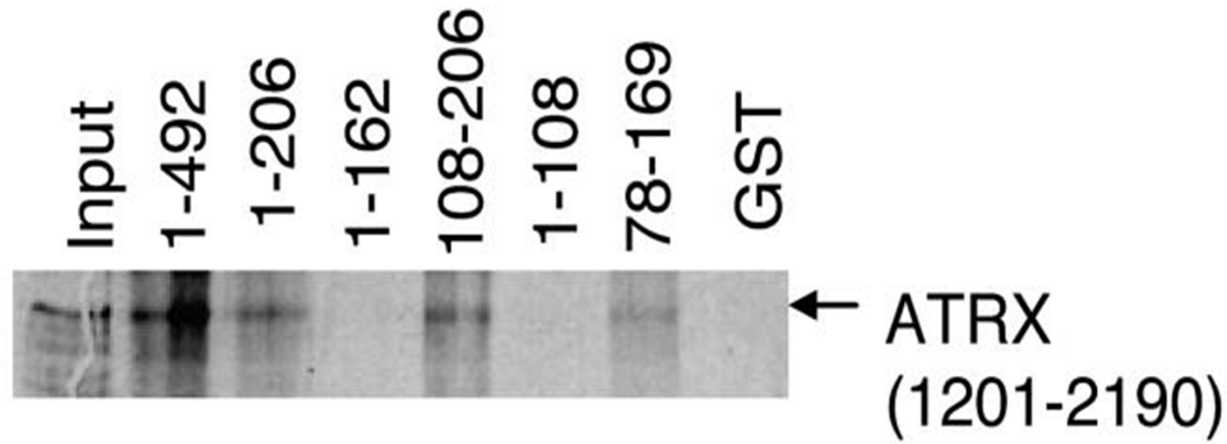
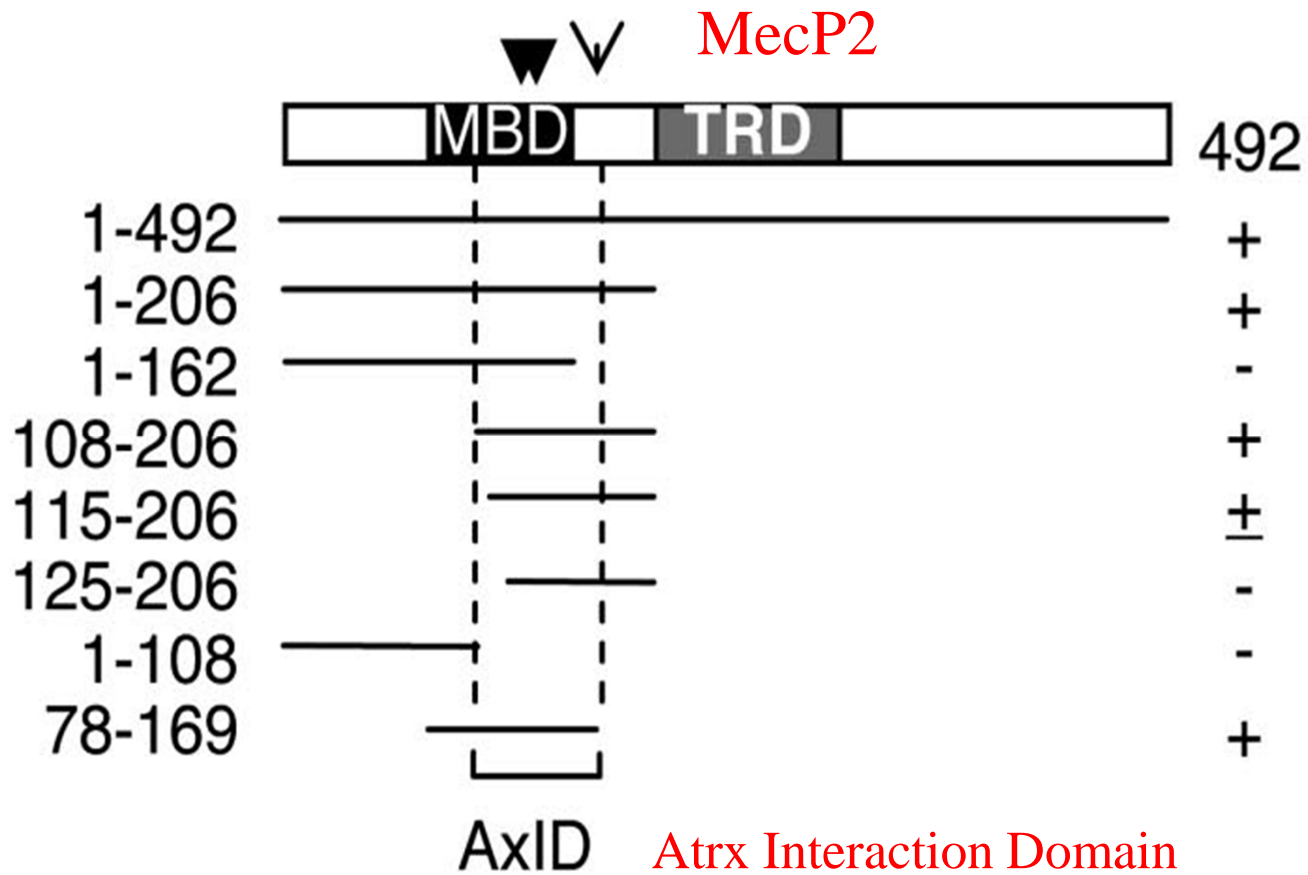
- (b) The ATRX-interaction region overlaps the MBD of MeCP2. The MeCP2 region that binds ATRX was identified by GST pulldown by using a deletion series of MeCP2-GST fusion proteins incubated with *in vitro*-translated [³⁵S]-labeled ATRX fragment 1201–2190 (bracket; *a*). The input lane (*Top*) contained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

GST pull-down assay



Run Western blot

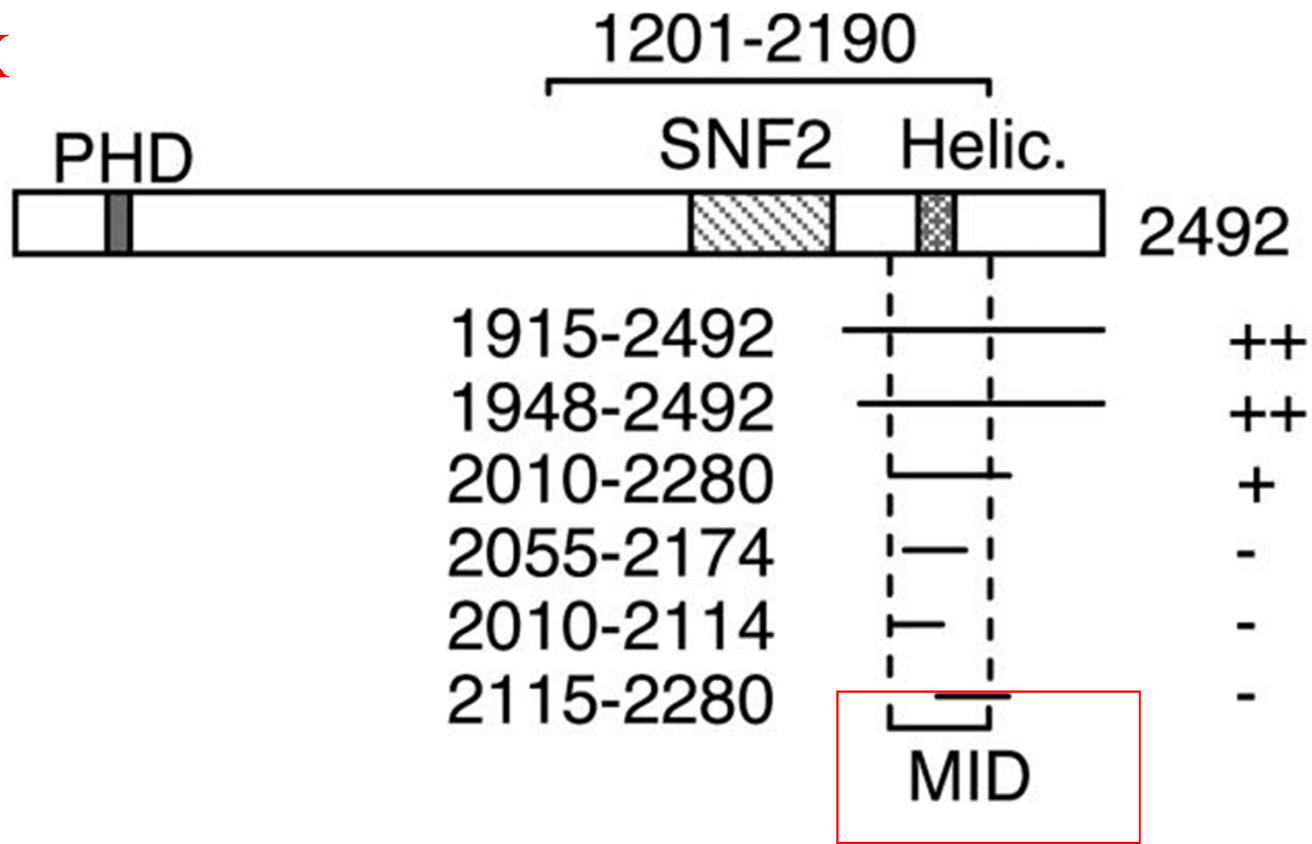


b**c**

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

ATRX



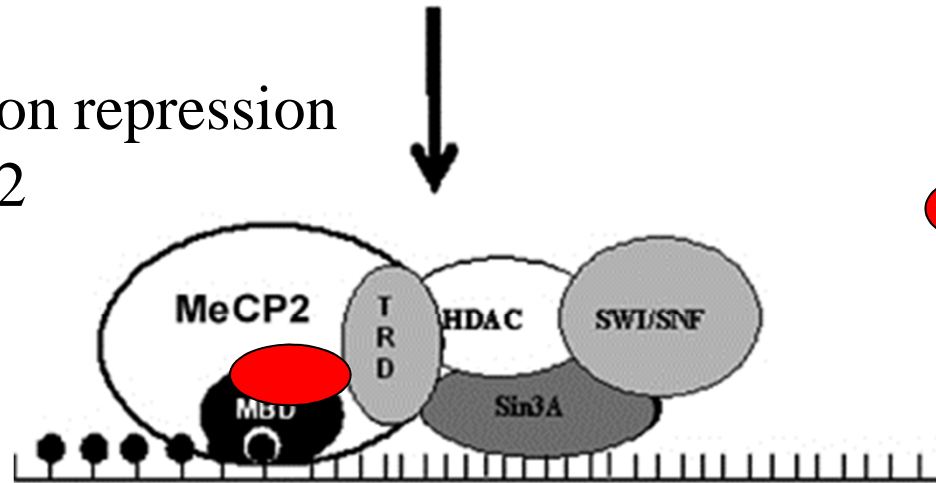
MecP2 Interaction Domain



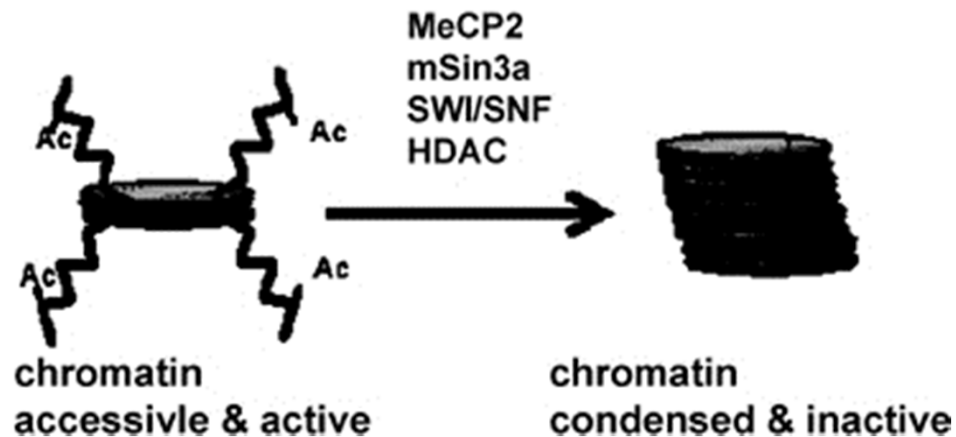
methyl-binding domain (MBD)
transcription repression domain (TRD)

