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The archaeal Xpf/Mus81/FANCM homolog Hef and the Holliday junction resolvase Hjc define alternative pathways that are essential for cell viability in *Haloferax volcanii*

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ABSTRACT

The XPF/MUS81 family of endonucleases is found in eukaryotes and archaea, in the former they play a critical role in DNA repair and replication fork restart. Hef is a XPF/MUS81 family member found in Euryarchaea and is related to the Fanconi anemia protein FANCM. We have studied the role of Hef in the euryarchaeon *Haloferax volcanii*. Unlike Xpf in eukaryotes, Hef is not involved in nucleotide excision repair; instead, this function is encoded by the *uvrABC* genes. Similarly, deletion of *hef* confers only moderate sensitivity to DNA crosslinking agents, whereas mutation of FANCM in leads to hypersensitivity in eukaryotes. However, Hef is essential for cell viability when the Holliday junction resolvase Hjc is absent, and both the helicase and nuclease activities of Hef are indispensable. By contrast, single mutants of *hjc* and *hef* display no significant defects in growth or homologous recombination. This suggests that Hef and Hjc are redundant for the resolution of recombination intermediates, and that Hef is the functional homolog of eukaryotic Mus81. Furthermore, deletion of *hef* in a recombination-deficient Δ*radA* background is highly deleterious but deletion of *hjc* has no effect. Therefore, Hjc acts exclusively in homologous recombination whereas Hef, in addition to its role in resolving recombination intermediates, can act in a pathway that avoids the use of homologous recombination. We propose that Hef and Hjc provide alternative means to restart stalled DNA replication forks.

1. Introduction

DNA damage is unavoidable, it occurs during normal metabolic processes and continually threatens genome integrity. Unrepaired lesions can stall DNA replication forks, which must be restarted to ensure cell viability. Links between genome rearrangements and cellular defects that impair DNA replication have been described in bacteria [1], yeast [2] and higher eukaryotes [3]. Investigating these links in *Escherichia coli* has shown that homologous recombination (HR) is used to rescue arrested replication forks [4,5]. HR promotes strand exchange between DNA sequences and is catalyzed by proteins of the RecA family. Holliday junctions (HJs) are four-way branched recombination intermediates that are formed by strand exchange, they must be resolved to allow the formation of two recombinant duplexes. In *E. coli*, the RuvAB complex binds to HJs and promotes branch migration, the RuvC endonuclease is then recruited to resolve the junction [6,7]. HJ resolvases are found in bacteria, archaea and eukarya, although bacterial RuvC, eukaryal GEN1 and archaeal Hjc are not related [8,9]. Hjc was first identified in the euryarchaeon *Pyrococcus furiosus*, it binds specifically to HJs and cleaves two opposing strands symmetrically to generate two recombinant duplexes, similar to *E. coli* RuvC [10]. Hjc is conserved throughout archaea and biochemical studies of the *Sulfolobus solfataricus* homolog have shown that it interacts with PCNA to activate its resolvase activity [11,12]. The structure of Hjc is distinct from other resolvases and instead resembles the architecture of type II restriction endonucleases [13,14].

HJ processing can also be performed by members of the XPF/MUS81 family of endonucleases, which are found in eukaryotes and archaea. Eukaryotes encode several members of this family, which act on branched DNA structures and are involved in DNA repair, recombination and replication restart [15]. Defects in XPF/MUS81 proteins are associated with diseases such as xeroderma pigmentosum (XPF–ERCC1) and Fanconi anemia (FANCM), while Mus81 plays an important role in processing of stalled DNA replication forks by HR [16]. By contrast, only one member of XPF/MUS81 family is found in archaea. Euryarchaeota have a structure-specific endonuclease called Hef (*h*elicase-associated

Abbreviations: HJ, Holliday junction; HR, homologous recombination; NER, nucleotide excision repair; MMC, mitomycin C; WT, wild-type; SE, standard error; NCO, noncrossover; CO, crossover.

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*e*ndonuclease *f*ork-structure DNA) [17], it is a homolog of human FANCM that is involved in the Fanconi anemia pathway of DNA repair [18,19]. Hef comprises two distinct domains: an N-terminal domain of the DEAH helicase family [20] and a C-terminal domain of the XPF endonuclease family [21,22]. Hef acts on nicked, flapped and forked DNA, and dimerisation of the nuclease domain is needed for efficient activity [21,22]. Collaboration of the nuclease and helicase domains allows Hef to convert a HJ to a forked structure and introduce an incision near the branch point [23]. Based on these results, it has been proposed that Hef acts to restore stalled DNA replication forks. The XPF homolog found in Crenarchaeota contains only the C-terminal nuclease domain, it is a 3′-flap endonuclease similar to the eukaryotic XPF–ERCC1 complex that is involved in nucleotide excision repair (NER). Crenarchaeal XFP requires an interaction with PCNA for nuclease activity on flapped and forked DNA [24,25], but also targets D-loops and nicked HJs. It has been suggested that crenarchaeal XPF acts on stalled replication forks and/or recombination intermediates, similar to eukaryotic Mus81 [26].

To investigate the role of Hef *in vivo*, we have used the genetically tractable halophile *Haloferax volcanii*, a member of the Euryarchaeota [27]. We show that Hef is essential for cell viability in the absence of the HJ resolvase Hjc, suggesting that they are redundant for the resolution of recombination intermediates that arise from stalled DNA replication forks. Hjc appears to act in the same pathway as RadA, whereas Hef may have an additional role that is independent of HR.

2. Materials and methods

Unless stated otherwise, chemicals were from Sigma and restriction enzymes from New England Biolabs. Standard molecular techniques were used.

