Roles of Nucleoid-Associated Proteins in Stress-Induced Mutagenic Break Repair in Starving *Escherichia coli*

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ABSTRACT The mutagenicity of DNA double-strand break repair in *Escherichia coli* is controlled by DNA-damage (SOS) and general (RpoS) stress responses, which let error-prone DNA polymerases participate, potentially accelerating evolution during stress. Either base substitutions and indels or genome rearrangements result. Here we discovered that most small basic proteins that compact the genome, nucleoid-associated proteins (NAPs), promote or inhibit mutagenic break repair (MBR) via different routes. Of 15 NAPs, H-NS, Fis, CspE, and CbpA were required for MBR; Dps inhibited MBR; StpA and Hha did neither; and five others were characterized previously. Three essential genes were not tested. Using multiple tests, we found the following: First, Dps, which reduces reactive oxygen species (ROS), inhibited MBR, implicating ROS in MBR. Second, CbpA promoted F' plasmid maintenance, allowing MBR to be measured in an F'-based assay. Third, Fis was required for activation of the SOS DNA-damage response and could be substituted in MBR by SOS-induced levels of DinB error-prone DNA polymerase. Thus, Fis promoted MBR by allowing SOS activation. Fourth, H-NS represses ROS detoxifier *sodB* and was substituted in MBR by deletion of *sodB*, which was not otherwise mutagenic. We conclude that normal ROS levels promote MBR and that H-NS promotes MBR by maintaining ROS. CspE positively regulates RpoS, which is required for MBR. Four of five previously characterized NAPs promoted stress responses that enhance MBR. Hence, most NAPs affect MBR, the majority via regulatory functions. The data show that a total of six NAPs promote MBR by regulating stress responses, indicating the importance of nucleoid structure and function to the regulation of MBR and of coupling mutagenesis to stress, creating genetic diversity responsively.

KEYWORDS double-strand break repair; evolution; mutagenesis; nucleoid-associated proteins (NAPs); stress response

In *Escherichia coli*, the structure of the bacterial chromosome or nucleoid is governed by the action of small, abundant DNA-binding nucleoid-associated proteins (NAPs) (Ali Azam *et al.* 1999). NAPs bind dynamically to the genome

both sequence specifically and sequence nonspecifically, causing a variety of genomic conformations (Azam *et al.* 2000). Through this action, several NAPs also act as global transcriptional regulators, with some of their regulons containing hundreds of genes (Oberto *et al.* 2009; Kahramanoglou *et al.* 2011; Chib and Mahadevan 2012). There are 15 NAPs: Hfq, IHF-A and IHF-B (which constitute IHF), HU- α and HU- β (which constitute HU), Fis, H-NS, CbpA, Lrp, CspC, CspE, Dps, StpA, Hha, IciA (ArgP), DnaA, and CbpB (Ali Azam *et al.* 1999). The NAP composition of the nucleoid changes in a growth-phase-dependent fashion, with Hfq, IHF, HU, Fis, and H-NS controlling genomic compaction primarily in actively growing cells (Ali Azam *et al.* 1999). Dps takes over in stationary phase, and CbpA expression peaks in late stationary phase (Ali Azam *et al.* 1999).

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Fis, like most NAPs, is small, basic, and abundant in cells. Fis levels peak at 60,000 molecules per cell in mid-log phase (Ali Azam *et al.* 1999). Fis functions in site-specific recombination and also controls transcription of DNA recombination and growth genes (Ueguchi and Mizuno 1993; Esposito and Gerard 2003; Flatten and Skarstad 2013). During the transition to stationary phase, Fis drops to undetectable levels and is thought to be absent from starving cells (Ali Azam *et al.* 1999). H-NS functions largely as a transcription repressor, silencing especially genes recently acquired through horizontal transfer (Ueguchi and Mizuno 1993; Perez *et al.* 2008; Sharadamma *et al.* 2010; Dorman 2014). H-NS reaches 40,000 molecules per cell during growth phase but falls sharply on entry into stationary phase (Ali Azam *et al.* 1999). H-NS is also a global regulator controlling the transcription of a few hundred genes, including those required for protection from osmotic and acid stresses (Barth *et al.* 1995; Bouvier *et al.* 1998; Chib and Mahadevan 2012). In growing cells, Fis and H-NS work together to repress the transcription of *dps* (Grainger *et al.* 2008). Fis also represses *cbpA* transcription (Chintakayala *et al.* 2013).

When IHF, HU, H-NS, and Fis levels fall, the nucleoid becomes compacted by Dps (Akerlund *et al.* 1995). In stationary phase, Dps levels can reach as high as 180,000 molecules per cell. Dps binds sequences nonspecifically (Ali Azam *et al.* 1999) and causes the nucleoid to assume a unique state with DNA cocrystallized with Dps protein molecules (Schnetz 2008). Whereas there is no direct evidence that Dps is a global transcriptional regulator, Dps plays important roles in both protection against reactive oxygen species (ROS) and regulation of replication initiation (Chodavarapu *et al.* 2008b; Calhoun and Kwon 2011). Little is known about the other stationary-phase-specific NAP, CbpA. CbpA has sequence similarity to the DnaJ cochaperone and has a DNA binding motif similar to that of Dps. CbpA is not known to be a global transcription regulator.

HU and IHF are the other primary growth-phase NAPs, and they function with Fis and H-NS to compact the replicating genome (Ali Azam *et al.* 1999). As cells reach stationary phase, HU and IHF levels drop (Ali Azam *et al.* 1999). DnaA is an essential replication-initiation protein that binds to the replication origin, *oriC*, and promotes open-complex formation for replication initiation (Ozaki and Katayama 2009). IciA, also known as ArgP, inhibits replication initiation by binding to conserved sequences within *oriC*, preventing DnaA from inducing open-complex formation (Hwang and Kornberg 1990; Thony *et al.* 1991; Hwang *et al.* 1992). CbpB, also known as Rob, is similar to CbpA in that it binds preferentially to curved DNA structures (Azam *et al.* 2000). StpA is a homolog of H-NS and can silence horizontally acquired DNA when H-NS is absent from cells (Sonnenfield *et al.* 2001; Uyar *et al.* 2009). Unlike the other NAPs, Hha does not bind directly to DNA but rather binds a protein that binds and sequesters DNA (Madrid *et al.* 2007). Lrp is the closest analog to a eukaryotic histone. Lrp forms octomeric structures around which DNA winds, similarly to DNA wrapping

around histones (de los Rios and Perona 2007). HU, IHF, Lrp, DnaA, and IciA are also global transcription regulators (Bouvier *et al.* 1998; Oberto *et al.* 2009; Prieto *et al.* 2012).

CspC and CspE, although belonging to the cold-shock-protein regulon, are constitutively expressed in cells even at 37° (Czapski and Trun 2014). CspC and CspE promote genomic compaction and are also required for stability of *rpoS* transcript levels (Cohen-Or *et al.* 2010) and for resistance to different environmental stressors (Cohen-Or *et al.* 2010; Shenhar *et al.* 2012), possibly by controlling levels of *rpoS* transcript. *rpoS* encodes σ^S (or RpoS protein), the transcriptional activator (RNA polymerase sigma factor) of the general/starvation stress response.

Here we address the roles of NAPs in mutagenesis, specifically mutations formed by mutagenic break repair (MBR), which happens in stressed, starving cells. Repair of DNA double-stranded breaks (DSBs) in *E. coli* is switched from high fidelity to a mutagenic mode during starvation stress under control of the RpoS general/starvation stress response (Ponder *et al.* 2005; Shee *et al.* 2011; Rosenberg *et al.* 2012). DSB repair switches either to a mutagenic homologous recombination (HR)-mediated repair pathway, using error-prone translesion DNA polymerases IV (DinB or Pol IV) (Ponder *et al.* 2005), Pol V (Petrosino *et al.* 2009; Shee *et al.* 2011), or Pol II (Frisch *et al.* 2010), which creates base substitution and indel mutations [single-nucleotide variations (SNVs)], or to a “micro-homologous” pathway that causes genome rearrangement [gross chromosomal rearrangements (GCRs)] using DNA Pol I [reviewed in Rosenberg *et al.* (2012) and Rogers *et al.* (2015)]. Both SNV and GCR pathways require RecA, RuvC, and RecBC HR proteins.

The general/starvation (RpoS) stress response (Layton and Foster 2003; Lombardo *et al.* 2004) and the unfolded periplasmic protein (RpoE) response (Gibson *et al.* 2010) are also required for both SNV and GCR mechanisms. RpoE promotes MBR at some genomic sites by promoting formation of DSBs by an as yet undetermined mechanism (Gibson *et al.* 2010). RpoS licenses the use of DinB and other error-prone DNA polymerases in break repair by an unknown mechanism (Ponder *et al.* 2005; Frisch *et al.* 2010; Shee *et al.* 2011). The SOS DNA-damage response is required for SNV formation (McKenzie *et al.* 2000) and promotes MBR by its induction of the DinB error-prone DNA polymerase (McKenzie *et al.* 2001; Galhardo *et al.* 2009). Neither SOS nor DinB plays a role in GCR formation (McKenzie *et al.* 2001). Formation of GCRs, but not SNVs, requires DNA Pol I (Slack *et al.* 2006).

