Critical Reviews in Biochemistry and Molecular Biology, 42:67–93, 2007

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230701279118

informa
healthcare

Nucleases of the Metallo-β-lactamase Family and Their Role in DNA and RNA Metabolism

Zbigniew Dominski
Department of Biochemistry and Biophysics and Program in Molecular Biology and Biotechnology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

ABSTRACT Proteins of the metallo-β-lactamase family with either demonstrated or predicted nuclease activity have been identified in a number of organisms ranging from bacteria to humans and has been shown to be important constituents of cellular metabolism. Nucleases of this family are believed to utilize a zinc-dependent mechanism in catalysis and function as 5′ to 3′ exonucleases and/or endonucleases in such processes as 3′ end processing of RNA precursors, DNA repair, V(D)J recombination, and telomere maintenance. Examples of metallo-β-lactamase nucleases include CPSF-73, a known component of the cleavage/polyadenylation machinery, which functions as the endonuclease in 3′ end formation of both polyadenylated and histone mRNAs, and Artemis that opens DNA hairpins during V(D)J recombination. Mutations in two metallo-β-lactamase nucleases have been implicated in human diseases: tRNase Z required for 3′ processing of tRNA precursors has been linked to the familial form of prostate cancer, whereas inactivation of Artemis causes severe combined immunodeficiency (SCID). There is also a group of as yet uncharacterized proteins of this family in bacteria and archaea that based on sequence similarity to CPSF-73 are predicted to function as nucleases in RNA metabolism. This article reviews the cellular roles of nucleases of the metallo-β-lactamase family and the recent advances in studying these proteins.

KEYWORDS 3′ end processing, tRNase Z, CPSF-73, RC-68, Integrator, Snm1A, Apollo, Artemis, β-CASP

1. INTRODUCTION

The metallo-β-lactamase family consists of a large number of prokaryotic and eukaryotic enzymes that vary in biological functions and substrate specificities but fold into a common 3-dimensional structure, referred to as the $\alpha\beta/\beta\alpha$ sandwich, consisting of two β-sheets at the core and a number of external α-helices (Aravind, 1999; Daiyasu *et al.*, 2001). The name of this family is derived from class B β-lactamases, the bacterial proteins in which the $\alpha\beta/\beta\alpha$ fold was identified for the first time (Carfi *et al.*, 1995). Class B β-lactamases utilize zinc ions to hydrolyze and thus inactivate β-lactams, a group of powerful antibiotics that inhibit bacterial growth by affecting cell wall biosynthesis (Wang *et al.*, 1999;

Address correspondence to Zbigniew Dominski, Program in Molecular Biology and Biotechnology, CB #3280, University of North Carolina, Chapel Hill, NC 27599. Email: dominski@med.unc.edu

Heinz and Adolph, 2004). β-lactamases of the other three classes (A, C, and D) utilize a serine-containing active site in hydrolyzing β-lactam antibiotics and belong to a different group of enzymes. In addition to adopting the same fold, proteins of the metallo-β-lactamase family share a conserved pattern of histidines and aspartates located in five separate sequence motifs, I–V (Aravind, 1999; Daiyasu *et al.*, 2001). Motif II, which has the consensus sequence HxHxDH (where x indicates any amino acid), is referred to as the histidine motif and is the signature sequence of the entire metallo-β-lactamase family (Aravind, 1999). As determined by crystallographic studies, the conserved histidine and aspartate residues of all five motifs form a catalytic center containing typically two metal ions and located at the bottom of a wide shallow groove between the two β-sheets. The broad substrate specificity of the metallo-β-lactamase family can be explained by the accessibility of the active site to a number of different molecules.

Most metallo-β-lactamase proteins are hydrolases that utilize zinc ions in catalysis. In addition to class B β-lactamases, other family members include glyoxylase II, arylsulfatase, alkylsulfatase, and 3′, 5′-cyclic-nucleotide phosphodiesterase (Daiyasu *et al.*, 2001). Most substrates hydrolyzed by the family members are characterized by the presence of an ester linkage and at least one positive charge (Aravind, 1999). Although hydrolytic enzymes are the most common, the family also includes proteins with redox activity that utilize iron instead of zinc during catalysis (Silva *et al.*, 2001).

A small number of hydrolases of the metallo-β-lactamase family have been implicated in metabolism of nucleic acids as nucleases. The best characterized among them is tRNase Z, an endonuclease required for 3′ end processing of tRNA precursors, a group of transcripts generated by RNA polymerase III (Vogel *et al.*, 2005). Another member of the family, CPSF-73, was isolated 15 years ago as a component of the cleavage/polyadenylation machinery (Bienroth *et al.*, 1991; Murthy and Manley, 1992) but was only recently shown to be the endonuclease that cleaves mRNA precursors downstream of the AAUAAA sequence (Ryan *et al.*, 2004; Mandel *et al.*, 2006). The same protein is also likely responsible for the generation of the unpolyadenylated 3′ end of histone mRNAs using a distinct 3′ end processing machinery (Dominski *et al.*, 2005a). Identification of CPSF-73 as the 3′ endonuclease in formation of both polyadenylated mRNAs and histone mRNAs filled an important gap in our knowledge of RNA metabolism (Wickens and Gonzalez, 2004; Weiner, 2005) and provided the first indication that the two 3′ processing machineries are evolutionarily related (Gilmartin, 2005; Dominski *et al.*, 2005a). A protein highly similar to CPSF-73, designated RC-68 (Dominski *et al.*, 2005b) or Int11, is in turn part of a large complex called "Integrator" and may function as an endonuclease in 3′ end processing of precursors to snRNAs, a separate class of RNA polymerase II transcripts (Baillat *et al.*, 2005). Artemis is involved in repair of DNA double strand breaks and V(D)J recombination (Moshous *et al.*, 2001; Ma *et al.*, 2002), whereas a protein named "Apollo," which only recently joined the family performs essential functions at telomeres and also plays a role in repair of DNA interstrand cross-links (van Overbeek and De Lange, 2006; Lenain *et al.*, 2006; Freibaum and Counter, 2006). Repair of DNA cross-links is the main but likely not the only task of a similar protein, Snm1A (Dronkert *et al.*, 2000; Ishiai *et al.*, 2004). The genomes of prokaryotes encode other predicted nucleases of the metallo-β-lactamase family that await functional characterization.

2. tRNase Z: 3′ END PROCESSING OF tRNA PRECURSORS

Transfer RNAs (tRNAs) are generated from longer precursors (pre-tRNAs), which contain additional sequences on both the 5′ and 3′ ends referred to as a 5′ leader and a 3′ trailer (Figure 1). Removal of these terminal sequences occurs during the process of tRNA maturation and is necessary to form functional tRNA molecules. A number of pre-tRNAs also contain introns, which are removed through splicing. All mature tRNAs are terminated at the 3′ end with the CCA triplet that is essential for both aminoacylation and recognition of the tRNA by the ribosome (Green and Noller, 1997). For many bacterial and some archaeal tRNAs, the CCA is encoded by their respective genes and therefore is already present in the precursor (Marck and Grosjean, 2002). In other cases, including all eukaryotic tRNAs, the CCA is added post-transcriptionally as a part of the maturation process (Deutscher, 1990; Weiner, 2004).

Two major Pathways for tRNA 3′ End Formation

In virtually all organisms with only a few exceptions (Willkomm *et al.*, 2002; Marszalkowski *et al.*, 2006), the

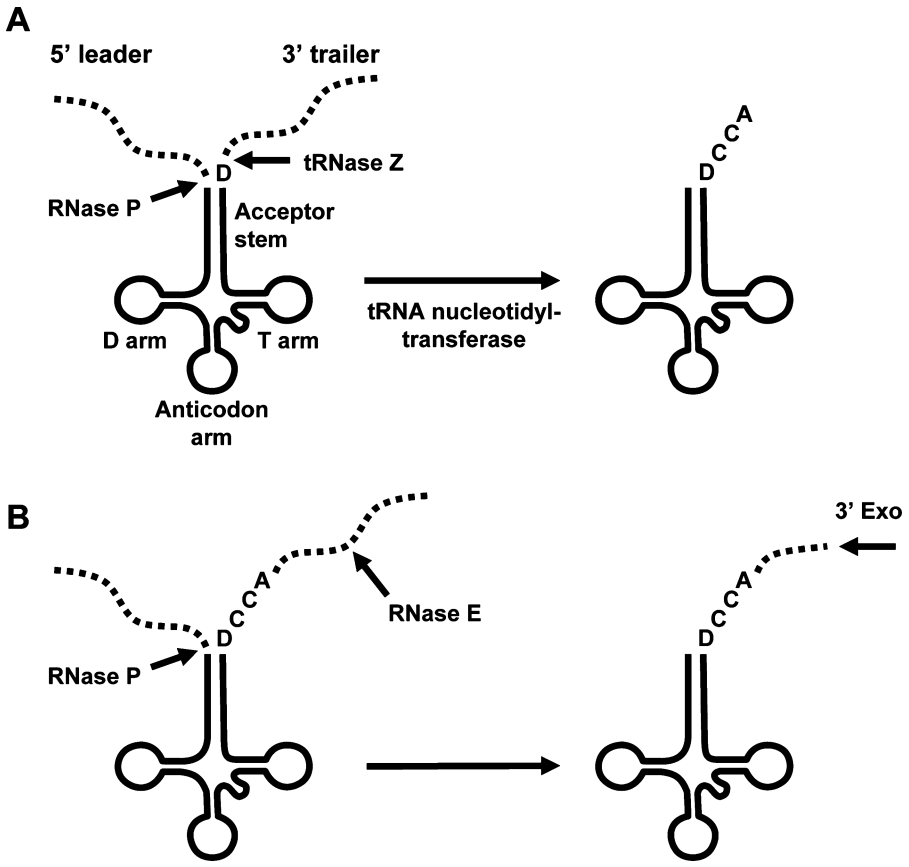

FIGURE 1 **Two major pathways for 3′ end processing of tRNA precursors. *A.* 3′ end processing of tRNA precursors lacking CCA. The 3′ trailer is removed by tRNase Z, which cleaves immediately after the discriminator nucleotide (*D*), as indicated with an arrow. RNase P removes the 5′ leader. *B.* 3′ end processing of tRNA precursors containing CCA. The 3′ trailer is first cleaved by RNase E and then trimmed by a number of redundant 3′ exonucleases to expose the CCA. RNase P removes the 5′ leader.**

5′ leader in pre-tRNAs is removed by a ubiquitous endonuclease, RNase P, which consists of the RNA catalytic subunit and one or more protein components (Walker and Engelke, 2006). RNase P cleaves the pre-tRNA between the 5′ trailer and the first nucleotide of the mature tRNA (Figure 1). 3′ end processing of tRNA precursors is more variable and occurs through one of two major pathways. Which of the two pathways is involved largely depends on the presence or absence of the CCA sequence in pre-tRNA. The 3′ trailer of pre-tRNA lacking the CCA is removed by an endonucleolytic cleavage catalyzed by tRNase Z, the first studied and so far best characterized nuclease of the metallo-β-lactamase family (Vogel *et al.*, 2005). tRNase Z, also known as RNase Z, 3′ tRNase or ELAC, cleaves pre-tRNAs immediately after the discriminator, i.e., the first unpaired nucleotide located 3′ of the acceptor stem (Figure 1A). The CCA sequence is subsequently added to the discriminator nucleotide by tRNA nucleotidyltransferase. Removal of the 3′ trailer by tRNase Z followed by the CCA addition is the most common mechanism leading to generation of the mature 3′ end of tRNAs.

In *E. coli*, the CCA is encoded by all tRNA genes and hence is present in all pre-tRNAs. In these pre-tRNAs, the 3′ trailer is removed by a combined action of an endonuclease, RNase E, which cleaves within the 3′ trailer, followed by exonucleolytic trimming of the remaining nucleotides to expose the CCA (Figure 1B) (Li and Deutscher, 2002). The trimming is carried out by a number of redundant bacterial 3′ exonucleases with the most pronounced being RNase T and RNase PH (Zuo and Deutscher, 2001; Deutscher and Li, 2001). Surprisingly, given the lack of the CCA-less tRNA genes in *E. coli*, the genome of this bacterial species contains a gene for tRNase Z. The *E. coli* tRNase Z was initially designated ElaC and recently renamed ZiPD (for zinc phosphodiesterase) based on its ability to catalyze a zinc-dependent hydrolysis of bpNPP and TpNPP, two small artificial substrates containing one phosphodiester bond (Vogel *et al.*, 2002). Recent studies have demonstrated that *E. coli* tRNase Z is in

fact identical to RNase BN, previously shown to be required for propagation of bacteriophage T4 (Ezraty *et al.*, 2005). In contrast to its host cells, T4 bacteriophage encodes CCA-less tRNAs and therefore vitally depends on the bacterially encoded tRNase Z. Deletion of the tRNase Z gene does not have any effects on cell viability and causes no changes the intracellular concentration of mature tRNA species, demonstrating that in *E. coli* tRNase Z is nonessential and as expected does not play a role in 3′ end processing of endogenous pre-tRNAs (Schilling *et al.*, 2004; Perwez and Kushner, 2006). Interestingly, the lack of tRNase Z activity in *E. coli* results in a significant increase in the steady-state levels of a subset of cellular mRNAs and this effect is greatly enhanced by concurrent inactivation of RNase E (Perwez and Kushner, 2006). Thus, in *E. coli* tRNase Z likely supports the endonucleolytic activity of RNase E in mRNA decay instead of participating in 3′ end processing of pre-tRNAs, raising an interesting possibility that tRNase Z in other organisms may perform more general functions in RNA metabolism than initially anticipated (Perwez and Kushner, 2006).

In *Bacillus subtilis*, approximately one third of the tRNA genes do not encode CCA and the tRNA transcripts generated from these genes are processed at the 3′ end by tRNase Z (Pellegrini *et al.*, 2003). As expected from the presence of the CCA-less tRNA genes, the *Bacillus* tRNase Z gene is essential. The remaining two thirds of pre-tRNAs in *B. subtilis* are processed using the 3′ exonuclease pathway (Wen *et al.*, 2005). A unique pathway of 3′ end processing of pre-tRNAs has been identified in *Thermotoga maritima* where nearly all pre-tRNAs contain the CCA sequence. Surprisingly, 3′ end processing of these pre-tRNAs depends on tRNase Z, which cleaves immediately after the CCA rather than after the discriminator, leading in one step to the generation of the mature 3′ end of tRNA (Minagawa *et al.*, 2004).

Two Forms of tRNase Z and Substrate Requirements

tRNase Z exists in two different forms, short and long, referred to as tRNase Z^S (or ELAC1) and tRNase Z^L (or ELAC2), respectively. The short form, exemplified by the enzymes from *E. coli*, *B. subtilis* and *T. maritima*, usually consists of 300 to 400 amino acids, while the longer form is approximately 2 to 3 times that size. The intact metallo-β-lactamase domain with strong similarity to tRNase Z^S is located in the C-terminal portion of tRNase Z^L, indicating that this portion is involved in catalysis (Tavtigian *et al.*, 2001). Interestingly, the N-terminal portion also shows a limited similarity to tRNase Z^S, suggesting that the gene encoding tRNase Z^L has evolved from tandem duplication of the gene encoding the short form. However, the key catalytic residues of the N-terminal portion, including those present in the histidine motif, have been altered during evolution.

Bacteria and archaea contain only tRNase Z^S, whereas eukaryotes contain either only tRNase Z^L or both forms. Human cells contain one long and one short form of tRNase Z. There is only the long form of the enzyme in *S. cerevisiae*, *C. elegans*, and *D. melanogaster* and two short and two long forms in *Arabidopsis thaliana* (Vogel *et al.*, 2005). The reasons for this variable distribution of tRNase Z in different eukaryotes are not fully understood. At least in some organisms different versions of the enzyme may be specialized to function in different cellular compartments and/or may be regulated developmentally. Interestingly, human tRNase Z^L contains a strong mitochondrial transport signal, suggesting that it may also function in this compartment, and in vitro processes nuclear and mitochondrial pre-tRNAs much more efficiently than tRNase Z^S (Yan *et al.*, 2006). Therefore, the short version of tRNase Z in human cells may represent a biochemical relic, or might have gained during evolution additional functions, possibly in processing of other RNA precursors (Yan *et al.*, 2006), as supported by the role of *E. coli* tRNase Z in mRNA degradation (Perwez and Kushner, 2006).

tRNase Z purified from a broad range of organisms either as an endogenous protein or recombinant variants has been shown to efficiently and accurately cleave tRNA precursors *in vitro* (Vogel *et al.*, 2005). 3′ end processing activity has been shown for tRNase Z^S from a number of species, including *Haloferax volcanii* (Schierling *et al.*, 2002), *Methanococcus janaschii* and *Arabidopsis thaliana* (Schiffer *et al.*, 2002), *B. subtilis* (Pellegrini *et al.*, 2003) and *T. maritima* (Minagawa *et al.*, 2004). Finally, the accurate processing activity was shown for tRNase Z^S from *E. coli* (Takaku *et al.*, 2004; Minagawa *et al.*, 2004; Perwez and Kushner, 2006), which, besides cleaving pre-tRNAs encoded by T4 phage has no apparent endogenous pre-tRNA substrates. The ability to faithfully process tRNA precursors in vitro was also demonstrated for the long form of tRNase Z from cells of *S. cerevisiae* (Takaku *et al.*, 2003), *Drosophila*

melanogaster (Dubrovsky *et al.*, 2004), pig, and human (Takaku *et al.*, 2003). The C-terminal half of human tRNase Z^L containing the intact metallo-β-lactamase domain retains a partial processing activity, whereas the N-terminal half is inactive, consistent with the lack of the key catalytic residues in this portion of the protein (Takaku *et al.*, 2003; Takaku *et al.*, 2004).

