Functions and regulation of the multitasking FANCM family of DNA motor proteins

Xiaoyu Xue,[1] Patrick Sung,[1] and Xiaolan Zhao[2]

[1]Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06520, USA; [2]Molecular Biology Program, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA

Members of the conserved FANCM family of DNA motor proteins play key roles in genome maintenance processes. FANCM supports genome duplication and repair under different circumstances and also functions in the ATR-mediated DNA damage checkpoint. Some of these roles are shared among lower eukaryotic family members. Human FANCM has been linked to Fanconi anemia, a syndrome characterized by cancer predisposition, developmental disorder, and bone marrow failure. Recent studies on human FANCM and its orthologs from other organisms have provided insights into their biological functions, regulation, and collaboration with other genome maintenance factors. This review summarizes the progress made, with the goal of providing an integrated view of the functions and regulation of these enzymes in humans and model organisms and how they advance our understanding of genome maintenance processes.

Vertebrate FANCM proteins and their influence in genome repair, replication, and surveillance

The Fanconi anemia (FA) pathway and replicative traversal of DNA interstrand cross-links (ICLs)

FANCM is one of the 17 proteins found to be mutated in FA patients (for review, see Soulier 2011; Wang and Smogorzewska 2015). These FA proteins constitute the core of the FA pathway that serves to eliminate DNA ICLs (for review, see Deans and West 2011; Kim and D'Andrea 2012; Kottemann and Smogorzewska 2013). ICLs interfere with DNA replication by blocking the progression of both leading and lagging strand DNA synthesis. These obstacles are removed during replication via the FA pathway. Within this pathway, FANCM collaborates with two obligate partners; namely, FAAP24 (FA-associated protein of 24 kDa) and MHF (a histone-fold complex consisting of MHF1 and MHF2) (Ciccia et al. 2007; Kim et al. 2008; Singh et al. 2010; Yan et al. 2010). The FANCM–FAAP24–MHF complex, which recognizes different DNA structures, provides an important means to target the other core FA proteins to ICL sites (Fig. 1). Upon association with the DNA lesion, the ubiquitin ligase FANCL within the FA core, in conjunction with the ubiquitin-conjugating enzyme UBE2T, mediates the monoubiquitination of FANCI and FANCD2 (Fig. 1). These ubiquitination events lead to the recruitment of several enzymatic entities to incise the ICL so as to "unhook" it, replicatively bypass the unhooked lesion, and complete the repair process via homologous recombination (Fig. 1). For details of the FA pathway, we recommend several comprehensive reviews (Deans and West 2011; Kim and D'Andrea 2012; Kottemann and Smogorzewska 2013).

Interestingly, a recent study suggests that FANCM and MHF promote replicative traversal across ICLs (Fig. 1; Huang et al. 2013). This function requires DNA binding by both FANCM and MHF and also the DNA motor activity of FANCM, suggesting that translocation of FANCM–MHF on the damaged DNA template somehow allows DNA synthesis to continue past ICLs, leaving the lesion to be removed by another mechanism post-replicatively. The investigators showed that this is a FA-independent mechanism and is used in several organisms. While the mechanism underlying ICL traversal remains to be determined, the study by Huang et al. (2013) raises several interesting questions. Most notably, what are the cues that prompt FANCM–MHF to initiate repair via the FA pathway versus lesion traversal? Moreover, as DNA cross-linking agents are commonly used in cancer treatment, how does pathway choice affect the efficacy of cancer therapy?

[*Keywords*: FANCM; Mph1; Fml1; MHF; BLM; replication fork regression; crossover control]
Corresponding author: zhaox1@mskcc.org
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.266593.115.

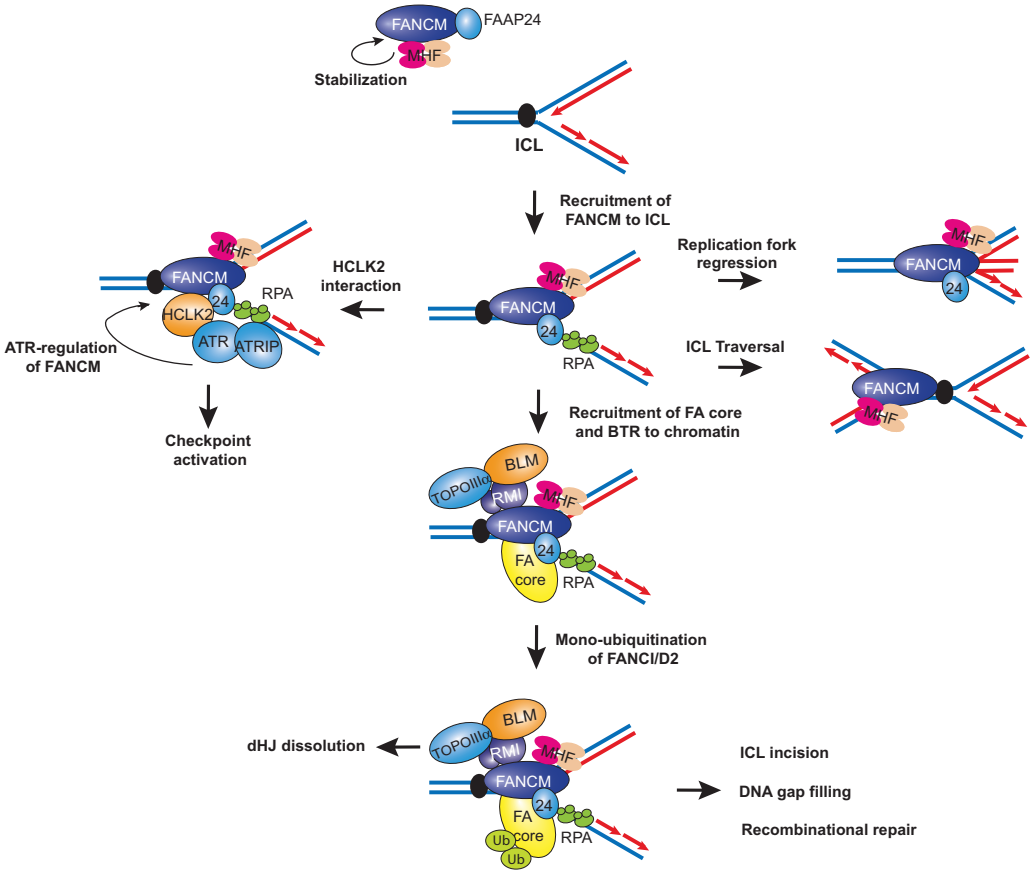

Figure 1. The role of FANCM and partner proteins in coping with template lesions during DNA replication. MHF forms a complex with FANCM and FAAP24 and stabilizes FANCM. This complex localizes to ICL sites through its DNA-binding attribute. FANCM carries out several FA-independent functions at ICL sites and in other replication blockage situations. FANCM–MHF promote ICL traversal, allowing replication to proceed past the lesion without repair. FANCM also can catalyze replication fork regression under certain circumstances. In addition, FANCM and FAAP24 interact with the checkpoint protein HCLK2 to activate the ATR checkpoint signaling pathway (other means by which FANCM can promote checkpoint activation are not depicted here). In the FA pathway, FANCM–MHF–FAAP24 recruits the FA core complex to the ICL sites. The subsequent monoubiquitination of FANCI and FANCD2 leads to multiple repair steps, including ICL incision, DNA gap filling, and recombinational repair. The recruitment of the BLM–topoisomerase IIIα (Topo IIIα)–RMI (BTR) complex by FANCM enables double Holliday junction (dHJ) dissolution.

The role of FANCM in DNA replication and the replication stress response

Aside from promoting ICL tolerance and removal, FANCM has a role in DNA replication under normal growth conditions and upon exposure of cells to clastogens such as the topoisomerase I poison camptothecin, ultraviolet light, and the DNA methylating agent methylmethane sulfonate (MMS) (Rosado et al. 2009; Singh et al. 2009; Luke-Glaser et al. 2010; Schwab et al. 2010; Blackford et al. 2012). From these studies in human and chicken DT40 cells, FANCM and its ATPase activity have been shown to prevent replication forks from stalling and/or allow damaged forks to restart. Two distinct mechanisms have been invoked to explain the involvement of FANCM in this capacity. One model posits that FANCM catalyzes replication fork regression, whereby the newly synthesized strands are dissociated from their template strands and anneal to form a partial duplex with a primer–template junction suitable for priming new DNA synthesis, and this is accompanied by reannealing of template strands (Figs. 1, 2A; Gari et al. 2008a,b). This process can help restart the stalled forks by several means (for review, see Yeeles et al. 2013). For example, it allows the use of the sister strand to template DNA synthesis (Fig. 2A). In addition, it leads to the relocation of the template lesion to a duplex region, which renders it amenable to repair (Fig. 2A). The fork regression function of FANCM appears to be independent of other FA proteins but is likely facilitated by MHF, which enhances the fork regression reaction in vitro (Luke-Glaser et al. 2010; Schwab et al. 2010; Singh et al. 2010; Yan et al. 2010).

