The RNA-Binding Protein *Whi3* Is a Key Regulator of Developmental Signaling and Ploidy in *Saccharomyces cerevisiae*

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ABSTRACT In *Saccharomyces cerevisiae*, the RNA-binding protein *Whi3* controls cell cycle progression, biofilm formation, and stress response by post-transcriptional regulation of the *Cdc28-Cln3* cyclin-dependent protein kinase and the dual-specificity protein kinase *Yak1*. Previous work has indicated that *Whi3* might govern these processes by additional, yet unknown mechanisms. In this study, we have identified additional effectors of *Whi3* that include the G₁ cyclins *Cln1/Cln2* and two known regulators of biofilm formation, the catalytic PKA subunit *Tpk1* and the transcriptional activator *Tec1*. We also provide evidence that *Whi3* regulates production of these factors by post-transcriptional control and might exert this function by affecting translational elongation. Unexpectedly, we also discovered that *Whi3* is a key regulator of cellular ploidy, because haploid *whi3Δ* mutant strains exhibit a significant increase-in-ploidy phenotype that depends on environmental conditions. Our data further suggest that *Whi3* might control stability of ploidy by affecting the expression of many key genes involved in sister chromatid cohesion and of *NIP100* that encodes a component of the yeast dynactin complex for chromosome distribution. Finally, we show that absence of *Whi3* induces a transcriptional stress response in haploid cells that is relieved by whole-genome duplication. In summary, our study suggests that the RNA-binding protein *Whi3* acts as a central regulator of cell division and development by post-transcriptional control of key genes involved in chromosome distribution and cell signaling.

EUKARYOTIC organisms have evolved highly complex signaling networks to control cell division and development. The functionality of signal transduction pathways crucially depends on the timely availability of individual components at sufficient levels to ensure adequate signaling capacity. A number of studies in different eukaryotes have shown that the production of individual signaling proteins is under the control not only of transcriptional regulators, but also of RNA-binding proteins (Lasko 2003; Sugiura *et al.* 2003; Prinz *et al.* 2007; Stewart *et al.* 2007; Malcher *et al.* 2011). This suggests that RNA-binding proteins could regulate the performance of whole signaling networks, but a precise view of key interconnections is often lacking.

In the budding yeast *Saccharomyces cerevisiae*, the Pumilio family (PUF) protein *Mpt5/Puf5* is a good example of an RNA-binding protein that controls cellular development by targeting signaling components. *Mpt5* has been found to negatively affect the performance of at least two different mitogen-activated protein kinase (MAPK) signaling pathways that regulate adhesion/biofilm formation and cell wall integrity (Prinz *et al.* 2007; Stewart *et al.* 2007). In general, PUF proteins are known to bind to the 3'-UTR of target mRNAs and reduce their stability or translatability (Quenault *et al.* 2011). This is also true for *S. cerevisiae* *Mpt5*, which represses the protein levels of key components of the MAPK cascade that regulates adhesion and biofilm formation. Specifically, *Mpt5* targets 3'-UTR sequences of the mRNAs for the MAPK kinase *Ste7* and the TEA family transcription factor *Tec1* (Prinz *et al.* 2007). In the case of the cell wall integrity MAPK pathway, *Mpt5* negatively regulates the mRNA levels of *LRG1*, which encodes a Rho GTPase activating protein that acts at the top of the pathway (Stewart *et al.* 2007).

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The RNA recognition motif (RRM) protein *Whi3* is another RNA-binding protein that controls *S. cerevisiae* adhesion and biofilm formation. In contrast to *Mpt5*, *Whi3* activates these processes and targets the dual-specificity tyrosine-regulated protein kinase *Yak1* (Mösch and Fink 1997; Malcher *et al.* 2011). Specifically, *Whi3* controls production of *Yak1* at the post-transcriptional level, which in turn activates expression of *FLO11*, a cell surface flocculin for adhesion and biofilm formation, via the transcription factors *Sok2* and *Phd1* (Gimeno and Fink 1994; Ward *et al.* 1995; Lo and Dranginis 1998; Guo *et al.* 2000; Pan and Heitman 2000; Reynolds and Fink 2001; Malcher *et al.* 2011). *Yak1*-dependent functions of *Whi3* further include control of stress responses via the transcription factors *Msn2* and *Msn4* (Lee *et al.* 2008) and acidic stress resistance mediated by the transcriptional regulator *Haa1* (Fernandes *et al.* 2005; Malcher *et al.* 2011). However, *Whi3* also seems to control filamentation and biofilm formation by yet unknown *Yak1*-independent mechanisms (Malcher *et al.* 2011), which might include other components of the complex signaling network that controls *FLO11* (Brückner and Mösch 2012).

In *S. cerevisiae*, *Whi3* also regulates cell division. Cells lacking *WHI3* enter S phase at a smaller volume than wild-type cells (Nash *et al.* 2001) and *WHI3* negatively regulates *CLN3* encoding a G₁ cyclin (Gari *et al.* 2001), indicating that *Whi3* controls cell size at the G₁/S transition. Specifically, *Whi3* binds *CLN3* mRNA by the RRM domain and interacts with *Cdc28* via its C-terminal region (Wang *et al.* 2004). It has thus been proposed that *Whi3* acts as a cytoplasmic retention factor for *Cdc28-Cln3* complexes in late G₁ phase and thereby controls passage through START, the point of cell cycle commitment. Global studies have identified a large number of mRNAs that are bound by the *Whi3* RRM domain *in vivo* (Colomina *et al.* 2008). A significant fraction of these *Whi3* targets encode proteins involved in transport and cell wall biogenesis, indicating that *Whi3* regulates cell morphogenesis (Colomina *et al.* 2009). In addition, *Whi3* binds to *CLN2* mRNA encoding another G₁ cyclin and to mRNAs for *Mpt5* and *Tec1*. This opens the possibility that *Whi3* controls additional key regulators of cell division and development and might therefore be connected to the respective signaling pathways by multiple links.

In this study, we characterized the role of *Whi3* in regulating cell division and development with the goal to identify novel targets. We find that *Whi3* controls not only production of the *Yak1* kinase, but also the expression of a number of other regulatory genes at the post-transcriptional level. Specifically, we show that in addition to its known effect on *Cdc28-Cln3*, *Whi3* is linked to the cell cycle by controlling the production of the G₁ cyclins *Cln1* and *Cln2*. We also find that *Whi3* targets the catalytic PKA subunit *Tpk1* and the transcription factor *Tec1*, showing that *Whi3* governs biofilm formation through different signaling cascades. Finally, we discovered that loss of *Whi3* in haploid cells results in rapid whole-genome duplication and affects

the expression of several genes involved in chromosome segregation. This suggests that *Whi3* fulfills a previously unknown role in ploidy control.

Materials and Methods

Yeast strains, plasmids, and growth conditions

All yeast strains used in this study are of the Σ 1278b genetic background (Table 1). Plasmids and primers are listed in Table 2 and [Supporting Information, Table S1](#), respectively. Yeast strain YHUM468 is identical to MY1398 and is a kind gift of Microbia (Lexington, MA). Yeast strain YHUM1920 was obtained by (i) deletion of *WHI3* in YHUM468, using plasmid pUG72 as a template and primers *Whi3_Kofw* and *Whi3_Korev*, and (ii) removal of the *URA3* selection marker by expression of Cre recombinase from plasmid pSH63 (Güldener *et al.* 2002). Strain YHUM2152 was obtained by passaging YHUM1920 every second day to fresh YEPD agar for 10 days and analysis of single colonies for diploid DNA content by FACS. Genomic GFP- and 6HA-fusion genes analyzed in this study were generated using plasmids pYM12 or pYM14 and gene-specific primers as described in Janke *et al.* (2004). For full-length gene fusions, S2- and S3-type primers were used, and S2 and S3b primers were used for truncated versions consisting of the first 99 bp of the analyzed genes fused to GFP. Yeast culture medium was prepared as described in Guthrie and Fink (1991). YNB media were supplemented with 0.4 mM tryptophan and 0.2 mM uracil (YNB + Trp + Ura). For sensitivity tests, yeast cultures were grown in liquid YEPD medium to logarithmic phase and serial dilutions were spotted on YEPD agar plates with or without inhibitors. Adhesive growth tests were described earlier (Roberts and Fink 1994). Crosses, sporulation, and tetrad dissection were performed according to standard methods (Guthrie and Fink 1991).

Transcript analysis

For transcript analysis, cultures of 25 ml were grown in YNB + Trp + Ura media to an OD₆₀₀ of 1 and cells were disrupted with glass beads in 400 μ l phenol:chloroform:isoamyl alcohol (25:24:1) and 400 μ l cross RNA buffer I [0.3 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA (pH 8.0), 0.2% (w/v) SDS] on a multivortexer for 10 min at 4°. The supernatant was transferred to a fresh tube and total RNA was precipitated by addition of 1 ml ice-cold ethanol for 10 min at −70° and centrifugation for 5 min at 13,000 rpm and 4°. Pellets were resuspended in 30 μ l of cross RNA buffer II [1× TE, 0.2% (w/v) SDS]. For each sample, 17 μ g of total RNA was mixed with 2.4 μ l of 10× MOPS (200 mM MOPS, 80 mM sodium acetate, 10 mM EDTA), 2.4 μ l of 8 M Glyoxal, 12 μ l of 100% (v/v) DMSO, and 4 μ l loading dye [1× MOPS, 50% (v/v) glycerol, 0.5% (w/v) bromophenol blue, 0.5% (w/v) xylencyanol blue]; separated on 1.2% (w/v) agarose gels containing 1× MOPS; and transferred to positively charged nylon membranes in 10×SSC (1.5 M

