Published in final edited form as:

Science. 2017 January 06; 355(6320): 40–45. doi:10.1126/science.aak9704.

A global view of meiotic double-strand break end resection

Eleni P. Mimitou¹, Shintaro Yamada¹, and Scott Keeney^{1,2,*}

¹Molecular Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, 10065

²Howard Hughes Medical Institute, Memorial Sloan Kettering Cancer Center, New York, NY, 10065

Abstract

DNA double-strand breaks that initiate meiotic recombination are exonucleolytically processed. This 5'→3' resection is a central, conserved feature of recombination but remains poorly understood. To address this lack, we mapped resection endpoints genome-wide at high resolution in *Saccharomyces cerevisiae*. Full-length resection requires Exo1 exonuclease and the DSB-responsive kinase Tel1, but not Sgs1 helicase. Tel1 also promotes efficient and timely resection initiation. Resection endpoints display pronounced heterogeneity between genomic loci that reflects a tendency for nucleosomes to block Exo1, yet Exo1 also appears to digest chromatin with high processivity and at rates similar to naked DNA in vitro. This paradox points to nucleosome destabilization or eviction as a defining feature of the meiotic resection landscape.

Introduction

Meiotic recombination promotes proper segregation of homologous chromosomes (1) and initiates with double-strand breaks (DSBs) formed by the topoisomerase-like Spo11, which remains covalently attached to DSB 5' ends (Fig. 1A). Endonucleolytic cleavage by the Mre11–Rad50–Xrs2 (MRX) complex plus Sae2 generates nicks on Spo11-bound strands that serve as entry points for modest 3'→5' Mre11 exonuclease activity and robust 5'→3' Exo1 exonuclease activity (2–5). These release Spo11 bound to short oligonucleotides (oligos) and generate 3' single-stranded DNA (ssDNA) tails, which are substrates for strand-exchange proteins (Dmc1 and/or Rad51) that search for homology and invade a homologous repair template (1). A similar nick-plus-exonuclease mechanism operates in vegetative cells (6), except that Exo1 is partially redundant with Sgs1–Top3–Rmi1 plus Dna2 for extensive 5'→3' resection (7, 8). In meiosis, available data implicate Exo1 but suggest dispensability of Sgs1 (4, 9).

*Correspondence: s-keeney@ski.mskcc.org.

Correspondence and requests for materials should be addressed to S.K. (s-keeney@ski.mskcc.org).

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Any Additional Author notes: EPM developed S1Seq and performed experiments. SY developed in silico modeling of resection length and speed. EPM and SK designed the study, analyzed data and wrote the paper with contributions from SY.

Although meiotic resection was first demonstrated experimentally 25 years ago and has long been known to be a fundamental step in recombination (10, 11), it remains poorly understood. In fact, detailed data are available only for one side of a single artificial DSB hotspot (4); whether this extrapolates to natural hotspots is unknown. Here, we map resection endpoints genome-wide with high specificity, sensitivity, and spatial resolution. These maps answered longstanding questions about genetic control of resection; the handoff from MRX–Sae2 to Exo1; locus-to-locus variation in resection; resection kinetics; and interaction of resection machinery with chromatin.

Mapping DSB resection endpoints

We digested DSB 3' tails with ssDNA-specific nucleases to generate sequencing libraries of ssDNA–dsDNA junctions that we compared to DSB maps from Spo11-oligo sequencing (12) (Fig. 1, A and B and table S1). Two biological replicates were pooled for each strain or time point (Pearson's $r = 0.95$ – 0.99) (fig. S1, A and B).

Reads from resection endpoints should be meiosis-specific and Spo11-dependent and should flank DSB hotspots with defined polarity (fig. S1C). S1 sequencing (S1Seq) reads were hotspot-enriched with the expected polarity, and enrichment was absent from premeiotic samples and meiotic samples from the catalytically inactive *spo11-Y135F* mutant (Fig. 1, B and C and fig. S1, D to F). S1Seq signal correlated well with Spo11 oligos (Fig. 1D), and reads spread further from hotspots over time in *dmc1* (Fig. 1B and fig. S1, E, G and H), reflecting known hyper-resection (13). Thus, S1Seq is a sensitive and quantitative measure of DSB resection endpoints.

