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Conjugative DNA Transfer Induces the Bacterial SOS Response and Promotes Antibiotic Resistance Development through Integron Activation

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Abstract
Conjugation is one mechanism for intra- and inter-species horizontal gene transfer among bacteria. Conjugative elements have been instrumental in many bacterial species to face the threat of antibiotics, by allowing them to evolve and adapt to these hostile conditions. Conjugative plasmids are transferred to plasmidless recipient cells as single-stranded DNA. We used lacZ and gfp fusions to address whether conjugation induces the SOS response and the integron integrase. The SOS response controls a series of genes responsible for DNA damage repair, which can lead to recombination and mutagenesis. In this manuscript, we show that conjugative transfer of ssDNA induces the bacterial SOS stress response, unless an anti-SOS factor is present to alleviate this response. We also show that integron integrases are up-regulated during this process, resulting in increased cassette rearrangements. Moreover, the data we obtained using broad and narrow host range plasmids strongly suggests that plasmid transfer, even abortive, can trigger chromosomal gene rearrangements and transcriptional switches in the recipient cell. Our results highlight the importance of environments concentrating disparate bacterial communities as reactors for extensive genetic adaptation of bacteria.

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Introduction
Free-living bacteria commonly face changing environments and must cope with varying conditions. These adaptive strategies involve temporary physiological responses through various groups of genes gathered in regulons that are induced or repressed according to the surrounding conditions. This is the case for the quorum sensing regulon [1,2], the stringent response and catabolite repression systems, which allow adjustment of gene expression according to the growth conditions [3–5]. In other instances, the only adaptive solution requires a genetic change, and bacteria have developed mechanisms that favour genome modifications either by transiently increasing their mutation rates, inducing re-arrangements, or lateral (horizontal) gene transfer (HGT). One of the better known responses of this kind is the trigger of the SOS regulon, which controls DNA repair and recombination genes [6].

SOS is a bacterial stress response induced when an abnormal rate of single stranded DNA (ssDNA) is present in the cell. ssDNA is the substrate for RecA polymerization. The formation of a ssDNA/RecA nucleofilament stimulates auto-protolysis of the LexA repressor, leading to de-repression of genes composing the SOS regulon. The SOS response is triggered by the accumulation of ssDNA, for example when cells try to replicate damaged DNA, after UV irradiation or treatment with antibiotics (fluoroquinolones, β-lactams) or mitomycin C (MMC), a DNA cross-linking agent. In addition to these endogenous sources, ssDNA is also produced by several mechanisms of exogenous DNA uptake involved in lateral gene transfer, namely by conjugation, transformation and occasionally transduction.

Conjugation is indeed one mechanism of lateral transfer that leads to the transient occurrence of ssDNA in the recipient cell [7,8]. The presence of anti-SOS factors in some conjugative plasmids, such as the psiB gene of R6K<sub>ads</sub> and R100-1 [9], suggests that conjugative DNA transfer can induce SOS. In plasmid R100-1, <i>psiB</i> (plasmic SOS inhibition) was shown to be transiently expressed during the first 20 to 40 minutes of conjugation [10,11] from a ssDNA promoter [12], and inhibited the bacterial SOS response [13,14]. Plasmids carrying <i>psiB</i> do not all express it at levels sufficient to alleviate SOS, as seems to be the case in F plasmids for instance [9,10,13,15,16].

Conjugation is a widespread mechanism in the intestinal tract of host animals where there is a high concentration of bacterial populations [17–22]. Lateral gene transfer plays a large role in the evolution of genomes and emergence of new functions, such as antibiotic resistance, virulence and metabolic activities in bacterial species [23].

Bacteria can also possess other internal adaptive genetic resources. <i>Vibrio cholerae</i> carries a superintegron (SI), that can be used as a reservoir of silent genes that can be mobilized when needed. Integrons are natural gene expression systems allowing the integration of an ORF by site-specific recombination, transform-
Author Summary

Bacteria exchange DNA in their natural environments. The process called conjugation consists of DNA transfer by cell contact from one bacterium to another. Conjugative circular plasmids have been identified as shuttles and reservoirs for adaptive genes. It is now established that such lateral gene transfer plays an essential role, especially for the antibiotic resistance development and dissemination among bacteria. Moreover, integrons, platforms of mobile gene cassettes, have been instrumental in this phenomenon, through their successful association with conjugative resistance plasmids. We demonstrate in this study that the conjugative transfer of plasmids triggers a bacterial stress response—the SOS response—in recipient cells and can impact the cassette content of integrons. The SOS response is already known to induce various genome modifications. Human and animal pathogens cohabit with environmental bacteria, in niches which will favor DNA exchange. SOS induction during conjugation is thus most probably able to impact a wide range of genomes. Bacterial SOS response could then be a suitable target for co-treatment of infections in order to prevent exchange of antibiotic resistance/adaptation genes.

