Actin-Related Protein Arp6 Influences H2A.Z-Dependent and -Independent Gene Expression and Links Ribosomal Protein Genes to Nuclear Pores

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Abstract
Actin-related proteins are ubiquitous components of chromatin remodelers and are conserved from yeast to man. We have examined the role of the budding yeast actin-related protein Arp6 in gene expression, both as a component of the SWR1 complex (SWR-C) and in its absence. We mapped Arp6 binding sites along four yeast chromosomes using chromatin immunoprecipitation from wild-type and swr1 deleted (swr1Δ) cells. We find that a majority of Arp6 binding sites coincide with binding sites of Swr1, the catalytic subunit of SWR-C, and with the histone H2A variant Htz1 (H2A.Z) deposited by SWR-C. However, Arp6 binding detected at centromeres, the promoters of ribosomal protein (RP) genes, and some telomeres is independent of Swr1 and Htz1 deposition. Given that RP genes and telomeres both show association with the nuclear periphery, we monitored the ability of Arp6 to mediate the localization of chromatin to nuclear pores. Arp6 binding is sufficient to shift a randomly positioned locus to nuclear periphery, even in a swr1Δ strain. Arp6 is also necessary for the pore association of its targeted RP promoters possibly through cell cycle-dependent factors. Loss of Arp6, but not Htz1, leads to an up-regulation of these RP genes. In contrast, the pore-association of GAL1 correlates with Htz1 deposition, and loss of Arp6 reduces both GAL1 activation and peripheral localization. We conclude that Arp6 functions both together with the nucleosome remodeler Swr1 and also without it, to mediate Htz1-dependent and Htz1-independent binding of chromatin domains to nuclear pores. This association is shown to have modulating effects on gene expression.

Introduction
Genomic DNA is complexed with histones and non-histone proteins to form chromatin, which is organized into active and inactive domains within the interphase nucleus [1–3]. Histone tail modifications, chromatin compaction, and the subnuclear positioning of chromatin domains contribute epigenetic information that helps to determine gene expression patterns. While the enzymology of histone modification has been well characterized, little is known about the mechanisms that determine the spatial organization of chromatin in interphase nuclei.

In both vertebrates and yeast, transcriptionally inactive heterochromatin is enriched around the nucleolus or at the nuclear envelope (NE). In vertebrates, perinuclear anchoring appears to require the nuclear lamina, while in yeast integral proteins of the inner nuclear membrane tether repressed chromatin domains peripherally [reviewed in 4]. Recent work has shown that in addition to silent heterochromatic loci, some euchromatic yeast genes are found at the NE as well. Indeed, inducible budding yeast genes such as INO1, GAL1, and HXK1 form a stable association with the nuclear pore complex (NPC) upon activation. In some cases, this interaction ensures maximal expression and fine-tuning of induction rates [5–7]. The up-regulated X chromosome in male flies may also be associated with nuclear pores [8], as are the highly transcribed ribosomal protein (RP) genes of yeast [9].

Besides nuclear pore proteins, little is known about the components that position active chromatin domains within the nucleus. Nuclear actin and myosin, as well as myosin-like and actin-related proteins have been proposed as candidates that could contribute to the organization of transcription in the interphase nucleus [8, 10–17]. Indeed, actin itself is not only found as part of the filamentous cytoskeleton, but in various large chromatin modifying complexes, which are exclusively nuclear.

In all organisms from yeast to man, the actin family includes a number of proteins that are structurally similar to actin, called actin-related proteins or ARPs. The yeast S. cerevisiae alone harbors...
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Author Summary

Actin and the structurally similar actin-related proteins (ARPs) are major components of nucleosome remodeling complexes in the nucleus. Here we show that budding yeast Arp6 has functions independent of its catalytic chromatin remodeling partner, Swr1. Arp6 binds to multiple promoters and subtelomeric zones at which Swr1 does not bind, and this association increases in strains lacking an intact SWR-C remodeling complex. We show that Arp6 can mediate the association of the ribosomal protein gene promoters to which it binds, with the nuclear envelope. The loss of Arp6 led to an up-regulation of these ribosomal protein genes, yet had the opposite effect on the galactose-induced GAL1 promoter, where it binds together with Swr1 and the histone H2A variant H2AZ. Indeed, loss of Arp6 and nuclear envelope binding delays galactose-induced activation of GAL1. The two opposing functions of Arp6 correlate with different pathways of anchoring mediated by Arp6: one requires that the intact SWR-C deposits H2AZ, while in the second Arp6 may mediate promoter binding to nuclear pore baskets directly. In both cases, Arp6 is implicated in the spatial localization of chromatin within the interphase nucleus, which has functional consequences on expression. This identifies a novel function for actin-related proteins.

In vitro analyses have indicated that the phenotypes associated with Arp gene deletion are more extensive than those provoked by loss of a complex-specific ATPase subunit. Indeed, Arp4 is essential for viability in yeast, and the protein is a component of both INO80- and SWR-C remodeling complexes and the NuA4 histone acetyltransferase, which carry out distinct nuclear functions [22,39,40]. Intriguingly, biochemical fractionation suggests that even these three complexes do not account for the entire nuclear complement of Arp4 [22,39]. Other support for independent functions for ARPs comes from genome-wide screens for synthetic lethality. The 125 gene deletions that are lethal for cells lacking Arp6, for instance, are not necessarily lethal for cells lacking the Swr1 ATPase subunit [41]. Finally, the human Arp8 (hArp8) was implicated in mitotic chromosome phenotypes that could not be attributed to the hINO80 chromatin remodeling complex to which it belongs [42].

Here we have localized Arp6 along budding yeast chromosomes, both in the absence and presence of Swr1. We find that most Arp6 co-localizes with Swr1, being enriched in the promoters of divergently transcribed genes. This correlates with the deposition of the histone H2A variant H2AZ/Htz1, and is consistent with the proposal that Arp6, as part of SWR-C, contributes to transcriptional regulation by exchanging H2A for Htz1 [33,35]. Intriguingly, however, Arp6 binds some promoters in a Swr1-independent manner, including promoters of ribosomal protein genes. Indeed, transcript measurements show that Arp6 alters RP gene regulation independently of Swr1-mediated Htz1 deposition. We find that Arp6 can relocate chromatin to the NE independently of Swr1, and that arp6 deletion reduces the association of RP genes with the NPC. This leads to a slight elevation in RP gene expression. We argue that Arp6 not only modulates local chromatin organization by facilitating Htz1 deposition, but also contributes to long-range chromatin organization that can fine-tune expression levels independently of SWR-C.

