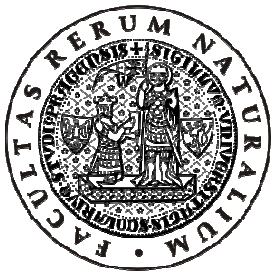

Charles University in Prague, Faculty of Science
Department of Cell Biology

The Role of FBH1 in Maintenance of Genome Stability

Bachelor thesis

Jitka Šimandlová

Supervisor: Dr. Igor Chevelev

Institute of Molecular Genetics AS CR
Laboratory of Chromosomal Stability
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Abstract

The genome is constantly threatened by various damaging agents and maintaining its integrity is crucial for all organisms. Several repair pathways have been implicated in the removal of different types of lesions from DNA. Among them, homologous recombination (HR) plays a key role in repair of double-strand breaks. HR is a highly important repair mechanism which has to be tightly regulated to prevent excessive HR events. These events could interfere with other DNA repair pathways, generate toxic intermediates, or block the progression of the replication fork. Therefore, it is not surprising that cells have evolved mechanisms that counteract inappropriate HR events. As it has been shown recently, cells possess DNA helicases capable of preventing excessive recombination. A novel human DNA helicase, hFBH1, belonging to the superfamily I has been shown to function as pro- and anti-recombinase. Similar to the two members of RecQ family, BLM and RECQL5, FBH1 disrupts Rad51 from nucleofilament. However, FBH1 might also promote initiation of HR. The FBH1 helicase possesses additional high conserved F-box motif which allows it to act within a Skp1-Cullin-F-box, SCF, complex as ubiquitin ligase and target proteins for degradation.

Key words: FBH1, homologous recombination, SCF complex, F-box protein, anti-recombinase, double-strand breaks, genome stability

Abstrakt

Genomová DNA je vystavena neustálému působení škodlivých faktorů, které mohou způsobit různé typy jejího poškození. Odstranění těchto poruch je nezbytné pro udržování celistvosti genomu a tudíž i pro přežití celého organismu. V buňkách se proto vyvinuly určité mechanismy, jak tato poškození opravovat. Homologní rekombinace (HR) je důležitým procesem pro odstraňování nejnebezpečnější poruchy DNA, kterou je dvouvláknový zlom. Naproti tomu, spontánní a nežádoucí HR se může prolínat s jinými opravnými drahami, tvořit toxické meziprodukty či způsobit zablokování replikační vidlice. Proces homologní rekombinace proto musí být regulován. Jedním z možných modulátorů jsou DNA helikázy, které jsou schopné zabraňovat nežádoucí rekombinaci. Nově objevená DNA helikáza hFBH1 patřící do rodiny SF1 helikáz se nejspíše účastní regulace HR. Obdobně jako BLM a RECQL5 DNA helikázy z RecQ rodiny, i FBH1 zabraňuje tvorbě, pro zahájení HR nezbytného, presynaptického filamentu. Na druhou stranu, FBH1 se také účastní iniciace HR. FBH1 má tedy jak prorekombinázovou, tak i antirekombinázovou roli. Ve své primární struktuře obsahuje FBH1 mimo helikázové domény také F-box motiv. Díky němu se jako F-box protein váže v Skp1-Cullin-F-box, SCF, komplexu, který specificky rozeznává proteiny určené pro degradaci v proteasomu.

Klíčová slova: FBH1, homologní rekombinace, SCF komplex, F-box protein, antirekombináza, dvouvláknové zlomy, stabilita genomu

Outline

Abbreviations

ADP	**Adenosine diphosphate**
APC	**Anaphase promoting complex**
AppNp	**5′-Adenylylimidodiphosphate**
ATP	**Adenosine triphosphate**
DNA	**Deoxyribonucleic acid**
dsDNA	**Double-stranded DNA**
ssDNA	**Single-stranded DNA**
DSB	**Double strand break**
FBP	**F-box protein**
HR	**Homologous recombination**
MAPK	**Mitogen-activated protein kinase**
NF	**Nucleofilament**
NLS	**Nuclear localization signal**
RPA	**Replication protein A**
SCF	**Skp1-Cullin-F-box complex**
SCF^{Fbh1}	**SCF complex with Fbh1 as F-box protein**
SDSA	**Synthesis-dependent strand-annealing**
SF	**Superfamily**
Ub	**Ubiquitin**
WD	**Tryptophan-aspartic acid**

1. Introduction

DNA in cells is maintained from generation to generation just with little change and the DNA sequences can occasionally be altered. In a population, certain type of genetic variation is crucial to allow organisms to evolve in response to changing environmental conditions over time. Such DNA rearrangements are caused by a set of mechanisms that are collectively called genetic recombination, in which are involved both homologous recombination and site-specific recombination.

Homologous recombination (HR) is a fundamental process of DNA metabolism which is used in all forms of life. In this action, nucleotide sequences of two homologous DNA strands are exchanged. In most eukaryotic cells, HR is essential during meiosis and mitosis. In meiosis, HR facilitates chromosomal crossover and thus contributes to generating genetic diversity. In mitosis, HR is the major DNA repairing pathway.

Genome integrity is constantly challenged by DNA damage. The most toxic chromosomal lesion represent DNA double-stranded breaks (DSBs) induced by exogenous insults such as ionizing radiation and chemical exposure. Certain types of DNA damage constitute a strong hindrance to the DNA replication machinery and can lead to arrest or collapse of DNA replication fork. To counteract the potential deleterious effects of DNA lesions, cells have evolved several DNA repair mechanisms. Among them a HR-based DNA repair pathway is crucial for preventing genome instability. On the other hand, untimely and unscheduled HR events might interfere with other DNA repair pathways, generate toxic intermediates, or block the progression of the replication fork (Sung and Klein, 2006).

An accumulation of numerous genetic changes that would lead to cancer in normal cell is quite rare. Less than 1 mutation per genome (~3.3×10^9 base pair) per cell division is happened in human cell (Drake et al., 1998, Chen et al., 2003). Disruption in DNA repair pathway leads to genomic instability and an increased susceptibility to certain types of cancer. Several cancer-prone genetic diseases such as Bloom´s and Werner´s syndrome are associated with HR dysfunction or deficiency. Overall, during DNA metabolism, cell DNA has to be maintained by tight control (Sung and Klein, 2006).

