10

Archaeal DNA Repair Nucleases

Roxanne Lestini[2], Christophe Creze[1], Didier Flament[1],
Hannu Myllykallio[2] and Ghislaine Henneke[1]
[1]*Ifremer, Centre National de la Recherche Scientifique, Université de Bretagne Occidentale, Laboratoire de Microbiologie des Environnements Extrêmes*
[2]*Centre National de la Recherche Scientifique, Laboratoire d'Optique et Biosciences*
France

1. Introduction

DNA is the carrier of genetic information, but is constantly assaulted by endogenous and exogenous genotoxic attacks in all living organisms. If left unrepaired damaged or structurally altered DNA can impede pathways of DNA metabolism and maintenance of genomic stability and lead to cell death or uncontrolled proliferation. Archaea comprise diverse microorganisms that can thrive in harsh environments like hydrothermal vents and acidic hot springs. They can live without sunlight or organic carbon as food, and instead survive on sulfur hydrogen, and other materials that most bacteria and eukaryotes can not metabolize. Considering the extreme environmental niches inhabited by archaeal species, DNA lesions could be massively induced by exposure to hazardous environmental factors, (e.g., ultraviolet, X- and γ-rays, elevated temperatures and endogenous mutagens, e.g., reactive oxygen/nitrogen species, alkylating agents and toxic metals), and very high rates of potentially mutagenic DNA lesions (deamination, depurination, oxidation by hydrolytic mechanisms, alkylations and subsequent strand breakage) are expected to arise. However, and interestingly, it was demonstrated that the hyperthermophilic crenarchaeota *Sulfolobus Acidocaldarius* exhibited a modest rate of spontaneous mutations nearly close to that of the mesophilic bacteria, *Escherichia Coli* (*E. coli*) (Grogan et al., 2001). Similarly, the euryarchaeota *Pyrococcus abyssi* can survive high doses of ionizing gamma irradiation (Jolivet et al., 2003b) and abasic sites formation in the hyperthermophilic chromosome was found to occur at a rate moderately higher than in *E. coli* (Palud et al., 2008). Thus, archaeal organisms seem to evolve efficient strategies for repairing DNA damage and thus avoiding mutations.

Like bacteria and eukaryotes, archaeal repair mechanisms seem to include nucleolytic processing of DNA. Consequently, this article sets out to review archaeal DNA nucleases based on current knowledge of sequence, structure and mechanism. We have focused on recent work on several DNA repair nucleases, with a detailed description of substrate preference and cleavage specificity of these archaeal enzymes. Crystal structures, when available, are discussed in the context of biochemical data to outline mechanistic features, such as enzymatic DNA cleavage, DNA binding, and sometimes, although not always, functions. This review stresses the molecular mechanisms which have been conserved throughout evolution with reference to eukaryotic DNA nucleases and, in some cases, to

bacterial counterparts. On the other hand, DNA nucleases which appears unique to archaea are emphasized with the aim to describe novel aspects of repair mechanisms.

2. Type 2 Ribonuclease H, a structure-specific DNA repair nuclease

2.1 RNase HII/2: a ubiquitous enzyme

Ribonucleases H (RNases H) catalyse the cleavage of the RNA portion of RNA/DNA hybrid molecules that are ubiquitously present in cells (Stein and Hausen, 1969). RNases H are classified into two major families, type 1 and type 2, based on amino acid sequence identities and distinct biochemical properties. Genes encoding RNases H are found in viruses, archaea, bacteria and eukaryotes and, at least, one RNase H is present within a single cell. Furthermore, type 2 RNases H are more widely distributed than type 1 RNases H in prokaryotic and eukaryotic genomes (Ohtani et al., 1999b). Biological roles, including DNA replication, DNA repair, and transcription have been assigned for these RNases H, as recently reviewed (Cerritelli and Crouch, 2009; Tadokoro and Kanaya, 2009). Here, we report recent progress in the structural and functional characterization of type 2 ribonucleases H (RNase HII/2) presumed to be involved in an excision repair system for the removal of ribose residues with a particular accent on archaeal enzymes.

2.2 RNases HII/2 orthologs

2.2.1 Distribution and amino acid sequence identities

In the process of analysing the 95 sequenced archaeal genomes, type 2 RNases H (RNases HII) have been detected among the five archaeal phyla: Euryarchaeota, Crenarchaeota, Korarchaeota, Thaumarchaeota and Nanoarchaeota. In contrast to the type 1 enzymes, archaeal RNases HII appear universally distributed, and most organisms only contain RNase HII, with the exception of few archaea, such as *Sulfolobus tokodaii, Haloferax volcanii*, *Halobacterium* sp. NRC-1 and *Pyrobaculum aerophilum* which possess both types. Despite the multiplicity of *rnh* genes within a single cell, the ubiquitous occurrence of RNase HII suggests that type 2 may provide the major RNase H activity in archaea cells, as recently proposed for eukaryotes (Bubeck et al., 2011; Frank et al., 1998b).

Sequence comparison within archaeal RNases HII has revealed a high degree of amino acid sequence identity (Chai et al., 2001; Haruki et al., 1998; Le Laz et al. 2010; Muroya et al., 2001). For instance, *Pyrococcus abyssi* (*Pab*RNase HII) shows amino acid sequence identities of 64% to *Thermococcus kodakaraensis* RNase HII (*Tko*RNase HII), 49% to *Archaeoglobus fulgidus* RNase HII (*Afu*RNase HII) and 40% to *Methanocaldococcus jannaschii* RNase HII (*Mja*RNase HII). Likewise, archaeal RNases HII have highly sequence similar orthologs in bacteria and eukaryotes. *Afu*RNase HII shows amino acid sequence identities of 31% to *Thermotoga maritima* RNase HII (*Tma*RNase HII) and 30% to the catalytical subunit of *Mus musculus* RNase H2A (*Mmu*RNase H2A), the latter composed of three distinct subunits (Shaban et al., 2010).

2.2.2 Biochemical characterization

The apparent sequence conservation among RNases HII/2 orthologs would indicate that these enzymes have biochemical properties in common. Interestingly, archaeal RNases HII display activity at alkaline pH (Chai et al., 2001; Haruki et al., 1998; Le Laz et al., 2010), and this property seems to be a hallmark of type 2 RNases H (Chon et al., 2009; Rohman et al., 2008; Rychlik et al., 2010). As first reported by Haruki, *et al*., the archaeal *Tko*RNase HII

activity was not salt-dependent, but was greatly attenuated by salt-concentrations higher than 100 mM (Haruki et al., 1998). More recent biochemical characterizations, however, seem to indicate that most of type 2 RNase H activities are salt-dependent (Chon et al., 2009; Ohtani et al., 2000; Rohman et al., 2008; Rychlik et al., 2010). All archaeal RNases HII studied to date have been shown to be strictly metal-dependent nucleases. *Pab*RNase HII prefers Mg^{2+} to Mn^{2+} or Co^{2+} for activity, *Tko*RNase HII shows preference for Co^{2+} over Mg^{2+}, Mn^{2+} or Ni^{2+}, and *Afu*RNase HII mostly prefers Mg^{2+} and Mn^{2+} to other metals (Chai et al., 2001; Haruki et al., 1998; Le Laz et al., 2010). Thus, metal ion usage by archaeal RNases HII may be a consequence of the environmental conditions under which they thrive. It may also dictate the substrate requirement for hydrolysis and confer a specialized function on the enzyme in the maintenance of genome integrity. This concern is nicely exemplified by bacterial RNases HII, for which Mg^{2+}- or Mn^{2+}- dependent activities are imposed by the nature of the substrate. This is in contrast to the eukaryotic RNases HII which appear more active in the presence of Mg^{2+} (Chon et al., 2009; Frank et al., 1994; Rohman et al., 2008).

Cleavage specificities for substrates containing single or few ribonucleotides embedded in double-stranded DNA (dsDNA) of type 2 RNases H are now well documented. Such structural substrates can arise *in vivo* during Okazaki fragment processing from intrinsic RNA ligation activity (Rumbaugh et al., 1997) or erroneous nucleotide incorporation by DNA polymerases (Nick McElhinny et al., 2010a; Nick McElhinny et al., 2010b), and during exposure to external damaging agents (Von Sonntag and Schulte-Frohlinde, 1978). Initial studies revealed that the archaeal *Tko*RNase HII was active on four ribonucleotides embedded in dsDNA (DNA-RNA_4-DNA/DNA). In the presence of Co^{2+} and 50 mM NaCl, the enzyme specifically cleaves at the phosphodiester bond between the third and fourth ribonucleotides, which is one ribonucleotide upstream of the RNA.DNA junction (Haruki et al., 1998). This cleavage specificity is consistent with what has been recently reported using the same substrate, but Mg^{2+} instead of Co^{2+} (Rohman et al., 2008). Similarly, *Tko*RNase HII exhibited a unique cleavage site on single ribonucleotides embedded in dsDNA (DNA-RNA_1-DNA/DNA) and specifically cut at the 5′ side of the ribonucleotide. When kinetic parameters of *Tko*RNase HII were determined in the presence of both DNA-RNA_1-DNA/DNA and DNA-RNA_4-DNA/DNA, substrate binding and turnover number of proteins were found to be comparable. Thus, one or few ribonucleotides embedded in dsDNA must be uniformly recognised and hydrolysed with similar efficiency in this archaeal organism. *Afu*RNase HII also possesses such catalytic specificities. In the presence of Mg^{2+} and 50 mM KCl, *Afu*RNase HII is shown to be active on DNA-RNA_1-DNA/DNA, unless the ribonucleotide is positioned<4 bases from the 5′ end or <2 bases from the 3′ end, and cleavage occurs at the phosphodiester bond 5′ of the junctional ribonucleotide (Bubeck et al., 2011). In addition, PCNA (Proliferating Cell Nuclear Antigen), described as a scaffold for DNA repair and replication enzymes (Maga and Hubscher, 2003; Meslet-Cladiere et al., 2007), enhances cleavage activity of *Afu*RNase HII on DNA-RNA_1-DNA/DNA, with the exception of ribonucleotide located within the first ten 5′-bases of the strand containing it. Interestingly, this result is consistent with that observed previously for *Pab*RNase HII (Meslet-Cladiere et al., 2007). *Afu*RNase HII also shows cleavage specificity on DNA-RNA_4-DNA/DNA, cutting 5′ of the last ribonucleotide of the junction in the presence of Mg^{2+} (Chai et al., 2001). Similarly, the archaeal *Pab*RNase HII acts as a specific nuclease on single embedded ribonucleotides, exhibiting cleavage activity in the presence of Mg^{2+} (Le Laz et al., 2010). Bacterial and eukaryotic type 2 RNase H enzymes share comparable substrate specificity for single or few ribonucleotides embedded in dsDNA. Substrate and cleavage

specificities for bacterial RNases HII, e.g. *Escherichia coli* RNase HII (*Eco*RNase HII) and *Tma*RNase HII, on DNA-RNA_1-DNA/DNA were found to be identical in the presence of Mg^{2+} and 50 mM NaCl (Chon et al., 2009; Ohtani et al., 2008; Rychlik et al., 2010). All bacterial enzymes specifically cleaved at the 5′ side of the ribonucleotide of the RNA.DNA junction. Similar cleavage specificities of few ribonucleotides embedded in dsDNA were also observed, leaving a mono-ribonucleotide at the 5′ terminus of the RNA.DNA junction (Ohtani et al., 2008; Rychlik et al., 2010). Thus, bacterial RNases HII share common features on junction substrates, in which Mg-dependent cleavage likely dominates over Mn-dependent hydrolysis. This statement can also be applicable to the eukaryotic RNases H2. Indeed, both mammalian and yeast enzymes displayed a unique cleavage site on DNA-RNA_1-DNA/DNA, cutting at the 5′-deoxyribonucleotide–ribonucleotide bond at the RNA.DNA junction in the presence of Mg^{2+} (Bubeck et al., 2011; Chon et al., 2009; Jeong et al., 2004; Rohman et al., 2008; Shaban et al., 2010). In contrast to *Afu*RNase HII, cleavage efficiency of *Human sapiens* RNase H2 (*Hsa*RNase H2) was not stimulated by its cognate PCNA, although they have been shown to co-localize and to interact *in vivo* (Bubeck et al., 2011; Chon et al., 2009). Under the same reaction conditions than those described for hydrolysis of single embedded ribonucleotides, cleavage specificity of *Saccharomyces cerevisiae* RNase H2 (*Sce*RNase H2) on DNA-RNA_4-DNA/DNA took place at the phosphodiester bond between the third and fourth ribonucleotides (Chai et al., 2001; Jeong et al., 2004; Rohman et al., 2008). Moreover, kinetic parameters indicated that substrate binding and turnover number of proteins were found equivalent for both DNA-RNA_1-DNA/DNA and DNA-RNA_4-DNA/DNA, as also observed for *Tko*RNase HII (Rohman et al., 2008).

