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Non-paradoxical evolutionary stability of the recombination initiation landscape in yeast†

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Abstract

The nonrandom distribution of meiotic recombination shapes heredity and genetic diversification. Theoretically, hotspots — favored sites of recombination initiation — either evolve rapidly toward extinction or are conserved, especially if they are chromosomal features under selective constraint, such as promoters. We tested these theories by comparing genome-wide recombination initiation maps from widely divergent *Saccharomyces* species. We find that hotspots frequently overlap with promoters in the species tested and, consequently, hotspot positions are well conserved. Remarkably, the relative strength of individual hotspots is also highly conserved, as are larger-scale features of the distribution of recombination initiation. This stability, not predicted by prior models, suggests that the particular shape of the yeast recombination landscape is adaptive, and helps in understanding evolutionary dynamics of recombination in other species.

Introduction

DNA double-strand breaks (DSBs) generated by the Spo11 protein initiate meiotic recombination, which alters genetic linkage and promotes pairing and accurate chromosome segregation (1). DSBs are distributed nonrandomly across genomes, occurring often within narrow regions called hotspots (2). Theoretical work exploring evolutionary dynamics of recombination has led to a prevailing hypothesis, the “hotspot paradox”, that predicts rapid hotspot extinction (3–7). This view rests on biased gene conversion, in which the broken

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chromosome copies genetic information from its uncut homolog, possibly generating an extra copy of a genetic variant (Fig. 1A). Consequently, hotspot alleles with different DSB activity deviate from a Mendelian segregation ratio, with less recombinationally active alleles overrepresented among the offspring. This type of meiotic drive is observed in yeast (8) and humans (9) and predicts that mutations that reduce or eliminate hotspot activity will be rapidly fixed in populations, while hotspot-activating mutations are rapidly extinguished (3, 5, 10). The paradox is that hotspots exist at all despite this drive against them.

One answer to this paradox comes from PRDM9, a mammalian histone methyltransferase with an array of Zn-finger modules that rapidly evolve new DNA binding specificity (11). PRDM9 targets DSB formation near its binding sites, thus dictating hotspot positions. PRDM9 recognition motifs, which have no known intrinsic function, are lost quickly from genomes of humans and mice because of meiotic drive from biased gene conversion (12–14), but appearance of new *PRDM9* alleles with different sequence specificity creates new hotspots and redraws the recombination landscape (11). This hotspot-targeting mechanism confirms the rapid extinction predicted by the hotspot paradox and explains how hotspots can nonetheless exist. However, most taxa (including yeast and some mammals) lack such a system, so it has remained unclear how generalizable this solution is.

An alternative view predicts that hotspot positions can be evolutionarily stable if Spo11 targets genomic features that are under selective constraint for functions unrelated to their roles as hotspots (8, 15). This hypothesis derives from correspondence of most hotspots in *S. cerevisiae* with promoter-containing intergenic regions (IGRs) (15). However, theoretical studies have considered this implausible as a mechanism to preserve hotspots (3, 10). Instead, many studies start from the assumption that hotspot lifespan must always be short and that the fine-scale recombination initiation landscape will always be highly dynamic over evolutionary scales (4, 6, 7, 16). This assumption is appropriate for primates and mice because they use PRDM9, but has not been evaluated for other taxa.

High resolution double-strand break maps in Saccharomycetes

To distinguish between these models, we asked whether the DSB landscape is conserved in yeast. Previously, population genetic data were used to deduce a recombination map in *S. paradoxus* and compare it to *S. cerevisiae* (17). Partial conservation was inferred, but the data had insufficient resolution to detect individual hotspots (15). We overcame these limitations by comparing high-resolution, whole-genome DSB maps between widely diverged *Saccharomyces* species and between *S. cerevisiae* strains (the laboratory strain SK1 and wild-derived strains YPS128 and UWOPS03-461.4) (18) (Fig. 1B, Table S1). DSB maps were generated by deep-sequencing of DNA oligonucleotides (oligos) covalently bound to Spo11 as a byproduct of DSB formation (15, 19) (Fig. 1A, Table S2).

The *Saccharomyces sensu stricto* clade last shared a common ancestor ~20 million years ago (20). We examined species ranging from *S. paradoxus*, with coding sequence divergence from *S. cerevisiae* comparable to that between humans and mice (~100 million years divergence), to *S. kudriavzevii*, roughly as distant as mammals from birds (~300 million years divergence) (21) (Fig. 1B). The *S. cerevisiae* strains chosen display 0.5–0.7%

sequence divergence, comparable to the polymorphism density between humans and chimpanzees. Most differences are simple sequence polymorphisms (SNPs and small indels), with few large-scale structural differences aside from one discussed below (18, 22).

