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What is the DNA repair defect underlying Fanconi anemia?

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Fanconi anemia (FA) is a rare human genetic disease characterized by bone marrow failure, cancer predisposition, and genomic instability. It has been known for many years that FA patient-derived cells are exquisitely sensitive to DNA interstrand cross-linking agents such as cisplatin and mitomycin C. On this basis, it was widely assumed that failure to repair endogenous interstrand cross-links (ICLs) causes FA, although the endogenous mutagen that generates these lesions remained elusive. Recent genetic evidence now suggests that endogenous aldehydes are the driving force behind FA. Importantly, aldehydes cause a variety of DNA lesions, including ICLs and DNA protein cross-links (DPCs), re-kindling the debate about which DNA lesions cause FA. In this review, we discuss new developments in our understanding of DPC and ICL repair, and how these findings bear on the question of which DNA lesion underlies FA.

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Introduction

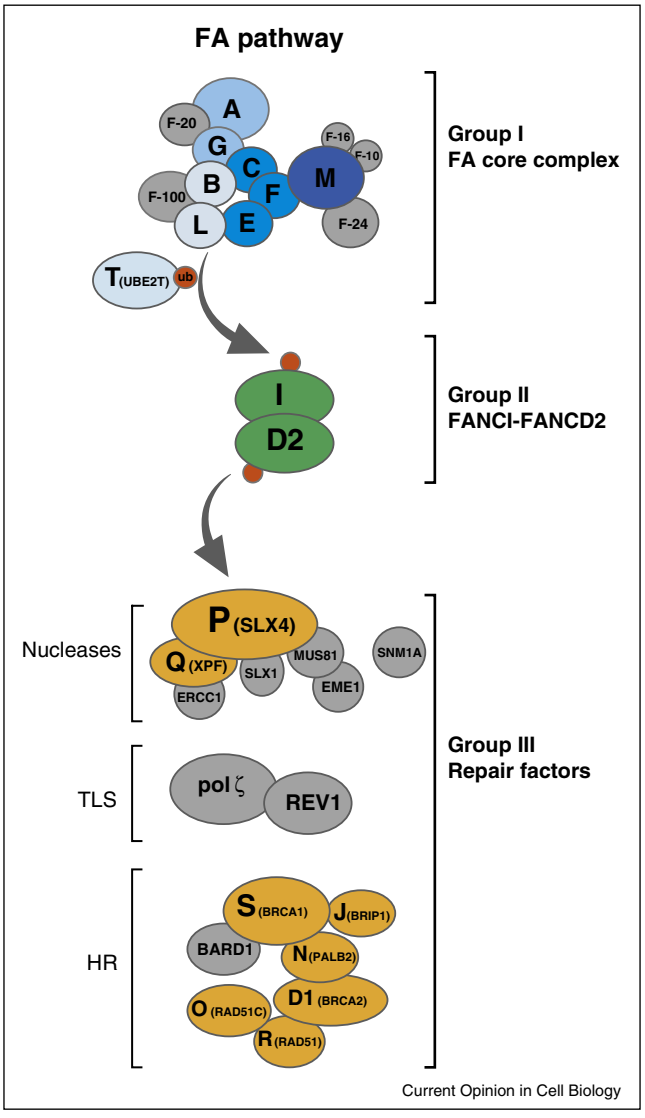
In 1927, the Swiss pediatrician Guido Fanconi described three brothers who presented with developmental birth defects and died of a condition resembling pernicious anemia [1,2]. He soon realized that the disease affected all blood lineages, and that it also involves cancer predisposition. Fanconi's anemia (now referred to as Fanconi anemia; FA) was subsequently recognized as a rare genetic disorder inherited as a Mendelian recessive trait that affects 1 in every ~100 000 births. So far, 19 FANC genes have been identified, mutations in which cause FA. While mutations in most complementation groups cause the full spectrum of FA-associated phenotypes (congenital abnormalities, early onset bone marrow failure (BMF), predisposition to acute myeloid leukemia and solid tumors), some complementation groups (e.g. FANCD1, N, O, S and R) exhibit a subset of these features.

In the 1970s, researchers discovered that FA cells undergo chromosome breakage upon treatment with crosslinking agents such as mitomycin C (MMC) or diepoxybutane [3••,4], suggesting that an inability to repair DNA interstrand cross-links (ICLs) underlies FA. ICLs are cytotoxic lesions that covalently link the two strands of the double helix, thereby inhibiting any process that requires DNA unwinding, including DNA replication and transcription. Two distinct mechanisms of ICL repair have been described. One mechanism is tightly coupled to DNA replication and requires the FANC proteins [5•,6••,7,8,9•]. The other operates outside of S phase, involves nucleotide excision repair but not the FANC proteins, and may sometimes be coupled to transcription [10,11].