Resection endpoints extended ~200–2000 nt from hotspot centers (mean 822 nt) with a positive skew (Fig. 1, C and E). This pattern resembles Southern blotting at *HIS4LEU2* (4) (Fig. 1E, range 350–1550, mean 800 nt), but S1Seq captured less abundant species at the distribution extremes. Genome-average profiles were smooth and left–right symmetric (Fig. 1C), but individual hotspots were heterogeneous with peaks and valleys differing between hotspots or sides of the same hotspot (Fig. 1B and fig. S1E).

S1Seq profiles affirmed Sgs1 dispensability (Fig. 1, B and F and fig. S1E). In contrast, the nuclease-defective mutation *exo1-D173A* (*exo1-nd*) (14) reduced resection lengths to <1100 nt (mean 373 nt), comparable to *HIS4LEU2* in *exo1* (mean 270 nt) (4) (Fig. 1, B, E and F and fig. S1E). Resection endpoints in *exo1-nd* likely are the most distal Mre11-dependent nicks formed in wild type. If so, the difference between Spo11-oligo lengths and *exo1-nd* endpoints indicates that multiple nicks are formed or that 3'→5' digestion averaging 335 nt accompanies a single distant nick (fig. S1I).

Tel1 promotes resection initiation and extension

Absence of Tel1 (orthologous to human ATM) decreased resection length at *HIS4LEU2* for early DSBs (15). This and other findings led to the proposal that Tel1 controls resection when DSB numbers are still low whereas higher DSB numbers later allow Mec1 (ATR in humans) to substitute (15). S1Seq data allowed us to test this model and uncovered that Tel1 acts at multiple steps.

S1Seq reads in *tel1*⁻ fell closer to DSB hotspots at early (2 h) and later (4 h) time points, indicating shorter resection, but with peaks similar to wild type (Fig. 2, A and B). Although some tracts at 4 h in *tel1*⁻ matched the longest in wild type (Fig. 2B), DSBs remained hypo-resected over all (Fig. 2, A and B). Therefore Tel1 modulates resection length throughout meiosis, not just early.

DSBs in wild type have appeared to be maximally resected as soon as they are detectable (13, 15). S1Seq profiles at individual hotspots seemed stable over time in wild type (Fig. 2A), especially compared to *dmc1*⁻ (Fig. 1B), but genome-wide averages revealed slightly shorter resection tracts at 2 h than at 4–6 h (Fig. 2, B to D, fig. S1J and S2E). Locus-to-locus variability and greater sensitivity of S1Seq are nonexclusive possibilities for why this change was previously unseen. Shorter tracts may reflect partially resected DSBs, but we favor that later-forming DSBs tend toward longer resection, perhaps via increasing Tel1 activity (15).

The *tel1*⁻ mutant showed higher S1Seq signal within hotspots, accounting for a greater fraction of reads at 2 h than 4 h in *tel1*⁻, but also present in wild type at lower levels (Fig. 2, A and B and fig. S1E). We hypothesized that this signal reflects unresected DSBs. Indeed, within-hotspot S1Seq signal displayed peaks overlapping strong Spo11-oligo clusters (Fig. 2A) and correlated with hotspot strength in *tel1*⁻ and wild type (fig. S2, A and B). Fine-scale patterns matched expectation for preferred Spo11 cleavage 3' of C residues and for the 2-nt 5' overhang of Spo11 primary cleavage products (fig. S2, C and D). We conclude that unresected DSBs are present in wild type at levels difficult to detect by Southern blotting; higher levels in *tel1*⁻ indicate that Tel1 promotes normal resection initiation, possibly via Sae2 phosphorylation (16).

