Epigenetics in Yeast

Dom Helmlinger
CRBM, Montpellier
Outline

• Genetic and epigenetic regulation of gene expression.

• Mating-type switching in budding yeast.

• Positive and negative regulation of mating-type switching.

• Epigenetics and heterochromatin formation in fission yeast.

• Non-coding RNAs and gene regulation in fission yeast.
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Gene expression regulation

• The study of how genes are turned ‘ON’ or ‘OFF’ in response to a signal.

• The Operon model (Jacob & Monod, 1961):

  “the gene was something in the minds of people…which was as inaccessible, by definition, as the material of the galaxies. That experiments we were doing would involve an actual physical interaction between a compound in the cell and actually the gene itself, was something extremely difficult to come to.”
Gene regulation is fundamental

Genetic / Epigenetic control of gene expression is important for many fundamental processes:

• Embryonic development.

• Maintenance and differentiation of pluripotent stem cells.

Dysfunctional in:

• Cancer.

• Mental retardation.

• ...
Genetic regulation of gene expression

Mechanisms:

• Promoter / enhancer.
• Transcription factor binding sites.
Definition of Epigenetics

• Heritable differences in genome function that occur without a change in DNA sequence.

• “… all the weird and wonderful things that can’t be explained by genetics.”

Denise Barlow
Epigenetic regulation of expression

Mechanisms:

• DNA methylation.
• Histone modifications.
• Nucleosome positioning and remodelling.
• Non-coding RNAs.
• Prions.
Models to study epigenetic regulation of gene expression

- **Budding yeast (S. cerevisiae):** repression of telomeres, mating-type switching.
- **Fission yeast (S. pombe):** repression of centromeres & telomeres, RNAi, mating-type switching.
- **Fungus (Neurospora):** DNA methylation.
- **Ciliates (Tetrahymena):** Histone modifications + variants.
- **Flies (Drosophila):** Histone modifications, chromatin domains.
- **Plants + worms:** DNA methylation, RNAi.
Example of a phenotype revealing epigenetic regulation of gene expression: variegation

Fly eye

Budding yeast
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Mating-type switching in the budding yeast *S. cerevisiae*

= 

An example of both genetic and epigenetic regulation
Key concept

- Allele translocation (= gene conversion) between a transcriptionally silent and an active locus.
- States are determined by chromatin structure and epigenetic modifications.

The study of this phenomenon, particularly using classical genetics starting in the early ‘80s, has led to the discovery of several fundamental, conserved mechanisms and tools, and is continuing to do so.
S. cerevisiae life cycle

- Nutrients
  PROLIFERATION
  a haploid
  Mitosis
  budding
  a haploid
  Conjugation
  Meiosis
  a/α diploid
  spore
  - Nutrients
  DIFFERENTIATION
  α haploid
Requirements to switch mating-type

- One active copy of mating-type genes (‘playback’)
- One silent copy of 2 other mating-type genes (‘storage’).
- Regulated, targeted homologous recombination.
- Mix progeny after division to allow different mating types to ‘meet’.
- *Mechanisms to allow directional switching and to choose the correct ‘storage’ copy.*
Mating-type is determined by the presence of a specific allele present at the *MAT* locus, which is expressed.
**MAT** locus alleles

- Additional, but **silent**, copies of this locus (= cassettes), 5’ and 3’ of **MAT**.
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Controlling mating-type switching

• A yeast is able to switch mating-type, after each division (aka. homothallism),

• Through homologous recombination (= DNA damage repair mechanism).
Regulation of the switch

- Tight regulation: happens after each mitosis but only in one of the 2 cells (= the mother).

S = spore  M = mother cell  D = daughter cell
Mechanism of the switch

- Double-strand break at the \textit{MAT} locus, generated by an endonuclease (HO).
- Homologous recombination using \textit{HMLa} or \textit{HMRa} as donor DNA.
Therefore:

Regulation of mating type identity depends on the regulation of *HO* expression:

- Expressed in mother cell, not in daughter cell;
- Expressed in haploid cell, not in diploid cell;
- Expressed in late G1 phase of the cell cycle (before DNA is replicated).
Molecular mechanism of \textit{HO} induction: genetic regulation
How is expression regulated at the ‘playback’, *MAT* locus?

