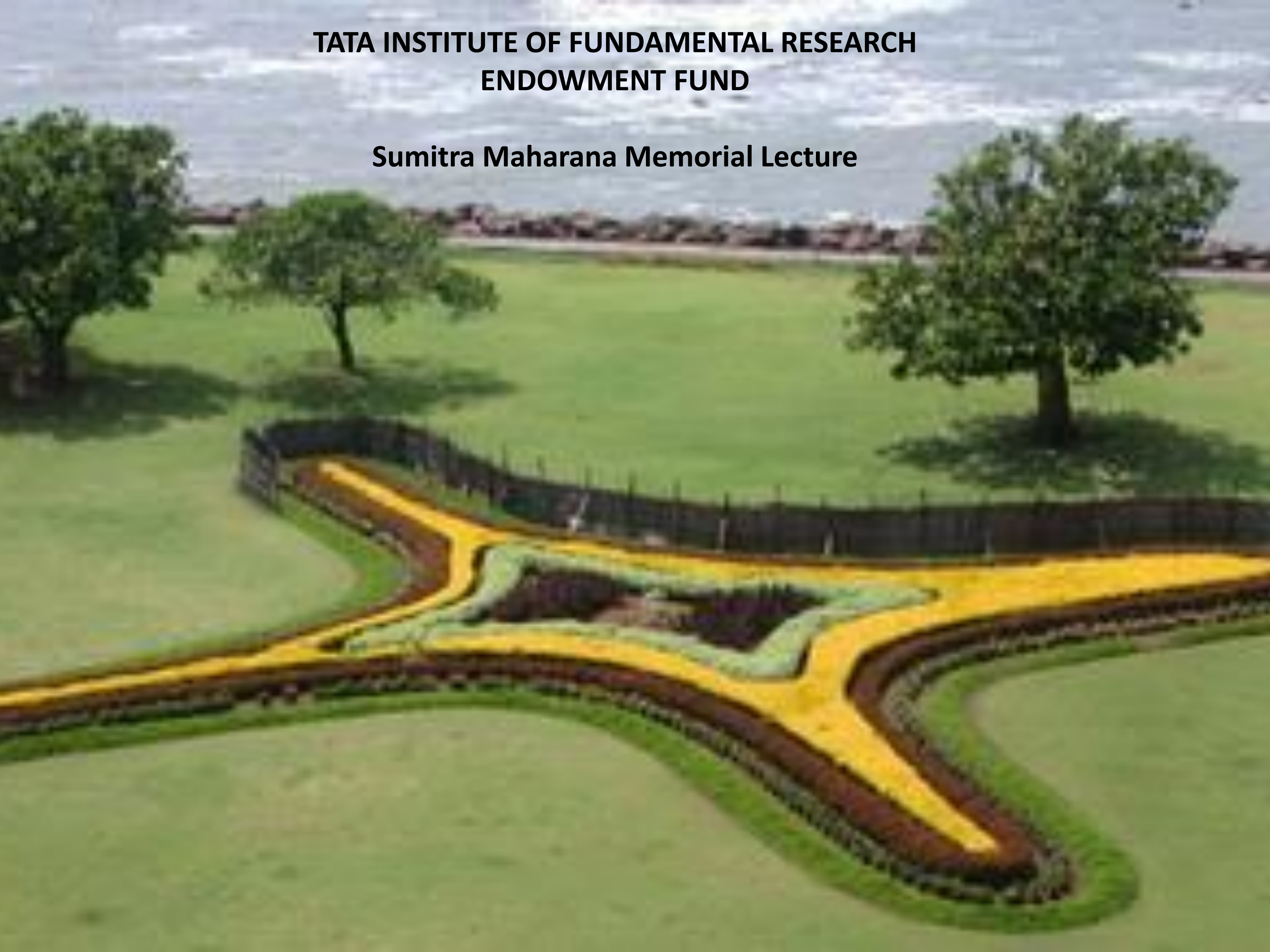
TATA INSTITUTE OF FUNDAMENTAL RESEARCH ENDOWMENT FUND

Sumitra Maharana Memorial Lecture



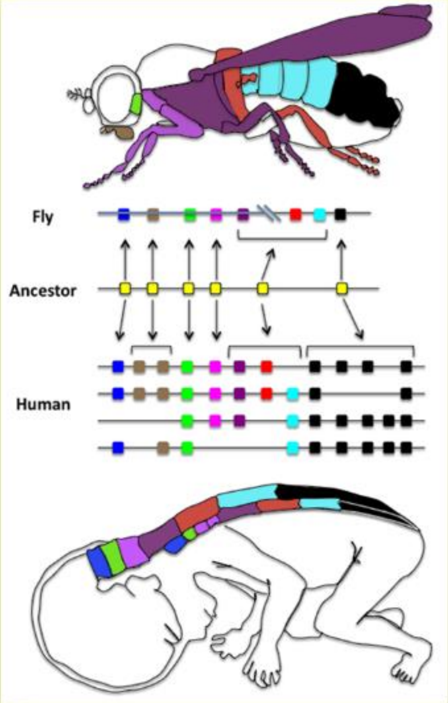
TATA INSTITUTE OF FUNDAMENTAL RESEARCH
2ND SUMITRA MEMORIAL LECTURE

Determining the details of the antero-posterior body axis

Dr. Rakesh Mishra *is a Senior Scientist at the* ***CCMB, Hyderabad, India****.*

An accomplished developmental biologist and molecular geneticist, he made outstanding contributions in the field of developmental patterning by the homeobox family of genes using multiple organisms.

Outline of an adult body pattern is laid at the embryonic stage. The ***homeobox (hox)*** *family of genes are known to determine the anteroposterior body pattern. In this lecture, Dr. Mishra will discuss how the* ***hox*** *gene expression pattern is regulated by the chromosomal organization and its impact on the development of some of the hitherto unknown features.*



RAKESH MISHRA
Centre For Cellular and Molecular Biology,
Hyderabad, India

Monday, January 25, 2016 at 5 p.m.
Lecture Theatre, AG66, TIFR
1-Homi Bhabha Road, Colaba, Mumbai 400005

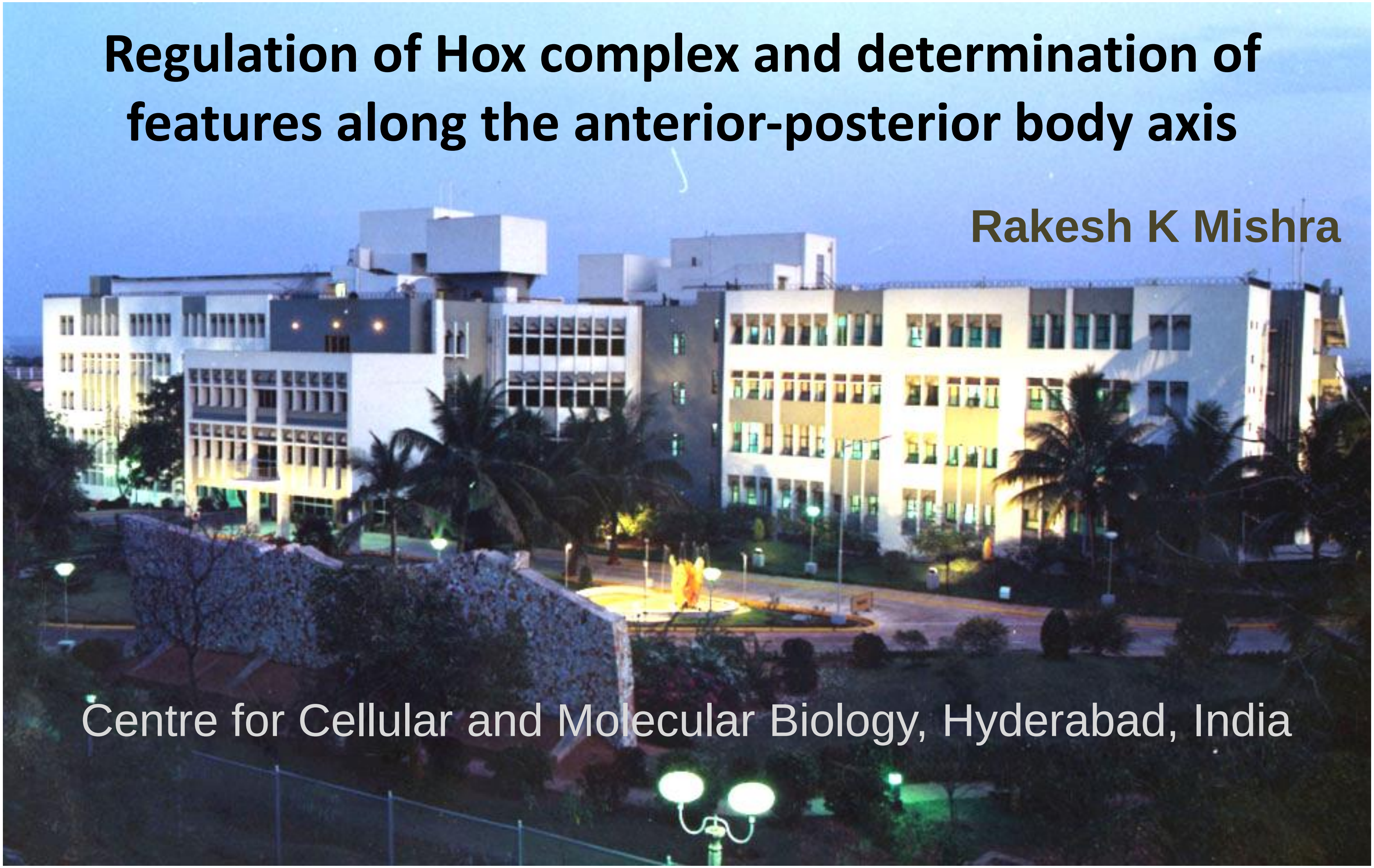
For details:
22782500, 22782235, 22782089
Email: pro@tifr.res.in

Talk is open to all.
Non TIFR members are requested to carry valid photo ID card in person.

Dr. Sumitra Maharana
1951-2012

Sumitra Maharana, born on April 17, 1951 had her early education at Baripada and Rourkela. She completed her Pre-professoinal degree from Fakir Mohan College, Balasore, and received her M.B.B.S. and M.D.(Pathology) degrees from S.C.B. Medical College (Utkal University). Her M.D. thesis was on Hepatitis-B virus and liver cancer which was published subsequently. Instead of opting for regular medical practice, she worked in Regional Center for Medical Research (under ICMR), Bhubaneswar, on Lymphatic Filariasis and its eradication and wrote a couple papers on that topic. She was suffering from incurable ailment and faced death bravely.

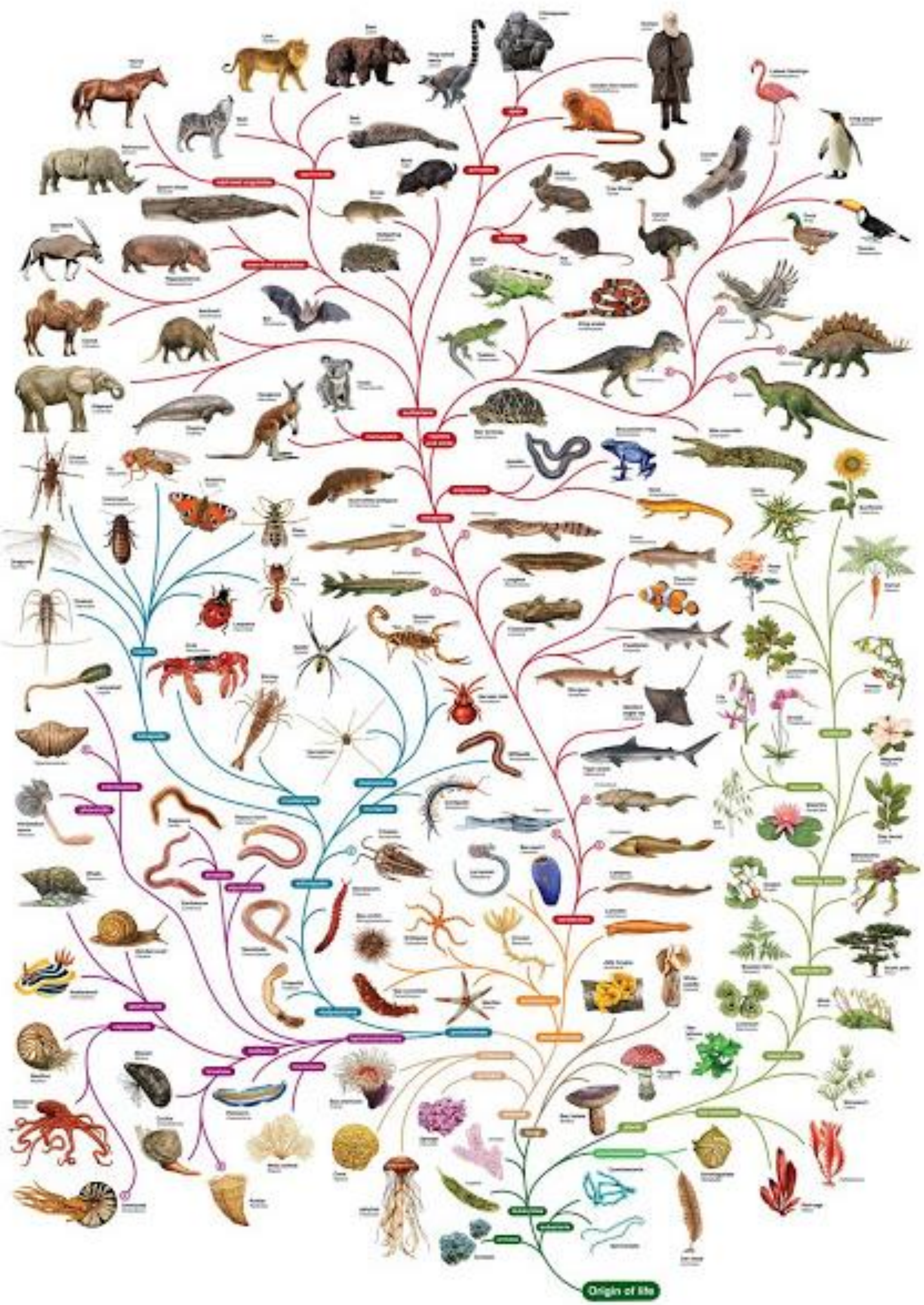
TIFR – Sumitra Memorial Lecture, Mumbai. January 25, 2016



Regulation of Hox complex and determination of features along the anterior-posterior body axis

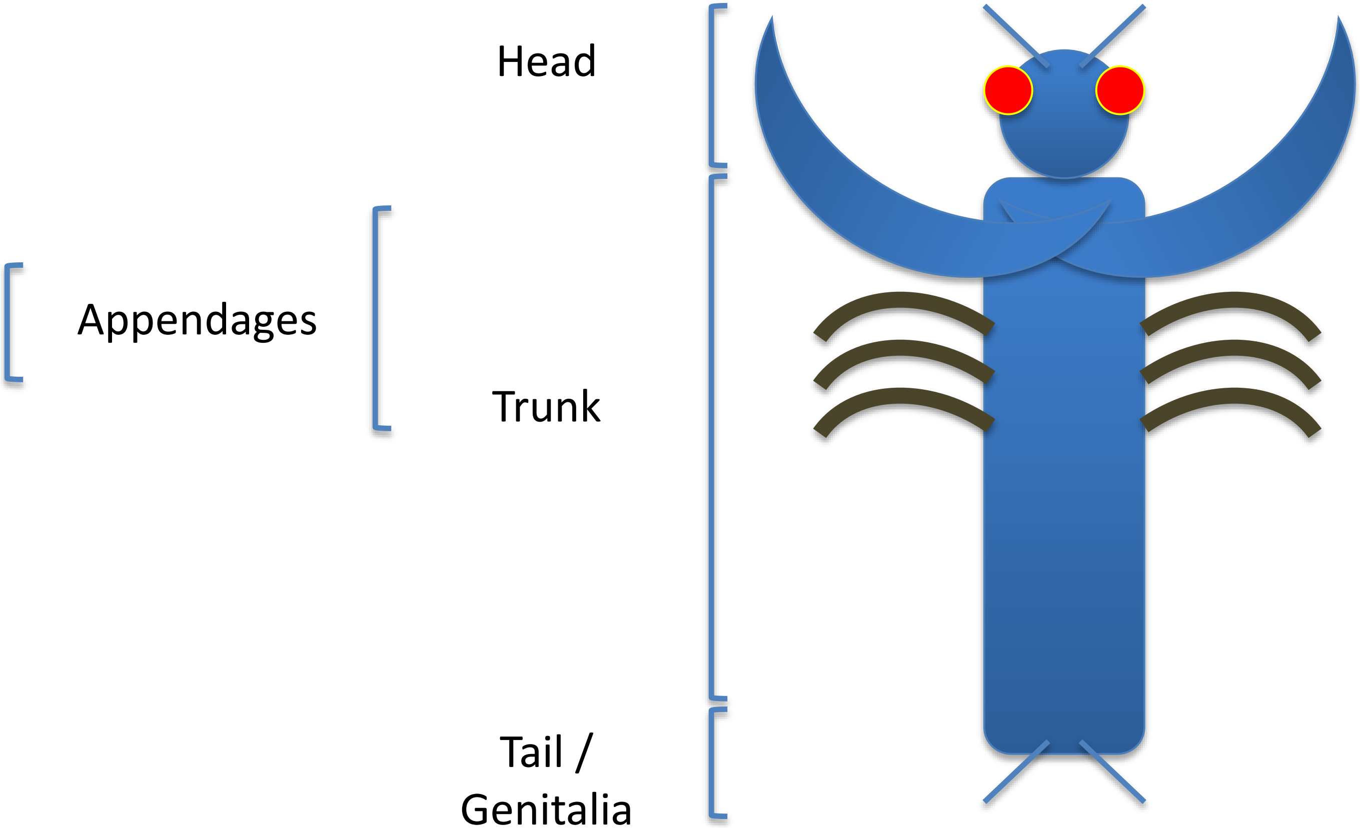
Rakesh K Mishra

Centre for Cellular and Molecular Biology, Hyderabad, India



Relatedness and variety/complexity of life forms

Complexity in body plan
Variety of features across the anterior-posterior body axis