The FA pathway

The proteins encoded by the 19 FANC genes coordinate different steps of ICL-repair and can be placed into 3 groups based on their functions [12]. The group I proteins FANCA, B, C, E, F, G, L, and M, together with three Fanconi associated proteins (FAAP20, FAAP24 and FAAP100), assemble into a large FA core complex, which functions as an E3 ubiquitin ligase (Figure 1, group I). The core complex associates with chromatin upon DNA damage or replication stress [13,14], and it mono-ubiquitylates the group II proteins FANCI and FANCD2, which form a heterodimer called the ID_2 complex [15••,16••] (Figure 1, group II). Mono-ubiquitylated ID_2 binds to chromatin and is required to suppress ICL sensitivity. Extensive evidence suggests the existence of distinct functional modules within the core complex. FANCM interacts with FAAP24 and a dimer of histone-fold containing proteins, MHF1 and MHF2 (also known as FAAP16 and FAAP10) [17–19]. This heterotetrameric FANCM subcomplex recognizes model DNA structures that resemble replication forks [17], and this binding is thought to recruit the core complex to chromatin [14,20]. The FANCM subcomplex also regulates downstream repair and checkpoint signaling [21,22], presumably by remodeling stalled replication forks through FANCM's ATPase activity [23,24]. FANCB, FANCL, and FAAP100 form a minimal catalytic module in which the RING domain of FANCL ubiquitylates ID_2 [25•,26–28,29•]. UBE2T (recently identified as FANCT

Figure 1



The Fanconi anemia pathway. The FA pathway comprises 19 proteins that have been classified into three groups [12]. Upon detection of the crosslink, the FA core complex (group I, blue spheres) ubiquitylates the heterodimer FANCI-FANCD2 (ID_2) (group II, green spheres). Ubiquitylated ID_2 then coordinates processing by downstream repair factors (group III, orange spheres). Proteins shaded in grey are important for ICL repair and can be classified as group I–III, but they have not been found to be mutated in patients with FA. Although BRCA1 and RAD51 are considered to fall into group III, they also have functions upstream of ID_2 ubiquitylation [40•,61].

[30–32]) functions as the E2 ubiquitin-conjugating enzyme, and its interaction with FANCL is required for ID_2 monoubiquitination [33,34]. FANCA, FANCG, FAAP20 and FANCC, FANCE, FANCF form two other subcomplexes that are proposed to assist the catalytic subcomplex in binding to chromatin [26].

Mono-ubiquitylated ID_2 promotes repair of the ICL by group III proteins, which include the nuclease XPF (FANCQ) [35], the scaffolding protein SLX4 (FANCP) [36,37], and the homologous recombination (HR) factors PALB2 (FANCN) [38], BRCA2 (FANCD1) [39••], RAD51 (FANCR) [40•], RAD51 C (FANCO) [41], BRCA1 (FANCS) [42] and FANCJ (BRIP1) [43–45] (Figure 1, group III). Finally, the modified ID_2 complex is deubiquitinated by the ubiquitin specific peptidase 1 (USP1) [46] and its activating partner UAF1 [47]. Importantly many other factors participate in ICL repair including the nucleases SNM1A, SNM1B, FAN1, MUS81-EME1, SLX1, MRN and CTIP, and translesion (TLS) polymerases REV1 and polymerase ζ (pol ζ) [11]. In most cases, these factors were identified because their deficiency causes cellular sensitivity to ICLs. Whether all the above proteins actually operate in the FA pathway of ICL repair is presently unclear. For example, current evidence suggests that FAN1, originally identified as a nuclease that is recruited to sites of damage by ubiquitylated ID_2 [48–50], probably does not operate in the FA pathway of ICL repair [51•]. Consistent with this view, mutations in FAN1 are associated with karyomegalic interstitial nephritis, a form of chronic kidney disease, instead of FA [52•].

