Functional genomics in model organisms

April 13, 2009

Advantages to functional genomics in model organisms

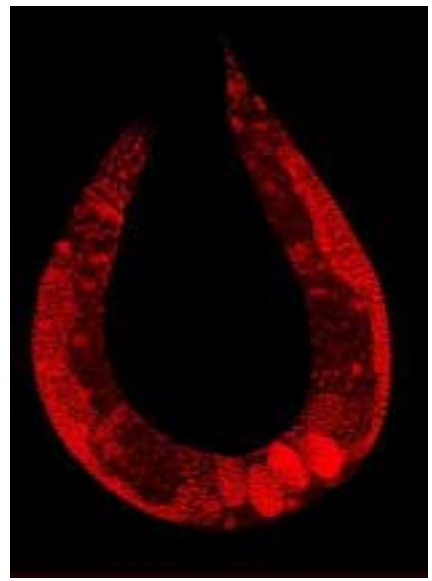
- smaller genome than human
- compact gene structure with local regulation
- conserved gene regulatory mechanisms
- functional conservation of many genes involved in cellular and developmental processes, including disease genes

Advantages to functional genomics in model organisms

- can perform analyses in vivo
  - developmental processes
  - tissue-specific regulation
- can analyze data in context of existing genetic and developmental pathways
- follow-up functional validation of genome-wide data can be performed fast, cheap and in vivo

Multicellular model organisms

- The nematode Caenorhabditis elegans
  - soil dwelling
  - hermaphrodite/male
  - 1 mm long
  - 3d life cycle
  - 300 progeny/hermaphrodite
  - defined cell lineage during development



- The fruit fly Drosophila melanogaster
  - commensal with humans
  - male/female
  - 10d life cycle
  - 800 progeny/female
  - undergo metamorphosis
  - many advanced tissues (brain, eyes, etc)

Experimental advantages

- cheap to culture/maintain
- high fecundity
- possible to generate large quantities
- relatively pure genetic backgrounds
- extensive and sophisticated genetic tricks and tools

The genomes of worm and fly

- The *C. elegans* genome (published in 1998):
  - 100Mb
  - 20% of sequence is genic
  - ~20,000 genes
  - no cytologically visible heterochromatin

- The Drosophila genome (published in 2000):
  - 180Mb
  - 20% of sequence is genic
  - ~14,000 genes
  - extensive visible heterochromatic regions (1/3 genome)

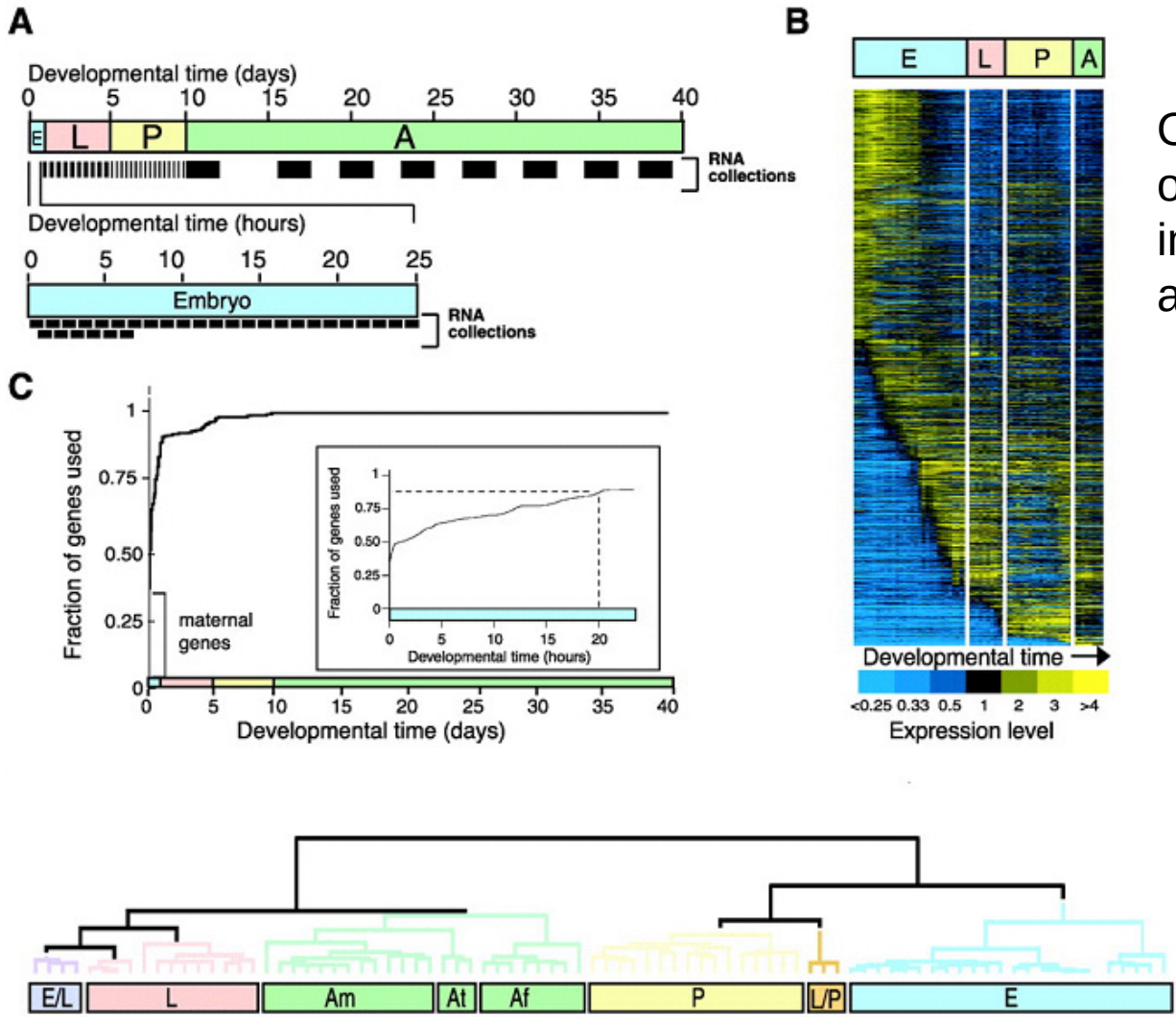
Functional genomic studies in worms and flies

- RNA expression profiling by microarray analysis
  - developmental timecourses
  - mutant analysis
  - tissue-specific profiling
  - gene annotation improvement
- genome-wide RNAi screening
  - cell culture (drosophila)
  - in vivo (C. elegans)
- genome location studies (chromatin immunoprecipitation)
  - DNA binding proteins (transcription factors)
  - chromosome and chromatin factors
  - histone modifications
- systematic imaging
  - temporal
  - spatial

Gene expression studies

- Small genomes and compact genes facilitate microarray analyses
- Can decipher spatial and temporal gene expression patterns
- Can follow up with genetic and developmental biology analyses to put gene regulation in phenotypic context

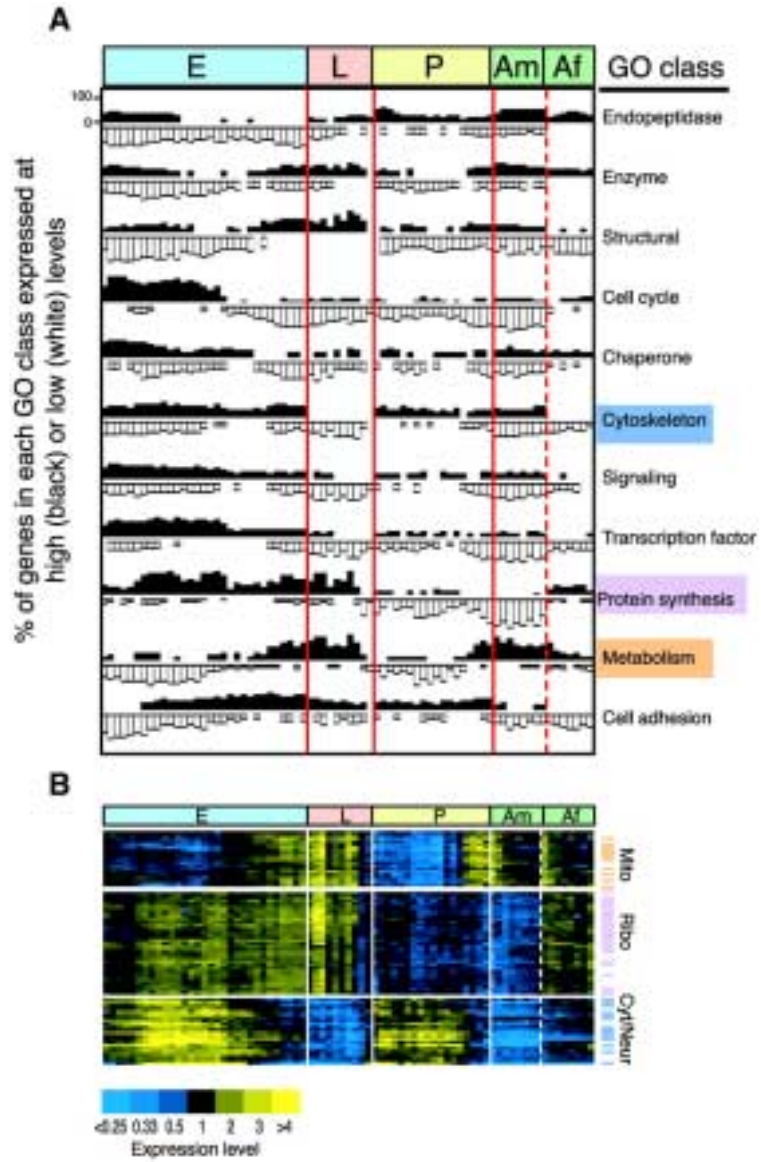
Drosophila Developmental Timecourse



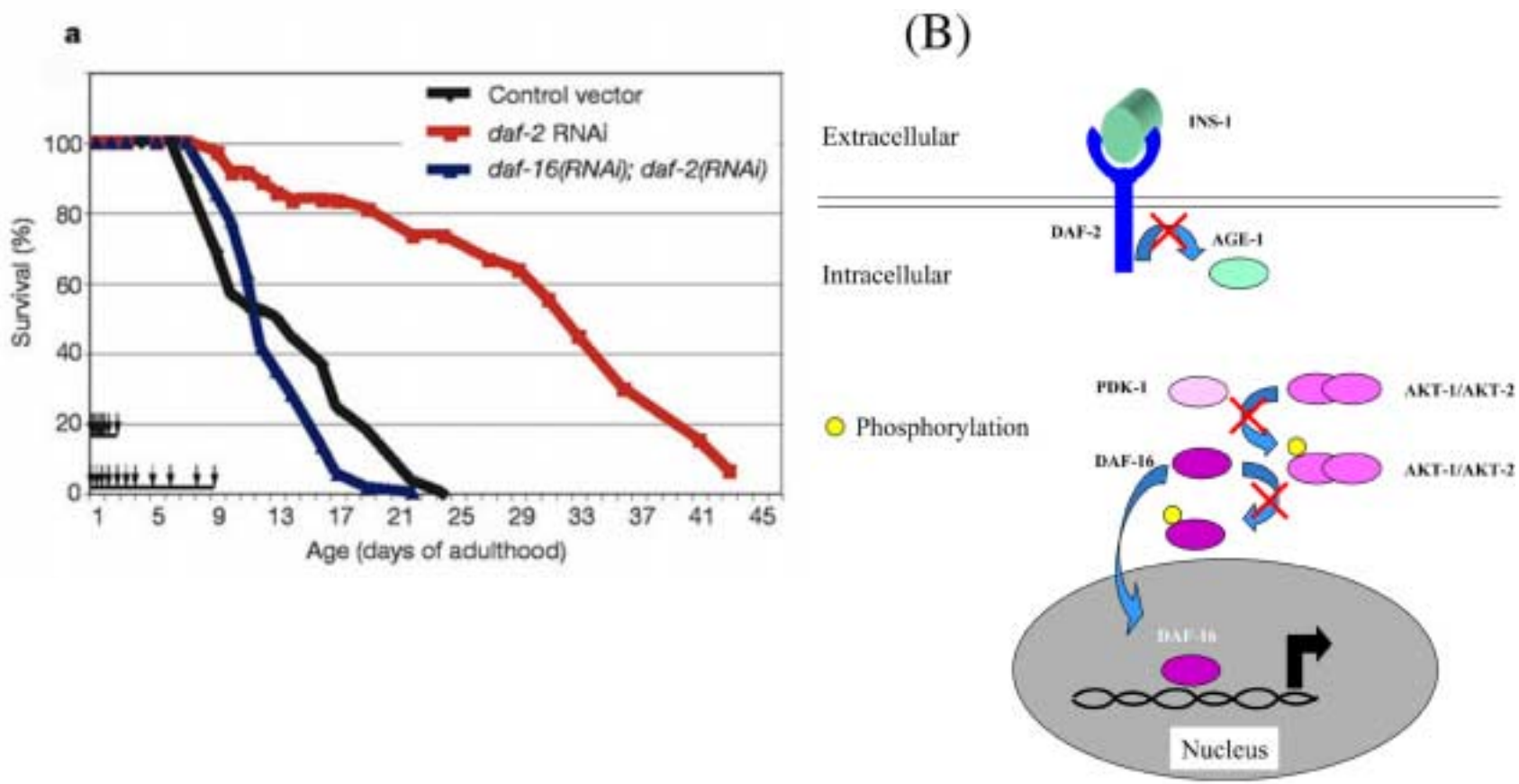
Ordered by onset of first increase in txpt abundance

Arbeitman et al., Science 2002

Drosophila Developmental Timecourse

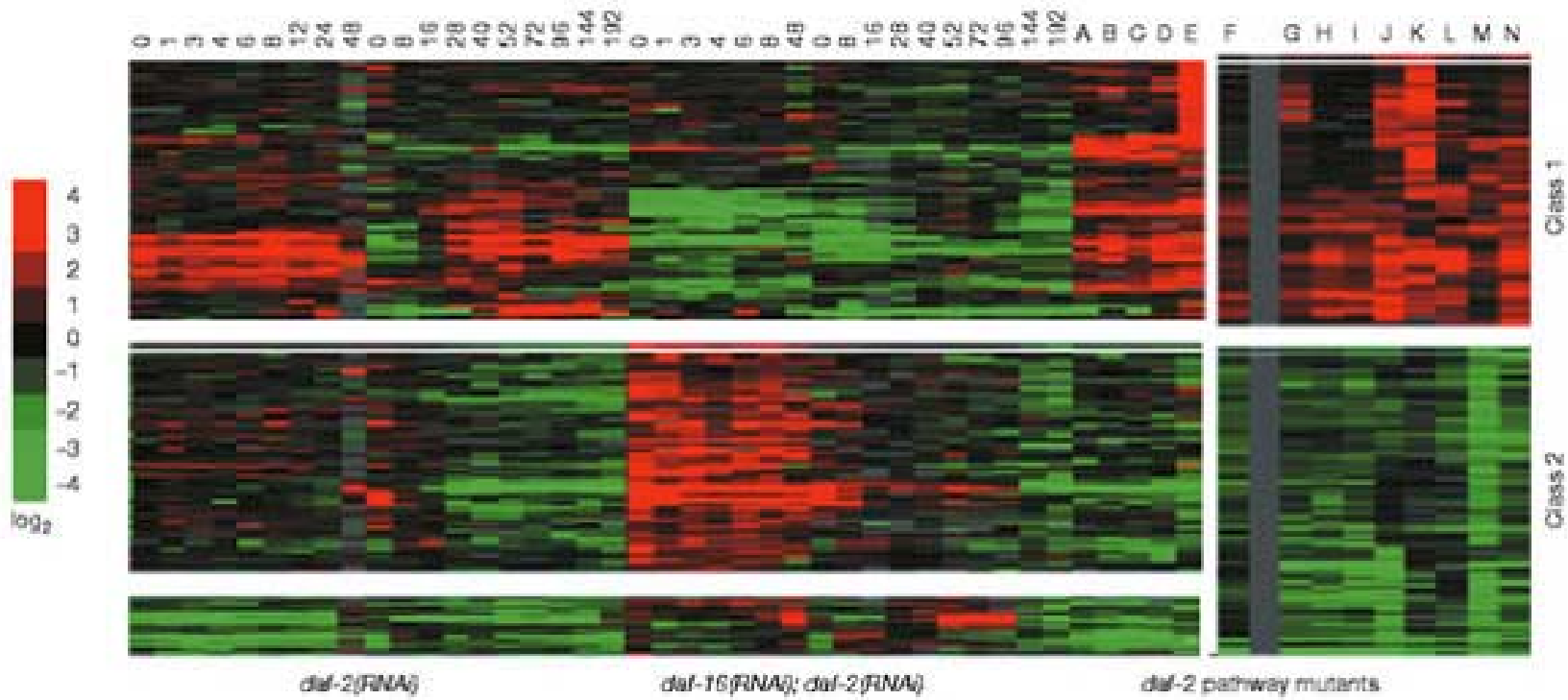


C. elegans aging (mutant analysis of transcription factor DAF-16)



Murphy et al., Nature 2003

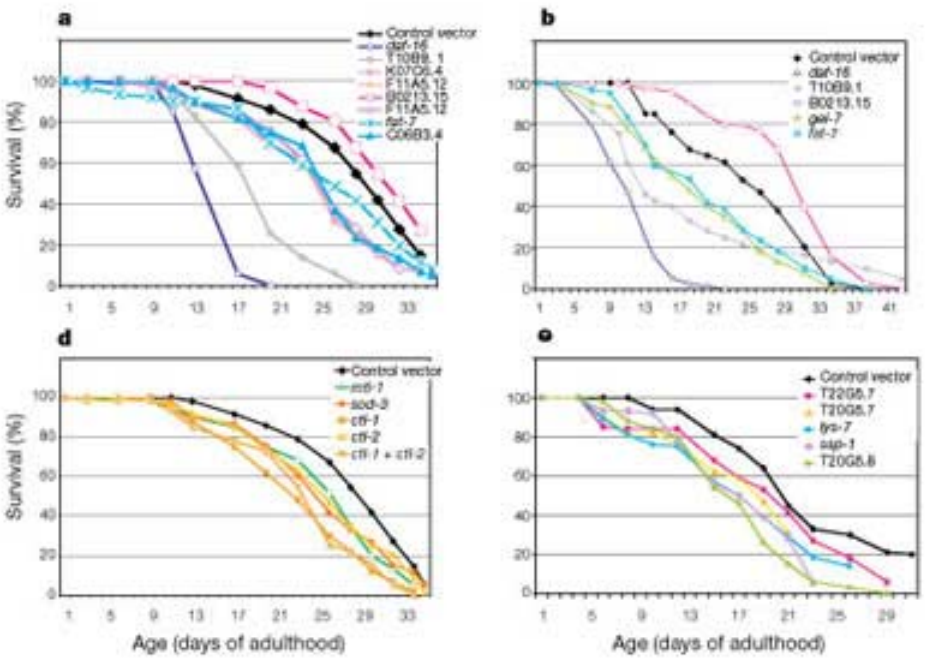
C. elegans aging (mutant analysis of transcription factor DAF-16)



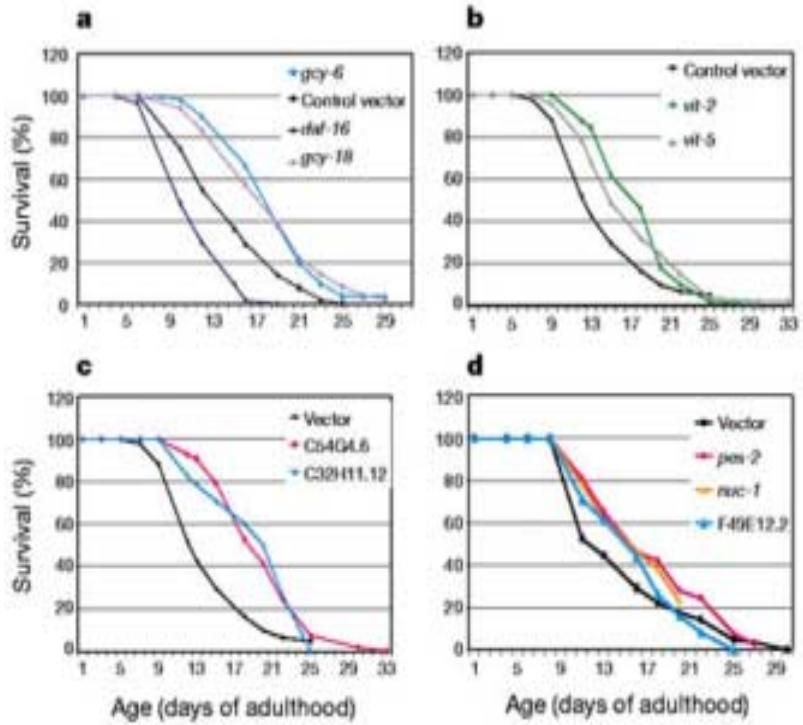
The first cluster contains genes induced in DAF-2 pathway mutants but repressed in *daf-16(RNAi)*; *daf-2(RNAi)* animals (class 1). These are candidates for genes that extend lifespan. The second cluster contains genes that displayed the opposite profile are candidates for genes that shorten lifespan (class 2).