Overall, it appears that Mg-dependent hydrolysis of single or few ribonucleotides embedded in dsDNA along with the unique substrate specificity are a hallmark of type 2 RNases H, implying that key structural elements necessary for activity must be conserved among eukaryotes and prokaryotes.

2.3 Structure and catalysis by RNases HII/2

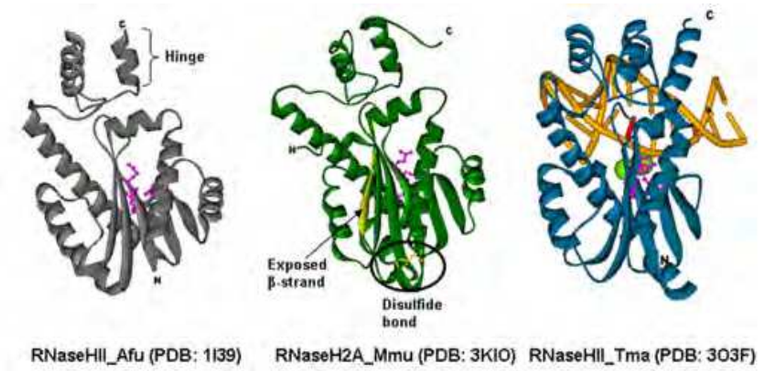
2.3.1 Overall topology

Structural comparison of three type 2 RNases H from archaea (*Afu*RNase HII: PDB code 1I39), bacteria (*Tma*RNase HII: PDB code 3O3F) and eukaryotes (*Mmu*RNase H2A: PDB code 3KIO) identifies a conserved catalytic core, termed RNase H fold, consisting of a five-stranded β sheet with three antiparallel and two parallel strands (54123, ↑↑↑↓↑) surrounded by α-helices (Fig. 1a)(Nowotny and Yang, 2009; Yang and Steitz, 1995). Concomitant with high sequence similarity, the three-dimensional structures of single polypeptide archaeal RNases HII from *A. fulgidus*, *M. jannaschii* and *T. kodakaraensis* share analogous topology and fold (Chapados et al., 2001; Lai et al., 2000; Muroya et al., 2001). They are composed of two distinct domains. The N-terminal domain comprises the central catalytic core flanked by seven α-helices (α1- α7). The C-terminal domain mainly consists of two parallel α-helices (α8- α9). Unlike *Tko*RNase HII, the helix α9 is incomplete in *Afu*RNase HII, *Mja*RNase HII and *Pab*RNase HII, as recently reported (Le Laz et al., 2010). This secondary structure element is important for *Tko*RNase HII to bind substrate (Muroya et al., 2001). On the other hand, a flexible hinge region (residues $_{195}$SNLR$_{198}$ in helix α9) in *Afu*RNase HII allows a wide range of motion when bound to its cognate PCNA (Bubeck et al., 2011)(Fig. 1a). Likewise, the structures of archaeal RNases HII are very similar to the bacterial ortholog, *Tma*RNase HII. For instance, the structures of *Afu*RNase HII and *Tma*RNase HII are superimposable

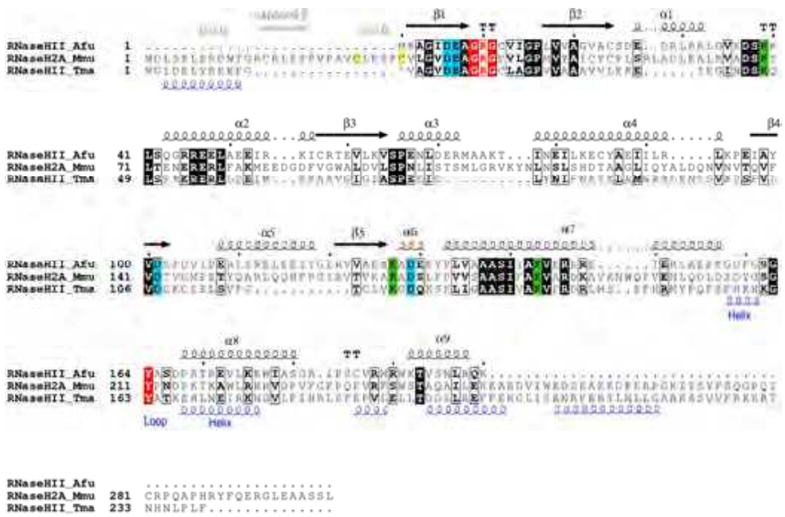
with root-mean-square deviations (RMSD) of 2.6 Å over 128 Cα atoms (SuperPose V1.0 server (Maiti et al., 2004)). *Tma*RNase HII consists of the N-terminal domain with the five-stranded β sheet flanked by two helices on one side and three helices on the other side. The C-terminal domain contains two helices constituting a helix-loop-helix motif (Rychlik et al., 2010)(Fig. 1b). Additionally, *Tma*RNase HII possesses approximate 39-amino acids C-terminal and 12-amino acids N-terminal extensions that are not found in the archaeal RNase HII (Fig. 1b). Recently, the crystal structure of the heterotrimeric mouse RNase H2 (*Mmu*RNase H2) has been solved, consisting of the RNase H2B-RNase H2C subcomplex that interfaces with the catalytic RNase H2A protein (Shaban et al., 2010). The RNase H2B-RNase H2C dimer is reported to provide a structural support for RNase H2A to become active and also acts as a platform for interactions with other proteins, such as PCNA (Bubeck et al., 2011; Chon et al., 2009). *Mmu*RNase H2A shares significant structural similarity to the archaeal *Afu*RNase HII and the bacterial *Tma*RNase HII. For example, the structures of *Afu*RNase HII and *Mmu*RNase H2A are superimposable with RMSD of 3.6 Å over 196 Cα atoms (SuperPose V1.0 server (Maiti et al., 2004)). *Mmu*RNase H2A contains the conserved RNase H fold surrounded by α-helices (Shaban et al., 2010). Moreover, it comprises approximate 30-amino acids N-terminal and 50-amino acids C-terminal extensions that are absent in *Afu*RNase HII (Fig. 1b). Unlike *Tma*RNase HII that contains a helix-loop-helix motif at its extended C-terminal domain (Rychlik et al., 2010), this region appears disordered in the *Mmu*RNase H2A structure (Fig. 1b) (Shaban et al., 2010). *Mmu*RNase H2A N-terminal extension is structurally organised and forms a β-strand (called β1 or exposed β-strand) held rigidly by a disulfide bond (Fig. 1). This exposed β-strand is thought to act as an additional protein-protein interface (Shaban et al., 2010).

2.3.2 Active site, substrate binding and catalytic mechanism

Because the structures of type 2 RNases H contain a common RNase H fold, the catalytic center may be similar among archaea, bacteria and eukaryotes. Comparison of secondary structure among *Afu*RNase HII, *Mmu*RNase H2A and *Tma*RNase HII for which crystal structures have been solved (Bubeck et al., 2011; Chapados et al., 2001; Shaban et al., 2010) points out that these three enzymes have remarkably similar active sites, consisting of four highly conserved carboxylates (DEDD motif) (Fig. 1b). The spatial clustering of these carboxylates is preserved among archaeal enzymes (Chapados et al., 2001; Lai et al., 2000; Muroya et al., 2001), and forms a small catalytic pocket composed of strands β1, β4 and helix α6. This active site geometry has been functionally analysed in *Afu*RNase HII and *Tko*RNase HII (Chapados et al., 2001; Muroya et al., 2001). Mutations of D6, E7, and D101 abolished the nucleolytic activity, while mutation of D129 showed 50% reduced activity in *Afu*RNase HII. Corresponding residues (D34, E35, D142, and D170) have been mutated in *Mmu*RNase H2A, and the resulting heterotrimeric mutant RNase H2 exhibited no detectable activity (Shaban et al., 2010). Similarly, conservative mutations and subsequent functional analyses of three potential active site carboxylates in yeast RNase H2A led to a loss of substrate cleavage (Jeong et al., 2004). The four carboxylates are positionally conserved in *Mmu*RNase H2A, and form a catalytic pocket composed of strands β2 and β5, and helices α4 and α5 (Shaban et al., 2010). The active site of *Tma*RNase HII is also composed of four conserved carboxylates that are structurally similar to *Afu*RNase HII, *Mmu*RNase H2A. These residues (D18, E19, D107, and D124) are located in the catalytic cleft that is lined by strands β1 and β4, and a loop before the last helix of the RNase H fold (Rychlik et al., 2010). Thus, the similar active site geometry observed in eukaryotes and prokaryotes suggests a conserved two-metal ion catalytic mechanism in type 2 RNases H.



(a)



(b)

Fig. 1. Structure and structure-based sequence alignment of *Archaeoglobus fulgidus* (Afu), *Mus Musculus* (Mmu) and *Thermotoga maritima* (Tma) type 2 RNases H. (a) Ribbon diagrams of RNaseHII_Afu (PDB ID: 1I39), RNaseH2A_Mmu (PDB: 3KIO) and RNaseHII_Tma (PDB: 3O3F). The active-site carboxylates are shown as magenta ball-and-stick models. Exposed β-sheet and disulfide bonds are shown in *yellow* in RNaseH2A_Mmu. The flexible hinge is highlighting ($_{195}$SNLR$_{198}$). The two magnesium ions are shown as *green* spheres in RNaseHII_Tma complexed with the DNA-RNA$_1$-DNA/DNA substrate, (b) Sequence alignment of the three type 2 RNases H based on the three-dimensional structure of RNaseHII_Afu. Conserved active site residues are highlighted in *blue*. Conserved residues that contact the nucleic acid backbone in the co-crystal structure of RNaseHII_Tma are highlighted in *green*. Conserved GRG motif and tyrosine residue involved in junction sensing coupled to catalysis in RNaseHII_Tma are highlighted in *red*. Cysteine residues forming a disulfide bond in RNaseH2A_Mmu are highlighted in *yellow*.

In the crystal structure of *Tma*RNase HII in complex with single embedded ribonucleotide, the substrate is bound to the protein, such that the noncleaved strand fits in a groove on the protein surface at the C-terminal domain, and the cleaved strand containing the single ribonucleotide interacts with the catalytic site (Rychlik et al., 2010). Several basic residues, e.g., K47, K122, and K138, are involved in substrate binding, and contact with the phosphate

groups of the nucleic acid backbone in *Tma*RNase HII. These three lysines are strictly conserved in *Afu*RNase HII and *Mmu*RNase H2A (Fig. 1b), which strongly supports a putative role for these residues in substrate binding. In fact, mutational studies indicated that K138 equivalent in *Afu*RNase HII is important for nucleic acid binding (Chapados et al., 2001). Furthermore, a substrate recognition motif (G21, R22, and G23) and Y163 have been identified as key structural elements responsible for the detection and cleavage of single embedded ribonucleotides in *Tma*RNase HII (Rychlik et al., 2010). Accordingly, mutational analyses of Y163 equivalent in *Afu*RNase HII suggested that this residue may intercalate into the duplex to stabilize a bent conformation required for substrate recognition and catalysis (Chapados et al., 2001). The highly conserved GRG motif and tyrosine residue (Fig. 1b) convincingly suggests that the specific recognition mechanism for single embedded ribonucleotides must be similar among type 2 RNases H. Besides, it has been recently described that the four conserved carboxylates of the DEDD motif were directly involved in coordinating the metal ions in *Tma*RNase HII, the distance between the two metal ions (A and B) imposed by the geometry of the active site carboxylates and substrate (Rychlik et al., 2010). The influence of nucleic acid on the metal-ion coordination ensures the catalytic specificity of *Tma*RNase HII. One of the most important features observed in the co-crystal structure of *Tma*RNase HII is that the tyrosine required for 2′-OH detection of single embedded ribonucleotides also participates in metal ion positioning, because it induces a slight deformation of the nucleic backbone at the RNA.DNA junction, and renders possible the coordination of metal ion A by the phosphate group. Moreover, a conserved DSK motif (D45, S46, and K47) located in the vicinity of the catalytic pocket has been proposed to participate in the active site formation of *Tma*RNase HII (Rychlik et al., 2010). Based on strictly conserved key structural elements responsible for substrate binding, metal ion coordination, and formation of the catalytic center in *Tma*RNase HII, the cleavage mechanism of single ribonucleotides embedded in dsDNA likely proceeds in a similar fashion in archaeal, bacterial and eukaryotic type 2 RNases H.

