Scavenger Decapping Activity Facilitates 5' to 3' mRNA Decay

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mRNA degradation occurs through distinct pathways, one primarily from the 5' end of the mRNA and the second from the 3' end. Decay from the 3' end generates the m'GppN cap dinucleotide, which is subsequently hydrolyzed to m'Gp and ppN in Saccharomyces cerevisiae by a scavenger decapping activity termed Dcs1p. Although Dcs1p functions in the last step of mRNA turnover, we demonstrate that its activity modulates earlier steps of mRNA decay. Disruption of the DCS1 gene manifests a threefold increase of the TIF51A mRNA half-life. Interestingly, the hydrolytic activity of Dcs1p was essential for the altered mRNA turnover, as Dcs1p, but not a catalytically inactive Dcs1p mutant, complemented the increased mRNA stability. Mechanistic analysis revealed that 5' to 3' exoribonucleolytic activity was impeded in the des3Δ strain, resulting in the accumulation of uncapped mRNA. These data define a new role for the Dcs1p scavenger decapping enzyme and demonstrate a novel mechanism whereby the final step in the 3' mRNA decay pathway can influence 5' to 3' exoribonucleolytic activity.

A unique characteristic of eukaryotic mRNA is the N7-methylated guanosine cap structure that is cotranscriptionally added to the 5' terminus of nascent RNA (40). The cap serves to protect the 5' end of the mRNA from exoribonucleolytic degradation (49). It also functions in the transport of mature mRNA from the nucleus to the cytoplasm (18, 20), in translation initiation (12), and in pre-mRNA splicing (24). The cap is bound by distinct cap-binding proteins in each cellular compartment. A heterodimeric complex of CBC20 and CBC80 binds the cap in the nucleus (19), while eIF4E, the translation initiation factor, associates with the cap in the cytoplasm (12). In addition to these major cap-binding proteins, several other factors have also been demonstrated to bind the mRNA cap, including the mammalian poly(A)-binding protein (22), the cold shock protein YB-1 (10), and the scavenger decapping protein (28, 37).

Decay of mRNA is not a randomized process and proceeds through at least two major decay pathways, both of which are initiated by removal of the poly(A) tail (31). Following deadenylation, the mRNA is decapped and degraded by a 5' to 3' exonucleolytic activity in the 5' decay pathway. In the 3' decay pathway, the mRNA is continuously degraded from the 3' end following deadenylation, generating an m'GpppN cap dinucleotide that is hydrolyzed by a scavenger decapping activity (27, 48).

Each mRNA decay pathway utilizes a unique decapping activity with a distinct substrate specificity (7). In the 5' pathway, both the yeast and human Dcp2 proteins utilize capped mRNA as a substrate and hydrolyze the cap structure to generate m'GDP and RNA with a monophosphate at its 5' end (29, 41, 43, 47). The exposed 5' end of the RNA is degraded by Xrn1p, the 5' to 3' exoribonuclease, leading to rapid degradation of the RNA body (4, 17, 25). A scavenger decapping activity termed DcpS in mammals and Dcs1p in budding yeast functions in the final step of the 3' decay pathway. This activity hydrolyzes the residual cap structure following 3' to 5' exoribonucleolytic decay by the exosome complex to release m'GMP and nucleotide diphosphate (27, 48). Characterization of scavenger decapping activity indicates its strong preference for binding and hydrolyzing cap structure linked to an RNA of less than 10 nucleotides in mammals and 3 nucleotides in Caenorhabditis elegans (6, 27, 28).

Recent structural analysis of DcpS reveals that it can form either an asymmetric dimer bound to two cap dinucleotides (13) or a symmetric dimer in the ligand-free protein (5). The dimers contain distinct amino-terminal and carboxyl-terminal domains separated by a hinge region (13). The structure indicates that the N terminus can flip back and forth, alternating on each side from a productive closed to a nonproductive open conformation (13). An interesting property of DcpS is its almost exclusive utilization of the residual cap dinucleotide following 3' exonucleolytic decay of the RNA (27, 28). This substrate specificity is due in part to a higher affinity of DcpS for the cap structure (28) and more significantly to both entropic and steric constraints in the formation of a closed decapping-competent complex in the presence of an mRNA moiety on the cap (13).

Saccharomyces cerevisiae contains two proteins termed Dcs1p and Dcs2p that are equally homologous to the human DcpS. Curiously, only Dcs1p possesses intrinsic decapping activity analogous to that of DcpS (27). The enzymatic activity and substrate specificity for Dcs2p remain unknown, although its ability to heterodimerize with Dcs1p suggests that it might be a modulator of Dcs1p decapping activity (30). Dcs1p is a member of the histidine triad (HIT) family of nucleotide binding proteins and contains the characteristic HIT motif (His-X-His-X-His-X, where X is a hydrophobic amino acid) which is required for its cap hydrolysis activity (27). By virtue of its ability to hydrolyze the cap dinucleotide (27), DcpS and Dcs1p are postulated to function during the terminal phase of mRNA decay. Here we demonstrate that Dcs1p also functions to impact the 5' mRNA decay pathway.
**TABLE 1. Strains used in this study**

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</tr>
<tr>
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<td>This study</td>
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**MATERIALS AND METHODS**

Strains. Disruption of the DCS1 gene within the Y262, yRP1192, and yJC135 backgrounds was carried out by homologous recombination using a cassette of the neomycin resistance gene flanked by DCS1 intragenic sequences. The cassette was PCR amplified using the 5’ primer (5’ TCTAGTCCCAAGACATCGAAGAC 3’) and the 3’ primer (5’ ATTCCTACAACTATGCGATCT 3’) from the genomic DNA of strain Y15179 (ResGen Invitrogen Corporation, Huntsville, AL) containing a substitution of the neomycin resistance gene in the DCS1 gene. All the strains used in this report are listed in Table 1.

Plasmids. The plasmid pRS426-DCS1, containing the full-length DCS1 gene including 342 bp upstream of the translation start site and 488 bp downstream of the mRNA 3’ end of Zhang et al. (50) with minor modifications. Cells were disrupted with glass beads by vortexing sequentially for 30 s each time followed by a 2-min cooling on ice repeated six times. After the extract was centrifuged at 10,000 × g for 10 min to remove cellular debris, the supernatant was subjected to ultracentrifugation at 50,000 × g for 45 min. The resulting supernatant was collected and supplemented with 10% glycerol, and protein concentrations were determined by Bio-Rad assay reagent (Bio-Rad Laboratories Inc., Hercules, CA).

**Extraction of RNA and cDNA synthesis.** Preparation of total RNA was performed according to the method of Zhang et al. (50) with minor modifications. Cells were disrupted with glass beads by vortexing sequentially for 30 s each time followed by a 2-min cooling on ice repeated six times. After the extract was centrifuged at 10,000 × g for 10 min to remove cellular debris, the supernatant was subjected to ultracentrifugation at 50,000 × g for 45 min. The resulting supernatant was collected and supplemented with 10% glycerol, and protein concentrations were determined by Bio-Rad assay reagent (Bio-Rad Laboratories Inc., Hercules, CA).

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**Real-time PCR.** mRNA levels were quantified from reverse-transcribed cDNA by real-time PCR using SYBR green PCR core reagent (Applied Biosystems, Foster City, CA), and TIFS1A, HTBI, or PGKI mRNA abundance was quantitated using the standard curve method according to the recommendation of the manufacturer. Values were normalized to the stable U3 RNA. Each gene was amplified using the appropriate specific primers, 5’ ACCATT ACCGCA GAGAGATGATG 3’ and 5’ TGAACATGGACGGTGACACT 3’ for TIFS1A, 5’ CAACATCCACTCCGACGAC 3’ and 5’ TACGCGACCAATT CAAGAAC 3’ for HTBI, 5’ CTCTTAGAAGAGTGATCTGTA 3’ and 5’ CGGCAGCTCCGCGGCCGTTT TTTTTTTTT 3’ was used for reverse transcription. Amplification of the TIFS1A 3’ primer was carried out by PCR with the 5’ GTATTTTATCATCATATA GAAAC 3’ primer, the primer, and the oligo(dT)-anchored 3’ primer in the presence of [α-32P]dATP. PCR amplified material was visualized by autoradiography following electrophoresis on a 6% T/3 M urea denaturing polyacrylamide gel.