Thus, there are three main hubs of stress-response regulation of MBR (Al Mamun *et al.* 2012; Rosenberg *et al.* 2012): (1) formation of a DSB, which RpoE promotes (Gibson *et al.* 2010), and its repair, (2) SOS induction, which upregulates DinB about 10-fold transcriptionally (Galhardo *et al.* 2009), and (3) activation of the RpoS general stress response, which allows DinB and other low-fidelity DNA polymerases to participate in DSB repair by as yet unknown means (Ponder *et al.* 2005; Frisch *et al.* 2010; Shee *et al.* 2011; Rosenberg *et al.* 2012).

Table 1 *Escherichia coli* K12 trains used in this study

Strain/plasmid	Genotype	Source/reference
CAG12080	<i>zah281::Tn10</i>	Singer <i>et al.</i> (1989)
FC36	$\Delta(lac\ proB)XIII\ thi\ ara\ Rif^R$	Cairns and Foster (1991)
FC40	FC36 [F' $\Delta 45 = F'$ <i>proAB</i> ⁺ <i>lac</i> ^R <i>lacI33</i> Ω <i>lacZ</i>]	Cairns and Foster (1991)
PJH18	FC40 [F' <i>lac</i> -amplified]	Hastings <i>et al.</i> (2000)
PJH33	FC40 [F' <i>lac</i> -amplified]	Hastings <i>et al.</i> (2000)
PJH51	FC40 [F' <i>lac</i> -amplified]	Hastings <i>et al.</i> (2000)
PJH1242	SMR4562 $\Delta lrp::KanFRT$	SMR4562 $\times$ P1(JW0802)
PJH1390	SMR4562 $\Delta rpoS::KanFRT$	SMR4562 $\times$ P1(SMR10336)
PJH1399	SMR4562 $\Delta rpoS::FRT$	PJH1390 $\times$ pCP20
PJH1428	SMR4562 $\Delta leuO::KanFRT$	SMR4562 $\times$ P1(JW0075) ^a
PJH1438	SMR4562 $\Delta hns::KanFRT$	SMR4562 $\times$ P1(JW1225) ^a
PJH1440	SMR4562 $\Delta hha::KanFRT$	SMR4562 $\times$ P1(JW0449) ^a
PJH1442	SMR4562 $\Delta hfg::KanFRT$	SMR4562 $\times$ P1(JW4130) ^a
PJH1444	SMR4562 $\Delta stpA::KanFRT$	SMR4562 $\times$ P1(JW2644) ^a
PJH1446	SMR4562 $\Delta hfb::KanFRT$	SMR4562 $\times$ P1(JW0895) ^a
PJH1448	SMR4562 $\Delta fis::KanFRT$	SMR4562 $\times$ P1(JW3229) ^a
PJH1948	SMR4562 $\Delta cbpA::KanFRT$	SMR4562 $\times$ P1(JW0985) ^a
PJH1952	SMR4562 $\Delta dps::KanFRT$	SMR4562 $\times$ P1(JW0797) ^a
PJH1966	SMR4562 $\Delta cspE::KanFRT$	SMR4562 $\times$ P1(JW618) ^a
PJH2602	SMR4562 $\Delta dps::KanFRT\ \Delta rpoS::FRT$	PJH1399 $\times$ P1(JW0797) ^a
PJH2608	SMR4562 $\Delta dps::FRT$	PJH1952 $\times$ pCP20
PJH2610	SMR4562 $\Delta hns::FRT$	PJH1438 $\times$ pCP20
PJH2673	SMR4562 [F' <i>zah281::Tn10 Lac</i> ⁺] $\Delta fis::KanFRT$	PJH1448 $\times$ P1(SMR8847)
PJH2677	SMR4562 [F' <i>zah281::Tn10 Lac</i> ⁺] $\Delta cbpA::KanFRT$	PJH1948 $\times$ P1(SMR8847)
PJH2715	FC36 $\Delta hns::KanFRT$	FC36 $\times$ P1(JW1225) ^a
PJH2716	FC36 $\Delta fis::KanFRT$	FC36 $\times$ P1(JW3229) ^a
PJH2732	FC36 $\Delta cbpA::KanFRT$	FC36 $\times$ P1(JW0985) ^a
PJH2790	SMR4562 $\Delta fis::KanFRT$ [F' <i>lac</i> -amplified]	PJH2716 $\times$ PH18
PJH2792	SMR4562 $\Delta fis::KanFRT$ [F' <i>lac</i> -amplified]	PJH2716 $\times$ PJH33
PJH2794	SMR4562 $\Delta fis::KanFRT$ [F' <i>lac</i> -amplified]	PJH2716 $\times$ PJH51
PJH2804	SMR4562 $\Delta cbpA::KanFRT$ [F' <i>lac</i> -amplified]	PJH2732 $\times$ PH18
PJH2806	SMR4562 $\Delta cbpA::KanFRT$ [F' <i>lac</i> -amplified]	PJH2732 $\times$ PJH33
PJH2841	SMR4562 $\Delta hns::KanFRT$ [F' <i>lac</i> -amplified]	PJH2715 $\times$ PJH18
PJH2843	SMR4562 $\Delta hns::KanFRT$ [F' <i>lac</i> -amplified]	PJH2715 $\times$ PJH33
PJH2845	SMR4562 $\Delta hns::KanFRT$ [F' <i>lac</i> -amplified]	PJH2715 $\times$ PJH51
PJH2863	SMR4562 $\Delta hns::KanFRT$ [F' <i>zah281::Tn10 Lac</i> ⁺]	PJH1438 $\times$ P1(SMR8847)
PJH2867	SMR4562 $\Delta dps::FRT\ recA::Tn10dCam$	PJH2608 $\times$ P1(SMR4610)
PJH2874	SMR4562 $\Delta cbpA::KanFRT$ [F' <i>lac</i> -amplified]	PJH2732 $\times$ PJH51
PJH2947	SMR4562 <i>soxR104 zjc2206::Tn10Kan</i>	SMR4562 $\times$ P1(JTG1048) (Nunoshiba and Demple 1994)
PJH2961-4	SMR4562 $\Delta att\lambda::P_{sula-gfp}\ \Delta hns::KanFRT$	SMR6039 $\times$ P1(JW1225) ^a
PJH2971-2974	SMR4562 $\Delta att\lambda::P_{sula-gfp}\ \Delta cbpA::KanFRT$	SMR6039 $\times$ P1(JW0985) ^a
PJH2977	SMR4562 $\Delta dps::KanFRT\ \Delta dinB50::FRT$ [F' $\Delta dinB50::FRT$]	SMR4562 $\times$ P1(JTG1048) (Nunoshiba and Demple 1994)
PJH3019	SMR4562 $\Delta rssB::tet\ \Delta cbpA::KanFRT$	SMR6039 $\times$ P1(JW1225) ^a
PJH3092	SMR4562 $\Delta rssB::tet\ \Delta fis::KanFRT$	SMR6039 $\times$ P1(JW0985) ^a
PJH3114	SMR4562 [F' <i>lafU2::FRTcatFRT\ dinB(o^c)</i>] $\Delta fis::KanFRT$	SMR5889 $\times$ P1(JW0797) ^a
PJH3122	SMR4562 [F' <i>lafU2::FRTcatFRT\ dinB(o^c)</i>] $\Delta cbpA::KanFRT$	SMR12566 $\times$ P1(JW0985) ^a
PJH3215	SMR4562 $\Delta sodB::KanFRT$	SMR12566 $\times$ P1(JW3229) ^a
PJH3217	SMR4562 $\Delta hns::FRT\ \Delta sodB::KanFRT$	PJH2610 $\times$ P1(JW1648) ^a
PJH3219-3222	SMR4562 $\Delta att\lambda::P_{sula-gfp}\ \Delta fis::KanFRT$	SMR6039 $\times$ P1(JW3229) ^a
PJH3245	SMR4562 $\Delta dps::FRT\ ruvC53\ eda::Tn10dCam$	PJH2608 $\times$ P1(SMR6906)
PJH3267	SMR4562 $\Delta dps::FRT\ soxR104\ zjc2206::Tn10Kan$	PJH2608 $\times$ P1(JTG1048)
SMR601	<i>ruvC53\ eda51::Tn10</i>	Lopez <i>et al.</i> (2005)
SMR789	FC40 <i>ruvC53\ eda51::Tn10</i>	FC40 $\times$ P1(SMR601)
SMR4562	Independent construction of FC40	McKenzie <i>et al.</i> (2000)
SMR4610	SMR4562 <i>recA::Tn10dCam</i>	Bull <i>et al.</i> (2001)
SMR5889	SMR4562 $\Delta dinB50::FRT$ [F' $\Delta dinB50::FRT$]	Galhardo <i>et al.</i> (2009)
SMR6039	SMR4562 $\Delta att\lambda::P_{sula-gfp}$	Hastings <i>et al.</i> (2004)
SMR6178	SMR4562 $\Delta att\lambda::P_{sula-gfp}\ sula::Tn5\ lexA3(lnd^-)\ malB::Tn9$	Al Mamun <i>et al.</i> (2012)
SMR6263	MG1655 <i>leuB::Tn10</i>	Gibson <i>et al.</i> (2010)

(continued)

Table 1, continued

Strain/plasmid	Genotype	Source/reference
SMR6906	SMR4562 <i>ruvC53 eda::Tn10dCam</i>	By linear replacement (Datsenko and Wanner 2000) into SMR789
SMR8847	SMR4562 [F' <i>zah281::Tn10 Lac⁺</i>]	SMR4562 × P1(CAG12080) (Singer <i>et al.</i> 1989)
SMR10308	SMR4562 [F' <i>lafU2::FRTcatFRT dinB21(o^c)</i>]	Galhardo <i>et al.</i> (2009)
SMR12566	SMR4562 Δ <i>rssB::tet</i>	Al Mamun <i>et al.</i> (2012)

^a All JW strains are from the Keio Collection, described in Baba *et al.* (2006).

