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

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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
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. 1Cii; "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 arrow). The resulting abnormal primer template is then extended by DNA polymerase η (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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the incisions is processed by a nuclease. Either way, the final adduct is not removed in egg extracts.

Certain features of the dual fork mechanism likely apply to the single fork model (Fig. 1B). Thus, when a single fork strikes an ICL, the leading strand probably also stalls 20 nucleotides from the lesion due to the MCM2-7 footprint (Fig. 1B), and incisions are likely to require eviction of the MCM2-7 complex (Fig. 1Bii). Whether incisions occur in the leading (Fig. 1Aii) or lagging strand template (Fig. 1Bii) of a single stalled fork remains unclear. However we favor the latter scenario because this allows extension past the lesion using the existing replication apparatus (Fig. 1Bii). In addition, as discussed below, it is not obvious which endonuclease would cleave the leading strand template.

4. Merits of the single versus dual fork collision models

Which of the two collision models applies in cells is subject to lively debate [39,45,56]. The single fork mechanism has at least one potential disadvantage. Unlike bacteria [26] and budding yeast [3], metazoans do not contain a known pathway for replicative DNA helicase loading in S phase. Therefore, the restored fork is unlikely to resume synthesis and would have to await the arrival of a converging fork, as seen after HU-induced replication fork collapse [54]. Such a helicase-deficient, stationary fork might be unstable and cause genomic rearrangements. The major criticism of the dual fork model is that it was observed in the context of a small plasmid where two forks inevitably converge on the ICL. In vivo, where origins are spaced 100 kb apart and forks move at a rate of 1.5 kb/min [16], most of the time one fork is expected to strike an ICL well before a converging fork arrives. Nevertheless, two forks will converge on an ICL when the lesion is located midway between two origins that fire contemporarily. Moreover, given that fork processing requires MCM2-7 dissociation, leading strand approach to the ICL, and nuclease recruitment, which together takes at least 30–40 min [20,36,56], there will exist a significant temporal window during which a second fork can arrive before incisions take place. Thus, based on first principles, it seems likely that both single and dual fork collisions will occur at significant frequencies, necessitating pathways to resolve both structures.

5. The traverse model

The single versus dual fork debate recently took an unexpected turn when Seidman and colleagues investigated the encounter of DNA replication forks with fluorescently tagged ICLs in vivo using DNA combs [29]. The work showed that single and dual fork collisions each comprise 15–20% of ICL encounters. Surprisingly, in ~60% of cases, DNA replication forks bypass or “traverse” ICLs without unhooking them (Fig. 1Dii). An earlier study inferred that forks generally stall at ICLs, but in this case, the location of ICLs relative to DNA replication tracts was not determined [67]. Traverse requires the translocase activity of FANCm but not other FANC proteins and is very rapid, taking only a few minutes. When FANCm is defective, the frequency of traverse events goes down while single fork collisions go up, indicating that single fork collisions are followed by traverse. It is unclear whether MCM2-7 jumps over the ICL during traverse, a new MCM2-7 molecule is recruited on the other side of the ICL (unlike given the absence of known S phase MCM2-7 loading mechanisms), or a different DNA helicase loads distal to the ICL. Interestingly, FANCm has been shown to recruit RPA to an ICL in duplex DNA, suggesting it helps to melt DNA in the vicinity of these structures [30], which might facilitate traverse. In summary, it appears that the most common substrate for incisions during ICL repair is an X-shaped DNA molecule, which is generated by some combination of fork traverse and fork convergence events.

6. Can a single fork trigger ICL repair?

The apparent preponderance of X-shaped structures at ICLs in vivo raises the important question of whether a single fork that has not traversed an ICL can trigger repair. Replication of a psoralen ICL in Xenopus egg extracts suggested that sometimes, a single fork can trigger incisions, but it was unclear whether the observed incisions led to a productive repair outcome [41]. In mammalian cells, repair of an ICL flanked by a replication roadblock suggested that a single fork can trigger repair [49]. However, the possibility of traverse was not considered and the efficiency of the roadblock was not determined, leaving open the possibility that an X-shaped structure was the substrate for repair. In the future, it will be critical to directly compare the efficiency of processing and repair of single stalled forks, converged forks, and traversed forks.

