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A role for the nucleoporin Nup170p in chromatin structure and gene silencing

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Abstract

Embedded in the nuclear envelope, nuclear pore complexes (NPCs) not only regulate nuclear transport, but also interface with transcriptionally active euchromatin, largely silenced heterochromatin, as well as the boundaries between these regions. It is unclear what functional role NPCs play in establishing or maintaining these distinct chromatin domains. We report that the yeast NPC protein Nup170p interacts with regions of the genome containing ribosomal protein and subtelomeric genes. Here, it functions in nucleosome positioning and as a repressor of transcription. We show that the role of Nup170p in subtelomeric gene silencing is linked to its association with the RSC chromatin-remodeling complex and the silencing factor Sir4p, and that the binding of Nup170p and Sir4p to subtelomeric chromatin is cooperative and necessary for the association of telomeres with the nuclear envelope. Our results establish the NPC as an active participant in silencing and the formation of peripheral heterochromatin.

Keywords

nuclear pore complex; chromatin-remodeling; telomere; heterochromatin; ribosomal protein genes; RSC; Sth1p; Sir4p; Rap1p; Nup170p

INTRODUCTION

The nuclear envelope (NE) functions as a barrier between the genome and the cytoplasm. To regulate the exchange of macromolecules between the cytoplasm and the nucleus, molecules

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must traverse large, proteinaceous channels, termed nuclear pore complexes (NPCs), that extend across the NE. The role of NPCs in nuclear transport has been extensively studied (reviewed in: Aitchison and Rout, 2012). NPCs are cylindrical channels composed of repetitive subunits organized with 8-fold symmetry around the central axis. Thus, despite its considerable mass, ~50 MDa in yeast, an NPC is composed of only ~30 proteins, termed nucleoporins or Nups, present in multiple copies (Rout et al., 2000). The NPC is composed of membrane proteins, a symmetrical core scaffold, containing the Nup84p and Nup170p-Nup157p complexes, and FG-Nups that populate the central channel and form cytoplasmic fibrils and a nuclear basket structure. The FG-Nups interact with transport factors and directly facilitate nucleocytoplasmic transport.

The NPC and other features of the NE also provide attachment sites for the underlying chromatin, and each establishes a distinct environment for chromatin. For example, transcriptionally silenced heterochromatin preferentially associates with the inner nuclear membrane (INM; reviewed in: Taddei et al., 2010) whereas active genes appear associated with the NPC. In *S. cerevisiae*, the silent mating type loci and telomeres are associated with the INM, and anchoring chromatin at the INM promotes silencing (Andrulis et al., 1998). A contributing factor to this peripheral gene silencing is the local concentration of the silent information regulator (SIR) complex, comprising Sir2p, Sir3p, and Sir4p (Gotta et al., 1996; Gasser et al., 2004) at the NE. These proteins bind subtelomeric and telomeric chromatin, and with the yKu70/yKu80 dimer, mediate telomere binding to the INM proteins Mps3p and Esc1p (Reviewed in: Taddei et al., 2010).

NPCs have long been postulated to tether chromosomal loci at the NE. Morphological observations of metazoan cells have revealed euchromatin channels extending from NPCs into the nucleoplasm and penetrating peripheral heterochromatin, positioning NPCs at the interface between these two chromatin states. Recent analyses of NPC-chromatin interactions in yeast have identified NPCs in association with transcriptionally active chromatin, silenced chromatin, and boundary chromatin, which displays characteristics of both states. Multiple Nups have been detected in association with active genes and certain genes exhibit Nup-dependent recruitment to the NE upon transcriptional activation (reviewed in: Van de Vosse et al., 2011). Moreover, Nup2p has been shown to play a role in demarcating boundaries between active and silenced chromatin (Ishii et al., 2002; Dilworth et al., 2005). NPCs have also been proposed to organize silent chromatin domains at the NE, and two NPC-associated proteins, Mlp1p and Mlp2p, were implicated in telomere tethering at the NE (Galy et al., 2001; Feuerbach et al., 2002). However, these results have been contested (Hediger et al., 2002a and 2002b). More recently, members of the Nup84 complex have been implicated in both subtelomeric silencing and telomere tethering (Therizols et al., 2006).

How NPCs influence chromatin structure remains unknown, in large part due to a limited understanding of the molecular basis for their interactions. We investigated the role of a core component of the yeast NPC, Nup170p, in chromatin structure. Nup170p has been implicated in the maintenance of heterochromatin structure in centromeric regions (Kerscher et al., 2001), and counterparts of Nup170p in higher eukaryotes are physically linked to chromatin-modifying complexes (Mendjan et al., 2006; Kehat et al., 2011). Here, we report physical and functional interactions between Nup170p and specific chromatin domains including RP genes and subtelomeric and telomeric regions. We show that Nup170p, through its interactions with the RSC chromatin remodeling complex and the silencing factor Sir4p, is required for the formation of subtelomeric chromatin and its NE association.

RESULTS

Nup170p functionally interacts with chromatin-modifying complexes

The combined functions of yeast Nup170p and its paralogue, Nup157p, are essential for cell viability (Aitchison et al., 1995; Makio et al., 2009); however, strains lacking each protein are viable. Thus, we used synthetic genetic array (SGA) analysis (Tong et al., 2001) to screen a non-essential gene deletion library for mutants displaying epistatic interactions with null mutants of *NUP170* (*nup170*Δ). These interacting mutations are interpreted to compromise an essential structure or network, or two functionally redundant pathways contributing to an essential cellular function. 73 gene deletions displayed reduced fitness in combination with *nup170*Δ, predictably including NPC components (Figure 1 and Table S1). Notably, a significant proportion of the interacting genes (27 of 73; p-value = 1.99×10^{-15}) encode subunits of complexes that function in chromatin organization including chromatin-remodeling (SWR1), histone deacetylation (Rpd3L and Set3C), and histone H2BK123 ubiquitination (Figure 1 and Table S1). These interactions appeared specific for *NUP170*; SGA analysis of *nup53*Δ, *nup60*Δ, or *kap123*Δ did not yield similar results (Table S1; Ptak et al., 2009) and the cohort of *NUP170* interacting genes did not show interactions with various other Nup genes (*NUP188, NUP157, NUP53*, or *NUP2*) (Tables S1 and S2). Importantly, this role for Nup170p in chromatin organization is unlikely to be linked to nucleocytoplasmic transport, as the *nup170*Δ mutant has no detected defects in active transport (Aitchison et al., 1995; Makio et al., 2009), and its previously detected contribution to the NPC diffusion barrier is phenocopied by the *nup188*Δ mutant (Shulga et al., 2000), which did not display similar genetic interactions.

Nup170p physically interacts with the RSC complex

We investigated the physical basis for the genetic interactions between mutations in *NUP170* and the chromatin-modifying complexes. Nup170-protein A (pA) was purified from strains producing GFP-tagged versions of representative members of the genetically interacting complexes. As expected, the Nup170p binding partner Nup53p was detected (Figure 2A; Lusk et al., 2002). However, with the exception of low-levels of Rpd3-GFP, none of the GFP-tagged proteins were bound to Nup170-pA. These results implied a role for Nup170p in a functionally overlapping pathway. Such a pathway is predicted to exhibit similar genetic interactions as the *nup170*Δ mutant. Analysis of databases revealed two chromatin-remodeling complexes, INO80 and RSC, showing similar genetic interactions with the SWR1, Rpd3L, and Set3C complexes (Figure 1 and BioGRID; Stark et al., 2006). An examination of Nup170-pA bound proteins revealed Sth1p, the ATPase subunit of the RSC complex, but not INO80 subunits (Figure 2). By contrast, Sth1p was not detected bound to Nup84-pA or Nup188-pA. Reciprocal experiments using Sth1-pA also detected associated with Nup170p and Nup53p, as well as RSC complex members. Notably, Nup53p binding to Sth1-pA was dependent on Nup170p. We conclude that Nup170p physically associates with the RSC complex, potentially through its binding to Sth1p.

Nup170p is required for silencing of subtelomeric genes

RSC catalyzes nucleosome restructuring events that play a role in DNA double-strand break repair, telomere structure, and gene expression (Angus-Hill et al., 2001; Askree et al., 2004; Shim et al., 2005). To begin to assess the role of Nup170p in these functions, we examined the cellular transcription profile of the *nup170*Δ mutant using DNA microarrays. This analysis revealed 424 ORFs up-regulated and 59 ORFs down-regulated greater than 2-fold in the *nup170*Δ mutant. Strikingly, the up-regulated genes were enriched for ribosomal protein (RP) genes (109 of 138 RP genes, p-value 1.85×10^{-105}; p-values by one-tailed Fisher's exact test) and genes positioned in subtelomeric regions (i.e. within 25 kb of telomeres). The smaller number of down-regulated ORFs was randomly distributed

throughout the genome (Figure 3A). While housing only ~6% of all ORFs, subtelomeric regions contained ~28% of the genes up-regulated in *nup170*Δ cells (119 of 424, p-value 1.94×10^{-56}), with ~34% of subtelomeric ORFs (119 of 347) showing alleviated repression (see Table S3). By contrast, gene expression changes in *nup157*Δ and *nup188*Δ cells showed no enrichment of subtelomeric regions (Figure 3A).

