Supplemental Methods

Growth conditions

S. pombe was grown at 32 ºC in rich medium for the MNase experiments and for chromatin immunoprecipitation or in minimal medium to select for *ura4* transformants. *S. cerevisiae* was grown at 30 ºC in YP medium supplemented with 2% glucose. *S. pombe* strains 1_3 and Shuffled grew in minimal medium without uracil at a rate comparable to wild type cells. Strain 1_6 grew at a slightly lower rate in the same medium probably due to differences in the stability or efficiency of translation of the mRNA due to its higher G+C content (51.7%) relative to the wild type (42.9%). All the *S. pombe* strains grew at a comparable rate in rich medium.

Chromatin immunoprecipitation and qPCR

ChIP analysis in Supplemental Fig S5 was performed as described by Pidoux et al. (2004) with some modifications. Exponential *S. pombe* cells were fixed with 1% formaldehyde for 20 min at room temperature. Cells were disrupted in Fast Prep (3 pulses of 1 minute at speed 4.5) and chilled on ice for 2–3 min between each disruption step. Cell extracts were sonicated to shear chromatin to a size range of 200-600 nucleotides using a Diagenode Bioruptor Sonicator (5 cycles of 10 min each with alternating pulses of 30 sec on/off). Samples were incubated overnight at 4 ºC with 1 μg of a monoclonal antibody (8WG16-Abcam) against the CTD repeat (YSPTSPS) of the largest subunit of eukaryotic RNA polymerase II. Samples were purified with the GFX PCR DNA and Gel Band kit (GE Healthcare). Immunoprecipitated chromatin and whole cell extract control samples were resuspended in 70 μl of sterile water before being used as a template for qPCR analysis.

Digestion with MNase and indirect end-labelling analyses

400 ml exponential cultures of *S. pombe* cells were processed as described by de Castro et al. (2012) except that spheroplasts were resuspended to a final volume of 7 ml in NP-buffer and split into seven fractions of 1 ml. Six samples were digested with 0, 1.5, 3, 7, 15 and 30 units/ml of MNase at 37 °C for 10 minutes. DNA was isolated directly from the remaining sample (naked DNA control) and digested with 0.15 units of MNase under the same conditions. For *S. cerevisiae*, cells from 200 ml exponential cultures were permeabilized as for *S. pombe* excluding the preincubation step, and treated with 10 mg Zymoliase 20T for 10 min at 37 ºC. Chromatin was digested with 0, 0.5, 1.5, 3, 4.5 and 6 units/ml of MNase. DNA was purified from the MNase treated samples, digested with the appropriate restriction enzymes, electrophoresed, Southern blotted and hybridized with end terminal probes as indicated in the corresponding Figures. Some examples of the whole range of digestions with MNase are shown in Supplemental Fig S8.

Preparation of mononucleosomal DNA

S. pombe cultures of 200 ml at 0.8 x 10^7 cells/ml were collected for preparation of mononucleosomal DNA. Cells were treated with 8 mg of Zymolyase 20T during 30 minutes at 30 ºC to generate spheroplasts. Mononucleosomal fragments were generated by digesting DNA with 150 units/ml of micrococcal nuclease (MNase) at 37 ºC during 45 minutes. The amount of MNase was optimized experimentally to generate a 80:20 ratio of mononucleosomes to dinucleosomes (Lantermann et al. 2010).

S. cerevisiae cultures of 200 ml at 0.8 x 10^7 cells/ml were collected for preparation of mononucleosomal DNA. Cells were treated with 10 mg of Zymolyase 20T during 5 minutes at 30 ºC to generate spheroplasts. Mononucleosomal fragments were generated by digesting DNA with 300 units/ml of micrococcal nuclease (MNase) at 37 ºC during 10 minutes. The

amount of MNase was optimized experimentally to generate a 80:20 ratio of mononucleosomes to dinucleosomes, as described by Soriano et al. (2014). In the two cases, mononucleosomal DNA was recovered from 1.5% agarose gels.

References

Lantermann AB, Straub T, Strålfors A, Yuan G-C, Ekwall K & Korber P. 2010. *Schizosaccharomyces pombe* genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of *Saccharomyces cerevisiae*. *Nat. Struct. Mol. Biol.* **17:** 251–257

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