Rrp47 and the function of the Sas10/C1D domain

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Abstract

The Sas10/C1D domain is found in a small group of eukaryotic proteins that have functions in RNA processing events, translational control and DNA repair mechanisms. The domain is predicted to be -helical in nature and comprises approximately 80 amino acid residues. While the Sas10/C1D domain has yet to be functionally characterised, available data suggest that this domain forms a binding surface for specific interactions with other proteins and can concomitantly interact with RNA or DNA. This property of the Sas10/C1D domain may facilitate this family of proteins to dock other proteins onto nucleic acid substrates.

Introduction

The budding yeast (*Saccharomyces cerevisiae*) protein Rrp47 (also known as Lrp1 or yC1D) and its mammalian homologue C1D are nuclear proteins that bind both DNA and RNA [1-3]. Most work on the yeast protein is based on its physical association with the exosome nuclease complex [4-6] and the role of this complex in RNA processing and degradation pathways (for recent reviews of exosome structure and function, see [7-11]), while C1D was initially characterised as a DNA repair factor. Nevertheless, there is strong evidence of functional conservation of Rrp47/C1D; both proteins interact directly with the catalytic exosome component Rrp6 (known as PM-Scl100 in humans) [2,3] and similar defects in 5.8S rRNA maturation are observed in yeast *rrp47-Δ* or *rrp6-Δ* mutants and upon depletion of C1D in mammalian cells [3,5,12], while yeast *rrp47-Δ* mutants show defects in DNA repair [13,14]. Bioinformatical analyses [15] have identified a domain within Rrp47/C1D, known as the Sas10/C1D domain, that is also found in the U3 small nucleolar RNA (snoRNA)-associated proteins Sas10 (also known as Utp3) and Lcp5, as well as the metazoan protein neuroguidin. Sas10 and Lcp5 are required for the synthesis of 18S rRNA [16,17], while neuroguidin is an eIF4E-binding protein that is required for cytoplasmic polyadenylation-dependent translational control in neuronal cells [18]. Here, I review the data available for the function of members of this protein family and discuss data pertaining to the molecular function of the Sas10/C1D domain.

Structure and expression of Rrp47/C1D

Rrp47 and C1D are small, basic proteins of 21kDa and 16kDa, respectively. There is no detailed structural information currently available but sequence homology and secondary structure prediction

programmes suggest that the N-terminal ~120 residues of Rrp47 comprise a conserved -helical domain, while the C-terminal region is less structured and more variable. The N-terminal domain includes residues 10-89 of Rrp47 and shows homology with other members of the Sas10/C1D family (Fig. 1). The C-termini of Rrp47 homologues are typically very rich in basic residues, particularly lysine. C1D can be phosphorylated in vitro by the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and is associated with this protein in cell lysates [19]. However, the importance of C1D phosphorylation to its function in vivo remains unclear. Similarly, the yeast protein can also be phosphorylated by the protein kinases Atg1 and Yck1 in vitro [20] but is not observed as a phosphoprotein in yeast cell extracts, either in mass spectrometric analyses of exosome complexes [21] or by in vivo orthophosphate labelling experiments (our unpublished data). Recombinant Rrp47 is expressed in *Escherichia coli* as a multimeric complex [2]. Consistent with roles in RNA surveillance and DNA repair, both yeast and mammalian proteins are localised to the cell nucleus [1,14,22].

Protein interactions involving Rrp47/C1D

Rrp47 is associated with Rrp6-containing exosome complexes [5,6] and interacts directly with Rrp6 in vitro [2]. The interaction occurs between the N-terminal region of Rrp47 that encompasses the Sas10/C1D domain and the N-terminal PMC2NT domain of Rrp6 [2](our unpublished data). Rrp47 levels are severely depleted in the absence of Rrp6 and are normalised upon expression of the PMC2NT domain alone [2], suggesting that Rrp47 stability is dependent upon its interaction with Rrp6. Thus, the depletion of Rrp47 should be taken into account when interpreting data from experiments using *rrp6-Δ* alleles. An interaction is also observed between the mammalian proteins C1D and PMScl-100 [3].

C1D has been identified through two hybrid screens as an interactor of the nuclear hormone receptor RevErb [23], DNA-dependent protein kinase [19] and the translin-associated factor X [24], while the homologous protein from *Schizosaccharomyces pombe*, Cti1, was shown to interact with condensin [25]. Analyses of proteins copurifying with TAP-tagged Rrp47 from yeast cell lysates solely identified known exosome components [6], suggesting that the exosome is the only protein complex with which yeast Rrp47 is stably associated. However, this does not eliminate the possibility of additional, transient interactions or complexes involving lower abundance species. Indeed, protein capture experiments using recombinant Rrp47 and yeast cell lysates, in association with analysis of RNA processing phenotypes of *rrp47* mutants, demonstrate functionally relevant interactions between Rrp47 and the snoRNP proteins Nop56 and Nop58 (our unpublished data).

Rrp47/C1D function as an RNA-binding protein

Rrp47 binds structured RNA such as poly(A)/(U) or tRNA in vitro but does not bind poly(A) [2]. Similarly, C1D interacts with tRNA and poly(G), but does not bind poly(A), poly(U) or poly(C) [3]. Rrp47 and C1D lack functionally characterised domains or motifs that are known to have RNA binding activity [26,27] and so the molecular basis of this interaction is of particular interest. The highly conserved basic nature of the C-terminus probably makes key interactions in RNA binding [2,28] but would be unable in itself to mediate specific binding to structured RNA. This suggests that RNA binding involves contacts with both the Sas10/C1D domain and the basic C-terminus. It has been shown that Rrp47 can bind both RNA and the PMC2NT domain of Rrp6 concomitantly, demonstrating that the binding surfaces for these ligands do not physically overlap [2].

What is the molecular function of Rrp47 in RNA processing and RNA surveillance? Yeast strains lacking Rrp47 or Rrp6 accumulate cellular RNAs that, in wild-type cells, are normally processed efficiently to mature RNAs or rapidly degraded. These include 3' extended, incompletely processed precursors to 5.8S rRNA, snoRNAs and small nuclear RNAs (snRNAs), excised RNA fragments generated during RNA processing reactions such as the pre-rRNA 5' external transcribed spacer (5' ETS) region, and cryptic unstable transcripts (CUTs) [5,6,29-31]. Rrp47 is not required for the expression of Rrp6 or the binding of Rrp6 with exosome complexes [5]. The patterns of stable RNA precursors observed in *rrp47-Δ* and *rrp6-Δ* strains by northern blot analyses are largely indistinguishable [5] while distinct effects are seen in these mutants upon analysis of global mRNA profiles [14,32], suggesting that Rrp47 functions together with Rrp6 on some, but not all, of its substrates.

Purified recombinant Rrp6 degrades RNA in a distributive manner and degrades stem loop structures slowly [33,34], whilst Rrp47 binds specifically to structured RNA [2]. A plausible mechanism is that Rrp47 promotes the progression of Rrp6 through structured RNA elements (Fig. 2A). By allowing Rrp6 to interact indirectly with structured RNAs, Rrp47 might increase the retention time of substrates on Rrp6. Alternatively, through interacting with the adjacent PMC2NT domain, Rrp47 might position 3' single-stranded extensions of structured RNA elements close to the catalytic centre of Rrp6. Such a function has been proposed for the C'-terminal HRDC domain of RNase D [35]. Alternatively, but not mutually exclusive with the possible mechanisms noted above, interactions between Rrp47 and other

RNA processing factors may contribute to substrate recognition or processing (Fig. 2B).

