RecA bundles mediate homology pairing between distant sisters during DNA break repair

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DNA double-strand break (DSB) repair by homologous recombination has evolved to maintain genetic integrity in all organisms. Although many reactions that occur during homologous recombination are known, it is unclear where, when and how they occur in cells. Here, by using conventional and super-resolution microscopy, we describe the progression of DSB repair in live Escherichia coli. Specifically, we investigate whether homologous recombination can occur efficiently between distant sister loci that have segregated to opposite halves of an E. coli cell. We show that a site-specific DSB in one sister can be repaired efficiently using distant sister homology. After RecBCD processing of the DSB, RecA is recruited to the cut locus, where it nucleates into a bundle that contains many more RecA molecules than can associate with the two single-stranded DNA regions that form at the DSB. Mature bundles extend along the long axis of the cell, in the space between the bulk nucleoid and the inner membrane. Bundle formation is followed by pairing, in which the two ends of the cut locus relocate at the periphery of the nucleoid and together move rapidly towards the homology of the uncut sister. After sister locus pairing, RecA bundles disassemble and proteins that act late in homologous recombination are recruited to give viable recombinants 1–2-generation-time equivalents after formation of the initial DSB. Mutated RecA proteins that do not form bundles are defective in sister pairing and in DSB-induced repair. This work reveals an unanticipated role of RecA bundles in channelling the movement of the DNA DSB ends, thereby facilitating the long-range homology search that occurs before the strand invasion and transfer reactions.

Although repair of DSBs by homologous recombination is efficient in E. coli, it is not known whether homologous recombination events are restricted to the ∼15 min period in which sister loci remain in close association after replication¹,², or whether homologous recombination can occur between distant segregated sister loci (∼1.3 μm apart). To test whether segregated distant sisters can undergo recombination when a site-specific DSB was introduced into one sister ∼1 megabase pairs (Mb) from the replication oriC, we expressed small amounts of unstable I-SceI endonuclease³ so that most cells had only one of two sisters cleaved, and repaired the DSB efficiently in reactions that required RecA(C1 and Extended Data Fig. 1).

Fluorescent ParB protein binding to DNA on either side of the cut site allowed us to monitor the fate of cut and uncut loci. After DSB induction, loss and randomized positioning of foci occurred when compared to the uncut control, in which one-focus cells had the focus displaced slightly from mid-cell and two-focus cells had the foci on either side of mid-cell. Time-lapse analysis showed focus pairing, accompanied by increased fluorescence and focus loss dependent on RecA Bundles. RecA bundles disassembled rapidly and unidirectionally before sister re-segregation (Fig. 1A). The two ends of the DSB remained in proximity after cutting and during subsequent processing, supporting schemes in which both DNA ends are engaged in homologous recombination repair (Fig. 1C and Extended Data Fig. 2A), as reported for eukaryotic repair. The behaviour of fluorescent foci marking each of the cut ends was similar, with the foci moving to the cell periphery after cutting (compare Fig. 1A and Extended Data Fig. 1b, d). RecBCD processing of the DSB was bidirectional, consistent with efficient loading of RecBCD complexes onto both ends of the break. The rate of DNA degradation in the absence of RecA was ∼190 bp s⁻¹ per RecBCD complex, eventually leading to complete nucleoid degradation (Fig. 1C, D, Extended Data Fig. 2B, C, Supplementary Video 1 and Supplementary Discussion).

RecBCD-dependent recruitment of RecA to the DSB was assayed in cells expressing a carboxy-terminal fusion of RecA to green fluorescent protein (GFP) and wild-type RecA, both expressed from wild-type chromosomal recA promoters. These cells exhibited wild-type repair and recombination, whereas those expressing RecA–GFP alone were not fully repair proficient (Extended Data Fig. 3A). Before DSB induction, ∼95% of cells showed RecA–GFP fluorescence that was uniformly distributed throughout the cell, with ∼5% of cells having fluorescent spots that were not associated with a marked locus (21% colocalization) (Extended Data Fig. 3B). After DSB induction, fluorescent RecA spots appeared close to or coincident with one of the two marked sister loci (64% colocalization; Fig. 2a, b). The transient RecA spots nucleated rapidly into filamentous structures that we term RecA bundles, which had formed their maximum length by ∼13 min and most often extended along the cell (Fig. 2c, d and Supplementary Video 2). The DSB-induced stimulation of RecA spot and bundle formation required RecBCD processing of the cut ends (Fig. 2e).

