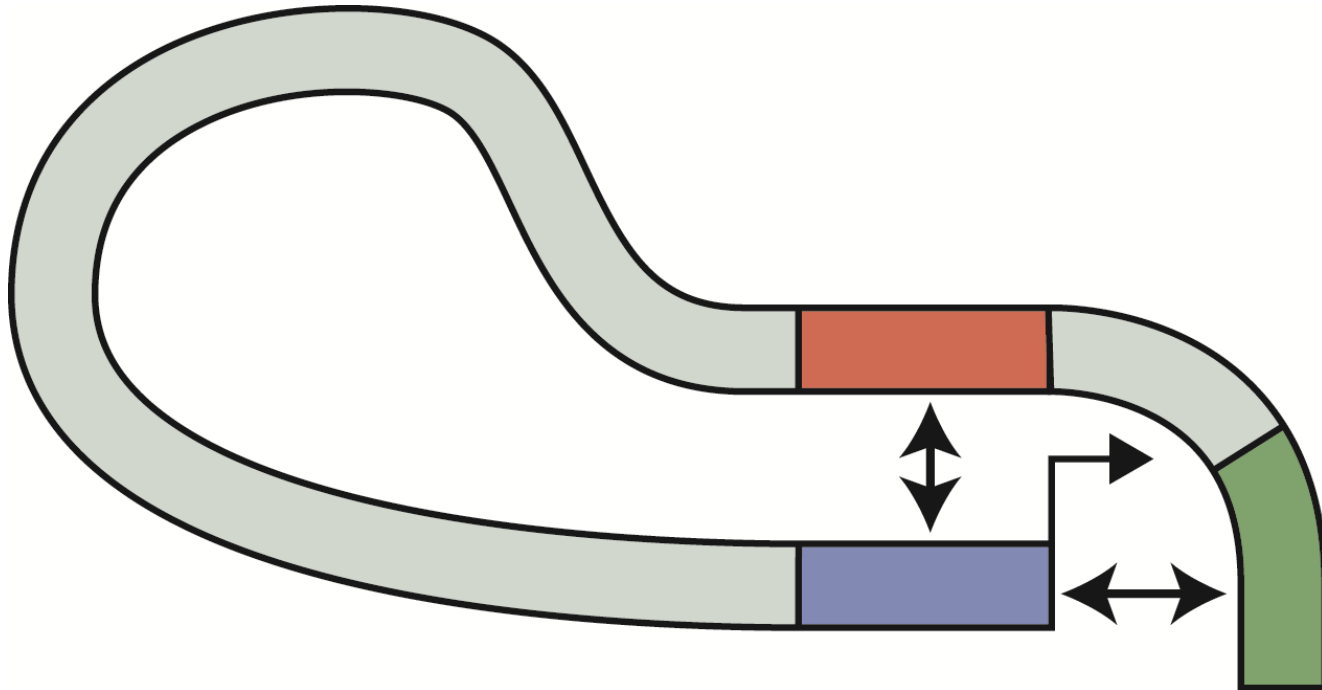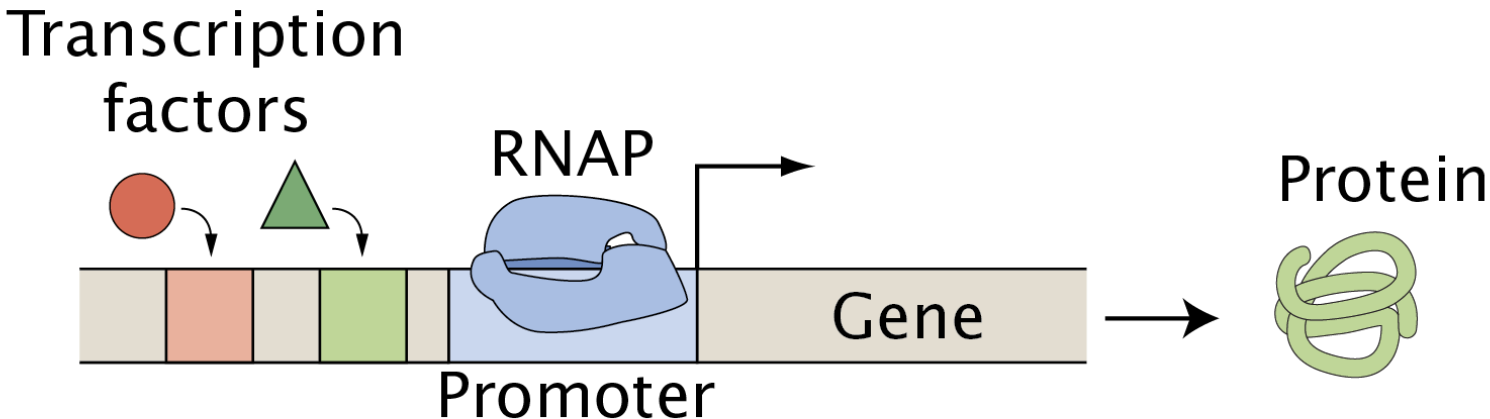# How, when and where in pattern formation:
## The regulatory knobs of evolution

**Jacques P. Bothma and Hernan G. Garcia**
Department of Molecular and Cell Biology
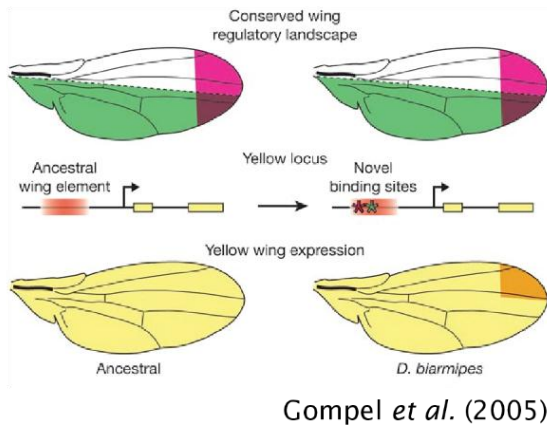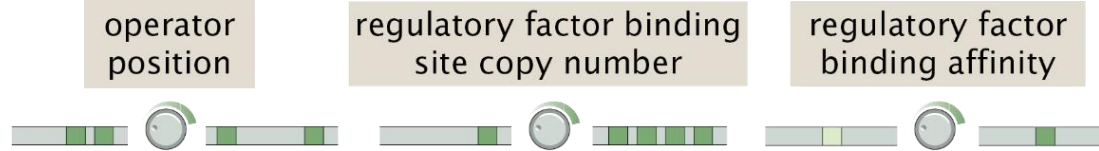Department of Physics
UC Berkeley

# Evolution of proteins and the sequences regulating their expression

# Different scenarios for regulatory region evolution

Modify enhancers to create new gene expression patterns

Regulatory knobs

operator position

regulatory factor binding site copy number

regulatory factor binding affinity

Conserved wing regulatory landscape

Yellow locus

Ancestral wing element

Novel binding sites

Yellow wing expression

Ancestral

D. biarmipes

Gompel *et al.* (2005)

This talk: A molecular exploration of the mechanisms underlying promoter-enhancer interactions and their potential role as an evolutionary substrate
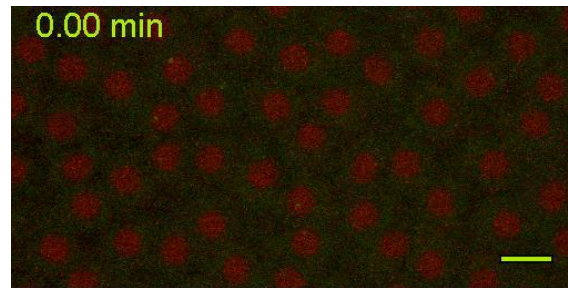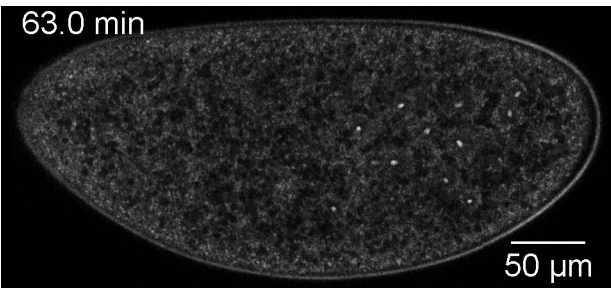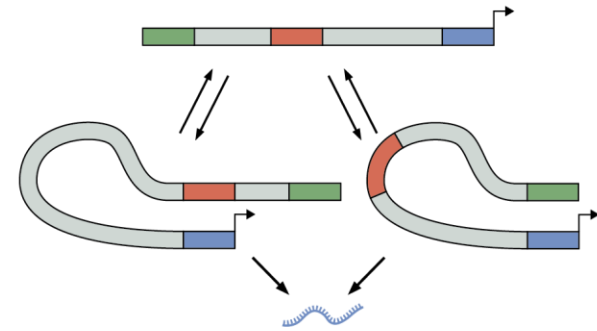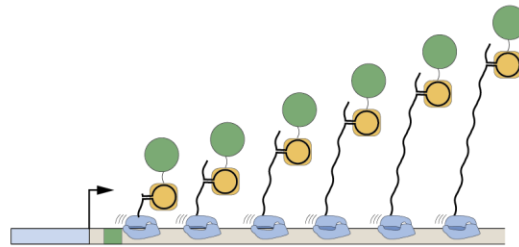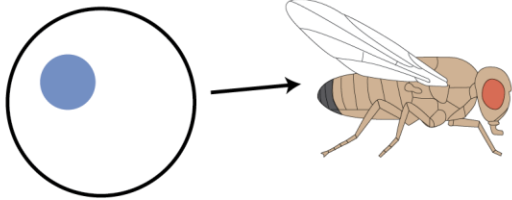
# Outline



Fruit fly development to study the evolution of gene regulation

Enabling technologies for precise measurements in embryonic development

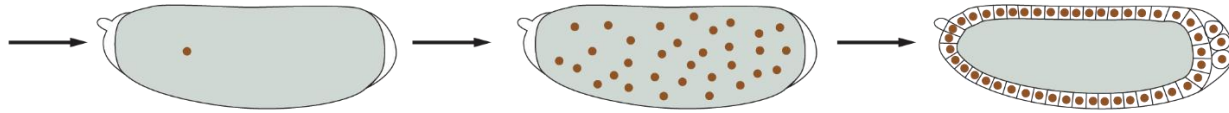Redundant enhancers as a substrate for evolutionary novelty?

