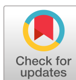

Carcinogenic bacterial pathogen *Helicobacter pylori* triggers DNA double-strand breaks and a DNA damage response in its host cells

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The bacterial pathogen *Helicobacter pylori* chronically infects the human gastric mucosa and is the leading risk factor for the development of gastric cancer. The molecular mechanisms of *H. pylori*-associated gastric carcinogenesis remain ill defined. In this study, we examined the possibility that *H. pylori* directly compromises the genomic integrity of its host cells. We provide evidence that the infection introduces DNA double-strand breaks (DSBs) in primary and transformed murine and human epithelial and mesenchymal cells. The induction of DSBs depends on the direct contact of live bacteria with mammalian cells. The infection-associated DNA damage is evident upon separation of nuclear DNA by pulse field gel electrophoresis and by high-magnification microscopy of metaphase chromosomes. Bacterial adhesion (e.g., via blood group antigen-binding adhesin) is required to induce DSBs; in contrast, the *H. pylori* virulence factors vacuolating cytotoxin A, γ-glutamyl transpeptidase, and the cytotoxin-associated gene (Cag) pathogenicity island are dispensable for DSB induction. The DNA discontinuities trigger a damage-signaling and repair response involving the sequential ataxia telangiectasia mutated (ATM)-dependent recruitment of repair factors—p53-binding protein (53BP1) and mediator of DNA damage checkpoint protein 1 (MDC1)—and histone H2A variant X (H2AX) phosphorylation. Although most breaks are repaired efficiently upon termination of the infection, we observe that prolonged active infection leads to saturation of cellular repair capabilities. In summary, we conclude that DNA damage followed by potentially imprecise repair is consistent with the carcinogenic properties of *H. pylori* and with its mutagenic properties in vitro and in vivo and may contribute to the genetic instability and frequent chromosomal aberrations that are a hallmark of gastric cancer.

DNA damage signaling | genomic instability | gastric tumorigenesis | chromosome breaks

Chronic infection with the human bacterial pathogen *Helicobacter pylori* causes gastritis and peptic ulceration (1) and increases the carrier's risk of developing gastric cancer (2) or gastric mucosa-associated lymphoid tissue lymphoma (3). The epidemiological association between *H. pylori* infection and gastric adenocarcinoma has been confirmed experimentally in rodent models using Mongolian gerbils (4) and C57BL/6 mice (5–7). Both epidemiological and experimental data suggest that bacterial virulence factors, host genetic traits, and environmental influences determine whether *Helicobacter*-induced gastritis will progress to gastric cancer (6, 8). In particular, *H. pylori*'s cytotoxin-associated gene (Cag) pathogenicity island (PAI), certain proinflammatory cytokine promoter polymorphisms, and a high-salt diet have been identified as cofactors affecting gastric cancer risk (2, 4, 5, 9). We have shown previously that gastric cancer precursor lesions induced by *Helicobacter felis* or *H. pylori* infection in C57BL/6 mice (which manifest histologically as atrophic gastritis, hyperplasia, and metaplasia) arise as a consequence of a T-helper type 1 cell-driven immunopathological response to the infection (10, 11). Mice lacking functional α/β^+ T cells are protected from precancerous lesions, and the adoptive transfer of $CD4^+CD25^-$ effector T cells is sufficient to sensitize mice to *Helicobacter*-induced preneoplasia (10).

In addition to the pathological effects of the *Helicobacter*-specific immune response on the gastric mucosa, several lines of evidence indicate that the bacteria may promote gastric carcinogenesis by jeopardizing the integrity and stability of their host's genome (12). *H. pylori* infection of cultured gastric epithelial cells down-regulates the components of the mismatch repair (MMR) and base excision repair machineries at the RNA and protein levels and impairs the efficiency of DNA repair as judged by MMR activity assay (13, 14). The down-regulation of MMR proteins was confirmed in experimentally infected murine gastric mucosa (14) and can be reversed by *Helicobacter* eradication in patients (15), suggesting that impaired DNA repair also is a hallmark of *H. pylori* infection in vivo. Touati et al. (16) reported an infection-induced increase in gastric mutation frequencies in the Big Blue transgenic model. The genotoxicity of *H. pylori* in this model has been attributed to oxidative damage of the DNA by reactive oxygen species (ROS) based on a high frequency of AT → CG and GC → TA transversions (16). Furthermore, mice deficient for the repair enzyme alkyladenine DNA glycosylase are more sensitive than wild-type animals to *H. pylori*-induced gastric preneoplasia (17).

