A multiprotein complex in DNA damage response network of Fanconi anemia, Bloom syndrome and Breast cancer

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A Multi-protein Complex Connects Two Genomic Instability Diseases: Bloom Syndrome and Fanconi Anemia

**Bloom Syndrome**

- Genomic Instability:  
  -sister-chromatid exchange  

- Cancer predisposition  

- Mutation in BLM, a RecQ DNA Helicase  

- BLM participates in:  
  - HR-dependent DSB repair  
  - Recovery of stalled replication forks  

- BLM works with Topo IIIa and RMI to Suppress crossover recombination  

Courtesy of Dr. Ian Hickson
A Multi-protein Complex Connects Two Genomic Instability Diseases: Bloom Syndrome and Fanconi Anemia

Meeteei et al. MCB 2003
A Multi-protein Complex Connects Two Genomic Instability Diseases: Bloom Syndrome and Fanconi Anemia

Meetei et al. MCB 2003
BRAFT-a Multisubunit Machine that Maintains Genome Stability and is defective in Fanconi anemia and Bloom syndrome

**BRAFT Super-complex**

- **Fanconi Anemia Core Complex:**
  - FANCA
  - FANCC
  - FANCE
  - FANCF
  - FANCG
  - FANCM
  - FANCB
  - FANCL
  - FANCM
  - FAAP24
  - MHF1
  - MHF2
  - FAAP100

- **Bloom Syndrome Complex:**
  - BLM
  - Topo IIIα
  - BLAP75
  - RMI1
  - RMI2
  - RPA70
  - RPA34
  - RPA14
  - BLAP250

**12 polypeptides:**
- Ubiquitin ligase
- Binds and translocates Branch point (4 WJ, fork) dsDNA translocase
- Targets FANCM to ssDNA
- Targets FANCM to dsDNA

**7 polypeptides:**
- Helicase (HJ, fork, D-loop), fork regression, dHJ dissolution
- Topoisomerase, dHJ dissolution
- Stimulates dHJ dissolution
- Mediates protein-protein interactions
- Binds ssDNA
- Stimulates BLM helicase
- Stimulates dHJ dissolution
Fanconi Anemia: A multi-Gene Disease Model to Study Repair of Crosslinked DNA Damage and Function of BRCA Proteins

- 13 complementation groups and genes
  - FANC-A,B,C,D1,D2,E,F,G,I, J, L,M,N

- BRCA1 and BRCA2 connection
  - BRCA2=FANCD1
  - PALB2=FANCN
  - BACH1/BRIP1=FANCJ

- Cellular Hypersensitivity to DNA-crosslinking drugs
  - A model for studying repair of crosslinked DNA damage

- Genomic Instability:
  - Chromosomal breaks and interchanges

- Cancer predisposition:
  - Myeloblastic leukemia
  - Squamous cell carcinomas

- Aging:
  - Bone Marrow Failure
  - Skin abnormalities—look older

Courtesy of Dr. Johan de Winter
Group I FA proteins constitute the FA core complex that has a E3 ubiquitin ligase and a DNA remodeling enzyme.
Group II FA proteins are monoubiquitinated by the FA core complex; Group III may act downstream of the FA pathway.
Fanconi anemia core complex participates in monoubiquitination of FA ID complex through FANCL and DNA repair via FANCM
Vertebrate FANCM interacts with multiple repair and signaling partners to integrate signals from several pathways.
FANCM-MHF may represent a minimal version of the FA core complex conserved in all eukaryotes.

**Yeast and all eukaryotes**

- Fanconi Anemia Core Complex
- BLM/Sgs1 Complex

- FANCM/MPH1/FML1
  - MHF1
  - MHF2

- BLM
  - SGS1
  - Topo IIIα
  - BLAP75
  - RMI1

??
MHF1 and MHF2 Are New Components of the FA Core Complex

MHF1/2: FANCM-associated Histone Fold protein 1 and 2.

(Yan et al. Mol Cell in press)
MHF1 and MHF2 Are New Components of the FA Core Complex

HeLa Nuclear Extract → Superose 6 fractionation → MHF IP

MHF IP:
- FANCM
- BLM
- FANCA
- TOPIIIα
- FAAP100
- BLAP75
- RPA70
- FANCG
- FANCC&E
- FANCF
- RPA32
- FAAP24
- MHF1
- MHF2

FANCM-associated Proteins:
- A
- BLM
- TopIIIα
- C
- RMI1
- E
- RMI2
- F
- RPA
- B
- 100

FA-core complex

BLM complex
MHF1 and MHF2 can also form a complex without FANCM
MHF is identical to the CENP-S complex and binds dsDNA.

