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Nucleases of Metallo-β-Lactamase and Protein Phosphatase Families in DNA Repair

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1. Introduction

All living organisms must struggle to maintain genomic integrity and long-term stability in the face of the lesions that are constantly inflicted upon the genome by environmental factors, e.g., genotoxic chemicals, UV light, ionizing radiation (IR), and endogenous factors, e.g., during DNA replication. These various DNA lesions (or injuries) encompass a bewildering array of chemical and physical modifications to the DNA structure that must be repaired to preserve the faithful maintenance of the genome. A prevalent class of DNA lesion consists of a break across both DNA strands, termed double strand break (DSB) (Fig. 1 and Table 1). Only of endogenous origin, about 50 DSBs have been calculated to occur per human cell division (Vilenchik and Knudson 2003). Many of these DSBs are generated by IR, reactive oxygen species, and DNA replication across a nick (Ma, J.L. et al. 2003). If left unrepaired, DSBs can cause dire effects such as gene loss during cell division, chromosomal translocations, increased mutation rates, and carcinogenesis (Khanna and Jackson 2001). The various cellular mechanisms that are collectively referred to as DNA repair include DNA damage detection (or sensing), binding and recruitment of specialized protein complex machinery to the site of damage, signaling, initiation of repair, repair, and resolution of the lesion (Fig. 1).

Central to all DNA repair processes are nucleases, enzymes and enzyme complexes that can cleave DNA either in a sugar specific fashion (e.g., DNA and RNA nucleases) or in a sugar unspecific fashion (Marti and Fleck 2004). Nucleases can be further divided into exonucleases, which remove nucleotides from a free 5′ or 3′ end, and endonucleases, which hydrolyze internal phosphodiester bonds without the requirement for a free end. DNA nucleases, which can cleave single stranded (ss) or double stranded (ds) DNA, cleave a phosphodiester bond between a deoxyribose and a phosphate group, thus producing one cleavage product with a 5′ terminal phosphate group and another product with a 3′ terminal hydroxyl group.

Two kinds of DNA lesions, double strand breaks (DSBs) and interstrand crosslinks (ICLs) (Fig. 1), are significantly dependent on the timely action of DNA nucleases, since the initiating step in the repair pathways of DSBs and ICLs often consists of an exonucleolytic or endonucleolytic cleavage that exposes the substrate for the next DNA repair activity. Without the action of a nuclease, the DNA lesion would stay unrepaired because of chemically inaccessible or sterically blocked DNA intermediates. Therefore, nucleases are an integral part of the cellular mechanisms that have evolved to handle DNA damage. Indeed,

quality repair mechanisms that strive to reconstitute the undamaged, original DNA structure imply that DNA lesion repair, after the initial nucleolytic processing, requires additional factors, minimally DNA synthesis and ligation, but it also can involve a complex sequence of molecular events.

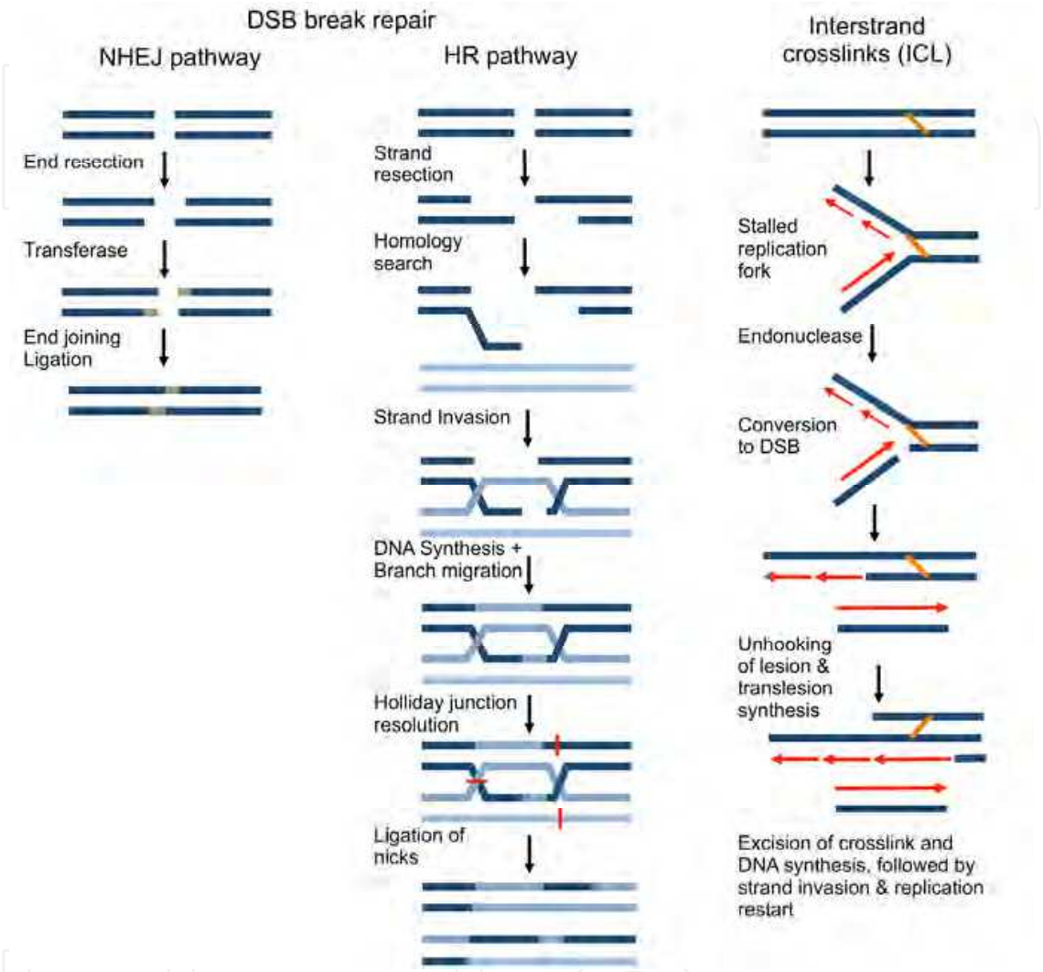

Fig. 1. Schematic of the DNA repair pathways wherein nucleases of the metallo-β-lactamase and protein phosphatase 2B families have been shown to participate.

DNA lesion	DNA Repair Pathway	Nucleases	Fold
DSB	Non-homologous end joining (NHEJ)	Mre11	PP2B
		Artemis	MBL
DSB	Homologous recombination (HR)	Mre11	PP2B
DSB	Microhomology-mediated end joining	Mre11	PP2B
ICL	Repair of interstrand crosslinks (ICL)	SNM1A/B	MBL

Table 1. Nucleases in DNA repair span a growing number of prokaryotic, archeal and eukaryotic exo- and endonucleolytic enzymes with specialized roles in different repair pathways. DSB, double strand break; ICL, interstrand crosslink; MBL, metallo-β-lactamase; PP2B, protein phosphatase 2B.

DSBs are repaired in human cells mainly by two alternative mechanisms, non-homologous end joining (NHEJ) and homologous recombination (HR) (Fig. 1). While HR occurs mostly in S/G2 phase (Takata et al. 1998), when a sister chromatid is available to provide a template to replace the damaged nucleotides, NHEJ, which does not require a template (Ma, J.L. et al. 2003), is prevalent during G1/early S phase (Takata et al. 1998). The sequential steps necessary for NHEJ comprise synapsis (the protein-mediated structure whereby the two ends of a DSBs are tethered, or held together in close proximity, to allow successful repair), end resection (catalyzed by nucleases), DNA synthesis, and ligation (Fig 1). HR requires too an initial exonucleolytic step that consists of the resection of both strands at the DSB end, thus preparing them for the invasion of the neighboring, intact chromatid DNA (Fig. 1). DNA synthesis, branch migration, Holliday junction resolution, and ligation of remaining nicks, are the next steps needed to complete DNA repair by HR. Regardless of the repair pathway used, an exonucleolytic step is always required to provide the DNA substrates for the subsequent repair processes.