Rrp47/C1D as a DNA-binding protein

C1D was isolated as a protein with "exceptional DNA affinity", since it remained bound to DNA under denaturing conditions (alkaline extraction and phenol extraction) [1], and Rrp47 and C1D both bind to circular plasmid or linear double-stranded DNA in vitro [1,2]. The dissociation constant of yeast Rrp47 for DNA is comparable to that of RNA, both being approximately 1μM (calculating the protein concentration based on the predicted molecular weight of the monomeric protein) [2]. RNA is an effective competitor in a DNA binding assay [2], strongly suggesting that the ligands occupy the same binding site on Rrp47. The expression level of Rrp47 is comparable to that of Rrp6 [2], which is reported to be approximately 2,000 molecules per yeast cell [36]. Given the volume of a yeast cell nucleus is approximately 3μm^3 [37], the intracellular concentration of Rrp47 is close to its dissociation constant. Changes in the effective concentration of Rrp47 would therefore have a large impact on its ability to bind substrates.

Rrp47/C1D is well annotated as a DNA repair factor. A key observation is that C1D physically interacts with the catalytic subunit of the major sensor of DNA double strand breaks, DNA-PK, and stimulates its protein kinase activity [19]. DNA-PK binds to the ends of double-stranded DNA and promotes their joining through the nonhomologous end-joining pathway [38]. DNA-PK is thought to trigger a kinase signalling pathway that allows the recruitment of DNA repair factors to the double-strand break (DSB), and C1D is very efficiently phosphorylated by DNA-PK. Yeast *rrp47-Δ* mutants were subsequently shown to be defective in plasmid-based assays for DNA repair [13]. However, the details of the proposed DNA-PK-dependent signalling pathway are yet to be determined and the role of C1D in DSB repair remains unclear. Moreover, recombinant Rrp47 and C1D bind relaxed and supercoiled, circularised plasmid DNA, just as well as linearised DNA [1,2], and C1D does not require DNA termini to bind to DNA-PKcs or to stimulate its kinase activity [19]. Yeast strains lacking Rrp47 or Rrp6 were reported to be defective in the degradation of specific mRNA transcripts upon exposure to UV irradiation [14]. The *rrp6-Δ* and *rrp47-Δ* mutants are defective in the repair of UV irradiation-induced cyclobutane dimers, and *rrp6-Δ* mutants are synergistically sick in combination with *rad26-Δ* mutants [14]. One possibility is that the sensitivity of *rrp6-Δ* and *rrp47-Δ* mutants to UV irradiation may reflect a quality control function of the exosome to eliminate aberrant transcripts that arise as a result of DNA

damage in transcription-coupled DNA repair [39].

Other members of the Sas10/C1D family

The Sas10/C1D domain is a bioinformatically defined protein domain (Pfam domain PF04000)[15] of approximately 80 amino acid residues that is found in organisms throughout the eukaryotic kingdom (Fig. 1). Almost all of the members of this family contain one or two Sas10/C1D domains and most members lack any other functionally characterised domain. This protein family comprises Sas10, Lcp5 and neuroguidin, in addition to Rrp47/C1D.

Sas10 was initially characterised as a yeast protein that, when overexpressed, partially suppressed silencing of transcriptionally repressed chromatin [40]. Genetic analyses revealed that this suppression functions independently of the Sir protein silencing machinery. Mutants of the exosome complex and its associated cofactor TRAMP also show increased expression of genes encoded by repressed chromatin in a Sir-independent manner [41,42]. One explanation is that overexpression of Sas10 may (partially) titrate out the complex between Rrp47 and Rrp6. Sas10 has been shown to be a component of the U3 snoRNA-containing “small subunit processome” that is required for processing of 18S rRNA from the 35S pre-rRNA [17]. Lcp5 is also required for 18S rRNA processing [16] and is a component of the same 90S preribosomes [43-45].

