

Supplemental Data

Nicotinamide Riboside Promotes Sir2

Silencing and Extends Lifespan via Nrk

and Urh1/Pnp1/Meu1 Pathways to NAD⁺

Peter Belenky, Frances G. Racette, Katrina L. Bogan,
Julie M. McClure, Jeffrey S. Smith, and Charles Brenner

Supplemental Experimental Procedures

Plasmid Construction

Primer sequences are provided in Table S1. pPAB01 was constructed by amplifying the *PNP1* gene from wild-type yeast genomic DNA with primers 14061 and 14060. The PCR product was inserted into pRS416 with Xho1 and BamH1. pPAB02 was constructed by amplifying the *URH1* gene using primers 14051 and 14050. The PCR product was inserted into pRS416 with Kpn1 and Xba1. Plasmids were confirmed by DNA sequencing.

S. cerevisiae Strains

All yeast strains used in this study are listed in Table S2. Strains were created in the BY4742 background, unless otherwise indicated. Strains from the consortium deletion library (Winzeler et al., 1999) were verified by PCR. New deletions were introduced by direct transformation with PCR products (Brachmann et al., 1998) and were verified by PCR.

Supplemental References

Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., and Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14, 115-132.

Buck, S. W., Sandmeier, J. J., and Smith, J. S. (2002). RNA polymerase I propagates unidirectional spreading of rDNA silent chromatin. *Cell* 111, 1003-1014.

Gallo, C. M., Smith, Jr., D. L., and Smith, J. S. (2004). Nicotinamide clearance by Pnc1 directly regulates Sir2-mediated silencing and longevity. *Mol Cell Biol* 24, 1301-1312.

Sandmeier, J. J., Celik, I., Boeke, J. D., and Smith, J. S. (2002). Telomeric and rDNA Silencing in *Saccharomyces cerevisiae* Are Dependent on a Nuclear NAD(+) Salvage Pathway. *Genetics* 160, 877-889.

Smith, J. S., Caputo, E., and Boeke, J. D. (1999) A genetic screen for ribosomal DNA-silencing defects identifies multiple DNA replication and chromatin-remodeling factors. *Mol Cell Biol* 19, 3184-3197.

Smith, J. S., Brachmann, C. B., Celic, I., Kenna, M. A., Muhammad, S., Starai, V. J., Avalos, J. L., Escalante-Semerena, J. C., Grubmeyer, C., Wolberger, C., and Boeke, J. D. (2000). A phylogenetically conserved NAD⁺-dependent protein deacetylase activity in the Sir2 protein family. *Proc Natl Acad Sci U S A* 97, 6658-6663.

Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., *et al.* (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285, 901-906.

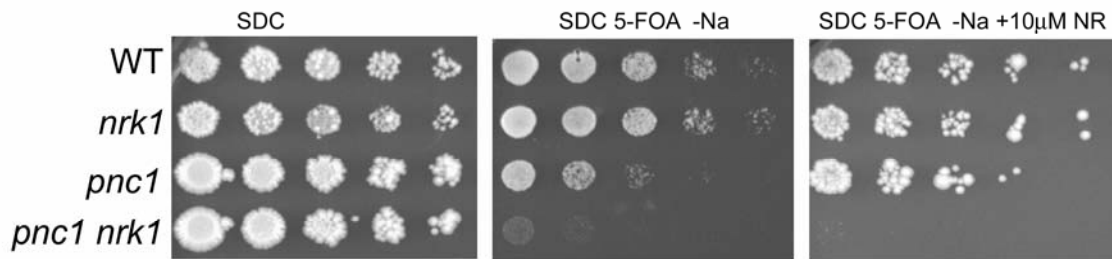


Figure S1. Pnc1 Acts Second in the Urh1/Pnp1/Meu1 Pathway

Isogenic strains carrying a telomeric *URA3* construct were assayed for gene silencing on 5-FOA media with no vitamin supplement or with addition of 10μM nicotinamide riboside (NR). Sir2-dependent telomeric gene silencing, scored as 5-FOA resistance, of the WT, *nrk1*, and *pnc1* strains was improved by the addition of NR. However, the addition of NR to the *nrk1 pnc1* strain exacerbated a silencing defect. In this strain, NR cannot be converted past nicotinamide, leading to Sir2 inhibition.

Table S1. Oligodeoxynucleotide Primers

Primer Number	Sequence
14050	gctctagaCAGACAAGTGGTATGCATATCC
14051	cggggtaccGATGTGCTGTGACTGGG
14060	gccgctgagCTTCCCGCTATGTAATAAATAGAGG
14061	cgcgatccGCATCATCTGTCAATTCCTTG

Table S2. Yeast Strains

Strain	Genotype (method)	Reference	Figures
BY4734	<i>MATα his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0</i>	Brachmann, 1998	
BY4741	<i>MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Brachmann, 1998	
BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Brachmann, 1998	2D, 3ABC, 4A, 6, 7A
PAB015	BY4742 <i>nrk1Δ::HIS3</i> (direct transformation)	This study	2D, 4ABC, 5, 6, 7B
PAB016	BY4742 <i>nrk1Δ::HIS3 pnp1Δ::kanMX4</i> (direct transformation)	This study	4C
PAB037	BY4742 MAT α <i>nrk1Δ::HIS3</i> (segregant of PAB015 x BY4741)	This study	
PAB038	BY4742 <i>nrk1Δ::HIS3 pnp1Δ::kanMX4 urh1::NAT</i> (direct transformation)	This study	4CD, 5, 7D
PAB039	BY4742 <i>nrk1Δ::HIS3 meu1Δ::kanMX4</i> (segregant of PAB037 x <i>meu1Δ::kanMX4</i>)	This study	4C
PAB040	BY4742 <i>nrk1Δ::HIS3 urh1Δ::kanMX4</i> (segregant of PAB037 x <i>urh1Δ::kanMX4</i>)	This study	4C
PAB041	BY4742 <i>pnc1Δ::kanMX4</i>	Winzeler, 1999	4B
PAB045	BY4742 <i>pnc1Δ::kanMX4 nrk1Δ::HIS3</i> (segregant of PAB037 x <i>pnc1Δ::kanMX4</i>)	This study	4B
PAB046	BY4742 <i>pnp1Δ::kanMX4 urh1::NAT</i> (segregant of PAB038 x BY4741)	This study	4C, 6, 7C
PAB052	BY4742 <i>pnp1Δ::kanMX4 urh1::NAT nrk1Δ::HIS3 meu1Δ::LEU2</i>	This study	6
PAB057	JS807 <i>nrk1Δ::HIS3</i> (direct transformation)	This study	S1
KB008	BY4742 <i>npt1Δ::kanMX4</i>	Winzeler, 1999	2D
KB011	BY4742 <i>npt1Δ::kanMX4 nrk1Δ::HIS3</i> (direct transformation)	This study	2D
JS799	<i>MATα his3Δ200 trp1Δ63 leu2Δ1::TRP1 ura3-52 ADH4::URA3-TEL</i>	This study	

	<i>npt1Δ::kanMX4</i> (segregant of JS641 X BY4734)		
YCB647	<i>MATa his3Δ200 lys2Δ202 trp1Δ63 leu2Δ1::TRP1 ura3-52 ADH4::URA3-TEL</i>	Smith, 2000	2C
JS641	YCB647 <i>npt1Δ::kanMX4</i> (direct transformation)	Smith, 2000	2C
JS942	YCB647 <i>nrk1Δ::kanMX4</i> (direct transformation)	This study	2C
JS961	<i>MATa his3Δ200 trp1Δ63 leu2Δ1::TRP1 ura3-52 ADH4::URA3-TEL npt1Δ::kanMX4 nrk1Δ::kanMX4</i> (segregant of JS942 X JS799)	This study	2C
YSB348	<i>MATα his3Δ200 leu2Δ1 ura3-167 RDN1(50L)::mURA3-HIS3</i>	Buck et al. 2002	2B
YSB408	YSB348 <i>sir2Δ::kanMX4</i> (direct transformation)	Buck et al. 2002	2B
CGY145	YSB348 <i>npt1Δ::kanMX4</i> (direct transformation)	This study	2B
CGY153	YSB348 <i>bna1Δ::kanMX4</i> (direct transformation)	This study	2B
JS306	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 RDN1(NTS2)::Ty1-MET15 RDN1(18S)::mURA3-HIS3</i>	Smith, 1999	2AB
JS576	JS306 <i>sir2Δ::kanMX4</i> (direct transformation)	Smith, 1999	2AB
JS673	JS306 <i>npt1Δ::kanMX4</i> (direct transformation)	Smith, 2000	2AB
JS807	YCB 647 <i>pnc1Δ::kanMX4</i> (direct transformation)	Sandmeier, 2002	S1
JJSy143	<i>MATa his3Δ200 leu2Δ0 ura3-52 trp1Δ63 lys2Δ202 ADH4::URA3-TEL</i>	Gallo et al, 2004	
JM84	PAB038 <i>ADH4::URA3-TEL</i> (segregant of JJSy143 X PAB038)	This study	6
JM85	JM84 <i>nrk1Δ::HIS3</i> (segregant of JJSy143 X PAB038)	This study	6
JM88	JM84 <i>pnp1Δ::kanMX4 urh1Δ::NAT</i> (segregant of JJSy143 X PAB038)	This study	6
JM89	JM84 <i>nrk1Δ::HIS3 pnp1Δ::kanMX4 urh1Δ::NAT</i> (segregant of JJSy143 X PAB038)	This study	6