Head

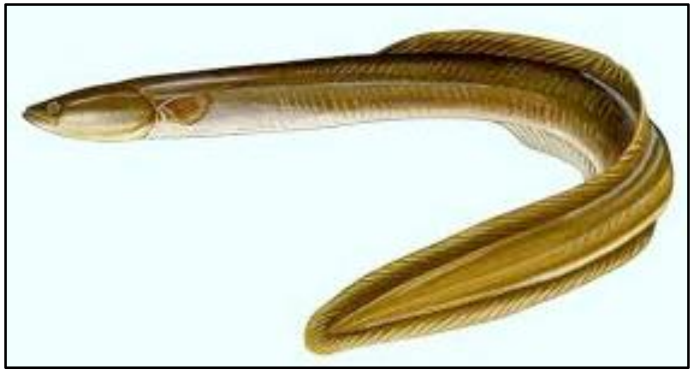
Digits

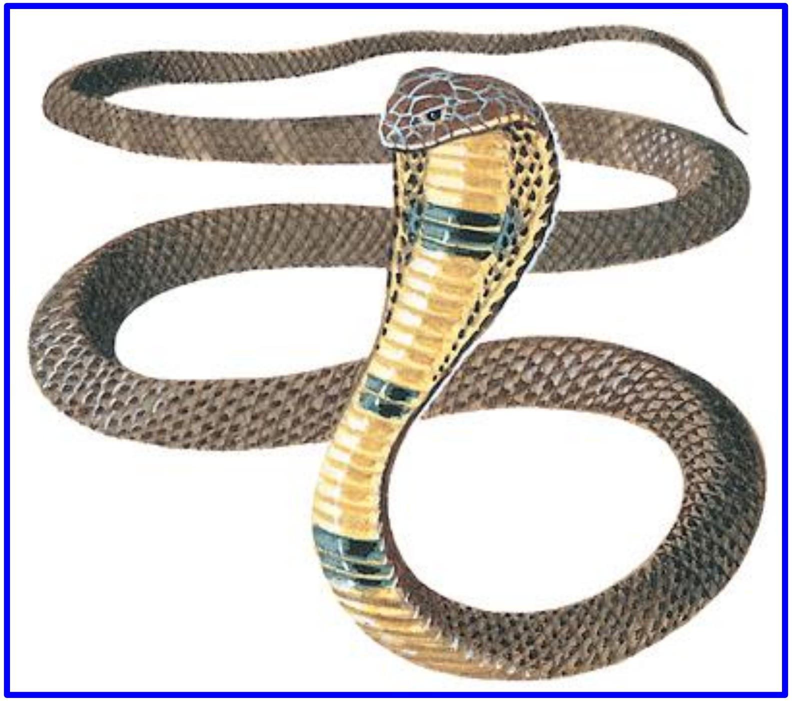
Appendages

Trunk

Tail / Genitalia

Variation in body form along the AP axis





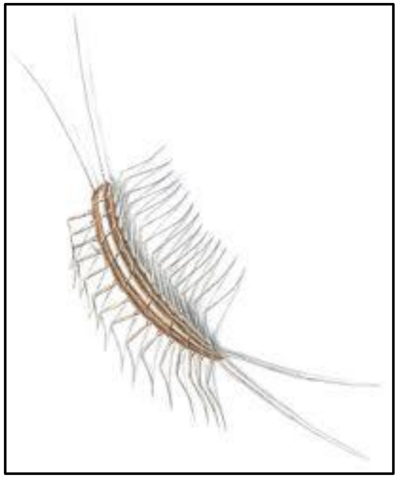
Losing appendages multiple times to go for elongated body forms

Gaining appendages to go for elongated body form

Use of appendages :walk (legs)

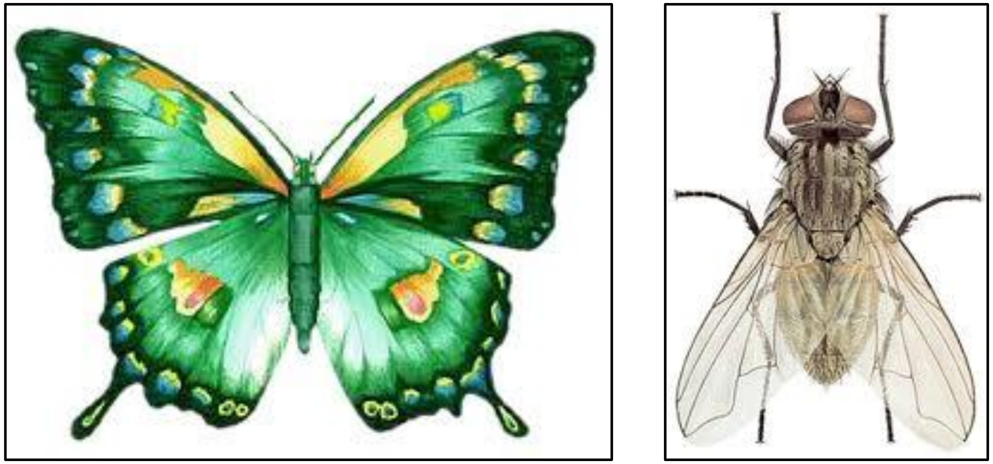
:swim (fins)

:fly (wings)

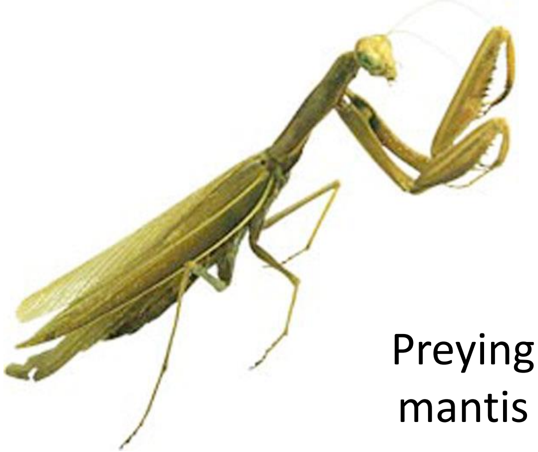


Variation in body form along the AP axis

Trading off wing to haltare
4 → 2 wing shift



Special use of appendages
Leafy / sticky deception



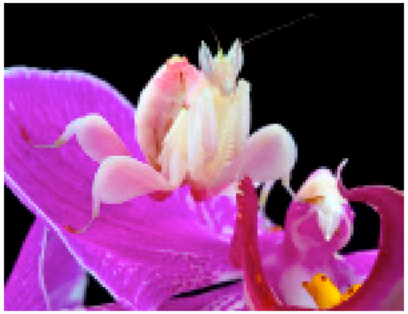
Preying mantis

Mantis species: a remarkable variety in use of appendages

Brut force to deadly deception

Violin mantis

Spiny flower mantis



Orchid mantis

Budwing mantis

So much variation in appendages and digits (shape/size/usage in different species)

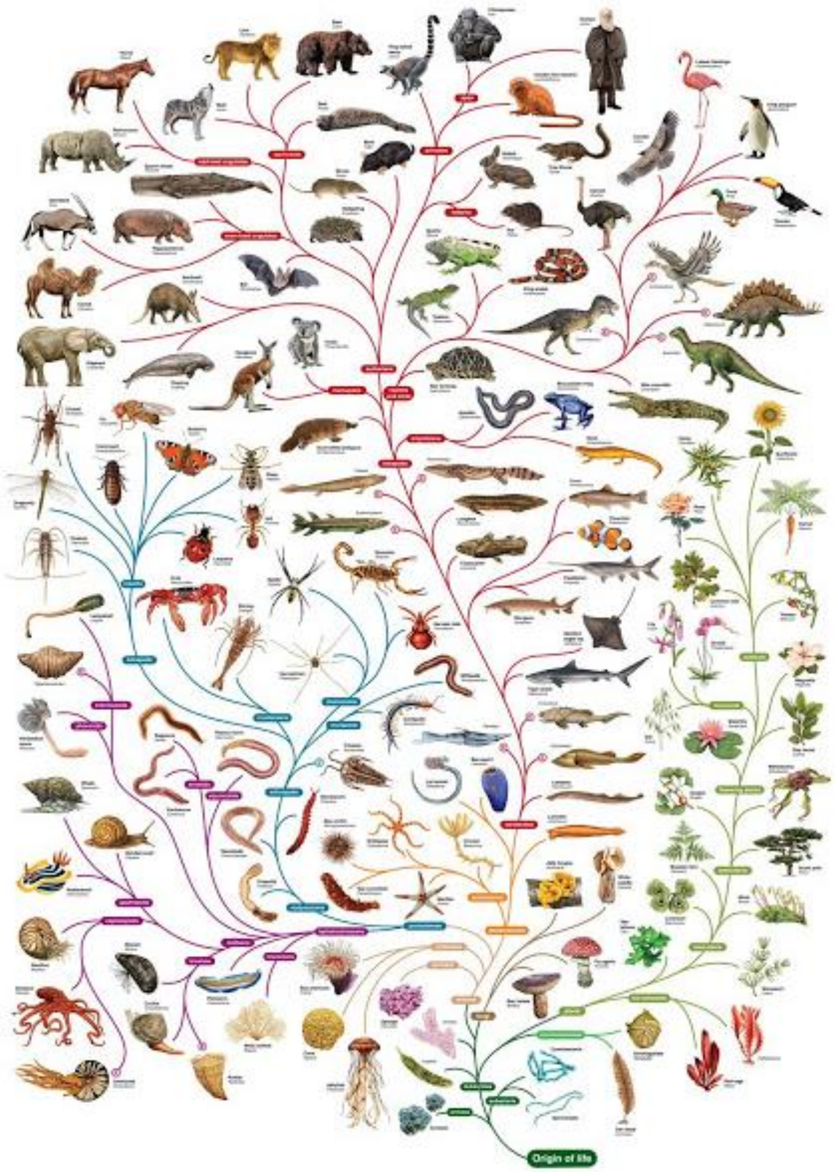
What determines the number and shape of appendages along the AP axis?

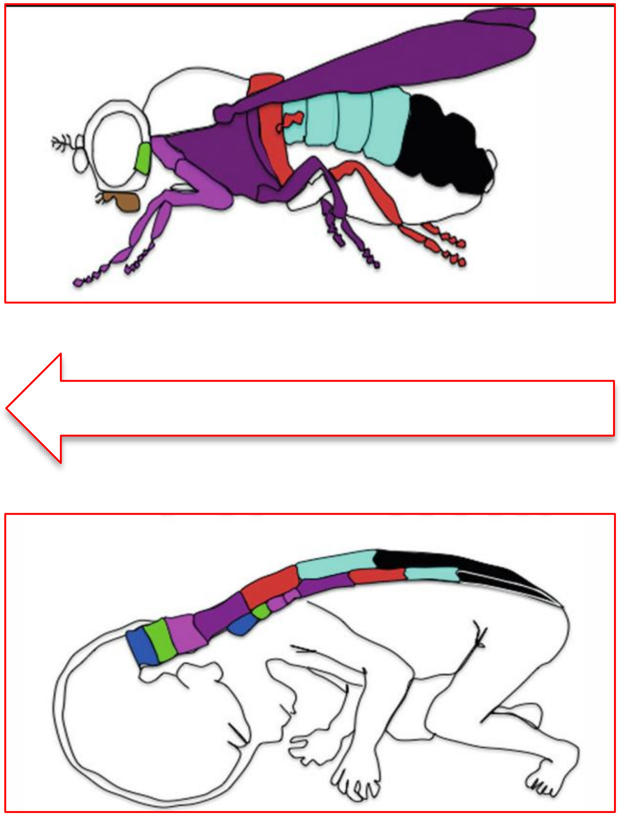
Hox genes

The only way to define A-P body axis

in all bilaterians

Variety and complexity of life forms is built upon a basic common theme of body plan

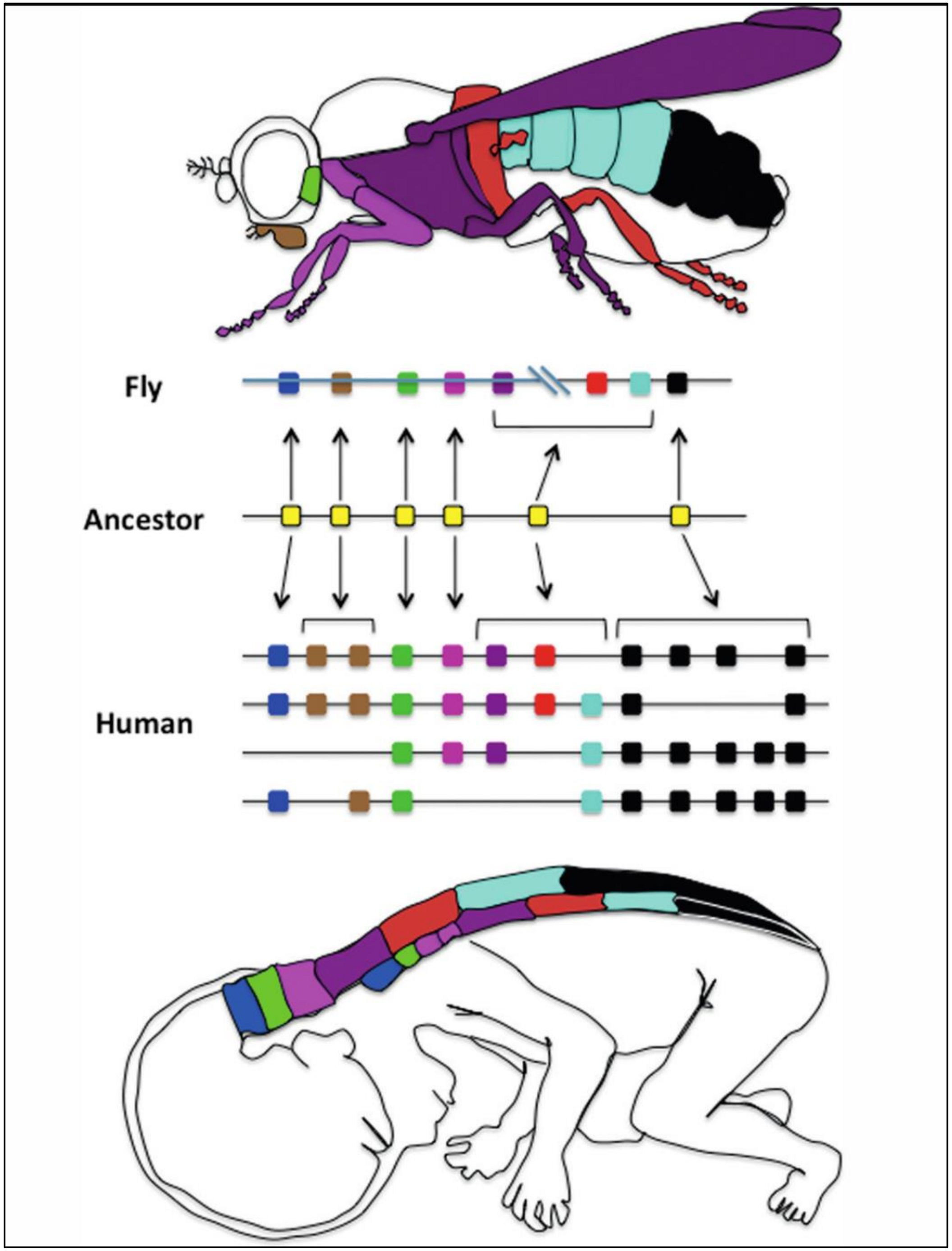




Hox genes exist in a cluster

Their organization and expression pattern is conserved during evolution

While invertebrate have one set of hox genes, vertebrates have at least four Hox complexes