**Generation of cap-labeled RNA and detection of decapping products.** Un- capped TIFS1A 3’UTR was transcribed in vitro with T7 polymerase from a PCR-generated template using the following primers: 5’ CGTATAGACGACT CACTATAGGGACCGGTTAACATCATGGCATGAGTT GTTGAAGATTACATAAAGAG 3’. The cap-labeled RNA containing the label on the first phosphoate following the methyl guanosine (m5GpppG) was generated with the vaccinia virus capping enzyme utilizing [α-32P]GTP and S-adenosyl-methionine, followed by gel purification as described previously (46). Decapping assays were carried out as previously described (27), and decapping products were resolved by polyethyleneimine-cellulose thin-layer chromatography (TLC) and developed in 0.45 M (NH4)2SO4. The TLC plates (Sigma) were dried and detected with a Molecular Dynamics PhosphorImager (Storm 860).

**Western blot analysis.** Protein samples were resolved on 12.5% SDS gels and transferred to a nitrocellulose membrane (Schleicher & Schuell BioScience, Inc., Keene, NH) by use of a semidry blotting apparatus. Hybridizations were performed with affinity-purified human DcpS antibody (1:200) (27) and visualized by enhanced chemiluminescence using horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Cappel, West Chester, PA) (1:10,000 dilution).

**[35S]methionine incorporation.** Yeast cells (100 ml culture) were grown in medium lacking methionine at 25°C to an optical density at 600 nm (OD600) of 0.5, followed by supplementation with 50 μM unlabeled methionine and 1 μCi/ml of [35S]methionine (NEN). At each indicated time point (see Fig. 5), 1 ml of culture was removed for OD600 analysis, and another 1 ml of cells was used for protein analysis by lysis and precipitation with 10% ice-cold trichloroacetic acid (TCA). The samples were filtered through 0.2 μM nitrocellulose filters (Milli-
FIG. 1. Disruption of the Dcs1p scavenger decapping enzyme in Saccharomyces cerevisiae increases the stability of TIF51A mRNA. Degradation of TIF51A mRNA was monitored following transcriptional shutoff with thiolutin (20 μg/ml), and RNA was isolated at the indicated time points. (A) Degradation of TIF51A transcripts was detected by Northern blot analysis. Time points following transcriptional repression are shown above the lanes. The top panel shows the results for the TIF51A mRNAs from isogenic wild-type (WT) (BY4742) and dcs1Δ (Y15179) cells. The blot was probed with 32P-labeled TIF51A 3’UTR. The stable U3 RNA was used as an internal control and, as shown in the bottom panel, probed with the entire coding sequence. (B) Half-life measurements of TIF51A transcripts were carried out by quantitative real-time PCR using RNA derived from wild-type and dcs1Δ strains as described for panel A. The numbers at the bottom indicate minutes after transcription repression. The percentage of RNA remaining at each time point was calculated, normalized to U3 RNA levels by a standard curve method, and plotted for each time point. The average value and standard derivation at each time point were obtained from at least four independent experiments, each carried out in triplicate.

pore, Billerica, MA) prewashed with 5% TCA to retain the proteins. The filters were subsequently washed twice with 5 ml ice-cold 5% TCA to remove unincorporated [35S]methionine and air dried. The amount of bound newly synthesized proteins was determined with a liquid scintillation counter.

RESULTS

Disruption of DCS1 results in stabilization of the TIF51A mRNA. The scavenger decapping activity functions in the last step of the 3’ mRNA decay pathway (27, 48). To address whether the last step of mRNA decay can influence the overall rate of mRNA turnover, we determined the consequence of the absence of Dcs1p on mRNA decay in a DCS1-disrupted yeast strain. The half-life of the TIF51A mRNA was monitored following transcriptional arrest with thiolutin. Interestingly, the stability of TIF51A mRNA was different in the two strains and threefold greater in the dcs1Δ strain, as determined by Northern blot analysis (Fig. 1A). The half-life increased from 13 min in the wild-type strain to 40 min in the dcs1Δ strain. Similar results were also obtained using a quantitative real-time PCR approach with primers that amplified a fragment within the coding region of the TIF51A mRNA (Fig. 1B). These data demonstrate that the TIF51A mRNA is more stable in the dcs1Δ strain and suggest that Dcs1p contains a novel function that influences the efficiency of mRNA decay. Furthermore, the correlation between the Northern blot analysis and the quantitative real-time PCR analysis demonstrates the validity of the PCR approach, which was used in subsequent experiments.

