MRN1 implicates chromatin remodeling complexes and architectural factors in mRNA maturation.

Düring, Louis; Thorsen, Michael; Petersen, Darima; Køster, Brian; Jensen, Torben Heick; Holmberg, Steen

Published in:
P L o S One

DOI:
10.1371/journal.pone.0044373

Publication date:
2012

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Düring, L., Thorsen, M., Petersen, D., Køster, B., Jensen, T. H., & Holmberg, S. (2012). MRN1 implicates chromatin remodeling complexes and architectural factors in mRNA maturation. P L o S One, 7(9). https://doi.org/10.1371/journal.pone.0044373
MRN1 Implicates Chromatin Remodeling Complexes and Architectural Factors in mRNA Maturation

Louis Düring¹², Michael Thorsen¹, Darima Sophia Njama Petersen¹, Brian Køster¹³, Torben Heick Jensen², Steen Holmberg¹

1 Department of Biology, Copenhagen BioCenter, University of Copenhagen, Copenhagen, Denmark, 2 Centre for mRNP Biogenesis and Metabolism, Department of Molecular Biology, Aarhus University, Aarhus, Denmark

Abstract

A functional relationship between chromatin structure and mRNA processing events has been suggested, however, so far only a few involved factors have been characterized. Here we show that rsc nhp6Δ1Δ mutants, deficient for the function of the chromatin remodelling factor RSC and the chromatin architectural proteins Nhp6A/Nhp6B, accumulate intron-containing pre-mRNA at the restrictive temperature. In addition, we demonstrate that rsc8-ts16 nhp6Δ1Δ cells contain low levels of U6 snRNA and U4/U6 di-snRNA that is further exacerbated after two hours growth at the restrictive temperature. This change in U6 snRNA and U4/U6 di-snRNA levels in rsc8-ts16 nhp6Δ1Δ cells is indicative of splicing deficient conditions. We identify MRN1 (multi-copy suppressor of rsc nhp6Δ1Δ) as a growth suppressor of rsc nhp6Δ1Δ synthetic sickness. Mrn1 is an RNA binding protein that localizes both to the nucleus and cytoplasm. Genetic interactions are observed between 2 μm-MRN1 and the splicing deficient mutants snt309 Δ, prp3, prp4, and prp22, and additional genetic analyses link MRN1, SNT309, NHP6A/B, SWI/SNF, and RSC supporting the notion of a role of chromatin structure in mRNA processing.

Introduction

In eukaryotes, DNA is packaged into chromatin, which can inhibit the accessibility of DNA binding factors to their cognate sites in vivo. Thus, chromatin structural changes play a central role in controlling gene transcription as the formation of transcripts must contend with the repressive chromatin [1]. For active transcription to take place nucleosomes, the basic units of nucleosomes, the basic units of transcription to take place nucleosomes, the basic units of chromatin, need to be remodeled. ATP-dependent remodelers containing a catalytic subunit belonging to the Swi2/Snf2 family of ATPasers, induce conformational changes in nucleosomes by interacting with nucleosome-DNA interaction. In the Swi2/Snf2 family four different subclasses of remodelers are recognized: SWI/SNF, ISWI, CHD and INO80, that are all conserved from yeast to metazoans [2]. The yeast Saccharomyces cerevisiae contains the founding family member, SWI/SNF, and the highly related RSC (remodels the structure of chromatin) complex. RSC is abundant and holds fifteen-subunits with central roles in transcription [3,4], DNA repair [5] and chromosome segregation [6]. Moreover, a genome-wide location analysis indicated that RSC is recruited to both RNA polymerase II (RNAPII) and RNA polymerase III (RNAPIII) promoters [7] and recently it was shown that RSC regulates nucleosome positioning at RNAPII genes and nucleosome density at RNAPIII genes [8].

The S. cerevisiae chromatin architectural factors and histone modifiers Nhp6A/B are related to the high-mobility group 1 (HMG1) family of small, abundant chromatin proteins that lack sequence specificity of DNA binding, but bend DNA sharply and modulate gene expression [9]. Nhp6 is encoded by two genes, NHP6A and NHP6B, which are functionally redundant. Consequently, only the nhp6Δ1 nhp6Δ2 double deletion mutant (nhp6Δ1Δ2 mutant) is temperature sensitive for growth [10]. Nhp6p is important for activation and repression of transcription of several RNAPII genes [11] and promote transcriptional elongation as part of the FACT complex [12]. Of significance in the context of this paper, Nhp6 is important for expression of the SNR6 gene, encoding the U6 snRNA transcribed by RNAPII [13,14].

The human SWI/SNF subunit BAF57 contains a HMG box domain similar to the one present in Nhp6, which is not found in the yeast complex [15] and the Drosophila BRM component Bap111 is also a HMG-domain protein [16]. In yeast, NHP6 interacts genetically with both SWI/SNF and RSC [17], both RSC and Nhp6 have a repressive effect on the expression of CHA1 [3,11] and data from transcriptome analysis of swi/snf and nhp6Δ1Δ2 mutants, partly overlap [11]. Furthermore, RSC components interact with Nhp6A in vitro and facilitate the loading of Nhp6A onto nucleosomes [17].
A connection between chromatin dynamics and mRNA maturation has previously been suggested [18]. The SWI/SNF complex has been linked to alternative pre-mRNA splicing [19,20]. In higher eukaryotes pre-mRNA splicing is suggested to be a co-transcriptional event [21,22]. In yeast, splicing mainly occurs post-transcriptionally, but initiation of spliceosome assembly and removal of introns from genes with long second exons are probably co-transcriptional events [23,24]. The spliceosome consists of 5 snRNPs (small nuclear ribonucleoprotein particles (U1, U2, U4, U5, U6)) as well as non-snRNP proteins [25,26].

Brml, a subunit of the mammalian orthologue of the yeast SWI-SNF complex interacts with HfPp4, a U5 snRNP-associated kinase [27]. Brm, also a subunit of the mammalian orthologue of the yeast RSC (SWI/SNF) complex, was found upon over-expression to favor inclusion of variant exons in the mRNA and to associate with both U1- and U5-snRNP as well as with coding regions of intron-containing genes [20]. Brm in insect cells was shown to be associated with nascent pre-mRNA's and to regulate the type of alternative transcripts produced [19]. Brml, Brml and additional SWI/SNF-related polypeptides associate with chicken supraspliceosomes [20]. Included in the supraspliceosome is the NineTeen Complex (NTC), which functions in spliceosome activation by specifying the interaction of U5 and U6 with pre-mRNA for their stable association with the spliceosome after U1 and U4 dissociation [29,30].

Here we take a genetic approach and discover an interplay between HMG proteins, chromatin remodeling factors and mRNA maturation. We show that rsc nhp6 triple mutants accumulate pre-mRNA and demonstrate that rsc8-ts16 nhp6ΔA cells display low levels of the U4/U6 snRNA dimer and of total U6 snRNA. Thus, a link between chromatin remodelers, architectural factors and mRNA maturation is established.