7. What DNA structures are formed at ICL-stalled forks?

A detailed knowledge of the DNA structures created after forks encounter an ICL is critical to understand the mechanism of incisions. During repair of ICLs in egg extracts, incisions occur only after one leading strand has advanced to within 1 nucleotide of the ICL (−1 position) [56]. Thus, on the 5′ side of the ICL (left side in Fig. 1C–D), nucleases act on a replication fork whose leading strand abuts the lesion. The structure on the 3′ (right) side is less clear. After MCM2-7 eviction from converged forks [20], the ssDNA between the ICL and the 3′ end of the leading strand (located 20–40 nucleotides from the ICL) might not re-anneal due to RPA binding (Fig. 1Dii, left inset). Since RPA’s preferred binding mode involves a 30 nucleotide footprint [70], it is likely to preferentially occupy the lagging strand template where more ssDNA is available. In this case, incisions by a 3′ flap endonuclease would likely occur close to the ICL (Fig. 1Dii, left inset, red arrowhead). Alternatively, after MCM2-7 departure, the parental strands might re-anneal up to the 3′ end of the leading strand (Fig. 1Dii, right inset). In this case, incisions would likely occur near the dsDNA–ssDNA junction (right inset, pink arrowhead), further away from the ICL, but as discussed below, this is not an ideal substrate for 3′ flap endonucleases. The structure generated during traverse would be similar to the structure that results when two forks converge. The only possible difference is that if DNA synthesis re-initiates just beyond the ICL, the 5′ end of the new leading strand after traverse will be closer to the lesion than at a converged fork (compare green strands in Fig. 1C and D). This would affect which endonucleases are able to cut the structure (see below). For single fork collisions without traverse, the 3′ side of the ICL will be double-stranded (Fig. 1A and B), and therefore not recognized by structure specific endonucleases, unless the ICL is distorting, in which case there could be a limited amount of single-stranded DNA 3′ to the ICL. Most likely, a diversity of structures exists at ICL-stalled forks, which might help explain the many endonucleases that have been implicated in ICL repair.

8. Endonucleases

Six different nucleases (XPF-ERCC1, MUS81-EME1, SLX1-SLX4, FAN1, SNM1A, and SNM1B) have been implicated in ICL repair. For each nuclease, we discuss the evidence linking it to the incision step, its specificity, and how it might act on the structures shown in Fig. 1.

9. XPF-ERCC1

The best candidate for an incision endonuclease is XPF-ERCC1, which is best known for its role in nucleotide excision repair (NER) [11]. XPF contains an excision repair cross-complementation
group 4 (ERCC4) endonuclease domain and a Helix-hairpin-Helix (HhH) DNA binding domain. ERCC1 contains the same domains but the endonuclease domain has acquired mutations that render it catalytically inactive. The preferred substrate of XPF-ERCC1 is a “splayed arm” structure, which it cuts at the base of the 3′ arm, a few nucleotides internal to the DNA duplex (Fig. 2A). XPF-ERCC1 also cuts the 3′ flap structure depicted in Fig. 2B, but less efficiently than splayed arms [13], and it does not cut the 3′ arm of 5′ flap structures (Fig. 2C) [57]. During NER, XPF-ERCC1 helps remove bulky lesions by cutting on the 5′ side of an open bubble structure surrounding the lesion (Fig. 2E). XPF-ERCC1 is unique among NER proteins in conferring cellular resistance to ICLs [15,40,51]. Mutations in ERCC1 that disrupt its interaction with XPA, another NER factor, prevent NER but not ICL repair, showing that the two functions of this nuclease can be uncoupled [52]. Mutations in mouse Ercc1 cause a spectrum of phenotypes reminiscent of Fanconi anemia [28,47,55], and recently, XPF mutations were discovered in Fanconi patients [7,33]. Together, the data strongly suggest that XPF-ERCC1 operates in the FA-dependent ICL repair pathway.