Separation of the complementary strands of the DNA duplex is required to provide single-stranded DNA (ssDNA) templates for DNA transactions such as those involved in recombination, repair and replication. For this purpose cells posses an important enzymatic

tool, DNA helicases. They move along the DNA and catalyze breaking hydrogen of bonds that hold the DNA strands together. Thus, DNA helicases are required for many cellular processes, such as DNA replication, transcription, recombination, DNA repair and chromosome segregation. DNA helicases can be characterized as DNA dependent ATPases because energy from ATP hydrolysis is necessary for the unwinding of double stranded DNA (dsDNA). Helicases exhibit specific directionality (3´→5´or 5´→3´) of the DNA unwinding activity with respect to the DNA strand to which it is bound. Sequence alignments have revealed that many DNA helicases carry 7 distinct conserved motifs. Based on the presence and the form of these helicase motifs, these enzymes have been sorted out to several superfamilies (SF): SF1, SF2, SF3 (Tuteja and Tuteja, 2004).

A novel human DNA helicase, called hFbh1, is a member of SF1 (Kim et al., 2002). The hFBH1 seems to be homologous and/or functionally related to those DNA helicases which play a crucial role in maintenance of genome stability. Recent studies suggest that Fbh1 acts as both an anti-recombinase by dissociating Rad51 from nucleofilament (NF) and as a pro-recombinase by facilitating ssDNA overhang production that is further used in DNA repair (Fugger et al., 2009). Moreover, a highly conserved F-box motif was found in primary structure of hFBH1 and thus, hFBH1 is able to act as F-box protein (FBP) in Skp1-Cullin-F-box (SCF) complex (Kim et al., 2004).

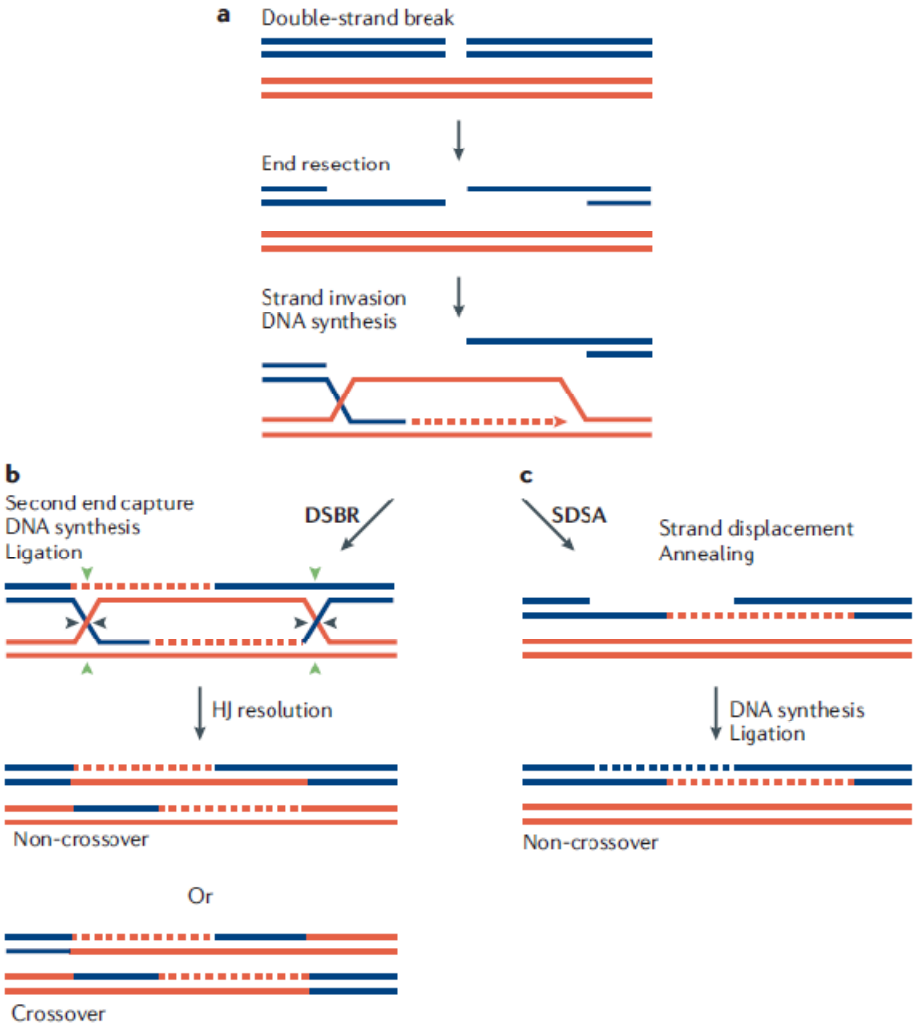
In this work, I will give an overview of published results and proposals for a possible role of human FBH1 helicase in process of DNA repair and its contribution to maintenance of genome stability.

2. DNA repair

The repair of DNA lesions such as base damage, inter- and intra-strand DNA crosslinks and single- and double-strand DNA breaks is essential for the survival of an organism. Especially detrimental to the cell are DSBs. To remove unwanted DSBs and protect cells from their deleterious consequences, two general strategies of DSBs repair have been evolved.

Both of them are initiated by generating of 3´ssDNA overhangs at the broken DNA ends (Fig.1). The overhanging ssDNA stretches are promptly coated by replication protein A (RPA). The key step in HR is the nucleofilament (NF; also known as presynaptic filament) formation by loading of Rad51 (*Escherichia coli* RecA, *Schizosaccharomyces pombe* Rhp51) onto the coated ssDNA molecule. To overcome the inhibitory effect of RPA on Rad51 nucleofilament assembly, the mediator proteins, such as Rad52 (*S. pombe* Rad22) and heterodimer Rad55-Rad57, promote delivery of Rad51 to RPA-covered DNA. The Rad51 NF catalyses search for a homologous region on the sister chromatid followed by D-loop formation which promotes DNA strand invasion (Raji and Hartsuiker, 2006, Sung and Klein, 2006).

The first strategy, is called “DSB repair”. In this process, double Holliday junction is formed. Resolution of this Holliday junction leads to a gene conversion, with or without associated crossovers. The second strategy is synthesis-dependent strand-annealing (SDSA) when only one strand of non-damaged homologous DNA is used for reparative synthesis. After resolving a D-loop, newly synthesized strand uses itself as a template for reparative synthesis of the second broken strand of DNA. In this mechanism, no Holliday junction formation occurs and resulting products are always non-crossover (Sung and Klein, 2006).



<u>Figure 1</u> HR-based repair of DNA DSBs by DSB repair and SDSA.
A) Initiation of the repair process by generating 3´ssDNA overhangs at the broken DNA, *blue filaments*. Strand invasion and D-loop formation by the overhanging ssDNA into homologous sequence, *red filaments*, is followed by DNA synthesis, *dotted filament*. B) DSB repair by double Holliday junction formation, that leads to non-crossover or crossover products. C) Synthesis-dependent strand-annealing (SDSA) is repair mechanism when new synthesized strand uses itself as a template for reparative synthesis of the second broken strand of DNA. Resulting products are always non-crossover (adapted from Sung and Klein, 2006).