2.1. Strains and plasmids

H. volcanii strains (Table 1) were grown at 45 °C on complete (Hv-YPC), casamino acids (Hv-Ca) or minimal (Hv-Min) agar, or in Hv-YPC or Hv-Ca broth, as described previously [28,29]. Isolation of genomic and plasmid DNA, and transformation of *H. volcanii* were carried out as described previously [28,30].

2.2. Construction of mutant strains

Deletion mutants were constructed as described previously [28,31]. Plasmids for gene deletion and complementation are shown in Table 2 and were generated by PCR using the primers in Table 3, the template DNA used was isolated from genomic DNA

Table 1
Haloferax volcanii strains used.

Strain	Relevant genotype	Source or reference
H26	Δ*pyrE2*	[28]
H97	Δ*pyrE2* Δ*radA*	H26 pTA80
H178	Δ*pyrE2* Δ*hjc*	H26 pTA225
H195	Δ*pyrE2* Δ*trpA* Δ*hdrB*	[29]
H282	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hjc*	H195 pTA225
H358	Δ*pyrE2* Δ*hef*	H26 pTA370
H364	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef*	H195 pTA370
H387	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*radA*	[32]
H509	Δ*pyrE2* Δ*uvrA*	H26 pTA595
H514	Δ*pyrE2* Δ*uvrD*	H26 pTA597
H515	Δ*pyrE2* Δ*hef* Δ*uvrD*	H358 pTA597
H938	Δ*pyrE2* Δ*fen1*	H26 pCN6
H939	Δ*pyrE2* Δ*hef* Δ*fen1*	H358 pCN6
H1012	Δ*pyrE2* Δ*hef hjc*$^+$::[Δ*hjc pyrE2*$^+$]	H358, pTA225 pop-in[a]
H1049	Δ*pyrE2* Δ*hjc* Δ*trpA*	H178 pTA95
H1051	Δ*pyrE2* Δ*hef* Δ*trpA*	H358 pTA95
H1052	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hjc radA*$^+$::[Δ*radA::trpA*$^+$ *pyrE2*$^+$]	H282, pTA324 pop-in
H1054	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef radA*$^+$::[Δ*radA::trpA*$^+$ *pyrE2*$^+$]	H364, pTA324 pop-in
H1064	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef hjc*$^+$::[Δ*hjc::trpA*$^+$ *pyrE2*$^+$]	H364, pTA1062 pop-in
H1069	Δ*pyrE2* Δ*hjc* Δ*trpA hef*$^+$::[Δ*hef::trpA*$^+$ *pyrE2*$^+$]	H1049, pTA1064 pop-in[a]
H1070	Δ*pyrE2* Δ*hef* Δ*trpA hjc*$^+$::[Δ*hjc::trpA*$^+$ *pyrE2*$^+$]	H1051, pTA1062 pop-in[a]
H1071	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hjc radA*$^+$::[Δ*radA::trpA*$^+$ *pyrE2*$^+$] p[*radA*$^+$ *pyrE2*$^+$ *hdrB*$^+$]	H1052 transformed with pTA411
H1073	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef radA*$^+$::[Δ*radA::trpA*$^+$ *pyrE2*$^+$] p[*radA*$^+$ *pyrE2*$^+$ *hdrB*$^+$]	H1054 transformed with pTA411
H1084	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef hjc*$^+$::[Δ*hjc::trpA*$^+$ *pyrE2*$^+$] p[*hef*$^+$ *hdrB*$^+$]	H1064 transformed with pTA1088
H1104	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef* Δ*hjc::trpA*$^+$ p[*hef*$^+$ *hdrB*$^+$]	H1084, pTA1062 pop-out[b]
H1109	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef* p[*hdrB*$^+$]	H364 transformed with pTA356
H1110	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef* p[*hef*$^+$ *hdrB*$^+$]	H364 transformed with pTA1088
H1115	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hjc* Δ*radA::trpA*$^+$	H1071, pTA324 pop-out, pTA411 lost
H1120	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef*	H1109, pTA1088 lost
H1145	Δ*pyrE2 hef*-K48A	H358 pTA1110
H1146	Δ*pyrE2 hef*-D679A	H358 pTA1112
H1158	Δ*pyrE2 hef*-K48A-D679A	H358 pTA1132
H1164	Δ*pyrE2 hef*-K48A *hjc*$^+$::[Δ*hjc::trpA*$^+$ *pyrE2*$^+$]	H1145, pTA1062 pop-in[a]
H1165	Δ*pyrE2 hef*-D679A *hjc*$^+$::[Δ*hjc::trpA*$^+$ *pyrE2*$^+$]	H1146, pTA1062 pop-in[a]
H1179	Δ*pyrE2 hef*-K48A-D679A *hjc*$^+$::[Δ*hjc::trpA*$^+$ *pyrE2*$^+$]	H1158, pTA1062 pop-in[a]
H1181	Δ*pyrE2* Δ*uvrB*	H26 pTA1139
H1182	Δ*pyrE2* Δ*hef* Δ*uvrB*	H358 pTA1139
H1187	Δ*pyrE2* Δ*uvrC*	H26 pTA1158
H1188	Δ*pyrE2* Δ*hef* Δ*uvrC*	H358 pTA1158
H1240	Δ*pyrE2* Δ*trpA* Δ*hdrB* Δ*hef* Δ*radA::trpA*$^+$	H1073, pTA324 pop-out, pTA411 lost

[a] Pop-in strains from which pop-out strains could not be obtained.
[b] Complementing plasmid essential for viability.