A putative condensin loading factor that controls yeast chromosome III architecture

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Abstract

Condensin plays an important and evolutionarily conserved role in mitotic chromosome condensation, three-dimensional genome organization and regulation of gene expression. Condensin is a multi-subunit complex that has natural affinity for the promoters of highly transcribed genes, and also associates with specific transcription factors. However, a general mechanism for functional loading of the complex onto chromatin remains elusive. Our lab previously demonstrated that in haploid *MATa* yeast cells, condensin and Sir2 (a histone deacetylase) both associate with the recombination enhancer (RE), a cis-acting element on chromosome III that directs donor preference of mating-type switching. Here, Sir2 locally regulates transcription of a small gene called *RDT1*, while condensin contributes to the 3-dimensional organization of chromosome III, as well as donor preference, indicating that the *RDT1* promoter region acts as a locus control region (Li et al., 2019). We have now further characterized the mechanism of condensin recruitment to the *RDT1* promoter LCR, and uncovered a critical role for a non-meiotic version of the monopolin complex, known as cohibin (Lrs4 and Csm1 subunits), analogous to its known role in recruiting condensin to the rDNA locus. To test if cohibin functions more generally in condensin loading, or as a condensin accessory factor, we have performed ChIP-seq for genome-wide condensin binding sites in WT and *lrs4*Δ strains. Numerous Brn1 peaks (including *RDT1* and the rDNA) were eliminated or significantly reduced by *lrs4*Δ, consistent with the condensin loader hypothesis. Micro-C XL was then used to characterize the general effects of defective condensin recruitment on genomic conformation in a *lrs4*Δ mutant, or when the Brn1 condensin subunit was depleted using an auxin-inducible degron system. Alterations were observed on multiple chromosomes, though the most severe changes occurred on chromosome III, which had significant negative effects on the efficiency of mating-type switching, as well as donor preference. We therefore hypothesize that cohibin (Lrs4/Csm1) is indeed a condensin loader at the *RDT1* promoter region, where it establishes chromosome III conformation, potentially through a loop extrusion mechanism. Evidence for direct recruitment of condensin by the cohibin complex at other genomic sites and any possible role for cohibin in chromosome condensation are currently under investigation.

Aim

Establish whether Lrs4 is a condensin loader at *RDT1* promoter region

1. Mechanism of mating type switching in budding yeast

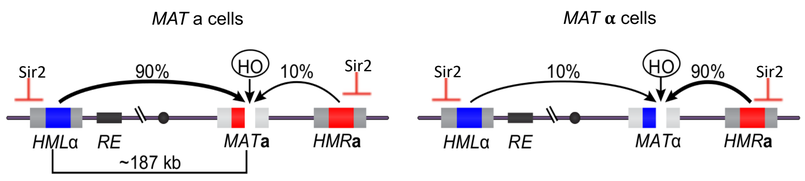


Fig 1. Donor preference during mating-type switching is regulated by recombination enhancer (RE). When the HO endonuclease cuts at *MAT* locus, *MAT*a cells primarily use *HML*α as a donor, while *MAT*α cells use *HMR*a as a donor, resulting in a high frequency of mating-type switching. Dummer et al., PLOS Genetics |DOI:10.1371/journal.pgen.1006094 June 3, 2016.

2. ChIP-seq analysis of Sir2 and condensin, revealed closely overlapping binding sites within the promoter of a gene called *RDT1* in *MAT*a cells

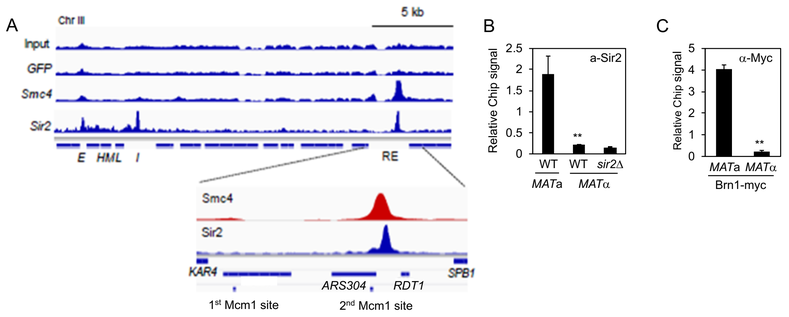


Figure 2. *MAT*a-specific binding of Sir2 and condensin to the recombination enhancer (RE). (A) ChIP-seq of Smc4-myc, Sir2-myc, and nuclear localized GFP in WT and *sir2*Δ backgrounds. Inset: The minimal 700bp RE element required for donor preference is indicated, as are the two Mcm1/ α2 binding sites (DPS1 and DPS2) and *RDT1*. (B) ChIP showing *MAT*a-specific binding of Sir2 to the RE. (C) ChIP showing *MAT*a-specific binding of Brn1-myc to the RE. ChIP signal relative to input is plotted as the mean of three replicates. Error bars = standard deviation. (**p<0.005). Li et al., Plos Genetics, 2019.