Table 1 Yeast strains used in this study

Strain	Description	Source
YHUM468	<i>MATa ura3Δ trp1Δ</i>	Microbia
YHUM1920	YHUM468 with <i>whi3Δ::loxP</i>	This study
YHUM2152	<i>MATa/MATa ura3Δ/ura3Δ trp1Δ/trp1Δ whi3Δ::loxP/whi3Δ::loxP</i>	This study
YHUM492	<i>MATa/MATα ura3Δ/ura3Δ trp1Δ/trp1Δ leu2Δ/LEU2 his3Δ/HIS3</i>	Microbia Inc.
YHUM1313	YHUM468 with <i>yak1Δ::kanMX4</i>	Malcher et al. (2011)
YHUM2045	YHUM468 with <i>TPK1-GFP::kanMX4</i>	This study
YHUM2046	YHUM1920 with <i>TPK1-GFP::kanMX4</i>	This study
YHUM1755	YHUM468 with <i>YAK1-GFP::kanMX4</i>	This study
YHUM1757	YHUM1920 with <i>YAK1-GFP::kanMX4</i>	This study
YHUM2041	YHUM468 with <i>PHD1-GFP::kanMX4</i>	This study
YHUM2042	YHUM1920 with <i>PHD1-GFP::kanMX4</i>	This study
YHUM1937	YHUM468 with <i>TPK2-GFP::kanMX4</i>	This study
YHUM1939	YHUM1920 with <i>TPK2-GFP::kanMX4</i>	This study
YHUM1886	YHUM468 with <i>FLO8-GFP::kanMX4</i>	This study
YHUM1935	YHUM1920 with <i>FLO8-GFP::kanMX4</i>	This study
YHUM1745	YHUM468 with <i>TEC1-GFP::kanMX4</i>	This study
YHUM1746	YHUM1920 with <i>TEC1-GFP::kanMX4</i>	This study
YHUM1885	YHUM468 with <i>MPT5-GFP::kanMX4</i>	This study
YHUM1932	YHUM1920 with <i>MPT5-GFP::kanMX4</i>	This study
YHUM1884	YHUM468 with <i>MIG2-GFP::kanMX4</i>	This study
YHUM1929	YHUM1920 with <i>MIG2-GFP::kanMX4</i>	This study
YHUM1888	YHUM468 with <i>CLN1-GFP::kanMX4</i>	This study
YHUM1926	YHUM1920 with <i>CLN1-GFP::kanMX4</i>	This study
YHUM1921	YHUM468 with <i>CLN2-GFP::kanMX4</i>	This study
YHUM1923	YHUM1920 with <i>CLN2-GFP::kanMX4</i>	This study
YHUM2213	YHUM468 with <i>NIP100-6HA::kanMX4</i>	This study
YHUM2214	YHUM1920 with <i>NIP100-6HA::kanMX4</i>	This study
YHUM2215	<i>MATa/MATa ura3Δ/ura3Δ trp1Δ/trp1Δ whi3Δ::loxP/whi3Δ::loxP NIP100-6HA::kanMX4/ NIP100-6HA::kanMX4</i>	This study
YHUM2047	YHUM468 with <i>TPK1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM2048	YHUM1920 with <i>TPK1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1760	YHUM468 with <i>YAK1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1762	YHUM1920 with <i>YAK1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM2043	YHUM468 with <i>PHD1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM2044	YHUM1920 with <i>PHD1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1938	YHUM468 with <i>TPK2¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1940	YHUM1920 with <i>TPK2¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1934	YHUM468 with <i>FLO8¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1936	YHUM1920 with <i>FLO8¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1747	YHUM468 with <i>TEC1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1748	YHUM1920 with <i>TEC1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1931	YHUM468 with <i>MPT5¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1933	YHUM1920 with <i>MPT5¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1928	YHUM468 with <i>MIG2¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1930	YHUM1920 with <i>MIG2¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1925	YHUM468 with <i>CLN1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1927	YHUM1920 with <i>CLN1¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1922	YHUM468 with <i>CLN2¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM1924	YHUM1920 with <i>CLN2¹⁻⁹⁹-GFP::kanMX4</i>	This study
YHUM2151	<i>MATa/MATa ura3Δ/ura3Δ trp1Δ/trp1Δ MIG2-GFP::KanMX4/MIG2</i>	This study
L6438	<i>MATa/MATa ura3Δ/ura3Δ his3Δ/his3Δ leu2Δ/leu2Δ</i>	Galitski et al. (1999)
L6441	<i>MATα ura3Δ his3Δ leu2Δ</i>	Galitski et al. (1999)
L6442	<i>MATα/MATα ura3Δ/ura3Δ his3Δ/his3Δ leu2Δ/leu2Δ</i>	Galitski et al. (1999)

NaCl, 150 mM trisodium citrate dihydrate, pH 7). To visualize rRNA, membranes were stained with 0.04% (w/v) methylene blue. Transcripts of *GFP*-fusion constructs, *NIP100-6HA* and *ACT1* (loading control) were quantified using the Lab Image 1D software (Kapelan Bio-imaging, Leipzig, Germany) after hybridization with ³²P-labeled DNA probes according to the Megaprime DNA Labeling System and detection by a Storm860 Phosphorimager (GE

Healthcare Life Sciences, Munich, Germany). Transcripts of *FLO11* together with *ACT1* as loading control were analyzed using the DIG-labeling kit (Roche, Mannheim, Germany).

Microarray analysis

Transcriptional profiling of yeast strains YHUM468 (*WHI3*), YHUM1920 (*whi3Δ*), and YHUM2152 (*whi3Δ/whi3Δ*) was

Table 2 Plasmids used in this study

Plasmid	Description	Source
pUG72	Vector containing <i>loxP</i> -flanked <i>URA3</i> cassette	Güldener <i>et al.</i> (2002)
pSH63	Vector containing Cre recombinase	Güldener <i>et al.</i> (2002)
pYM12	Vector containing <i>yeGFP</i> for C-terminal GFP fusion	Knop <i>et al.</i> (1999)
pYM14	Vector containing <i>6HA</i> for C-terminal 6HA fusion	Janke <i>et al.</i> (2004)

performed in duplicate, using Yeast Genome 2.0 expression arrays (Affymetrix, High Wycombe, UK) following standard protocols. Strains were grown to logarithmic phase in YNB + Trp + Ura medium, harvested, and rapidly frozen in liquid nitrogen. Total RNA was extracted with the QIAGEN RNeasy Mini Kit, using Yeast Protocol 1c for mechanical disruption (QIAGEN, Hilden, Germany), and all subsequent steps were conducted following the GeneChip 3'IVT Express Kit manual (Affymetrix). Briefly, cDNAs were synthesized from 250 µg of total RNA. *In vitro* transcription labeling was carried out for 16 hr and fragmented samples were controlled with an Agilent 2100 Bioanalyzer (Agilent Technologies, Böblingen, Germany). For each sample, 5 µg of labeled amplified RNA was hybridized for 16 hr on Affymetrix GeneChip Yeast Genome 2.0 expression arrays (169 format) followed by washing and staining steps using an Affymetrix Fluidics 450 station. Arrays were scanned on an Affymetrix 3000 7G GeneArrayScanner and the data were processed and analyzed with the Affymetrix GeneChip Operating software. Genes were defined as differentially expressed when exhibiting a difference in average signal intensity of >15 and a fold change in expression of >1.99 (calculated as the fold change of average expression in duplicate measurements). Array data are available from the ArrayExpress database (<http://www.ebi.ac.uk>), under accession no. E-MEXP-3610.

Quantitative real-time PCR

Quantitative real-time PCR was performed by reverse transcription of 500 ng of DNase-treated total RNA into cDNA, using the iScript cDNA Synthesis Kit (Bio-Rad, Munich, Germany), and subsequent PCR reactions were performed in 96-well plates, using the MyiQ single-color real-time PCR detection system (Bio-Rad). Independent PCRs were performed using the same cDNA for both the gene of interest and *ACT1* as a reference. Gene-specific primers are shown in Table S1. The reaction mix (25 µl final volume) consisted of 12.5 µl of 2× iQ SYBR green supermix (Bio-Rad), 1 µl of each primer (0.4 µM final concentration), 0.5 µl fluorescein isothiocyanate (FITC) (0.02 µM final concentration), 9 µl H₂O, and 1 µl of cDNA. A control without template was incorporated in each assay. The thermocycling program consisted of one hold at 95° for 3 min, followed by 45 cycles of 15 sec at 95°, 30 sec at 60°, and 30 sec at 72°. After completion of these cycles, melting curve data were collected to determine PCR specificity, contamination, and the absence of primer dimers. The detection of the threshold cycle was done automatically by the cycler software. Quantification of gene expression was carried out with the Gene

Expression Analysis for iQ real-time PCR detection system software (Bio-Rad).

Preparation of total yeast cell extracts and immunoblot analysis

Total yeast cell extracts were prepared as described in Riezman *et al.* (1983). Briefly, 1 ml of yeast cultures grown in YNB + Trp + Ura medium to an OD₆₀₀ of 1 was mixed with 150 µl lysis buffer [1.85 M NaOH, 7.5% (v/v) β-mercaptoethanol] and incubated on ice for 10 min. After addition of 150 µl 50% (w/v) trichloroacetic acid and 10 min incubation on ice, samples were centrifuged for 10 min at 13,000 rpm and 4°. Pellets were dissolved in 50 µl of urea buffer [5% (w/v) SDS, 8 M urea, 92.6 mM Na₂HPO₄, 107.4 mM NaH₂PO₄, 1.5% (w/v) dithiothreitol, 0.1 mM EDTA (pH 6.8), 0.03% (w/v) bromophenol blue], separated by SDS-PAGE, and transferred to nitrocellulose membranes by electrophoresis. GFP fusion and 6HA-tagged proteins were detected by enhanced chemiluminescence (ECL) technology (Amersham, Buckinghamshire, UK), using the sc9969 monoclonal mouse anti-GFP (Santa Cruz Biotechnology, Heidelberg, Germany) or the H3663 monoclonal mouse anti-HA antibody (Sigma Aldrich, Munich, Germany). The monoclonal rabbit anti-Tub1 antibody cp06 (Calbiochem, Darmstadt, Germany) and polyclonal goat anti-Cdc28 antibodies (Santa Cruz; sc6709) were used for loading controls. As secondary antibodies, horseradish peroxidase-coupled variants of goat anti-mouse (Dianova, Hamburg, Germany), goat anti-rabbit (Santa Cruz; sc2054), or mouse anti-goat (Santa Cruz; sc2354) antisera were used. Signals were detected with the Chemo Star Imager (INTAS Science Imaging Instruments, Göttingen, Germany) and quantified using the Lab Image 1D software (Kapelan Bio-imaging).