3. SCF ubiquitin ligase

The ubiquitin (Ub) system of intracellular protein degradation controls the abundance of almost all proteins in the cell. In this process, proteins destined for degradation are covalently attached to ubiquitin, a highly conserved 76 amino-acid protein. These polyubiquitinated substrates are targeted to S26 proteasom which degrades the protein to short peptides and free amino acids. Ub-mediated degradation of regulatory proteins plays an important role in the control of numerous process, including cell-cycle progression, signal transduction, transcriptional regulation, receptor down-regulation, and endocytosis.

The attachment of the Ub to the protein is usually catalysed by three enzymes (Fig. 2). An E1 (Ub-activating enzyme) uses ATP to form a thioester bond between itself and Ub. E1 then transfers the Ub to an E2 (Ub-conjugating enzyme). Finally, E3 (Ub protein ligase) attaches Ub to the substrate (Willems et al., 1999, Baumeister et al., 1998, Bai et al., 1996).

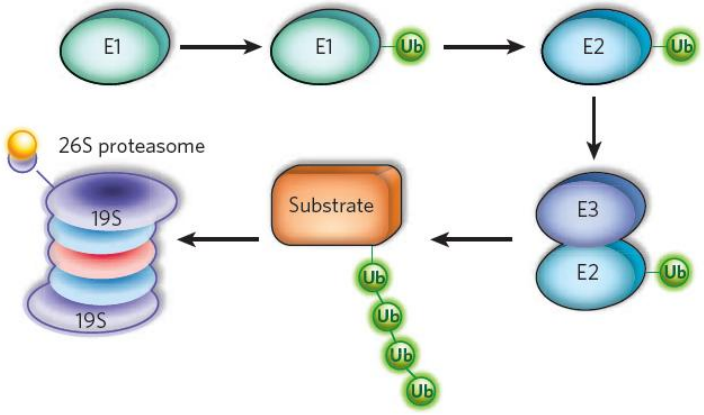


Figure 2 A scheme of the ubiquitination process.
E1 (Ub-activating enzyme) attaches Ub (ubiquitin) and transfers to an E2 (Ub-conjugating enzyme). Ub is transferred to the substrate by E3 (Ub protein ligase). Finally, the poly-ubiquitinated substrate is targeted to 26S proteasom and degradated (adapted from Hoeller and Dikic, 2009).

Two types of multisubunit E3 enzymes that mediate ubiquitination of many cell cycle proteins are the APC, anaphase-promoting complex, and SCF, Skp1 – Cullin - F-box protein complex (Hershko and Ciechanover, 1998).

The SCF complex is a multisubunits enzyme composed of an invariant core, containing three components (Fig.3):

- Skp1 is an adaptor protein, essential in the recognition and binding of the F-box motif
- Cullin (Cdc53 ortholog) functions as an scaffold protein, linking the Skp1 protein with Rbx1 protein
- Rbx1 (or Roc1,Hrt1) protein, containing a RING finger motif, mediates binding to the E2 enzyme

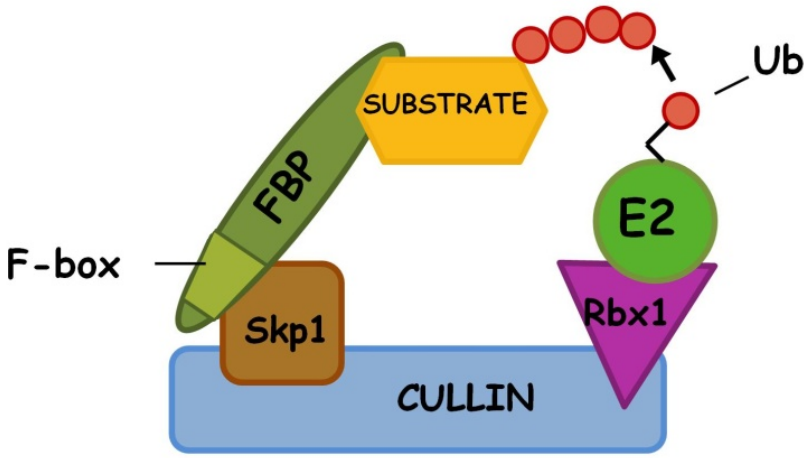


Figure 3 A schematic model of SCF complex.
Invariable subunits of SCF complex are Skp1, Cullin and Rbx1. F-box protein (FBP) is linked to the core of the complex through the interaction of its F-box motif and Skp1. On the other end of the FBP is attached a specific substrate, that is ubiquitinated and targeted for degradation. Ub, ubiquitin; E2, Ub-conjugating enzyme.

The substrate specificity of the SCF complex is governed by the interchangeable F-box protein subunit that recruits a specific set of substrates for ubiquitination to the SCF core complex. Simultaneously, the capability of the SCF backbone to recruit multiple F-box proteins with distinct substrate specificities, substantially increases the substrate repertoire (Cardozo and Pagano, 2004).

4. F-box proteins

The unique feature of the FBPs is presence of the F-box motif which was named after the first defined FBP, cyclin F (Bai et al., 1996). The F-box motif constitutes a conserved sequence, that consists of approximately 50 amino acids (Fig. 4). In general, this motif is located at the N-terminal part of the protein (Fig. 5). Moreover, FBPs often possess additional C-terminal motifs such as WD (tryptophan-aspartic acid) repeats, leucine-rich repeats or a wide range of other motifs including zinc fingers, leucine zipper, ring fingers or proline rich regions (Cenciarelli et al., 1999, Hermand, 2006)

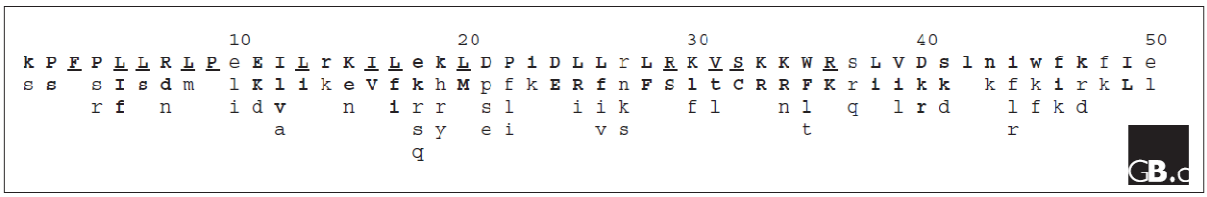


Figure 4 Consensus sequence of F-box motif.
Bold and underlined capital letters signify residues found in over 40% of the F-box sequences; *bold, non-underlined, capital letters* signify residues found in 20-40% of the F-boxes; *bold lower case letters* indicate residues found in 15-19% of the F-boxes; and *non-bold lower case letters* indicate residues found in 10-14% of the F-boxes (adapted from Kipreos and Pagano, 2000).

