View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

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

Moira Cockell, Hubert Renauld*, Paul Watt† and Susan M. Gasser

Several regions of the *Saccharomyces cerevisiae* genome are subject to position-dependent transcriptional repression mediated by a multi-component nucleosome-binding complex of silent information regulator proteins (Sir2p, Sir3p and Sir4p). These proteins are present in limiting amounts in the nucleus and are targeted to specific chromosomal regions by interaction with sequence-specific DNA-binding factors. Different sites of repression compete for Sir complexes, although it is not known how Sir distribution is regulated. In a screen for factors that interact with the Sir4p amino terminus, we have cloned *SIF2*, which encodes a WD40-repeat-containing factor that disrupts telomeric silencing when overexpressed. In contrast to deletion of *SIR4*, *SIF2* deletion improved telomeric repression, suggesting that under normal conditions Sif2p antagonizes Sir4p function at telomeres. Sif2p overexpression altered the subnuclear localization of Sir4p, but not its protein expression level, suggesting that Sif2p may recruit Sir4p to nontelomeric sites of repression. The *sif2* mutant strains were hypersensitive to a range of stress conditions, but did not have decreased viability and did not alter repression in the rDNA. In conclusion, Sif2p resembles the Sir4p regulatory proteins Sir1p and Uth4p in that it competes for the functional assembly of Sir4p at telomeres, yet unlike Sir1p or Uth4p, it does not target Sir4p to either mating-type or rDNA loci.

Address: Swiss Institute for Experimental Cancer Research, Chemin des Boveresses 155, CH-1066, Epalinges/Lausanne, Switzerland.

Present addresses: *Kansai Advanced Research Center, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe 65124, Japan. †Institute of Child Health Research, Subiaco, Western Australia, 6008 Australia.

Correspondence: Susan M. Gasser
E-mail: sgasser@eliot.unil.ch

Received: **27 January 1998**
Revised: **6 April 1998**
Accepted: **11 May 1998**

Published: **8 June 1998**

Current Biology 1998, 8:787–790
http://biomednet.com/elecref/0960982200800787

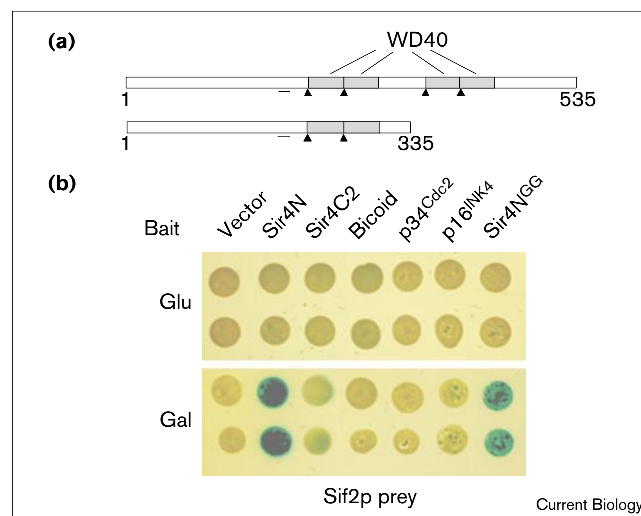
© Current Biology Ltd ISSN 0960-9822

Results and discussion

The dosage and balance of the proteins encoded by *SIR2*, *SIR3* and *SIR4* are critical for the maintenance of position-dependent transcriptional silencing in yeast [1,2]. We have shown recently that overexpression of amino acids 9–271 of Sir4p (Sir4N) derepresses both telomere-proximal repression (TPE) and silencing at the mating-type locus *HML*. In a two-hybrid screen, we identified three novel proteins that interacted exclusively with Sir4N bait, activating two different reporter constructs (see Supplementary material). One of the proteins has been identified as a ligand of the 72 kDa subunit of the origin recognition complex (Orc2p, D. Pederson, personal communication), and a second was a membrane-associated protein. Deletion of the genes encoding either of these two proteins was lethal (data not shown), and further characterization of their roles in silencing will require isolation of conditional mutants.

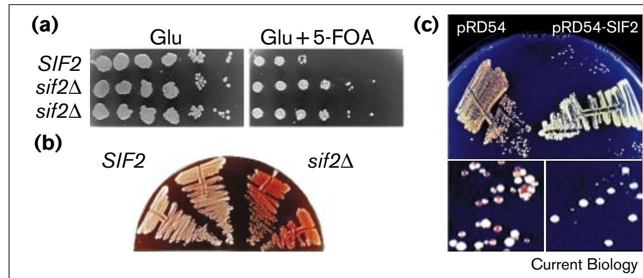
Here we describe the third Sir4N ligand, Sir4p-interacting factor 2 (Sif2p), which was recovered as the amino-terminal 335 amino acids of a novel protein (Figure 1a). The

Figure 1



(a) The full-length Sif2p (EMBL accession number Z35972) and the amino-terminal domain (amino acids 1–335) that was recovered in the two-hybrid screen. WD40 repeats are shaded and a nuclear localization signal is underlined. **(b)** Sif2p is a specific ligand for the Sir4p amino terminus. Duplicate transformants of the strain EGY48 were patched onto selective media (ura$^-$ his$^-$ trp$^-$) containing X-gal indicator and either 2% glucose (glu) or 2% galactose and 1% raffinose (gal). Blue color indicates a two-hybrid interaction between bait and prey. All transformants carried the reporter plasmid pSH18-34, the galactose-inducible plasmid pJG45-SIF2 as prey, and one of the following baits: pEG202 (vector) or LexA fusions to either Sir4N (residues 9–271), Sir4C2 (731–1358), Bicoid, p34^{Cdc2}, p16^{INK4} or Sir4N^{GG} (9–271). Sir4N^{GG} carries mutations of serines at positions 63 and 84 to glycines.

Figure 2



Deletion of *SIF2* enhances telomeric silencing and overexpression of Sif2p derepresses telomeric silencing. **(a)** The growth of isogenic *SIF2* and two independently derived *sif2*::*kan*MX2 deletion strains (*sif2Δ*) carrying *URA3* at Tel VIIL (strain GA492) and *ADE2* at Tel VR (MC118) was compared in the presence or absence of 5-FOA by plating 10-fold serial dilutions [4]. **(b)** *ADE2* repression in the same strains was compared by streaking independent single colonies onto synthetic media with limiting adenine concentrations. A higher frequency of red colonies indicates improved telomeric repression. **(c)** The effect of overexpression of HA–Sif2p (pRD54-SIF2) was compared with the effect of the vector alone (pRD54) in a Tel VR::*ADE2* strain (UCC3107). Transformants were streaked onto synthetic selective medium lacking uracil, but with limiting adenine and with 2% galactose and 1% raffinose to induce transcription of HA–Sif2p from the *GAL1* promoter. In addition, the effect of Sif2p on *HML* silencing was monitored using strain EG30 [6] with and without a *sif2*::*kan*MX2 deletion. The normalised β-galactosidase values from three separate experiments were 9.8 ± 1.0 and 12.6 ± 1.9 units for strains with or without the deletion, respectively, indicating ~20% increase in repression.

interaction between Sir4N and Sif2p was specific (Figure 1b). Moreover, mutations in the amino terminus of Sir4p that improve TPE (M.C., unpublished observations) resulted in a less efficient interaction with Sif2p by the two-hybrid assay (Figure 1b). Consistent with a direct interaction taking place between the proteins, Sif2p could be immunoprecipitated with affinity purified anti-Sir4p antibodies when it was overexpressed in yeast (data not shown).

The full-length *SIF2* gene encodes a protein of 535 amino acids that contains a nuclear localization signal and four tandem WD40-repeat motifs [3]. Sif2p bears no significant homology to other sequences in the database, including other WD40-repeat-containing proteins. Strains carrying a complete deletion of the *SIF2* locus (*sif2*::*kan*MX2) were viable, indicating that *SIF2*, like the *SIR* genes, is not essential for mitotic growth.

Sif2p competes for recruitment of Sir4p to telomeres

The relevance of the Sif2p–Sir4N interaction for silencing was analyzed in isogenic *SIF2* and *sif2*::*kan*MX2 strains using telomere-proximal reporters [4]. When *SIF2* cells were plated on 5-fluoro-orotic acid (5-FOA), a compound toxic to cells that express *URA3*, a telomere-proximal *URA3* gene was efficiently repressed in 0.5% of the cells, whereas in *sif2*::*kan*MX2 strains, repression increased to 12.5% or roughly 25-fold (Figure 2a). Improved telomeric repression was neither strain-specific nor promoter-specific (Figure 2b). The enhanced repression reflects the Sir-mediated pathway, because the *sif2*::*kan*MX2 deletion did not improve repression in a *sir3* or *sir4* deletion background (data not shown).

Deletion of *SIR1* similarly improves TPE, although *HM* repression is decreased [5] (H.R., M.C., unpublished observations). To see whether *SIF2* deletion affects the *HM* loci, we deleted *SIF2* in a strain carrying a *LEU lacZ* reporter at *HML* flanked by only one silencer [6], which allows a maximum degree of sensitivity for detecting modulation of repression. Complete deletion of *SIF2* slightly improved repression at *HML* (by ~20%, see Figure 2 legend), showing that Sif2p, unlike Sir1p, is not necessary for *HM* silencing. Together these results suggest that Sif2p competes for Sir4p recruitment or assembly at both telomeric and *HM* loci, with a more pronounced effect at telomeres. Consistent with this, the overexpression of *SIF2* significantly weakened telomeric repression. When either full-length Sif2p tagged with the hemagglutinin epitope (HA–Sif2p) or the amino-terminal half of Sif2p were overexpressed in a Tel VR::*ADE2* strain, the sectored colonies became completely white, reflecting increased *ADE2* expression or loss of TPE (Figure 2c and data not shown).

Elevated levels of Sif2p lead to mislocalization of Sir4p

Western analysis and immunofluorescence showed that the levels of Sir proteins were not altered when *SIF2* was overexpressed or deleted (Figure 3), indicating that Sif2p does not regulate *SIR* gene expression or protein turnover. Alternatively, Sif2p could interfere with silencing by regulating the assembly of Sir4p into a Sir complex or by directly disrupting silent chromatin. To test this we evaluated the subnuclear localization of Sif2p and Sir4p under conditions in which Sif2p interfered with telomeric repression. After induction for at least 1 hour, we detected the tagged Sif2p protein as a diffuse nuclear staining (Figure 3a,d). This pattern is distinctly unlike the punctate patterns of Rap1, Sir3p and Sir4p staining in wild-type yeast nuclei that have been shown to coincide with telomeric clusters [7,8] (Figure 3g), indicating that Sif2p does not preferentially localize to telomeres.

Strikingly, the foci of Sir4p staining were lost after a brief induction of HA–Sif2p, resulting in a diffuse rather than punctate Sir4p staining pattern (Figure 3d–f). Due to plasmid loss or poor induction, HA–Sif2p was not expressed in all cells, and when no Sif2p was present, the Sir4p foci remained (Figure 3e,f). Consistent with the ensuing loss of TPE, Rap1 and Sir3p signals also became more diffuse in the nucleus when Sif2p was overexpressed (Figure 3h,i). Because Sif2p bound Sir4p directly but did not accumulate at telomeres, these results are most consistent with a model in which Sif2p sequesters Sir4p away from telomeric foci, leading to the dispersal of the other silencing factors.

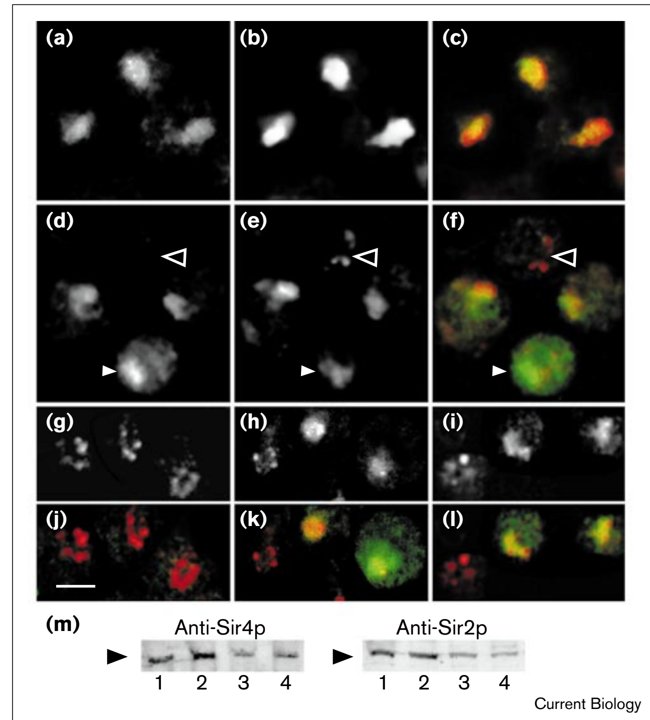
Mutations of *SIF2* and *UTH4* are synergistic for TPE but not for stress sensitivity

Recently it was shown that two related yeast genes, *UTH4* and *YGL023*, are necessary for the accumulation of both Sir3p and a truncated form of Sir4p in the yeast nucleolus [9,10]. Moreover, overexpression of *UTH4*, like overexpression of *SIF2*, reduces telomeric silencing, whereas *UTH4* deletion increases telomere-proximal repression [11]. In view of these similar phenotypes, we asked whether *SIF2* and *UTH4* or *YGL023* cooperate in Sir targeting by screening for synergism in strains containing mutations in both *SIF2* and *UTH4*. As the effect of *UTH4* mutation on TPE is only evident when *YGL023* is disrupted, these analyses were done in a *ygl023Δ* background [11]. Deletion of *SIF2* improved TPE more significantly than deletion of *UTH4*, and the effects of deleting both *SIF2* and *UTH4* were cumulative (Figure 4a), suggesting that they act on separate pathways to antagonize telomeric silencing.

Strains deficient for *UTH4* exhibit a rapid senescence that correlates with their inability to target Sir4p to the nucleolus [9,10], and have an increased sensitivity to environmental stresses [11]. To compare *uth4* and *sif2* strains, *sif2* strains were tested for stress sensitivity and aging phenotypes. In at least two backgrounds, strains carrying the *sif2*::*kan*MX2 disruption showed enhanced lethality under conditions of nitrogen or carbon source starvation (data not shown). This sensitivity was even more pronounced for heat-induced stress: after 15 minutes at 50°C we saw 10^3-fold increased lethality in a *sif2*::*kan*MX2 strain, compared with its isogenic wild-type strain (see Supplementary material). Using double and single deletions of *UTH4* and *SIF2* in a *ygl023* background, we saw that either *SIF2* or *UTH4* deletion reduced viability after heat shock by at least two orders of magnitude whereas the *ygl023* mutation alone had no effect (Figure 4a). The viability of the strain carrying both *sif2* and *uth4* mutations was not lower than either of the single mutations, however, suggesting that these genes might lie on the same pathway in establishing resistance to stress.

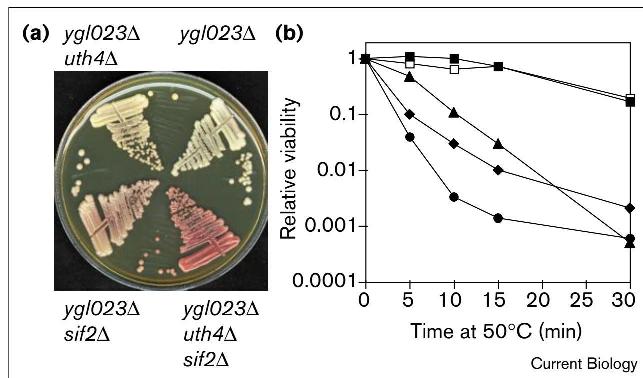
To test whether the *sif2* stress sensitivity correlated with effects on lifespan and rDNA instability, we monitored the lifespans of isogenic strains carrying *sif2*, *uth4* and *ygl023* mutations by separating mother cells from their buds and counting the cell divisions completed [11]. In an isogenic background we confirmed that *uth4* strains had a shortened lifespan, whereas *sif2* and *ygl023* strains had the same average lifespan as the parental strain. Similar conclusions were drawn when viability after 7 days growth at 30°C was assayed (data not shown). Finally, we examined the effects of *SIF2* deletion on the repression of a *URA3* reporter inserted in the rDNA repeat. *SIF2*-deleted strains, unlike *uth4* strains, had a wild-type level of Sir2p-dependent silencing in the nucleolus (data not shown). In conclusion, whereas Sif2p appeared to compete directly and efficiently for Sir4p recruitment at telomeres, it was not essential for the targeting of Sir proteins to the *HM* loci or to rDNA, nor did it play a role in the *UTH4* pathway of lifespan modulation. *SIF2* did participate in a pathway of stress resistance that relies in part on *UTH4*, implicating proteins that influence chromatin organization in the cellular response to stress.

Figure 3



Cells overexpressing HA–Sif2p displace Sir4p from telomeric foci without increasing Sir4p or Sir2p levels. The haploid strain GA24 carrying pRD54-SIF2 (HA–Sif2p under *GAL1* promoter control) was grown overnight on glycerol and lactate, then on 2% galactose for various times. Immunofluorescence was performed as published [7] using affinity purified antibodies and the mouse anti-HA monoclonal antibody 12CA5. **(a)** Detection of HA–Sif2p after 10 h induction on galactose; **(b)** detection of genomic DNA in the same cells; **(c)** an overlay of (a) in green with (b) in red; yellow indicates colocalization. **(d)** Detection of HA–Sif2p after galactose induction for 1 h. The open triangle indicates a cell with no *SIF2* expression; **(e)** detection of Sir4p in the same cells shown in (d); **(f)** an overlay of (d) in green with (e) in red. Cells not expressing HA–Sif2p have Sir4p foci (open triangle). The closed triangle indicates a cell expressing HA–Sif2p. **(g)** Detection of Sir4p in GA24 carrying pRD54-SIF2 without Sif2p induction. **(h)** Detection of Rap1p and **(i)** detection of Sir3p in GA24 carrying pRD54-SIF2 after Sif2p induction for 1 h. **(j–l)** An overlay of HA–Sif2p expression (green) with either (j) Sir4p expression (red), (k) Rap1p expression (red), or (l) Sir3p expression (red) in the same cells as (g–i), respectively. Focal perinuclear staining coincides with telomeric DNA [7]. The bar is equivalent to 2 μm. **(m)** Chemiluminescent western blotting using affinity purified anti-Sir4p and anti-Sir2p antibodies was performed on equal amounts of nuclear proteins from the following strains: lane 1, GA492 (*SIF2*+); lane 2, MC118 (*sif2*::*kan*MX2); lane 3, GA24; lane 4, GA24 containing pRD54-SIF2. Cells for lanes 3 and 4 were grown on galactose for 10 h.

Figure 4



Strains deleted for *SIF2* show increased TPE and heat sensitivity. The effect of the *sif2*::*kan*MX2 deletion was examined in isogenic strains carrying *ADE2* at Tel VIIL and the following single, double and triple gene disruptions: *ygl023*::*his*G (GA543); *ygl023*::hisG *uth4*::*LEU2* (GA542); *ygl023*::*his*G *sif2*::*kan*MX2 (MC248); and *ygl023*::*his*G *uth4*::*LEU2 sif2*::*kan*MX2 (MC184). **(a)** Red pigment accumulation indicates improved TPE after the strains were grown on YPD. **(b)** Representative viability curves of the strains in (a) after exposure to 50°C heat shock for the time intervals indicated (see Supplementary material). Open squares represent the *YGL023 UTH4 SIF2* background strain (PSY316AT); filled squares, *ygl023*; filled diamonds, *ygl023 uth4*; filled circles, *ygl023 sif2*; and filled triangles, *ygl023 uth4 sif2*. Viability is expressed as the fraction of cells able to form colonies. The relative viability at time *t* is the ratio of viability measured at that time versus viability at time 0.

Competition among nuclear domains for silencing factors

The competition between *HM* and subtelomeric loci for silencing factors [12,13] supports the model that a hierarchy of transcriptional silencing pathways co-exist in yeast [4]. The rDNA and sites of double-strand break repair are additional sites of Sir protein action [14–17]. Although Sir1p appears to target the Sir complex to *HM* silencers [18], other mechanisms that distribute silencing factors within the nucleus are unknown. Uth4p is needed to direct Sir3p and Sir4p to the nucleolus, where they accumulate in both aging cells and in mutants with extended lifespans [9–11], although there is no evidence as yet that Uth4p binds Sir proteins directly. *UTH4* overexpression, like *SIF2* overexpression, competes for TPE and causes a general delocalization of Sir4p (B. Kennedy and S.M.G., unpublished observations). Based on these analogies, we propose that Sif2p interacts with the Sir4 amino-terminal domain to target Sir4p from telomeres to other sites of action. As the strains that lacked Sif2p showed hypersensitivity to a range of stress conditions, Sif2p may be involved in a general, coordinated regulation of gene transcription in response to stress, possibly regulated by signaling cascades.

Supplementary material

Additional methodological detail, yeast genotypes and figures showing the Sif2p sequence and a viability curve for *sif2::kanMX2* are published with this paper on the internet.

Acknowledgements

We would like to thank R. Brent, E. Golemis, D. Gottschling and A. Reymond for gifts of plasmids and strains, T. Laroche for help with microscopy and M. Gotta, F. Palladino and B. Kennedy for sharing unpublished results and materials. This work was supported by grants from the Swiss National Science Foundation and the Human Frontiers Research Program (to S.M.G.).

References

1. Grunstein M: **Molecular model for telomeric heterochromatin in yeast.** *Curr Opin Cell Biol* 1997, **9:**383-387.
2. Shore D: **Telomere position effects and transcriptional silencing in the yeast *Saccharomyces cerevisiae*.** In *Telomeres.* Edited by Blackburn EH, Greider CW. New York: Cold Spring Harbor Press; 1995:139-192.
3. Neer EJ, Schmidt CJ, Nambudripad R, Smith TF: **The ancient regulatory-protein family of WD-repeat.** *Nature* 1994, **371:**297-300.
4. Aparicio OM, Billington BL, Gottschling DE: **Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*.** *Cell* 1991, **66:**1279-1287.
5. Fox CA, Ehrenhofer-Murray AE, Loo S, Rine J: **The origin recognition complex, SIR1, and the S phase requirement for silencing.** *Science* 1997, **276:**1547-1551.
6. Boscheron C, Maillet L, Marcand S, Tsai-Pflugfelder M, Gasser SM, Gilson E: **Cooperation at a distance between silencers and proto-silencers at the yeast *HML* locus.** *EMBO J* 1996, **15:**2184-2195.
7. Gotta M, Laroche T, Formenton A, Maillet L, Scherthan H, Gasser SM: **The clustering of telomeres and colocalization with Rap1, Sir3 and Sir4 proteins in wild-type *Saccharomyces cerevisiae*.** *J Cell Biol* 1996, **134:**1349-1363.
8. Maillet L, Boscheron C, Gotta M, Marcand S, Gilson E, Gasser SM: **Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression.** *Genes Dev* 1996, **10:**1796-1811.
9. Kennedy BK, Gotta M, Sinclair DA, Mills K, McNabb DS, Murthy M, *et al.*: **Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension in life span in *S. cerevisiae*.** *Cell* 1997, **89:**381-391.
10. Gotta M, Strahl-Bolsinger S, Renauld H, Laroche T, Kennedy BB, Grunstein M, Gasser SM: **Sir2 localization: the nucleolus as a compartment of silencing regulatory factors.** *EMBO J* 1997, **16:**3243-3255.
11. Kennedy BK, Austriaco NR Jr, Zhang J, Guarente L: **Mutation in the silencing gene SIR4 can delay aging in *S. cerevisiae*.** *Cell* 1995, **80:**485-496.
12. Buck SW, Shore D: **Action of a RAP1 carboxy-terminal silencing domain reveals an underlying competition between HMR and telomeres in yeast.** *Genes Dev* 1995, **9:**370-384.
13. Hardy CF, Sussel L, Shore D: **A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation.** *Genes Dev* 1992, **6:**801-814.
14. Gottlieb S, Esposito RE: **A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA.** *Cell* 1989, **56:**771-776.
15. Bryk M, Banerjee M, Murphy M, Knudsen KE, Garfinkel DJ, Curcio MJ: **Transcriptional silencing of Ty elements in the RDN1 locus of yeast.** *Genes Dev* 1997, **11:**255-269.
16. Smith JS, Boeke JD: **An unusual form of transcriptional silencing in yeast ribosomal DNA.** *Genes Dev* 1997, **11:**241-254.
17. Tsukamoto Y, Kato J-I, Ikeda H: **Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*.** *Nature* 1997, **388:**900-903.
18. Triolo T, Sternglanz R: **Role of interactions between the origin recognition complex and SIR1 in transcriptional silencing.** *Nature* 1996, **381:**251-253.

Supplementary material

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

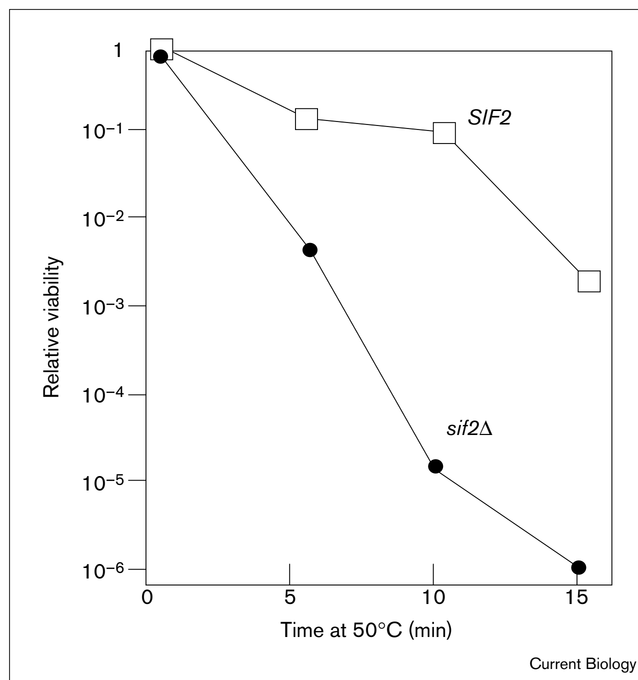
Moira Cockell, Hubert Renauld, Paul Watt and Susan M. Gasser

Current Biology 8 June 1998, 8:787-790

Figure S1

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.

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).

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.

Plasmids and PCR primers

A 1.6 kb *Hin*dIII-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)*