DNA microarray analysis was also performed on cells depleted of Sth1p. *STH1* is an essential gene. Thus, to regulate its expression, the *STH1* promoter was replaced with *MET3* promoter (P_{MET3}-*STH1*) allowing rapid depletion of Sth1p to barely detectable levels 4 h after methionine repression (Figure 3B). We interrogated the transcriptome of P_{MET3}-*STH1* cells 2 h after repression, at which point *STH1* mRNA levels were reduced ~4.4-fold and Sth1p levels were down ~2-fold (data not shown). Similar to *nup170*Δ cells, a pattern of subtelomeric derepression was observed (Figure 3C), which could be reversed by reinduction of *STH1*.

Our microarray data are consistent with a role for Nup170p and RSC in subtelomeric gene silencing. This function was further evaluated using cell growth assays that provide a readout for the transcriptional state of two reporter genes, *URA3* and *ADE2*, inserted in subtelomeric regions adjacent to telomeres VII-L (Tel7L) and V-R (Tel5R) (Figure 3D). Repression of these genes prevents growth in the absence of uracil and adenine, but allows cells to grow in the presence of 5-FOA, whereas loss of a silencing factor, (e.g. Sir3p), favors derepression of *URA3* and *ADE2* allowing cell growth on medium lacking adenine and uracil, but rendering them sensitive to 5-FOA. *nup170*Δ cells showed similar silencing defects, which were reversed by an exogenous copy of *NUP170*. No silencing defects were detected in a *nup188*Δ mutant.

While certain subunits of the RSC complex are essential, others are not, allowing us to test the effects of mutations in several RSC components, including *rsc1*Δ, *rsc3*Δ, *rsc7*Δ, and *htl1*Δ, on silencing of the *URA3* and *ADE2* reporter genes. Loss of silencing was most evident and reproducibly detected in the *rsc3*Δ and *htl1*Δ null mutants (Figure 3D and Figure S1), albeit to a lesser extent than either *nup170*Δ or *sir3*Δ. The milder silencing phenotypes of these *rsc* mutants may be explained by the fact that they do not compromise the essential function of RSC.

Loss of Nup170p alters nucleosome positioning in subtelomeric chromatin

RSC is an important contributor to nucleosome structure including the organization of a nucleosome free region (NFR) adjacent to transcriptional start sites (TSS) (Hartley and Madhani, 2009). The interactions of Nup170p with RSC and its role in transcription led us to examine subtelomeric chromatin structure in the absence of Nup170p using genome-wide nucleosome mapping. Across the genome, WT cells have a canonical positioning of the −1 and +1 nucleosomes flanking the NFR followed by periodic phasing of downstream nucleosomes (Figure 4 and Figure S2; see Weiner et al., 2010). By contrast, *nup170*Δ cells had reduced occupancy of the +1 nucleosome and, to a lesser extent, the −1 nucleosome (Figure 4A). The reduction in −1 and +1 nucleosome occupancy was more prominent within subtelomeric regions (0–25 kb), while adjacent regions (25–50 kb) showed little difference from the WT control. As with the subtelomeric regions, nucleosome profiles were also altered at RP genes (Figure 4A). These data further pointed to a role for Nup170p in defining chromatin structure within subtelomeric regions and RP genes.

The consequence of Sth1p depletion on nucleosome position was also examined following *STH1* repression (Figure 4). As in previous reports (Hartley and Madhani, 2009), our genome-wide analysis revealed shrinkage of the NFR and a reduced occupancy of the +1 and −1 nucleosomes following Sth1p depletion. Clustering of subtelomeric and RP genes

revealed similar changes (Figure 4B; data not shown). Of the subtelomeric genes containing a recognizable TSS, 47 of 149 ORFs exhibited altered +1 nucleosome structure. Significantly, 42 ORFs showed similar changes in the *nup170*Δ mutant (p-value of 1.05×10^{-3}), consistent with a functional relationship between Nup170p and RSC.

Nup170p is enriched at RP genes and subtelomeric DNA

To understand how Nup170p contributes to transcriptional repression, we investigated the physical interactions of Nup170p with chromatin. Chromatin immunoprecipitation (ChIP) analysis was performed on strains producing Nup170p tagged with 9xMyc, and Nup170p-associated DNA was analyzed using DNA microarrays (ChIP-chip). Predominant among bound DNA fragments were regions adjacent to genes exhibiting increased expression in the absence of *NUP170*; of the 424 up-regulated genes, 378 had probes on the microarray within 0.5 kb of the ORF, and 178 were detected in association with Nup170p (p-value 1.8×10^{-49}; Table S3). Prominent were regions of DNA near chromosome ends (Nup170p-associated DNA bound 117 of 439 probes within 10 kb of telomeres; p-value = 1.14×10^{-48}), corresponding to subtelomeric regions of 23 of 26 telomeres analyzed (Figure 5A, 5B, and S3). Here our ChIP-chip analysis detected a specific enrichment of Nup170p at many of the genes up-regulated upon loss of Nup170p. Of the 119 genes within 25 kb of telomeres that exhibited increased expression upon deletion of *NUP170*, our microarrays contained probes for 75 of these within 0.5 kb of the ORF, of which, 57 were detected in association with Nup170p by ChIP-chip (p-value 8.1×10^{-3}; Table S3 and S4). By contrast, ChIP-chip analysis did not reveal similar levels of Nup157p enrichment at specific sites within the genome. However, we observed modest binding to chromatin regions showing the highest levels of Nup170p enrichment (see Figure 5 and Figure S3) likely reflecting the physical association of Nup157p with Nup170p (Alber et al. 2007; Amlacher et al., 2011) and chromatin interactions mediated by Nup170p. These results were supported by quantitative, real-time PCR (qPCR) assays, with subtelomeric DNA adjacent to a representative telomere (Tel6R), which was enriched with Nup170p but not Nup157p or Nup188p (Figure 5D).

In addition to subtelomeric DNA, 131 of 138 RP genes were significantly enriched with Nup170p (p-value 3.3×10^{-112}; see Figure S4). These associations strongly correlated with the observed up-regulation of 109 of 138 RP genes in the *nup170*Δ mutant (Figure S4). Thus, our results are consistent with Nup170p functioning as a transcriptional repressor that physically interacts with subtelomeric chromatin and RP genes.

Nup170p association with subtelomeric DNA is mediated by Sir4p

Transcriptional repression of subtelomeric genes has been extensively studied and various factors that contribute to this process have been identified. The yKu70/yKu80 heterodimer and the SIR complex are essential for subtelomeric chromatin structure and silencing. Since Nup170p lacks a detectable DNA binding motif, we hypothesized that its role in silencing may be linked to these key silencing factors. Therefore, we examined whether Nup170p physically interacts with members of the yKu and SIR complexes. Analysis of Nup170-pA purified from cell extracts revealed Sir4–13xMyc, but not yKu70-13xMyc bound to Nup170-pA (Figure 6A and 6B). Furthermore, we detected Nup170-13xMyc bound to purified Sir4-pA (Figure 6C). Since Sir4p binding to subtelomeric chromatin is mediated by the DNA binding protein Rap1p, we also purified Rap1-pA. As with Sir4-pA, Nup170-13xMyc bound to Rap1-pA (Figure 6D). The specificity of the Sir4p–Nup170p interaction was also supported by experiments showing Nup157p, a binding partner of Nup170p, bound to Sir4p while Nup84p, a member of a distinct NPC subcomplex did not (Figure 6E and 6F).

The association of Nup170p with Sir4p led us to examine whether Nup170p binding to subtelomeric chromatin required Sir4p. In the absence of Sir4p (*sir4*Δ) the enrichment of Nup170p at many of its subtelomeric chromatin binding sites (most dramatically at 19 of 23 analyzed) was reduced, while its association with non-subtelomeric DNA was largely unaffected (Figure 6H and Figure S5). By contrast, the loss of yKu70p (*yKu70*Δ) did not affect the DNA binding profile of Nup170p. Similarly, a *sir2*Δ mutation did not appear to alter the subtelomeric association of Nup170p (Figure S6). These results are consistent with Sir4p functioning, directly or indirectly, to facilitate Nup170p binding to subtelomeric chromatin.