Cell size analysis

Yeast cultures grown to logarithmic phase were diluted in 10 ml of Isoton II Diluent and cell size was measured using the Multisizer 3 Particle Analyzer (Beckman Coulter, Krefeld, Germany). For each strain, the median cell size of at least 100 cells was independently determined by using differential interference contrast (DIC) microscopy on a Axiovert 200 microscope (Zeiss, Jena, Germany), an Orca ER digital camera (Hamamatsu, Bridgewater, NJ), and the Openlab (Perkin-Elmer, Waltham, MA) and ImageJ software (Schneider *et al.* 2012).

Flow cytometry

For flow cytometry, cells were prepared as described in Costello *et al.* (1986). Briefly, 5 ml of cultures grown to logarithmic

phase in YNB + Trp + Ura were harvested for 3 min at 2,000 rpm, washed with 3 ml of PBS buffer, and fixed overnight in 70% (v/v) ethanol at 4°. After washing, cells were resuspended in 5 ml of PBS and separated using a Sonoplus sonicator (Bandelin, Berlin). The pelleted cells were resuspended in 1 ml of 50 mM sodium citrate buffer (pH 7.4) and digested with 0.25 mg/ml RNAse for 12 hr at 37°. After incubation of the samples in 25 µl proteinase K solution (Roche, Mannheim, Germany) for 1 hr at 50°, cells were washed with 50 mM sodium citrate (pH 7.4) and stained with 3 µg/ml propidium iodide (AppliChem, Darmstadt, Germany) for 3 hr in the dark. After washing, cells were 5× diluted in FACS buffer [PBS, 3% (v/v) fetal calf serum] and analyzed with a BD-FACS Calibur Flow Cytometer (Becton Dickinson, Heidelberg, Germany), using the red FL3 channel.

Results

Whi3 is a post-transcriptional regulator of G₁ cyclin gene expression

Previous studies suggested that *Whi3* regulates cell size by controlling *Cln3* (Gari *et al.* 2001). However, *Whi3* is also able to bind *CLN2* mRNA (Colomina *et al.* 2008), indicating that *Whi3* might control other G₁ cyclins. We therefore tested the possibility that *Whi3* controls *CLN1* and *CLN2* gene expression at the post-transcriptional level. For this purpose, we created *WHI3* and *whi3Δ* yeast strains carrying endogenous *CLN1-GFP* or *CLN2-GFP* fusion genes. Functionality of these fusion genes was first determined by analyzing the resulting strains for their cell size, because this phenotype is influenced by *Cln1* and *Cln2* (Hadwiger *et al.* 1989). As expected, deletion of *WHI3* led to a significant decrease of the average cell size as measured by Coulter counter particle analysis and microscopy (Figure 1A). However, no difference was observed between control and *CLN1-GFP* or *CLN2-GFP* strains, indicating that fusion genes are functional (Figure 1A). We then performed quantitative analysis of transcript and protein levels of the two cyclins in *WHI3* and *whi3Δ* strains (Figure 1B) and calculated the protein-to-mRNA ratio for each gene, to determine the post-transcriptional influence of *Whi3* (Figure 1C). We found that absence of *Whi3* led to a reduction of the protein/mRNA ratios of the full-length *CLN1-GFP* (2.3-fold) and *CLN2-GFP* (1.4-fold) (Figure 1C). We also generated strains carrying truncated *CLN1-GFP* and *CLN2-GFP* fusion genes that lack most of the cyclin ORF sequences (*ORF¹⁻⁹⁹-GFP*), to determine whether *Whi3* is able to target the isolated 5'-UTR. Here, we found that in the absence of *Whi3* the protein/mRNA ratio was reduced 2.3-fold for *CLN1¹⁻⁹⁹-GFP* and 1.6-fold for *CLN2¹⁻⁹⁹-GFP*, respectively (Figure 1, B and C). These results indicate that *Whi3* is able to regulate expression of *CLN1* and *CLN2* at the post-transcriptional level via the 5'-UTR, suggesting that *Whi3* might affect cell size by targeting not only *Cln3*, but also additional G₁ cyclins.

Whi3 controls key regulators of yeast development at the post-transcriptional level

We have previously shown that *Whi3* is able to control biofilm development by both post-transcriptional control of *Yak1* and yet unknown, *Yak1*-independent mechanisms (Malcher *et al.* 2011). We therefore wanted to know whether *Whi3* is able to control additional yeast developmental regulators at the post-transcriptional level. For this purpose, we determined the influence of *Whi3* on the protein/mRNA ratio of selected biofilm regulators including components of the cAMP-PKA pathway (*Tpk1*, *Tpk2*, and *Flo8*), the PKA-regulated *Yak1* branch (*Yak1* and *Phd1*), and the *Fus3/Kss1* MAPK cascade (*Tec1*) as well as the RNA-binding protein *Mpt5*. We also included *Mig2*, which has not been associated with biofilm formation, but whose mRNAs are bound by *Whi3* *in vivo* (Colomina *et al.* 2008). To determine the effect of *Whi3* on production of these regulators, we used the same experimental approach as for the analysis of G₁ cyclins and created a series of *WHI3* and *whi3Δ* yeast strains that carry endogenous fusions of corresponding genes to GFP. Resulting strains were assayed for biofilm formation, showing that all fusion genes are fully functional except *FLO8-GFP* that conferred partially reduced functionality (Figure S1). We then performed quantitative analysis of transcript and protein levels of the tested regulators in *WHI3* and *whi3Δ* strains (Figure S2A). We found that absence of *Whi3* led to significantly reduced (>2-fold) protein/mRNA ratios for *TPK1-GFP*, *YAK1-GFP*, and *TEC1-GFP* (Figure 2). This corroborates our previous finding that *Whi3* controls *Yak1* at the post-transcriptional level (Malcher *et al.* 2011) and identifies *Tpk1* and *Tec1* as additional targets for *Whi3*. In the case of GFP fusions for full-length *PHD1* and *MIG2*, respectively, we observed a notable, but less pronounced reduction (~1.5-fold) of protein/mRNA ratios in *whi3Δ* strains, identifying these regulators as possible minor *Whi3* targets.

To determine whether *Whi3*-mediated regulation of putative target mRNAs is conferred by the 5'-UTR or sequences within the ORF, we also created truncated GFP fusion genes that carry the full 5'-UTR region, but lack most of the ORF sequences (*ORF¹⁻⁹⁹-GFP*). Here, we found that *Whi3*-mediated regulation of the protein/mRNA ratio was reduced in the case of *YAK1¹⁻⁹⁹-GFP* (1.6-fold) and *MIG2¹⁻⁹⁹-GFP* (1.5-fold), indicating that the ORF regions of the corresponding mRNAs mediate *Whi3* control (Figure S2B and Figure 2). In contrast, *Whi3*-dependent protein/mRNA ratios of *TPK1¹⁻⁹⁹-GFP*, *PHD1¹⁻⁹⁹-GFP*, and *TEC1¹⁻⁹⁹-GFP* did not differ from the corresponding full-length versions (Figure 2), indicating that *Whi3* control is exerted by the 5'-UTRs of these mRNAs. In the case of *MPT5*, we uncovered a *Whi3*-mediated regulation of the protein/mRNA ratio after deletion of the ORF region, suggesting that *Whi3* could target the 5'-UTR of the *MPT5* mRNA.

In summary, these results show that *Whi3* is able to control a number of key regulators of yeast development at the post-transcriptional level by targeting 5'-UTR or ORF regions of corresponding mRNAs.

Figure 1 Post-transcriptional regulation of G₁ cyclins by *WHI3*. (A) Influence of *WHI3* and *CLN1*-GFP or *CLN2*-GFP fusions on cell size was measured using *WHI3* yeast strains YHUM468 (no tag), YHUM1888 (*CLN1*-GFP), and YHUM1921 (*CLN2*-GFP) and *whi3Δ* strains YHUM1920 (no tag), YHUM1926 (*CLN1*-GFP), and YHUM1923 (*CLN2*-GFP) by Coulter counter particle analysis and microscopy. Absolute particle volumes are displayed in the top graph. The table below shows relative cell size values for Coulter counter (no parentheses) and microscopic measurements (parentheses) with values for control strain YHUM468 set to 100%. (B) Influence of *WHI3* on expression of full-length and truncated versions of *CLN1* and *CLN2* was measured using yeast strains YHUM1888 (*CLN1*-GFP), YHUM1926 (*CLN1*-GFP *whi3Δ*), YHUM1921 (*CLN2*-GFP), YHUM1923 (*CLN2*-GFP *whi3Δ*), YHUM1925 (*CLN1*¹⁻⁹⁹-GFP), YHUM1927

Absence of *WHI3* increases sensitivity toward inhibitors of translational elongation

Our finding that *Whi3* affects expression of several genes at the post-transcriptional level by targeting the 5'-UTR or ORF regions suggests a putative role of *Whi3* during translation. We therefore determined the sensitivity of *whi3* mutant strains toward low doses of diverse inhibitors of translational elongation including Cycloheximide, Geneticin, and Hygromycin B (Adoutte-Panvier *et al.* 1980; Jimenez and Davies 1980). We found that absence of *Whi3* resulted in significantly lower resistance to Geneticin and Hygromycin B (Figure 3). We also detected a slightly increased sensitivity of the *whi3* mutant strain toward Cycloheximide, which corroborates earlier observations (Nash *et al.* 2001). These data demonstrate that *Whi3* confers higher resistance toward several inhibitors of translation elongation, suggesting that *Whi3* might positively affect this process. This would explain the reduced mRNA/protein ratios that we detected for G₁ cyclins and biofilm regulators in the absence of *Whi3* (Figures 1C and 2).

WHI3 is required for maintenance of the haploid state

In the course of chromosomal gene tagging of *whi3Δ* mutant strains (*MATa*) with *GFP*, we noted that a significant fraction of resulting clones contained not only the tagged but also the native, untagged gene version (Figure S3A). Further analysis revealed that clones carrying both tagged and untagged gene copies also contained a diploid DNA content (Figure S3B), but were still able to mate, indicating that they originated from diploid *MATa/MATa whi3Δ/whi3Δ* cells and that absence of *WHI3* might have caused a higher frequency of whole-genome duplication (WGD).