**Diagram:**

- **HMLα**
  - 200 kb
  - ‘Storage’ locus
  - OFF
  - Heterochromatin

- **MAT**
  - 150 kb
  - ‘Playback’ locus
  - ON
  - Euchromatin

- **HMRα**
  - ‘Storage’ locus
  - OFF
  - Heterochromatin
Two genes at the *MAT* locus

- Gene structure of the expressed, *MAT* locus:
Mating-type identity in haploids

MATα cells

a-specific genes
Mcm1

α-specific genes

MATα cells

Mα2-Mcm1 repressor

Mα1-Mcm1 activator
Mating-type identity in diploids

**a-specific genes**
- **MATA cells**: Mcm1
- **MATα cells**: Matα2-Mcm1 repressor
- **MATA/MATα cells**: Matα2-Mcm1 repressor

**α-specific genes**
- **MATA cells**: 
- **MATα cells**: Matα1-Mcm1 activator
- **MATA/MATα cells**: Mata1-Matα2 repressor

**haploid-specific genes**
- **MATA cells**: 
- **MATα cells**: 
- **MATA/MATα cells**: Mata1-Matα2 repressor
Examples of such downstream targets

- \textit{a/\alpha\textendash specific genes}: pheromones and their receptors (discovery of GPCR signaling).

- \textit{Haploid-specific}: HO endonuclease (= tool to study DNA repair after DBSs).
Impact of these studies

• Role of transcription factors in cell fate decisions.

• Transcription factors act by binding specific DNA sequences (promoters).

• Their functions can be modulated through heterodimerization (repressors – activators).

• Recently: role of non-coding RNAs in repression of meiosis-specific genes in haploid cells.

• GPCRs + kinases (MAPK) transmit the pheromone signal to induce mating (cell fusion).
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Repression of the storage ‘cassettes’: epigenetic regulation

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  - OFF
  - Heterochromatin

- **MAT**
  - ‘Playback’ locus
  - ON
  - Euchromatin

- **HMRα**
  - ‘Storage’ locus
  - OFF
  - Heterochromatin

200 kb

150 kb
Identification of other factors controlling silent cassettes = identification of an important, conserved epigenetic regulator

Genetic screen: identify mutations that allow mating even in the absence of a functional (expressed) \( MAT \) locus.

\[ \downarrow \]

\( SIR \) genes (Silent Information Regulator): Sir1-4
**SIR** mutant phenotypes

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Sir2

• A very important and well-studied epigenetic regulator.

• The first HDAC (Histone Deacetylase) identified with a role in silencing:
  De-acetylates histones H3 and H4 = repression of transcription.

• = human SIRT1 (sirtuin), involved in aging, metabolic diseases, inflammation, Alzheimer’s, …
Making a silent domain = making heterochromatin

• Precise positioning of nucleosomes within the silent locus (example of $HMRa$):

![Diagram showing precise positioning of nucleosomes within the silent locus](image-url)
Establishment of heterochromatin

- Sir1 is recruited to an ‘initiation site’ (\textit{HMR-E} and \textit{HMR-I}):
Spreading, maintenance, and inheritance of heterochromatin

- Sir1 recruits Sir2 (deacetylate H3/H4), then Sir3/Sir4 recognize deacetylated H3/H4, bringing new Sir2, …
Heterochromatin properties

$HML/HMR$

- Limited to 3 kb (2 flanking silencer regions).
- No transcription.
- No access to $HO$, but to homologous recombination factors.
- Same properties mark / same factors form heterochromatin at telomeres in $S.\ cerevisiae$. 
Conclusion: working model
But: is silent chromatin really inert and unchanging?
But: is silent chromatin really inert and unchanging?
Sectoring assays in WT cells: rare, but detectable loss of silencing
Uncovering novel regulators of silencing maintenance/inheritance
Role of these factors?

Required for proper maintenance / inheritance, re-establishing heterochromatin after disruptions:

- Cell cycle stage (replication).
- Dynamic recycling of nucleosomes / silencing factors.
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What is fission yeast?
Similarities budding vs fission yeast

- Unicellular yeast.
- Very easy to manipulate for genetics, biochemistry, and (epi)genomics.