Nup170p facilitates Sir4p binding to subtelomeric DNA

We hypothesized that the function of Nup170p in subtelomeric gene silencing is linked to its physical association with Sir4p. Because chromatin association of Sir4p is considered a prerequisite for its role in silencing, we examined the effect of Nup170p depletion on the association of Sir4p with its chromatin-binding partner Rap1p. In WT cells, Sir4–9xMyc is detected bound to isolated Rap1-pA, but this interaction was greatly reduced in the absence of Nup170p (Figure 7A). ChIP analysis also revealed that Sir4p binding to DNA regions adjacent to a representative telomere (Tel6R) were reduced approximately 3-fold in the absence of Nup170p (Figure 7B). Similarly, the Sir4p binding partners Sir2–9xMyc and Sir3–9xMyc also showed reduced association with Tel6R (Figure S6), consistent with the established role of Sir4p in facilitating Sir2p and Sir3p binding to subtelomeric chromatin (reviewed in: Rusche et al., 2003). These observations were in sharp contrast to Rap1p, which showed enhanced binding to Tel6R and its subtelomeric regions in the absence of Nup170p (Figure S6). While the basis for the increased binding of Rap1p is unclear, this phenotype further supports the conclusion that Nup170p plays a physiological role at telomeres.

The role of Nup170p in Sir4p localization was also examined by fluorescence microscopy. Sir4p is generally detected in 6–8 telomere clusters positioned along the nuclear periphery (Gotta et al., 1996). Mutations that reduce Sir4p binding to telomeres impair clustering and Sir4p foci redistribute to the nuclear interior (Cockell et al., 1995). We observed 67% of Sir4-GFP foci at the NE in WT and *nup157*Δ cells (Figure 7C). However, cells lacking Nup170p exhibited a diffuse intranuclear Sir4-GFP signal and the peripheral localization of Sir4-GFP foci was reduced to 41%, similar to that observed in the tethering deficient *yku70*Δ mutant (Figure 7C). Importantly, the decreased NE association of Sir4p in the *nup170*Δ mutant did not appear to be due to changes in the localization of its NE receptors Esc1p or Mps3p (Figure S7B), nor did loss of *NUP170* impair the ability of Esc1p to tether a tagged chromosomal locus to the NE (Figure S7C).

Our results support a model in which Nup170p facilitates Sir4p association with subtelomeric chromatin binding sites, including the telomere-associated protein Rap1p. Unlike Nup170p, the Rap1-interacting factor, Rif1p, antagonizes Sir4p binding to subtelomeric chromatin by competing with Sir4p for binding to Rap1p (Buck and Shore, 1995). We hypothesized that removing Rif1p and suppressing its antagonistic function would compensate for the loss of Nup170p and restore subtelomeric association of Sir4p. In support of this idea, the introduction of a *rif1*Δ mutation into a *nup170* null mutant (*nup170*Δ *rif1*Δ) rescued the NE localization of Sir4-GFP to WT levels (Figure 7C). Moreover, deletion of *RIF1* suppressed the silencing defect of the *nup170*Δ mutant, further linking the loss of silencing phenotype of *nup170*Δ cells to compromised Sir4p function (Figure S1).

Telomere tethering to the NE requires Nup170p

As Sir4p supports telomere tethering to the NE, we examined whether Nup170p also played a role in telomere positioning. Several commonly studied telomeres, Tel6R, Tel8L, and Tel14L, were tagged with 256 *lac* operators (*lacO*) in cells producing the *lacO*-binding protein GFP-LacI (Hediger et al., 2002b). Telomere position relative to the NE marker Sec63-GFP was examined, and foci scored for their localization within three concentric zones of equal area (Figure 7D and S7; Table S5). As previously observed (Hediger et al., 2002b), in WT cells Tel6R, Tel8L, and Tel14L were detected in zone 1 immediately underlying the NE (~70% of foci) during G1- and S-phase. Similar results were obtained in *nup2*Δ or *nup157*Δ mutants (Figure 7D and S7; Table S5). However, the NE association of all three telomeres was compromised in *nup170*Δ cells during G1-phase, similar to that detected in *yku70*Δ and *sir4*Δ mutants (also see Hediger et al., 2002b; Hiraga et al., 2008). In contrast to *sir4*Δ mutants, *nup170*Δ cells showed no defects in telomere localization in S-phase cells. Consistent with the effect of Nup170p loss on these individual telomeres, Rap1 foci, representative of telomere clusters, showed a similar G1-phase specific decrease in NE association in the *nup170*Δ cells (Figure 7E). Thus, we conclude that Nup170p plays an essential role in the localization of telomeres during G1-phase of the cell cycle.

DISCUSSION

The inner nuclear membrane in most cell types interacts with condensed and/or silenced chromatin. By contrast, studies in yeast and metazoan cells have established functional links between specific Nups and transcriptionally active genes. Thus, the interfaces between NPCs and the inner nuclear membrane are likely transitional zones between chromatin states. We have uncovered functional interactions between Nup170p and chromatin domains that generally reside adjacent to the NE, including subtelomeric and telomeric regions. We propose that Nup170p establishes a platform at the NPC that interacts with these chromatin regions and promotes transcriptional repression of RP genes and subtelomeric chromatin. In the context of subtelomeric chromatin, Nup170p functionally interacts with RSC and Sir4p, facilitating Sir4p assembly on subtelomeric heterochromatin, chromatin association with the NE, and repression of resident gene expression.

The functional links between Nup170p and various chromatin-modifying factors suggest that Nup170p contributes to a distinct, but related, function to that of its genetically interacting partners, including transcriptional repression and heterochromatin assembly. Set3C and Rpd3L are HDACs with roles in silencing (Ehrentraut et al., 2010), and Bre1p and its interactors mediate silencing through their downstream effects on histone H3 methylation (Sun and Allis, 2002). SWR1 also contributes to silencing by defining heterochromatin regions through exchange of canonical histone H2A for the histone variant H2A.Z (Htz1p), notably near telomeres (Mizuguchi et al., 2004). The specific genetic interactions discovered for *NUP170*, as well as other data present here, lead us to conclude that Nup170p contributes to chromatin structure, such as heterochromatin formation and resulting gene silencing, through parallel activities with its genetically interacting complexes.

This concept is supported by results documenting a role for Nup170p in the organization of nucleosomes *in vivo* (Figure 4), the first evidence that a Nup functions in this capacity. Analysis of the *nup170*Δ mutant revealed a decrease in −1 and +1 nucleosome occupancy, as well as a broadening of the NFR. These changes were nonrandom and most prominent in subtelomeric chromatin and at RP genes. While the mechanistic role of Nup170p in nucleosome positioning is unclear, its physical interaction with Sth1p implies a functional link to RSC. Consistently, loss of RSC activity also leads to aberrant −1 and +1 nucleosome positioning (Badis et al., 2008; Hartley and Madhani, 2009; Figure 4). Moreover, many of

the subtelomeric genes showing altered nucleosome structure in Sth1p depleted cells are also altered in the *nup170*Δ mutant.

Nup170p-associated RSC is also envisaged to contribute to transcriptional repression and the maintenance of subtelomeric and telomeric chromatin. Several observations support this idea. In addition to its role in promoting transcription, RSC also plays a role in transcriptional repression (Angus-Hill et al., 2001; Badis et al., 2008). Notably, loss of Rsc30p function leads to up-regulation of RP genes (Angus-Hill et al., 2001), strikingly similar to the loss of Nup170p (Figure S4). Moreover, RSC components are required for normal telomere length (Rsc2p, Rsc4p, Rsc14p, and Htl1p; Askree et al., 2004), Rap1 localization (Rsc1p and Rsc2p; Hiraga et al., 2008), and depletion of Sth1p derepresses multiple subtelomeric genes and alters Nup170p interactions with subtelomeric chromatin (Figure 3 and data not shown). These various functions of RSC underscore what are likely context-dependent roles defined by its subunits and accessory binding factors such as Nup170p. For example, Nup170p could influence RSC function by altering its activity and/or by positioning RSC at defined locations, both at specific chromatin sites as well as at the NPC.

Numerous observations point to interconnected pathways functioning to control ribosome biogenesis and telomere maintenance, including the identification of shared factors such as Rap1p, which functions in RP gene expression and subtelomeric gene silencing (Bosio et al., 2011). Our results place Nup170p in a similar regulatory network. Nup170p interacts with virtually all RP genes (131 of 138 meet statistical cut-offs; Figure S4), potentially through its interaction with Rap1p (Figure 6) and represses their expression. This is in contrast to previous studies that concluded NPC association of RP genes was a consequence of the transcriptional ‘on’ state of these genes (Casolari et al., 2004; Yoshida et al., 2010); however, whether this interaction promotes RP gene expression was not investigated. Interestingly, the localization of one NE-associated RP gene showing increased expression in the absence of Nup170p, *RPL9A*, was not altered in the *nup170*Δ mutant (Figure S4C). This result implies that increased *RPL9A* expression did not arise from a change in the gene’s association with the NE but rather the loss of a Nup170p-mediated repressive function. We infer that binding sites for activated genes at the NPC are distinct from chromatin binding regions established by Nup170p and its associated repressive activities.