Another means by which FANCM could help to cope with replication stress stems from its ability to activate the ATR-mediated DNA damage checkpoint (Collis et al. 2008; Sobeck et al. 2009; Huang et al. 2010; Luke-Glaser et al. 2010; Schwab et al. 2010; Wang et al. 2013b), which stabilizes replication forks and inhibits the firing of dormant replication origins (for review, see

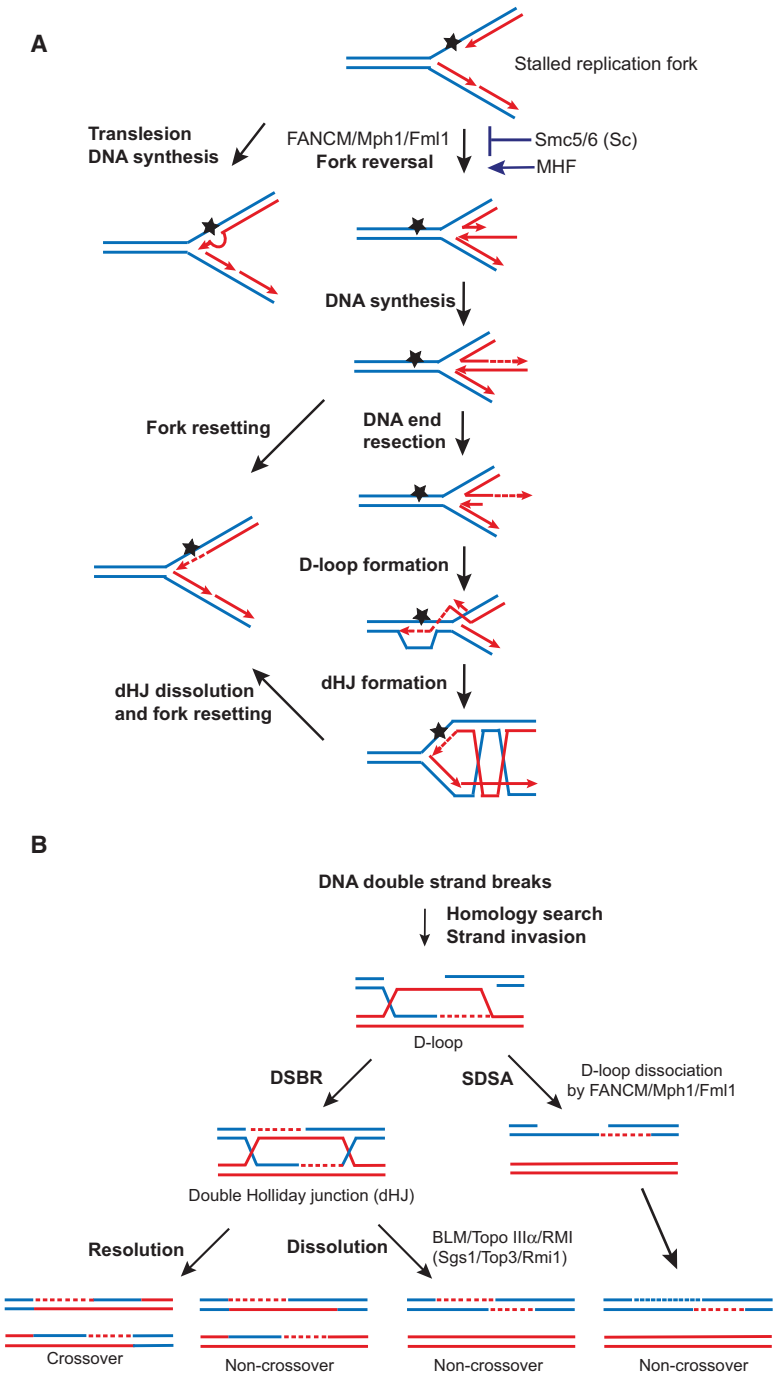

Figure 2. The roles of FANCM family proteins in replication fork repair and crossover control. (*A*) Pathways that cope with replication blockage (denoted by the star), including error-prone translesion DNA synthesis and FANCM/Fml1/Mph1-mediated fork regression and subsequent recombination, are depicted. Note that Smc5/6 (structural maintenance of chromosomes 5/6) down-regulates Mph1's replication fork regression (or fork reversal) and DNA branch migration activities to prevent the generation of potentially harmful recombination intermediates, and *Saccharomyces cerevisiae* MHF (*Sc*MHF) helps overcome this effect of Smc5/6. Two scenarios following fork reversal are shown. Following DNA synthesis, where one nascent strand uses the other to prime DNA synthesis, the fork can be reset, allowing replication resumption. Alternatively, the dsDNA ends can be resected to generate a substrate for invading the template strands that share sequence homology, generating a dHJ that requires resolution to allow replication resumption. Note that fork reversal can also lead to other outcomes that are not depicted here. (*B*) Model showing how FANCM family proteins promote DNA double-strand break repair via the synthesis-dependent strand annealing (SDSA) pathway that generates noncrossover products only. Repair via the double-strand break repair pathway (DSBR) leads to the formation of a dHJ intermediate that is resolved nucleolytically into crossover or noncrossover products. Alternatively, the dHJ can be dissolved by a helicase/topoisomerase complex composed of BTR or the yeast counterpart Sgs1/Top3/Rmi1 (STR), to yield noncrossover products. The dotted line denotes newly synthesized DNA.

Labib and De Piccoli 2011; Errico and Costanzo 2012). The checkpoint function of FANCM requires FAAP24 but not other FA proteins and entails the association with and regulation of other checkpoint factors (Fig. 1; more below). How FANCM-mediated replication fork regression and ATR activation contribute to replication fork stabilization and restart and how these attributes may be related to the lesion traversal function of the protein remain open questions. It is worth noting that several other FA proteins also appear to possess FA pathway-independent roles in DNA replication (Sobeck et al. 2006; Schlacher et al. 2012; Lossaint et al. 2013; Luebben et al. 2014). Investigating the molecular events that dictate the participation of FANCM and other FA proteins in FA-dependent versus FA-independent functions will be informative to understand the mechanisms of replication fork protection and restart.

Regulation of recombinational repair outcome

Another FANCM function important for genome maintenance pertains to the regulation of the outcome of

recombinational repair. Homologous recombination repairs both DNA double-strand breaks and structures formed during perturbed replication, such as single-strand gaps and regressed replication forks. The DNA intermediates resulting from strand invasion and subsequent DNA synthesis, such as the displacement loop (D loop), Holliday junction (HJ), and double HJ (dHJ), can be resolved into either a crossover or noncrossover configuration (Fig. 2B). While resolvase-mediated resolution leads to both product types at similar frequencies, DNA helicase-dependent DNA strand displacement or dHJ dissolution by a helicase–topoisomerase complex generates exclusively noncrossover products (Fig. 2B). FANCM and its orthologs possess an anti-crossover function, as their loss in human, mouse, and chicken DT40 cells leads to elevated sister chromatid exchanges (SCEs), a crossover indicator (Mosedale et al. 2005; Bakker et al. 2009; Deans and West 2009; Rosado et al. 2009). This function requires the DNA translocase activity of FANCM as well as FAAP24 and MHF but is independent of other FA proteins (Niedzwiedz et al. 2004; Bakker et al. 2009; Rosado et al. 2009; Yan et al. 2010; Suhasini et al. 2011; Wang et al. 2013b). The anti-crossover function of FANCM and its orthologs are discussed in detail in a later section. As crossovers can lead to chromosome translocations and a loss of heterozygosity (for review, see Wyatt and West 2014), the anti-crossover attribute of FANCM helps preserve genomic integrity during recombination-mediated chromosomal repair.

In summary, recent progress has shed light on the role of vertebrate FANCM in DNA replication and repair. Besides its well-known function in the FA pathway, FANCM exerts FA-independent roles in ICL traversal, replication fork restart, ATR checkpoint activation, and anti-crossover control. While the role of FANCM in the FA pathway stems from its ability to recruit other FA proteins to the DNA lesion independently of its DNA translocase activity, all of the other known functions of FANCM require this activity. The distinct functions fulfilled by FANCM implicate it in multiple genome maintenance processes but also render the delineation of its biological roles a challenging task. In this regard, insights into some of the highly conserved FANCM functions have been gained from studying its orthologs in lower eukaryotic cells or plants as described below.