To test this hypothesis, we directly measured the increase in ploidy rate of haploid *whi3Δ* mutant strains. For this purpose, haploid *WHI3* control and freshly generated *whi3Δ* mutant strains were cultivated in YNB (minimal) and YEPD (rich) liquid media as well as on solid YNB and YEPD agar (Figure 4A). Cultures were grown for up to 72 days by passaging aliquots to fresh media every second day and analyzed for DNA content at different time points by FACS analysis. As expected, ploidy of a haploid *WHI3* control strain was highly stable on all tested media and no significant amount of 4n cells was detected after 72 days, which corresponds to 36 passages and ~250 generations (Figure

(*CLN1*¹⁻⁹⁹-GFP *whi3Δ*), YHUM1922 (*CLN2*¹⁻⁹⁹-GFP), and YHUM1924 (*CLN2*¹⁻⁹⁹-GFP *whi3Δ*) by quantitative transcript and protein analysis after growth in liquid YNB + Trp + Ura medium. Transcript and protein levels of GFP fusions were determined by Northern (top) and Western (bottom) blot analyses in a minimum of three independent measurements, respectively. Numbers indicate relative transcript or protein levels using *ACT1* transcript or Cdc28 protein levels as internal standards. Values for the respective *WHI3* strains were set to 100%. (C) Relative transcript and protein levels obtained in B were combined to calculate the protein/mRNA ratio of GFP fusions in *whi3Δ* mutants normalized to the *WHI3* strains.

Figure 2 Post-transcriptional control of developmental regulators by *WHI3*. Effect of *WHI3* deletion on expression of full-length and truncated versions of *TPK1*, *YAK1*, *PHD1*, *TPK2*, *FLO8*, *TEC1*, *MPT5*, and *MIG2* was measured by transcript and protein analysis as described in Figure 1 and Figure S2. Data were used to calculate the relative protein/mRNA ratios for the different GFP fusions in the *whi3Δ* genetic background normalized to the values in the *WHI3* control strains.

4B). In contrast, *whi3Δ* strains displayed a significant WGD rate, which was dependent on the growth conditions. When using minimal medium, we detected a small amount of 4n cells in liquid cultures after 72 days and a complete shift to diploid cells on solid agar (Figure 4B). When using rich medium, the WGD rate was even higher, because cultures had completely shifted to the diploid state after 72 days in liquid medium and after only 14 days (~50 generations) on solid agar (Figure 4B). WGD of haploid *whi3Δ* strains was also verified by genetic crosses (Figure S4). These results indicate that *Whi3* is crucial for the maintenance of the haploid state and that this previously unknown function depends on the growth conditions, such as the nutritional supply and the physical environment.

Whole-genome duplication in haploid *whi3Δ* strains does not rescue defects in biofilm formation and cell size, but leads to apparent stability of ploidy

We next wanted to know how WGD affects the phenotypes that are caused by deletion of *WHI3* in haploids. For this purpose, we first analyzed *MATa whi3Δ* and *MATa/MATa whi3Δ/whi3Δ* strains with respect to *FLO11*-mediated biofilm formation, cell size, and maintenance of ploidy. As previously shown, ploidy did not significantly affect *FLO11* expression and biofilm formation when comparing haploid *MATa* and diploid *MATa/MATa* control strains (Figure 5A) (Galitski *et al.* 1999). Also, a *MATa/MATa* control strain was significantly reduced for *FLO11* expression, however, not due to its diploid state, but owing to its mating type (Rupp *et al.* 1999). Furthermore, we found that diploid *whi3Δ/whi3Δ* strains were repressed for *FLO11* expression and biofilm formation similar to haploid *whi3Δ* mutants (Figure 5A). This indicates that *Whi3* is essential for *FLO11*-mediated biofilm formation in both haploids and diploids and that WGD of haploid *MATa* strains does not significantly affect this developmental phenotype. Furthermore, WGD in a *whi3Δ* strain led to an ~1.3-fold increase in cell size,

which is, however, comparable to the difference observed for haploid and diploid *WHI3* control strains (Figure 5B). This indicates that cell size control by *Whi3* and ploidy are conferred by independent mechanisms. Finally, we found that the ploidy of *whi3Δ/whi3Δ* diploids was highly stable even after 72 days on solid YEPD medium (Figure 5C), the condition where we observed the highest WGD rate for haploid *whi3Δ* strains. Thus, WGD in a *whi3Δ* haploid is sufficient to rescue the ploidy control defect or tetraploid *whi3Δ* mutants are not observed due to decreased viability.

***Whi3* regulates expression of *NIP100* encoding a component of the dynactin complex at the post-transcriptional level**

Our finding that absence of *Whi3* causes increased WGD rates prompted us to look for genes that genetically interact with *WHI3* and affect chromosome segregation. One gene that fulfills these criteria is *NIP100*, encoding a component of the yeast dynactin complex (Costanzo *et al.* 2010). It was shown that *nip100Δ* deletion strains are viable, but exhibit a high frequency of failed mitoses, in which chromatid separation and mitotic spindle elongation are restricted to mother cells (Kahana *et al.* 1998). Therefore, we tested the possibility that *WHI3* affects the cellular content of *Nip100*. For this purpose we tagged *NIP100* with the 6HA epitope in the genome of control and *whi3Δ* strains and compared corresponding transcript and protein levels (Figure 6A). These measurements were performed in liquid YNB medium that causes only a minimal increase in the WGD rate of haploid *whi3Δ* mutants and with solid YEPD agar, on which we observed the highest WGD rate (see Figure 4). When growing strains in liquid YNB, we found an ~20% decrease in *Nip100* protein/mRNA ratios in the absence of *WHI3* (Figure 6B). On solid YEPD, we already measured a significant reduction of *Nip100* protein levels and corresponding protein/mRNA ratios in the presence of *WHI3*. Under these conditions, the deletion of *WHI3* led to an additional threefold decrease in the *Nip100* protein/mRNA ratio. These results indicate that post-transcriptional expression of *Nip100* is under control of both *Whi3* and growth conditions.

***WHI3* is a global regulator of genes involved in cell cycle regulation, sister chromatid cohesion, and stress response**

To better understand how loss of *Whi3* in haploids and subsequent WGD affect gene expression and cell physiology on a global scale, we compared transcriptional profiles of *MATa WHI3* control, *MATa whi3Δ* haploid, and *MATa/MATa whi3Δ/whi3Δ* diploid strains, using high-density oligonucleotide arrays (Affymetrix GeneChips). Analysis of these profiles revealed that 319 genes are regulated at least twofold by *Whi3* (Figure 7A). Interestingly, expression of most of these genes (309) was elevated in the *whi3Δ* mutant, showing that *Whi3* has a predominantly repressing influence on transcription. Furthermore, we found 38 genes that are

Figure 3 Requirement of *WHI3* for resistance against inhibitors of translational elongation. Yeast strains YHUM468 (*WHI3*) and YHUM1920 (*whi3Δ*) were grown to logarithmic phase and serial dilutions were spotted on solid YEPD

medium (control) or YEPD plates supplemented with different inhibitors of translational elongation (3 μ g/ml Cycloheximide, 20 μ g/ml Geneticin, or 65 μ g/ml Hygromycin B). Pictures were taken after 2 days of growth at 30°. Spots correspond to serial dilutions (from left to right: undiluted, 5 $\times$, 25 $\times$, 125 $\times$, 625 $\times$).

differentially expressed in *whi3Δ* haploid and *whi3Δ/whi3Δ* diploid strains, identifying targets that are regulated by ploidy in the absence of *Whi3*. Remarkably, 20 of these genes were also regulated by *Whi3*. A representation factor (RF) of 9.4 (a measure of the observed number of overlapping genes compared with the expected number) was calculated for this overlap ($P < 6.4 \times 10^{-16}$), indicating that *Whi3* has an above average impact on ploidy-regulated transcription.

To identify *Whi3*-regulated cellular processes and transcription factors that might mediate *Whi3* control, we performed concurrent enrichment analysis for Gene Ontology (GO) terms and transcription factor binding sites (Abascal *et al.* 2008; Morris *et al.* 2010). This analysis revealed that *Whi3*-regulated genes were significantly enriched for functions associated with the cell cycle, DNA replication, and sister chromatid cohesion as well as for binding by the cell cycle regulators MBF (*Mbp1-Swi6*), SBF (*Swi4-Swi6*), *Fkh1*, and *Fkh2* (Figure 7B). This finding underpins the role of *Whi3* in cell cycle control. We further noted that absence of *Whi3* in haploids causes a roughly twofold induction of many genes involved in sister chromatid condensation and cohesion, including *SMC1*, *SMC3*, *MCD1*, and *IRR1*, which encode core components of the cohesion complex and are bound by *Mbp1* and *Swi6* (Figure 7C and Table S2). This finding might further explain the increased WGD rate of haploid *whi3Δ* mutants. However, none of these genes were regulated by ploidy in the *whi3Δ* genetic background, indicating that WGD does not rescue their increased transcription. We also found that *Whi3*-regulated genes were enriched for mitochondrial and TCA cycle functions. Most of these genes were expressed at higher levels in *whi3Δ* mutant strains, indicating that *Whi3* might negatively control mitochondrial functions. Finally, we found that the class of 20 genes that are regulated by both *Whi3* and ploidy was enriched for functions associated with stress response and for binding by the stress transcription factors *Msn2* and *Msn4* (Figure 7D). Remarkably, increased transcription caused by the deletion of *WHI3* was reversible by WGD for most genes of this class (Figure 7D and Table S2). Thus, absence of *Whi3* appears to trigger a stress response that leads to increased transcription of *Msn2/Msn4*-regulated genes and that is alleviated by WGD.

Discussion

In this study, we present evidence that the RRM domain protein *Whi3* not only regulates cell size, biofilm formation,

and stress response, but also controls maintenance of cellular ploidy. We have also identified a number of regulatory proteins involved in cell cycle progression, signal transduction, and sister chromatid distribution, whose production is controlled by *Whi3* at the post-transcriptional level. As such, our data provide new insights into the regulatory pathways and mechanisms by which RNA-binding proteins control cell growth and development.