Single cell

63.0 min

50 μm

0.00 min

# Fruit fly development as a case study in enhancer-promoter choice



Make lots of cells

fertilization

3 hours

# Development is dynamic!
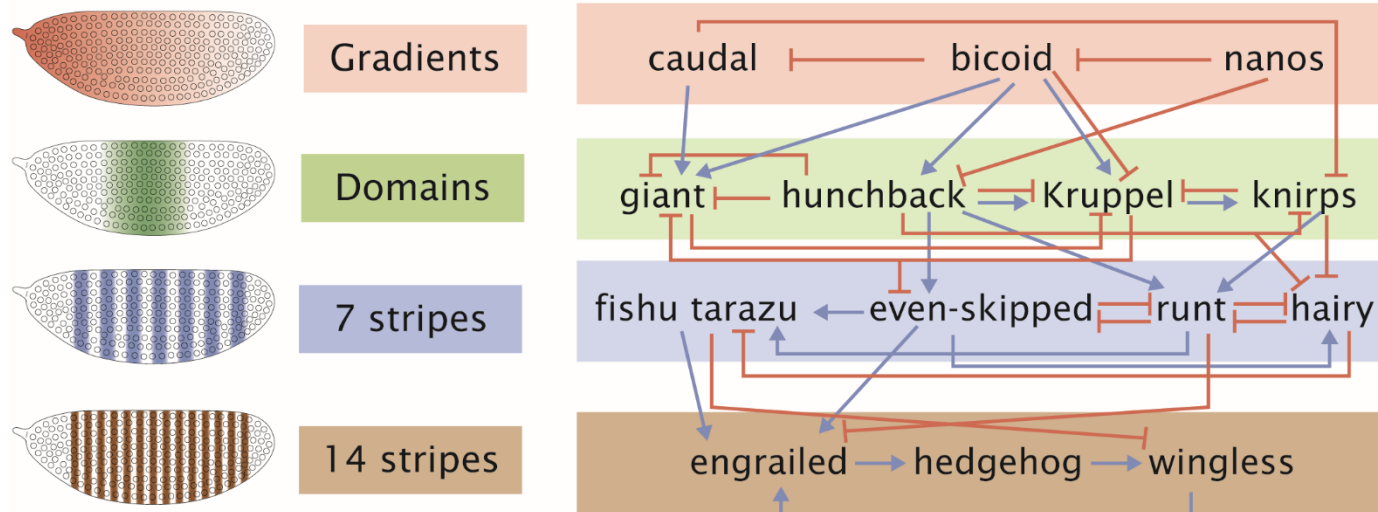
Make lots of cells

Make body segments



63.0 min

50 μm

ventrolateral view                02:51:20
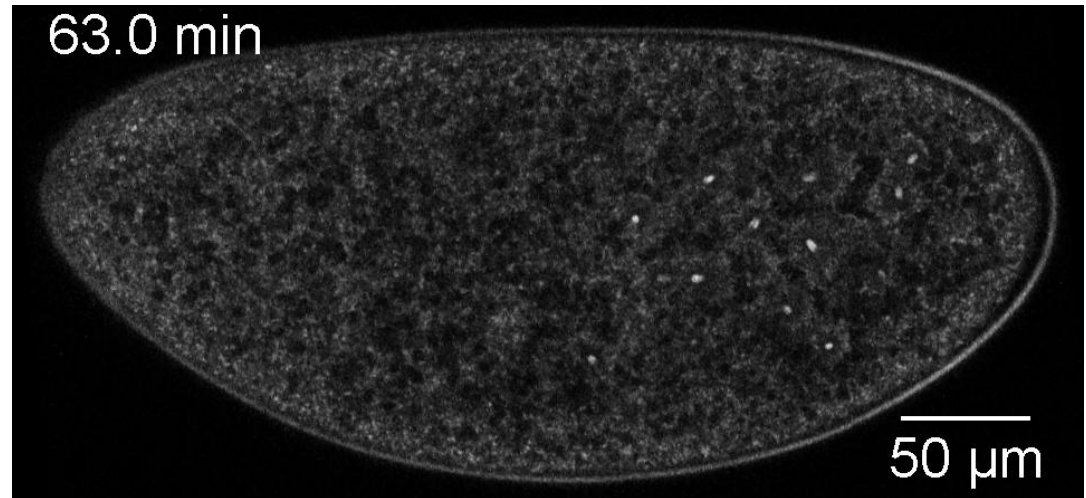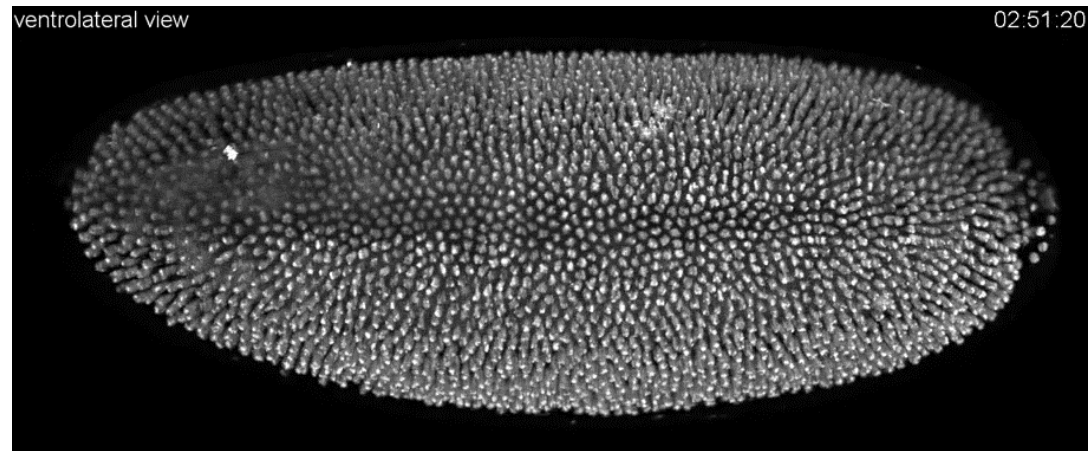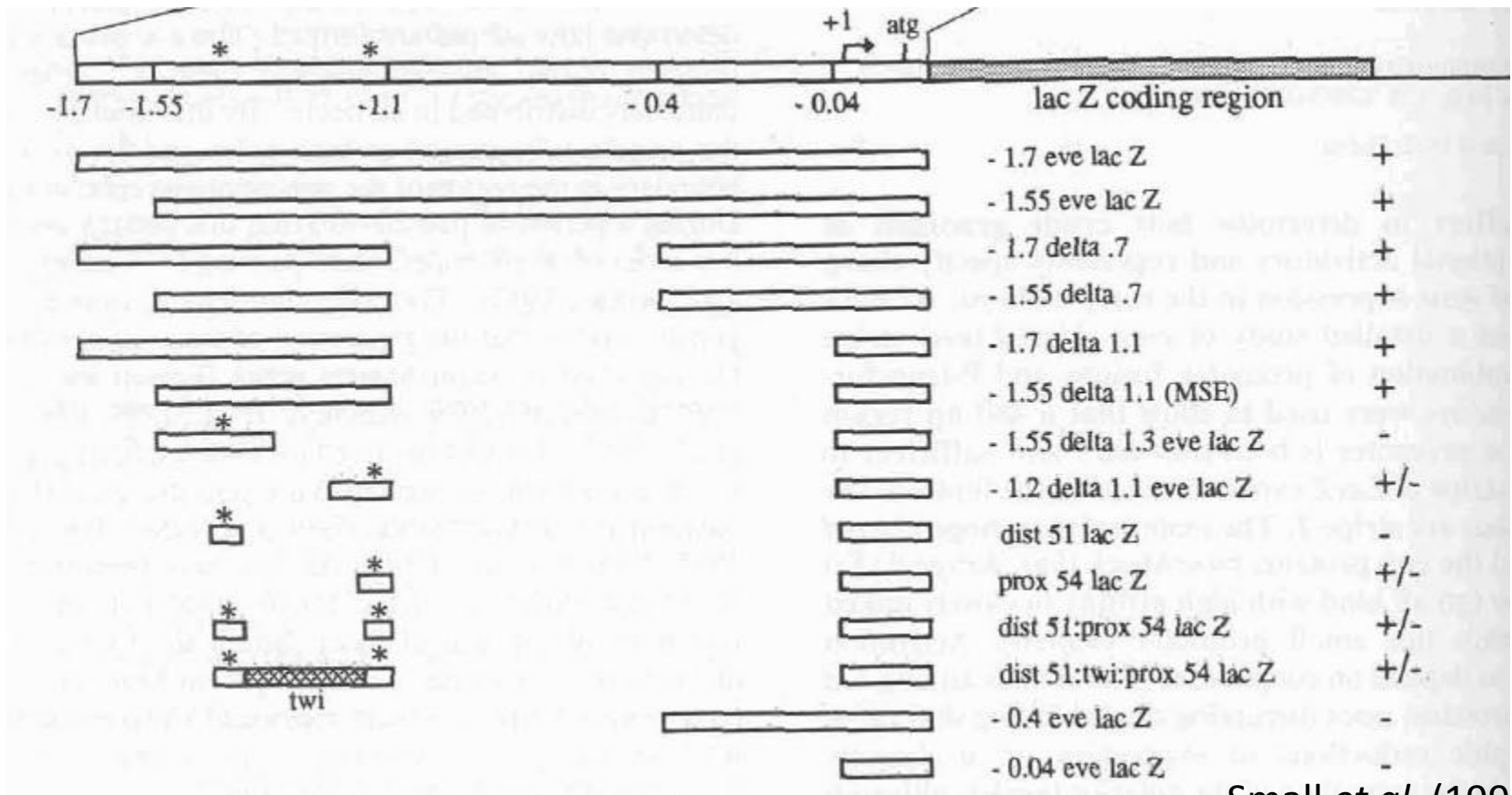
Tomer … Keller, *Nat Methods* (2012)

# The hydrogen atom of developmental biology

*"There's a reason physicists are so successful with what they do, and that is they study the hydrogen atom and the helium ion and then they stop"*,
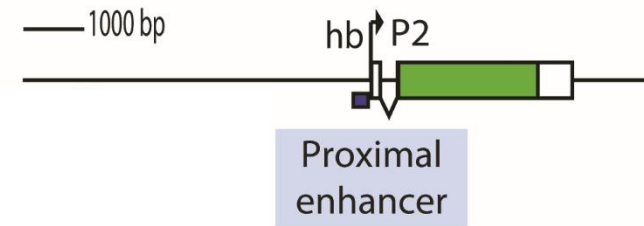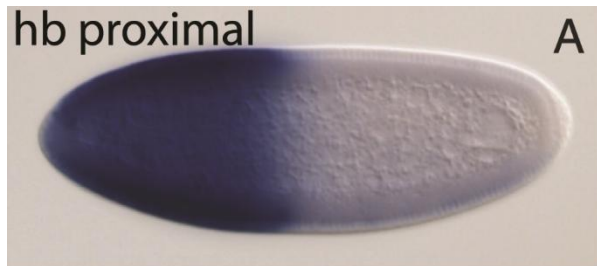Richard Feynman

# Finding enhancers can be laborious!



Small *et al.* (1992)

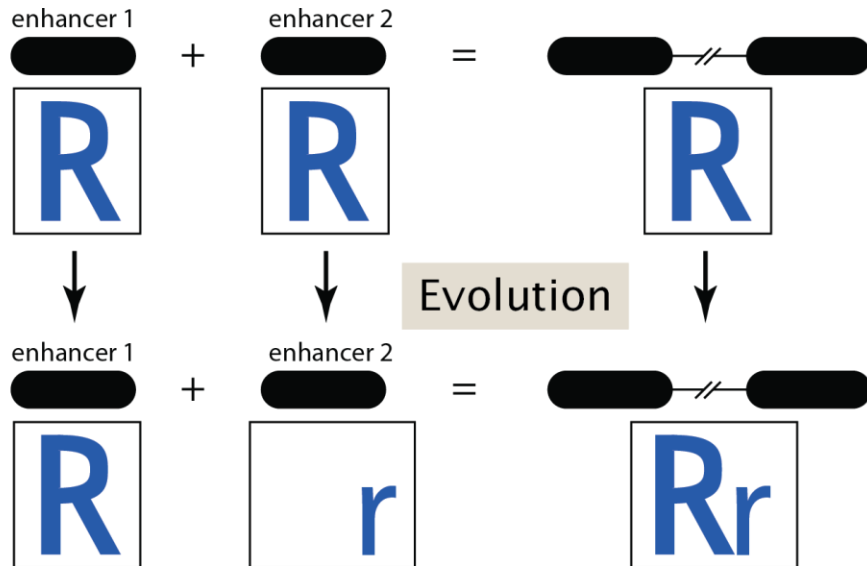- Once you find your enhancer you stop!

