Chromosomal Inheritance of Epigenetic States in Fission Yeast During Mitosis and Meiosis

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Epigenetics

• **Hereditary changes** that affect gene expression without changing the DNA sequence

• **Epigenetic silencing** regulates important biological processes
  
  – **Imprinting**: alleles are expressed in a parent-of-origin-specific manner
  – **X-chromosome inactivation**: random inactivation of one female X chromosome
  – **Silencing of repetitive sequences** (centromeres, telomeres,...)
Epigenetic control of gene expression

Different epigenetic marks affect gene expression

DNA methylation
Histone modifications
Histone variants
Small RNAs

Position effect variegation (PEV) in Drosophila

- PEV first described by **Muller in 1930**
- Gene is placed **near centromere or telomere** (heterochromatin)
- The imprinted ON and OFF states are **inherited for several generations**

How the heterochromatin spreads and causes silencing?
How this information is inherited during cell division?

https://smallscienceworks.com/tag/position-effect-variegation/
Position effect variegation in Drosophila

- **Suppressor of PEV (SU(VAR)):** HP1, SU(VAR)3-9

- Several SU(VAR) regulate expression by affecting heterochromatin structure or histone modifications
The regulation of heterochromatin spreading

- Multiple proteins recruited
- Addition of silencing marks
- Removal of activating marks

Heterochromatin .......... Euchromatin

Allshore and Madhani, 2017
Fission yeast is an important model for epigenetic regulation

- **Position Effect Variegation** similar to Drosophila when reporters are integrated near centrome telomeres and the mating-type regions

- These regions show **heterochromatin properties**: Silenced, No recombination, spread of silencing to reporter

- **Research in Fission yeast** has led to **fundamental discoveries** about heterochromatin formation and inheritance and discovery of RNAi-dependent heterochromatin formation.
  
  - Shares many chromatin modifications with higher organisms but,
  - Unicellular organism, easy to grow, unlimited material
  - Only 3 chromosomes
  - Simpler centromeric regions
  - In many cases only one copy of regulatory genes

Allshire, and Ekwall 2015  Cold Spring Harb
Life cycle of *S. pombe*

Wild-type homothallic *h*\(^90\) cells, switch mating type to opposing type 75-90% of cell divisions

N starvation triggers the sexual phase
Mating type locus in *S. pombe*

- Transcriptionally active *mat1* and transcriptionally silent *mat2-P* and *mat3-M*

![Diagram of mating type locus](image)

- *mat2-mat3* region has heterochromatic features: Silent, no recombination, silent reporters

- Recombination between *mat1-P* and *mat3-M* or *mat1-M* and *mat2-P*

- Position of *mat2* and *mat3*, rather than genetic information conditions the recognition of the donor in each switch: specific chromatin organization.

Thor and Klar, 1993
Regulators of silencing over the mating-type region

- Genetic screens identified loci that cause \textit{mat2-P/mat3-M} derepression and allow recombination in the \textit{mat2-P/mat3-M} region (suggests loss of heterochromatin).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Function</th>
<th>Motifs/similarity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{clr1}</td>
<td>Putative DNA-binding protein</td>
<td>3 zinc fingers</td>
<td>Than and Klar, 1992; G. Than and A. Klar (personal communication)</td>
</tr>
<tr>
<td>\textit{clr2}</td>
<td>Unknown</td>
<td>No similarities in database</td>
<td>Ekw'all and Ruusala, 1994; Than et al., 1994</td>
</tr>
<tr>
<td>\textit{clr3}</td>
<td>Putative histone deacetylase</td>
<td>Human HDAC4 and HDAC5, \textit{S. cerevisiae} Hda1</td>
<td>Ekw'all and Ruusala, 1994; Than et al., 1994; Grewal et al., 1998</td>
</tr>
<tr>
<td>\textit{clr4}</td>
<td>Chromatin modifier</td>
<td>SET and Chromo domains/\textit{Drosophila} Suvar3-9 and Polycomb, human SUV39H1</td>
<td>Ekw'all and Ruusala, 1994; Than et al., 1994; Ivanova et al., 1998</td>
</tr>
<tr>
<td>\textit{clr6}</td>
<td>Putative histone deacetylase</td>
<td>Human HDAC1 and HDAC2, \textit{S. cerevisiae} Rpd3 and Chromo and Shadow domains/Polycomb and heterochromatin protein HP1 from \textit{Drosophila} mouse and humans</td>
<td>Grewal et al., 1998</td>
</tr>
<tr>
<td>\textit{swi6}</td>
<td>Chromatin modifier</td>
<td>Xeroderma pigmentosum group E(XP-E) DNA repair protein</td>
<td>Lorentz et al., 1994</td>
</tr>
<tr>
<td>\textit{rik1}</td>
<td>Putative DNA binding protein</td>
<td>Xeroderma pigmentosum group E(XP-E) DNA repair protein</td>
<td>Egel et al., 1989; O. Nielsen (GenBank Accession #AP136156)</td>
</tr>
</tbody>
</table>

- These genes are conserved and were identified as factors involved in heterochromatin formation during Position Effect Variegation in Drosophila.