Recombination intermediates

Accompanying resection signal was weaker S1Seq signal with the “wrong” polarity, e.g., top-strand reads mapping left of hotspots (Fig. 1B, and fig. S1, D and E). This signal was not resection from neighboring hotspots, it correlated with hotspot strength, it was meiosis-specific and Spo11-dependent, and it was essentially absent in *dmc1*⁻ (Fig. 1, B and C, Fig. 3A; fig. S1, E to H, and fig. S3A). We conclude that this signal derives from S1-sensitive recombination intermediates (RIs), probably displacement (D) loops from strand exchange (fig. S3, B and C). Throughout this study, resection profiles were corrected by subtracting an estimate for the small amount of RI signal with the “correct” polarity that presumably lies under the resection signal (fig. S3, B and C; see Supplementary Text).

At 4 h in wild type, RI reads had a positively skewed distribution with a mean of 606 bp from hotspot centers, i.e., ~25% shorter than resection length (Fig. 3B). Wild type and *sgs1*⁻ were indistinguishable (fig. S3D), but RI distances were shorter in *tel1*⁻ consonant with modestly shorter resection tracts (Fig. 3B), suggesting that resection length influences RI position. In *exo1-nd* RI distances were even shorter (Fig. 1F, 3B, and fig. S3E). The similarity of RI distances (mean 350 bp) to resection lengths in *exo1-nd* suggests that much of the resection tract is used up making RIs, whereas RIs in wild type usually form within a DSB-proximal subregion of resection tracts.

We reasoned that quantitative differences between Spo11 oligos and S1Seq can reveal differences for lifespans of recombination steps, because turnover of Spo11-oligo complexes is tied to meiotic prophase exit (17) whereas lifespans of resection and RI signals reflect recombination progression more directly. Smaller chromosomes tend to incur more DSBs per kb because of negative feedback tied to engagement of homologous chromosomes (12, 17). It was proposed that smaller chromosomes take longer on average to engage homologous partners because multiple DSBs per chromosome are usually needed for successful engagement (17, 18). This hypothesis predicts that resected DSBs should persist longer on smaller chromosomes. Indeed, the ratio of S1Seq resection signal to Spo11-oligo counts at 4 h correlated negatively with chromosome size, i.e., the yield of resected DSBs (S1Seq) per DSB formed (Spo11 oligos) was higher on smaller chromosomes (Fig. 3C). Anticorrelation was not seen at 2 h (Fig. 3C), as expected because early DSBs have not had time to progress in recombination regardless of chromosome size. The ratio of RI signal to Spo11-oligo counts was uncorrelated with chromosome size (fig. S3F), thus RI lifespan is governed by local features rather than chromosome pairing kinetics.

The ratio of S1Seq resection reads to Spo11 oligos was lower for pericentromeric hotspots than for other sub-chromosomal domains (Fig. 3D). This apparently shorter lifespan could reflect delayed DSB formation and/or more rapid DSB repair, consistent with kinetochore components suppressing DSB formation and promoting sister chromatid recombination (19).

Modeling Exo1 mechanism and speed

To gain further insight into Exo1 resection, we tested a model in which Exo1 enters DNA at an Mre11-generated nick and digests DNA until dissociation. This model predicts that Exo1 run lengths follow a geometric distribution defined by the average probability of dissociation at each nucleotide step. We determined the geometric distribution providing the best fit to wild-type resection when combined with the distribution of presumptive Exo1 entry points (i.e., the endpoint distribution in *exo1-nd*) (Fig. 4A). This geometric model could be ruled out because it fit the data poorly, but shifting the geometric distribution provided a better fit (Fig. 4A). This alternative can be conceptualized in terms of a high probability of Exo1 resecting for a minimum distance (best-fit estimate of 220 nt), after which resection termination is geometrically distributed.