C. elegans aging (mutant analysis of transcription factor DAF-16)

Reduced lifespan of *daf-2* mutants when fed dsRNAi of class I genes



Increased lifespan when fed dsRNAi of class II genes



Upregulated genes are required for longevity, downregulated genes shorten lifespan

Tissue-specific profiling

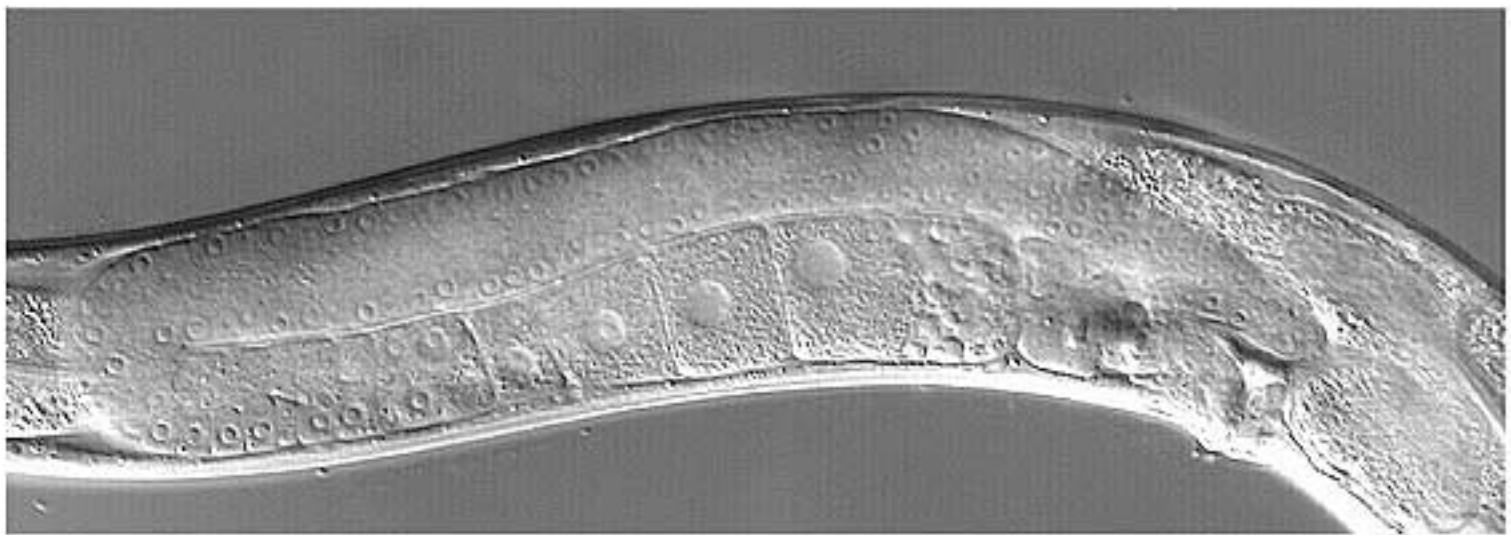
–Mutants to produce more or less of a given tissue

–Dissecting (when possible)

–Cell sorting (from embryos)

–RNA tagging

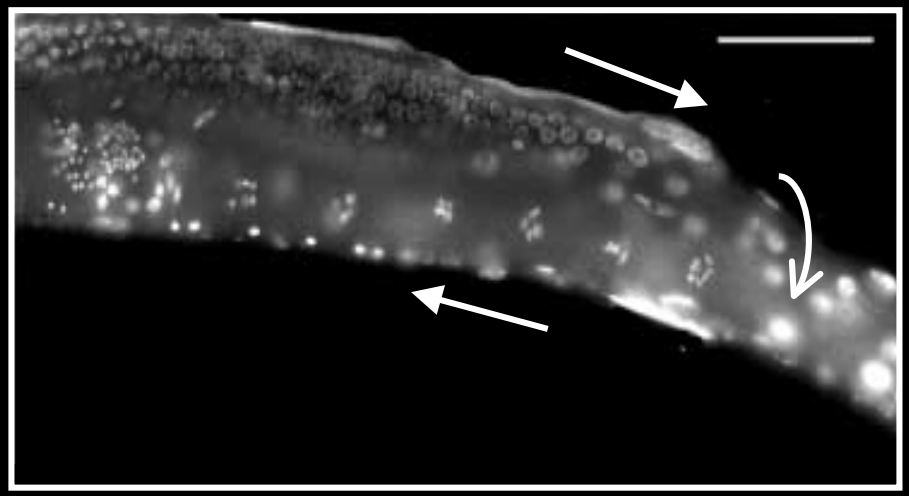
Tissue-specific profiling - mutant analyses

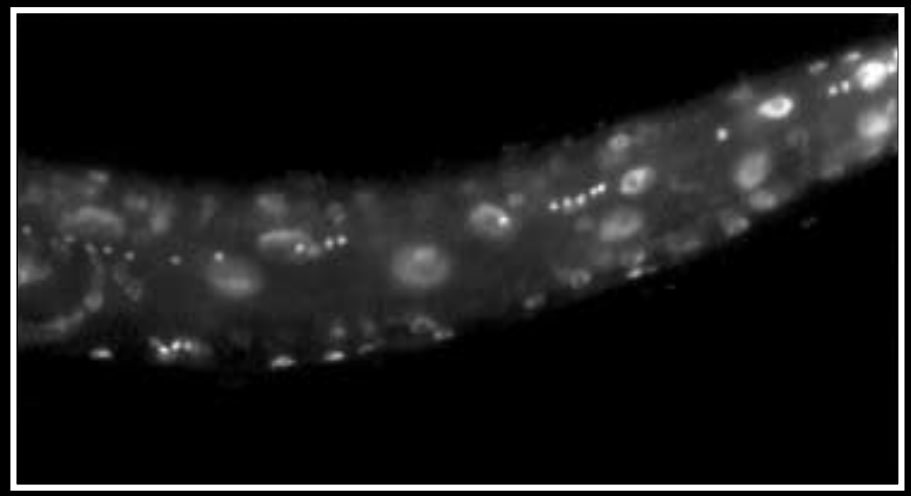


***C. elegans* germline**

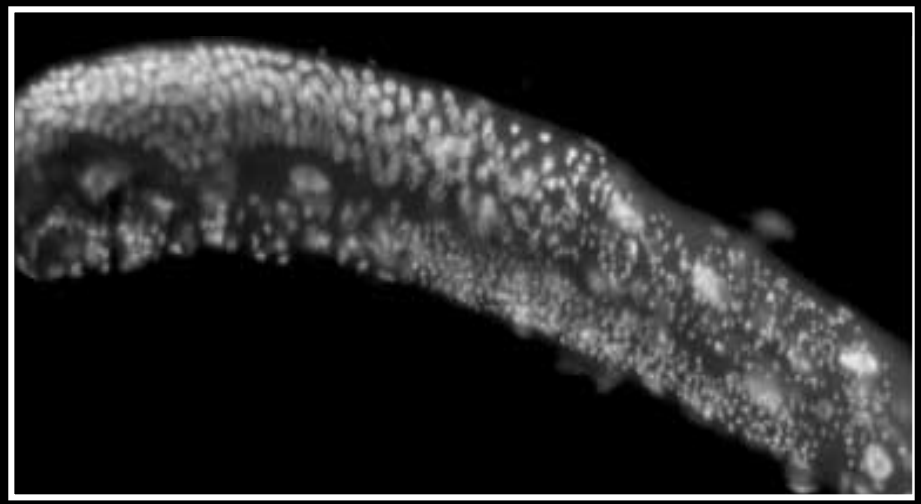
Germline mutant comparisons

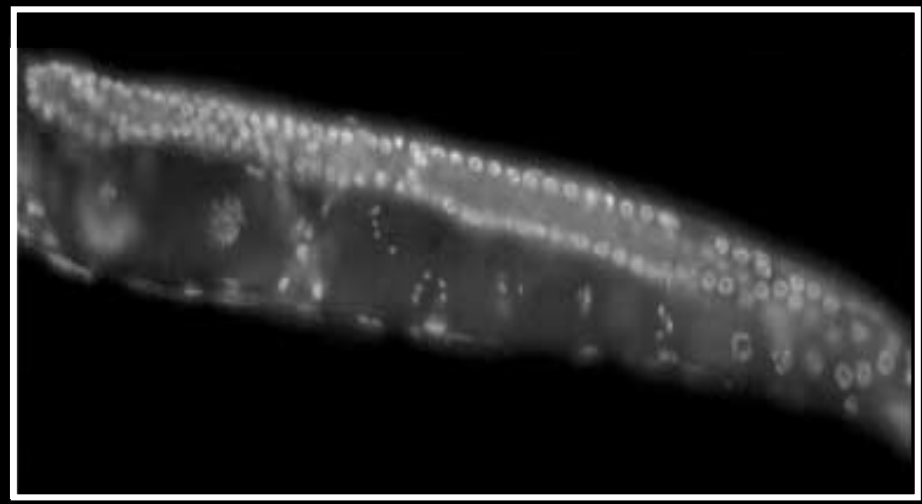
wild type **vs.** **no germ line (*glp-4*)**





sperm only *(fem-3gf)* **vs.** **oocytes only *(fem-1lf)***

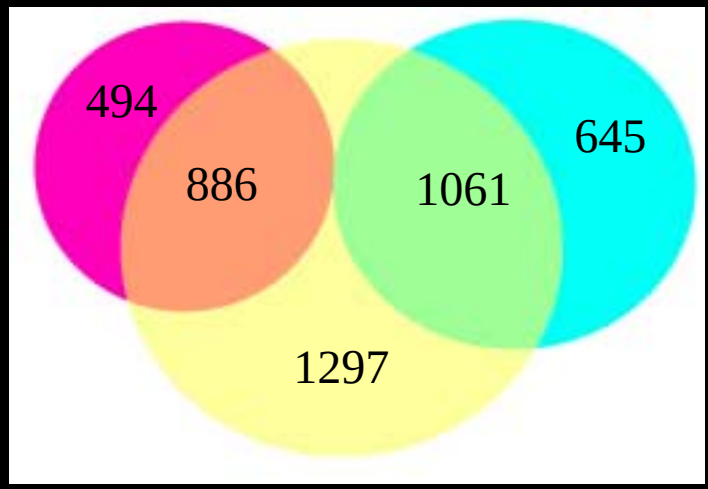




Reinke et al., Mol. Cell 2000

Germline gene categories

1380 sperm-enriched

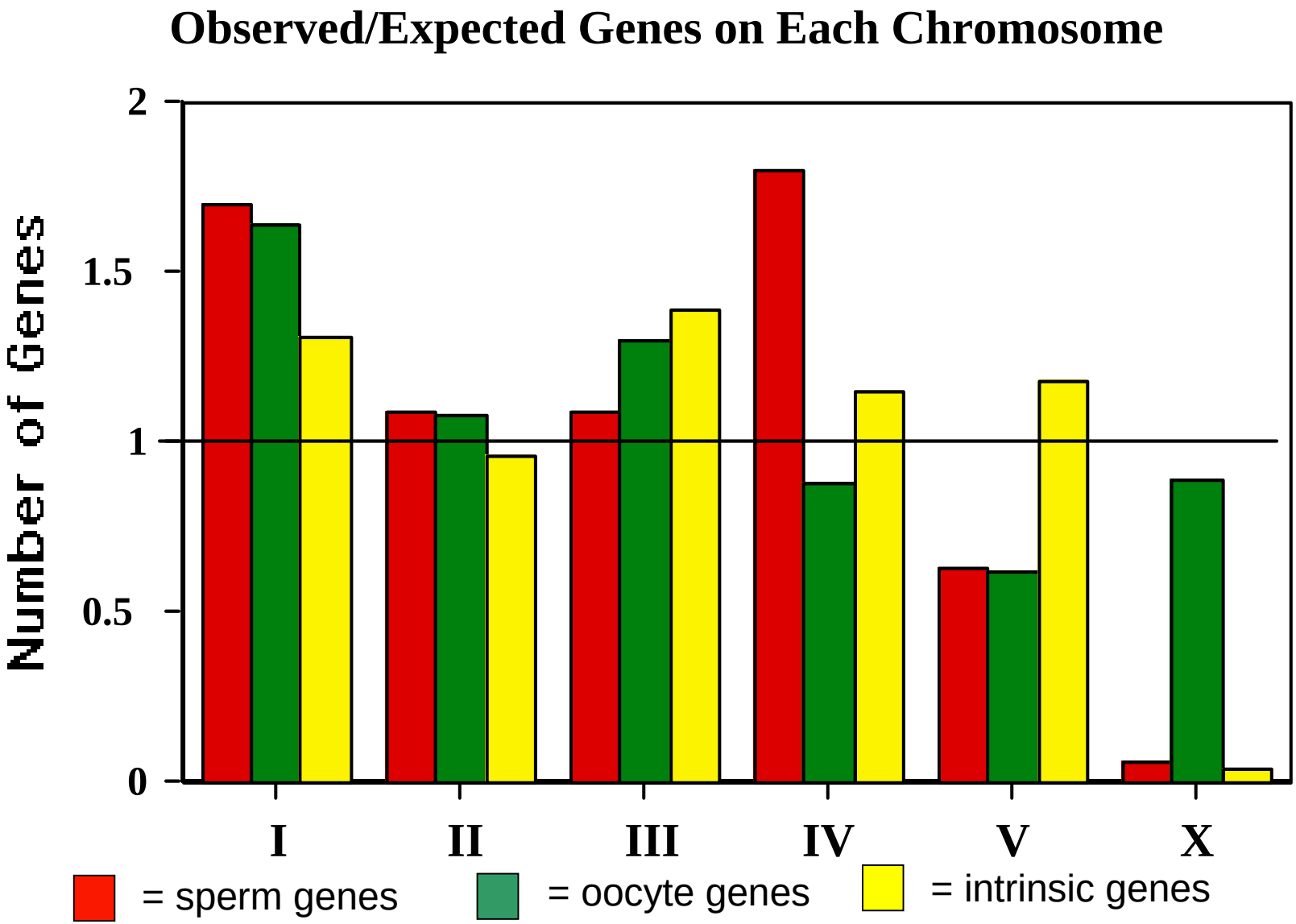


1706 oocyte-enriched

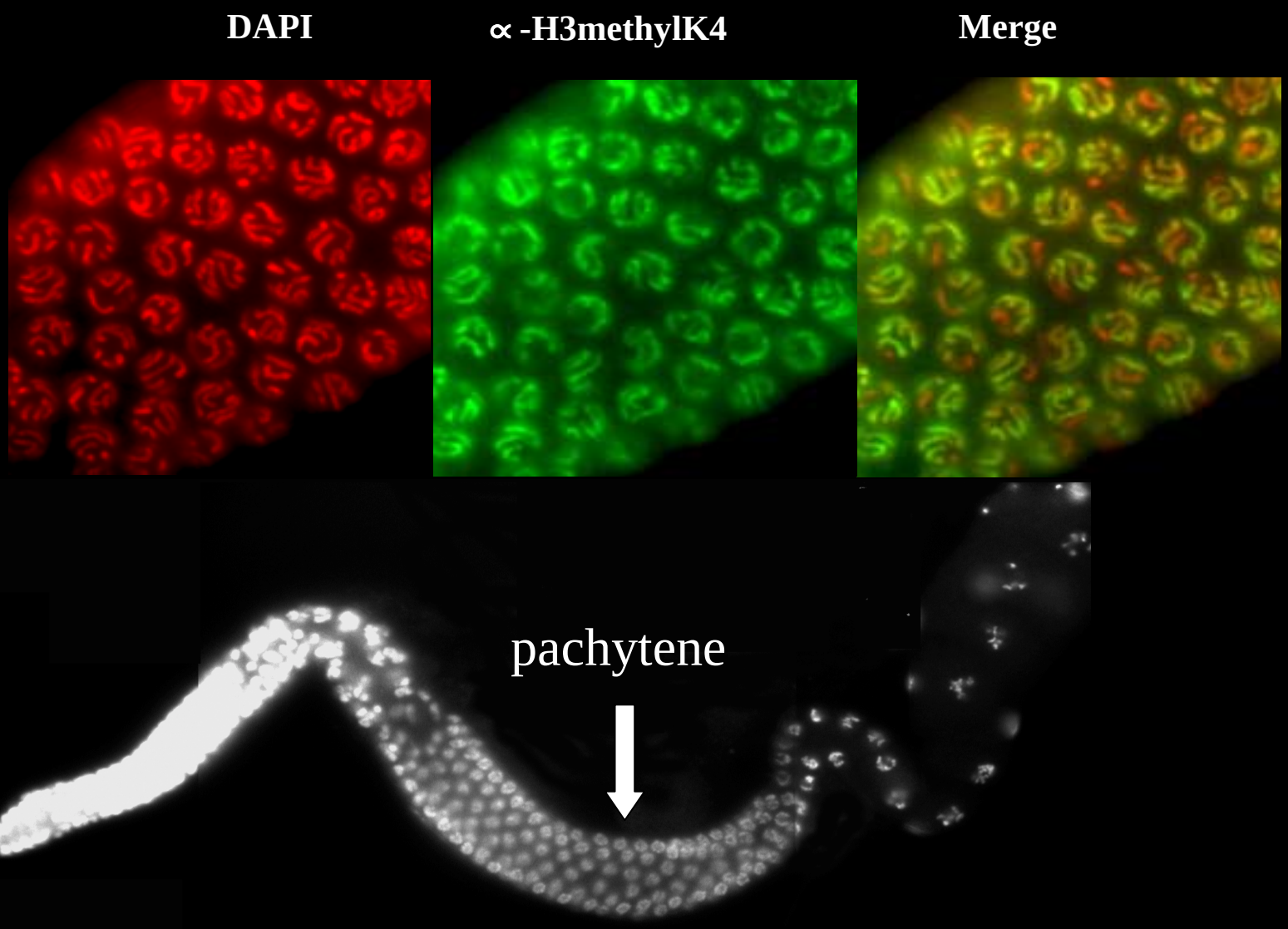
1297 intrinsic
(stem cells, transition, pachytene)

>2fold, p<0.01

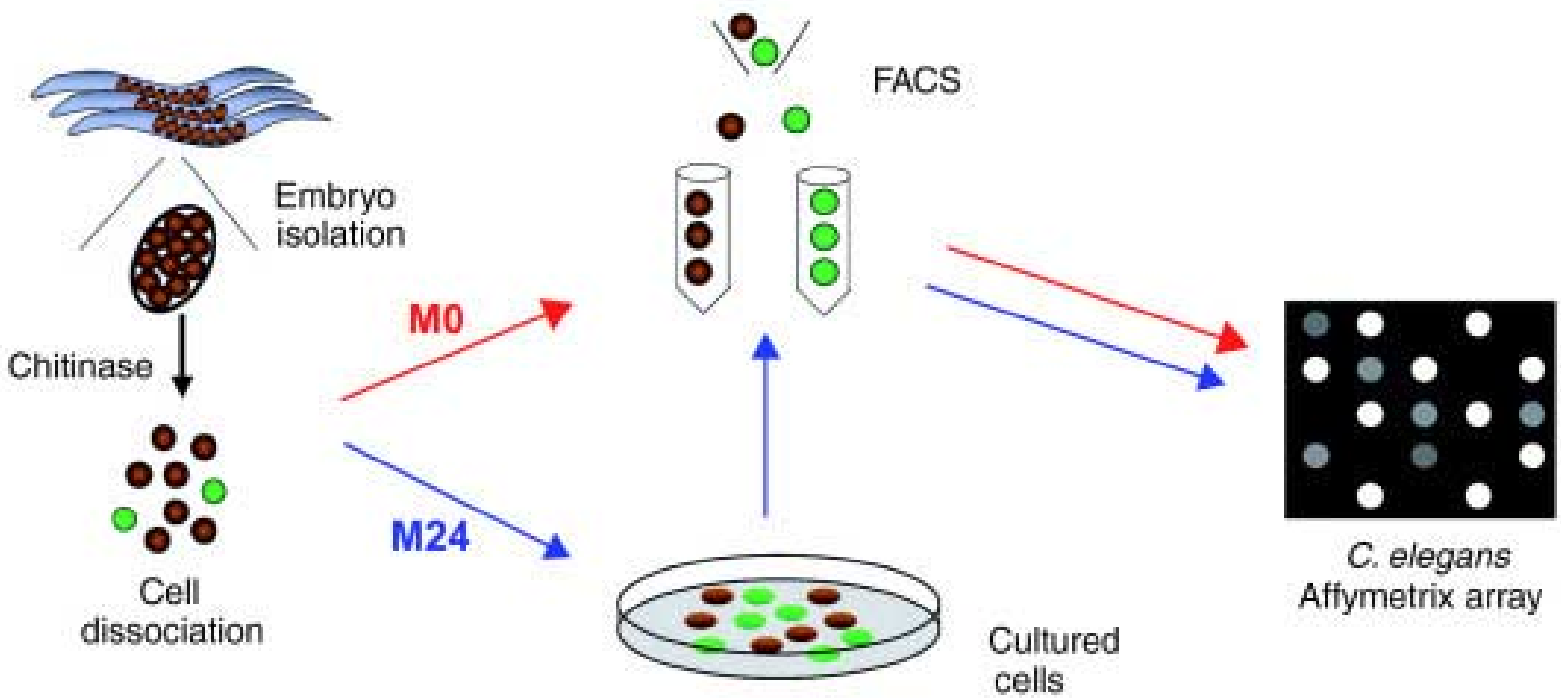
Germline genes exhibit a bias against the X chromosome



