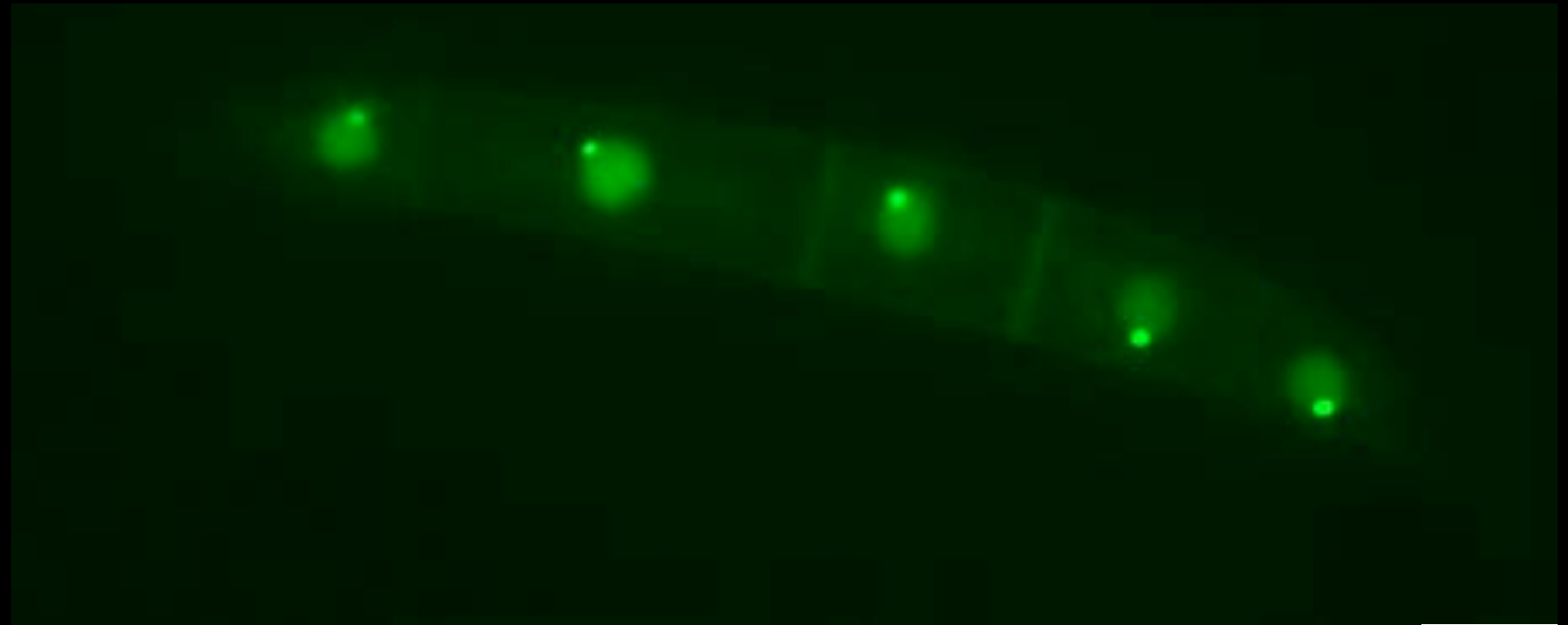


ChIP-sequencing: Centromeres and Circadian Rhythms



Freitag Lab
Oregon State University



Outline

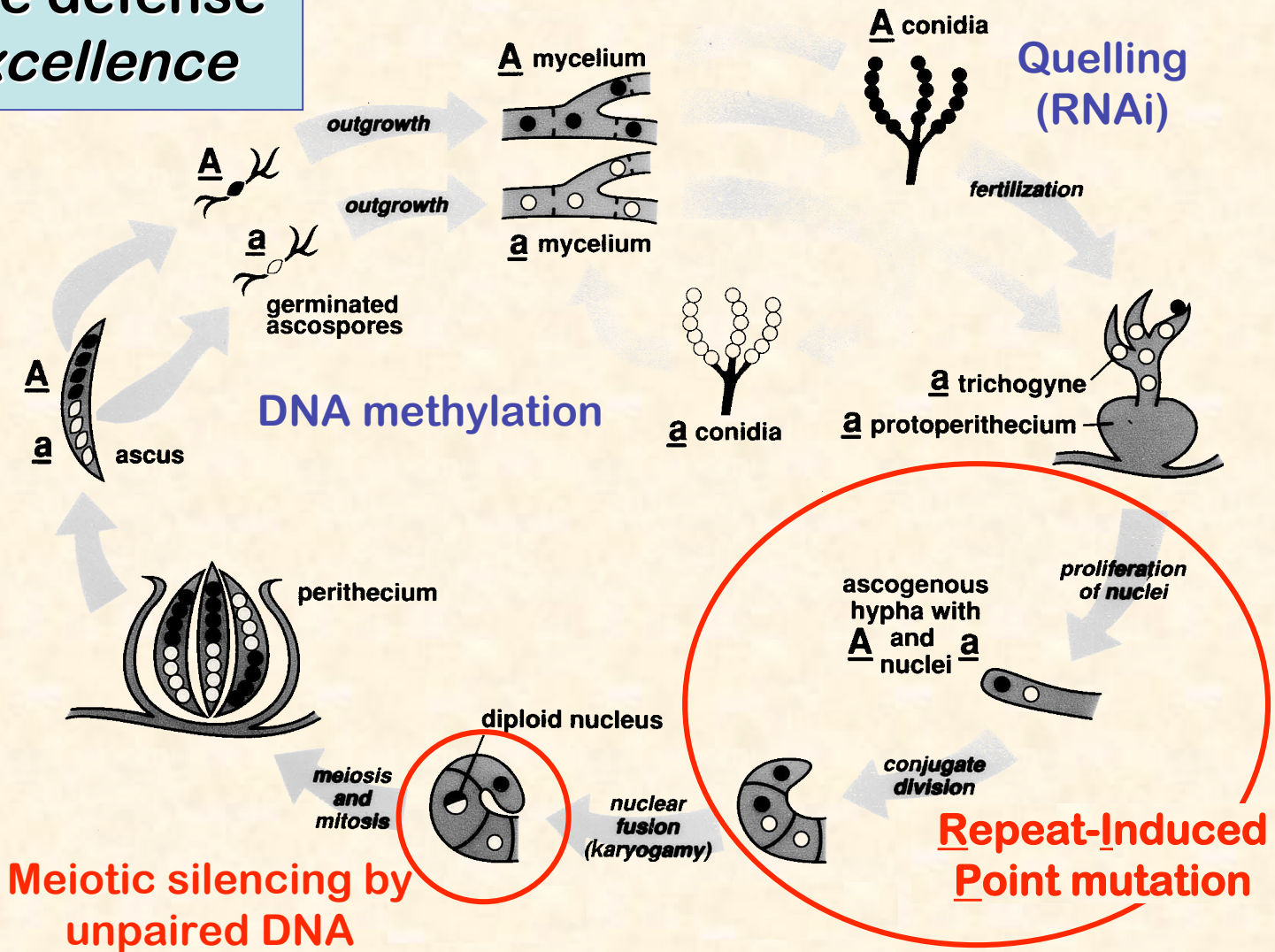
Formation of heterochromatin in Neurospora

Centromeres: Constitutive heterochromatin?

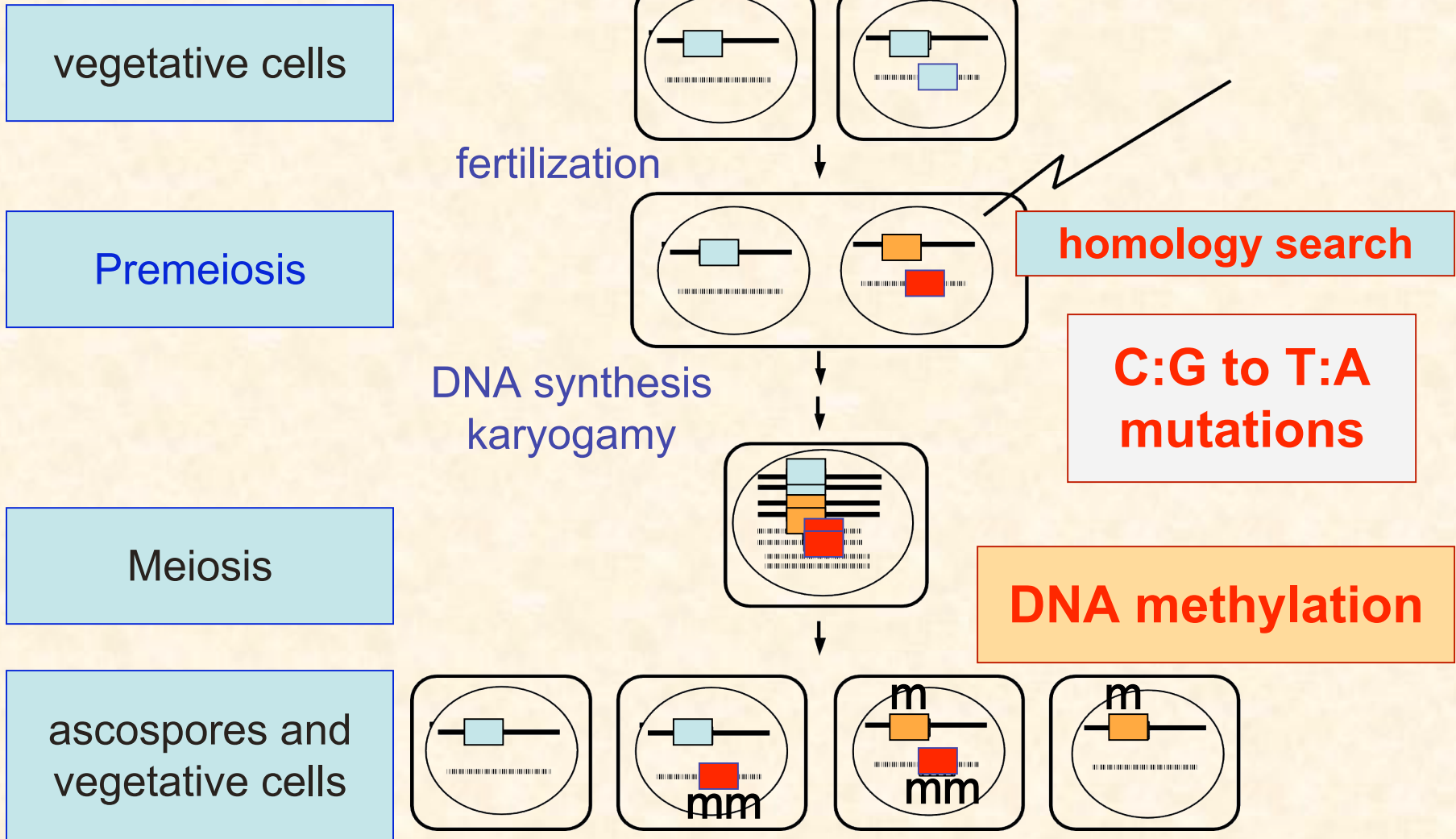
**ChIP-seq of transcription factors involved in
light regulation and the circadian clock**

