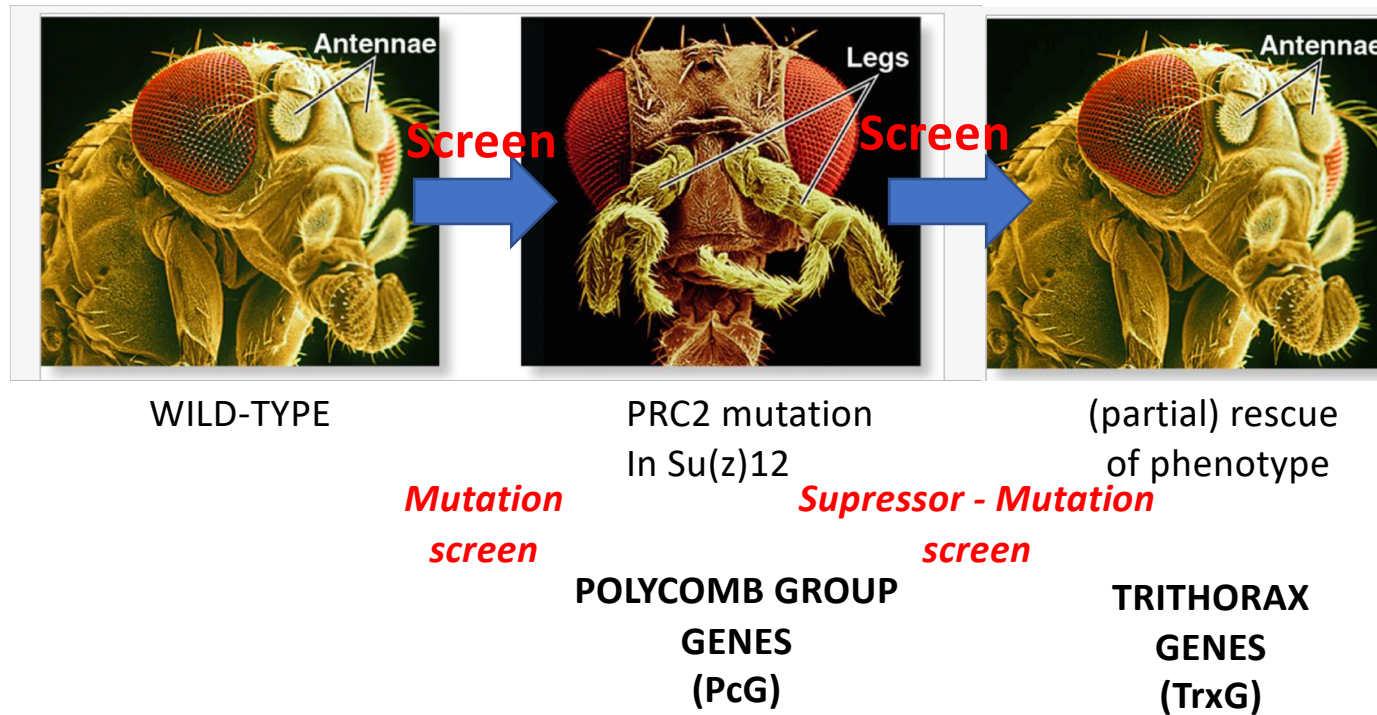


EXPERIMENTAL APPROACH IN DROSOPHILA:

**A SUPPRESSOR MUTATION SCREEN TO IDENTIFY
EPIGENETIC REGULATORS THAT ACTIVATE GENE EXPRESSION
--- TRITHORAX GROUP GENES ---**



A SUPPRESSOR MUTATION SCREEN TO IDENTIFY EPIGENETIC REGULATORS THAT ACTIVATE GENE EXPRESSION --- TRITHORAX GROUP GENES ---

