How modENCODE informs us about human chromatin

Jason Lieb
Carolina Center for Genome Sciences
University of North Carolina at Chapel Hill

What chromatin data did we generate?

A total of 291 Worm datasets,
mostly over 3 developmental timepoints:
Early embryo, 3rd Larval stage, and adult

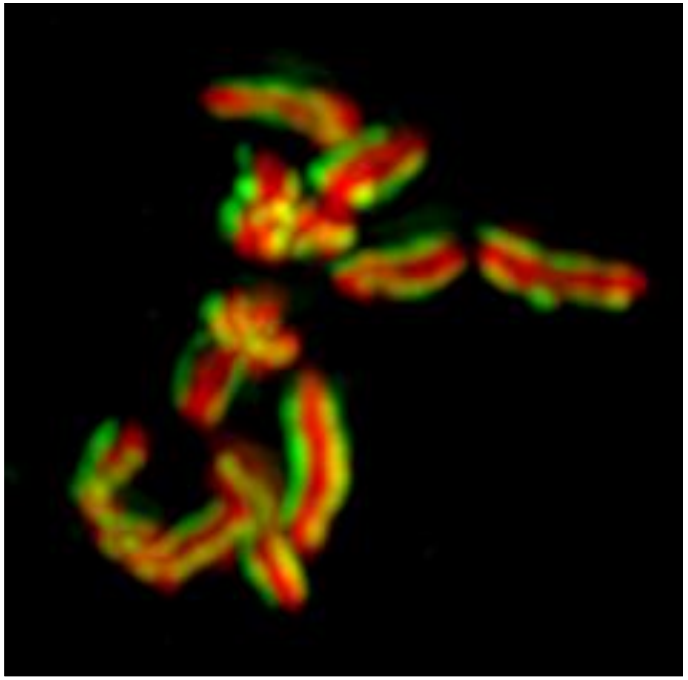
133 **profiles of 30 different histone marks**
147 **profiles of 72 different non-histone chromosomal proteins**
451 **polyclonal antibodies, 288 validated by at least one assay**

A total of 601 Fly datasets
mostly over 3 cell lines
plus early embryo, 3rd Larval stage, and adult
288 **profiles of 28 different histone marks**
313 **profiles of ~50 non-histone chromosomal proteins**

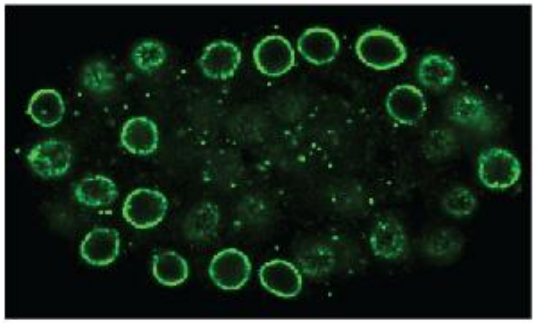
See talks by Gerstein (networks) & Kellis (states)

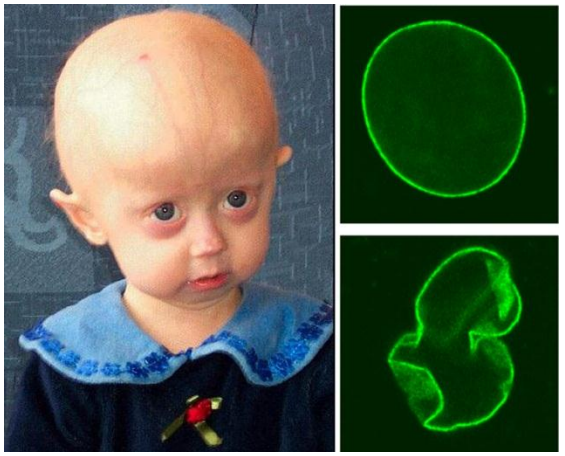
2 stories: Unique insights gained by modENCODE

(1) Centromere specification



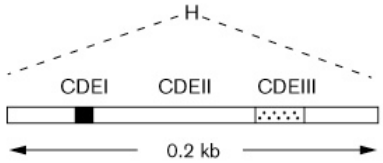
(2) Chromosome-membrane interactions



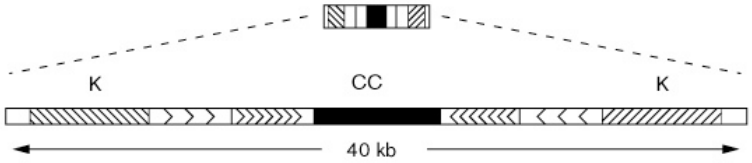


PLoS Biology Vol. 3/11/2005, e395

Centromeric DNA is not conserved

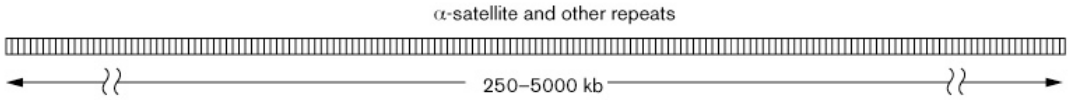


Budding Yeast: 125 bp



Fission Yeast: 40,000-100,000 bp

Fruit Fly: ~400,000 bp

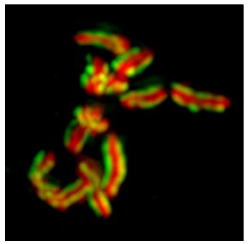


Humans: up to 5 million bp

α-satellite: NEITHER NECESSARY NOR SUFFICIENT

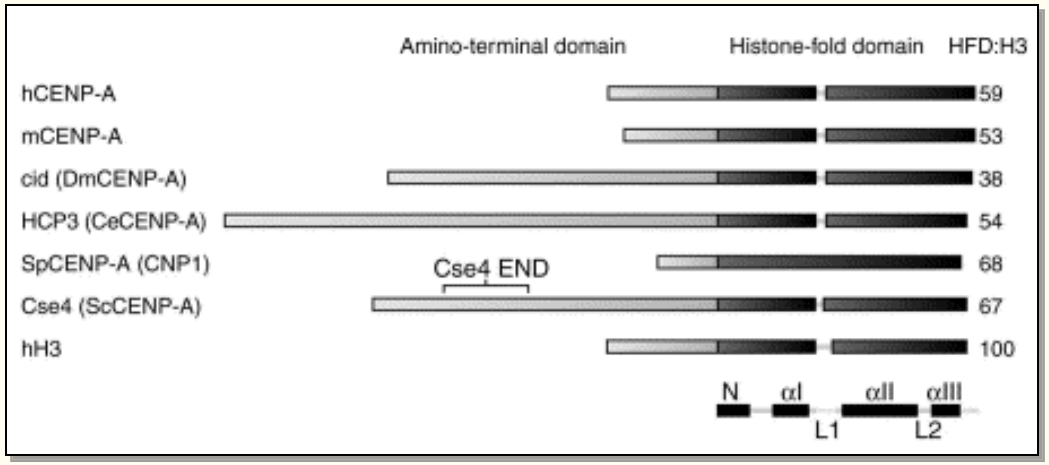
HOLOCENTRICS

some
Nematodes, Insects, and Plants



***C. elegans*: ~15 Mb Chromosomes**

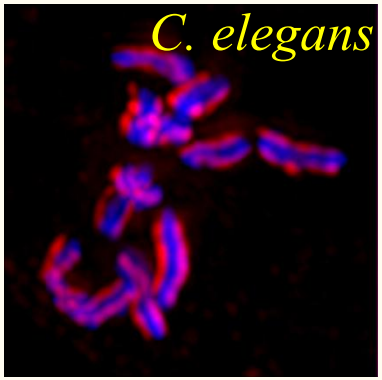
Kinetochores assemble on chromatin containing the histone H3 variant CENP-A (CenH3)

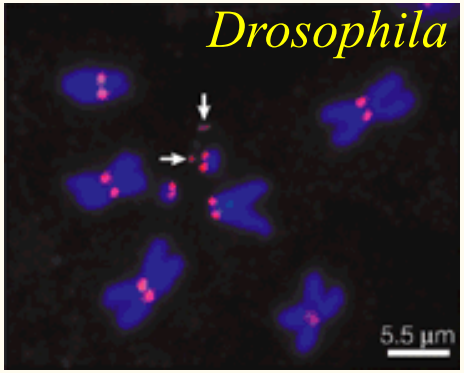


CENP-A and its chaperone HJURP over-expressed in many human cancers.

In flies, CENP-A over-expression results in ectopic sites, ectopic kinetochores, missegregation and aneuploidy.

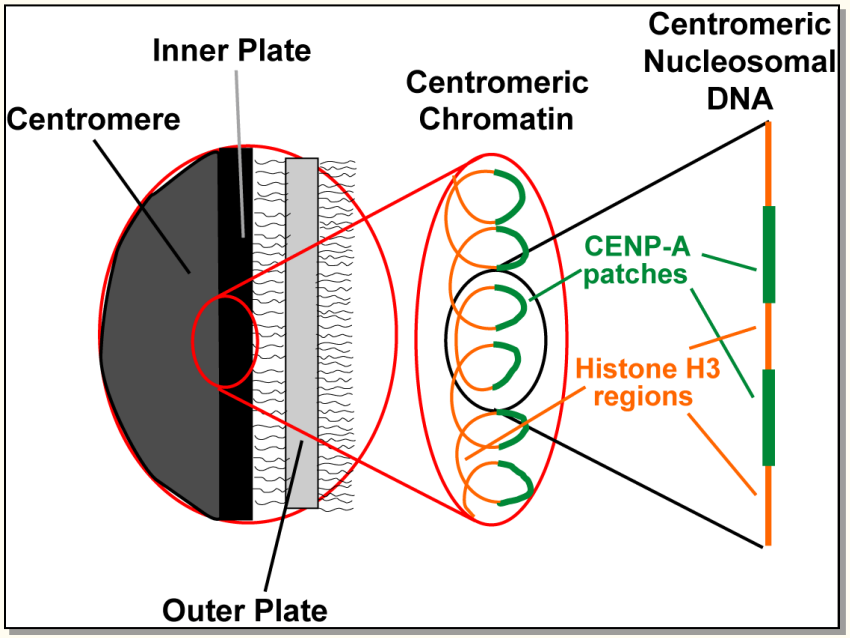
DNA/CENP-A

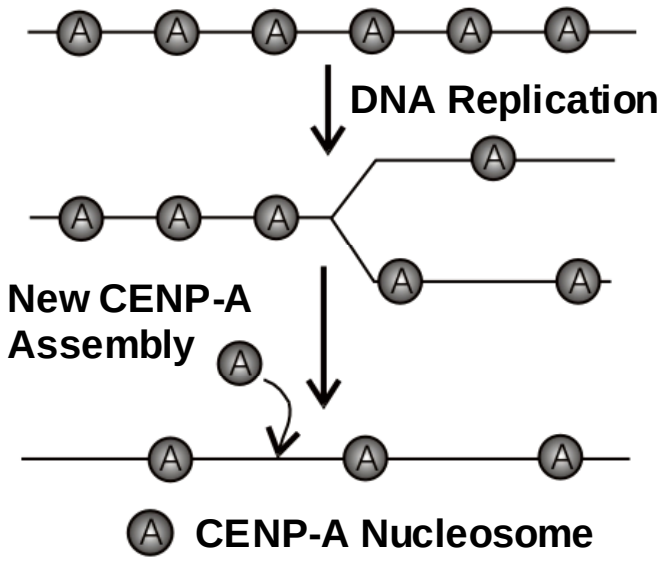




- **substitutes for H3 in (altered) nucleosomes**
- **located at the base of the kinetochore**

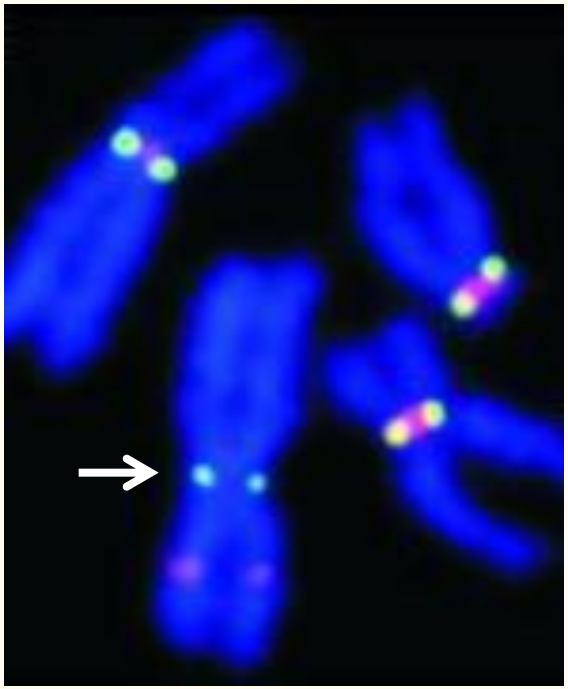
Inheritance of CENP-A nucleosome domains is postulated to propagate centromere identity ("OLD" → "NEW")

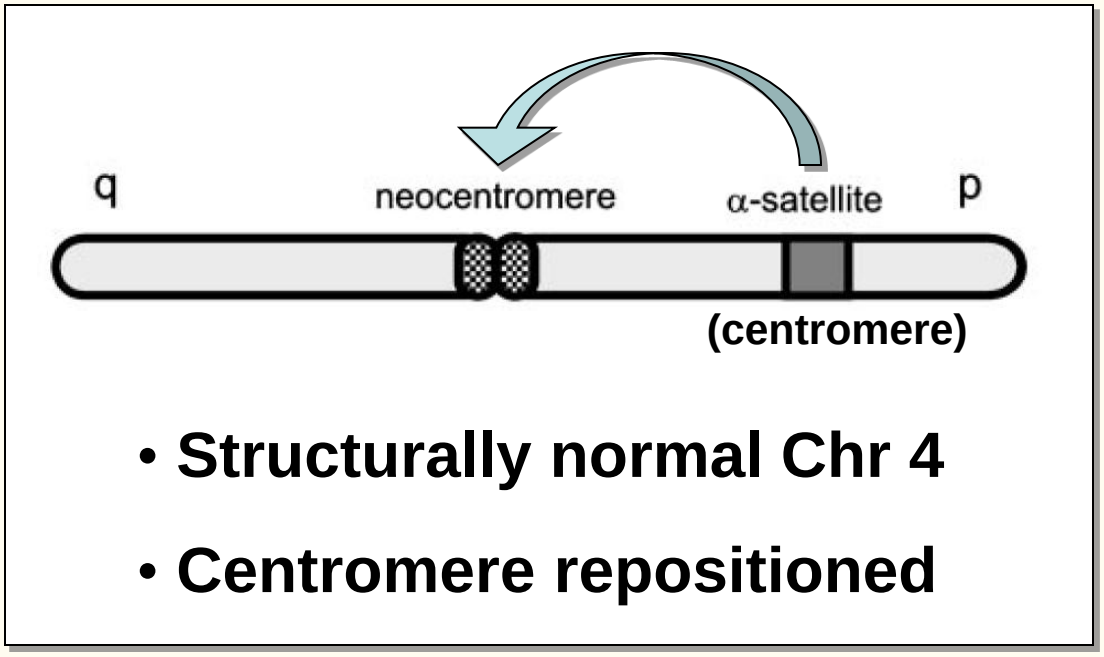




- Uncoupled from DNA replication
- Requires specialized machinery (e.g. KNL-2, HJURP/Scm3)
- Occurs in late anaphase-early G1 in mammals, *Drosophila*

Human Neocentromeres: Evidence for rare *de novo* CENP-A chromatin domain establishment





- Structurally normal Chr 4
- Centromere repositioned

CENP-A

Alpha-satellite (CEN DNA)

