

Genome-Wide Mapping of the Cohesin Complex in the Yeast *Saccharomyces cerevisiae*

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<http://research.stowers-institute.org/jeg/2004/cohesin/>

Stowers Core Facility Journal Club

Earl F. Glynn

9 May 2005

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Overview

1. Background:
Cohesion / Cohesin Complex / Cohesin Sites
2. Previous Studies
3. Focus of this Study
4. Approach
5. PeakFinder
6. Results
7. Take Home Message

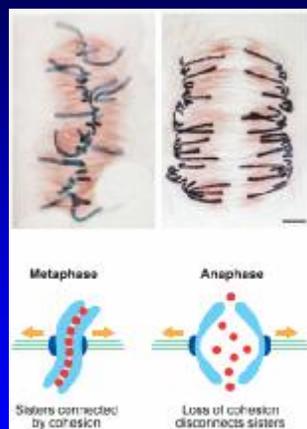
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Background

- Continuity of life depends on cell division with correct duplication and segregation of genetic materials
- Improper segregation can result in an abnormal number of chromosomes in daughter cells (aneuploidy)
 - Mitosis: condition associated with cancer
 - Meiosis: congenital disorders, e.g., Down's syndrome

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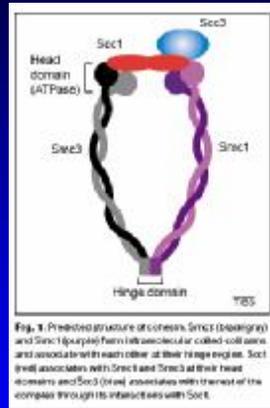
Cohesion Between Sister Chromatids



Source: Nasmyth, et al. Science, 288, May 2000

Cohesion mediated by protein complex called cohesin. 4

Cohesin Complex: Smc1, Smc3, Scc1, Scc3

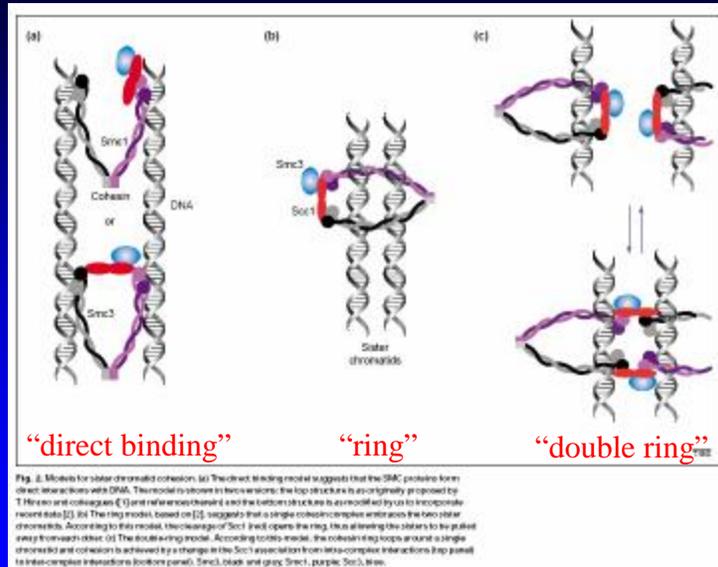


Source: Joseph L. Campbell and Orna Cohen-Fix. "Chromosome cohesion: ring around the sisters?", *TRENDS in Biochemical Sciences*, Vol 27, No. 10, Oct 2002, p. 493

Smc = structural maintenance of chromosomes
Scc = sister chromatid cohesion

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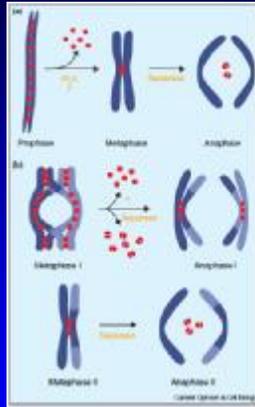
Models of Sister Chromatid Cohesion



Source: Joseph L. Campbell and Orna Cohen-Fix. "Chromosome cohesion: ring around the sisters?", *TRENDS in Biochemical Sciences*, Vol 27, No. 10, Oct 2002, p. 494