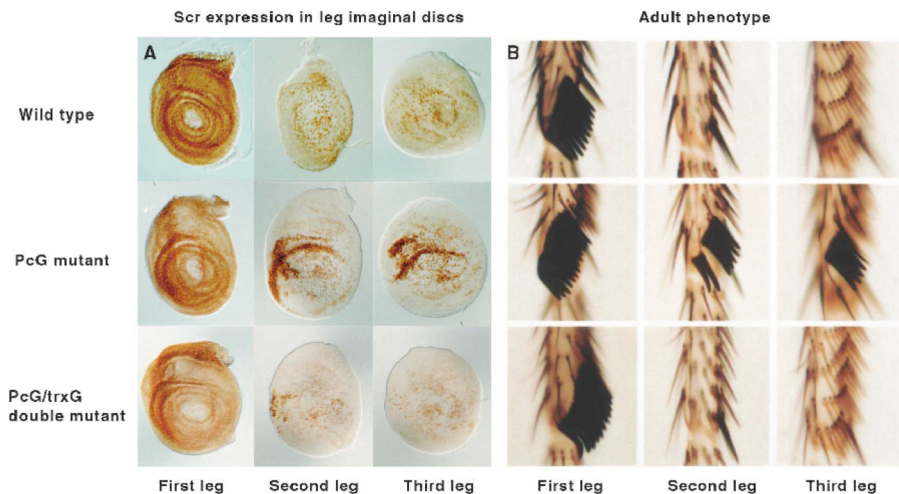


Figure 4. trxB mutations block the derepression of Hox genes in PcG mutants. (A) Leg imaginal discs stained with antibodies against the protein encoded by the Hox gene, *Scr*, which specifies the identity of the labial and first thoracic segments, including the first leg. (B) Basitarsal segments of the legs of wild-type and mutant adults. Note the presence of sex comb teeth on the first leg, but not the second and third legs of wild-type adults. The *Scr* gene is partially derepressed in the second and third leg discs, in which it is normally silent, in individuals heterozygous for mutations in PcG genes leading to the appearance of ectopic sex comb teeth on the second and third legs. These phenotypes are suppressed by mutations in *brm* and many other trxB genes. (A, Reprinted, with permission, from [Tamkun et al. 1992](#), © Elsevier; B, portion modified, with permission, from Kennison 2003, © Elsevier.)

Table 1. Biochemical functions of trxB proteins

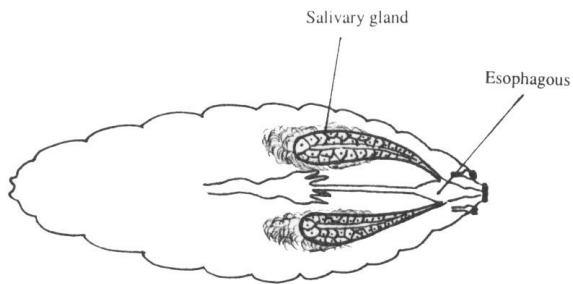
Known function	Organism			Complexed with non-trxB proteins?
	<i>Drosophila</i>	Human	Yeast	
ATP - dependent chromatin remodeling	BRM	BRG1/HBRM	Swi2/Snf2, Sth1	Yes (5–10) ^a
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	Kismet (KIS)	CHD7	–	NK
Histone methyltransferases	Trithorax (TRX)	MLL1, MLL3	MLL2, Set1	Yes (5–20)
	Absent, small or homeotic 1 (ASH1)	MLL4, hASH1	hSET1 –	NK
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Cohesin subunit	Verthandi (VTD)	Rad21	Scc1/Rad21	Yes (>3)
Transcription factor	Trithorax-like (TRL)	BTBD14B	–	No
Growth factor receptor	Breathless (BTL)	FGFR3	–	NK
Other	Sallimus (SLS)	Titin	–	NK

Drosophila males use their sex combs to grasp the females' abdomen and genitalia and to spread their wings prior to copulation.

