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MINIREVIEW

The RuvABC Proteins and Holliday Junction Processing in *Escherichia coli*

STEPHEN C. WEST*

Genetic Recombination Laboratory, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, United Kingdom

Until five years ago, little was known about the way in which bacteria processed recombination intermediates into mature recombinant products. Genetic studies of recombination, in combination with detailed biochemical studies of the *Escherichia coli* recombination protein RecA indicated that recombining chromosomes (and plasmids) were linked by a crossover, or Holliday junction (Fig. 1), and it was reasonable to assume that these junctions served as a substrate for a specific junction-resolving endonuclease. Unfortunately, biochemical attempts to detect this endonuclease had been unsuccessful. Similarly, genetic screens had failed to identify a mutant that exhibited a clear resolution-defective phenotype. The prospects were not looking good.

DISCOVERY OF THE *E. COLI* RESOLVASE

The key observation which led to the identification of the *E. coli* Holliday junction resolvase was made by Bernadette Connolly while she was a postdoc in this laboratory. Using recombination intermediates made in vitro with RecA protein, Connolly identified a weak activity in fractionated cell extracts that appeared to produce recombinant products (9). The results would not have been convincing but for the fact that the same fractions resolved small synthetic Holliday junctions into nicked duplex products. Cleavage occurred by the introduction of symmetrically related nicks at the site of the junction, as expected of a resolvase on the basis of studies of the bacteriophage T4 gene *49*-encoded resolvase (endonuclease VII). Gel filtration studies indicated that the *E. coli* resolvase was a small protein with a molecular mass of approximately 20 kDa. To identify its gene, extracts were prepared from various recombination-defective mutants, and one extract prepared from a *ruvC* strain was found to be deficient in cleavage activity (8). These observations, in combination with bacterial genetics, have since led to the discovery of a number of proteins that interact with Holliday junctions.

The first *ruv* mutants were identified by Otsuji et al. (29) in 1974 and exhibited phenotypes characterized by increased sensitivity to UV light and to mitomycin and an associated cell division defect which led to the formation of nonseptate filamentous cells after DNA-damaging treatments. In certain genetic backgrounds, *ruv* mutants also showed defects in recombination (22, 46). Subsequent studies showed that the *ruv* operon, which maps at 41 min on the *E. coli* linkage map, actually encodes three genes and that either *ruvA*, *ruvB*, or *ruvC* mutants display the same phenotypic properties as the original mutant (7, 40, 45). Otsuji's mutation is now known to map within *ruvB*.

* Phone: (44)-171-269-3868. Fax: (44)-171-269-3811.

One significant clue to the role of the *ruv* gene products in recombination was provided by work from Robert Lloyd's laboratory. When they failed to recover F-prime plasmid transconjugants from *rec*$^+$ *ruv* strains, Benson et al. (6) quite rightly proposed that the defect was due to abortive recombination and that the Ruv proteins must act late in recombination. Soon the *ruvC* gene was cloned and sequenced (42, 48), and the protein was purified from overexpressed plasmids. The purified 19-kDa RuvC protein was found to resolve Holliday junctions (11, 19). In retrospect, the *ruv* genes were in fact prime candidates for encoding the Holliday junction resolvase.

IDENTIFICATION OF THE RuvA AND RuvB PROTEINS AS CATALYSTS OF BRANCH MIGRATION

The identification of RuvC as the *E. coli* Holliday junction resolvase led to new efforts being exerted toward a study of the *ruvA* and *ruvB* gene products. The RuvA (22-kDa) and RuvB (37-kDa) proteins had been purified by Hideo Shinagawa and colleagues; Iwasaki et al. (17) showed that RuvB was a DNA-dependent ATPase and Shiba et al. (44) showed that its activity was stimulated by RuvA. They also found that the combined action of RuvAB resulted in the reabsorption of an extruded cruciform structure from plasmid DNA (44). Since cruciforms are structurally related to Holliday junctions, the possibility that cruciform loss occurred by a process akin to branch migration was discussed. Irina Tsaneva, a visiting scientist in my laboratory, used recombination intermediates to show directly that RuvA and RuvB were able to promote ATP-dependent branch migration in vitro (51, 52). These studies were reinforced by experiments with synthetic Holliday junctions which demonstrated that RuvA and RuvB interact and that RuvA specifically targets RuvB to the Holliday junction where they cooperate to promote branch migration (18, 34, 35).

The concept that branch migration was a protein-catalyzed process, rather than something that occurred spontaneously, caused some surprise, since studies in the mid-1970s (50, 55) had suggested that Holliday junctions were capable of rapid spontaneous branch migration (step rate, 10,000 bp/s at 30°C). However, more-recent studies were beginning to question the validity of these experiments, and using well-defined systems, Müller et al. (28) and Panyutin and colleagues (30, 31) demonstrated that the rate of spontaneous branch migration was much slower than originally thought.

STUDIES OF THE RuvAB BRANCH MIGRATION MOTOR

Since the RuvAB complex hydrolyzes ATP, initially it was reasonable to imagine models in which the protein complex, once targeted to the Holliday junction, would translocate and

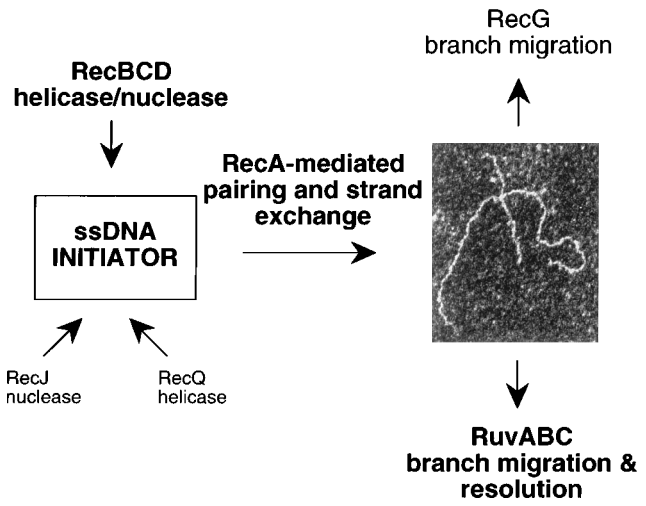

FIG. 1. Scheme for recombination in *E. coli*. Recombination events are initiated by single-stranded DNA (ssDNA) or partially single-stranded DNA; in conjugal recombination single-stranded DNA is produced by RecBCD enzyme, although other proteins can take over this function when RecBCD is inactivated by mutation. Single strands are then recognized by RecA protein which promotes homologous pairing and strand exchange, leading to the formation of recombination intermediates containing Holliday junctions. Holliday junction intermediates are then further processed by RuvABC proteins, or alternatively by RecG, to produce mature recombinants. The dominant activities in wild-type cells are indicated in bold type.

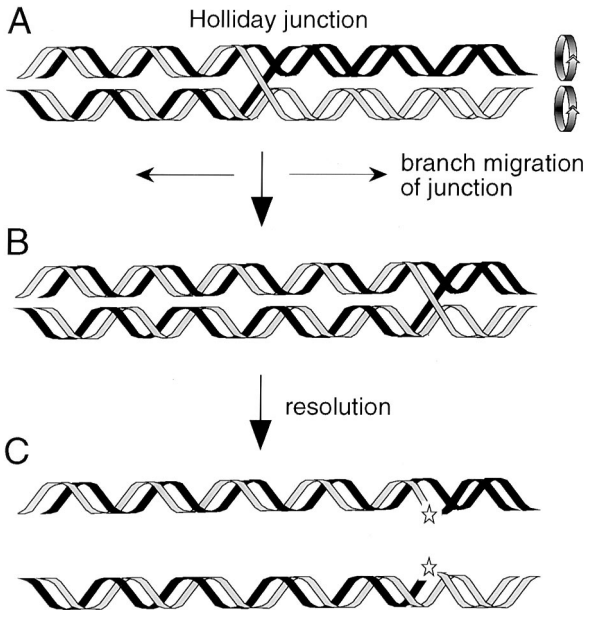

FIG. 2. Role of RuvABC proteins in the processing of recombination intermediates. (A) Holliday junctions made by RecA are recognized and bound by RuvAB. These proteins promote movement of the junction in either direction to facilitate the formation of heteroduplex DNA by forward branch migration (B) or to drive branch migration backwards (not shown). Early models for branch migration suggested that the transfer of strands could occur via helical rotation of the two interlinked DNA molecules (as shown). (C) The Holliday junction is then resolved by RuvC protein to produce nicked duplex products that can be repaired by DNA ligase.

push the junction along the DNA (57). Translocation of the protein could lead to rotation of the two interlinked DNA helices such that strands would pass from one molecule to another, so forming heteroduplex DNA (Fig. 2).

This concept of a translocating branch migration motor was supported by observations indicating that RuvA and RuvB together exhibit DNA helicase activity (1, 15, 53, 54). However, the remarkable mechanism of action of this branch migration motor was not appreciated until RuvAB-Holliday junction complexes were directly visualized by electron microscopy. In collaboration with Andrzej Stasiak and Edward Egelman, we observed that RuvB protein forms hexameric rings around DNA (47). Furthermore, Parsons et al. (32) showed that RuvAB proteins form a tripartite complex at the Holliday junction. Two features about the structure of this complex were unexpected. First, binding of the junction by RuvA resulted in its unfolding into a fourfold symmetric structure (Fig. 3A). This result provided the first direct evidence that Holliday junction structure is modified by protein binding. Second, rather than seeing a polar branch migration motor, we observed a twofold symmetric structure with RuvA sandwiched between two rings of RuvB. This configuration of protein and DNA immediately suggested an elegant mechanism of branch migration.

A working model for RuvAB-mediated branch migration is shown in Fig. 3B (32). It is proposed that the two RuvB rings are functional helicases capable of moving DNA through the central hole in their hexameric structures. Assuming that the rings are oriented in opposite directions, each will apply an equal and opposite force to the DNA, such that homoduplex DNA will be drawn into the RuvAB complex and heteroduplex DNA will exit from RuvB. This can be imagined as a mechanism in which the two helicases are trying to push toward each other.

The mechanism of branch migration catalyzed by the two ring helicases may be analogous to certain reactions that occur during the formation of a replication bubble. For example, the simian virus 40 replication origin is bound by simian virus 40 T antigen, another hexameric ring helicase (26). Because of the palindromic nature of the origin sequence, two rings are thought to bind facing in opposite orientations, and their DNA helicase activity will lead to opening of the DNA between the two rings (32, 56). Several known hexameric ring helicases (e.g., *E. coli* DnaB protein and bacteriophage T7 gene 4 helicase/primase) may play related roles in DNA replication (14, 36). Other aspects of DNA metabolism, such as transcription and DNA repair, may also involve the targeting of polar ring helicases to specific sites.

MECHANISM OF HOLLIDAY JUNCTION RESOLUTION BY RuvC

The mechanism of Holliday junction resolution can be subdivided into a number of experimentally separable steps including (i) DNA binding, (ii) modification of DNA structure, and (iii) cleavage. By using synthetic Holliday junctions, it has been shown that RuvC forms a defined protein-DNA complex in the absence of divalent metal ions (3, 11, 49). Resolution-defective mutant proteins retain the ability to bind junctions (2, 37, 43). When the wild-type protein binds to a Holliday junction, it induces a sensitivity of the DNA to attack by hydroxyl radicals. The hypersensitivity maps to sites located 1 or 2 nucleotides to the 3′ side of the junction (3). RuvC, like RuvA, unfolds the junction, but in this case the junction exhibits twofold symmetry (5), consistent with RuvC functioning as a dimer (Fig. 4A). Whether RuvC can act in conjunction with RuvA remains to be determined. Within the RuvC-Holliday

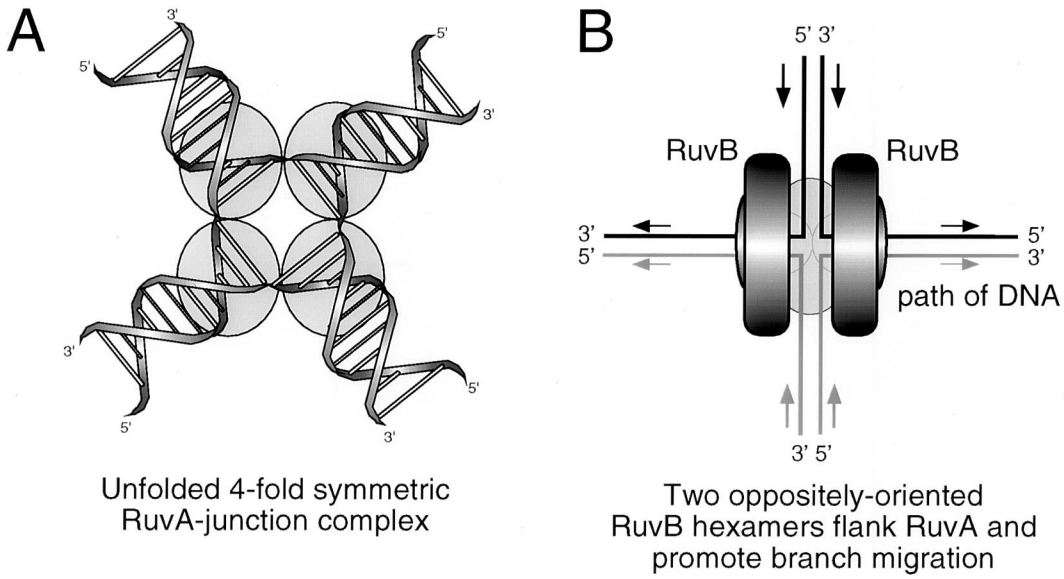

FIG. 3. Models for the structure of RuvA and RuvAB-Holliday junction complexes. (A) Ribbon model of the RuvA-Holliday junction complex showing a structure that exhibits approximate fourfold symmetry (32). (B) The RuvAB complex assembles in the form of a tripartite structure. In this complex, the junction lies unfolded (as shown in panel A) and RuvA is sandwiched between two hexameric rings of RuvB. The rings are oriented in opposite directions, and each ring encompasses a DNA duplex. Branch migration occurs as the RuvB ring motors promote the passage of DNA. Because of the symmetry of a Holliday junction, RuvAB-mediated branch migration can occur in either direction, depending upon which pair of arms are bound by RuvB. This diagram shows the forward branch migration reaction which leads to the formation of heteroduplex DNA. Branch migration is likely to involve the ATP-dependent DNA helicase activity of RuvAB. Panel B is adapted from *Nature* with permission of the publisher (32).

junction complex, base pairing at the crossover appears to be disrupted because the DNA becomes sensitive to chemical probes such as permanganate ions (5).

In the presence of divalent metal ions (Mg^{2+} or Mn^{2+}), RuvC catalyzes resolution of the junction. This occurs by the introduction of symmetrically related nicks in a pair of strands of like polarity (3, 11, 38). By using junctions in which the arms are tethered to adopt one isomeric form or the other, Bennett and West (4) showed that RuvC promotes cleavage in the pair of strands defined as the continuous (i.e., noncrossover) strands. These are also the strands that are sensitive to hydroxyl radical attack. Unexpectedly, the cleavage reaction exhibits sequence specificity, with a requirement for the tetranucleotide sequence 5′-WTT ↓ S-3′, where W is A or T and S is G or C (39). The sequence specificity is relaxed by the presence of Mn^{2+} ions. The cleavage sequence must be present in both junction arms (39a), and the efficiency of resolution is optimal when the cleavage site is located precisely at the crossover (5a). Following cleavage of the Holliday junction by RuvC, the resolution process is completed by DNA ligase

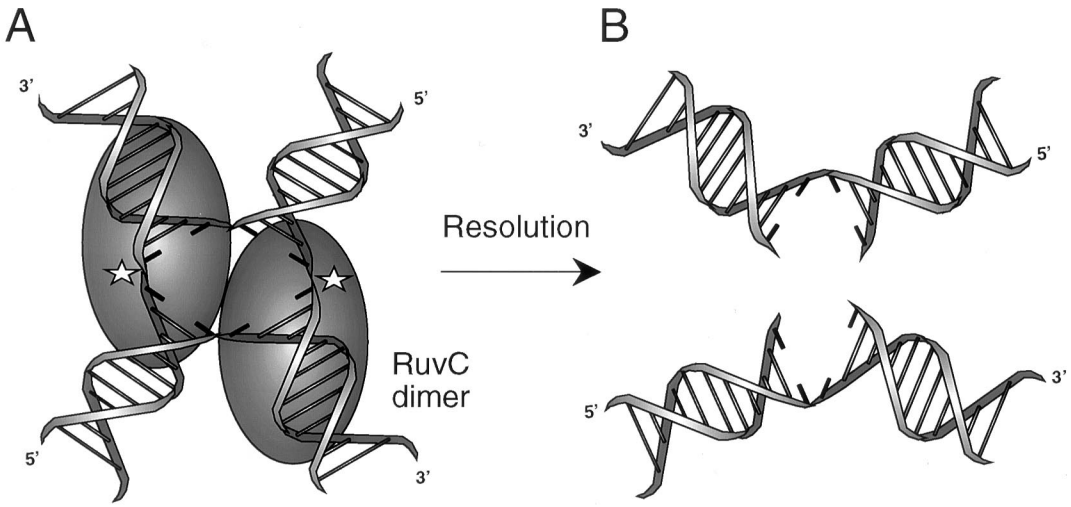

FIG. 4. Schematic model of the RuvC-Holliday junction complex. (A) Ribbon model of the structure of a RuvC-Holliday junction complex. The junction adopts an unfolded, twofold symmetric structure in which base pairing at the crossover is disrupted. The RuvC dimer is shown schematically, with the monomer subunits facing opposite directions, as indicated by crystallographic studies (2). RuvC cleaves the pair of strands that form the wide angles of the junction (often defined as the continuous or noncrossover strands). (B) Since the cleavage sites are positioned with perfect symmetry, resolution leads to the formation of nicked duplex products which can be repaired by DNA ligase. This figure is adapted from the *Journal of Molecular Biology* with permission of the publisher (5).

which rejoins the 5′-P and 3′-OH termini in the nicked duplex products (3, 12) (Fig. 4B).

X-RAY STRUCTURE OF RuvC

The crystal structure of RuvC was recently determined at a resolution of 2.5 Å (0.25 nm) and indicates a dimer in which the subunits are related by twofold symmetry (2). In combination with mutational analysis, the active site was identified as containing four acidic residues which are located at the bottom of a DNA binding cleft within each monomer (37). In the dimer, the two active sites are located approximately 30 Å (3 nm) apart. The structure of RuvC shows a striking similarity to RNase H1 despite the lack of any sequence homology. The similarity is shared with the RNase H domain of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (10) and the HIV-1 integrase protein (13). It is possible that the nucleolytic activity of these enzymes evolved from some common ancestor. Sequence or structural homologies between RuvC and the bacteriophage T4 and T7 resolvases have not been observed (2).

REDUNDANCY IN HOLLIDAY JUNCTION PROCESSING PATHWAYS?

Until recently, the apparent lack of a profound recombination deficiency in *ruv* mutants was a concern. One way to rationalize their lack of a *rec* mutant phenotype was to assume either that Holliday junctions could be processed by alternative proteins or that certain recombination events did not proceed via Holliday junction intermediates. The puzzle was solved by the demonstration that RecG protein has activities that partially overlap those of the Ruv proteins. Key genetic observations showed that conjugal recombination was severely depressed in *ruv recG* double mutants and that these strains were more UV sensitive than either *ruv* or *recG* single mutants (21). Further studies showed that the *recG* gene encodes a 76-kDa protein that like RuvAB, binds Holliday junctions and promotes ATP-dependent branch migration (23, 24). One possibility that has been suggested is that RecG protein promotes resolution by driving branch migration in the reverse direction, thus dissociating recombination intermediates without the need for nucleolytic cleavage (58, 59).

While it is possible that bacteria have evolved two equal and overlapping systems for the late steps of recombination, it is also possible that the Ruv and RecG systems will have specialized functions. The SOS-inducible RuvAB proteins may play a particularly important role in recombination and DNA repair by promoting the formation of heteroduplex DNA containing insertions and deletions (27). In support of this, RuvAB proteins promote branch migration through quite extensive regions of heterology in vitro (20, 33). In contrast, *recG* mutations have a less significant effect than *ruvAB* mutations on the formation of recombinants between two diverged DNAs (27). Conversely, mutations in *recG*, but not those in *ruvA* or *ruvB*, affect constitutive stable DNA replication, a process which occurs in *E. coli rnhA* mutants (defective in RNase HI). Remarkably, cells carrying mutations in both *rnhA* and *recG* were found to be inviable, leading to the suggestion that RecG protein plays an important role in the removal of the R loops made by RecA, which are involved in the initiation of constitutive stable DNA replication (16).

At the present time it is not known whether RuvC protein represents the only functional Holliday junction resolvase in *E. coli*. Mandal et al. (25) identified a mutation which suppresses the UV-sensitive phenotype and restores recombination proficiency to *ruvC* mutants. The suppressor, defined as *rus-1*, was found to be an IS*2* insertion that activates the expression of a 14-kDa protein (24a). Sharples et al. (41) recently demonstrated that the purified protein cleaves Holliday junctions in a manner very similar to that catalyzed by RuvC.

Although these results indicate that *E. coli* may have a second Holliday junction resolvase, it is currently unclear whether the gene encoding the 14-kDa Rus protein is expressed in normally growing *E. coli* (21a). New studies indicate that the gene encoding Rus forms part of the defective prophage DLP12 which maps at 12.5 min on the *E. coli* genetic map (43a). Interestingly, the *rus* gene is 98% identical to an open reading frame in bacteriophage 82. Strains carrying *ruvC rus* mutations exhibit a genetic phenotype similar to that of *ruvC* single mutants.

In conclusion, the initial discovery of resolvase activity in *E. coli* has led to many surprises and several new proteins. Certainly, few would have predicted that simple organisms would contain specialized branch migration proteins, since this role was thought to be carried out by RecA itself. Now that the key players responsible for Holliday junction processing appear to have been identified, it will be possible to further develop in vitro systems for branch migration and resolution and hopefully define whether RuvAB and RecG functions truly overlap. Other key questions, such as whether there is a functional analog of RuvC waiting to be discovered, need to be answered, and it is likely that a combination of molecular genetics and biochemistry will reveal further insights into the complexity of the process. I suspect that it is unlikely that all the secrets of Holliday junctions and their suitors have now been revealed.

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Without the efforts and insights of my colleagues, both past and present, this minireview would have little to say. I am therefore indebted to them for making the discoveries that have led to the burst of activity in this area of research. Thanks go to Robert Lloyd and Andrzej Stasiak for enlightening discussions and to Hideo Shinagawa for communication of results prior to publication.

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