Supplementary information

Supplementary materials and methods

Table I. List of strains used in this study

Strain	Genotype	Reference
A2	*h$^+$ ade6-M216 his3-D1 leu1 1-32 ura4-D*	lab stock
972	*h$^-$*	lab stock
oca2Δ	*h$^+$ ade6-M216 his3-D1 leu1 1-32 ura4-D18 oca2Δ::kanMX6*	lab stock
oca2-HA	*h$^+$ ade6-M216 his3-D1 leu1 1-32 ura4-D18 oca2-HA::kanMX6*	lab stock
oca2Δ-20	*h$^-$ oca2Δ::kanMX6*	this study
cha4Δ	*h$^+$ ade6-M216 his3-D1 leu1 1-32 ura4-D18 cha4Δ::kanMX6*	this study
ago1Δ	*h$^+$ ade6-M216 his3-D1 leu1 1-32 ura4-D18 ago1 Δ::kanMX6*	this study
hat1Δ	*h$^+$ ade6-M216 his3-D1 leu1 1-32 ura4-D18 hat1 Δ::kanMX6*	this study
clr6-1	*h$^-$ clr6-1*	(S4)
clr4Δ	*h$^+$ clr4::hph$^+$otr1R(SphI)::ade6-M210*	Robin Allshire
oca2Δ clr6-1	*h? ade6-M216 leu1 1-32 oca2 Δ::kanMX6 clr6-1*	this study
wt	*h$^+$ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI*	Marc Buehler
rrp6Δ	*h$^+$ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI rrp6Δ::Nat^R*	Marc Buehler
Flag-ago1	*h$^+$ otr1R(SphI)::ura4$^+$ ura4-DS/E leu1-32 ade6-M210 Nat^R-nmt1-3xFlag::ago1*	(S1)

Table II. Genes used in this study

Name	Systematic name
oca2	SPCC1020.10
ppk8	SPAC22G7.08
cha4	SPBC1683.13c
hat1	SPAC139.06
prw1	SPAC29A4.18
per1	SPAP7G5.06
put4	SPAC869.10

Table III. List of primers used in this study

Name	sequence/reference	purpose
1F	TGTGTCTCGGCTTACCCTTC	ChIP *per1*
1R	GCGGAAAGAGACGGATAACA	ChIP *per1,* 5'RACE
1BR	GTGCAATCTACTAGCAACCAAG	5'RACE
2F	GCTCTCCAAGTGCCAGTTTC	ChIP *per1*
2R	GCCGCACAATATGGATAAGG	ChIP *per1*
3F	CGCATCTTGCATTATTTCACC	ChIP *per1*
3R	AAACGATTGGGAAACACTCG	ChIP *per1*, 5'RACE
4F	GTCGCCAGATCGCTCTATTG	ChIP *per1*
4R	TGGGATGAATGTCGAAAACA	ChIP *per1*, 5'RACE
5F	GCGGTGGTGTTCCTACTGAT	ChIP, qRT-PCR
5R	GCACGAGGGAGAGACTTTTG	ChIP, qRT-PCR
6F	TGATTTAGACACGGGACTTCG	ChIP *per1*
6R	CAAAGAGATTGCCAAATCCA	ChIP *per1*
7F	CGTTGTAAGTTTATATGTTGAAGCA	ChIP *per1*
7R	TGCGAATGCAAGGCATAATA	ChIP *per1*
8F	ATATAATGTTGAGCTCCTTGGTTAGC	ChIP *per1*
8R	TGAGCTTGATAAGGCGGTCT	ChIP *per1*
Put4BF	AAAAAGGCGTTGCAGTATGA	ChIP *put4*
Put4BR	TTTCTCCGTACTTCTTTTCAACG	ChIP *put4*
Adh1PF	CTTCCGCGTCTCATTGGT	ChIP *adh1*
Adh1PR	TTGCTTAAAGAAAAGCGAAGG	ChIP *adh1*
dhF	(20)	ChIP *dh*
dhR	(20)	ChIP *dh*
Put4F	ACATGATCGCTTGGGTTTTC	qRT-PCR *put4*
Put4R	TTAGGGATGTTACGCCTTGG	qRT-PCR *put4*
Adh1F	CGTATTGACTCTATCGAGGCTCTT	qRT-PCR *adh1*
Adh1R	CTTGGAAAGGTCCAAGACGA	qRT-PCR *adh1*
RT-PCR1	(50)	RT-PCR *dh*
RT-PCR2	(50)	RT-PCR *dh*
ACTF	(50)	RT-PCR *act1*
ACTR	(50)	RT-PCR *act1*
ORFT7	TAATACGACTCACTATAGGGAGAGCGGTGGTGTTCCTACTGAT	Northern *per1*
ORFT3	AATTAACCCTCACTAAAGGGAGACCAACACGAAGGGAGAGGTA	Northern *per1*
US2T7	TAATACGACTCACTATAGGGAGAGCCCATTCCCATTCAATTTT	Northern *per1*
US2T3	AATTAACCCTCACTAAAGGGAGAATGTTTGAGCGCGTGTATGT	Northern *per1*
US1T7	TAATACGACTCACTATAGGGAGACTGCTGCAAAACTTTGGTTG	Northern *per1*
US1T3	AATTAACCCTCACTAAAGGGAGATTAGTTACCCTATTTGGAAG	Northern *per1*
RplAT7		Northern *rpl1002*