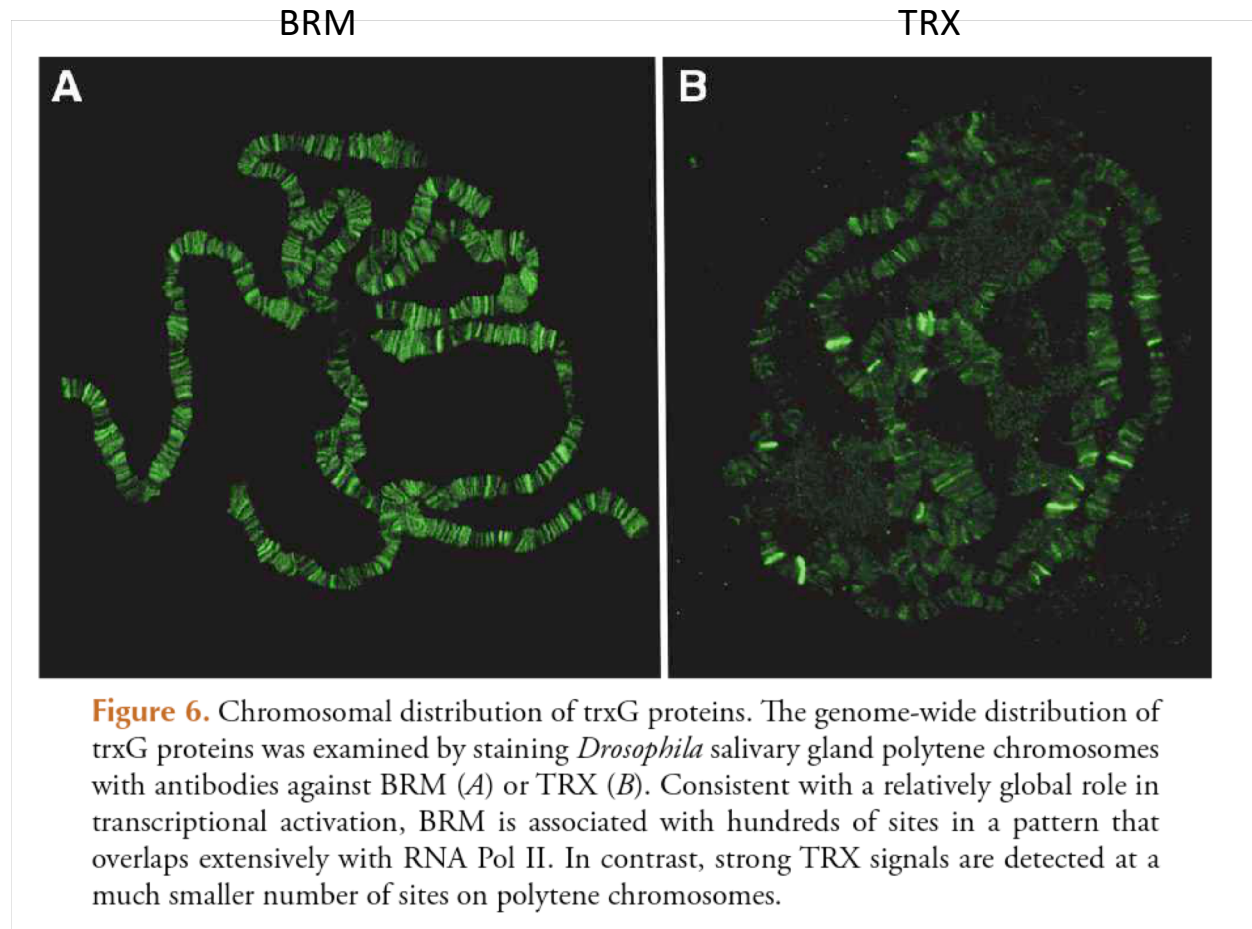
TRITHORAX GROUP GENES TrxG

Selected <i>D. melanogaster</i> TrxG proteins	Mammalian homologues	Biochemical role(s)	
Trithorax	Myeloid/lymphoid or mixed-lineage leukaemia proteins 1–3	SET domain subunit of a methyltransferase for H3K4	CHROMATIN MODIFICATION H3K4 METHYLATION
Absent small and homeotic disks protein 1 (ASH1)	ASH1-like	SET domain subunit of a methyltransferase for H3K4 and/or H3K36	CHROMATIN MODIFICATION H3K4 – H3K36 METHYLATION
Brahma (BRM)	BRM (also known as SMARCA2) and BRG1 (also known as SMARCA4)	ATPase subunit of a SWI/SNF-type nucleosome remodelling complex	CHROMATIN REMODELLING
Kismet	Chromodomain helicase DNA-binding protein 7	ATPase subunit of a presumed chromodomain-type nucleosome remodelling complex, which functions in transcription elongation	CHROMATIN REMODELLING
Nejire (also known as CBP)	?	Subunit of the TAC1 complex, which mediates histone acetylation	CHROMATIN MODIFICATION