Interestingly, early precancerous lesions in patient samples, as well as specific oncogene activation in different tumor models, have been linked to the formation of DNA double-strand breaks (DSBs) and the activation of DNA-damage checkpoints (18, 19). Thus, in this study, we examined the possibility that *H. pylori* directly damages DNA and triggers a DNA-damage response (DDR) in infected cells. We find that DSBs accumulate in various cell lines and in primary gastric epithelial cells upon infection with *H. pylori* in a time- and dose-dependent manner. The fragmentation of host nuclear DNA requires direct contact of live bacteria with their host cells, is independent of the *H. pylori* virulence determinants vacuolating cytotoxin A (VacA) and the Cag PAI, and does not require ROS-mediated DNA damage. Furthermore, infected cells display ataxia telangiectasia mutated-dependent phosporylated histone H2A variant X (γ-H2AX), mediator of DNA damage checkpoint protein 1

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(MDC1), and p53-binding protein 1 (53BP1) nuclear foci indicative of *H. pylori*-induced DNA-damage signaling. Efficient repair of *H. pylori*-induced DSBs is apparent upon antibiotic killing of the bacteria, but prolonged infections lead to residual unrepaired breaks and negatively affect cell viability. In conclusion, we show here that *H. pylori* has the unique ability to induce host cellular DNA damage directly, providing a mechanistic explanation for the carcinogenic properties of this bacterial pathogen.

Results

***H. pylori* Infection of Cultured Cells Induces DSBs in Nuclear DNA That Trigger a DNA-Damage and Repair Response.** To assess a possible effect of *H. pylori* infection on the integrity of host cellular DNA, we subjected cultured gastric adenocarcinoma cells (AGS) to a pulse field gel electrophoresis (PFGE) approach that visualizes fragmented DNA ranging in size from 0.5–2.5 Mb (20). Infection of AGS cells with *H. pylori* G27 for 6 h results in a dose-dependent fragmentation of the DNA that is consistent with DSB induction (Fig. 1*A*). DSB induction leads to the recruitment of the early repair factor 53BP1 (21) to microscopically visible nuclear foci that form at the sites of DSBs in approximately one-third of infected AGS cells at 6 h postinfection as well as in all γ-irradiated cells at 1 h postirradiation (Fig. 1*B*). To assess whether this effect is restricted to cancer cells, we cultured murine primary gastric epithelial cells and infected them with *H. pylori*. Indeed, 53BP1 foci also could be detected readily in primary cells (Fig. 1*C*). Finally, we tested U2OS osteosarcoma cells, a convenient and commonly used system for DDR studies (18). *H. pylori* adheres well to these cells despite their fibroblastic origin and induces DNA fragmentation and 53BP1 foci to a similar extent as in gastric epithelial cells (Fig. 1*D* and Fig. S1*A*). *H. pylori*-induced foci also contain MDC1 (Fig. S1*B*), which binds to phosphorylated H2AX and directs the recruitment of DDR factors to damaged chromatin (22). The utility of U2OS cells as target cells of *H. pylori* allowed us to determine the kinetics of foci formation in U2OS cells stably expressing MDC1 as an EGFP fusion protein; EGFP-MDC1 is recruited rapidly to the sites of DSBs (Fig. S1*C*) and is retained in DSB-induced foci for at least 16 h as determined by time-lapse video microscopy (Fig. S1*D*). Indeed, the proportion of cells with clearly discernible repair foci increases over time and is greater than 50% after 48 h of infection (Fig. 1*E* and Fig. S2*A*). To determine whether 53BP1 foci form as the result of a physiological response to DSBs, which is known to be mediated by the activation of the ATM kinase at broken DNA (23), we treated U2OS cells with the ATM inhibitor KU-55933 during the 6-h time course of *H. pylori* infection. ATM inhibition strongly reduced the number of γH2AX/53BP1$^+$ cells, indicating that *H. pylori*-induced DSBs elicit a canonical DDR including the activation of the ATM kinase signaling pathway (Fig. 1 *F* and *G*).

The colocalization of 53BP1 and γH2AX foci in *H. pylori*-infected cells suggests strongly that the fragmented DNA observed by PFGE analysis results from DSBs formed in the host cells. To rule out that the "fragmented DNA" is of bacterial origin (the *H. pylori* genome is 1.6 Mb in size and should migrate in the same band), we performed Southern blots using a probe hybridizing to *H. pylori* 16S rRNA (Fig. S2*B*). Southern detection of 16S rRNA revealed that the bacterial genome indeed migrates similarly as broken DNA under the PFGE conditions used; however, the amount of bacterial DNA present in our infected cell cultures is not sufficient to be detected by ethidium bromide staining (or Southern hybridization; Fig. S2*B*). Therefore, we can rule out the possibility that the band corresponding to broken DNA in the pulse field gels is of bacterial rather than host cell origin. Another concern was related to the viability of the infected cells; therefore we checked parameters of apoptotic cell death by Western blotting for activated caspase-3 and cleaved poly-(ADP-ribose) polymerase 1 (PARP-1) and by flow cytometric analysis. No indication of apoptotic cell death was obtained at 6 and 16 h postinfection, confirming that the infected cells are viable despite sustaining considerable DNA damage (Fig. S2 *C* and *D*). In conclusion, *H. pylori* acts as an efficient inducer of DSBs in the nuclear DNA of its host cells, thereby triggering the recruitment of DDR factors to DSB sites in various primary and transformed mammalian cells.