We also detected physical interactions between Nup170p and subtelomeric regions of one or both ends of all chromosomes analyzed (Figure 5). The idea that NPCs interact with telomeres was previously suggested but has been controversial, as data supporting this claim were indirect. Mlp2p was suggested to anchor telomeres to NPCs (Galy et al., 2000; Feuerbach et al. 2002), but others did not detect these activities (Hediger et al., 2002a and 2002b). More recently, mutations in Nup84 complex members were shown to alter the NE association of Tel11L and Sir3p, and suppress subtelomeric silencing (Therizols et al., 2006). However, the mechanistic link between the Nup84 complex and telomeres is unclear, and the interpretation of these observations are confounded by pleiotropic effects of mutations in the Nup84 complex on NPC structure and function. By comparison, loss of Nup170p does not appear to alter NE structure or active transport (Aitchison et al., 1995; Makio et al., 2009).

Our analysis of Nup170p has also provided a molecular basis for its contribution to subtelomeric gene silencing. We show that Nup170p physically and functionally interacts with Sir4p and Rap1p (Figure 6), and our data support a model where the interaction of Nup170p with Sir4p promotes their binding to subtelomeric chromatin (Figures 6, 7, and Figure S5). For example, most subtelomeric binding sites for Nup170p (at least 19 of 23) show dramatic sensitivity to the loss of Sir4p. Similarly, Nup170p is required for the

association of Sir4p with Rap1p and the NE, as well as Sir4p binding to a representative telomere, Tel6R (Figure 7). Consistent with this latter observation, in *nup170*Δ cells most Sir4p is intranuclear while telomeres are NE-bound during S-phase.

The interactions of Nup170p with Sir4p and telomeres are likely regulated and may occur during distinct molecular events such as Sir4p assembly on telomeres. This is based, in part, on observations that telomeres exhibit limited colocalization with NPCs when asynchronous cell populations are examined by fluorescence microscopy (Taddei et al., 2004; and data not shown). This lower frequency of colocalization is perhaps not surprising when one considers that telomere association with the NE is transient, with resident times ranging from seconds to several minutes (Hediger et al., 2002b; Hiraga et al., 2006; Ebrahimi and Donaldson, 2008). This is interpreted to reflect the dynamic nature of telomere binding to NE tethering factors including Esc1p and Mps3p, and may explain the observations that the bulk of Esc1p fails to colocalize with Sir4p and telomeres (Taddei et al., 2004), and that Mps3p is present predominantly at spindle pole bodies (Jaspersen et al., 2002).

Built upon the dynamic nature of telomere interactions with the NE are cell cycle specific mechanisms that mediate these interactions. While chiefly NE-associated in interphase, during the later stages of DNA replication telomeres and Sir4p are dispersed throughout the nucleoplasm until the latter stages of mitosis when they again relocalize to the NE (Laroche et al., 2000; Ebrahimi and Donaldson, 2008). During interphase, Sir4p and the yKu70/yKu80 heterodimer play distinct roles in telomere binding to the NE (reviewed in: Taddei et al., 2010). For example, yKu70 is required for NE anchoring of Tel14L only during S-phase while loss of Sir4p disrupts Tel14L localization during G1-and S-phase (Hediger et al., 2002b; Figure 7). We also detect a role for Nup170p in telomere localization to the NE that appears linked to the cell cycle, with the loss of Nup170p reducing telomere association with the NE during G1-phase (Figure 7). Moreover, we observed that the interactions of Nup170p with Sir4p are most robust in M-phase arrested cells (Figure S7D). These results suggest that Nup170p may function primarily in mitosis or during G1-phase to position telomeres at the NE. Importantly, the timing of these interactions approximately coincide with mitotic events proposed to establish of subtelomeric gene silencing (see Young and Kirchmaier, 2012). This would explain the loss of subtelomeric silencing observed in *nup170*Δ cells despite the ability of telomeres to reassociate with the NE during S-phase.

The interactions of Nup170p with Rap1p, Sir4p, and the RSC complex provide molecular insight into the composition of what is likely one of several chromatin binding platforms at the NPC. We envisage Nup170p positioned adjacent to the pore membrane (Alber at al., 2007; Makio et al., 2009) with domains extending into the nucleoplasm. However, a clear picture of this molecular interface awaits a higher resolution structure for what is likely a very dynamic and flexible NPC. While Nup170p contributes to gene silencing and heterochromatin structure, other Nup platforms are predicted to interface with activated genes to augment transcription, mRNA export, and to facilitate transcriptional memory (Reviewed in: Taddei et al., 2010; Van de Vosse et al., 2011). Still other Nups may function in establishing boundaries between silenced and transcriptionally active chromatin (Ishii et al., 2002; Dilworth et al., 2005) or assist in DNA double-strand break repair (Therizols et al., 2006). Our analysis of Nup170p highlights the ability of NPCs to bind, and potentially regulate, chromatin modifiers (e.g. RSC) that, in turn, define local chromatin structure. While our data have concentrated on these functions, a less clear function is the potential role played by chromatin and chromatin modifiers in NPC structure and assembly. For example, recent evidence suggests that RSC contributes to the structural organization of the NPC (Titus et al., 2010), and we have detected similar functions for other chromatin modifiers (data not shown).

The molecular interactions between the yeast Nup170p-containing complex and Sir4p, as well as the interactions of Sir4p with Mps3p, may be conserved at the interface between NPCs and heterochromatin in higher eukaryotes. Intriguingly, Sun1, a mammalian protein that shares homology with Mps3p, localizes to the inner nuclear membrane and NPCs (Liu et al., 2007) where it interacts with heterochromatin and, in cells undergoing meiotic division, contributes to telomere tethering at the NE (Ding et al., 2007). At the NPC, Sun1 appears to interact with Pom121 (Talamas and Hetzer, 2011), which would place Sun1 in close proximity to another binding partner of Pom121, Nup155 (the mammalian counterpart of Nup170p; Mitchell et al, 2010). Strikingly, Sun1, Nup155, and Nup53 all interact with nuclear lamins, with these Nups proposed to sit at the interface between the NPC and the nuclear lamina (Hawryluk-Gara et al., 2005; Crisp et al., 2006; J. Mitchell and R. Wozniak unpublished data). When comparing the yeast and vertebrate complexes, Sir4p and vertebrate lamins appear unrelated. However, these proteins may, in fact, share similar functions. Over two decades ago Diffley and Stillman (1989) reported on structural similarities between Sir4p and lamin A/C. While the similarities between these proteins lie within commonly found coiled-coil motifs, their conserved binding partners, association with the inner nuclear membrane, and links to silencing suggest Sir4p and the lamins share, at least a subset, of functional properties. We also speculate that Nup155 functions as a chromatin binding platform similar to Nup170p, an idea consistent with a reported interaction between Nup155 and the histone deacetylase HDAC4 (Kehat et al., 2011).

EXPERIMENTAL PROCEDURES

A detailed description of the methods employed in this study is provided in the Extended Experimental Procedures.

Yeast Strains

Yeast strains used in this study are listed in Table S6.

Synthetic Genetic Arrays

Synthetic genetic array (SGA) analyses were performed as previously described (Tong et al., 2001), except, to minimize genome instability associated with a *nup170*Δ null mutant (Kerscher et al., 2001), a plasmid borne wild type copy of *NUP170* was maintained throughout the screen until removal at the final step of single and double mutant selection by addition of 5-FOA.

Affinity Purifications

Yeast cells expressing protein A-tagged fusion proteins were frozen in liquid nitrogen and lysed using a planetary ball mill (Retsch). Affinity purification of pA fusion proteins from cell lysates was performed using IgG-coupled magnetic beads.

Gene Expression Profiling

Two-color microarrays were performed using Agilent whole-genome *Saccharomyces cerevisiae* arrays (Agilent). All experiments were performed with duplicate experimental and duplicate technical replicates of each condition as previously described (Wan et al., 2009).

Subtelomeric Silencing Assay

10-fold serial dilutions of cell cultures were spotted onto one of three sets of plates: 1) synthetic complete medium (SC), SC-ura-ade, and SC + 1 mg/mL 5-FOA, 2) SC-leu, SC-

leu-ura-ade, and SC-leu + 1 mg/mL 5-FOA, or 3) YPD medium. Plates were incubated for 2–5 d at 30°C and then placed at 4°C for an additional 10 d to permit color development.

Genome-wide Nucleosome Positioning Analysis

Mononucleosomal DNA was isolated as previously described (Yuan et al., 2005) and subjected to Illumina sequencing.