The hermaphrodite X chromosome lacks active chromatin

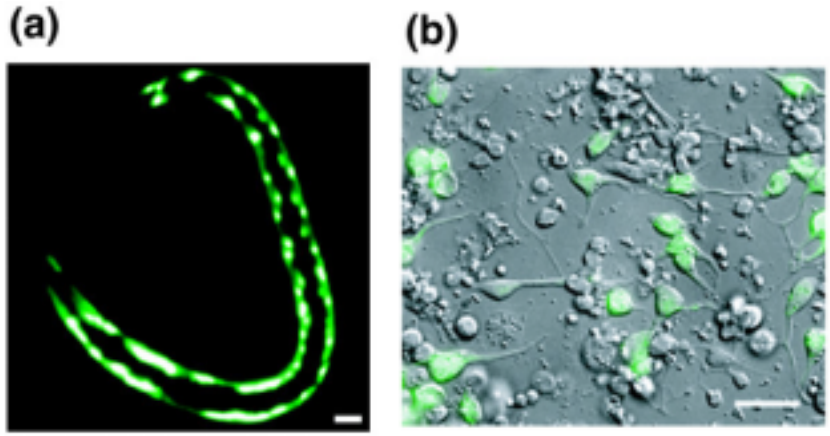


Tissue-specific profiling - cell sorting



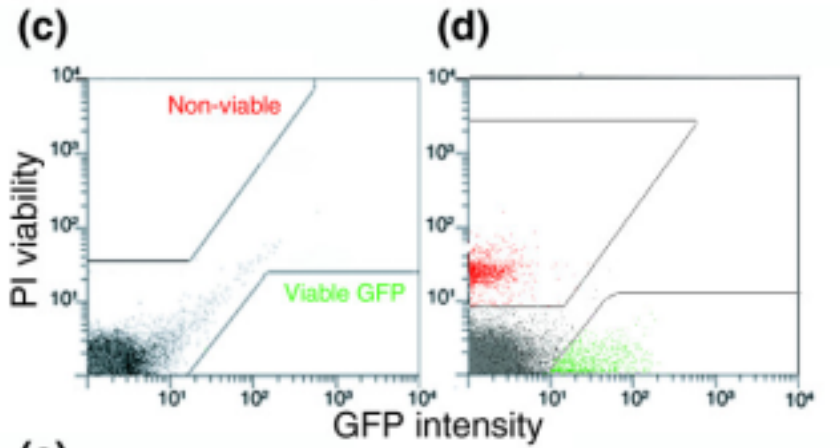
Fox et al., Genome Biology 8(9): R188 2007

muscle specific reporter



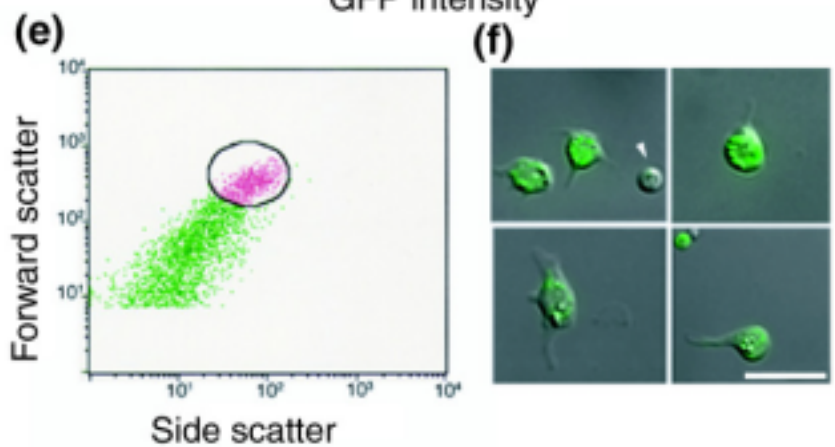
dissociated embryonic cells from strain carrying reporter

non-GFP cells



sorted GFP+ cells

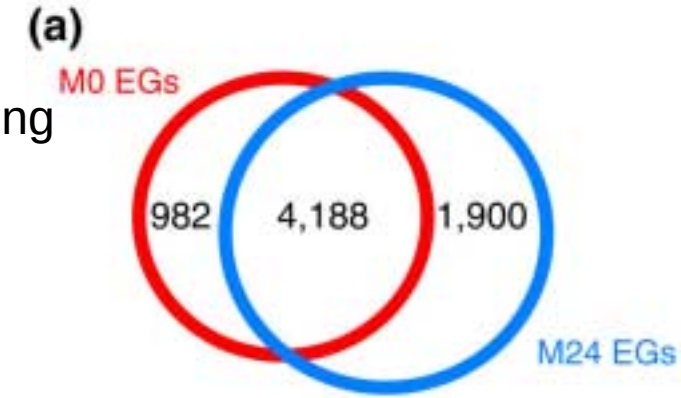
sorted to exclude clumps and debris



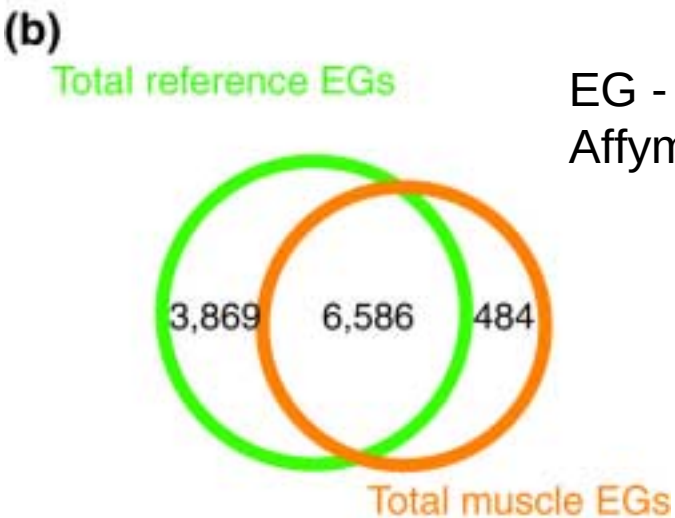
sorted GFP+ cells

Defining sets of muscle expressed genes

M0 -
No culture prior to sorting
“young” muscle cells



M24 -
24h culture prior to sorting
“differentiated” muscle cells

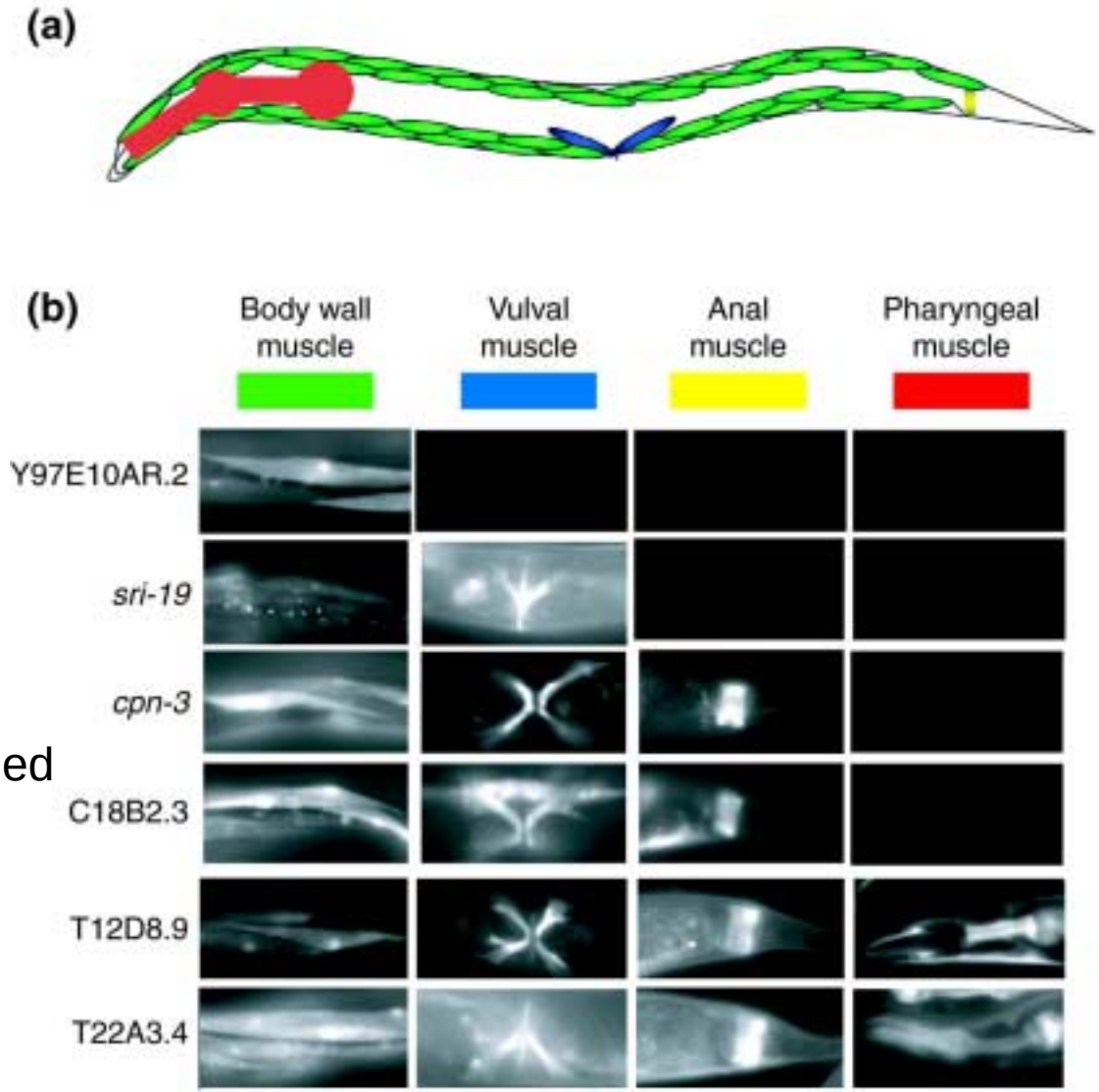


EG - expressed gene based on Affymetrix analysis

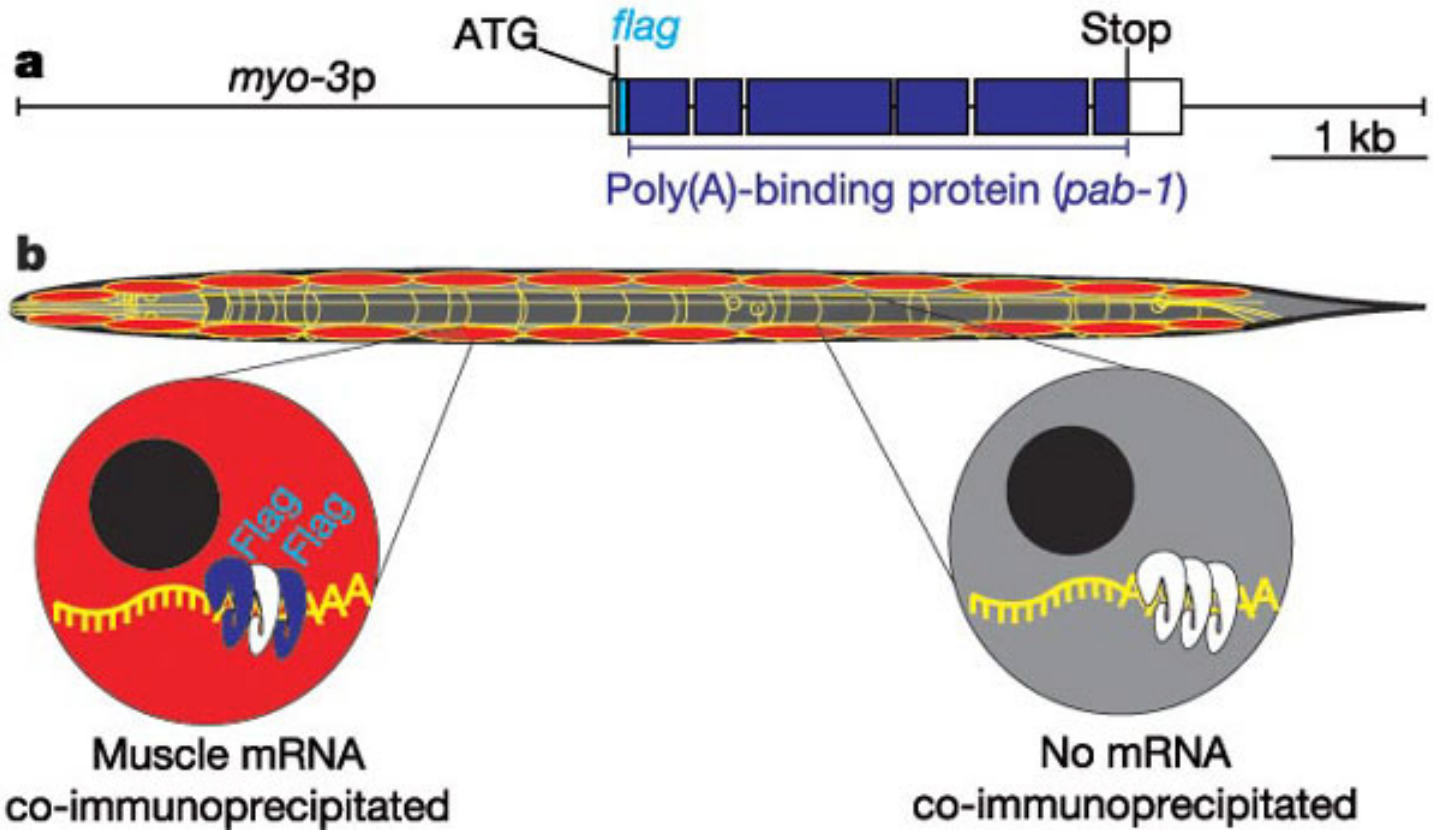
Reference:
all dissociated embryo cells after 24h in culture

Biological validation of muscle expression

reporter gene analysis of muscle-enriched genes



Tissue-specific profiling - RNA IPs



- muscle (Roy et al., Nature 2002)
- neurons (von Stetina et al., Genes Dev 2007)
- intestine (Pauli et al., Development 2006)

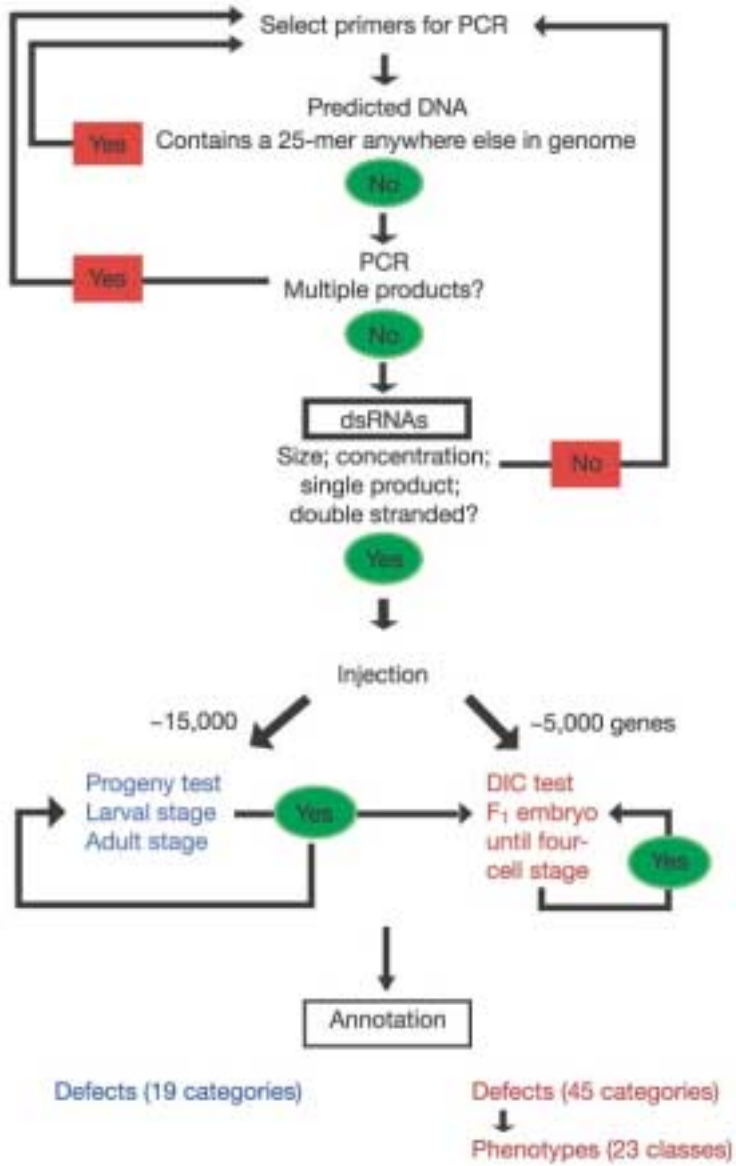
RNAi studies

- RNAi is a mechanism for knocking down the expression of a specific gene
- depending on the assay, different phenotypes can be monitored
- in *C. elegans*, RNAi can be induced simply by feeding worms bacteria expressing a dsRNA corresponding to the gene of interest (systemic)
- in Drosophila, no systemic RNAi, so express RNAi using transgenic system or in cell culture
- For both species, a “library” of RNAi vectors that cover most predicted genes exists and is generally available

RNAi screen for factors required for *C. elegans* embryogenesis

Insert movie of C. elegans embryogenesis

Systematic RNAi in *C. elegans*



Sonnischen et al., Nature 434: 462-9 2005

Results from RNAi screen

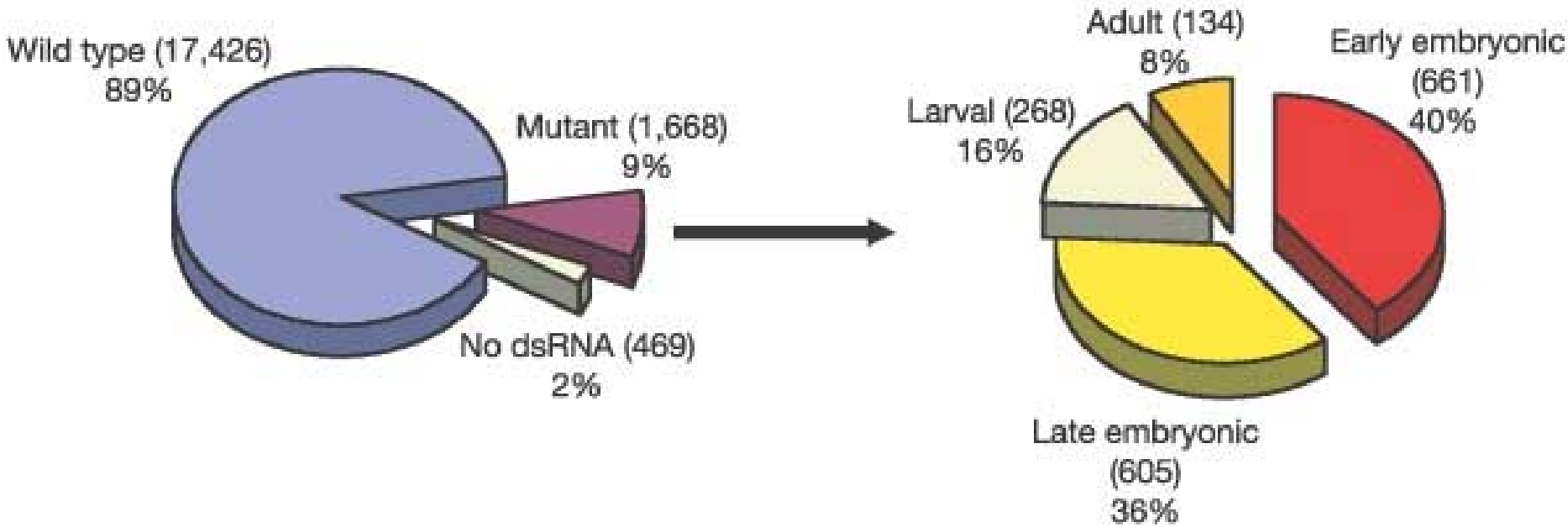
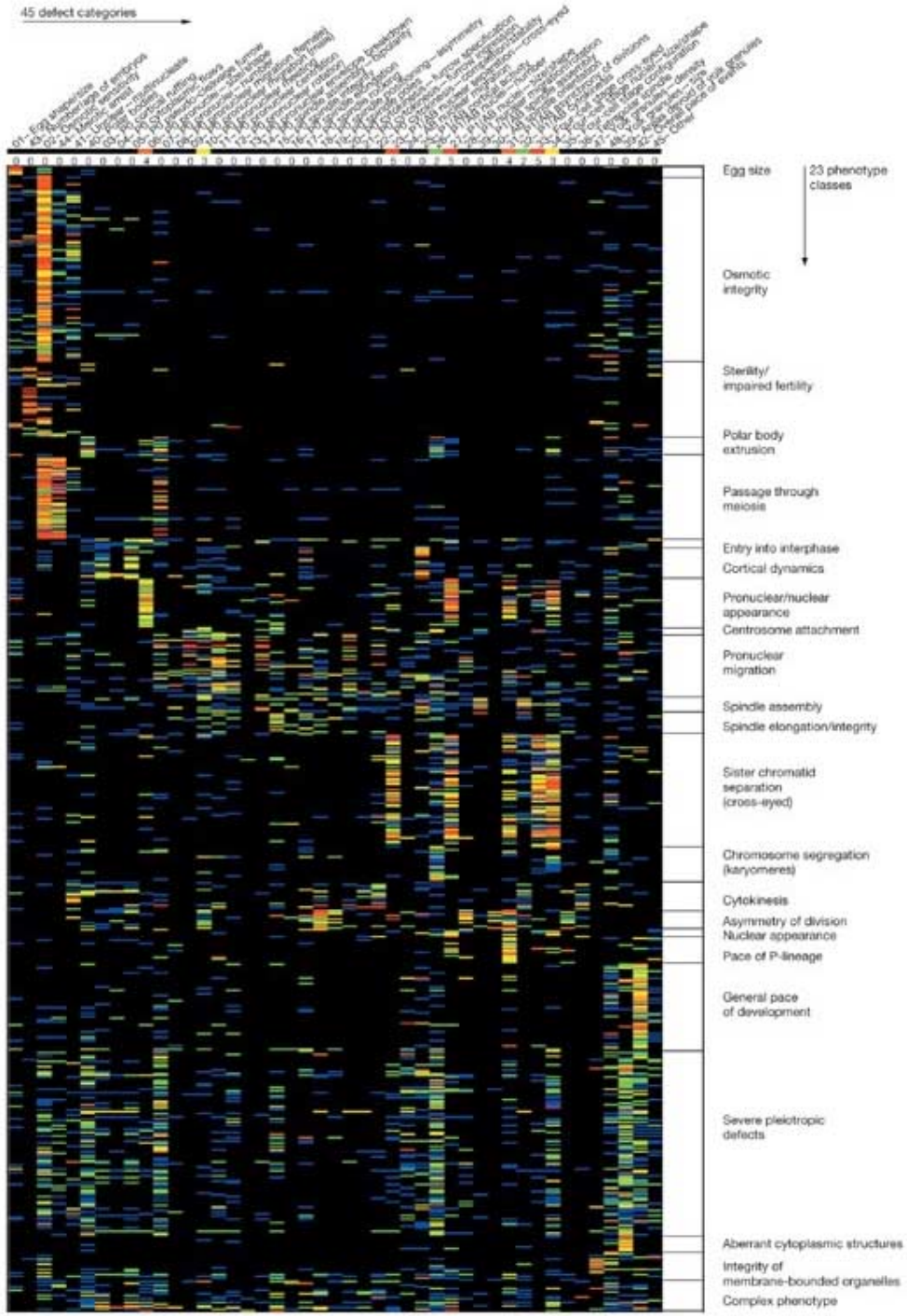