All yeasts examined underwent synchronous and efficient meiosis (fig. S1A), hence the strain SK1 is not anomalous in this regard. As in *S. cerevisiae*, two major size classes of Spo11-oligo complexes were observed (Figs. 1C, S1B), reflecting oligos of similar length distributions (Fig. 1D). Each oligo is a tag recording where Spo11 generated a DSB, and maps based on deep sequencing (23) agree spatially and quantitatively with direct detection of DSBs by Southern blot (15). Biological replicate maps were highly reproducible (Figs. 1E, S2) and most sequenced reads (>98%) were mapped uniquely (Table S2).

Targeting of breaks to promoters is conserved

We asked whether targeting of promoters is conserved among yeast. We mapped nucleosomes by sequencing micrococcal nuclease-resistant DNA (MNase-seq) from meiotic cultures (23). In *S. cerevisiae*, DSBs form preferentially in promoter-associated nucleosome-depleted regions (NDRs) (15, 24), and promoter chromatin structure during mitotic growth is conserved among other *Saccharomyces* species (25). Spo11 oligos were highly enriched in promoter NDRs in all species tested, whether examined at individual locations (Figs. 2A, S3A), or averaged across annotated genes (Figs. 2B, S3B). Many Spo11 oligos mapped to promoter-containing IGRs (i.e., IGRs flanked by divergent or tandemly oriented genes), whereas few mapped to convergent IGRs (i.e., lacking promoters) or within genes (Figs. 2D, S3D). We conclude that the Spo11 preference for promoters is a stable feature of the *Saccharomyces* DSB landscape.

Similar numbers of Spo11-oligo hotspots (~4000) were identified in all species (Table S3). When ranked by Spo11-oligo count, hotspots formed a smooth continuum over a wide range, with nearly superimposable cumulative curves in all species (Figs. 2E, S3E). Hence, the distribution of DSBs among hotspots is the same. Hotspots had low average nucleosome occupancy (Figs. 2C, S3C) consistent with open chromatin structure providing a window of opportunity for Spo11 (26). The distribution of hotspot width was also nearly identical, with wider hotspots tending to have more Spo11 oligos (Figs. 2F, S3F). Conserved hotspot width agrees with conservation of NDR width observed previously (25). Importantly, most hotspots overlapped the same promoter-containing IGRs in all species examined (Figs. 2G, S3G). The low frequency of sex and outcrossing in yeasts could slow hotspot extinction compared to obligately outcrossed species (17), but the yeasts examined here have had ample sexual generations to allow biased gene conversion to erode hotspots. For example, there have been an estimated >200,000 outcrossed sexual generations since divergence of *S. cerevisiae* from *S. kudriavzevii*, comparable to the number of human sexual generations since divergence from chimpanzees (23). Thus, as predicted (8), DSB hotspot locations can be preserved when the targeted chromosome architecture is conserved.

Conservation of DSB frequency in hotspots

The hotspot paradox predicts that hotspot strength should vary widely even if their locations are conserved. Furthermore, the rate of hotspot extinction should scale with hotspot heat,

because alleles that experience frequent DSBs provide more chances for loss (3–5, 10, 13). The selective constraint model is agnostic in this regard: if cis-acting sequence polymorphisms can quantitatively modulate DSB formation without ablating Spo11 targeting (which has been experimentally shown (e.g., 27)), then hotspot heats will change rapidly. On the other hand, if DSB frequency (not just position) is tied to selectively constrained features, or if DSB frequency is itself constrained, then hotspot heats will tend to be conserved.

To address this question, we summed Spo11 oligos within 3426 promoter-containing IGRs that could be stringently and unambiguously matched between species on the basis of conservation of flanking coding sequences (Fig. 3A, Table S4). This group contains 81% of divergent and tandem IGRs and accounts for 83% of promoter-proximal hotspots in *S. cerevisiae*, thus most of the relevant Spo11-targeted genomic space is included. An IGR-centric approach is preferable to relying on more arbitrary hotspot definitions (23). Within-IGR Spo11-oligo counts were highly similar between *S. cerevisiae* strains: we observed correlation coefficients (0.89–0.92) that were nearly as high as for comparisons between biological replicates (0.97–1.00) (Figs. 3B–D, S4A, Table S4). Thus, intra-species variation of DSB heat within these IGRs is low despite ~0.7–1% median sequence divergence.

Strong correlations were also found between species, with little change in correlation strength over large evolutionary distances (Figs. 3B–D, S4A). Moreover, the hottest 1% of promoter IGRs in *S. cerevisiae* SK1 were enriched among the hottest IGRs in other species, with a median percentile ranking within the top 5% even in *S. kudriavzevii* (Fig. 3E, F). This was only modestly greater than the extent of conservation of the coldest IGRs (Fig. 3E). Theoretical modeling of biased gene conversion predicts that strong hotspots are less likely to be shared between species than weak ones (5). We found specific examples where strong hotspots in one species were substantially weaker in other species (Fig. 3G), so there is no absolute barrier to evolutionary changes. But the behavior of most IGRs leads us to conclude that the hottest hotspots present in the last common ancestor of *Saccharomyces* tended to retain high Spo11 target activity, and that it has been rare for ancestrally cold promoters to acquire strong hotspot activity.