→ Conservation across eukaryotes.
## Differences budding vs fission yeast

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Epigenetic regulation of chromatin states in *S. pombe*

- Discovery of a **Position Effect Variegation** phenotype in *S. pombe*, similar to *Drosophila*.
Epigenetic regulation of chromatin states in *S. pombe*

- Discovery of a **Position Effect Variegation** phenotype in *S. pombe*, similar to *Drosophila*.
- Transgene expression state is variable.
- Stably transmitted in subsequent generations.
- Happens if transgene inserted near/in heterochromatic regions.

➤ Exploited for understanding establishment, maintenance and repression mechanisms.

➤ Found conserved properties and mechanisms.
Heterochromatin / silenced regions in *S. pombe*
Functional roles of heterochromatin in *S. pombe*

- Chromosome segregation (mitosis, meiosis)
- Chromosome integrity
- Mating-type identity
- Genome integrity?
Defective heterochromatin leads to abnormal centromere structures.
Epigenetic regulators identified

Identified as mating-type region silencer mutants.

Mutants also defective for centromeric silencing (distinction from *S. cerevisiae*).

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Key findings

Heterochromatin silencing in *S. pombe* is different from *S. cerevisiae*, but similar to plants and metazoan:

- Histone modifications (deposition & recognition of H3-K9me3).
- RNA interference (RNAi) machinery.
- RNA Pol II transcription.
- *But without DNA methylation.*
Assembly of heterochromatic domains at centromeres

Heterochromatin

CENP-A chromatin

Heterochromatin

RDRC

CLRC

RITS

Stc1

Tas3

AGO1

Chp1

Cir4

Me

Me

Me

Me

Me

Me

Me

Me

Me

Me

Me

Me

Me

Pol II

Cir3

SHREC

Ac

Kinetochore

Outer repeat

Central domain

Outer repeat

dh
dg
dh
dg
cnt

dh
dg
dh
dg
Establishment vs maintenance

Two ‘establishment’ models ongoing:

- siRNAs recognize nascent transcripts, loading RITS.
- siRNAs originate from degradation or Dicer activity.
- Supported by genetics: Clr4 tethering bypass requirement for RNAi.
Establishment vs maintenance

Two ‘establishment’ models ongoing:

• siRNAs recognize nascent transcripts, loading RITS.
• siRNAs originate from degradation or Dicer activity.
• Supported by genetics: Clr4 tethering bypass requirement for RNAi.
• BUT: no siRNAs in clr4Δ!
Establishment vs maintenance

Second ‘establishment’ model:

- H3K9me required for siRNA generation.
- Feedback amplification.

➔ *Chicken vs egg?*
Establishment vs maintenance

Maintenance through cell divisions (epigenetic):

- Swi6 detached during mitosis.
Establishment vs maintenance

Maintenance through cell divisions (epigenetic):

- Swi6 detached during mitosis.
- Heterochromatin decondensation during S phase allows RNA Pol II transcription.
- siRNA production.
- RITS-RDRC-CLRC recruitment.
Cell-cycle regulation of centromere heterochromatin assembly
Summary

What we have learned from *S. pombe*:

- Heterochromatin is found *mainly* at centromeres, telomeres, ribosomal DNA, and mating-type regions.
- Heterochromatin assembly requires RNAi machinery, H3K9me3 mark, and transcription (!).
Summary

What we have learned from *S. pombe*:

• Heterochromatin is found *mainly* at centromeres, telomeres, ribosomal DNA, and mating-type regions.

• Heterochromatin assembly requires RNAi machinery, H3K9me3 mark, and transcription (!).

• Boundaries (role Epe1 and Cullin ligases).

• Epigenetic inheritance of centromere identity through deposition of CENP-A.
Epigenetic regulation of chromatin states in *S. pombe*
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• Genetic and epigenetic regulation of gene expression.

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• Positive and negative regulation of mating-type switching.

• Epigenetics and heterochromatin formation in fission yeast.