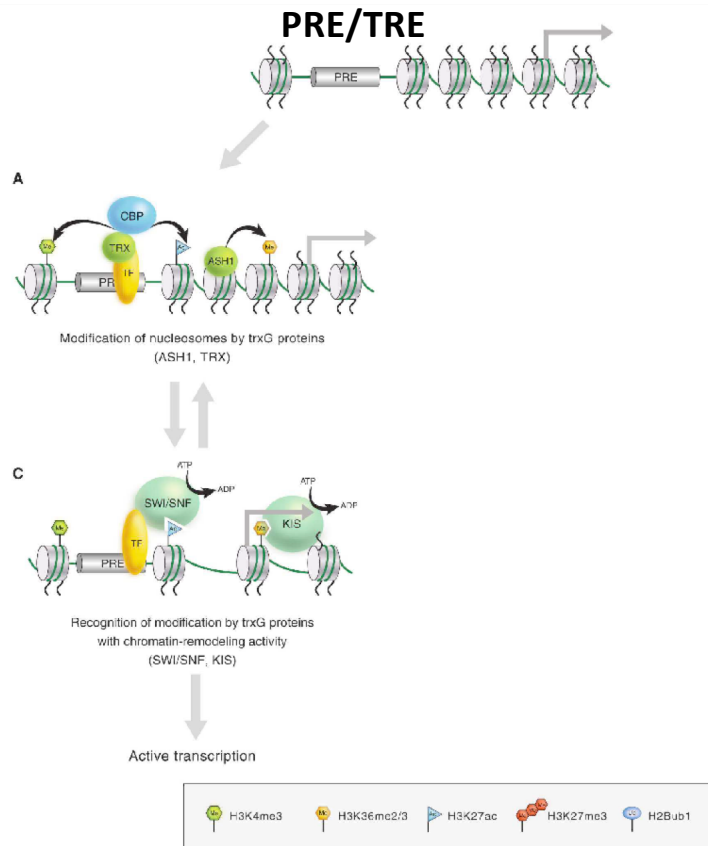
TRITHORAX GROUP GENES ARE LOCALIZED TO DEFINED POSTIONS IN THE GENOME



Un cromosoma politenico è un cromosoma gigante. I cromosomi politenici si formano in seguito a vari cicli di replicazione che producono molte copie (anche centinaia) di cromatidi fratelli che rimangono uniti. La formazione dei cromosomi politenici ha la funzione di aumentare il volume cellulare ma può anche comportare un vantaggio metabolico dato che l'elevato numero di copie di geni permette un alto livello di espressione genica. In *Drosophila melanogaster*, per esempio, i cromosomi delle ghiandole salivari delle larve subiscono numerosi cicli di endoreplicazione, e questo consente di produrre grandi quantità di secreto prima dell'impupamento.



1. TRITHORAX GROUP GENES COVALENTLY MODIFY HISTONES



Fly **TRX** has 6 HKMTs homologs in humans:

- hSET1A
 - hSET1B
 - MLL1
 - MLL2
 - MLL3
 - MLL4
- COMPASS complex components: H3K4 methylation and promotion of shift from transcriptional initiation to elongation; in general H3K4me associates with active transcription
- H3K4me associates with active transcription

Fly **ASH1** has 1 HKMT homolog in humans:

- hASH1
- H3K4me and H3K36 methylation → activation of transcription

REMEMBER THE LINK BETWEEN H3K4me and DNA methylation

2. A LINK BETWEEN TrxG PROTEINS AND HISTONE ACETYLATION

Fly: -

TRX is associated with dCBP: a histone lysine acetyl transferase (dCBP is not a TrxG gene) (humans: CBP/p300 HAT complex!!!). Recruitment of TrxG proteins by transcription factor brings HAT and leads to:

- TrxG protein kismet (kis) contains a helicase domain is recruited after ASH1 activity → chromatin remodeling

- H3K4 methylation, H3K36 methylation
- H3 and H4 acetylation
- Reduced H3K9 methylation
- Reduced H3K27 methylation
- Reduced DNA methylation (humans)

EPIGENETIC ACTIVATION OF GENE EXPRESSION

Figure 7. trxG and PcG functions and interactions. Both trxG and PcG families include proteins that covalently modify histones and those that noncovalently modify chromatin. Covalent modifications on histones can promote or block the binding or activity of trxG complexes (e.g., SWI/SNF and KIS), PcG complexes (e.g., PRC1 and PRC2), or other factors involved in the maintenance of active or repressed states. Binding by these latter complexes has the potential to lead to further covalent modification, thus leading to iterative cycles of covalent modification and recognition of the covalent marks.

3. TRITHORAX GROUP PROTEINS ARE CENTRAL CHROMATIN REMODELERS

Trithorax group proteins BRM (fly) BRG1 (human) belong to the SWI/SNF family of chromatin remodelers.

SWI/SNF family chromatin remodeling complexes contain up to 18 subunits and can shift nucleosomes to increase the accessibility of regulatory sequences

Chromatin remodelers are recruited by transcription factors but are also recruited during transcriptional elongation.

