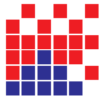

REVIEW

Overview of *Saccharomyces cerevisiae* Ribonucleases

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Summary

Ribonucleases (RNases) are hydrolytic enzymes that hydrolyze single-stranded RNA, double-stranded RNA, and RNA hybridized with DNA. Cells produce some specific ribonucleases that are needed in a variety of cellular processes, but also a set of general RNases that are secreted or targeted to the lysosome or vacuole. Eukaryotes have developed highly complex RNA processing and multiple turnover pathways. In general, each type of RNA has a distinctive complement of components that accomplish and regulate its biosynthesis and turnover, though there are components that are adapted to act along the multiple pathways. In this paper overview of Saccharomyces cerevisiae RNases is given along with short description of their functions in RNA processing and turnover in the cell.

Key words: *yeast RNases, RNA processing, RNA turnover*

Sažetak

Ribonukleaze (RNaze) su hidrolitički enzimi koji hidroliziraju jednolančanu RNA, dvolančanu RNA i RNA hibridiziranu s DNA. Stanice proizvode specifične ribonukleaze koje su potrebne u različitim staničnim procesima, kao i nespecifične RNaze koje se izlučuju iz stanice ili se lokaliziraju u lizosome ili vakuole. Eukarioti su razvili vrlo složene mehanizme za procesiranje RNA te različite puteve za razgradnju RNA. U pravilu za svaku vrstu RNA u stanici postoji set različitih komponenata koji provode njenu biosintezu, regulaciju biosinteze i razgradnju, iako neke od komponenata sudjeluju u više različitih puteva. U ovom radu je dan pregled RNaza iz kvasca Saccharomyces cerevisiae i kratak opis njihovih uloga u procesiranju i razgradnji RNA u stanicama.

Ključne riječi: *RNaze kvasca, procesiranje RNA, razgradnja RNA*

Introduction

Ribonucleases (RNases) are hydrolytic enzymes that hydrolyze single-stranded RNA, double-stranded RNA, and RNA hybridized with DNA. Cells produce some specific ribonucleases that are needed in a variety of cellular processes, but also a set of general RNases that are secreted or targeted to the lysosome or vacuole. Eukaryotes have developed highly complex RNA processing and multiple turnover pathways. In general, each type of RNA has a distinctive complement of components that accomplish and regulate its biosynthesis and turnover, though there are components that are adapted to act along the multiple pathways. Although the incorporation of ribonucleotides in place of deoxyribonucleotides within genomic DNA is rare, there are occasions when rNTPs can be linked to DNA chains. Ribonucleases H (RNases H) specifically hydrolyze the RNA moiety of RNA-DNA hybrids. In eukaryotes primary tRNAs, transcribed by RNA polymerase III, undergo extensive post-transcriptional modifications. Ribosomal RNAs (rRNAs) are transcribed as large precursors by RNA polymerases I and III and also undergo processing, including 5' and 3' cleavages, removal of introns, and nucleotide modifications through the action of a series of RNA/protein complexes. Pre-mRNA processing is a multi-step process coupled to transcription that involves RNA binding proteins, snRNAs (small nuclear RNAs), endo- and exo-nucleases, and other specific factors. In this paper overview of *Saccharomyces cerevisiae* RNases is given along with short description of their functions in RNA processing and turnover in the cell (Table 1).

RNase families

Ribonucleases (RNases) could be generally divided into the RNase A, RNAse T1 and RNase T2 families. The Ribonucleases from T2 (EC 3.1.27.1) family are found in all organisms so far examined (Deshpande and Shankar, 2002), while RNase T1 enzymes exist only in bacterial and fungal organisms, and RNase A family enzymes are highly represented in animals. The optimal pH of activity of many T2 ribonucleases is between 4 and 5, contrasting with the alkaline (pH 7–8) or weakly acidic (pH 6.5–7) activities of enzymes of the RNase T1 and RNase A families respectively (Deshpande and Shankar, 2002; Irie, 1999). Furthermore, T2 ribonucleases generally cleave at all four bases, whereas RNase A and RNase T1 family members tend to be specific for pyrimidines or guanosine, respectively (Deshpande and Shankar, 2002; Irie, 1999).

RNase A is small pancreatic ribonuclease, consist of 124 amino acid residues. RNase A contains 19 of the 20 natural amino acids, lacking only tryptophan. The predominant elements of secondary structure are a long four-stranded antiparallel β-sheet and three short α-helixes. The enzyme is cross-linked by four disulfide bonds, which involve all eight of its cysteine residues. RNase A catalyzes the cleavage of the P-O5' bond of RNA *via* 2',3'-cyclic phosphodiester intermediate and hydrolysis of this cyclic intermediate to form a 3'-phosphomonoester. The amino acid sequences of RNase A have been obtained from over 40 different vertebrates. RNase A homologues are pyrimidine base-specific RNases with a molecular mass of 13–14 kDa and have optimal pHs between 7–8. The RNase A family of RNases function mainly as intra and extracellular

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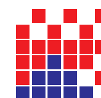
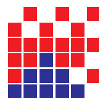
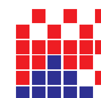


Table 1. *Owerviev of Saccharomyces cerevisiae RNases*

Ribonuclease	Gene(s)	Description	Function	Localizaton
Ribonuclease H1	*RNH1*	Binds double-stranded RNAs and RNA-DNA hybrids; associates with RNA polymerase I	Ribonuclease H1 removes a mismatch within an RNA-DNA heteroduplex region.	Nuclear
Ribonuclease H2	*RNH202*	Substrate-binding subunit	Ribonuclease H2 removes RNA primers during Okazaki fragment synthesis and errant ribonucleotides misincorporated during DNA replication.	Nuclear
	RNH203	Required for RNase H2 activity		
	RNH201	Catalytic subunit		
Nuclear RNase P	*RPR2*	Subunit of nuclear RNase P	Nuclear RNase P cleaves tRNA precursors to generate mature 5' ends and facilitates turnover of nuclear RNAs.	Nuclear
	POP1 *POP3* *POP4* *POP5* *POP6* *POP7* *POP8* *RPP1*	Subunits of both nuclear RNase P and RNase MRP		
	RPR1	Catalytic RNA component of nuclear RNase P, may be responsible for recognition of substrate tRNAs		
Mitochondrial RNase P	*RPM2*	Protein subunit of mitochondrial RNase P; has roles in nuclear transcription, cytoplasmic and mitochondrial RNA processing, and mitochondrial translation	RNase P removes 5' extensions from mitochondrial tRNA precursors.	Mitochondrial RNase P is localized in mitochondria, while Rpm2p is distributed to mitochondria, cytoplasmic processing bodies, and the nucleus.
	RPM1	RNA component of mitochondrial RNase P		
RNase MRP	*RMP1*	Catalytic subunit of RNase MRP	RNase MRP processes pre-rRNA and has a role in cell cycle-regulated degradation of daughter cell-specific mRNAs.	Nucleolus, mitochondria and TAM body.
	POP1 *POP3* *POP4* *POP5* *POP6* *POP7* *POP8* *RPP1*	Subunits of both nuclear RNase P and RNase MRP		
	SNM1	Subunit of RNase MRP		
	NME1	RNA component of RNase MRP		
Xrn1p	*XRN1*	Major cytoplasmic 5'-3' exoribonuclease	RNase Xrn1p is exoribonuclease involved in turnover of pre-rRNA and mRNA. Xrn1p also plays a role in microtubule-mediated processes, filamentous growth and telomere maintenance.	Cytoplasm
Rny1p	*RNY1*	Endoribonuclease of the T2 family	Rny1p cleaves tRNAs upon oxidative or stationary phase stress; promotes apoptosis under stress conditions and this function is independent of its catalytic activity.	Vacuoles, relocalizes to the cytosol upon oxidative or stationary phase stress.
Nuc1p	*NUC1*	Major mitochondrial exonuclease	Nuc1p has RNAse and DNA endo- and exonucleolytic activities; has roles in mitochondrial recombination, apoptosis and maintenance of polyploidy	Mitochondria



Ribonuclease	Gene(s)	Description	Function	Localizaton
Trz1p	*TRZ1*	Endonuclease, interacts genetically with Rex2 exonuclease.	Trz1p is involved in tRNA 3'-end processing	Mitochondria
Rex1p	*REX1*	3'-5' exoribonuclease	Rex1p is required for maturation of 3' ends of 5S rRNA and tRNA-Arg3 from dicistronic transcripts	Nucleus
Rex2p	*REX2*	3'-5' RNA exonuclease	Rex2p is involved in 3'-end processing of U4 and U5 snRNAs, 5S and 5.8S rRNAs, and RNase P and RNase MRP RNA.	Mitochondria
Rex3p	*REX3*	3'-5' RNA exonuclease	Rex3p is required for maturation of the RNA component of RNase MRP; functions redundantly with Rnh70p and Rex2p in processing of U5 snRNA and RNase P RNA.	Nucleus and cytoplasm
Rex4p	*REX4*	3'-5' RNA exonuclease	Rex4p is possibly involved in pre-rRNA processing and ribosome assembly.	Nucleus
Pan2p	*PAN2*	3'-5' RNA exonuclease, essential subunit of the Pan2p-Pan3p poly(A)-ribonuclease complex	Pan2p-Pan3p controls poly(A) tail length and regulate the stoichiometry and activity of postreplication repair complexes.	Cytoplasm
Pan3p	*PAN3*			
Cytoplasmic exosome complex	*DIS3*	Cytoplasmic and nuclear exosome core complex catalytic subunit; possesses both endonuclease and 3'-5' exonuclease activity.	Exosome is involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm. In the cytoplasm, the exosome catalyzes the 3'-5' turnover of mRNAs and participates in mRNA quality control pathways.	Cytoplasm
	CSL4 *RRP4* *RRP40*	RNA binding subunits		
	RRP41 *RRP42* *RRP43* *RRP45* *RRP46* *MTR3*	Cytoplasmic and nuclear exosome non-catalytic core components		
Nuclear exosome complex	*DIS3*	Cytoplasmic and nuclear exosome core complex catalytic subunit; possesses both endonuclease and 3'-5' exonuclease activity.	In the nucleus the exosome is responsible for the degradation of incorrectly processed pre-mRNA precursors, pre-mRNA turnover, as well as for the maturation of stable RNAs such as rRNA, snoRNA, and snRNA. Rrp6p also performs the final trimming step in the maturation of pre-5.8S rRNA and certain pre-snoRNAs that have already been processed by the core exosome	Nucleus
	RRP6	Nuclear exosome exonuclease component; possesses 3'-5' exonuclease activity.		
	CSL4 *RRP4* *RRP40*	Cytoplasmic and nuclear RNA binding subunits		
	RRP41 *RRP42* *RRP43* *RRP45* *RRP46* *MTR3*	Cytoplasmic and nuclear exosome non-catalytic core components		



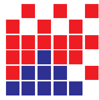
digestive enzymes. However, bovine and human angiogenins, members of the RNase A family, have been reported to accelerate angiogenesis related to metastasis of tumor cells.

Ribonuclease T1, (RNase T1) of the slime mould *Aspergillus oryzae* is the best known representative of a large family of homologues microbial ribonucleases with members in the prokaryotic and the eukaryotic world. The enzyme consists of a single polypeptide chain of 104 residues (molecular weight 11 kDa) and contains two disulfide bridges. RNase T1 is a stable acidic protein with pI 2.9; it is fairly resistant to heat, acids, and bases. It consists of a 4.5-turn *a* helix and two antiparallel β sheets, connected through a series of wide loops. The residues implicated in catalysis are placed in the major β sheet, while guanosine specificity is defined by residues in loop regions. RNase T1, cleaves the P-O5' ester bond of GpN sequences of single-stranded RNA by a transphosphorylation reaction yielding a 2',3'-cyclophosphate. In a second, separate step, this cyclic product is hydrolyzed to yield 3'- guanylic acid. All members of the RNase T1 family are guanyl specific or preferential, except RNase U_2. It is believed that the highly conserved region from Tyr_{42} to Glu_{46} is primarily responsible for guanine binding (Yoshida, 2001). Divalent cations Ag^{2+}, Zn^{2+}, Cu^{2+}, and Hg^{2+} at 1 x 10^{-3} M concentration are potent inhibitors, while histidine and EDTA acts as activators.

Ribonucleases of the T2 family are transferase type RNases and are classified by their similarity to the RNase T2 from *A. oryzae.* T2 ribonucleases have been suggested to perform a variety of functions in different organisms, including scavenging of nucleic acids, degradation of self-RNA, modulating host immune responses, and serving as extra- or intracellular cytotoxins. They catalyze the cleavage of single-stranded RNA through a 2',3'–cyclic phosphate intermediate, producing mono- or oligonucleotides with a terminal 3'-phosphate. The base specificity of the many enzymes in this group is purine-nucleotide preferential, especially adenylic acid, followed by guanylic acid, but there are also some guanylic acid preferential enzymes. Most of RNase T2 family members are acidic proteins with pI in the range of 4.0 to 6.0, consist of a single polypeptide chain with molecular weight in the range of 24 to 36 kDa, and are typically secreted from the cell, or localized to internal compartments such as the lysosome or vacuole. As these proteins enter the secretory pathway, they are generally glycosylated in eukaryotic cells. The pH optima of majority of T2 family RNases are in the range of 4.0 to 6.0, and in general, exhibit wide pH stability (from pH 3.0 to 11.0). The temperature optima of majority of T2 family RNases have not been reported, but in the case of some of the well-characterized enzymes they are in the range of 45^0C to 60^0C (Deshpande and Shankar, 2002). T2 family RNases are neither metalloenzymes nor metal-requiring enzymes, and they are not inhibited by metal chelators such as EDTA. However, divalent cations like Cu^{2+}, Zn^{2+}, and Hg^{2+} are potent inhibitors and in a few cases Mg^{2+}, Ca^{2+}, and Cd^{2+} were also found to be inhibitory (Deshpande and Shankar, 2002). Various mononucleotides, products and their analogs, inhibited competitively RNase T2 from *A. oryzae*, RNase Rh from *Rhizopus niveus*, and RNase M from *Aspergillus saitoi* (Uchida and Egami, 1971; Komiyama and Irie, 1972). The structure and mechanism of RNA cleavage by T2 ribonucleases is well understood. A variety of crystal structures of T2 family members from bacteria, plants, and fungi have revealed a conserved α/β core structure. Catalysis is promoted by one to three histidine residues (His_{46}, His_{104}, His_{109}) and Glu_{105} (RNase Rh numbering) that are found in two blocks of conserved amino acids (designated CASI and CASII) located in the active site of the enzyme (Irie, 1999). In general, fungal RNases have 10, bacterial RNases 6, while animal and plant RNases have 8 cysteine residues. The 4 cysteine residues forming disulfide bonds, Cys_8-Cys_9 and Cys_{11}- Cys_{14} are common to all RNases of T2 family and hence might have a fundamental importance for maintaining the active conformation (Irie, 1999). RNA cleavage by T2 enzymes occurs in two steps: transphosphorylation and hydrolysis. The participation of Glu residues in enzymatic activities of fungal RNases was at first revealed by analysis of the pH profiles of the kinetic parameters, and the participation of two His residues by chemical modification of RNases with iodoacetate at pH 5.0 (Irie *et al.*, 1986; Kawata *et al.*, 1991). Two histidines are required to perform acid-base catalysis at each of these steps, with these histidines reversing roles as acid and base at each step. Incorporation of a carboxymethyl group into either one of these His residues inactivates the enzyme. The majority of RNases in the RNase T2 family contain 5–7 tryptophan residues, and at least one of those is essential for enzyme activity as it was shown by experiment in which the tryptophan residues were oxidized by N-bromosuccinimide at pH 2.0 with concomitant loss of enzymatic activity (Ohgi and Irie, 1977). Later the protein engineering studies done by Ohgi *et al.* (1991, 1992, 1993,1995, 1996) in which the cDNA of RNase Rh was cloned and expressed in yeast cells confirmed those earlier results. In this experiments several site-directed mutants of the amino acid residues presumed to constitute the active site were introduced (Phe, three His residues, several Lys residues, Asp_{51} and Glu_{105}). Results showed that the mutants of three His residues (His_{46}, His_{104}, and His_{109}) were virtually inactive, but the degree of inactivation was more marked for His_{46} and His_{109} and it was tentatively concluded that His_{46} and His_{109} are general acid / base catalysts at the first stage of the reaction (Ohgi *et al.*, 1992). The Glu_{105} mutant was markedly inactivated, while the Asp_{51} mutant was fairly active, thus Glu_{105} was thought to be involved in catalysis. Glu_{105} is catalytically crucial and probably operates to polarize the P=O bond or to stabilize a pentacovalent intermediate (Ohgi *et al.*, 1993).

Hydrolysis of the RNA of RNA-DNA hybrids

Ribonucleases H (RNases H) specifically hydrolyze the RNA moiety of RNA-DNA hybrids and are present in all living organisms. Although the incorporation of ribonucleotides (rNTPs) in place of deoxyribonucleotides (dNTPs) within genomic DNA is generally avoided by the high selectivity of DNA polymerases, there are occasions when rNTPs can be linked to DNA chains, such as during the synthesis of Okazaki fragments or possibly during repair of double strand DNA breaks in G1 (Nick McElhinny and Ramsden, 2003; Zhu and Shuman, 2008). Recent work indicates that during normal DNA replication, DNA polymerases can also incorporate rNTPs in place of dNTPs (Nick McElhinny *et al*., 2010). rNMPs embedded in DNA sensitizing the DNA backbone to spontaneous and/or enzymatic nicking, so rNMPs must be removed prior to the next cell cycle. A recent study has shown that single rNMPs



in DNA templates impede DNA synthesis by the yeast replicases (Watt *et al*., 2011). Most organisms have more than one type of RNases H and they are classified into two distinct groups, RNase H1 and RNase H2, based on amino acid sequence similarity (Ohtani *et al*., 1999). *Saccharomyces cerevisiae* Ribonuclease H1, coded by *RNH1* binds to double-stranded RNAs and RNA-DNA hybrids and associates with RNA-polymerase I (Huet *et al*., 1977). *Saccharomyces cerevisiae* RNase H2 comprises three polypeptides coded by *RNH201* (catalytic subunit), *RNH202* and *RNH203* (Jeong *et al*., 2004). The loss of any of the three subunits of RNase H2 results in the absence of RNase H2 activity (Jeong *et al*., 2004). Rnh202p and Rnh203p first form a subcomplex, followed by the recruitment of Rnh201p to complete complex formation. Rnh201p alone or in combination with Rnh203p showed neither substrate-binding, nor catalytic activity, indicating that both activities of Rnh201p are latent until it becomes an integral part of the complex. However, Rnh202p by itself showed substrate-binding activity (Nguyen *et al*., 2011). There is a wide variation in specific activity, divalent metal ion preference and sites at which the enzymes are capable of cleaving RNA-DNA hybrids (Ohtani *et al*., 1999.). RNase H1 and RNase H2 activities have partially overlapping substrate specificity but while RNase H1 requires at least four rNMPs to cleave, RNase H2 can incise 5′ to a single rNMP incorporated within a DNA molecule (Cerritelli and Crouch, 2009). RNase H1 is the preferential enzyme for processing RNA-DNA hybrids where more than four rNMPs are present and plays a crucial role in the repair of rNMPs incorporated by replicative DNA polymerases (Lazzaro *et al.,* 2011). Mispaired rNMPs in chromosomal DNA are removed by the mismatch repair system in competition with RNase H2. However, a mismatch within an RNA-DNA heteroduplex region requires RNase H type 1 for removal (Shen *et al*., 2012). RNase H2 removes RNA primers during Okazaki fragment synthesis.

tRNA, rRNA and mRNA processing in yeast

In eukaryotes primary tRNAs, transcribed by RNA polymerase III, undergo extensive post-transcriptional modifications including trimming of the 5′ leader and 3′ trailer sequences, the addition of the CCA sequence to the 3′ terminus, nucleotide modification and, in the case of about 20% of yeast tRNA, intron removal in the nucleus before export to the cytosol (Hopper and Phizicky, 2003). It is known that there are more than 55 proteins involved in tRNA splicing, modification, degradation and export. Most tRNA modifications are introduced in the tRNA precursor form (pre-tRNA) prior to export to the cytoplasm. However, in *S. cerevisiae*, the tRNA-splicing endonuclease is associated with the mitochondrial outer membrane (Yoshihisa *et al.*, 2003) and some of the RNA-modifying enzymes are located in the cytoplasm (Hopper and Phizicky, 2003). Thus, the intron-bearing pre-tRNAs must be transported into the cytoplasm to continue maturation. Finally, cytoplasmic mature tRNAs are imported back into the nucleus (Shaheen and Hopper, 2005) probably for the quality check of the mature tRNAs.

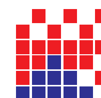
Ribosomal RNAs (rRNAs) are transcribed as large precursors by RNA polymerases I and III in nucleoli (Perry, 1981). These RNAs also undergo processing, including 5' and 3' cleavages, removal of introns, and nucleotide modifications through the action of a series of RNA/protein complexes. The mature rRNAs complex with about 80 proteins to form ribosomes. Many of the modifications and splicing events require small nucleolar RNAs (snoRNAs) and specific protein factors. Notably, some proteins are shared between the tRNA and rRNA maturation complexes (Kressler *et al.*, 1999). The RNase MRP (ribonuclease MRP) complex is responsible for the majority of 5' maturation steps and the exosome complex is responsible for 3' rRNA maturation.

Pre-mRNA processing is a multi-step process coupled to transcription through the involvement of the unique C-terminal domain (CTD) of the large subunit of RNA polymerase II. The processing involves RNA binding proteins, snRNAs (small nuclear RNAs), endo- and exo-nucleases, and other specific factors. Eukaryotic mRNAs are primarily degraded by removal of the 3' poly(A) tail, followed either by cleavage of the 5' cap structure (decapping) and 5'-3' exonucleolytic digestion, or by 3' to 5' degradation (Coller and Parker, 2004).

YEAST RNases INCLUDED IN 5' PROCESSING OF RNA

Nuclear RNase P

Nuclear RNase P is responsible for 5' tRNA processing (Xiao *et al.,* 2001). Nuclear Ribonuclease P (RNase P) is an essential endoribonuclease that is localized in the yeast nucleolus (Schmitt and Clayton, 1993; Bertrand *et al*., 1998) and catalyzes the cleavage of the 5′ leader of pre-tRNAs (Xiao *et al*., 2001). In *S. cerevisiae* nuclear RNase P is composed of ten subunits: a single RNA unit coded by *RPR1* (Lee *et al*., 1991) and nine essential proteins range in size from 15.5 to 100 kDa coded by *POP1, POP3, POP4, POP5, POP6, POP7, POP8, RPP1* and *RPR2* (Chamberlain *et al.*, 1998; Lygerou *et al.*, 1994; Dichtl and Tollervey, 1997; Stolc and Altman, 1997; Chu *et al.*, 1997; Stolc *et al*., 1998). The spatial organization of these components within the enzyme is not yet fully understood. Houser-Scott *et al.* (2002) showed that Pop1p and Pop4p specifically bind the RNA subunit Rpr1r. Pop4p also interacted with seven of the other eight protein subunits. The remaining protein subunits all showed one or more specific protein–protein interactions with the other integral protein subunits. Rpr2p, the only protein subunit found in RNase P but not in the closely related enzyme RNase MRP, interacts strongly with itself as well as with Pop4p. RNA component of nuclear RNase P Rpr1r have catalytic role and may be responsible for recognition of substrate tRNAs (Srisawat *et al*., 2002). After a mutation in the catalytic RNA subunit of nuclear RNase P in *S. cerevisiae* a wide variety of noncoding RNAs were shown to accumulate, suggesting that nuclear RNase P participates in the turnover of normally unstable nuclear RNAs (Marvin *et al.*, 2011). Pre-mRNAs containing introns also accumulated broadly, consistent with either compromised splicing or failure to efficiently turn over pre-mRNAs that do not enter the splicing pathway. Taken together with the high complexity of the nuclear RNase P holoenzyme and its relatively nonspecific capacity to bind and cleave mixed sequence RNAs, these data suggest that nuclear RNase P facilitates turnover of nuclear RNAs in addition to its role in pre-tRNA biogenesis (Marvin *et al.*, 2011).



Mitochondrial RNase P

In the yeast *S. cerevisiae* mitochondrial RNase P is coded in both mitochondrial and nuclear genomes. Mitochondrial DNA (mtDNA) codes for Rpm1r, the RNA subunit of mitochondrial RNase P, and nuclear DNA codes for the protein subunit, Rpm2p (Hollingsworth and Martin, 1986). *RPM1* encodes an RNA of about 450 nucleotides, also known as the 9S RNA (Miller and Martin, 1983), which removes the 5' leaders from mitochondrial tRNA precursors (Hollingsworth and Martin, 1987). *RPM1* is conserved in the mitochondrial genomes of other yeasts, but its size varies widely. Some homologs are only about 140 nucleotides in length, suggesting that *S. cerevisiae RPM1* may contain nonessential regions (Wise and Martin, 1991). That observation is supported with finding that *RPM1* RNA becomes fragmented during purification of RNase P from mitochondria, but the enzyme containing RNA fragments remains active (Morales *et al.*, 1989).

RPM2 is a nuclear gene encoding a multifunctional protein that can localize to both mitochondria, cytoplasmic processing bodies, and the nucleus. Rpm2p has roles in nuclear transcription, cytoplasmic and mitochondrial RNA processing, and mitochondrial translation. Rpm2p is required for the tRNA processing activity of the mitochondrial RNase P, and is also required for maturation of the *RPM1* RNA, which does not have the normal 5' or 3' ends in a strain carrying a C-terminally truncated variant of Rpm2p (Stribinskis *et al*., 2001). Independent of RNase P activity, Rpm2p has a role in translation of mitochondrially encoded cytochrome c oxidase subunits Cox1p, Cox2p and Cox3p (Stribinskis *et al*., 2001a) and acts as transcriptional activator in the nucleus when not targeted to the mitochondria (Kassenbrock *et al*., 1995).

RNase MRP

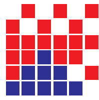
In the yeast *S. cerevisiae*, RNase MRP is an essential endoribonuclease that consists of one RNA unit coded by *NME1* (Schmitt and Clayton, 1992; Chu *et al*., 1994) and ten essential proteins coded by *POP1, POP3, POP4, POP5, POP6, POP7, POP8, RPP1, SNM1* and *RMP1* (Chamberlain *et al.,*1998; Dichtl and Tollervey, 1997; Stolc and Altman, 1997; Chu *et al.*, 1997; Stolc *et al*., 1998; Schmitt and Clayton, 1994; Salinas *et al.*, 2005). RNase MRP is highly conserved throughout evolution and, although orthologs of the Snm1p subunit have not been identified outside of the fungi, the *NME1* RNA has both sequence and structural similarity to the RNA components of RNases P and MRP from a variety of organisms, while orthologs of the Rmp1p subunit have been identified in fungi and *Arabidopsis* (Rosenblad *et al*., 2006). RNase MRP participates in the major pre-rRNA maturation pathway (Chamberlain *et al.,*1998; Dichtl and Tollervey, 1997; Stolc and Altman, 1997; Chu *et al.*, 1997; Schmitt and Clayton, 1992; Schmitt and Clayton, 1993; Chu *et al.*, 1994), processes mitochondrial RNA primers, which serve as primers for the leading strand of mitochondrial DNA replication (Chang and Clayton 1987a; Lee and Clayton, 1998), and has a role in cell cycle-regulated degradation of daughter cell-specific mRNAs (Gill *et al.*, 2006; Cai *et al*., 2002,_Gill *et al.,* 2004). Rmp1p subunit of RNase MRP is essential for the rRNA processing activity of the complex, and essential for viability (Salinas *et al*., 2005). Unlike most subunits, it is not shared between RNase MRP and nuclear RNase P. A temperature-sensitive *rmp1* mutant was generated and found to exhibit rRNA processing defect identical to that seen in other RNase MRP mutants, whereas no defect in tRNA processing was observed (Salinas *et al*., 2005). More recent research has uncovered a novel function for RNase MRP in cell cycle regulation (Cai *et al*., 2002). Mutations in several RNase MRP components cause a cell cycle delay in late mitosis and the accumulation of cells in telophase, with large buds and dumbbell-shaped nuclei (Cai *et al*., 2002). Analyses determined that RNase MRP directly cleaves the 5′-untranslated region (UTR) of the yeast B-type cyclin, *CLB2* mRNA, allowing for rapid degradation by the 5′-3′ exoribonuclease Xrn1p, which must occur in order for mitosis to be completed (Gill *et al*., 2004). Cleavage of the 5′-UTR of *CLB2* mRNA and subsequent degradation by Xrn1p is a unique mode of mRNA turnover in *S. cerevisiae* (Gill *et al*., 2006). It is thought that RNase MRP is predominantly localized to the nucleolus (Schmitt and Clayton, 1993; Bertrand *et al.*, 1998). However, degradation of *CLB2* mRNA is presumed to occur in the cytoplasm, and fraction of MRP RNase is localized to the mitochondrion (Chang and Clayton, 1987; Topper *et al.*, 1992). Gill *et al.* (2006) find that MRP RNase localization is cell cycle controlled, exiting the nucleolus during mitosis and localizing to a single cytoplasmic foci in daughter cells, called the TAM body (temporal asymmetric MRP body) that is present in a single copy in the cytoplasm of daughter cells. They also proposed that in these foci the *CLB2* mRNA is being degraded. Asymmetric localization of the TAM body to daughter cells is dependent on the locasome, which is a protein-mRNA complex that transports at least 30 different mRNAs specifically to daughter cells (Gill *et al.*, 2006). This degradation of cell cycle-regulated, daughter cell-specific mRNAs is required for completion of mitosis (Gill *et al.*, 2006; Cai et al., 2002, Gill *et al.,* 2004).

RNase Xrn1p

Xrn1p is the major cytoplasmic 5'-3' exoribonuclease involved in RNA turnover (Caponigro and Parker, 1996; Heyer *et al.*, 1995). After decapping of mRNA, 5' to 3' mRNA degradation is accomplished by Xrn1. The 5'-3' exonuclease activity of Xrn1p is capable of degrading single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) as well (Johnson and Kolodner, 1991). Mutations in *XRN1* are viable and result in defects in the turnover of pre-rRNA (Henry *et al.*, 1994; Stevens *et al.*, 1991) and mRNA (Caponigro and Parker, 1996). Xrn1p also plays a role in microtubule-mediated processes (Kim *et al.*, 1990), filamentous growth (Kim and Kim, 2002) and telomere maintenance (Askree *et al.,* 2004).

Endoribonuclease Rny1p

Rny1p is nonspecific endoribonuclease and the only member of the T2 RNase family present in *S. cerevisiae*. Rny1 is a 48-kDa protein with an amino acid sequence containing the two active-site motifs characteristic for the T2 RNase family. The N-terminal 293 amino acids of the Rny1p can be aligned to the sequence of T2 RNase from *Aspergillus oryzae*, which is the archetypal enzyme in this family (Irie, 1997). In addition, amino acids 294–434 of the Rny1p form a C-terminal extension that is not found in other T2 RNase family members. Enzyme



has four potential glycosylation sites and a putative secretion signal of 18 amino acids at the N terminus. MacIntosh *et al.* (2000) showed that Rny1 is an active, secreted RNase and that *RNY1* expression is rapidly regulated in response to certain environmental and stress conditions. They predicted that Rny1p may regulate membrane permeability or stability. Cells that lack Rny1 activity (*rny1*) are larger than wild-type cells and have impaired growth at 37°C (MacIntosh *et al.*, 2000). During oxidative stress cytosolic RNAs are cleaved by release of the Rny1p from the vacuole into the cytosol. Rny1p also modulates cell survival during oxidative stress, but this effect is independent of its catalytic ability. These observations argue that release of Rny1p from the vacuole both promotes tRNA cleavage and separately activates a downstream pathway that promotes cell death (Thompson and Parker, 2009).

Exonuclease Nuc1p

In yeast more than 50% of all cellular nuclease activity is due to a Nuc1p, a single polypeptide bound to the mitochondrial inner membrane with an apparent molecular weight of 38 kDa. This enzyme possesses both RNase and DNase 5' exonuclease activity (Vincent *et al.*, 1988; Dake *et al.,* 1988). Nuc1p can efficiently trigger apoptotic cell death when excluded from mitochondria independently of metacaspase or of apoptosis inducing factor. Deletion of *NUC1* diminishes apoptotic death when mitochondrial respiration is increased but enhances necrotic death when oxidative phosphorylation is repressed, pointing to dual, lethal and vital, roles for Nuc1p (Büttner *et al.,* 2007).

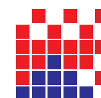
YEAST RNases INCLUDED IN 3' PROCESSING OF RNA

RNase Trz1p

Two proteins, Trz1p (Takaku *et al.*, 2003) and Lhp1p (Yoo and Wolin, 1997) have been shown to be involved in 3' tRNA maturation in yeast. Lhp1p stabilizes pre-tRNAs as an RNA chaperone, while Trz1p has tRNase Z activity. Trz1p (tRNase Z1), coded by *TRZ1*, is an essential protein of 838 amino acids that can be divided into amino- and carboxy- domains, which are structurally homologous. The carboxydomain possesses tRNase Z activity, while the amino domain is enzymatically inactive (since most of the key zinc coordinating histidines have changed to some other polar amino acid) but may modulate the specificity of carboxy domain. Although Trz1p is required for growth, yeast is quite insensitive to the absolute level of its expression. *TRZ1* genetically interacts with *REX2*, suggesting a role for Trz1p in RNA processing and mitochondrial maintenance (Chen *et al.*, 2005).

RNases of exosome complex

In the 3' to 5' pathway, RNA degradation is catalyzed by a multisubunit 3' to 5' exoribonuclease and endoribonuclease complex termed the RNA exosome (Mitchell *et al.*, 1997; Hilleren and Parker, 1999; Wang and Kiledjian, 2001). The exosome also participates in RNA processing of small nucleolar and small nuclear RNAs (snoRNAs, snRNAs) and ribosomal RNAs (rRNAs) (Allmang *et al.*, 1999; van Hoof *et al.*, 2000). The nine-subunit yeast exosome core consists of Rrp40p, Rrp41p/Ski6p, Rrp45p, Mtr3p, Rrp42p, Rrp46p, Rrp43p, Rrp4p, and Csl4p. Six exosome subunits, Rrp41p/Ski6p, Rrp42p, Rrp43p, Rrp45p, Rrp46p, and Mtr3p, share 20%–30% sequence identity to *E. coli* RNase PH and PNPase PH domains, two phosphorolytic 3'-5' exoribonucleases that activate inorganic phosphate as a nucleophile to release ribonucleoside 5' diphosphate products. Three additional exosome subunits, Csl4p, Rrp4p, and Rrp40p are postulated to bind RNA. The tenth yeast exosome subunit is Dis3p/Rrp44p, the catalytic subunit of the core exosome complex, which possesses both 3'-5' exonuclease activity and endoribonuclease activity (Dziembowski *et al.,* 2007). In addition to its nuclease activity, Dis3p/Rrp44p is required for recognition of certain exosome substrates (Schneider *et al.*, 2007). Dis3p/Rrp44p shares sequence similarity to *E. coli* RNase R and RNase II (Mitchell *et al*., 1997; Cheng and Deutscher, 2005) and associates with the exosome through its N-terminus, which mediates interactions with other exosome subunits such as Rrp45p, Rrp43p, and Ski6p (Schneider *et al.,* 2009). The 10-subunit core exosome complex (Csl4p, Rrp4p, Rrp40p, Ski6p, Rrp42p, Rrp43p, Rrp45p, Rrp46p, Mtr3p, Dis3p) is the same in nuclear and cytoplasmic exosome, but the nuclear exosome contains an additional subunit Rrp6p (Liu *et al.*, 2006) and two additional accessory factors Lrp1p and Mpp6p (Synowsky *et al.*, 2009). Rrp6 is a metal-dependent exoribonuclease with distributive hydrolytic activity, similar to bacterial RNase D. In addition to its involvement in processes mediated by the nuclear exosome, Rrp6p also performs the final trimming step in the maturation of pre-5.8S rRNA and certain pre-snoRNAs that have already been processed by the core exosome (Briggs *et al.*, 1998). In the cytoplasm, the exosome catalyzes the 3'-5' turnover of mRNAs and participates in mRNA quality control pathways (Schaeffer *et al.*, 2008). In the nucleus the exosome is responsible for the degradation of incorrectly processed pre-mRNA precursors (LaCava *et al.*, 2005), pre-mRNA turnover (Bousquet-Antonelli *et al.*, 2000), as well as for the maturation of stable RNAs such as rRNA, snoRNA, and snRNA (Allmang *et al.,* 2000; Vanacova and Stefl, 2007). Exosome activities are presumably regulated through association with complexes such as the Ski7p and Ski2p/Ski3p/Ski8p complex (Jacobs Anderson and Parker, 1998) and TRAMP (the Trf4p/Air2p/Mtr4p polyadenylation complex containing RNA helicase Mtr4p; a poly(A) polymerase, Trf4p; and a zinc knuckle protein, Air2p), a multisubunit polymerase complex that primes structured RNA for degradation (LaCava *et al*., 2005; Vanacova *et al*., 2005; Wyers *et al*., 2005). The nuclear exosome is associated with the TRAMP complex, and the cytoplasmic exosome is associated with Ski7p and the Ski2p/Ski3p/Ski8p complex. Ski2p/Ski3p/Ski8p complex may act to recruit the exosome to mRNA or act on the mRNP to allow access by the exosome (Jacobs Anderson and Parker, 1998) and Ski7p is coupling protein that mediates interactions between the Ski complex and the cytoplasmic exosome during 3'-5' RNA degradation. *In vivo* analyses of yeast strains defective in various combinations of TRAMP components suggest that TRAMP recognizes a variety of nuclear transcripts produced by RNA polymerase I, RNA polymerase II, and RNA polymerase III (LaCava *et al*., 2005; Wyers *et al*., 2005). Furthermore, these analyses show that TRAMP contributes little to exosome mediated RNA



processing but rather serves as an activating cofactor for the nuclear exosome during RNA decay.

RNase D family

A second class of interesting 3' exonucleases consists of five related yeast proteins that are part of a large family of 3' exoribonucleases - RNase D family. One of these five yeast proteins, Pan2p, has been shown to play a role in initial shortening of the poly(A) tails of mRNA (Brown and Sachs, 1998). Other four proteins are Rex1p, Rex2p, Rex3p and Rex4p. Rex1p has previously been identified as Rnh70p, a 70 kDa protein that copurifies with RNase H activity. However, Rex1p/Rnh70p does not show any sequence similarity to known RNase H proteins and *rex1/rnh70* mutants do not show reduced RNase H activity, nor the expected phenotype for an RNase H mutant (Frank *et al.*, 1999; Qiu *et al.*, 1999). Each of these exonucleases is required for the processing of distinct RNAs. Rex1p is required for 5S rRNA and tRNA-Arg3 trimming, Rex2p is required for U4 snRNA trimming and Rex3p is required for trimming of the RNA subunit of RNase MRP. Rex1p and Rex2p function redundantly in 5.8S rRNA maturation, Rex1p, Rex2p and Rex3p are redundant for the processing of U5 snRNA and RNase P RNA, and Rex1p and the exonuclease Rrp6p have an unknown redundant essential function (van Hoof *et al*., 2000).

Conclusions

Saccharomyces cerevisiae has developed highly complex RNA processing and turnover pathways, and each type of RNA has a distinctive complement of components that accomplish and regulate its biosynthesis and turnover, although there are components that are adapted to act along the multiple pathways. The bulk of RNA turnover takes place in the nucleus, although in the *S. cerevisiae* only 4% of all genes produce intron-containing transcripts. Precursors to rRNAs, tRNAs, sno- and snRNAs are all processed into smaller species in the nucleus and the trimmings are recycled. In addition, all nuclear ribonucleoproteins (RNPs) are subject to quality control, with the extent of turnover depending on the condition of the cell. Some tRNA and rRNA precursors, as well as ribonucleotide subunits of some enzymes are processed by mitochondrial RNases, while mRNA that have done their translational duty are degraded in the cytoplasm. In this paper present knowledge about *S. cerevisiae* RNases is presented, including genetic data, physiological functions and their localization in the cell.

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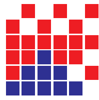

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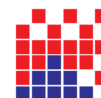

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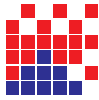

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