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# Mechanism and regulation of incisions during DNA interstrand cross-link repair

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ABSTRACT

A critical step in DNA interstrand cross-link repair is the programmed collapse of replication forks that have stalled at an ICL. This event is regulated by the Fanconi anemia pathway, which suppresses bone marrow failure and cancer. In this perspective, we focus on the structure of forks that have stalled at ICLs, how these structures might be incised by endonucleases, and how incision is regulated by the Fanconi anemia pathway.



## 1. Introduction

DNA interstrand cross-links (ICLs) are cytotoxic lesions that covalently link the Watson and Crick strands of DNA. From a human health perspective, there are two primary motivations to study ICL repair. First, ICL repair is defective in Fanconi anemia (FA), a human genetic disease caused by biallelic mutations in any one of 16 different FANC genes [7,33,38]. FA is characterized by congenital abnormalities, bone marrow failure, and cancer predisposition. If ICL repair defects indeed cause FA, as is widely believed, understanding how ICL repair normally occurs and why it fails in patients might point the way to a cure for FA. Second, ICL-inducing agents are widely used in cancer chemotherapy. However, cancers almost invariably become resistant to these agents, in some cases due to up-regulation of repair. Novel inhibitors of ICL repair might augment the efficacy of ICL-inducing agents for chemotherapy, although this might also cause enhanced toxicity.

The major ICL repair pathway operating in proliferating cells is coupled to DNA replication [1,56,58,65]. When forks collide with an ICL, repair is initiated through the excision of the ICL from one parental strand (Fig. 1A). This releases or "unhooks" one daughter duplex from the ICL, forming a double-stranded DNA break that must subsequently be repaired. ICL repair is thus a rare instance in which stalled replication forks undergo programmed collapse, and recent evidence suggests this process is dependent on the FANC proteins [37]. As such, programmed fork collapse can be regarded as a unique event that distinguishes ICL removal from other forms of DNA repair. To shed light on the mechanisms by which forks are processed during ICL repair, we consider here the possible structures of stalled forks prior to collapse and how diverse endonucleases might act on these structures. We also consider the regulation of fork collapse by the FANC proteins.

## 2. Early models of ICL repair

Genetic analysis has identified four major classes of gene products that confer resistance to ICLs. (1) Structure-specific endonucleases, which recognize and incise specific DNA structures. (2) Translesion DNA synthesis (TLS) polymerases, error prone polymerases that are able to tolerate DNA damage in the template strand. (3) DNA recombinases, proteins that mediate strand exchange during homologous recombination. (4) 16 FANC proteins, which are mutated in FA. In the FA "pathway," eight "group I" FANC proteins assemble into a core complex that mono-ubiquitylates a

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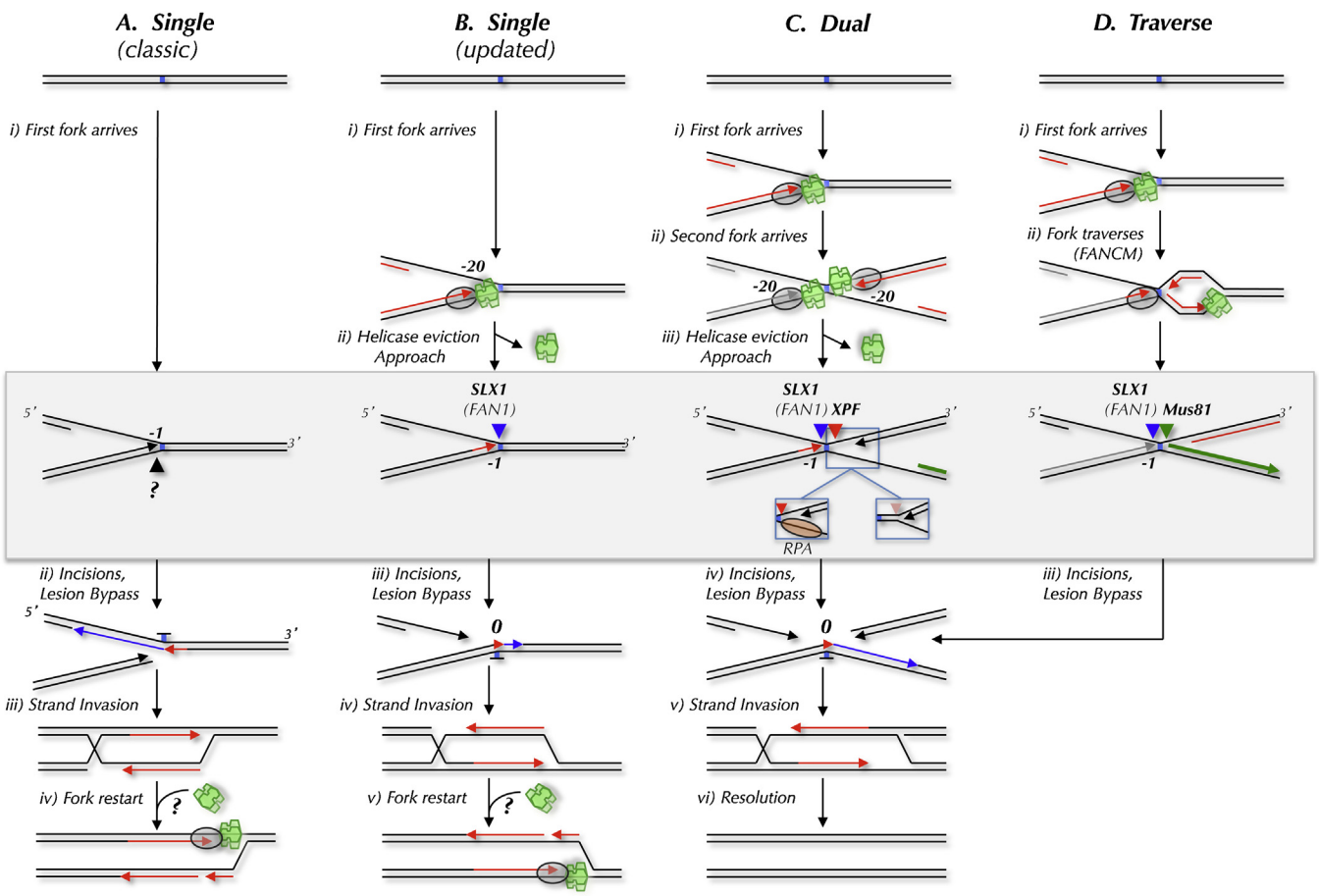


**Fig. 1.** Possible mechanisms of replication-coupled ICL repair. Four mechanisms of replication-dependent ICL repair are depicted. The DNA structures acted on by endonucleases in each model are highlighted by a gray box. Incisions are represented by black, blue, red, and green arrowheads. The proposed nuclease(s) that performs each incision is indicated above the arrowhead. (A) The classic ICL repair model, in which a single replication fork collides with the ICL and the leading strand template is incised [50]. (B) The classic model, but taking into account the observation that leading strands initially stall 20 nucleotides from the ICL due to the MCM2-7 complex, and that incision occurs on the lagging strand template [56]. In models A and B, fork restart would require re-loading of the MCM2-7 complex, for which there is no known mechanism. (C) The dual fork convergence model [56]. Left inset, 3′ incision substrate if RPA binds the lagging strand template after MCM2-7 removal. Right inset, 3′ incision substrate if parental strands re-anneal after MCM2-7 removal. (D) Traverse model [29]. The only difference in the incision substrate in the dual fork and traverse models is the location of the 5′ end of the nascent strand on the right side of the ICL (green strand).

heterodimer of two "group II" FANC proteins, FANCI and FANCD2 (the "ID" complex) [2,21,61]. The mono-ubiquitylated ID complex (ID-Ub) is essential for ICL repair [21,37]. The six remaining "group III" FANC proteins fall into the recombinase and nuclease categories. Given the four classes of proteins implicated in ICL repair and the coupling of repair to DNA replication, the following model crystallized several years ago [50,69]. Repair is triggered when a DNA replication fork collides with the ICL (Fig. 1Ai). This creates a substrate for structure-specific endonucleases, which incise the fork, unhooking the cross-link and generating a double-stranded DNA break (DSB) (Fig. 1Aii). The unhooked ICL is bypassed by translesion DNA polymerases (Fig. 1Aii). Finally, the fork is restored via homologous recombination (Fig. 1Aiii). Although this model accounted for the different gene products implicated in ICL repair and the S phase dependence of repair, it lacked molecular detail. Thus, the precise nature of the DNA intermediates involved remained unclear, making it difficult to understand how the endonucleases and other proteins participate in repair. In addition, it was unknown how the FA pathway promotes repair.

## 3. The dual fork convergence model

More recently, replication-dependent ICL repair was recapitulated in *Xenopus* egg extracts, allowing a more detailed description of repair intermediates [56]. When a 6 kb plasmid carrying a single, site-specific ICL is incubated in egg extract, a significant fraction of the lesions is repaired in a replication-dependent manner. Repair begins when two replication forks converge on the ICL (Fig. 1Ci and Cii). The 3′ ends of both converged leading strands initially stall 20–40 nucleotides from the ICL due to steric hindrance by the MCM2-7 helicase, which translocates along the leading strand template ahead of the polymerase [20]. Upon collision with the ICL, the 5′ ends of lagging strands are located 50–300 nucleotides from the lesion, and they subsequently undergo resection. Concurrent with MCM2-7 release from the ICL, one leading strand advances to within 1 nucleotide of the ICL (Fig. 1Ciii; "Approach"). After Approach, the opposing parental strand is incised on either side of the ICL, leading to unhooking of the ICL and formation of a DSB (Fig. 1Civ). In the absence of ID-Ub, incisions are severely impaired and the leading strand remains stuck 1 nucleotide from the lesion [37]. After incisions, the lesion is bypassed in two steps. First, a nucleotide is inserted across from the damaged base by an unknown translesion DNA polymerase (Fig. 1Civ, red arrowhead). The resulting abnormal primer template is then extended by DNA polymerase ζ (Fig. 1Civ, blue arrow). Finally, the DSB is repaired via Rad51 dependent strand exchange with the intact sister chromatid [43]. In the *Xenopus* system, a vestige of the ICL remains attached to one parental strand. This observation implies either that the incisions occur very close together, or that the oligonucleotide between

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