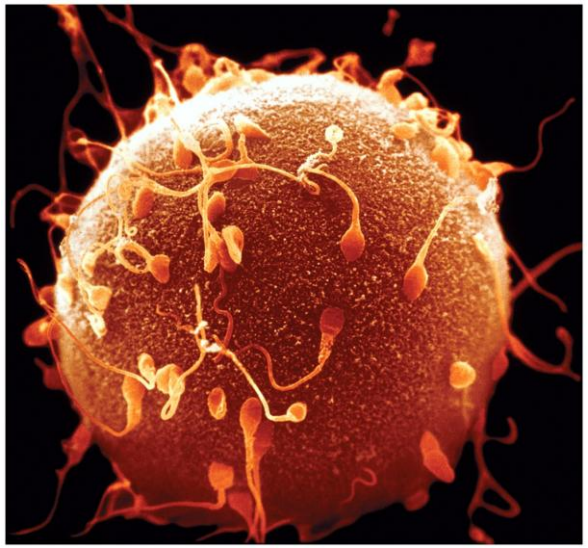
AP Body patterning in flies: Experimental tests of the morphogen hypothesis

KITP: Dynamics of Development
8/17/2011

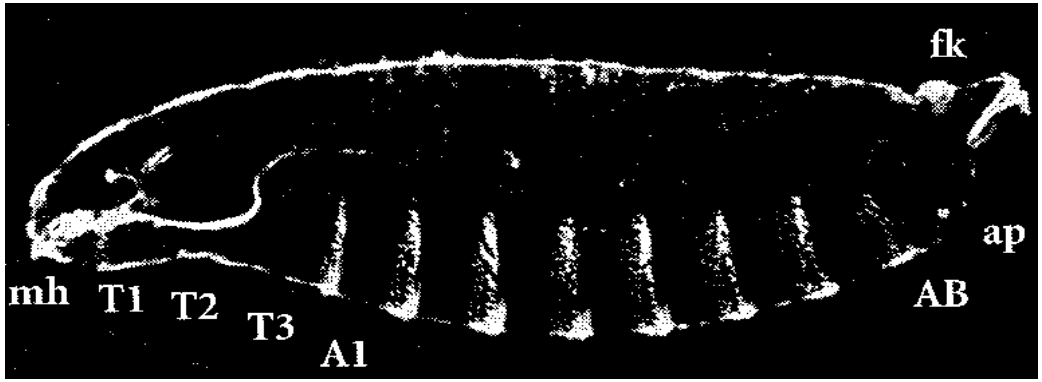
Steve Small
NYU Biology



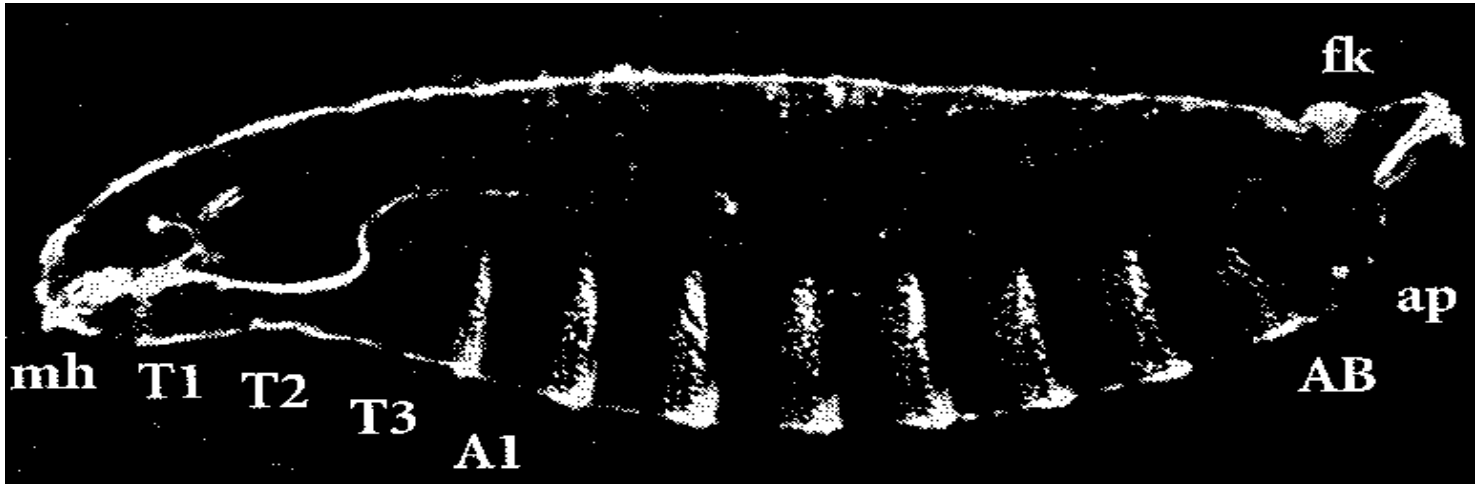
?

?

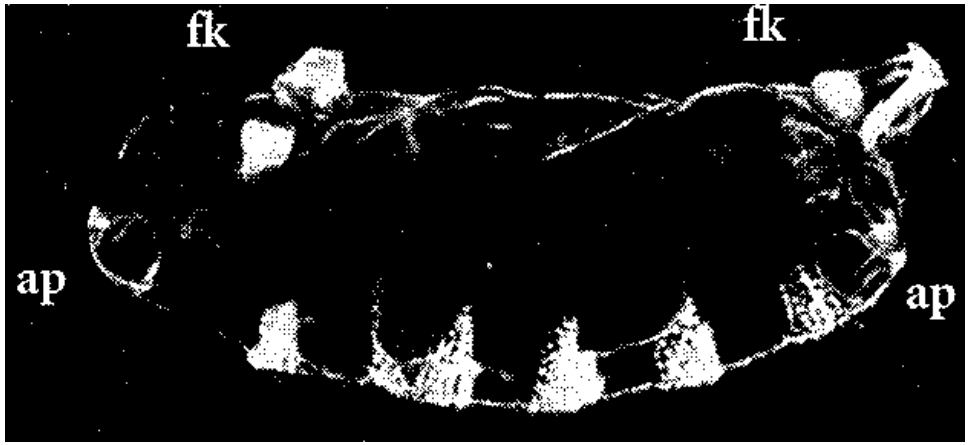
24 hours



wild-type:



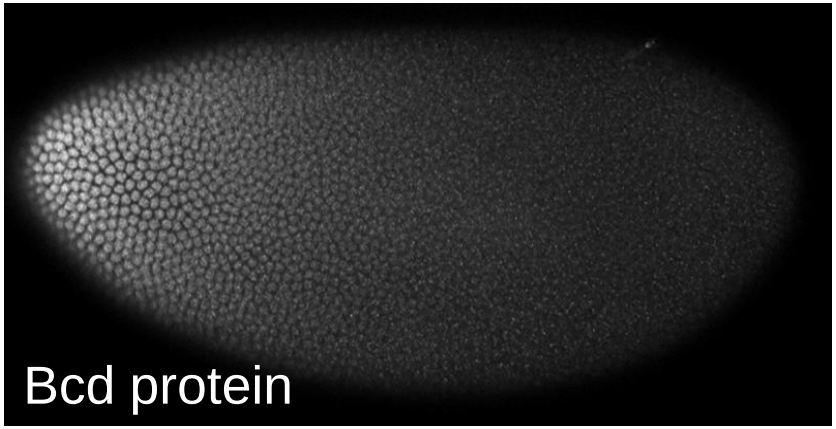
bicoid mutant:

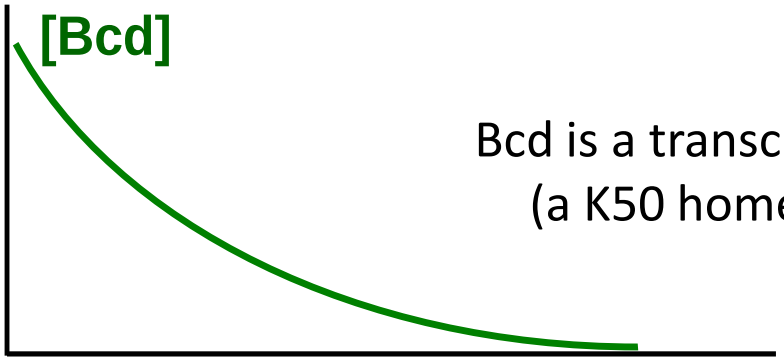


No head or thorax

Bcd is required for specifying and positioning many different cell types.

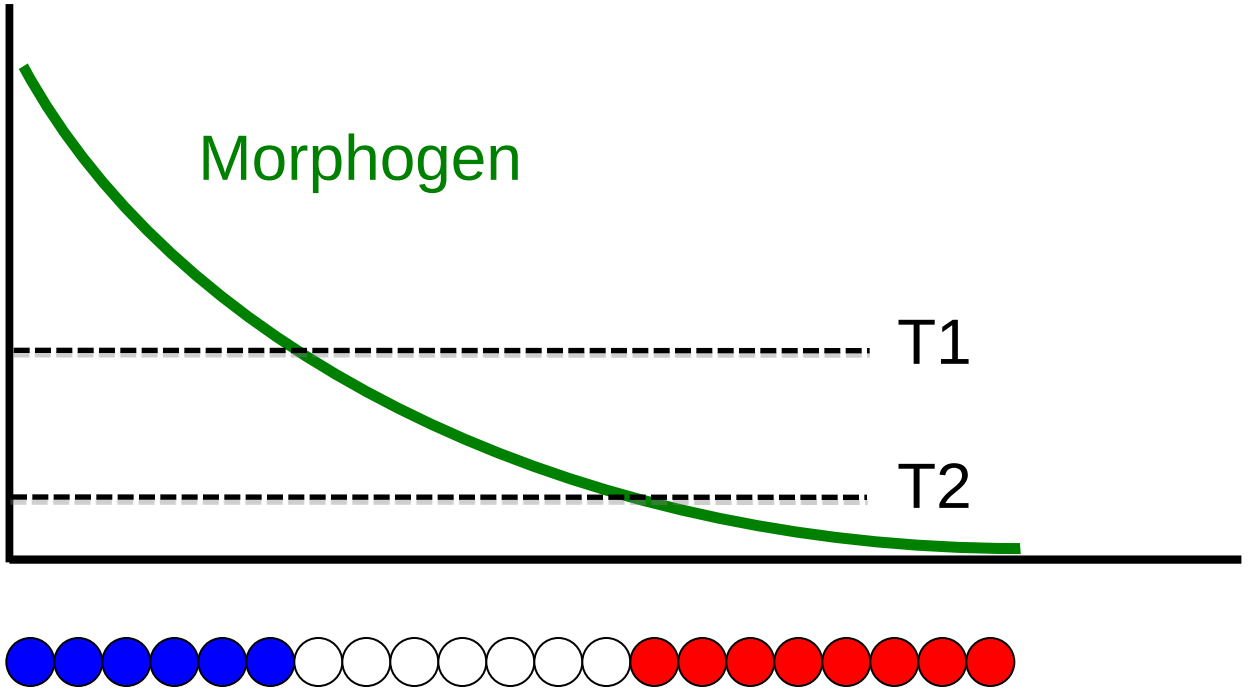
The Bcd Gradient



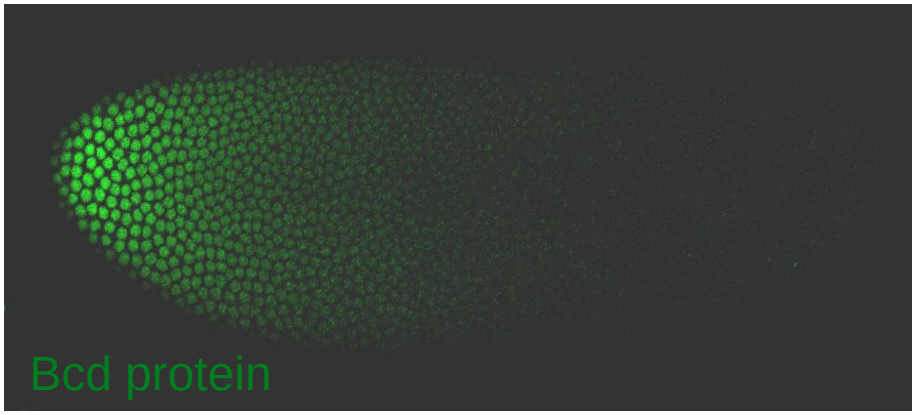


Bcd is a transcription factor
(a K50 homeodomain)

French Flag Model (Wolpert)



Question 1: Does Bcd function as a morphogen?


Bcd protein

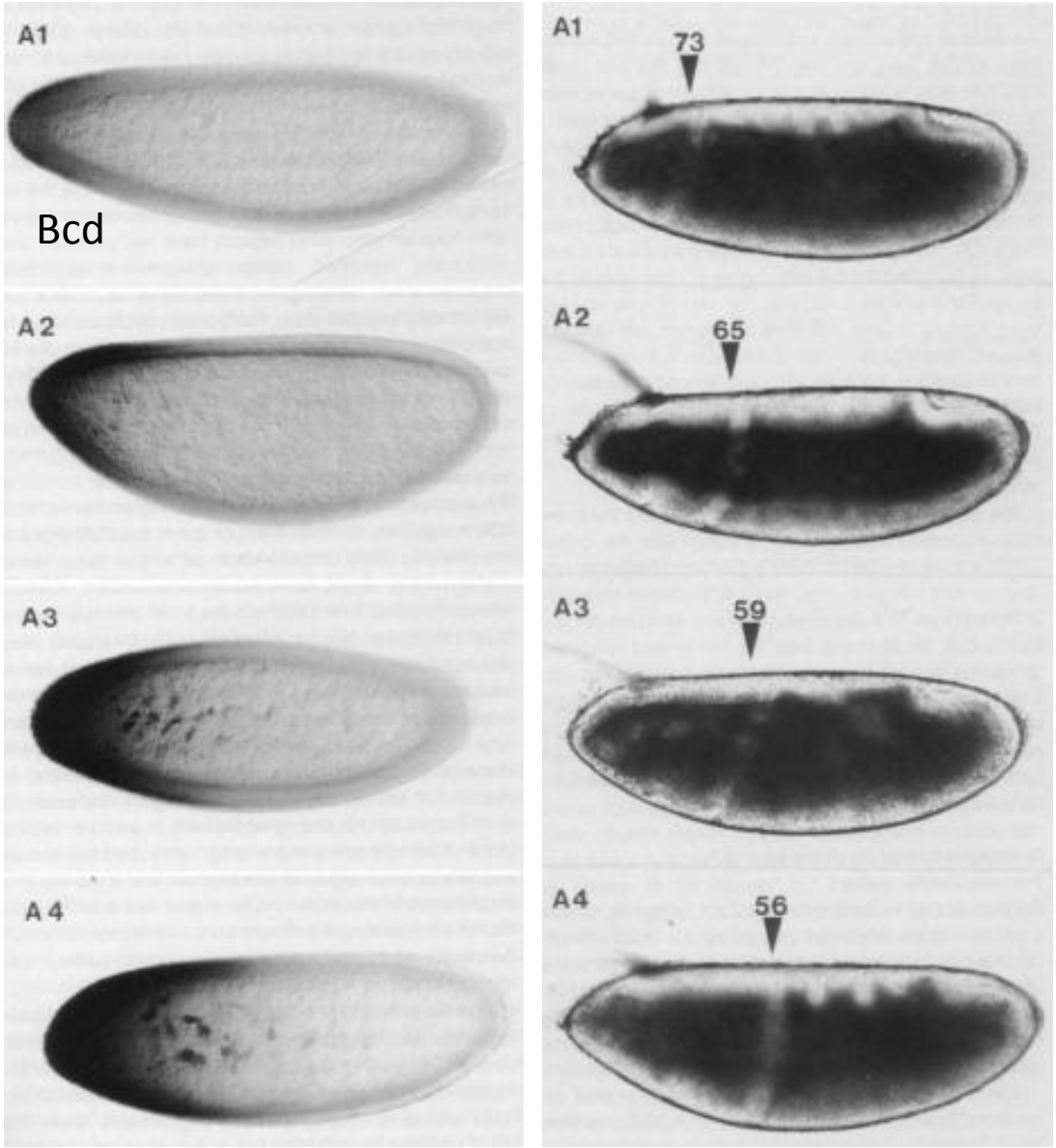
Changing *bcd* copy number shifts the head furrow position.

1x bcd

2x bcd

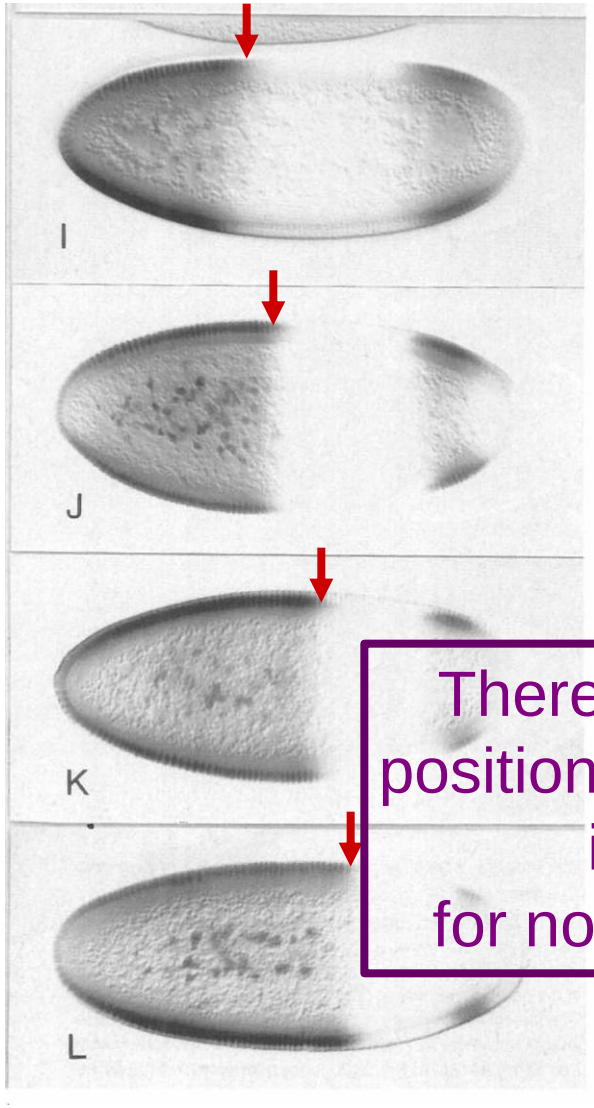
3x bcd

4x bcd



Driever et al, 1988

Changing *bcd* copy number moves the *hb* boundary.

