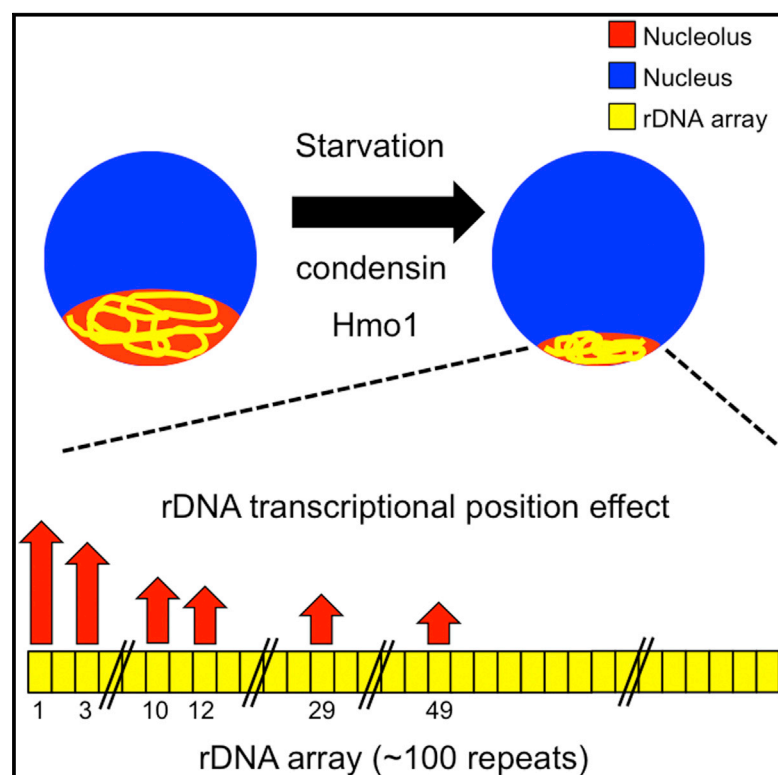Report

Condensin and Hmo1 Mediate a Starvation-Induced Transcriptional Position Effect within the Ribosomal DNA Array

Graphical Abstract

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In Brief

The regulation of individual repeats within a tandem DNA array remains poorly understood. Wang et al. use repeat-specific reporters in the highly repetitive rDNA of *Saccharomyces cerevisiae* to show that RNAPII activity in starved cells depends on relative repeat position. They also identify two nucleolar factors controlling this position effect.

Highlights

- Starvation induces a transcriptional position effect in the yeast rDNA

- The position effect arises during condensin-dependent nucleolar contraction

- Nucleolar contraction also requires the HMG-protein Hmo1

- The position effect is reduced upon disruption of condensin or Hmo1

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Condensin and Hmo1 Mediate a Starvation-Induced Transcriptional Position Effect within the Ribosomal DNA Array

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SUMMARY

Repetitive DNA arrays are important structural features of eukaryotic genomes that are often heterochromatinized to suppress repeat instability. It is unclear, however, whether all repeats within an array are equally subject to heterochromatin formation and gene silencing. Here, we show that in starving _Saccharomyces cerevisiae_, silencing of reporter genes within the ribosomal DNA (rDNA) array is less pronounced in outer repeats compared with inner repeats. This position effect is linked to the starvation-induced contraction of the nucleolus. We show that the chromatin regulators condensin and Hmo1 redistribute within the rDNA upon starvation; that Hmo1, like condensin, is required for nucleolar contraction; and that the position effect partially depends on both proteins. Starvation-induced nucleolar contraction and differential desilencing of the outer rDNA repeats may provide a mechanism to activate rDNA-encoded RNAPII transcription units without causing general rDNA instability.

INTRODUCTION

Repetitive DNA arrays are important constituents of eukaryotic genomes and affect many aspects of basic cell physiology (Richard et al., 2008). A prominent example is the ribosomal DNA (rDNA), which is found in large tandem-repeat arrays in most eukaryotes. In the yeast _Saccharomyces cerevisiae_, the rDNA locus consists of more than 100 copies of a 9.1-kb repeat unit and accounts for nearly 10% of the genome. The sheer size of this array raises intriguing questions about its spatial organization and whether all repeats are subject to the same regulation.

Gene expression in the yeast rDNA is tightly controlled. Each repeat encodes an RNAPI-transcribed _35S_ rRNA gene and an RNAPIII-transcribed _5S_ rRNA gene. In addition, RNAPII-transcribed sequences are encoded in the intergenic spacer sequences, _IGS1_ and _IGS2_, as well as on the anti-sense strand of the _35S_ rRNA gene (Coelho et al., 2002; Kobayashi and Ganley, 2005). A specialized chromatin state selectively silences RNAPII-dependent gene expression within the rDNA to protect repeats from non-allelic recombination, which is stimulated by intergenic transcription (Mekhail and Moazed, 2010; Ganley and Kobayashi, 2014). Silencing is controlled by several histone modifiers, including the sirtuin Sir2 (Huang, 2002; Kueng et al., 2013; Ryu and Ahn, 2014). In addition, the conserved condensin complex (comprising Smc2, Smc4, Brn1, Ycs4, and Ycg1) has been identified as a modulator of both Sir2-dependent and Sir2-independent silencing pathways in the rDNA (Machín et al., 2004; Ryu and Ahn, 2014).

