Epigenetic cellular memory:
Polycomb and Trithorax group paradigm in development

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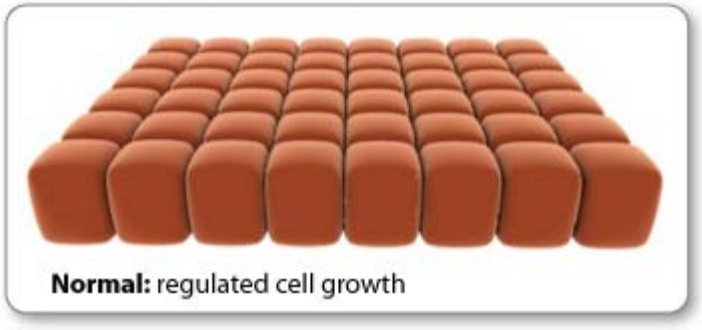
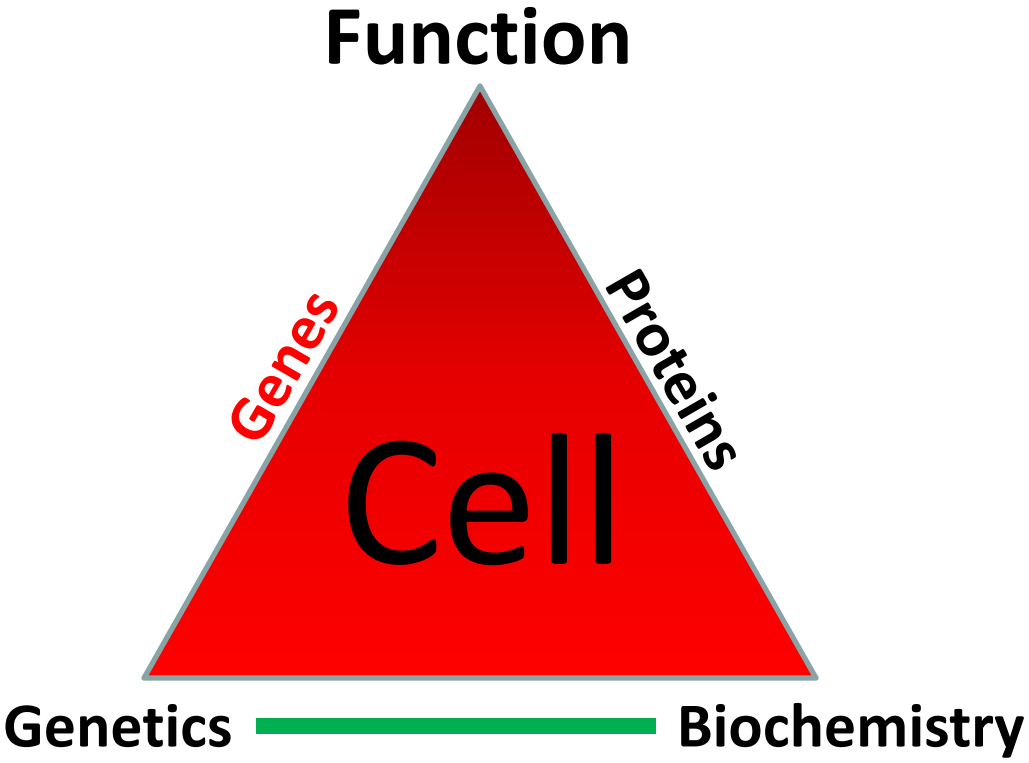
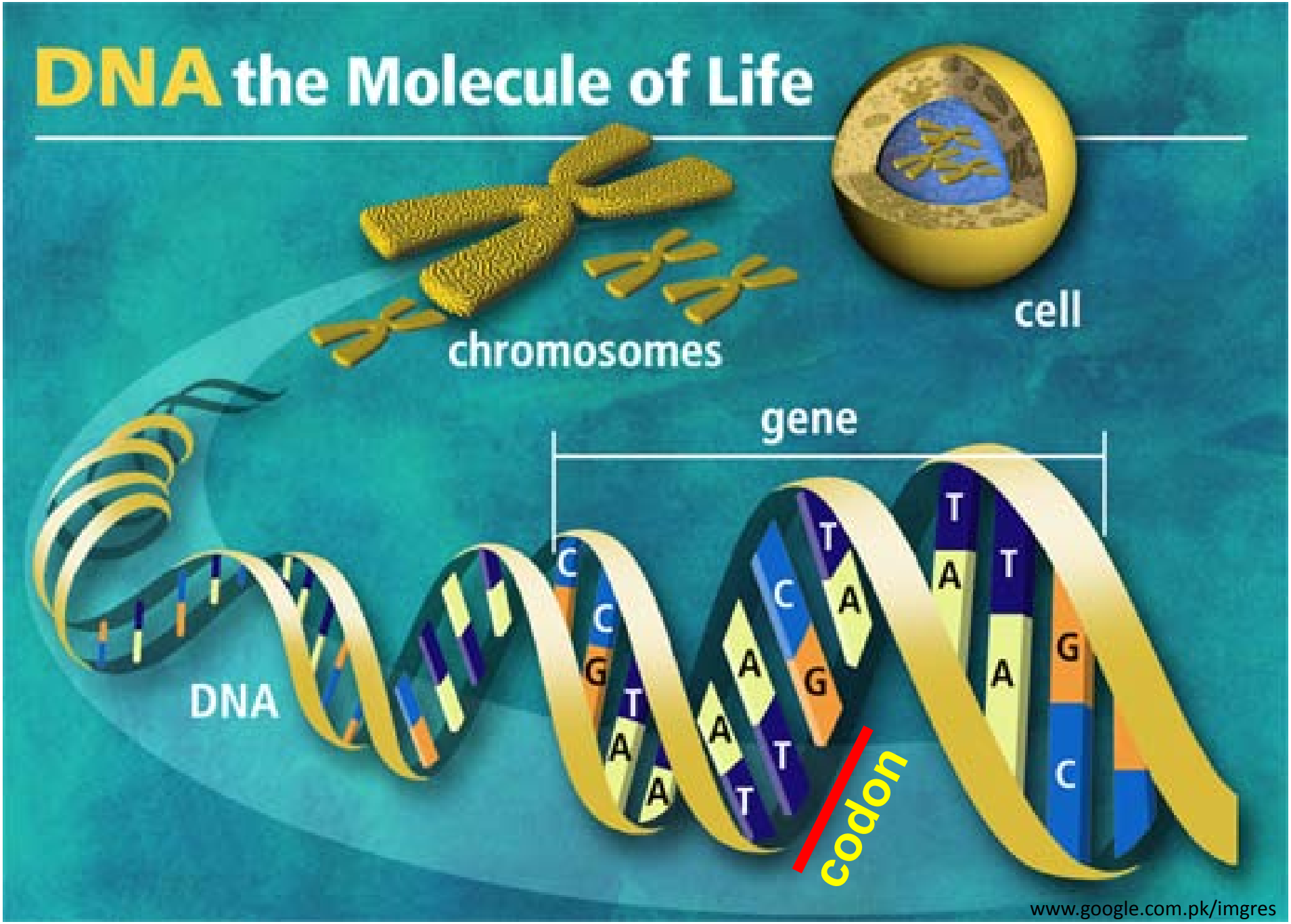
From normal to abnormal



Normal: regulated cell growth


Function
Genes
Proteins
Cell
Genetics
Biochemistry
Molecular Biology
(Recombinant DNA technology, gene cloning etc)



www.google.com.pk/imgres

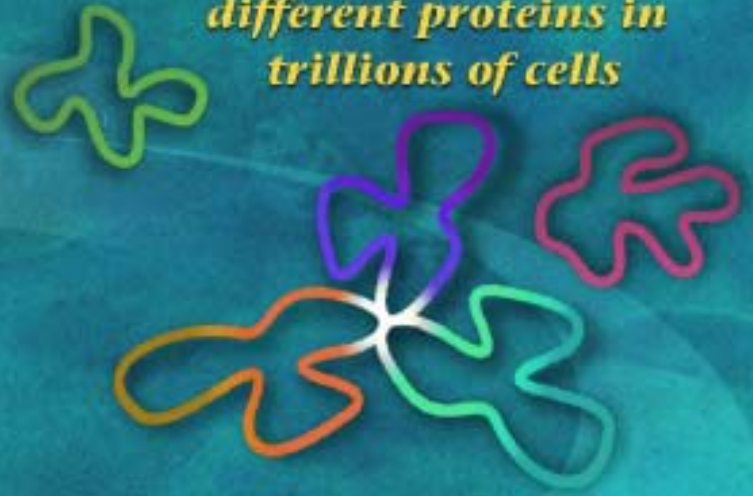
From DNA to Humans

CGTTCTCTATTAACA...

GCAAGAGATAATTGT...

3 billion DNA subunits in the cell nucleus

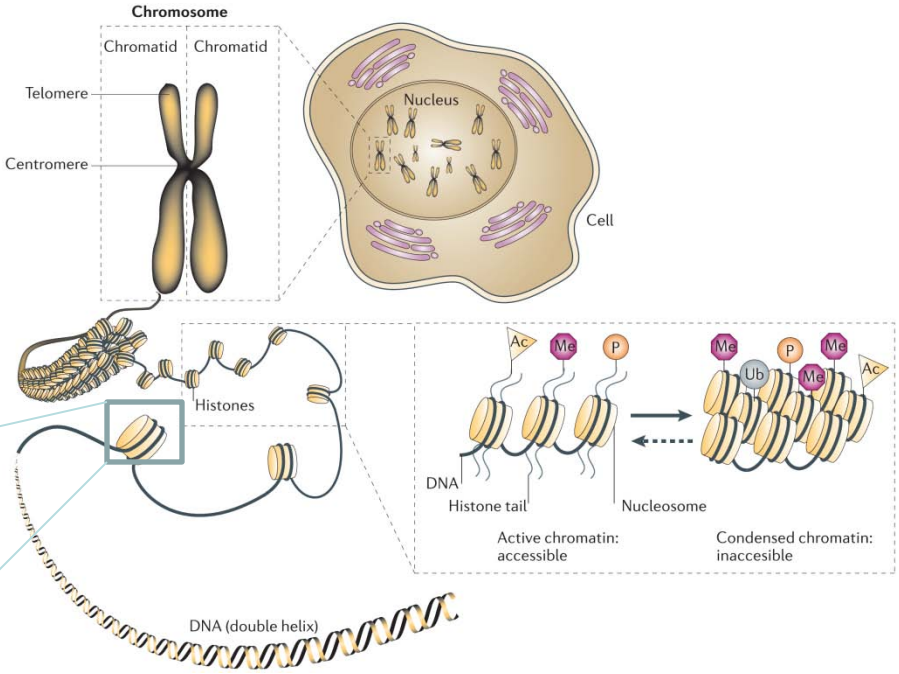
DNA Codes for ~80,000 different proteins in trillions of cells

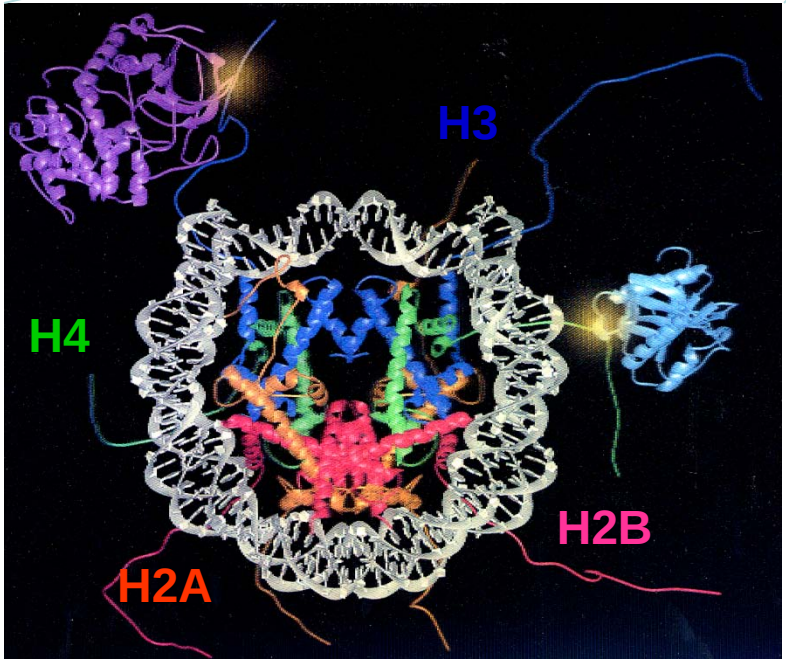


Cells respond to environment

YGG-00-0482

Eukaryotic chromatin organization





Covalent modifications of DNA and histones constitute Epigenome

Epigenetic modifications of chromatin

Cytosine methylation of DNA

M
CpG
Plants
mammals

M
CpNpG
Plant specific
(mammals)

M
CpNpN
Plant specific
(mammals)

Histone tail modifications

Genetic effect:

ATG CGA CTG C**A**A GAT ACG CAT
TAC GCT GAC G**T**T CTA TGC GTA

Normal

ATG CGA CTG C**G**A GAT ACG CAT
TAC GCT GAC G**C**T CTA TGC GTA

Mutant

Epigenetic effect:

