Distribution and Dynamics of Chromatin Modification Induced by a Defined DNA Double Strand Break

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Supplemental Experimental Procedures

Culture Conditions for *HO* Induction

For *HO* induction in growing cultures, cells were grown to $0.5 - 10^7$ cells/ml in YEPL broth [S3] with vigorous aeration. A 10 ml sample was collected, D-galactose (Sigma, 20% w/v) was added (final concentration, 2% w/v), and $\leq$ 10 ml samples were collected at appropriate times. For induction of *HO* in G1-arrested cultures, *bar1* strains were grown to 0.5×10^7 cells/ml in YEPL broth, α-mating pheromone (Sigma) was added to a final concentration of 200 nM, and cells were incubated for 3 hr, at which point >95% of cells were unbudded schmoos. *HO* was then induced as above.

Chromatin Immunoprecipitation

ChIP was performed as described [S4], with the following changes. Lysis buffer contained 1 M NaCl to help block reassociation of free histones with DNA. Cells were agitated for 5 min with glass beads in a Mini-Bead Beater-8 (Bio Spec). Lysates were sonicated to an average fragment size of 0.5 1 kb. Primary antibodies were against γ-H2AX [S5], Mre11p [S6], and the HA epitope (monoclonal antibody 12CA5, Roche). Later experiments used rabbit polyclonal antiserum against the yeast-specific phosphopeptide, with no significant change in relative ChIP ratios. Immunocomplexes were bound to immobilized Protein G (Pierce), washed five times in lysis buffer with 1 M NaCl, three times with lysis buffer with 0.5 M NaCl, and twice with the final wash as described [S4], and were eluted in 10 mM Tris and 1 mM EDTA (pH 8.0). Eluates were incubated at 65°C for $\geq$6 hr and then at 95°C for 20 min to dissociate cross-linked protein from DNA. Genomic DNA was prepared as described [S7]. All samples for a given experiment were processed in parallel.

PCR and Data Analysis

One to ten percent of each immunoprecipitate or 250 pg of purified DNA was used in multiplex PCR reactions containing two primer pairs: a pair specific to a sequence of interest near *MAT* and a control pair specific to *YCL011c* (Table S2). PCR reactions contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 25 mM MgCl2, 200 mM each dNTP, 25 pM each oligonucleotide, and 2.5 units of Taq polymerase (Promega). PCR reactions were 25 cycles of: 95°C, 15 s; 55°C, 15 s; 72°C, 30 s. Products were displayed on 10% polyacrylamide gels, stained with SYBR Green 1 (Molecular Probes), imaged on a Fuji LAS1000 imaging system, and quantified with Image Gauge V4.0 (Fuji).

Table S1. Yeast Strains

Strain Name	Relevant Genotype
JKM179 [S1]	*MAT*α
CY3265	*MAT*α *dun1::KAN*
YAA2	*MAT*α *chk1::KAN*
JKM187	*MAT*α *mre11::hisG*
YAA25	*MAT*α *sml1::KAN mec1::NAT*
H987	*MAT*α *tel1::URA3*
YAA45	*MAT*α *sml1::KAN mec1::NAT tel1::TRP1*
YMV73-3	*MAT*α *rad53::NAT sml1::KAN*
JKM139 [S2]	*MAT*a
H1069	*MAT*a *HA-6xHis- HTB2-URA3*
yXW1 [S2]	*MAT*a *bar1::ADE3*
R664	*MAT*a *bar1::ADE3 tel1::TRP1*
H1073	*MAT*a *bar1::ADE3 mre11::hisG*
H1081	*MAT*a *bar1::ADE3 sml1::KAN mec1::NAT*
H1132	*MAT*a *bar1::ADE3 sml1::KAN tel1::TRP1 mec1::NAT*

All strains are *ho hml::ADE1 leu2–3, 112 hmr::ADE1 ade1 lys5 trp1:: hisG ura3-52 ade3::GAL10::HO*. Parent strains are underlined; derivates are indented. H1069 contains an *HA-6xHis* immunotag fused in-frame to the *HTB2* N-terminus and the *URA3* gene inserted in noncoding sequences 3′ to *HTB2* (gift from Namrita Dhillon).

DSB Analysis

DNA samples were digested with SspI (New England Biolabs) and displayed on alkaline agarose gels as described [S8]. Gel blots were probed with a single-strand probe complementary to the unresected DSB strand, prepared by single-primer linear amplification [S7] with primer 7 (Table S2) and a PCR product (with primer 7 and HO-R, 5′-GAAACACCAAGGGAGAGAAGAC-3′). This probe contains sequences 20–715 nucleotides to the right of the HO-cut site. Blot radioactivity was detected with a Fuji BAS2000 phosphorimager and quantified with ImageGuage 4.0 software (Fuji).

Supplemental References

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S3. Rudin, N., and Haber, J.E. (1988). Efficient repair of HO-induced chromosomal breaks in *Saccharomyces cerevisiae* by recombination between flanking homologous sequences. Mol. Cell. Biol. *8*, 3918–3928.

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S7. Liu, J., Wu, T.C., and Lichten, M. (1995). The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. EMBO J. *14*, 4599–4608.

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Table S2. PCR Primers

Primer Name	Sequence	kb from *MAT* DSB
a. 25	5′-GTATCACGAGCCCATCTCCAATAG-3′	
b. 26	5′-CATCCCTCACCAAGGAAGAAAGAG-3′	−46.37
a. 23	5′-CTCTTTCTTCCTTGGTGAGGGATG-3′	
b. 24	5′-GTTGTGTCCTTAGTTAGGTTCAGCG-3′	−40.44
a. 19	5′-TCGTCGTCGCCATCATTTTC-3′	
b. 20	5′-GCCCAAGTTTGAGAGAGGTTGC-3′	−29.61
a. 17	5′-GGAGTCAGATGGAACTGGGGTAAC-3′	
b. 18	5′-TGCCGCTAAGTGTAAGGAAGTCG-3′	−23.04
a. 15	5′-CGTCTTCTCAGCGAACAACAGC-3′	
b. 16	5′-GCAATAACCCACGGAAACACTG-3′	−16.44
a. 13	5′-TCAGGGTCTGGTGGAAGGAATG-3′	
b. 14	5′-CAAAGGTGGCAGTTGTTGAACC-3′	−9.32
a. 1	5′-ATTGCGACAAGGCTTCACCC-3′	
b. 2	5′-CACATCACAGGTTTATTGGTTCCC-3′	−5.08
a. 39	5′-TGTCGGTAGTGTCAGCAAACACG-3′	
b. 40	5′-CCTGAAATTCAAGCATTGCCTC-3′	−4.18
a. 3	5′-ATTCTGCCATTCAGGGACAGCG-3′	
b. 4	5′-CGTGGGAAAAGTAATCCGATGC-3′	−3.43
a. 43	5′-CCAGATTTGTATTAGACGAGGGACG-3′	
b. 44	5′-AAGATGCTGCCGCACAACTCTCAC-3′	−1.90
a. 3-1	5′-ATGTCCTGACTTCTTTTGACGAGG-3′	
b. 4-1	5′-ACGACCTATTTGTAACCGCACG-3′	−1.35
a. 45	5′-CCACATTAAATACCAACCCATCCG-3′	
b. 46	5′-TAGTGATGAGGAGAAGAAGTTGTTGC-3′	−0.77
a. 5′BreakF	5′-AAGAGTTTGGGTATGTAATATGAG-3′	
b. 5′BreakR	5′-CTGAAACTAAAAGAAAAACCCGAC-3′	−0.01
a. 7	5′-CCTGGTTTTGGTTTTGTAGAGTGG-3′	
b. 8	5′-GTACAAACACATCTTCCCAATA-3′	0.02
a. 49	5′-CATGCGGTTCACATGACTTTTGAC-3′	
b. 50	5′-GGAAGTAACCTCTACTGTGGAGGCAC-3′	0.48
a. 51	5′-TGGACGGAGGACTTAATATCGTCAC-3′	
b. 52	5′-AGGATGCCCTTGTTTTGTTTACTG-3′	1.02
a. 9	5′-AACGCTCGTCGATCGCCGTTCTAA-3′	
b. 10	5′-AATGGATTTGCCAAATGCACAT-3′	1.81
a. 53	5′-CGGAGTACCATCGTGTTCATGG-3′	
b. 54	5′-CTGGGTATAAGGCAAGTTCAAGATG-3′	2.29
a. 11	5′-GCGGTTTCTCGAAGTCTTGTTCCA-3′	
b. 12	5′-ACGAATGAGTTAGCATGGGCACTT-3′	2.80
a. 55	5′-CCTCTTCCTCTTCATCGTGCTCAG-3′	
b. 56	5′-GCAGTTCTGCATCATATTGGGTCC-3′	3.72
a. 27	5′-CAGGTTTATATCCACCTTCATCGG-3′	
b. 28	5′-TTTGGGGCAACAGTAGGCAGTG-3′	5.67
a. 29	5′-GCAATCGTGTCAATGTGGTCATC-3′	
b. 30	5′-GTTTCAGGAGCCCCATAATCAAC-3′	7.77
a. 31	5′-CGTTGTCTTTTCGTTTGGTGTCTG-3′	
b. 32	5′-GCTCTTTGCCCCTGTCTTTGAC-3′	12.84
a. 33	5′-GAACCAAACGCCAGCCATTC-3′	
b. 34	5′-GCCAAAGTAAAGTGTGTCACCTTCC-3′	21.41
a. 35	5′-TCCAGGCGGGTGTGAAAAAC-3′	
b. 36	5′-ATGGGGAATACGGAAGTGGGTC-3′	29.43
a. YCR011CF	5′-CGTTACTTTCCTCATCACCTTCGC-3′	
b. YCR011CR	5′-ACAGAGAGAGTGGGCTCATCTTGC-3′	−65.93

Left-hand and right-hand primers in each pair are labeled “a” and “b.” All distances are calculated from the closest point of the PCR product to the HO-cut site in a *MAT*a strain. Primers with negative values are between the HO-cut site and *CEN3*. PCR product sizes range from 143 to 204 bp, with the exception of the YCR011CF/YCR011CR (217 bp) and MAT3-1/MAT4-1 (246 bp).