→ Chromatin remodeling is essential during all phases of transcription

→ #25000 complexes in a human cell: highly important for gene expression control

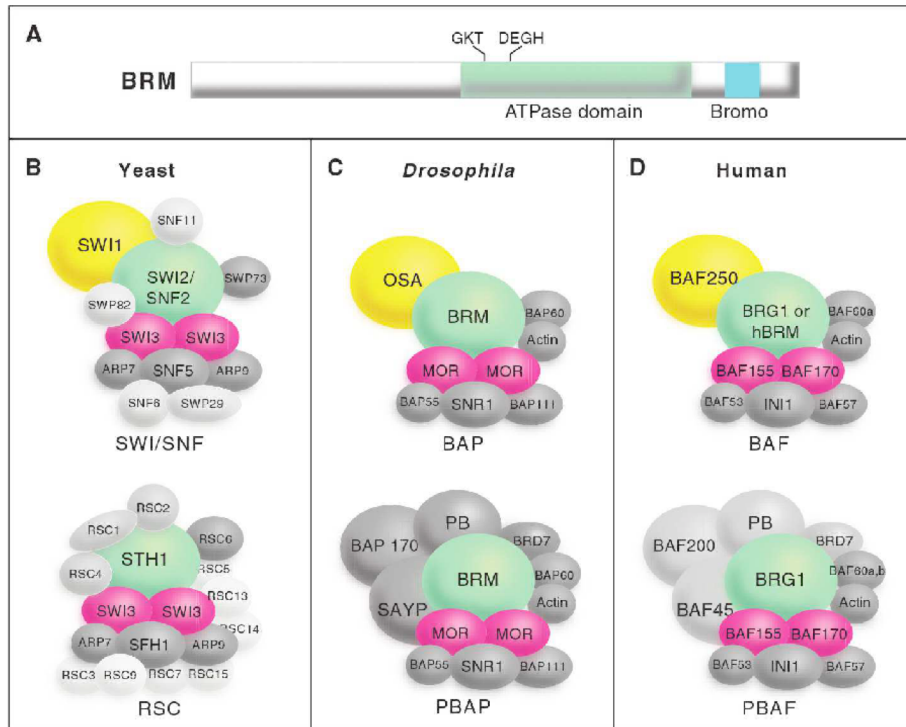


Figure 5. The SWI/SNF family of remodeling complexes. Each complex contains a member of SNF2/SWI2 family of ATPases and at least eight other subunits. (A) Schematic diagram of the BRM protein showing the location of the ATPase domain and carboxy-terminal bromodomain (which shows affinity to acetylated lysine residues in histone tails), which are conserved in all SNF2/SWI2 family members. SWI/SNF complexes in yeast (B), *Drosophila* (C), and humans (D) are shown. *Drosophila* trxG proteins (BRM, MOR, and OSA) and their counterparts in other organisms are shown in color. Further information about these complexes and their subunits may be found in [Mohrmann and Verrijzer \(2005\)](#).

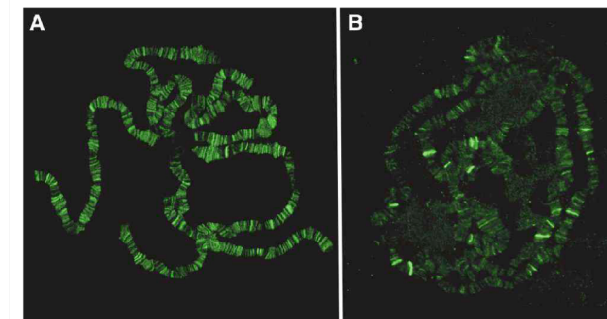


Figure 6. Chromosomal distribution of trxG proteins. The genome-wide distribution of trxG proteins was examined by staining *Drosophila* salivary gland polytene chromosomes with antibodies against BRM (A) or TRX (B). Consistent with a relatively global role in transcriptional activation, BRM is associated with hundreds of sites in a pattern that overlaps extensively with RNA Pol II. In contrast, strong TRX signals are detected at a much smaller number of sites on polytene chromosomes.