		TAATACGACTCACTATAGGGAGAGCTCGTATCTGTGCCAACAA	
	RplAT3		Northern *rpl1002*
		AATTAACCCTCACTAAAGGGAGAGTAGCAACCGTCGGGAATAA	
	RplBT7		Northern *rpl1002*
		TAATACGACTCACTATAGGGAGATGGTGTTGGATGAATCGGTA	
	RplBT3		Northern *rpl1002*
		AATTAACCCTCACTAAAGGGAGACGTGGAAGTAGATGCGGAGT	
	Adh1T7		Northern *adh1*
		TAATACGACTCACTATAGGGAGATTCAAGGTGACTGGCCTCTT	
	Adh1T3		Northern *adh1*
		AATTAACCCTCACTAAAGGGAGACAAGGCACGATAGCAAGTGA	
	Prw1FT7		*in vitro* translation
		GTGATAACTACTAATACGACTCACTATAGGGAGAATGGC	
		TGTATCAGCTGTTC	
	Prw1R	TTAACTTAAATATGCCGTAG	*in vitro* translation
	Cha4FT7		*in vitro* translation
		GTGATAACTACTAATACGACTCACTATAGGGAGAAT	
		GCAAATGAAACCCCGAC	
	Cha4R	TTAATATTTTACATTGGGAG	*in vitro* translation
	Hat1F	ATGAGTGCTG TTGATGAATG	pBS-*hat1*
	Hat1R	TTAGGAAGAAGATTGAGCAAG	pBS-*hat1*
	Mis16F	ATGTCAGAGGAAGTAGTCC	pBS-*mis16*
	Mis16R	TTACTCCAGATCCCTAGGAG	pBS-*mis16*
	Ago1F	GATGTCGTATAAACCAAGCTCAG	pBS-*ago1*
	Ago1R	TTACATATACCACATCTTTGTTTTC	pBS-*ago1*
	P14	ACCCCCGGGCATGTCTGTCACCCT	pGEX4T1-*oca2*
	P15	GGACCCGGGATGCTTTGCAGGTGG	pGEX4T1-*oca2*
	Hat1KanF		*hat1Δ*
		GAGCTAGAAATCTATATAATAGTAAATATTTTTTAATAA	
		TAACAGGTGTAGCACGTGAAAGCGGATCCCCGGGTTAATTAA	
	Hat1KanR		*hat1Δ*
		TAAATTTTGGAAAAAGCAGTTCATTATGAGGAATTGTTTGA	
		ATTTTTATAAGGTGCCTTTGAATTCGAGCTCGTTTAAAC	

Kinase assays using recombinant proteins

For expression of Oca2-His_6 in Sf21 insect cells, a C-terminal His_6-tag was first introduced into pGEX4T1-Oca2 by PCR with primers P16 and P17, followed by cloning of the Oca2-His_6 ORF into pTriEx1 (Novagen) using *EcoR*V and *Sma*I sites. Sf21 insect

