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Chapter 3
Fungal Pre-mRNA 3′-End Processing

Aurelia Vavasseur and Yongsheng Shi

Abstract 3′ end processing of messenger RNAs (mRNAs) is not only an essential step in eukaryotic gene expression, but it also impacts many other aspects of mRNA maturation and decay. A large portion of eukaryotic genes produce multiple mRNAs with different 3′ ends through alternative cleavage/polyadenylation (APA). mRNA 3′ processing and especially APA has been increasingly recognized as an important mechanism for gene regulation. Much of what we currently know about eukaryotic mRNA 3′ processing came from studies using the genetically tractable yeast systems. Here we review the fungal mRNA 3′ processing system by describing both the evolutionarily conserved mechanisms as well as the fungus-specific features.

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Pre-mRNA 3′-End Processing: An Overview

The vast majority of eukaryotic mRNAs have a polyadenosine (poly(A)) tail at their 3′ ends (Chan et al. 2011; Zhao et al. 1999; Colgan and Manley 1997; Proudfoot 2011). The poly(A) sequences are not encoded in the genome, but are added posttranscriptionally through two chemical reactions, an endonucleolytic cleavage and the addition of a string of adenosines by the poly(A) polymerase (PAP). The poly(A) tails are critical for mRNA export, stability, and translation (Zhao et al. 1999; Colgan and Manley 1997; Chan et al. 2011; Proudfoot 2011). The 3′ end formation process itself is required for transcription termination and it significantly impacts other mRNA processing steps, including splicing (Kim et al. 2004; West et al. 2004; Connelly and Manley 1988; see Chap. 2 for more details). Mutations that disrupt the mRNA 3′ processing of critical genes and mutations in mRNA 3′ processing factors cause a number of diseases, including thalassemias, thrombophilia, and oculopharyngeal muscular dystrophy (Danckwardt et al. 2008; Chan et al. 2011).

Pre-mRNA 3′ end formation involves the assembly of several multisubunit complexes on specific cis-element that defines the polyadenylation site (PAS) (Chan et al. 2011; Shi et al. 2009; Skolnik-David et al. 1987; Humphrey et al. 1987). The majority of the 3′ processing factors are conserved throughout eukaryotic evolution (Darmon and Lutz 2012; Mandel et al. 2008). Interestingly, however, there are also a number of lineage-specific essential 3′ processing factors (Zhao et al. 1999). The loss and/or gain of these factors during evolution might be correlated with changes in the cis-element of PAS as described below.

APA is the phenomenon in which a gene can produce multiple mRNA isoforms with distinct 3′ ends through using alternative PAS (Shi 2012; Di Giammartino et al. 2011; Tian and Manley 2013; Elkon et al. 2013). It is estimated that over half of the eukaryotic genes produce alternatively polyadenylated transcripts (Shi 2012). Unlike alternative splicing, which has expanded dramatically during eukaryotic evolution, the prevalence of APA in yeast is comparable to that in metazoans (Ozsolak et al. 2010; Derti et al. 2012). APA isoforms may encode different proteins and/or have different 3′ untranslated regions (UTRs). In yeast, many APA events involve PAS found within the coding sequences (CDS) as well (Sparks and Dieckmann 1998; Yoon and Brem 2010; Mayer and Dieckmann 1989, 1991).

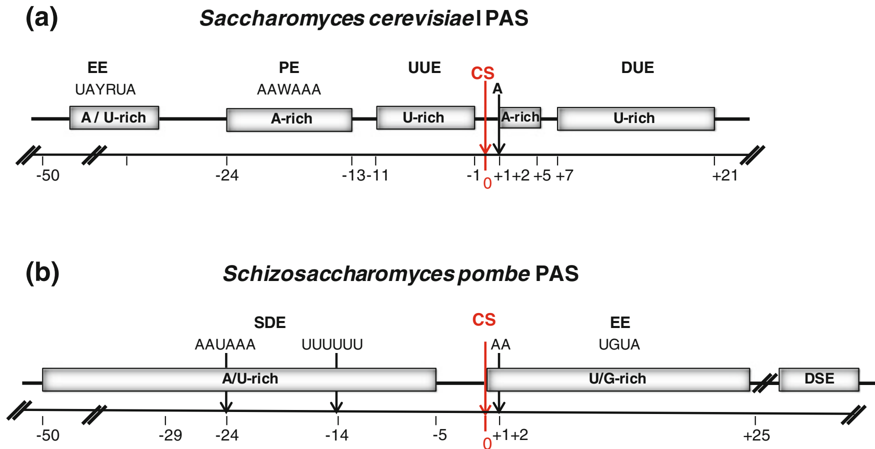

Fig. 3.1 Key cis-element for yeast poly(A) sites. The names, positions, consensus sequences of the known cis-element in *S. cerevisiae* (**a**) and *S. pombe* (**b**) PAS. *EE* Efficiency element, *PE* Positioning element, *UUE* Upstream U-rich element, *DUE* Downstream U-rich element, *SDE* Site determining element, *DSE* Downstream sequence element. See details in the text

Such APA isoforms are predicted to produce either truncated proteins or no protein product. Thus, APA significantly expands the proteome diversity and mRNA regulatory potential. The global APA profile is highly dynamic and regulated during development and in response to environmental cues (Shepard et al. 2011; Sandberg et al. 2008; Flavell et al. 2008; Graber et al. 2013). Aberrant APA regulation has also been implicated in a number of diseases, including cancer (Mayr and Bartel 2009; Jenal et al. 2012; Shi 2012; Di Giammartino et al. 2011).

The 3′ ends of mRNAs can be further processed in the cytoplasm (Richter 1999). For example, poly(A) tails can be extended or trimmed (Richter 1999). Other nucleotides, such as uracil, can be added (Rissland et al. 2007). These modifications play important roles in regulating the stabilities of target mRNAs (Scott and Norbury 2013). But for this article, we will focus on the 3′ end processing in the nucleus.

Fungal Poly(A) Site Sequence Features

Single gene studies and global analyses of *Saccharomyces cerevisiae* PAS have identified five key sequence elements (Fig. 3.1): (1) the A/U-rich Efficiency Element (EE) located at variable positions upstream of the cleavage site (CS) with the nucleotide consensus sequence UAYRUA (with Y: pyrimidine, and R: purine); (2) the A-rich Positioning Element (PE) located 10–30 nucleotides (nt) upstream of the CS. AAWAAA (W: A or U) is one of the most frequently found motifs in PE;

(3) the Upstream U-rich Element (UUE); (4) the CS; and (5) the Downstream U-rich Element (DUE) (Zhao et al. 1999). Recently, a short A-rich region from +2 to +5 nt (relative to the CS) has been suggested to influence the strength of the PAS (Fig. 3.1) (Moqtaderi et al. 2013). Functionally, the PE is most closely related to the AAUAAA hexamer in mammalian PAS (Zhao et al. 1999).

The *Schizosaccharomyces pombe* PAS have also been studied in some detail (Fig. 3.1) (Hansen et al. 1998; Birse et al. 1997; Humphrey et al. 1994). *S. pombe* PAS have an A-rich upstream sequence (called Site Determining Element (SDE)), equivalent of the aforementioned PE in *S. cerevisiae* PAS, and a UG-rich downstream element called Efficiency Element (EE) (Hansen et al. 1998; Birse et al. 1997; Humphrey et al. 1994). Genome-wide analyses suggest that AAUAAA is present in about 20 % of SDEs and UGUA is found in 24 % of EE in *S. pombe* PAS (Mata 2013; Schlackow et al. 2013; Ozsolak et al. 2010). Based on the high frequency of AAUAAA in SDEs, it has been suggested that *S. pombe* PAS are more similar to mammalian PAS (Humphrey et al. 1994; Chakraborty et al. 2002; Schlackow et al. 2013; Mata 2013). The distance between SDE and EE influences the efficiency of 3′ end formation, and the EE was proposed to enhance the binding of specific factors to SDE (Humphrey et al. 1994). An additional element located further downstream of the PAS, called the downstream element (DSE), also plays an important role in the transcription termination, most likely by inducing RNA pol II pausing (Birse et al. 1997; Hansen et al. 1998).

The specificity and activity of mRNA 3′ processing machineries in different yeast species seem quite similar. For example, *S. cerevisiae* PAS can be correctly processed in *S. pombe* and vice versa (Humphrey et al. 1991). A global comparison of mRNA polyadenylation in three yeast species, *S. cerevisiae*, *Kluyveromyces lactis*, and *Debaryomyces hansenii*, revealed several similarities (Moqtaderi et al. 2013). First, PAS in all these species share highly similar nucleotide composition and motifs (Fig. 3.1). Second, the position of the CS is highly heterogeneous. There are on average over 60 distinct CS located with a ~200 nt “end zone” for each PAS. In contrast, CS in mammalian PAS tend to cluster within 40 nt (Lee et al. 2007). Thus, the high heterogeneity of CS may be a widespread feature of fungal mRNA 3′ processing. Third, secondary structures may play an important role in determining PAS strength. RNA folding analyses predicted that the predominant PAS in these yeast species adopt a common configuration characterized by a double-stranded stem with the CS adjacent to a single-stranded domain. The weaker PAS seem less associated with such structures (Moqtaderi et al. 2013). On the other hand, species-specific differences in mRNA 3′ processing machinery also exist. For example, when large chromosomal fragments from *Debaryomyces hansenii* were introduced into *S. cerevisiae*, the polyadenylation within this region, especially the distribution of the CS within the end zone, adopted a pattern similar to that of the host strain. This observation suggests that the mRNA 3′ processing factors contribute to the species-specific polyadenylation profiles (Moqtaderi et al. 2013).

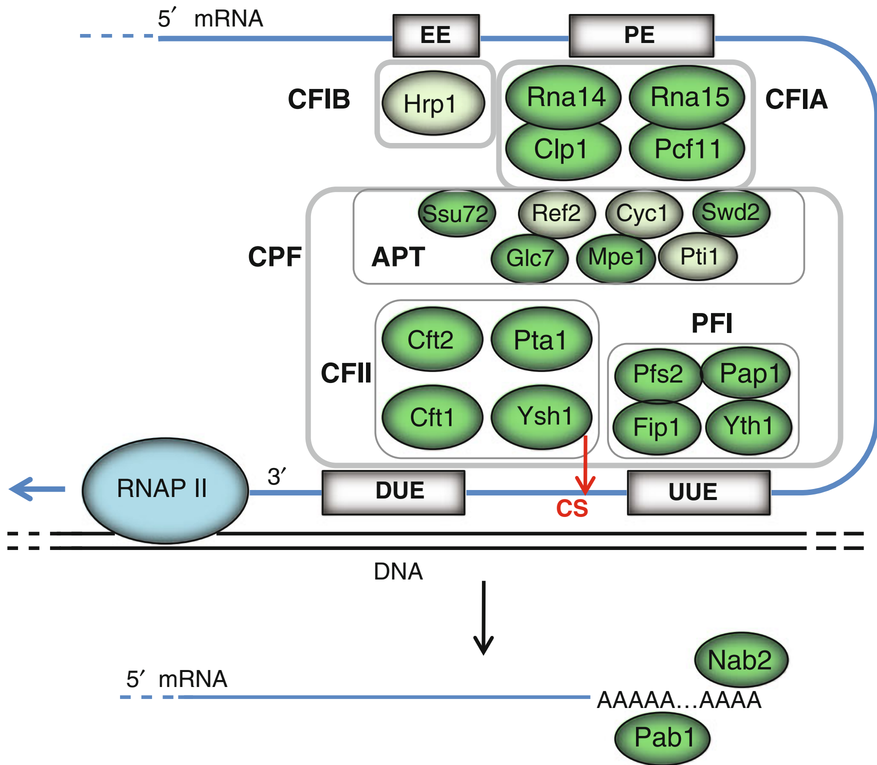

Fig. 3.2 The mRNA 3′ processing machinery in *Saccharomyces cerevisiae*. DNA templates are shown as *black lines* and RNA as a *blue line*. The mRNA 3′ processing factors conserved between yeast and mammals are marked as *dark green circles*. Subunits of complexes and subcomplexes are enclosed in *gray circles*

Fungal mRNA 3′ Processing Factors

There are over 20 known mRNA 3′ processing factors in *S. cerevisiae* (Fig. 3.2) (Zhao et al. 1999; Proudfoot 2004). At least 13 of them have been conserved up to mammals (Table 3.1). Interestingly, although *S. cerevisiae* and *S. pombe* do not express homologues of the mammalian Cleavage Factor I complex (CFIm), a putative homologue of the CFIm subunit CFIm25 can be found in filamentous fungus *Aspergillus oryzae*, and in the plant pathogens *Ustilago maydis* and *Magnaporthe oryzae* (Munsterkotter and Steinberg 2007; Franceschetti et al. 2011). There are no clear orthologs of CFIm68 in *M. oryzae* based on primary amino acid sequence. However, the *M. oryzae* protein Rbp35 interacts with the CFIm25 homologue in vivo (Franceschetti et al. 2011), and its RRM domain shares structural similarity with the metazoan CFIm68 RRM (Yang et al. 2010).

Table 3.1 Major 3′ processing factors in *S. cerevisiae* and *S. pombe*

Saccharomyces cerevisiae factor	*Schizosaccharomyces pombe* homologue	Domains	Functions	Mamalian homologue
CFIA				
Rna14	Rna14	HAT (Half A Tetratricopeptide repeat) : protein-protein interactions	Required for the specificity of Rna15 recognition of the PE	CstF77
Rna15	Ctf1	RRM : RNA binding	Binding to the PE	CstF64
Pcf11	Pcf11	CID: CTD interaction; Zinc fingers	Interaction with RNA PolI CTD. Transcription termination	Pcf11
Clp1		Walker A : ATP binding	CFIA Scaffolding; CPF /CFIA bridging	Clp1
CFIB				
Nab4/Hrp1		Two RRMs	Binding to EE. Important for NMD, APA, Copper stress response	
CFII				
Cft1/Yhh1	Cft1	Beta-Propeller repeats	Binding at the CS	Cpsf160
Cft2/Ydh1	Cft2	Metallo-Beta-Lactamase domain; Beta-CASP domain	Binding at the CS	Cpsf100
Ysh1/Brr5	Ysh1	Metallo-Beta-Lactamase domain; Beta-CASP domain	Putative endoribonuclease	Cpsf73
Pta1	Pta1		APT /CFII bridging	Symplekin
PFI				
Yth1	Yth1	CCCH zinc finger domain	Binds U-rich elements surrounding CS	Cpsf30
Pfs2	Pfs2	WD-40 repeat : protein-protein interaction	PFI /CFIA /CFII /APT bridging	Wdr33
Fip1	Fip1		Potential regulator of Pap1	Fip1

(continued)

Table 3.1 (continued)

Saccharomyces cerevisiae factor	*Schizosaccharomyces pombe* homologue	Domains	Functions	Mamalian homologue
Other CPF subunits				
Pap1	Pla1		Catalyses adenosine addition to the 3′ cleavage product	PAP
Glc7	Dis2; Sds21		Type 1 protein phosphatase	PP1
Mpe1	SPBP8B7.15c	Zinc knuckle		Rbbp6
Ssu72	Ssu72		Phosphatase	Ssu72
Swd2	Swd2.2 (SPAC824.04) : CPF; Swd2 : Set1 complex	WD40 repeat	Links 3′ processing with chromatin modifications?	Wdr82 : Set1 complex
			Increases weak PAS usage	
Ref2				
Pti1		RRM	snoRNA 3′ formation	
Syc1			Negative regulator?	

These structural and functional similarities raise the possibility that *RBP35* is the functional ortholog of CFIm68 in filamentous fungi. Below we discuss the structure and functions of the major fungal 3′ processing factors in detail.

mRNA 3′ Processing Factors in S. cerevisiae

Most mRNA 3′ processing factors in *S. cerevisiae* are essential for viability and they form three major complexes: *Cleavage Factor IA (CFIA), Cleavage Factor IB (CFIB), and Cleavage and Polyadenylation Factor (CPF)* (Zhao et al. 1999). CPF consists of three subcomplexes: Cleavage Factor II (*CFII*), Polyadenylation Factor I (*PFI*), and Associated with Pta1 (*APT*). Additionally, the C-terminal domain (*CTD*) of RNA polymerase II (RNA pol II) binds to many mRNA 3′ processing factors and facilitates the recruitment of these factors to nascent RNAs in a co-transcriptional manner (see also Chap. 1).

CFIA

The CFIA complex contains four subunits, Rna14, Rna15, Pcf11, and Clp1. *RNA14* and *RNA15* are the homologs of the mammalian CstF77 and CstF64, respectively. *S. cerevisiae* does not appear to encode a homolog of the third mammalian CstF subunit, CstF50 (Zhao et al. 1999). Depletion of Rna14 or Rna15 leads to global shortening of poly(A) tails and a defect in PAS recognition (Minvielle-Sebastia et al. 1994; Mandart and Parker 1995). Similar to its mammalian homolog CstF77, Rna14 seems to serve as a scaffold through interactions with Rna15, CFIB (see below), and RNA POL II CTD (Noble et al. 2004). Also similar to its mammalian homolog CstF64, Rna15 contributes to PAS recognition by directly binding to RNA via its RRM domain (Gross and Moore 2001). However, Rna15 binds to the A-rich PE upstream of the CS while CstF64 binds specifically to the U/GU-rich regions downstream of CS (MacDonald et al. 1994; Takagaki and Manley 1997; Yao et al. 2012). Recognition of the PE by Rna15 also requires CFIB (Hrp1/Nab4) (Leeper et al. 2010).

PCF11 and *CLP1* are also conserved from yeast to human (Darmon and Lutz 2012). Pcf11 interacts with the RNA pol II CTD through its N-terminal CTD Interacting Domain (CID) (Meinhart and Cramer 2004; Barilla et al. 2001; Licatalosi et al. 2002). RNA pol II CTD is a unique protein domain that consists of 26 (yeast) to 52 (human) highly conserved heptapeptide repeats of the consensus sequence: YSPTSPS (Buratowski 2003; Hirose and Manley 2000; Bentley 2005). The CTD is highly phosphorylated and its phosphorylation is tightly regulated during the transcription cycle. For example, Ser5 is phosphorylated early in transcription and helps to recruit the capping enzymes. On the other hand, Ser2 phosphorylation is low at the promoter regions, but accumulates during transcription

elongation and peaks near the 3′ ends of genes and is important for recruiting mRNA 3′ processing factors (Buratowski 2003; Hirose and Manley 2000; Bentley 2005). For example, Pcf11 CID specifically interacts with CTD phosphorylated at Ser2 (Licatalosi et al. 2002; Meinhart and Cramer 2004). In addition to its role in mRNA 3′ processing, Pcf11 functions in transcription termination by bridging the RNA pol II CTD to the nascent transcripts and dismantling the transcription elongation complex (Zhang et al. 2007; Zhang and Gilmour 2006).

Clp1 interacts with Pcf11 and the CFII subunits Ysh1/Brr5, thereby linking CFIA to CPF (Minvielle-Sebastia et al. 1997; Kessler et al. 1996). Clp1 contains a Walker A motif, a known ATP/GTP-binding domain, but no ATPase activity has been detected (Noble et al. 2007). The human Clp1 protein has been shown to possess RNA-specific 5′-OH polynucleotide kinase activity (Weitzer and Martinez 2007). However, yeast Clp1 seems to lack this activity and mutations in the kinase domain do not affect viability (Ramirez et al. 2008), indicating that the RNA kinase activity is not required for mRNA 3′ processing in yeast.

CFIB

CFIB is composed of one unique subunit, *Hrp*1/*Nab*4 (16, 62, 160). Although Hrp1/Nab4 has no sequence homology with any mammal protein, it may share similar structures with the mammal splicing factor hnRNP A1 (Kessler et al. 1997). *HRP*1/*NAB*4 is essential for cell viability and is required for cleavage and polyadenylation. Hrp1/Nab4 directly binds to the U-rich EE via its two RRM domains (Chen and Hyman 1998; Perez-Canadillas 2006). Its depletion in vivo leads to a global decrease in poly(A) tail length (16, 62). It interacts with Rna14 and Rna15, and shuttles between the nucleus and the cytoplasm (Kessler et al. 1997). Moreover, Hrp1/Nab4 was shown to regulate APA and stress response (further discussed in the APA section) (Kim Guisbert et al. 2007).

CPF

CPF contains homologues of all the major subunits of mammal Cleavage and Polyadenylation Specificity Factor (CPSF) and they comprise three subcomplexes: CFII, PFI, and APT (Zhao et al. 1999).

The CFII subcomplex. CFII contains four proteins: Cft1/Yhh1 (Cleavage Factor Two 1), Cft2/Ydh1 (Cleavage Factor Two 2), Ysh1/Brr5 (Yeast 73 kDa Homolog 1), and Pta1 (Pre-Trna Accumulation 1). They are homologous to the mammalian CPSF160, CPSF100, CPSF73, and symplekin, respectively (Zhao et al. 1999; Darmon and Lutz 2012). The CFII subunits are functionally similar to their mammalian counterparts as well. Cft1/Yhh1 binds to the mRNAs in the vicinity of the CS via a Beta-propeller repeat domain (Fig. 3.2) (Stumpf and Domdey 1996; Dichtl

et al. 2002b). Moreover, Cft1/Yhh1 interacts with RNA pol II CTD and is essential for mRNA transcription termination (Dichtl et al. 2002b). Similar to CPSF 100 and CPSF73, Cft2/Ydh1 and Ysh1/Brr5 both contain a putative metallo-beta-lactamase domain and a beta-CASP domain. However, Cft2/Ydh1 is not able to bind metal ions (Mandel et al. 2006). Cft2/Ydh1 binds the mRNA at a region encompassing the CS (Zhao et al. 1997). Cft2/Ydh1 interacts with other CFII subunits, Pfs2 (PFI subunit), Ssu72 (APT subunit), and the RNA pol II CTD (Kyburz 2003). Similar to CPSF73, Ysh1/Brr5 is believed to be the endonuclease for mRNA 3′ processing in budding yeast. Consistent with this conclusion, mutations in Ysh1/Brr5 that disrupt zinc binding are lethal (Mandel et al. 2006; Ryan et al. 2004). Pta1 shares homology with Symplekin, a scaffolding factor in the mammalian CPSF complex (Takagaki and Manley 2000). Pta1 is believed to bridge the APT complex with CFII complex through multiple interactions (Nedea et al. 2008).

The CPF: PFI complex. The PFI subcomplex contains Yth1 (Yeast 30 kDa Homolog 1), Pfs2 (Polyadenylation Factor Subunit 2), Fip1 (Factor Interacting with Poly(A) polymerase 1), Mpe1 (Mutant PCF11 Extragenic suppressor 1), and Pap1 (Zhao et al. 1999).

Yth1 is related to the mammal RNA-binding zinc finger protein CPSF30 (64 % similarity and is essential for in vitro cleavage and polyadenylation (Barabino et al. 1997). Yth1 contains five CCCH zinc finger domains, and the second one has been shown to be critical for mRNA 3′ processing. Yth1 binds to the U-rich element surrounding the CS: the UUE and DUE sequences (Barabino et al. 1997). Yth1 interacts with Fip1 and Ysh1/Brr5, subunit of CFII subcomplex (Barabino et al. 1997; Tacahashi et al. 2003; Helmling et al. 2001).

Fip1 is an intrinsically disordered/unstructured protein that shares 52 % similarity with mammal RNA-binding protein Fip1 (Meinke et al. 2008; Darmon and Lutz 2012). Although Fip1 is not required for cleavage, elimination of the C-terminal half leads to a general shortening of poly(A) tail in vivo (Preker et al. 1995). Fip1 interaction with Pap1 was suggested to regulate Pap1 poly(A) polymerase activity (Preker et al. 1995; Helmling et al. 2001). Moreover, as mutations specifically disrupting Fip1–Pap1 interactions are lethal, it is likely that Fip1 mediates the recruitment of Pap1 to the PAS (Helmling et al. 2001). Fip1 also interacts with Rna14 and Pfs2 (Ohnacker et al. 2000).

Pfs2 is a WD-40 repeat protein and the homolog of the mammal WD40 repeat protein Wdr33 (58 % similarity) (Darmon and Lutz 2012). Pfs2 is required for cleavage and polyadenylation. Pfs2 links PFI with CFIA, CFII, and APT subcomplexes through its interaction with Fip1, Rna14, Ysh1/Brr5, and Swd2 (Ohnacker et al. 2000).

Pap1 is required for polyadenylation but not for cleavage in vitro (Lingner et al. 1991). Structural and enzymatic properties of Pap1 are highly conserved in fungi, as exemplified by studies of *Candida albicans* and *S. pombe* poly(A) polymerase (Bougie and Bisaillon 2007). Pap1 is posttranslationally modified by phosphorylation and ubiquitylation during the cell cycle. Phosphorylation of Pap1 occurs during S and G2 phases, and this modification inhibits Pap1 activity (Mizrahi and Moore 2000). Several proteins, including Fip1, Cft1, and Pta1, interact with Pap1

and could potentially modulate its activity (Nedea et al. 2003; Ezeokonkwo et al. 2012). Pap1 also interacts with the RNA-binding protein Nab6, which was suggested to bind poly(A) mRNA to increase their stability, and to target more specifically mRNAs encoding for proteins of the cell wall (Ezeokonkwo et al. 2012). Pap1 also influences PAS choice (Mandart and Parker 1995).

The CPF: APT subcomplex. CPF contains additional factors that form a third subcomplex called the APT complex (Associated with Pta1) (Nedea et al. 2003). Some of these factors have homologs in metazoan, including Glc7 (GLyCogen 7, homolog of the mammalian phosphatase PP1), Ssu72, and Swd2 (Darmon and Lutz 2012). The other APT component appear to be specific to yeast, including Ref2 (RNA End Formation 2), Pti1 (PTa1p Interacting protein), and Syc1 (Similar to Ysh1 C-terminal 1) (Darmon and Lutz 2012).

Ssu72 is required for cleavage but not for polyadenylation (He et al. 2003). Ssu72 is a protein phosphatase that specifically dephosphorylates RNA pol II CTD at Ser5 (Krishnamurthy et al. 2004; Hausmann et al. 2005). It has been suggested that Ssu72 functions to regenerate hypophosphorylated RNA pol II for new rounds of transcription. However, Ssu72 phosphatase activity seems not required for mRNA 3′ processing itself (Krishnamurthy et al. 2004; Hausmann et al. 2005). Besides Pta1, Ssu72 interacts with Cft2/Ydh1 and with RNA pol II subunit, Rpb2 (Krishnamurthy et al. 2004; Hausmann et al. 2005; Dichtl et al. 2002a). Even though the *Kluyveromyces lactis* Ssu72 protein shares 76 % identity with its *S. cerevisiae* counterpart, it cannot functionally complement *S. cerevisiae* Ssu72, indicating functional divergence during fungal evolution (Rodriguez-Torres et al. 2013).

Glc7 is the homolog of the mammalian protein phosphatase PP1 (Darmon and Lutz 2012). Glc7 is specifically required for cleavage, but not for polyadenylation (He and Moore 2005). Pta1 was identified as the Glc7 substrate in the mRNA 3′ processing machinery. It has been proposed that Pta1 goes through a phosphorylation-dephosphorylation cycle during mRNA 3′ processing and Glc7-mediated dephosphorylation is essential for the transition between cleavage and polyadenylation (He and Moore 2005). Glc7 is regulated by another APF subunit, Ref2 (RNA end formation 2) (Nedea et al. 2008). Ref2 directly binds to RNA and is required for the efficient processing at weak poly(A) sites (Russnak et al. 1995). Additionally, Ref2 mediates Glc7 association with the CPF complex. In the absence of Ref2, Glc7 dissociates from CPF, which results in defects in transcription termination at snoRNA genes (Nedea et al. 2008).

Swd2 is a WD-40 repeat protein essential for cell viability. It was first identified as part of Set1 (SET (Su(var)3-9; Enhancer of zeste; Trithorax) domain containing 1) /COMPAS (Complex Proteins Associated with Set1), which is essential for histone H3 methylation at lysine 4 (H3K4me) (Roguev et al. 2001; Miller et al. 2001). This epigenetic mark is important for gene expression and also for rDNA and telomeric heterochromatin silencing (Eissenberg and Shilatifard 2010). The presence of this epigenetic mark necessitates Swd2 (Cheng et al. 2004). Swd2 was also identified as part of the APT complex and functionally interacts with Ref2 (Nedea et al. 2003). Even though Swd2 is not essential for in vitro cleavage and polyadenylation, it is

required for RNA pol II transcription termination (Cheng et al. 2004). However, these two functions of Swd2 do not seem to be tightly coupled (Cheng et al. 2004).

Mpe1 has a putative zinc knuckle domain that may mediate RNA interactions and plays critical roles in mRNA 3′ processing by promoting the specific interactions between CPF and the pre-mRNAs (Vo et al. 2001). Mpe1 shares limited homology with the mammalian protein Rbbp6, which interacts with Rb and p53 and has been implicated in cancer (Shi et al. 2009; Pugh et al. 2006; Sakai et al. 1995). The functions of Mpe1 in mRNA 3′ processing remain poorly understood.

Syc1 shares homology with the C-terminal domain of Ysh1/Brr5 (Zhelkovsky et al. 2006). As mentioned above, *YSH1/BRR5* is essential for cell viability and mRNA 3′ processing. *SYC1* is not essential, but its deletion rescues the growth and mRNA 3′ processing defects in ysh1/brr5 mutant, indicating that Syc1 is a negative regulator of mRNA 3′ processing (Zhelkovsky et al. 2006).

Pti1 shares homology with Rna15 and the mammalian CstF64 and interacts with Pta1, but Pti1functions in mRNA 3′ processing have not been characterized in detail (Qu et al. 2007).

Poly(A) Binding Proteins

Poly(A) **B**inding **P**roteins (Pabps) play important roles in poly(A) tail length control (Mangus et al. 2003). *S. cerevisiae* encodes two main Pabps, Pab1 and Nab2, and they are homologous to the mammalian proteins PABPC1 and ZC3H14 (Soucek et al. 2012). Both proteins are essential for cell viability and depletion of either proteins leads to a global lengthening of poly(A) tails in vivo (Sachs and Davis 1989; Anderson et al. 1993). Nab2 is believed to be the major Pabp in the nucleus. It is co-transcriptional recruited to the nascent transcripts (Soucek et al. 2012). Nab2 physically interacts with Hrp1 and genetically interacts with Pap1, Rna15, and Syc1 (Soucek et al. 2012; Yu et al. 2008; Kerr et al. 2013). Nab2 interacts with RNAs through its zinc finger domains (Anderson et al. 1993; Marfatia et al. 2003). However, it remains poorly understood how Nab2 contributes to poly(A) tail length control. Additionally, Nab2 interacts with the Mlp1 (Myosin Like Protein 1), a factor involved in the nuclear retention of unspliced mRNAs and the nuclear exosome subunit Rrp6 (Green et al. 2003). These observations suggest that Nab2 contributes to mRNA quality control by targeting misprocessed RNAs to the exosome for degradation (Schmid et al. 2012; Soucek et al. 2012). Following mRNA export, Nab2 is believed to be replaced by Pab1 during the mRNP remodeling (Soucek et al. 2012). Pab1 contains four RRM domains and is associated with CFIA through the interaction with Rna15 (Amrani et al. 1997). In addition to its role in nuclear mRNA 3′ processing, Pab1 also mediates poly(A) shortening to promote translation in vivo (Sachs and Davis 1989). Pab1 recruits the Pab1-dependent Poly(A) Nuclease (PAN) to trim the poly(A) tails (Mangus et al. 2004). Because overexpression of Pab1 cannot rescue the hyperadenylation defect in nab2-deficient cells, these proteins have nonoverlapping functions (Hector et al.

2002). For example, Pab1 is able to bind mRNA with poly(A) tails as short as 10 nt, whereas Nab2 associates mainly with mature poly(A) tails (60–80 nt) (Hector et al. 2002).

S. pombe 3′ Processing Factors

Despite the fact that *S. pombe* and *S. cerevisiae* are evolutionary rather distant, their mRNA 3′ processing machineries are more similar to each other than to the mammalian system. For example, the *S. pombe* poly(A) polymerase Pla1 shows a higher sequence similarity with *S. cerevisiae* Pap1 (55 % identity) than with its mammalian homologues (88). Consistently, *S. cerevisiae* Pap1 can be functionally substituted both in vivo and in vitro by Pla1, whereas Pla1 cannot replace mammalian Pap1 in vitro (Ohnacker et al. 1996). These results suggest that Pla1 is able to interact with *S. cerevisiae* mRNA 3′ processing factors efficiently enough to correctly process pre-mRNAs. Due to these similarities, the *S. pombe* ura4 transcript can be cleaved and polyadenylated in vitro in *S. cerevisiae* cell extracts, and conversely, the *S. cerevisiae* cyc1 mRNA is correctly processed in vivo when expressed in *S. pombe* (Humphrey et al. 1991).

On the other hand, some of 3′ processing factors in *S. pombe* seem functionally closer to their mammalian homologs than to their budding yeast counterparts. For example, *S. pombe* Ctf1 (also called spCstF-64) is homolog to *S. cerevisiae* Rna15 and mammalian CstF64 (Aranda and Proudfoot 2001). Unlike Rna15, which recognizes the PE upstream of CS (39), Ctf1 binds to the EE downstream to the CS (Dichtl and Keller 2001). This is similar to its mammalian homolog CstF64, which has been shown to bind specifically to U/GU-rich sequences downstream of the CS (MacDonald et al. 1994; Takagaki and Manley 1997; Yao et al. 2012)(Table 3.1).

Interestingly, mRNA 3′ processing defects have been shown to manifest in some unexpected phenotypes in *S. pombe*. For example, mutations in *PFS2* gene cause chromosome segregation defects, which are believed to be downstream effect of mRNA 3′ processing and transcription termination malfunctions (Wang et al. 2005). Supporting this conclusion, transcription termination defects are observed in cells deficient for Pfs2 and the chromosome segregation defects are suppressed by overexpression of another mRNA 3′ processing factor Cft1(Wang et al. 2005). In keeping with the link between transcription termination and cell cycle , Dhp1, a 5′–3′ exonuclease homologous to Rat1 in *S. cerevisiae*, is also required for chromosome segregation (Shobuike et al. 2001; Sugano et al. 1994).

Although most mRNA 3′ processing factors in *S. pombe* have not been studied in detail, the Pabps have been characterized. *S. pombe* encodes for two RRM-containing Pabps, Pabp and Pab2 (Perreault et al. 2007; Thakurta et al. 2002). These proteins are nonessential for cell viability, suggesting functional redundancy. Pabp is the homolog of *S. cerevisiae* Pab1, and has been shown to be involved in mRNA export (Thakurta et al. 2002). Pab2 shares 47 % identity and 66 % similarity with human PABPN1, and possess a coiled-coil region, an RRM,

and a C-terminal arginine-rich domain. Pab2 binds to RNA poly(A) tails in vitro, and regulates mRNA poly(A) length in vivo (Perreault et al. 2007). Indeed, the maximum length of the poly(A) tail exceeds 226 nt in pab2-deficient cells as compared to 120 nt in wild type cells. Pab2 has been shown to self-associate in an RNA-independent manner. Similar to PABPN1, Pab2 is methylated at the R residues of the R-rich domain by Rmt1, a type I protein arginine N-methyltransferase (Perreault et al. 2007). R methylation is important for Pab2 oligomerization, but not for its nuclear localization or its function in regulation of poly(A) tail length. Mutations in the human *PABPN1* gene are linked to oculopharyngeal muscular dystrophy (OMPD), a disease characterized by fibrous inclusions in the nuclei of skeletal muscle fibers (Jenal et al. 2012; de Klerk et al. 2012). Interestingly, Pab2 overexpression leads to growth defects mediated by the R-rich domain, exacerbated when rmt1 is deleted, suggesting that elevated levels of unmethylated Pab2 is toxic for the cells (Perreault et al. 2007). Altogether these data suggest that Pab2 is functionally similar to its human homolog. Chromatin immunoprecipitation data indicate that although Pab2 binds to the poly(A) tail of mRNA, it might be recruited at earlier steps of transcription through its interaction with the large RNA pol II subunit Rpb1 (Perreault et al. 2007).

Regulators of mRNA 3′ Processing

Several factors have been identified as regulators of mRNA 3′ processing. One of the negative regulators is the RNA-binding protein Npl3 (Nuclear Protein Localization 3) (Bucheli and Buratowski 2005). Npl3 contains two RRMs and a domain that is rich in Serine/Arginine (SR) dipeptide repeats, a domain structure that is similar to the SR family splicing regulators in higher eukaryotes (Graveley 2000). Npl3 is required for the correct splicing of several mRNAs by mediating the recruitment of splicing factors via direct interaction (Kress et al. 2008). In addition to its functions in splicing, Npl3 stimulates transcription elongation through interactions with RNA pol II and impedes efficient transcription termination by competing with Rna15 for binding to the A-rich PE of the PAS (Dermody et al. 2008; Deka et al. 2008). Phosphorylation of Npl3 by Casein Kinase 2 (CK2) decreases its interaction with the RNA pol II and its ability to bind RNA, thereby promoting transcription termination (Dermody et al. 2008). Moreover, Npl3 phosphorylation stimulates a negative autoregulation by promoting the distal PAS usage of *NPL3* transcript, which leads to a decrease in Npl3 protein level (Lund et al. 2008).

The mRNA export adaptor Yra1 has also been shown to regulate mRNA 3′ processing. Yra1 negatively regulates 3′ end formation by competing with Clp1 for interaction with Pcf11, and depletion of Yra1 leads to changes in the global APA profile (Johnson et al. 2009, 2011).

Factors Required for S. cerevisiae Histones mRNA 3′ Processing

The expression of replication-dependent histone genes is highly regulated during the cell cycle to allow accumulation of histone mRNAs specifically during the S phase (Marzluff et al. 2008). In metazoans, the 3′ ends of replication-dependent histone mRNAs are formed by an endonucleolytic cleavage step without polyadenylation (Marzluff et al. 2008). This process involves the recognition of a highly conserved stem-loop by the SLBP protein and the downstream sequences by the U7 snRNP at the 3′ ends of the mRNAs. But mRNA 3′ processing factors, such as CPSF and CstF, are also required. The metazoan histone mRNA 3′ processing activities are regulated in a cell cycle-specific manner (Marzluff et al. 2008).

In contrast to metazoans, the 3′ ends of histone mRNAs in fungi, plants, and protozoa generated through the regular cleavage/polyadenylation mechanism and the 3′ processing of these mRNA requires the canonical mRNA 3′ processing factors, including Rna14, Pcf11, Rna15, and Pap1 (Fahrner et al. 1980; Canavan and Bond 2007). However, recent studies have implicated Sen1, a putative helicase required for the 3′ processing of many nonpolyadenylated RNAs, in yeast histone mRNA 3′ processing (Beggs et al. 2012). Additionally, in *S. cerevisiae*, the poly(A) tails of histone mRNAs, which are 20–50 nt, are shorter than the average length (70–90 nt), and their poly(A) tails shorten during the *S* phase (Beggs et al. 2012). *S* phase-specific inhibition of Pap1 activity by phosphorylation might be a potential mechanism for histone mRNA poly(A) tail length control. Further studies are needed to understand how histone mRNAs levels are regulated by the cell cycle and how such mechanisms evolved during evolution.

Release of mRNA 3′ Processing Factors After Polyadenylation

Although the assembly of the mRNA 3′ processing machinery has been studied extensively, how these factors are released following polyadenylation remains poorly understood. An important insight came from the observation that mutations in factors involved in mRNA export or the assembly of export-competent mRNPs (such as Mex67), lead to a defect in the release of mRNA 3′ processing factors from mRNAs (Qu et al. 2009). This suggest that a remodeling of mRNPs takes place during which mRNA export factors may replace 3′ processing factors on polyadenylated mRNAs (Qu et al. 2009). This functional coupling may help to ensure that only fully processed mRNAs are targeted for export, but the mechanistic details of this mRNP remodeling step remain unclear.

Alternative Polyadenylation and its Regulation in Fungi

Recent global studies have revealed that APA is surprisingly widespread in yeast. For example, it is estimated that 40–70 % of the *S. cerevisiae* and *S. pombe* genes produce alternatively polyadenylated mRNAs whose CS are separated by 50 nt or more (Ozsolak et al. 2010; Mata 2013; Schlackow et al. 2013; Moqtaderi et al. 2013; Yoon and Brem 2010). However, as mentioned earlier, there seems to be a high level of heterogeneity in the position of the CS in yeast (Moqtaderi et al. 2013). Rather than one or a few distinct CS, cleavage/polyadenylation occurs in a ~200 nt "end zone." Thus, it may be difficult to distinguish between CS heterogeneity from the same PAS and APA. However, recent studies clearly demonstrated that APA is widespread in fungi (Ozsolak et al. 2010; Mata 2013; Schlackow et al. 2013; Moqtaderi et al. 2013; Yoon and Brem 2010). In *S. cerevisiae*, more than 600 genes use PAS within the CDS, producing truncated transcripts. Interestingly, a motif, GAAGAAGA, is enriched in the 50 nucleotides upstream of the intragenic CS. These truncated transcripts originate mainly from genes involved in stress response and meiosis (Yoon and Brem 2010). Indeed, APA has been implicated in cellular responses to many types of stress and in the regulation of meiotic gene expression, which are discussed in details below.

APA Regulation and Metabolism

One of the first examples of APA regulation in budding yeast was described for the gene *CBP1* (Cytochrome b processing 1) (Mayer and Dieckmann 1989). Cbp1 is required for the expression of the mitochondrial gene encoding Cytochrome B, a component of the electron transport chain in respiration. In fermenting cells, two *CBP1* APA isoforms are produced (Mayer and Dieckmann 1989). The short isoform uses a PAS within the CDS and the resulting truncated mRNA does not code for any protein product. The longer isoform encodes the functional Cbp1 protein. Following induction of respiration by switching to a nonfermentable carbon source, there is a shift in *CBP1* APA pattern from the long to the short isoform while the total mRNA level remains unchanged (Sparks and Dieckmann 1998; Mayer and Dieckmann 1989). Three additional mRNAs were later shown to undergo the same type of respiration-dependent APA change: *AEP2/ATP13*, which is also necessary for respiration, and *RNA14* and *SIR1*. Like *CBP1*, *AEP2* encodes a factor required for the expression of a mitochondrial respiration gene (*ATP9*). Rna14 is a mRNA 3′ processing factor (see "CFIA"), and *SIR1* encodes a mating type locus silencing factor. Suppression of the short *CBP1* transcript leads to the constitutive production of elevated levels of the long *CBP1* transcript independently of respiration induction, which in turn results in the accumulation of the mitochondrial *CYTOCHROME B* mRNAs (Sparks and Dieckmann 1998). These

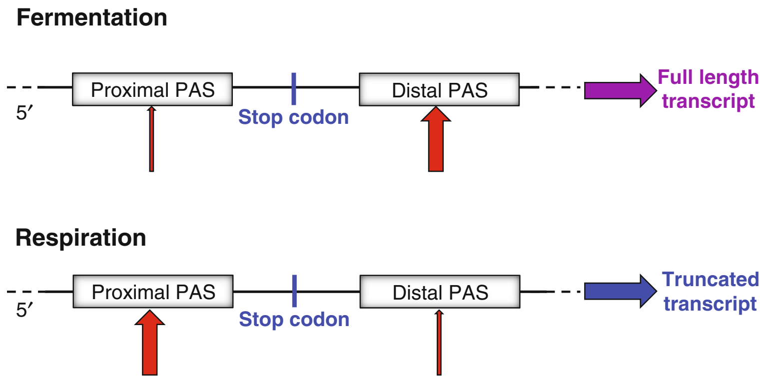

Fig. 3.3 Metabolism-mediated APA regulation. In fermenting *Saccharomyces cerevisiae* cells, polyadenylation occurs mainly at the distal poly(A) site downstream of the coding sequences, leading to production of the full-length mRNAs. In respiring cells, polyadenylation shifts to the proximal poly(A) sites in the coding sequences, leading to the production of truncated transcripts

observations indicate that the APA may be a mechanism for respiration-dependent regulation of gene expression (Fig. 3.3). However, the mechanism and biological significance of APA regulation by metabolism remain to be determined.

APA Regulation and DNA Damage Response

APA changes have been observed in response to DNA damage (Fig. 3.4). For example, polyadenylation of *RPB2* (RNA Polymerase B 2) and *CBP1* mRNAs switches from the proximal to the distal PAS upon UV irradiation (Yu and Volkert 2013). A global study detected similar APA changes for over 2,000 genes under similar conditions (Graber et al. 2013). Two possible mechanisms have been proposed, which are not mutually exclusive. First, the transcription elongation rate has been suggested to play an important role (Yu and Volkert 2013). A pharmacologically induced decrease in transcription elongation rate abolishes the APA changes in *RPB2* mRNAs following DNA damage (Yu and Volkert 2013), indicating that fast transcription elongation rate promotes skipping of the proximal PAS. Second, UV-induced DNA damage has been shown to cause a reduction in the protein levels of CPF subunits and in turn lower mRNA 3′ processing activity. The decrease in the 3′ processing activity results in the preferential recognition of the distal PAS as they are intrinsically stronger and have higher affinity for the mRNA 3′ processing machinery (Graber et al. 2013) (Fig. 3.4). Interestingly, a transient inhibition of the mRNA 3′ processing machinery has been observed in mammalian cells (Kleiman and Manley 2001). This is mediated by sequestration of CstF50 by the BRCA1-BARD1 complex following DNA damage (Kleiman and

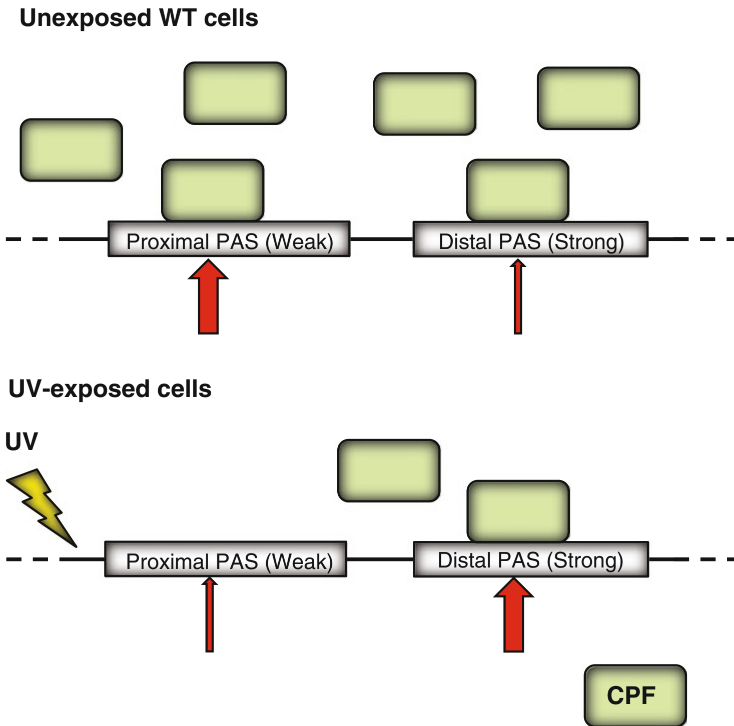

Fig. 3.4 APA regulation in DNA damage response. In unstressed cells, high levels of CPF allows for the recognition of weak poly(A) sites in the coding sequences, leading to the production of truncated transcripts. Following UV-induced DNA damage, CPF levels decrease and the remaining CPF preferentially binds to the strong distal poly(A) sites downstream of the coding sequences, leading to production of full-length mRNAs

Manley 2001). It will be of great interest to determine how CPF levels are regulated and what the functional importance of these APA changes is for cellular survival after DNA damage.

Nab4/Hrp1-Mediated APA Regulation and Copper Stress

The general 3′ processing factor Nab4/Hrp1 has been identified as an important APA regulator (Kim Guisbert et al. 2007). The *SUA7* gene (Suppressor of Upstream AUG 7) produces two APA isoforms. The long isoform is more abundant in exponentially growing cells while the short isoform accumulates during stationary phase (Hoopes et al. 2000). Nab4/Hrp1 was found to be critical for regulating the cell cycle-dependent ratio of the isoforms (Kim Guisbert et al. 2007). Nab4/Hrp1 binds to a UA-rich motif and promote the usage of adjacent

PAS. Additionally, the protein level of Nab4/Hrp1 is also important: high levels of Nab4/Hrp1 promote the usage of the proximal PAS in *SUA7* mRNA (Kim Guisbert et al. 2007). However, it has not been determined whether and how Nab4/Hrp1 protein level is regulated in cell cycle. Interestingly, nab4/hrp1 mutant stains are extremely resistant to high concentration of copper. This is due to the APA change in the *CTR2* gene, which encodes a copper transporter. In nab4/hrp1 mutant strain, the level of the *CTR2* mRNA isoforms with the longest 3′ UTR increases (Kim Guisbert et al. 2007). These observations suggest that APA regulation plays an important role in cellular stress induced by copper and perhaps other metals.

APA Regulation of Meiotic Gene Expression

A subset of meiotic genes in *S. pombe* was also shown to undergo APA upon meiosis induction, and it was suggested to be important for the regulation of their expression (McPheeters et al. 2009; Cremona et al. 2011; Potter et al. 2012). The mechanisms involved are discussed in a following paragraph.

The first example of meiosis-dependent APA regulation was demonstrated for the meiotic gene *CRS1* (McPheeters et al. 2009). *CRS1* mRNAs are polyadenylated at two PAS. In vegetative cells, *CRS1* mRNAs are actively degraded by the Mmi1 pathway as described later ("mRNA 3′ Processing in the Regulation of S. pombe Meiotic Genes"). Upon meiosis, Mmi1-mediated repression is alleviated and *CRS1* mRNA undergoes splicing-coupled polyadenylation at both proximal and distal PAS. Even though the ratio between the short and the long isoforms slightly changes during the time course of meiosis, the distal PAS is always more predominantly used over the proximal PAS (McPheeters et al. 2009; Chen et al. 2011). Later studies identified additional meiotic transcripts using meiosis specific 3′ processing-dependent regulation (Cremona et al. 2011; Potter et al. 2012). These transcripts were found to utilize more than one PAS upon meiosis induction, but again in this case the proximal PAS usage relatively to the distal PAS was not studied in detail. Although the biological consequences of meiotic-dependent APA are still not clear, APA might be an additional way to regulate the proper timing of activation of these genes during sexual differentiation progression.

Other Examples of Alternative Polyadenylation in Fungi

Kluyveromyces lactis (*K. lactis*) *CYC1* mRNA (*KLCYC1*) was shown to use two distinct PAS, whereas *S. cerevisiae CYC1* mRNA has only one PAS (Freire-Picos et al. 1995). *CYC1* encodes the iso-1-cytochrome c factor, and *KLCYC1* is essential for respiratory growth in *K. lactis*. Specifically the longer APA isoform has been suggested to be responsible for an increase biomass production during respiration, and an inhibition of ethanol production during fermentation of *K. lactis* (Seoane et al.

2005). Comparison of the sequences surrounding the two CS revealed a common feature. There is an AACAA motif a few nucleotides upstream of the CS, and an AU-rich region just upstream of the AACAA motif only for the proximal PAS. The ratio between the two isoforms changes according to cellular growth conditions: the distal PAS usage increases with the optical density of the culture (OD) when the proximal PAS usage remains constant. *KLCYC1* mRNA was correctly processed at the two PAS when transformed into *S. cerevisiae*, but the growth-dependent APA change was not observed, indicating the lack of a specific regulatory factor in *S. cerevisiae* (Freire-Picos et al. 2001). The mechanism for *KLCYC1* APA regulation remains unclear, but Pta1 and Pcf11 seem to be involved (Seoane et al. 2009).

Gene Regulation at the 3′ End

In addition to APA, mRNA 3′ processing can participate in gene regulation in other ways. The efficiency of mRNA 3′ processing plays an important role in controlling the mRNA abundance. mRNA 3′ processing factors can modulate other cellular processes to influence gene expression. In this section, we discuss a couple of such examples.

mRNA 3′ Processing in the Regulation of S. pombe Meiotic Genes

In *S. pombe*, the mRNAs for meiotic genes are not detectable in vegetative cells (Harigaya et al. 2006). However, several lines of evidence suggest that meiotic genes are transcribed, but are actively degraded. First, depletion of the exosome subunit Rrp6 results in the accumulation of hyperpolyadenylated meiotic mRNAs, and hyperpolyadenylation of these mRNAs in the Rrp6-depleted cells depends on Mmi1 (Meiotic mRNA interception 1), the poly(A) polymerase Pla1 (Harigaya et al. 2006; Yamanaka et al. 2010), and Red1, a CCCH zinc-finger-containing protein interacting with Mmi1 (Sugiyama and Sugioka-Sugiyama 2011). Second, depletion of the mRNA 3′ processing factors Rna15, Pla1 Pab2, Pfs2, and Dhp1, as well as several transcription termination factors all induce the accumulation of meiotic mRNAs (McPheeters et al. 2009; Yamanaka et al. 2010; St-Andre et al. 2010; Chen et al. 2011). Lastly, polyadenylation of meiotic mRNAs was shown to be required for their elimination in vegetative cells (McPheeters et al. 2009; Yamanaka et al. 2010).

Mmi1 plays a central role in this regulation (Fig. 3.5) (Harigaya et al. 2006). In mitotic cells, Mmi1 interacts with meiotic mRNAs with a specific cis-element containing the degenerate hexanucleotide motif UNAAAC (Zhang et al. 2010; Hiriart et al. 2012; Yamashita et al. 2012). Mmi1 recruits mRNA 3′ processing

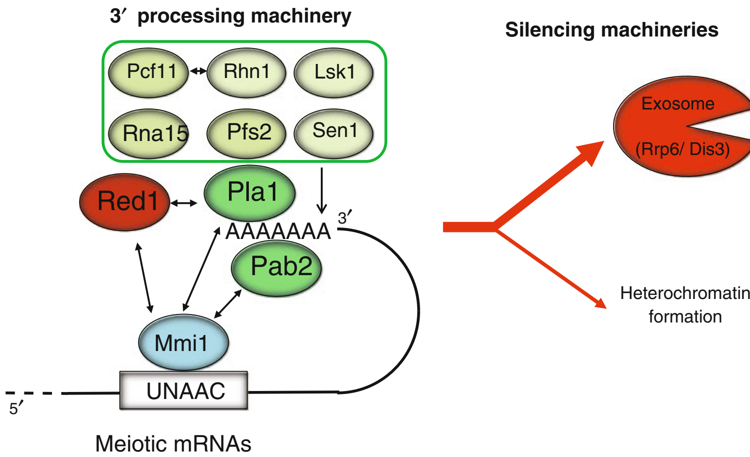

Fig. 3.5 mRNA 3′ processing factors are involved in the suppression of meiotic mRNAs. In vegetative cells, Mmi1 binds to UNAAC motifs in its target meiotic mRNAs and recruits mRNA 3′ processing factors, leading to polyadenylation of its target mRNAs and their degradation by the exosome. Mmi1-mediated meiotic mRNA degradation also promotes the formation of heterochromatin at meiotic gene loci

factors including Rna15 and Pla1 to hyperpolyadenylate its mRNA targets, which in turn are degraded by the exosome (Yamanaka et al. 2010). Additionally, Mmi-mediated meiotic mRNA degradation promotes the formation of heterochromatin at meiotic genes, which also contributes to meiotic gene silencing (Zofall et al. 2012). During meiosis, Mmi1 is sequestered by the master meiotic regulator Mei2 and the meiotic mRNAs are derepressed (Harigaya et al. 2006). Although mRNA 3′ processing factors are clearly involved in this regulatory pathway, their specific functions in this process remain unclear.

mRNA 3′ Processing Regulates the Expression of Neighboring Genes

Given the compressed nature of the yeast genomes, transcription read-through due to inefficient mRNA 3′ processing is likely to interfere with the expression of neighboring genes. When adjacent genes are arranged in tandem, transcription read-through from upstream genes may inhibit the transcription of downstream genes (Shearwin et al. 2005). On the other hand, when the neighboring genes are convergent, 3′ processing defects may lead to the collision of the transcription machinery (Prescott and Proudfoot 2002). Additionally, a recent study revealed an additional mechanism in *S. pombe* that regulate convergent gene expression in a cell cycle-dependent manner (Gullerova and Proudfoot 2008, 2012). During G1-S

phases, inefficient transcription termination leads to transcription read-through. The resulting double-stranded RNAs formed between the transcripts of convergent genes lead to the activation of the RNAi pathway. A transient RNAi-dependent heterochromatin structure is formed in the intergenic region between the convergent genes, characterized by the histone modification H3K9me3 and Swi6 binding, both hallmarks of heterochromatin (for more details see Chap. 13). Through a direct interaction, Swi6 induces the recruitment of cohesins at the chromatin of these convergent genes in the G2 phase. Cohesins are proteins involved in the regulation of sister chromatid separation during cell division. The presence of cohesins between convergent genes blocks transcription read-through and promotes transcription termination at the proper PAS, thereby restoring the mRNA levels of the convergent genes. After mitosis, cohesins are released and the heterochromatin structure at these loci is relieved (Gullerova and Proudfoot 2008, 2012). In *S. cerevisiae*, cohesins were also shown to concentrate at intergenic regions of convergent genes (Lengronne et al. 2004), but as H3K9me3 and RNAi are not conserved in budding yeast, this process might involve a different mechanism. This cell cycle-dependent gene regulation involving transcription termination regulation has been shown to be particularly important for the regulation of RNAi genes as 80 % of RNAi genes are convergent. This process of autoregulation may be important for the regulation of heterochromatin formation during different phases of the cell cycle (Gullerova and Proudfoot 2012; Zofall et al. 2012).

Conclusion /Future Directions

Studies in fungi have made tremendous contribution to our understanding of eukaryotic mRNA 3′ processing. Given the genetic tractability and the advent of high throughput analysis approaches, fungi will prove highly useful in addressing the remaining important questions in the field. First, although the list of essential mRNA 3′ processing factors is nearly complete, the functions of each factor in 3′ processing remains poorly characterized. The combination of genetic and biochemical analyses will be key to address this question. Second, as mentioned earlier, the mRNA 3′ processing machinery has evolved quite significantly in eukaryotes. Both PAS sequences and mRNA 3′ processing factors have diverged in different lineages and species. Given the increasingly number of species with their genomes sequenced, fungi provide a unique system to study the evolution of the mRNA 3′ processing system. Third, APA has increasingly been recognized as an important mechanism for gene regulation. Since APA is widespread in yeast and some of the regulatory mechanisms seem highly conserved between yeast and mammals, fungi again will be very useful in deciphering the “polyadenylation code,” the rules by which PAS selection is regulated.

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