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The Fanconi Anemia Pathway in Replication Stress and DNA Crosslink Repair

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Abstract

Interstrand crosslinks (ICLs) are DNA lesions where the bases of opposing DNA strands are covalently linked, inhibiting critical cellular processes such as transcription and replication. Chemical agents that generate ICLs cause chromosomal abnormalities including breaks, deletions and rearrangements, making them highly genotoxic compounds. This toxicity has proven useful for chemotherapeutic treatment against a wide variety of cancer types. The majority of our understanding of ICL repair in humans has been uncovered through analysis of the rare genetic disorder Fanconi anemia, in which patients are extremely sensitive to crosslinking agents. Here, we discuss recent insights into ICL repair gained through new ICL repair assays and highlight the role of the Fanconi Anemia repair pathway during replication stress.

Keywords

Fanconi anemia; interstrand crosslink repair; Translesion synthesis; Homologous recombination repair; genome stability

Introduction to ICLs

ICLs prevent the separation of the Watson and Crick strands of the double helix, inhibiting critical cellular processes such as transcription and replication. ICLs cause a range of structural changes in the DNA. Platinum compounds, such as cisplatin, generate large structural distortion to the DNA. These distorting ICL lesions are recognized directly by the DNA repair machinery. Other ICL inducing agents, such as mitomycin C, do not generate distorting lesions (Noll et al. 2006; Smeaton et al. 2008). Instead, these non-distorting ICLs act as barriers to processes that require translocation along the DNA and their detection depends on the genomic transactions that occur in their vicinity. For example, in cells undergoing replication, the replication machinery serves as the sensor for non-distorting ICLs. In non-proliferating cells (such as post-mitotic differentiated cells, quiescent or senescent cells) non-distorting ICLs are detected through the transcription machinery encountering ICLs present in actively transcribed genes (transcription-coupled ICL repair). In mammalian cells, ICL repair is generally considered to be predominantly replication-

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predict the patients' response to crosslinking drugs

dependent, although ICL repair in G1 does occur (Nojima et al. 2005; Patel and Joenje 2007; Ben-Yehoyada et al. 2009). In this review, we discuss the role of the Fanconi Anemia pathway in the detection and repair of ICL lesions in mammalian cells and *Xenopus* cell free extracts. For reviews of ICL repair in lower organisms, please see (Lage et al. 2003; Noll et al. 2006; Lehoczky et al. 2007).

The Fanconi Anemia Pathway

Major advances in our understanding of ICL repair have come from the molecular analysis of the rare genetic disorder known as Fanconi anemia, a disorder in which patients are extremely sensitive to crosslinking agents (Grompe and D'Andrea 2001; de Winter and Joenje 2009). Fanconi anemia (FA) is a rare disease associated with congenital abnormalities, progressive bone marrow failure and a predisposition to cancers, such as acute myeloid leukemia and squamous cell carcinomas (Alter et al. 2003). Cells derived from FA patients are severely sensitive to DNA crosslinking agents, including mitomycin C (MMC), psoralen-UV-A, cisplatin (CDDP) and diepoxybutane (DEB) (D'Andrea and Grompe 2003). The ability of DEB to induce chromosomal breakages in FA lymphocytes serves as a functional diagnostic test for FA (Auerbach 1993).

The underlying gene mutations responsible for FA have been identified through functional complementation of ICL sensitivity, positional cloning, biochemical purification, and more recently through direct sequencing of candidate genes (Kee and D'Andrea 2010; Wang and Gautier 2010). Currently, fourteen FA genes have been identified (FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, P) (Table 1). This number excludes the *RAD51* paralog, *RAD51C*, where inactivation caused a FA-like disorder lacking symptoms of bone marrow failure (Vaz et al. 2010) (Table 2). The FA pathway consists of three distinct functional groups: the FA core complex (FANCA, B, C, E, F, G, L, M and N), the ID complex (FANCI/FANCD2) and the downstream effector proteins (FANCD1, J, N and P) (Table 1). The FA core complex functions as a ubiquitin ligase that monoubiquitinates both ID complex proteins FANCD2 and FANCI.

Monoubiquitination of FANCD2 and FANCI is critical for their retention on chromatin and localization at nuclear foci with the downstream BRCA-related FA effector proteins (FANCD1, J, N and P) (Garcia-Higuera et al. 2001; Taniguchi et al. 2002; Sims et al. 2007; Smogorzewska et al. 2007). These downstream components are not required for FANCD2/I monoubiquitination and instead participate directly in the final stages of ICL repair via homologous recombination (Figure 1).

The FA Core Complex

The FA core complex consists of eight FA proteins and three Fanconi anemia-associated proteins: FAAP20, FAAP24 and FAAP100 (Moldovan and D'Andrea 2009; Singh et al. 2009; Kim et al. 2012; Leung et al. 2012) (Table 2). For more comprehensive reviews on the FA core complex, please see (Grompe and D'Andrea 2001; de Winter and Joenje 2009; Moldovan and D'Andrea 2009). Activation of the FA pathway in response to ICLs requires the targeting components of the FA core complex, FANCM and FAAP24 (Ciccia et al. 2007; Kim et al. 2008). FANCM is the only FA core component with DNA binding activity.

In vitro, FANCM can specifically bind to Holliday junctions and replication fork structures (Gari et al. 2008a; Gari et al. 2008b). Once bound to chromatin, FANCM/FAAP24 recruits the FA core complex through a direct interaction between FANCM-FANCF (Figure 1) (Deans and West 2011). FANCM is a highly conserved protein, with an ortholog, Hef1 (helicase associated endonuclease for forked-structures), present in *archaeobacteria* (Meetei et al. 2005; Mosedale et al. 2005). FANCM is the only core complex member that is not strictly required for the stability of the complex and as a result FANCD2 is still partially monoubiquitinated in its absence (Singh et al. 2009). FANCM/FAAP24 instead are required for the chromatin localization of the core complex and resistance to ICLs (Ciccia et al. 2007; Kim et al. 2008; Singh et al. 2009).

The FA accessory protein, FAAP20, is the newest member of the FA core complex. It was identified as a direct interactor of FANCA and is required for the integrity of the FA core complex and for crosslink repair (Ali et al. 2012; Kim et al. 2012; Leung et al. 2012). FAAP20 contains a RAD18-like ubiquitin-binding zinc-finger 4 domain that appears to be dispensable for FANCD2 monoubiquitination and crosslink repair (Leung et al. 2012). Instead, the UBZ4 domain of FAAP20 binds to the monoubiquitinated form of Rev1, an important component of the error-prone Translesion Synthesis pathway (TLS), a key DNA damage tolerance pathway (discussed in more detail below). FAAP20 stabilizes Rev1 nuclear foci and provides a critical link between the FA core complex and TLS polymerase activity that may explain why FA core deficient cell lines are hypomutable for point mutations (Papadopoulou et al. 1990; Mirchandani et al. 2008; Kim et al. 2012).

The FA core complex ubiquitinates FANCI and FANCD2 through its catalytic subunits, FANCL (the E3 ligase) and UBE2T (the ubiquitin E2 ligase) (Meetei et al. 2003; Machida et al. 2006). Insights into the ubiquitination of the ID complex have come from reconstructing the ubiquitination of FANCD2 *in vitro*; various forms of DNA including single-stranded, double-stranded and branched DNA are capable of stimulating FANCD2 monoubiquitination *in vitro* (Sato et al. 2012). Notably, the DNA-binding activity of FANCI is required for the DNA dependent stimulation of FANCD2 monoubiquitylation (Sato et al. 2012). FANCI also imparts specificity to the ubiquitination reaction by limiting monoubiquitination to K561 of FANCD2 (Alpi et al. 2008). It was also reported that monoubiquitinated PCNA stimulates FANCD2 and FANCI monoubiquitination *in vitro*, strengthening independent findings that RAD18-mediated monoubiquitination of PCNA is an important regulator of the ID complex (Geng et al. 2010; Williams et al. 2011). *In vivo*, it is still unclear what factors regulate and participate in targeting the core complex to its substrates, FANCD2 and FANCI. One possibility is that FANCE bridges the FA core complex and FANCD2 (Pace et al. 2002).