Neurospora crassa

Genome defense
par excellence



Repeat-induced point mutation (RIP)



Example of RIP

CAA GAA GAC **I** ATG ATG GAC GAC **S-L** AAG ATG AGC TAC ACA AAG TTG CGC **A-V** GAG CAA 180
T T T

AGG CTT GCC GAG GAA GAG GAA AAG GCT CGC **V** GAA AAC GAA AAG **A-V** GAG GAA AGG CGC 240
T T

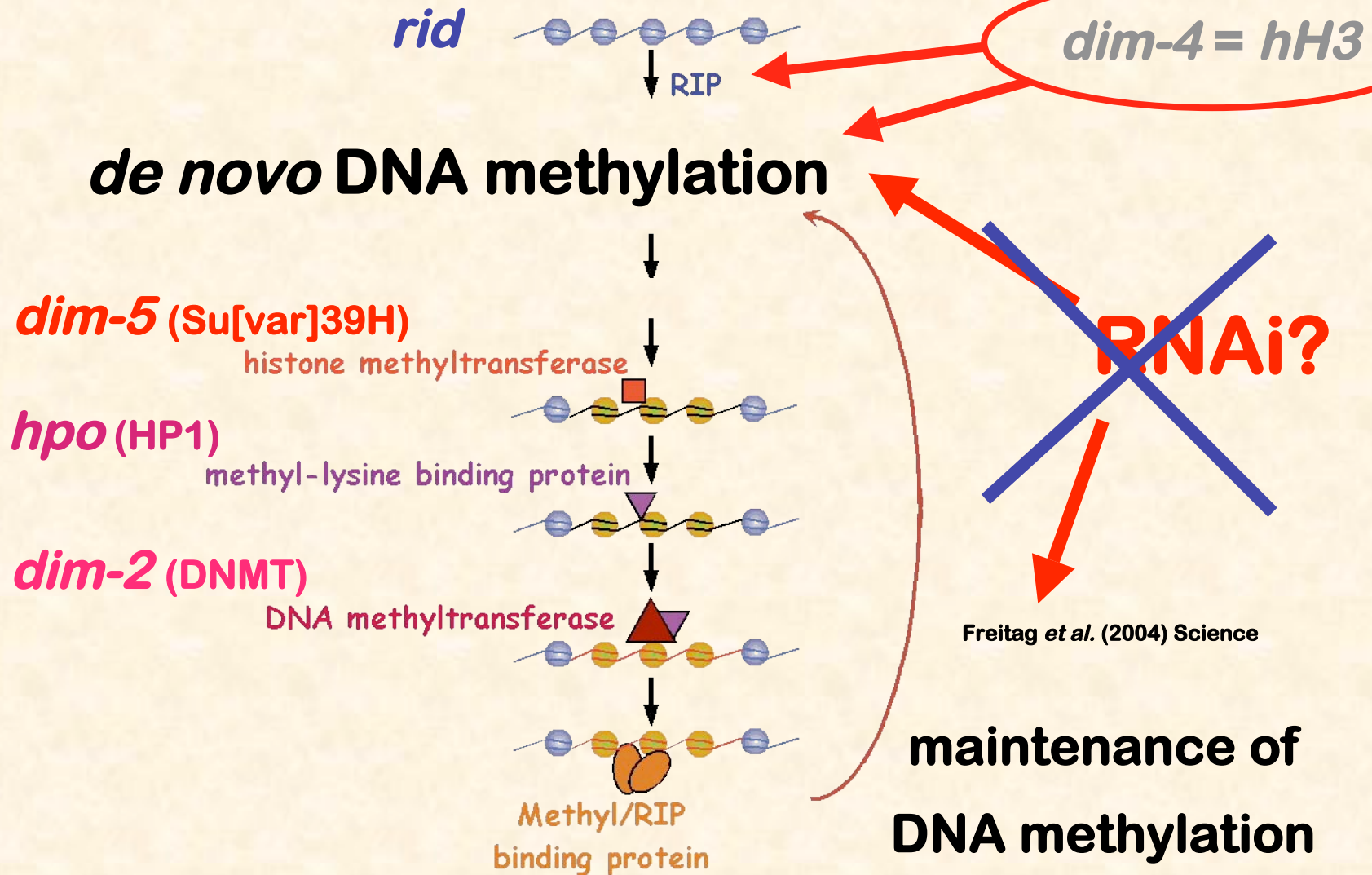
AAA AAG AAG TGG AAA AAG AAG GTT CTG **S** AAA GAG GAG CGC GAT GCA AAG GCA AAG GAT 300
T

CTG GAC CGG CTT CTT GCT ~~⊘~~ **A-V** **F** **S** **S** **L** **N** **T-I** AAG AAG 360
T T T T T T T

GTT CTT **G-S** CGT GTG GGA **T-I** **G-S** CTG GAT **H-Y** GAG TTG **A** 420
A T A T T

Neurospora crf5-1 (homologue of Arabidopsis DDM1)

Control of DNA methylation



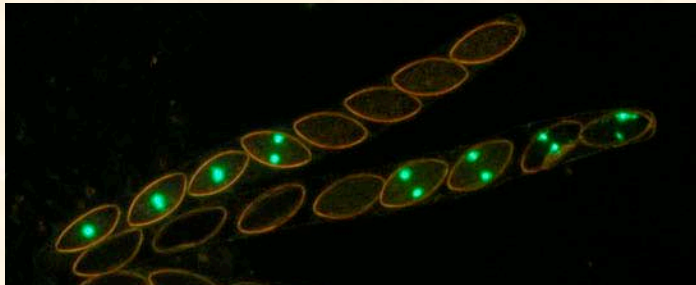
Questions about RIP and heterochromatin

What controls pairing in RIP?

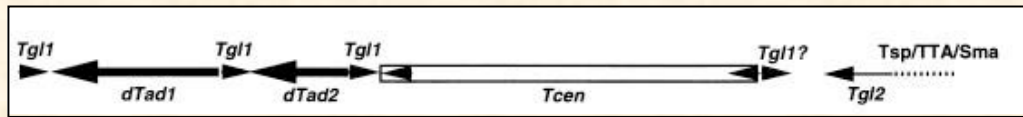
What is the mechanism for RIP?