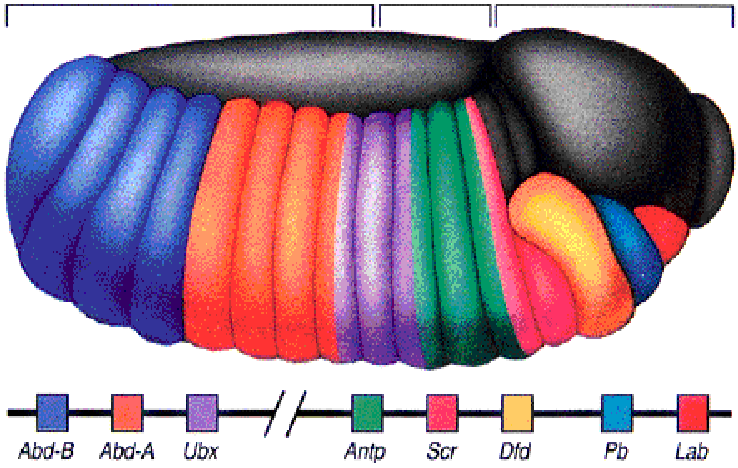
Mechanisms of regulation of *Hox* clusters enforce the conservation of their organization

- setting up of expression pattern along the A-P axis

- maintenance of the expression patterns

Clustering of hox genes / regulatory elements

Collinearity of organization and expression

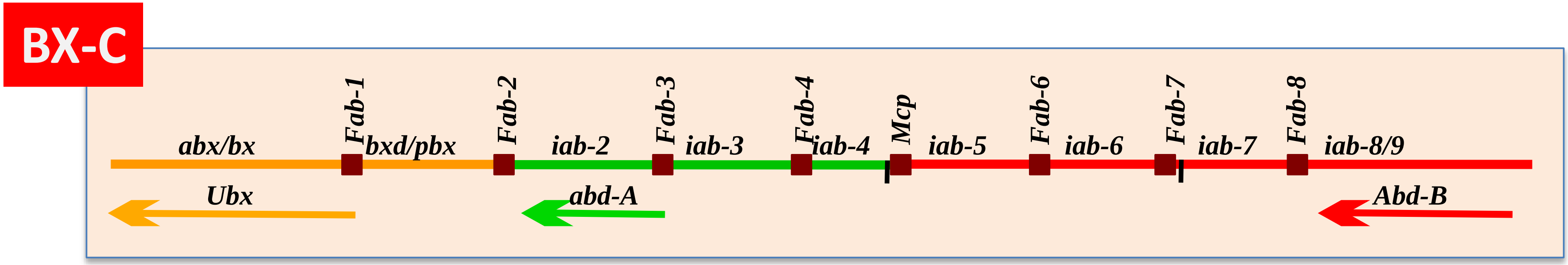


Boundaries define the functional domains of gene expression

Maintenance of the patterns after differentiation

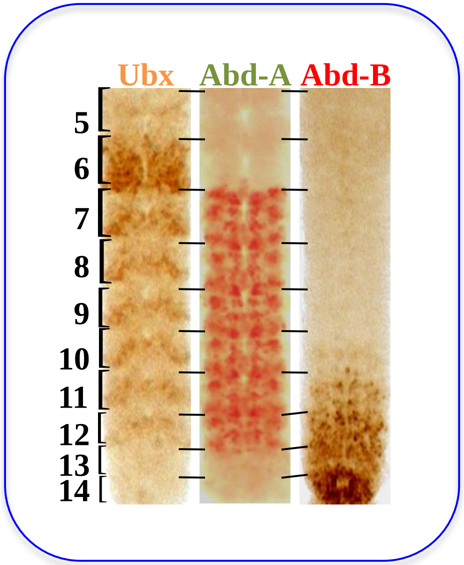
Features discovered at Hox loci are relevant across the genome

Boundaries define the regulatory domains of the bithorax complex



Boundaries define the limits of *iab*'s

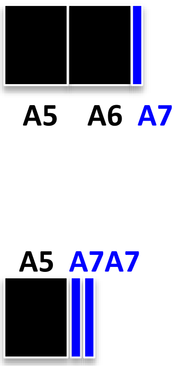
Collineairty of regulatory domains and the homeotic genes

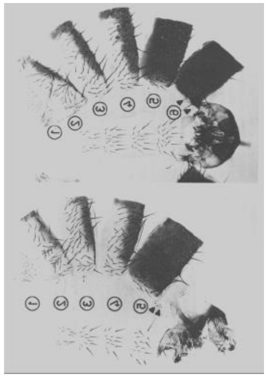


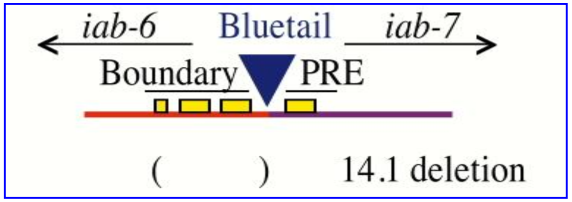
Boundary elements separate the cis-regulatory domains of the bithorax complex

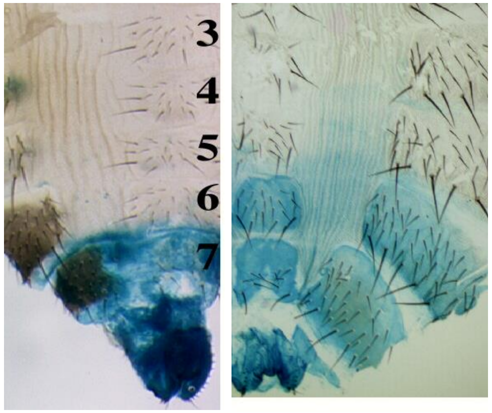
iab-5 (A5) *Fab-6* *iab-6* (A6) *Fab-7* *iab-7* (A7) *wt*

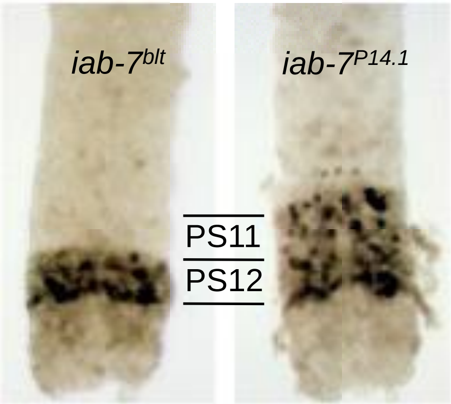
iab-5 (A5) *Fab-6* *iab-6* (A7) ( ) *iab-7* (A7) *Fab-7*











chromatin domain Boundary Element Search Tool

cdBEST


A Srinivasan

Bioinformatics tool to locate boundaries across the *Drosophila* genome

>4500 boundary elements predicted in *Drosophila melanogaster*

Great majority locating in the intergenic regions

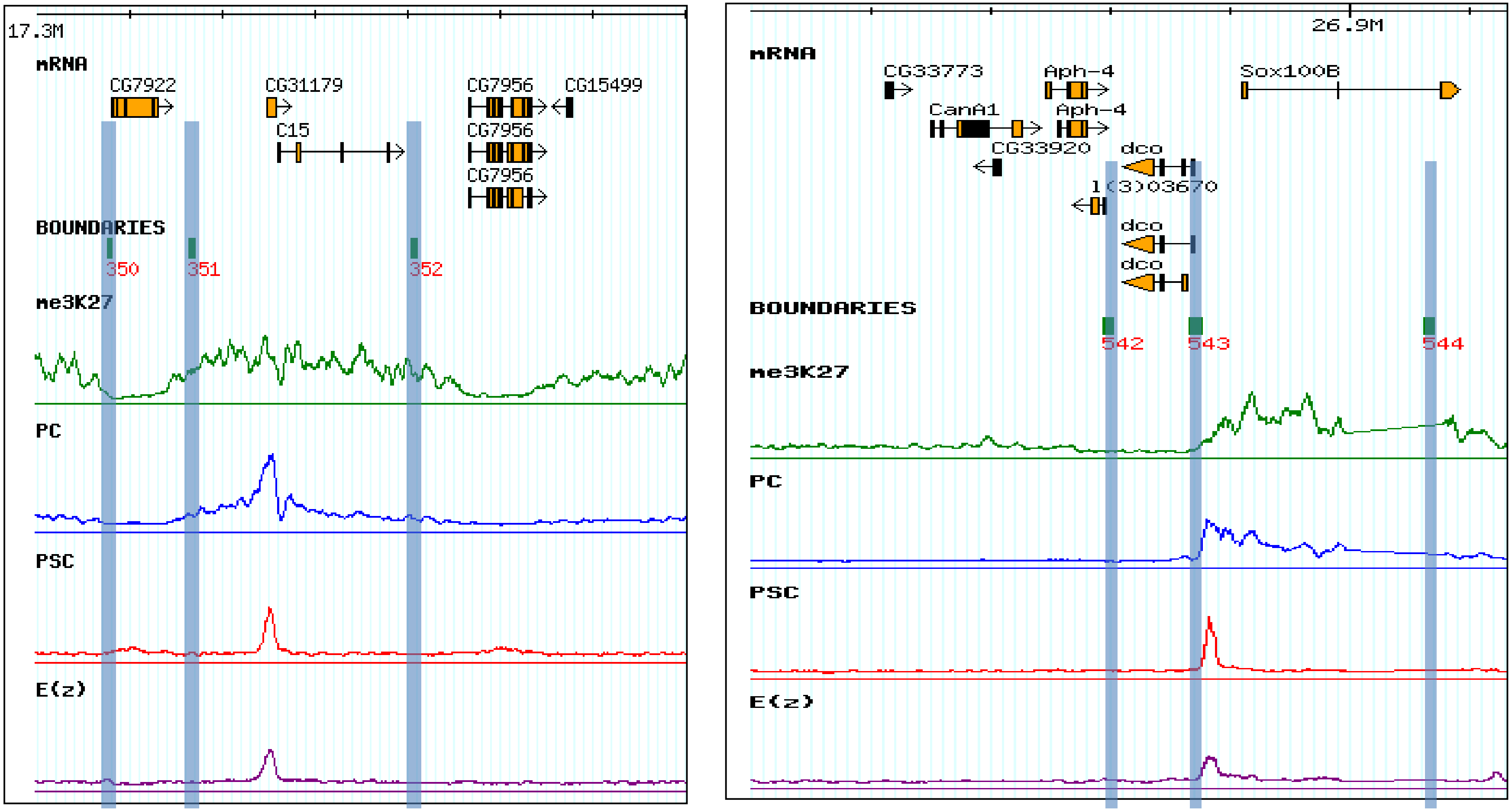
Genomes of 12 species of *Drosophila* analysed give similar results

Transposable elements as boundaries is common feature in all drosophilids

Srinivasan & Mishra, Nucleic Acids Res. 2012

cdBEST – marking borders of genes and epiprofiles

[separating functional domains of genome]

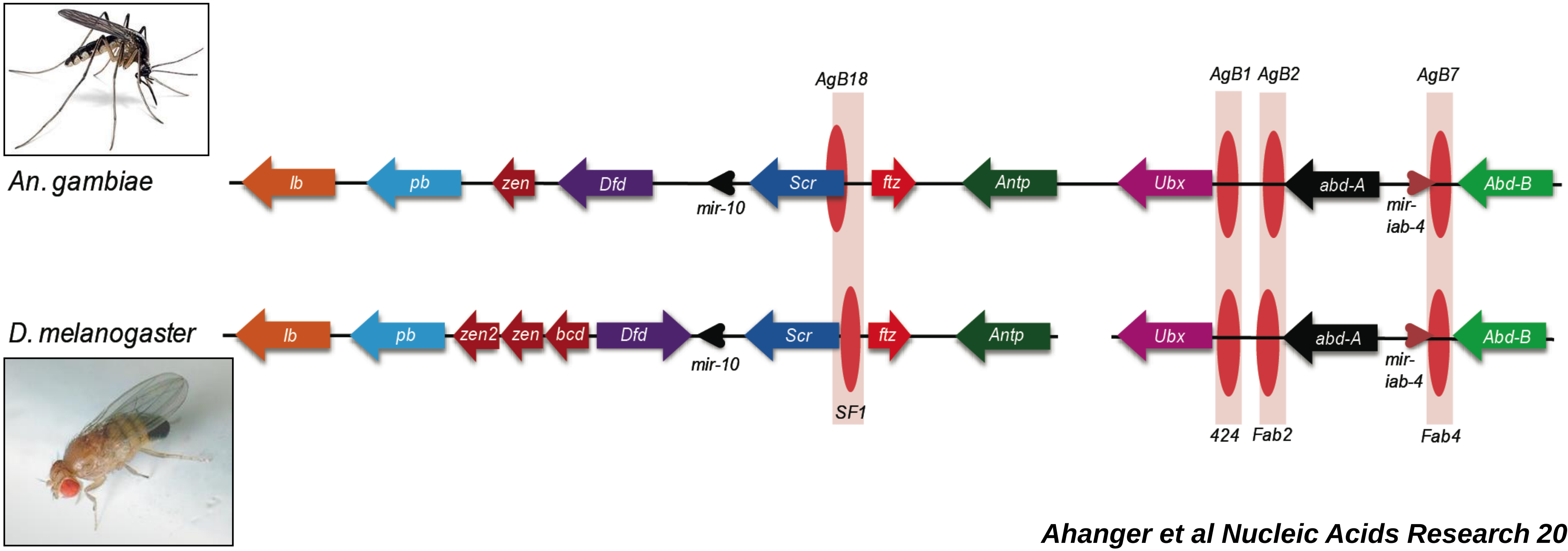


Srinivasan & Mishra, Nucleic Acids Res. 2012

cdBEST

Accounts for ~3% of *Drosophila* genome
>80% of cdBEST predictions function as boundaries S2 cells
Applicable to other insects (e.g., malaria mosquito *An. gambiae*)

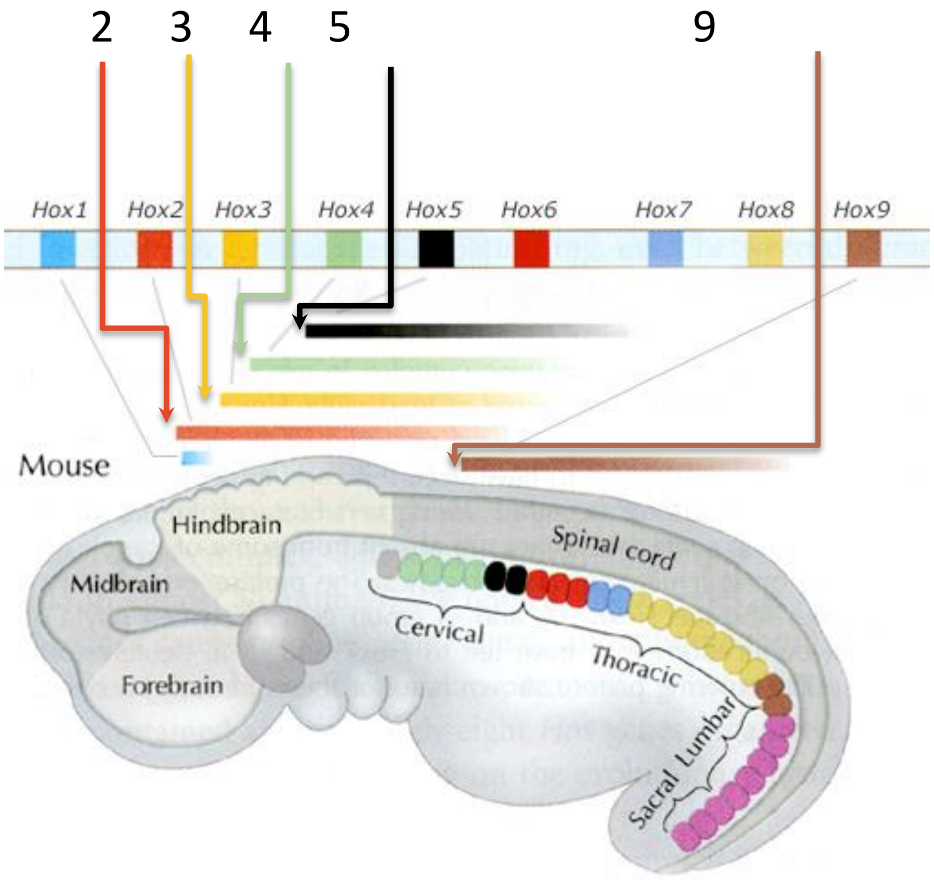
Boundary elements in the Hox complex of mosquito, *An. gambiae*



Ahanger et al Nucleic Acids Research 2013

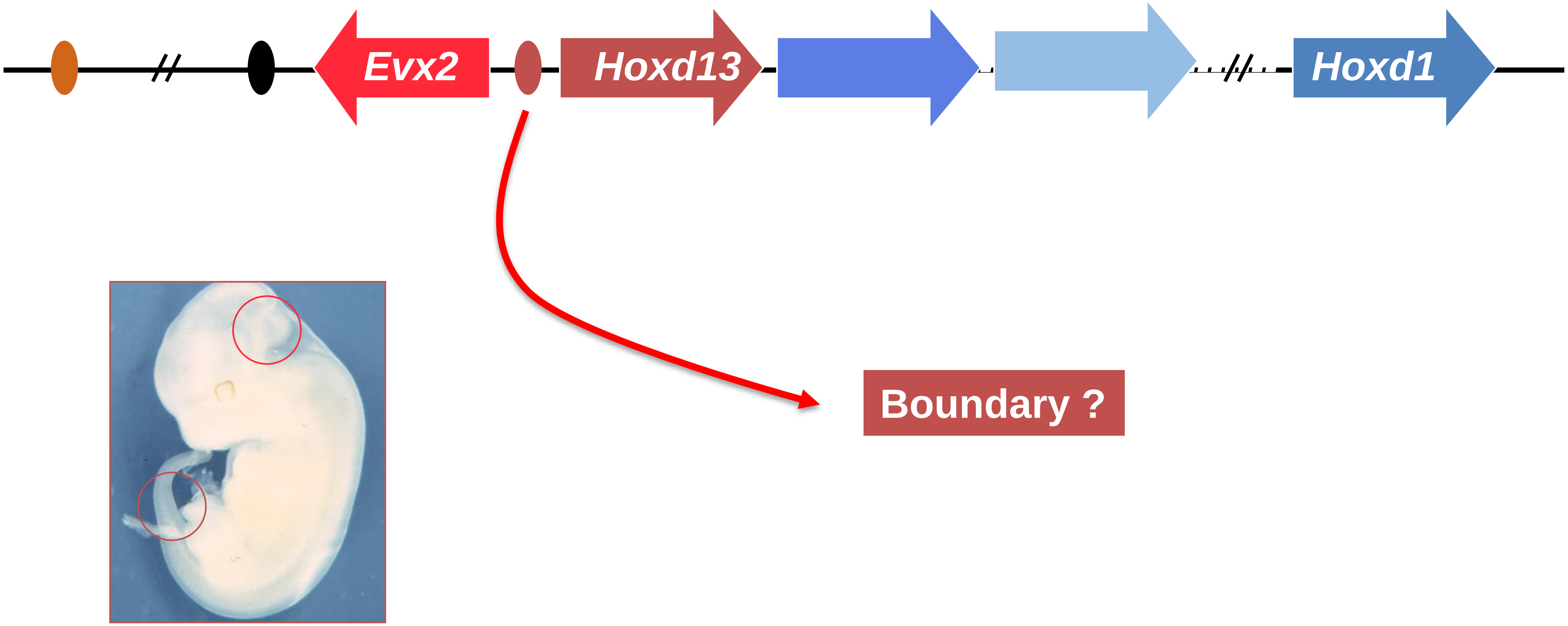
Boundaries needed to explain the regulation of hox genes in vertebrates too?