The FANCI/FANCD2 (ID) Complex

Monoubiquitination of the ID complex serves as a marker for activation of the FA core complex. The monoubiquitination of both FANCI (K523) and FANCD2 (K561) enables the complex to associate with chromatin through an unknown mechanism (Garcia-Higuera et al. 2001; Timmers et al. 2001; Taniguchi et al. 2002; Dorsman et al. 2007; Sims et al. 2007; Smogorzewska et al. 2007). Monoubiquitination of the ID complex can be induced by

numerous types of DNA damage in addition to ICLs, including ionizing radiation, ultraviolet radiation and replication stress generated by inhibitors of replication, such as hydroxyurea and aphidicolin, as well as endogenous sources of replication stress such as re-replication (Garcia-Higuera et al. 2001; Howlett et al. 2005; Zhu and Dutta 2006).

Until recently it was unclear whether FANCD2 and FANCI contained any additional domains other than their monoubiquitination sites that were important for promoting DNA repair. Both FANCI and FANCD2 contain EDGE motifs (defined by the EDGE amino acid sequence), which is a cluster of acidic residues, essential for the correction of MMC sensitivity in FA-I or FA-D2 deficient fibroblasts (Montes de Oca et al. 2005; Colnaghi et al. 2011). The precise role of the EDGE motif in DNA repair is unclear but it appears to act downstream of FANCI/FANCD2 monoubiquitination and may participate in recruiting additional repair factors to ICLs, or alternatively may be a critical structural component of both proteins (Colnaghi et al. 2011).

The crystal structure of the unmodified ID complex was recently solved providing much needed structural insight into how these proteins interact (Joo et al. 2011). The structure demonstrated that the two proteins interact in an antiparallel manner, with the regulatory monoubiquitination sites mapped to the interface of the complex, suggesting that monoubiquitination occurs on monomeric proteins or an opened complex. Since this structure does not include the ubiquitin modifications, it is still unclear what role these modifications play in the assembly of the ID complex. Given their location in this complex, though, it suggests that they may serve to stabilize the ID heterodimer.

FAN1

Ubiquitination of the ID complex not only serves to localize the complex to chromatin, it also plays a key role in the repair process. The structure specific endonuclease, FAN1 (FANCD2-associated nuclease), contains an amino-terminal ubiquitin binding zinc finger (UBZ) domain that targets FAN1 to monoubiquitinated FANCD2 (Figure 1) (Kratz et al. 2010; Liu et al. 2010; MacKay et al. 2010; Shereda et al. 2010; Smogorzewska et al. 2010). FAN1 possesses 5' flap endonuclease activities and FAN1 recruitment to FANCD2 is required for resistance to ICLs induced by MMC and cisplatin. Precisely how FAN1 contributes to ICL repair is unclear and remains an interesting subject for future investigation using defined ICL substrates.

USP1

Monoubiquitination of the ID2 complex is dynamic, with deubiquitylation playing a critical role in ICL repair. Both FANCI and FANCD2 are deubiquitylated by the ubiquitin specific protease, USP1 (Nijman et al. 2005). Deubiquitination of the ID complex is crucial for ICL repair, with loss of USP1 resulting in ICL sensitivity and increased genomic instability (Oestergaard et al. 2007; Murai et al. 2011; Jones et al. 2012). Interestingly, USP1 knockout mice display a FA-like phenotype (Kim et al. 2009). In addition to its role in the FA pathway, USP1 also deubiquitinates a second monoubiquitinated substrate, PCNA, inhibiting repair through the TLS pathway (Huang et al. 2006). Therefore, USP1 may play

an important role in coordinating DNA repair by promoting the FA pathway and inhibiting the TLS pathway.

USP1 activity is under tight control regulated by multiple pathways. The first of these pathways acts directly on the enzymatic activity of USP1, which is stimulated by binding to a co-factor, USP1 associated factor 1 (UAF1), also known as WDR48 (Cohn et al. 2007). Secondly, USP1 expression is repressed by p21 upon exposure to DNA damaging agents. In the absence of p21 the persistent expression of USP1 can interfere with the accumulation of FANCD2 and FANCI monoubiquitination (Rego et al. 2012). Finally, two separate pathways target the USP1 protein for degradation. Upon UV DNA damage, USP1 is auto-cleaved and degraded by the proteasome (Huang et al. 2006). USP1 protein levels are also regulated in a cell cycle dependent manner through the APC/C^{Cdh1} ubiquitin ligase, which maintains low levels of USP1 in G1 (Cotto-Rios et al. 2011a; Cotto-Rios et al. 2011b). The activity and tight regulation of USP1 ensures that both the FA and TLS repair pathways function correctly to maintain genome stability.

ICL Repair

Until recently, it has been difficult to study the repair of ICL lesions exclusively since many crosslinking agents also generate intra-strand crosslinks and other forms of DNA damage. For this reason, strategies to introduce sequence specific synthetic ICLs into mammalian cells and cell free *Xenopus* extracts have been developed to study ICL repair and checkpoint activation (Raschle et al. 2008; Ben-Yehoyada et al. 2009; Knipscheer et al. 2009; Shen et al. 2009). These approaches have provided insight into how different ICL lesions are detected and repaired.

As mentioned previously, ICL lesions can be simplified into two classes: lesions that distort the DNA helix considerably, such as psoralen or cisplatin, and lesions that are less distorting (non-distorting) MMC and nitrogen mustards ICLs. The degree of helix distortion influences how the ICL is recognized and the repair pathways utilized (Smeaton et al. 2008). The chemical structures of common chemotherapeutic ICL agents and their impact on the DNA helix have been discussed in detail elsewhere (Noll et al. 2006; Hlavín et al. 2010). Distorting ICL lesions are repaired through both replication-dependent and independent pathways (Ben-Yehoyada et al. 2009; Shen et al. 2009). In contrast, non-distorting lesions are primarily considered to be repaired through replication-dependent pathways (Raschle et al. 2008; Knipscheer et al. 2009). The replication-dependent repair of ICLs is performed using the error-free homologous recombination (HR) pathway. Since cells in G1/G0 have not yet acquired sister chromatids, they are forced to repair ICL lesions using the error-prone recombination-independent pathway, which employs nucleotide excision (NER) and translesion synthesis (TLS) repair pathways (Wang et al. 2001; Zheng et al. 2003; Sarkar et al. 2006).

Replication-Independent ICL Repair

In mammalian cells, ICL repair is primarily considered to be a replication-dependent process, therefore the majority of studies have focused on understanding this pathway. However, there is growing interest in how ICLs are repaired in a replication-independent

manner, since replication-independent repair is likely to be crucial for the tolerance of non-dividing or terminally differentiated cells to ICLs.

Many reports of replication-independent repair exist, yet the repair pathway remains poorly defined (Sarkar et al. 2006; Ben-Yehoyada et al. 2009; Muniandy et al. 2009; Shen et al. 2009). Replication-independent ICL repair occurs in two stages (Figure 2A). First, the NER pathway proteins unhook and excise the crosslink (Muniandy et al. 2009). Second, the TLS pathway performs DNA repair synthesis (Ho and Scharer 2010). Of the NER proteins, XPA and XPC are known to be required for the recruitment of the FA core component, FANCA, to ICLs (Shen et al. 2009). This places XPA and XPC upstream of the FA pathway in the detection of ICLs, suggesting that the FA core complex is recruited to an ICL repair intermediate generated by the NER pathway (Shen et al. 2009). How the FA pathway participates in replication-independent ICL repair pathways is still unclear.