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Separating Sister Chromatids



Separating sister chromatids. (a) During mitosis, cohesin is removed from chromosomes in two steps. During condensation in prophase, cohesin is removed along the chromosome arms by a pathway that probably involves the phosphorylation of cohesin subunits. At the metaphase to anaphase transition the remaining cohesin, mostly concentrated at the centromeres, is cleaved by separase. (b) During meiosis, cohesin along chromosome arms is dissolved in the first reductional division to allow homologue segregation. Separase cleavage of cohesin is required for this step in budding yeast, but may not be in *Xenopus*. For separation of sister chromatids in the second equational division, cohesin left at the centromeres must probably be cleaved by separase.

Source: Frank Uhlmann, Chromosome cohesion and segregation in mitosis and meiosis, *Current Opinion in Cell Biology*, 2001, 13:754-761

Different cohesin mechanisms exist for mitosis and meiosis. 7

Cohesin Behavior During Cell Cycle

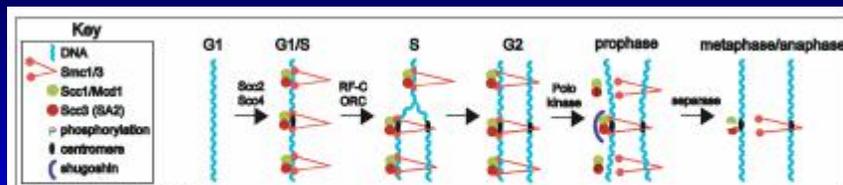


Figure 2. Behavior of Cohesin during the Cell Cycle. One cohesin complex is depicted at each site for the sake of simplicity; at the centromere especially there are likely to be many complexes. Cohesion is represented as occurring via the "entwining" model; other models have been proposed.

Chromosome Cohesion: A Cycle of Holding Together and Falling Apart

McEwen | Molecular Biology

Cohesion established in S phase; dissolved completely in metaphase. 8

Cohesin Sites for Sister Chromatids



DOI: 10.1371/journal.pbio.0030094.g001

Figure 1. Cohesin Sites for Sister Chromatids of Chromosome I in *S. cerevisiae*

Cohesin sites (red ovals) are concentrated at the centromere/pericentric region (where the two chromatids are "pinched"), but also occur along the arms of the chromatids.

Source: Jennifer Gerton, Chromosome Cohesion: A Cycle of Holding Together and Falling Apart, *PLoS Biology*, March 2005, p. 371

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Features of Cohesin Sites

1. Large, dense areas of cohesin at centromeres
2. Not associated with origins of replication
3. Can be located at telomeres
4. No consensus sequence in arms
5. Bias toward regions of high AT content
6. Bias toward intergenic regions with convergent transcription
7. Average distance between sites is 18 kb

Source: Jen Gerton's Science Club Talk at Stowers

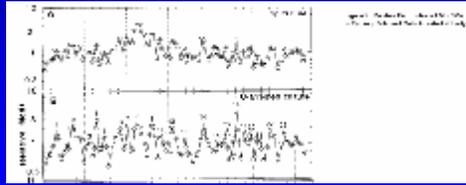
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Previous Studies

Cell, Vol. 98, 249-259, July 23, 1999, Copyright © 1999 by Cell Press

Cohesins Bind to Preferential Sites along Yeast Chromosome III, with Differential Regulation along Arms versus the Centric Region

Yuval Blat* and Nancy Kleckner
Department of Molecular and Cellular Biology
Harvard University
Cambridge, Massachusetts 02138



“Each peak may represent a single unique consensus binding determinant or a cluster ...”

Low resolution (3 kb) study of entire length of chromosome III.

Chromosome III is sex chromosome in budding yeast; has unusual properties.

Did not address questions about position of cohesin to smaller-scale features.