**How does heterochromatin affect
the life cycle and evolution?**

Most RIPed DNA is centromeric

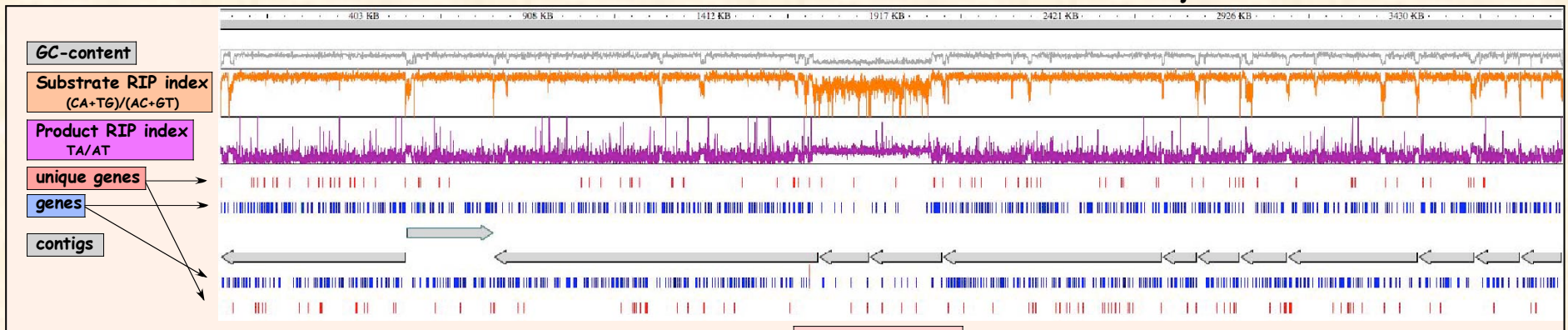


hH1-sgfp at the *his-3* locus, linked to *Cen-I*



Cambareri *et al.* (1998) MCB

Neurospora crassa LG VII



centromere

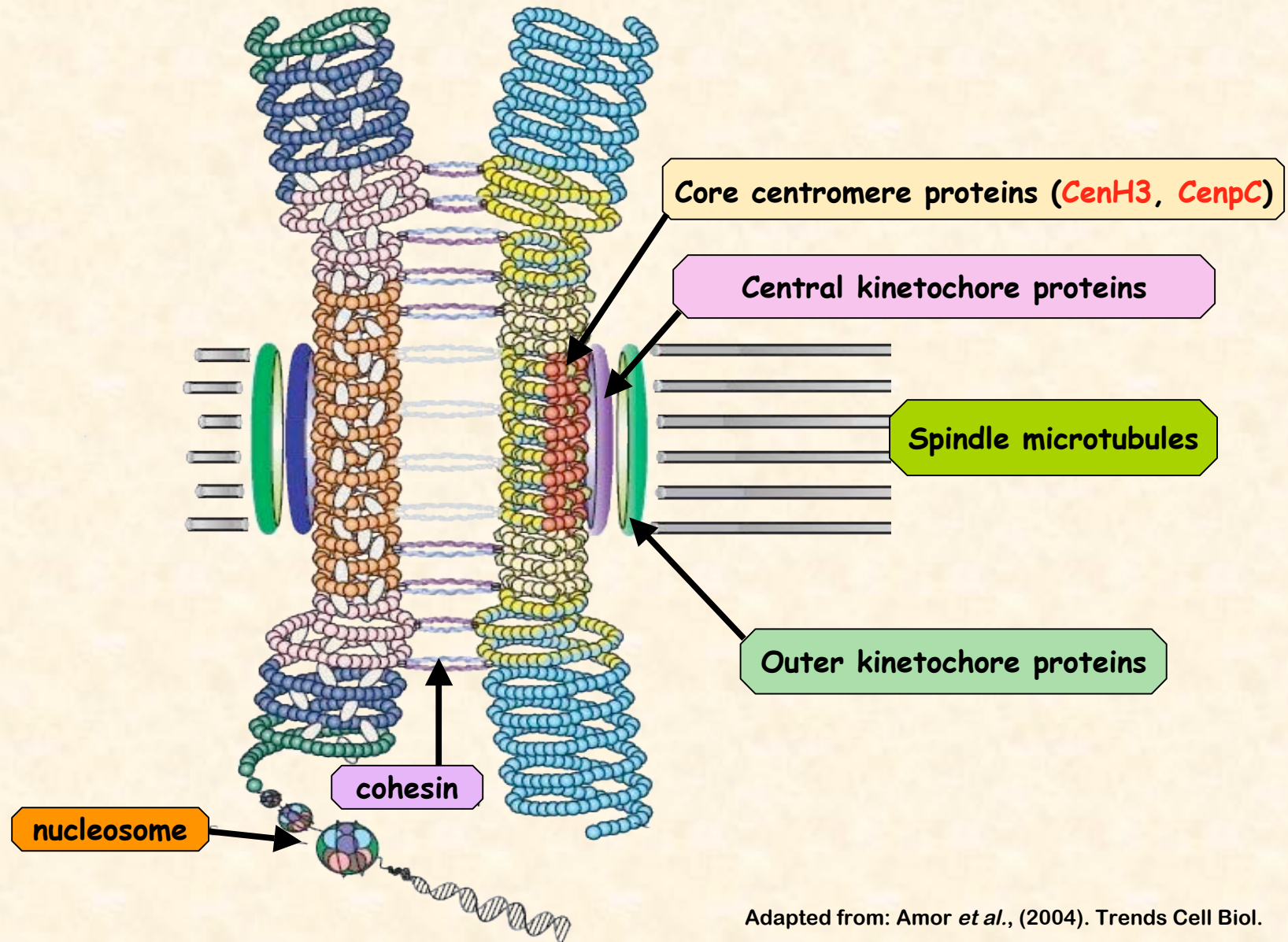


Markers

met-7 |
hH2B,A

|| *ars-1*
qa cluster

Centromere and kinetochore proteins



Adapted from: Amor *et al.*, (2004). Trends Cell Biol.

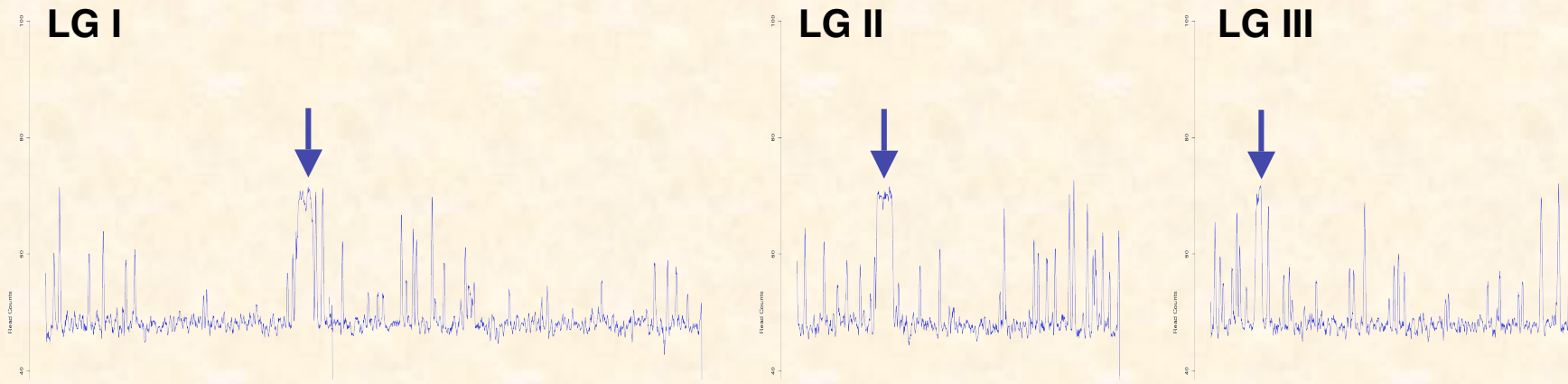
Questions relating to **centromere assembly, maintenance** and evolution

Where are the Neurospora centromere cores?

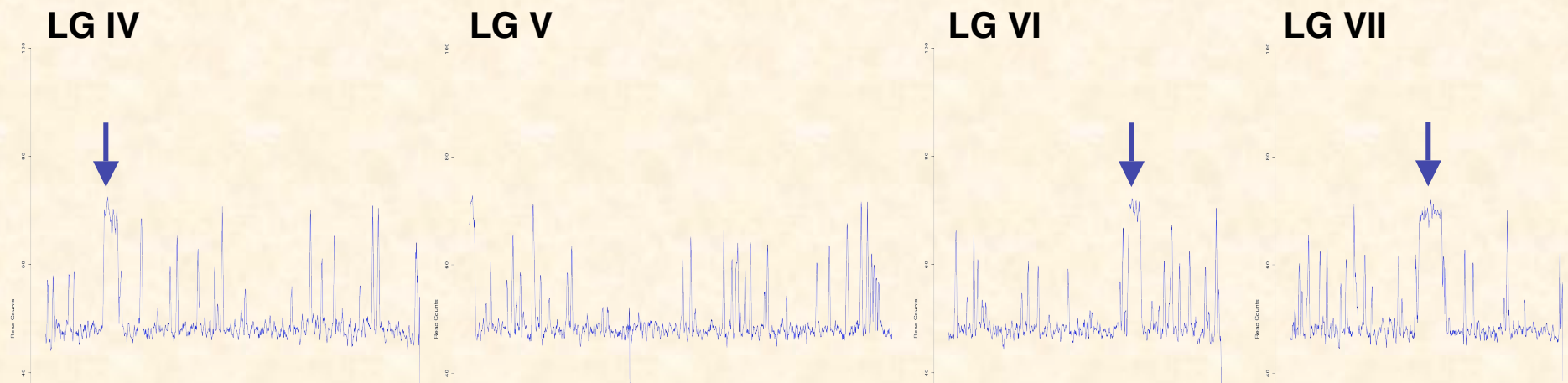
What is required for centromere assembly?
(heterochromatin? siRNA?)

What is required for centromere inheritance?
(same as above and kinetochore?)

Where are the Neurospora centromere cores?

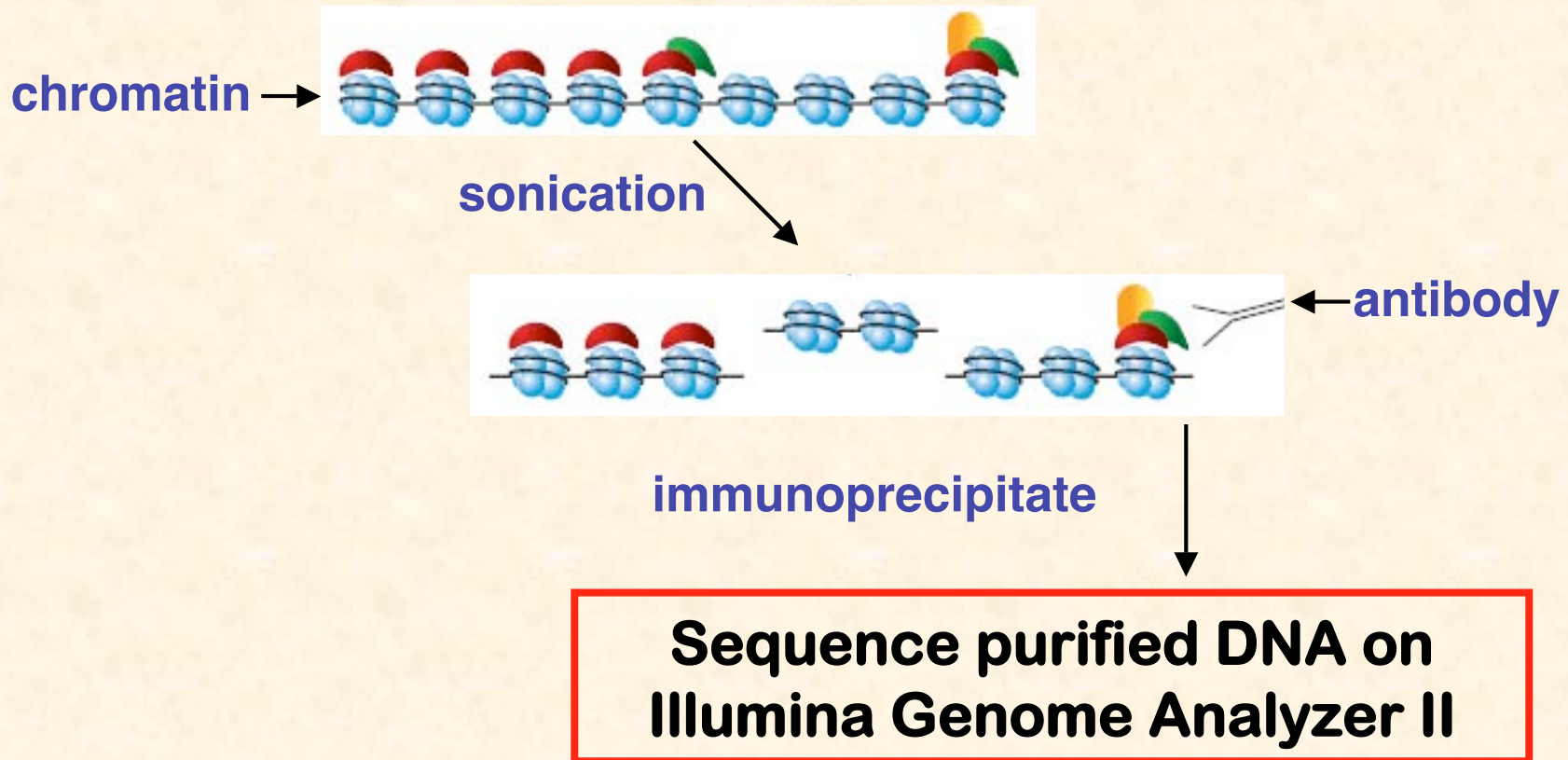


Is AT percentage predictive for centromeric regions?