cells were transfected with pTriEx1-Oca2-His_6 according to manufacturers instructions and the viral titer amplified over 3 rounds. To induce protein expression of Oca2-His6, 500 ml of Sf21 cells were transfected with 5 ml of viral supernatant and incubated for 4 days at 27°C. Cells were resuspended in lysis buffer (20mM Tris-HCl pH 8.0, 10% glycerol, 200mM KCl, 0.01% NP40) containing complete protease inhibitor cocktail (Roche) and lysed by sonication on ice. After centrifugation the supernatant was incubated with Ni-NTA beads (Qiagen) for 1 hr at 4°C. Beads were washed twice with 20 bed volumes of lysis buffer, once with 5 bed volumes of lysis buffer containing 500mM NaCl, once with 5 bed volumes of lysis buffer containing 20mM Imidazole and once with 5 bed volumes of lysis buffer. Oca2-His_6 was eluted with elution buffer (25mM HEPES pH 7.6, 10% glycerol, 100mM KCl, 250mM Imidazole). Purified recombinant proteins were incubated in kinase buffer (25 mM HEPES pH 7.6, 10% glycerol, 30 mM KCl, 10 mM MgCl2, 1 mM DTT) containing 0.05 mM ATP and 0.5 µl ^{32}P-ATP for 1hr at 30°C. Proteins were precipitated with TCA, resolved by SDS-PAGE and stained with Coomassie brilliant Blue. Dried gels were subjected to autoradiography.

PepChip Kinase microarray

Two separate PepChip kinase arrays (Pepscan Systems) were incubated with 100ng baculoexpressed Oca2-His_6 under *in vitro* phosphorylation conditions. Slides were washed, dried and exposed on a phosphoimager screen. Both slides gave similar results and the 30 strongest signals were analysed. Amino acid sequences of the corresponding peptides were compared by eye to define an Oca2 phosphorylation consensus motif.

Supplementary References

S1 **Buker, S. M., Iida, T. , Bühler, M., Villén, J. Gygi, S.P., Nakayama, J.-I. and D. Moazed.** 2007. Two different Argonaute complexes are required for siRNA generation and heterochromatin assembly in fission yeast. Nature Struct Mol Biol **14**: 200 – 207.

S2 **Irvine, D. V., M. Zaratiegui, N. H. Tolia, D. B. Goto, D. H. Chitwood, M. W. Vaughn, L. Joshua-Tor, and R. A. Martienssen.** 2006. Argonaute slicing is required for heterochromatic silencing and spreading. Science **313**:1134-7.

S3 **Win, T. Z., A. L. Stevenson, and S. W. Wang.** 2006. Fission yeast Cid12 has dual functions in chromosome segregation and checkpoint control. Mol Cell Biol **26:**4435-47.

S4 **Wiren, M., R. A. Silverstein, I. Sinha, J. Walfridsson, H. M. Lee, P. Laurenson, L. Pillus, D. Robyr, M. Grunstein, and K. Ekwall.** 2005. Genomewide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. EMBO J **24:**2906-18.

Supplementary Figure Legends

Figure S1

(A) Oca2 has kinase activiy *in vitro*. 30ng of baculo-expressed Oca2-His_6 were incubated either alone (lane 1) or with increasing amounts (200, 400, 800 ng) of MBP (Upstate; lanes 2-4) and proteins were analysed on 18% SDS-PAGE. Lane 5 contains 800

ng of MBP alone. (B) Oca2 phosphorylates serines and threonines surrounded by basic amino acids. A peptide micro-array containing 1400 peptides with known phosphorylation sites was incubated with recombinant Oca2-His_6 under *in vitro* kinase conditions followed by exposure on a phosphoimager screen. Amino acid sequence analysis of phosphorylated peptides from two independent experiments gave a consensus phosphorylation motif for Oca2 (shown below). (C) Oca2 is a phosphoprotein. Whole cell extracts prepared from *HA-oca2* cells was bound to HA-beads. After washing the beads with lysis buffer proteins were incubated with alkaline phosphatase (AP, lane 3), shrimp phosphatase (SP, lane 4) or buffer alone (mock, lane 2). Lane 1: untreated *HA-oca2* whole cell extract. Proteins were eluted with SDS sample buffer and analysed by Western blotting.

Figure S2

(A) *oca2Δ*, *cha4Δ* and *ago1Δ* cells are rapamycin resistant. Wt, *oca2Δ, cha4Δ* and *ago1Δ* cells were grown in YE, diluted into EMM with ammonia containing 0.3 μM Rapamycin or vehicle, and OD600 was measured at the indicated time points. (B) 5' extended transcripts of *per1* are rapamycin sensitive. Northern Blot analysis of total RNA isolated from wild type, *oca2Δ* and *cha4Δ* cells grown in EMM medium containing ammonia and rapamycin (even numbered lanes) or vehicle alone (uneven numbered lanes). (A) Hybridization was with probes against the ORF (Probe ORF, left) and upstream regions of *per1* (probe US2, middle; probe US1 right). (B) Same Northern blot as in (A) hybridized with a probe against *adh1* ORF.

