

How Xer-exploiting mobile elements overcome cellular control

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Most strains of *Neisseria gonorrhoeae* (Ng), the causative agent of the sexually transmitted disease gonorrhoea, and a few strains of *Neisseria meningitidis* (Nm), which is responsible for a large number of meningitides, harbor a 57-kb horizontally acquired genetic element, the gonococcal genomic island (GGI) (1–3). Certain versions of the GGI are associated with disseminated gonococcal infection (1, 4). In addition, the GGI encodes numerous homologs of type IV secretion system genes, which are necessary for DNA secretion and facilitate natural transformation of the *Neisseria* (1, 2, 4). GGI are found integrated at the chromosomal dimer resolution site of their host chromosome, *dif*, and are flanked by a partial repeat of it, *dif*_{GGI} (Fig. 1A) (1, 5). The *dif* site is the target of two highly conserved chromosomally encoded tyrosine recombinases, XerC and XerD, which normally serve to resolve dimers of circular chromosomes through the addition of a crossover between directly repeated *dif* sites (6). This reaction raises questions on how GGI could be stably maintained (5). The results presented by Fournes et al. (7) in PNAS shed a new light on this apparent paradox.

The Xer machinery is highly conserved in bacteria. The *dif* sites consist of 11-bp XerC- and XerD-binding motifs, separated by an overlap region at the border of which recombination occurs (Fig. 1B). Recombination is under the control of a hexameric DNA pump, FtsK (Fig. 1C) (8). FtsK is a powerful translocase (9) and strips DNA from most proteins (10). However, a direct interaction between its extreme C-terminal domain, FtsKy, and the Xer recombinases stops it (Fig. 1C) (11, 12) and activates the exchange of a pair of strands by XerD catalysis when in the presence of a synaptic complex (Fig. 1C) (8, 11, 13). The exchange of a second pair of strands by XerC catalysis converts the resulting Holliday junction into product (Fig. 1C) (8, 13). FtsK belongs to the cell division machinery. It assembles at midcell when most of the chromosomal DNA has been replicated and segregated, which restricts recombination at *dif* to the time of cell division (14, 15) and to the chromosome replication terminus region (16, 17).

Numerous mobile elements have been shown to exploit Xer recombination. Plasmids use it for the

resolution of multimers, the formation of which compromises vertical transmission from mother to daughters by reducing the number of independently segregating plasmid units (18). Integrating mobile element exploiting Xer (IMEX) use it to insert into the *dif* site of one of the chromosomes of their host (19). In both cases, the FtsK control imposed on Xer recombination must be overcome, because the replication/segregation cycle of plasmids and the integration/excision cycle of IMEX should be independent from the cell cycle. Moreover, Xer recombination leads to the formation of plasmid multimers when they harbor a *dif* site (17, 20) and to the excision of the intervening DNA between directly repeated *dif* sites (17, 21). Correspondingly, the central region of plasmid sites seems to prevent FtsK-dependent XerD catalysis (Fig. 1B) (22), and the central region of the attachment sites of most IMEX lacks the necessary homology to stabilize XerD-mediated strand exchanges with *dif* (Fig. 1B) (23, 24). This is not the case for the central region of the different alleles of *dif*_{GGI} (Fig. 1D). The problem was most striking for the most common of these alleles, *dif*_{GGI1}, which differs from the neisserial *dif* by only 4 bp (Fig. 1D).

In PNAS, Fournes et al. (7) observe that the Ng Xer recombinases efficiently bound to *dif*_{GGI1}, synapsed it with *dif*_{Ng}, and catalyzed complete recombination reactions between the two sites when activated by Ng FtsKy. However, they noticed that recombination was reduced in the presence of the FtsK translocation module. The authors smartly hypothesize that FtsK translocation inhibited recombination by stripping Ng XerD from *dif*_{GGI1}, which they successfully verified in vitro.

It was previously suggested that GGI initially harbored true neisserial *dif* sites and that their stabilization resulted from mutations that occurred after their integration (5). Many different types of mutations, including mutations in the central region of the *dif* sites and mutations abolishing the binding of the recombinases to them, could impede Xer recombination. Why, then, should *dif*_{GGI1} harbor mutations that blocked FtsK-dependent recombination without affecting XerC and XerD binding and synapse formation? One of the *dif*_{GGI} alleles found in Nm strains, *attP*_{GGI2}, harbors

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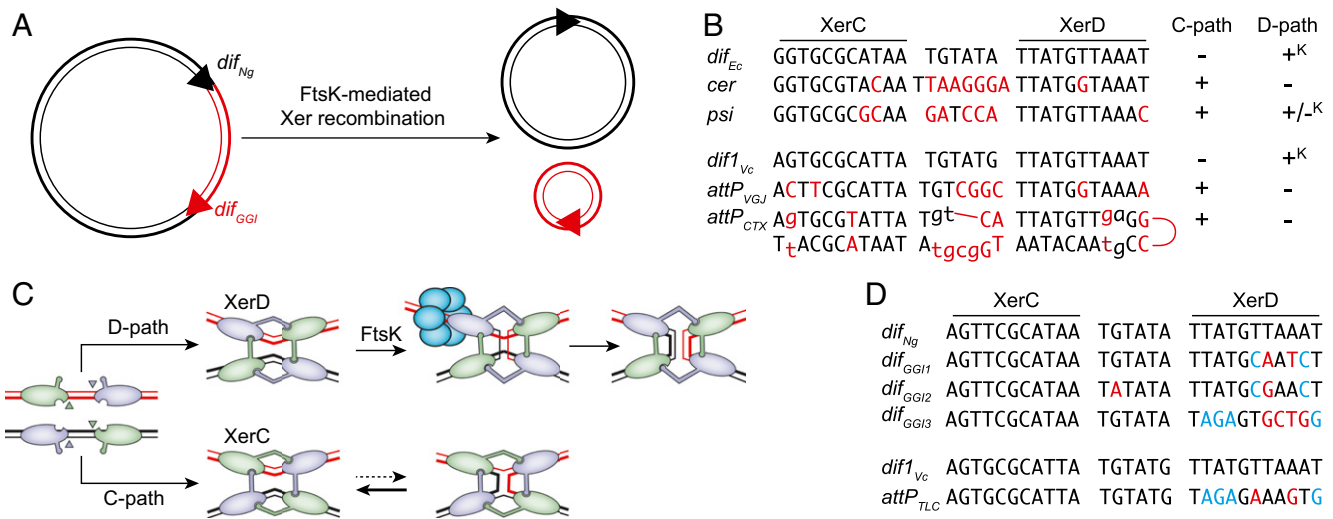


Fig. 1. (A) Schematic of XerCD-mediated excision of the GGI. Black double lines, *N. gonorrhoeae* chromosomal DNA; red double lines, GGI DNA; black triangles, *dif_{Ng}* and red triangle, *dif_{GGI}*. **(B)** Sequence alignment of the Xer recombination site of mobile elements and their cognate partner chromosomal *dif* site; *attP_{CTX}*, CTX ϕ attachment site; *attP_{VGJ}*, VGJ ϕ attachment site; *cer*, core dimer resolution site of plasmid ColE1; *dif_{Ec}*, *E. coli dif*; *dif1_{Vc}*, *V. cholerae* chr1 *dif*; and *psi*, core dimer resolution site of plasmid pSC101. Apart from *attP_{CTX}*, which is the stem of a forked hairpin from the single-stranded form of the genome of CTX ϕ , a single of the two DNA strands is represented in the 5' to 3' orientation from left to right. Bases of *cer* and *psi* that differ from *dif_{Ec}* and bases from *attP_{CTX}* and *attP_{VGJ}* that differ from *dif1_{Vc}* are indicated in red. Plus (+) and minus (-) signs indicate whether the sites can engage in recombination pathways initiated by XerC or XerD strand exchanges; +^K denotes FtsK-dependent recombination pathways. **(C)** Schematic of Xer recombination. XerD and XerC are represented in magenta and green, respectively. Following the Cre paradigm, the active pair of recombinases are drawn with their extreme C-terminal domains contacting the partner recombinases in *cis*. Blue circles represent the hexamer of FtsK. **(D)** Sequence alignment of *dif_{Ng}*, the three different types of *dif_{GGI}*, *dif1_{Vc}* and *attP_{TLC}*. Bases of the *dif*-like site of mobile elements that differ from their cognate *dif* partner are highlighted in color, with blue highlighting those that are identical in *dif_{GGI1}* and *dif_{GGI2}* and in *attP_{GGI3}* and *attP_{TLC}*.

two out of four of the bases that differentiate *dif_{GGI1}* from *dif_{Ng}*, which suggests that these changes were not randomly picked up (Fig. 1D, blue bases of *dif_{GGI1}* and *dif_{GGI2}*). Indeed, it is striking to note that *dif_{GGI2}* is fully palindromic and carries two XerC-binding arms (Fig. 1D). In contrast, 8 out of 11 of the bases of the XerD-binding arm of *dif_{GGI3}* differentiate it from the XerD arm of *dif* sites (Fig. 1D). The attachment site of a *V. cholerae* IMEX, the toxin-linked cryptic phage (TLC ϕ) harbors four of these bases (Fig. 1D, blue bases of *dif_{GGI3}* and *attP_{TLC}*) (25). We previously demonstrated that XerD poorly bound to *attP_{TLC}*, which is sufficient to prevent XerD-mediated FtsK-dependent recombination (25). Thus, it is tempting to propose that GGI are IMEX and *dif_{GGI}* sites were selected not only to escape but also to overcome the normal cellular control imposed on Xer recombination by FtsK. GGI harboring *dif_{GGI3}* probably belong to the TLC ϕ class of

IMEX, which integrate into and excise from the genome of their host via a XerD-first FtsK-independent recombination pathway (25). GGI harboring *dif_{GGI1}* and *dif_{GGI2}* probably define a new class of IMEX. Future work will need to address the Xer recombination pathway they exploit and if they can truly integrate independently of FtsK. In addition, it will be interesting to determine which factors encoded in the genome of GGI IMEX and/or in the genome of their host help them overcome the cellular control that is normally imposed on Xer recombination, as observed for plasmids (18) and the CTX ϕ class of IMEX (26).

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