Role of inner nuclear membrane protein complex Lem2-Nur1 in heterochromatic gene silencing in *S. pombe*

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**Running title:** Lem2-Nur1 in heterochromatic gene silencing

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Abstract

Heterochromatin in the fission yeast *Schizosaccharomyces pombe* is clustered at the nuclear periphery and interacts with a number of nuclear membrane proteins. However, the significance, and the factors that sequester heterochromatin at the nuclear periphery are not fully known. Here, we report that an inner nuclear membrane protein complex Lem2-Nur1, is essential for heterochromatin-mediated gene silencing. We found that Lem2 is physically associated with another inner nuclear membrane protein, Nur1, and deletion of either *lem2* or *nur1* causes silencing defect at centromeres, telomeres and at rDNA loci. We analyzed the genome-wide association of Lem2 using ChIP-seq and found that it binds to the central core region of centromeres, in striking contrast to Chp1, a component of pericentromeric heterochromatin, which binds H3K9me-rich chromatin in neighboring sequences. The recruitment of Lem2 and Nur1 to silent regions of genome is dependent on H3K9 methyltransferase, Clr4. Finally, we show that Lem2-Nur1 complex regulates the local balance between the SHREC histone deacetylase complex and the anti-silencing protein Epe1. These findings uncover a novel role for Lem2-Nur1 as a key functional link between localization at the nuclear periphery and heterochromatin-mediated gene silencing.
Introduction

Regulation of eukaryotic genome function involves the differential organization of chromatin into euchromatic and heterochromatic domains distinguished on the basis of their appearance, structure, localization and function (1-5). Heterochromatin is a silent chromatin structure that represses gene expression and recombination to maintain genome integrity. In the fission yeast Schizosaccharomyces pombe heterochromatic loci are restricted to the pericentromeric DNA regions, mating type loci and telomeres. The centromeres are surrounded by DNA repeats that comprise an outer repeat (otr) regions (containing dg and dh repeats) and innermost repeat (imr) regions, which flank the central core (cnt) domain. These repeats are thought to be transposon remnants and are required for centromere assembly and proper chromosome segregation (6-8). Assembly of heterochromatin in fission yeast requires methylation of histone H3 at lysine 9 (H3K9) by the conserved Clr4 methyltransferase. H3K9 methylation requires components of the RNA interference (RNAi) machinery. RNA transcripts act as scaffold for the assembly of RNAi and chromatin modifying factors that initiate the formation of heterochromatin. Methylation of H3K9 recruits chromodomain containing HP1 proteins, Swi6 and Chp2, which are critical for heterochromatic gene silencing (9-15). Cytological studies have shown that heterochromatin has a tendency to associate with the nuclear periphery, raising the possibility that proximity to the nuclear envelope facilitates heterochromatin-mediated gene silencing (3,16). In Schizosaccharomyces pombe, for example, telomeres form clusters at the nuclear periphery. Localization of transcriptionally silent domains towards the nuclear periphery has also been observed in budding yeast, flies and plants (17). Observations in higher eukaryotes also suggest a role for nuclear periphery in
gene silencing. In human cells, gene poor chromosomes as well as the inactive X chromosome or Barr body tend to localize at the nuclear periphery. In metazoans, lamina associated domains (lamina-associated domains or LADs) which lies beneath the inner nuclear membrane are enriched for repressed chromatin state (18). This peripheral association of these domains is linked to repressive H3K9 methylation, a hallmark of repressive chromatin. In humans lamina-associated domains constitute around 40% of mammalian genome (19-20). Similarly in *Caenorhabditis elegans* lamina-associated domains constitute repeat rich regions and knockdown of lamina-associated proteins EMR-1 and LEM-2 leads to derepression of perinuclear heterochromatin (21-22). These studies suggest that attachment of chromosome domains to the nuclear periphery can affect its expression. In support of this, artificial tethering of transcriptionally active loci in yeast and mammals become repressed when tethered to nuclear periphery (2). Lamins have been shown to interact with several inner nuclear membrane proteins (INM). Several lamin-associated proteins (LAPs) contain a LEM domain (LAP2—Emerin—MAN1), a 40-amino-acid helix–extension–helix (HEH) motif that is conserved from yeast to humans (23-24). *Schizosaccharomyces pombe* contain three INM (Lem2, Man1, and Ima1) proteins that show homology with lamin-associated proteins (25). Lem2 and Man1 contain HEH motif homologous to metazoan LEM domain. Recently, Lem2 and Man1 have been shown to be important for nuclear structure integrity and telomere anchoring with nuclear membrane in fission yeast (26). Furthermore, in *Saccharomyces cerevisiae* Lem2-Nur1 homologue, Heh1-Nur1 form CLIP complex (chromosome linkage INM proteins) that physically link rDNA repeats to nuclear periphery. CLIP is required for the maintenance of rDNA repeat stability and not for their silencing. Deletion of either
heh1 or nur1 causes release of rDNA repeats from the nuclear periphery and leads to chromosome instability by promoting aberrant recombination events in the rDNA repeats. In addition, artificial tethering of rDNA repeats to nuclear periphery partially suppresses repeat instability in heh1 or nur1 mutant cells (27). Although the localization of fission yeast heterochromatin at the nuclear periphery is thought to be important for gene regulation the functional significance and the factors that sequester heterochromatin at the nuclear periphery are not fully known. Here, we show that inner nuclear membrane protein Lem2 and its interacting partner Nur1 are required for heterochromatin silencing in S. pombe. We found that deletion of lem2 or nur1 causes silencing defects at centromeres, telomeres and at rDNA loci. We further determined the genome-wide localization of Lem2 and demonstrate that it associates specifically with silent regions of the genome in heterochromatin dependent manner. Furthermore, we show that Lem2-Nur1 complex regulates the balance between chromatin binding of RNAi/histone deacetylase complex SHREC and anti-silencing protein Epe1. Overall, our results uncover a novel role for Lem2-Nur1 complex in heterochromatin gene silencing in S. pombe. These findings add a new perspective to the evolutionarily conserved LEM domain proteins and suggests that the mammalian homologs might also play crucial roles in heterochromatin gene silencing.
RESULTS