Locate centromeres by “ChIP-seq”

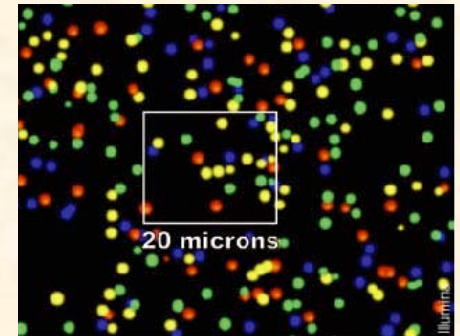
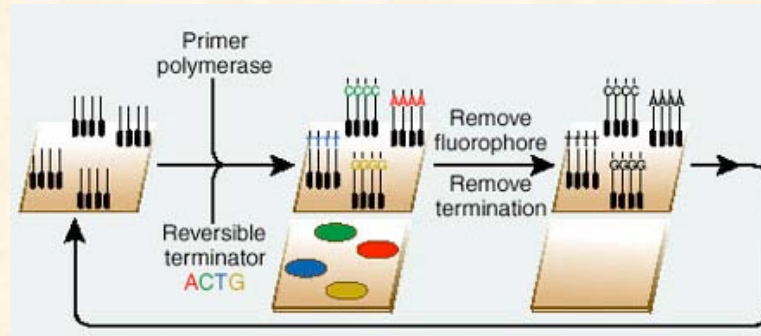
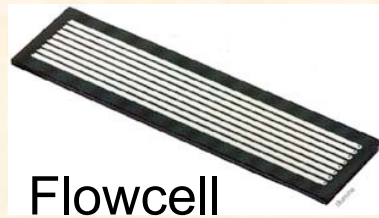
chromatin immunoprecipitation (ChIP) of CenH3 and CenpC followed by high-throughput sequencing



High-throughput ChIP sequencing

Illumina/Solexa GAI analyzer: "Sequencing by synthesis"

• 3-4 days (~\$5k)



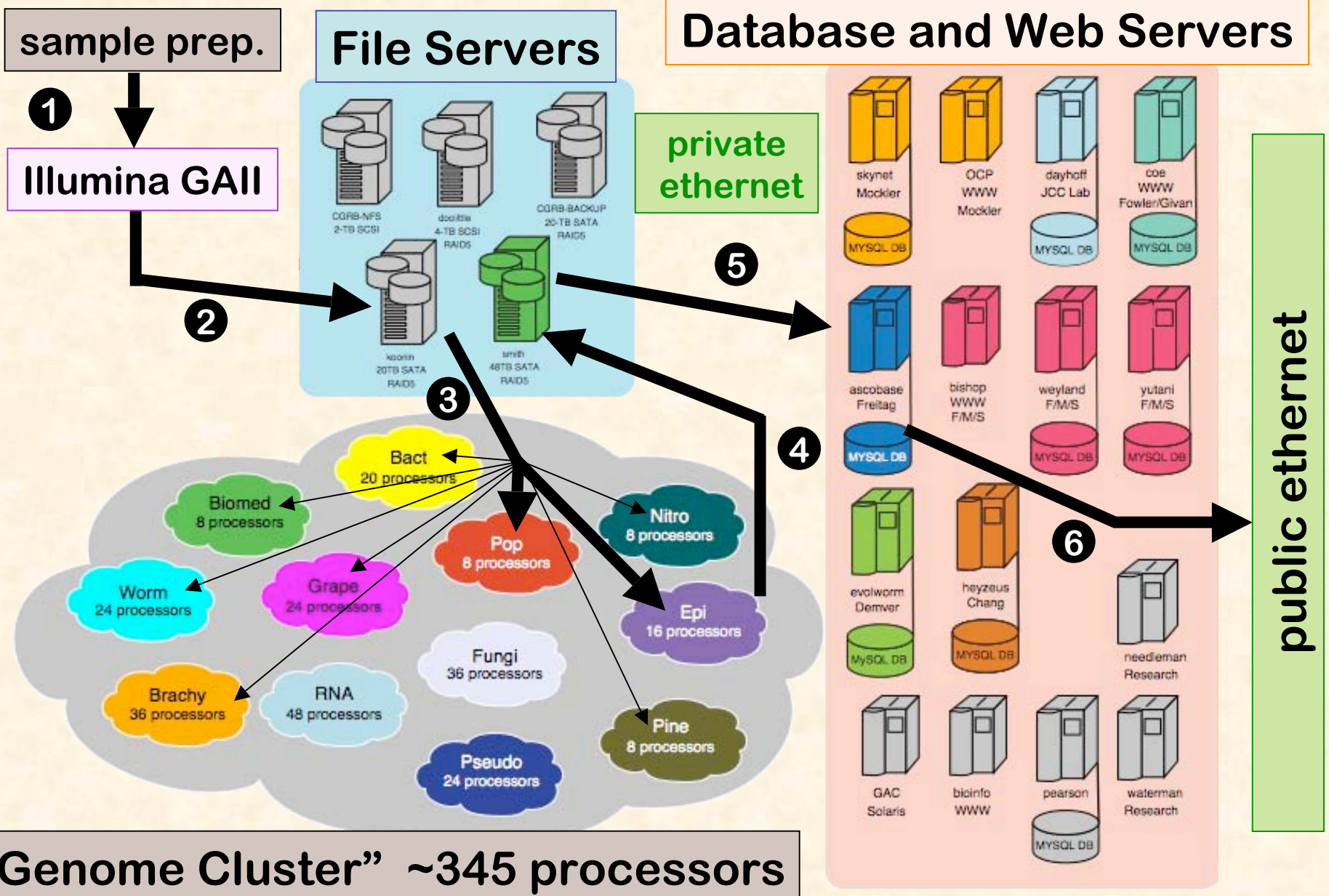
~200,000 clusters/tile x 100 tiles = 20 million reads/lane

x 8 lanes x 36 nt "reads" = ~5.7 Gb of sequence/flowcell

Neurospora genome: ~40 Mb

(~18x coverage/lane; ~140x coverage/flowcell)