Rapid sister locus pairing occurred ∼47 min after RecA bundle formation (Fig. 2f, g). During this period, no consistent changes in bundle architecture occurred and no significant turnover of RecA within the mature bundle structure was observed by fluorescence recovery after photobleaching (FRAP; Extended Data Fig. 3C and Supplementary Video 3). Once initiated, sister focus pairing was rapid (within 5 min in 69% of events) and was asymmetric, with the cut sister travelling ∼84% of the ∼1.3 μm distance between sister loci (Fig. 2h). Although the cut and uncut loci had similar diffusion coefficients (Dₜₚmissão), the movement of the cut locus was 5–7-fold more directional than the uncut locus, indicative of channelling of the movement of the cut locus (Fig. 2h and Extended Data Fig. 4A).

We propose that nucleation of RecA onto the single-stranded (ss)DNA ends formed by RecBCD processing at the DSB triggers polymerization of RecA into bundles, which are required for pairing between distant sisters. RecA bundles disassembled rapidly and unidirectionally ∼17 min after pairing of the sister loci, possibly triggered by the loss of the ssDNA–RecA ends upon strand invasion (Fig. 2l and Supplementary Video 2). After pairing, sisters re-segregated and cells re-established normal growth, cell division and chromosome segregation (Fig. 2k, summary diagram). In parallel, we have shown that DSB induction and bundle formation are followed by the recruitment of PriA and RecG recombination proteins, which promote replication restart and recombination intermediate processing. By contrast, RecN was recruited early to the break (Extended Data Fig. 4B, C and Supplementary Discussion). Bundles of wild-type RecA that formed in response to DSBs were also observed in fixed cells using immunocytchemistry and in live cells with a more functional

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fusion between RecA803 and red fluorescent protein (RFP) (Extended Data Fig. 5). Furthermore, repair-deficient cells expressing RecA(K72A)–GFP, which fails to bind ATP, did not form spots or bundles, whereas cells expressing RecA(K72R)–GFP, which binds ATP but is hydrolysis impaired, formed weak spots close to the cut locus (0.49 ± 0.2 μm), consistent with RecA(K72R) forming filaments on ssDNA and initiating strand transfer in vitro. Nevertheless, the RecA(K72R)–GFP spots failed to nucleate bundles, and recA K72R cells did not show sister pairing and were repair-deficient (Extended Data Fig. 5). These data demonstrate that RecA bundles are physiologically relevant forms of RecA that are critical for homologous recombination repair between distant regions of homology.

To gain more insight into RecA bundle architecture and function, we used super-resolution three-dimensional structured illumination microscopy (3D-SIM) and live-cell time-lapse imaging (Fig. 3). RecA bundles most frequently adopted overall longitudinal trajectories, with some radial writhing (Supplementary Video 4) and were located between the surface of the nucleoid and the inner membrane. Bundles may be excluded from the nucleoid because of their size, as are 20-nm-diameter ribosomes, and their localization at the cell membrane could arise because of association of activated forms of RecA with phospholipid membranes. 3D-SIM showed that 91% of bundles were associated with at least one sister focus, confirming the earlier data (Fig. 3A, Extended Data Fig. 3B and Supplementary Video 5). Bundles were on average 1.4-μm long and typically consisted of a 160-nm-thick central region bounded by thinner (≤120 nm) extensions (Extended Data Fig. 3B). 3D-SIM time-lapse analysis revealed that the central region was relatively immobile whereas the thin extensions moved rapidly and appeared to probe the inner cell compartment (Fig. 3A and Supplementary Videos 6, 7).