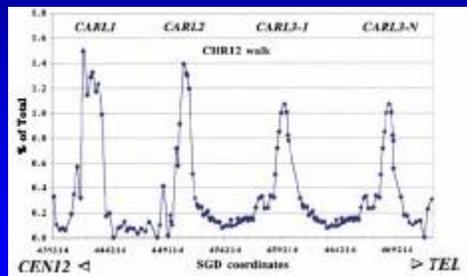
Previous Studies

The Journal of Cell Biology, Volume 151, 2000

Chromosomal Addresses of the Cohesin Component Mcd1p

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¹Howard Hughes Medical Institute, ²Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210, and ³Basic Science Division, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111



CAR = cohesin-associated region; CEN = centromere; TEL = telomere

CHR = chromosome; SGD = Saccharomyces Genome Database

High resolution (300 bp) over limited regions on certain chromosomes. ¹²

Focus of this Study

- Attempt to resolve some discrepancies between low resolution and high resolution studies, e.g., whether cohesin is found at telomeres.
- Look at cohesin binding across the whole genome with medium resolution (1-2 kb), since many aspects of cohesin-DNA interaction remain obscure.
- Looking for potential correlations between cohesin binding and genome features, such as base composition and transcriptional state.

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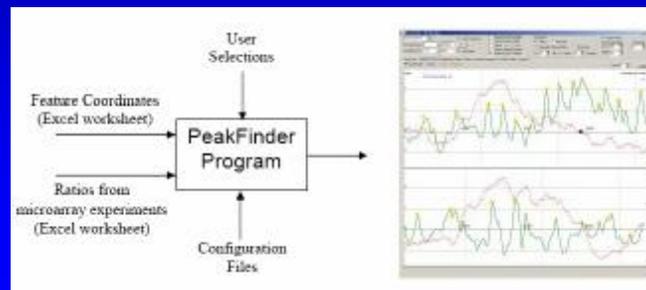
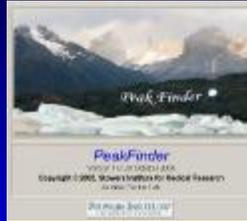
Approach

1. Use genome-wide application of chromatin immunoprecipitation with microarray technology (ChIP chip)
2. Study several different growth conditions and three different strains
3. Develop “PeakFinder” software to analyze genome in a consistent way

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PeakFinder

<http://research.stowers-institute.org/jeg/2004/cohesin/peakfinder/index.html>



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PeakFinder

The screenshot shows the PeakFinder application window with several panels:

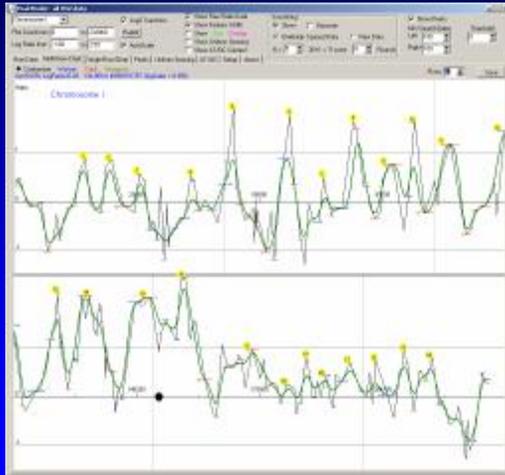
- Chromosome 1:** Includes checkboxes for 'Log2 Transform', 'Show Row Ratio Data', 'Show Feature Width', 'Show Color Bands', 'Show Uniform Spacing', and 'Show ATGC Profile'.
- Smoothing:** Includes checkboxes for 'Show', 'Gaussian', 'Uniformly Spaced Data', and 'Fixed Data'.
- Show Peaks:** Includes checkboxes for 'Show Peaks', 'Min Smooth Data', and 'Threshold'.
- File List:** Shows a list of files including '1. Feature Index file', '2. Sequence Descriptor', '3. Coordinates', and '4. Ratios'.
- Data Table:** A table with columns: Name, Coord, Coord2, Length, Chromosome, ChrCoord, ChrCoord2, Median, Step/Width, Ratio. The table contains several rows of data, with some highlighted in yellow.