\textit{clr4} = \textit{SU(VAR)3-9}  
\textit{swi6} = \textit{HP1}

- These loci also affect silencing of reporter genes near telomeres or centromeres
Chromosomal Inheritance of Epigenetic States in Fission Yeast During Mitosis and Meiosis

- Reporters inserted adjacent to mat2-P and mat3-M become silent: heterochromatin spread

Figure 1

KΔ::ura4

- Donor loci remain silent: no RNA expression of mat2-P/mat3-M by Northern blot.
Variegation of ura4 expression in \( K\Delta::ura4 \)

- Single colony of \( h90; K\Delta::ura4 \) with Ura+ phenotype (grow in URA-) grown in rich medium and plated on URA- and FOA medium

Although all cells are \textit{genetically identical}...

...they observed \textit{variegation} in the expression of the Ura reporter

Silencing of the \textit{ura4} reporter
The Ura phenotype correlates with the mating-type switching

Efficiency of mating-type interconversion:

- Pedigree analyses of haploid cells
- Iodine staining of individual colonies

Switched type to ~30% of cell division
(80% in WT cells)
Colony stained lightly: LIGHT form

Switched type to ~65% of cell division
Colony stained darkly: DARK form

Ura4+ expression reduces the efficiency of mating type switch

Diagram:
(URA- plates)
(Ura+)
Individual haploid cells
mitosis
how many generations?

(FOA plates)
(Ura-)

\[ \text{Ura}^+ \] \[ \text{Ura}^- \]
Light and dark states correlate with *ura4* expression

- To test if the URA+ and URA- phenotypes were the results of differences in *ura4* expression

---

**Figure 1**

<table>
<thead>
<tr>
<th>Northern analysis</th>
<th>ura4Δ</th>
<th>ura4</th>
<th>Light</th>
<th>Light from dark</th>
<th>Dark</th>
<th>Dark from light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YEA</td>
<td>YEA</td>
<td>YEA</td>
<td>YEA</td>
<td>YEA</td>
<td>FOA</td>
</tr>
</tbody>
</table>

*cdc2*  
*ura4*  
Internal loading  
Medium
An epigenetic-based change is responsible for the light and dark phenotypes.

- Is the variegation of light and dark states controlled by:
  - **mutational alterations**: extremely stable and low interconversion rate
  - **an epigenetic mechanism**: higher rates of interconversion

**Mitotic stability assay:**
25 generation, non selective medium
Plate on URA- and FOA

- URA- \[\rightarrow\] FOA
  - 98-99% Ura+
  - 1-2% Ura-

- FOA \[\rightarrow\] URA-
  - 98-99% Ura-
  - 1-2% Ura+

- URA- \[\rightarrow\] FOA
  - 98-99% Ura+ 1-2% Ura-

- FOA \[\rightarrow\] URA-
  - 98-99% Ura- 1-2% Ura+

Light and dark states are **mitotically metastable**
Frequency much **higher than expected** for spontaneous mutations
Mutations in Trans-Acting regulators suppress Variegation

- Since \textit{clr}- and \textit{swi6}- have been implicated in silencing of mating-type loci, they tested their effect on variegation of the \textit{KΔ::ura4} reporter

- Introgressed the different mutations into Ura+ cells

**Figure 2**

<table>
<thead>
<tr>
<th>Background</th>
<th>N/S</th>
<th>URA(^-)</th>
<th>FOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td><img src="image" alt="WT N/S" /></td>
<td><img src="image" alt="WT URA(^-)" /></td>
<td><img src="image" alt="WT FOA" /></td>
</tr>
<tr>
<td>\textit{clr1}(^-)</td>
<td><img src="image" alt="clr1 N/S" /></td>
<td><img src="image" alt="clr1 URA(^-)" /></td>
<td><img src="image" alt="clr1 FOA" /></td>
</tr>
<tr>
<td>\textit{clr2}(^-)</td>
<td><img src="image" alt="clr2 N/S" /></td>
<td><img src="image" alt="clr2 URA(^-)" /></td>
<td><img src="image" alt="clr2 FOA" /></td>
</tr>
<tr>
<td>\textit{clr3}(^-)</td>
<td><img src="image" alt="clr3 N/S" /></td>
<td><img src="image" alt="clr3 URA(^-)" /></td>
<td><img src="image" alt="clr3 FOA" /></td>
</tr>
<tr>
<td>\textit{clr4}(^-)</td>
<td><img src="image" alt="clr4 N/S" /></td>
<td><img src="image" alt="clr4 URA(^-)" /></td>
<td><img src="image" alt="clr4 FOA" /></td>
</tr>
<tr>
<td>\textit{swi6}(^-)</td>
<td><img src="image" alt="swi6 N/S" /></td>
<td><img src="image" alt="swi6 URA(^-)" /></td>
<td><img src="image" alt="swi6 FOA" /></td>
</tr>
<tr>
<td>\textit{ura4Δ} (Control)</td>
<td><img src="image" alt="ura4Δ N/S" /></td>
<td><img src="image" alt="ura4Δ URA(^-)" /></td>
<td><img src="image" alt="ura4Δ FOA" /></td>
</tr>
<tr>
<td>\textit{ura4}(^+) (Control)</td>
<td><img src="image" alt="ura4(^+) N/S" /></td>
<td><img src="image" alt="ura4(^+) URA(^-)" /></td>
<td><img src="image" alt="ura4(^+) FOA" /></td>
</tr>
</tbody>
</table>