Previous studies showed that the NAP HU was required for MBR, possibly by promoting HR (Williams and Foster 2007). Another four NAPs (Hfq, CspC, Lrp, and IhfA) were shown to promote MBR (Al Mamun *et al.* 2012), the majority associated with regulation of stress responses required for MBR.

Here we show that although most NAPs affect MBR, most do so via regulation, and they do so by different mechanisms.

Materials and Methods

Bacterial strains and media

E. coli K12 strains used in this work and their origins are outlined in Table 1. Mutations were introduced using standard P1-mediated transduction (Miller 1992) and/or recombineering methods (Datsenko and Wanner 2000). Genotypes were confirmed by PCR and various phenotypic tests. The media used were M9 minimal medium (Miller 1992) with 10 μ g/ml vitamin B₁ and 0.1% glycerol or 0.1% lactose and Luria-Bertani-Herskowitz (LBH) medium (Torkelson *et al.* 1997).

Determination of Lac⁺ mutation rates

Experimental procedures are described in Harris *et al.* (1996) and Hastings *et al.* (2000). In all experiments reported, Lac⁺ viable cell counts did not vary significantly over the course of the experiment, and mutations introduced by transduction did not affect colony formation time, measured in reconstruction experiments [reviewed by Rosenberg (2001)]. The proportion of *lac*-amplified and Lac⁺ point mutants (carrying –1-bp indels) was determined based on Hastings *et al.* (2000) by replating samples of colonies on rich medium plates containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (x-gal) dye and scoring for stable blue phenotype (Lac⁺ indels) vs. sectorized-colony morphology (*lac* amplification). Absolute values for mutation rates (Lac⁺ colonies per cell per day) vary from day to day, but relative values remain constant (Lombardo *et al.* 2004). Errors bars shown are 1 SEM of three or more experiments.

Chromosome and F' plasmid copy-number analyses

DAPI staining and flow cytometry were used to determine numbers of chromosomes per cell. Aliquots of saturated cultures grown for 3 days at 32° in M9 glycerol medium were fixed in ethanol and stained with 1 mg/ml DAPI in PBS. The average number of chromosomes per cell was calculated by measuring the DAPI fluorescence per cell and normalizing to the fluorescence value of the single-chromosome peak in the

flow cytometry DAPI histogram for each sample. F' plasmid copy number relative to the chromosome was calculated in the same cells by quantitative PCR (qPCR). Portions of cell cultures were collected, and genomic DNA was isolated using a modified cetyltrimethyl ammonium bromide (CTAB) method (Wilson 2001). Reactions contained 10 ng of genomic DNA and 350 μ M primer in 1× KAPA SYBR FAST ABI Prism qPCR mix and were run on an Applied Biosystems 7900 HT. F' copy number relative to the chromosome was determined using the $\Delta\Delta$ Ct method (Livak and Schmittgen 2001), and from these measurements, F' plasmid content per cell was calculated. The F'-specific primer sequences used were (5'–3'): F1: GATGGGACTTGATGTCTGTTAGG/CAGAGGAAGCAGAGGAGATAAATG; F2: GCTATAGCTGGCTCAAGT TAGG/CATGCAGACCTCACGAGTTAAT; and F3: GATGGGACTTGATGTCTGTTAGG/CAGAGGAAGCAGAGGAGATAAAT. *oriC*-specific primers were TTCGATCACCCCTGCGTACA/CGCAACAGCATGGCGATAAC. *ter*-specific primers were AATGATGCCGGTTACCCAAAGC/AGTTGCGTTTCGACGGTCATTC.

Quantitative P1 phage transduction assay for HR

Five-milliliter cultures of SMR4562 (Rec⁺), isogenic NAP deletion mutants, and an isogenic Δ *recA* control strain were grown in triplicate to saturation at 37° in LBH medium, as indicated by Gibson *et al.* (2010). Dilutions of each culture were plated on LBH medium to determine viable cell titers. P1 transduction (Miller 1992) was performed with 50 μ l of either a 10^{–8}, 10^{–7} dilution of P1 lysate from *E. coli* strain SMR6263 of known titer containing the mutation *leuB::Tn10* (Tet^R) or 50 ml of LBH medium and 100 μ l of each saturated culture. Then 1.5 × 10⁷ phage particles and 1.5 to 2.1 × 10⁹ cells were used in each reaction to ensure a multiplicity of infection of <0.1. Entire reaction mixtures were plated on solid LBH medium containing 3.3 μ g/ml tetracycline and 20 mM sodium citrate. Using the titer of Tet^R transductant colonies and calculated phage particles added, frequencies of transductant colony-forming units (cfu) per phage were calculated.

UV-sensitivity assays

Five-milliliter cultures of repair-proficient SMR4562, isogenic NAP deletion mutants, and Δ *recA* control strain were grown in triplicate to saturation at 37° in LBH medium. Serial dilutions in M9 salts were plated in duplicate on LBH medium to determine viable cell titer, and appropriate dilutions were plated on LBH solid medium and exposed to UV light at 20,

Figure 1 NAPs are required for mutagenic break repair. (A) Representative Lac assay experiment (left) showing requirements for NAPs for MBR and summary of MBR rates from three experiments (right) (mean rates $\pm$ SEM) compared with the wild-type protein. Deletion of *hns*, *fis*, *cbpA*, and *cspE* reduced MBR rates ($P = 0.005$, 0.03 , 0.03 , and 0.049 , respectively, Student's two-tailed t -test), whereas Δ *dps* increased the mutation rate. (B) Both point mutation and amplification are affected. Relative rates of point mutant (left) and *lac*-amplified colony formation (right). (C) Average time to colony formation in reconstruction experiments. These measure the time taken for strains carrying either the Lac⁺ point mutant or *lac*-amplified alleles to form colonies under MBR experimental conditions. *hns*, *fis*, and *cbpA* deletion strains formed colonies as rapidly as NAP⁺ cells and so are defective in mutagenesis, not growth of mutant colonies afterward. Strain designations relevant to the genotypes in all figures are given in Table 1.

40, 60, and 80 mJ/cm². Irradiated plates were incubated in the dark at 37° overnight. Colonies were counted the next day, and survival was calculated.

Flow cytometric assay of spontaneous DNA damage and SOS response

All strains used for SOS induction determination carried the SOS-regulated *PsuA-gfp* allele (Pennington and Rosenberg 2007). *PsuA-gfp* is inserted in a nongenic chromosomal site in *sulA*⁺ cells (Pennington and Rosenberg 2007). Five-milliliter cultures of isogenic SMR6039 (SOS-proficient control), SMR6178 [*lexA3*(Ind) SOS-defective], and an NAP deletion mutant were grown for 3 days at 32° in M9 minimal glycerol medium as in MBR starvation assay conditions. Controls were grown in duplicate with four independently isolated strains of each NAP deletion derivative. Gates for GFP fluorescence in flow cytometry were set by using the parent strain (SMR6039) and the SOS induction-deficient control (SMR6178). Then 10⁵ cells were measured per determination and the fraction of GFP⁺ cells reported. UV induction of the SOS response was measured at 80 mJ/cm².

Results

We tested NAPs for possible roles in MBR. Only two, *StpA* and *Hha*, had no effect, and one, *Dps*, inhibited MBR (Figure 1A).

Of the 10 that influenced MBR, 5 had been shown previously to be wholly or partly required for MBR, and their functions were partly characterized (Williams and Foster 2007; Al Mamun *et al.* 2012). We investigated the roles of the others.

We used the Lac assay for MBR (Cairns and Foster 1991). *E. coli* cells carrying a leaky *lacIZ* +1-bp frameshift allele can revert either by a single-base deletion compensatory frameshift mutation (SNV) or by amplification of the leaky *lac* allele to give a tandem array of 20 or more copies (GCR) (Hastings *et al.* 2000, 2004; Hersh *et al.* 2004; Rogers *et al.* 2015). TraI single-strand endonuclease makes nicks in the F' that become DSBs (Ponder *et al.* 2005; Wimberly *et al.* 2013), which are required for both SNV and GCR (Harris *et al.* 1994; Wimberly *et al.* 2013).

