Supplementary material

Sif2p interacts with the Sir4p amino-terminal domain and antagonizes telomeric silencing in yeast

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Figure S1

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  1 MSITSEELNYLIWRYCQEMGHEVSALALQDETRVLEFDEKYKEHIPLGTL
 51 VNLVQRGILYTESELMVDSKGDISALNEHHLSEDFNLVQALQIDKEKFPE
101 ISSEGRFTLETNSESNKAGEDGASTVERETQEDDTNSIDSSDDLDGFVKI
151 LKEIVKLDNIVSSTWNPLDESILAYGEKNSVARLARIVETDQEGKKYWKL
201 TIIAELRHPFALSASSGKTTNQVTCLAWSHDGNSIVTGVENGELRLWNKT
251 GALLNVLNFHRAPIVSVKWNKDGTHIISMDVENVTILWNVISGTVMQHFE
301 LKETGGSSINAENHSGDGSLGVDVEWVDDDKFVIPGPKGAIFVYQITEKT
351 PTGKLIGHHGPISVLEFNDTNKLLLSASDDGTLRIWHGGNGNSQNSFYGH
401 SQSIVSASWVGDDKVISCSMDGSVRLWSLKQNTLLALSIVDGVPIFAGRI
451 SQDGQKYAVAFMDGQVNVYDLKKLNSKSRSLYGNRDGILNPLPIPLYASY
501 QSSQDNDYIFDLSWNCAGNKISVAYSLQEGSVVAI
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Deduced amino-acid sequence of Sif2p. Sif2p corresponds to the hypothetical 59.1 kDa protein encoded by locus *YBR103W* on *S. cerevisiae* chromosome II (EMBL accession number Z35972). The positions of four WD40 repeats [3] are indicated by shaded boxes, with the initial amino acid indicated by a triangle. Sif2p is not closely related to the Wtn family of WD40 proteins implicated in Gal11p-mediated gene repression [S1], nor with the WD40-repeat-containing subunit of the chromatin assembly complex of yeast [S2]. A putative bipartite nuclear localization signal is underlined. The black dot indicates the 3′ boundary of the fragment recovered in the two-hybrid screen for Sir4N interaction. This fragment contains two of the four WD40 repeats and confers derepression of TPE upon overexpression.

Materials and methods

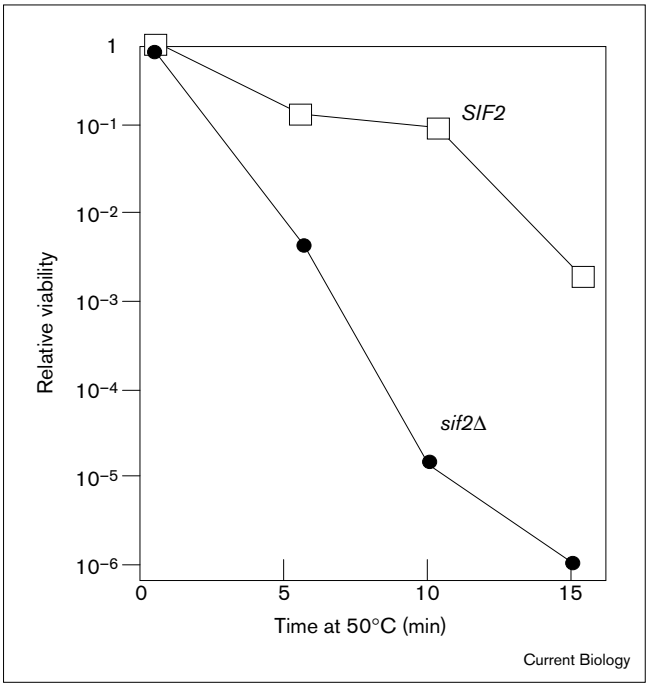
Two hybrid screen

Plasmid pEG202-SIR4N, encoding a fusion of amino acids 9–271 of Sir4p to the LexA DNA-binding domain was used to screen a yeast two-hybrid library in the strain EGY48 for Sir4N ligands using protocols described in Golemis *et al.* [S3]. Primary transformants ($2–3 \times 10^5$) of the pJG45 library were harvested and stored at −70°C. Approximately 2×10^6 of the stored transformants were screened for activation of integrated *LEU2* and plasmid-borne *lacZ* reporters and 20 independent colonies that appeared positive for galactose-dependent activation of both reporters were obtained. Restriction analysis and sequencing showed them to be multiple isolates of four different clones. Three of the four different clones interacted with only the Sir4N fusion protein when tested with a variety of other baits.

Yeast media and strain construction

The genotypes of yeast strains used in this study are described in Table S1. Standard culture media were used [S3]. A PCR-based gene disruption technique was employed to delete *SIF2* in strains GA492, GA542 and GA543 as described by Wach *et al.* [S4]. *SIF2* disruption was verified by PCR on whole cells.

Figure S2



The effect of *sif2*::*kan*MX2 in the same heat-shock assay as in Figure 4 performed in strain background that is *YGL023*. *(SIF2* is strain UCC3505 and *sif2* is strain MC83).

Plasmids and PCR primers

A 1.6 kb *Hind*III-flanked fragment containing the full-length *SIF2* gene was obtained by PCR of genomic DNA. The fragment was cloned into pRD54 to create an HA-epitope tagged *SIF2* gene under control of the *GAL1-10* promoter. The expression of fusion proteins of the appropriate predicted sizes from all pJG45-derived and pRD54-derived plasmids was assessed by western blots with an anti-HA monoclonal antibody (12CA5) on whole cell extracts prepared from yeast grown under inducing and non-inducing conditions, as described in the Figure legends.

Repression, heat shock and aging assays

Repression of *URA3* at the VIIL telomere in strains GA492 and MC118 was monitored by measuring the frequencies of 5-FOA-resistant cells as previously described [4]. Individual colonies from strains carrying the *ade2*-1 mutation and an intact *ADE2* gene integrated close to the telomere on the right arm of chromosome V (strains GA492 and MC118) or the left arm of chromosome VII (strains GA542, GA543, MC184 and MC248) [11], were streaked onto media containing 5 μg/ml adenine. Colonies were grown for several days at 30°C and then stored at 4°C for up to one week to allow pigment accumulation.

Cells grown at 30°C in synthetic complete medium to stationary phase, were transferred to 50°C. Samples were taken at various time intervals

(0, 5, 10, 15 and 30 min) and serially diluted into sterile water. The total number of cells was counted under a hemacytometer and various dilutions were plated onto synthetic medium and incubated at 30°C. A viability of 100% was calculated as the number of colonies grown when not exposed to heat shock. Repeated assays showed that the lethality curve of the *sif2 uth4* double mutant was not significantly different from that of the *uth4* and *sif2* single mutants, although the kinetics of death varied somewhat among assays. An identical protocol was followed for monitoring survival after 7 days growth in rich media. This assay provides a rough measure of lifespan in yeast, since it reflects the population of dead cells in a culture in which a high proportion of cells have divided many times. More accurate lifespan determination was performed as described [11].

Immunofluorescence and antibodies

Immunofluorescence was performed as described [7], using affinity purified rabbit antibodies to the Sir4C-terminus, Sir3p and Rap1, and the anti-HA monoclonal antibody 12AC5. Secondary antibodies coupled to the fluorochromes DTAF and CY3, were visualised on a Zeiss Axiovert 100 microscope (Zeiss Laser Scanning Microscope 410) with a 63× or 100× Plan-Apochromat objective (1.4 oil). Under standard imaging conditions no signal from one fluorochrome could be detected on the other filter set. Standardized conditions for the image capture and subtraction of a background value taken from outside the yeast cells (about 15% of the maximum signal) were carried out uniformly on all images.

References

S1. Pemberton LF, Blobel G: **Characterisation of the Wtm proteins, a novel family of *Saccharomyces cerevisiae* transcriptional modulators with roles in meiotic regulation and silencing.** *Mol Cell Biol* 1997, **17:**4830-4841.

S2. Kaufman PD, Kobayashi R, Stillman B: **Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cells lacking chromatin assembly factor-I.** *Genes Dev* 1996, **11:**345-357.

S3. Golemis EA, Gyuris J, Brent R: **Interaction trap/ two hybrid system to identify interacting proteins.** In *Current Protocols in Molecular Biology*. Edited by Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. John Wiley Sons Inc; 1996; 20.1.1-20.1.23.

S4. Wach A, Brachat A, Pohlmann R, Philippsen P: **New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*.** *Yeast* 1994, **10:**1793-1808.

S5. Stone EM, Pillus L: **Activation of an MAP kinase cascade leads to Sir3p hyperphosphorylation and strengthens transcriptional silencing.** *J Cell Biol* 1996, **135:**571-583.

Table S1

Yeast strains.

GA492	*(MATα his3 leu2 trp1 ade2-1 ura3-52 adh4::URA3-TEL VII-L lys2::LYS2-dam+ TEL V-R::ADE2)*
MC118	*(MATα his3 leu2 trp1 ade2-1 ura3-52 adh4::URA3-TEL VII-L lys2::LYS2-dam+ TEL V-R::ADE2 sif2::kanMX2)*
PSY316AT	*(MATα ade2-101 his3Δ200 leu2-3,2-112 lys2-801 ura3-52 TEL VII-L::ADE2)*
GA542	*(MATα ade2-101 his3Δ200 leu2-3,2-112 lys2-801 ura3-52 TEL VII-L::ADE2 ygl023::hisG-URA3-hisG uth4::LEU2);* [10]
GA543	*(MATα ade2-101 his3Δ200 leu2-3,2-112 lys2-801 ura3-52 TEL VII-L::ADE2 ygl023::hisG-URA3-hisG);* [10]
MC184	*(MATα ade2-101 his3Δ200 leu2-3,2-112 lys2-801 ura3-52 TEL VII-L::ADE2 ygl023::hisG-URA3-hisG uth4::LEU2 sif2::kanMX2)*
MC248	*(MATα ade2-101 his3Δ200 leu2-3,2-112 lys2-801 ura3-52 TEL VII-L::ADE2 ygl023::hisG-URA3-hisG sif2::kanMX2)*
EGY48	*(MATα his3 trp1 ura3-52 leu2::pLEU2-lexAop6);* [S3]
GA24	*(MATa ura3 GAL⁺ his3 bar1 suc2Δ9 pep4-3)*
UCC3107	*(MATa ade2::hisG can1::hisG his3-11 leu2 trp1Δ ura3-52 TEL V-R::ADE2);* [S5]
UCC3505	*(MATa ura3-52 lys2-801 ade2-101 trp1-63, his3Δ200 leu2-1 ppr1::HIS3 adh4::URA3-TEL VII-L; TEL V-R::ADE2)*
MC83	*(MATa ura3-52 lys2-801 ade2-101 trp1-63, his3Δ200 leu2-1 ppr1::HIS3 adh4::URA3-TEL VII-L; TEL V-R::ADE2 sif2::kanMX2)*