• Non-coding RNAs and gene regulation in fission yeast.
IncRNA recruits RNAi and the exosome to dynamically regulate *pho1* expression in response to phosphate levels in fission yeast
noncoding RNAs:

- Genomes are highly transcribed, including many noncoding RNAs.
- From short to very long ncRNAs (21nt. to ~300 bp, up to >10 kb).
- Typically rapidly processed (eg. exosome degradation).
- Noise or functional roles: still poorly understood.
Examples of regulatory roles:

Several examples, different mechanisms:

- Cis-acting, transcriptional interference (*SER3, IME4*).
- dsRNA-mediated silencing (sense/anti-sense).
- Recruitment and establishment of epigenetics marks (HOTAIR-PRC2 at HOX loci).
- Silencing: piRNAs in germ line cells in animals.
- Constitutive repression: RNAi-dependent heterochromatin formation in fission yeast.
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- Recruitment and establishment of epigenetics marks (HOTAIR-PRC2 at HOX loci).
- Silencing: piRNAs in germ line cells in animals.
- **Constitutive repression**: RNAi-dependent heterochromatin formation in fission yeast.
At facultative heterochromatin?

This is the question that this paper asks.

Key findings:

- Repressing by heterochromatin formation.
- Repression is RNAi-dependent.
- Recognized by RNA binding protein (Mmi1).
- Degraded (and terminated?) by exosome.
Figure 1: Two overlapping transcripts detected at *pho1.*

Caution: examples of bad Photoshop cropping!

Upstream ncRNA confirmed by 5' + 3' RACE.
Figure 2: upstream ncRNA represses downstream mRNA.
Figure 1 + 5: transcription of pho1 locus.

ChIP + Run-on (TRO) experiments:
Figure 4: Deposition of H3K9me2 marks across *pho1*,

ChIP

- **pho1** (1362bp)
  - pA site

**PCR products**
1  2  3  4  5  6

**E**

**H3K9me2**

- WT
- *rrp6Δ*

10mM KH$_2$PO$_4$, 12h

0mM KH$_2$PO$_4$, 12h

**α-H3K9me2/α-H3 (IP/input)**

1  2  3  4  adh1

**PCR products**
Figure 4: Deposition of H3K9me2 marks across *pho1*, upon ncRNA expression.

Depends also on RNAi (Ago1, Dcr1) and on Red1 (degradation machinery for meiotic transcripts).
Figure 3: kinetics of \textit{pho1} expression.

3 important observations:

1. Rrp6 promotes induction:

2. Clr4 (H3K9me) inhibits induction:

3. Rrp6 and Clr4 act independently (problem!)
Another problem:

- Two overlapping transcripts: one ncRNA regulating one mRNA.
- Share the same 3’ end, differ 5’.
- Degradation (and termination) only of the ncRNA depends on exosome.

How does the exosome know which is the ncRNA?
Figure 6: 5’ sequence motif suggest role of Mmi1. (Found in Yamamoto lab: Harigaya Y, *Nature*, 2006)
Working model

**pho1 ON:**

**pho1 OFF:**

Transcriptional silencing

Transcription termination

RITS

Mmi1

Rrp6

DSR

Clr4

HP1

Pol II

pA site

Pho1 protein

(A)n

5'
Conclusions

- Describes new role for a ncRNA.
- Mechanism of transcriptional repression at constitutive heterochromatin may be more general.
- Confirmed by several other studies at meiotic genes.

- Poor choice of inducible gene: strongly Rrp6-dependent, but not so much phosphate-regulated.
- Some contradictory results ignored.
- Writing…
Thank you!

Now – which one is the ‘higher’ eukaryote?!
If they ask you anything you don’t know, just say it’s due to epigenetics.
Learn more

- Ask me:
  dom@helmlinger.com

- Reference articles:
  http://elifesciences.org/content/early/2015/01/12/eLife.05007
Figure 1 + 5: transcription of *pho1* locus.

- Discrepancy ChIP / TRO.
- Termination defect?

Unclear…
Transcription run-on assay

(a) Isolate Nuclei
(b) DOT Blot Assay

membranes with gene of interest DNA.

Hybridize to run-on transcripts

Incubate with nucleotides including $^{32}$P-GTP

Isolate $^{32}$P-RNA (Run-on RNA)