Supporting Material

A Polar Barrier to Transcription can be Circumvented by Remodeler-induced Nucleosome Translocation

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Supporting Experimental Procedures

Yeast Strains. To replace the entire *CUP1* locus with *URA3*, p391 was constructed by insertion of *URA3* as a *Pme*I-*Sma*I fragment from pNEB-URA3 into the *Pme*I site in pΔCup2A (1). To replace *URA3* at the *CUP1* locus (*cup1Δ::URA3*) with a single *CUP1* gene and *LEU2* in the same orientation, p416 was constructed by insertion of *CUP1* as a filled *Eco*RI fragment from pGEM-TAC into pΔCup3 (1). A unique *Xho*I site was engineered in the *CUP1* promoter at -9 with respect to the start codon at +1 in p416 to create p538. The 147 bp 603-sequence was obtained as an *Xho*I fragment by PCR using pGEM-3z-603 as template with primers 9 and 10 (Table S2). It was inserted at the *Xho*I site in p538 in the non-permissive orientation with respect to the transcription start site of *CUP1* to obtain p543, and in the permissive orientation to obtain p544. Insert sequences were verified. The parent strain is BJ5459 (2) which carries mutations in two of the vacuolar protease genes (***MATa*** *ura3-52 trp1 lys2-801 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL cir+*). The entire *CUP1* locus was deleted and replaced with *URA3* by transforming BJ5459 with an *Avr*II-*Swa*I digest of p391 to create BJ-*cup1Δ* For BJ-CUP1, BJ-603-NP and BJ-603-P, BJ-*cup1Δ* was transformed with an *Avr*II-*Swa*I digest of p416, p543 or p544, respectively, resulting in the replacement of *URA3* with *CUP1* and *LEU2*. Leu^+

transformants were selected and confirmed to be ura$^-$. Strains were verified using Southern blot hybridisation and PCR.

Northern Blots. RNA blots were hybridized simultaneously with a *CUP1* probe (the 459 bp *Pac*I-*Kpn*I fragment from pCPIA (1)) and an *ACT1* probe (a 799-bp fragment corresponding to +277 to +1075 relative to the start codon made by PCR using yeast genomic DNA and primers 11 and 12).

Supporting References

1. Shen, C.H., Leblanc, B.P., Alfieri, J.A. and Clark, D.J. (2001) Remodeling of yeast *CUP1* chromatin involves activator-dependent repositioning of nucleosomes over the entire gene and flanking sequences. *Mol. Cell. Biol.*, **21**, 534-547.

2. Jones, E.W. (1991) Tackling the protease problem in *Saccharomyces cerevisiae*. *Methods Enzymol.*, **194**, 428-453.

Legends to Supporting Figures

Supporting Figure S1. The experimental approach for analysis of the nucleosomal barrier to Pol II transcription *in vitro*. Pol II elongation complex (EC9, with 9-nt RNA) was assembled on the 50-bp DNA fragment and annealed to DNA or nucleosomes (before or after remodeling by ISW2) at 300 mM KCl (ISW2 dissociates from the templates). The immobilized complexes were washed, ligated, and transcribed in the presence of limited NTPs to produce pulse-labeled EC45. EC45 was released into solution by adding 0.1 M imidazole and transcription was resumed in the presence of all unlabeled NTPs.

Supporting Figure S2. ISW2-induced nucleosome translocation relieves the strong nucleosomal barrier on the 603-NP template. 603-NP DNA (D), nucleosome (N) or nucleosomes remodeled with ISW2 (N*ISW2) were transcribed by Pol II in 40 mM KCl without NTPs or in 40 mM, 150 mM, 300 or 1000 mM KCl with 0.4 mM NTPs. Pulse-labeled RNA was analyzed by denaturing PAGE. Nucleosome positions before (black) and after remodeling (grey) and H3-H4 tetramer-binding regions are indicated. The PBS sequence is indicated by the red line. Note that after ISW2 remodeling the +45 barrier is considerably decreased and the yield of run-off transcripts increased. Arrows on the right (DNA) indicate positions of non-ligated and ligated nucleosome-positioning DNA fragments.