rRNA production in _S. cerevisiae_ is downregulated in response to gradual exhaustion of nutrients during saturating growth and upon acute shift to various low-nutrient conditions (Conrad et al., 2014). Although there are regulatory differences between the respective starvation responses (Klosinska et al., 2011), most involve signaling by TOR kinase, a conserved regulator of cell growth and ribosome biogenesis (Loewith and Hall, 2011). Inhibition of TOR kinase by rapamycin leads to reduced rRNA expression, increased rDNA stability, and enhanced Sir2 binding in the rDNA (Ha and Huh, 2011). Altered transcriptional activity of the rDNA is also reflected in the size of the nucleolus, the subnuclear structure organized by the rDNA. The nucleolus occupies approximately one-third of the nucleus in metabolically active yeast, but shrinks upon nutrient depletion or rapamycin treatment (Ashrafi et al., 1999; Sinclair and Guarente, 1997; Tsang et al., 2003). This nucleolar reorganization is condensin dependent and involves a physical compaction of the rDNA array (Tsang et al., 2007).

Evidence suggests that there are physiological differences between individual rDNA repeats despite their identical DNA sequence. Repeats coexist in two chromatin states, harboring either dormant _35S_ rRNA genes with stable nucleosome occupancy (Dammann et al., 1993) or actively transcribed _35S_ genes enriched for the HMG protein Hmo1 (Merz et al., 2008). Furthermore, rDNA repeats either fire their replication origins or get passively replicated (Pasero et al., 2002). In both cases, this physiological heterogeneity appears to be independent of repeat position (Dammann et al., 1995; Pasero et al., 2002). In contrast, position effects are apparent in some meiotic mutants, in which rDNA recombination is specifically elevated in the outer repeats of the rDNA (Vader et al., 2011). Here, we investigate the
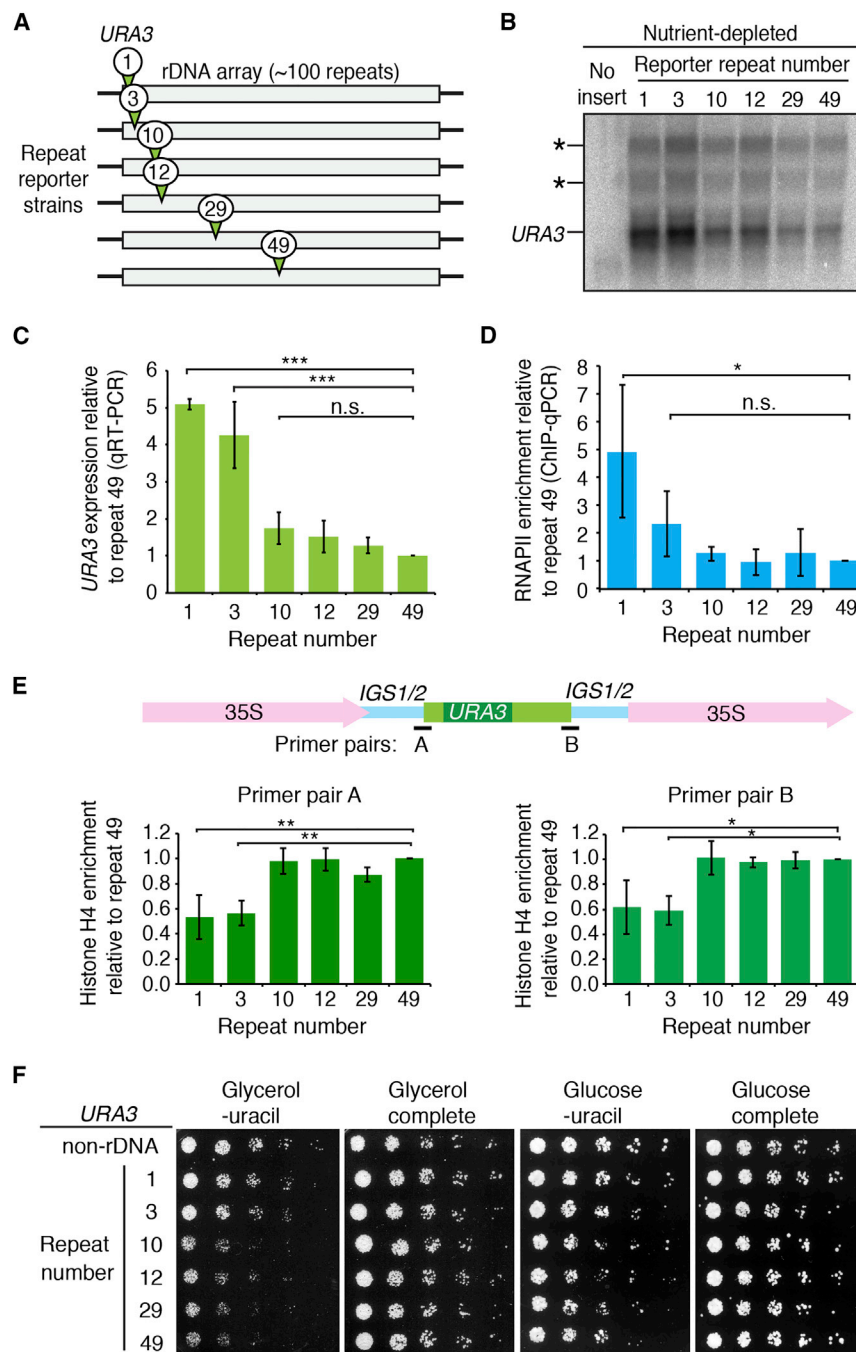
Figure 1. Reporter Gene Expression in Nutrient-Depleted Cells Depends on Position within the rDNA Array