Table 2 **Defect categories and associated scoring criteria**

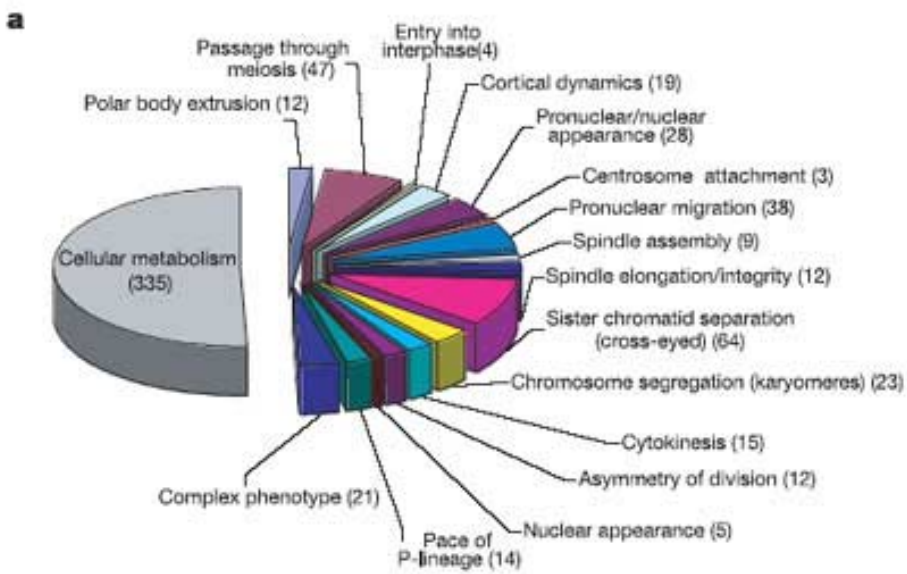
No.	Defect category	Scoring criteria
1	Egg shape/size	Eggs small (less than 70% of wild type), large (more than 130% of wild type) or irregularly shaped
2	Osmotic sensitivity	Swelling of the embryo to fill the egg shell and/or irregular granule movements
3	P_0 cortical ruffling	Excessive or no early cortical ruffling (wild type: wave of membrane ruffling from posterior to anterior resulting in pseudo-cleavage furrow)
4	P_0 cytoplasmic flows	Lack of granular flows towards the male PN (wild type: directional flow of yolk granules towards the male PN
5	P_0 pseudo-cleavage furrow	No or excessive furrowing (wild type: covering 20–30% the width of the embryo)
6	P_0 pronuclei – size/shape	Size more than 30% smaller or larger than wild type (wild type: diameter approx. 25% the width of the embryo at onset of PN migration), or irregular shape of PNs
7	P_0 pronuclei – number	Numbers of PNs above or below 2 (wild type: one female, one male PN)
8	P_0 pronuclear migration (female)	No or little migration of female PN towards the male PN (wild type: directional movement of the female towards the male PN)
9	P_0 pronuclear migration (male)	No migration of male PN from posterior cortex towards centre of the embryo
10	P_0 pronuclear meeting	Position near the cortex or centrally (wild type: near the centre of the posterior half of the embryo)
11	P_0 pronuclear centration	Lack of centration
12	P_0 pronuclear rotation	Rotation takes place after PN envelope breakdown (wild type: approx. 2–3 min before Pronucleur Envelope Breakdown)
13	P_0 pronuclear envelope breakdown	Lag time between PN meeting and PN envelope breakdown exceeds 8 min (wild type: 4–5 min)
14	P_0 spindle assembly – bipolarity	Lack of two visible poles, that is, two regularly shaped sites of yolk granule exclusion
15	P_0 spindle integrity	Irregular length (wild type: 25–30% the length of the embryo during metaphase), or thickness (wild type: 20–25% the width of the embryo at metaphase), or lack of rigidity
16	P_0 spindle elongation	Spindle is shorter or longer than 50–60% the length of the embryo at telophase
17	P_0 spindle rocking	No or excessive spindle rocking
18	P_0 spindle positioning – asymmetry	Aberrant spindle positioning at telophase (wild type: along the longitudinal axis with the posterior pole shifted posteriorly approx. 10–15%)
19	P_0 spindle poles	Irregular shape, in particular lack of flattening of posterior pole in telophase
20	P_0 cytokinesis – furrow specification	Fewer or more than two sites of furrowing and/or aberrant positioning (wild type: two sites, intersecting the long axis by a ratio of approx. 3:2)
21	P_0 cytokinesis – furrow ingression	Little or no ingression, or uneven ingression from the two sites
22	P_0 cytokinesis – completion/stability	Regression of the furrow
23	P_1/AB nuclear separation – cross-eyed	Reforming daughter nuclei stay closely attached to the central cortex
24	AB nuclear migration	Time for centration of AB nucleus exceeds 7–8 min, AB nucleus migrates towards the cortex before centration (wild type: AB nucleus usually centres directly after cytokinesis)
25	P_1/AB cortical activity	Excessive membrane ruffling and blebbing
26	P_1/AB nuclei – number	Numbers of nuclei in daughter cells below or above 1
27	P_1/AB nuclei – size/shape	Size >30% smaller or larger than WT (diameter approx. 25% of AB blastomere), or irregular shape of nuclei
28	P_1 nuclear migration/rotation	Lack of migration of P1 nucleus towards posterior cortex, lack of rotation of P1 spindle before nuclear envelope breakdown
29	P_1/AB spindle assembly	Aberrant bipolarity or length or thickness
30	AB spindle orientation	Rotation of AB spindle (wild type: AB spindle keeps orientation, whereas P1 spindle rotates)
31	P_1/AB asynchrony of divisions	Delay between AB and P1 cleavage furrow initiation is either shorter than 2 min or exceeds 5 min (wild type: approx. 2–3 min)
32	P_1/AB cytokinesis	Aberrant furrow initiation or ingression or completion
33	Four-cell stage cross-eyed	Reforming daughter nuclei stay closely attached to the central cortex
34	Four-cell stage nuclei – size/shape	Irregular size and/or shape of nuclei in daughter cells
35	Four-cell stage configuration	PAR-like configurations of blastomeres
36	Tetrapolar spindle	Four poles, visible by exclusion of yolk granules
37	Yolk granules – density	Reduction more than 30%
38	Yolk granules – size	Aberrant size of individual or all granules
39	Areas devoid of yolk granules	Aberrant cytoplasmic structures excluding yolk granules
40	Polar bodies	Aberrant number (fewer or more than two) or size (matches or exceeds size of early PNs) or internalization of polar bodies
41	Unclear – multinucleate	Aberrant numbers of nuclei
42	Overall pace of events	Time span between pronuclear meeting and initiation of AB cleavage furrow exceeded 30 min (wild type: 18–22 min)
43	Number/age of embryos	Absence or reduction of one-cell and two-cell stage embryos, suggesting reduced fertility of the parent worm
44	Meiotic arrest	No visible PNs, few or no cytoplasmic events, embryo often fills egg shell
45	Other	Any other observation deferring from wild-type events
46	Inadequate test	Technical inadequacy, (focusing, coverage of recordings, etc.)

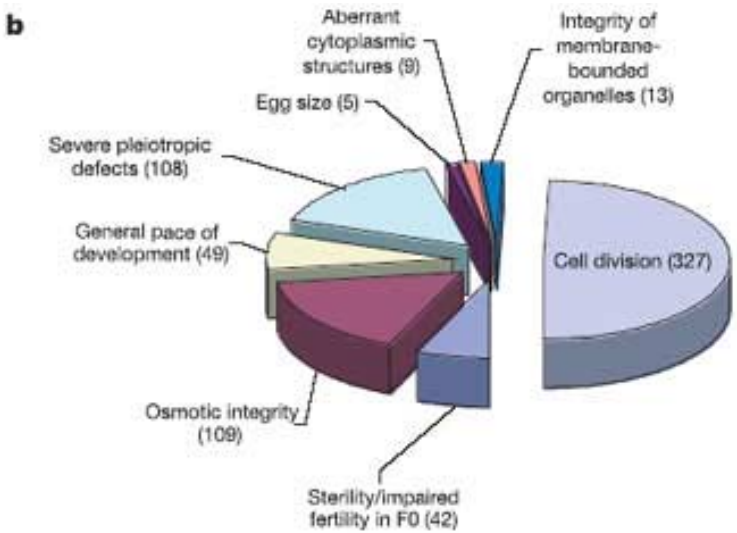
AB and P_1, anterior and posterior blastomere; PAR genes, genes involved in polarity of the embryo; PN, pronucleus.

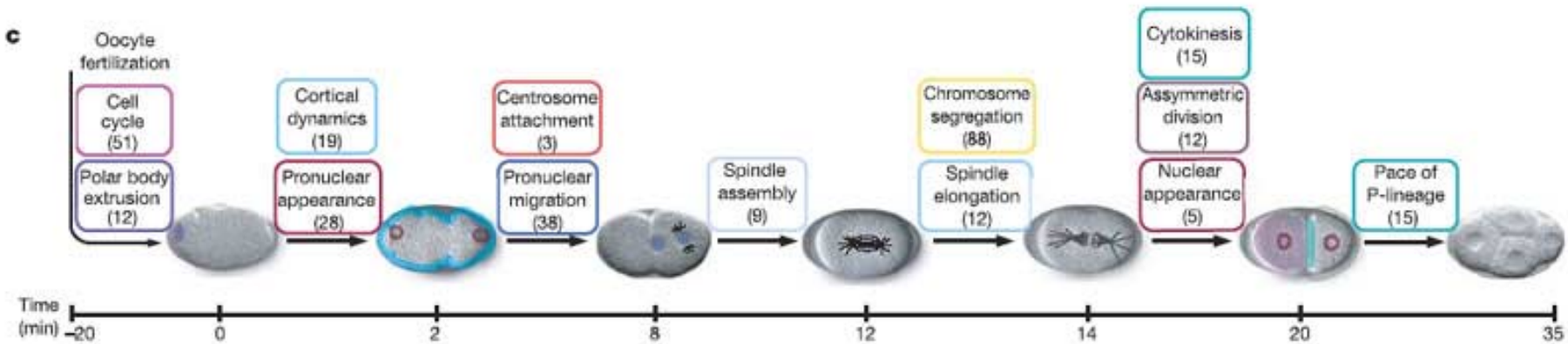
Clustering of phenotypes



Assignment of genes to embryonic processes

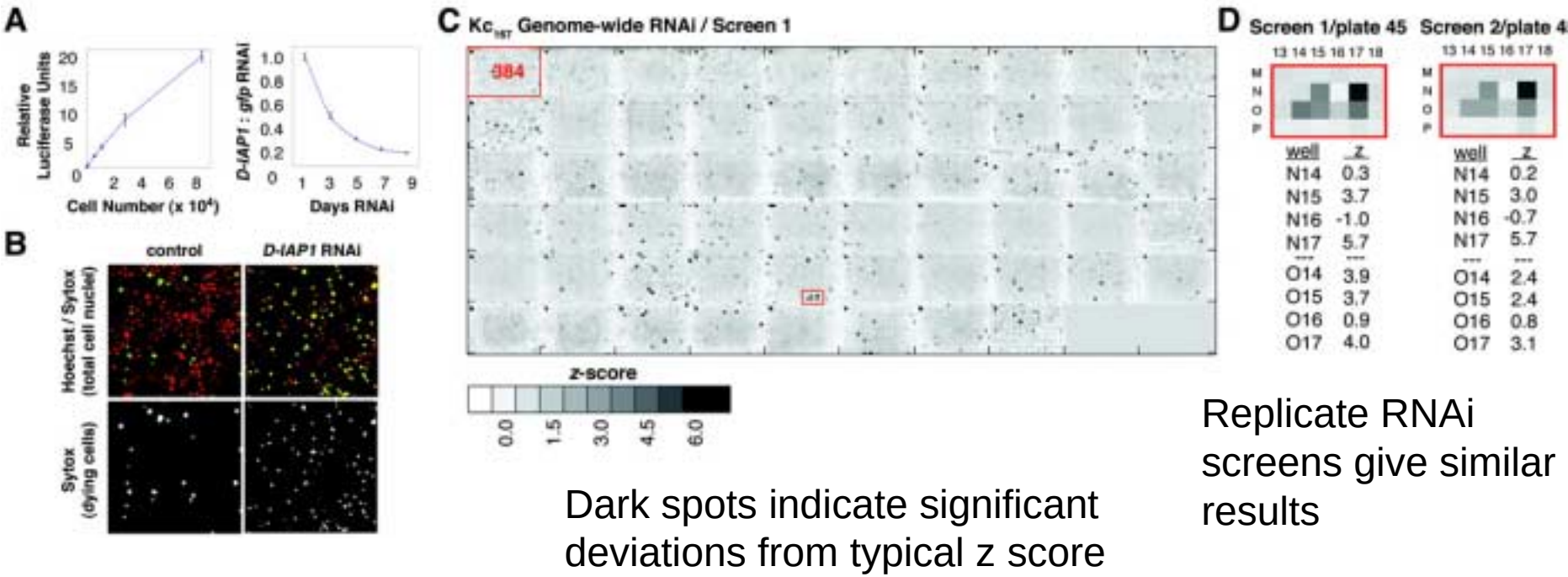






Drosophila RNAi screens in cells

Cell viability screen - quantitative assay of cell number that correlated the reduction of signal to dying cells (based on luciferase levels



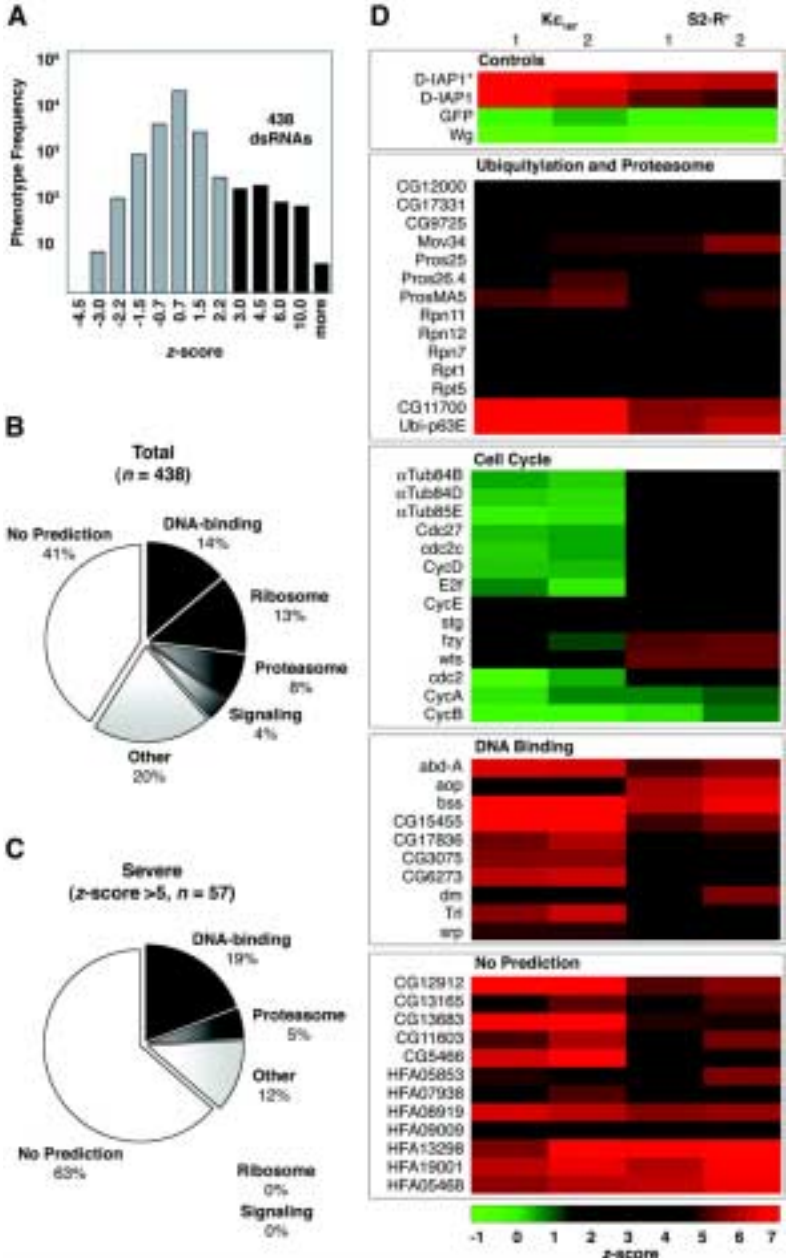
Dark spots indicate significant deviations from typical z score

Replicate RNAi screens give similar results

Two cell lines, each done in duplicate
438 genes with z score >3, taken as core “cell viability” genes