**Lem2-Nur1 complex is essential for heterochromatic gene silencing**

To identify novel factors required for heterochromatin gene silencing and its localization towards nuclear periphery, we identified two inner nuclear membrane proteins in fission yeast, Lem2, a conserved LEM domain protein, and Nur1, a nuclear rim protein. Homologs of Lem2 and Nur1 in *S.cerevisiae* have been shown to be required for peripheral localization of heterochromatin but not for silencing (27). We used co-immunoprecipitation assays to demonstrate that the fission yeast Lem2 and Nur1 were physically associated. We performed immunoprecipitation experiments from cells that expressed functional C-terminally TAP-tagged Lem2 and C-terminally myc-tagged Nur1 expressed under the control of their endogenous promoters. As shown in Fig. 1A, Nur1-myc co-immunoprecipitated with Lem2-TAP. To gain insight about the role of Lem2-Nur1 complex in heterochromatin gene silencing, we deleted either *lem2* or *nur1* or both in a strain that has *ura4* reporter inserted at the centromere (Fig. 1B). Deletion of either *lem2* or *nur1* leads to a loss of gene silencing which is observed with an *imr1R::ura4* reporter gene and a loss of growth on 5-FOA medium (Fig. 1C, 5-FOA is toxic to cells that express *ura4*). The silencing phenotypes were verified by quantitative RT-PCR (qRT-PCR) to examine the levels of inserted *ura4* reporter gene (*imr1::ura4*) or endogenous heterochromatic transcripts originating from the dg elements of centromeres. *clr4Δ* in which heterochromatin is completely disrupted was used as a positive control. We found that inserted *ura4* and centromeric dg transcripts were derepressed in *lem2 Δ* and *nur1 Δ* cells, although to a lesser extent than in *clr4Δ* cells (Fig. 1D and E). Consistent with the silencing and qRT-PCR results, occupancy of Pol II at the centromeric dg region increased in *lem2* and *nur1*
mutant cells (Fig. 1F). Centromeric heterochromatin is important for chromosome segregation and any perturbation of centromeric heterochromatin causes defects in chromosome segregation (31-32). We observed that like \textit{clr4Δ} cells, \textit{lem2 Δ} and \textit{nur1 Δ} cells showed hypersensitivity to microtubule destabilizing drug thiabendazole (TBZ) (Fig. 1G). These results indicate that Lem2-Nur1 complex is required for heterochromatic gene silencing at pericentromeric heterochromatin.

