Supporting Information

Park et al. 10.1073/pnas.1105262108

SI Materials and Methods

Yeast Mating Assays to Monitor Silencing. *MAT*α yeast cells of the relevant genotypes were grown in either YPD, Casamino acid, or synthetic medium (to select for plasmids, as appropriate) and maintained in log phase for at least 24 h. At A600 of ~1.0 they were incubated with an excess of *MAT*a cells and plated on selective agar medium that allowed only diploids to grow. Tenfold serial dilutions of mating mixtures were plated to obtain quantitative data about mating efficiencies. In these experiments, reduced mating is equivalent to reduced silencing.

Quantitative PCR to Measure Levels of a1 mRNA. To determine the levels of a1 mRNA transcribed from *HMR-SS*a, total RNA was prepared from cells grown to an OD of 0.6 to 0.8 using standard hot phenol extraction methods. Total RNA concentration was determined by spectroscopic analysis. For reverse transcription (RT), 5 μg of total RNA was used in a 20-μL reaction mixture using oligo$(dT)_{12–18}$ primers (Invitrogen) and SuperScript III (Invitrogen) following the manufacturer's protocol. cDNA was purified from each RT mixture using QIAquick PCR purification kit (Qiagen) and eluted in 50-μL TE. Twofold serial dilutions were used in 25-cycle PCR reactions as follows: for measuring *ACT1* expression, 1 and 2 μL of 1:10 diluted cDNA were used with *ACT1*-specific primers or for a1 mRNA, with a1-specific primers (Table S2). The PCR products were resolved on a 1% agarose gel stained with GelRed dye (Biotium). The bands were quantified using video densitometry analysis and Labworks analysis software (UVP Inc.).

Acyl-Biotin Exchange Assay to Monitor S-Acylation. Cells were harvested in log-phase in liquid YPD. Biotin switch assay uses a modified version of acyl-biotinyl exchange (ABE) chemistry (1). ABE substitution of biotin for palmitoyl modification that we used involves three steps (2) with some modification: (*i*) proteins are denatured by 6 M urea and free cysteines are blocked using *N*-ethyl maleimide (Fluka), (*ii*) palmitoyl groups are cleaved using hydroxylamine (Sigma), and (*iii*) the liberated cysteines are tagged with biotin. Biotinylated proteins are subsequently precipitated with neutravidin coupled to agarose beads (Pierce). Gnp1 has been previously shown to be palmitoylated and thus serves as a positive control (3).

Measuring Rif19xMyc Solubility. Whole-cell extracts were made from cells growing in log-phase, harvested at A600 = OD. 1, and fractionated as described (4).

ChIP Experiments. ChIPs were performed using standard procedures and as described (5, 6) using anti-Sir3 antibodies to monitor Sir3 binding or anti-Myc antibodies (to monitor Rif1-9xMyc binding). *HMR* was detected using primers described previously (7). Telomeric loci were detected with primers (Table S2). Signals were normalized to the *ADH4* locus (7).

Telomere-Length Measurements. DNA was prepared from cells in log-phase and telomere-length measurements were performed exactly as described (8).

Cytology and Cell Imaging. Cells were grown in liquid YPD media overnight and subcultured the following morning to 0.1 to 0.2 OD_{600}. Subcultured cells were grown additional 5 h at 30 °C and washed with water. Cells containing Rif1-GFP and HDEL-DsRED were visualized by serial sectioning and confocal microscopy microscopy (Nikon Ti-E, equipped with a Roper CoolSnap HQ2 CCD camera). Confocal sections were acquired at 300-nm intervals through the nucleus. Foci were counted manually in images of live cells.

To visualize Sir3-GFP and telomere VIR positioning in the nucleus of live cells, the following procedures were followed.

Cell growth and imaging. Freshly streaked cells were grown in well-aerated liquid YPDA media overnight, subcultured, and regrown to 0.2 to 0.3 OD_{600}. Cells were washed with water before mounting on slides containing 1.4% agarose plugs. Fields of about 15 to 50 cells were imaged with a Zeiss Axioplan II fluorescence microscope (100× Plan-Apochromat objective, NA = 1.4) and Axiocam HR camera. *Z*-stacks were composed of 17 elevations, each separated by 250 nm and an acquisition time of 250 ms. The cell-cycle stage was categorized morphologically: G1, unbudded cells; G2, large budded cells with nuclei that are distorted or adjacent to the bud neck; S, small-to-medium budded cells that retain a spherical nucleus that has not yet moved to the bud neck.

Foci counting. Fluorescent spots per nucleus were counted manually in images of live cells. Datasets for each strain consist of three to four trials of about 50 to 150 cells, which were pooled after the average values of spots per nucleus were found to lie within the same 95% confidence interval. The pooled data for each mutant strain were evaluated relative to wild-type by the Student *t* test. *P* values are noted in the figure legends.

Measurement of foci intensity. Nuclei with bright GFP foci were selected manually for more rigorous analysis. Zeiss Axiovision software was used to obtain a unitless intensity value I = (Sf – Bf)/Bf, where Sf equals signal intensity of an area encompassing the bright spot and Bf equals the signal intensity of a comparable nuclear area lacking a fluorescent spot. An arbitrary cutoff of I > 1.1 was chosen to define an SBF (single bright focus). By this criterion, roughly half of the spots selected manually for analysis were eventually classified numerically as SBFs. Three to four independent trials satisfied χ^2 tests of homogeneity of proportions and were pooled. Error bars represent the SE of proportion. All values for mutants were found to be significantly different from wild-type by χ^2 tests.

Perinuclear position of TelVIR. GFP positions were determined according to the method of Hediger et al. (9), as described briefly here. In the focal plane with the brightest GFP fluorescence, the shortest distance from the spot to the nuclear membrane (marked with Nup49-GFP) was measured relative to the diameter of the nucleus in that plane using the Zeiss Axiovision software package. Cells with GFP spots at the top or bottom of the nucleus were ignored. The values were categorized as occupying one of three concentric zones of the nucleus, each corresponding to an equal surface area. Zone 1 represents the nuclear volume immediately adjacent to the nuclear envelope. For statistical analyses, zones II and III were combined. G1 and early to mid S-phase cells were used exclusively. Datasets for each strain consist of four to five independent trials of about 20 to 50 G1 cells each, which were pooled because they satisfied χ^2 tests of homogeneity of proportions. The pooled data for each strain were evaluated relative to the random scenario and to wild-type by χ^2 analyses. Significance was determined with a 95% confidence interval.

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8. Askree SH, et al. (2004) A genome-wide screen for *Saccharomyces cerevisiae* deletion mutants that affect telomere length. *Proc Natl Acad Sci USA* 101:8658–8663.
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Fig. S1. (*A*) Protein immunoblots with anti-HA antibody (Covance) showing expression of *PFA4-HA* and *pfa4(C108A)-HA* from CEN plasmids (1) and *pfa4 (C108A)-HA* from a 2-μm plasmid. (*B* and *C*) Quantitative mating assays were performed with *MATα HMRa sir1*Δ *pfa4*Δ cells transformed with the indicated plasmids. *MATα HMRa sir1*Δ cells were transformed with empty vector for a control. The cells were grown under conditions that selected for plasmids.

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Fig. S2. (*A*) Outline of the ABE protocol (1) used to identify S-acylation of cysteines on proteins. (*B*) ABE analysis of Rif1-9xMyc and Gnp1-3xHA, Orc1 and Fkh1 in wild type (*PFA4*) and *pfa4*Δ mutant cells. The same extracts were examined for all four proteins by protein immublotting with α-Myc, α -HA, α-Orc1 (2), or α –Fkh1 (3).

1. Drisdel RC, Green WN (2004) Labeling and quantifying sites of protein palmitoylation. *Biotechniques* 36:276–285.
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Fig. S3. (*A*) Anti-GFP protein immunoblot was performed to detect levels of Rif1-GFP in cells with the indicated genotypes. (*B*) Images were acquired on a swept field confocal microscope (Nikon Ti-E) equipped with a Roper CoolSnap HQ2 CCD camera using a Nikon 60×, 1.4NA Planapo oil objective lens, and a 1.5× Optivar auxillary magnifier. Rif1-GFP was detected in the indicated cells. (*C*) Quantification of GFP foci in cells present in *B*. (*D*) Anti-GFP protein immunoblot was performed to detect levels of Sir3-GFP in cells with the indicated genotypes.

Table S1. Yeast strains used in this study

Strain	Genotype	Reference
JRY2334	*MAT*a *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 (W303-1A*)	Thomas and Rothstein, 1989 (1)
JRY3009	MATα ade2-1 his3-11, 15 leu2-3, 112, trp1-1, ura3-1, can1-100 (W303-1B)	
JRY4012	*JRY2334,* ADE2 lys2∆	Herman and Rine, 1997 (2)
CFY774	JRY2334, TEL VII-L*::TRP1::URA3 ura3∆::LEU2*	Gottschling et al., 1990 (3)
CFY775	JRY2334, TEL VII-L*::TRP1::URA3 ura3∆::LEU2 sir2∆::TRP1*	
CFY1649	JRY3009, *HMR-SS*a *sir1∆::TRP1*	
CFY1781	JRY3009, *HMR-SS*a *sir1∆::LEU2 sir3∆::HIS3*	
CFY2374	JRY3009, *HMR-SS*a *sir1∆::TRP1 pfa4∆::KanMX4*	
CFY2670	JRY3009, *sir1∆::TRP1*	
CFY2887	JRY3009, *sir1∆::LEU2 rif1∆::KanMX4*	
CFY2888	JRY3009, *sir1∆::TRP1 pfa4∆::KanMX4*	
CFY2890	JRY3009, *sir1∆::LEU2 rif1∆::KanMX4 pfa4∆::KanMX4*	
CFY2891	JRY3009, *sir1∆::LEU2 rif1∆::KanMX4 rif2∆::KanMX4*	
CFY2892	JRY3009, *sir1∆::TRP1 rif2∆::KanMX4*	
CFY2893	JRY2334, *SIR3::GFP::URA3 ADE2 lys2∆*	Hoppe et al., 2002 (4)
CFY2896	JRY3009, *RIF1::9XMYC::TRP1*	Mishra and Shore 1999 (5)
CFY2897	JRY3009, *RIF1::9XMYC::TRP1 pfa4∆::KanMX4*	
CFY2899	JRY2334, TEL VII-L*::TRP1::URA3 ura3∆::LEU2 pfa4∆::KanMX4*	
CFY3006	JRY2334, *SIR3::GFP::URA3 rif1∆::KanMX4 pfa4∆::KanMX4 ADE2 lys2∆*	
CFY3011	JRY2334, *SIR3::GFP::URA3 pfa4∆::KanMX4 ADE2 lys2∆*	
CFY3013	JRY2334, *SIR3::GFP::URA3 rif1∆::KanMX4 ADE2 lys2∆*	
CFY3048	JRY3009, *RIF1:: 9XMYC::TRP1* TEL VII-L*::TRP1::URA3 ura3∆::LEU2*	
CFY3049	JRY3009, *RIF1:: 9XMYC::TRP1 pfa4∆::KanMX4* TEL VII-L*::TRP1::URA3 ura3∆::LEU2*	
CFY3066	JRY2334 *RIF1:: 9XMYC::TRP1 sir1∆::LEU2*	
CFY3068	JRY2334, *RIF1:: 9XMYC::TRP1 sir1∆::LEU2 pfa4∆::KanMX4*	
CFY3072	JRY2334, *rif1∆::KanMX4 sir1∆::LEU2 pfa4∆::KanMX4*	
CFY3221	JRY4012, *RIF1::*GFP*::URA3*	
CFY3296	JRY4012, *GNP1::3XHA::KanMX4*	
CFY3297	JRY4012, *rif1∆::KanMX4 sir1∆::LEU2*	
CFY3310	JRY4012 *RIF1::*GFP*::cURA3 pfa4∆::KanMX4*	
CFY3312	JRY4012 *pfa4∆::KanMX4*	
CFY3346	JRY2234 *ADE2-1 rif1∆::KanMX4*	
CFY3347	JRY3009 *ADE2-1 rif2∆::KanMX4*	
CFY3348	JRY2234 *ADE2-1 rif2∆::KanMX4 pfa4∆::KanMX4*	
CFY3351	JRY2234 *ADE2-1 rif1∆::KanMX4 rif2∆::KanMX4*	
CFY3353	JRY2234 *ADE2-1 rif1∆::KanMX4 pfa4∆::KanMX4*	
CFY3400	JRY2234 TEL VII-L*::TRP1::URA3 ura3∆::LEU2*	
CFY3401	JRY2234 TEL VII-L*::TRP1::URA3 ura3∆::LEU2 rif1∆::KanMX4*	
CFY3403	JRY2234 TEL VII-L*::TRP1::URA3 ura3∆::LEU2 pfa4∆::KanMX4*	
CFY3406	JRY2234 TEL VII-L*::TRP1::URA3 ura3∆::LEU2 rif1∆::KanMX4 pfa4∆::KanMX4*	

All yeast strains were congenic to W303 and were generated by standard yeast genetic and molecular techniques.

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Table S2. Primers used in this study

Purpose	Primers	
	Forward	Reverse
ACT1 expression	OCF2006 caagaaatgcaaaccgctgc	OCF2007 ggtcaataccggcagattcc
a1 expression	OCF2002 ggcggaaaacataaacagaactctg	OCF2003 ccgactatgctattttaatcattgaaaacg
ChIP primers for *TELVII-L*	OCF363 gtgctgctactcatcctagt	OCF369 gttcaccctctaccttagcatc
ChIP primers for *TELIII-R*	OCF3204 tcaaagtgtttatgtatt	OCF3205 ccaccacatgccatactcac
ChIP primers for *TELVI-R*	OCF2436 ctgtgcatccactcgttaggatca	OCF2437 atatgcactagtgcactaggcgc
A telomeric probe (1)	OCF2863 TGTGGGTGTGGTGTGTGGGTGTGGTG	

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