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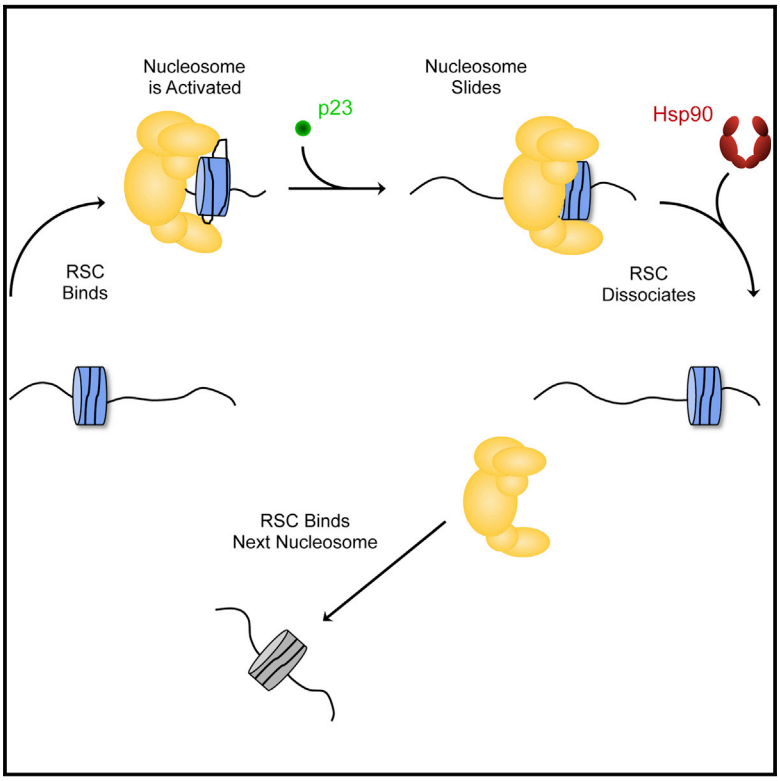
Molecular Cell

Hsp90 and p23 Molecular Chaperones Control Chromatin Architecture by Maintaining the Functional Pool of the RSC Chromatin Remodeler

Graphical Abstract



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In Brief

The Hsp90 and p23 molecular chaperones contribute to proteostasis by facilitating the function of fully assembled RSC chromatin remodeler complexes. In brief, p23 promotes RSC remodeling activity, whereas Hsp90 fosters the transition of RSC between nucleosomal targets. Our studies demonstrate that a chromatin landscape is dependent on molecular chaperones.

Highlights

- Chromatin architecture is actively maintained via an Hsp90-dependent mechanism
- Hsp90 releases RSC from a target, thereby terminating remodeling and mobilizing RSC
- P23 fosters RSC-DNA dynamics, facilitating nucleosome sliding and histone eviction
- Hsp90 and p23 support proteostasis by enabling the activities of protein complexes

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Hsp90 and p23 Molecular Chaperones Control Chromatin Architecture by Maintaining the Functional Pool of the RSC Chromatin Remodeler

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SUMMARY

Molecular chaperones govern protein homeostasis, being allied to the beginning (folding) and ending (degradation) of the protein life cycle. Yet, the Hsp90 system primarily associates with native factors, including fully assembled complexes. The significance of these connections is poorly understood. To delineate why Hsp90 and its cochaperone p23 interact with a mature structure, we focused on the RSC chromatin remodeler. Both Hsp90 and p23 triggered the release of RSC from DNA or a nucleosome. Although Hsp90 only freed bound RSC, p23 enhanced nucleosome remodeling prior to discharging the complex. In vivo, RSC mobility and remodeling function were chaperone dependent. Our results suggest Hsp90 and p23 contribute to proteostasis by chaperoning mature factors through energetically unfavorable events, thereby maintaining the cellular pool of active native proteins. In the case of RSC, p23 and Hsp90 promote a dynamic action, allowing a limited number of remodelers to effectively maintain chromatin in a pliable state.

INTRODUCTION

The protein homeostasis or proteostasis process safeguards a cell's polypeptide population by continuously scrutinizing the conformational status of each protein. Persistent monitoring facilitates the forward flow of biological pathways, including the folding of nascent polypeptide chains and the removal of damaged proteins (Hartl et al., 2011). This high-volume work is predominately carried out by members of the diverse molecular chaperone family, and disruptions in the process correlate with the onset of a variety of diseases, including neurodegeneration, type II diabetes, and heart failure (Balch et al., 2008; Powers et al., 2009). The promiscuous, transient-binding activities of the abundant chaperones are well-suited to perform such high-volume tasks, which are further challenged by the vast sequence and structural variety of a proteome. Adding to the complexity is the range of conformations any protein can assume and still be within the scope of a native factor (Bartlett and Radford, 2009). Likely, different structural forms distinguish distinct functional states of each factor. Here, we suggest that the Hsp90 and p23 chaperones contribute to proteostasis by manipulating fully assembled protein structures, thereby fostering the available pool of functional complexes (i.e., facilitating specific activities).

In eukaryotes, the central molecular chaperones are Hsp90 and Hsp70, along with their respective cochaperones (Röhl et al., 2013). Although the Hsp70 hub manages early and late events (i.e., folding and degradation), the Hsp90 system preferentially associates with near native or native proteins (Dezwaan and Freeman, 2008). Although Hsp90 can form long-lived interactions with metastable proteins (e.g., unactivated kinases and steroid hormone receptors), Hsp90 components generally associate weakly with their protein targets (Zhao et al., 2005; Millson et al., 2005; McClellan et al., 2007; Sharma et al., 2012; Taipale et al., 2012, 2014). However, the relevance of transient chaperone interactions with seemingly native clients is yet to be fully understood.

Because numerous Hsp90 targets are part of large multi-subunit structures, an influence in the assembly and/or disassembly of protein machines has been proposed (Makhnevych and Houry, 2012). In addition to Hsp90, its cochaperones often connect to the same complexes, albeit through distinct subunits, suggesting a joint chaperone action with protein structures (Echtenkamp and Freeman, 2012). Yet, studies outlining the roles of Hsp90 and any of its cochaperones on the same client are limited. Here, we investigated the impact of the p23 cochaperone and Hsp90 on the remodel the structure of chromatin (RSC) complex, which is an ~1.5-MDa protein structure composed of 17 subunits (Cairns et al., 1996). Our prior work genetically connected the yeast p23 gene with *RSC7*, and two different studies linked *HSP90* with *RSC14* (Zhao et al., 2005; McClellan et al., 2007; Echtenkamp et al., 2011).

