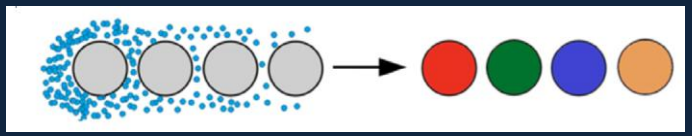
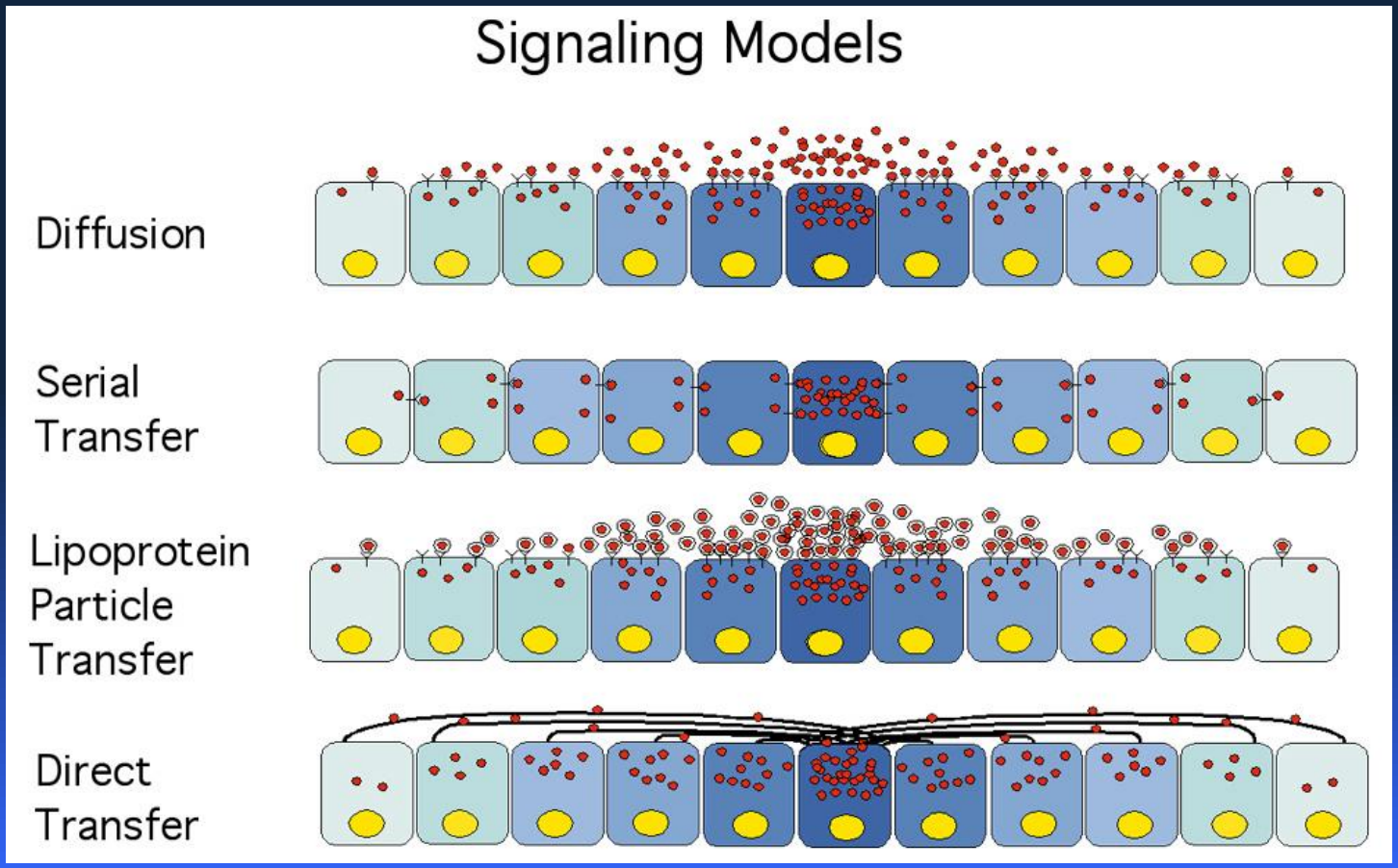
Direct delivery mechanisms of morphogen dispersion

Thomas Kornberg, UCSF

How do signaling proteins like Hh and Dpp move across fields of cells?

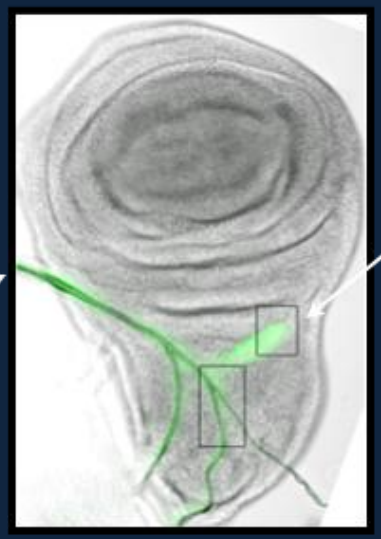
Signaling protein gradients elicit concentration-dependent responses from target cells





Wing disc and trachea make specific contacts

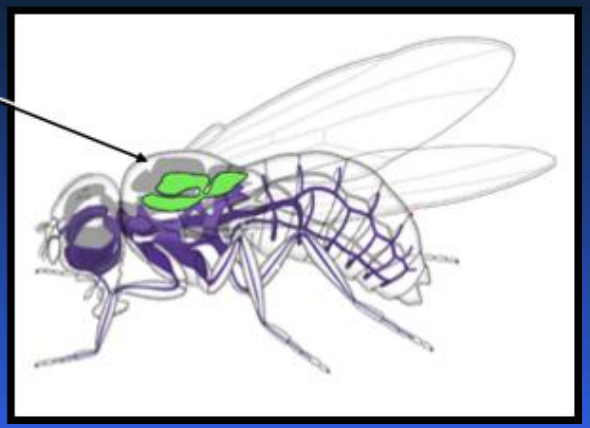
wing disc



Air sac primordium (FGF-induced tube)

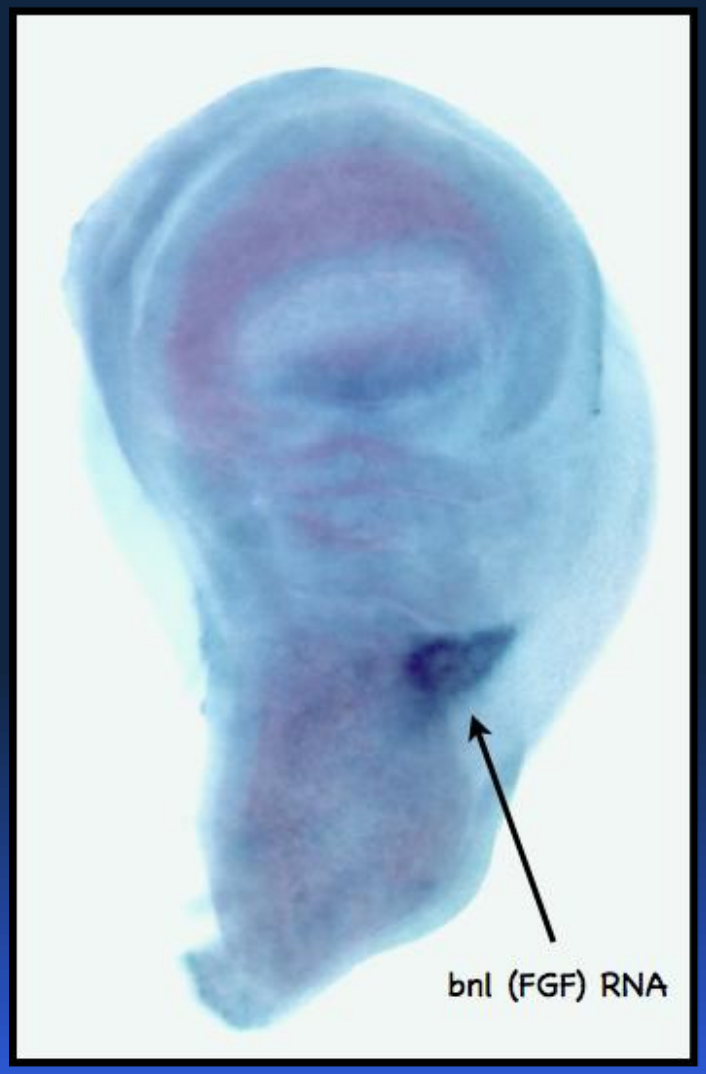
trachea
(Tr2 transverse connective)

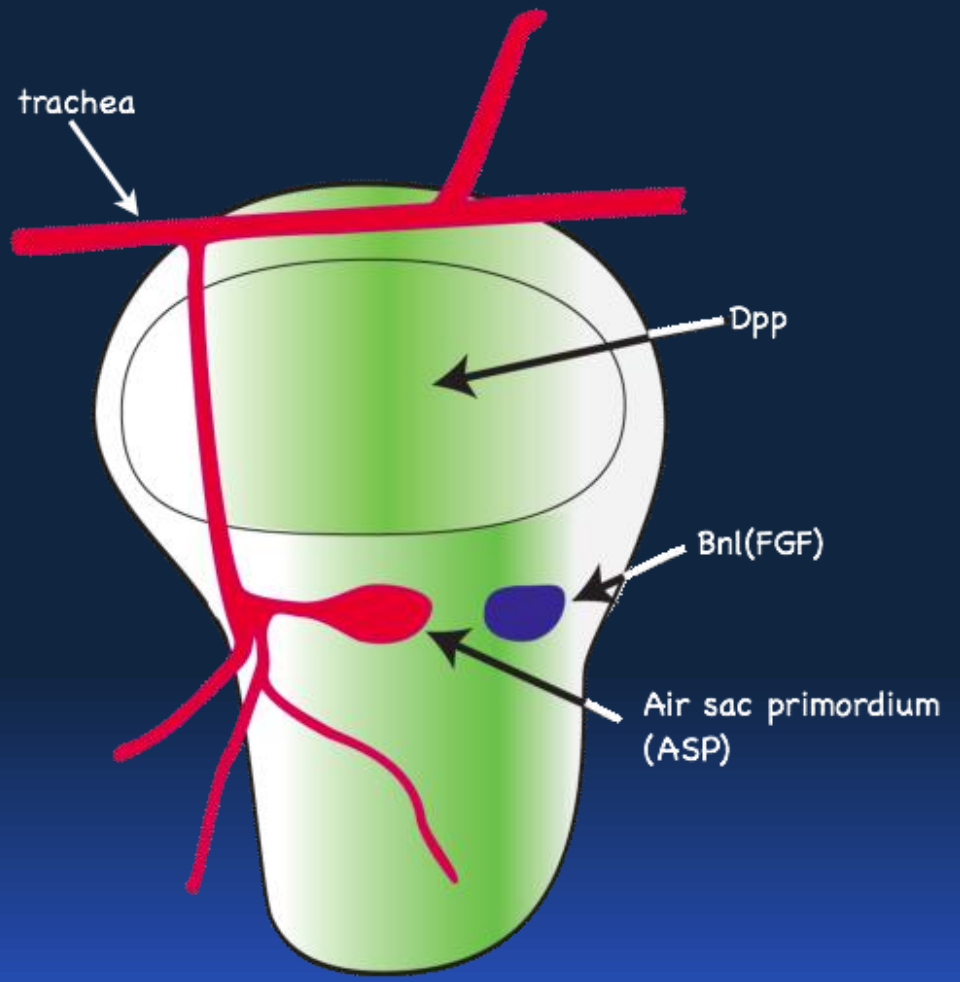
Dorsal Air Sacs



Dorsal air sacs - multi-lobed organs that function in gas exchange, supplying oxygen to the flight muscles

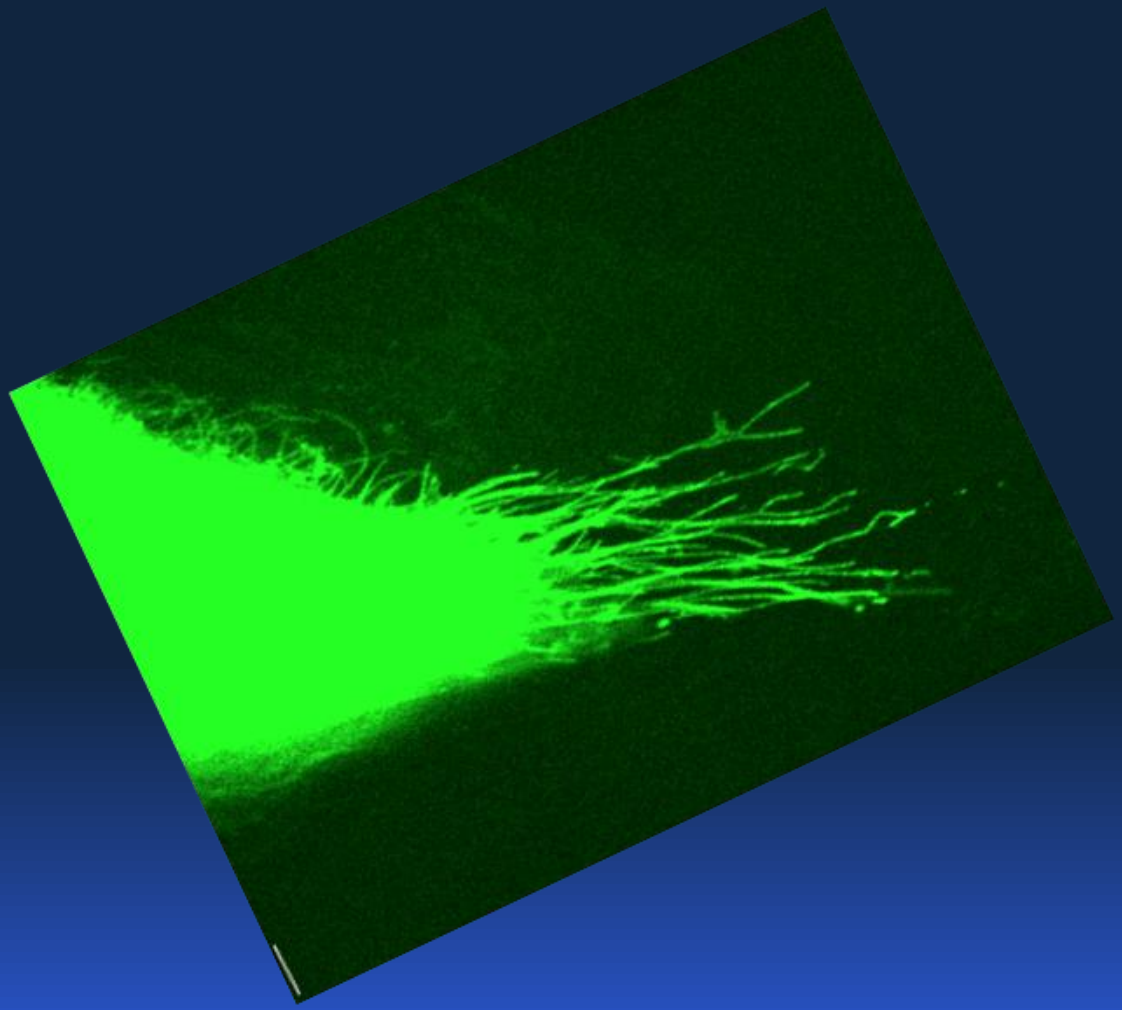
Bnl(FGF) expression in the wing disc



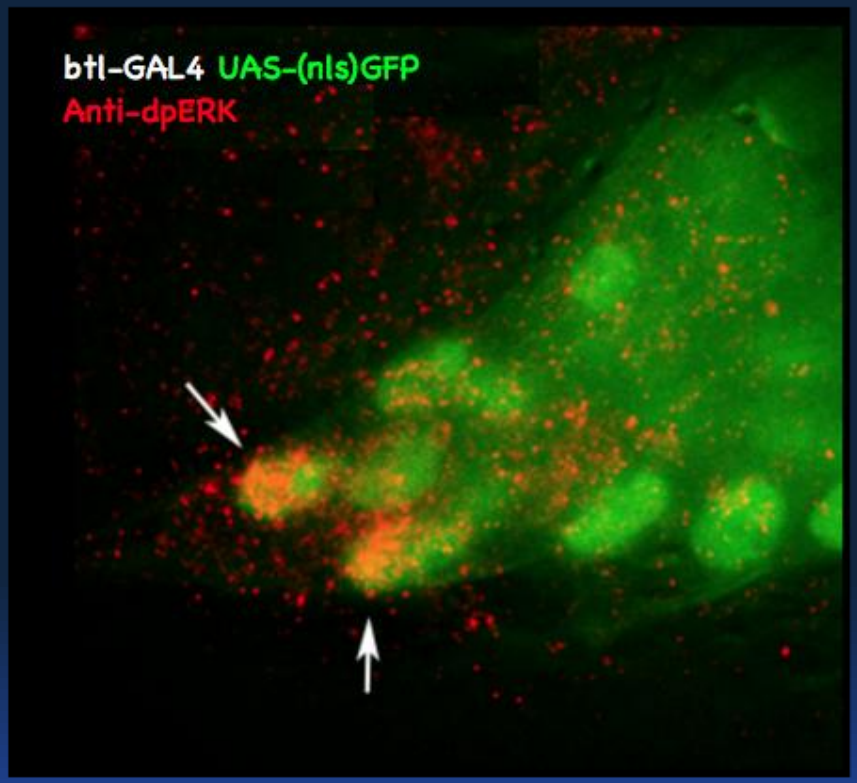


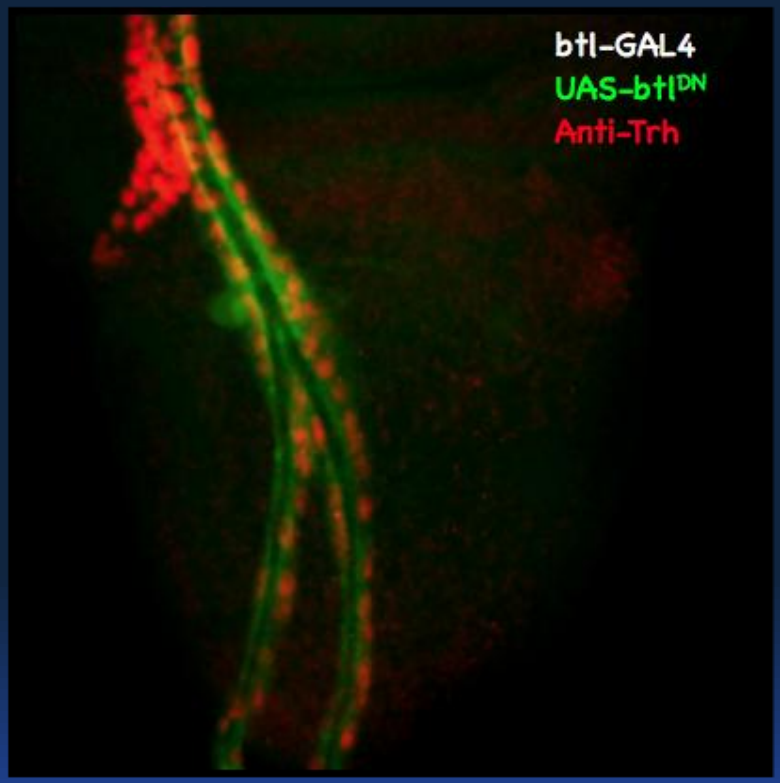
adapted from:
Sato and Kornberg, (2002)

Cytonemes extend from the tip of the ASP to the disc cells expressing Bnl(FGF)



ASP growth and morphogenesis requires Bnl(FGF) signaling

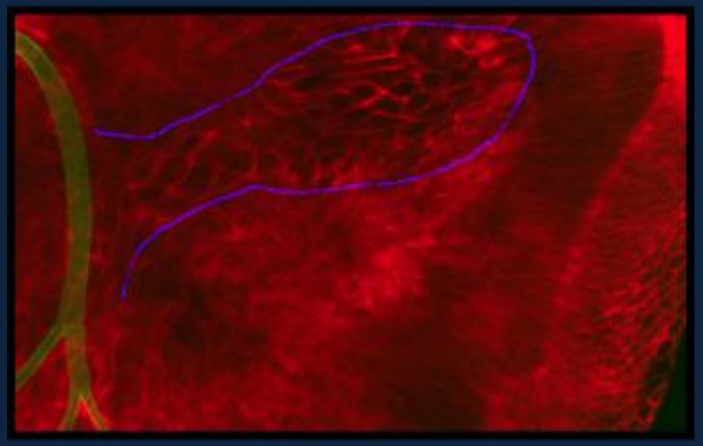




adapted from:
Sato and Kornberg, (2002)

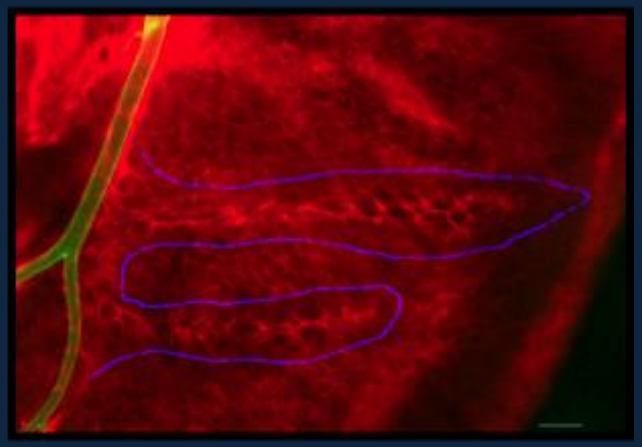
Dpp is required for ASP morphogenesis

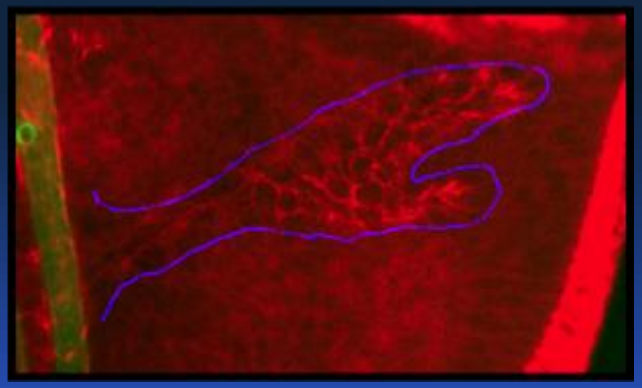
WT



Anti-Discs large

dpp^{ts}

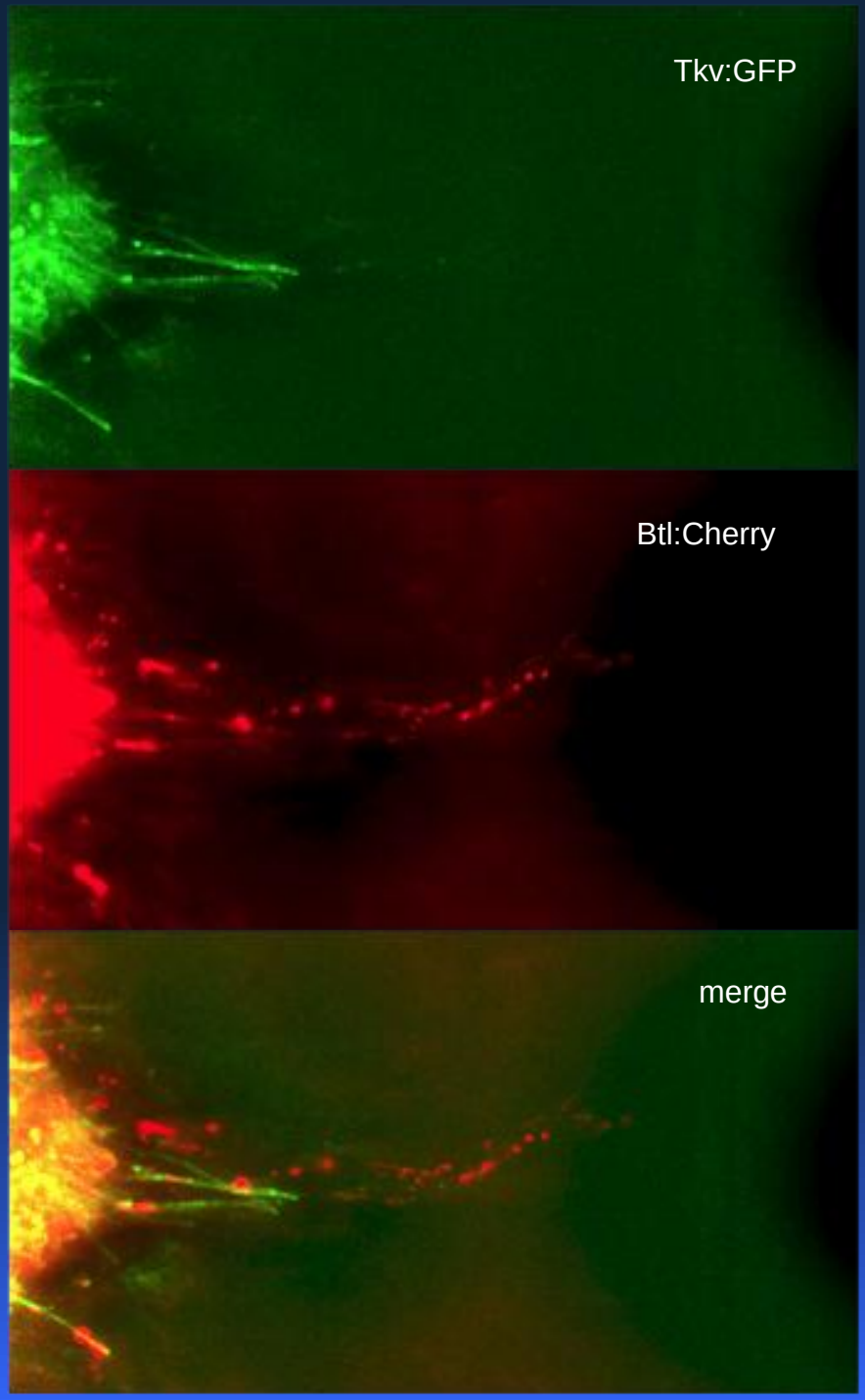




Dpp receptor Tkv localizes to ASP cytonemes

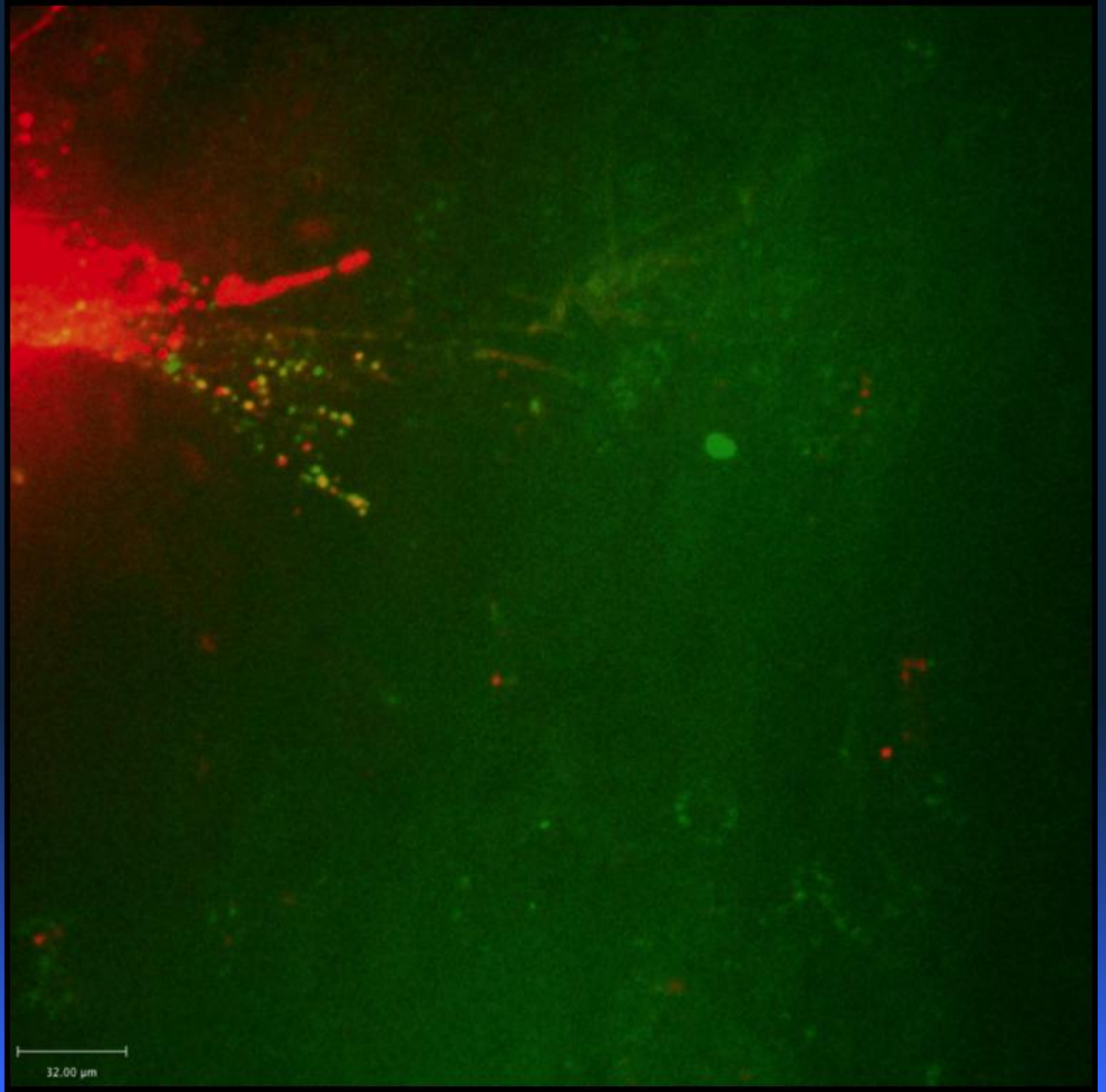
FGF receptor Btl localizes to ASP cytonemes

Dpp and FGF receptors segregate to different cytonemes

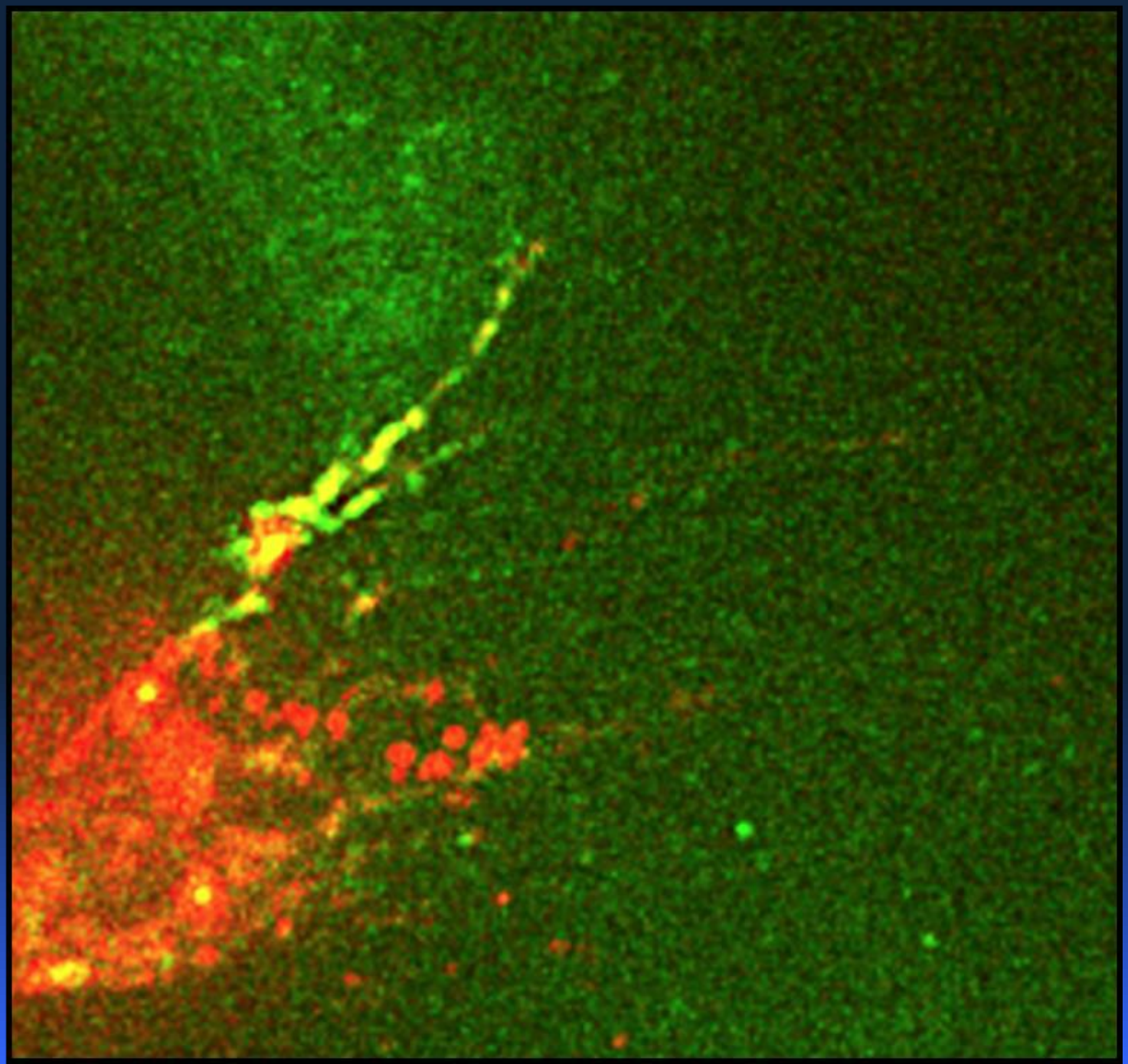


adapted from:
Roy et al, (2011)

LexA>Dpp:GFP
Gal4>Tkv:Cherry

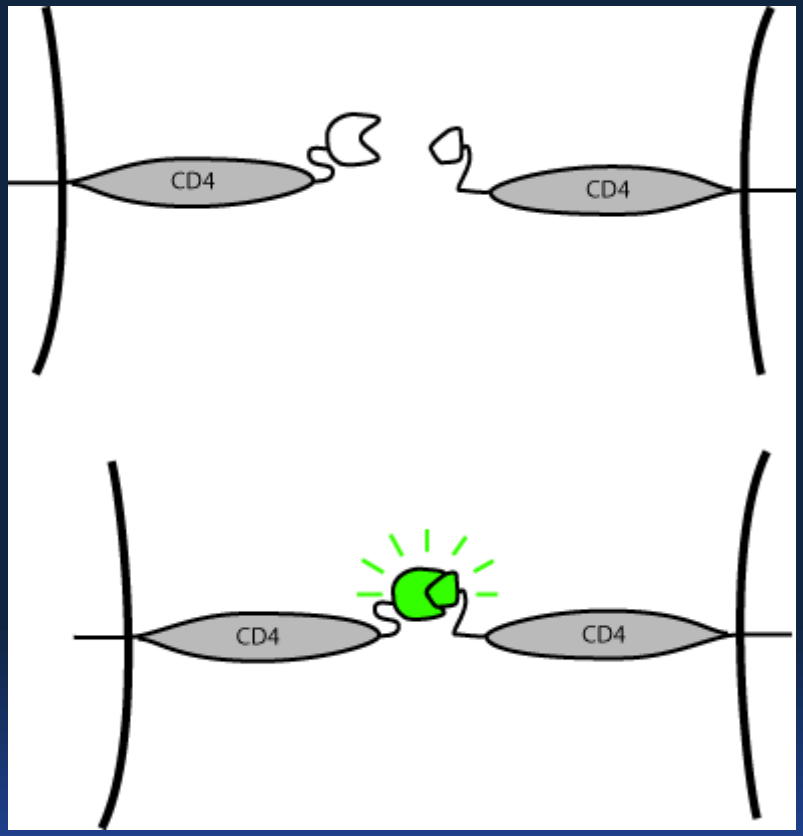


LexA>Dpp:GFP
Gal4>CD8:Cherry



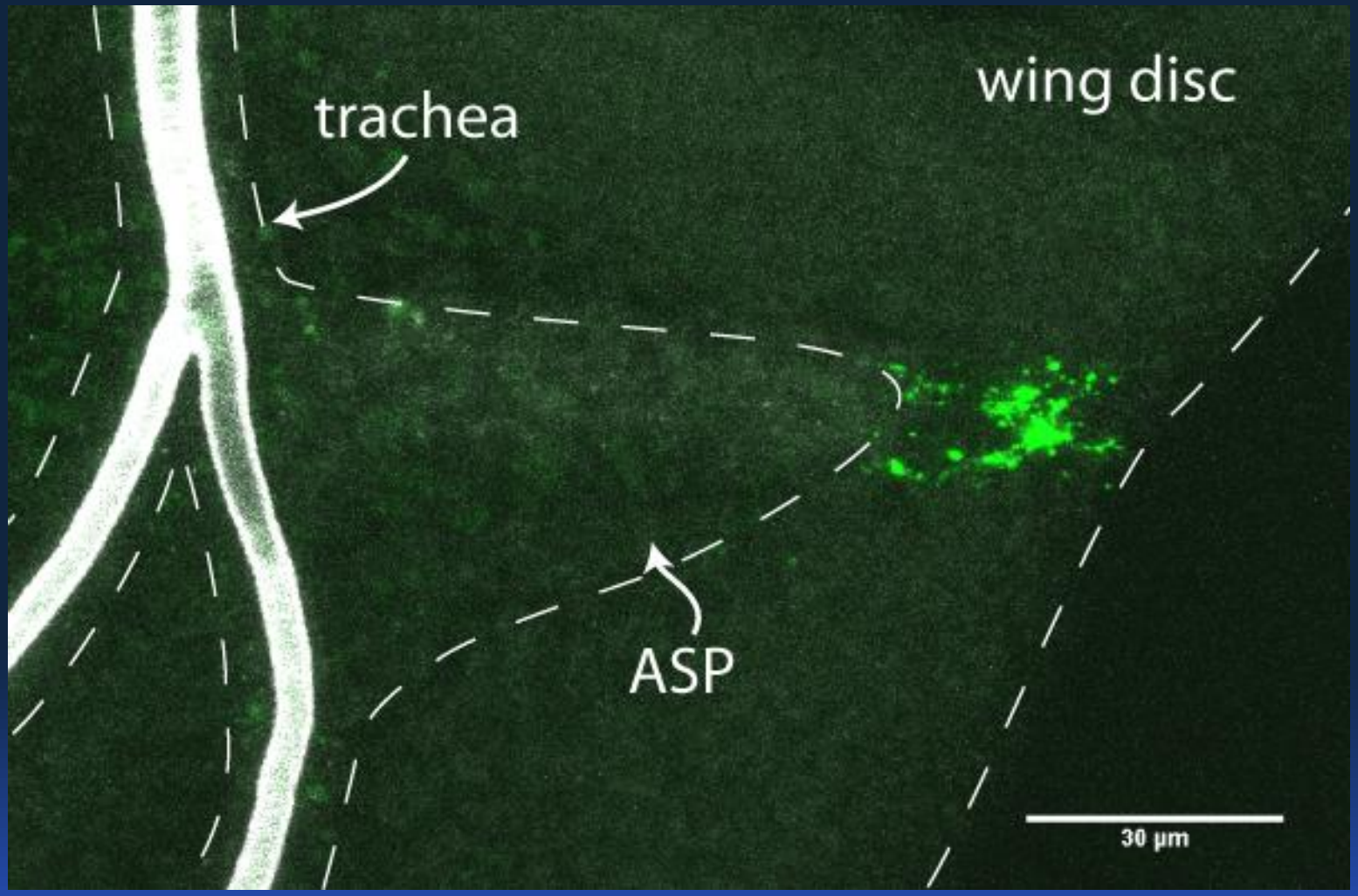
GRASP – split GFP complementation

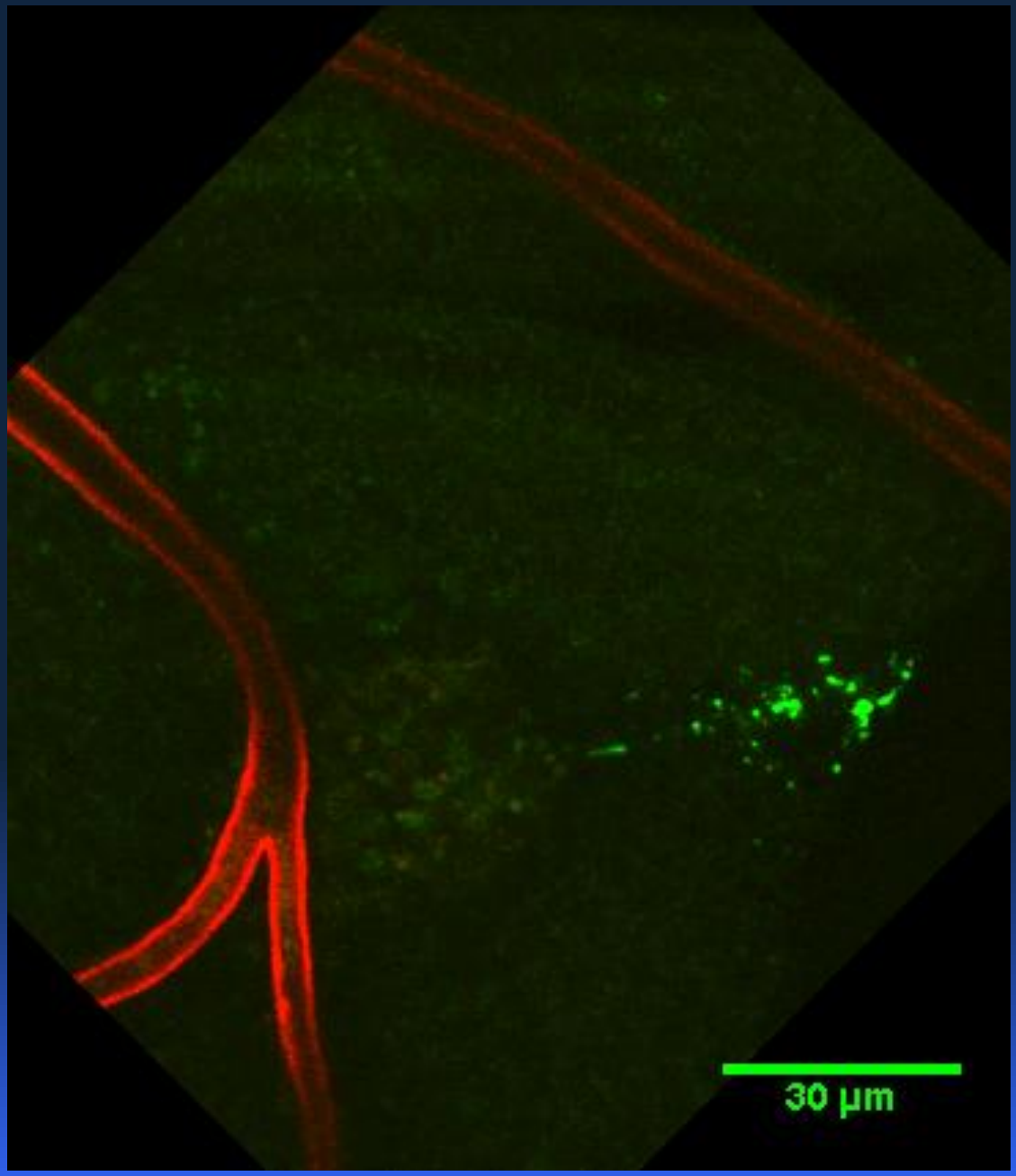
CD4:GFP1-10



CD4:GFP11

Feinberg, Bargmann et al (2008)
Neuron 57, 353-363


wing disc
trachea
ASP
30 µm


30 µm

Conclusions

- Cytonemes are ligand-specific signaling filopodia.
- Morphogen signaling proteins can disperse by moving along cytonemes after transfer from producing to target cells at points of direct contact.
- Asymmetric cell processes that traffic signals are a feature common to many (all?) cell types and they are not unique to neurons.

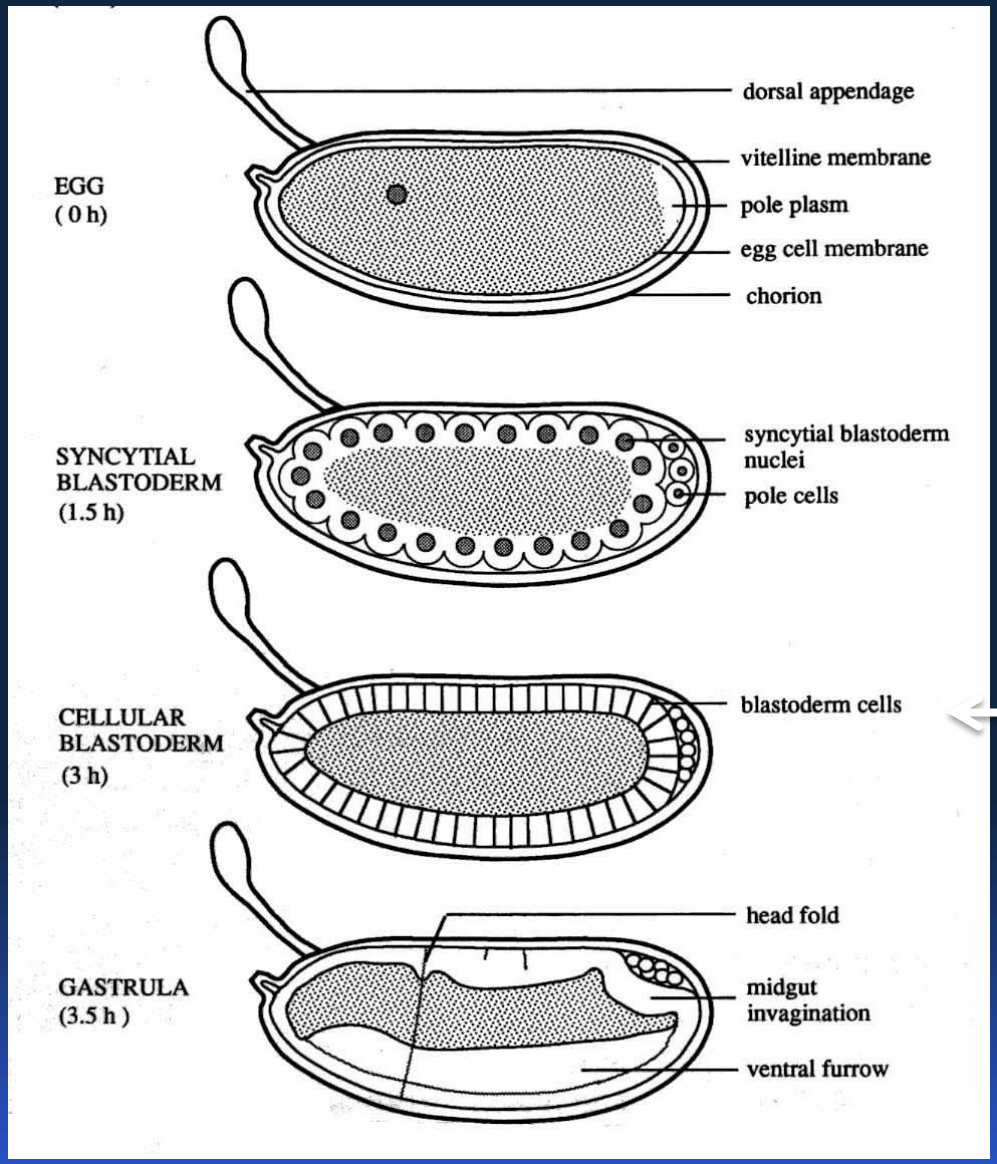
People who did the work:

Felipe-Andrés Ramírez-Weber
Frank Hsuing
Makoto Sato
Sougata Roy

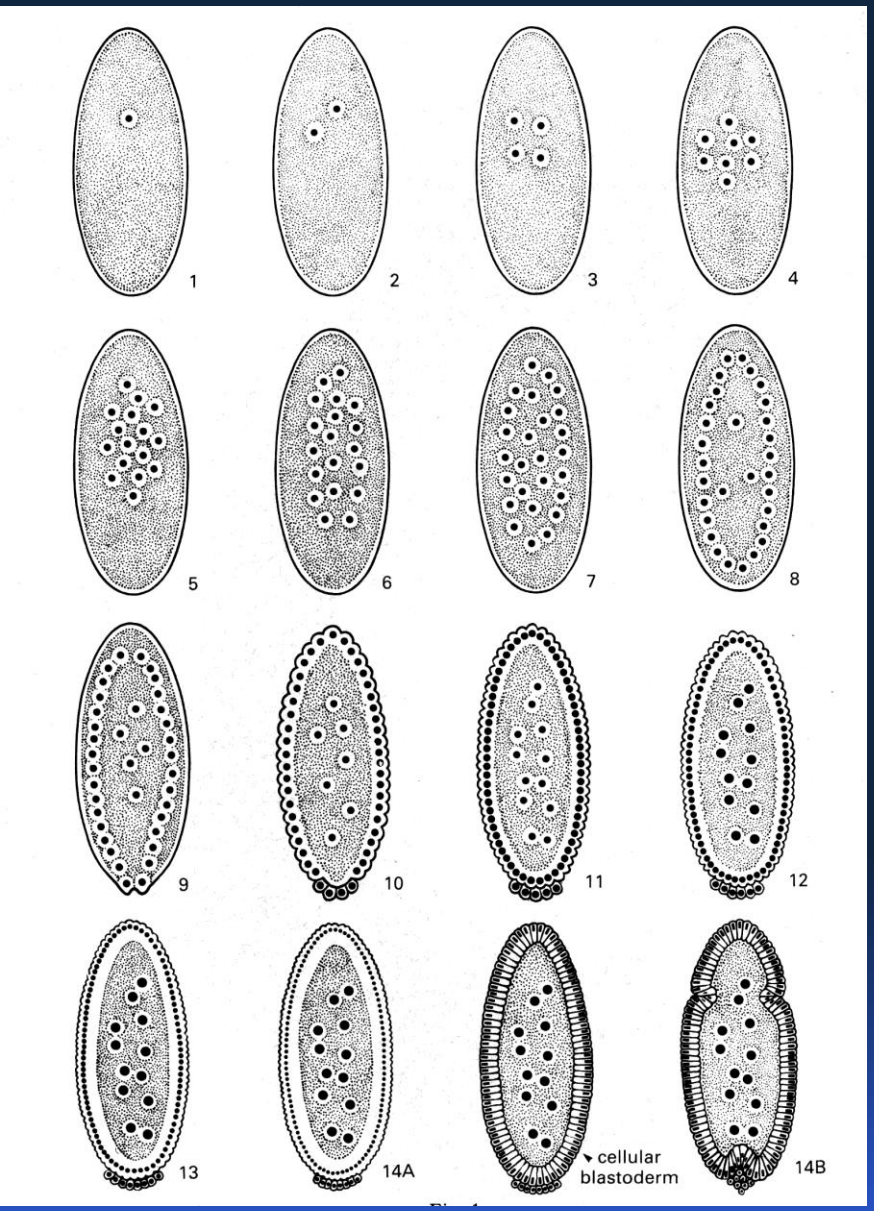
Regulation and gene expression in the (early) Drosophila embryo

"Wolpert doctrine"

"It is not birth, marriage or death [or the kids moving out of the house or the dog dying] but *gastrulation* which is truly the most important time of your life."



mid-bastoderm transition (MBT)



The cell cycles in the early Drosophila embryo are rapid (9.6 minutes) and proceed synchronously without cytokinesis in a syncytium.

The cell cycle times begin to slow after nine cycles, but synchrony remains remarkably strict until after the 14th cycle when cells form and gastrulation begins.

Dogma: the interphase period of the rapidly dividing syncytial nuclei (3.4 min) is too short for functional transcripts to be made or for mRNA to be made and translated.

Development 104, 495–509 (1988)
Printed in Great Britain

Requirements for autosomal gene activity during precellular stages of *Drosophila melanogaster*

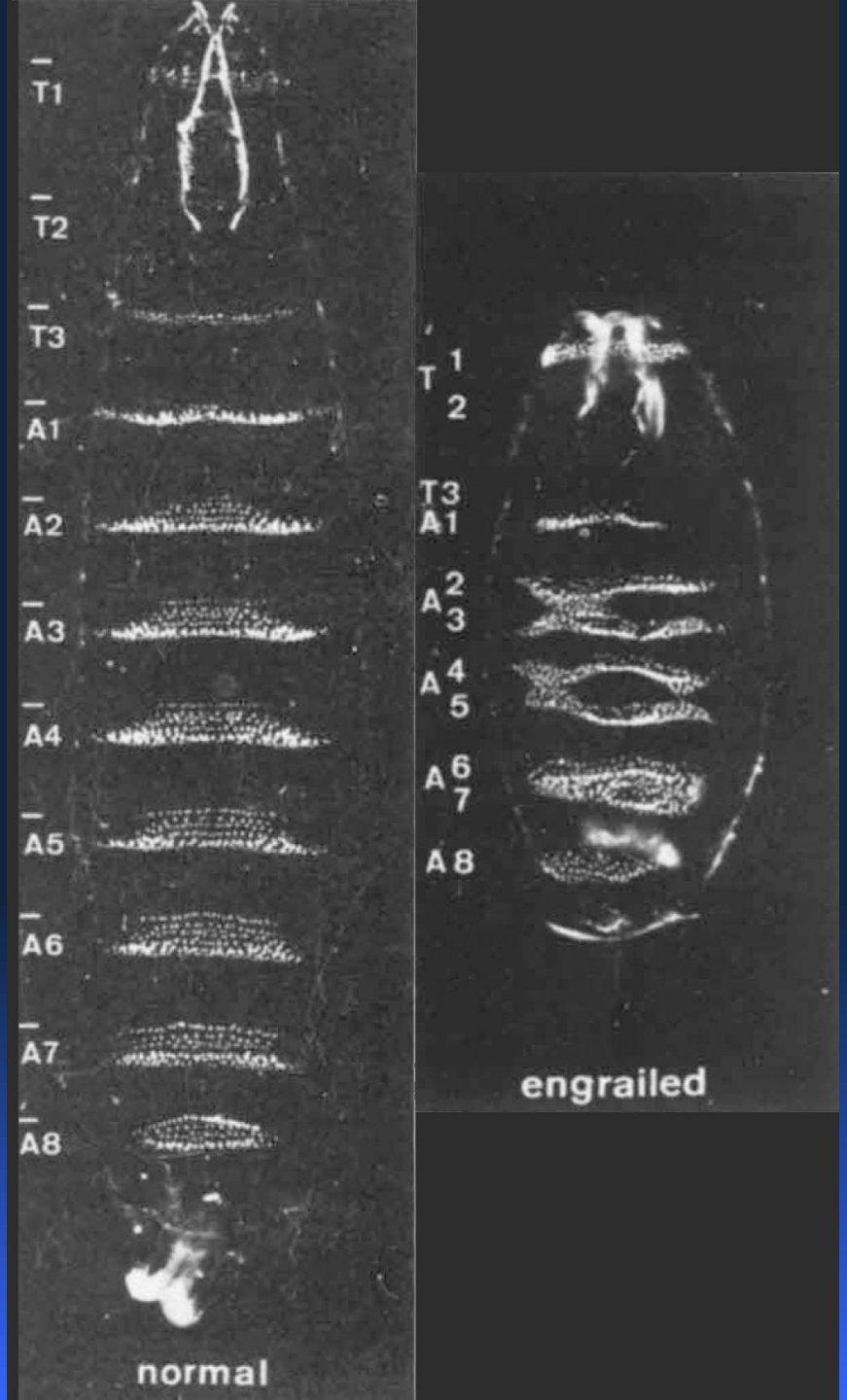
PAULINE T. MERRILL, DARI SWEETON and ERIC WIESCHAUS

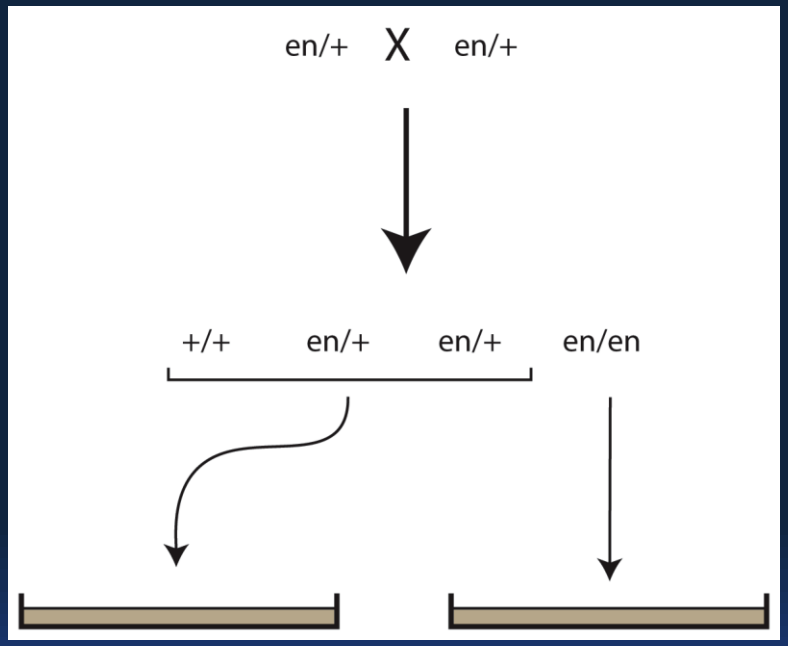
Department of Biology, Princeton University, Princeton, NJ 08540, USA

Summary

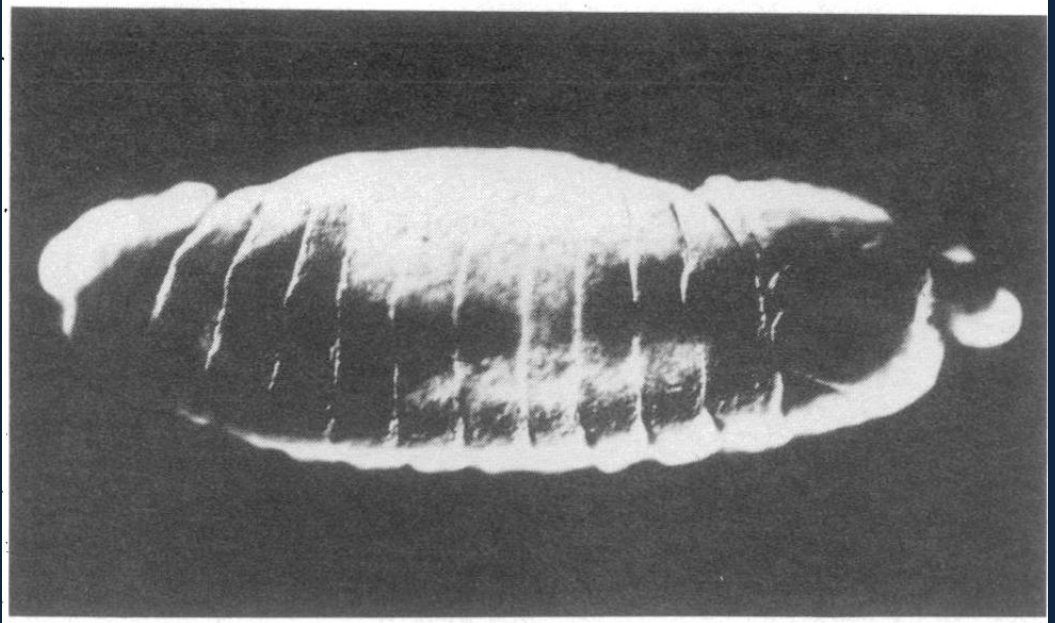
To identify early requirements for zygotic gene activity in *Drosophila*, we used compound autosomes and autosome-Y translocations to generate embryos deficient for cytologically defined portions of the genome. No obvious gross morphological defects were observed in any deficiency class until the beginning of cycle 14. Only seven autosomal regions were identified with discrete effects visible prior to the onset of gastrulation. These regions include genes with locus-specific effects on the clearing of the cortical cytoplasm during early cycle 14, (22AB), the initiation of the slow and fast phases of cellularization (26BF and 40AC, respectively), the apical–basal distribution of nuclei during cycle 14 (71C–75C) and the closing off of furrow canals during cellularization (100AC). The distal tip of the third chromosome also contains two loci (99DF and 100AC) whose deletion causes multiple nuclei to be cellularized into single cells, a phenotype similar to that produced in embryos totally lacking the X-chromosome.

Key words: *Drosophila melanogaster*, autosomal gene activity, precellular stages.

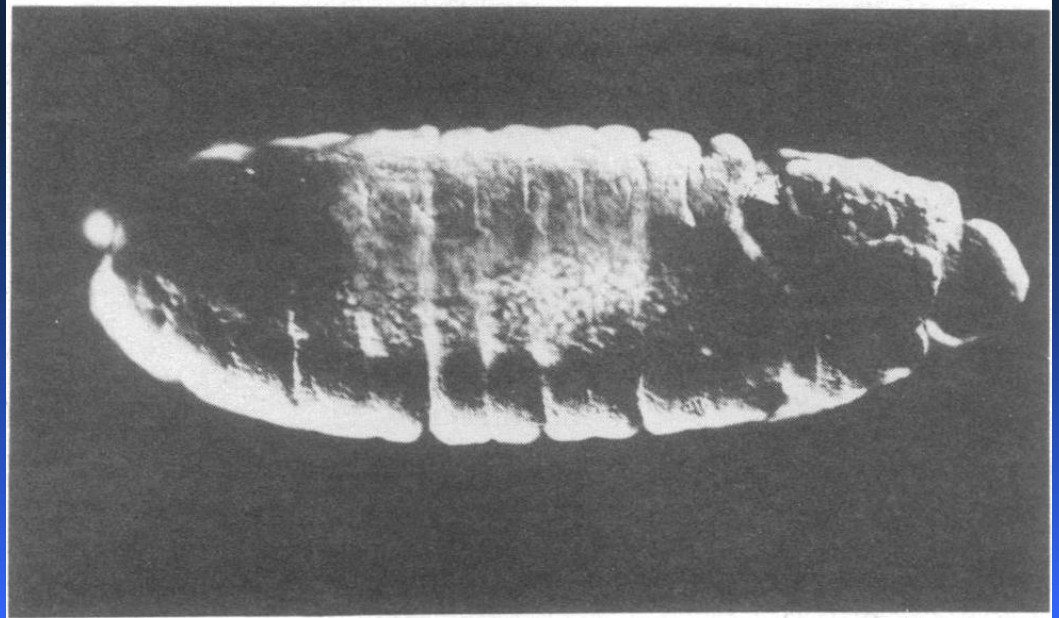

T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
normal
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
engrailed


en/+ X en/+
+/+ en/+ en/+ en/en

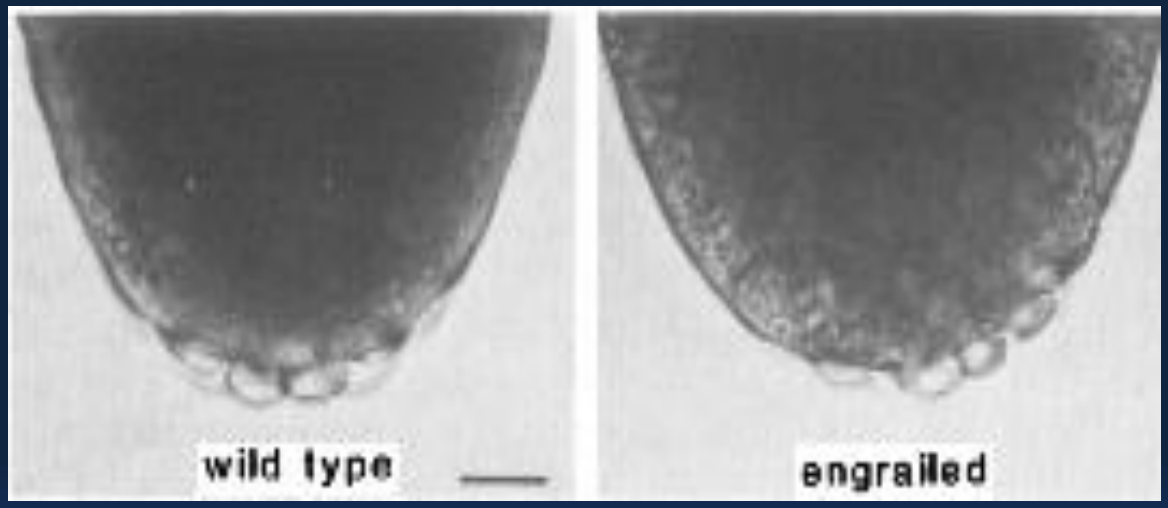
Wild type



engrailed



Zehra Ali


wild type
engrailed

Cell, Vol. 43, 591–601, December 1985 (Part 2), Copyright © 1985 by MIT

The *engrailed* Locus of D. melanogaster Provides an Essential Zygotic Function in Precellular Embryos

Timothy L. Karr, Zehra Ali, Barry Drees, and Thomas Kornberg
Department of Biochemistry and Biophysics
University of California
San Francisco, California 94143

Summary

Early embryonic development in Drosophila depends on genes expressed during oogenesis or after zygote formation. We show that the *engrailed* gene is needed for the processes that organize the embryo during the nuclear divisions that precede cellularization. During the precellular blastoderm stages *engrailed* mutant embryos show several notable anomalies: the pole cells form at a position slightly displaced from the posterior pole; yolk nuclei continue to divide after the tenth nuclear division cycle, when wild-type yolk nuclei have stopped dividing mitotically; and somatic nuclei are not positioned uniformly along the embryo periphery and do not undergo mitotic divisions in regular waves. This early requirement for engrailed does not appear to be a maternal function, and only genetically *engrailed* embryos displayed these precellular phenotypes. Synthesis of a 2.7 kb poly(A)$^+$ transcript of the *engrailed* region was found in precellular embryos.

Development 104, 495–509 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

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Key words: *Drosophila melanogaster*, autosomal gene activity, precellular stages.

Embryos deficient for 2R cellularize normally but fail to make ventral furrows at gastrulation

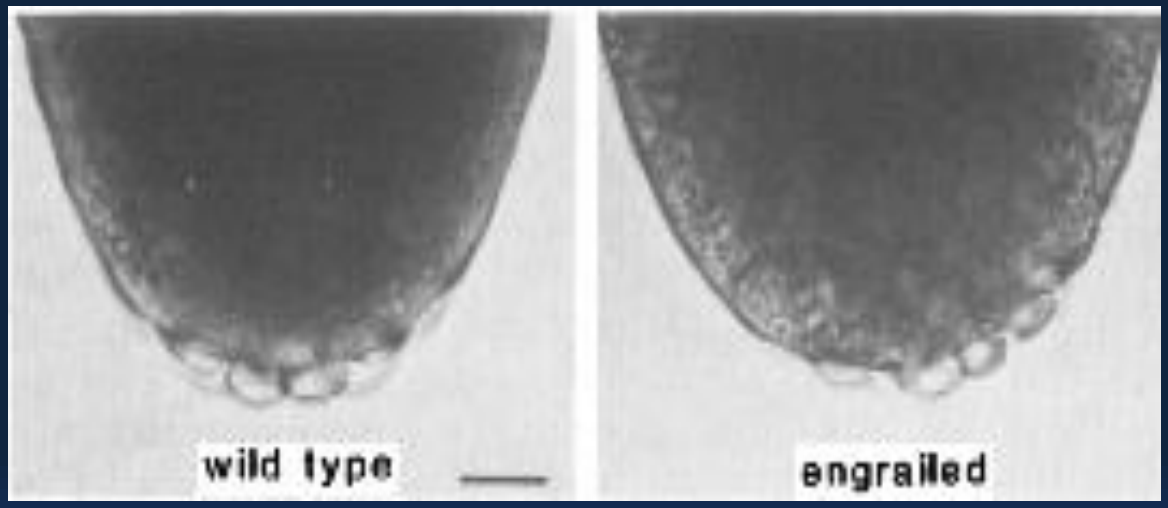
The normal appearance of $2R^-$ embryos through cellularization was unexpected, given the observation by Karr *et al.* (1985) that embryos homozygous for a mutation on 2R (*engrailed*) become abnormal during cleavage divisions. Using our mounting procedures, we could not detect the reported right–left asymmetry of the pole cells in living embryos shown subsequently deficient for 2R, nor have we observed any other consistent pregastrulation defects in the embryos we videotaped (Fig. 2, Table 2).

Zehra takes maternity leave

No takers

20+ years later ….

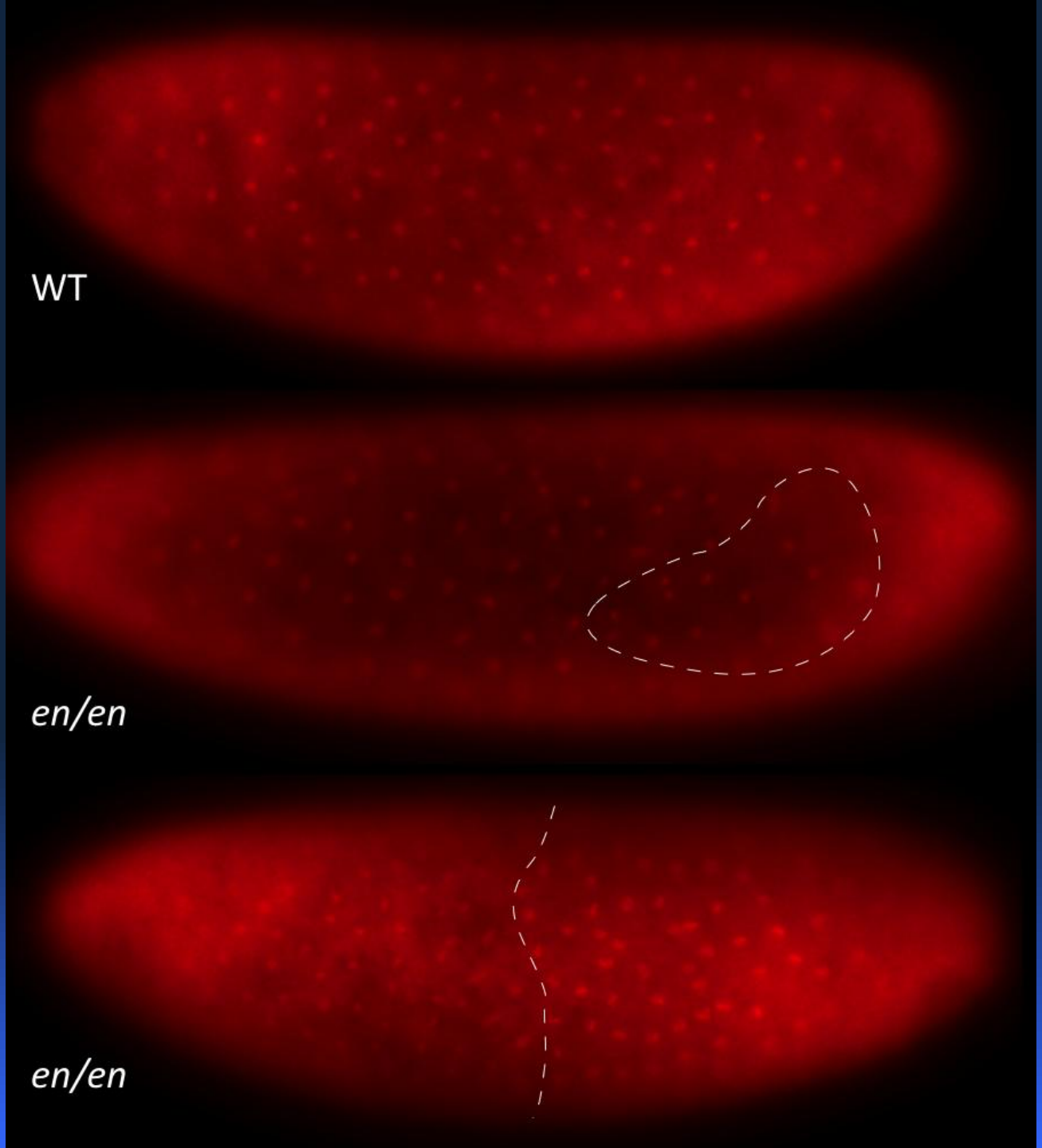
Zehra returns from maternity leave


wild type
engrailed

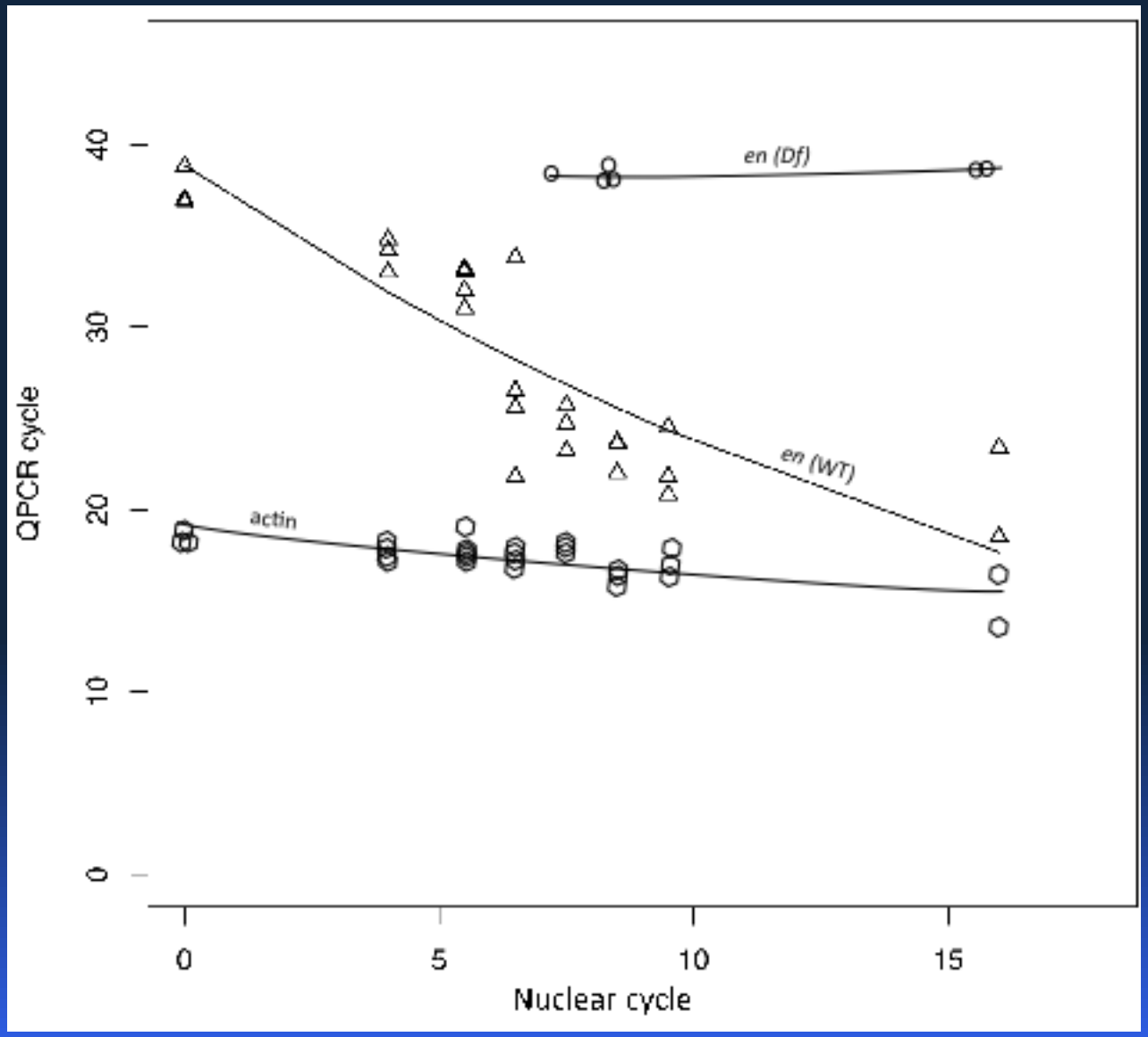
20+ years later …

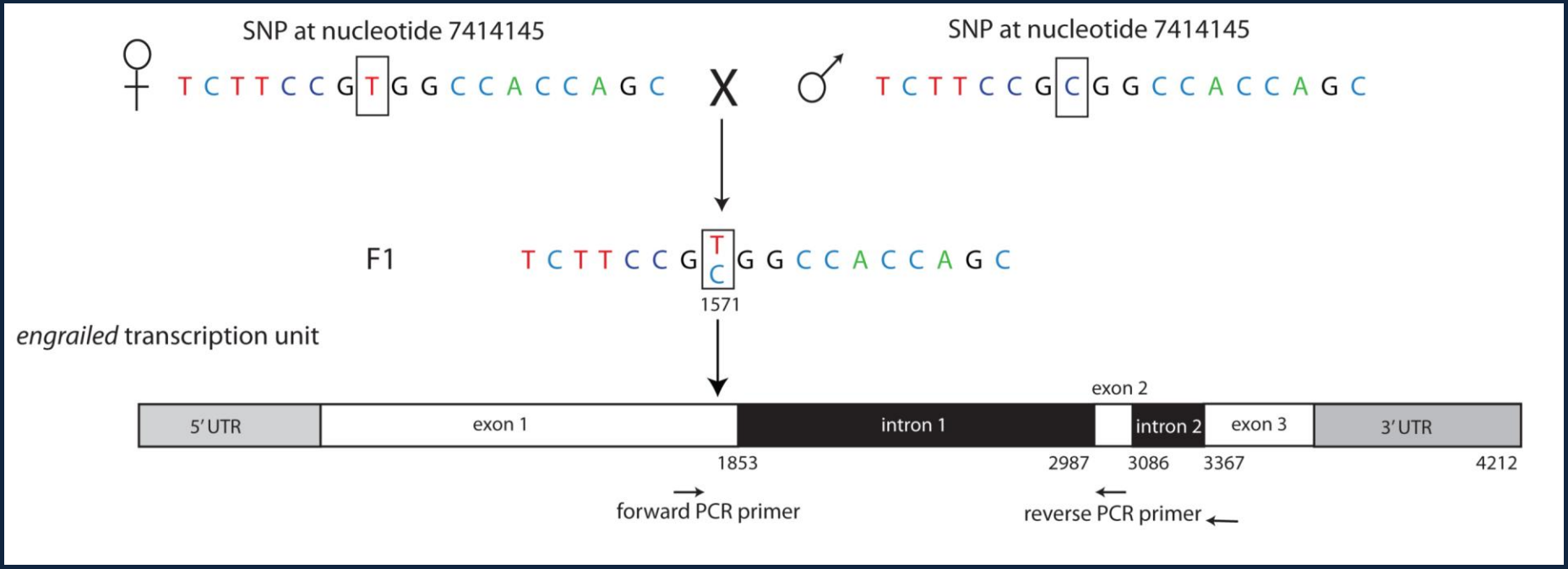
Many new techniques: fluorescent proteins, PCR, genomic analysis

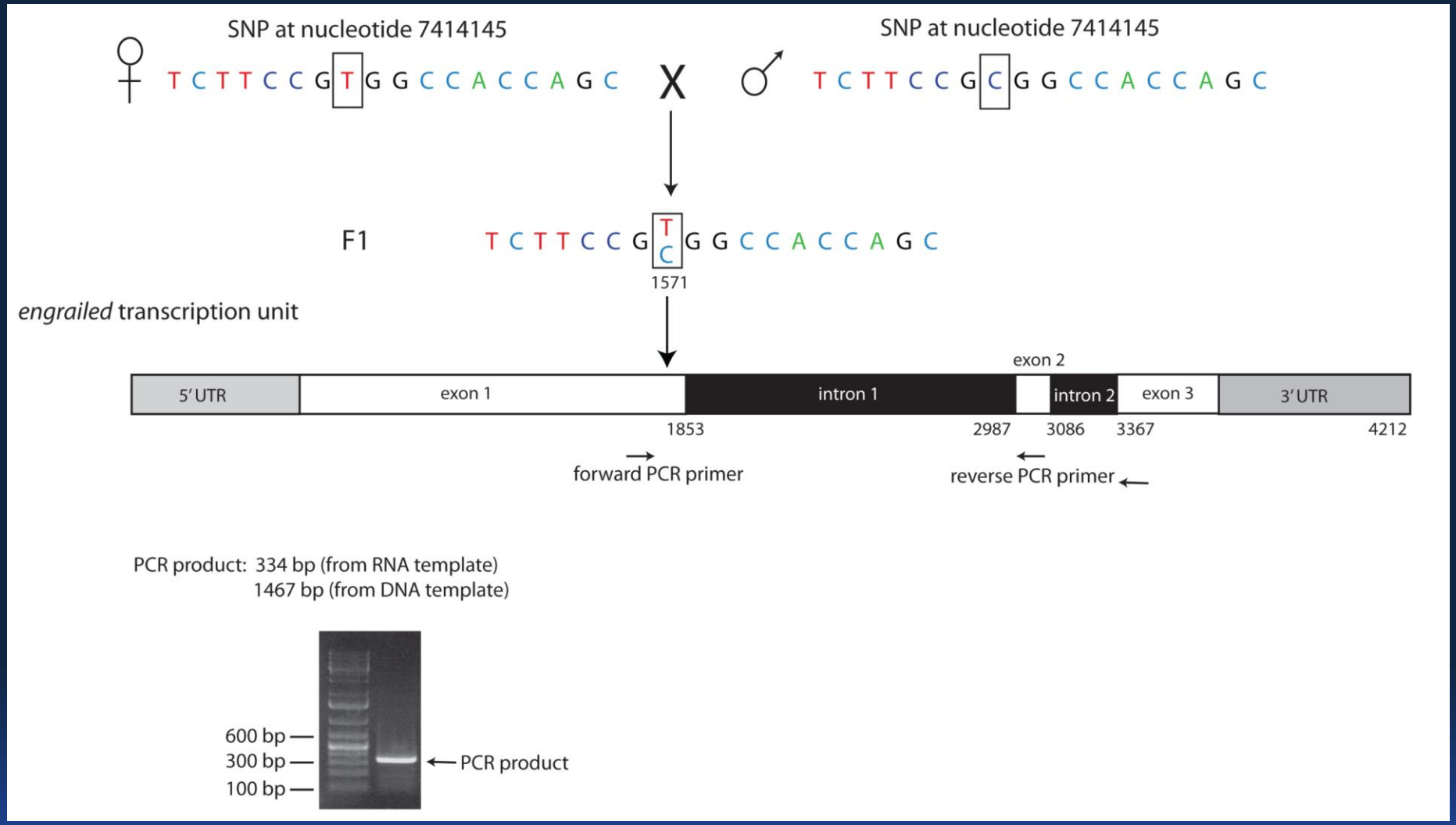
Histone:RFP

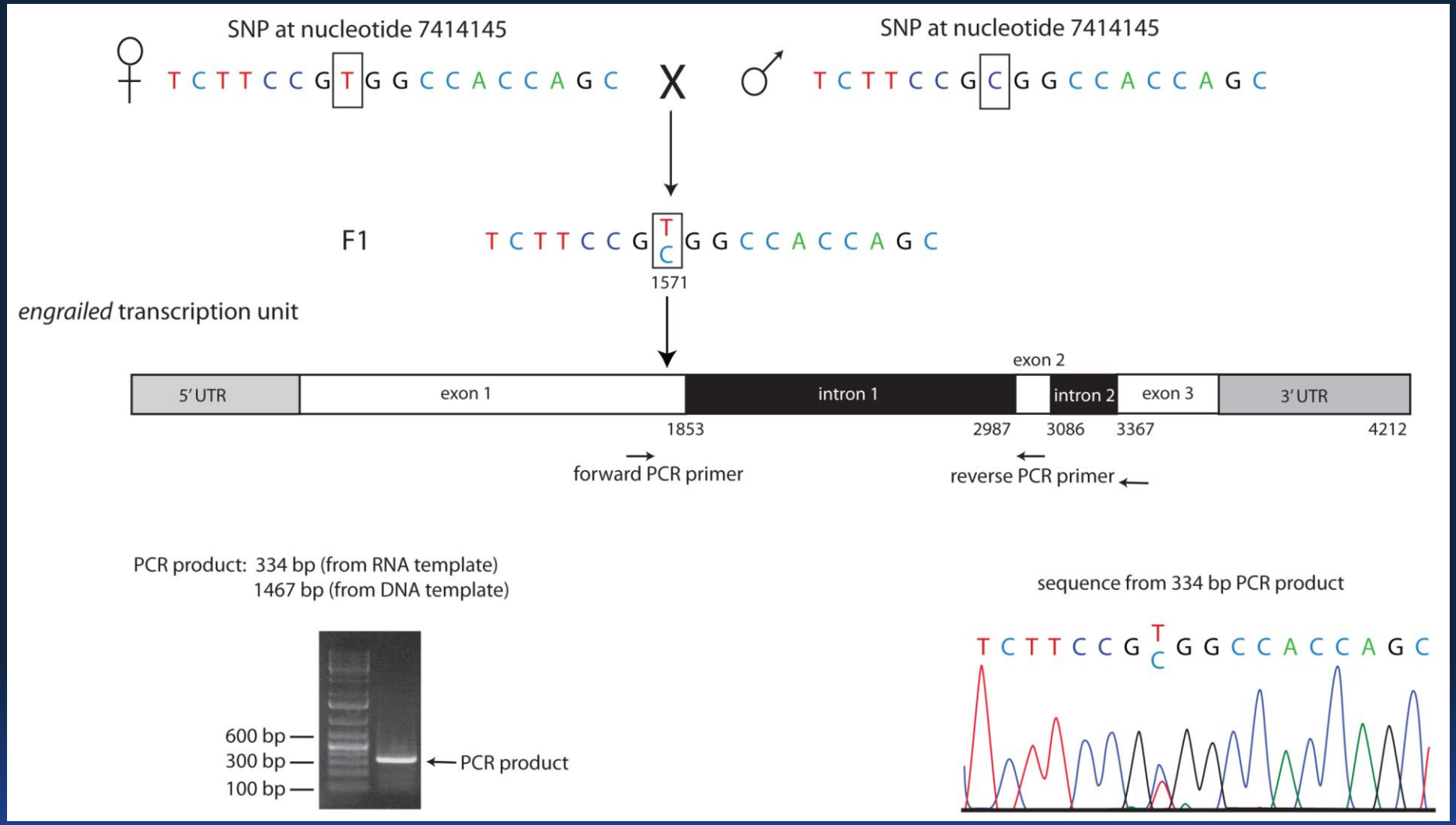


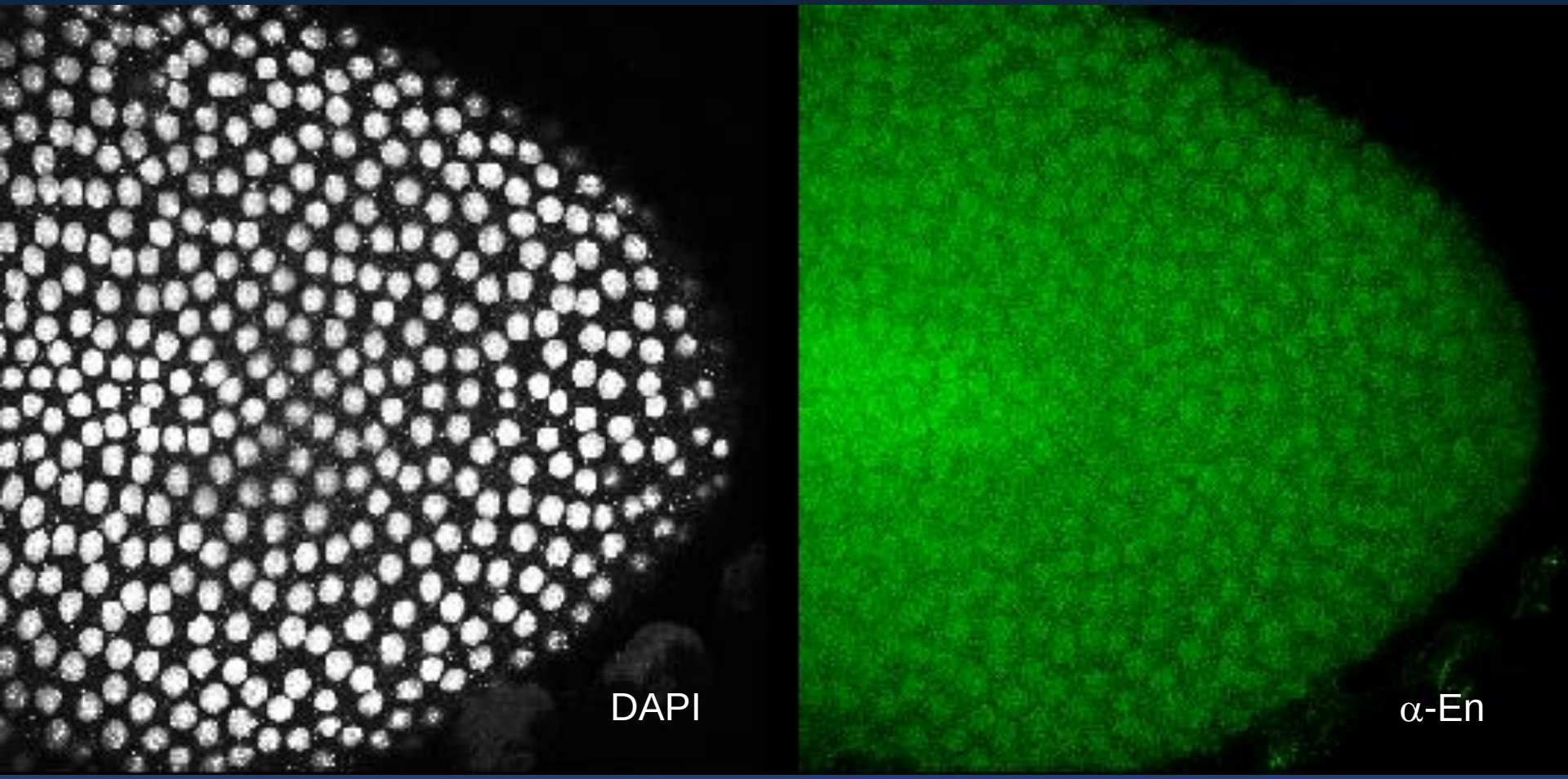
Q-PCR



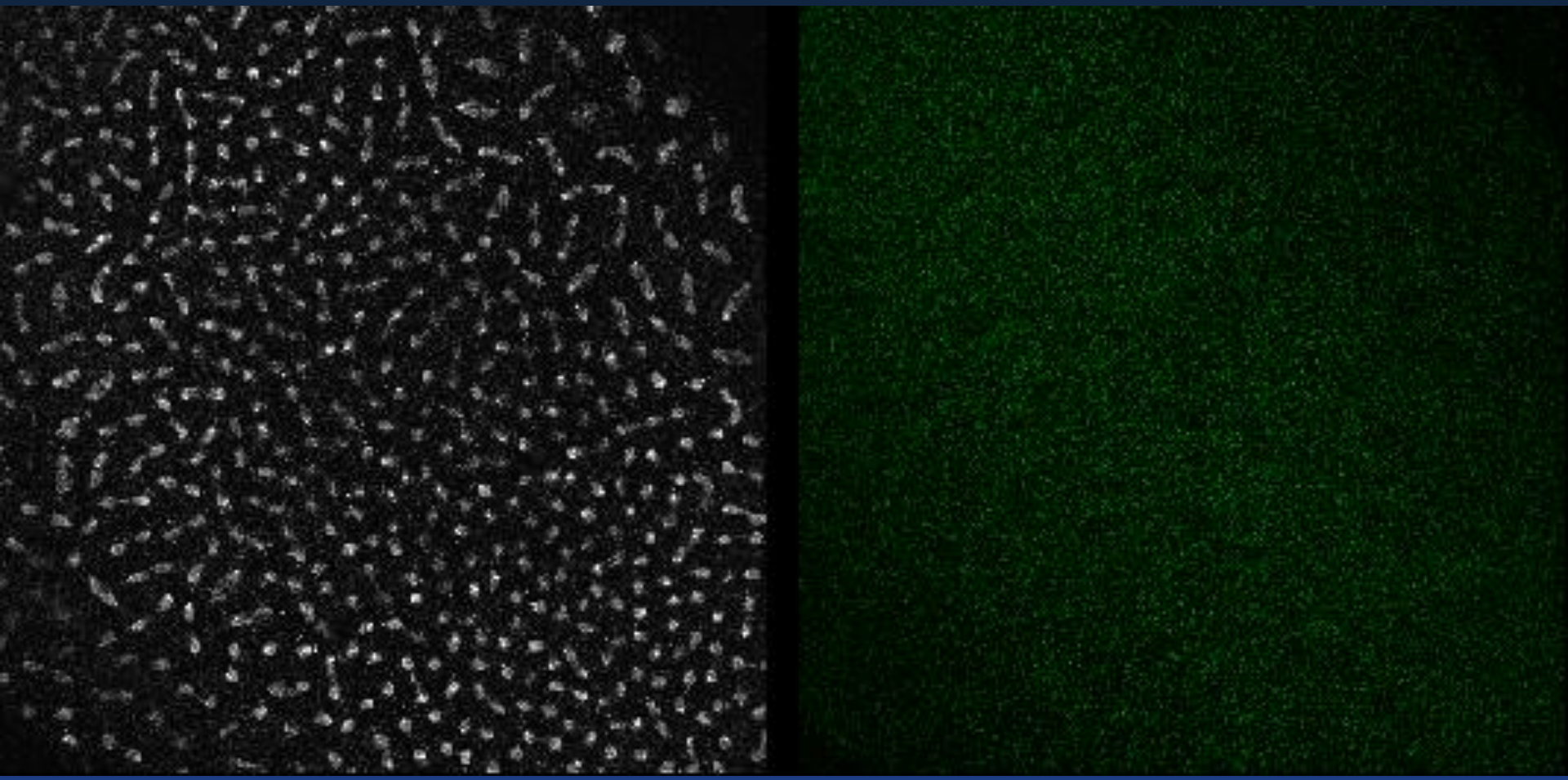

SNP at nucleotide 7414145
T C T T C C G T G G C C A C C A G C
X
SNP at nucleotide 7414145
T C T T C C G C G G C C A C C A G C
F1
T C T T C C G T/C G G C C A C C A G C
1571
engrailed transcription unit
exon 2
5′ UTR
exon 1
intron 1
intron 2
exon 3
3′ UTR
1853
2987
3086
3367
4212
forward PCR primer
reverse PCR primer


SNP at nucleotide 7414145
T C T T C C G T G G C C A C C A G C
X
SNP at nucleotide 7414145
T C T T C C G C G G C C A C C A G C
F1
T C T T C C G T/C G G C C A C C A G C
1571
engrailed transcription unit
5′ UTR
exon 1
intron 1
exon 2
intron 2
exon 3
3′ UTR
1853
2987
3086
3367
4212
forward PCR primer
reverse PCR primer
PCR product: 334 bp (from RNA template)
1467 bp (from DNA template)
600 bp
300 bp
100 bp
PCR product


SNP at nucleotide 7414145
T C T T C C G T G G C C A C C A G C
X
SNP at nucleotide 7414145
T C T T C C G C G G C C A C C A G C
F1
T C T T C C G T/C G G C C A C C A G C
1571
engrailed transcription unit
exon 2
5′ UTR
exon 1
intron 1
intron 2
exon 3
3′ UTR
1853
2987
3086
3367
4212
forward PCR primer
reverse PCR primer
PCR product: 334 bp (from RNA template)
1467 bp (from DNA template)
600 bp
300 bp
100 bp
PCR product
sequence from 334 bp PCR product
T C T T C C G T/C G G C C A C C A G C



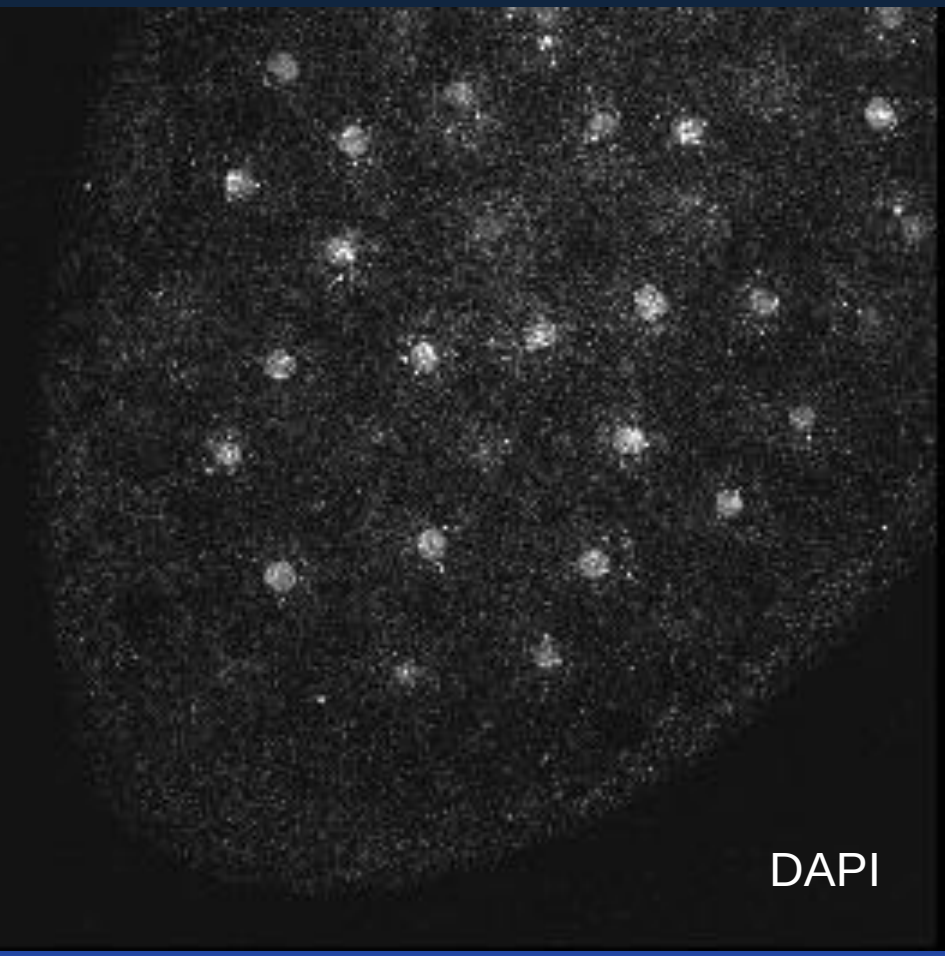
WT

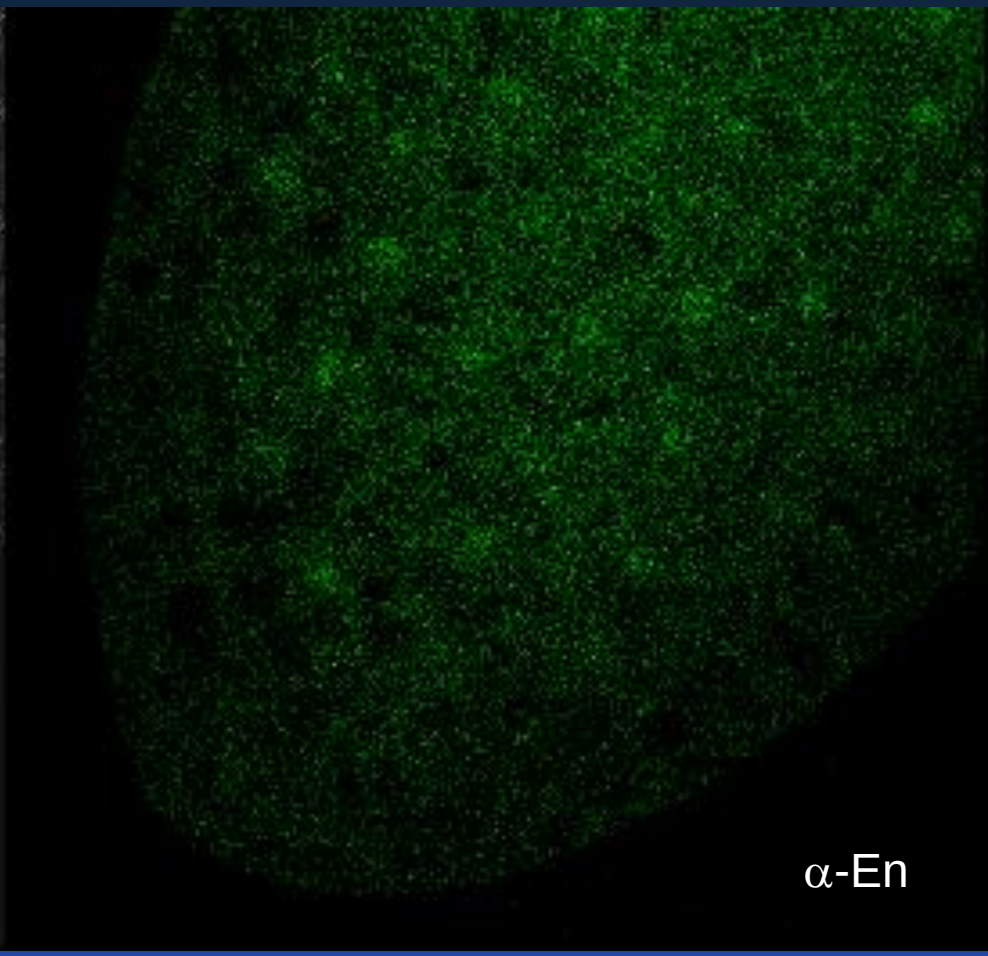


DAPI

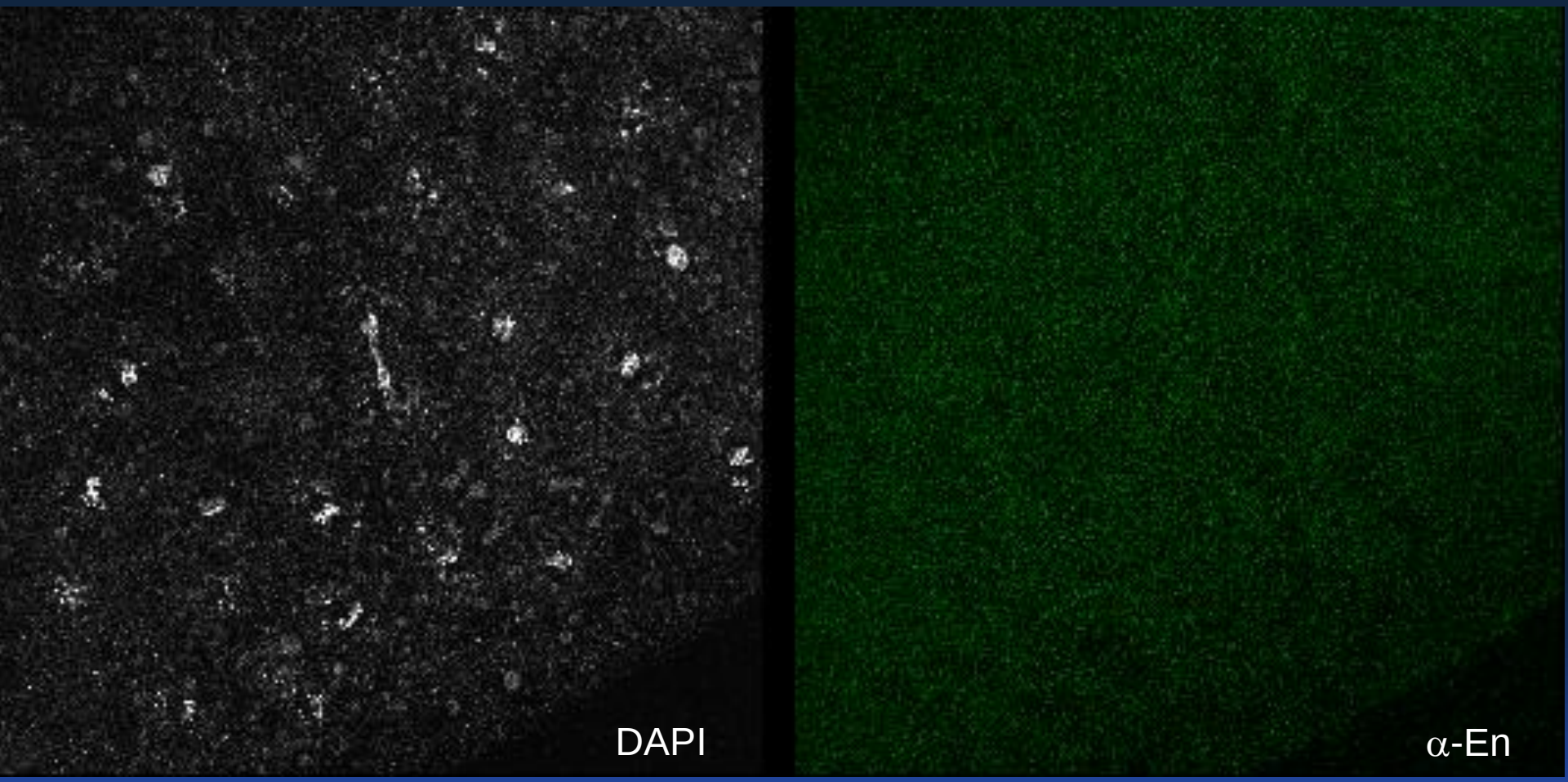
α-En

mutant *en/en*





WT



mutant *en/en*

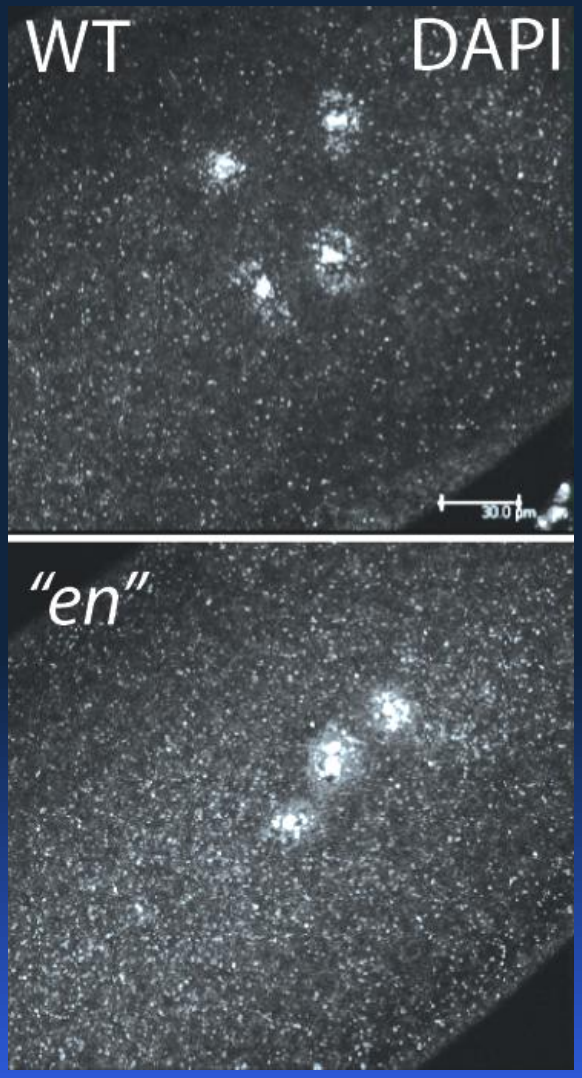
Cycle 3

WT

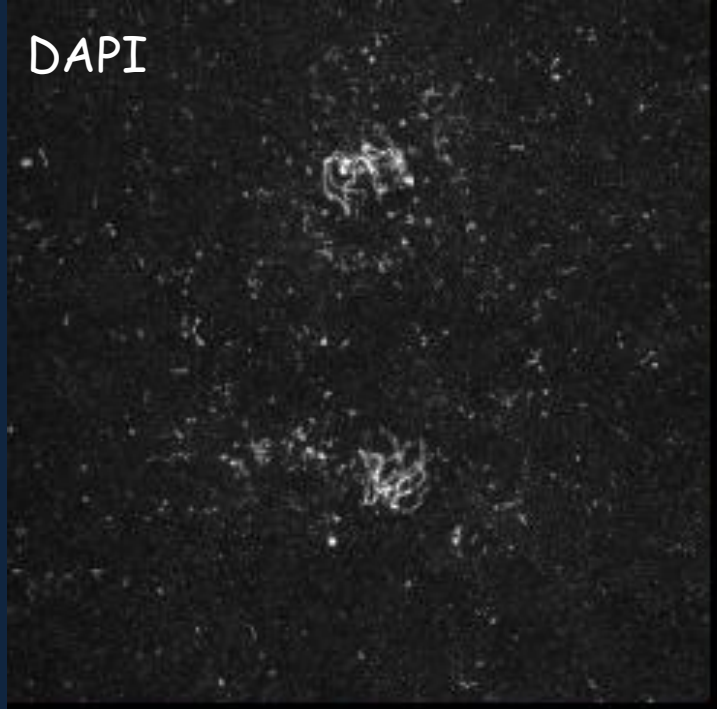
461/461 cycle 2-7 embryos
nuclei = 2^n

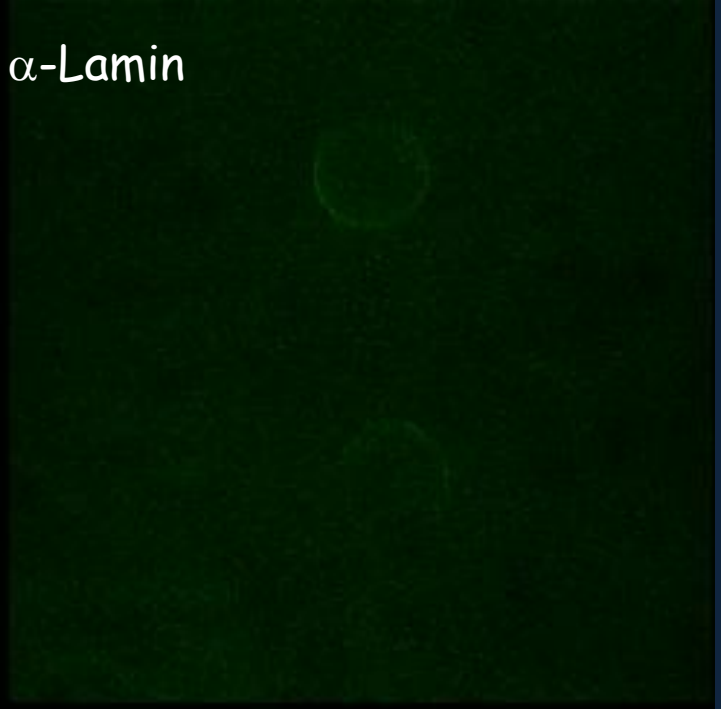
***en*/+ X *en*/+**

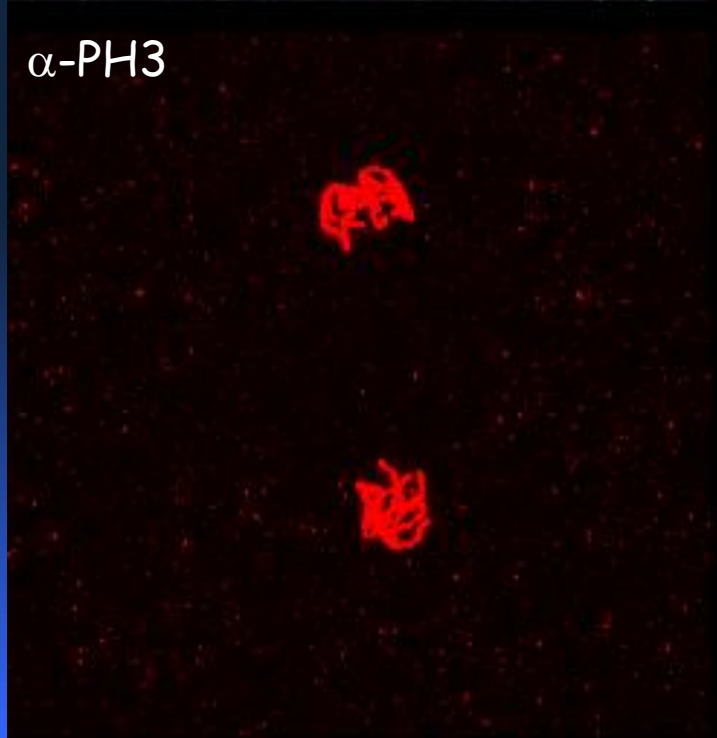
16/18 cycle 3 embryos = 4 nuclei
2/18 cycle 3 embryos = 3 nuclei

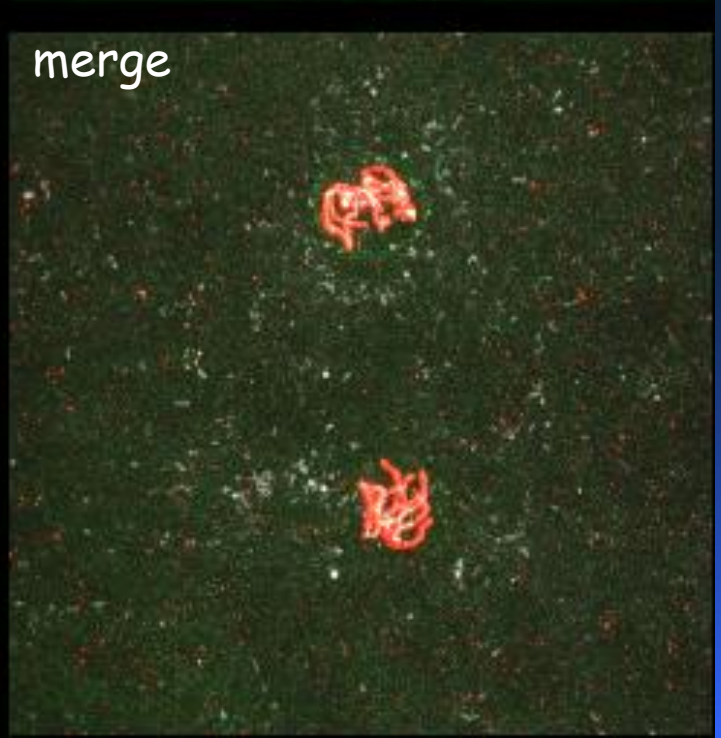


nuclear cycle 2
wild type

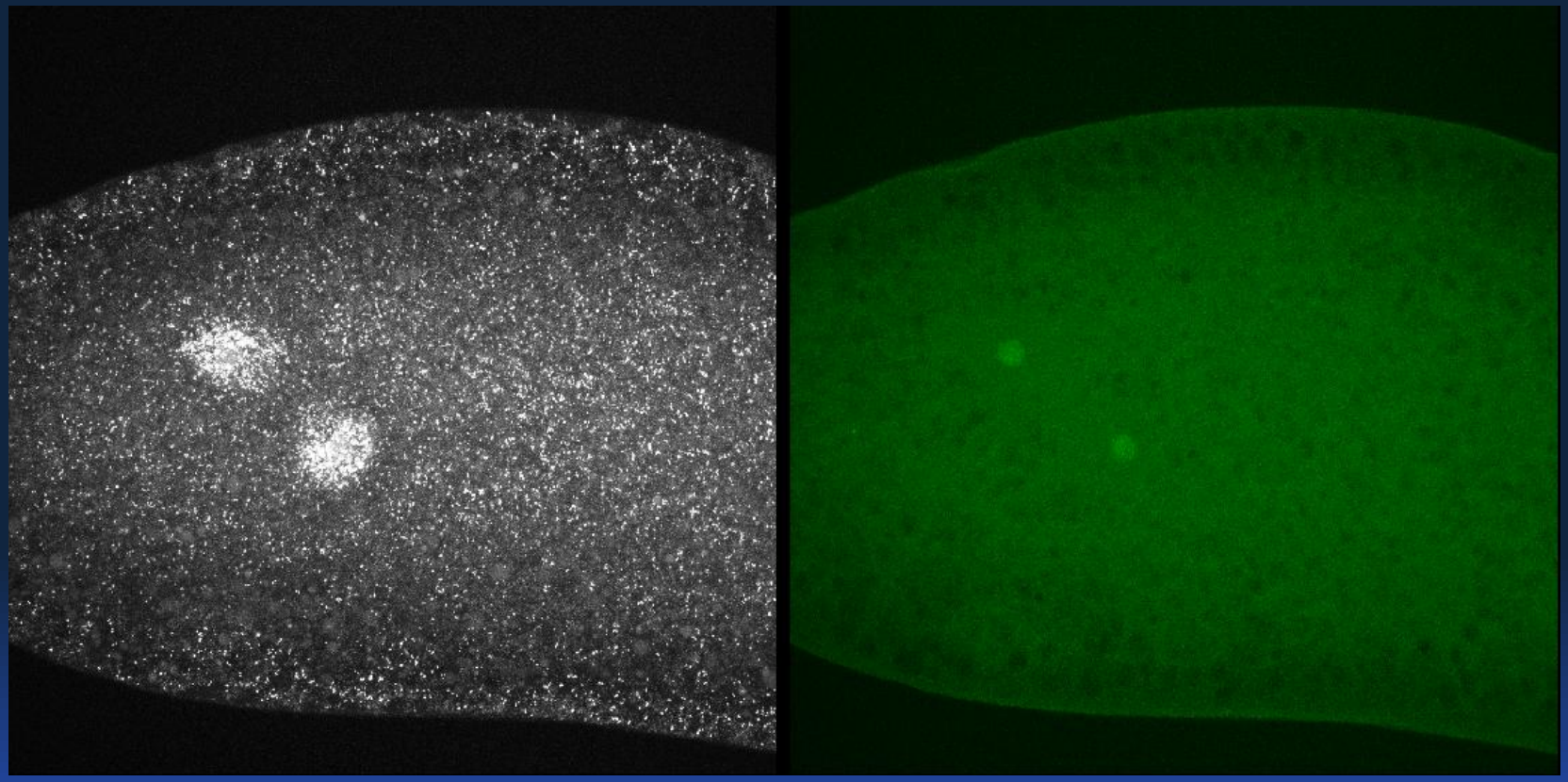








nuclear cycle 2
wild type



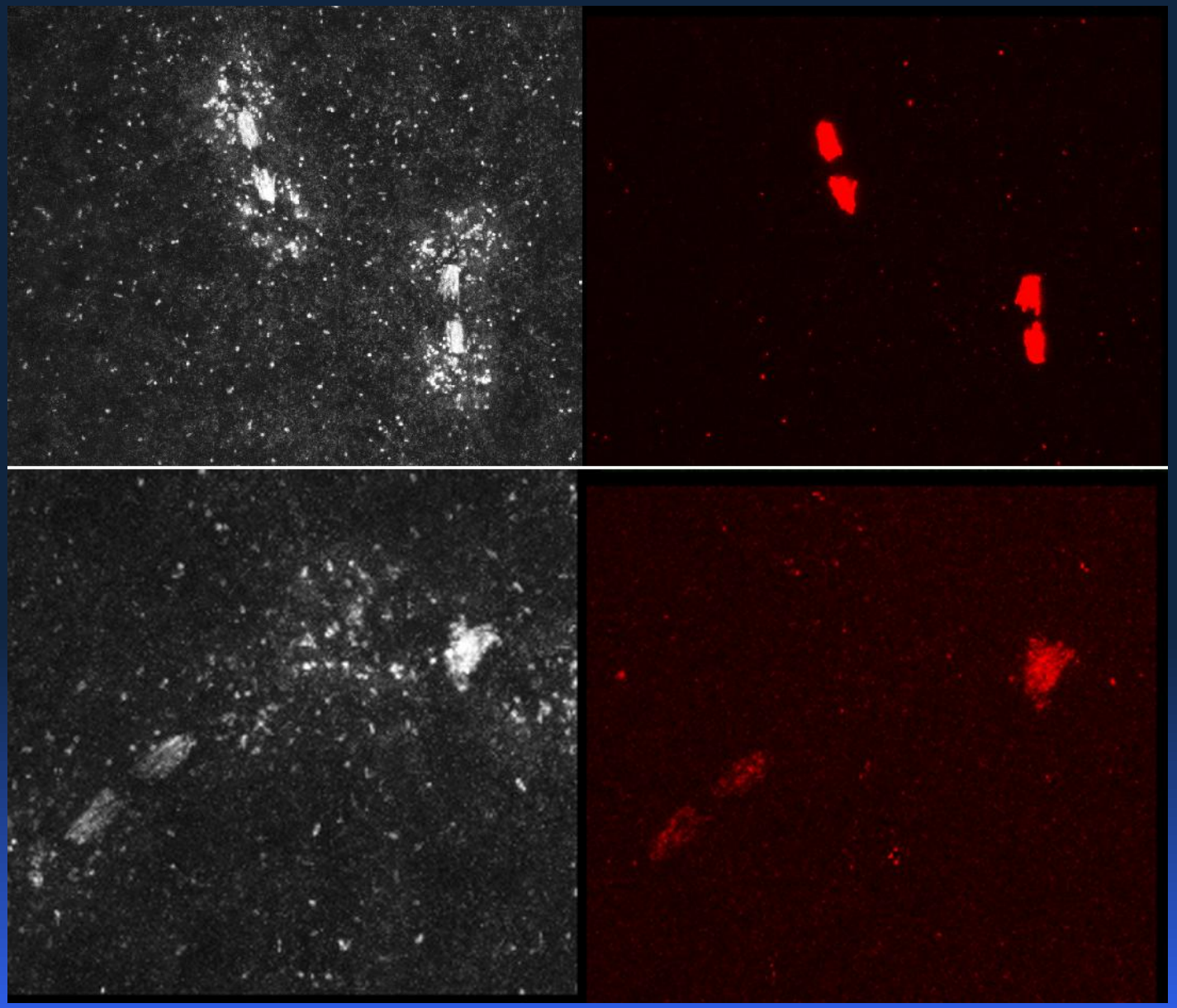
nuclear cycle 2-3

DAPI

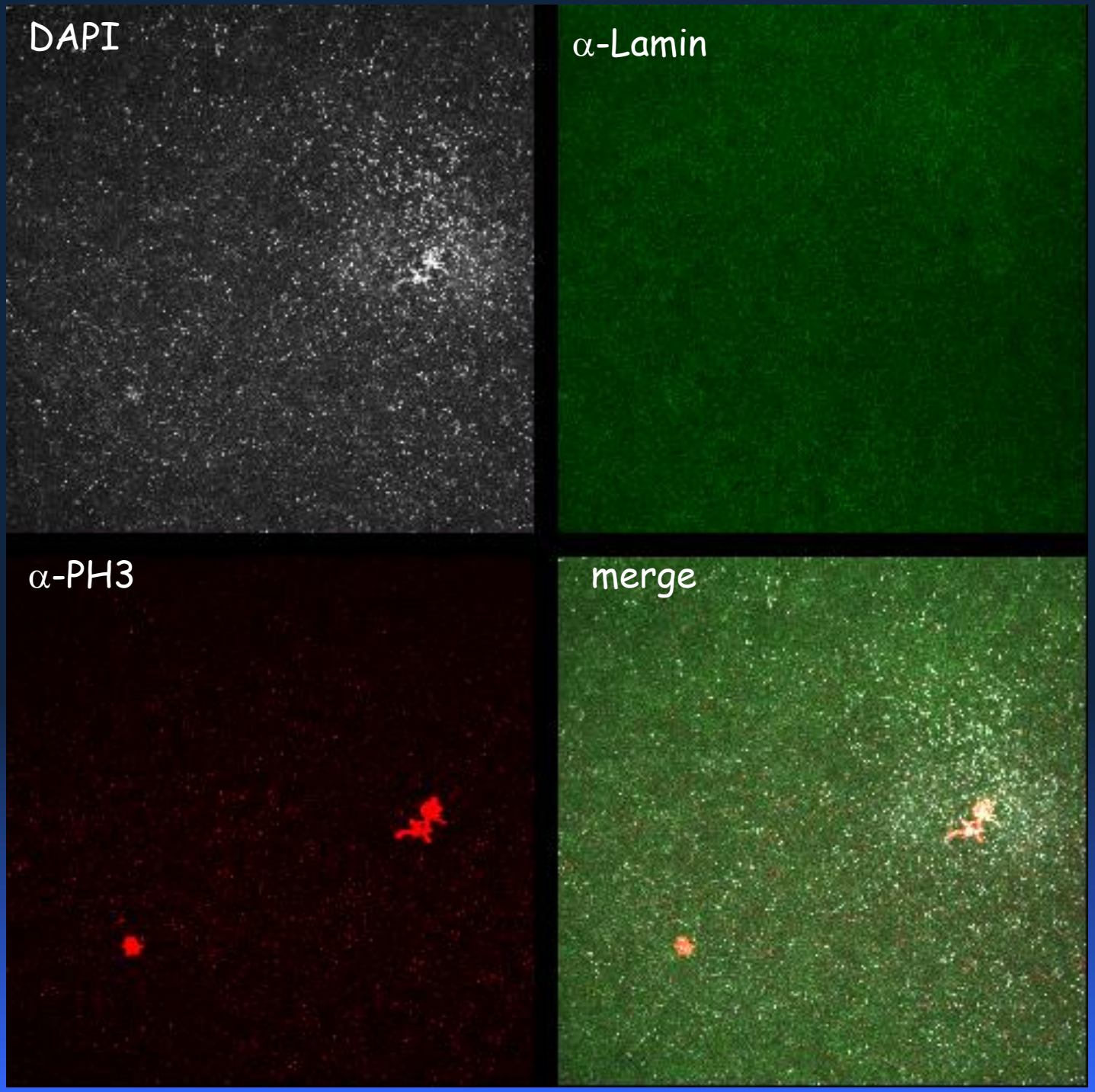
α-PH3

wild type

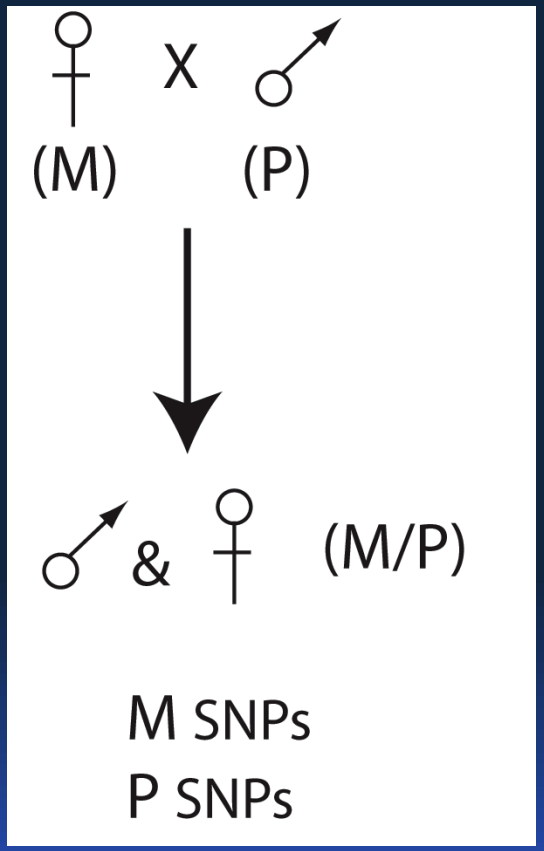
en/en



nuclear cycle 2-3
en/en



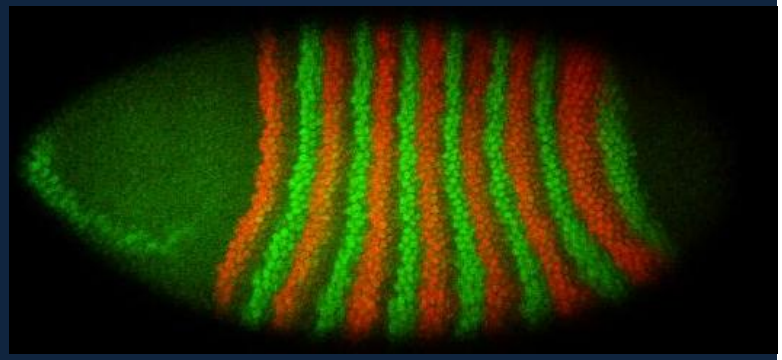
RNA-seq from early cycle embryos



Mike Eisen, Susan Lott
UCB

60 X 10^6 reads

RNA-seq identified a group of about 50 genes that produce transcripts in pre-syncytial blastoderm embryos.



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Repression of the *Drosophila fushi tarazu (ftz)* segmentation gene

J.Lesley Brown[1], Sandra Sonoda[2], Hitoshi Ueda[1,3], Matthew P.Scott[2,4], and Carl Wu[1,5]

The striped expression of the *Drosophila* segmentation gene *fushi tarazu* in alternate parasegments of the early embryo is controlled by the 740 bp zebra element. Among multiple protein factors that bind to the zebra element, FTZ-F2 behaves as a transcriptional repressor of *ftz*. Point mutations in the zebra element which disrupt FTZ-F2 binding to DNA cause ectopic expression of zebra-*lacZ* activity in transformed embryos. The mutant constructs are expressed from the zygotic genome in preblastoderm embryos as early as the third nuclear division cycle. This unprecedented early transcription suggests that *ftz* requires active repression during initial nuclear division cycles, a novel type of embryonic gene regulation. A putative FTZ-F2 cDNA clone isolated by recognition site screening of an expression library was found to be identical in sequence with the zinc finger protein *tramtrack* (Harrison and Travers, 1990).