OSU Center for Genome Research and Biocomputing Infrastructure



“Genome Cluster” ~345 processors

ChIP-seq read mapping by CASHX

Fahlgren *et al.*, (2009) RNA

LG IV



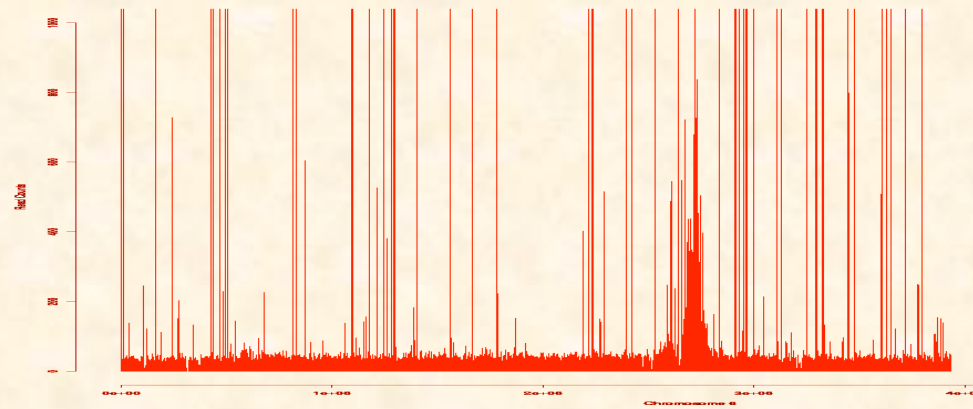
Blue = single read

Green = 2-10 identical reads

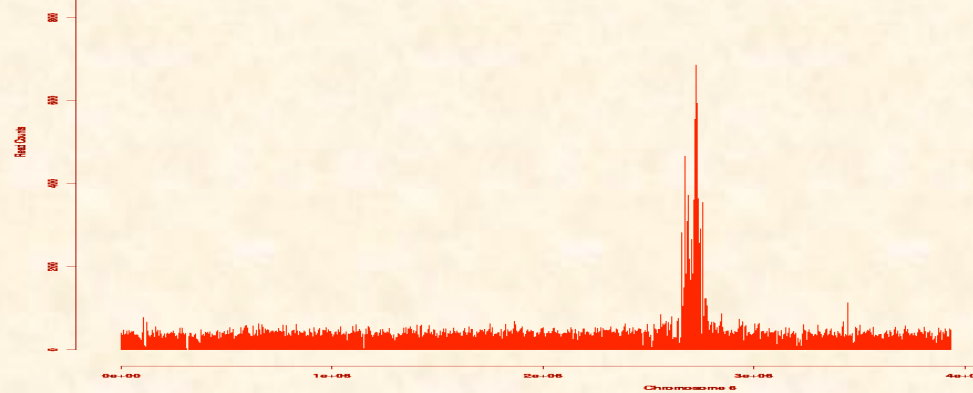
Data filtering

CenpC-GFP

Read counts/500bp



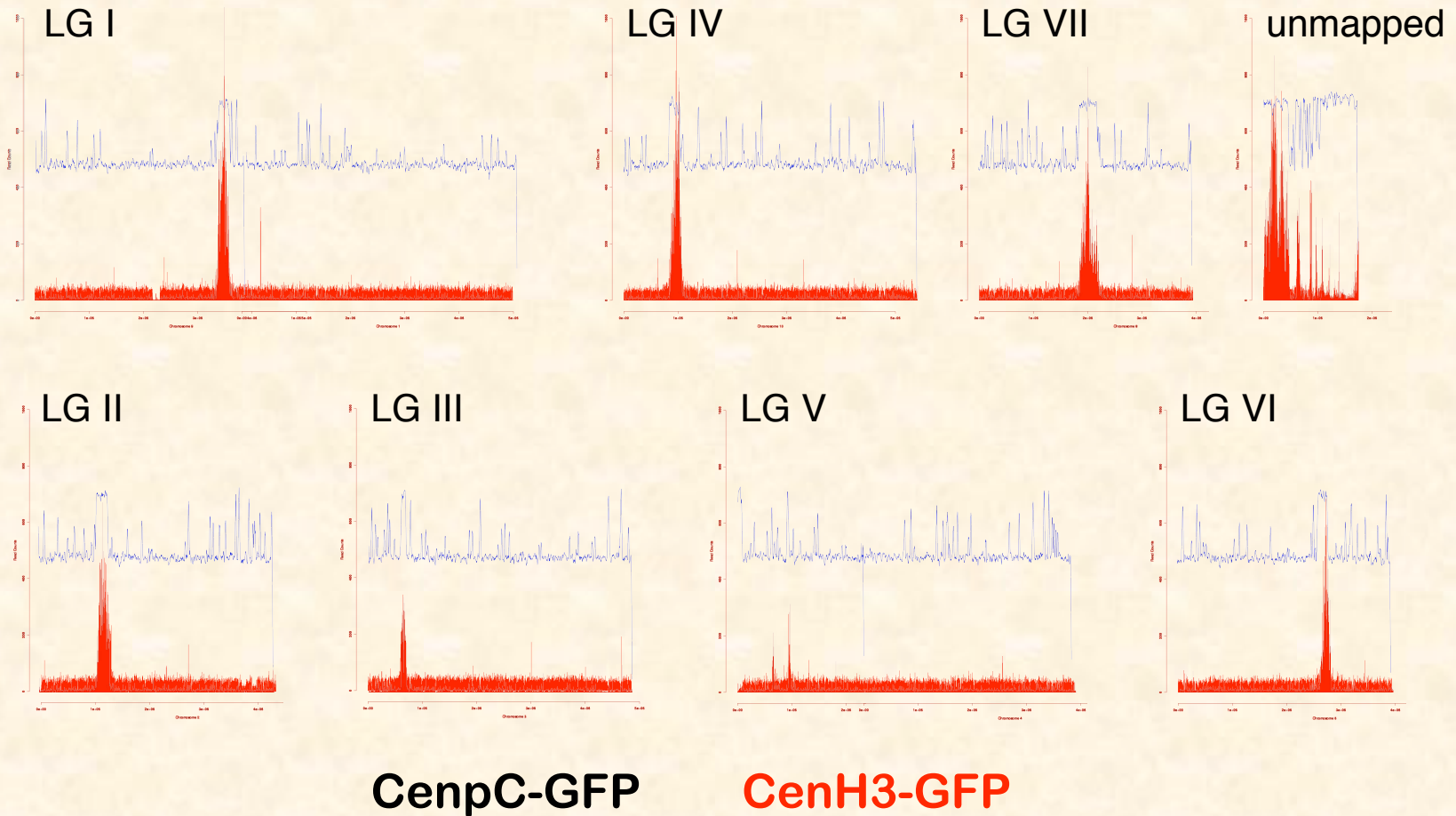
All reads



<1000 hits
(normalized)

LG VII *CEN*

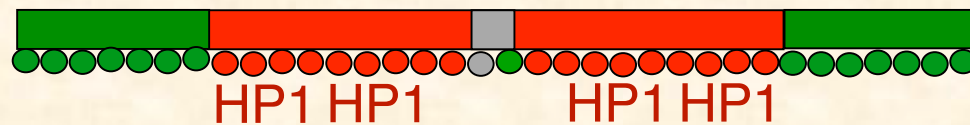
CenH3 and CenpC localize at predicted centromeric regions



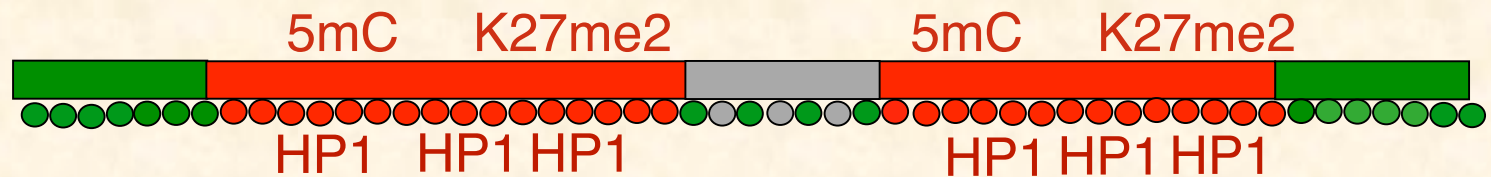
What is required for centromere assembly and maintenance?







Histone modifications?

Fission yeast



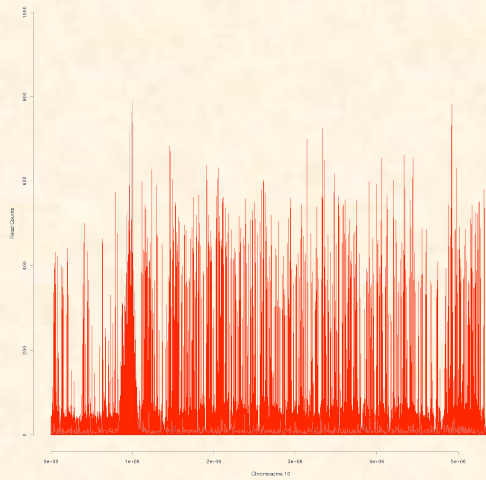
Animals



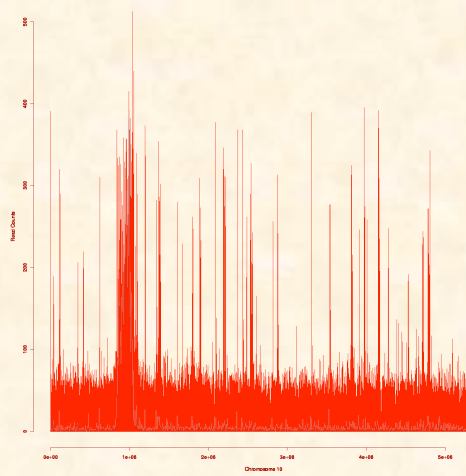
- | | |
|---|---|
|  centrochromatin |  CenH3 |
|  heterochromatin |  H3 K9me |
|  euchromatin |  H3 K4me |