Results

Conjugative transfer of plasmids R388, R6Kdrd, and RP4 induces the SOS response in E. coli and V. cholerae

During conjugation, plasmid DNA enters the host cell in a single stranded fashion [7,8]. In order to test whether conjugation induces the SOS response in the recipient cell, we used reporter E. coli and V. cholerae strains carrying yfdA::lacZ (7631) and recN::lacZ (7453) β-galactosidase fusions, respectively. yfdA (cell division in E.coli) and recN (recombinational repair) genes belong to the SOS regulon of E.coli. We also identified a LexA binding box upstream of recN in V. cholerae. We confirmed that induction of SOS in these strains results in expression of the β-galactosidase (β-gal) enzyme (not shown). Table 1 summarizes the conjugative plasmids belonging to several incompatibility groups we used in this study [32]. The donor (DH5α) strain was recBAD and ΔlacZ.

The conjugation rates of these plasmids were first measured at various time points after donor and recipient cells were mixed (Figure 1A). In E. coli, all plasmids conjugate approximately at the same rate so that nearly all recipients have received a plasmid after 60 min of conjugation. In V. cholerae transfer rates vary considerably, only 1 in 100 cells have received a plasmid after 4h of mating with R6Kdrd and R388, while RP4 has a transfer rate similar to that of E. coli (10⁻⁴ to 1). Neither R6Kdrd nor R100-1 replicate in V. cholerae. In order to address whether R100-1 actually transfers from E. coli into V. cholerae, we used pSU19-oriTF plasmids containing the oriTF (72 bp) of plasmid F. Plasmid F does not replicate in V. cholerae and oriTF is 98% identical to oriTR100. The high oriTF transfer rate observed at 1h of mating confirms that plasmids F and R100-1 (and presumably R6Kdrd) can indeed transfer into V. cholerae and that the lack of R100/R64 transconjugants is due to their inability to establish themselves in this bacterium.

SOS induction linked to conjugation was measured in the total recipient population by counting the actual number of recipient cells plated on selective medium instead of using OD units (Materials and Methods), to obtained an induction value per potential recipient cell. Mating was interrupted at various time points (t₀, t₄₀, t₆₀, t₁₂₀, t₁₈₀, t₂₄₀) and β-gal activity was measured in both E. coli and V. cholerae recipients (Figure 1B). The results are represented on the graph as the induction ratios at times t₀, t₆₀ and t₂₄₀ over the induction at t₀. When the recipient strain was mixed with empty donor, no SOS induction was observed. A peak of SOS induction in E. coli was detected after 40 min to 60 min of mating with a conjugation proficient donor, 1.7 fold induction for RP4 and R6Kdrd and 2.3 fold for R388. The induction peak was also observed in V. cholerae (2.3 fold for R6Kdrd, 2.7 fold for RP4 and 3.4 fold for R388). To verify that the β-gal activity was due to the SOS induction, we deleted the recN gene in the recipient E. coli strain. No induction of β-gal activity was observed in the ArcA strain after conjugation with RP4, R6Kdrd and R388. This confirms that the β-gal induction observed in recA+ strain indeed reflects the SOS induction by RP4, R6Kdrd and R388.
As described above, β-gal induction peaks between τ40 and τ60 minute of mating. The induction then decreases to reach the level shown at τ240, forming bell shaped curves (data not shown). This induction pattern reflects the SOS induction in an asynchronous population of bacteria. It can be explained by the fact that plasmids RP4, R6Kdrd and R388 replicate in recipient cell. Once mating has started and as time goes by, there tends to be less plasmidless recipient cells. Indeed, entry of the plasmid DNA induces SOS, the incoming plasmidic ssDNA then replicates in the conjugant cell and the entry exclusion systems prevents entry of another plasmid [33,34]. However, cells continue to divide so that the population of kanamycin resistant (kanR) host cells increases. Accordingly, even when the transfer rate remains constant (especially for low rated plasmids), the increase in the number of kanR cells can explain the drop of activity per recipient in the curve. The SOS response is expected to return to normal once all the cells have acquired the plasmid.