Results

Chromatin remodeling complexes and Nhp6 interact genetically

In Saccharomyces cerevisiae, the remodeling complex RSC and the architectural factors Nhp6 have a repressive effect on the chromatin structure at the CHA1 locus [3,11]. Release of both RSC- and Nhp6-dependent repression results in increased transcript levels at CHA1 mRNA, suggesting that RSC and Nhp6 co-operate in CHA1 repression [3,11]. To identify further relationships between RSC and Nhp6, we tested whether Nhp6 genetically interacts with RSC or SWI/SNF and found that the swi2Δ nhp6ΔA and rsc8-ts21 nhp6ΔA triple mutants exhibited a synthetic sickness phenotype compared to their cognate single and double mutants (Figure 1A and Figure 1B). The combination of rsc8-ts16, yfj1-1, sot1-3u, rsc1Δ or rsc2Δ and swi1Δ or swi5Δ mutations swi3Δ, swi5Δ or nhp6Δ with nhp6ΔA also resulted in reduced growth (Table 1). Thus, the architectural factor Nhp6 shares functionality with RSC and SWI/SNF.

Multi-copy growth suppression screen of rsc8-ts16 nhp6ΔA,Δ yields MRN1

Next we performed a suppression screen of the rsc8-ts16 nhp6ΔA synthetic sickness phenotype. Using a Ycp24-based (2 μm) genomic library [31] we isolated YPL184c as a multi-copy suppressor (Figure 1C) and named it MRN1 for multi-copy suppressor of rsc nhp6. Western blot analysis of Myc-tagged 2 μm-MRN1 confirmed increased levels of the Mrn1 protein (Figure 1D). Multi-copy MRN1 was able to suppress the growth defect of all tested rsc nhp6 and swi1Δ nhp6 triple mutants except rsc2Δ nhp6ΔA (Table 1). The latter result is likely indicative of the inability of the rsc2Δ mutation to contain 2 μm plasmids [32].

The Mrn1 protein is predicted to be 612 amino acids long and to contain as many as five RNA Recognition Motifs (RRMs, Figure 1E). Four of these are arranged in pairs and within each pair the RRMs are separated by a short linker (~25 amino acids, Figure 1E). Present in all kingdoms of life, and most abundantly in eukaryotes, RRM domains are able to bind RNA and also DNA and protein(s) [33]. In addition to the predicted RRM domains, Mrn1 contains an N-terminal region rich in asparagine (91% between amino acids 6 and 28, Figure 1E), a region rich in glutamine (44% between amino acids 98 and 125, Figure 1E) and two regions rich in alanine (40–55% between amino acids 171–189 and amino acids 407–422 respectively, Figure 1E).

Cellular location of Mrn1

The cellular location of Mrn1-GFP expressed from its genomic location has been reported to be cytoplasmic [34]. Using the same Mrn1-GFP tagged strain we also observed Mrn1 located primarily in the cytoplasm both at 25°C and 37°C (Figure 2A). However, in these cells we estimated that approximately 5% of Mrn1 is nuclear (M. Lisby, personal communication). To unambiguously detect Mrn1 in the nucleus, we analyzed Mrn1 localization in the temperature-sensitive mex67-5 mRNA export deficient mutant [35]. In the mex67-5 genetic background we detected Mrn1-GFP accumulation in the nucleus at 37°C in approximately 95% of the cells (Figure 2A and Figure 2B). This demonstrates that Mrn1 is located both in the nucleus and the cytoplasm.

Genetic link between chromatin, MRN1 and mRNA processing

To substantiate the genetic link between MRN1 and chromatin remodeling complexes, we combined mnr1A with swi2A or nhp6ΔA, respectively. We found that the mnr1A swi2A combination resulted in synthetic sickness on plates containing 3% formamide (Figure 3A), which is known to cause transcriptional stress, and that the mnr1A nhp6ΔA triple deletion was sick at 37°C (Figure 3B). In contrast, the mnr1A snf5Δ or mnr1A rsc2Δ combinations did not reveal enhanced growth defects (data not shown).

The presence of RRM domains in Mrn1 could suggest a possible role of the protein in mRNP maturation. Interestingly, an ongoing Synthetic Genetic Array (SGA) screen with mnr1A as query linked MRN1 genetically to several splicing deficient mutants (SGA screen to be published elsewhere). Thus, combining mnr1A and the snl309A mutant deleted of the NineTeen Complex (NTC) subunit Snt309 resulted in synthetic sickness (Figure 3C). Snl309 associates with the spliceosome simultaneously with or immediately after dissociation of U4 [36] and the snl309A mutant has a splicing defect that results in the accumulation of intron-containing pre-mRNA at the non-permissive temperature in vivo [36]. Also, we found that 2 μm-MRN1 interacted genetically with snl309Δ as 2 μm-MRN1 suppressed the ts-phenotype of the snl309Δ mutant strain (Figure 3D). The synthetic sickness of mnr1A snl309Δ indicated that multi-copy MRN1 suppression of the ts-phenotype of snl309Δ mutant reflects relevance for endogenous MRN1 function. Constanzo et al. recently reported that mnr1A interacts genetically with pop6-1, pop22, and snl309A [37]. Interestingly, 2 μm-MRN1 also suppressed the ts-phenotype of pop22 (Figure 3E), pop6-1 (Figure 3F) and pop3-1 (data not shown). As 2 μm-MRN1 suppressed the swi1Δ nhp6ΔA and the rsc nhp6ΔA triple mutants as well as snl309A mutant, we examined whether rsc, swi1 nhp6 or nhp6ΔA interacted genetically with snl309Δ and found that both nhp6ΔA snl309A and rsc2Δ snl309A double mutants were
synthetic sick (Figure 3G). In agreement with this, Cairns and co-workers reported genetic interaction between \( snt309D \) and \( rsc7D \) \cite{38}. We also revealed a synthetic lethal interaction between \( snt309D \) and \( nhp6DD \) by tetrad analysis. Out of 21 tetrads 12 did not contain the triple mutant and the remaining 9 each had 3 viable spores and the missing spore would have been the triple mutant (data not shown). To establish that \( snt309D \) and \( nhp6DD \) indeed are synthetic lethal, an \( snt309D \) \( nhp6DD \) heterozygous diploid was transformed with a \( URA3 \) containing plasmid expressing \( NHP6B \). After dissection, genotype verification, and spot assay we found that \( snt309D \) \( nhp6DD \) spores were unable to grow on 5-FOA (Figure 3H). However, Snt309p and Mrn1p are not functionally redundant as high-copy \( SNT309 \) did not suppress the growth defect of the \( rsc8-ts16 nhp6DD \) triple mutant (data not shown).

Figure 1. Synthetic sickness of \( swi/snf nhp6DD \) and \( rsc nhp6DD \) triple mutants is suppressed by \( 2\mu\text{-MRN1} \). (A) and (B) Cells ten-fold serially diluted, spotted on SC plates and incubated for four days. Wild type: SG632; \( swi2D \); SG418; \( nhp6DD \); SG722; \( swi2D nhp6DD \); SG759; wild type: SG358; \( rsc8-ts21 \) SG359; \( nhp6DD \); SG394; \( rsc8-ts21 nhp6DD \); SG658. Colony rows compared in the same panel derives from one plate. (C) Ability of \( 2\mu\text{-MRN1} \) to suppress \( rsc8-ts16 nhp6DD \). Cells streaked on SC-His plates and incubated for four days. Shown on the plates are two transformants containing \( 2\mu\text{-vector} \) and four transformants containing \( 2\mu\text{-MRN1} \). \( rsc8-ts16 nhp6DD \); SG657; \( 2\mu\text{-vector} \); pTK839; \( 2\mu\text{-MRN1} \); pTK1395. (D) Western blot analysis to visualize levels of endogenously expressed and \( 2\mu\) expressed Mrn1-Myc. Rpb3-HA serves as a loading control. Untagged strain TG694 and tagged strain SG40 containing either pTK839 or pTK1423. Two \( \mu\)g of whole cell extract was separated on a SDS-PAGE and immunoblotted with anti-HA or anti-Myc antibody as indicated. (E) A schematic representation of the predicted domains and identified regions in Mrn1 (See text for details).