Bundles contained ~70% of the normal pool of cellular RecA, estimated to be ~4.640 ± 1.908 molecules per cell, independently of the presence of spots or bundles (Fig. 3B, C, Extended Data Fig. 3D and Supplementary Discussion). The rate of RecA incorporation into DSB-induced bundles was ~4 monomers per second, which compares with an in vitro rate of 0.5–7 monomers per second per DNA end or nucleation point. We estimate that >85% of RecA molecules present in bundles are likely to be DNA free and additional to the two presynaptic ssDNA–RecA filaments that we propose nucleate bundle formation (Supplementary Discussion). DNA-free RecA filaments and bundles can form in vitro and, have a similar architecture to that of RecA–DNA filaments. The observed 1.4-μm length of RecA bundles is less than the 2.5-μm-long twin filament that would form from ~3,200 RecA molecules nucleating onto the two ssDNA ends at the break. Thus, RecA bundles may contain lateral filaments, potentially explaining the thicker central regions of the bundles. RecA bundles have been observed in previous light microscopy and electron microscopy studies, and have been shown to appear in response to DNA damage in E. coli, and in response to DSBs in Bacillus subtilis. In the latter case, spots appeared close to the site of the DSB and extended into...
Figure 2 | RecA bundle formation and disassembly, and RecA-mediated sister locus pairing. a, RecA–GFP spot formation in relation to the cut locus during sister pairing using wide-field microscopy. b, Histograms of the mean distance (D) between the centres of RecA spots and the closest DSe focus with the percentage of colocalization (when D < 0.5 μm). Spont., spontaneous. c, Wide-field imaging of nucleation of RecA bundles from RecA–GFP spots at the DSe. d, Time-lapse images (time in minutes indicated by prime symbol) and analysis of RecA–GFP bundle formation. e, Histograms of the timing of bundle formation and disassembly as respect to sister pairing (time-lapse analysis; n events). f, Mobility of sister loci during sister pairing. DSe focus positions over 300 s (n events). g, Histograms of the timing of bundle formation and disassembly as respect to sister pairing (time-lapse analysis; n events). h, Time-lapse images and analysis of RecA–GFP bundle disassembly. i, Schematic of DSB-end and RecA dynamics during DSB repair by *E. coli* homologous recombination, based on integration of all data. Error bars indicate standard deviations.

Our data demonstrate conclusively that DSB-induced homology pairing can occur between distant segregated sister loci, and therefore homologous recombination is not restricted to the ~15 min cohesion period after replication. We propose that RecA bundles actively facilitate the process of locus pairing between distant sisters, thereby leading to eventual homology-directed strand invasion. The observation that sister pairing is not necessarily accompanied by bundle shortening suggests that the DNA ends move along the bundle. This could be driven by sliding between RecA filaments within the bundle, as proposed for ParM-mediated DNA movement, a treadmilling mechanism in which RecA filament growth at the leading edge and disassembly in the region of the cut locus could lead to rapid pairing, or a process in which a motor-like protein associated with the cut ends moves along the bundle, akin to other motor proteins moving along cytoskeletal elements. Mechanisms in which pairing arises in part as ‘passive’ one-dimensional diffusion along a restraining bundle would still require a process that directs the cut locus towards the opposite side of the cell. In any of these scenarios, the channelled movement of the cut ends would be ‘blind’ to the uncut sister until a local homology search accompanied by strand invasion generated the strand transfer complex whose processing leads to completed recombination products. It follows, therefore, that nucleation of a RecA spot into a bundle should occur irrespective of whether sister homology is present. Consistent with this, RecA bundles formed at similar frequencies regardless of whether sister homology was present (Extended Data Fig. 3E and Supplementary Discussion). The two-step mechanism we propose only needs to be used when sister homology is distant from the cut locus; immediately after replication, when sister loci remain in close proximity, DSB repair by homologous recombination might occur simply by the canonical mechanism of strand invasion and transfer.
The formation of RecA bundles and equivalent structures in other organisms may be a general mechanism for channelling the movement of damaged DNA loci, thereby enabling a genome-wide homology search in a cell compartment at the periphery of the nucleoid. As other repair proteins are known to interact with RecA and its orthologues, bundles may promote the binding and movement of other players in DNA repair. In eukaryotes, homologous recombination may also fall into two classes, defined by the distance the homology search needs to take. For example, allelic events between cohesed sisters and between paired homologues in meiosis do not involve distant pairing events. By contrast, non-allelic homologous recombination events, or those between homologous mitotic chromosomes, may use processes that involve the equivalent of RecA bundles to facilitate the homology search, a process that may take longer, as observed for homologous recombination between homologues intermediates7.