3. Sir2 regulates transcription of *RDT1* while condensin contributes to 3-dimensional organization of chromosome III, bilaterally regulating the mating-type switching in yeast by binding within the *RDT1* promoter region

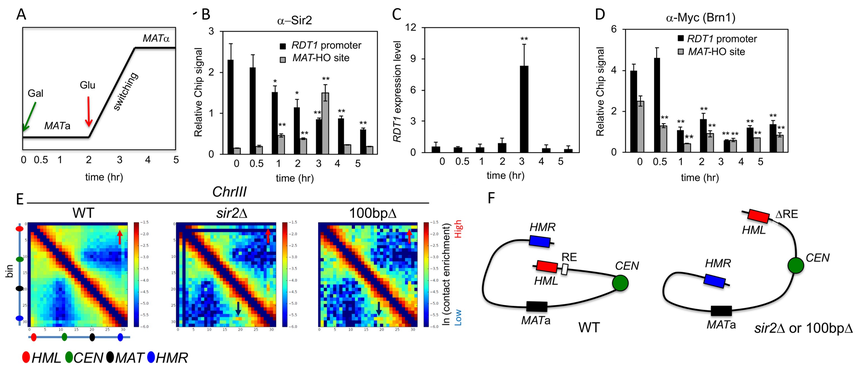


Figure 3. Sir2 and condensin are displaced from the *RDT1* promoter during mating-type switching and the Sir2/condensin binding site controls chromosome III architecture in the process. (A) Schematic of a mating-type switching time course where HO was induced by galactose at time 0, then glucose added at 2 hr to stop HO expression and allow for break repair. Switching is maximal at 3 hr. (B) ChIP of Sir2 at the *RDT1* promoter and the HO-induced DSB site (*MAT*-HO). (C) qRT-PCR of *RDT1* expression relative to *ACT1* across the mating-type switching time course. (D) ChIP of Brn1-myc at the *RDT1* promoter and *MAT*-HO break site across the same time course. (*p<0.05, **p<0.005 compared to time 0). (E) Iteratively corrected and read-normalized Hi-C heat maps revealing an interaction between *HMR* and *MAT*a in the *sir2*Δ and 100bpΔ mutants (black arrow). Solid black lines indicate bins with insufficient read coverage post-filtering. Red arrows indicate the *HML*-*HMR* interaction. (F) Summary of large-scale changes in chromosome III architecture. Δ indicates the 100bp deletion. Li et al., Plos Genetics, 2019.

4. A putative physical interaction between Mcm1 and Lrs4 shown from *Saccharomyces* Genome Database (SGD)

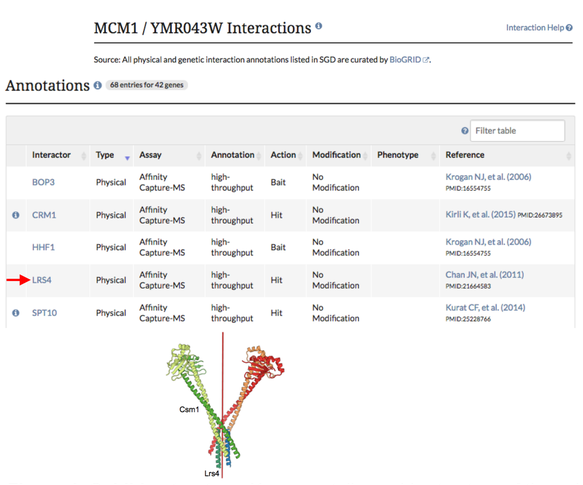


Table S3, related to Figure 2. Results of LC-MS/MS analysis of TAP purifications.