***H. pylori*-Induced DSBs Appear at Mitosis as Chromosomal Breaks and Depend on Direct Host–Pathogen Contact but Not on the Cag PAI or VacA.** To detect DSBs microscopically and quantify them in the chromosomes of infected cells, we examined their condensed metaphase chromosomes (Fig. 2*A*). Although control cells with more than one broken chromatid were observed only rarely, ∼30% of infected cells exhibited two or more breaks (Fig. 2 *A* and *B* and Fig. S3*A*). In fact, we found a substantial proportion of infected cells in which at least three and up to seven chromosomes had sustained microscopically evident chromatid breaks; such severely damaged cells were not detectable in the absence of *H. pylori* infection (Fig. 2 *A* and *B*). To examine whether the ability to induce DSBs is specific to (live) *H. pylori*, we compared live and dead bacteria and also included a laboratory strain of *Escherichia coli*. Only live *H. pylori* efficiently induced DSBs in AGS cells as determined by PFGE; *E. coli* or *H. pylori* bacteria killed by ethanol had no discernible effects (Fig. 2*C*). Inhibition of bacterial protein synthesis by chloramphenicol or gentamycin during the coculture with AGS cells also reduced DSB induction (Fig. S3*B*). DSB induction further required the direct interaction between the bacteria and their host cells and was abolished by their separation with a Transwell filter (Fig. 2*D*). We also assessed the effects of preconditioned medium (i.e., supernatants of AGS cells that had been infected for 16 h) with respect to DSB induction in fresh cells. No breaks were detected (Fig. 2*D*), ruling out the possibility that DSBs are induced by secreted bacterial or host cell factors.

Having obtained evidence that direct binding of *H. pylori* is a prerequisite for DSB induction, we next examined the dependence of DSB induction on the *H. pylori* blood-group antigen-binding adhesin BabA, which targets human Lewis (b) surface epitopes and has been tightly associated with duodenal ulcers and gastric cancer (24). We took advantage of the Kato III gastric epithelial cell infection model, in which we found *H. pylori* adhesion and *H. pylori*-specific IL-8 induction to be mediated predominantly by BabA (Fig. S4 *A*–*C*). Kato III cells were infected with either wild-type *H. pylori* or an isogenic Δ*babA* mutant and were assessed for DSB induction by PFGE. The Δ*babA* mutant was clearly less capable of inducing DSBs than the corresponding parental strain; this result was obtained with two different *H. pylori* strain backgrounds, J99 and G27 (Fig. 2*E* and Fig. S4*D*). Interestingly, preincubation of wild-type bacteria with increasing amounts of soluble Lewis (b) reduced DSB induction by *H. pylori* in a dose-dependent manner (Fig. S4*E*). The results confirm that tight attachment of the bacteria to their host cells, e.g., through the BabA/Lewis (b) interaction, is a prerequisite for infection-induced DNA damage. We next sought to determine whether well-known *H. pylori* virulence factors such as Cag PAI or VacA contribute to DSB induction. Isogenic Δ*PAI* or Δ*vacA* mutants induced DSBs to a similar extent as wild-type bacteria (Fig. 2 *F*–*H*), suggesting that neither VacA nor the PAI-encoded type IV secretion system of *H. pylori* is essential for DSB induction. Finally, we assessed whether a suspected *H. pylori* virulence factor that previously was reported to induce oxidative DNA damage (25), the γ-glutamyl transferase (γGT), is involved in DSB induction. This clearly was not the case; furthermore, a double mutant lacking both γGT and VacA also did not differ in this respect from the corresponding wild-type bacteria (strain P12) (Fig. S5). In summary, *H. pylori*, but not *E. coli*, induces DSBs in