\textbf{Lem2-Nur1 binds to central core region of centromeres, telomers and rDNA repeats}

To gain further insight into the role of Lem2-Nur1 in heterochromatin organization, we constructed strains expressing Lem2 or Nur1 with a C-t TAP-tag. We performed ChIP-seq analysis of Lem2 and Chp1. Interestingly, Lem2 was strictly confined to the central core cnt1 domain and is excluded from the pericentromeric regions, while Chp1 is enriched at the outer repeats (Fig. 2B and C). The ChIP-seq data was further validated by chromatin immunoprecipitation (ChIP-qPCR) experiments at dg and central core regions (Fig. 2D and E). Furthermore, ChIP analysis of Nur1 showed similar binding pattern as that of lem2 (Fig. 2F and G). In addition to centromeres, genome wide distribution of Lem2 showed that it also associates with other silent regions of the genome like telomeres and rDNA repeats (Fig. 3A-D). Similar to pericentromeric heterochromatin, deletion of either Lem2 or Nur1 leads to derepression of telomeres (Fig. 3E). Interestingly, we did not see any enrichment of Lem2 or Nur1 to euchromatin regions of the genome. These findings indicate that regulation of heterochromatin gene silencing by Lem2 and Nur1 is through direct association with centromeric as well as with other silent regions of the genome.

\textbf{Chromatin binding of Lem2-Nur1 is dependent on Clr4}

Heterochromatin has been shown to serve as a platform for the recruitment of diverse pool of cellular factors (33-34). Because Lem2-Nur1 complex specifically
localizes to heterochromatic loci, we explored whether Lem2-Nur1 recruitment to heterochromatic loci is dependent on heterochromatin. We constructed strains expressing Lem2 or Nur1 with a C-t TAP-tag in clr4 deletion background. ChIP-seq analysis revealed deletion of H3K9-specific methyltransferase Clr4 abolished lem2 localization at centromeres, (Fig. 4 A and B), telomeres and the rDNA repeats (data not shown). The ChIP-seq experiments were further verified by chromatin immunoprecipitation (ChIP) experiments, which showed complete loss of Lem2-Nur1 binding in clr4 deleted cells (Fig. 4 C and D). Thus, effects of mutations in heterochromatin assembly factor Clr4 impairs Lem2-Nur1 recruitment. These observations suggest that heterochromatin serves as a platform for binding of Lem2 and Nur1 complex, which tether heterochromatin towards nuclear periphery that ensures proper silencing.

Lem2-Nur1 regulates SHREC and Epe1 localization to heterochromatin

In S. pombe H3K9 methylation is a key event in the formation of heterochromatin. This mark serves as a binding site for Swi6, which plays a critical role in maintaining chromatin structure and ensuring faithful chromosome segregation during cell division (15-18). To gain insight into the molecular mechanism of Lem2-Nur1 complex in heterochromatin gene silencing, we looked at the effect of deleting lem2 on H3K9me2 and Swi6 enrichment at centromeric heterochromatin. Cells lacking Lem2 displayed a drop in levels of H3K9me2 and Swi6 (Fig. 5A and B). Similar effect was seen on levels of H3K9me2 and Swi6 in nur1 deleted cells. Since deletion of lem2 and nur1 did not greatly affect H3K9me and Swi6 levels, we speculated whether deletion of lem2 or nur1 impairs the recruitment of other factors that are important for silencing. To test this, we took a candidate based approach and determined
the recruitment of RNAi-linked Snf2/HDAC repressor complex SHREC and anti-silencing protein Epe1 in lem2 deletion cells, because these factors have previously been shown to work antagonistically in regulation of heterochromatin gene silencing downstream of H3K9me (35-36). SHREC belongs to Snf2/HDAC repressor complex family and contains the class II HDAC Clr3 that targets lysine 14 of histone H3. Epe1 is a conserved Jumonji C (JmjC) domain nuclear protein that antagonizes heterochromatization. Inactivation of Epe1 enhances reporter gene silencing and promotes heterochromatin spreading across boundary elements, while its overexpression abrogates heterochromatin structure and impairs centromere functions (36). We constructed strains expressing either FLAG Tagged Clr1, a component of SHREC or FLAG Tagged Epe1 in lem2 deletion cells and looked at binding of SHREC or Epe1 in lem2 deleted cells by chromatin immunoprecipitation. Interestingly, we found increased association of Epe1 and decreased SHREC /Clr1 binding in lem2 deleted cells (Fig. 5C and D). Consistent with the ChIP data, the acetylation levels were greatly increased in cells lacking either lem2 or nur1 (Fig. 5E). These results suggest that lem2-Nur1 promotes SHREC binding while preventing Epe1 localization at centromeres.

DISCUSSION

The eukaryotic genome within the nucleus is spatially segregated into euchromatin and heterochromatin, with heterochromatin often associated with the nuclear envelope (2-3). These specific spatiotemporal distributions correlate with the cell's functional state (37-39). In fission yeast heterochromatin domains are localized towards nuclear periphery and interacts with a number of nuclear membrane proteins (2-3,6). This localization is thought to present a sub-compartment enriched for factors required for heterochromatin silencing and with properties distinct from the nuclear interior (12,40). This
perinuclear sequestration of heterochromatin is conserved from yeast to humans (41-44). However, the significance of this peripheral localization in terms of heterochromatin stability and the proteins responsible for this perinuclear sequestration is not fully understood. The present study identified and characterized a novel inner nuclear membrane protein complex, Lem2-Nur1 as being essential for heterochromatin-mediated gene silencing in fission yeast. Deletion of either lem2 or nur1 causes silencing defects both at centromeres and at telomeres. Compared to clr4 deletion, where heterochromatin is completely disrupted, lem2 and nur1 deleted cells show significant derepression of transcription within centromeric and telomeric heterochromatin (Fig. 1 and 3). Interestingly, ChIP and ChIP-Seq data showed that Lem2 and Nur1 strictly bind to the central core cnt1 domain of centromeres, which is involved in kinetochore assembly, and deletion of Lem2 and Nur1 causes segregation defects possibly through defect in kinetochore assembly (Fig 1). Interestingly, unlike lem2 and nur1 which are important for tethering and silencing of all the heterochromatin domains in S pombe, the budding yeast homolog Heh1 and Nur1, which form the CLIP complex are implicated in tethering and stability of rDNA repeats and not in silencing. It would be interesting to investigate if lem2-Nur2 complex in S pombe does play a role in stability of rDNA repeats.

Even though, tethering and silencing have been mechanistically separated from each other, both processes reinforce each other (16, 45). Indeed, the mechanisms used for the recruitment are often epigenetic marks established during heterochromatin formation. In case of budding yeast, deacetylation of histone H4 is necessary for SIR protein recruitment. Similarly in C. elegans methylation of H3K9 is required for SET-25 localization with peripheral heterochromatin (2, 46). In tune with above findings, Lem2-Nur1 recruitment
to repressive chromatin depends on heterochromatin (Fig. 4).

On the mechanistic level the function of Lem2-Nur1 involves the recruitment of the repressor complex SHREC to heterochromatin, which contributes to repression SHREC is the Snf2/HDAC-containing Repressor Complex, which deacetylates histone H3 and H4 and remodels chromatin structure at heterochromatic regions, resulting in transcriptional gene silencing and heterochromatin spreading (Fig. 6) (35). Deletion of lem2 or nur1 causes increase in binding of Epe1, a JmjC domain protein that blocks spreading of H3K9me at heterochromatic barriers (36). Epe1 counteracts SHREC and targeting of Epe1 causes an increase in transcription at heterochromatin and a defect in heterochromatin spreading. Furthermore, loss of lem2 or nur1 increases the acetylation levels at the silent regions (Fig. 5C). A recent study by Barrales et al (34) shows that chromatin association and tethering of centromeres to the periphery are mediated by the N-terminal domain of Lem2, while, as telomere anchoring and silencing require its conserved C-terminal domain. Regulation of SHREC and Epe1 binding by Lem2-Nur1 adds an unexpected functional layer in the regulation of heterochromatin silencing. We speculate that Clr4 mediated methylation of H3K9 and subsequent binding of Swi6 to methylated histones localizes heterochromatin to nuclear periphery. Localization to nuclear periphery depends on interaction with inner nuclear membrane protein complex Lem2-Nur1, which then regulate binding of Snf2/HDAC-containing Repressor Complex, SHREC (Fig. 6). Given the evolutionary conservation of Lem2-Nur1, the mechanism described here may represent a widespread strategy for fine-tuning transcriptional gene silencing by localization of heterochromatin at the nuclear periphery.

In summary, we uncovered a multi-step mechanism of heterochromatin gene silencing mediated by Clr4 dependent chromatin binding of Lem2-Nur1. Lem2-Nur1 regulates silencing by
ensuring appropriate balance between binding of repressing, SHREC and activating, Epe1 factors. These factors modulate the levels of repressive and activating histone post-translational modifications, which in turn affect gene silencing.
MATERIALS AND METHODS

Yeast Strains. Strains and plasmids used in this study are in table 1 and were made by a PCR based gene targeting procedure (28).

Quantitative RT-PCR

Yeast cultures were grown in YES medium at 32°C to an OD\textsubscript{600} of 0.5. Total RNA was isolated by hot phenol procedure and cleaned by using RNeasy kit (Qiagen) to remove potential genomic DNA contamination. Gene specific primers for dg and tlh1 were used to prepare cDNA followed by quantitative PCR using Light Cycler. Relative RNA levels were calculated from C\textsubscript{T} values according to the ∆C\textsubscript{T} method (Applied Biosystems) and normalized to act1\textsuperscript{+} RNA levels.

Chromatin immunoprecipitation (ChIP) and Chromatin immunoprecipitation Sequencing (ChIP-Seq) assays

ChIP assays were performed as described previously (29). Cells were cross-linked with 10 mM dimethyl adipimidate and subsequently with 1% formaldehyde for 20 min at room temperature and quenched with 125mM glycine for 5 min. Cross-linked chromatin was sonicated to yield DNA fragments of an average size of 200-500 base pairs. Immunoprecipitations were carried out with anti TAP (applied Biosystems), anti- Flag M2 (Sigma) and diMeH3K9 antibody (Abcam). Primers used in the PCR reactions were analyzed for linearity range and efficiency with a LightCycler (Applied Biosystems) in order to accurately evaluate occupancy (% of IP/input). The results shown with standard errors are based on three independent experiments. For ChIP-Seq experiments libraries for Illumina sequencing were constructed following the manufacturer's protocols, starting with ~5 ng of immunoprecipitated DNA fragments. Each library was generated with custom-made adapters carrying unique barcode sequences at the ligating end (30). Barcoded
libraries were mixed and sequenced with Illumina HiSeq 2000. Raw reads were separated according to their barcodes and mapped to the S. pombe genome using Bowtie. Mapped reads were normalized to reads per million and visualized in IGV.

Silencing Assays
Silencing assays were performed from overnight cultures grown in 5ml YEA (yeast extract supplemented with adenine). Ten fold serial dilutions were made so that highest density spot contained 1.2 x 10^5 cells. Cells were spotted on normal YEA, 5-FOA and TBZ plates (same as normal plates with the addition of 1g/lit 5-fluoro-orotic acid and 10 µg/ml thiabendazole). The plates were then incubated at 32°C for 3 days.

Immunoprecipitations
For immunoprecipitations non-tagged control and Lem2 Tap -Nur1 myc cells were harvested at OD600 = 1.5-2 and resuspended in lysis buffer (20 mM Hepes–NaOH at pH 7.5, 100 mM NaCl, 5 mM MgCl2, 1 mM EDTA pH 8.0, 0.5 mM DTT, 10% glycerol, 0.25% Triton X-100, 1 mM PMSF, complete Protease Inhibitor Cocktail). Cells were disrupted by bead beating and were cleared by centrifugation at 13,000 rpm for 5 min. Protein concentrations were normalized using the BioRad protein assay, and the supernatant was incubated with 30 ul of IgG-conjugated Dynabeads for 2 hr at 4°C. Beads were washed four times with ice-cold lysis buffer and bound proteins were analyzed by SDS-PAGE followed by western using anti-myc antibody.
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Author contributions:

S.B., Z.F., and MA designed research. S.B., Z.F., R.R., EA., and MA performed experiments and analyzed data. SB and MA wrote the paper.
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Figure Legends

Figure 1
Lem2-Nur1 complex is essential for heterochromatic gene silencing. (A) Lem2 interacts with Nur1 in vivo. Western blot analysis of coimmunoprecipitation experiments showing that myc Nur1 immunoprecipitates with Lem2-TAP. Whole cell extracts prepared from cells expressing TAP-Lem2 and myc-Nur1 or from a control strain were incubated with anti-TAP antibody, and immunoprecipitated fractions were analyzed by Western blotting with anti-myc antibodies. (B) Schematic representation of the pericentromeric DNA repeats of the *S. pombe* centromere 1, central core region (cnt-blue box), the *imr1R* (light grey arrow), and the *dg* and *dh* elements of *otr1R* are shown as light green and light blue arrows, respectively. The site of the ectopic ura4+ insert is shown as black line. (C) Tenfold serial dilution plating on counter-selective fluoroorotic acid (FOA) medium used to examine gene silencing of ura4+ reporter gene inserted at the centromeric *imr* (*imr1R::ura4+*). (D and E) Quantitative RT-qPCR was used to assay expression of *imr1::ura4+* and centromeric dg repeats. RT-qPCR shows these transcripts are derepressed in *lem2* and *nur1* deletion strains; *clr4Δ* was used as a positive control. The error bars represent standard deviations from three independent experiments. All values were normalized to act1+ transcript levels. (F) Chromatin immunoprecipitation experiments showing Pol II enrichment at centromeric dg repeats in the indicated strains. (G) TBZ sensitivity assay. Tenfold serial dilution of wild type or mutant cells were plated on media with or without 15 mg/lit microtubule-destabilizing drug thiabendazole (TBZ). *Lem2* and *nur1* deleted cells show hypersensitivity to the microtubule destabilizing compound TBZ.

Figure 2
Lem2 and Nur1 bind to central core region of centromeres. (A) Schematic representation of centromere. (B and C) ChIP Seq experiments showing the binding profile of Lem2 and Chp1 at the centromere of chromosome 3. Libraries were sequenced on the Illumina platform and normalized to reads per million (y axis). Chromosome coordinates are indicated above the reads. (D and E) ChIP-qPCR experiments showing the binding of Lem2 and Chp1 to central core region of centromeres and centromeric dg repeats respectively. (F and G) ChIP-qPCR assays showing the association of Nur1 to central core and dg repeats of centromeres.
Figure 3
Lem2 and Nur1 binding to other silent regions of the genome. (A and B) ChIP Seq data showing association of Lem2 and Chp1 to telomers and rDNA repeats of Chr 3L and 3R. Libraries were sequenced on the Illumina platform and normalized to reads per million (y axis). Chromosome coordinates are indicated above the reads. (C and D) ChIP-qPCR analysis of Lem2 and Nur1 at the telomeric tlh1 gene. (E) Quantitative RT-PCR showing derepression of telomeres (tlh1 gene) in lem2 and Nur1 deleted cells. The error bars represent standard deviations for three independent experiments. All values were normalized to act1+ transcript levels.

Figure 4
Lem2 and Nur1 bind repressive chromatin in heterochromatin dependent manner. (A and B) ChIP Seq experiments showing loss of Lem2 association to repressive chromatin in the wild type and clr4Δ cells. (C and D) ChIP-qPCR analysis of lem2 and nur1 binding for the indicated wild-type and mutant cells. Error bars reflect SD. Error bars represent the standard deviations from three independent biological experiments.