Growth on FOA in \textit{clr2}- and \textit{swi6}- is due to rearrangements where the K-region is lost.

how would it be if the mutations were introgressed in Ura- Cells?
Are the Dark and Light states chromosomally inherited and meiotically stable?

If states are meiotically stable and chromosomally inherited, they should co-segregate with the HIS marker.
Light and Dark states are linked to the K-Region and inherited as a Mendelian genetic marker.

Figure 3

<table>
<thead>
<tr>
<th>SPG27 (L) ($h^{90}$, $K\Delta::ura4$, his2, ade6-210)</th>
<th>SPG51 (D) ($h^{90}$, $K\Delta::ura4$, ade6-216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td>Light</td>
<td>Dark</td>
</tr>
</tbody>
</table>

- Reverse the phenotypes of SPG27(L) and SPG51(D) to SPG27(D) and SPG51(L) and cross...
- Analyzed 18 tetrads, all of which showed a 2:2 segregation, supporting their conclusions.
Main findings

• An epigenetic mechanism regulates the efficiency of mating-type interconversion and silencing of a marker gene in the K-region.

• Generation or propagation of these epigenetic states is under the control of *clr1, clr2, clr3, clr4*, and *swi6*

• Epigenetic states remain stable and are chromosomally inherited during mitosis and meiosis as Mendelian genetic markers
Model of chromosomal inheritance of the Light and Dark Epigenetic States

- States highly stable: able to self-perpetuate probably during DNA synthesis
- Some of the genes that affect k-region silencing (clr, swi6,…) can directly bind to chromatin
- Nucleoprotein complexes might remain bound to chromatin during S-phase to self-replicate
Further studies

• What is the mechanism of silencing over the reporter?
  – spread of silencing from the \textit{mat2-P} and \textit{mat3-P} loci

• What is the mechanism of epigenetic inheritance?
  – Why the states change from light/dark or vice versa? Error in assembly? Regulated?
Further studies

• What is the mechanism of silencing/heterochromatin formation over the reporter?
  – spread of silencing from the $mat2$-$P$ and $mat3$-$P$ loci

Model of RNAi-mediated heterochromatin assembly

- siRNA-mediated silencing
- Histone binding proteins
- Histone modifiers

Further studies

- What is the mechanism of epigenetic inheritance?
- Why the states change from light/dark or vice versa? Error in assembly? Regulated?

Allshore and Madhani, 2017
Conservation of factors implicated in heterochromatin formation

<table>
<thead>
<tr>
<th>Component</th>
<th>S. pombe</th>
<th>Neurospora</th>
<th>Drosophila</th>
<th>Mouse</th>
<th>Arabidopsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitious DNA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DNA methylation</td>
<td>No</td>
<td>Yes</td>
<td>No*</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>H3K9 methylation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>HP1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Small RNAs</td>
<td>Yes</td>
<td>No*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Pol II</td>
<td>Yes</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RDR</td>
<td>Yes</td>
<td>No*</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Yes indicates that the factor has been implicated to have a role in heterochromatin formation in the given organism. No indicates that the factor is not present in the organism. No* indicates that the organism has the factor but that it seems not to have a role in heterochromatin formation. ND means that the organism has the factor but whether it has a role in heterochromatin formation is unknown. Arabidopsis, Arabidopsis thaliana; Neurospora, Neurospora crassa; RDR, RNA-dependent RNA polymerase. (Table adapted from ref. 19.)
## Conservation of RNAi pathway