Chromatin Immunoprecipitation

ChIP was performed as described by Wan et al., 2009. Real-time qPCR was performed as previously described (Wan et al., 2009). Genome-wide chromatin localization analysis (ChIP-chip) was performed using Agilent whole-genome *S. cerevisiae* arrays and processed with the Agilent Feature Extraction and Agilent ChIP Analytics software (Agilent).

Fluorescence Microscopy

Yeast strains producing GFP-tagged and/or mCherry-tagged fusion proteins were immobilized on 2% agarose pads containing SC medium. Images were acquired as a series of 14 section z-stacks and processed using *ImageJ* software (National Institutes for Health). Analysis of the subnuclear positioning of Sir4-GFP and Rap1-GFP foci was aided by iterative deconvolution of the Sec63-mCherry signal to enhance visualization of the NE. Subnuclear positioning of 256 x *lacO*-tagged telomeres was determined as previously described (Hediger et al., 2002b).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Nup170p physically interacts with Sir4p, Rap1p, and the chromatin remodeler RSC
- Nup170p binds and represses expression of subtelomeric and ribosomal protein genes
- Nup170p and Sir4p bind cooperatively to subtelomeric DNA
- Tethering of subtelomeric chromatin to the NE during G1-phase requires Nup170p

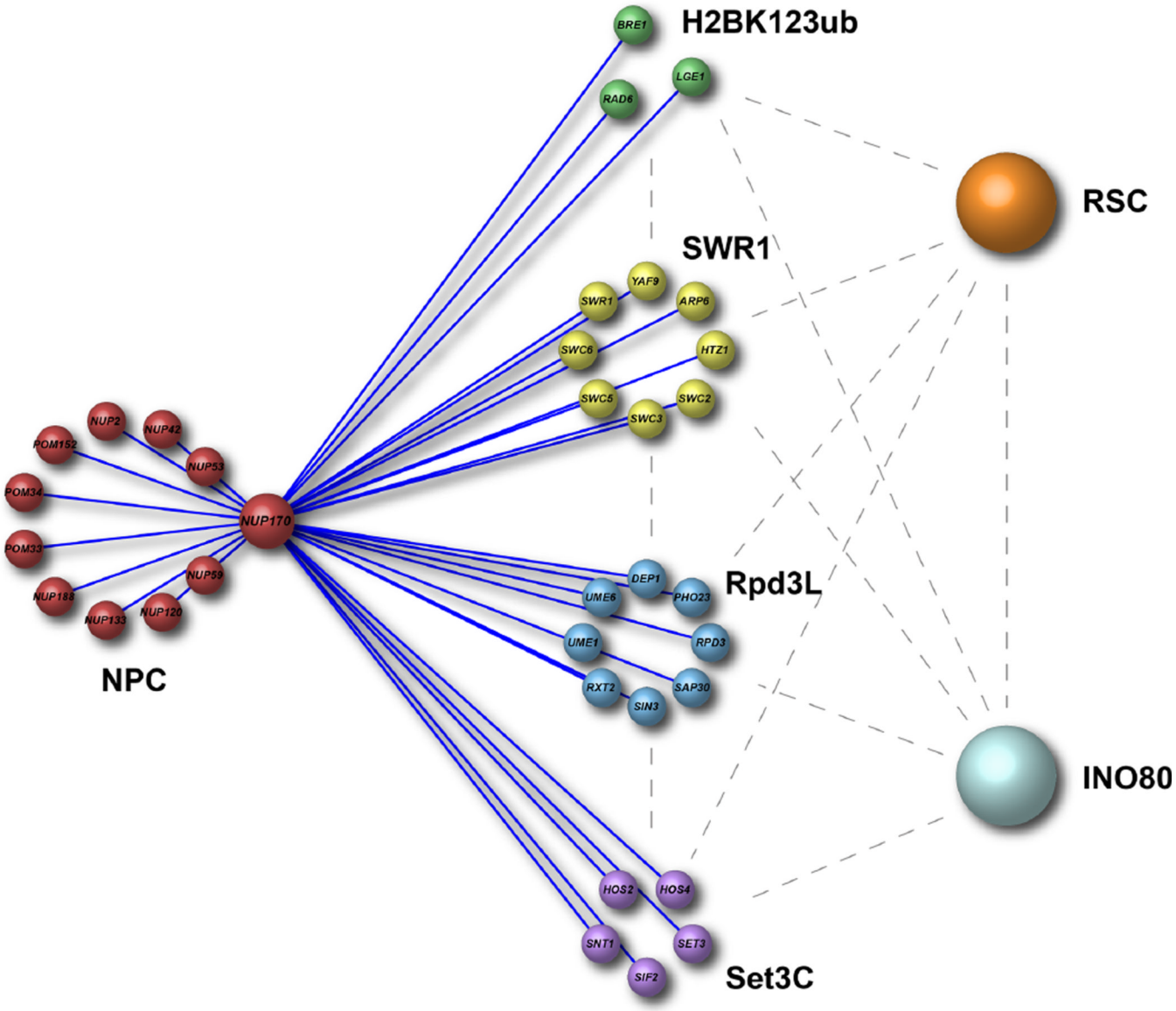

Figure 1. NUP170 functionally interacts with chromatin modifying complexes

Cytoscape depicts genetic interactions between *NUP170* and genes encoding chromatin-modifying complexes. Nodes represent genes grouped by functional complexes. Edges connecting nodes represent synthetic genetic interactions. Only those interactions of *NUP170* with multiple components of a subcomplex are shown. Blue edges represent interactions identified by SGA analysis of *NUP170*, grey dashed edges represent previously reported genetic interactions among chromatin complexes as denoted by the *Saccharomyces* Genome Database. Also see Tables S1 and S2.

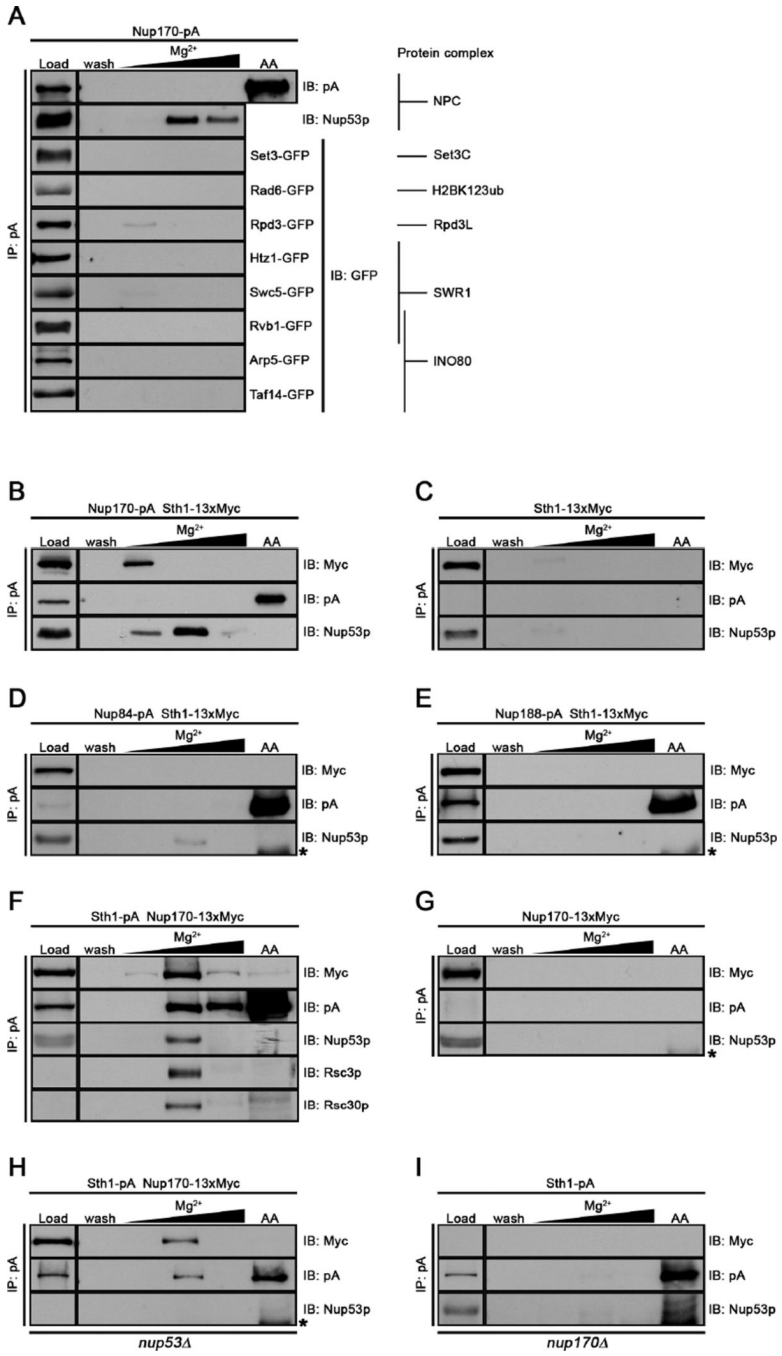

Figure 2. Nup170p binds the RSC complex

(A) The Nup170-pA fusion was affinity purified from cells producing the indicated GFP fusion proteins. Bound complexes were released by step-wise elution using increasing concentrations of $MgCl_2$ (Mg^{2+}) and a final acetic acid wash (AA). Eluted proteins were analyzed by SDS-PAGE and western blotting to detect the indicated proteins. Samples of the total cell lysates (load) and the final wash (wash) prior to elution are shown. (B–I) Similar experiments as described in A were performed using strains producing the indicated pA- and/or 13xMyc-tagged proteins. Asterisks indicate IgG cross-reacting species in AA fractions.