FANCM orthologs in lower eukaryotes and plants

FANCM orthologs, which are members of the SF2 helicase family, have been identified in many organisms outside the vertebrate realm, such as the Hef protein in archaea and Mph1 and Fml1 in budding and fission yeasts, respectively (Singleton et al. 2007). All of these proteins share significant homology at their helicase domains and regions C-terminal to the helicase domain (Fig. 3; Meetei et al. 2005; Mosedale et al. 2005). We recommend a detailed description of the FANCM family of proteins in a review by Whitby (2010). As lower eukaryotes appear to lack many FA proteins, they are useful models for deriving mechanistic information regarding

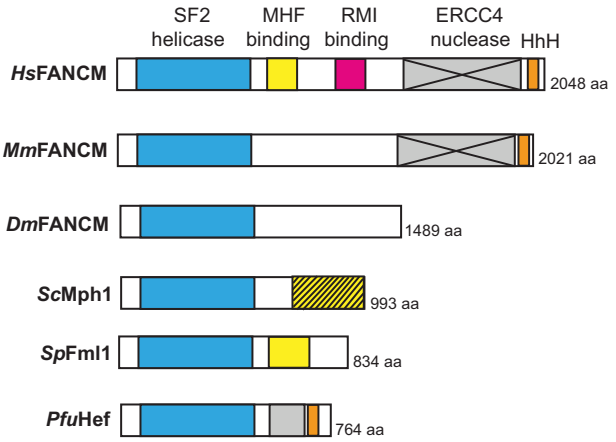

Figure 3. The FANCM family of DNA motor proteins. The domain structures of selected FANCM family proteins are indicated. These domains include the SF2 helicase domain (blue), MHF-binding region (yellow), RMI interaction region (pink), ERCC4 nuclease domain (gray; cross indicates the inactive nature of the domain), and HhH (a tandem helix–hairpin–helix; orange) domain. The MHF-binding domain on Mph1 (hatched) also interacts with Smc5/6. (*Hs*) *Homo sapiens*; (*Mm*) *Mus musculus*; (*Dm*) *Drosophila melanogaster*; (*Sc*) *S. cerevisiae*; (*Sp*) *Schizosaccharomyces pombe*; (*Pfu*) *Pyrococcus furiosus*.

the FA-independent functions of FANCM. Below, we summarize the progress in understanding the biological functions of FANCM orthologs in yeasts and other model systems.

Roles of Schizosaccharomyces pombe *Fml1 and* Saccharomyces cerevisiae *Mph1 in DNA replication*

Like FANCM, Fml1 and Mph1 play a role in DNA replication, particularly under stress conditions. Based on their ability to catalyze replication fork regression (Sun et al. 2008; Zheng et al. 2011) and on a replication-coupled recombination assay in *S. pombe*, a model has been proposed in which nucleolytic processing of the 5′ terminus of the regressed fork enables the formation of Rad51 recombinase-coated ssDNA that can invade the intact template DNA followed by DNA synthesis and resolution of the DNA joint (Fig. 2A; Sun et al. 2008). In this manner, a damaged or stalled replication fork is channeled into the homologous recombination pathway for repair. Also in support of this model, Mph1-made recombination intermediates have been detected by two-dimensional DNA gel analysis upon replication fork stalling (Chen et al. 2009; Choi et al. 2010; Chavez et al. 2011). However, even though fork regression is clearly useful for restarting replication, it may dislodge the replisome or generate hard-to-resolve DNA structures (for review, see Yeeles et al. 2013). Eukaryotic cells possess at least one other means for DNA damage tolerance; namely, translesion DNA synthesis (TLS) that is catalyzed by specialized and generally error-prone DNA polymerases (Fig. 2A). That the Fml1/Mph1-mediated pathway and TLS act on

the same or a derivative DNA structure is made evident by the observation that the mutation rate via TLS becomes elevated upon the loss of Mph1 (Scheller et al. 2000; Schurer et al. 2004).

Unlike in vertebrates, where FANCM regulates the ATR checkpoint, Mph1 loss does not reduce the checkpoint function in *S. cerevisiae* (Chen et al. 2013). Nevertheless, Mph1 may also affect replication by additional means. Recent genetic evidence suggests that Mph1 functions with the DNA motor protein Rad54 in extending the Rad51-made D loop to promote sister chromatid association (Ede et al. 2011). The sensitivity of *fml1* and *mph1* mutants to replication-blocking agents likely stems from the simultaneous ablation of multiple mechanisms of fork preservation and recombinational repair (Scheller et al. 2000; Sun et al. 2008).

The role of Fml1 and Mph1 in crossover suppression and DNA cross-link repair

As in the case of FANCM, Fml1 and Mph1 serve to suppress crossover during homologous recombination (Sun et al. 2008; Prakash et al. 2009; Lorenz et al. 2012). While this function of Fml1 is evident in both mitotic and meiotic cells, such a role for Mph1 has only been demonstrated in mitotic cells, which may be due to its low expression in meiosis (Primig et al. 2000). Crossover suppression by these motor proteins likely stems from their ability to dissociate D loops made by Rad51 (Sun et al. 2008; Prakash et al. 2009; Sebesta et al. 2011; Lorenz et al. 2012). Recent studies provide further insights into the anti-crossover role of Mph1. For example, findings from Mazon and Symington (2013) suggest that Mph1 recognizes an early recombination intermediate, such as the D loop, and channels it into the synthesis-dependent strand annealing (SDSA) recombination pathway that yields only noncrossover products (Fig. 2B). In the absence of Mph1, the nascent D loop is stabilized by DNA synthesis. It is then channeled into either the double-strand break repair (DSBR) pathway, which generates both crossover and noncrossover products due to resolvase-mediated resolution of the dHJ, or the break-induced replication mechanism, which can lead to extensive acquisition of genetic information from the donor chromosome or chromosome translocation (Fig. 2B; Luke-Glaser and Luke 2012; Stafa et al. 2014).

Both Fml1 and Mph1 are involved in ICL repair (Daee et al. 2012; McHugh et al. 2012; Ward et al. 2012; Fontebasso et al. 2013), but the repair function of Mph1 is only apparent in the absence of an exonuclease capable of degrading the oligonucleotide stemming from ICL unhooking. The fact that *S. pombe* and *S. cerevisiae* contain only a few equivalents of FA proteins suggests a major difference in the mechanism of ICL repair in these organisms compared with higher eukaryotes. On the other hand, as discussed above, Fml1 and Mph1 clearly possess at least some of the FA-independent functions shared among FANCM family members. Thus, it is quite possible that the FA pathway of ICL repair is a relatively recent acquisition in vertebrate species.

Progress made on other FANCM family proteins

Drosophila melanogaster FANCM (*Dm*FANCM) was recently shown to function in crossover control in both mitotic and meiotic cells (Kuo et al. 2014). Similar to what was found in *S. cerevisiae*, genetic evidence suggests that the role of *Dm*FANCM is to channel a critical DNA intermediate into the SDSA pathway of homologous recombination. This study also demonstrates that *Dm*FANCM is required for cellular resistance to not only ICL-inducing agents but also MMS and ionizing radiation, while the FANCL homolog is only required for ICL resistance. This finding provides evidence that *Dm*FANCM also possesses FA-dependent and FA-independent functions, as seen in the vertebrate counterparts. Two studies of the *Arabidopsis thaliana* FANCM suggest that this protein appears to be more specialized, controlling crossover and chromosome synapsis during meiosis but not functioning in DNA replication or repair (Crismani et al. 2012; Knoll et al. 2012).

Summary

All of the FANCM family members examined to date possess an anti-crossover function in mitotic cells, with some members also serving the same role during meiosis. In addition, most family members are also involved in replication fork repair such as by catalyzing fork regression. In contrast, their involvement in ICL repair appears to vary among species. Another important difference is that the FANCM counterpart in lower eukaryotes does not appear to possess a DNA damage checkpoint function. Among family members, human FANCM seems to fulfill the most diverse biological roles, which likely stems from the acquisition of additional protein domains and interactors. For example, human FANCM contains a large C-terminal region that is absent in Fml1, Mph1, and *D. melanogaster* and *A. thaliana* FANCM (Fig. 3). In addition, FAAP24 and most other FA proteins appear to be unique to vertebrate species. Despite this divergence, the presence of MHF and several FA protein equivalents in yeasts, such as Chl1 (FANCJ) and Slx4 (FANCP) in *S. cerevisiae* (McHugh et al. 2012; Ward et al. 2012), is aptly reflective of conserved functions of these motor proteins. Below, we summarize the biochemical attributes of several of the FANCM family members and how their biological functions are regulated.

