EMBO reports

scientific report

Conserved motifs in both CPSF73 and CPSF100 are required to assemble the active endonuclease for histone mRNA 3′-end maturation

Nikolay G. Kolev[1,2], *Therese A. Yario*[1,2], *Eleni Benson*[2†] & *Joan A. Steitz*[1,2+]

[1]Howard Hughes Medical Institute, and [2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, USA

In eukaryotes, the process of messenger RNA 3′-end formation involves endonucleolytic cleavage of the transcript followed by synthesis of the poly(A) tail. The complex machinery involved in this maturation process contains two proteins of the metallo-β-lactamase (MBL) superfamily, the 73 and 100 kDa subunits of the cleavage and polyadenylation specificity factor (CPSF). By using an *in vitro* system to assess point mutations in these two mammalian proteins, we found that conserved residues from the MBL motifs of both polypeptides are required for assembly of the endonuclease activity that cleaves histone pre-mRNAs. This indicates that CPSF73 and CPSF100 act together in the process of maturation of eukaryotic pre-messenger RNAs, similar to other members of the MBL family, RNases Z and J, which function as homodimers.

Keywords: CPSF; metallo-β-lactamase; 3′-end processing; endonuclease; histone mRNA

EMBO reports (2008) **9**, 1013–1018. doi:10.1038/embor.2008.146

INTRODUCTION

One step in the maturation of eukaryotic messenger RNAs is endonucleolytic cleavage of the RNA polymerase II transcript to form the 3′-end. For most mRNAs, the cleavage is coupled to a reaction catalysed by poly(A) polymerase to produce a polyadenylate tail that is not encoded in the genome (reviewed by Mandel *et al*, 2008). The molecular machinery required for the correct execution of this biogenesis step is rather complex (Mandel *et al*, 2008) and at least some components are shared with the machinery that is involved in the maturation of 3′-ends of histone mRNAs (Dominski *et al*, 2005a; Kolev & Steitz, 2005; Wagner *et al*, 2007). Metazoan histone mRNAs are the only examples of eukaryotic mRNAs that do not have poly(A) tails (reviewed by Dominski & Marzluff, 2007). Among the common protein factors are cleavage and polyadenylation specificity factor (CPSF)73 and CPSF100, which are both members of the metallo-β-lactamase (MBL) superfamily (Aravind, 1999; Callebaut *et al*, 2002; Dominski, 2007). Members of this group of enzymes that act on nucleic acids also include RNase Z, which catalyses the removal of the 3′-trailer sequences of tRNA precursors before the addition of the terminal CCA (reviewed by Redko *et al*, 2007), and other members of the so-called β-CASP (metallo-β-lactamase-associated CISF Artemis SNM1/PSO2) group (Callebaut *et al*, 2002): Int11 and Int9, which function in the 3′-end formation of small nuclear RNAs (snRNAs; Baillat *et al*, 2005); RNase J, which acts on mRNAs in bacteria (Mathy *et al*, 2007; de la Sierra-Gallay *et al*, 2008) and certain DNA-metabolizing proteins (Callebaut *et al*, 2002; Dominski, 2007).

Although typical MBLs have five conserved motifs (1–5) containing histidine and aspartate residues (Aravind, 1999) that usually coordinate two metal ions (most often Zn^{2+}), the members of the β-CASP group lack motif 5 and instead contain three other motifs, A–C (Callebaut *et al*, 2002). Recently, X-ray crystal structures have been solved for human CPSF73 (Mandel *et al*, 2006), yeast CPSF100/Ydh1 (Mandel *et al*, 2006), *Thermus thermophilus* RNase J (de la Sierra-Gallay *et al*, 2008) and another bacterial protein from the β-CASP group, Ttha0252 (Ishikawa *et al*, 2006). In all cases, the sequence that forms the MBL domain is interrupted by a large segment that comprises the β-CASP domain. The active site is positioned deep in a cleft between the MBL and β-CASP domains, and the available structures do not predict how a substrate RNA might be accommodated for endonucleolytic cleavage.

Most of the signature residues of the MBL motifs are not conserved in yeast CPSF100/Ydh1 (Aravind, 1999) and no bound metal atoms were observed in the solved structure (Mandel *et al*, 2006). However, these residues are significantly conserved in other organisms—mammals, insects and plants—and we investigated whether mammalian CPSF100 is really an 'inactive' member of the MBL family, as suggested by bioinformatic studies (Aravind, 1999; Callebaut *et al*, 2002). We tested point mutations

[1]Howard Hughes Medical Institute, and [2]Department of Molecular Biophysics and Biochemistry, Yale University, 295 Congress Avenue, New Haven, Connecticut 06519, USA
[†]Present address: Baylor College of Medicine, BCM368, One Baylor Plaza, Houston, Texas 77030, USA
[+]Corresponding author. Tel: +1 203 737 4418; Fax: +1 203 624 8213; E-mail: joan.steitz@yale.edu

Received 7 May 2008; revised 18 June 2008; accepted 1 July 2008; published online 8 August 2008

in conserved residues of the mammalian CPSF73 and CPSF100 polypeptides for their effect on the processing of histone mRNA precursors and found that alterations in crucial MBL residues of both proteins are deleterious for the endonuclease activity that creates the 3′-end.

RESULTS

Zn^{2+} chelator does not block histone mRNA maturation

For *in vitro* processing analysis, we used a segment of the mouse histone H4–12 pre-mRNA (Fig 1A). This substrate generates two 5′ fragments as a result of processing (Streit *et al*, 1993), the minor product being 2 nt longer (Fig 1A). *In vitro* maturation of histone mRNAs is resistant to high concentrations of EDTA (Gick *et al*, 1986), in contrast to cleavage associated with polyadenylation (Ryan *et al*, 2004). This difference might reflect either a metal-containing processing component that is required only for polyadenylation-associated cleavage or a differential sensitivity of a Zn^{2+}-dependent endonuclease.

We investigated whether the presence of *o*-phenanthroline (a potent Zn^{2+} chelator) affects histone pre-mRNA processing. Preincubation of HeLa nuclear extract with up to 20 mM *o*-phenanthroline had little effect on the overall efficiency of processing (Fig 1B) but, in contrast to EDTA, use of the minor cleavage site increased significantly. This shift in the sequence specificity of cleavage was reproducible and unaffected by the addition of Zn^{2+} at concentrations higher than those of *o*-phenanthroline either before or after treatment of the nuclear extract with the chelator. Thus, the increase in cleavage at the minor site is not the result of Zn^{2+} depletion. X-ray crystallographic analysis of human CPSF73 (Mandel *et al*, 2006) at a resolution of 2.1 Å showed high occupancy of the Zn-binding site in the crystals, suggestive of tight metal binding. By contrast, the 2.5 Å structure of yeast CPSF100/Ydh1 (Mandel *et al*, 2006) did not detect these Zn atoms, which is consistent with poor conservation of the crucial residues in the metal-binding motifs of the *Saccharomyces cerevisiae* protein. However, as there is reasonably good conservation of amino acids with metal-chelating potential in mammalian CPSF100 (Fig 2; supplementary Figs 1,2 online), perhaps both mammalian proteins bind to Zn so tightly that the reaction is immune to inhibition by *o*-phenanthroline.

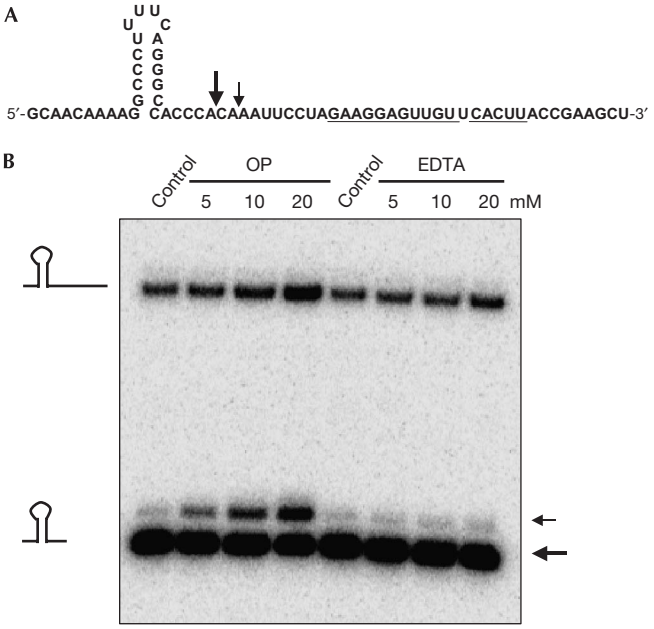


Fig 1 | The Zn^{2+}-specific chelator *o*-phenanthroline does not inhibit histone pre-mRNA processing. (**A**) The histone H4–12 pre-mRNA substrate used for *in vitro* processing. The underlined sequence is complementary to the 5′-end of U7 snRNA. Two alternative positions (major and minor) of endonucleolytic cleavage are indicated by large and small arrows, respectively. (**B**) *In vitro* processing reactions in HeLa nuclear extract in the presence of 10 mM EDTA and the indicated increasing concentrations of 1,10-phenanthroline (*o*-phenanthroline, OP), or additional EDTA (10 mM is included in the standard reaction). The extract was preincubated with the chelators for 10 min at 32 °C before the addition of RNA substrate. The positions of the 5′-end-labelled substrate and products are indicated on the left; arrows are as in (**A**). snRNA, small nuclear RNA.