The increase in TIF51A mRNA stability in dcs1Δ was not unique to thiolutin-mediated shutdown of transcription. A similar threefold difference was also observed in the rpb1-1 strain background (Table 2; Fig. 2A), which contains a thermostable allele of the largest subunit of RNA polymerase II and allows inhibition of transcription under nonpermissive conditions (37°C) (16, 34). These data also demonstrate that the observed increase in TIF51A mRNA half-life upon disruption of the DCS1 gene is not strain specific. The rpb1-1 strain background is different from that used in Fig. 1. Furthermore, the increase in mRNA stability in the dcs1Δ strain was not restricted to the TIF51A mRNA only and was also observed with at least two other transcripts tested. The stability of the HTB1 mRNA was also increased by threefold in the dcs1Δ strain relative to that of the parental strain from a half-life of 8 min to 23 min, respectively (Table 2). Similarly, the half-life of the stable PGK1 transcript was greater than the 60-min duration of the experiment compared to 20 min seen with the parental strain (Table 2). These data suggest the observed increase of mRNA stability in the dcs1Δ strain is not restricted to a single transcript and could be a more general property.

Regulation of TIF51A mRNA stability is dependent on Dcs1p hydrolysis activity. To address whether the difference in TIF51A mRNA half-life was a function of the presence of Dcs1p, the dcs1Δ strain was complemented with the DCS1 gene. As expected, the threefold increase of TIF51A mRNA half-life in the DCS1-disrupted background was reversed upon complementation with the DCS1 gene (Fig. 2A). However, reversal of the increased half-life was not detected with a catalytically inactive Dcs1p mutant (Dcs1pM) (Fig. 2A). Decapping assays using extract from cells complemented with the wild-type and mutant DCS1 genes confirmed that strains complemented with the wild-type gene but not those complemented with the mutant gene contained decapping activity (data not shown). The mutant protein used harbored an asparagine substituted in place of histidine 268 within the HIT hydrolase motif to generate a decapping-deficient protein. Western blot analysis was carried out to confirm that the wild-type and mutant exogenous Dcs1p proteins were expressed at

<table>
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</tr>
<tr>
<td>dcs1Δ</td>
<td>23</td>
</tr>
<tr>
<td>HTB1</td>
<td>8</td>
</tr>
<tr>
<td>TIF51A</td>
<td>10</td>
</tr>
<tr>
<td>PGK1</td>
<td>20</td>
</tr>
<tr>
<td>rpb1-1</td>
<td>23</td>
</tr>
<tr>
<td>dcs1Δ</td>
<td>30</td>
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</table>
FIG. 2. Hydrolysis activity of Dcs1p is required to regulate the rate of TIF51A mRNA degradation. (A) Half-life measurements were assessed for the temperature-sensitive rpb1-1 (Y262) strain. Transcription was repressed by a shift to the nonpermissive temperature of 37°C, and RNA was isolated at the indicated time points. The four different strains used are shown in the boxed insert. Dcs1pM denotes the catalytically inactive mutant Dcs1p harboring a histidine-to-asparagine amino acid substitution (H268N) in the HIT motif. The average value and standard deviations for each time point were obtained from at least three independent experiments, each carried out in triplicate. (B) Expression of exogenous wild-type and inactive Dcs1p mutant was detected by Western blot analysis, utilizing primary antibody directed against human DcpS. With the amount of extract used in this experiment, there is enough cross-reactivity with the yeast Dcs1 protein to detect the overexpressed but not the endogenous protein. Lane 1 is the positive control detecting the human DcpS protein in HeLa cell extract. (C) Expression of both wild-type and mutant Dcs1p complements the glycerol lethality phenotype. Dilutions of the indicated cells were spotted onto plates lacking uracil and containing either glucose (left panel) or glycerol (right panel) as the carbon source. Rows rpb1-1 and rpb1-1 dcs1Δ contained the pRS426 empty vector with URA3 marker. The rpb1-1 dcs1Δ Dcs1p and rpb1-1 dcs1Δ Dcs1pM cells expressed the pRS426 plasmid encoding the wild-type and the mutant DCS1 gene, respectively.

equivalent levels (Fig. 2B). Judging on the basis of the similarity of the crystal structures for the wild-type and the HIT motif mutant-containing mammalian DcpS proteins (5, 14), the structure of the mutant yeast protein should not be altered by the single amino acid substitution. This point was further substantiated by the ability of the Dcs1pM expression to suppress the glycerol lethality of the dcs1Δ strain. Under glycerol conditions, both the wild-type and mutant Dcs1 proteins can complement the growth defect (Fig. 2C), further demonstrating that the overall structural integrity of the mutant protein is maintained. Taken together, these results indicate that functional Dcs1p was required for the regulation of TIF51A mRNA stability and strongly suggest that the hydrolytic activity, rather than the Dcs1p protein per se, serves the regulatory role.

The regulation of mRNA stability mediated by Dcs1p is independent of translation. Several observations argue that increasing mRNA association with ribosomes inhibits 5′ decapping rates, which in turn slows mRNA decay. For example, inhibition of translation elongation by mutations or by elongation inhibitors significantly decreases the rates of 5′ decapping (3, 36). To exclude the possibility that stabilization of TIF51A mRNA is a consequence of altered mRNA translation, we examined the global translation efficiency by determining the [35S]methionine incorporation in cells possessing and lacking Dcs1p activity. Yeast cells were pulsed with [35S]methionine, and newly synthesized proteins were detected. As shown in Fig. 3, the pattern of methionine incorporation over time in dcs1Δ was analogous to that obtained from the parental strain, while treatment with cycloheximide showed the expected inhibition of protein synthesis. These data demonstrate that there was no detectable defect in translation efficiency when Dcs1p was removed. dcs1Δ expressing the catalytically inactive Dcs1pM also displayed normal translation (Fig. 3), confirming that loss of cap hydrolysis activity does not influence translation under these assay conditions. We conclude that the increase of mRNA stability observed in the dcs1Δ strain is not due to altered translation and is a direct consequence of scavenger decapping activity manifesting a novel regulatory mechanism.

Dcs1p activity influences 5′ to 3′ degradation of TIF51A mRNA. In an attempt to address the mechanism by which TIF51A mRNA became stabilized in the dcs1Δ background, we determined which step of mRNA decay was negatively impacted. The three most likely events include either the initial
deadenylation step or one of the subsequent 5' to 3' or 3' to 5' decay steps. As shown in Fig. 4A, comparable deadenylation rates were detected for the \textit{TIF51A} mRNA following transcriptional shutoff between strains expressing and lacking \textit{DCS1}. The decrease in poly(A) tail length over time was indistinguishable between the wild-type and \textit{dcs1\Delta} cells by a poly(A) assay (38), suggesting that there was no significant effect on deadenylation in the absence of Dcs1p.

Having ruled out an influence at the level of deadenylation as the cause for the greater stability of the \textit{TIF51A} mRNA, we next determined whether decay from either the 5' or 3' end was affected. Several complementary approaches were used to address this question. A modification of an approach we previously used to simultaneously monitor the disappearance of both termini of an mRNA was employed (48). The presence of sequences at the 5' end of \textit{TIF51A} mRNA relative to sequences corresponding to the 3' end was detected using quantitative real-time PCR. We would anticipate a ratio of the two termini to be approximately 1, since the majority of mRNAs would be either full length or completely degraded. However, a small percentage of mRNAs that were in the midst of decay from one end or the other would be trapped upon RNA isolation. These intermediates would skew the ratio to more than 1 under conditions that stabilize the 5' end or to less than 1 when the 3' end is more stable. A comparison of the 5'-to-3' ratio between the \textit{rpb1-1} and the \textit{rpb1-1 dcs1\Delta} strains revealed that a ratio greater than 1 (Fig. 4B, bars 1 and 2) indicated that the 5' end of the \textit{TIF51A} mRNA was more stable in the \textit{dcs1\Delta} cells as determined by the same approach used as described for panel C. The transcriptional block and normalization relative to the U3 RNA for panels C, D, and E were carried out as described in the legend to Fig. 1. Note that the differences in \textit{TIF51A} mRNA half-lives observed in Fig. 1, 2, and 4 are a function of the different strain backgrounds used.

![Fig. 4](image)

**FIG. 4.** Disruption of \textit{DCS1} impedes 5' to 3' degradation of the \textit{TIF51A} mRNA. (A) The length of the poly(A) tail in cells expressing or lacking Dcs1p was analyzed by the poly(A) assay. RNA was isolated from the two different strains at the indicated times following transcriptional repression as described for Fig. 2. Migration of unadenylated (A-) RNAs is denoted on the left side of the panel. (B) A schematic of the \textit{TIF51A} mRNA is shown at the bottom of the panel, with the translation start (AUG) and stop (UAA) sites denoted and the relative position of the 5' and 3' fragments of the RNA indicated. The bar graph represents the relative steady-state ratios of the 5' to 3' fragment ratios are presented relative to the ratio for the \textit{rpb1-1} strain, which was arbitrarily set to 1. The values were derived from three independent experiments carried out in triplicate, with the error bars shown. (C) The half-life of the \textit{TIF51A} mRNA measured by real-time PCR is shown in the isogenic wild-type (WT) (yRP684) and \textit{dcs1\Delta} strains. (D) The half-life of the \textit{TIF51A} mRNA for \textit{ski2\Delta} (yRP1192) and \textit{ski2\Delta dcs1\Delta} strains is shown as determined by the same approach used as described for panel C. (E) Half-life measurements for the \textit{TIF51A} mRNA are shown for \textit{dhh1\Delta} (yJC135) and \textit{dhh1\Delta dcs1\Delta} cells as determined by the same approach used as described for panel C. The transcriptional block and normalization relative to the U3 RNA for panels C, D, and E were carried out as described in the legend to Fig. 1. Note that the differences in \textit{TIF51A} mRNA half-lives observed in Fig. 1, 2, and 4 are a function of the different strain backgrounds used.
capping step is severely compromised in a \textit{dhh1}Δ background (8, 11). If the repression of \textit{TIF51A} mRNA degradation were mediated through the 5’ decay pathway, the \textit{TIF51A} mRNA stability in cells harboring either a \textit{dhh1}Δ disruption or a \textit{dhh1}Δ \textit{dcs1}Δ double disruption should remain the same, since the 5’ decay pathway is already compromised. Conversely, a double disruption of \textit{dcs1}Δ with \textit{ski2}Δ, which is essential for the decay of an mRNA from the 3’ end (2), would be expected to result in an additive stabilization.

We first confirmed that disruption of the \textit{DCS1} gene, within the parental strain from which the \textit{DHHL1} and \textit{SKI2} gene lesions were derived, maintained the observed increase in \textit{TIF51A} mRNA stability. As shown in Fig. 4C, a threefold-greater half-life was detected in this strain background upon disruption of the \textit{DCS1} gene. Consistent with 5’ end decay being a critical determinant for the \textit{TIF51A} mRNA decay (45), a block of the 3’ decay pathway in the \textit{ski2}Δ strain did not significantly alter the \textit{TIF51A} mRNA half-life relative to that of the parental strain (3.5 min compared to 2.5 min, respectively). As expected, the stability of \textit{TIF51A} mRNA was increased in the \textit{ski2}Δ \textit{dcs1}Δ double-disruption cells compared to the \textit{ski2}Δ strain results (Fig. 4D). These data demonstrate an additive effect of \textit{dcs1}Δ and \textit{ski2}Δ on \textit{TIF51A} mRNA stability. Consistent with an influence of Dcs1p on 5’ end decay, the \textit{TIF51A} mRNA half-life was not significantly altered in the presence or absence of the \textit{DCS1} gene within the \textit{dhh1}Δ background (Fig. 4E). Collectively, these data indicate that the Dcs1p influence is exerted through the 5’ decay pathway.

\textbf{Dcs1p activity regulates 5’ to 3’ exoribonucleolytic activity.}

Having determined that Dcs1p imparts a regulatory influence on the 5’ decay pathway, we next addressed which step was altered. The influence of Dcs1p could be through either the 5’ decapping step or the subsequent 5’ to 3’ exoribonucleolytic step, both of which were tested. Extract derived from either wild-type or \textit{dcs1}Δ cells was utilized in an in vitro decapping assay using cap-labeled \textit{TIF51A} 3’ UTR RNA substrate. Dcp1p/2p decapping activity was detected in the cytoplasmic 50,000 \times \textit{g} supernatant fraction (SS0) (Fig. 5A, lane 3). The presence of m’GDP was confirmed by its conversion to m’GTP by nucleotide diphosphate kinase (NDPK) (48) (lanes 5 and 6). A comparison of lanes 3 and 4 demonstrates similar levels of decapping efficiency, as determined by the generation of m’GDP. Therefore, the presence or absence of Dcs1p did not appear to have a significant effect on mRNA decapping under these in vitro assay conditions.

Following decapping of the deadenylated mRNA, the RNA is degraded by the 5’ to 3’ exoribonuclease Xrn1p in yeast (17). To address whether this exoribonucleolytic step is hindered in the \textit{dcs1}Δ cells, we determined whether uncapped RNA accumulated in the disrupted strain. Uncapped RNA would not be detected under wild-type conditions due to efficient exoribonucleolytic decay, while accumulation of this substrate was detected in an \textit{xrn1}Δ background (15, 32, 51). Therefore, if the exoribonucleolytic step was slowed in the \textit{dcs1}Δ strain, we would expect uncapped RNA to accumulate. Capped RNA was separated from uncapped RNA by immunoprecipitation with an anticap antibody, and the presence of \textit{TIF51A} RNA was monitored. The reaction was spiked with \textit{TIF51A} cap-labeled RNA to determine the efficiency of capped RNA immunoprecipitation. As shown in the control panel on the bottom of Fig. 5B, capped RNA was detected only in the immunoprecipitated fraction but not in the supernatant fraction. This observation confirms the efficiency of the capped RNA immunoprecipitation and demonstrates that the antibody was not saturated with capped RNA under these assay conditions.

The immunoprecipitated RNAs were next detected by Northern blot analysis to detect the proportion of capped versus uncapped \textit{TIF51A} RNA. In wild-type cells, 98% of \textit{TIF51A} RNA was capped and present in the immunoprecipitated lane.
whereas the supernatant fraction, corresponding to uncapped RNA, contained only a trace amount of TIF51A RNA (Fig. 5B; compare lane 2 to 3). In contrast, 10% of uncapped RNA was detected in the dcs1Δ cells (lane 6), demonstrating that uncapped RNA accumulates in the dcs1Δ strain. This result was reproducible with three independent experiments. Collectively, our results imply that 5’ exoribonucleolytic activity is positively regulated by Dcs1p in yeast cells. Therefore, stability of the TIF51A mRNA is increased due to a hindrance of the 5’ to 3’ exoribonucleolytic activity in the absence of the scavenger decapping function.

**DISCUSSION**

We present a novel role for the scavenger decapping enzyme in mRNA degradation in *Saccharomyces cerevisiae*. Des1p, which was previously thought to function primarily in the last step of mRNA decay (27, 44), also influences overall mRNA stability (Fig. 1, 2A, and 4C; Table 2). Furthermore, we demonstrate that this regulation is at the level of 5’ to 3’ exoribonucleolytic decay (Fig. 4 and 5). Although our analysis focused on the TIF51A mRNA, a similar stabilization was observed in the dcs1Δ strain for two other mRNAs tested, the PGK1 and HTB1 mRNAs, demonstrating that this regulation is not restricted to TIF51A. Our data support the hypothesis that Des1p functions in a general regulatory mechanism whereby its activity influences the degradation of mRNA in the 5’ decay pathway.

Several lines of evidence support the role of Des1p in a potential feedback mechanism regulating the stability of mRNA by influencing 5’ end decay. First, strains disrupted for *DCS1* contain mRNAs that are more stable than strains that express *DCS1* (Fig. 1, 2A, and 4C; Table 2), indicating that Des1p influences mRNA decay. Second, the 5’ end of the TIF51A mRNA persisted longer than the 3’ end in the absence of functional Des1p, a result which was subsequently reversed upon expression of active Des1p (Fig. 4B). Third, genetic inactivation of the 5’ degradation pathway abolished the Des1p-mediated effect on mRNA decay (Fig. 4D). Lastly, uncapped TIF51A mRNA accumulated in the absence of Des1p activity, indicating that the 5’ to 3’ exoribonucleolytic activity involved in the decay of uncapped mRNA is compromised (Fig. 5B). Considering that Xrn1p is the cytoplasmic exoribonuclease responsible for 5’-end decay of mRNA in yeast (17, 21, 31), it is most likely that Des1p activity influences Xrn1p function (Fig. 6).

In addition to the interesting finding that a Des1p-mediated function exists to regulate 5’ exoribonucleolytic decay, an unexpected result was that this regulation was dependent on the decapping activity of Des1p but not directly dependent on the Des1p protein. This was made evident by the observed complementation of the dcs1Δ strain with Des1p but not the catalytically inactive Des1pM protein containing a single amino acid substitution in the HIT motif active site (Fig. 2A and 4B). These data indicate that the regulator is not a protein that is directly controlled by Des1p through protein-protein interactions; rather, either the substrate or product of Des1p could be functioning as a signal to influence overall mRNA decay.