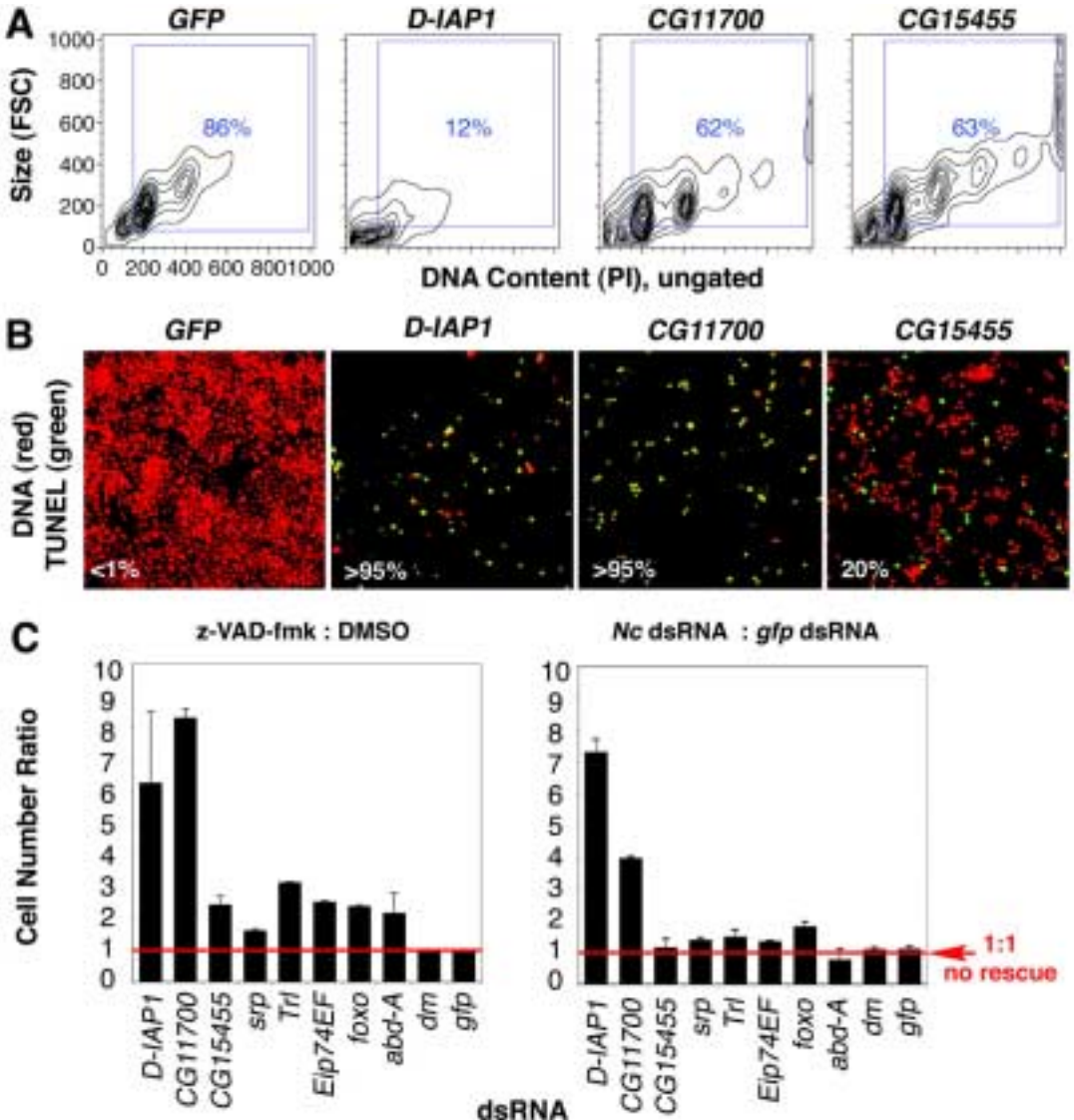
Boutros et al., Science 303: 832-5 2004

Drosophila RNAi screens in cells



Drosophila RNAi screens in cells

Analysis of candidate apoptosis genes

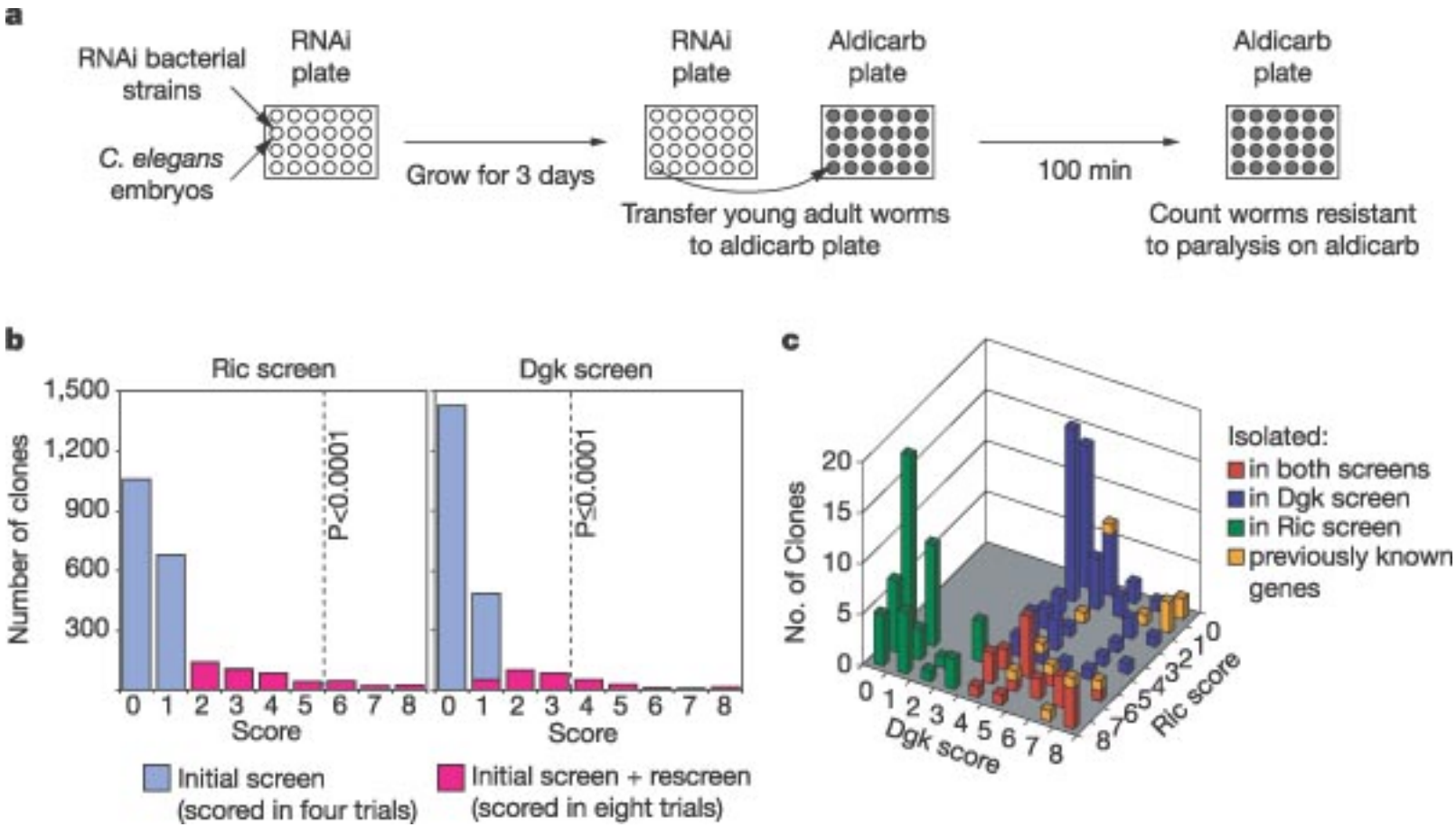


C. elegans screen for synaptic genes

- treatment with aldicarb interferes with acetylcholine uptake at neuronal synapses, resulting in paralysis/death
- screened for genes that rescue the paralysis
- neurons are resistant to RNAi, so used a double mutant background with enhanced RNAi sensitivity
- Loss of *dgk-1* (DAG kinase) enhances sensitivity to aldicarb (might be more sensitive to modest reductions of ACh secretion produced by RNAi treatments)

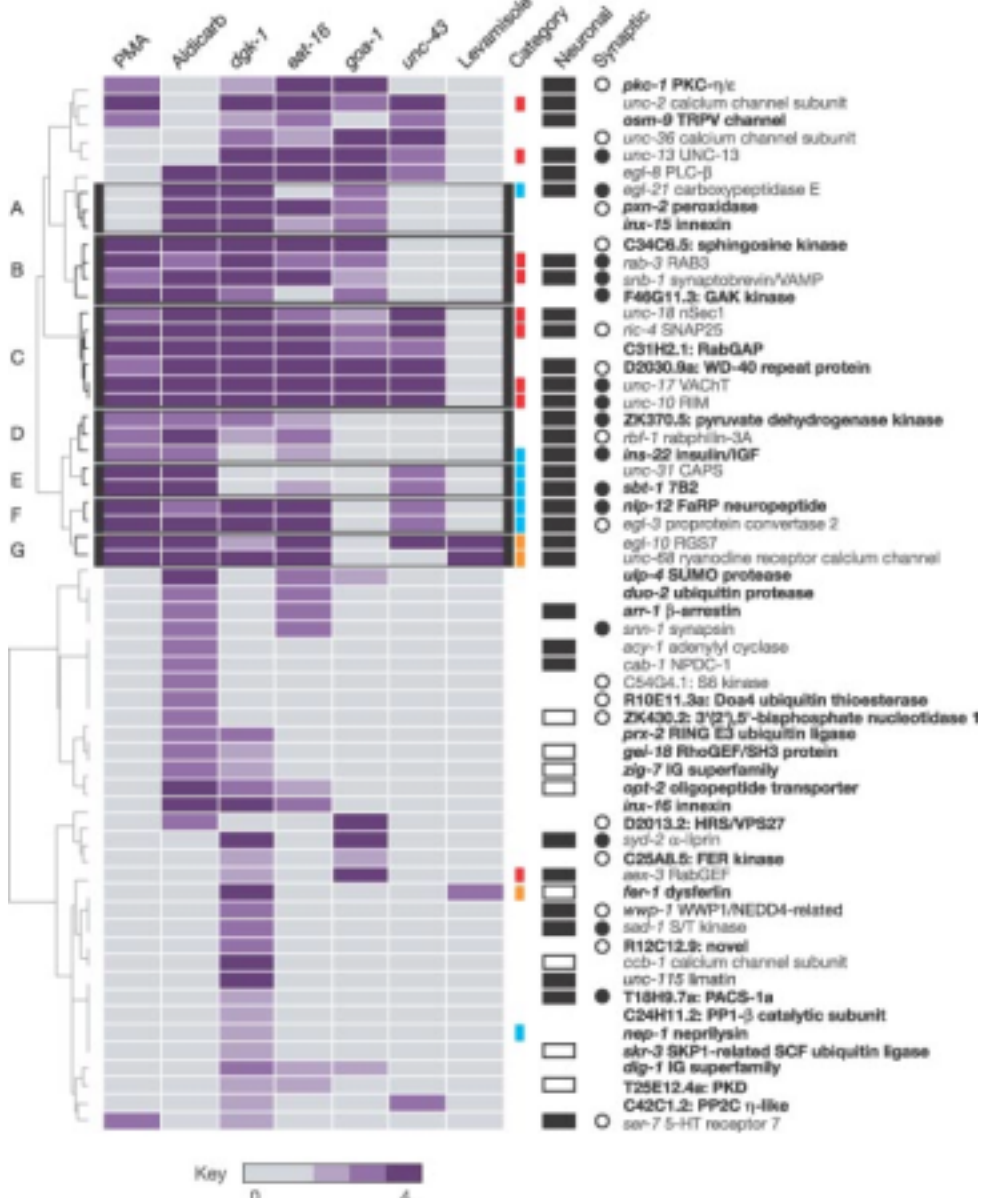
Sieburth et al., Nature 436: 510-7 2005

Screen design



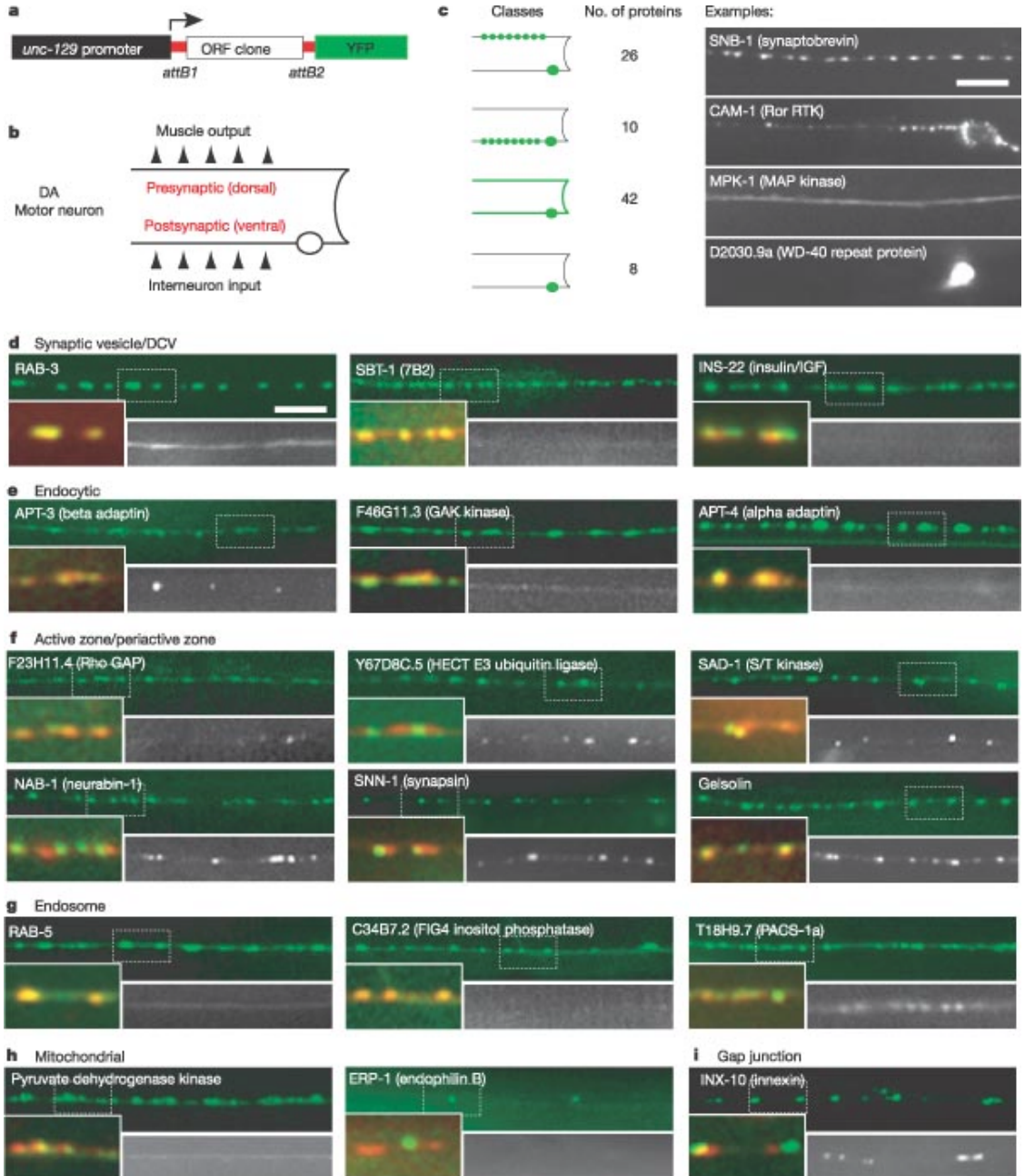
Sieburth et al., Nature 436: 510-7 2005

Secondary screens (“functional profiling”) to classify candidate genes

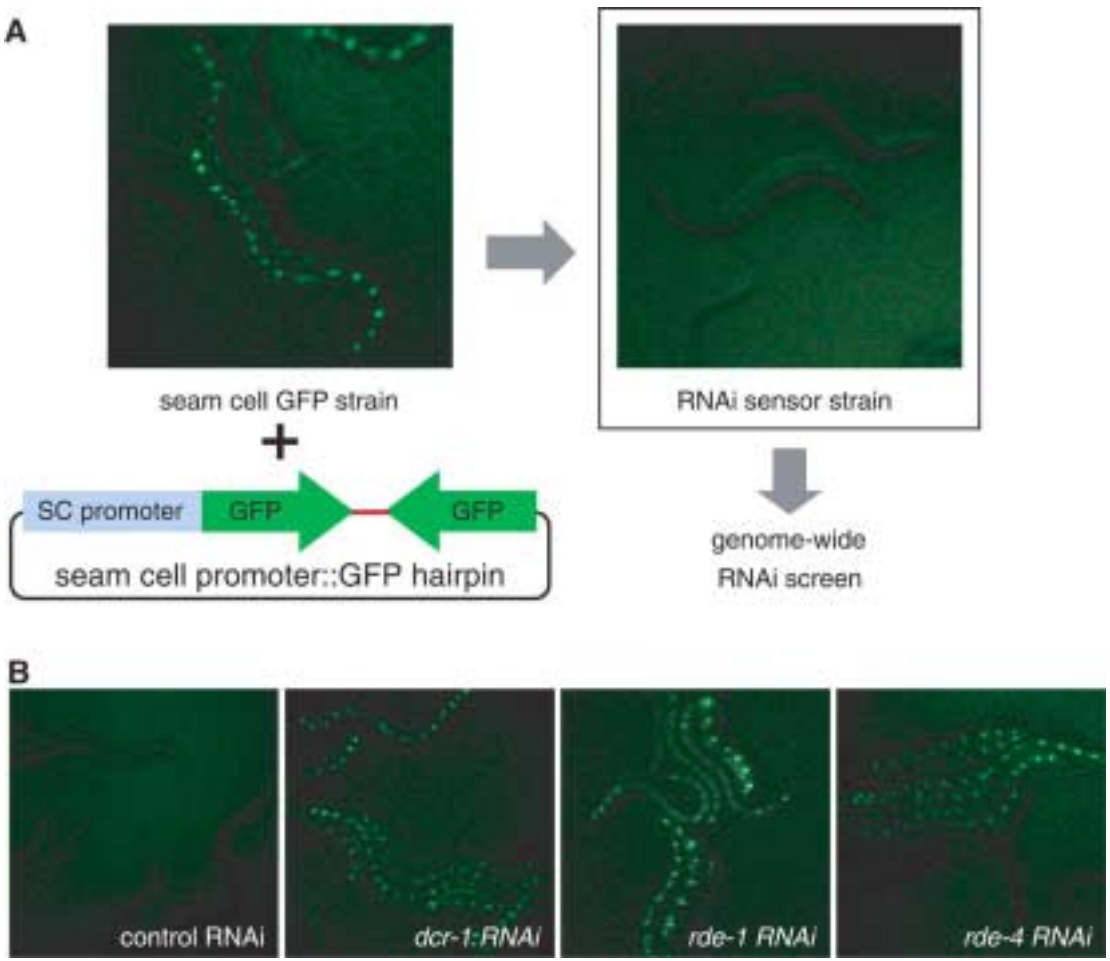


Analysis of sub-cellular localization of candidates

Of 100 proteins analysed, ten were localized in a punctate pattern in the ventral cord (candidate dendritic proteins) and 26 were punctate in the dorsal cord (candidate axonal proteins)



RNAi screen for factors involved in RNAi



identified 90 genes that disrupted RNAi when RNAi'd

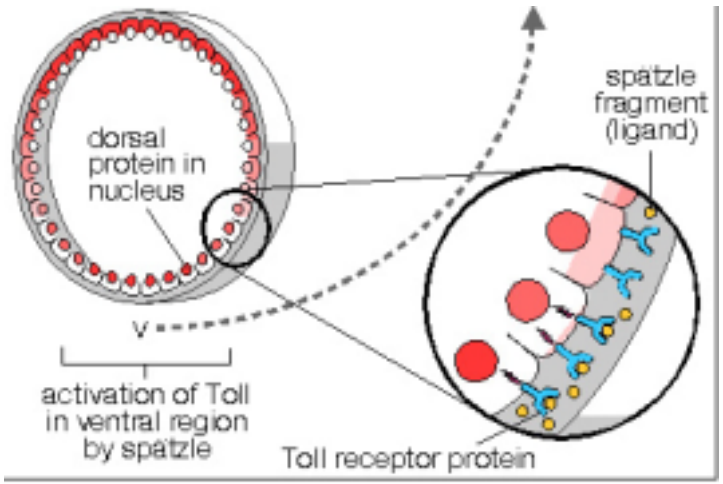
confirmed by testing on standard RNAi of other genes, as well as in other RNA silencing mechanisms

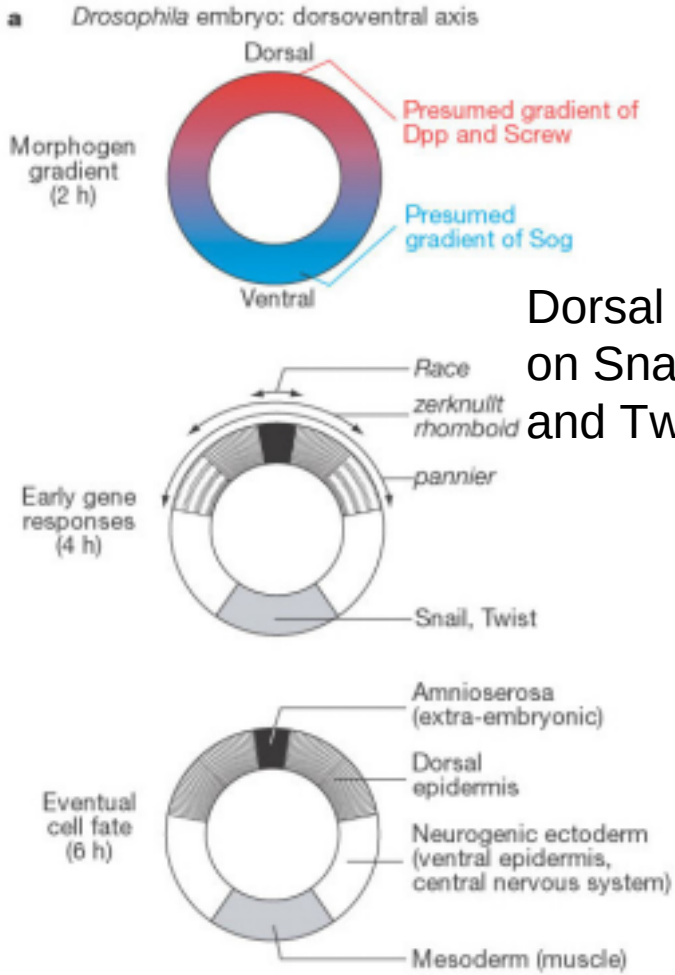
Kim et al., Science 308:1164-7 2005

Chromatin immunoprecipitation in model organisms

- Identify protein-DNA interactions in vivo
- Define transcriptional regulatory networks in the context of developmental events
- Define chromatin/chromosome regulation in developmental context
- Previously done using ChIP followed by microarray analysis, now switching to ChIP followed by deep sequencing.

Dorsal/ventral polarity in Drosophila





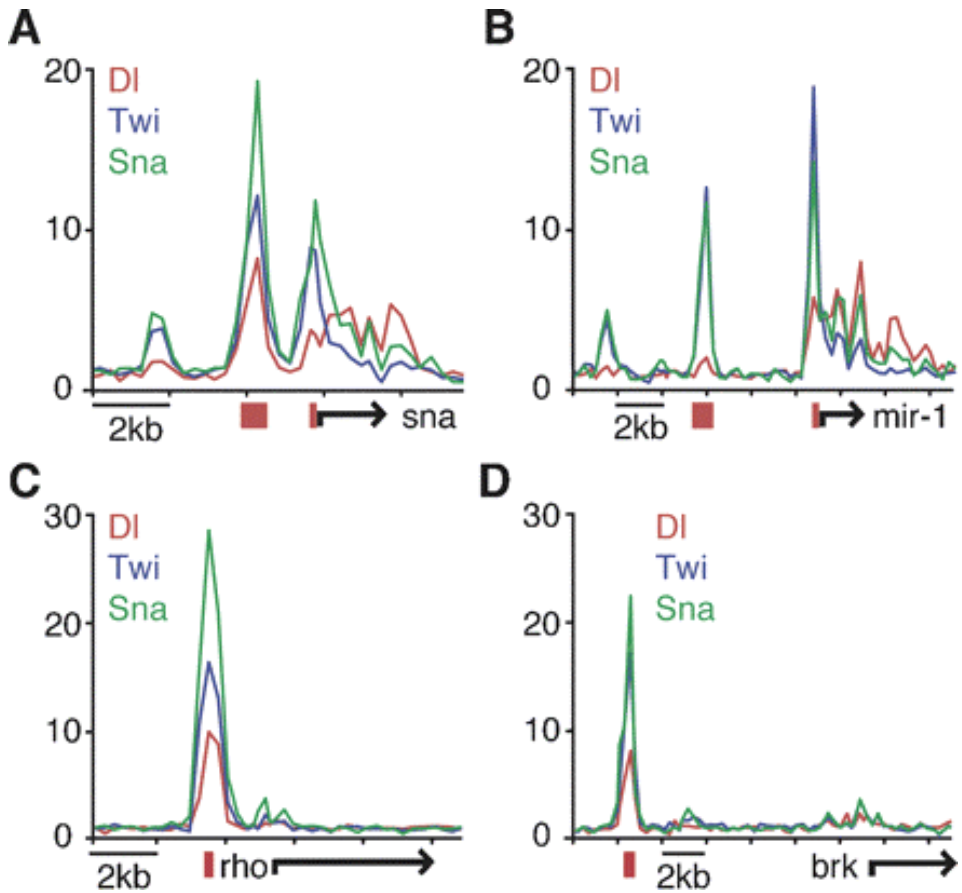
Dorsal turns on Snail and Twist

- Transcription factors dorsal, twist and snail all contribute to establishment of dorsal-ventral polarity of the Drosophila embryo, and seem to share target genes

Zeitlinger et al., Genes Dev 21: 385-390 2007

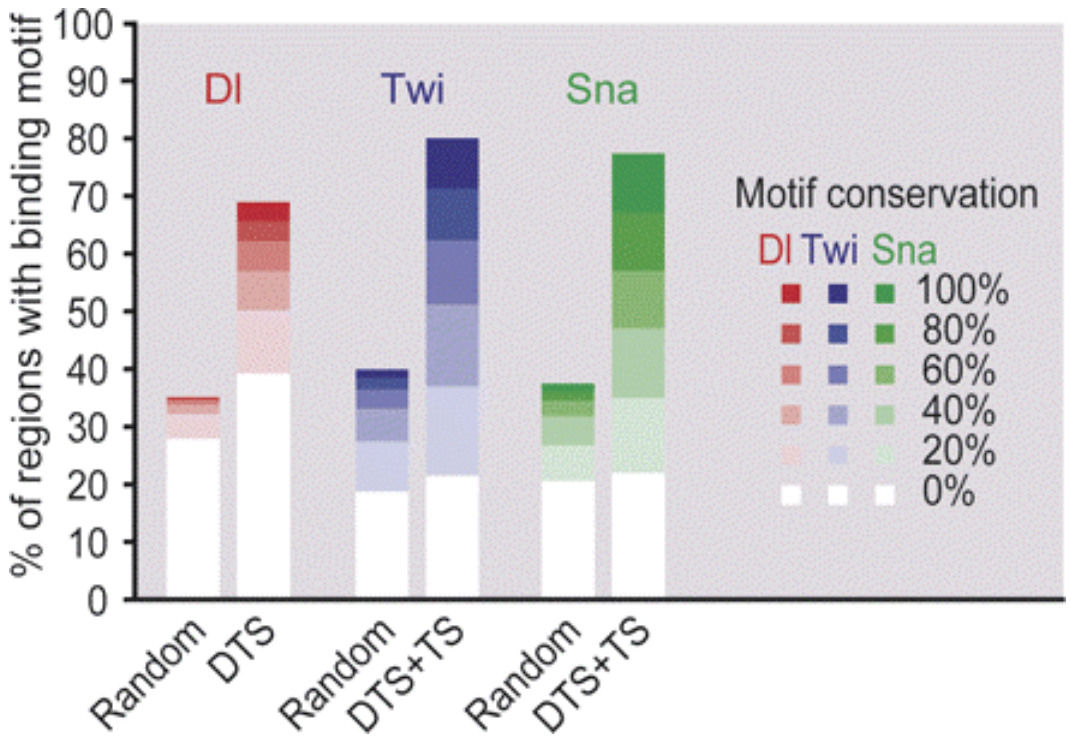
Dorsal, Snail and Twist binding sites exhibit substantial overlap

428 high-confidence DTS and 433 high-confidence TS regions across the genome

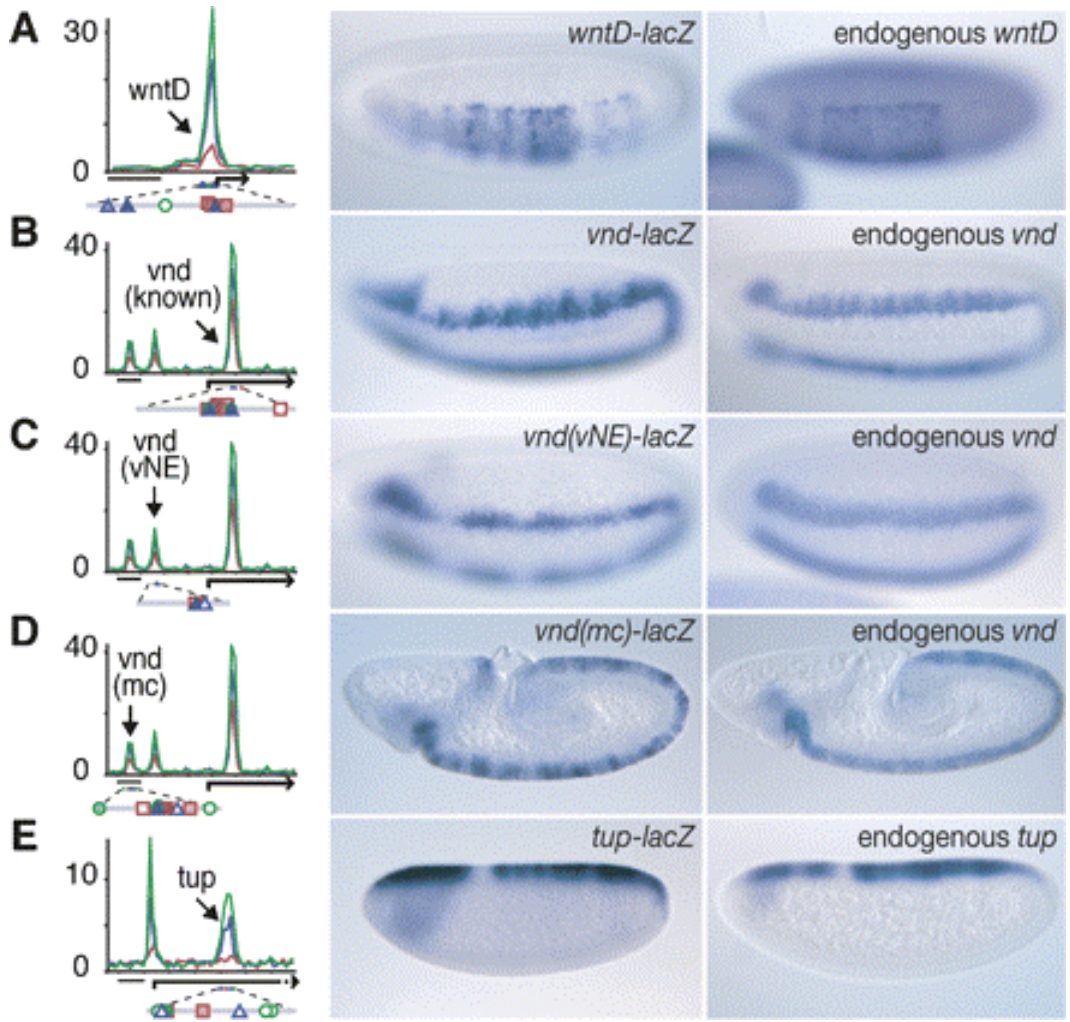


Performed in ventralized embryos, which provide uniform substrate

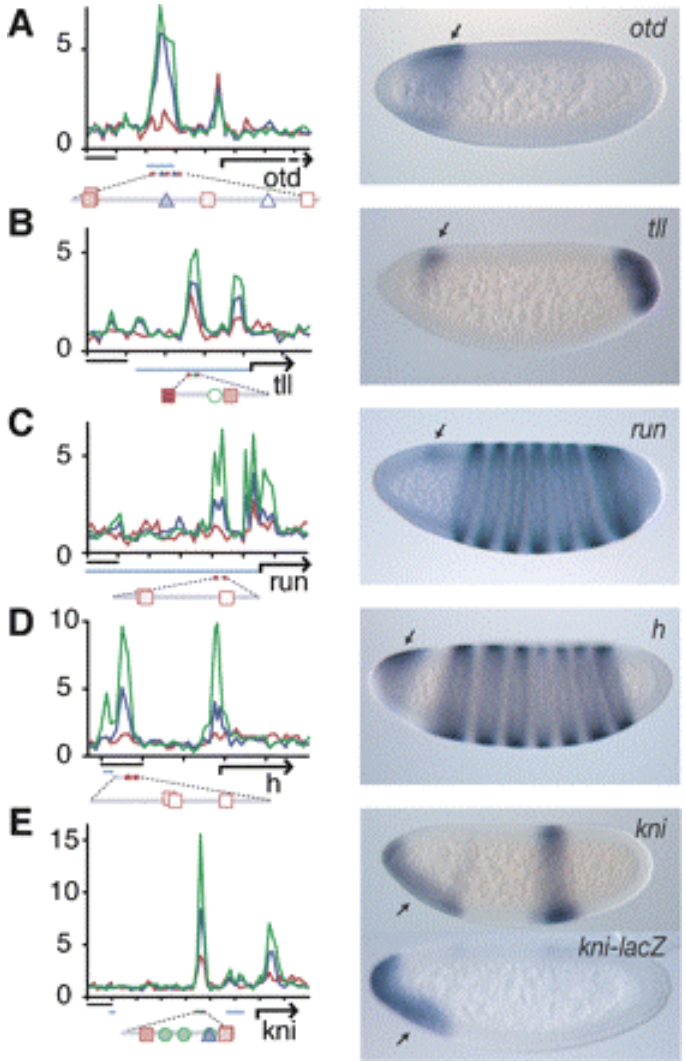
Binding sites contain known binding sequence motifs



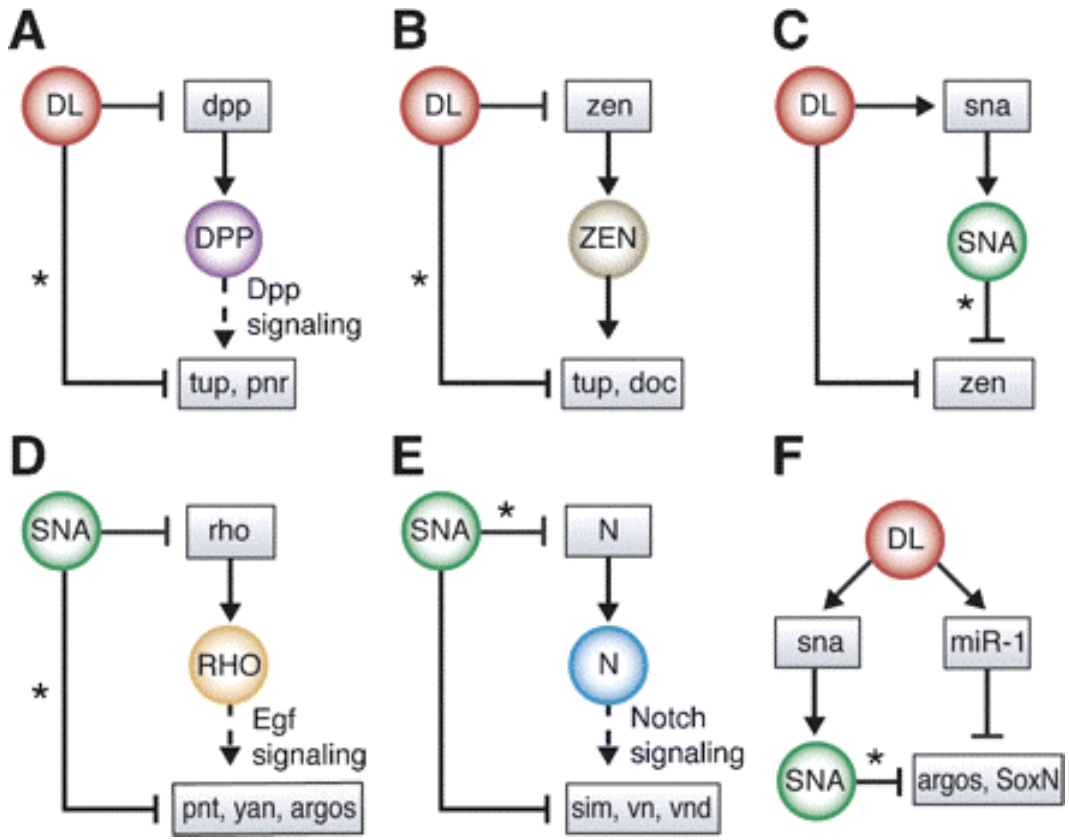
Confirmation of ventral expression of novel candidate genes by in situ



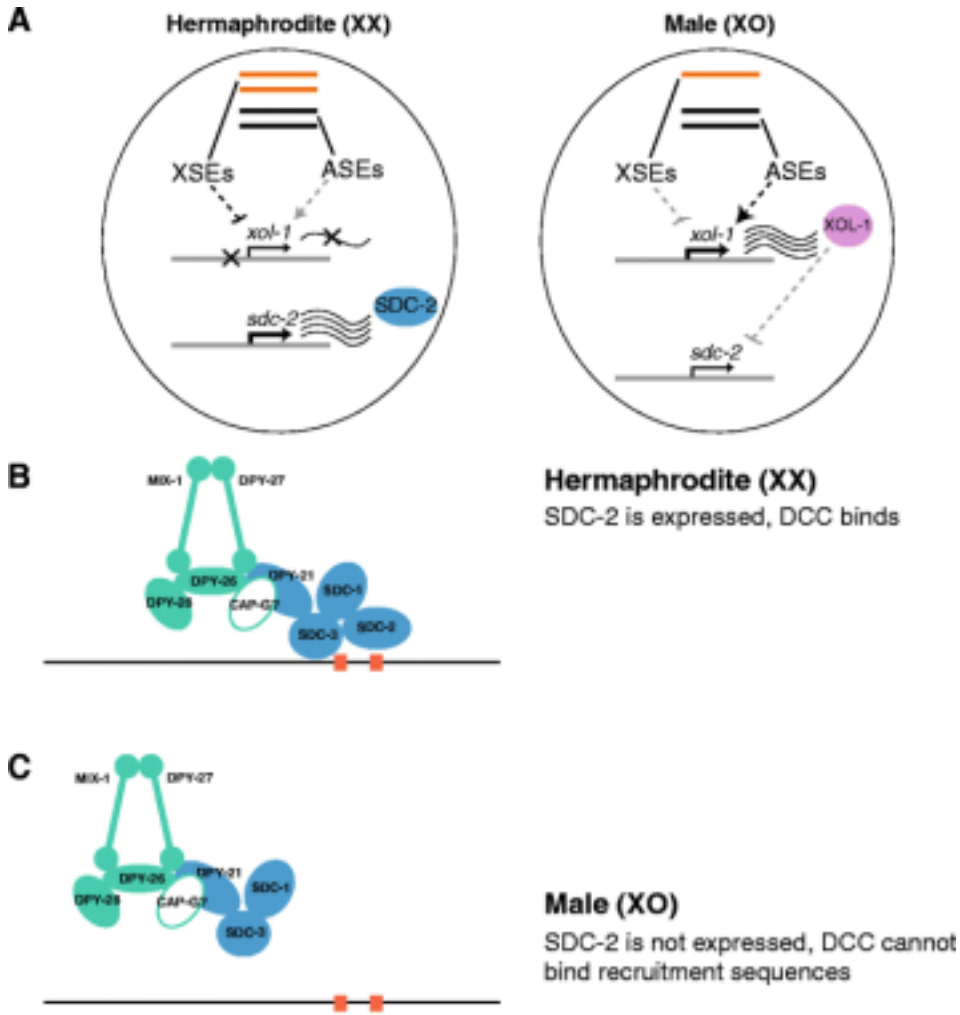
Some (D)TS sites regulate genes that direct AP polarity



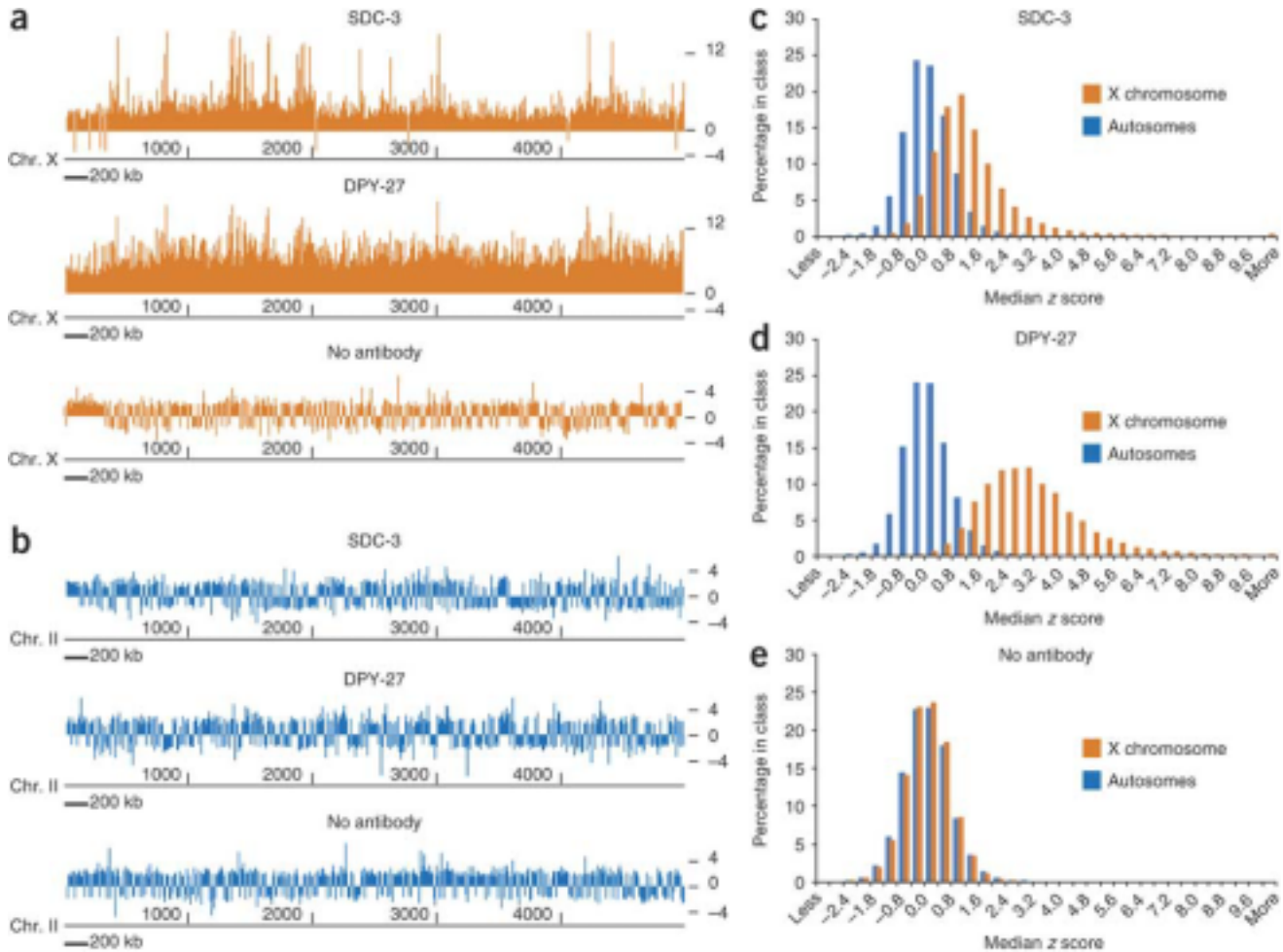
Integration of new target genes into the Dorsal network



Dosage compensation in *C. elegans*



ChIP of two dosage compensation components

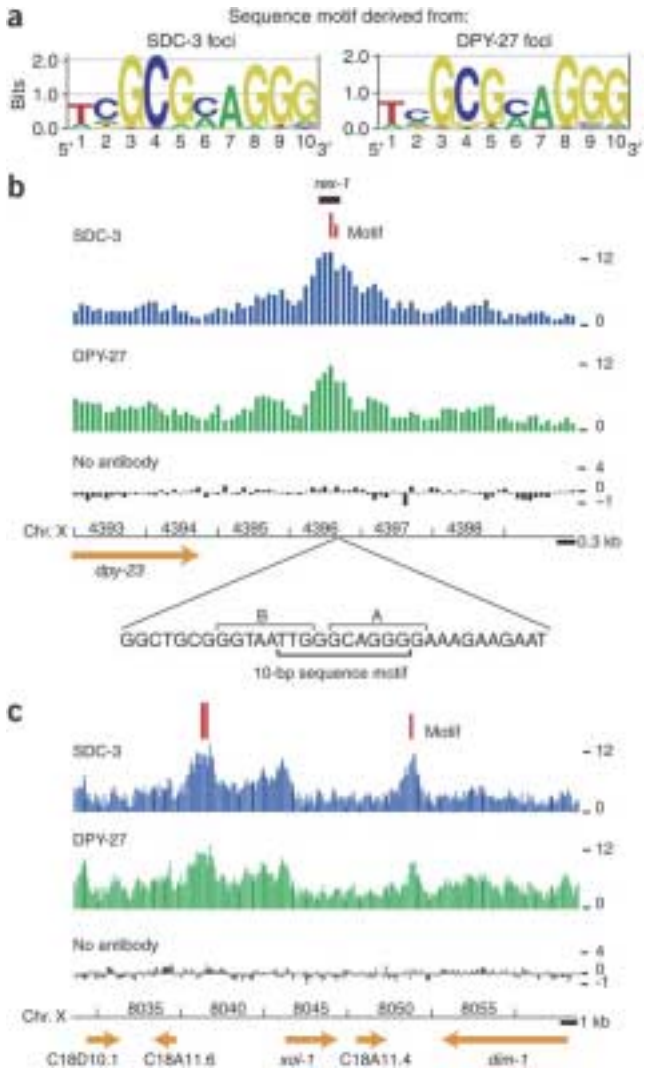


Ercan et al., Nature Genetics 39:403–8 2007

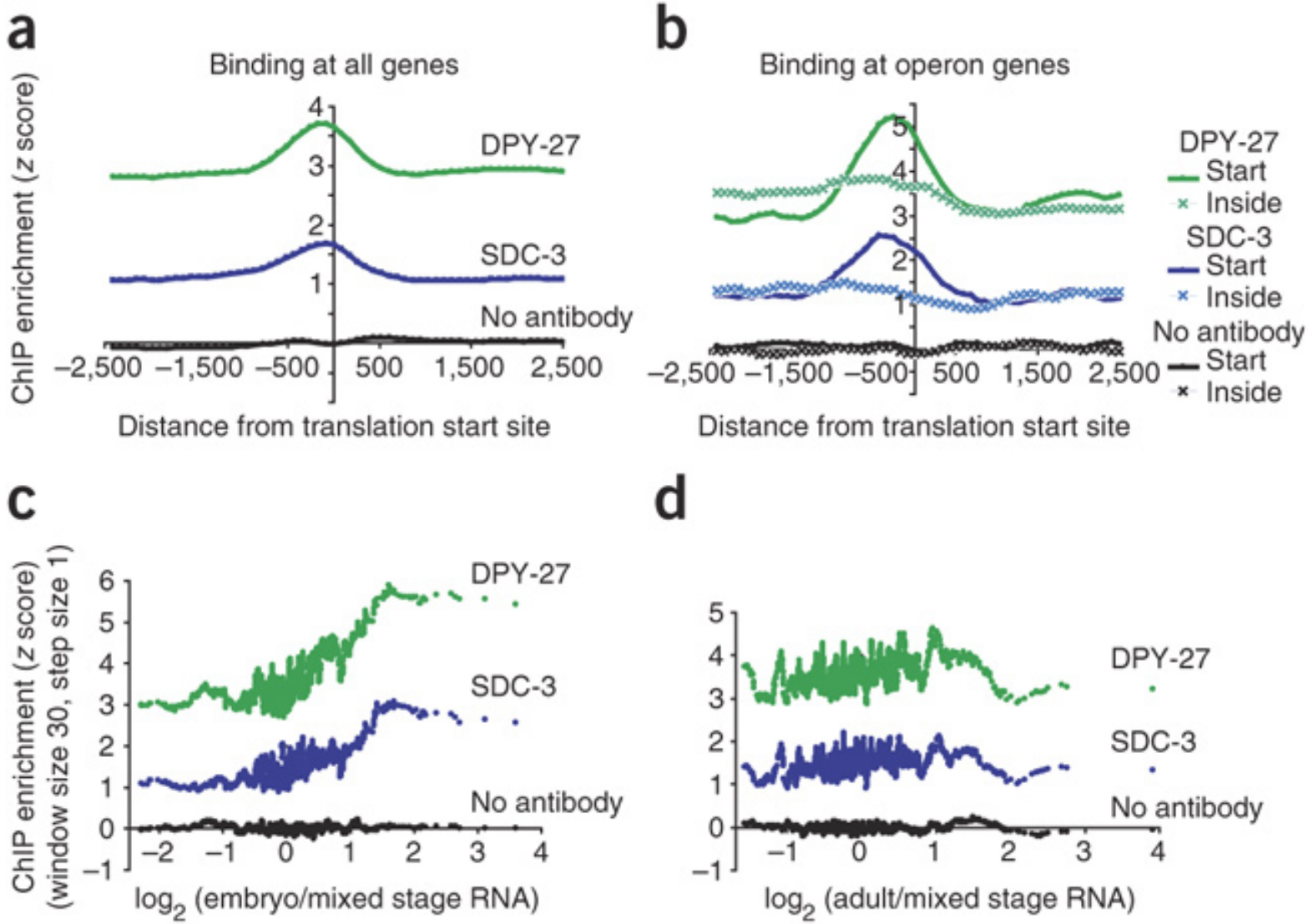
Derivation of DCC binding site sequence

Notably, sequence is enriched on X, but not exclusive to X.

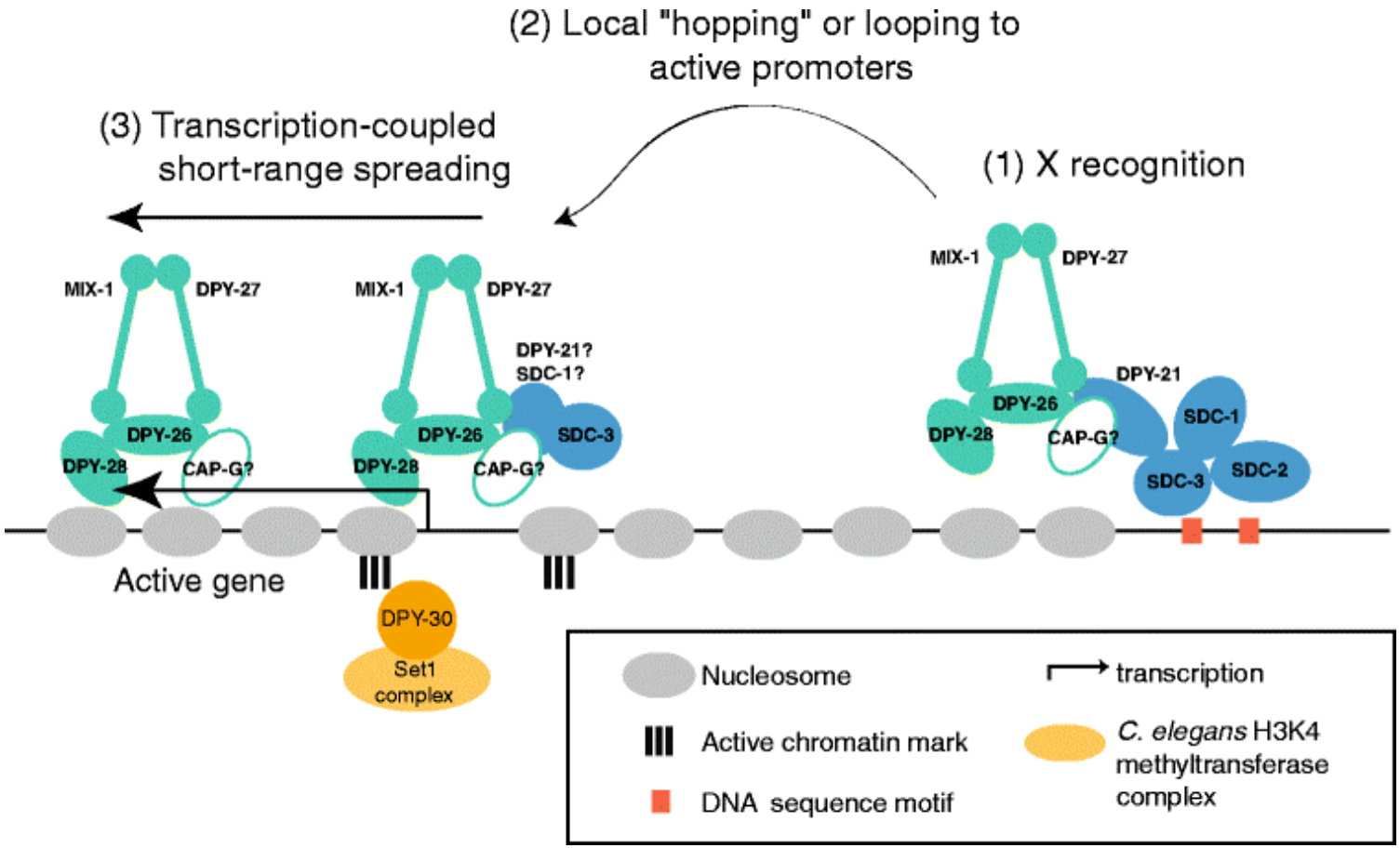
Had to be experimentally defined.



DCC binds preferentially at promoters

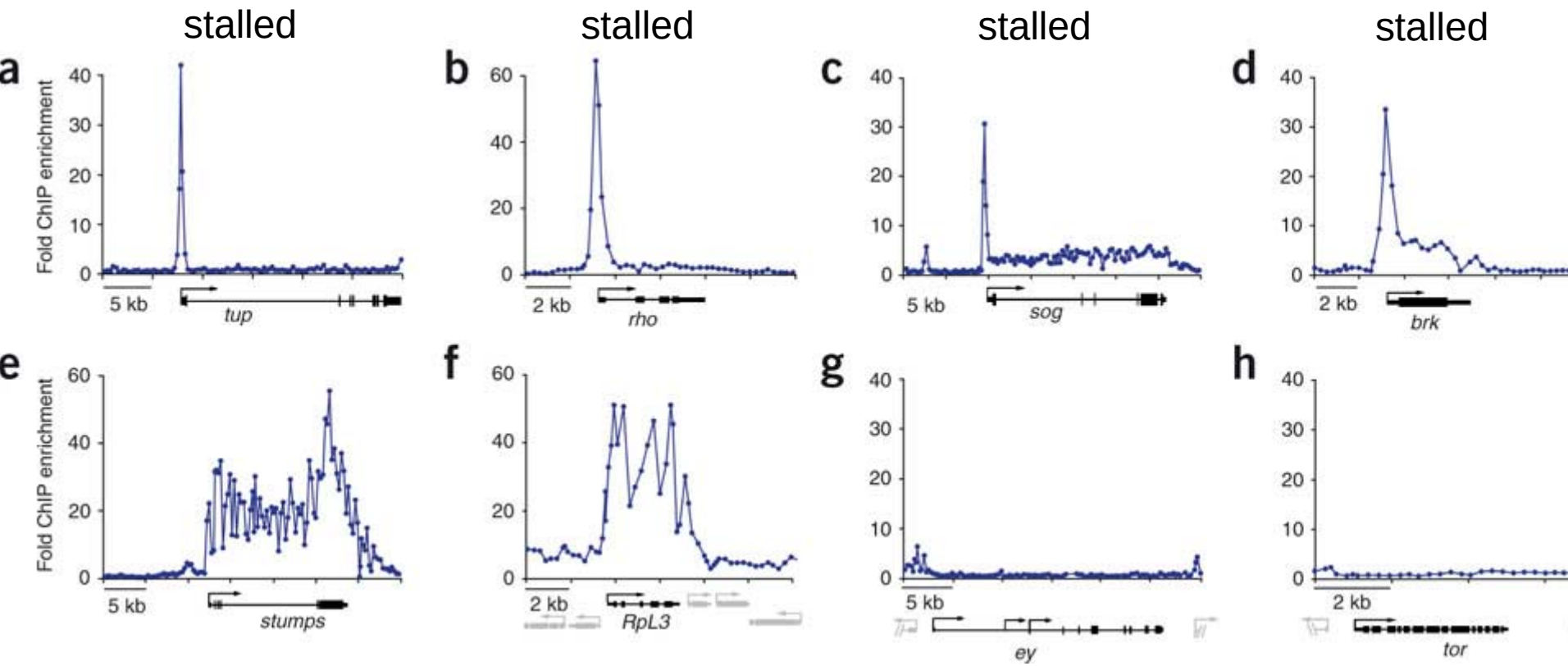


Revised model for DCC action



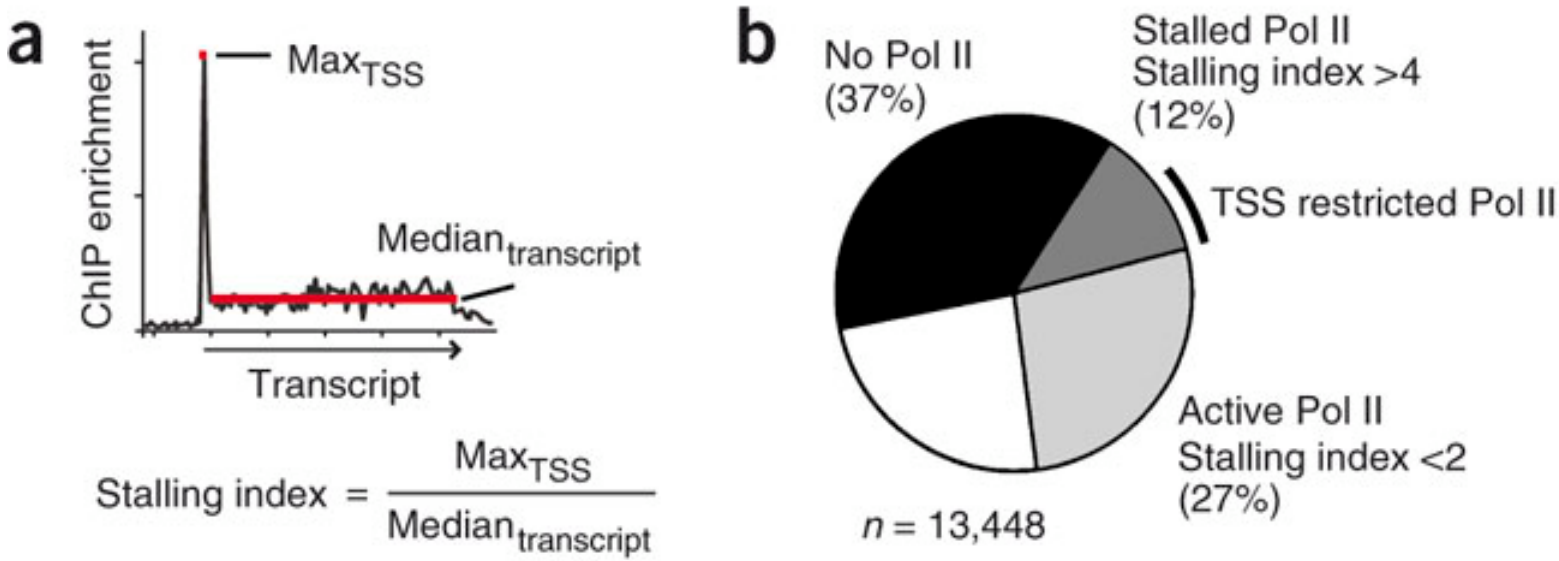
RNA pol II ChIP in Drosophila reveals polymerase stalling

a-d: txly repressed genes, e,f: active genes g-h: inactive genes

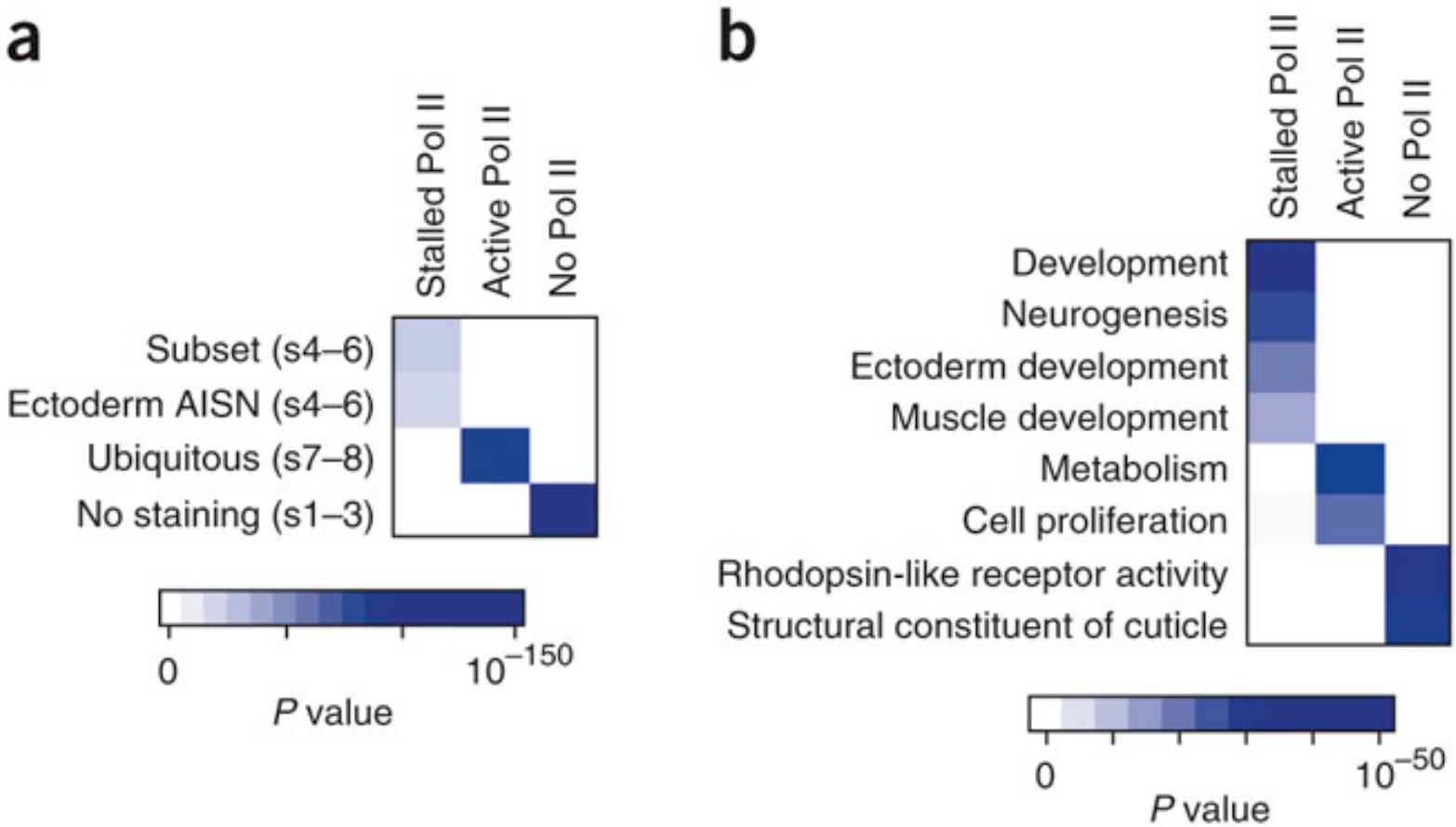


Zeitlinger et al., Nature Genetics 39: 1512-6 2007

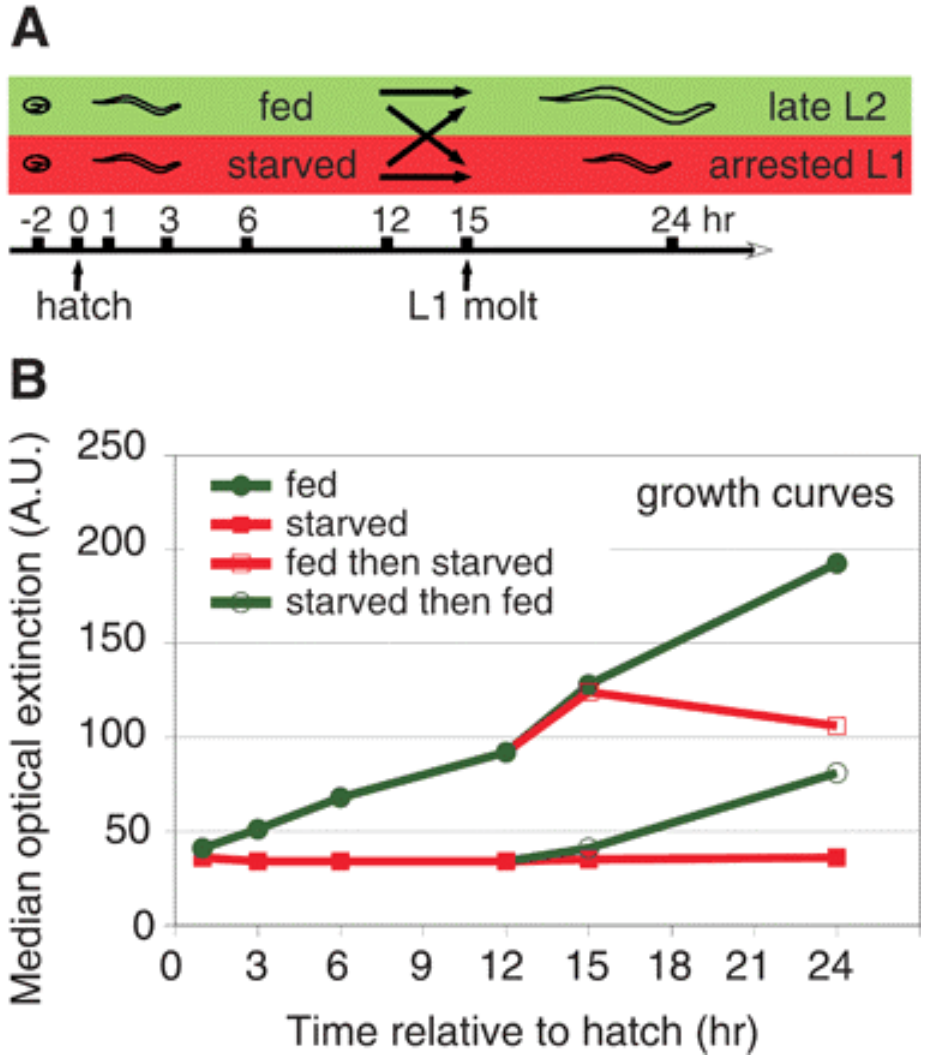
Establishment of “stalling index”