Biochemical attributes of FANCM family proteins and their relevance for biological functions

The biochemical attributes of Fml1, Mph1, and human FANCM have been examined in detail, and these FANCM family members have been found to share several common features. All three proteins bind to ssDNA and structured DNA, with a clear preference for branched DNA structures, including the HJ, DNA fork, and D loop (Mosedale et al. 2005; Gari et al. 2008b; Sun et al. 2008; Xue et al. 2008, 2014; Prakash et al. 2009; Nandi and Whitby 2012). The DNA-binding ability is attributed to the conserved

helicase domain, while the region distal to the helicase domain confers the ability to interact with other protein factors and serves a regulatory role (Ciccia et al. 2007; Sun et al. 2008; Bhattacharjee et al. 2013; Xue et al. 2014). Some of the partner proteins of FANCM/Fml1/Mph1 also possess a DNA-binding ability and exert an influence on the DNA-binding specificity of complexes harboring the FANCM orthologs. For example, FAAP24 bestows the ability to bind the 3′ DNA flap and splayed arm DNA (Ciccia et al. 2007), while human MHF facilitates the engagement of other branched DNA species (Zhao et al. 2014). As such, the partner proteins facilitate the targeting of complexes of FANCM orthologs to specific DNA structures. Below, we summarize several major activities of human FANCM, Fml1, and Mph1 and their biological relevance. These features are also summarized in Table 1.

DNA translocase and unwinding activities

Human FANCM and Fml1 exhibit ssDNA- and dsDNA-dependent ATPase activities (Meetei et al. 2005; Nandi and Whitby 2012), while Mph1 possesses ssDNA-dependent ATPase activity only (Prakash et al. 2005). ATP hydrolysis by these proteins can lead to the unwinding of duplex DNA or dissociation of DNA triplex as observed for Fml1/Mph1 and human FANCM, respectively (Table 1; Meetei et al. 2005; Prakash et al. 2005; Sun et al. 2008; Kang et al. 2012). These functions support different DNA transactions as described below.

DNA fork regression and branch migration

Human FANCM, Fml1, and Mph1 not only regress the DNA replication fork but can also catalyze translocation of the branch point (i.e., branch migration) in the regressed fork (Table 1; Gari et al. 2008a,b; Sun et al. 2008; Zheng et al. 2011; Kang et al. 2012; Xue et al. 2014). These activities have been demonstrated in vitro using DNA oligonucleotide or plasmid-based replication fork substrates. In all cases, DNA replication fork regression and branch migration are fueled by ATP hydrolysis. Genetic studies, DNA fiber analysis, and two-

Table 1. *Biochemical activities of the FANCM family of DNA motor proteins*

DNA substrate	Activity	FANCM	Mph1	Fml1	Hef	In vivo implications	References
Triple helix	Translocase	✓	ND	ND	ND	Translocate along dsDNA	Meetei et al. 2005
3′ overhang	Unwinding	×	✓	ND	ND	3′–5′ DNA helicase	Meetei et al. 2005; Prakash et al. 2005
Flap 	Unwinding	×	✓	✓	✓	Lagging strand unwinding	Nishino et al. 2005; Sun et al. 2008; Zheng et al. 2011
Movable replication fork 	Fork reversal	✓	✓	✓	✓	Replication fork repair	Komori et al. 2002; Gari et al. 2008a,b; Sun et al. 2008; Zheng et al. 2011; Xue et al. 2014
Movable Holliday junction 	Branch migration	✓	✓	✓	ND	Replication fork repair	Gari et al. 2008b; Sun et al. 2008; Zheng et al. 2011; Xue et al. 2014
σ structure 	Branch migration	✓	✓	✓	ND	Replication fork repair	Gari et al. 2008a; Zheng et al. 2011; Nandi and Whitby 2012
Synthetic D loop	D-loop unwinding	×	✓	✓	ND	Crossover control	Sun et al. 2008; Prakash et al. 2009
D loop 	D-loop dissociation	✓	✓	✓	ND	Crossover control	Gari et al. 2008a; Sun et al. 2008; Prakash et al. 2009

dimensional DNA gel analysis have linked these activities to the recombinational repair of stalled replication forks (Sun et al. 2008; Chen et al. 2009; Choi et al. 2010; Blackford et al. 2012). On the other hand, since this repair mechanism could generate hard-to-resolve DNA structures or replisome destabilization, tight regulation is thought to be necessary to minimize potential harm (see below).

D-loop dissociation

During recombinational repair, ssDNA associated with a lesion becomes coated by the recombinase Rad51, which then mediates the search for a homologous duplex target, leading to invasion of the latter to form a D loop (Fig. 2B; Sung and Klein 2006). The D loop can be either extended via DNA synthesis or dissolved by rejecting the invading strand after limited DNA synthesis has occurred (Sung and Klein 2006). Biochemical assays have shown that human FANCM, Mph1, and Fml1 can dismantle D loops in an ATP hydrolysis-dependent manner, and Mph1 is capable of doing so even with Rad51 remains bound to the D loop (Table 1; Fig. 2B; Gari et al. 2008a; Sun et al. 2008; Prakash et al. 2009). This shared activity likely provides a means for channeling the D loop into the noncrossover SDSA pathway as described above. It is noteworthy that some of the aforementioned attributes of eukaryotic FANCM family members are conserved in the archaeal Hef protein, which also exhibits DNA unwinding and replication fork regression activities (Table 1). However, it uniquely possesses a structure-specific nuclease activity that is thought to collaborate with the other Hef activities in replication fork remodeling (Komori et al. 2002, 2004; Nishino et al. 2005).

Partners and regulators of the FANCM family proteins

The FANCM family members depend on partner proteins in the execution of biological functions (Table 2). This is best demonstrated in the FA pathway, in which FANCM, in partnership with FAAP24 and MHF, interacts with FANCF to recruit the FA core complex to DNA lesions (Fig. 1). A growing number of additional interactors have been identified, and the biological consequences of these interactions are being elucidated. While some interactors facilitate reactions catalyzed the FANCM family members, others exert negative regulation. There are differences in how the FANCM family members are regulated that likely reflect the specific genome maintenance needs of various organisms. Below, we discuss recent progress on how the functions of this family of enzymes are regulated in the cellular context, with a focus on regulatory events mediated by non-FA proteins. We recommend recent reviews on the regulation of FANCM within the context of the FA pathway (Deans and West 2011; Kim and D'Andrea 2012; Kottemann and Smogorzewska 2013).

Table 2. *Partners of FANCM and its orthologs*

Partners	Biological functions	References
*Hs*MHF	FANCM stabilization; DNA damage localization; assisting FANCM in RF repair; replicative ICL traverse; crossover control	Singh et al. 2010; Yan et al. 2010
*Sc*MHF	Assisting Mph1 in RF repair; relieving the inhibitory effect of Smc5	Xue et al. 2015
*Sp*MHF	Assisting Fml1 in RF repair, ICL repair, and crossover control	Yan et al. 2010; Bhattacharjee et al. 2013
*Sc*Smc5	Inhibiting Mph1's fork reversal and branch migration activity; preventing the accumulation of toxic recombination intermediates	Chen et al. 2009; Chavez et al. 2011; Xue et al. 2014
FAAP24	Recruitment of the FA core complex to chromatin; checkpoint activation; crossover control	Ciccia et al. 2007; Collis et al. 2008; Kim et al. 2008
BLM–Topo IIIα–RMI	DNA break repair; dHJ dissolution; crossover control	Deans and West 2009; Hoadley et al. 2012

(RF) Replication fork.

The histone-fold MHF complex and the Smc5/6 complex

As mentioned earlier, MHF is a conserved partner of FANCM family members and generally acts to enhance the activities of the latter (Table 2). Important progress has been made to elucidate the structure, biochemical attributes, and cellular roles of MHF in human and yeast cells. MHF from these organisms, a dimer of the heterodimer composed of the histone-fold proteins MHF1 and MHF2, resembles the histone H3/H4 tetramer in structure (Nishino et al. 2012; Tao et al. 2012; Yang et al. 2012; Wang et al. 2013a; Fox et al. 2014; Zhao et al. 2014). Human MHF binds various types of DNA and promotes FANCM functions in DNA binding, replication fork reversal, and DNA branch migration (Singh et al. 2010; Yan et al. 2010; Tao et al. 2012; Fox et al. 2014; Zhao et al. 2014). Cells lacking MHF or harboring mutations affecting its DNA binding or FANCM interaction are compromised for FANCM stability and FA core complex recruitment to damaged chromatin and are prone to chromosome aberrations and loss of viability upon exposure to clastogens (Singh et al. 2010; Yan et al. 2010; Tao et al. 2012; Fox et al. 2014; Zhao et al. 2014). Human MHF also aids FANCM in SCE suppression (Yan et al. 2010; Fox et al. 2014). Biochemical and structural studies have suggested possible modes of DNA interaction for MHF and the MHF–FANCM complex. A crystal structure

of an MHF–DNA complex showed that MHF can bind a pair of DNA duplexes, while a small-angle X-ray-scattering study of MHF raised the possibility that it may form an elongated octamer on duplex DNA (Wang et al. 2013a; Zhao et al. 2014). These findings suggest that MHF engages DNA through different binding modes. How these DNA-binding modes facilitate FANCM functions remains to be determined.