Since all cells in the recipient population have not received a conjugating DNA at the time of the β-gal assay, we calculated the SOS induction per conjugant, i.e. per recipient cell that has actually undergone DNA uptake (Materials and Methods). The results are represented as ratios over τ0 in Figure 1C. As expected, the induction signal is amplified when one takes into account the conjugation rate for each plasmid. This amplification of several orders of magnitude is likely to be an effect of unsuccessful conjugation: SOS is induced by incoming DNA, that is not always converted into a replicating plasmid. The induction profiles, however, are compatible with Figure 1B: R388 and R6Kdrd strongly induce SOS, RP4 also shows a high induction, however it is lower than the former two plasmids. Once again, no (or very weak) induction was observed in E. coli and V. cholerae (Figure 1C and data not shown), confirming that SOS induction can be triggered by this plasmid. Interestingly, Rs-a conjugates at a rate of 10⁻¹ and yields an intermediate induction level (like RP4). Even though we have a small sample of plasmids, SOS induction during mating seems to inversely correlate with conjugation rate (at 1h of mating) or replication of plasmids, except for R6Kdrd. Further study is needed to verify this observation. On the other hand, RIP113 (IncN) induced SOS in V. cholerae only (Figure S2).

An increasing number of non-replicative conjugative elements, generally named ICE, have been described in bacteria. One of the best studied is the SXT element discovered in V. cholerae [35–37]. We addressed if conjugative transfer of an SXT element integrated in the chromosome of E. coli [38] to V. cholerae also induces the SOS response. We observed a similar induction profile as for the conjugative plasmids with a peak of induction at τ100 (Figure 1B). The delay can likely be explained by the very low transfer rate (10⁻⁶ after 6 six hours of mating).

Our results show that plasmids lacking the psiB gene (here RP4, R388, R6Kdrd and Rs-a) induce SOS upon conjugation into the recipient cell. On the other hand, RIP113 (IncN) induced SOS in V. cholerae only (Figure S2), thus behaving like R64drd and R100-1 plasmids.

PsiB strongly alleviates the SOS response in E. coli but only very weakly in V. cholerae: conjugation with plasmids R64drd and R100-1 induces SOS in V. cholerae. R64drd and R100-1 plasmids do not induce (or very poorly) the SOS response in E. coli (Figure 1B and 1C). This was expected as these plasmids carry a psiB anti-SOS gene. Plasmid RIP113 behaves like R64drd and R100-1 in terms of SOS induction in E. coli. We thus suspected RIP113 to carry a psiB gene as 64drd and R100-1 plasmids. This was confirmed by PCR amplification with psiB-specific primers (data not shown). This finding is supported by another IncN plasmid which has been sequenced: the R64 plasmid carries a gene named sth4 (locus R46_027), presenting 42% DNA sequence identity with psiB.

We observed a strong induction of SOS by the same 3 plasmids in V. cholerae (Figure 1C), suggesting that the psiB gene is either not expressed in V. cholerae or that its product is not active in this species (R64drd and R100-1 do not replicate in V. cholerae, thus no activity per conjugant could be calculated). Moreover, SOS induction is continuously high for R64drd and R100-1 plasmids after ~60 min, whereas SOS induction declined after 60 min for RP4, R6Kdrd and R388, as mentioned above. We were unable to delete psiB from R64drd and thus could not check if in its absence SOS induction would be restored in E. coli. The reason for the unsuccessful cloning attempts could be the presence of several genes (such as ssb coding the single strand binding protein, anti-restriction gene anti and flm/ hok) in the same region where ORFs and regulatory regions overlap [9,11,39–41], such that deletion of psiB could have unpredicted consequences on plasmid transfer and replication. Instead, psiB from R64drd was cloned and over-expressed from a pBAD plasmid, under the control of the arabinose inducible promoter. SOS induction after mitomycin C (MMC) treatment was measured in E. coli sftC::lacZ and V. cholerae recN::lacZ containing either empty pBAD or pBAD-PsiB plasmids. As previously published [31], MMC treatment induced SOS in E. coli and V. cholerae (Figure 2). SOS induction was strongly reduced in E. coli when PsiB was expressed from pBAD (6 fold induction instead of 11.6 fold, Figure 2A) whereas SOS induction was insensitive to PsiB expression in V. cholerae (~60 fold induction with and without PsiB over-expression, Figure 2B). These results show that the psiB(R64drd) and presumably the psiB(R100-1) which presents 85% identity to psiB(R64) is expressed during conjugation in E. coli and inhibits the SOS response, whereas...
Figure 1. Conjugation induces SOS in *E. coli* and *V. cholerae*. Shaded bars: *E. coli*. Dotted bars: *V. cholerae*. Grey: values at peak of induction t40–t60 min (for SXT, the peak is shown at t210); White: values at t240. A: conjugation rates in *E. coli* recA+ and recA (strains 7651 and 7713) and *V. cholerae* (7453); B: SOS induction in total population of recipient *E. coli* and *V. cholerae* measured by β-gal tests; C: SOS induction ratio in conjugants only, for *E. coli* and *V. cholerae*. Induction was calculated as described in Materials and Methods. Induction ratios are units at time t/x/units at time t0.