We propose that Des1p can influence mRNA decay by controlling the availability of its m7GpppN cap dinucleotide substrate or its hydrolyzed methylated guanosine product (Fig. 6). Yeast disrupted for the *DCS1* gene accumulate m7GpppN cap dinucleotide (27), which could serve as a negative effector of 5’ to 3’ exoribonucleolytic activity. A role for Des1p as a regulator of dinucleotide levels is consistent with previous suggestions that HIT protein family members could be mediators of dinucleotide signaling molecules (23, 26). Conversely, m7GMP, the Des1p decapping product, or m7G, the subsequent dephosphorylated product, could serve as a positive effector of exoribonucleolytic activity. In the absence of Des1p, the lack of m7GMP (or m7G) could lead to a slowing of exoribonucleolytic activity.

It is presently unclear whether the cap dinucleotide or the decapping product serves as the signal influencing mRNA decay. However, since m7GMP is a general mRNA decay product, it could function as the signal. m7GMP can be formed by Des1p in cells by the hydrolysis of m7GpppN (27), m7GDP (44), and m7GTP (unpublished observations). Further support for m7GMP, rather than the cap dinucleotide, as the mediator comes from Fig. 4D, in which it is shown that stability of the TIF51A mRNA was greater in the ski2Δ dcs1Δ double disruption than in the ski2Δ single disruption. Considering that the ski2Δ strain is compromised in 3’-end exonuclease decay, we would expect minimal, if any, cap dinucleotide accumulation. However, m7GMP would still be generated from the Dcp2p product of m7GDP, which can be hydrolyzed to m7GMP by Des1p (44). Therefore, the observed additive effect in the ski2Δ dcs1Δ double-disruption strain supports the hypothesis that mRNA stabilization results from the lack of m7GMP formation.

One surprising finding of our work is that the feedback regulation is at the level of exoribonucleolytic decay rather than at the level of the initial decapping step. Although at present the physiological implication is unclear, a coordination bei-
 tween the two exonucleolytic decay pathways appears most likely. The ability of the scavenger decapping activity to enhance 5' exonucleolytic decay could serve to facilitate the rapid clearing of uncapped mRNAs. Accumulation of uncapped mRNA could otherwise sequester exosome protein components and prevent their function on capped mRNAs that are destined for decay and need to be cleared. The activity of the exosome on capped mRNAs, which are still translationally competent, might be more critical than its function on uncapped RNAs that are translationally incompetent. Whether Dcs1p can coordinate this interplay remains to be determined.

Although it has long been thought to be among the least-regulated steps of mRNA decay, several examples exist showing that the 5' to 3' exonucleolytic step is regulated. First, the TIF51A gene product has been shown to specifically influence 5' exonucleolytic activity following decapping (51). Second, accumulation of pAp in cells results in the inhibition of Xrn1p exonucleolytic activity (9). Further underscoring the significance of 5' exonuclease activity, the expression of Xrn1 is developmentally controlled in Drosophila melanogaster (42) and critical for ventral epithelial enclosure during embryogenesis in C. elegans (33). Modulation of 5' to 3' exonucleolytic activity could also regulate the decay of mRNA fragments generated by small interfering RNA-mediated cleavage which involves Xrn1 in both C. elegans (33) and D. melanogaster (35). Our study provides additional evidence that the 5' to 3' exonuclease activity can be modulated and is more regulated than previously appreciated.

A second surprising finding of this work is the demonstration that Dcs1p is a multifunctional protein that functions in a role independent of its decapping activity. Disruption of the DCS1 gene results in a slow-growth phenotype under glucose conditions and a lethal phenotype when glycerol is used as the carbon source (Fig. 2C). Interestingly, the catalytically inactive Dcs1pP434, which is unable to reverse the increased mRNA stability in the dcs1Δ cells, was nevertheless able to complement the glycerol lethality (Fig. 2C). These data demonstrate that the decapping activity of Dcs1p and its influence on mRNA decay can be uncoupled from novel function in glycerol-dependent growth.

Our data support a more general role for the yeast scavenger decapping activity in mRNA turnover. In addition to its function in clearing the cap structure during terminal stages of mRNA decay, it also impacts earlier steps involving 5' cap-dependent elongation inhibition in cis and in trans on the decay of the unstable yeast MFA2 mRNA. J. Biol. Chem. 269:9067–9069.

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