S. pombe MHF (*Sp*MHF) also enhances Fml1 functions in ICL repair, crossover control, and replication fork repair (Table 2; Yan et al. 2010; Bhattacharjee et al. 2013). Interestingly, a new role of *Sp*MHF in conjunction with Fml1 in the resolution of DNA bridges between sister chromatids has been reported (Bhattacharjee et al. 2013). Unlike its human and fission yeast counterpart, *S. cerevisiae* MHF (*Sc*MHF) has no DNA-binding capability and does not affect DNA binding by Mph1 (Table 2; Xue et al. 2015). The absence of a DNA-binding attribute in *Sc*MHF may account for the lack of effect on Mph1-mediated reactions in vitro and Mph1-dependent crossover control in vivo (Xue et al. 2015). Despite these differences, genetic evidence supports a role of *Sc*MHF in Mph1-mediated DNA damage repair and tolerance, partly by helping overcome the negative regulation imposed on Mph1 by the Smc5/6 complex (Fig. 2A; Xue et al. 2015).

Smc5/6 is a conserved SMC family complex with a role in the regulation of recombination intermediate levels. Several lines of evidence support the notion that Smc5/6 counteracts the prorecombinogenic activity of Mph1 through physical interaction with the C-terminal region of the latter (Table 2; Chen et al. 2009; Choi et al. 2010; Xue et al. 2014). For example, removing Mph1 or abolishing its DNA motor function alleviates multiple defects of *smc5/6* mutants, including the increased level of recombination intermediates seen in these mutants (Chen et al. 2009; Choi et al. 2010; Chavez et al. 2011). Importantly, purified Smc5/6 selectively inhibits Mph1's replication fork regression and branch migration activities (which can lead to the generation of recombination intermediates) without affecting its D-loop dissociative function (Xue et al. 2014). Mechanistically, through physical interaction with Mph1, Smc5 prevents the assembly of Mph1 oligomers at the junction of DNA forks (Xue et al. 2014). The observation that negative regulation of specific Mph1 activities can limit the formation of cytotoxic recombination intermediates reinforces the concept that although replication fork regression is beneficial for lesion removal or tolerance, it could also increase the risk of undesirable chromosomal rearrangements. The existence of a restraining mechanism provides flexibility in the repair response so that other less risky repair mechanisms, such as TLS, may be efficiently engaged. In principle, this negative regulation must be overcome when replication fork regression or HJ migration is needed, such as when a particular template lesion cannot be dealt with by TLS. Our recent findings show that by competing with Smc5 for Mph1 binding, *Sc*MHF liberates Mph1 from Smc5 inhibition (Xue et al. 2015). The antagonistic action of Smc5/6 and MHF provides a cellular mechanism to attenuate or activate the DNA replication fork regression and branch migration activities of Mph1, respectively, thus allowing repair pathway choice to be made at a damaged replication fork (Fig. 2A). Considering the high degree of conservation of Smc5/6 and MHF, it will be of considerable interest to test whether a similar antagonistic relationship exists between these proteins in other organisms.

FANCM regulation by FAAP24 and kinases

The vertebrate-specific FAAP24 helps target the FA core complex to ICLs and also collaborates with FANCM to promote ATR kinase-mediated checkpoint signaling by several mechanisms (Fig. 1; Table 2; Collis et al. 2008; Huang et al. 2010; Luke-Glaser et al. 2010; Schwab et al. 2010; Wang et al. 2013b). First, FAAP24 and FANCM interact with the HCLK2 kinase, an ATR–ATRIP-associated protein required for the S-phase checkpoint function (Collis et al. 2007, 2008). In addition, FAAP24 leads to the recruitment of the ssDNA-binding protein RPA to ICLs and activation of the ATR checkpoint pathway (Huang et al. 2010). FANCM also influences another checkpoint kinase, Chk1, as the two proteins appear to stabilize each other in HeLa cells (Luke-Glaser et al. 2010). Moreover, the FANCM DNA motor activity contributes to optimal ATR/Chk1 signaling, likely through generating long stretches of ssDNA that are recognized as a DNA stress signal by the checkpoint machinery (Collis et al. 2008; Schwab et al. 2010). Indeed, the loss of FANCM reduces chromosomal association of the ATR activator TopBP1 in chicken DT40 cells (Schwab et al. 2010). Future studies will be needed to elucidate the molecular mechanisms through which FANCM/FAAP24 triggers the different ATR activation modes under distinct cellular stress situations.

While the aforementioned studies have established a role of FANCM upstream of the ATR checkpoint, recent work has also revealed a dependence of FANCM on ATR. Specifically, ATR is responsible for FANCM phosphorylation at Ser1045 in response to genotoxic stress, and this modification is important for the recruitment of FANCM to ICL sites; FA pathway activation, as indicated by FANCD2 monoubiquitination; and ATR/CHK1 checkpoint function (Singh et al. 2013b). Taken together, these findings suggest the existence of a positive feedback loop between FANCM and ATR (Fig. 1). Further examination of the molecular details of this feedback loop and its connection with the ATR-mediated regulation of other FA proteins will be important for understanding the intricate role of ATR in the regulation of the FA pathway.

FANCM phosphorylation also occurs in the absence of genotoxic stress, and the cell cycle stage has a major influence in this regard, with the level of phosphorylation rising when cells transit from S phase into mitosis and declining after mitotic exit (Kim et al. 2008). FANCM phosphorylation in mitosis is mediated by the Polo-like kinase PLK1, and this modification leads to SCF-mediated degradation of FANCM (Kee et al. 2009). This phosphorylation-triggered FANCM degradation provides a neat mechanism for releasing the FA core complex from

chromatin during mitosis (Kee et al. 2009). It will be interesting to test whether other kinases are also involved in regulating the abundance and activity of FANCM throughout the cell cycle and also determine how dephosphorylation is achieved after mitotic exit.

Crossover control and the BLM—topoisomerase IIIαTopo IIIα—RMI (BTR) complex

The BTR complex—composed of BLM (a member of the RecQ helicase family), Topo IIIα, and the OB-fold RMI complex (a heterodimer of RMI1 and RMI2)—is the structural and functional equivalent of the Sgs1–Top3–Rmi1 (STR) complex in *S. cerevisiae* (Table 2). These protein complexes can dissolve the dHJ to yield noncrossover recombinants exclusively (Fig. 2B). This activity stems from the HJ branch migration activity of BLM/Sgs1 followed by DNA decatenation by Topo IIIα/Top3, while RMI/Rmi1 stimulates BLM/Sgs1 and Topo IIIα/Top3 activities (Wu and Hickson 2003; Plank et al. 2006; Raynard et al. 2006; Wu et al. 2006; Singh et al. 2008; Xu et al. 2008).

The role of STR in crossover control is mechanistically distinct from the anti-crossover role of Mph1/Fml1 and that of another helicase, Srs2, which acts early in the recombination process by dismantling the Rad51–ssDNA nucleoprotein filament (Krejci et al. 2003; Veaute et al. 2003). Genetic analyses have shown that simultaneously removing any two of these helicases increases crossover level more than ablating just one of them (Prakash et al. 2009; Mitchel et al. 2013). Nonoverlapping DNA repair roles between Mph1 and either Sgs1 or Srs2 lead to synthetic sickness or lethality of double mutants upon DNA damage occurrence (St Onge et al. 2007; Chen et al. 2009; Panico et al. 2010). Similarly, Mph1 and STR orthologs in fission yeast and *A. thaliana* also have independent functions in crossover control and/or DNA repair (Sun et al. 2008; Knoll et al. 2012; Seguela-Arnaud et al. 2015). In *D. melanogaster*, FANCM and BTR appear to be epistatic for crossover control but additive for DNA repair (Kuo et al. 2014).