(A) Cartoon depicting the six isogenic reporter lines used in this study. Each line carries a *URA3* reporter insertion at a homologous position within a different rDNA repeat.

(B) Northern blot detecting repeat-specific *URA3* expression in nutrient-depleted cells (for the corresponding log-phase cultures, see Figure S1B). Asterisks indicate rDNA-derived read-through transcripts observed in nutrient-depleted cells.

(C) Repeat-specific *URA3* expression in nutrient-depleted cells measured by qRT-PCR and normalized within strains to *UBC6* before normalization to repeat 49.

(D) ChIP-qPCR detecting RNAPII (8WG16) occupancy in six repeat-specific *URA3* reporters.

(E) ChIP-qPCR detecting histone H4 occupancy at the junctions of the repeat-specific reporter constructs. Primer pair A amplifies the junction between the reporter sequence and *IGS1*. B amplifies the junction with *IGS2*. Error bars in (C)–(E) show SD of three independent experiments, each analyzed in triplicate qPCRs. Significance was determined by a Welch two-sample t test. For all tests, *p < 0.05, **p < 0.01, ***p < 0.001, n.s., not significant.

(F) Plate growth assays of the repeat-specific reporter lines. 3.5-fold serial dilutions were plated onto synthetic glycerol (left) and glucose (right) media supplemented with either uracil (complete) or lacking uracil (−ura). The "Non-rDNA" control strain has *URA3* integrated on Chr. XI between *YKL075C* and *YKL077W*.

See also Figure S1.

possibility that RNAPII silencing may similarly depend on position within the rDNA.

RESULTS

To test whether transcriptional silencing differs between individual repeats of the rDNA array, we took advantage of an existing collection of isogenic rDNA insertion lines in *S. cerevisiae* (Vader et al., 2011). These lines were created by random pop-in recombination of a *URA3* reporter construct into the *IGS1/2* region of

the rDNA repeats (Figure S1A). Individual insertions were mapped to repeats 1, 3, 10, 12, 29, and 49, respectively, allowing us to assay gene expression at defined points within the left half of the approximately 100 repeats of the rDNA array (Figure 1A). As the reporters are inserted at homologous positions within the different repeats, any differential behavior of the reporters must be due to their relative position within the array.

To probe for position-dependent gene expression within the rDNA, the six reporter lines were grown in parallel and analyzed by northern blotting. Reporter gene expression was measured for logarithmically proliferating (log phase) and saturated cultures (hereafter referred to as nutrient depleted). Log-phase cells produced mostly read-through transcripts (likely due to transcription initiation by RNAPI from the adjacent *35S* rRNA promoter; Figures S1A and S1B). In contrast, nutrient-depleted cells expressed a distinct transcript of the expected length of the *URA3* reporter construct (Figure 1B). The transcript first became unambiguously detectable upon exit from log phase (Figures S1C and S1D) and was produced at higher levels

from the outer repeats of the rDNA (repeats 1 and 3) than from more central repeats (29 and 49). Thus, under conditions of nutrient depletion, reporter gene expression within the rDNA is influenced by the relative position of the reporter within the array.

Several findings indicate that this position effect is due to differential activity of RNAPII. First, the *URA3* transcripts can be enriched using an oligo-dT primer and are thus polyadenylated. Quantification of these polyadenylated transcripts by quantitative RT-PCR (qRT-PCR) revealed at clear position dependency (Figure 1C). Second, the position effect is largely abolished upon disruption of Sir2 (Figures S1E and S1F), which specifically silences RNAPII-dependent transcription (Smith and Boeke, 1997). In this experiment, however, loss of position dependency may partly result from the increased reporter instability caused by loss of Sir2 (Gottlieb and Esposito, 1989). To further test the role of RNAPII, we performed repeat-specific ChIP-qPCR of RNAPII in nutrient-depleted WT cells. RNAPII occupancy was higher for reporters in the border repeats than in the central repeats (Figure 1D), indicating that RNAPII activity in the rDNA array is under positional control. In contrast, there was no overt positional bias for RNAPII occupancy in log-phase cells (Figure S1G), despite up to 8-fold higher enrichment (Figure S1H). Notably, in nutrient-depleted cells, histone occupancy next to the reporter constructs was lowest in the repeats with the highest reporter gene activity (Figure 1E). The functional significance of this anti-correlation is currently unclear, but these data indicate that chromatin states may differ between individual rDNA repeats in nutrient-depleted cells.