Neurospora centromeres are heterochromatic

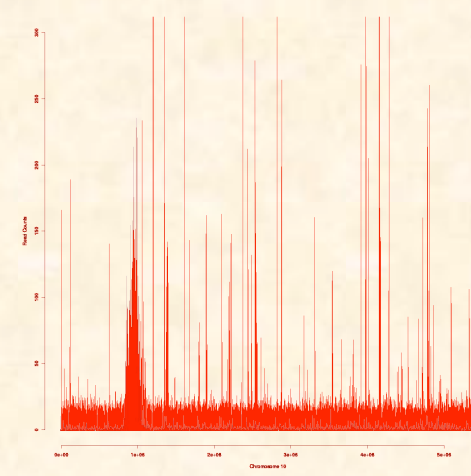
LG IV



CenH3-GFP
H3 K4me2



CenH3-GFP
H3 K9me3



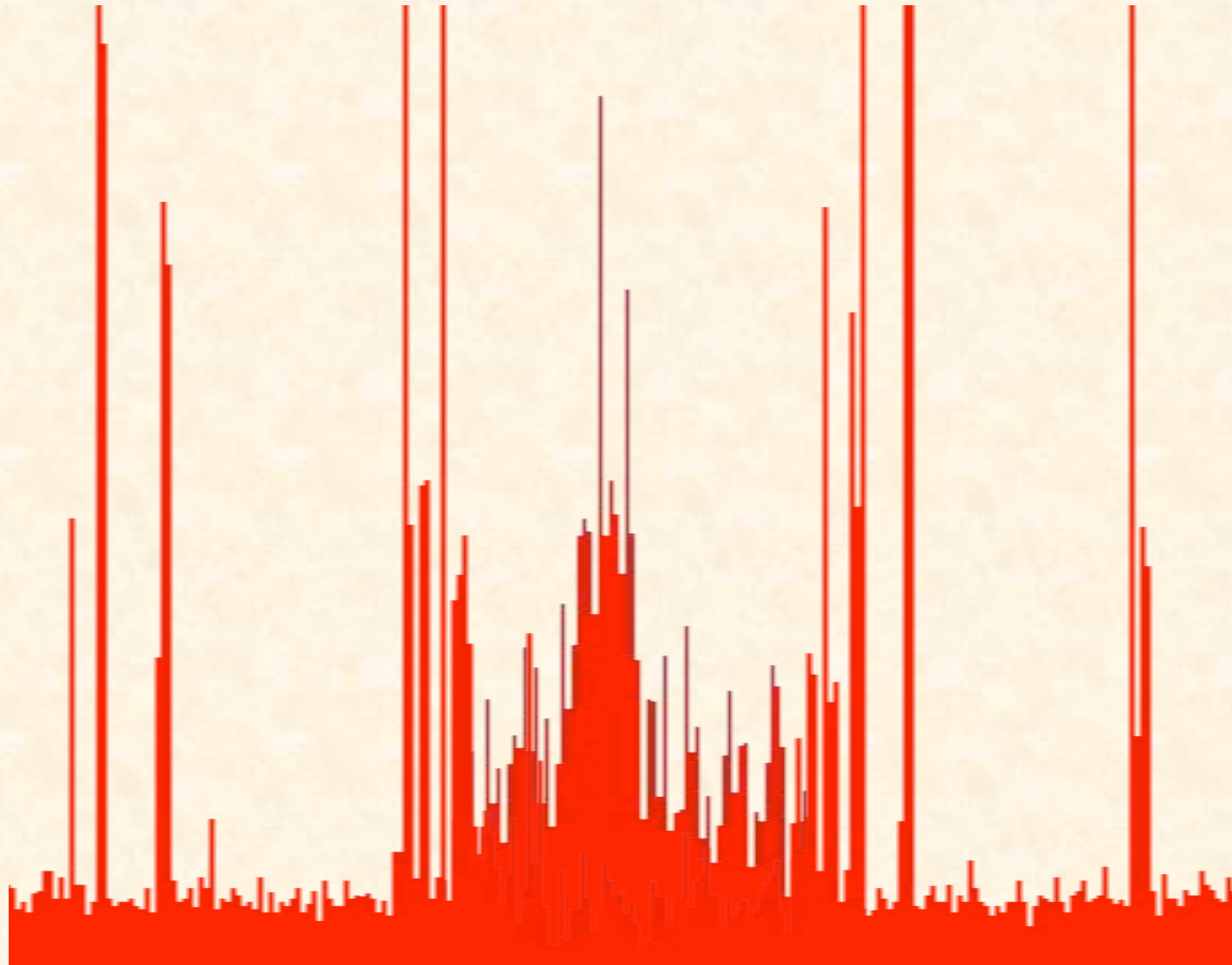
CenH3-GFP
5MeC

DNA methylation mostly in pericentric regions

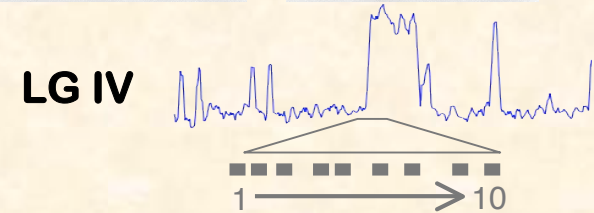
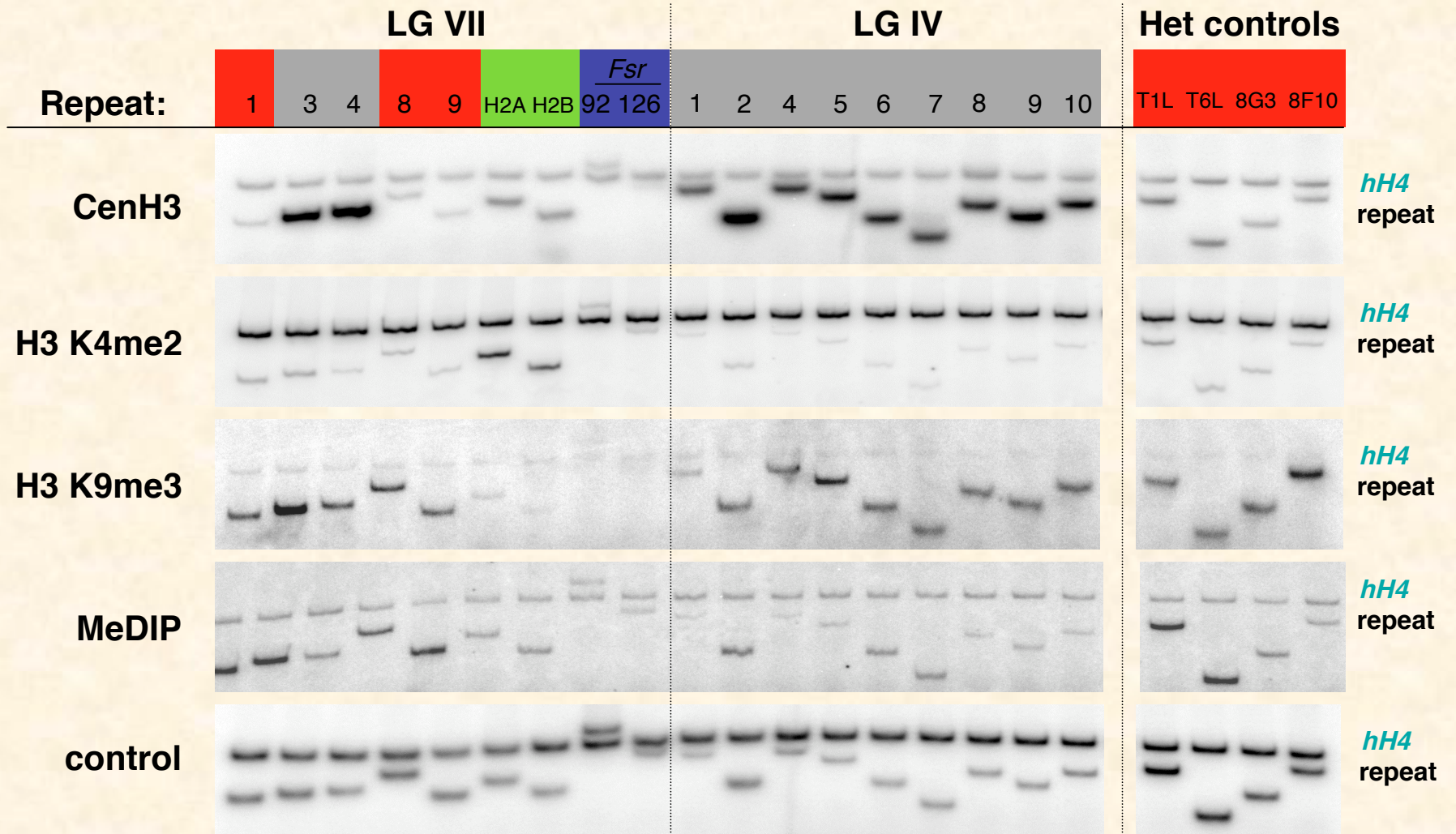
Cen VII