2.4 Physiological roles for RNases HII/2

Type 2 RNases H are represented in organisms across domains and exhibit a conserved core structure. Moreover, they have been identified as the sole enzymes able to recognise and cleave a single ribonucleotide embedded in dsDNA (Eder and Walder, 1991), thereby contrasting to type 1 RNases H that requires at least four ribonucleotides for cleavage (Ohtani et al., 1999a). As mentioned earlier, single ribonucleotides embedded in dsDNA can arise from external damaging agents (Von Sonntag and Schulte-Frohlinde, 1978), and can occur by intrinsic RNA ligation (Rumbaugh et al., 1997) or erroneous nucleotide incorporation during DNA replication (Nick McElhinny et al., 2010a; Nick McElhinny et al., 2010b). The presence of riboses in DNA has been shown to induce a helical alteration, promoting a B- to A-form transition in DNA (Horton and Finzel, 1996). This result is consistent with the local DNA backbone distortion recently observed in the structure of *Tma*RNase HII bound to single embedded ribonucleotide substrates (Rychlik et al., 2010). If left unrepaired, such structural alterations could be mutagenic for the cells, given that accurate DNA synthesis by replicative DNA polymerases depends on DNA helix geometry. Since type 2 RNases H specifically cleave single embedded ribonucleotides, they can be considered to be involved in DNA repair. However, as the enzymes cut the phosphodiester bond 5′ of the junctional ribonucleotide, other components are required to eliminate the remaining ribonucleotide. To clarify the physiological role of type 2 RNases H in bacteria, archaea and eukaryotes, mutant strains containing one or two RNases H-encoding genes

have been constructed. In single-celled species, deletions of all RNase H genes were not lethal, but showed modest sensitivity to DNA-damaging agents, indicating their requirement in DNA repair (Arudchandran et al., 2000; Fukushima et al., 2007; Itaya et al., 1999; Meslet-Cladiere et al., 2007). Conversely, in a multicellular organism, both type 1 and type 2 RNases H were shown to be essential. Deletion of type 1 RNases H causes embryonic lethality in mouse and *Drosophila Melanogaster* (Cerritelli et al., 2003; Filippov et al., 2001), the former impairing mitochondrial DNA replication (Cerritelli et al., 2003). On the other hand, type 2 RNases H have been described as the major source of RNase H activity in eukaryotes (Eder and Walder, 1991; Frank et al., 1998a), and mutations in human RNase H2 can cause the neurological disorder, Aicardi-Goutières Syndrome (AGS). The pathogenesis of AGS is linked to the activation of innate immune system, likely because of accumulation of normally degraded RNA/DNA nucleic acids (Crow et al., 2006). Likewise, defect of the catalytic mutant *Mmu*RNase H2 to hydrolyse single embedded ribonucleotides pointed toward a role for eukaryotic RNase H2 in DNA repair (Shaban et al., 2010). Furthermore, based on genetic and biochemical results, removal of single embedded ribonucleotides seems to involve at least type 2 RNases H, Fen1 (Flap Endonuclease 1) and PCNA (Bubeck et al., 2011; Meslet-Cladiere et al., 2007; Nick McElhinny et al., 2010a; Rumbaugh et al., 1997; Rydberg and Game, 2002), with PCNA:RNase HII/2 complex acting as a sensor of erroneous ribonucleotides. The association of such protein components likely suggests a role of type 2 RNases H in base excision repair (BER) to accomplish the removal of mutagenic ribonucleotides. In this pathway, type 2 RNase H would act initially by recognising and incising the damaged strand at the 5′ side of the ribonucleotide. However, further studies are now required to identify and reconstitute the sequential enzymatic steps involved in this repair process.

3. Endonucleases of the XPF/MUS81 family

DNA repair and replication restart pathways generate a variety of branched structures such as four-way DNA junctions (Holliday junctions, HJs), fork structures and 5'- or 3'-flaps, all of which are substrates for structure-specific endonucleases. Many nucleases that act upon 3'-flap structures belong to the XPF/MUS81 family of proteins, which are present throughout eukarya and archaea but are not found in bacteria. Defects in XPF/MUS81-family members are associated with human disease such as Xeroderma pigmentosum (XPF-ERCC1) (Sijbers et al., 1996) or Fanconi anemia (FANCM) (Meetei et al., 2005).

3.1 Eucaryal members of the XPF/MUS81 family of endonucleases

The human XPF-ERCC1 complex and its counterpart RAD1-RAD10 in *Saccharomyces cerevisiae* have been thoroughly characterized for their role in nucleotide excision repair (NER), cleaving damaged DNA 5′ to the lesion (Bardwell et al., 1994; Sijbers et al., 1996). Moreover RAD1-RAD10 acts in DNA double-strand break repair (Ma et al., 2003; Schiestl and Prakash, 1988), a role also conserved in mammalian cells (Ahmad et al., 2008; Al-Minawi et al., 2008). Notably, the XPF-ERCC complex has been directly observed in living cells using fluorescence resonance energy and spectral imaging techniques (Dinant et al., 2008).

MUS81 complexes are distinct to XPF, and initial work in *Schizosaccharomyces pombe* led to the proposal that Mus81-Eme1 is a Holliday junction resolvase (Boddy et al., 2001). However, *in vitro* work has shown that recombination intermediates such as D-loops and nicked HJs are cleaved by Mus81-Eme1, and are likely to represent its main substrates *in*

vivo (Gaillard et al., 2003; Gaskell et al., 2007; Osman et al., 2003). By contrast the *S. cerevisiae* ortholog Mus81-Mms4, which displays similar substrate specificity *in vitro* (Ehmsen and Heyer, 2008; Gaskell et al., 2007; Whitby et al., 2003), is not the principal HJ resolvase (de los Santos et al., 2003) but instead resolves aberrant joint molecules in meiosis (Jessop and Lichten, 2008; Oh et al., 2008). The recent discovery in humans and *S. cerevisiae* of the Hen1/Yen1 Holliday junction resolvase, which is absent from *S. pombe*, suggests that this enzyme is a meiotic HJ resolvase, and that in its absence Mus81 can fulfill that role (Ip et al., 2008). Nevertheless, in both *S. cerevisiae* and *S. pombe*, the primary function of Mus81 appears to be the restart of collapsed-replication forks by homologous recombination (Doe et al., 2004; Froget et al., 2008; Kai et al., 2005; Matulova et al., 2009; Roseaulin et al., 2008), a role that is functionally redundant with the Sgs1-Top3 and the Rqh1-Top3 complexes, respectively (Doe et al., 2004; Kaliraman et al., 2001). The MUS81 complex is also found in humans (Chen et al., 2001), and appears to promote replication fork restart by homologous recombination (Ciccia et al., 2003; Franchitto et al., 2008; Hanada et al., 2007; Shimura et al., 2008).

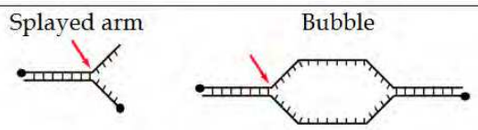
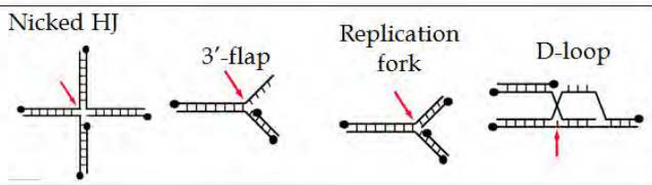
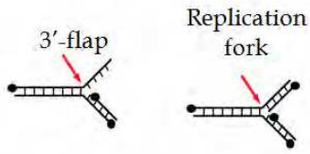
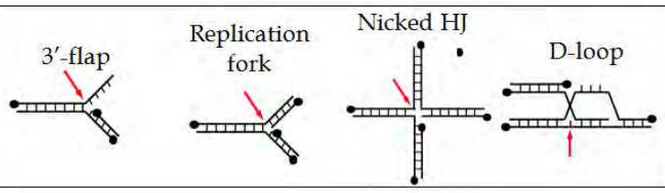
Human FANCM was identified thanks to the archaeal ortholog Hef, an XPF/MUS81 family protein featuring a helicase:nuclease fusion (Meetei et al., 2005; Mosedale et al., 2005). The FANCM-FAAP24 complex is a XPF/MUS81 member found in humans (Ciccia et al., 2007) that possesses two separate functions: (i) to recruit the Fanconi anemia core complex to the repair of DNA interstrand crosslinks (Ciccia et al., 2007; Kim et al., 2008; Meetei et al., 2005; Mosedale et al., 2005), and (ii) to facilitate the response to replication stress by the ATR pathway, *via* its ATP-dependent translocase activity (Collis et al., 2008; Gari et al., 2008; Xue et al., 2008). Since FANCM promotes fork reversal *in vitro*, it has been proposed that FANCM ATP-dependent activity at stalled forks is needed to allow processing for replication restart (Gari et al., 2008; Xue et al., 2008). The recently-identified *S. pombe* FANCM ortholog Fml1 appears to promote homologous recombination at stalled forks, suggesting that the ATP-dependent helicase activity of FANCM at DNA replication forks is conserved in *S. pombe* (Sun et al., 2008).

3.2 Archaeal members of the XPF/MUS81 family of endonucleases

All archaea encode a protein of the XPF/MUS81/FANCM family of endonucleases except the *Thermoplasmatales*. Archaeal XPF exists in two forms: the long form consisting of an N-terminal helicase domain fused to a C-terminal nuclease domain, specific to the euryarchaea, and a short form lacking the helicase domain found in the crenarchaea and thaumarchaea.

3.2.1 Hef

Hef (helicase-associated endonuclease fork-structure DNA) is present only in euryarchaeota and was identified in *Pyrococcus furiosus* due to its activity on branched DNA structures (Komori et al., 2002). Similarly to for instance eukaryotic FANCM proteins, Hef has both an active helicase domain and an active nuclease domain. The C-terminal fragment of *Pyrococcus furiosus* Hef adopts a similar domain organization to those in the XPF/MUS81 proteins, corresponding to the nuclease domain containing the $ERKX_3D$ signature sequence involved in nuclease activity and the helix-hairpin-helix motifs of the HhH domain (Nishino et al., 2003). Mutational analyses showed that residues in the $ERKX_3D$ motif are indeed involved in the cleavage of Hef endonuclease. The Hef nuclease domain adopts a type II

	Substrate specificity *in vitro* (nuclease activity)	Function
S. cerevisae Rad1/Rad10 *H. sapiens* XPF/ERCC1		NER, ICL repair
Yeast Mus81/Eme1 and Mus81/mms4		Stalled fork repair, meiotic recombination
P. furiosus Hef		Stalled fork repair, NER (?)
S. solfataricus XPF		Stalled fork repair (?), NER (?)

Abbreviations: HJ, Holliday junction; NER, Nucleotide excision repair; *S. cerevisae, Saccharomyces cerevisae; H. sapiens, Homo sapiens; P. furiosus, Pyrococcus furiosus; S. solfataricus, Sulfolobus solfataricus.*

Table 1. Substrate specificities and functions of members of the XPF/MUS81 family. Schematic representation of the various substrates that are cleaved *in vitro* by various XPF/MUS81 family proteins. A red arrow indicates the approximate sites of cleavage within each DNA structure. Black circle indicates 5′ termini.

restriction endonuclease fold, indicating that Hef nuclease belongs to this restriction endonuclease family (Nishino et al., 2003). Accordingly, the Hef nuclease activity is strictly dependent on Mg^{2+} or Mn^{2+} whereas Ca^{2+} cannot substitute.