METHODS SUMMARY

Bacteria were derivatives of E. coli K-12 TB28 (MG1655; AlacZYA). Mutations were introduced by λ-Red recombination or P1 transduction. Cells were grown at 30 °C in M9 0.2% glucose and antibiotics when required. Exponentially growing cells (Act-red 0.1–0.2) were used for microscopy. Full details of bacteria and plasmids used, as well as protocols for sample preparation, imaging and analyses can be found in Methods.

Online Content Any additional Methods, 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 Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to D.J.S. (david.sherratt@bioch.ox.ac.uk).
METHODS

Bacterial strains and growth. All strains are derivatives of E. coli K12 TB28 (MG1655; *lacIZYA*). Cells were grown at 30 °C in M9 media supplemented with 0.2% glucose. Ampicillin (100 µg ml⁻¹), kanamycin (50 µg ml⁻¹), chloramphenicol (20 µg ml⁻¹) and tetracycline (10 µg ml⁻¹) were added when required. Expression of I-SceI endonuclease from an arabinose-inducible promoter25 was induced by the addition of 0.2% arabinose. Intracellular localization of chromosomal loci flanking the I-SceI cassette was visualized by fluorescent wide-field microscopy using two distinct ParB/ParS systems27. Mutations were generally constructed by λ-red recombination29 and further introduced into the strain of interest by P1 transduction. When needed, the DNA region between the two fts sites (*canK/kan*) resistance genes) was removed using FLP recombinase expressed from pCP20 (ref. 26).

Microscopy sample preparation. All microscopy experiments were performed on live cells growing in exponential phase (A₆₀₀nm ~ 0.1–0.2). Cells were transferred from liquid culture to a slide mounted with 1% agarose in M9 glucose 0.2% medium and incubated during microscopy at the required temperature using an incubation chamber. 3-D SIM snapshots were performed at 24 °C with cells covered with precision cover glass, thickness no. 1.5H (170–200 µm). For time lapses after DSB induction, arabinose 0.2% was added to the liquid culture for 30 min before the cell were mounted on the slide. Nucleoids were visualized using 4 µg ml⁻¹ 4',6-diamidino-2-phenylindole (DAPI), and cell membranes were visualized using 1 µg ml⁻¹ FM4-64 (Life Technologies).

Wide-field microscopy, 3-D SIM and FRAP imaging. Conventional wide-field fluorescence microscopy was carried out on an Eclipse TE2000-U microscope (Nikon), equipped with a ×100/1.4 oil planapo objective and either a Cool-Snap HQ CCD or a QuantEM camera (Photometrics), and using MetaMorph software for image acquisition. Super-resolution 3-D SIM imaging as well as conventional wide-field imaging was performed on a DeltaVision OMX V3 (Applied Precision/GE Healthcare) equipped with a Blaze SIM module, a wide-field imaging was performed on a DeltaVision OMX V3 (Applied Precision/Applied Precision) or a DeltaVision OMX V4 (Applied Precision/Applied Precision) using a 100/1.4 oil PlanApo objective and either a Cool-Snap HQ CCD or a QuantEM camera (Photometrics). The fast-live mode enabled ultra-high-speed illumination and simultaneous or sequential acquisition of multiple-color three-dimensional stacks of RecA–GFP/DAPI, RecA–GFP/FM4-64, RecA–GFP/mCherry or RecA–GFP alone. For each colour, the raw 3-D SIM stacks were composed of 225 images (512 × 512 pixels) consisting of 15 ×-2 sections (125 nm z-distance, sample thickness of 1.750 µm), with 15 images per z-section with the striped illumination pattern21−25 rotated to the three angles (−60°, 0°, +60°) and shifted in five phase steps. Acquisition settings were as follows: RecA–GFP, 2−5 ms exposure with 488 nm laser (attenuated to 10% transmission); DAPI, 20 ms exposure with 405 nm laser (100% transmission); FM4-64, 30 ms exposure with 593 nm laser (100% transmission). Total acquisition times per stack were 1.8 s for RecA–GFP, and −10 s for RecA–GFP/ DAPI and RecA–GFP/FM4-64. Note that simultaneous imaging of RecA–GFP and Dse foci (ParB–mCherry) was performed in 3-D SIM mode for RecA–GFP and conventional wide-field mode for ParB-mCherry. The 3-D SIM raw data was computationally reconstructed with SoftWoRx 6.0 (Applied Precision) using a Wiener filter setting of 0.002 and channel specifically measured optical transfer functions to generate a super-resolution three-dimensional image stack with a lateral (x−y) resolution of 100−130 nm (wavelength dependent) and an axial (z) resolution of ~300 nm. In the reconstruction process the pixel size is halved from 80 nm to 40 nm and the pixel number doubled in order to meet the Nyquist sampling criterion. The number of pixels in the conventional images of Dse foci were doubled using Pism (Image Visualization Environment; http://msg.ucsf.edu/IVE) in order to merge with 3-D SIM images. A constrained iterative three-dimensional image deconvolution was applied to conventional wide-field data in SoftWoRx 6.0. Images from the different colour channels were registered with alignment parameters obtained from calibration measurements with 0.2-µm-diameter TetraSpeck beads (Life Technologies) using the OMX Editor software (C. Weisgerber and J. Sedat, unpublished). FRAP experiments were performed with an UltraVIEW VoX spinning disk confocal system with Photokinesis module (PerkinElmer) assembled on an IX8 microscope (Olympus) equipped with an CF100-13 EMCCD camera (Hamamatsu) and a ×100/1.4 oil planapo objective (Olympus). Photo-bleaching of a small diffraction-limited spot was carried out with a focused 488 nm laser beam (100 ns with AOTF set to 50% transmission). Fluorescence intensity measurements of the unbleached and bleached regions were performed using ImageJ (http://rsweb.nih.gov/ij/). Values were normalized to those of initial pre-bleaching images. Snapshots and time-lapse analysis. Snapshot analysis was performed with the MicroscopeTracker suite28 extended custom MATLAB routines that we specifically developed to generate focus positioning dotplots, histograms of 0/1/2-focus cell fractions, two-color cell-type counting, ISD, USe–DSe focus distance, focus position along the cell long x-axis (length) and short y-axis (width). Distributions of DSe foci along the cell diameter presented Fig. 1B were determined by subdividing cells into five cell slices of equivalent areas as previously described22. Stoichiometry of RecA was determined by convolving the total fluorescence in recA-GFP cells and comparing it to that of mukB-GFP cells for which MukB stoichiometry is known23. Total intracellular fluorescence intensity distribution analysis was performed on images resulting from the average projection of 15 z-sections (125 nm z-distance) corresponding to a sample thickness of 1.750 µm, using MicrobeTracker. Our estimate of RecA stoichiometry is in the range of previous estimates23,25. Fluorescent particle tracking during long time lapses (>30 s per frame) was performed using a semi-automated custom MATLAB routine as previously described22. Mean square displacements (MSD) in x and y were calculated on single frames that were obtained by running the ViewSDK plug-in (ImageJ) on short time lapses (300 x 5 s per frame). The apparent two-dimensional diffusion coefficient (D_app) was calculated from the initial slope of MSD plotted against time (t) using MSDxy = 4D_app t. The directionality of movement reflects the fraction of the overall displacement (Δd1−t), compared to the total distance travelled by the focus (Δd1−t + Δd−1−t + Δd−1−t + ΣΔd−t + Δd−1−t). Immunocytochemistry. Cells were fixed in phosphate buffered saline with paraformaldehyde 5% (pH 7.2) and glutaraldehyde 0.06% and immobilized on poly-L-lysine slides (Poly-prep, Sigma-Aldrich), followed by treatment with lysozyme 1 mg ml⁻¹. Immunocytochemistry was performed using rabbit anti-RecA polyclonal antibodies (Abnova), which were revealed using anti-rabbit secondary antibodies conjugated to Alexa 594 (Life Technologies). Coverslips were mounted with Vectashield mounting medium (Vector Laboratories).

Flow cytometry. Cells were grown in M9 glucose at 30 °C and sampled in late stationary phase, exponential phase (A600nm ~ 0.15) and 3 h after incubation with cephalexin and rifampicin (run-out). Cell samples were prepared as described26 except that Syto16 (Life Technologies) was used to stain DNA. Analysis was performed on FACScalibur flow cytometer (BD Biosciences). Data files were analysed using CellQuest (BD Biosciences) and Weasel (WEHI; http://www.wehi.edu.au/).

Introduction of DSBs by I-SceI. The recA- strain, in which DSBs were initially introduced to TB28 and the I-SceI cut site was visualized by fluorescent wide-field microscopy using two antibodies (Abnova), which were revealed using anti-rabbit secondary antibodies conjugated to Alexa 594 (Life Technologies). Coverslips were mounted with Vectashield mounting medium (Vector Laboratories).

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Extended Data Figure 1 | DSB induction. a, DSBs were introduced using the I-SceI endonuclease. The I-SceI cutting site (I-SceI<sup>+</sup>) is an 18 bp site (5'-TAGGGATAACAGGGTAAT-3') that was inserted 1,067 kb from oriC on the right replichore (codA-cynR intergenic region). I-SceI<sup>−</sup> is flanked by an upstream <i>parS-P1</i> site (USe; 1.2 kb upstream of the cut) and a downstream <i>parS-PMT1</i> site (DSe; 0.6 kb downstream of the cut) that binds different fluorescent ParB proteins. In <i>vivo</i> localization of USe and DSe was monitored in live cells by expressing the fluorescent fusions of the corresponding ParBP1- and ParBPMT1-binding proteins from pFHC2973 or pMS11. The I-SceI gene controlled by the arabinose-inducible promoter was expressed from the <i>araB</i> locus of the chromosome (782 kb from oriC), or alternatively expressed from a plasmid when a higher level of cutting was required. Two alleles of the I-SceI gene were used, namely the <i>wt</i> allele and an unstable allele carrying a degron tag (I-SceIMycDas). The construction of the system is detailed in Methods. 

b, Cell viability of <i>Rec</i><sup>1</sup> and <i>recA</i> cells was assayed by plating efficiency after induction of I-SceIMycDas. Colony-forming units (c.f.u. ml<sup>−1</sup>) were counted 0, 30, 60, 120 and 180 min after addition of 0.2% arabinose in a steady-state population (OD<sub>600 nm</sub> = 0.2). Values at t = 0 were normalized to 1. Bottom, Southern blot shows the efficiency of DSB induction for the same time points, and the level of DSBs measured from three independent experiments. A <i>cynT</i> probe (500 bp) was used to visualize the substrate: a 12 kb <i>SacII</i> fragment containing the I-SceI cut site, or the product of restriction by I-SceI (downstream 3.1 kb fragment). Conditions were used in which 60 min after I-SceI was induced, most cells with segregated sisters received no more than a single DSB, consistent with the overall level of cutting observed biochemically (~57%) and the dependence on RecA for survival.

c, Flow cytometry showing the DNA content distribution of TB28 strains in the stationary phase, during exponential growth (M9 glucose 30 °C) and after rifampicin/cephalexin run-out. Subpopulations harbouring 1 or 2 chromosome equivalents after run-out indicate the number of replication origins per cell (1.91), which was used to calculate the C (DNA synthesis period) + D (time between completion of DNA replication and cell division) period (74 min) using Helmstetter’s model (origin per cell = 2<sup>(C+D)/2</sup>) (ref. 43). A summary of the cell cycle parameters is shown. All experiments were performed in identical growth conditions in which cells harbour only one replicating chromosome, with initiation of replication occurring ~6 min after birth in the T = 80 min cell cycle. d, Snapshot analysis of USe localization before (left) and after (right) 60 min of DSB induction showed an identical pattern compared with DSe (Fig. 1A). From left to right: ratio of cells with 0, 1 or 2 foci; dot plots of USe foci positions as a function of cell length; histograms of USe position along the cell long axis and the ISD (in μm) are shown. Bottom, a plot of ISD against cell length shows that DSB induction triggers the global decrease of ISD values and generates a population of long cells with reduced ISD (framed in green), revealing the pairing of sister loci.
**Extended Data Figure 2 | Consequences of DSB induction.** A, Analysis of DSB-induced DSe sister pairing in Rec<sup>+</sup> cells. a, Time-lapse particle tracking of DSe foci (5 min per frame) with indicative images (every 20 min) showing pairing between segregated sister loci (DSB was induced 30 min before the beginning of time-lapse acquisition). The red dashed line indicates the sister-pairing event and the grey area indicates the pairing period. b, DSe focus fluorescence intensity quantification performed on two consecutive frames—that is, before and after pairing—shows that the one focus observed after pairing retained 104 ± 15% of the added fluorescence of the previous sister focus (normalized at 100%), consistent with colocalization of the two localization arrays. c, Distances between DSe and USe focus centres remained unchanged upon DSB induction in Rec<sup>+</sup> and recA cells, indicating that both DNA ends remain coincident throughout cutting, pairing and repair. d, Summary diagram of sister pairing that occurred 103 ± 46 min after DSB induction and lasted for 50 ± 34 min (grey area). Once initiated, pairing occurred rapidly (<5 min) and engaged both DSB ends. B, a, DSB induction generated a variety of cell types harbouring 0, 1 or 2 DSe foci with 0, 1 or 2 USe due to loss of the DSe and/or USe foci. One image for each of the nine cell types is shown with their quantification 0, 60 and 120 min after DSB induction in Rec<sup>+</sup> and recA strains. These data are displayed using a square density representation. This was obtained with a custom MATLAB code that uses the data generated by the spotfinderM function of MicrobeTracker software. Only cells >3.3 μm are considered in order to focus the analysis on the fraction of cells in which the I-SceI cut site region (codA-cynR) has already segregated. b, Two-colour time-lapse analysis after DSB induction showed that DSe and USe foci belonging to the same DSB disappeared within 0–5 min from one another in 71% of cases (green and red lines), and recovered within 0–10 min from one another in 87% of cases (green and red arrows), consistent with bidirectional processing of the two ends of the DSB by RecBCD (see Supplementary Discussion). C, Time-lapse analysis of RecBCD-mediated complete degradation of the nucleoid after DSB induction in recA cells. One example is shown with nucleoid DNA stained with DAPI (green), cell membranes stained with FM4-64 (red). The decrease in fluorescence intensity of nucleoids undergoing degradation (red curve) was measured and reflects the rate of DNA degradation in vivo (Supplementary Video 1). Decrease in fluorescence of non-degraded nucleoids is accounted for by photobleaching of the DAPI (blue curve). The overall rate of DNA degradation was 23.2 kb min<sup>−1</sup> (386 bp s<sup>−1</sup>); that is, 193 bp s<sup>−1</sup> per RecBCD complex (n = 38 cells analysed).
Extended Data Figure 3 | RecA bundle characterization. A, Survival after ultraviolet (UV) irradiation shows complementation of recA-gfp allele sensitivity (SS0385 strain) by expression of a wild-type recA gene in the fhuB ectopic locus of the chromosome or from a plasmid (pSC101-recA). RecA–GFP strain (TB28, recA-gfp, fhuB::recA) showed a level of UV resistance similar to the wild-type (wt) strain. B, a, Snapshot analysis of RecA–GFP structures induced by DSBs. Each image was obtained by projection of a three-dimensional stack (15 z-sections of 125 nm z-distance) of 3D-SIM images for RecA–GFP (green) merged with wide-field images for DSe (red). Before DSB induction, RecA is diffuse in most cells (95%) and spontaneous spots are distant from DSe foci. Sixty minutes after DSB induction, RecA spots form in close proximity to a DSe focus and RecA bundles are observed in 29% of total cells, regardless of whether cells have one or two DSe foci. b, Bundle length (nm), and the thickness of the large central body and the thinner extensions is shown in the histogram on the left (nm). The width of the thinner extensions (≤100 nm) corresponds to the lateral resolution limit of 3D-SIM (see Supplementary Videos 2–7). c, In cells with two DSe foci and a RecA bundle, 47% of RecA–GFP bundles were associated with one DSe focus and 44% were associated with both DSe foci. C, FRAP of RecA bundles. Fluorescence intensities of bleached (green curve) and non-bleached regions (blue curve) of RecA bundles were monitored up to 1,500 s after bleaching. No fluorescence recovery was observed throughout this period, as shown by the indicative time-lapse images below. The green and blue boxes indicate the bleached and non-bleached regions of the bundle, respectively (time in s). Initial fluorescence was normalized to 1 (Supplementary Video 3). D, RecA bundle formation after UV irradiation. The ratio of cells with RecA bundle(s) after irradiation with UV light (50 J m⁻²) was measured, as was the total fluorescence intensity of RecA–GFP. The level of the SOS response, induced in response to DNA damage, was reported by the expression of the m-cherry gene controlled by the PsulA SOS-inducible promoter. Time-lapse images of RecA–GFP after UV irradiation confirms rapid bundle formation after UV treatment and snapshot images show multiple bundles present in most cells 45 min after UV irradiation (Supplementary Video 8). E, Relative frequencies of RecA bundles and RecA spots 60 min after DSB induction at codA (356.6 kb) or ydeO (1,581.7 kb) cut sites, ydeO and codA loci have different replication–segregation timings as manifested by the fractions of two-focus cells (8% and 69%, respectively). Images below show the morphology of RecA bundles observed in those two cell populations (see Supplementary Discussion).
Extended Data Figure 4 | Movement of loci after DSB induction and recruitment of recombination proteins. 