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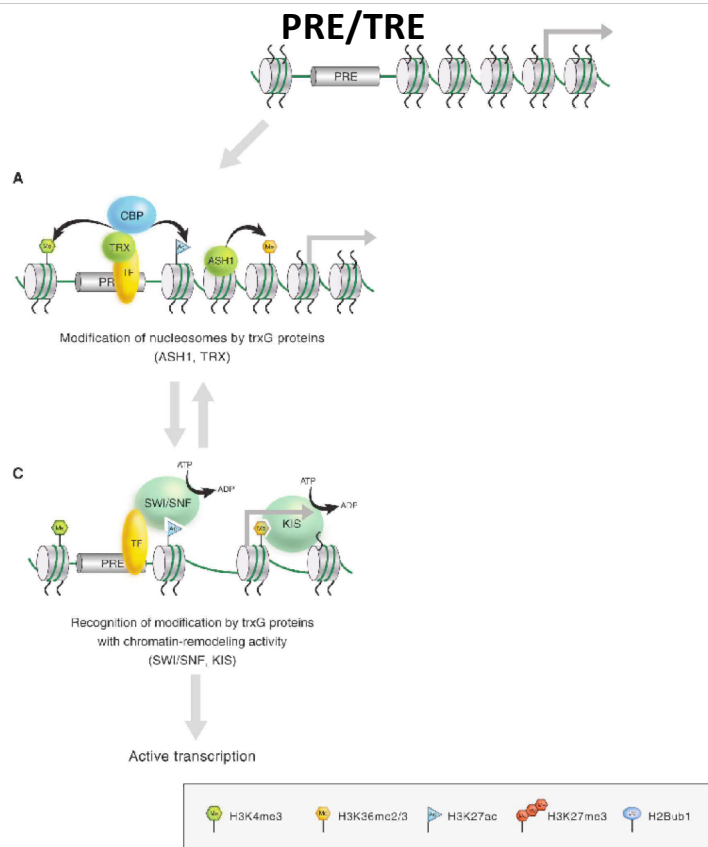


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Kismet (fly) CHD7 (human) has affinity for H3K36methylated chromatin
 → Chromatin remodelling complex (multiprotein)
 → Associated with RNA polymerase

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CHARGE SYNDROME IS CAUSED BY MUTATION IN HUMAN *CHD7* (CHD7)

CHARGE syndrome (formerly known as CHARGE association), is a syndrome caused by a genetic disorder. It was first described in 1979. In 1981, the term "CHARGE" came into use as an acronym for the set of unusual congenital features seen in a number of newborn children.[1] The letters stand for: coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness. These features are no longer used in making a diagnosis of CHARGE syndrome, but the name remains. CHARGE syndrome is the leading cause of congenital deafblindness.

CHARGE syndrome was formerly referred to as CHARGE association, which indicates a non-random pattern of congenital anomalies that occurs together more frequently than one would expect on the basis of chance. Very few people with CHARGE will have 100% of its known features. In 2004, mutations on the CHD7 gene (located on Chromosome 8) were found in 10 of 17 patients in a study conducted in the Netherlands, making CHARGE an official syndrome. A further study in the US of 110 individuals with CHARGE syndrome showed that 60% of those tested had a mutation of the CHD7 gene.[6] CHD7 is a member of the chromodomain helicase DNA-binding (CHD) protein family that plays a role in transcription regulation by chromatin remodeling.[7]

1/10000 births

500 different mutations in CHD7 identified

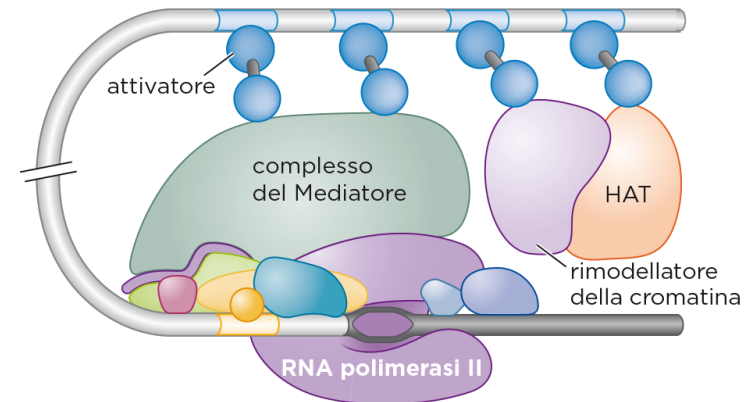


Major Features of CHARGE Syndrome (very common in CHARGE and relatively rare in other conditions)