Previous studies have suggested that *Whi3* controls cell size by regulating the timing of G₁/S transition (Gari *et al.* 2001; Nash *et al.* 2001). Specifically, *Whi3* has been proposed to inhibit *Cdc28-Cln3*, which activates SBF- and MBF-regulated transcription (Dirick *et al.* 1995; de Bruin *et al.* 2004; Wang *et al.* 2004; Ferrezuelo *et al.* 2010). Our transcriptional profiling supports these studies by showing that *Whi3* represses many MBF- and SBF-regulated genes and supports the view that *Whi3* controls cell size by negative regulation of G₁/S transition (Figure 8). However, we found that *Whi3* positively controls expression of *CLN1* and *CLN2* at the post-transcriptional level. This indicates that *Whi3* might also be able to promote G₁/S transition, because *Cln1* and *Cln2* positively act on cell cycle passage through START (Enserink and Kolodner 2010). Thus, *Whi3* seems to be able to control cell size by two independent mechanisms that have opposite effects on cell size. Whereas inhibition of *Cdc28-Cln3* by *Whi3* causes a larger cell size, its post-transcriptional activation of G₁ cyclin production would be expected to promote smaller cells. We can only speculate on the physiological role of this dual regulation by *Whi3*, but it might contribute to ensuring that the cell size is kept within certain boundaries and cells do not become too small or too big.

A novel finding of this study is the involvement of the RNA-binding protein *Whi3* in maintenance of ploidy. A seminal screen has identified several genes, whose inactivation causes an increase-in-ploidy phenotype (Chan and Botstein 1993). These include *CDC31* coding for a component of the spindle pole body or *IPL1* encoding the enzymatic component of the Aurora kinase complex that regulates chromosome segregation (Biggins *et al.* 1999; Ruchaud *et al.* 2007). Other mutations causing an increase-in-ploidy phenotype were described, for example for *MOB1* encoding a component of the mitotic exit network (Luca and Winey 1998) or *NDC1* coding for a subunit of the nuclear pore complex that contributes to spindle pole body duplication (Chial *et al.* 1999). In our study, we found that ploidy stability is affected by *WHI3* encoding a regulatory RNA-binding protein and we

Figure 4 Requirement of *WHI3* for maintenance of ploidy under different growth conditions. (A) Scheme for experimental setup. The *MATa* haploid *WHI3* (YHUM468) and the freshly generated *whi3Δ* (YHUM1920) strain were cultivated in liquid or on solid media for up to 72 days by passing cultures to fresh media every second day before DNA contents were analyzed by FACS. Arrows indicate time points of FACS measurements for each culture. (B) DNA content of *WHI3* and *whi3Δ* strains grown in liquid or on solid media (YNB + Trp + Ura or YEPD). Shown are the DNA contents after cultivation for 1 day (black), 6 days (green), and 72 days (red). For the *whi3Δ* culture on solid YEPD medium, the DNA content is shown for day 14 (red), because it already consisted of mostly diploid cells after this time period.

have uncovered two different pathways that could explain the increase-in-ploidy phenotype of haploid *whi3Δ* mutant strains (Figure 8). First, our transcript profiling shows that *Whi3* negatively controls expression of most genes encoding components and regulators of the yeast cohesin complex, which is required for sister chromatid cohesion (Nasmyth and Haering 2009). In addition, we found that a large fraction of cohesin genes regulated by *Whi3* have been associated with binding and regulation by MBF (Lee *et al.* 2002; Harbison *et al.* 2004). Thus, *Whi3* might control adequate expression of cohesin genes during G₁ through *Cdc28-Clb3* and MBF. Moreover, reduction of *Whi3* might cause concerted overproduction of cohesin complex subunits and regulators during G₁ and consequently lead to inefficient separation of sister chromatids during M phase, thereby resulting in an increased WGD rate. This conclusion is supported by the fact that loading of cohesin onto chromatin

occurs before S phase and that cohesin components such as *Smc1/Smc3* fail to establish cohesion when expressed at later stages (Nasmyth and Haering 2009). A second pathway by which *Whi3* appears to control ploidy involves the large subunit of the dynactin complex, *Nip100*, which is involved in partitioning the mitotic spindle between mother and daughter cells (Muhua *et al.* 1994; Yeh *et al.* 1995; Kahana *et al.* 1998). It has also been shown that strains lacking *Nip100* display an enhanced frequency of internal mitosis (Kahana *et al.* 1998). Thus, absence of *Whi3* might result in inefficient distribution of sister chromatids due to a reduction of functional dynactin complex. Here, we have shown that *Whi3* is able to efficiently control *Nip100* expression at the post-transcriptional level, but only under environmental conditions that favor whole-genome duplications. In addition, we found evidence that *Nip100* expression is regulated by environmental conditions independently of *Whi3*.

Figure 5 Effects of *WHI3* and ploidy on *FLO11*-mediated biofilm formation, cell size, and maintenance of ploidy. (A) Formation of adhesive biofilms of yeast strains YHUM468 (*MATa WHI3*), YHUM1920 (*MATa whi3Δ*), YHUM2152 (*MATa/MATa whi3Δ/whi3Δ*), YHUM492 (*MATa/MATa WHI3/WHI3*), and L6438 (*MATa/MATa WHI3/WHI3*) was measured by a washing assay after growth on solid YEPD medium for 5 days. Plates were photographed before (total growth) and after (adhesive growth) washing nonadhesive cells off the surface. FACS analysis of biofilms produced by the haploid *whi3Δ* strain indicated that the population contained <5% diploid cells (data not shown). Expression of *FLO11* was measured in all strains by quantitative transcript analysis after growth in YEPD medium using *ACT1* as an internal standard. Numbers indicate relative values with the haploid *WHI3* strain set to 100%. (B) Influence of *WHI3* and ploidy on cell size was measured by Coulter counter particle analysis and microscopy, using strains YHUM468, YHUM1920, YHUM2152, and YHUM492. Absolute particle volumes are displayed in the graph and relative cell size values were calculated for Coulter counter

Thus, *Whi3*-dependent and environment-controlled pathways seem to exist that control *Nip100* protein levels and provide an explanation for our finding that increased rates of whole-genome duplications depend on both absence of *Whi3* and appropriate growth conditions. How exactly *Nip100* expression is regulated by *Whi3* and the environment remains to be elucidated.

An interesting question with respect to ploidy control arises from our observation that diploid strains that lack *Whi3* do not exhibit an increase-in-ploidy phenotype even under favorable environmental conditions. This is surprising, given the fact that diploid *whi3Δ* mutants do not differ from haploid *whi3Δ* mutant strains with respect to elevated cohesion gene expression or reduced *Nip100* protein levels. One explanation for this observation might be that tetraploid cells do arise from diploid cells with enhanced frequency in the absence of *Whi3*, but that tetraploids do not accumulate in the population due to decreased viability. Indeed, a previous study has shown that a major defect arising from whole-genome duplication in *S. cerevisiae* is a striking decrease of tetraploids to survive during stationary phase, because such cells continue to bud and form mitotic spindles (Andalis *et al.* 2004). An alternative explanation might be that *Whi3*-mediated ploidy control is masked in diploids by other, ploidy-dependent regulatory mechanisms. It has been shown that ploidy regulates a specific set of genes that are involved in cell division or polarization (Galitski *et al.* 1999). Thus, defects in chromosome segregation caused by the absence of *Whi3* might be compensated by ploidy-specific alterations in the expression of genes that are not under control of *Whi3*.

Our study provides new insights into the regulation of adhesion and biofilm formation by *Whi3*. We have previously shown that *Whi3* regulates expression of the adhesin gene *FLO11* by post-transcriptional control of the *Yak1* kinase (Malcher *et al.* 2011). We now found evidence that *Whi3* is also a post-transcriptional regulator of the TEA transcription factor *Tec1* that is well known to activate *FLO11* expression (Rupp *et al.* 1999; Heise *et al.* 2010; Brückner *et al.* 2011). This suggests that *Whi3* is able to control *Flo11*-mediated biofilm formation by at least two independent signaling cascades involving *Yak1* or *Tec1* (Figure 8). Interestingly, *Whi3* is the second RNA-binding protein that has been identified to affect *TEC1* expression at the post-transcriptional level. In contrast to the PUF protein *Mpt5*, however, which negatively affects *TEC1* expression (Prinz *et al.* 2007), the RRM protein *Whi3* is a positive regulator of *Tec1* protein levels. Moreover, both *Mpt5* and *Whi3* bind to the *TEC1* mRNA (Prinz *et al.* 2007; Colomina *et al.* 2008), but

(no parentheses) and microscopic measurements (parentheses) as described in Figure 1A. (C) Ploidy stability of diploid *WHI3/WHI3* (YHUM492) and *whi3Δ/whi3Δ* (YHUM2152) yeast strains was determined by FACS analysis after growth on solid YEPD medium for different time points as described in Figure 4. Shown are the DNA contents after cultivation for days 1 (black), 14 (green), and 72 (red).

Figure 6 Post-transcriptional control of the dynactin complex component Nip100 by *WHI3*. (A) *NIP100-6HA*-tagged yeast strains YHUM2213 (*WHI3*), YHUM2214 (*whi3Δ*), and YHUM2215 (*whi3Δ/whi3Δ*) were cultivated in liquid YNB + Trp + Ura or on solid YEPD medium for 4 days and passaged to fresh media after 2 days. Transcript (top) and protein (bottom) levels were determined by Northern and Western analysis, using *18S rRNA* or tubulin as internal standards. Numbers indicate relative expression levels with the value obtained for the control strain YHUM2213 in liquid YNB set to 100%. (B) Values from A were combined to generate relative protein/mRNA ratios.