A. a, Mobility analysis of uncut loci. b, Comparative mobilities of long-travelling and the short-travelling sister foci during DSB-induced sister pairing. c, Mean square displacement values (MSDs), and MSD scaling parameters ($\alpha$); $\alpha = 0.5$ for the uncut locus compared with 0.69 for the long-travelling sister and 0.41 for the short-travelling one. This compares with $\alpha = 1$ for diffusive movement and $\alpha = 2$ for processive movement\(^+$). d, Summary showing the diffusion coefficients $D_{\text{app}}$, the directionality of movement and the scaling factor values for uncut loci, and long- and short-travelling loci. 

B, Survival after UV irradiation of cells expressing RecN–YPet, PriA–YPet and RecG–YPet fluorescent proteins expressed from their endogenous promoters in their normal chromosomal position. All four fusion proteins were recombination proficient, as demonstrated by resistance to UV irradiation. C, Histogram of the fraction of cells harbouring RecN–YPet, RecG–YPet or PriA–YPet fusions showing DSB-induced focus formation in Rec\(^+\), but not in recB cells. Averages and standard deviations are calculated from snapshot images of three independent experiments. Bottom, the percentage of RecN–YPet, PriA–YPet and RecG–YPet foci that colocalized (<0.5 $\mu$m apart) with a DSe focus is shown with indicative images. Residence times (s) of these foci, estimated by time-lapse analysis, are indicated (see Supplementary Discussion).
Extended Data Figure 5 | RecA bundles are not artefacts and require that RecA can bind ATP. a, Immunocytochemistry using anti-RecA antibodies and Alexa-594-conjugated secondary antibodies. Left, bundles formed by wild-type RecA 60 min after DSB induction (in >25% of analysable cells) or 10 min after UV induction (in >80% of analysable cells). In the recA<sup>wt</sup>/recA<sup>–gfp</sup> diploid strain, bundles were visualized both by immunocytochemistry and by direct observation of RecA–GFP. b, Visualization of RecA<sup>803–RFP</sup> upon DNA damage induction. Merge of phase contrast and RFP images acquired with conventional fluorescent microscopy are shown. RecA<sup>803–RFP</sup> appeared diffuse in the cell before damage induction, and showed bundle formation similar to that observed with the RecA–GFP fusion after DSB induction (same conditions as in Figs 1 and 2) or UV irradiation (10 J m<sup>–2</sup>). Histogram presents the fractions of cells with RecA<sup>803–RFP</sup> bundles in corresponding conditions. c, Survival of cells carrying different recA alleles after DSB induction and UV irradiation (10 J m<sup>–2</sup>), estimated by a plating assay. d, 3D-SIM images of RecA–GFP, RecA<sup>(K72A)</sup>–GFP and RecA<sup>(K72R)</sup>–GFP fusions after DSB induction (same conditions as in Figs 1 and 2) or UV induction (10 J m<sup>–2</sup>). Histograms present the fraction of cells with diffuse fluorescence, cells with RecA spots or RecA bundles ('others' corresponds to cells with saturated signal or aberrant morphologies).