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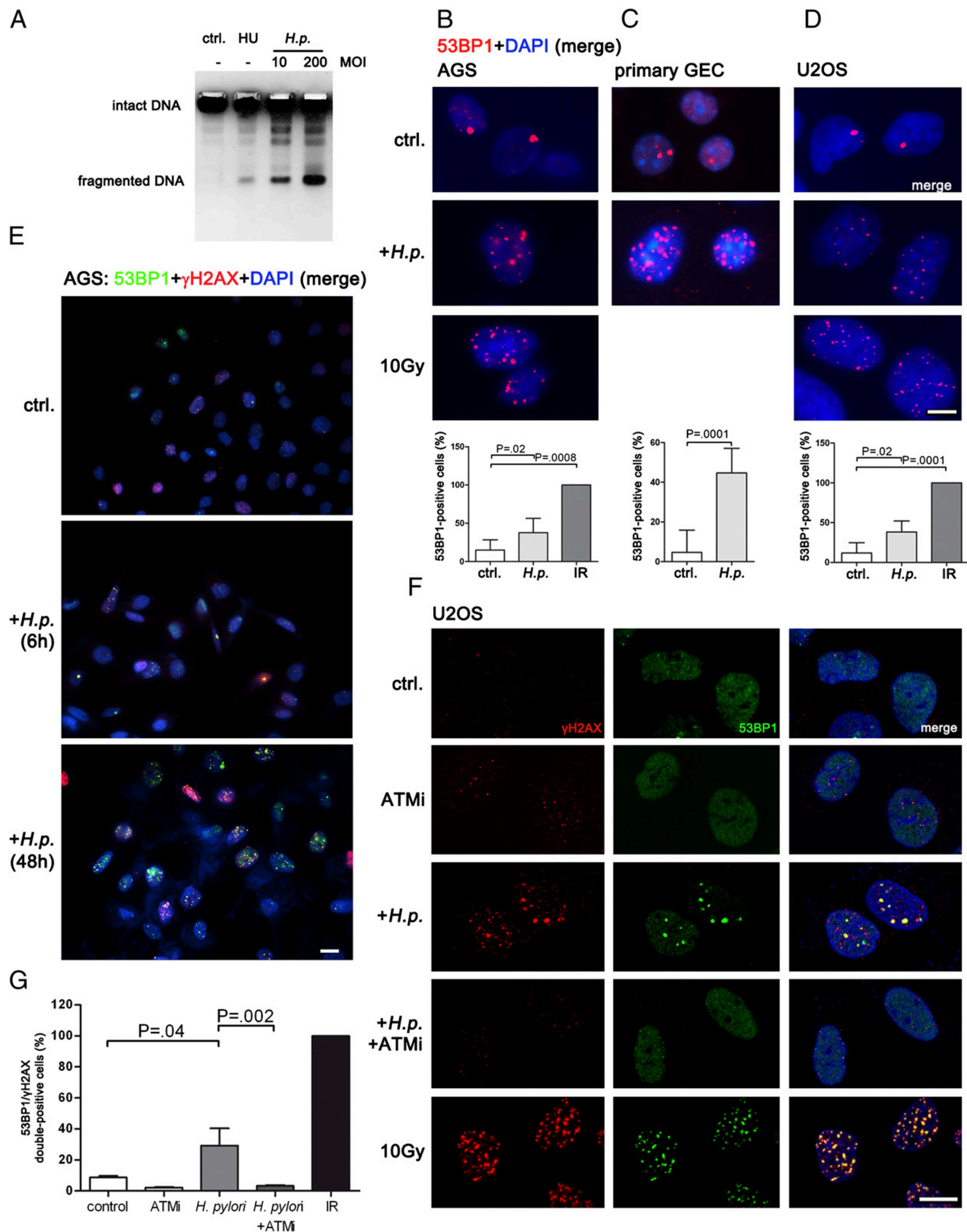

Fig. 1. *H. pylori* infection of cultured cells induces DSBs and DNA damage-response pathways. (*A*) AGS cells were infected with *H. pylori* strain G27 for 6 h at MOIs of 10 and 200. Hydroxyurea (HU) treatment (5 mM) served as positive control. DNA integrity was assessed by PFGE. (*B*–*D*) AGS cells (*B*), primary murine gastric epithelial cells (GEC) (*C*), and U2OS cells (*D*) were infected with *H. pylori* strain G27 (MOI 200) for 6 h and immunostained for 53BP1 and stained with DAPI for nuclear DNA. Cells irradiated with 10 Gy served as positive control as indicated. (*E*) AGS cells were infected for 6 or 48 h with G27 and stained for 53BP1, γH2AX, and nuclear DNA. (*F* and *G*) U2OS cells were infected with *H. pylori* G27 (MOI 200) for 6 h and/or treated with the ATM inhibitor KU-55922 before staining for 53BP1 and γH2AX. (Scale bars: 10 μm.) In *B*, *C*, *D*, and *G*, cells with more than four foci per nucleus were scored as positive; 150 cells were scored per condition. SEM and *P* values (Student's two-tailed *t* test) were calculated from three independent experiments.

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a contact/BabA-dependent but VacA-, Cag PAI-, and γGT-independent manner.