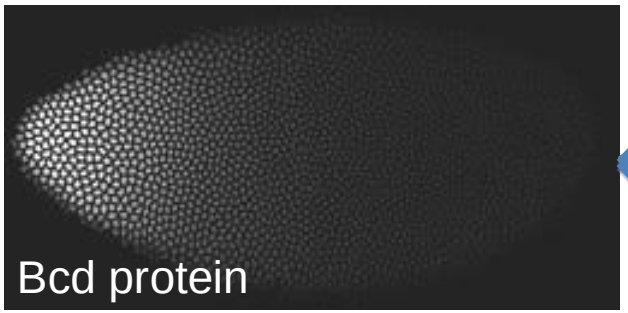


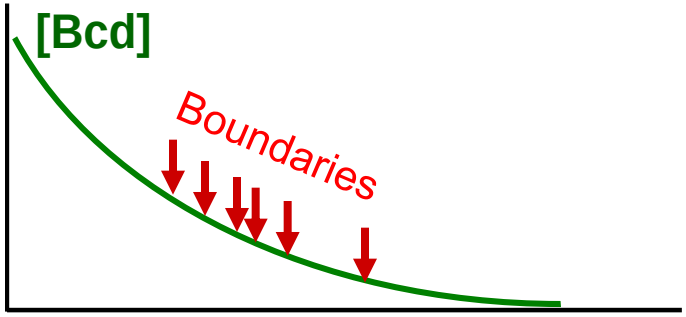
All these embryos become fertile adults in the lab!

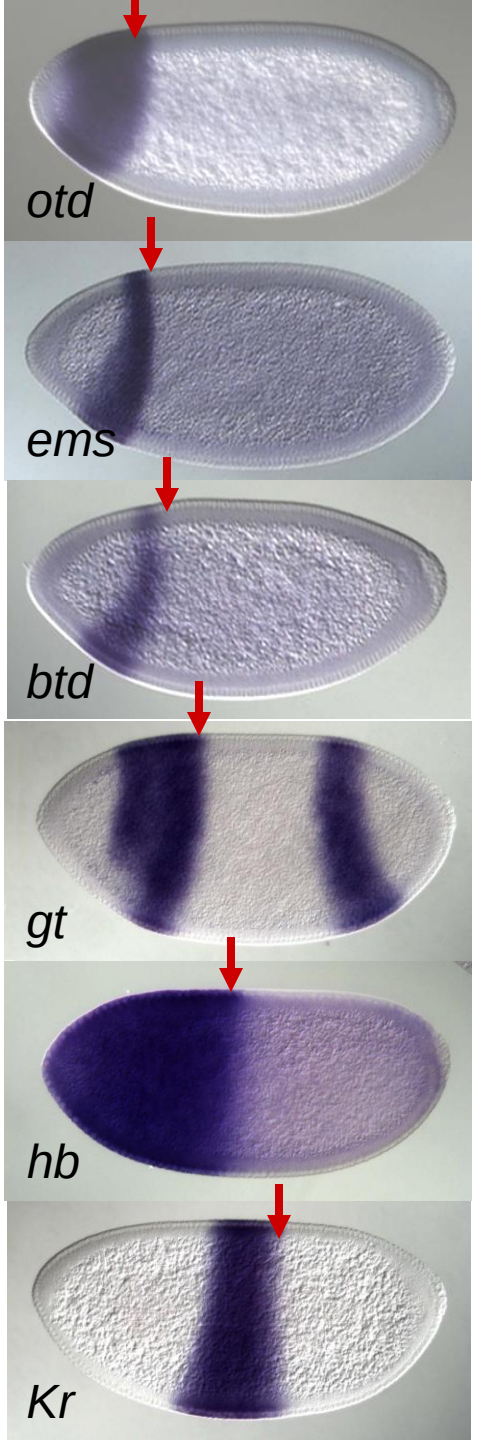
Therefore, the absolute positioning of this boundary is **not** critical for normal development.

Struhl et al., 1989

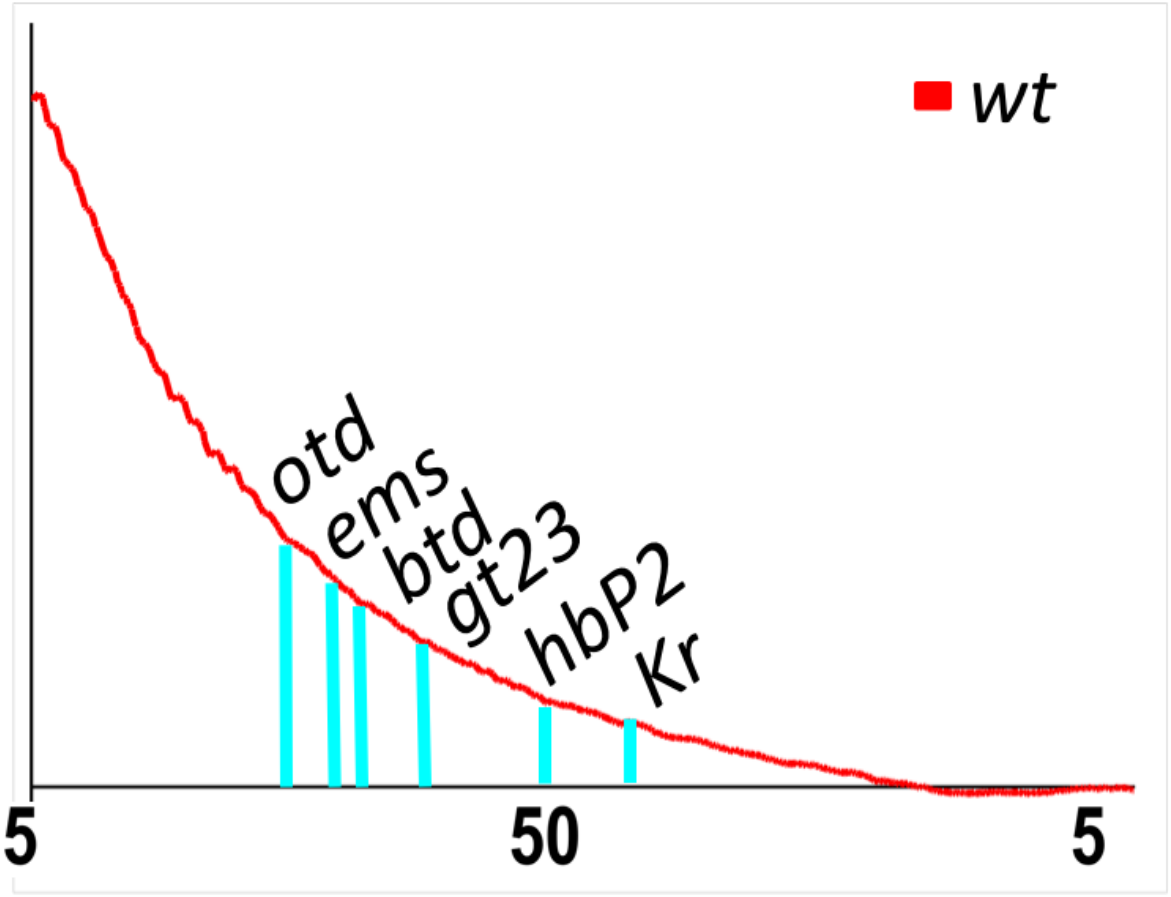
The relative positioning
of multiple gene boundaries
TO EACH OTHER
is most important.





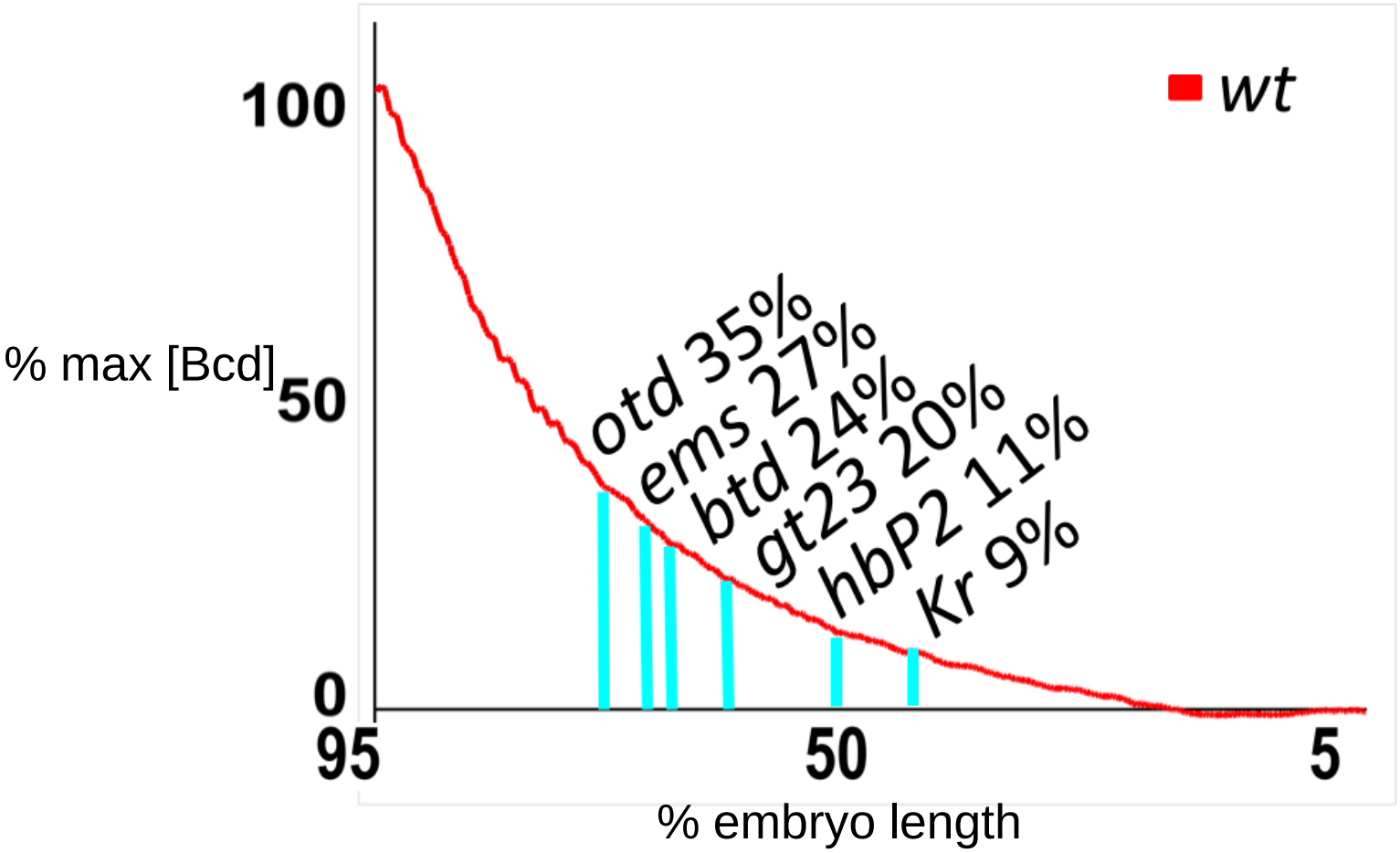


Posterior boundary positions in wild type embryos



Danyang Yu

Relative [Bcd] at positions of posterior boundaries



Hypothesis: Specific levels of [Bcd] position on/off boundaries.

Danyang Yu

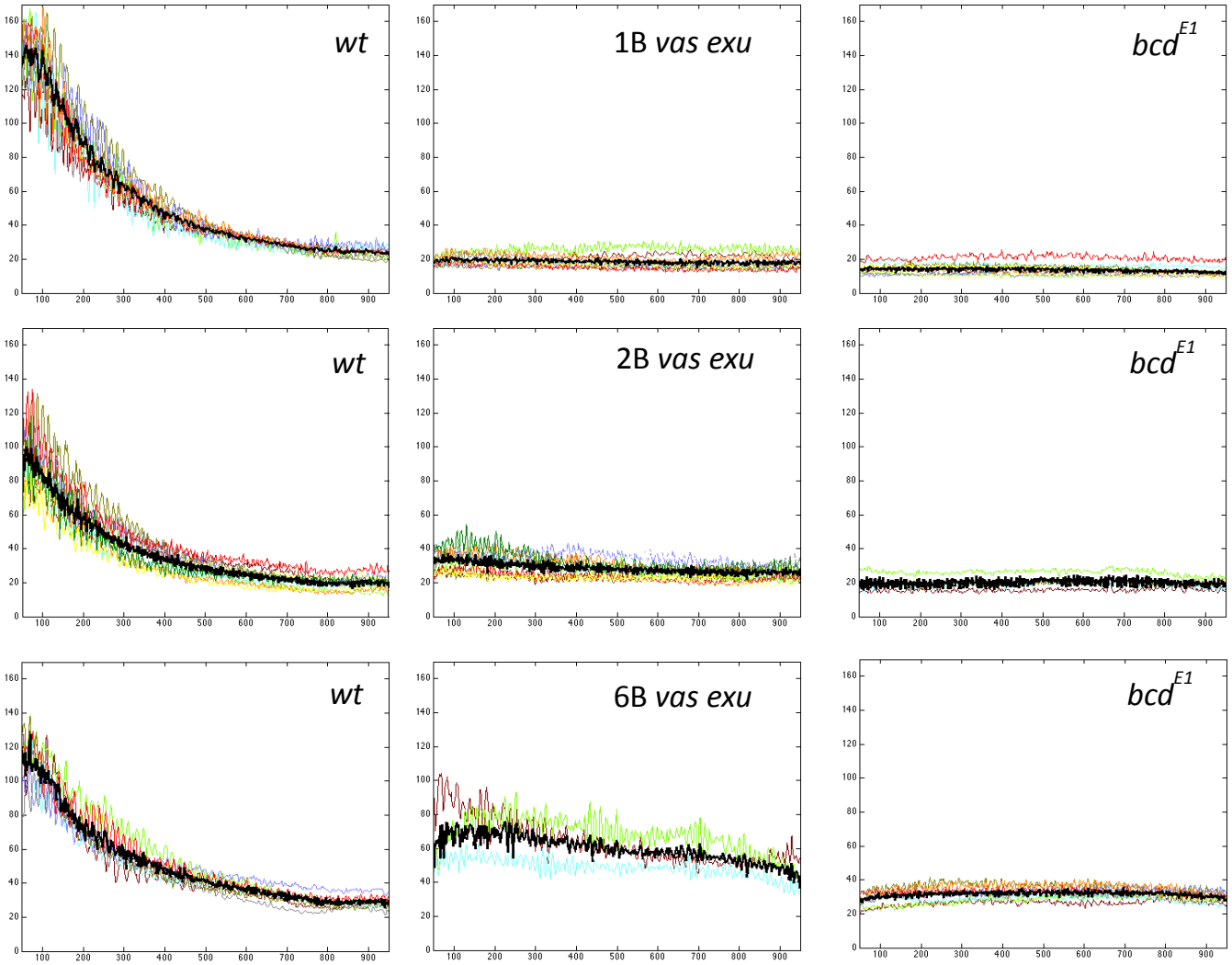
Genetic manipulations to “FLATTEN” the Bcd gradient

exuperantia (exu) -required for anchoring *bcd* mRNA
vasa (vas) -no translation repression from the posterior

Use a *bcd* rescue transgene to generate embryos with different levels of “flat” Bcd.