Strategy

We used various assays that dissect known components of MBR under stress to distinguish how various NAPs affect MBR: (1) a quantitative P1 phage-mediated transduction test of HR proficiency at DSB ends, a proxy for DSB repair by HR, (2) sensitivity to UV light, which requires functional DSB repair (Khan and Kuzminov 2012), (3) a flow-cytometric assay for the ability to induce the SOS response, which uses an SOS-regulated promoter fused to *gfp* (Pennington and Rosenberg 2007), and (4) an assay for genes that promote MBR by contributing to induction of the RpoS general stress response

Figure 2 Dps inhibits the canonical MBR pathway. (A) Hypermutation in Δ *dps* cells requires known MBR proteins RpoS, RecA, RuvC, and DinB. Thus, the MBR pathway (Rosenberg *et al.* 2012; Rogers *et al.* 2015), not some other mutagenic mechanism, was increased in Δ *dps* cells. Rates of three experiments (mean $\pm$ SEM) compared with those of Dps-proficient and Δ *dps* single-mutant cells. (B) Δ *dps* cells are sensitive to hydrogen peroxide. Relative survival of Dps-proficient and Δ *dps* cells after short-term exposure to 100 mM H₂O₂ showed 70% lower viability of Δ *dps* than Dps-proficient cells (72 $\pm$ 0.5%; *n* = 3 experiments). (C) Constitutive activation of the SoxR (anti-ROS) regulon reduces MBR. The *soxR104* allele constitutively activates genes encoding ROS-detoxifying enzymes, reduces basal intracellular ROS content (Nunoshiba and Demple 1994), and here reduced MBR rate in Δ *dps* and Dps-proficient strains (240 $\pm$ 30 vs. 18 $\pm$ 2 Lac⁺ cfu/10⁸ cells/day for Δ *dps* vs. *soxR104* Δ *dps*; 169 $\pm$ 25 vs. 17 $\pm$ 8 Lac⁺ cfu/10⁸ cells/day for wild-type cells vs. *soxR104*; *n* = 3 experiments in both cases). These data imply that ROS promote MBR (explored in a separate study: J. M. Moore, S. M. Rosenberg, and P. J. Hastings, unpublished manuscript).

(Al Mamun *et al.* 2012), which measures the ability to bypass a specific requirement for an MBR gene by artificial upregulation of RpoS (Al Mamun *et al.* 2012), achieved by deletion of the RpoS negative regulator *rssB* (Battesti *et al.* 2013). Finally, we tested for genes that promote MBR via activation of the SOS response, as in Al Mamun *et al.* (2012). SOS functions in the indel and base-substitution pathway of MBR by transcriptional upregulation of DinB error-prone DNA polymerase (Galhardo *et al.* 2009). Because the requirement for SOS is fully suppressed by constitutive expression of *dinB* at SOS-like levels, using a *dinB* operator-constitutive (*o^c*) allele (Galhardo *et al.* 2009), genes that promote MBR solely via SOS induction are not needed for MBR in *dinB*(*o^c*) cells. In addition, we determined relative changes in F' plasmid copy number by qPCR, comparing the ratio of each three sites in the F' plasmid with a chromosomal position.

Stationary-phase NAP Dps inhibits mutagenesis

We found that Dps inhibited mutagenesis (Figure 1A). Deletion of *dps* led to a 3.5-fold increase in Lac⁺ mutation rates

(Figure 1A). Dps-mediated inhibition of MBR was seen for both point (indel) mutation and amplification rates (Figure 1B). The increased mutagenesis in Δ *dps* cells required known MBR proteins RpoS, RecA, RuvC, and DinB [reviewed in Rosenberg *et al.* (2012)] (Figure 2A). We conclude that increased mutagenesis in Δ *dps* cells reflects increased MBR, not an increase in an alternative mutagenesis mechanism/pathway.

We investigated whether Dps inhibited mutagenesis by protection from ROS (reviewed earlier). If it did, we would expect that reduction in ROS would decrease MBR at least in Δ *dps* cells and perhaps generally. In support of this possibility, we found that constitutive activation of the *sox* regulon via the *soxR104* allele (Nunoshiba and Demple 1994), which reduces ROS levels, reduced MBR in otherwise wild-type cells and in Δ *dps* cells (Figure 2C). The *sox* regulon includes *sodA* and *sodB*, which encode superoxide dismutases (Nunoshiba and Demple 1994). This result implies that MBR generally and increased MBR in Δ *dps* cells specifically require ROS. We conclude that Dps inhibits MBR and suggest that MBR is promoted by ROS and that Dps inhibits MBR by

Figure 3 CbpA is required for F' plasmid maintenance. (A) HR proficiency of $\Delta cbpA$ strains in P1-transduction HR assays ($2.6 \times 10^{-6} \pm 7 \times 10^{-7}$ Tet^R phages for wild-type cells vs. $2.6 \times 10^{-6} \pm 8 \times 10^{-7}$ for $\Delta cbpA$; $n = 3$ experiments). (B) UV resistance of $\Delta cbpA$ cells ($P = 0.98$, Student's two-tailed t -test for significant difference between survival curves). Representative experiment of three cultures per strain. (C) Deletion of RpoS-negative regulator *rssB* does not restore MBR $\Delta cbpA$ cells. MBR rates from three experiments $\pm$ SEM. (D) $\Delta cbpA$ cells are SOS response-induction proficient. Percentage of $\Delta cbpA$ cells showing green fluorescence in a population of 10^5 cells in strains with *gfp* fused to an SOS response-inducible promoter. *lexA3* (*lex*-noninducible) was included as a positive control. Data from three experiments $\pm$ SEM. (E) *dinB(oc)* did not suppress the $\Delta cbpA$ MBR phenotype, showing that CbpA is not required in MBR for upregulation of the SOS response. Data from three experiments $\pm$ SEM. (F) $\Delta cbpA$ cells contain roughly 2.3 chromosomes per cell compared with 1.3 in wild-type cells. Comparison of fluorescence intensity due to DAPI staining. Data from three experiments $\pm$ SEM. (G) $\Delta cbpA$ strains are defective in maintaining the F' plasmid. qPCR at *oriC* on the chromosome and at three sites on the F' conjugative plasmid shows one F' per chromosome in wild-type cells and 0.38 F' per chromosome in $\Delta cbpA$ cells. This equates to an average of 0.87 F' per cell. Data from three experiments $\pm$ SEM.

protection against ROS. Further work is required to test this hypothesis.

Canonical NAPs H-NS, Fis, CspE, and CbpA are required for stress-induced MBR

Deletion of the genes encoding four NAPs, H-NS, Fis, CspE, and CbpA, caused significant reductions in mutagenesis, showing that they are wholly or partially required for MBR. Deletions of *hns*, *fis*, *cspE*, and *cbpA* resulted in 90, 50, 50, and 60% reduction in MBR rates, respectively (Figure 1A, $P = 0.005$, 0.03, 0.03, and 0.049, Student's two-tailed t -test). Point mutation and amplification mechanisms were reduced roughly equally (Figure 1B). Further, we showed that the reduction in mutant colonies was not caused by a slow colony-growth phenotype of these mutants under these experimental conditions [reviewed in Rosenberg (2001)] (Figure 1C). We conclude that these NAPs are needed for mutagenesis, not for colony formation.

CspE is a known upstream regulator of RpoS stability (Shenhar *et al.* 2012), which is required for MBR (Layton and Foster 2003; Lombardo *et al.* 2004; Ponder *et al.* 2005; Shee *et al.* 2011; Al Mamun *et al.* 2012) and so was not studied further here.

CbpA promotes F' plasmid maintenance

The following data imply that CbpA is not required for the DSB repair or SOS or RpoS response components of MBR but rather contributes to F' plasmid maintenance. We assayed for defects in HR at DSB ends by quantitative P1 transduction (Gibson *et al.* 2010) (Figure 3A), a HR-dependent process (Rouviere-Yaniv *et al.* 1979). We infected $\Delta cbpA$ cells with P1 grown on donor bacteria carrying a tetracycline (Tet) resistance cassette and then determined the frequency of Tet^R recombinant colonies. $\Delta cbpA$ did not affect Tet^R transductant frequency, whereas the HR-blocking *recA* mutation did (Figure 3A), indicating that $\Delta cbpA$ cells are HR proficient. DSB repair proficiency also was supported by the finding that $\Delta cbpA$ strains were UV resistant (Figure 3B), unlike the DSB repair-defective $\Delta recA$ strain (Figure 3B) or *recB* (Kushner 1974). These data rule out the possibility that CbpA promotion of MBR is via promotion of HR, which is required for MBR.

The following data suggest that CbpA promotes MBR other than or in addition to by promoting the RpoS stress response. RssB is a negative regulator of RpoS that targets RpoS for proteolytic degradation (Battesti *et al.* 2013).

Figure 4 Fis promotes MBR by allowing the SOS response and DinB induction. (A) Δfis cells were as proficient as wild-type cells at recombinational DSB repair, as assayed by quantitative P1 transduction. Data from three experiments $\pm$ SEM. (B) Similar survival of Δfis cells and wild-type cells after UV irradiation. Representative experiment of three experiments using three cultures per strain. (C) The MBR defect of Δfis cells is not alleviated by $\Delta rssB$ upregulation of RpoS. Deletion of the *rssB*, a negative regulator of RpoS, did not suppress the *fis* MBR phenotype. Data from three experiments $\pm$ SEM. (D) Δfis cells did not alter chromosomal copy number, showing the same fluorescence intensity due to DAPI strain as wild-type cells. Data from three experiments $\pm$ SEM. (E) Δfis cells are not defective in plasmid maintenance, determined by qPCR at *oriC* and at three plasmid locations. Three experiments $\pm$ SEM. (F) Fis is required for SOS induction. UV irradiation induced GFP under an SOS-inducible promoter in Δfis cells only about one-quarter as well as in wild-type cells. (G) SOS-like levels of DinB provided by the *dinB(o^c)* mutation completely suppresses the *fis* MBR defect. We conclude that Fis is required in MBR for induction of the SOS response that upregulates DinB. Data from 3 experiments $\pm$ SEM.