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SOS induction following MMC treatment. A:
V. cholerae
but not in
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from Salmonella, an enterobacterium, also carries the
and behaves like R64
bacterial species where its carrier plasmids normally reside (here
Altogether, these data suggest that PsiB is functional only in
strain, PsiB does not alleviate SOS (Figure 2A, note that RecAVch
over-expressed, we hypothesized that PsiB would be deficient in
plasmids does not inhibit the SOS response in
V. cholerae
when over-expressed, we hypothesized that PsiB would be deficient in
interacting with RecACVch. Our β-gal tests show that when expressed in
V. cholerae
 PsiB reduces the SOS response in V. cholerae when over-expressed, we hypothesized that PsiB would be deficient in
interacting with RecACVch. Our β-gal tests show that when expressed in
V. cholerae
 together with RecACeco, PsiB reduces the SOS response from 60 fold to 24 fold induction (Figure 2B). Consistently, when co-expressed with RecACVch in an E. coli ΔrecA
strain, PsiB does not alleviate SOS (Figure 2A, note that RecACVch is active in E. coli). Finally, expression of RecACeco in the E. coli ΔrecA
strain complements SOS induction alleviation by PsiB. Altogether, these data suggest that PsiB is functional only in bacterial species where its carrier plasmids normally reside (here E. coli), thus antagonising RecA in a species-specific manner.

On the other hand, we showed that the RIP113 plasmid isolated from Salmonella, an enterobacterium, also carries the psiB gene and behaves like R64drd and R100-1 in inhibiting SOS in E. coli but not in V. cholerae. Unlike these two plasmids, RIP113 replicates in V. cholerae but since it was isolated in Salmonella, and to our
knowledge IncN plasmids have not been described in V. cholerae so far, we considered that V. cholerae is not one of its usual hosts. To our
knowledge PsiB is present only in narrow host range plasmids. We conclude that PsiB functions in a species-specific manner.

Conjugation induces the integron integrase

It was recently shown that the integron integrase is regulated by the SOS response [31]. We showed above that conjugal DNA transfer induces SOS. We then addressed whether conjugation affects V. cholerae IntIA SI integrase expression levels. To do this, we constructed a V. cholerae reporter strain containing a translational fusion between intI and gfp (7093::p4640), and used flow cytometry to determine the fraction of cells where the integrase-GFP fusion was induced. As expected, no induction was observed in the ΔrecA control strain (Figure 3). In the recA+ strain we observe no induction after conjugation with RP4 and R6Kdrd (Figure 3). Alternatively, the integrase expression increased 2.8 fold when the strain is conjugated with R388, and 5.3 and 6.2 fold with R6Kdrd and R100-1, respectively. In β-gal SOS induction tests shown earlier, RP4 and R6Kdrd also yielded a lower induction in total population graphs (Figure 1B). Note that β-gal induction reflects the recN promoter, which is more strongly expressed than the intI promoter. Our results imply that the SOS induction during RP4/R6Kdrd conjugation may not reach sufficiently high levels to induce the integrase reporter used in flow cytometry experiments.

Finally we tested mating of E. coli carrying an SXT element with V. cholerae. SXT transfer is induced through induction of SOS when the donor is treated with MMC [37]. Transfer of the SXT element into V. cholerae increased intI1 promoter activity 12 fold compared to a plasmidless control and was 2 fold higher than uninduced cells (i.e. without MMC treatment of donor).

