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Spontaneous DNA breakage in single living *Escherichia coli* cells

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Abstract

Spontaneous DNA breakage is predicted to be a frequent, inevitable consequence of DNA replication and is thought to underlie much of the genomic change that fuels cancer and evolution^{1–3}. Despite its importance, there has been little direct measurement of the amounts, types, sources and fates of spontaneous DNA lesions in living cells. We present a direct, sensitive flow cytometric assay in single living *Escherichia coli* cells for DNA lesions capable of inducing the SOS DNA damage response, and we report its use in quantification of spontaneous DNA double-strand breaks (DSBs). We report efficient detection of single chromosomal DSBs and rates of spontaneous breakage ~ 20- to 100-fold lower than predicted. In addition, we implicate DNA replication in the origin of spontaneous DSBs with the finding of fewer spontaneous DSBs in a mutant with altered DNA polymerase III. The data imply that spontaneous DSBs induce genomic changes and instability 20–100 times more potently than previously appreciated. Finally, FACS demonstrated two main cell fates after spontaneous DNA damage: viability with or without resumption of proliferation.

DNA DSBs are important instigators of genomic instability that provoke mutation, genome rearrangement and chromosome aberrations important in evolution, oncogenesis and genetic disease^{3–5}. Frequent DSBs are thought to arise spontaneously and to be repaired accurately when normal DNA replication encounters damage from endogenous causes^{1–3}. Indirect estimates of rates of spontaneous DSB formation have been derived from inviability, chromosome loss or cytogenetic phenotypes of cells lacking DNA repair proteins, some of which affect processes other than repair^{1–3}. These estimates are ambiguous, ranging from 0.2–1 per genome replication in *Escherichia coli*^{1,2} and 50 per human genome replication³, and the extent to which the phenotypes underlying these estimates are attributed to DSBs

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AUTHOR CONTRIBUTIONS

S.M.R. conceived the study; J.M.P. and S.M.R. designed the study, analyzed the data and wrote the paper and J.M.P. performed the experiments.

COMPETING INTERESTS STATEMENT

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versus other lesions also varies^{1–3}. More direct measurement of spontaneous DNA breakage in wild-type living cells has not been achieved.

We describe a direct flow cytometric assay in single living *E. coli* cells for rapid quantification of cells with DNA damage that induces the SOS DNA damage response (including single-stranded (ss) DNA gaps, DSBs and double-strand ends (DSEs)⁴). RecA bound to ssDNA promotes proteolysis of the LexA transcriptional repressor, increasing expression of more than 40 SOS damage response genes⁴. We engineered wild-type *E. coli* to carry a chromosomally located *gfp* gene encoding green fluorescent protein (GFP) controlled by the SOS-inducible *sulA* promoter⁶, which causes cells with SOS-inducing DNA damage to fluoresce green (that is, these cells are GFP⁺). Because it is not plasmid borne and does not inactivate any gene (including *sulA*), damage is detected without perturbing levels of proteins that affect DNA damage processing (Supplementary Methods online). The emission wavelength, rapid expression (8 min after induction) and ≥ 24 -h half-life of this GFP⁷ allow sensitive flow cytometric detection ($\sim 10^5$ cells per minute) and isolation of living cells in which SOS has been induced.

We show that in this system, green fluorescence in cells results from SOS induction (Fig. 1). In one positive control, *lexA*(Def) (SOS repressor–null) cultures fluoresce green quantitatively (Fig. 1a)⁶, and two negative controls, SOS-uninducible *lexA*(Ind[–]) or $\Delta recA$ cells, show $2 \times 10^{-4} \pm 0.1 \times 10^{-4}$ and $7 \times 10^{-4} \pm 4 \times 10^{-4}$ green cells (mean $\pm$ s.e.m.), respectively (Fig. 1a,d). Thus, green cells observed at frequencies above 2×10^{-4} to 7×10^{-4} per culture reflect genuine SOS induction, not spurious promoter firing. Moreover, fluorescence intensity correlated with exposure to ultraviolet C light (Fig. 1b), demonstrating a dose response to a known SOS-inducing, DNA-damaging treatment. Thus, this assay is sensitive and responsive to SOS induction.