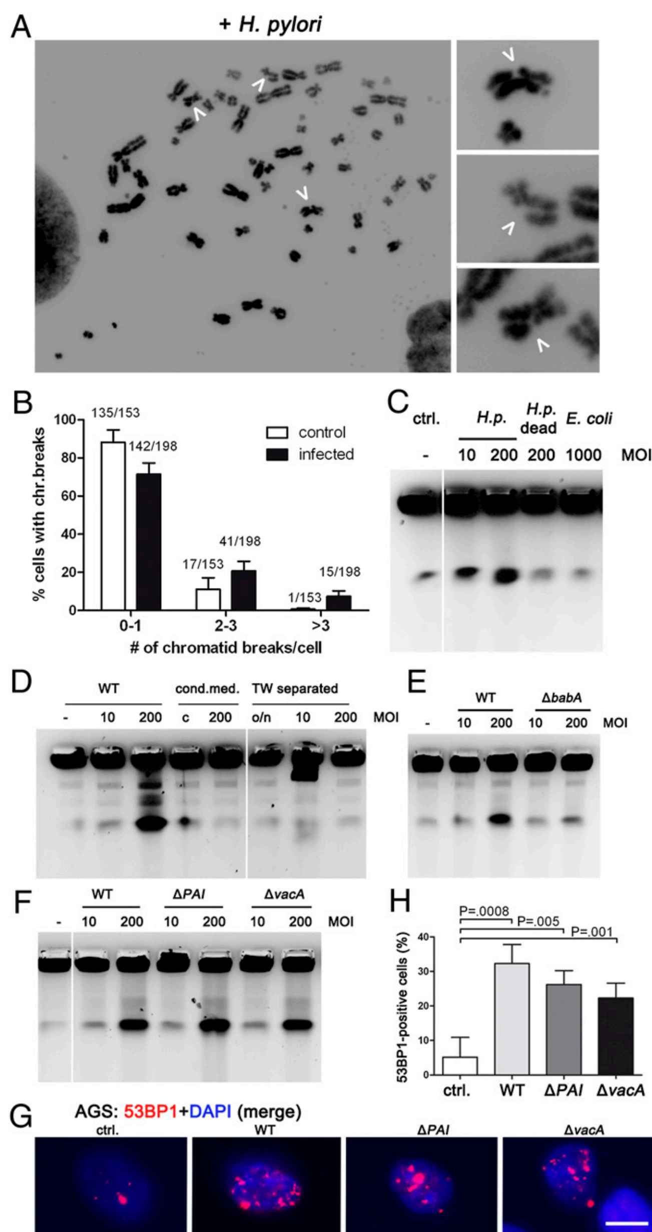

Fig. 2. DSB induction is *H. pylori* specific and depends on the direct contact of live bacteria with their host cells. (*A* and *B*) U2OS cells were infected with *H. pylori* strain G27 (MOI 200) followed by visualization of chromatid breaks by chromosome spreading. The integrity of chromatids was assessed by visual inspection of the chromosome spreads of 153 control cells and 198 infected cells in three independent experiments. The number of chromatid breaks per cell and number of cells with chromatid breaks (%) is indicated in *B*; error bars indicate the variation within the three experiments. (*C*) AGS cells were cocultured with live *H. pylori* G27, ethanol-killed bacteria, or live *E. coli* DH10B at the indicated MOIs for 16 h. DNA fragmentation was assessed by PFGE. (*D*) AGS cells were infected with *H. pylori* G27 for 6 h at the indicated MOIs (lanes 1–3). In lanes 4 and 5, cells were treated with sterile-filtered cell-culture supernatants obtained from 16-h control- or *H. pylori*-infected (MOI 200) AGS cells. In lanes 6–8, Transwell filters were used to separate AGS cells physically from a concentrated overnight *H. pylori* culture supernatant (lane 6) or from living bacteria at MOIs of 10 (lane 7) or 200 (lane 8). DNA fragmentation was assessed by PFGE. (*E*) Kato III cells were infected with *H. pylori* J99 or the isogenic mutant Δ*babA* for 6 h at the indicated MOIs. DNA integrity was assessed by PFGE. (*F*–*H*) AGS cells were infected with wild-type G27 or Δ*PAI* or Δ*vacA* isogenic mutants and assessed by PFGE (*F*) or staining for 53BP1 (*G* and *H*). In *H*, cells with more than four foci per nucleus were scored as positive; SEM and *P* values were calculated from three independent experiments.

Neither DNA Synthesis nor Oxidative Damage Is Required for *H. pylori*-Induced DSBs. Only a fraction of infected cells exhibits nuclear foci and chromatid aberrations (Figs. 1 and 2 *A* and *B*), suggesting a cell-cycle dependence of DSB induction. Because oncogene-induced DSBs have been attributed to DNA replication stress, and most spontaneous DSBs are caused by collapsed replication forks (18, 19), a possible explanation would be that cells are sensitive only in S-phase. To determine whether DNA replication is required for DNA fragmentation, we synchronized U2OS cells in G2/M by nocodazole treatment before infection with *H. pylori*. Cells were infected for 6 h at 2 h after release from G2/M arrest. Their DNA integrity was assessed by PFGE at the end point 8 h after release, i.e., at a time when the cells had not yet reentered S-phase (as documented by flow cytometric cell-cycle analysis; Fig. S6). As shown in Fig. 3*A*, synchronized cells sustained a level of DSBs similar to that in asynchronous cultures infected in parallel for the same period. Thus, unlike DSBs that are caused by alkylating agents, intrastrand crosslinking, and topoisomerase I inhibitors, *Helicobacter*-induced DSBs do not require bulk DNA replication.

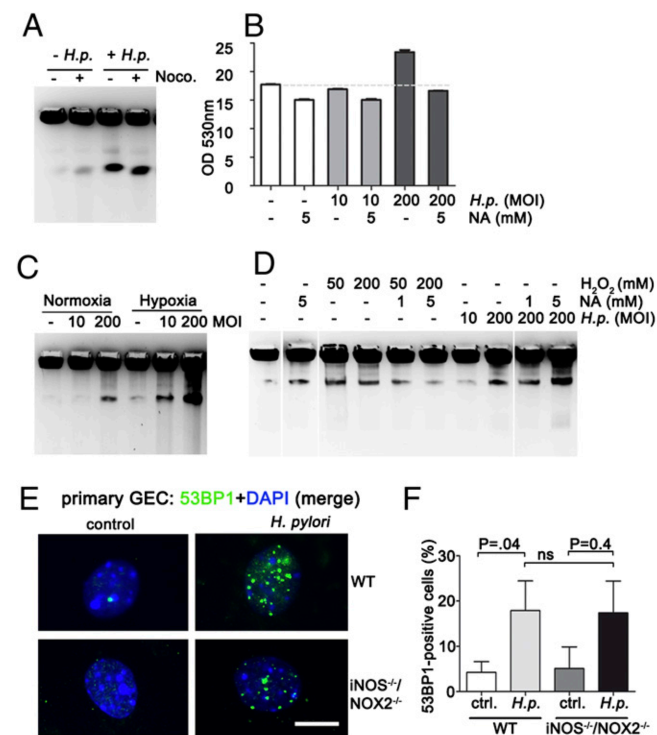

Fig. 3. *H. pylori*-associated DSBs occur independently of cell-cycle phase and oxidative DNA damage. (*A*) U2OS cells were arrested in G2/M by 12-h treatment with 250 ng/mL nocodazole, released for 2 h, and infected with *H. pylori* G27 for 6 h. DNA integrity was assessed by PFGE. (*B*) AGS cells were left untreated, were treated with 5 mM *N*-acetyl-cysteine (NA), and/or were infected with *H. pylori* G27 at MOIs of 10 and 200. Relative induction of ROS was assessed by oxidation of 2,7-dichlorofluorescein diacetate (DCF-DA) to fluorescent DCF. (*C*) AGS cells were infected with *H. pylori* G27 for 6 h at the indicated MOIs in 20% oxygen (normoxia) or 1% oxygen (hypoxia). (*D*) AGS cells were infected at the indicated MOIs for 6 h. *N*-acetyl-cysteine (NA) was added at 1 or 5 mM as indicated. H_2O_2 treatment for 1 h served as positive control. In *C* and *D*, DNA fragmentation was visualized by PFGE. (*E* and *F*) Primary gastric epithelial cells explanted from wild-type C57BL/6 or iNOS$^{-/-}$/NOX2$^{-/-}$ mice were infected with *H. pylori* for 6 h and immunostained for 53BP1. Cells with more than four 53BP1 foci per nucleus were graded as positive; SEM and *P* values were calculated from three independent experiments.

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The mutations detected in the chronically *H. pylori*-infected stomach are predominantly AT > CG and GC > TA transversions indicative of oxidative DNA damage (16). *H. pylori* efficiently induces reactive oxygen and nitrogen species (RONS) in AGS cells, which can be scavenged by *N*-acetyl-cysteine (Fig. 3*B*). To determine whether *H. pylori*-associated DSB induction is dependent on RONS, we used three complementary approaches in which the production of RONS was minimized by growth under hypoxic conditions, pharmacological scavenging of RONS, or a combined targeted deletion of the genes encoding inducible nitric oxide synthase (iNOS) and NADPH oxidase 2 (NOX2). Remarkably, AGS cells grown under hypoxic conditions (1% oxygen) suffered more, rather than fewer, DSBs upon infection with *H. pylori*, arguing against the idea that *H. pylori*-induced DSBs are mediated by RONS (Fig. 3*C*). Similarly, addition of the RONS scavenger *N*-acetyl-cysteine to infected cells aggravated *H. pylori*-induced DSBs rather than preventing them (Fig. 3*D*); in contrast, the same scavenger concentrations efficiently reduced H_2O_2-induced DSBs (Fig. 3*D*). Finally, infection of primary gastric epithelial cells isolated from iNOS/NOX2$^{-/-}$ mice induced DSBs with a similar efficiency as infection of wild-type cells (Fig. 3 *E* and *F*), suggesting that the RONS produced through the combined activity of both enzymes are not essential for DSB induction. In summary, the results suggest that RONS produced by gastric epithelial cells upon *H. pylori* infection are not mechanistically involved in DSB induction despite a temporal correlation of RONS production and DSB induction.

Infection-Induced DSBs Are Continuously Repaired. We next assessed whether DSBs accumulate during infection and/or whether they are resolved continuously by the cellular DNA repair machinery. We observed no PFGE-detectable difference in DSB levels comparing short (6-h) and prolonged (54-h) infections (Fig. 4*A*), suggesting that infected cells may reach an equilibrium in which DSBs are repaired continuously even as new breaks are incurred. Indeed, we find that the PFGE band corresponding to fragmented DNA persists for at least 48–54 h in the continuous presence of live bacteria in two independent cell types tested (Fig. S7). To test whether cells are capable of efficient DSB repair, we infected AGS cells for 6 h, eradicated the bacteria by antibiotic therapy, and allowed the cells to recover for 48 h. Antibiotic eradication resulted in the subsequent efficient repair of DSBs as judged by the disappearance of fragmented DNA (Fig. 4*A*) as well as by the disappearance of 53BP1/MDC1 foci (Fig. 4 *B* and *C*). However, in the presence of continuous infection for 48 h or longer, ∼70% of the cells lose their proliferative capacity (i.e., fail to form colonies in a clonogenic assay) (Fig. 4*D*). We conclude from these results that *H. pylori*-induced DSBs are addressed efficiently by the host cell repair machinery, with the results of the repair process being evident only after the de novo induction of DSBs is prevented by inactivation of the bacteria. However, prolonged continuous infection at high multiplicities of infection (MOIs) may saturate the repair mechanisms, lead to unrepaired breaks, and cause cell lethality.

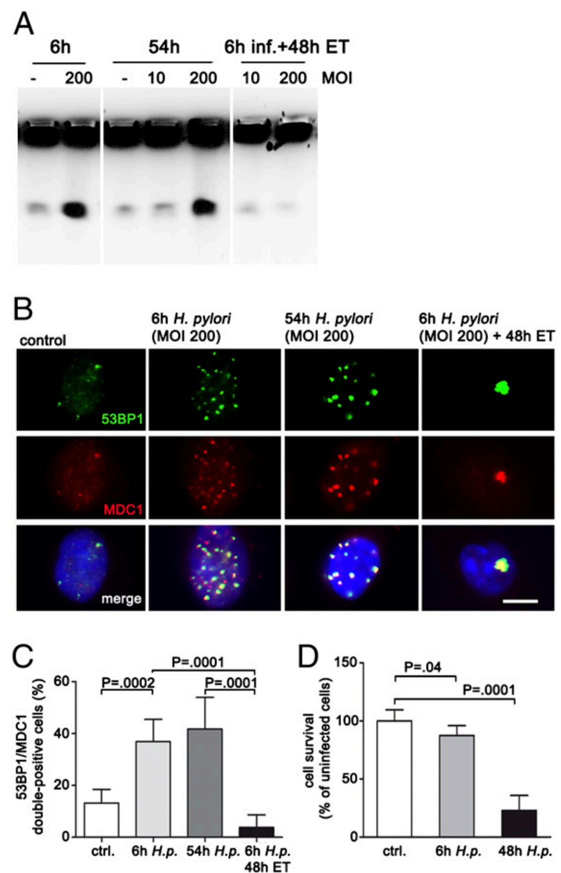

Fig. 4. *H. pylori*-induced breaks are repaired only after bacterial killing. (*A*–*C*) AGS cells were infected with *H. pylori* G27 at the indicated MOIs for 6 h before antibiotic killing of the bacteria (eradication therapy, ET). Cells then were grown in full medium containing penicillin/streptomycin for 48 h to allow DNA repair to occur, were harvested, and were subjected to PFGE (*A*) or to immunostaining for 53BP1 and MDC1 (*B*) and compared with control cells that had been infected either for 6 h or continuously for 54 h. In *C*, cells with more than four foci per nucleus were graded as positive; SEM and *P* values were calculated from three independent experiments. (*D*) Cells were infected with *H. pylori* G27 for 6 and 48 h, subjected to *H. pylori* eradication, and cultured for another 10 d. Proliferative capacity was assessed by clonogenic assay; the survival and clonogenic capacity of never-infected (ctrl) cells is set at 100%.

Discussion

We describe here the phenomenon of chromosomal DSB induction in mammalian cells upon infection with the carcinogenic bacterium *H. pylori*. This process requires viable bacteria in close contact with their host cells. We provide evidence that bacterial adhesion, for instance via the BabA adhesin, is required for DSB induction; in contrast, the *H. pylori* virulence factors VacA, γGT, and the Cag PAI are dispensable for DSB induction. Host cellular ROS and reactive nitrogen species production is triggered by the infection but appears to not contribute to DSB formation. The ability to induce DSBs is shared by the three *H. pylori* isolates examined in the course of our studies (G27, J99, and P12) and occurs with similar kinetics and efficiency in primary and transformed, murine and human, epithelial and mesenchymal cells. *H. pylori*-induced DSBs are evident upon separation of nuclear DNA by PFGE and by high-magnification microscopy of metaphase chromosomes. The damaged DNA triggers a damage-signaling and repair response involving the sequential ATM-dependent recruitment of repair factors (53BP1, MDC1) and the phosphorylation of histone H2AX. *H. pylori*-induced DSBs can be repaired efficiently by the damaged cells, but prolonged bacterial infection at high MOIs saturates cellular repair mechanisms and impairs proliferation.

Very few reports are available on bacterially induced DNA damage of host cell nuclear DNA. Interestingly, most bacterial species with demonstrated genotoxic properties are gastrointestinal pathogens. In pathogenic strains of *E. coli*, a genomic island encoding modular nonribosomal peptide and polyketide synthases has been linked to DSB induction and DNA-damage checkpoint activation (26). The hybrid peptide/polyketide genotoxin produced by these enzymes arrests the DNA-damaged cells at the G2/M transition; the decelerated self-renewal of the intestinal epithelium resulting from cell-cycle arrest has been

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proposed to benefit the bacteria by facilitating their persistence (26). The only other bacterial virulence factor known to affect the integrity of the host genome is produced by the enteropathogenic species *Helicobacter hepaticus* and *Campylobacter jejuni*, close relatives of *H. pylori*. The so-called "cytolethal distending toxins" (CDTs) exhibit structural and functional homology to mammalian phosphodiesterases such as DNase I (21) and cause cell-cycle arrest and cell death in intestinal epithelial cells (27). In murine models of *H. hepaticus* infection, CDT triggers the development of chronic intestinal inflammation mimicking inflammatory bowel disease in humans (28) and promotes hepatocellular dysplasia (29). Similarly, CDT is required for the induction of gastroenteritis by *C. jejuni* (30). No orthologs of CDT are present in any of the *H. pylori* genomes sequenced to date, including the genome of G27, which we predominantly used here.

DNA DSBs are the most harmful lesions a cell can encounter, and efficient DSB repair is crucial for the maintenance of genomic integrity and viability (31). Cells have evolved mechanistically distinct strategies for DSB repair (32). These strategies include minor mutagenic pathways that recently have generated great interest in the field as a possible source of the chromosomal aberrations observed in many cancers (33). Induction of DSBs followed by imprecise repair would be consistent with the carcinogenic properties of *H. pylori* (as well as those of other enteropathogenic species capable of inducing DSBs, such as *H. hepaticus*) and with its mutagenic properties in vitro and in vivo (16). Importantly, we observe that prolonged bacterial infection leads to saturation of the repair capabilities of the host cells, possibly leading to ineffective and mutagenic DSB repair attempts. We consider a comprehensive analysis of the DSB repair pathways activated by *H. pylori* infection to be an important challenge for future studies. In conclusion, we propose here that DSB induction contributes to the genetic instability and frequent chromosomal aberrations that are a hallmark of gastric cancer, indicating that as yet unidentified *H. pylori*-derived oncoproteins encoded outside the Cag PAI must be considered to gain a better understanding of *H. pylori*-induced gastric carcinogenesis.

Materials and Methods

Mouse and Bacterial Strains, Cell Lines, Infection Conditions, and Pharmacological Treatments. C57BL/6 (Charles River Laboratories) and iNOS$^{-/-}$/NOX2$^{-/-}$ mice (generously provided by Wolf-Dietrich Hardt, Eidgenössische Technische Hochschule, Zurich) were used to generate primary gastric epithelial cells as described (10). *H. pylori* was grown as described (10). AGS cells (ATCC CRL 1739, a human gastric adenocarcinoma cell line), immortalized murine primary gastric epithelial cells (IMPGE) (10), U2OS cells, and a stable U2OS line expressing EGFP-MDC1 were grown and infected with *E. coli* DH10B or *H. pylori* strains as specified in *SI Materials and Methods*.

PFGE, Chromosome Spreads, Quantification of RONS, Cell-Cycle Analysis, and Clonogenic Assays. Chromosome spreads, PFGE experiments, quantification of RONS, cell-cycle analysis, clonogenic assays are specified in *SI Materials and Methods*.

Immunofluorescence Microscopy, Western and Southern Blotting. For immunostaining, cells were fixed in methanol, stained with 53BP1 (sc-22760; Santa Cruz), MDC1 (3835), or γH2AX (05-636; Upstate), quantified and analyzed as specified in *SI Materials and Methods*. Western and Southern blotting protocols are specified in *SI Materials and Methods*.

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