The C-terminal fragment of *Pyrococcus furiosus* Hef and the entire Hef protein form dimers through a combination of two interfaces, one in the nuclease domain and one in the HhH domain, which function independently with each other. It appears that simultaneous dimer formation in both the nuclease and the HhH domains is crucial to substrate recognition specificity (Nishino et al., 2003). In the homodimer, both HhH domains are equally important for substrate recognition while at least one of the nuclease active site is required for cleavage of the fork-structured DNA. The active site of the catalytic domain is positioned near the cleavage site, two to three bases away from the junction, and has the potential to introduce unpairing near the junction center. The HhH region is bound to duplex regions and is not directly involved in the recognition of the fork structure but dramatically enhanced the catalytic site unpairing.

The substrate specificity for the cleavage activity of the Hef protein is contained in the C-terminal domain as both the C-terminal fragment and the entire Hef protein recognize and

cleave nicked, flapped and fork-structured DNAs at the 5′ side of the nicked position. *P. furiosus* Hef thus displays XPF/Mus81-like specificity, suggesting that Hef is involved in NER (Nishino et al., 2005a; Nishino et al., 2005b).

The N-terminal fragment of *P. furiosus* Hef containing all the conserved helicase motifs consist of three structural subdomains. Domains 1 and 3 are each folded into the RecA-like architectures with the conserved helicase motifs. Domain 2 is a relatively mobile domain with a positively charged surface inserted into domain 3 (Nishino et al., 2005b). Hef domain 2 exhibits architectural similarity with the thumb domain of *Taq* DNA polymerase being involved in double-stranded DNA binding. Mutational analyses show that the domain 2 dictates the recognition of specific DNA structures, especially fork-structured DNA, while domains 1 and 3 are crucial for the structure-specific helicase activity. Interestingly, two other SF2 helicase members recognizing branched structures, RecQ and RecG, contain an insertion, after and before the helicase core respectively. It remains unclear how Hef domain 2 participates in branched structure recognition.

The N-terminal domain of Hef displays a DNA structure-specific helicase activity as the most prominent enhancement of the ATPase activity is observed with fork-structured DNAs. Interestingly *in vitro* experiments suggest that the N-terminal domain binds to the fork-structured DNA and process the DNA to increase cleavage of the substrate by the endonuclease domain. These observations have led to the proposal that Hef also acts at stalled replication forks, both the helicase and the nuclease activities being required for the rearrangement of forked-structure DNA (Komori et al., 2004).

The genetic study of Hef mutant in the euryarchaea *Haloferax volcanii* suggest that Hef is involved in the restart of arrested replication forks as an alternative pathway to homologous recombination-dependent pathway (Lestini et al., 2010). In this organism Hef is not involved in nucleotide excision repair but *Haloferax volcanii* possesses bacterial homologs of NER proteins which may have displaced the original archaeal NER proteins. Therefore the absence of an NER function of Hef cannot be generalized to all euryarchaea. In support to this, a recent genetic analysis of *hef* mutants in the hyperthermophilic archaeon, *Thermococcus Kodakaraensis*, has demonstrated that Hef is involved in the repair of a wide variety of DNA damages. In addition, the higher sensitivity of Δ*hef* mutants to methyl sulfonate and mitomycin C, suggests a central role for Hef protein in the archaeal NER and/or ICL repair pathways (Fujikane et al., 2010).

3.2.2 XPF in Crenarcheota

By contrast, the XPF ortholog found in crenarchaeota contains only the C-terminal nuclease domain. The structure of XPF from the crenarchaea *Aeropyrum pernix* reveals two domains, a N-terminal nuclease domain and a $(HhH)_2$ domain (Newman et al., 2005). As expected by analogy with Hef, dimers are formed by interaction of the two nuclease domains and by the interaction of the two $(HhH)_2$ domains from each monomer. Comparaison of the structure of the protein in the presence and absence of dsDNA reveals that the $(HhH)_2$ domain plays a major role in interacting with DNA. Upon binding to DNA a domain movement allow the coupling between the $(HhH)_2$ domain and the nuclease domain to recognize and cleave the DNA thanks to the flexibility of the connecting linker sequence between the two domains. It seems that dimer interaction with DNA involves two binding sites that can only both interact with DNA if the substrate is bent by around 90°, suggesting that XPF may recognize ds/ssDNA junctions by their susceptibility to distorsion. The structural data suggest that only one monomer is catalytically active at a time in a dimer bound to DNA.

The studies of XPF from *Sulfolobus solfataricus* have shown that XPF strickly requires interaction with PCNA to show any nuclease activity and it has been suggested that PCNA directly stimulates the nuclease activity without changing the binding affinity of XPF for its substrate (Hutton et al., 2008). XPF preferentially cleaves 3′-flap but the presence of downstream duplex influences the choice of position cleavage, *Sso*XPF appearing to act as a processive nuclease *in vitro* by processing 3′-flap into gapped duplex products. In this respect, it is noteworthy that recent fluorescence quenching and FRET studies have indicated that PCNA and XPF cooperate to distort DNA substrates (Hutton et al., 2009). It has also been observed that SsoXPF can act on substrates containing a variety of types of DNA damage or modification, suggesting a role in the removal of these lesions *in vivo* (Roberts and White, 2005). Altogether these data suggest that crenarchaeal XPF is recruited by PCNA to act in NER and replication fork restart, but to date this scenario has not yet been addressed using *in vivo* data.

4. The Mre11-Rad50 complex in Archaea

The processing of DNA double strand breaks (DSBs) is a crucial mechanism for genomic integrity. DNA breaks can arise during replication as intermediates in programmed DNA rearrangements including meiosis and immune system development or can be caused by oxidative damages and exposure to ionizing radiations. Double strand break repair (DSBR) is an essential repair pathway in the three domains of life, and plays a major role in the rescue of stalled or collapsed replication forks. In bacteria, DSBs are processed *via* homologous recombination, whereas, in eukarya, they are repaired by homologous or non-homologous recombination (Kowalczykowski et al., 1994; Sonoda et al., 2006). In archaea, the picture is not clear. Homologous recombination (HR) is presumably the preferred process of DSBR as proteins involved in HR have been identified whereas Ku homologs have not (White, 2011); yet a recent genetic study on *Haloferax volcanii* demonstrate that DSBR by HR is restrained, likely because this species is highly polyploid (Delmas et al., 2009). Nevertheless, the study of such processes in hyperthermophilic archaea is of importance, given that they are exposed to DNA damaging temperatures and are among the most radioresistant organisms, repairing fragmented chromosomes efficiently (DiRuggiero et al., 1997; Gerard et al., 2001; Jolivet et al., 2003a; Jolivet et al., 2003b). The core component in charge of the early steps of this critical event in eukaryotic cells is the Mre11-Rad50 (MR) complex, associated with a third component, Xrs2 in yeast and Nbs1 in higher eukaryotes. Homologs of the Mre11-Rad50 complex have been found in the three domains of life; however, Xrs2/Nbs1 additional component has not been found in bacteria and archaea, it appears thus that Mre11 and Rad50 comprise the core enzymatic members of this conserved multiprotein machine.

4.1 Catalytic activities and DSB ends processing

The archaeal homologs of Mre11 and Rad50 were initially identified in *Pyrococcus furiosus* (Hopfner et al., 2000a). *Pfu*Mre11 shares significant similarities with eukaryotic counterparts, particularly in the N-terminal region, which contains five phosphoesterase motifs that form the nuclease domain of the protein (Williams et al., 2008). The enzyme is endowed with ssDNA endonuclease and manganese-dependent 3′-5′exonuclease activities (Hopfner et al., 2000a). However, at temperature closer to physiological conditions (55°C), *Pfu*Mre11 displays an ATP dependent nuclease activity in magnesium. This activity consists

of both a weak 3′-5′ exonuclease activity as well as endonucleolytic cleavage activity on the 5′ strand at a break (Hopkins and Paull, 2008). *Pfu*Rad50 is related to the Structural Maintenance of Chromosomes (SMC) family and displays the classical modular structure composed of N- and C- terminal head domains, each bearing the Walker A and B motifs, respectively, separated by a coiled-coil region with a zinc hook. Rad50 has ATPase and adenylate kinase activities (Bhaskara et al., 2007; Hopfner et al., 2000b). Consistent with the model based on the crystallographic structure of *Pfu*Rad50 catalytic domain, the MR complex of *P. furiosus* likely exhibits ATP-dependent DNA binding activity. Thus, Rad50 may regulate DNA binding and release after proper DNA end processing in conjunction with Mre11 (Hopfner et al., 2000b). All the archaeal genomes sequenced to date contain clear homologs of eukaryal Mre11 and Rad50 and the initial biochemical characterization of the *P. furiosus* homologs indicate that the archaeal MR complex is functionally similar to those from Bacteria and Eukarya. However, the 3′-5′ exonuclease activity described for the complex is opposite to the polarity required for the 5′-3′ resection of DSB ends necessary for the initiation of HR, suggesting the involvement of additional components to catalyse efficient DSB resection. As in the other thermophilic archaea, Mre11 and Rad50 are commonly found in an operon that frequently includes the *herA* and *nurA* genes and the four genes are co-transribed in the crenarchaeon *Sulfolobus acidocaldarius* (Constantinesco et al., 2002; Constantinesco et al., 2004). HerA is an ATP-dependent helicase, which is strikingly bidirectional and can thus unwind DNA from both 3′ and 5′ single-stranded overhangs. NurA defines a new nuclease family exhibiting both a single strand endonuclease activity and a 5'-3' exonuclease activity on single and double stranded DNA (Constantinesco et al., 2002; Constantinesco et al., 2004; Manzan et al., 2004). The cooperation of the four proteins for the 5′ strand resection at DSB has been demonstrated by Hopkins and Paull (Hopkins and Paull, 2008). This process depends on the enzymatic activities of HerA, NurA and Rad50, Mre11 activity being partially dispensable. The *P. furiosus* MR complex generates short 3′ single stranded overhangs through limited degradation of the 5′ strand at a DSB. This specific structure allows the entry of the complex HerA-NurA and the NurA nuclease together with HerA helicase activities generate the long 3′ single strand suitable for RadA-catalysed strand exchange. The role of Mre11 nuclease activity may be confined to the removal of short 5′ terminal oligonucleotides, which could be essential for the clearance of covalently attached proteins at the 5′ strand. HerA and NurA have not been detected in eukaryotes, however, recent studies in budding yeast demonstrate that while MRX complex is involved in DSBs processing initiation, the functional homologs Sgs1 and Exo1 nucleases and Dna2 nuclease/helicase, are necessary for the extensive 5′ strand resection (Mimitou and Symington, 2008; Zhu et al., 2008). In bacteria, the DSB recognition and 5′strand resection to produce the 3′-OH overhang for RecA mediated strand exchange is performed by a single RecBCD complex (Kowalczykowski et al., 1994). SbcCD, the bacterial homolog of MR complex, has been shown to cleave hairpin DNA, which can block stalled replication fork, prior to homologous recombination rescue of the fork (Connelly et al., 1998) and to be implicated in the removal of covalently attached protein to promote repair (Connelly et al., 2003).

4.2 Structural insight into the Mre11-Rad50 complex

The archaeal MR complex is structurally very similar to their eukaryal counterparts, and has proven very useful for crystallographic and biophysical studies (Arthur et al., 2004; Hopfner et al., 2002a; Hopfner et al., 2001; Hopfner et al., 2000a; Hopfner et al., 2000b; Hopfner et al.,

2002b; Williams et al., 2008). Indeed, with the exception of the recent description of the first eubacterial Mre11 nuclease, the bulk of structural data have been obtained from analysis of *P. furiosus* Mre11-Rad50 complex (Das et al., 2010).