We examined logarithmic-phase wild-type cells grown aerobically (Supplementary Methods) for spontaneous SOS-inducing DNA damage. The previous estimate of 0.2–1 DSB or DSE per genome replication (for example, see refs. 1,2) might have been expected to produce a unimodal distribution of cells shifted toward higher GFP intensity from the position of the peaks from SOS-uninducible *lexA*(Ind[–]) and $\Delta recA$ (non-green) control cultures, as was seen with varying doses of ultraviolet C light (Fig. 1b). Notably, untreated, log-phase wild-type cells show a small subpopulation ($\sim 0.9\%$) of spontaneously green cells (Fig. 1c,d). This is well above the limit established in our controls of fluorescence independent of the SOS response, so it does not reflect spurious promoter firing (Fig. 1a,c). Moreover, this green signal is RecA dependent, indicating a genuine SOS response (Fig. 1c,d).

To determine the fraction of this spontaneous SOS signal caused by DSBs or DSEs, we quantified the fraction dependent on functional RecBCD enzyme. RecB is required for SOS induction resulting from treatments that produce DSEs but not for SOS induction in the absence of DSEs⁴. RecB was required for $62\% \pm 3\%$ of spontaneous SOS induction in wild-type cells (Fig. 1c,d). The remaining GFP signal in *recB* cells was RecA dependent (Fig. 1c,d), as expected, implying that other non-DSE lesions (such as ssDNA gaps) account for the remaining $\sim 38\%$ of spontaneous SOS induction.

To determine the assay's sensitivity to DSBs, we created single, chromosomal DSBs *in vivo* using a regulatable I-SceI double-strand endonuclease and a chromosomal cut site shown to linearize chromosomal DNA efficiently⁸ (Supplementary Methods and Fig. 2). Notably, essentially all of the cells fluoresced green (Fig. 2a) after I-SceI induction, with $87\% \pm 5\%$ of cells falling within a stringently green 'gate' (Fig. 2a,d and Supplementary Methods).

This response requires endonuclease induction, a cut site, RecA and RecB (Fig. 2d). Thus, the assay was able to detect a model chromosomal DSB efficiently.

Most log-phase cells have two or more chromosomes⁹ (Supplementary Fig. 1 online), both of which might have been cleaved by I-SceI (Fig. 2a). To address whether a single DSB per cell is detected, we quantified fluorescent cells after induction of DSBs by I-SceI in cells with their chromosome number reduced to one using a *dnaA*(TS) mutation. *dnaA*(TS) cells cannot initiate replication at the restrictive temperature but continue to divide until most carry only one chromosome (Supplementary Fig. 1 and references therein). First, a temperature shift reduced the chromosome number to one in $61\% \pm 4\%$ of cells (Supplementary Fig. 1 and Supplementary Note online). Second, $84 \pm 4\%$ of shifted cells fell within the green gate after DSB induction (Fig. 2c,f), compared with $95\% \pm 2\%$ of *dnaA*⁺ cells (Fig. 2f). Thus, the assay was able to detect a single DSB (two DNA ends or DSEs) per cell efficiently.

Because most growing cells have two or more chromosomes⁹, most spontaneous DSBs should be repaired via homology-mediated DSB repair using a sister chromosome. Thus, most cells might have triggered the SOS response after I-SceI induction (Fig. 2a,c,d,f) because attempted repair using the sister restores an I-SceI cut site, making these DSBs irreparable, in effect. We modeled reparable DSBs using I-SceI in cells carrying an F' episome with ~ 20 kb of DNA identical to the region spanning the chromosomal I-SceI cut site but with no I-SceI site itself⁸ (Supplementary Methods). This allows healing of the chromosomal break upon repair, as expected for natural DSBs. Of these cells, $27\% \pm 2\%$ were GFP⁺ upon I-SceI induction (Fig. 2b,e); this response to I-SceI requires RecA and RecB (Fig. 2e). Thus, the assay was able to detect a transient, reparable DSB with ~ 27% efficiency. The decreased efficiency of detection of these reparable DSBs (27%, Fig. 2e) versus irreparable DSBs (87–84%; Fig. 2d,f) results from repair, not from the F' episome *per se* (Fig. 2e and Supplementary Note), and might occur either because (i) most or all DSBs induce SOS but at a level lower than that which activates the *sulA* promoter controlling GFP (a mid-range SOS promoter¹⁰), or (ii) many DSBs are repaired quickly and do not induce SOS. Either way, the 27% figure indicates that about four times more ($1 / 0.27$) cells with reparable DSBs are present than are detected, thus providing a correction factor with which to calculate spontaneous DSB levels from the spontaneous green signal.