# Multiple enhancers drive overlapping expression patterns in fly development
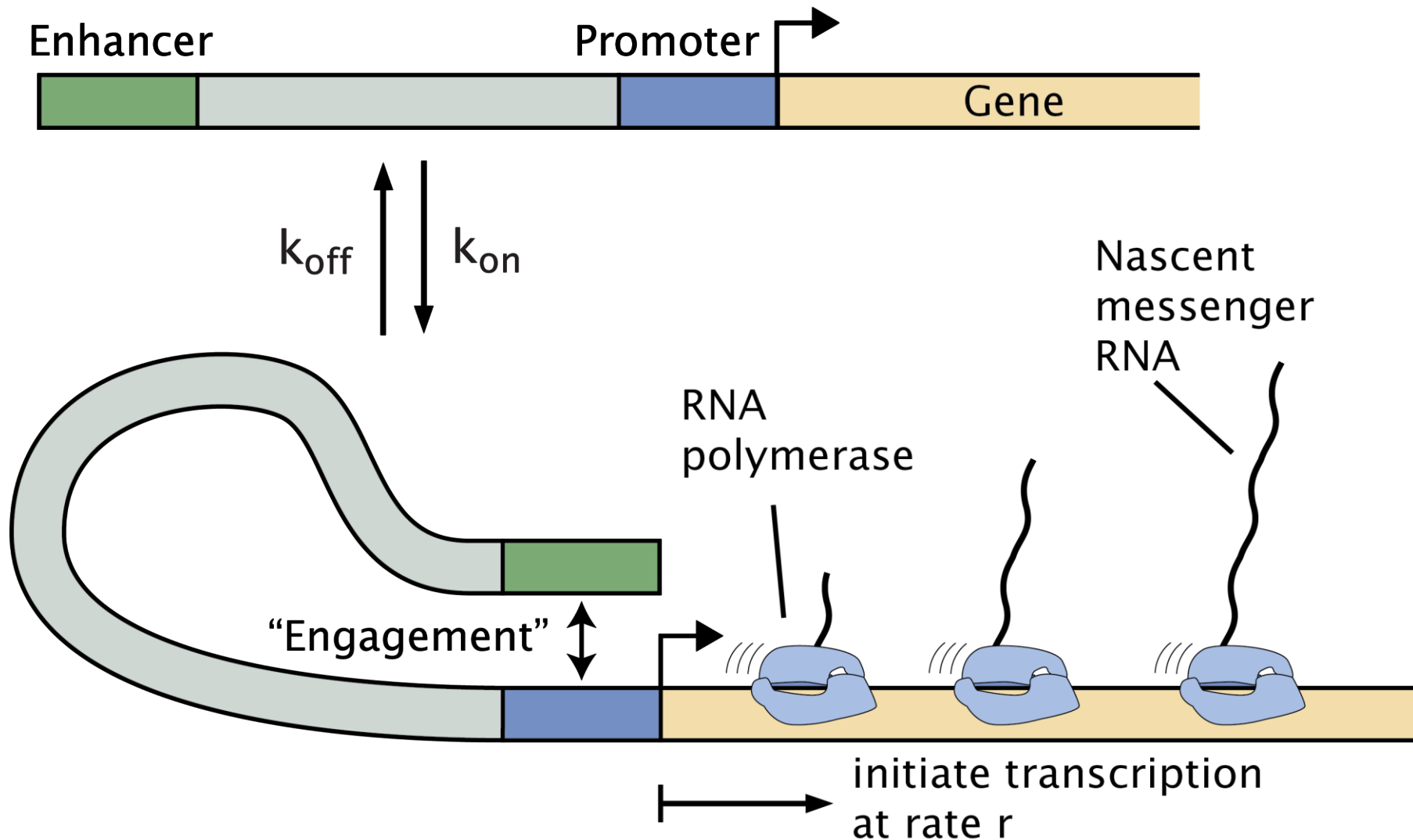


Perry *et al.*, PNAS (2011)

# Why have multiple enhancers?



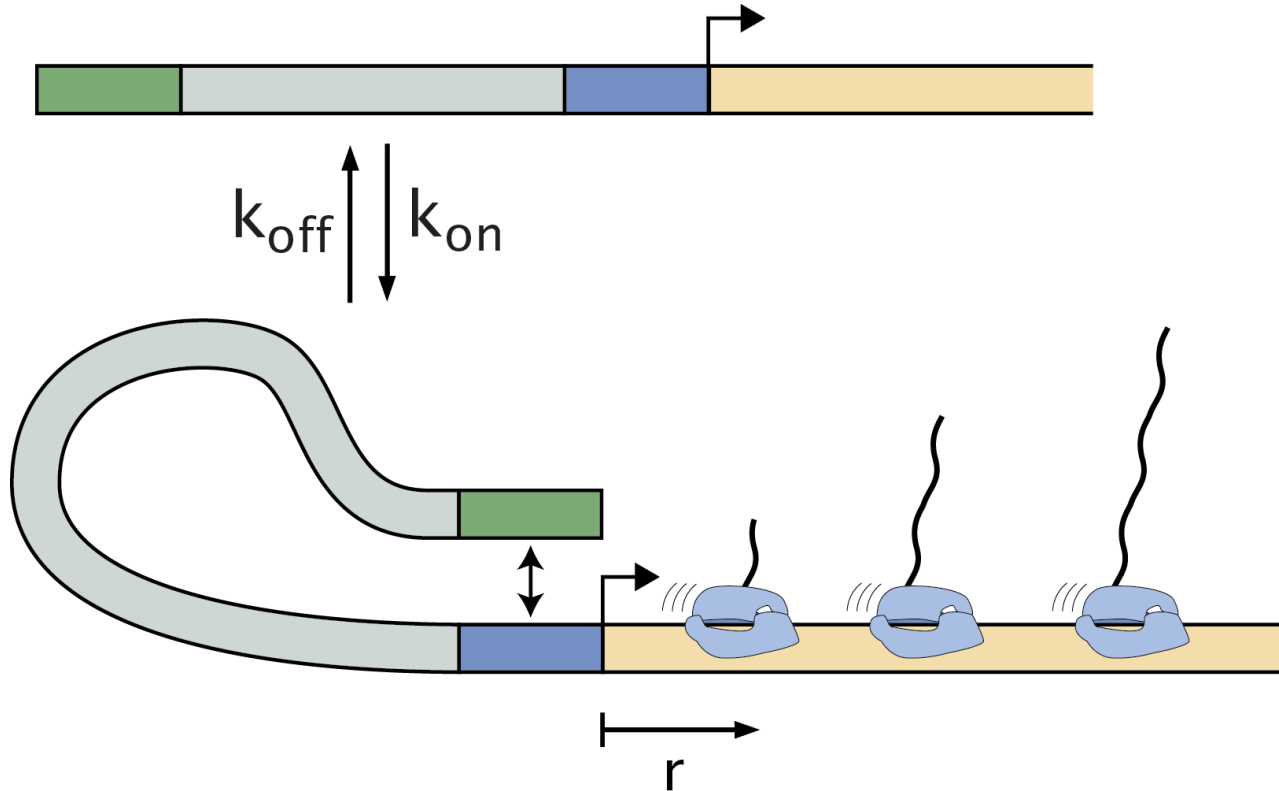Are these multiple enhancers the equivalent of a gene duplication event?

Shadow enhancers as case study in enhancer-promoter interaction and specificity
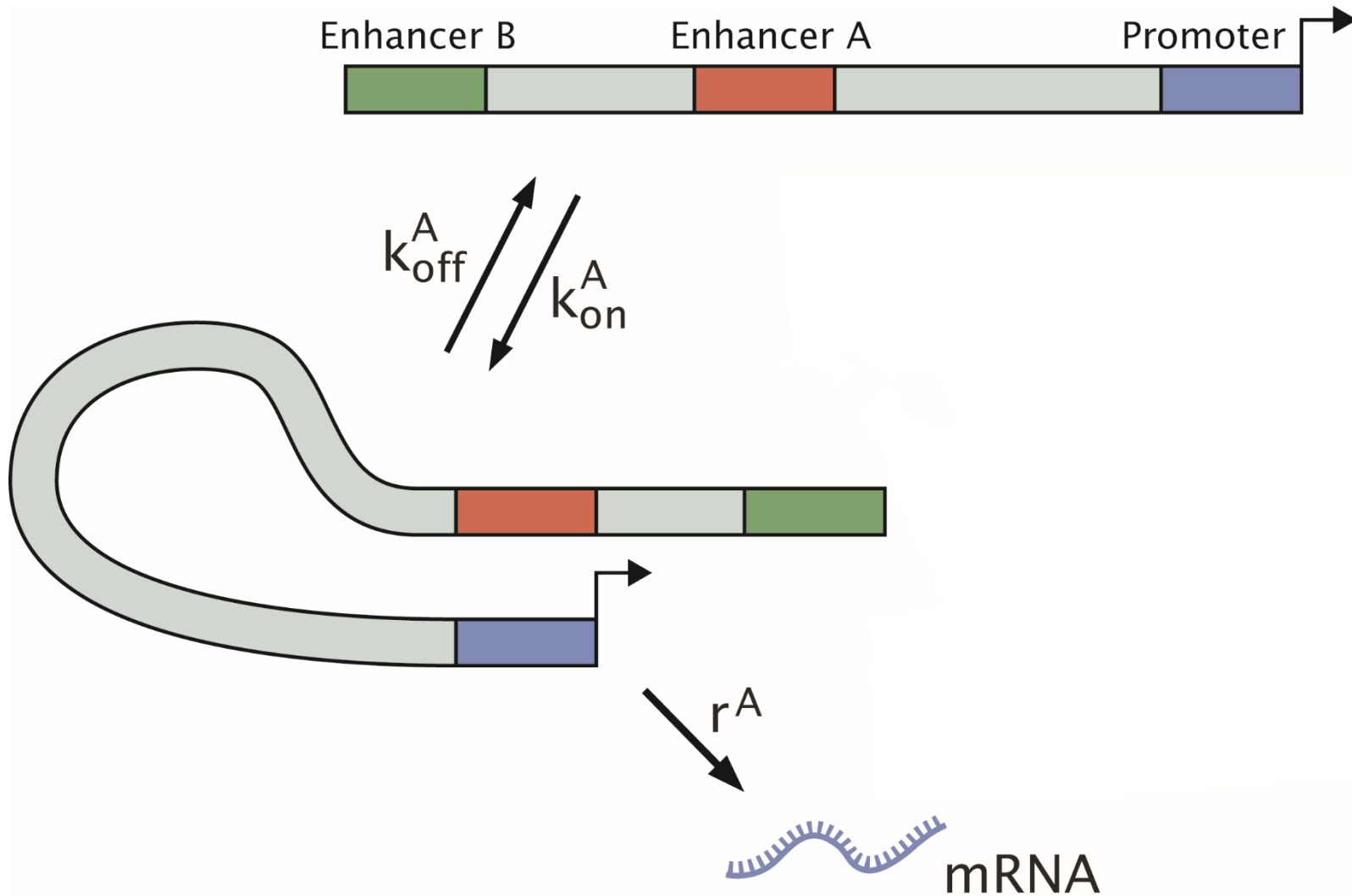
# A simple model of enhancer action