Figure S3

Northern blot analysis of the ribosomal protein gene *rpl1002* located upstream of *per1*. Total RNA was isolated from wt and *oca2Δ* grown in EMM medium containing either ammonia or proline. Northern blots were probed with single-stranded RNA probes specific for sense transcripts mapping to the ORF (probe A) or immediately downstream of *rpl1002* (Probe B) and to the ORF of *adh1*.

Figure S4

5'RACE of *per1* transcripts. Total RNA was reverse transcribed using random hexamers, and cDNA was amplified with two nested PCR reactions. The *per1* locus is shown schematically as a black line with the *per1* ORF indicated with a white arrow and the positions of the ChIP primers and Northern probes indicated with black boxes. The 5'RACE products are shown as grey boxes with the nested 5' RACE primers indicated by duplicated black arrows.

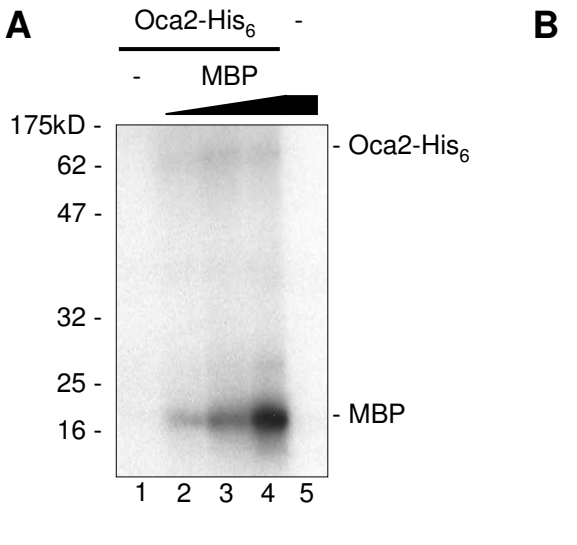
Figure S5

H3 ChIP of wt and *oca2Δ* cells grown in EMM medium containing either ammonia or proline. The positions of the PCR primers across *per1* are indicated. ChIP signal values are expressed as % of input DNA corrected for the no antibody control. The data shown represent the average and SEM of three independent experiments.

Figure S6

Northern blot analysis of *per1* in strains lacking *snf22*. Total RNA was isolated from *snf22Δ* cells grown in EMM medium containing either NH_4 or proline and analysed using single-stranded RNA probes mapping to the ORF of *per1*.

Figure S1


A
Oca2-His6
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MBP
175kD -
62 -
47 -
32 -
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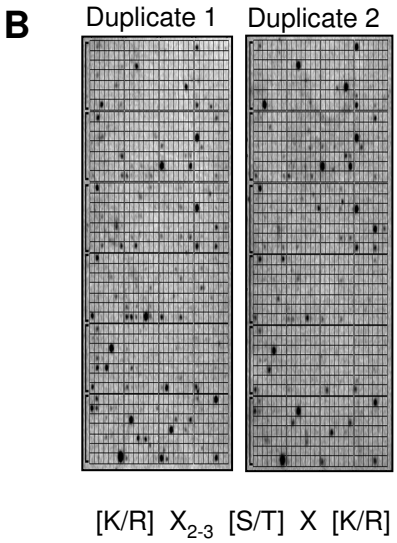
16 -
- Oca2-His6
- MBP
1 2 3 4 5


B
Duplicate 1
Duplicate 2
[K/R] X2-3 [S/T] X [K/R]