This high degree of yeast hotspot conservation differs markedly from that in humans: DSB hotspot heat between men sharing the same or similar *PRDM9* alleles (28) was less conserved than between *S. cerevisiae* strains despite much greater sequence identity (Fig. 3C, D, fig. S4B). This difference is consistent with PRDM9 motif erosion contributing to variation in hotspot strength between individuals (28).

Conservation of the DSB landscape over larger size scales

Hotspots are only one level of non-randomness in the DSB landscape in that they reside within larger domains of greater or lesser DSB potential (2, 15). In several taxa, conservation has been noted for the distribution of crossover recombination over broad genomic regions (16), but conservation of DSB distributions has not been evaluated. We therefore investigated if large-scale features of the DSB landscape are also conserved in yeast. Spo11-oligo maps demonstrated that DSB suppression observed near telomeres and

centromeres (15, 26) is preserved (Figs. 4A, B, S5A, B). This result is not surprising, as recombination in these subchromosomal regions can interfere with genome integrity: subtelomeric regions are rife with repetitive DNA elements that can undergo nonallelic homologous recombination (29), and crossing over that occurs close to centromeres can cause segregation errors (30)).

More remarkably, however, Spo11-oligo counts were also well correlated between species when we compared ~20-kb segments in syntenic regions across interstitial (i.e., non-telomeric and non-centromeric) portions of the chromosomes (Figs. 4C, D, S5C–F, Table S5). This scale is comparable to the average length of the chromatin loops of meiotic chromosomes, and DNA segments of this size typically encompass multiple hotspots (1, 15). These findings indicate that the larger-scale domain structure of the DSB landscape is also evolutionarily stable. Spo11-oligo counts were correlated with G+C content of DNA in each species tested, with weaker correlation over short distances (~1 kb) and stronger correlations over large distances (Fig. 4E). This scale-dependent pattern is consistent with the hypothesis that large-scale DSB domains, like hotspots, reflect selective constraint on the underlying chromosomal architecture (15). Furthermore, such large-scale domains presumably reflect factors — such as attachment of chromatin loops to chromosome axes — that work in cis but at a distance from DSB hotspots. Because such factors are too far to be frequently included in gene conversion tracts and are thus not subject to loss through biased gene conversion, they are not expected to evolve as rapidly as hotspots (3, 5, 16, 31).

Chromosome length affects DSB frequency

In *S. cerevisiae*, DSB density is anti-correlated with chromosome size, i.e., smaller chromosomes on average incur more DSBs per kb than larger ones (15). This relationship is conserved in other Saccharomycetes (Figs. 5A, S6). This whole-chromosome control of DSB density is in large part a patterning effect of a negative feedback circuit in which homologous chromosomes that have successfully engaged one another stop making additional DSBs (32). Perhaps smaller chromosomes tend to take more time to engage their homologs and thus enjoy a longer window of opportunity to make DSBs. It has been argued (15) that this form of DSB regulation can account for the earlier finding that smaller chromosomes undergo more crossing over per kb than larger chromosomes (33). However, whereas chromosome bisection and fusion experiments have demonstrated that difference in chromosome length is the cause of variation in crossover density (33), this has not been formally tested for DSBs.

S. mikatae provides a natural experiment, as reciprocal translocations have placed parts of ancestral chromosome VI onto longer chromosomes in that species (23) (Fig. 5B). DNA segments syntenic with the left and right arms of ancestral chromosome VI had a Spo11-oligo density predicted by their chromosome length: density was higher when the segments resided on the short chromosome VI in *S. cerevisiae* but lower when on longer chromosomes in *S. mikatae* (Fig. 5B). Syntenic segments on similar-length chromosomes exhibited matched Spo11-oligo densities (Fig. 5C). These findings indicate that whole-chromosome variation in DSB density is a direct consequence of chromosome size per se and is thus in large part extrinsic to the DNA sequence.

Conclusions

Our observations fit the hypothesis that hotspots tend to be stable if Spo11 targets functional genomic elements that are evolutionarily constrained (8). Conversely, evolutionary stability of DSB hotspots may indicate constrained function(s), even if that function is presently unknown. Interestingly, DSB hotspots are well conserved between the *Schizosaccharomyces* species *S. pombe* and *S. kambucha* (~0.5% sequence divergence) (34) despite mapping to sites without known function (35). In contrast, in *Drosophila*, which lacks a PRDM9-like system but also does not preferentially target recombination to promoters or known functional elements, the fine-scale distribution of recombination appears to evolve rapidly (36).