(Proteins in untagged cells were removed)

(Part A) 0.5% NP40:

Purification A1 – Lrs4–TAP

ORF	NP	ID
YDR439W	47	LRS4
YCR086W	14	CSM1
YML034W	6	HEH1
YLR150W	4	STM1
YMR043W	3	MCM1
YDL089W	3	NUR1
YBL002W	2	H2B
YBR009C	2	H4
YJL076W	2	NET1
YFL037W	2	TUB2
YHR121W	2	LSM12
YBR017C	2	KAP104
YDL088C	2	ASM4
YHR045W	2	YHR045W
YDL072C	1	YDL072C
YPL200W	1	CSM4

Chan et al., Developmental Cell. 20, 867–879, June 14, 2011

Figure 4. Cohibin structure. X-ray crystallographic structure of the cohibin complex consisting of 4 Csm1 subunits and 2 Lrs4 subunits that form a v-shaped conformation through interactions of the coiled-coil domains. Vertical red line indicates the axis of symmetry.

5. Proposed model of condensin and Sir2 recuitment to the *RDT1* promoter by Lrs4 and Mcm1

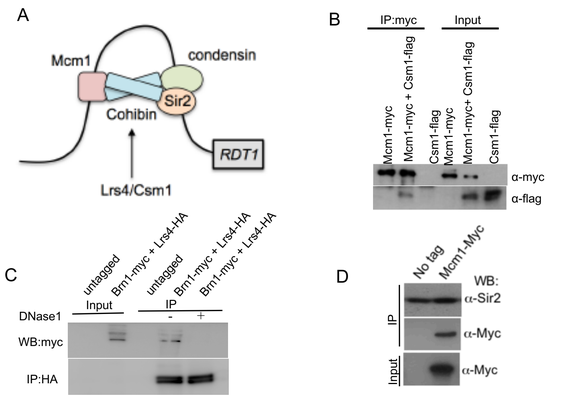


Figure 5. (A) Model of condensin and Sir2 recuitment to the *RDT1* promoter by Mcm1. The cohibin complex (Lrs4/Csm1) bridges interaction between the Mcm1 binding site and the condensin/Sir2 site. (B) Cohibin physically interacts with Mcm1. (C) Cohibin physically interacts with condensin. Interaction between Lrs4 and condensin is disrupted in presence of DNase1 (D) Mcm1 physically interacts with Sir2. Loaded inputs are 2.5-5% of cell lysate used for IP.

6. *lrs4* Δ reduces condensin binding at the *RDT1* promoter region

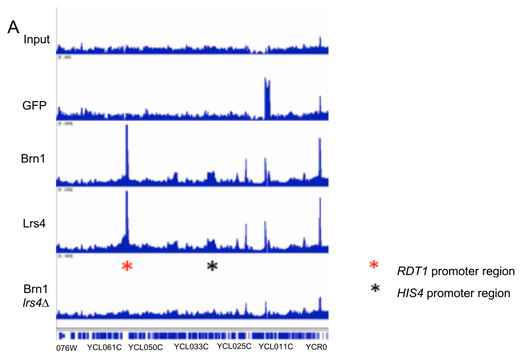


Figure 6. ChIP-seq to identify the altered level of condensin binding between wild type and *lrs4*Δ (A) ChIP-seq screenshot of the Chr III left arm. Brn1 and Lrs4 are 13x-myc tagged. (B) MA plot showing the binding affinity of condensin between wild type and *lrs4*Δ. X-axis shows the log2 of the average condensin binding (in counts per million, cpm). Y-axis shows the log2 fold-change in condensin binding between Wt and *lrs4*Δ. Each dot represents a binding site, with red dots representing a differentially bound site (831 sites, FDR < 0.1). The condensin binding sites lose binding affinity in *lrs4*Δ as evidenced by red dots above the center line which indicates condensin loading dependent on Lrs4 than gain in binding affinity shown by red dots below the center line. Blue dots representing peaks not differentially bound with blue smears indicating overrepresentation of blue dots.