The core Mre11-Rad50 complex forms a large globular complex at the root of an elongated coiled-coil structure. The complex exists as a heterotetrameric assembly (M_2R_2) and the globular head is composed of two Mre11 and two Rad50 ATPase domains, both of which bind DNA (Hopfner et al., 2001). This bipolar structure of the MR complex is consistent with both the enzymatic role in DNA end processing and structural function in DNA end joining. Indeed the M_2R_2 heterotetramer contains two DNA binding/processing active sites, which could be important in the alignment of DNA ends in NHEJ or of DNA ends and sister chromatids in HR (Hopfner et al., 2002a). X-ray crystallographic data from the *P. furiosus* Rad50 coiled-coil region reveals a hook structure that caps the distal end of the coiled-coils with a conserved Cys-X-X-Cys motifs that mediates Rad50-Rad50 dimerization through this motif by coordinated binding of a zinc atom (Hopfner et al., 2002b). The ability to interact through coiled coil ends in supra-molecular complexes is proposed to be necessary for the mechanistic role of MR complex (de Jager et al., 2004). The crystallographic structure of *Pfu*Mre11 reveals a two-domain architecture consisting of a protein-phosphatase-like domain and a small capping domain that interact at the active site (Fig.2).

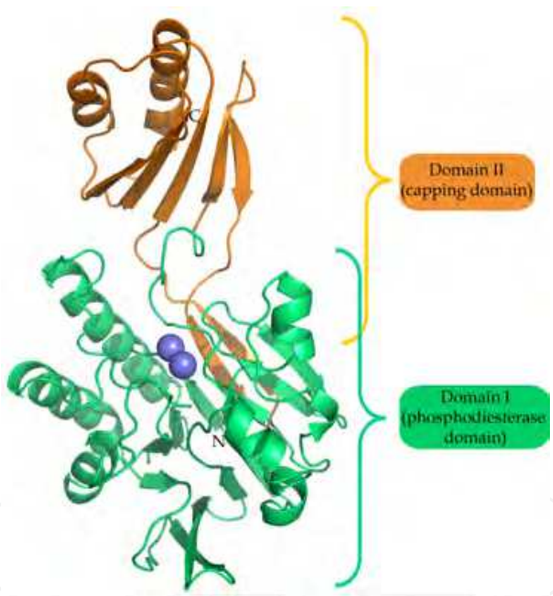


Fig. 2. Crystal structure of *Pfu*Mre11. Ribbon diagram of the two domain fold of *P. furiosus* Mre11 (1-342, PDB_1II7). Two Mn^{2+} ions (purple spheres) are coordinated by seven conserved residues of the phosphodiesterase motifs (Hopfner et al., 2001).

The first domain contains the five phosphoesterase motifs which form the nuclease active site. This domain is composed of two parallel mixed β sheets that are flanked by seven α helices. The capping domain consists of a 5-stranded β sheet and two helices and partially caps the nuclease catalytic motifs of the N-terminal domain, suggesting that the capping domain might be involved in DNA-binding specificity (Hopfner et al., 2001). X-ray structure of *Pfu*Mre11 bound to DNA reveals that Mre11 dimerization is critical for efficient DNA binding and is mediated by N-terminal conserved domains (Williams et al., 2008). Six DNA

recognition loops promote DNA binding and assemble into a contiguous DNA interaction surface. The interaction with DNA is mediated by contacts to the sugar-phosphate backbone, which is consistent with the lack of sequence specificity for Mre11 in DNA binding. The structure of the archaeal Mre11 was used to map eukaryotic Mre11 mutations linked to human disease and yeast DSBR defect and helped define a large surface area outside the nuclease motif, which may be an important protein-protein or protein-DNA interface (Hopfner et al., 2001).

4.3 Physiological roles of Mre11-Rad50 complex

Genetic studies in eukaryotes indicate that the MR complex is required for genomic stability and is involved in a large variety of different functions in response to DSBs (Stracker and Petrini, 2011). *In vivo* studies, in budding and fission yeast, have demonstrated a critical role for Mre11 and the other subunits of the MRX complex, for survival of DSBs caused by ionizing radiations and genotoxins (Chahwan et al., 2003; D'Amours and Jackson, 2002). In addition, hypomorphic mutations in the human *mre11* and *nsb1* genes lead to ataxia telangiectasia-like disorder and Nijmegen Breakage syndrome, respectively. The cellular features resulting from these mutations include hypersensitivity to ionizing radiation, radioresistant DNA synthesis, and abrogation of ATM-dependent events (Stewart et al., 1999; Williams et al., 2007). These phenotypes emphasize the importance of the eucaryotic Mre11 complex in a large variety of DNA metabolic pathways. The bacterial homologue of Mre11-Rad50 is SbcCD, and it has been shown that *sbcCD* mutants of *Deinococcus radiodurans* display reduced survival and present a delay in kinetics of DSB repair (Bentchikou et al., 2007). In *Bacillus subtilis* and *D. radiodurans*, the deletion of *sbcC* results in an increased sensitivity of the cells to ionizing radiation (Mascarenhas et al., 2006).

In archaea, the first evidence of the involvement of Mre11 in DNA repair process was demonstrated by Quaiser et al. (Quaiser et al., 2008), using an immunodetection approach to determine the roles of Mre11, Rad50, NurA and HerA proteins, in post-irradiation DNA repair in *S. acidocaldarius*. They observe that a complex of the three proteins HerA, Mre11 and Rad50 is formed constitutively *in vivo*. Rad50 is constitutively associated with DNA and upon chromosome fragmentation, Mre11 proteins is recruited to the DNA or to Rad50 DNA-bound proteins, suggesting that Mre11 is actively involved in DNA repair processes and/or acts as an inducible damage sensor. The analyses of *mre11 rad50* deletion mutants of the halophilic *Halobacterium sp.* strain NRC1 also suggest that Mre11 and Rad50 may have independent functions outside the MR complex in archaea, since the absence of Rad50 has no effect on the repair of DSBs, whereas the loss of Mre11 results in a decrease rate of DSBR, due to the loss of either nuclease activity or the DNA damage-sensing activity of Mre11 (Kish and DiRuggiero, 2008). Surprinsingly, the *mre11 rad50* deletion mutant of *Haloferax volcanii* displays an enhanced resistance to DNA damage that correlates with a higher level of homologous recombination in the mutant, suggesting that Mre11-Rad50 restrains the use of HR for repair (Delmas et al., 2009). The unrestrained use of HR in *mre11 red50* mutant enhances cells survival but leads to a slower recovery presumably due to difficulties in the resolution of repair intermediates. Two non-exclusive hypotheses are proposed to account for that increased resistance observed in *mre11 rad50* mutants: (i) Mre11-Rad50 binds to double-strand breaks and prevents HR, and/or (ii) Mre11-Rad50 stimulates an alternative pathway of double-strand breaks repair. To add some complexity, a recent genetic analysis in the hyperthermophilic archaeon *Thermococcus kodakaraensis*, has shown that the *mre11*, *rad50*, *herA*, *nurA* and *radA* genes are essential for *T. kodakaraensis*, which is in contradiction

with the previous genetic analyses in archaea, yeast and bacteria. This result could reflect the importance of HR to repair DNA damage caused by the high temperature required for *T. kodakaraensis* for viability (Fujikane et al., 2010).
Contrasting with the wealth of structural and biochemical data gained from the study of archaeal MR complex, the paucity and the conflicting nature of the genetic analyses underscore the importance to develop more effective genetic tools, for hyperthermophilic archaea in particular, to improve our knowledge on the functions of the complex in response to DSBs. Biochemical and *in vivo* investigations of the functions of HerA, NurA and the single stranded DNA binding proteins, RadA and SSB/RPA, required for strand exchange, should also improve our knowledge of the HR/DSBR in archaea and potentially shed light on the eukaryal pathway.

5. The Pab2263-NucS protein

5.1 Identification of a novel nuclease

Many nucleases are highly regulated by the sliding clamp PCNA (Proliferating Cell Nuclear Antigen). For instance, PCNA increases the affinity of Fen-1 for its substrate (Hutton et al., 2008) as well as catalytic rate of *Sso*XPF (Hutton et al., 2009). Interaction of Fen-1 and other proteins with PCNA is mediated by the so-called PIP-motif (PCNA Interacting Motif), a relatively short peptide motif found in a large number of PCNA-interacting proteins (Vivona and Kelman, 2003; Warbrick, 1998). The sequence motif in *Pyrococcus abyssi* species has been experimentally defined and corresponds to the peptide motif QX_2LX_2[WLFT][LFT] (Meslet-Cladiere et al., 2007). Among others, previously uncharacterized protein encoded by the *pab2263* gene was shown to carry this peptide motif at its carboxy-terminus.
Pab2263 belongs to the DUF91 family (Domain of Unknown Function 91) and, as many members of this family, contains the C-terminal domain that carries the characteristic residues forming the active site of the RecB family nucleases. This nuclease domain is found in many enzymes with potential functions in DNA replication and/or repair (Aravind et al., 2000). For instance, the DUF91 family members are found in euryarchaeota (59 homologues annotated in 2011), crenarchaeota (33 homologues), actinobacteria (259 homologues), and proteobacteria (41 homologues). Up to date, no eukaryotic member of the DUF91 family has been identified.

5.2 Structure of Pab2263-NucS

Crystallographic structure of Pab2263 has been solved (Ren et al., 2009; Ren et al., 2007) and is the first representative of the DUF91 family. Pab2263 is composed of two independent domains, separated by a long and flexible linker (~28Å). This multi-domain organisation is common for many nuclease domains which are often associated with helicase domains (Rouillon and White, 2010).
The C-terminal domain of this protein family displays the minimal endonuclease fold (Pingoud et al., 2005): an α/β structure composed of a five-stranded β-sheet and four flanking α helices. Active site is represented by a sequence motif conserved in the RecB-like nucleases (Aravind et al., 2000): E----[Gh]xxD----hxhh[ED]hK---QhxxY, where 'h' refers to hydrophobic residues (YFWLIVMA) and '---' indicates that the characteristic residues are not consecutive in the sequence. A conserved patch of four basic residues flanks one side of the cleft of the active site and might be involved in the binding of nucleic acids.
Strikingly, the N-terminal domain of Pab2263 displays a half-closed β-barrel, composed of height β-strands arranged in two antiparallel β-sheets. This fold was never previously

described, but can remotely be seen as a structural homologue of the OB- or the Sm-folds, two folds that are involved in the binding of nucleic acid (Kambach et al., 1999; Theobald et al., 2003). In Pab2263, the potential binding site involves two patches of three consecutive basic residues, two conserved aromatic residues and a conserved arginine. High affinity ssDNA binding activity of the N-terminal domain was desmonstrated using site-directed mutagenesis and EMSA experiments (Ren et al., 2009).

The N-terminal domain displays a large hydrophobic patch exposed to the C-terminal domain, and is involved in the dimerisation of the protein. Dimer formation brings one extra residue of one monomer to the active site of the second monomer and the flexible linker cap the active site. As a result, the active site becomes a 'closed' channel, which indicates that the substrate for the enzyme must have a free end.

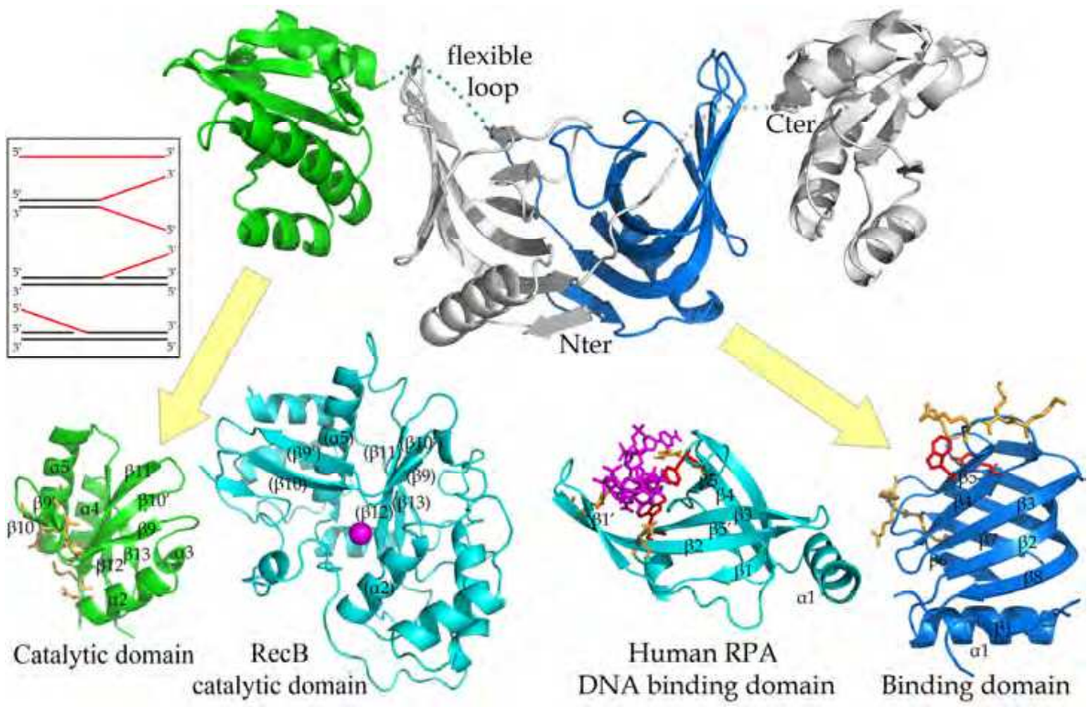


Fig. 3. The structure of Pab2263-NucS. Up: overall organisation of the NucS dimer, one protein is depicted in grey, the other in shades of blue. Down: comparison of the fold of each domain with the RecB one and the RPA one. Structural features of RecB are numbered in the same scheme as NucS. Residues of RPA and NucS involved in the binding of the nucleic acids are detailed; orange and red are respectively basic and aromatic residues. Inlet: range of substrates processed by NucS, cleaved part is indicated in red.

5.3 Activity of the Pab2263-NucS protein

Tests of various substrates on Pab2263 reveal its surprisingly broad range of substrate specificity. In agreement with the structural data indicating that the active site of the Pab2263 is located in the closed channel, this protein preferentially cleaves single stranded DNA, and was thus renamed 'NucS', for 'NUClease specific for single-stranded DNA'. Under stoechiometric binding conditions, single-stranded regions of splayed arms, 3' flaps and 5' flaps are all cleaved by NucS, leaving only double-stranded products. Long single

stranded DNA substrates are cleaved to regularly spaced products, which suggests that the protein could somehow measure its distance to the DNA end.
In high concentration of NucS, nuclease activity can invade to the double stranded DNA regions, suggesting that NucS proteins carry a weak helicase and/or unmelting activity of dsDNA. Important observation is that addition of PCNA directs the cleavage activity of *P. abyssi* NucS towards the ss/ds DNA junctions, thus increasing the cleavage specificity (Ren et al., 2009).
Pab2263-NucS is a founding member of a new family of structure-specific DNA endonuclease. The discovery of this novel nuclease family thus further indicates that archea contain many more nucleases than previously expected on the basis of search of homologous of 'conventional' eukaryotic or bacterial nucleases. For example, the Bax1 and GAN (GINS Associated Nuclease) nucleases from the DUF790 and Phosphoesterase RecJ-like families, respectively, have been identified in a similar manner (Li et al., 2011; Richards et al., 2008; Roth et al., 2009; Rouillon and White, 2010).

6. Conclusion

DNA repair pathways require the function of nucleases to ensure the removal of damages in DNA and thus integrity of the genetic information. The molecular mechanisms of the archaeal DNA nucleases reviewed in this work clearly underscore the conservation of the genetic information processing in archaea and eukarya. Whereas considerable amount of biochemical and structural data are available for archaeal DNA repair nucleases, their physiological roles remain less understood. More biochemical and genetic studies to investigate physiological functions of classical and recently discovered archaeal nucleases have clearly a strong potential to contribute to understanding complexity of eukaryotic DNA repair in the cellular context.

7. Acknowledgments

D.F., H.M, R.L., and C.R., were supported by grant ANR-07-BLAN-0371 from the National Research Agency. G.H., was supported by grant ANR-10-JC from the National Research Agency. H.M and R.L. also acknowledge support from the ANR Project RETYD(Y)DNA.

8. References

Ahmad, A., Robinson, A.R., Duensing, A., van Drunen, E., Beverloo, H.B., Weisberg, D.B., Hasty, P., Hoeijmakers, J.H., and Niedernhofer, L.J. (2008). ERCC1-XPF endonuclease facilitates DNA double-strand break repair. *Mol Cell Biol* 28, 5082-5092.

Al-Minawi, A.Z., Saleh-Gohari, N., and Helleday, T. (2008). The ERCC1/XPF endonuclease is required for efficient single-strand annealing and gene conversion in mammalian cells. *Nucleic Acids Res* 36, 1-9.

Aravind, L., Makarova, K.S., and Koonin, E.V. (2000). SURVEY AND SUMMARY: holliday junction resolvases and related nucleases: identification of new families, phyletic distribution and evolutionary trajectories. *Nucleic Acids Res* 28, 3417-3432.

Arthur, L.M., Gustausson, K., Hopfner, K.P., Carson, C.T., Stracker, T.H., Karcher, A., Felton, D., Weitzman, M.D., Tainer, J., and Carney, J.P. (2004). Structural and functional analysis of Mre11-3. *Nucleic Acids Res* 32, 1886-1893.

Arudchandran, A., Cerritelli, S., Narimatsu, S., Itaya, M., Shin, D.Y., Shimada, Y., and Crouch, R.J. (2000). The absence of ribonuclease H1 or H2 alters the sensitivity of Saccharomyces cerevisiae to hydroxyurea, caffeine and ethyl methanesulphonate: implications for roles of RNases H in DNA replication and repair. *Genes Cells* 5, 789-802.

Bardwell, A.J., Bardwell, L., Tomkinson, A.E., and Friedberg, E.C. (1994). Specific cleavage of model recombination and repair intermediates by the yeast Rad1-Rad10 DNA endonuclease. *Science* 265, 2082-2085.

Bentchikou, E., Servant, P., Coste, G., and Sommer, S. (2007). Additive effects of SbcCD and PolX deficiencies in the in vivo repair of DNA double-strand breaks in Deinococcus radiodurans. *J Bacteriol* 189, 4784-4790.

Bhaskara, V., Dupre, A., Lengsfeld, B., Hopkins, B.B., Chan, A., Lee, J.H., Zhang, X., Gautier, J., Zakian, V., and Paull, T.T. (2007). Rad50 adenylate kinase activity regulates DNA tethering by Mre11/Rad50 complexes. *Mol Cell* 25, 647-661.

Boddy, M.N., Gaillard, P.H., McDonald, W.H., Shanahan, P., Yates, J.R., 3rd, and Russell, P. (2001). Mus81-Eme1 are essential components of a Holliday junction resolvase. *Cell* 107, 537-548.

Bubeck, D., Reijns, M.A., Graham, S.C., Astell, K.R., Jones, E.Y., and Jackson, A.P. (2011). PCNA directs type 2 RNase H activity on DNA replication and repair substrates. *Nucleic Acids Res*. 39, 3652-3666

Cerritelli, S.M., and Crouch, R.J. (2009). Ribonuclease H: the enzymes in eukaryotes. *Febs J* 276, 1494-1505.

Cerritelli, S.M., Frolova, E.G., Feng, C., Grinberg, A., Love, P.E., and Crouch, R.J. (2003). Failure to produce mitochondrial DNA results in embryonic lethality in Rnaseh1 null mice. *Mol Cell* 11, 807-815.

Chahwan, C., Nakamura, T.M., Sivakumar, S., Russell, P., and Rhind, N. (2003). The fission yeast Rad32 (Mre11)-Rad50-Nbs1 complex is required for the S-phase DNA damage checkpoint. *Mol Cell Biol* 23, 6564-6573.

Chai, Q., Qiu, J., Chapados, B.R., and Shen, B. (2001). Archaeoglobus fulgidus RNase HII in DNA replication: enzymological functions and activity regulation via metal cofactors. *Biochem Biophys Res Commun* 286, 1073-1081.

Chapados, B.R., Chai, Q., Hosfield, D.J., Qiu, J., Shen, B., and Tainer, J.A. (2001). Structural biochemistry of a type 2 RNase H: RNA primer recognition and removal during DNA replication. *J Mol Biol* 307, 541-556.

Chen, X.B., Melchionna, R., Denis, C.M., Gaillard, P.H., Blasina, A., Van de Weyer, I., Boddy, M.N., Russell, P., Vialard, J., and McGowan, C.H. (2001). Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. *Mol Cell* 8, 1117-1127.

Chon, H., Vassilev, A., DePamphilis, M.L., Zhao, Y., Zhang, J., Burgers, P.M., Crouch, R.J., and Cerritelli, S.M. (2009). Contributions of the two accessory subunits, RNASEH2B and RNASEH2C, to the activity and properties of the human RNase H2 complex. *Nucleic Acids Res* 37, 96-110.

Ciccia, A., Constantinou, A., and West, S.C. (2003). Identification and characterization of the human mus81-eme1 endonuclease. *J Biol Chem* 278, 25172-25178.

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

Collis, S.J., Ciccia, A., Deans, A.J., Horejsi, Z., Martin, J.S., Maslen, S.L., Skehel, J.M., Elledge, S.J., West, S.C., and Boulton, S.J. (2008). FANCM and FAAP24 function in ATR-

mediated checkpoint signaling independently of the Fanconi anemia core complex. *Mol Cell* 32, 313-324.

Connelly, J.C., de Leau, E.S., and Leach, D.R. (2003). Nucleolytic processing of a protein-bound DNA end by the E. coli SbcCD (MR) complex. *DNA Repair (Amst)* 2, 795-807.

Connelly, J.C., Kirkham, L.A., and Leach, D.R. (1998). The SbcCD nuclease of Escherichia coli is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proc Natl Acad Sci U S A* 95, 7969-7974.

Constantinesco, F., Forterre, P., and Elie, C. (2002). NurA, a novel 5'-3' nuclease gene linked to rad50 and mre11 homologs of thermophilic Archaea. *EMBO Rep* 3, 537-542.

Constantinesco, F., Forterre, P., Koonin, E.V., Aravind, L., and Elie, C. (2004). A bipolar DNA helicase gene, herA, clusters with rad50, mre11 and nurA genes in thermophilic archaea. *Nucleic Acids Res* 32, 1439-1447.

Crow, Y.J., Leitch, A., Hayward, B.E., Garner, A., Parmar, R., Griffith, E., Ali, M., Semple, C., Aicardi, J., Babul-Hirji, R., *et al.* (2006). Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutieres syndrome and mimic congenital viral brain infection. *Nat Genet* 38, 910-916.

D'Amours, D., and Jackson, S.P. (2002). The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. *Nat Rev Mol Cell Biol* 3, 317-327.

Das, D., Moiani, D., Axelrod, H.L., Miller, M.D., McMullan, D., Jin, K.K., Abdubek, P., Astakhova, T., Burra, P., Carlton, D., *et al.* (2010). Crystal structure of the first eubacterial Mre11 nuclease reveals novel features that may discriminate substrates during DNA repair. *J Mol Biol* 397, 647-663.

de Jager, M., Trujillo, K.M., Sung, P., Hopfner, K.P., Carney, J.P., Tainer, J.A., Connelly, J.C., Leach, D.R., Kanaar, R., and Wyman, C. (2004). Differential arrangements of conserved building blocks among homologs of the Rad50/Mre11 DNA repair protein complex. *J Mol Biol* 339, 937-949.

de los Santos, T., Hunter, N., Lee, C., Larkin, B., Loidl, J., and Hollingsworth, N.M. (2003). The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. *Genetics* 164, 81-94.

Delmas, S., Shunburne, L., Ngo, H.P., and Allers, T. (2009). Mre11-Rad50 promotes rapid repair of DNA damage in the polyploid archaeon Haloferax volcanii by restraining homologous recombination. *PLoS Genet* 5, e1000552.

Dinant, C., van Royen, M.E., Vermeulen, W., and Houtsmuller, A.B. (2008). Fluorescence resonance energy transfer of GFP and YFP by spectral imaging and quantitative acceptor photobleaching. *J Microsc* 231, 97-104.

DiRuggiero, J., Santangelo, N., Nackerdien, Z., Ravel, J., and Robb, F.T. (1997). Repair of extensive ionizing-radiation DNA damage at 95 degrees C in the hyperthermophilic archaeon Pyrococcus furiosus. *J Bacteriol* 179, 4643-4645.

Doe, C.L., Osman, F., Dixon, J., and Whitby, M.C. (2004). DNA repair by a Rad22-Mus81-dependent pathway that is independent of Rhp51. *Nucleic Acids Res* 32, 5570-5581.

Eder, P.S., and Walder, J.A. (1991). Ribonuclease H from K562 human erythroleukemia cells. Purification, characterization, and substrate specificity. *J Biol Chem* 266, 6472-6479.

Ehmsen, K.T., and Heyer, W.D. (2008). Saccharomyces cerevisiae Mus81-Mms4 is a catalytic, DNA structure-selective endonuclease. *Nucleic Acids Res* 36, 2182-2195.

Filippov, V., Filippov, M., and Gill, S.S. (2001). Drosophila RNase H1 is essential for development but not for proliferation. *Mol Genet Genomics* 265, 771-777.

Franchitto, A., Pirzio, L.M., Prosperi, E., Sapora, O., Bignami, M., and Pichierri, P. (2008). Replication fork stalling in WRN-deficient cells is overcome by prompt activation of a MUS81-dependent pathway. *J Cell Biol* 183, 241-252.

Frank, P., Albert, S., Cazenave, C., and Toulme, J.J. (1994). Purification and characterization of human ribonuclease HII. *Nucleic Acids Res* 22, 5247-5254.

Frank, P., Braunshofer-Reiter, C., and Wintersberger, U. (1998a). Yeast RNase H(35) is the counterpart of the mammalian RNase HI, and is evolutionarily related to prokaryotic RNase HII. *FEBS Lett* 421, 23-26.

Frank, P., Braunshofer-Reiter, C., Wintersberger, U., Grimm, R., and Busen, W. (1998b). Cloning of the cDNA encoding the large subunit of human RNase HI, a homologue of the prokaryotic RNase HII. *Proc Natl Acad Sci U S A* 95, 12872-12877.

Froget, B., Blaisonneau, J., Lambert, S., and Baldacci, G. (2008). Cleavage of stalled forks by fission yeast Mus81/Eme1 in absence of DNA replication checkpoint. *Mol Biol Cell* 19, 445-456.

Fujikane, R., Ishino, S., Ishino, Y., and Forterre, P. (2010). Genetic analysis of DNA repair in the hyperthermophilic archaeon, Thermococcus kodakaraensis. *Genes Genet Syst* 85, 243-257.

Fukushima, S., Itaya, M., Kato, H., Ogasawara, N., and Yoshikawa, H. (2007). Reassessment of the in vivo functions of DNA polymerase I and RNase H in bacterial cell growth. *J Bacteriol* 189, 8575-8583.

Gaillard, P.H., Noguchi, E., Shanahan, P., and Russell, P. (2003). The endogenous Mus81-Eme1 complex resolves Holliday junctions by a nick and counternick mechanism. *Mol Cell* 12, 747-759.

Gari, K., Decaillet, C., Delannoy, M., Wu, L., and Constantinou, A. (2008). Remodeling of DNA replication structures by the branch point translocase FANCM. *Proc Natl Acad Sci U S A* 105, 16107-16112.

Gaskell, L.J., Osman, F., Gilbert, R.J., and Whitby, M.C. (2007). Mus81 cleavage of Holliday junctions: a failsafe for processing meiotic recombination intermediates? *EMBO J* 26, 1891-1901.

Gerard, E., Jolivet, E., Prieur, D., and Forterre, P. (2001). DNA protection mechanisms are not involved in the radioresistance of the hyperthermophilic archaea Pyrococcus abyssi and P. furiosus. *Mol Genet Genomics* 266, 72-78.

Grogan, D.W., Carver, G.T., and Drake, J.W. (2001). Genetic fidelity under harsh conditions: analysis of spontaneous mutation in the thermoacidophilic archaeon Sulfolobus acidocaldarius. *Proc Natl Acad Sci U S A* 98, 7928-7933.

Hanada, K., Budzowska, M., Davies, S.L., van Drunen, E., Onizawa, H., Beverloo, H.B., Maas, A., Essers, J., Hickson, I.D., and Kanaar, R. (2007). The structure-specific endonuclease Mus81 contributes to replication restart by generating double-strand DNA breaks. *Nat Struct Mol Biol* 14, 1096-1104.

Haruki, M., Hayashi, K., Kochi, T., Muroya, A., Koga, Y., Morikawa, M., Imanaka, T., and Kanaya, S. (1998). Gene cloning and characterization of recombinant RNase HII from a hyperthermophilic archaeon. *J Bacteriol* 180, 6207-6214.

Hopfner, K.P., Craig, L., Moncalian, G., Zinkel, R.A., Usui, T., Owen, B.A., Karcher, A., Henderson, B., Bodmer, J.L., McMurray, C.T., *et al.* (2002a). The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature* 418, 562-566.

Hopfner, K.P., Karcher, A., Craig, L., Woo, T.T., Carney, J.P., and Tainer, J.A. (2001). Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* 105, 473-485.

Hopfner, K.P., Karcher, A., Shin, D., Fairley, C., Tainer, J.A., and Carney, J.P. (2000a). Mre11 and Rad50 from Pyrococcus furiosus: cloning and biochemical characterization reveal an evolutionarily conserved multiprotein machine. *J Bacteriol* 182, 6036-6041.

Hopfner, K.P., Karcher, A., Shin, D.S., Craig, L., Arthur, L.M., Carney, J.P., and Tainer, J.A. (2000b). Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* 101, 789-800.

Hopfner, K.P., Putnam, C.D., and Tainer, J.A. (2002b). DNA double-strand break repair from head to tail. *Curr Opin Struct Biol* 12, 115-122.

Hopkins, B.B., and Paull, T.T. (2008). The P. furiosus mre11/rad50 complex promotes 5' strand resection at a DNA double-strand break. *Cell* 135, 250-260.

Horton, N.C., and Finzel, B.C. (1996). The structure of an RNA/DNA hybrid: a substrate of the ribonuclease activity of HIV-1 reverse transcriptase. *J Mol Biol* 264, 521-533.

Hutton, R.D., Craggs, T.D., White, M.F., and Penedo, J.C. (2009). PCNA and XPF cooperate to distort DNA substrates. *Nucleic Acids Res* 38, 1664-1675.

Hutton, R.D., Roberts, J.A., Penedo, J.C., and White, M.F. (2008). PCNA stimulates catalysis by structure-specific nucleases using two distinct mechanisms: substrate targeting and catalytic step. *Nucleic Acids Res* 36, 6720-6727.

Ip, S.C., Rass, U., Blanco, M.G., Flynn, H.R., Skehel, J.M., and West, S.C. (2008). Identification of Holliday junction resolvases from humans and yeast. *Nature* 456, 357-361.

Itaya, M., Omori, A., Kanaya, S., Crouch, R.J., Tanaka, T., and Kondo, K. (1999). Isolation of RNase H genes that are essential for growth of Bacillus subtilis 168. *J Bacteriol* 181, 2118-2123.

Jeong, H.S., Backlund, P.S., Chen, H.C., Karavanov, A.A., and Crouch, R.J. (2004). RNase H2 of Saccharomyces cerevisiae is a complex of three proteins. *Nucleic Acids Res* 32, 407-414.

Jessop, L., and Lichten, M. (2008). Mus81/Mms4 endonuclease and Sgs1 helicase collaborate to ensure proper recombination intermediate metabolism during meiosis. *Mol Cell* 31, 313-323.

Jolivet, E., L'Haridon, S., Corre, E., Forterre, P., and Prieur, D. (2003a). Thermococcus gammatolerans sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *Int J Syst Evol Microbiol* 53, 847-851.

Jolivet, E., Matsunaga, F., Ishino, Y., Forterre, P., Prieur, D., and Myllykallio, H. (2003b). Physiological responses of the hyperthermophilic archaeon "Pyrococcus abyssi" to DNA damage caused by ionizing radiation. *J Bacteriol* 185, 3958-3961.

Kai, M., Boddy, M.N., Russell, P., and Wang, T.S. (2005). Replication checkpoint kinase Cds1 regulates Mus81 to preserve genome integrity during replication stress. *Genes Dev* 19, 919-932.

Kaliraman, V., Mullen, J.R., Fricke, W.M., Bastin-Shanower, S.A., and Brill, S.J. (2001). Functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease. *Genes Dev* 15, 2730-2740.

Kambach, C., Walke, S., Young, R., Avis, J.M., de la Fortelle, E., Raker, V.A., Luhrmann, R., Li, J., and Nagai, K. (1999). Crystal structures of two Sm protein complexes and their implications for the assembly of the spliceosomal snRNPs. *Cell* 96, 375-387.

Kim, J.M., Kee, Y., Gurtan, A., and D'Andrea, A.D. (2008). Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24. *Blood* 111, 5215-5222.

Kish, A., and DiRuggiero, J. (2008). Rad50 is not essential for the Mre11-dependent repair of DNA double-strand breaks in Halobacterium sp. strain NRC-1. *J Bacteriol* 190, 5210-5216.

Komori, K., Fujikane, R., Shinagawa, H., and 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., and 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.

Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D., and Rehrauer, W.M. (1994). Biochemistry of homologous recombination in Escherichia coli. *Microbiol Rev* 58, 401-465.

Lai, L., Yokota, H., Hung, L.W., Kim, R., and Kim, S.H. (2000). Crystal structure of archaeal RNase HII: a homologue of human major RNase H. *Structure* 8, 897-904.

Le Laz, S., Le Goaziou, A., and Henneke, G. (2010). Structure-specific nuclease activities of Pyrococcus abyssi RNase HII. *J Bacteriol* 192, 3689-3698.

Lestini, R., Duan, Z., and Allers, T. (2010). 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. *DNA Repair (Amst)* 9, 994-1002.

Li, Z., Pan, M., Santangelo, T.J., Chemnitz, W., Yuan, W., Edwards, J.L., Hurwitz, J., Reeve, J.N., and Kelman, Z. (2011). A novel DNA nuclease is stimulated by association with the GINS complex. *Nucleic Acids Res*.

Ma, J.L., Kim, E.M., Haber, J.E., and Lee, S.E. (2003). Yeast Mre11 and Rad1 proteins define a Ku-independent mechanism to repair double-strand breaks lacking overlapping end sequences. *Mol Cell Biol* 23, 8820-8828.

Maga, G., and Hubscher, U. (2003). Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *J Cell Sci* 116, 3051-3060.

Maiti, R., Van Domselaar, G.H., Zhang, H., and Wishart, D.S. (2004). SuperPose: a simple server for sophisticated structural superposition. *Nucleic Acids Res* 32, W590-594.

Manzan, A., Pfeiffer, G., Hefferin, M.L., Lang, C.E., Carney, J.P., and Hopfner, K.P. (2004). MlaA, a hexameric ATPase linked to the Mre11 complex in archaeal genomes. *EMBO Rep* 5, 54-59.

Mascarenhas, J., Sanchez, H., Tadesse, S., Kidane, D., Krisnamurthy, M., Alonso, J.C., and Graumann, P.L. (2006). Bacillus subtilis SbcC protein plays an important role in DNA inter-strand cross-link repair. *BMC Mol Biol* 7, 20.

Matulova, P., Marini, V., Burgess, R.C., Sisakova, A., Kwon, Y., Rothstein, R., Sung, P., and Krejci, L. (2009). Cooperativity of Mus81.Mms4 with Rad54 in the resolution of recombination and replication intermediates. *J Biol Chem* 284, 7733-7745.

Meetei, A.R., Medhurst, A.L., Ling, C., Xue, Y., Singh, T.R., Bier, P., Steltenpool, J., Stone, S., Dokal, I., Mathew, C.G., *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.

Meslet-Cladiere, L., Norais, C., Kuhn, J., Briffotaux, J., Sloostra, J.W., Ferrari, E., Hubscher, U., Flament, D., and Myllykallio, H. (2007). A novel proteomic approach identifies new interaction partners for proliferating cell nuclear antigen. *J Mol Biol* 372, 1137-1148.

Mimitou, E.P., and Symington, L.S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455, 770-774.

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

Muroya, A., Tsuchiya, D., Ishikawa, M., Haruki, M., Morikawa, M., Kanaya, S., and Morikawa, K. (2001). Catalytic center of an archaeal type 2 ribonuclease H as revealed by X-ray crystallographic and mutational analyses. *Protein Sci* 10, 707-714.

Newman, M., Murray-Rust, J., Lally, J., Rudolf, J., Fadden, A., Knowles, P.P., White, M.F., and McDonald, N.Q. (2005). Structure of an XPF endonuclease with and without DNA suggests a model for substrate recognition. *EMBO J* 24, 895-905.

