The transcription fidelity factor GreA impedes DNA break repair

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Homologous recombination repairs DNA double-strand breaks and must function even on actively transcribed DNA. Because break repair prevents chromosome loss, the completion of repair is expected to outweigh the transcription of broken templates. However, the interplay between DNA break repair and transcription processivity is unclear. Here we show that the transcription factor GreA inhibits break repair in Escherichia coli. GreA restarts backtracked RNA polymerase and hence promotes transcription fidelity. We report that removal of GreA results in markedly enhanced break repair via the classic RecBCD–RecA pathway. Using a deep-sequencing method to measure chromosomal exonucleolytic degradation, we demonstrate that the absence of GreA limits RecBCD-mediated resection. Our findings suggest that increased RNA polymerase backtracking promotes break repair by instigating RecA loading by RecBCD, without the influence of canonical Chi signals. The idea that backtracked RNA polymerase can stimulate recombination presents a DNA transaction conundrum: a transcription fidelity factor that compromises genomic integrity.

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recA, break repair deficiency caused persistent DNA fragmentation (Extended Data Fig. 3c, e). Enhanced DNA repair was also observed in the ΔgreA single mutant compared with WT cells, albeit at a less pronounced level (Extended Data Fig. 3d), possibly because of the contribution of DksA to DSB repair. In agreement with the PFGE results, the increased survival of the ΔgreA mutant after phleomycin treatment was almost completely dependent on RecA (Fig. 1h). These data indicate that GreA impedes DSB repair via the canonical RecA-dependent homologous recombination pathway.

To confirm that the phleomycin resistance of ΔgreA cells was specific to the repair of DSBs, we used an inducible DNA break system consisting of the I-SceI endonuclease41 along with I-SceI recognition sites engineered at various chromosomal loci (Fig. 2a). Induction of I-SceI resulted in roughly 100-fold killing in WT cells (Fig. 2b). Deletion of greA increased survival upon I-SceI break induction by about tenfold (Fig. 2b), indicating enhanced repair of a single DSB using sister homology in the absence of GreA. Survival of the ΔdksA mutant was reduced compared with WT cells (Fig. 2b), whereas deletion of greA restored viability to the level of WT but not to that of the ΔgreA mutant (Fig. 2b), implying again that DksA may independently promote DSB repair. The increased viability after I-SceI-induced DSBs in the ΔgreA mutant required recA and recB (Fig. 2c). Elimination of RecD, which abolishes S‘–3′ helicase and RecB nuclease activities but increases homologous recombination proficiency28,29, did not affect the DSB resistance of ΔgreA mutants (Fig. 2c and Extended Data Fig. 4a). Thus, RecB (Figs 1h and 2c) but not RecD functions are involved in the repair phenotype of the ΔgreA mutant. Additionally, the GreA effect on break repair did not require the RecA exonuclease or the RecA loading RecFOR complex, which function in a secondary pathway of recombination20,21 (Extended Data Fig. 4b-d and Supplementary Discussion).

The influence of GreA on I-SceI DSB survival was not locus-specific, as we obtained similar results at the yjaZ site (Fig. 2a, b). Both the yjaZ and lac loci are located in highly transcribed regions. By contrast, deletion of greA produced no survival advantage over WT cells when the I-SceI site was engineered within the lambda prophage, a predominantly transcriptionally silent region18 (Extended Data Fig. 4e, f), suggesting that transcription is required in areas surrounding the break site to promote DSB resistance in absence of GreA.

**Backtracked RNAP increases DSB repair**

To understand how the absence of GreA promotes break repair, we first determined that the DNA damage response (Extended Data Fig. 4g–i) and R-loop formation (Extended Data Fig. 5a) were not involved in the DSB resistance of ΔgreA mutants (Supplementary Discussion). Next, we tested whether the phleomycin resistance of ΔgreA mutants was dependent on known factors that control RNAP backtracking. Deletion of mfd, a TCR-dependent helicase that reactivates backtracked RNAP by pushing it forward42, produced no significant change in the phleomycin resistance of the ΔgreA mutant (Fig. 2d). Removal of UvrD, which opposes GreAB action by pulling RNAP backwards in the secondary TCR pathway43, reduced the phleomycin resistance of ΔgreA cells (Fig. 2d), implying a function for UvrD in promoting DSB repair in the absence of GreA. This role is probably independent of the mismatch repair and nucleotide excision repair functions of UvrD19 (Extended Data Fig. 5c). The small molecule ppGpp acts in concert with UvrD to promote RNAP backtracking and facilitate TCR20. Cells lacking ppGpp are sensitive to phleomycin, whereas overproduction of ppGpp by the spoT203 allele rendered cells resistant to DSBs with the help of UvrD (Extended Data Fig. 5b). Thus, as in the TCR pathway20, the combined action of UvrD and ppGpp on RNAP backtracking may also stimulate DSB repair. Along with DksA, ppGpp also regulates the stringent response through binding to RNAP44. We examined the phleomycin phenotype of RNAP mutations that disrupt ppGpp–RNAP
interactions\(^2,^3\) and found that the site 2 mutant, which abolishes the stringent response, behaved like WT cells (Extended Data Fig. 5b), suggesting that stringent response activation by ppGpp and DksA was not required for phleomycin resistance. The site 1 mutant and ppGpp null cells were equally sensitive to phleomycin (Extended Data Fig. 5b), suggesting that this site could be involved in DNA break repair.

We also examined the phleomycin survival phenotypes of RNAP mutants that are more or less susceptible to backtracking and found that accumulation of UvrD-dependent backtracked RNAP resulted in DNA break resistance (Extended Data Fig. 5b, d, e and Supplementary Discussion).

**XO-seq captures features of DSB repair**

To determine at what stage GreA inhibits DSB repair, we developed an assay to analyse the different steps of DSB repair in living cells. Our method combined massive parallel sequencing with I-SceI DSB induc- tion (exonuclease sequencing, XO-seq) to generate a genome-wide landscape of DNA loss from exonucleolytic resection of break ends during repair (Fig. 3a, b). In WT cells, XO-seq read counts revealed a decrease in DNA content around the break site (Fig. 3b and Extended Data Fig. 6e), which far exceeded the boundaries of many transcription units (Extended Data Fig. 6e). Read counts reached a steady state after 30 min, reflecting a balance between I-SceI cutting, resection, and repair synthesis (Fig. 3b). However, read loss was asymmetrical around the break site, extending less than 100 kb in the (oriC)-proximal side, but more than 200 kb in the (oriC)-distal direction (Fig. 3b and Extended Data Fig. 6f). This skewing can be mainly attributed to the disproportionate number of active Chi sites upstream of the break and can be switched by inverting a portion of the chromosome (Extended Data Fig. 7a, b), validating the broken fork repair model\(^24\) (Supplementary Discussion).

The pattern of sequencing reads around the break site provides a way of analysing resection and recombination activity (Supplementary Methods). The experimental curves were reproduced using a mathematical model, which describes the stochastic process of RecBCD-dependent DNA resection (Fig. 3c, Extended Data Fig. 7d and Supplementary Methods). Furthermore, the model parameters used in fitting the experimental data inform us about key physiological parameters (RecBCD processivity and Chi site sensitivity; Extended Data Fig. 7c).

We validated the observation that DNA attrition in the vicinity of the DSB represents recombination processes by analysing the behaviour of \(\Delta\text{recD}\) mutants that showed reduced DNA degradation (Fig. 3d and Extended Data Fig. 6b) and \(\Delta\text{recA}\) mutants that displayed massive degradation (Fig. 3e and Extended Data Figs 6c, 7e), and conclude that XO-seq captures known features of break repair (Supplementary Discussion).

**RNAS backtracking reduces RecBCD resection**

Next, we used XO-seq to evaluate how GreA inhibits DSB repair. Read counts in \(\Delta\text{greA}\) cells also reached a steady state. However, at 30 and 60 min after DSB induction, \(\Delta\text{greA}\) cells had significantly more reads around the break site compared with WT cells (Fig. 4a, b), which could reflect either reduced resection or increased repair synthesis following recombination. To differentiate between these possibilities, we first tested resection dynamics in the repair-impaired \(\Delta\text{recA}\Delta\text{greA}\) mutant. The \(\Delta\text{recA}\Delta\text{greA}\) cells displayed slightly less degradation than the \(\Delta\text{recA}\) single mutant (Extended Data Fig. 7f), suggesting that GreA promotes resection to some extent even in the absence of RecA. To alleviate the analytical complications of massive chromosome loss in the \(\Delta\text{recA}\) mutant, we used a thermosensitive allele of DNA polymerase III (\(\text{dnaEts}\)), which abolishes repair synthesis when inactivated\(^25\) (Extended Data Figs 1a, 6d and 8a). At the restrictive temperature, we observed significantly less resection in the \(\text{dnaEts}\Delta\text{greA}\) mutant compared with the \(\text{dnaEts}\) single mutant (Fig. 4c). Thus, resection but not repair synthesis is reduced in the absence of GreA. Moreover, removal of \(\text{greA}\) produced no degradation change in the exonuclease mutants \(\Delta\text{recB}\) (Fig. 4d) or \(\Delta\text{recB}\Delta\text{recE}\) (Extended Data Fig. 8b–d), indicating that GreA impedes RecB-dependent resection (Supplementary Discussion).

The reduced DNA resection in the absence of GreA suggests that the accumulation of backtracked RNAP on DNA may slow RecBCD processing. Thus, inhibition of transcription should increase DNA resection. Treatment of WT cells with the transcription
Figure 5 | Backtracked RNAP promotes recombination by increasing RecA loading.

**a**, c, Representative (one of two) XO-seq plots (DSB at lacA and rifampicin [rif] addition) in the indicated strains; inset plots are n ≥ 2; mean ± s.d.; ***P < 0.001; **P < 0.01 (two-tailed two sample t-test).

**b**. Phleomycin (1 μg ml⁻¹) survival of indicated mutants, n ≥ 3 biological replicates (cultures); mean ± s.e.m.; **P < 0.01 (Kruskal–Wallis test with multiple testing correction).

**c**. Model describing how backtracked RNAP formation can enhance DSB repair by altering RecBCD function and promoting RecA loading.

**d**. Backtracked RNAPs that lack nuclease and Chi-dependent RecA loading activities.

**e**. Backtracked RNAPs that can form stable complexes on DNA could provoke RecBCD pausing, which in turn can stimulate RecA loading and enhance DSB repair.

**f**. Interestingly, in eukaryotes, mutations in the THO/TREX transcription elongation complex result in phenotypes similar to the *ΔgreA* mutant including hyper-recombination, which may in part be due to increased backtracked RNAP.

**g**. This separation-of-function experiment suggests that, although removing GreA can suppress the instability of recB*ΔrecF* mutants to load RecA, RecJ exonuclease activity is required for DSB repair in the recB*ΔgreA* mutant. Thus, enhanced repair in the *ΔgreA* mutant requires the helicase and nuclease activities of resection, but can bypass the Chi-dependent RecA loading function required for repair, suggesting that the absence of GreA can promote RecA loading independent of Chi sites.

**Transcription fidelity impedes DSB repair**

Our results show that increasing backtracked RNAP promotes DSB repair by the RecBCD–RecA pathway. We found that the build-up of backtracked RNAP that occurs in the absence of GreA reduces RecBCD resection, as well as the requirement for specific RecA loading activity of RecB. We propose that backtracked RNAPs can instigate recombination in a manner similar to Chi sites (Fig. 5e). Backtracked RNAPs that can form stable complexes on DNA could provoke RecBCD pausing, which in turn can stimulate RecA loading and enhance DSB repair. Interestingly, in eukaryotes, mutations in the THO/TREX transcription elongation complex result in phenotypes similar to the *ΔgreA* mutant including hyper-recombination, which may in part be due to increased backtracked RNAP.

**Code Availability** MATLAB and R code are available on reasonable request.

**Data Availability** Source data for Figs 1–5 are provided with the paper. Sequencing data are available through the Sequence Read Archive under accession number SRP091869. All other data are available from the corresponding author upon reasonable request.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions P.S. and C.H. conceived the study. P.S., J.A.H., and J.L. performed the experiments. M.A.B.N. and L.A.S. analysed the sequencing data. P.S. and C.H. conceived the study. P.S., J.A.H., and J.L. performed the experiments. M.A.B.N. and L.A.S. analysed the sequencing data. M.A.B.N. and L.A.S. performed the mathematical modelling of RecBCD. P.S., C.H., L.A.S., I.G., and S.M.R. wrote the manuscript.

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Extended Data Figure 1 | DSB repair by the RecBCD–RecA pathway, TCR, and RNAP backtracking. **a,** The RecBCD tri-subunit complex binds to blunt DSB ends with high affinity. The enzyme uses translocase, helicase, and nuclease activities to move along the DNA while unwinding and degrading the DNA. RecB is a helicase with 3′→5′ translocation polarity, whereas RecD acts on the other strand. Initially, the 3′ end of DNA is degraded more vigorously than the 5′ end. When RecBCD encounters an 8-bp Chi site that is recognized by the RecC subunit, the nuclease polarity is switched and unwinding proceeds at a slower rate resulting in the formation of ssDNA overhangs onto which RecA is loaded. The RecA–ssDNA filament performs the homology search and invades the homology donor (blue). DNA from the donor is copied by DNA polymerase III (DnaE). **b,** TCR is initiated when RNAP is stalled at a bulky or helix-distorting lesion. UvrD and ppGpp together can pull RNAP backwards, or alternatively Mfd can promote RNAP forward translocation. Either of these processes expose the DNA lesion, allowing it to be accessed and repaired by UvrA (blue) and other proteins (not shown) in the nucleotide excision repair pathway. **c,** Elongating RNAP can undergo reverse translocation or backtracking along the DNA template. When this happens, the 3′ end of the nascent transcript, which was originally within the active site of RNAP, gets extruded into the secondary channel and transcription elongation cannot continue. GreA and GreB can independently stimulate the internal hydrolytic cleavage of the RNA, realigning the 3′ end within the active site of RNAP, allowing the restart of transcription elongation.

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Extended Data Figure 2 | DSB generation and phleomycin phenotype of GreB overexpression. a, DSB formation is unaffected by removal of greA. Mu GamGFP (Gam protein from phage Mu fused to GFP, ref. 37) foci formation in WT and ΔgreA cells after treatment with 20 μg ml⁻¹ phleomycin (PHL) compared with untreated cells (no PHL). Enlarged cells are shown in the upper right corner. Without treatment, 3–5% of WT and ΔgreA cells have only one GamGFP focus. After phleomycin treatment, 87% of WT cells and 77% of ΔgreA cells have multiple GamGFP foci.

b, GreB, when present at the appropriate levels, can also modulate phleomycin sensitivity. Representative (one of three) semiquantitative spot assay of tenfold serially diluted log-phase cultures from strains overexpressing GreB from its native promoter on a high copy plasmid at the indicated phleomycin concentrations. GreB overexpression, but not the pBA169 plasmid-only control (−), suppresses the phleomycin resistance of the greA deletion.

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Extended Data Figure 3 | PFGE analysis. a, b, Absorption at 600 nm (OD$_{600}$) (a) and survival (b) measured at each of the time points at which samples were obtained for PFGE in the $\Delta$dksA, $\Delta$dksA$\Delta$greA, and $\Delta$dksA$\Delta$recA$\Delta$greA mutants, described in Fig. 1f, g. c, Intensity of DNA within the well, which is composed of intact circular E. coli genomes, as determined by densitometric analysis. For a–c, n ≥ 3 biological replicates (cultures), data are mean ± s.e.m. d, Representative PFGE of WT and $\Delta$greA strains before (lanes 1, 7), after 60 min of treatment with 20 ng ml$^{-1}$ phleomycin (lanes 2, 8), and at indicated times after washing out the drug (lanes 3–6, 9–12). Black bars highlight the observable DNA fragmentation difference between $\Delta$greA and WT cells at 30 min after removing phleomycin. e, Representative PFGE of the $\Delta$dksA$\Delta$recA$\Delta$greA and $\Delta$dksA$\Delta$recA mutants after treatment with 20 ng ml$^{-1}$ phleomycin (lanes 2, 7) and at indicated times after washing out the drug (lanes 3–5, 8–10), showing that unrepaired fragmented DNA is eventually degraded in $\Delta$recA mutants both in the presence and in the absence of GreA. For source data see Supplementary Fig. 1.
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | Factors influencing DSB resistance in the ΔgreA mutant. a, Deletion of recD does not alter the phleomycin resistance of ΔgreA cells (1 μg ml⁻¹ phleomycin). b, In the absence of RecBCD, an alternative repair mode involving the RecQ helicase, RecJ exonuclease, and RecF-assisted RecA-ssDNA filament formation (by displacing SSB) can repair DSBs. c, RecF is not required for the phleomycin (1.5 μg ml⁻¹) resistance of ΔgreA strains. d, RecJ is also not required for the phleomycin (1 μg ml⁻¹) resistance of ΔgreA strains. e, Schematic representation of the I-SceI cutting site engineered within the phage lambda genome (prophage). The I-SceI enzyme is expressed from the doxycycline-induced P<sub>N25-tetO</sub> promoter. f, Generation of a DSB in the prophage genome reduces the survival of WT and ΔgreA cells equally. g, The DNA damage (SOS) response is activated when RecA bound to ssDNA (RecA*) stimulates the self-proteolytic cleavage of the transcriptional repressor LexA, which is part of the SOS regulon. The SOS response can be monitored using a transcriptional fusion of the promoter of sulA (P<sub>sulA</sub>) to GFP (ref. 39). h, Flow cytometry analysis of P<sub>sulA</sub>-GFP expression shows no significant difference between WT and ΔgreA cells, before (0 min) and 60 min after I-SceI DSB induction at the lacA locus. Gating was performed using the lexA3 allele; n = 3, data are mean ± s.e.m.; P ≥ 0.05 (Mann–Whitney U-test). i, Phleomycin resistance of the ΔgreA mutant is unaffected by the inability of cells to activate the SOS response using the lexA3 allele (phleomycin used at 1 μg ml⁻¹). For a, c, d, f, and i, n ≥ 3 biological replicates (cultures), mean ± s.e.m.; **P ≤ 0.01; *P ≤ 0.05; NS, P ≥ 0.05 (Kruskal–Wallis test with multiple testing correction).

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Extended Data Figure 5 | Pathways of RNAP backtracking and their influence on phleomycin resistance. a, Overexpression of RnaseH does not affect the phleomycin phenotype of WT or ΔgreA mutants; RnaseH plasmid (pSK760 rnhA) and control plasmid (pSK762 –) (one of three representative biological replicates (cultures)). Plasmid pSK760 was confirmed as RnaseH overexpressing by transforming into a dnaAtsΔrnhA mutant, growth of which would be rescued only if the plasmid expressed WT rnhA. b, Phleomycin survival phenotypes of the indicated mutants at 1.5 μg ml⁻¹ phleomycin; n ≥ 3 biological replicates (cultures); mean ± s.e.m. c, Resistance of ΔgreA mutants to 1.5 μg ml⁻¹ phleomycin is not affected by deletion of uvrA (nucleotide excision repair pathway) or mutS (mismatch repair); n ≥ 2 biological replicates (cultures); mean ± s.e.m. d, e, Representative (one of three) semiquantitative spot assay of cultures grown to absorbance at 600 nm ~ 0.4 in LB, tenfold serially diluted, and plated on LB agar at the indicated phleomycin concentrations. In d, rpoB8, a slow transcribing RNAP mutant prone to backtracking⁴¹, is resistant to phleomycin whereas rpoB2, a mutation that makes RNAP elongate faster⁴², is sensitive to phleomycin. In e, phleomycin resistance of rpoB8 is suppressed by deletion of uvrD.

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Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Genome levels effects of XO-seq.
a. Normalized read counts of WT cells across the 4.6 Mb E. coli genome at the indicated times after I-SceI DSB generation at lacA. Read loss is restricted to regions on either side of the break site. However, DNA content changes at oriC and ter can be seen, caused by normalization of each time point relative to the uninduced (0 min) sample. DNA degradation seen around the break site is greater than previously reported for in vivo DSB generation at lacA. This discrepancy could arise from the Tagmentation procedure (Illumina) used to fragment and tag DNA, which captures only double-stranded DNA and may miss ssDNA substrates formed after Chi recognition, or from the differences in the repairability of the two systems. 

b, c. Normalized read counts of ΔrecD and ΔrecA mutants across the genome at the indicated times after I-SceI induction at lacA, showing reduced DNA degradation in the absence of RecD (b), but extensive 'Rec-less' DNA loss without RecA (c).
d. Whole-genome reads from the dnaEts mutant at the indicated times showing read pattern changes at oriC (higher copy number than WT) and ter (lower copy number than WT) consistent with the inactivation of replication by DNA polymerase III (dnaE). 

e. Position and orientation of transcribed genes around the lacA::I-SceI cutsite.
f. Quantification of asymmetry in the indicated strains and times after DSB induction. Window size is ±150 kb for all strains and times except for ΔrecD, where window size is ±50 kb.

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Extended Data Figure 7 | Chi asymmetry effects, mathematical modelling, and extensive DNA degradation identified by XO-seq.

a. Location of 920 kb inversion that surrounds the yjaZ::I-SceI cutsite, but does not include oriC. b, Representative (one of two biological replicates) curves of sequencing in WT and ΔrecD strains compared with strains after inversion of part of the genome (WT and ΔrecD), 60 min after DSB generation at yjaZ. c, Parameters of the mathematical model of RecBCD resection obtained from fitting the degradation curves at 15 min for WT, ΔrecD, and ΔrecA mutants. Also shown are the Chi sensitivities (ε) from each fit; n = 2 biological replicates (cultures); data are mean ± s.d. d, The mathematical model of RecBCD resection fits well with the degradation curve obtained 60 min after I-SceI break induction at lacA in the dnaEts background. e, Representative (one of four) plot of read patterns obtained after I-SceI break induction at lacA in the ΔrecA mutant at the indicated time points. f, XO-seq plots comparing ΔrecA and ΔrecAΔgreA mutants; representative (one of four) plot shown, inset bar plot is from n ≥ 4 biological replicates (cultures); data are mean ± s.d.; NS, P ≥ 0.05.
Extended Data Figure 8 | Resection and DNA damage response influence on XO-seq read patterns. a, Read patterns in WT and the dnaEts mutant 120 min after 1-SceI induction at lacA and temperature shift to 42 °C to inactivate dnaE (ref. 25), showing that dnaE function is removed at 120 min. b, Read count patterns in WT and ΔrecB showing greater DNA degradation in the absence of recB. c, Degradation is reduced in the ΔrecBΔrecJ background, suggesting that RecJ contributes to resection when RecB is not present. d, Degradation is similar in ΔrecBΔrecJ and ΔrecBΔrecJΔgreA cells. For a–d, representative plots are shown after inducing 1-SceI DSB at lacA for the indicated times, the inset bar plots are from n ≥ 2 biological replicates (cultures); data are mean ± s.d.; **P ≤ 0.01; NS, P ≥ 0.05 (two-tailed two sample t-test). e, f, Read count patterns in WT after treatment with streptomycin (strep) compared with treatment with streptomycin and rifampicin (strep and rif) (e) and WT after treatment with tetracycline (tet) compared with treatment with tetracycline and rifampicin (tet and rif) (f). g, WT after treatment with rifampicin, 15 min after 1-SceI induction.
Extended Data Figure 9 | See next page for caption.
Extended Data Figure 9 | Effect of RNAP backtracking on DSB repair in a ‘Chi-free’ region and mechanism of reduced resection in ΔgreA mutants. a, Comparison of read count patterns after 60 min of DSB induction at the lacA and mhpC loci. Vertical red and green lines show positions of the I-SceI recognition site at lacA and mhpC, respectively, and the pink and green bars show the positions of correctly oriented Chi sites for lacA and mhpC, respectively. b, Read count patterns 60 min after DSB induction at mhpC in WT compared with ΔgreA, and rpoB8ΔgreA mutants, in which increasing backtracked RNAP complexes are formed. c, The rpoB8ΔgreA mutant shows an increased effect on survival after DSB induction at mhpC compared with ΔgreA and WT cells; n = 4 biological replicates (cultures); data are mean ± s.e.m. d, The ΔrecDΔgreA mutant has reduced degradation compared with the ΔrecD mutant alone 60 min after DSB induction at lacA; representative (one of two) plot shown, inset bar plot is from n ≥ 2 biological replicates (cultures); data are mean ± s.d.; **P ≤ 0.01 (two-tailed two sample t-test). e, Substitution of aspartic acid with alanine at position 1080 in the nuclease domain of recB (recBD1080A or recB*) abolishes the ability of RecB to perform nuclease and RecA loading activities16,27,28. RecJ can compensate for the nuclease function and RecF aids in RecA filament formation, resulting in a hybrid resection machine composed of RecB helicase, RecJ exonuclease, and RecF-assisted RecA loading28,45. Suppression of each individual function by the ΔgreA mutant is indicated. f, Phleomycin survival of the indicated mutants as graphed in Fig. 5d. The recB*ΔrecJ and recB*ΔrecJΔgreA mutants produced no countable colonies on phleomycin; n ≥ 3 biological replicates (cultures); data are mean ± s.e.m.; **P ≤ 0.01 (Kruskal–Wallis test with multiple testing correction). g, Representative (one of four) semiqualitative spot assay showing that the ΔgreA deletion can suppress the phleomycin sensitivity of the recB*ΔrecF mutant but not the recB*ΔrecJ mutant at a low concentration of phleomycin (0.5 μg ml⁻¹).

45. Jockovich, M. E. & Myers, R. S. Nuclease activity is essential for RecBCD recombination in Escherichia coli. Mol. Microbiol. 41, 949–962 (2001).
## Extended Data Table 1 | Plasmids and primers used in this study

### a

| Plasmid       | Features     | Reference |
|---------------|--------------|-----------|
| pKD3          | FRTcamFRT    | 56        |
| pKD4          | FRTkanFRT    | 56        |
| pcp20         | flipp⁺, cI857 λPr | 57        |
|               | ampR camR    |           |
| pBR322        | ampR         | 58        |
| pBR322-DksA   | ampR         |           |
| pH169         | ampR         |           |
| pH169-ΩreB    | ampR         | 58        |
| pSK760-RnhA   | ampR         | 40        |
| pK763-c       | ampR         | 40        |
| pUA66         | pshU::gfpflu2 | 59        |
| pshuA::gfpflu2 | kanR         |           |
| pTSA29-CXI    | ci857-PR-(λxis-λint) | 60 |

1 See supplementary references

### b

| Primer | Sequence |
|--------|----------|
| OC 170 | 5’ ggcctatcgcaccacatgtcagaacaggtctgctgactgtctgctgc 3’ |
| OC 171 | 5’ ttaaagctttgatcatggtaagactgtctgctgc 3’ |
| OC 436 | 5’ atggctctactgcatctcttcaagagatagactgctgtctgctgc 3’ |
| OC 437 | 5’ agaatttcggagcgacacggcctgctgactgtctgctgc 3’ |
| OC 780 | 5’ agaatttcggagcgacacggcctgctgactgtctgctgc 3’ |
| OC 781 | 5’ gacgagccaggtttacctactgcagctgctgactgtctgctgc 3’ |
| OC 1097 | 5’ cgagctgttacctgactgctgactgtctgctgc 3’ |
| JL 118 | 5’ atagtaagagacgctc 3’ |
| JL 119 | 5’ gcgagacgcagttg 3’ |
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Experimental design

1. Sample size
   - Describe how sample size was determined.

2. Data exclusions
   - Describe any data exclusions.

3. Replication
   - Describe whether the experimental findings were reliably reproduced.

4. Randomization
   - Describe how samples/organisms/participants were allocated into experimental groups.

5. Blinding
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   - A statement indicating how many times each experiment was replicated
   - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
### Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Described in Supplementary Methods.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

### Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

N/A

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

N/A

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

N/A

b. Describe the method of cell line authentication used.

N/A
c. Report whether the cell lines were tested for mycoplasma contamination.

N/A
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

### Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

N/A

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A