doi:10.1371/journal.pone.0044373.g001

Table 1. \( 2\mu\text{-MRN1} \) Suppresses \( rsc nhp6 \) and \( swi/snf nhp6 \) Synthetic Sickness.

| Complex | Genotype | Synthetic sick | \( 2\mu\text{-MRN1} \) suppression | Restrictive temperature |
|---------|----------|----------------|--------------------------------|------------------------|
| RSC     | \( rsc1D nhp6DD \) | Yes | Yes | 36\degree C |
|         | \( rsc2D nhp6DD \) | Yes | No | 36\degree C |
|         | \( rsc8-ts16 nhp6DD \) | Yes | Yes | 34\degree C |
|         | \( rsc8-ts21 nhp6DD \) | Yes | ND | 31\degree C |
|         | \( sfh1-1 nhp6DD \) | Yes | ND | 32\degree C |
|         | \( sfh1-3D nhp6DD \) | Yes | Yes | 35\degree C |
| SWI/SNF | \( swi2D nhp6DD \) | Yes | Yes | 35\degree C |
|         | \( swi3D nhp6DD \) | Yes | ND | 35\degree C |
|         | \( snf5D nhp6DD \) | Yes | ND | 36\degree C |
|         | \( snf6D nhp6DD \) | Yes | ND | 31\degree C |

Strains used in Table 1: SG759 (\( snf5D nhp6DD \)), SG469 (\( swi2D nhp6DD \)), SG476 (\( rsc2D nhp6DD \)), SG518 (\( rsc1D nhp6DD \)), SG657 (\( rsc8-ts16 nhp6DD \)), SG658 (\( rsc8-ts21 nhp6DD \)), SG659 (\( sfh1-1 nhp6DD \)), SG661 (\( sfh1-3D nhp6DD \)), SG662 (\( snf5D nhp6DD \)), SG742 (\( swi3D nhp6DD \)) and SG759 (\( swi2D nhp6DD \)). ND = Not determined.
doi:10.1371/journal.pone.0044373.t001
In wild type cells U6 snRNP is in excess of U4 snRNP, but in mutant strains the U4/U6 complex is destabilized. Specifically, in mutant strains including Prp3, Prp4, Prp19, Prp24, Prp38 and Lsm proteins [40,41,42,43,44,45,46]. Apparently, in these mutant components including Prp3, Prp4, Prp19, Prp24, Prp38 and Lsm proteins [40,41,42,43,44,45,46]. Apparently, in these mutant cells accumulate intron-containing pre-mRNA at 25°C and exceedingly more so after incubation at 37°C for two hours (Figure 4C). Again, the relative increase in total RNA levels at 37°C was lower (2–5-fold) than the relative increase in pre-mRNA levels (14–60-fold) (compare Figure 4D and Figure 4E). Furthermore, overexpressed Mrn1 modestly suppressed the accumulation of ACT1, ASC1, and RPS11B pre-mRNA at 37°C (Figure 4C). Analyses of all four intron-containing transcripts in the rsc8-ts16 nhp6ΔAΔ cells indicated a similar accumulation of pre-mRNA at 25°C and exceedingly more so after incubation at 37°C for two hours (Figure S2). Importantly, accumulation of ECM33, ACT1, ASC1, RPS11B pre-mRNAs did not generally occur in single rsc mutants or in the double nhp6 deletion strain (Figure S3). Thus, rsc nhp6 triple mutants exhibited an mRNA maturation deficiency, which was aggravated after a two hour incubation at 37°C. In addition to suppression of the temperature sensitivity of the rsc nhp6 triple and snl309A strains, Mrn1 over-expression also modestly suppressed the pre-mRNA accumulation exhibited by the mutants.

Reduced U4/U6 dimer snRNA levels in rsc nhp6ΔAΔ cells

In wild type cells U6 snRNP is in excess of U4 snRNP, but reduced levels of U6 is a common phenotype in strains with mutations in genes encoding U6, U4/U6, or tri-snRNP components including Prp3, Prp4, Prp19, Prp24, Prp38 and Lsm proteins [40,41,42,43,44,45,46]. Apparently, in these mutant strains the U4/U6 complex is destabilized. Specifically, in snl309A mutant cells the U4/U6 dimer is destabilized, resulting in accumulation of free U4 and decreased levels of total U6 and in failure of spliceosome recycling due to impaired U4/U6 biogenesis [47]. This is underscored as over-expressed U6 suppresses the ts-phenotype of snl309A [47]. In addition, we found that 2 μm-SNR6
restored growth of the rsc2-ts16 nhp6ΔΔ triple mutant (data not shown). To determine the levels of the U4/U6 dimer, free U4 and total U6 in rsc2-ts16 nhp6ΔΔ cells total RNA was fractionated both on non-denaturing and denaturing polyacrylamide gels for Northern analysis with U4 and U6 specific probes, respectively. The rsc2-ts16 nhp6ΔΔ cells had decreased amounts of the U4/U6 dimer and accumulated free U4 at 25°C (Figure 5A). Interestingly, the amount of U4/U6 dimer was further decreased after a two-hour incubation at 37°C. In the mutant total U6 snRNA levels were also reduced after two hours at 37°C (Figure 5B). To quantify the snRNA levels we performed five independent experiments and normalized the U4 and U6 data to the same blots re-probed for U1 snRNA. First, the rsc2-ts16 nhp6ΔΔ strain showed a more than 5-fold increase in free U4; second, at 25°C the mutant strain had a 2-fold decrease in the levels of U4/U6 and total U6; third, a two-fold decrease in the levels of U4/U6 and total U6 snRNA; fourth, a two-fold decrease in the levels of U4/U6 and total U6; fifth, a ten-fold increase in free U4. To determine the levels of U4/U6 dimer, free U4 and total U6 snRNA, of total U6 snRNA and accumulate free U4 snRNA at 25°C and that the low levels of U4/U6 dimer and of total U6 is further aggravated after a two-hour shift at 37°C.