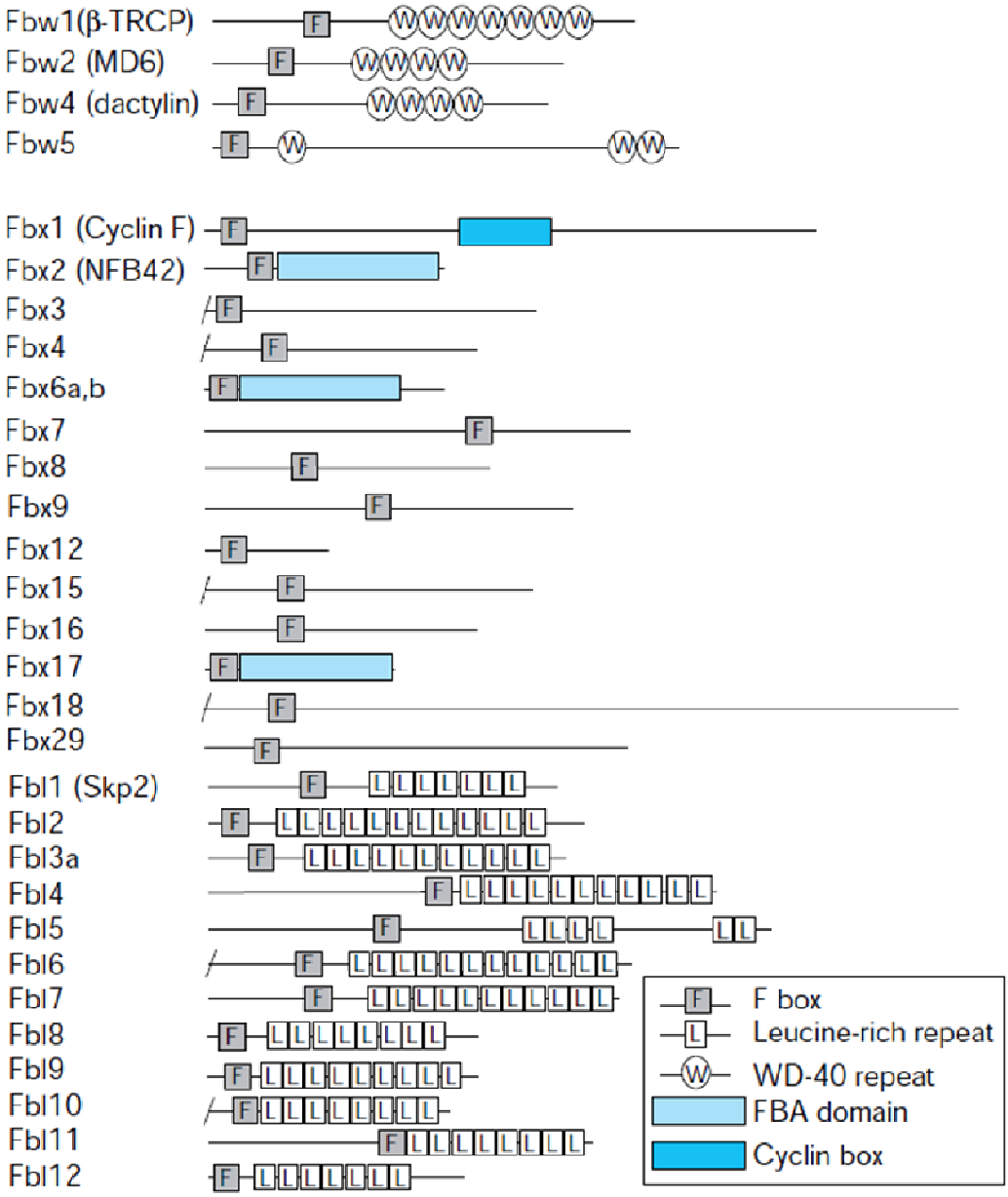


Figure 5 Domain structure of several FBPs

Comparison of domain structure and distribution in several mammalian F-box proteins (FBPs). General feature of all FBPs is the F-box motif located at the N-terminal part. WD, (tryptophan-aspartic acid) repeats; FBA (F-box associated) domain (adapted from Winston et al., 1999).

Substrate phosphorylation is one common prerequisite for the recognition by FBP in the majority of the model organisms. Thus, it is crucial, that one or more of the substrate specific epitopes are phosphorylated prior to the interaction with the FBP. Although, not all FBP selectively recognize phosphorylated substrate, no example of negative effect of phosphorylation to the interaction with FBP has been identified until recently (Lawrence et al., 2009, Ho et al., 2008, Deshaies, 1999, Skowyra et al., 1997).

In spite of the huge number of identified FBPs, there are still many of them, whose substrate and function have not been defined yet. 11 FBPs have been determinated in *Saccharomyces cerevisiae,* about 30 in *Drosophila,* 326 in *Caenorhabditis elegans,* about 600 in *Arabidopsis thaliana,* and at least 38 in human. However, there is not known example in prokaryotes (Ho et al., 2008, Kipreos and Pagano, 2000).

In general, FBPs are suggested to be short-lived proteins. Consistent with this, some of the FBPs seem to be tagged with Ub and subjected to degradation by SCF core complex in absence of a substrate protein. This mechanism of autoubiquitination has been proposed to control SCF ubiquitin ligase activity when the substrate protein is not available and allows the cell to promptly adapt to different phases of cell cycle or environmental conditions (Koepp, 2010, Zhou and Howley, 1998, Galan and Peter, 1999).

Recent evidences suggest that some FBPs act independently on SCF complex (Zhou and Howley, 1998). Skp1 associated with certain FBPs can interact with other proteins to set up non SCF complex, which has no ubiquitin ligase activity and thus no degradation occurs. In several cases FBP also functions without association with Skp1 and binds to another proteins. Moreover, as alluded below, one of FBPs was found, surprisingly, to possess intrinsic enzymatic activity.