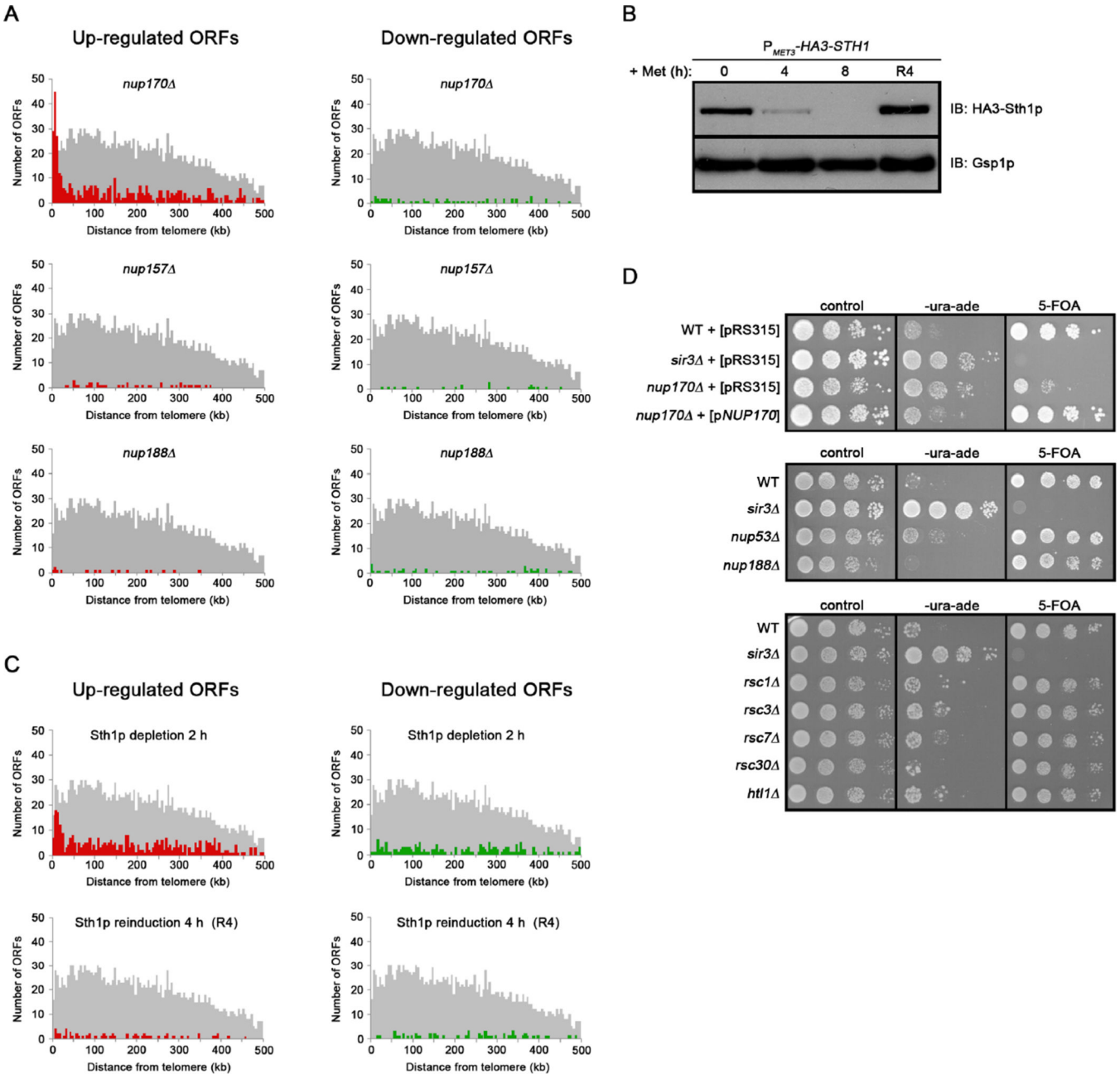

Figure 3. Nup170p is required for subtelomeric gene silencing

(A) Gene expression profiles of *nup170*Δ, *nup157*Δ, and *nup188*Δ cells were determined by DNA microarray analysis. The positions of ORFs identified as differentially expressed (lambda ≥ 100) and exhibiting a 2-fold increase (red) or decrease (green) in transcript levels along the length of the chromosomes were determined and the number of altered genes within 5 kb bins (y-axis) were plotted versus their distance from chromosome ends (x-axis). The distribution of all ORFs within these 5 kb bins are represented in grey, with the number of ORFs in each bin three times that shown on the y-axis. (B and C) Cells containing P_{MET3}-*HA3-STH1* were depleted of Sth1p by addition of methionine for the indicated times. Western blotting (B) was used to detect HA3-Sth1p (IB: HA) and a loading control (IB: Gsp1p). (C) Microarray analysis was performed on Sth1p depleted cells (2h) and then

following reinduction of *STH1* for 4 h (R4) and data were analyzed as described in panel A. (D) Silencing of the subtelomeric reporter genes *URA3* and *ADE2* was examined in the indicated haploid strains containing either no plasmid (*middle and bottom panels*), empty vector (pRS315), or pHNP170 (*pNUP170*) (top panel). Cell growth was examined under nonselective (control) and selective (lacking uracil and adenine [-ura-ade] or plus 5-FOA [5-FOA]) conditions. See also Figure S1.