7. Lrs4 helps to recruit condensin at *RDT1* promoter in *MAT*a cells

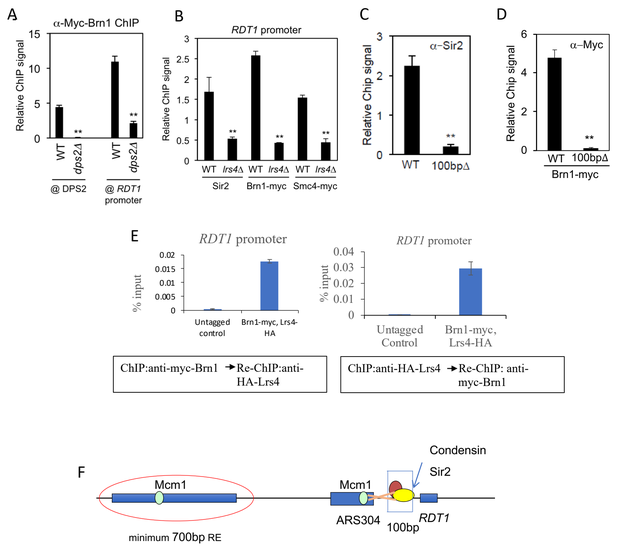


Figure 7. Sir2 and condensin recruitment to the RE requires cohibin. (A) Brn1-myc enrichment at DPS2 and the *RDT1* promoter in wild type (WT) and *dps2*Δ strains. (B) Sir2, Brn1-myc, and Smc4-myc enrichment at the *RDT1* promoter in WT and *lrs4*Δ strains. (C) ChIP of Sir2 in the 100bpΔ mutant. (D) ChIP of Brn1-Myc in the 100bpΔ mutant. (*p < 0.05, **<0.005). (E) Re-ChIP showing the co-occupancy of Lrs4 and condensin at the *RDT1* promoter region. (F) Schematic of inferred bridging of Mcm1 with condensin and Sir2 around 100 bp region via the cohibin complex (Lrs4/Csm1).

8. Association of Lrs4 with *RDT1* promoter is cell cycle regulated

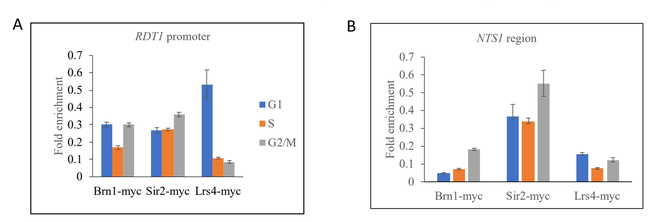
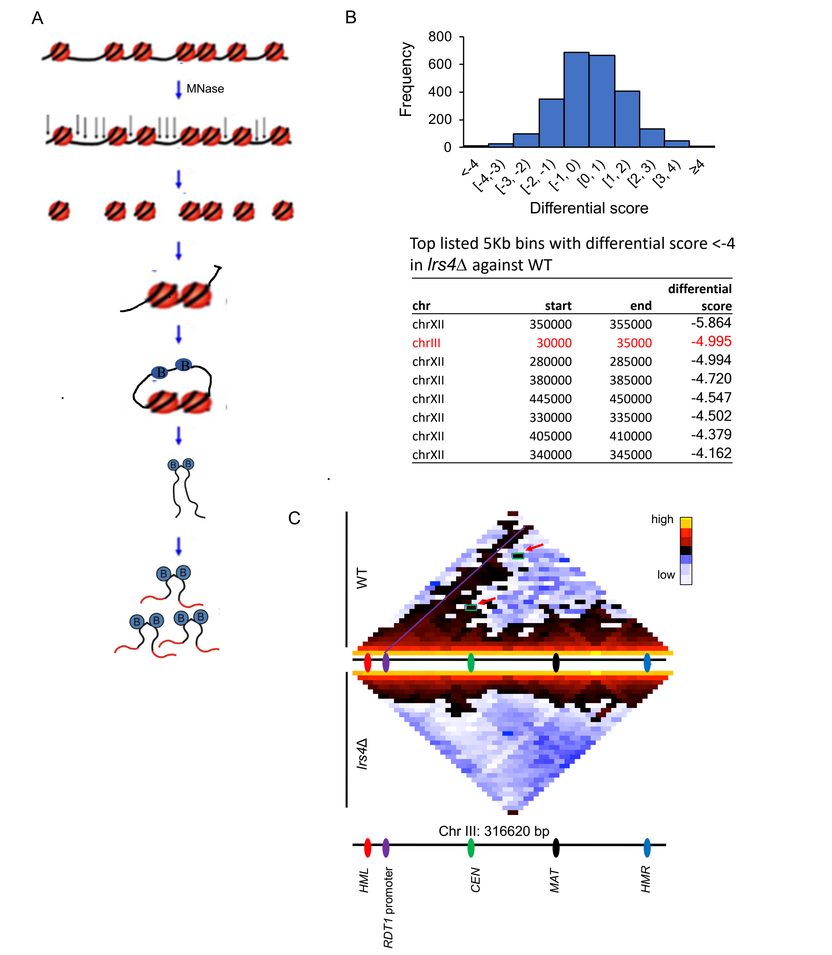