Amanda Ochoa-Espinosa

Raw data from one experiment



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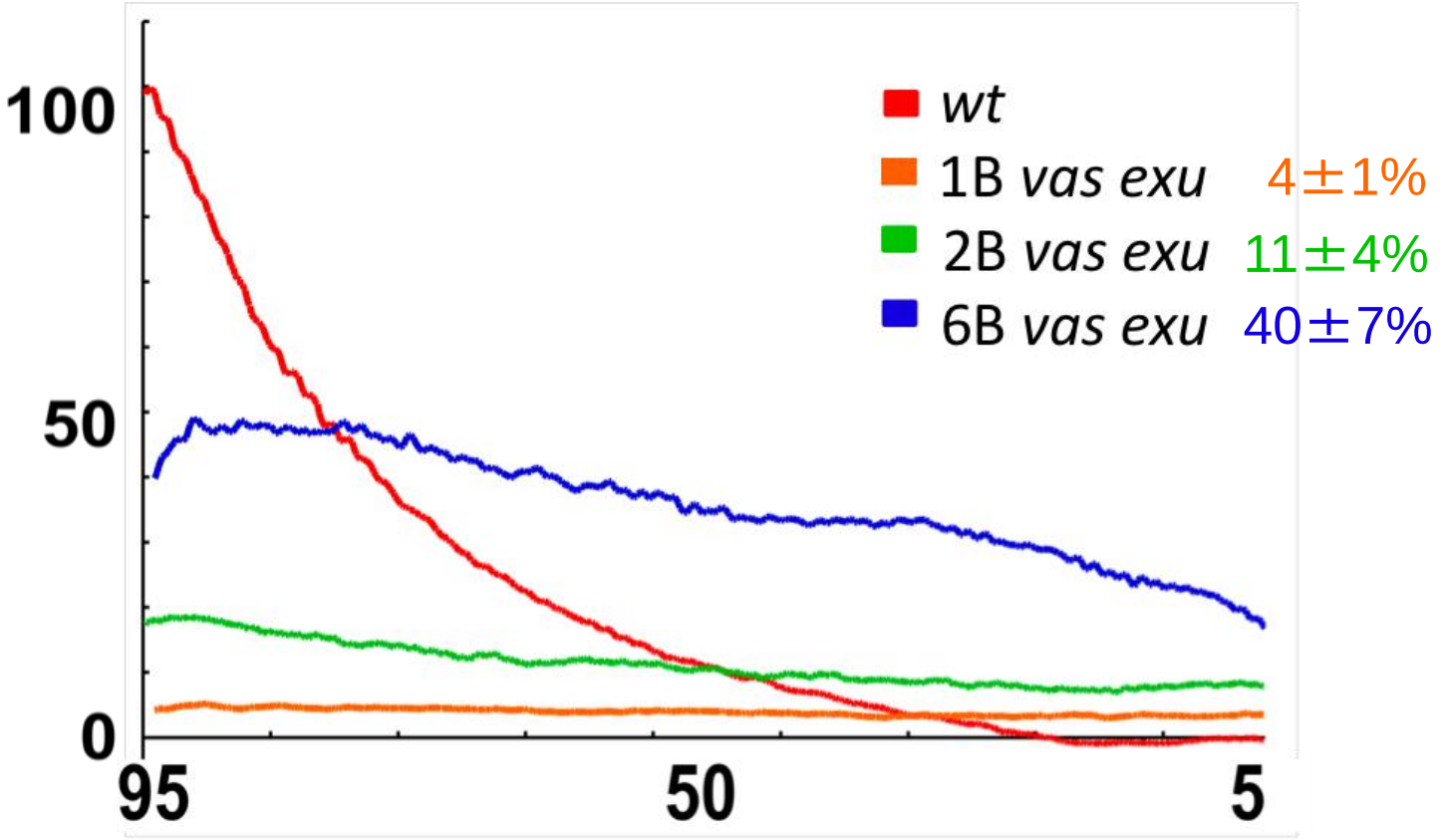
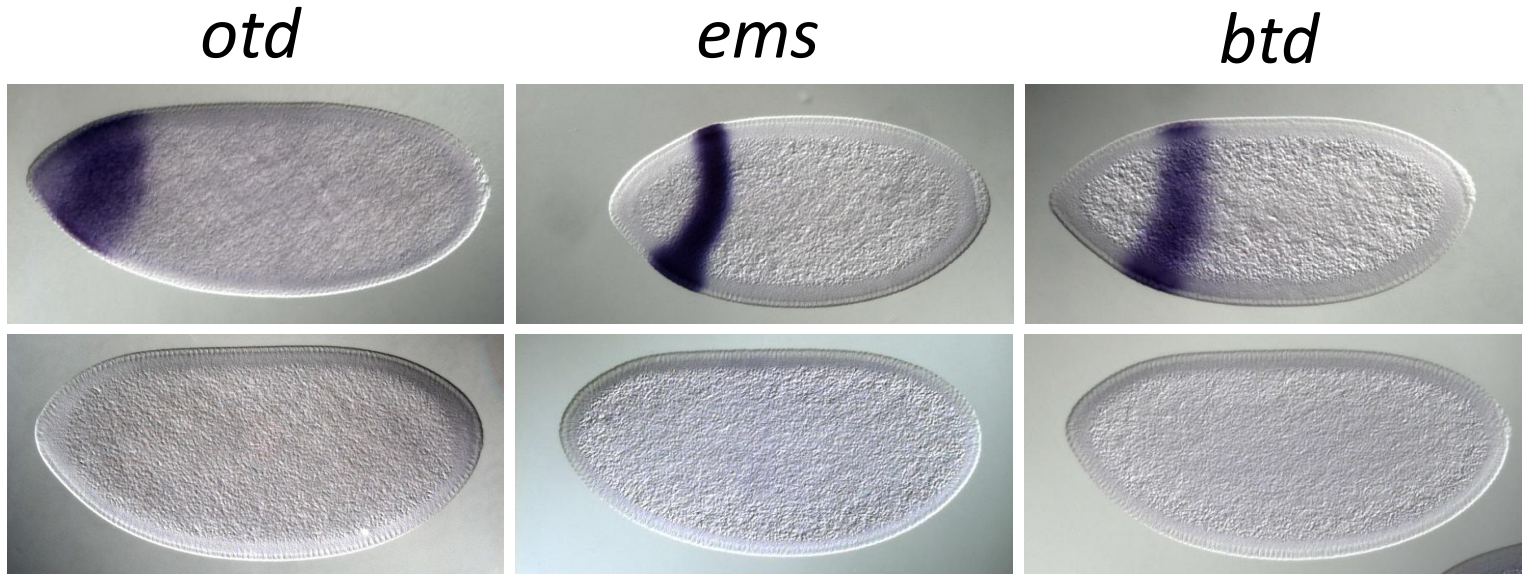
Average expression profiles of flattened Bcd gradients

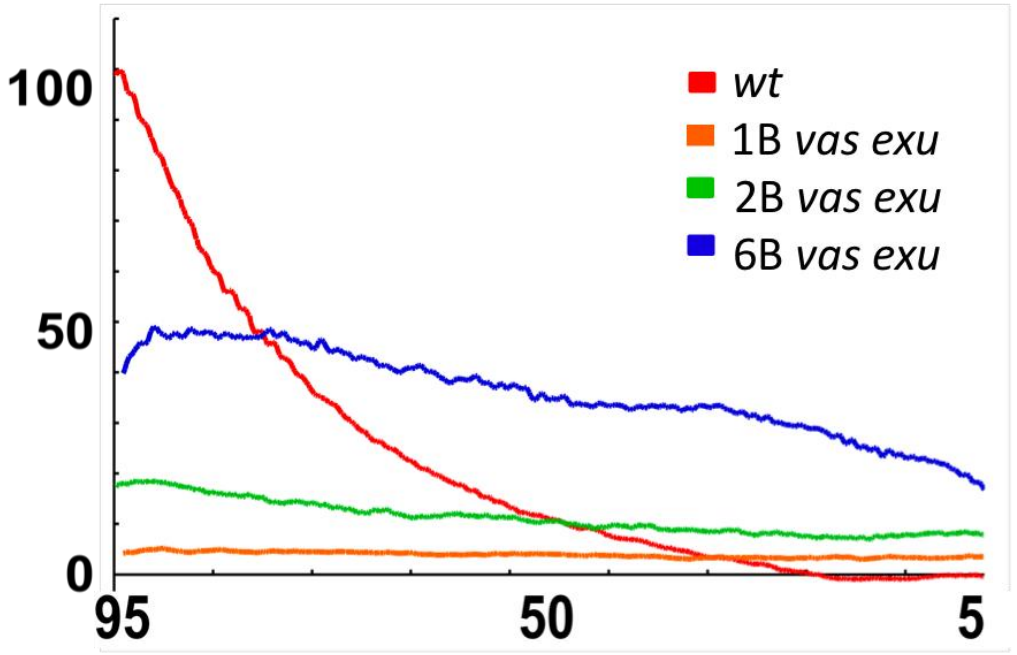


Danyang Yu

Activation of head gap genes by flattened Bcd gradients

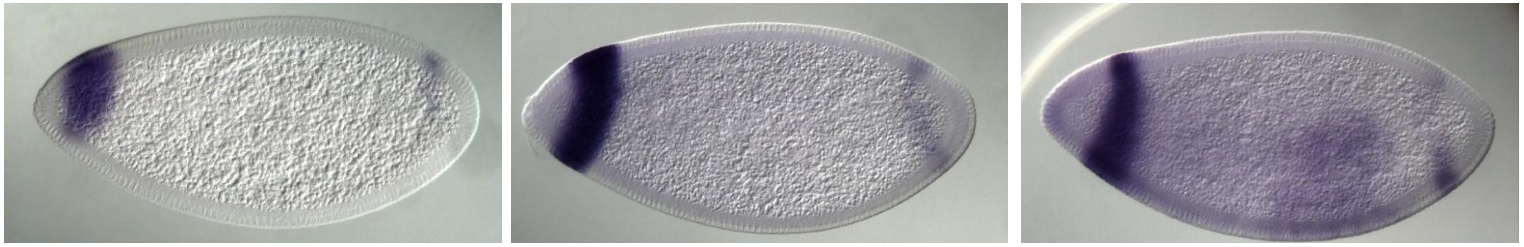


Activation of head gap genes by flattened Bcd gradients

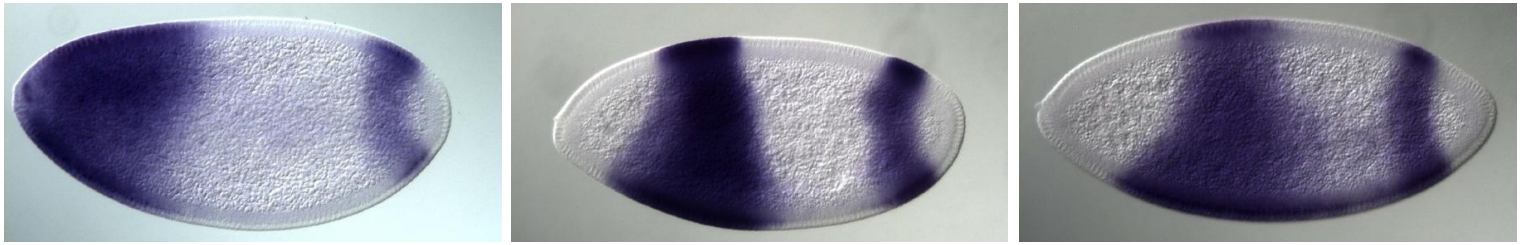


otd *ems* *btd*

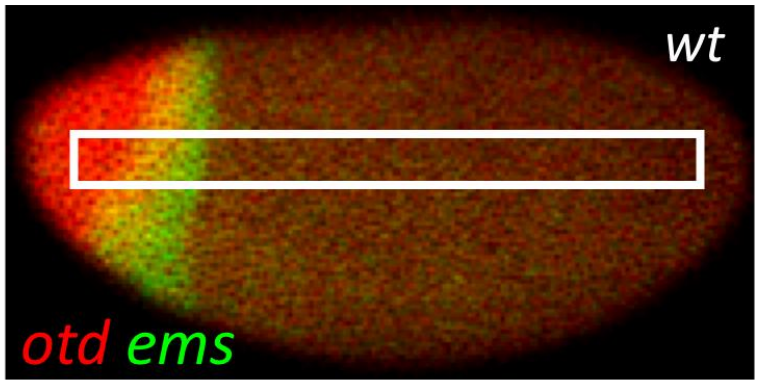
vas exu 2B

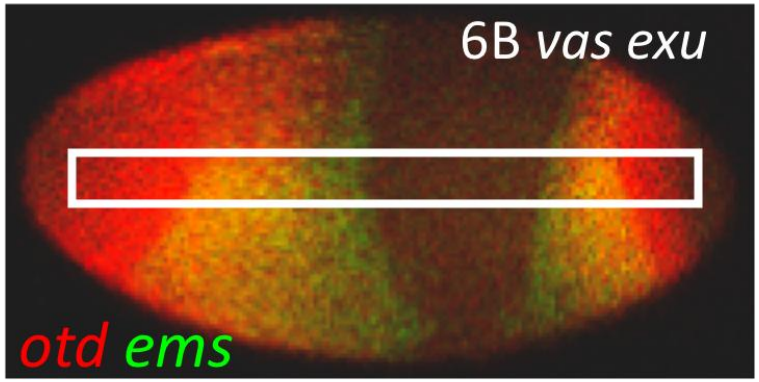


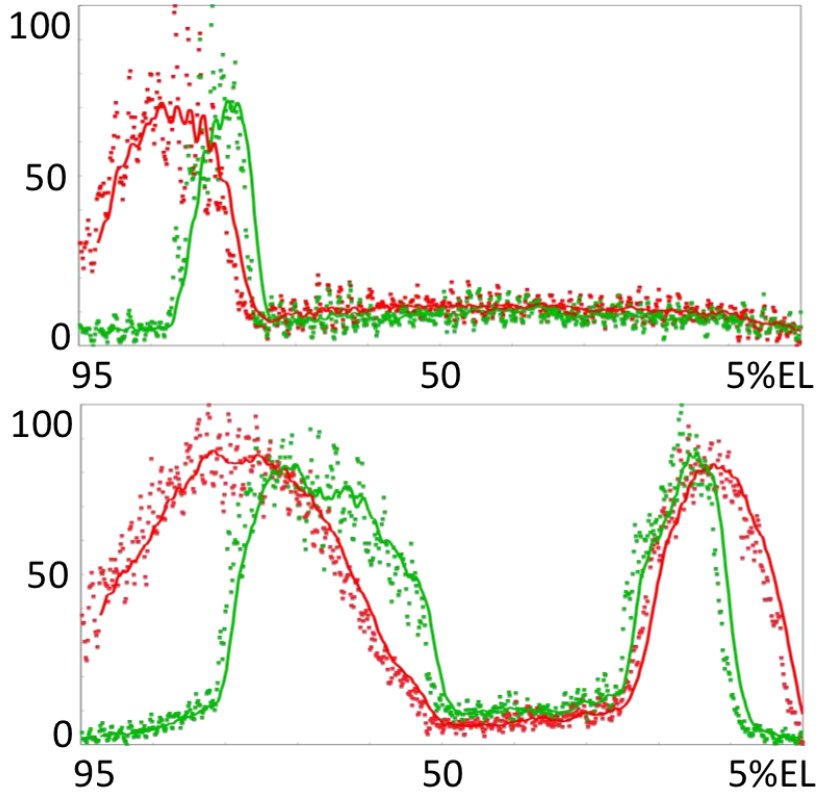
vas exu 6B



Head gap gene boundaries are correctly placed in embryos with flattened Bcd gradients. Posterior stripes are in **reverse** order.

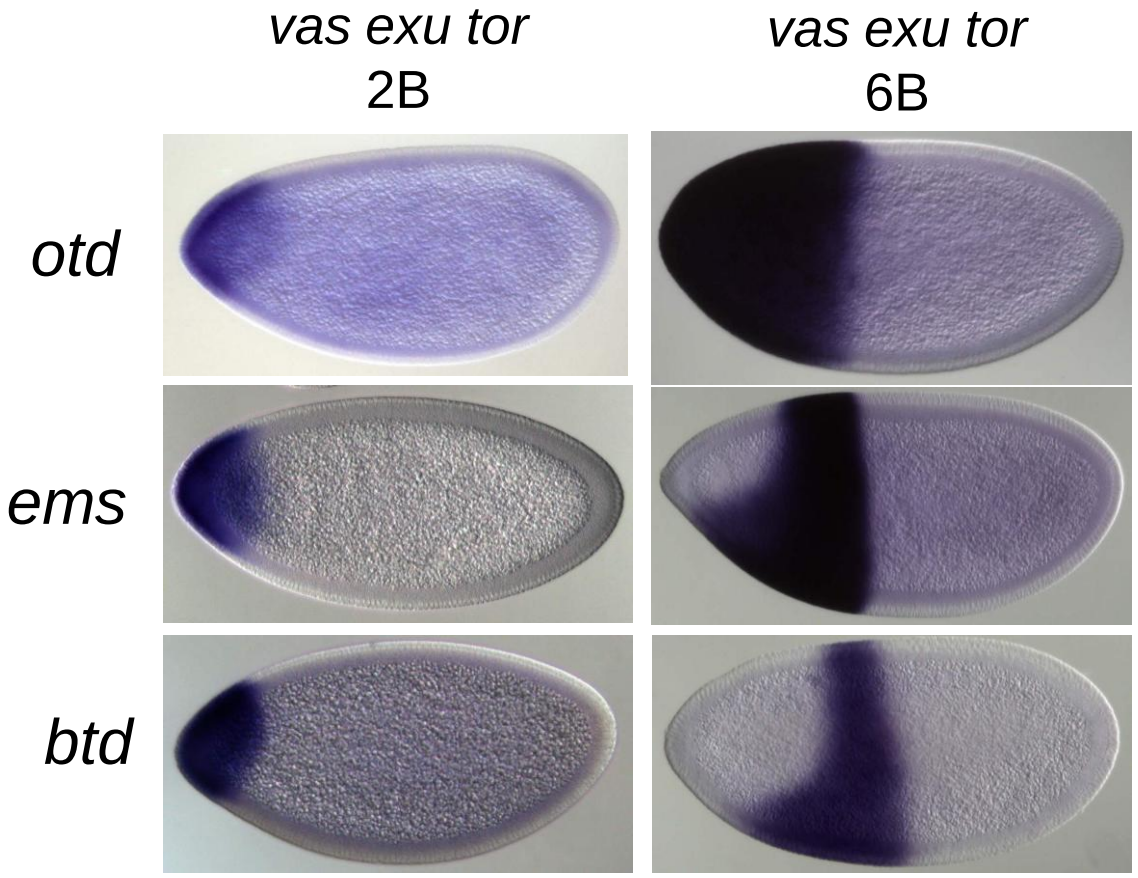






Is the terminal system involved?

Removing the terminal system does not prevent boundary formation.



Conclusion 1:
Neither a steep Bcd gradient nor the terminal system is required for making boundaries of the head gap genes.