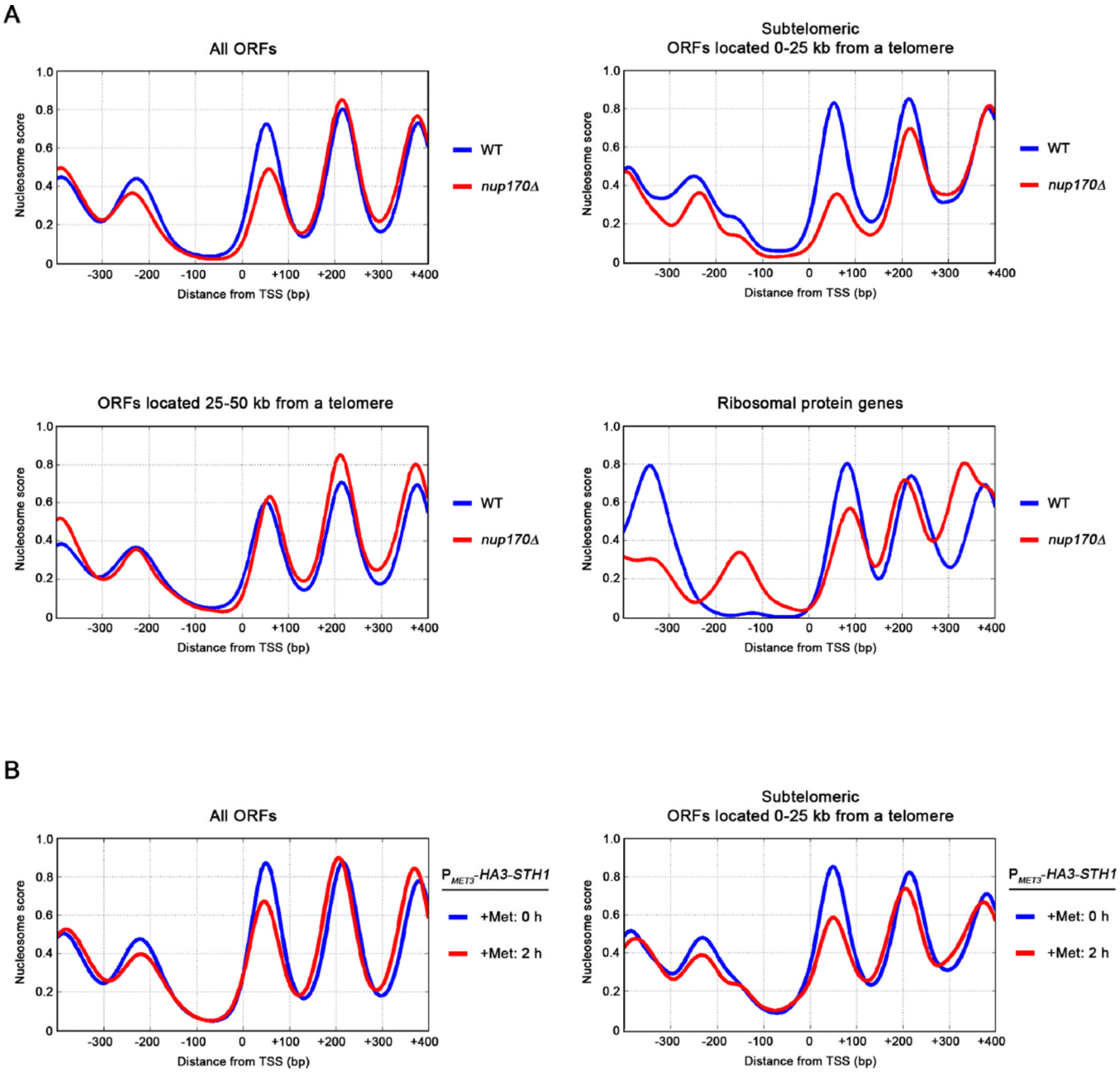

Figure 4. Nup170p is required for nucleosome positioning

(A) TSS-aligned average nucleosome scores were derived from the analysis of WT (blue) and *nup170*Δ (red) cells. Shown is the genome-wide profile (5419 ORFs) (*top left*) and profiles for ORFs located within 0–25 kb (149 ORFs; *top right*) and 25–50 kb (343 ORFs; *bottom left*) from chromosome ends. Similar analysis was performed on 138 RP genes (*bottom right*). (B) TSS-aligned average nucleosome scores were also obtained from cells depleted of Sth1p for 0 h (blue) and 2 h (red). All ORFs (5419; *left panel*) and ORFs located within 0–25 kb regions (*right panel*) are shown. See also Figure S2.

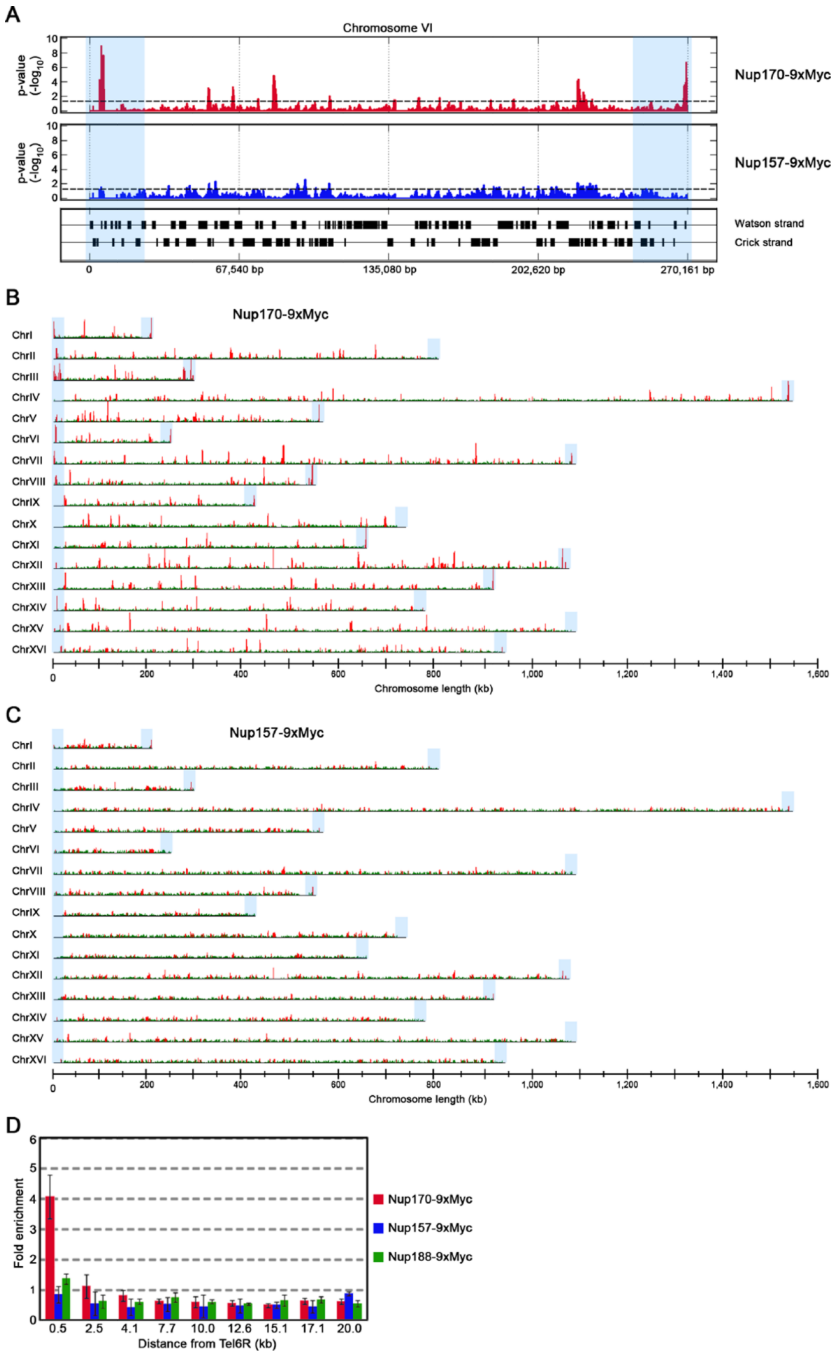

Figure 5. Nup170p is enriched at subtelomeric DNA

(A) Binding profiles, as determined by ChIP-chip analysis, of Nup170-9xMyc (red) and Nup157-9xMyc (blue) along chromosome VI are plotted as a logarithmic function of their p-values. Black rectangles represent ORFs. The complete genome-wide DNA binding profiles of Nup170-9xMyc and Nup157-9xMyc are shown in panels B and C. Red peaks mark statistically significant binding sites with p-values ≤ 0.05 and green peaks mark statistically insignificant binding sites. Peak height is inversely proportional to p-value (y-axis; -log10 scale= 10). In panels A, B, and C subtelomeric regions are highlighted by blue shading. Note, too few probes were available on chips to accurately evaluate significant binding to six subtelomeric regions (0–10 kb), Tel9L, Tel10L, Tel10R, Tel16L (0 probes), Tel15R (1

probe), and Tel4L (2 probes). (D) ChIP of Nup170-9xMyc (red), Nup157-9xMyc (blue), and Nup188-9xMyc (green) followed by qPCR using primer sets positioned along a 20 kb subtelomeric region of the right arm of chromosome VI (x-axis). Mean relative enrichment of three independent ChIP experiments is plotted on the y-axis with standard error. See also Figure S3 and S4.