A second class of DNA lesions, ICL, can be generated exogenously by mono- or bifunctional alkylating agents (crosslinkers), IR, and endogenously by the collapse of replication forks. A crosslink at a replication fork leads to stalling, since the individual DNA strands can no longer be unwound for DNA synthesis. Repair of an ICL-induced stalled replication fork can be achieved by the series of steps outlined in Fig. 1. Here again, the initial, critical step is an endonucleolytic cleavage catalyzed by DNA nucleases, which helps convert the stalled fork to a DSB-like lesion that is susceptible to repair.

In the following we will survey two families of DNA nucleases that play significant roles in one or various processes involved in the repair of DSB and ICL lesions, from the indispensable initiation of end resection of broken DNA ends to other specialized DNA repair processes such as those in ICL repair. These families, which are structurally and functionally related, are the metallo-β-lactamase (MBL) and the protein phosphatase 2B (PP2B) families. Members of those two families, such as Artemis (MBL) and Mre11 (PP2B), provide essential activities for DNA repair (Table 1). Here, we will discuss the structural and functional properties of the MBL and PP2B nucleases with roles in DNA repair, and will draw a comprehensive portrait of the structural, biochemical, cellular, and evolutionary framework wherein they function.

2. Nucleases of the MBL and PP2B families in DNA repair

Here we summarize current knowledge on two related families of DNA repair nucleases that share significant similarity at the topology, fold structure, active site composition and metal-ion binding: the metallo-β-lactamase (MBL) and the protein phosphatase 2B (PP2B) families (Tables 1, 2 & 3). Despite some degree of functional redundancy, the two families are distinguished by the exact composition and location of the catalytic residues, which explain their distinct functional roles. Likewise, differences in the insertion of accessory domains have allowed the incorporation of substrate-binding domains that widen, or restrict, the range of potential substrates that can be recognized and processed by either class of nucleases.

Both the MBL and PP2B families of nucleases belong to the two-metal-ion-dependent nucleases (Yang 2010), an operational class that encompasses the largest variety of tertiary folds and the broadest range of biological outcomes among the nucleases. The defining feature of these enzymes is the absolute dependence for catalytic competence of an active

center with two metal ions, one of which acts by polarizing the substrate phosphoester whereas the second is more commonly associated with the stabilization of the nascent negative charge on the leaving group. Given the enormous variety of folds and substrate structures, this simple principle (that this class of nucleases are unified by their two-metal-ion dependence) provides an appropriate framework for discussing their structural and mechanistic properties (Yang 2010). Nucleases from the MBL and PP2B families have been implicated in two specific repair pathways of DNA lesions, the repair of DSBs by non-homologous end joining (NHEJ) and the repair of interstrand crosslinks (ICL) (Fig. 1 and Table 1).

2.1 Metallo-β-lactamase fold nucleases

The metallo-β-lactamase (MBL) fold is characterized by a four-layered αβ/βα fold with a wide, shallow active site that is always located on the same side of the fold and that ligands one or, usually, two catalytic metal ions (Aravind 1999; Daiyasu et al. 2001; Callebaut et al. 2002; Dominski 2007). The core of the MBL domain is formed by a β-sandwich of eight β-strands with α-helices packing on both sides. The degree of sequence conservation between MBLs tends to be so low that novel proteins are often only confidently assigned as an MBL once the crystal structure is solved. Despite the low sequence identity between its members, the MBL family presents five sequence motifs that define the active site and provide a signature for the metal binding center (Fig. 2). Motifs 1 and 2 are located in the first β-sheet, and motif 2 comprises the HXHXDH sequence motif that is nearly absolutely conserved among all of the MBLs, and where the first His and Asp residues are completely invariable (Fig. 2). The histidine residues belonging to motifs 3 and 4 are located in the second β-strand; and motif 5 is a cysteine or an acidic residue located at the C terminus.

The MBL family encompasses a large number of enzymes with hydrolytic activities toward a variety of different substrates and, less frequently, oxidorreductases. The best-known hydrolytic MBLs include the zinc-dependent β-lactamases (class B β-lactamases) that hydrolyze β-lactam and non-β-lactam antibiotics (Wang, Z. et al. 1999). Examples of other hydrolytic MBL subfamilies are glyoxylase II, methyl parathion hydrolase, Pce-catalytic domain-like, alkylsulfatase, PqsE-like (all of them zinc-dependent enzymes) and L-ascorbate-6-phosphate lactonase (UlaG) (which is manganese-dependent) (Garces et al. 2010). The ROO N-terminal domain family represents an MBL with oxidorreductase activity. Some MBL families with hydrolase activity act on nucleic acid substrates, predominantly on RNA, such as the β-CASP, arylsulfatase (or RNAse Z-like) and YhfI-like families. Of those, only certain members of the β-CASP family have been demonstrated to use DNA as a substrate, whereas the remaining enzymes display RNase activity. A common trait among the β-CASP MBLs is the presence of an additional domain inserted into the MBL canonical fold, which itself contains unique sequence and structural features (Fig. 2).

The β-CASP motif was first defined by Callebuet et al. (2002), and the name was inspired by the common features that displayed the C termini of certain MBLs that were capable of binding nucleic acid substrates [CPSF-73 (Cleavage and Polyadenylation Specificity Factor-73), Artemis, SNM1 and Pso2]. Interestingly, of the four β-CASP founder sequences three (Artemis, SNM1, and Pso2) are known to bind DNA and to possess nuclease activity that is relevant in DNA repair processes. The β-CASP domain is characterized by three motifs (Fig. 2): (1) motif A, consisting of an acidic residue (D or E) preceded by hydrophobic residues (ϕ) in a pattern typical of β-strands (ϕ-ϕ-ϕ-(D,E)-(T,S)-T); (2) motif B (His); and (3) motif C,

which is a His residue in all the proteins with exception of Artemis/SNM1/Pso2 where it is a Val (Fig. 2). Since the three motifs are located near the active center, the functions of β-CASP are associated with the specific recognition and binding of the substrate. Other proteins from the β-CASP family are CPSF-100, RC-47, RC-68, Snm1B (Apollo) and the ribonucleases RNases J1 and J2 from *Bacillus* spp. (Callebaut et al. 2002; Dominski et al. 2005; Even et al. 2005; Dominski 2007). Several recently published crystal structures of members of the β-CASP family have further confirmed the insertion of the β-CASP domain inside the MBL fold as a α/β domain, which resembles a helicase-like α/β domain without the P-loop motif (Table 2 and Fig. 3).

		Metallo-β-lactamase			
		I	II	III	IV
Human	SNM1A	FTV**D**AF	LT**H**F**H**S**DH**YA	AN**H**CP	ILHTG**D**FR
Yeast	SNM1/Pso2	IVV**D**GF	LS**H**F**H**S**DH**YI	AN**H**CP	ILHTG**D**FR
Human	SNM1B/Apollo	IAV**D**FW	LS**H**F**H**S**DH**YT	AN**H**CP	ILYTG**D**FR
Human	SNM1C/Artemis	ISI**D**RF	LS**H**C**H**K**DH**MK	AG**H**CP	VLYTG**D**FR
Human	CPSF-73	IML**D**CG	IS**H**F**H**L**DH**CG	AG**H**VL	LLYTG**D**FR
E. coli	UlaG	VCV**D**FW	AT**H**D**H**N**DH**ID	AF**D**RT	LYHSG**D**SH
B. cereus	BLM	VLV**D**SS	IT**H**A**H**A**D**RIG	KG**H**TE	ILVCG**C**LV

		β-CASP domain		
		A	B	C
Human	SNM1A	LYL**D**TT	E**H**SSY	IPT**V**N
Yeast	SNM1/Pso2	LYL**D**TT	E**H**SSF	IPT**V**N
Human	SNM1B/Apollo	LYL**D**NT	D**H**SSY	VPI**V**S
Human	SNM1C/Artemis	VYL**D**TT	F**H**SSY	YPN**V**I
Human	CPSF-73	LII**E**ST	A**H**TDY	ILV**H**G

Fig. 2. Characteristic sequence motifs of DNA nucleases of the MBL/β-CASP family.