Neuroguidin, like maskin, binds to both the translation initiation factor eIF4E and the cytoplasmic polyadenylation element (CPE)-binding protein CPEB, and inhibits translation of specific mRNAs in a CPE-dependent manner [18]. Mutational analyses of sequence motifs within neuroguidin that are required for eIF4E binding demonstrate that the Sas10/C1D homology region is not responsible for interaction with eIF4E. Whether neuroguidin binds CPEB via the Sas10/C1D domain is not clear. By analogy with maskin, translation activation is coupled to polyadenylation by Gld2 [46]. Gld2 is a noncanonical poly(A) polymerase related to the TRAMP components Trf4 and Trf5 that promote RNA degradation by the exosome. Thus, both Rrp47 and neuroguidin function in concert with related poly(A) polymerases via bridging proteins (Rrp6 or CPEB). Rrp6, like CPEB, is phosphorylated [47] but the functional relevance of this event is unclear.

Sas10, Lcp5 and neuroguidin all function with RNA but whether these proteins interact directly with RNA requires further direct experimentation. Notably, Sas10, Lcp5 and neuroguidin also have basic

regions at the C-terminus in addition to the Sas10/C1D domain that may contribute to RNA binding.

Conclusions

The Sas10/C1D domain is found in eukaryotic proteins that function in aspects of RNA processing, RNA quality control and translational control. At least in the case of Rrp47, this domain provides contacts in both protein-protein interactions and in the binding of nucleic acids that are key to protein function. Future research will address whether the Sas10/C1D domain represents a general dual functional domain for protein- and RNA interactions, and discern the molecular nature of these interactions and their potential regulation through phosphorylation events.

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References

1 Nehls, P., Keck, T., Greferath, R., Spiess, E., Glaser, T., Rothbarth, K., Stammer, H. and Werner, D. (1998) cDNA cloning, recombinant expression and characterization of polypetides with exceptional DNA affinity. Nucleic Acids Res **26**, 1160-1166

2 Stead, J., Costello, J., Livingstone, M. and Mitchell, P. (2007) The PMC2NT domain of the catalytic exosome subunit Rrp6p provides the interface for binding with its cofactor Rrp47p, a nucleic acid-binding protein. Nucleic Acids Res **35**, 5556-5567

3 Schilders, G., van Dijk, E. and Pruijn, G. (2007) C1D and hMtr4p associate with the human exosome subunit PM/Scl-100 and are involved in pre-rRNA processing. Nucleic Acids Res **35**, 2564-2572

4 Gavin, A., Bösche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J., Michon, A., Cruciat, C., Remor, M., H√∂fert, C., Schelder, M., Brajenovic, M., Ruffner, H., Merino, A., Klein, K., Hudak, M., Dickson, D., Rudi, T., Gnau, V., Bauch, A., Bastuck, S., Huhse, B., Leutwein, C., Heurtier, M., Copley, R., Edelmann, A., Querfurth, E., Rybin, V., Drewes, G., Raida, M., Bouwmeester, T., Bork, P., Seraphin, B., Kuster, B., Neubauer, G. and Superti-Furga, G. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415**, 141-147

5 Mitchell, P., Petfalski, E., Houalla, R., Podtelejnikov, A., Mann, M. and Tollervey, D. (2003) Rrp47p is an exosome-associated protein required for the 3' processing of stable RNAs. Mol Cell Biol **23**, 6982-6992

6 Peng, W., Robinson, M., Mnaimneh, S., Krogan, N., Cagney, G., Morris, Q., Davierwala, A., Grigull, J., Yang, X., Zhang, W., Mitsakakis, N., Ryan, O., Datta, N., Jojic, V., Pal, C., Canadien, V., Richards, D., Beattie, B., Wu, L., Altschuler, S., Roweis, S., Frey, B., Emili, A., Greenblatt, J. and Hughes, T. (2003) A panoramic view of yeast noncoding RNA processing. Cell **113**, 919-933

7 Vanacova, S. and Stefl, R. (2007) The exosome and RNA quality control in the nucleus. EMBO Rep **8**, 651-657

8 Lebreton, A. and Séraphin, B. (2008) Exosome-mediated quality control: substrate recruitment

and molecular activity. Biochim Biophys Acta **1779**, 558-565

9 Schmid, M. and Jensen, T. (2008) The exosome: a multipurpose RNA-decay machine. Trends Biochem Sci **33**, 501-510

10 Belostotsky, D. (2009) Exosome complex and pervasive transcription in eukaryotic genomes. Curr Opin Cell Biol **21**, 352-458

