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The mRNA Transcription/Processing Factor Ssu72 Is a Potential Tyrosine Phosphatase*

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Ssu72 is an essential and highly conserved protein involved in mRNA transcription and 3′-end processing. The biochemical function of Ssu72 was so far unknown. We report here evidence that Ssu72 is a phosphatase that resembles protein tyrosine phosphatases (PTPases). First, recombinant Ssu72 cleaves the phosphotyrosine analogue *p*-nitrophenylphosphate, and this catalytic activity is impaired by PTPase-inhibiting agents. Second, the Ssu72 sequence contains the CX_5R signature motif of PTPases; mutation of the catalytic cysteine in this motif abolishes Ssu72 activity *in vitro* and has been shown to confer lethality *in vivo*. Third, secondary structure prediction and site-directed mutagenesis predict that Ssu72 adopts the fold of PTPases of the low molecular weight family. Distinguishing features, such as a short "aspartate loop" at the active site, suggest however that Ssu72 is the founding member of a new phosphatase subfamily. The novel Ssu72 activity may regulate coupling events during mRNA biogenesis.

Over the last few years a wealth of biochemical and genetic data have revealed extended coupling between nuclear events of gene expression (1, 2). In particular, transcription by RNA polymerase II (pol II)[1] is coupled to mRNA processing, including 5′ capping and splicing and 3′-end formation of the transcript. This coupling is achieved by the binding of mRNA processing factors to the phosphorylated C-terminal repeat domain (CTD), a mobile extension of the catalytic core of pol II (3). The CTD becomes phosphorylated during transcription initiation and remains phosphorylated during RNA chain elongation. Several CTD kinases have been described (4), but only one CTD phosphatase, called Fcp1, is known (5, 6). In addition to pol II, several other proteins of the transcription machinery are phosphorylated (7), and RNA processing complexes contain phosphoproteins as well. Phosphorylation and dephosphorylation events are thus crucial for the regulation of transcription-coupled mRNA processing.

Ssu72 was originally identified in a yeast genetic screen. A mutation in the *ssu72* gene enhances a defect in the general transcription factor IIB (TFIIB) that confers a shift in the transcription start site (8). Indeed, Ssu72 binds directly to TFIIB (9), and it also interacts with pol II, both genetically and physically (10, 11). Yeast Ssu72 is a subunit of the cleavage and polyadenylation factor (CPF), which together with cleavage factor I and poly(A) polymerase is sufficient for mRNA 3′-end formation (11, 12). Because RNA 3′-end formation and transcription termination are interlinked, and because Ssu72 functions also during transcription initiation, Ssu72 may be involved in a possible coupling between transcription termination and initiation (1). Recent data show that Ssu72 is also involved in transcription elongation (11). The Ssu72 mutation can increase pol II pausing and can counteract the toxicity of 6-azauracil, an inhibitor of pol II elongation (11). Yeast Ssu72 is essential for viability (8) and shares 44% identical amino acid residues with its human homologue. The high degree of conservation suggests that Ssu72 has a similar function in all eukaryotic cells.

Thus Ssu72 is an essential and highly conserved protein involved in eukaryotic mRNA biogenesis. However, the biochemical function of Ssu72 remained unknown. Here we provide evidence that Ssu72 is a phosphatase that resembles protein tyrosine phosphatases (PTPases). PTPases can be divided in four families, including receptor-like, intracellular, dual specificity, and low molecular weight (low M_r) PTPases (13, 14). We demonstrate that Ssu72 shows similarities to the low M_r PTPase family but that it also has distinguishing features. Possible functions of this novel enzymatic activity during mRNA biogenesis are discussed.