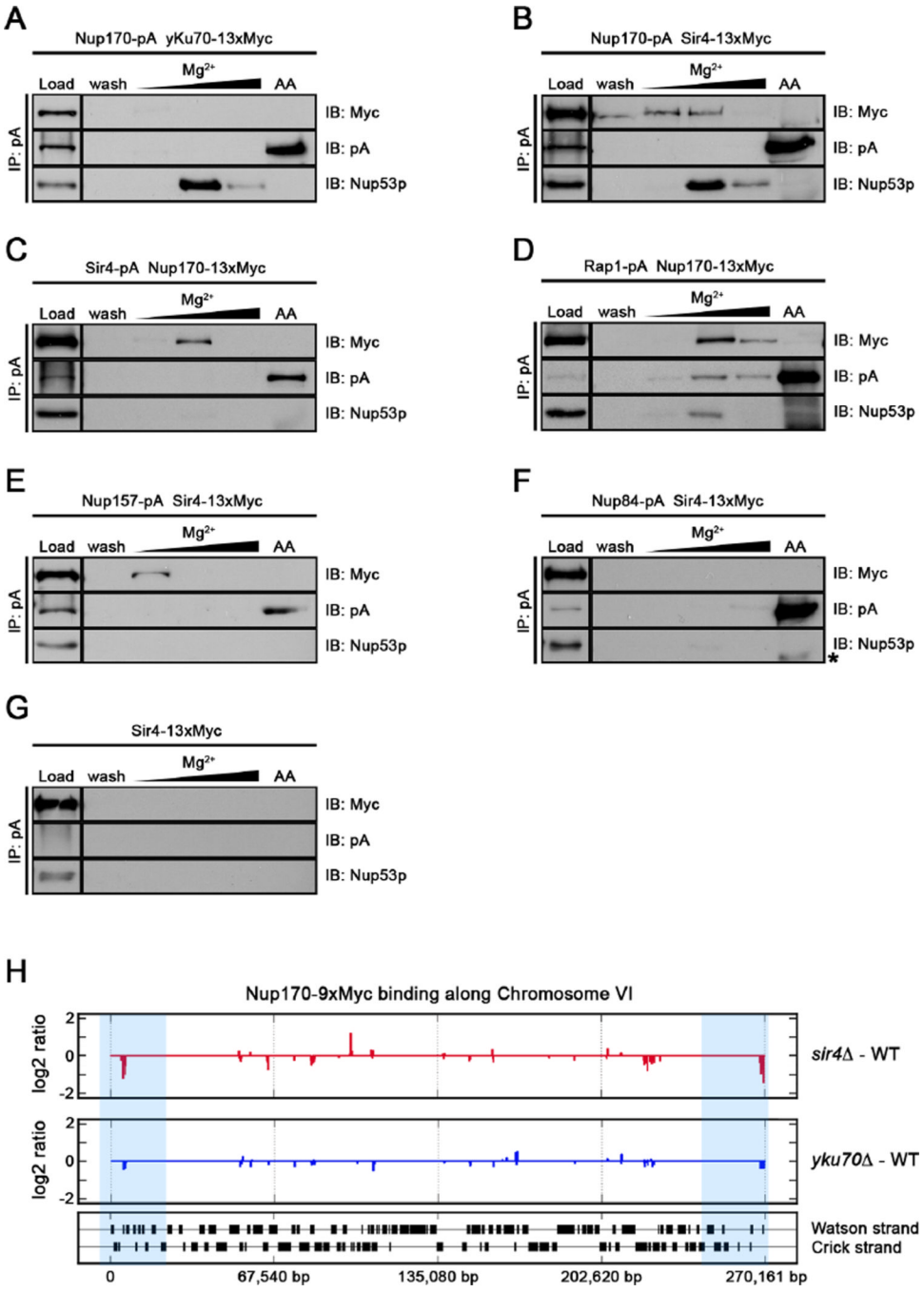

Figure 6. Nup170p association with subtelomeric DNA is mediated by Sir4p

(A–G) Protein A-tagged chimeras were affinity-purified from lysates containing the indicated 13xMyc-tagged proteins as described in Figure 2. The asterisk indicates an IgG cross-reacting band. (H) Genome-wide binding profiles of Nup170-9xMyc in WT, *sir4*Δ, and *yku70*Δ cells were determined by ChIP-chip and the binding profiles from WT cells were compared to *sir4*Δ (red) and *yku70*Δ (blue) along chromosome VI (x-axis). Plotted is fold-enrichment of DNA associated with Nup170-9xMyc isolated from mutant cells minus that from WT cells where p-values ≤ 0.05 (y-axis; $-\log_2$). Values plotted below the red or blue horizontal line indicate the degree of decreased binding in the mutant strain. Subtelomeric regions are indicated by blue shading. See also Figure S5 and S6.

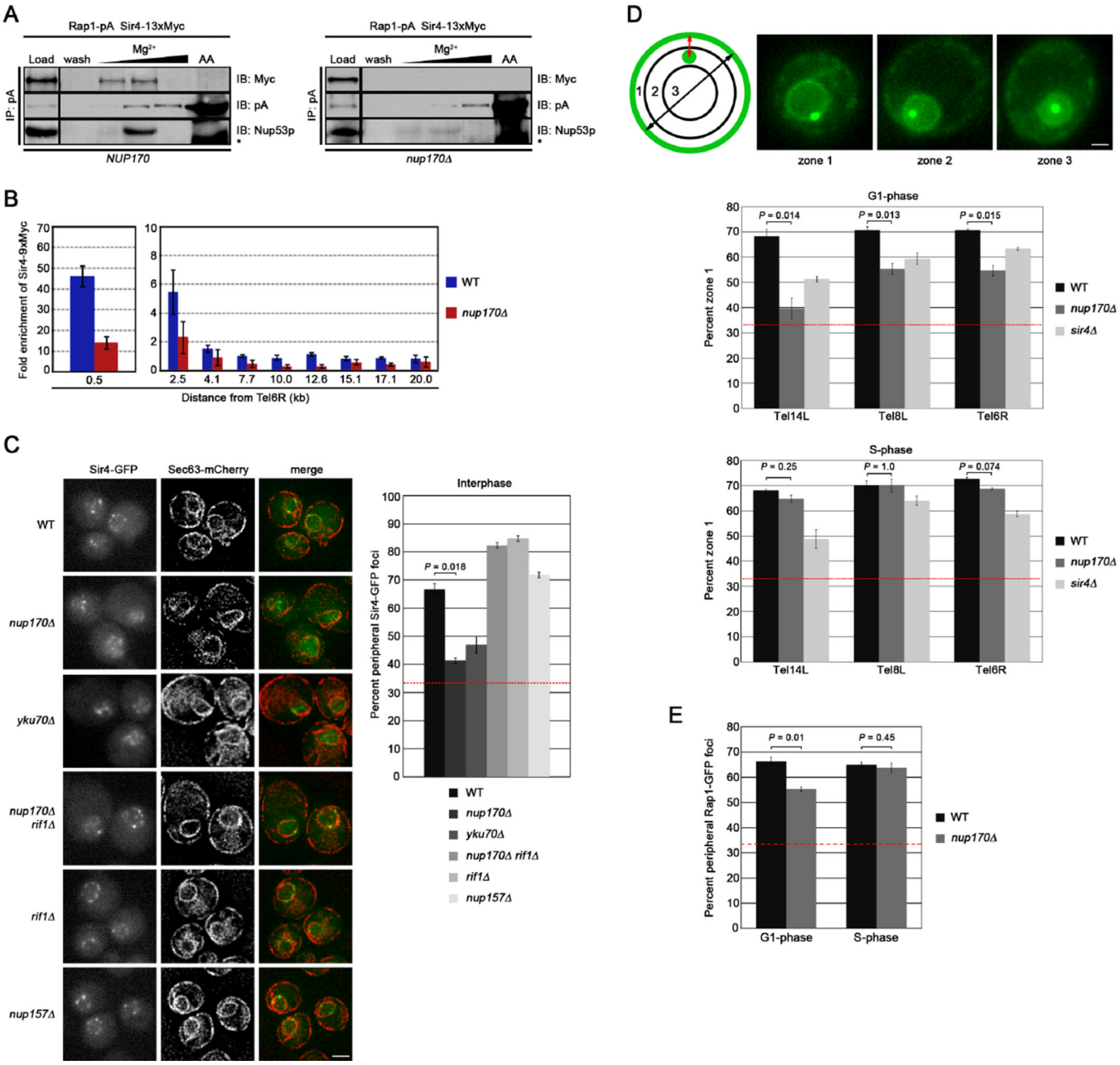

Figure 7. Nup170p facilitates Sir4p binding to subtelomeric DNA

(A) Rap1-pA was affinity-purified from cells producing Sir4–13xMyc and containing (*NUP170*) or lacking (*nup170*Δ) Nup170p (*right panel*) and analyzed as in Figure 2. Asterisks indicate IgG cross-reacting bands. (B) ChIP of Sir4–9xMyc followed by qPCR was performed on samples from WT and *nup170*Δ strains as described in Figure 5. (C) The indicated strains expressing *SIR4-GFP* and *SEC63-mCherry* were analyzed by fluorescence microscopy. A deconvolved Sec63-mCherry signal was used to demarcate the NE. Histograms show the percentage of Sir4-GFP foci (based on > 400 foci counted per strain) localized at the NE in interphase cells. Mean percentage and standard error are shown (n=3). Scale bar, 2 μm. (D) Telomere positioning was analyzed in asynchronous cultures of WT, *nup170*Δ, and *sir4*Δ cells. Tel6R, Tel8L, and Tel14L were visualized using integrated *lacO* repeats and GFP-LacI. The sub-nuclear position of GFP foci in a single focal plane was

determined relative to the NE marker Sec63-GFP and assigned to one of three concentric nucleoplasmic zones of equal area (see schematic and representative single focal plane images). The subnuclear positions of 100 foci for Tel14L and 50 foci for Tel6R and Tel8L were determined in unbudded (G1-phase) and small budded (S-phase) cells. The mean percentages of telomere foci localized in zone 1 and the standard error are shown (n=3). Scale bar, 1 μm. (E) The percentage of Rap1-GFP foci localized at the NE in G1- and S-phase was determined in WT and *nup170*Δ cells. For each experiment, > 200 foci were counted per strain. The mean percentage and standard error of three independent experiments are shown. In panels C-E, random-distributions are indicated by a red dashed-line at 33% and the statistical significance of the difference between *nup170*Δ and WT cells is indicated (Student's t-Test). See also Figure S6, S7, and Table S5.