Short tRNase Z enzymes from archaea (Schierling *et al.*, 2002), bacteria (Minagawa *et al.*, 2004), and plant mitochondria (Schiffer *et al.*, 2001) require the entire structure of pre-tRNA substrates for optimal activity. In contrast, the long form from a variety of organisms has relatively relaxed structural requirements and, in addition to complete pre-tRNAs, can cleave a number of artificial substrates that only partially resemble pre-tRNA although the efficiency of processing for some of them is drastically reduced (Levinger *et al.*, 1998; Nashimoto *et al.*, 1999; Schiffer *et al.*, 2001). The minimal substrate for tRNase Z^L encompasses the 3′ portion of pre-tRNA, consisting of the 3 trailer followed by the 7-base-pair acceptor stem and the T arm containing a 5-base-pair stem and a 7-nucleotide loop (Figure 1). The efficiency of processing of this minimal substrate depending on the source of tRNase Z^L varies between 10 and 60% of the efficiency for the full length pre-tRNA (Takaku *et al.*, 2004). Both tRNase Z^S and tRNase Z^L have relaxed sequence requirements for their substrates consistent with the variability of pre-tRNA sequences processed by the enzyme. The only known restriction is that tRNase Z from many organisms strongly disfavors a cytidine immediately following the discriminator (Nashimoto, 1997; Mohan *et al.*, 1999; Pellegrini *et al.*, 2003). *Drosophila* tRNase Z^L expressed in the baculovirus system processes mature tRNA with CCA at the 3′ end almost 100-fold less efficiently than a pre-tRNA containing the natural trailer (Zareen *et al.*, 2006). This feature ensures that the mature tRNA is not cleaved by tRNase Z as the precursor and can progress to aminoacylation instead of taking part in repeating cycles of removing and adding the CCA. Consistently, the discriminator is usually not followed by a cytosine (Mohan *et al.*, 1999).

Structural Features of tRNase Z

A unique feature of tRNase Z from many organisms that distinguishes this enzyme from other members of the metallo-β-lactamase family is the presence of an insertion within the metallo-β-lactamase domain, referred to as exosite (Schilling *et al.*, 2005) or external binding domain (EBD) (Zareen *et al.*, 2006). The exosite is proposed to function as a clamp to stabilize binding of the pre-tRNA substrate to the enzyme and properly orient the cleavage site near the catalytic center. At least three different types of exosite have been identified in the short and long forms of tRNase Z (Schilling *et al.*, 2005). The exosite in the *E. coli* tRNase Z^S consists of about 50 amino acids. Deletion of the entire region abolishes the ability of *E. coli* tRNase Z^S to cleave pre-tRNA in vitro but does not affect hydrolysis of a small artificial substrate bpNPP containing one phosphodiester bond, indicating that the mutated enzyme is catalytically active. A similar exosite also exists in tRNase Z^S from many other organisms but is absent from the C-terminal portion of tRNase Z^L. This portion retains all key residues required for metal binding and catalysis and therefore is considered a functional equivalent of tRNase Z^S. Interestingly, the exosite similar to that present in *E. coli* tRNase Z^S can be found in the N-terminal portion of tRNase Z^L containing a degenerate and enzymatically inactive metallo-β-lactamase domain (Schilling *et al.*, 2005).

Recently solved crystal structures of the short form of tRNase Z from three bacterial species–*B. subtilis* (de la Sierra-Gallay *et al.*, 2005), *T. maritima* (Ishii *et al.*, 2005) and *E. coli* (Kostelecky *et al.*, 2006)–provided important insight into the mechanism of catalysis and substrate recognition by this endonuclease. All three enzymes are homodimers with monomers exhibiting the typical metallo-β-lactamase $\alpha\beta/\beta\alpha$ fold and a head-to-head orientation within each homodimer. In addition to striking similarities, the structural analysis also demonstrates significant conformational differences between the three enzymes. The *E. coli* homodimer consists of two functionally identical subunits, each containing the zinc-loaded active site and the exosite that protrudes from the protein core as a long flexible arm (Kostelecky *et al.*, 2006). The active site of one subunit faces the flexible arm of the other subunit. This configuration suggests a model of action in which the pre-tRNA substrate is bound by one subunit and cleaved by the other. It is predicted that the *E. coli* enzyme due to its symmetrical structure is capable of simultaneously cleaving two pre-tRNA molecules (Kostelecky *et al.*, 2006). In *B. subtilis* tRNase Z^S, the two subunits of the homodimer are not equivalent in the crystallographic structure (de la Sierra-Gallay *et al.*, 2005). Only one subunit referred to as the A subunit contains two zinc ions and thus

represents an active conformation of the enzyme. The B subunit lacks zinc ions due to a distortion of the active site, indicating that it is catalytically inactive. Interestingly, the crystal structure of the complex between the *B. subtilis* tRNase Z^S and tRNA suggests that binding of the pre-tRNA substrate to the enzyme triggers a cascade of structural rearrangements that relieve the distortion of the active site resulting in zinc binding and capability of cleaving pre-tRNA also by the B subunit (de la Sierra-Gallay *et al.*, 2006). As a result of adopting the active conformation by this subunit, the homodimer of *B. subtilis* tRNase Z^S is predicted to cleave two molecules of pre-tRNA at the same time thus resembling in the mode of action tRNase Z^S from *E. coli*.

The structural studies of *B. subtilis* tRNase Z^S led to a model of how its conserved residues participate in zinc binding (de la Sierra-Gallay *et al.*, 2005). The two zinc ions are coordinated by five conserved histidines and two conserved aspartates distributed in the five conserved motifs of the metallo-β-lactamase domain (Figure 2). Additional points of coordination are provided by a water molecule and a phosphate ion. The same coordination sphere has been identified in the crystal structure of tRNase Z^S from *E. coli* (Kostelecky *et al.*, 2006). As predicted, the critical role in catalysis is played by the highly conserved HxHxDH cluster (motif 2), which in the *B. subtilis* protein occupies positions 63 to 68 (de la Sierra-Gallay *et al.*, 2005). The first two histidines of this motif (positions 63 and 65) together with histidine at position 140 (motif 3) coordinate one zinc ion, whereas the two remaining residues of the motif 2 (aspartate 67 and histidine 68) and histidine at position 269 (motif V) coordinate the other zinc ion (Figure 2). An additional aspartate located at position 211 (motif 4) bridges the two zinc ions. Histidine 68 is stabilized by hydrogen bonds with aspartate 37 (motif 1), which plays a key role in maintaining the correct conformation of the catalytic center.

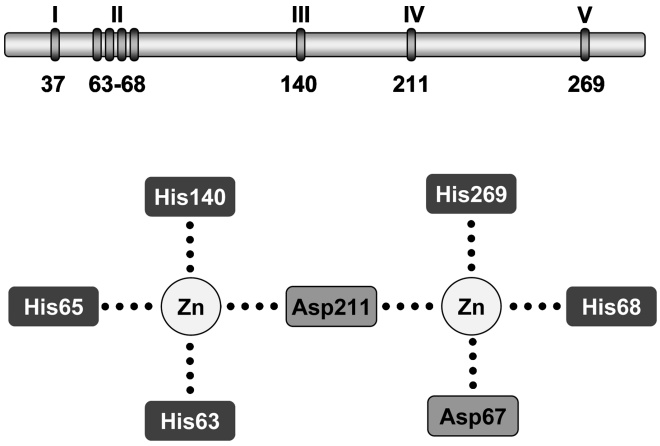

FIGURE 2 **Zinc coordination in *B. subtilis* tRNase Z^S. Schematic representation of the metallo-β-lactamase domain in *B. subtilis* tRNase Z^S depicting positions of the five conserved sequence motifs (I–V). Amino acids involved in coordination of the two zinc ions in the catalytic center are shown below.**

Other Functions for tRNase Z^L?

Surprisingly, the gene encoding human tRNase Z^L, referred to as ELAC2, has been linked to familial form of prostate cancer (Tavtigian *et al.*, 2001). Subsequent studies demonstrated that one mutation identified in high risk pedigrees resulted in a truncated form of the protein that lacked the ability to process pre-tRNAs *in vitro* (Takaku *et al.*, 2003). Remaining amino acid alterations associated with the familial form of prostate cancer did not detectably affect the catalytic activity of the protein *in vitro*. It is unclear at present how these mutations can result in increased occurrence of prostate cancer. One possibility is that tRNase Z^L performs other functions in the cell that are unrelated to RNA processing. In support of this possibility, human tRNase Z^L was shown to interact with the γ-tubulin complex and when overexpressed leads to delay in cell cycle progression (Korver *et al.*, 2003). In *C. elegans,* tRNase Z is required for germline proliferation and reduced expression of this protein results in sterility (Smith and Levitan, 2004).

3. CPSF-73: 3′ END PROCESSING OF mRNA PRECURSORS

3′ end processing of mRNA precursors (pre-mRNAs) results in the formation of the mature 3′ end in mRNAs, which affects all downstream processes in mRNA metabolism, including mRNA export, stability, and translation (Wickens *et al.*, 1997; Mangus *et al.*, 2003; Kuhn and Wahle, 2004; Marzluff, 2005). The vast majority of eukaryotic pre-mRNAs are processed at the 3′ end by a two-step reaction in which cleavage within the 3′ untranslated region is followed by addition of a poly(A) tail to the newly formed 3′ end (Colgan and Manley, 1997; Zhao *et al.*, 1999a). The only exceptions to this rule are metazoan replication dependent histone pre-mRNAs, which are cleaved by a distinct processing machinery and the resulting histone mRNAs are not polyadenylated (Dominski and Marzluff, 1999).

Cleavage Coupled to Polyadenylation

3′ end processing of pre-mRNAs that occurs through cleavage and polyadenylation has been extensively studied in a number of eukaryotes using both biochemical and genetic approaches and was a subject of a number of detailed reviews (Colgan and Manley, 1997; Minvielle-Sebastia and Keller, 1999; Wahle and Ruegsegger, 1999; Zhao *et al.*, 1999a). In mammalian cells, cleavage coupled to polyadenylation depends on the presence of a highly conserved polyadenylation signal, AAUAAA, and a loosely defined downstream G/U-rich sequence. Cleavage occurs between the two sequence elements, usually 10 to 30 nucleotides downstream of the AAUAAA and is followed by addition of the poly(A) tail to the newly generated 3′ end.

Although cleavage and polyadenylation are intimately associated *in vivo*, these two steps can be uncoupled *in vitro* by a number of reagents, including EDTA, which at low concentrations inhibits polyadenylation but has no major effect on cleavage. The cleavage reaction *in vitro* requires assembly of a large complex consisting of at least 12 protein components (Figure 3). The AAUAAA is recognized by cleavage/polyadenylation specificity factor (CPSF), whereas the downstream G/U-rich sequence is recognized by cleavage stimulation factor (CstF). CPSF consists of five subunits: CPSF-160, CPSF-100, CPSF-73, CPSF-30 and Fip1 (Kaufmann *et al.*, 2004). CPSF-160 makes direct contacts with the AAUAAA element (Murthy and Manley, 1995) although the binding of this protein to pre-mRNA may be assisted by two additional CPSF subunits, CPSF-30 and Fip1 that preferentially bind U-rich sequences (Kaufmann *et al.*, 2004). CstF contains three subunits of 77, 64, and 50 kDa, with the CstF-64 subunit binding the G/U-rich downstream element (MacDonald *et al.*, 1994). Fractionation of nuclear extracts initially indicated that CPSF and CstF exist as separate entities that sequentially bind to pre-mRNA substrate during 3′ end processing. However, more recent purification studies suggest that these two factors may in fact form a larger complex that also includes symplekin (Takagaki and Manley, 2000). Symplekin is a protein of unknown function that was initially identified as a component of intercellular junctions called tight junctions, but is also present in the nucleus of all tested cell types (Keon *et al.*, 1996). Symplekin displays low but significant similarity to the yeast protein PTA1 shown to be an essential component of the yeast cleavage/polyadenylation machinery (Zhao *et al.*, 1999b). It is unclear whether symplekin is directly required for cleavage/polyadenylation in the mammalian system or whether it only plays the role of a scaffold, facilitating the assembly of the processing complex (Takagaki and Manley, 2000). The mammalian processing machinery includes two other factors required only for the cleavage step; mammalian cleavage factor I (CF I_m) consisting of at least two subunits (Ruegsegger *et al.*, 1996; Ruegsegger *et al.*, 1998), and the poorly characterized mammalian cleavage factor II (CF II_m) (De Vries *et al.*, 2000). CF I_m binds to sequences upstream of the AAUAAA element and stimulates binding of CPSF to pre-mRNA (Brown and Gilmartin, 2003; Venkataraman *et al.*, 2005). Finally, under some *in vitro* conditions cleavage depends on the C-terminal domain (CTD) of the large subunit of RNA polymerase II (Hirose and Manley, 1998), and for most pre-mRNAs, on poly(A) polymerase (PAP) (Takagaki *et al.*, 1988; Christofori and Keller, 1989; Terns and

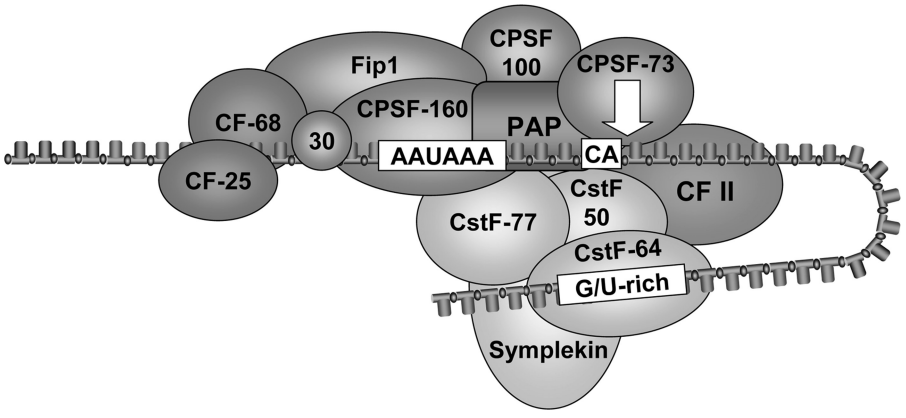

FIGURE 3 **Known components of the cleavage complex assembled on a pre-mRNA containing the AAUAAA. The locations of only some proteins within the complex are supported experimentally. The arrow indicates the site of cleavage by CPSF-73 that is preferentially located after a CA. The C-terminal domain of the large subunit of RNA polymerase II is not shown.**

Jacob, 1989). Following cleavage, the newly formed 3′ end is extended by synthesis of a poly(A) tail. This step of 3′ end processing is catalyzed by PAP and also requires CPSF, which stabilizes PAP on the upstream cleavage product, and poly(A)-binding protein II, which associates with the poly(A) sequence (Wahle, 1995).

Individual components of the cleavage/polyadenylation machinery engage in a network of interactions spanning the cleavage site (Colgan and Manley, 1997; Zhao *et al.*, 1999a). Among them, CPSF-160 interacts with CstF-77 resulting in the cooperative binding of CPSF and CstF to pre-mRNA (Murthy and Manley, 1995). CPSF-160 also interacts with PAP (Murthy and Manley, 1995) and both proteins form a ternary complex with Fip1 (Kaufmann *et al.*, 2004), whereas the CTD interacts with CstF-50 (McCracken *et al.*, 1997). The main function of these interactions is to form a stable processing complex on pre-mRNA and to properly juxtapose catalytic components of the complex, the 3′ endonuclease and PAP, with the site of cleavage and polyadenylation.

Despite the identification of numerous proteins involved in the cleavage reaction, the nature of the nuclease responsible for cleaving pre-mRNAs downstream of the AAUAAA sequence remained elusive until recently (Wickens and Gonzalez, 2004). In 1999, this function was tentatively assigned to CPSF-73, owing to its predicted structure typical of hydrolytic enzymes of the metallo-β-lactamase family (Aravind, 1999). However, a few years elapsed before this hypothesis was tested experimentally. Strong support for the role of CPSF-73 as an endonuclease in 3′ end processing came from studies of the yeast CPSF-73 homologue, Ysh1p. Mutations in the key residues of the metallo-β-lactamase domain of this protein resulted in lethality, thus confirming that the predicted catalytic activity of the protein is essential for its function (Ryan *et al.*, 2004). In addition, a protein of molecular size consistent with that of CPSF-73 was cross-linked in an AAUAAA-dependent manner to the vicinity of the cleavage site (Ryan *et al.*, 2004). Although immunoprecipitation experiments did not conclusively prove that the cross-linked protein was CPSF-73, these studies left little doubt that CPSF-73 is indeed the endonuclease in formation of polyadenylated mRNAs (Figure 3).

Interestingly, CPSF-73 is related to CPSF-100 (Jenny *et al.*, 1994), suggesting that their genes have evolved through duplication of a common ancestral gene. CPSF-100 shares typical features of the metallo-β-lactamase fold with other members of the family but contains alterations within the histidine motif and other conserved residues and therefore is most likely catalytically inactive (Aravind, 1999; Callebaut *et al.*, 2002).

Cleavage of Histone Pre-mRNAs

Metazoan replication-dependent histone pre-mRNAs are processed at the 3′ end by a unique processing machinery that until recently appeared to be entirely distinct from the cleavage/polyadenylation machinery (Dominski and Marzluff, 1999; Jaeger *et al.*, 2005). Replication dependent histone pre-mRNAs contain two sequence elements required for processing; a highly conserved stem-loop structure located less than 50 nucleotides 3′ to the stop codon, and a purine-rich sequence referred to as the histone downstream element (HDE) that begins 15 to 20 nucleotides downstream of the stem-loop (Marzluff *et al.*, 2002). Cleavage occurs between the two sequence elements, usually four to five nucleotides after the stem, and is not followed by polyadenylation (Figure 4). The stem-loop consists of a six-base-pair stem and a four-nucleotide loop and is a binding site for a protein termed the stem-loop binding protein (SLBP) (Wang *et al.*, 1996) or the hairpin binding factor (HBF) (Martin *et al.*, 1997). The HDE interacts with the U7 small nuclear ribonucleoprotein (snRNP) that contains an approximately 60-nucleotide U7 snRNA (Galli *et al.*, 1983; Mowry and Steitz, 1987). The interaction involves the formation of a duplex between the 5′ end of the U7 snRNA and the HDE. In the *in vitro* system based on mammalian nuclear extracts, the cleavage site is located at a fixed distance from the HDE suggesting that the U7 snRNP plays a key role in specifying the site of cleavage, most likely by directly recruiting the processing endonuclease (Scharl and Steitz, 1994). Preventing the interaction between the U7 snRNP and the pre-mRNA by using oligonucleotides complementary to either HDE or the 5′ end of U7 snRNA leads to complete inhibition of cleavage (Cotten *et al.*, 1991).