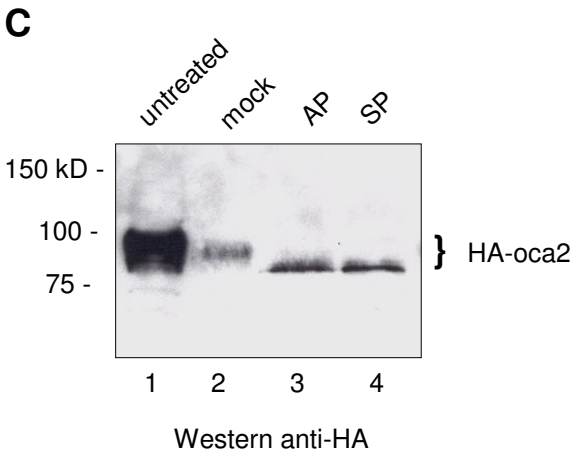
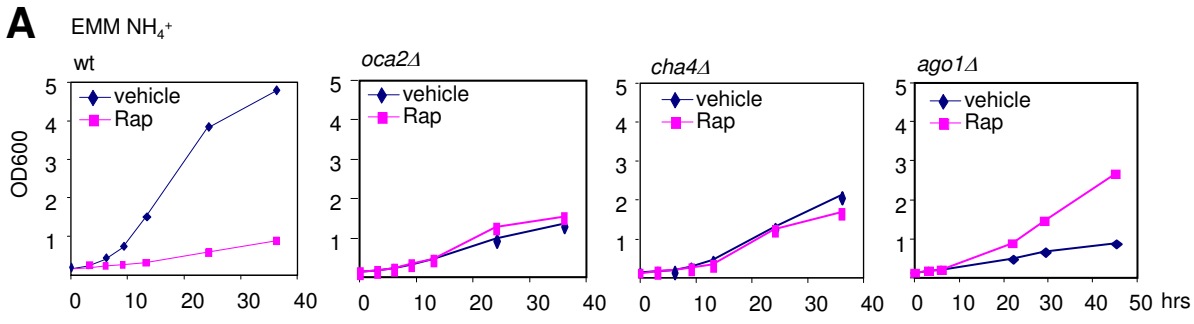

C
untreated
mock
AP
SP
150 kD -
100 -
75 -
} HA-oca2
1 2 3 4
Western anti-HA

Figure S2


A
EMM NH4+
wt
oca2Δ
cha4Δ
ago1Δ
vehicle
Rap
OD600
hrs

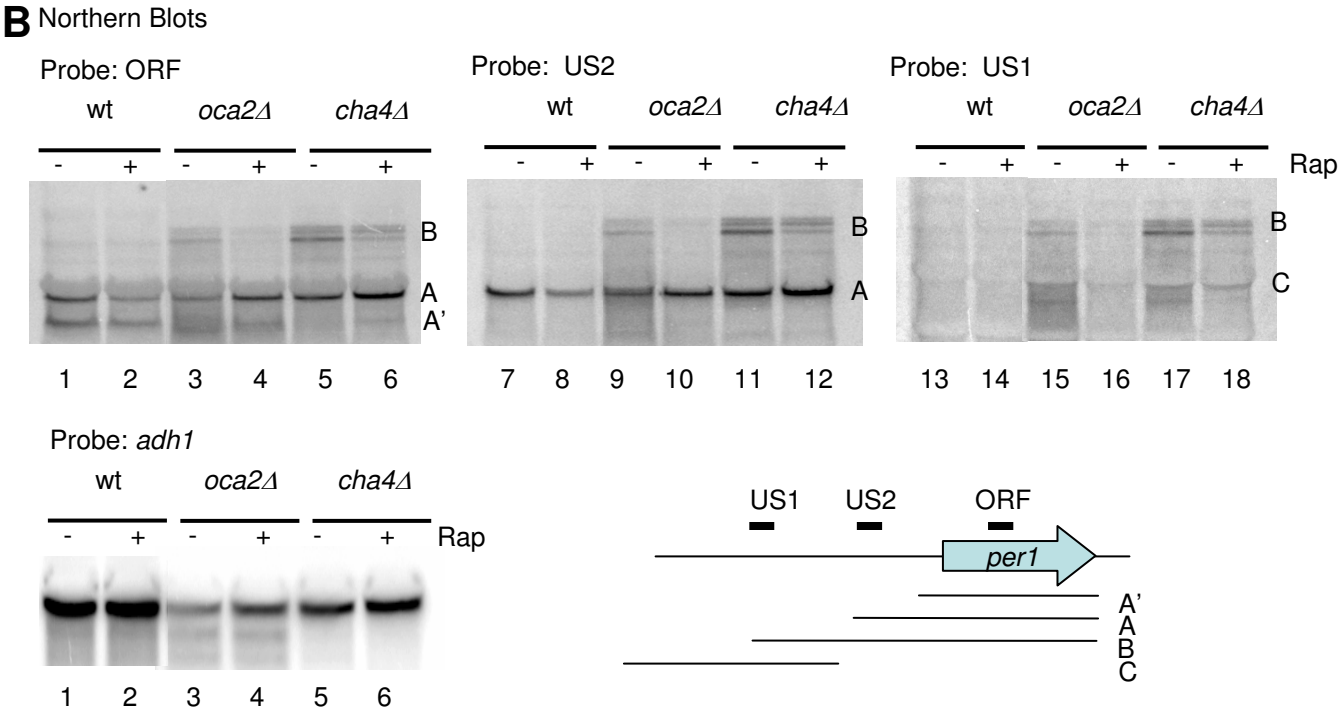
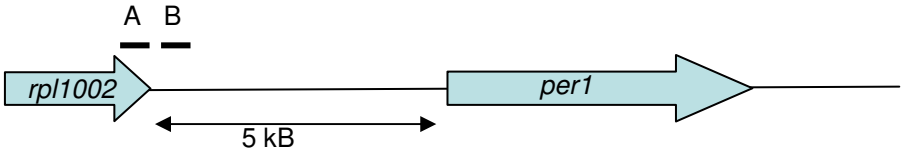

B Northern Blots
Probe: ORF
Probe: US2
Probe: US1
wt
oca2Δ
cha4Δ
Rap
B
A
A'
C
Probe: adh1
US1
US2
ORF
per1

Figure S3

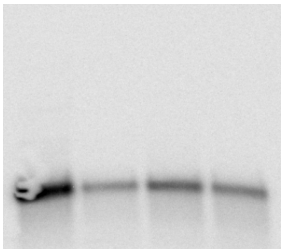
A



Probe: *rpl1002* A

wt | *oca2Δ*

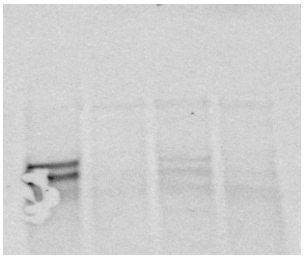
NH_4^+ Pro NH_4^+ Pro



Probe: *rpl1002* **B**

wt | *oca2Δ*

NH_4^+ Pro NH_4^+ Pro



Probe: *adh1*

wt | *oca2Δ*

NH_4^+ Pro NH_4^+ Pro

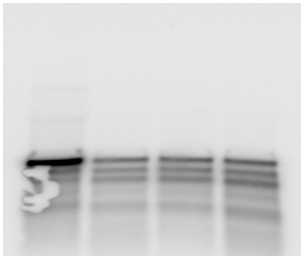
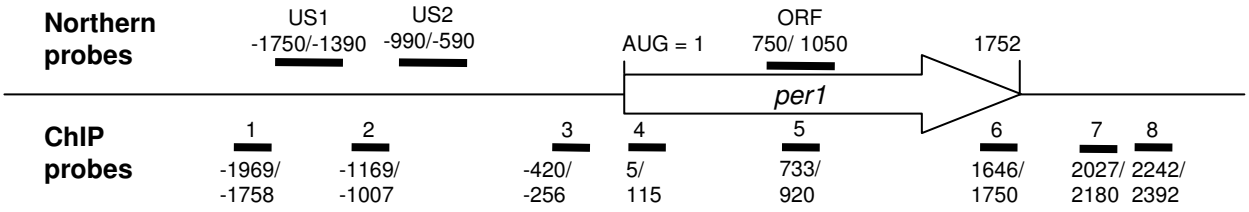