Name	Coord	Coord2	Length	Chromosome	ChrCoord	ChrCoord2	Median	Step/Width	Ratio
1 YAL059W	335	649	314	1	335	649	492.0	1.25413	
2 Pk001	645	1587	1190	1	645	1587	1226.0	0.141702	
3 YAL090C	1607	2169	562	1	1607	2169	1586.0	0.123735	
4 Pk003	2123	3425	1306	1	2123	3425	2022.0	0.100820	
5 Pk008	3435	4711	1276	1	3435	4711	4058.0	0.02646	
6 Pk009	4301	5587	1286	1	4301	5587	5734.0	0.02687	
11903 YPC26W	11158475	11158888	2605	16	33240	37845	36744.5	55	0.713688
11904 Pk018	11159033	11159357	324	16	37794	38118	37956.0	-55	0.80340
11905 YPL257W	11159412	11160041	629	16	38173	38802	38487.5	55	0.57241
11906 Pk019	11159986	11160309	323	16	38747	39070	38908.5	-55	0.70083

Feature "points" have variable "width."
Smooth data and set thresholds interactively.

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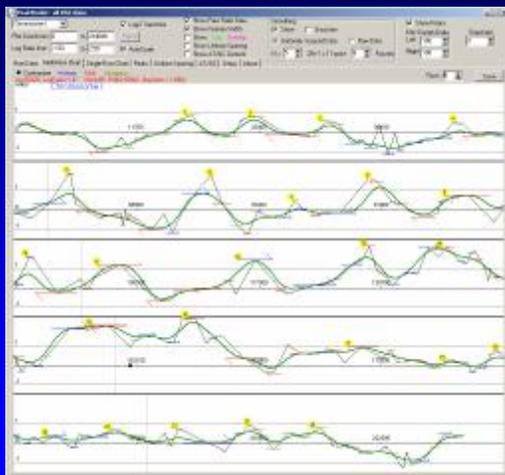
PeakFinder



Features: Watson, Crick, Intergenic

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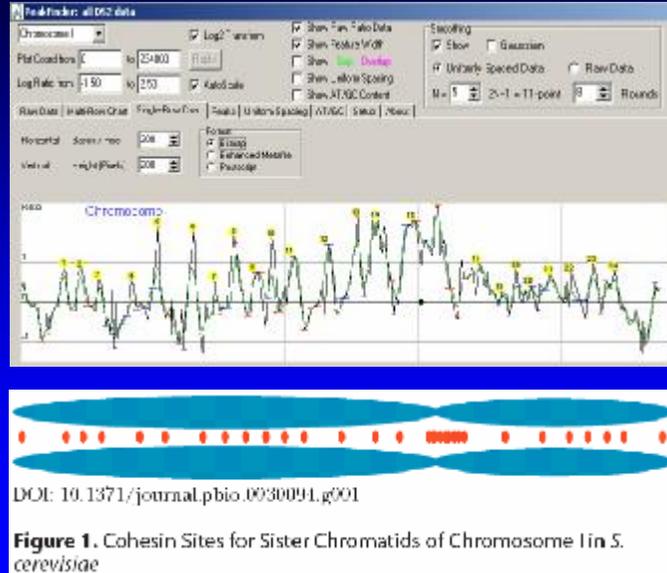
PeakFinder



Limitation: Did not attempt to detect sites near telomeres

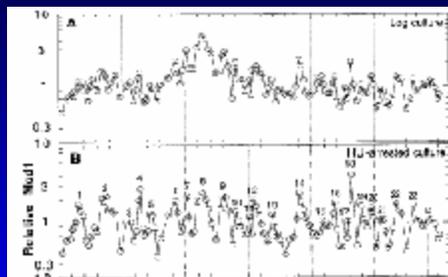
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PeakFinder