11 Lykke-Andersen, S., Brodersen, D. and Jensen, T. (2009) Origins and activities of the eukaryotic exosome. J Cell Sci **122**, 1487-1494

12 Briggs, M., Burkard, K. and Butler, J. (1998) Rrp6p, the yeast homologue of the human PM-Scl 100-kDa autoantigen, is essential for efficient 5.8 S rRNA 3' end formation. J Biol Chem **273**, 13255-13263

13 Erdemir, T., Bilican, B., Cagatay, T., Goding, C. and Yavuzer, U. (2002) Saccharomyces cerevisiae C1D is implicated in both non-homologous DNA end joining and homologous recombination. Mol Microbiol **46**, 947-957

14 Hieronymus, H., Yu, M. and Silver, P. (2004) Genome-wide mRNA surveillance is coupled to mRNA export. Genes Dev **18**, 2652-2662

15 Finn, R., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J., Gavin, O., Gunasekaran, P., Ceric, G., Forslund, K., Holm, L., Sonnhammer, E., Eddy, S. and Bateman, A. (2009) The Pfam protein families database. Nucleic Acids Res **38** (Database issue), D211-D222

16 Wiederkehr, T., Prétôt, R. and Minvielle-Sebastia, L. (1998) Synthetic lethal interactions with conditional poly(A) polymerase alleles identify LCP5, a gene involved in 18S rRNA maturation. RNA **4**, 1357-1372

17 Dragon, F., Gallagher, J., Compagnone-Post, P., Mitchell, B., Porwancher, K., Wehner, K., Wormsley, S., Settlage, R., Shabanowitz, J., Osheim, Y., Beyer, A., Hunt, D. and Baserga, S. (2002) A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. Nature **417**, 967-970

18 Jung, M., Lorenz, L. and Richter, J. (2006) Translational control by neuroguidin, a eukaryotic initiation factor 4E and CPEB binding protein. Mol Cell Biol **26**, 4277-4287

19 Yavuzer, U., Smith, G., Bliss, T., Werner, D. and Jackson, S. (1998) DNA end-independent activation of DNA-PK mediated via association with the DNA-binding protein C1D. Genes Dev **12**, 2188-2199

20 Ptacek, J., Devgan, G., Michaud, G., Zhu, H., Zhu, X., Fasolo, J., Guo, H., Jona, G., Breitkreutz, A., Sopko, R., McCartney, R., Schmidt, M., Rachidi, N., Lee, S., Mah, A., Meng, L., Stark, M., Stern, D., De Virgilio, C., Tyers, M., Andrews, B., Gerstein, M., Schweitzer, B., Predki, P. and Snyder, M. (2005) Global analysis of protein phosphorylation in yeast. Nature **438**, 679-684

21 Synowsky, S., van den Heuvel, R., Mohammed, S., Pijnappel, P. and Heck, A. (2006) Probing genuine strong interactions and post-translational modifications in the heterogeneous yeast exosome protein complex. Mol Cell Proteomics **5**, 1581-1592

22 Kumar, A., Agarwal, S., Heyman, J., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., Cheung, K., Miller, P., Gerstein, M., Roeder, G. and Snyder, M. (2002) Subcellular localization of the yeast proteome. Genes Dev **16**, 707-719

23 Zamir, I., Dawson, J., Lavinsky, R., Glass, C., Rosenfeld, M. and Lazar, M. (1997) Cloning and characterization of a corepressor and potential component of the nuclear hormone receptor repression complex. Proc Natl Acad Sci U S A **94**, 14400-14405

24 Erdemir, T., Bilican, B., Oncel, D., Goding, C. and Yavuzer, U. (2002) DNA damage-dependent interaction of the nuclear matrix protein C1D with Translin-associated factor X (TRAX). J Cell Sci **115**, 207-216

25 Chen, E., Sutani, T. and Yanagida, M. (2004) Cti1/C1D interacts with condensin SMC hinge and supports the DNA repair function of condensin. Proc Natl Acad Sci U S A **101**, 8078-8083

26 Chen, Y. and Varani, G. (2005) Protein families and RNA recognition. FEBS J **272**, 2088-2097

27 Stefl, R., Skrisovska, L. and Allain, F. (2005) RNA sequence- and shape-dependent recognition by proteins in the ribonucleoprotein particle. EMBO Rep **6**, 33-38

28 Spriggs, R., Murakami, Y., Nakamura, H. and Jones, S. (2009) Protein function annotation from sequence: prediction of residues interacting with RNA. Bioinformatics **25**, 1492-1497

29 Wyers, F., Rougemaille, M., Badis, G., Rousselle, J., Dufour, M., Boulay, J., Régnault, B., Devaux, F., Namane, A., Séraphin, B., Libri, D. and Jacquier, A. (2005) Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. Cell **121**, 725-737

30 Arigo, J., Eyler, D., Carroll, K. and Corden, J. (2006) Termination of cryptic unstable transcripts is directed by yeast RNA-binding proteins Nrd1 and Nab3. Mol Cell **23**, 841-851

31 Milligan, L., Decourty, L., Saveanu, C., Rappsilber, J., Ceulemans, H., Jacquier, A. and Tollervey, D. (2008) A yeast exosome cofactor, Mpp6, functions in RNA surveillance and in the degradation of noncoding RNA transcripts. Mol Cell Biol **28**, 5446-5457

32 Houalla, R., Devaux, F., Fatica, A., Kufel, J., Barrass, D., Torchet, C. and Tollervey, D. (2006) Microarray detection of novel nuclear RNA substrates for the exosome. Yeast **23**, 439-454

33 Burkard, K. and Butler, J. (2000) A nuclear 3'-5' exonuclease involved in mRNA degradation interacts with Poly(A) polymerase and the hnRNA protein Npl3p. Mol Cell Biol **20**, 604-616

34 Liu, Q., Greimann, J. and Lima, C. (2006) Reconstitution, activities, and structure of the eukaryotic RNA exosome. Cell **127**, 1223-1237

35 Zuo, Y., Wang, Y. and Malhotra, A. (2005) Crystal structure of Escherichia coli RNase D, an exoribonuclease involved in structured RNA processing. Structure **13**, 973-984

36 Ghaemmaghami, S., Huh, W., Bower, K., Howson, R., Belle, A., Dephoure, N., O'Shea, E. and Weissman, J. (2003) Global analysis of protein expression in yeast. Nature **425**, 737-741

37 Jorgensen, P., Edgington, N., Schneider, B., Rupes, I., Tyers, M. and Futcher, B. (2007) The size of the nucleus increases as yeast cells grow. Mol Biol Cell **18**, 3523-3532

38 Weterings, E. and Chen, D. (2008) The endless tale of non-homologous end-joining. Cell Res **18**, 114-124

39 Mirkin, N., Fonseca, D., Mohammed, S., Cevher, M., Manley, J. and Kleiman, F. (2008) The 3' processing factor CstF functions in the DNA repair response. Nucleic Acids Res **36**, 1792-1804

40 Kamakaka, R. and Rine, J. (1998) Sir- and silencer-independent disruption of silencing in Saccharomyces by Sas10p. Genetics **149**, 903-914

41 Vasiljeva, L., Kim, M., Terzi, N., Soares, L. and Buratowski, S. (2008) Transcription termination and RNA degradation contribute to silencing of RNA polymerase II transcription within heterochromatin. Mol Cell **29**, 313-323

42 Houseley, J., Kotovic, K., El Hage, A. and Tollervey, D. (2007) Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. EMBO J **26**, 4996-5006