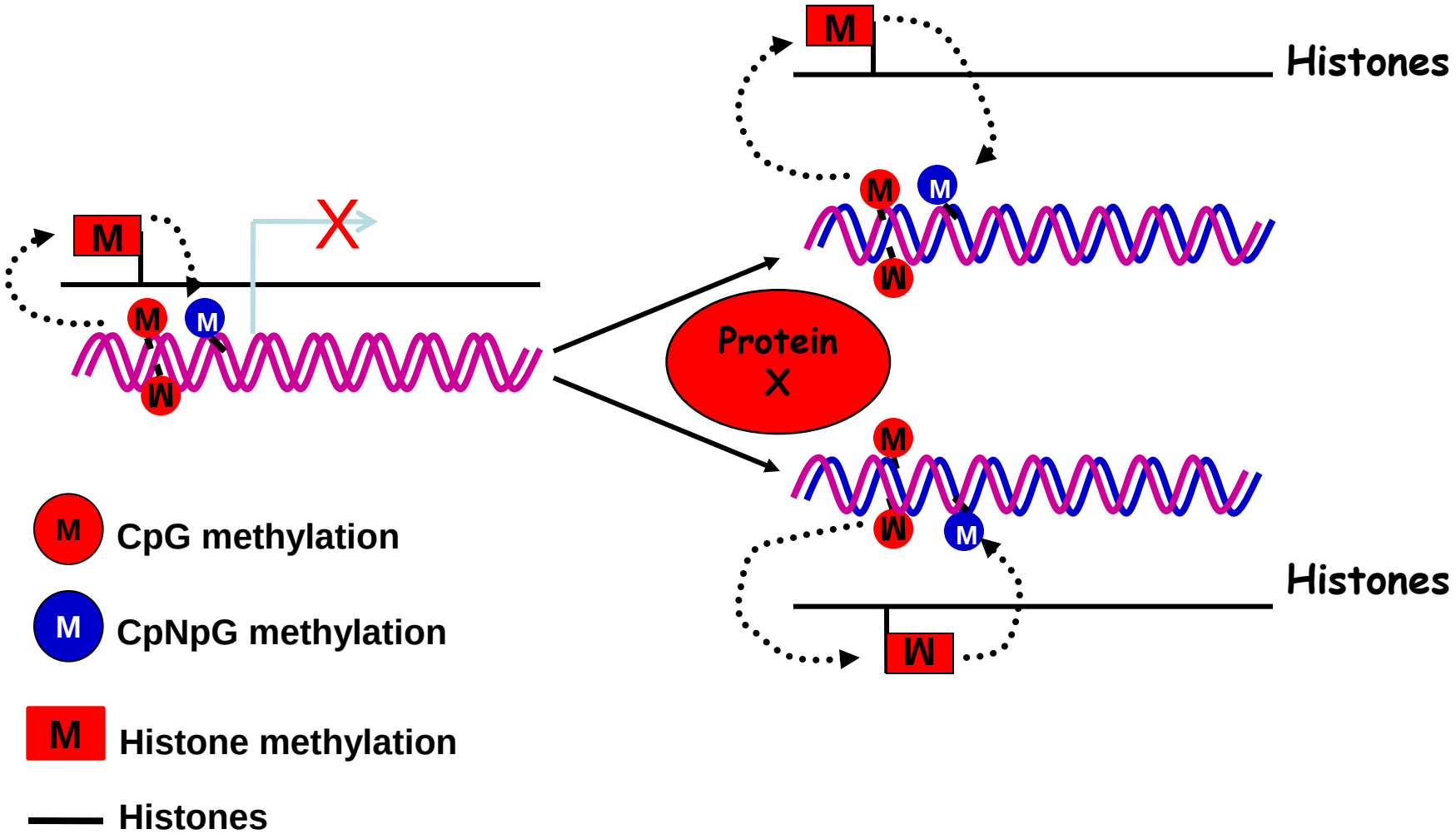
ATG CGA CTG CAA GAT ACG CAT
TAC GCT GAC GTT CTA TGC GTA

Normal

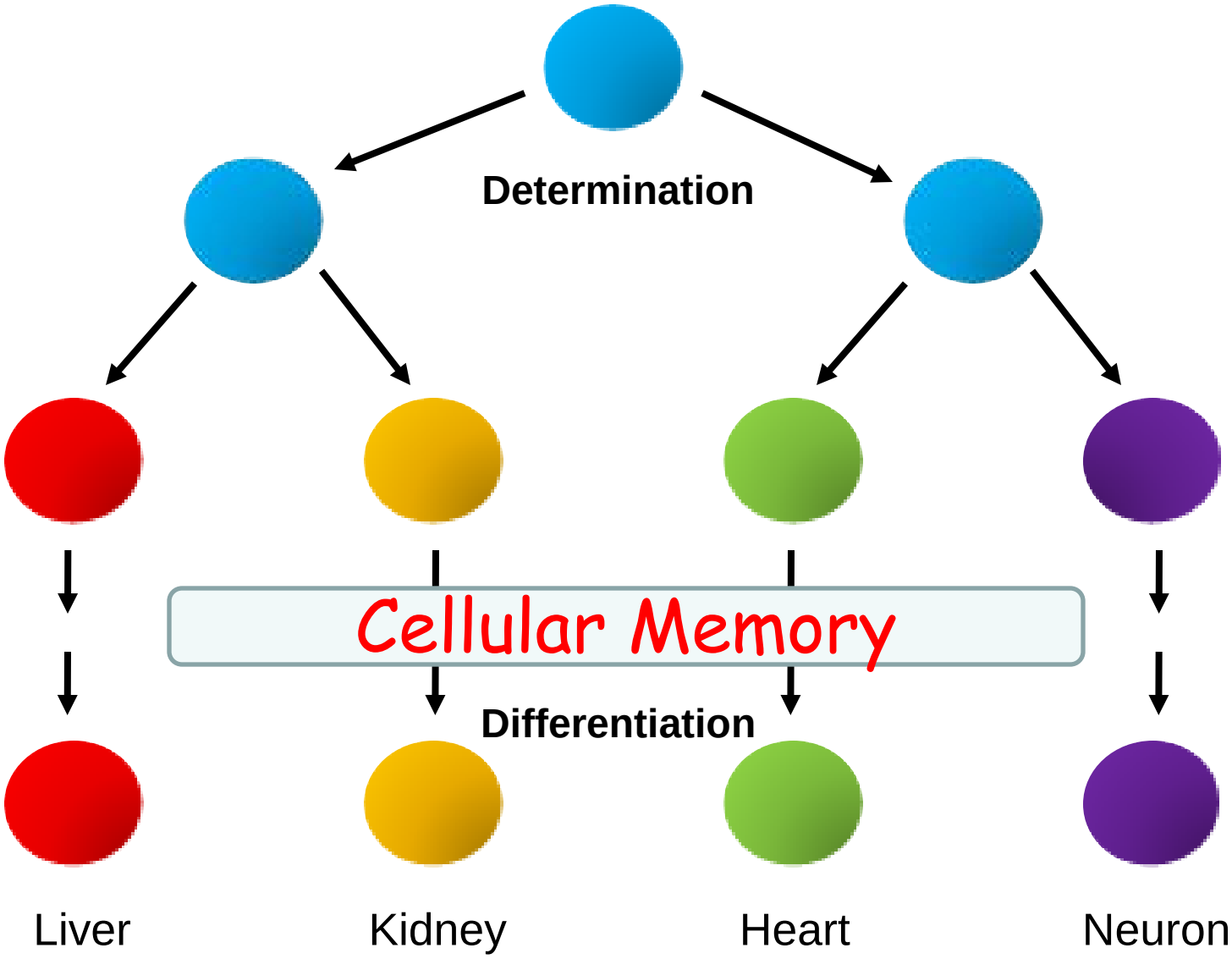
ATG CGA CTG CAA GAT ACG CAT
TAC GCT GAC GTT CTA TGC GTA

Epi-mutant

Epigenetic inheritance



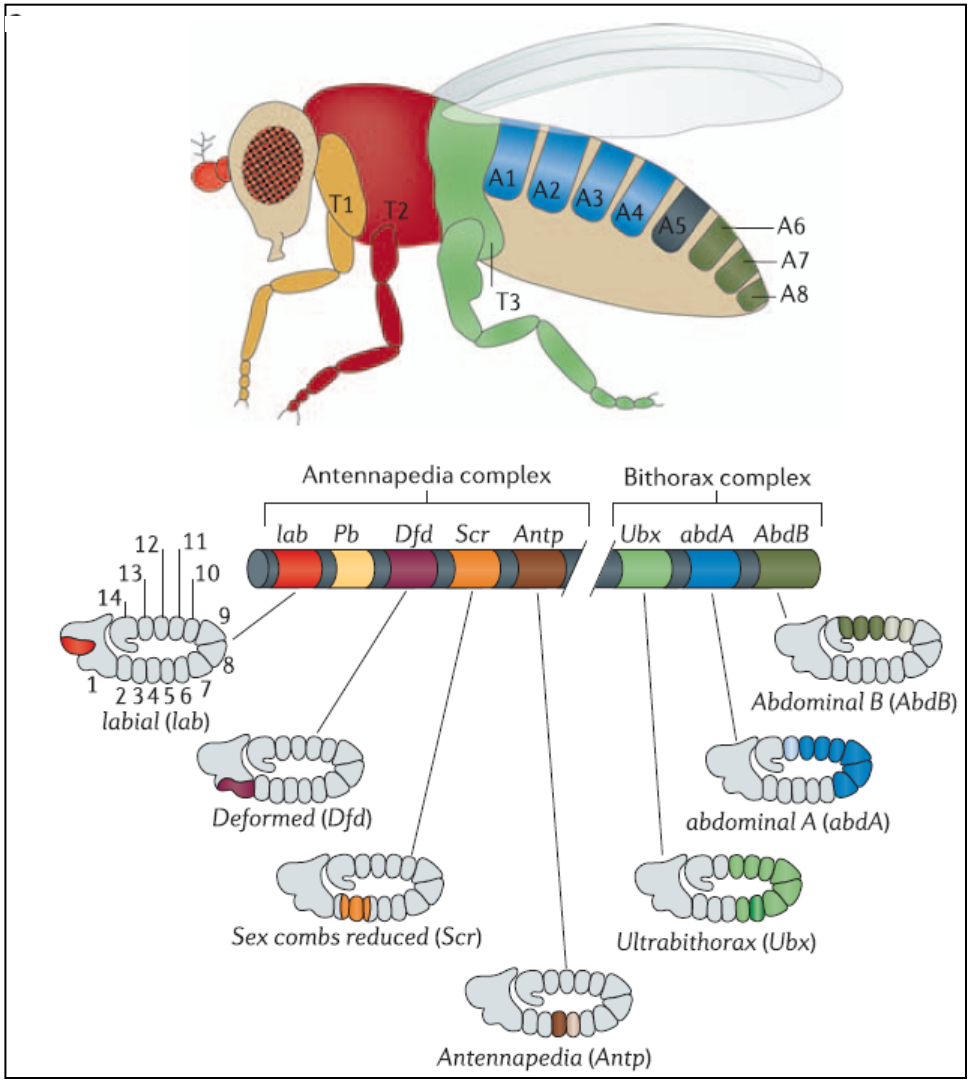
Cellular memory in eukaryotic development

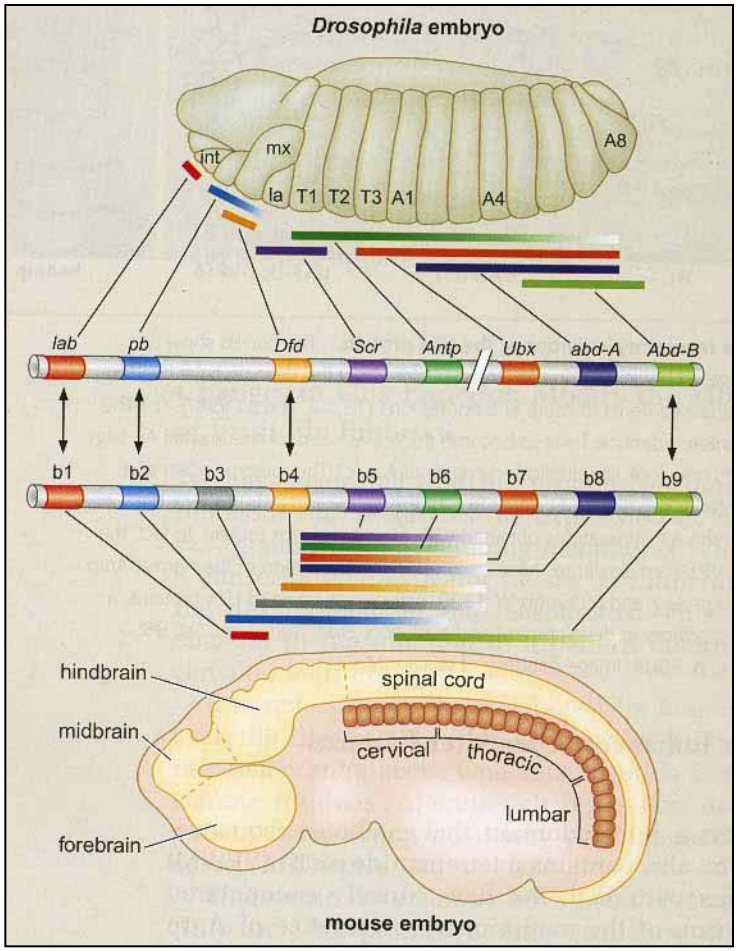


Cells must remember their identity

Cellular memory in eukaryotic development: lessons from fly

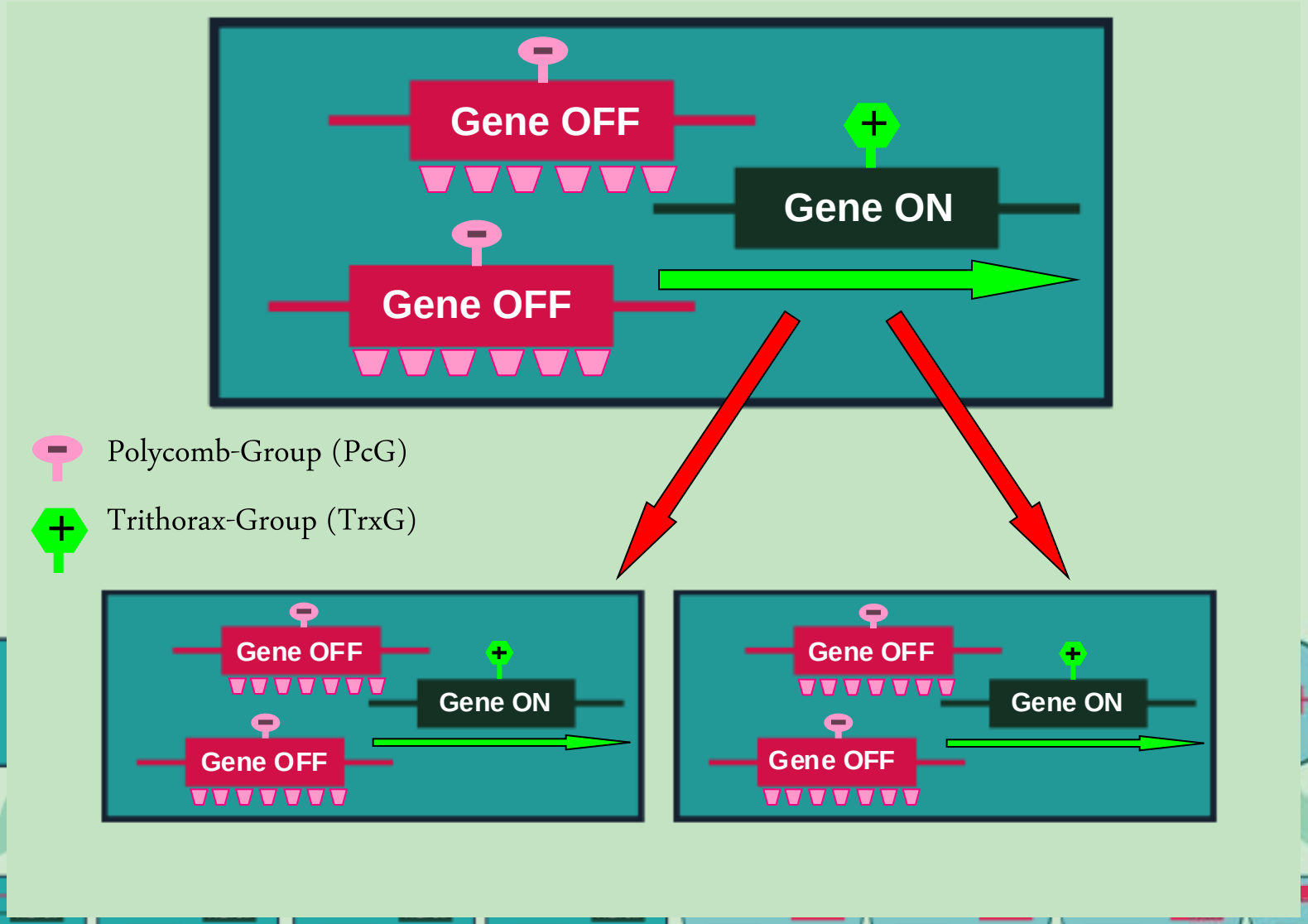
Anterior-posterior pattern formation



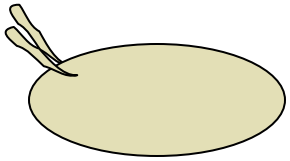


Cellular memory is maintained at <u>epigenetic</u> level

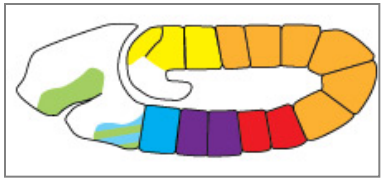
Cellular Memory



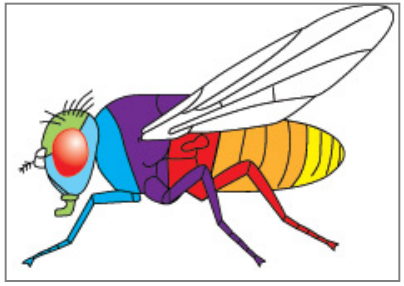
Polycomb group (PcG) and trithorax group (trxG) proteins: Gate keepers of cellular memory



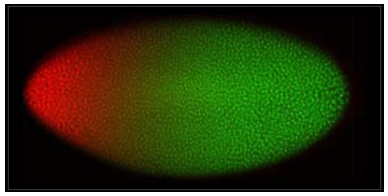
Determination



Differentiation

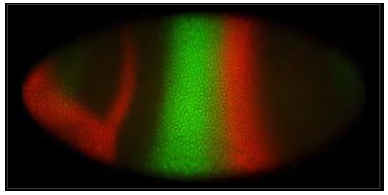


maternal genes



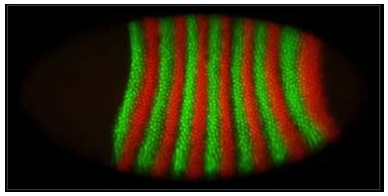
Bcd Cad

gap genes



Kni Kr

pair-rule genes



Eve Ftz

homeotic genes

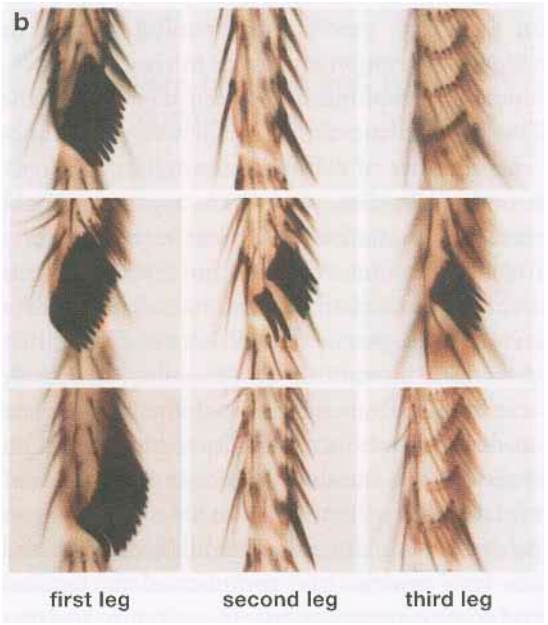
PcG / trxG

PcG/trxG paradigm

Wild type

PcG mutant

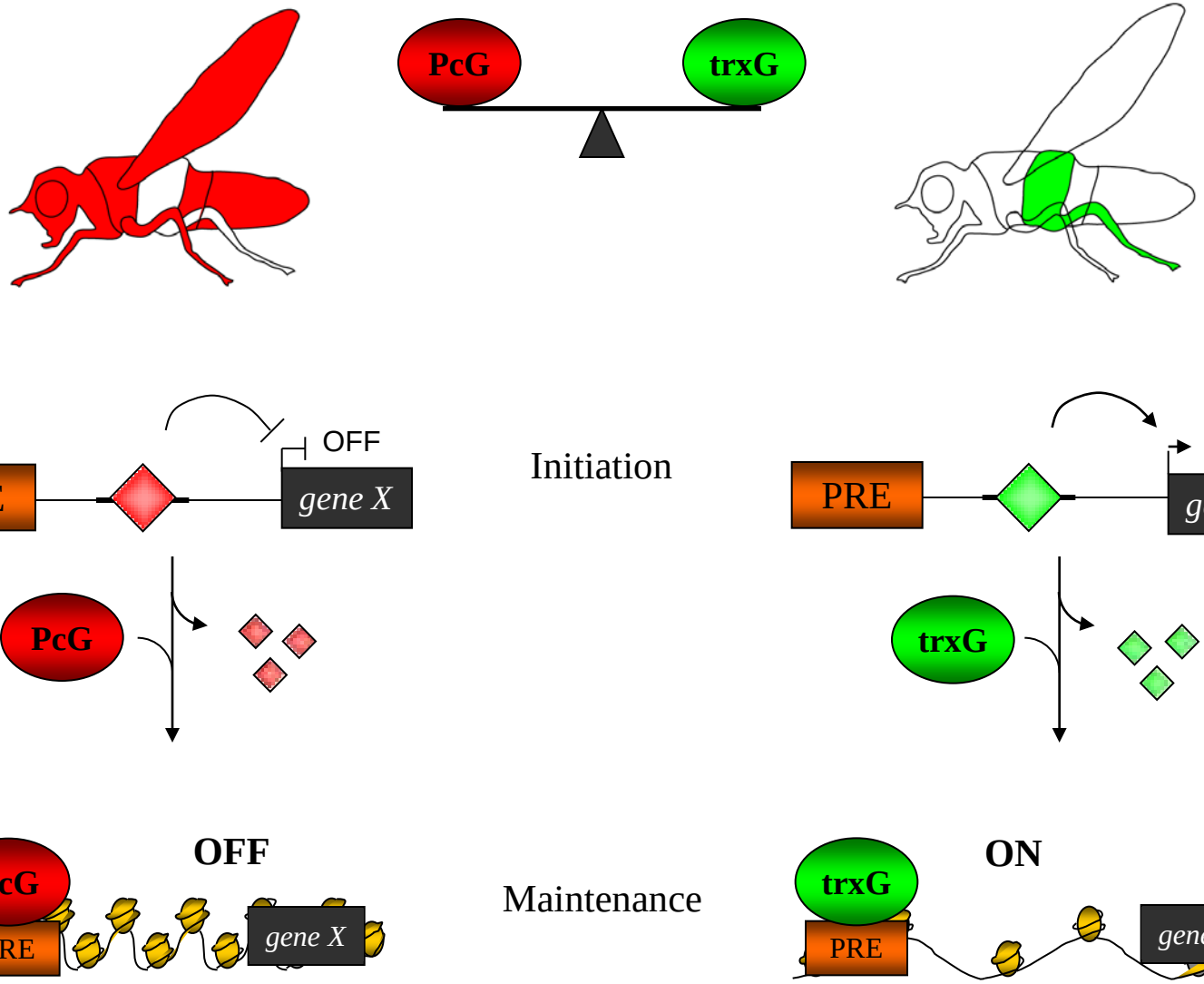
PcG/trxG mutant



trxG counteracts PcG

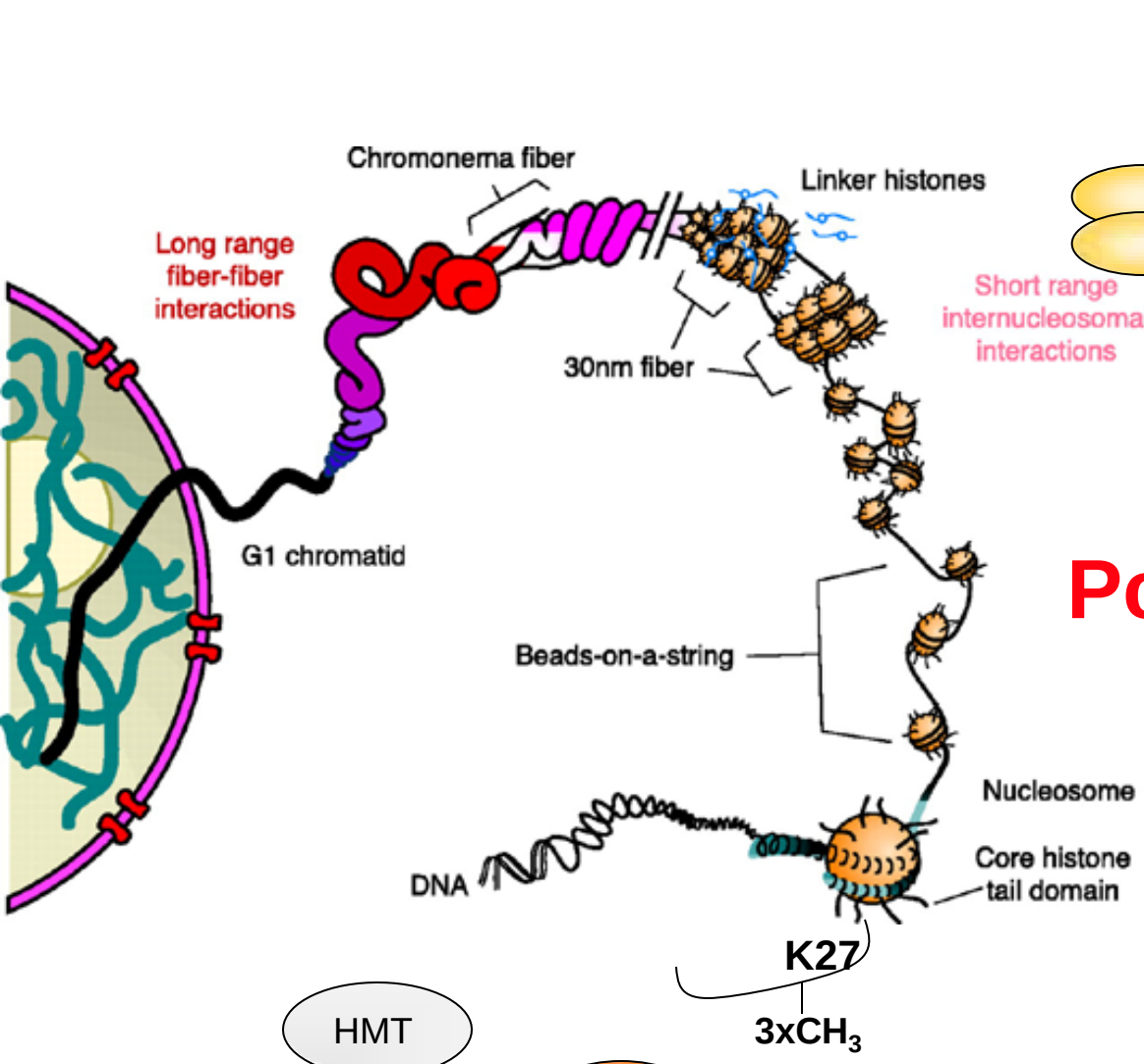
Kingston and Tamkun 2006

Mechanism of Cellular memory mediated by PcG and trxG

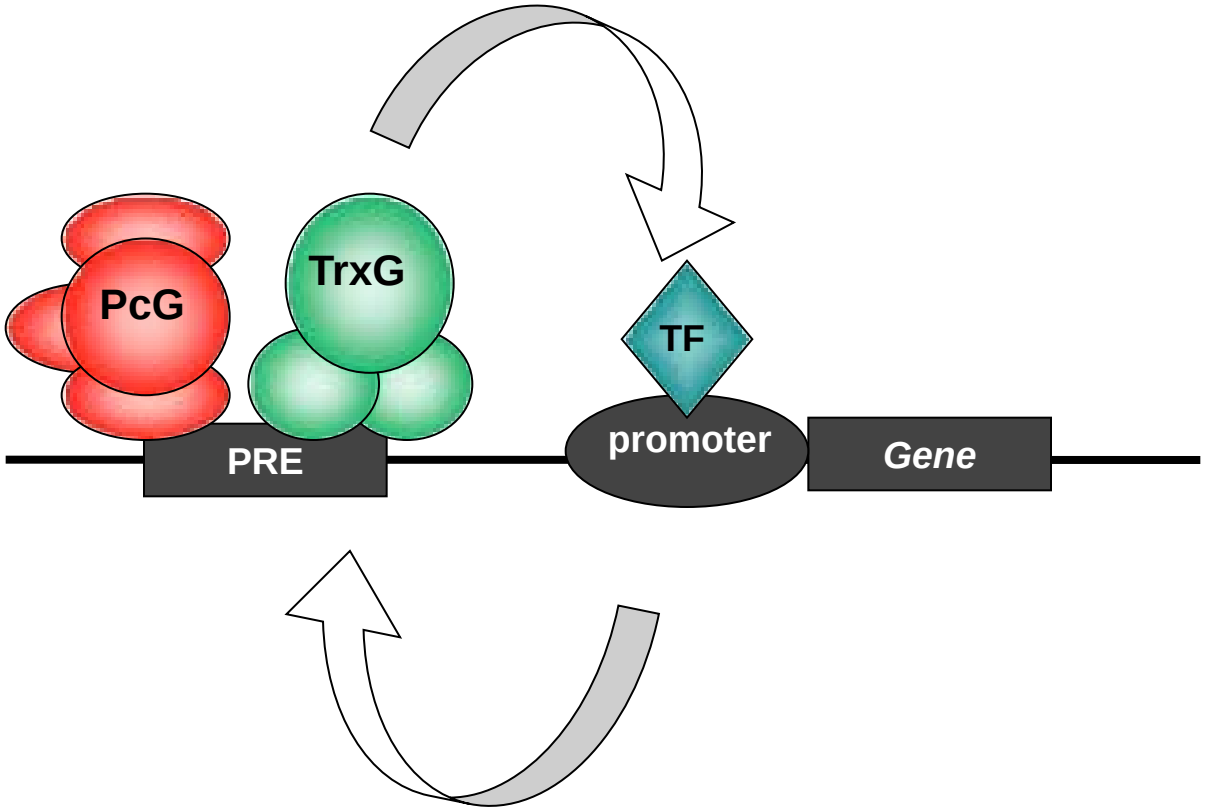


ΔPcG or ΔtrxG leads to homeotic phenotypes

PcG/TrxG proteins act at the level of chromatin



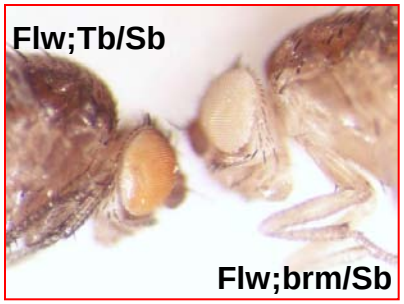
PcG/TrxG proteins act through PREs



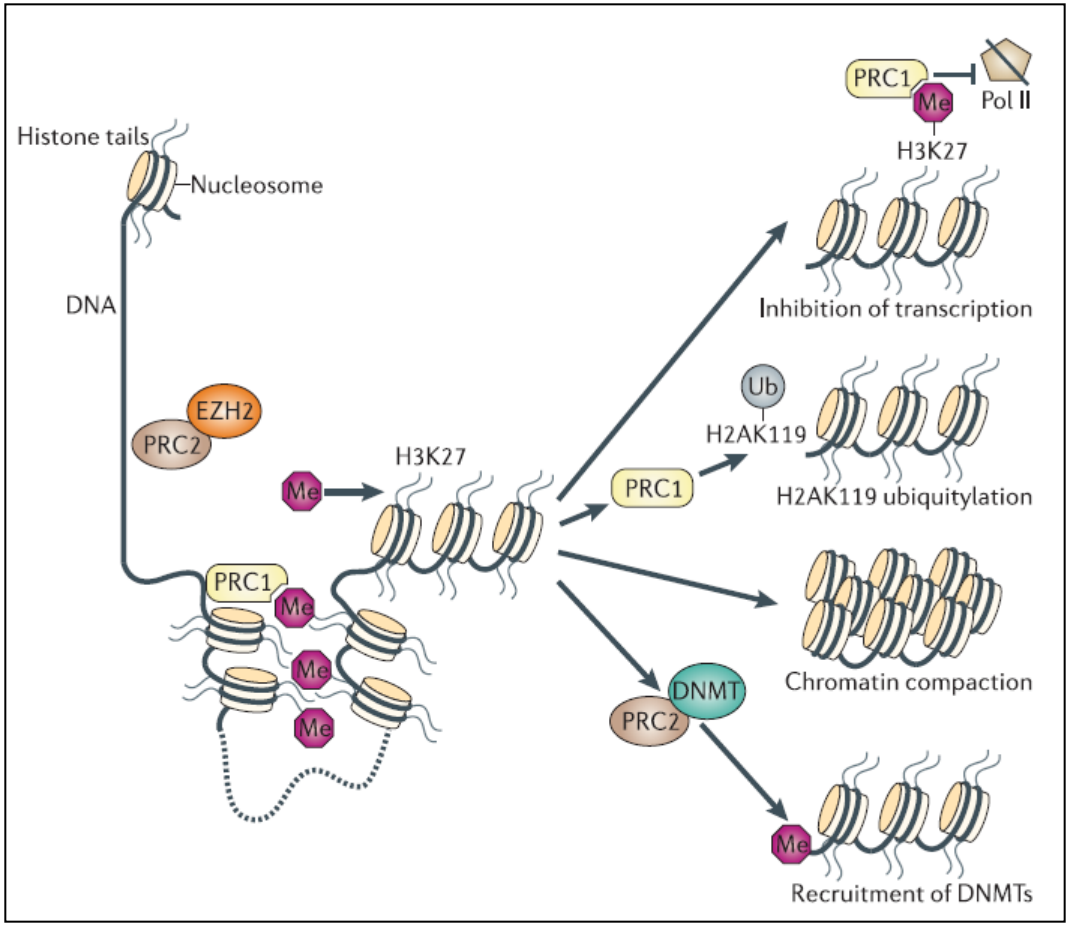
PcG/TrxG gene regulation in a transgene:

Analysis of FLW transgene

brm : brahma
trx: trithorax



Mechanism of PcG action



PcG proteins are conserved across species

Proteins			Protein domains	Functions
Drosophila melanogaster	Human	Mouse		
PRC2 initiation complex				
ESC	EED	EED	WD40 repeats*	
E(Z)	EZH1	EZH1/ENX2	SET domain‡	Histone methyltransferase
	EZH2	EZH2/ENX1		
SU(Z)12	SUZ12	SUZ12	Zinc-finger domain	
PRC1 maintenance complex				
PC	CBX2/HPC1	CBX2/M33	Chromodomain	Methyl-lysine binding
	CBX4/HPC2	CBX4/MPC2		
	CBX8/HPC3	CBX8/PC3		
PH	EDR1/HPH1	EDR1/MPH1/RAE28	Zinc-finger SPM domain§	
	EDR2/HPH2	EDR2/MPH2		
	EDR3/HPH3	EDR3		
RING	RING1/RNF1/RING1A	RING1/RING1A	RING-finger domain‖	Ubiquitin ligase
	RNF2/RING1B	RNF2/RING1B		
PSC	BMI1	BMI1	RING-finger domain‖	
	RNF110/ZFP144/ PCGF2	RNF110/ZFP144/ MEL18		
	ZNF134	ZNF134		
PHO	YY1	YY1	Zinc-finger domain	Sequence-specific DNA binding
PHO-like				DNA binding
SCM	SCML1	SCMH1	Zinc-finger SPM domain§	
PCL	PHF1	PHF1/PCL1	PHD-finger domain	

Human cancers with altered expression of PcG proteins

Protein	Cancer type
EZH2	B-cell non-Hodgkin lymphoma
	Bladder
	Breast
	Colon
	Hodgkin lymphoma
	Liver
	Mantle cell lymphoma
	Melanoma
	Prostate
SUZ12	Breast
	Colon
	Liver
BMI1	B-cell non-Hodgkin lymphoma
	Leukaemia
	Mantle cell lymphoma
	Medulloblastoma
	Neuroblastoma
	Non-small cell lung cancer
PCL3	Colon
	Skin
	Lung
	Rectal
	Cervical
	Uterus
	Liver
RAE28	Acute lymphoblastic leukaemia

Environment interacts with Epigenome

Hsp90 mediated homeotic phenotypes

- Hsp90 has over 100 client proteins
- conformational maturation of diverse set of proteins

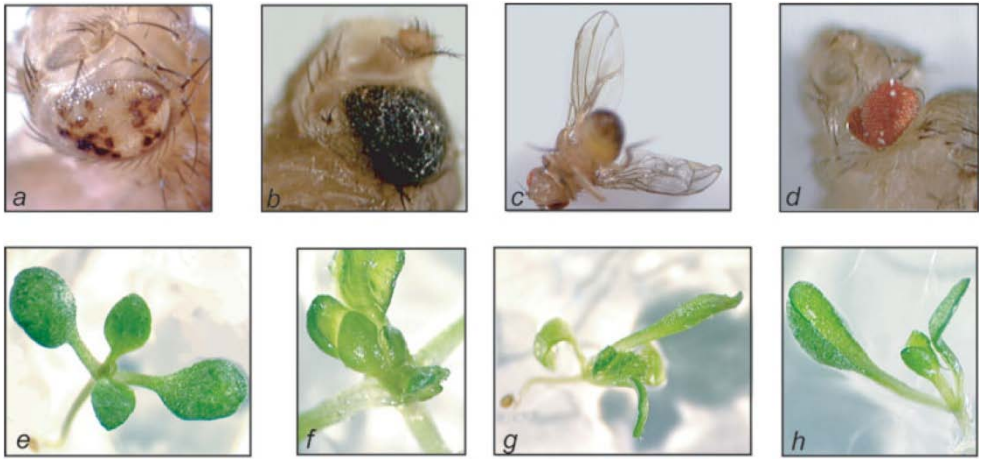


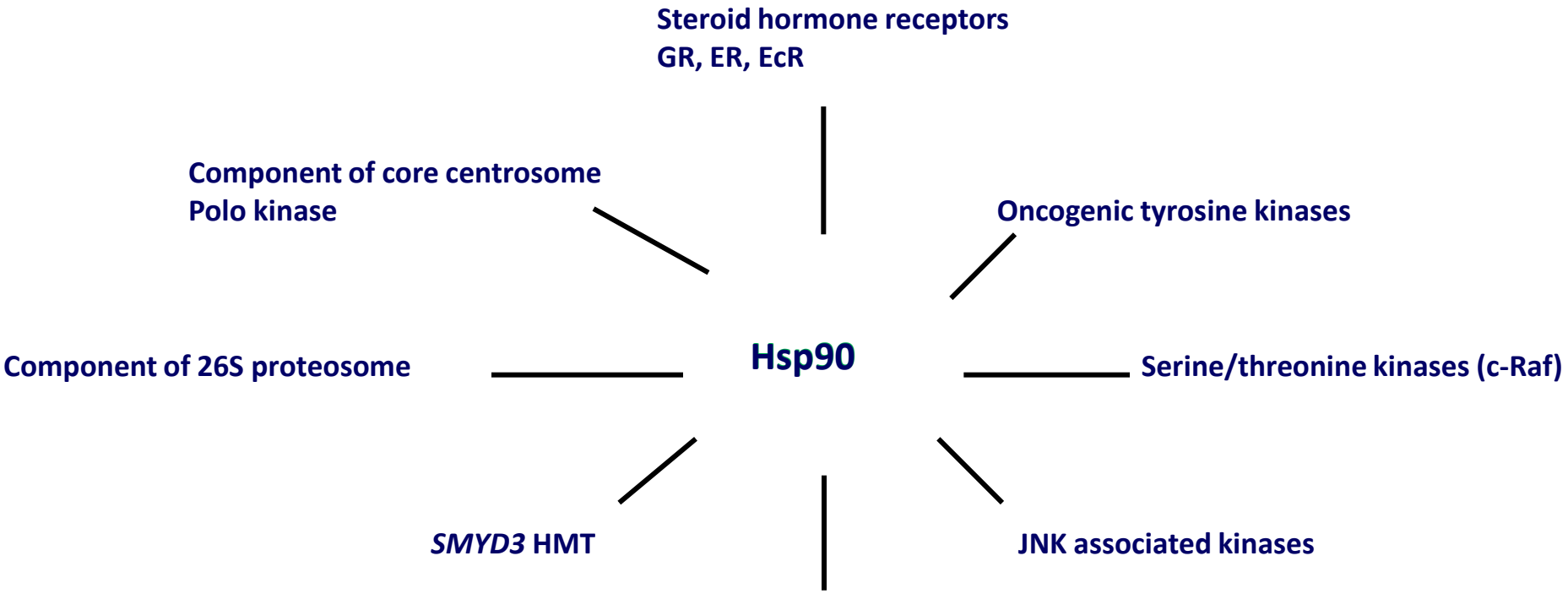
Table 1 Developmental defects associated with Hsp90 deficit

Body part	Code	Description	No. of observations	Temperature (°C)	F_1?	F_2?
Abdomen	A1	Disorganized tergites	14	25	Yes	–
	A2	External trachea?	7	25	No	n.d.
Bristles	B1	Duplications	36	30, 18	Yes	Yes
	B2	Extra scutellar bristles	48	18	Yes	n.d.
	B3	Split scutellars	8	18	Yes	n.d.
	B4	Forked	5	25	No	n.d.
Eyes	E1	Deformed	22	30	Yes	Yes
	E2	Transformed	7	18	No	–
	E3	Smooth	18	18	Yes	Yes
	E4	Rough	16	25	Yes	Yes
	E5	Black facets	24	18	Yes	Yes
	E6	Eyes absent	3	18	Yes	n.d.
Halteres	H1	Ubx transformations	9	25	Yes	Yes
Legs	L1	Deformed	28	18	Yes	Yes
	L2	Transformed	3	18	No	–
Thorax	T1	Disc eversion	12	25	No	–
	T2	Humeral 'balls'	5	26	Yes	n.d.
	T3	Duplication	6	25	No	n.d.
Wings	W1	Small round	26	18	Yes	Yes
	W2	Notched	6	18	Yes	–
	W3	Wing veins	7	18	No	Yes
	W4	Wing border	5	25	No	–
	W5	Transformed	9	18	Yes	n.d.

Developmental abnormalities produced in *Hsp83* mutants, coded according to the part of the fly affected. The approximate number of observations of, and the temperature most frequently producing, each trait are indicated. The observation of at least one cross producing multiple F_1 flies with a given trait is indicated by 'Yes', as is any instance of transmission of the trait to the F_2 generation. A dash indicates not observed; n.d., not done. Many fewer flies were tested at 30 °C, so this situation is under-represented.

Rutherford and Lindquist 1998
Queitsch et al 2002

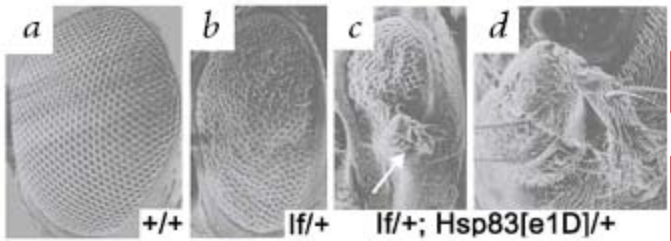
Molecular chaperone Hsp90 and Development



Epigenetics

Hsp90 and PcG or trxG???

Hsp90 and epigenetics



Inheritance of Hsp90

Mutant **Phenotype** in

subsequent Generations

was **Independent** of Hsp90

Mutation

Table 1 • *Me(Kr$^{lf\text{-}1}$)* mutations identified in a genetic screen

Me(Kr$^{lf\text{-}1}$)	Ectopic outgrowth*	Protein product
brahma^{2} (brm^{2})	5 ± 1%	Chromatin binding
devenir^{2} (dev^{2}/btl^{2})	10 ± 3%	FGF receptor
fs(1)h1^{1}	10 ± 2%	Chromatin binding
hsp83^{e3A}	11 ± 3%	Chaperone
hsp83^{e4A}	6 ± 2%	Chaperone
hsp83^{e6A}	6 ± 2%	Chaperone
hsp83^{e6D}	13 ± 4%	Chaperone
hsp83^{e1D}	15 ± 4%	Chaperone
khotalo^{1} (kto^{1})	15 ± 4%	Chromatin binding
kismet^{1} (kis^{1})	<0.1%	Chromatin binding
osa^{2}	10 ± 2%	Chromatin binding
skuld^{2} (skd^{2})	2 ± 1%	Unknown
Trithorax-like^{85} (Trl^{85})	10 ± 2%	Chromatin binding
urdur^{2} (urd^{2})	5 ± 2%	Unknown
verthandi^{3} (vtd^{3})	55 ±8%	Unknown
zeste^{V778} (z^{V778})	5 ± 2%	Chromatin binding
iso-1,2,3	<0.1%	Isogenic strain
iso-Kr$^{lf\text{-}1}$	<0.1%	Isogenic strain with *Kr$^{lf\text{-}1}$*

*Percentages are the mean ± s.d. for ten replicate vials at 25 °C crossing mutation/balancer virgin female × *iso-Kr$^{lf\text{-}1}$* male. In all cases, the number of F1 progeny scored was >500. The strains *kis^{1}*, *iso-1,2,3* and *iso-Kr$^{lf\text{-}1}$* did not show any transformation phenotype in over 500 F1 progeny. As *kis* is a TrxG gene, this indicates that not all mutations in TrxG genes induce ectopic outgrowth. All strains have mutations in TrxG genes except those with the *hsp83* alleles and the last two.

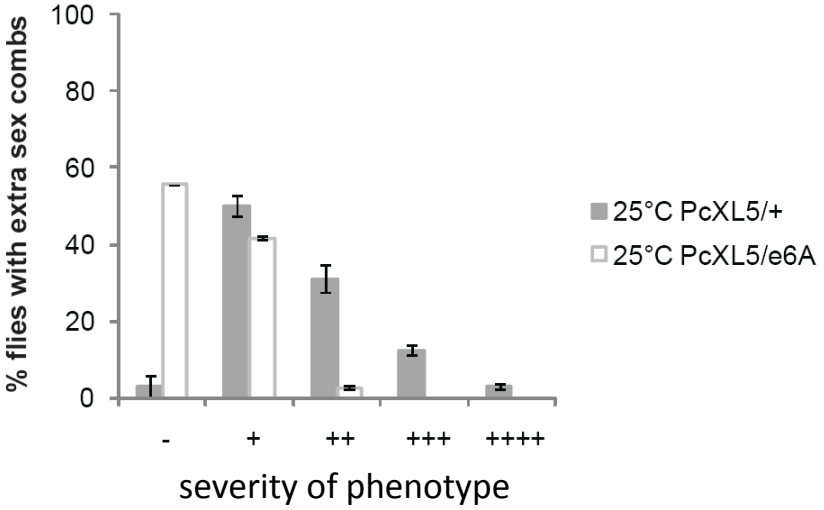
Sollars et al 2002

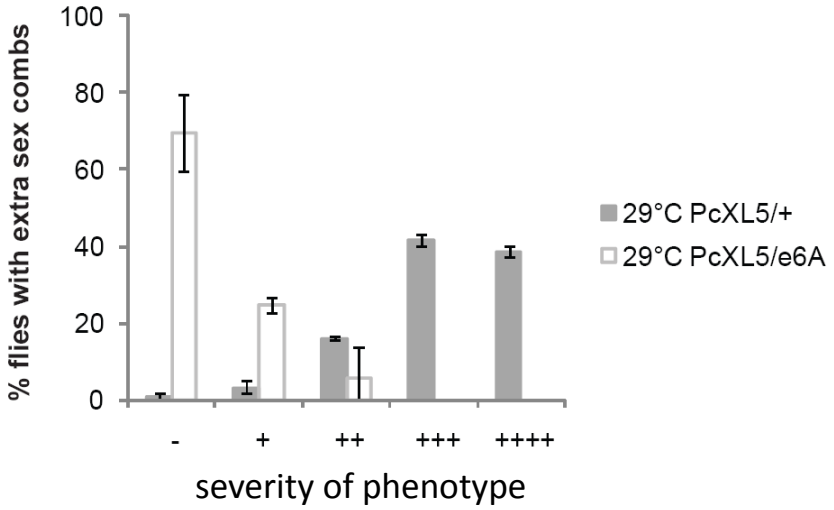
Mutations in Hsp90 behave similar to trxG mutations

Analysis of extra sex comb phenotype in different Pc alleles at different temperatures

Hsp90 allele: e6A

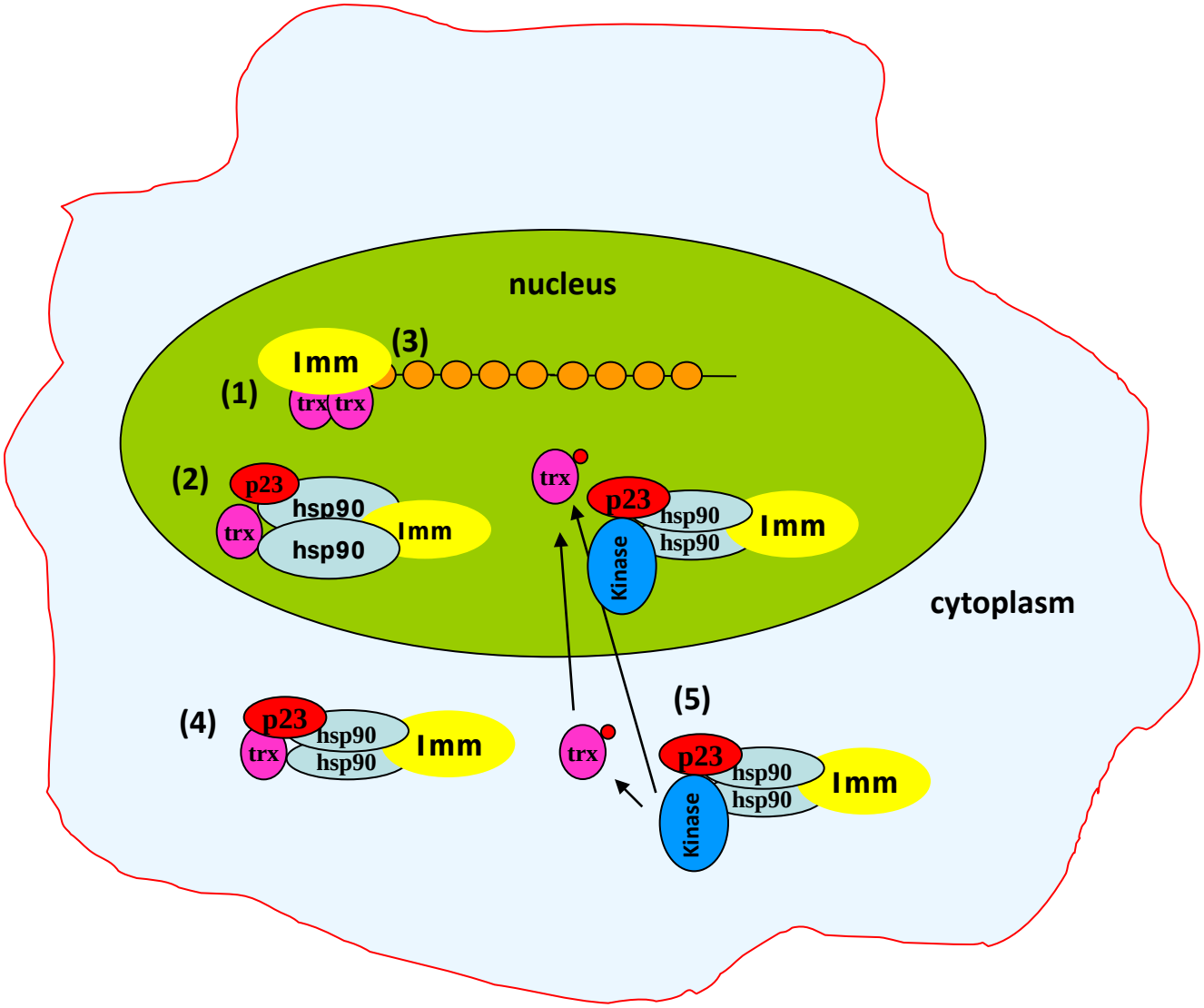
Pc allele : PcXL5



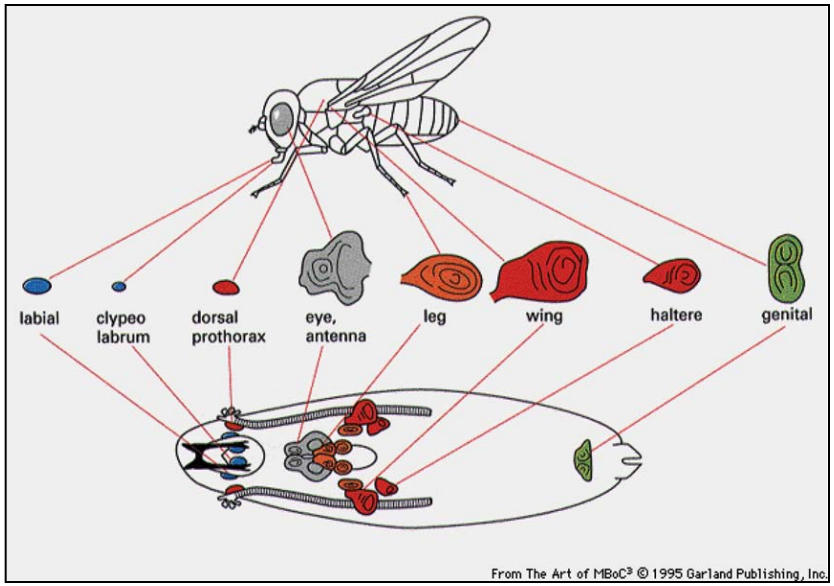


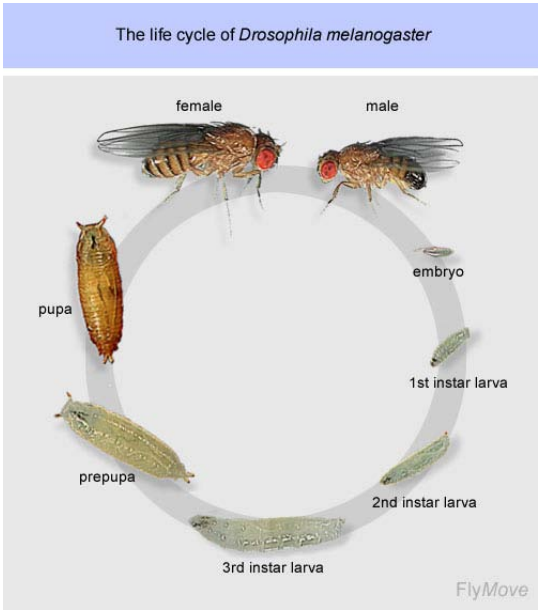
Hsp90 heterozygous mutants strongly suppress extra sex combs by various Pc mutations

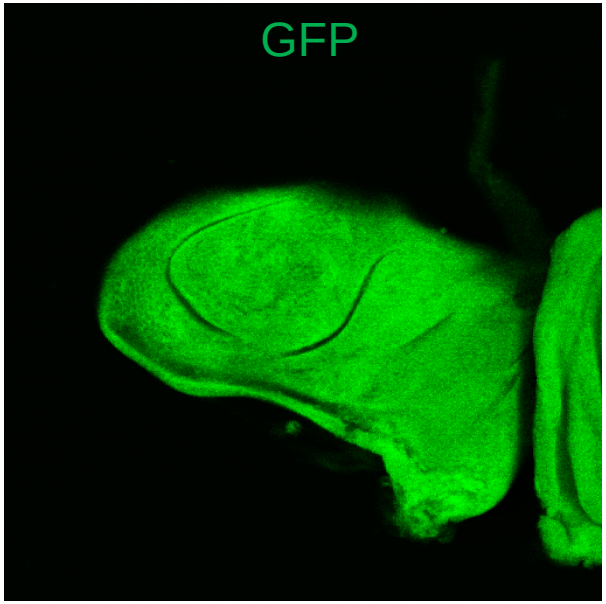
Interaction between Hsp90 and PcG or trxG?

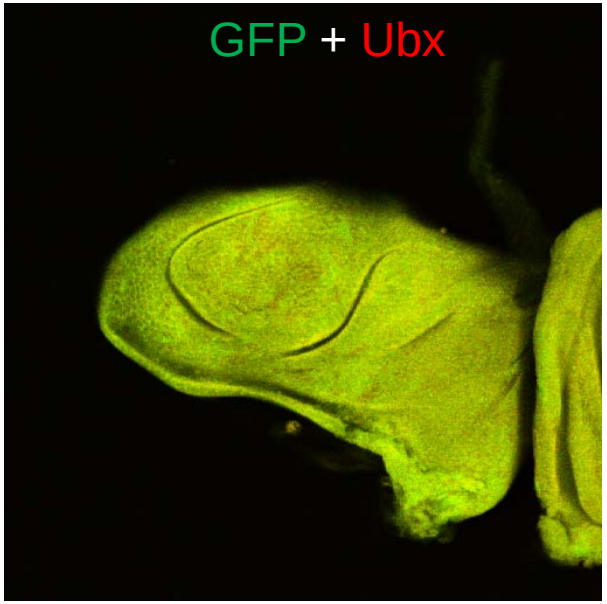


Drosophila Development



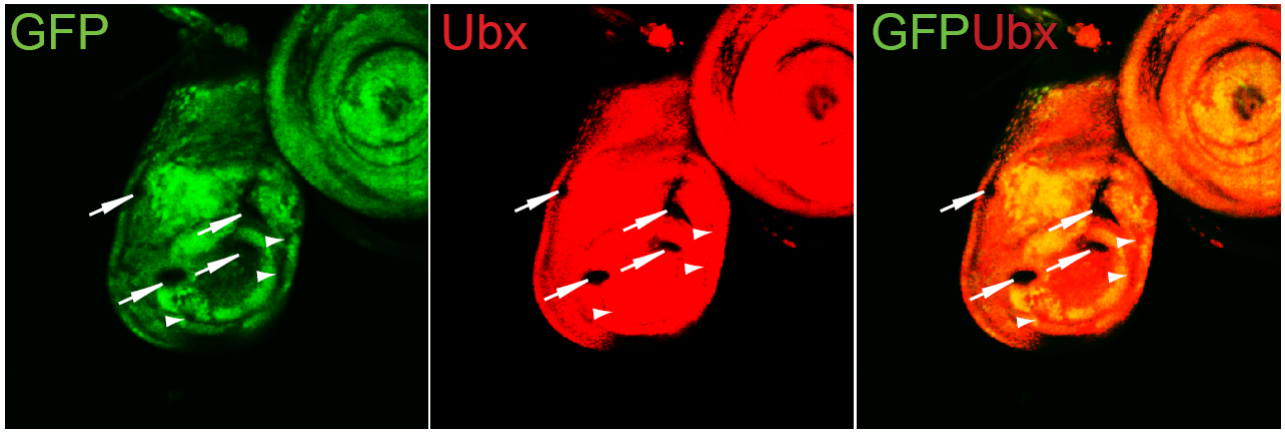






Mutations in Hsp90 behave similar to trxG mutations-III

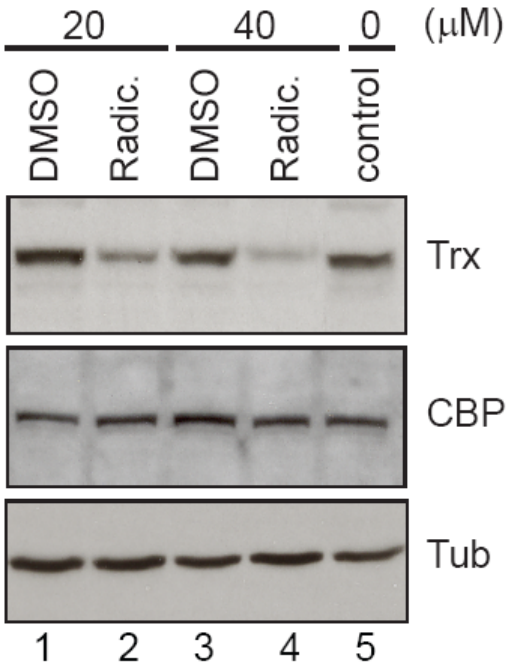
Analysis of *Ubx* expression in Hsp90 somatic clones in the haltere discs



Hsp90 homozygous somatic clones show loss of Ubx expression

Inhibition of Hsp90 leads to depletion of Trx

Trx in Kc cells after drug treatment

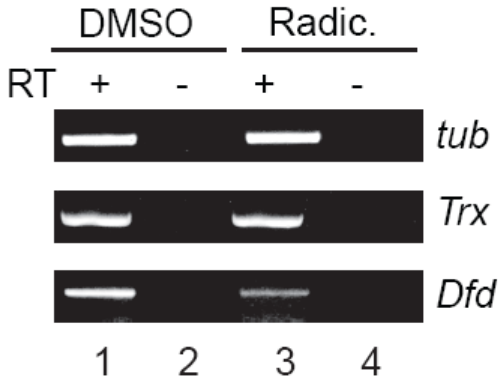


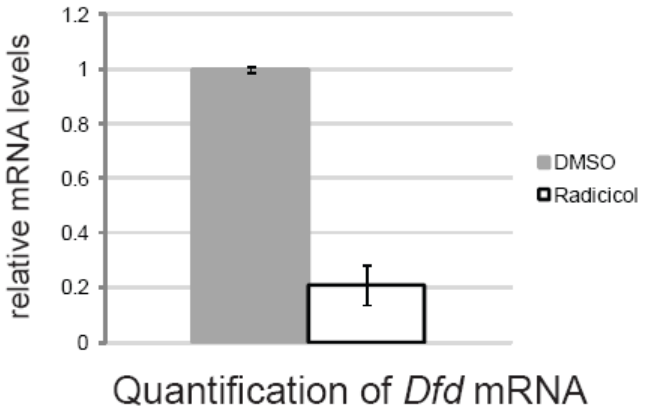
•Radicicol treatment of Kc cells leads to specific depletion of Trx protein within 4 hrs

•CBP, a member of TAC1 trxG complex, shows no effect

Hsp90 inhibition results in down-regulation of Trx targets

Analysis of mRNA levels for *Trx*, *tub* and *Dfd* after drug treatment

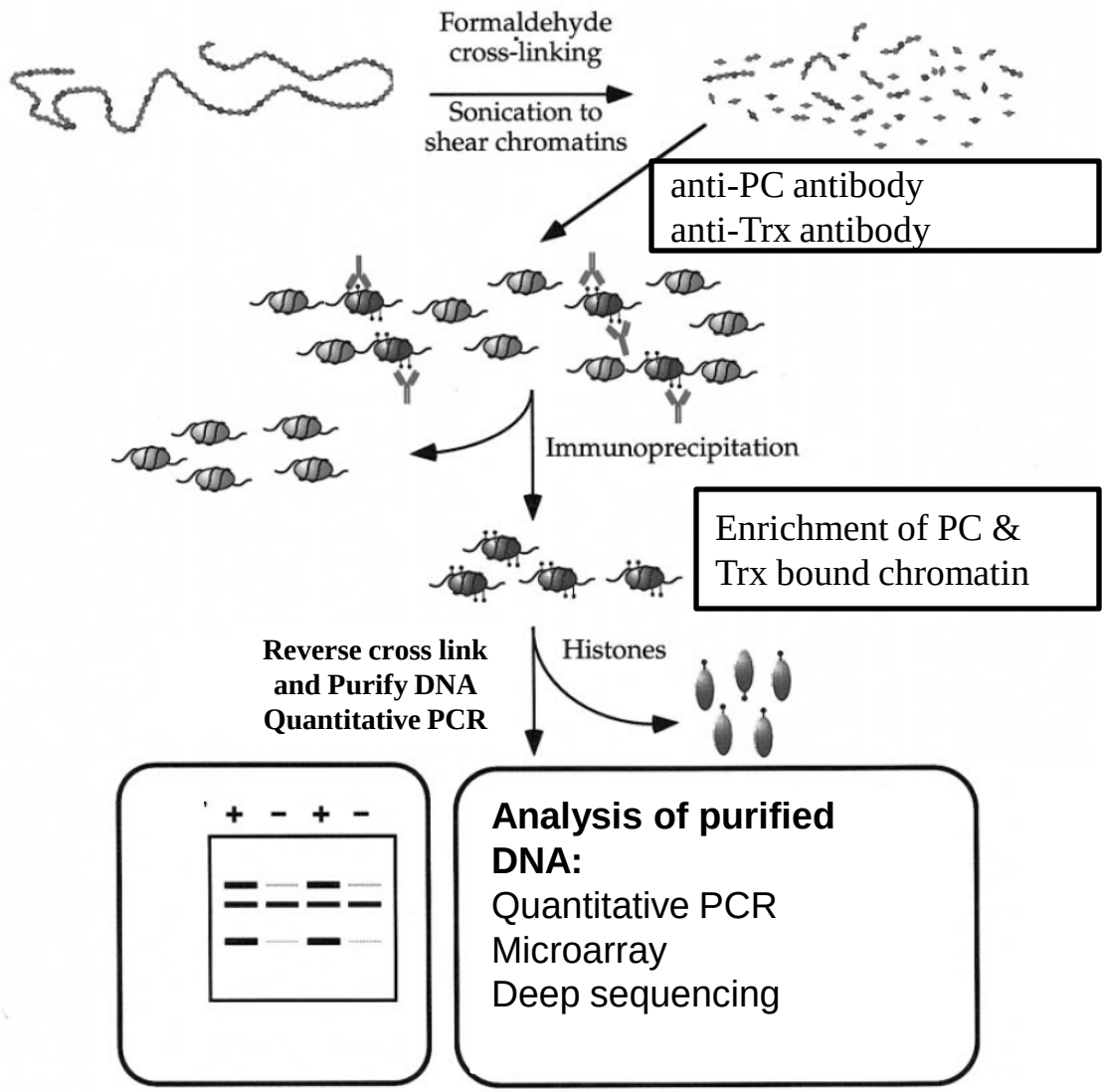




Hsp90 inhibition results in down regulation of *Dfd* in Kc cells

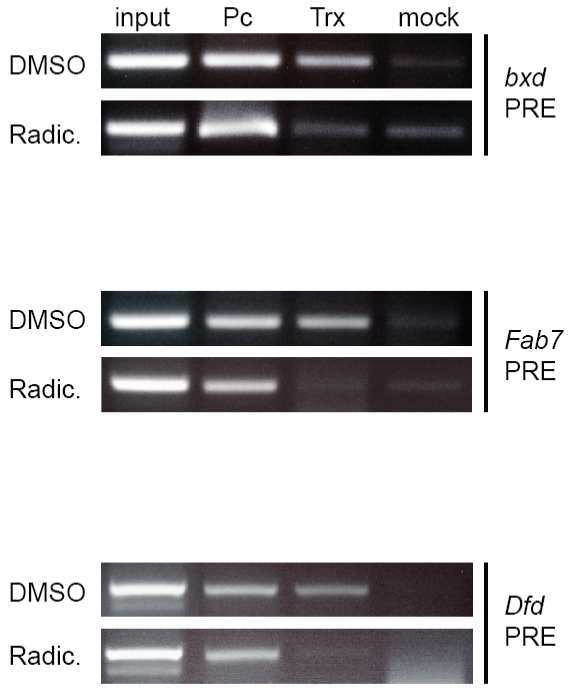
Chromatin Immunoprecipitation (ChIP) :

Specifically purify chromatin associated proteins and map underneath DNA

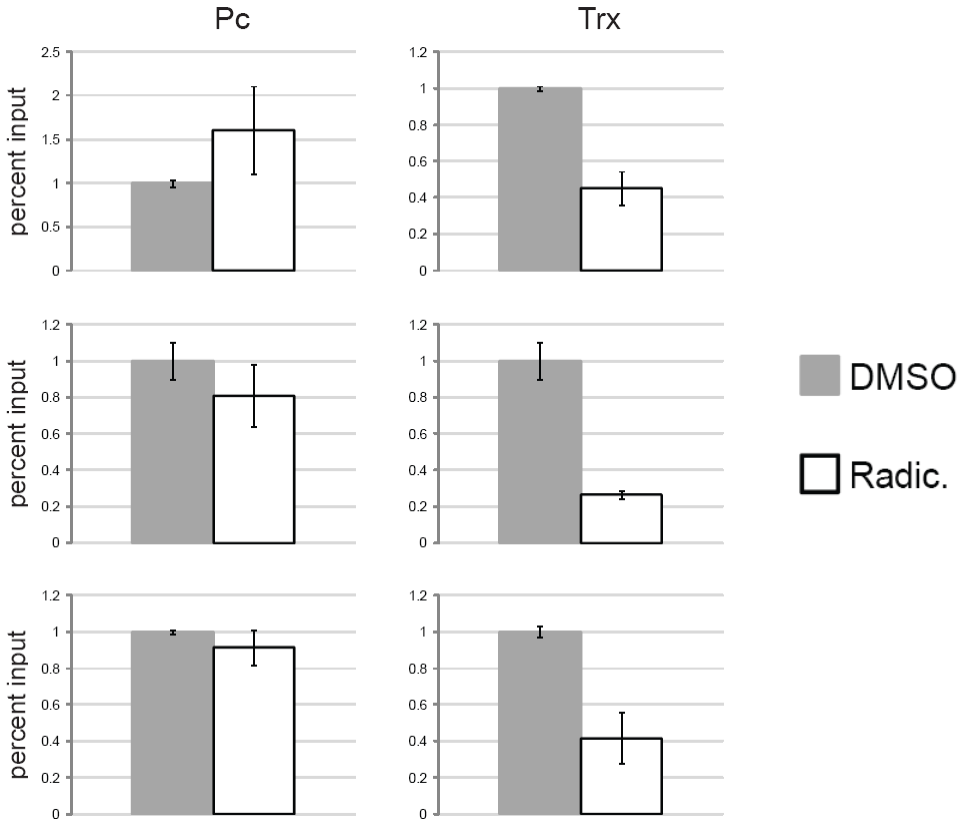


Inhibition of Hsp90 leads to depletion of Trx:

ChIP from Kc cells treated with 40µM radicicol

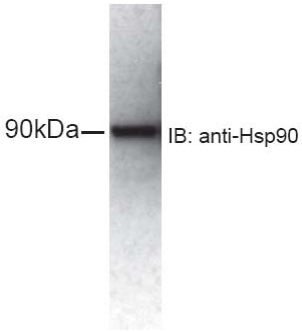


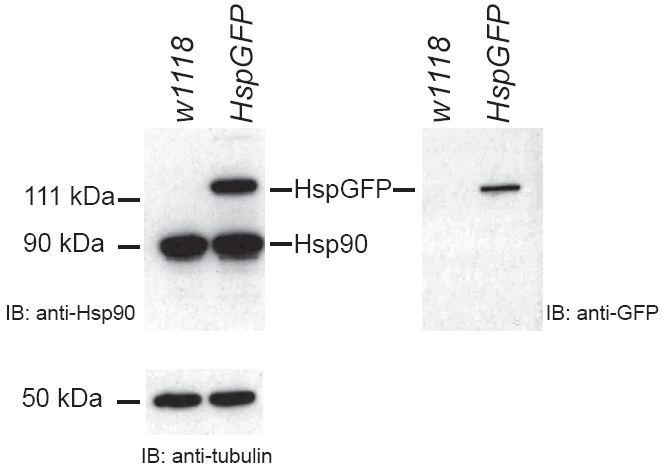
Quantification of ChiP from Kc cells after drug treatment



Generation of HspEGFP transgenic flies:

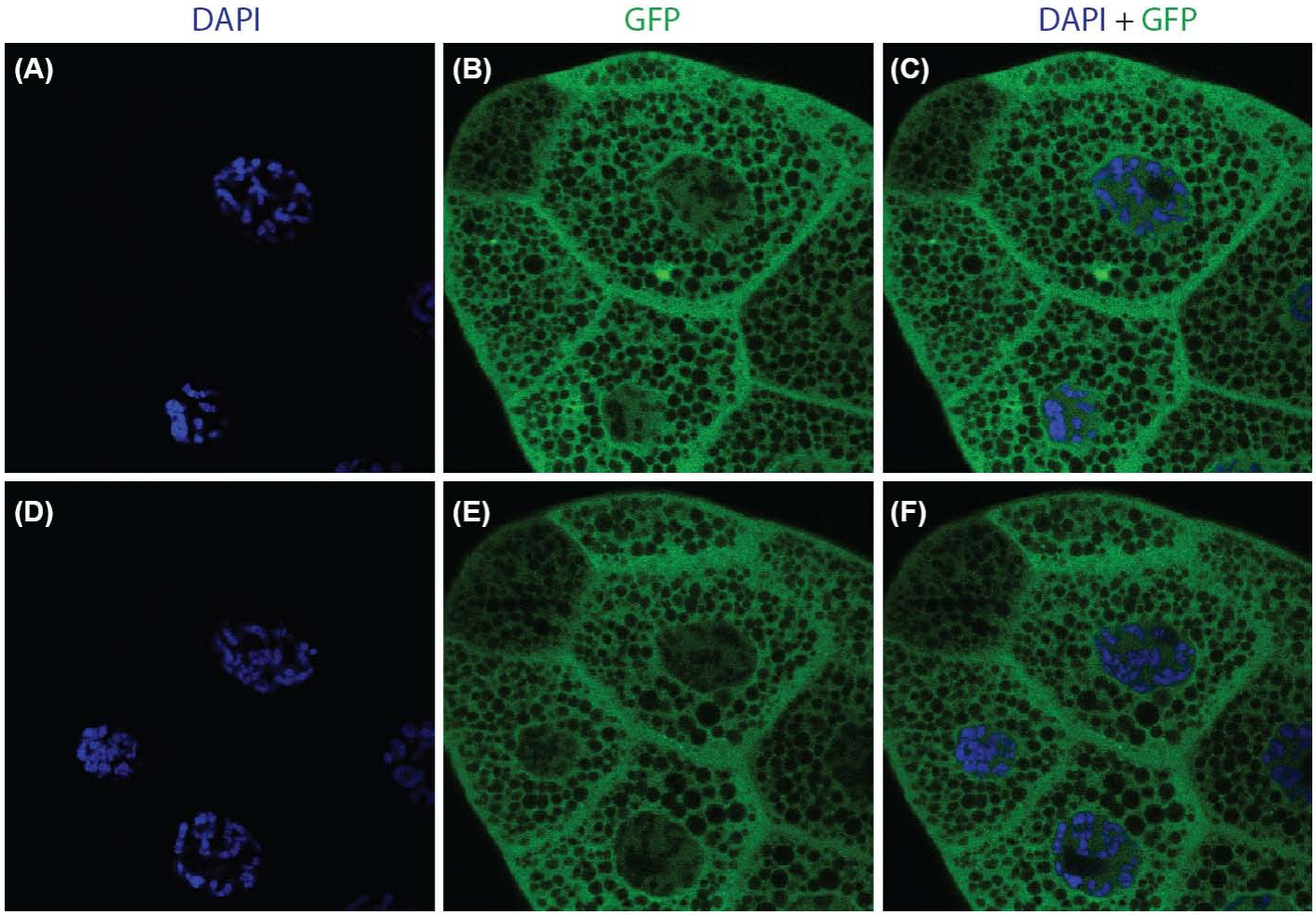
Expression of HspEGFP fusion protein in fly heads:



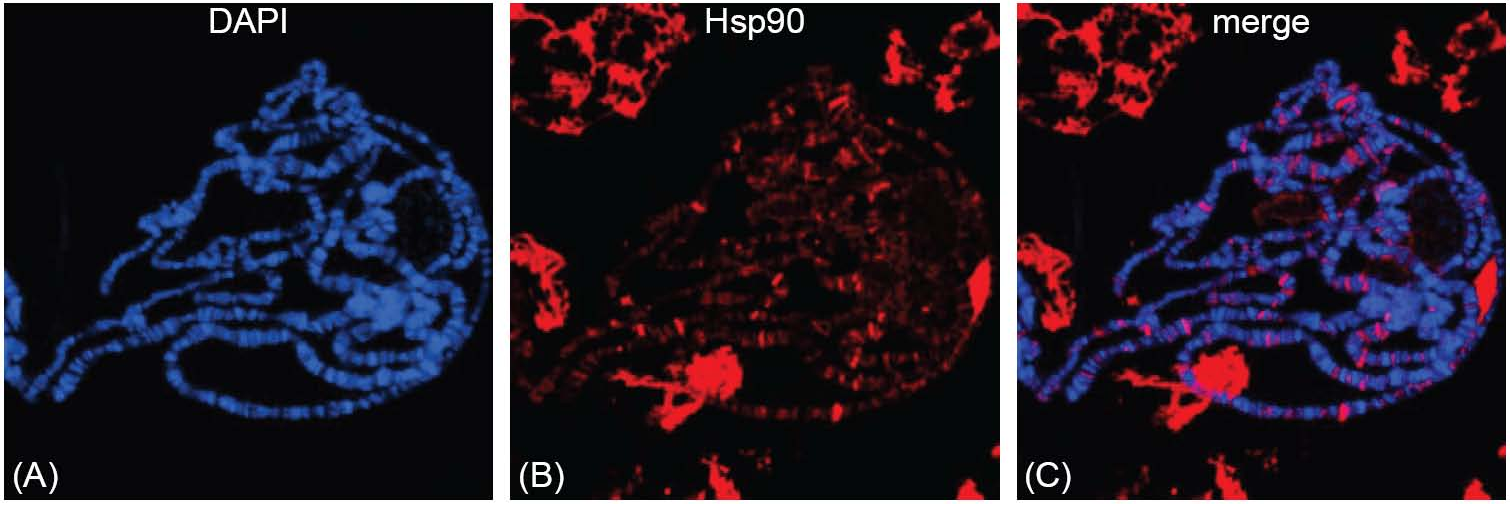


Localization of HspEGFP in un-fixed Salivary glands

2 independent confocal frames

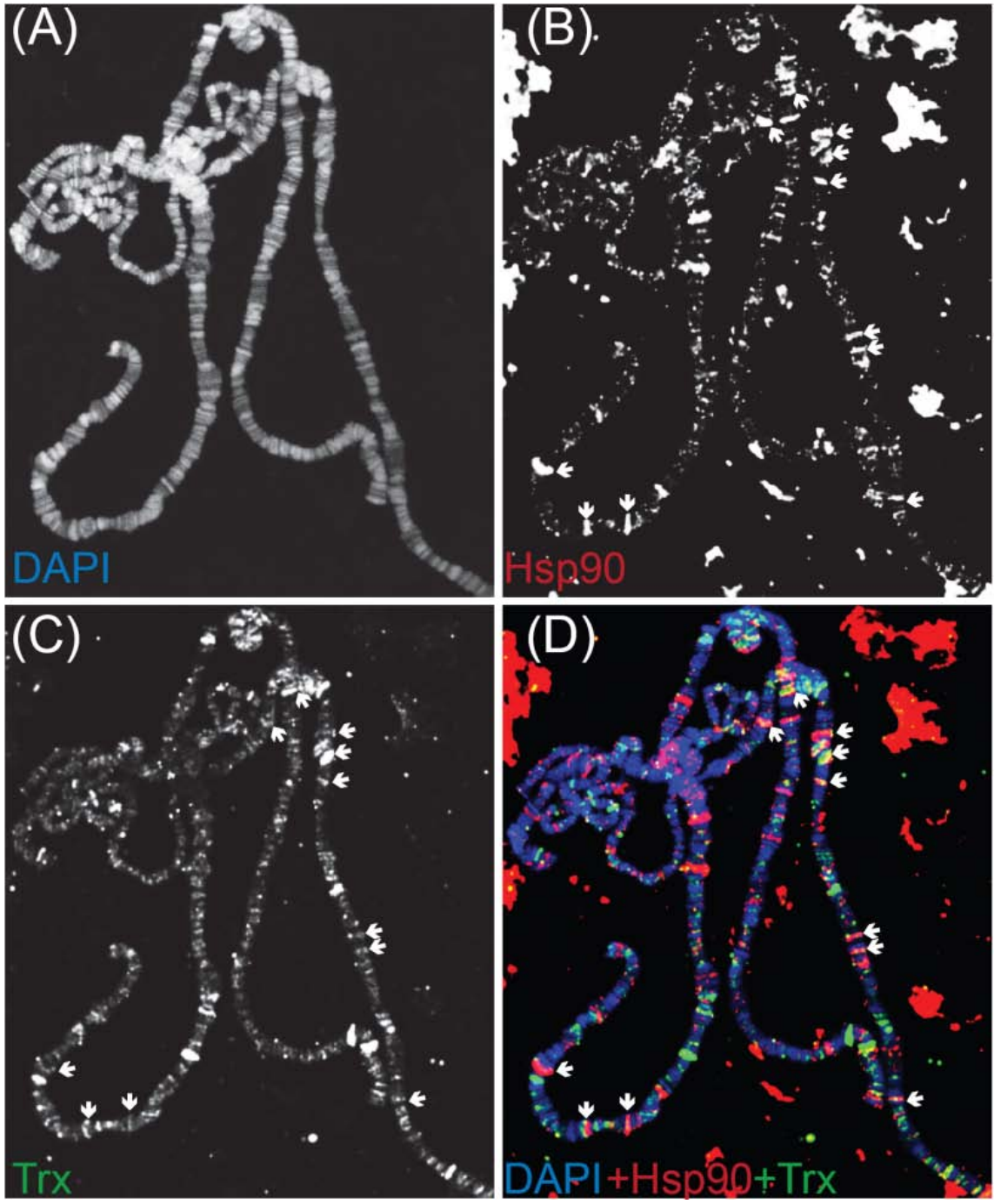


Hsp90 binds to polytene chromosomes

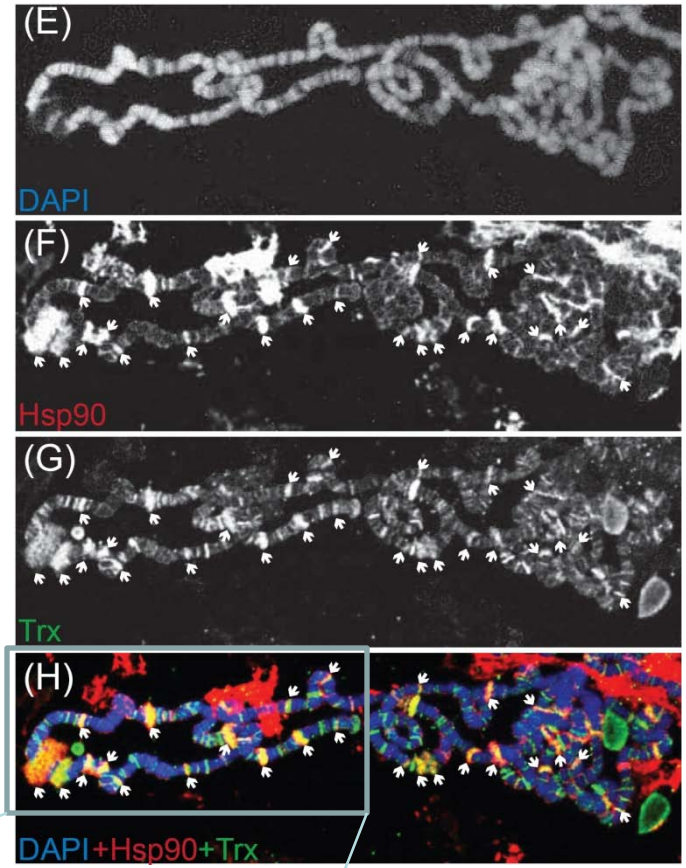


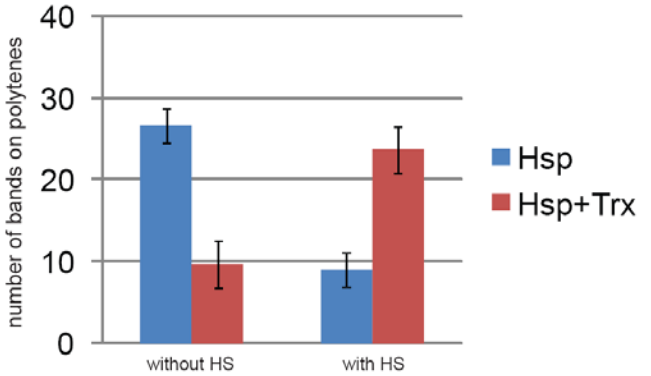
Hsp90 and Trx colocalization:
(without heat shock)

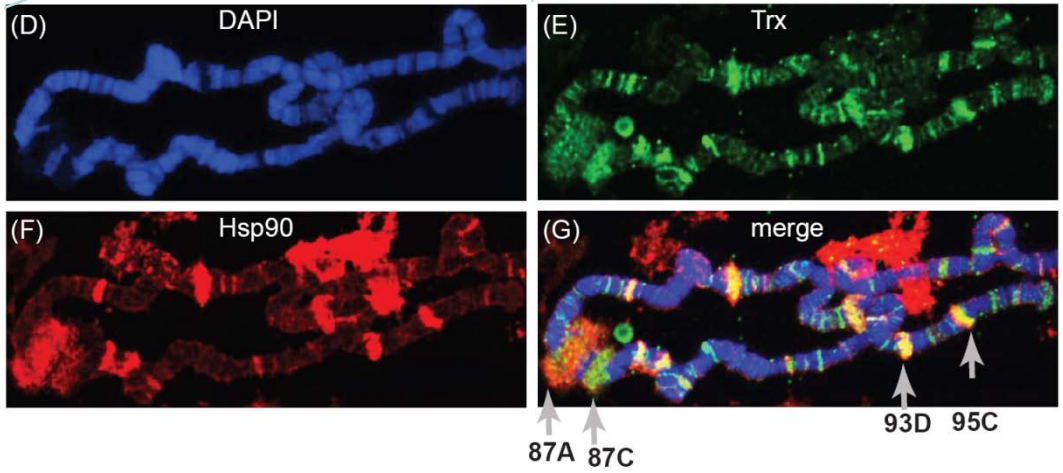
Limited overlap between Hsp90 and Trx at polytene chromosomes



Hsp90 and Trx colocalization: (with heat shock)

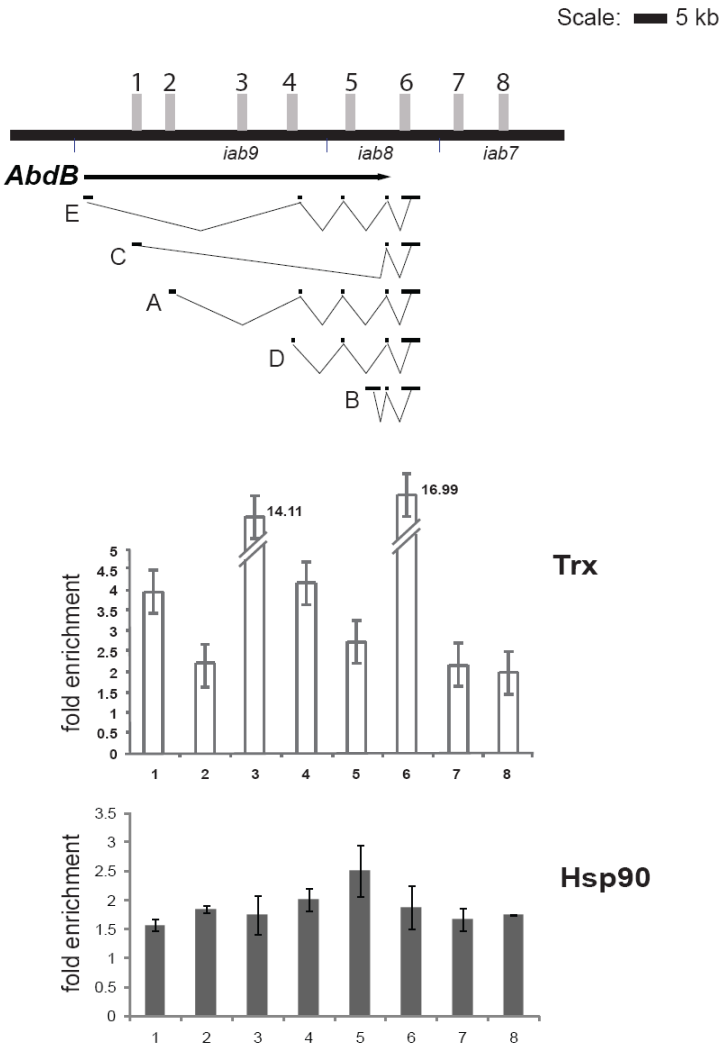




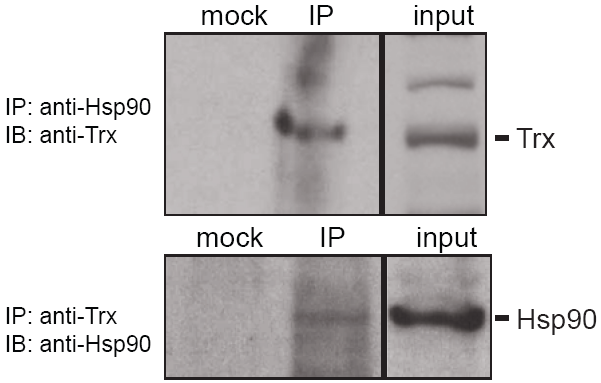


Hsp90 interacts with Trx protein

ChIP in SF4 cells



Embryonic nuclear extract: co-ip

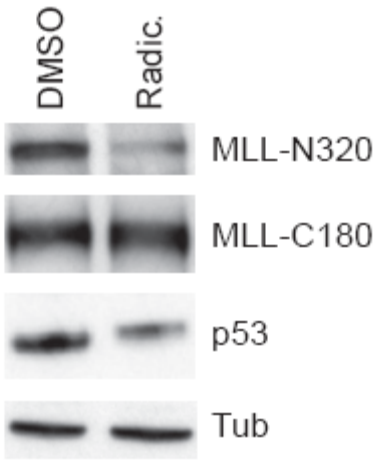


Hsp90 binds to Trx binding sites across *Abd-B* region

Inhibition of Hsp90 leads to depletion of MLL

MLL : Mixed lineage leukemia
Trx homologue in mammals

MLL in HEK293 cells after drug treatment



•Radicicol treatment of HEK293 cells leads to specific depletion of MLL-N within 4 hrs

•MLL-C shows no effect
•p53, a known Hsp90 client

Conclusions

Mutations in Hsp90 behave like trxG mutations

- FLW transgene
- extra sex comb analysis
- loss of Ubx expression in somatic clones in haltere discs

Pharmacological inhibition of Hsp90 affects Trx protein levels

Radicicol treatment of Kc cells leads to:

- Depletion of Trx within 4 hrs of treatment
- Dissociation of Trx from chromatin (70% reduction) but does not affect PC levels
- Down-regulation of *AbdB* and *Dfd* genes
- Global H3Ac levels are reduced by radicicol treatment
- Inhibition of Hsp90 leads to specific MLL-N320 degradation in HEK293 cells

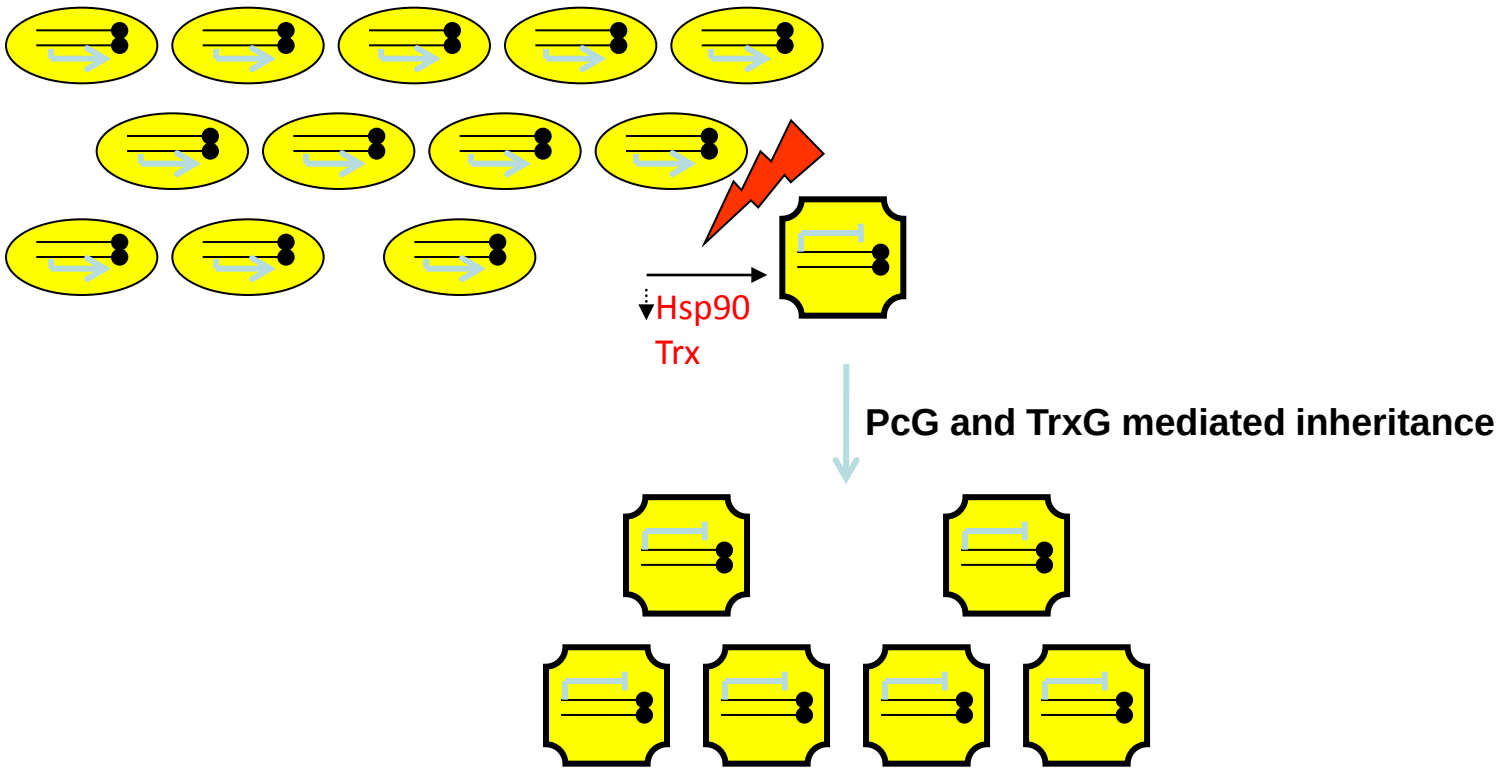
- Co-immunoprecipitation of endogenous Hsp90 and Trx, from embryonic nuclear extracts

Hsp90 binds to chromatin

- Polytene chromosome staining with anti-GFP and anti-Hsp shows binding in interband regions
- Hsp90 co-localizes with Trx at a number of sites including heat shock puffs after heat shock
- Hsp90 and Trx co-localize at heat-shock puffs in both wild type and HspEGFP transgenic flies

- Hsp90 associates with *Abd-B* region in SF4 cells

Epigenetic progenitor model:



Modulation of cellular memory by Hsp90-Trx nexus may lead to cells with altered identity, eventually leading to beginning of disease