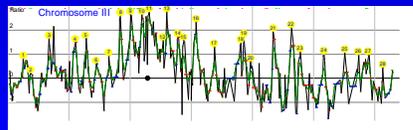


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Results: Validation



Low-Resolution Data
Blat, et al, 1999

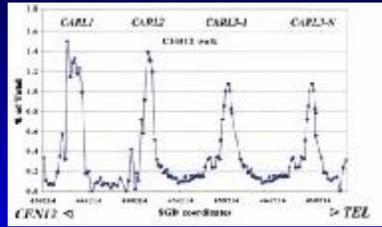


Our Medium Resolution Data

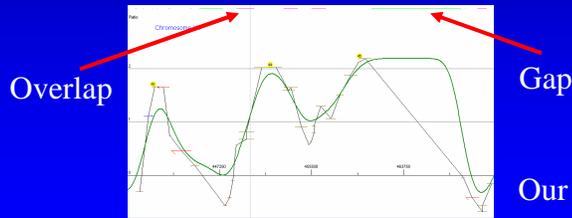
“Qualitatively the results are comparable”

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Results: Validation



High-Resolution Data
Laloraya, et al, 2000



Our Medium Resolution Data

“Qualitatively the results are comparable”

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Results: Internal Consistency and Reproducibility

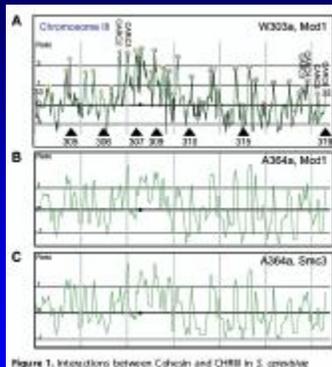


Figure 1. Interactions between Cohesin and CHR8 in *S. cerevisiae*

“There is good agreement between the location of cohesin peaks in different strain backgrounds.”

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Results: Genomic Distribution of Cohesin

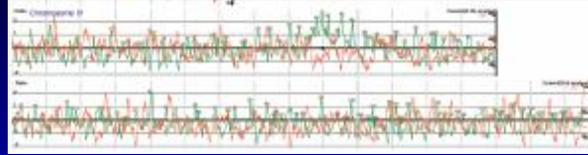
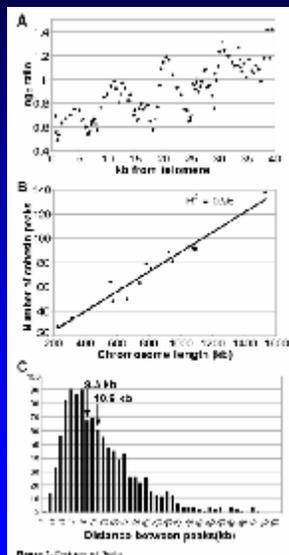


Figure 2. Visual Representation of the Interactions between Mcd1-18Myc and the *S. cerevisiae* Genome in W303a
For each of the 16 chromosomes the centromere is indicated with a black circle, the smoothed data (based on the log₂ of the ratio) is indicated with a green line (left y-axis), and the percent GC is indicated by a red line (right y-axis). Vertical grey bars mark 50-kb intervals. Peaks are located and numbered by PeakFinder (with the exception of telomeres).

- Large regions of intense binding in the pericentric domain
- Less intense, smaller regions distributed in semiperiodic manner throughout the arms
- 9 of 32 telomeres associated with cohesin
- Chromosome III cohesin peaks appear to be associated with origins of replication
- Usually show GC content since it is low when AT content and cohesin binding is high

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Results: Peak Statistics



- Less intense, smaller regions of binding are distributed in a semiperiodic manner throughout the arms
- Lower intensity of cohesin binding near telomeres

- Number of cohesin-binding peaks per chromosome correlated with chromosome length ($R^2 = 0.96$)

- Mean distance between peaks is 10.9 kb
- Cohesin distribution appears to be nonrandom with tendency for even distribution over the genome
- Large gaps appear randomly scattered in arms of larger chromosomes

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Results: Peaks and AT Content