URA3 reporter gene activity in the rDNA is typically measured by growth assays on medium lacking uracil (−ura), although this assay is complicated by the fact that position-dependent *URA3* expression is restricted to nutrient depletion. Accordingly, when colony growth was analyzed on −ura plates containing glucose as a favorable carbon source, we observed little difference in growth between repeat-specific reporter strains (Figure 1F). When cells were spotted on −ura plates containing a nonfermentable carbon source (glycerol), a position-dependent difference in the rate of colony growth became apparent that correlated with the differences in *URA3* expression observed by northern and qRT-PCR (Figures 1B and 1C). Thus, position-dependent expression of reporter genes in the rDNA is linked to unfavorable nutrient conditions as well as nutrient depletion.

As nutrient stress causes a contraction of the nucleolus, we tested whether the appearance of the transcriptional position effect was linked to nucleolar reorganization. To establish the timing of nucleolar contraction, we followed relative nucleolar volume in a time course as cells exhausted their nutrients, using immunofluorescence (IF) of Nop1 to mark the nucleolus (Sinclair and Guarente, 1997) and a nuclear GFP construct to control for the reduced average nuclear volume of starved cultures (Jorgensen et al., 2007). Relative nucleolar volume abruptly decreased at the earliest stages of exit from log phase (Figures 2A–2C), whereas further nucleolar shrinkage was more gradual. *URA3* transcription became detectable shortly after the initial volume decrease (Figure S1D). A correlation with nucleolar contraction was also apparent when cells were treated with rapamycin to induce nucleolar shrinkage in rich medium (Tsang et al., 2003). *URA3* expression from repeat 1 was observed by northern blot-

ting within 1 hr of rapamycin addition (Figure S2A). qRT-PCR analysis (Figure 2D) revealed higher expression levels of *URA3* at the border repeats relative to repeat 49 upon rapamycin treatment, but not in pretreatment or mock-treated control cells. A similar effect was also observed upon acute shift to low glucose (Figure S2B). We conclude that multiple conditions triggering nucleolar contraction render rDNA reporter gene expression dependent on repeat position.

To probe a possible mechanistic link between the transcriptional position effect and nucleolar remodeling, we investigated the genetic requirements for nucleolar contraction upon log-phase exit. Consistent with previous findings (Tsang et al., 2007), nucleolar contraction was largely abolished in temperature-sensitive mutants of the condensin subunit Ycs4 (*ycs4-2*) after 2 hr at the restrictive temperature (37°C; Figures 2E and 2F). Notably, *ycs4-2* mutants also exhibited a defect in nucleolar contraction at the permissive temperature (23°C). Such temperature independence is in line with previous observations showing that some nucleolar phenotypes of condensin mutants can be uncoupled from the temperature-sensitive growth defect (Haeusler et al., 2008; Machín et al., 2004).

A search for additional factors involved in starvation-induced nucleolar contraction uncovered a role for the HMG protein Hmo1, which binds to open rDNA chromatin and responds to nutrient signaling by TOR kinase (Berger et al., 2007; Merz et al., 2008; Xiao and Grove, 2009). Analysis of Nop1 staining revealed that the relative nucleolar volume of *hmo1Δ* mutants did not significantly change upon nutrient depletion (Figures 2G and 2H) and remained at log-phase levels even after 48 hr of nutrient depletion to account for possible effects from the slower growth of *hmo1Δ* mutants. Thus, like condensin, Hmo1 promotes nucleolar contraction in response to starvation.

Loss of Hmo1 also led to changes in nucleolar morphology not observed in the condensin mutant. In contrast to the crescent-shaped nucleolus observed in WT and condensin mutants, *hmo1Δ* mutants exhibited globular or ring-like Nop1 staining (Figure 2G). Moreover, the Nop1 domain was often separated from the nuclear chromatin by a DAPI-negative gap (arrow, Figure 2G), which increased the distance between the centroid of the nuclear chromatin and the centroid of the nucleolus by approximately 50% in log-phase *hmo1Δ* mutants compared with WT (Figure 2I). However, because this gap was also observed in nutrient-depleted *hmo1Δ* mutants (Figure 2I) and was not apparent in condensin mutants, it is unlikely to be related to nucleolar contraction.