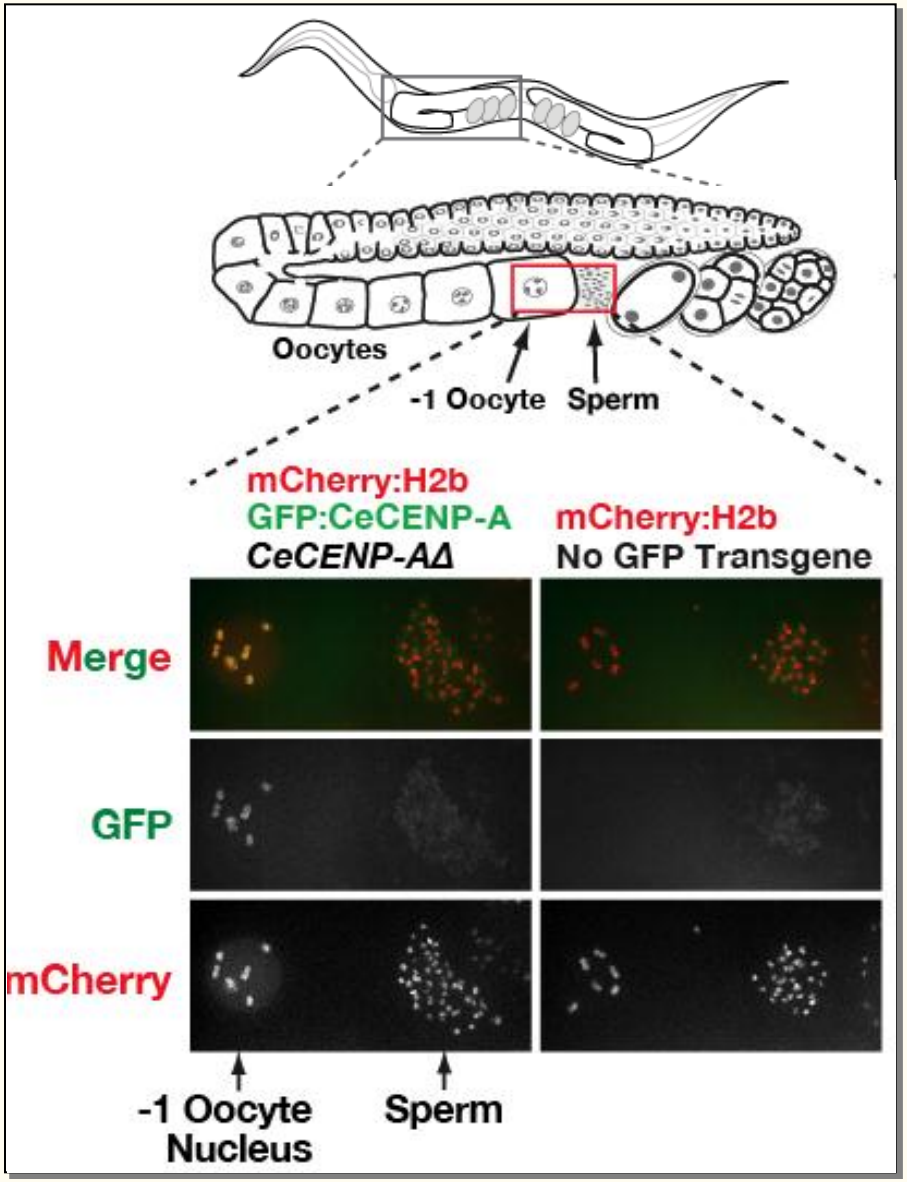
Andy Choo & colleagues

Amor et al. *PNAS* (2004)

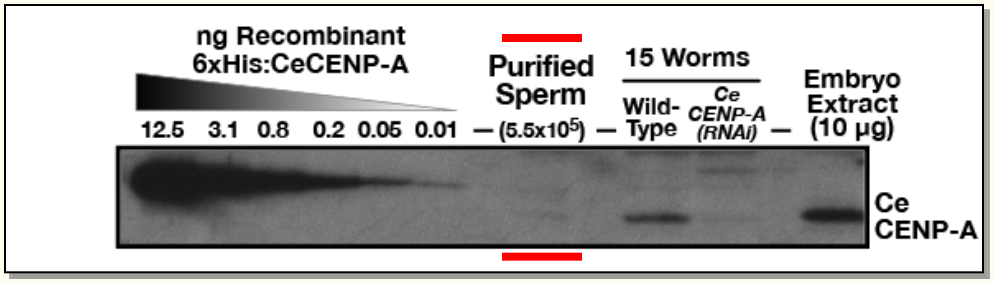
- Neocentromerization observed in diverse species
- Centromere repositioning common among related species

CENP-A is not detectable in *C. elegans* sperm

Microscopy

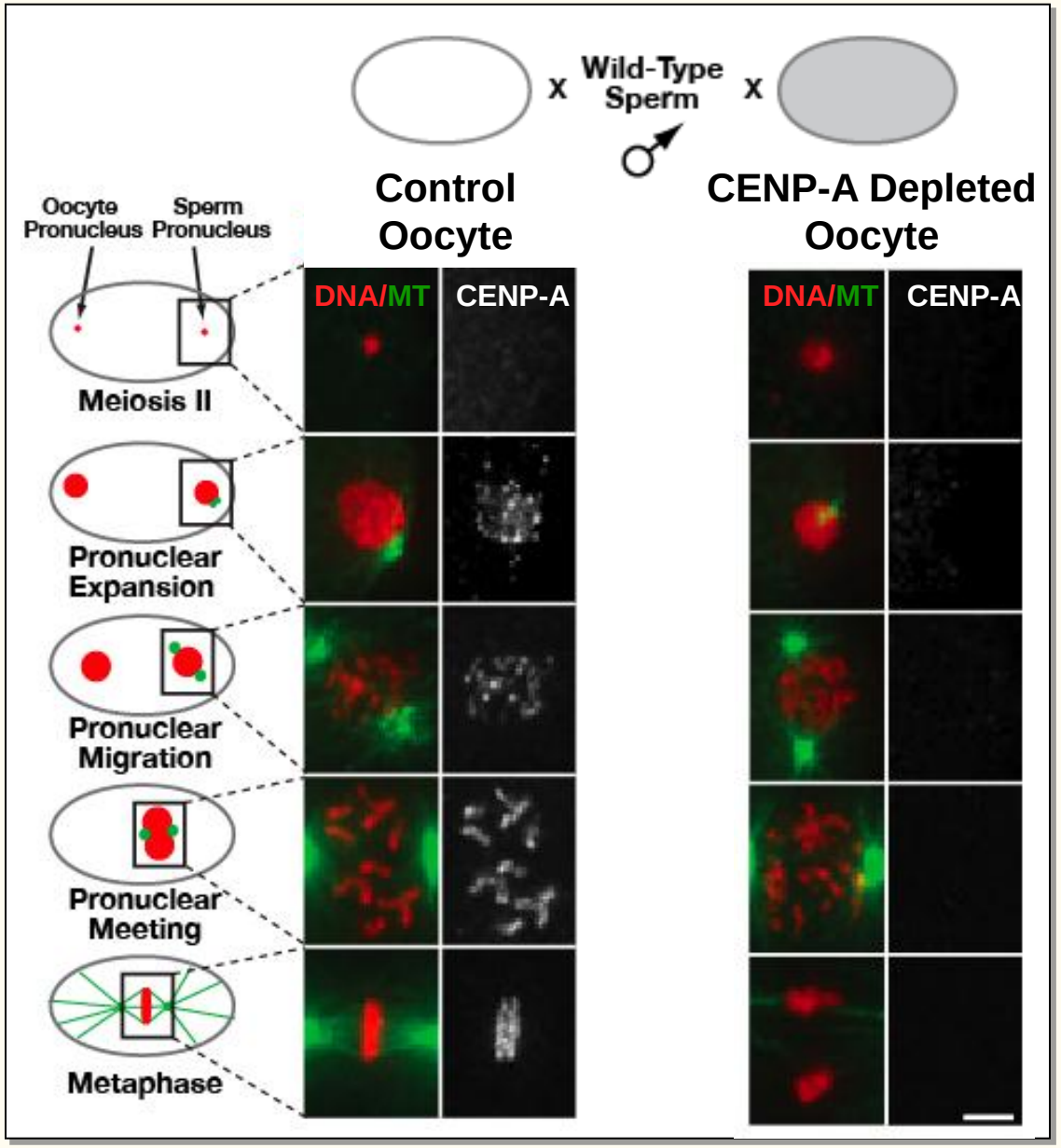


Quantitative immunoblotting



- **<300 CENP-A molecules / sperm**
- **Embryos have ~130,000 CENP-A molecules per nucleus (purified nuclei)**

Sperm chromatin recruits CENP-A from oocyte cytoplasm

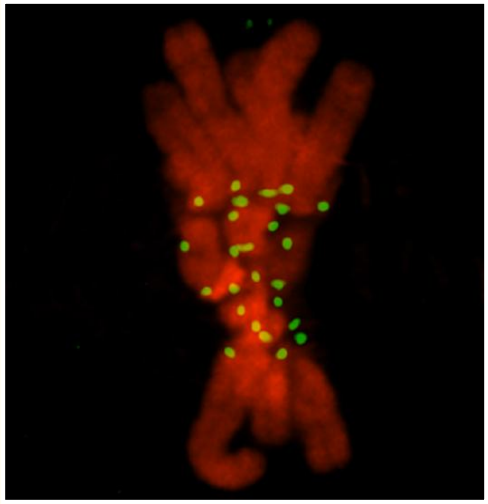


- **CENP-A Absent from Mature Sperm & Recruited From Oocyte Cytoplasm at Fertilization**
- **CENP-A Removal & Reloading in Meiotic Prophase**

Inconsistent with "OLD" →"NEW" Model

If not OLD CENP-A nucleosomes, what epigenetic features restrict targeting of CENP-A?

Monocentric



Repetitive Architecture of human centromeres has prevented mapping of CENP-A location

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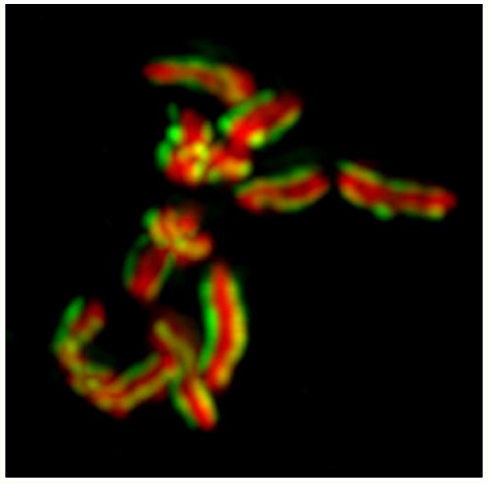
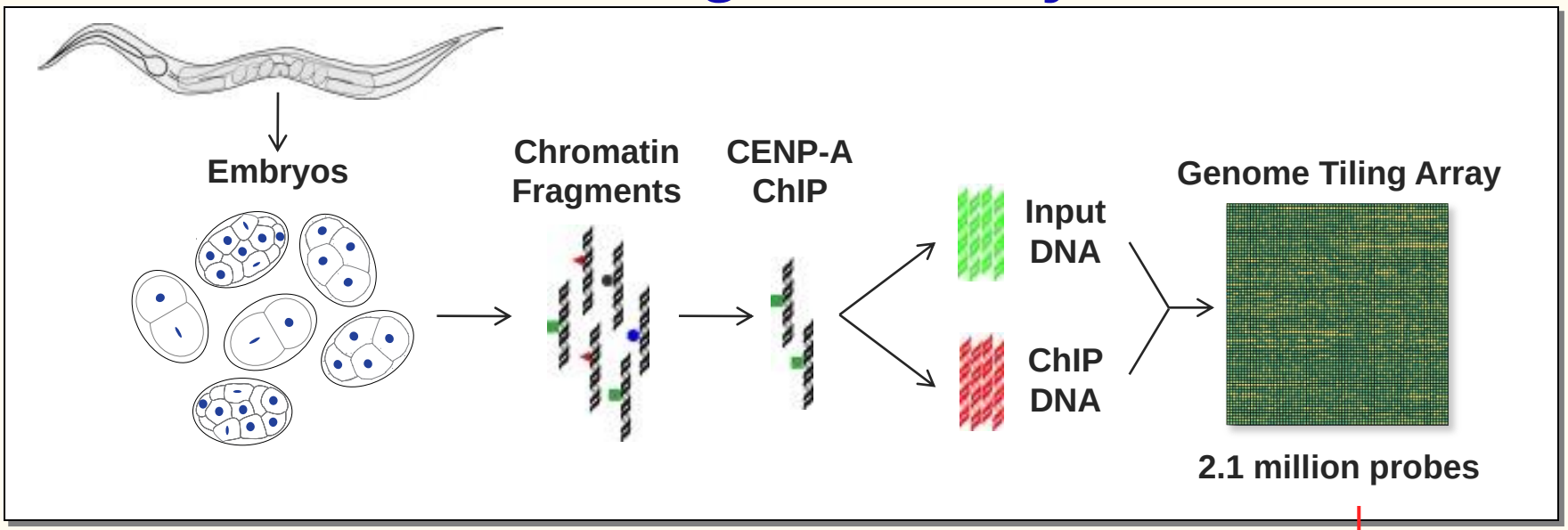
Holocentric

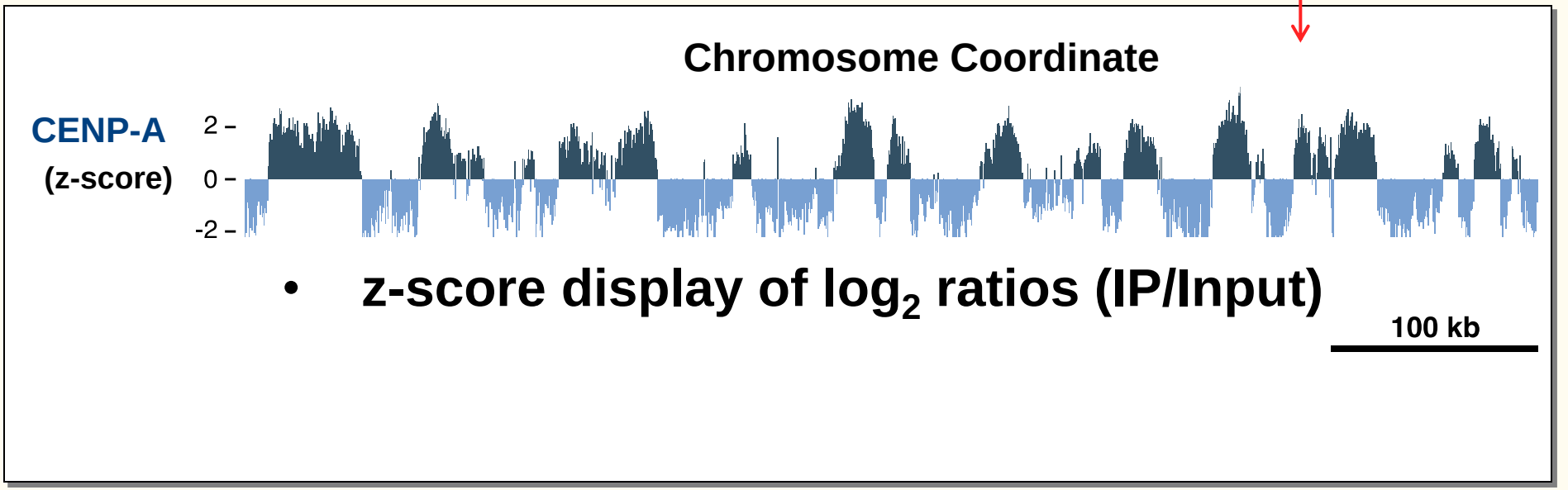


CENP-A Chromatin IP

Genomic Tiling Microarrays (Nimblegen)

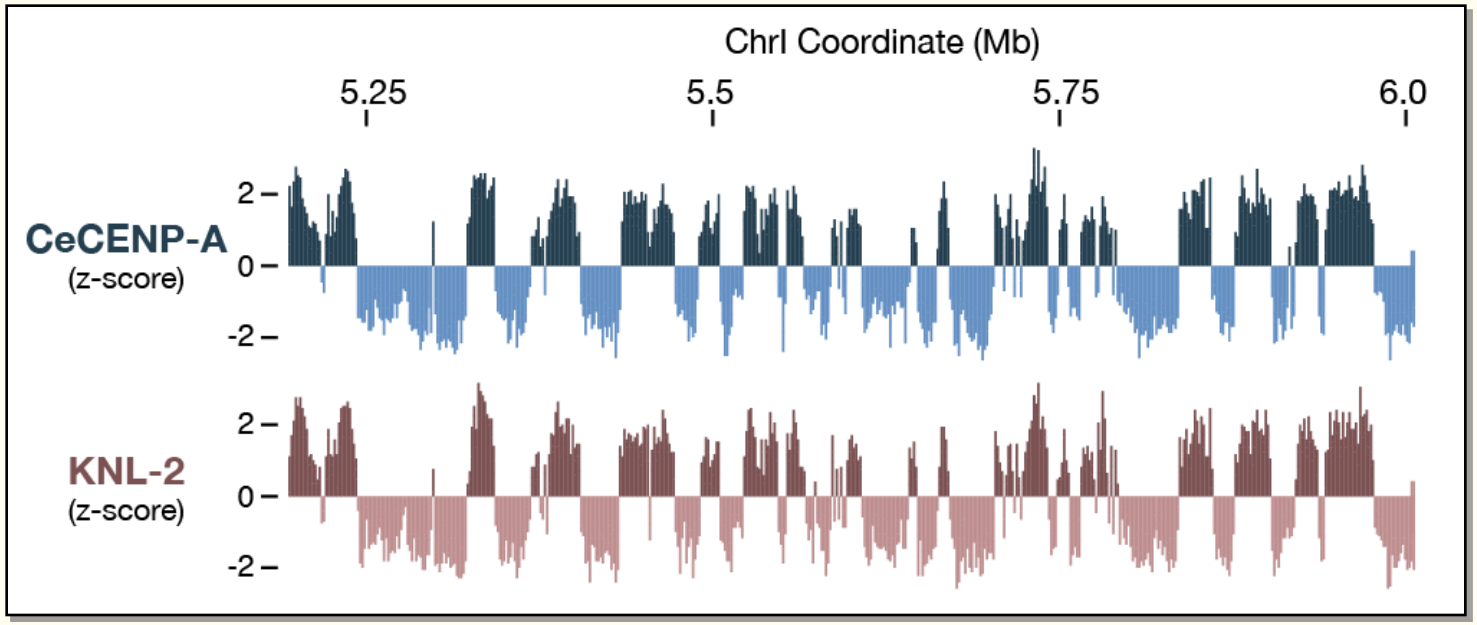
Mapping CENP-A (& KNL-2) location genome-wide in *C. elegans* embryos