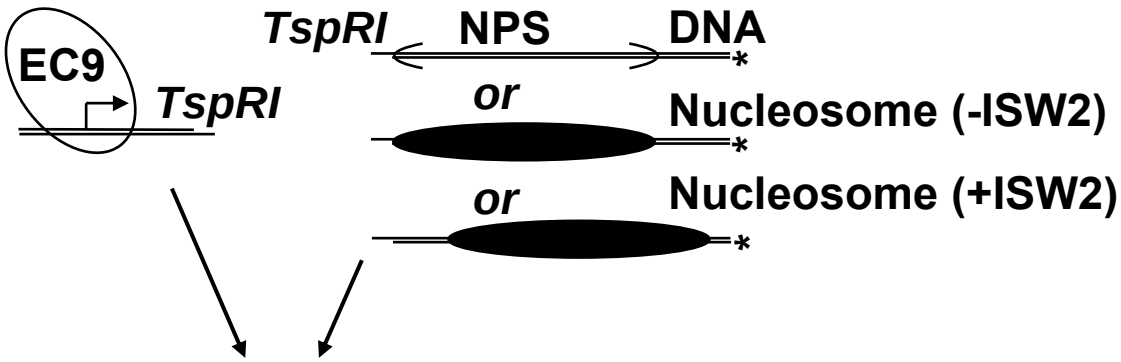
Supporting Figure S3. The efficiency of transcription through permissive 603-P nucleosome. 603-P DNA (D), nucleosome (N) or nucleosomes remodeled with ISW2 (N*ISW2) were transcribed by Pol II in 40 mM KCl without NTPs or in 40 mM, 150 mM, 300 or 1000 mM KCl

with 0.4 mM NTPs. Pulse-labeled RNA was analyzed by denaturing PAGE. Designations are as in Fig. S2. The position of the +15 nucleosomal barrier is indicated.

Supporting Figure S4. The DNA from nucleosome core particles is fully trimmed and is not significantly nicked after repair. (**A**) Native gel to ascertain the size and quality of repaired nucleosome core particle DNA: WT (lanes 1 and 2), 603-NP (lanes 3 and 4), 603-P (lanes 5 and 6). Each pair of lanes represents core particle DNA prepared from the same strain using different MNase concentrations. For each strain, the best trimmed sample with the least nicking was used in the occupancy assay. Core particle DNA was end-labelled using γ-^{32}P-ATP and resolved in an 8% polyacrylamide gel. The core particles were well trimmed: the DNA is ~ 150bp in size. M: Radiolabelled low molecular weight DNA ladder (NEB). A phosphorimage is shown. (**B**) Denaturing gel to test for nicking in the same repaired core particle DNA samples analysed in A: 10% polyacrylamide gel containing 8 M urea. The great majority of the core particles are the correct size (not nicked). M: Radiolabelled low molecular weight DNA ladder (NEB). A phosphorimage is shown.

Supporting Figure S5. Evidence for nucleosomes positioned over the *CUP1*-603 junction sequences in copper-induced cells. Cells were induced with 50 μM $CuSO_4$ for 20 minutes. Detection of nucleosomes positioned over the *CUP1*-603 junctions in **(A)** 603-NP cells, and **(B)** 603-P cells. The schematics shows a nucleosome correctly positioned on the 603-insert. The dotted ovals represent nucleosomes in positions which overlap the junctions of 603 with *CUP1*. The locations of the upstream and downstream *CUP1* primers and of the internal 603 primers 1 and 2 are indicated (small arrows) with their respective amplicon sizes. Phosphorimages of

quantitative PCR assays are shown. NCP = nucleosome core particles; gen. DNA = genomic DNA; M = end-labeled marker showing bands at 100 and 75 bp. Lanes 1 to 6: multiplex PCR with the upstream junction primer pair and *PHO5* promoter primers (internal control). Lanes 7 to 12: PCR with the downstream junction primer pair and *PHO5* primers.



Annealing at 300 mM KCl

↓

Immobilization, wash to remove ISW2

↓

Ligation

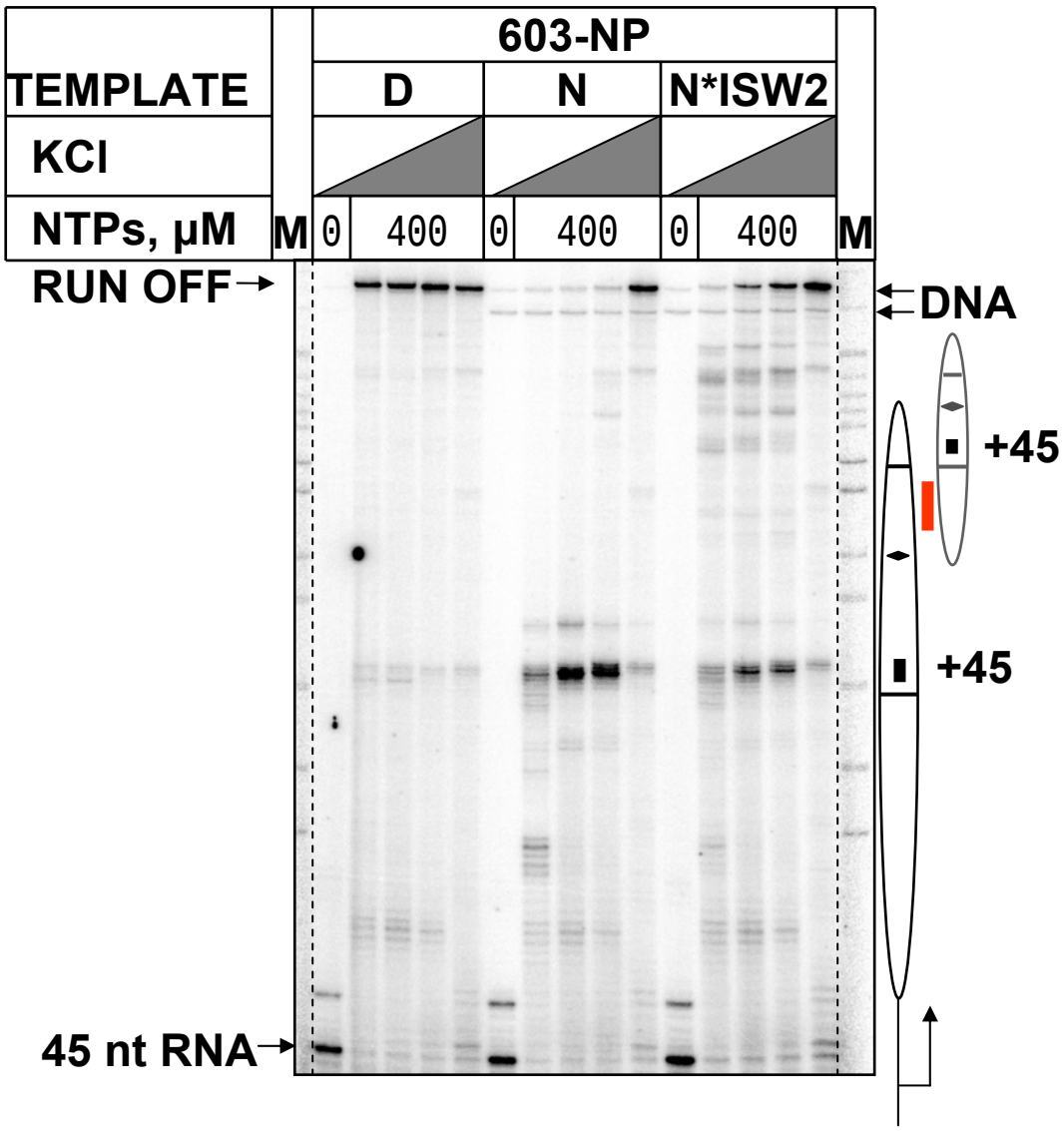
↓

Limited transcription to form pulse-labeled EC45

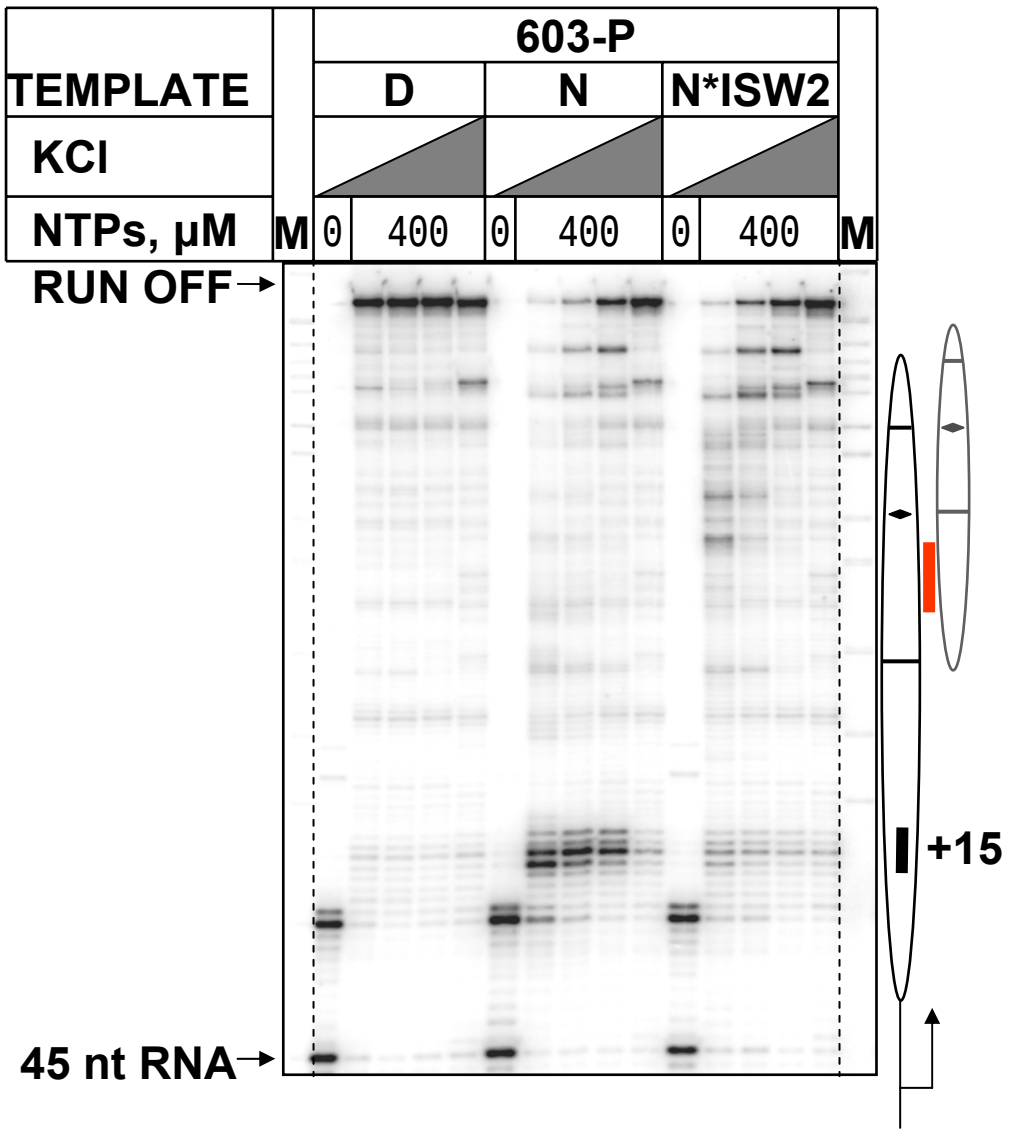
↓

Elution, transcription in the presence of all NTPs

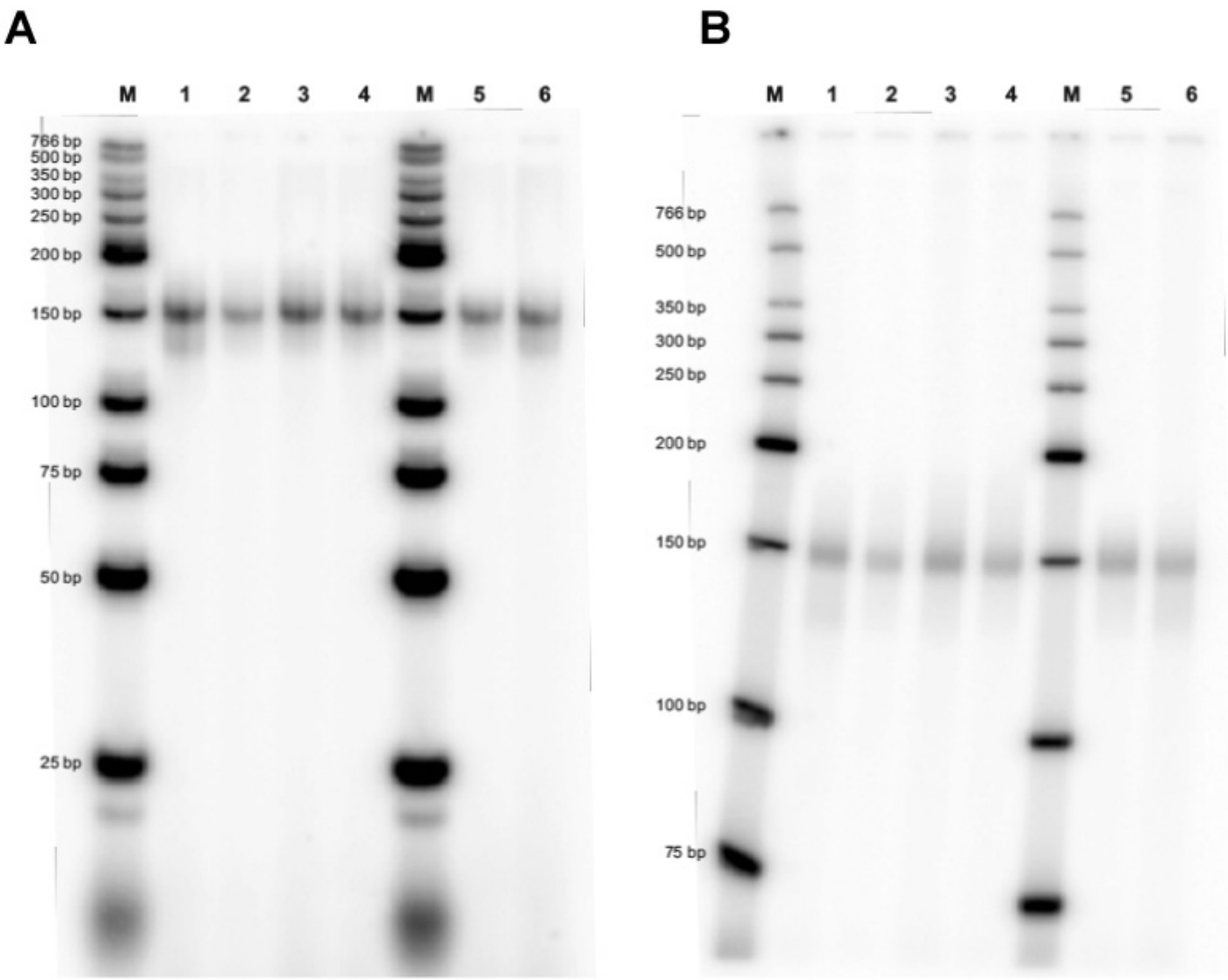
Gaykalova et al. Fig. S1



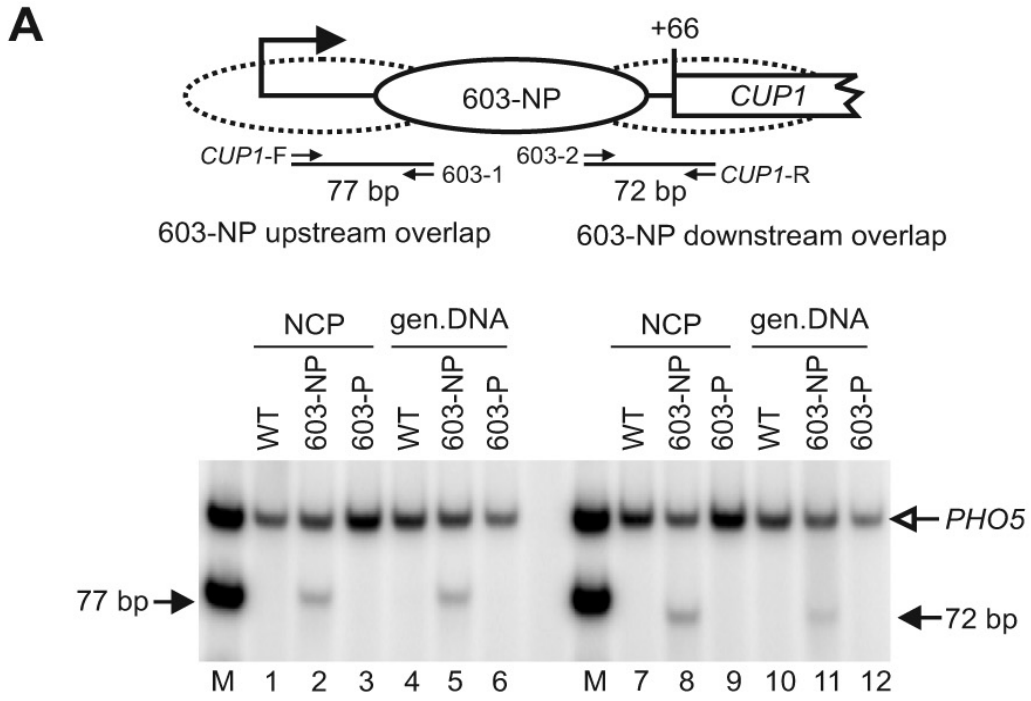
Gaykalova et al. Fig. S2

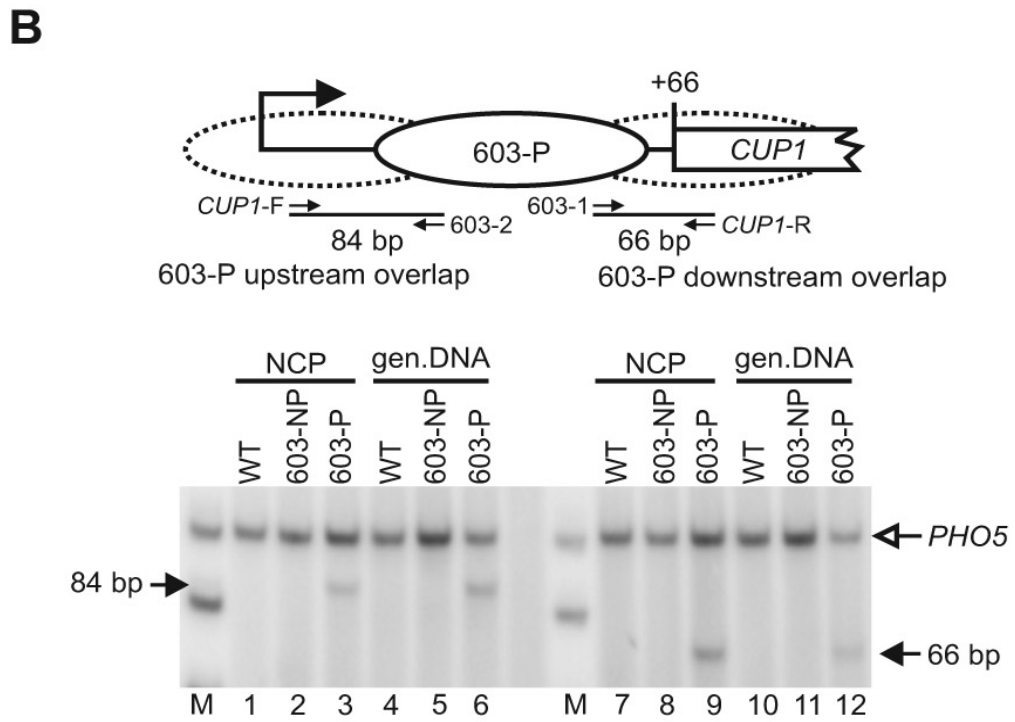


Gaykalova et al. Fig. S3



Gaykalova et al. Fig. S4





Gaykalova et al. Fig. S5