<table>
<thead>
<tr>
<th>Schizosaccharomyces pombe</th>
<th>Arabidopsis thaliana</th>
<th>Caenorhabditis elegans</th>
<th>Drosophila</th>
<th>Homo sapiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dcr1</td>
<td>DCL1 to 4</td>
<td>Dcr-1</td>
<td>Dcr1 and 2</td>
<td>Dcr-1</td>
</tr>
<tr>
<td>Agol</td>
<td>AG01 to 10</td>
<td>Rde-1, Alg-1, and -2</td>
<td>Agol to 3, Piwi</td>
<td>Agol to Ago4</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Prg-1 and 2, and 19 others</td>
<td>Aubergine/Sting</td>
<td>Piwi1 to Piwi4</td>
</tr>
<tr>
<td>Chp1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CMT3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tas3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>AIN-1</td>
<td>GW182</td>
<td>TNRC6</td>
</tr>
<tr>
<td>Rdpl</td>
<td>RDR1 to 6</td>
<td>Ego-1, Rrf-1 to -3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hrr1</td>
<td>SGS2/SDE3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ZK1067.2</td>
<td>GH20028p</td>
<td>KIAA1404</td>
</tr>
<tr>
<td>Cid12</td>
<td>—</td>
<td>Rde-3, Trf-4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CG11265&lt;sup&gt;c&lt;/sup&gt;</td>
<td>FOLC&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Swi6</td>
<td>LHP1 (TFL2)</td>
<td>Hpl-1, Hpl-2, F32E10.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>HP1a, b</td>
<td>HP1α, β, γ</td>
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<td>Clr4</td>
<td>SUVH2 to 6</td>
<td>Su(var)3-9</td>
<td>Su(var)39h1 and 2</td>
<td></td>
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<tr>
<td>Rik1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>DDB1</td>
<td>M18.5</td>
<td>Ddb1</td>
<td>Ddb1</td>
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<td>Cu14</td>
<td>CUL4</td>
<td>Cu14</td>
<td>Cu14</td>
<td>Cu14</td>
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<tr>
<td>Sir2</td>
<td>SIR2</td>
<td>Sir2-1</td>
<td>Sir2</td>
<td>SirT1</td>
</tr>
<tr>
<td>Clr3</td>
<td>HDA6</td>
<td>Hda-1</td>
<td>Rpd3</td>
<td>HDAC1</td>
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<tr>
<td>Clr6</td>
<td>—</td>
<td>DDM1</td>
<td>CG6393</td>
<td>THEX1</td>
</tr>
</tbody>
</table>

Martienssen R and Moazed, D, 2015
How this information is inherited during cell division?

Epigenetic inheritance of CG methylation

Semiconservative inheritance of CG methylation

Bostic, M et al, Science 2007
How the heterochromatin spreads and causes silencing?

The regulation of heterochromatin spreading

a Writer
Reader

b Nucleosome depletion

c Nucleosome turnover

d Opposing PTMs

Nature Reviews | Molecular Cell Biology
Allshore and Madhani, 2017
<table>
<thead>
<tr>
<th>Strain Number</th>
<th>mat Region</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP1000</td>
<td>$h^{90}$</td>
<td>$\text{swi6-115, leu1-32, ura4, his2, ade6-210}$</td>
</tr>
<tr>
<td>SPG16</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{swi6-115, leu1-32, ura4, his2, ade6-210}$</td>
</tr>
<tr>
<td>SPG27</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{leu1-32, ura4, his2, ade6-210}$</td>
</tr>
<tr>
<td>SPG51</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{leu1-32, ura4, ade6-216}$</td>
</tr>
<tr>
<td>SPG60</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{clr1-5, leu1-32, ura4, his2, ade6-216}$</td>
</tr>
<tr>
<td>SPG62</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{clr3-735, leu1-32, ura4, his2, ade6-210}$</td>
</tr>
<tr>
<td>SPG64</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{clr4-681, leu1-32, ura4, his2, ade6-210}$</td>
</tr>
<tr>
<td>SPG66</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{clr2-760, leu1-32, ura4, his2, ade6-216}$</td>
</tr>
<tr>
<td>SPG74</td>
<td>$h^{90}$, $K\Delta:ura4$</td>
<td>$\text{leu1-32, ura4-D18, his2, ade6-210}$</td>
</tr>
</tbody>
</table>
Swi6 co-localizes with centromeres, telomeres and mating-type regions

Ekwall et al, 1995, Science
What constitutes a good presentation?

• **Introduction** (10 min):

  • Explain the *relevant background/techniques* needed to understand the paper.
  
  • Use material from other papers and reviews on the reading list or other papers.

• **Paper** (20-30 min):

  • Don’t have to explain every figure of the paper in great detail.
  
  • Convey the *main message* of the paper concisely, while *critically analyzing* the validity of the data presented.
  
  • Try to understand the *materials and methods* but you don’t need to introduce every detail of the techniques used.
  
  • Try to end with a *statement of the main findings* of the paper, and what would be the *next logical questions* to ask in this field.