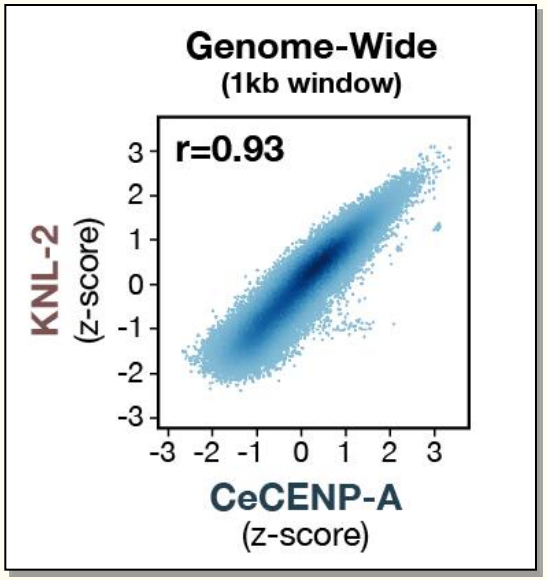




- z-score display of $\log_2$ ratios (IP/Input)

CENP-A & KNL-2 exhibit identical genomic distribution

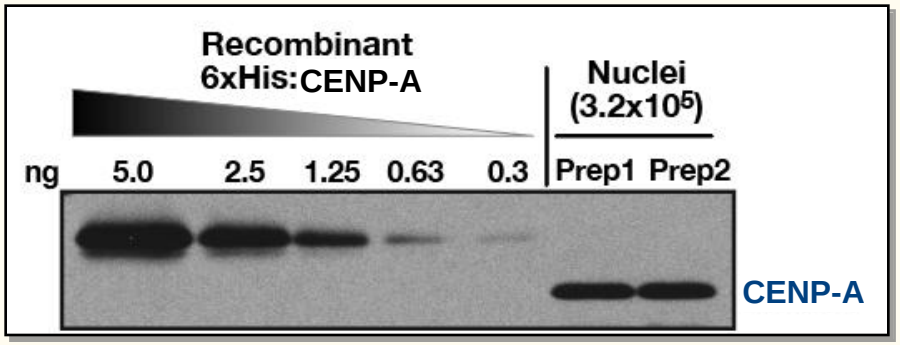




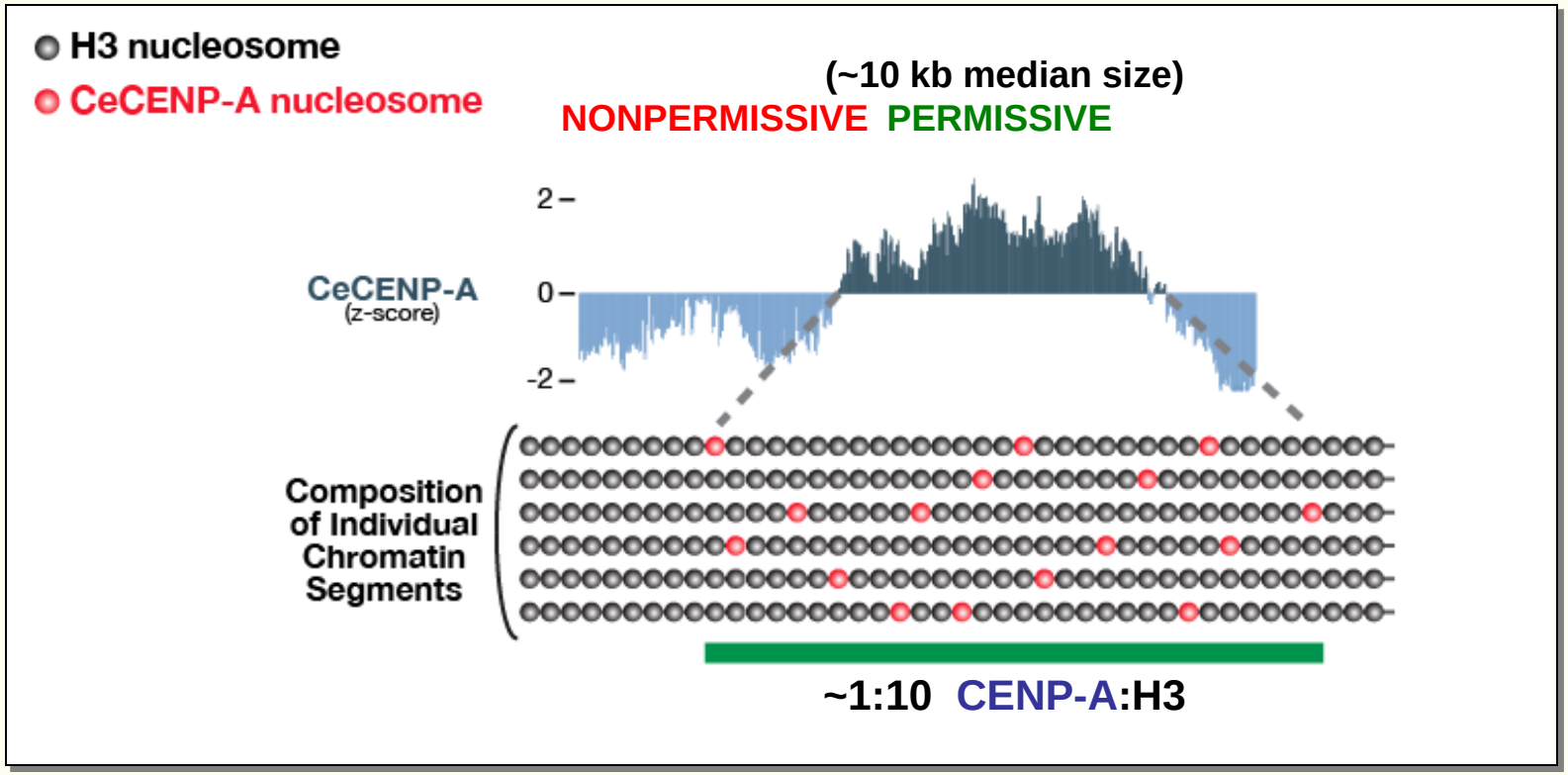
- **Broad Distribution (47% of Genome)**

Counting CENP-A molecules per nucleus to interpret structure of CENP-A enriched domains

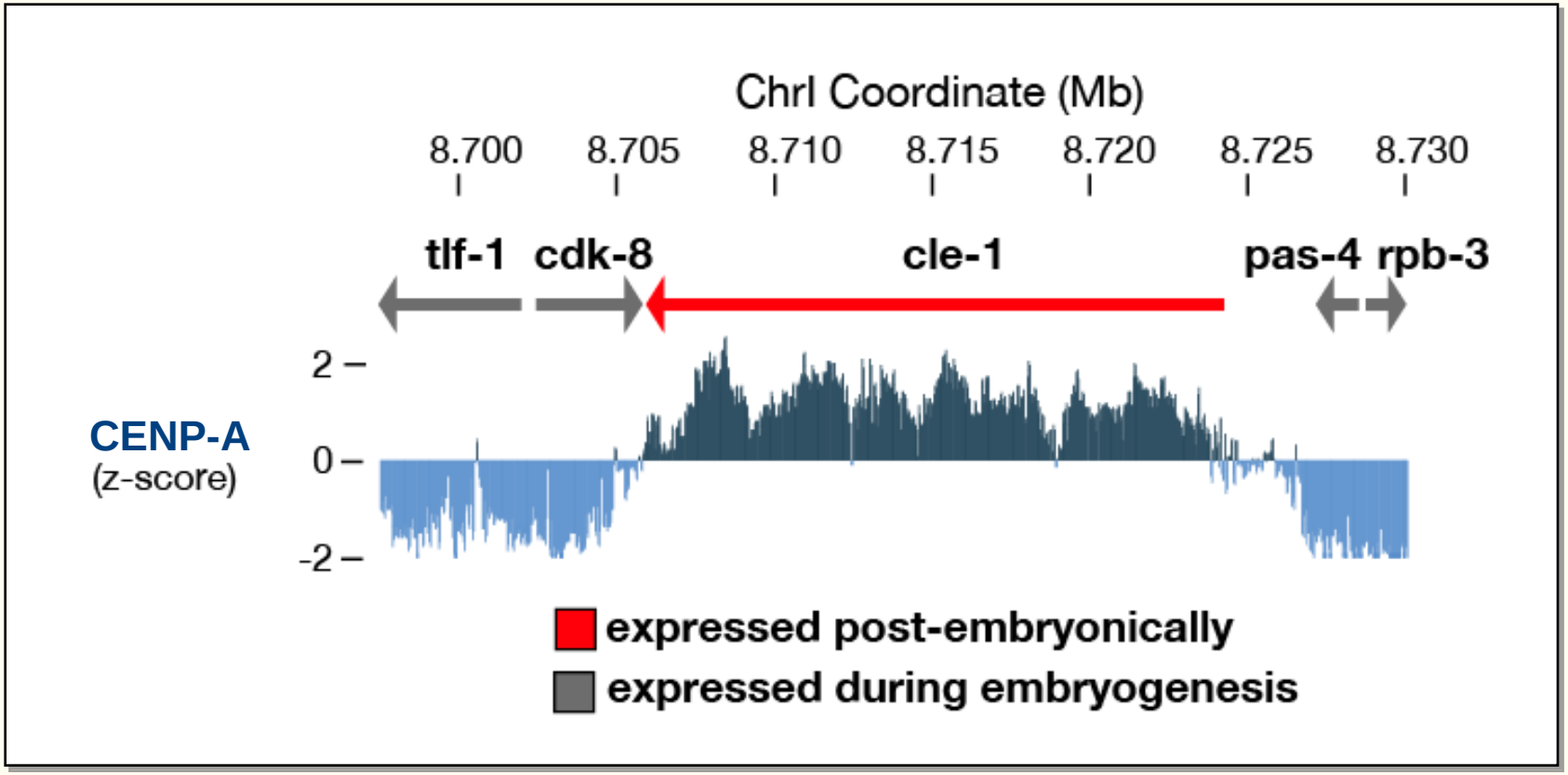
Maximally, 4% of the nucleosomes CAN be CENP-A nucleosomes



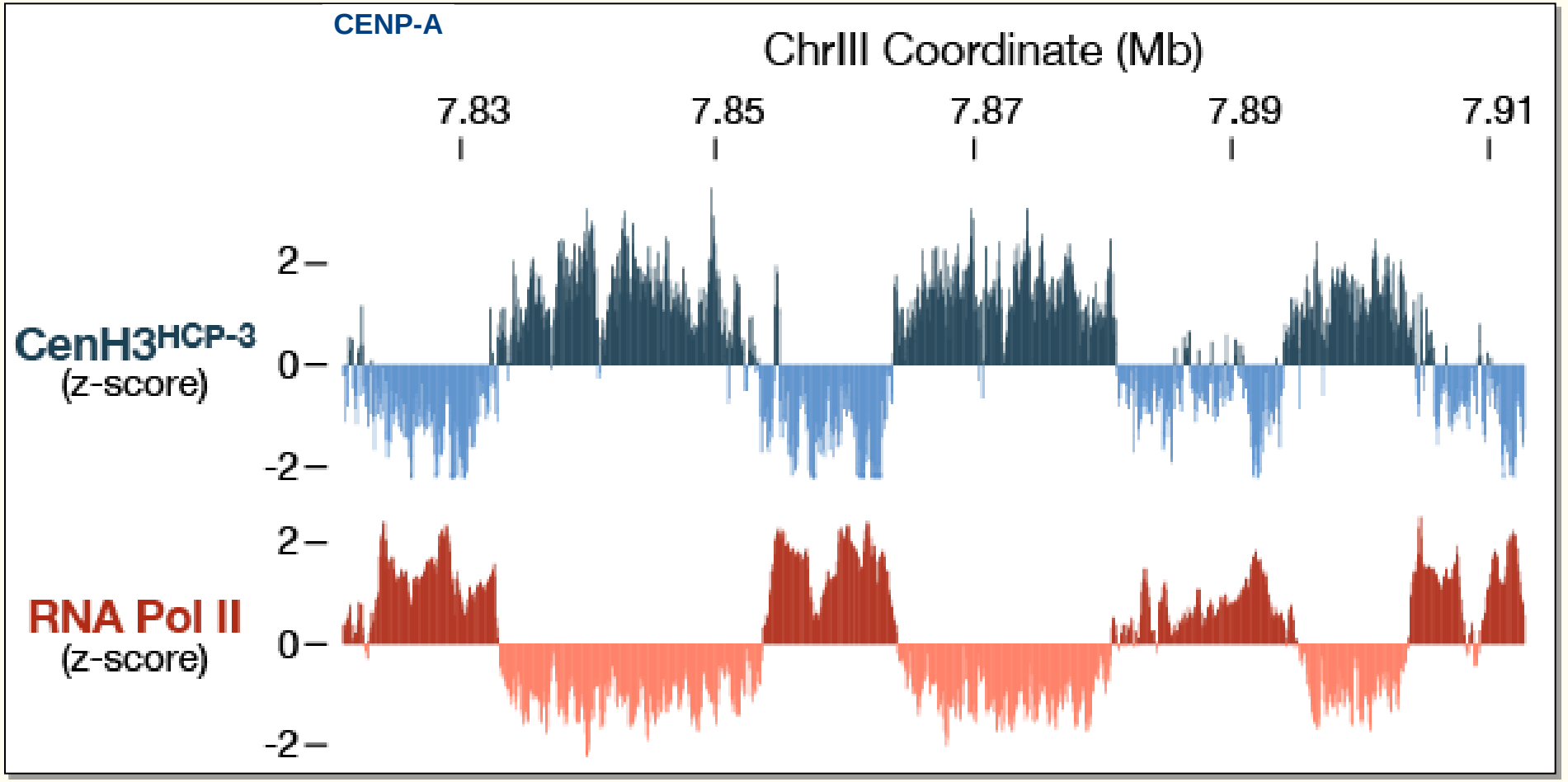
1.3 x 10^5 CENP-A molecules/nucleus



In embryos, CENP-A domains appear to be delimited by gene expression



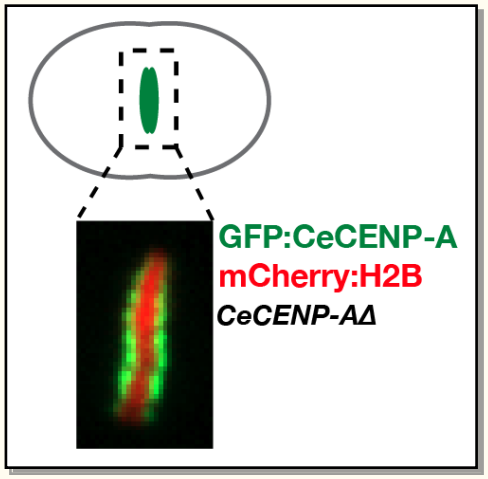
CENP-A & RNA Polymerase II Occupancy are Inversely Correlated



Is CENP-A simply excluded from regions of active transcription?