Results

Co-localization of Arp6 and Swr1 at yeast promoter regions

We determined the localization of Arp6 and Swr1 along budding yeast chromosomes by chromatin immunoprecipitation using high-density microarrays (ChIP-chip assay). A 3FLAG epitope tag was introduced at the 3’ end of the genomic copy of either Arp6 or SWR1, and the functionality of the fusion constructs was confirmed by monitoring growth rates and sensitivity to DNA damaging agents (Figure S1). Genomic DNA fragments (mean size $\equiv 100$ bp) were recovered, together with either Arp6 or Swr1 by anti-FLAG immunoprecipitation from formaldehyde-fixed and sonicated cells grown asynchronously in glucose-containing media (YPD). The fragments isolated by ChIP, as well as those in the total extract, were labeled with fluorescent dyes and hybridized to a high-density oligonucleotide array covering chromosomes 3, 4, 5, and 6R at either 100-bp or 300-bp resolution. With eleven 25-nucleotide probes for each 300-bp locus, the reliability of signal strength could be evaluated robustly by calculating the p-value for each locus ($p<0.025$ [43]). Reliability of the log2 ratio of the ChIP fraction recovery to the supernatant fraction was scored, and allowed discrimination of significant positive (yellow bars) and negative signals (open bars) for binding with overall resolution of $\sim 300$ bp [43] (data available at www.ncbi.nlm.nih.gov/geo/under the access number GSE9213).

Arp6 binding sites were found widely dispersed among chromosomes 3, 4, 5, and 6R, occasionally spreading over regions of several kb (Figures S2, S3, S5, and Figure 1, respectively).
Among 8441 detectable chromosomal loci, 1498 loci were evaluated as positive for Arp6 binding (Table 1). Among the Arp6-binding sites, 71% also tested positive for Swr1, suggesting that Arp6 co-localizes with Swr1 at the majority of its chromosomal binding sites. The frequency of co-localization does not vary significantly among the chromosomes tested (Table 1). In Figure 1A, vertical arrows indicate the top ten peaks (signal log ratio >0.8) for Arp6 binding on Chr 6. Interestingly, one of the Arp6 peaks encompasses the centromere (arrow I), and a second is subtelomeric, containing unique sequences adjacent to the TG-rich repeats (arrow X). Arp6 binding sites generally coincided well with those of Swr1 (Figure 1A and 1B), except in subtelomeric zones where Arp6 binds alone (arrow X). Co-localization patterns were similar on all chromosome arms analysed (Figures S2, S3, S5). A closer examination of Arp6 promoter binding on Chr 6, reveals that Arp6 and Swr1 bind preferentially at the promoters of divergently transcribed genes (Figure S6, gray shade). These sites also contain Htz1 (H2A.Z), and the coincidence of these three signals suggests that SWR-C stays bound after depositing Htz1 (H2A.Z), presumably to modulate promoter accessibility. In conclusion, our ChIP-chip analysis suggests that Arp6 binds chromatin as a component of SWR-C at most euchromatic sites, and in particular in the promoters of divergently transcribed genes. However, at centromeres, some telomeres and select promoters, Arp6 appears to bind independently of Swr1 (Figure 1C, Table S1, and below).

Comparison of Arp6- and Swr1-containing complexes

The fact that Arp6 ChIP recovered the sites where Swr1 does not bind suggests that Arp6 may associate with other nuclear proteins or complexes, although to date it has only been reported to be a component of SWR-C. To test this possibility, we analyzed the native molecular masses of Swr1- and Arp6-containing complexes using gel filtration chromatography (Figure 2). Swr1 is recovered almost exclusively in fractions of ~1–2 MDa (Figure 2A, second panel, lanes 5 and 6), and fractionates similarly to the catalytic ATPase of the SWI/SNF remodeler Snf2 (Figure 2A, first panel). In the presence of Swr1, Arp6 was distributed in both the 1–2 MDa Swr1-containing fractions, and in fractions that correspond to a molecular weight of 100–250 kDa (Figure 2A, third panel). In this latter fraction the complexes are still likely to be larger than the 57-kDa Arp6 monomer. Upon deletion of Swr1, the presence of Arp6 in the high MW fractions was significantly reduced (Figure 2A, fourth panel, and Figure 2B), and its abundance in the lower MW fractions increased. Our observations are consistent with the observation that Swr1 serves as platform for the assembly of SWR-C subunits, and that in the absence of Swr1, Arp6 only retains association with Swc6 [28,44]. Nonetheless, a small amount of Arp6 was still recovered in a high MW complex in the swr1Δ strain (>1 MDa, Figure 2A, fourth panel). This may reflect participation of Arp6 in another large complex, albeit one of lower abundance. Quantitation of Arp6 recovery in both wild-type and swr1Δ cells, suggests that 30% of Arp6 is part of SWR-C or another large complex (Figure 2B), while the majority of Arp6 self-dimerizes or forms a complex with other small proteins. The nature of these is unknown, but the only reported partners of Arp6 in a swr1Δ strain are Swc6 and nucleosomes [28].

SWR-C independent chromatin association of Arp6

To examine the ability of Arp6 to bind chromatin with or without Swr1, we fractionated yeast cells into a chromatin and a soluble protein fraction, using the well-established TritonX-100 lysis procedure [45]. This analysis confirmed that the majority of Arp6 is associated with chromatin in wild-type cells (Figure 3B). This is also true for tightly bound chromatin proteins like Ino80, topoisomerase II and ORC (Figure 3B and data not shown). Consistent with the finding that most Arp6-binding sites coincide with those of Swr1, we found that the association of Swr1 with chromatin required the presence of Arp6 (Figure 3B, arp6, lane Chr). This is unlikely to reflect an indirect effect on chromatin, since the association of Ino80 or topoisomerase II with chromatin was unaffected by arp6 deletion (Figure 3B). In contrast, a large fraction of Arp6 (39% compared to wild-type) remained associated with chromatin even in the absence of Swr1 (Figure 3B, swr1, lane Chr). This result suggests that the physical association of Arp6 with chromatin is at least in part independent of SWR-C, and is consistent with the ChIP-chip data which show partially non-overlapping distributions of Arp6 and Swr1 (Figure 1).

Detection of the Arp6 binding loci in the absence of Swr1

To elucidate the SWR-C-dependent and -independent functions of Arp6, we performed ChIP-chip analysis for Arp6 in a strain lacking SWR1 entirely (swr1Δ). Consistent with the reduced level of chromatin-bound Arp6 (Figure 3B), fewer Arp6-binding sites were found in swr1Δ cells (Table 1). The major binding sites lost were those where both Arp6 and Swr1 colocalize in 5′ promoter regions (Figure 1, Figure S6, and Table 1), including the intergenic region 5′ of the SWR1 gene itself (Figure S4).

