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Abstract

DNA repair is crucial to the survival of all organisms. The bacterial RecA protein is a central component in the SOS response and in recombinational and SOS DNA repairs. The RecX protein has been characterized as a negative modulator of RecA activity in many bacteria. The *recA* and *recX* genes of *Herbaspirillum seropedicae* constitute a single operon, and evidence suggests that RecX participates in SOS repair. In the present study, we show that the *H. seropedicae* RecX protein (RecXₖs) can interact with the *H. seropedicae* RecA protein (RecAₖs) and that RecAₖs possesses ATP binding, ATP hydrolyzing and DNA strand exchange activities. RecXₖs inhibited 90% of the RecAₖs DNA strand exchange activity even when present in a 50-fold lower molar concentration than RecAₖs. RecAₖs ATP binding was not affected by the addition of RecX, but the ATPase activity was reduced. When RecXₖs was present before the formation of RecA filaments (RecA-ssDNA), inhibition of ATPase activity was substantially reduced and excess ssDNA also partially suppressed this inhibition. The results suggest that the RecXₖs protein negatively modulates the RecAₖs activities by protein-protein interactions and also by DNA-protein interactions.

Key words: RecA; RecX; *Herbaspirillum seropedicae*; SOS repair

Introduction

Bacterial genomic DNA is constantly subject to chemical and physical damage. The repair of such damages is essential since DNA is the template for its own duplication as well as the production of diverse types of RNA and proteins (1). Specialized enzymatic complexes are designed to avoid genetic alterations or to reduce their frequency (2).

RecA is a key enzyme in the DNA repair process in bacteria. RecA promotes the expression of proteins involved in the non-mutagenic and mutagenic DNA repair by inducing LexA repressor autocleavage (3) and promotes recombinational DNA repair by catalyzing the DNA strand exchange reaction (4). In addition, RecA-ATP binds to the UmuD₂C complex to form the active PolV polymerase responsible for the DNA translesion synthesis or SOS mutagenesis (5).

The RecA protein is highly conserved among bacteria, and homologues of RecA are also found in bacteriophages, Archaea and Eucarya (4). RecA activity is induced upon binding to single-stranded DNA (ssDNA), a process named nucleation, followed by rapid 5’ to 3’ extension, in such a way that additional RecA monomers are only added at the 3’ filament end (6). Disassembly of RecA filaments is also unidirectional (5’ to 3’) and end-dependent, occurring on the end opposite to that where filament extension occurs (6-8).

To mediate a broad spectrum of biological events the RecA protein needs to be bound to the DNA and also requires the participation of other proteins that interact with RecA and/or modulate RecA activity (RecFOR, RecBCD, SSB, LexA, UmuD, DinI, RdgC, PsiB, and RecX proteins) (9-15).

The RecX protein has attracted much interest because the *recX* gene is located downstream from *recA*, forming a single operon in many bacteria (16-21). Knock-out mutants
of recX caused a great variety of phenotypes associated with RecA functions (17-26). Protein analyses revealed that RecX interacts directly with the RecA protein and that RecX negatively modulates the recombinase, ATPase, and co-protease activities of RecA in Mycobacterium tuberculosis (27), Escherichia coli (25) and Deinococcus radiodurans (26). These inhibitory activities are performed by block- ing RecA filament extension (28,29). In vivo studies in Neisseria gonorrhoeae (24) and Xanthomonas oryzae pv. oryzae (18) suggested an alternative positive modulation of RecA by RecX.

In Herbaspirillum seropedicae the recAX genes constitute a single operon. Physiological analyses of the mutant recX of H. seropedicae have suggested the participation of the RecX protein in the SOS response and homologous recombination (21), indicating involvement of the RecX protein in the modulation of RecA activity. In this report, we demonstrate that the H. seropedicae RecX (RecXHis) interacts with the homologous RecA protein (RecAHis) and inhibits RecA recombinase and ATPase activities but not ATP binding activity. These results, together with previous reports, which showed that RecX is capable of forming DNA-protein complexes, suggest that the mechanism of RecA modulation by RecX might involve not only protein-protein interactions but also DNA-RecX interactions.

Material and Methods

Plasmid construction

The fragment Ndel/Xhol containing the recX gene of H. seropedicae from the plasmid pCWG3 (30) was cloned into the Ndel/SalI digested vector pT7-7 (New England Biolabs, USA), yielding the plasmid pXT7. The recA gene of H. seropedicae was amplified using the plasmid pBMR503 (31) as the template together with the primers recA1 (5'-GAGAGAG ACATATG GCGAGA-3') and recA2 (5'-AGGGAGCGGATCC TCAGGAGGCCTCG-3'). The Ndel and BamHI sites (underlined) were used to clone the 1.1-kbp PCR fragment into the Ndel/BamHI-digested vector pET28b+HMK (constructed and donated by P. Bordes, Université de Toulouse, France), yielding the plasmid pAEWT-HTMK. The amino-terminal hexaHis sequence of the vector was deleted by digesting with NdeI and XbaI enzymes, producing the plasmid pCWG9. The His-tagged RecX (RecXHis), native RecX, aminointerminal His-tagged RecA (RecAHis) and the native RecA proteins were overexpressed from the plasmids pCWG3, pXT7, pAEWT-HTMK, and pCWG9, respectively.

Protein expression

E. coli strains BL21 (DE3) pLyS or B834 (DE3) (Merck KGaA, Germany) containing the correct plasmid were grown in Terrific Broth medium (32) on a rotary shaker to an OD_{600} of 0.3 at 37°C. At this point, the culture was maintained at 25°C for 30 min before adding 1 mM isopropyl-β-D-thiogalactopyranoside and incubated for a further 3-4 h. Cells were harvested by centrifugation and stored at -20°C. All subsequent purification steps were at 4°C.

Native RecA protein purification

The native RecA protein was purified as described by Steffen and Bryant (33) with a few modifications. Briefly, the soluble protein extract was treated with polymyxin-P, followed by ammonium sulfate (four times, at 5, 34, 54, or 58% saturation). The final sample was dialyzed against buffer A (10 mM Tris-HCl pH 8.0, 50 mM NaCl, and 1 mM DTT) containing 10% glycerol and then loaded onto a DEAE sepharose column (28 mL, 1.6 x 21 cm, GE Healthcare, USA). After being submitted to an NaCl gradient (0.05-1 M) in buffer A containing 10% glycerol, the fractions containing the native RecA were dialyzed and stored at -80°C.

Native RecX protein purification

The frozen cells were thawed on ice, suspended in 25 mL buffer A containing 10% glycerol and 1 mM phenylmethylsulfonylfluoride and lysed by sonication (Ultrasonic Processor XL, Qsonica, LLC, USA) on ice. The soluble protein extract was loaded onto an SP-sepharose column (50 mL, 1.6 x 25.5 cm, GE Healthcare). After being submitted to an NaCl gradient (0.05-1 M) in buffer A containing 10% glycerol the fractions containing the native RecX were dialyzed and stored at -80°C.

His-tagged RecA and RecX proteins purification

The frozen cells were thawed on ice and lysed in a cell disruptor (Constant Systems Ltd., UK) in 25-50 mL buffer A (25 mM NaH_2PO_4 pH 7.0, 5% glycerol, and 0.5 M NaCl) containing a cocktail tablet of protease inhibitors (F. Hoffmann-La Roche Ltd., Switzerland, Cat. No. 11836153001). The soluble protein extract was loaded onto a 5 mL Hi-Trap Chelating column (GE Healthcare) charged as indicated by the manufacturer. After being submitted to an imidazole gradient (0.04-0.8 M) in buffer A, the fractions containing the RecAHis or RecXHis protein were dialyzed and stored at -80°C.

Ni^{2+} bead immobilization

RecXHis (~5 µM-8.4 µg) or DraGHis (~2.5 µM-8.4 µg) was immobilized on Sepharose-chelating FF beads (Ni^{2+} beads, 15 µL) following manufacturer instructions (GE Healthcare). After centrifugation (500 g, 5 min) and washing the pellet with buffer A containing 5% glycerol, 2.5 µM (8.4 µg) native RecA was added. The system was then incubated for a further 30 min, centrifuged (500 g, 5 min) and the pellet was washed with buffer A containing 5% glycerol. The immobilized proteins were eluted by the addition of buffer A containing 5% glycerol and 0.5 M imidazole. An aliquot from each fraction was analyzed by SDS-PAGE and the protein content visualized by staining with Coomassie blue.
DNA strand exchange assay

The DNA strand assay was adapted from Drees et al. (28). The RecA protein (6.7 µM) was pre-incubated with 22.6 µM φX174 circular ssDNA (New England Biolabs) and an ATP-regenerating system [10 U/mL pyruvate kinase (Sigma-Aldrich, USA) and 5 mmol phosphoenolpyruvate (Sigma-Aldrich)] for 10 min in 20 µL buffer E (25 mM Tris-acetate pH 7.5, 1 mM DTT, 5% (w/v) glycerol, 3 mM potassium glutamate, and 10 mM magnesium acetate) at 37°C. SSB protein (2 µM) and ATP (3 mM) were then added, followed by another 6 min of incubation. The RecX protein was added at the concentrations given in the figure legends, incubated for 6 min before the addition of 15.6 µM φX174 linear double-stranded DNA (dsDNA) and incubated for a further 90 min before stopping the reaction by adding 5 µL equilibrated phenol and 3 µL 10% SDS to each reaction. Samples were electrophoresed on 0.8% agarose gels in TAE buffer, stained with ethydium bromide, and exposed to ultraviolet light. The E. coli RecA (RecAEC, GE Healthcare) was used as control.

ATP binding assay

ATP binding assays by UV crosslinking were conducted essentially as described by Schumacher et al. (34) with some modifications. Standard reaction mixtures (20 µL) containing ATP binding buffer (20 mM Tris-HCl pH 7.5, 20 mM potassium chloride and 10 mM magnesium chloride), RecAHs (2.5 µM) and/or RecXHs (2.5 or 10 µM), 6.5 µM φX174 circular ssDNA and 37 kBq [α32P]-ATP (111 TBq/ mmol) were placed on a glass plate, which was covered with a plastic film (Parafilm™) and maintained on ice. The reactions were then irradiated with a UV lamp (254 nm, UVG-54; UVP, USA) at a distance of 3 cm for 20 min and quenched by the addition of 3 µL 6X SDS-PAGE loading dye. After heating at 95°C for 3 min, the samples were analyzed on SDS-PAGE gels (12.5% (35)). Gels were stained with Coomassie blue to detect the proteins and, after drying, the cross-linked products were visualized with a FLA-5000 Phosphor Imager and the images were analyzed using the AIDA densitometry program.

ATPase assay

ATPase assays were performed in ATPase buffer (25 mM Tris-acetate pH 7.5, 1 mM DTT, 3 mM potassium glutamate, 10 mM magnesium acetate and 5% (w/v) glycerol), with φX174 circular ssDNA (44 µM, unless otherwise indicated), RecA protein (1.2 µM), RecX protein (5 µM, unless otherwise indicated), SSB protein (0.2 µM), and labeled ATP (0.4 mM). The components of each reaction system (20 µL) are described in the legends to the figures. Reactions were started by the addition of the SSB protein and labeled ATP, which was prepared by mixing 1.1 MBq [α32P]-ATP (111 TBq/mmol), 50 µL cold ATP (4 mM) and 97 µL ultrapure water. Reactions were stopped at different times (see figure legends for details) by the addition of 10 µL 2 M cold formic acid to an aliquot (2 µL) of the reaction and kept on ice. Aliquots (1 or 2 µL) of each reaction were analyzed by thin layer chromatography as described by Bochner and Ames (36). The amounts of labeled ATP and ADP present in each reaction were determined after imaging with a FLA-5000 Phosphor Imager and analyzed as before.

Results

Interaction of H. seropedicae RecA and RecX proteins

A bead immobilization assay was carried out to determine whether the RecA and RecX proteins of H. seropedicae could interact. Co-elution of tethered RecXHs and native RecA from Sepharose beads indicated interaction of the two proteins (Figure 1A). In contrast, when the DraGHis protein of Azospirillum brasilense was bound to the Sepharose beads it failed to bind RecA (Figure 1B). Native RecA was unable to bind to the beads per se. These results suggest a specific binding of RecX to RecA in vitro.

RecX prevents strand exchange promoted by RecA

Since RecXHs protein was shown to bind high and low molecular weight DNA molecules (30; data not shown), we examined the effect of RecX on RecA DNA strand exchange activity. In this reaction, the circular ssDNA (S1) and the homologous linear dsDNA (S2) are recombined to form a nicked circular dsDNA (P) and a linear ssDNA as products. The SSB was included in the reaction to melt out the secondary structure in the circular ssDNA substrate and to stabilize the linear ssDNA product. The RecAHs was able to promote strand exchange (Figure 2A, lane 1). The addition of 0.05 µM RecXHs protein to the RecAHs reaction resulted in a substantial decrease of product formation (Figure 2A, lane 2) and the reaction was abolished with 2 µM RecXHs (Figure 2A, lane 5). The RecXHs protein also inhibited the RecAEC-mediated DNA strand exchange (Figure 2B). The inhibition of the RecA DNA strand exchange activity by stoichiometric RecX concentrations suggests that RecX does not have to interact with every RecA monomer to exert its effect.

RecX does not change RecA ATP binding activity

To test whether RecX influences the binding of ATP to RecA, [32P]-labeled ATP was incubated with RecAHs in the presence or the absence of RecXHs, and the bound [32P]-labeled ATP was subsequently cross-linked to RecAHs. The extent of the ATP crosslinking to RecAHs was not altered by incubation with RecXHs at concentrations of 2.5 or 10 µM (Figure 2C), suggesting that ATP binding is not affected by the protein interaction. At high RecXHs concentration (10 µM; Figure 2C, lanes 5 and 7), a shorter and weaker labeled band migrating close to the RecXHs molecular weight was observed, suggesting that RecXHs has a low affinity ATP binding activity or nonspecific ATP binding. Cold ADP was
added to the reactions as an ATP competitor.

RecX inhibits ATP hydrolysis by RecA

The quantification of RecA ATPase activity was used as an indirect measure of the amount of RecA bound to DNA. In the absence of agents that affect the intrinsic RecA ATPase activity, this has proven to be a reliable measure of RecA binding (6,28,29). φX174 circular ssDNA was used as the template to form the RecA filament since the RecA protein did not possess ATPase activity in the absence of DNA or in the presence of φX174 linear dsDNA at pH 7.5 (data not shown).

ATP hydrolysis by the H. seropedicae RecA protein in the presence of ssDNA was slightly less efficient than the E. coli RecA. RecX did not show any ATPase activity (Figure 3A), but inhibited RecA ATPase activity in a concentration-dependent manner (Figure 3A): 10 µM RecX inhibited 90% of the RecA ATPase activity. The effect of the order of addition of the RecX protein on the ATPase activity of the RecA protein was also investigated. When RecX was added to the system after pre-incubation of RecA with ssDNA (RecA + ssDNA → RecX), the ATP hydrolysis rate was reduced by 35%. When RecX was incubated with RecA before the addition of ssDNA (RecA + RecX → ssDNA), the reduction was 50% (Figure 3B). The reduction was even larger (60%) when RecX was incubated together with RecA and ssDNA (RecA + RecX + ssDNA).

The pre-incubation of RecX with DNA before the addition of RecA (RecX + DNA → RecA) almost abolished ATP hydrolysis and this inhibition was partially reversed by the addition of excess ssDNA (132 µM) together with RecA (RecX + ssDNA → RecA + ssDNA) or 20 min after RecA (Figure 3C). Furthermore, reducing the RecX concentration to 0.5 µM caused reduction of the inhibition level (20%, contrasting with the 88% inhibition with 5 µM RecX).

Figure 1. The binding of Herbaspirillum seropedicae RecA to RecX. The interaction between the H. seropedicae RecA and RecX proteins was determined by a Sepharose-Ni2+ bead immobilization assay as described in Methods. The HisTagged protein immobilized on Ni2+ beads was incubated with the native protein and then eluted with 0.5 M imidazole. A, Lane M = molecular weight markers; lane 1 = native RecA (4.2 µg) and RecXHis (4.2 µg) control; lane 2 = fraction eluted with 0.5 M imidazole from RecXHis-charged Ni2+ beads after the addition of native RecA. B, Lane 1 = DraGHis (4.2 µg) and native RecA (4.2 µg); lane 2 = washing fraction from DraGHis-charged Ni2+ beads; lane 3 = washing fraction of the system DraGHis-Ni2+ beads after the addition of native RecA; lane 4 = fraction eluted with 0.5 M imidazole from DraGHis-charged Ni2+ beads after the addition of native RecA; lane 5 = native RecA (4.2 µg); lane 6 = fraction eluted with 0.5 M imidazole from native RecA-charged Ni2+ beads. The histidine-tagged proteins were immobilized on Sepharose-chelating FF beads (Ni2+ beads, 15 µL) and washed with buffer A (10 mM Tris-HCl, pH 8.0, 50 mM NaCl and 1 mM DTT) containing 5% glycerol in the presence or absence of 2.5 µM (8.4 µg) of native RecA as indicated. The immobilized proteins were eluted with buffer A containing 5% glycerol and 0.5 M imidazole.
RecX inhibits RecA ATPase activity more efficiently when RecX is pre-incubated with ssDNA, indicating that the RecX-ssDNA binding might be important in the mechanism of inhibition of the RecA filament formation.

**Discussion**

In most bacterial species studied to date, including *H. seropedicae*, recX is found downstream and co-transcribed with the recA gene (17,20,21), but exceptions to the typical recA-recX genomic organization are found including overlapping (16) or separated genes on the chromosome regulated by their own promoters (24; Table S1). A role for RecX as a negative regulator of RecA was initially hypothesized based on the observation that RecX is required to overcome the harmful effects of RecA overexpression in *Pseudomonas aeruginosa* (22), *Mycobacterium smegmatis* (23), *Streptomyces lividans* (17), *X. oryzae pv. oryzae* (18) and *H. seropedicae* (21). Such phenotype was not observed in *E. coli* and *D. radiodurans* recX mutants in which RecA overexpression was not lethal (20,26; Table S1). Contrasting phenotypes were also found in *D. radiodurans* and *X. oryzae pv. oryzae* when the recX gene was deleted, resulting in constitutive expression and repression of recA, respectively (26,18; Table S1).

In this study, we show that RecX modulates negatively the RecA recombinase (Figure 2A) and ATPase activities in vitro (Figure 3A) without affecting its ATP binding activity (Figure 2B). Analyses of the effect of RecX on RecA activities showed that a 30-fold molar excess of the *H. seropedicae* RecX protein was necessary to inhibit completely RecA ATPase activity (Figure 3A). In *E. coli*, *M. tuberculosis* and *N. gonorrhoeae*, substoichiometric concentrations of RecX were capable to completely abolish homologous RecA ATPase activity (28,27,37) but higher levels of RecX were also required to inhibit RecA from *D. radiodurans* (26; Table S1). On the other hand, *H. seropedicae* RecA DNA strand exchange activity was inhibited by RecX at
Figure 3. Effect of the RecX<sub>Hs</sub> protein concentration (A), the order of its addition (B) and the effect of ssDNA (C) on the ATP hydrolysis mediated by RecA<sub>Hs</sub>. A, The RecA<sub>Hs</sub> protein, φX174 circular ssDNA and increasing concentrations of RecX<sub>Hs</sub> protein (0 µM, filled triangles; 0.1 µm, filled squares; 0.5 µm, open squares; 2 µm, crosses; 5 µm, open triangles; 10 µM, filled circles) were incubated in ATPase buffer at 37°C for 10 min. After this period, the SSB protein and [α<sup>32</sup>P]-ATP were added. As a control without RecA, 5 µM RecX<sub>Hs</sub> was incubated with ssDNA (open lozenges). B, Two of the following components: φX174 circular ssDNA, RecA<sub>Hs</sub> and RecX<sub>Hs</sub> proteins were pre-incubated in ATPase buffer at 37°C for 10 min. After this period, the third component (RecX, ssDNA or RecA) was added together with the SSB protein and [α<sup>32</sup>P]-ATP (filled squares, filled circles, open squares). Alternatively, all three components were pre-incubated together under the same conditions, before the addition of the SSB protein and [α<sup>32</sup>P]-ATP after 10 min (crosses). As controls, RecA<sub>Hs</sub> was incubated with ssDNA in the absence of RecX (filled triangles) or incubated with RecX<sub>Hs</sub> in the absence of ssDNA (open lozenges). C, RecX<sub>Hs</sub> (0.5 or 5 µM) and φX174 circular ssDNA were pre-incubated in ATPase buffer at 37°C for 10 min. After this period, RecA alone (filled lozenges, open triangles), RecA plus ssDNA (132 µM, open squares) or RecA and then ssDNA (132 µM, added just 20 min later, as indicated by the gray arrow; filled squares) were added together with the SSB protein and [α<sup>32</sup>P]-ATP. The concentration of RecA<sub>Hs</sub> was unchanged and ssDNA<sub>1</sub> and RecX<sub>1</sub> indicate the following unusual concentrations: 132 µM ssDNA and 0.5 µM RecX, respectively. In all of the assays after the addition of [α<sup>32</sup>P]-ATP (zero time), aliquots were collected and stopped.
substoichiometric concentrations (Figure 2A), as reported for *M. tuberculosis* and *D. radiodurans* (27,26). In contrast, in *E. coli*, stoichiometric levels of RecX were required to inhibit strand DNA exchange by RecA (28; Table S1). The affinities of RecX for ssDNA and dsDNA also vary among the different organisms. While ssDNA binding of *E. coli* and *N. gonorrhoeae* RecX is observed at ssDNA concentrations lower than 1 µM (37), RecX-ssDNA binds ssDNA only at higher concentrations (≥1 µM; 30). In sharp contrast, *M. tuberculosis* RecX did not show any DNA binding activity (27; Table S1).

The negative effect of *H. seropedicae* RecX on RecA activities may be partially due to their direct interaction as shown in Figure 1A. *In vitro* studies revealed that RecX and RecA from several organisms such as *E. coli* (25), *M. tuberculosis* (27) and *D. radiodurans* (26) interact with each other, and therefore the direct binding of these two proteins is functionally relevant (Table S1). However, to further investigate the mechanism of RecX action, the ATPase activity of *H. seropedicae* RecA was studied under several conditions. The RecAΔHs protein has a low DNA-independent ATPase activity, which is not stimulated by dsDNA at pH 7.5 as it was reported for the *E. coli* RecA protein (38). Addition of ssDNA greatly stimulated the ATPase activity. Interestingly, the inhibition of RecAΔHs ATPase activity by RecXΔHs was influenced by the order of addition of the proteins in a pattern suggesting competition between RecA and RecX for DNA binding. Previously, Drees et al. (28,29) suggested that the RecX inhibition mechanism involved the binding of RecX to RecA filaments thus blocking the filament extension, a mechanism consistent with the observed interaction of the two proteins. However, if this RecX only interacted with the RecA filament, the same level of ATP hydrolysis by RecA should occur when RecX was pre-incubated with DNA before the addition of RecA and when RecX was incubated together with RecA and DNA. However, this was not observed: the inhibition of ATPase activity was strongest when RecX was pre-incubated with ssDNA, therefore before RecA filaments were formed on ssDNA (Figure 3B). We have shown previously that RecXΔHs has ssDNA binding activity (30), together with the results reported here, strongly suggests that RecXΔHs inhibits RecA filament formation by a mechanism involving direct interaction with RecA and also by controlling the availability of the ssDNA, thus inhibiting the nucleation of RecA or the extension of the RecA filament. This suggestion is strengthened by the observation that the addition of excess ssDNA to the system overrides RecX inhibition of ATP hydrolysis (Figure 3C). Since the concentration of ssDNA expressed in nucleotides was greater than the concentration of RecX, a mechanism whereby RecX inhibits RecA solely by direct protein-protein interaction would be insensitive to extra ssDNA being added to the reaction system. Detailed studies in *E. coli* have revealed that RecX acts by binding to the end of the growing RecA filament, thus limiting filament growth and leading to net disassembly (28). However, the observation that RecXΔEc competes with SSB implied that the mechanism of control of RecA activity by RecX in *E. coli* may involve RecX binding to both RecA and ssDNA (39), which is in agreement with our results.

The variety of recA-recX genomic organizations, recX mutant phenotypes, RecA ATPase and strand DNA exchange inhibition level by RecX, RecX ssDNA and dsDNA affinities suggests that specific mechanisms to control RecA activity operate in different bacteria. Furthermore, accessory protein may play an important role in the regulation of RecA activities. For example, in *E. coli* the RecF and DinI proteins antagonize the negative modulation of RecX (11,10). The absence of the recF, dinl, dinb, recBCD, sbcA, sbcCD genes, and the presence of a truncated umuC gene in the *H. seropedicae* genome (40) suggest that DNA recombination and repair systems of this bacteria and the control of RecA activities may present novel features.

The results presented here show that RecX of *H. seropedicae* negatively modulates the RecA strand exchange and ATPase activities but not ATP binding. The results also support the suggestion that RecX-ssDNA interactions are relevant for this negative regulatory activity of RecX in addition to protein-protein interactions.

### Supplementary material

**Table S1.**

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