CPSF73 and CPSF100 MBL motifs are needed for activity

Therefore, we decided to test the effect of point mutations in the MBL motifs of both CPSF subunits on cleavage of the histone pre-mRNA. To assess the activity of wild-type and mutant CPSF73, we used a complementation assay that had previously enabled the identification of symplekin as a required processing factor (Kolev & Steitz, 2005). As the addition of symplekin restores processing in heat-inactivated HeLa nuclear extract, the heat-treated extract presumably contains all other required components of the processing machinery, which are still active. As symplekin exists in a complex with CPSF73 and CPSF100, we reasoned that exogenously expressed CPSF subunits might co-purify with sufficient endogenous symplekin to rescue activity of a heat-treated extract.

Tagged CPSF73, carrying both an amino-terminal Myc tag and a carboxy-terminal Flag tag, was expressed in human embryonic kidney (HEK)293 cells in parallel with mutants in MBL motif 2 (H73A, D75A and H76A), motif B (H396A) and a control mutation outside the conserved motifs (S334A; Figs 2,3A). The tagged proteins were purified on anti-Flag resin and eluted with 3 × Flag peptide. Western blot analysis showed that the wild-type and control mutant S334A co-purified with significantly larger amounts of symplekin, CPSF100 and CstF64 than the MBL motif mutants of CPSF73 (Fig 3B). Blotting with a CPSF73 antibody indicated that endogenous CPSF73 is not co-immunoselected with the tagged CPSF73 construct (supplementary Fig 3 online). When the eluates were tested for their ability to rescue the processing activity of heat-inactivated nuclear extract, wild-type and S334A CPSF73 successfully restored endonucleolytic processing, whereas the MBL motif mutants failed to restore cleavage appreciably (Fig 3C).

Similar complementation experiments showed that changes in the MBL motifs of CPSF100 are also deleterious for assembly and processing. Point mutations in MBL motifs 2 and B (H67A and R543A) were compared with wild-type and the control mutation, D289A (Figs 2,4A). Wild-type and D289A CPSF100 were better expressed in HEK293 cells and co-purified with significantly more symplekin, CPSF73 and CstF64 (Fig 4B) than H67A and R543A. The wild-type and D289A eluates restored processing in heat-inactivated extract to levels more than twofold above the H67A and R543A mutants (Fig 4C).

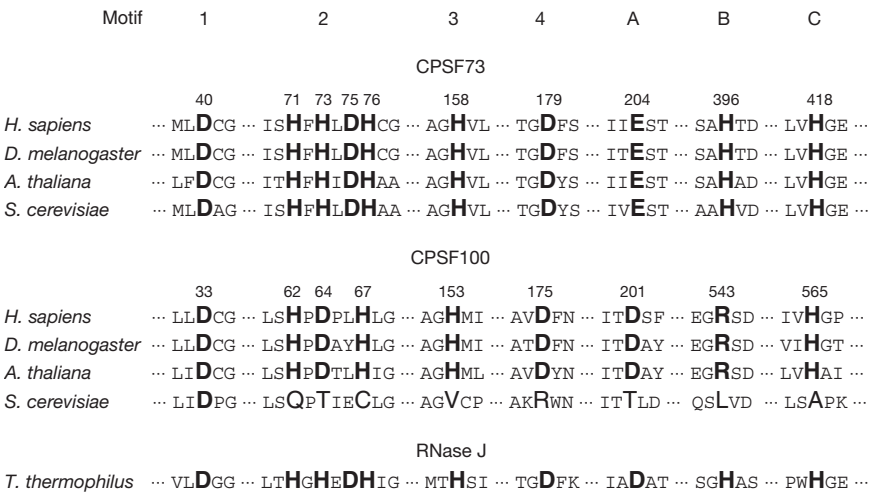


Fig 2 | Conserved MBL motif residues in CPSF73, CPSF100 and RNase J. Signature residues (in larger font) in motifs 1, 2, 3, 4, A, B and C are aligned with two amino acids on each side for *Homo sapiens*, *Drosophila melanogaster*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae* CPSF73 and CPSF100, and for bacterial RNase J. The amino-acid positions are indicated for human CPSF73 and CPSF100 only. CPSF, cleavage and polyadenylation specificity factor.

Triple fusion confirms requirement for MBL motifs

The complementation assay is dependent on the amount of symplekin added to the heat-treated extract. Interpretation of the results is therefore complicated by the fact that some CPSF73 and CPSF100 mutants are expressed and thus purified in lower amounts than the wild-type proteins. Furthermore, there is a potential for rearrangement of the purified complexes after their addition to inactivated extract, which presumably contains active endogenous CPSF73 and CPSF100. To avoid these concerns, we designed a triple-fusion polypeptide containing symplekin, CPSF73 and CPSF100 (Fig 5A).

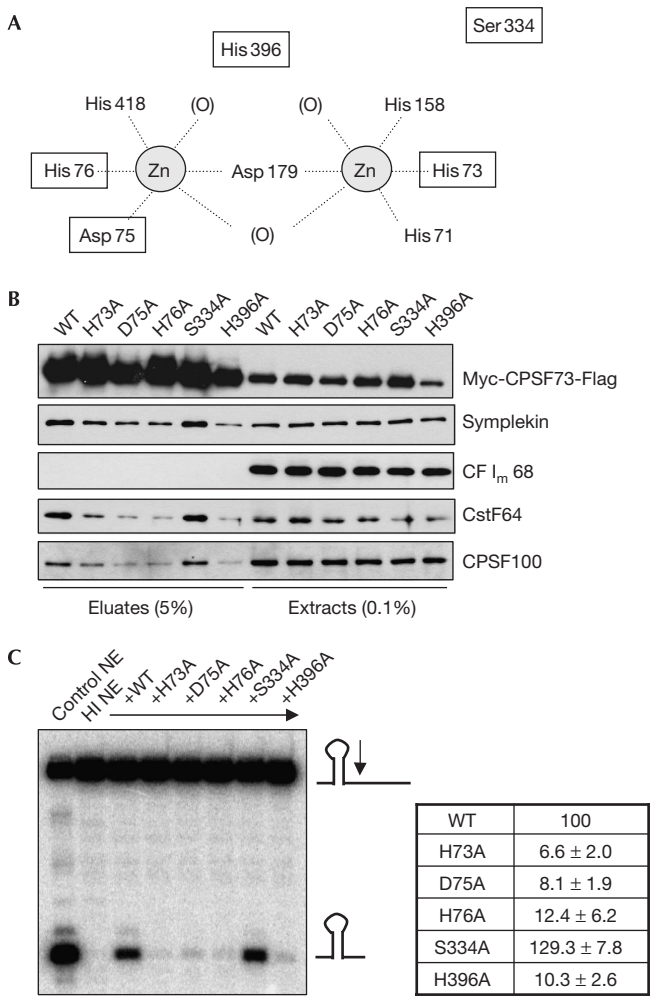
The same MBL motif mutations as described above in CPSF73 and CPSF100, plus another mutation D64A in CPSF100 motif 2 (Fig 2), were inserted into the triple-fusion construct. All fusion proteins were expressed and selected at comparable levels (Fig 5B), ensuring the presence of stoichiometric ratios of symplekin and the two CPSF subunits in the complementation assays (Fig 5C). Although the activity of the wild-type triple fusion was relatively low, it was clear that mutations in the conserved MBL motifs of both CPSF73 (H73A, D75A, H76A and H396A) and CPSF100 (D64A, H67A and R543A) inhibited the ability to activate heat-treated extract by about threefold; by contrast, mutations lying outside those motifs (S334A for CPSF73 and D289A for CPSF100) did not (see Fig 5C, table). The slightly higher activity of MBL mutations in the triple fusion (particularly in the case of CPSF73) is probably due to the relatively low activity of this construct compared with the activities of the individual CPSF subunits (Figs 3C,4C), which are comparable with background restoration of activity, probably owing to trace amounts of co-purifying endogenous symplekin.

DISCUSSION

Recent evidence has shown that CPSF73 is likely to be the endoribonuclease involved in 3′-end formation of eukaryotic mRNAs. Initially, this protein was categorized by bioinformatics as a member of the MBL family (Aravind, 1999; Callebaut *et al*, 2002). Later, it was shown to UV crosslink to precursors of both polyadenylated and histone mRNAs in the vicinity of the site for endonucleolytic attack (Ryan *et al*, 2004; Dominski *et al*, 2005a). More recently, the solved X-ray crystal structure of human CPSF73 showed two Zn atoms bound by the MBL motifs, and recombinant CPSF73 expressed in bacteria was reported to have weak nonspecific endoribonucleolytic activity (Mandel *et al*, 2006).

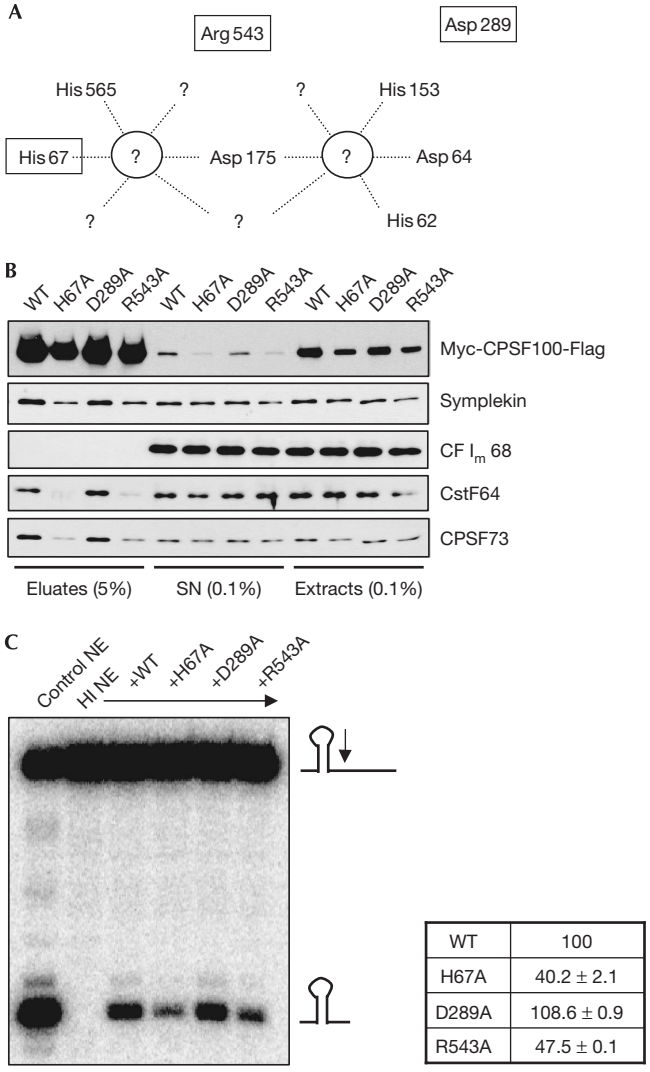
Here, we provide the first biochemical evidence, to our knowledge, that changes in the MBL motifs of CPSF73 are deleterious for its endonucleolytic activity in the processing of histone pre-mRNAs. Obtaining direct evidence for the specific catalytic function of CPSF73 on the basis of active site mutations has been hampered by the lack of antibodies that successfully deplete the native protein from nuclear extracts—the *in vitro* system of choice for studying pre-mRNA processing; as the protein functions in the context of large complexes containing several subunits, it has not been possible to avoid depletion of other factors. Instead, we used a complementation assay with heat-inactivated HeLa nuclear extract, which relies on the addition of wild-type or mutant CPSF73 complexed or fused to symplekin, the protein that is inactivated by the heat treatment (Kolev & Steitz, 2005).

To our surprise, experiments with both CPSF73 and the presumably inactive CPSF100 MBL subunit yielded similar results. In each case, changing residues within the MBL motifs (Fig 2) significantly reduced endonucleolytic activity, whereas mutations outside these motifs had minimal effect (Figs 3–5). In the complementation assays of the individual CPSF subunits, the MBL motif mutants of either CPSF73 or CPSF100 co-purify with significantly lower amounts of other components of the cleavage apparatus (symplekin, CPSF and CstF subunits). This indicates that the conserved motifs of both the 73 and 100 kDa subunit of CPSF are required for correct assembly of the endonuclease complex. Lower levels of these other cleavage complex components can be visualized by Western blotting (Figs 3,4) but not by staining of polyacrylamide gels with Coomassie stain (supplementary Fig 4 online). The assembly does not depend on the presence of RNA,



WT	100
H73A	6.6 ± 2.0
D75A	8.1 ± 1.9
H76A	12.4 ± 6.2
S334A	129.3 ± 7.8
H396A	10.3 ± 2.6

Fig 3 | Changes in the MBL motifs of CPSF73 affect assembly and activity of the histone pre-mRNA cleavage complex. (**A**) Schematic diagram of the Zn-binding site of human CPSF73. A water molecule oxygen and (substrate) phosphate oxygens are depicted as (O). Boxed residues were individually changed to alanine; Ser 334 resides outside the putative active site. (**B**) Myc-CPSF73-Flag, containing the WT sequence or alanine mutations, was immunoprecipitated with anti-Flag resin from HEK293 cell extracts, eluted with 3 × Flag peptide and visualized on a Western blot with anti-Myc. Co-immunoselected proteins were detected with antibodies. (**C**) Complementation assay for *in vitro* processing of the H4–12 substrate in heat-inactivated (50 °C for 15 min) nuclear extract (HI NE). The first lane is a reaction with the untreated extract. The immunopurified complexes containing WT or alanine mutants of the tagged CPSF73 were used to supplement the heat-inactivated extract. The positions of the substrate and product are indicated on the right. The percentage processing was determined with ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). The recovery of processing efficiency for each mutant was normalized to that of the WT protein, set at 100%. The table shows the average of two independent experiments performed with several preparations of HeLa nuclear extract and Myc-CPSF73-Flag proteins. CPSF, cleavage and polyadenylation specificity factor; HEK, human embryonic kidney; MBL, metallo-β-lactamase; WT, wild type.