CenH3

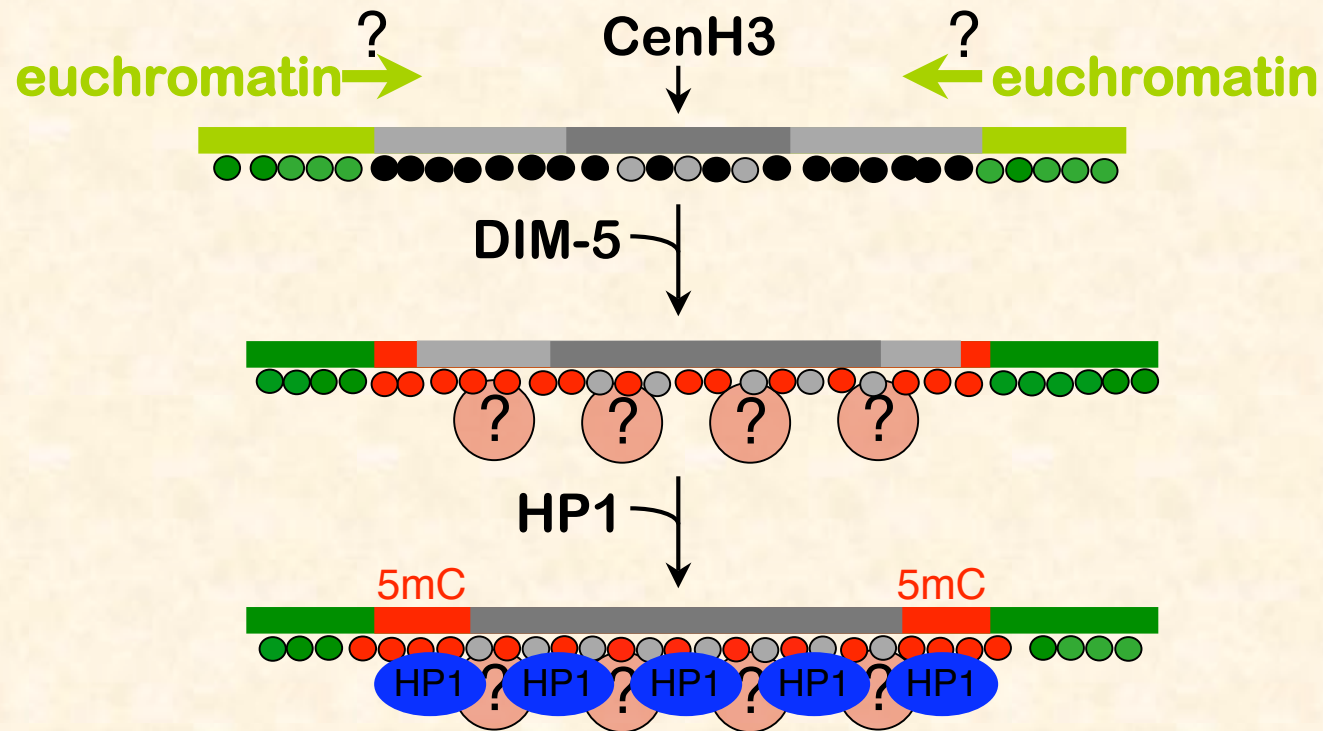
5mC



ChIP PCRs confirm ChIP-seq

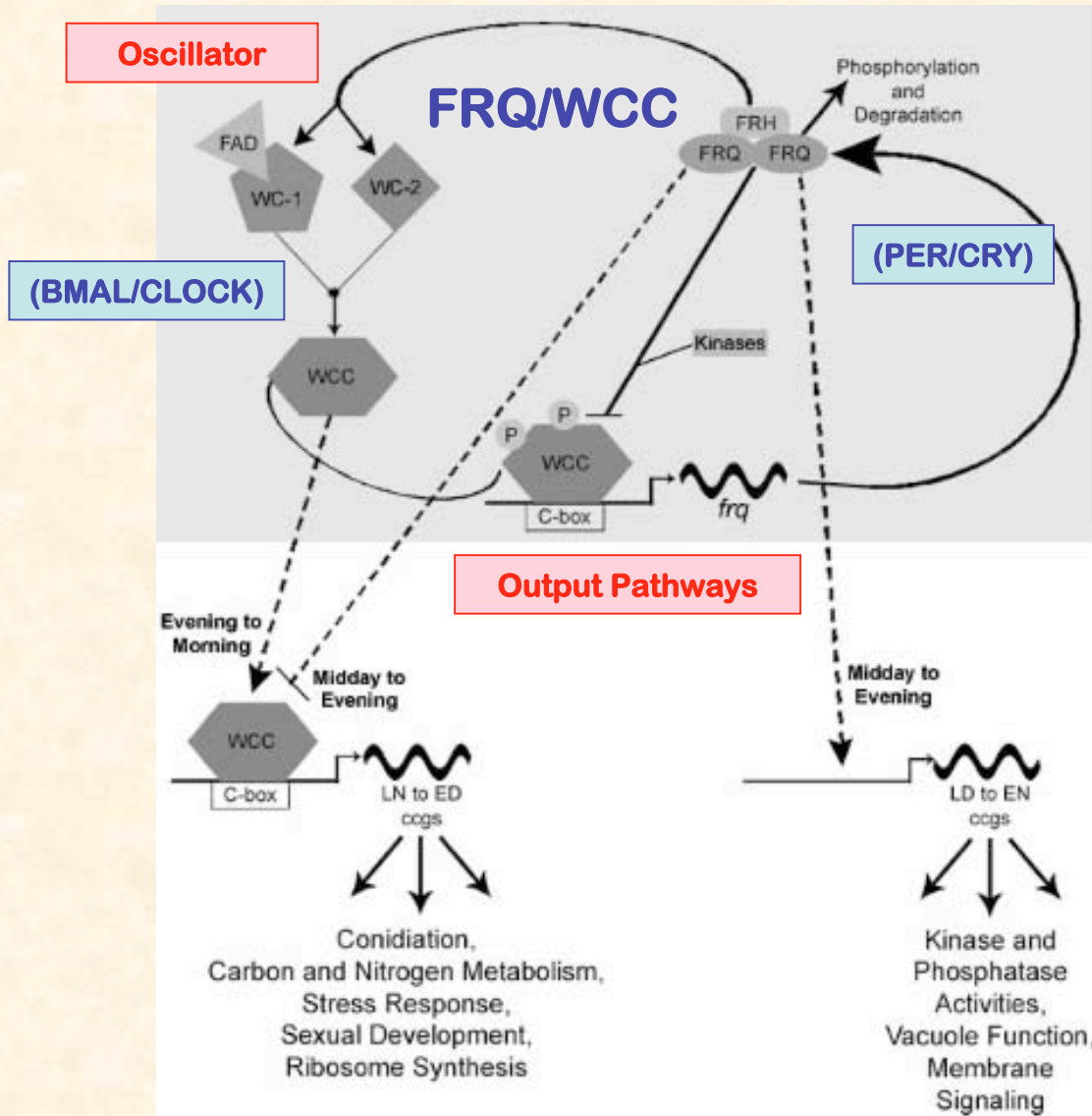


Model of Neurospora centromeres

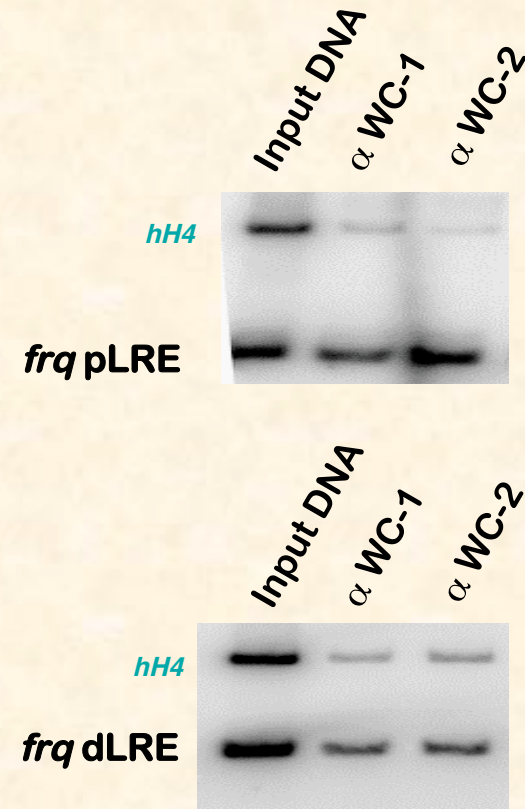


- | | |
|-------------------|-----------------|
| ■ centromatin | ● CenH3 / CenpC |
| ■ heterochromatin | ● H3 K9me3 |
| ■ euchromatin | ● H3 K4me2 |
| | ● unknown |

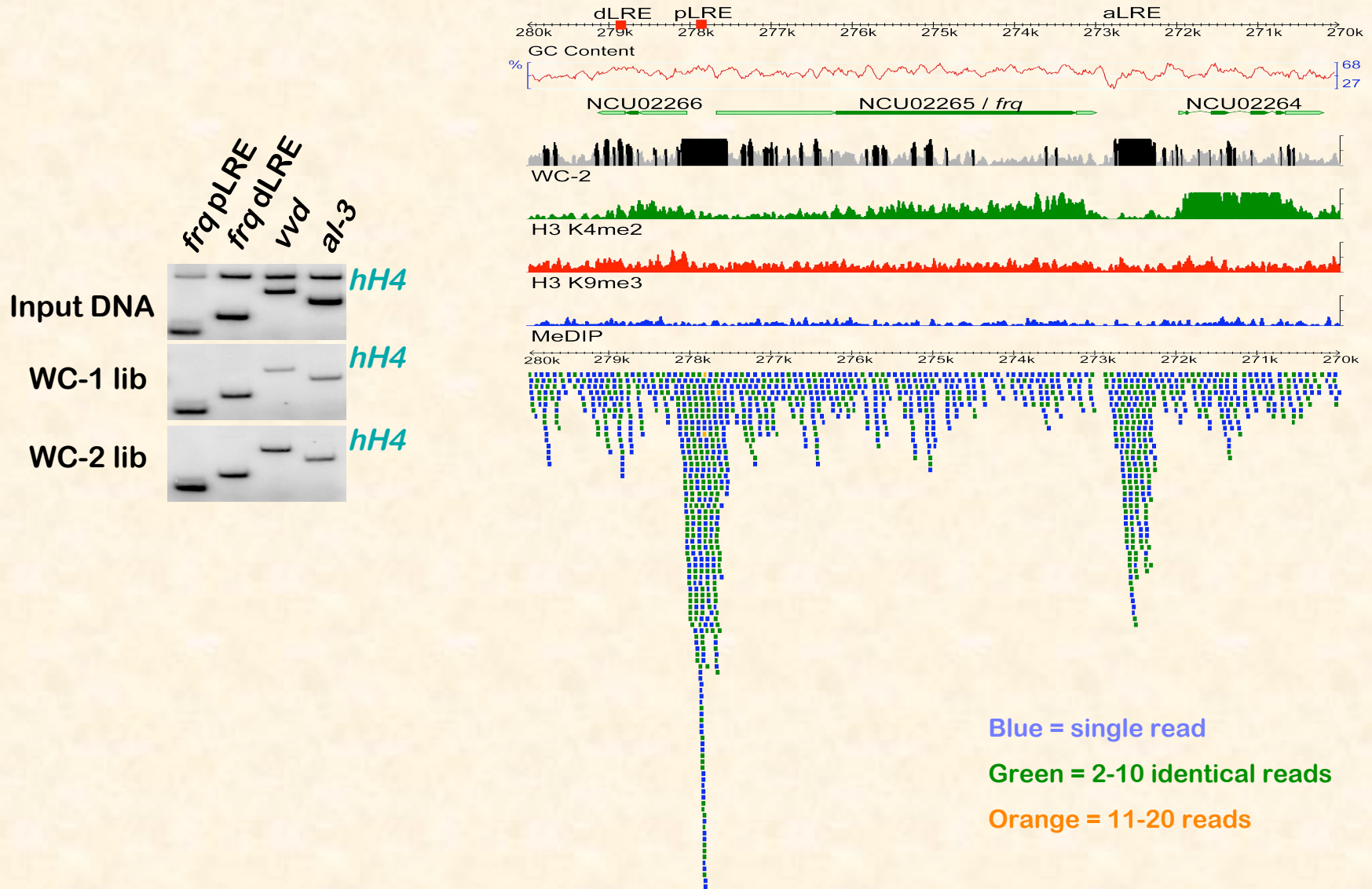
Light and circadian regulation



ChIP of White Collar Complex

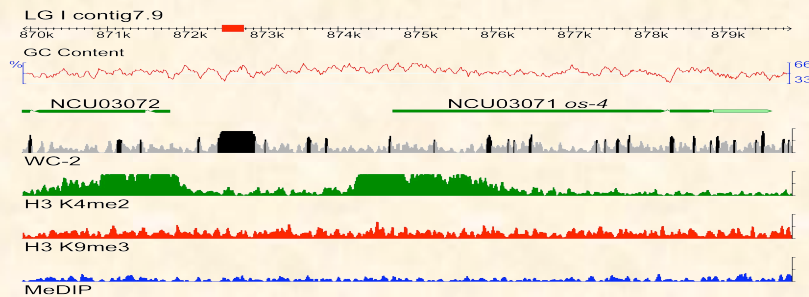


ChIP-seq of WC-1 and WC-2 (WCC)