RSC is one of eight chromatin remodelers in budding yeast, which are conserved through humans, and is responsible for depositing histones and positioning nucleosomes across the

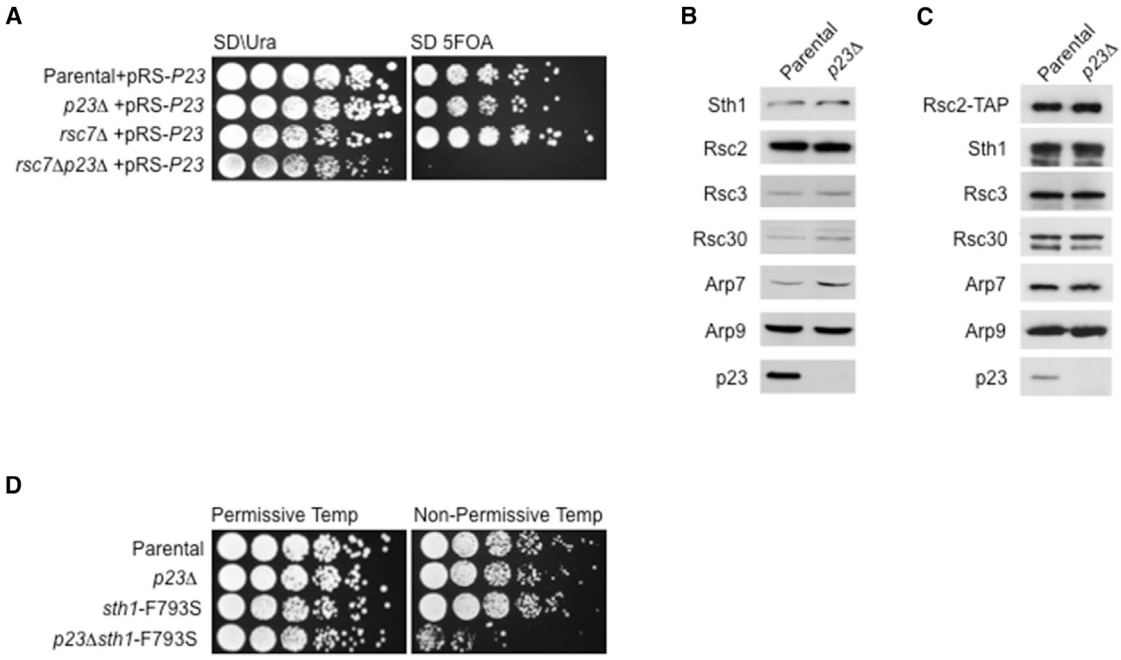


Figure 1. The p23 Chaperone Is Physiologically Linked to RSC, but Not as a Quality Control Factor

(A) The viability of single (*p23*Δ and *rsc7*Δ) or double (*p23*Δ*rsc7*Δ) gene knockout yeast was evaluated with a plasmid dropout test. The yeast carry an *URA3*-marked covering plasmid with the endogenous *p23* locus, and the vector was either positively (SD\−Ura) or negatively (SD\+5FOA) selected.

(B) Steady-state protein levels of the indicated RSC subunits were assayed by immunoblot analysis using whole cell extracts prepared from logarithmically growing parental or *p23*Δ cells.

(C) Assembly of the RSC protein complex is p23 independent. Using the standard tandem affinity pull-down assay, RSC complexes were isolated from extracts prepared from *TAP-RSC2* parental or *p23*Δ*TAP-RSC2* yeast, and the relative levels of the marked RSC subunits was checked.

(D) Cell viability of a temperature-sensitive *sth1* allele is p23 dependent. Parental, *p23*Δ, *sth1-F793S*, and *p23*Δ*sth1-F793S* yeast were grown at 30°C (permissive) or exposed to 37°C (non-permissive) for 24 hr, and viability was then checked using a spot-test assay and incubation at 30°C.

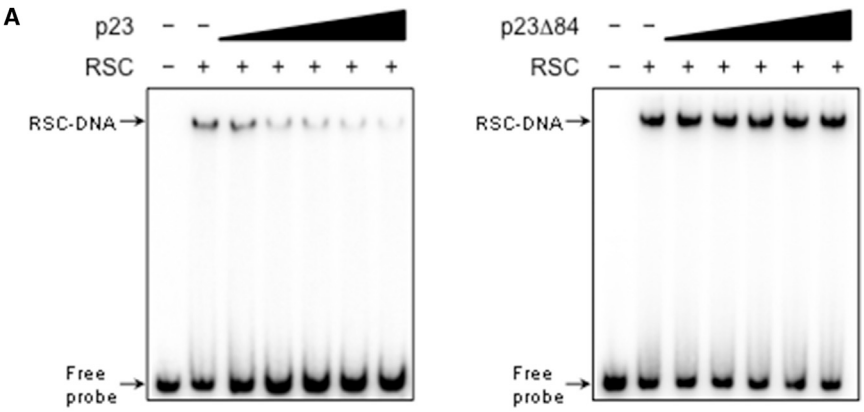
genome (Clapier and Cairns, 2009). RSC, however, is the only chromatin remodeler essential for growth (Cairns et al., 1996). In brief, remodelers provide a critical means to regulate genomic activities since these complexes alter nucleosome spacing to control access to the underlying DNA (Venkatesh and Workman, 2015). A key feature of the chromatin process is the fast kinetics of histone repositioning/removal because many events (e.g., DNA repair or transcription) must be undertaken promptly to maintain cellular homeostasis.

Challenging the effectiveness of remodeling pathways is the overabundance of nucleosomes relative to the total number of remodelers (Ghaemmaghami et al., 2003; Brogaard et al., 2012; Chong et al., 2015). Although a division of labor among the remodelers may alleviate some of the burden, it likely doesn't account for the efficiency of the remodeling system. For instance, RSC favors nucleosomes at RNAP II gene promoters, helping to maintain promoter-associated nucleosome depleted regions (NDRs) (Parnell et al., 2008; Yen et al., 2012). Yet, within minutes of a signaling event, RSC can redistribute to hundreds of new gene promoters to create NDRs at these loci (Damelin et al., 2002; Ng et al., 2002). Although nucleation of RSC to certain promoters is mediated by select transcription factors, along with the RSC DNA-binding subunits Rsc3 and Rsc30 (Angus-Hill et al., 2001; Badis et al., 2008), the events triggering the release of RSC to allow the rapid relocation are not understood. Given the genetic links between both chaperones and RSC subunits, along with the functional dependence of chromatin on p23 (Wilson et al., 2006; Zelin et al., 2012), we felt that the RSC complex served as an ideal model to understand how molecular chaperones modulate an assembled multi-subunit protein structure.

RESULTS

p23 Is Not a Classic Proteostasis Factor of RSC Proteins and Complex Assembly

The connection between p23 and RSC was initially discovered in a synthetic genetic array (SGA) screen (Echtenkamp et al., 2011). We validated the genetic interaction between *SBA1* (yeast p23 gene) and the non-essential RSC subunit gene *RSC7* using a plasmid dropout spot-test assay (Figure 1A). The only known role for Rsc7 is in the assembly of the RSC structure, as incorporation of Rsc3 and Rsc30 is impaired in *rsc7*Δ yeast (Wilson et al., 2006). Because construction of protein complexes is a common molecular chaperone function, we checked whether p23 mediates classic RSC proteostasis events. We found that steady-state RSC protein levels and assimilation of the subunits into the RSC structure were similar in parental and *p23*Δ yeast (Figures 1B and 1C). Thus, p23 is not a typical proteostasis factor of individual RSC proteins or the RSC subunit assembly.



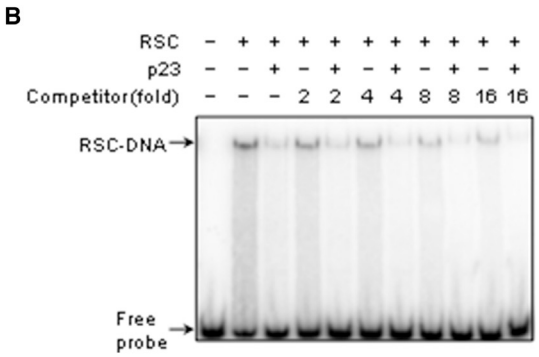


Figure 2. p23 Dissociates RSC-DNA Complexes In Vitro

(A) RSC DNA-binding activity was monitored by EMSA using purified RSC (5 nM) and radiolabeled *PHO8* promoter DNA (12.5 nM). The influence of p23 on RSC-DNA structures was determined using a titration (0, 1, 2, 4, 8, and 16 μM) of full-length p23 or the carboxyl-terminal truncation mutant p23Δ84.