5. *S. pombe* F-box DNA helicase 1

F-box DNA helicase 1 (Fbh1) was originally identified from a biochemical screening for novel *S. pombe* DNA helicases by Park and colleagues (Park et al., 1997). Firstly, it was named Fdh1, however this name is also used for a formate dehydrogenase gene from *Candida boidinii* (Sakai et al., 1997). Therefore, it was later renamed Fbh1 after its human homolog (Osman et al., 2005). Human homolog of *S. pombe* Fbh1 (spFbh1) was identified by Kim and colleagues and named human F-box DNA helicase 1, hFBH1 (Kim et al., 2002).

As a member of FBP family, Fbh1 contains F-box motif at the N-terminal part (Fig. 6 and 7) that is responsible for binding to the SCF complex. What makes this protein unique among FBPs is the helicase domain at its C-terminal part (Kim et al., 2002). Any of the additional C-terminal motifs of FBPs, such as WD or leucine-rich repeats, have not been described for Fbh1 yet.

```
hFBH1      (141-183)..LPSEVLRHVFAFLPVEDLYWNLSLVCHLWREII---SDPLFIPWKK
mFBH1      (76-118)...LPSEVLRHIFAFLPVEDLYWNLSLVCHLWREII---NDPLFIPWKK
SpFBH1     (14-55)....LPLEIIPLICRFLSVQDIQSFIR-VFPSFQTILDSSNDLFWKK---
hCyclinF   (35-77)....LPEDVLFHILKWLSVEDILAVRA-VHSQLKDLVD-NHASVWACAS-
mSKP2      (100-142)..LPDELLLGIFSCLCLPELLRVSG-VCKRWYRLS--LDESLWQSLDL
yCDC4      (278-320)..LPFEISLKIFNYLQFEDIINSLG-VSQNWNKIIR-KSTSLWKKLL-
F-box consensus       LP EIL  I   L   DL      V      I       LW
```

Figure 6 The F-box amino acids alignment between hFBH1 and other FBPs
Identical and conserved amino acids are demonstrated in *red* and *blue*, respectively (adapted from Kim et al., 2002).

Homologs of the spFbh1 are found in human, monkey, chicken and mouse. However, Fbh1 is absent in organisms such as budding yeast *S. cerevisiae*, fruit fly, frog, fish and plants (Kim et al., 2002, Park et al., 1997, Chiolo et al., 2007).

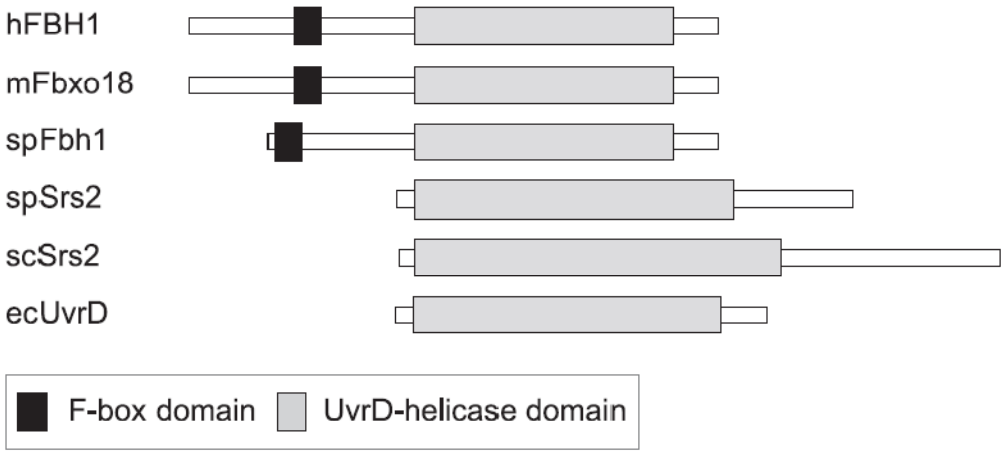


Figure 7 A schematic diagram of several helicases from UvrD family.
Conserved domains are shown for human FBH1, mouse mFbox18, *S. pombe* spFbh1, *S. pombe* and *S. cerevisiae* Srs2, and *E. coli* ecUvrD helicases (adapted from Chiolo et al., 2007).

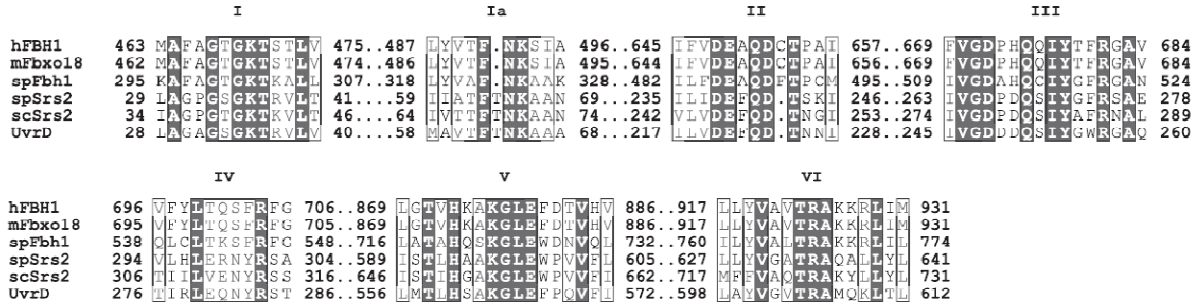
5.1 F-box motif and helicase domain

A number of mutants of *S. pombe* Fbh1 were investigated to determine the functional roles of its F-box motif and the helicase domain. It was shown that both domains play indispensable but distinct roles in Fbh1 function (Sakaguchi et al., 2008).

5.1.1 The helicase domain

Fbh1 belongs to the SF1 family of DNA helicases that is conserved from bacteria to human. They are characterized by seven short conserved helicase motifs, I, Ia, II, III, IV, V, VI (Fig. 8). Well known members of this family are *E. coli* UvrD and yeast Srs2 helicases that are involved in recombination and DNA repair pathways (Matson, 1991).

Studies of the helicase mutants of spFbh1 showed that the helicase domain is involved in controlling of the Rhp51 action (*S .cerevisiae* Rad51). In the process of DSB repair, Rad51 is believed to be responsible for NF formation, the central step leading to the D-loop assembly (Sakaguchi et al., 2008).



<u>Figure 8</u> Amino acid sequence alignments of SF1 helicase domains.
Sequence alignments were performed with CLUSTALW, using the seven helicase motifs found in *E. coli* (ec) UvrD, *S. cerevisiae* (sc) Srs2, *S. pombe* (sp) Srs2, *S. pombe* Fbh1, mouse (m) Fbox18, and hFBH1. Identical and conserved amino acids are indicated within *gray* and *white boxes*, respectively (adapted from Chiolo et al., 2007).