- **No significant zygotic transcription until 30-cell stage**

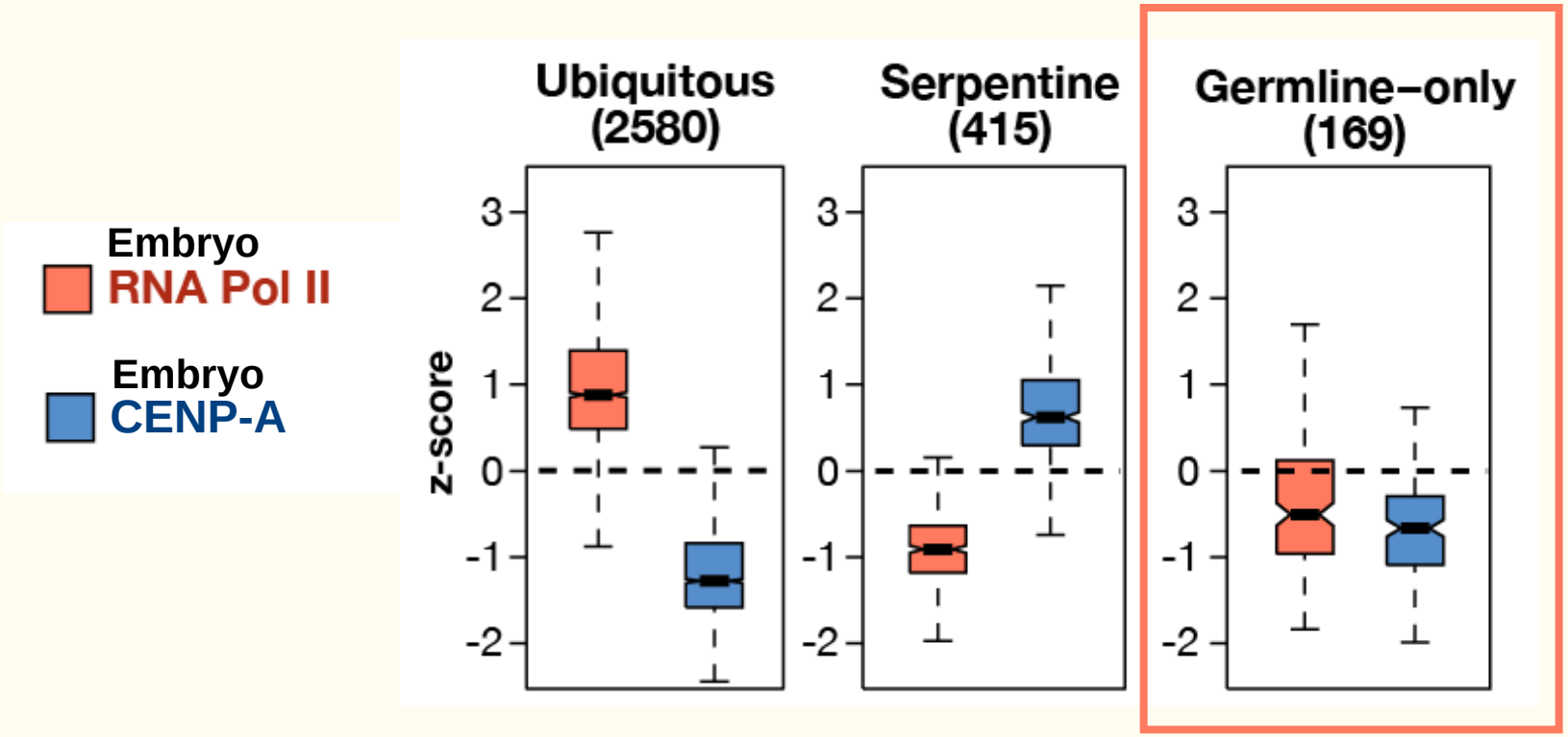
1-Cell Embryo



- **Early divisions are normal in pol II-inhibited embryos**

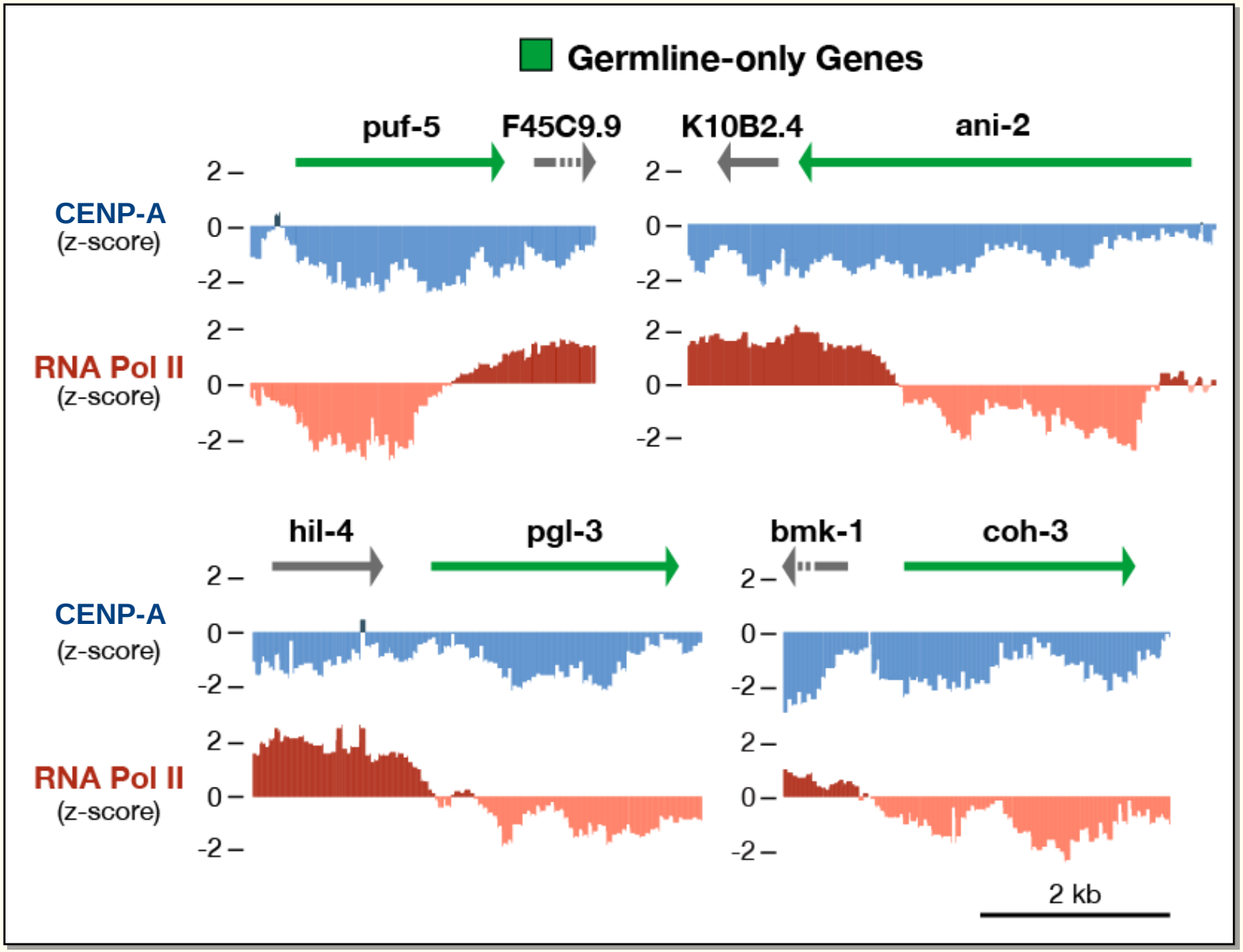
- **No change in CENP-A pattern during development (from 8-cell stage to >250 cell stage)**

Gene set analysis: A clue to the origin of the underlying pattern?

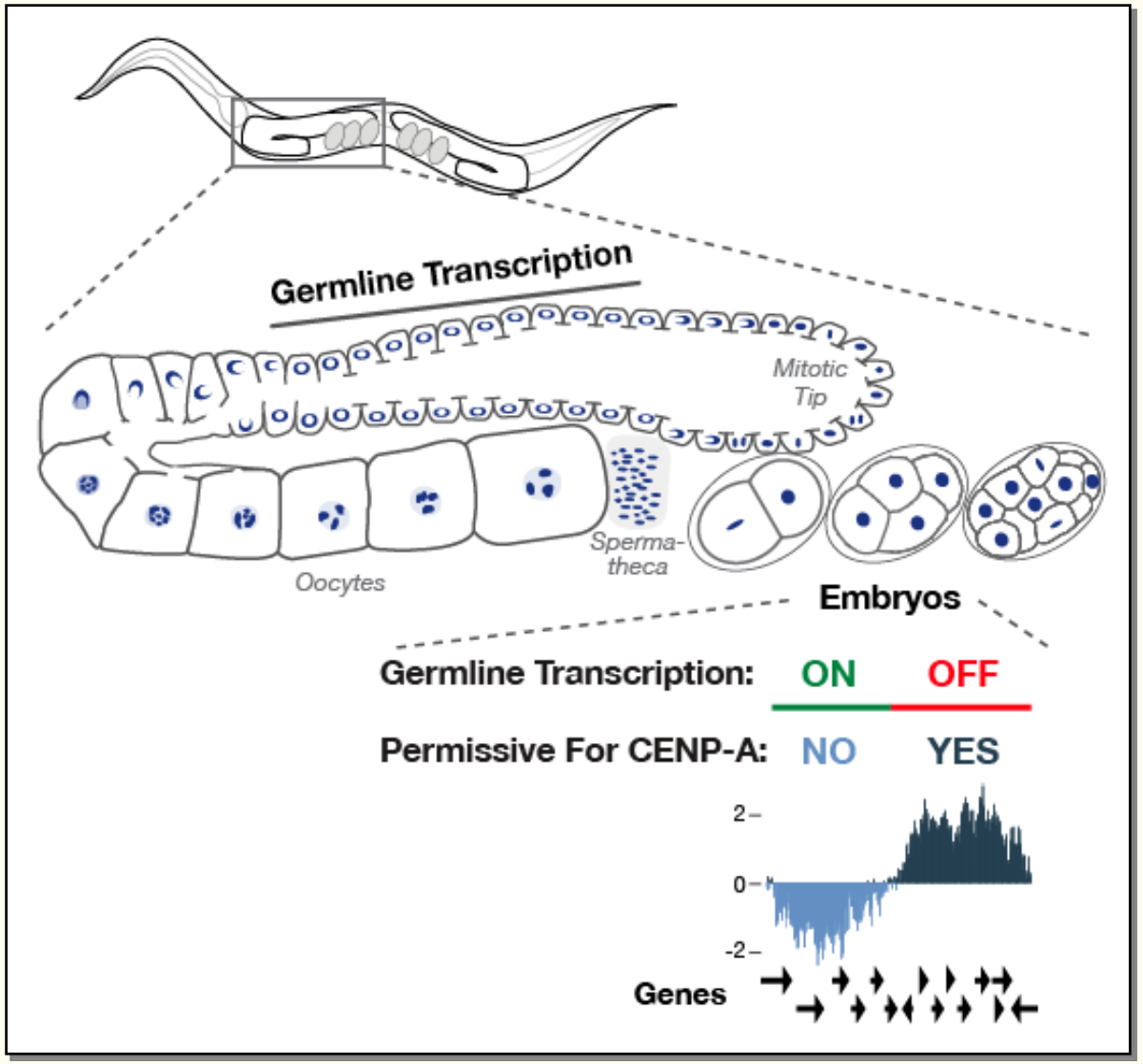


Germline-only = expressed in germline but not in embryos or other tissues

Example “DOUBLE NEGATIVE” germline-only genes

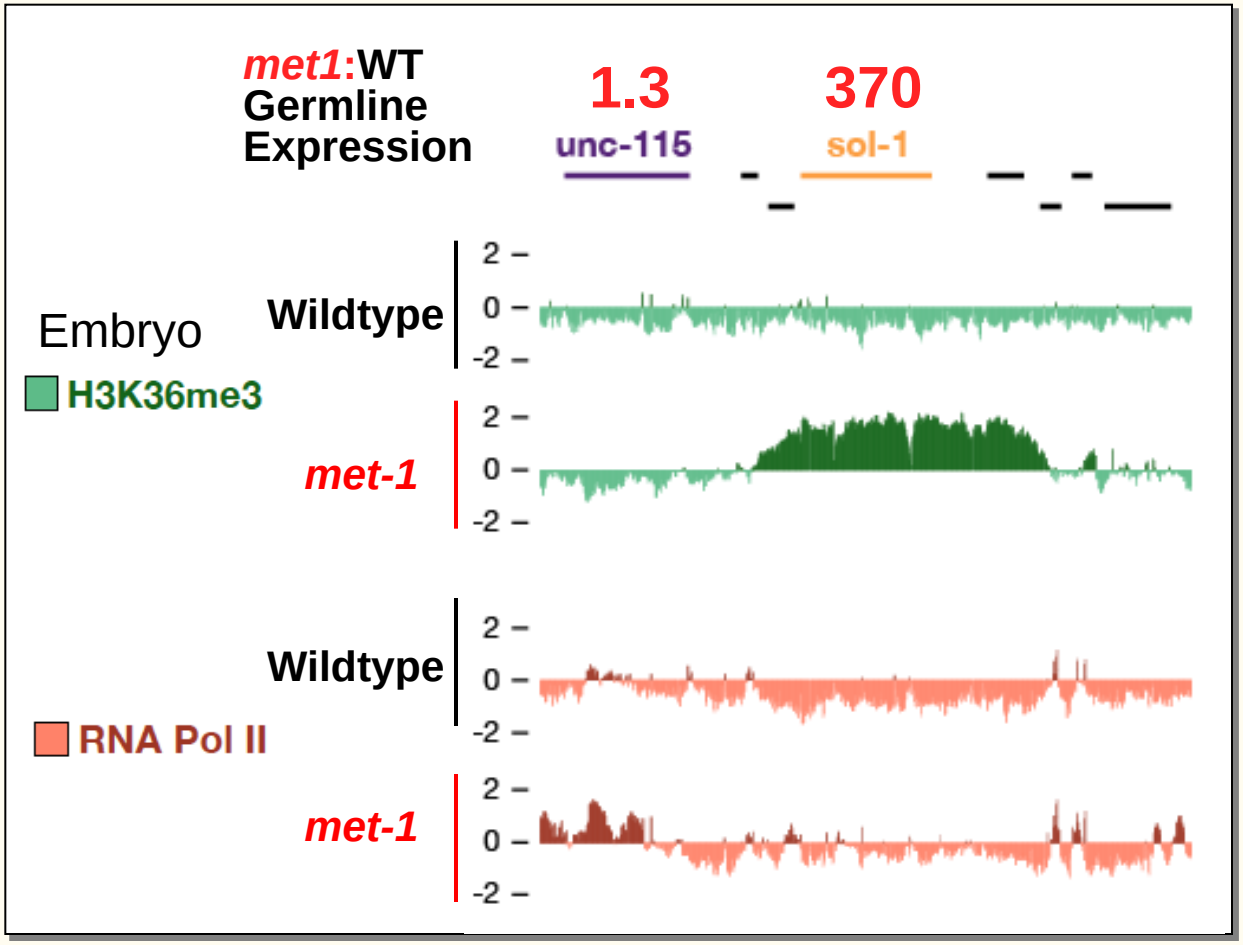


Model: Germline transcription defines regions of CENP-A exclusion throughout embryogenesis



Testing the Model: Does ectopic expression in the germline convert a CENP-A^{+} region into CENP-A^{-} in embryos?