Enhancer

Promoter

Gene

$k_{off}$   $k_{on}$

"Engagement"

RNA polymerase

Nascent messenger RNA

initiate transcription at rate r

# Our model predicts the rate of mRNA production



$$\frac{d\text{ mRNA}}{dt} = r \underbrace{\frac{k_{on}}{k_{on} + k_{off}}}_{\substack{\text{Fraction of time} \\ \text{in on-state}}}$$
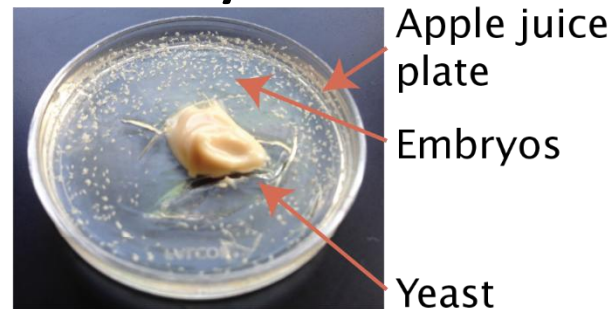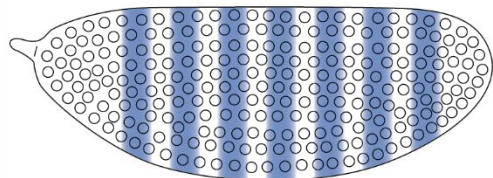
# Accounting for the competition of multiple enhancers

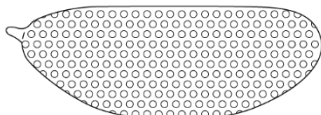# Combined enhancer action cannot be higher than the sum of their individual activities

$$k_{off} = k_{off}^{A} = k_{off}^{B}$$

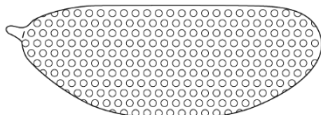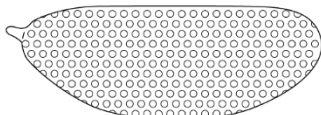$$k_{on} = k_{on}^{A} = k_{on}^{B}$$
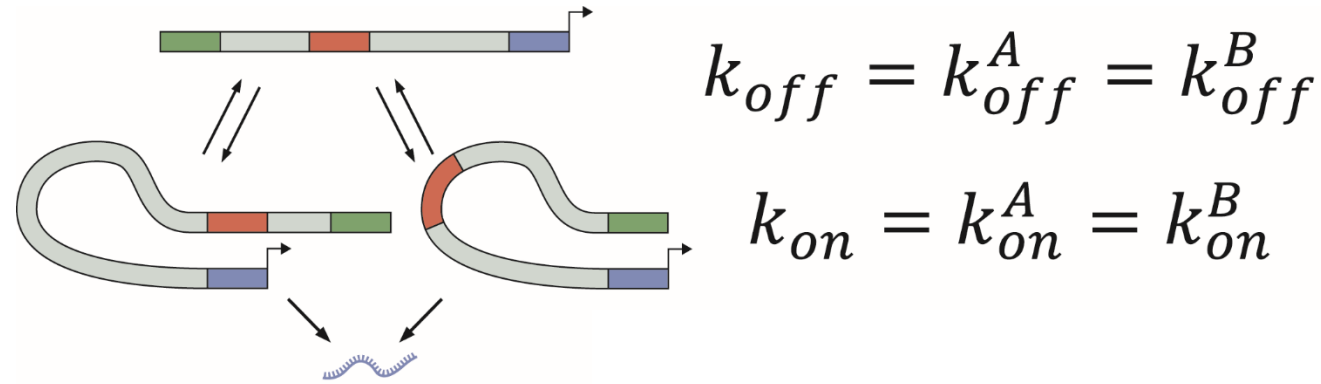
# The state of the art in the field: Inferring dynamics from static pictures of dead embryos
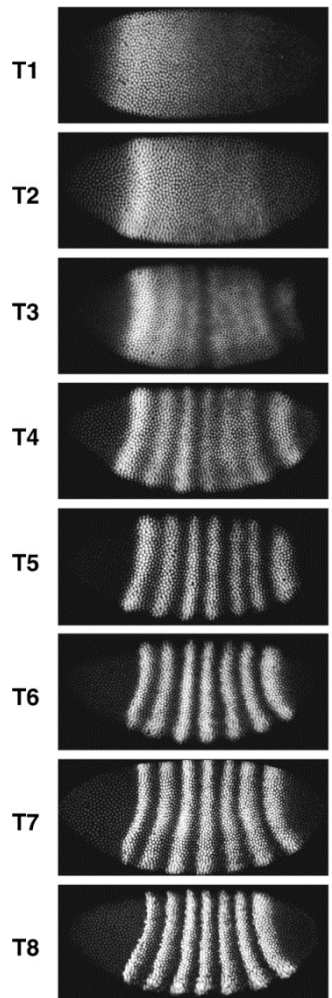
How is this pattern made?

Apple juice plate

Embryos

Yeast

Collect unsynchronized embryos
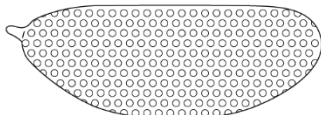
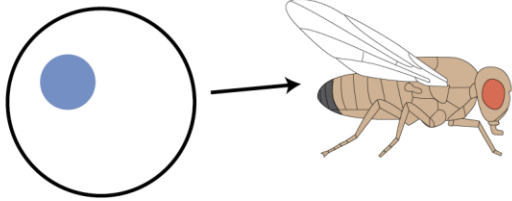# A stop motion movie with a new actor in each frame
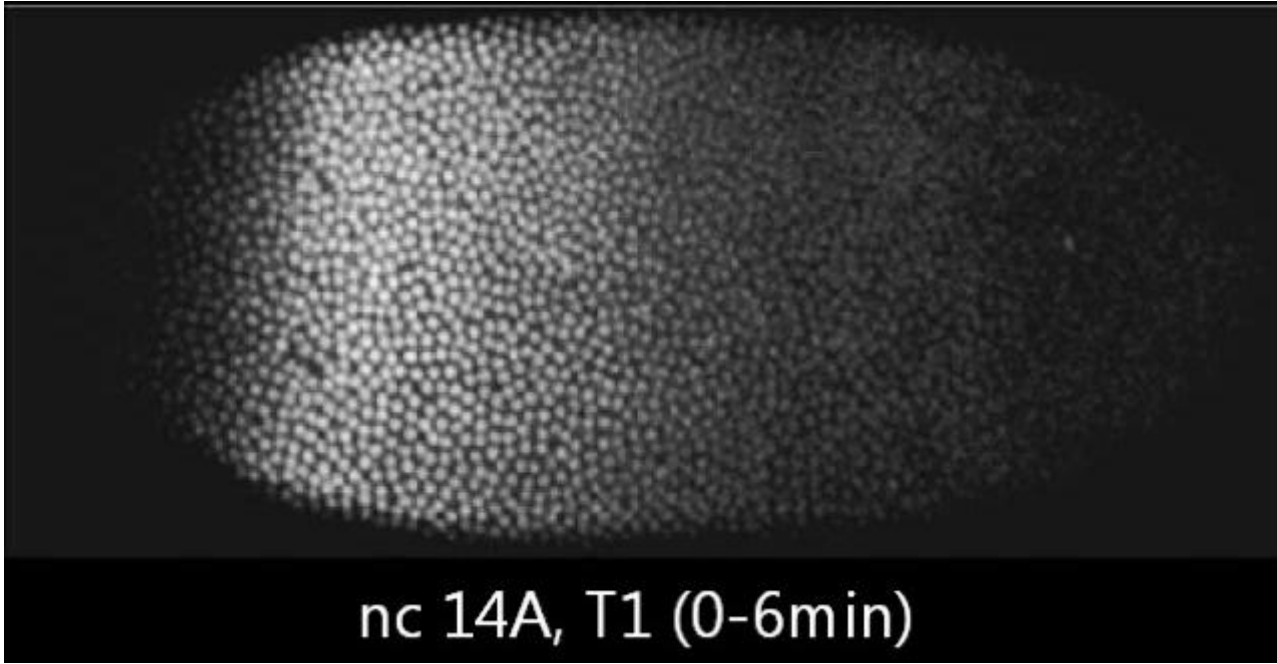


Surkova *et al.,* Dev. Bio. (2008)

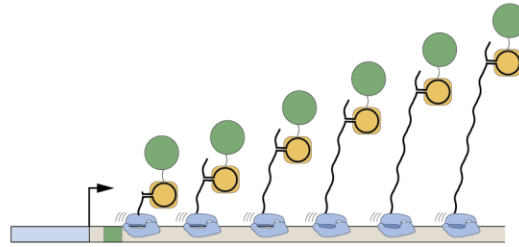- Create a "movie" by pooling together embryos of different time classes.

# Outline



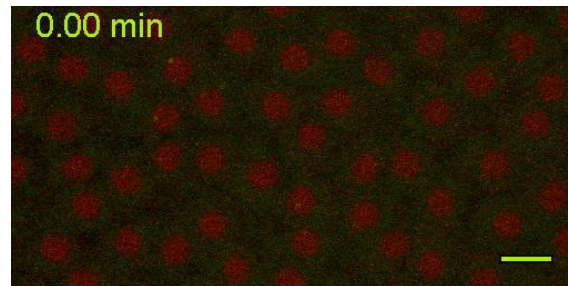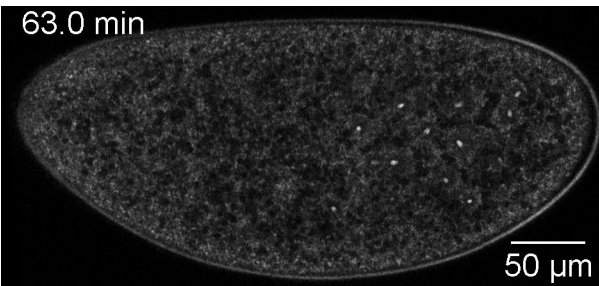Fruit fly development to study the evolution of gene regulation
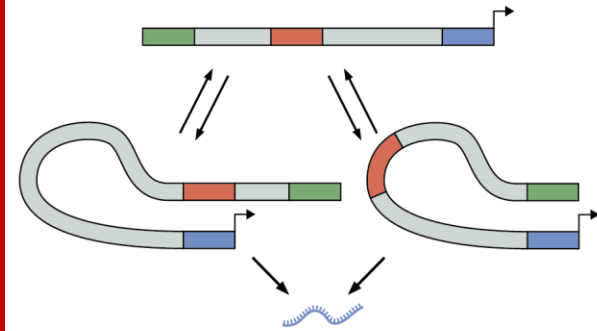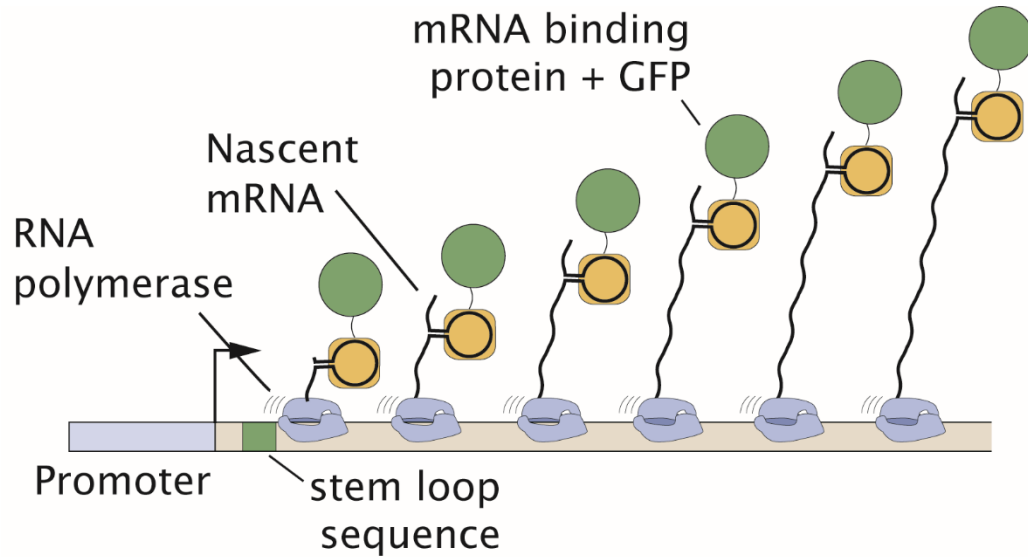
Single cell

63.0 min

50 μm

Enabling technologies for precise measurements in embryonic development

0.00 min

Redundant enhancers as a substrate for evolutionary novelty?

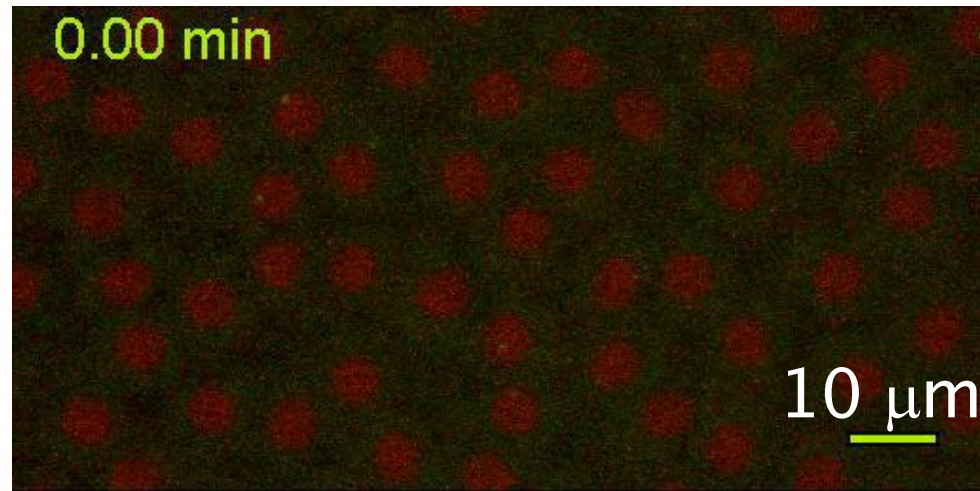# Enabling technology to spy on development in real time



mRNA binding protein + GFP

Nascent mRNA
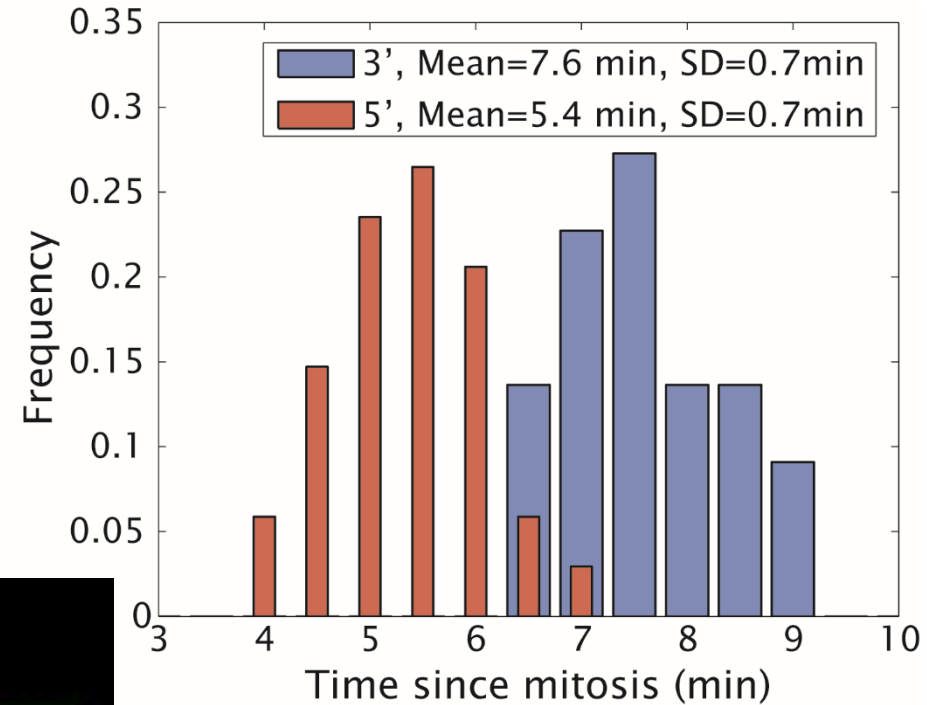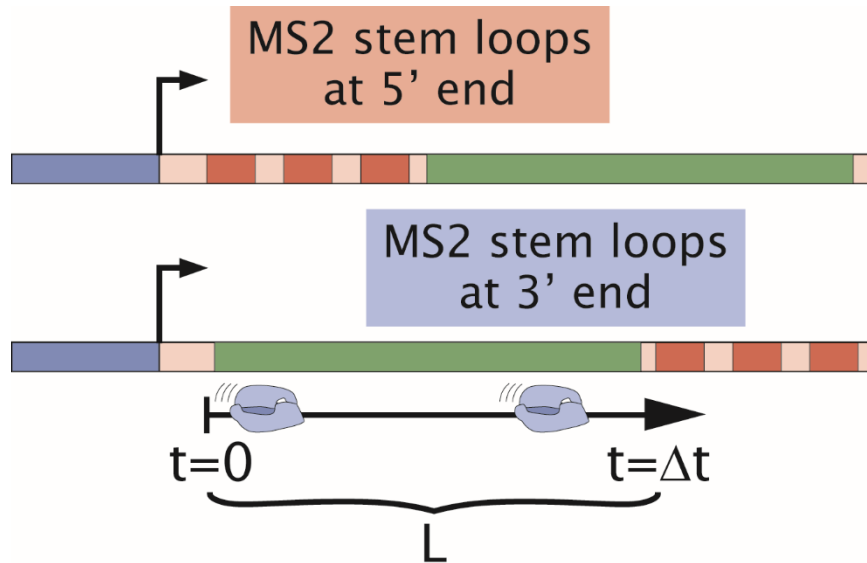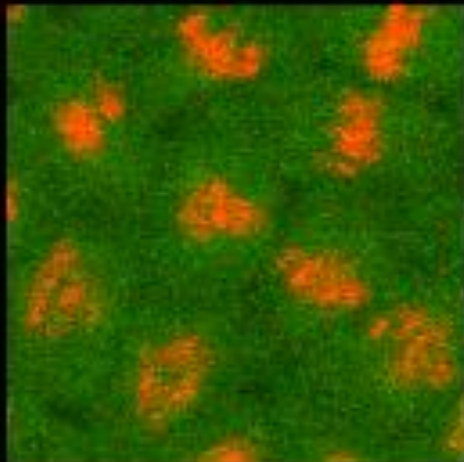
RNA polymerase

Promoter

stem loop sequence

mRNA    DNA

0.00 min

10 μm

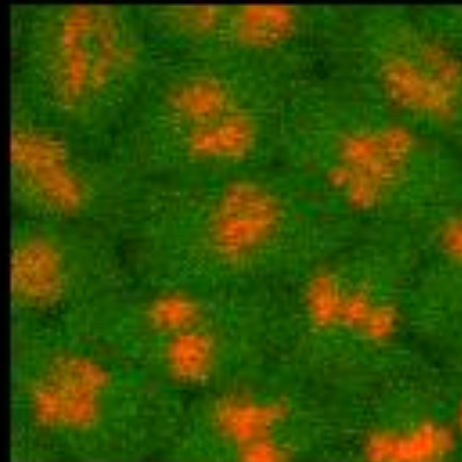# We can precisely measure timing of transcription
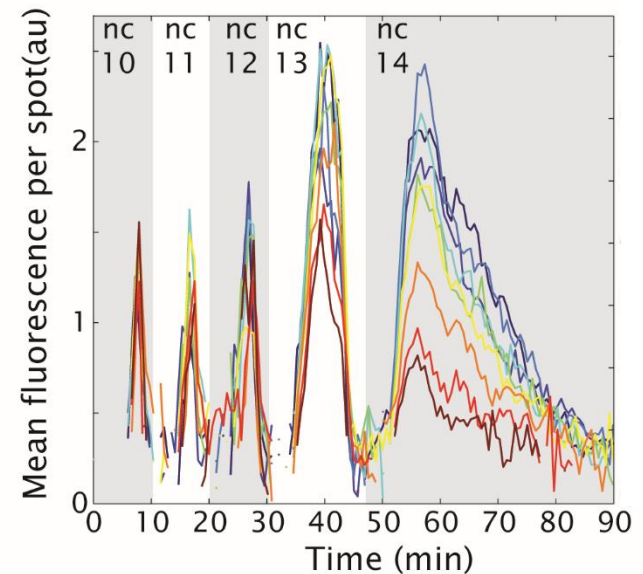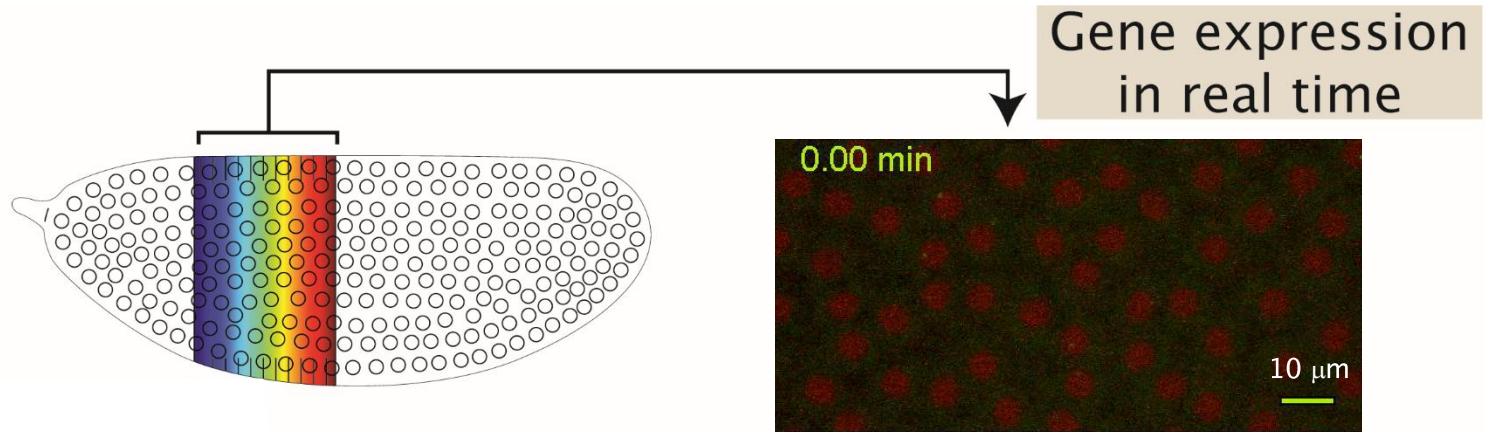


- Elongation rate of (1.54 ± 0.14) kb/min, consistent with single cell and fixed embryo results.

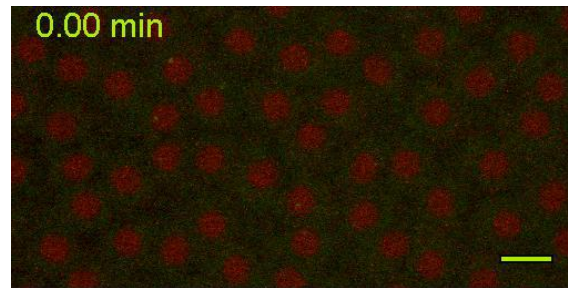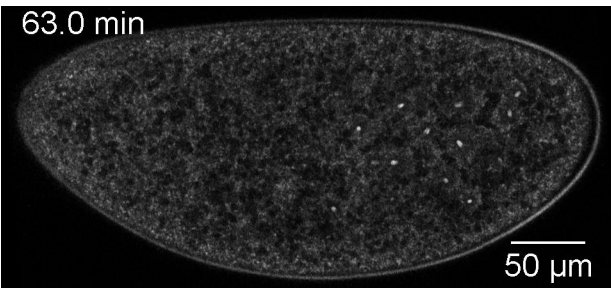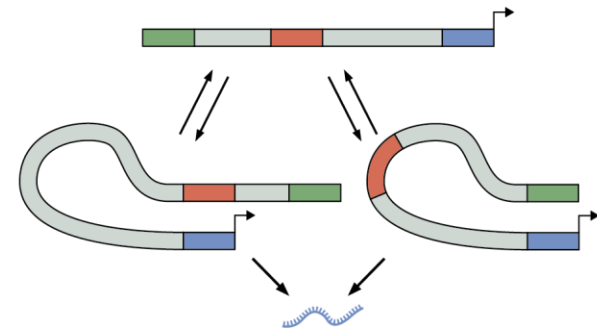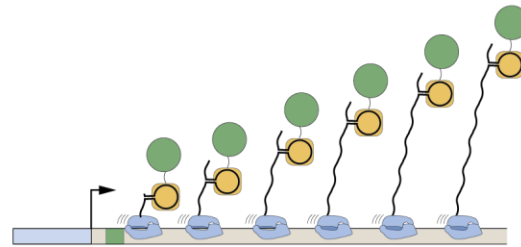# The best of both worlds: Using dead embryos to count single molecules

# Outline



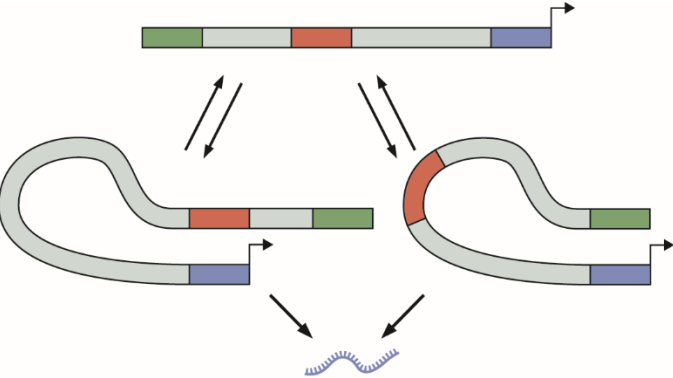**Fruit fly development to study the evolution of gene regulation**

Single cell

63.0 min

50 μm

**Enabling technologies for precise measurements in embryonic development**

0.00 min

**Redundant enhancers as a substrate for evolutionary novelty?**
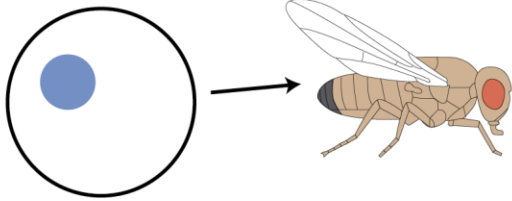
# Combined enhancer action cannot be higher than the sum of their individual activities



$$k_{off} = k_{off}^A = k_{off}^B$$

$$k_{on} = k_{on}^A = k_{on}^B$$

Activity of enhancer A alone

$$\frac{d[\text{mRNA}]}{dt} = \left( \overbrace{\frac{d[\text{mRNA}]^A}{dt}} + \frac{d[\text{mRNA}]^B}{dt} \right) \frac{1 + \frac{k_{on}}{k_{off}}}{1 + 2\frac{k_{on}}{k_{off}}}$$

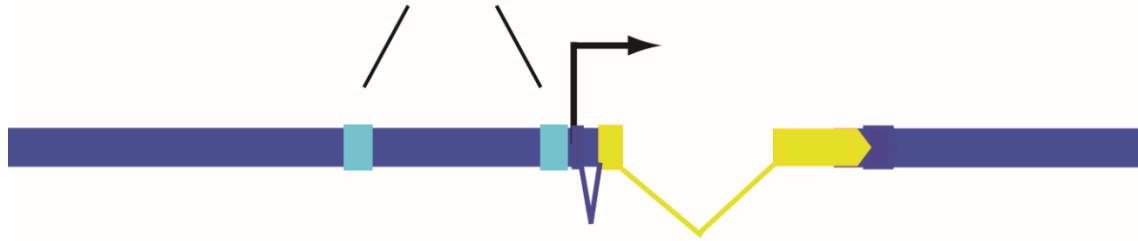**Weak promoter engagement** $\quad k_{on} \ll k_{off}$

$$\left( \frac{d[\text{mRNA}]^A}{dt} + \frac{d[\text{mRNA}]^B}{dt} \right)$$

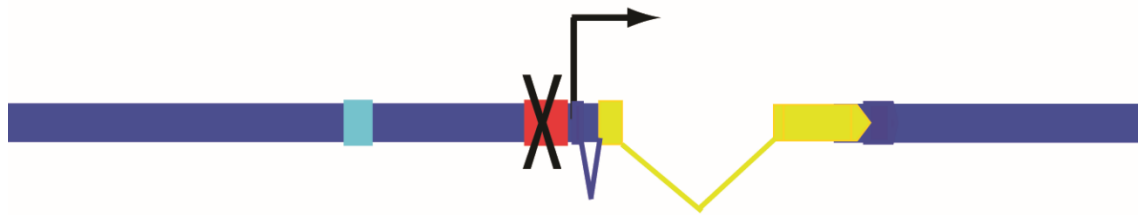**Strong promoter engagement** $\quad k_{on} \gg k_{off}$

$$\frac{1}{2}\left( \frac{d[\text{mRNA}]^A}{dt} + \frac{d[\text{mRNA}]^B}{dt} \right)$$

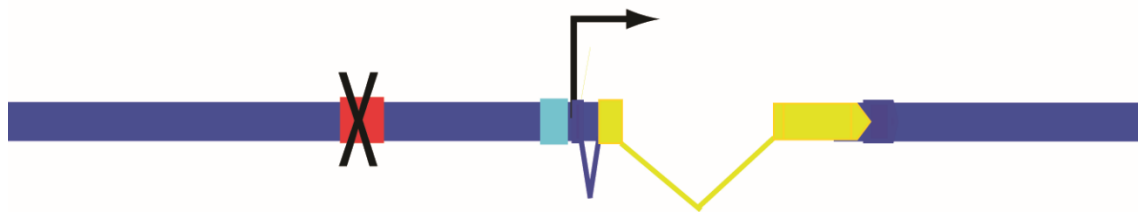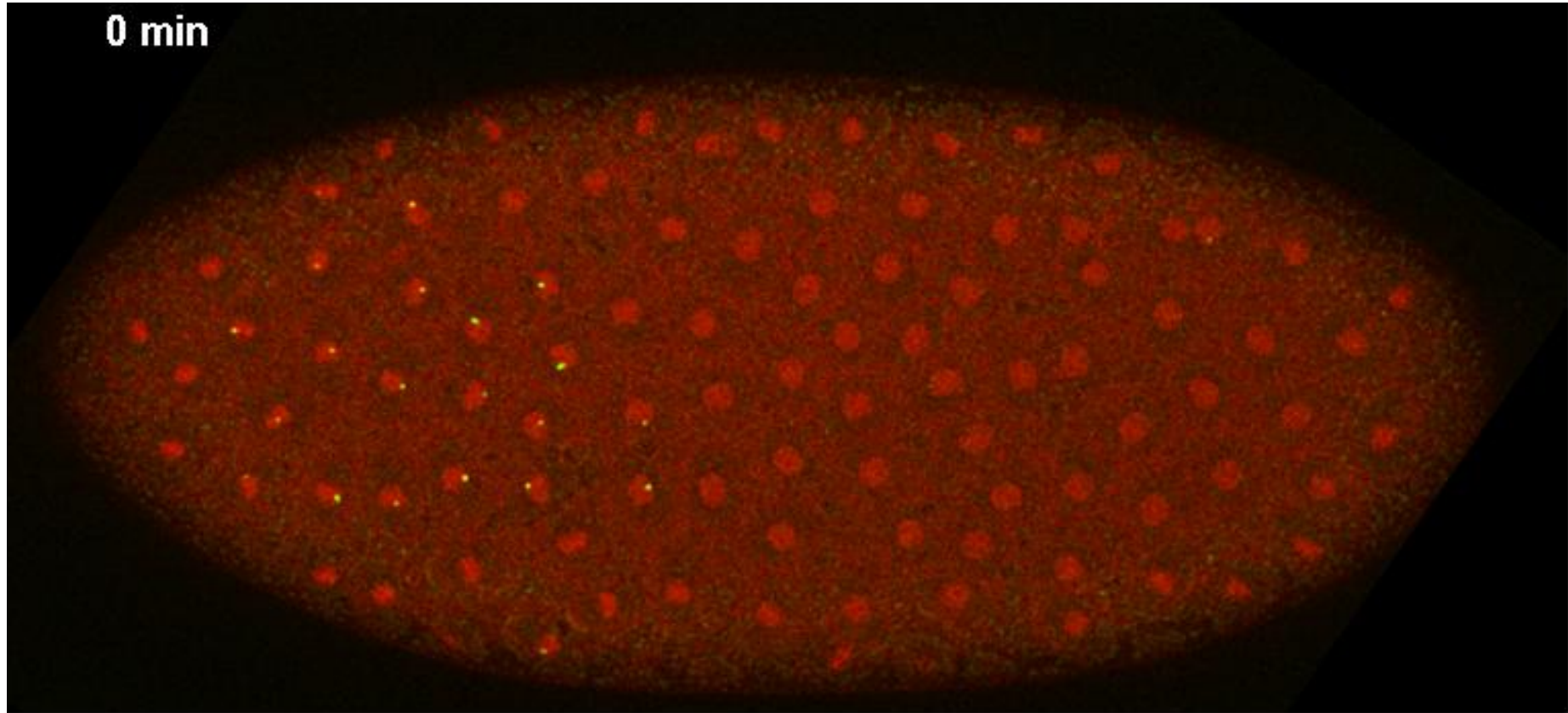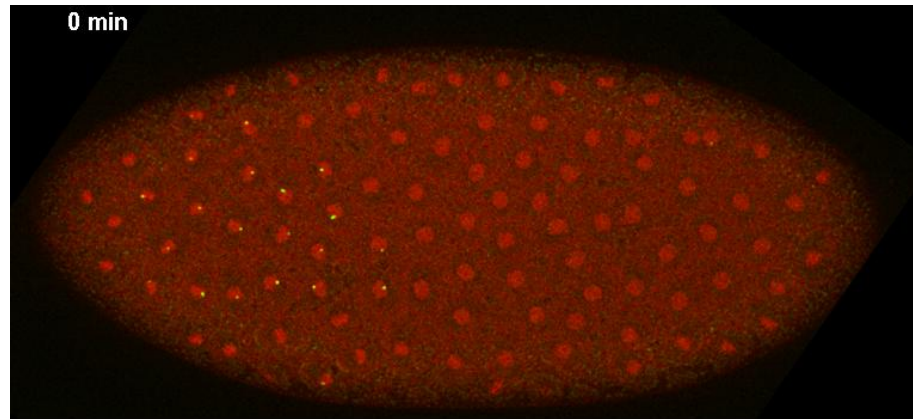# An experiment to uncover the contribution of "redundant" enhancers

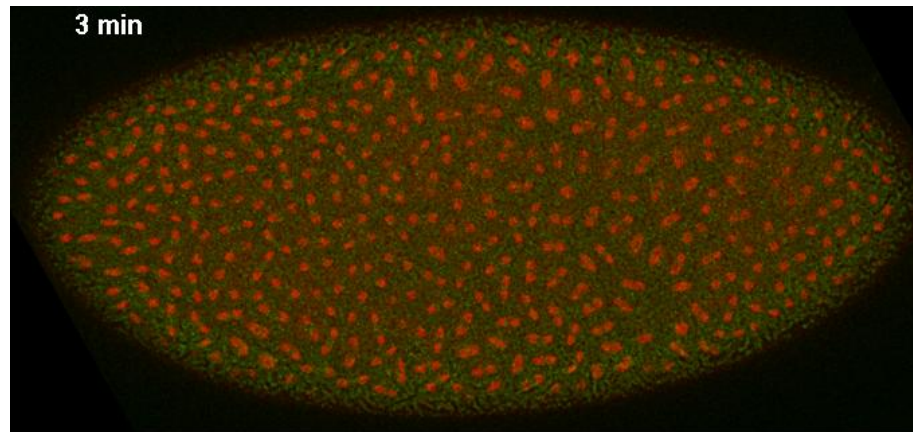# Wild-type *hunchback* expression dynamics
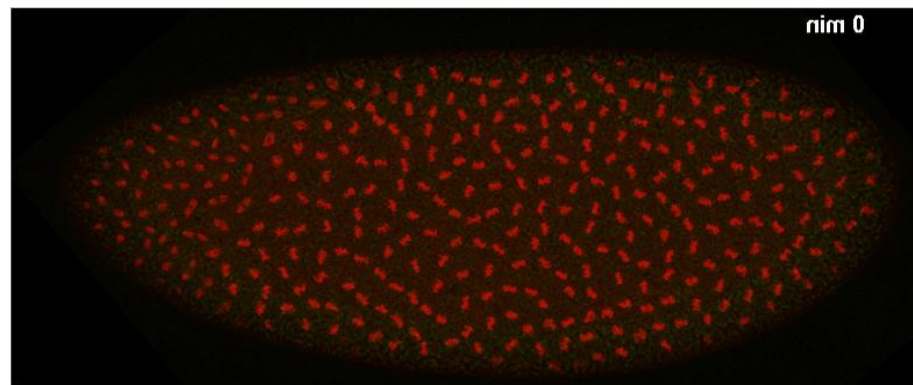


Histone RFP
MCP-GFP

# *hunchback*: multiple enhancer action is not redundant
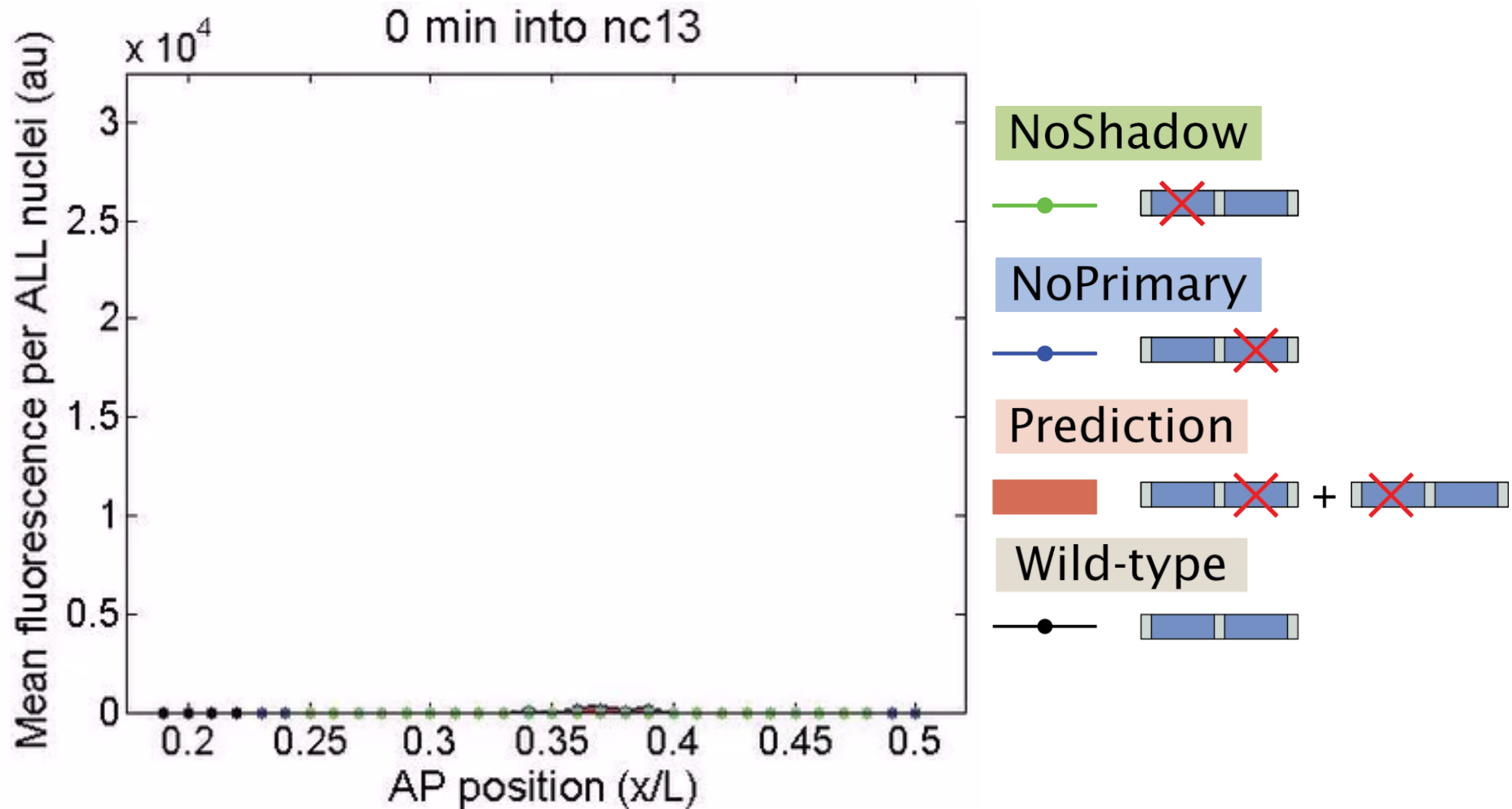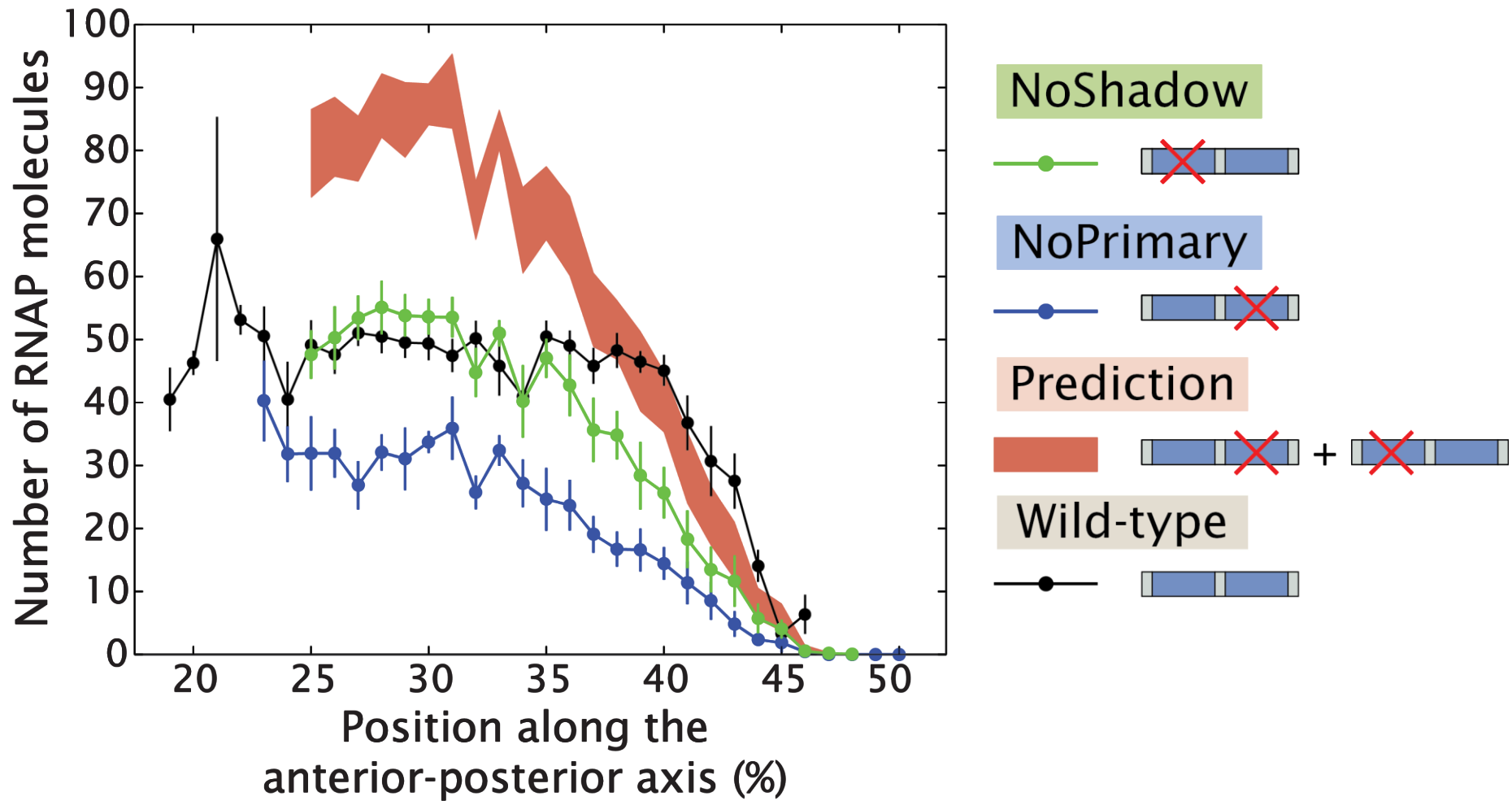