Some eukaryotes preferentially repair from sister chromosomes rather than homologs¹¹, as modeled here. If present in *E. coli*, sister preference might prolong the time before productive repair using the F' episome, possibly artificially elevating the green signal from reparable DSBs. In eukaryotes, sister preference is thought to result from sister cohesion after replication¹². In *E. coli*, not all investigators observe chromosome cohesion during growth^{13,14}, and when observed¹⁴, cohesion is minimal in cells with rapid, 30- to 60-min generation times (as in this study) during which only ~ 10% of each sister is predicted to be cohered, with cohesion at DNA near the replication origin (ref. 14 and D. Bates, personal communication). Thus, given the current data, sister preference (at least caused by chromosome cohesion) seems unlikely, although more data are needed to address this point conclusively. Moreover, the repair partner used, an F', is carried in 1.4- to 2.3-fold excess of the chromosome¹⁵. Thus, repair might be biased toward the F' and toward more rapid repair (a lower green signal) than when spontaneous chromosomal DSBs repair using a sister chromosome.

The signal from the model reparable DSB also is unlikely to result from repeated cycles of nonhomologous end-joining (NHEJ) and recleavage, because NHEJ is exceedingly rare to absent in *E. coli* (Supplementary Note¹⁶). Supporting the idea that our model of a reparable

DSB is realistic, we note efficient RecA-dependent, RecB-dependent and homology-dependent repair and survival of the reparable DSB (Fig. 3b).

Reparable DSBs have been thought not to activate the DNA damage response in yeast¹⁷, but they did activate it here (Fig. 2b,e). This apparent difference might result from different quantitative levels of detection in the yeast assay versus the *E. coli* assay, rather than a qualitative difference (Supplementary Note). SOS is required for maximum survival of DSBs, including reparable DSBs (Fig. 3), suggesting that SOS is a normal response to DSBs.

We estimated the rate of cells experiencing one or more spontaneous DSBs as follows. Considering that 0.9% of growing cells experienced SOS induction (Fig. 1c,d), 62% of which is RecB dependent (Fig. 1c,d), and given that this assay can detect 27% of reparable DSBs (Fig. 2b,e), the fraction of cells with one or more DSBs at steady state is approximately $0.9\% \times 62\% / 27\% = 2.1\%$. We assume that neither dilution by cell division nor degradation of GFP contributes significantly to the net change in GFP⁺ cells per generation because (i) the GFP half-life is ≥ 24 h (ref. 7) (which is long, relative to the *E. coli* generation time of 0.5–1 h; Supplementary Methods); (ii) only ~35% of GFP⁺ cells can divide to form colonies (below) and (iii) cell division normally lags after SOS induction⁴. Because the frequency of GFP⁺ cells is unchanged during early, late and mid-log phase (Supplementary Fig. 3 online), we can assume a constant rate of addition of new green cells each generation. Thus, the maximum number of GFP⁺ cells added per cell doubling is half the total number of GFP⁺ cells in culture (to achieve a steady state). Therefore, the maximum rate of addition of cells with one or more DSBs is $2.1\% \times 0.5 = 1\%$ of cells per generation. This rate is 20- to 100-fold lower than previous estimates for *E. coli*^{1,2} and, proportionately to DNA content, approximately fourfold lower than the 50 per generation estimated for human cells³. These previous estimates probably reflect real DNA problems, but our data imply that most are not DSBs.

Breakage of DNA replication forks is postulated to underlie most spontaneous DSBs^{1,18}. To test this idea, after trying several imperfect strategies (Supplementary Note and Supplementary Fig. 2 online), we examined an altered function allele affecting the catalytic subunit of DNA polymerase (Pol) III, the main replicative polymerase. The *dnaE915* allele causes a reduced spontaneous mutation rate¹⁹. Although DnaE915 has not been characterized biochemically, genetic evidence suggests that it may exclude lower-fidelity DNA polymerases, possibly owing to increased processivity²⁰, which might reduce replication fork breakage. We observed a $48\% \pm 4\%$ decrease in levels of spontaneous SOS induction in *dnaE915* cells, mostly in the RecB-dependent component (Fig. 4), implying a reduction of spontaneous DSBs or DSEs, or a reduction in their detection, in this mutant. These data implicate the activity of the wild-type Pol III catalytic subunit, and thus implicate replication, in generation of most of the spontaneous DSB- or DSE-induced SOS response.

Replication by Pol III could either promote DNA breakage by any of several hypothesized routes involving replication into sites of DNA damage and/or fork stalling^{1,2,21}, or it could, in principle, affect SOS induction after breakage. Either way, association of the replisome with spontaneous DSBs and/or DSEs is supported. The data also suggest an alternative hypothesis for the *dnaE915* ‘antimutator’ phenotype: reduction of spontaneous SOS induction and, thus, SOS-associated mutagenesis⁴.

Finally, we used FACS to explore the fates of cells that sustained spontaneous DNA damage. We isolated spontaneously green cells ($97\% \pm 0.5\%$ purity; Supplementary Note) and assayed for colony formation. Approximately 35% of GFP⁺ cells produced colonies ($29\% \pm 0.6\%$ of green cells forming colonies, normalized by $82\% \pm 1\%$ of non-green cells

forming colonies, which controls for FACS-induced effects on colony formation; values represent mean $\pm$ range from two experiments). The GFP⁺ cells that did not form colonies seemed to be viable (as determined by their exclusion of propidium iodide; Supplementary Methods, Supplementary Note and Supplementary Fig. 4 online) but non-culturable. Therefore, many of the SOS-induced cells detected were not *en route* to death, but rather at least 35% (and perhaps most) of them succeeded at repair and survived. In addition, the remaining ~ 65% (non-colony formers) appear to be analogous to senescent human cells—alive but not proliferating—and, similarly, seem to have entered this state in response to DNA damage²². We excluded alternative possibilities, such as that these cells were under typical cell-cycle arrest with recovery or that they were in a transient growth slowdown called ‘persistence’ in bacteria (Supplementary Note and Supplementary Fig. 4).

The data reported provide four important conclusions. First, SOS is a relatively robust detector of reparable DSBs (Fig. 2b,e). Second, the data support the hypothesis^{1–3,21} that DNA replication contributes to formation of most spontaneous DSBs and/or DSEs (Fig. 4). Third, the rate of spontaneous double-strand breakage is 20- to 100-fold lower than predicted. Fourth, the main fates awaiting cells that sustain spontaneous SOS-inducing DNA damage, including DSBs, are viability with rapid proliferation or viability without rapid proliferation.

Previous studies that predicted 20- to 100-fold higher rates of spontaneous DNA breakage in bacteria and approximately fourfold higher rates of breakage per human DNA equivalent than estimated here relied on viability or cytogenetic phenotypes of repair-defective mutants: *E. coli* *recA* and *recB* and human *blm*^{1–3}. These estimations assume that loss of the repair protein merely allows accumulation of spontaneous DSBs and DSEs and does not cause additional DNA damage. This assumption is likely to be incorrect or incomplete. If these mutant backgrounds increase DNA damage levels, this might partially account for the discrepancies between previous estimates and ours; for example, RecA promotes replication fork regression, which could exacerbate or ameliorate damage²³. In addition, cleft, regressed replication forks are more abundant in cells lacking RecBCD, such that RecBCD might prevent fork cleavage and DSE formation normally²¹. Moreover, all of these proteins affect processes other than repair, including, at the very least, damage-response induction (RecA and RecB⁴) and probably DNA replication as well (BLM²⁴). Thus, in their absence, DNA damage might arise and produce the phenotypes noted in earlier studies^{1–3} as a result of lesions other than DSBs and DSEs being processed in unusual ways. Our estimates are based more specifically on the contribution of DSBs and are from wild-type cells, thereby avoiding potential damage generated only in repair mutants. Additionally, many different kinds of DNA lesions should cause viability and cytogenetic phenotypes. Whereas it is likely that replication is perturbed frequently^{1,18}, our data establish an upper limit on how often the lesion resulting from replication arrest is a DSB.

Likewise, our measurements pertain specifically to DSBs. RecBCD enzyme is expected to respond to a single DSE in addition to a DSB, which can be regarded as two DSEs in a cell. Whereas *I-SceI* experiments established that the SOS-GFP assay detects as few as two DNA ends in a cell (Fig. 2c,f), it remains formally possible that a single DSE per cell is detected inefficiently. The data cannot rule out the possibility that one replication fork collapse per replication produces one DSE², but they can exclude the possibility that an equivalent frequency of replication fork stoppages or disruptions creates DSBs (reviewed in ref. 18). Such disruptions probably occur¹⁸ but generate DNA lesions or structures other than DSBs and thus have different consequences for genome instability.

These data alter our view of DNA replication and the origins of spontaneous genomic change in two ways. First, the apparent potency of DSBs in inducing spontaneous genome

rearrangements is 20–100 times higher than estimated previously. For example, DSBs probably underlie spontaneous duplicated chromosome segments^{8,25} present in $\sim 10^{-3}$ to 10^{-4} bacteria. If that frequency approximates the formation rate, with $\sim 10^{-2}$ cells with one or more DSBs per genome replication, this implies that as many as 10% of DSBs are repaired mutagenically, producing rearrangements. Each DSB has an extremely high probability of destabilizing the genome. Because similar methods have been used previously to estimate spontaneous DSBs in humans³, these are also likely to be overestimates. DSB repair underlies some breast cancers⁴, highlighting the importance of more accurate estimates. The strategy used in our assay could be adapted for use in eukaryotic cells.

Second, the lower rate of DSBs implies that the first defense against their genotoxic effects is break avoidance, not break repair, at replisomes. Break avoidance is probably an active function, and we suggest that many good candidates for its enforcement are already identifiable. Many conditional replication mutants show chromosome breakage²¹, implying that altering the encoded proteins negates the break prevention functions that they normally possess. Replication appears dangerous, but break prevention may be a first defense limiting genome instability, and repair may be a second, riskier stratagem used when prevention fails.

We found the apparent senescence-like state of some cells that experienced SOS-inducing damage to be unexpected. Senescence in multicellular animals is regarded as an evolutionarily selected response to DNA damage that (temporarily) allows cancer avoidance²². It and apoptosis are p53-dependent alternatives that suppress proliferation of cells that have experienced DNA damage and thus might carry mutations²². Although *E. coli* has no p53 homolog, it undergoes both programmed cell death, perhaps in response to DNA damage²⁶, and also apparent senescence in response to aging²⁷ and nutritional depletion²⁸. The connection of DNA damage to a senescence-like state in a bacterium might, like senescence in multicellular animals, be selected to suppress proliferation of less-fit cells, which is useful to the clone. Mathematical modeling indicates an advantage for diminishing the contribution of less-fit cells to clones during rapid growth (the conditions of this study) but not during a stationary phase²⁹. SOS might provide a mechanism that stops proliferation of less-fit cells during rapid growth (as the results of this work imply) and instead promotes mutagenesis⁸ in the stationary phase, both as adaptive strategies^{8,29}. Our findings suggest that the evolution of such stasis responses preceded the evolution of p53 and p53-dependent senescence mechanisms.

METHODS

Bacterial strains, genetic constructs and mutant alleles

Bacterial strains used in each figure are described in detail in Supplementary Methods and Supplementary Table 1 online.

Flow cytometry analysis

Log-phase cells cultured in M9 glucose medium were analyzed using the Epics XL-MCL flow cytometer (Beckman Coulter) as described (Supplementary Methods and ref. 30). FlowJo software was used for data analysis (Treestar). *P* values were calculated by single-factor analysis of variance (ANOVA) and the Tukey test using SigmaStat software (Systat Software).

Cell sorting

GFP⁺ cells were sorted from wild-type cultures and counted using the Epics Altra cell sorter equipped with a Forward Fluorescence Detector (Beckman Coulter) as described in

Supplementary Methods and ref. ³⁰. Further details on sorting and sorting purity are given in the Supplementary Note.

Survival after I-SceI induction of DSBs

Survival is described in detail in Supplementary Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Steady-state levels of spontaneous RecB-dependent SOS induction demonstrate the presence of spontaneous DSBs and/or DSEs in a small cell subpopulation. **(a–c)** Representative flow cytometry histograms. **(a)** Positive control, GFP⁺ *lexA51(Def)* cells, and negative controls, *lexA3(Ind[−])* and Δ *recA* control cells. **(b)** Wild-type cells after exposure to ultraviolet C light. **(c)** Steady-state levels of SOS induction in wild-type, *recA*, *recB* and *recB recA* cells (full genotypes of all strains are given in Supplementary Methods and Supplementary Table 1). Horizontal bars represent the GFP⁺ gate. For **b**, ~0.9% of these cells are spontaneously green (see **c,d**), but the y-axis scale hides this shoulder to the main peak. For **c**, the *recA* and *recB recA* curves are both shown in gray, as the lines superimpose. **(d)** Quantification of steady-state levels of green wild-type cells (within the GFP⁺ gate) (mean $\pm$ s.e.m. of 18, 15, 7 and 4 experiments for wild-type, *recB*, *recA* and *recB recA* cells, respectively). In all figures (except where specified), sample groups with stars indicate statistical significance among all other starred sample groups using single-factor ANOVA and Tukey test, $P < 0.01$. Additionally, in Figure 1d, all strains are significantly different from all others ($P < 0.01$), except for *recA* and *recB recA*, which are not.