RNA pol II stalls at developmentally regulated genes

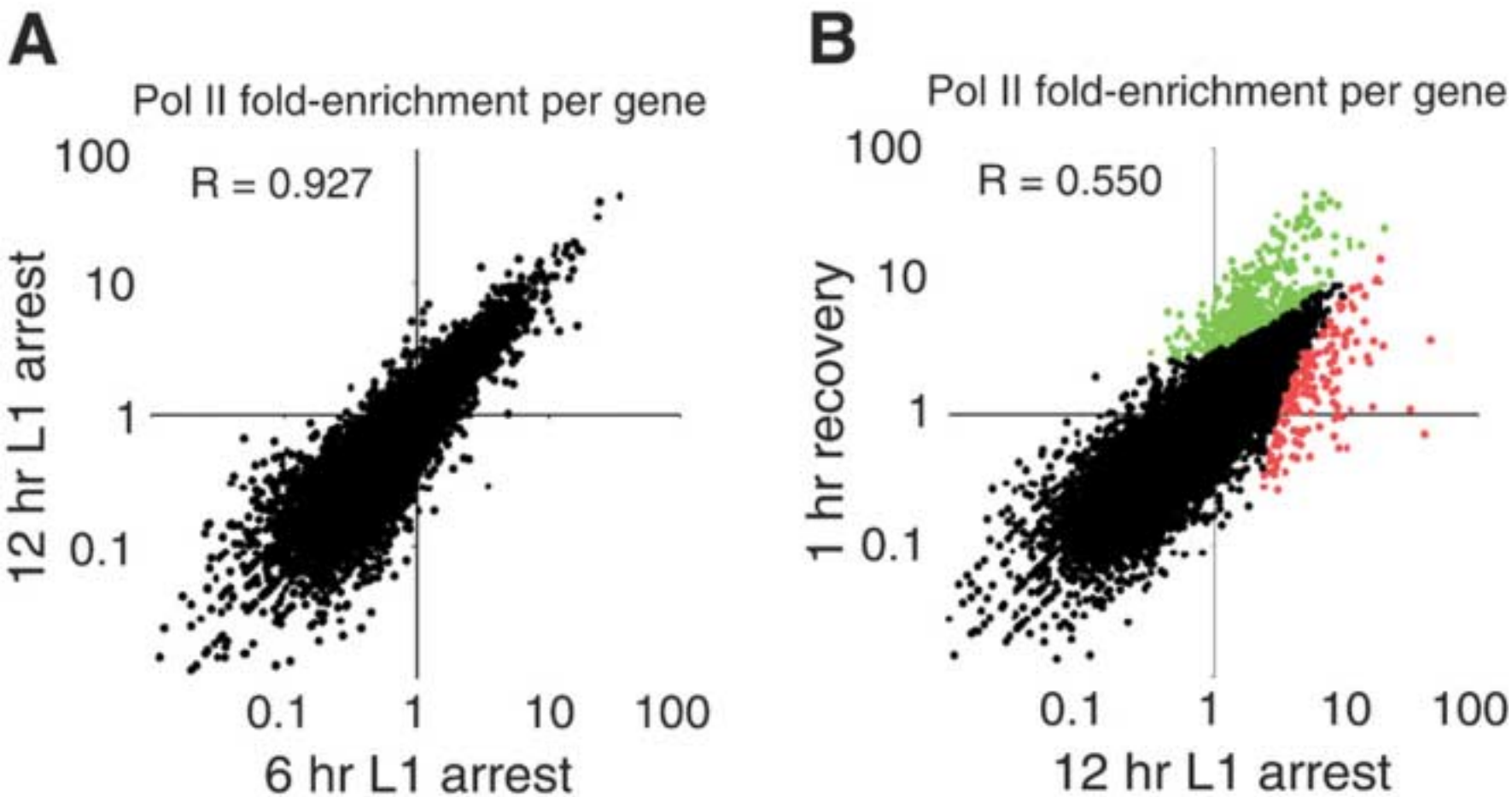


C. elegans RNA pol II ChIP under starvation conditions

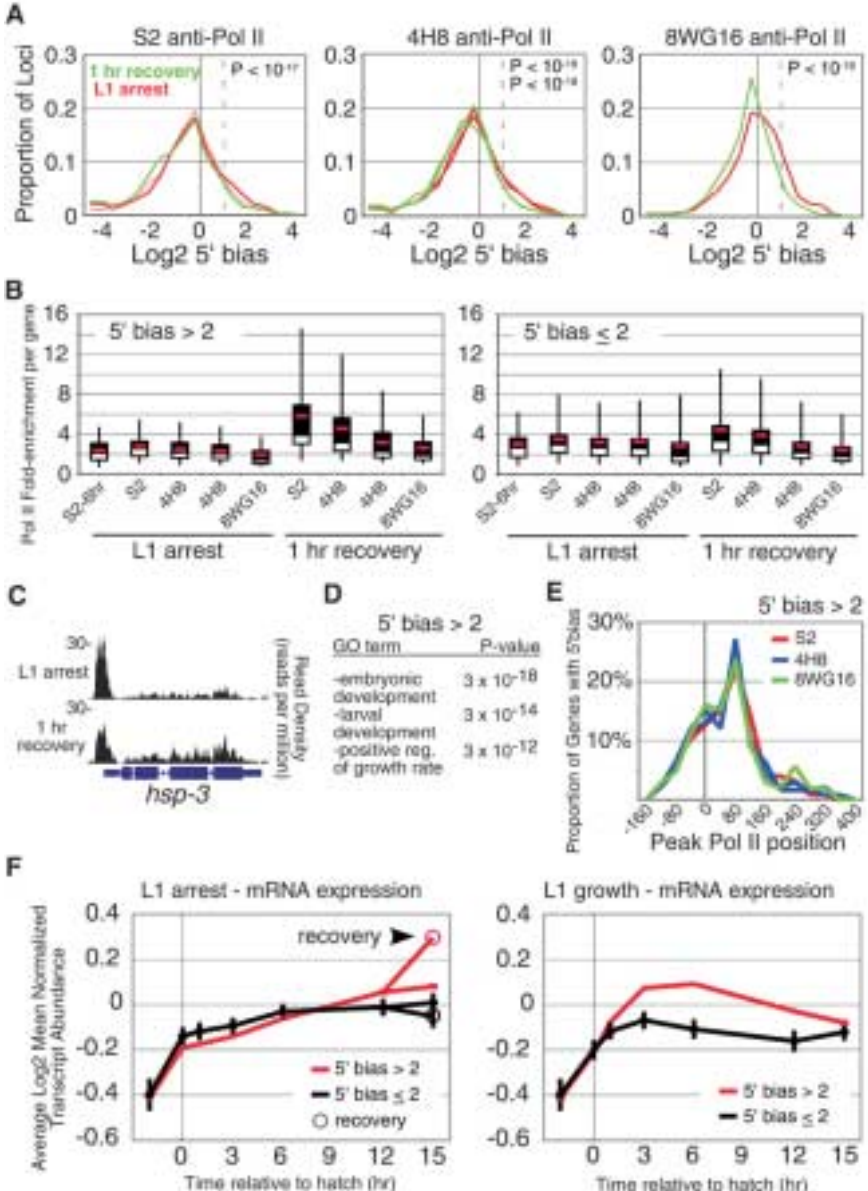


Baugh et al., Science 324: 92-4 2009

RNA pol II distribution changes dramatically upon feeding



Pol II pausing/stalling seen at growth genes in arrest conditions



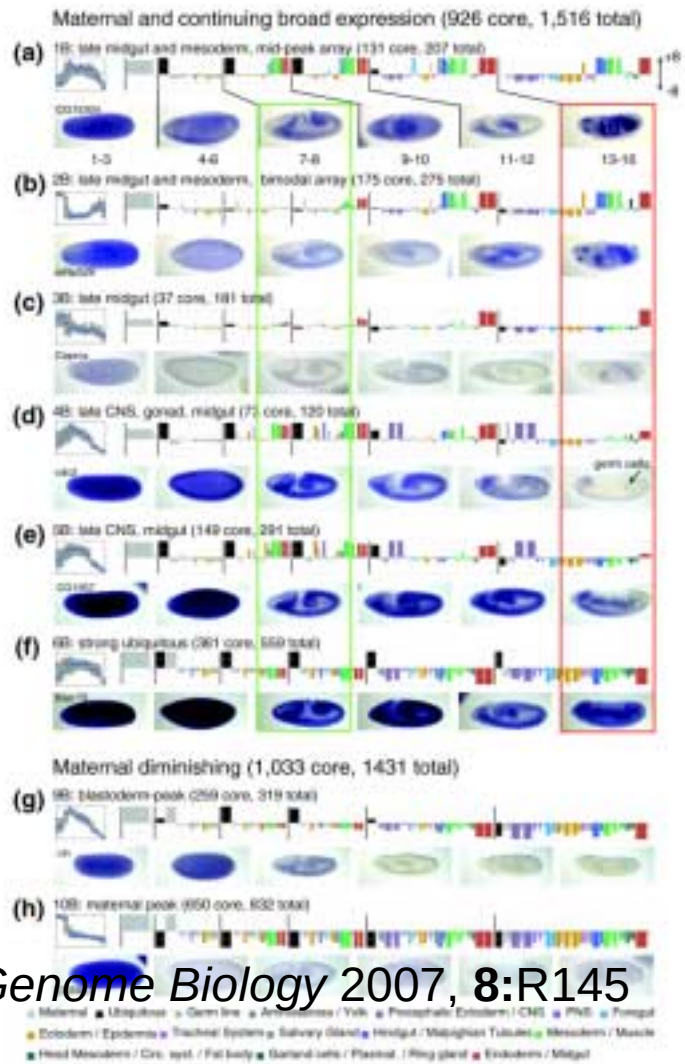
Deep sequencing

- Identify mutants
- Improve annotation (RNA seq)
- Improve data quality of ChIP experiments

Systematic imaging

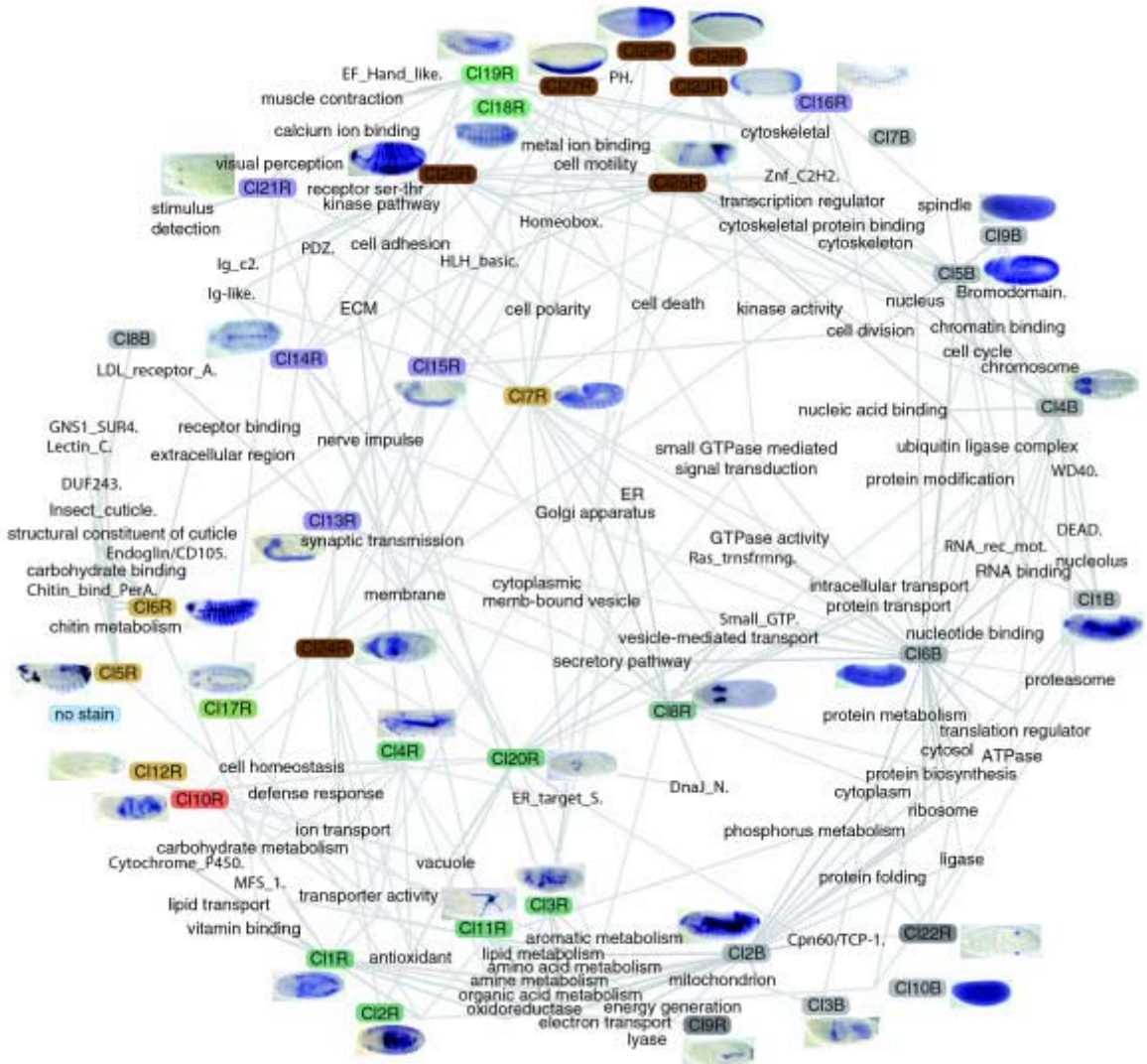
- In situ hybridization
- Reporter/lineage analysis

In situ hybridization in Drosophila embryogenesis

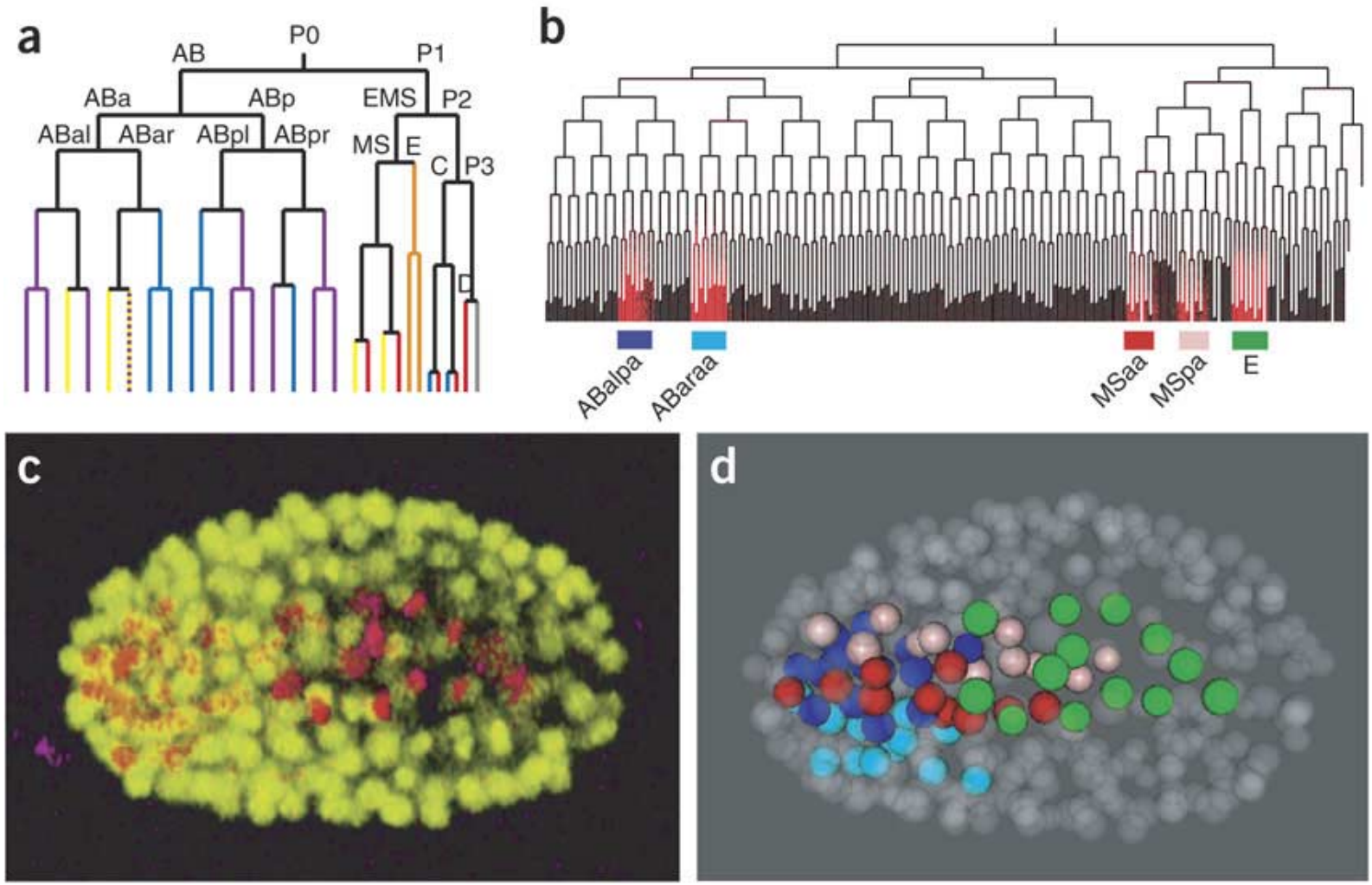


Tomancek et al., Genome Biology 2007, **8:**R145

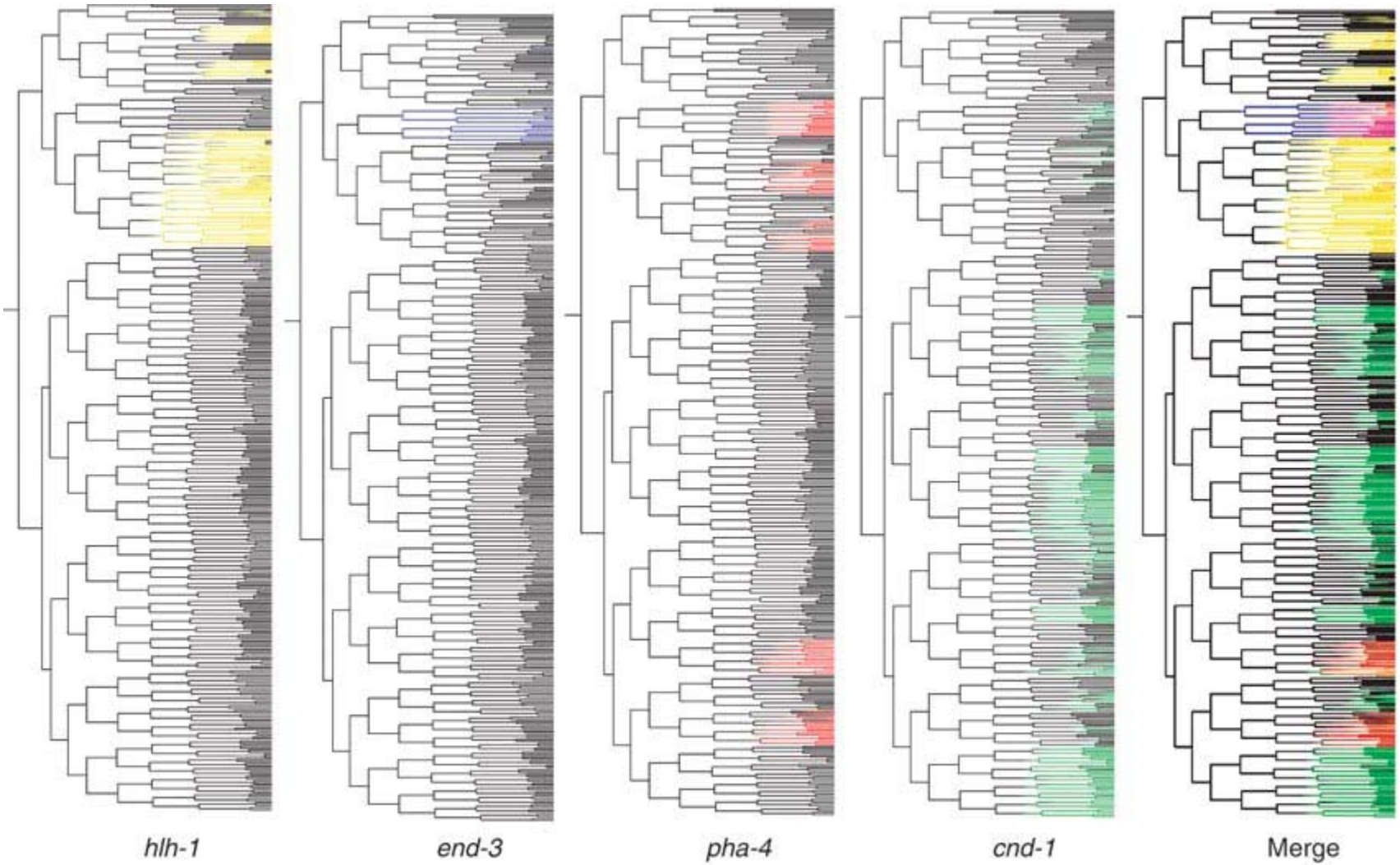
In situ hybridization in Drosophila embryogenesis



Reporters + lineage in C. elegans embryogenesis



Reporters + lineage in C. elegans embryogenesis



Reporters + lineage in C. elegans embryogenesis