REVIEW

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Fanconi anaemia and the repair of Watson and Crick DNA crosslinks

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The function of Fanconi anaemia proteins is to maintain genomic stability. Their main role is in the repair of DNA interstrand crosslinks, which, by covalently binding the Watson and the Crick strands of DNA, impede replication and transcription. Inappropriate repair of interstrand crosslinks causes genomic instability, leading to cancer; conversely, the toxicity of crosslinking agents makes them a powerful chemotherapeutic. Fanconi anaemia proteins can promote stem-cell function, prevent tumorigenesis, stabilize replication forks and inhibit inaccurate repair. Recent advances have identified endogenous aldehydes as possible culprits of DNA damage that may induce the phenotypes seen in patients with Fanconi anaemia.

Fanconi anaemia occurs in about 1 in every 100,000 births[1]. Biallelic mutations in Fanconi anaemia genes lead to bone-marrow failure and susceptibility to both acute myeloid leukaemia (AML) and solid tumours, congenital abnormalities and infertility[2]. The function of the pathway, on the other hand, is anything but rarefied: Fanconi anaemia proteins participate in the repair of extraordinarily deleterious lesions, interstrand crosslinks, and in maintaining genomic stability during DNA replication.

Fanconi anaemia is genetically heterogeneous. So far, 15 genes have been identified as mutated in patients (Fig. 1 and Table 1), and many more interacting genes have been discovered. Furthermore, there are still patients in whom a mutation has yet to be identified. The known Fanconi anaemia genes work together in interstrand crosslink repair to coordinate the actions of multiple repair processes, in particular nucleases that are necessary for cutting out the interstrand crosslink and for other nucleolytic processing needed for repair, trans-lesion synthesis (TLS) (a mode of damage tolerance that uses specialized polymerases to insert a base across from a lesion or abasic site) and homologous recombination (the pathway best known for its role in repairing double-strand breaks). Fanconi anaemia proteins may also counteract some of the activities of the non-homologous end joining (NHEJ) pathway, an error-prone repair pathway that is used to directly religate DNA ends. Overall, the Fanconi anaemia pathway interacts with many of the genome maintenance pathways, and studying it provides a unique window into the elaborate interplay of multiple cellular networks.

As new functions come to light, the list of biological processes in which the Fanconi anaemia proteins are intimately involved is expanding (Fig. 2). The bone-marrow failure seen in patients with the disorder suggests a function for the pathway in stem-cell biology, and recent work has begun to gain tantalizing insight into not only Fanconi anaemia pathogenesis, but also the intersection between DNA repair and stem-cell maintenance. Several genes involved in the Fanconi anaemia pathway also predispose individuals to breast and ovarian cancer, which, together with the cancer susceptibility of patients with Fanconi anaemia, suggests Fanconi anaemia proteins have an important role in suppressing tumorigenesis. Recent work has also described an unanticipated role for Fanconi anaemia proteins in replication-fork stabilization. Finally, the identification of aldehydes as endogenous genotoxins in Fanconi anaemia provides an exciting glimpse into the challenges in the cellular environment that are important in human disease. In this Review, we highlight these topics and discuss the current understanding of the molecular details of the Fanconi anaemia pathway.

Stem-cell function in Fanconi anaemia pathway absence

One of the clinical identifiers of Fanconi anaemia is bone-marrow failure, which can be treated by bone-marrow transplantation, suggesting that disrupting the Fanconi anaemia pathway leads to dysfunction of the haematopoietic stem cells and progenitor cells themselves. Although the bulk of Fanconi anaemia research so far has concentrated on the DNA repair functions of the pathway in terminally differentiated cells, this clinical manifestation points to a crucial role in stem-cell development that must be explored. A clear connection between the cellular and disease phenotypes in Fanconi anaemia has been elusive, owing to the difficulty in establishing mouse models that parallel human disease and because the limited proliferative potential of primary haematopoietic stem cells makes patient-derived *in vitro* systems unfeasible. Understanding the interactions between the function of the Fanconi anaemia pathway and stem-cell regulation and maintenance will probably prove to be key to elucidating the functional link between the pathway and the disease.

Recent research provides a glimpse into a functional link between defects in genome maintenance and bone-marrow failure in Fanconi anaemia. Levels of p53, one of the central 'guardians' of the genome, and its transcriptional target p21 are elevated in primary blood, bone marrow and even in the livers of fetuses with Fanconi anaemia during haematopoietic stem-cell expansion in the organ. Perhaps because of this hyperactivation, the pool of available haematopoietic stem cells and progenitor cells is already compromised at birth in individuals with Fanconi anaemia, and the bone marrow of these patients is less proliferative than the bone marrow of healthy individuals — with more cells in the G0 and G1 cell-cycle stages and an increase in DNA damage signalling, as indicated by the presence of phosphorylated histone H2AX. Not only are there fewer haematopoietic stem cells and progenitor cells in Fanconi anaemia bone marrow, but those stem cells are also unable to produce progenitors *in vitro*[3].

Disrupting the Fanconi anaemia pathway is itself sufficient to impair haematopoietic development *in vitro*. Short-hairpin RNA knockdown of either *FANCA* or *FANCD2* in human embryonic stem cells leads to significantly reduced production of haematopoietic stem and progenitor cells and their differentiated daughter populations[4]. Exposing progenitor cells to crosslinking agents exacerbates the proliferation deficiency. These findings link the canonical interstrand crosslink repair function of the Fanconi anaemia pathway to its role in stem-cell development. In murine haematopoietic stem cells and human bone-marrow stromal cells, Fanconi anaemia pathway deficiency leads to an

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increase in binucleated cells, suggesting that cytokinesis failure may also contribute to bone-marrow failure[5].

An important role for the Fanconi anaemia pathway in stem-cell function was uncovered when researchers attempted to derive induced pluripotent stem (iPS) cells from Fanconi anaemia fibroblasts. They discovered that fibroblasts that are deficient for the Fanconi anaemia pathway are refractory to reprogramming[6,7]. It has been proposed, although not shown, that Fanconi anaemia proteins participate directly in reprogramming. Alternatively, the increase in DNA damage signalling in Fanconi anaemia cells may preclude the normal cell-cycle progression (division) necessary for reprogramming. It is also possible that the Fanconi anaemia pathway is required to repair DNA damage that reprogramming itself induces. Discriminating between these possibilities will be key to understanding the role of the Fanconi anaemia pathway in stem cells.

Taken together, recent data suggest a connection between DNA repair defects and haematopoietic stem cell and progenitor cell failure, although many mysteries remain. It is intriguing, for example, that Fanconi anaemia pathway deficiency leads to problems with both quiescent haematopoietic stem cells and their rapidly dividing progenitors — two very different cellular landscapes. An appreciation of the underlying molecular functions of Fanconi anaemia proteins in haematopoietic stem-cell populations will provide a better understanding of the pathophysiology of the disease. Furthermore, teasing out how a pathway that is traditionally associated with DNA repair is important to the normal function of a stem-cell population will provide insight into stem-cell biology in general.

Fanconi anaemia pathway and tumour suppression

Fanconi anaemia has a complicated relationship with tumorigenesis. Although susceptibility to cancer, particularly AML and squamous cell carcinomas, is endemic in patients with homozygous mutations in Fanconi anaemia genes, four out of five downstream members of the pathway — which function in homology-directed repair of DNA ends — also confer susceptibility to breast cancer and ovarian cancer when mutated in only one copy[8–14] (Table 1). The best known breast cancer predisposition gene *BRCA2* (also known as *FANCD1*)[15] is involved in the Fanconi anaemia pathway, as is its partner *PALB2* (also known as *FANCN*)[16], as well as *BRIP1* (also known as *FANCJ*)[17,18]. Defects in *RAD51C*, one of the RAD51 paralogues important for homologous recombination, are the cause of a Fanconi-anaemia-like disorder, and *RAD51C* is provisionally called *FANCO*[19]. Patients with biallelic *BRCA2* and *PALB2* mutations evince more severe phenotypes with more prevalent and earlier onset of leukaemia and appearence of embryonal tumours in the first 2 years of life.

The cancers that develop in patients with Fanconi anaemia are indicative of the complex landscape that is required for tumorigenesis to progress. Predisposition to AML may derive from the same haematopoietic stem-cell instability that contributes to bone-marrow failure. The types of squamous cell carcinoma of the head and neck that are common to patients with Fanconi anaemia are also associated with human papilloma virus, raising the possibility that these tumours develop in the context of infection with an oncogenic virus.

The correlation between downstream members of the Fanconi anaemia pathway and cancer is intuitive when one considers the mechanics of the pathway. The upstream members that belong to the core and FANCI–FANCD2 (I–D2) complex are more self-contained, functioning in pathway activation and early coordination steps, whereas BRCA2 and PALB2 are intimately associated with homology-directed repair (homologous recombination), which is used more broadly outside and independently of the Fanconi anaemia pathway.

Fanconi anaemia proteins in interstrand crosslink repair

Crosslinked DNA impedes both transcription and replication; interstrand crosslinks, therefore, need to be removed during all stages of the cell cycle. Indeed, there is evidence for both replication-dependent and replication-independent repair. In the absence of damage sensing by active replication forks and a homologous template, repair depends on nucleotide excision repair, especially transcription-coupled nucleotide excision repair and TLS proteins (including polymerase κ)[20,21]. The involvement of Fanconi anaemia and Fanconi-anaemia-associated proteins in G1 repair remains to be elucidated, although the core complex components and the I–D2 complex are able to bind to interstrand crosslinks independently of replication and their absence results in inefficient repair[22].

During S phase, the replication fork encounters a lesion and is forced to stall, activating a cascade of events that leads to initiation of the DNA damage response. Current models favour a structure in which two replication forks converge on a single lesion, which is most likely to occur in late S phase[23]; however, it is likely that a replication fork approaching the lesion from only one side would also elicit repair. Replication-fork stalling at the site of damage is followed by an unhooking step, in which incisions are made on either side of the covalently linked nucleotides (Fig. 1). On the strand that is incised, TLS polymerases are recruited to bypass the unhooked crosslink. The incision event also effectively creates

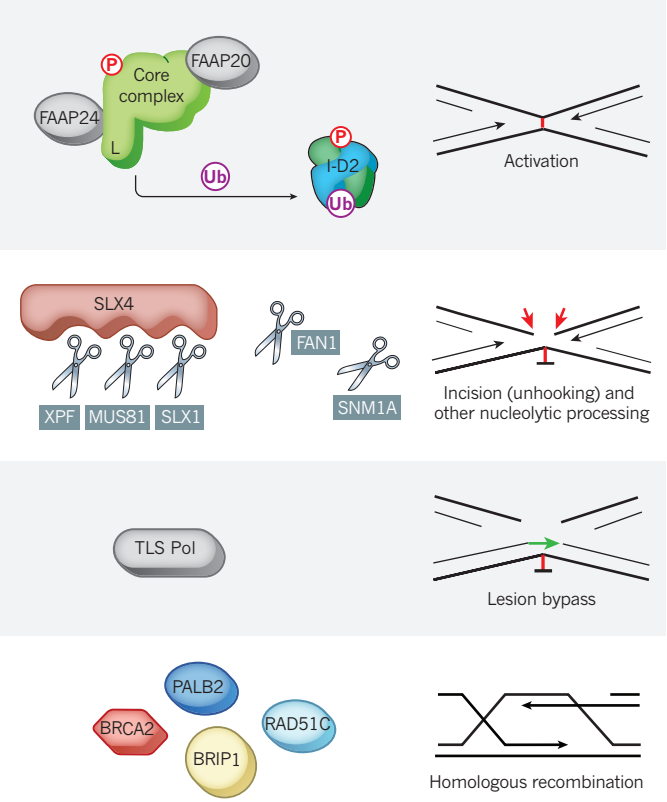

Figure 1 | The Fanconi anaemia pathway and interstrand crosslink repair. The core complex is made up of Fanconi anaemia proteins and accessory proteins (such as FAAP20 and FAAP24, shown here). On detection of the crosslink, the complex is activated by ATR-mediated phosphorylation (P), and one component, FANCL, ubiquitinates (Ub) the I–D2 complex. The I–D2 complex then coordinates the action of downstream repair factors. SLX4 functions as a scaffold for the three nucleases XPF, MUS81 and SLX1 that function at the site of DNA damage to make incisions either side of two covalently linked nucleotides. FAN1 and SNM1A also have a role in processing the crosslink after incision, although the precise mechanism is unclear. On the incised strand, TLS polymerases (TLS Pol) are recruited to bypass the unhooked crosslink. The break is then repaired through homologous recombination involving the Fanconi anaemia proteins BRCA2, BRIP1, PALB2 and RAD51C. SLX4 may also have a role here by regulating the nucleases MUS81 and SLX1. BRIP1 is implicated in homologous recombination but is also required at an earlier step for pathway activation. The proteins shown in colour are Fanconi anaemia proteins. Those in grey are important for interstrand crosslink repair, but their genes have not been found to be mutated in patients with Fanconi anaemia.

Table 1 | Fanconi anaemia and Fanconi-anaemia-associated DNA repair factors and human disease

Genes	Human phenotypes	Cancer type in patients with Fanconi anaemia and in mutation carriers (italics)	Pathway and function	Link to Fanconi anaemia
FANCA, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL** and *FANCM**	BMF, growth retardation, thumb and radial ray defects, developmental abnormalities, hypogonadism, microcephaly, infertility and hyperpigmentation	AML, HNSCC, oesophageal cancer, gynaecological cancers and liver cancers. *No cancers in mutation carriers for most groups, but FANCC carriers show increased risk of breast cancer*[75]	Fanconi anaemia; core complex; FANCL is an E3 ubiquitin ligase	Genes mutated in Fanconi anaemia
BRCA2 (also known as *FANCD1*)	BMF, growth retardation, thumb and radial ray defects and microcephaly	AML, ALL, medulloblastoma, neuroblastoma and Wilms tumour† *Breast, ovarian and prostate cancer*	Fanconi anaemia, homologous recombination; loads RAD51 onto DNA	Gene mutated in Fanconi anaemia
BRIP1 (also known as *FANCJ*)	BMF, growth retardation, thumb and radial ray defects, severe developmental abnormalities and hyperpigmentation	AML, HNSCC *Breast and ovarian cancer*	Fanconi anaemia, homologous recombination; 3′ to 5′ helicase	Gene mutated in Fanconi anaemia
PALB2 (also known as *FANCN*)	Anaemia, growth retardation, thumb radial ray defects, hyperpigmentation and microcephaly	AML, medulloblastoma, neuroblastoma, Wilms tumour and haemangioendothelioma† *Breast, ovarian and pancreatic cancer*	Fanconi anaemia, homologous recombination; promotes BRCA2 function	Gene mutated in Fanconi anaemia
RAD51C (provisionally known as *FANCO*‡)	Growth retardation, thumb and radial ray defects, hypogonadism, cystic kidneys and renal failure	*Breast and ovarian cancer*	Homologous recombination	Gene mutated in Fanconi-anaemia-like syndrome
SLX4 (also known as *FANCP*)	BMF, growth retardation, microcephaly, developmental defects including thumb and radial ray defects	HNSCC (one patient)	Fanconi anaemia; coordinates XPF–ERCC1, MUS81–EME1 and SLX1 nucleases	Gene mutated in Fanconi anaemia
BLM	Growth retardation, sun sensitivity, COPD, diabetes, recurrent infections and infertility	Broad predisposition including non-Hodgkin's lymphoma, AML, ALL, skin cancer, colon cancer and breast cancer	5′-3′ helicase, anti-recombinase	Interacts with core complex, BRIP1; genetic interaction with SLX4
BRCA1	No patients with biallelic mutations reported yet	*Breast and ovarian cancer*	Homologous recombination, interstrand crosslink repair	Interacts with BRIP1, BRCA2; necessary for Fanconi anaemia pathway activation
LIG4, DNA-PK$_{cs}$, Ku70 and Ku80§	Immune deficiency, pancytopaenias, growth retardation, microcephaly and skin photosensitivity	T-cell ALL (one report)	NHEJ	Genetic interaction with Fanconi anaemia proteins (see Table 2)
FAN1	KIN and renal failure	None reported	Interstrand crosslink repair	Interacts with I–D2 complex
XPF	Sun sensitivity, neurodegeneration and accelerated ageing	Basal and squamous cell carcinoma of the skin	NER, Fanconi anaemia nuclease	Interacts with SLX4

ALL, acute lymphocytic leukaemia; AML, acute myelogenous leukaemia; BMF, bone-marrow failure; COPD, chronic obstructive pulmonary disease; HNSCC, head and neck squamous cell carcinoma; I–D2, FANCI–FANCD2; KIN, karyomegalic interstitial nephritis; NER, nucleotide excision repair; NHEJ, non-homologous end joining. *No cancers have been reported in patients with *FANCL* or *FANCM* mutations. And the FANCM phenotype is unknown because the only patient described in the literature also has *FANCA* mutation. †In patients with *BRCA2* and *PALB2* mutations, the growth, bone-marrow and developmental phenotypes are as in other Fanconi anaemia complementation groups, but the probability of malignancy is higher, the spectrum is different and the age of onset is lower, indicative of a more extreme subtype of Fanconi anaemia. ‡FANCO is a provisional term, as patients with *RAD51C* mutations do not thus far display bone-marrow failure or cancer, and the chromosomal breakage in their cells is not as high as seen in individuals with Fanconi anaemia. §No mutations in *Ku70*, *Ku80* or *DNA-PK$_{cs}$* have ever been observed in patients.

a double-strand break, which is repaired by homologous recombination[23–25]. The multistep nature of interstrand crosslink repair requires the tight coordination of several different repair pathways, and it is within this delicately balanced system that the Fanconi anaemia pathway has its regulatory role.

An important step in the Fanconi anaemia pathway is the monoubiquitination of two components that form the I–D2 complex. Monoubiquitination of FANCD2 at Lys 561, and to a lesser extent of FANCI at Lys 523, represents the activation step of the Fanconi anaemia pathway[26,27]. This step is dependent on the Fanconi anaemia core complex, which comprises FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM, and accessory proteins, including FAAP20, FAAP24 and FAAP100. With the exception of FANCM, every member of the core complex is essential for successful monoubiquitination of I–D2. The ubiquitin ligase function of the core complex depends on the E3 ligase FANCL, with UBE2T lending its services as an E2 ligase[28–30]. Ubiquitination of FANCI and FANCD2 is mutually interdependent in mammalian cells, and also require discrete phosphorylation events: phosphorylation of FANCI at SQ or TQ sites close to the targeted lysine is necessary for specific ubiquitination of the I–D2 complex[31]. Recent evidence that FANCD2 ubiquitination is robustly stimulated by the presence of DNA suggests that this event may occur on chromatin[32]. Ubiquitinated I–D2 complex that is localized to chromatin orchestrates the actions of downstream repair proteins. Interestingly, the I–D2 complex may also have a direct role in the regulation of nucleosome assembly at sites of damage: the turnover of histone H3 after treatment with a crosslinker is markedly slowed after the gene encoding FANCD2 is knocked down[33].

The downstream components — those that are dispensable for monoubiquitination of the I–D2 complex — are BRCA2, BRIP1, PALB2 and RAD51C, which are the products of cancer predisposition genes necessary for homologous recombination, and SLX4 (also known as FANCP), which interacts with multiple nucleases (discussed in greater detail later). BRCA2 and PALB2 work in later stages of repair, in which they complete it through homologous recombination. The function of BRIP1 largely remains a mystery, and its role as a Fanconi anaemia protein needs to be explored further, although it has been implicated both in the activation of the Fanconi anaemia pathway as well as in the homologous recombination.

Although the details of how the Fanconi anaemia pathway is turned off have not been fully elucidated, one protein that clearly participates is the deubiquitinating enzyme USP1, which removes the ubiquitin from FANCD2 (ref. 34). In its absence, ubiquitinated FANCD2 is elevated, but the cells are sensitive to crosslink damage[35,36]. As such, it is clear that both appropriate activation and deactivation of the pathway are important for cell viability when challenged with interstrand crosslink damage.

The Fanconi anaemia pathway coordinates nuclease action

A key step in most repair pathways is the physical removal of the damaged bases, as well as the processing of the DNA by nucleases. In the case of interstrand crosslinks, the lesion affects both the Watson strand and the Crick strand, and its repair requires the collaboration of several processing enzymes. Each incision event requires a nuclease with the correct structure specificity to yield a repair intermediate that can correctly feed into the subsequent step. For example, crosslink unhooking must precede the resection steps that are necessary to provide a substrate for homologous recombination. Therefore, the removal steps are a complex choreography of nuclease action. The Fanconi anaemia pathway has been implicated in the recruitment and regulation of several different nucleases, including XPF–ERCC1, MUS81–EME1, SLX1 and FAN1 (refs 37–42).

SLX4 is a recently identified Fanconi anaemia protein that functions as a scaffold, modulator and cofactor for the three structure-specific nucleases XPF–ERCC1, MUS81–EME1 and SLX1 (refs 43–46). Essentially modular in nature, SLX4 is unique in that it can bring a veritable 'tool belt' of DNA-processing enzymes to the site of DNA damage, ensuring the presence of the best repair nuclease for the job. Of the three nucleases associated with SLX4, XPF–ERCC1 is the most important for resistance to interstrand crosslinks, with MUS81–EME1 and SLX1 having less prominent roles[47,48].

The exonuclease SNM1A is also biochemically active at sites of crosslinks. It is required for interstrand crosslink resistance, consistent with an *in vivo* function in repair of these lesions. Although its exact interaction with Fanconi anaemia proteins remains largely unclear, it is thought to act in concert with SLX4-associated XPF–ERCC1 to process the crosslink after the initial incision[49]. *In vitro*, SNM1A can digest interstrand crosslink containing DNA past the lesion, creating a preferred substrate for downstream TLS polymerases[49]. Understanding precisely how SNM1A, as well as other nucleases, is coordinated and controlled by, or independent of, Fanconi anaemia proteins will be crucial to understanding this multifaceted repair pathway.

Multiple research groups have identified the Fanconi-associated nuclease FAN1 to be required for interstrand crosslink resistance[38–41], implicating it in interstrand crosslink repair. FAN1 can be recruited to the sites of DNA damage in a manner dependent on the ubiquitinated I–D2 complex and on its own ubiquitin-binding domain. The precise mechanism of action of FAN1 on crosslinked DNA remains elusive; however, it is clear that the nuclease participates in a separate, but as yet uncharacterized, branch of crosslink repair in addition to the Fanconi anaemia pathway.

This function of FAN1 has recently been illuminated in the study of a rare genetic disease called karyomegalic interstitial nephritis (KIN), which is clinically characterized by progressive renal failure and histologically characterized by enlarged and hyperchromatic nuclei within the kidney tubular epithelium. A cohort of patients with KIN had mutations in *FAN1*. Interestingly, patients with KIN have none of the hallmarks of Fanconi anaemia, but their cells did have significant sensitivity to crosslinking agents. This sensitivity was complemented with wild-type FAN1, suggesting that an interstrand crosslink repair defect conferred by FAN1 deficiency does, at least in part, underlie the syndrome[50]. Deficiency of FAN1, which results in KIN but not Fanconi anaemia — despite the fact that FAN1 and FANCD2 interact — suggests that FAN1 might be redundant with another nuclease in the Fanconi anaemia pathway. It also implies that FAN1 has a function that is independent of the pathway in the kidney, in which FAN1 may repair lesions that the Fanconi anaemia pathway cannot. It is of interest that FAN1 expression is very high in the kidney. The deviation between KIN and Fanconi anaemia phenotypes is a fascinating puzzle that might help to understand different modes of interstrand-crosslink repair, as well as be a clue to the endogenous lesions that give rise to disease, not only in patients with rare disorders but also during normal ageing.

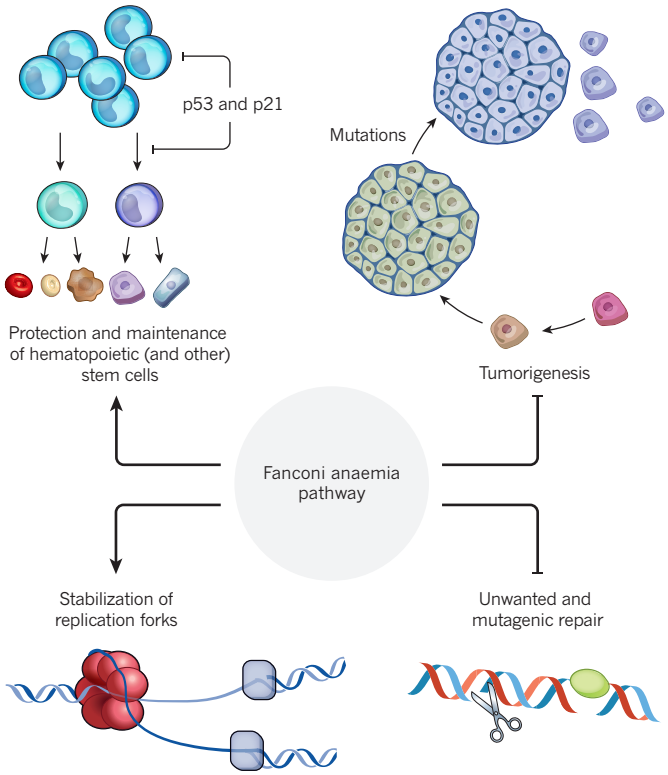

Figure 2 | The diverse functions of the Fanconi anaemia pathway. The Fanconi anaemia pathway has many roles in human biology. Disrupting the Fanconi anaemia pathway leads to dysfunction of haematopoietic stem and progenitor cells as a result of elevated levels of p53 and p21 in stem cells, leading to impaired differentiation and a compromised pool of cells at birth. This could lead to stochastic developmental abnormalities and infertility in individuals with Fanconi anaemia. Several genes in the Fanconi anaemia pathway predispose individuals to some cancers. Fanconi anaemia proteins could have a role in suppressing tumorigenesis, the sequential accrual of mutations that transforms a healthy cell into a rapidly dividing cancer cell and finally into a metastatic tumour. The Fanconi anaemia pathway preserves replication-fork stability during S phase. The pathway also functions as a barrier against unwanted mutagenic repair processes, for example NHEJ.

Fanconi anaemia pathway and replication stress

In addition to an indispensable role at DNA crosslinks, the Fanconi anaemia pathway is required for the protection of replication-fork stability under stress. The I–D2 complex is monoubiquitinated during S phase even in unchallenged cells[15]. The pathway is also activated by depletion of nucleotide pools with hydroxyurea, and even by ultraviolet light[51]. This is puzzling because the cell lines of patients with Fanconi anaemia are not sensitive to either of these agents. However, recent data suggest that FANCA, ubiquitinated FANCD2, BRCA1 and BRCA2 are necessary for the protection of the stalled replication fork after hydroxyurea treatment. It has been proposed that when these proteins are functionally absent, RAD51 is not deposited onto the fork under stress, and the nascent DNA is degraded in an MRE11-dependent manner[52,53]. The outcome seems to be an increase in chromosomal instability, which may not lead directly to immediate cellular demise, can still be mutagenic in the long term, thus contributing to the increase in tumorigenesis seen in patients with Fanconi anaemia. Importantly, the protective function

of the Fanconi anaemia–BRCA axis, which has been uncovered by these experiments, seems to be separate from the canonical function of BRCA2 in homologous recombination. It will be important to address whether any of the interstrand crosslink sensitivity of Fanconi anaemia cell lines might be secondary to a defect in fork-protection function, and whether the stem-cell failure in Fanconi anaemia can be mitigated by fork stabilization.

Cooperation with other DNA repair pathways

At its heart, repair of interstrand crosslinks mediated by the Fanconi anaemia pathway is a highly regulated ensemble of other repair pathways: a nuclease borrowed from canonical nucleotide excision repair makes incisions, trans-lesion synthesis polymerases are responsible for filling in the gap opposite the unhooked crosslink, and homologous recombination is co-opted to resolve the double strand breaks that result from the encounter between replication forks and the lesion. It is becoming clear that — in addition to using other pathways for discrete repair steps — the Fanconi anaemia pathway is intimately entwined with the DNA damage response as a whole, engaging in collaboration and antagonism with almost all of the classic repair pathways in the fight for survival after genotoxic insult. The most notable of these are the mismatch repair pathway components implicated in FANCJ[54] and FANCD2 (ref. 55) function, and the helicase BLM, which seems to intersect with the Fanconi anaemia pathway at multiple levels. *BLM* mutations are found in Bloom syndrome, an autosomal recessive disease of growth retardation, immunodeficiency, sun sensitivity and profound cancer predisposition. The BLM protein is responsible for unwinding a variety of DNA structures and together with DNA topoisomerase IIIα is able to remove the double Holliday junctions that form during homologous recombination[56]. This process, called double Holliday junction dissolution, prevents the inappropriate engagement of nucleolytic Holliday junction resolvases, whose action may lead to deleterious exchanges of DNA between sister chromatids.

Table 2 | Summary of genetic interactions between Fanconi anaemia and non-homologous end joining

Fanconi anaemia gene	NHEJ gene	Outcome
Caenorhabditis elegans		
fcd-2 (*FANCD2*) (germline mutation)	*lig-4* (germline mutation)	Rescue of crosslink sensitivity and aberrant meiotic crossovers[63]
Chicken DT40 cells		
FANCC (knockout)	*Ku70* (knockout)	Partial rescue of crosslink sensitivity[64]
FANCC (knockout)	*LIG4* or *DNA-PK*$_{cs}$ (knockout)	No rescue[64]
Mouse embryonic fibroblasts		
Fanca$^{-/-}$, *Fancc*$^{-/-}$ (germline mutation)	*DNA-PK*$_{cs}$ (NU7026 inhibitor)	Partial rescue of crosslink sensitivity[63]
Fancd2$^{-/-}$ (germline mutation)	*Prkdc*$^{sc/sc}$ (*scid* mouse)	No rescue[65]
Fancd2$^{-/-}$ (germline mutation)	*Ku80*, 53bp1 (germline mutations)	No rescue. Increase in chromosomal instability and sensitivity[66]
Human cells		
M059J cells, *FANCD2* siRNA	*DNA-PK*$_{cs}$ deficient cell line	Rescue of crosslink (MMC) sensitivity[63]
HeLa cells, *FANCA* siRNA	*DNA-PK*$_{cs}$ (NU7026 inhibitor)	Partial rescue of crosslink (MMC) sensitivity[63]
HeLa cells, *FANCD2* siRNA	*DNA-PK*$_{cs}$ inhibitor	Rescue of crosslink sensitivity[63]
PD773 (*FANCD2*), PD331 (*FANCC*), patient mutations	*KU80* siRNA	Partial rescue of crosslink (MMC) sensitivity[63]

MMC, mitomycin C; siRNA, short interfering RNA.

Two connections between Fanconi anaemia proteins and BLM have recently been uncovered. One pertains to ultrafine anaphase bridges, which are thread-like DNA structures that form between masses of condensed DNA during anaphase and are thought to arise from persistent, unresolved DNA catenanes or recombination intermediates. Both BLM and the I–D2 complex localize to these microbridges, although FANCI and FANCD2 travel only to a subset of them — probably those caused by replication stress at fragile sites[57]. These microbridges may underlie the cytokinesis failure in Fanconi anaemia bone-marrow cells. The second connection is with SLX4, which collaborates with BLM during Holliday junction resolution. Co-depletion of these two proteins leads to a uniquely aberrant chromosome morphology, for which sister chromatids seem to stay linked and the chromosomes themselves adopt an odd, segmented, appearance[58]. As with many of the apparent interactions that the Fanconi anaemia pathway has with other repair pathways, the underlying mechanisms and relationships must still be elaborated, but taken together, the data reiterate how the Fanconi anaemia pathway has a far-reaching influence on general genome maintenance.

Fanconi anaemia and non-homologous end joining

The DNA repair pathway that has a particularly fraught relationship with the Fanconi anaemia pathway is NHEJ. This is an error-prone mechanism for fixing double-strand breaks for which no sister chromatid is available to be the template for homologous recombination, and DNA ends are ligated directly together without regard for homology. The heterodimer Ku70–Ku80 (Ku) binds to DNA ends, where it recruits the catalytic subunit of DNA-dependent protein kinase (DNA-PK$_{cs}$). DNA ligase-4 (LIG4) is then brought in to carry out the ligation (reviewed in ref. 59).

NHEJ is the default double-strand break repair pathway in the G0 and G1 phases. In other phases of the cell cycle, the cell faces a choice of whether to use NHEJ or homologous recombination to restore the break. Recent work has uncovered a dynamic shift between preferred pathways throughout the cell cycle, with homologous recombination reaching a peak during late S phase[60]. The decision also hinges on which factors access the free DNA end. The pattern of resection at the site of the double-strand DNA break provides a particular substrate for each type of repair. Homologous recombination requires extensive resection to create a long 3′ overhang that — once coated with RAD51 — can initiate homology search, although NHEJ requires little, if any, resection. Competition between the binding of homologous recombination and NHEJ factors influences the degree of resection at the break, and only once the 3′ overhang has been successfully resected is the cell fully committed to homologous recombination[61].

The Fanconi anaemia pathway is thought to have a role in pathway choice, funnelling the double-strand breaks created by interstrand crosslink processing or other forms of replication stress into high-fidelity homologous recombination repair. Therefore, NHEJ-mediated repair in the absence of a functional Fanconi anaemia pathway is speculated to be an underlying cause of the gross chromosomal abnormalities observed in Fanconi anaemia cell lines. Without a functional Fanconi anaemia pathway, perhaps the breaks become subject to the profligate end-pairing of NHEJ and thereby generate chromosomal rearrangements. Animal and human cell models have been developed to test this hypothesis, and so far the findings are contradictory (Table 2).

In the nematode *Caenorhabditis elegans*, the basic components of the Fanconi anaemia pathway are conserved, including the homologues *fcd-2* (*FANCD2*), *fnci-1* (*FANCI*), *fncm-1* (*FANCM*) and *dog-1* (*FANCJ*)[62]. The *C. elegans* I–D2 complex is ubiquitinated and travels to sites of damage similar to its human counterpart, and knockout of all four genes leads to hypersensitivity to crosslinking agents. *C. elegans* is an excellent model system to observe the effects of knocking out NHEJ components in a Fanconi-anaemia-deficient background, as it mimics relevant phenotypes with a pared-down pathway. When LIG4, the DNA ligase important for NHEJ, is mutated in *fcd-2* knockout worms, the sensitivity to crosslinking agents is corrected to near wild-type levels.

These data suggest that NHEJ does in fact have a deleterious effect when the Fanconi anaemia pathway is deficient[63].

The *C. elegans* model is also useful for studying the interplay between the Fanconi anaemia pathway and NHEJ during meiosis. Although patients with Fanconi anaemia, as well as knockout mice, exhibit fertility defects, the mechanisms underlying this loss of fertility are poorly understood. The regulation and resolution of meiotic crossovers derived from recombination of programmed double-strand breaks is mediated by many proteins that also serve in a DNA repair capacity, raising the possibility that the Fanconi anaemia pathway might also have a role. In the *fcd-2* mutant, RAD51 foci persist during meiosis, which are indicative of unresolved homologous recombination intermediates, and inappropriate non-homologous chromosome engagements are observed after diakinesis[63]. Similar to what is seen in somatic cells, the deletion of *lig-4* ameliorates the aberrant crossovers observed in the *fcd-2* mutant.

In cells of the chicken DT40 cell line, the interstrand crosslink hypersensitivity and chromosome breakage that are endemic in *FANCC* knockout cell lines can be suppressed by also knocking out *Ku70* (ref. 64). Interestingly, knocking out either *DNA-PK*$_{cs}$ or *LIG4* in the DT40 cells effects no such rescue, in contrast with the observations in *C. elegans*. In this case, the specificity of Ku70 rescue may point to a direct role for Fanconi anaemia proteins, possibly the I–D2 complex, in antagonizing Ku attachment at DNA ends.

The implication that NHEJ underlies the hypersensitivity and chromosomal breakage phenotypes in Fanconi anaemia is of great clinical interest because it points to a putative pharmaceutical target. If the cellular phenotypes that underlie the disease are caused not by interstrand crosslinks themselves but by aberrant repair processes that occur in the absence of the Fanconi anaemia pathway, perhaps NHEJ inhibition could serve as a highly specific treatment. It is therefore crucial to investigate the relationship between these two pathways in mammalian cell lines. RNA interference (RNAi) knockdown of *Ku80* in *FANCC*-deficient and *FANCD2*-deficient patient cell lines partially suppresses sensitivity to the killing effects of crosslinking agents. Furthermore, the addition of a DNA-PK$_{cs}$ inhibitor mitigates the hypersensitivity that results from knockdown of *FANCA* or *FANCD2* by RNAi in HeLa cells, or by knockout of *Fanca* or *Fancc* in mouse embryonic fibroblasts[63]. This again is in contrast to genetic experiments that show that the crosslink sensitivity of the *Fancd2* knockout is not changed in the setting of decreased DNA-PK$_{cs}$ activity (*Prkdc*$^{sc/sc}$)[65] and that loss of p53-binding protein 1 (53BP1) or Ku80 renders FANCD2-deficient cells even more sensitive to DNA-crosslinking agents than FANCD2 deficiency alone[66].

The inconsistencies between these studies raise questions about how to parse data from different organisms, genetic backgrounds and experimental set-ups. As hinted by these experiments, different components of the NHEJ pathway may have different roles in different organisms and even in different cells of the same organism; indeed, fibroblasts may not accurately reflect how cells that are more relevant to Fanconi anaemia, such as haematopoietic stem cells and progenitor cells, might behave. Furthermore, interactions between components of the two pathways may be exquisitely sensitive to the spatial and temporal relationships between them. When considering the possible therapeutic implications of these findings, it is important to remember that full inhibition of the Ku proteins is not feasible in human cells because they have an essential function at human telomeres[67]. Overall, well-controlled work, especially in human haematopoietic cells, will be required to understand the interplay of the Fanconi anaemia and NHEJ pathways. It will also be necessary to determine how these extremely toxic interstrand crosslinks are repaired if both pathways are compromised.

Alcohol metabolism and Fanconi anaemia deficiency

The average human is not exposed to the clastogenic agents that are used in the laboratory to model DNA repair defects. However, the Fanconi anaemia pathway is required for dealing with replication stresses that can arise during unchallenged cell-cycle progression, as shown by the activation of the pathway during normal S phase. The pathway also probably evolved to maintain genome integrity in the face of mutagenic agents that arise endogenously from processes of cellular metabolism. Oxidative stress within the cell can yield nucleophilic crosslinking agents such as malondialdehyde and acrolein, which derive from lipid peroxidation. Nitric oxide, another endogenous genotoxin that can promote the formation of crosslinks, is generated independently of lipids[68]. Aldehydes (discussed in greater detail later) are also a major source of endogenous crosslink damage. Understanding how damage from these endogenous agents feeds into the Fanconi anaemia pathway is crucial to understanding the disease.

Ethanol metabolism has long been known to produce reactive aldehydes that can function as carcinogens: acetaldehyde, an intermediate in the metabolic processing of ethanol, is particularly implicated as a genotoxic agent. Acetaldehyde stimulates monoubiquitination of FANCD2 *in vitro*, and Fanconi anaemia cells are sensitive to formaldehyde[69,70]. Until recently, however, no *in vivo* studies functionally linked these cellular by-products to concrete phenotypes in a Fanconi anaemia model. The first indication that such cellular by-products may indeed pose a challenge to Fanconi-anaemia-pathway-deficient systems came from a genetic interaction between Fanconi anaemia genes and superoxide dismutase, an enzyme that decreases the load of oxidative damage in the cell as a whole. *Fancc*$^{-/-}$ and *Sod*$^{-/-}$ mice display bone-marrow hypocellularity, which is not present in either of the single mutants[71]. Studies investigating the effects of interfering with acetaldehyde catabolism have uncovered a strong connection to the Fanconi anaemia pathway. Chicken DT40 cells with Fanconi anaemia genes knocked out, including *FANCB*, *FANCC*, *FANCL* and *BRCA2*, are sensitive to treatment with acetaldehyde[72]. In addition, DT40 cells with Fanconi anaemia knockouts are synthetic lethal with mutations in the formaldehyde catabolism gene *ADH5*, suggesting that toxicity is generated when the cells permit natural toxic aldehydes to build up[73].

A double mutant for *Fancd2* and the aldehyde dehydrogenase *Aldh2* was created to test the relevance of acetaldehyde sensitivity in the developing mouse. The double mutant did not survive, with embryos dying between embryonic day 9.5 and 13.5, but only if the mother was homozygous for the *Aldh2* mutation. By contrast, pregnant female mice with one wild-type *Aldh2* allele could carry pups to term. When heterozygous mothers were fed ethanol during pregnancy, the double-mutant pups exhibited drastic developmental abnormalities, such as exencephaly and eye defects[72]. These data suggest that the developing mouse is exquisitely sensitive to toxic aldehydes, casting light onto the significance of aldehyde catabolism in Fanconi-anaemia-pathway deficient backgrounds.

A more important question to ask is whether toxic aldehydes can produce symptoms in the mouse that mimic the human disease phenotype. Chronic alcohol abuse is correlated with bone-marrow problems, reminiscent of the bone-marrow failure observed in patients with Fanconi anaemia. Indeed, *Fancd2*$^{-/-}$:*Aldh2*$^{-/-}$ mice display increased levels of ethanol-induced bone-marrow failure compared with wild-type mice or single mutants. Even without administration of ethanol, the double mutants die early, owing to abnormalities that closely resemble acute lymphoblastic leukaemia.

Most tellingly, the double mutant mice that do not suffer from leukaemia develop aplastic anaemia that is similar to that seen in patients with Fanconi anaemia. The bone-marrow failure in these mice can be traced back to a DNA repair deficiency in the haematopoietic stem-cell and progenitor-cell pool, suggesting that endogenous aldehyde may be particularly harmful to haematopoietic stem cells, and providing the first direct link between acetaldehyde-related toxicity and bone-marrow failure phenotypes in Fanconi anaemia[74].

Animal, particularly mouse, models of Fanconi anaemia allow us to explore what genetic and environmental factors contribute to disease pathogenesis and, as such, can lead to better disease management and treatment plans. As sequencing becomes more affordable, understanding what genetic factors modify and shape the progress of Fanconi

anaemia symptoms will offer better diagnostics and tailored treatment. Discovering what commonly encountered genotoxic agents contribute to the clinical manifestations of Fanconi anaemia will provide therapeutic targets and lifestyle modifications, not only for patients with Fanconi anaemia but also for those with many diseases that are derived from DNA repair defects.

Looking forward

As a multifunctional, highly connected pathway, the Fanconi anaemia pathway is well poised to be a very important topic in DNA repair and in biology in general. Although there is evidence of functional relationships between the Fanconi anaemia pathway and virtually all of the cellular DNA repair pathways, many of the details remain murky. Understanding the mechanisms underlying these connections will foster understanding of DNA repair as a whole, rather than as a mixed bag of disparate pathways. The genetic link between Fanconi anaemia and breast cancer will also be an important topic as research progresses, shedding light on the nature of tumour suppressors, and the enigma of genotype–phenotype correlations. As technologies make it easier to manipulate stem cells, the function of Fanconi anaemia in haematopoietic stem-cell lineages and other stem cells will become a growing subfield within Fanconi anaemia research. We may finally gain insight into the developmental abnormalities that are seen in patients. Combining studies of Fanconi anaemia function and stem-cell development will allow us to create a powerful model of DNA repair within stem-cell and progenitor-cell populations. Another pressing issue is the identification of the endogenous damage that the Fanconi anaemia pathway responds to. It is likely that the lesions arise from multiple sources and that there will be nuances to how the Fanconi anaemia pathway responds to each of them. Finally, there are many details about the molecular mechanism of interstrand crosslink repair that are still missing. Coordination and regulation of the nucleases identified as necessary for repair is a topic of intense interest. As new players in the pathway are identified, we can only expect that it will become more complex, but also more revealing. Deciphering the mechanics of Fanconi anaemia pathway function will help with efforts to understand both a fascinating disease, and basic human biology. ■

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1. Rosenberg, P. S., Tamary, H. & Alter, B. P. How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi anemia in the United States and Israel. *Am. J. Med. Genet. A* **155A,** 1877–1883 (2011).
2. Auerbach, A. D. Fanconi anemia and its diagnosis. *Mutat. Res.* **668,** 4–10 (2009).
3. Ceccaldi, R. *et al.* Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. *Cell Stem Cell* **11,** 36–49 (2012).
This study identifies activation of the p53–p21 axis as a major contributing factor to loss of haematopoietic stem cells in Fanconi anaemia.
4. Tulpule, A. *et al.* Knockdown of Fanconi anemia genes in human embryonic stem cells reveals early developmental defects in the hematopoietic lineage. *Blood* **115,** 3453–3462 (2010).
5. Vinciguerra, P., Godinho, S. A., Parmar, K., Pellman, D. & D'Andrea, A. D. Cytokinesis failure occurs in Fanconi anemia pathway-deficient murine and human bone marrow hematopoietic cells. *J. Clin. Invest.* **120,** 3834–3842 (2010).
6. Muller, L. U. *et al.* Overcoming reprogramming resistance of Fanconi anemia cells. *Blood* **119,** 5449–5457 (2012).
7. Raya, A. *et al.* Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* **460,** 53–59 (2009).
This paper demonstrates that human fibroblasts deficient in the Fanconi anaemia pathway are refractory to reprogramming into iPS cells unless they are first corrected with an appropriate gene, and that Fanconi anaemia-corrected cells can be reprogrammed into iPS cells that give rise to phenotypically normal lineages.
8. Deans, A. J. & West, S. C. DNA interstrand crosslink repair and cancer. *Nature Rev. Cancer* **11,** 467–480 (2011).
9. Meindl, A. *et al.* Germline mutations in breast and ovarian cancer pedigrees establish *RAD51C* as a human cancer susceptibility gene. *Nature Genet.* **42,** 410–414 (2010).
10. Rafnar, T. *et al.* Mutations in *BRIP1* confer high risk of ovarian cancer. *Nature Genet.* **43,** 1104–1107 (2011).
11. Rahman, N. *et al. PALB2*, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nature Genet.* **39,** 165–167 (2007).
12. Seal, S. *et al.* Truncating mutations in the Fanconi anemia J gene *BRIP1* are low-penetrance breast cancer susceptibility alleles. *Nature Genet.* **38,** 1239–1241 (2006).
13. Tischkowitz, M. *et al.* Analysis of PALB2/FANCN-associated breast cancer families. *Proc. Natl Acad. Sci. USA* **104,** 6788–6793 (2007).
14. Walsh, T. *et al.* Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc. Natl Acad. Sci. USA* **108,** 18032–18037 (2011).
15. Howlett, N. G. *et al.* Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* **297,** 606–609 (2002).
This paper identifies the breast cancer predisposition gene *BRCA2* as mutated in a very severe form of Fanconi anaemia, firmly establishing Fanconi anaemia as a DNA repair deficiency disease.
16. Xia, B. *et al.* Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. *Nature Genet.* **39,** 159–161 (2007).
17. Levran, O. *et al.* The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nature Genet.* **37,** 931–933 (2005).
18. Levitus, M. *et al.* The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. *Nature Genet.* **37,** 934–935 (2005).
19. Vaz, F. *et al.* Mutation of the *RAD51C* gene in a Fanconi anemia-like disorder. *Nature Genet.* **42,** 406–409 (2010).
20. Enoiu, M., Jiricny, J. & Schärer, O. D. Repair of cisplatin-induced DNA interstrand crosslinks by a replication-independent pathway involving transcription-coupled repair and translesion synthesis. *Nucleic Acids Res.* **40,** 8953–8964 (2012).
21. Wang, X. *et al.* Involvement of nucleotide excision repair in a recombination-independent and error-prone pathway of DNA interstrand cross-link repair. *Mol. Cell. Biol.* **21,** 713–720 (2001).
22. Shen, X. *et al.* Recruitment of Fanconi anemia and breast cancer proteins to DNA damage sites is differentially governed by replication. *Mol. Cell* **35,** 716–723 (2009).
23. Knipscheer, P. *et al.* The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. *Science* **326,** 1698–1701 (2009).
This work uses a cell-free system to demonstrate the requirement for FANCD2 and FANCI in the replication-dependent repair of interstrand crosslinks.
24. Raschle, M. *et al.* Mechanism of replication-coupled DNA interstrand crosslink repair. *Cell* **134,** 969–980 (2008).
25. Long, D. T., Raschle, M., Joukov, V. & Walter, J. C. Mechanism of RAD51-dependent DNA interstrand cross-link repair. *Science* **333,** 84–87 (2011).
26. Smogorzewska, A. *et al.* Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* **129,** 289–301 (2007).
27. Taniguchi, T. *et al.* S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. *Blood* **100,** 2414–2420 (2002).
28. Alpi, A. F., Pace, P. E., Babu, M. M. & Patel, K. J. Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol. Cell* **32,** 767–777 (2008).
29. Machida, Y. J. *et al.* UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Mol. Cell* **23,** 589–596 (2006).
30. Meetei, A. R. *et al.* A novel ubiquitin ligase is deficient in Fanconi anemia. *Nature Genet.* **35,** 165–170 (2003).
31. Ishiai, M. *et al.* FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway. *Nature Struct. Mol. Biol.* **15,** 1138–1146 (2008).
32. Sato, K., Toda, K., Ishiai, M., Takata, M. & Kurumizaka, H. DNA robustly stimulates FANCD2 monoubiquitylation in the complex with FANCI. *Nucleic Acids Res.* **40,** 4553–4561 (2012).
33. Sato, K. *et al.* Histone chaperone activity of Fanconi anemia proteins, FANCD2 and FANCI, is required for DNA crosslink repair. *EMBO J.* **31,** 3524–3536 (2012).
34. Nijman, S. M. *et al.* The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol. Cell* **17,** 331–339 (2005).
35. Kim, J. M. *et al.* Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. *Dev. Cell* **16,** 314–320 (2009).
36. Oestergaard, V. H. *et al.* Deubiquitination of FANCD2 is required for DNA crosslink repair. *Mol. Cell* **28,** 798–809 (2007).
37. Kim, Y. *et al.* Mutations of the *SLX4* gene in Fanconi anemia. *Nature Genet.* **43,** 142–146 (2011).
38. Kratz, K. *et al.* Deficiency of FANCD2-associated nuclease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents. *Cell* **142,** 77–88 (2010).
39. Liu, T., Ghosal, G., Yuan, J., Chen, J. & Huang, J. FAN1 acts with FANCI–FANCD2 to promote DNA interstrand cross-link repair. *Science* **329,** 693–696 (2010).
40. MacKay, C. *et al.* Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell* **142,** 65–76 (2010).
41. Smogorzewska, A. *et al.* A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. *Mol. Cell* **39,** 36–47 (2010).
42. Stoepker, C. *et al.* SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. *Nature Genet.* **43,** 138–141 (2011).
43. Fekairi, S. *et al.* Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. *Cell* **138,** 78–89 (2009).
44. Svendsen, J. M. *et al.* Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. *Cell* **138,** 63–77 (2009).
45. Andersen, S. L. *et al. Drosophila* MUS312 and the vertebrate ortholog BTBD12 interact with DNA structure-specific endonucleases in DNA repair and recombination. *Mol. Cell* **35,** 128–135 (2009).

46. Munoz, I. M. *et al.* Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. *Mol. Cell* **35,** 116–127 (2009).
References 43–46 have identified SLX4 as a partner to multiple DNA repair nucleases necessary for interstrand crosslink repair.
47. Bhagwat, N. *et al.* XPF–ERCC1 participates in the Fanconi anemia pathway of cross-link repair. *Mol. Cell. Biol.* **29,** 6427–6437 (2009).
48. Kim, Y., Spitz, G. S., Veturi, U., Lach, F. P., Auerbach, A. D. & Smogorzewska, A. Regulation of multiple DNA repair pathways by the Fanconi anemia protein SLX4. *Blood* http://dx.doi.org/10.1182/blood-2012-07-441212 (23 October 2012).
49. Wang, A. T. *et al.* Human SNM1A and XPF–ERCC1 collaborate to initiate DNA interstrand cross-link repair. *Genes Dev.* **25,** 1859–1870 (2011).
50. Zhou, W. *et al. FAN1* mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. *Nature Genet.* **44,** 910–915 (2012).
51. Howlett, N. G., Taniguchi, T., Durkin, S. G., D'Andrea, A. D. & Glover, T. W. The Fanconi anemia pathway is required for the DNA replication stress response and for the regulation of common fragile site stability. *Hum. Mol. Genet.* **14,** 693–701 (2005).
52. Schlacher, K. *et al.* Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* **145,** 529–542 (2011).
53. Schlacher, K., Wu, H. & Jasin, M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell* **22,** 106–116 (2012).
This work identifies an important role for FANCD2, BRCA1 and BRCA2 in protecting the stability of replication forks, illuminating a repair-independent function for these proteins in genome maintenance.
54. Peng, M. *et al.* The FANCJ/MutLα interaction is required for correction of the cross-link response in FA-J cells. *EMBO J.* **26,** 3238–3249 (2007).
55. Williams, S. A. *et al.* Functional and physical interaction between the mismatch repair and FA-BRCA pathways. *Hum. Mol. Genet.* **20,** 4395–4410 (2011).
56. Wu, L. & Hickson, I. D. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* **426,** 870–874 (2003).
57. Chan, K. L., Palmai-Pallag, T., Ying, S. & Hickson, I. D. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. *Nature Cell Biol.* **11,** 753–760 (2009).
58. Wechsler, T., Newman, S. & West, S. C. Aberrant chromosome morphology in human cells defective for Holliday junction resolution. *Nature* **471,** 642–646 (2011).
59. Lieber, M. R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* **79,** 181–211 (2010).
60. Karanam, K., Kafri, R., Loewer, A. & Lahav, G. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. *Mol. Cell* **47,** 320–329 (2012).
61. Symington, L. S. & Gautier, J. Double-strand break end resection and repair pathway choice. *Annu. Rev. Genet.* **45,** 247–271 (2011).
62. Youds, J. L., Barber, L. J. & Boulton, S. J. *C. elegans*: a model of Fanconi anemia and ICL repair. *Mutat. Res.* **668,** 103–116 (2009).
63. Adamo, A. *et al.* Preventing nonhomologous end joining suppresses DNA repair defects of Fanconi anemia. *Mol. Cell* **39,** 25–35 (2010).
This work investigates the relationship between the Fanconi anaemia pathway and NHEJ, suggesting that the Fanconi anaemia cellular phenotypes derive from aberrant repair by NHEJ.
64. Pace, P. *et al.* Ku70 corrupts DNA repair in the absence of the Fanconi anemia pathway. *Science* **329,** 219–223 (2010).
65. Houghtaling, S. *et al.* Fancd2 functions in a double strand break repair pathway that is distinct from non-homologous end joining. *Hum. Mol. Genet.* **14,** 3027–3033 (2005).
66. Bunting, S. F. *et al.* BRCA1 functions independently of homologous recombination in DNA interstrand crosslink repair. *Mol. Cell* **46,** 125–135 (2012).
67. Wang, Y., Ghosh, G. & Hendrickson, E. A. Ku86 represses lethal telomere deletion events in human somatic cells. *Proc. Natl Acad. Sci. USA* **106,** 12430–12435 (2009).
68. Pang, Q. & Andreassen, P. R. Fanconi anemia proteins and endogenous stresses. *Mutat. Res.* **668,** 42–53 (2009).
69. Marietta, C., Thompson, L. H., Lamerdin, J. E. & Brooks, P. J. Acetaldehyde stimulates FANCD2 monoubiquitination, H2AX phosphorylation, and BRCA1 phosphorylation in human cells *in vitro*: implications for alcohol-related carcinogenesis. *Mutat. Res.* **664,** 77–83 (2009).
70. Ridpath, J. R. *et al.* Cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde. *Cancer Res.* **67,** 11117–11122 (2007).
71. Hadjur, S. *et al.* Defective hematopoiesis and hepatic steatosis in mice with combined deficiencies of the genes encoding Fancc and Cu/Zn superoxide dismutase. *Blood* **98,** 1003–1011 (2001).
72. Langevin, F., Crossan, G. P., Rosado, I. V., Arends, M. J. & Patel, K. J. Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. *Nature* **475,** 53–58 (2011).
73. Rosado, I. V., Langevin, F., Crossan, G. P., Takata, M. & Patel, K. J. Formaldehyde catabolism is essential in cells deficient for the Fanconi anemia DNA-repair pathway. *Nature Struct. Mol. Biol.* **18,** 1432–1434 (2011).
74. Garaycoechea, J. I. *et al.* Genotoxic consequences of endogenous aldehydes on mouse haematopoietic stem cell function. *Nature* **489,** 571–575 (2012).
References 72 and 74 demonstrate that increasing aldehyde load in Fanconi anaemia mutant mice leads to phenotypes that are strikingly similar to those of the human disease, including developmental abnormalities, leukaemia and bone-marrow failure.
75. Berwick, M. *et al.* Genetic heterogeneity among Fanconi anemia heterozygotes and risk of cancer. *Cancer Res.* **67,** 9591–9596 (2007).

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