U4/U6 dimer and total U6 snRNA levels are unchanged after a two-hour transcriptional shutdown

Both Rsc and Nhp6 are involved in transcriptional regulation of RNPIII transcribed genes and high-copy SNR6 suppresses the growth defect of nhp6ΔΔ double mutants [14,48]. Therefore, it was important to determine if the observed reduction in U4/U6 dimer and total U6 snRNA levels in the triple mutant was an effect of the rsc nhp6 mutations to reduce SNR6 transcription. In this case the drop in U4/U6 and total U6 snRNA content would just reflect SNR6 RNA turnover. We addressed this question by determining U4/U6 dimer, free U4 and total U6 stability in rsc2-ts16 nhp6ΔΔ cells after growth for two hours in the presence of thiolutin. The antifungal agent thiolutin efficiently inhibits all three yeast polymerases both in vivo and in vitro [49,50]. Total RNA was

Figure 3. Genetic interactions linking MRN1 and chromatin mutants to pre-mRNA splicing. (A) mrn1Δ is synthetic sick with swi2Δ. Cells ten-fold serially diluted, spotted on SC plates and incubated for four days at 30°C. swi2Δ: SG418; swi2Δ Δ: SG520; wild type: SG632 and mrn1Δ swi2Δ: SG766. (B) mrn1Δ is synthetic sick with nhp6ΔΔ. Cells ten-fold serially diluted, spotted on SC plates and incubated for four days at the indicated temperatures. mrn1Δ: SG520; wild type: SG632; nhp6Δ Δ: SG727 and mrn1Δ nhp6Δ Δ: SG762. (C) mrn1Δ snt309Δ cells are synthetic sick. Cells ten-fold serially diluted, spotted on SC plates and grown at the indicated temperatures for four days. mrn1Δ: SG912; wild type: SG632; snt309Δ: SG648; mrn1Δ snt309Δ: SG920. (D) The temperature sensitivity of snt309Δ is suppressed by 2 μM-MRN1. Cells ten-fold serially diluted, spotted on SC-His plates and incubated for four days at the indicated temperatures. Wild type: SG632; snt309Δ: SG648; 2 μM-vector: pTK839; 2 μM-MRN1: pTK1395. (E) The temperature sensitivity of prp22 is suppressed by 2 μM-MRN1. Cells ten-fold serially diluted, spotted on SC-Ura plates and incubated for four days at the indicated temperatures. prp4-1: SG845; 2 μM-vector: pTK51; 2 μM-MRN1: pTK1386. (G) snf5Δ and rsc2Δ genetically interacts with snt309Δ. Cells ten-fold serially diluted, spotted on SC plates and incubated for four days at the indicated temperatures. rsc2Δ: SG417; snf5Δ: SG420; wild type: SG632; snt309Δ: SG729; rsc2Δ snt309Δ: SG773 and snf5Δ snt309Δ: SG774. (H) snt309Δ is synthetic lethal with nhp6ΔΔ. Cells ten-fold serially diluted, spotted on SC-Ura plates or 5-FOA plates and incubated for four days at 30°C. Wild type: SG865; nhp6Δ Δ: SG867; snt309Δ: SG868; snf5Δ nhp6Δ Δ: SG869; 2 μM-NHP6-Ura3: pTK1382. Colony rows compared in the same panel derives from one plate.

doi:10.1371/journal.pone.0044373.g003
Figure 4. rsc8-ts16 nhp6ΔΔ cells accumulate unspliced transcripts. (A) Northern blot analysis was done with total RNA isolated from logarithmically SC-His growing cells at 25°C or after a two hour shift at 37°C. Total RNA was electrophoresed in a 0.25 M formaldehyde agarose gel, blotted and hybridized with specific 32P-labeled probes. The probe was either intron-specific or 3′ exon-specific, respectively, for the ECM33 RNA (see Figure S1). Ethidium bromide staining of the 18S and 25S rRNA is shown as a loading control. (B) Northern blot analysis was done with total RNA isolated from logarithmically SC-His growing cells at 30°C or after a two hour shift at 37°C. The probe was specific for both the RPS11B pre-mRNA and for the RPS11B mRNA (see Figure S1). Ethidium bromide staining of the 18S and 25S rRNA is shown as a loading control. (C), (D) and (E) Total RNA isolated from logarithmically SC-His growing cells at 25°C or after a two-hour shift at 37°C amplified by RT-qPCR with ECM33-, ACT1-, ASC1-, RPS11B- or RDN25-specific primers. (C) The ratio intron-3′exon junction RT-PCR-amplificate/3′exon RT-PCR-amplificate. (D) The ratio 3′exon RT-PCR-amplificate/RDN25 RT-PCR-amplificate. (E) The ratio intron-3′exon junction RT-PCR-amplificate/RDN25 PCR-amplificate. The ratio in wild type cells at 25°C was arbitrarily set to 1. Wild type: SG632; rsc8-ts16 nhp6ΔΔ: SG657; 2 μ-vector: pTK839; 2 μ-MRN1: pTK1423.

doi:10.1371/journal.pone.0044373.g004
isolated from cells treated with thiolutin for two hours and the levels of U4/U6, free U4, and total U6 was determined by Northern blotting. The amount of U4/U6 dimer, free U4 and total U6 snRNA was unchanged after two hours of incubation with thiolutin both in the wild type and more importantly, also in the rsc8-ts16 nhp6 DD strain (Figure 6A and Figure 6B). Quantification of four independent experiments confirmed this result (Figure 6C). In contrast, as expected the levels of three tested mRNA’s were drastically reduced under the same growth conditions (Figure 6D).

Thus, efficiently shutting down RNA polymerase III transcription by the polymerase inhibitor thiolutin for two hours did not influence the levels of U4/U6 dimer or total U6 snRNA neither in the wild type nor in the rsc8-ts16 nhp6DD strain indicating that the decrease in snRNA levels is not due to impaired transcription of the SNR6 gene, but only observed in cells with specific splicing-deficient conditions.

**Discussion**

In this study we have utilized a genetic approach to study the functional interplay between the chromatin remodeling complexes RSC or SWI/SNF and the architectural factors Nhp6. We found that rsc- or swi/snf mutations in combination with nhp6 double deletion results in synthetic sickness. Interestingly, we found that rsc nhp6 triple mutants accumulate pre-mRNA, strongly suggesting a defect in pre-mRNA maturation. The defect in pre-mRNA maturation is underscored as rsc8-ts16 nhp6DD cells contained low levels of U4/U6 dimer and total U6 snRNA as well as high amounts of free U4 snRNA. Further, incubation at 37°C for two hours dramatically enhanced the accumulation of pre-mRNA in rsc nhp6DD cells. This is substantiated as rsc8-ts16 nhp6DD cells contained significantly reduced amounts of U4/U6 dimer and total U6 after two hours incubation at 37°C. The reduction in U4/U6 dimer and total U6 was not due to deficient SNR6 transcription as a two-hour shutdown of SNR6 transcription induced by thiolutin did not result in reduced amounts of U4/U6 dimer or total U6 indicating that these RNAs are very stable. In agreement with this result, U6 snRNA have previously been reported to be very stable unless in a splicing deficient mutant background [40]. For example, temperature inactivation of the known U6 (or U4/U6) snRNP associated factors Prp3, Prp4, Prp6, Prp24 or the NTC or Prp38 splicing factors lead to a decrease in U6 snRNA levels [40,43,44,45,46,47]. Apparently, in these mutant strains the U4/U6 complex is destabilized, perhaps exposing the U6 snRNA to intracellular nuclease attack. Furthermore, Moenne et al. [51] observed only a slight decrease in total U6 snRNA level after a five hour inactivation of a temperature-sensitive RNAPIII mutant. Accordingly, a two-hour shift to 37°C reduces U4/U6 dimer and total U6 snRNA levels in rsc8-ts16 nhp6DD cells as a consequence of their mRNA processing defect and not as a consequence of deficient transcription of SNR6. We did not see a general accumulation of unspliced mRNA for the tested transcripts after a two hour incubation at 37°C in the rsc1A or rsc8-ts16 single mutants, or in the nhp6DD double mutant. However, the rsc and swi/snf single mutants, and the nhp6DD double mutant might harbor potential splicing defects. In support of this notion we observed that combining rsc2A or snf5A with the NTC splicing mutant sn309A resulted in synthetic sickness and that a sn309A nhp6DD triple mutant is synthetic lethal. In conclusion, the
Figure 6. U4/U6 dimer, free U4 and total U6 snRNA levels remain stable after two hours of transcriptional shutdown. Total RNA prepared from logarithmically SC-His growing cells at 25°C or after two hours growth in the presence of 5 μg/ml Thiolutin at 25°C. (A) RNA was fractionated on a non-denaturing 6% polyacrylamide gel, blotted and hybridized with a U4 specific probe. After analysis the membrane was stripped and re-probed with a U1 specific probe. (B) RNA was fractionated on a denaturing 6% polyacrylamide gel, blotted and hybridized with a U4 specific probe. After analysis the membrane was stripped and re-probed with a U1 specific probe. (C) Quantification of U4/U6 dimer, free U4 and Total U6 snRNA amounts relative to U1 snRNA based on quantification of Storm Images from at least four individual experiments. (D) Total RNA was isolated from logarithmically SC-His growing rsc8-ts16 nhp6ΔΔ cells at 25°C and electrophoresed in a 0.25 M formaldehyde agarose gel or in a denaturing 6% polyacrylamide gel, blotted and hybridized with gene-specific 32P-labeled probes. rsc8-ts16 nhp6ΔΔ : SG657. doi:10.1371/journal.pone.0044373.g006