The central part of the U7 snRNA contains a unique Sm binding site that interacts with a heteroheptameric Sm complex (Grimm *et al.*, 1993). The Sm complex in the U7 snRNP differs from that found in the spliceosomal snRNPs as it lacks D1 and D2 Sm proteins and instead contains two Sm-like proteins, Lsm10, and Lsm11 (Pillai *et al.*, 2001; Pillai *et al.*, 2003). The cleavage reaction also requires a 100 kDa zinc finger protein

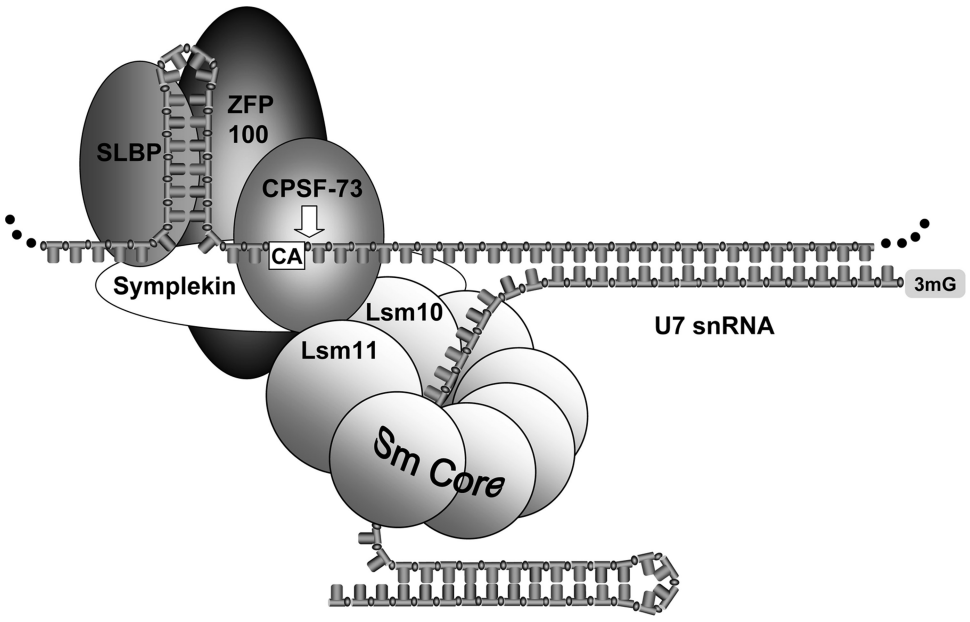

FIGURE 4 **Known components of the cleavage complex assembled on a histone pre-mRNA. The arrow indicates the site of cleavage by CPSF-73 that is preferentially located after a CA. The presented model does not include CPSF-100 and other polyadenylation factors, which together with symplekin may constitute an integral part of the heat labile factor. The location of symplekin in the complex is hypothetical.**

(ZFP100), which interacts both with SLBP bound to the stem-loop (Dominski *et al.*, 2002) and with Lsm11 (Azzouz *et al.*, 2005). This bridging interaction is believed to stabilize the U7 snRNP on the pre-mRNA and is particularly important in processing substrates unable to efficiently recruit U7 snRNP solely by base pairing (Streit *et al.*, 1993; Dominski *et al.*, 1999). Pre-mRNAs that form strong duplexes with the HDE are cleaved in nuclear extracts from mammalian cells in the absence of SLBP, indicating that at least under *in vitro* conditions SLBP is an auxiliary factor required for maximum efficiency rather than an essential component of the processing machinery.

A distinct component of nuclear extracts required for 3′ end processing of histone pre-mRNAs, referred to as heat labile factor (HLF), was defined based on its sensitivity to mild heat treatment (Gick *et al.*, 1987). This factor has been recently identified as symplekin (Kolev and Steitz, 2005). Interestingly, chromatographic fractions of a HeLa nuclear extract capable of restoring processing activity to a heat treated extract in addition to symplekin contained all five CPSF subunits and two out of three CstF subunits, CstF-77 and CstF-64 (Kolev and Steitz, 2005). However, it has not been yet determined whether these proteins are essential for 3′ end processing of histone pre-mRNAs.

Following cleavage of histone pre-mRNA by an endonuclease, the upstream product terminated with the stem-loop corresponds to the mature histone mRNA, whereas the downstream product is degraded in the 5′–3′ orientation by an exonuclease that is also dependent on the U7 snRNP (Walther *et al.*, 1998). The degradation is believed to release the U7 snRNA from its association with the HDE, hence allowing recycling of the U7snRNP for another round of processing. It remains to be determined whether the U7-dependent 5′ exonuclease additionally functions in termination of transcription of histone genes through the "torpedo" mechanism (Rosonina *et al.*, 2006; Luo *et al.*, 2006). Until recently the endonuclease remained unknown, although a number of observations suggested that it may be related to the enzyme involved in formation of polyadenylated mRNAs (Dominski *et al.*, 2005b). In support of this notion, both *in vitro* cleavage reactions share an unusual combination of features, *i.e.*,the ability to proceed in the presence of EDTA and to generate a 3′ hydroxyl and a 5′ phosphate, the termini typical of metal-dependent catalysis. In addition, both processing reactions have a strong preference for cleaving after a CA, although this is not an absolute requirement and other sites can be selected with lower efficiency.

An important clue to the identity of the endonuclease was provided by UV cross-linking studies with a site-specifically labeled histone pre-mRNA containing a phosphorothioate modification at the cleavage

site (Dominski *et al.*, 2005a). These studies identified a protein of approximately 85 kDa that forms a UV-induced cross-link with the RNA in the vicinity of the cleavage site in a U7-dependent manner. Changes to the *in vitro* processing reaction that resulted in either partial or complete inhibition of cleavage had a comparable effect on the efficiency of cross-linking, strongly suggesting that the 85 kDa protein is the endonuclease. Indeed, UV cross-linking of this protein absolutely required the presence of the phosphorothioate modification at the cleavage site, which likely slowed down catalysis thus extending the time window when the protein was in contact with the RNA (Dominski *et al.*, 2005a). A protein of the same molecular mass was also cross-linked to the downstream cleavage product during its degradation by a U7-dependent 5′ exonuclease (Dominski *et al.*, 2005a). Surprisingly, immunoprecipitation experiments identified the 85 kDa cross-link formed on each substrate as CPSF-73, thus suggesting that the same protein functions as both the endonuclease and 5′ exonuclease in 3′ end processing of histone pre-mRNAs (Dominski *et al.*, 2005a). This finding provided additional support for the notion that CPSF-73 is the endonuclease in cleavage/polyadenylation, and together with the demonstration that symplekin is involved in 3′ end formation of both histone mRNAs and polyadenylated mRNAs also revealed an unanticipated evolutionary link between the two types of 3′ end processing (Gilmartin, 2005).

CPSF-73 is an Endonuclease *In Vitro*

While a number of studies supported the notion that CPSF-73 is the key catalytic component that cleaves mRNA precursors at the 3′ end, direct evidence for its endonuclease activity was missing. This ultimate proof has been recently provided by the demonstration that the bacterially expressed N-terminal portion of human CPSF-73 (amino acids 1 to 460) can endonucleolytically cleave single stranded RNA substrates *in vitro* (Mandel *et al.*, 2006). Interestingly, the detection of the endonuclease activity requires pre-incubation of the recombinant CPSF-73 with calcium ions. Calcium ions per se are not required for catalysis but they most likely stimulate structural changes of CPSF-73 resulting in the activation of its enzymatic activity and/or increase in the accessibility of the catalytic site for the substrate. A mutant of CPSF-73, containing alanine substitutions of two conserved residues of the histidine motif was enzymatically inactive. No activity *in vitro* was also detected for CPSF-100, consistent with the predicted inability of this protein to bind zinc ions (Mandel *et al.*, 2006). It has not yet been conclusively determined whether CPSF-73 can also function *in vitro* as a 5′ exonuclease, as suggested by cross-linking studies on 3′ end processing of histone pre-mRNAs (Dominski *et al.*, 2005a) and the mode of action of some other metallo-β-lactamase nucleases (see below).

Potential Mechanisms for Recruitment and Activation of CPSF-73

An intriguing property of CPSF-73 is that in cleavage/polyadenylation, and presumably in 3′ end processing of histone pre-mRNAs, it co-exists with CPSF-100. The two proteins directly interact with each other and the interaction involves the C-terminal portion of CPSF-73, which is not a part of the catalytic domain (Dominski *et al.*, 2005b). The same structural arrangement has been identified in the highly related heterodimer consisting of RC-68 and RC-74 (Dominski *et al.*, 2005b) (see below). The biological sense of this arrangement is unknown. One possibility is that CPSF-100 functions as a repressor of the nucleolytic activity of CPSF-73 and relieves the repression only when CPSF-73 is recruited to the vicinity of the cleavage site. An alternative although not mutually exclusive possibility is that CPSF-100 serves as an adaptor subunit, whereas CPSF-73 is involved only in catalysis. Such a functional arrangement would be reminiscent of that existing in the long form of tRNase Z. This form has likely evolved from duplication of tRNase Z^S followed by catalytic inactivation of the N-terminal copy and its specialization toward substrate binding and regulatory functions, allowing the C-terminal portion to perform solely catalytic functions (Takaku *et al.*, 2004; Schilling *et al.*, 2005). Interestingly, in the homodimer of *B. subtilis* tRNase Z^S one subunit adopts the catalytically active configuration only upon binding of the pre-tRNA substrate. This enzyme may therefore represent a transitional state between dimers containing two fully active subunits, like in *E. coli* tRNase Z^S, and those in which one domain (or subunit) is permanently inactive, like in the long form of the tRNase Z and the CPSF-73/CPSF-100 heterodimer.

The involvement of CPSF-73 in 3′ end processing of histone pre-mRNAs, in addition to cleavage/

polyadenylation, raises an intriguing question of how the same endonuclease is recruited to the two vastly different processing machineries. In cleavage/polyadenylation, CPSF-73 is brought to the pre-mRNA by CPSF-160, which binds the AAUAAA upstream of the cleavage site. Histone pre-mRNAs do not contain the AAUAAA sequence and hence it is unlikely that CPSF-73 is brought to these substrates by CPSF-160. CPSF-73 may be recruited to the vicinity of the cleavage site through interaction with Lsm11, ZFP100, or with yet unknown components of the processing machinery. Or perhaps, as previously proposed (Kolev and Steitz, 2005), an important role in the recruitment of CPSF-73 is played by symplekin, CstF-77 and CstF-64; components of the cleavage/polyadenylation machinery that are potentially shared with 3′ end processing of histone pre-mRNAs. Although it has not yet been experimentally demonstrated, CPSF-73 is likely partnered with CPSF-100 also during 3′ end processing of histone pre-mRNAs and this catalytically inactive subunit may mediate these interactions, allowing CPSF-73 to engage solely in catalysis.

4. RC-68: 3′ END PROCESSING OF snRNA PRECURSORS

Small nuclear RNAs (snRNAs) are a large group of noncoding and metabolically stable RNAs that play important roles in processing of mRNA precursors (Soller, 2006). Most snRNAs are involved in splicing and are referred to as spliceosomal snRNAs. The spliceosomal snRNA are divided into two groups, major and minor, dependent on the type of splicing they participate in and their intracellular concentration (Wu and Krainer, 1999; Will and Luhrmann, 2001; Will and Luhrmann, 2005). The major spliceosomal snRNAs, U1, U2, U4, U5, and U6, are abundant and take a part in splicing of conventional introns, whereas the minor snRNAs, U11, U12, U4atac and U6atac exist in relatively low concentrations and are involved in splicing of AT-AC introns. U7 snRNA is the only known snRNA that does not participate in splicing and instead it is an essential component of 3′ end processing of histone pre-mRNAs (Schumperli and Pillai, 2004). Most snRNAs are synthesized by RNA polymerase II (Henry *et al.*, 1998; Hernandez, 2001). The only exceptions are U6 and U6atac snRNAs, which are synthesized by RNA polymerase III.

3′ End Processing of Pre-snRNA Depends on snRNA Promoter

Formation of the mature 3′ end in snRNAs synthesized by RNA polymerase II in vertebrates depends on the presence of a 13- to16-nucleotide conserved sequence element in the snRNA genes called the 3′ box located several nucleotides downstream relative to the mature snRNA 3′ end (Hernandez, 1985; Ach and Weiner, 1987). Efficient recognition of the 3′ box depends on initiation of transcription from a compatible promoter of the snRNA type. Transcription initiated from promoters of protein-encoding genes leads to generation of snRNAs lacking a properly formed 3′ end (Neuman de Vegvar *et al.*, 1986; Hernandez and Weiner, 1986). Based on these observations, formation of the 3′ end of snRNAs was initially believed to occur through accurate transcription termination directed by the 3′ box. More recent studies provided evidence that snRNAs are initially generated as longer precursors (pre-snRNAs) that extend far beyond the 3′ box and acquire the mature 3′ end through an endonucleolytic cleavage in the nucleus (Cuello *et al.*, 1999). Correct 3′ end processing *in vivo* depends on the C-terminal domain (CTD) of the large subunit of RNA polymerase II, likely reflecting the tight coupling between transcription and 3′ end processing in biogenesis of snRNAs (Medlin *et al.*, 2003; Jacobs *et al.*, 2004; Medlin *et al.*, 2005). As an important step towards studying the mechanism of 3′ end processing of snRNA precursors, a suitable *in vitro* system based on nuclear extracts and pre-synthesized RNA substrates containing the 3′ box has been developed (Uguen and Murphy, 2003). *In vitro* cleavage of synthetic snRNA precursors in crude preparations of nuclear extracts occurs with relatively low efficiency, most likely due to the inability to completely uncouple 3′ end processing from transcription *in vitro*, but can be significantly improved by partial purification of the 3′ box-dependent processing activity. The active fractions are not enriched in factors involved in cleavage/polyadenylation, leading to the important conclusion that 3′ end processing of precursors to snRNAs and mRNAs is carried out by two separate machineries (Uguen and Murphy, 2003). *In vitro* processing strongly depends on the wild-type sequence of the 3′ box, and for at least some snRNA precursors is stimulated by an additional sequence located several nucleotides upstream of the cleavage site (Uguen and Murphy, 2004). This bipartite organization of the processing elements is found in the U2 snRNA

precursors and resembles that existing in all pre-mRNA in which the downstream and the upstream sequences act in synergistic fashion to specify the cleavage site. Consistent with the *in vivo* results, *in vitro* 3′ end processing of snRNA precursors is stimulated by phosphorylated CTD and to a lesser extent, by non-phosphorylated CTD (Uguen and Murphy, 2003). The reaction resembles processing of histone pre-mRNAs in that it also requires an RNA component, possibly another snRNA, and a heat labile factor (Uguen and Murphy, 2004). Despite these important findings, the identity of these factors and in particular, the nature of the endonuclease cleaving the snRNA precursors remained unknown until recently.

Identification of a Potential Endonuclease in 3′ End Processing of Pre-snRNA

Known genomes of plants and animals encode a protein that is highly similar to CPSF-73 and retains all key residues of the metallo-β-lactamase fold necessary for metal binding and catalysis. The human CPSF-73 and its homologue share 40% identity and 60% similarity within the first 450 amino acids. The CPSF-73-like protein was first described in *Arabidopsis thaliana* where it was designated AtCPSF-II and shown to be essential for early stages of plant development (Xu *et al.*, 2004). The mammalian orthologue of AtCPSF-II was named RC-68, after a 68 kDa protein related to CPSF-73 (Dominski *et al.*, 2005b). In mammalian cells RC-68 forms a tight complex with a closely related protein, RC-74, which shares typical features of the metallo-β-lactamase fold but lacks some of the critical residues involved in metal binding. The similarity between the RC-68/RC-74 heterodimer and the complex of CPSF-73 and CPSF-100 suggested that RC-68 functions as an endonuclease in 3′ end processing of a subset of cellular RNA precursors, with histone pre-mRNAs being considered as the most likely candidate (Dominski *et al.*, 2005b). Since this task was later shown to be carried out by CPSF-73, one possibility was that substrates for RC-68 include precursors to small nuclear RNAs (snRNAs), which are also generated by RNA polymerase II.

Indeed, both RC-68 and RC-74 were found to exist in a multi-subunit complex designated Integrator, which is associated with snRNA genes but not protein-encoding genes (Baillat *et al.*, 2005). In addition to RC-68 and RC-74, the Integrator complex contains at least 10 other previously uncharacterized proteins and all the subunits of RNA polymerase II, including the large subunit (Figure 5). The presence of this subunit is consistent with earlier *in vitro* and *in vivo* studies demonstrating an essential role of the CTD in 3′ end processing of pre-snRNAs (Uguen and Murphy, 2003; Medlin *et al.*, 2003; Jacobs *et al.*, 2004; Medlin *et al.*, 2005). Depletion

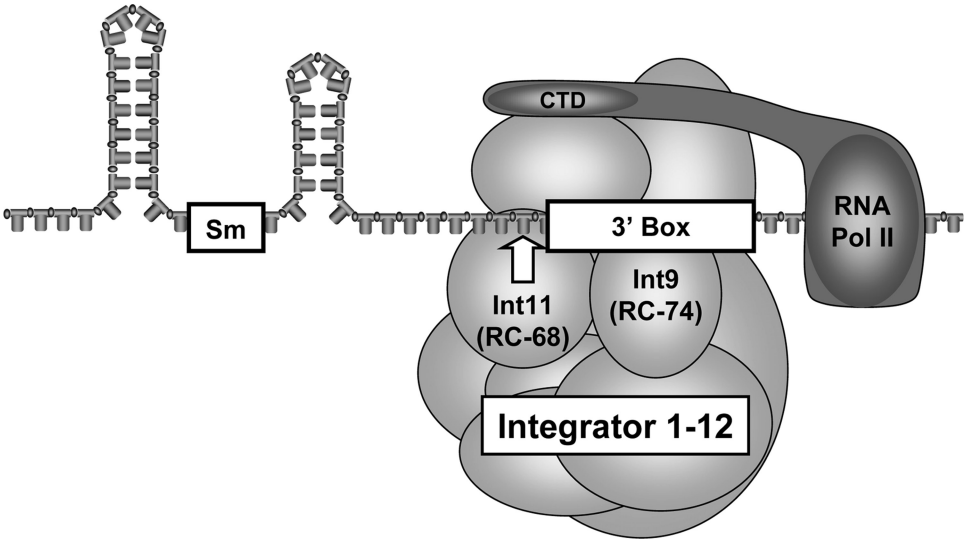

FIGURE 5 **Known components of the cleavage complex assembled on a pre-snRNA generated by RNA polymerase II. A schematic pre-snRNA with two stem-loops, the Sm binding site and the 3′ box is shown. The site of cleavage upstream of the 3′ box is indicated with an arrow. The cleavage complex consists of Integrator and all subunits of RNA Pol II, including the large subunit and its CTD. Integrator contains 12 subunits (not all subunits are depicted), of which RC-68/Int11 is the catalytic component and directly interacts with RC-74/Int9. Other interactions and positions of individual components within the complex are unknown. The complex also contains an essential RNA component, and a heat labile factor (not shown), which could be a separate entity or a subunit of the Integrator/Pol II complex.**

of individual subunits of Integrator, including RC-68 and RC-74, referred to as Int11 and Int9, respectively, results in a small but detectable increase in the amount of read-through transcripts that lack proper processing at the 3′ end. It is predicted that the Integrator complex is assembled at the promoter of U snRNA genes through its association with the CTD and travels with RNA polymerase II along the gene until the 3′ box in the nascent transcript is synthesized. The role of individual components of Integrator in 3′ end processing of pre-snRNAs is unknown (Baillat *et al.*, 2005). The 3′ box most likely serves as a processing signal recognized by at least one subunit of Integrator, which positions the catalytic component RC-68 at the cleavage site (Figure 5). It is possible that another set of components is required for initiation of transcription at specific promoters of the snRNA genes, whereas others may play regulatory roles or recognize additional signals for 3′ end processing in snRNA precursors.

Other Potential Substrates and Functions for RC-68 (Int11)

Although Integrator so far has only been detected in the vicinity of the U1 and U2 genes (Baillat *et al.*, 2005), it is anticipated that all vertebrate snRNAs generated by RNA polymerase II are processed by the same assembly of proteins. Moreover, an attractive possibility is that while CPSF-73 is the endonuclease in formation of all cellular mRNAs, both polyadenylated and histone mRNAs, RC-68, and the Integrator complex may play the same role in 3′ end processing of all non-coding RNAs that are synthesized by RNA Pol II. Among potential substrates for Integrator are the RNA component of telomerase (Feng *et al.*, 1995; Fu and Collins, 2003) and individually transcribed small nucleolar RNAs (snoRNA), including U3 and U8 snoRNAs (Watkins *et al.*, 2004). The substrates may also include primary transcripts lacking open reading frames and instead containing micro RNAs (Cullen, 2004). At least some of these transcripts do not contain the AAUAAA, suggesting that they are not processed by CPSF-73 (Bartel, 2004; Ohler *et al.*, 2004). Finally, recent studies of animal and plant genomes using high-resolution tiling arrays identified a plethora of transcripts synthesized from strands antisense to known genes and in intergenic regions (Bertone *et al.*, 2004). Little is known about the RNA polymerases involved in this previously unanticipated transcriptional activity or the role of the generated transcripts. However, most of these transcripts of unknown function (TUFs) are not polyadenylated and contain only short open reading frames (Mattick, 2003; Frith *et al.*, 2005) thus suggesting that they may undergo 3′ end processing carried out by RC-68 and Integrator.

The genomes of most fungi do not contain the RC-68 gene (Dominski *et al.*, 2005b). In *S. cerevisiae*, precursors to snRNAs are processed by a homologue of RNase III (Chanfreau *et al.*, 1997; Chanfreau *et al.*, 1998; Abou and Ares, Jr., 1998; Perumal and Reddy, 2002). In addition, a second processing pathway for pre-snRNAs likely exists in yeast cells that utilizes some components of the cleavage/polyadenylation machinery and a number of other proteins (Steinmetz *et al.*, 2001; Morlando *et al.*, 2002; Vasiljeva and Buratowski, 2006). The only known fungus that contains RC-68 is *Encephalitozoon cuniculi* (Katinka *et al.*, 2001; Vivares and Metenier, 2001), an intracellular parasite of various mammals that belongs to a fungal group called microsporidia (Keeling and Fast, 2002). *E. cuniculi* contains two highly homologous proteins, one being clearly more similar to CPSF-73 and the other being more similar to RC-68. Interestingly, there is only one identifiable homologue of CPSF-100/RC-74 with the disrupted histidine motif. This protein shares more similarity with CPSF-100 than with RC-74, suggesting that the genome of *E. cuniculi* does not encode an orthologue of RC-74. Consistent with this observation, the *E. cuniculi* RC-68 lacks the C-terminal domain that in the mammalian protein is involved in the interaction with RC-74 (Dominski *et al.*, 2005b).

The genome of *E. cuniculi* is the smallest among known eukaryotic genomes and is extremely compacted, due to gene overlapping as well as to loss of non-coding sequences and regions encoding nonessential protein domains (Keeling, 2001; Vivares *et al.*, 2002). Thus, the presence of RC-68 in *E. cuniculi*, in conjunction with its absence in other species of fungi, is striking and indicates that RC-68 plays an essential role in this organism. In human cells, RC-68 localizes to both the nucleus and the cytoplasm while its binding partner RC-74 is present only in the nucleus (Dominski *et al.*, 2005b). It is therefore possible that the role of RC-68 is not limited to 3′ end processing of snRNA precursors and includes another process that takes place exclusively in the cytoplasm. The involvement of RC-68 in this process rather than in 3′ end processing of pre-snRNAs may be of vital importance for *E. cuniculi*. Consistent with this hypothesis, *E. cuniculi* appears to

lack almost all subunits of Integrator, although these subunits are conserved in metazoa and plants (Baillat *et al.*, 2005).

5. Snm1A: REPAIR OF DNA INTERSTRAND CROSS-LINKS

DNA interstrand cross-links (ICLs) prevent strand separation during transcription and replication and constitute one of the most severe types of DNA damage (Dronkert and Kanaar, 2001). ICLs can be generated by a number of chemicals including photoactivated psoralens, nitrogen mustard, mitomycin C and cis-platin. Repair of ICLs is initiated by the introduction of incisions in the vicinity of the damage, leading to formation of DNA double strand breaks.

Yeast Snm1/Pso2

A search for *S. cerevisiae* mutants sensitive to nitrogen mustard or psoralen resulted in the identification of a gene, *SNM1* (after "sensitive to nitrogen mustard"), also known as *PSO2* (after "psoralen"), required for normal ability to repair ICLs. Mutants in the *SNM1* gene are specifically disabled in repair of ICLs and are not sensitive to agents causing other types of DNA damage such as ionizing radiation, UV and monoalkylating chemicals. The yeast *SNM1* gene is not essential and encodes a 661-amino acid protein containing the metallo-β-lactamase domain. Yeast *snm1* mutants treated with nitrogen mustard retain normal ability to incise DNA in the vicinity of cross-links but are impaired in restoration of high molecular weight chromosomal DNA. This phenotype demonstrates that Snm1 protein is not involved in endonucleolytic cleavage of DNA in the vicinity of ICLs. This task is likely carried out by components of the nucleotide excision repair (NER) system (Dronkert and Kanaar, 2001). Mutation of a single conserved amino acid within the histidine motif of Snm1 was sufficient to abolish its activity in repair of ICLs, demonstrating that Snm1 is functionally dependent on the catalytically active metallo-β-lactamase domain (Li and Moses, 2003).

Vertebrate Snm1A

While both *D. melanogaster* and *C. elegans* contain only one Snm1 homologue, in vertebrates three proteins designated Snm1A (Snm1), Snm1B (Apollo), and Snm1C (Artemis) share significant sequence similarity with the yeast Snm1/Pso2 protein (Dronkert *et al.*, 2000; Ishiai *et al.*, 2004). Snm1A is the longest of the three proteins and has the highest degree of similarity to yeast Snm1/Pso2 (Dronkert *et al.*, 2000; Ishiai *et al.*, 2004). Human Snm1A is a nuclear protein expressed at a very low level in all tested cell types (Dronkert *et al.*, 2000; Richie *et al.*, 2002). Deficiency of Snm1A causes increased sensitivity of the DT40 chicken cells to both mitomycin C and cisplatin (Ishiai *et al.*, 2004) and only moderately sensitizes mouse cells to mitomycin C but not to other cross-linking agents (Dronkert *et al.*, 2000; Akhter *et al.*, 2005). This incomplete phenotype strongly contrasts with the effects of disrupting the *SNM1* gene in *S. cerevisiae*, which sanitizes cells to a broad spectrum of cross-linking agents, and likely results from the existence of Snm1B (see below) and redundancy of ICL repair pathways in vertebrates (Dronkert *et al.*, 2000). Knock out mice lacking the Snm1A gene are viable and fertile, indicating that Snm1A protein is not essential for development (Akhter *et al.*, 2005). In mammalian cells Snm1A changes its intra-nuclear localization shortly after the treatment with cross-link inducing agents and accumulates in multiple foci reflecting the recruitment of Snm1A to the sites of DNA damage (Richie *et al.*, 2002).

It has not yet been determined how Snm1A contributes to repair of DNA interstrand cross-links. Snm1A may function as a nuclease in post-incision steps, most likely in processing of ICL-specific double strand breaks prior to final restoration of the DNA integrity. Consistent with this role, recent *in vitro* studies demonstrated that the bacterially expressed yeast Snm1 is capable of degrading single and double stranded DNA substrates in the 5′–3′ direction (Li *et al.*, 2005). Mutation of a key catalytic residue within the metallo-β-lactamase domain severely interfered with this activity, supporting the notion that Snm1 functions in repair of ICLs as a 5′ exonuclease.

6. ARTEMIS (Snm1C): V(D)J RECOMBINATION AND DNA DOUBLE-STRAND BREAK REPAIR BY NHEJ

The extraordinary diversity of immunoglobulins and T-cell receptors is achieved by V(D)J recombination, a process of DNA rearrangements that involves formation of DNA double strand breaks between each variable (V), diversity (D) and joining (J) segment of immunoglobulins and T-cell receptor genes (Bassing *et al.*,

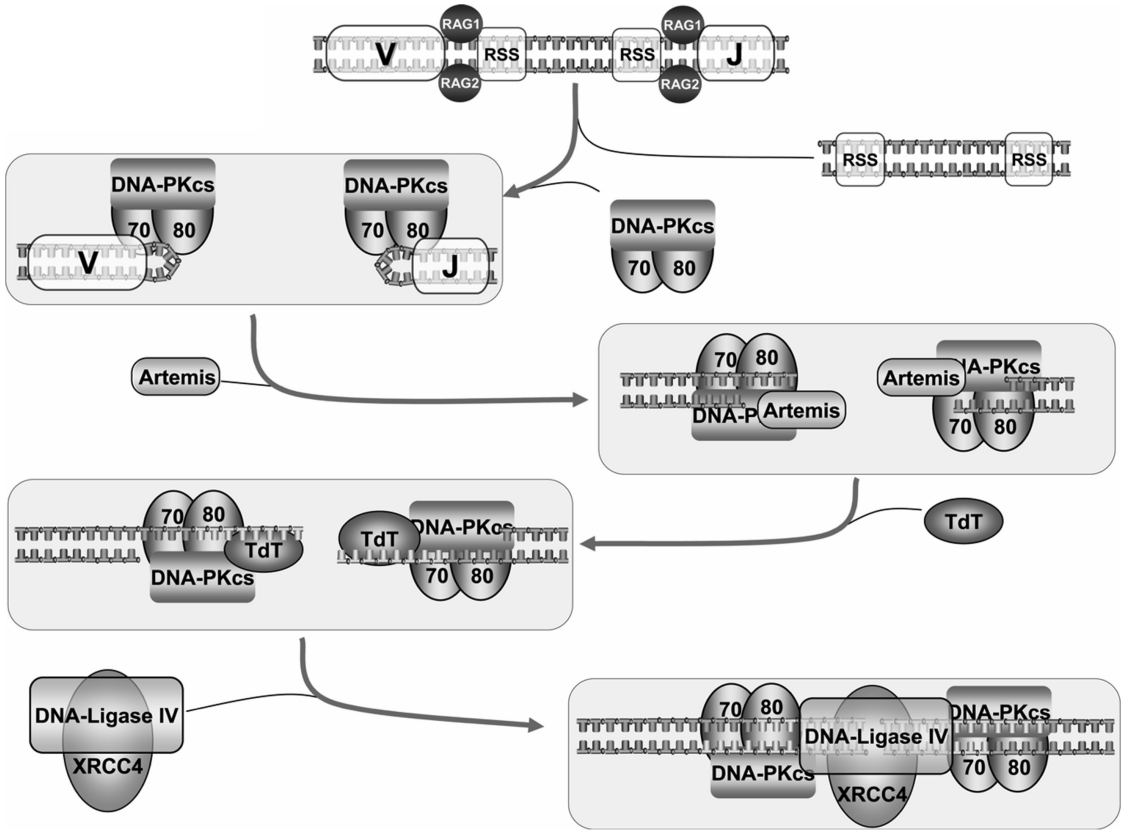

FIGURE 6 **The role of Artemis in V(D)J recombination. The model depicts direct recombination between V and J segments that occurs in the light chain loci. The difference in the sequence between the RSSs that flank V and J segments is not schematically indicated. DNA hairpins created near V and J segments by RAG1 and RAG2 are first recognized by the Ku70/80 heterodimer and subsequently endonucleolytically cleaved by the Artemis/DNA-PK$_{cs}$ complex. Artemis may also participate in an additional processing of DNA overhangs using its endo- and/or 5′–3′ exonucleolytic activities. The ends are extended by terminal deoxynucleotidyl transferase (TdT) that adds non-template nucleotides. The ligation step is carried out by the DNA-ligase IV/XRCC4 complex. The ligase complex also contains a newly identified component XLF/Cernunnos, which is not shown.**

2002; De Villartay *et al.*, 2003). V(D)J recombination is initiated by the introduction of single stranded breaks (nicks) by RAG-1 and RAG-2 proteins within recombination signal sequences (RSS) flanking each V, D, or J segment (Figure 6). The 3′ hydroxyl of each nick is subsequently involved in a transesterification reaction with the complementary strand, leading to formation of a DNA hairpin, an unusual DNA double strand break in which the 3′ and 5′ ends are covalently closed. The next step in V(D)J recombination is endonucleolytic cleavage within the hairpins, which results in formation of open ends typically containing 3′ overhangs. The open ends are subsequently processed and fused by nonhomologous end joining (NHEJ), a pathway ubiquitously used in higher eukaryotes to repair DNA double strand breaks (Lieber *et al.*, 2003; Lieber *et al.*, 2004). In vertebrates, NHEJ requires DNA-dependent protein kinase (DNA-PK), DNA-ligase IV, XRCC4 and a recently identified component called XLF or Cernunnos (Ahnesorg *et al.*, 2006; Buck *et al.*, 2006; Sekiguchi and Ferguson, 2006). DNA-PK consists of the catalytic subunit (DNA-PK$_{cs}$) and a complex of two regulatory subunits, Ku70 and Ku80, which bind to the DNA ends and stimulate catalytic activity of DNA-PKcs. In higher eukaryotes, NHEJ is also required for repair of pathological double strand breaks caused by exposure to chemical and physical agents (Lieber *et al.*, 2003; Lieber *et al.*, 2004). Mutations in all known components of NHEJ lead to RS-SCID (radiosensitive severe combined immunodeficiency), a phenotype characterized by a lack of mature B and T cells caused by impaired V(D)J recombination, and increased radiosensitivity resulting from reduced efficiency of repair of pathological DNA double strand breaks (Schwarz *et al.*, 2003).

The Role of Artemis in V(D)J Recombination

Until recently, opening of DNA hairpins presented a major unresolved question in V(D)J recombination.

A number of *in vivo* and *in vitro* studies suggest that this step is carried out by Artemis, also known as Snm1C, a member of the metallo-β-lactamase family of proteins that is ubiquitously expressed at low levels in all tissues analyzed (Moshous *et al.*, 2001; Ma *et al.*, 2002). Human Artemis is a 692-amino acid protein and contains the metallo-β-lactamase domain in the N-terminal portion (Moshous *et al.*, 2001). *In vitro*, Artemis is a 5′–3′ exonuclease capable of degrading single stranded DNA fragments, with the activity strongly depending on the presence of a 5′ phosphate on the DNA substrate (Ma *et al.*, 2002). Interestingly, Artemis is also efficient in degrading single stranded RNAs, indicating that under *in vitro* conditions Artemis has a general specificity toward single-stranded nucleic acids. Upon association with DNA-PK_{cs}, Artemis is phosphorylated and becomes predominantly an endonuclease capable of opening DNA hairpins and cleaving 3′ and 5′ overhangs (Ma *et al.*, 2002). In DNA hairpin opening, the Artemis/DNA-PK_{cs} complex preferentially cleaves 3′ to the tip of the hairpin substrate, consistent with the specificity of the hairpin opening activity *in vivo*. Additional support for the role of Artemis in opening DNA hairpins that are generated during V(D)J recombination comes from analysis of Artemis-deficient mice, which accumulate these unprocessed intermediates in thymocytes (Rooney *et al.*, 2002). This defect is strikingly similar to that found in mice lacking DNA-PKcs providing strong *in vivo* evidence that these two proteins work together to open covalently sealed DNA ends. The mechanism by which DNA-PK_{cs} confers the endonuclease activity on Artemis has not yet been fully elucidated, although an attractive model has emerged from recent studies. According to this model, the endonuclease activity of Artemis is suppressed by the C-terminal part of the protein and its phosphorylation in multiple sites by DNA-PK_{cs} (Ma *et al.*, 2005a; Soubeyrand *et al.*, 2006) relieves the autoinhibition (Niewolik *et al.*, 2006). Consistent with this model, deletion variants of Artemis lacking the C-terminal domain are capable of cleaving DNA hairpins independent of DNA-PK_{cs} (Niewolik *et al.*, 2006).

Based on a sequence comparison with other members of the metallo-β-lactamase family, nine amino acids in Artemis have been predicted to participate in metal binding and catalysis (Pannicke *et al.*, 2004). As expected, most mutant proteins with either single or double substitutions of these residues lacked endonuclease activity in hairpin opening both *in vitro* and *in vivo*. However, one mutant protein containing a substitution of the last histidine in the highly conserved HxHxDH motif retained nearly normal endonucleolytic activity (Pannicke *et al.*, 2004; Poinsignon *et al.*, 2004). In tRNase Z, the corresponding residue is involved in coordination of the second zinc ion and substitutions of this residue nearly abolish the enzymatic activity of *D. melanogaster* tRNase Z^L (Zareen *et al.*, 2005). Perhaps a histidine in this position in Artemis plays a less important role or its function can be partially replaced by another residue. Surprisingly, all tested mutant proteins of Artemis that were inactive in opening of DNA hairpins retained normal 5′ exonuclease activity *in vitro* (Pannicke *et al.*, 2004). Further studies are required to determine how this intriguing and unexpected result can be reconciled with the fact that metallo-β-lactamase fold enzymes contain only one active site that is expected to function in hydrolysis of all types of substrates.

The detailed *in vitro* and *in vivo* analysis of Artemis suggests a likely sequence of events that follows the formation of DNA hairpins in V(D)J recombination (Figure 6). The DNA double strand breaks are first recognized by a heterodimer of Ku70 and Ku80, which recruits a complex of DNA-PK_{cs} and Artemis. Within the complex Artemis is phosphorylated by DNA-PK_{cs} and acquires endonucleolytic activity capable of opening DNA hairpins 3′ to their tip thus creating 3′ overhangs on each end. The ends are further processed by the same endonucleolytic and/or 5′ exonucleolytic activity of Artemis and, if necessary, extended by terminal deoxynucleotidyl transferase (TdT) that additionally contributes to the diversity of antibodies and T-cell receptors. The final step in V(D)J recombination is joining the processed ends by a complex of DNA-ligase IV with two regulatory proteins, XRCC4 and XLF/Cernunnos, resulting in physical association of V, D, and J segments.

Artemis in Repair of DNA Double Strand Breaks

In addition to causing SCID, mutations in Artemis result in increased sensitivity to X-rays, suggesting that Artemis also participates in repair of pathological DNA double strand breaks (Moshous *et al.*, 2001). Since pathological DNA double strand breaks do not end with hairpins, one role of Artemis in repair of these lesions may be to process 3′ and 5′ overhangs prior to the ligation phase (Ma *et al.*, 2002; Le Deist *et al.*, 2004). According to other studies, Artemis is not directly

involved in repair of DNA double-strand breaks and instead may function as a check point protein required for proper cell cycle arrest in response to DNA damage (Zhang *et al.*, 2004). In support of this model, it has been shown that the N-terminal catalytic half of Artemis containing the metallo-β-lactamase domain alone can support V(D)J recombination but it does not complement the radiosensitive phenotype of Artemis-deficient cells (Poinsignon *et al.*, 2004). This result suggests that the C-terminal half of the protein while dispensable for V(D)J recombination, may have a unique function in sensing pathological DNA double strand breaks.

7. Snm1B (APOLLO): PROCESSING OF CHROMOSOME ENDS

Human Snm1B protein consists of 532 amino acids and resembles Artemis in that it also contains the metallo-β-lactamase domain in the N-terminal region. In both proteins the histidine motif HxHxDH begins about 30 nucleotides from the N-terminus. Based on this structural similarity, Snm1B has recently been renamed Apollo, after the twin brother of Artemis in Greek mythology (van Overbeek and De Lange, 2006; Lenain *et al.*, 2006). Chicken DT40 cells lacking the gene encoding Snm1B display increased sensitivity to cisplatin and mitomycin C, although the phenotype is weaker than that caused by a deficiency of Snm1A (Ishiai *et al.*, 2004). Depletion of Snm1B by RNA interference sensitizes Hela cells to ionizing radiation, suggesting that Snm1B may be involved in repair of pathological double strand breaks in addition to playing a role in repair of ICLs (Demuth *et al.*, 2004).

Recent studies from three different laboratories resulted in the surprising finding that a fraction of the cellular Snm1B pool associates with telomeres (van Overbeek and De Lange, 2006; Lenain *et al.*, 2006; Freibaum and Counter, 2006). Snm1B is recruited to telomeres through interaction with TRF2, which directly binds to the telomeric DNA repeats (Smogorzewska and De Lange, 2004). Snm1B is much less abundant at telomeres than TRF2, suggesting that it is an accessory factor only transiently associated with these structures (van Overbeek and De Lange, 2006). Reduction of the cellular level of Snm1B by RNAi results in the accumulation of DNA-damage response factors at chromosome ends. This effect is predominantly observed in S phase cells, indicating that under normal conditions Snm1B is active at telomeres only during DNA replication (van Overbeek and De Lange, 2006). The telomere dysfunction caused by depletion of Snm1B is intensified by the mutations within TRF2 (Lenain *et al.*, 2006). Cells lacking both proteins at telomeres display a number of abnormalities including increased DNA-damage response, a high frequency of chromosome fusions, and severe growth defects.

Consistent with the results obtained for Artemis/Snm1C and Snm1A, recombinant Snm1B/Apollo was also shown to function *in vitro* as a 5′ exonuclease on single stranded DNA oligonucleotides (Lenain *et al.*, 2006). Snm1B is also capable of removing the 5′ nucleotides from a blunt end or a 3′ overhang. However, in these cases the efficiency of degradation is relatively low. It has not yet been determined how the 5′ exonuclease activity of Smn1B contributes to the stability of telomeres. One possibility is that Smn1B is involved in nucleolytic processing of DNA ends in postreplication chromosomes and allows generation of stable telomere structures that differ from typical double strand breaks and therefore are not recognized by cellular DNA repair mechanisms (Szilard and Durocher, 2006).

8. *BACILLUS* RNases J1 AND J2: CLEAVAGE OF THE T-BOX TRANSCRIPTS

A group of genes involved in biosynthesis of amino acids and tRNA aminoacylation are regulated in *Bacillus subtilis* and other Gram-positive bacteria by a common mechanism of tRNA-mediated antitermination (Putzer *et al.*, 2002; Henkin and Yanofsky, 2002; Grundy and Henkin, 2003). Transcripts generated from these genes contain an approximately 300-nucleotide 5′ leader (an untranslated region that spans between the 5′ end and the translation initiation codon) that can fold into two competing and mutually exclusive structures, the terminator or antiterminator. Folding of the nascent mRNA into the terminator results in premature transcription termination and prevents expression of the gene, while folding into the antiterminator eliminates this negative regulation and allows transcription to continue into the coding region. Formation of the antiterminator depends on the interaction of the nascent mRNA with the uncharged cognate tRNA, which functions as a sensor to monitor the availability of the cognate amino acid. In the absence of the cognate amino acid due to amino acid starvation, the uncharged tRNA accumulates to higher concentrations and interacts with the

leader, thus stabilizing the antiterminator structure at the expense of the terminator and increasing the expression of the gene specific to the limiting amino acid. The specificity of the interaction is provided by base pairing between the tRNA anticodon and complementary nucleotides in the leader. An additional interaction occurs between the last four nucleotides of the tRNA (the discriminator and the CCA) and nucleotides of the leader located within the T-box, a highly conserved sequence present in all genes regulated by amino acid starvation. It is believed that the charged tRNA is not capable of interacting with the T-box nucleotides due to steric hindrance imposed by the amino acid covalently attached to the last nucleotide of the tRNA.

It has been observed that following successful transcription, the full length transcript is endonucleolytically cleaved within the antiterminator resulting in the generation of a 5′ truncated transcript that has a longer half life than the full length transcript (Condon *et al.*, 1996). Thus, formation of the tRNA-mediated antiterminator contributes in two ways to increased expression of the genes involved in amino acid metabolism: first, by allowing synthesis of the full length transcript and second, by facilitating its processing to result in increased stability. Biochemical fractionation of a high salt wash of *Bacillus* ribosomes resulted in identification of two related and previously uncharacterized proteins responsible for cleaving T-box leaders (Even *et al.*, 2005). The two proteins referred to as ribonucleases (RNases) J1 and J2 were classified based on sequence analysis as members of the metallo-β-lactamase family. Both RNase J1 and J2 consist of 555 amino acids and are 49% identical. RNases J1 and J2 purified from *B. subtilis* ribosomes or overexpressed as recombinant proteins cleave the T-box transcripts at the same sites as those utilized *in vivo*, supporting the notion that the two ribonucleases are involved in the tRNA-mediated regulation of gene expression.

RNase J1 is essential, whereas the gene encoding RNase J2 can be deleted from the *Bacillus* genome without causing any obvious growth defects (Even *et al.*, 2005). This result suggests that RNase J1and J2 have overlapping rather than redundant functions. Indeed, it has recently been demonstrated that RNase J1 in addition to cleaving 5′ leaders of the T-box transcripts also catalyzes the final step in maturation of 16S rRNA (Britton *et al.*, 2007). Depletion of RNase J1 or disruption of its histidine motif results in an accumulation of 16S rRNA precursors in *B. subtilis*. It is yet unclear whether the role of RNase J1 in the maturation of 16S rRNA is essential for *B. subtilis* and explains the lethal effect caused by the deficiency of this protein. The unprocessed precursors to 16S rRNA are found in functional ribosomes consistent with the possibility that RNase J1 performs other vital functions in RNA metabolism in *B. subtilis* (Britton *et al.*, 2007).

Reduced expression of RNase J1 in conjunction with deletion of the RNase J2 gene also results in a moderate increase in half life of selected messages, raising the possibility that the two *B. subtilis* nucleases may participate in mRNA degradation (Even *et al.*, 2005). In *E. coli* a key role in both mRNA degradation and 16S rRNA maturation is played by RNase E, an endonuclease unrelated to RNases J1 and J2 that exists in many bacterial species but is absent from *B. subtilis* (Condon and Putzer, 2002). Interestingly, RNase E can also efficiently cleave *B. subtilis* T-box transcripts when expressed in *E. coli* (Condon *et al.*, 1997). Thus, RNases J1/J2 and RNase E despite of sharing no sequence or structural similarity perform numerous equivalent functions in these two bacterial species. RNases J1 and J2 are present in many organisms of both bacteria and archaea kingdoms and in some of them often exist together with RNase E. Many species encode only a single RNase J whereas others can have as many as four copies of RNase J (Even *et al.*, 2005). Further studies are required to identify additional functions for RNase J in these organisms and determine the extent of functional overlap between this group of nucleases and RNase E.

9. THE β-CASP DOMAIN

Typical members of the metallo-β-lactamase family are characterized by the presence of five short sequence motifs that contain highly conserved residues, histidines, and acidic amino acids, which are involved in metal binding and catalysis (Aravind, 1999; Daiyasu *et al.*, 2001). A structure-based sequence comparison of CPSF-73, Artemis and the yeast Snm1/Pso2, led to the conclusion that these three proteins display a number of structural features, which distinguish them from other members of the metallo-β-lactamase family (Callebaut *et al.*, 2002). The most characteristic feature of these three proteins is the lack of a readily identifiable motif 5 and instead the presence of a larger domain called β-CASP appended to the metallo-β-lactamase domain and three conserved motifs A–C. Motif A consists of a stretch of hydrophobic residues followed by an acidic

residue. Motif B contains a conserved histidine, whereas motif C varies between different proteins and contains either a histidine (CPSF-73) or a valine (Artemis and Snm1/Pso2). All three conserved amino acids are predicted to be positioned at the end of β-strands, in the vicinity of the catalytic center formed by the metal binding residues of the canonical metallo-β-lactamase domain (Callebaut *et al.*, 2002).

The β-CASP domain is not limited to the founding members of the group and its presence is also predicted in other metallo-β-lactamase proteins acting on nucleic acids, including RC-68, Snm1B (Apollo) (Callebaut *et al.*, 2002), and the two *Bacillus* ribonucleases, RNase J1 and J2 (Even *et al.*, 2005). The characteristic structural features of the β-CASP domain are also found in CPSF-100 and RC-74, the two members of the metallo-β-lactamase family that are predicted to be catalytically inactive due to the lack of some highly conserved residues in motifs 1 to 4 and A-C (Callebaut *et al.*, 2002). Attempts were made to classify tRNase Z^S and tRNase Z^L as members of the β-CASP group (Dumont *et al.*, 2004). However, these two proteins do not fall into the same category as β-CASP proteins (Ishikawa *et al.*, 2006) and in fact, based on sequence similarities were initially classified as arylsulfatases and included in a separate group of the metallo-β-lactamase family (Daiyasu *et al.*, 2001).

Important clues to the structure of the β-CASP proteins have been provided by the recent crystallographic studies on human CPSF-73 (amino acids 1 to 460), yeast CPSF-100 (Mandel *et al.*, 2006) and a bacterial homologue of these two proteins from *Thermus thermophilus*, TTHA0252 (Ishikawa *et al.*, 2006). As previously predicted based on sequence analysis (Callebaut *et al.*, 2002), the crystal structure of the N-terminal portion of CPSF-73 containing the first 460 amino acids revealed the presence of two closely integrated domains: the metallo-β-lactamase domain and the β-CASP domain (Mandel *et al.*, 2006). The metallo-β-lactamase domain folds into the typical $\alpha\beta/\beta\alpha$ sandwich and occupies residues 1 to 208, whereas the β-CASP domain is located between amino acids 209–394 (Figure 7). Interestingly, amino acids C-terminal to the β-CASP domain contribute three additional β-strands to the $\alpha\beta/\beta\alpha$ sandwich, indicating that the β-CASP domain forms a cassette inserted into the metallo-β-lactamase domain (Figure 7). The β-CASP domain consists of a central β-sheet flanked by α-helices and structurally resembles the nucleotide binding fold (NBF), although it lacks the Walker A motif and is unlikely to bind nucleotides. It is unknown at present what role is played by the β-CASP domain. Potential functions may include substrate binding and/or regulation of enzymatic activity. In the crystal structure of CPSF-73, the active site is located deep in the interface between the two domains and under normal conditions appears to be inaccessible to the RNA substrate, suggesting that the protein may require a structural rearrangement prior to catalysis (Mandel *et al.*, 2006). The active site of CPSF-73 contains two zinc ions, which are bound by the protein with extremely high affinity. Yeast CPSF-100 folds into the same overall structure as CPSF-73 although it does not contain zinc ions, consistent with the lack of conserved amino acids in the metallo-β-lactamase

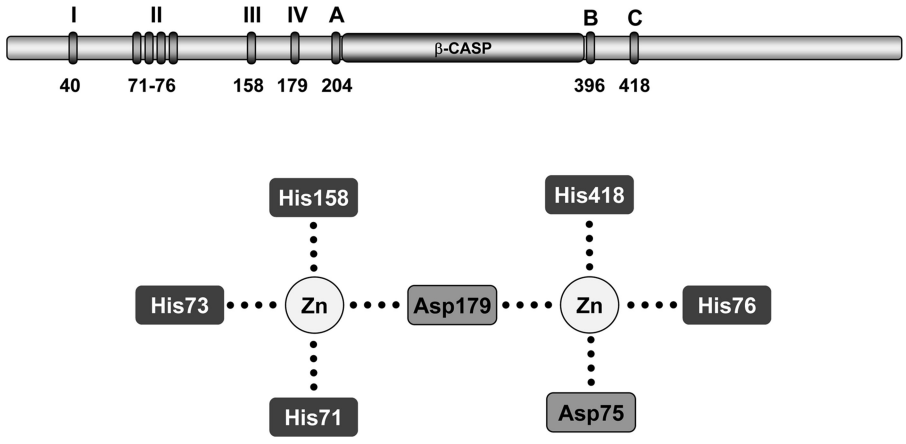

FIGURE 7 **CPSF-73 as a member of the β-CASP group of the metallo-β-lactamase family. The unusual domain arrangement in human CPSF-73 and positions of the conserved motifs I–IV and A–C are shown at the top. The β-CASP domain is inserted as a cassette into the metallo-β-lactamase domain between motifs A and B. Amino acids involved in coordination of the two zinc ions in the catalytic center are shown below.**

and β-CASP domains of this protein (Mandel *et al.*, 2006).

Motifs A (Glu 204) and B (His 396) are located on the C- and N-terminal edges of the split metallo-β-lactamase domain, respectively, and flank the intervening β-CASP domain. The histidine of motif C is located in position 418 (Figure 7). The structure of CPSF-73 demonstrated that one zinc ion is coordinated by His 71 and by His 73 of motif 2, and His 158 of motif 3. Amino acid ligands of the second zinc ion include Asp 75 and His 76 of motif 2 and His 418 of motif C. The two zinc ions are bridged by Asp 179 from motif 4 (Figure 7). The conserved amino acids of motif 1 and A-B are required for stabilization of the active center and catalysis (Mandel *et al.*, 2006). Virtually the same structure has been solved for TTHA0252 from *Thermus thermophilus*, which shares strong sequence similarity with CPSF-73 (Ishikawa *et al.*, 2006).

One of the amino acids coordinating the second zinc ion in CPSF-73 and TTHA0252 is histidine in motif C, indicating that in β-CASP proteins this residue functionally substitutes for the conserved histidine of motif 5 found in the canonical metallo-β-proteins. Interestingly, the corresponding position in Artemis and other DNA-specific β-CASP proteins is occupied by valine, suggesting that the second zinc ion in these proteins is coordinated by a different amino acid. Previously it has been proposed that the presence of either a histidine or a valine in motif C may determine substrate specificity of the β-CASP proteins towards either RNA (histidine) or DNA (valine) (Callebaut *et al.*, 2002). This hypothesis was not supported by subsequent studies on Artemis, which despite having a valine in motif C can act as a 5′ exonuclease on both single stranded DNA and RNA substrates (Ma *et al.*, 2002). In addition, replacement of this residue with alanine, histidine or serine did not abolish the nuclease activity of Artemis, suggesting that this residue is not essential (Poinsignon *et al.*, 2004).

10. *IN VITRO* NUCLEASE ACTIVITY AND METAL REQUIREMENTS

A major effort has been undertaken in recent years to prove that selected members of the metallo-β-lactamase family are capable of hydrolyzing DNA and/or RNA substrates *in vitro* and thus display genuine nuclease activity. This ultimate proof of activity was first provided for the long and short forms of tRNase Z that had been highly purified from a number of different organisms representing all three kingdoms; bacteria, archaea and eukaryota (Vogel *et al.*, 2005). tRNase Z^L and tRNase Z^S behave *in vitro* as typical endonucleases, cleaving tRNA precursors immediately after the discriminator. Following identification of the corresponding genes, the same results were reproduced with recombinant proteins purified using various expression systems. The nuclease activity was later demonstrated for other members of the metallo-β-lactamase family, including RNases J1 and J2 (Even *et al.*, 2005), Artemis (Ma *et al.*, 2002; Pannicke *et al.*, 2004; Ma *et al.*, 2005b) and more recently the yeast Snm1/Pso2 (Li *et al.*, 2005) and human Smn1B/Apollo (Lenain *et al.*, 2006). RNases J1 and J2 are endoribonucleases whereas Artemis, Snm1/Pso2 and Snm1B/Apollo have an intrinsic 5′ exonuclease activity on single stranded DNA substrates. Artemis also has specificity towards single stranded RNA species and, when complexed with and phosphorylated by DNA-PK_{cs}, functions as an endonuclease for single stranded DNA regions, including overhangs and loops (Ma *et al.*, 2002).

UV cross-linking studies suggest that CPSF-73 functions in 3′ end processing of histone pre-mRNAs as both an endonuclease that hydrolyzes the phosphodiester bond at the cleavage site and a 5′ exonuclease that is responsible for the subsequent degradation of the downstream cleavage product (Dominski *et al.*, 2005b). A detailed analysis of products generated during the cleavage reaction that precedes polyadenylation demonstrated that the downstream cleavage product is converted to a family of progressively shortened RNAs that differ in the position of the 5′ end (Moore and Sharp, 1985; Moore *et al.*, 1986; Sheets *et al.*, 1987). This observation strongly supports the notion that CPSF-73 also functions as both an endonuclease and a 5′ exonuclease during formation of polyadenylated mRNAs. Most recent studies demonstrated that a bacterially expressed 460-amino acid version of human CPSF-73 lacking the C-terminal portion acts *in vitro* as a nonspecific endonuclease (Mandel *et al.*, 2006). Further studies are required to determine whether CPSF-73 also possesses 5′ exonuclease activity. Under *in vivo* conditions, CPSF-73 is a part of a multi-component complex and at least some of these components might be required to observe the full spectrum of CPSF-73 activities *in vitro*.

Although the majority of hydrolytic enzymes of the metallo-β-lactamase family are believed to be specific for zinc ions, at least some of them can use other metals in catalysis. The recent structural studies on

phosphorylcholine esterase revealed that this hydrolytic enzyme contains two iron ions in the active site (Garau *et al.*, 2005). On the other hand, glyoxalase II from *A. thaliana* has unusually relaxed metal requirements, yielding comparable catalytic efficiencies independent of the ratios of *in vivo* bound iron, manganese and zinc ions (Schilling *et al.*, 2003). The most compelling evidence for the exclusively zinc-dependent catalysis exists for tRNase Z from *E. coli*. Both structural (Kostelecky *et al.*, 2006) and spectroscopic studies (Vogel *et al.*, 2004) have demonstrated that each monomer of *E. coli* tRNase Z^S binds two atoms of zinc and that substitution of zinc with other metals including magnesium, manganese, and iron drastically reduced the activity of the enzyme (Vogel *et al.*, 2002). The presence of zinc ions has also been demonstrated in the crystal structure of tRNase Z^S from *B. subtilis* (de la Sierra-Gallay *et al.*, 2005) although additional studies using alternative methods are required to verify this result.

Surprisingly, a number of reports indicated that, depending on the type of substrate and the source of tRNase Z, *in vitro* processing of pre-tRNA is optimal in the presence of magnesium ions varying in concentration between 0.3 and 5 mM, (Mohan *et al.*, 1999; Schierling *et al.*, 2002). The enzymatic activity of tRNase Z purified from various sources was also shown to be stimulated by different concentrations of manganese (Spath *et al.*, 2005; Shibata *et al.*, 2006; Minagawa *et al.*, 2006). One possible explanation to this apparent contradiction is that magnesium or manganese ions are required for the proper RNA folding and/or binding of the substrate to the enzyme while catalysis depends solely on zinc ions (Mohan *et al.*, 1999; Vogel *et al.*, 2005). The activity of other nucleases of the family was also routinely tested in the presence of magnesium ions and addition of EDTA in at least some cases inhibits the activity. Artemis was active only in magnesium-containing buffers and changing this cofactor to manganese or zinc abolishes the nuclease activity (Ma *et al.*, 2002). The presence of magnesium ions was also necessary to detect endonuclease activity for RNases J1 and J2, and 5′ exonuclease activity for Snm1 (Li *et al.*, 2005) and Snm1B (Lenain *et al.*, 2006), although in these cases it has not been tested whether other metals can substitute for magnesium (Even *et al.*, 2005). It should be noted that, at least for some proteins of the family, the nuclease activity was tested using unstructured RNAs and hence the requirement for divalent cations cannot be solely explained by its role in substrate folding. Clearly, further structural and enzymatic studies are required to fully understand the importance and the role of magnesium ions in nucleolytic activity of the family members.

Cleavage that precedes polyadenylation occurs efficiently in the presence of 5 mM EDTA, although it is sensitive to higher concentrations of EDTA (Hirose and Manley, 1997; Ryan *et al.*, 2004). The resistance to EDTA rules out the involvement of magnesium ions in catalysis and was the source of the long-standing view that cleavage is metal-independent. It is now well documented that in fact catalysis depends on zinc ions. Among observations that support this notion, it was shown that zinc-specific chelators are more effective than EDTA in inhibiting the cleavage reaction and zinc ions can restore high efficiency cleavage to nuclear extracts that have lost the processing activity due to prolonged dialysis (Ryan *et al.*, 2004). More importantly, the recent structural studies of human CPSF-73 provided the strongest so far argument for the presence of zinc ions in the catalytic center (Mandel *et al.*, 2006). The catalytic center of CPSF-73 is positioned deep inside the protein and binds zinc ions with extremely high affinity, explaining the inability of EDTA to inhibit the enzymatic activity of CPSF-73 during 3′ end processing of pre-mRNAs (Mandel *et al.*, 2006).

It is intriguing that 3′ end processing of histone pre-mRNAs despite using the same endonuclease is routinely carried out in the presence of 20 mM EDTA (Gick *et al.*, 1986) and is not significantly affected by 40 mM EDTA (Dominski *et al.*, 2005b). The explanation may lie in the fact that each processing complex is composed of a number of unique factors, some possibly still unknown. Perhaps the cleavage/polyadenylation machinery, but not 3′ end processing of histone pre-mRNAs, contains another essential zinc-dependent component that binds zinc ions relatively weakly and is responsible for the higher sensitivity to EDTA.

Cleavage of mRNA precursors seems to be the only reaction that utilizes a nuclease of the metallo-β-lactamase family and proceeds in the presence of EDTA. Cleavage of snRNA precursors, another processing reaction that utilizes a metallo-β-lactamase nuclease and occurs in a multi-component assembly, requires magnesium or manganese ions (Uguen and Murphy, 2003). Perhaps at least one component of Integrator requires these cofactors for the activity while the catalytic activity of RC-68 may solely depend on zinc ions.

11. CONCLUSIONS AND QUESTIONS FOR THE FUTURE

Proteins of the metallo-β-lactamase family with either demonstrated or predicted nuclease activities have emerged in recent years as important constituents of DNA and RNA metabolism. Members of the family that act as endonucleases due to employing metal dependent catalysis generate a 3′ hydroxyl and a 5′ phosphate on the two resulting products. The generation of a 3′ hydroxyl on the upstream cleavage product allows direct modification of the newly formed 3′ end without the necessity for additional processing steps. The most prevalent 3′ modification reactions are the CCA addition to tRNAs and the extension of mRNAs by the poly(A). Although histone mRNAs are typically not further processed, in *Xenopus* oocytes a short tract of adenosines is added to the 3′ end as a mechanism for translational repression of histone mRNAs during oogenesis (Sanchez and Marzluff, 2004). One of the steps in V(D)J recombination following DNA hairpin opening is addition of nucleotides to the 3′ end by terminal deoxynucleotidyl transferase. This step facilitates ligation of V, D, and J segments and at the same time, by creating imprecise ends, increases genetic variability of immunoglobulins and T-cell receptors. The presence of a 3′ hydroxyl is also likely important for the formation of snRNAs, since 3′ exonucleolytic trimming in the cytoplasm follows the initial endonucleolytic cleavage in the nucleus (Perumal and Reddy, 2002).

CPSF-73 and RC-68 are the only known nucleases of the metallo-β-lactamase family that under biologically relevant conditions act as site-specific nucleases in large complexes containing many additional subunits. As indicated throughout this review, the functional and structural organization of these macromolecular machineries is only poorly understood. Among the most important questions to answer in the future are how CPSF-73 and RC-68 are recruited to the cleavage site in their respective RNA substrates and what role is played by their binding partners, CPSF-100 and RC-74 that have lost critical catalytic residues. Do all β-CASP proteins function as both an endonuclease and a 5′ exonuclease, and what features determine which mode of activity is used during processing of their substrates?

The role of at least some metallo-β-lactamase nucleases may extend beyond their enzymatic activity in RNA or DNA metabolism and include other important functions. A notable example is the possible role of Artemis as a checkpoint protein that participates in proper G2/M transition in cells exposed to various DNA-damaging agents (Zhang *et al.*, 2004). Snm1A, in addition to its role in repair of DNA interstrand cross-links, has been shown to function as a mitotic checkpoint protein required for arresting cells in response to spindle poisons (Akhter *et al.*, 2004) and knockout mouse lacking this protein exhibit accelerated tumorogenesis (Akhter *et al.*, 2005). As pointed out above, RC-68 may also perform additional functions beyond its role in 3′ end processing of snRNA precursors. Strikingly, HeLa cells depleted of this protein by RNA interference arrest in G1 phase but continue growing until reaching the size typical of G2 cells (Dominski *et al.*, 2005b). This rare phenotype of uncoupling cell growth from cell cycle progression occurs before any changes in the level of snRNAs can be detected (author's unpublished results), indicating that downregulation of RC-68 must also affect some other cellular process. Finally, there is an unresolved link between mutations within the human gene ELAC2, which encodes the long form of tRNase Z and an increased rate of prostate cancer. Further studies will determine whether the role of nuclease of the metallo-β-lactamase family in cellular metabolism is more complex than initially anticipated.

Interestingly, genome sequencing projects reveal that proteins closely related to CPSF-73 and RC-68 exist in many representatives of bacteria and archaea. The presence of these members of the β-CASP group in prokaryotes was initially noticed 10 years ago as a result of identifying the amino acid sequence of CPSF-73 (Jenny *et al.*, 1996). The archaeal *Methanococcus* contains as many as three proteins homologous to CPSF-73, each sharing with the human protein remarkable 30% identical and 50% similar residues over the region of 450 amino acids. In one of these proteins, the catalytic domain exists together with the KH domain known to mediate RNA binding, suggesting that at least some prokaryotic homologues of CPSF-73 function in RNA metabolism. Indeed, preliminary studies on a bacterial homologue of CPSF-73 from *Thermus thermophilus*, TTHA0252, determined that this protein can degrade RNA substrate but shows no activity against DNA (Ishikawa *et al.*, 2006). Detailed characterization of the CPSF-73 homologues in prokaryotes may provide interesting clues to understanding the origin of the highly regulated and complex mechanism of pre-mRNA 3′ end processing in eukaryotes, and shed some

light on other processes that involve nucleases of the metallo-β-lactamase family.

ACKNOWLEDGMENTS

I am very grateful to L. Levinger (York College, CUNY) and S. Murphy (Oxford University) for critical comments on the manuscript and valuable scientific feedback. I also thank my colleagues from UNC at Chapel Hill for their contribution to my work, and J. Spychala (VisiScience, Inc.) for help with the figures. This work is supported by a grant GM29832 from the National Institute of Health.

REFERENCES

Abou, E.S. and Ares, M., Jr. 1998. Depletion of yeast RNase III blocks correct U2 3′ end formation and results in polyadenylated but functional U2 snRNA. *EMBO J* 17:3738–3746.

Ach, R.A., and Weiner, A.M. 1987. The highly conserved U small nuclear RNA 3′-end formation signal is quite tolerant to mutation. *Mol Cell Biol* 7:2070–2079.

Ahnesorg, P., Smith, P., and Jackson, S.P. 2006. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* 124:301–313.

Akhter, S., Richie, C.T., Deng, J.M., Brey, E., Zhang, X., Patrick, C., Jr., Behringer, R.R., and Legerski, R.J. 2004. Deficiency in SNM1 abolishes an early mitotic checkpoint induced by spindle stress. *Mol Cell Biol* 24:10448–10455.

Akhter, S., Richie, C.T., Zhang, N., Behringer, R.R., Zhu, C., and Legerski, R.J. 2005. Snm1-deficient mice exhibit accelerated tumorigenesis and susceptibility to infection. *Mol Cell Biol* 25:10071–10078.

Aravind, L. 1999. An evolutionary classification of the metallo-beta-lactamase fold proteins. *In Silico Biol* 1:69–91.

Azzouz, T.N., Gruber, A., and Schumperli, D. 2005. U7 snRNP-specific Lsm11 protein: dual binding contacts with the 100 kDa zinc finger processing factor (ZFP100) and a ZFP100-independent function in histone RNA 3′ end processing. *Nucleic Acids Res* 33:2106–2117.

Baillat, D., Hakimi, M.A., Naar, A.M., Shilatifard, A., Cooch, N., and Shiekhattar, R. 2005. Integrator, a multiprotein mediator of small nuclear RNA processing, associates with the C-terminal repeat of RNA polymerase II. *Cell* 123:265–276.

Bartel, D.P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281–297.

Bassing, C.H., Swat, W., and Alt, F.W. 2002. The mechanism and regulation of chromosomal V(D)J recombination. *Cell* 109:S45–S55.

Bertone, P., Stolc, V., Royce, T.E., Rozowsky, J.S., Urban, A.E., Zhu, X., Rinn, J.L., Tongprasit, W., Samanta, M., Weissman, S., Gerstein, M., and Snyder, M. 2004. Global identification of human transcribed sequences with genome tiling arrays. *Science* 306:2242–2246.

Bienroth, S., Wahle, E., Suter-Crazzolara, C., and Keller, W. 1991. Purification of the cleavage and polyadenylation factor involved in the 3′-processing of messenger RNA precursors. *J Biol Chem* 266:19768–19776.

Britton, R.A., Wen, T., Schaefer, L., Pellegrini, O., Uicker, W.C., Mathy, N., Tobin, C., Daou, R., Szyk, J., and Condon, C. 2007. Maturation of the 5′ end of Bacillus subtilis 16S rRNA by the essential ribonuclease YkqC/RNase J1. *Mol Microbiol* 63:127–138.

Brown, K.M. and Gilmartin, G.M. 2003. A mechanism for the regulation of pre-mRNA 3′ processing by human cleavage factor Im. *Mol Cell* 12:1467–1476.

Buck, D., Malivert, L., De Chasseval, R., Barraud, A., Fondaneche, M.C., Sanal, O., Plebani, A., Stephan, J.L., Hufnagel, M., Le Deist, F., Fischer, A., Durandy, A., De Villartay, J.P., and Revy, P. 2006. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* 124:287–299.

Callebaut, I., Moshous, D., Mornon, J.P., and De Villartay, J.P. 2002. Metallo-beta-lactamase fold within nucleic acids processing enzymes: the beta-CASP family. *Nucleic Acids Res* 30:3592–3601.

Carfi, A., Pares, S., Duee, E., Galleni, M., Duez, C., Frere, J.M., and Dideberg, O. 1995. The 3-D structure of a zinc metallo-beta-lactamase from Bacillus cereus reveals a new type of protein fold. *EMBO J* 14:4914–4921.

Chanfreau, G., Abou, E., Ares, M., Jr., and Guthrie, C. 1997. Alternative 3′-end processing of U5 snRNA by RNase III. *Genes Deve* 11:2741–2751.

Chanfreau, G., Legrain, P., and Jacquier, A. 1998. Yeast RNase III as a key processing enzyme in small nucleolar RNAs metabolism. *J Mol Biol* 284:975–988.

Christofori, G. and Keller, W. 1989. Poly(A) polymerase purified from HeLa cell nuclear extract is required for both cleavage and polyadenylation of pre- mRNA in vitro. *Mol Cell Biol* 9:193–203.

Colgan, D.F. and Manley, J.L. 1997. Mechanism and regulation of mRNA polyadenylation. *Genes Dev* 11:2755–2766.

Condon, C. and Putzer, H. 2002. The phylogenetic distribution of bacterial ribonucleases. *Nucleic Acids Res* 30:5339–5346.

Condon, C., Putzer, H., and Grunberg-Manago, M. 1996. Processing of the leader mRNA plays a major role in the induction of thrS expression following threonine starvation in Bacillus subtilis. *Proc Natl Acad Sci U S A* 93:6992–6997.

Condon, C., Putzer, H., Luo, D., and Grunberg-Manago, M. 1997. Processing of the Bacillus subtilis thrS leader mRNA is RNase E-dependent in Escherichia coli. *J Mol Biol* 268:235–242.

Cotten, M., Oberhauser, B., Brunar, H., Holzner, A., Issakides, G., Noe, C.R., Schaffner, G., Wagner, E., and Birnstiel, M.L. 1991. 2′-O-methyl, 2′-O-ethyl oligoribonucleotides and phosphorothioate oligodeoxyribonucleotides as inhibitors of the *in vitro* U7 snRNP-dependent mRNA processing event. *Nucleic Acids Res* 19:2629–2635.

Cuello, P., Boyd, D.C., Dye, M.J., Proudfoot, N.J., and Murphy, S. 1999. Transcription of the human U2 snRNA genes continues beyond the 3′ box *in vivo*. *EMBO J* 18:2867–2877.

Cullen, B.R. 2004. Transcription and processing of human microRNA precursors. *Mol Cell* 16:861–865.

Daiyasu, H., Osaka, K., Ishino, Y., and Toh, H. 2001. Expansion of the zinc metallo-hydrolase family of the beta-lactamase fold. *FEBS Lett* 503:1–6.

de la Sierra-Gallay, I.L., Mathy, N., Pellegrini, O., and Condon, C. 2006. Structure of the ubiquitous 3′ processing enzyme RNase Z bound to transfer RNA. *Nat Struct Mol Biol* 13:376–377.

de la Sierra-Gallay, I.L., Pellegrini, O., and Condon, C. 2005. Structural basis for substrate binding, cleavage and allostery in the tRNA maturase RNase Z. *Nature* 433:657–661.

De Villartay, J.P., Fischer, A., and Durandy, A. 2003. The mechanisms of immune diversification and their disorders. *Nat Rev Immunol* 3:962–972.

De Vries, H., Rüegsegger, U., Hübner, W., Friedlein, A., Langen, H., and Keller, W. 2000. Human pre-mRNA cleavage factor II_m contains homologs of yeast proteins and bridges two other cleavage factors. *EMBO J* 19:5895–5904.

Demuth, I., Digweed, M., and Concannon, P. 2004. Human SNM1B is required for normal cellular response to both DNA interstrand crosslink-inducing agents and ionizing radiation. *Oncogene* 23:8611–8618.

Deutscher, M.P. 1990. Ribonucleases, tRNA nucleotidyltransferase, and the 3′ processing of tRNA. *Prog Nucleic Acid Res Mol Biol* 39:209–240.

Deutscher, M.P. and Li, Z. 2001. Exoribonucleases and their multiple roles in RNA metabolism. *Prog Nucleic Acid Res Mol Biol* 66:67–105.

Dominski, Z., Erkmann, J.A., Yang, X., Sanchez, R., and Marzluff, W.F. 2002. A novel zinc finger protein is associated with U7 snRNP and

interacts with the stem-loop binding protein in the histone pre-mRNP to stimulate 3′-end processing. *Genes Dev* 16:58–71.
Dominski, Z. and Marzluff, W.F. 1999. Formation of the 3′ end of histone mRNA. *Gene* 239:1–14.
Dominski, Z., Yang, X.C., and Marzluff, W.F. 2005a. The Polyadenylation Factor CPSF-73 Is Involved in Histone-Pre-mRNA Processing. *Cell* 123:37–48.
Dominski, Z., Yang, X.C., Purdy, M., Wagner, E.J., and Marzluff, W.F. 2005b. A CPSF-73 homologue is required for cell cycle progression but not cell growth and interacts with a protein having features of CPSF-100. *Mol Cell Biol* 25:1489–1500.
Dominski, Z., Zheng, L.X., Sanchez, R., and Marzluff, W.F. 1999. Stem-loop binding protein facilitates 3′-end formation by stabilizing U7 snRNP binding to histone pre-mRNA. *Mol Cell Biol* 19:3561–3570.
Dronkert, M.L., de Wit, J., Boeve, M., Vasconcelos, M.L., Van Steeg, H., Tan, T.L., Hoeijmakers, J.H., and Kanaar, R. 2000. Disruption of mouse SNM1 causes increased sensitivity to the DNA interstrand cross-linking agent mitomycin C. *Mol Cell Biol* 20:4553–4561.
Dronkert, M.L. and Kanaar, R. 2001. Repair of DNA interstrand cross-links. *Mutat Res* 486:217–247.
Dubrovsky, E.B., Dubrovskaya, V.A., Levinger, L., Schiffer, S., and Marchfelder, A. 2004. Drosophila RNase Z processes mitochondrial and nuclear pre-tRNA 3′ ends in vivo. *Nucleic Acids Res* 32:255–262.
Dumont, M., Frank, D., Moisan, A.M., Tranchant, M., Soucy, P., Breton, R., Labrie, F., Tavtigian, S.V., and Simard, J. 2004. Structure of primate and rodent orthologs of the prostate cancer susceptibility gene ELAC2. *Biochim Biophys Acta* 1679:230–247.
Even, S., Pellegrini, O., Zig, L., Labas, V., Vinh, J., Brechemmier-Baey, D., and Putzer, H. 2005. Ribonucleases J1 and J2: two novel endoribonucleases in B.subtilis with functional homology to E.coli RNase E. *Nucleic Acids Res* 33:2141–2152.
Ezraty, B., Dahlgren, B., and Deutscher, M.P. 2005. The RNase Z homologue encoded by Escherichia coli elaC gene is RNase BN. *J Biol Chem* 280:16542–16545.
Feng, J., Funk, W.D., Wang, S.S., Weinrich, S.L., Avilion, A.A., Chiu, C.P., Adams, R.R., Chang, E., Allsopp, R.C., Yu, J., and . 1995. The RNA component of human telomerase. *Science* 269:1236–1241.
Freibaum, B.D. and Counter, C.M. 2006. hSnm1B is a novel telomere-associated protein. *J Biol Chem* 281:15033–15036.
Frith, M.C., Pheasant, M., and Mattick, J.S. 2005. The amazing complexity of the human transcriptome. *Eur J Hum Genet* 13:894–897.
Fu, D. and Collins, K. 2003. Distinct biogenesis pathways for human telomerase RNA and H/ACA small nucleolar RNAs. *Mol Cell* 11:1361–1372.
Galli, G., Hofstetter, H., Stunnenberg, H.G., and Birnstiel, M.L. 1983. Biochemical complementation with RNA in the Xenopus oocyte: a small RNA is required for the generation of 3′ histone mRNA termini. *Cell* 34:823–828.
Garau, G., Lemaire, D., Vernet, T., Dideberg, O., and Di Guilmi, A.M. 2005. Crystal structure of phosphorylcholine esterase domain of the virulence factor choline-binding protein e from streptococcus pneumoniae: new structural features among the metallo-beta-lactamase superfamily. *J Biol Chem* 280:28591–28600.
Gick, O., Kramer, A., Keller, W., and Birnstiel, M.L. 1986. Generation of histone mRNA 3′ ends by endonucleolytic cleavage of the pre-mRNA in a snRNP-dependent in vitro reaction. *EMBO J* 5:1319–1326.
Gick, O., Kramer, A., Vasserot, A., and Birnstiel, M.L. 1987. Heat-labile regulatory factor is required for 3′ processing of histone precursor mRNAs. *Proc Natl Acad Sci USA* 84:8937–8940.
Gilmartin, G.M. 2005. Eukaryotic mRNA 3′ processing: a common means to different ends. *Genes Dev* 19:2517–2521.
Green, R., and Noller, H.F. 1997. Ribosomes and translation. *Annu Rev Biochem* 66:679–716.
Grimm, C., Stefanovic, B., and Schümperli, D. 1993. The low abundance of U7 snRNA is partly determined by its Sm binding site. *EMBO J* 12:1229–1238.
Grundy, F.J. and Henkin, T.M. 2003. The T box and S box transcription termination control systems. *Front Biosci* 8:d20–d31.
Heinz, U., and Adolph, H.W. 2004. Metallo-beta-lactamases: two binding sites for one catalytic metal ion? *Cell Mol Life Sci* 61:2827–2839.
Henkin, T.M., and Yanofsky, C. 2002. Regulation by transcription attenuation in bacteria: how RNA provides instructions for transcription termination/antitermination decisions. *Bioessays* 24:700–707.
Henry, R.W., Ford, E., Mital, R., Mittal, V., and Hernandez, N. 1998. Crossing the line between RNA polymerases: transcription of human snRNA genes by RNA polymerases II and III. *Cold Spring Harbor Symp Quant Biol* 63:111–120.
Hernandez, N. 1985. Formation of the 3′ end of U1 snRNA is directed by a conserved sequence located downstream of the coding region. *EMBO J* 4:1827–1837.
Hernandez, N. 2001. Small nuclear RNA genes: a model system to study fundamental mechanisms of transcription. *J Biol Chem* 276:26733–26736.
Hernandez, N., and Weiner, A.M. 1986. Formation of the 3′ end of U1 snRNA requires compatible snRNA promoter elements. *Cell* 47:249–258.
Hirose, Y., and Manley, J.L. 1997. Creatine phosphate, not ATP, is required for 3′ end cleavage of mammalian pre-mRNA *in vitro*. *J Biol Chem* 272:29636–29642.
Hirose, Y., and Manley, J.L. 1998. RNA polymerase II is an essential mRNA polyadenylation factor. *Nature* 395:93–96.
Ishiai, M., Kimura, M., Namikoshi, K., Yamazoe, M., Yamamoto, K., Arakawa, H., Agematsu, K., Matsushita, N., Takeda, S., Buerstedde, J.M., and Takata, M. 2004. DNA cross-link repair protein SNM1A interacts with PIAS1 in nuclear focus formation. *Mol Cell Biol* 24:10733–10741.
Ishii, R., Minagawa, A., Takaku, H., Takagi, M., Nashimoto, M., and Yokoyama, S. 2005. Crystal structure of the tRNA 3′ processing endoribonuclease tRNase Z from Thermotoga maritima. *J Biol Chem* 280:14138–14144.
Ishikawa, H., Nakagawa, N., Kuramitsu, S., and Masui, R. 2006. Crystal structure of TTHA0252 from Thermus thermophilus HB8, a RNA degradation protein of the metallo-beta-lactamase superfamily. *J Biochem (Tokyo)* 140:535–542.
Jacobs, E.Y., Ogiwara, I., and Weiner, A.M. 2004. Role of the C-terminal domain of RNA polymerase II in U2 snRNA transcription and 3′ processing. *Mol Cell Biol* 24:846–855.
Jaeger, S., Barends, S., Giege, R., Eriani, G., and Martin, F. 2005. Expression of metazoan replication-dependent histone genes. *Biochimie* 87:827–834.
Jenny, A., Hauri, H.-P., and Keller, W. 1994. Characterization of cleavage and polyadenylation specificity factor and cloning of its 100-kilodalton subunit. *Mol Cell Biol* 14:8183–8190.
Jenny, A., Minvielle-Sebastia, L., Preker, P.J., and Keller, W. 1996. Sequence similarity between the 73-kilodalton protein of mammalian CPSF and a subunit of yeast polyadenylation factor I. *Science* 274:1514–1517.
Katinka, M.D., Duprat, S., Cornillot, E., Metenier, G., Thomarat, F., Prensier, G., Barbe, V., Peyretaillade, E., Brottier, P., Wincker, P., Delbac, F., El Alaoui, H., Peyret, P., Saurin, W., Gouy, M., Weissenbach, J., and Vivares, C.P. 2001. Genome sequence and gene compaction of the eukaryote parasite Encephalitozoon cuniculi. *Nature* 414:450–453.
Kaufmann, I., Martin, G., Friedlein, A., Langen, H., and Keller, W. 2004. Human Fip1 is a subunit of CPSF that binds to U-rich RNA elements and stimulates poly(A) polymerase. *EMBO J* 23:616–626.
Keeling, P.J. 2001. Parasites go the full monty. *Nature* 414:401–402.
Keeling, P.J. and Fast, N.M. 2002. Microsporidia: biology and evolution of highly reduced intracellular parasites. *Annu Rev Microbiol* 56:93–116.
Keon, B.H., Schafer, S., Kuhn, C., Grund, C., and Franke, W.W. 1996. Symplekin, a novel type of tight junction plaque protein. *J Cell Biol* 134:1003–1018.
Kolev, N.G. and Steitz, J.A. 2005. Symplekin and multiple other polyadenylation factors participate in 3′–end maturation of histone mRNAs. *Genes & Development* 19:2583–2592.

Korver, W., Guevara, C., Chen, Y., Neuteboom, S., Bookstein, R., Tavtigian, S., and Lees, E. 2003. The product of the candidate prostate cancer susceptibility gene ELAC2 interacts with the gamma-tubulin complex. *Int J Cancer* 104:283–288.

Kostelecky, B., Pohl, E., Vogel, A., Schilling, O., and Meyer-Klaucke, W. 2006. The crystal structure of the zinc phosphodiesterase from Escherichia coli provides insight into function and cooperativity of tRNase Z-family proteins. *J Bacteriol* 188:1607–1614.

Kuhn, U. and Wahle, E. 2004. Structure and function of poly(A) binding proteins. *Biochim Biophys Acta* 1678:67–84.

Le Deist, F., Poinsignon, C., Moshous, D., Fischer, A., and De Villartay, J.P. 2004. Artemis sheds new light on V(D)J recombination. *Immunol Rev* 200:142–155.

Lenain, C., Bauwens, S., Amiard, S., Brunori, M., Giraud-Panis, M.J., and Gilson, E. 2006. The Apollo 5′ exonuclease functions together with TRF2 to protect telomeres from DNA repair. *Curr Biol* 16:1303–1310.

Levinger, L., Bourne, R., Kolla, S., Cylin, E., Russell, K., Wang, X., and Mohan, A. 1998. Matrices of paired substitutions show the effects of tRNA D/T loop sequence on Drosophila RNase P and 3′-tRNase processing. *J Biol Chem* 273:1015–1025.

Li, X., Hejna, J., and Moses, R.E. 2005. The yeast Snm1 protein is a DNA 5′-exonuclease. *DNA Repair (Amst)* 4:163–170.

Li, X. and Moses, R.E. 2003. The beta-lactamase motif in Snm1 is required for repair of DNA double-strand breaks caused by interstrand crosslinks in S. cerevisiae. *DNA Repair (Amst)* 2:121–129.

Li, Z. and Deutscher, M.P. 2002. RNase E plays an essential role in the maturation of Escherichia coli tRNA precursors. *RNA* 8:97–109.

Lieber, M.R., Ma, Y., Pannicke, U., and Schwarz, K. 2003. Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev Mol Cell Biol* 4:712–720.

Lieber, M.R., Ma, Y., Pannicke, U., and Schwarz, K. 2004. The mechanism of vertebrate nonhomologous DNA end joining and its role in V(D)J recombination. *DNA Repair (Amst)* 3:817–826.

Luo, W., Johnson, A.W., and Bentley, D.L. 2006. The role of Rat1 in coupling mRNA 3′-end processing to transcription termination: implications for a unified allosteric-torpedo model. *Genes Dev* 20:954–965.

Ma, Y., Pannicke, U., Lu, H., Niewolik, D., Schwarz, K., and Lieber, M.R. 2005a. The DNA-dependent Protein Kinase Catalytic Subunit Phosphorylation Sites in Human Artemis. *J Biol Chem* 280:33839–33846.

Ma, Y., Pannicke, U., Schwarz, K., and Lieber, M.R. 2002. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 108:781–794.

Ma, Y., Schwarz, K., and Lieber, M.R. 2005b. The Artemis:DNA-PKcs endonuclease cleaves DNA loops, flaps, and gaps. *DNA Repair (Amst)* 4:845–851.

MacDonald, C.C., Wilusz, J., and Shenk, T. 1994. The 64-kilodalton subunit of the CstF polyadenylation factor binds to pre-mRNAs downstream of the cleavage site and influences cleavage site location. *Mol Cell Biol* 14:6647–6654.

Mandel, C.R., Kaneko, S., Zhang, H., Gebauer, D., Vethantham, V., Manley, J.L., and Tong, L. 2006. Polyadenylation factor CPSF-73 is the pre-mRNA 3′-end-processing endonuclease. *Nature* 444:953–956.

Mangus, D.A., Evans, M.C., and Jacobson, A. 2003. Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol* 4:223.

Marck, C. and Grosjean, H. 2002. tRNomics: analysis of tRNA genes from 50 genomes of Eukarya, Archaea, and Bacteria reveals anticodon-sparing strategies and domain-specific features. *RNA* 8:1189–1232.

Marszalkowski, M., Teune, J.H., Steger, G., Hartmann, R.K., and Willkomm, D.K. 2006. Thermostable RNase P RNAs lacking P18 identified in the Aquificales. *RNA* 12:1915–1921.

Martin, F., Schaller, A., Eglite, S., Schümperli, D., and Müller, B. 1997. The gene for histone RNA hairpin binding protein is located on human chromosome 4 and encodes a novel type of RNA binding protein. *EMBO J* 16:769–778.

Marzluff, W.F. 2005. Metazoan replication-dependent histone mRNAs: a distinct set of RNA polymerase II transcripts. *Curr Opin Cell Biol* 17:274–280.

Marzluff, W.F., Gongidi, P., Woods, K.R., Jin, J.P., and Maltais, L. 2002. The human and mouse replication-dependent histone genes. *Genomics* 80:487–498.

Mattick, J.S. 2003. Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. *Bioessays* 25:930–939.

McCracken, S., Fong, N., Yankulov, K., Ballantyne, S., Pan, G.H., Greenblatt, J., Patterson, S.D., Wickens, M., and Bentley, D.L. 1997. The C-terminal domain of RNA polymerase II couples mRNA processing to transcription. *Nature* 385:357–361.

Medlin, J., Scurry, A., Taylor, A., Zhang, F., Peterlin, B.M., and Murphy, S. 2005. P-TEFb is not an essential elongation factor for the intronless human U2 snRNA and histone H2b genes. *EMBO J* 24:4154–4165.

Medlin, J.E., Uguen, P., Taylor, A., Bentley, D.L., and Murphy, S. 2003. The C-terminal domain of pol II and a DRB-sensitive kinase are required for 3′ processing of U2 snRNA. *EMBO J* 22:925–934.

Minagawa, A., Takaku, H., Ishii, R., Takagi, M., Yokoyama, S., and Nashimoto, M. 2006. Identification by Mn2+ rescue of two residues essential for the proton transfer of tRNase Z catalysis. *Nucleic Acids Res* 34:3811–3818.

Minagawa, A., Takaku, H., Takagi, M., and Nashimoto, M. 2004. A novel endonucleolytic mechanism to generate the CCA 3′ termini of tRNA molecules in Thermotoga maritima. *J Biol Chem* 279:15688–15697.

Minvielle-Sebastia, L. and Keller, W. 1999. mRNA polyadenylation and its coupling to other RNA processing reactions and to transcription. *Curr Opin Cell Biol* 11:352–357.

Mohan, A., Whyte, S., Wang, X., Nashimoto, M., and Levinger, L. 1999. The 3′ end CCA of mature tRNA is an antideterminant for eukaryotic 3′-tRNase. *RNA* 5:245–256.

Moore, C.L. and Sharp, P.A. 1985. Accurate cleavage and polyadenylation of exogenous RNA substrate. *Cell* 41:845–855.

Moore, C.L., Skolnik-David, H., and Sharp, P.A. 1986. Analysis of RNA cleavage at the adenovirus-2 L3 polyadenylation site. *EMBO J* 5:1929–1938.

Morlando, M., Greco, P., Dichtl, B., Fatica, A., Keller, W., and Bozzoni, I. 2002. Functional analysis of yeast snoRNA and snRNA 3′-end formation mediated by uncoupling of cleavage and polyadenylation. *Mol Cell Biol* 22:1379–1389.

Moshous, D., Callebaut, I., De Chasseval, R., Corneo, B., Cavazzana-Calvo, M., Le Deist, F., Tezcan, I., Sanal, O., Bertrand, Y., Philippe, N., Fischer, A., and De Villartay, J.P. 2001. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 105:177–186.

Mowry, K.L. and Steitz, J.A. 1987. Identification of the human U7 snRNP as one of several factors involved in the 3′ end maturation of histone premessenger RNA's. *Science* 238:1682–1687.

Murthy, K.G. and Manley, J.L. 1992. Characterization of the multisubunit cleavage-polyadenylation specificity factor from calf thymus. *J Biol Chem* 267:14804–14811.

Murthy, K.G.K. and Manley, J.L. 1995. The 160-kD subunit of human cleavage polyadenylation specificity factor coordinates pre-mRNA 3′-end formation. *Genes and Development* 9:2672–2683.

Nashimoto, M. 1997. Distribution of both lengths and 5′ terminal nucleotides of mammalian pre-tRNA 3′ trailers reflects properties of 3′ processing endoribonuclease. *Nucleic Acids Res* 25:1148–1154.

Nashimoto, M., Tamura, M., and Kaspar, R.L. 1999. Minimum requirements for substrates of mammalian tRNA 3′ processing endoribonuclease. *Biochemistry* 38:12089–12096.

Neuman de Vegvar, H.E., Lund, E., and Dahlberg, J.E. 1986. 3′ end formation of U1 snRNA precursors is coupled to transcription from snRNA promoters. *Cell* 47:259–266.

Niewolik, D., Pannicke, U., Lu, H., Ma, Y., Wang, L.C., Kulesza, P., Zandi, E., Lieber, M.R., and Schwarz, K. 2006. DNA-PKcs dependence of artemis endonucleolytic activity: Differences between hairpins and 5′ or 3′ overhangs. *J Biol Chem* 281:33900–33909.

Ohler, U., Yekta, S., Lim, L.P., Bartel, D.P., and Burge, C.B. 2004. Patterns of flanking sequence conservation and a characteristic upstream motif for microRNA gene identification. *RNA* 10:1309–1322.

Pannicke, U., Ma, Y., Hopfner, K.P., Niewolik, D., Lieber, M.R., and Schwarz, K. 2004. Functional and biochemical dissection of the structure-specific nuclease ARTEMIS. *EMBO J* 23:1987–1997.

Pellegrini, O., Nezzar, J., Marchfelder, A., Putzer, H., and Condon, C. 2003. Endonucleolytic processing of CCA-less tRNA precursors by RNase Z in Bacillus subtilis. *EMBO J* 22:4534–4543.

Perumal, K. and Reddy, R. 2002. The 3′ end formation in small RNAs. *Gene Expr* 10:59–78.

Perwez, T. and Kushner, S.R. 2006. RNase Z in Escherichia coli plays a significant role in mRNA decay. *Mol Microbiol* 60:723–737.

Pillai, R.S., Grimmler, M., Meister, G., Will, C.L., Luhrmann, R., Fischer, U., and Schumperli, D. 2003. Unique Sm core structure of U7 snRNPs: assembly by a specialized SMN complex and the role of a new component, Lsm11, in histone RNA processing. *Genes Dev* 17:2321–2333.

Pillai, R.S., Will, C.L., Lührmann, R., Schümperli, D., and Müller, B. 2001. Purified U7 snRNPs lack the Sm proteins D1 and D2 but contain Lsm10, a new 14 kDa Sm D1-like protein. *EMBO J* 20:5470–5479.

Poinsignon, C., Moshous, D., Callebaut, I., De Chasseval, R., Villey, I., and De Villartay, J.P. 2004. The Metallo-{beta}-Lactamase/{beta}-CASP Domain of Artemis Constitutes the Catalytic Core for V(D)J Recombination. *J Exp Med* 199:315–321.

Putzer, H., Condon, C., Brechemier-Baey, D., Brito, R., and Grunberg-Manago, M. 2002. Transfer RNA-mediated antitermination in vitro. *Nucleic Acids Res* 30:3026–3033.

Richie, C.T., Peterson, C., Lu, T., Hittelman, W.N., Carpenter, P.B., and Legerski, R.J. 2002. hSnm1 colocalizes and physically associates with 53BP1 before and after DNA damage. *Mol Cell Biol* 22:8635–8647.

Rooney, S., Sekiguchi, J., Zhu, C., Cheng, H.L., Manis, J., Whitlow, S., DeVido, J., Foy, D., Chaudhuri, J., Lombard, D., and Alt, F.W. 2002. Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. *Mol Cell* 10:1379–1390.

Rosonina, E., Kaneko, S., and Manley, J.L. 2006. Terminating the transcript: breaking up is hard to do. *Genes Dev* 20:1050–1056.

Ruegsegger, U., Beyer, K., and Keller, W. 1996. Purification and characterization of human cleavage factor Im involved in the 3′ end processing of messenger RNA precursors. *J Biol Chem* 271:6107–6113.

Ruegsegger, U., Blank, D., and Keller, W. 1998. Human pre-mRNA cleavage factor Im is related to spliceosomal SR proteins and can be reconstituted in vitro from recombinant subunits. *Molecular Cell* 1:243–253.

Ryan, K., Calvo, O., and Manley, J.L. 2004. Evidence that polyadenylation factor CPSF-73 is the mRNA 3′ processing endonuclease. *RNA* 10:565–573.

Sanchez, R. and Marzluff, W.F. 2004. The oligo(A) tail on histone mRNA plays an active role in translational silencing of histone mRNA during Xenopus oogenesis. *Mol Cell Biol* 24:2513–2525.

Scharl, E.C. and Steitz, J.A. 1994. The site of 3′ end formation of histone messenger RNA is a fixed distance from the downstream element recognized by the U7 snRNP. *EMBO J* 13:2432–2440.

Schierling, K., Rosch, S., Rupprecht, R., Schiffer, S., and Marchfelder, A. 2002. tRNA 3′ end maturation in archaea has eukaryotic features: the RNase Z from Haloferax volcanii. *J Mol Biol* 316:895–902.

Schiffer, S., Helm, M., Theobald-Dietrich, A., Giege, R., and Marchfelder, A. 2001. The plant tRNA 3′ processing enzyme has a broad substrate spectrum. *Biochemistry* 40:8264–8272.

Schiffer, S., Rosch, S., and Marchfelder, A. 2002. Assigning a function to a conserved group of proteins: the tRNA 3′-processing enzymes. *EMBO J* 21:2769–2777.

Schilling, O., Ruggeberg, S., Vogel, A., Rittner, N., Weichert, S., Schmidt, S., Doig, S., Franz, T., Benes, V., Andrews, S.C., Baum, M., and Meyer-Klaucke, W. 2004. Characterization of an Escherichia coli elaC deletion mutant. *Biochem Biophys Res Commun* 320:1365–1373.

Schilling, O., Spath, B., Kostelecky, B., Marchfelder, A., Meyer-Klaucke, W., and Vogel, A. 2005. Exosite modules guide substrate recognition in the ZiPD/ElaC protein family. *J Biol Chem* 280:17857–17862.

Schilling, O., Wenzel, N., Naylor, M., Vogel, A., Crowder, M., Makaroff, C., and Meyer-Klaucke, W. 2003. Flexible metal binding of the metallo-beta-lactamase domain: glyoxalase II incorporates iron, manganese, and zinc in vivo. *Biochemistry* 42:11777–11786.

Schumperli, D., and Pillai, R.S. 2004. The special Sm core structure of the U7 snRNP: far-reaching significance of a small nuclear ribonucleoprotein. *Cell Mol Life Sci* 61:2560–2570.

Schwarz, K., Ma, Y., Pannicke, U., and Lieber, M.R. 2003. Human severe combined immune deficiency and DNA repair. *Bioessays* 25:1061–1070.

Sekiguchi, J.M., and Ferguson, D.O. 2006. DNA double-strand break repair: a relentless hunt uncovers new prey. *Cell* 124:260–262.

Sheets, M.D., Stephenson, P., and Wickens, M.P. 1987. Products of in vitro cleavage and polyadenylation of simian virus 40 late pre-mRNAs. *Mol Cell Biol* 7:1518–1529.

Shibata, H.S., Minagawa, A., Takaku, H., Takagi, M., and Nashimoto, M. 2006. Unstructured RNA is a substrate for tRNase Z. *Biochemistry* 45:5486–5492.

Silva, G., LeGall, J., Xavier, A.V., Teixeira, M., and Rodrigues-Pousada, C. 2001. Molecular characterization of Desulfovibrio gigas neelaredoxin, a protein involved in oxygen detoxification in anaerobes. *J Bacteriol* 183:4413–4420.

Smith, M.M. and Levitan, D.J. 2004. The Caenorhabditis elegans homolog of the putative prostate cancer susceptibility gene ELAC2, hoe-1, plays a role in germline proliferation. *Dev Biol* 266:151–160.

Smogorzewska, A. and De Lange, T. 2004. Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 73:177–208.

Soller, M. 2006. Pre-messenger RNA processing and its regulation: a genomic perspective. *Cell Mol Life Sci* 63:796–819.

Soubeyrand, S., Pope, L., De Chasseval, R., Gosselin, D., Dong, F., De Villartay, J.P., and Hache, R.J. 2006. Artemis phosphorylated by DNA-dependent protein kinase associates preferentially with discrete regions of chromatin. *J Mol Biol* 358:1200–1211.

Spath, B., Kirchner, S., Vogel, A., Schubert, S., Meinlschmidt, P., Aymanns, S., Nezzar, J., and Marchfelder, A. 2005. Analysis of the functional modules of the tRNA 3′ endonuclease (tRNase Z). *J Biol Chem* 280:35440–35447.

Steinmetz, E.J., Conrad, N.K., Brow, D.A., and Corden, J.L. 2001. RNA-binding protein Nrd1 directs poly(A)-independent 3′-end formation of RNA polymerase II transcripts. *Nature* 413:327–331.

Streit, A., Koning, T.W., Soldati, D., Melin, L., and Schümperli, D. 1993. Variable effects of the conserved RNA hairpin element upon 3′ end processing of histone pre-mRNA *in vitro*. *Nucleic Acids Res* 21:1569–1575.

Szilard, R.K. and Durocher, D. 2006. Telomere protection: an act of God. *Curr Biol* 16:R544–R546.

Takagaki, Y. and Manley, J.L. 2000. Complex protein interactions within the human polyadenylation machinery identify a novel component. *Mol Cell Biol* 20:1515–1525.

Takagaki, Y., Ryner, L.C., and Manley, J.L. 1988. Separation and characterization of a poly(A) polymerase and a cleavage/specificity factor required for pre-mRNA polyadenylation. *Cell* 52:731–742.

Takaku, H., Minagawa, A., Takagi, M., and Nashimoto, M. 2003. A candidate prostate cancer susceptibility gene encodes tRNA 3′ processing endoribonuclease. *Nucleic Acids Res* 31:2272–2278.

Takaku, H., Minagawa, A., Takagi, M., and Nashimoto, M. 2004. The N-terminal half-domain of the long form of tRNase Z is required for the RNase 65 activity. *Nucleic Acids Res* 32:4429–4438.

Tavtigian, S.V., Simard, J., Teng, D.H., Abtin, V., Baumgard, M., Beck, A., Camp, N.J., Carillo, A.R., Chen, Y., Dayananth, P., Desrochers, M., Dumont, M., Farnham, J.M., Frank, D., Frye, C., Ghaffari, S., Gupte, J.S., Hu, R., Iliev, D., Janecki, T., Kort, E.N., Laity, K.E., Leavitt, A., Leblanc, G., McArthur-Morrison, J., Pederson, A., Penn, B., Peterson, K.T., Reid, J.E., Richards, S., Schroeder, M., Smith, R., Snyder,

S.C., Swedlund, B., Swensen, J., Thomas, A., Tranchant, M., Woodland, A.M., Labrie, F., Skolnick, M.H., Neuhausen, S., Rommens, J., and Cannon-Albright, L.A. 2001. A candidate prostate cancer susceptibility gene at chromosome 17p. *Nat Genet* 27:172–180.

Terns, M.P. and Jacob, S.T. 1989. Role of poly(A) polymerase in the cleavage and polyadenylation of mRNA precursor. *Mol Cell Biol* 9:1435–1444.

Uguen, P., and Murphy, S. 2003. The 3′ ends of human pre-snRNAs are produced by RNA polymerase II CTD-dependent RNA processing. *EMBO J* 22:4544–4554.

Uguen, P., and Murphy, S. 2004. 3′-box-dependent processing of human pre-U1 snRNA requires a combination of RNA and protein co-factors. *Nucleic Acids Res* 32:2987–2994.

van Overbeek, M., and De Lange, T. 2006. Apollo, an Artemis-related nuclease, interacts with TRF2 and protects human telomeres in S phase. *Curr Biol* 16:1295–1302.

Vasiljeva, L., and Buratowski, S. 2006. Nrd1 interacts with the nuclear exosome for 3′ processing of RNA polymerase II transcripts. *Mol Cell* 21:239–248.

Venkataraman, K., Brown, K.M., and Gilmartin, G.M. 2005. Analysis of a noncanonical poly(A) site reveals a tripartite mechanism for vertebrate poly(A) site recognition. *Genes Dev* 19:1315–1327.

Vivares, C.P., Gouy, M., Thomarat, F., and Metenier, G. 2002. Functional and evolutionary analysis of a eukaryotic parasitic genome. *Curr Opin Microbiol* 5:499–505.

Vivares, C.P., and Metenier, G. 2001. The microsporidian Encephalitozoon. *Bioessays* 23:194–202.

Vogel, A., Schilling, O., and Meyer-Klaucke, W. 2004. Identification of metal binding residues for the binuclear zinc phosphodiesterase reveals identical coordination as glyoxalase II. *Biochemistry* 43:10379–10386.

Vogel, A., Schilling, O., Niecke, M., Bettmer, J., and Meyer-Klaucke, W. 2002. ElaC encodes a novel binuclear zinc phosphodiesterase. *J Biol Chem* 277:29078–29085.

Vogel, A., Schilling, O., Spath, B., and Marchfelder, A. 2005. The tRNase Z family of proteins: physiological functions, substrate specificity and structural properties. *Biol Chem* 386:1253–1264.

Wahle, E. 1995. Poly(A) tail length control is caused by termination of processive synthesis. *J Biol Chem* 270:2800–2808.

Wahle, E. and Ruegsegger, U. 1999. 3′-End processing of pre-mRNA in eukaryotes. *FEMS Microbiol Rev* 23:277–295.

Walker, S.C. and Engelke, D.R. 2006. Ribonuclease P: the evolution of an ancient RNA enzyme. *Crit Rev Biochem Mol Biol* 41:77–102.

Walther, T.N., Wittop, K.T., Schümperli, D., and Muller, B. 1998. A 5′–3′ exonuclease activity involved in forming the 3′ products of histone pre-mRNA processing in vitro. *RNA* 4:1034–1046.

Wang, Z., Fast, W., Valentine, A.M., and Benkovic, S.J. 1999. Metallo-beta-lactamase: structure and mechanism. *Curr Opin Chem Biol* 3:614–622.

Wang, Z.-F., Whitfield, M.L., Ingledue, T.I., Dominski, Z., and Marzluff, W.F. 1996. The protein which binds the 3′ end of histone mRNA: a novel RNA- binding protein required for histone pre-mRNA processing. *Genes Dev* 10:3028–3040.

Watkins, N.J., Lemm, I., Ingelfinger, D., Schneider, C., Hossbach, M., Urlaub, H., and Luhrmann, R. 2004. Assembly and maturation of the U3 snoRNP in the nucleoplasm in a large dynamic multiprotein complex. *Mol Cell* 16:789–798.

Weiner, A.M. 2004. tRNA maturation: RNA polymerization without a nucleic acid template. *Curr Biol* 14:R883–R885.

Weiner, A.M. 2005. E Pluribus Unum: 3′ end formation of polyadenylated mRNAs, histone mRNAs, and U snRNAs. *Mol Cell* 20:168–170.

Wen, T., Oussenko, I.A., Pellegrini, O., Bechhofer, D.H., and Condon, C. 2005. Ribonuclease PH plays a major role in the exonucleolytic maturation of CCA-containing tRNA precursors in Bacillus subtilis. *Nucleic Acids Res* 33:3636–3643.

Wickens, M., Anderson, P., and Jackson, R.J. 1997. Life and death in the cytoplasm: messages from the 3′ end. *Curr Opin Genet Dev* 7:220–232.

Wickens, M. and Gonzalez, T.N. 2004. Molecular biology. Knives, accomplices, and RNA. *Science* 306:1299–1300.

Will, C.L. and Luhrmann, R. 2001. Spliceosomal UsnRNP biogenesis, structure and function. *Curr Opin Cell Biol* 13:290–301.

Will, C.L. and Luhrmann, R. 2005. Splicing of a rare class of introns by the U12-dependent spliceosome. *Biol Chem* 386:713–724.

Willkomm, D.K., Feltens, R., and Hartmann, R.K. 2002. tRNA maturation in Aquifex aeolicus. *Biochimie* 84:713–722.

Wu, Q., and Krainer, A.R. 1999. AT-AC Pre-mRNA splicing mechanisms and conservation of minor introns in voltage-gated ion channel genes. *Mol Cell Biol* 19:3225–3236.

Xu, R., Ye, X., and Li, Q.Q. 2004. *AtCPSF73-II* gene encoding an *Arabidopsis* homolog of CPSF 73 kDa subunit is critical for early embryo development. *Gene* 324:35–45.

Yan, H., Zareen, N., and Levinger, L. 2006. Naturally occurring mutations in human mitochondrial pre-tRNASer(UCN) can affect the transfer ribonuclease Z cleavage site, processing kinetics, and substrate secondary structure. *J Biol Chem* 281:3926–3935.

Zareen, N., Hopkinson, A., and Levinger, L. 2006. Residues in two homology blocks on the amino side of the tRNase Z His domain contribute unexpectedly to pre-tRNA 3′ end processing. *RNA* 12:1104–1115.

Zareen, N., Yan, H., Hopkinson, A., and Levinger, L. 2005. Residues in the conserved His domain of fruit fly tRNase Z that function in catalysis are not involved in substrate recognition or binding. *J Mol Biol* 350:189–199.

Zhang, X., Succi, J., Feng, Z., Prithivirajsingh, S., Story, M.D., and Legerski, R.J. 2004. Artemis is a phosphorylation target of ATM and ATR and is involved in the G2/M DNA damage checkpoint response. *Mol Cell Biol* 24:9207–9220.

Zhao, J., Hyman, L., and Moore, C. 1999a. Formation of mRNA 3′ ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis. *Microbiol Mol Biol Rev* 63:405–445.

Zhao, J., Kessler, M., Helmling, S., O'Connor, J.P., and Moore, C. 1999b. Pta1, a component of yeast CFII, is required for both cleavage and poly(A) addition of mRNA precursor. *Mol Cell Biol* 19:7733–7740.

Zuo, Y. and Deutscher, M.P. 2001. Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. *Nucleic Acids Res* 29:1017–1026.

Editor: Judith Campbell