(B) P23 and competitor DNA jointly contribute to dissociating RSC-DNA complexes. Preformed RSC-DNA assemblies were challenged with the indicated fold excess of unlabeled PHO8 DNA (competitor) in the absence or presence of p23 (10 μM).

Next, we checked whether the p23 link might entail an undetermined, specialized role of Rsc7 or if it involved the RSC chromatin remodeler. Typically, remodeling function is tied to the catalytic motor of each remodeler (Clapier and Cairns, 2009). Complicating our analysis is the essential nature of the RSC ATPase encoding gene *STH1* (Cairns et al., 1996). To circumvent this difficulty, we took advantage of the *sth1* temperature-sensitive derivative Sth1-F793S. Unlike other *sth1* allelic protein products, Sth1-F793S degrades at restrictive temperatures (> 37°C) and therefore serves as an effective null (Titus et al., 2010). Because *STH1* is essential, the final growth test is not performed at the restrictive temperature, but rather the cells are exposed and then grown at a permissive temperature. In brief, exponentially growing parental, *p23Δ*, *sth1-F793S*, and *p23Δsth1-F793S* yeast were shifted to 37°C for 24 hr, and cells were then serially diluted, spotted onto media, and incubated at 30°C. The double mutant *p23Δsth1-F793S* survived poorly relative to the other strains, indicating a possible physiological connection between p23 and the remodeling function of RSC (Figure 1D).

p23 Triggers Release of RSC from Promoter DNA

We have shown that the maintenance of NDRs is contingent upon p23 (Zelin et al., 2012). We had postulated that p23's ability to regulate the DNA-binding activities of numerous transcription factors accounted for the NDR effects; however, transcription factors do not directly move nucleosomes. Besides transcription factors, an increase in the DNA occupancy of sites associated with chromatin modifiers, including Rsc3 and Rsc30 elements, was apparent (Zelin et al., 2012). Of note, the majority of the genetic links between p23 and the various chromatin modifiers involve DNA-binding subunits, suggesting a common basis for the connections (Echtenkamp et al., 2011). Hence, we checked the influence of p23 on the DNA-binding activity of RSC.

RSC uses multiple subunits to bind comparably to naked or nucleosomal DNA, with the Rsc3/30 heterodimer providing sequence selectivity (Angus-Hill et al., 2001; Sengupta et al., 2001; Damelin et al., 2002; Titus et al., 2010). To monitor RSC DNA interactions, we used a probe representing a section of the *PHO8* promoter that has two Rsc3/30 binding sites. RSC positions nucleosomes at the *PHO8* promoter in vivo and in vitro (Wippo et al., 2011). Purified RSC bound the PHO8 probe, and the addition of recombinant p23 dissociated the RSC-DNA structure (Figure 2A). Dissolution of the complex was dependent on p23 chaperone activity because the defective mutant p23Δ84 had no apparent impact on RSC binding (Figure 2A) (Toogun et al., 2007). Although the effective p23 amounts were seemingly high, the applied levels are comparable to the nuclear concentration of p23 (~4 μM) (Figure S1A). Of note, p23 does not itself interact with DNA (Figure S1B).

To further mimic in vivo settings, in which numerous RSC binding sites are available, we challenged the radiolabeled DNA-RSC complex with unlabeled PHO8 probe and observed an additive effect of p23 and competitor DNA on loss of the radiolabeled RSC structure (Figure 2B). To further understand the impact of p23 on RSC, we utilized fluorescence anisotropy. We found that p23 accelerates the off rate of RSC from DNA alone or in the presence of competitor DNA (Figure S2). Hence, p23 disassembles RSC-DNA complexes, thereby fostering the transition of RSC to new sites.

Nucleosome Remodeling Activity of RSC Is Advanced by p23

Although RSC binds naked and nucleosomal DNA with comparable affinities, the target of interest is a nucleosome (Sengupta

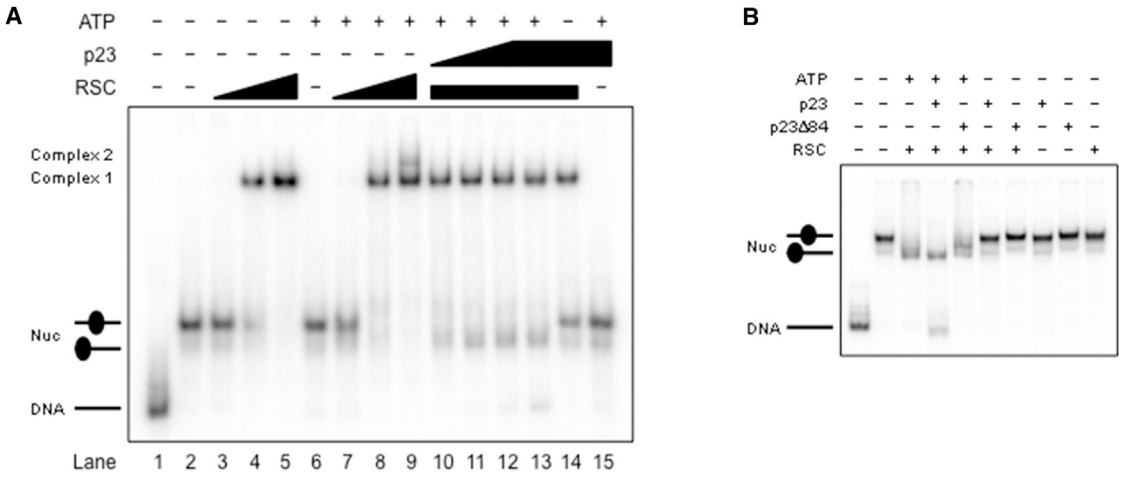


Figure 3. p23 Promotes RSC-Mediated Nucleosome Remodeling In Vitro

(A) RSC nucleosome binding and remodeling activity was checked by EMSA using nucleosomes prepared with radiolabeled 601-positioning DNA and titrations of purified RSC (1, 5, and 10 nM). The influence of ATP (1 mM) and p23 (1, 3, 5, and 15 μM) were assessed, as indicated. The migration positions of free DNA, nucleosomes with a centrally or terminally positioned histone, and RSC-nucleosome structures (complex 1 and 2) are marked.

(B) A nucleosome-sliding assay was used to confirm the influence of p23 on RSC remodeling activity and to check if p23 chaperone function was required using the chaperone-deficient mutant p23Δ84.

et al., 2001). To test whether p23 influences RSC-nucleosome interactions, we built a mononucleosome with a centrally localized histone octamer using a 601 nucleosome-positioning DNA as the probe in an EMSA-based assay. RSC bound the nucleosome independent of ATP (Figure 3A; complex 1) and formed an activated nucleosome structure upon ATP addition (complex 2) (Lorch et al., 1998). In the presence of p23, the remodeling reaction was driven to completion, as evidenced by the release of terminally localized mononucleosomes and/or free DNA (Figure 3A; lanes 10–13). Of note, p23 promoted the release of slid nucleosomes or free DNA, even in the absence of competitor DNA, which is typically added to remove RSC from its modified target (Figure S3A). In the absence of ATP, p23 triggered dissociation of RSC from the unremodeled mononucleosomes (Figure 3A; lane 14). Thus, p23 differentially impacts RSC in an ATP-dependent manner (i.e., in the presence of ATP, p23 fosters the remodeling reaction, but in the absence of ATP, p23 dissociates the idle complex).