Deleting *rssB* increases RpoS levels and can suppress the MBR deficiencies of genes required in MBR for RpoS upregulation (Al Mamun *et al.* 2012). We found that removal of RssB from $\Delta cbpA$ strains did not suppress their MBR deficiency (Figure 3C), suggesting that CbpA does not promote MBR by upregulation of RpoS. MBR rates were 3.6 times higher in wild-type cells than $\Delta cbpA$ cells and 1.7 times higher in *rssB* cells than in $\Delta rssB \Delta cbpA$ cells—ratios that did not quite differ significantly ($P = 0.052$, Student's *t*-test).

Loss of CbpA did not affect the frequencies of spontaneous DNA damage or SOS induction, as shown with the flow cytometric assay of Pennington and Rosenberg (2007) for fluorescent SOS-induced cells (Figure 3D) carrying a chromosomal SOS-regulated *Psula-gfp* transgene. Isogenic *Psula-gfp* cells with the SOS-blocking *lexA3*(Ind[−]) mutation demonstrate the SOS dependence of fluorescence in this assay (Figure 3D) (Pennington and Rosenberg 2007). These data imply that CbpA is not required generally for SOS-response activation. Further, upregulation of *dinB* can substitute for the SOS response in MBR (Galhardo *et al.* 2009) such that *dinB(o^c)* mutant alleles, which express SOS-induced levels of *dinB* constitutively, suppress the MBR deficiency of SOS-noninducible *lexA*(Ind[−]) mutants (Galhardo *et al.* 2009). We found that the *dinB(o^c)* mutation did not

alleviate the requirement for CbpA in MBR (Figure 3E). We conclude that CbpA promotes MBR other than or in addition to by upregulation of the SOS response.

We found that CbpA promotes maintenance of the F' plasmid carrying the *lac* mutation-reporter gene as follows: in wild-type cells, chromosome copy number is two in $\sim 40\%$ and one in $\sim 60\%$ of stationary-phase cells (Akerlund *et al.* 1995). We used DAPI staining and flow cytometry to determine chromosome copy number and qPCR to determine the ratio of F' plasmids to chromosomes. In wild-type cells, chromosome copy number averaged 1.3 chromosomes per cell (Figure 3F), similar to that seen previously (Akerlund *et al.* 1995), and the F'-to-chromosome ratio was $\sim 1:1$ (Figure 3G; compare three F' loci with the chromosomal locus). Whereas deletion of *cbpA* increased chromosome copy number to ~ 2.3 chromosomes per cell (Figure 3F), $\Delta cbpA$ significantly reduced the F'-to-chromosome ratio to 0.38 (Figure 3, F and G, $P = 0.00003$, Student's *t*-test). These data indicate that $\Delta cbpA$ cells had, on average, only 0.9 copies of the F' per cell ($2.3 \times 0.38 = 0.87$), suggesting that reduced MBR in $\Delta cbpA$ cells may result from an inability to maintain the F' plasmid carrying the reporter allele either via fewer mutation targets or via fewer cells with a sister F' plasmid to engage in DSB repair. We infer that reduced MBR in $\Delta cbpA$ cells probably

Figure 5 H-NS promotes MBR via maintenance of normal ROS levels. (A) Δhns cells are not defective in homologous recombination, as determined by quantitative P1 transduction. The mean of three experiments ($\pm$ SEM) shows no significant difference from wild-type cells ($P = 0.198$, Student's two-tailed t -test). (B) UV resistance of Δhns cells confirms no difference from wild-type cells in homologous recombination. Representative example of three experiments with three cultures per strain. (C) Δhns cells are proficient at SOS induction. Percentage of green fluorescent cells in populations of 10^5 cells of Δhns and wild-type cells with *gfp* fused to an SOS response-inducible promoter. Δhns cells showed no significant deficiency in spontaneous activation of the SOS response ($P = 0.167$, Student's two-tailed t -test) measured by flow cytometry. (D and E) Δhns cells are not defective in plasmid maintenance. Deletion of *hns* led to an increase in both the average chromosomal content per cell (2.3 chromosomes per cell shown by DAPI staining) and F'-to-chromosome ratio (1.7 F' per chromosome). qPCR was used to measure copy number of the chromosomal *oriC* region compared with multiple F' sites. (F) H-NS promotes MBR by repressing *SodB* and maintaining normal ROS levels required for mutagenesis. Deletion of superoxide dismutase gene *sodB* completely suppressed the MBR defect of Δhns cells, implying that deletion of *sodB* and the resulting increase in ROS promotes MBR. Data are from three experiments $\pm$ SEM.

results from reduced F' copy number, which reduces MBR opportunities. The slight and not quite significant restoration of MBR in RpoS-elevated $\Delta rssB$ cells (Figure 3C) might affect maintenance of plasmid copy number—a hypothesis that remains to be tested.

Fis is required for SOS induction in MBR

We found that Fis promotes SOS induction, which upregulates DinB error-prone DNA polymerase during MBR. First, loss of Fis did not confer a significant reduction in HR assayed via quantitative P1 transduction (Figure 4A), which implies that HR at DSB ends is unaffected. Similarly, Δfis strains were not UV sensitive (Figure 4B), also indicating HR proficiency. Second, deletion of *rssB* did not restore MBR to Δfis cells in that Δfis conferred similar MBR reductions in *rssB* and wild-type cells (Figure 4C, Fis/WT = 0.53, Fis *RssB*/*RssB* = 0.62, $P = 0.119$, Student's two-tailed t -test). Third, Δfis caused

an insignificant increase in chromosome copy number (Figure 4D) and an increased F'-to-chromosome ratio (Figure 4E), which indicated more, not fewer, F' plasmids per cell. Thus, Fis promotes MBR other than by decreasing homologous recombination, RpoS upregulation, or F' plasmid copy number. However, Δfis conferred a threefold decrease in both spontaneous and UV-induced SOS induction, measured as GFP⁺ cells by flow cytometry of the fluorescent SOS-reporter strain (Figure 4F). Spontaneous SOS/GFP⁺ cells were $\leq 0.1\%$ of Δfis cells compared with 0.45% of wild-type cells (Figure 4F, $P = 0.012$, Student's two-tailed t -test). After UV irradiation, only $\sim 20\%$ of Δfis cells showed SOS induction, whereas $\sim 77\%$ of wild-type cells did (Figure 4F, $P = 0.031$, Student's two-tailed t -test). Moreover, the *dinB*(o^c) allele completely restored MBR to Δfis cells (Figure 4G), indicating that SOS-induced levels of DinB created by this mutant allele (Galhardo *et al.* 2009) substituted fully for the role of Fis in

Table 2 Summary of NAP effects in MBR

Mutant gene(s)	Effect on MBR	RpoS activation ^d	SOS activation ^e	RpoE activation ^f	HR proficiency ^g	UV	F' copy number ^h	ROS levels
<i>hns</i>	Down ^a	ND	Down ^a	NC ^a	Down ^a	NC ^a	Up ^a	Down^a
<i>fis</i>	Down ^a	NC ^a	Down^a	NC ^a	Down ^a	NC ^a	NC ^a	
<i>cbpA</i>	Down ^a	NC ^a	NC ^a	NC ^a	NC ^a	NC ^a	Down^a	
<i>dps</i>	Up ^a	NC ^a	NC ^a	NC ^a	NC ^a	NC ^a		Up^a
<i>ihfA</i>	Down ^b	NC ^b		S^b		NC ^b		
<i>hupA hupB</i>	Down ^c	NC ^c	NC ^c				NC ^c	
<i>lrp</i>	Down ^b	NC		S^b		NC ^b		
<i>cspC</i>	Down ^b	Down^b		NC ^b		NC ^b		
<i>cspE</i>	Down ^a	Downⁱ						
<i>hfq</i>	Down ^b	Down^b	NC ^b	NC ^b		S ^b		
<i>hha</i>	No effect ^a							
<i>stpA</i>	No effect ^a							

Bold text, probable cause of MBR phenotype; NC, not changed; S, sensitive; UV, ultraviolet light sensitivity.

^a This work.

^b Al Mamun *et al.* (2012).

^c Williams and Foster (2007).

^d Epistasis with *rssB*.

^e Determined by flow cytometry.

^f Detergent sensitivity.

^g Quantitative transduction.

^h qPCR.

ⁱ Shenhar *et al.* (2012).

MBR. These data indicate that Fis promotes MBR by allowing activation of the SOS response, which upregulates DinB.

H-NS can be substituted in MBR by increasing ROS levels

Following the findings with Dps reported earlier, we discovered that H-NS also affects MBR by controlling ROS levels. Δhns cells showed no significant reduction in HR measured by quantitative transduction (Figure 5A) and were UV resistant (Figure 5B), indicating that the Δhns MBR defect is not due to loss of DSB repair ability. Similarly, the proportion of spontaneously SOS-induced cells was unchanged in Δhns cells, which indicates an intact SOS response (Figure 5C). Δhns caused higher chromosome copy numbers (Figure 5D) and higher F'-to-chromosome ratios (Figure 5E), which show that neither F' maintenance nor availability of sister DNA molecules for repair account for H-NS function in MBR.