5.1.2 <u>The F-box motif</u>

spFbh1 is predominantly detected in the nucleus where it forms foci at sites of DNA damage (Morishita et al., 2005). Mutation in the F-box motif disables the foci formation, even after exposure to damaging agents. Moreover, these Fbh1 mutants with an additional NLS (nuclear localization signal) enter the nucleus, but fail to form foci in response to DNA damage. This indicates that the F-box motif is crucial for the nuclear localization and DNA damage-induced focus formation of Fbh1 (Sakaguchi et al., 2008).

5.2 The role of spFbh1

S. pombe Srs2 helicase is known to displace Rad51 from NF (Krejci et al., 2003, Veaute et al., 2003) as well as bacterial UvrD helicase dissociates RecA from NF. So it tempts to speculate that spFbh1 as a member of the same protein family could also have such anti-recombinogenic role in disrupting Rhp51 from NF. Experiments with synthetic lethality of mutations in *Srs2* and *spFbh1* genes have revealed that spFbh1 functions in recombination repair on the Rhp51 pathway and plays a role in processing recombination intermediates. In the absence of Fbh1, the Rhp51 NF formation occurs spontaneously and further processing

of the DNA lesion is defective. This leads to toxic accumulation of recombination intermediates (Morishita et al., 2005).

Further analyses showed that spFbh1 prevents Rhp51-dependent recombination in the absence of mediator protein, Rad22 (*S. cerevisiae* Rad52). It is believed that the mediator proteins might make the Rhp51 NF formation more efficient and fast but they may also contribute to increased chance of „inappropriate“ filament assembly. Therefore, such inefficiently formed Rhp51 NF may be disrupted by Fbh1. Altogether, the Rhp51 might be controlled by a balance between Fbh1 and the mediator proteins, such as Rad22 (Osman et al., 2005).

In *S. pombe*, *Fbh1* mutants exhibit increased formation of spontaneous Rhp51 NF and elevated sensitivity to DNA damaging agents. Furthermore, spFbh1 is essential for viability in absence of Rqh1 (human RecQ) and this lethality is suppressed by additional inactivation of Rph51. These data suggests that spFbh1 works in parallel with Srs2 and Rqh1 to prevent the formation of toxic recombination intermediates. Thus, the Fbh1 function is probably to promote processing of recombination intermediates (Kohzaki et al., 2007, Osman et al., 2005, Morishita et al., 2005). Further experiments have provided evidence that spFbh1 functions in opposition to Rad22 to restrain the Rhp51 NF assembly. Thus, Fbh1 acts as a Rhp51 disruptase and the balance between spFbh1 and Rad22 is critical to appropriate Rhp51 NF formation (Lorenz et al., 2009). In light of the similarity to Srs2, Fbh1 could use its helicase/translocase activity to displace the Rad51 NF. However, it is still not certain, how exactly Fbh1 controls the Rhp51 activity.

Interestingly, *Fbh1* mutation in chicken DT40 cells displays a normal phenotype. Hence, *S. pombe* Fbh1, in contrast to its chicken ortholog, has a prominent function in the DNA damage response. This could suggest that either vertebrate cells have a reduced requirement for this helicase or that the function of Fbh1 could be covered by other helicase, such as BLM (Kohzaki et al., 2007). The absence of Fbh1 or BLM may cause an accumulation of some recombination intermediates that are normally resoluted by these helicases. Thus, there is possibility that Fbh1 acts in parallel with BLM helicase to control recombination-mediated DSB repair at replication blocks and to reduce the frequency of crossovers (Kohzaki et al., 2007).

6. FBH1 related genes and other Rad51 disruptases

A critical point at which recombination can be regulated is a removal of the Rad51 NFt (Symington and Heyer, 2006). In *S. cerevisiae*, Srs2 DNA helicase can disrupt Rad51 NF and regulate HR by this way (Krejci et al., 2003). In additional to hFBH1, three other DNA helicases have been implicated in the regulation of HR in mammalian cells, two members of the RecQ family, BLM (Bugreev et al., 2007) and RECQL5 (Hu et al., 2007), and recently discovered RTEL1 (Barber et al., 2008). BLM, RECQL5 and hFBH1 seem to be involved in regulation of NF formation by displacement of Rad51. In contrast, RTEL1 acts as a D-loop disruptase.

6.1 BLM

The BLM DNA helicase is believed to play many roles in HR and DNA repair. The human disorder caused by mutation in this gene is called Bloom´s syndrome. Specific hallmark of this disease is strong hyper-recombination between sister chromatids and homologous chromosomes, and subsequent high degree of genome instability leading to cancer (Sung and Klein, 2006). The BLM helicase is suggested to dissociate the Rad51 from the NF and thus work as anti-recombinase (Wu and Hickson, 2006). Conversely, there are also pro-recombination activities proposed for this enzyme, such as presence during resection of DSB ends (Zhu et al., 2008, Gravel et al., 2008) or promotion of replication fork regression (Ralf et al., 2006). Taken together, BLM displays a broad spectrum of activities that either negatively or positively regulate HR events (Bugreev et al., 2007).

6.2 RECQ5

According to the current model, RECQ5 disrupts Rad51 NF formation through its interaction with Rad51. Although RECQ5 has not been associated with a human disease, it is suggested to be an important tumour suppressor by preventing unscheduled HR events via its anti-recombinase activity (Schwendener et al., 2010, Hu et al., 2007).

6.3 RTEL1

Recent studies have revealed that RTEL1, regulator of telomere elongation helicase 1, suppress HR through disassembling D-loop-recombination intermediates during DNA repair. Moreover, RTEL1 is crucial for regulation of telomere length in mice, and its loss has been associated with shortened telomere length, chromosome breaks, and translocations. Defects in its function are connected with glioma predisposition (Barber et al., 2008, Ding et al., 2004).

6.4 Srs2

Genetic analysis in yeast model has revealed an important enzyme in HR process, Srs2 helicase. These studies have shown that Srs2 plays a crucial role in the maintenance of genome stability by regulating DNA recombination. The Srs2 protein was identified as a 3´→5´ helicase (Rong and Klein, 1993) that is structurally and functionally related to bacterial UvrD helicase family (Veaute et al., 2005). Srs2 acts as an anti-recombinase by displacement of Rad51 from ssDNA. Therefore, Srs2 prevents spontaneous and unscheduled HR events in yeast (Veaute et al., 2003, Krejci et al., 2003).

Until recently, no ortholog of *S. cerevisiae* Srs2 has been described in human. However, further experiments provided evidences that Fbh1 has such a function related to those of Srs2. Both enzymes share 3´→5´ helicase activity and the amino acids identity of their helicase domains (Fig. 8) is about 20% (Chiolo et al., 2007).