Histone-fold

MHF1/CENP-S

\[
\begin{array}{c}
\alpha_1 \\
\alpha_2 \\
\alpha_3 \\
\end{array}
\]

138 aa

MHF2/CENP-X

\[
\begin{array}{c}
\alpha_1 \\
\alpha_2 \\
\alpha_3 \\
\end{array}
\]

81 aa

MHF complex

(CENP-S complex)

Amano et al. JCB 2009
MHF provides a complementary DNA binding interface for FANCM-FAAP24 to recognize stalled forks
MHF recruits FANCM to dsDNA
FANCM and MHF binds DNA synergistically.
Most FANCM-MHF is present in a complex that is largely free of other FA core complex components.

[Diagram showing HeLa Nuclear Extract flowing through an IP step to result in MHF1 IP, with FANCM, MHF1, and MHF2 bands on the gel.]
A large percentage of FANCM-MHF is present in a complex independent of the FA core complex.
MHF and FANCM Form a DNA remodeling Complex that Protects Genome Stability from Human to Yeast

<30% of FANCM-MHF

Vertebrates Only

Ub-FANCD2-FANCI

DNA repair

Most FANCM-MHF

All eukaryotes

DNA repair
Reconstituted FANCM-MHF has Higher Replication Fork Reversal Activity than FANCM alone

FANCM can catalyze fork regression

( Dr. Angelos Constantinou Lab )
MHF Is Required for stability and chromatin association of FANCM
MHF Is Required for Normal Monoubiquitination of FANCD2-FANCI and cellular resistance to MMC in human HeLa cells

<table>
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 MMC Cisplatin

Survival (%) vs. MMC (ng/ml)

siControl
siMHF2
FANCM and MHF Are Rapidly Recruited to DNA Interstrand Crosslinks induced by Laser-activated Psoralen in S-phase cells

**S-phase HeLa cells + laser-activated psoralen**

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<th>min</th>
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<th>30</th>
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- FANCM-MHF is recruited to replication forks blocked by ICLs

Mike Seidman’s group
FANCM Recruitment to replication forks stalled by laser-induced ICLs is disrupted in MHF-depleted cells
eCHIP—A new method to directly detect proteins recruited to DNA interstrand crosslinks in cells

(Lei Li’s group; Shen et al. Mol. Cell 2009)
MHF1 is recruited to DNA interstrand crosslinks; and its recruitment is stimulated by replication.
MHF and FANCM Work in the Same Pathway to Promote FANCD2 Monoubiquitination and to Suppress SCE in chicken DT40 cells

DT40 cells:  
- WT
- MHF1\(-/-\)
- FANCM\(-/-\)
- MHF1\(-/-\) FANCM\(-/-\)

L/S:  
- WT: 1.54
- MHF1\(-/-\): 0.34
- FANCM\(-/-\): 0.16
- MHF1\(-/-\) FANCM\(-/-\): 0.16

SCEs per Metaphase

WT  
SCE: 2.5

MHF1\(-/-\)  
SCE: 9.7

FANCM\(-/-\)  
SCE: 18.3

FANCM\(-/-\) MHF1\(-/-\)  
SCE: 17.9
MHF complex containing mutant A lacks DNA binding activity

mut AB

mut A  mut B

MHF1

Histone-fold

MHF complex

WT  Mut A

MHF1

His-MHF2

MHF
MHF containing mutant A lacks DNA binding activity and fails to recruit FANCM to fork DNA.
The MHF1 mutant A has normal association with MHF2 and FANCM, whereas mutant B and AB have reduced association.
MHF1 Requires its DNA binding activity to Promote normal FANCD2 Monoubiquitination
MHF1 Requires its DNA binding activity to efficiently Suppress Sister-Chromatid Exchange
Yeast MHF and FANCM Work in the Same Pathway to resist MMS-induced cell killing

*S. cerevisiae*

Cell number

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*(srs2Δ strain)*

*(Dr. Kyungjae Myung Lab)*
The FANCM ortholog in *S. Pombe* is required for HR-dependent gene conversion at stalled replication forks.
FANCM and MHF work in the same pathway to promote gene conversion at stalled replication forks in *S. Pombe*.
A large portion of FANCM-MHF is present independent of the FA core complex; it may exist and protect genome stability in all eukaryotes.
Summary

• FANCM-MHF is a conserved DNA remodeling complex required for:
  – Resistance to replication stress
  – Suppression of crossover recombination
  – Activation of FA-BRCA signaling pathway

• MHF is an essential partner of FANCM:
  – Provides a unique DNA binding surface
  – Promotes DNA binding and remodeling activity of FANCM in vitro
  – Required for stability, chromatin-association, and recruitment of FANCM to stalled forks in vivo

Yan et al. Mol. Cell in press; Meetei and Sung’s group, same issue
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