Conjugation triggers IntI1 integrase-dependent recombination

We have shown that conjugation induces SOS in the recipient bacteria and flow cytometry analysis clearly shows that the

![Figure 2. PsiB alleviates SOS induction in E. coli but not in V. cholerae because of impaired interaction with RecA]
Integron integrase is induced during conjugation in *V. cholerae*. In a first set of experiments, we wanted to test if the SOS induction leads to a higher activity of the integron integrase in *E. coli*, using the class 1 integrase IntI1. We developed an experimental strategy in an *E. coli* strain that contains an insertion in the *dapA* gene (7949). This strain is unable to synthesize DAP (2,6-diaminopimelic acid), and as a result is not viable without DAP supplemented in the medium. The insertion in *dapA* is flanked by two specific recombination sites, *attI* and *attC*. Integrase expression causes site-specific recombination and excision of the synthetic cassette, restoring a functional *dapA* gene and allowing the strain to grow on DAP-free medium (Figure 4A). We transformed in this *dapA*- strain a multi-copy plasmid (p7755) carrying the *intI1* gene under the control of its natural SOS regulated promoter. The recombination rate due to integrase expression is calculated as the ratio of the number of cells growing in the absence of DAP over the total number of cells. Figure 4B shows the cassette excision rate in *E. coli* 7949 p7755 after conjugation with different conjugative plasmids. In the absence of a conjugative plasmid in the donor cell, the spontaneous excision rate is about 10−3, which reflects the stringency of the *intI1* promoter. Conjugation with R6K*drd* and R388 increases excision rate to 10−3 and 10−2 respectively, whereas conjugation with R64*drd* does not increase significantly beyond the basal recombination level. RP4 yields an intermediate level of DAP+ cells, which is compatible with its intermediate SOS induction level in *E. coli*. These results are consistent with SOS induction results in *E. coli*, and as expected, there is a correlation between SOS induction and integrase induced cassette recombination. To confirm that cassette recombination is due to integrase expression, we performed the same experiment in strain 7949 lacking the integrase carrying plasmid p7755, and no cassette excision was observed (<10−6). We conclude that conjugation with *psiB* deficient plasmids in *E. coli* induces the expression of the integrase from the *intI1* promoter, and thus triggers cassette recombination.

Conjugation Induces SOS and Cassette Recombination

Figure 3. Conjugation induces *V. cholerae* integron integrase *intI1* expression. Donor and recipient (7093:p4640 recA+ and ΔrecA) strains were grown until OD 0.2, mixed at a 1:1 ratio and incubated overnight on filter. % of GFP-induced cells was measured by flow cytometry. doi:10.1371/journal.pgen.1001165.g003

Conjugation increases *intI1*-dependent cassette excision rate in *E. coli*. A: experimental setup. 7949 strain contains plasmid p7755 carrying *intI1* under the control of its natural LexA-regulated promoter. B: cassette excision rate was calculated by counting recombinated *cfu* (Dap+) over total *cfu*. “No plasmid” means that recipient 7949 p7755 was mixed with empty donor. “No integrase” means that recipient 7949 without p7755 was conjugated with donor containing a conjugaive plasmid. doi:10.1371/journal.pgen.1001165.g004

Conjugation triggers *IntI1*–mediated cassette recombination in the *V. cholerae* superintegron

In the cassette excision experiment described above, we used a multicopy plasmid expressing the *intI1* integrase in *E. coli*. Since conjugation induces the SOS response and in turn expression of the integron integrase in *V. cholerae*, we addressed in a second set of experiments whether conjugation in wild type *V. cholerae* can trigger recombination events in the superintegron. The *V. cholerae* SI carries a promoterless *catB* cassette that is not expressed in *V. cholerae* laboratory strain N16961 because it is located 7 cassettes (approximately 5000 bp) downstream of the Pc promoter [44]. When expressed, the *catB* gene confers resistance to chloramphenicol (Cm). We tested if conjugation can spontaneously yield Cm-resistant (Cm-R) *V. cholerae* cells, i.e. if *IntI1* is induced and recombines the *catB* cassette to a location allowing its expression (Figure 5A).

Our results show that when the donor strain does not carry any conjugative plasmid, the rate of CmR cells is about 7.10−11 (Figure 5B). Consistent with the *intI1* induction results, conjugation with RP4 and R6K*drd* did not increase this frequency (6.10−11). Conjugation with R388, R64*drd* and R100-1 increased the CmR *cfu* appearance rate 28 fold, 280 fold and 140 fold, respectively. To verify that this increase was dependent on SOS,
Conjugative plasmids yielded a rate of CmR lower than 10^{-11}.

To determine if these events corresponded to IntIA mediated cassette rearrangement, we performed a PCR analysis with primers in the Pc promoter and at the beginning of the catB cassette. In the wild type strain, this PCR amplifies a band of about 5000 bp. In the CmR colonies, the PCR amplifies a band of 1432 bp (Figure S2A). Sequencing confirmed that the cassette was now moved to 2^{nd} position on the SI.

