



Sup. Fig 4. ISW2 dependent nucleosome positioning at other Ssn6-Tup1 regulated genes.

The chromatin structure of STE6, ANB1, and HUG1 loci was examined by MNase mapping. Black triangles point to the hypersensitive sites that are unique or enhanced under the repressed state, while the asterisks indicate the hypersensitive sites exposed in mutants or under the derepressed condition. (A) Chromatin structure at the STE6 locus in repressed Mat α cells compared to that in derepressed Mat a cells and in Mat α cell containing the $\Delta isw2$ mutation. Notice that deletion of ISW2 in $Mat\alpha$ cell results a digestion pattern similar to that of $Mat\alpha$ cell or naked DNA, and that ISW2-dependent nucleosome positioning extends far into the ORF. The open reading frame and the α2/MCM1-binding sites are indicated on the left. DNA was digested with Bgl II (at -678) and a probe corresponding to -685—444 was used for Southern Blotting. Our data agrees with reports published after the submission of this manuscript showing that deleting ISW2 derepresses mating type specific genes and affects the chromatin structure over the promoter of STE6 (Ruiz et al. 2003; McConnell et al., 2004). However, the mapping of the coding sequence of STE6 or comparison to the derepressed state (Mat a) was not presented in the later publication (McConnell et al., 2004). (B) Deleting ISW2 disrupts nucleosome positioning over ANB1. ANB1 locus was mapped by indirect end-labeling after digestion with EcoR I (at -964) and using a probe specific for bp -975—643. The locations of the TATA-box and Rox1 binding sites are indicated on the left. (C) Δ isw2 mutants have disrupted chromatin over HUG1. HUG1 was mapped by indirect end-labeling after digestion with Sac II (at +398) and using a probe specific to bp +267-+394. The putative Crt1 binding sites (DREs) and TATA box of HUG1 gene are indicated on the left. The ORF of HUG1 is only 207 bp long, approximately 1 nuc, and the underlying DNA is relatively insensitive to MNase so assigning a nucleosome position is not possible. However, changes in the digestion pattern over the TATA box and promoter are observed and are highlighted by asterisks.