Figure 8. Lrs4 is maximally enriched with *RDT1* promoter during G1 phase of the cell cycle. Cells were synchronized in G1 phase by α-factor and released into S and G2/M phase. (A) Brn1-myc, Sir2-myc and Lrs4-myc enrichment at the *RDT1* promoter in wild type. (B) Brn1-myc, Sir2-myc and Lrs4-myc enrichment at the *NTS1* region of rDNA in wild type.

9. Micro-C XL reveals a change in chromosomal architecture genome-wide in *lrs4*Δ



Top listed 5Kb bins with differential score <-4 in *lrs4*Δ against WT

chr	start	end	differential score
chrXII	350000	355000	-5.864
chrIII	30000	35000	-4.995
chrXII	280000	285000	-4.994
chrXII	380000	385000	-4.720
chrXII	445000	450000	-4.547
chrXII	330000	335000	-4.502
chrXII	405000	410000	-4.379
chrXII	340000	345000	-4.162

Figure 9. Effect of *lrs4*Δ on chromatin organization. (A) Overview of the Micro-C XL method. (B) Distribution of differential scores of all 5Kb bins in the genome which is quantified from paired t-test comparing chromatin interactions of target bin with its nearby bins (+/- 200Kb) in *lrs4*Δ against WT. Negative score indicates loss of local chromatin interaction between two loci in *lrs4*Δ. (C) Micro-C XL contact map at 10 kb resolution shown for chromosome III. Red arrowheads indicate looping interactions between *MAT*a-*HML*α and *HML*α-*HMR*a. Purple line showing the interaction of the RDT1 promoter region with the rest of chromosome arm.

10. Condensin depletion leads to loss of interaction between *MAT*a-*HML*α, *HML*α-*HMR*a in ChrIII during mating type switching

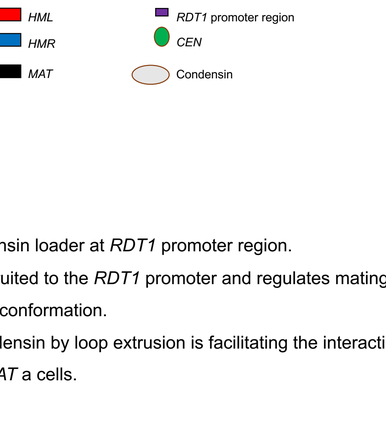
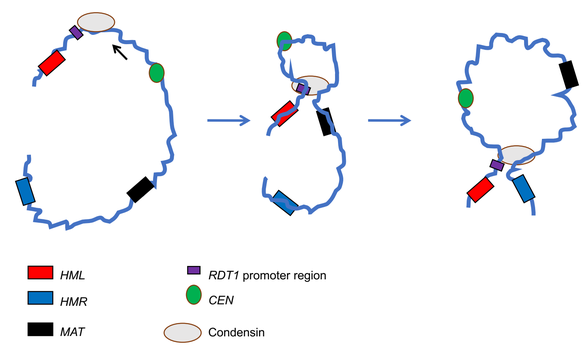


Figure 10. Effect of condensin depletion on chromosome III during mating-type switching. Micro-C XL contact map at 5 kb resolution shown for chromosome III during mating type switching in wild-type and condensin depletion strain. Condensin is depleted using auxin-inducible degron (AID) system. Red arrowheads indicate looping interactions between *MAT*a-*HML*α, *HML*α-*HMR*a and *MAT*a-*HMR*a. Purple line indicates the interaction of *RDT1* promoter region with the rest of chromosome region.

11. Hypothetical loop extrusion model after binding of condensin to the *RDT1* promoter



12. Conclusion

- Lrs4 is the condensin loader at *RDT1* promoter region.
- Condensin is recruited to the *RDT1* promoter and regulates mating type switching by maintaining the chromosome conformation.
- Presumably, condensin by loop extrusion is facilitating the interaction of *HML* with *MAT* to repair DNA breaks in *MAT* a cells.

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