	β-CASP protein	**Organism**	**PDB ID (Reference)**
EUKARYA	CPSF-73	Human	2I7T (Mandel et al. 2006)
	CPSF-100 (Ydh1p)	Yeast	2I7X (Mandel et al. 2006)
ARCHEA	CPSF subunit PH1404	*P. horikoshii*	3AF5 (Nishida et al. 2010)
	CPSF homolog	*M. mazei*	2XR1 (Mir-Montazeri et al. 2011)
EUBACTERIA	TTHA0252	*Th. termophilus*	2DKF (Ishikawa et al. 2006)
	EF2904	*E. faecalis*	2AZ4 (MSDG)

Table 2. Nucleases of the β-CASP family with known crystal structure. PDB ID, Protein Data Bank Identification code.

β-CASP nucleases, in contrast to conventional MBL amidohydrolases, which degrade the β-lactam moiety of many antibiotics that inhibit cell wall biosynthesis (Wang, Z. et al. 1999),

are nucleases that hydrolyze the phosphodiester bond in RNA or DNA. The nuclease activity can be either endonuclease or 5′- to 3′-exonuclease. Despite the fact that the overall β-CASP fold is reminiscent of the DNase I fold (Callebaut et al. 2002), the number of β-strands and the relative ordering of them in the central β-sheet is different between the β-CASP and the DNase I folds. In keeping with conventional MBLs, β-CASP nucleases appear to have a strong preference for the tight binding of two Zn^{2+} ions regardless of substrate, a selectivity enhanced by the presence of multiple conserved His residues in the catalytic center (Baldwin et al. 1979). As of recent, however, a new MBL has been discovered whose preferred catalytic metal ion is Mn^{2+} rather than Zn^{2+} and that shows some limited phosphodiesterase activity toward cyclic nucleotides (Garces et al. 2010). This raises the interesting possibility that other nucleases with the MBL fold may tolerate (or even prefer) Mn^{2+} in their active sites, a well-known metal catalyst in DNA-dependent nucleases such as Mre11 (a nuclease with the PP2B fold; Table 1).

Even though there is no known crystal structure for DNA nucleases of the β-CASP family, sequence conservation patterns and the accumulated knowledge on structure/function of diverse MBL enzymes lend support to the notion that the overall fold and domain arrangements of β-CASP DNA nucleases will be similar to those of β-CASP RNases (Callebaut et al. 2002). These shared features would include the presence of a β-CASP domain inserted into the two-metal-ion MBL fold scaffold, with motifs A and B of the β-CASP domain lying near the canonical motifs I-IV of MBLs, which configure the active site (Figs. 2-3 and Table 2).

In Archea, homologs of the β-CASP-containing MBL protein CPSF-73 contain a specific N-terminal domain that precedes the MBL domain. This extra N-terminal domain, termed CPSF-KH domain (Nishida et al. 2010; Mir-Montazeri et al. 2011), is composed of two type-II KH-domains (N-KH and C-KH domains) linked by α-helices. As in other proteins where the type-II KH domains are found, the CPSF-KH domain is involved in RNA binding. In *M. mazei* CPSF-KH domain, the N-KH domain is not canonical and therefore shows very low sequence identity to other KH domains (Nishida et al. 2010; Mir-Montazeri et al. 2011); in particular, the signature motif in type-II KH domains (VIGXXG) is only fully conserved in the C-KH domain but not in N-KH domain (Fig. 3).

The first β-CASP/MBL that was found to be involved in DNA repair pathways was the yeast protein Pso2/Snm1 (heretofore Pso2), which was identified using genetic screenings designed to specifically isolate mutants hypersensitive to interstrand crosslinking (ICL) (Henriques and Moustacchi 1980, 1981; Ruhland et al. 1981a; Ruhland et al. 1981b). The two mutant strains found, *SNM1* (Sensitivity to Nitrogen Mustard) and *PSO2* (sensitivity to PSOralen), turned out to be allelic (Cassier-Chauvat and Moustacchi 1988). Several homologous proteins to yeast Pso2 have subsequently been found in higher eukaryotes, all of which constitute the SNM1 family. Two of these proteins, CPSF-73 and ELAC2, are involved in RNA maturation, whereas three additional proteins are known to participate in DNA repair pathways and are DNA nucleases: SNM1A, SNM1B (also known as Apollo), and SNM1C (also known as Artemis) (Demuth and Digweed 1998; Aravind 1999; Dronkert et al. 2000). As evidence accumulates, it points to a functional compartmentalization of SNM1A and SNM1B (being involved in ICL resolution), like yeast Snm1/Pso2, whereas SNM1C has been involved in certain steps of DSB repair (Cattell et al. 2010).

2.1.1 SNM1A/Pso2

In yeast, Pso2 levels are strictly conserved with less than one mRNA molecule per cell; however, upon induction of interstrand crosslinks (ICLs) the amount of *PSO2* message