7. Role of SCF complex containing spFbh1 as a FBP

Until recently, no possible target of the SCF^{Fbh1} (spFbh1 in complex SCF) complex has been identified. But in recent past, Lawrence et al. detected one potential substrate in *S. pombe*. It is a transcription factor Atf1 which plays a vital role in stress-induced response in *S. pombe*. Upon exposure to stress, Atf1 is phosphorylated by mitogen-activated protein kinase (MAPK) Sty1 which causes its stabilization. The resulting increase of Atf1 leads to stress-activated expression of its target genes (Lawrence et al., 2009, Lawrence et al., 2007, Wilkinson et al., 1996, Chen et al., 2003).

Fbh1 physically interacts with Atf1 and this interaction occurs only under basal conditions. By other words (Fig. 9), in absence of stress, Atf1 is basally phosphorylated by Sty1 and thus SCF^{Fbh1} complex is able to bind Atf1 as a substrate and attach to Ub. In contrast, upon the stress exposure, Atf1 is hyper-phosphorylated and no recognition and subsequent degradation occurs (Lawrence et al., 2009).

Since most FBP-substrate interactions described to date are mediated positively by phosphorylation, this kind of interaction is very untypical among FBPs. This is the first *in vivo* evidence demonstrating that substrate phosphorylation can negatively regulate its interaction with FBP.

Atf1 is the first discovered example of a substrate for any SCF^{Fbh1} complex in any organism. In common with other FBPs, it seems likely that Fbh1 will target multiple proteins for ubiquitination. Due to its detection at the sites of DSBs (Morishita et al., 2005), potential substrates could be proteins involved in the HR pathway of DNA repair. However, no target of the SCF complex with human FBH1 has been identified yet. Since the mammalian homolog of Atf1, ATF-2, exists, it is tempting to speculate that it might be target of hFBH1 for Ub-mediated degradation.

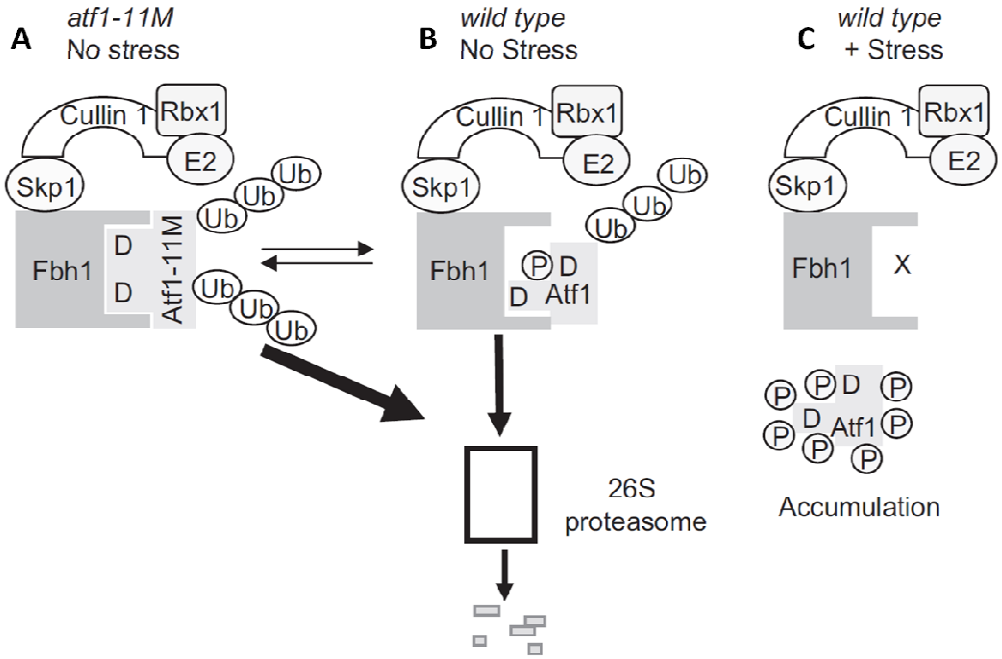


Figure 9 Model for regulation of Atf1 level in different stress conditions.
(A) The mutant Atf1 lacking all eleven phosphorylation-sites cannot be phosphorylated in presence of stress and thus Atf1 is degraded in proteasome. (B) In absence of stress, Atf1 is not hyper-phosphorylated and Fbh1 recognizes it for subsequent degradation. (C) In response to stress exposure, Atf1 is hyper-phosphorylated and stabilized (adapted from Lawrence et al., 2009).

8. Human F-box DNA helicase 1

As a result of searching for a human homolog of *S. pombe* DNA helicase I, Kim et al. identified in 2002 a novel DNA helicase. Due to its additional F-box motif, they have named this enzyme hFBH1, human F-box DNA helicase 1. hFBH1 shares 28% of identity and 44% of similarity with its *S. pombe* ortholog. The sequence analysis showed that the F-box motif is situated at the N-terminal part, as in other FBPs (Fig. 10). Conversely, helicase domain is located at the C-terminal part (Kim et al., 2002).

Figure 10 A schematic outline
The F-box motif is shown as *hatched box* and the helicase motifs as *solid boxes* (adapted from Kim et al., 2002).

Biochemical properties of hFBH1, meaning helicase and ATPase activities, are similar to those of spFbh1, although there are at least two differences between these two enzymes. Firstly, hFBH1 is neither stimulated nor inhibited by hRPA, whereas SpFbh1 is stimulated by SpRPA. Second, hFBH1 can utilize ADP and nonhydrolyzable ATP analog AppNp (5′-Adenylylimidodiphosphate), although the efficiencies are relatively poor (25 and 45%, respectively) in respect to ATP, whereas SpFBH1 cannot use ADP or AppNp as an energy source (Kim et al., 2004).

8.1 Biochemical properties of the SCF complex containing hFBH1 as a FBP

To find out if hFBH1 binds to Skp1 forming functional SCF complex, SCF^{hFBH1} complex (hFBH1 in SCF complex) was established *in vivo* and *in vitro*, and the characterization of enzymatic activities was performed.

The observation showed, that in presence of monomeric Ub and E1 and E2 enzymes, the SCF^{hFBH1} complex exhibits not only supposed E3 Ub ligase activity, but also DNA helicase and DNA-dependent ATPase activity. Both hFBH1 and SCF^{hFBH1} act in a distributive manner. Hence, the enzyme may be involved in a DNA transaction that requires unwinding of short stretches of DNA such as DNA repair or recombination. The poly-Ub chain formation by the SCF^{hFBH1} complex was not changed in presence of ssDNA or dsDNA. This could indicate that the multiple activities present in this complex act independently of each other and thus, the SCF^{hFB1} complex can catalyze the ubiquitination reaction as well as DNA unwinding (Kim et al., 2004).