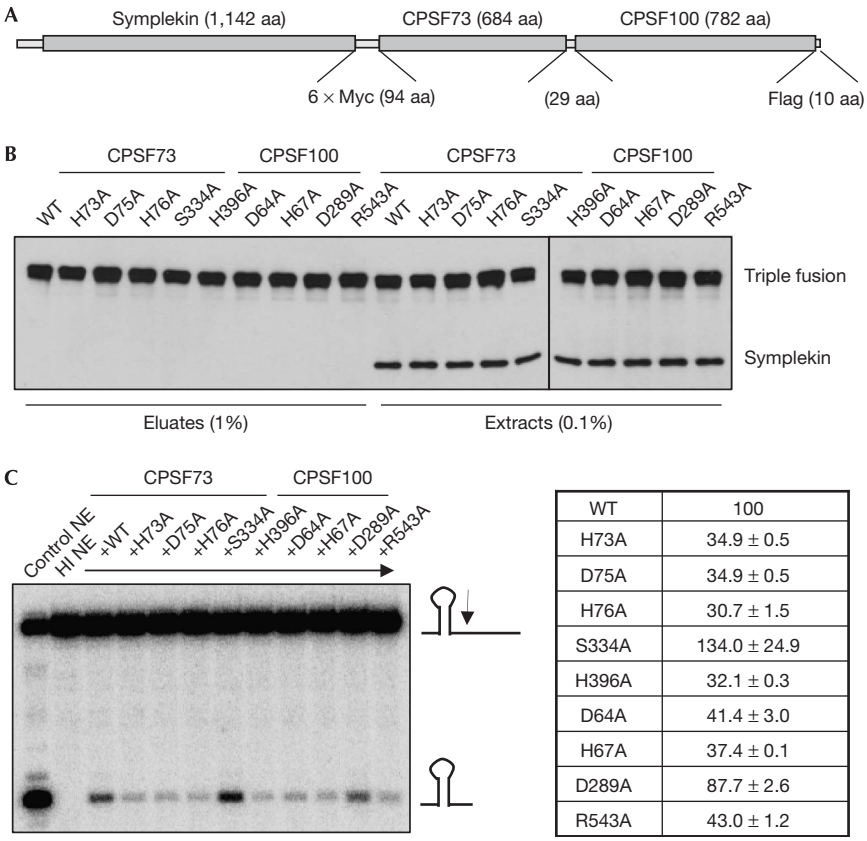


WT	100
H67A	40.2 ± 2.1
D289A	108.6 ± 0.9
R543A	47.5 ± 0.1

Fig 4 | Changes in conserved MBL residues in CPSF100 inhibit complex assembly and histone pre-mRNA cleavage. (**A**) Schematic diagram of the putative Zn-binding site of mammalian CPSF100. Question marks depict unknown components in positions comparable with those in Fig 3A. Boxed residues were changed to alanine; Asp 289 is positioned outside the putative Zn-binding site. (**B**,**C**) Myc-CPSF100-Flag, containing the WT sequence or alanine mutations, was assayed for interactions and processing activity as in Fig 3B and C. CPSF, cleavage and polyadenylation specificity factor; MBL, metallo-β-lactamase; WT, wild type.

as it was insensitive to RNase A (data not shown). A complementation assay with the triple fusion of symplekin, CPSF73 and CPSF100 showed that the defect occurs even when the proteins are stoichiometric (Fig 5; supplementary Fig 4 online).

How do changes in the MBL domains of CPSF100 and CPSF73 affect assembly of the cleavage machinery? At present, we cannot distinguish the effects on the interaction between these two subunits from that of interactions with other proteins. Limited proteolysis with trypsin and proteinase K indicated that CPSF100 MBL motif mutants show alterations in the structure of the



WT	100
H73A	34.9 ± 0.5
D75A	34.9 ± 0.5
H76A	30.7 ± 1.5
S334A	134.0 ± 24.9
H396A	32.1 ± 0.3
D64A	41.4 ± 3.0
H67A	37.4 ± 0.1
D289A	87.7 ± 2.6
R543A	43.0 ± 1.2

