Chapter 7
5′-3′ Exoribonucleases

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Contents

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Abstract The 5′-3′ exoribonucleases have important functions in RNA processing, RNA degradation, RNA interference, transcription, and other cellular processes. The Xrn1 and Xrn2/Rat1 family of enzymes are the best characterized 5′-3′ exoribonucleases, and there has been significant recent progress in the understanding of their structure and function. Especially, the first structural information on Rat1 just became available. Other 5′-3′ exoribonucleases have been identified recently, including yeast Rrp17 and *B. subtilis* RNase J1, the first enzyme with 5′-3′ exoribonuclease activity found in prokaryotes. This review will summarize our current understanding of these enzymes, focusing on their sequence conservation, molecular structure, biochemical and cellular functions.

7.1 Introduction

Exoribonucleases are involved in RNA processing, RNA degradation, RNA interference, transcription, modulation of gene expression, antiviral defense, and other cellular processes. These enzymes can be simply classified based on the direction of their activity, hence 5′-3′ or 3′-5′ exoribonucleases. While a large number of 3′-5′ exoribonucleases have been identified, in bacteria and eukaryotes (Zuo and Deutscher 2001) (see also Chap. 8), few 5′-3′ exoribonucleases are currently known. The best characterized 5′-3′ exoribonucleases are the Xrn1/Xrn2 family of enzymes (to be referred to as XRNs here), which have only been found in eukaryotes.

Recently, Rrp17 was identified as another 5′-3′ exoribonuclease, with an important role in the 5′-end processing of pre-ribosomal RNAs (Oeffinger et al. 2009). Several enzymes that possess both endo- and 5′-3′ exoribonuclease activity have also been reported, including *B. subtilis* RNase J1 (Condon 2010), the first enzyme with 5′-3′ exoribonuclease activity found in prokaryotes (see also Chap. 10). RNase J1 is structurally homologous to human CPSF-73 (Mandel et al. 2006), which has also been suggested to have 5′-3′ exoribonuclease activity (Dominski et al. 2005) in addition to its endonuclease activity.

In this chapter, we will focus on the sequence conservation, structure, and function of the XRNs (Sects. 7.2–7.11). We will also discuss the other 5′-3′ exoribonucleases, including Rrp17 (Sect. 7.12) and RNase J1/CPSF-73 (Sect. 7.13).

7.2 Sequence Conservation of the XRNs

Yeast and most metazoans have two XRNs, with Xrn1 (175 kD) primarily in the cytoplasm and Xrn2 (115 kD, more commonly known as Rat1 in yeast) primarily in the nucleus. *RAT1* is an essential gene in yeast, while deletion of *XRN1* in yeast leads to slow growth, sporulation defect, DNA recombination defect, and other phenotypes. The plant *Arabidopsis* has three XRNs, two of which (AtXRN2 and

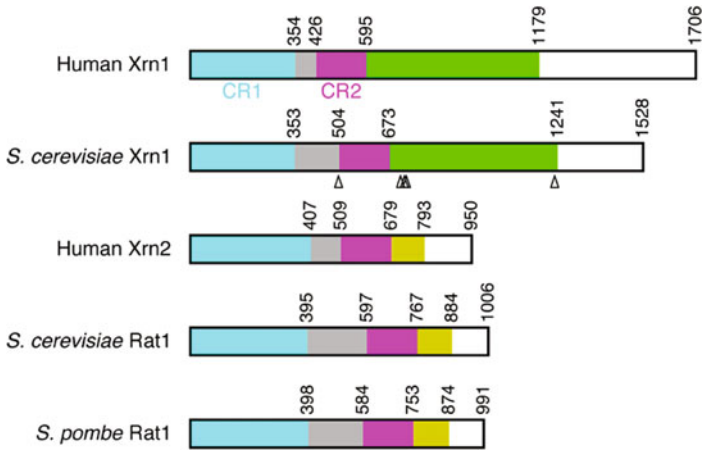

Fig. 7.1 Sequence conservation of XRNs. Schematic drawing of the domain organization of human Xrn1, *S. cerevisiae* Xrn1, human Xrn2, *S. cerevisiae* Rat1, and *S. pombe* Rat1. The two highly conserved regions (CR1 and CR2) are labeled. The 570-residue weakly conserved segment in Xrn1 and a 120-residue segment in Xrn2/Rat1 are indicated. Small triangles in *S. cerevisiae* Xrn1 indicate protease-sensitive sites. The segment at the extreme C-terminus of these proteins is not required for activity

AtXRN3) are in the nucleus, while the third (AtXRN4) is in the cytoplasm (Kastenmayer and Green 2000). However, all three *Arabidopsis* XRNs are Xrn2 homologs, and a sequence homolog of Xrn1 may not exist in higher plants.

The amino acid sequences of the XRNs contain two highly conserved regions (CR1 and CR2) in their N-terminal segment (Fig. 7.1). The sequence identity among Xrn2 homologs for these two regions is 50–60%, while that between Xrn1 and Xrn2 homologs is about 40–50%. In comparison, conservation of sequences outside of these two regions is much lower, especially between Xrn1 and Xrn2. In fact, the larger size of Xrn1 is due to an extensive C-terminal segment that is absent in Xrn2. The linker between CR1 and CR2 is also poorly conserved among the XRNs, both in sequence and in length (Fig. 7.1). Several protease-sensitive sites identified in *S. cerevisiae* Xrn1 map to the boundaries of these segments (Fig. 7.1) (Page et al. 1998).

CR1 covers residues 1–354 of human Xrn1 and residues 1–407 of human Xrn2 (Fig. 7.1), as the latter has three small inserted segments. CR1 contains seven strictly conserved acidic residues (Asp35, Asp86, Glu176, Glu178, Asp206, Asp208, and Asp292 in human Xrn1), and it was recognized that these residues may be homologous to those in the active site of several other Mg^{2+}-dependent nucleases (Solinger et al. 1999), even though CR1 shares little overall sequence conservation with these other enzymes. Therefore, CR1 may have a crucial role in the active site of the XRNs, which is supported by the fact that mutations of these acidic residues abolish the exonuclease activity (Johnson 1997; Page et al. 1998; Solinger et al. 1999). It is expected that the seven conserved acidic residues can coordinate two Mg^{2+} ions for catalysis (Yang et al. 2006).

CR2 covers residues 426–595 of human Xrn1 and residues 509–679 of human Xrn2 (Fig. 7.1). This segment appears to be unique to the XRNs, and has an important role in defining the overall landscape of the active site of the XRNs (see Sect. 7.9).

A 570-residue segment directly following CR2 shows weak sequence conservation among the Xrn1 enzymes (Fig. 7.1). For example, human and yeast Xrn1 share 26% sequence identity for this segment. In contrast, the remaining C-terminal segments of the Xrn1 enzymes have little sequence conservation. This C-terminal segment of yeast Xrn1 is dispensable for its exoribonuclease activity and in vivo function, while the 570-residue segment, though weakly conserved, is required for activity (Page et al. 1998).

The Xrn2 enzymes have a roughly 240-residue C-terminal segment following CR2 (Fig. 7.1). Human Xrn2 and yeast Xrn2/Rat1 share 24% sequence identity for this segment. The last 125 residues of *S. pombe* Rat1 can be deleted without affecting its in vivo function at the permissive temperature (the truncation does lead to a *ts* phenotype). Further deletions, removing the C-terminal 204 residues, inactivated the protein (Shobuike et al. 2001).

Observations on the C-terminal deletion mutants of Xrn1 and Xrn2/Rat1 described above suggest that CR1 and CR2, while highly conserved among the XRNs, are not sufficient for the activity of these enzymes. A segment following CR2 (roughly 570 residues for Xrn1 and 120 residues for Xrn2/Rat1) is required for activity, even though it is only weakly conserved.

The segment of the XRNs containing CR1 and CR2 is generally acidic in nature, with a pI of 5.6 for this segment of yeast Xrn1. In contrast, the remaining C-terminal segments of the Xrn1 enzymes are much more basic, with a pI of 9.4 for yeast Xrn1 (Page et al. 1998).

7.3 5′-3′ Exonuclease Activity of XRNs

The XRNs are Mg^{2+}-dependent, processive 5′-3′ exoribonucleases (Stevens 1978, 1980; Stevens and Poole 1995). Mn^{2+} can also support the catalytic activity of these enzymes. They generally prefer single-stranded RNA substrates with a 5′-end monophosphate group. RNAs with a hydroxyl, cap, or triphosphate group at the 5′-end are poor substrates for XRNs (Stevens 1978; Stevens and Poole 1995). Yeast Xrn1 and Rat1 also have weak exonuclease activity toward single-stranded DNA (Page et al. 1998; Solinger et al. 1999; Stevens and Poole 1995). Yeast Xrn1 can cleave G4 tetraplex DNA derived from guanine-rich sequences that are normally found in telomeres (Liu and Gilbert 1994), while mouse Xrn1 can also cleave G4 tetraplex RNA (Bashkirov et al. 1997).

The presence of strong secondary structures in the RNA substrate can block or stall the exoribonuclease activity of yeast Xrn1 and Rat1 (Poole and Stevens 1997; Stevens and Poole 1995). A strong stem loop at the 5′-end of the genome of

Narnavirus 20 S RNA, a persistent virus in yeast, protects it from degradation by Xrn1 (Esteban et al. 2008).

The exoribonuclease activity of yeast Xrn1 and Rat1 is inhibited by adenosine 3′,5′ bisphosphate (pAp) (Dichtl et al. 1997). Nearly 80% inhibition of both Xrn1 and Rat1 can be achieved with 1 mM pAp. The inhibition of Xrn1 is not affected by the concentration of the RNA substrate, suggesting that pAp may not compete against RNA. pCp and pUp are as potent as pAp in inhibiting Xrn1, while 5′ or 3′ AMP is essentially inactive. pAp is a byproduct of the sulfate assimilation pathway, and is normally converted to 5′ AMP and P_i by the enzyme 3′,5′ bisphosphate nucleotidase, Hal2/Met22 in yeast. Hal2 is inhibited by submillimolar concentrations of Li^+, and the resulting increase in cellular pAp concentration (up to 3 mM) and the consequent inhibition of Xrn1 and Rat1 may be part of the mechanism for Li^+ toxicity in yeast. A similar mechanism may contribute to the physiological effects of Li^+ in other organisms, including the therapeutic effects of Li^+ for the treatment of various neurological diseases in humans.

The cellular functions of the XRNs are primarily linked to their exoribonuclease activity. Therefore, these enzymes are involved in the turnover of mRNAs and degradation of aberrant mRNAs (quality control) (Fig. 7.2). They are also involved in the maturation (5′ trimming) of ribosomal RNAs (rRNAs), small nucleolar RNAs (snoRNAs), and others, as well as the degradation of hypomodified mature tRNAs and spacer RNA byproducts from rRNA processing. The exoribonuclease activity of Xrn2/Rat1 also contributes to transcription termination by nuclear RNA polymerases I and II (Pol I and Pol II). The physiological functions of Xrn1 and

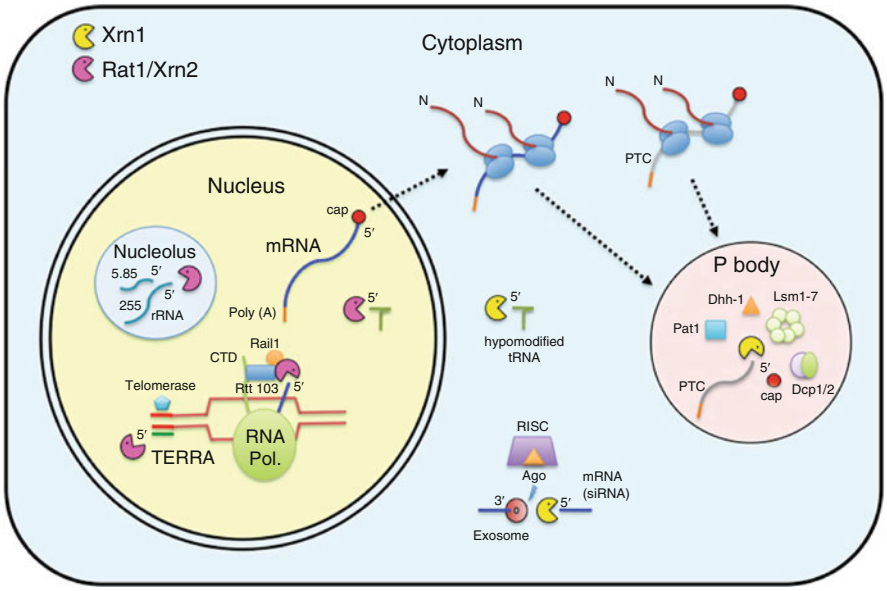

Fig. 7.2 Schematic drawing of the functions of XRNs

Xrn2/Rat1 will be described in more detail in the following two sections, and the functions of the plant XRNs are decribed in Sect. 7.7.

Some of the functional differences between Xrn1 and Xrn2/Rat1 are due to their different cellular localizations. However, a nuclear-targeted Xrn1 can rescue the lethal phenotype of *rat1-1* (carrying a *ts* mutation in *RAT1*) yeast cells, suggesting that Xrn1 can complement the essential function of Rat1 (Johnson 1997). Conversely, *RAT1* expressed from a high copy-number plasmid, as well as Rat1 without its nuclear localization sequence (NLS), can rescue the defects due to the loss of Xrn1 (Johnson 1997).

The XRNs may have other functions that are independent of their exonuclease activity. For example, they may mediate protein–protein interactions to recruit other proteins or to be recruited by other proteins and/or RNA to proper locations in the cell. Especially, yeast Rat1 is known to form a stable complex with Rai1 (Rat1 interacting protein 1), which can stimulate the exoribonuclease activity of Rat1. Rat1 may also interact with other protein factors that are important for Pol II termination, including Rtt103. Yeast Xrn1 may interact directly with microtubules. The protein complexes for Xrn1 and Xrn2/Rat1 are described in a Sect. 7.6.

7.4 Functions of Xrn1

Xrn1 nuclease activity was first identified in yeast (Larimer et al. 1992; Stevens 1978). Later studies showed that the enzyme is identical to several other proteins isolated based on other biochemical and functional properties (Kearsey and Kipling 1991), DNA strand exchange protein 1 including (Sep1) (Tishkoff et al. 1991), DNA strand transferase 2 (Dst2) (Dykstra et al. 1991), Kar^{-} enhancing mutant 1 (Kem1) (Kim et al. 1990), and radiation-resistant on 5 (Rar5) (Kipling et al. 1991). Xrn1 may also be identical to the antiviral superkiller 1 (Ski1) protein (Johnson and Kolodner 1995). This illustrates the various functions for this enzyme other than RNA metabolism, such as DNA recombination, chromosome stability, microtubule association, nuclear fusion, meiosis, telomere maintenance, and cellular senescence. Defects in many of these processes are observed in cells lacking Xrn1 (Larimer and Stevens 1990).

Xrn1 homologs in *S. pombe* (also known as Exo II) (Kaslin and Heyer 1994) and higher eukaryotes have also been cloned, including *C. elegans* (Newbury and Woollard 2004), *Drosophila* (Pacman) (Till et al. 1998), mouse (Bashkirov et al. 1997), and humans (Sato et al. 1998; Shimoyama et al. 2003).

7.4.1 Functions of Xrn1 in RNA Degradation and Turnover

Xrn1 has important roles in mRNA degradation and turnover. This subject has been reviewed extensively over the past few years (Conti and Izaurralde 2005; Doma and

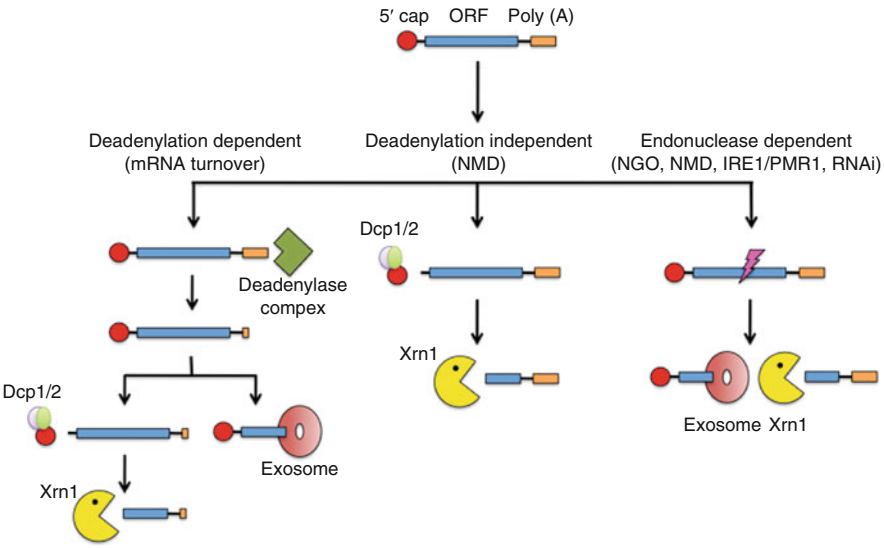

Fig. 7.3 Schematic drawing of mRNA turnover and mRNA degradation pathways

Parker 2007; Houseley and Tollervey 2009; Isken and Maquat 2007; Parker and Song 2004), and will only be discussed briefly here, focusing on the functions of Xrn1 in these processes. The basic mode of action is that RNAs with a 5′-end monophosphate are generated by decapping of mRNAs (possibly preceded by deadenylation) or by endonucleolytic cleavage, which are then rapidly degraded by Xrn1 (Fig. 7.3). The 3′-5′ exosome also plays an important role in mRNA metabolism (see Chap. 9), although Xrn1 is the primary enzyme for mRNA degradation and turnover in yeast. The rate of mRNA turnover is reduced in yeast cells lacking Xrn1, leading to accumulation of non-polyadenylated mRNAs that also partially lack the 5′-end cap structure (Hsu and Stevens 1993).

Xrn1 is predominantly localized to cytoplasmic foci known as P-bodies (processing bodies/GW bodies), which are the major location for mRNA decapping and 5′-3′ degradation as well as for temporary storage of mRNAs during translation repression (Kulkarni et al. 2010; Parker and Sheth 2007). Recent studies show that decapping and Xrn1-mediated degradation of mRNAs can also occur on actively translating ribosomes (Hu et al. 2009), as does deadenylation-independent decapping initiated by nonsense-mediated decay (NMD) (Hu et al. 2010).

Endonucleolytic cleavage of mRNAs can be initiated by no-go decay (NGD) and by the RNA-induced silencing complex (RISC) for RNA interference (RNAi) (Fig. 7.3) (Orban and Izaurralde 2005). In addition, endonucleolytic cleavage during maturational processing of many RNA precursors can produce byproducts that are degraded by Xrn1. For example, Xrn1 degrades the internal transcribed spacer ITS1 generated from pre-ribosomal RNA processing in yeast (Fig. 7.4) (Stevens et al. 1991).

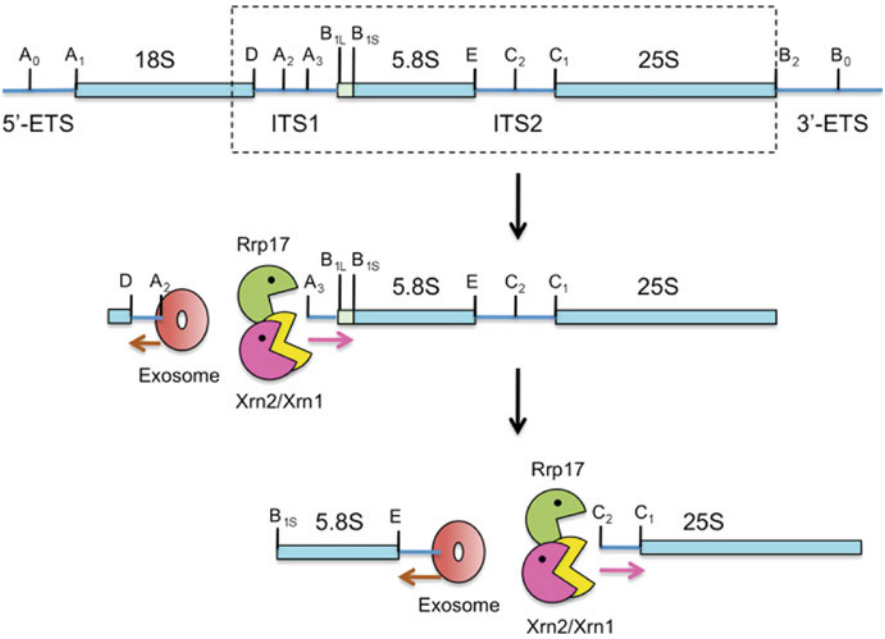

Fig. 7.4 Schematic drawing of the pre-ribosomal RNA processing pathways. The extent of the exonuclease trimming is indicated by the *arrows*

Recently, it has been found that Xrn1 and Rat1 can degrade hypomodified mature tRNAs in yeast, in the rapid tRNA decay (RTD) pathway (Chernyakov et al. 2008).

7.4.2 *Functions of Xrn1 in RNA Maturation*

Xrn1 plays a role in pre-ribosomal RNA processing and maturation, which may be especially important in the absence of Rat1 activity in yeast. This will be discussed in more detail in Sect. 7.5.1.

7.4.3 *Functions of Xrn1 in DNA Recombination and Chromosome Stability*

Xrn1 was identified in a biochemical search for DNA recombination proteins (and hence named Sep1 and Dst2) (Dykstra et al. 1991; Tishkoff et al. 1991). It has homologous pairing and strand exchange activities in vitro. Yeast cells lacking Xrn1 are defective for intrachromosomal recombination, sporulation, and trigger

arrest at pachytene stage in the meiotic cell cycle (Solinger et al. 1999; Tishkoff et al. 1995). On the other hand, Xrn1 may not be involved in mitotic recombination or mating-type switching.

Xrn1 was identified from a genetic screen for mutants that can enhance the nuclear fusion defect of yeast cells carrying the *kar1-1* mutation (hence named Kem1) (Kim et al. 1990). Kem1 mutants also have reduced chromosome stability and are hypersensitive to the microtubule-destabilizing drug benomyl. Defective interactions with microtubules may be the basis of these phenotypes (see Sect. 7.6).

Yeast cells lacking Xrn1 also show cellular senescence and telomere shortening (Liu et al. 1995), which may be related to the nuclease activity of this enzyme toward G4 tetraplex DNA (Liu and Gilbert 1994).

Most of the defects in these nuclear processes (sporulation defect, arrest at pachytene, chromosome instability) due to loss of Xrn1 can be rescued by targeting Rat1 to the cytoplasm (Johnson 1997); consistent with the fact that Xrn1 is primarily a cytoplasmic protein. This also suggests the possibility that the effects of Xrn1 on these processes may not be direct.

7.4.4 Other Functions of Xrn1

Human Xrn1 may function as a tumor suppressor in osteogenic sarcoma, and its expression level is reduced in these tumors (Zhang et al. 2002). Mouse Xrn1 is highly expressed in testis, suggesting a functional role in gametogenesis (Shobuike et al. 1997). *Drosophila* Xrn1/Pacman is required for male fertility (Zabolotskaya et al. 2008). The expression level of Pacman is correlated with developmental stages in *Drosophila* (Till et al. 1998), and *C. elegans* Xrn1 is critical for ventral epithelial enclosure during embryogenesis (Newbury and Woollard 2004).

Xrn1 is also involved in host antiviral response. It can suppress viral RNA recombination (Cheng et al. 2006), and down-regulate replication by HIV (Chable-Bessia et al. 2009) and HCV (Jones et al. 2010).

7.5 Functions of Xrn2/Rat1

Like Xrn1, Xrn2 was first identified from several independent studies, due to its different functions. It was found from a screen for ribonucleic acid trafficking defects in yeast, and hence named Rat1 (Amberg et al. 1992), and from a screen for protein translation defects (Hke1, homology to Kem1), which are more likely due to defects in RNA processing or trafficking (Kenna et al. 1993). It was also found to have functions in transcription activation (Tap1) (Aldrich et al. 1993; di Segni et al. 1993).

In contrast to *XRN1*, *RAT1* is an essential gene in yeast, although the exact function (or the substrate) of this protein that is required for cell viability is currently not known.

Homologs of Rat1/Xrn2 in other organisms have also been cloned, including *S. pombe* (also named Dhp1) (Shobuike et al. 2001; Sugano et al. 1994), mouse (Dhm1) (Shobuike et al. 1995), and humans (Zhang et al. 1999).

7.5.1 Functions of Xrn2/Rat1 in RNA Processing and Degradation

Rat1 is required for 5′-end trimming during the maturation of the 5.8 S and 25 S rRNA, and Xrn1 can support this activity in the absence of Rat1 (El Hage et al. 2008; Fang et al. 2005; Fatica and Tollervey 2002; Geerlings et al. 2000; Henry et al. 1994). The 5.8 S, 18 S and 25 S ribosomal RNAs are made in a single transcript by Pol I in eukaryotes, which undergoes extensive endo and exonucleolytic processing (Fig. 7.4). The primary transcript includes two external transcribed spacers (5′- and 3′-ETS) and two internal transcribed spacers (ITS1 and ITS2) (Fig. 7.4). Rat1/Xrn1 is involved in the degradation of a fragment of ITS1 that is released during pre-rRNA processing. Recent studies identified Rrp17 as an independent 5′-3′ exoribonuclease that can also process the 5′-ends of 5.8 S and 25 S rRNA (see Sect. 7.12) (Oeffinger et al. 2009).

Rat1 is required for 5′-end processing of polycistronic and some intronic snoRNAs in yeast, and Xrn1 can (at least partially) support this activity (Lee et al. 2003; Petfalski et al. 1998; Qu et al. 1999; Villa et al. 1998). Rat1 and Xrn1 are involved in the degradation of some intron-containing unspliced pre-mRNAs and intron lariats (Danin-Kreiselman et al. 2003). The entry sites for the XRNs are produced by prior endonucleolytic cleavage or by debranching of the intron lariat.

Rat1 degrades telomeric repeat-containing RNA (TERRA) in yeast (Luke et al. 2008). Telomeres are transcribed by Pol II and polyadenylated, and cells lacking Rat1 accumulate TERRA and have short telomeres. Therefore, Rat1 promotes telomere elongation and is important for telomerase regulation.

7.5.2 Functions of Xrn2/Rat1 in RNA Polymerase Transcription Termination

Xrn2/Rat1 has a central role in the torpedo model for transcription termination by RNA polymerases I and II. This area has been reviewed extensively over the past few years (Buratowski 2005; Ghazal et al. 2009; Gilmour and Fan 2008; Luo and Bentley 2004; Richard and Manley 2009; Rondon et al. 2009), and will only be briefly discussed here.

The torpedo model suggests that the downstream RNA product, with a 5′-monophosphate, produced by an endonucleolytic cleavage of the primary transcript serves as the entry point for a 5′-3′ exoribonuclease, which degrades this downstream RNA, catches up to the elongating (or paused) polymerase, and

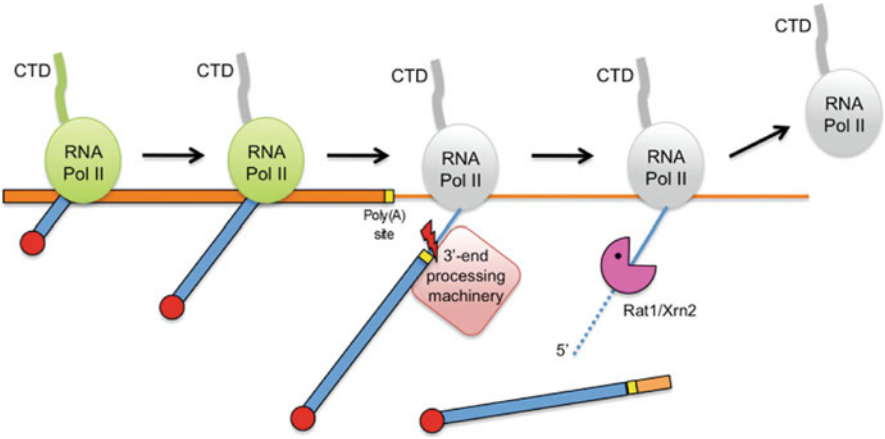

Fig. 7.5 Schematic drawing of the allosteric-torpedo (unified) model of Pol II termination. Changes in the phosphorylation state of the CTD and in the body of Pol II are indicated (Modified from Luo et al. 2006)

causes transcription termination (Connelly and Manley 1988). The 5′-3′ exoribonuclease for Pol II termination was identified as Rat1 in yeast and Xrn2 in mammalian cells (Kim et al. 2004; West et al. 2004). It was shown more recently that Pol I transcription termination is also mediated by the torpedo model, with Rat1 being the 5′-3′ exoribonuclease for this function in yeast (Fig. 7.5) (El Hage et al. 2008; Kawauchi et al. 2008).

The molecular mechanism for how Xrn2/Rat1 brings about transcription termination once it catches up to the polymerase is still not clearly understood. Degradation of the downstream product is not sufficient for termination. Nuclear-targeted Xrn1 can degrade the downstream product in yeast cells lacking Rat1, but nuclear Xrn1 cannot cause Pol II termination (Luo et al. 2006). In addition, Rat1 alone is not sufficient for Pol II termination in an in vitro transcription system (Dengl and Cramer 2009). Therefore, other factors are also required for transcription termination by Rat1/Xrn2. The pre-mRNA 3′-end processing factor Pcf11 may be important for dismantling the polymerase elongation complex (Luo et al. 2006; West and Proudfoot 2008; Zhang et al. 2005).

7.5.3 Other Functions of Xrn2/Rat1

Xrn2 is a candidate gene for spontaneous lung tumor susceptibility based on a genome-wide association study in mice (Lu et al. 2010). In addition, polymorphisms in human Xrn2 are associated with human lung cancer, and over-expression of human Xrn2 can affect the differentiation of a leukemia cell line (Park et al. 2007).

7.6 Protein Partners of XRNs

Xrn1 is associated with the decapping machinery in yeast and may directly interact with several of its components, including Dcp1/Dcp2, Pat1, Dhh1, and the Lsm1–7 complex (Coller and Parker 2004). This may facilitate the degradation of RNAs once they are decapped by this machinery. The region(s) of Xrn1 that is required for these interactions has not been identified.

Yeast Xrn1 interacts directly with tubulin and promotes microtubule assembly (Interthal et al. 1995). Cells lacking Xrn1 show increased chromosome loss, defects in spindle pole body separation and karyogamy, and hypersensitivity to benomyl (Kim et al. 1990). The exonuclease activity of Xrn1 is not required for this interaction (Solinger et al. 1999). The benomyl sensitivity of cells lacking Xrn1 can be rescued by targeting Rat1 to the cytoplasm, although cytoplasmic Rat1 does not appear to be associated with microtubules (Johnson 1997).

In yeast, Rat1 has direct and strong association with Rai1, and the Rat1-Rai1 complex was first purified from *S. cerevisiae* extract (Stevens and Poole 1995). A stable Rat1-Rai1 complex was also observed in *S. pombe* (Shobuike et al. 2001). Rai1 (45 kD) has orthologs in most eukaryotes, including plants, and the mammalian homolog is known as Dom3Z (Xue et al. 2000). The sequences of these orthologs are highly divergent, however, with only a few conserved residues. In contrast to Rai1, Dom3Z does not appear to interact with Xrn2.

Rai1 is not essential for yeast cell viability, and does not have any nuclease activity (Xue et al. 2000). However, Rai1 can moderately stimulate the exoribonuclease activity of Rat1 (Xiang et al. 2009; Xue et al. 2000). This may be due in part to the stabilization of Rat1 by Rai1. Rat1 is unstable and quickly loses activity upon pre-incubation at 30 °C, whereas the Rat1-Rai1 complex is able to retain most of its nuclease activity during this pre-incubation (Xue et al. 2000). Like Rat1, Rai1 is also required for 5.8 S rRNA processing. However, while Rat1 is only involved in the 5′-end processing of this RNA, Rai1 is also needed for 3′-end processing (Fang et al. 2005; Xue et al. 2000).

The *Drosophila* genome contains two homologs of Rai1/Dom3Z: CG9125 and CG13190. CG13190, also known as Cutoff (Cuff), was first identified in a female-sterile screen. *cuff* mutations affect germline cyst development, produce ventralized eggs, and reduce female fecundity (Chen et al. 2007). Over-expressed Cuff is localized in the cytoplasm and in perinuclear puncta, and Cuff does not interact with *Drosophila* Xrn2.

S. cerevisiae also has a homolog of Rai1, Ydr370c, which is poorly conserved with Rai1 at the sequence level (Xue et al. 2000). The function of this protein is currently not known. Sequence analysis suggests that this homolog is restricted to only a few of the fungal species, while most other eukaryotes contain only one homolog of Rai1/Dom3Z.

Rtt103 (*r*egulation of *T*y1*t*ransposition 103) can interact with the Rat1-Rai1 complex in yeast (Dengl and Cramer 2009; Kim et al. 2004). Rtt103 was originally found by a screen for mutants that increased Ty1 transposon's mobility (Scholes

et al. 2001). Rtt103 has a RNA Pol II carboxy-terminal domain (CTD)-interacting domain (CID), and recognizes Ser2 phosphorylated CTD. Rtt103 may be involved in nuclear pre-mRNA regulation (Kim et al. 2004), and it localizes at the 3′-end of transcribing genes together with Rat1-Rai1 in vivo (Kim et al. 2004) and in vitro (Dengl and Cramer 2009).

A functional interaction between Rat1 and the pre-mRNA 3′-end processing factor Pcf11 has been suggested (Luo et al. 2006; West and Proudfoot 2008), although currently there is no biochemical evidence for direct interaction between these two proteins. Pcf11 may be responsible for the recruitment of Rat1 to the 3′-end of pre-mRNAs and/or *vice versa*.

7.7 Functions of XRNs in Plants and Other Organisms

In *Arabidopsis*, AtXRN2 is involved in 5′-end processing of 5.8 S and 25 S rRNAs (Zakrzewska-Placzek et al. 2010), a function similar to that of Rat1. In addition, both AtXRN2 and AtXRN3 can degrade miRNA loop and transgene for suppressing endogenous post-transcriptional gene silencing (Gy et al. 2007).

The cytoplasmic AtXRN4 can degrade specific RNA transcripts but may not be a general RNA degradation enzyme, in contrast to Xrn1. It degrades 3′-end mRNA products derived from miRNA-mediated cleavage (Souret et al. 2004). Mutation of AtXRN4 leads to accumulation of decapped mRNAs that could be templates for facilitating the RNAi pathway, and AtXRN4 may link mRNA degradation and RNA silencing (Gazzani et al. 2004; Gregory et al. 2008). AtXRN4 also contributes to the regulation of the ethylene response pathway (and hence is also known as EIN5, ETHYLENE-INSENSITIVE5) (Olmedo et al. 2006; Potuschak et al. 2006).

In *Trypanosoma brucei* and other kinetoplastids, four XRN-related proteins have been identified, XRNA, XRNB, XRNC, and XRND (Li et al. 2006). XRND is nuclear, XRNB and XRNC are cytoplasmic, and XRNA is present in both compartments. XRNA and XRND are essential for growth, and XRNA is required for degrading highly unstable, developmentally regulated mRNAs, while having little effect on more stable, unregulated mRNAs (Li et al. 2006).

7.8 Overall Structure of Xrn2/Rat1

Crystal structure of the *S. pombe* Rat1-Rai1 complex is the first structural information on the XRNs (Xiang et al. 2009). The structure of Rat1 indicates that CR1 and CR2 form a single, large domain (Fig. 7.6a). CR1 is composed of a seven-stranded (β1 through β7) mostly parallel β-sheet sandwiched by α-helices on both faces. Strands β2 through β7 are arranged similar to those in the Rossmann fold, but with strand β7 running in the opposite direction. A helix is inserted after β2 (αB) and β7 (αD). CR2 contains several helices and long loops, which wrap around the base

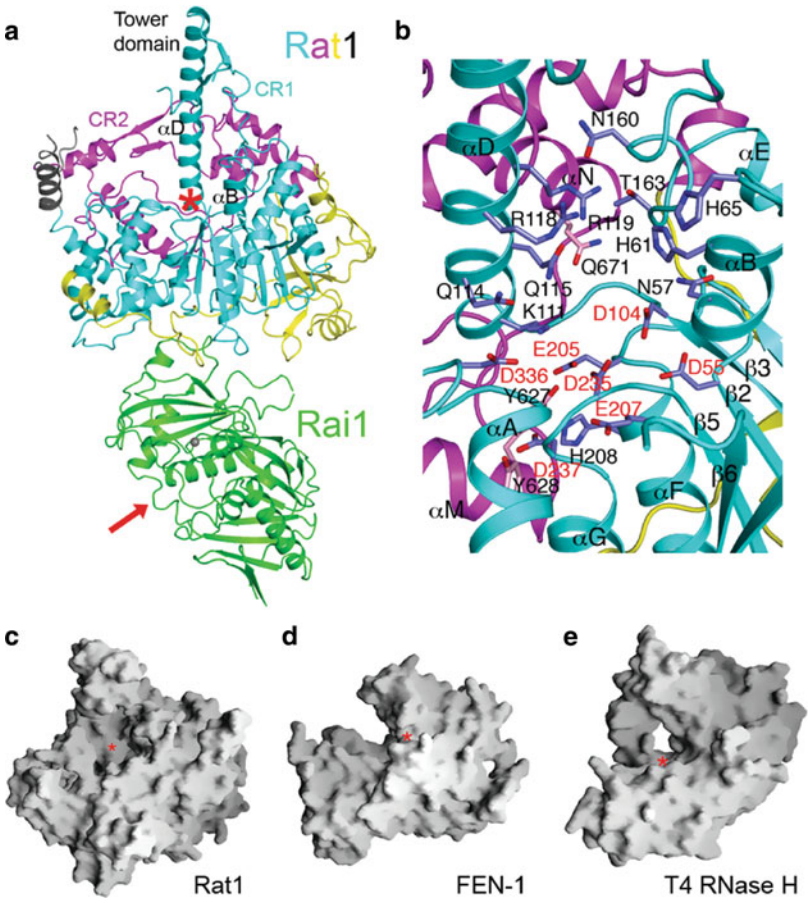

Fig. 7.6 Structure of the *S. pombe* Rat1–Rai1 complex. (**a**) Schematic drawing of the structure of *S. pombe* Rat1–Rai1 complex (Xiang et al. 2009). The active site of Rat1 is indicated with the star, and the arrow points to the opening of the Rai1 active site pocket. A bound divalent metal cation in the active site of Rai1 is shown as a sphere. (**b**) Schematic drawing of the active site of *S. pombe* Rat1. Side chains of residues in the active site are shown and labeled. Overall molecular surface of (**c**) Rat1, (**d**) FEN-1 (Chapados et al. 2004), and (**e**) T4 RNase H (Devos et al. 2007). The active site is indicated with the star

(N-terminal end) of the αD helix. Residues in the linker between CR1 and CR2 are mostly disordered in the structure. The N- and C-termini of this segment are located within 10 Å of each other, suggesting that it is likely an inserted cassette between the two conserved regions (Fig. 7.6a).

A striking feature of the *S. pombe* Rat1 structure is the long αD helix, with its C-terminus projected 30-Å away from the rest of the structure (Fig. 7.6a). This feature has been named the "tower domain." The N-terminal residues of helix αD are strongly conserved among XRNs, and they contribute to the formation of the active site (see Sect. 7.9). The C-terminal residues of this helix are poorly

conserved, and sequence analysis indicates that this helix is much shorter in Xrn1. Two temperature-sensitive mutations in XRNs, P90L in Xrn1 (Page et al. 1998), and Y657C in Rat1 (the *rat1-1* mutation) (Luo et al. 2006), are located near the N-terminal end of helix αD. Both mutations may destabilize this helix at the non-permissive temperature, supporting the functional importance of the tower domain.

The structure of CR1 has many homologs, most of which are nucleases. These include the FEN-1 family of endonucleases (Chapados et al. 2004; Hwang et al. 1998; Sakurai et al. 2005; Sayers and Artymiuk 1998), the 5′ exonuclease from the phage T5 (Ceska et al. 1996), RNase H from the phage T4 (Devos et al. 2007; Mueser et al. 1996), the 5′ nuclease domain of Taq DNA polymerase (Kim et al. 1995; Murali et al. 1998), and other PIN domain-containing nucleases (Clissold and Ponting 2000; Glavan et al. 2006). The sequence conservation between Rat1 and these other enzymes is very low, between 8% and 15%. The structural homology is limited to strands β2-β7 in the central β-sheet and a few of the flanking helices. The tower domain in Rat1 is equivalent to the helical clamp in *A. fulgidus* FEN-1 (Chapados et al. 2004) and the helical arch in T5 exonuclease (Ceska et al. 1996), but the equivalent region is a long loop in *M. jannaschii* FEN-1 (Hwang et al. 1998) and is disordered in T4 RNase H (Devos et al. 2007; Mueser et al. 1996).

The Rat1 structure covers residues 1–874, which are sufficient for the activity of this protein at the permissive temperature (Shobuike et al. 2001). The 120-residue segment following CR2 forms three distinct structural features (Fig. 7.6a). The N-terminal region (residues 752–840) of this segment adds four anti-parallel strands (β8–β11) to the central β-sheet of CR1, producing a highly twisted 11-stranded β-sheet. Residues 841–863 form a long loop that traverses the entire bottom face of the central β-sheet of CR1. Finally, the C-terminal region of this segment (residues 864–874) forms an α-helix that interacts with helices αA and αH in CR1. Therefore, despite being poorly conserved among XRNs, this segment has an important structural role, which may explain why it is required for the function of Rat1.

The strong sequence conservation for CR1 and CR2 suggests that these two segments should have a similar structure in Xrn1 (with the exception of the tower domain). On the other hand, most of the 570-residue segment following CR2 is unique to Xrn1 and forms several distinct structural domains, as revealed by the structure of Xrn1 (unpublished data).

7.9 Active Site of Xrn2/Rat1

The active site of Rat1 is located at the top of the central β-sheet of CR1, with contributions from residues at the base of the αD helix (Fig. 7.6a). The seven conserved acidic residues in CR1 form a cluster, and are located in the center of the active site (Fig. 7.6b). In the structure of bacteriophage T4 RNase H, two metal ions are associated with these acidic residues (Mueser et al. 1996), consistent with the hypothesis that the two metal ions mediate the nuclease activity (Yang et al. 2006). Metal ions were not observed in the structure of Rat1, and there are some

noticeable differences in the conformations of some of these acidic side chains between Rat1 and T4 RNase H.

Three positively-charged (Lys111, Arg118, Arg119) and two polar (Gln114, Gln115) residues at the base of the αD helix, as well as His61, His65, and Asn57 in helix αB contribute their side chains to the active site (Fig. 7.6b). These residues form a steep wall at one side of the active site, and may be important for interacting with the phosphate backbone of the RNA substrate. Mutations of these residues, as well as several of the conserved acidic residues, disrupt Rat1's exonuclease activity.

Residues in CR2 encircle the base of helix αD, but contribute few residues to the Rat1 active site. The side-chain hydroxyl groups of Tyr627 and Tyr628 hydrogen-bond with the acidic residues Glu205 and Asp237 in the active site, respectively, and the side chain of Gln671 is located in the cluster of polar side chains from the αB and αD helices (Fig. 7.6b).

However, CR2 introduces a dramatic difference in the overall landscape of the active site of Rat1 as compared to other related nucleases. Due to the presence of CR2, the Rat1 active site is a pocket (Fig. 7.6c), while the active sites of related nucleases are more open (Figs. 7.6d,e). It has been suggested that the ssDNA substrate threads through the helical arch in T5 nuclease (Ceska et al. 1996). In T4 RNase H, a single-stranded DNA portion of its forked DNA substrate is also bound in this region (Devos et al. 2007). However, such a binding mode would not be possible in Rat1, as the substrate would clash with residues in CR2. This may be the explanation why Rat1 is an exonuclease.

The poorly conserved C-terminal segment of Rat1, following CR2, is located away from the active site and does not have any direct contributions to it. However, this segment is important for recruiting Rai1, which can (indirectly) stimulate the exoribonuclease activity of Rat1.

7.10 Structure of the Rat1-Rai1 Complex

The structure of the Rat1-Rai1 complex shows that Rai1 is bound on the opposite face from the Rat1 active site (Fig. 7.6a), interacting primarily with the poorly conserved C-terminal loop that traverses the bottom of CR1 (Xiang et al. 2009). The Rat1-Rai1 interface buries approximately 800 $Å^2$ of surface area of each protein, consistent with the stability of this complex. Ion-pair, hydrogen-bonding, as well as van der Waals interactions contribute to the formation of this complex. Mutations introduced in this interface can abolish the interaction as well as the stimulation of Rat1 by Rai1 (Xiang et al. 2009).

Rai1 does not directly contribute to the active site of Rat1. Structural and biochemical studies indicate that Rai1 enhances Rat1′s exonuclease activity at least in part by increasing the enzyme's stability (Xue et al. 2000). This is also supported by the observation that over-expressing Rai1 can rescue a temperature-sensitive phenotype of Rat1 (Shobuike et al. 2001). On the other hand, real-time

measurements of exoribonuclease activities of Rat1 and Rat1-Rai1 complex suggest that the Rat1 enzyme is inherently less active (Sinturel et al. 2009). Therefore, Rai1 may also indirectly help to organize the active site of Rat1. The structure of Rat1 alone, and comparison with the Rat1–Rai1 complex, may reveal any changes in the active site that is induced by Rai1 binding.

Residues at the Rat1–Rai1 interface are generally conserved among the fungal proteins, consistent with the observations that Rat1 and Rai1 form tight complexes in both *S. cerevisiae* and *S. pombe* (Shobuike et al. 2001; Stevens and Poole 1995). However, Rai1 residues that interact with Rat1 are not conserved in the mammalian Rai1 homolog Dom3Z, and Dom3Z does not interact with mammalian Xrn2. Therefore, the Rat1–Rai1 interaction appears to be unique to the fungal species. Whether mammalian Xrn2 also has a protein partner that can stimulate its activity is currently not known.

7.11 Rai1/Dom3Z and RNA 5′-End Capping

An unexpected discovery from the structure of Rai1 is that it contains a large pocket (Figs. 7.7a,b), and the few residues that are highly conserved among Rai1 orthologs are all located in this pocket (rather than in the interface with Rat1) (Xiang et al. 2009). Moreover, three conserved acidic side chains, Glu150, Asp201, and Glu239 (*S. pombe* Rai1 numbering), together with the main-chain carbonyl of Leu240 and two water molecules octahedrally coordinate a divalent cation (Mg^{2+} or Mn^{2+}) (Fig. 7.7c), and this metal ion is located near the bottom of the pocket (Fig. 7.7b). Therefore, the structural information strongly suggests that Rai1 and its mammalian homolog Dom3Z may have enzymatic activity of its own.

Biochemical studies demonstrate that Rai1 possesses RNA 5′-end pyrophosphohydrolase activity, being able to remove a pyrophosphate group from RNA with 5′-end triphosphate (pppRNA) (Xiang et al. 2009). Such an enzyme (RppH) was first characterized in bacteria (Deana et al. 2008), which is a member of the Nudix family of enzymes. Rai1/Dom3Z shares neither sequence nor structural homology with RppH. Remarkably, Rat1 can stimulate this pyrophosphohydrolase activity of Rai1, even though the binding site is located far from the active site of Rai1 (Fig. 7.7a) (Xiang et al. 2009).

Further biochemical studies showed that Rai1 can also remove unmethylated 5′-end cap of RNAs (GpppRNA) (Jiao et al. 2010). This activity is however distinct from the classical decapping enzymes. First, Rai1 has much lower activity toward methylated 5′-end cap. Second, the product released by Rai1 is GpppN, while the classical decapping enzymes release m^7GDP. Therefore, Rai1 appears to have two distinct enzymatic activities.

The amino acid sequence of the *Drosophila* Rai1 homolog Cuff contains mutations at several of the conserved acidic residues that are important for metal ion binding. It is possible that Cuff does not have RNA 5′-end pyrophosphohydrolase and decapping activities.

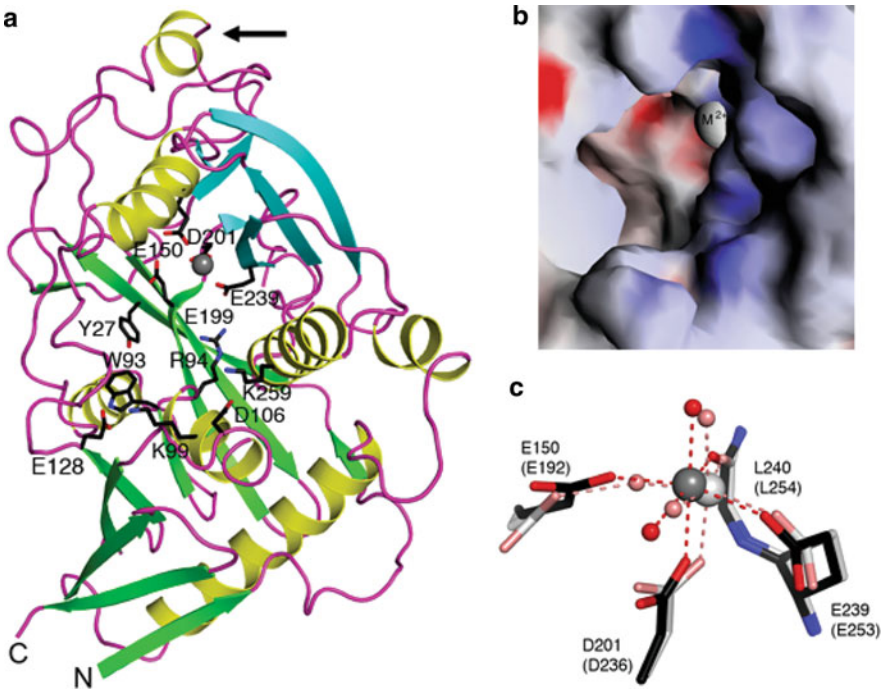

Fig. 7.7 Rai1 possesses an active site of its own. (**a**) Schematic drawing of the structure of *S. pombe* Rai1 (Xiang et al. 2009). A bound divalent cation in the active site is shown as a sphere. The arrow points to residues in the interface with Rat1. (**b**) Molecular surface of the active site region of Rai1, showing a large pocket. The metal ion is located at the bottom of the pocket. (**c**) Overlays of the metal ion binding site in the structure of Rai1 (in *black*) and mouse Dom3Z (in *gray*). Residue numbers in parenthesis are for Dom3Z. The interaction between Glu192 in Dom3Z and the metal ion is mediated by a water molecule

The biochemical activities of Rai1 suggest a physiological function for this enzyme. Rai1 may be an mRNA 5′-end capping quality checkpoint. Both Rai1 substrates (pppRNA and GpppRNA) are intermediates in the mRNA 5′-end capping pathway. mRNAs with defective 5′-end capping cannot serve as template for translation. At the same time, these defective mRNAs cannot be degraded by Xrn1/Rat1, due to the protected 5′-end. Therefore, Rai1 can remove the 5′-end from such mRNAs, and the products can then be rapidly degraded by Xrn1/Rat1. Studies in yeast show that mRNAs with 5′-end capping defects are stabilized in cells lacking Rai1, consistent with this 5′-end capping quality checkpoint model (Jiao et al. 2010). In addition, mRNAs with aberrant 5′-end capping also accumulate under stress conditions (glucose deprivation or amino acid starvation) in cells lacking Rai1. Moreover, defective capping in yeast cells is linked to enhanced recruitment of Rat1 throughout the transcribing unit, and promotes Pol II termination upstream of the poly(A) site (Jimeno-Gonzalez et al. 2010). This suggests that Rai1 can remove the defective cap in such conditions, which then allows Rat1 to

function as a torpedo to induce Pol II termination before the completion of transcription, in a mechanism equivalent to that of transcription termination at the 3′-end of the pre-mRNA.

These studies provide the first demonstration of an mRNA 5′-end capping checkpoint (Jiao et al. 2010; Xiang et al. 2009). It was generally believed in the field that 5′-end capping always proceeds to completion. The data on Rai1 convincingly demonstrate such a checkpoint in yeast. Dom3Z has a conserved active site, and it remains to be seen whether such a checkpoint also functions in metazoans.

7.12 The 5′-3′ Exoribonuclease Rrp17

Rrp17 (*r*ibosomal *R*NA *p*rocessing) is associated with pre-ribosomes and the nuclear pore complex (Oeffinger et al. 2009). It is an independent nuclease for the 5′-end trimming of the 5.8 S and 25 S rRNAs. Rrp17 is an essential gene in yeast, and has highly conserved orthologs in most eukaryotes. Rrp17 has 5′-3′ exoribonuclease activity, with preference for a phosphate group at the 5′-end of the substrate, while a triphosphate group or cap structure inhibits the nuclease activity. In comparison to the XRNs, Rrp17 also has activity toward RNAs with a 5′-end hydroxyl group. The activity requires Mg^{2+} ions, while the enzyme is inactive with Mn^{2+}.

7.13 RNase J1/CPSF-73

Earlier studies have only identified 5′-3′ exoribonucleases in eukaryotes, leading to the general belief that these enzymes are not present in prokaryotes. However, it was recently discovered that the *B. subtilis* endoribonuclease RNase J1 also has 5′-3′ exoribonuclease activity, establishing for the first time the presence of such activity in bacteria (Condon 2010; Mathy et al. 2007). The exoribonuclease activity is required for mRNA degradation and for 5′-end maturation of 16 S rRNA in *B. subtilis*. Structural studies show that the endo- and exonuclease activities share the same active site, and suggest that RNase J1 may switch from an endo mode to exo mode on the same RNA substrate (de la Sierra-Gallay et al. 2008).

The exoribonuclease activity of RNase J1 is more permissive toward 5′-end modification of the RNA substrate as compared to Xrn1 (Mathy et al. 2007). The highest activity is observed for RNA with a 5′-end monophosphate or 5′-end hydroxyl group, although this activity is roughly tenfold lower than that of Xrn1, leading to the suggestion that RNase J1 may require a cofactor for full activity. RNA with a 5′-end triphosphate group can also be degraded, but with roughly fourfold weaker activity. The activity toward RNA with a 5′-end cap is even lower (Mathy et al. 2007).

RNase J1 exists in a complex with RNase J2, which is a sequence homolog of RNase J1 but with little nuclease activity. RNase J1 homologs are found in bacteria (but not in *E. coli*), archaea (Clouet-d'Orval et al. 2010; de la Sierra-Gallay et al.

2008), and they may also be present in the chloroplasts of plants (de la Sierra-Gallay et al. 2008).

RNase J1 is a structural homolog of CPSF-73 (Mandel et al. 2006), the endoribonuclease for the cleavage step in eukaryotic pre-mRNA 3′-end processing (Mandel et al. 2008; Proudfoot 2004; Wahle and Ruegsegger 1999; Zhao et al. 1999). Recent studies suggest that CPSF-73 may also have an exoribonuclease activity, degrading the downstream cleavage product of histone pre-mRNAs (Dominski and Marzluff 2007; Dominski et al. 2005; Yang et al. 2009). Analogous to the RNase J1/J2 heterodimer, the CPSF complex also contains CPSF-100, an inactive sequence homolog of CPSF-73. It may be possible that mammalian CPSF-73/CPSF-100 and *B. subtilis* RNase J1/J2 share a common evolutionary origin.

7.14 Perspectives

Studies over the past few years have greatly enhanced our understanding of the structure and function of 5′-3′ exoribonucleases, as well as identified new proteins that possess this activity. It is anticipated that further characterization of these enzymes in the coming years, especially in higher eukaryotes, will lead to significant new insights into the biological significance of these enzymes. Moreover, studies on Rat1–Rai1 complex led to the discovery of a novel mRNA 5′-end capping quality checkpoint. There may be further exciting surprises from the studies of these exoribonucleases and their interaction partners.

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