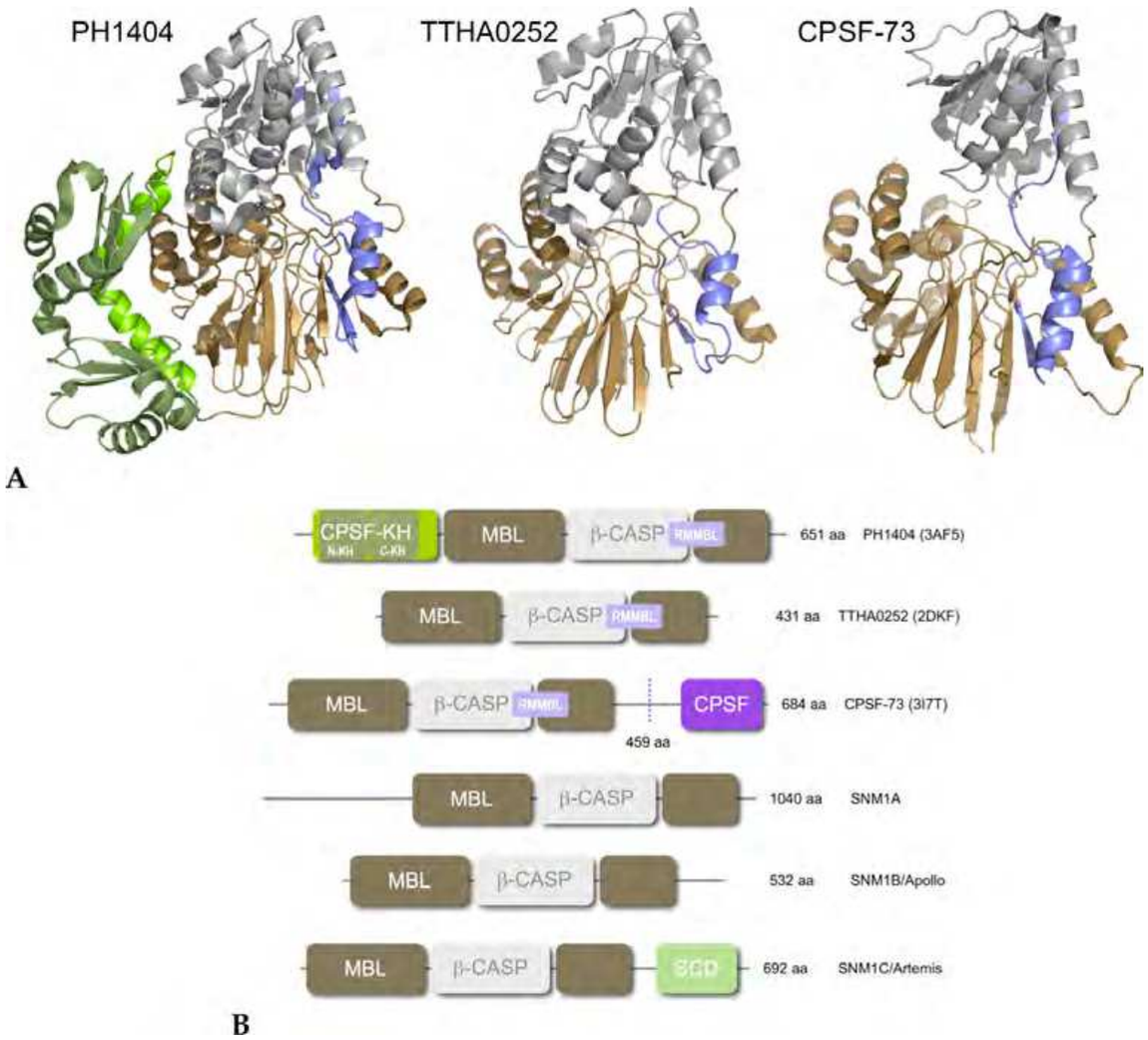

Fig. 3. *A*, Crystal structures of representative nucleases of the β-CASP family (human CPSF-73 and two hypothetical proteins, PH1404 and TTHA0252, from Archea and Bacteria, respectively), shown in ribbons. Different colors highlight the domains and motifs characteristic of the β-CASP nucleases. *B*, Domain organization, including β-CASP DNA nucleases involved in DNA repair, e.g., SNM1A, Apollo, and Artemis. Color coding as in *A*.

becomes dramatically upregulated (Wolter et al. 1996; Lambert et al. 2003). Accordingly, *pso2* mutants exhibit elevated sensitivity to a wide spectrum of crosslinking agents. The nuclease activity most convincingly shown for Pso2 is a 5′- to 3′-exonuclease acting on ssDNA and dsDNA with little preference (Li et al. 2005). The MBL domain of Pso2 is crucial for this activity, since mutation of D252 in motif II (Fig. 2) completely ablates Pso2 function in a manner indistinguishable from the null mutant (Li and Moses 2003). Interestingly, Pso2 appears to play a role in the repair of DSBs associated with ICLs, which result, e.g., from the collapse of stalled replication forks, since *pso2* mutants are greatly incapacitated to repair ICL-associated DSBs (Li and Moses 2003; Barber et al. 2005; Dudas et al. 2007). Furthermore, Pso2, together with two other nucleases, Exo1 and Mre11, have been involved in the processing of IR-induced DSBs (Lam et al. 2008), a function that is provided for partially by

all three nucleases. Since attempts to find potential Pso2 binding partners by two-hybrid screen have thus far failed (Dudas et al. 2007), the functional context where Pso2 acts remains speculative (Cattell et al. 2010).

Of the higher eukaryotic homologs of Pso2, the slightly greater sequence identity and comparatively longer N terminus of SNM1A makes it the closest in terms of sequence and domain structure (Fig. 3). This similarity suggested that SNM1A could be, too, the closest vertebrate ortholog to yeast Pso2, and therefore exhibit similar functions in ICL repair. Even though current evidence partly supports that proposal, one must caution that ICL processing is significantly more complex in vertebrates than in yeast, in part because of the concourse of two complexes [XPF-ERCC1 (De Silva et al. 2000, 2002) and Fanconi anemia proteins (Niedernhofer et al. 2005; Taniguchi and D'Andrea 2006)] that are lacking in yeast. Like yeast Pso2, SNM1A shows 5′- to 3′-exonuclease activity on ssDNA (slightly preferred) and dsDNA and importantly can complement *pso2* mutants in yeast (Hejna et al. 2007; Hazrati et al. 2008). In mammals, the precise role of SNM1A in ICL repair appears to restrict to a specific kind of ICL that originates from mytomicin C (MMC) treatment but not other ICL-inducing agents. Although currently unknown, this selectivity might arise from the fact that these ICLs do not induce large structural distortions of the DNA and could therefore be better detected by transcriptional or replicative stalling, which would render SNM1A's function cell cycle phase and checkpoint arrest specific (Cattell et al. 2010).

2.1.2 SNM1B/APOLLO

Apollo is termed after Artemis (SNM1C; see section 2.1.3) because of their structural and gene sequence similarities (Demuth et al. 2004) (Fig. 2-3). There are two splice variants of *APOLLO*, with the longer Apollo protein sharing 33% sequence identity with yeast Pso2; the second, shorter splice variant has so far resisted functional assignment. Full-length Apollo has 5′- to 3′-exonuclease activity. Aside from, but connected with, Apollo's role in DNA repair, its best-studied role is in telomere maintenance, mainly by interacting with the telomere-associated factor TRF2 (Freibaum and Counter 2006; Lenain et al. 2006; van Overbeek and de Lange 2006). TRF2 is responsible for the recruitment of several protective factors to the telomeres, and forms part of the six-subunit complex Shelterin (consisting of TRF1, TRF2, Rap1, TIN2, TPP1, and POT1), which protects the telomeres from the DNA damage response and therefore maintains their length (van Overbeek and de Lange 2006). In DNA repair, cells depleted of Apollo show various defects upon exposure to ionizing radiation and in the subsequent cellular response to DSBs, including attenuation in the autophorphorylation of ATM and in the phosphorylation of downstream ATM target proteins (Demuth et al. 2008). Part of this effect has an interesting connection with telomere maintenance, since cells depleted of both Apollo and TRF2 suffer increased DNA damage response and growth abnormalities than any of the single mutants (Lenain et al. 2006). Defects in Apollo are felt mainly during S phase, thereby suggesting a role for Apollo principally linked to DNA replication (van Overbeek and de Lange 2006) and associated with the repair of fork-stalling ICLs, much as Pso2 and SNM1A.

2.1.3 SNM1C/ARTEMIS

Artemis is a third vertebrate β-CASP DNA nuclease with roles in DNA repair by the NHEJ pathway (Ma, Y. et al. 2002; Rooney et al. 2003) and, in addition, in V(D)J recombination, a process indispensable for acquired immunity (Rooney et al. 2002). In contrast to Pso2,

SNM1A, and Apollo, Artemis does not appear to be involved in the repair of ICL lesions. As Apollo and SNM1A, Artemis has been described as a 5′- to 3′-exonuclease toward ssDNA and dsDNA (Ma, Y. et al. 2002) in a DNA PKcs independent manner, as well as an endonuclease whose activity is directed toward DNA hairpins and as a 3′- to 5′-exonuclease on DNA overhangs (Ma, Y. et al. 2002; Niewolik et al. 2006). The endonuclease activity on ssDNA appears to be intrinsic to Artemis (Gu et al. 2010; Pawelczak and Turchi 2010) and regulatable by DNA PKcs (Huang et al. 2009; Gu et al. 2010; Pawelczak and Turchi 2010).
An area that is hotly debated concerns the activation of Artemis upon DNA damage. Early studies suggested that the activation of Artemis depended on its phosphorylation by DNA PKcs, on the basis that Artemis has eleven Ser and Thr residues that are phosphorylatable in vitro (Ma, Y. et al. 2002; Niewolik et al. 2006). More recently, it has been shown that DNA cleavage by Artemis could be facilitated by a hypothetical DNA conformational change upon DNA PKcs autophosphorylation (Goodarzi et al. 2006; Yannone et al. 2008; Gu et al. 2010). Another element of discussion is whether Artemis has one single active site that is responsible for both the exonucleolytic and the endonucleolytic activity, or there are two separate, though partially overlapping, active sites for each of these activities. This question is based on the fact that mutants of Artemis impair only the endonuclease activity but have no consequences for Artemis exonuclease activity (Ma, Y. et al. 2002; Pannicke et al. 2004); strikingly, not even an Asp736 mutant of Artemis, a mutant that losses activity in all other SNM1 family members, compromises the 5′- to 3′-exonuclease activity. The latter, and the fact that two (even partially) separated active sites could coexist in a β-CASP nuclease, would make Artemis completely unique in the MBL superfamily. Even more recently, the assignment of 5′- to 3′-exonuclease activity has been called into question, as this activity could not be detected in further purified samples of Artemis (Pawelczak and Turchi 2010).
Efforts to clarify which roles does Artemis play in DNA repair have provided two sound answers. First, failure of Artemis deficient cells to show defects upon exposure to ICL inducing chemicals dispels a potential role for Artemis in the repair of ICL lesions. Instead, Artemis nuclease activity has been shown to be involved in the repair of a subgroup of DSBs (10–15%) produced by IR that contain covalently modified ends refractory to direct repair by other nucleases (Riballo et al. 2004; Wang, J. et al. 2005; Darroudi et al. 2007). The processing by Artemis of those 'blocked' DSBs so that they become accessible to downstream DNA repair machinery would be fitting with the known ability of Artemis-DNA PKcs to process 5′ or 3′ overhangs, hairpins, loops, gaps, or flaps, within DNA (Ma, Y. et al. 2005), as well as oxidation lesions at DNA ends (Povirk et al. 2007). In fact, DNA PKcs has been demonstrated to recruit Artemis to DSB sites, especially in heterochromatin, where DNA PKcs could modify the DNA structure so as to facilitate cleavage by Artemis (Goodarzi et al. 2006). At DSBs, Artemis collaborates with ATM to promote DSB repair by two different pathways, NHEJ at G0/G1 phase and HR at G2 phase (Goodarzi et al. 2006).

2.2 Protein phosphatase fold nucleases (Mre11)

The most prominent DNA nuclease of the protein phosphatase 2B (PP2B) fold is Mre11 (Meiotic recombination 11) (Gueguen et al. 2001), which is one of the central nucleases for the repair of DSBs by the non-homologous end joining (NHEJ) and homologous recombination (HR) repair pathways. Phylogenetic analyses show that Mre11 is conserved across the tree of life, likely because of its vital functionality in DNA repair. Mre11 contains five conserved motifs (shared with some structurally related polymerase small subunits), including a two-metal-ion-binding site that has a strong preference for manganese (Gueguen et al. 2001) and is

essential for catalysis in the archeal, yeast, and human enzymes. At least in vitro, Mre11 exhibits the following enzymatic activities: ssDNA endonuclease, dsDNA 3′- to 5′-exonuclase, DNA hairpin cleaving (Hopfner et al. 2001), and activation of DNA checkpoint kinase (ATM in humans, Tel1 in yeast) (Williams, R.S. et al. 2008). Mn^{2+} is required for all these activities, and interaction of Mre11 with Rad50 is necessary for dsDNA 3′- to 5′-exonuclease and cleaving DNA hairpins (Hopfner et al. 2001; Ghosal and Muniyappa 2005; Williams, R.S. et al. 2008). Besides, Mre11 has been observed to participate in 5′ to 3′ end resection of DSBs in vivo (Williams, R.S. et al. 2007), although the precise mechanism remains to be completely elucidated. A current working model involves other enzymes with nuclease or helicase activity in addition to Mre11, like Sae2, Exo1, Dna2, or Sgs1 (Zhu et al. 2008). The cooperation between these enzymes is supported by the observation of a reduced 5′- to 3′-exonucleolytic activity in cells lacking Exo1 and a complete ablation of this activity when Exo1, Sae2, and the MRX complex are all absent (Zhu et al. 2008). It appears that Mre11, together with Sae2, initiates DSB resection by facilitating trimming of 5′ ends, which can then be degraded by Exo1 or Dna2, in collaboration with the Sgs1 helicase, thus generating long single-stranded overhangs (Mimitou and Symington 2008).

As many other DNA processing enzymes, Mre11 is part of a multisubunit complex whose core is composed of four subunits, two subunits of Mre11 and two of Rad50 (Table 1). In this four-subunit MR complex, Mre11 acts as the nuclease engine, whereas Rad50 contributes localization and tethering specific functions. In eukaryotic organisms, as opposed to the simpler archeal and bacterial MR complex, there is a third subunit associated with Mre11 and Rad50, Nbs1 (or Xrs2 in yeast). Nbs1 is an integral part of the eukaryotic complex, which is named MRN (in yeast, MRX) (Hopfner et al. 2001). The MRN complex participates in various DNA repair processes such as in DNA damage detection, HR (Williams, R.S. et al. 2008), telomere maintenance, or checkpoint signaling, meiotic recombination, NHEJ and MMEJ (Lammens et al. 2011). Through its capacity to activate the ATM kinase, the MRN complex participates in the cell cycle (Lammens et al. 2011).

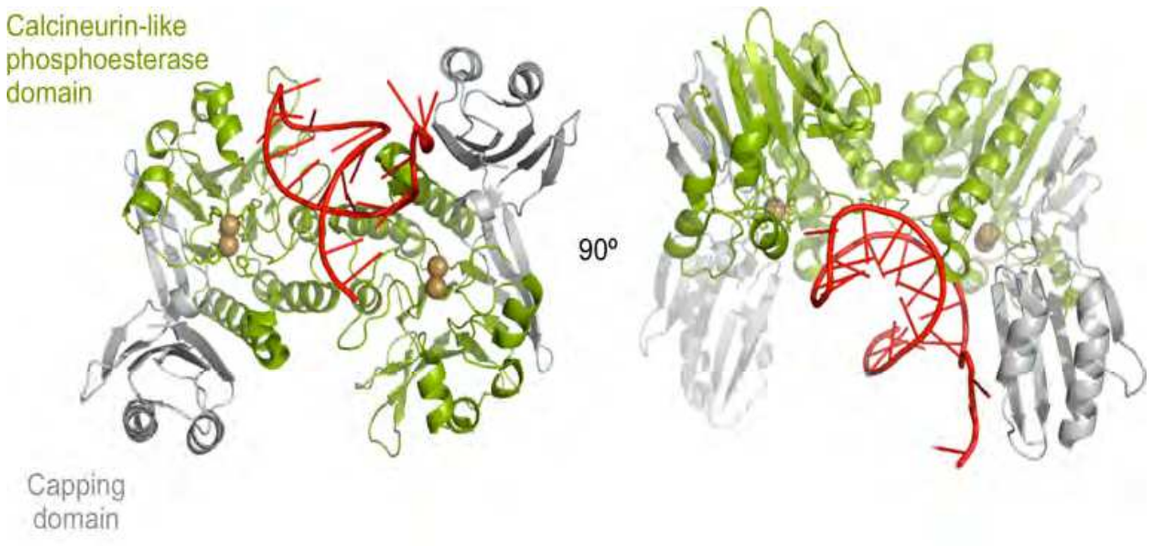

Fig. 4. Crystal structure of *Pf*Mre11 (two views 90º apart) in complex with branched DNA (Williams, R.S. et al. 2008), an intermediate during DSB repair.

	PP2B protein	Organism	PDB ID (Reference)
ARCHEA	Mre11:Rad50	*P furiosus*	3QKR (Williams, G.J. et al. 2011)
	Mre11	*P. furiosus*	3DSD, 3DSC (Williams, R.S. et al. 2008)
	Mre11	*P. furiosus*	1II7 (Hopfner et al. 2001)
	Mre11-3	*P. furiosus*	1S8E (Arthur et al. 2004)
EUBACTERIA	Mre11:Rad50	*T. maritima*	3QF7, 3QG5 (Lammens et al. 2011)
	Mre11	*T. maritima*	2Q8U (Das et al. 2010)

Table 3. X-ray crystal structures of archeal and eubacterial Mre11.

