Holliday junction-binding peptides inhibit distinct junction-processing enzymes

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Holliday junctions (HJ) are the central intermediates in both homologous recombination and site-specific recombination performed by tyrosine recombinases such as the bacteriophage λ Integrase (Int) protein. Previously, our lab identified peptide inhibitors of Int-mediated recombination that prevent the resolution of HJ intermediates. We now show that two of these inhibitors bind HJ DNA in the square-planar conformation even in the absence of Int protein. The peptides prevent unwinding of branched DNA substrates by the RecG helicase of *Escherichia coli* and interfere with the resolution of HJ substrates by the RuvABC complex. Our results suggest that these peptides target all proteins that process HJ in the square-planar conformation. These inhibitors should be extremely useful for dissecting homologous recombination and recombination-dependent repair *in vitro* and *in vivo*.

homologous recombination | recombination-dependent repair | RecG helicase | RuvABC resolvasome | tyrosine recombinase

Holliday junctions (HJ), or four-way junctions, are central intermediates in homologous recombination, repair of collapsed replication forks, and reactions performed by the tyrosine recombinase family of site-specific enzymes (1–5). The first two processes are important in all organisms and are involved in the maintenance of chromosome integrity and repair of DNA damage. In the case of diploid organisms, faithful chromosome segregation depends on homologous recombination (6). Site-specific recombination reactions performed by tyrosine recombinases are also very widespread and control gene expression, regulate plasmid and bacterial chromosome copy number, and mediate lysogeny (3). The presence and disappearance of HJ, their level within cells, and the enzymes that both generate and resolve them are of intense interest. More tools would be extremely useful for both *in vitro* and *in vivo* dissection of homologous recombination and repair processes in all organisms.

Phage λ integrase (Int) binds to and mediates strand exchange between pairs of *att* sites. During recombination, one round of DNA cleavage, strand exchange, and ligation of the top strands of each partner DNA molecule generates a HJ intermediate, which is resolved by a second round of the same catalytic steps (3). These reactions are both rapid and highly reversible, making intermediates very difficult to study.

We have identified peptides that inhibit recombination by trapping the protein-bound HJ intermediate and preventing its resolution either to substrates or to products (7–9). The most potent inhibitory peptide, WRWYCR, traps virtually all HJ formed during a reaction and has an IC_{50} of 5–20 nM (9). A related peptide, KWWCRW, is very similar to WRWYCR in potency (8). These peptides also inhibit the mechanistically related Cre, XerC and D, and Flp proteins (ref. 9; A.M.S., unpublished results; A. Conway and P. A. Rice, personal communication). Because these proteins share little primary sequence identity, we reasoned that these peptides might interact with free HJ.

HJ adopt one of two conformations in solution, depending on the concentration of cations (10). In the absence of metal ions, the junction arms are extended into a square-planar conformation that minimizes the phosphate repulsion of the backbone. The central bases at the junction point are unstacked and sensitive to oxidation by osmium tetroxide or potassium permanganate, which recognize unstacked bases (10). Cations shield the negatively charged DNA backbone and permit the HJ to adopt an X-like structure in which the junction arms stack on each other. In this conformation, the central region of the HJ is much less susceptible to oxidation. The crystal structures of the related Cre and Flp recombinases show junctions held in an extended conformation with ≈170 $Å^2$ of base-pair surface that is accessible to solvent in the central opening formed by the Cre or Flp tetramer (4, 5). This space is the target for peptide binding (11).

Here we examine the interactions between peptide WRWYCR and KWWCRW and HJ. We show that these peptides specifically bind square-planar HJ and inhibit the RecG helicase and RuvABC resolvase of *Escherichia coli*.

Materials and Methods

Peptides and Generation of HJ. Peptides WRWYCR and KWWCRW were synthesized with a C-terminal amide group at Sigma–Genosys and dissolved in 50% DMSO. Phage λ excision reactions were performed in the presence of peptide, as described, to generate large HJ (ref. 9; *Supporting Text*, which is published as supporting information on the PNAS web site). Synthetic junctions were assembled as described (10) from oligonucleotides whose sequence is shown in *Supporting Text*. The oligos were purchased from Integrated DNA Technologies (Coralville, IA).

Peptide/HJ Gel Mobility-Shift Assays. Standard binding reactions in Fig. 1*A* were performed similarly to λ excision recombination reactions, excluding nonspecific DNA and protein (9). Peptide was added at the indicated concentrations to 2 nM radiolabeled excision HJ in the presence or absence of 2 mM spermidine. For the competition experiment in Fig. 1*B*, reactions were premixed in binding buffer [10 mM Tris·HCl (pH 7.8)/1 mM EDTA/5% glycerol] with 2 nM radiolabeled excision HJ and the indicated competitor, peptide was then added, and reactions incubated 10 min on ice. Reactions were electrophoresed, without loading dye, through a 5% native polyacrylamide gel in 0.5× TBE buffer. EDTA was replaced with 80 μM $MgCl_2$ in the electrophoresis buffer for the binding experiment in Fig. 1*A* and Fig. 4*C*, which is published as supporting information on the PNAS web site.

$KMnO_4$ Footprinting Analysis of HJ Conformation. Oligonucleotides 1–4 (see *Supporting Text*) were used to make synthetic HJ substrates (with a frozen branch point) for footprinting (details in *Supporting Text*).

RecG Helicase Activity Assays. Oligonucleotides 5–8 were used to make HJ substrates, and combinations of oligonucleotides 5 and 9–11 were used to make replication fork substrates for RecG

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Abbreviations: Int, integrase; HJ, Holliday junction(s).

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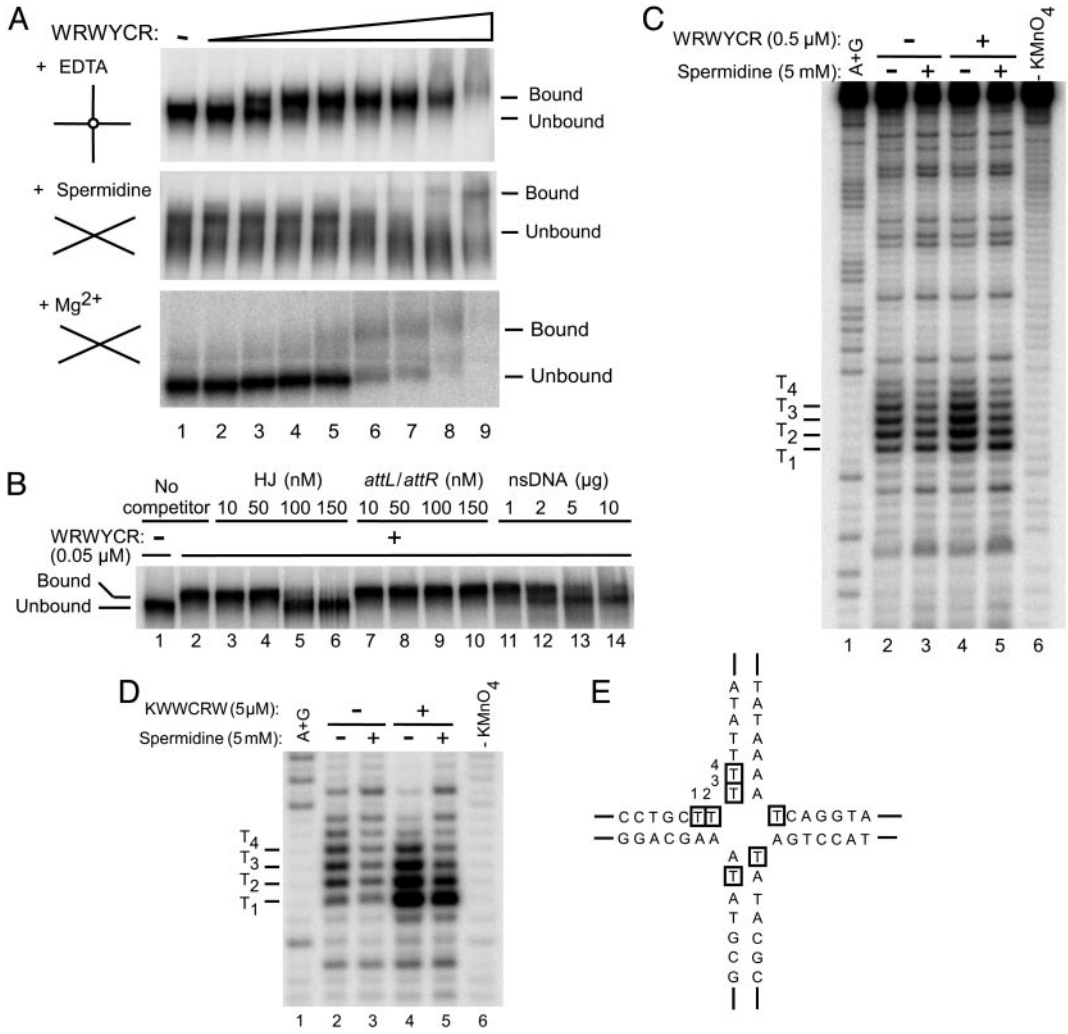

Fig. 1. Peptide WRWYCR binds specifically to HJ DNA. (*A*) Band-shift assay demonstrating binding of peptide WRWYCR to HJ DNA (2 nM) in the absence of cations or presence of spermidine (2 mM) or Mg^{2+} (80 μM $MgCl_2$ in buffer). Reaction mixtures contained no peptide (lane 1) or increasing amounts of WRWYCR (0.01, 0.025, 0.05, 0.1, 0.5, 1, 2.5, and 5 μM; lanes 2–9). At the highest concentrations, the peptide exhibits nonspecific DNA binding, resulting in the apparent loss of labeled DNA from the gel; in fact, the counts accumulate in the well (not shown; ref. 9). (*B*) Competition between HJ and competitor DNA. The indicated amounts of junctions (lanes 3–6), *attL* and *attR* (lanes 7–10), and salmon sperm DNA (nsDNA, lanes 11–14) were preincubated with 2 nM ^{32}P-labeled junction before addition of peptide WRWYCR. (*C*) $KMnO_4$ footprinting of HJ (2 nM) bound with peptide WRWYCR (0.5 μM) in the presence or absence of spermidine in the reaction buffer and incubated for 10 min. (*D*) $KMnO_4$ footprint of peptide KWWCRW at 5 μM concentration. (*E*) Summary of the affected thymines (boxed) for all DNA strands in the junction substrate.

(see *Supporting Text*). Unwinding assays were performed as detailed (ref. 12; *Supporting Text*).

RecG Band Shift and Footprinting. Oligonucleotides 1–4 (see *Supporting Text*) were used to make synthetic HJ substrates for RecG band shift and footprinting experiments (ref. 12; *Supporting Text*).

RuvABC Resolvase Assays. Assays were performed as described (13) by using oligonucleotides 12–15 (see *Supporting Text*) to make substrates for RuvABC cleavage assays.

Results

Peptide WRWYCR Binds Free HJ DNA to Form Square-Planar Complexes. We tested whether the peptides bind free HJ both in the absence and presence of spermidine, a trivalent cation, which stimulates λ site-specific recombination 5- to 10-fold and which is present in all cells. The peptides bind to square-planar junctions, in the absence of cations, with 10–100× higher apparent affinity than to stacked-X junctions formed in the presence of Mg^{2+} or spermidine (Fig. 1*A*). Presumably, the square-planar conformation provides more space in the center of the junction for the peptides. The conformation of the bound junction is dominated by the peptide rather than by spermidine (data not shown). Addition of DTT reverses interactions between the peptides and HJ (Fig. 4 and data not shown), suggesting that the peptides bind free junctions as a dimer, just as they inhibit Int-mediated recombination (9).

Competition assays show that the peptide prefers HJ DNA over a 240-fold excess (by weight) of nonspecific double-stranded DNA, and we have seen no sequence specificity yet (Fig. 1*B* and data not shown). The global conformation of HJ (10) remains square-planar even in the presence of peptide WRWYCR (Fig. 4), resembling proteins that convert the junction to a square-planar conformation upon binding (RuvA and RecG).

$KMnO_4$ footprinting showed that thymines in the center of the junction are more sensitive to modification in the presence than in the absence of peptide (Fig. 1*C*, lanes 2 and 4). Addition of

Table 1. Summary of IC_{50} values for peptide inhibition of HJ- and replication fork-processing enzymes

Protein (pathway/substrate)	Activity*	IC_{50}, μM
λ Int (excision)	Res.	0.02[†]
RecG[‡] (HJ)	Unw.	0.12
RecG (fork, leading + lagging)	Unw.	0.12
RecG (fork, lagging strand only)	Unw.	0.025
RecG (fork, leading strand only)	Unw.	0.2
RuvABC, top product[§]	Clvg.	0.064 (0.18)[§]
RuvAC	Clvg.	1.3
RuvBC, top product[§]	Clvg.	2.8 (11)[§]
RuvC	Clvg.	34
RuvAB	Unw.	20
T7 endonuclease I	Clvg.	50

*Activity being inhibited: Res., resolution; Unw., unwinding; Clvg., cleavage. Resolution of the junctions by Int gives two religated products, whereas cleavage gives two nicked products.

[†]IC_{50} values differ depending on recombination pathway (9).

[‡]The RecG and Ruv proteins used are from *E. coli.* The substrate used for each assay is a HJ, unless specified otherwise. The presence of the leading or the lagging strand is indicated in parentheses.

[§]The top products result from RuvC cleavage directed by the binding of RuvB. The bottom products, shown in parentheses, result from undirected cleavage.

5 mM spermidine, roughly the same concentration present in cells (14), protects the center of the junction from permanganate modification even in the presence of the peptide (Fig. 1*C*, lane 5). Addition of 5 μM instead of 0.5 μM peptide rendered the peptide more dominant over 5 mM spermidine (Fig. 1*D*). Quantitating the T1–T4 bands from Fig. 1 *C* and *D* showed that the peptide-induced hypersensitivity of T_3 and T_4 is completely reversed by spermidine, and T_2 is slightly protected by spermidine, but the peptides make T_1 hypersensitive to permanganate both in the presence and absence of spermidine (Fig. 5, which is published as supporting information on the PNAS web site). In summary, the peptide and spermidine affect the isomerization state of HJ in opposite ways.

The footprinting experiment shown used an artificial junction that is unable to branch migrate, but the same results were obtained with junctions that can branch migrate over the seven central base pairs. In the latter case, all seven central base pairs were sensitive to permanganate (not shown). The peptides may trap several independent junctions "centered" at different base pairs, or the junctions may still branch migrate in the presence of the peptides. We favor the former possibility, because "frozen" junctions also display several permanganate-sensitive nucleotides (Fig. 1 *C* and *D*).

We conclude that the peptides interact best with square-planar junctions and exacerbate the disruption of the center of these structures. Spermidine or Mg^{2+} compact the junction into a stacked-X structure, closing off the center and preventing access to the peptide at low peptide concentrations. In the presence of a sufficient concentration of peptides, the junction is converted into a square-planar conformation with a disrupted central region.

Inhibition of the RecG Helicase of *E. coli*. HJ or four-way junctions are substrates for several helicases and junction resolvases in all organisms (15, 16). Some of these enzymes, for example phage T7 endonuclease I and phage T4 endonuclease VII, recognize junctions largely in the stacked-X form (12, 17, 18). Our peptides inhibit the cleavage of HJ by phage T7 endonuclease I poorly (Table 1). Other junction-processing enzymes, including the *E. coli* RecG helicase and RuvABC enzyme complex, act on junctions in the square-planar conformation (15, 16). RecG and RuvABC have been implicated in the rescue of collapsed replication forks and thus are very important to the survival of bacteria. RecG is highly conserved among bacteria but absent from most eukaryotes, where RecQ helicase homologs may fulfill a similar function (19–22). Enzymes related to RuvC are found in most bacteria, archaea, and some eukaryotic viruses (15, 16, 23). Although RuvC homologs are not present in eukaryotes, HJ-resolving enzymes from eukaryotes are of great interest (22, 24–28).

RecG is a monomeric helicase that interconverts branched DNA structures such as replication forks and HJ (2, 15). RecG unwinds the leading and lagging strands simultaneously, which then reanneal to each other to form a HJ. RecG "resolves" junctions via branch migration. Mg^{2+} and ATP are required for the unwinding reaction but not for binding to three-way or HJ substrates (12).

To test whether peptide WRWYCR inhibits RecG activity, we used synthetic HJ and substrates that mimic replication forks (12). Peptide binding does not depend either on branch migration potential or the specific bases at the junction point (data not shown). RecG unwinds the short HJ substrates into partially single-stranded "flayed duplex" products (Fig. 2*A*, lane 2) and removes both leading and lagging strands from replication fork substrates to generate flayed duplexes (Fig. 2*B*, lane 2). Addition of peptide WRWYCR (or peptide KWWCRW; data not shown) prevents both the unwinding of the HJ substrate by RecG (Fig. 2*A*) and the unwinding of the replication fork-like substrates (Fig. 2*B*). The peptide inhibits RecG unwinding of fork substrates containing only the lagging strand better than substrates containing only the leading strand or both strands (Fig. 2*C* and Table 1). These differences may reflect the affinity of the peptide for the different DNA substrates or the interactions of peptide with different RecG–substrate complexes. However, the peptides do not gel-shift the replication fork-type substrates, perhaps because these complexes are unstable during electrophoresis. Thus, the degree of peptide inhibition of RecG activities in various complexes is more likely due to the nature and/or stability of interactions between RecG and the substrates in question. Further analyses of structure–activity relationships of the peptides (A. Flores, S. Patra, K.V.K., and A.M.S., unpublished results) support our conclusion that the peptides inhibit both Int and RecG via their interactions with HJ substrates.

To investigate the mechanism of RecG inhibition further, we tested the effect of peptides on RecG–substrate interactions using gel mobility-shift assays and footprinting. Either peptide WRWYCR or RecG shifts the HJ (Fig. 2*D*, lane 2 vs. lane 3). In the presence of both peptide and RecG, the peptide–junction complex predominates (Fig. 2*D*, lane 4), indicating that the peptide prevents interactions between HJ and RecG. The HJ center is slightly more susceptible to $KMnO_4$ cleavage in the presence of RecG alone (Fig. 2 *E* and *F*), consistent with crystal structure data indicating that the wedge domain of RecG interacts with the junction point (29). The presence of both RecG and peptide WRWYCR in the binding reaction results in a $KMnO_4$ cleavage pattern that closely matches the 3- to 4-fold increase in $KMnO_4$ sensitivity induced by peptide alone (Fig. 2 *E* and *F*). Taken together, the results show that peptide binding to the junction center prevents binding of RecG (Fig. 2*G*).

The crystal structure of RecG bound to a fork substrate suggests how competition may occur (29). The 98-aa wedge domain of RecG makes intimate contact with the replication fork substrate, inserting between the two arms of the replication fork. In the crystal structure, Phe-204 and Tyr-208 of RecG contact the central bases in a manner that mimics base stacking and probably provide stacking partners for bases at the junction point during unwinding (29). The peptides may occupy the same space as the RecG wedge domain, causing distortion of the central bases and disrupting interactions between the HJ or replication fork substrate and RecG.

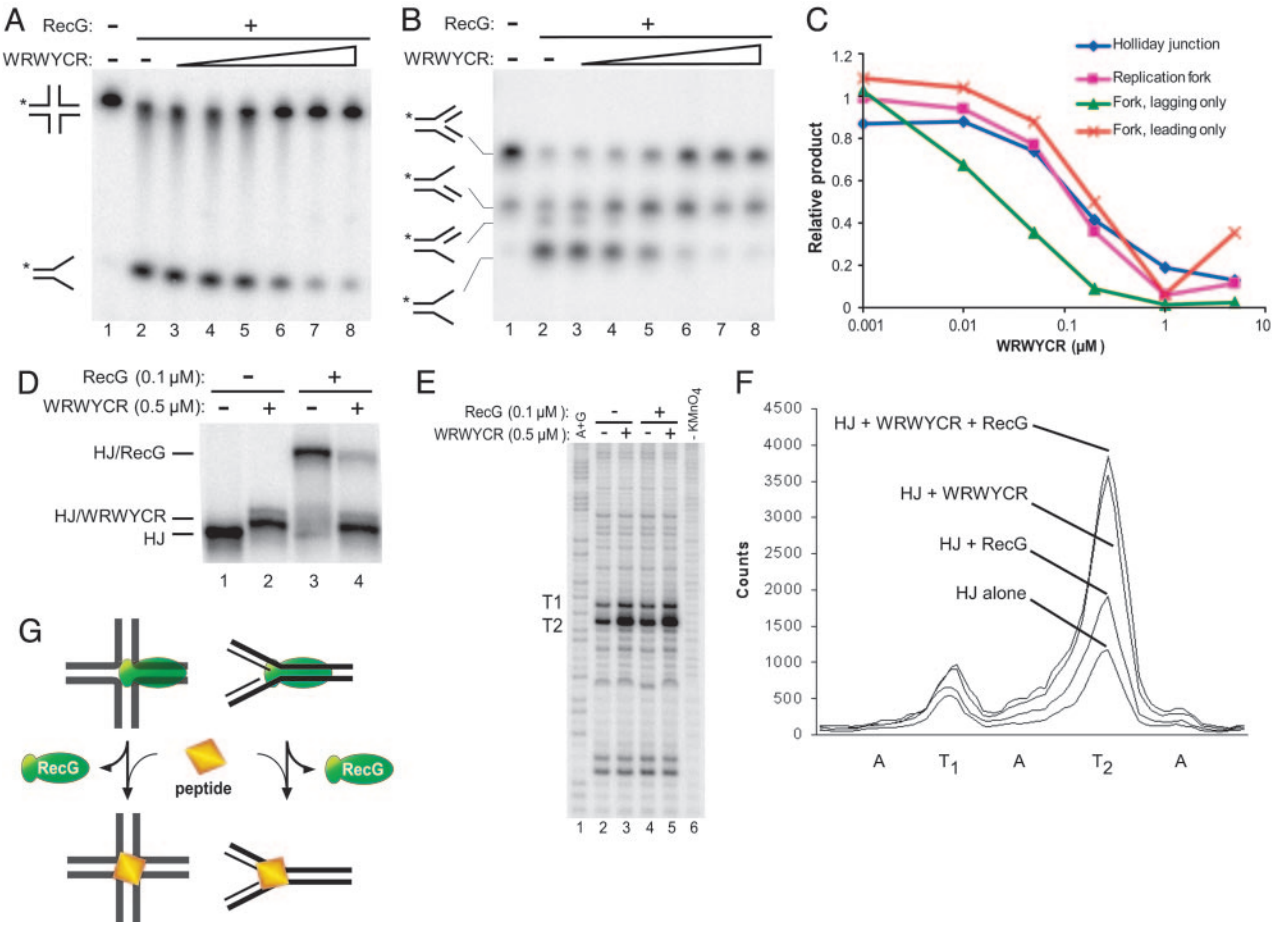

Fig. 2. Peptide WRWYCR inhibits unwinding of branched DNA substrates by RecG. In *A* and *B*, reactions contain 0.5 nM RecG where indicated and 0.001, 0.01, 0.05, 0.2, 1, and 5 μM peptide WRWYCR (lanes 3–8). WRWYCR was added to the reaction buffer with 2 nM synthetic junction DNA (*A*) or replication forks (*B*), followed by addition of RecG. (*C*) Graphical representation of RecG inhibition data. Relative product denotes the ratio of RecG product produced in the presence of peptide to product in the absence of peptide. (*D*) Band-shift assay showing the interactions of peptide WRWYCR and/or RecG with 2 nM synthetic junction DNA. In *D–F* are shown the effects of peptide WRWYCR on RecG binding by bandshift assays (*D*) and $KMnO_4$ footprinting (*E*; graphical representation of band intensity is shown in *F*). WRWYCR (0.5 μM) was added to reaction buffer with 2 nM synthetic junction DNA, followed by addition of RecG (0.1 μM). (*G*) Model of RecG activity inhibition by peptide.

Peptide WRWYCR Inhibits the RuvABC Complex. As mentioned above, the RuvABC complex also processes HJ, in a way distinct from the mechanism of RecG and with different mechanisms depending on which of the three proteins are acting on the junction. A tetramer of the RuvA protein recognizes and binds to the junction center (30, 31). It recruits the RuvB helicase, which "pulls" the junction arms through its "donut-like" structure and catalyzes branch migration (32, 33). Finally, the RuvC junction resolvase binds as a dimer to the junction–RuvA-RuvB complex on the opposite side of RuvA and cleaves two of the four strands to leave ligatable nicks (34, 35). In addition to the RuvABC complex, several subcomplexes also have partial activities, at least *in vitro*. The RuvAB complex can branch migrate a junction in the absence of RuvC (30, 32). A RuvAC or a RuvBC complex can catalyze junction cleavage, albeit less efficiently than the RuvABC complex (13, 36, 37). A hallmark of the RuvABC complex is that the RuvA protein opens the junction into a square-planar conformation, the preferred substrate of the RuvC resolvase.

We investigated how peptides affect the junction processing activities of the RuvABC complex as well as those of various subcomplexes (Fig. 3*A*). The junction substrates we used constrain RuvABC to assemble in a defined manner and direct cleavage of the junction predominantly to one pair of strands, because RuvB is reported to bind only to the long arms of the junction (13). Peptide WRWYCR inhibits junction cleavage by RuvC with the following hierarchy of potencies: RuvABC>RuvAC≅RuvBC>RuvC (Fig. 3*B* and Table 1). Surprisingly, we observed two products in the RuvBC reaction: the bottom product is the same product generated by the RuvC protein alone, whereas the top product corresponds to the major product of the RuvABC complex, and its presence suggests that RuvB may bind transiently to the short arms of the junction. The appearance of the directed product is inhibited ≈4× more than the undirected product (Table 1); thus, even the transient presence of RuvB sensitizes the junction to peptide inhibition. In conclusion, the peptide inhibits most effectively those complexes in which the junction is in the most open conformation by virtue of being bound by RuvA and/or RuvB. These observations suggest a model in which RuvAB are required to open the junction structure to provide the optimal target for peptide binding (Fig. 3*C*). The bound peptide then blocks cleavage by RuvC, preventing resolution of the junction.

Discussion

We have presented results that show that peptide WRWYCR and KWWCRW interact directly with HJ DNA substrates. These interactions are the basis for peptide inhibition not only of

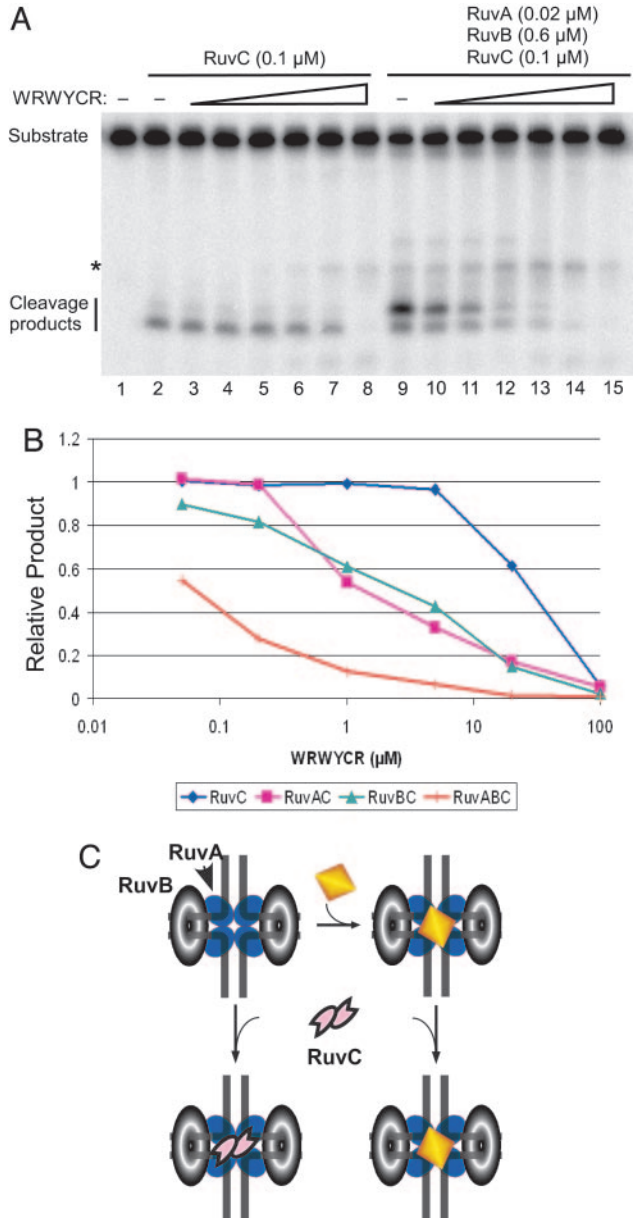

Fig. 3. Peptide WRWYCR inhibits HJ resolution by RuvABC. (*A*) WRWYCR at 0.05, 0.2, 1, 5, 20, or 100 μM (lanes 3–8; refs. 10–15) was added to reaction buffer with 1 nM synthetic junction DNA, followed by addition of the indicated amounts of protein. The top bands represent RuvC cleavage directed by assembly of RuvB hexameric rings; the bottom bands result from undirected cleavage (13). The asterisk on the left side of gel represents an unknown peptide-dependent product. (*B*) Quantification of RuvABC inhibition by peptide WRWYCR. Only the RuvA and/or RuvB-directed cleavage products are represented. (*C*) Model of RuvABC complex inhibition. Alternatively, RuvC may bind in the presence of peptide but may be unable to cleave the junction.

site-specific tyrosine recombinases like phage λ Int but also of enzymes involved in DNA repair and homologous recombination. We have demonstrated that the helicase activity of the RecG protein is inhibited by peptides, as is the resolution activity of the RuvC HJ nuclease when the HJ is bound by the RuvA and RuvB proteins. RuvABC and RecG proteins do not share primary sequence or structure similarities either with each other or with the tyrosine recombinases, and thus their inhibition by our peptides strongly suggests that these inhibitors primarily target a structure, the HJ. The peptides bind to junctions in the open conformation with much greater affinity than to junctions in the stacked-X conformation and cause their center to be even more sensitive to $KMnO_4$ than it is in the absence of peptide. Although peptide WRWYCR and KWWCRW are the most potent of this inhibitor class, several related peptides (7–9, 38, 39) and small molecules (D. Ranjit, C. Pinilla, and A.M.S., unpublished results) have similar activities.

We do not know whether the peptide interacts exclusively with DNA or makes additional contacts to the proteins in the junction complexes. RecG interactions with HJ and replication fork substrates are not very stable in the presence of Mg^{2+}, and the enzyme is not processive (12, 40). Our results indicate that the peptides and RecG cannot both bind to a junction substrate, supporting our proposal that the peptides and the RecG wedge domain interact with junctions in a similar way.

We are not yet certain of the mechanism of peptide inhibition of the RuvABC complex activity, and we do not yet know whether the peptides contact any members of this complex. RuvA has four acidic pins that are probably involved in strand separation, although they do not take up space in the center of the HJ (41). Peptide WRWYCR does not prevent a RuvA tetramer from binding to HJ substrates (K.V.K. and A.M.S., unpublished results). RuvB does not interact with the center of junctions (33, 42), and it is highly unlikely that the peptide interferes directly with its binding. Indeed, the helicase activity of the RuvAB complex is only weakly inhibited by the peptide (Table 1). Cleavage activity of the RuvC protein by itself is not strongly inhibited by the peptide either, suggesting that, in the presence of Mg^{2+}, the peptide does not bind free junctions as well as RuvC. The peptide inhibits the RuvABC complex most, presumably because the peptide has the easiest access to the junction center in this complex. Because nearly all of the RuvC-containing complexes are inhibited more than the RuvAB complex, the peptides probably interfere with cleavage by the RuvC resolvase, which itself is most active when the junction conformation is closest to square-planar (13, 36, 37). The peptide may inhibit RuvC activity by distorting the central base pairs of the junction and either sterically hindering its binding or interfering with catalysis. The Phe-69 residue of RuvC is involved in DNA binding and catalysis, probably by stacking with a nucleotide near the junction center and producing a conformational change that is necessary for cleavage (43). The peptide prevents binding of RuvC by itself to junctions in the absence of cations (K.V.K. and A.M.S., unpublished results), supporting our hypothesis that the peptide and RuvC compete for the center of the open junction.

Three factors have contributed to the model describing peptide–HJ interactions shown in Fig. 2*G*: (*i*) the presence of aromatic amino acids in all of the most active peptides; (*ii*) the crystal structure of the Cre-*lox*-peptide ternary complex; and (*iii*) the structure of the RecG protein. Although the peptide [in this case, WKHYNY (7, 38)] is not resolved in the Cre-*lox*-peptide structure, peptide-dependent extra electron density is seen in the junction center, and two of the four base pairs closest to the central opening are unstacked with respect to base pairs further along the arms (11). Aromatic amino acids in the peptide may stack with the central base pairs in the junction, rendering these more susceptible to oxidation by permanganate. Such stacking interactions may resemble those seen between two aromatic amino acids present in the wedge domain of the *Thermotoga maritima* RecG protein and nucleotides in the center of its replication fork substrate (29). Such interactions would be possible only with junctions in the extended, or open, conformation. However, peptide WKHYNY does not bind protein-free junctions stably (evidenced by lack of gel shifts with junctions and no inhibition of RecG; data not shown), whereas peptides WRWYCR and KWWCRW do (refs. 9 and 38; this work); thus

we do not yet know how similar the details of peptide–DNA interactions will be.

The bacterial HU protein and the eukaryotic high-mobility group proteins (HMG) also interact with HJ in the open conformation (44–46), but structural evidence shows the interactions are quite different and do not include the junction center (reviewed in refs. 47 and 48). In contrast to the peptides, neither HU nor HMG accumulate HJ during phage λ site-specific recombination; instead, they replace IHF due to their DNA-bending abilities (49, 50). Clearly, the high-affinity stable interactions of peptides WRWYCR and KWWCRW with the junctions are concentrated in a much smaller "package," a hexapeptide dimer (or tetramer) with a mass of <2 kDa.

In vivo, protein-free junctions are most likely found in a "stacked-X" conformation due to charge neutralization of the backbone phosphates by Mg^{2+} and other cations like spermidine. Such junctions are relatively "inert," because they cannot branch migrate (51). Almost all proteins that act on HJ impose an open conformation. These proteins will generate a better target for peptide binding by opening the junction center, but their activities may be inhibited in consequence.

In fact, peptides that block Int-mediated recombination most potently *in vitro* are also bactericidal, promote defects in cell division and chromosome partitioning, and cause accumulation of free 3′-OH ends (C. Gunderson and A.M.S., unpublished results). These effects may be explained by peptide stabilization of HJ and the consequent inhibition of recombination-dependent repair of collapsed replication forks and/or chromosome segregation.

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