Comparable to the EMSA results, p23 enhanced RSC-mediated histone repositioning and eviction using a mononucleosome sliding assay (Figures S3B and S3C). Importantly, the chaperone activity of p23 was required to induce RSC-dependent mononucleosome sliding and histone eviction (Figure 3B). Besides RSC, we assessed the chaperone impact on ISW2, which is one of two remodelers without an apparent genetic connection to p23 (Echtenkamp et al., 2011). P23 did not alter ISW2 remodeling activity (Figure S3D). Overall, p23 appears to selectively modulate RSC by targeting the remodeler-nucleosome complexes at potentially unproductive stages (i.e., post-remodeling or in the absence of ATP) and either drives the reaction to completion (+ATP) or releases the stalled complex (−ATP). In both cases, p23 contributes to RSC action by recycling otherwise inert RSC-nucleosome assemblies.

Rsc3 Is Released from DNA by p23

To identify the RSC components influenced by p23, we screened the subunits by yeast two-hybrid. Of the 17, 3 displayed a relationship with p23 (Figure 4A). Rsc30 had a standard positive two-hybrid result because the *HIS3* reporter was active upon coexpression of the bait (DBD-p23) and prey (AD-Rsc30). On the other hand, AD-Rsc3 and AD-Sfh1 both autoactivated the *GAL1-HIS3* reporter, yet coexpression of DBD-p23 abrogated the autoactivation. Because we used a standard Gal4-based two-hybrid strategy and RSC is known to modulate the endogenous *GAL1* promoter in an Rsc3-dependent manner (James et al., 1996; Floer et al., 2010), we suspected that the DNA-binding activity of Rsc3 drove the autoactivation. By increasing the local concentration of p23 through the expression of DBD-p23, AD-Rsc3 is dissociated from the *GAL1* promoter. Because Sfh1 and Rsc3 have been directly linked, it is possible that AD-Sfh1 joins the endogenous Rsc3 to activate the reporter or Sfh1 itself might bind the promoter DNA (Sengupta et al., 2001; Campsteijn et al., 2007).

We checked our premise that p23 targets Rsc3 to disassemble protein-DNA complexes by EMSA using purified recombinant p23, Rsc3, and Rsc30. Although p23 removed Rsc3 from DNA, it did not affect Rsc30 (Figures 4B and 4C). By anisotropy, it was apparent that p23 accelerated the off rate of Rsc3 but does not appreciably impact Rsc30 (Figure S4). Significantly, p23 dissociated the Rsc3/Rsc30 heterodimer from DNA, indicating that the presence of Rsc3 is sufficient for p23 to act on a DNA-bound protein complex (Figure 4D).

Hsp90 Disassembles RSC-Nucleosome Structures and Impedes Remodeling

Besides p23, Hsp90 shares a genetic connection with RSC through the *RSC14/LDB7* gene (Zhao et al., 2005; McClellan et al., 2007). Rsc14 works in conjunction with Rsc7 to assimilate

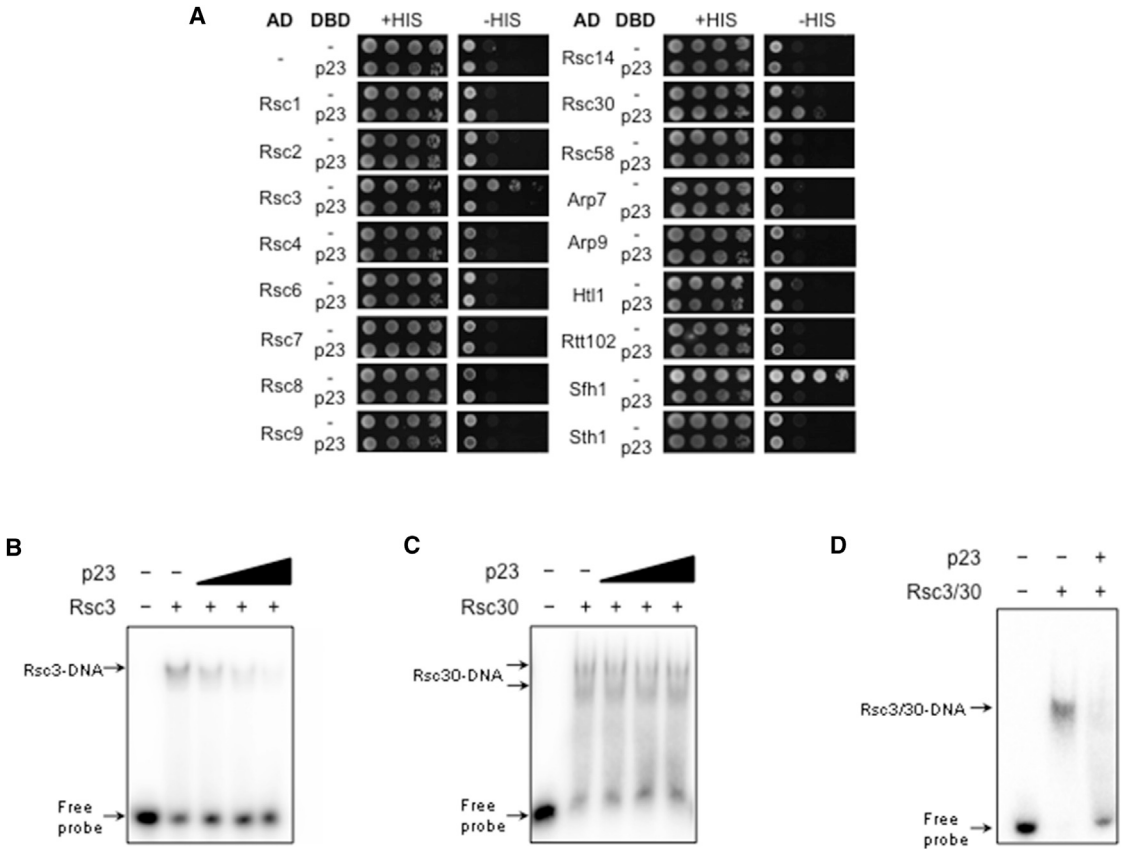


Figure 4. Rsc3 Is Directly Targeted for Dissociation by p23

(A) Interactions between p23 and each RSC subunit were monitored by yeast 2-hybrid.

(B and C) The influence of a p23 titration (0, 4, 12, and 32 μM) on the DNA-binding activities of purified Rsc3 (90 nM) (B) or Rsc30 (90 nM) (C) was checked by EMSA using a radiolabeled Rsc3/30 element from the *ACC1/TIM23* loci as a probe (Zhu et al., 2009).

(D) The DNA-binding capacity of the Rsc3/30 heterodimer (50 nM) in the presence or absence of p23 (6 μM) was checked by EMSA using radiolabeled *PHO8* promoter DNA as a probe.

the Rsc3/30 heterodimer into the RSC complex (Wilson et al., 2006). Because Hsp90 and p23 are also considered partners, we checked whether yeast Hsp90 affects the DNA-binding activity of RSC and found that Hsp90 effectively disengaged RSC from naked DNA (Figure 5A). Of note, Hsp90 and p23 were only additive in their abilities to dislodge RSC from DNA and were not synergistic (data not shown). In examining RSC-nucleosome binding and remodeling activities, we discovered a difference in chaperone effects on RSC. Although p23 fostered completion of the remodeling reaction (Figure 2B), Hsp90 simply freed RSC from unremodeled nucleosomes, even in the presence of ATP (Figure 5B; lanes 10–13). By two-hybrid, Hsp90 relieved the reporter auto-activation by AD-Rsc3 and AD-Sfh1, but Hsp90 did not interact with AD-Rsc3 (Figure S5). Hence, Hsp90 can serve as a general release factor of RSC-DNA/nucleosome complexes, but unlike p23, it does not impart a stimulatory influence on RSC remodeling activity.