- **Mutant with Altered Germline Expression**



(met-1)

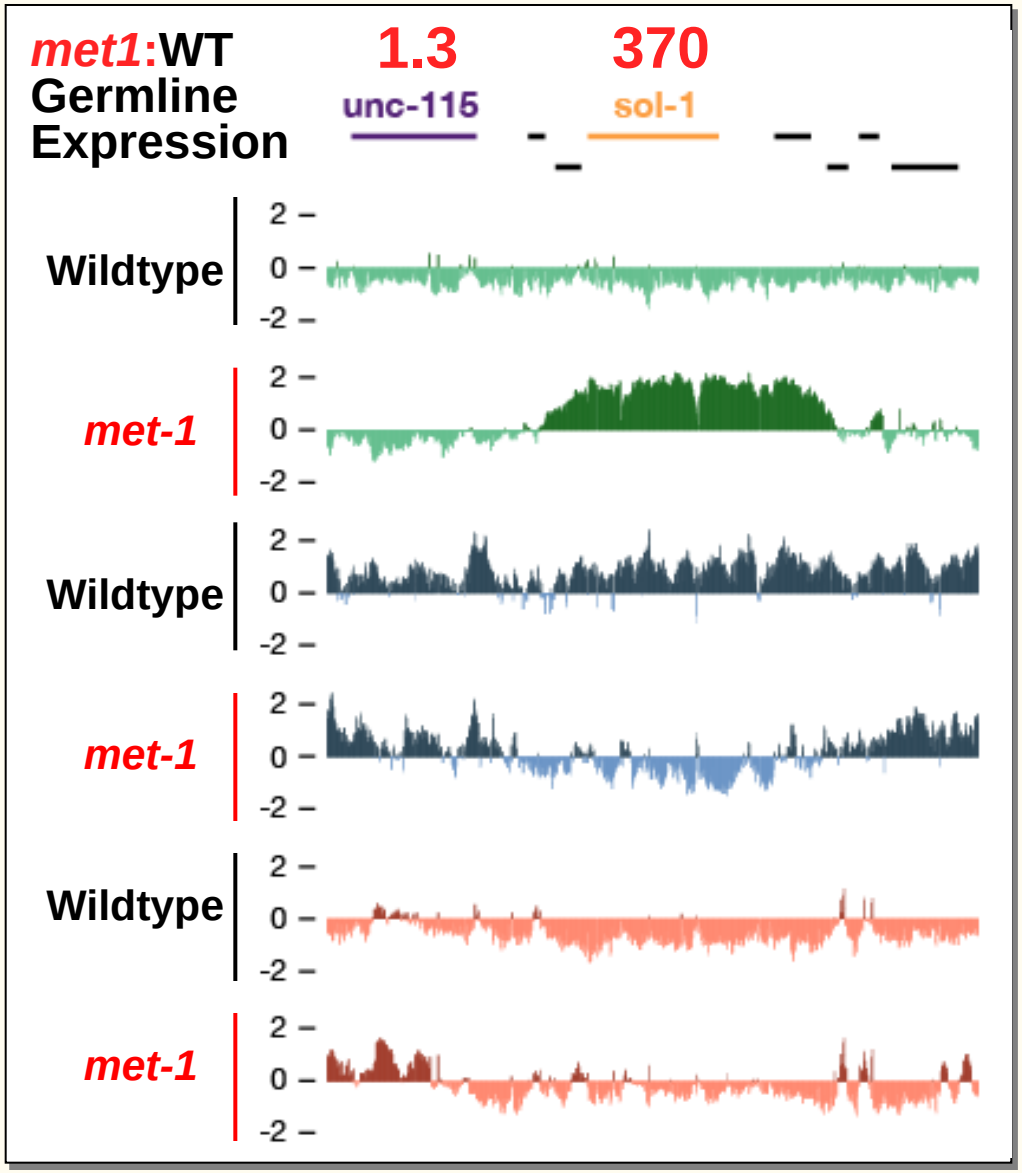
non-essential H3K36 methyltranferase

- **Germline Expression**
  Measured in Dissected Gonad Tissue

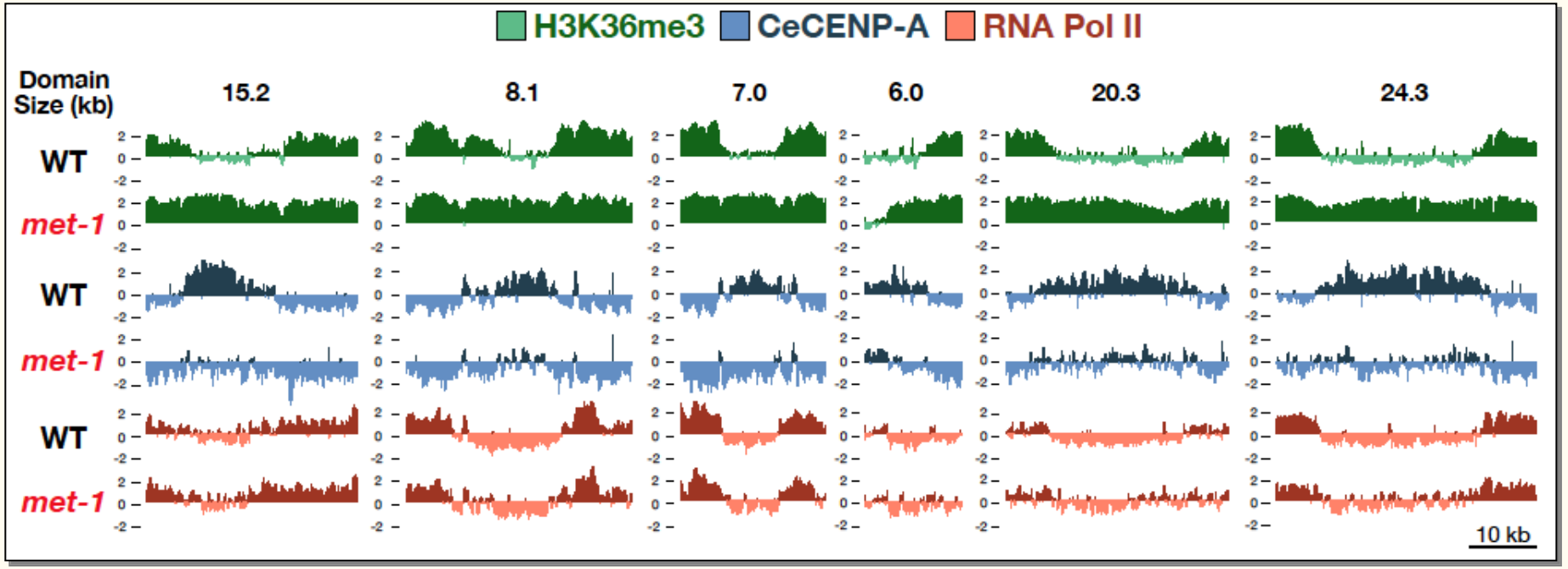
- **H3K36me3**
  Marks Germline-Expressed Genes Throughout Embryogenesis
  (Strome & Kelly Labs)

- **RNA Pol II: Marks Active Transcription in Embryos**

Ectopic germline expression leads to CENP-A loss without active transcription in embryos



Ectopic germline expression leads to CENP-A loss without active transcription in embryos



WT vs. *met-1*:

132 domains > 5kb size with H3K36me3 increase > 1 z-score in *met-1* vs WT

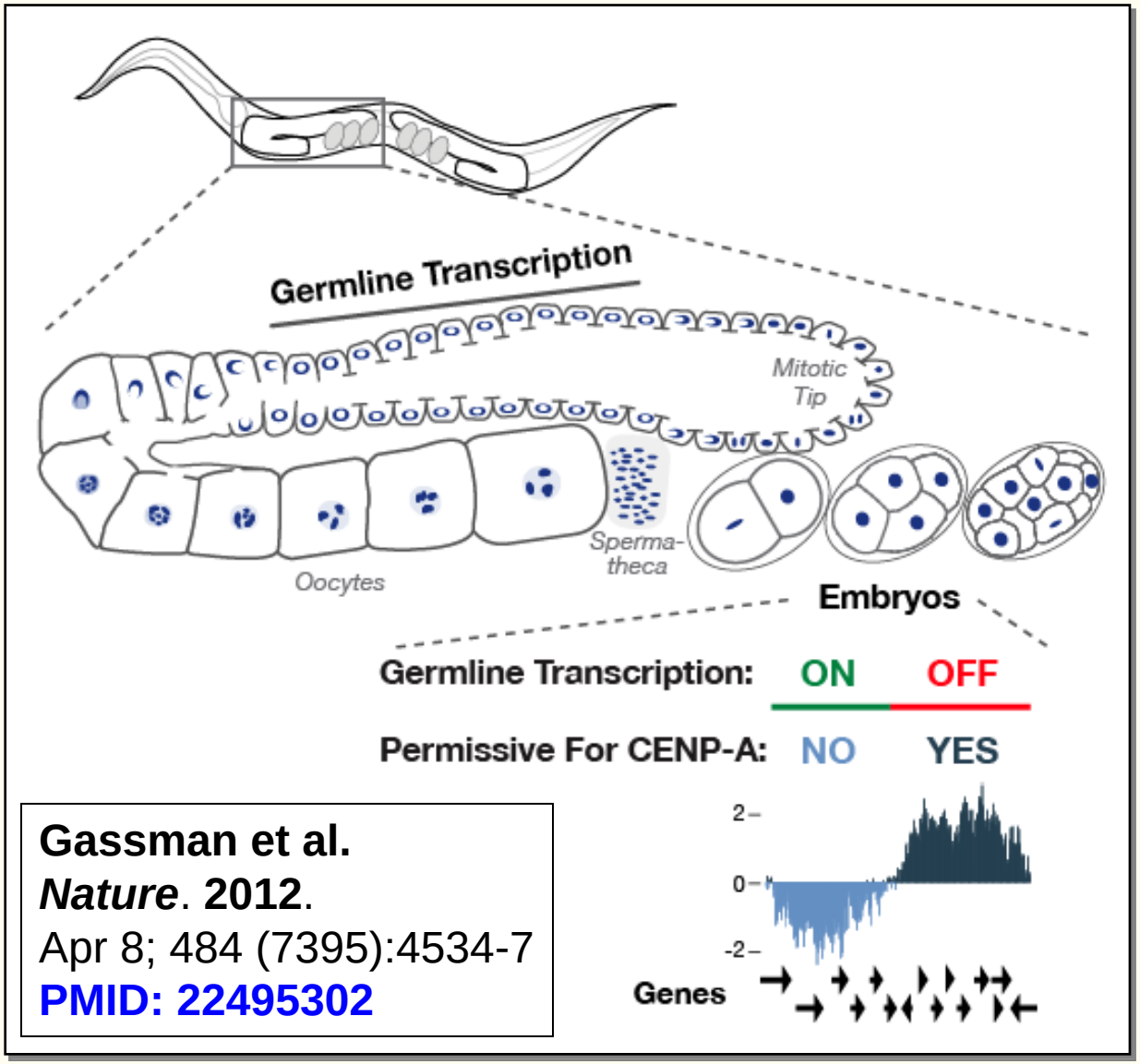
↓

75 domains with RNA Pol II z-score < 0.1 in *met-1*

↓

Average ΔCENP-A$^{met\text{-}1\ -\ WT}$ z-score = - 0.8

Model: Germline transcription defines regions of CENP-A exclusion throughout embryogenesis



Gassman et al.
***Nature*. 2012**.
Apr 8; 484 (7395):4534-7
PMID: 22495302

Mechanism Underlying "Memory"?

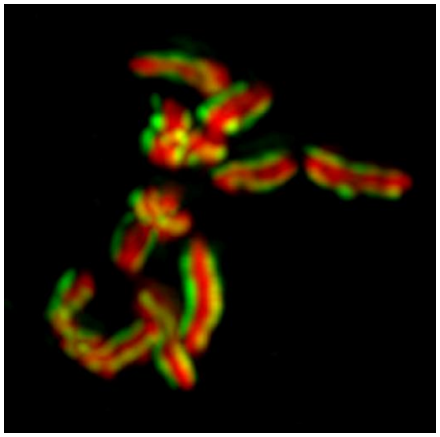
i) MES-4 (Major H3K36 Methyltransferase)
Strome & Kelly Labs

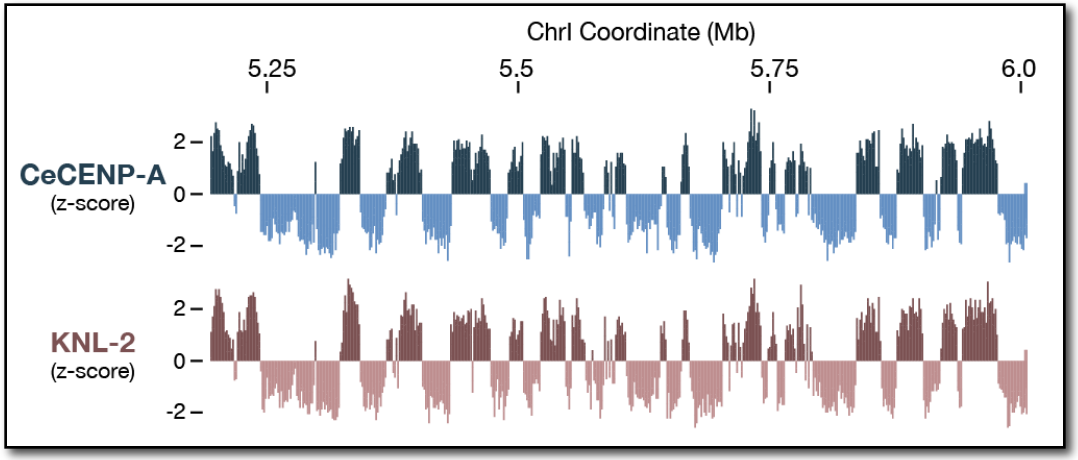
PLoS Genetics. 2010 Sep 2;6(9). e1001091.
PMID: 20824077

ii) CSR-1 Argonaute & 22G small RNAs
Mello Lab

Implications

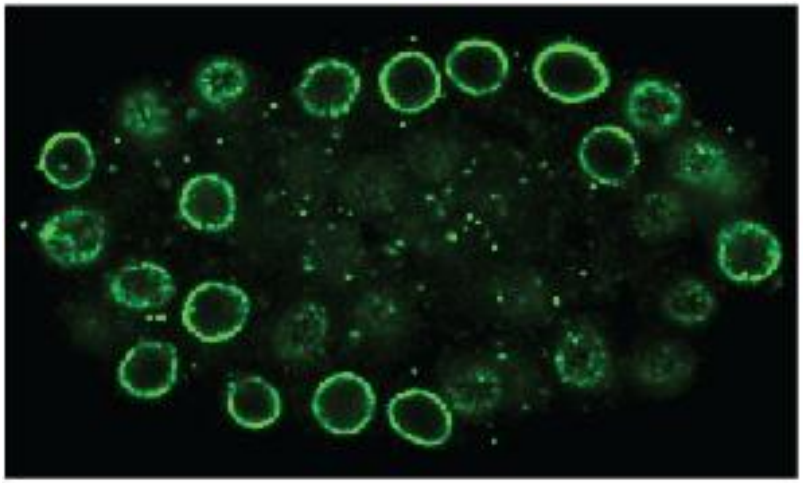
- **CENP-A nucleosomes can be "guided" by cues that are not pre-existing CENP-A nucleosomes.**
- **Trans-generational epigenetic memory of gene expression regulates histone variant incorporation.**
- **Germline expression may influence sites chosen for centromere repositioning during evolution.**



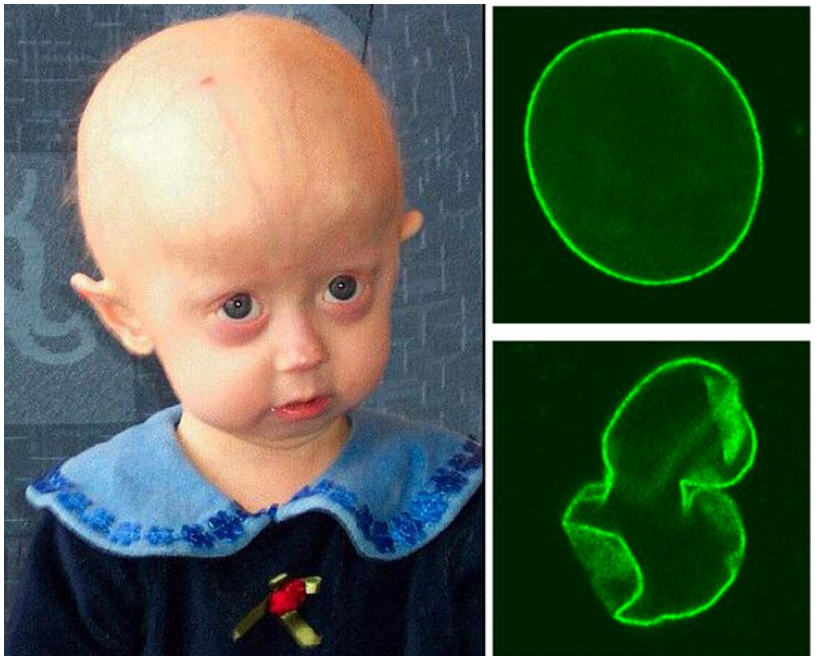


(2) Chromosome-membrane interactions

LEM-2 at the *C. elegans* nuclear envelope



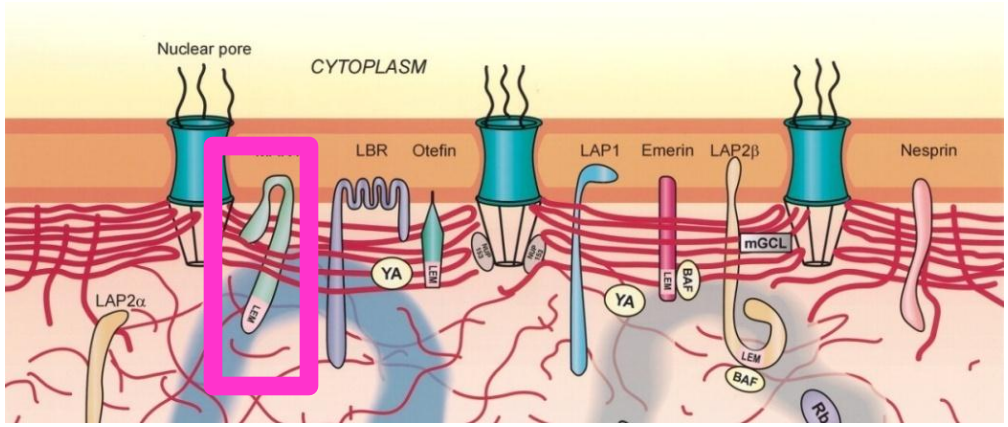
Hutchinson-Gilford Progeria, with aberrant nuclear morphology



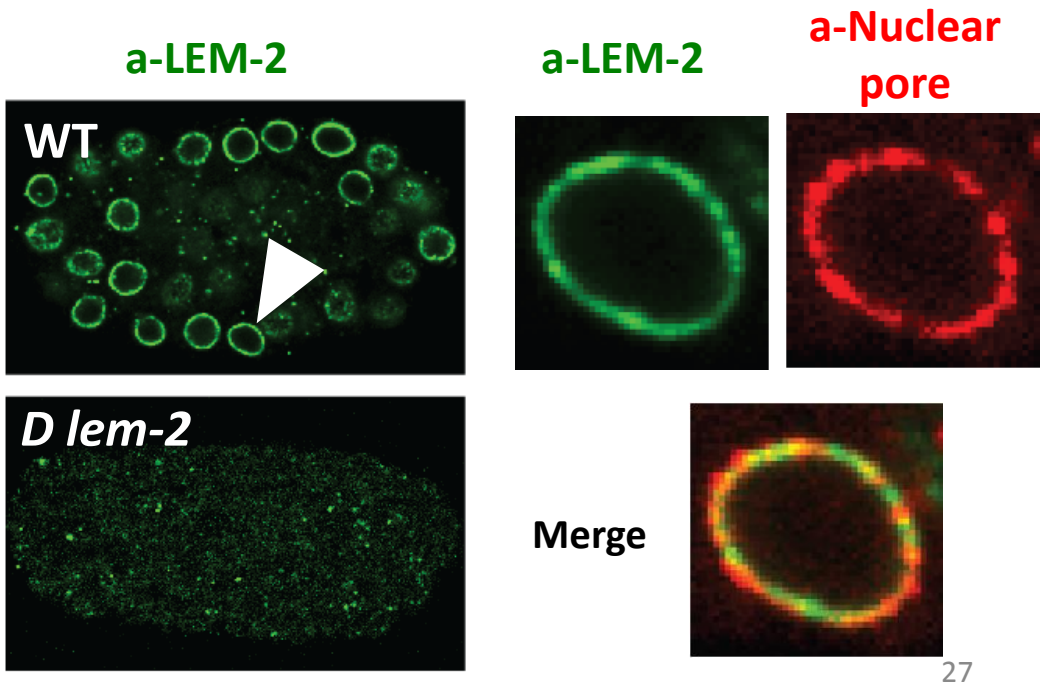
PLoS Biology Vol. 3/11/2005, e395

LEM-2, a nuclear membrane protein

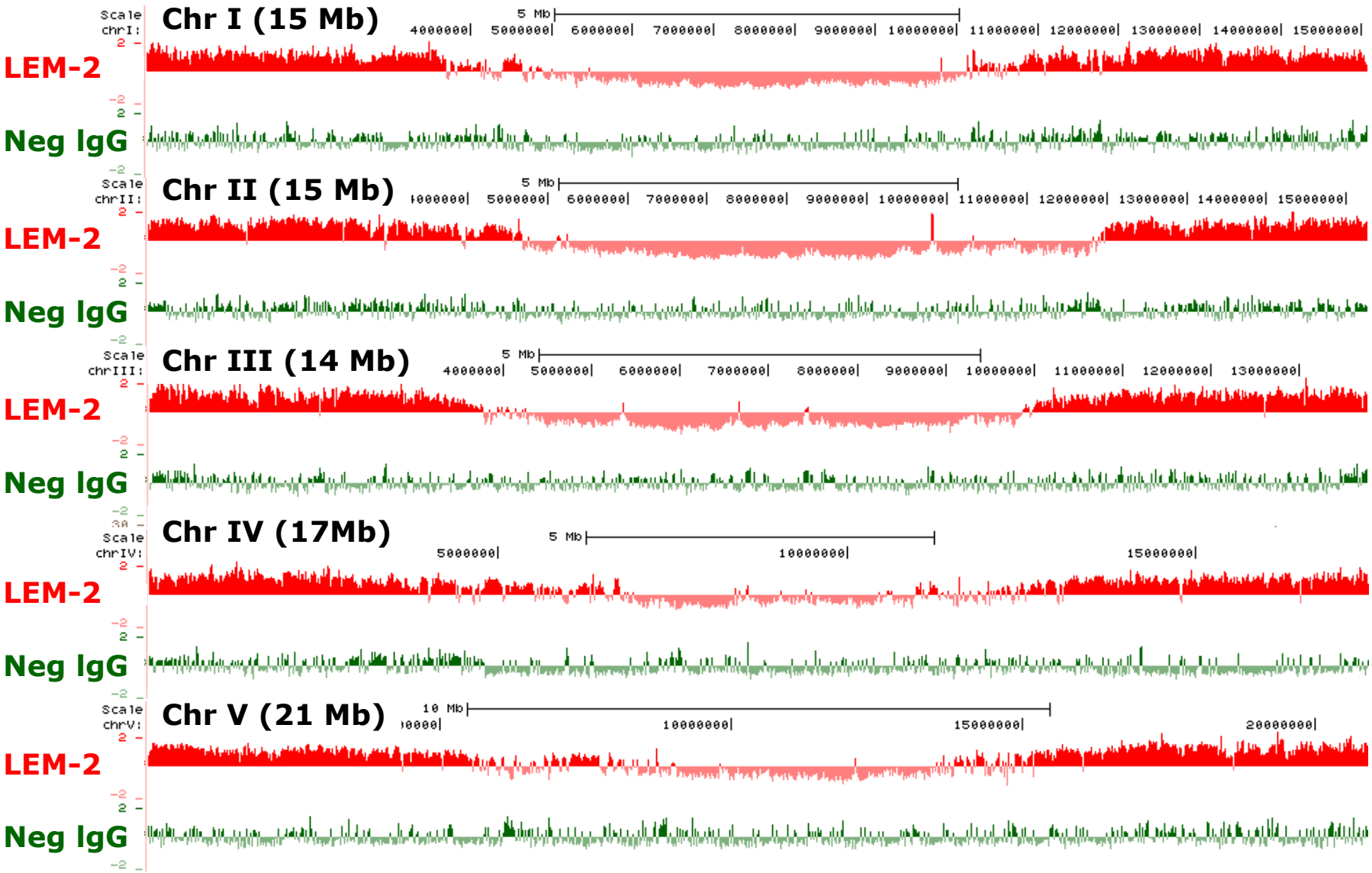
- Localized at the nuclear envelope; dependant on:
  - transmembrane domains
  - lamin A
- Conserved among yeast, *C. elegans* & mammals
- Ubiquitously expressed
- Not essential, but co-RNAi with emerin causes embryonic lethality in *C. elegans*
- Interacts w/DNA-binding protein BAF-1
- Associates w/ yeast chromatin



Goldman et al. *Genes Dev*. (2002)

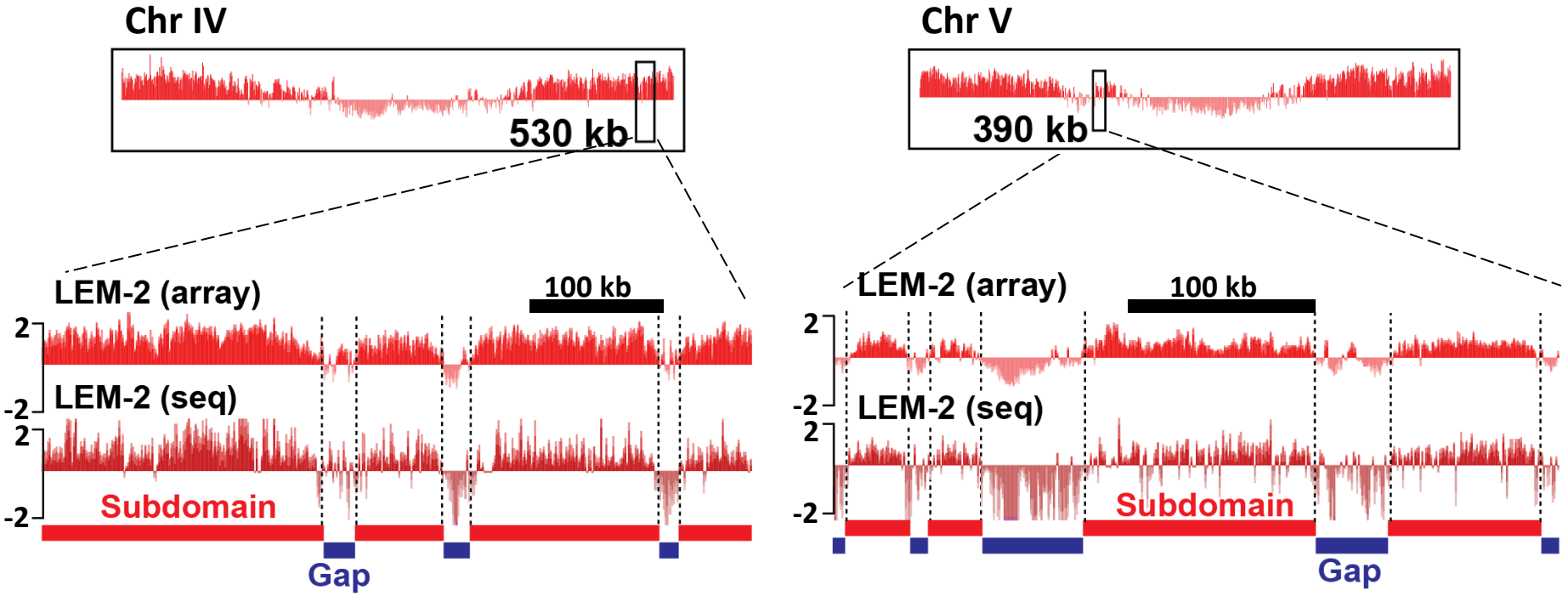


LEM-2 associates with chromosome arms



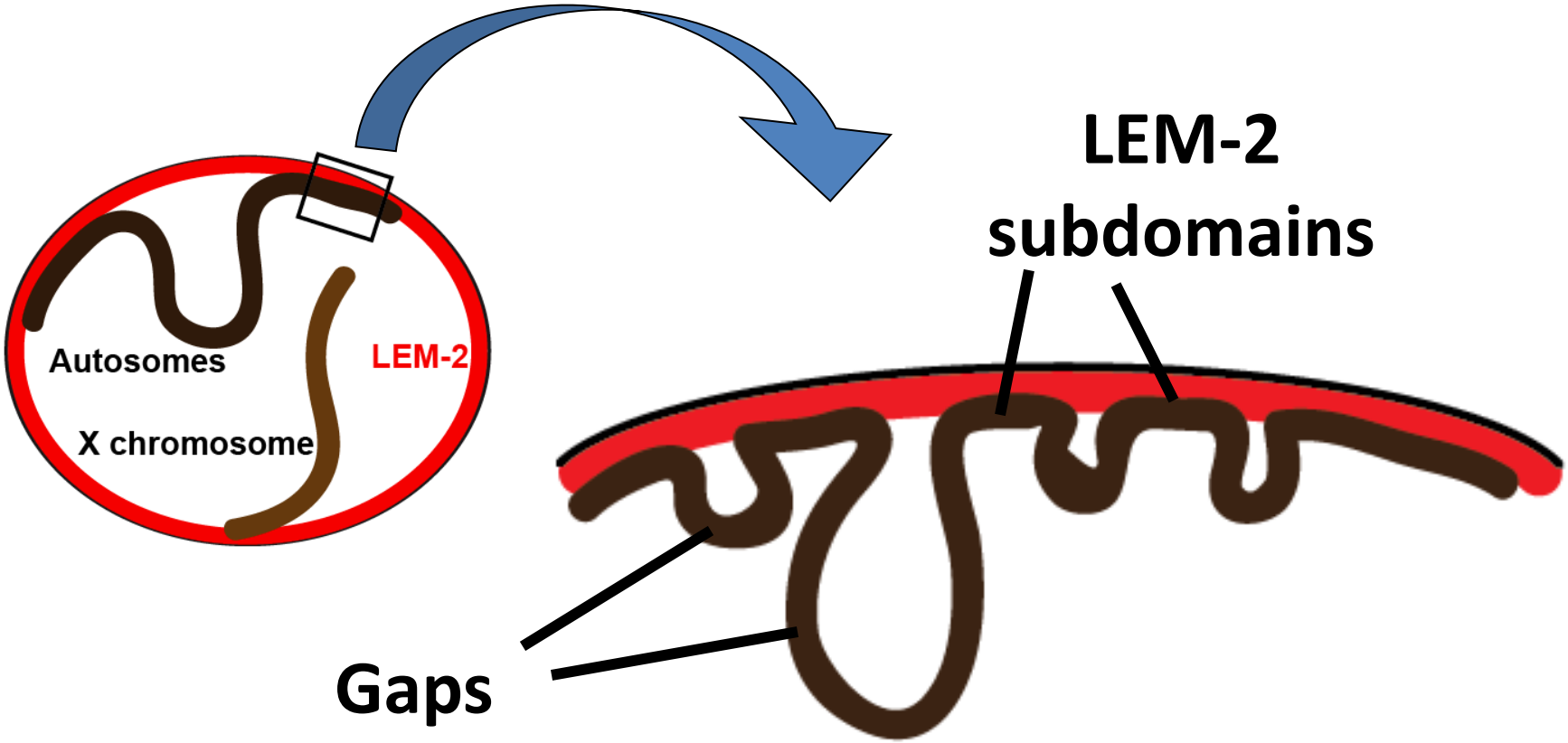
Ikegami *et al.*, Genome Biology (2010)

Large LEM-2 domains consist of small subdomains

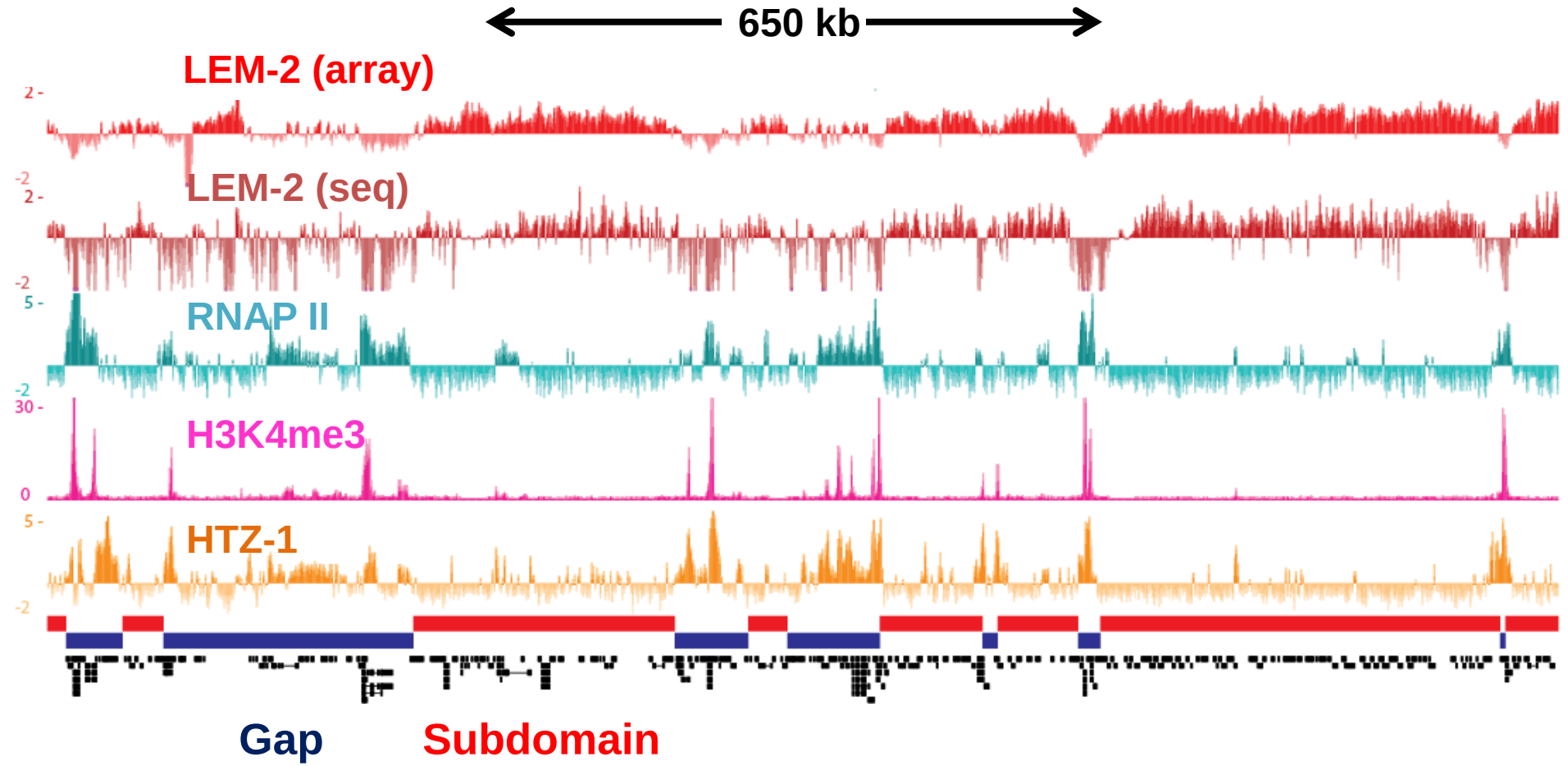


LEM-2 domain: ~4 Mb
LEM-2 subdomain: ~60 kb
Gap: ~12 kb

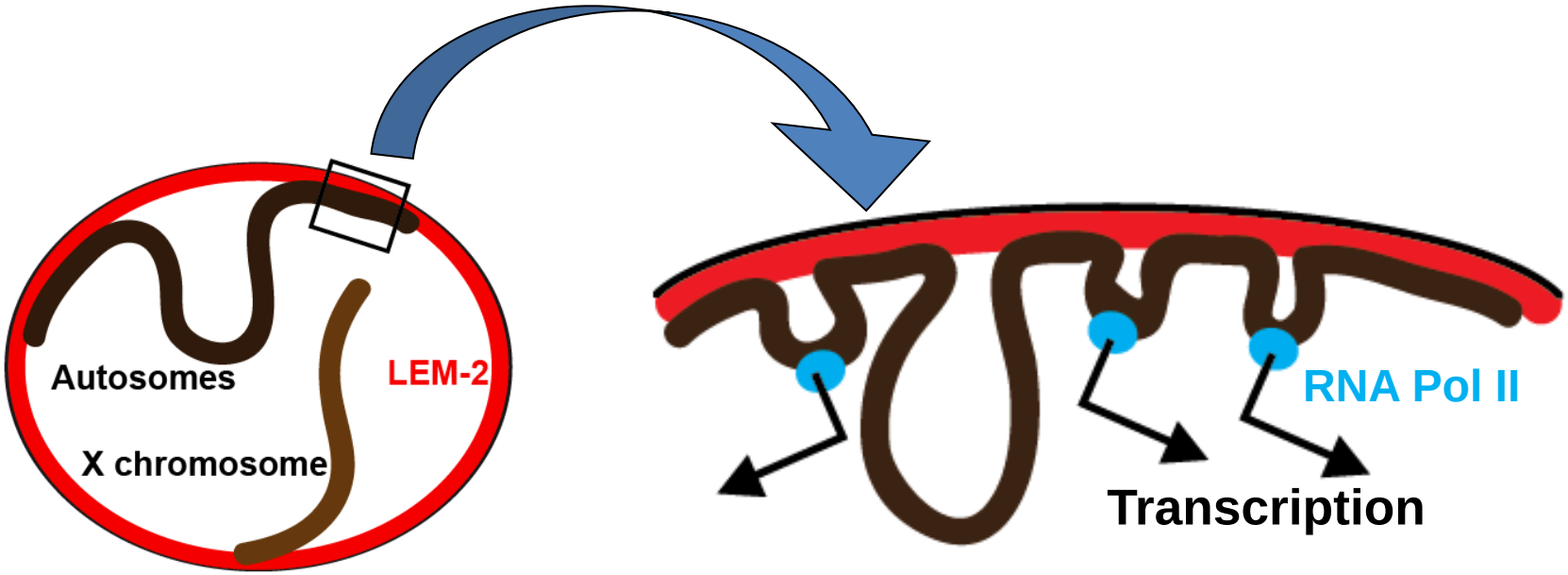
Domain-subdomain organization of nuclear membrane association



High RNA polymerase II, H3K4me3 and HTZ-1 (H2A.Z) levels at gaps between LEM-2 subdomains



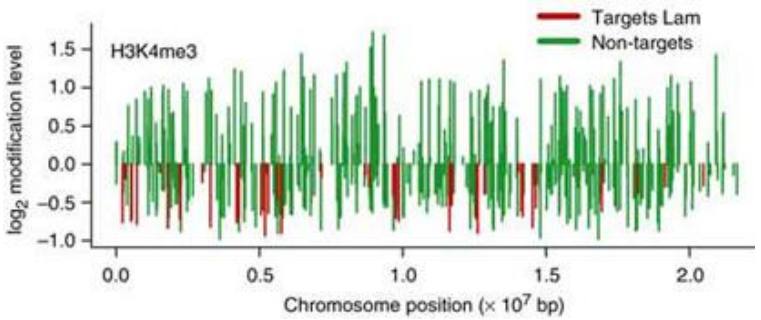
Small loops emerging from the nuclear membranes are transcriptionally active



- Small loops may be “wells” to concentrate factors, for higher residency times and component recycling
- Development of protocols for nuclear membrane genomics

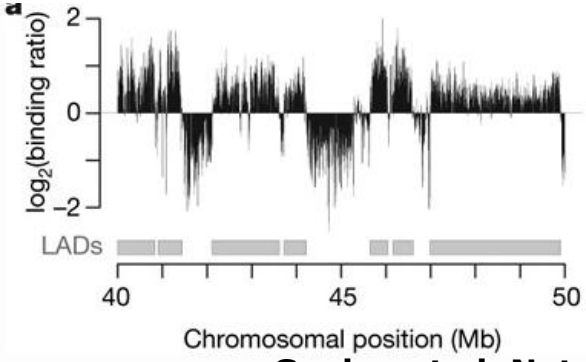
Chromosome interaction with the nuclear envelope

***Drosophila*:**
Repressive environment in Lamin B associated regions



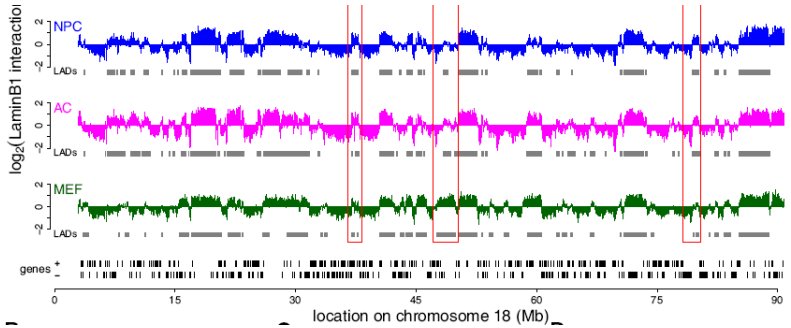
Pickersgill et al. Nat Genet 2006

Human:
Domain-wide (100 kb-10 Mb) Lamin B1 association



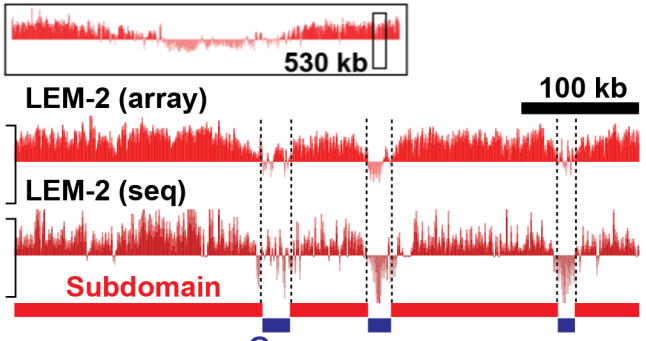
Guelen et al. Nature 2008

Mouse:
Cell-type specific Lamin B1 association



Peric-Hupkes et al., Mol Cell 2010

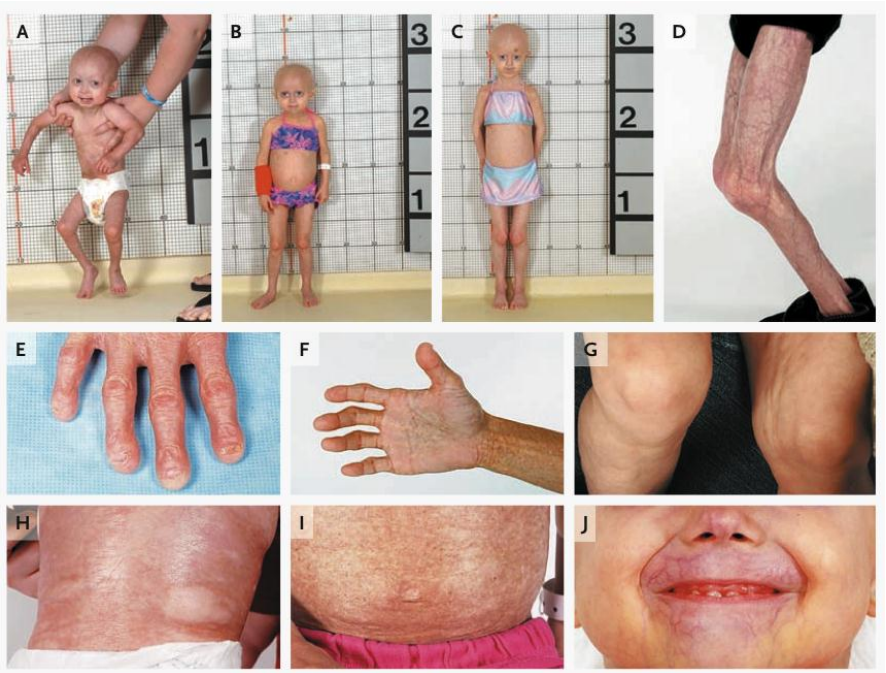
C. elegans
Domain-subdomain organization;
Active small loops; Local determinates



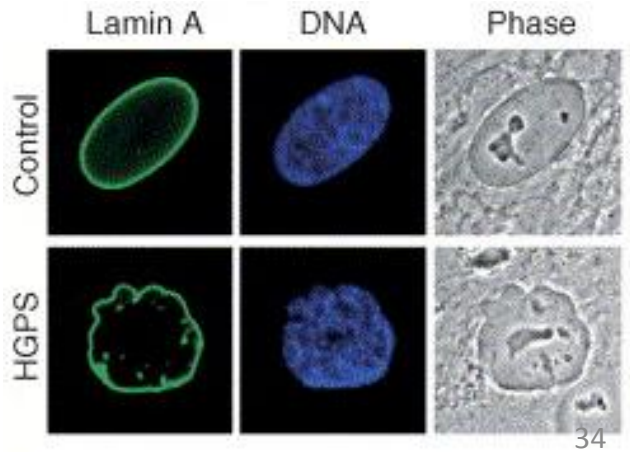
Ikegami et al. 2010

Hutchinson-Gilford Progeria Syndrome (HGPS)

- Phenotypes
  - Segmental premature aging
  - **Average lifespan of 13 years**
  - Affected tissues:
    - Bone (decreased bone mineral)
    - Fat (decreased body fat)
    - Cardiovascular system (elevated blood pressure)
    - Skin (dimpling, mottling (spots))
  - Unaffected tissues:
    - Immune system
    - Gastrointestinal system
  - **Major cause of death**
    - **Coronary atherosclerosis (heart attack)**
    - **Cerebrovascular arteries (stroke)**
- Rare disease
  - 1 in 4 million
  - 64 living patients identified world-wide (April, 2010)
- Cellular phenotypes
  - Gene expression changes
  - Aberrant nuclear membrane

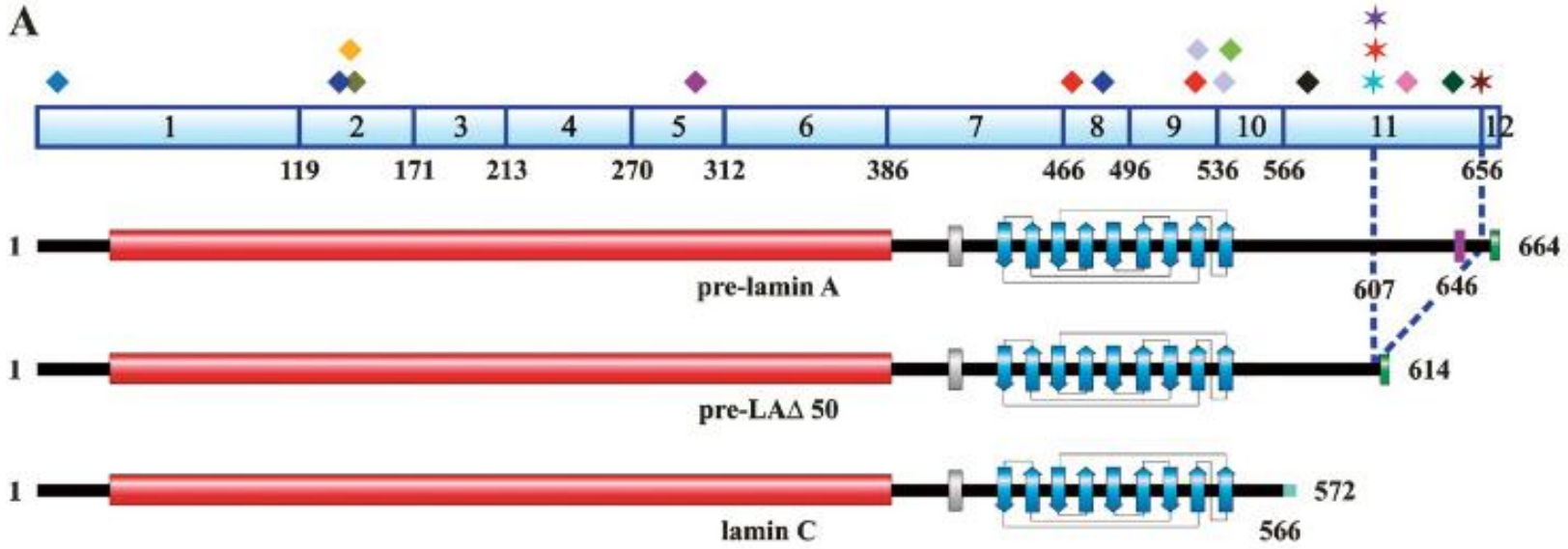


Merideth et al., N Engl J Med 2008



Mattout et al., Curr Opin Cell Biol 2006

HGPS is caused by mutations of the lamin A/C gene



Dechat et al., Gene Dev 2008

- Lamin A/C is one of two major lamin proteins
  - A-type: lamin A and C; B-type: lamin B1 and B2
- Mostly dominant *de novo* point mutations; C->T mutation (G608G) in exon11
  - Activation of a cryptic splicing site
  - Deletion of 50 amino acids => a mutant pre-lamin A, called “Progerin”
  - Progerin retains farnseylation

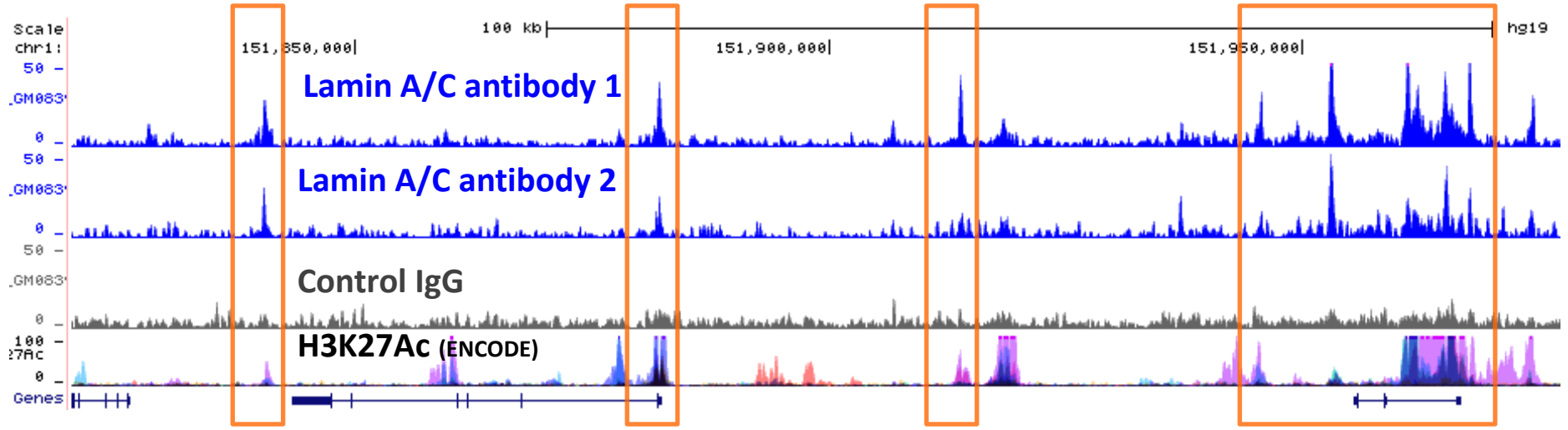
Many other LMNA mutations linked to diseases

Table 1
Diseases caused by mutations in genes encoding lamins and associated proteins

Disease	Mutation	Major disease phenotypes
Striated muscle diseases		
Autosomal dominant EDMD	*LMNA*	Muscle weakness and wasting in scapulohumeral-peroneal distribution; early joint contractures; dilated cardiomyopathy
Autosomal recessive EDMD	*LMNA*	Muscle weakness and wasting in scapulo-humeral peroneal distribution; early joint contractures; dilated cardiomyopathy
Cardiomyopathy dilated 1A	*LMNA*	Cardiomyopathy with minimal to no skeletal muscle involvement
Limb-girdle muscular dystrophy type 1B	*LMNA*	Muscle weakness and wasting in limb-girdle distribution; dilated cardiomyopathy
Congenital-type muscular dystrophy	*LMNA*	Severe relatively diffuse myopathy presenting in first year of life; later cardiomyopathy
"Heart-hand" syndrome (with limb defects)	*LMNA*	Brachydactyly with mild hand and more severe foot involvement; cardiomyopathy
X-linked EDMD	*EMD*	Muscle weakness and wasting in scapulo-humeral peroneal distribution; early joint contractures; and dilated cardiomyopathy
Partial lipodystrophy syndromes		
FPLD2	*LMNA*	Loss of subcutaneous fat from the extremities at puberty, followed by increased fat accumulation in the face and neck; insulin resistance; diabetes mellitus; hyptertriglyceridemia; hepatic steatosis
Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules	*LMNA*	Generalized fat loss; insulin-resistant diabetes, hypertriglyceridemia, hepatic steatosis, hypertrophic cardiomyopathy; disseminated whitish papules
Mandibuloacral dysplasia (also has features of progeria)	*LMNA*	Hypoplastic mandible with dental crowding, acroosteolysis, stiff joints, atrophy of the skin over hands and feet, hypoplastic clavicles; "Andy Gump" appearance; persistently wide cranial sutures and multiple wormian bones; alopecia and short stature; and partial lipodystrophy
Acquired partial lipodystrophy (Barraquer-Simons syndrome)	*LMNB2*	Progressive, sporadic lipodystrophy with phenotype similar to FPLD2 (above)

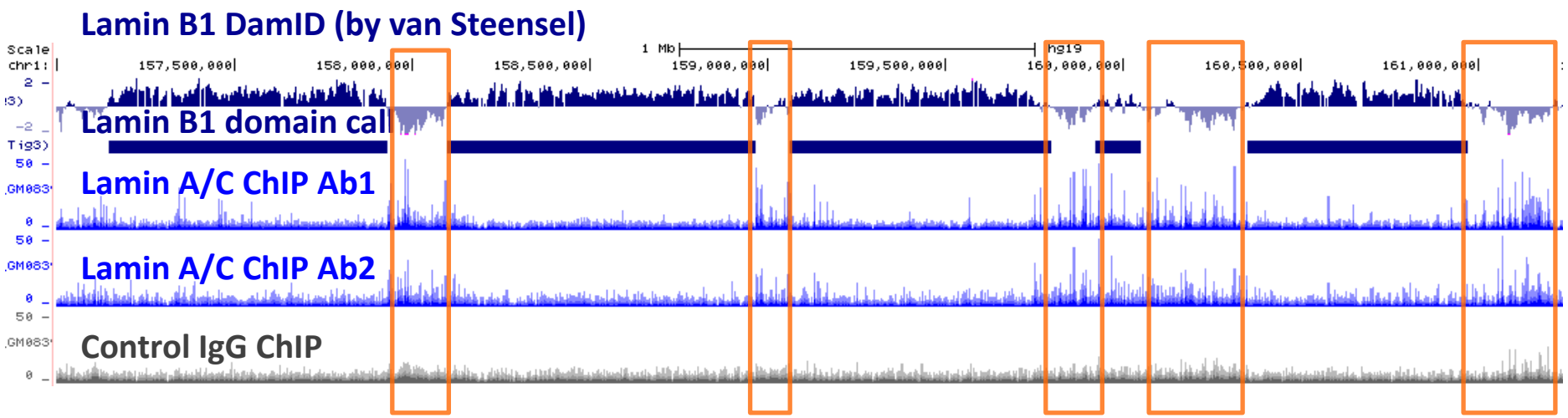
Worman et al., J. Clin. Invest 2009

Lamin A/C associates with active regulatory sites



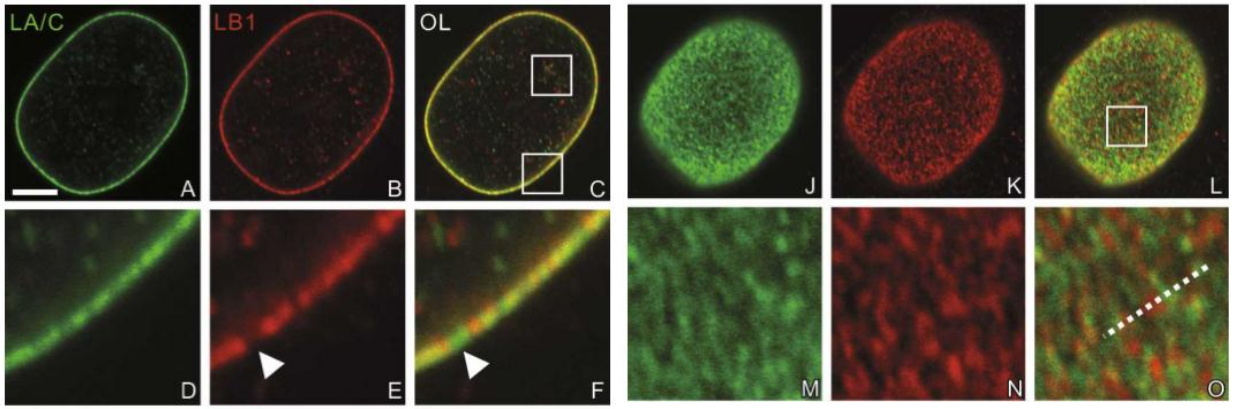
ChIP in primary human fibroblast cells

Lamin A/C distribution is inversely correlated with lamin B

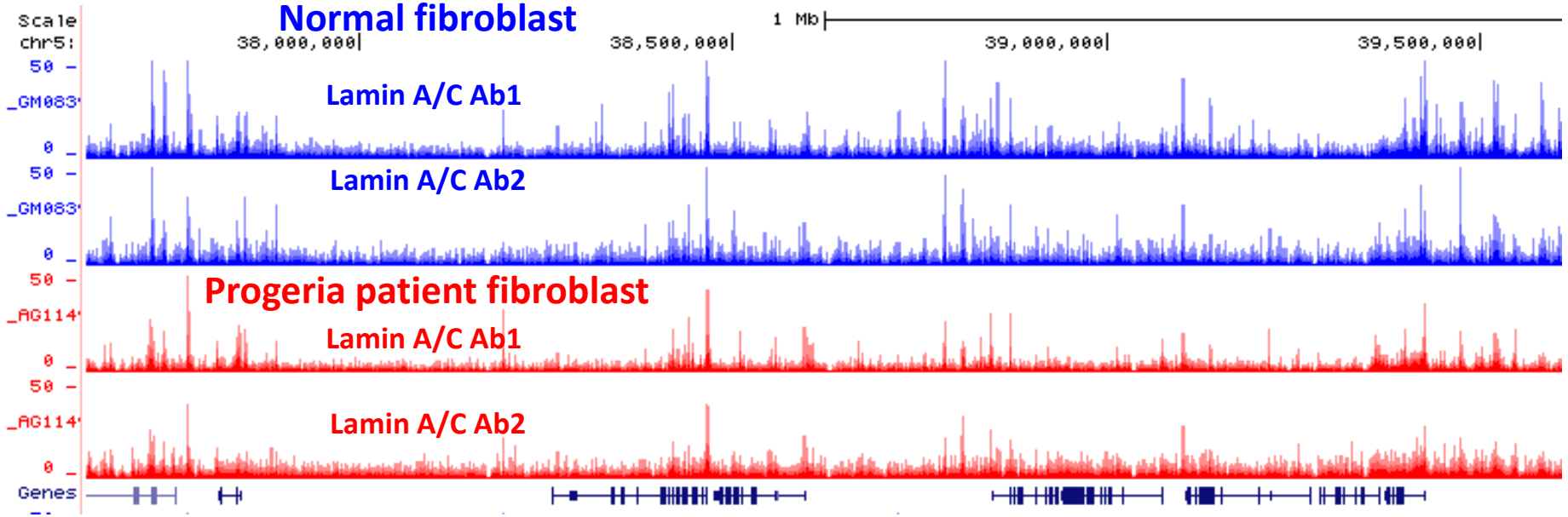


Both ChIP and DamID (Guelen et al., Nature 2008) are performed in primary human fibroblast cells

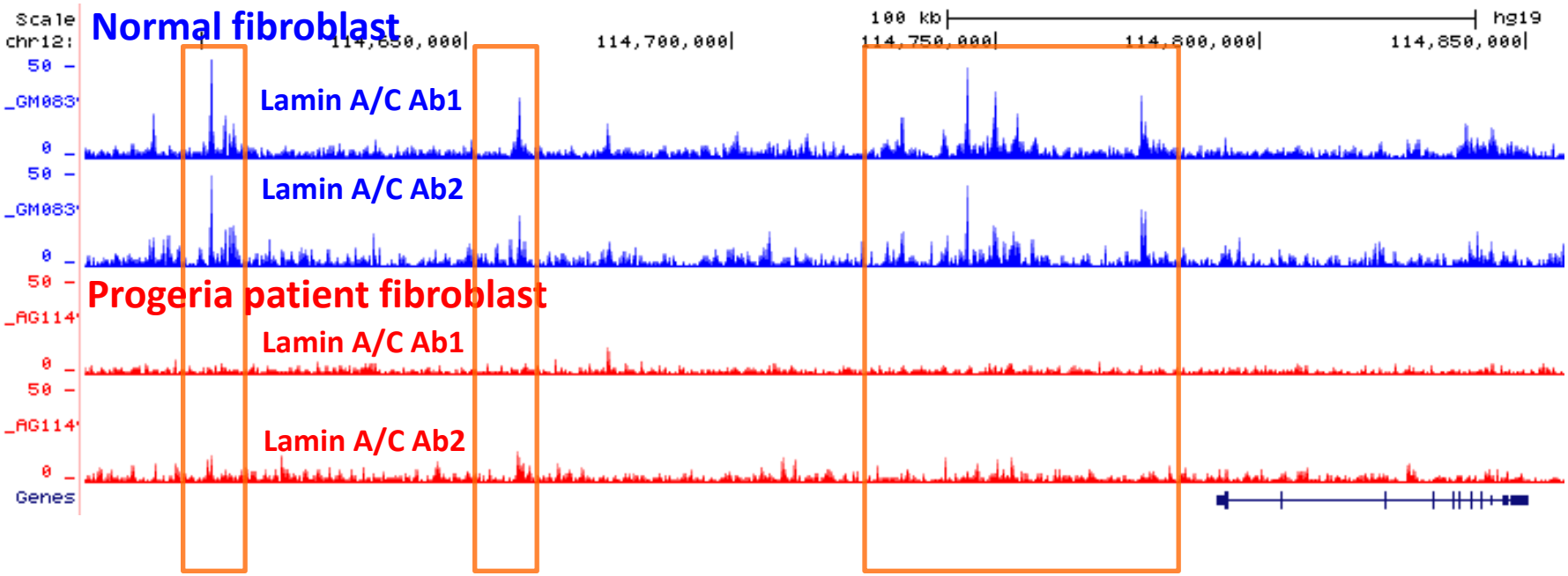
Lamin A/C and lamin B1 are separately localized in the lamina
(Shimi et al., Genes Dev 2008)



The pattern of lamin A/C association is largely maintained in progeria cells



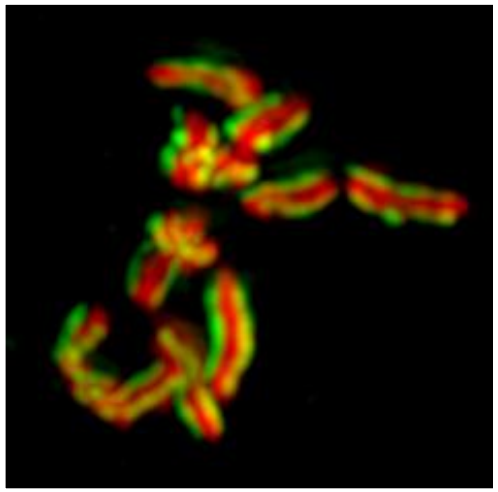
Some regions lose lamin A/C associations in progeria cells



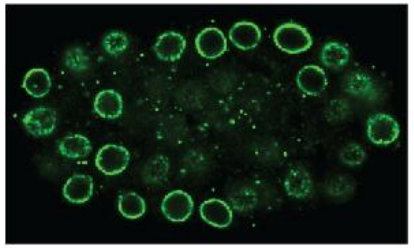
Currently ChIPing from cells treated with Farnesyltransferase Inhibitor (FTI)

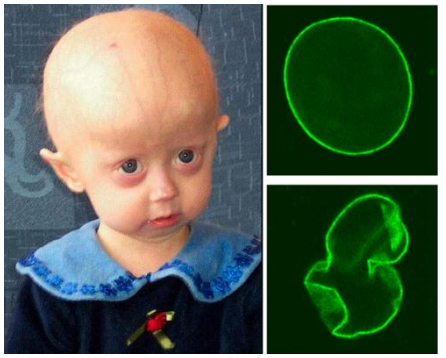
Unique insights gained by modENCODE

(1) Centromere specification



(2) Chromosome-membrane interactions





…and many other discoveries (exon marking by H3K36me3, dosage compensation insights in worms and flies, new functions for H4K20me1, principles of metazoan nucleosome organization…)

Acknowledgements

CENP-A

Reto Gassmann, Andreas Rechtsteiner, Karen W. Yuen, Andrew Muroyama, Francie Barron, Paul Maddox, Joost Monen, Thea Egelhofer, Sevinc Ercan, Karen Oegema, Jason Lieb, Susan Strome, **Arshad Desai**

An Inverse Relationship to Germline Transcription Defines the *C. elegans* Holocentromere in Progeny

Nature. 2012. Apr 8; 484 (7395):4534-7 PMID: 22495302

LEM-2 and Progerin

The Progeria Research Foundation

Kohta Ikegami, Thea Egelhofer, Susan Strome, Jason Lieb

C. elegans chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2.

Genome Biology. 2010;11(12):R120. PMID: 21176223

Worm Chromatin Group Labs

Julie Ahringer
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Eran Segal

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Sally Elgin
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