Figure 5
Lem2-Nur1 regulates SHREC and Epe1 binding to heterochromatin. (A and B) Chromatin immunoprecipitation experiments showing that loss of heh1 or nur1 causes decrease in levels of H3K9me2 and Swi6 at the centromeric dg repeats. (C and D) ChIP-qPCR analysis of Clr1-Flag and Epe1-Flag at the centromeric dg repeats in wild type and lem2 Δ cells. Immunoprecipitations were performed using wild type, lem2Δ, and nur1Δ cells carrying either Clr1-Flag or Epe1-Flag gene. Untagged cells were used as a control. (E) ChIP-qPCR experiments showing H3K14 acetylation levels in the wild type, lem2 Δ and nur1 Δ cells. The error bars represent standard deviations from three independent experiments.
Figure 6

Model for Lem2-Nur1 in heterochromatin gene silencing. Lem2-Nur1 is recruited to silent regions of genome in heterochromatin dependent manner and promotes SHREC binding which causes deacetylation of histones and subsequent repression. Methylation of H3K9 by Clr4 and subsequent recruitment of Swi6 localizes heterochromatin to nuclear periphery. Tethering to nuclear periphery and maintenance of silencing depends on interaction of heterochromatin domains with inner nuclear membrane proteins like lem2 and Nur1. Lem2 and Nur1 regulate SHREC binding and subsequent deacetylation of histones causes repression.
Figure 1

A. Western blot showing Nur1 myc and Lem2 TAP proteins.

B. Schematic diagram of the imr1::ura4+ pathway.

C. Yeast transformation assay results for Wt, lem 2Δ, nur 1Δ, Δlem2 Δnur1, and clr 4Δ strains in YEA and FOA media.

D. Graph showing relative expression of RT-PCR for Wt, lem 2Δ, nur 1Δ, Δlem2 Δnur1, and clr 4Δ strains.

E. Graph showing relative expression of RT-PCR for dg dosage.

F. ChIP-qPCR results for Wt, lem 2Δ, nur 1Δ, Δlem2 Δnur1, and clr 4Δ strains.

G. Yeast surface transformation assay results for Wt, lem 2Δ, nur 1Δ, Δlem2 Δnur1, and clr 4Δ strains in YEA and TBZ media.
Figure 2
Figure 3

A

Chr 3 L

18kb

2 kb

4 kb

6 kb

8 kb

12 kb

14 kb

16 kb

Reads per million

Chp1

Lem2

Telomeres

rDNA repeats

B

Chr 3 R

2443 kb

2446 kb

2448 kb

2450 kb

Chp1

Lem2

C

D

Telomeres rDNA repeats

ChIP-qPCR

ChIP-qPCR

ChIP-qPCR

E

Relative expression

RT-PCR

Tlh1

Tlh1

Wt

lem2

nur1

clr4

lem2

nur1

clr4

lem2Δ

nur1Δ

clr4Δ

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Figure 4

A

Lem2

B

Lem2 - clr4 Δ

C

ChIP-qPCR

D

ChIP-qPCR

Figure 4
Figure 5
Recruitment

Silencing

SHREC

Epe1

Figure 6
Table 1: List of Strains used in the paper.

| Strain   | Genotype                                      |
|----------|-----------------------------------------------|
| SPY137   | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 |
| SPY 815  | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 clr4Δ::kanMX |
| SPY 4000 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 heh1Δ::kanMX |
| SPY 4001 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 nur1Δ::kanMX |
| SPY 4002 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 heh11Δ::kanMX nur11Δ::hphMX |
| SPY 4003 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 Kan-TAP-Heh1 |
| SPY 4004 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 Kan-TAP-Nur1 |
| SPY33    | h+ leu1-32 ad6-216 ura4DS/E imr1R(Nc o1):ura4+orl1 chp1-TAP-kanMX6 |
| SPY 4005 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 heh1Δ::kanMX Clr1 Flag::hphMX |
| SPY 4006 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 heh1Δ::kanMX Epe1 Flag::hphMX |
| SPY 4007 | h+ otr1R(Sp hijo):ura4+ ura4-DS/E leu1-32 ade6-M210 Kan-TAP-Heh1 Nur1 myc::hphMX |
Role of inner nuclear membrane protein complex Lem2-Nur1 in heterochromatic gene silencing in S. pombe

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