Strong conservation in Saccharomycetes of DSB frequencies within hotspots, across subchromosomal domains, and even across whole chromosomes supports the hypothesis that this conservation traces back to the DSB landscape being shaped by selectively constrained chromosomal features that work combinatorially, hierarchically, and over multiple size scales (15). For example, transcription, telomere and centromere function, and sister chromatid cohesion rely on and shape chromosome structures over scales ranging from tens to millions of base pairs. Because these structures in turn mold the DSB landscape, selective pressure to maintain them for gene expression, cell division, and other processes imposes a tendency to conserve the DSB landscape. However, the remarkable strength of conservation across millions of years of evolution leads us to speculate that the specific shape of the yeast DSB landscape may confer fitness benefits. The recombination distribution is a heritable trait subject to selection (16, 37), so we speculate that selective pressures may operate more directly on the DSB landscape genome-wide, perhaps related to accurate meiotic chromosome segregation and/or beneficial effects of disrupting or maintaining linkage groups at various size scales (37, 38).

Finally, we note that available evidence in plants, birds, and canids — all apparently lacking a PRDM9-like hotspot targeting mechanism — point to Spo11 acting preferentially at promoters, CpG islands, and/or other genomic elements that are under selective constraint to maintain functions separate from being Spo11 targets (39–41). In finches, high-resolution recombination maps inferred from population genetic data reveal evolutionary stability of recombination hotspots, analogous to *Saccharomyces* but wholly unlike PRDM9-reliant apes or mice (41). Thus, not only is it untrue that recombination initiation landscapes inevitably evolve rapidly, but conservation is likely to be a common pattern for many sexual species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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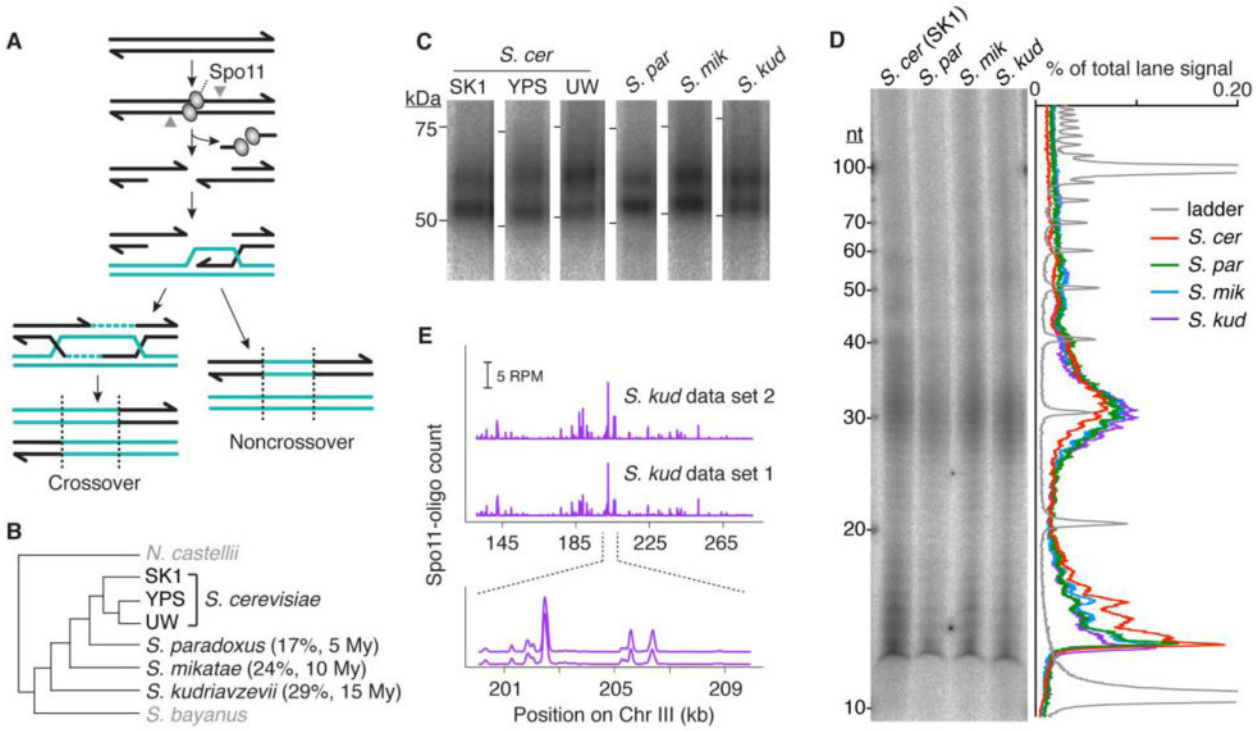

Fig. 1.

Generation of Spo11-oligo maps. (A) Meiotic recombination. Spo11 generates covalent protein-linked DSBs; endonucleolytic cleavage (grey arrowheads) liberates Spo11 bound to short oligos. DSB ends are resected and repaired to yield crossover or non-crossover products. The broken chromosome (black) copies information from the uncut allele (teal). (B) Schematic of *Saccharomyces* phylogeny (18, 20). Black, species/strains in this study; genic sequence divergence from *S. cerevisiae* (42) and estimated time since last common ancestor (20) are shown. YPS, YPS128; UW, UWOPS03-461.4. (C,D) Conserved sizes of Spo11 oligos. Immunoprecipitated, radiolabelled Spo11-oligo complexes were separated by SDS-PAGE (C), or were digested with proteinase K and resolved on a denaturing polyacrylamide gel (D). Autoradiographs (with lane traces in D) are shown. (E) Reproducibility of *S. kudriavzevii* Spo11-oligo maps. RPM, reads per million mapped; profiles were smoothed with 201-bp Hann window.

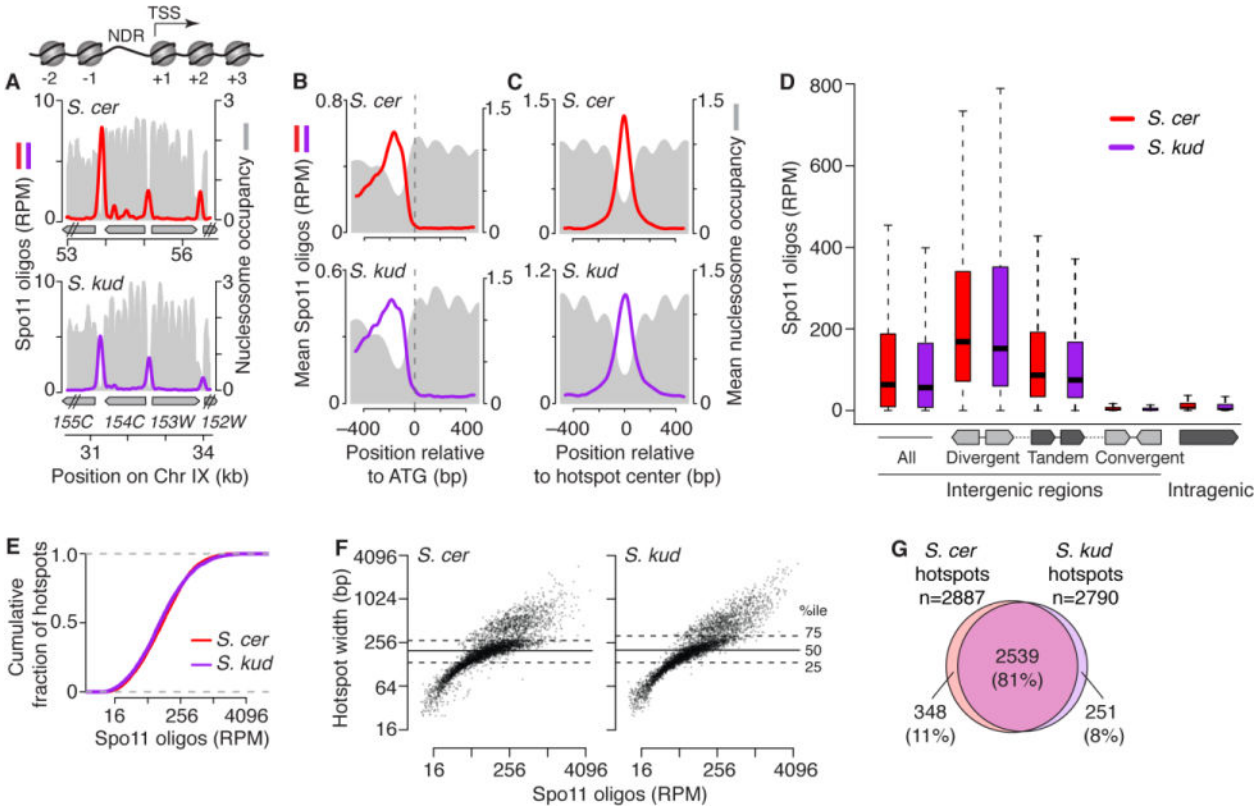

Fig. 2.