Several crystal structures of Mre11 in several functionally relevant complexes have been solved in two extremophilic microorganisms: the Archea *Pyrococcus furiosus* (Hopfner et al. 2001) (Fig. 4 and Table 3) and the Eubacteria *Thermotoga maritima* (Das et al. 2010) (Table 3). *Pf*Mre11 is composed of two domains, domain I (or nuclease domain) and domain II (or capping domain). Domain I is formed by two parallel β-sheets surrounded by seven α-helices, and is characterized by five conserved phosphodiesterase motifs that confer nuclease activity. These conserved motifs are the blueprint of the PP2B superfamily to which Mre11 belongs. In addition, the conserved five motifs are very similar, at the structural level, to those found in Ser/Thr phosphatases (although the latter phosphatases show preference for different metal ions, such as Zn^{2+} and Fe^{2+}). The capping domain is composed of five β-strands and two α-helices, and its function is to specifically bind DNA (Hopfner et al. 2001). Like in many two-domain proteins, there is flexibility in the relative orientation around the linker that connects the two domains, and it has been suggested that this flexibility enables Mre11 to efficiently bind widely different substrates (dsDNA, ssDNA, or hairpin DNA). In addition, this flexibility allows the capping domain to rotate while bound to DNA thus facilitating the opening of the DNA substrate (Hopfner et al. 2001)

Similarly to other nucleases of the MBL fold, archeal and human Mre11 are homodimeric enzymes. Two chains of Mre11 assemble in a homodimer with an interface composed of α-helices that are stabilized by apolar interactions between hydrophobic and aromatic residues, as Leu61, Ile65, Pro92, Leu97, and Phe101 (Williams, R.S. et al. 2008). This dimerization interface appears to be conserved at the sequence level in Bacteria, Archea, fungi, and humans (Das et al. 2010), and sequence alignments with *Trypanosoma brucei* or *Arabidopsis thaliana* lend support to the notion that Mre11 may share its quaternary structure even in paramecia and plants (Tan et al. 2002). Despite its evolutionary conservation, the integrity of the dimer structure does not appear to be required for the nuclease activity, at least in *P. furiosus* Mre11 (*Pf*Mre11). Instead, the dimer structure seems to be essential for binding the DNA substrate in the correct orientation. The dimeric architecture of Mre11 enables it to simultaneously bind to the two ends of a DSB in a synaptic complex whereby the two ends of a DSB are drawn near one another (or 'tethered') (Hopfner et al. 2001; Williams, R.S. et al. 2008). This tethering represents an essential prerequisite for the successful Mre11-mediated processing of a DSB lesion, since close proximity of the two DSB ends is necessary for ligation.

As explained above, the presence of two metal ions is an absolute requirement for the nuclease activity of Mre11. In *Pf*Mre11, residues Asn84 and His85 have been shown to be essential for catalysis. In fact, the residues implicated in metal coordination are conserved, at least in *P. furiosus*, yeast and humans (Hopfner et al. 2001). This feature has not been proven in *T. maritima* Mre11 (*Tm*Mre11) because of the absence of any bound metals in the crystal structure (Das et al. 2010); this does not necessarily imply that *Tm*Mre11 has no requirement

for Mn^{2+}, since metalloenzymes can become depleted of the metal they need for catalysis during purification. Mutations in *Pf*Mre11 of two catalytic His residues of the nuclease domain, His85 and His52, eliminate the 3′-5′ exonuclease activity (Williams, R.S. et al. 2008). Mutations in His85 abolish endonuclease activity, while a H52S mutant affects only weakly to the endonuclease activity (Williams, R.S. et al. 2008), therefore indicating that His85 is the most crucial residue since it is required to maintain both the exonuclease and the endonuclease activities. Some authors propose that the major function of Mre11 is endonucleolytic, as needed for homologous recombination (Williams, R.S. et al. 2008). His85 is believed to act as a proton donor to the 3′-OH group of the substrate (Hopfner et al. 2001), while His52 facilitates the rotation of the phosphate group required for 3′-5′ exonucleolytic activity (Williams, R.S. et al. 2008). Given the conservation of these two His residues, this mechanism assumes that the nuclease activity is highly conserved among Mre11 orthologs, including *T. maritima* and yeast (Das et al. 2010). Assays with dAMP (deoxyadenosine monophosphate) proved that the interaction of Mre11 with the substrate occurs via the phosphate group of dAMP, without a specific recognition of the adenine base (i.e., non-specifically) (Hopfner et al. 2001). This observation is in agreement with the sequence non-specificity of Mre11, which has been shown to interact mostly with the minor groove of DNA (Williams, R.S. et al. 2008). This property may further enhance Mre11's capacity to recognize widely different DNA sequences and to accomplish diverse activities.

*Pf*Mre11 interacts with its DNA substrate in two different ways, both of which are biologically relevant (Williams, R.S. et al. 2008): A synaptic DNA complex, which simulates dsDNA end joining of two DSB products, a fundamental process in DSB repair; and a branched DNA complex (Fig. 4), wherein the joined ssDNA-dsDNA structure sits at the interface between the nuclease and the capping domains of Mre11. The branched DNA complex, as opposed to the synaptic complex, is an asymmetric complex (Fig. 4).

In *T. maritima*, *Tm*Mre11 forms a crystallographic homodimer of slightly smaller subunits, and the nuclease domain is composed of 12 β-strands and five α-helices (Das et al. 2010). The C-terminal, DNA binding domain contains three β-strands and two α-helices, and is equivalent to the capping domain of *Pf*Mre11. Unlike *Pf*Mre11, solution analyses (size-exclusion chromatography and static light scattering) indicate that *Tm*Mre11 can be monomeric. In support of a physiologic homodimeric structure, conserved residues at the dimerization interfaces of *Pf*Mre11, yeast, and human Mre11, are also conserved in *Tm*Mre11, including Leu75, Leu78, Lys79, and Ile113. Phe102 and Phe105, two hydrophobic residues at the putative dimerization interface of *Tm*Mre11 that would be exposed in the monomeric structure, have too equivalent residues in *Pf*Mre11, yeast, and human Mre11. Although there are many shared features in the DNA binding domain of *Tm*Mre11, the specific configuration of this domain may explain the differential functionality among these species. In keeping with the conservation of the nuclease domain, enzymatic studies have shown that *Tm*Mre11 possesses both exonuclease and endonuclease activities. Furthermore, His94 in *Tm*Mre11 seems to carry out the same function as His85 in *Pf*Mre11, and His61 (*Tm*Mre11) is functionally equivalent to His52 (*Pf*Mre11) (Das et al. 2010).

The MR complex from *T. maritima*, like that of *P. furiosus*, comprises four subunits, two each of Mre11 and Rad50, the core of the complex being an Mre11 homodimer that is stabilized by a core of hydrophobic interactions. Each Mre11 subunit in the core dimer contacts one Rad50 subunit (Lammens et al. 2011), with an overall organization of the bacterial MR complex that is identical to that of the archeal MR complex. This notion that not only the constituent subunits but also their mode of interaction inside a seemingly isofunctional

complex is a fundamental aspect of protein complex evolution, which has been elegantly shown too for other DNA and chromatin binding complexes, e.g. the histone acetylation and deacetylation complexes (Doyon et al. 2006). The human MR complex has been studied by SFM (scanning force microscopy), revealing a large central globular domain whence two long, flexible projections emerge (de Jager et al. 2001), which has led to the proposal of an evolutionarily conserved architecture for the MR complex. In contrast to the prokaryotic MR complexes, the human MR complex seems to bind DNA preferably at the end of linear dsDNA, whereas in circular DNA the MR complex binds almost exclusively as monomers. The yeast MR complex has been studied as well, with the result that mutations that perturb the complex lead to genome aberrations, loss of cell viability, problems in recombination, and mistakes in telomere maintenance; many of these defects stem from a reduced DSB repair (Ghosal and Muniyappa 2005). In yeast, as in humans, a third subunit (Xrs2) is associated with Mre11 and Rad50 in a so-called MRX complex, which has roles mostly in DSB repair by the HR pathway (Yamaguchi-Iwai et al. 1999).

Telomere ends are a special class of DSBs, and in this context it has been shown that yeast Mre11 (*Sc*Mre11) is implicated in the removal of Spo11p, a protein that generates DSB during meiotic homologous recombination (Diaz et al. 2002). Apparently, *Sc*Mre11 has high affinity for parallel G-quadruplex (G4) DNA, a feature of yeast telomeres. Indeed, *Sc*Mre11 can cleave G4 DNA as well as G-rich ssDNA. This activity of *Sc*Mre11 toward G4 DNA and related DNAs is thought to facilitate the action of telomerase and the binding of other proteins to the telomeres. *Sc*Mre11 can also bind ssDNA and dsDNA, though with lower affinity (Ghosal and Muniyappa 2005). More recently, some studies have shown a relationship between *Sc*Mre11 and topoisomerases (Hamilton and Maizels 2010), whereby two known topoisomerase inhibitors (camptothecin and etoposide) show differential effects in an *mre11Δ* knockout. In turn, an H59A mutant is affected in the resistance to hydroxyurea and IR, whereas it is not affected by camptothecin (Hamilton and Maizels 2010). Thus, these results suggest an interaction, whether direct or indirect, between Mre11 and topoisomerases, both of which carry out functions on a DNA substrate. Like *Sc*Mre11, *Tb*Mre11 is thought to participate in telomere maintenance (Tan et al. 2002), an observation that raises the possibility that the conservation of telomeric repeat structure from yeast to vertebrates may be linked to the conservation of Mre11.

3. Structural and catalytic parallels

The MBL and PP2B families have a number of significant similarities at different, functionally relevant levels, and therefore many parallels can be drawn between the two nuclease families, structurally and catalytically. First, the core of either fold consists of β-sheets with analogous topology between the β-CASP nucleases and Mre11. Second, their tertiary structures are similar. Third, both nuclease folds use a two-metal-ion binding site for catalysis (Zn^{2+} in the β-CASP nucleases, and Mn^{2+} by Mre11), and have similar active-site residues to perform catalysis. The differences in substrate specificity and reactivity can be rationalized, very preliminary in the absence of additional structural information for the β-CASP DNA nucleases, as stemming from the different localization of the active-site residues in either fold, and in the prominent role of the accessory or inserted domains in modulating catalysis by the nuclease domain.

Several crystal structures of archeal Mre11 alone and in complex with DNA reveal a conserved homodimer with a tertiary structure and active sites that are reminiscent to those

of β-CASP nucleases (Hopfner et al. 2001; Williams, R.S. et al. 2008). In both fold families, the core of each β-sheet is composed of seven strands ordered as (↑↓↑↑↑↑↑), and the active site is surrounded by many conserved His residues and carboxylates that bind the catalytic metal ions. The two Mn^{2+} ions in Mre11 are located in equivalent positions to the Zn^{2+} ions in the β-CASP nucleases. Are the catalytic roles of Zn^{2+} in β-CASP nucleases transferable to Mre11 Mn^{2+} ions? Even though the DNA co-crystal structures of Mre11 have not captured the DNA substrate within coordination distance of the two Mn^{2+} ions, there is some experimental evidence from structural and mutagenic analyses that indicate that one conserved His residue is active in orienting DNA substrate for the exonuclease cleavage, while a second His residue could be involved in either metal-ion binding or catalysis (Williams, R.S. et al. 2008). This behavior might indeed bear some resemblance to the classical catalytic mechanism of MBLs, whereby one Zn^{2+} ion binds and orientates the substrate, whereas a second Zn^{2+} ion fulfills a distinct, but essential role, in, e.g., shielding the negative charge that develops in the leaving group during the transition state.

This array of similarities between Mre11 and the β-CASP family, spanning the overall structure, active site configuration, and the presence of two tightly bound metal ions, has been suggested as the underlying cause of the partially overlapping functions of Mre11 and the MBL nucleases Snm1 and Pso2 (Lam et al. 2008; Yang 2010). Indeed, having more than one enzyme to fulfill a vital function is a well-established cellular strategy to preserve viability in the event that one of the enzymes is lost due to mutation or deletion, and the fact that Mre11 and Snm1/Pso2 exhibit partial functional redundancy illustrates how important these functions are.

4. Nucleases at the heart of DNA repair complexes

Protein complexes, rather than isolated proteins, carry out the immense majority of cellular functions, and the intricate processes of DNA repair are no exception. Even though there are nucleases that perform catalysis in the absence of physically associated protein partners, the highly regulated and exquisitely orchestrated process of DNA repair requires protein multisubunit complexes able to sense inputs and effect biological outcomes via the nucleosome engine subunit. A conspicuous example is the MRN complex, which has been described as an analog computer molecular machine.

All of the β-CASP DNA nucleases establish stable or transient interactions with other proteins or protein complexes. Even though no stable interaction partners for Pso2/SNM1A have thus far been found, both yeast Pso2 and mammalian SNM1A participate in complex processes that require the intervention of other proteins, in particular other nucleases, and it is then plausible that transient interactions play a major role in the correct orchestration of, e.g., repair of ICLs. In contrast, SNM1B/Apollo has been demonstrated to form several stable complexes. For example, Apollo can associate with the six-subunit Shelterin complex that protects the telomeres likely through an interaction between its C terminus and TRF2, an interaction that has been shown in a co-crystal structure of TRF2 and a C-terminal Apollo peptide (Chen et al. 2008). Another functionally important interaction of Apollo, this time via the MBL domain, involves Astrin; the disruption of this interaction by mutations in the MBL domain of Apollo causes deficient prophase checkpoint (Liu et al. 2009).

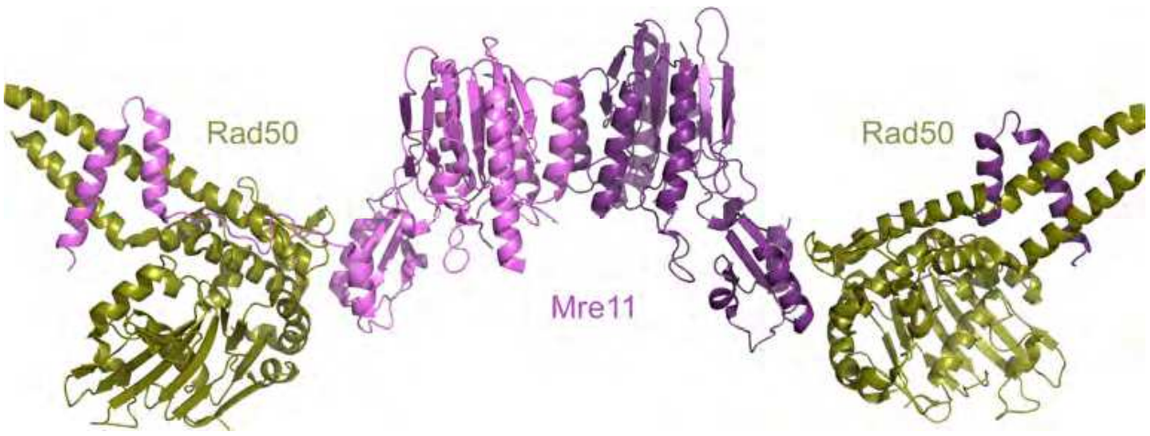

Fig. 5. Crystal structure of the bacterial MR complex (Mre11-Rad50) (Lammens et al. 2011) (PDB ID 3QG5).

As has been pointed out above (Section 2), Mre11 acts in the context of multisubunit complexes with Rad50 and/or Nbs1/Xrs2 (MR and MRN/MRX complexes, respectively) that provide expanded functionality in the recognition and tethering of DSBs and the sensing of cellular stress signals via its non-nuclease subunits. These extra capabilities are essential to target DSBs and avoid wasteful scanning and/or enzymatic processing by Mre11. Small-angle x-ray scattering (SAXS) and analytical ultracentrifuge (AUC) experiments have provided compelling evidence that the MR complex is a heterotetramer formed by two subunits each of Mre11 and Rad50; corroborative evidence of the subunit composition of the MR complex has been obtained by electron microscopy (EM) (Hopfner et al. 2001). Furthermore, the tethering of DNA by the MRX complex has been shown by atomic force microscopy (AFM) (Williams, R.S. et al. 2008). Perhaps the most convincing evidence is the direct observation of the interaction surfaces between Mre11 with the nucleotide-binding domain of Rad50 (Fig. 5), and of the coiled coil segment of Rad50 and an Mre11-derived peptide, both captured by x-ray crystallography from the archeal and the eubacterial MR complexes (Williams, G.J. et al. 2011).

Apart from its role as the nucleolytic engine of the MRN complex, Mre11 acts jointly with other nucleases in what may be described as a functional cooperation. A chief example of this comes from the observation made in yeast that both Mre11 and another exonuclease, Exo1, are both required for the efficient initiation and processivity of resection at specific DSBs generated during meiosis (Hodgson et al. 2011). Loss of function of either Mre11 or Exo1 causes severe delay in resection, therefore suggesting that Mre11 and Exo1 are the major nucleases involved in creating the resection tracts typical of meiotic recombination (Hodgson et al. 2011).

5. Evolution of DNA repair nucleases

MBL fold nucleases involved in DNA repair have most likely evolved from precursor enzymes with the capacity to act upon RNA substrates, which are widespread across the tree of life and include enzymes that can recognize either sequence features, structure, or combined sequence-structure signatures on RNA substrates. Changing the substrate specificity from RNA to DNA should have been easily achieved during evolution, as the same fold scaffolds have been proven to catalyze either reaction. Many of these MBL RNases

utilize inserted domains to assist recognition and binding of RNA molecules, which are large and very densely charged molecules. One outstanding example concerns RNases from the MBL superfamily, which often possess a β-CASP domain for recognition and binding of the nucleic acid. Although no structure of a DNA nuclease of the MBL fold is available to date, it is conceivable that an RNA binding MBL enzyme might support sequence modifications that allow it to bind DNA, either ssDNA or dsDNA. Actually, binding ssDNA in the context of melted dsDNA molecules is not only conceivable but also most likely true, since many of the MBL enzymes have been convincingly shown to act upon ssDNA segments. Artemis, for example, is activated by DNA-PKcs, and one way this could happen is by the melting action of DNA PKcs upon a dsDNA substrate, which would be sufficient to provide ssDNA substrate to Artemis. However, a complete clarification of the involved processes will have to wait until more careful experiments are conducted.

PP2B nucleases, like Mre11, are also widespread across the tree of life and have been identified in Bacteria, Archea, and Eukarya. Crystal structures of the eubacterial and archeal enzymes are available in the Protein Data Bank for comparison, and they have been shown to be of different length while maintaining all of the conserved key residues for catalysis, as well as the identity of the catalytic metal ion (manganese). Therefore, it is quite plausible that there existed an Mre11-like enzyme in the last universal common ancestor (LUCA) of all extinct life forms with, potentially, similar roles in DSB repair and maintenance of genome integrity. Further support for this idea is derived from the clear assumption that the selection pressure for sophisticated and efficient DNA repair machinery for LUCA must have been even stronger than at present.

6. Disease states and mutations in nucleases

There is a plethora of mutation studies in MBL and PP2B nucleases carried out in model organisms that can be related to human pathophysiology linked to DNA repair and genome stability. These disease-associated mutations provide a wealth of information on function, specificity, and redundancy of the DNA repair nucleases.

Among the nucleases from the MBL family, a well-known syndrome is radiation sensitive severe combined immunodeficiency (RS-SCID), a disease condition that arises from defects in Artemis and is truly the result of impaired function of Artemis in DNA repair and in V(D)J recombination (Dominski 2007). Another striking example comes from patients with Hoyeraal-Hreidarsson (HH) syndrome, a severe form of *dyskeratosis congenita* caused by impaired telomere protection. Patients with HH syndrome suffer of premature aging and are immunodeficient. At the molecular level, the HH syndrome is characterized by a unique *APOLLO* splice variant that lacks the (TRFH)-binding motif (TBM) to TRF2 (Touzot et al. 2010). In addition to the roles involved in DNA repair and telomeric protection, Apollo deficient cells present a deficient prophase checkpoint increased when the interaction of Astrin and the MBL domain of Apollo is disrupted by mutations in such domain (Liu et al. 2009). Fanconi anemia (FA) is another example. Mutations in up to 14 different FANC genes have been associated with FA, a DNA repair disorder that dramatically enhances predisposition to cancer and is characterized by progressive bone marrow failure, congenital development defects, chromosomal abnormalities, and cellular hypersensitivity to ICL agents. Although none of the FANC genes are MBLs or PP2B fold nucleases, functional associations with MBL nucleases have been described, therefore FA has interconnections

with MBL nucleases of DNA repair that underlie the complex network of functions that is disrupted by FA.

The PP2B family protein Mre11 has a vital role across phylogenetically diverse organisms ranging from Bacteria to vertebrates, on the basis of its crucial role in DNA repair. Well-established links between *MRE11* mutations and disease states exist, e.g., in yeast, where the N113S mutation (in the nuclease domain) causes an enhanced sensitivity to ionizing radiation (IR). In humans, the same mutation (N117S) has been implicated in the onset of ataxia-telangiectasia disorder (ATLD) (Hopfner et al. 2001). Another yeast mutation, P162S, affects the repair of DSBs carried out by Mre11 (Hopfner et al. 2001), which in metazoans would likely affect genome stability and increase the chances of neoplastic transformation. Other mutations in Mre11, like H129N, confer early embryonic lethality in homozygosis in mice (Buis et al. 2008), and depletion of chicken Mre11 appears critical in the survival of animal cells through its participation in homologous recombination repair, leading to the accumulation of DSBs and increased of radiosensitivity (Yamaguchi-Iwai et al. 1999). Other studies show that animal cells deficient in MRE11 seem to be non-viable, whereas in *Trypanosoma brucei Tb*Mre11 is not so critical for cell maintenance, but this discrepancy could be reconciled with most of the current knowledge on Mre11 if other, functionally redundant mechanisms were found to repair DSBs in *T. brucei* (Tan et al. 2002). In agreement with the important role of multisubunit complexes in DNA repair, mutations in another component of the human MRN complex, Nbs1, give rise to Nijmegen breakage syndrome, a paradigm of a disease linked to DNA repair defects (de Jager et al. 2001) that has been associated with an enhanced predisposition to colorectal cancer.

7. Conclusions and future outlook

There are many standing issues in the field of DNA repair nucleases whose elucidation awaits further research. Some of these issues include the complete biochemical and structural characterization of DNA nucleases of the MBL fold family, which are known to play key roles in DNA repair but which have thus far proved hard to reveal their substrate specificities (e.g., ssDNA versus dsDNA), activities (e.g., controversies over the 3′-5′ and 5′-3′ exonuclease activities of Artemis), or even the requirement for post-translational modifications (such as DNA PKcs mediated phosphorylation of Artemis). In protein phosphatase nucleases, the best-known example is archeal and eukaryotic Mre11 and the architecture of the MRN complex. There, one crucial aspect is to decipher how the MRN complex processes all its inputs and delivers a comprehensive functional outcome. In more applied science, there is always the wide-ranging and crucial question of how can the tremendous amount of basic science results be put to clinical use. In DNA repair, the identification of mutations that cause, or predispose, to acquire certain diseases must advance to the point that early diagnosis becomes feasible for many. Cures to these diseases may be far into the future, but the current and near future research is providing sure steps toward this much-longed end.

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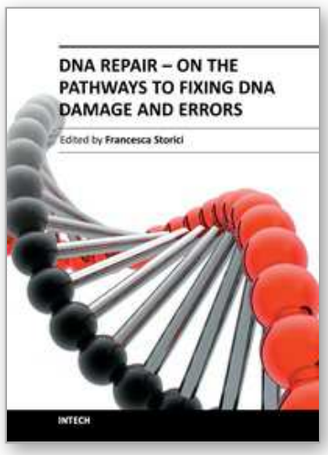

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DNA repair is fundamental to all cell types to maintain genomic stability. A collection of cutting-edge reviews, DNA Repair - On the pathways to fixing DNA damage and errors covers major aspects of the DNA repair processes in a large variety of organisms, 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.

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