How fast is Exo1? In vegetative cells, unrepairable DSBs are continuously resected at ~4.4 kb/h (8, 20, 21). Long-range resection by Exo1 only (i.e., in *sgs1*) is slower, ~1 kb/h (8). To test whether the vegetative rate can explain meiotic resection, we performed Monte Carlo simulations to generate populations of resected DNA molecules to compare with S1Seq patterns (Fig. 4, B and C, fig. S4 and movies S1 and S2). Rates of 4 or 8 kb/h were not fast enough: these speeds predicted that DSBs should be resected less than observed, particularly at early times. But simulations matched observations well when we used rates from 16 to 40 kb/h, the latter being the value from single-molecule studies for Exo1 resecting naked DNA (22). Since rates ~8 kb/h appear implausibly slow, we conclude that meiotic resection is faster than long-range resection in vegetative cells. Because ~16 kb/h is plausible, it suggests that Exo1 processes meiotic DSBs in vivo nearly as quickly as it degrades naked DNA in vitro. Hyper-resection in *dmc1* was slower, with an estimated average speed of 0.19 kb/h

(Fig. 4D). These findings illuminate differences between the extreme rapidity but limited length of wild-type meiotic resection, the slow continuity of hyper-resection in the absence of Dmc1, and the moderate pace but unconstrained distance of long-range resection of unrepairable breaks in vegetative cells.

Chromatin shapes the resection landscape

Verifying that strong peaks in S1Seq resection profiles were not a sequencing artifact, S1 treatment converted resected DSB fragments at the *GAT1* hotspot from the usual featureless smears into discrete banding patterns on Southern blots (Fig. 5A and fig. S5A). Prominent bands occupied similar positions in mutants with different average resection lengths, so banding is a reproducible feature at *GAT1*. In contrast, S1 generated no discrete banding at *HIS4LEU2* (fig. S5B), explaining why preferred resection endpoints were not previously uncovered (4). We also note that the spatial resolution of resection analyses in vegetative cells would have been inadequate to detect such heterogeneity if it were present.

Stereotyped nucleosome positions around hotspots allowed us to test the hypothesis that this heterogeneity reflects chromatin structure. Most yeast promoters have a nucleosome depleted region (NDR), where many DSBs form, flanked by positioned nucleosomes with the transcription start site (TSS) in the first (+1) nucleosome (12, 23) (Fig. 5B). When S1Seq data from wild type were averaged around +3 nucleosomes, the broad peak showed modest scalloping in register with average nucleosome occupancy (Fig. 5C). Speculating that variation in nucleosome positions blurs the chromatin signature, we compiled S1Seq averages for three groups of genes divided by linker width between +3 and +4 nucleosomes (Fig. 5D). An S1Seq peak overlapping left edges of +4 nucleosomes moved progressively rightward with increasing linker width (arrows, Fig. 5D). The widest group also accumulated reads within linkers. Similar patterns for the shorter resection tracts in *tel1* were seen for +2 and +3 nucleosomes (fig. S5, C and D), and +1 and +2 nucleosomes yielded similar patterns for Mre11-dependent clipping in *exo1-nd* (Fig. 5, E and F). These findings establish a spatial correlation between nucleosomes and preferred endpoints for both the Mre11 and Exo1 resection steps. Patterns for Exo1 fit with digestion proceeding partway into nucleosomes, with increasing likelihood of resection termination as Exo1 approaches the nucleosome dyad, possibly reflecting greater stability of histone-DNA binding near the dyad (24). RIs tended to peak within nucleosome linkers (fig. S5E), indicating that RIs are also influenced by chromatin, possibly the recombination partner's.

To test if this correlation reflects causality, we examined mutants lacking transcription factors Bas1 or Ino4, in which specific hotspots experience altered chromatin structure nearby while retaining promoter-associated DSBs (25). In *ino4* the *CHO2* nucleosome array shifts toward the promoter as revealed by micrococcal nuclease (MNase) digestion of chromatin (Fig. 5G). In *bas1* the normally variable nucleosome positioning in *SHM2* (a shallow nucleosome ladder in wild type) becomes sharper (Fig. 5H). Concordantly in both cases, S1Seq reads shifted closer to promoters, whereas neither chromatin structure nor resection were affected in the adjacent *PTII* or *REX2* genes or a control locus (Fig. 5, G and H and fig. S5F). Thus, S1Seq heterogeneity is not solely from DNA sequence biases, and chromatin directly affects Exo1 endpoints.

Conclusions

Given chromatin structure at hotspots, Mre11 incision often occurs in +1 or +2 nucleosomes and Exo1 then traverses several nucleosomes' worth of DNA (Fig. 5B). This creates an apparent paradox: feeble activity of Exo1 on nucleosomal substrates in vitro (26) and the tendency for resection to stop near nucleosome boundaries in vivo indicate that nucleosomes are a potent block to Exo1. Yet Exo1 resects several hundred nucleotides quickly and with high apparent processivity in vivo, as if nucleosomes are initially little or no barrier at all.

To resolve this paradox, we propose that nucleosomes are destabilized or removed prior to digestion by Exo1, with a constraint on resection length being how many nucleosomes are removed (fig. S5G). Additional factors may include inhibitors of Exo1, spatially regulated Exo1 activity, and/or iterative Exo1 loading (fig. S5H). Nucleosome eviction during resection has been proposed for vegetative yeast and somatic mammalian cells, but experimental support is indirect and conflicting and has been unable to distinguish whether chromatin changes are prerequisite or consequence of resection (27–29). Our parameterization of resection speed, apparent processivity, and preferred endpoint positions clearly favor this model. Chromatin immunoprecipitation also supports nucleosome disassembly near unresected DSBs being repaired by non-homologous end joining in human cells (29). Chromatin remodelers implicated in vegetative resection (30) are candidates for nucleosome destabilization in meiosis. Interestingly, DSB resection in mouse meiosis extends a similar average distance as in yeast and is inferred to traverse multiple nucleosomes (31). Conservation of the scale of DSB-provoked chromatin remodeling would parsimoniously unify resection mechanisms in these distant species.

If nucleosomes are evicted, we infer that this occurs after DSB formation because Spo11 rarely cuts within nucleosomes (12). Furthermore, the chromatin signature in *exo1-nd* implies that full eviction occurs after Mre11-dependent incision positions are established. Unresected DSBs in wild type may indicate that Mre11 incision is rate-limiting, as in *Schizosaccharomyces pombe* (32). Tel1 may promote Mre11-dependent incision given that there are more unresected DSBs in its absence. Tel1 also regulates resection distance without changing preferred endpoints. A minor role of Tel1 in mitotic DSB resection has been reported (33) and ATM is proposed to destabilize chromatin during non-homologous end joining in human cells (29). We speculate that Tel1 controls efficiency or distance over which nucleosome destabilization occurs via effects on chromatin remodelers, histone modifications, or both. S1Seq gives a selective, sensitive and quantitative measure of DSB resection tracts and should be applicable to other settings and organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank A. Viale (MSKCC Integrated Genomics Operation) for sequencing and N. Socci (MSKCC Bioinformatics Core) for mapping S1Seq reads. Core facilities were supported by NIH grant P30 CA008748. We thank M. Neale for discussions, N. Hunter and R. Liskay for strains, and S. Tischfield for discussions and help with base composition analysis. This work was supported by NIH grants R01 GM058673 and R35 GM118092. E.P.M.

was supported by a Helen Hay Whitney Foundation Fellowship and S.Y. was supported by a Kuro Murase MD-JMSA Scholarship. Sequencing data are at GEO (accession GSE85253). The authors declare no competing financial interests.

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Fig. 1. Genome-wide mapping of meiotic DSB resection

(A) Early recombination steps and S1Seq (boxed). DNA from meiotic cells was embedded in agarose plugs to protect from shearing, digested with ssDNA nucleases, and ligated to a 5'-biotinylated adaptor. After extraction from plugs and sonication, biotinylated fragments were purified, ligated to a second adaptor, amplified by low-cycle PCR, deep sequenced, and mapped. (B) DSBs (Spo11 oligos (12)) and strand-specific S1Seq surrounding the *CCT6* hotspot (51-bp smoothing). *spo11-YF* is catalytically inactive *spo11-Y135F*; *sgs1-ΔC* lacks the C-terminal helicase domain. (C) Genome-average, strand-specific S1Seq (101-bp smoothing) centered on Spo11-oligo hotspots ($n=3908$). (D) S1Seq correlates well with Spo11 oligos. S1Seq signal (wild type, 4 h) was summed from +200 to +1600 bp (top strand) and -200 to -1600 bp (bottom strand) relative to hotspot midpoints for "loners", i.e., no other hotspot within 3 kb. (E) Comparing genome-wide S1Seq resection endpoints with Southern blotting at *HIS4LEU2* (4). (F) Genome averages as in panel C.