The situation in vertebrates is different. First, human BTR forms a higher-order ensemble with the FA core complex, termed the BRAFT supercomplex (Meetei et al. 2003; Wang 2007). FANCM is responsible for recruiting the BTR complex to ICL sites and to the FA core complex through two domains called MM1 and MM2 (Fig. 1; Deans and West 2009). While MM1 mediates association with the FA core complex through FANCF, MM2 binds RMI1 and Topo IIIα (Deans and West 2009; Hoadley et al. 2012). A deletion of either MM1 or MM2 in FANCM engenders ICL sensitivity and elevates SCEs (Deans and West 2009). Interestingly, MM1 deletion impairs FANCD2 foci formation after treatment of cross-link agents or irradiation, while MM2 deletion or mutation affects BLM foci formation under replication stress (Deans and West 2009). This finding is consistent with the idea that FANCM possesses FA-dependent and FA-independent functions, the latter of which requires BTR. Based on these observations, FANCM appears to collaborate with BTR in crossover control and thus SCE suppression. A similar conclusion has been drawn in chicken DT40 cells (Rosado et al. 2009). As *BLM* mutations underlie Bloom's syndrome (BS), which is characterized by high levels of SCEs (Chaganti et al. 1974; Ellis et al. 1995; Cheok et al. 2005), it will be interesting to examine the possible involvement of FANCM in BS and define the mechanism by which the two DNA motor proteins functionally collaborate to suppress SCEs.

Concluding remarks

The past few years have witnessed rapid progress in understanding the role of the FANCM family of DNA motor proteins in genome replication and repair and the DNA damage response. Members of this family share common biochemical activities, with some possessing unique attributes. The studies of human FANCM and its orthologs in model systems have provided a framework for deciphering their roles in ICL repair and tolerance, replication fork repair, DNA damage checkpoints, and crossover control. Many important questions regarding these DNA motor proteins remain. Most notably, how do the regulators of FANCM family proteins act in different cellular contexts to selectively affect enzyme functions? Besides the interactors described above, other potential partners and regulators have been identified, but the roles that they fulfill have not yet been defined. For example, Mph1 associates with RPA in affecting gross chromosomal rearrangements via an unknown mechanism (Banerjee et al. 2008). Likewise, the manner in which Mph1 functions with Fkh1, Msh2–Msh6, and Mgm101 in ICL repair or with Elg1 possibly in lagging strand DNA synthesis remains to be delineated (Ho et al. 2002; Gavin et al. 2006; Kang et al. 2012; Ward et al. 2012; Singh et al. 2013a). In addition, recent studies suggest that FANCM family proteins function in additional processes; e.g., in nucleotide excision repair to remove bulky DNA adducts induced by ultraviolet light treatment (Kelsall et al. 2012). Finally, even though FANCM phosphorylation is clearly important for its cellular functions, there is a paucity of information regarding how the biochemical attributes of FANCM family proteins are influenced by phosphorylation and other post-translational modifications. Ongoing studies that entail biochemistry, genetics, cell biology, and structural biology will undoubtedly provide mechanistic answers to these questions and will continue to yield insights into the multifaceted role of these DNA motor proteins in genome maintenance.

Acknowledgments

We thank members of our laboratories, in particular James Daley, Simonne Longerich, Youngho Kwon, and Koyi Choi, for helpful discussions and editorial comments. The studies in our laboratories have been supported by National Institutes of Health grants GM057814, ES007061, ES015632, CA92584, and GM080670; American Cancer Society grant RSG-12-013-01-CCG; and a Leukemia and Lymphoma Society Scholar Award.

References

Bakker ST, van de Vrugt HJ, Rooimans MA, Oostra AB, Steltenpool J, Delzenne-Goette E, van der Wal A, van der Valk M, Joenje H, te Riele H, et al. 2009. Fancm-deficient mice reveal unique features of Fanconi anemia complementation group M. *Hum Mol Genet* **18:** 3484–3495.

Banerjee S, Smith S, Oum JH, Liaw HJ, Hwang JY, Sikdar N, Motegi A, Lee SE, Myung K. 2008. Mph1p promotes gross chromosomal rearrangement through partial inhibition of homologous recombination. *J Cell Biol* **181:** 1083–1093.

Bhattacharjee S, Osman F, Feeney L, Lorenz A, Bryer C, Whitby MC. 2013. MHF1–2/CENP-S-X performs distinct roles in centromere metabolism and genetic recombination. *Open Biol* **3:** 130102.

Blackford AN, Schwab RA, Nieminuszczy J, Deans AJ, West SC, Niedzwiedz W. 2012. The DNA translocase activity of FANCM protects stalled replication forks. *Hum Mol Genet* **21:** 2005–2016.

Chaganti RS, Schonberg S, German J. 1974. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proc Natl Acad Sci* **71:** 4508–4512.

Chavez A, Agrawal V, Johnson FB. 2011. Homologous recombination-dependent rescue of deficiency in the structural maintenance of chromosomes (Smc) 5/6 complex. *J Biol Chem* **286:** 5119–5125.

Chen YH, Choi K, Szakal B, Arenz J, Duan X, Ye H, Branzei D, Zhao X. 2009. Interplay between the Smc5/6 complex and the Mph1 helicase in recombinational repair. *Proc Natl Acad Sci* **106:** 21252–21257.

Chen YH, Szakal B, Castellucci F, Branzei D, Zhao X. 2013. DNA damage checkpoint and recombinational repair differentially affect the replication stress tolerance of Smc6 mutants. *Mol Biol Cell* **24:** 2431–2441.

Cheok CF, Bachrati CZ, Chan KL, Ralf C, Wu L, Hickson ID. 2005. Roles of the Bloom's syndrome helicase in the maintenance of genome stability. *Biochem Soc Trans* **33:** 1456–1459.

Choi K, Szakal B, Chen YH, Branzei D, Zhao X. 2010. The Smc5/6 complex and Esc2 influence multiple replication-associated recombination processes in *Saccharomyces cerevisiae*. *Mol Biol Cell* **21:** 2306–2314.

Ciccia A, Ling C, Coulthard R, Yan Z, Xue Y, Meetei AR, Laghmani el H, Joenje H, McDonald N, de Winter JP, et al. 2007. Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Mol Cell* **25:** 331–343.

Collis SJ, Barber LJ, Clark AJ, Martin JS, Ward JD, Boulton SJ. 2007. HCLK2 is essential for the mammalian S-phase checkpoint and impacts on Chk1 stability. *Nat Cell Biol* **9:** 391–401.

Collis SJ, Ciccia A, Deans AJ, Horejsi Z, Martin JS, Maslen SL, Skehel JM, Elledge SJ, West SC, Boulton SJ. 2008. FANCM and FAAP24 function in ATR-mediated checkpoint signaling independently of the Fanconi anemia core complex. *Mol Cell* **32:** 313–324.

Crismani W, Girard C, Froger N, Pradillo M, Santos JL, Chelysheva L, Copenhaver GP, Horlow C, Mercier R. 2012. FANCM limits meiotic crossovers. *Science* **336:** 1588–1590.

Daee DL, Ferrari E, Longerich S, Zheng XF, Xue X, Branzei D, Sung P, Myung K. 2012. Rad5-dependent DNA repair functions of the *Saccharomyces cerevisiae* FANCM protein homolog Mph1. *J Biol Chem* **287:** 26563–26575.

Deans AJ, West SC. 2009. FANCM connects the genome instability disorders Bloom's syndrome and Fanconi anemia. *Mol Cell* **36:** 943–953.

Deans AJ, West SC. 2011. DNA interstrand crosslink repair and cancer. *Nat Rev Cancer* **11:** 467–480.

Ede C, Rudolph CJ, Lehmann S, Schurer KA, Kramer W. 2011. Budding yeast Mph1 promotes sister chromatid interactions by a mechanism involving strand invasion. *DNA Repair (Amst)* **10:** 45–55.

Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S, Proytcheva M, German J. 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* **83:** 655–666.

Errico A, Costanzo V. 2012. Mechanisms of replication fork protection: a safeguard for genome stability. *Crit Rev Biochem Mol Biol* **47:** 222–235.

Fontebasso Y, Etheridge TJ, Oliver AW, Murray JM, Carr AM. 2013. The conserved Fanconi anemia nuclease Fan1 and the SUMO E3 ligase Pli1 act in two novel Pso2-independent pathways of DNA interstrand crosslink repair in yeast. *DNA Repair (Amst)* **12:** 1011–1023.

Fox D III, Yan Z, Ling C, Zhao Y, Lee DY, Fukagawa T, Yang W, Wang W. 2014. The histone-fold complex MHF is remodeled by FANCM to recognize branched DNA and protect genome stability. *Cell Res* **24:** 560–575.

Gari K, Decaillet C, Delannoy M, Wu L, Constantinou A. 2008a. Remodeling of DNA replication structures by the branch point translocase FANCM. *Proc Natl Acad Sci* **105:** 16107–16112.

Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. 2008b. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. *Mol Cell* **29:** 141–148.

Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, Marzioch M, Rau C, Jensen LJ, Bastuck S, Dumpelfeld B, et al. 2006. Proteome survey reveals modularity of the yeast cell machinery. *Nature* **440:** 631–636.

Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutilier K, et al. 2002. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* **415:** 180–183.

Hoadley KA, Xue Y, Ling C, Takata M, Wang W, Keck JL. 2012. Defining the molecular interface that connects the Fanconi anemia protein FANCM to the Bloom syndrome dissolvasome. *Proc Natl Acad Sci* **109:** 4437–4442.

Huang M, Kim JM, Shiotani B, Yang K, Zou L, D'Andrea AD. 2010. The FANCM/FAAP24 complex is required for the DNA interstrand crosslink-induced checkpoint response. *Mol Cell* **39:** 259–268.

Huang J, Liu S, Bellani MA, Thazhathveetil AK, Ling C, de Winter JP, Wang Y, Wang W, Seidman MM. 2013. The DNA translocase FANCM/MHF promotes replication traverse of DNA interstrand crosslinks. *Mol Cell* **52:** 434–446.

Kang YH, Munashingha PR, Lee CH, Nguyen TA, Seo YS. 2012. Biochemical studies of the *Saccharomyces cerevisiae* Mph1 helicase on junction-containing DNA structures. *Nucleic Acids Res* **40:** 2089–2106.

Kee Y, Kim JM, D'Andrea AD. 2009. Regulated degradation of FANCM in the Fanconi anemia pathway during mitosis. *Genes Dev* **23:** 555–560.

Kelsall IR, Langenick J, MacKay C, Patel KJ, Alpi AF. 2012. The Fanconi anaemia components UBE2T and FANCM are functionally linked to nucleotide excision repair. *PLoS One* **7:** e36970.

Kim H, D'Andrea AD. 2012. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. *Genes Dev* **26:** 1393–1408.

Kim JM, Kee Y, Gurtan A, D'Andrea AD. 2008. Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24. *Blood* **111:** 5215–5222.

Knoll A, Higgins JD, Seeliger K, Reha SJ, Dangel NJ, Bauknecht M, Schropfer S, Franklin FC, Puchta H. 2012. The Fanconi anemia ortholog FANCM ensures ordered homologous recombination in both somatic and meiotic cells in *Arabidopsis*. *Plant Cell* **24:** 1448–1464.

Komori K, Fujikane R, Shinagawa H, Ishino Y. 2002. Novel endonuclease in *Archaea* cleaving DNA with various branched structure. *Genes Genet Syst* **77:** 227–241.

Komori K, Hidaka M, Horiuchi T, Fujikane R, Shinagawa H, Ishino Y. 2004. Cooperation of the N-terminal Helicase and C-terminal endonuclease activities of archaeal Hef protein in processing stalled replication forks. *J Biol Chem* **279:** 53175–53185.

Kottemann MC, Smogorzewska A. 2013. Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature* **493:** 356–363.

Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, Klein H, Ellenberger T, Sung P. 2003. DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* **423:** 305–309.

Kuo HK, McMahan S, Rota CM, Kohl KP, Sekelsky J. 2014. *Drosophila* FANCM helicase prevents spontaneous mitotic crossovers generated by the MUS81 and SLX1 nucleases. *Genetics* **198:** 935–945.

Labib K, De Piccoli G. 2011. Surviving chromosome replication: the many roles of the S-phase checkpoint pathway. *Philos Trans R Soc Lond B Biol Sci* **366:** 3554–3561.

Lorenz A, Osman F, Sun W, Nandi S, Steinacher R, Whitby MC. 2012. The fission yeast FANCM ortholog directs non-crossover recombination during meiosis. *Science* **336:** 1585–1588.

Lossaint G, Larroque M, Ribeyre C, Bec N, Larroque C, Decaillet C, Gari K, Constantinou A. 2013. FANCD2 binds MCM proteins and controls replisome function upon activation of s phase checkpoint signaling. *Mol Cell* **51:** 678–690.

Luebben SW, Kawabata T, Johnson CS, O'Sullivan MG, Shima N. 2014. A concomitant loss of dormant origins and FANCC exacerbates genome instability by impairing DNA replication fork progression. *Nucleic Acids Res* **42:** 5605–5615.

Luke-Glaser S, Luke B. 2012. The Mph1 helicase can promote telomere uncapping and premature senescence in budding yeast. *PLoS One* **7:** e42028.

Luke-Glaser S, Luke B, Grossi S, Constantinou A. 2010. FANCM regulates DNA chain elongation and is stabilized by S-phase checkpoint signalling. *EMBO J* **29:** 795–805.

Mazon G, Symington LS. 2013. Mph1 and Mus81–Mms4 prevent aberrant processing of mitotic recombination intermediates. *Mol Cell* **52:** 63–74.

McHugh PJ, Ward TA, Chovanec M. 2012. A prototypical Fanconi anemia pathway in lower eukaryotes? *Cell Cycle* **11:** 3739–3744.

Meetei AR, Sechi S, Wallisch M, Yang D, Young MK, Joenje H, Hoatlin ME, Wang W. 2003. A multiprotein nuclear complex connects Fanconi anemia and Bloom syndrome. *Mol Cell Biol* **23:** 3417–3426.

Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, Bier P, Steltenpool J, Stone S, Dokal I, Mathew CG, et al. 2005. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat Genet* **37:** 958–963.

Mitchel K, Lehner K, Jinks-Robertson S. 2013. Heteroduplex DNA position defines the roles of the Sgs1, Srs2, and Mph1 helicases in promoting distinct recombination outcomes. *PLoS Genet* **9:** e1003340.

Mosedale G, Niedzwiedz W, Alpi A, Perrina F, Pereira-Leal JB, Johnson M, Langevin F, Pace P, Patel KJ. 2005. The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nat Struct Mol Biol* **12:** 763–771.

Nandi S, Whitby MC. 2012. The ATPase activity of Fml1 is essential for its roles in homologous recombination and DNA repair. *Nucleic Acids Res* **40:** 9584–9595.

Niedzwiedz W, Mosedale G, Johnson M, Ong CY, Pace P, Patel KJ. 2004. The Fanconi anaemia gene FANCC promotes homologous recombination and error-prone DNA repair. *Mol Cell* **15:** 607–620.

Nishino T, Komori K, Ishino Y, Morikawa K. 2005. Structural and functional analyses of an archaeal XPF/Rad1/Mus81 nuclease: asymmetric DNA binding and cleavage mechanisms. *Structure* **13:** 1183–1192.

Nishino T, Takeuchi K, Gascoigne KE, Suzuki A, Hori T, Oyama T, Morikawa K, Cheeseman IM, Fukagawa T. 2012. CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. *Cell* **148:** 487–501.

Panico ER, Ede C, Schildmann M, Schurer KA, Kramer W. 2010. Genetic evidence for a role of *Saccharomyces cerevisiae* Mph1 in recombinational DNA repair under replicative stress. *Yeast* **27:** 11–27.

Plank JL, Wu J, Hsieh TS. 2006. Topoisomerase IIIα and Bloom's helicase can resolve a mobile double Holliday junction substrate through convergent branch migration. *Proc Natl Acad Sci* **103:** 11118–11123.

Prakash R, Krejci L, Van Komen S, Anke Schurer K, Kramer W, Sung P. 2005. *Saccharomyces cerevisiae MPH1* gene, required for homologous recombination-mediated mutation avoidance, encodes a 3′ to 5′ DNA helicase. *J Biol Chem* **280:** 7854–7860.

Prakash R, Satory D, Dray E, Papusha A, Scheller J, Kramer W, Krejci L, Klein H, Haber JE, Sung P, et al. 2009. Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. *Genes Dev* **23:** 67–79.

Primig M, Williams RM, Winzeler EA, Tevzadze GG, Conway AR, Hwang SY, Davis RW, Esposito RE. 2000. The core meiotic transcriptome in budding yeasts. *Nat Genet* **26:** 415–423.

Raynard S, Bussen W, Sung P. 2006. A double Holliday junction dissolvasome comprising BLM, topoisomerase IIIα, and BLAP75. *J Biol Chem* **281:** 13861–13864.

Rosado IV, Niedzwiedz W, Alpi AF, Patel KJ. 2009. The Walker B motif in avian FANCM is required to limit sister chromatid exchanges but is dispensable for DNA crosslink repair. *Nucleic Acids Res* **37:** 4360–4370.

Scheller J, Schurer A, Rudolph C, Hettwer S, Kramer W. 2000. *MPH1*, a yeast gene encoding a DEAH protein, plays a role in protection of the genome from spontaneous and chemically induced damage. *Genetics* **155:** 1069–1081.

Schlacher K, Wu H, Jasin M. 2012. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51–BRCA1/2. *Cancer Cell* **22:** 106–116.

Schurer KA, Rudolph C, Ulrich HD, Kramer W. 2004. Yeast *MPH1* gene functions in an error-free DNA damage bypass pathway that requires genes from Homologous recombination, but not from postreplicative repair. *Genetics* **166:** 1673–1686.

Schwab RA, Blackford AN, Niedzwiedz W. 2010. ATR activation and replication fork restart are defective in FANCM-deficient cells. *EMBO J* **29:** 806–818.

Sebesta M, Burkovics P, Haracska L, Krejci L. 2011. Reconstitution of DNA repair synthesis in vitro and the role of polymerase and helicase activities. *DNA Repair (Amst)* **10:** 567–576.

Seguela-Arnaud M, Crismani W, Larcheveque C, Mazel J, Froger N, Choinard S, Lemhemdi A, Macaisne N, Van Leene J, Gevaert K,

et al. 2015. Multiple mechanisms limit meiotic crossovers: TOP3α and two BLM homologs antagonize crossovers in parallel to FANCM. *Proc Natl Acad Sci* **112:** 4713–4718.

Singh TR, Ali AM, Busygina V, Raynard S, Fan Q, Du CH, Andreassen PR, Sung P, Meetei AR. 2008. BLAP18/RMI2, a novel OB-fold-containing protein, is an essential component of the Bloom helicase-double Holliday junction dissolvasome. *Genes Dev* **22:** 2856–2868.

Singh TR, Bakker ST, Agarwal S, Jansen M, Grassman E, Godthelp BC, Ali AM, Du CH, Rooimans MA, Fan Q, et al. 2009. Impaired FANCD2 monoubiquitination and hypersensitivity to camptothecin uniquely characterize Fanconi anemia complementation group M. *Blood* **114:** 174–180.

Singh TR, Saro D, Ali AM, Zheng XF, Du CH, Killen MW, Sachpatzidis A, Wahengbam K, Pierce AJ, Xiong Y, et al. 2010. MHF1–MHF2, a histone-fold-containing protein complex, participates in the Fanconi anemia pathway via FANCM. *Mol Cell* **37:** 879–886.

Singh S, Shemesh K, Liefshitz B, Kupiec M. 2013a. Genetic and physical interactions between the yeast *ELG1* gene and orthologs of the Fanconi anemia pathway. *Cell Cycle* **12:** 1625–1636.

Singh TR, Ali AM, Paramasivam M, Pradhan A, Wahengbam K, Seidman MM, Meetei AR. 2013b. ATR-dependent phosphorylation of FANCM at serine 1045 is essential for FANCM functions. *Cancer Res* **73:** 4300–4310.

Singleton MR, Dillingham MS, Wigley DB. 2007. Structure and mechanism of helicases and nucleic acid translocases. *Annu Rev Biochem* **76:** 23–50.

Sobeck A, Stone S, Costanzo V, de Graaf B, Reuter T, de Winter J, Wallisch M, Akkari Y, Olson S, Wang W, et al. 2006. Fanconi anemia proteins are required to prevent accumulation of replication-associated DNA double-strand breaks. *Mol Cell Biol* **26:** 425–437.

Sobeck A, Stone S, Landais I, de Graaf B, Hoatlin ME. 2009. The Fanconi anemia protein FANCM is controlled by FANCD2 and the ATR/ATM pathways. *J Biol Chem* **284:** 25560–25568.

Soulier J. 2011. Fanconi anemia. *Hematology Am Soc Hematol Educ Program* **2011:** 492–497.

Stafa A, Donnianni RA, Timashev LA, Lam AF, Symington LS. 2014. Template switching during break-induced replication is promoted by the Mph1 helicase in *Saccharomyces cerevisiae*. *Genetics* **196:** 1017–1028.

St Onge RP, Mani R, Oh J, Proctor M, Fung E, Davis RW, Nislow C, Roth FP, Giaever G. 2007. Systematic pathway analysis using high-resolution fitness profiling of combinatorial gene deletions. *Nat Genet* **39:** 199–206.

Suhasini AN, Rawtani NA, Wu Y, Sommers JA, Sharma S, Mosedale G, North PS, Cantor SB, Hickson ID, Brosh RM Jr. 2011. Interaction between the helicases genetically linked to Fanconi anemia group J and Bloom's syndrome. *EMBO J* **30:** 692–705.

Sun W, Nandi S, Osman F, Ahn JS, Jakovleska J, Lorenz A, Whitby MC. 2008. The FANCM ortholog Fml1 promotes recombination at stalled replication forks and limits crossing over during DNA double-strand break repair. *Mol Cell* **32:** 118–128.

Sung P, Klein H. 2006. Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nat Rev Mol Cell Biol* **7:** 739–750.

Tao Y, Jin C, Li X, Qi S, Chu L, Niu L, Yao X, Teng M. 2012. The structure of the FANCM–MHF complex reveals physical features for functional assembly. *Nat Commun* **3:** 782.

Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, Fabre F. 2003. The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* **423:** 309–312.

Wang W. 2007. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat Rev Genet* **8:** 735–748.

Wang AT, Smogorzewska A. 2015. SnapShot: Fanconi anemia and associated proteins. *Cell* **160:** 354–354.

Wang W, Guo Q, Shtykova EV, Liu G, Xu J, Teng M, Liu P, Dong Y. 2013a. Structural peculiarities of the (MHF1–MHF2)4 octamer provide a long DNA binding patch to anchor the MHF–FANCM complex to chromatin: a solution SAXS study. *FEBS Lett* **587:** 2912–2917.

Wang Y, Leung JW, Jiang Y, Lowery MG, Do H, Vasquez KM, Chen J, Wang W, Li L. 2013b. FANCM and FAAP24 maintain genome stability via cooperative as well as unique functions. *Mol Cell* **49:** 997–1009.

Ward TA, Dudasova Z, Sarkar S, Bhide MR, Vlasakova D, Chovanec M, McHugh PJ. 2012. Components of a Fanconi-like pathway control Pso2-independent DNA interstrand crosslink repair in yeast. *PLoS Genet* **8:** e1002884.

Whitby MC. 2010. The FANCM family of DNA helicases/translocases. *DNA Repair (Amst)* **9:** 224–236.

Wu L, Hickson ID. 2003. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* **426:** 870–874.

Wu L, Bachrati CZ, Ou J, Xu C, Yin J, Chang M, Wang W, Li L, Brown GW, Hickson ID. 2006. BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. *Proc Natl Acad Sci* **103:** 4068–4073.

Wyatt HD, West SC. 2014. Holliday junction resolvases. *Cold Spring Harb Perspect Biol* **6:** a023192.

Xu D, Guo R, Sobeck A, Bachrati CZ, Yang J, Enomoto T, Brown GW, Hoatlin ME, Hickson ID, Wang W. 2008. RMI, a new OB-fold complex essential for Bloom syndrome protein to maintain genome stability. *Genes Dev* **22:** 2843–2855.

Xue Y, Li Y, Guo R, Ling C, Wang W. 2008. FANCM of the Fanconi anemia core complex is required for both monoubiquitination and DNA repair. *Hum Mol Genet* **17:** 1641–1652.

Xue X, Choi K, Bonner J, Chiba T, Kwon Y, Xu Y, Sanchez H, Wyman C, Niu H, Zhao X, et al. 2014. Restriction of replication fork regression activities by a conserved SMC complex. *Mol Cell* **56:** 436–445.

Xue X, Choi K, Bonner JN, Szakal B, Chen YH, Papusha A, Saro D, Niu H, Ira G, Branzei D, et al. 2015. Selective modulation of the functions of a conserved DNA motor by a histone fold complex. *Genes Dev* **29:** 1000–1005.

Yan Z, Delannoy M, Ling C, Daee D, Osman F, Muniandy PA, Shen X, Oostra AB, Du H, Steltenpool J, et al. 2010. A histone-fold complex and FANCM form a conserved DNA-remodeling complex to maintain genome stability. *Mol Cell* **37:** 865–878.

Yang H, Zhang T, Tao Y, Wu L, Li HT, Zhou JQ, Zhong C, Ding J. 2012. *Saccharomyces cerevisiae* MHF complex structurally resembles the histones (H3-H4)(2) heterotetramer and functions as a heterotetramer. *Structure* **20:** 364–370.

Yeeles JT, Poli J, Marians KJ, Pasero P. 2013. Rescuing stalled or damaged replication forks. *Cold Spring Harb Perspect Biol* **5:** a012815.

Zhao Q, Saro D, Sachpatzidis A, Singh TR, Schlingman D, Zheng XF, Mack A, Tsai MS, Mochrie S, Regan L, et al. 2014. The MHF complex senses branched DNA by binding a pair of crossover DNA duplexes. *Nat Commun* **5:** 2987.

Zheng XF, Prakash R, Saro D, Longerich S, Niu H, Sung P. 2011. Processing of DNA structures via DNA unwinding and branch migration by the *S. cerevisiae* Mph1 protein. *DNA Repair (Amst)* **10:** 1034–1043.