43 Uetz, P., Giot, L., Cagney, G., Mansfield, T., Judson, R., Knight, J., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S. and Rothberg, J. (2000) A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature **403**, 623-627

44 Hoang, T., Peng, W., Vanrobays, E., Krogan, N., Hiley, S., Beyer, A., Osheim, Y., Greenblatt, J., Hughes, T. and Lafontaine, D. (2005) Esf2p, a U3-associated factor required for small-subunit processome assembly and compaction. Mol Cell Biol **25**, 5523-5534

45 Krogan, N., Cagney, G., Yu, H., Zhong, G., Guo, X., Ignatchenko, A., Li, J., Pu, S., Datta, N., Tikuisis, A., Punna, T., Peregr√≠n-Alvarez, J., Shales, M., Zhang, X., Davey, M., Robinson, M., Paccanaro, A., Bray, J., Sheung, A., Beattie, B., Richards, D., Canadien, V., Lalev, A., Mena, F., Wong, P., Starostine, A., Canete, M., Vlasblom, J., Wu, S., Orsi, C., Collins, S., Chandran, S., Haw, R.,

Rilstone, J., Gandi, K., Thompson, N., Musso, G., St Onge, P., Ghanny, S., Lam, M., Butland, G., Altaf-Ul, A., Kanaya, S., Shilatifard, A., O'Shea, E., Weissman, J., Ingles, C., Hughes, T., Parkinson, J., Gerstein, M., Wodak, S., Emili, A. and Greenblatt, J. (2006) Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. Nature **440**, 637-643

46 Barnard, D., Ryan, K., Manley, J. and Richter, J. (2004) Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell **119**, 641-651

47 Synowsky, S., van Wijk, M., Raijmakers, R. and Heck, A. (2008) Comparative Multiplexed Mass Spectrometric Analyses of Endogenously Expressed Yeast Nuclear and Cytoplasmic Exosomes. J Mol Biol **385**, 1300-1313

Figures

Figure 1. Alignment of the Sas10/C1D domain.

Sequences were taken from the full alignment for Pfam family PF04000 (http://pfam.sanger.ac.uk). Rrp47 is synonymous with Lrp1. Residues that showed conservation within the full alignment were colour-coded by hand, using the following key: glycine, brown; proline, yellow; small or hydrophobic residues (A,V,L,I,M,F,W), turquoise; charged amino acids (D,E,R,K), dark green; histidine or tyrosine, bright green.

Figure 2. Roles of Rrp47 in Rrp6-mediated RNA degradation.

(A) Rrp6 is able to degrade nonstructured RNA efficiently but progresses slowly through structural elements. Rrp47 is able to interact with structured elements within RNA whilst remaining bound to Rrp6. Rrp47 may enable Rrp6 to progress more readily through structural elements by retaining the enzyme on the RNA and/or by directly presenting the free 3' end of the RNA to the catalytic domain of Rrp6. (B) Protein-protein interactions between Rrp47 and other processing factors (labelled "X") allow targeting of Rrp6 to the 3' end of its substrates or to recognise assembled ribonucleoprotein (RNP) complexes.

Figure 1:

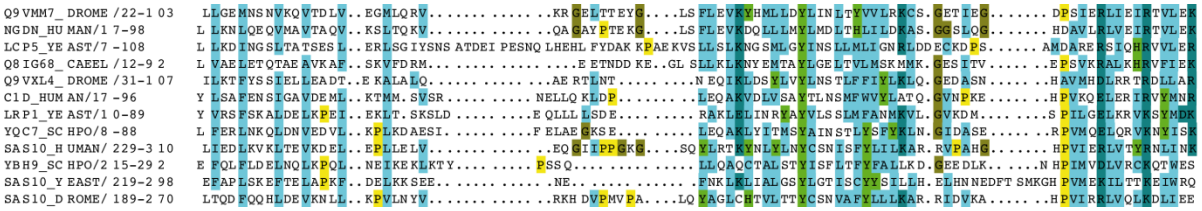

Figure 2A and 2B: