

Supplementary Information for:

***Schizosaccharomyces pombe* Grx4 Regulates the Transcriptional Repressor
Php4 via [2Fe-2S] Cluster Binding**

Adrienne C. Dlouhy,^a Jude Beaudoin,^b Simon Labbé^b and Caryn E. Outten^{a*}

^a Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, South Carolina 29208, United States

^b Département de Biochimie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Pavillon Z-8, 3201, Jean Mignault, Sherbrooke, QC, J1E 4K8, Canada

Supplementary Methods

Plasmids for homologous integration of S. cerevisiae FRA2 at the chromosomal locus of fra2⁺.

A *fra2⁺* promoter region encompassing positions -1 to -366 (with respect to A of the initiator codon of *fra2⁺*) was amplified by PCR. After purification, the DNA fragment was cloned into pKSloxP-KAN-loxP, creating plasmid pKSfra2prom-loxP-KAN-loxP. *S. cerevisiae FRA2* coding regions corresponding to amino acid residues 1-120 (long form) and 36-120 (short form) were isolated by PCR and cloned downstream of the *fra2⁺* promoter region, generating plasmids pKSfra2prom-longFRA2-loxP-KAN-loxP and pKSfra2prom-shortFRA2-loxP-KAN-loxP, respectively. A 363-bp DNA fragment from the *fra2⁺* 3' untranslated region (UTR) was amplified by PCR and cloned at the 3' end of the loxP-KAN-loxP cassette, creating plasmids pKSfra2prom-longFRA2-loxP-KAN-loxP-*fra2*-3'UTR and pKSfra2prom-shortFRA2-loxP-KAN-loxP-*fra2*-3'UTR. Each plasmid was subsequently digested to isolate the two DNA fragments (*fra2*prom-longFRA2-loxP-KAN-loxP-*fra2*-3'UTR and *fra2*prom-shortFRA2-loxP-KAN-loxP-*fra2*-3'UTR) that allowed homologous recombination of *FRA2* at the chromosomal locus of *fra2⁺*, thereby replacing the endogenous *fra2⁺* allele by the *S. cerevisiae FRA2* gene. *S. pombe* cells expressing the long or short version of *S. cerevisiae FRA2* were spotted onto yeast extract plus supplement (YES) medium that was either supplied or not with 170 μ M 2,2'-dipyridyl (Dip).

CD-monitored titrations of Php4 and Grx4. Several titration methods were used in an attempt to convert between Grx4 homodimer and Php4-Grx4 heterocomplexes. All samples were prepared and scanned under anaerobic conditions with 1 mM GSH, and the [2Fe-2S] content was kept constant at 50 μ M. (1) Purified [2Fe-2S] Php4-Grx4 was incubated with a 2.5-fold excess of Grx4 monomer. The mixed sample was scanned after five and ten minutes. (2) Apo-Php4-Grx4 was added to [2Fe-2S] Grx4, in 1:1, 1:2, and 1:4 ratios of [2Fe-2S]:Php4-Grx4.

Supplementary Results

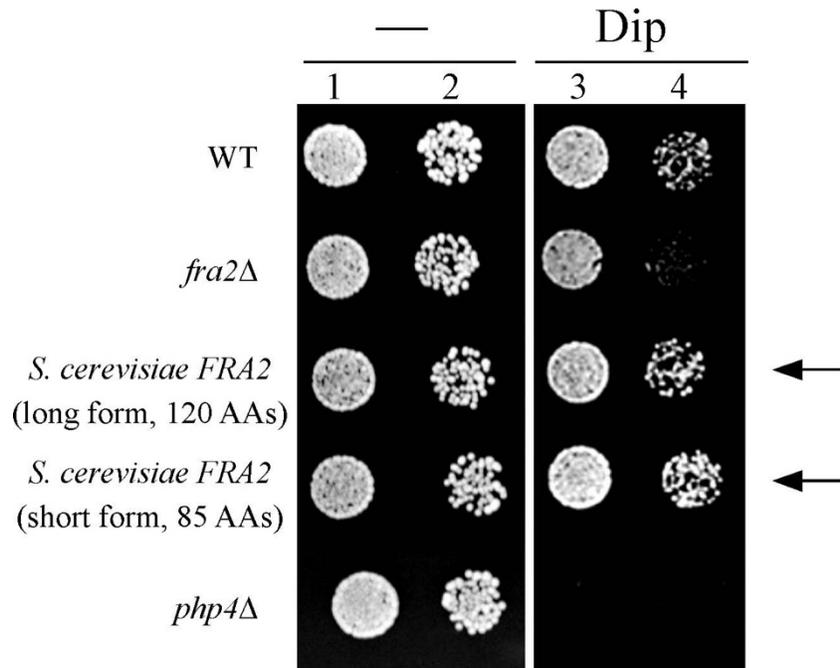


Fig. S1. *S. cerevisiae* Fra2 complements the function of *S. pombe* Fra2 *in vivo*. *S. pombe fra2Δ* mutant cells were spotted onto a medium depleted of iron by addition of the iron chelator 2,2'-dipyridyl (Dip) (170 μ M, columns 3 and 4). *fra2Δ* cells exhibit a much slower growth on low iron medium in comparison to wild-type cells (column 4) as previously demonstrated.¹ In contrast, *S. pombe* cells expressing *S. cerevisiae fra2⁺* gene (short or long form) integrated at the chromosomal locus of *fra2⁺* regained the ability to grow in medium containing Dip (column 4) Note: *S. cerevisiae* genes (short and long) are under the control of the *S. pombe fra2⁺* promoter.

A *php4Δ* mutant was used as a control strain as it was previously shown to be unable to grow on low iron medium.¹ Columns 1 and 2 are cells that were spotted on control medium (no chelator).

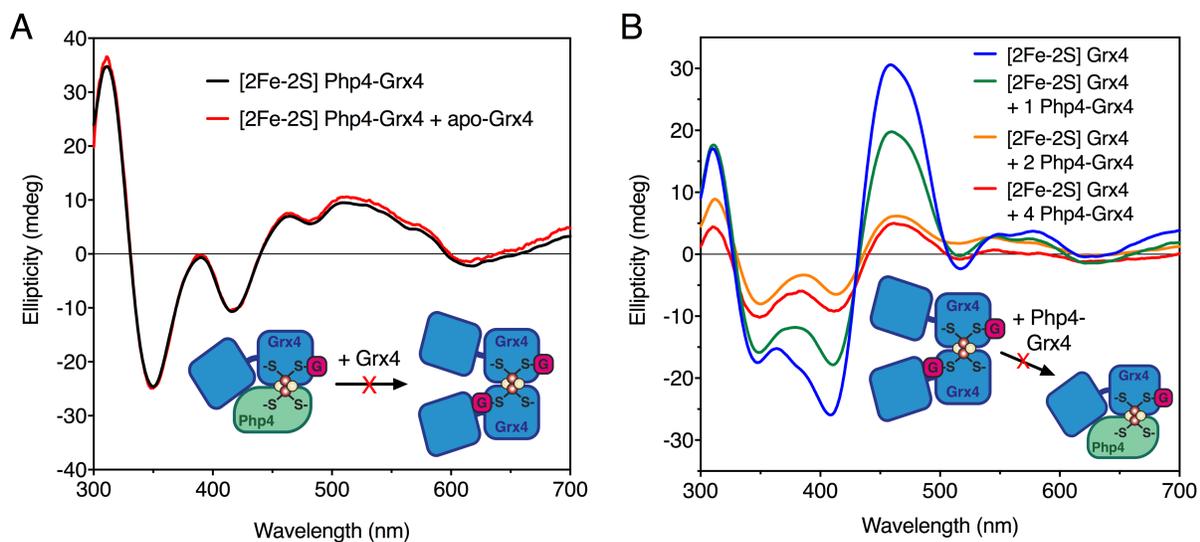


Fig. S2. (A) CD spectra for as-purified [2Fe-2S] Php4-Grx4 (black line) incubated with 2.5-fold molar excess apo-Grx4 (red line). The [2Fe-2S] concentration was kept constant at 50 μ M. **(B)** CD-monitored titration of apo-Php4-Grx4 into [2Fe-2S] Grx4. As-purified [2Fe-2S] Grx4 before any addition is shown as a blue line. The mixtures of [2Fe-2S] Grx4 and Php4-Grx4 (at 1:1, 1:2, and 1:4 ratio of [2Fe-2S]:Php4-Grx4) are shown as green, orange, and red lines. The decreasing CD intensity with increasing apo-Php4-Grx4 added is due to precipitation of the sample with addition of the apo-proteins. There is no clear shift in the CD wavelengths indicative of [2Fe-2S] Php4-Grx4 formation.

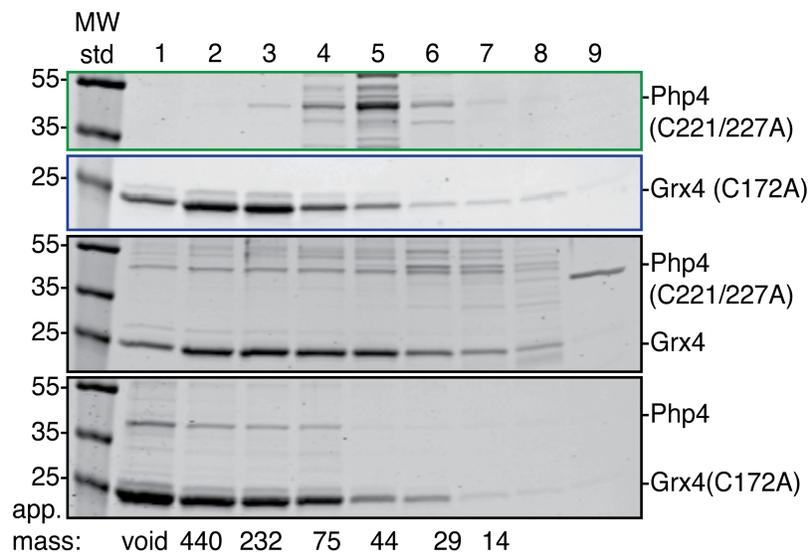


Fig. S3. SDS-PAGE analysis of gel filtration fractions collected for purified Php4(C221/C227A) expressed alone (1st panel), Grx4(C172A) expressed alone (2nd panel), Php4(C221/C227A) coexpressed with Grx4 (WT) (3rd panel), and Php4 (WT) coexpressed with Grx4(C172A) (4th panel). Identical fractions from each analytical gel filtration experiment were loaded for comparison of elution volumes. Masses (in kD) of MW standards used in the SDS-PAGE gel are shown on the left. The elution position and apparent mass (in kD) of the molecular weight standards used to calibrate the gel filtration column are shown across the bottom.

References

1. J. F. Jacques, A. Mercier, A. Brault, T. Mourer and S. Labbé, Fra2 is a co-regulator of Fep1 inhibition in response to iron starvation, *PLoS One*, 2014, **9**, e98959.