Given the requirement of condensin and Hmo1 for nucleolar contraction, we asked whether their binding patterns in the rDNA change following nutrient depletion. Condensin and Hmo1 are enriched on the rDNA, but localize to different parts within the repeats (Berger et al., 2007; Johzuka et al., 2006; Kasahara et al., 2007; Wang et al., 2004). We performed ChIP-qPCR for the condensin subunit Smc4 tagged with Pk9 in log-phase and nutrient-depleted cells using a previously published set of primers spanning an entire rDNA repeat (Figure 3A) (Huang and Moazed, 2003). Log-phase cells exhibited strong enrichment of Smc4 at the replication fork barrier (Figures 3B and 3C; *RFB*, primer pair 15), as observed previously for other condensin subunits (Johzuka et al., 2006). In nutrient-depleted cells,
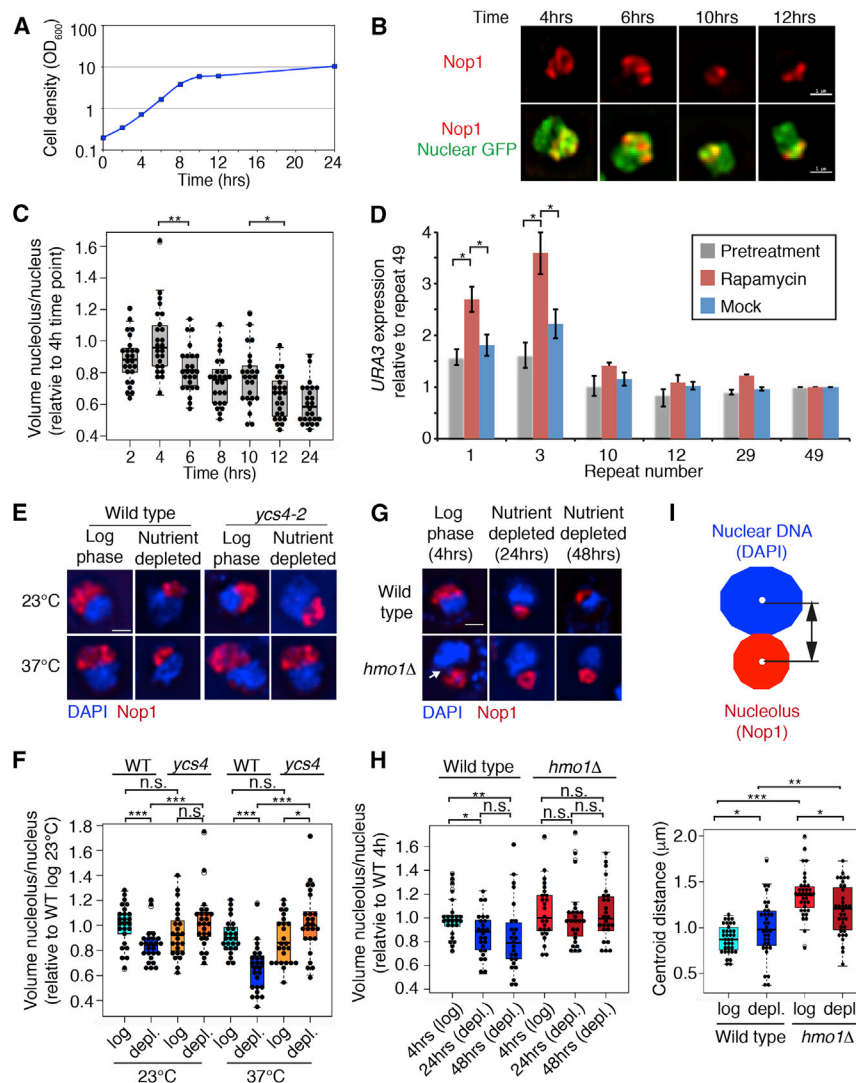
Figure 2. Nucleolar Contraction upon Nutrient Depletion Requires Condensin and Hmo1

(A) Cell density of WT cells growing in rich medium (YPD) over time. Cells were seeded at low density (8.5×10^5 cells/ml), OD_{600} = 0.2. Arrow indicates exit from log phase.

(B and C) Relative nucleolar volume from the same time course. Samples were taken at the indicated time points, fixed, and stained for Nop1 and nuclear GFP (tetR-NLS-GFP) to determine nucleolar and nuclear volume, respectively. (B) Representative images of Nop1 (red) and nuclear GFP (green) staining. (C) Relative nucleolar volume was determined for 25 cells for each time point and was normalized to the 4-hr time point.

(D) Repeat-specific URA3 expression before and after treatment with rapamycin or ethanol (mock) was measured by qPCR. Values are normalized against UBC6 and repeat 49 per treatment. Error bars indicate SD of three independent experiments, each analyzed in triplicate.

(E) Representative IF images of cells stained for Nop1 (red) and DAPI (blue) to label the nucleolus and non-nucleolar chromatin. Scale bar represents 1 μm.

(F) Relative nucleolar volume compared with nuclear GFP normalized to WT log phase at 23°C; n = 25 for each condition.

(G) Representative IF images of hmo1Δ mutants grown at 30°C in synthetic complete medium. Cells were stained for Nop1 (red) and DAPI (blue). Arrow indicates DAPI-negative gap in hmo1Δ mutants.

(H) Relative nucleolar volume as in (F); n = 25. Nucleolar volume was normalized to WT log phase.