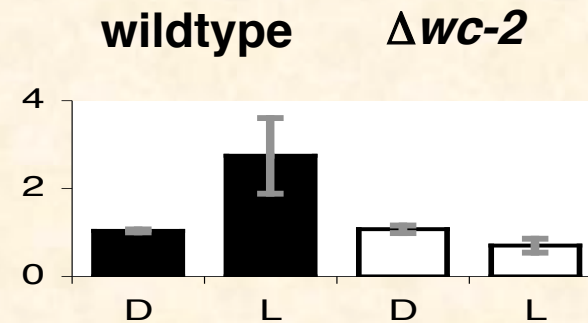


Examples of interesting targets: MAPKKK and Sirtuin

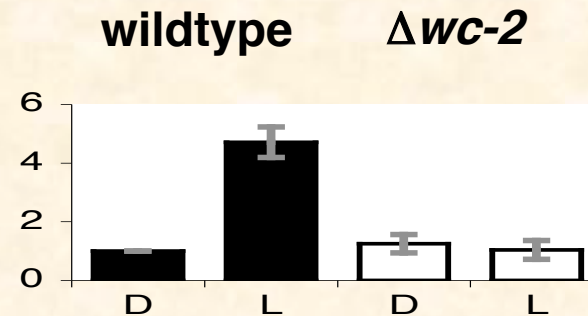
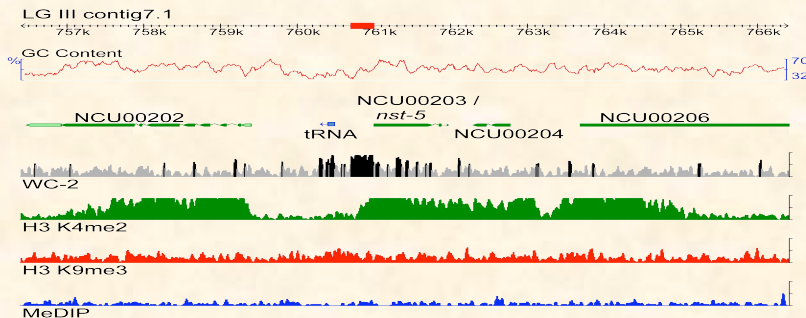
NCU03071 *os-4* MAPKKK



light-induction
dependent on WC-2

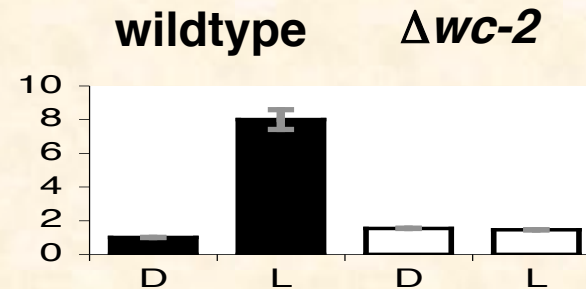
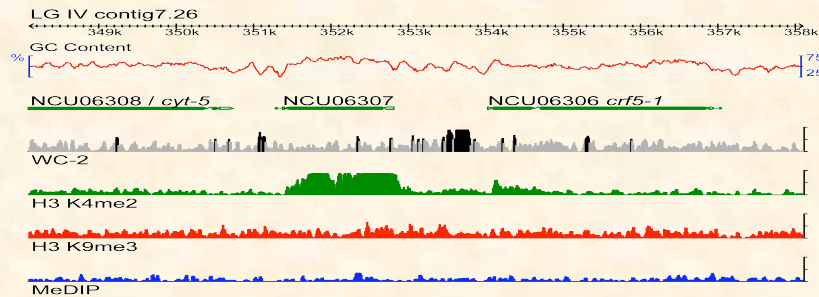


NCU00203 *nst-5* Sirtuin

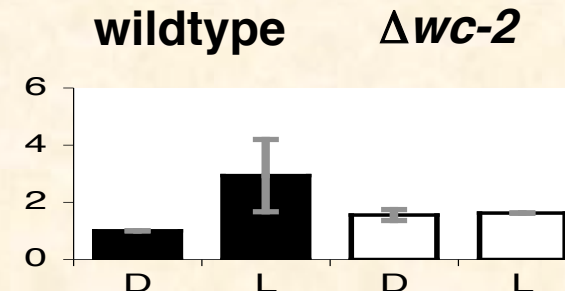
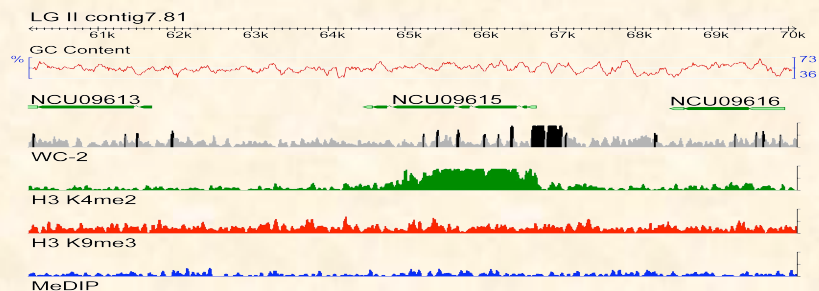


Chromatin remodelers and transcription factors

NCU06306 *crf5-1* AtDDM1



NCU09615 Transcription Factor



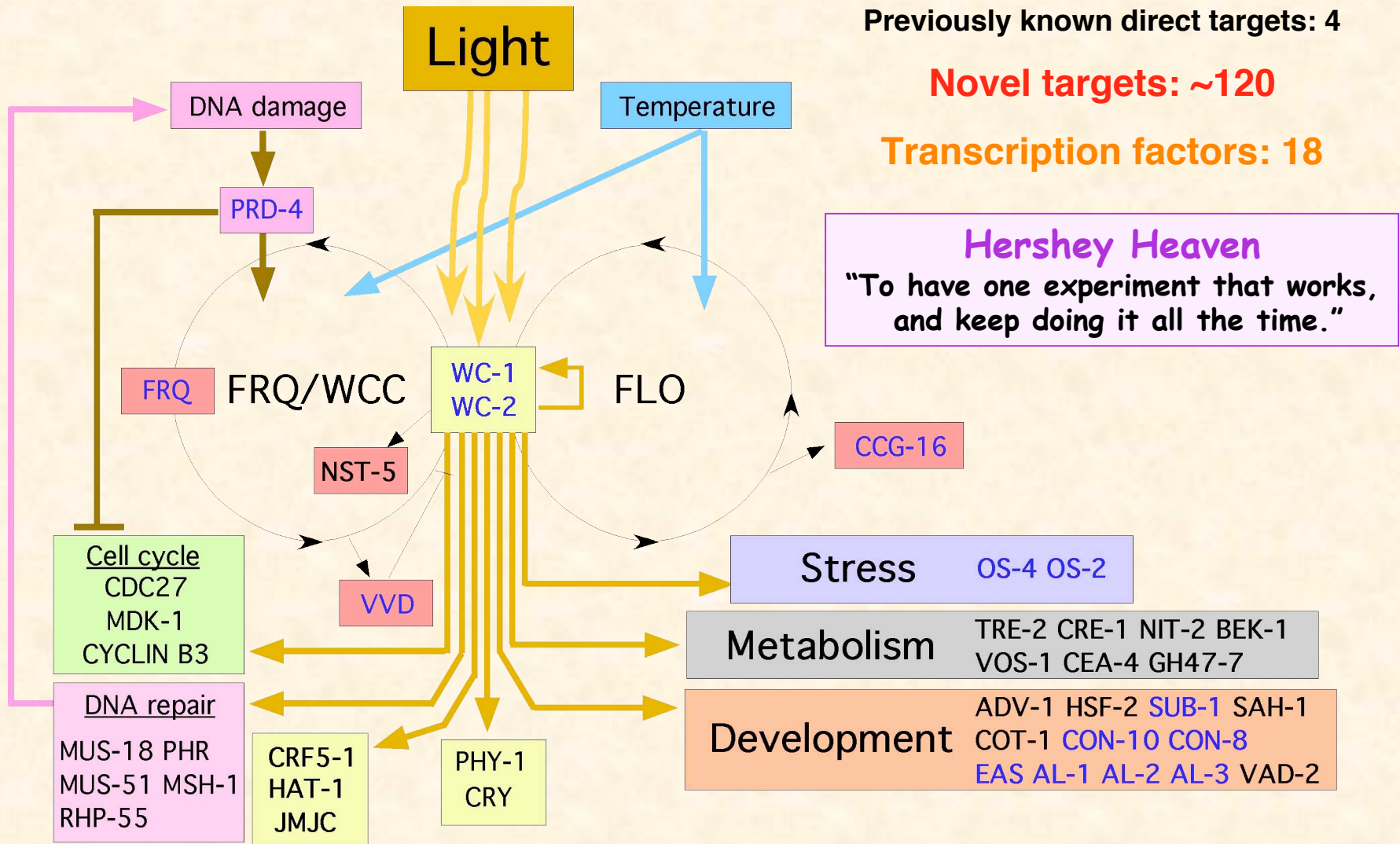
Updated model for light regulation

White Collar Complex

Previously known direct targets: 4

Novel targets: ~120

Transcription factors: 18



Collaborators

OSU & CGRB

Michael Gross
Chris Mathews Lab
Chris Sullivan
Jim Carrington Lab
Mark Dasenko
Steve Drake
Scott Givan

Poplar epigenomics

Steve Strauss Lab
Todd Mockler Lab

Small RNA in fungi

Jason Stajich
(UC Riverside)

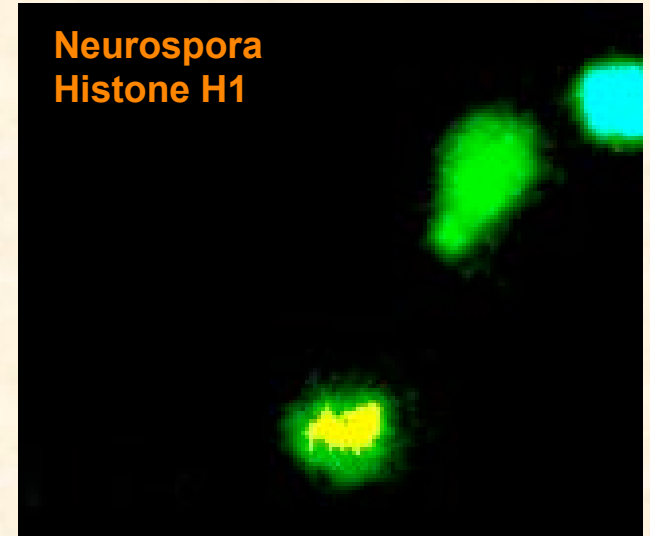
Light regulation

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Shinji Honda, Eric Selker (University of Oregon)

Neurospora
Histone H1



Gabriela Roca



American Cancer Society, OHSU MRF (centromeres)
NIH (light induction)
DOE/USDA (poplar epigenomics)
OSU - CGBI, BB, CoS (start-up funds)

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