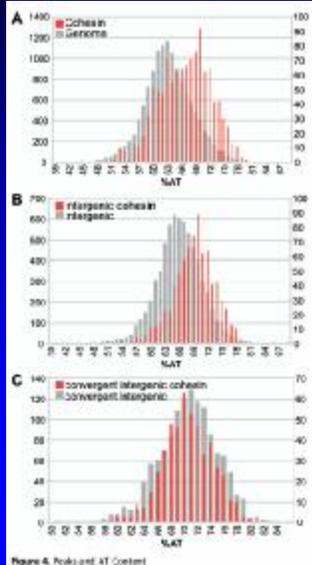


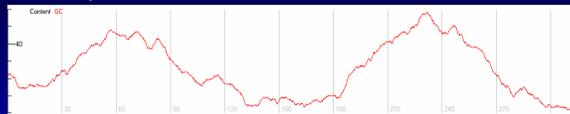
Figure 4. Peaks and AT Content

- Cohesin peaks strongly associated with AT-rich regions
- 810 of 1095 array elements defined as cohesin-binding sites have AT content above yeast median
- 70% of cohesin peaks are associated with intergenic regions (27% of genome)
- Intergenic regions in yeast even more AT rich than open reading frames (ORFs)
- AT content major determinant for cohesin association

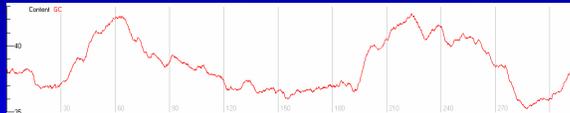
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GC Content Variation by Moving Average Window Size

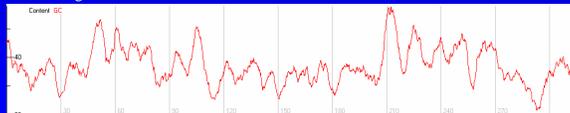
50 kb sliding window



30 kb sliding window: Matches output from Blat and Kleckner, 1999, Fig. 5A



5 kb sliding window



"We observed local oscillations of AT content in a 5-kb sliding window, which corresponded to cohesin-binding peaks in chromosome arms..." ²⁶

Results: Distribution of Cohesin on a Yeast Artificial Chromosome (YAC)

“The semiregular spacing of cohesin and the correlation with local oscillations of base composition suggested that AT content and/or a measuring mechanism might control cohesin distribution ...”

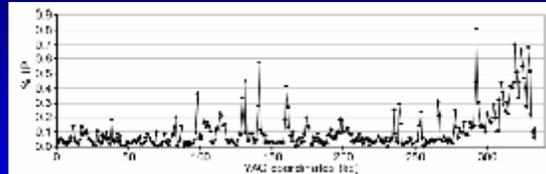


Figure 5. Cohesin Sites Mapped Using ChIP Followed by Semi-quantitative PCR with Primers at 1-kb Intervals in a YAC Containing Human DNA. Cohesin binding for the entire YAC is shown.

In YAC composed of Human DNA fragment:

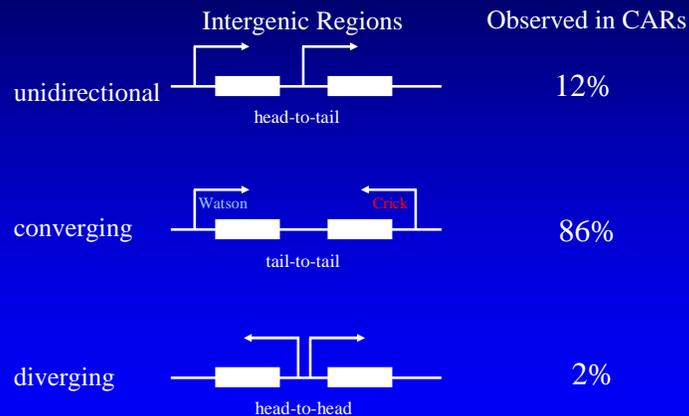
- Pericentric region does show broad, intense association with cohesin
- Oscillations of AT content not similar to yeast
- Pattern of cohesin does not appear to reflect base composition

“some property of the sequence, rather than precise location or context, was responsible for cohesin binding”

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Results: Transcription and Cohesin

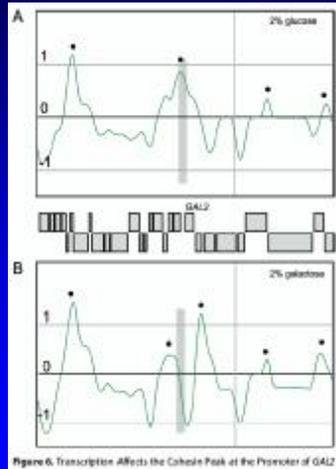
Cohesin-Associated Regions (CARs) are biased towards intergenic regions with convergent transcription



“bias of the intergenic sequences associated with cohesin can be partly explained by the AT bias of convergent intergenic regions ...”