Elegant research from the Gautier laboratory using both *Xenopus* egg extracts and mammalian cells revealed that the Fanconi pathway performs a critical replication-independent checkpoint signaling function in response to ICLs (Ben-Yehoyada et al. 2009). Using a site-specific ICL lesion and monitoring DNA damage signaling under restrictive replication conditions, it was demonstrated that the FA pathway acts upstream of ATR activation. This is surprising since ATR activation is achieved when RPA coated ssDNA is generated in response to uncoupling of the DNA helicase and the replisome (Zou and Elledge 2003; Byun et al. 2005). Moreover, ATR and RPA were previously shown to be important for FANCD2 monoubiquitination (Andreassen et al. 2004). The study from the Gautier laboratory also concluded that replication-independent (and replication dependent) ICL repair involves extensive DNA synthesis, consistent with models implicating translesion synthesis polymerases in ICL repair (Ben-Yehoyada et al. 2009).

Translesion Synthesis (TLS)

TLS is a major DNA damage tolerance mechanism whereby alternative error-prone TLS polymerases bypass DNA lesions that would otherwise cause replication arrest and cell death (Friedberg 2005). TLS Polymerases possess low fidelity and can introduce mutations when replicating undamaged DNA templates; therefore they must be tightly regulated. Dysregulation of these error-prone enzymes can cause genomic instability and tumorigenesis (Bavoux et al. 2005; Lange et al. 2011; Jones et al. 2012). PCNA, the replicative sliding clamp, recruits TLS polymerases to DNA lesions (Prakash et al. 2005; Lehmann et al. 2007). The Y-family of polymerases contains two domains important for their interaction with PCNA. The first of these domains is the PCNA interacting motif or PIP box (Haracska et al. 2001a; Haracska et al. 2001b), which has the consensus sequence Q-X-X-(ϕ)-X-X-(A)-(A), where (X) can be any residue, (ϕ) represents hydrophobic residues (I, L, M) and (A) represents residues with aromatic side chains (e.g. F, Y) (Warbrick 1998; Maga and Hubscher 2003). Importantly, the PIP box present in Y family polymerases has relatively low affinity for PCNA in comparison to the p21 PIP box (Hishiki et al. 2009; Jones et al. 2012).

The majority of the binding affinity for PCNA comes from the second PCNA binding domain found in Y-family TLS polymerases, their ubiquitin-binding domains (UBM or UBZ domains). These domains interact with ubiquitinated PCNA to strengthen their interaction and facilitate the switch between replicative and TLS polymerases (Kannouche et al. 2004; Bienko et al. 2005). Since PCNA is monoubiquitinated by Rad6-Rad18 at sites of stalled replication forks, this enables TLS polymerases to be recruited specifically to where they are most needed (Hoegge et al. 2002; Kannouche et al. 2004). In both yeast and mammalian cells, studies specifically focused on replication-independent ICL repair have clearly demonstrated Rad18 dependent monoubiquitination of PCNA is necessary for the recruitment of polymerase ζ to bypass ICLs (Sarkar et al. 2006; Shen et al. 2006). It is unclear whether PCNA monoubiquitination performs a similar role during replication-dependent ICL repair.

Replication Dependent ICL Repair

The majority of ICL repair in actively dividing cells is coupled to DNA replication. This was classically demonstrated in synchronized human fibroblasts, where ICL repair occurred exclusively during S-phase, regardless of which cell-cycle phase the ICL was induced (Akkari et al. 2000). In order to discuss replication-dependent ICL repair, it is important to provide a brief overview of DNA replication licensing and initiation.

In human cells, genome replication begins at tens of thousands of replication origins distributed throughout the genome (Mechali 2010). Failure to initiate replication from a sufficient number of sites would leave regions of the genome unreplicated prior to mitosis. DNA replication licensing occurs in G1 with the assembly of pre-replicative complexes (pre-RCs), consisting of Cdc45, MCM2-7 and the GINS (collectively referred to as the CMG complex) at origins of replication. The pre-RCs are recruited by the origin recognition complex (ORC), Cdc6 and Cdt1. Subsequent phosphorylation of the Pre-RC by two S-phase promoting kinases, the cyclin-dependent (CDK) and Cdc7-Dbf4 (DDK), facilitates origin unwinding and the recruitment of the DNA polymerases and additional accessory factors, collectively known as the replisome complex (Zhu et al. 2005; Sclafani and Holzen 2007).

The MCM complex is an important component of the replisome and functions as the replicative helicase necessary for both origin unwinding and replication fork progression (Labib et al. 2000; Lei and Tye 2001; Takahashi et al. 2005). The MCM complex unwinds the parental DNA duplex ahead of the replication fork, providing the single stranded DNA template for the DNA polymerases of the leading and lagging strands (Fu et al. 2011). Since the MCM complex is positioned ahead of the replication fork, it is likely to be the first replisome component to encounter ICL lesions during DNA replication. This is supported by the enrichment of MCM7 at a site-specific psoralen crosslink (Shen et al. 2009). It will be interesting to determine whether MCMs play an important role in signaling and/or recruitment of repair proteins to ICLs, although admittedly, given their role in DNA replication, it will be difficult to separate these functions.

Detailed models of replication-dependent ICL repair are beginning to emerge from the use of site-specific ICL templates in *Xenopus* extracts (Figure 2B). In this cell-free system, two

replication forks converge on the ICL, with the leading strand polymerases initially pausing 20 - 24 nucleotides from the crosslink (Raschle et al. 2008). This distance is likely dictated by the inherent size of the replisome's footprint (including polymerase + CMG complex) on DNA. The lagging strand polymerases were located at a greater and more variable distance from the lesion. After the initial fork pause, lesion bypass is initiated when the leading strand of a single fork advances to within 1 nucleotide of the ICL lesion. A dual incision process surrounding the ICL then unhooks the parental strands, and TLS incorporates a nucleotide across from the ICL lesion in a polymerase ζ dependent process. Both the incision step and the insertion of a nucleotide opposite the ICL lesion is mediated by FANCI and FANCD2, since these events are absent when the ID complex is immunodepleted from extracts (Knipscheer et al. 2009).

Once the ICL is unhooked and the nucleotide opposite the lesion is inserted, the leading strand is extended and ligated to the first downstream Okazaki fragment. The final stage of ICL repair restores the broken sister chromatids (generated by the incision step) using the restored sister as a template for homologous recombination (Figure 2A). The repair of the broken chromatid by homologous recombination is Rad51 dependent with Rad51 binding to the ICL independently of the ID complex and prior to the formation of the double stranded DNA break (Long et al. 2011).

Overall, the convergent replication model differs from earlier models that suggested a single replication fork collides with the ICL lesion (Niedernhofer et al. 2005). The small size of the plasmid used in these studies may not accurately reflect the repair scenario on chromosomal DNA, where the average replicon is 100-120 kb (Mechali 2010). However, it is very likely that additional origins are fired within replicons containing ICL lesions. In any case, ICL repair was recently shown to occur whether one or two forks reached the ICL (Le Breton et al. 2011). Mammals possess 15 unique DNA polymerases and from these, Pol ζ and Rev1 stand out as the most important for ICL repair (Ho and Scharer 2010). Although, there may also be requirements for additional TLS polymerases since studies have demonstrated roles for other TLS polymerases during crosslink repair including Rev1, Pol ν , Pol κ and Pol η (Shen et al. 2006; Minko et al. 2008; Mogi et al. 2008; Ho and Scharer 2010; Moldovan et al. 2010). A remaining challenge will also be to determine the identity of the structure specific nuclease required for the dual incisions. A number of endonucleases have been implicated in the incision events of ICL repair including XPF-ERCC1, MUS81-EME1, SLX1-SLX4 and the Fanconi anemia associated nuclease 1 (FAN1). The biochemical activities of these nucleases and their possible roles in ICL repair have been discussed elsewhere (Ciccio et al. 2008; Sengerova et al. 2011).

In contrast to the detailed analysis of ICL repair obtained in cell free *Xenopus* extracts (described above) up until recently, it has been difficult in mammalian cells to demonstrate a direct role for the FA pathway in HR, using the classic I-Sce endonuclease HR assay. Deficiency in either the FA core or ID complexes in this assay leads to a relatively mild HR defect compared to downstream FA pathway components, such as BRCA2 (Nakanishi et al. 2005; Moynahan and Jasin 2010). Recently, an improved assay specific for ICL-induced HR repair has been used successfully to demonstrate a defect in ICL induced HR repair in a FANCA patient cell line. Importantly, this repair defect was only observed when the

reporter was able to replicate (Nakanishi et al. 2011). Using this improved assay to study ICL-induced HR repair in mammalian cells may provide further insights into replication-independent and replication-coupled ICL repair.

FA pathway and Replication Stress

In addition to its role in ICL repair, the FA pathway also contributes to the maintenance of genome stability by protecting against replication stress. ICL lesions may be viewed as a severe form of replication stress. The FA pathway is important for maintaining genome stability in response to many forms of replication stress, including damaging agents (such as aphidicolin (APH) or hydroxyurea), and endogenous sources of replication stress (including re-replication, oncogene induced replication stress (E7 oncoprotein protein expression) and dysregulation of error-prone polymerases) (Garcia-Higuera et al. 2001; Howlett et al. 2005; Zhu and Dutta 2006; Spardy et al. 2007; Jones et al. 2012). In particular, the FA pathway protects specific regions of the genome, termed common Fragile sites, which display gaps and breaks on metaphase chromosomes in response to replication stress (Howlett et al. 2005; Chan et al. 2009; Naim and Rosselli 2009). What makes these regions “fragile” is not entirely clear; the consensus is that these regions are inherently difficult to replicate.

There are two main theories as to why fragile sites are difficult to replicate. The first suggests that fragile sites possess structurally distinct properties including CGG expansions and AT-rich repeats that can form secondary structures (when unwound by the DNA helicase), such as hairpins or cruciform structures making them vulnerable to replisome stalling and collapse (Hewett et al. 1998; Durkin and Glover 2007). The second theory suggests that factors affecting replication dynamics in these regions, including the organization and selection of replication origins, makes these sites fragile. Fragile sites are often located within late replicating regions that possess a low density of replication origins, and therefore they are prone to incomplete replication in response to replication stress (Letessier et al. 2011). These two theories are not necessarily mutually exclusive and each or both may be relevant to a particular fragile site.

Replication stress induced DNA breaks at fragile sites are particularly important since chromosome breakage and rearrangement at common fragile sites are early events in tumorigenesis (Durkin and Glover 2007). Rearrangements at fragile sites also inactivate their associated genes. In the case of FRA3B and FRA16D, both reside within large tumor suppressor genes, *FHIT* and *WWOX* (Durkin and Glover 2007). These DNA breaks at fragile sites are thought to arise when incompletely replicated regions are hyper-condensed during mitosis. Recently, the formation of mitotic double strand breaks in response to replication stress was shown to be dependent on the condensin subunit SMC2, which is required for the mechanical stability of condensed chromatin (Gerlich et al. 2006; Vagnarelli et al. 2006; Lukas et al. 2011). The SMC2 dependence for mitotic DSBs strongly suggests that mitotic chromosome condensation may trigger the DSB formation at fragile sites. Interestingly, both FANCI and FANCD2 localize to fragile sites during mitosis (Chan et al. 2009; Naim and Rosselli 2009). This localization may be important for protecting these sites from condensation induced breakage or may facilitate their repair after mitosis.

Future Perspectives

Much of what we know about ICL repair in mammalian cells has come from investigating the FA pathway. In this respect, there is still a lot to be learned about key steps within this pathway that contribute to the repair of ICLs. Understanding how ICLs are repaired will improve the use of ICL inducing agents in cancer treatment (Deans and West 2011).

Activation of the FA pathway

How is the interaction between the FA core complex and the ID complex regulated? FANCE directly binds to FANCD2 and it has been suggested that this interaction is the bridge between the FA core complex and FANCD2 (Pace et al. 2002). This finding predates the discovery of FANCI and many of the core complex components, as well as their accessory proteins. It will be interesting to re-investigate whether FANCE-FANCD2 is the only direct interaction between these two complexes.

How does monoubiquitination of the ID complex facilitate its association with chromatin? It is possible that ubiquitin binding domains within unknown proteins associated with chromatin recruit the monoubiquitinated ID complex onto chromatin. This may specifically involve monoubiquitinated FANCI since FANCD2 monoubiquitination was shown to bind the FAN1 nuclease (Kratz et al. 2010; Liu et al. 2010; MacKay et al. 2010; Shereda et al. 2010; Smogorzewska et al. 2010).

The FA pathway and DNA Replication

The coordination between the FA pathway and DNA replication is still unclear. There is an increasing amount of evidence linking replisome components with members of the FA pathway. For example, PCNA interacts with FANCD2, and is thought to function as a platform to facilitate the mono-ubiquitination of FANCD2 (Howlett et al. 2009; Geng et al. 2010). The FA core complex component, FANCF, physically interacts with PSF2, a member of the GINS complex involved in both the initiation and elongation steps of DNA replication (Tumini et al. 2011). These interactions potentially places the FA core and ID complexes at the replisome which could have important implications for how the FA pathway is activated in response to fork stalling at ICLs and other DNA damaging lesions. A better understanding of how the FA pathway protects against replications stress is also needed. The FA pathway may protect cells from replication stress by participating in the activation/regulation of dormant origin firing. The activation of dormant origins is a major pathway protecting cells from replication stress and contributes to the recovery of stalled replication forks (Woodward et al. 2006; Ge et al. 2007; Kawabata et al. 2011). Currently, how these back up origins are regulated is unclear and it will be interesting to determine whether the FA pathway participates in their selection.

In summary, continued analysis of the Fanconi anemia pathway will provide valuable knowledge on how cells respond to ICLs and other forms of replication stress. The recent development of defined ICL substrates and their use in mammalian cells and *Xenopus* extracts has provided an extremely detailed model of the FA pathway's role in ICL repair.

The challenge remains to use this knowledge to improve on current therapies for Fanconi Anemia patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

(FA)	Fanconi anemia
(USP1)	Ubiquitin specific protease 1
(DUBs)	deubiquitinating enzymes
(PCNA)	Proliferating Cell Nuclear Antigen

References

- Akkari YM, Bateman RL, Reifsteck CA, Olson SB, Grompe M. DNA replication is required To elicit cellular responses to psoralen-induced DNA interstrand cross-links. *Mol Cell Biol.* 2000; 20(21): 8283–8289. [PubMed: 11027296]
- Ali AM, Pradhan A, Singh TR, Du C, Li J, Wahengbam K, Grassman E, Auerbach AD, Pang Q, Meetei AR. FAAP20: a novel ubiquitin-binding FA nuclear core-complex protein required for functional integrity of the FA-BRCA DNA repair pathway. *Blood.* 2012; 119(14):3285–3294. [PubMed: 22343915]
- Alpi AF, Pace PE, Babu MM, Patel KJ. Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol Cell.* 2008; 32(6):767–777. [PubMed: 19111657]
- Alter BP, Greene MH, Velazquez I, Rosenberg PS. Cancer in Fanconi anemia. *Blood.* 2003; 101(5): 2072. [PubMed: 12584146]
- Andreassen PR, D'Andrea AD, Taniguchi T. ATR couples FANCD2 monoubiquitination to the DNA-damage response. *Genes Dev.* 2004; 18(16):1958–1963. [PubMed: 15314022]
- Auerbach AD. Fanconi anemia diagnosis and the diepoxybutane (DEB) test. *Exp Hematol.* 1993; 21(6):731–733. [PubMed: 8500573]
- Bavoux C, Hoffmann JS, Cazaux C. Adaptation to DNA damage and stimulation of genetic instability: the double-edged sword mammalian DNA polymerase kappa. *Biochimie.* 2005; 87(7):637–646. [PubMed: 15989980]
- Ben-Yehoyada M, Wang LC, Kozekov ID, Rizzo CJ, Gottesman ME, Gautier J. Checkpoint signaling from a single DNA interstrand crosslink. *Mol Cell.* 2009; 35(5):704–715. [PubMed: 19748363]
- Bienko M, Green CM, Crosetto N, Rudolf F, Zapart G, Coull B, Kannouche P, Wider G, Peter M, Lehmann AR, et al. Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science.* 2005; 310(5755):1821–1824. [PubMed: 16357261]
- Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev.* 2005; 19(9): 1040–1052. [PubMed: 15833913]
- Chan KL, Palmai-Pallag T, Ying S, Hickson ID. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. *Nat Cell Biol.* 2009; 11(6):753–760. [PubMed: 19465922]
- Ciccio A, Ling C, Coulthard R, Yan Z, Xue Y, Meetei AR, Laghmani el H, Joenje H, McDonald N, de Winter JP, et al. Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Mol Cell.* 2007; 25(3):331–343. [PubMed: 17289582]
- Ciccio A, McDonald N, West SC. Structural and functional relationships of the XPF/MUS81 family of proteins. *Annu Rev Biochem.* 2008; 77:259–287. [PubMed: 18518821]

- Cohn MA, Kowal P, Yang K, Haas W, Huang TT, Gygi SP, D'Andrea AD. A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. *Mol Cell*. 2007; 28(5):786–797. [PubMed: 18082604]
- Colnaghi L, Jones MJ, Cotto-Rios XM, Schindler D, Hanenberg H, Huang TT. Patient-derived C-terminal mutation of FANCI causes protein mislocalization and reveals putative EDGE motif function in DNA repair. *Blood*. 2011; 117(7):2247–2256. [PubMed: 20971953]
- Cotto-Rios XM, Jones MJ, Busino L, Pagano M, Huang TT. APC/CCdh1-dependent proteolysis of USP1 regulates the response to UV-mediated DNA damage. *J Cell Biol*. 2011a; 194(2):177–186. [PubMed: 21768287]
- Cotto-Rios XM, Jones MJ, Huang TT. Insights into phosphorylation-dependent mechanisms regulating USP1 protein stability during the cell cycle. *Cell Cycle*. 2011b; 10(23):4009–4016. [PubMed: 22101265]
- D'Andrea AD, Grompe M. The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer*. 2003; 3(1):23–34. [PubMed: 12509764]
- de Winter JP, Joenje H. The genetic and molecular basis of Fanconi anemia. *MutatRes*. 2009; 668(1-2):11–19.
- Deans AJ, West SC. DNA interstrand crosslink repair and cancer. *Nat Rev Cancer*. 2011; 11(7):467–480. [PubMed: 21701511]
- Dorsman JC, Levitus M, Rockx D, Rooimans MA, Oostra AB, Haitjema A, Bakker ST, Steltenpool J, Schuler D, Mohan S, et al. Identification of the Fanconi anemia complementation group I gene, FANCI. *Cell Oncol*. 2007; 29(3):211–218. [PubMed: 17452773]
- Durkin SG, Glover TW. Chromosome fragile sites. *Annu Rev Genet*. 2007; 41:169–192. [PubMed: 17608616]
- Friedberg EC. Suffering in silence: the tolerance of DNA damage. *Nat Rev Mol Cell Biol*. 2005; 6(12):943–953. [PubMed: 16341080]
- Fu YV, Yardimci H, Long DT, Ho TV, Guainazzi A, Bermudez VP, Hurwitz J, van Oijen A, Scharer OD, Walter JC. Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. *Cell*. 2011; 146(6):931–941. [PubMed: 21925316]
- Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol Cell*. 2001; 7(2):249–262. [PubMed: 11239454]
- Gari K, Decaillet C, Delannoy M, Wu L, Constantinou A. Remodeling of DNA replication structures by the branch point translocase FANCM. *Proc Natl Acad Sci U S A*. 2008a; 105(42):16107–16112. [PubMed: 18843105]
- Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. *Mol Cell*. 2008b; 29(1):141–148. [PubMed: 18206976]
- Ge XQ, Jackson DA, Blow JJ. Dormant origins licensed by excess Mcm2-7 are required for human cells to survive replicative stress. *Genes Dev*. 2007; 21(24):3331–3341. [PubMed: 18079179]
- Geng L, Huntoon CJ, Karnitz LM. RAD18-mediated ubiquitination of PCNA activates the Fanconi anemia DNA repair network. *J Cell Biol*. 2010; 191(2):249–257. [PubMed: 20937699]
- Gerlich D, Hirota T, Koch B, Peters JM, Ellenberg J. Condensin I stabilizes chromosomes mechanically through a dynamic interaction in live cells. *Curr Biol*. 2006; 16(4):333–344. [PubMed: 16488867]
- Grompe M, D'Andrea A. Fanconi anemia and DNA repair. *Hum Mol Genet*. 2001; 10(20):2253–2259. [PubMed: 11673408]
- Haracska L, Johnson RE, Unk I, Phillips B, Hurwitz J, Prakash L, Prakash S. Physical and functional interactions of human DNA polymerase eta with PCNA. *Mol Cell Biol*. 2001a; 21(21):7199–7206. [PubMed: 11585903]
- Haracska L, Johnson RE, Unk I, Phillips BB, Hurwitz J, Prakash L, Prakash S. Targeting of human DNA polymerase iota to the replication machinery via interaction with PCNA. *Proc Natl Acad Sci U S A*. 2001b; 98(25):14256–14261. [PubMed: 11724965]

- Hewett DR, Handt O, Hobson L, Mangelsdorf M, Eyre HJ, Baker E, Sutherland GR, Schuffenhauer S, Mao JI, Richards RI. FRA10B structure reveals common elements in repeat expansion and chromosomal fragile site genesis. *Mol Cell*. 1998; 1(6):773–781. [PubMed: 9660961]
- Hishiki A, Hashimoto H, Hanafusa T, Kamei K, Ohashi E, Shimizu T, Ohmori H, Sato M. Structural basis for novel interactions between human translesion synthesis polymerases and proliferating cell nuclear antigen. *J Biol Chem*. 2009; 284(16):10552–10560. [PubMed: 19208623]
- Hlavin EM, Smeaton MB, Miller PS. Initiation of DNA interstrand cross-link repair in mammalian cells. *Environ Mol Mutagen*. 2010; 51(6):604–624. [PubMed: 20658650]
- Ho TV, Scharer OD. Translesion DNA synthesis polymerases in DNA interstrand crosslink repair. *Environ Mol Mutagen*. 2010; 51(6):552–566. [PubMed: 20658647]
- Hoegge C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature*. 2002; 419(6903):135–141. [PubMed: 12226657]
- Howlett NG, Harney JA, Rego MA, Kolling F.W.t. Glover TW. Functional interaction between the Fanconi Anemia D2 protein and proliferating cell nuclear antigen (PCNA) via a conserved putative PCNA interaction motif. *J Biol Chem*. 2009; 284(42):28935–28942. [PubMed: 19704162]
- Howlett NG, Taniguchi T, Durkin SG, D'Andrea AD, Glover TW. The Fanconi anemia pathway is required for the DNA replication stress response and for the regulation of common fragile site stability. *Hum Mol Genet*. 2005; 14(5):693–701. [PubMed: 15661754]
- Huang TT, Nijman SM, Mirchandani KD, Galaray PJ, Cohn MA, Haas W, Gygi SP, Ploegh HL, Bernards R, D'Andrea AD. Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat Cell Biol*. 2006; 8(4):339–347. [PubMed: 16531995]
- Jones MJ, Colnaghi L, Huang TT. Dysregulation of DNA polymerase kappa recruitment to replication forks results in genomic instability. *EMBO J*. 2012; 31(4):908–918. [PubMed: 22157819]
- Joo W, Xu G, Persky NS, Smogorzewska A, Rudge DG, Buzovetsky O, Elledge SJ, Pavletich NP. Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway. *Science*. 2011; 333(6040):312–316. [PubMed: 21764741]
- Kannouche PL, Wing J, Lehmann AR. Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. *Mol Cell*. 2004; 14(4):491–500. [PubMed: 15149598]
- Kawabata T, Luebben SW, Yamaguchi S, Ilves I, Matise I, Buske T, Botchan MR, Shima N. Stalled fork rescue via dormant replication origins in unchallenged S phase promotes proper chromosome segregation and tumor suppression. *Mol Cell*. 2011; 41(5):543–553. [PubMed: 21362550]
- Kee Y, D'Andrea AD. Expanded roles of the Fanconi anemia pathway in preserving genomic stability. *Genes Dev*. 2010; 24(16):1680–1694. [PubMed: 20713514]
- Kim H, Yang K, Dejsuphong D, D'Andrea AD. Regulation of Rev1 by the Fanconi anemia core complex. *Nat Struct Mol Biol*. 2012; 19(2):164–170. [PubMed: 22266823]
- Kim JM, Kee Y, Gurtan A, D'Andrea AD. Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24. *Blood*. 2008; 111(10):5215–5222. [PubMed: 18174376]
- Kim JM, Parmar K, Huang M, Weinstock DM, Ruit CA, Kutok JL, D'Andrea AD. Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. *Dev Cell*. 2009; 16(2):314–320. [PubMed: 19217432]
- Knipscheer P, Raschle M, Smogorzewska A, Enoiu M, Ho TV, Scharer OD, Elledge SJ, Walter JC. The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. *Science*. 2009; 326(5960):1698–1701. [PubMed: 19965384]
- Kratz K, Schopf B, Kaden S, Sandoel A, Eberhard R, Lademann C, Cannavo E, Sartori AA, Hengartner MO, Jiricny J. Deficiency of FANCD2-associated nuclease KIAA1018/FANL1 sensitizes cells to interstrand crosslinking agents. *Cell*. 2010; 142(1):77–88. [PubMed: 20603016]
- Labib K, Tercero JA, Diffley JF. Uninterrupted MCM2-7 function required for DNA replication fork progression. *Science*. 2000; 288(5471):1643–1647. [PubMed: 10834843]
- Lage C, de Padula M, de Alencar TA, da Fonseca Goncalves SR, da Silva Vidal L, Cabral-Neto J, Leitao AC. New insights on how nucleotide excision repair could remove DNA adducts induced

- by chemotherapeutic agents and psoralens plus UV-A (PUVA) in *Escherichia coli* cells. *Mutat Res.* 2003; 544(2-3):143–157. [PubMed: 14644316]
- Lange SS, Takata K, Wood RD. DNA polymerases and cancer. *Nat Rev Cancer.* 2011; 11(2):96–110. [PubMed: 21258395]
- Le Breton C, Hennion M, Arimondo PB, Hyrien O. Replication-fork stalling and processing at a single psoralen interstrand crosslink in *Xenopus* egg extracts. *PLoS One.* 2011; 6(4):e18554. [PubMed: 21525992]
- Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, Kannouche PL, Green CM. Translesion synthesis: Y-family polymerases and the polymerase switch. *DNA Repair (Amst).* 2007; 6(7):891–899. [PubMed: 17363342]
- Lehoczyk P, McHugh PJ, Chovanec M. DNA interstrand cross-link repair in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev.* 2007; 31(2):109–133. [PubMed: 17096663]
- Lei M, Tye BK. Initiating DNA synthesis: from recruiting to activating the MCM complex. *J Cell Sci.* 2001; 114(Pt 8):1447–1454. [PubMed: 11282021]
- Letessier A, Millot GA, Koundrioukoff S, Lachages AM, Vogt N, Hansen RS, Malfoy B, Brison O, Debatisse M. Cell-type-specific replication initiation programs set fragility of the FRA3B fragile site. *Nature.* 2011; 470(7332):120–123. [PubMed: 21258320]
- Leung JW, Wang Y, Fong KW, Huen MS, Li L, Chen J. Fanconi anemia (FA) binding protein FAAP20 stabilizes FA complementation group A (FANCA) and participates in interstrand cross-link repair. *Proc Natl Acad Sci U S A.* 2012; 109(12):4491–4496. [PubMed: 22396592]
- Liu T, Ghosal G, Yuan J, Chen J, Huang J. FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. *Science.* 2010; 329(5992):693–696. [PubMed: 20671156]
- Long DT, Raschle M, Joukov V, Walter JC. Mechanism of RAD51-dependent DNA interstrand cross-link repair. *Science.* 2011; 333(6038):84–87. [PubMed: 21719678]
- Lukas C, Savic V, Bekker-Jensen S, Doil C, Neumann B, Pedersen RS, Grofte M, Chan KL, Hickson ID, Bartek J, et al. 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. *Nat Cell Biol.* 2011; 13(3):243–253. [PubMed: 21317883]
- Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D'Andrea AD, Dutta A. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Mol Cell.* 2006; 23(4):589–596. [PubMed: 16916645]
- MacKay C, Declais AC, Lundin C, Agostinho A, Deans AJ, MacArtney TJ, Hofmann K, Gartner A, West SC, Helleday T, et al. Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell.* 2010; 142(1):65–76. [PubMed: 20603015]
- Maga G, Hubscher U. Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *J Cell Sci.* 2003; 116(Pt 15):3051–3060. [PubMed: 12829735]
- Mechali M. Eukaryotic DNA replication origins: many choices for appropriate answers. *Nat Rev Mol Cell Biol.* 2010; 11(10):728–738. [PubMed: 20861881]
- Meetei AR, de Winter JP, Medhurst AL, Wallisch M, Waisfisz Q, van de Vrugt HJ, Oostra AB, Yan Z, Ling C, Bishop CE, et al. A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat Genet.* 2003; 35(2):165–170. [PubMed: 12973351]
- Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, Bier P, Steltenpool J, Stone S, Dokal I, Mathew CG, et al. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat Genet.* 2005; 37(9):958–963. [PubMed: 16116422]
- Minko IG, Harbut MB, Kozekov ID, Kozekova A, Jakobs PM, Olson SB, Moses RE, Harris TM, Rizzo CJ, Lloyd RS. Role for DNA polymerase kappa in the processing of N2-N2-guanine interstrand cross-links. *J Biol Chem.* 2008; 283(25):17075–17082. [PubMed: 18434313]
- Mirchandani KD, McCaffrey RM, D'Andrea AD. The Fanconi anemia core complex is required for efficient point mutagenesis and Rev1 foci assembly. *DNA Repair (Amst).* 2008; 7(6):902–911. [PubMed: 18448394]
- Mogi S, Butcher CE, Oh DH. DNA polymerase eta reduces the gamma-H2AX response to psoralen interstrand crosslinks in human cells. *Exp Cell Res.* 2008; 314(4):887–895. [PubMed: 18068156]
- Moldovan GL, D'Andrea AD. How the fanconi anemia pathway guards the genome. *Annu Rev Genet.* 2009; 43:223–249. [PubMed: 19686080]

- Moldovan GL, Madhavan MV, Mirchandani KD, McCaffrey RM, Vinciguerra P, D'Andrea AD. DNA polymerase POLN participates in cross-link repair and homologous recombination. *Mol Cell Biol*. 2010; 30(4):1088–1096. [PubMed: 19995904]
- Montes de Oca R, Andreassen PR, Margossian SP, Gregory RC, Taniguchi T, Wang X, Houghtaling S, Grompe M, D'Andrea AD. Regulated interaction of the Fanconi anemia protein, FANCD2, with chromatin. *Blood*. 2005; 105(3):1003–1009. [PubMed: 15454491]
- Mosedale G, Niedzwiedz W, Alpi A, Perrina F, Pereira-Leal JB, Johnson M, Langevin F, Pace P, Patel KJ. The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nat Struct Mol Biol*. 2005; 12(9):763–771. [PubMed: 16116434]
- Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nat Rev Mol Cell Biol*. 2010; 11(3):196–207. [PubMed: 20177395]
- Muniandy PA, Thapa D, Thazhathveetil AK, Liu ST, Seidman MM. Repair of laser-localized DNA interstrand cross-links in G1 phase mammalian cells. *J Biol Chem*. 2009; 284(41):27908–27917. [PubMed: 19684342]
- Murai J, Yang K, Dejsuphong D, Hirota K, Takeda S, D'Andrea AD. The USP1/UAF1 complex promotes double-strand break repair through homologous recombination. *Mol Cell Biol*. 2011; 31(12):2462–2469. [PubMed: 21482670]
- Naim V, Rosselli F. The FANCD1 pathway and BLM collaborate during mitosis to prevent micro-nucleation and chromosome abnormalities. *Nat Cell Biol*. 2009; 11(6):761–768. [PubMed: 19465921]
- Nakanishi K, Cavallo F, Perrouault L, Giovannangeli C, Moynahan ME, Barchi M, Brunet E, Jasin M. Homology-directed Fanconi anemia pathway cross-link repair is dependent on DNA replication. *Nat Struct Mol Biol*. 2011; 18(4):500–503. [PubMed: 21423196]
- Nakanishi K, Yang YG, Pierce AJ, Taniguchi T, Digweed M, D'Andrea AD, Wang ZQ, Jasin M. Human Fanconi anemia monoubiquitination pathway promotes homologous DNA repair. *Proc Natl Acad Sci U S A*. 2005; 102(4):1110–1115. [PubMed: 15650050]
- Niedernhofer LJ, Lalai AS, Hoeijmakers JH. Fanconi anemia (cross)linked to DNA repair. *Cell*. 2005; 123(7):1191–1198. [PubMed: 16377561]
- Nijman SM, Huang TT, Dirac AM, Brummelkamp TR, Kerkhoven RM, D'Andrea AD, Bernards R. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol Cell*. 2005; 17(3):331–339. [PubMed: 15694335]
- Nojima K, Hochegger H, Saberi A, Fukushima T, Kikuchi K, Yoshimura M, Orelli BJ, Bishop DK, Hirano S, Ohzeki M, et al. Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells. *Cancer Res*. 2005; 65(24):11704–11711. [PubMed: 16357182]
- Noll DM, Mason TM, Miller PS. Formation and repair of interstrand cross-links in DNA. *Chem Rev*. 2006; 106(2):277–301. [PubMed: 16464006]
- Oestergaard VH, Langevin F, Kuiken HJ, Pace P, Niedzwiedz W, Simpson LJ, Ohzeki M, Takata M, Sale JE, Patel KJ. Deubiquitination of FANCD2 is required for DNA crosslink repair. *Mol Cell*. 2007; 28(5):798–809. [PubMed: 18082605]
- Pace P, Johnson M, Tan WM, Mosedale G, Sng C, Hoatlin M, de Winter J, Joenje H, Gergely F, Patel KJ. FANCE: the link between Fanconi anaemia complex assembly and activity. *EMBO J*. 2002; 21(13):3414–3423. [PubMed: 12093742]
- Papadopoulou D, Guillouf C, Mohrenweiser H, Moustacchi E. Hypomutability in Fanconi anemia cells is associated with increased deletion frequency at the HPRT locus. *Proc Natl Acad Sci U S A*. 1990; 87(21):8383–8387. [PubMed: 2236046]
- Patel KJ, Joenje H. Fanconi anemia and DNA replication repair. *DNA Repair (Amst)*. 2007; 6(7):885–890. [PubMed: 17481966]
- Prakash S, Johnson RE, Prakash L. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annu Rev Biochem*. 2005; 74:317–353. [PubMed: 15952890]
- Raschle M, Knipscheer P, Enoiu M, Angelov T, Sun J, Griffith JD, Ellenberger TE, Scharer OD, Walter JC. Mechanism of replication-coupled DNA interstrand crosslink repair. *Cell*. 2008; 134(6):969–980. [PubMed: 18805090]

- Rego MA, Harney JA, Mauro M, Shen M, Howlett NG. Regulation of the activation of the Fanconi anemia pathway by the p21 cyclin-dependent kinase inhibitor. *Oncogene*. 2012; 31(3):366–375. [PubMed: 21685936]
- Sarkar S, Davies AA, Ulrich HD, McHugh PJ. DNA interstrand crosslink repair during G1 involves nucleotide excision repair and DNA polymerase zeta. *EMBO J*. 2006; 25(6):1285–1294. [PubMed: 16482220]
- Sato K, Toda K, Ishiai M, Takata M, Kurumizaka H. DNA robustly stimulates FANCD2 monoubiquitylation in the complex with FANCI. *Nucleic Acids Res*. 2012
- Sclafani RA, Holzen TM. Cell cycle regulation of DNA replication. *Annu Rev Genet*. 2007; 41:237–280. [PubMed: 17630848]
- Sengerova B, Wang AT, McHugh PJ. Orchestrating the nucleases involved in DNA interstrand cross-link (ICL) repair. *Cell Cycle*. 2011; 10(23):3999–4008. [PubMed: 22101340]
- Shen X, Do H, Li Y, Chung WH, Tomasz M, de Winter JP, Xia B, Elledge SJ, Wang W, Li L. Recruitment of fanconi anemia and breast cancer proteins to DNA damage sites is differentially governed by replication. *Mol Cell*. 2009; 35(5):716–723. [PubMed: 19748364]
- Shen X, Jun S, O’Neal LE, Sonoda E, Bemark M, Sale JE, Li L. REV3 and REV1 play major roles in recombination-independent repair of DNA interstrand cross-links mediated by monoubiquitinated proliferating cell nuclear antigen (PCNA). *J Biol Chem*. 2006; 281(20):13869–13872. [PubMed: 16571727]
- Shereda RD, Machida Y, Machida YJ. Human KIAA1018/FAN1 localizes to stalled replication forks via its ubiquitin-binding domain. *Cell Cycle*. 2010; 9(19):3977–3983. [PubMed: 20935496]
- Sims AE, Spiteri E, Sims RJ 3rd, Arita AG, Lach FP, Landers T, Wurm M, Freund M, Neveling K, Hanenberg H, et al. FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. *Nat Struct Mol Biol*. 2007; 14(6):564–567. [PubMed: 17460694]
- Singh TR, Bakker ST, Agarwal S, Jansen M, Grassman E, Godthelp BC, Ali AM, Du CH, Rooimans MA, Fan Q, et al. Impaired FANCD2 monoubiquitination and hypersensitivity to camptothecin uniquely characterize Fanconi anemia complementation group M. *Blood*. 2009; 114(1):174–180. [PubMed: 19423727]
- Smeaton MB, Hlavin EM, McGregor Mason T, Noronha AM, Wilds CJ, Miller PS. Distortion-dependent unhooking of interstrand cross-links in mammalian cell extracts. *Biochemistry*. 2008; 47(37):9920–9930. [PubMed: 18702509]
- Smogorzewska A, Desetty R, Saito TT, Schlabach M, Lach FP, Sowa ME, Clark AB, Kunkel TA, Harper JW, Colaiacovo MP, et al. A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. *Mol Cell*. 2010; 39(1):36–47. [PubMed: 20603073]
- Smogorzewska A, Matsuoka S, Vinciguerra P, McDonald ER 3rd, Hurov KE, Luo J, Ballif BA, Gygi SP, Hofmann K, D’Andrea AD, et al. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell*. 2007; 129(2):289–301. [PubMed: 17412408]
- Spardy N, Duensing A, Charles D, Haines N, Nakahara T, Lambert PF, Duensing S. The human papillomavirus type 16 E7 oncoprotein activates the Fanconi anemia (FA) pathway and causes accelerated chromosomal instability in FA cells. *J Virol*. 2007; 81(23):13265–13270. [PubMed: 17898070]
- Takahashi TS, Wigley DB, Walter JC. Pumps, paradoxes and ploughshares: mechanism of the MCM2-7 DNA helicase. *Trends Biochem Sci*. 2005; 30(8):437–444. [PubMed: 16002295]
- Taniguchi T, Garcia-Higuera I, Andreassen PR, Gregory RC, Grompe M, D’Andrea AD. S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. *Blood*. 2002; 100(7):2414–2420. [PubMed: 12239151]
- Timmers C, Taniguchi T, Hejna J, Reifsteck C, Lucas L, Bruun D, Thayer M, Cox B, Olson S, D’Andrea AD, et al. Positional cloning of a novel Fanconi anemia gene, FANCD2. *Mol Cell*. 2001; 7(2):241–248. [PubMed: 11239453]
- Tumini E, Plevani P, Muzi-Falconi M, Marini F. Physical and functional crosstalk between Fanconi anemia core components and the GINS replication complex. *DNA Repair (Amst)*. 2011; 10(2):149–158. [PubMed: 21109493]

- Vagnarelli P, Hudson DF, Ribeiro SA, Trinkle-Mulcahy L, Spence JM, Lai F, Farr CJ, Lamond AI, Earnshaw WC. Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis. *Nat Cell Biol.* 2006; 8(10):1133–1142. [PubMed: 16998479]
- Vaz F, Hanenberg H, Schuster B, Barker K, Wiek C, Erven V, Neveling K, Endt D, Kesterton I, Autore F, et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. *Nat Genet.* 2010; 42(5):406–409. [PubMed: 20400963]
- Wang LC, Gautier J. The Fanconi anemia pathway and ICL repair: implications for cancer therapy. *Crit Rev Biochem Mol Biol.* 2010; 45(5):424–439. [PubMed: 20807115]
- Wang X, Peterson CA, Zheng H, Nairn RS, Legerski RJ, Li L. Involvement of nucleotide excision repair in a recombination-independent and error-prone pathway of DNA interstrand cross-link repair. *Mol Cell Biol.* 2001; 21(3):713–720. [PubMed: 11154259]
- Warbrick E. PCNA binding through a conserved motif. *Bioessays.* 1998; 20(3):195–199. [PubMed: 9631646]
- Williams SA, Longerich S, Sung P, Vaziri C, Kupfer GM. The E3 ubiquitin ligase RAD18 regulates ubiquitylation and chromatin loading of FANCD2 and FANCI. *Blood.* 2011; 117(19):5078–5087. [PubMed: 21355096]
- Woodward AM, Gohler T, Luciani MG, Oehlmann M, Ge X, Gartner A, Jackson DA, Blow JJ. Excess Mcm2-7 license dormant origins of replication that can be used under conditions of replicative stress. *J Cell Biol.* 2006; 173(5):673–683. [PubMed: 16754955]
- Zheng H, Wang X, Warren AJ, Legerski RJ, Nairn RS, Hamilton JW, Li L. Nucleotide excision repair- and polymerase eta-mediated error-prone removal of mitomycin C interstrand cross-links. *Mol Cell Biol.* 2003; 23(2):754–761. [PubMed: 12509472]
- Zhu W, Abbas T, Dutta A. DNA replication and genomic instability. *Adv Exp Med Biol.* 2005; 570:249–279. [PubMed: 18727504]
- Zhu W, Dutta A. An ATR- and BRCA1-mediated Fanconi anemia pathway is required for activating the G2/M checkpoint and DNA damage repair upon rereplication. *Mol Cell Biol.* 2006; 26(12):4601–4611. [PubMed: 16738325]
- Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science.* 2003; 300(5625):1542–1548. [PubMed: 12791985]

Figure 1. The Fanconi Anemia Repair Pathway

The **FA Core Complex** consisting of FANCA, B, C, D, E, F, G, L and M + accessory components FAAP20, FAAP24 and FAAP100 recognize ICL lesions through FANCM. Recognition of the ICL triggers the core complex to monoubiquitinate the **ID Complex** (FANCI + FANCD2). Once the ID complex becomes monoubiquitinated FAN1 binds to the ID complex which localizes with the **Downstream Effector Proteins** FANCN, FANCI, RAD51C, FANCD1 and SLX4. USP1/UAF1 deubiquitinate the ID complex upon completion of ICL repair.

Figure 2. Replication Dependent and Independent ICL Repair Pathways

A. Replication-independent ICL repair. The ICL is recognized and unhooked by the NER pathway. TLS polymerase synthesis passed the unhooked ICL filling the gap generated by the unhooking. The ICL remnant is excised and repair is complete **B.** Replication-dependent ICL repair. Replication forks converge towards the ICL. The replication forks stall 20–40 nucleotides before the ICL. Then the leading strand of one fork advances towards the ICL and pauses 1 nucleotide from the ICL. Dual incisions are made on the non-template strand and a TLS polymerase extends past the unhooked ICL. The ICL remnant is removed and HR repairs the double strand break.

Table 1

The Fanconi Anemia Genes and Associated Proteins

Chromosomal locations were identified in Ensembl and associated proteins were collected from UniProtKB.

Fanconi Anemia Genes	Chromosomal Location	Associated Proteins	Gene Function
FANCA	16q24.3	FAAP20, FANCC, FANCG	FA core complex stability
FANCB	Xp22.31	FAAP100	FA core complex stability
FANCC	9q22.3	FANCA, FANCG	FA core complex stability
FANCE	6p21.3	FANCD2	FA core complex stability
FANCF	11p15	FANCM	FA core complex stability
FANCG	9p13	FANCA, FANCC, FANCD1/BRCA2, FANCD2, XRCC3	FA core complex stability
FANCL	2p16.1	FAAP100, UBE2T	E3 Ligase for the ID complex
FANCM	14q21.2	FAAP24, FANCF	Targeting component of the FA core complex Binds to DNA directly Translocase activity
FANCD2	3p25.3	FANCI, FANCE, PCNA USP1, BRCA1, BLM, BRCA2, FAN1, RAD51, MEF1, SNM1B,	ID complex is required for both the incision step and the insertion of a nucleotide opposite the ICL lesion
FANCI	15q26.1	FANCD2, USP1	Binds to DNA directly
FANCD1/BRCA2	13q12.3	FANCN/PALB2, BRCA1	HR repair
FANCI/BACH1	17q23.2	BRCA1	Helicase/Translocase
FANCN/PALB2	16p12.2	FANCD1/BRCA2, BRCA1	HR repair
FANCP/SLX4	16p13.3	XPF-ERCC1, MUS81-EME1, SLX1	HR repair