Nick McElhinny, S.A., Kumar, D., Clark, A.B., Watt, D.L., Watts, B.E., Lundstrom, E.B., Johansson, E., Chabes, A., and Kunkel, T.A. (2010a). Genome instability due to ribonucleotide incorporation into DNA. *Nat Chem Biol* 6, 774-781.

Nick McElhinny, S.A., Watts, B.E., Kumar, D., Watt, D.L., Lundstrom, E.B., Burgers, P.M., Johansson, E., Chabes, A., and Kunkel, T.A. (2010b). Abundant ribonucleotide incorporation into DNA by yeast replicative polymerases. *Proc Natl Acad Sci U S A* 107, 4949-4954.

Nishino, T., Komori, K., Ishino, Y., and Morikawa, K. (2003). X-ray and biochemical anatomy of an archaeal XPF/Rad1/Mus81 family nuclease: similarity between its endonuclease domain and restriction enzymes. *Structure* 11, 445-457.

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

Nishino, T., Komori, K., Tsuchiya, D., Ishino, Y., and Morikawa, K. (2005b). Crystal structure and functional implications of Pyrococcus furiosus hef helicase domain involved in branched DNA processing. *Structure* 13, 143-153.

Nowotny, M., and Yang, W. (2009). Structural and functional modules in RNA interference. *Curr Opin Struct Biol* 19, 286-293.

Oh, S.D., Lao, J.P., Taylor, A.F., Smith, G.R., and Hunter, N. (2008). RecQ helicase, Sgs1, and XPF family endonuclease, Mus81-Mms4, resolve aberrant joint molecules during meiotic recombination. *Mol Cell* 31, 324-336.

Ohtani, N., Haruki, M., Morikawa, M., Crouch, R.J., Itaya, M., and Kanaya, S. (1999a). Identification of the genes encoding Mn2+-dependent RNase HII and Mg2+-dependent RNase HIII from Bacillus subtilis: classification of RNases H into three families. *Biochemistry* 38, 605-618.

Ohtani, N., Haruki, M., Morikawa, M., and Kanaya, S. (1999b). Molecular diversities of RNases H. *J Biosci Bioeng* 88, 12-19.

Ohtani, N., Haruki, M., Muroya, A., Morikawa, M., and Kanaya, S. (2000). Characterization of ribonuclease HII from Escherichia coli overproduced in a soluble form. *J Biochem* 127, 895-899.

Ohtani, N., Tomita, M., and Itaya, M. (2008). Junction ribonuclease: a ribonuclease HII orthologue from Thermus thermophilus HB8 prefers the RNA-DNA junction to the RNA/DNA heteroduplex. *Biochem J* 412, 517-526.

Osman, F., Dixon, J., Doe, C.L., and Whitby, M.C. (2003). Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. *Mol Cell* 12, 761-774.

Palud, A., Villani, G., L'Haridon, S., Querellou, J., Raffin, J.P., and Henneke, G. (2008). Intrinsic properties of the two replicative DNA polymerases of Pyrococcus abyssi

in replicating abasic sites: possible role in DNA damage tolerance? *Mol Microbiol* 70, 746-761.

Pingoud, A., Fuxreiter, M., Pingoud, V., and Wende, W. (2005). Type II restriction endonucleases: structure and mechanism. *Cell Mol Life Sci* 62, 685-707.

Quaiser, A., Constantinesco, F., White, M.F., Forterre, P., and Elie, C. (2008). The Mre11 protein interacts with both Rad50 and the HerA bipolar helicase and is recruited to DNA following gamma irradiation in the archaeon Sulfolobus acidocaldarius. *BMC Mol Biol* 9, 25.

Ren, B., Kuhn, J., Meslet-Cladiere, L., Briffotaux, J., Norais, C., Lavigne, R., Flament, D., Ladenstein, R., and Myllykallio, H. (2009). Structure and function of a novel endonuclease acting on branched DNA substrates. *EMBO J* 28, 2479-2489.

Ren, B., Kuhn, J., Meslet-Cladiere, L., Myllykallio, H., and Ladenstein, R. (2007). Crystallization and preliminary X-ray analysis of a RecB-family nuclease from the archaeon Pyrococcus abyssi. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 63, 406-408.

Richards, J.D., Cubeddu, L., Roberts, J., Liu, H., and White, M.F. (2008). The archaeal XPB protein is a ssDNA-dependent ATPase with a novel partner. *J Mol Biol* 376, 634-644.

Roberts, J.A., and White, M.F. (2005). An archaeal endonuclease displays key properties of both eukaryal XPF-ERCC1 and Mus81. *J Biol Chem* 280, 5924-5928.

Rohman, M.S., Koga, Y., Takano, K., Chon, H., Crouch, R.J., and Kanaya, S. (2008). Effect of the disease-causing mutations identified in human ribonuclease (RNase) H2 on the activities and stabilities of yeast RNase H2 and archaeal RNase HII. *Febs J* 275, 4836-4849.

Roseaulin, L., Yamada, Y., Tsutsui, Y., Russell, P., Iwasaki, H., and Arcangioli, B. (2008). Mus81 is essential for sister chromatid recombination at broken replication forks. *EMBO J* 27, 1378-1387.

Roth, H.M., Tessmer, I., Van Houten, B., and Kisker, C. (2009). Bax1 is a novel endonuclease: implications for archaeal nucleotide excision repair. *J Biol Chem* 284, 32272-32278.

Rouillon, C., and White, M.F. (2010). The evolution and mechanisms of nucleotide excision repair proteins. *Res Microbiol* 162, 19-26.

Rumbaugh, J.A., Murante, R.S., Shi, S., and Bambara, R.A. (1997). Creation and removal of embedded ribonucleotides in chromosomal DNA during mammalian Okazaki fragment processing. *J Biol Chem* 272, 22591-22599.

Rychlik, M.P., Chon, H., Cerritelli, S.M., Klimek, P., Crouch, R.J., and Nowotny, M. (2010). Crystal structures of RNase H2 in complex with nucleic acid reveal the mechanism of RNA-DNA junction recognition and cleavage. *Mol Cell* 40, 658-670.

Rydberg, B., and Game, J. (2002). Excision of misincorporated ribonucleotides in DNA by RNase H (type 2) and FEN-1 in cell-free extracts. *Proc Natl Acad Sci U S A* 99, 16654-16659.

Schiestl, R.H., and Prakash, S. (1988). RAD1, an excision repair gene of Saccharomyces cerevisiae, is also involved in recombination. *Mol Cell Biol* 8, 3619-3626.

Shaban, N.M., Harvey, S., Perrino, F.W., and Hollis, T. (2010). The structure of the mammalian RNase H2 complex provides insight into RNA.NA hybrid processing to prevent immune dysfunction. *J Biol Chem* 285, 3617-3624.

Shimura, T., Torres, M.J., Martin, M.M., Rao, V.A., Pommier, Y., Katsura, M., Miyagawa, K., and Aladjem, M.I. (2008). Bloom's syndrome helicase and Mus81 are required to induce transient double-strand DNA breaks in response to DNA replication stress. *J Mol Biol* 375, 1152-1164.

Sijbers, A.M., de Laat, W.L., Ariza, R.R., Biggerstaff, M., Wei, Y.F., Moggs, J.G., Carter, K.C., Shell, B.K., Evans, E., de Jong, M.C., *et al.* (1996). Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. *Cell* 86, 811-822.

Sonoda, E., Hochegger, H., Saberi, A., Taniguchi, Y., and Takeda, S. (2006). Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. *DNA Repair (Amst)* 5, 1021-1029.

Stein, H., and Hausen, P. (1969). Enzyme from calf thymus degrading the RNA moiety of DNA-RNA Hybrids: effect on DNA-dependent RNA polymerase. *Science* 166, 393-395.

Stewart, G.S., Maser, R.S., Stankovic, T., Bressan, D.A., Kaplan, M.I., Jaspers, N.G., Raams, A., Byrd, P.J., Petrini, J.H., and Taylor, A.M. (1999). The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* 99, 577-587.

Stracker, T.H., and Petrini, J.H. (2011). The MRE11 complex: starting from the ends. *Nat Rev Mol Cell Biol* 12, 90-103.

Sun, W., Nandi, S., Osman, F., Ahn, J.S., Jakovleska, J., Lorenz, A., and Whitby, M.C. (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.

Tadokoro, T., and Kanaya, S. (2009). Ribonuclease H: molecular diversities, substrate binding domains, and catalytic mechanism of the prokaryotic enzymes. *Febs J* 276, 1482-1493.

Theobald, D.L., Mitton-Fry, R.M., and Wuttke, D.S. (2003). Nucleic acid recognition by OB-fold proteins. *Annu Rev Biophys Biomol Struct* 32, 115-133.

Vivona, J.B., and Kelman, Z. (2003). The diverse spectrum of sliding clamp interacting proteins. *FEBS Lett* 546, 167-172.

Von Sonntag, C., and Schulte-Frohlinde, D. (1978). Radiation-induced degradation of the sugar in model compounds and in DNA. *Mol Biol Biochem Biophys* 27, 204-226.

Warbrick, E. (1998). PCNA binding through a conserved motif. *Bioessays* 20, 195-199.

Whitby, M.C., Osman, F., and Dixon, J. (2003). Cleavage of model replication forks by fission yeast Mus81-Eme1 and budding yeast Mus81-Mms4. *J Biol Chem* 278, 6928-6935.

White, M.F. (2011). Homologous recombination in the archaea: the means justify the ends. *Biochem Soc Trans*. 39, 5-9.

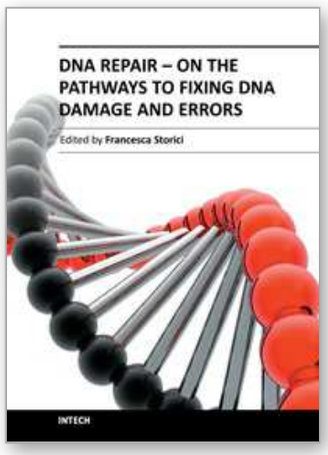
Williams, R.S., Moncalian, G., Williams, J.S., Yamada, Y., Limbo, O., Shin, D.S., Groocock, L.M., Cahill, D., Hitomi, C., Guenther, G., *et al.* (2008). Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell* 135, 97-109.

Williams, R.S., Williams, J.S., and Tainer, J.A. (2007). Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochem Cell Biol* 85, 509-520.

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

Yang, W., and Steitz, T.A. (1995). Recombining the structures of HIV integrase, RuvC and RNase H. *Structure* 3, 131-134.

Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. (2008). Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134, 981-994.



DNA Repair - On the Pathways to Fixing DNA Damage and Errors
Edited by Dr. Francesca Storici

ISBN 978-953-307-649-2
Hard cover, 380 pages
Publisher InTech
Published online 09, September, 2011
Published in print edition September, 2011

DNA repair is fundamental to all cell types to maintain genomic stability. A collection of cutting-edge reviews, DNA Repair - On the pathways to fixing DNA damage and errors covers major aspects of the DNA repair processes in a large variety of organisms, emphasizing foremost developments, questions to be solved and new directions in this rapidly evolving area of modern biology. Written by researchers at the vanguard of the DNA repair field, the chapters highlight the importance of the DNA repair mechanisms and their linkage to DNA replication, cell-cycle progression and DNA recombination. Major topics include: base excision repair, nucleotide excision repair, mismatch repair, double-strand break repair, with focus on specific inhibitors and key players of DNA repair such as nucleases, ubiquitin-proteasome enzymes, poly ADP-ribose polymerase and factors relevant for DNA repair in mitochondria and embryonic stem cells. This book is a journey into the cosmos of DNA repair and its frontiers.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Roxanne Lestini, Christophe Creze, Didier Flament, Hannu Myllykallio and Ghislaine Henneke (2011). Archaeal DNA Repair Nucleases, DNA Repair - On the Pathways to Fixing DNA Damage and Errors, Dr. Francesca Storici (Ed.), ISBN: 978-953-307-649-2, InTech, Available from: http://www.intechopen.com/books/dna-repair-on-the-pathways-to-fixing-dna-damage-and-errors/archaeal-dna-repair-nucleases

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen