Supplemental material

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Spatial organization and dynamics of the association of Rec102 and Rec104 with meiotic chromosomes

Supplemental Table. Yeast strains

Strain	Genotype[a]
NKY611	*MAT**a**/ MATα ho::LYS2 ura3 lys2 leu2::hisG*
SKY212	*REC102-myc9::URA3*
SKY428	*REC102-myc9::URA3 spo11Δ::hisG-URA3 -hisG*
SKY507	*REC102-myc9::URA3 rec104Δ*
SKY510	*REC102-myc9::URA3 rad50Δ::hisG-URA3-hisG*
SKY513	*REC102-myc9::URA3 mre11Δ::hisG-URA3-hisG*
SKY516	*REC102-myc9::URA3 hop1Δ::URA3*
SKY519	*REC102-myc9::URA3 mei4Δ::URA3*
SKY568	*trp1::hisG arg4-Bgl/arg4-Nsp his4B::LEU2/his4X::LEU2 REC102-Flag3::TRP1*
SKY670	*REC102-myc9::URA3 rec114Δ::KanMX4*
SKY673	*REC102-myc9::URA3 ski8Δ::KanMX4*
SKY676	*REC102-myc9::URA3 mer2Δ::LYS2*
SKY788	*REC102-myc9::URA3 REC8-HA3::URA3*
SKY791	*myc8-REC104*
SKY807	*myc8-REC104 rec102Δ::URA3*
SKY810	*myc8-REC104 spo11Δ::hisG-URA3-hisG*
SKY813	*myc8-REC104 mre11Δ::hisG-URA3-hisG*
SKY819	*myc8-REC104 mer2Δ::LYS2*
SKY825	*myc8-REC104 REC102-Flag3::TRP1*
SKY893	*myc8-REC104 ski8Δ::KanMX4*
SKY896	*myc8-REC104 rec114Δ::KanMX4*
SKY962	*myc8-REC104 mei4Δ::URA3*

[a] All strains are *MAT**a**/MATα* diploids and are homozygous for *ho::LYS2*, *lys2*, *ura3*, and *leu2::hisG.* Strain NKY611 was provided by N. Kleckner; all other strains were derived in this laboratory. All alleles are homozygous unless otherwise specified.

Supplementary Materials and Methods

Construction of *mycREC104*

Two cassettes were constructed, each containing eight repeats of the myc epitope, one cassette ending with a stop codon. These cassettes were ligated to a 1.1 kb *URA3* fragment, creating a ~1.7 kb *myc8(stop)-URA3-myc8* fragment in pBSII KS+ (pKK16). This construct can be targeted to the 5′ end, 3′ end, or defined internal positions within any gene. To tag *REC104*, ~500 bp fragments upstream and downstream of the first coding nucleotide were amplified from SK1 genomic DNA. A third fragment containing the *myc8(stop)-URA3-myc8* cassette was PCR amplified from pKK16 using primers containing 50 bp of sequence upstream and downstream of the *REC104* start codon. This fragment was then re-amplified using the two 500 bp fragments as megaprimers. The resulting ~2.8 kb fragment was gel purified and used to transform an SK1 strain. Ura- subclones in which intrachromosomal recombination had left only one *myc8* cassette fused to 5′ end of *REC104* were selected on 5-FOA. The final construct was verified by sequencing. MycRec104 protein migrated on SDS-PAGE with an apparent molecular weight of 58,000 (55,000 for the hypophosphorylated form), significantly larger than the 30,000 predicted from its amino acid sequence. We have noted similar anomalous migration for other proteins tagged with multiple epitopes (unpublished observations).

Chromosome spreads

Five ml of meiotic culture (~1.6 OD_{600}) was harvested and washed in 1 ml prespheroplast buffer (100 mM Tris-HCl, pH 9.4, 10 mM DTT). Cells were resuspended in 1 ml spheroplast buffer (50 mM potassium phosphate, pH 7.4, 0.6 M sorbitol, 10 mM DTT), 10 μl of 10 mg/ml zymolyase 100T was added, the suspension was incubated 10 min at 30°C, then the spheroplasts were pelleted and washed in 50 mM HEPES-NaOH, pH 7.5, 100 mM KCl, 2.5 mM $MgCl_2$, 0.4 M sorbitol. The pellet was resuspended in 1 ml of 0.1 M MES-NaOH. pH 6.8, 1 mM EDTA, 0.5 mM $MgCl_2$, 0.5% (v/v) Lipsol, plus protease inhibitors (0.4 mM pefabloc-SC, 1 mM PMSF, 1 μg/ml each of leupeptin, pepstatin A, and chymostatin). After ~15 min on ice, 50 μl of the suspension was placed on a glass slide and 150 μl of 4% (w/v) paraformaldehyde was dripped onto it. A glass rod was used to distribute the mixture evenly. Slides were dried overnight at 4°C and stored at -20°C. Staining was performed with mouse monoclonal anti-myc (1:500 dilution, Covance); rat monoclonal anti-HA (1:100, Boehringer); or guinea pig polyclonal anti-GST-Zip1 (1:1000; K. Henderson, this laboratory). Secondary antibodies from Molecular Probes were used at 1:500 dilution: goat anti-mouse Alexa-488; goat anti-rat Alexa-594; goat anti-guinea pig Alexa-546.

Evaluating Rec102-Rec8 overlap

Because the staining patterns of both Rec8 and Rec102 are often irregular rather than being limited to discrete foci, we evaluated overlap between these proteins on a pixel-by-pixel basis in the CCD-captured images using the mask features of the Slidebook software package. First, a threshhold fluorescence intensity was assigned based on visual inspection of the Rec8 immunofluorescence channel. This threshhold served as a cutoff to distinguish Rec8-positive and Rec8-negative pixels. The process was then repeated separately for the Rec102 immunofluorescence channel to divide the image into Rec102-positive and Rec102-negative groups of pixels. The Rec102-positive pixels were then

further subdivided into those that were also Rec8-positive and those that were Rec8-negative. The total Rec102 fluorescence was measured by calculating the sum of the signals in all Rec102-positive pixels, corrected for the background fluoresence in the image (estimated by measuring the average fluorescence intensity in all Rec102-negative pixels). Total signals in the Rec8-positive (overlapping) and Rec8-negative (non-overlapping) subpopulations of Rec102-positive pixels were summed in the same manner.

To evaluate the possible contribution of fortuitous overlap, we selected 7 symmetrically spread leptotene nuclei, rotated the Rec102 fluorescence channel 180° relative to the Rec8 channel, and measured the overlap using the same threshhold settings as for the unrotated image.

To assess colocalization, proper alignment of the images in the two fluorescence channels was essential. We gauged alignment for the light path in our microscope-camera setup by imaging 0.2 μm diameter fluorescent beads (Tetraspek, Molecular Probes) and comparing the alignment of the bead images in the red and green fluorescence channels. Discrepancies of 1-2 pixels in the X and Y axes were observed; all chromosome spread images were therefore corrected prior to quantification using the Align Channel function in the Slidebook software.

Phosphatase treatment

~4 × 10^8 cells were collected at 4 hr in meiosis and lysed by agitation with glass beads in 20% TCA. Precipitated material was dissolved in SDS buffer, then diluted 10-fold with 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl plus protease inhibitors. Myc-tagged protein was immunoprecipitated with rabbit polyclonal anti-myc antibody (Covance) and protein G agarose (Roche), then the immunoprecipitate was equilibrated in phosphatase buffer (New England BioLabs) and divided into three aliquots. One aliquot was left untreated, one was treated with 50 units lambda phosphatase, and the third was treated with phosphatase plus phosphatase inhibitors (50 mM EDTA, 50 mM NaF, 10 mM Na_3VO_4). Samples were then boiled in SDS buffer and analyzed by western blotting.