All or none responses to flattened Bcd gradients

gt

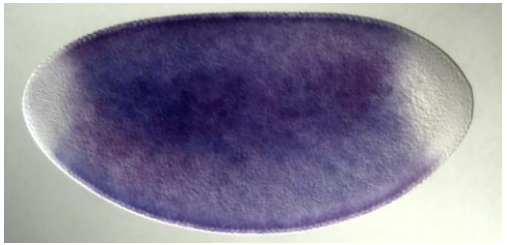
wild type

bcd-

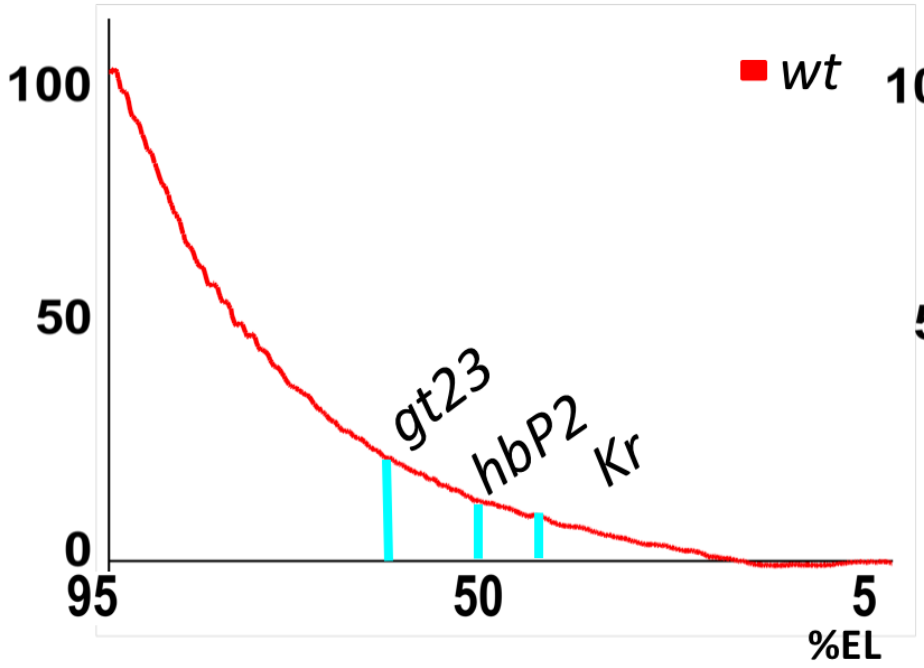
vas exu
1B

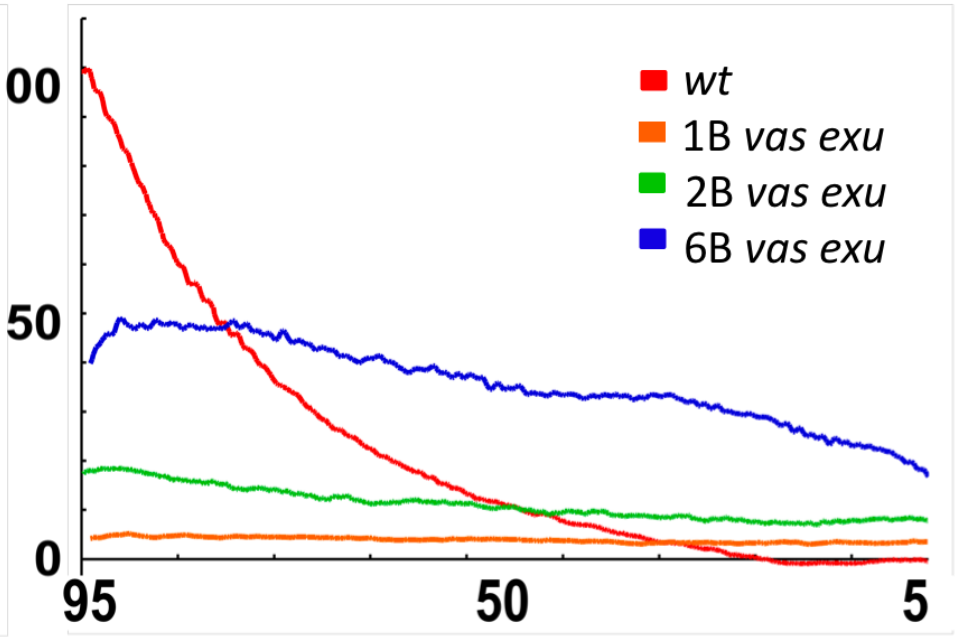
vas exu
2B

vas exu
6B

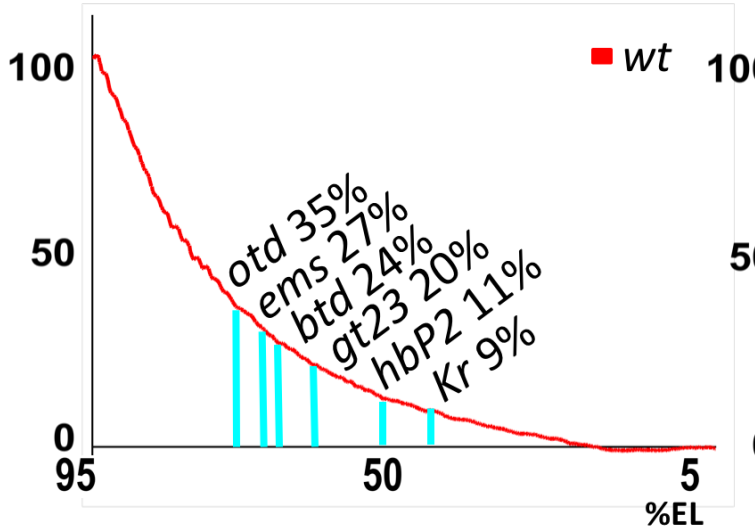


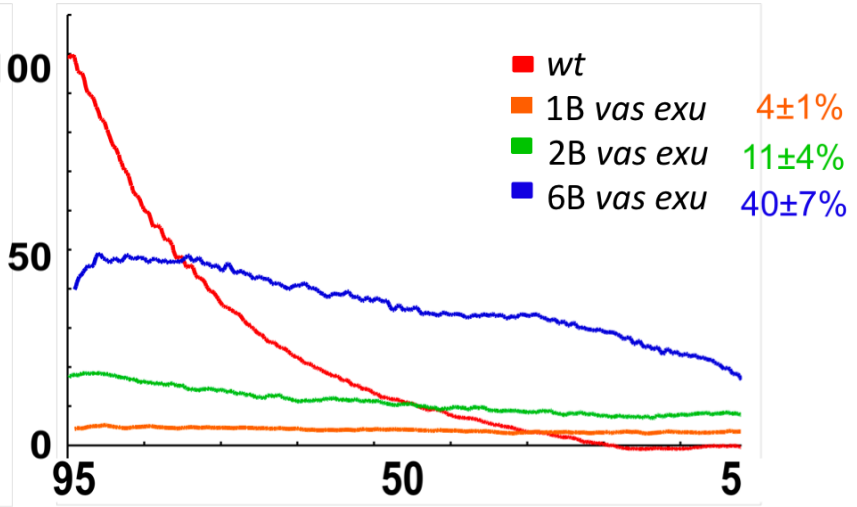
hb and *gt* require less Bcd for activation than the amounts predicted by the morphogen model.





Target gene:	PBP in wt:	Flat [Bcd] required for activation:
hb P2	11%	1B (4±1%)
gt 23	20%	2B (11±4%)



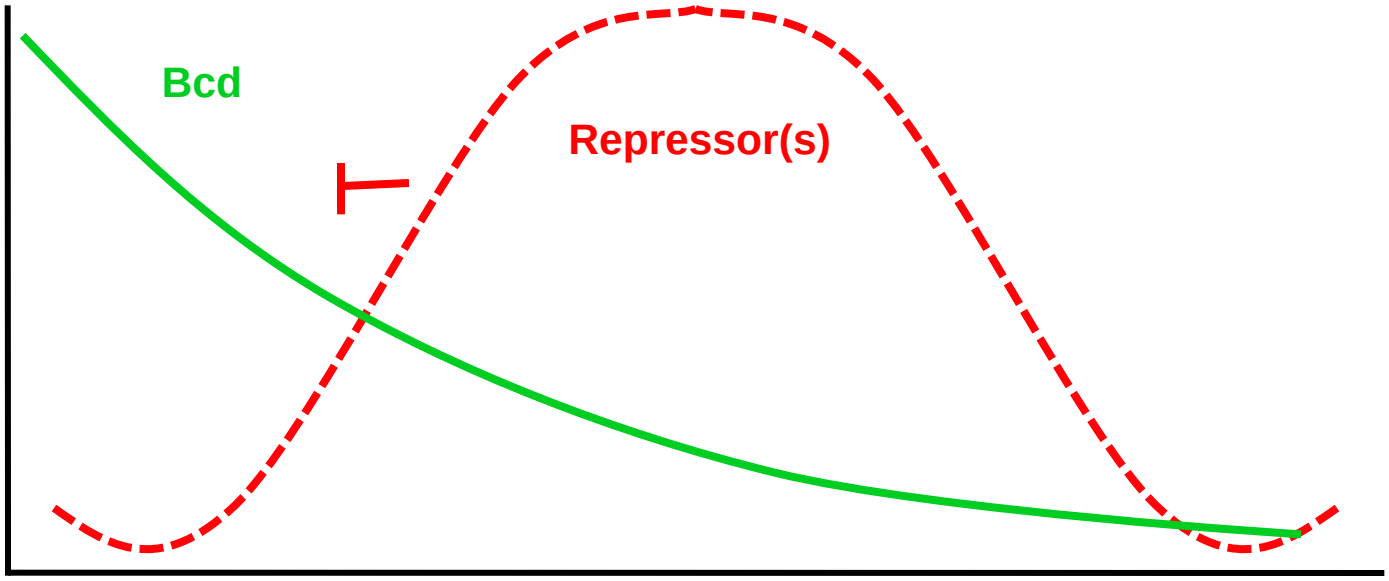


Target gene:	PBP in wt:	Flat [Bcd] required for activation:
Kr	9%	4%
hb P2	11%	4%
gt 23	20%	11%
btd	24%	< 20%
ems	27%	< 20%
otd	35%	< 20%

Conclusion 2:

The Bcd concentrations required for activation are less than the amounts predicted by the morphogen model.

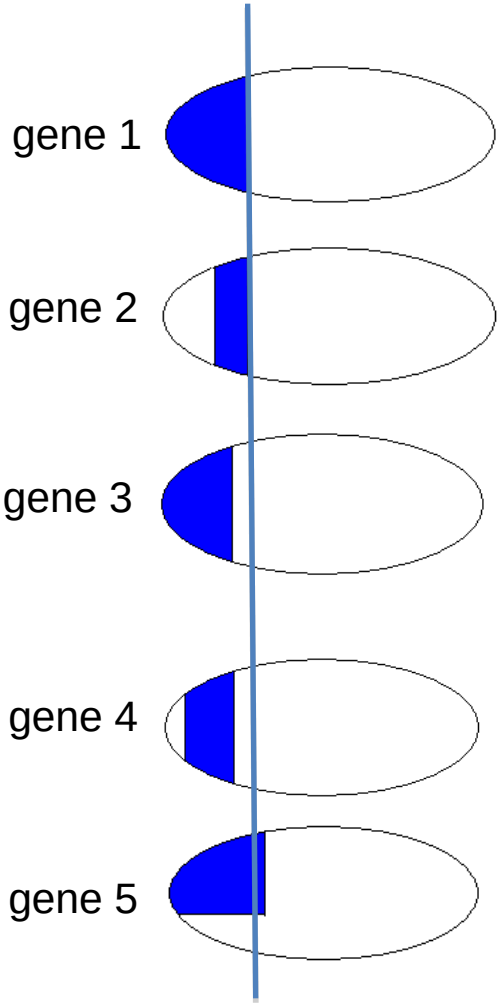
Hypothesis: Repressors limit Bcd-dependent activation



How do we find the repressors?

A method for finding repressors:

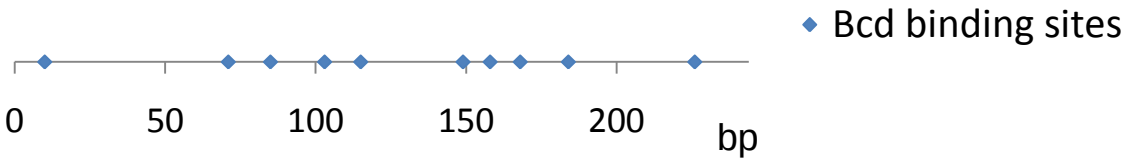
Genes expressed in similar patterns might share common regulatory motifs.



First step:
Find lots of enhancers

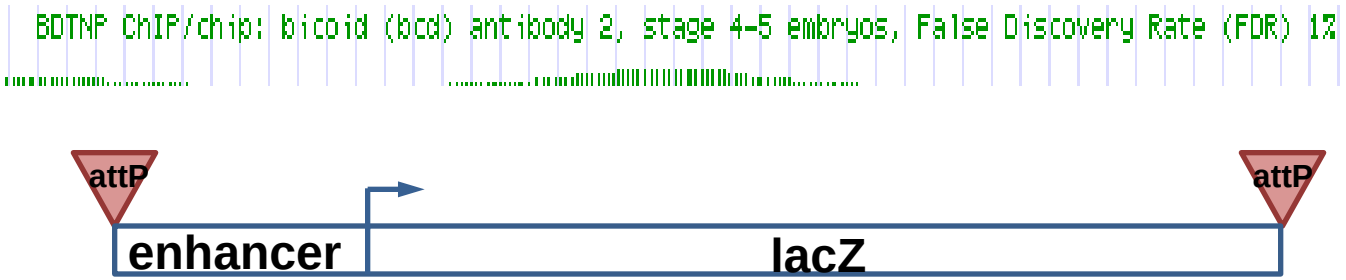
Identifying Bcd dependent enhancers: two methods

Bcd binding site cluster prediction



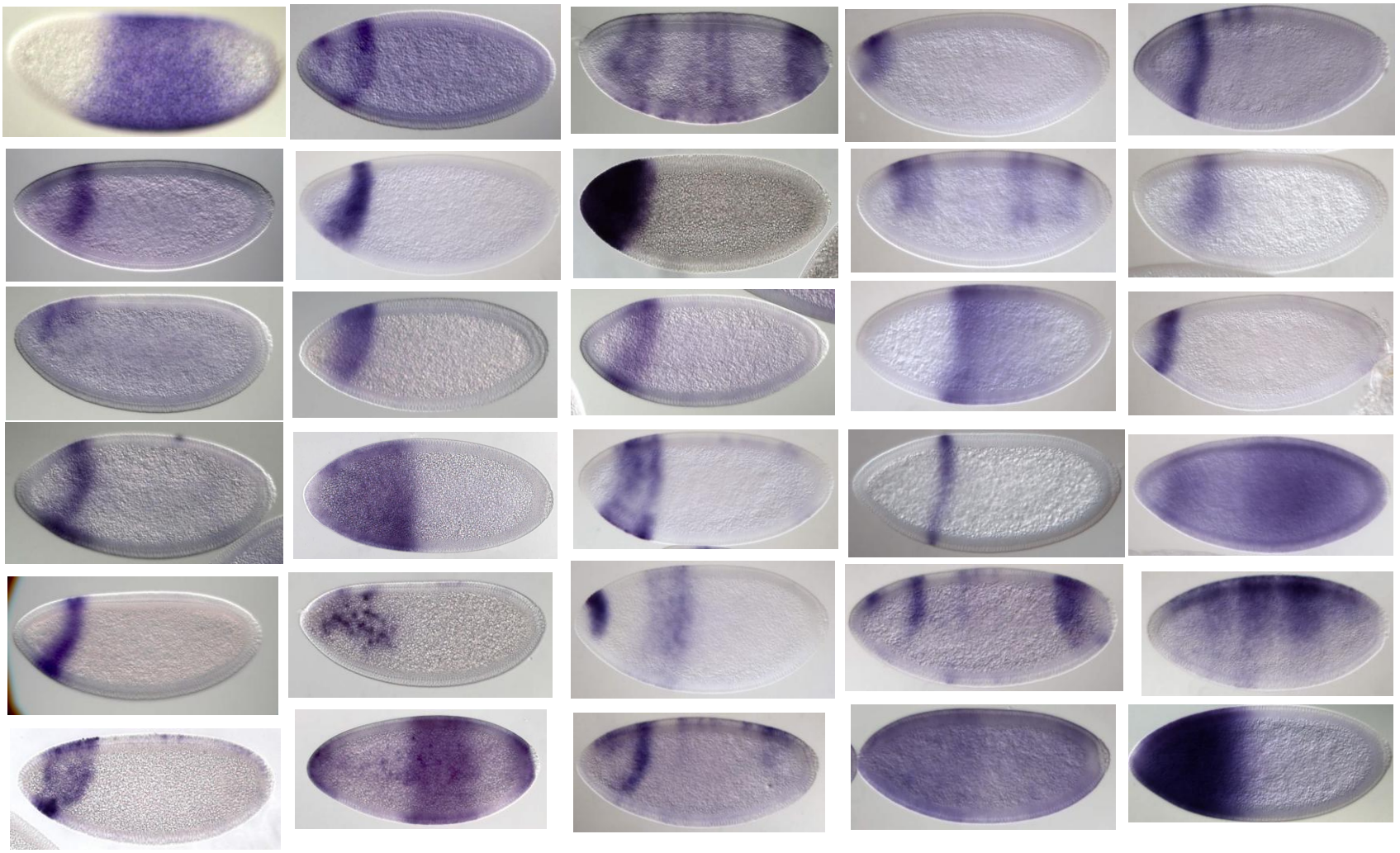
Bcd binding in vivo (ChIP/chip)

(Li et al., 2008)