To investigate the influence of Hsp90 on chromatin architecture in vivo, we used the DNase-Seq assay to map DNase I hypersensitive sites (DHSs) across the genome in yeast expressing either wild-type (WT) Hsp90 or the temperature-sensitive mutant G170D, which inactivates in ~5 min at temperatures > 37°C (Nathan and Lindquist, 1995). DHSs mark areas of open chromatin, including active gene promoters, terminators, and enhancers (Bell et al., 2011). Analysis of the DHS patterns following a 15-min incubation at 37°C revealed a dependence on Hsp90 because ~27% of the DHSs in the WT background were lost in G170D and 585 (~10%) novel sites appear (Figure 5C). For comparison, ~50% of the DHSs are reduced in the p23 null cells, with a gain of ~25% unique sites (Zelin et al., 2012). Additionally, the average DHS width doubled in *p23Δ* relative to parental cells, yet the DHS lengths in G170D and WT were comparable (data not shown). Likely, the differences are attributable to the ability of p23 to modulate both RSC mobility and remodeling, whereas Hsp90 only controls the release of RSC from a target.

Besides detecting open chromatin, DNase-Seq can gauge the relative DNA occupancy of select proteins because cognate DNA elements are protected from DNase I cleavage (Hesselberth et al., 2009). In cells expressing WT Hsp82 136 Rsc3 and 137 Rsc30, consensus sites were bound, whereas in G170D, the numbers rose to 275 and 272, respectively. We believe that in the absence of Hsp90, RSC associates more stably with

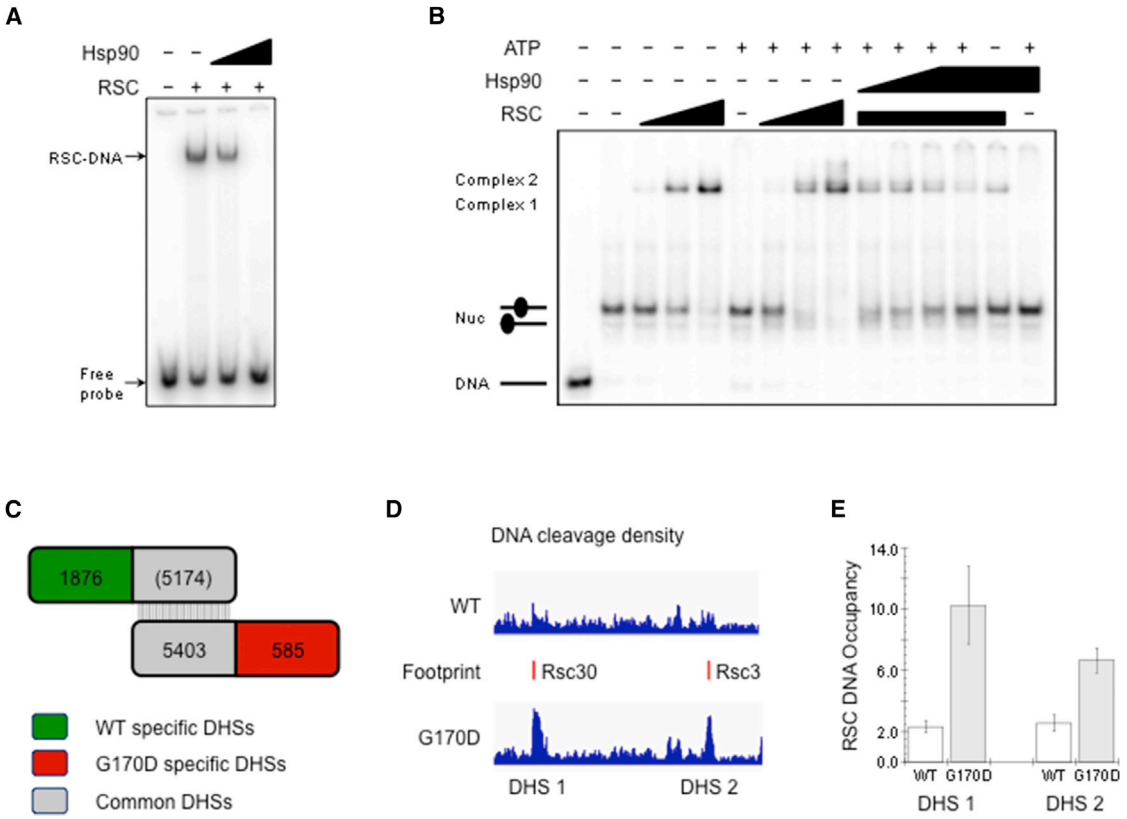


Figure 5. Hsp90 Dissociates RSC from DNA and/or Nucleosomes but Does Not Enhance Remodeling Activity

(A) RSC DNA-binding activity was monitored by EMSA using purified RSC and radiolabeled *PHO8* promoter DNA. The influence of Hsp90 on RSC-DNA structures was determined by titrating in Hsp90 (0, 2, or 20 μM).

(B) RSC nucleosome binding and remodeling activity was checked by EMSA using nucleosomes prepared with radiolabeled 601-positioning DNA and titrations of purified RSC (1, 5, and 10 nM). The influence of ATP (1 mM) and Hsp90 (4 μM) were assessed, as indicated. The migration positions of free DNA, nucleosomes with a centrally or terminally positioned histone, and RSC-nucleosome structures (complex 1 and 2) are marked.

(C) DHSs were identified by deep-sequencing samples following limited DNase I digestion of chromatin within nuclei prepared from yeast expressing either wild-type Hsp82 or the G170D *ts* mutant as the sole source of Hsp90. Prior to collecting the yeast, the cells were incubated at 37°C for 15 min, which inactivates G170D (Nathan and Lindquist, 1995). The green bar represents DHSs unique to wild type, red is DHSs specific to G170D, and gray bars are the remaining sites, with the number of overlaps indicated in parentheses.

(D) At shared DHSs containing either an occupied Rsc30 (DHS 1) or Rsc3 (DHS 2) consensus element, an increase in the levels of DNase I cleavage events was observed. A representative example of the DNA cleavage density taken from chromosome XI (60,938–109,058) is shown with the positions of the occupied Rsc30 or Rsc3 sites marked.

(E) RSC DNA occupancy increased at the DHSs with enhanced nuclease sensitivity. ChIP was used to measure the relative association of RSC at DHS1 and DHS2 in the parental and G170D yeast incubated at 37°C for 15 min. The ChIP data represent the averages of three independent replicas, and error bars are SEM.

DNA. A remarkable aspect of the DNase-Seq data is how quickly the DHS and footprint changes were detected (15 min), indicating that the chromatin landscape is actively maintained by an Hsp90-dependent mechanism.

At DHSs with Rsc3/30 elements occupied in both yeast backgrounds, the DNase I cleavage levels were elevated in G170D, suggesting RSC continued remodeling at the sites, thus lowering nucleosome levels and increasing access to the DNA (Figure 5D). Supporting this contention was the finding that RSC DNA occupancy increased at these DHSs following Hsp90 inactivation (Figure 5E). Because p23 is present in both strains, chaperone assistance for the remodeling reaction is available. Reinforcing a continued activity of RSC in the absence of Hsp90 is the finding that few DHSs with Rsc3/30 footprints were lost in the G170D background (3 of the 1876; Figure 5C). Overall, the DNase-Seq data support our contention that Hsp90 dissociates RSC from DNA and that RSC nucleosome remodeling activity is not Hsp90 dependent. Perhaps the ability of Hsp90 to mobilize remodelers accounts for the previous observation that Hsp90 generally localizes to NDRs (Sawarkar et al., 2012).