FEATURE	INCLUDES	FREQUENCY
Coloboma of the eye	Coloboma (sort of like a cleft) of the iris, retina, choroid, macula or disc (not the eyelid); microphthalmos (small eye) or anophthalmos (missing eye): CAUSES VISION LOSS Pictures	80%-90%
Choanal atresia or stenosis	The choanae are the passages that go from the back of the nose to the throat. They can be narrow (stenosis) or blocked (atresia). It can be unilateral (one-sided) or bilateral (both sides), bony or membranous. Unilateral atresia or stenosis can be difficult to diagnose Pictures	50%-60%
Cranial nerve abnormality	I - Missing or decreased sense of smell	90-100%
	IX/X - Swallowing difficulties, aspiration - Pictures	70%-90%
	VII - Facial palsy (one side or both) - Pictures	40%
CHARGE outer ear	Short, wide ear with little or no lobe, "snipped off" helix (outer fold), prominent antihelix (inner fold) which is discontinuous with tragus, triangular concha, decreased cartilage (floppy), often stick out, usually asymmetric - Pictures	>50%
CHARGE middle ear	Malformed bones of the middle ear (ossicles): CAUSES CONDUCTIVE HEARING LOSS	Common
CHARGE inner ear	Malformed cochlea (Mondini defect); small or absent semicircular canals: CAUSE HEARING LOSS AND BALANCE PROBLEMS - Pictures	90%

4. TRITHORAX GROUP PROTEINS ARE ASSOCIATED WITH THE TRANSCRIPTIONAL COMPLEX

- The mediator complex is a large protein complex (<20 proteins) that communicates between the basal transcription factors and activating regulatory elements.
- Essential for the initiation of transcription!!



5. TRITHORAX GROUP PROTEINS ARE LINKED WITH LONG-RANGE CHROMATIN INTERACTION

Rad21 is member of trithorax group genes!
Has a role in long range chromatin interaction
Links enhancers with promoters via loop formation

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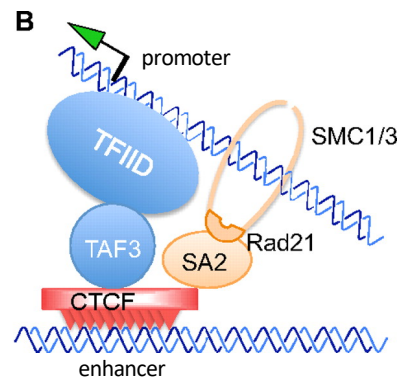


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Trithorax group proteins are recruited by TRE – Trithorax response elements

DROSOPHILA

TRE elements are concentrated DNA binding sites for Trithorax group proteins

→ Recruitment function

NOTE: MOST TRE elements overlap with PRE elements → PRE/TRE motifs

For example: GAF/Psq and Zeste have an important role in Trithorax recruitment
And gene activation.

However, sites are located in PREs

→ Cell type specific transcription program (type and dosage of expressed transcription factors
co-ordinate balance of Polycomb/Trithorax function

Development 134, 223-232 (2007) doi:10.1242/dev.02723

Polycomb/Trithorax response elements and epigenetic memory of cell identity

Leonie Ringrose¹ and Renato Paro²

PRE/TRE motifs and flexibility of PRE/TRE design.

- (A) DNA motifs shown to be important for PRE/TRE function. The Grh (Grainy head) protein binds to several different PRE/TRE sites. The motif shown is that found in PRE/TREs by Blastyak et al. (Blastyak et al., 2006). The Dsp1 protein also has broad DNA-binding specificity (Brickman et al., 1999). The motif shown is that used by Dejardin et al. (Dejardin et al., 2005). Gaf binds the same target sequence as Pipsqueak (Psq), suggesting that the two proteins may compete or cooperate at closely spaced sites.
- (B) Many of these motifs are important for regulating genes that do not have PRE/TREs, for example the *Drosophila white* gene which is regulated by the Zeste protein (600 bp of upstream regulatory region are shown). These motifs are also short and occur randomly in DNA, such as in the bacterial *LacZ* gene (the first 600 bp of the coding sequence are shown).
- (C) PRE/TREs have different combinations of motifs, with no preferred order or number. Shown here are ~600 bp of the *bxd* and *Fab-7* PREs from the *Drosophila* Bithorax complex, and of PRE/TREs from the *Drosophila* engrailed (*en*), vestigial (*vg*) and homothorax (*hth*) loci. **Grey boxes show minimal PRE/TREs where these have been defined (Dejardin et al., 2005; Brown et al., 2005).** Flanking sequences contain additional motif clusters which may contribute to the function of these PRE/TREs in their endogenous context.