Figure 2. Efficient SOS induction by chromosomal DSBs. **(a–c)** Representative flow cytometry histograms and **(d–f)** quantification of multiple experiments, showing GFP⁺ cells after induction of I-SceI endonuclease in the presence of a single chromosomal I-SceI cut site. Horizontal bars in **a–c** represent GFP⁺ gate (Supplementary Methods). **(a)** SOS induction by I-SceI in log-phase cells with a single chromosomal cut site. **(b)** Efficient detection of a chromosomal DSB repaired (and removed) by homologous recombination with an F' episome. **(c)** Detection of cells with only two DSEs (a single chromosome with an I-SceI-induced DSB) in *dnaA*(TS) cells with chromosome counts reduced to one (Supplementary Fig. 1 and Supplementary Note). Isogenic *dnaA*⁺ cells assayed in parallel produced profiles similar to those in **a**. Here and in **a**, there is a small enzyme- and cut site-dependent contribution to green cells independent of intentional induction, which probably reflects difficulty of repressing I-SceI expression⁸. In **b**, there is a low-level, repeatable F'-specific and I-SceI induction-specific SOS induction in all cells, perhaps suggesting F'-specific binding of I-SceI that induces a low-level response. **(d–f)** Multiple experiments assessing SOS and GFP induction and their dependence on RecA and RecB in response to I-SceI-mediated DSBs. Strains as in **a–c**, carrying a single chromosomal I-SceI cut site in the absence **(d)** or in the presence **(e)** of the F' episome carrying the 20-kb homologous region for recombinational repair or in *dnaA46*(TS) cells **(f)** at restrictive temperature (one chromosome per cell; Supplementary Fig. 1). White and gray bars indicate percentages of

cells within the green gate (shown in **a–c**) after repression and induction, respectively, of I-*SceI*. ‘DSB’ indicates that both I-*SceI* and a cut site were present. Shown are mean $\pm$ s.e.m. of three (**d**), three to seven (**e**) and three (**f**) experiments. Strain designated ‘F’ cut site’ (**e**) has a cut site disrupting the homology on the F’. This control shows that the reduction of green signal when homology is supplied for repair is caused by repair, not extraneous aspects of the F’.

Figure 3.

Induction of the LexA regulon of the SOS response is required for survival of cells (as determined by colony formation) with induced DSBs. **(a)** Irreparable DSBs. **(b)** Repairable DSBs. Note the much greater survival with homologous DNA present for repair **(b)**. The fraction of cells surviving I-SceI induction in the presence of a single chromosomal I-SceI site is reduced in cells unable to induce expression of SOS genes, carrying the *lexA3*(Ind⁻) allele, and in cells lacking RecA and RecB proteins required for DSB repair (in addition to their roles in SOS induction). When irreparable DSBs are induced with I-SceI **(a)**, constitutive high expression of LexA and SOS genes in *lexA*(Def) repressor-null mutant cells also improves survival (to 13% ± 1%) relative to wild-type (4% ± 0.4%). Data represent mean ± s.e.m. from four or five experiments. Stars indicate statistical significance calculated as in Figure 1 but versus wild-type cells only. In addition, the surviving fraction of wild-type cells with both I-SceI and cut site differs significantly from the surviving fraction of I-SceI-only cells and the surviving fraction of cut site-only cells, in both **a** and **b**. CFU, colony-forming units.

Figure 4.

Reduction of spontaneous SOS induction from DSEs in cells having DNA Pol III with altered function. Labels are as in Figure 1. **(a)** Representative flow cytometry histograms of the isogenic strains indicated (Supplementary Table 1 for full genotypes). The *dnaE915* antimutator allele¹⁹ of the gene encoding the DNA Pol III catalytic subunit is reviewed in the text. **(b)** Data represent mean $\pm$ s.e.m. from four experiments. As in Figure 1, sample groups marked with an asterisk differ significantly from all other sample groups with an asterisk ($P < 0.01$). Additionally, here, all sample groups differ significantly from all others ($P < 0.01$) except for *recB* versus *dnaE915 recB*, and *recA* versus *dnaE915 recA*, which do not differ significantly.