Supporting Table S1. Normalized fractional distribution of nucleosomes over the 603 insert and neighbouring *CUP1* sequences.

	Upstream junction	Central 603	Downstream junction
Non-induced 603-NP	0.20 +/- 0.03	0.40 +/- 0.01	0.40 +/- 0.04
Non-induced 603-P	0.28 +/- 0.02	0.41 +/- 0.09	0.31 +/- 0.10
Induced 603-NP	0.23 +/- 0.05	0.47 +/- 0.05	0.30 +/- 0.00
Induced 603-P	0.22 +/- 0.11	0.40 +/- 0.13	0.38 +/- 0.02

Core particle DNA signals were normalized to the *PHO5* promoter internal control and then normalized to the ratios for genomic DNA. The central 603 primers and the upstream and downstream junction primers should together detect all nucleosomes formed over the 603-insert. Therefore, the data are presented as fractions of the total normalized nucleosome signal: the sum of the normalized values for the upstream and downstream junction sequences and the central-603 sequence was taken as 1. Values represent the means and standard deviations of 2 independent experiments.

Supporting Table S2. PCR primers used in this study.

Primers used to prepare templates for *in vitro* transcription:

1. 601 forward primer	GTCGCTGTTCGGCACTGGGACAGGATGTATATATCTGAC
2. 601 reverse primer	GCATGATTCTTCACACCGAG
3. 603 forward primer	AAAATAATCGACAGGCACTGGGGCCCAGTTCGCGCGCCC
4. 603 reverse primer	ATTATATCCTCTAGAGTGGGAGCTCGGAACACTATCCG
5. 605 forward primer	ATAGCGACAGGTCAAGGTGGCACTGGGGCGGGATATGGG
6. 605 reverse primer	AGTTAGAGCTCGGAACACTATCCGACTGG
7. 603R forward primer	AGTCTCAAACCTTATGGCACTGGGTACCCCAGGGACTTGAAG
8. 603R reverse primer	GCCAAGCTATTTAGGTGACACTATAGA

Primers used for experiments *in vivo*:

9. 603 forward (*Xho*I)	GGCGCGCTCGAGCCCCAGGGACTTGAAG
10. 603 reverse (*Xho*I)	GGCGCGCTCGAGGCCCAGTTCGCGC
11. *ACT1* forward	ATCGATTGCTTCATTCTTTT
12. *ACT1* reverse	CTGAATCTTTCGTTACCAAT
13. *PHO5* internal control forward primer	GCAAGGCATATACCCATTTGG
14. *PHO5* internal control reverse primer	GCCATACTAACCTCGACTTAG
15. 603 nucleosome primer 1	CCCCAGGGACTTGAAG
16. 603 nucleosome primer 2	GCCCAGTTCGCGCGC
17. Central 603 primer 1	GTCACTCGGGCTTCTAAGTAC
18. Central 603 primer 2	CCTCTTTCAACATCGATGCAC
19. Upstream *CUP1* primer (+16 to +44 relative to the major transcription site of *CUP1*)	GATATTAAGAAAAACAAACTGTACAATC
20. Downstream *CUP1* primer (+244 to +217 relative to the major transcription site of *CUP1*)	TTAATTAATTCGCTGAACATTTTATGTG
21. Internal 603 primer 1	CTCCGTCCTTATTACTTCAAG
22. Internal 603 primer 2	GACGACTTCACACGGTAG