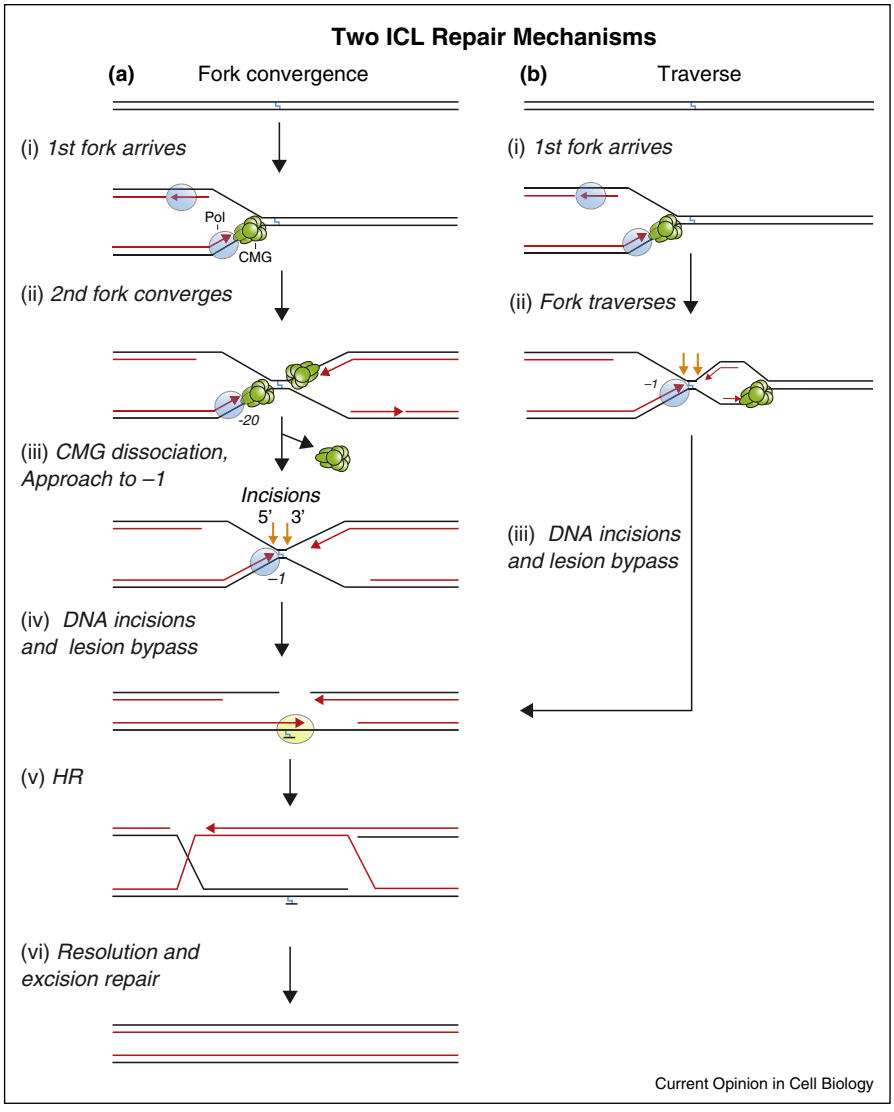
Some Fanconi proteins stabilize stressed replication forks. A new report identified a dominant negative RAD51 (FANCR) mutation that causes a Fanconi-like phenotype [40•]. While FANCR patient cells are HR proficient, they are sensitive to crosslinking agents, apparently due to over-resection of nascent strands by DNA2 [40•,53–55]. Interestingly, when forks are arrested with hydroxyurea, nascent strands are protected from Mre11-dependent degradation by BRCA1, BRCA2, and RAD51 [56,57], as well as FANCA and ubiquitylated FANCD2 [58•]. However, it seems unlikely that all FA genes, particularly those required for endonucleoytic cleavage of DNA (SLX4 and XPF), will be required to protect stalled forks in the absence of damage. As such, it seems unlikely that fork protection by FA proteins in the absence of damage is central to suppression of the FA phenotype.

Replication-coupled ICL repair: how does it work?

The participation of structure-specific endonucleases, TLS polymerases, and the HR machinery led to a model of ICL repair in which collision of a replication fork triggers incisions on either side of the ICL, followed by TLS, and HR-mediated fork restart [12,59]. However, a detailed mechanism, as well as the function of the FA pathway, remained elusive until replication-coupled ICL repair was recapitulated in *Xenopus* egg extracts [6••]. When a plasmid containing a site-specific ICL is incubated in egg extract, replication initiates at a random location on the plasmid, and two replisomes quickly converge on

the ICL (Figure 2a, i-ii). Upon collision with the ICL, leading strands of each replisome initially stall ~20 nucleotides from the lesion due to steric hindrance by the CMG helicase (a complex of Cdc45, MCM2-7, and GINS), which travels along the leading strand template and thus stalls at the lesion [60]. The first detectable event in repair is the active unloading of the stalled CMGs, which requires ubiquitin signaling and the BRCA1 (FANCS)-BARD1 tumor suppressor complex [60,61]. The mechanism of CMG unloading is still unknown but may involve ubiquitylation of the MCM2-7 complex [62,63] by BRCA1-BARD1. Once CMG is unloaded, leading strands are extended to within 1 nucleotide of the crosslink (-1 position), probably by the leading strand DNA polymerase ε [64] (Figure 2a, iii). Concurrent with this 'approach' of the leading strand, ubiquitylated ID_2 localizes to the ICL and promotes dual incisions on either side of the ICL ('unhooking') [65••] by recruiting a complex of the scaffolding protein SLX4 and the 3′ flap endonuclease XPF (FANCQ)-ERCC1 [66,67•] (Figure 2a, iv). Interestingly, in the absence of XPF, neither the 5′ nor the 3′ incision takes place [67•], suggesting that XPF might perform both incisions, as recently proposed [68•]. Alternatively, one or more 5′ flap endonucleases might incise the 5′ side but depend on prior action of XPF on the 3′ side. Indeed, the 5′ flap endonuclease SLX1 could be recruited through an interaction with SLX4 [69–72] while CTIP, a newly recognized 5′ flap endonuclease [73,74], might be recruited directly via ID_2 [75,76]. Analogous coupling between 3′

Figure 2



Mechanisms of ICL repair. Two mechanisms of ICL repair are depicted. **(a)** The fork convergence pathway, in which ICL repair is triggered when two forks converge on the lesion. **(b)** The fork traverse pathway, in which a single fork bypasses an ICL without unhooking the parental strands. Incisions are represented by orange arrows.

and 5′ flap endonucleases has been observed between XPF and XPG during nucleotide excision repair (NER) [77]. Dual incisions generate a two-ended double strand break (DSB) in one of the sister chromatids while leaving a DNA adduct on the other sister. The adducted chromatid is restored by TLS in a two-step reaction. First a nucleotide is inserted across from the damage base by an unknown polymerase. The abnormal primer template is then extended by a complex of REV1 and pol ζ, whose recruitment to chromatin requires the FA core complex [6••,64,78] (Figure 2a, iv). Finally, the DSB is repaired by Rad51-mediated HR utilizing the intact sister chromatid as a homology donor [79] (Figure 2a, v). In egg extracts, the remaining mono-adduct persists, but in cells, the adduct is likely removed by excision repair (Figure 2a, vi).

An important question is whether ICL repair requires the convergence of two forks on the lesion or whether one fork suffices, as proposed in most classical models of ICL repair [59]. Indeed, during chromosomal DNA replication, where the inter-origin distance is roughly 100 kb [80], one fork will often encounter an ICL long before a second fork arrives. Importantly, when only one fork was allowed to strike the ICL in egg extracts (due to a barrier that prevented arrival of the second fork), the lone fork did not initiate ICL repair and its CMG was never unloaded [81•]. However, CMG unloading and repair were restored when the second fork arrived as much as one hour after the first fork (due to timed dissolution of the barrier). Although the requirement for two forks needs to be confirmed in cells, the data in egg extracts show that a single fork, while inactive for ICL repair, remains stable and competent for repair until a second fork arrives. Consistent with this view, stalled replication forks are generally very stable *in vivo*, and collapse only after prolonged treatment with hydroxyurea [82•] or following global exhaustion of the single strand DNA binding protein, RPA [83•]. The advantage of coupling CMG unloading to fork convergence is that it avoids inadvertent replisome disassembly at single forks that have stalled transiently. This is especially important given the absence of *de novo* CMG assembly pathways in the S phase of the cell cycle [84]. In summary, it appears that waiting for two forks to converge on an ICL is a viable strategy to initiate ICL repair.

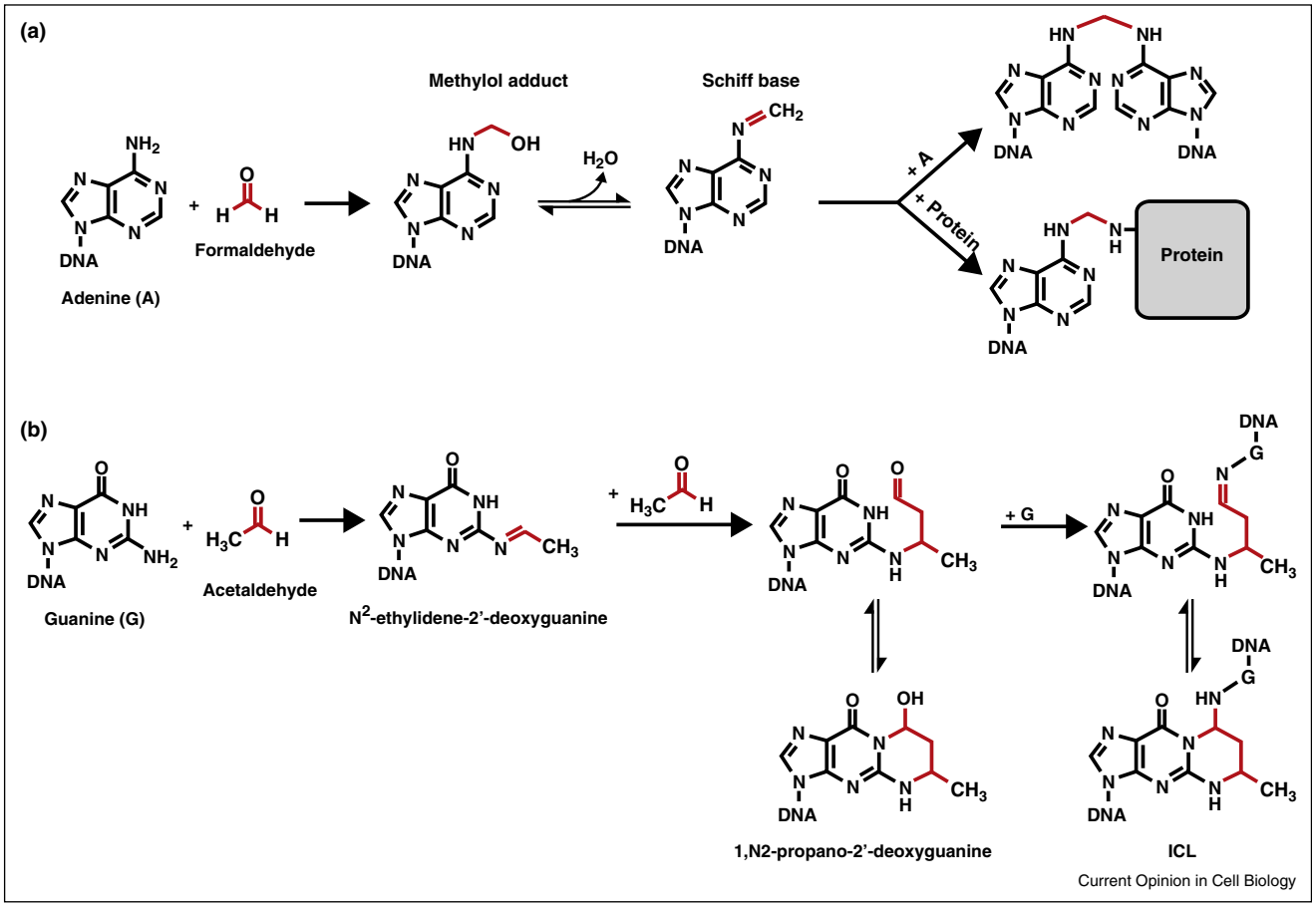
Recently, Seidman and colleagues investigated the collision of replication forks with fluorescently marked psoralen ICLs in cells using DNA combing [85••]. Although they observed many instances of fork convergence, more often single forks bypassed ICLs *without* unhooking them (Figure 2b, i-ii, 'traverse'). In this scenario, ICL repair is thought to occur after the traversed fork has moved beyond the lesion. The mechanism of traverse, including the identity of the helicase that unwinds DNA distal to the ICL, is currently unclear. However, one attractive possibility is that the CMG ring transiently opens and is pushed past the ICL by FANCM, whose translocase activity is required for traverse [85••]. In a mechanism that may be analogous to ICL traverse, the large T antigen DNA helicase was shown to bypass a covalent DNA–protein cross-link (DPC) on the translocation strand, probably via ring opening [86]. Importantly, whether two forks converge on an ICL (Figure 2a, iii) or a single fork undergoes traverse (Figure 2b, ii), a similar X-shaped DNA structure is generated around the lesion, which may be the critical trigger for ICL unhooking [87]. At present, it is unclear how the balance between traverse and fork convergence in cells is governed. Nevertheless, the work in extracts and cells suggests that a single fork stalled at an ICL is unable to promote repair and that an X-shaped structure, generated by fork convergence or traverse, is the key substrate of the endonucleases that unhook the ICL.

FA and reactive aldehydes

Based on the sensitivity of FA cells to agents such as cisplatin and mitomycin C, it has been widely assumed that FA is caused by defective repair of endogenously produced ICLs. However, which metabolites produce such ICLs has been a matter of conjecture. The best candidates are reactive aldehydes, since they are known to form a wide variety of DNA adducts including ICLs [88]. For example, formaldehyde is generated during histone demethylation and dealkylation of methylated DNA [89,90]. It is also an intermediate required for the biosynthesis of purines and certain amino acids and is found at high concentration in human plasma (~30–90 μM) [91,92]. Formaldehyde generates ICLs via a methylene bridge formed between the exocyclic amino groups of adjacent DNA bases [93,94] (Figure 3a, top pathway). Acetaldehyde is a byproduct of ethanol oxidation and an intermediate of carbohydrate metabolism [95], and it forms ICLs, mainly by reacting with guanines (Figure 3b) [96–98]. Other reactive aldehydes including 4-hydroxynonenal (4-HNE), acrolein, malondialdehyde and crotonaldehyde are produced through lipid peroxidation [88], and most of these agents are able to form ICLs [98–102]. Despite evidence that reactive aldehydes induce ICLs in the test tube, for many years there was no evidence to suggest they underlie the etiology of FA.

This picture has changed dramatically, as recent genetic experiments provide powerful evidence for a connection between endogenous aldehydes and FA. Initially, it was reported that chicken cells deficient in FANC proteins are highly sensitive to low doses of formaldehyde [103]. Patel and colleagues then explored the interplay of aldehyde metabolism and the Fanconi pathway in mice, by deleting the acetaldehyde detoxifying enzyme, aldehyde dehydrogenase 2 (ALDH2). While *Aldh2*$^{-/-}$ and *Fancd2*$^{-/-}$ single mutant mice were viable and exhibited no severe phenotypes, *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice were born only from mothers carrying at least one wild-type allele of Aldh2,

Figure 3



Aldehyde mediated DNA cross-links. Reactions of DNA with formaldehyde **(a)** and acetaldehyde **(b)** are depicted. (a) Formaldehyde reacts with primary amines of DNA bases to form a methylol adduct. Dehydration results in the formation of a Schiff base intermediate that can react with another base to form an interstrand cross-link (top) or a lysine to form a DNA–protein cross-link (bottom). (b) Acetaldehyde mainly reacts with the exocyclic amine of deoxyguanine to form N^2-ethylidene-2′-deoxyguanine adducts [97]. Through the reaction of a second acetaldehyde molecule N^2-ethylidene-2′-deoxyguanine is converted to 1,N^2-propano-2′-deoxyguanine [97,132]. 1,N^2-propano-2′-deoxyguanine adducts exist in equilibrium between the open and closed form. The ring open form which is favored in double strand DNA can induce ICLs by reacting with another dG on the complementary strand [96–98]. It can also form DPCs by reacting with primary amines of proteins (not depicted) [111].

demonstrating that aldehyde catabolism *in utero* is essential for embryonic development [104••]. Viable *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice displayed developmental abnormalities and died of acute lymphoblastic leukemia within the first 6 months of life. The few mice that did not get leukemia eventually developed spontaneous BMF [105••]. When challenged with ethanol soon after birth, mice rapidly developed severe BMF [104••]. Underlying these phenotypes was a profound reduction in the hematopoietic and progenitor stem cell pool as observed in FA patients [105••]. More recently, Patel and colleagues also examined the connection of the FA pathway with alcohol dehydrogenase 5 (ADH5), thought to be the major formaldehyde catabolizing enzyme [106]. By contrast to the situation for ALDH2, *Adh5*$^{-/-}$ *Fancd2*$^{-/-}$ mice were born irrespectively of maternal *Adh5* status. However, these mice developed BMF and hematopoietic stem cell depletion much earlier than *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice, suggesting that endogenous formaldehyde is more cytotoxic than acetaldehyde. Accordingly, in chicken cells mutations in FANC genes are synthetically lethal with *Adh5* but not *Aldh2* mutations [104••,107•]. The greater toxicity of formaldehyde could be due to a higher reactivity of formaldehyde and/or a greater abundance of endogenous formaldehyde versus acetaldehyde. The fact that *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ and *Adh5*$^{-/-}$ *Fancd2*$^{-/-}$ double mutant mice recapitulate the key phenotypes of FA suggests that BMF in FA patients is caused by aldehyde toxicity. Consistent with this view, in Japanese FA patients, the severity of the disease correlates with the Asian flushing mutation, a dominant negative allele of *Aldh2* present in 36% of the population in East Asia [108•]. In the future, it will be

important to track the maternal *Aldh2* status for a wider cohort of FA patients to determine whether maternal aldehyde detoxification protects human FA patients from DNA damage [104••,109]. Together, these experiments indicate that eukaryotic cells use a two-pronged approach to avoid the genotoxic effects of reactive aldehydes [110]. On the one hand, their levels are kept in check by aldehyde catabolizing enzymes. On the other hand, the damage created by these agents is neutralized by the FA pathway. In mice, only the absence of both pathways causes severe toxicity, whereas in humans, neutralizing the FA pathway alone is sufficient to cause disease.

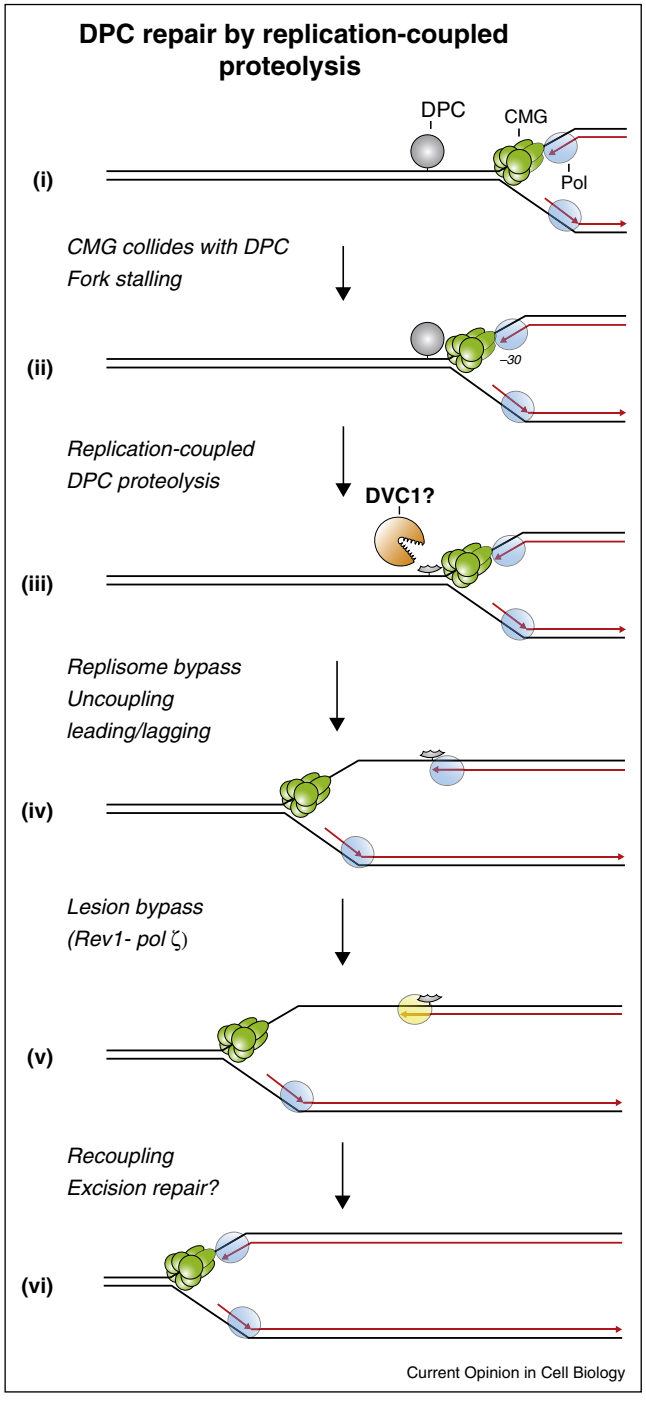
What do these studies teach us about the endogenous lesions that cause FA? The first question is which specific aldehyde(s) are relevant to FA. Since mutations in *Aldh2* and *Adh5* both cause synthetic sickness with mutations in *FANC* genes, both acetaldehyde and formaldehyde might be able to cause the offending lesions. Alternatively, mutations in *Aldh2* and *Adh5* may lead to an increase in a common, unique metabolite that drives FA. Given its greater toxicity in chicken cells and mice, formaldehyde might be the primary culprit [106,107•]. Even if this is the case, there is a further ambiguity, since formaldehyde causes not only ICLs, but also DPCs [111,112]. In fact, formaldehyde is known to favor DPC lesions [92] by forming a methylene bridge between nucleophilic amino acid side chains and exocyclic amines of DNA bases [113] (Figure 3a, bottom pathway). To complicate matters further, most chemicals traditionally considered to be ICL-inducing agents such as nitrogen mustards or platinum compounds can also cause DPCs [114–117]. Thus, when FA cells are treated with aldehydes, cisplatin, or other bifunctional compounds, both ICLs and DPCs are likely to form, making it impossible to determine which lesion drives toxicity. In conclusion, although the identification of aldehydes as the endogenous metabolites underlying FA represents an important breakthrough, the nature of the offending DNA lesions remains unknown.

The FA pathway and DPC repair

One approach to resolve the above ambiguity is to examine the requirement for the FA pathway in the repair of *chemically-defined* DNA lesions. Importantly, extract and cell-based studies showed that the FA pathway is essential for the repair of synthetic ICLs [9•,65••]. What about DPCs?

The repair of chemically-defined DPC lesions was recently recapitulated in egg extracts [118••]. A bacterial DNA methyltransferase (M. HpaII) was covalently linked to a specific location in a plasmid (via the C6 position on cytosine), and the resulting DPC was incubated in egg extracts, whereupon it was repaired in a replication-dependent manner. When the DPC is encountered on the leading strand template, replication stalls due to collision of CMG with the lesion, as seen during ICL repair (Figure 4, i-ii). However, in contrast to ICL repair, DPC repair does not require fork convergence, and CMG is not unloaded. Instead, the DPC is degraded

Figure 4



DNA–protein cross-link repair by replication-coupled proteolysis. A replication fork stalls when it encounters a DNA–protein cross-link (DPC). This stalling can be relieved by the degradation of the DPC to a peptide by a replication-dependent protease. A potential candidate for this protease is DVC1, which has homology to the yeast DPC protease Wss1.

on DNA via a replication-coupled protease, yielding a peptide–DNA adduct that is bypassed by CMG (Figure 4, iii-iv). The leading strand is subsequently extended and stalls again at the peptide adduct. Finally, a complex of REV1 and Pol ζ allows the leading strand to bypass the peptide adduct (Figure 4, v). A DPC on the lagging strand template only transiently stalls the replisome, but it too is degraded to a peptide, allowing Okazaki fragment bypass. Importantly, DPC repair proceeds without incision of parental strands and therefore does not involve a DSB intermediate. Consistent with this observation, M. HpaII DPC-repair does not require the ID_2 complex. If DPCs are formed by reactive aldehydes *in vivo*, the chemistry of the protein–DNA linkage will be different from that of the M. HpaII DPC. However, the attachment chemistry is unlikely to affect the initial processing of the DPC, but rather dictate which translesion DNA polymerase is employed to bypass the remaining peptide adduct. To test this assumption, it will be critical to examine the role of FANC proteins in the repair of aldehyde-induced DPCs. In conclusion, when the repair of chemically defined DPCs and ICLs is compared in the same cell-free system, only the latter requires the FA pathway. This observation supports the notion that failure to repair ICLs created by endogenous aldehydes is the underlying cause of FA.

DPC repair and human disease

To determine how defective DPC repair impacts human health, it will be critical to identify factors that are specifically dedicated to DPC repair. In an elegant study, Jentsch and colleagues identified budding yeast Wss1 as a DPC protease [119••]. Wss1 contains an N-terminal metalloprotease domain, SHP and VIM domains that mediate binding to the Cdc48 ATPase (known as p97 or VCP in higher eukaryotes), and tandem SUMO interaction motifs (SIMs). Wss1 removes covalently trapped topoisomerase I complexes and it confers formaldehyde resistance. Both of these functions require the metalloprotease and Cdc48 binding domains, and to a lesser extent, the SIMs. Interestingly, purified Wss1 contains DNA binding activity and it degrades proteins only when these are bound to DNA. Collectively, these and other data indicate that Wss1 functions as a DPC protease that removes DPCs during replication [119••]. Further analysis will be required to elucidate the role of Cdc48 in this process, and to determine how Wss1's activity is regulated to avoid degrading DNA binding proteins that are not covalently linked to DNA. One possibility is that Wss1 activity is dependent on replication fork stalling, as suggested by the work in egg extracts [118••].

Before the discovery of Wss1 and a replication-coupled DPC repair mechanism [118••,119••], the main pathways implicated in DPC repair were NER and HR. NER provides resistance to DPC-inducing agents such as formaldehyde, and is proposed to remove small (<11 kDa) DPCs outside of S phase [120–123]. By contrast, larger DPCs, which evade NER, were thought to depend on HR during replication [120,121]. In yeast, Wss1 and the recombinase Rad52 are not epistatic with regard to formaldehyde sensitivity, and in the absence of Wss1, Rad52 repair foci and gross chromosomal rearrangements increase, arguing that DPC proteolysis and HR represent alternative mechanisms of DPC repair during S phase [119••]. Given that it does not involve a DSB intermediate [118••], proteolysis-dependent DPC repair probably represents the preferred means of eliminating DPCs, while HR might act on a subset of DPCs that cannot be degraded. Alternatively, the requirement for HR in formaldehyde resistance might involve the repair of formaldehyde-induced ICLs [93,94], or result from such a large load of DPCs that Wss1 becomes limiting.

The closest vertebrate homolog of Wss1 is DVC1 (also known as Spartan), which also contains an N-terminal metalloprotease domain and an SHP p97 binding motif [124,125]. Instead of SIM domains, DVC1 contains a ubiquitin binding motif and a PCNA-interaction protein (PIP) motif, which it uses to bind ubiquitylated PCNA [126]. DVC1 participates in the response to UV irradiation [124,126,127]. Although this may reflect a role for DVC1 in regulating TLS at UV-induced lesions, it is also consistent with a role for DVC1 in the repair of DPCs, which can be caused by UV light [128,129]. Consistent with the latter view, DVC1 knockdown causes sensitivity to camptothecin, a drug that traps topoisomerase I on DNA [127]. Strikingly, DVC1 was recently identified as the causative mutation in an atypical Werner-like progeroid syndrome with clinical features distinct from those of FA [130••]. Patients harboring biallelic germline mutations in DVC1, including one that resides in the protease domain, exhibited premature aging features such as graying hair, muscular atrophy and cataracts. The patients also developed early onset hepatocellular carcinomas. Cells from these patients contained DNA damage and signs of replication stress, consistent with DVC1 acting in a replication-coupled repair pathway. In mice, DVC1 null mutations cause embryonic lethality, implying that DVC1 is essential to repair a highly toxic endogenous lesion. Consistent with the human phenotypes, hypomorphic DVC1 alleles cause premature aging in mice, although no cancers were detected [131•]. DVC1 conditional knockout MEFs do not proliferate. Before they die, they display replication stress, which is rescued by introduction of wild-type DVC1 but not DVC1 harboring a mutation in its conserved protease domain. Given the parallels between DVC1 and Wss1, and the requirement for DVC1s protease domain to relieve replication stress and suppress aging and cancer, the simplest interpretation of these results is that DVC1 functions as a DPC protease. If this is the case, it would show that failure to repair DPCs causes a disease that is phenotypically distinct from FA, further

disfavoring the idea that defective DPC repair underlies FA. The question then arises whether endogenous aldehydes also underlie the DVC1-deficiency syndrome, which can be addressed by crossing DVC1 hypomorphic mice with Aldh2 deficient mice.

Conclusions

Nearly a century after Guido Fanconi's description of FA, the field has made great progress in identifying and understanding the properties of 19 FANC gene products and their roles in repairing ICLs. The recent identification of reactive aldehydes as the likely mutagen underlying FA is a major advance, but in itself, does not identify the offending DNA lesion. Importantly, in DNA repair assays using a limited number of chemically defined DNA lesions, the FA pathway is required for ICL repair but not DPC repair, supporting the original idea that failure to repair ICLs causes FA. This conclusion is further strengthened by emerging evidence that defective DPC repair causes a genetic disease that is phenotypically distinct from FA. One plausible scenario is that reactive aldehydes cause ICLs and DPCs, and failure to repair each class of lesion causes a different clinical manifestation. However, the real situation may be more complex. For example, aldehydes might link a protein to both strands of the DNA, generating a DPC that effectively mimics an ICL. If the protein is attached to the two DNA strands at closely spaced amino acids, the lesion may not be amenable to DVC1 processing and thus may have to be dealt with via ID_2-dependent incisions. To further clarify these issues, it will be important to better define the aldehydes whose upregulation in $Aldh2^{-/-}$ and $Adh5^{-/-}$ cells cause toxicity. In addition, more sensitive analytical tools will also have to be developed to identify endogenous lesions that accumulate on the chromosomes of FA cells. Ultimately, understanding the molecular etiology of FA will not only enhance our view of the cell's varied DNA repair pathways but also lay the foundation for targeted therapies. Thus, researchers may one day be able to neutralize the offending mutagen or shunt the lesion it causes into an alternative DNA repair pathway that is still intact in FA patients.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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This study described for the first time that FA patient derived cells are susceptible to chromosome breakage when treated with interstrand crosslinking agents such as nitrogen mustard or mitomycin C.

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This study demonstrates that DNA replication is required to trigger a cellular response to ICLs. The authors induced ICLs in human cells at different stages of the cell cycle. ICLs induced in G1 or S phase lead to a late S phase arrest. By contrast ICLs introduced in G2 did not cause arrest until cells entered the next cell cycle, where they again accumulated in late S phase. This was the first indication for a replication-coupled mechanism of ICL repair.

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