Fig. 2. Tel1 and the evolution of resection tract lengths over time in meiosis

(A) S1Seq (51-bp smoothing) at *CCT6*. (B) Genome averages as in Fig. 1C. (C) Average resection tracts are longer later in wild type (loner hotspots; $p < 2.2 \times 10^{-16}$, two-sample Kolmogorov-Smirnov test). (D) Mean resection lengths over time.

Figure 3. Recombination intermediates detected by S1Seq

(A) S1Seq RIs correlate with hotspot heat and are Dmc1-dependent. S1Seq (101 bp smoothing, top- and bottom-strand reads co-oriented and averaged) is shown for loner hotspots divided according to Spo11-oligo counts. Arrows, RI regions. (B) RI distribution around hotspots. Arrows, means (bp). (C) Longer DSB lifespan on smaller chromosomes. S1Seq signal (as in Fig. 1D) per DSB (Spo11 oligos) correlates negatively with chromosome size at 4 h but not 2 h. (D) S1Seq signal per DSB is lower for pericentromeric hotspots ($n=82$) than subtelomeric ($n=60$) or all other hotspots (interstitial, $n=3744$). Boxplots are as defined in fig. S2C except outliers are shown.

Figure 4. Modeling resection

(A) Exo1 run lengths. The observed resection distribution (right) was modeled by combining theoretical Exo1 run lengths (middle) with observed resection endpoints in *exo1-nd* (i.e., Exo1 entry points; left). Solid red lines: geometric model for Exo1, equivalent to single turnover with uniform termination probability at each exonucleolytic step. Dashed red lines: shifted geometric model, i.e., zero probability of Exo1 ceasing resection within some minimum distance. (B,C) Exo1 speed. Monte Carlo simulations generated populations of resected DSBs using empirical timing of DSB formation and Exo1 entry points, plus the shifted geometric model for Exo1 run lengths. Panel B, simulated and observed resection at 2 h (10-bp binned data, 11-bin smoothing). Panel C, median deficits for simulated resection tracts over time in meiosis. See fig. S4 and movies S1 and S2. (D) Slow hyper-resection in *dmc1Δ*, measured by tracking the leading edge (filled circles; 80th percentile) of resection endpoints (10-bp binned data, 11-bin smoothing; loner hotspots).

Figure 5. Chromatin structure shapes the resection landscape

(A) S1 converts smears of resected DSBs into discrete bands on Southern blots. (B) Resection endpoints within a nucleosome array. Top-strand S1Seq reads (51-bp smoothing) are superimposed on a nucleosome map (12). The schematic illustrates to scale positions of Mre11 clipping and Exo1 digestion. (C) Average S1Seq resection signal from wild type at 4 h (41-bp smoothed) compared with average nucleosome occupancy, centered on midpoints of +3 nucleosomes with no other hotspot 3 kb downstream (n=1815). (D) Correlating resection endpoints with nucleosome edges. Pairs of +3 and +4 nucleosomes with robust H3 occupancy (n=962) were ordered by linker width. Horizontal lines demarcate nucleosome

positions, shaded by H3 occupancy. Nucleosome pairs were divided into three groups and averaged S1Seq signal (31-bp smoothed) is shown. **(E,F)** Comparing *exo1-nd* resection endpoints at 4 h with chromatin structure. Data are as for panels C and D, except centered on +1 nucleosomes (n=1832 for panel E, n=944 for panel F). **(G,H)** Altering chromatin structure alters resection endpoints accordingly. Spo11 oligos and Southern blots of MNase-digested chromatin are from (25).

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