Boundaries setting the anterior limits of expression of hox genes

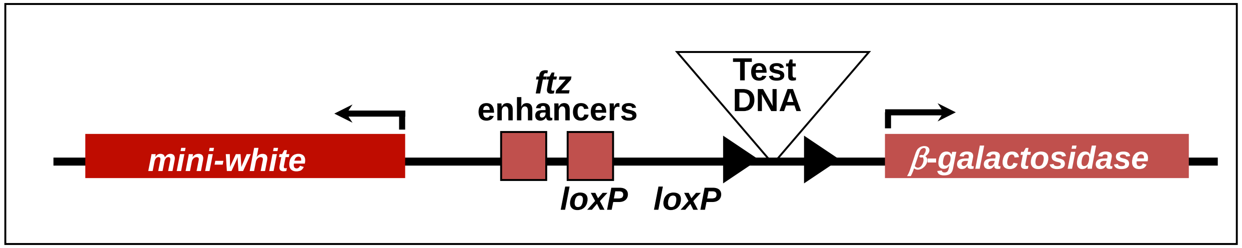


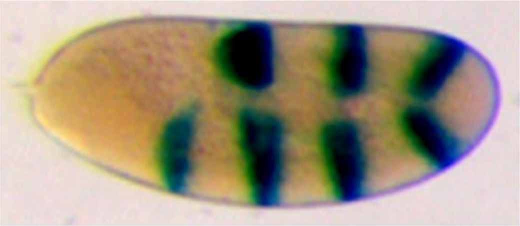
Boundary that separate Evx2 and Hoxd13 domains are functionally conserved in flies

Vasanthi

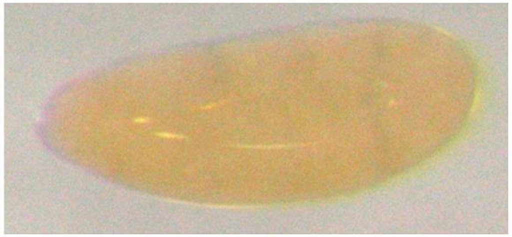


Mouse element functions as boundary in *Drosophila*

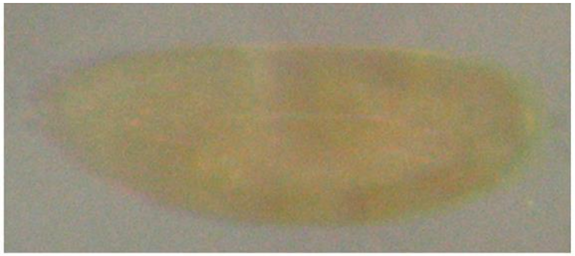




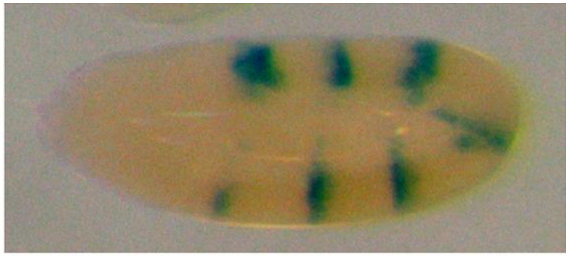
Phage DNA



Evx2-d13



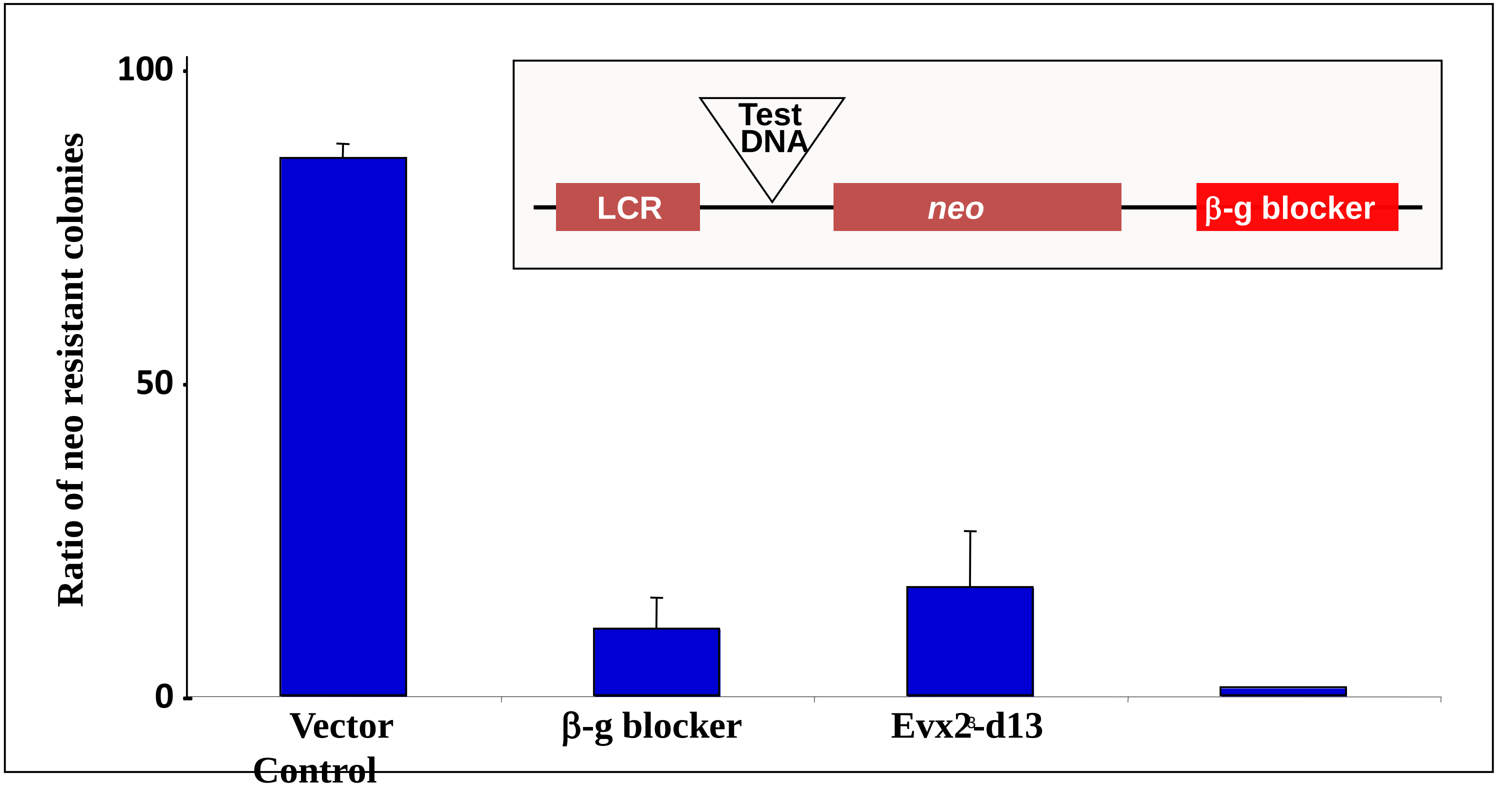
Initial line



Flipped out line

Vasanthi et al. Development 2010

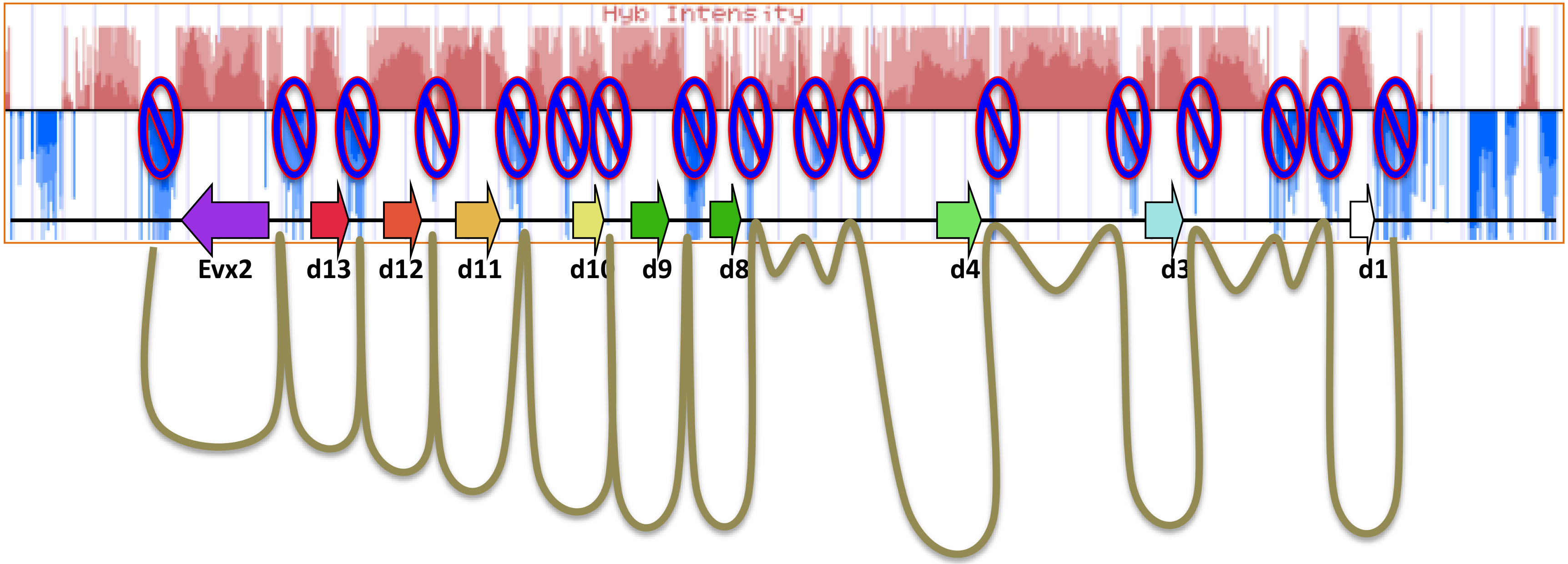
Enhancer blocking activity of Evx2-d13 boundary in human cells also



Multiple boundaries across the hox complex

Organizing for sequential / colinear expression along the A-P body axis

Surabhi



Srivastava et al. Epigenetics & Chromatin, 2013

Maintenance of expression of hox genes

i. Polycomb response elements (PRE), a.k.a. Cellular memory modules (CMM), are the cis-elements for this purpose.

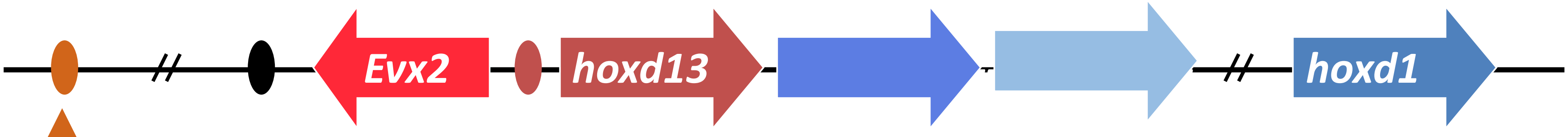
i. PREs ‘sense’ the expression state of the locus and recruit ‘appropriate’ PcG/trxG proteins/complexes.

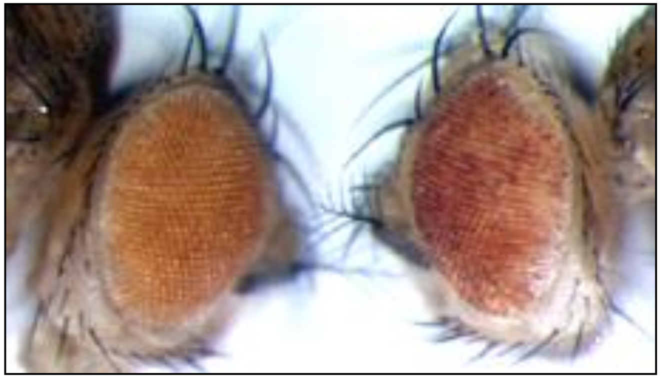
ii. This system is not exclusive to Hox loci, it is a genome level mechanism.

- much of our understanding about PcG system comes from studies in *Drosophila*.

- the Polycomb system is conserved during evolution.

Mouse repressor element is functional in fly





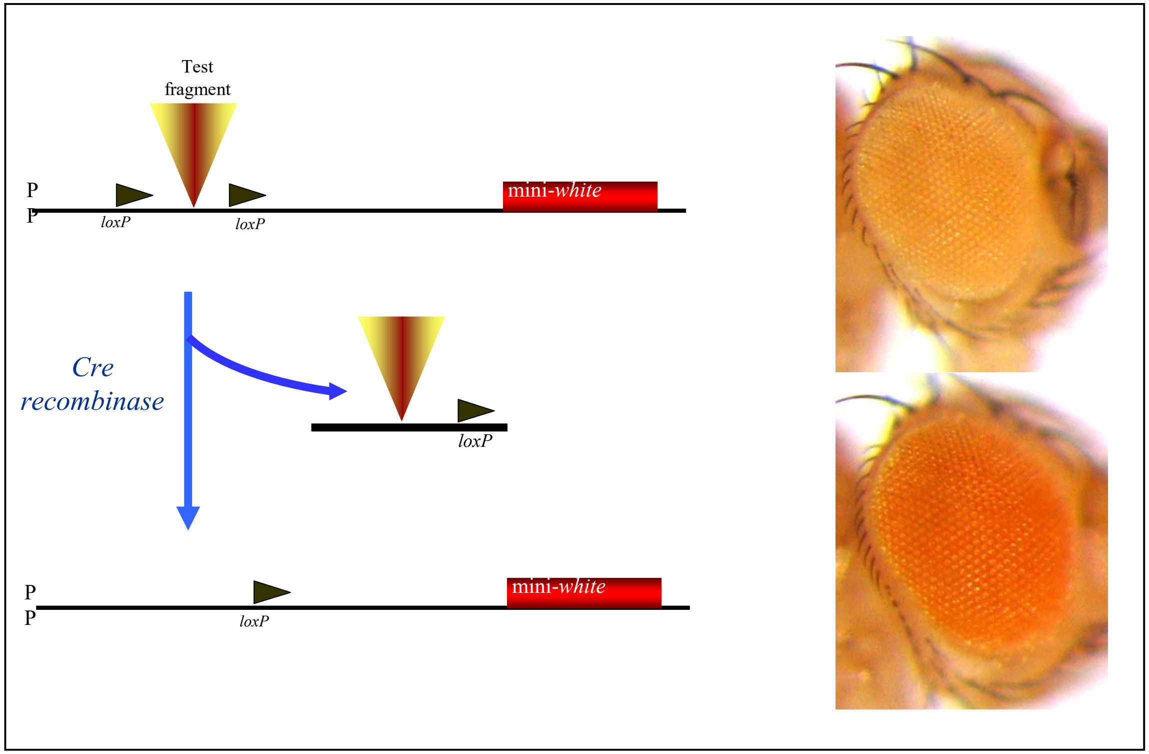
Functions as repressor
Associates with H3K27Me3
Responds to PcG / trxG mutations

The first vertebrate PRE !