methylated CpG 

transcription repression
by MeCP2

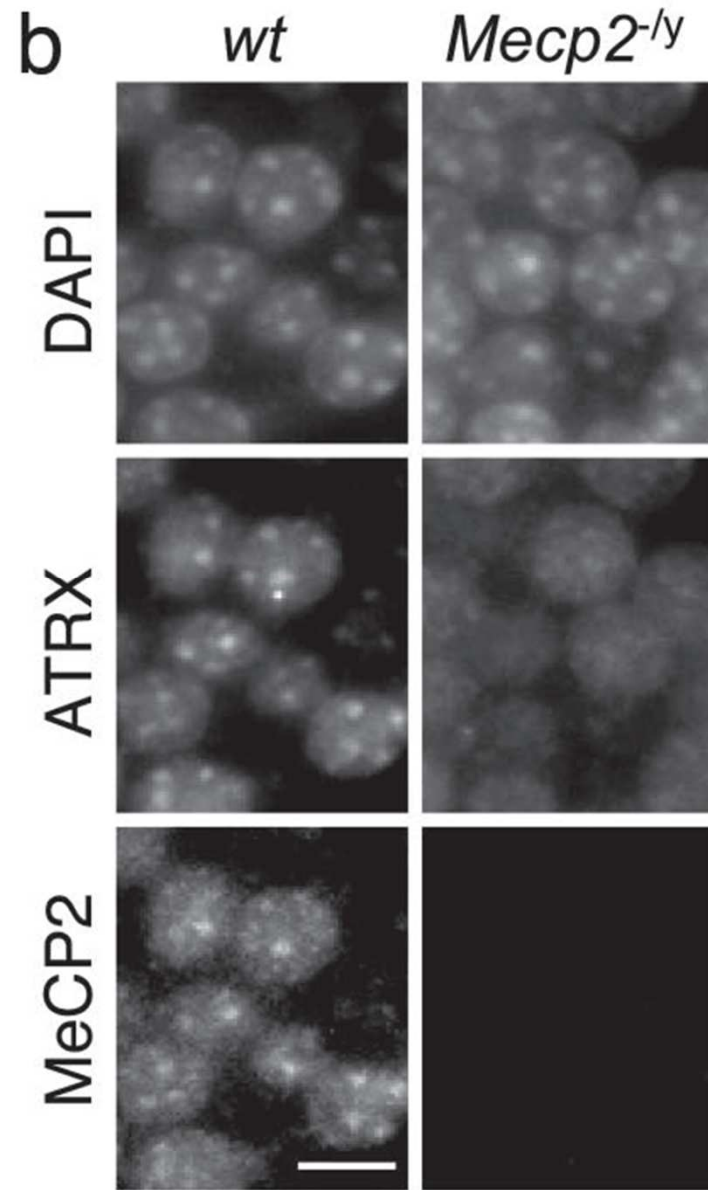


 ATRX



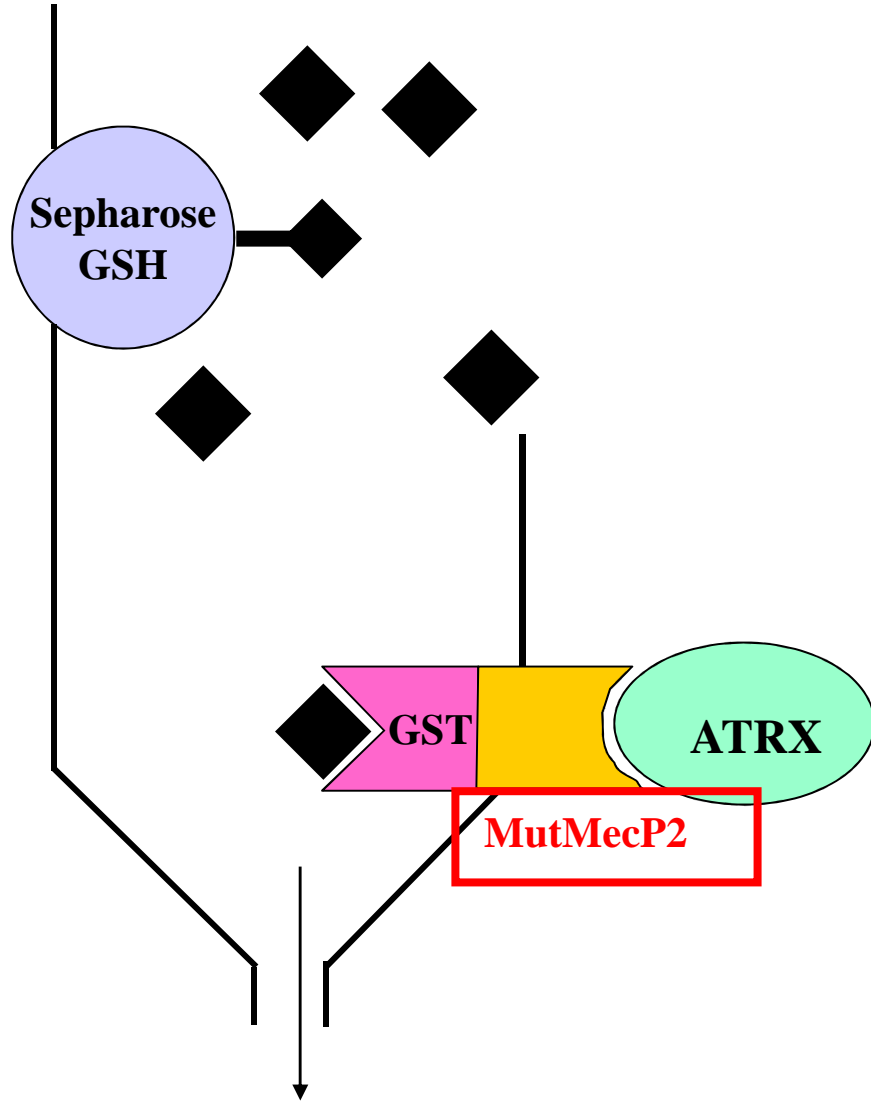
**ATRX localization in neurons of
Mecp2-null mice**

