J. Biochem. **136**, 805–812 (2004)
DOI: 10.1093/jb/mvh190

Decapping Reaction of mRNA Requires Dcp1 in Fission Yeast: Its Characterization in Different Species from Yeast to Human

Takeshi Sakuno*, Yasuhiro Araki*, Yuriko Ohya, Satoshi Kofuji, Shinya Takahashi, Shin-ichi Hoshino and Toshiaki Katada†

Department of Physiological Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033

Cleavage of the 5′-cap structure is involved in the major 5′-to-3′ and nonsense-mediated mRNA decay pathways, and the protein complex consisting of Dcp1 and Dcp2 has been identified as the species responsible for the decapping reaction in *Saccharomyces cerevisiae* and human. Although *in vitro* studies indicate that Dcp2 is catalytically an active component, the role of Dcp1 in the decapping reaction remains to be explored in organisms other than budding yeast. To elucidate the Dcp1-dependent decapping mechanisms, we identified the homologues of *S. cerevisiae* Dcp1 (ScDcp1) in higher eukaryotes and analyzed their functions in the different species. The phenotypes of slow growth and mRNA stabilization induced by *Scdcp1*-gene disruption in budding yeast could be suppressed by the *Shizosaccharomyces pombe* SpDcp1 but not by the human homologue hDcp1. In contrast, the same phenotypes caused by *Spdcp1*-gene disruption in fission yeast were effectively complemented by hDcp1 and its partial sequence comparable to SpDcp1. These results indicate that not only Dcp2 but also Dcp1 plays an indispensable role in mRNA-decay pathway and that the characteristics of Dcp1-dependent decapping reaction in fission yeast hold an intermediate position in the evolution of mRNA-decay machinery from budding yeast to mammals.

Key words: 5′-cap structure, decapping complex (Dcp1/2), fission yeast, mRNA decay.

Abbreviations: EMM, Edinburgh-minimal medium; EVH1, ena, Vasp and Homer-homology domain 1; 5FOA, 5-fluororotic acid; NMD, nonsense-mediated decay; PCR, polymerase chain reaction; ts, temperature-sensitive; WT, wild type.

Messenger RNA degradation is a key step in the proper control of gene expression (*1*, *2*). Work in budding yeast, *Saccharomyces cerevisiae*, has demonstrated that mRNAs are degraded via two general pathways. Both pathways usually begin with deadenylation, the shortening of the poly (A) tail at the 3′ end of mRNAs. After deadenylation, the 5′-cap structure can be removed by decapping enzymes, exposing the transcript to be digested by a 5′-to-3′ exonuclease Xrn1. Alternatively, mRNAs can be degraded in a 3′-to-5′ direction by a cytoplasmic exosome (*3*). The nonsense-mediated decay (NMD) pathway that rapidly degrades mRNAs containing premature-termination codons also involves either deadenylation-independent decapping followed by 5′-to-3′ decay or accelerated deadenylation followed by 3′-to-5′ exonucleolytic digestion (*4*–*7*).

Decapping is a critical step in the 5′-to-3′ decay pathway, because it initiates the degradation of both normal and aberrant mRNAs. Decapping enzymes cleave capped mRNA to release 7-methyl GDP and yield 5′-phosphorylated products that serve as a substrate for the downstream Xrn1 5′-to-3′ exonuclease (*8*). Recent studies have indicated that the protein complex consisting of Dcp1 and Dcp2 is responsible for the decapping reaction in *S. cerevisiae* and human. The Dcp1 protein was initially thought to mediate the decapping activity. However, Dcp2 was recently shown to catalyze this reaction (*9*–*12*). The Dcp2 contains a MutT motif, which is a catalytic domain found in a class of pyrophosphatases designated as the Nudix hydrolases (*13*). In human cells, the down-regulation of Dcp2 protein by RNA-interference methods abrogates NMD (*14*). In addition to Dcp1 and Dcp2, another decapping activity was also identified in yeasts and human (*15*, *16*). The enzyme, named DcpS, has the ability to hydrolyze the residual 7-methyl GpppN-cap structure, which is a product of the decapping reaction catalyzed by Dcp1 and Dcp2 or the complete 3′-to-5′ degradation by the exosome. It thus appears that DcpS acts as a downstream enzyme of Dcp1 and Dcp2 or the exosome.

In budding yeast, the gene disruption of not only *DCP2* but also *DCP1* leads to growth arrest and failure to remove the 5′-cap structure, resulting in the stabilization of numerous mRNAs that include aberrant mRNAs degraded by the NMD pathway (*8*, *13*). Although the previous *in vitro* studies suggest that Dcp1 primarily acts to enhance the catalytic activity of Dcp2, the role of Dcp1 in the mRNA-decay pathway remains to be explored in organisms other than budding yeast. To elucidate the Dcp1-dependent decapping mechanisms, we identified the functional homologues of *S. cerevisiae* Dcp1 (ScDcp1) in higher eukaryotes and analyzed their complementary actions in different species. We found that the phenotypes of slow growth and mRNA stabilization induced by *Scdcp1*-gene disruption in budding yeast could be sup-

*The first two authors contributed equally to this work.
†To whom correspondence should be addressed. Tel: +81-3-5841-4750, Fax: +81-3-5841-4751, E-mail: katada@mol.f.u-tokyo.ac.jp

Table 1. **Yeast strains used in this study.**

Name	Relevant genotype
JY746	*h⁺ ura4-D18 leu1-32 ade6-M210*
JY741	*h⁻ ura4-D18 leu1-32 ade6-M216*
YSP006	*h⁺/h⁻ ura4-D18/ura4-D18 leu1-32/leu1-32 ade6-M210/ade6-M216*
YSP058	*h⁺ dcp1-GFP≪kan^r ura4-D18 leu1-32 ade6-M210*
YSP075	*h⁺/h⁻ dcp1⁺/dcp1::hyg.Br ura4-D18/ura4-D18 leu1-32/leu1-32 ade6-M210/ade6-M216*
YSP078	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r pREP2(dcp1⁺) ura4-D18/ura4-D18 leu1-32/leu1-32 ade6-M210/ade6-M216*
YSP079	*h⁺ dcp1::hyg.B^r pREP2 (FLAG-dcp1⁺) ura4-D18 leu1-32 ade6-M210, M216*
YSP120	*h⁺ dcp1::hyg.B^r pREP2 (FLAG-dcp1⁺) pREP1(–) ura4-D18 leu1-32 ade6-M210, M216*
YSP121	*h⁺ dcp1::hyg.B^r pREP2 (FLAG-dcp1⁺) pREP1(FLAG-dcp1⁺) ura4-D18 leu1-32 ade6-M210, M216*
YSP122	*h⁺ dcp1::hyg.B^r pREP2 (FLAG-dcp1⁺) pREP1(Sc DCP1) ura4-D18 leu1-32 ade6-M210, M216*
YSP123	*h⁺ dcp1::hyg.B^r pREP2 (FLAG-dcp1⁺) pREP1(h DCP1) ura4-D18 leu1-32 ade6-M210, M216*
YSP124	*h⁺ dcp1::hyg.B^r pREP2 (dcp1⁺) pREP1(h DCP1ΔC) ura4-D18 leu1-32 ade6-M210, M216*
YSP086	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r leu1/leu1::Padh1-FLAG-dcp1⁺≪kan^r ura4-D18/ura4-D18 ade6-M210/ade6-M216*
YSP087	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r leu1/leu1::Padh1-Sc DCP1≪kan^r ura4-D18/ura4-D18 ade6-M210/ade6-M216*
YSP088	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r leu1/leu1::Padh1-h DCP1≪kan^r ura4-D18/ura4-D18 ade6-M210/ade6-M216*
YSP089	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r leu1/leu1::Padh1-h DCP1ΔC≪kan^r ura4-D18/ura4-D18 ade6-M210/ade6-M216*
YSP090	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r leu1/leu1::Padh1-FLAG-dcp1-P74S≪kan^r ura4-D18/ura4-D18 ade6-M210/ade6-M216*
YSP091	*h⁺/h⁻ dcp1⁺/dcp1::hyg.B^r leu1/leu1::Padh1-FLAG-dcp1-L69S≪kan^r ura4-D18/ura4-D18 ade6-M210/ade6-M216*
YSP125	*h⁻ dcp1::hyg.B^r leu1::Padh1-FLAG-dcp1⁺≪kan^r ura4-D18 leu1–32 ade6-M210, M216*
YSP126	*h⁻ dcp1::hyg.B^r leu1::Padh1-Sc DCP1≪kan^r ura4-D18 leu1-32 ade6-M210, M216*
YSP127	*h⁻ dcp1::hyg.B^r leu1::Padh1-h DCP1≪kan^r ura4-D18 leu1-32 ade6-M210, M216*
YSP128	*h⁻ dcp1::hyg.B^r leu1::Padh1-h DCP1ΔC≪kan^r ura4-D18 leu1-32 ade6-M210, M216*
YSP129	*h⁻ dcp1::hyg.B^r leu1::Padh1-FLAG-dcp1-P74S≪kan^r ura4-D18 leu1-32 ade6-M210, M216*
YSP130	*h⁻ dcp1::hyg.B^r leu1::Padh1-FLAG-dcp1-L69S≪kan^r ura4-D18 leu1-32 ade6-M210, M216*
YSP131	*h⁺ dcp2⁺-13 Myc≪kan^r pREP2 ura4-D18 leu1-32 ade6-M210*
YSP132	*h⁺ dcp2⁺-13 Myc≪kan^r pREP2 (FLAG-dcp1⁺) ura4-D18 leu1-32 ade6-M210*
YSP133	*h⁺ dcp2⁺-13 Myc≪kan^r pREP2 (FLAG-dcp1 L74S) ura4-D18 leu1-32 ade6-M210*
YSP134	*h⁺ dcp2⁺-13 Myc≪kan^r pREP2 (FLAG-dcp1 P69S) ura4-D18 leu1-32 ade6-M210*
W303-1A	*MATa ura3 leu2-3,112 ade2-1 lys2 his311,15 trp1-1 can1-100*
YSC100	*Δdcp1::C.g. HIS3*
YSC101	*Δdcp1::C.g. HIS3 YCp-URA3 (GAL1p-FLAG-ScDCP1)*
YSC102	*Δdcp1::C.g. HIS3 YCp-URA3 (GAL1p-FLAG-ScDCP1) YCp-TRP1(–)*
YSC103	*Δdcp1::C.g. HIS3 YCp-URA3 (GAL1p-FLAG-ScDCP1) YCp-TRP1(GAL1p-FLAG-DCP1)*
YSC104	*Δdcp1::C.g. HIS3 YCp-URA3 (GAL1p-FLAG-ScDCP1) YCp-TRP1(GAL1p-FLAG-Spdcp1⁺)*
YSC105	*Δdcp1::C.g. HIS3 YCp-TRP1(GAL1p-FLAG-Spdcp1⁺)*
YSC106	*Δdcp1::C.g. HIS3 YCp-URA3 (GAL1p-FLAG-DCP1) YCp-TRP1(GAL1p-FLAG-h DCP1)*

pressed by the *Shizosaccharomyces pombe* SpDcp1 but not by the human homologue hDcp1. In contrast, the same phenotypes caused by *Spdcp1*-gene disruption or mutation in fission yeast were effectively complemented by hDcp1 and its partial sequence comparable to SpDcp1. These results indicate that not only Dcp2 but also Dcp1 plays an indispensable role in the mRNA-decay pathway, and that the characteristics of the Dcp1-dependent decapping reaction in fission yeast are intermediate in position in the evolution of mRNA-decay machinery from budding yeast to mammals.

MATERIALS AND METHODS

Yeast Strains—All yeast strains used in this study are listed in Table 1. The yeast cells were grown in standard culture media and transformed with DNA by the lithium acetate method. Disruption and epitope tagging of fission yeast genes were performed by the one-step method (*17*, *18*). They were confirmed by phenotypic analysis and/or polymerase chain reaction (PCR) with primers specific for the genes. Unless otherwise indicated, strains of fission yeast and budding yeast were derivatives of JY746 and W303-1A, respectively.

Temperature-sensitive mutants were prepared by slight modification of the gap repair procedure (*19*). Briefly, *pTRPGAL1*-His_6-FLAG-*Spdcp1* was used as a template for PCR. A pair of oligonucleotide primers for *GAL1* promoter (at –300 to –320 bp position from the start codon) and *CMK1* terminator (at +300 to +320 bp position from the stop codon) was used for mutagenic PCR. The *pTRPGAL1*-His_6-FLAG plasmid was gapped between *Nde*I and *Sal*I. Transformants were replica-plated onto a medium containing 5-fluororotic acid (5FOA) to remove the wild-type *URA3* plasmid, then onto a warm plate at 37°C. Temperature-sensitive colonies were selected, and the plasmid was recovered. Plasmids conferring a temperature-sensitive phenotype were transferred to the integration plasmid pKLA (a gift from Dr. M. Yoshida, Riken, Saitama, Japan) and transformed into fission yeast. The constructs were integrated into the genome at the *leu1* locus, in which the mutant genes were expressed from the *leu1* locus under the control of the *adh1⁺* promoter. Finally, the *dcp1⁺* of these strains was deleted.

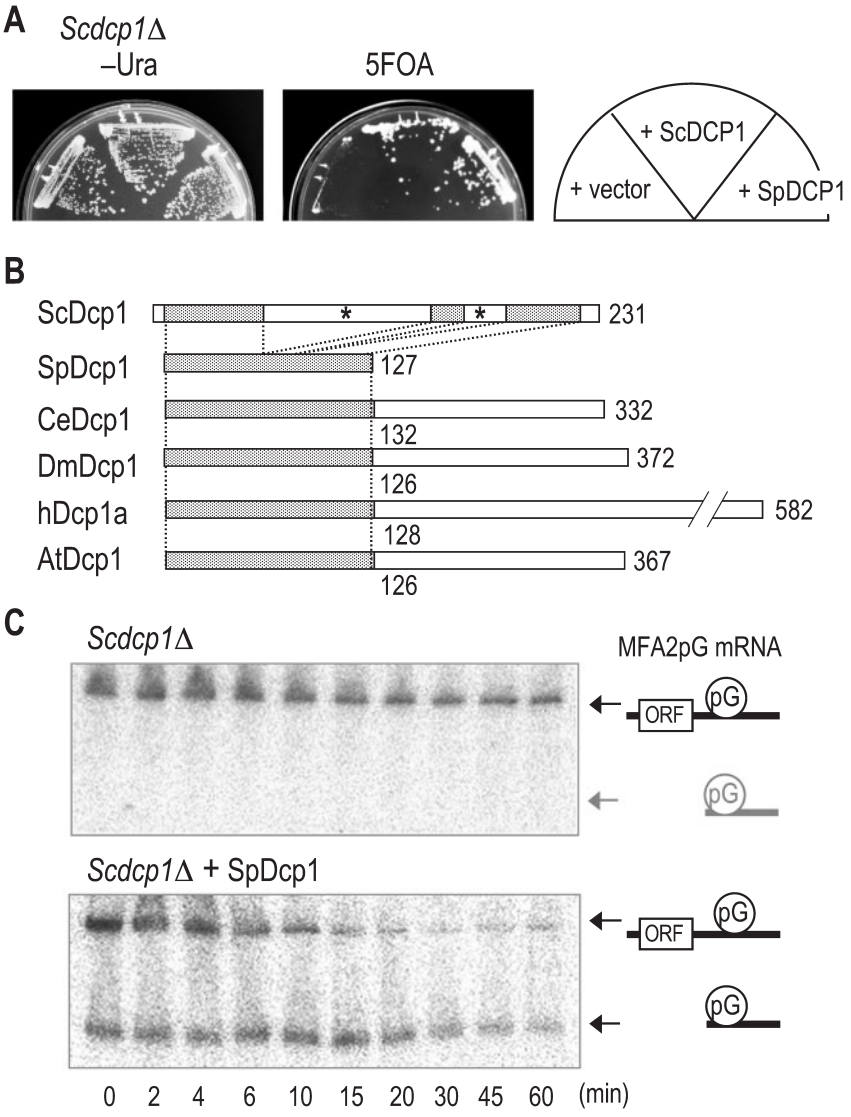

Fig. 1. **Isolation of *S. pombe* Dcp1 that complements slow-growth and mRNA-stabilization phenotypes observed in *dcp1*Δ budding yeast.** (A) *S. cerevisiae dcp1*Δ strain carrying *pURAGAL1*-Sc*DCP1* was transformed with *pTRPGAL1* alone (+ vector; YSC102), ScDcp1 (+ Sc*DCP1*, YSC103) or SpDcp1 (+ Sp*DCP1*, YSC104) and grown at 30°C for 3 days on plates either lacking uracil (–Ura, left panel) or containing 5-FOA (5FOA, middle panel). (B) Schematic representation of the structure of Sc Dcp1 and other eukaryotic Dcp1s. Sc, *S. cerevisiae*; Sp, *S. pombe* (SPBC3B9.21); Ce, *Caernohabditis elegans*; Dm, *Drosophila melanogaster*; h, *Homo sapience* (hDcp1a); At, *Arabidopsis thaliana*. The *asterisks* show sequences that exist only in ScDcp1. (C) *S. cerevisiae* strains, which lack the *DCP1* gene but carry the *MFA2pG* reporter and either the empty vector alone (*Scdcp1*Δ, YSC100) or the vector containing *SpDCP1* gene (*Scdcp1*Δ + SpDcp1, YSC105), were grown to mid-log phase in the galactose medium and shifted to a glucose medium to repress transcription of the reporter mRNA. After incubation for the indicated times, Northern blot analysis was performed with the end-labeled oligonucleotide specific for the MFA2pG mRNA. Positions of the full-length and poly(G) to 3′-end fragments of MFA2pG mRNA are indicated at right.

Two temperature-sensitive alleles of *Spdcp1* were referred to as *Spdcp1-P74S* and *Spdcp1-L69S*, respectively.

Plasmid Construction—pREP1, pREP2, *pURAGAL1*-His_6-FLAG, *pTRPGAL1*-His_6-FLAG and pKLA were used for expressing the various proteins of Dcp1 in yeast cells (*20*, *21*). The DNA fragments of the various Dcp1 versions were inserted into the region between the *Nde*I and *Sal*I sites of all the above vectors. pREP1-*MFA2pG* was constructed by inserting *MFA2pG* into pREP1. *MFA2pG* was isolated from *pGAL1-MFA2pG* as a *Nde*I fragment and inserted into pREP1 previously digested with *Nde*I.

RNA Analysis—Yeast cells were transformed with the pREP1-*MFA2pG* plasmid encoding the MFA2pG reporter mRNAs under the control of the *nmt1*$^+$ promoter. Degradation of these reporters was detected as follows. The strains were grown at 28°C to mid-log phase (OD_{600} = 0.6–0.8) in Edinburgh-minimal medium (EMM) and further incubated at 37°C. After 3 h, transcription of the reporters was blocked by washing once and addition of appropriate medium containing 10 μg/ml of thiamine; then cells were further incubated at 37°C. At the indicated times, cells were removed, pelleted down, and immediately frozen in liquid nitrogen. RNA that had been extracted by the hot-phenol procedure was separated by either 1.25% agarose-gel or 4% polyacrylamide/7.5 M urea-gel electrophoresis and transferred to Hybond XL (Amersham Pharmacia). The reporters were detected by Northern blotting using the oligonucleotide oRP121 (5′-AAT TCC CCC CCC CCC CCC CA-3′). The degradation of p*GAL-MFA2pG* reporter in *S. cerevisiae* cells was assayed as described previously (*21*).

Immunoprecipitation of Epitope-Tagged Proteins—Immunoprecipitation of FLAG-tagged proteins and subsequent detection of co-precipitated proteins were performed as follows. The strains were first grown in selective media at 28°C and further incubated at 37°C for 3 h. The cells were resuspended in 250 μl of a lysis buffer consisting of 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, 0.1% NP-40, and protease inhibitors. The cells were mixed with glass beads (1 g) and disrupted by 10 cycles of vortexing for 30 s followed by incubating on ice for 1 min. The cell extracts were obtained by two consecutive runs of centrifugation (14,000 × *g* for 10 min). After addition of anti-FLAG anti-

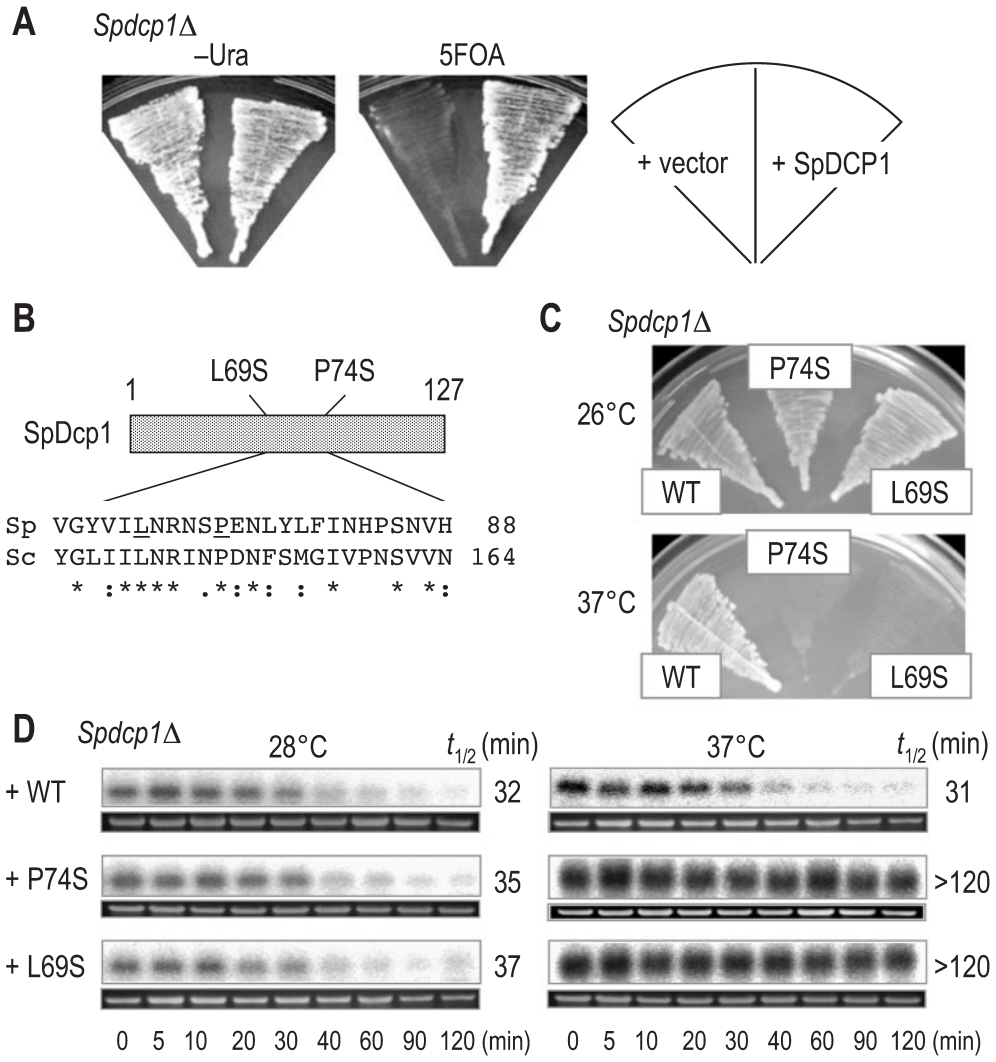

Fig. 2. **SpDcp1 is required for both cell viability and mRNA decay in fission yeast.** (A) *S. pombe* strains, which lack the *SpDCP1* gene but carry pREP1 alone (vector; YSP120) or pREP1–Sp*DCP1* (*SpDCP1*; YSP121), were grown at 30°C for 5 days on plates either lacking uracil (–Ura, left panel) or containing 5-FOA (5FOA, middle panel). (B) The *SpDCP1* mutants, *L69S* and *P74S*, generated in this study. The amino acid residues mutated in *SpDCP1* are underlined and compared with the sequence of *ScDCP1*. (C) Temperature-sensitive growth defect in *SpDCP1* mutants. *S. pombe dcp1*Δ strains carrying pKLA-*SpDCP1* WT (YSP125), *P74S* (YSP129), or *L69S* (YSP130) were grown on the complete plate at 26°C (top) or 37°C (bottom) for 5 days. (D) *S. pombe dcp1*Δ strains, which carry the MFA2pG transcript (pREP1-MFA2pG) and pKLA-*SpDCP1* WT (YSP125), *P74S* (YSP129) or *L69S1* (YSP130), were grown to mid-log phase in EMM at 28°C. They were shifted to EMM containing thiamine to repress transcription of the reporter mRNA and further incubated at 28°C (left panels) for the indicated times. The same strains were cultured at 37°C for 3 h, shifted to the thiamine-containing medium, and further incubated at 37°C (right panels). Northern blot analysis was performed with the end-labeled oligonucleotide specific for the MFA2pG mRNA (upper panels). The signals were quantified using a phosphor-imager and corrected for the loading amounts of 18S rRNA (lower panels). The half-lives of MFA2pG mRNA are shown as the average values obtained from three independent experiments.

body-conjugated beads (M2-Agarose-Afinity, Sigma), the extracts were incubated on a rotator at 4°C for 2 h. The beads were sedimented and washed extensively with the lysis buffer without protease inhibitors. Proteins binding to the beads were eluted with an SDS-PAGE sample buffer by boiling for 5 min. The cell extracts (5% of total extracts) and one-half of the eluted fractions were subjected to SDS-PAGE and immunoblotted with anti-Myc (9E10) and anti-FLAG (M2) monoclonal antibodies.

RESULTS

Identification of S. pombe Dcp1 That Complements Slow-Growth and mRNA-Stabilization Phenotypes Observed in dcp1Δ Budding Yeast—To identify a functional homologue of *S. cerevisiae DCP1* (*ScDCP1*) gene in fission yeast, we searched through the genome database and found several hypothetical gene products that have relevant similarity to the open reading frame of *ScDCP1*. The candidate genes were examined for the ability to complement the slow-growth phenotype observed in budding yeast lacking *DCP1* gene, *S. cerevisiae dcp1*Δ strain (*8*). As shown in Fig. 1A (middle panel), expression of the *S. pombe* gene *SPBC3B9.21* (Gene Bank accession No. CAB69661.1) was effective in complementing the slow-growth phenotype of *dcp1*Δ strain as had been observed with *ScDCP1*. The deduced 127–amino acid product of *SPBC3B9.21* cDNA clone has a sequence quite similar to the homologues of other eukaryotic species (Fig. 1B). Therefore, we designated *SPBC3B9.21* gene as *Spdcp1*$^+$.

*S. cerevisiae dcp1*Δ strain has another phenotype, which is characterized by slow degradation of various mRNAs (*8*). To examine if SpDcp1 also suppresses this phenotype, we measured the decay of a MFA2 transcript, MFA2pG, which contains a poly(G) tract in its 3′-untranslated region in *S. cerevisiae dcp1*Δ strain. The reporter mRNA was under the control of the *GAL1* UAS, and the *de novo* induction was repressed in a glucose medium to allow measurement of the decay rate (*21*). As shown in Fig. 1C (upper panel), there were no detectable intermediates of MFA2pG mRNA in *S. cerevisiae dcp1*Δ strain, consistent with the previous results. On the other

hand, the 3′-end decay intermediates were clearly observed when SpDcp1 had been introduced into the *dcp1*Δ strain (lower panel). The mRNA decay accelerated by SpDcp1 was comparable to that observed with ScDcp1 (data not shown), suggesting that SpDcp1 functions as a decapping factor just like ScDcp1 in *S. cerevisiae*.

SpDcp1 Is Required for Both Cell Viability and mRNA Degradation in Fission Yeast—To elucidate the function of SpDcp1 in fission yeast, the *Spdcp1* gene was disrupted in a wild-type diploid. A heterozygous diploid strain (YSP075, see Table 1) was sporulated, and the resulting tetrads were dissected. Tetrad analyses of the asci reproducibly yielded two viable spores, both of which were *Spdpc1*$^+$ (all of 7 tetrads tested, data not shown). Furthermore, the *Spdcp1*Δ-haploid strain into which both pREP2-*Spdcp1*$^+$ (YSP079, see Table 1 and "MATERIALS AND METHODS") and vector pREP1 (YSP120, see Table 1) were introduced could not grow on a plate containing 5-fluororotic acid (5FOA) at 28°C, in contrast to the *Spdcp1*Δ-haploid strain containing both pREP2-*Spdpc1*$^+$ and pREP1-*Spdpc1*$^+$ (Fig. 2A). These results indicate that *Spdcp1*$^+$ is an essential gene for cell viability in fission yeast.

To further explore the role of SpDcp1 in the regulation of mRNA degradation, we constructed two temperature-sensitive alleles referred to as *Spdcp1 L74S* and *P69S*. These mutations were C-G to T-A (L74S) and T-A to C-G (P69S) transitions, resulting in the alteration of Leu74 to Ser and Pro69 to Ser, respectively. Both Leu74 and Pro69 were conserved residues between *Sc*Dcp1 and *Sp*Dcp1 (see Fig. 2B). At 28°C, *Spdcp1 L74S* and *P69S* mutants were indistinguishable from the wild-type strain in both growth rate (Fig. 2C, upper panels) and cell morphology (data not shown). Shifted to 37°C, however, the two mutants showed almost no growth on a plate containing complete medium (Fig. 2C, lower panels). To determine whether SpDcp1 has a direct role in mRNA decay in fission yeast, the decay rate of a reporter was assayed in the *Spdcp1* temperature-sensitive mutants and the wild-type strain. The reporter used was pREP1-*MFA2pG*. This construct is derivative of the gene for budding yeast *MFA2*, which contains a poly(G) tract in its 3′ UTR. The transcription of MFA2pG mRNA under the control of *nmt1* promoter is induced by EMM and repressed by adding thiamine in fission yeast cells (*20*). In budding yeast, MFA2pG mRNA was degraded by exonucleolytic digestion in both 5′-to-3′ and 3′-to-5′ directions (*22*, *23*). However, in fission yeast, MFA2pG mRNA derived from pREP1-*MFA2pG* appeared to be degraded mainly in the 5′-to-3′ direction, and the contribution of the 3′-to-5′ pathway was negligible. This is because the 3′-end decay intermediates of MFA2pG mRNA corresponding to fragments from poly(G) to the 3′ end were scarcely degraded (data not shown). Therefore, the decay rate of full-length MFA2 mRNA after repressing transcription is indicative of the contribution of mRNA degradation from the 5′-to-3′ pathway.

As shown in Fig. 2D, *Spdcp1*$^+$ and the two *Spdcp1* temperature-sensitive (ts) mutants were equally efficient in the degradation of full-length MFA2pG mRNA at permissive temperature (28°C, left panels). This is consistent with the growth phenotype observed in Fig. 2C. On the other hand, the decay rates of MFA2pG mRNA in the two ts mutants were both exceedingly slow ($t_{1/2}$ > 120 min) at restrictive temperature (37°C, right panels), compared with the wild-type strain ($t_{1/2}$ = 18 min). These results strongly suggest that SpDcp1 regulates mRNA decay by the 5′-to-3′ pathway due to its decapping action in fission yeast.

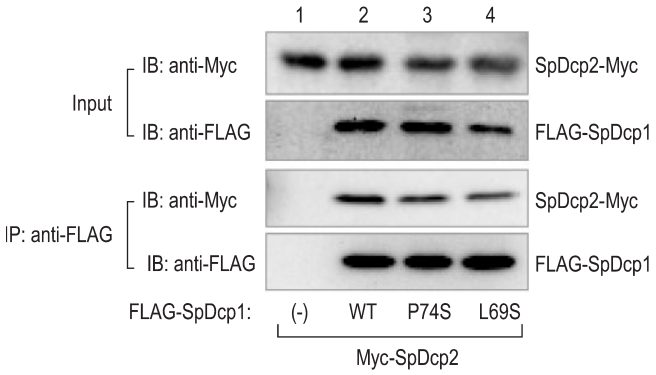

Fig. 3. **Biochemical properties of temperature-sensitive mutants of SpDcp1.** Cell extracts were prepared from *S. pombe* strains that carry SpDcp2-Myc and pREP2 vector alone (lane 1; YSP131), pREP2-FLAG-*SpDCP1* WT (lane 2, YSP132), *P74S* (lane 3, YSP133), or *P69S* (lane 4, YSP134). The extracts were immunoprecipitated (IP) with an anti-FLAG antibody. The precipitated proteins (the third and fourth panels) and whole-cell extracts (the first and second panels) were immunoblotted (IB) with anti-FLAG and anti-Myc antibodies to detect SpDcp1 and SpDcp2, respectively.

Biochemical Properties of Temperature-Sensitive Mutants of SpDcp1—It has been reported that Dcp1 and Dcp2 proteins physically interact with each other in *S. cerevisiae* and human (*9*, *10*, *13*). Therefore, we examined whether the two SpDcp1 mutants exert any influence on the interaction between Dcp1 and Dcp2 in *S. pombe*. A gene product encoded by *SPAC19A8.12* was first identified as a Dcp2 homologue of *S. pombe* (Gene Bank accession No. NP 593780 and designated as *Spdcp2*), based on the homology of the primary sequence, especially in the conserved Nudix/MutT domain (*13*). We introduced plasmids expressing FLAG-tagged wild-type (WT) SpDcp1 or SpDcp1 ts mutants into a strain whose chromosomal copy of *Spdcp2* was tagged with Myc at its C terminus. There was no obvious difference in the amounts of expressed proteins between the mutants and wild-type SpDcp1 in fission yeast cells (Fig. 3, upper two panels). Immunoprecipitation assay demonstrated that approximately 15–20% of total Myc-SpDcp2 was detected in the fraction precipitated with either FLAG-SpDcp1 WT or the mutants (Fig. 3, lower two panels). These findings indicate that the interaction between Dcp1 and Dcp2 was also conserved in *S. pombe* and that Leu74 and Pro69 residues of SpDcp1 are not required for the interaction.

Characteristics of Human Dcp1 in a Comparison with ScDcp1 and SpDcp1—In human, two Dcp1 proteins, hDcp1a and hDcp1b, have been isolated (*9*, *10*). Human Dcp1a consisting of 582 amino acids has two domains, the N-terminal region (1–128 amino acid sequence) similar to SpDcp1 (Fig. 1B) and the C-terminal region that does not exist in SpDcp1. SpDcp1 (127 amino acids) and the 128-amino acid N terminus of hDcp1a share 31% identity over their entire sequences. Human Dcp2 has

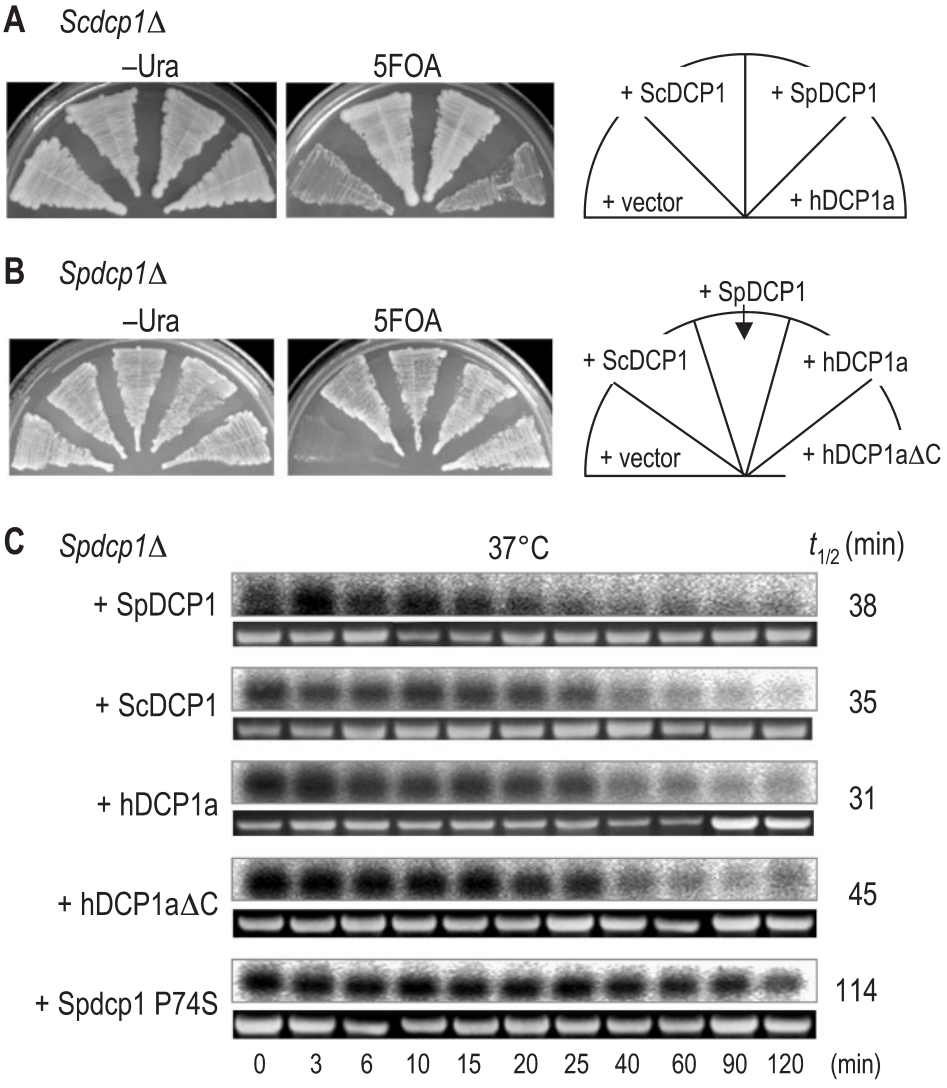

Fig. 4. **Characteristics of hDcp1: Its differential ability to complement for SpDCP1 and ScDCP1.** (A) *S. cerevisiae dcp1*Δ strains carrying *pURAGAL1-ScDCP1* and *pTRPGAL1* vector alone (YSC102), *ScDCP1* (YSC103), *SpDCP1* (YSC104), or *hDCP1a* (YSC106) were grown at 30°C for 5 days on plates either lacking uracil (–Ura, *left panel*) or containing 5-FOA (5FOA, middle panel). (B) The N-terminal domain of hDcp1 is sufficient to complement for SpDcp1. *S. pombe dcp1*Δ strains carrying pREP2-*SpDCP1* and pREP1 vector alone (YSP120), *SpDCP1* (YSP121), *ScDCP1* (YSP122), *hDCP1a* (YSP123), or the N terminal domain (1–127) of *hDCP1a* (*hDCP1a*ΔC; YSP124) were grown at 30°C for 5 days on plates either lacking uracil (–Ura, left panel) or containing 5-FOA (5FOA, left panel). (C) *S. pombe* strains, which lack the *DCP1* gene but carry the MFA2pG reporter and pKLA-*SpDCP1* WT (YSP125), *ScDCP1* (YSP126), *hDCP1a* (YSP127), the N-terminal domain of *hDcp1a* (*hDCP1a*ΔC; YSP128), or Sp*dcp1 P74S* (YSP129), were grown to mid-log phase in EMM at 28°C and shifted at 37°C for 3 h. They were then transferred to the thiamine-containing EMM medium and further incubated at 37°C for the indicated times. Northern blot analysis was performed as described in Fig. 2D.

been established as an mRNA-decapping enzyme, based on the recent reports showing that hDcp2 protein has intrinsic decapping activity and is required for mRNA decay in human cells (*9*, *10*, *14*). In contrast, it remains unclear whether the hDcp1s are involved in the regulation of mRNA degradation in living cells, though the hDcp1s interact with hDcp2 and localize in the cytoplasm (*9*, *10*, *24*). To investigate if hDcp1a functions as a regulator of mRNA decay in living cells, we constructed *S. pombe* and *S. cerevisiae* strains that carry hDcp1a instead of their native Dcp1s and observed their growth phenotypes. As shown in Fig. 4A, hDcp1a failed to complement the slow-growth phenotype observed in *S. cerevisiae dcp1*Δ strain, in sharp contrast to the action of ScDcp1 (see Fig. 1A). Thus, hDcp1a could not substitute for ScDcp1 in *S. cerevisiae* cells. In contrast, introducing of either the full length (hDcp1a) or the N-terminal region (1–128, hDcp1aΔC) of hDcp1a rescued the lethality of *S. pombe dcp1*Δ strain at the same level as that observed with SpDcp1 (Fig. 4B). Interestingly, the lethality of *S. pombe dcp1*Δ strain was also rescued by full-length hDcp1b (data not shown), indicating functional similarity between *S. pombe* and human Dcp1s.

We next determined whether expression of hDcp1a could also complement the mRNA-stabilization phenotype of *S. pombe dcp1*Δ strain. To measure mRNA-decay rate, yeast cells were grown at the permissive temperature and shifted to the restrictive temperature (37°C). As shown in Fig. 4C, the full-length form of hDcp1a, together with the N domain (hDcp1aΔC), restored the decay rate of mRNA to the same level as that observed with SpDcp1 or ScDcp1. In contrast, the temperature-sensitive mutant SpDcp1 (L74S) again failed to restore the slow decay phenotype, which was used as a negative control for hDcp1a. These results were consistent with the suppression of slow-growth phenotype observed in Figs. 4B and 2C. Thus, we concluded that hDcp1a regulates the mRNA-decay rate of the 5′-to-3′ pathway in living cells and that the N-terminal domain of hDcp1a, which has similarity to SpDcp1, is sufficient for this function.

DISCUSSION

To elucidate the mechanisms of the decapping reaction involved in the mRNA decay of higher eukaryotes, we

identified the homologues of *S. cerevisiae* Dcp1 and investigated the function of human Dcp1 (hDcp1) in fission yeast. Our present study clearly indicates that *S. pombe* Dcp1 (SpDcp1) is an indispensable component of the decapping machinery in fission yeast and that hDcp1 is capable of regulating the 5′-to-3′ route of mRNA decay in living yeast cells. The major findings obtained here are summarized as follows. First, expression of SpDcp1 suppressed the phenotypes of slow growth and delay of mRNA decay in budding yeast lacking the *ScDCP1* gene (Fig. 1). Second, two *Spdcp1*-ts mutants (*L74S*, *P69S*) conferred the delay of mRNA degradation at a temperature conducive to cell growth (Fig. 2). Third, hDcp1 was capable of complementing both phenotypes of growth arrest and mRNA stabilization observed in fission yeast lacking the *Spdcp1* gene (Fig. 4).

Recent study indicates that ScDcp1 structurally resembles the EVH1 (ena, Vasp and Homer-homology domain 1) family of protein domains (*25*). One of the notable differences between ScDcp1 and the other EVH1-related structures is the presence of two short α helices (α1 and α2), which have no counterparts in the previous EVH1 domains. Another difference is an insert (the amino-acid residue 80–135) in ScDcp1 that is largely disordered in its crystal structure. However, the short α helices and the insert exist only in *S. cerevisiae* Dcp1 (see asterisks in Fig. 1B). Therefore, it is unlikely that these sequences are critical for Dcp1 function. The residues L69 and P74 of SpDcp1, whose mutations show thermosensitive phenotypes in fission yeast (Fig. 2), are conserved in *S. cerevisiae*. The sequence alignment of ScDcp1 and SpDcp1 indicated that residues L69 and P74 of SpDcp1 might be located at a specific surface of the ScDcp1 structure named the "hydrophobic patch" (*25*). The conserved residues in this domain appear to be required for the enhancement of decapping activity of ScDcp2, but not for the interaction between Dcp1 and Dcp2. This is consistent with our present result that both L69S and P74S SpDcp1-mutant proteins could interact with SpDcp2 in yeast at the restrictive temperature (Fig. 3). Thus, the failure of mRNA decay obtained with SpDcp1 ts mutations seems to be independent of the ability to interact with SpDcp2. Our results strongly suggest that SpDcp1 is analogous to *S. cerevisiae* and human Dcp1s, although we could not address the molecular mechanisms of the Dcp1-dependent decapping reaction.

Messenger RNA is normally degraded in both 5′-to-3′ and 3′-to-5′ directions in the cytoplasm of *S. cerevisiae* (*1*, *2*), and a strain lacking both pathways exhibits no growth (*23*, *26*). Thus, both 5′-to-3′ and 3′-to-5′ pathways of mRNA degradation are required for cell survival in *S. cerevisiae*. In contrast, the fission yeast strain lacking SpDcp1 appears to be lethal (Fig. 2A). This strongly suggests that 5′-to-3′ mRNA decay is likely to be essential for cell viability in *S. pombe* cells, and that the contribution of the two mRNA-decay directions is different between *S. cerevisiae* and *S. pombe*.

Human Dcp1 has a large extra C-terminal domain, which is not present in ScDcp1 or SpDcp1. It has been reported that the N-terminal region of hDcp1a, which has homology to ScDcp1 and SpDcp1, is not sufficient for full-decapping activity of hDcp2, even though they could interact with each other (*9*). Our study demonstrates that an elevated dosage of not only the full-length form of hDcp1 but also its N terminus suppresses the phenotypes caused by disruption of *S. pombe dcp1* gene (Fig. 4). Therefore, the C-terminal domain of hDcp1 has little involvement in the decapping, and the N terminus is sufficient for decapping reaction to regulate the stability of mRNA in fission yeast cells. However, we can not totally exclude the possibility that the C-terminal domain of hDcp1 plays some role in human cells. In this regard, it has been reported that the C domain of hDcp1 associates with the transcription factor Smad4 in transforming growth factor β-signaling pathway (*27*).

Regulators of the decapping reaction, such as Lsm proteins, Pat1, Xrn1 and Edc proteins, have been identified in *S. cerevisiae*, and their homologues appear to exist in other eukaryotes (*28*). Nevertheless, hDcp1 could suppress the lethality observed in *S. pombe Spdcp1*Δ strain, but not the slow-growth phenotype of *S. cerevisiae Scdcp1*Δ strain (Fig. 4). Moreover, Dcp1s of both budding yeast and human suppress the defect of mRNA decay in *Spdcp1*Δ strain (Fig. 4). These findings suggest that the characteristics of the Dcp1-dependent decapping reaction in fission yeast are intermediate in position in the evolution of mRNA-decay machinery from budding yeast to mammals. Thus, analyses in fission yeast are promising as a means to elucidate the process of mRNA decay mediated through the decapping complex and its regulatory factors in higher eukaryotes.

We are grateful to Drs. Yoshinori Watanabe and Minoru Yoshida for generous gifts of various plasmids and yeast strains. This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government, Japan Society for the Promotion of Science (JSPS), the Mitsubishi Foundation, and the Uehara Memorial Foundation.

REFERENCES

1. Mitchell, P. and Tollervey, D. (2001) mRNA turnover. *Curr. Opin. Cell Biol.* **13**, 320–325
2. Parker, R. and Song, H. (2004) The enzymes and control of eukaryotic mRNA turnover. *Nat. Struct. Mol. Biol.* **11**, 121–127
3. Butler, J.S. (2002) The yin and yang of the exosome. *Trends Cell Biol.* **12**, 90–96
4. Muhlrad, D. and Parker, R. (1994) Premature translational termination triggers mRNA decapping. *Nature* **370**, 578–581
5. Cao, D. and Parker, R. (2003) Computational modeling and experimental analysis of nonsense-mediated decay in yeast. *Cell* **113**, 533–545
6. Mitchell, P. and Tollervey, D. (2003) An NMD pathway in yeast involving accelerated deadenylation and exosome-mediated 3′–5′ degradation. *Mol. Cell* **11**, 1405–1413
7. Takahashi, S., Araki, Y., Sakuno, T., and Katada, T. (2003) Interaction between Ski7p and Upf1p is required for nonsense-mediated 3′-to-5′ mRNA decay in yeast. *EMBO J.* **22**, 3951–3959
8. Beelman, C.A., Stevens, A., Caponigro, G., LaGrandeur, T.E., Hatfield, L., Fortner, D.M., and Parker, R. (1996) An essential component of the decapping enzyme required for normal rates of mRNA turnover. *Nature* **382**, 642–646
9. Lykke-Andersen, J. (2002) Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay. *Mol. Cell. Biol.* **22**, 8114–8121
10. van Dijk, E., Cougot, N., Meyer, S., Babajko, S., Wahle, E., and Seraphin, B. (2002) Human Dcp2: a catalytically active mRNA

decapping enzyme located in specific cytoplasmic structures. *EMBO J.* **21**, 6915–6924
11. Wang, Z., Jiao, X., Carr-Schmid, A., and Kiledjian, M. (2002) The hDcp2 protein is a mammalian mRNA decapping enzyme. *Proc. Natl Acad. Sci. USA* **99**, 12663–12668
12. Steiger, M., Carr-Schmid, A., Schwartz, D.C., Kiledjian, M., and Parker, R. (2003) Analysis of recombinant yeast decapping enzyme. *RNA* **9**, 231–238
13. Dunckley, T. and Parker, R. (1999) The DCP2 protein is required for mRNA decapping in Saccharomyces cerevisiae and contains a functional MutT motif. *EMBO J.* **18**, 5411–5422
14. Lejeune, F., Li, X., and Maquat, L.E. (2003) Nonsense-mediated mRNA decay in mammalian cells involves decapping, deadenylating, and exonucleolytic activities. *Mol. Cell* **12**, 675–687
15. Wang, Z. and Kiledjian, M. (2001) Functional link between the mammalian exosome and mRNA decapping. *Cell* **107**, 751–762
16. van Dijk, E., Le Hir, H., and Seraphin, B. (2003) DcpS can act in the 5′-3′ mRNA decay pathway in addition to the 3′-5′ pathway. *Proc. Natl Acad. Sci. USA* **100**, 12081–12086
17. Bahler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14**, 943–951
18. Knop, M., Siegers, K., Pereira, G., Zachariae, W., Winsor, B., Nasmyth, K., and Schiebel, E. (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast* **15**, 963–972
19. Muhlrad, D., Hunter, R., and Parker, R. (1992) A rapid method for localized mutagenesis of yeast genes. *Yeast* **8**, 79–82
20. Maundrell, K. (1993) Thiamine-repressible expression vectors pREP and pRIP for fission yeast. *Gene* **123**, 127–130
21. Araki, Y., Takahashi, S., Kobayashi, T., Kajiho, H., Hoshino, S., and Katada, T. (2001) Ski7p G protein interacts with the exosome and the Ski complex for 3(-to-5(mRNA decay in yeast. *EMBO J.* **20**, 4684–4693
22. Decker, C.J. and Parker, R. (1993) A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. *Genes Dev.* **7**, 1632–1643
23. Jacobs Anderson, J.S. and Parker, R.P. (1998) The 3′ to 5′ degradation of yeast mRNAs is a general mechanism for mRNA turnover that requires the SKI2 DEVH box protein and 3′ to 5′ exonucleases of the exosome complex. *EMBO J.* **17**, 1497–1506
24. Cougot, N., Babajko, S., and Seraphin, B. (2004) Cytoplasmic foci are sites of mRNA decay in human cells. *J. Cell Biol.* **165**, 31–40
25. She, M., Decker, C.J., Sundramurthy, K., Liu, Y., Chen, N., Parker, R., and Song, H. (2004) Crystal structure of Dcp1p and its functional implications in mRNA decapping. *Nat. Struct. Mol. Biol.* **11**, 249–256
26. Tucker, M., Valencia-Sanchez, M.A., Staples, R.R., Chen, J., Denis, C.L., and Parker, R. (2001) The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in *Saccharomyces cerevisiae*. *Cell* **104**, 377–386
27. Bai, R.Y., Koester, C., Ouyang, T., Hahn, S.A., Hammerschmidt, M., Peschel, C., and Duyster, J. (2002) SMIF, a Smad4-interacting protein that functions as a co-activator in TGFβ signalling. *Nat. Cell Biol.* **4**, 181–190
28. Coller, J. and Parker, R. (2004) Eukaryotic mRNA decapping. *Annu. Rev. Biochem.* **73**, 861–890