RSC and p23 Colocalize along the Genome

Given the dual impact of p23 on RSC in vitro, we focused on p23 to better understand how a chaperone might influence a remodeler in vivo. To start, we checked whether p23 is in position to regulate RSC across the yeast genome. We mapped the genomic sites associated with p23 by ChIP-seq and found 572 high-quality peaks (p values < 0.001). We compared the p23

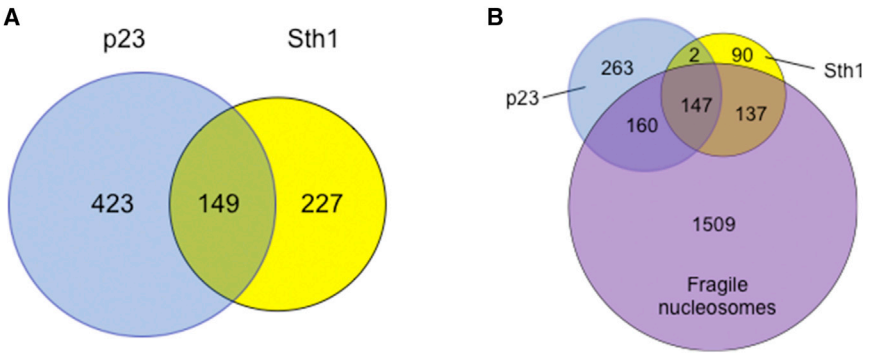


Figure 6. The Genomic Localization Profiles of RSC and p23 Overlap

The genomic association pattern of yeast p23 was determined using α-p23 antibody, chromatin immunoprecipitation, high-throughput sequencing, and bioinformatics.

(A) The identified genomic sites bound by p23 were compared to an established map of the RSC motor subunit Sth1 (Parnell et al., 2015). Of the 149 overlapping sites, 60 contain Rsc3/30 consensus elements.

(B) The overlap between p23 and the known patterns of Sth1 and fragile nucleosomes across the yeast genome was determined (Kubik et al., 2015).

pattern to a recent Sth1 map (Parnell et al., 2015) and found 149 sites in common or ~40% of the Sth1 peaks (Figure 6A). Comparison to another RSC map, which was made by targeting five different RSC subunits, along with microarrays composed of intergenic DNA (Ng et al., 2002), showed that p23 intersected with ~36% of the RSC sites (Figure S6A). Although Fisher exact tests using 2×2 contingency tables indicated high correlations between the p23 and RSC peaks (p values $< 1 \times 10^{-104}$ or $< 1 \times 10^{-71}$, respectively), the overlap between the RSC sites was limited to 80 (Figure S6B). Although the larger overlap between both RSC maps and p23 relative to the two RSC patterns was unexpected, we suspect it reflects the dynamic nature of the RSC complex. Likely, RSC repositions in response to the growth conditions of a given experiment. Because we believe p23 transiently, but repeatedly, interacts with RSC to promote a rapid exchange with chromatin, the p23-RSC association would be independent of the experimental status.

Regardless of the analyzed RSC dataset, commonalities in the shared p23-RSC peaks were apparent. For example, 46 of the 149 shared sites between p23 and Sth1 were at tRNA genes, where RSC continuously depletes nucleosomes to maintain some of the lowest histone occupancies along the genome (Rao et al., 2005; Parnell et al., 2008). Recently, it was shown that RSC is also associated with fragile nucleosomes, which are found at highly expressed genes (Kubik et al., 2015). Of the Sth1 peaks, 284 overlapped with a fragile nucleosome, and p23 was at 307 in total, with 147 being coassociated with Sth1 (Figure 6B); a comparable pattern was found with the other RSC genomic dataset (Figure S5C). Relative to the other DNA-binding protein or DNA elements known to be associated with p23 (Figure S5D), RSC areas displayed a greater overlap with p23 sites. Taken together, our genomic data demonstrate that p23 localizes across the genome at sites of RSC action.

RSC Activities Are p23 Dependent In Vivo

To address whether p23 impacts the cellular function of RSC, we exploited the common use of RSC within signal transduction pathways (Clapier and Cairns, 2009). Prompt reactions to diverse physiological cues necessitate the rapid mobilization of RSC to chromatin areas in need of remodeling in order to support growth (Damelin et al., 2002; Ng et al., 2002). For example, mutations in the RSC motor Sth1 sensitize cells to biological stressors, including ethanol contact (Du et al., 1998). Significantly, loss of *p23* in the *sth1-2* allelic background resulted in yeast hyper-sensitized to ethanol exposure (Figure 7A). Given the capacity of p23 to dissociate RSC from DNA/nucleosomes in vitro, we checked whether p23 altered RSC mobility in vivo. Because RSC does not appear to have a set chromatin localization pattern under normal conditions (Ng et al., 2002; Parnell et al., 2015), we focused on monitoring the arrival kinetics of RSC at ethanol-regulated genes.

RSC nucleation at ethanol-responsive loci occurred in ~1 min in parental cells, but slowed to ~15 min in *p23*Δ (Figures 7B and S7A), and gene activation was delayed even further in the absence of p23 (Figures 7C and S7B). We assessed the local chromatin structure using a micrococcal nuclease mapping assay (Bai et al., 2011). In parental yeast, NDRs near the transcription start sites were apparent, along with well-positioned −1/+1 nucleosomes, whose occupancies declined after ethanol addition (Figures 7D and S7C). In contrast to parental cells, nucleosome levels at the promoters reduced slowly, correlating with the kinetics of gene activation (Figures 7D and S7C). Hence, in the absence of p23, there is a sequential lag in the nucleation of RSC at target promoters, followed by a belated remodeling of nearby nucleosomes. Thus, the in vivo data correlate well with our biochemical studies, showing a dual role of p23 in promoting the transition of RSC between target sites and in fostering nucleosome remodeling. Overall, p23 associates with RSC across the genome, where it fosters a dynamic action critical for manipulating RSC conformations during remodeling and for rapidly relocating RSC in response to fluctuating physiological conditions.

DISCUSSION

Proteins are core units for generating and maintaining life. As such, significant resources are directed toward supporting protein health, including an integrated network of pathways mediating polypeptide synthesis, folding, assembly, and degradation that collectively form the proteostasis process (Hartl et al., 2011). Proficiency of the system is reliant on a continuous sampling of each factor, with variances in conformational states dictating protein fates. Here, we suggest that the persistent activities of p23 and Hsp90 elevate the functionality of assembled protein complexes by exploiting the RSC chromatin remodeler as a molecular model. In addition to the proteostatic contributions, our studies reveal a significant dependency of chromatin architecture on Hsp90.