The observed pre-mRNA accumulation in the rsc nhp6 triple mutants can be explained in several ways. The lack of RSC/Nhp6 activity concomitantly might influence transcription of splicing factor-encoding genes leading to the observed pre-mRNA accumulation and U4/U6 destabilization. It is also possible that the primary splicing block imposed by the rsc8-ts16 nhp6ΔΔ mutant results from the failure of splicing complexes to assemble or function properly. Thus, RSC and Nhp6 might be required for generating the correct chromatin state required for proper spliceosome assembly thereby affecting mRNA processing. Recent studies document connections between chromatin and splicing. The mammalian orthologue of the RSC complex, hSWI/SNF subunit Brm, was found to associate with several components of the spliceosome as a regulator of alternative splicing in several mammalian cell types [20]. Likewise, Brm and several hSWI/SNF subunits were shown to associate with chicken supraspliceosomes [28]. In yeast only very few genes contain more than one intron, and although it has been reported that most splicing is post-transcriptional, recruitment of U1 is a co-transcriptional event at probably all genes [24]. One possibility is that rsc nhp6 and swi/snf nhp6 cells are deficient in the process of co-transcriptional recruitment of the pre-spliceosome. Batschê et al. [20] showed that Brm interacts in vivo with both U1 and U5 snRNPs and suggested that hSWI/SNF is involved in recruitment of the splicing machinery. Tyagi et al. [19] recently showed that Brm interacts directly with nascent pre-mRNPs and suggest that Brm post-transcriptionally regulates the type of alternative transcript produced. Whether RSC, SWI/SNF and/or Nhp6 factors can be loaded onto pre-mRNA in yeast remains to be elucidated.
**Table 2. Yeast Strains Used in This Study.**

| Strain   | Genotype                                                                 | Source or reference |
|----------|---------------------------------------------------------------------------|---------------------|
| SG304    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG306    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG312    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG350    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG358    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG359    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG360    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG394    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG416    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG417    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG418    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG420    | BY4741 MATa leu2·10 his3·11 ura3·30 met15·10 rsc1·1::KANMX               | Euroscarf           |
| SG452    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG476    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG488    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG518    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG520    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG655    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG659    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG661    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG662    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG668    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG672    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG676    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG677    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG678    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG680    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG684    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG685    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG686    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG687    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG688    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG689    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG690    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG691    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG692    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG693    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |
| SG694    | MAT\(^{\text{d}}\) his3·11 ura3·30 met15·10 rsc1·1::KANMX               | This study          |

\(^{\text{d}}\): The mating type has not been determined.

doi:10.1371/journal.pone.0044373.t002
mTK1395 was transformed into strain SG657 and was able to digested pRS423 (pTK839) resulting in plasmid pTK1395.

YPL184c the 2357 bp fragment containing complete ORF. pTK1386 was digested with synthetic sickness. pTK1386 contained YPL184c re-ligated) containing the genomic sequence from 194878 to 198486 of chromosome XVI. Subcloning revealed that plasmid non-permissive temperature (34°C)

Construction of MRN1-MYC

The endogenous MRN1-MYC was constructed by inserting a Myc-Tag C-terminally on the MRN1 gene by transformation and homologous recombination in yeast strain TG694 with two PCR fragments using homologous recombination in yeast with a Myc-Tag containing PCR fragment. DNA was amplified with oligonucleotides MYCa, sfh1d, sfh1e and MYCab as the selection marker. DNA was amplified with oligonucleotides MYCc and MYCab using pTK1259 as the template. Plasmid pTK1423 was rescued in this study.

Table 3. Plasmids Used in This Study.

| Name       | Genotype         | Source or reference |
|------------|------------------|--------------------|
| pTK151     | Yep24: 2 μm-URA3-Ampr' | [63]               |
| pTK639     | pRS423: 2 μm-HIS3-Ampr' | [64]               |
| pTK1259    | pFA6a: 13Myc-KANMX6 | [65]               |
| pTK1382    | 2 μm-URA3-Ampr'-NHPSB | This study         |
| pTK1385    | 2 μm-URA3-Ampr'-MRN1 | This study         |
| pTK1386    | 2 μm-URA3-Ampr'-MRN1 | This study         |
| pTK1395    | 2 μm-HIS3-Ampr'-MRN1 | This study         |
| pTK1423    | 2 μm-HIS3-Ampr'-MRN1-MYC | This study |
| pML96      | URA3-Ampr'-NLS-yEmRFP | M. Lisby           |

Materials and Methods

Media, strains and genetic methods

Yeast extract-peptone-dextrose (YPD) medium, synthetic minimal (SD) medium, synthetic complete (SC) and SC lacking specific amino acids were prepared as described previously [53]. Standard yeast methods were used for dissection, sporulation, mating and replica plating. Lithium acetate transformation was used for chromosome XVI. Subcloning revealed that plasmid pTK1386 digested with NheI and SacI, blunt ended and re-ligated) containing the genomic sequence from 194878 to 198486 of chromosome XVI was a suppressor of ts8-ts16 nhp6ΔA synthetic sickness. pTK1386 contained TPI184c as the only complete ORF, pTK1386 digested with SmaI and EcoRI and the 2357 bp fragment containing TPI184c, from 198277 to 195919 of the genomic sequence, was cloned into SmaI and EcoRI digested pRS423 (pTK839) resulting in plasmid pTK1395. pTK1395 was transformed into strain SG657 and was able to suppress its growth defect, and accordingly, we concluded that 2 μm-TPI184c is a suppressor of the synthetic sickness of the ts8-ts16 nhp6ΔA triple mutant.