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

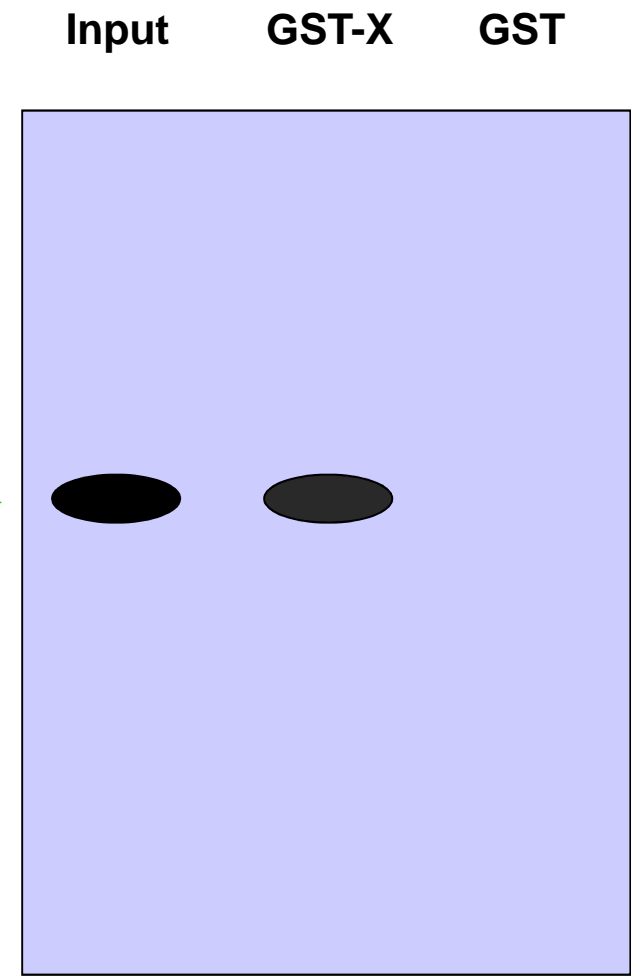


Mutations in human MeCP2 cause mental
retardation

GST pull-down assay















Run Western blot

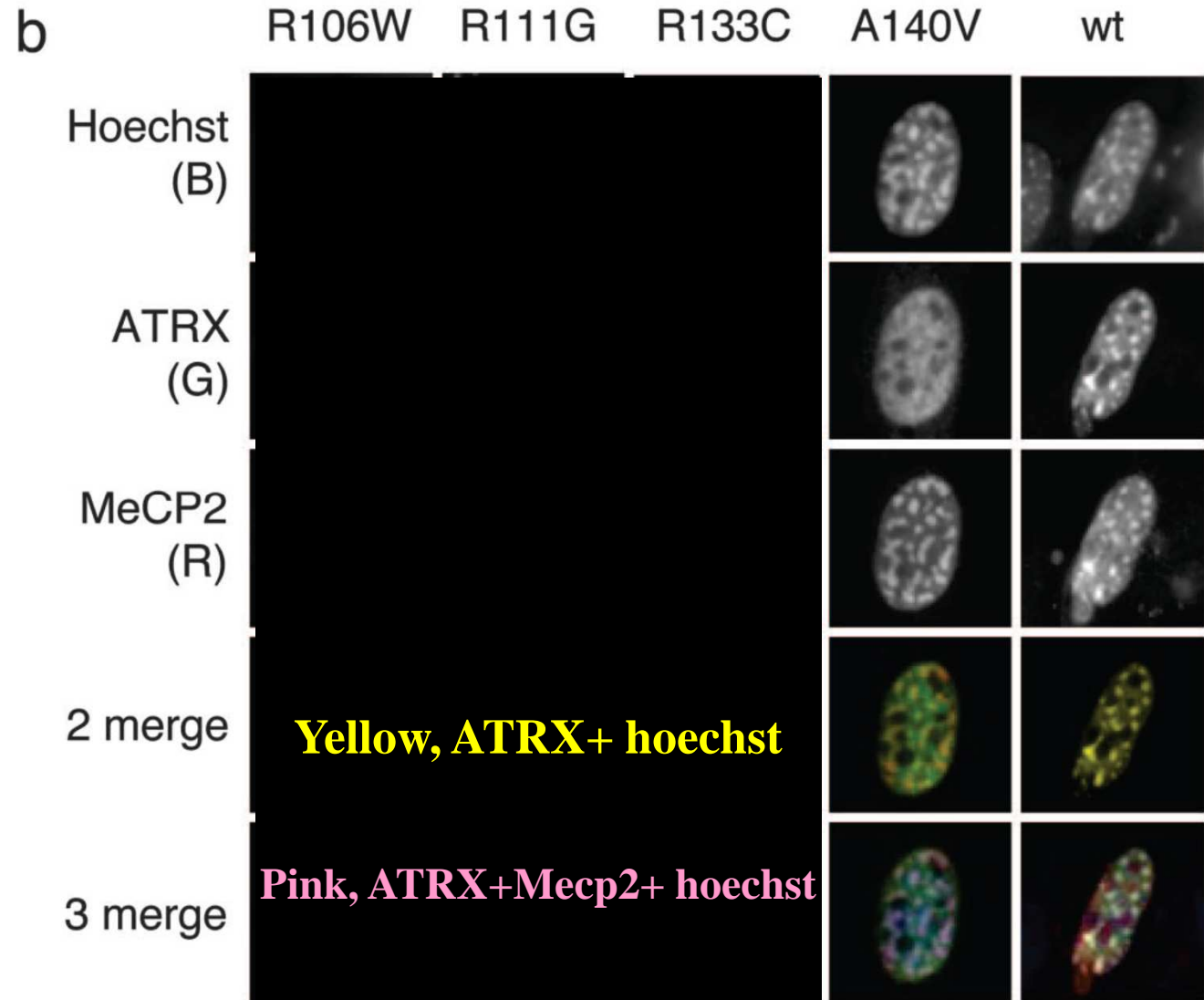


Mutations in human in the MBD of human MeCP2 that cause mental retardation disturb the MeCP2–ATRAX interaction without affecting methyl- CpG binding.

a

	Input	ATRAX	GST	<u>% bound relative to <i>wt</i></u>	<u>P value</u>	<u>mCpG binding</u>
WT				100	-	+
R133C				46 \pm 37	0.0557	+
A140V				39 \pm 23	0.0065	+
R168X				19 \pm 18	0.0006	+

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.**