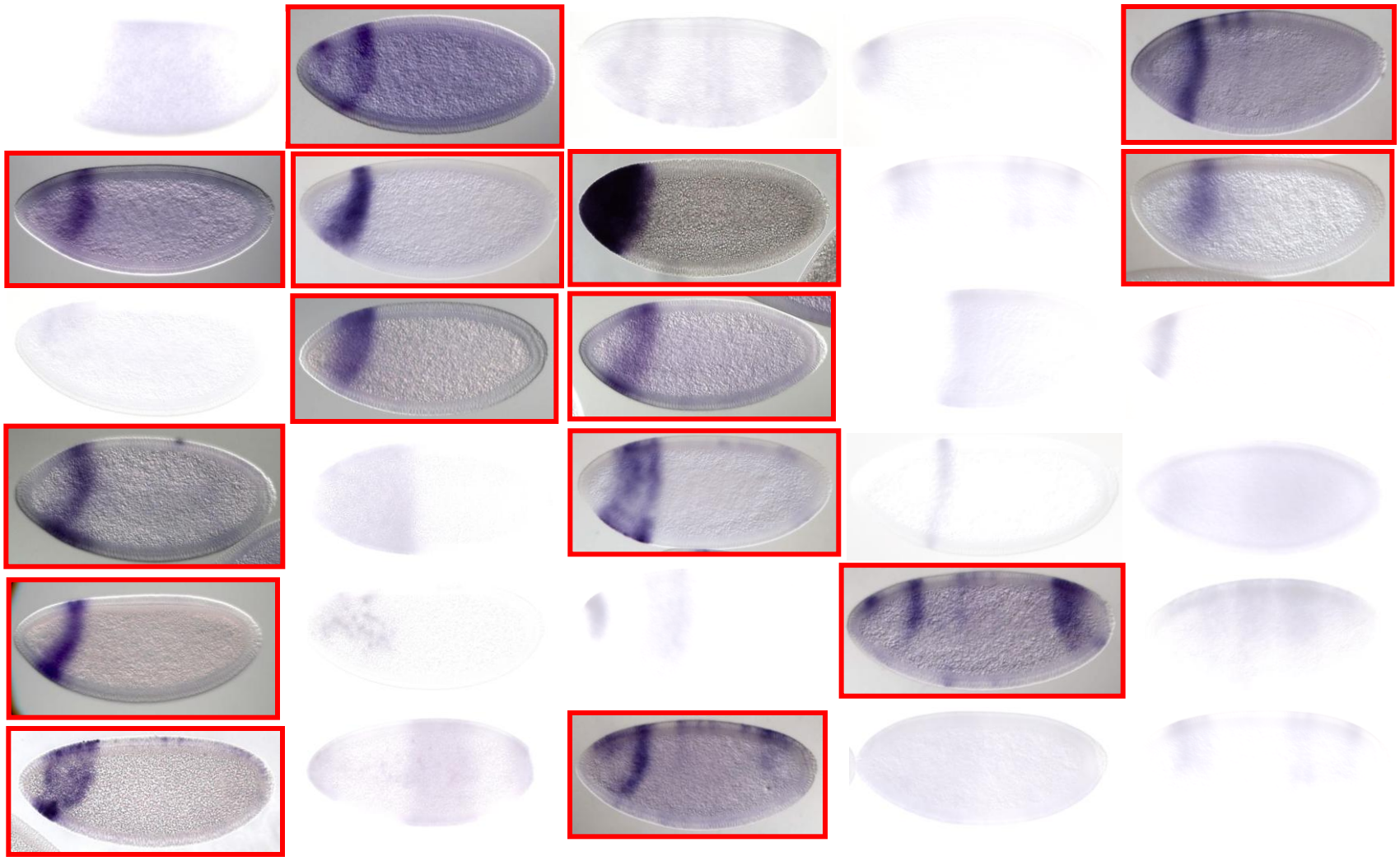
78 predicted enhancers were tested by reporter genes.

Thirty novel validated Bcd-dependent enhancers.

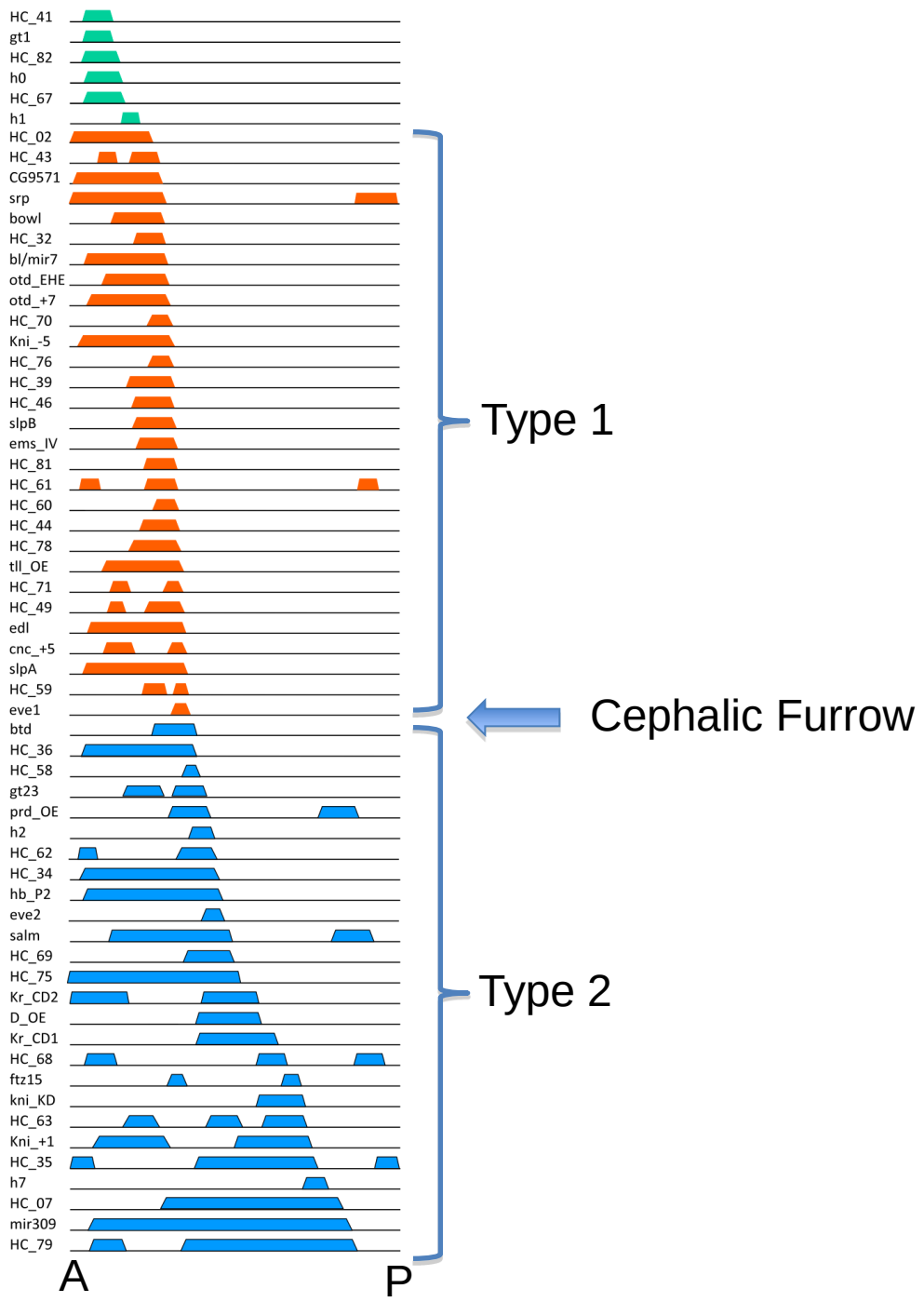


Total number of confirmed Bcd target enhancers: 58

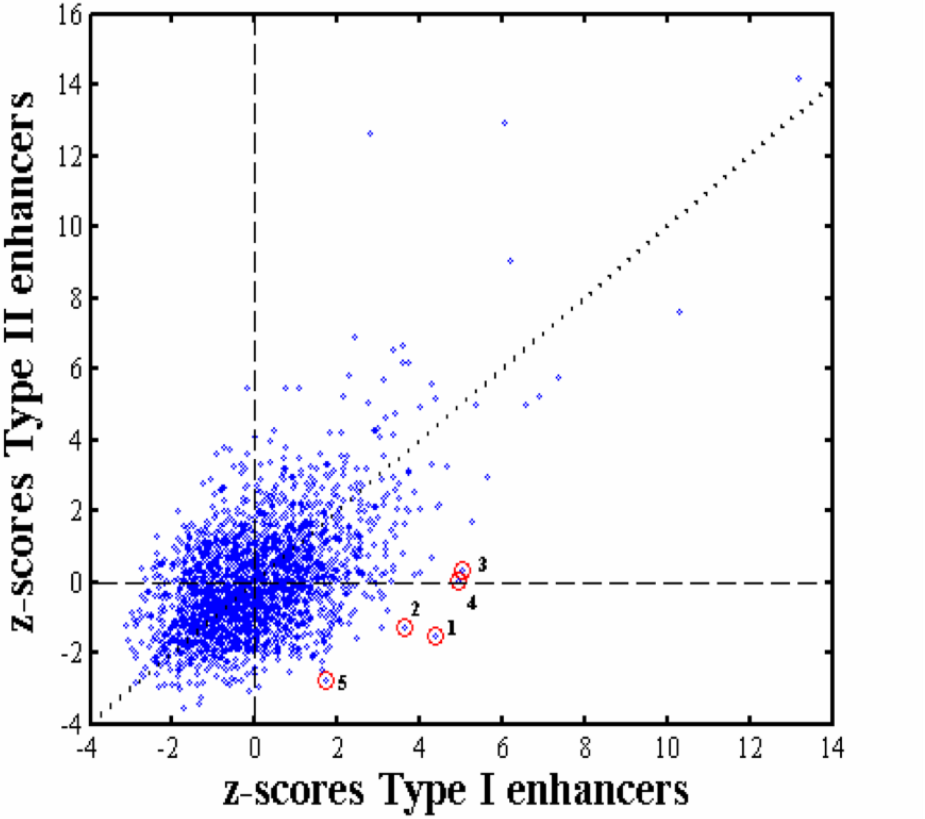
Fourteen enhancers with similar posterior boundaries.



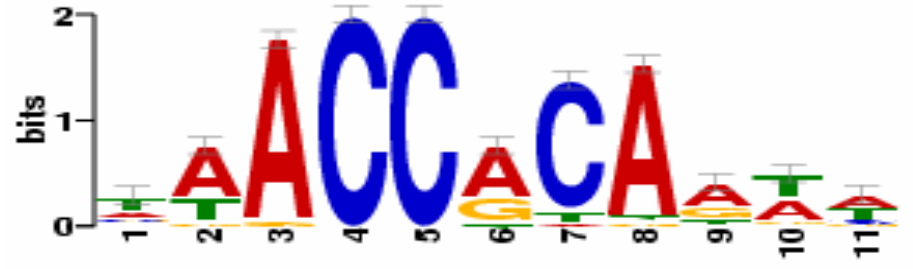
Summary of Bcd-dependent patterns



Searching for over-represented motifs

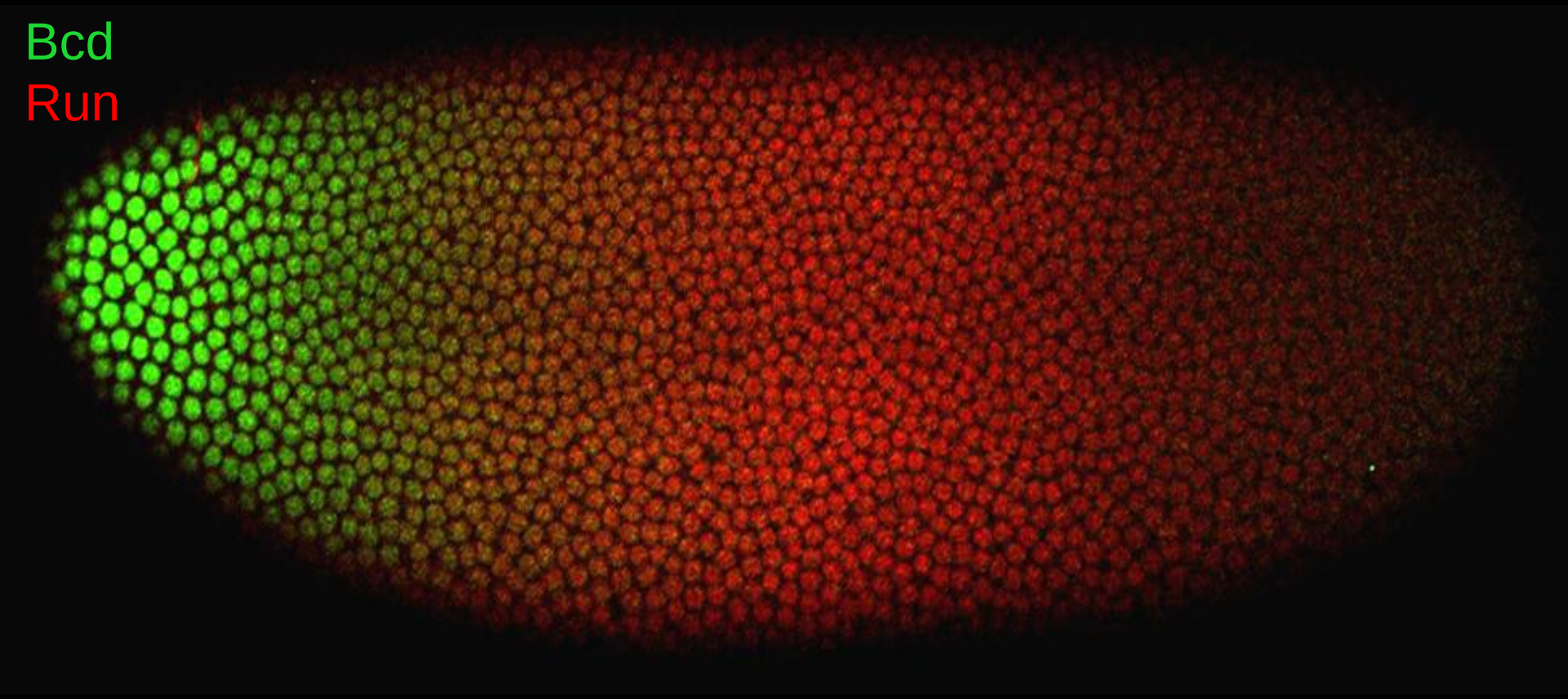


Matrix from the top 5 over-represented sequences:



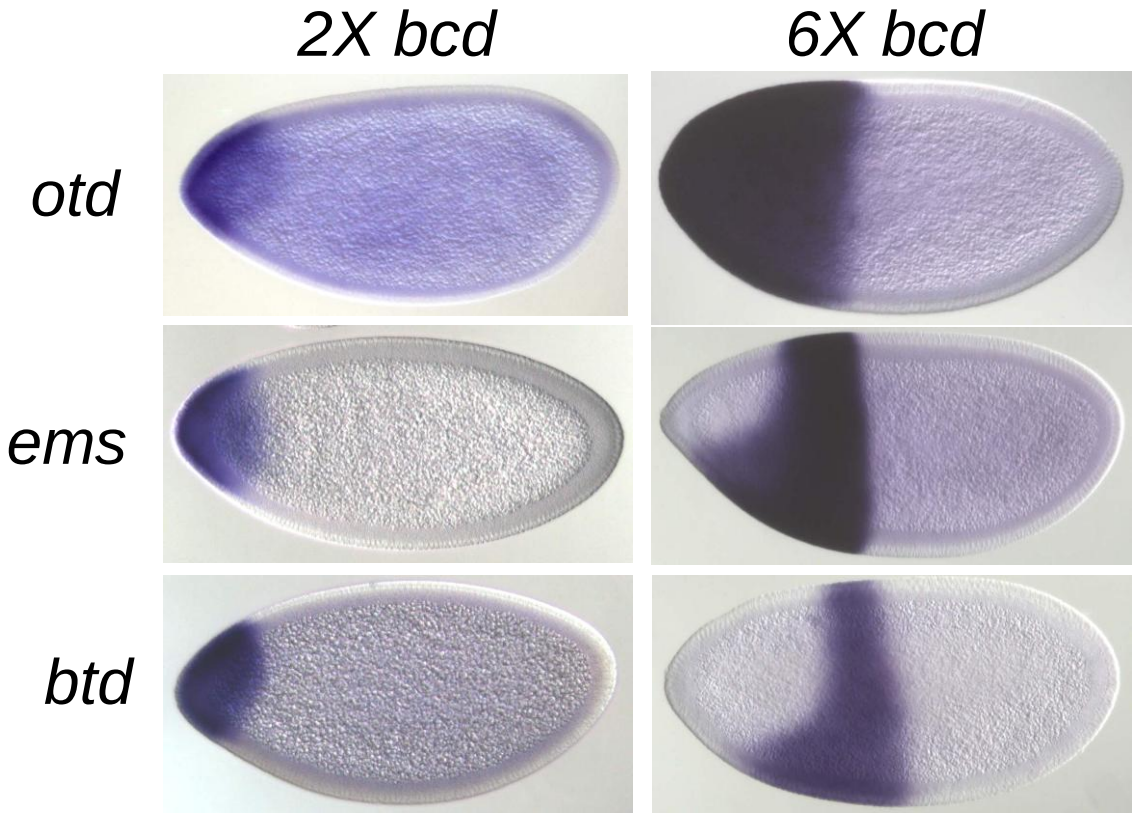
***Dm* Run::Bgb**
(*p*-value 0.00017)

***Hs* Runx1**
(*p*-value 0.00039)

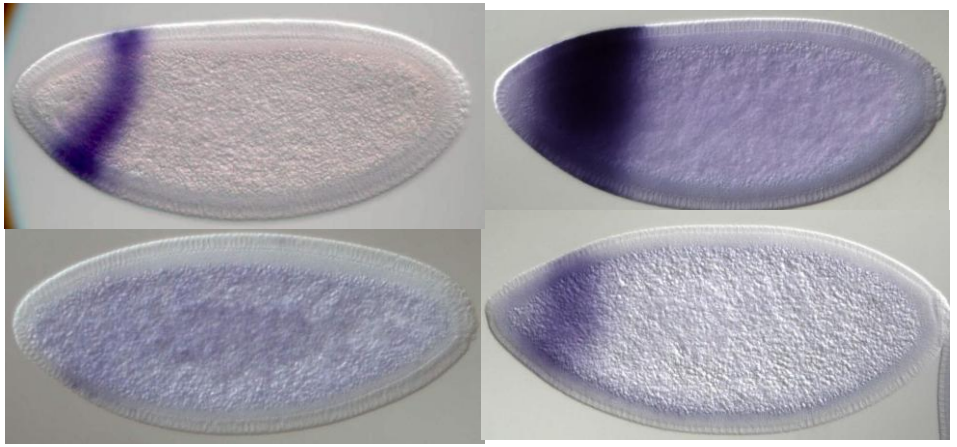


Hypothesis: Repression by Run limits Bcd-dependent activation.

vas exu tor embryos



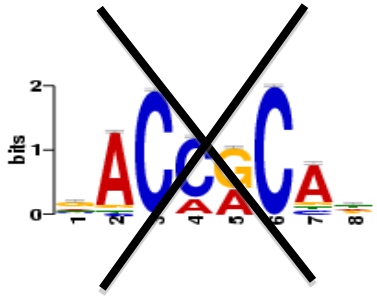
Overexpressing Run represses enhancers that contain the motif.