In order to determine if cassettes between the Pc promoter and catB gene were deleted after rearrangement – i.e. if catB moved because cassettes were deleted or because it was re-integrated – we performed PCR analysis with several oligonucleotides amplifying cassettes located between attI and catB in V. cholerae N16961. We found that these cassettes were still present in the genome of the Cm-resistant clones (data not shown), showing that they were not deleted, and indicating that catB was relocated by recombination events.

Discussion

We showed that conjugation of RP4, R6Kдрд, R388 and Rs-a plasmids, that do not carry any anti-SOS function, induces the SOS response in recipient E. coli and V. cholerae cells. Alternatively, plasmids R6Kдрд, R100-1 and RIP113 that do carry the anti-SOS ψΔ4 gene do not induce SOS when the recipient cell is E. coli, while the SOS response is induced in V. cholerae. Finally, the SXT element (here integrated in the E. coli chromosome) is also able to induce SOS when it transfers to recipient V. cholerae.

Induction of SOS during conjugation

It has been shown that during inter-species Hfr conjugation, SOS is induced in the host cell [46,47]. It was proposed that the low level of homology prevents rapid recombination of incoming DNA into the chromosome and thus dramatically enhances the SOS induction. This suggests that SOS induction levels may reflect the ability of RecA to find homologous DNA and initiate strand exchange [48]. In the case of plasmid conjugation, there is no homology with the bacterial chromosome, explaining the very high SOS induction levels we observe. Moreover, SOS induction proceeds as a wave. At the early stages of SOS induction, the concentration of LexA decreases as it is self-cleaved, then LexA synthesis is induced at later stages, and if ssDNA does not persist, the SOS induction level gradually decreases. This is what happens when RP4, R388 and R6Kдрд conjugate into E. coli cells. Alternatively, R388 and Rs-a conjugate into V. cholerae, and explains the bell-shaped induction curves we obtained. After 1h, nearly all the recipient cells are conjugants and no new conjugation is initiated because of plasmidic entry exclusion systems [49]. As conjugation or establishment rates are very low for R388 and R6Kдрд in V. cholerae, plasmidless host cells are always present in the total population so we observe a plateau reflecting new rounds of conjugation during the course of the experiment. The fact that R6Kдрд and R100-1 are the strongest inducers in V. cholerae could thus be due to the inability of these plasmids to synthesize the complementary DNA strand and establish themselves in V. cholerae, increasing the prevalence of ssDNA accessible to RecA binding. Moreover, this strongly suggests that abortive conjugation induces SOS, which explains the fact that we are able to detect SOS induction in the total population despite the lack of transconjugants in V. cholerae. This is consistent with data showing very high induction values when we
calculate the SOS induction in conjugants only. The plateau of induction in the whole population points to a "permanent conjugation state" where ssDNA enters the host cell, induces SOS, but does not replicated, and a new round of conjugation begins.

Another interesting observation is that SOS induction does not seem to prevent conjugation. Indeed, the conjugation rate for all plasmids (in E. coli for instance) is approximately the same after 2h of mating even though they induce SOS differently at the beginning of mating. To test this point, we used recipient cells already induced for SOS by pre-incubation with MMC, and these cells yielded the same conjugation rates in E. coli for a given plasmid regardless of the SOS induction level (Figure S1).

Narrow host range plasmids inhibit the SOS response in their natural host

SOS induction is due to RecA binding to ssDNA. We have shown above that plasmids R6Kdrd, R100-1 and RIP113 that carry the anti-SOS pslB gene do not induce SOS when the recipient cell is E. coli. PsiB has been shown to interact with RecA in vitro [43] and in vivo (Figure 2), preventing it from binding to ssDNA and inducing the SOS response. Even though RecA_Vch and RecA_Vco show 79% protein identity, our data suggests that PsiB is impaired in its interaction with RecA_Vch in vitro (Figure 2), explaining why PsiB does not strongly reduce the SOS induction in V. cholerae as it does in E. coli. The presence of the pslB anti-SOS function in narrow host range plasmids such as R64 and R100 suggests that the dissemination strategies of narrow and broad host range plasmids could be distinct. Induction of the SOS response can be potentially detrimental to the host cell because of the induction of mutagenic polymerases or cell division arrest (like E. coli fcfb) [50]. Thus, it is tempting to speculate that narrow host range plasmids use their anti-SOS gene as a furtive strategy to hide from their customary host and thus prevent the host cell from being stressed and change its own or the incoming plasmid DNA. Note that by narrow host range plasmids, we mean plasmids that only replicate in a restricted number of bacteria (such as R64) but also plasmids that are found only in a few kinds of hosts in nature, even though they are able to replicate in others, such as the RIP113 originally in Salmonella.