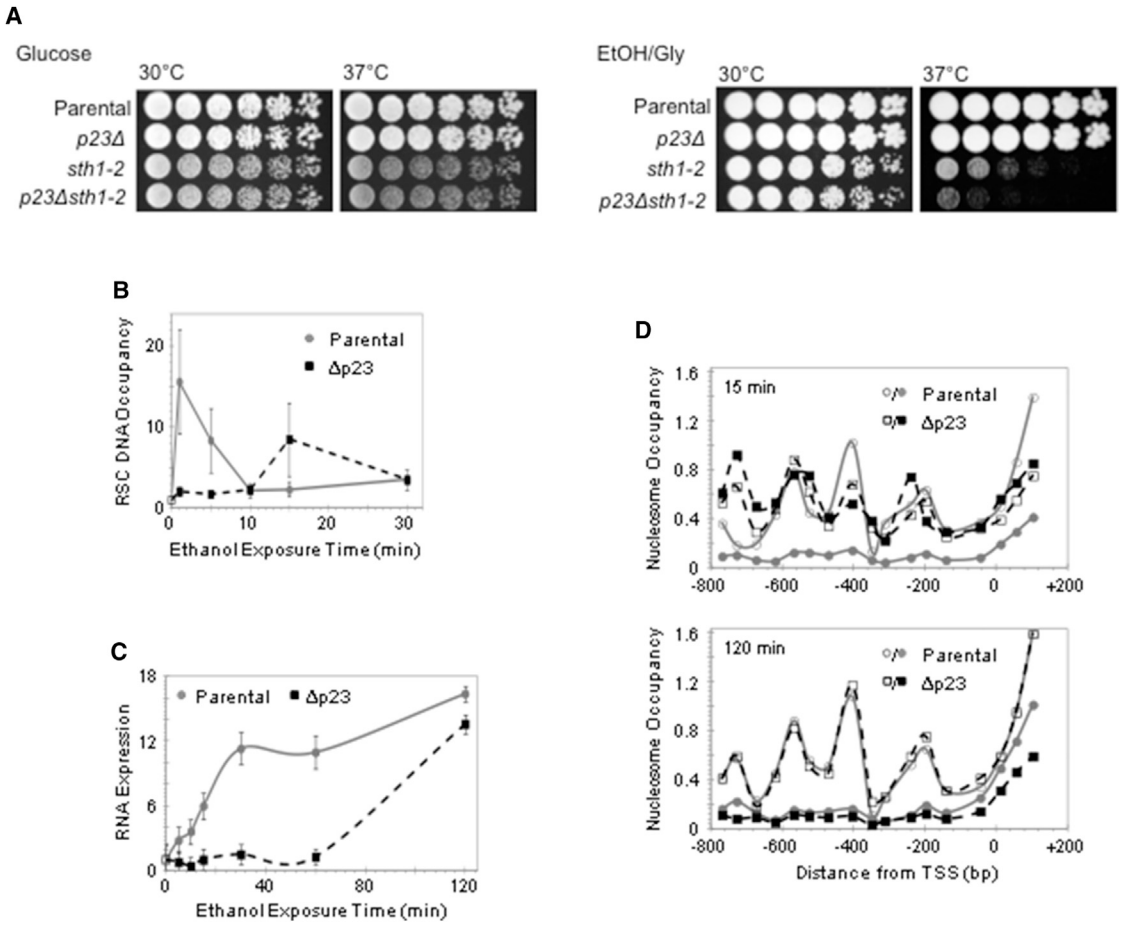


Figure 7. RSC Promoter Recruitment and Remodeling Activity Is Compromised in *p23Δ* Cells

(A) Loss of p23 increases the temperature sensitivity of *sth1-2* yeast grown on ethanol/glycerol (EtOH/Gly), but not on glucose. Logarithmic phase yeast were serially diluted, spotted onto the indicated media, and grown at 30°C or 37°C.

(B) The temporal occupancy of RSC at the *HXK1* ethanol-responsive promoter was determined by ChIP using parental *TAP-RSC3* (solid gray) or *p23ΔTAP-RSC3* (dashed black) yeast. The time course was initiated by the addition of ethanol (7.5% final) to logarithmically growing cells.

(C) The ethanol-induced RNA levels of *HXK1* was monitored by RT-qPCR in parental *TAP-RSC3* (solid gray) and *p23ΔTAP-RSC3* (dashed black) at the noted times following addition of ethanol (7.5%). ACT1 RNA levels were used for normalization.

(D) The relative nucleosome positions and densities at the *HXK1* gene were determined in parental (gray) and *p23Δ* (black) yeast without (open) or with (closed) exposure to ethanol (7.5%) for 15 or 120 min. The ChIP and RT-qPCR data represent the averages of seven independent replicas, the MNase mapping is the average of three replicas, and the error bars are SEM.

Although the use of molecular chaperones in building protein structures has long been appreciated (Ellis, 1987), a post-assembly role is typically not considered. Yet, the overall goal of proteostasis is the upkeep of a healthy proteome (Balch et al., 2008). Because the maintenance of any system is more efficient than de novo synthesis and destruction, a continuous chaperone action with mature factors would be beneficial for perpetuating an active native protein landscape. In this scenario, the binding energy of a chaperone-client interaction dissuades non-native forms, thereby retaining the target within its ensemble of native configurations (Bartlett and Radford, 2009). Hence, the sizable Hsp90 chaperone system provides a low energy mechanism to habilitate a healthy proteome.

Besides proteostatic contributions, we believe Hsp90 and p23 are further used to meet a specialized need of the nucleus (Sawarkar and Paro, 2013; Echtenkamp and Freeman, 2014). The nuclear compartment is usually not associated with proteostasis because conventional protein life cycle events happen in the cytosol (e.g., translation and degradation). Yet, the upkeep of polypeptides in the nucleus is essential for vitality. Highlighting this point is the occurrence of nuclear protein inclusions in about a third of the known aggregation diseases (Woulfe, 2007). Hence, maintaining properly functioning nuclear factors has significant long-term implications, in addition to the immediate actions of these proteins (e.g., remodel chromatin).

In housing a genome, the nucleus encounters unique challenges centering on the need to package chromosomes into a confined space, while allowing "on call" access to the entire length of the DNA. The basic process of condensing chromosomes with histones, while controlling access to the underlying

DNA by altering nucleosome spacing using remodelers, is straightforward (Clapier and Cairns, 2009). Yet, the typical chromatin model does not take into account the disparity in the copy numbers of nucleosomes and remodelers. For example, yeast use ~70,000 nucleosomes to condense their genomes, yet there are only ~200–2,000 RSC particles (Ghaemmaghami et al., 2003; Brogaard et al., 2012; Chong et al., 2015). Even though seven additional remodelers exist, the sum of the assembled particles is still substoichiometric, totaling ~1,300–14,000. Hence, remodelers must be able to rapidly transition between targets in order to effectively manage the overall chromatin architecture in response to fluctuating physiological conditions.

RSC mobility is vital because RSC fosters many genomic pathways requiring quick access to the underlying DNA, including DNA repair, stress, and cell-cycle transcription programs (Clapier and Cairns, 2009; Sinha and Peterson, 2009). For example, exposure to ethanol, rapamycin, or hydrogen peroxide triggers the redistribution of RSC to hundreds of new sites, including promoter nucleosomes that are remodeled to allow gene expression within minutes (Damelin et al., 2002; Ng et al., 2002). In the absence of p23, the ethanol-induced translocation timing of RSC was impaired (Figure 7A). Even after RSC associated with a promoter, the kinetics of nucleosome remodeling were further delayed in *p23*Δ yeast (Figures 7D and S5C). Hence, by modulating multiple steps in the remodeling pathway, the contribution of p23 is considerable.

Why might a chromatin remodeler depend on molecular chaperones? Standard remodeling models advocate that the energy of ATP hydrolysis drives the various structural changes occurring in either the remodeler complex or nucleosome during the course of the reaction (Clapier and Cairns, 2009). Yet, sizeable conformational shifts follow the initial binding event between RSC and a nucleosome. Electron micrograph studies revealed a bowl-like shape of RSC, with a central depression holding the nucleosome (Asturias et al., 2002; Leschziner et al., 2007). In the EM structures, the nucleosomal DNA is disordered because RSC-binding energy, independent of ATP, separates a large section of the DNA from the histones (Asturias et al., 2002; Lorch et al., 2010). Crosslinking studies identified four RSC subunits in close proximity to the DNA, including Sth1, Rsc2, Rsc3, and Rsc4 (Sengupta et al., 2001). We suggest that the binding energy of chaperone interactions shifts the conformational status of the RSC proteins bound to DNA, which triggers the release of the complex. In a manner comparable to the dissociation of the DNA-Rsc3/Rsc30 heterodimer by p23, we favor a model, in which select RSC subunits are targeted by the chaperones to trigger release of the entire complex.