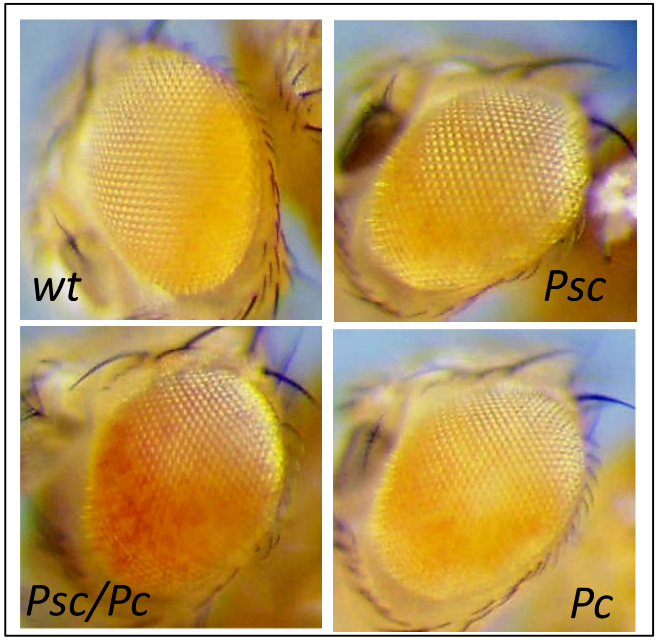
Mishra et al., Genesis 2007

Mouse *HoxD PRE* in *Drosophila*

Vasanthi

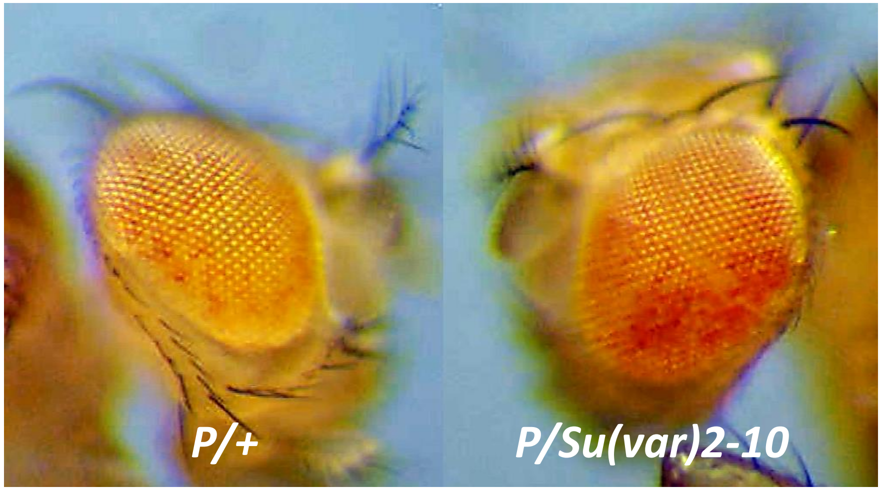


Mild but cumulative effect of PcG mutations



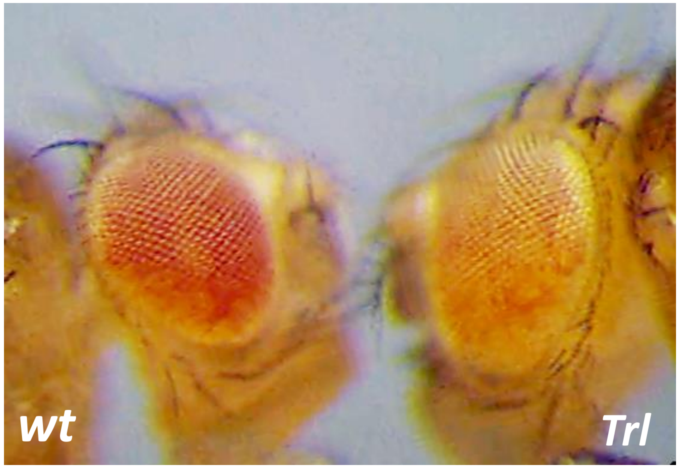
Vasanthi et al. Sci. Reports 2013

Differences between mouse and fly PREs



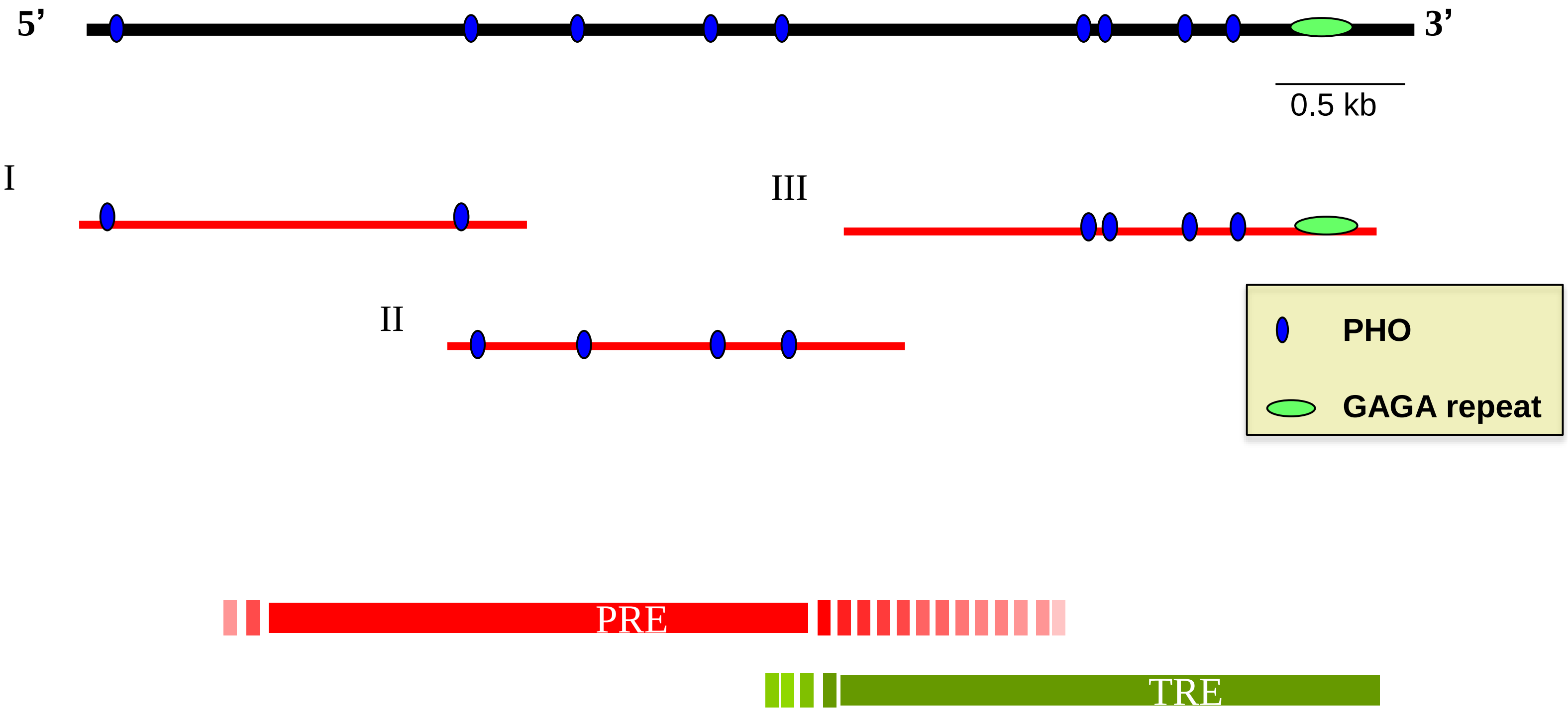
Mouse PRE responds to Su(var)s

Trl **behaves like trxG member**



Vasanthi et al. Sci. Reports 2013

Dissection of the mouse HoxD-PRE



Closely spaced PRE and TRE

Vasanthi et al. Sci. Reports 2013

Mouse PRE: a complex element

Functionally conserved in fly but differs in several aspects

no pairing dependent silencing
more variegation
mild but cumulative effect of PcG mutations
***Trl* effect is like trxG member**
***Su(var)* mutations have effect on this PRE**

greater variety of genetic interactions

Mouse PRE receives input from larger number and variety of factors

Expansion of the epigenetic tool kit and evolution of complexity

While invertebrates have one set of hox genes, vertebrates have $\geq$4 Hox clusters

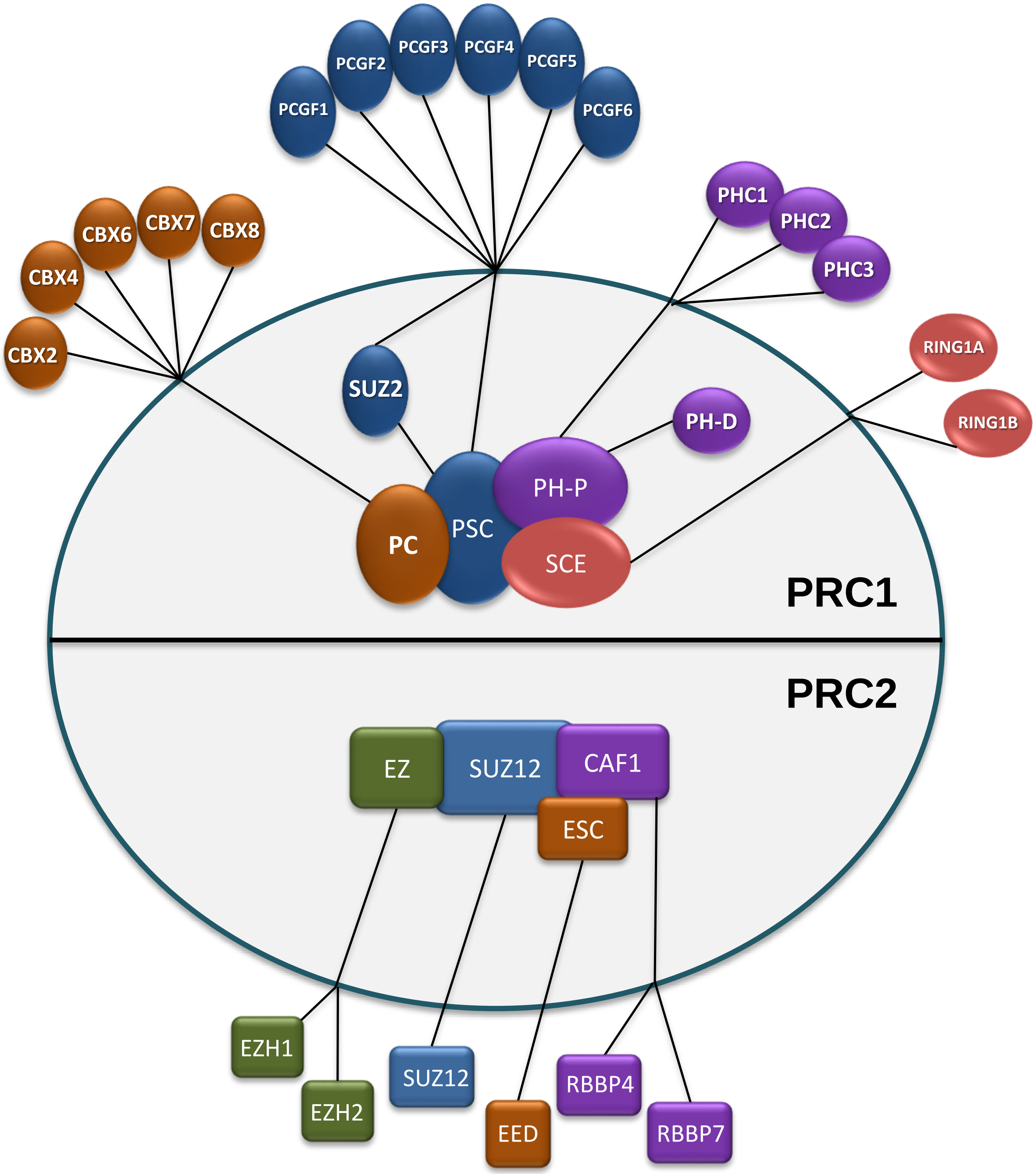
This Evolution of complexity coincides with the expansion of Polycomb system

Tej

Senthil

Bioinformatic approach to explore PcG genes

Expansion of the epigenetic tool kit



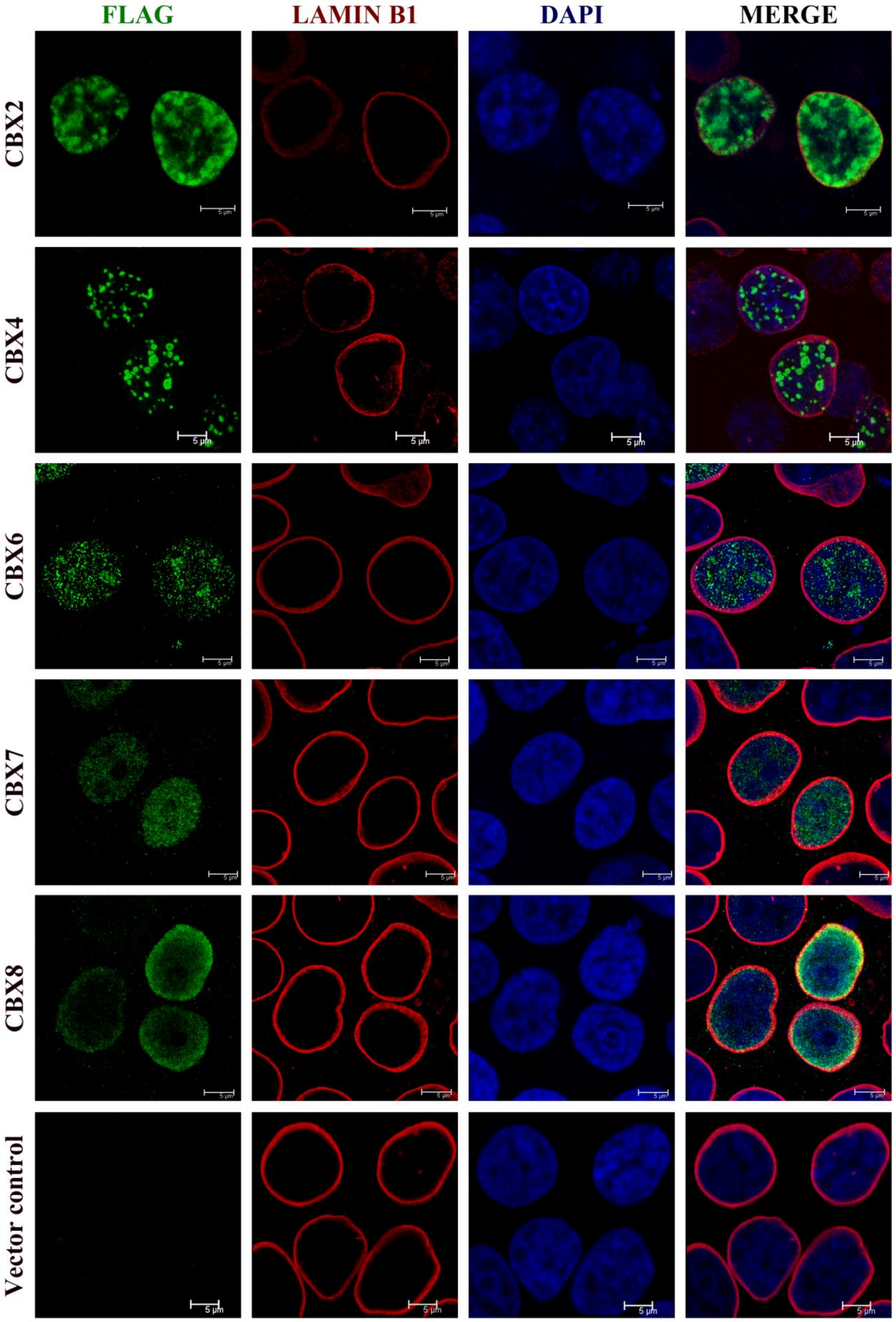
Polycomb system consists of two kinds of complexes:

PRC2 - writers of the epigenetic code

PRC1 - readers of the epigenetic code

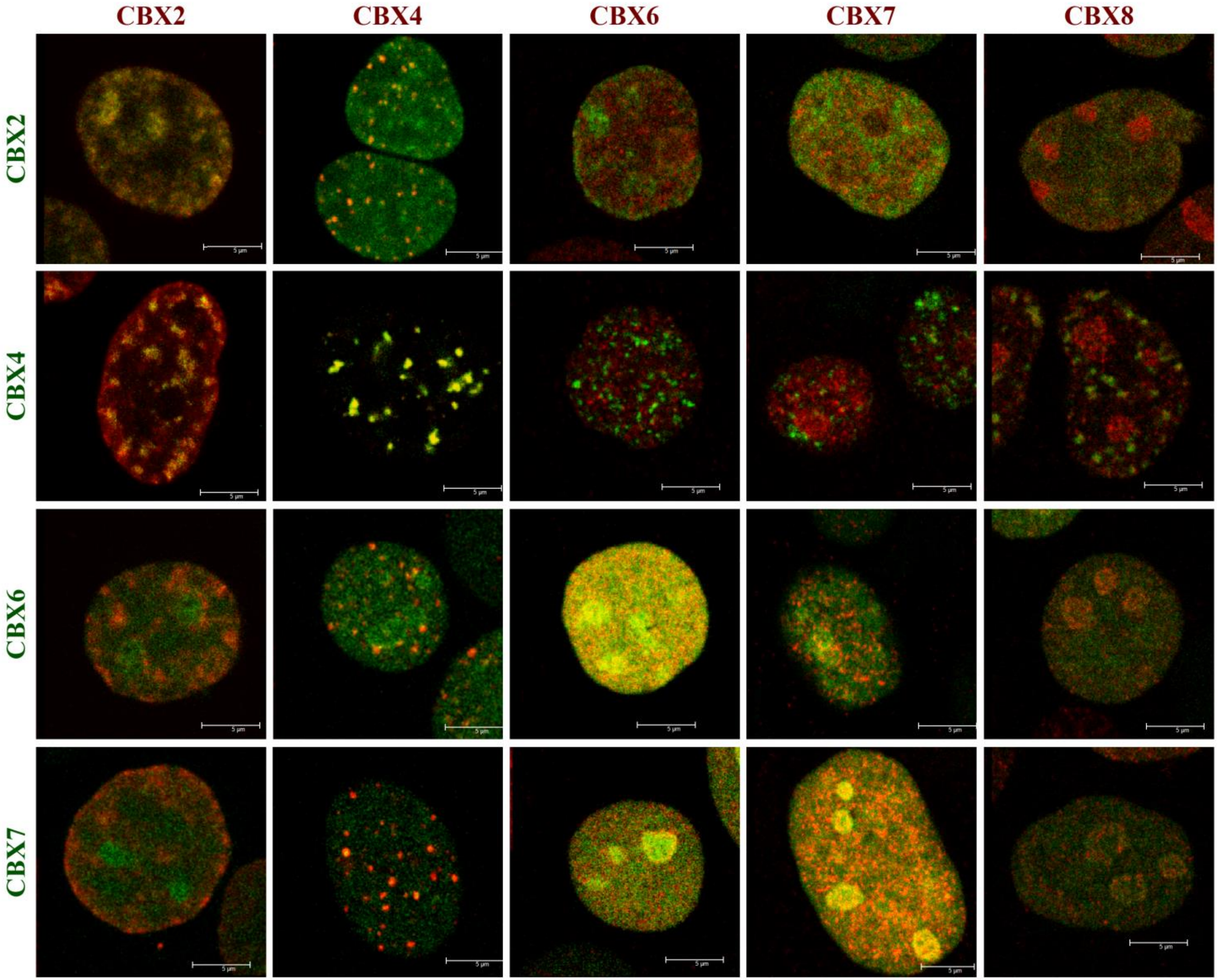
In mammalian cells

Pc homologues have distinct nuclear localization pattern



The nuclear localization pattern of FLAG tagged mouse homologues. Scale 5μM.

Vertebrate Pc homologues have only limited co-localization



Our findings suggest:

PRC1 members expanded early during vertebrate evolution

PRC2 members remain relatively unchanged: more ancient & fixed

Most of expanded members have gained paralogue specific motifs that may reflect their novel functions

Constant selection pressure for 500 million years

Non-overlapping localization of different Cbx members

We propose distinct complex (PRC1s) formation by the PcG members.

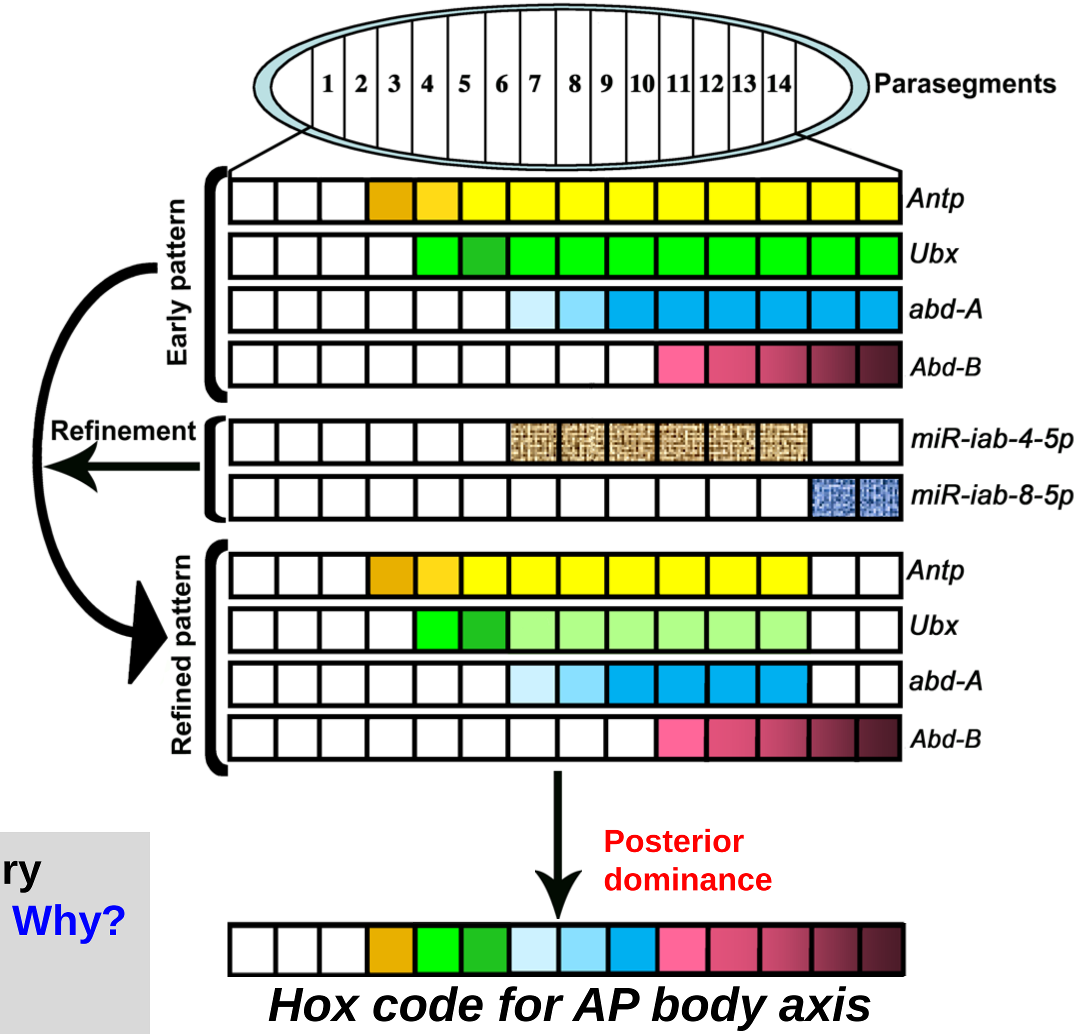
Expansion of PcG, preceded by the emergence of vertebrates,
contributed as enhanced epigenetic resource for the evolution of complexity.

Narendra Pratap Singh

Hox code for determining AP body axis

Hox genes show overlapping expression pattern

Posterior dominance to rescue?



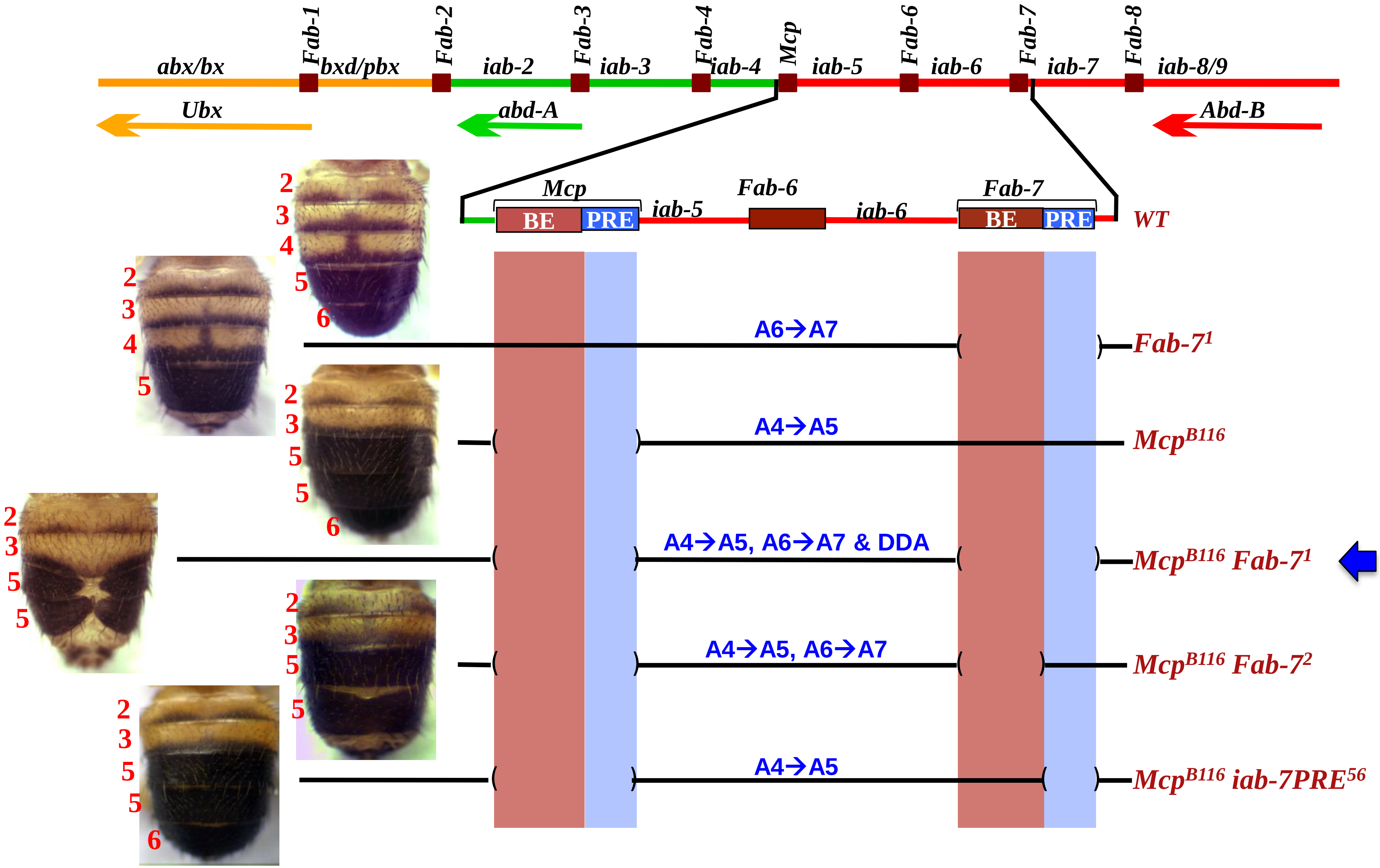
Hox code for AP body axis

Boundaries that define the regulatory domains are associated with PREs. Why?

Hox genes are regulated with great precision, still express in overlapping domains for posterior dominance! Why?

Singh & Mishra, BioEssays 2008

Boundary-PRE combinations of the BX-C interact genetically



abx/bx *Fab-1* *bxd/pbx* *Fab-2* *iab-2* *Fab-3* *iab-3* *Fab-4* *iab-4* *Mcp* *iab-5* *Fab-6* *iab-6* *Fab-7* *iab-7* *Fab-8* *iab-8/9*

Ubx *abd-A* *Abd-B*

Mcp BE PRE *iab-5* *Fab-6* *iab-6* *Fab-7* BE PRE *WT*

A6→A7 — *Fab-7*^{1}

A4→A5 — *Mcp*^{B116}

A4→A5, A6→A7 & DDA — *Mcp*^{B116} *Fab-7*^{1}

A4→A5, A6→A7 — *Mcp*^{B116} *Fab-7*^{2}

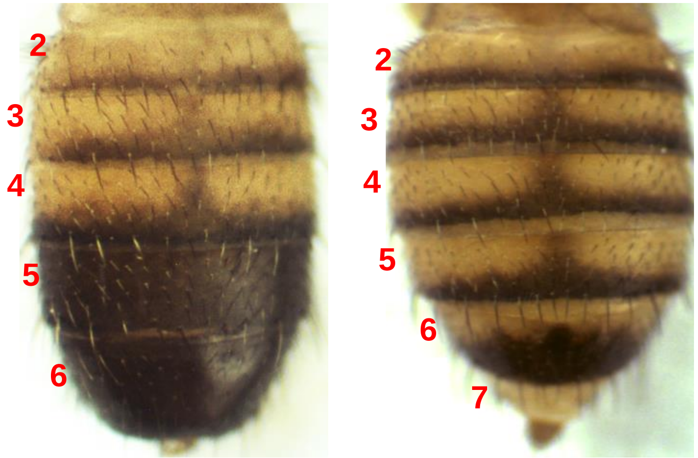
A4→A5 — *Mcp*^{B116} *iab-7PRE*^{56}

Dorsal closure defect in the adult abdominal phenotype

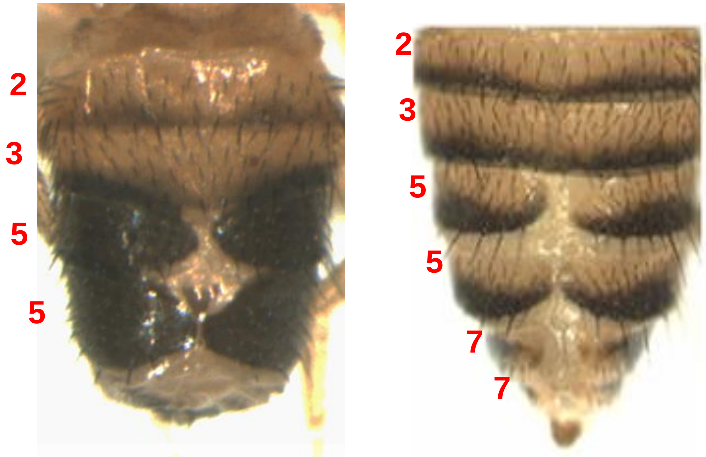
Deletion of boundary/PRE combinations lead to more than additive phenotype

Mcp **and** ***Fab7*** **interact in the context of** ***Abd-B***

Non-homeotic DDA phenotype seen in the region where identity is determined by ***Abd-B***



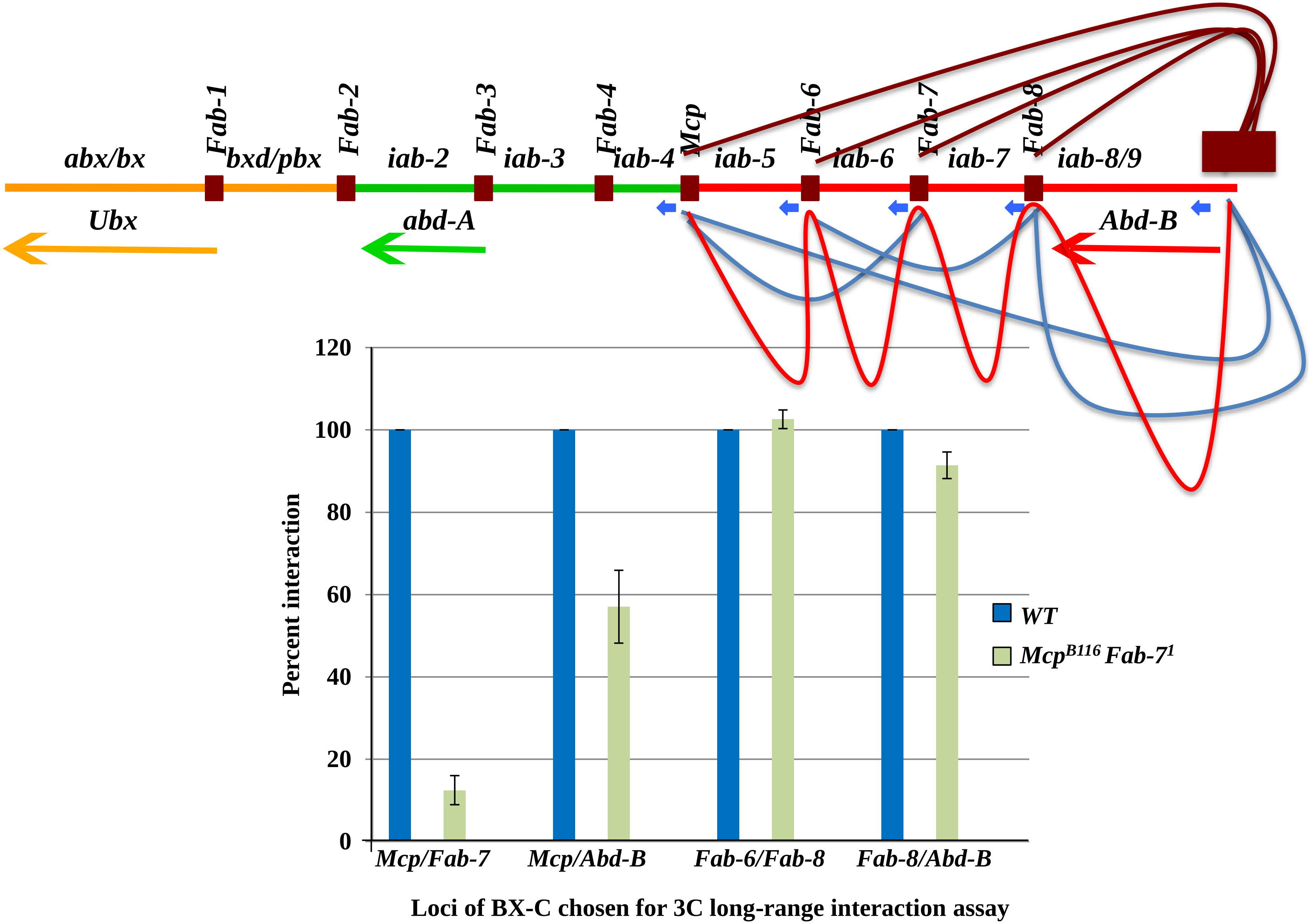
Wild type



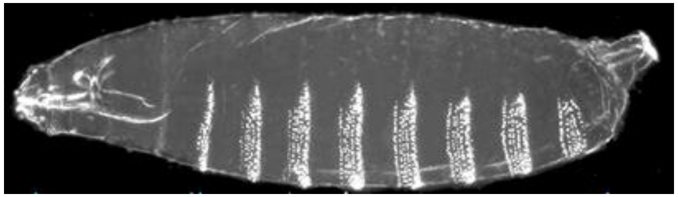
Mcp^{B116} $Fab\text{-}7^{1}$

Homozygous viable and fertile

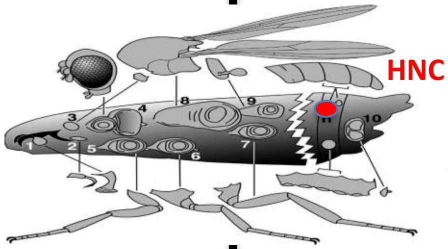
Mcp and *Fab-7* regions physically interact with each other and with *Abd-B* promoter region