Fig 5 | A triple-fusion protein containing the two MBL subunits and symplekin shows that both subunits are required for histone pre-mRNA endonucleolytic activity. (**A**) Diagram of the symplekin–CPSF73–CPSF100 fusion. The symplekin sequence contains 1,142 aa, previously annotated as the full-length protein (Keon *et al*, 1996; accession AAC50667), and a further 103 aa at the N terminus (see the supplementary information online). (**B**) The Flag-tagged construct, containing the WT sequence or alanine mutations in the indicated CPSF subunit, was purified with anti-Flag resin from HEK293 cell extracts, eluted with 3 × Flag peptide and visualized by anti-symplekin Western blotting. The positions of the triple fusion and endogenous symplekin are indicated. (**C**) Complementation assay for *in vitro* processing of the H4–12 substrate in heat-inactivated HeLa nuclear extract (HI NE). The reactions were supplemented with the purified WT or alanine mutants. The percentage processing for each complementation reaction was determined with ImageQuant software (Molecular Dynamics). The recovery of processing efficiency for each mutant was normalized to that of the WT construct, set at 100%. The table shows the average of two experiments performed with several preparations of the triple-fusion constructs. CPSF, cleavage and polyadenylation specificity factor; HEK, human embryonic kidney; MBL, metallo-β-lactamase; WT, wild type.

C-terminal portion, whereas CPSF73 mutants yielded no changes detectable by using this method (supplementary Fig 5 online; data not shown). Alternative efforts to study the CPSF73–CPSF100 interaction directly by *in vitro* pull-down assays were inconclusive owing to their apparently weak association. It has been shown previously that the C-terminal 245 aa of mammalian CPSF73 interact weakly with full-length CPSF100 in a yeast two-hybrid assay (Dominski *et al*, 2005b). This finding is similar to the observation that RNase J—a β-CASP protein—requires its C-terminal domain for the formation of the active form of the enzyme, a homodimer (de la Sierra-Gallay *et al*, 2008). Interestingly, a single-point mutation of a metal-chelating residue in motif 2 (His 76, equivalent to His 73 in human CPSF73) of RNase J1 leads to a decreased ability to form a homodimer (Mathy *et al*, 2007), implying that its C-terminal domain is insufficient for dimerization. Our results indicate a similar situation for the two mammalian β-CASP subunits of CPSF—point mutations in the MBL motifs of either CPSF73 or CPSF100 affect the formation of a higher order complex that is essential for the activity of the enzyme.

Although we cannot conclude that the MBL motifs of mammalian CPSF100 bind metal ions, several residues with metal-binding potential are conserved (Fig 2; supplementary Fig 2 online). However, the lack of absolute evolutionary conservation of these amino acids (Fig 2; supplementary Fig 2 online) raises several questions. First, are both subunits in the CPSF73–CPSF100 dimer catalytically competent, as in the case of the RNase J homodimer, or is mammalian CPSF100 deficient in nuclease activity (as is suspected for yeast CPSF100/Ydh1 and mammalian Int9 in the Int11–Int9 dimer involved in the maturation of snRNAs)? We have not detected any specific or nonspecific nuclease activity (endo or exo) associated with our purified, tagged CPSF73, CPSF100 or triple-fusion proteins (data not shown). Second, how do CPSF73 and CPSF100

interact? Third, what is the function of the β-CASP domain and how is RNA accommodated in the dimer for cleavage? We believe that answers will be provided only by solving the structure of the CPSF73–CPSF100 heterodimer in the presence of bound substrate RNA. Although the effects of CPSF73 MBL motif mutations are slightly greater in our biochemical assays, the comparable residues of CPSF100 are also clearly required for the endonuclease activity in 3′-end formation of histone mRNAs.

We expect our conclusions from the analyses of histone pre-mRNA processing to also apply to the endonucleolytic 3′-end processing of polyadenylated mRNAs, as the components that we studied in this work—CPSF73, CPSF100 and symplekin—are common to both machineries. Although histone mRNA maturation uniquely involves the U7 snRNP, shared components with the polyadenylation machinery point to a common ancestry in early eukaryotic evolution. It is possible that both systems diverged from a primordial mechanism reminiscent of spliced leader snRNP-dependent *trans*-splicing of polycistronic transcripts, a process in which spliced leader snRNP couples *trans*-splicing at the 5′-end of the downstream mRNA with 3′-end processing of the upstream mRNA (Ullu *et al*, 1993; Evans *et al*, 2001).

METHODS

A detailed description of the experimental procedures is provided in the supplementary information online.