The capacity of p23 and Hsp90 to dissociate RSC from either free or nucleosomal DNA might rationalize why the timing and efficiency of remodeling is significantly different in vitro versus in vivo. For example, RSC redistributes across a genome within minutes and remodels numerous nucleosomes (Damelin et al., 2002; Ng et al., 2002), but under conditions mimicking the cellular state, in which nucleosomal arrays are in excess of RSC, only approximately two arrays are remodeled every 120 min in vitro (Logie et al., 1999). Attempts to enhance the efficiency with hyperacetylated histones resulted in a declined rate of ~0.6 array/120 min. Likely, the acetylation stabilizes the RSC-nucleosome interaction, thereby further slowing the reaction. The longevity of RSC binding is even sufficient to block ligation of nucleosomal DNA in vitro (Lorch et al., 2001). In vivo, however, chromatin remodelers dynamically associate with nucleosomes having half-lives in the range of seconds, even at gene promoters being actively remodeled (Johnson et al., 2008). We suspect the presence of p23 and Hsp90 account for the kinetic differences.

In general, genomic processes function through cooperative mechanisms to rapidly nucleate multi-component structures at precise locations. Although high-affinity interactions produce robust and select actions, the inherent stability of such assembled complexes would impede the timing of biological events. We believe Hsp90 and p23 limit the timing of protein-DNA interactions, thereby creating a highly dynamic nuclear environment to accelerate DNA-associated pathways (Echtenkamp and Freeman, 2014). Of note, an early discovery of yeast p23 showed that loss or overexpression of this chaperone results in chromosome instability, which led to its naming as chromosome stability 18 (*CST18*) (Ouspenski et al., 1999). Significantly, the combination of Hsp90 and histone deacetylase inhibitors has proven to be synergistic in clinical trials (Yang et al., 2008). Although a mechanism involving the acetylation status of Hsp90 had been suggested, our studies open an unexplored therapeutic possibility by showing that proper chromatin architecture relies on Hsp90. Perhaps in a manner analogous to the more common role of molecular chaperones in protein folding, Hsp90 and p23 contribute to nuclear function by circumventing otherwise long-lived, unproductive structures (i.e., pathway intermediates), thereby ensuring an effective forward flow of biological events, including pathways organizing chromatin.

EXPERIMENTAL PROCEDURES

Yeast

The utilized *Saccharomyces cerevisiae* strains are described in Table S1. BY4741 *rsc7::KanR p23::His3* pRS316 p23 locus was produced through tetrad dissection. All p23Δ strains were created using a PCR-based method (Longtine et al., 1998).

Genetic Interaction Assays

A 5-fold serial dilution spot-test assay determined the viability of single (*p23*Δ, *rsc7*Δ, and *sth1-F793S*) and double (*p23*Δ*rsc7*Δ and *p23*Δ*sth1-F793S*) mutants. The Rsc7-associated experiments used plates lacking uracil or containing 5-Fluoroorotic acid (5-FOA) and incubations at 30°C. The Sth1-F793S related tests incubated cells at the non-permissive temperature (37°C) for 24 hr prior to spotting and incubation at 22°C. A 3-fold serial dilution spot test checked the viability of single (*p23*Δ and *sth1-2*) and double (*p23*Δ*sth1-2*) mutants, along with growth on ethanol/glycerol or glucose-containing media.

Protein Purification

Tandem affinity purification (TAP)-tagged RSC complex was isolated as described (Sinha and Peterson, 2009). Recombinant *Xenopus laevis* histones, p23, p23Δ50, and p23Δ84 were purified as described (Luger et al., 1999; Toogun et al., 2007). Recombinant Rsc3 and Rsc30 were purified as His_6-SUMO fusion proteins (Butt et al., 2005).

Nucleosome Reconstitution

The 200-bp DNA fragment of the 601 positioning sequence was generated by PCR from pGem-3Z 601. Nucleosomes were assembled using purified recombinant histone octamers by salt dialysis (Luger et al., 1999).

EMSAs

RSC DNA-binding assays used a radiolabeled *PHO8* probe (7 nM). Reactions were resolved on pre-chilled 3.2% native polyacrylamide Glycerol Tolerant Gel (GTG). For competition assays, the reactions were incubated with 2-, 4-, 8-, or 16-fold unlabeled PHO8 promoter DNA for 5 min prior to resolution. Nucleosomes, RSC, and p23 were incubated for 10 min at r.t. in binding buffer (10 mM Tris, 40 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 5% glycerol, and 50 μg/ml BSA), along with 1 mM ATP, as marked.

Nucleosome Sliding Assay

Nucleosomes (5 nM) were incubated with RSC (1 nM), p23 (as marked) in sliding buffer (7 mM HEPES, 0.1 mM EDTA, 0.06% Tween, 55 mM NaCl, 1 mM DTT, 3 mM MgCl2, 1% glycerol, and 100 μg/ml BSA) at 30°C for 20 min.

Yeast 2-Hybrid

Screen was completed using the Matchmaker GAL4 2-hybrid system (Clontech). RSC subunits and p23 were cloned into pGAD-T7 and pGBK-T7 and expressed in AH109, as indicated. A 10-fold serial dilution spot-test assay was used. Cells were plated on SD+His or SD−His plates and incubated at 30°C.

Chromatin Immunoprecipitation

ChIP assay was performed as previously described (Kuras and Struhl, 1999). Briefly, Rsc3-TAP and *p23*ΔRsc3-TAP strains were growing logarithmically in YPD before ethanol (7.5%) treatment. Isolated DNA was analyzed by qPCR using primers selected to the ethanol responsive loci and the control locus *POL1* (Ng et al., 2002). RSC localization was measured by comparing the ratio of each locus over that of *POL1* for each time point, and then comparing the fold increase of each time point to the 0-min time point.

qPCR

Rsc3-TAP and *Δp23* Rsc3-TAP strains were grown as in the ChIP experiment. Cells were collected at the indicated time points and flash frozen. RNA was isolated using hot phenol extraction and treated with Turbo DNase (Life Technologies). cDNA was made using the SuperScript III First-Strand Synthesis SuperMix for qRT-PCR kit. All time points were normalized to ACT1 RNA levels.

Nucleosome Mapping

MNase digestion of chromatin was performed on spheroplasts, as described (Bai et al., 2011). Digested DNA and genomic DNA were analyzed with overlapping primer pairs for each gene promoter generating ~100-bp PCR products. Nucleosomal occupancy levels were calculated by the fold change between genomic DNA and MNase digested DNA normalized to the *PHO5* TATA (−1) nucleosome.

DNase-Seq Analysis

The DNase-seq protocol was adapted from an established method (Hesselberth et al., 2009), as described (Zelin et al., 2012).

ACCESSION NUMBERS

The accession numbers for the data reported in this paper are GEO: GSE88875, GSE88876, and GSE88877.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2016.09.040.

AUTHOR CONTRIBUTIONS

F.J.E., Z.G., N.L.A., M.L.-D., S.W., and B.C.F. conducted the experiments, Z.G. and Y.Z. analyzed the genomic data, and B.C.F. designed the experiments and wrote the paper. CL.P. designed the experiments.

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