From larva to adult

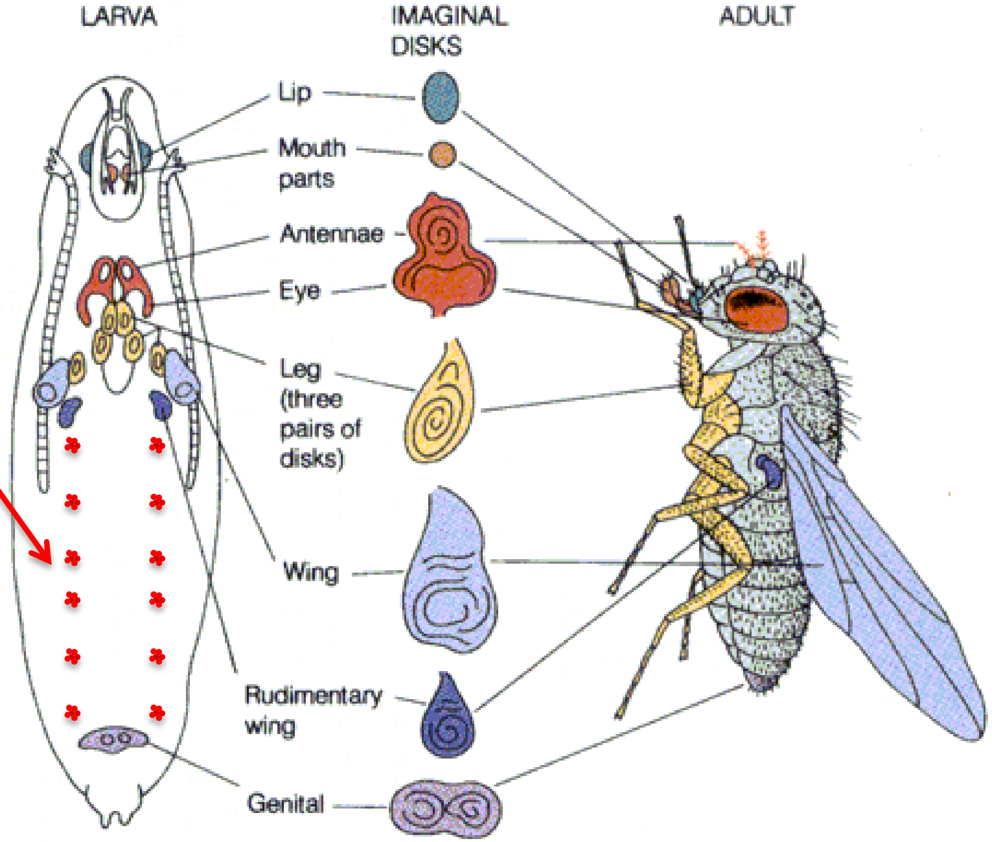


Larva



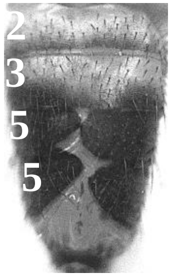
adult

Histoblast nest cells

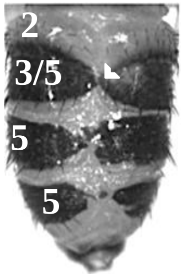


Reprogramming in a closed system:

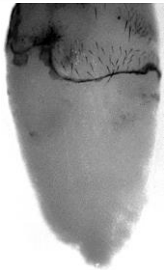
Massive cell death
Growth and differentiation



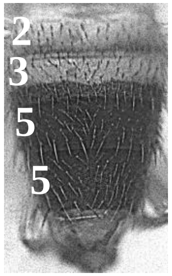
The ‘*Mcp-Fab7*’ DDA phenotype



enhanced by PRC1 mutants (readers) but not by PRC2 (writers), trxG, boundary factors



loss of abdominal epithelia upon over expression of *Abd-B* in LECs



rescued by RNAi of *Abd-B* in LECs

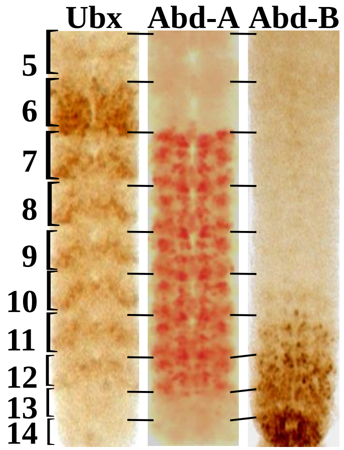
Interaction among *boundary-PRE* combinations is facilitated by PRC1

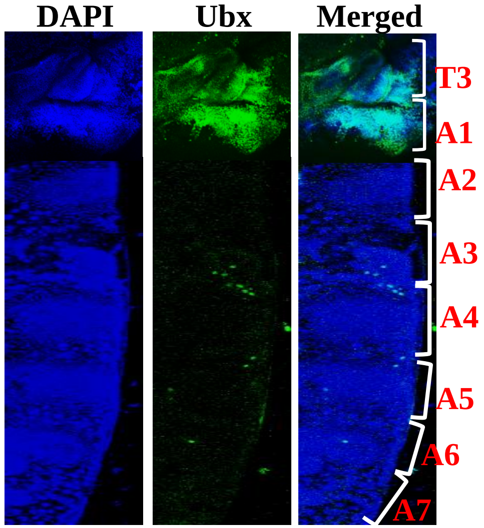
‘*Mcp-Fab7*’ DDA is due to ectopic activation of *Abd-B*

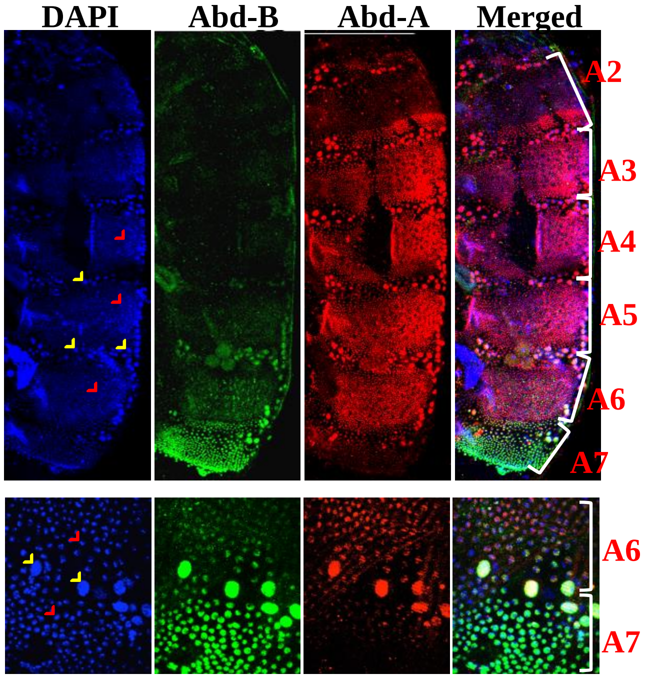
Ectopic activation of *Abd-B* suppresses *abd-A* which is needed for abdominal epithelia formation

Boundary-PRE combination is essential for precise regulation of homeotic genes.

Early and late expression patterns of the hox genes of the BX-C



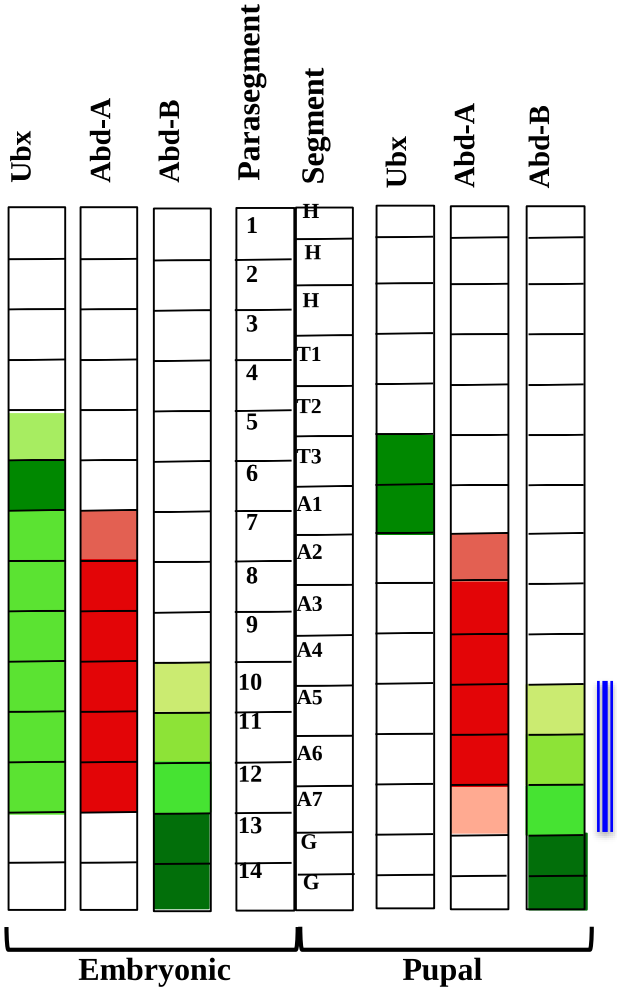




LECs and **HNCs**, both express hox genes.

In the overlapping domain of expression, *abd-A* and *Abd-B* are present even in the same nucleus.

Early and late expression patterns of the hox genes of the bithorax complex



***abd-A* and *Abd-B* expression continues to overlap in A5 to A7**

Role of *abd-A* in abdominal epithelia formation

abd-A RNAi

esg for HNC

Eip-71CD for LEC

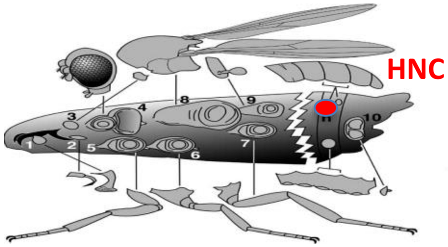
71B and pnr for HNC & LEC, both

esg-Gal4

Eip-71cd-Gal4

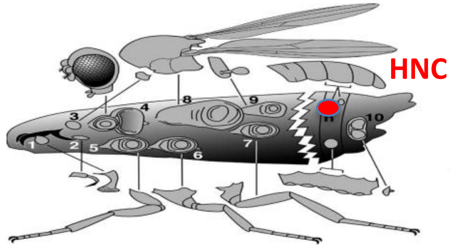
71B-Gal4

pnr-Gal4

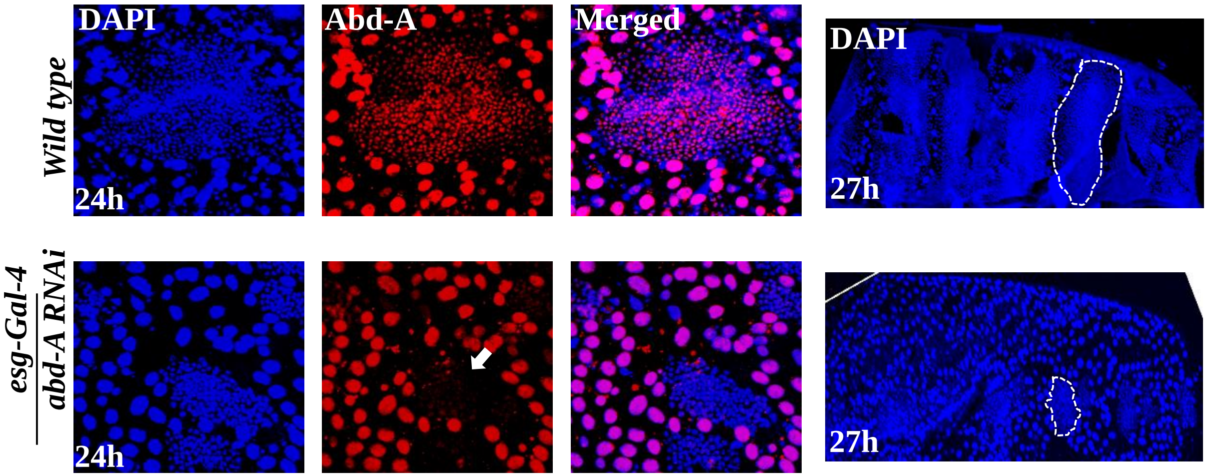


***abd-A* is essential for adult epithelia formation in A2-A7**

Role of *abd-A* in abdominal epithelia formation



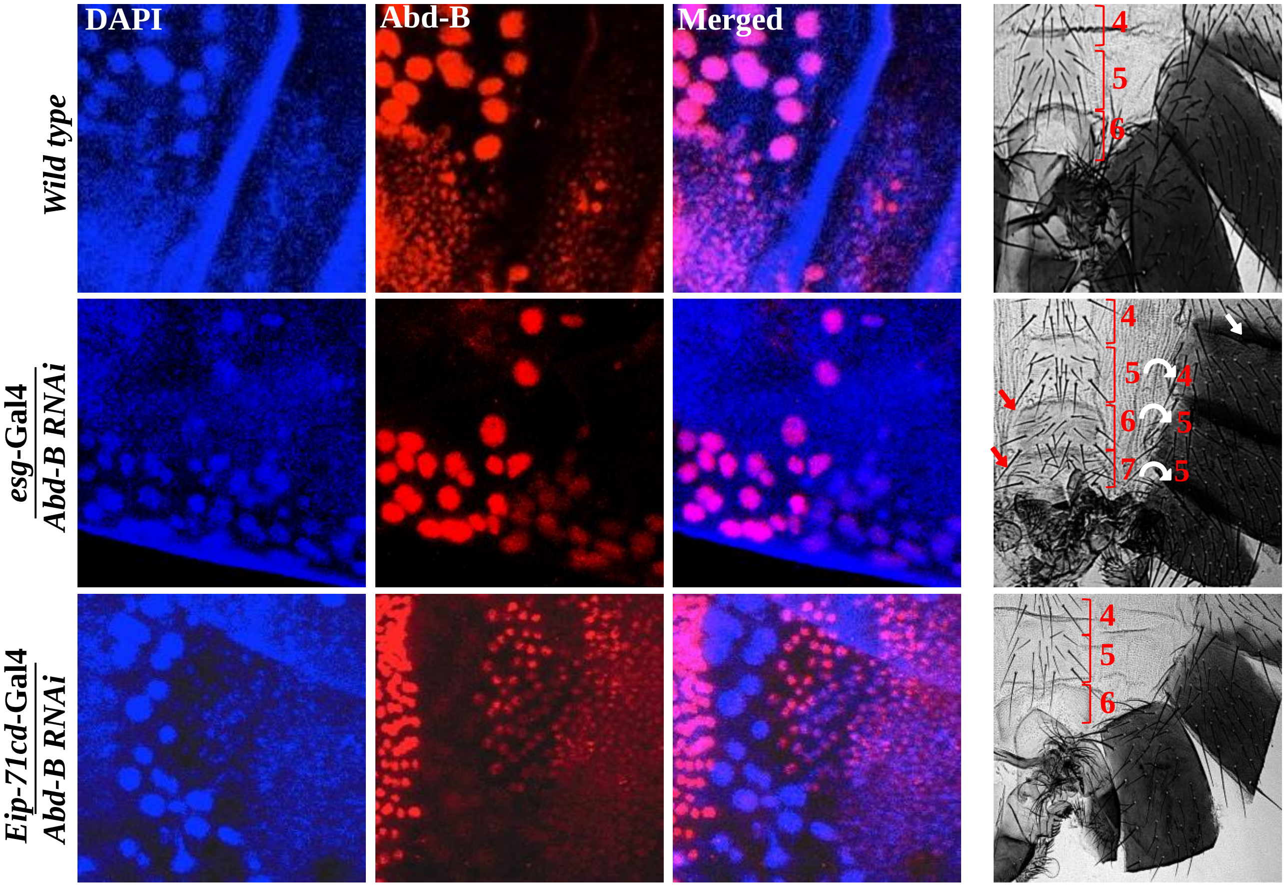
esg for HNC



Live cell imaging to analyze HNCs and LECs
24h and 27 h snap shots

***abd-A* required in HNCs for their proliferation while it is required in LECs for their removal**

Role of *Abd-B* in adult epithelia formation

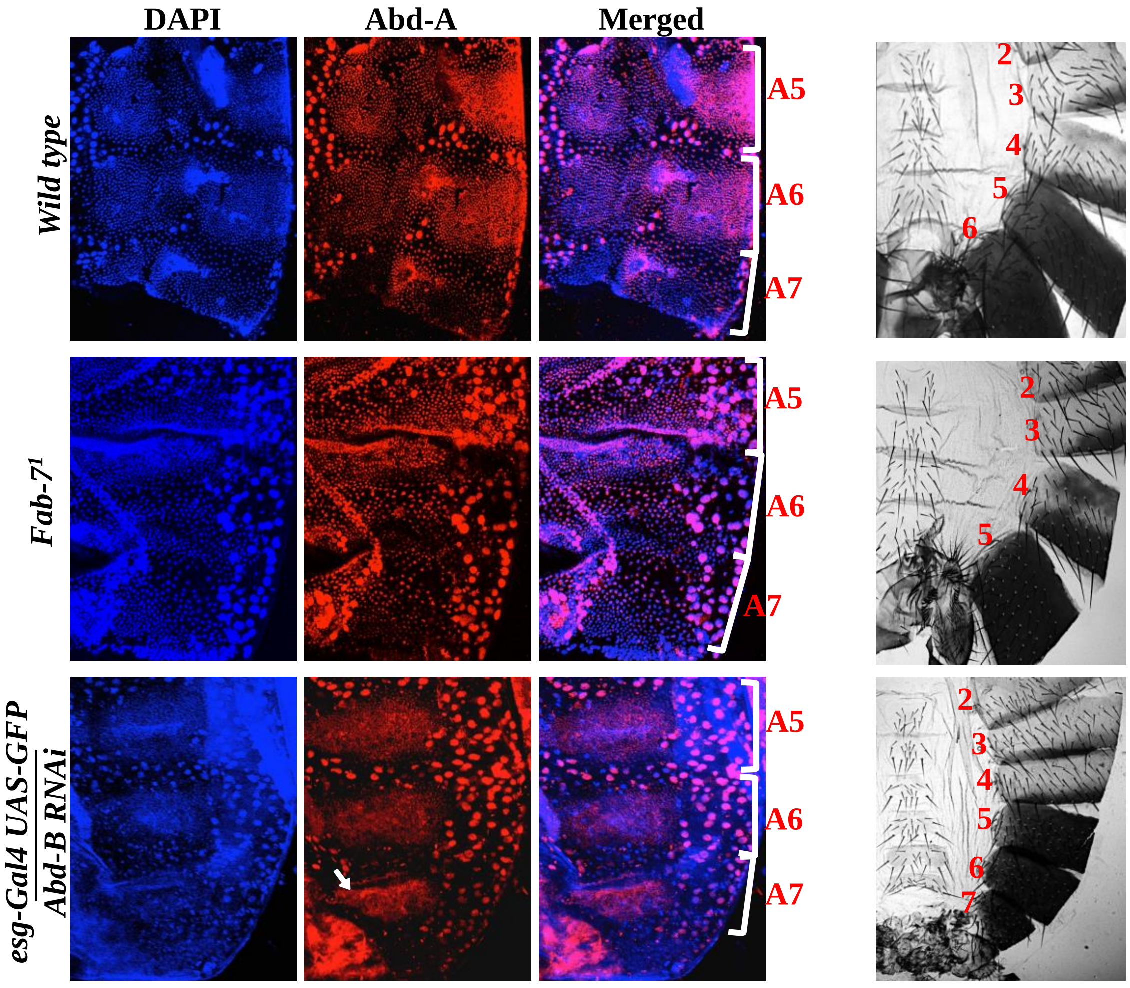


esg for HNC

Eip-71CD for LEC

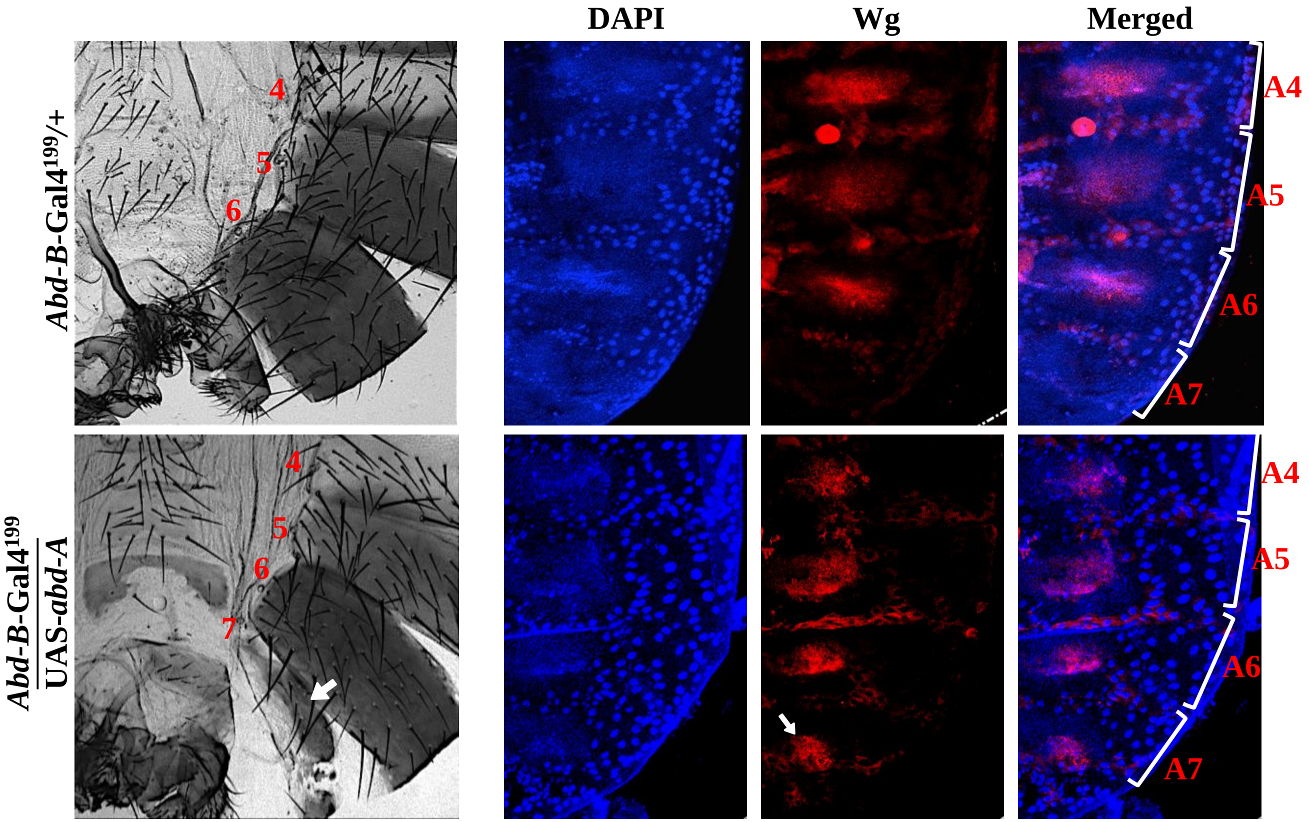
Abd-B **is required in HNCs for the identity of the adult abdominal epithelia**

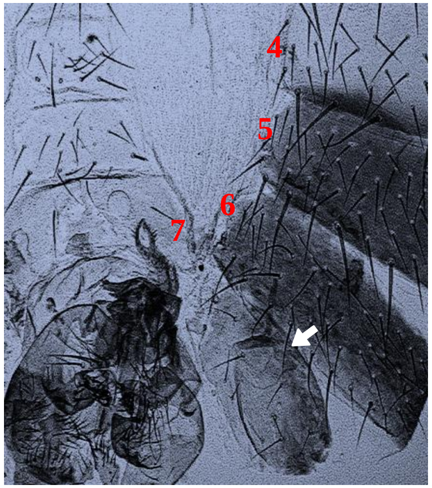
Higher level of *Abd-B* suppresses *abd-A* (in A7)



abd-A is proliferation promoter in HNCs.
When suppressed in A7 by higher level of *Abd-B*, the segment is lost.

wingless is downstream of *abd-A* in proliferation promotion pathway in A7





Abd-B-Gal4^{LDN}
UAS-*wg*

In A7

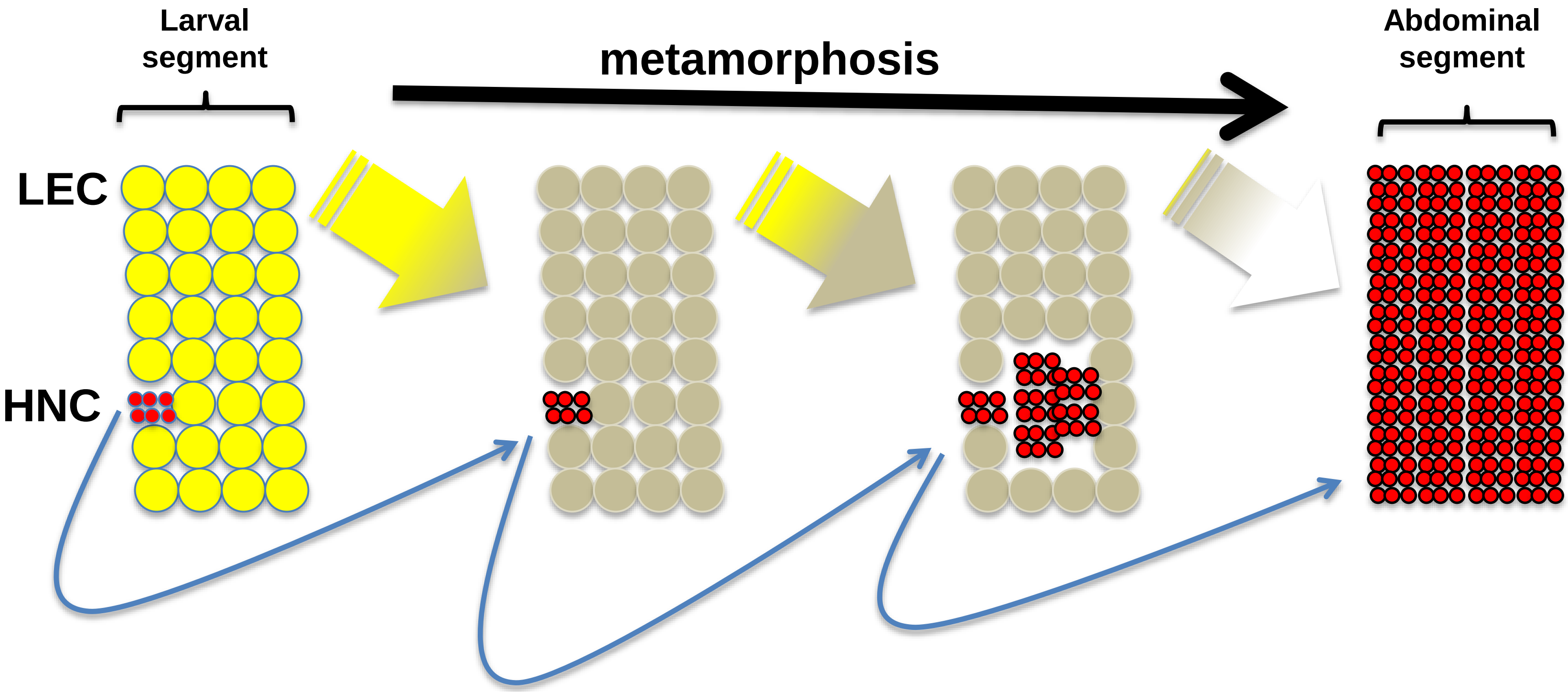
Abd-B **(when above certain level) down regulates *abd-A*.**

abd-A **up regulates *wg*.**

wg **promotes proliferation of HNCs / formation of adult epithelia.**

Identity of segment determined by *Abd-B*

Changed level of *Abd-B* → altered segmental identity



Growth of HNCs depends on abd-A

Remove abd-A from HNC → no adult cuticle formation

Singh & Mishra PLOS Genetics 2014

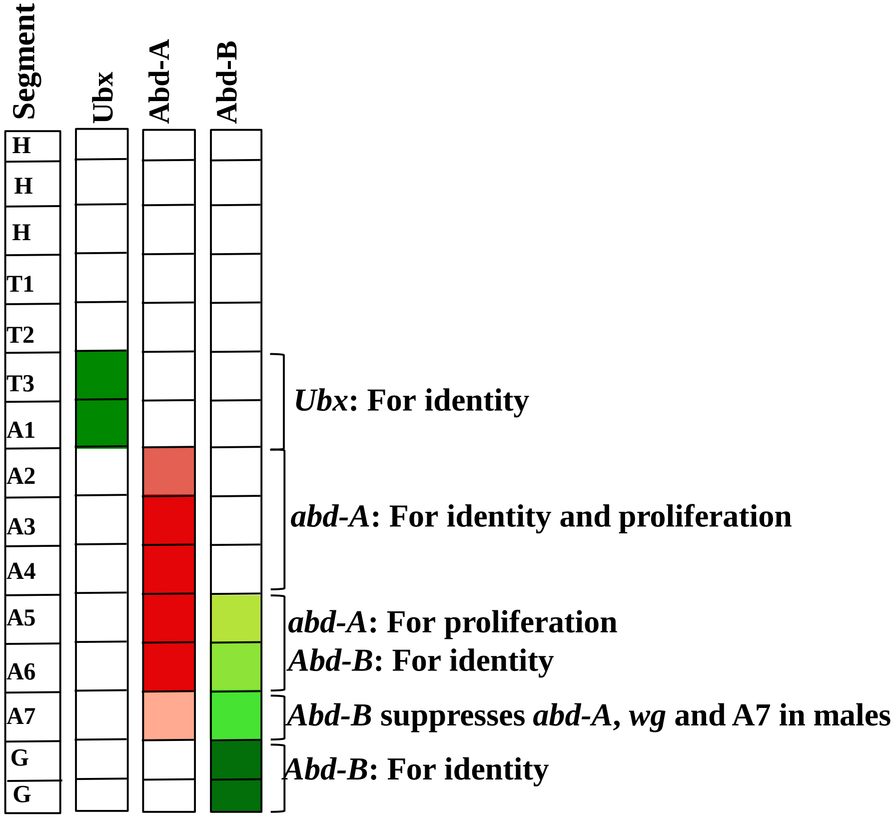
Precise pattern is set by multiple regulatory elements, long range interactions and 'boundary-PRE' combinations.

abd-A defies posterior prevalence rule and plays key role in adult epithelia formation in regions where identity is determined by *Abd-B*.

1. Boundary and PRE combination is essential for precise level of expression of Hox genes.

2. Hox genes have non homeotic role of cell proliferation.

3. In the domains of overlapping expression, anterior and posterior hox genes have distinct and essential function. This finding breaks the concept of 'posterior dominance'.



Segment	Ubx	Abd-A	Abd-B
H			
H			
H			
T1			
T2			
T3	🟩		
A1	🟩		
A2		🟥	
A3		🟥	
A4		🟥	
A5		🟥	🟩
A6		🟥	🟩
A7		🟥	🟩
G			🟩
G			🟩

Ubx: For identity

abd-A: For identity and proliferation

abd-A: For proliferation
Abd-B: For identity

Abd-B suppresses *abd-A*, *wg* and A7 in males

Abd-B: For identity

Take home:

Boundaries define regulatory domains of hox genes and are conserved during evolution

PREs maintain expression state of hox genes and polycomb system is not only conserved but expanded during emergence of vertebrates

Boundaries and PREs function across the genome, not only in the Hox context

Boundary and PRE combination is essential for precise level of expression of Hox genes.

Multiple regulatory elements (boundaries & PREs) show long range interaction and add up to provide novel features

In the domains of overlapping expression, ***abdA*** leads to cell proliferation/growth while ***AbdB*** determines the identity.

Thank You

our_lab@CCMB

bithorax complex	Titus Ponrathnam A Srinivasan
evo-devo of Hox	Gopal Kushwah Avinash Srivastava
chromatin & epigenome	Divya Tej Sowpati Shreekant Verma Parna Saha Amitha Sampath Shagufta Khan Mukesh K Yadav Shreyasi Mukherjee K Phanindhar Ved Dube Fathima Athar Herojeet Singh Philge Philip Nikhil Hajirnis Ishani Srivastava Sachin Gadakh
Nuclear architecture	

Rashmi U Pathak
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