Figure S4


Northern probes
US1
-1750/-1390
US2
-990/-590
AUG = 1
ORF
750/ 1050
1752
per1
ChIP probes
1
-1969/
-1758
2
-1169/
-1007
3
-420/
-256
4
5/
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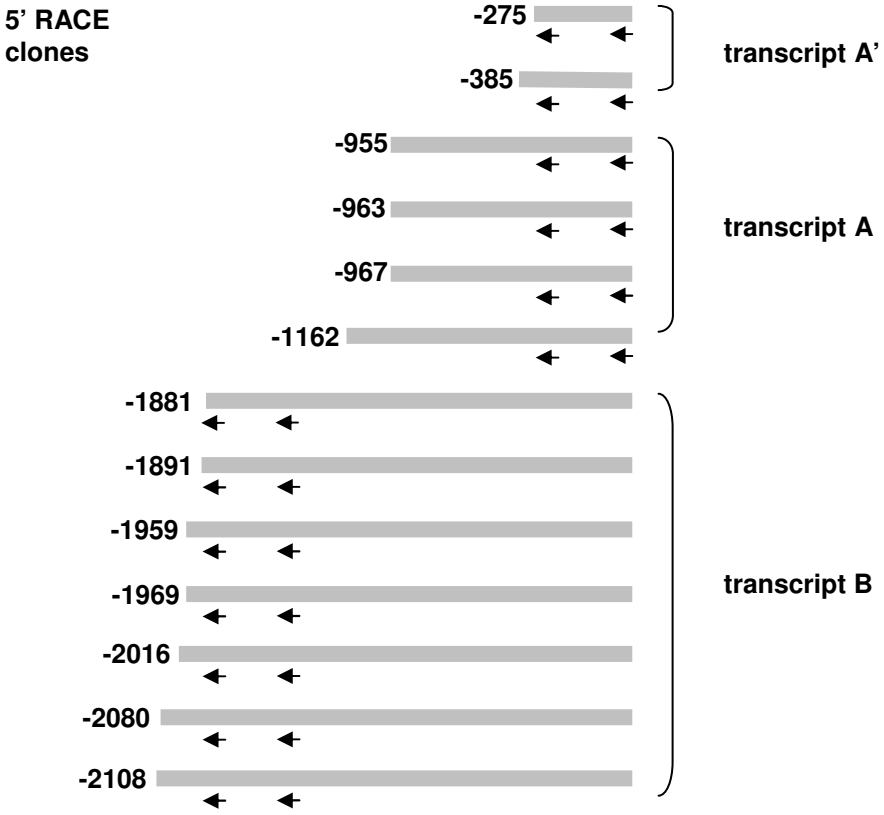
5
733/
920
6
1646/
1750
7
2027/
2180
8
2242/
2392


5' RACE clones
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-385
transcript A'
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transcript A
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-2080
-2108
transcript B

Figure S5

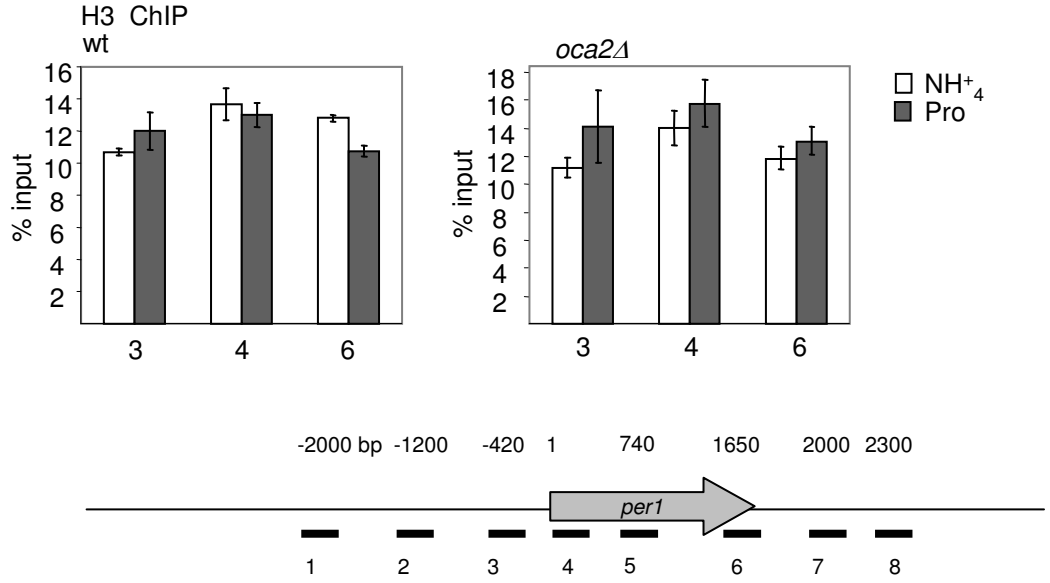
H3 ChIP
wt
oca2Δ
% input
NH+4
Pro
3
4
6
-2000 bp
-1200
-420
1
740
1650
2000
2300
per1
1
2
3
4
5
6
7
8

Figure S6