SOS induction during conjugation leads to chromosome rearrangements

One consequence of SOS induction during conjugative DNA transfer is the triggering of integron cassette recombination. Conjugation with strong SOS inducer plasmids R388 and R6Kdrd in E. coli increases expression of IntI1 from its SOS regulated natural promoter leading to an increased RecA-dependent cassette excision rate, whereas plasmids R6Kdrd and R100-1 that do not induce SOS in E. coli do not trigger cassette recombination in our E. coli cassette recombination assay. These results highlight the existence of a link between conjugation and site-specific recombination, leading to genome evolution. We also showed that conjugation triggers cassette recombination in the natural context of the SI carried in wild type V. cholerae. Plasmids R388, R6Kdrd and R100-1 strongly induce SOS (and intI1) in V. cholerae and significantly increases the cassette recombination rate.

Our results highlight the link between conjugative HGT and genome evolvability in V. cholerae. Since conjugation induces integrase activity, one can consider conjugative plasmids as both vehicles for cassette dissemination and cassette shuffling for those already present in the SI. Indeed, some plasmids such as R388 [51] and R64 [32] carry an RI platform that can acquire new cassettes and transmit them to a new host by conjugation. It was shown that R388 can incorporate the catB cassette from the V. cholerae SI and transfer it to other bacteria [44]. Here we observed the displacement of a cat cassette catalyzed by the V. cholerae IntI1 in its natural context. Conjugation can thus bring new cassettes but also favour their integration into the host chromosomal integron by inducing SOS.

Conjugation, SOS, and genome evolution

Cassette recombination upon conjugation could be a widespread mechanism, since conjugation is a naturally occurring phenomenon in highly concentrated bacterial environments, such as the host intestinal tract (see for example [17–19,21]), biofilms [33–55] forming in the aquatic environment where V. cholerae grows, or even on medical equipment in hospitals [56]. Moreover, no mutation has been found in the bacterial chromosome that can prevent the uptake of conjugative DNA, meaning that bacteria cannot avoid being used as recipient cells [57]. By inducing the SOS response, incoming DNA triggers its own recombination not only through integrase induction but also homologous recombination, promoting genomic rearrangements.

Another important effect of SOS induction is the derepression of genes implicated in the transfer of integrating conjugative elements (ICEs), such as SXT from V. cholerae, which is a ~100 kb ICE that transfers and integrates into the recipient bacterial genome, conferring resistance to several antibiotics [37]. Moreover, different ICEs are able to combine and create their own diversity in a RecA-dependent manner via homologous recombination [35,36], and also, as observed here for SXT transfer in V. cholerae, by inducing SOS following transfer. Thus, SOS induction leads to genetic diversification of these mobile elements and to their transfer to surrounding bacteria, spreading antibiotic resistance genes, among others.

Conjugation induced SOS is thus one of the mechanisms allowing bacteria to evolve in their natural niches, creating the diversity that allows them to adapt to new environments and survive. Under conditions where SOS is prevented (by bacterial means such as the PsiB system or exogenously), cassette recombination is decreased to experimentally undetectable levels, showing that SOS induction plays an important role in adaptation, and can be used by broad host range plasmids to adapt to a new host. Consistently, narrow host range plasmids that do not need to adapt to a new host, express an SOS inhibitor to maintain the integrity of the plasmid DNA and host genome. This connection between host range and SOS induction needs to be expanded to a larger range of plasmids to determine its general character. A significant association between laterally transferred genes and gene rearrangements was already suggested in [58], which is consistent with our data, when we consider that SOS induction plays a major role in gene rearrangements. Remarkably, induction of SOS considerably enhances genome duplications and mutagenesis [59]. Further work is needed to test whether other HGT mechanisms, such as transformation, also induces SOS; considering that many bacterial species, like V. cholerae for instance, are naturally competent [60]. It would be interesting to investigate if SOS is induced in the gut of the host animal. If this were the case, inhibiting the bacterial SOS response would become an ideal target to prevent the acquisition of antibiotic resistance genes, and could be used in combination with antibiotics for the treatment of infections.

Materials and Methods

For strain and plasmid constructions, and oligonucleotide list, see Text S1 and Tables S1 and S2.
Conjugation and β-galactosidase tests

Overnight cultures of donor and recipient cells were diluted 100× in LB and grown until OD~0.5. Donor and recipient cells were then mixed in 1:1 ratio on 0.045 mm conjugation filters on LB plates preheated at 37°C. At each time point, a filter was resuspended in 3ml LB and dilutions were plated on selective plates to count (i) conjugants and (ii) total number of recipients. For details, see Text S1.

β-gal tests were performed on these cultures as described ([61] and Text S1). According to the Miller formula:

\[
\text{specific activity for total population} = \frac{\text{measured units at } t_x}{\text{nb of recipient}}
\]

and

\[
\text{specific activity per conjugant} = \frac{\text{measured units at } t_x - (\text{units at } t_0 \times \text{nb of recipients at } t_0)}{\text{nb of conjugants at } t_x}
\]

where \(\text{units at } t_0 \times \text{nb of recipients at } t_0\) is the basal expression per cell when SOS is not induced. For details, see Text S1.

SOS induction tests using MMC were performed as published [31].

Flow cytometry

The same conjugation assay was performed overnight for the flow cytometry experiments. For each experiment, 100000 events were counted on the FACS-Calibur device. For details, see Text S1 and Figure S3.

Cassette excision measurements

Described in the Results section. For details, see Text S1.

Cassette displacement measurements

The recipient strain was \(V.\) cholerae N16961 (Cm sensitive 5μg/ml Cm). The donor strain was DH5α or a dap- derivative (T1) for counter selection of Cm-R plasmids. Conjugations were performed as described for 4h. Filters were resuspended, centrifuged and the pellet was plated on LB medium containing 25μg/ml Cm. PCR screenings were performed using oligonucleotides cat2/i4 and the GoTaq polymerase. Oligonucleotides 896 to 905 were used to verify the presence of other cassettes.

Supporting Information

Figure S1 SOS induction does not affect conjugation rate. Recipient \(E.\) coli and \(V.\) cholerae were grown in LB containing 0.2 μg/ml MMC up to OD ~0.5. Conjugations were performed for 1h as described in the Materials and Methods. Found at: doi:10.1371/journal.pgen.1001165.s001 (0.41 MB TIF)

Figure S2 Cassette displacement after conjugation within the \(V.\) cholerae SI: A: Displacement of catB cassette within the SI after R64/ R388 conjugation. Oligonucleotides used for PCR reactions were i4/ cat2. B: Southern blot visualization of the \(V.\) cholerae SI cassette array reorganization after SOS induction. gDNA from \(V.\) cholerae N16961 and CmR derivatives were digested by AccI and probed with a mix of oligonucleotides corresponding to different cassettes. Oligonucleotides anneal to cassettes VCA0291 to VCA0295, VCA0298 to 0300, VCA0329, VCA0343, VCA0354 to VCA0356, VCA0361, VCA0364 to VCA0366 and are listed in Table S2. Found at: doi:10.1371/journal.pgen.1001165.s002 (0.13 MB TIF)

Figure S3 Example of analysis of flow cytometry data. Y axis: cell count, X axis: fluorescence. Red curve is obtained by counting \(V.\) cholerae cells with constitutive GFP expression. Black curve represents a mating mixture of plasmid free \(E.\) coli and \(V.\) cholerae carrying the inf-gfp fusion. This mixture was taken as negative reference. Cells showing a fluorescence in the M1 region (intersection point of black and red curves and further right) are considered as induced cells. Green curve is an example of the data obtained: it represents a mixture of \(E.\) coli carrying plasmid R100-1 and \(V.\) cholerae.

Found at: doi:10.1371/journal.pgen.1001165.s003 (0.05 MB TIF)

Table S1 Bacterial strains and plasmids.

Found at: doi:10.1371/journal.pgen.1001165.s004 (0.09 MB DOC)

Table S2 Oligonucleotides used in this study.

Found at: doi:10.1371/journal.pgen.1001165.s005 (0.05 MB DOC)

Text S1 Supplementary experimental procedures.

Found at: doi:10.1371/journal.pgen.1001165.s006 (0.07 MB DOC)

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Author Contributions

Conceived and designed the experiments: ZB DM. Performed the experiments: ZB. Analyzed the data: ZB DB DM. Contributed reagents/materials/analysis tools: ZB DB. Wrote the paper: ZB DM.

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