The following data indicate that the known H-NS role in transcriptional repression of *sodB* (Niederhoffer *et al.* 1990; Dubrac and Touati 2002; Zhang *et al.* 2005), a superoxide dismutase that detoxifies ROS, can explain H-NS function in MBR. We found that deletion of *sodB*, which increases superoxide levels (Liochev and Fridovich 1997), completely substituted for H-NS in MBR (Figure 5F). The $\Delta hns \Delta sodB$ strains showed higher MBR than the Δhns single mutant (Figure 5F, $P = 0.00004$, Student's two-tailed *t*-test). Moreover, as observed previously (Farr *et al.* 1986), $\Delta sodB$ conferred only slight mutator activity in otherwise wild-type cells, which was not significant at the 5% level (less than twofold, $P = 0.06$, Student's two-tailed *t*-test). That is, $\Delta sodB$ is not a general mutator but rather specifically relieves the MBR defect of Δhns cells (Figure 5F). These data suggest that H-NS promotes MBR by repressing the ROS-detoxifying enzyme *sodB*, allowing ROS to persist within the cell, which

promotes mutagenesis (also shown in Figure 2C). How ROS promote MBR is explored in a separate work (J. M. Moore, S. M. Rosenberg, and P. J. Hastings, unpublished manuscript).

Discussion

We found that the canonical NAPs Fis, H-NS, CspE, and CbpA are required for MBR and that Dps, the major stationary-phase NAP, inhibits MBR. Of 15 NAPs, 7 studied here and 5 studied previously (reviewed later), only 2, StpA, an analog of H-NS (Lucchini *et al.* 2009), and Hha did not affect MBR (Figure 1). Surprisingly, the various NAPs appear to modulate MBR by different mechanisms (summarized in Table 2) nearly all positively, and many, but not all, centered on stress-response regulation. Regulation of stress responses comprised the major allocation of genes in a large 93-gene network that underlies MBR in *E. coli*, highlighting the importance of stress-response activation to MBR (Al Mamun *et al.* 2012) (extended later).

Perhaps the most surprising aspect of our results and those of another recent study (Warnecke *et al.* 2012) is the absence of detectable direct *cis* effects of NAPs on mutagenesis in the DNA sites that those NAPs bind. Instead, our study shows important *trans* effects on regulation of regulatory and other proteins and components of MBR (Table 2). Warnecke *et al.* (2012) used Chip-Seq to map chromosomal distributions of four NAPs—H-NS, Fis, IhfA, and IhfB—at different growth phases and compared those distributions with patterns of mutations across the genomes of 54 sequenced *E. coli* strains. They found a small but significant correlation of NAP occupancy with genome variation between strains (Warnecke *et al.* 2012). By contrast, we found robust evidence

Figure 6 The updated mutagenic break-repair network with NAPs. Protein-protein interactions: CytoScape 3.0.2 software, unweighted force-directed layout (Saito *et al.* 2012), links from String 9.1 (Franceschini *et al.* 2013). Proteins that promote RpoS, RpoE, SOS activation, DSBs, and DSB repair are shown as solid green, black circle, red circle, solid cyan, and solid pink, respectively. Downstream of SOS, solid red; DNA polymerases, green circle; ROS level maintenance, solid yellow; replicon copy number, solid salmon; NAPs, blue circle; unknown function in MBR, solid gray (Gibson *et al.* 2015). This rendition includes the NAPs shown here to promote MBR, and the other proteins discovered to promote MBR after the initial description of the MBR network by Al Mamun *et al.* (2012): Mfd (Wimberly *et al.* 2013) and PhoB and PhoR (Gibson *et al.* 2015).

that most NAPs modulate genome evolution/mutagenesis positively *in trans* by virtue of their regulatory functions (summarized next).

Dps, the sole NAP inhibitor of MBR (Table 2 and Figure 1B), compacts and cocrystalizes with DNA in the stationary-phase nucleoid and protects DNA/cells from ROS (Ali Azam *et al.* 1999). We discovered that ROS are required for MBR in that constitutive expression of the SoxR regulon, which encodes proteins that detoxify ROS, reduced MBR (Figure 2C). We suggest that Dps might inhibit MBR by reduction of ROS or their effects on DNA. The role of ROS in MBR is explored in a separate study (J. M. Moore, S. M. Rosenberg, and P. J. Hastings, unpublished manuscript).

We found that Fis promotes MBR by allowing activation of the SOS DNA-damage response, which promotes MBR by upregulation of DinB error-prone DNA polymerase (Figure 4, F and G). Providing SOS-like DinB levels with a *dinB* operator-constitutive allele (Galhardo *et al.* 2009) wholly substituted for Fis in MBR (Figure 4G).

CspE is required for MBR (Figure 1, A and B). CspE is a known positive regulator of RpoS (Shenhar *et al.* 2012), suggesting that CspE, like Fis, might exert its influence on MBR through stress-response regulation—SOS for Fis and RpoS for CspE.

H-NS is also required for MBR (Figure 1, A and B) apparently via its transcriptional repression of *sodB* (Niederhoffer *et al.* 1990; Dubrac and Touati 2002; Zhang *et al.* 2005), which encodes a superoxide dismutase that detoxifies ROS. Deletion of *sodB* completely and specifically alleviated the need for H-NS in MBR (Figure 5F), presumably by allowing the accumulation of ROS. Δ *sodB* caused no such increase in MBR in otherwise wild-type (H-NS⁺) cells, implying that sufficient ROS for MBR occur in H-NS-proficient cells.

The reduction in mutagenesis in Δ *cbpA* cells seems likely to result from decreased numbers of mutation reporter-bearing F' plasmids in those cells. The calculated average of 0.9 plasmids per Δ *cbpA* cell implies that many cells do not carry more than one copy of the plasmid. This would make HR repair of F'-located DSBs unlikely in most cells and thus re-

duce MBR in the F'. Increased F' copy number was previously proposed to cause increased MBR (Foster and Rosche 1999). Because the error-prone polymerase DinB is encoded in both the F' and the chromosome in these strains, it is possible that both chromosomal and F' mutations might be influenced by F' copy number, as seen previously by comparing mutations in F⁻ and F' cells—more copies of *dinB* gave more mutations additively (Shee *et al.* 2011).

Of the five NAPs previously shown to promote MBR [Lrp, Hfq, CspC, IHF (Al Mamun *et al.* 2012), and HU (Williams and Foster 2007); reviewed Table 2], Hfq, CspC, and Lrp were shown to act upstream of activation of stress-response regulators—RpoS for Hfq and CspC and RpoE for Lrp (Al Mamun *et al.* 2012). Additionally, *ihfA* mutants mimicked the sensitivity to detergent with EDTA of RpoE response-defective cells but showed normal expression of the RpoE-regulated *rpoH* P3 promoter (Al Mamun *et al.* 2012), perhaps because the *rpoH*-P3 assay is not conducted under similar starvation-stress conditions. The fifth NAP, HU, affected MBR possibly by promoting HR (Williams and Foster 2007). Thus, four of these five NAPs appear to promote MBR via stress-response regulation. Because of their essential nature, IciA and DnaA (Chodavarapu *et al.* 2008a) were not included in this study. CbpB, which, like IciA and DnaA, binds the replication origin (Skarstad *et al.* 1993), also was not studied.

Our results both support and reveal the incompleteness of our previous study of a large network of genes required for MBR (Al Mamun *et al.* 2012). First, the screen that identified most of the MBR network detected about half the then-known MBR genes, suggesting that additional MBR genes remain to be discovered. Our discovery here of four NAPs that promote MBR (Fis, H-NS, CspE, and CbpA) (Figure 1), in addition to the four NAPs identified in the previous screen [Lrp, Hfq, CspC, and IHF (Al Mamun *et al.* 2012)], implies that the full MBR network is likely to be about twice as large as the 93 genes of Al Mamun *et al.* (2012)—~180–190 genes. In addition to the four NAPs added in this work, we also subsequently identified the transcription protein Mfd (Wimberly *et al.* 2013) as MBR promoting. Also

discovered after the 93-gene network of Al Mamun *et al.* (2012), PhoB and PhoR promote MBR by an as yet unknown mechanism (Gibson *et al.* 2015). This adds 7 new genes to the MBR network for a total of 100 demonstrated MBR genes (Figure 6).

Second, more than 50% of the original network genes was implicated as acting upstream of activation stress responses, sensing and transducing stress signals to stress-response regulators that activate the effector proteins that cause mutations in DNA (Al Mamun *et al.* 2012). Downstream effectors constitute a small fraction of the network. As illustrated in Figure 6, our data show that most NAPs are upstream regulators. Most allow activation of stress responses (Table 2). Thus, the most important allocation of NAP genes to MBR, and of genes to MBR generally, is in the temporal regulation of mutagenesis to times of stress, when cells are poorly adapted to their environments. Modeling indicates that this strategy can accelerate evolution even in asexual bacterial populations, which do not easily lose deleterious mutations, and can be selected on this basis (Ram and Hadany 2012; Ram and Hadany 2014). The number of NAPs and other genes in this role indicates the importance of stress-response regulation to mutagenesis in *E. coli*.

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Literature Cited

- Akerlund, T., K. Nördstrom, and R. Bernander, 1995 Analysis of cell size and DNA content in exponentially growing and stationary-phase batch cultures of *Escherichia coli*. *J. Bacteriol.* 177: 6791–6797.
- Ali Azam, T., A. Iwata, A. Nishimura, S. Ueda, and A. Ishihama, 1999 Growth phase-dependent variation in protein composition of the *Escherichia coli* nucleoid. *J. Bacteriol.* 181: 6361–6370.
- Al Mamun, A. A., M. J. Lombardo, C. Shee, A. M. Lisewski, C. Gonzalez *et al.*, 2012 Identity and function of a large gene network underlying mutagenic repair of DNA breaks. *Science* 338: 1344–1348.
- Azam, T. A., S. Hiraga, and A. Ishihama, 2000 Two types of localization of the DNA-binding proteins within the *Escherichia coli* nucleoid. *Genes Cells* 5: 613–626.
- Baba, T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura *et al.*, 2006 Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* 2: 2006.0008.
- Barth, M., C. Marschall, A. Muffler, D. Fischer, and R. Hengge-Aronis, 1995 Role for the histone-like protein H-NS in growth phase-dependent and osmotic regulation of sigma S and many sigma S-dependent genes in *Escherichia coli*. *J. Bacteriol.* 177: 3455–3464.
- Battesti, A., J. R. Hoskins, S. Tong, P. Milanesio, J. M. Mann *et al.*, 2013 Anti-adaptors provide multiple modes for regulation of the RssB adaptor protein. *Genes Dev.* 27: 2722–2735.
- Bouvier, J., S. Gordia, G. Kampmann, R. Lange, R. Hengge-Aronis *et al.*, 1998 Interplay between global regulators of *Escherichia coli*: effect of RpoS, Lrp and H-NS on transcription of the gene *osmC*. *Mol. Microbiol.* 28: 971–980.
- Bull, H. J., M. J. Lombardo, and S. M. Rosenberg, 2001 Stationary-phase mutation in the bacterial chromosome: recombination protein and DNA polymerase IV dependence. *Proc. Natl. Acad. Sci. USA* 98: 8334–8341.
- Cairns, J., and P. L. Foster, 1991 Adaptive reversion of a frame-shift mutation in *Escherichia coli*. *Genetics* 128: 695–701.
- Calhoun, L. N., and Y. M. Kwon, 2011 The ferritin-like protein Dps protects *Salmonella enterica* serotype Enteritidis from the Fenton-mediated killing mechanism of bactericidal antibiotics. *Int. J. Antimicrob. Agents* 37: 261–265.
- Chib, S., and S. Mahadevan, 2012 Involvement of the global regulator H-NS in the survival of *Escherichia coli* in stationary phase. *J. Bacteriol.* 194: 5285–5293.
- Chintakayala, K., S. S. Singh, A. E. Rossiter, R. Shahapure, R. T. Dame *et al.*, 2013 *E. coli* Fis protein insulates the *cbpA* gene from uncontrolled transcription. *PLoS Genet.* 9: e1003152.
- Chodavarapu, S., M. M. Felczak, J. R. Yaniv, and J. M. Kaguni, 2008a *Escherichia coli* DnaA interacts with HU in initiation at the *E. coli* replication origin. *Mol. Microbiol.* 67: 781–792.
- Chodavarapu, S., R. Gomez, M. Vicente, and J. M. Kaguni, 2008b *Escherichia coli* Dps interacts with DnaA protein to impede initiation: a model of adaptive mutation. *Mol. Microbiol.* 67: 1331–1346.
- Cohen-Or, I., Y. Shenhar, D. Biran, and E. Z. Ron, 2010a CspC regulates *rpoS* transcript levels and complements *hfq* deletions. *Res. Microbiol.* 161: 694–700.
- Czapski, T. R., and N. Trun, 2014 Expression of *csp* genes in *E. coli* K-12 in defined rich and defined minimal media during normal growth, and after cold-shock. *Gene* 547: 91–97.
- Datsenko, K. A., and B. L. Wanner, 2000 One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* 97: 6640–6645.
- de los Rios, S., and J. J. Perona, 2007 Structure of the *Escherichia coli* leucine-responsive regulatory protein Lrp reveals a novel octameric assembly. *J. Mol. Biol.* 366: 1589–1602.
- Dorman, C. J., 2014 H-NS-like nucleoid-associated proteins, mobile genetic elements and horizontal gene transfer in bacteria. *Plasmid* 75: 1–11.
- Dubrac, S., and D. Touati, 2002 Fur-mediated transcriptional and post-transcriptional regulation of FeSOD expression in *Escherichia coli*. *Microbiology* 148: 147–156.
- Esposito, D., and G. F. Gerard, 2003 The *Escherichia coli* Fis protein stimulates bacteriophage lambda integrative recombination in vitro. *J. Bacteriol.* 185: 3076–3080.
- Farr, S. B., R. D'Ari, and D. Touati, 1986 Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. *Proc. Natl. Acad. Sci. USA* 83: 8268–8272.
- Flåtten, I., and K. Skarstad, 2013 The Fis protein has a stimulating role in initiation of replication in *Escherichia coli* in vivo. *PLoS One* 8: e83562.
- Foster, P. L., 2007 Stress-induced mutagenesis in bacteria. *Crit. Rev. Biochem. Mol. Biol.* 42: 373–397.
- Foster, P. L., and W. A. Rosche, 1999 Increased episomal replication accounts for the high rate of adaptive mutation in *recD* mutants of *Escherichia coli*. *Genetics* 152: 15–30.

- Franceschini, A., D. Szklarczyk, S. Frankild, M. Kuhn, M. Simonovic *et al.*, 2013 STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* 41: D808–D815.
- Frisch, R. L., Y. Su, P. C. Thornton, J. L. Gibson, S. M. Rosenberg *et al.*, 2010 Separate DNA Pol II- and Pol IV-dependent pathways of stress-induced mutation during double-strand-break repair in *Escherichia coli* are controlled by RpoS. *J. Bacteriol.* 192: 4694–4700.
- Galhardo, R. S., R. Do, M. Yamada, E. C. Friedberg, P. J. Hastings *et al.*, 2009 DinB upregulation is the sole role of the SOS response in stress-induced mutagenesis in *Escherichia coli*. *Genetics* 182: 55–68.
- Gibson, J. L., M. J. Lombardo, P. C. Thornton, K. H. Hu, R. S. Galhardo *et al.*, 2010 The sigma(E) stress response is required for stress-induced mutation and amplification in *Escherichia coli*. *Mol. Microbiol.* 77: 415–430.
- Gibson, J. L., M.-J. Lombardo, I. Aponyi, D. Vera Cruz, M. P. Ray *et al.*, 2015 Atypical role of PhoU in mutagenic break repair under stress in *Escherichia coli*. *PLoS One* 10: e0123315.
- Grainger, D. C., M. D. Goldberg, D. J. Lee, and S. J. Busby, 2008 Selective repression by Fis and H-NS at the *Escherichia coli* dps promoter. *Mol. Microbiol.* 68: 1366–1377.
- Harris, R. S., S. Longerich, and S. M. Rosenberg, 1994 Recombination in adaptive mutation. *Science* 264: 258–260.
- Harris, R. S., K. J. Ross, and S. M. Rosenberg, 1996 Opposing roles of the holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. *Genetics* 142: 681–691.
- Hastings, P. J., H. J. Bull, J. R. Klump, and S. M. Rosenberg, 2000 Adaptive amplification: an inducible chromosomal instability mechanism. *Cell* 103: 723–731.
- Hastings, P. J., A. Slack, J. F. Petrosino, and S. M. Rosenberg, 2004 Adaptive amplification and point mutation are independent mechanisms: evidence for various stress-inducible mutation mechanisms. *PLoS Biol.* 2: e399.
- Hersh, M. N., R. G. Ponder, P. J. Hastings, and S. M. Rosenberg, 2004 Adaptive mutation and amplification in *Escherichia coli*: two pathways of genome adaptation under stress. *Res. Microbiol.* 155: 352–359.
- Hwang, D. S., and A. Kornberg, 1990 A novel protein binds a key origin sequence to block replication of an *E. coli* minichromosome. *Cell* 63: 325–331.
- Hwang, D. S., B. Thony, and A. Kornberg, 1992 IciA protein, a specific inhibitor of initiation of *Escherichia coli* chromosomal replication. *J. Biol. Chem.* 267: 2209–2213.
- Kahramanoglou, C., A. S. Seshasayee, A. I. Prieto, D. Ibberson, S. Schmidt *et al.*, 2011 Direct and indirect effects of H-NS and Fis on global gene expression control in *Escherichia coli*. *Nucleic Acids Res.* 39: 2073–2091.
- Khan, S. R., and A. Kuzminov, 2012 Replication forks stalled at ultraviolet lesions are rescued via RecA and RuvABC protein-catalyzed disintegration in *Escherichia coli*. *J. Biol. Chem.* 287: 6250–6265.
- Kushner, S. R., 1974 In vivo studies of temperature-sensitive recB and recC mutants. *J. Bacteriol.* 120: 1213–1218.
- Layton, J. C., and P. L. Foster, 2003 Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli*. *Mol. Microbiol.* 50: 549–561.
- Liochev, S. I., and I. Fridovich, 1997 Lucigenin luminescence as a measure of intracellular superoxide dismutase activity in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 94: 2891–2896.
- Livak, K. J., and T. D. Schmittgen, 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔC_T} Method. *Methods* 25: 402–408.
- Lombardo, M. J., I. Aponyi, and S. M. Rosenberg, 2004 General stress response regulator RpoS in adaptive mutation and amplification in *Escherichia coli*. *Genetics* 166: 669–680.
- Lopez, C. R., S. Yang, R. W. Deibler, S. A. Ray, J. M. Pennington *et al.*, 2005 A role for topoisomerase III in a recombination pathway alternative to RuvABC. *Mol. Microbiol.* 58: 80–101.
- Lucchini, S., P. McDermott, A. Thompson, and J. C. Hinton, 2009 The H-NS-like protein StpA represses the RpoS (sigma 38) regulon during exponential growth of *Salmonella typhimurium*. *Mol. Microbiol.* 74: 1169–1186.
- Madrid, C., C. Balsalobre, J. Garcia, and A. Juárez, 2007 The novel Hha/YmoA family of nucleoid-associated proteins: use of structural mimicry to modulate the activity of the H-NS family of proteins. *Mol. Microbiol.* 63: 7–14.
- McKenzie, G. J., P. L. Lee, M. J. Lombardo, P. J. Hastings, and S. M. Rosenberg, 2001 SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol. Cell* 7: 571–579.
- McKenzie, G. J., R. S. Harris, P. L. Lee, and S. M. Rosenberg, 2000 The SOS response regulates adaptive mutation. *Proc. Natl. Acad. Sci. USA* 97: 6646–6651.
- Miller, J. H., 1992 *A Short Course in Bacterial Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Niederhoffer, E. C., C. M. Naranjo, K. L. Bradley, and J. A. Fee, 1990 Control of *Escherichia coli* superoxide dismutase (sodA and sodB) genes by the ferric uptake regulation (fur) locus. *J. Bacteriol.* 172: 1930–1938.
- Nunoshiba, T., and B. Dimple, 1994 A cluster of constitutive mutations affecting the C-terminus of the redox-sensitive SoxR transcriptional activator. *Nucleic Acids Res.* 22: 2958–2962.
- Oberto, J., S. Nabti, V. Jooste, H. Mignot, and J. Rouviere-Yaniv, 2009 The HU regulon is composed of genes responding to anaerobiosis, acid stress, high osmolarity and SOS induction. *PLoS One* 4: e4367.
- Ozaki, S., and T. Katayama, 2009 DnaA structure, function, and dynamics in the initiation at the chromosomal origin. *Plasmid* 62: 71–82.
- Pennington, J. M., and S. M. Rosenberg, 2007 Spontaneous DNA breakage in single living *Escherichia coli* cells. *Nat. Genet.* 39: 797–802.
- Perez, J. C., T. Latifi, and E. A. Groisman, 2008 Overcoming H-NS-mediated transcriptional silencing of horizontally acquired genes by the PhoP and SlyA proteins in *Salmonella enterica*. *J. Biol. Chem.* 283: 10773–10783.
- Petrosino, J. F., R. S. Galhardo, L. D. Morales, and S. M. Rosenberg, 2009 Stress-induced beta-lactam antibiotic resistance mutation and sequences of stationary-phase mutations in the *Escherichia coli* chromosome. *J. Bacteriol.* 191: 5881–5889.
- Ponder, R. G., N. C. Fonville, and S. M. Rosenberg, 2005 A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation. *Mol. Cell* 19: 791–804.
- Prieto, A. I., C. Kahramanoglou, R. M. Ali, G. M. Fraser, A. S. Seshasayee *et al.*, 2012 Genomic analysis of DNA binding and gene regulation by homologous nucleoid-associated proteins IHF and HU in *Escherichia coli* K12. *Nucleic Acids Res.* 40: 3524–3537.
- Ram, Y., and L. Hadany, 2012 The evolution of stress-induced hypermutation in asexual populations. *Evolution* 66: 2315–2328.
- Ram, Y., and L. Hadany, 2014 Stress-induced mutagenesis and complex adaptation. *Proc. Biol. Sci.* 281: pii: 20141025.
- Rogers, E., R. Correa, B. Barreto, M. A. Bravo Núñez, P. J. Minnick *et al.*, 2015 Double-strand-break repair mutagenesis and stress, in *Stress and Environmental Control of Gene Expression in Bacteria*, edited by F. DeBruijn. *In press*.
- Rosenberg, S. M., 2001 Evolving responsively: adaptive mutation. *Nat. Rev. Genet.* 2: 504–515.
- Rosenberg, S. M., C. Shee, R. L. Frisch, and P. J. Hastings, 2012 Stress-induced mutation via DNA breaks in *Escherichia*

- coli*: a molecular mechanism with implications for evolution and medicine. *BioEssays* 34: 885–892.
- Rouvière-Yaniv, J., M. Yaniv, and J. E. Germond, 1979 *E. coli* DNA binding protein HU forms nucleosomal-like structure with circular double-stranded DNA. *Cell* 17: 265–274.
- Saito, R., M. E. Smoot, K. Ono, J. Ruschinski, P. L. Wang *et al.*, 2012 A travel guide to Cytoscape plugins. *Nat. Methods* 9: 1069–1076.
- Schnetzer, K., 2008 Fine-tuned growth phase control of dps, encoding a DNA protection protein, by FIS and H-NS. *Mol. Microbiol.* 68: 1345–1347.
- Sharadamma, N., Y. Harshavardhana, P. Singh, and K. Muniyappa, 2010 *Mycobacterium tuberculosis* nucleoid-associated DNA-binding protein H-NS binds with high-affinity to the Holliday junction and inhibits strand exchange promoted by RecA protein. *Nucleic Acids Res.* 38: 3555–3569.
- Shee, C., J. L. Gibson, M. C. Darrow, C. Gonzales, and S. M. Rosenberg, 2011 Impact of a stress-inducible switch to mutagenic repair of DNA breaks on mutation in *E. coli*. *Proc. Natl. Acad. Sci. USA* 108: 13659–13664.
- Shenhar, Y., D. Biran, and E. Z. Ron, 2012 Resistance to environmental stress requires the RNA chaperones CspC and CspE. *Environ. Microbiol. Rep.* 4: 532–539.
- Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel *et al.*, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. *Microbiol. Rev.* 53: 1–24.
- Skarstad, K., B. Thony, D. S. Hwang, and A. Kornberg, 1993 A novel binding protein of the origin of the *Escherichia coli* chromosome. *J. Biol. Chem.* 268: 5365–5370.
- Slack, A., P. C. Thornton, D. B. Magner, S. M. Rosenberg, and P. J. Hastings, 2006 On the mechanism of gene amplification induced under stress in *Escherichia coli*. *PLoS Genet.* 2: e48.
- Sonnenfield, J. M., C. M. Burns, C. F. Higgins, and J. C. Hinton, 2001 The nucleoid-associated protein StpA binds curved DNA, has a greater DNA-binding affinity than H-NS and is present in significant levels in hns mutants. *Biochimie* 83: 243–249.
- Thöny, B., D. S. Hwang, L. Fradkin, and A. Kornberg, 1991 *iciA*, an *Escherichia coli* gene encoding a specific inhibitor of chromosomal initiation of replication in vitro. *Proc. Natl. Acad. Sci. USA* 88: 4066–4070.
- Torkelson, J., R. S. Harris, M. J. Lombardo, J. Nagendran, C. Thulin *et al.*, 1997 Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* 16: 3303–3311.
- Ueguchi, C., and T. Mizuno, 1993 The *Escherichia coli* nucleoid protein H-NS functions directly as a transcriptional repressor. *EMBO J.* 12: 1039–1046.
- Uyar, E., K. Kurokawa, M. Yoshimura, S. Ishikawa, N. Ogasawara *et al.*, 2009 Differential binding profiles of StpA in wild-type and hns mutant cells: a comparative analysis of cooperative partners by chromatin immunoprecipitation-microarray analysis. *J. Bacteriol.* 191: 2388–2391.
- Warnecke, T., F. Supek, and B. Lehner, 2012 Nucleoid-associated proteins affect mutation dynamics in *E. coli* in a growth phase-specific manner. *PLOS Comput. Biol.* 8: e1002846.
- Williams, A. B., and P. L. Foster, 2007 The *Escherichia coli* histone-like protein HU has a role in stationary phase adaptive mutation. *Genetics* 177: 723–735.
- Wilson, K., 2001 Preparation of genomic DNA from bacteria. *Curr. Protoc. Mol. Biol.* 2: 2.4.1–2.4.5.
- Wimberly, H., C. Shee, P. C. Thornton, P. Sivaramakrishnan, S. M. Rosenberg *et al.*, 2013 R-loops and nicks initiate DNA breakage and genome instability in non-growing *Escherichia coli*. *Nat. Commun.* 4: 2115.
- Zhang, Z., G. Gosset, R. Barabote, C. S. Gonzalez, W. A. Cuevas *et al.*, 2005 Functional interactions between the carbon and iron utilization regulators, Crp and Fur, in *Escherichia coli*. *J. Bacteriol.* 187: 980–990.

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