Multi-copy suppressor screen

Strain SG657 (ts8-ts16 nhp6ΔA) was transformed with a Yep24 based yeast genetic library [31]. Colonies able to grow at the non-permissive temperature (34°C) were selected. In total ~2 x 107 transformants were screened. Plasmids from ~40 colonies were rescued in Escherichia coli and 17 different plasmids were identified as suppressors. Sixteen contained either NHPS6A or NHPS6B. One plasmid, pTK1385, contained the genomic sequence from 190959 to 198486 of chromosome XVI. Subcloning revealed that plasmid pTK1386 (pTK1385 digested with NheI and SacI, blunt ended and re-ligated) containing the genomic sequence from 194878 to 198486 of chromosome XVI was a suppressor of ts8-ts16 nhp6ΔA synthetic sickness. pTK1386 contained TPI184c as the only complete ORF, pTK1386 digested with SmaI and EcoRI and the 2357 bp fragment containing TPI184c, from 198277 to 195919 of the genomic sequence, was cloned into SmaI and EcoRI digested pRS423 (pTK839) resulting in plasmid pTK1395. pTK1395 was transformed into strain SG657 and was able to suppress its growth defect, and accordingly, we concluded that 2 μm-TPI184c is a suppressor of the synthetic sickness of the ts8-ts16 nhp6ΔA triple mutant.

Fluorescence microscopy

Fluorescence microscopy was done with a Zeiss Imager Z1 using the channels for GFP, RFP and DIC. Logarithmically SC growing cells at 25°C or after a 30 min incubation at 37°C was harvested for microscopy. Strains SG1008 (MRN1-GFP ADH1p-NLS-yEmRFP::URA3) and SG1010 (me67-5 ADH1p-NLS-yEmRFP::URA3) were constructed by integrating plasmid pML96-IntADH1p-NLS-yEmRFP-1 in the uaa3 locus in strains SG737 and SG736 (Table 2), respectively, after digestion with NheI. For quantification of each genotype and growth condition, 100–200 cells were inspected. Error bars indicate 95% confidence intervals.

Measurement of pre-mRNA accumulation by RT-qPCR

RNA was electrophoresed in a 0.25 M formaldehyde agarose gel, transferred to a Hybond-NX (GE Healthcare) membrane by blotting overnight. RNA was cross-linked to the membrane in a Stratalinker (1200 J/cm²). Radioactively (32P) random primed labeled probes were produced with Prime-It II Random Primer Labeling Kit (Stratagene) and purified with ProbeQuant G-25 Micro Columns (Amersham), utilizing gel purified PCR product as the template. The templates were produced with specific primers (Table 4) utilizing genomic yeast DNA as the template. Membranes were hybridized over night in a Hybid oven at 42°C with Ultrahyb hybridization buffer (Ambion) and the membranes were washed as recommended by the manufacture. Hybridized probe were visualized and quantified using a Storm 840 Phosphorimager (Molecular Dynamics) and also visualized with Kodak BioMax MS Film when needed.
### Table 4. Oligonucleotides Used in This Study.

#### Oligonucleotides for RT-PCR and RT-qPCR

| Name | Sequence |
|------|----------|
| ACT1 (intron-exon2): Act1c | 5' GGTCGACATTGCTCGAGAGATTTC 3' |
| ACT1 (intron-exon2): Act1d | 5' CGGTTTACATACCAGAACCAG 3' |
| ACT1 (3' exon): Act1e | 5' GCCTTACATGATCCTGCAACCC 3' |
| ACT1 (3' exon): Act1f | 5' GGCGTGAGGTAGAGAGAAACCAG 3' |
| ASC1 (intron-exon2): Asc1a | 5' GTGATGATGAGAATTGAGC 3' |
| ASC1 (intron-exon2): Asc1b | 5' GCTGTTACAGGAGAAGGCTG 3' |
| ASC1 (3' exon): Asc1c | 5' ACCGAGAAGGACAATGACTTG 3' |
| ASC1 (3' exon): Asc1d | 5' ACCGAGAAGGACAATGACTTG 3' |
| ECM33 (intron-exon2): Ecm33a | 5' AACCGAGAAGGACAATGACTTG 3' |
| ECM33 (intron-exon2): Ecm33b | 5' ACCGAGAAGGACAATGACTTG 3' |
| ECM33 (3' exon): Ecm33c | 5' GCCGAGAAGGACAATGACTTG 3' |
| ECM33 (3' exon): Ecm33e | 5' GCCGAGAAGGACAATGACTTG 3' |
| RPS11B (intron-exon2): Rps11a | 5' AACCGAGAAGGACAATGACTTG 3' |
| RPS11B (intron-exon2): Rps11b | 5' AACCGAGAAGGACAATGACTTG 3' |
| RPS11B (3' exon): Rps11c | 5' AACCGAGAAGGACAATGACTTG 3' |
| RPS11B (3' exon): Rps11d | 5' AACCGAGAAGGACAATGACTTG 3' |
| RDN25: Rd25-1a | 5' GCGAGAAGGACAATGACTTG 3' |
| RDN25: Rd25-1b | 5' GCGAGAAGGACAATGACTTG 3' |

#### Oligonucleotides used in PCR amplification of a template for Northern blot probes

| Name | Sequence |
|------|----------|
| ECM33 (intron): Ecm33g | 5' CCTCAGAATTCGGAGGATCATG 3' |
| ECM33 (intron): Ecm33h | 5' TATGTAATCAGGAGAATGACTTG 3' |
| ECM33 (3' exon): Ecm33c | 5' GCCGAGAAGGACAATGACTTG 3' |
| ECM33 (3' exon): Ecm33f | 5' GCCGAGAAGGACAATGACTTG 3' |
| RPS11B: oMiT138 | 5' GCCGAGAAGGACAATGACTTG 3' |
| RPS11B: oMiT138 | 5' GCCGAGAAGGACAATGACTTG 3' |
| U1: Snr19a | 5' GCCGAGAAGGACAATGACTTG 3' |
| U1: Snr19b | 5' GCCGAGAAGGACAATGACTTG 3' |
| Cha1op | 5' GCCGAGAAGGACAATGACTTG 3' |
| Cha1ned | 5' GCCGAGAAGGACAATGACTTG 3' |
| Hta1e | 5' GCCGAGAAGGACAATGACTTG 3' |
| Hta1f | 5' GCCGAGAAGGACAATGACTTG 3' |
| Cyc1a | 5' GCCGAGAAGGACAATGACTTG 3' |
| Cyc1b | 5' GCCGAGAAGGACAATGACTTG 3' |

#### Oligonucleotides used directly as template for Northern blot probes

| Name | Sequence |
|------|----------|
| U4: Snr14 | 5' GCCGAGAAGGACAATGACTTG 3' |
| U6: Snr6a | 5' GCCGAGAAGGACAATGACTTG 3' |

#### Oligonucleotides used in the amplification of Myc-Tagged DNA fragment

| Name | Sequence |
|------|----------|
| MYCc | 5' TGGAGAAGGACAATGACTTG 3' |
| MYCAb | 5' TGGAGAAGGACAATGACTTG 3' |
| MYCa | 5' TGGAGAAGGACAATGACTTG 3' |
| Sfh1d | 5' TGGAGAAGGACAATGACTTG 3' |
| Sfh1e | 5' TGGAGAAGGACAATGACTTG 3' |
(BIO-RAD) was used for the RT-qPCR amplifications done with the iCycler iQ (BIO-RAD). Data was analyzed with the iCycler-iQ software (BIO-RAD). Standard deviations were calculated as suggested by Simon [56]. The sequence of used oligonucleotides is shown in Table 4.

U4/U6 Assay
To visualize U4/U6 dimer, U4 Free and total U6 exponentially growing cells were harvested and resuspended in 250 µl of RNA extraction buffer (100 mM LiCl, 1 mM EDTA, 100 mM Tris-Cl (pH 7.5), 0.2% SDS) and transferred to a tube containing 250 µl glass beads and 250 µl Phenol-chloroform-isooamyl alcohol (25:5:0.2). Then the cells were lysed in a bead mill for 3 x 15 sec at 4°C. For non-denaturing gels the aqueous phase containing the RNA was mixed with one-third volume of loading dye (50% glycerol, 0.02% bromophenol blue) and loaded on a 6% non-denaturing polyacrylamide (29:1) Tris-borate-EDTA gel containing 5% glycerol with 0.5 TBE as running buffer. The gel was run overnight at 80 V at 4°C. The gel was then soaked twice in 20 mM NaPO4 (pH 6.5), 8.3 M urea, 0.1% SDS at 37°C, for 45 min and once in 20 mM NaPO4 (PH 6.5) at 4°C for 1 hour. RNA was electrotransferred to a nylon membrane (Hybond-NX (GE Healthcare)) followed by UV cross-linking to the membrane in a Stratalinker (1200 Φ) for 5 min washes in 6xSSC, 0.2% SDS and one 15 min wash in 2xSSC, 0.2% SDS at 42°C. Hybridized probe were visualized and quantified using a Storm 840 Phosphorimager (Molecular Dynamics) and also visualized with Kodak BioMax MS Film when needed. This protocol was modified from Lygerou et al. [57].

References
1. Workman JL (2006) Nucleosome displacement in transcription. Genes Dev 20: 2009–2017.
2. Becker PB, Horz W (2002) ATP-dependent nucleosome remodeling. Annu Rev Biochem 71: 247–273.
3. Moreira JM, Hohlberg S (1999) Transcriptional repression of the yeast CHAI gene requires the chromatin-remodeling complex RSC. Embry 1: 2836–2844.
4. Angus-Hill ML, Schlacher A, Roberts D, Erdjument-Bromage H, Tempst P, et al. (2001) A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the chromatin-remodeling RSC in gene expression and cell cycle control. Mol Cell 7: 741–751.
5. Chai B, Huang J, Cairns BR, Laurent BC (2005) Distinct roles for the RSC and Swr1/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. Genes Dev 19: 105–110.
6. Hsu JM, Huang J, Meluh PB, Laurent BC (2003) The yeast RSC chromatin-remodeling complex is required for kinetochore function in chromosome segregation. Mol Cell Biol 23: 3202–3215.
7. Ny HH, Robert F, Young RA, Shroff K (2002) Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. Genes Dev 16: 806–819.
8. Parme TJ, Huff JT, Cairns BR (2008) RSC regulates nucleosome positioning at Pol-II genes and density at Pol III genes. Embry 17: 100–110.
9. Bustin M (1999) Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol 19: 5237–5246.
10. Costigan G, Kolodrubetz D, Snyder M (1994) NHP6a and NHP6b, which encode HMG1-like proteins, are candidates for downstream components of the yeast SLT2 mitogen-activated protein kinase pathway. Mol Cell Biol 14: 2403–2409.
11. Moreira JM, Hohlberg S (2000) Chromatin-mediated transcriptional regulation by the yeast architectural factors NHP6a and NHP6b. Embo J 19: 6804–6813.
12. Brevet D, Johnston GC, Singer RA (2001) A bipartite yeast SSRP1 analog comprised of Pol3 and Nhp6 proteins modulates transcription. Mol Cell Biol 19: 3191–3197.
13. Lopez S, Livingstone-Zatchej M, Jourdain S, Thoma F, Sentenac A, et al. (2001) High-mobility-group proteins NHP6a and NHP6b participate in activation of the RNA polymerase III SNR6 gene. Mol Cell Biol 21: 3096–3104.
14. Krapov M, Moir RD, Kolodrubetz D, Willie J (2001) Nhp6, an HMG1 protein, functions in SNR6 transcription by RNA polymerase III in S. cerevisiae. Mol Cell 7: 309–318.
15. Wang W, Chl T, Xue Y, Zhou S, Kao A, et al. (1998) Architectural DNA binding by a high-mobility-group/kinase-like subunit in mammalian SWI/SNF-related complexes. Proc Natl Acad Sci U S A 95: 492–498.
16. Papoulas O, Daubresse G, Armstrong JA, Jin J, Scott MP, et al. (2001) The HMG-domain protein BAP11 is important for the function of the BRM chromatin-remodeling complex in vivo. Proc Natl Acad Sci U S A 98: 5728–5733.

Supporting Information
Figure S1 Schematic representation of RPS11B, ASC1, ACT1, and ECM33 probes and qPCR primers. The relative position of the DNA fragments used as RPS11B and ECM33 Northern probes as well as the relative position of the primers used for the qPCR analyses are depicted. (TIF)
Figure S2 rsc1A nhp6ΔA and snr309A cells accumulate unspliced transcripts. Total RNA isolated from logarithmically SC-His growing cells at 25°C or after a 2 hour shift at 37°C amplified by RT-qPCR with ECM33-, ACT1-, ASC1- or RPS11B-specific primers. The ratio intron-3’exon junction RT-PCR-amplify/3’exon RT-PCR-amplify. The ratio in wild type cells at 25°C was arbitrarily set to 1. ND: Not determined. Wild type: SG632; rsc1A nhp6ΔA: SG318; snr309A: SG648. (TIF)
Figure S3 rsc1A and rsc8-ts16 or nhp6ΔA mutants do not generally accumulate unspliced mRNA at 37°C. Total RNA isolated from logarithmically SC-His growing cells at 25°C or after a 2 hour shift at 37°C amplified by RT-qPCR with ECM33-, ACT1-, ASC1- or RPS11B-specific primers. The ratio intron-3’exon junction RT-PCR-amplify/3’exon RT-PCR-amplify. The ratio in wild type cells at 25°C was arbitrarily set to 1. Wild type: SG632; rsc1A: SG416, rsc8-ts16: SG360 and nhp6ΔA: SG306. (TIF)

Acknowledgments
We are very grateful to Birgith Kolding and Perrille Roshof for excellent technical assistance, Christophe Concalves for help with the Robetta Server and Michael Libby for providing the ADHIP-l/Mx2EmRFP::URA4 construct and for critical reading of the manuscript. Soo-Chen Cheng is thanked for providing high copy YPT309 plasmids.

Author Contributions
Conceived and designed the experiments: LD MT DP BK SH. Performed the experiments: LD MT DP BK SH. Analyzed the data: LD MT DP BK SH. Contributed reagents/materials/analysis tools: LD BK THJ SH. Wrote the paper: LD THJ SH.
17. Szerlong H, Saha A, Cairns BR (2003) The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. Embryo J 22: 3173–3187.

18. Morillon A, Karabetsou N, O’Sullivan J, Keat N, Proudfoot N, et al. (2003) Iws1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. Cell 115: 425–435.

19. Tyagi A, Ryme J, Brodin D, Ostlund Farrants AK, Visa N (2009) SWI/SNF associates with nascent pre-mRNPs and regulates alternative pre-mRNA processing. PLoS Genet 5: e1000470.

20. Batsche E, Yaniv M, Muchir C (2006) The human SWI/SNF subunit Brm is a regulator of alternative splicing. Nat Struct Mol Biol 13: 22–29.

21. Allemand E, Batsche E, Muchir C (2006) Splicing, transcription, and chromatin: a menage a trois. Curr Opin Genet Dev 16: 145–151.

22. Neugebauer KM (2002) On the importance of being co-transcriptional. J Cell Biol 156: 571–576.

23. Moore MJ, Schwartzfarb EM, Silver PA, Yu MC (2006) Differential recruitment of the splicing machinery during transcription predicts genome-wide patterns of mRNA splicing. Mol Cell 24: 903–915.

24. Tardif DF, Lacadie SA, Robash M (2006) A genome-wide analysis indicates that yeast pre-mRNA splicing is predominantly posttranscriptional. Mol Cell 24: 917–929.

25. Jurica MS, Moore MJ (2005) Pre-mRNA splicing: awash in a sea of proteins. Mol Cell 22: 4–14.

26. Nilson TW (2000) The splicosome: the most complex macromolecular machine in the cell? BioEssays 22: 1147–1149.

27. Dellaire G, Makarov EM, Cowger JJ, Longman D, Sutherland HG, et al. (2002) Mammalian PRP4 kinase copurifies and interacts with components of both the U5 snRNP and the N-CoR deacetylase complex. Mol Cell Biol 22: 5141–5156.

28. Chen YI, Moore RE, Ge HY, Young MK, Lee TD, et al. (2007) Proteomic analysis of in vivo-assembled pre-mRNA splicing complexes expands the catalog of participating factors. Nucleic Acids Res 35: 3928–3944.

29. Chan SP, Cheng SC (2005) The PpRP19-associated complex is required for specifying interactions of U5 and U6 with pre-mRNA during splicing activation. J Biol Chem 280: 31190–31199.

30. Electra D, Batsche E, Muchir C, Muchir C (2006) Splicing, transcription, and chromatin: a menage a trois. Curr Opin Genet Dev 16: 145–151.

31. Carlson M, Botstein D (1982) Two differentially regulated mRNAs with different 5′ ends encode secreted with intracellular forms of yeast invertase. Cell 28: 145–154.

32. Wong MC, Scott-Drew SR, Hayes MJ, Howard PJ, Murray JA (2002) RSC2, an essential fungal-specific RSC nucleosome remodeling protein, binds to both poly(A) + mRNA and to core proteins of small nuclear ribonucleoproteins. Mol Cell Biol 22: 4210–4229.

33. Maris C, Dominguez C, Allain FH (2005) The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression. FEBS J 272: 2118–2131.

34. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, et al. (2003) Global analysis of yeast protein localization. Science 297: 511–518.

35. Mehta SC, Moore RE, Ge HY, Young MK, Lee TD, et al. (2006) Protocomic analysis of in vivo-assembled pre-mRNA splicing complexes expands the catalog of participating factors. Nucleic Acids Res 35: 3928–3944.

36. Cherepakhin KB, Khabibullina ON, Fateev AA, Krasilnikov VV, Yudin AM, et al. (2006) Identification and characterization of a novel chromatin remodeling protein, Swi5ip, that influences chromatin remodeling. Mol Cell 22: 2118–2131.

37. Costanzo M, Barchuk K, Marti R (2009) The Swi5ip transcription machine. FEBS J 276: 4198–4207.

38. Xie J, Beickmann K, Ott E, Rymond BC (1998) Progression through the splicesome cycle requires Ppp3c function for U4/U6 snRNA dissociation. EMBO J 17: 2938–2946.

39. Rymond BC (1995) Convergent transcripts of the yeast PRP38-SMD1 locus encode two essential splicing factors, including the D1 core polypeptide of small nuclear ribonucleoprotein particles. Proc Natl Acad Sci U S A 92: 848–852.

40. Hu J, Xu Y, Schappert K, Harrington T, Wang A, et al. (1994) Mutational analysis of the PRP4 protein of Saccharomyces cerevisiae suggests domain structure and snRNP interactions. Nucleic Acids Res 22: 1724–1734.

41. Cooper M, Johnston LH, Beggs JD (1995) Identification and characterization of Usp1p (Ssb2p): a novel U6 mRNA-associated protein with significant similarity to core proteins of small nuclear ribonucleoproteins. EMBO J 14: 2066–2075.

42. Anthony JG, Weidenshammer EM, Woolford JL (1997) The yeast Prp3 protein is a U4/U6 snRNP protein necessary for integrity of the U4/U6 snRNP and the U4/U6/U5 tri-snRNP. RNA 3: 1143–1152.

43. Chen CH, Kao DI, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkaline cations. J Bacteriology 153: 163–168.

44. Horwich P, Dicker R, Jacobson A (1990) Identification and comparison of stable and unstable mRNAs in Saccharomyces cerevisiae. Mol Cell Biol 10: 2269–2294.

45. Jimenez A, Tipper DJ, Davies J (1973) Mode of action of thiolutin, an inhibitor of macromolecular synthesis in Saccharomyces cerevisiae. Aminocyclot Agents Chemother 3: 729–738.

46. Mooren A, Camner S, Anderson G, Margotton F, Beggs J, et al. (1990) The U6 gene of Saccharomyces cerevisiae is transcribed by RNA polymerase III in vivo and in vitro. EMBO J 9: 271–277.

47. Hogan DJ, Rierdan DP, Gerber AP, Heschlag D, Brown PO (2008) Diverse RNA-Binding Proteins Interact with Functionally Related Sets of RNAs, Suggesting an Extensive Regulatory System. PLoS Biol 6: e255.

48. Sherman F (1991) Getting started with yeast. Methods Enzymol 194: 3–21.

49. Iro H, Fukuda Y, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkaline cations. J Bacteriology 153: 163–168.

50. Kim DE, Chivian D, Baker D (2004) Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 32: W526–331.

51. Simon P (2003) QGene: processing quantitative real-time RT-PCR data. Bioinformatics 19: 1439–1440.

52. Lygerou Z, Christophides G, Seraphin B (1999) A novel genetic screen for snRNP assembly factors in yeast identifies a conserved protein, Sad1p, also required for pre-mRNA splicing. Mol Cell Biol 19: 2008–2020.

53. Pauli TT, Carey M, Johnson RC (1996) Yeast HMG proteins NHP6A/B potentiate promter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev 10: 2769–2781.

54. De F, Flahavan P, Baker D, Baker D (2004) Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 32: W526–331.

55. Simon P (2003) QGene: processing quantitative real-time RT-PCR data. Bioinformatics 19: 1439–1440.

56. Lygerou Z, Christophides G, Seraphin B (1999) A novel genetic screen for snRNP assembly factors in yeast identifies a conserved protein, Sad1p, also required for pre-mRNA splicing. Mol Cell Biol 19: 2008–2020.

57. Pauli TT, Carey M, Johnson RC (1996) Yeast HMG proteins NHP6A/B potentiate promter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev 10: 2769–2781.

58. De F, Flahavan P, Baker D, Baker D (2004) Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 32: W526–331.

59. Simon P (2003) QGene: processing quantitative real-time RT-PCR data. Bioinformatics 19: 1439–1440.

60. Lygerou Z, Christophides G, Seraphin B (1999) A novel genetic screen for snRNP assembly factors in yeast identifies a conserved protein, Sad1p, also required for pre-mRNA splicing. Mol Cell Biol 19: 2008–2020.

61. Pauli TT, Carey M, Johnson RC (1996) Yeast HMG proteins NHP6A/B potentiate promter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev 10: 2769–2781.

62. De F, Flahavan P, Baker D, Baker D (2004) Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 32: W526–331.