Mpt5 acts via the 3'-UTR of the *TEC1* mRNA, whereas *Whi3* appears to target its 5'-UTR. Thus, *Mpt5* and *Whi3* might control translation efficiency of *TEC1* by independent mechanisms and together ensure appropriate production of the transcription factor. We further found that *Whi3* positively controls production of the catalytic PKA subunit *Tpk1* at the post-transcriptional level. This is unexpected, because we have previously shown that *Tpk1* is a negative regulator of *Yak1* and biofilm formation (Malcher *et al.* 2011). Thus, *Whi3* appears to be able to control *Yak1* and *Yak1*-mediated processes both positively and negatively. Interestingly, these connections between *Whi3*, *Tpk1*, and *Yak1* represent an incoherent feed-forward loop type I, which is common for transcriptional circuits and is able to function as a pulse generator (Mangan and Alon 2003; Basu *et al.* 2004). It is therefore possible that *Whi3* controls biofilm formation by

Figure 7 Identification of *Whi3*- and ploidy-regulated genes by transcriptional profiling. (A) Venn diagram showing the number of genes differentially expressed (at least twofold) in yeast strains YHUM468 (*MATa WHI3*) and YHUM1920 (*MATa whi3Δ*) (blue) and in yeast strains YHUM1920 (*MATa whi3Δ*) and YHUM2152 (*MATa/MATa whi3Δ/whi3Δ*) (red). Group-specific and overlapping gene numbers are indicated. The representation factor (RF), which describes the amount of overlap above that due to random sampling, and the probability for this enrichment (P -value) are depicted below. (B) Gene ontology (GO) term and transcription factor (TF) binding enrichment for the 299 genes regulated only by *Whi3* and for the 20 genes regulated by both *Whi3* and ploidy. Gene classes were analyzed using the Ceres Web-based software (Morris *et al.* 2010) and statistically overrepresented (P -value < 0.0001) GO terms and TFs are shown. (C) Subset of genes regulated by *Whi3*, but not by ploidy, which are involved in sister chromatid cohesion and enriched for binding by the transcription factors Mbp1 and Swi6 (blue boxes). Expression of the indicated genes is shown as fold changes, obtained by comparing haploid *WHI3* and *whi3Δ* strains (*Whi3*) or by comparing haploid *whi3Δ* and diploid *whi3Δ/whi3Δ* strains (ploidy). Fold changes of gene expression are shown in colors. (D) Class of 20 genes regulated by both *Whi3* and ploidy. Blue boxes indicate binding by the transcription factors Msn2 and Msn4.

Figure 8 Wiring diagram for Whi3 regulation of cell size, stability of ploidy, biofilm formation, and stress response. Whi3 positively controls post-transcriptional expression of effector proteins (indicated by stars), including Cln1 to affect cell size and Nip100 to control sister chromatid distribution as well as Tec1, Tpk1, and Yak1 to regulate biofilm formation and stress response. In addition, Whi3 affects activity of Cdc28-Cln3 (Wang *et al.* 2004) and MBF to control cell size and sister chromatid cohesion (for further details see text).

generating a pulse of *Yak1* activity in response to certain environmental signals.

Our data also provide new insights into the regulation of stress response by *Whi3*. Previous studies have suggested that *Whi3* affects stress response by targeting *Yak1* and the *Yak1*-regulated stress-responsive transcriptional activators *Msn2* and *Msn4* (Lee *et al.* 2008; Malcher *et al.* 2011). Here, we find that absence of *Whi3* in haploid strains causes increased expression of certain *Msn2/Msn4*-regulated stress genes and that whole-genome duplication of *whi3Δ* haploids is sufficient to revert this effect. This suggests that absence of *Whi3* might induce a stress signal that is relieved upon whole-genome duplication. However, such a stress signal does not appear to be mediated by the *Yak1-Msn2/Msn4* cascade, because this branch is under positive control of *Whi3* and expression of its target genes would thus be expected to decrease in *whi3Δ* strains. This indicates that *Whi3* is also able to negatively control stress-regulated genes via an additional route by activating the expression of a yet unknown repressor (Figure 8). In this scenario, reduced expression of such a repressor caused by the absence of *Whi3* could be balanced by an increased gene dosage upon WGD.

In summary, our data provide evidence that in *S. cerevisiae* the RNA-binding protein *Whi3* regulates not only cell division and biofilm formation, but also stability of ploidy. Moreover, *Whi3* appears to fulfill its diverse functions by controlling the cellular amounts of numerous regulatory

proteins at the post-transcriptional level. Homologs of *Whi3* are present in numerous other fungi, for example the human pathogenic yeasts *Candida glabrata* and *C. albicans* or the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa*. However, no functional studies have been performed with *Whi3* homologs in these organisms and it remains to be determined whether these putative RNA-binding proteins fulfill orthologous functions. It will be especially interesting to see whether *Whi3* homologs are also involved in the regulation of ploidy in these fungi, given the fact that *C. albicans* has been found to perform rapid changes in genome content during mitosis and that variable chromosome organization is thought to be a commonly used mechanism of pathogenic fungi for the adaptation to changing growth conditions (Selmecki *et al.* 2010).

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Literature Cited

- Abascal, F., P. Carmona-Saez, J. M. Carazo, and A. Pascual-Montano, 2008 ChIPCodis: mining complex regulatory systems in yeast by concurrent enrichment analysis of chip-on-chip data. *Bioinformatics* 24: 1208–1209.
- Adoutte-Panvier, A., J. E. Davies, L. R. Gritz, and B. S. Littlewood, 1980 Studies of ribosomal proteins of yeast species and their hybrids: gel electrophoresis and immunochemical cross-reactions. *Mol. Gen. Genet.* 179: 273–282.
- Andalis, A. A., Z. Storchova, C. Styles, T. Galitski, D. Pellman *et al.*, 2004 Defects arising from whole-genome duplications in *Saccharomyces cerevisiae*. *Genetics* 167: 1109–1121.
- Basu, S., R. Mehreja, S. Thiberge, M.-T. Chen, and R. Weiss, 2004 Spatiotemporal control of gene expression with pulse-generating networks. *Proc. Natl. Acad. Sci. USA* 101: 6355–6360.
- Biggins, S., F. F. Severin, N. Bhalla, I. Sassoon, A. A. Hyman *et al.*, 1999 The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev.* 13: 532–544.
- Brückner, S., and H.-U. Mösch, 2012 Choosing the right lifestyle: adhesion and development in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 36: 25–58.
- Brückner, S., S. Kern, R. Birke, I. Saugar, H. D. Ulrich *et al.*, 2011 The TEA transcription factor Tec1 links TOR and MAPK pathways to coordinate yeast development. *Genetics* 189: 479–494.
- Chan, C. S., and D. Botstein, 1993 Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. *Genetics* 135: 677–691.
- Chial, H. J., T. H. Giddings, Jr., E. A. Siewert, M. A. Hoyt, and M. Winey, 1999 Altered dosage of the *Saccharomyces cerevisiae* spindle pole body duplication gene, *NDC1*, leads to aneuploidy and polyploidy. *Proc. Natl. Acad. Sci. USA* 96: 10200–10205.
- Colomina, N., F. Ferrezuelo, H. Wang, M. Aldea, and E. Gari, 2008 Whi3, a developmental regulator of budding yeast, binds a large set of mRNAs functionally related to the endoplasmic reticulum. *J. Biol. Chem.* 283: 28670–28679.
- Colomina, N., F. Ferrezuelo, E. Verges, M. Aldea, and E. Gari, 2009 Whi3 regulates morphogenesis in budding yeast by enhancing Cdk functions in apical growth. *Cell Cycle* 8: 1912–1920.
- Costanzo, M., A. Baryshnikova, J. Bellay, Y. Kim, E. D. Spear *et al.*, 2010 The genetic landscape of a cell. *Science* 327: 425–431.
- Costello, G., L. Rodgers, and D. Beach, 1986 Fission yeast enters the stationary phase G0 state from either mitotic G1 or G2. *Curr. Genet.* 11: 119–125.
- de Bruin, R. A., W. H. McDonald, T. I. Kalashnikova, J. Yates, 3rd, and C. Wittenberg, 2004 Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. *Cell* 117: 887–898.
- Dirick, L., T. Bohm, and K. Nasmyth, 1995 Roles and regulation of Cln-Cdc28 kinases at the start of the cell cycle of *Saccharomyces cerevisiae*. *EMBO J.* 14: 4803–4813.
- Enserink, J. M., and R. D. Kolodner, 2010 An overview of Cdk1-controlled targets and processes. *Cell Div.* 5: 11.
- Fernandes, A. R., N. P. Mira, R. C. Vargas, I. Canelhas, and I. Sa-Correia, 2005 *Saccharomyces cerevisiae* adaptation to weak acids involves the transcription factor Haa1p and Haa1p-regulated genes. *Biochem. Biophys. Res. Commun.* 337: 95–103.
- Ferrezuelo, F., N. Colomina, B. Futcher, and M. Aldea, 2010 The transcriptional network activated by Cln3 cyclin at the G1-to-S transition of the yeast cell cycle. *Genome Biol.* 11: R67.
- Galitski, T., A. J. Saldanha, C. A. Styles, E. S. Lander, and G. R. Fink, 1999 Ploidy regulation of gene expression. *Science* 285: 251–254.
- Gari, E., T. Volpe, H. Wang, C. Gallego, B. Futcher *et al.*, 2001 Whi3 binds the mRNA of the G1 cyclin *CLN3* to modulate cell fate in budding yeast. *Genes Dev.* 15: 2803–2808.
- Gimeno, C. J., and G. R. Fink, 1994 Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Mol. Cell. Biol.* 14: 2100–2112.
- Güldener, U., J. Heinisch, G. J. Koehler, D. Voss, and J. H. Hegemann, 2002 A second set of *loxP* marker cassettes for Cre-mediated multiple gene knockouts in budding yeast. *Nucleic Acids Res.* 30: e23.
- Guo, B., C. A. Styles, Q. Feng, and G. R. Fink, 2000 A *Saccharomyces* gene family involved in invasive growth, cell-cell adhesion, and mating. *Proc. Natl. Acad. Sci. USA* 97: 12158–12163.
- Guthrie, C., and G. R. Fink (Editors), 1991 *Guide to Yeast Genetics and Molecular Biology*. Academic Press, San Diego.
- Hadwiger, J. A., C. Wittenberg, H. E. Richardson, M. de Barros Lopes, and S. I. Reed, 1989 A family of cyclin homologs that control the G1 phase in yeast. *Proc. Natl. Acad. Sci. USA* 86: 6255–6259.
- Harbison, C. T., D. B. Gordon, T. I. Lee, N. J. Rinaldi, K. D. Macisaac *et al.*, 2004 Transcriptional regulatory code of a eukaryotic genome. *Nature* 431: 99–104.
- Heise, B., J. van der Felden, S. Kern, M. Malcher, S. Brückner *et al.*, 2010 The TEA transcription factor Tec1 confers promoter-specific gene regulation by Ste12-dependent and -independent mechanisms. *Eukaryot. Cell* 9: 514–531.
- Janke, C., M. M. Magiera, N. Rathfelder, C. Taxis, S. Reber *et al.*, 2004 A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast* 21: 947–962.
- Jimenez, A., and J. Davies, 1980 Expression of a transposable antibiotic resistance element in *Saccharomyces*. *Nature* 287: 869–871.
- Kahana, J. A., G. Schlenstedt, D. M. Evanchuk, J. R. Geiser, M. A. Hoyt *et al.*, 1998 The yeast dynactin complex is involved in partitioning the mitotic spindle between mother and daughter cells during anaphase B. *Mol. Biol. Cell* 9: 1741–1756.
- Knop, M., K. Siegers, G. Pereira, W. Zachariae, B. Winsor *et al.*, 1999 Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast* 15: 963–972.
- Lasko, P., 2003 Gene regulation at the RNA layer: RNA binding proteins in intercellular signaling networks. *Sci. STKE* 2003: RE6.
- Lee, P., B. R. Cho, H. S. Joo, and J. S. Hahn, 2008 Yeast Yak1 kinase, a bridge between PKA and stress-responsive transcription factors, Hsf1 and Msn2/Msn4. *Mol. Microbiol.* 70: 882–895.
- Lee, T. I., N. J. Rinaldi, F. Robert, D. T. Odom, Z. Bar-Joseph *et al.*, 2002 Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* 298: 799–804.
- Lo, W. S., and A. M. Dranginis, 1998 The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 9: 161–171.
- Luca, F. C., and M. Winey, 1998 *MOB1*, an essential yeast gene required for completion of mitosis and maintenance of ploidy. *Mol. Biol. Cell* 9: 29–46.
- Malcher, M., S. Schladebeck, and H. U. Mösch, 2011 The Yak1 protein kinase lies at the center of a regulatory cascade affecting adhesive growth and stress resistance in *Saccharomyces cerevisiae*. *Genetics* 187: 717–730.
- Mangan, S., and U. Alon, 2003 Structure and function of the feed-forward loop network motif. *Proc. Natl. Acad. Sci. USA* 100: 11980–11985.
- Morris, R. T., T. R. O'Connor, and J. J. Wyrick, 2010 Ceres: software for the integrated analysis of transcription factor binding

- sites and nucleosome positions in *Saccharomyces cerevisiae*. *Bioinformatics* 26: 168–174.
- Mösch, H.-U., and G. R. Fink, 1997 Dissection of filamentous growth by transposon mutagenesis in *Saccharomyces cerevisiae*. *Genetics* 145: 671–684.
- Muhua, L., T. S. Karpova, and J. A. Cooper, 1994 A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration. *Cell* 78: 669–679.
- Nash, R. S., T. Volpe, and B. Futcher, 2001 Isolation and characterization of *WHI3*, a size-control gene of *Saccharomyces cerevisiae*. *Genetics* 157: 1469–1480.
- Nasmyth, K., and C. H. Haering, 2009 Cohesin: its roles and mechanisms. *Annu. Rev. Genet.* 43: 525–558.
- Pan, X., and J. Heitman, 2000 Sok2 regulates yeast pseudohyphal differentiation via a transcription factor cascade that regulates cell-cell adhesion. *Mol. Cell. Biol.* 20: 8364–8372.
- Prinz, S., C. Aldridge, S. A. Ramsey, R. J. Taylor, and T. Galitski, 2007 Control of signaling in a MAP-kinase pathway by an RNA-binding protein. *PLoS ONE* 2: e249.
- Quenault, T., T. Lithgow, and A. Traven, 2011 PUF proteins: repression, activation and mRNA localization. *Trends Cell Biol.* 21: 104–112.
- Reynolds, T. B., and G. R. Fink, 2001 Bakers' yeast, a model for fungal biofilm formation. *Science* 291: 878–881.
- Riezman, H., T. Hase, A. P. van Loon, L. A. Grivell, K. Suda *et al.*, 1983 Import of proteins into mitochondria: a 70 kilodalton outer membrane protein with a large carboxy-terminal deletion is still transported to the outer membrane. *EMBO J.* 2: 2161–2168.
- Roberts, R. L., and G. R. Fink, 1994 Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev.* 8: 2974–2985.
- Ruchaud, S., M. Carmena, and W. C. Earnshaw, 2007 Chromosomal passengers: conducting cell division. *Nat. Rev. Mol. Cell Biol.* 8: 798–812.
- Rupp, S., E. Summers, H. J. Lo, H. Madhani, and G. R. Fink, 1999 MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. *EMBO J.* 18: 1257–1269.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012 NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9: 671–675.
- Selmecki, A., A. Forche, and J. Berman, 2010 Genomic plasticity of the human fungal pathogen *Candida albicans*. *Eukaryot. Cell* 9: 991–1008.
- Stewart, M. S., S. A. Krause, J. McGhie, and J. V. Gray, 2007 Mpt5p, a stress tolerance- and lifespan-promoting PUF protein in *Saccharomyces cerevisiae*, acts upstream of the cell wall integrity pathway. *Eukaryot. Cell* 6: 262–270.
- Sugiura, R., A. Kita, Y. Shimizu, H. Shuntoh, S. O. Sio *et al.*, 2003 Feedback regulation of MAPK signalling by an RNA-binding protein. *Nature* 424: 961–965.
- Wang, H., E. Gari, E. Verges, C. Gallego, and M. Aldea, 2004 Recruitment of Cdc28 by Whi3 restricts nuclear accumulation of the G1 cyclin-Cdk complex to late G1. *EMBO J.* 23: 180–190.
- Ward, M. P., C. J. Gimeno, G. R. Fink, and S. Garrett, 1995 *SOK2* may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. *Mol. Cell. Biol.* 15: 6854–6863.
- Yeh, E., R. V. Skibbens, J. W. Cheng, E. D. Salmon, and K. Bloom, 1995 Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* 130: 687–700.

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GENETICS

Supporting Information

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The RNA-Binding Protein Whi3 Is a Key Regulator of Developmental Signaling and Ploidy in *Saccharomyces cerevisiae*

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Figure S1 Functionality of GFP-tagged developmental regulators in presence and absence of *WHI3*. Yeast strains YHUM2045 (*TPK1-GFP*), YHUM2046 (*TPK1-GFP whi3Δ*), YHUM1755 (*YAK1-GFP*), YHUM1757 (*YAK1-GFP whi3Δ*), YHUM2041 (*PHD1-GFP*), YHUM2042 (*PHD1-GFP whi3Δ*), YHUM1937 (*TPK2-GFP*), YHUM1939 (*TPK2-GFP whi3Δ*), YHUM1886 (*FLO8-GFP*), YHUM1935 (*FLO8-GFP whi3Δ*), YHUM1745 (*TEC1-GFP*), YHUM1746 (*TEC1-GFP whi3Δ*), YHUM1885 (*MPT5-GFP*), YHUM1932 (*MPT5-GFP whi3Δ*), YHUM1884 (*MIG2-GFP*), YHUM1929 (*MIG2-GFP whi3Δ*) carrying full length GFP fusion genes (upper panel), and yeast strains YHUM2047 (*TPK1¹⁻⁹⁹-GFP*), YHUM2048 (*TPK1¹⁻⁹⁹-GFP whi3Δ*), YHUM1760 (*YAK1¹⁻⁹⁹-GFP*), YHUM1762 (*YAK1¹⁻⁹⁹-GFP whi3Δ*), YHUM2043 (*PHD1¹⁻⁹⁹-GFP*), YHUM2044 (*PHD1¹⁻⁹⁹-GFP whi3Δ*), YHUM1938 (*TPK2¹⁻⁹⁹-GFP*), YHUM1940 (*TPK2¹⁻⁹⁹-GFP whi3Δ*), YHUM1934 (*FLO8¹⁻⁹⁹-GFP*), YHUM1936 (*FLO8¹⁻⁹⁹-GFP whi3Δ*), YHUM1747 (*TEC1¹⁻⁹⁹-GFP*), YHUM1748 (*TEC1¹⁻⁹⁹-GFP whi3Δ*), YHUM1931 (*MPT5¹⁻⁹⁹-GFP*), YHUM1933 (*MPT5¹⁻⁹⁹-GFP whi3Δ*), YHUM1928 (*MIG2¹⁻⁹⁹-GFP*), YHUM1930 (*MIG2¹⁻⁹⁹-GFP whi3Δ*) with corresponding truncated versions (lower panel) were assayed for adhesion on agar surfaces. Strains were patched on solid YPD medium and incubated for 5 days. Plates were photographed before (total growth) and after (adhesive growth) washing non-adhesive cells off the surface.

Figure S2 Posttranscriptional control of developmental regulators by *WHI3*. (A) Effect of *WHI3* deletion on expression of full length versions of *TPK1*, *YAK1*, *PHD1*, *TPK2*, *FLO8*, *TEC1*, *MPT5*, and *MIG2* was measured using the yeast strains described in Figure S1 by transcript (upper panel) and protein (lower panel) analysis in minimum 3 independent measurements as described in Figure 1B. Numbers indicate relative transcript or protein levels using *ACT1* transcript or *Cdc28* protein levels as internal standards. Values for respective *WHI3* strains were set to 100%. (B) Influence of *WHI3* on expression of truncated versions of *TPK1*, *YAK1*, *PHD1*, *TPK2*, *FLO8*, *TEC1*, *MPT5*, and *MIG2* was analyzed in yeast strains described in Figure S1 by transcript (upper panel) and protein (lower panel) analysis.

Figure S3 Analysis of ploidy of *WHI3* and *whi3Δ* strains carrying genomic *MIG2-GFP* fusions. (A) Detection of *MIG2* and *MIG2-GFP* alleles by PCR analysis. *MIG2* was chromosomally tagged with GFP in haploid yeast strain YHUM468 (*WHI3*) and YHUM1920 (*whi3Δ*) after approximately 25 generations on solid SC medium. At least 10 independent resulting clones together with the parental strains were analyzed for the presence of *MIG2* or *MIG2-GFP* using PCR. All clones derived from YHUM468 (*WHI3*) produced the same pattern represented by yeast strain YHUM1884 (*WHI3 MIG2-GFP*), whereas clones derived from YHUM1920 (*whi3Δ*) produced two distinct patterns represented by YHUM1929 (*whi3Δ MIG2-GFP*) or YHUM2151 (*whi3Δ/whi3Δ MIG2/MIG2-GFP*). (B) Ploidy of yeast strains YHUM492 (*WHI3/WHI3*), YHUM1920 (*whi3Δ*), and YHUM2151 (*whi3Δ/whi3Δ MIG2/MIG2-GFP*) was determined by FACS analysis as described in Figure 4B.

Figure S4 Analysis of ploidy of *whi3Δ* mutant strain YHUM2152 by genetic crosses. Yeast strain YHUM2152 was mated with haploid strain L6441 (cross 1) or diploid strain L6442 (cross 2), respectively, and resulting strains were subjected to sporulation. For each cross, 20 tetrads were analyzed for spore viability on rich medium. The clear difference in the number of viable spores obtained for the two crosses indicates that cross 1 resulted in a triploid and cross 2 in a tetraploid. This demonstrates that YHUM2152 is a diploid, a conclusion that is supported by the distribution of *TRP1*, *HIS3*, and *LEU2* marker genes within the 4 spore tetrads of cross 2.

Table S1 DNA primers used in this study

Primer	Sequence 5' to 3'
Act1_920	CAGGTATTGCCGAAAGAATGC
Act1_983	CTTCTGGAGGAGCAATGATC
Act1_MM1	GCTGCTTTGGTTATTGATAACGG
Act1_MM2	CAC TTGTGGTGAACGATAGATGG
Cln1tag1 (S3)	TAATAGAGCGTCTTCGATATCGTATCGTTAAGGAACCACAATAGCTCTCAACTGCGTACGCTGCAGGTCGAC
Cln1tag2 (S3b)	GCAGACATATTACCAATTGAATTGTCCAATGCAGAACTACTAACTCATTACGAACGTACGCTGCAGGTCGAC
Cln1tag3 (S2)	CGTAGTATTCCGTTATTAATTAAGTATATATGTAGGCTTGATGAGAAAATGGTCAATCGATGAATTCGAGCTCG
Cln2tag1 (S3)	ATCTAGCAGTGCCTCATCTTTAATTTCTTTTGGTATGGGCAATACCCAAGTAATACGTACGCTGCAGGTCGAC
Cln2tag2 (S3b)	TGCTAAACCGGACTACTATCCGATTGAGCTATCTAATGCAGAATTACTTTCTACCGTACGCTGCAGGTCGAC
Cln2tag3 (S2)	TTTTTGGTACGTTTGGCAAATGGCATTCTTTATCATGAAAAGAACAGGAACTAATCGATGAATTCGAGCTCG
Ddr2_78	CAACACCACGAGTAACGCTG
Ddr2_152	CAAAGCACCACCAACAGCAG
Flo11_MM1	TTGTCTCAACCACGTCGTTA
Flo11_MM2	AACTGGAAGAGCGAGTAGCAA
Flo8tag1 (S3)	TCTCAATTTACTGGATACAAATGAAAATGATTTCAATTTTATTAATTGGGAAGGCCGTACGCTGCAGGTCGAC
Flo8tag2 (S3b)	CACGGAACAACCGTACATGGCAAGCCAGTATAACAAGATTTGCAGAGTAATATTCGTACGCTGCAGGTCGAC
Flo8tag3 (S2)	TTTCTGTCTTAAGAGTTTTTATTTTTATTATAATACTCAACACGTGACTTCAATCGATGAATTCGAGCTCG
Hsp26_480	GTCATCACTTTGCCGACTAC
Hsp26_577	GGTTCCTACCATCCTTCTGAG
Irr1_3023	CGGAAACCGGTTTTTCGAG
Irr1_3121	GTTTAGTGCCACACGGGAAG
Mig2-52bp	ACGCTAAGACTATTGACCTCG
Mig2+1182bp	TTCATGACAGTTCGTTAGAGG
Mig2tag1 (S3)	GCCTGTACGTAACCTACTGAAACAAATTGATGTTTTCAACGGTCCCAAAAGAGTTCGTACGCTGCAGGTCGAC
Mig2tag2 (S3b)	ACCTTTTAGATGTGATACCTGTACCGTGGTTCCATCGGTTAGAACATAAAAAAGCGTACGCTGCAGGTCGAC
Mig2tag3 (S2)	TCATGACAGTTCGTTAGAGGAAAAATGGTGAGATAAAAAGGGGCCGTAAAGGTTAATCGATGAATTCGAGCTCG
Mpt5tag1 (S3)	TATGCCTGCCATGAATACCGCTAGAACATCTGATGAACTTCAATTCAC TTTGCCACGTACGCTGCAGGTCGAC
Mpt5tag2 (S3b)	TGGAGCATAAGCTTTAACGGGATCTTCTAACACAAATAGCATAATAACCAAAAACGTACGCTGCAGGTCGAC
Mpt5tag3 (S2)	GCGTAAATATTTGTACAGTAAGAAGGAAAGAAAAAGAAAGAAAAAAGTATTAATCGATGAATTCGAGCTCG
Nip100_S2	ATTTATTTACTGTATTTGAATGTTATAGACCTGCTAGAATCACTCAGTCAATCGATGAATTCGAGCTCG
Nip100_S3	AGAGGAAAAATATACGACTCAAATCTCAAATGTTGTTACATATTACAAACGTACGCTGCAGGTCGAC
Phd1_S2 (S2)	GAACTTCGGTTCTTGTTTCATAGAGCAAAGAGTTAACGGATTATGTTATGTGCTTAATCGATGAATTCGAGCTCG
Phd1_S3 (S3)	AATCAACCATATCGATACTGAGGCCCAAACAAGTAGAGCAAAAAATGAATTATCACGTACGCTGCAGGTCGAC
Phd1_S3b (S3b)	CACTCAATCTAACGCCGAATAACCCACCAGAAGTTACGACAATACCCTTCCTCGTACGCTGCAGGTCGAC
Smc1_3497	CTAACGTCCAGAGAATTGCTG
Smc1_3619	CGACGAGTTTTCTTGTTGCTG
Tec1-S2 (S2)	TATGCGTATTTATGTACGAGATGTATGTATGTATGTAGACATTTAATCGATGAATTCGAGCTCG
Tec1-S3 (S3)	CAAAGGAACTTTACGCCATCCAACCAATCGCATGGGAAC TTTTATCGTACGCTGCAGGTCGAC

Tec1tag2 (S3b)	TATAGAATCATATACTGGTAGAATTTTTGACGTATATATACAAAAAGATTTCGTATCGTACGCTGCAGGTCGAC
Tpk1_S2 (S2)	ATATGAAAAAAAAAATATAGATACGAGAGGAAAAATACAACAAACATTAGTCATTAATCGATGAATTCGAG CTCG
Tpk1_S3 (S3)	CATCAACTACGGTGTTCAAGGTGAAGACCCATATGCTGATCTTTCCGGGACTTCCGTACGCTGCAGGTCGAC
Tpk1_S3b (S3b)	TGATAGACAAGGTGAGGAATCACAAAAAGGTGAGACTAGTGAAAGGGAAACAACACGTACGCTGCAGGTCG AC
Tpk2gTagfw (S3)	GGTATTCAAGGCGATGATCCATATGCTGAATACTTTCAAGATTTCCGTACGCTGCAGGTCGAC
Tpk2gTagrv (S2)	AGAAAGTACTTGAAAATTGTTTTGTGTTTTTGGTTCATGGAACATCGATGAATTCGAGCTCG
Tpk2tag2 (S3b)	CCAGCAGCAAAATGTTAATACTTACGGGCAAGGCGTCCTACAACCGCATCATGATCGTACGCTGCAGGTCGAC
Whi3_Kofw	CTTTATCGATCAATATTTTCAGAGGGAAAAACCTGTATCTCTTAGCCAGCTGAAGCTTCGTACGC
Whi3_Korev	AATGTGATACATGCAAGGAAATCAGGTTTTTGC GGAACCATTTTTGCATAGGCCACTAGTGGATCTG
Yak1tag1 (S3)	ATTCTGTTCAAGAAGGGCCAACAAGCGCGTTCAATAAACTTCACATTGTCTGAAGAACGTACGCTGCAGGTCGA C
Yak1tag2 (S2)	AAGCCGTAATAAAATGAAATGGGTAGTTAAGAAAAGTCAGACAAAATAACGATTTAATCGATGAATTCGAGCT CG
Yak1tag3 (S3b)	ACATGAATAACTCCTTGAGCCCGACCCTTGAGCCACAGTGATGCTAGTATGGGCCGTACGCTGCAGGTCGAC

Table S2 Expression of selected Whi3 target genes¹

Gene	<i>whi3Δ</i> vs <i>WHI3</i>²	<i>whi3Δ/whi3Δ</i> vs <i>whi3Δ</i>³
<i>DDR2</i>	3,1 (4,2)	-2,5 (-3,3)
<i>HSP26</i>	5,3 (8,2)	-4,7 (-7)
<i>IRR1</i>	1,9 (2,5)	-1,3 (-1,1)
<i>SMC1</i>	1,7 (2,2)	-1,2 (-1,2)

¹Expression was measured by quantitative real time PCR and compared to values obtained by microarray array analysis shown in brackets

²Fold changes in gene expression obtained by comparison of yeast strains YHUM1920 (*MATa whi3Δ*) and YHUM468 (*MATa WHI3*)

³Fold changes in gene expression obtained by comparison of yeast strains YHUM2152 (*MATa/MATa whi3Δ/whi3Δ*) and YHUM1920 (*MATa whi3Δ*)