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Results: Negative Association between Transcription and Cohesin Binding

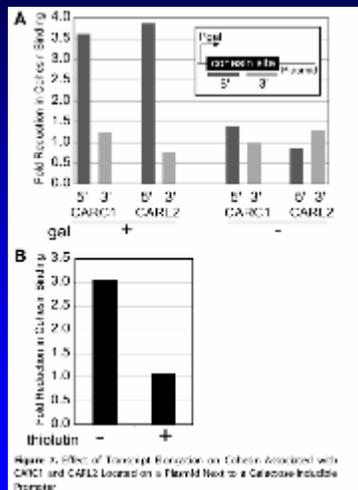


“One peak of cohesin binding in glucose was associated with the promoter of the GAL2 gene, which was induced 42-fold in galactose.”

“Promoter region of GAL2 became a trough of cohesin binding, and the single peak observed in glucose was split into two peaks.”

“high levels of transcription are incompatible with cohesin binding.” 29

Results: Mechanism of Negative Association between Transcription and Cohesin Binding



“Galactose-induced transcription ... disrupted cohesin associated with the 5' end of both CARC1 and CARL2”

“Transcription during G2 can displace cohesin”

“transcript elongation can displace cohesin within the G2 portion of a single cell cycle.”

Results: Meiotic Cohesin

Watanabe & Nurse (1999): "We propose that the persistence of *Rec8* at centromeres during meiosis I maintains sister-chromatid cohesion."

Klein et al (1999): *Rec8p* is a meiotic version of *Scclp*

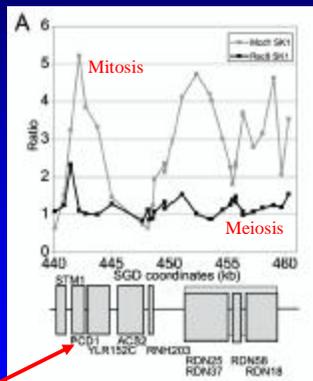


Figure 8. Meiotic Cohesin

(A) Ratios for meiotic cohesin are compared to mitotic cohesin in SK1 for kilobases 440-461 on Chromosome XII.

- The pattern of cohesin association in meiotic and mitotic cells appears to be similar (correlation coefficient = 0.77).
- PCD1 has shown to be a cohesin-binding site
- Transcription of PCD1 is induced in early meiosis
- Binding of the meiotic complex, like the mitotic complex, is not compatible with transcription

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Results: Meiotic Cohesin

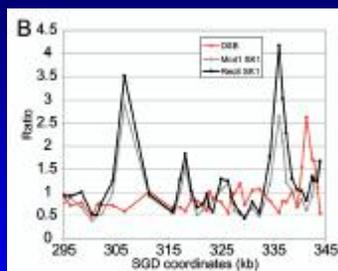


Figure 8. Meiotic Cohesin

(B) Ratios for meiotic cohesin are compared to mitotic cohesin and DSBs for kilobases 295-345 on Chromosome XII.

- Meiotic cohesin tends to be located in regions that contain low levels of double strand breaks (DSBs).
- DSBs initiate meiotic recombination
- Negative correlation (-0.26) between location of meiotic cohesin and location of DSBs.
- Cohesin has been shown to be required for formation of the synaptonemal complex (SC)

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Take Home Message

- Genome-wide map of chromosome cohesin is consistent with many known and predicted properties of chromatin.
- DNA sequences required for the replication and segregation of chromosomes must be protected from transcription to function properly.
- Cohesin sites are highly conserved in meiosis and mitosis suggesting common underlying structure during different developmental programs.
- Genome-wide analysis of cohesin in yeast provides useful framework to explore attributes of cohesin localization and cohesion in higher eukaryotes. ³³

Thank You

Jennifer Gerton