Since the efficiency of the SCF^{hFBH1} complex formation *in vitro* is very low (Kim et al., 2004) there is a possibility that the assembly of the SCF^{hFBH1} complex *in vivo* may be further regulated, for example by chaperones.

8.2 hFBH1 versus SCF^{hFBH1}

As mentioned above, the SCF^{hFBH1} complex retains all three activities: DNA helicase, ATPase and E3 Ub ligase activity, while hFBH1 alone exhibits only first two of them. Consistent with this, the enzymatic properties of the SCF^{hFBH1} complex were compared to those of the hFbh1 alone. The results demonstrate that helicase and ATPase activities of hFBH1 bound in the complex are indistinguishable from those of free hFBH1. DNA-unwinding activities of both enzymes are in 3´→5´ direction and dependent on both ATP and Mg^{2+}. The rate of ATP hydrolysis by both enzymes are nearly the same. Another similarity is that neither hFBH1 nor SCF^{hFBH1} complex showed any preference for a fork-like structure (Kim et al., 2004).

Taken together, hFBH1 alone or in complex with SCF retains similar helicase and ATPase activities. However, at present it is still not certain whether cells contain free hFBH1, a mixture of hFBH1 and SCF^{hFBH1} complex or the SCF^{hFBH1} complex only. Interestingly, purified hFBH1 deleted in the F-box motif exhibited significantly weaker helicase and ATPase activities. It is believed that this deletion could somehow damage the protein integrity required for the catalytic function (Kim et al., 2004).

8.3 The role of hFBH1

Fbh1 was found in *H. sapiens* and *S. pombe,* but not in *S. cerevisiae*, whereas Srs2 is conserved in *S. cerevisiae* and *S. pombe,* but not in mammals. In 2007 Chiolo at al. revealed evidences that hFBH1 may be a human ortholog of *S. cerevisiae* Srs2. This is supported by the finding that hFBH1 suppresses specific recombination defects of *S. cerevisiae* Srs2 mutants and that the hFBH1 F-box motif is necessary for its function(s) in substituting for Srs2. Moreover, it appears to be possible that hFBH1 in the SCF complex may control its own turnover through an autoubiquitination mechanism (Chiolo et al., 2007).

8.4 Pro- and Anti-recombinase activities

hFBH1 alone or bound in SCF complex is able to remove Rad51 from ssDNA suggesting that hFBH1 functions as an anti-recombinogenic factor in human cells through its ability to dismantle the Rad51 NF (Fugger et al., 2009). On the other hand, hFBH1 appears to possess additional pro-recombinase activity, by facilitating ssDNA production at sites of stalled replication fork or DSB and thereby promotes the loading of RPA (Fugger et al., 2009).

In summary, these data support the idea that hFBH1 functions as a regulator of HR repair in human cells. According to the proposed model (Fig. 11), hFBH1 is, recruited to replication block to help to facilitate ssDNA generation for consequent HR repair. Up this stage, hFBH1 can function to promote HR initiation. However, in later stages, hFBH1 can suppress HR events through its ability to dissociate the Rad51 NF. Thus, by possessing both pro- and anti-recombinogenic potential, hFBH1 is able to suppress or promote HR at different stages (Fugger et al., 2009).

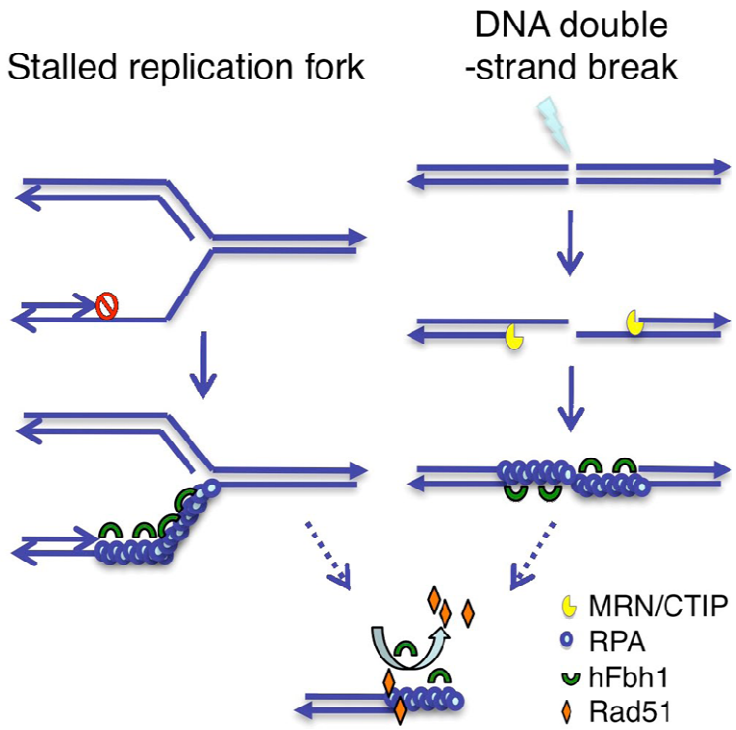


Figure 11 A hypothetical model of the hFBH1 pro- and anti-recombinase role.
hFBH1 might mediate initiation of HR repair at sites of stalled replication fork (left) and promotes ssDNA production at sites of DSB (right). At later stages, hFBH1 may use its anti-recombinase activity to disrupt the Rad51 from the NF (adapted from Fugger et al., 2009).

9. Conclusion

In the process of HR, human FBH1 appears to play both pro- and anti-recombinogenic roles. Thus, FBH1 may help to mediate HR-based DNA repair progression as well as supress the deleterious effect of excessive or unwanted HR events. Balance between these two pathways is essential for viability of all organisms. However, many important questions concerning the mechanism and regulation of HR remain still unanswered. FBH1 is proposed to have functional similarity to BLM helicase whose mutations are connected with susceptibility to cancer. It would be therefore interesting to determine whether a defect in FBH1 helicase function is also associated with cancer predisposition or even genetic disease similar to Bloom's and Werner's syndromes.

Feature that makes FBH1 as a F-box protein unique among all known FBPs, is presence of the helicase domain. In complex with SCF ubiquitin ligase, FBH1 may target specific proteins for proteasom degradation. However, the identification of the physiological substrates of the SCF^{FBH1} the complex is essential and still unresolved issue.

10. References

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