(I) Distance between the centroids of the nucleolus and the non-nucleolar chromatin in WT and hmo1Δ mutants as depicted in the schematic (top); n = 35. Significance tests were (D) Welch two-sample t test; (C, F, H, and I) Wilcoxon signed-rank test. See also Figure S2.

two changes were apparent. First, Smc4 binding was modestly increased over the 35S rRNA gene (Figure 3B), consistent with the increased binding of Ycs4-12xMYC observed upon TOR inhibition (Tsang et al., 2007). The degree of condensin enrichment seen here is substantially less dramatic, however, possibly due to experimental differences or as a result of the 12xMYC tag, which has been shown to alter Ycs4 functionality in starved cells (Yu and Koshland, 2003). Second, nutrient-depleted cells exhibited an additional strong peak of Smc4 enrichment near the origin of replication (Figure 3C; ARS, primer pair 22). These data indicate that nucleolar compaction is associated with a redistribution of condensin within the rDNA repeats.

ChIP-qPCR analysis also revealed altered rDNA binding of Hmo1 in nutrient-depleted cells. Previous studies found Hmo1 enriched across the 35S rRNA gene (Berger et al., 2007; Kasahara et al., 2007). Under our conditions, this enrichment pattern was barely detectable in log-phase cells (Figures 3D and S3). However, in nutrient-depleted cells, Hmo1 binding across the 35S rRNA gene was strongly increased, leading to

an overall 6-fold enrichment compared with other rDNA regions (IGS1+5S+IGS2; Figure 3D). Thus, the association patterns of both condensin and Hmo1 change upon nutrient depletion.

Despite their concurrent redistribution, neither factor influences the binding pattern of the other. Disruption of HMO1 does not alter Smc4 enrichment within IGS1 and IGS2 in log phase or nutrient-depleted cells (Figure S3A), and Hmo1 distribution is similarly unaffected in condensin (ycs4-2) mutants (Figures S3B–S3D). These data indicate that starvation-induced redistribution of condensin and Hmo1 occurs by independent mechanisms. Moreover, as loss of either factor prevents nucleolar contraction, nucleolar contraction occurs mechanistically downstream of the starvation-induced redistribution of condensin and Hmo1.

If nucleolar contraction is linked to position-dependent reporter gene expression in the rDNA, then this position effect should be reduced in the absence of condensin or Hmo1 function. Therefore, we introduced condensin and hmo1 mutations into the repeat-specific URA3 reporter lines. Reporter gene positions were in all
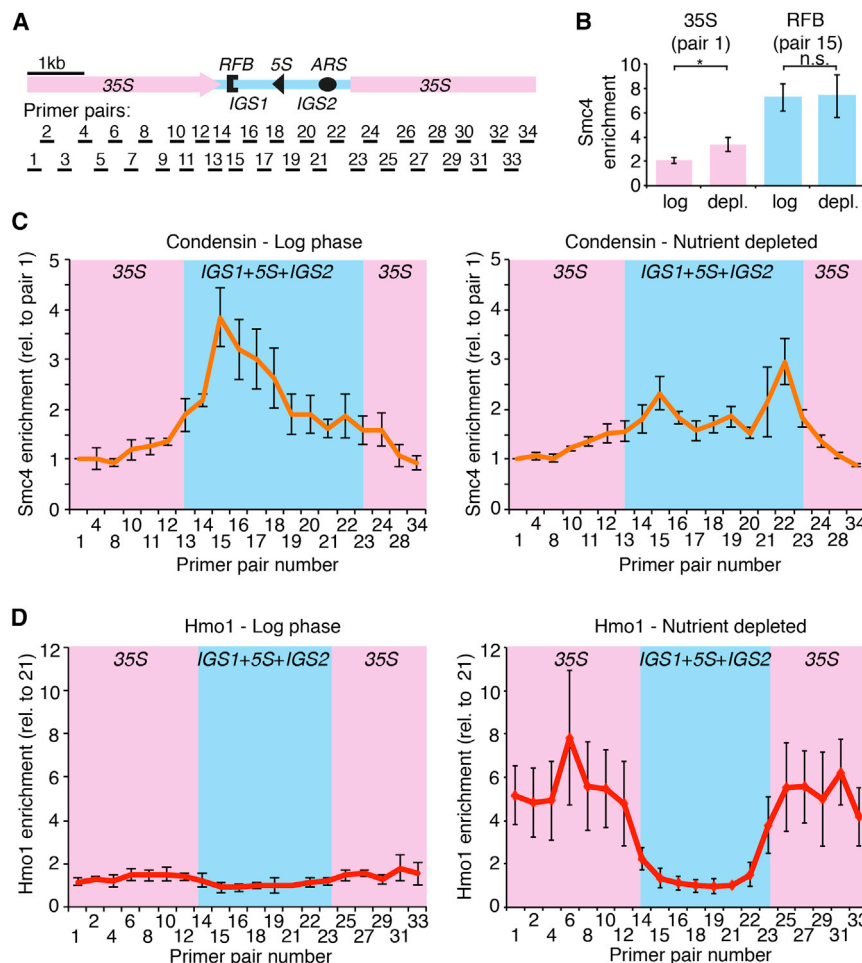
Figure 3. Nutrient Depletion Causes a Redistribution of Condensin and Hmo1 within the rDNA

(A) Schematic indicating the set of primers spanning an entire rDNA repeat (Huang and Moazed, 2003) used for the ChIP-qPCR experiments in (B)–(D).

(B) Smc4-Pk9 enrichment in the *35S* rDNA (primer pair 1) and near the *RFB* (primer pair 15) in log-phase and nutrient-depleted cells at 30°C. Samples were normalized to *CUP1*. Error bars indicate SD of three independent experiments, analyzed on the same qPCR plate. Significance was shown with Welch two-sample t test.

(C) Smc4-Pk9 enrichment across an rDNA repeat in log-phase cells (left) and nutrient-depleted cells (right). Signals were normalized to a lowly enriched position (primer pair 1) for easier visualization of fold differences.

(D) Hmo1 enrichment across an rDNA repeat in log-phase and nutrient-depleted cells. Signals are normalized to primer pair 21. Error bars in (C) and (D) indicate SD of three independent experiments, each analyzed in duplicate.

See also Figure S3.

ment of the transcriptional position effect within the rDNA, nucleolar contraction alone is not sufficient to maintain the position effect over extended periods of nutrient depletion.

DISCUSSION

Our results reveal unexpected positional differences in gene silencing within the rDNA of starved cells. Such position-dependent desilencing may result from a more permissive chromatin state spreading into the rDNA array upon nutrient depletion. It may also stem from increased exposure of the boundary-proximal repeats to a non-nucleolar environment that promotes gene activity or reduces silencing. Chromatin spreading may explain the repeat-dependent difference in histone occupancy (Figure 1E), although we note that we have so far failed to observe any difference in occupancy between the different reporter genes using ChIP analysis of Sir2, condensin, or Hmo1 enrichment (Figure S1I; data not shown). Conversely, increased residence outside the nucleolus would be akin to the previously described exit of rDNA double-strand breaks from the nucleolus during DNA repair (Torres-Rosell et al., 2007). Indeed, the genome of starved yeast cells undergoes substantial reorganization (Rutledge et al., 2015), which may drive changes in gene activity. The possibility of spatial regulation is currently being tested using fluorescently labeled repeats as well as repeat-specific chromosome conformation capture.

We note that position-dependent desilencing may provide a solution for activating rDNA-encoded genes without causing the severe array instability associated with a complete loss of

cases confirmed by pulsed-field gel analysis and were found to be stable over the time frame of the experiment (e.g., Figure S4A). qRT-PCR analysis of nutrient-depleted cells revealed that the rDNA repeats in *ycs4-2* mutants exhibited less profound positional differences in gene expression compared with WT (Figure 4A), a trend that was also apparent from colony growth (Figure 4B). Similar results were obtained for another temperature-sensitive condensin mutant (*ycg1-2*, Figures 4B and S4B). Thus, condensin is at least partly required for position-dependent reporter gene regulation in the rDNA. In both cases, the position effect was lost at the permissive temperature (23°C). Therefore, this defect, like that of *ycs4-2* mutants in nucleolar contraction, is largely temperature independent.

The position effect was also strongly reduced in the absence of Hmo1. qRT-PCR analysis revealed only small positional differences in repeat-specific reporter activity in *hmo1Δ* mutants after both 24 and 48 hr of nutrient depletion (Figure 4C). The same trend was apparent from colony growth (Figure 4D). Therefore, Hmo1 is required for efficient nucleolar contraction and establishment of the position effect. Unexpectedly, the position effect was also reduced in WT after 48 hr of nutrient depletion (Figure 4C), despite a persistently contracted nucleolus (Figure 2E). Thus, although nucleolar contraction is linked to the establish-
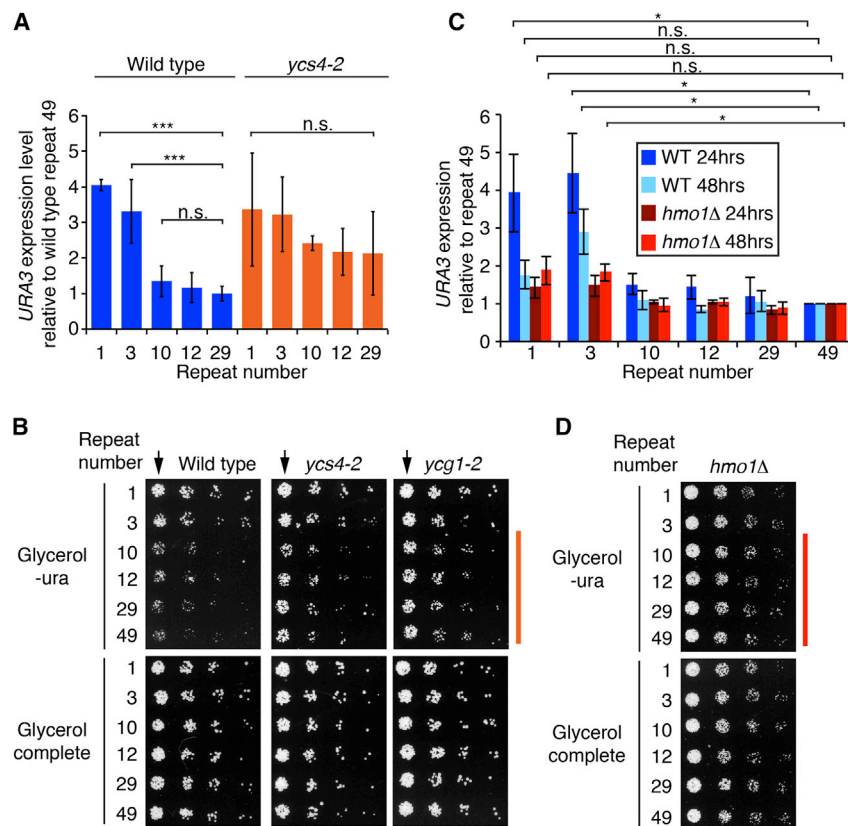
Figure 4. Condensin and Hmo1 Mediate the Transcriptional Position Effect in the rDNA Array

(A) Repeat-specific *URA3* expression was measured in nutrient-depleted *ycs4-2* mutants at 23°C by qRT-PCR. Signals were normalized to *UBC6* and repeat 29. Error bars indicate SD of three independent experiments, each analyzed in triplicate.

(B) Plate growth assays of WT, *ycs4-2* and *ycg1-2* cells carrying repeat-specific reporter cassettes. 3.5-fold serial dilutions were plated onto synthetic glycerol medium supplemented with uracil (complete) or lacking uracil (−ura) and grown at 23°C. Arrows highlight the dilutions with the most obvious position-dependent differences in colony size.

(C) Repeat-specific *URA3* gene expression was determined in nutrient-depleted *hmo1Δ* cells by qRT-PCR. Values are normalized against *UBC6* and repeat 49 for each condition. Because of the slow growth of *hmo1Δ* mutants, cells were harvested after 24 and 48 hr of nutrient depletion. 48 hr of nutrient depletion in *hmo1Δ* mutants roughly corresponds to 24 hr of nutrient depletion in WT, as determined by growth curves. Error bars are as in (A). Significance in (A) and (C) is shown with Welch two-sample t test.

(D) Plate growth assays of *hmo1Δ* mutants carrying repeat-specific reporter cassettes. Analysis is as in (B), but cells were grown at 30°C.

See also Figure S4.

silencing (Mekhail and Moazed, 2010). The rDNA of many organisms, including yeast and humans, encodes RNAPII-expressed transcripts that must be expressed at certain stages without causing general instability (Coelho et al., 2002; Kobayashi and Ganley, 2005; McStay and Grummt, 2008). In yeast, the *TAR1* gene, encoded in the anti-sense direction of the *35S* gene, is of particular interest because it is specifically expressed upon nutrient depletion (Bonawitz et al., 2008; Galopier and Hermann-Le Denmat, 2011), coincident with positional desilencing. Strikingly, nutrient-dependent regulation of *TAR1* is lost when the gene is analyzed on a plasmid (Galopier and Hermann-Le Denmat, 2011), suggesting that rDNA structure contributes regulatory control.

Our work adds to a growing body of evidence that position effects are inherent to the structural organization of repetitive DNA arrays. Previous analyses showed a position effect for meiotic recombination in the rDNA in specific mutants (Vader et al., 2011). We now demonstrate that position effects also affect reporter gene expression and occur in WT cells. An accompanying study by He et al. (2016 [this issue of *Cell Reports*]) also uncovered transcriptional position effects in the repetitive pericentromeric regions of *Schizosaccharomyces pombe*. Thus, position effects are not limited to the rDNA and occur in evolutionarily highly diverged organisms. Tandem repetitive DNA arrays occupy a significant fraction of the most eukaryotic genomes and play crucial roles in regulating gene expression, chromosome organization and disease (Neguem-

bor and Gabellini, 2010; Richard et al., 2008). We predict that higher order organization and internal position effects represent an important mechanism of epigenetic regulation for the repetitive genome.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions

Strains used in this study are of the SK1 background and are listed in Table S1. Stability of the reporter positions was reconfirmed after all genetic manipulations. Details on strain construction and culture conditions are provided in the Supplemental Experimental Procedures.

Estimation of Relative Nucleolar Volume

Nucleolar and nuclear volumes were estimated from deconvolved z stacks of fixed cells stained for Nop1 and nuclear GFP (*tetR-NLS-GFP*) using SoftWoRX2.50 software (Applied Precision).

Other Procedures

Conditions for IF, ChIP, northern blotting, reverse transcription, and qPCR are outlined in the Supplemental Experimental Procedures and Table S2.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.01.005.

AUTHOR CONTRIBUTIONS

D.W., A.M., and A.H. designed the study and acquired, analyzed, and interpreted the data. G.P. acquired initial data. All authors contributed to drafting and revision of the manuscript.

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