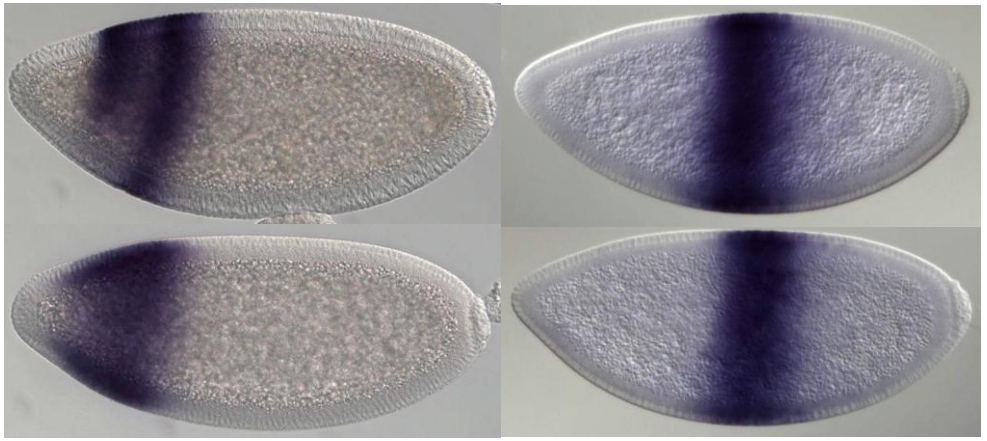


wt

heatshock
run

#32 #11(slpA)





wt

heatshock
run

gt 23 *Kr* CD1

wt

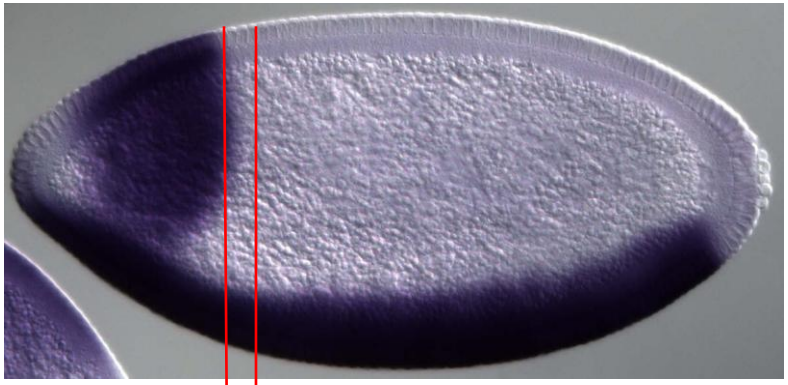
hb P2

45.1 ± 2.7 n=135

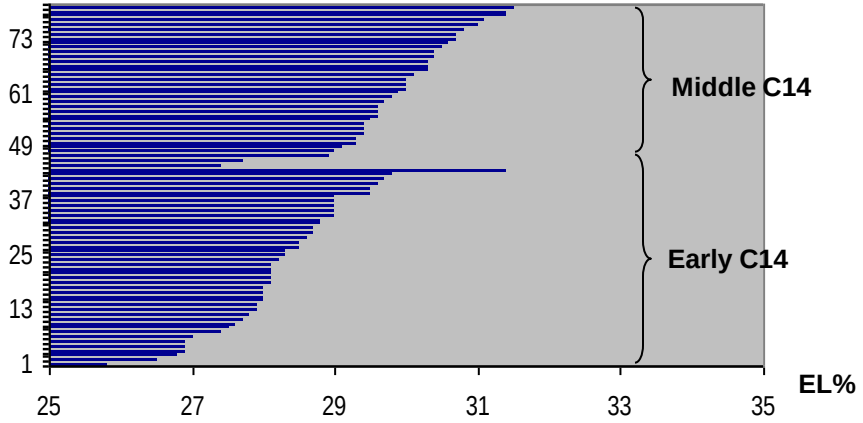
This is artificial.

Removing Runt expands expression posteriorly, but only a little bit.

run^{+}
yw

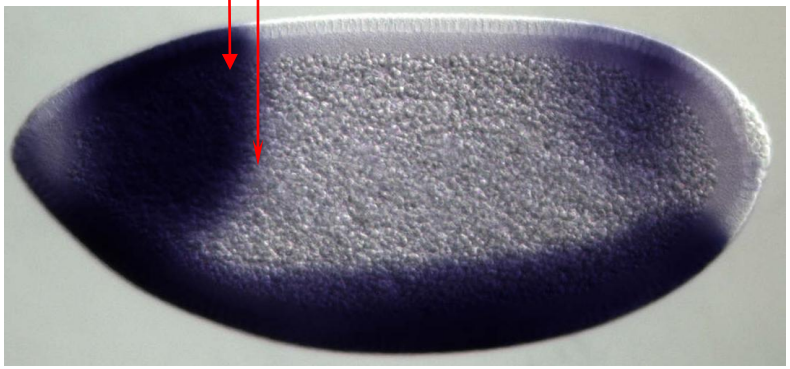


otd

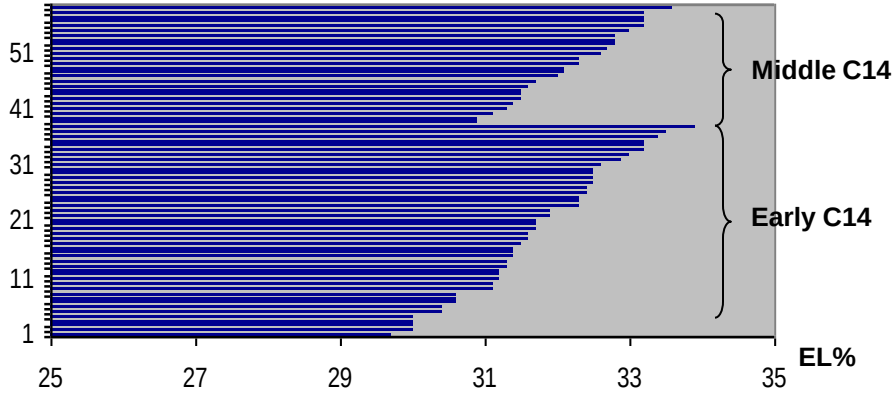


n=80, μ = 29.0, σ^2 = 1.61

run^{-}
M152



otd



n=59, μ = 31.9, σ^2 = 1.01

3% EL shift

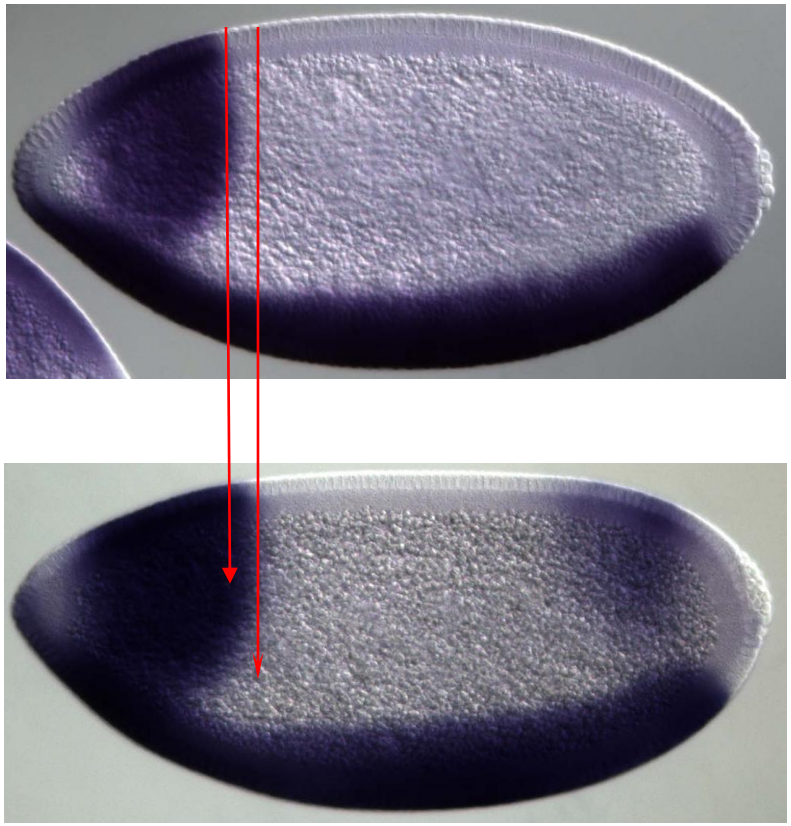
Why does otd shift only 3% EL in run$^-$ embryo?

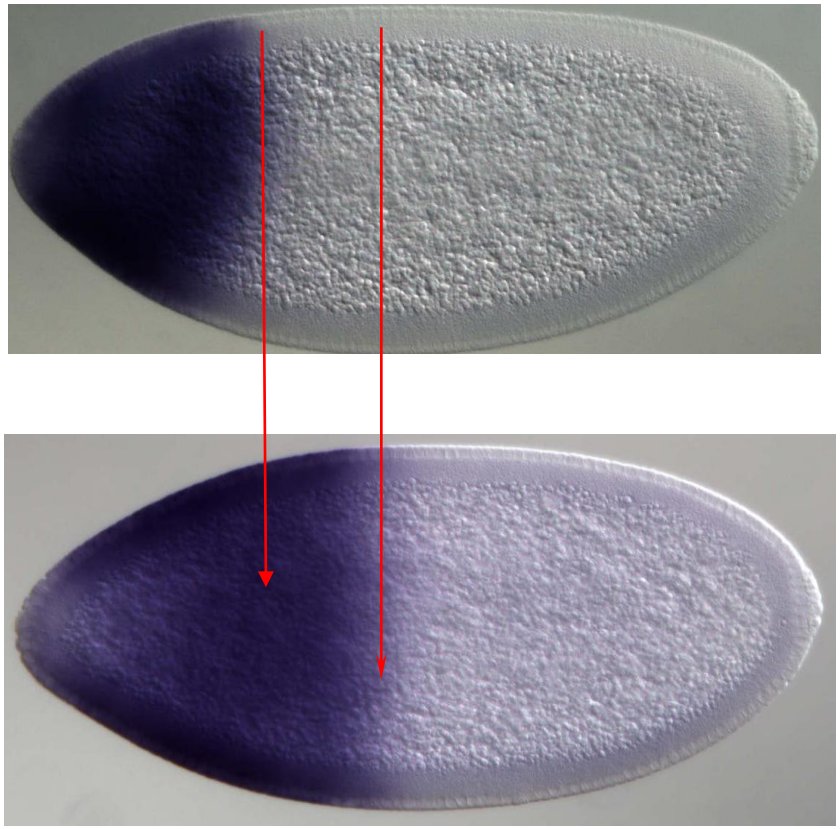
otd

hb P2 + 3 runt sites

run$^+$
wildtype

run$^-$
no Runt

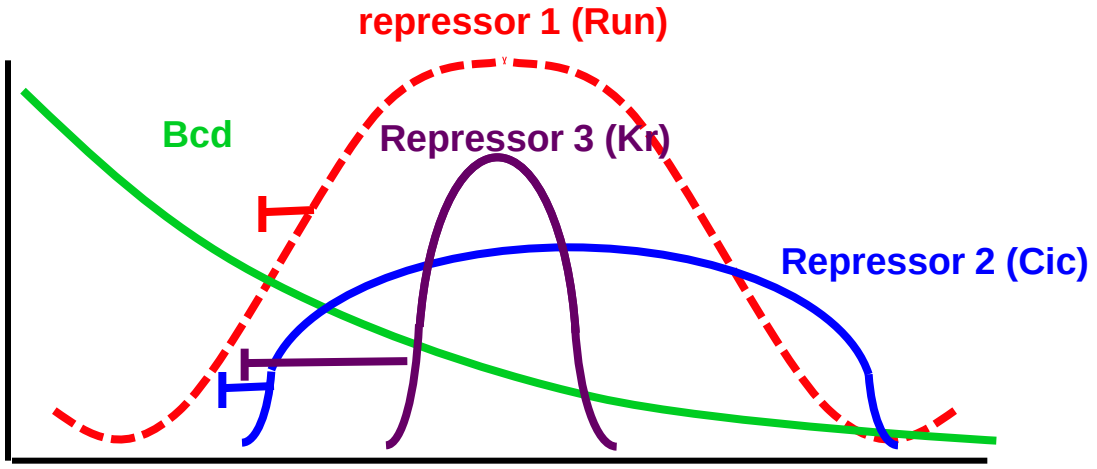


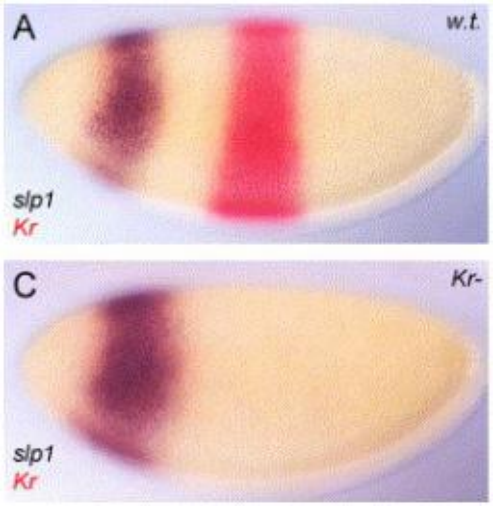


3% EL shift

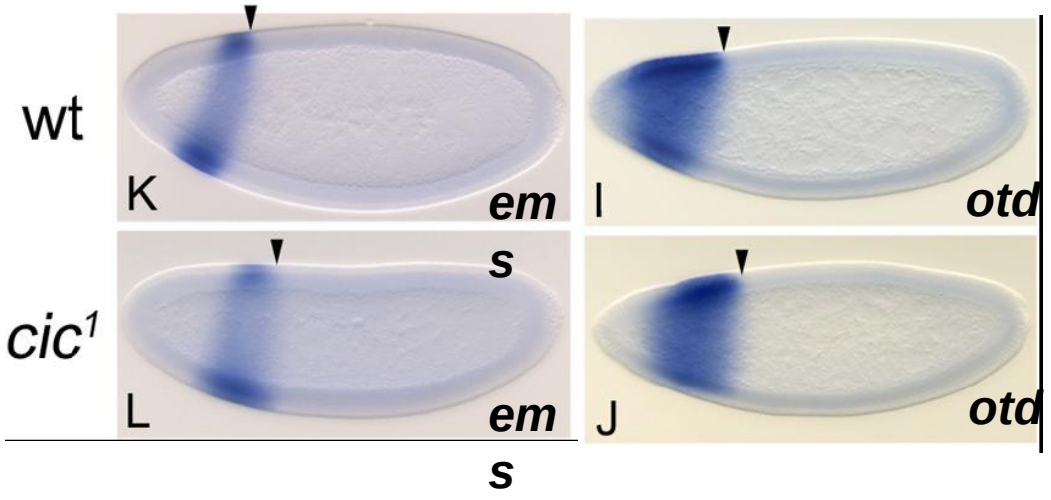
17% EL shift

Candidate repressors that interfere with Bcd activation.



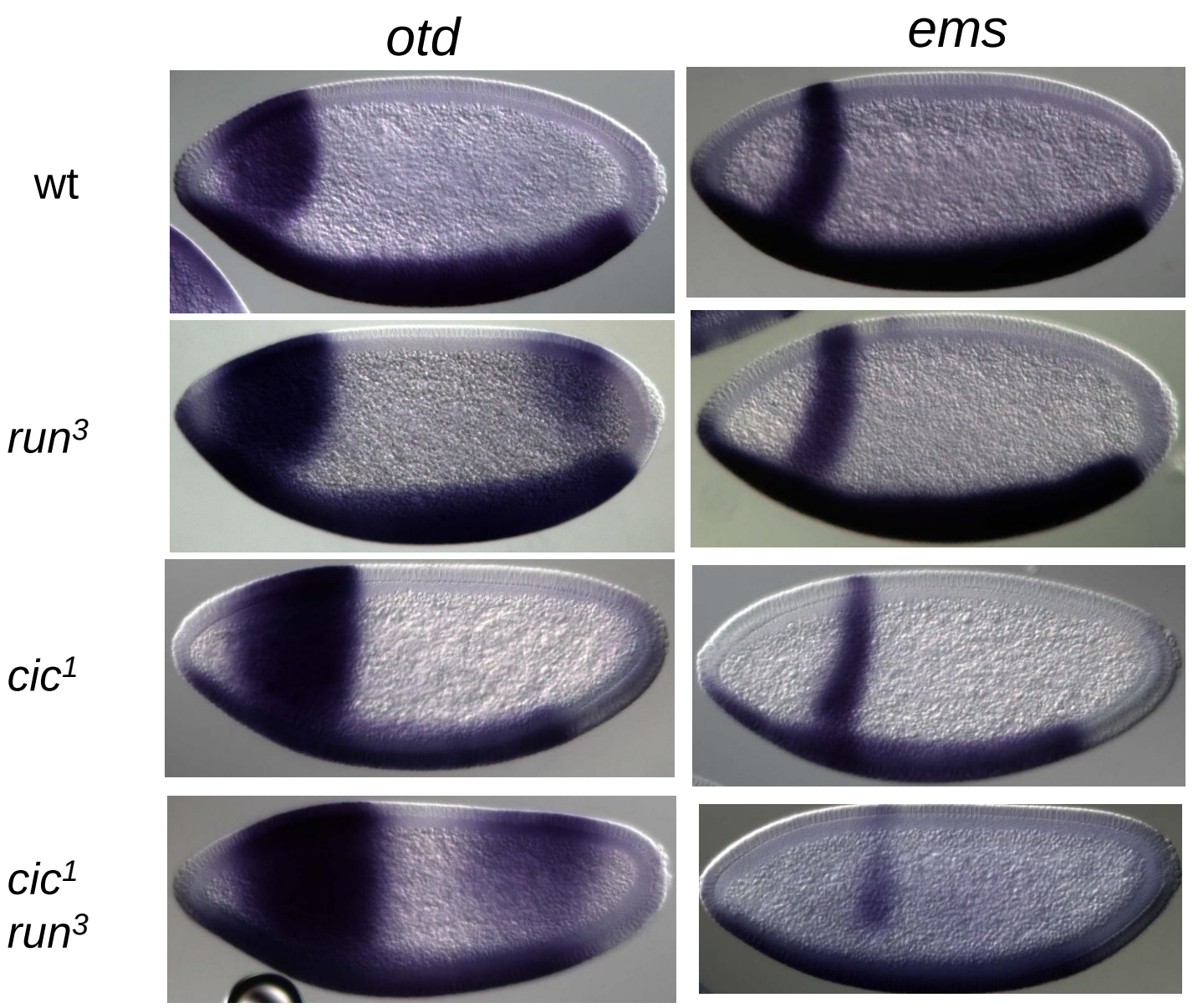


Andrioli *et al.*, 2004

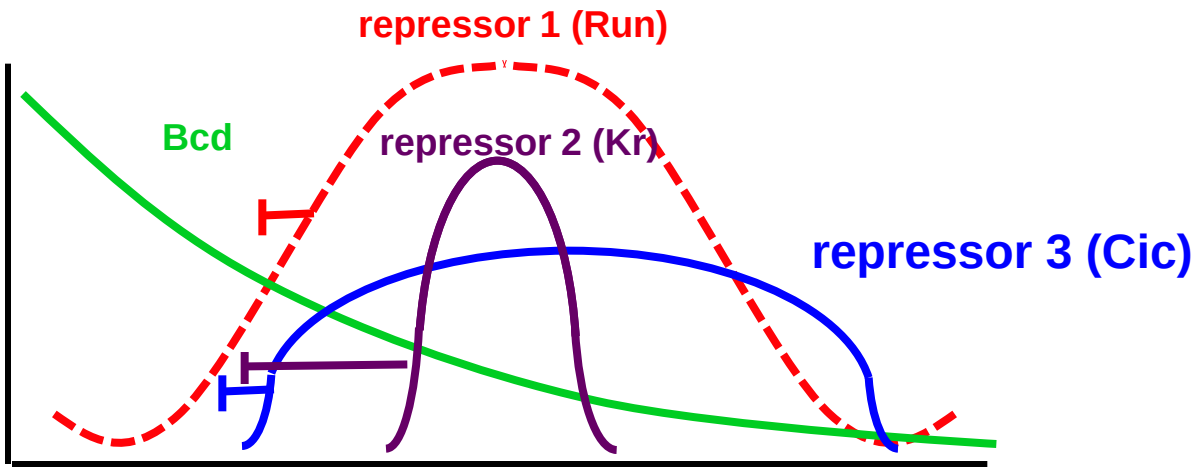


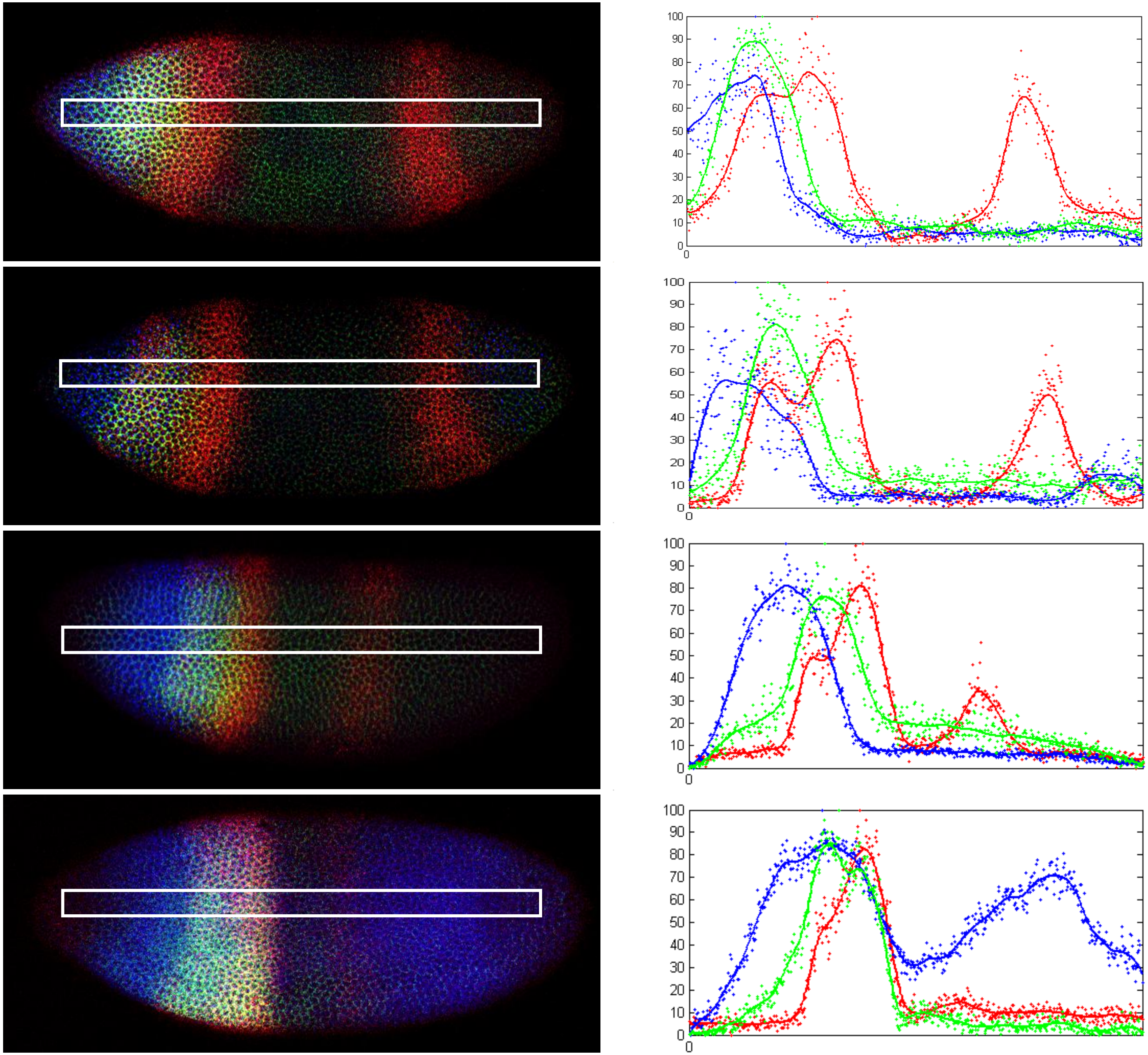
Lohr *et al.*, 2009

Removing Partially Redundant Repressors.



If we remove multiple repressors, can we collapse the proper registration of boundaries?




100
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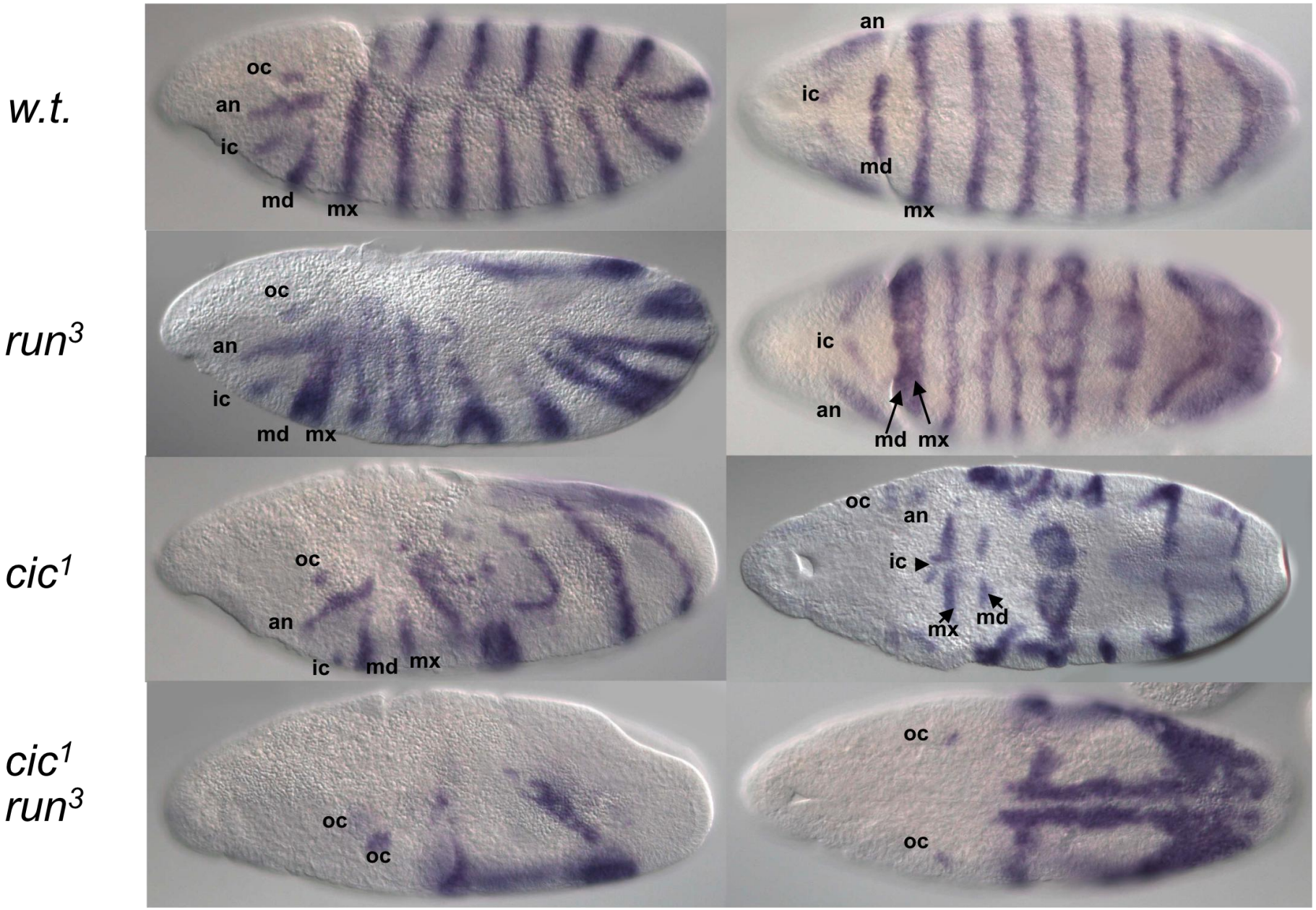
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oc
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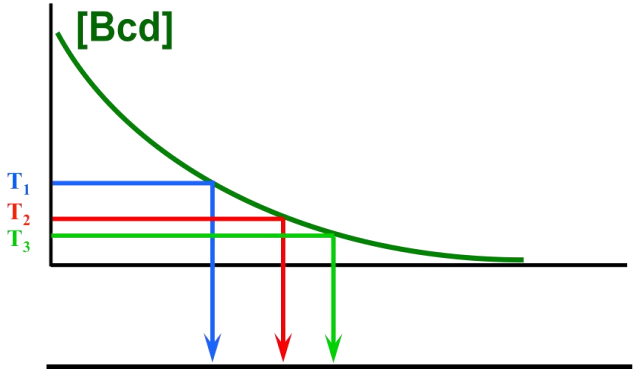
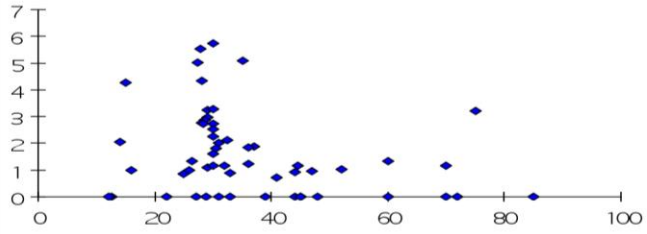
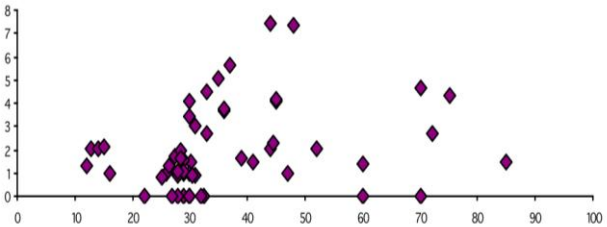
Repressor sites in Bcd-dependent enhancers



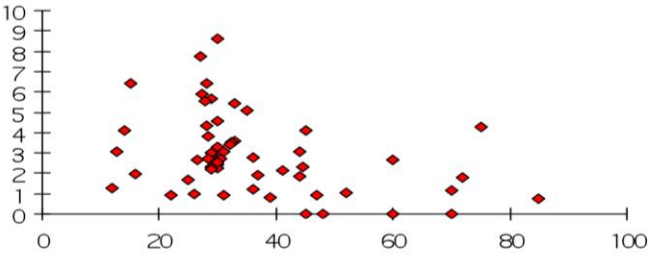
Run



Kr



Cic



Conclusions 3:

1. No target genes are patterned by Bcd alone.

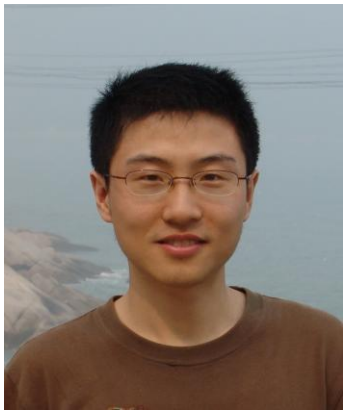
2. The Bcd gradient is part of a complex combinatorial system that integrates positive and negative inputs.

3. Boundary registration involves maternal and zygotic repressors that interfere with Bcd-dependent activation.

Acknowledgements

Bcd network

Amanda Ochoa-Espinosa



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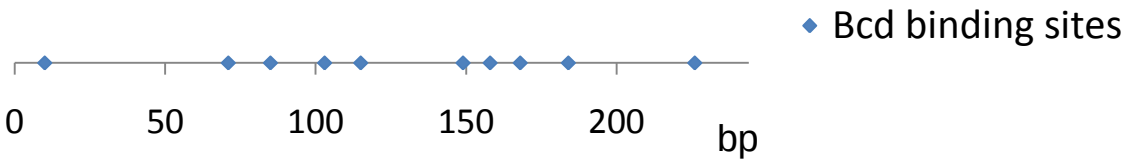
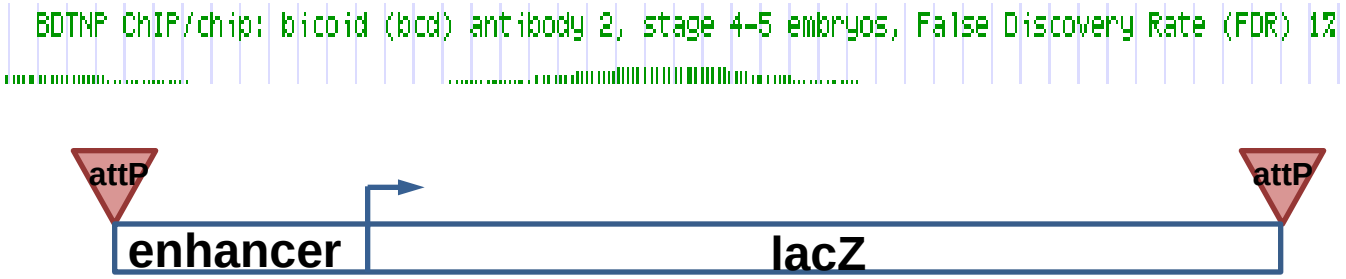
Identifying Bcd dependent enhancers: two criteria

Bcd binding site cluster prediction

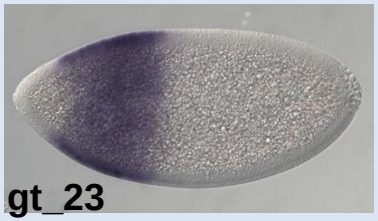
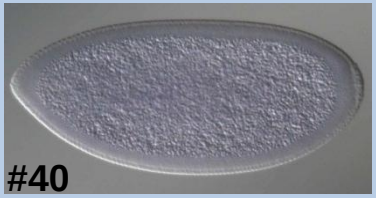


Bcd binding in vivo (ChIP/chip)

(Li et al., 2008)



78 predicted enhancers were tested by reporter genes, but only ~half work in the early embryo. Why?

	Expression pattern	Bcd clusters	Bcd binding in vivo
Positive enhancers	 gt_23	Yes	Yes
	 hb_P2	Yes	Yes
Negative enhancers	 #18	Yes	No
	 #40	Yes	V. Weak

Tao

Why do the negative enhancers not bind Bcd?

The zinc-finger protein Zelda is a key activator of the early zygotic genome in *Drosophila*

Hsiao-Lan Liang[1]*, Chung-Yi Nien[1]*, Hsiao-Yun Liu[1], Mark M. Metzstein[2], Nikolai Kirov[1] & Christine Rushlow[1]

Zelda binds TAGteam sites:

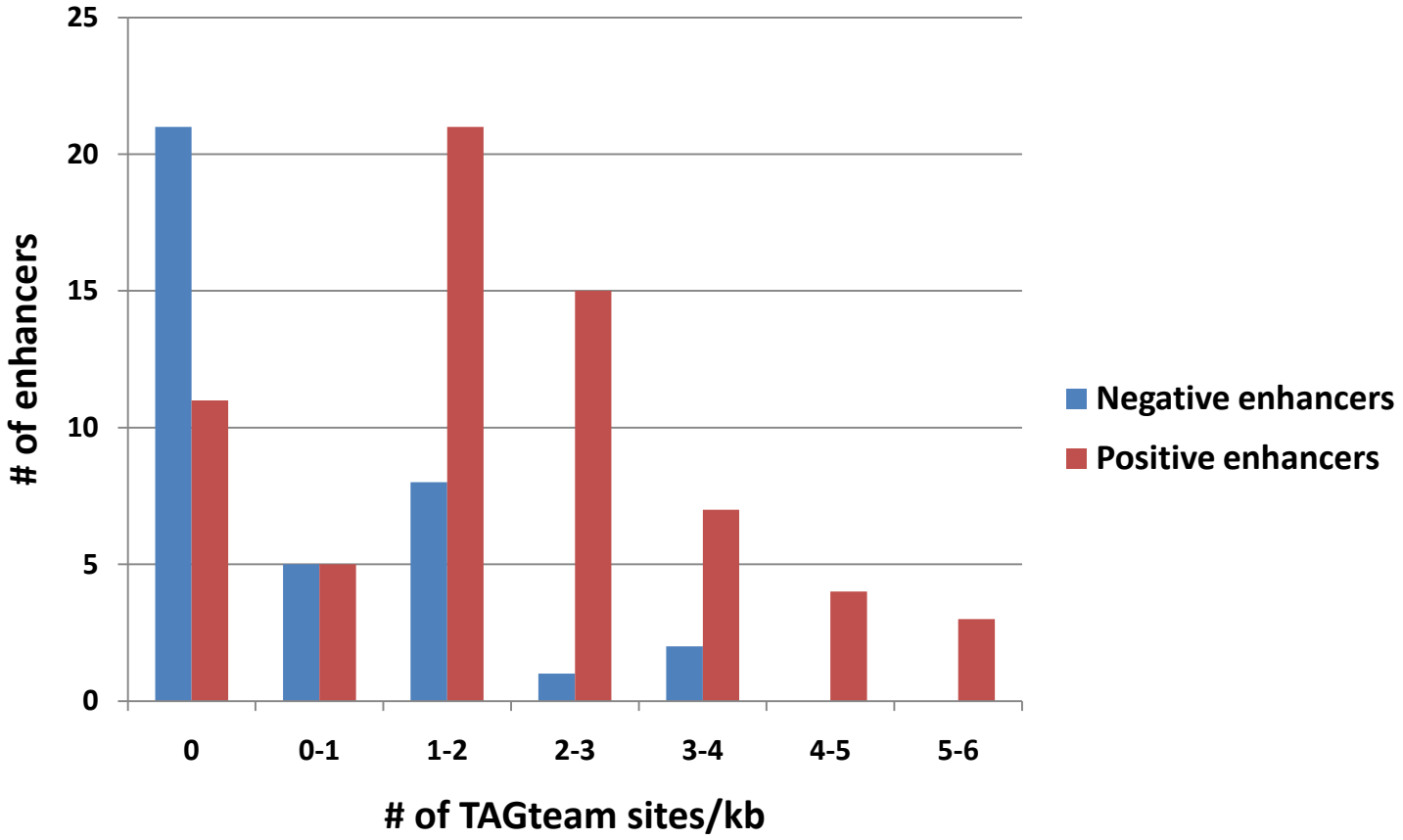
```
CAGGTAG
TAGGTAG
CAGGCAG
CAGGTAA
CAGGTAT
```

Overrepresented in EARLY zygotic genes

ten Bosch et al., 2006; De Renzis et al., 2007; Liang et al., 2008

Is there a correlation between TAGteam sites and embryonic enhancer activity?

TAGteam sites are underrepresented in negative enhancers.

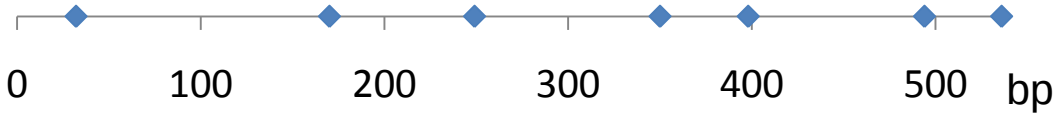


Tao; TAGteam PWM from Joey

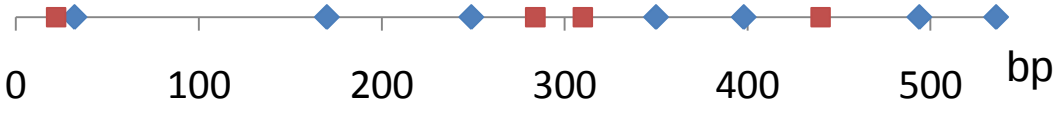
Add TAGteam sites to negative enhancers

CAGGTAG
TAGGTAG
CAGGCAG
CAGGTAA
CAGGTAT

#25

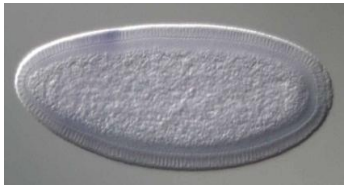


#25 + TAGteam sites

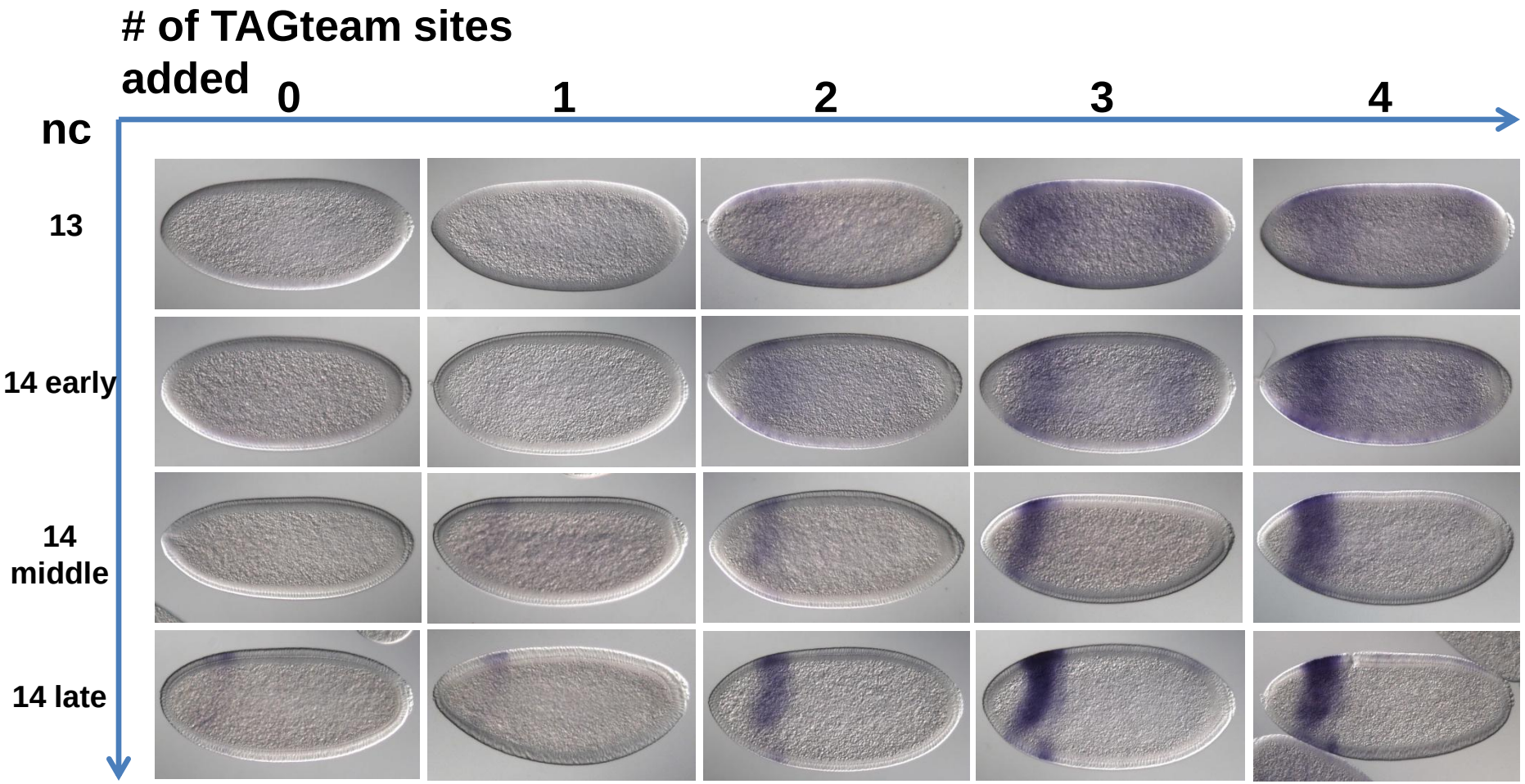


Bcd binding sites

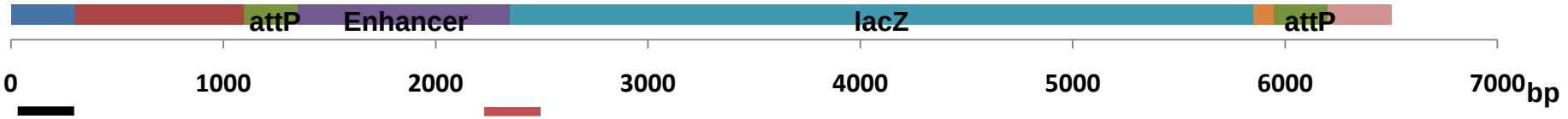
TAGteam sites

	#24	#25	#40	#18
wt Bcd cluster				

#40 enhancer



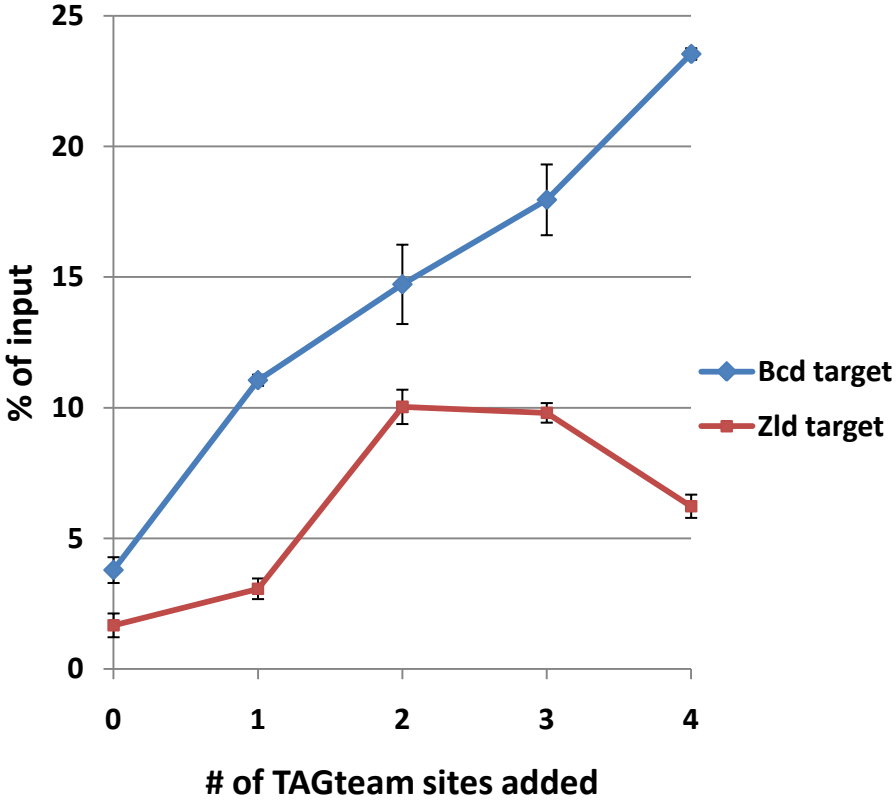
How does this work?



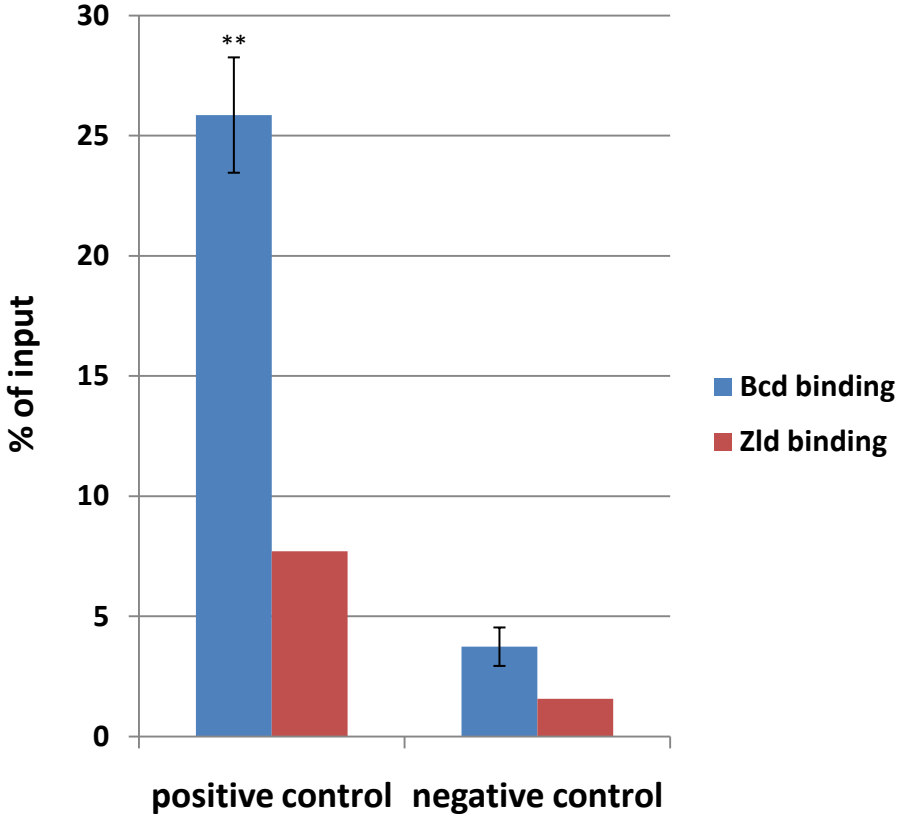
Negative control

Targeting region

Positive control = hb_P2



# of qPCR repeats = 3



# of qPCR repeats = 2

Conclusions 4:

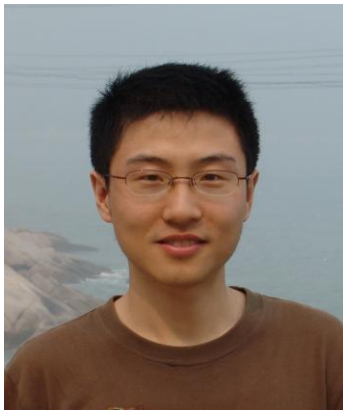
1. Zelda-binding can increase apparent binding activity of Bcd.

2. This may control which elements are available for activation in the early embryo.

Acknowledgements

Bcd network

Amanda Ochoa-Espinosa



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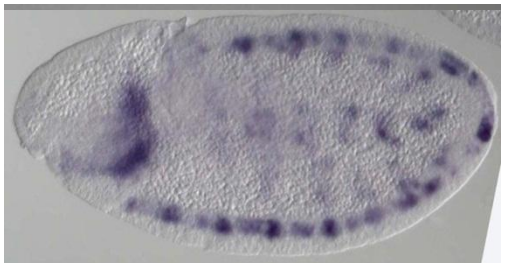
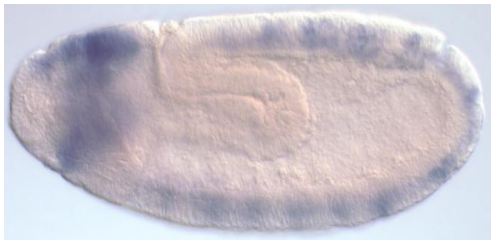
Negative enhancers have the potential to be bound and activated by Otd later in development.

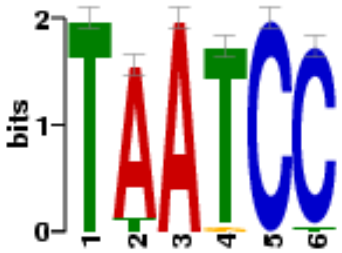
Enhancer #19

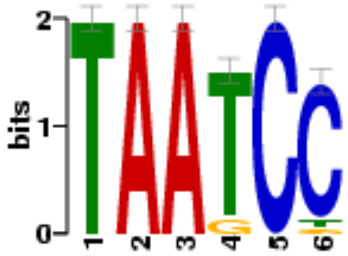
otd

late









Bcd site

Otd site

Maternal expression
PS4 stripe
Posterior stripe