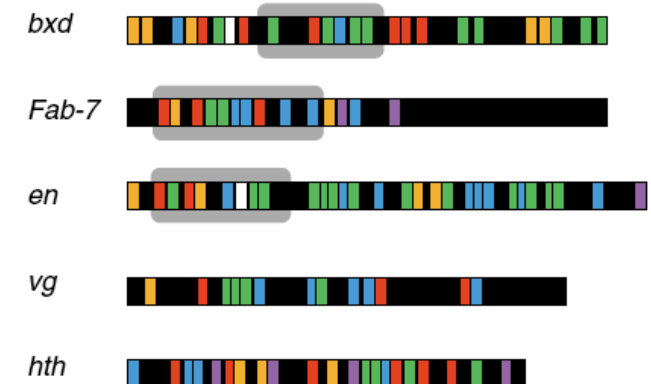
A PRE motifs

	Pho/Phol	GCCAT
	Dsp1	GAAAA
	GAF/Psq	GAGAG
	Zeste	YGAGYG
	Grh	TGTTTTT
	Sp1/KLF	RRGGYGY

B Motif occurrence in non-PREs



C Motif occurrence in PREs



COMPETING FUNCTION OF POLYCOMB AND TRITHORAX GROUP PROTEINS AT PRE/TREs

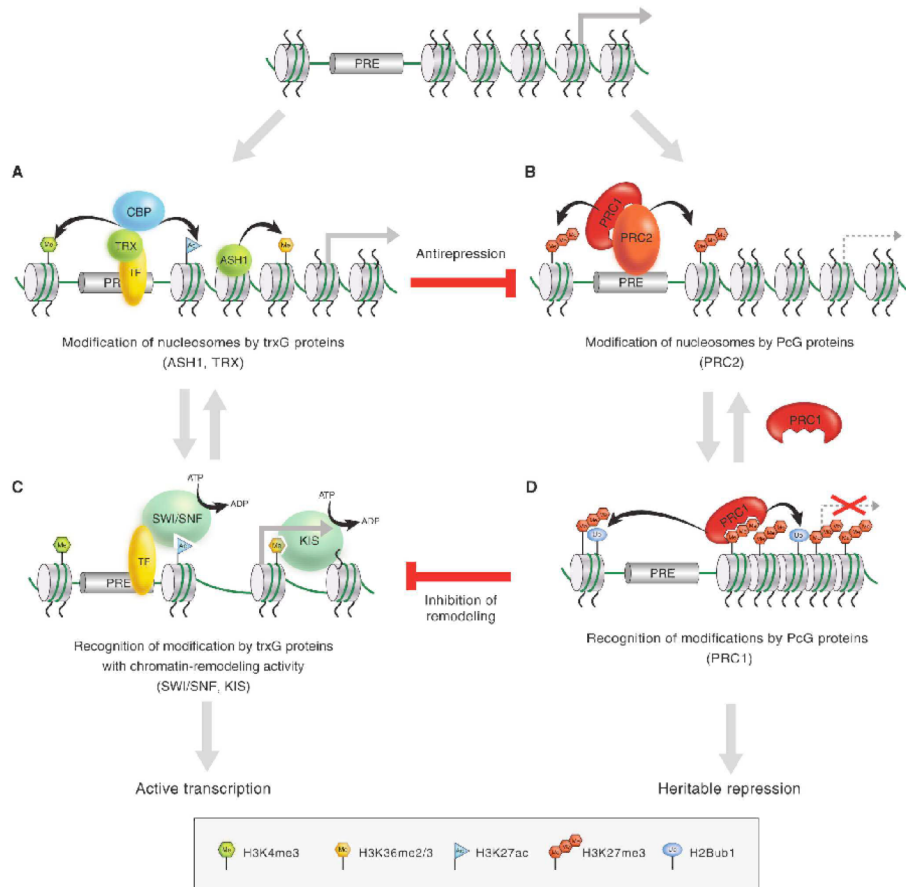


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- ASH1 mediates H3K4me3 and H3K36me3
- H3K36 methylation enhances transcriptional elongation
- TRX/CBP complex mediates H3K4methylation and H3K27acetylation
- H3K27acetylation prevents H3K27methylation by E(z)
- H3K4me3 inhibits the recruitment of PRC2
- H3K36methylation inhibits the activity of PRC2

FUNCTIONAL ANTAGONISM BETWEEN TRX and PcG

REMEMBER: TRANSCRIPTION FACTORS CONTROL THE EQUILIBRIUM BETWEEN PcG and TxG

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