VanDemark et al, Supplemental Materials

Additional methods for analysis of yFACT *in vitro*.

For Fig 6A, nucleosomes were prepared with a duplex DNA derived by PCR from the sea urchin 5S rDNA sequence (1). The product was digested with *Eco*RI and *Sca*I, yielding a 143 bp duplex with a 4 nucleotide single-stranded extension at the left end. The sequence before endonuclease digestion was:
...g·AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCC
TTTAAATAGCTTAACTTTCATCAAGCAAGAGCCTACGACCATACCATGCTGAATATACCGGT
TCTCGTCCGATCACCGAAGTCAAGT·act...

Nucleosomes were reconstituted by gradual dialysis from a high salt solution using chicken histone octamers (2). Native polyacrylamide gels for the electrophoretic mobility shift assay (EMSA) were as described previously (3).

For Figs 6B and 6C, the same 5S rDNA sequence was used, but it was amplified with primers that produce the following sequence after *Eco*RI digestion:
AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCCTT
TAAATAGCTTAACTTTCATCAAGCAAGAGCCTACGACCATACCATGCTGAATATACCGGTTC
TCGTCCGATCACCGAAGTCAAGCagatatcggctcggttagt

The 162 bp duplex with a 4 nucleotide single-stranded extension was assembled into nucleosomes using recombinant yeast histone octamers. The unique *Dra*I site used to probe accessibility is underlined and is centered about 10 bp from the center of the 147 bp nucleosome positioning sequence (capitalized).

The Spt16 NTD does not appear to be a binding module for N-terminal histone tails.

Genetic analysis reveals weak interactions between the Spt16 NTD and N-terminal histone tails.

We previously showed that the function of yFACT is influenced, both positively and negatively, by modifications of the H3 and H4 tails (5, 3). For example, methylation of H3-K4 by Set1 and acetylation at various sites by different HATs support the role of yFACT, whereas methylation of H3-K36 by Set2 opposes yFACT function (5). These interactions could be mediated through other proteins that recognize modified histones or they could be due to direct binding of the N-terminal tails by yFACT. None of the subunits of yFACT have known sequence motifs associated with recognition of modified residues such as bromodomains or chromodomains, but other domains could have this property.

To test this idea, we initially asked whether the Spt16 NTD contributes to recognition of histone N-terminal tail modifications genetically. If the Spt16 NTD is responsible for recognizing a modified histone tail, and if this interaction is the only role of both the binding domain and the modified tail, then deleting the Spt16 NTD or the histone tail completely should each have the same effect because each will disrupt the interaction completely. Further, a strain with both mutations should have the same phenotype as a strain with either single mutation, because no further disruption of the interaction should be possible. In contrast, mutations that partially inactivate either the ability to bind or the ability to be recognized can have additive effects because either single mutation only partly disrupts the binding, and combining mutations can therefore cause an additive decrease in the interaction.

Deleting the Spt16 NTD causes weak sensitivity to 120 mM HU at 30°, and deleting the N-terminal tail of histone H3 causes somewhat more severe sensitivity (Fig S5A). Combining these deletions causes an additive defect, as the double mutant does not grow under these conditions. This result suggests that Spt16 NTD is not simply a binding module for the H3 N-terminal tail. We were unable to do a similar test with the H4 N-terminal tail because deleting this tail is lethal in the strains we tested (6). We showed previously that some point mutations in yFACT cause strong synthetic defects when combined with point mutations in H4 that block the acetylation pattern that is associated with nucleosome deposition (6). For example, combining *pob3-Q308K* with H4-K5R, K12R caused decreased viability. Each of these mutations only partially disrupts the ability to deposit nucleosomes, as each single mutant is viable and healthy under normal conditions, so in this case additive sensitivity was interpreted