Conserved targeting of DSBs to promoters. (A) Overlap of DSB hotspots with promoter NDRs is evolutionarily conserved. The cartoon depicts typical yeast promoter chromatin structure, with an NDR upstream of the transcription start site (TSS). Sample region (around *YIL154C*) compares Spo11 oligos with the nucleosome map (MNase-seq read depth relative to genome average). (B) Average Spo11-oligo and nucleosome profiles around start codons (*S. cer* SK1, n=5766; *S. kud*, n=5578). (C) Average Spo11-oligo and nucleosome profiles at hotspots (*S. cer* SK1, n=4099; *S. kud*, n=3976). Spo11-oligo profiles were smoothed with 201-bp (A) or 75-bp (B,C) Hann window. (D) Spo11 oligos map preferentially to IGRs containing promoters. Box plots are as in fig. S3D. (E) Hotspot intensity varies over similar smooth continua in *S. cerevisiae* (SK1) and *S. kudriavzevii*. (F) Similar distributions of widths vs. Spo11-oligo counts in hotspots. (G) Conserved hotspot positions. Most promoter-containing IGRs hosting Spo11-oligo hotspots in *S. cerevisiae* (SK1) also had hotspots in *S. kudriavzevii*.

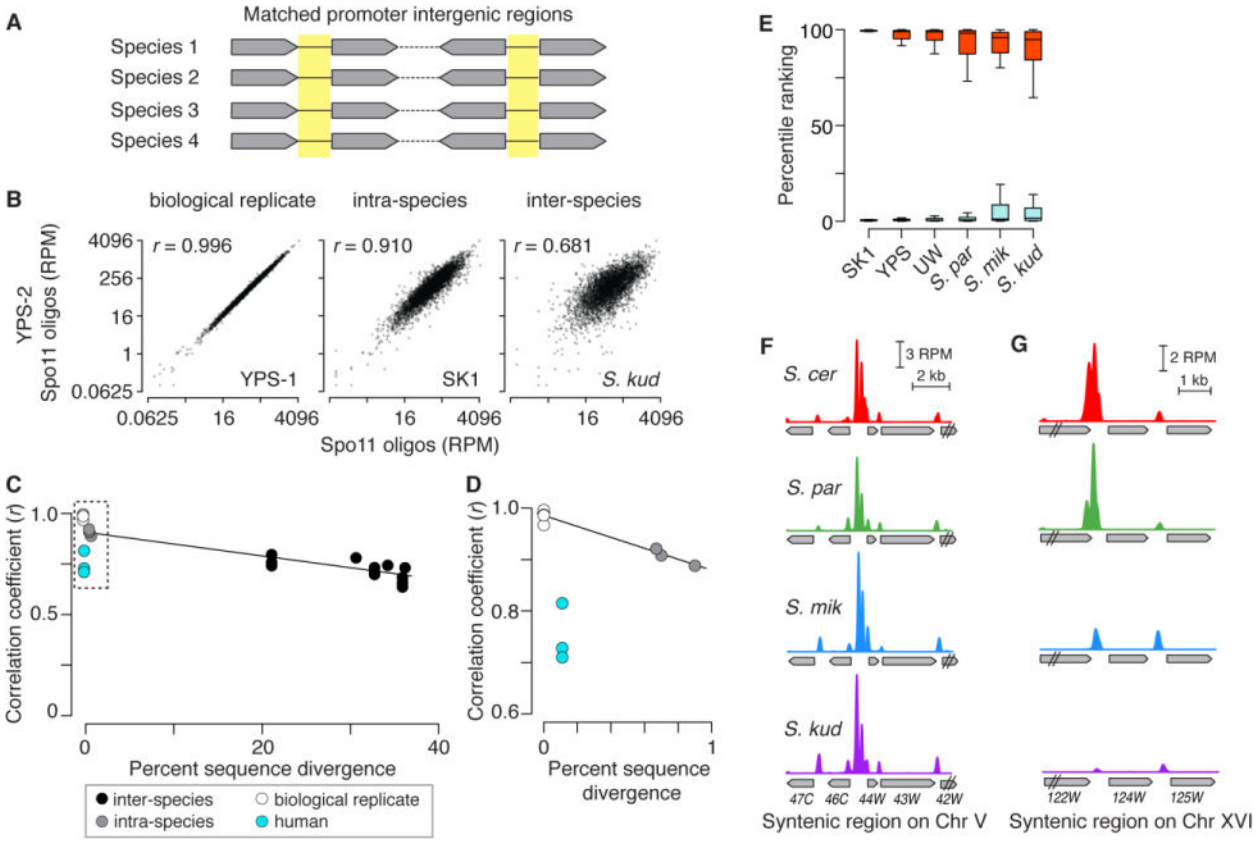

Fig. 3.

Conservation of hotspot strength. (A) Promoter-containing IGRs were matched between species using conservation of flanking genes. (B) Comparison of Spo11-oligo counts ($\log_2$ scale) within 3426 IGRs that were matched in all four species. Correlation coefficients for the $\log_2$-transformed data are shown (Pearson's *r*). (C, D) Spo11-oligo counts in promoter IGRs remain highly correlated despite wide sequence divergence. Correlation coefficients (as in B) are plotted against the median sequence divergence within IGRs, which is substantially greater than the coding sequence divergence in Fig. 1B (22). D is a zoomed view of the boxed region in C. Black lines highlight the yeast comparisons; they are not regression lines. Human data (from ref. 28) are for three men with identical or similar *PRDM9* alleles (37,345 hotspots (see fig. S4B); each had ~0.1% sequence difference from the reference genome (28)). (E) The hottest hotspots have stayed hot, and the coldest have stayed cold. Percentile rankings in other strains and species are shown for the matched promoter IGRs with the most (red) and least (cyan) Spo11 oligos in SK1 (top and bottom 1%). Box plots are as in fig. S3D. (F,G) Examples of a strong Spo11-oligo hotspot from SK1 whose heat is conserved (F, *YEL046C*) and one whose heat is not (G, *YPR124W*).

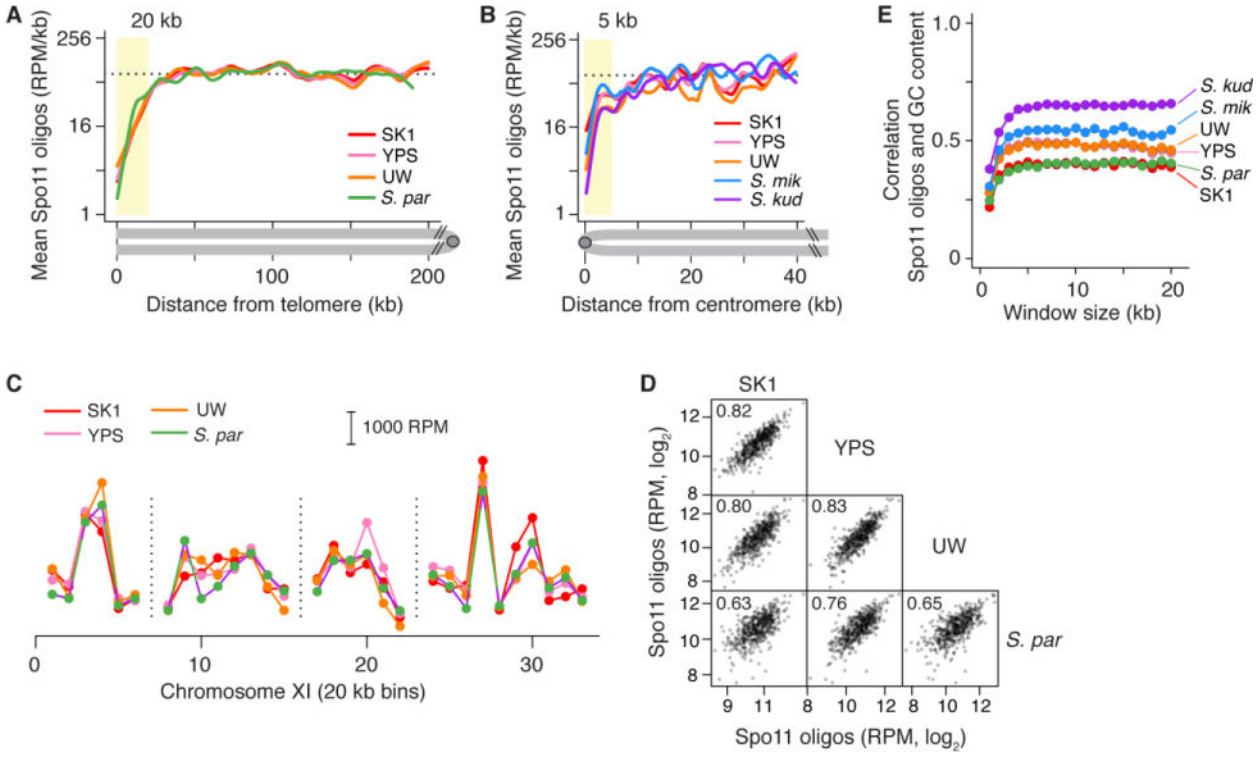

Fig. 4.

Conservation of large-scale features of the DSB landscape. (A,B) Telomere-proximal and pericentric DSB suppression. Lines are smoothed fit (Lowess) of Spo11-oligo densities in 500-bp bins averaged across 32 chromosome arms. Dashed line, genome average in SK1; yellow shading, DSB suppression zones. Genome assemblies are not complete enough to evaluate telomeres of *S. mikatae* or *S. kudriavzevii*. (C,D) Large-scale hot and cold interstitial domains are conserved. Interstitial segments (excluding subtelomeres and pericentromeres) were defined as syntenic between *S. cerevisiae* and *S. paradoxus* if orthologous genes were in the same order in both species. Spo11-oligo counts were summed in these syntenic segments divided into 20-kb bins (Table S5). A representative example is shown in panel C (vertical dashed lines denote synteny breaks, mostly from unresolved annotation discrepancies) and genome-wide scatter plots and correlation coefficients are in panel D. (E) Correlation (Pearson's *r*) between mean Spo11-oligo counts and GC content binned in windows of varying size.

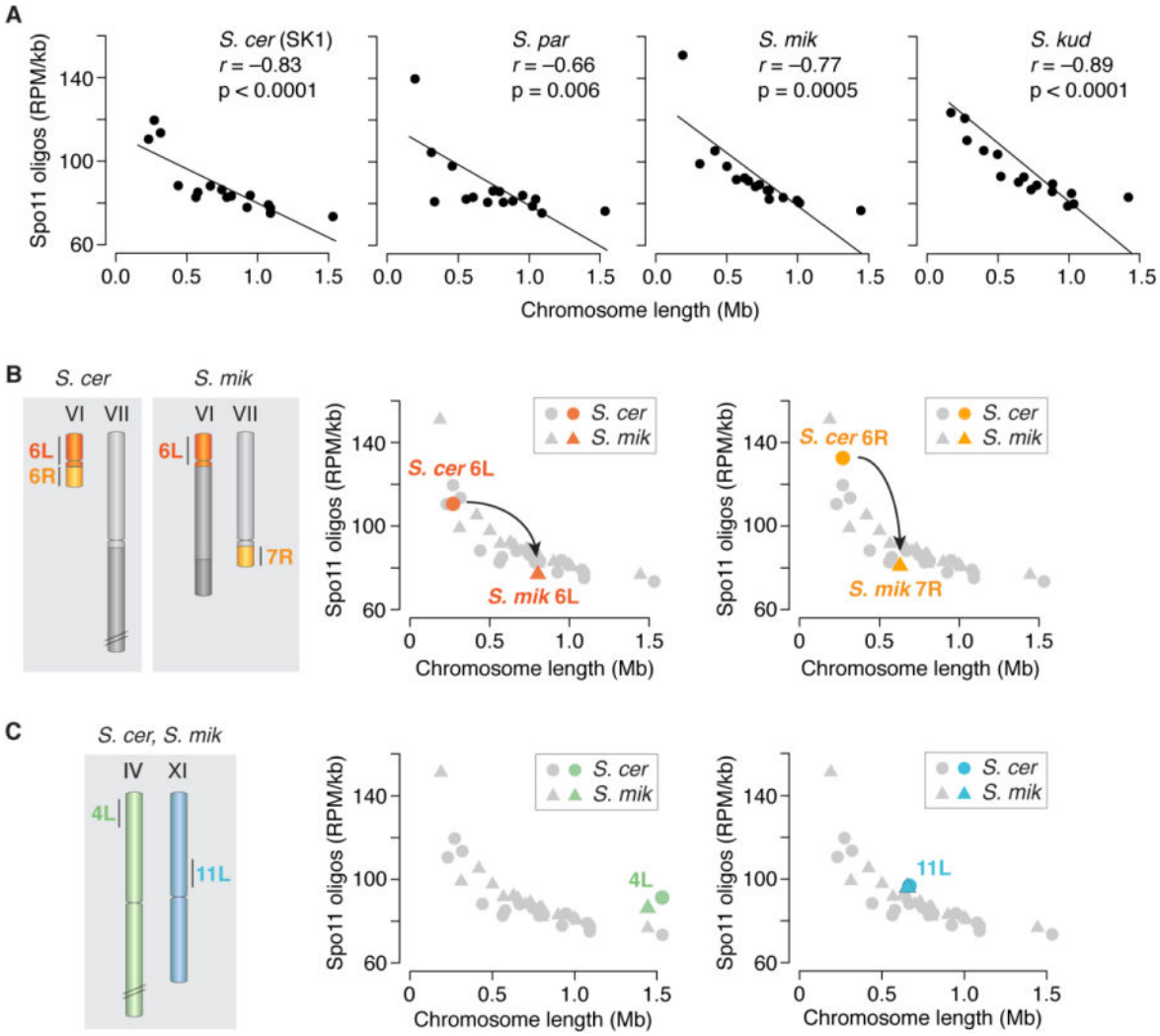

Fig. 5.

DSB density is influenced by chromosome length. (A) The anticorrelation between chromosome length and DSB density is conserved. Each point is one chromosome. (B) A natural experiment demonstrating chromosome length-dependent DSB control. The schematic illustrates syntenic segments on chromosomes of different size in *S. cerevisiae* and *S. mikatae*. The plots show that Spo11-oligo density is higher on these segments in *S. cerevisiae* (when on a short chromosome) than in *S. mikatae* (longer chromosomes). Gray symbols are whole-chromosome values from A for comparison. Note that the segments from ancestral chromosome VI display a Spo11-oligo density closely matched to the whole-chromosome value appropriate for the size of the chromosome on which they reside. (C) Control syntenic regions on similarly sized chromosomes have equivalent Spo11-oligo densities in both species.