Protein expression, purification and *in vitro* complementation assays. All proteins were expressed in HEK293 cells by transient transfection. The Flag-tagged proteins were purified on anti-Flag M2 affinity gel (Sigma, St Louis, MO, USA) and eluted with 3 × Flag peptide (Sigma). Western blots were performed with antibodies against c-Myc (clone 9E10; Sigma), CPSF73 (from David Bentley, University of Colorado School of Medicine), CPSF100 (Atlas Antibodies, Stockholm, Sweden), CF I_m 68 (from Walter Keller, University of Basel), CstF64 (from Clinton MacDonald, Texas Tech University) and symplekin (BD Biosciences, San Jose, CA, USA). HeLa nuclear extract was prepared and heat-inactivated as described previously (Kolev & Steitz, 2005), and processing reactions with the 5′-end-labelled H4–12 histone pre-mRNA substrate and heat-treated extract were complemented with the eluted Flag-tagged proteins.

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS

We thank D. Bentley, W. Keller and C. MacDonald for antibodies, K. Friend and K. Tycowski for comments on the manuscript, and A. Miccinello for editorial assistance. This study was supported by grant R01GM026154 from the National Institute of General Medical Sciences (NIGMS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIGMS or the National Institutes of Health. J.A.S. is an investigator of the Howard Hughes Medical Institute.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

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

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

Callebaut I, Moshous D, Mornon JP, de Villartay JP (2002) Metallo-β-lactamase fold within nucleic acids processing enzymes: the β-CASP family. *Nucleic Acids Res* **30:** 3592–3601

de la Sierra-Gallay IL, Zig L, Jamalli A, Putzer H (2008) Structural insights into the dual activity of RNase J. *Nat Struct Mol Biol* **15:** 206–212

Dominski Z (2007) Nucleases of the metallo-β-lactamase family and their role in DNA and RNA metabolism. *Crit Rev Biochem Mol Biol* **42:** 67–93

Dominski Z, Marzluff WF (2007) Formation of the 3′ end of histone mRNA: getting closer to the end. *Gene* **396:** 373–390

Dominski Z, Yang XC, Marzluff WF (2005a) The polyadenylation factor CPSF-73 is involved in histone-pre-mRNA processing. *Cell* **123:** 37–48

Dominski Z, Yang XC, Purdy M, Wagner EJ, Marzluff WF (2005b) A CPSF-73 homologue is required for cell cycle progression but not cell growth and interacts with a protein having features of CPSF-100. *Mol Cell Biol* **25:** 1489–1500

Evans D, Perez I, MacMorris M, Leake D, Wilusz CJ, Blumenthal T (2001) A complex containing CstF-64 and the SL2 snRNP connects mRNA 3′ end formation and *trans*-splicing in *C. elegans* operons. *Genes Dev* **15:** 2562–2571

Gick O, Krämer A, Keller W, Birnstiel ML (1986) Generation of histone mRNA 3′ ends by endonucleolytic cleavage of the pre-mRNA in a snRNP-dependent *in vitro* reaction. *EMBO J* **5:** 1319–1326

Ishikawa H, Nakagawa N, Kuramitsu S, Masui R (2006) Crystal structure of TTHA0252 from *Thermus thermophilus* HB8, a RNA degradation protein of the metallo-β-lactamase superfamily. *J Biochem* **140:** 535–542

Keon BH, Schäfer S, Kuhn C, Grund C, Franke WW (1996) Symplekin, a novel type of tight junction plaque protein. *J Cell Biol* **134:** 1003–1018

Kolev NG, Steitz JA (2005) Symplekin and multiple other polyadenylation factors participate in 3′-end maturation of histone mRNAs. *Genes Dev* **19:** 2583–2592

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

Mandel CR, Bai Y, Tong L (2008) Protein factors in pre-mRNA 3′-end processing. *Cell Mol Life Sci* **65:** 1099–1122

Mathy N, Bénard L, Pellegrini O, Daou R, Wen T, Condon C (2007) 5′-to-3′ exoribonuclease activity in bacteria: role of RNase J1 in rRNA maturation and 5′ stability of mRNA. *Cell* **129:** 681–692

Redko Y, Li de Lasierra-Gallay I, Condon C (2007) When all's zed and done: the structure and function of RNase Z in prokaryotes. *Nat Rev Microbiol* **5:** 278–286

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

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

Ullu E, Matthews KR, Tschudi C (1993) Temporal order of RNA-processing reactions in trypanosomes: rapid *trans* splicing precedes polyadenylation of newly synthesized tubulin transcripts. *Mol Cell Biol* **13:** 720–725

Wagner EJ, Burch BD, Godfrey AC, Salzler HR, Duronio RJ, Marzluff WF (2007) A genome-wide RNA interference screen reveals that variant histones are necessary for replication-dependent histone pre-mRNA processing. *Mol Cell* **28:** 692–699