Table 1. Correlation of localization of Arp6 and Swr1 on budding yeast chromosomes.

| Chr | SWR1 Arp6 binding loci | SWR1 coincidence with Swr1 binding | swr1 Arp6 binding loci |
|-----|------------------------|----------------------------------|------------------------|
| Chr3 | 383 (29%)              | 306 (80%)                        | 175 (13%)              |
| Chr4 | 1117 (23%)            | 817 (73%)                        | 671 (14%)              |
| Chr5 | 419 (23%)             | 324 (77%)                        | 293 (16%)              |
| Chr6 | 102 (25%)             | 81 (79%)                         | 73 (18%)               |
| Total | 2021 (24%)           | 1528 (76%)                       | 1216 (14%)            |

doi:10.1371/journal.pgen.1000910.t001
To quantify the effect of swr1 deletion on Arp6 binding patterns, we compared the log ratio of each Arp6-binding site in wild-type and swr1Δ cells (Figure S7A and S7B) with the log ratio of Swr1 binding at that locus. Generally, at the sites where both Arp6 and Swr1 were bound in wild-type cells, Arp6 binding was lost in swr1Δ cells (Figure 1 and Figures S2, S3, S5, S6). However, the overall distribution of Arp6 in the swr1Δ strain changes; notably, values increased at sites where Swr1 was not bound in the wild-type background (Figure S7B). This argues that in addition to an overall reduction in Arp6 binding, preferred Arp6 binding positions were altered in the absence of Swr1. This change in Arp6 binding suggests that SWR-C either competes for a limiting pool of Arp6 or alters chromatin such that some Arp6 binding sites are inaccessible, possibly reflecting indirect effects of Htz1 deposition.

Importantly, a subfraction of Arp6 binding sites persist in both wild-type and swr1Δ cells (see Figure 1). This argues, consistent with the fractionation data (Figure 3B), that Arp6 binds a subset of chromosomal loci independently of SWR-C, even in wild-type cells. Examples of this are RP gene promoters (e.g. RPL24, Figure 1, arrow V) and the Tel6R subtelomeric zone, which contains the inducible gene HXK1 (Figure 1, arrow X). Indeed, Swr1-independent binding of Arp6 was enriched generally in a number of subtelomeric regions (Table S1). Despite the difficulty of analyzing subtelomeric domains on microarrays due to the presence of repetitive sequences, persistent Arp6 binding could be confirmed at Tel6R, Tel3L, Tel3R, and Tel4R in the absence of Swr1 (Figure 1, Figures S2, S3).

**Involvement of Arp6 in the expression of RP genes independently of H2A.Z-deposition**

To examine the independent contributions of Arp6 and Swr1 to gene expression, we performed a yeast whole-genome microarray with wild-type, arp6Δ, and swr1Δ cells (Table S2) (details available at www.ncbi.nlm.nih.gov/geo/ under the access number GSE17780). This expression microarray analysis was repeated at least three times for each strain, and the statistical differences were determined by t-test. Changes with p,0.05 were considered significant. We found a larger number of genes to be differentially regulated in both arp6Δ and swr1Δ cells (Figure 4A). When we compared the misregulated genes between the two mutants, we found that 87 out of 506 genes repressed in swr1Δ (see down-regulated in swr1, Figure 4A) are also down-regulated in arp6Δ cells (17% overlap), and 56 out of 375 genes induced in swr1Δ (see up-regulated in swr1, Figure 4A) are also up-regulated in arp6Δ cells (15% overlap; Figure 4A and Table S2). This overlap of down- or up-regulated genes between swr1Δ and arp6Δ strains was less than that reported for down- or up-regulated genes between swr1 and htz1 mutants (44% and 38%, respectively) [30]. Our expression data are consistent with our biochemical and ChIP analyses, which suggest that a majority of Arp6 is not recovered with SWR-C by sedimentation analysis (Figure 2B).
Arp6 can influence gene expression independently of SWR-C.

To extract more information on the differential effects of Arp6 and Swr1 in gene expression, we compared the degree of change in transcript level for each gene whose misregulation was significant (p < 0.05) in both arp6Δ and swr1Δ strains (Figure 4B). Intriguingly, the degree of transcriptional change as a consequence of arp6 deletion was greater than that provoked by swr1 deletion. On the other hand, the deposition of Htz1 to promoters were similarly impaired in arp6Δ and swr1Δ cells, as previously reported [28,29] (Figure S8). This analysis further indicates that Arp6 contributes to gene expression not only through Htz1 deposition, but also through a Swr1-independent mechanism.

Strikingly, among the 40 most up-regulated genes in arp6Δ cells we found 21 ribosomal protein genes (Table 2). Some of these RP genes were also modestly up-regulated in swr1Δ cells. Consistently, the ChIP-chip analysis in chromosomes 3, 4, 5, and 6R (Figures S2, S3, S5, and Figure 1, respectively) revealed that Arp6 and Swr1 bind to 25, and respectively 24, of the 27 RP genes on these chromosomes (Table S3). Importantly, and in contrast to most other euchromatic loci, Arp6 remained bound to all of these RP genes (including RPS16B, RPL13A, RPP1A, RPL31A, and RPL2A), even after deletion of SWR1 (Figure 1, Figure 5, Figures S2, S3, S5, and Table S3). When we compared the transcription of RP genes between arp6Δ and swr1Δ cells, we find these genes more significantly up-regulated by loss of Arp6 than by loss of Swr1 (Figure 4B, red diamonds, and Table S4). From this we conclude that an Arp6-dependent, but Swr1- and Htz1-independent, mechanism modulates RP gene expression.

Figure 3. Arp6 partitions between soluble and insoluble chromatin fractions. (A) The fractionation protocol is shown. Yeast spheroplasts from appropriate strains were lysed with Triton-X100. A gentle centrifugation step separates a supernatant containing the bulk of cellular proteins from a chromatin pellet. (B) Wild-type, swr1, and arp6 cells were subjected to the fractionation protocol described in (A), and the spheroplast (T), soluble fraction (S), and chromatin-bound (Chr) samples were probed using Western blot for Arp6-FLAG, Swr1-FLAG, topoisomerase II (Top2), the enzymatic component of the INO80 chromatin remodeling complexes (Ino80-MYC), and the soluble non-chromatin protein, tubulin. Numbers under panels show the ratios of chromatin-bound Arp6 and Swr1 in the mutants relative to WT. Their intensities were normalized with chromatin-preparation efficiencies obtained by quantification of the Western blot for Ino80.

doi:10.1371/journal.pgen.1000910.g003
Consistent with our analysis, it was reported earlier that Htz1 is excluded from RP genes [34,35]. Moreover, microarray analyses have shown that the absence of Htz1 does not have any significant effects on RP gene expression [30,37,46]. To confirm this, we examined the expression of the relevant RP genes by quantitative rtPCR. We could confirm that transcript levels were not significantly altered by loss of Htz1, yet were increased in arp6Δ (Figure 6A). In contrast, other Arp6-bound promoters that are known to be regulated by SWR-C mediated deposition of Htz1 (e.g. GAL1 [31,47]) showed a reduction or delay in induction by galactose that was similar in both htz1Δ and arp6Δ cells (Figure 6B). This could be extended to several non-inducible genes, to which Arp6 binds in Swr1-dependent manner such as RDS1 (YCR106W) and UBX3 (YDL091C) (Figure S9). These genes, like the inducible GAL1, showed a similar decrease in expression in both htz1Δ and arp6Δ cells (Figure S9, filled and gray bars, respectively). Our observations argue that Arp6 is involved in gene expression in both the Htz1-dependent and Htz1-independent pathways. Moreover, Arp6 binding can both increase and lower transcript levels: at GAL1, where Htz1 is deposited in an Arp6- and Swr1-dependent manner, expression is less efficient in the absence of Arp6 or Htz1 deposition, while at RP genes, where Arp6 binding is independent of Swr1 and Htz1, its absence increases expression levels.

Arp6 mediates relocalization of chromatin in a Swr1-independent manner

Recent studies have suggested that not only local changes in chromatin organization, but also long-range chromatin organization can influence gene expression. Genome-wide ChIP-chip analysis for nuclear pore components has shown that RP genes associate with components of the NPC [9]. Given that Arp6 associates with most RP genes in the absence of Swr1, we wondered whether the Arp6-specific effect on transcription might be mediated through an interaction of the target gene with nuclear pores. To examine this possibility, we made use of an assay that scores for the ability of a protein fused to LexA to shift a randomly positioned chromosomal locus to the nuclear...
periphery [2]. This assay has been used to identify protein domains that are sufficient for interaction with structural components of the NE. The locus chosen is a constitutively expressed gene near ARS607 (PES4) at which we have inserted 4 LexA binding sites and a lac operator array that allows visualization with GFP-LacI (Figure 7A) [2]. Proteins that are to be tested for perinuclear relocalization activity are expressed as LexA fusions in a strain expressing GFP-Nup49 to tag the NE. Unlike the expression of LexA alone (Figure 7B, LexA), the LexA-Arp6 fusion protein led to an enrichment of the PES4 locus in the outermost nuclear zone (zone 1) in both G1- and S-phase cells (Figure 7B, LexA-Arp6 in WT). Importantly, the relocalization activity of LexA-Arp6 was independent of Swr1 (Figure 7B, LexA-Arp6 in swr1). Expression of LexA alone does not shift the position of the tagged locus, allowing us to conclude that LexA targeted Arp6 is sufficient to favor the association of a chromatin

### Table 2. Genes markedly up-regulated in arp6 cells.

| ORF name | arp6/wt log2 ratio | swr1/wt log2 ratio | Gene name | Description |
|----------|-------------------|--------------------|----------|-------------|
| YDL081C  | 2.31              | 0.75               | RPP1A    | Ribosomal protein |
| YOR167C  | 2.19              | 0.78               | RPS28A   | Ribosomal protein |
| YOR248W  | 2.19              | 0.72               | TOS11    | Questionable ORF |
| YGL030W  | 2.18              | 0.51               | RPL30    | Ribosomal subunit |
| YHR021C  | 2.18              | 0.69               | RPS27B   | Ribosomal protein |
| YHR143W-A| 2.03              | (0.03)             | RPC10    | Subunit of RNA polymerase II |
| YGL147C  | 2.00              | (0.06)             | RPL9A    | Ribosomal protein |
| YDL083C  | 1.91              | 0.22               | RPS16B   | Ribosomal protein |
| YJR123W  | 1.90              | 0.74               | RP55     | Ribosomal protein |
| YOL014W  | 1.89              | (0.32)             |         | Hypothetical protein |
| YPR078C  | 1.85              | 0.54               |         | Hypothetical protein |
| YLR185W  | 1.83              | (0.45)             | RPL37A   | Ribosomal protein |
| YDL130W  | 1.83              | 0.44               | RPP1B    | Ribosomal protein |
| YJR145C  | 1.77              | 0.41               | RPS4A    | Ribosomal protein |
| YPL163C  | 1.77              | 0.75               | SVS1     | Serine- and threonine-rich protein |
| YAL025C  | 1.74              | (−0.50)            | MAK16    | Putative nuclear protein |
| YLR264W  | 1.73              | 0.10               | RPS28B   | Ribosomal protein |
| YNL162W  | 1.73              | (0.08)             | RPL42A   | Ribosomal protein |
| YKL006W  | 1.70              | 0.28               | RPL14A   | Ribosomal protein |
| YHL001W  | 1.70              | 0.22               | RPL14B   | Ribosomal protein |
| YBL087C  | 1.69              | 0.39               | RPL23A   | Ribosomal protein |
| YBL071C  | 1.67              | 0.40               |         | Hypothetical protein |
| YPL220W  | 1.65              | 0.38               | RPL1A    | Ribosomal protein |
| YBR267W  | 1.63              | (−0.22)            | REI1     | Cytoplasmic pre-60S factor |
| YGL135W  | 1.60              | 0.47               | RPL1B    | Ribosomal protein |
| YNL333W  | 1.59              | (0.24)             | SNZ2     | Member of the stationary phase-induced gene family |
| YHR072W-A| 1.58              | (0.19)             | NOP10    | Component of H/ACA-box snoRNPs |
| YLR075W  | 1.58              | (0.11)             | RPL10    | Ribosomal protein |
| YOR292C  | 1.57              | 0.86               |         | Hypothetical protein |
| YAL012W  | 1.57              | (0.52)             | CYS3     | Cystathionine gamma-lyase |
| YAR009C  | 1.57              | 0.32               | TY1B     | Ty1B protein |
| YDL082W  | 1.55              | (−0.10)            | RPL13A   | Ribosomal protein |
| YDR101C  | 1.53              | (−0.22)            | ARX1     | Shuttling pre-60S factor |
| YNL255C  | 1.53              | (0.36)             | GIS2     | Contains seven cysteine-rich zinc finger motifs |
| YPL093W  | 1.53              | (−0.04)            | NOG1     | Nuclear G-protein (putative) |
| YDR184C  | 1.51              | (−0.21)            | ATC1     | Nuclear protein that interacts with Aip3 |
| YOR096W  | 1.51              | (−0.14)            | RPS7A    | Ribosomal protein |
| YJL136C  | 1.51              | (0.19)             | RPS21B   | Ribosomal protein |
| YLR110C  | 1.49              | (1.03)             | CCW12    | Cell wall mannoprotein |
| YLR157C-B| 1.48              | (0.52)             |         | Transposable element gene |

Parenthesis: change is not significant (p > 0.05). 
doi:10.1371/journal.pgen.1000910.t002
locus with the NE. This is independent of Htz1 deposition, since it requires the catalytic activity of Swr1. We note that there is a low level of endogenous Arp6 detected near the PES4 locus in swr1 mutant cells (Figure 1), yet this is insufficient to tether a significant fraction of the sites to the NE (see LexA alone).

**Figure 5. Swr1-independent binding of Arp6 to RP genes.** Vertical bars represent the binding ratio of proteins in each locus. The binding of Arp6-FLAG (top), Swr1-FLAG (middle), and Arp6-FLAG in swr1 cells (bottom) in the region 266K-353K of Chr 4L were compared. The positions of the RP genes (RPS16B, RPL13A, RPP1A, and RPL31A) in the region are shown with arrows and green lines. Red asterisks indicate those Arp6-gene promoter bindings which disappeared in the absence of Swr1.

doi:10.1371/journal.pgen.1000910.g005

Arp6 is required for the association of chromatin with the NPC

To confirm that the Arp6-bound locus associates with nuclear pores, as opposed to other perinuclear sites, we performed the relocation assay in a strain that expresses a nuclear pore
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protein Nup133 that lacks its N-terminal domain (nup133ΔN) [48]. In this mutant, functional NPCs cluster on one side of the nucleus allowing us to monitor whether a LexA-Arp6 targeted locus moves to pores or to other sites on the nuclear envelope (Figure 8A). Compared to cells expressing LexA alone, the L152 locus bound by LexA-Arp6 not only accumulated in the nuclear periphery zone like the PES4 locus (49% in zone 1 vs 34% for LexA alone in G1-phase cells, data not shown) but also colocalized significantly with clustered NPC (Figure 8A; 22.4%, n = 322, p<0.01). Previous studies have shown that a randomly distributed tagged locus would coincide with a pore cluster in 9% of the cells, while a locus that has a predisposition to be perinuclear (i.e. 60% occupation of zone 1) would coincide with a pore cluster in 10% of cell scored [49]. The 22% scored for Arp6 relocation versus the 8% scored for the control LexA is thus highly significant. It is comparable to the ~two-fold increase in colocalization achieved by targeting LexA-Nup84 vs LexA alone [49]. This rate of colocalization suggests that a component of the NPC is able to bind Arp6.

We next used quantitative ChIP analysis to test whether the loss of Arp6 influences the association of endogenous RP genes with the NPC. Immunoprecipitation of Nup133-Myc confirmed that the RP genes tested previously [9] are associated with pores and that deletion of *arp6* reduces the recovery of these genes with Nup133 (Figure 8B). *PES4*, a randomly positioned locus with no natural affinity for nuclear pores, did not precipitate significantly with Nup133 and was unaffected by *arp6* deletion (Figure 8B). From this we conclude that Arp6 is required for the RP gene-NPC interaction (Figure 8B).

We asked whether Nup133 was the only site of interaction for these genes with the nuclear envelope. In other words, we checked by lacO-tagging and scoring of subnuclear position, whether RP or GAL1 genes would lose all perinuclear localization in absence of Arp6. We found that the galactose-induced relocation of *GAL10* (which shares the *GAL1* promoter) to the NE was indeed lost in S-phase *arp6Δ* cells (Figure 9A), as was the constitutive association of the RP gene *RPL29A* (Figure 9B). Inexplicably, however, the loss of association provoked by *arp6* deletion was cell-cycle stage specific, arguing that an alternative, possibly redundant mechanism allowed loci to remain peripheral, although probably not associated with Nup133, in G1-phase cells. The effect was also at least partially locus- or context-specific, since a second tagged RP gene cluster at *RPP14A* was enriched at the nuclear periphery in both wild-type and *arp6Δ* strains (data not shown). Taken together our data argue that Arp6, while being sufficient to relocate loci to the NE (Figure 7), is not the only pathway that tethers active genes at nuclear pores. This was already suggested from the results from the Rosbash, Silver, Stutz, Hurt, Nehrbass, Brickner and Proudfoot laboratories, who have identified both SAGA-dependent and SAGA-independent pathways for locating active loci at nuclear pores [4]. The fact that *GAL10* and *RPL29A* association was ablated in S-phase by *arp6* deletion, suggests that a redundant pathway of anchoring functions primarily in G1 phase. Although it is unclear why transcription-regulated association with the NE, should be cell-cycle controlled, this is highly reminiscent of the distinct G1- and S-phase specific tethering mechanisms that mediate anchoring of telomeres [2,50] and DNA damage [49].

We next examined whether other pore-associated proteins, namely, Mlp1 and Mlp2, myosin-like proteins associated with the inner nuclear basket, are involved in either the Arp6–mediated pathway of gene anchoring. They were likely candidates due to their implication in the association of *GAL10* and *HSP104* with nuclear pores, through Mex67 and Yra1 [51–53]. To test this, the position of the tagged *PES4* locus bound by LexA-Arp6 or by LexA alone was determined in strains carrying *mlp1Δ mlp2Δ* deletions (Figure 7C, *mlp1Δ mlp2Δ*). Intriguingly, we again see that LexA-Arp6 anchoring activity was dependent on Mlp1 and Mlp2 exclusively in S-phase cells. We conclude that Arp6 is able to mediate association with nuclear pores in an Mlp1/Mlp2-dependent manner, yet again our data indicate that a second pathway for Arp6 binding is functional in G1 phase. Arp6 may interact with the coiled-coil proteins Mlp1 and Mlp2 directly, although it is more likely to bind through Yra1 (see Discussion). Overall, our results support the notion that Arp6 has a role both in local chromatin modulation through H2A.Z deposition, and in long-range chromatin organization through its ability to bind proteins associated with the NPC; an interaction which depends at least partially on the myosin-like proteins 1 and 2.

Discussion

**SWR-C–dependent and–independent binding of Arp6 to chromatin**

Our high resolution ChIP-chip assay has shown that Arp6 co-localizes with Swr1 at most of its euchromatic sites, presumably as a component of SWR-C [28,30]. No conserved sequence motif for
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B1 G1 phase

WT

\[ n = 374, p = 0.5 \]

\[ n = 362, p = 6 \times 10^{-14} \]

\[ n = 326, p = 5 \times 10^{-13} \]

\[ \text{lexA} \]

\[ \text{lexA-Arp6} \]

\[ \text{lexA-Arp6} \]

\[ \text{swr1}\Delta \]

\[ n = 192, p = 0.3 \]

\[ n = 166, p = 4 \times 10^{-3} \]

\[ n = 179, p = 3 \times 10^{-2} \]

\[ \text{lexA} \]

\[ \text{lexA-Arp6} \]

\[ \text{lexA-Arp6} \]

\[ \text{swr1}\Delta \]

\[ \text{S phase} \]

\[ \text{WT} \]

\[ \text{mlp1}\Delta \text{mlp2}\Delta \]

\[ n = 362, p = 6 \times 10^{-14} \]

\[ n = 231, p = 5 \times 10^{-9} \]

\[ n = 131, p = 0.3 \]

\[ \text{lexA-Arp6} \]

\[ \text{lexA-Arp6 / lexA} \]

\[ \text{swr1}\Delta \]

\[ \text{S phase} \]

\[ \text{WT} \]

\[ n = 166, p = 4 \times 10^{-3} \]

\[ n = 158, p = 0.1 \]

\[ n = 79, p = 0.6 \]

\[ \text{lexA-Arp6} \]

\[ \text{lexA-Arp6 / lexA} \]
the binding of Arp6 or Swr1 could be identified (data not shown), partly because the targeted regions are relatively large and transcription factor binding sites are often degenerate. Moreover, it is possible that Arp6 is targeted by recognition of a specifically modified nucleosome and not a DNA binding factor [28]. Interestingly, the highest Arp6 occupancy was detected within a 300-bp fragment containing the start ATG codon of the SWR1 gene. This coincides with a peak of Swr1 and argues for an auto-regulatory loop for SWR1 expression (Figure S4) [35].

More generally, the ChIP-chip assay showed a coordinated enrichment for both Arp6 and Swr1 in intergenic regions particularly near the 5’ ends of divergently transcribed genes (Figure 1A and 1B). Of the top ten loci for Arp6 binding on Chr 6 (arrows, Figure 1A), five contain the start ATG codon of genes, two are located within 200 bp of an ATG codon, and two others are within 400 bp of an ATG codon. Only at telomeres are Arp6 sites more than 1 kb from the nearest ATG. The localization of Htz1 on yeast chromosomes has been previously examined in detail, and was shown to be present at the 5’ ends of most genes [33–35]. This suggests that Arp6 and Swr1 remain chromatin-bound at sites where they incorporate Htz1.

Unexpectedly, we also detected numerous loci that were positive for Arp6 but negative for Swr1 interaction (Figure 1A and 1B, Table 1). A large fraction of Arp6 was shown not be integrated in the SWR-C complex by gel filtration analysis. Moreover, although Swr1 association with chromatin was dependent on Arp6, about 40% of total Arp6 remained chromatin-bound in the absence of Swr1 (Figure 2 and Figure 3). Swr1 contributes not only to the catalytic activity of SWR-C, but also provides critical protein-protein contacts that maintain the integrity of the holocomplex [28]. Importantly, in a swr1 deletion strain Swc2, the Htz1-binding module of SWR-C, dissociates from Arp6 and only Swc6 remains Arp6 bound [28]. Consistently, sites that bind Arp6 in the absence of Swr1 are anti-correlated with the presence of Htz1.

ChIP-chip analysis showed that the SWR-C-independent binding of Arp6 is observed at RP genes and in some subtelomeric zones, which are both depleted for Htz1 [37,38,54]. Intriguingly, we have also found that the perinuclear tethering of Tel6R is impaired in arp6, but not in swr1 mutants (K.S., A.T. and S.M.G. unpublished data). The role of Arp6 at telomeres is therefore not restricted to the deposition of Htz1.

Binding of Arp6 to RP genes

The RP genes are among the most important genes for cell metabolism, and the fine-tuning of RP gene transcription responds to a variety of environmental effects, ultimately coordinated by the TORC1 complex (Target of Rapamycin) [reviewed in 55]. However, exactly how these crucial genes are regulated at the transcriptional level is unclear. Several transcription factors, including Rap1, Fhl1, and the high mobility group protein, Hmo1, have been shown to play roles in the expression of RP genes. Previous studies suggested that Rap1 may recruit Fhl1 and Hmo1 to RP promoters [56,57]. As shown here, Arp6 binds to most of the RP genes present on the chromosomes we analyzed. Given that Rap1 has significant genetic interactions with Arp6 [58], we speculate that Arp6 may cooperate with Rap1 to regulate the association of other factors to RP gene promoters. A particular constellation of factors may also contribute to the association of these genes with nuclear pores.

Kasahara et al. [59] compared the binding of Hmo1 with those of Fhl1 and Rap1 to RP genes using ChiP-chip analysis, and divided RP genes into classes that have either Hmo1-dependent or Hmo1-independent binding of Fhl1 and Rap1. We find no correlation of either class with the presence of Arp6 (data not shown). Moreover, Arp6 is bound at the promoters of RPP1A, RPL4B, and RPP2B, which belong to a subgroup that binds neither Hmo1, Fhl1, nor Rap1 [59]. Thus, while it is possible that Arp6 influences the binding of these factors, the converse is not true. We also note that, unlike loss of Hmo1, Fhl1 or Rap1, the absence of Arp6 leads to an increase in the expression level of genes such as RPP1A (Table 2). This argues that the binding of Arp6 reduces rather than enhances RP gene expression. It is important to note that RP genes are highly expressed, and therefore even a 50% drop in expression means that the gene is still actively transcribed. Thus the localization of RP genes to pores by Arp6 binding reduces but does not eliminate expression. This is not the first report of pore association leading to reduced expression: a gene in the heat-shock family, HIS3, which is associated with the NPC by an mRNA- and Mlp1/Mlp2-dependent pathway, also had higher expression levels when its association with the NPC was impaired [51].

Arp6 is required for gene expression in a H2A.Z-dependent and -independent mechanism

We show here that the loss of Arp6 increases expression of RP genes by 1.5- to 2-fold, in a manner independent of Htz1 and SWR-C (Figure 4, Figure 6A, and Table 2). Arp6 binds many of these RP gene promoters and is required for their tight association with the NPC through their nuclear pore protein Nup133 (Figure 8B). Given that chromatin-bound Arp6 can relocate genes to pores, we can conclude that Arp6 either directly or indirectly mediates the association of RP genes with pores. In general, the RP and non-RP genes that are most activated by Arp6 deletion (Table 2, e.g. YOR248W) are among those associated with NPCs [9]. This establishes for the first time a strong correlation between association with the NPC and down-regulation for a class of coordinately regulated genes.

There have been several reports showing that genes induced by non-glucose carbon sources, inositol starvation or heat shock associate with the NPC for optimal induction [5–7,9,60]. We confirm here that the association of GAL1 with the NPC is Htz1- and Arp6-dependent [61] (Figures S8, S10), and that in the absence of either factor, induction occurs less rapidly, although the final mRNA level is unchanged (Figure 6B). Since Arp6 is required...
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Figure 8. Involvement of Arp6 in intranuclear organization through the NPC. (A) The positions of lacO-tagged LYS2 (red) and of CFP-Nup49 (green) were observed in a nup133ΔN background, in which nuclear pores cluster on one side of the nucleus. Bar graphs represent the percentage of complete red-green signal overlap counted in cells expressing LexA alone or a LexA-Arp6 fusion. The confidence values (p) for the χ² analysis between them is indicated. The predicted colocalization for a randomly positioned locus is 9% [49]. (B) Requirement of Arp6 for the interaction of RP genes with the NPC. The association of Nup133-Myc with RP genes, RPP1A, RPL13A, RPL2A, and RPL29, was quantified using ChIP analysis combined with quantitative PCR in wild-type (WT) and arp6 mutants, and is plotted over a background control of the TK gene [80]. The PES4 locus was analyzed as a control. The data points represent the mean ± SD for at least three experiments.

doi:10.1371/journal.pgen.1000910.g008

Arp6 and long-range chromatin organization

In addition to facilitating mRNA processing and export, the association of euchromatin domains with the NPC may facilitate the formation of nuclear subcompartments by creating boundaries [6] or by recruiting proteins required for genetic function or epigenetic control [reviewed in 4]. We note that a large fraction of Arp6 is chromatin-bound even in the absence of Swr1 (Figure 3), and that 25% of total Arp6 can be recovered in a nuclease-resistant nuclear scaffold fraction (data not shown). The association of Arp6 with an insoluble fraction of the nucleus, together with its ability to influence the localization of genes, argue that Arp6 can contribute to long-range organization of chromatin in the interphase nucleus. The ability of Arp6 to relocate chromatin to pores is not characteristic of all Arp proteins; the targeting of Arp5, a component of the INO80 chromatin remodeling complex with related molecular properties, does not change the random distribution of the tagged PES4 locus (H. van Attikum and S.M.G., personal communication). The perinuclear binding activity may thus reflect a unique domain of Arp6 or a binding partner with affinity for the NPC.

The positioning of chromatin in the interphase nucleus not only influences transcription, mRNA processing and export, but genome stability as well. Several laboratories have reported that critically short telomeres, irreparable DNA double-strand breaks and collapsed replication forks shift to the NPC for a repair pathway controlled by SUMO-dependent ubiquitin ligase [71–74]. Since arp6 mutants show hypersensitivity to various...
Figure 9. Arp6 is required for the peripheral association of galactose-induced GAL10 and constitutively expressed RP gene RPL9A. (A) The GAL1-GAL10 locus was tagged by inserting 256 lac operators in a haploid wild-type or arp6 deletion strain bearing GFP-lacI and Nup49-GFP fusions (wild-type; GA-4098, arp6; GA-6024) [81]. The position of the lacO arrays relative to the nuclear envelope was scored on images taken of living cells grown either on glucose (purple) or after 2 hours of gene induction on 2% galactose (black). Three zone scoring was carried out as in Figure 7. The number of cells analyzed for each stage of the cell cycle are indicated, and the confidence values (p) for the $\chi^2$ analysis between random and test distributions on galactose are: wild-type (G1, p = 4x10^{-10}; S, p = 6x10^{-9}) and arp6 (G1, p = 2.7x10^{-8}; S, p = 0.44) none of the values on glucose are significantly different from random (p > 0.05). The G1-S differences on galactose are not significant in WT, but are in the arp6 mutant (p = 0.024). Note that in this analysis we omitted the rare, very small budded cells. (B) The RPL9A locus was tagged by inserting 256 lac operators in a haploid wild-type or arp6 deletion strain bearing GFP-lacI and Nup49-GFP fusions (wild-type; GA-3635, arp6; GA-5132) [81]. The position of the lacO arrays relative to the nuclear envelope was scored as in Figure 7 on image stacks taken on living cells grown on SC. Symbols and quantitation are as in A. RPL9A locus p values for test vs random distributions are: wild-type (G1, p = 9.8x10^{-13}; S, p = 4.9x10^{-12}) and arp6 (G1, p = 7.6x10^{-9}; S, p = 0.07). The G1-S distributions in wild-type are not significantly different (p = 0.33) while in arp6 cells the difference is significant (p = 0.0018). An asterisk indicates that values that have a nonrandom distribution (p < 0.05). The number of cells analyzed (n) and the confidence values (p) for the $\chi^2$ analysis between random and test distributions are indicated. doi:10.1371/journal.pgen.1000910.g009
DNA damaging agents, Arp6 may also contribute to repair through its perinuclear relocalization activity.

We have recently analyzed chicken DT40 cells carrying a conditional knockout for Arp6, and found that the radial distribution of chromosome territories was altered in the absence of Arp6 (Ohfuchi et al., submitted). We therefore entertain the hypothesis that the contribution of Arp6 to long-range chromatin organization is evolutionarily conserved. In vertebrates there is as yet no compelling data implicating the NPC in gene expression or DNA repair, although other intranuclear structures such as PML bodies or transcription factories may replace pores in this function. We note that the reduction of human Arp4 by siRNA, unlike the loss of BRG-1, BRM, or Tip60, causes an expansion of the nuclear volume occupied by individual chromosomes (chromosome territories, [73]). While the mechanism remains obscure, this is consistent with the proposal that ARPs have roles in the long-range organization of chromatin that are independent of chromatin remodeling activities. The challenge remains to understand how cells regulate the interaction of chromatin with ARPs, nuclear actin, myosin and known structural proteins like lamin and nuclear pores.

**Materials and Methods**

**Plasmids, strains, and yeast imaging methods**

The LexA-Arp6 fusion was constructed as in Taddei et al. [7]. Yeast transformations were done using the lithium acetate procedure, and PCR-based gene deletions and tagging were performed as described [76]. The genotypes of all strains used in this study are listed in Table S6. Standard culture conditions at 30°C were used unless otherwise indicated. A 6His-3FLAG tag was fused to the C-terminus of Arp6 or Swr1 using the cassette amplified from pU6H3FLAG (gift from Dr. De Antoni) [43]. Live fluorescence microscopy and quantification was performed according to Hediger et al. [77] and Taddei et al. [2].

**Chromatin immunoprecipitation (ChIP)–chip analysis**

A chromosomal III, IV, V, and VI right-arm high-density oligonucleotide chip was produced by Affymetrix Custom Express Service (SC3456a520015F, P/N, Affymetrix). Sequence and position of oligonucleotides on the microarrays are available from Affymetrix. ChIP was carried out as previously described [43] with a few modifications. Yeast cells were grown in 200 ml YPD medium for 12 hr at 30°C, cross-linked, and disrupted using a multi-beads shocker (MB400C, Yasui Kikai), which was able to keep cells precisely at lower than 6°C during disruption by Zr beads. The anti-FLAG monoclonal antibody M2 (Sigma-Aldrich) was used for ChIP. ChIP DNA was purified and amplified by random priming as previously described [78]. The total of amplified DNA was digested with DNaseI to a mean size of 100 bp, purified, and the fragments were end-labeled with biotin-N6-ddATP. Hybridization, washing, scanning, and scanning were performed according to the manufacturer's instructions (Affymetrix). Data analyses were carried out as described previously [43].

**Microarray analysis**

For microarray analysis, total RNAs were prepared from cultures grown at 30°C in YPD medium to OD600 = 1.0 using TRIzol (Invitrogen). Microarray detection was performed as previously described [79], and carried out on at least three independent cultures.

**Gel filtration analysis**

The native molecular mass of complexes was monitored by gel filtration analysis according to Harata et al. [22] with modifications. Yeast extract from 100-ml culture of log-phase cells were applied to a Superose 6 column, and proteins were eluted at a flow rate of 0.2 ml/min. 1-ml fractions were collected and subjected to Western blot with the anti-FLAG M2 antibody to detect Arp6-Flag and Swr1-Flag. Suf2 was detected by using an anti-Suf2 antibody (Upstate).

**Chromatin fractionation assay**

The chromatin fractionation assay was performed as previously described [45] with the following modification. After spheroplasting, cells were washed twice in 50 mM Hepes-KOH pH 7.5, 20 mM KCl, 2 mM EDTA-KOH, 0.05 mM spermine, 0.125 mM spermidine, 1 M sorbitol, 1% Trasylol, and 1 mM PMSF. The pellet of spheroplasts (∼4×10⁶ cells) was then resuspended in 1 ml 50 mM Hepes-KOH pH 7.5, 2.5 mM MgCl2, 10 mM glycerol 2-phosphate, 0.1 mM Na2VO4, 0.25% Triton X-100, 300 µg/ml benzamidine, 1 µg/ml pepstatin A, 2 µg/ml antipain, 0.5 µg/ml leupeptin, 100 µg/ml TPCK, 50 µg/ml TLCK.

**Quantitative PCR and ChIP analysis**

For quantitative RT-PCR analysis, total RNAs were prepared from cultures grown at 30°C in YPD or YPG medium to OD600 = 1.0 by using RNeasy Mini Kit (Qiagen). Total RNAs were reverse-transcribed using the High Capacity cDNA Reverse Transcription kit (ABI), and subjected to quantitative real-time PCR with a SYBR Green Master Mix (ABI Prism 7000 Sequence Detector System and Software). ChIP was performed as for ChIP-chip analysis, but purified ChIP DNA was subjected to quantitative real-time PCR rather than microarray hybridization. For the primer sets, see Text S1. Real-time PCR monitors the threshold cycle at which the exponential curve of the accumulated product passes a threshold. PCR reactions were performed at least three times. TK normalization was performed as described in Shimada et al. [80].

**Supporting Information**

**Figure S1** The functionality of the tagged Arp6 and Swr1 was confirmed by monitoring cell growth and sensitivity to hydroxyurea (HU). Five-fold serial dilutions of each strain were plated on YPD with or without 50 mM HU and incubated at 30°C or 37°C for 3 days.

Found at: doi:10.1371/journal.pgen.1000910.s001 (1.09 MB TIF)

**Figure S2** Localization of Arp6 and Swr1 on chromosome 3. The binding of Arp6-FLAG (top), Swr1-FLAG (middle), and Arp6-FLAG in swr1 cells (bottom) are compared. The position of Tel 3L, Tel 3R, CEN3, and the RP gene are shown under the panels.

Found at: doi:10.1371/journal.pgen.1000910.s002 (9.62 MB TIF)

**Figure S3** Localization of Arp6 and Swr1 on chromosome 4. The binding of Arp6-FLAG (top), Swr1-FLAG (middle), and Arp6-FLAG in swr1 cells (bottom) in the whole chromosome region are compared. The position of Tel 4L, Tel 4R, CEN4, SWR1, and RP genes are shown under the panels.

Found at: doi:10.1371/journal.pgen.1000910.s003 (2.67 MB TIF)

**Figure S4** Localization of Arp6 and Swr1 on the region including the SWR1 gene of chromosome 4. The binding of Arp6-FLAG (top), Swr1-FLAG (middle), and Arp6-FLAG in swr1...
cells (bottom) are compared. The position and orientation of the SWR1 gene is shown.

Found at: doi:10.1371/journal.pgen.1000910.s004 (1.86 MB EPS)

**Figure S5** Localization of Arp6 and Swr1 on chromosome 5. The binding of Arp6-FLAG (top), Swr1-FLAG (middle), and Arp6-FLAG in swr1 cells (bottom) are compared. The position of Tel 5L, Tel 5R, CEN5, and the RP genes are shown under the panels.

Found at: doi:10.1371/journal.pgen.1000910.s005 (5.97 MB TIF)

**Figure S6** Preferential localization of Arp6 and Swr1 in the 5′ end of genes. Vertical bars represent the binding ratio of proteins in each locus. The binding of Arp6-Flag (Top), Swr1-Flag (middle), and Arp6-Flag in swr1 cells (bottom) are represented as scatterplots versus the Swr1 binding log2 ratio in each Arp6 binding locus of wild-type cells. The yellow lines represent the hypothetical pattern of the data if Arp6 and Swr1 bind equally on the chromosomes.

Found at: doi:10.1371/journal.pgen.1000910.s006 (1.29 MB EPS)

**Figure S7** Correlation of the localizations of Arp6 and Swr1. The Arp6-binding log2 ratios of Arp6-binding loci (change p-value < 0.025) in wild-type (A) and in swr1 cells (B) are represented as scatterplots versus the Swr1 binding log2 ratio in each Arp6 binding locus of wild-type cells. The values are indicated as percentage of input DNA obtained by ChIP with an anti-Htz1 antibody (abcam, ab4626) and quantified using real-time quantitative PCR in wild-type (WT), arp6, and swr1 cells. The values are indicated as percentage of input DNA obtained by ChIP with anti-Htz1 antibody. The data points represent the mean ± SD for at least three independent experiments.

Found at: doi:10.1371/journal.pgen.1000910.s007 (2.49 MB EPS)

**Figure S8** ChIP analysis for Htz1 in cells lacking Arp6 or Swr1. Htz1 association to the promoter of GAL1, SWR1, and ribosomal protein (RPL31a and RPS16B) genes was analyzed using ChIP with an anti-Htz1 antibody (abcam, ab4626) and quantified using real-time quantitative PCR in wild-type (WT), arp6, and swr1 cells. The values are indicated as percentage of input DNA obtained by ChIP with anti-Htz1 antibody. The data points represent the mean ± SD for at least three independent experiments.

Found at: doi:10.1371/journal.pgen.1000910.s008 (0.90 MB EPS)

**Figure S9** Quantitative analysis of RDS1 (YCR106W) and UBX3 (YDL091C) in arp6- and htz1-deletion mutants. The same amount of total RNA from wild-type, arp6, and htz1 cells was analyzed using real-time quantitative RT-PCR. The ACT1 gene was analyzed as a control. The relative amount of the transcript of the genes to ACT1 is shown. The data points represent the mean ± SD for at least three independent experiments.

Found at: doi:10.1371/journal.pgen.1000910.s009 (0.87 MB EPS)

**Figure S10** ChIP analysis for nuclear pore complex with GAL1 gene in arp6 cells. The association of GAL1 gene with NPC was analyzed using ChIP with an antibody against nuclear pore complex proteins (Mab414, abcam, ab24609) in wild-type (WT) and arp6 cells grown on the glucose- or galactose-containing media. Immunoprecipitated DNA was quantified using real-time PCR probed for GAL1 gene. The percentage of recovered DNA over input is plotted relative to wild-type cells on glucose as 1. The data points represent the mean ± SD for at least three independent experiments.

Found at: doi:10.1371/journal.pgen.1000910.s010 (0.85 MB EPS)

| Table S1 | Presence of Arp6 in nonrepetitive 10 kb subtelomere zones. |
| Table S2 | Microarray analysis in arp6Δ and swr1Δ cells. |
| Table S3 | Binding of Arp6 and Swr1 on ribosomal protein genes. |
| Table S4 | Expression of RP genes in arp6Δ and swr1Δ cells. |
| Table S5 | Genes markedly down-regulated in arp6Δ cells. |
| Table S6 | Strains used in this study. |

**Text S1** Primer sequences.

Found at: doi:10.1371/journal.pgen.1000910.s017 (0.04 MB DOC)

**Acknowledgments**

We thank F. Stutz, H. Renault, H. van Attikum, P. Heun, F. Neumann, H. Ferreira, and S. Nagai for yeast strains; I. Takehiko and K. Shirahige for technical advice; H. van Attikum for sharing unpublished results; and H. Ferreira and V. Dion for critically reading the manuscript.

**Author Contributions**

Conceived and designed the experiments: KS KO SMG MH. Performed the experiments: TY KS YO VK KA AT HI KK KO MH. Analyzed the data: TY KS YO VK KA AT HI KK KO SMG MH. Wrote the paper: TY KS SMG MH.

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