Wild-type

NoShadow

NoPrimary

# *hunchback* can be additive and sub-additive

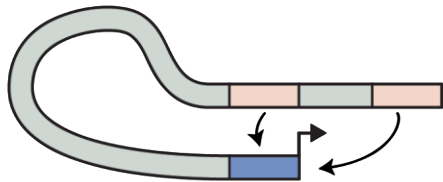# *hunchback* can be additive and sub-additive

# Regimes of additivity and sub-additivity consistent with enhancer competition for the promoter

# *knirps* enhancer activity



Wild-type

NoShadow

NoPrimary

# *knirps* can be super-additive!



0 min into nc14

Transcription activity (au)

15000
10000
5000
0

x/L
0.54   0.58   0.62   0.66   0.7

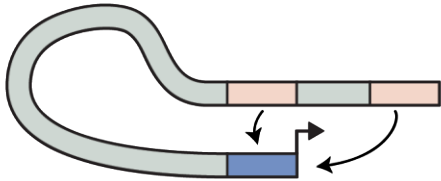NoShadow

NoPrimary

Prediction

Wild-type
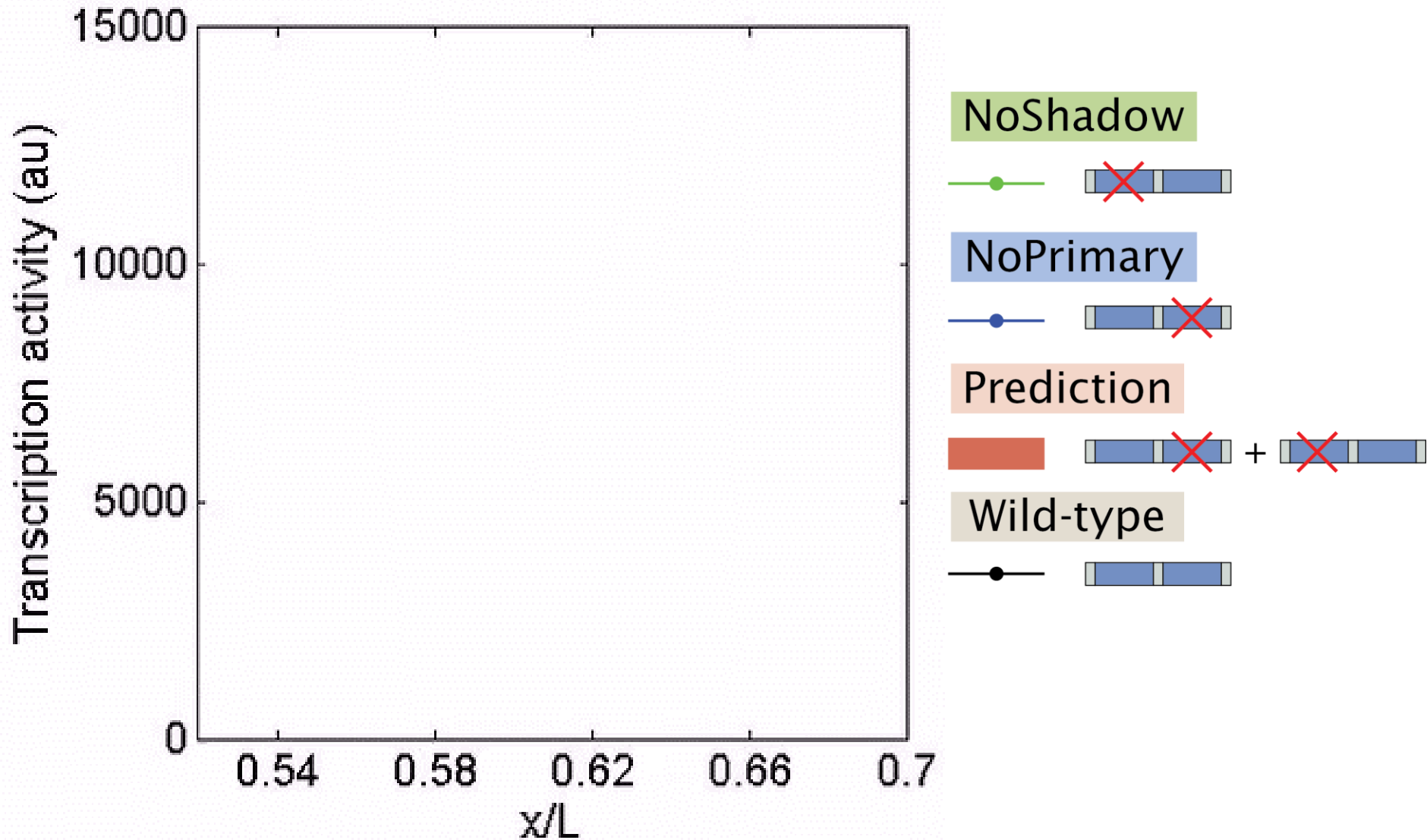
# Enhancer cooperativity is necessary to explain super-additive activity
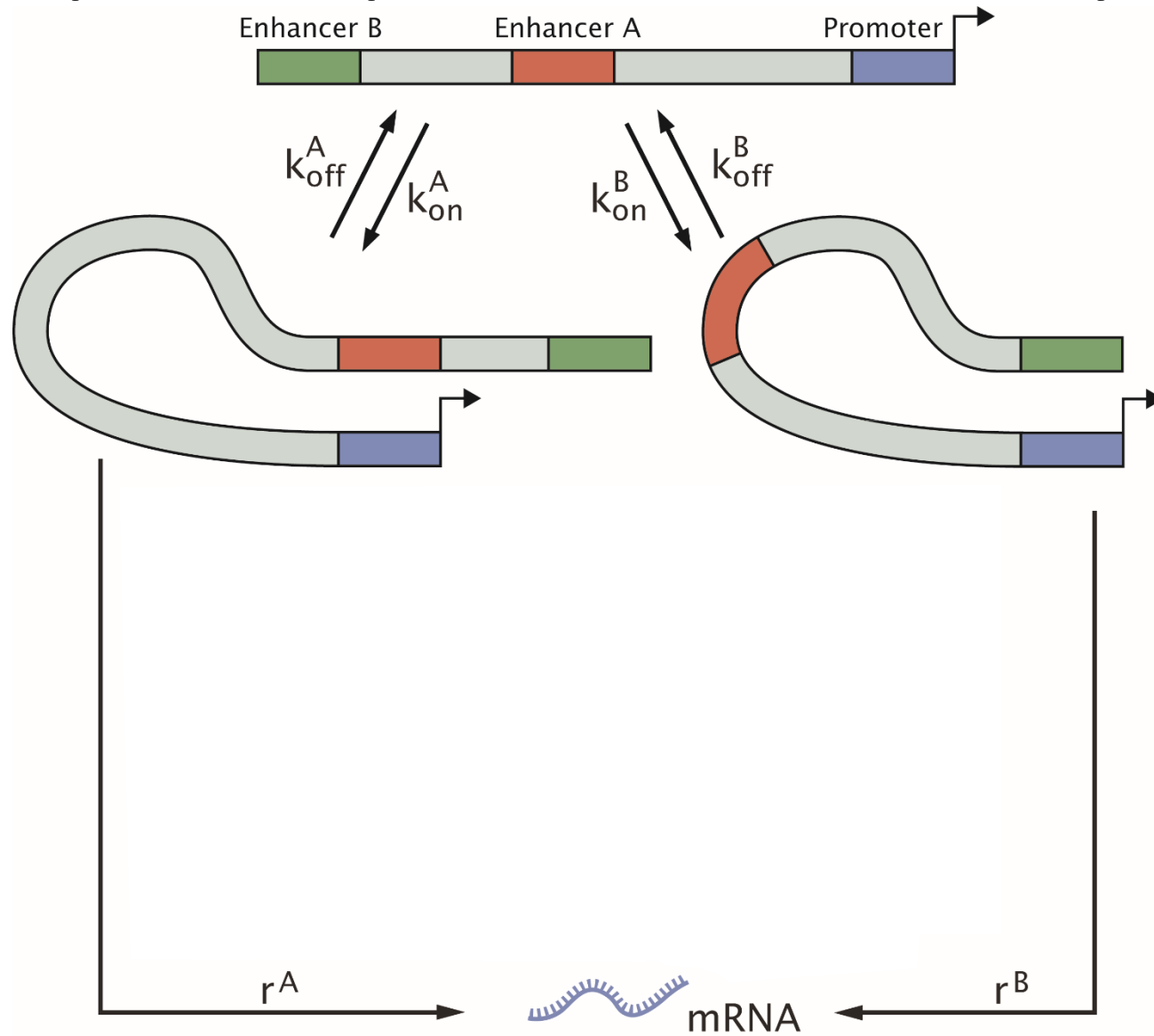
# Multiple regimes of enhancer action

# Why multiple enhancers?

Competition for the real
estate of the promoter

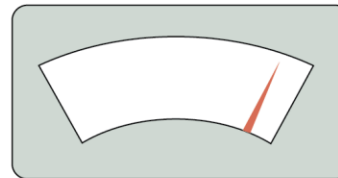Cooperative interaction
with the promoter

**Sub-additivity**
Strong enhancer interference

**Additivity**
Weak enhancers working
independently of each other

**Super-additivity**
Weak enhancers
working synergistically

multiple enhancer
activity

# Acknowledgements



Jacques Bothma



Thomas Gregor



Mike Levine

PRINCETON UNIVERSITY

Dicke Fellowship

Berkeley
UNIVERSITY OF CALIFORNIA