The specific function of XPF in ICL repair has been slow to emerge. In the absence of XPF or ERCC1, ICL-induced DSBS accumulate and persist, leading to the conclusion that XPF is not required for the first incision of ICL unhooking [15,51]. In contrast, recent results from the Knipscheer laboratory show that in XPF-depleted Xenopus egg extracts, forks that have converged on an ICL are not incised [36]. These apparently contradictory results can be reconciled by the proposal that XPF is responsible for initial ICL incision in both systems, but that in cells, forks persisting in the absence of XPF are aberrantly cleaved by MUS81 [68].

Given its preference for the 3′ arm of a splayed arm structure (Fig. 2A), XPF-ERCC1 probably cuts on the 3′ side of ICL-associated X-shaped structures (red arrowhead in Fig. 1C). XPF’s cleavage of 3′ splayed arms is dramatically enhanced by RPA binding to the 5′ arm [14], as depicted in Fig. 1Cii (left inset.) However, when the leading strand is present at the junction as depicted in Fig. 1Ciii (right inset), XPF may not cut efficiently [57], requiring another solution (see SNM1A section below). In Xenopus egg extracts, neither of the two incisions required for unhooking of converged forks occur in the absence XPF-ERCC1, or when XPF-depleted extracts are supplemented with catalytically inactive XPF-ERCC1 [36]. One explanation for this observation is that the 5′ incision cannot take place without the 3′ incision. A precedent for coupling between dual incisions is observed in NER, where incision by XPG 3′ to a lesion requires prior incision by XPF-ERCC1 on the 5′ side [62] (Fig. 2E). Another possibility is that XPF performs both incisions. Thus, in the context of a splayed arm structure containing an ICL, purified XPF-ERCC1 cuts on the 5′ and 3′ sides of the lesion (Fig. 2F) [18,40]). It is presently unclear how this activity would be affected by the presence of a fork on the other side of the ICL. In summary, although XPF-ERCC1 is almost certainly the primary 3′ incision endonuclease, the precise mechanism and circumstances of its action remain to be elucidated.

10. MUS81-EME1

Like XPF, the MUS81-EME1 heterodimer belongs to the XPF/MUS81 nuclease family, with MUS81 contributing the catalytic active ERCC4 domain [11], MUS81-EME1 greatly prefers 3′ flap structures that contain a 5′ end within 4 nucleotides of the flap junction (Fig. 2B) [6,10,11]. Mutations in MUS81-EME1 render cells sensitive to ICLs, but not as sensitive as mutations in XPF-ERCC1 [12,68]. This suggests that MUS81-EME1 plays a secondary role in ICL repair. While early experiments in mammalian cells suggested that MUS81 is required to generate ICL-induced DSBS [23], implying a role in incisions, more recent evidence indicates that MUS81 generates DSBS when normal fork processing is disrupted [68]. Consistent with the latter view, depletion of MUS81-EME1 had no effect on the incision of converged forks in Xenopus egg extracts.
[36]. We propose that MUS81 only acts on a subset of the intermediates generated when forks encounter ICLs. For example, if leading strand synthesis resumes immediately downstream of the ICL during fork traverse, a 5′ end is located near the flap junction (Fig. 1D; green arrow). The 5′ end disfavors cutting by XPF–ERCC1 [13,57], but creates an ideal substrate for MUS81–EME1. In summary, we propose that the primary role of MUS81–EME1 in ICL repair is to cut on the 3′ side of X-shaped structures in which a 5′ end abuts the ssDNA–dsDNA junction, possibly during a subset of traverse events.

Importantly, the structure generated during a single-fork collision lacks a 5′ end near the junction and therefore the leading strand template should not be cleaved efficiently by MUS81 (Fig. 1A–B). It may also not be cut efficiently by XPF, which is inhibited by the presence of a 3′ end at the junction [57]. Thus, no enzyme implicated in ICL repair is well suited to incise the leading strand template of a single stalled fork (Fig. 1A, black arrowhead). These considerations suggest that single forks should be cleaved on the lagging strand template by a 5′ flap endonuclease (Fig. 1B, blue arrowhead).

11. SLX1

SLX1 is a structure-specific endonuclease containing a UvrC-intron-endonuclease domain (URI) and a PHD-type zinc finger domain [5]. SLX1′s activity is dramatically stimulated through interaction with a non-catalytic subunit, SLX4. Homozygous deletion of the SLX1 gene in mice causes sensitivity to ICLs similar to that of MUS81, and rescue of this sensitivity requires the catalytic activity of SLX1 [8]; SLX1–SLX4 is a rather promiscuous endonuclease, in that it cleaves spliced arms, 5′ flaps, 3′ flaps, and Holliday junctions [19,71]. However, its preferred substrate is a 5′ flap, which it cuts at the ssDNA–dsDNA junction (Fig. 2C). In the context of ICL repair, a 5′ flap-like structure is generated on the 5′ side of the ICL when the leading strand of a stalled fork has been extended to the −1 position (Fig. 1A–D). Based on this and considerations discussed below, we propose that SLX1 is the primary nuclease that cleaves on the 5′ side of the ICL.

12. Scaffolding by SLX4

Accumulating evidence indicates that SLX4 serves as a master scaffold for incisions. Deletion of SLX4, the binding partner of SLX1, causes much greater sensitivity to ICLs than deletion of SLX1 or MUS81 [8], and it is required for incisions in Xenopus egg extracts [36]. Underscoring its importance for ICL repair, SLX4 is a Fanconi gene [34,63]. Strikingly, SLX4 co-precipitates with XPF–ERCC1, SLX1, and MUS81–EME1 [4,17,48,64]. SLX4 binds SLX1 via a helix-turn-helix motif (also referred as SBD: SLX1 binding domain), MUS81 via a SAP motif, and XPF via an MLR motif (Fig. 3). The binding of SLX4 to XPF–ERCC1 and SLX1 is critical for the action of these nucleases in ICL repair, probably by recruiting them to sites of damage [8,12,35,63]. In contrast, the interaction of MUS81 with SLX4 is dispensable for ICL repair [8]. Together, the data indicate that XPF–ERCC1–SLX4–SLX1 represents a core “XESS” complex that incises ICL-associated X-shaped structures (Fig. 3). MUS81 is employed for special scenarios, i.e. when the 3′ side of the ICL contains a true 3′ flap (Fig. 1D). Importantly, SLX4 appears to be more than just a recruitment platform, since it dramatically enhances XPF nuclease activity toward spliced arm and ICL-containing structures [27]. While SLX1 is probably the primary 5′ endonuclease for most situations, the mild ICL-sensitivity of SLX1 mutants suggests its function can be replaced by another endonuclease (next section).

13. FAN1

Fanconi Associated Nuclease 1 (FAN1) contains a UBZ4-type ubiquitin binding domain, a SAP DNA binding domain, and a PD-D/E/X/K nuclease motif, placing it in the same nuclease superfamily as XPF and MUS81 [39]. Like SLX1, FAN1 prefers 5′ flap structures. However, unlike SLX1, which cleaves at the ssDNA–dsDNA junction, FAN1 cleaves four nucleotides 3′ to the branch point (Fig. 2D) [39,42,45,60]. FAN1 also exhibits robust 5′→3′ exonuclease activity of 5′ recessed DNA ends, nicks, or gaps. FAN1 gene knockdown or knock-out selectively sensitizes cells to ICL-inducing agents, although not to the same extent as disruption of the FA pathway [39,42,45,60,66,73,76]. FAN1 and FA pathway mutations are not epistatic, suggesting distinct roles in ICL repair [73,76]. Indeed, FANC mutations cause much greater damage-induced chromosomal instability than FAN1 mutations [66,73,75]. Consistent with these observations, FAN1 and FANC mutations cause distinct clinical phenotypes, the former being associated with karyomegallic interstitialnephritis, a form of chronic kidney disease instead of FA [66,76]. Together, the data indicate that FAN1 participate in ICL repair, but via a distinct and secondary role relative to the FA pathway.

The function of FAN1 in ICL repair remains enigmatic. ICL-induced γH2AX foci still form in the absence of FAN1, leading to the proposal that FAN1 might function in the HR step of ICL repair, downstream of incisions [45]. However, γH2AX foci are caused by DNA damage other than dsDNA breaks [54], leaving open the possibility that FAN1 is required for incisions. When FAN1 is depleted from Xenopus egg extracts, there is no detectable repair or incision defect [36], but this might be due to redundancy with other nucleases such as SLX1. In the future, SLX1–FAN1 redundancy should be examined using biochemical and genetic ICL repair assays. A reasonable working hypothesis is that FAN1 plays a secondary role in cleaving the 5′ side of ICL-associated X-shaped structures (Fig. 3; light green line).

14. SNM1 family nucleases

SNM1 family nucleases (SNM1A, SNM1B, and SNM1C) belong to the β-CASP subfamily of metallo-beta-lactamases, which are DNA processing enzymes [9]. The single SNM1 family member in yeast, Pso2, exhibits singular sensitivity to ICLs but not other damaging agents. In vertebrates, SNM1A and SNM1B mutants are selectively sensitive to ICLs, with SNM1A showing the greatest sensitivity [9,31]. In contrast, SNM1C/Artemis mutants are not sensitive to ICLs but to ionizing radiation, consistent with this protein’s role in non-homologous end-joining. While SNM1A and B are reported to
exhibit non-epistasis with homologous recombination, translesion DNA synthesis, and FANC genes in chicken cells [31], there are hints of an epistatic relationship with XPF in mammalian cells [68]. Thus, although SNM1A and B appear to participate in ICL repair, whether they function in the FA-dependent pathway remains uncertain.

Given its greater sensitivity to ICLs, functional complementation of yeast pso2 mutants [24], and more robust nuclease activity [59], we will focus on SNM1A. Unlike the endonucleases discussed above, SNM1A functions as a 5′→3′ exonuclease with no obvious role in incisions [24,25]. Consistent with this, pso2 mutants in yeast still form ICL-induced dsDNA breaks [22,46]. Interestingly, SNM1A can use its 5′→3′ exonuclease activity to digest one DNA strand a few nucleotides past an ICL (Fig. 2G [68]). This suggests that as long as an incision is made on the 5′ side of an ICL (e.g. by SLX1 or FAN1), SNM1A might be able to complete the unhooking reaction without the need for 3′ endonucleolytic cleavage by XPF or MUS81.

Exonucleolytic degradation by SNM1A therefore might replace the 3′ incision in cases where the 3′ side of the ICL is not ideal for incision by XPF or MUS81 (as in Fig. 1Ciii, right inset). Another possible role for SNM1A is that after 5′ and 3′ incisions have occurred, SNM1A reduces the oligonucleotide between the incision points to a monoaduct that can be bypassed by translesion DNA polymerases.

15. Regulation of ICL processing

How does the FA pathway regulate incisions [37]? Upon exposure of cells to ICLs, the ID-Ub complex forms DNA damage foci that overlap with several DNA repair factors, including Rad51 and BRCA1, suggesting it binds to sites of damage [65]. Consistent with this idea, during repair of an ICL in egg extracts, ID-Ub localizes to the ICL immediately before incisions take place, as measured by chromatin immunoprecipitation [36]. Moreover, FANCi and FANCd2 bind preferentially to a variety of branched DNA structures [32,44,53,74], and the crystal structure of FANCi with DNA suggests that the ID complex should be able to accommodate an ICL-associated X-shaped structure (Fig. 3 [32]). Thus, ID-Ub likely binds directly to ICLs, allowing it to exert local control over the process of incisions (Fig. 3).

Several connections between the ID complex and the incision machinery have now been observed. First, the recruitment of FAN1 to damage foci in cells requires ubiquitylated FANCd2, as well as the UBZ domain of FAN1 [39,42,45,60]. On the other hand, FAN1 and the FANCi genes are not epistatic [73,76], and a FAN1 mutant disrupting the UBZ domain is functional for ICL resistance [76]. These data suggest that although FAN1 can be recruited to ICLs via ID-Ub, this is not essential in some cases, perhaps due to another, independent recruitment pathway (Fig. 3, dashed blue arrows). The connection between ID-Ub and XPF is more compelling. Most importantly, in Xenopus egg extracts, the binding of SLX4 and XPF to ICLs during repair requires ubiquitylated FANCd2 [36]. This observation is consistent with the earlier finding that SLX4 damage localization in cells depends on the SLX4 UBZ motif and ubiquitylated FANCd2 [172], but see [35]. Although the molecular details remain unclear, the data strongly suggest that ID-Ub recruits the XESS complex to sites of damage.

Why has a system as elaborate as the FA pathway evolved to regulate incisions? We propose this regulation arose to deal with the complexity inherent in ICL-associated X-shaped structures. In principle, these structures could be cleaved by structure-specific endonucleases in any one of the four arms that meet at the ICL. While several combinations of cuts are possible, the only one that yields a productive outcome involves incising the same parental strand on either side of the lesion (Fig. 3, red and green arrows), or incising on the 5′ side of the ICL and using an exonuclease to degrade past the ICL. We propose that ID-Ub has at least two functions. First, it recruits the XESS complex to the lesion to promote 5′ and 3′ incisions of one parental strand. Second, it embraces the ICL in such a manner as to suppress incisions on the other parental strand. Ultimately, to understand how ID-Ub controls incisions, the reaction will have to be reconstituted with purified components and a crystal structure of the ubiquitylated ID complex with an appropriate X-shaped structure will be needed. However, already one can envision that ID-Ub sequesters the lower strand of the X-shaped structure to protect it from cleavage (Fig. 3). It is important to note that the X-shaped DNA structure is not symmetrical, since a 3′ end abuts the ICL on just one side. This would allow the ID complex to interact differentially with the two parental strands.

16. Outlook

It is now clear that a signature event in ICL repair is the ID-Ub-dependent incision of stalled replication forks. The discovery of the XESS complex lays the foundation to understand how these incisions are carried out and regulated. Going forward, it will be critical to re-examine the specificities of all the relevant endonucleases on DNA substrates that better mimic the various structures predicted to exist at ICL-stalled DNA replication forks (Fig. 1A–D), including with distorting and non-distorting ICLs. This should help clarify which endonuclease is employed under which circumstances. A more ambitious goal is to reconstitute ID-Ub-dependent incisions with defined components so that we may precisely define the function of the Fanconi anemia pathway. These biochemical approaches will have to be complemented with incision assays under physiological conditions. Finally, it will be critical to understand why deficiencies in two endonucleases (FAN1 versus XPF-ERCC1-SLX4) that appear to resolve the same type of DNA damage cause such different clinical phenotypes. As we address these important issues, nature will undoubtedly serve up more surprises.

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References

DNA repair network, which is critical for maintaining genetic stability and preventing cancer. The discovery of the Fanconi Anemia (FA) pathway has revolutionized our understanding of the molecular mechanisms underlying DNA repair in human cells.

The FA pathway is a conserved genetic pathway that functions to coordinate DNA repair and cell cycle checkpoints in response to DNA damage. Mutations in the genes encoding FA proteins lead to a spectrum of clinical syndromes, including FA, anemia, and cancer susceptibility.

The FA pathway has been extensively studied in model systems, such as yeast and fruit flies, and in human cells. This research has provided valuable insights into the molecular mechanisms underlying DNA repair and cell cycle checkpoint regulation.

In this context, Jiri Zichner and colleagues have published a comprehensive review on the FA pathway in the journal Nature Reviews Genetics. Their work highlights the latest advances in our understanding of the FA pathway, including the identification of new FA genes, the molecular mechanisms underlying FA protein function, and the clinical implications of FA.

The FA pathway is mediated by a network of proteins that work in concert to repair DNA damage and maintain genomic stability. This network includes the FA core complex, which consists of FA proteins such as FANCD2 and FANCD20, as well as other proteins such as FANCI, FANCN, FANCQ, and FANCQ2.

The FA pathway is activated in response to DNA damage, such as DNA interstrand cross-links, which are formed when the DNA strands are covalently joined, preventing separation of sister chromatids. The FA pathway is involved in the repair of DNA interstrand cross-links, which are formed when the DNA strands are covalently joined, preventing separation of sister chromatids. The FA pathway is involved in the repair of DNA double-strand breaks, which are formed when both DNA strands are broken, and in the repair of DNA single-strand breaks, which are formed when only one DNA strand is broken.

In summary, the FA pathway is a critical component of the DNA repair network, which is essential for maintaining genetic stability and preventing cancer. The latest advances in our understanding of the FA pathway have provided valuable insights into the molecular mechanisms underlying DNA repair and cell cycle checkpoint regulation.

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