EXPERIMENTAL PROCEDURES

Cloning and Site-directed Mutagenesis—Gene *ssu72* encoding for the 194-amino acid residue human isoform 1 was amplified by PCR from human muscle cDNA using the oligonucleotide primers 5′-CGCATG-CC**ATGGCA**ATGCCGTCGTCCCCGCTGCGGG-3′ and 5′-GCTTTTC-CTG**GCGGCCGCG**TAGAAGCAGACGGTGTGC-3′ (mutated positions in bold, restriction sites underlined). Thereby, an *Nco*I restriction site and codons for two additional amino acids, methionine and alanine, were added to the 5′-end of the gene, and at the 3′-end of the gene, the stop codon was removed and a *Not*I site was added. PCR products were digested and ligated into pET28b (Novagen), resulting in a construct that encodes for the Ssu72 polypeptide chain plus a C-terminal hexahistidine tag. After transformation of ligation products into *Escherichia coli* XL1 blue cells, plasmid DNA was isolated, and DNA from positive clones was sequenced. The resulting DNA sequence of gene *ssu72* agrees with the published sequence (10) but deviates from the data base entry for PNAS-120 (NCBI accession number AAK07538) at two amino acid positions. Apparent mutations encode for residues Lys-182 and Ser-183 instead of Arg-182 and Val-183, respectively. Point mutations were introduced by site-directed mutagenesis with the two step PCR overlap extension method. The mutated gene *ssu72 C12S* was amplified from the cloned *ssu72* plasmid DNA in two steps. In the first step, two independent PCR reactions were carried out. The first reaction was carried out with a T7 promoter forward primer and the primer 5′-GCTCCGGTTCTGGTTGCTCGA**GCT**TCACCACCGCCACCCG-3′. In the second reaction we used the reverse complement counterpart to the latter primer and a T7 terminator reverse primer. In the second step, the entire mutated gene was amplified with both T7 primers from the PCR products of the first step. The resulting PCR product was digested

[1] The abbreviations used are: pol II, RNA polymerase II; CTD, C-terminal repeat domain; CPF, cleavage and polyadenylation factor; PTPase, protein tyrosine phosphatase; CV, column volume; DTT, dithiothreitol; pNPP, *p*-nitrophenylphosphate; TF, transcription factor.
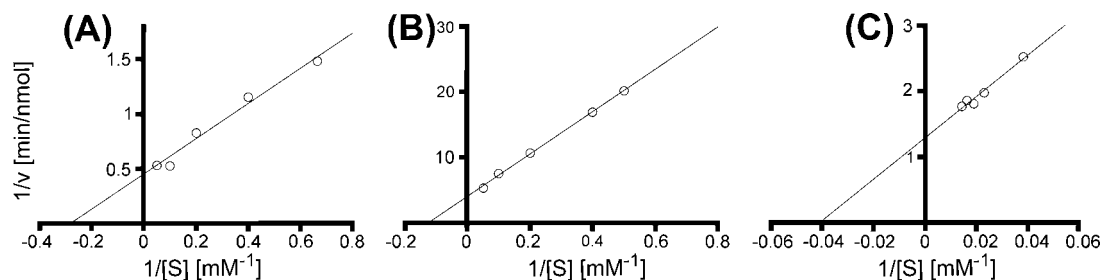
Ssu72, a Potential New Phosphatase

FIG. 1. **Phosphatase activity of Ssu72.** Lineweaver-Burk plots for the determination of K_m values for cleavage of pNPP by recombinant Ssu72 wild type (*A*), Ssu72 variant D140A (*B*), and Ssu72 variant D143A (*C*). Note the different scales used in *A*, *B*, and *C*. The K_m values obtained are listed in Table I.

TABLE I
Catalytic parameters of human Ssu72 variants and standard PTPases

Protein	PTPase family[a]	K_m	V_{max}	Specific activity	k_{cat}	k_{cat}/K_m	Reference
		mM	*nmol/min*	*units/mg*	*s^{-1}*	*10^3 M^{-1} s^{-1}*	
Ssu72 wild type		3.6	2.24	1	0.4	112	This work
Ssu72 D140A		7.9	0.24	0.1	0.04	5	This work
Ssu72 D143A		24	0.8	0.4	0.14	6	This work
Bovine PTPase	LMW	0.31	ND[b]	89	ND	ND	(35)
Human HCPTB	LMW	1.24	ND	70	ND	ND	(18)
LPT1	LMW	0.017	ND	3.2	ND	ND	(36)
VHR	DS	1.59	ND	ND	5.14	3240	(17)

[a] LMW, low M_r PTPase; DS, dual specificity PTPase.
[b] Not determined.

with *Nco*I and *Not*I and ligated into pET28b (Novagen). For amplification of mutated genes *ssu72 R18K, D140A,* and *D143A,* the primers 5′-GTTGTGCGCCTCCATGCTC**TT**GTTCTGGTTGCTCGAGCAC-3′, 5′-CCTGGAT**GG**CCACATTGACCACGTGC-3′, and 5′-GCCTCCTCGT-GGTT**CG**CCTGGATGTCCAC-3′ were instead used in the first step, respectively, together with their reverse complement counterparts.

Protein Expression and Purification—Plasmid DNA harboring the human gene *ssu72* was transformed into *E. coli* BL21 DE3 CodonPlus RIL cells (Stratagene). Cells were grown at 37 °C in LB medium supplemented with chloramphenicol and kanamycin, both at concentrations of 50 μg/ml. Once the cell culture reached an A_{600} of 0.5, temperature was reduced to 20 °C, and cells were induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside and grown over night. Cells were harvested by centrifugation and suspended in buffer A (50 mM Tris-Cl, pH 8.0, 500 mM NaCl, 10 mM β-mercaptoethanol), flash-frozen, and stored at −80 °C. All protein variants were purified as follows. Cell walls were broken with a French press followed by DNase I (Roche Applied Science) incubation on ice for 20 min. The slurry was cleared by centrifugation, and the supernatant was loaded onto a nickel-nitrilotriacetic acid-agarose column (Qiagen). The column was washed with 20 column volumes (CV) of buffer A followed by 5 CV of buffer B (buffer A but with 300 mM NaCl). Proteins were eluted with buffer B containing in addition 250 mM imidazole. Eluted proteins were diluted with 8 volumes of buffer C (50 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1 mM DTT) and bound to a MonoQ column (Amersham Biosciences). The column was washed with 2 CV of buffer C, and the protein was eluted in a gradient of 25 CV from 0 to 1 M NaCl in buffer C. Peak fractions were applied to a Superose 12 HR gel filtration column (Amersham Biosciences), equilibrated with 50 mM Tris-Cl, pH 8.0, 200 mM NaCl, 1 mM EDTA, 1 mM DTT. Protein purity was judged by SDS-PAGE. Recombinant Ssu72 migrated on SDS-PAGE with an apparent molecular mass of 30 kDa compared with a theoretical molecular mass of 24.0 kDa. In size exclusion chromatography, Ssu72 migrated like a globular protein of around 50 kDa molecular mass, suggesting that Ssu72 forms a stable homodimer. Protein concentration was determined with the Bradford assay (Bio-Rad). Under near physiological buffer conditions, Ssu72 was highly soluble and could be concentrated to 15 mg/ml. Pure protein samples were flash-frozen in liquid nitrogen and stored at −80 °C.

Phosphatase Assays—Cleavage of *p*-nitrophenylphosphate (pNPP) was used to characterize the enzymatic activity of Ssu72. Formation of the *p*-nitrophenylate product was followed in real time by measuring absorbance at 405 nm (ϵ_{405} = 18 mM^{-1}cm^{-1}) with an enzyme-linked immunosorbent assay reader. The pH optimum for pNPP cleavage was determined in steps of 0.5 pH units by measuring product absorbance after cleavage of 5 mM pNPP by 0.9 μM Ssu72 after a 15-min incubation at 37 °C. For assays between pH 4.5 and 7.5, 40 mM sodium citrate-HCl

buffer was used. Between pH 7.5 and 9.0, 40 mM Tris-Cl buffer was used. For determination of the temperature dependence of pNPP cleavage in the range of 25 to 50 °C, product formation was monitored in steps of 5° after a 20-min incubation of 5 mM pNPP and 0.9 μM Ssu72 in 40 mM sodium citrate-HCl buffer, pH 6.5. For reactions that included divalent metal ions, the buffer was replaced by 50 mM Bis-Tris-HCl, pH 6.5, to circumvent the chelating effect of citrate, and samples were incubated for 20 min. For all inhibitor studies, product formation after a 20-min incubation was measured for a substrate concentration of 5 mM. The inhibitory anions [BeF$_3$]$^-$ and [AlF$_4$]$^-$ were generated *in situ* by the addition of 1 mM NaF and 100 μM BeCl$_2$ or AlCl$_3$, respectively. The reaction was stopped by the addition of 9 volumes of 1 M Na$_2$CO$_3$. Michaelis-Menten kinetic parameters were determined by measuring initial reaction rates at various pNPP concentrations in 50 mM citrate-HCl, pH 6.5, 10 mM EDTA, 1 mM DTT, and 0.9 μM Ssu72. Data were fitted to Lineweaver-Burk equations with the program Origin. The inhibitory constant for phosphate was determined by measuring K_m values at various inhibitor concentrations followed by graphical evaluation.

RESULTS

Ssu72 Contains the Signature Motif of Protein Tyrosine Phosphatases—By visual inspection of the Ssu72 amino acid sequence, we identified the signature motif ^{11}VCX$_5$RS^{19}, which is typical for PTPases. This motif comprises a cysteine and an arginine residue that form part of the active site in all PTPases and are involved in catalysis (15). In PTPases, the cysteine residue is generally responsible for the nucleophilic attack of the substrate phosphorus atom, leading to the formation of a phosphoenzyme intermediate, whereas the arginine residue is involved in stabilization of the transition state (16). Except for the signature motif, there is no apparent sequence homology between Ssu72 and PTPases.

Ssu72 Cleaves a Phosphotyrosine Analogue—To test Ssu72 for a potential phosphatase activity, we cloned human Ssu72, over-expressed the protein in *E. coli*, and purified it to apparent homogeneity (see "Experimental Procedures"). We then subjected the purified recombinant protein to a colorimetric assay based on cleavage of the phosphotyrosine analogue pNPP (see "Experimental Procedures"). Highly purified recombinant Ssu72 could indeed cleave pNPP. Ssu72 activity was strongly dependent on the pH of the buffer and was highest at pH 6.5. At

pH 6.0, the protein showed 15% of its maximum activity, and below pH 5.5, protein precipitation essentially abolished activity. From pH 6.5 to 9.0, activity progressively decreased to about 10% of the maximum. The temperature optimum for Ssu72 activity was reached at 40 °C, but the protein showed 86% of its maximum activity at 37 °C. To minimize experimental errors and to avoid long incubation times that could lead to enzyme inactivation, further experiments were performed at pH 6.5 and 37 °C under near physiological conditions. Michaelis-Menten kinetics and Lineweaver-Burk analysis revealed a K_m value for pNPP of 3.6 mM (Fig. 1), comparable with values reported for the dual specificity phosphatase VHR (17) and the human low M_r PTPase HCPTB (18) but 1–2 orders of magnitude higher than that of two other low M_r PTPase (Table I).

Ssu72 Activity Is Impaired by Phosphatase Inhibitors—We next tested whether known phosphatase-inhibiting agents had an effect on Ssu72 activity (Fig. 2*A*). The anion $[BeF_3]^-$, a potent inhibitor of phosphatases that form phosphoaspartate intermediates (19, 20), did not show an effect on Ssu72 activity. Similar results were obtained with the anion $[AlF_4]^-$. In contrast, vanadate ions strongly inhibited Ssu72 activity. The addition of 1 mM orthovanadate reduced product formation below 5%. Phosphate ions also inhibited Ssu72 activity but not as strongly as vanadate. Michaelis-Menten analysis revealed that phosphate acts as a competitive inhibitor (Fig. 2*B*). The K_i value for phosphate, 4.3 mM, is close to the K_m value for pNPP. This shows that phosphate efficiently competes with pNPP for binding to the active site, suggesting that Ssu72 affinity for pNPP is governed by the binding of the phosphoryl group.

The influence of divalent metal ions on Ssu72 activity was also examined (Fig. 2*A*). The addition of 1 mM Mg^{2+}, Mn^{2+}, or Ca^{2+} had essentially no effect, but at 10 mM concentrations, product formation was reduced to 60, 45, and 80%, respectively. In contrast, 1 mM Co^{2+}, Ni^{2+}, or Cu^{2+} were sufficient to abolish activity. Consistently, Ssu72 was inactive after elution from a Ni-NTA-agarose column, but activity was rescued after removal of bound Ni^{2+} ions with a MonoQ column. In keeping with the negative effect of metal ions, EDTA enhanced Ssu72 activity slightly, most likely because of chelation of metal ion traces. Consequently, 10 mM EDTA was included in further experiments. These results showed that Ssu72 activity does not depend on metal ions, but is rather inhibited by them. The oxidizing agent H_2O_2 also impaired Ssu72 activity (Fig. 2*C*). Even at very low concentrations, H_2O_2 abolished catalysis after a 12-min incubation. Activity could however be recovered by adding the reducing agent DTT, demonstrating that Ssu72 oxidation is reversible (Fig 2*C*).

The Signature Motif Is Required for Ssu72 Activity—Inhibition of Ssu72 by metal ions and by oxidation suggested a catalytic role of the cysteine residue in the signature motif. Metal ions would mask the cysteine side chain, with the "soft" ions Co^{2+}, Ni^{2+} and Cu^{2+} being more effective because of their strong interaction with the "soft" cysteine sulfur atom or because of an oxidizing effect in the case of Cu^{2+}. Consistently, H_2O_2 would inactivate the cysteine side chain by oxidizing it to sulfenic acid (21). To test directly whether residues in the signature motif are required for Ssu72 activity, we mutated cysteine 12 and arginine 18 to serine and lysine, respectively, purified the resulting Ssu72 variants, C12S and R18K, and subjected them to pNPP cleavage assays. The variant C12S did not cleave pNPP even after incubation for 24 h, showing that cysteine 12 is essential for Ssu72 activity. The variant R18K retained only very low activity, and the catalytic reaction stopped after 10 min, so that kinetic parameters could not be determined. This points to a crucial catalytic role of arginine 18, which does not depend solely on the positive charge of the side chain.
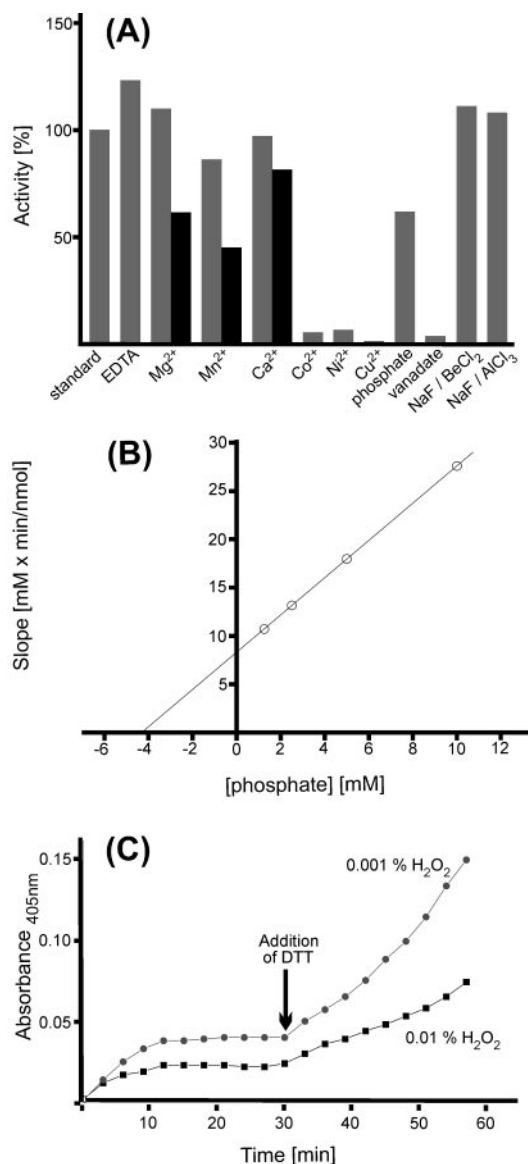

FIG. 2. **Inhibition of Ssu72 activity.** *A*, effects of EDTA (50 mM), metal ions (1 mM, *light gray*; 10 mM, *dark gray*), phosphate (5 mM), orthovanadate (1 mM), $[BeF_3]^-$, and $[AlF_4]^-$ on Ssu72 activity (compare "Experimental Procedures"). Data are shown as the percentage of Ssu72 activity in the absence of inhibitors. *B*, determination of the inhibitory constant K_i for phosphate ions. Slopes of four individual Lineweaver-Burk plots from K_m determinations at various phosphate concentrations were plotted against the four different phosphate concentrations used. The slope of the resulting plot corresponds to (K_m/V_{max}) + ([I]·K_m/K_i·V_{max}), where [I] is the phosphate inhibitor concentration. A linear regression for the values provides $-K_i$ as the intersection with the x axis, where the slope is zero. *C*, reversible oxidation and inactivation of Ssu72. Ssu72 was oxidized by adding 0.01 or 0.001% H_2O_2 to 5 mM pNPP and 0.9 μM Ssu72 in 50 mM citrate-HCl buffer, pH 6.5. After a 30-min incubation, DTT was added to reduce Ssu72 and restore catalytic activity.

Ssu72 Resembles PTPases of the Low Molecular Weight Family—The signature motif is found at different locations within the polypeptide sequence of various PTPase families. In receptor-like and intracellular PTPases, the signature motif is found in the second half of the catalytic domain, in dual specificity PTPases it is located near the middle of the sequence, and in low M_r PTPases it is located in the N-terminal region (22). Because the signature motif of Ssu72 is also found near the N terminus, and because an asparagine residue within the motif is conserved between Ssu72 and low M_r PTPases (23) but not in

FIG. 3. **Topological similarity of Ssu72 with low M_r PTPases.** A comparison of yeast and human Ssu72 sequences with the sequence of bovine low M_r PTPase is shown. Secondary structure elements as observed in the crystal structure of bovine low M_r PTPase are indicated above the sequences in *black* (*cylinders*, α-helices; *arrows*; β-strands; *lines*, loops). Below the human Ssu72 sequence, the predicted secondary structure elements (program *PHD* (24)) are shown in *gray*. Secondary structure elements with PHD scores below 6 are shown in *white*. Gaps introduced in the sequence alignments of yeast and human Ssu72 are indicated by a + sign and arbitrary gaps due to apparent insertions or deletions in the sequences of Ssu72 and bovine low M_r PTPase by a − sign. Catalytically important residues in the signature motif and the aspartate loop are *highlighted*. Note that the alignment between Ssu72 and bovine low M_r PTPase is tentative.

other PTPase families, Ssu72 may be related to low M_r PTPases. A relationship between Ssu72 and low M_r PTPases is strongly supported by secondary structure prediction (program PHD (24)), which revealed that Ssu72 is a mixed α/β protein that shows essentially the same succession of secondary structure elements as the low M_r PTPases (Fig. 3; Refs. 23, 25, and 26). A comparison of the sequences within the predicted secondary structure elements revealed weak homology between Ssu72 and enzymes of the low M_r family (Fig. 3), suggesting that the fold of the proteins is the same. The signature motif is predicted to form the loop region between the N-terminal β-strand and the subsequent α-helix, consistent with structures of low M_r PTPases (22).

An "Aspartate Loop" Contributes to Ssu72 Activity—In addition to the cysteine and arginine residues in the signature motif, the active site of PTPases comprises a crucial aspartate residue (15, 22). In sequences of the low M_r PTPase family, this aspartate is found about 110 residues C-terminal of the signature motif (Fig. 3). In the three-dimensional structure of PTPases, the aspartate is part of a flexible loop, here referred to as the aspartate loop, near the entrance to the active site (23). Based on the assumption that the fold of Ssu72 resembles that of low M_r PTPases, we predicted a region in Ssu72 that corresponds to the aspartate loop (Fig. 3). Indeed this region comprises the two aspartates 140 and 143. Whereas aspartate 143 is invariant, aspartate 140 is highly conserved and is a glutamate in *Arabidopsis thaliana* Ssu72.

To test whether one of the two aspartate residues corresponds to the active site aspartate in PTPases, we individually mutated the two residues to alanine, purified the resulting Ssu72 variants D140A and D143A, and quantified their activities with Michaelis-Menten kinetics. The variant D140A showed a K_m value of 7.9 mM, twice that of wild type Ssu72, and a V_{max} value 10-fold lower than that of wild type (Table I). The variant D143A showed a 7-fold higher K_m value, but its V_{max} value was 3-fold lower than that of wild type Ssu72 (Table I). In contrast to the differences in K_m values, Ssu72 wild type and variant D143A had comparable specific activities, but the variant D140A showed a 10-fold lower specific activity than wild type (Table I). Overall, both aspartate mutants of Ssu72 show a decreased catalytic activity, which is reflected in the low

k_{cat}/K_m values (Table I). The kinetic parameters suggest that aspartate 140 is important for transition state stabilization, whereas aspartate 143 is more important for substrate binding. These results establish the region of Ssu72 comprising aspartates 140 and 143 as the counterpart of the catalytically important aspartate loop of PTPases.

DISCUSSION

Ssu72 Is a Potential Tyrosine Phosphatase—We report here three lines of evidence suggesting that Ssu72 is a phosphatase that resembles PTPases. First, Ssu72 can cleave the phosphotyrosine analogue pNPP and its catalytic activity is impaired by known PTPase-inhibiting agents. Second, Ssu72 contains the signature motif found in all PTPases, and conserved residues in the signature motif are required for activity. Third, secondary structure prediction and site-directed mutagenesis suggest that the overall structure of Ssu72 resembles that of low M_r PTPases, including an aspartate loop that forms part of the active site.

The Phosphatase Signature Motif Is Essential for Cell Viability—Published data demonstrate an essential role of the signature motif in Ssu72 for cell viability. Truncation of an N-terminal protein part that harbors the catalytic cysteine residue is lethal to yeast (8). Most strikingly, a single point mutation of the catalytic cysteine residue to serine suffices to confer lethality (8). The gene encoding for Ssu72 was originally discovered as its *ssu72-1* allele, which enhances a genetic defect in TFIIB in yeast (27). The *ssu72-1* allele gives rise to a 10-amino acid duplication in the N-terminal region of Ssu72 (8). The duplication corresponds to residues 4–15 in human Ssu72 and comprises the essential cysteine residue.

Implications for the Catalytic Mechanism—The reaction catalyzed by PTPases generally involves two steps and three crucial active site residues. The CX_5R signature motif forms the phosphate-binding loop in the active site of all PTPases. The cysteine in the CX_5R motif acts as a nucleophile and accepts the PO_3 moiety from the phosphotyrosine, generating a phosphocysteine intermediate. In a second step, the PO_3 moiety is transferred to a water molecule, releasing phosphate and regenerating the enzyme. The arginine in the CX_5R motif is required for stabilization of the pentacovalent transition state.

In addition a flexible aspartate loop contributes an aspartate residue to the active site that serves as a general acid/base in both steps of the reaction (13, 25, 26).

Our mutational analysis and inhibitor studies suggest that the reaction mechanism and active site architecture of Ssu72 is similar to that of known PTPases. Similar to PTPases (15), mutation of the cysteine residues in the CX_5R motif abolishes Ssu72 activity, and mutation of the arginine residue dramatically reduces activity. Similar to low M_r PTPases, Ssu72 is inhibited by vanadate, which can mimic a pentacovalent transition state. In contrast, Ssu72 activity is not impaired by the $[BeF_3]^-$ anion, which inhibits phosphatases that form an aspartylphosphate intermediate (19, 20). The effects of metal ions on Ssu72 activity agree with similar studies of the PTPase VHR (28), except that Co^{2+} does not impair VHR activity, whereas it abolishes Ssu72 activity.

Differences Between Ssu72 and Low M_r PTPases—Despite these structural and mechanistic similarities, some substantial differences exist between Ssu72 and low M_r PTPases. Ssu72 differs from low M_r PTPases by the presence of a C-terminal extension, predicted to form a helix-loop-strand motif, and by an insertion of more than 10 residues after the second predicted α-helix (Fig. 3). Further, a counterpart of the aspartate loop in PTPases was identified in Ssu72, but it is about 10 residues shorter than in low M_r PTPases and comprises two catalytically important acidic residues instead of one. The shorter aspartate loop in Ssu72 may be less flexible than that of canonical PTPases, where residues in this loop can move up to 12 Å upon substrate binding (29). The structural differences in the aspartate loops are reflected in differences in their catalytic roles. Whereas the Ssu72 aspartate loop mutations D140A and D143A result mainly in a modest decrease in V_{max} and an increase in K_m, respectively, mutation of aspartate 129 in bovine low M_r PTPase did not change the K_m value but led to a more than 2000-fold decrease in V_{max} (30). Differences in the aspartate loop may also account for a weaker substrate affinity and a lower specific activity of recombinant Ssu72 when compared with some low M_r PTPases (Table I).

Possible Substrates and Functions of Ssu72—A candidate Ssu72 substrate is the phosphorylated pol II CTD, which coordinates mRNA transcription and processing. The CTD consists of YSPTSPS heptapeptide repeats, which can be phosphorylated at the tyrosine 1 in mammalian cells (31). In yeast, tyrosine 1 phosphorylation has not been reported, but it may occur, as substitution of tyrosine 1 by phenylalanine is lethal (32). Consistent with the idea that Ssu72 is a CTD tyrosine phosphatase, Ssu72 mutation impairs pol II transcription but does not affect pol I and pol III (11), both of which lack a CTD. Recombinant Ssu72, however, does not cleave synthetic CTD phosphotyrosine peptides or isolated phosphotyrosine (not shown). Thus Ssu72 could in principle function as a phosphotyrosine-binding protein, perhaps as an anchor that jams CPF to phosphorylated pol II, complementing known CTD-interacting subunits of CPF (33). Alternatively, Ssu72 phosphatase activity may depend on the complete target protein or may be stimulated by an activating factor, similar to the stimulation of the CTD phosphatase Fcp1 by TFIIF (34). Ssu72 activity may also depend on phosphorylation or on association with a small molecule, as shown for some low M_r PTPases (15). Proteins other than pol II could be the target of Ssu72 activity, but few tyrosine-phosphorylated nuclear proteins are known, perhaps because of the difficulty in detecting this transient modification. Finally, Ssu72 activity could also target nucleic acids.

Conclusion—We have discovered an essential phosphatase/phosphoprotein-binding activity residing in the Ssu72 protein that is intimately associated with the mRNA transcription/processing apparatus. It is likely that this novel Ssu72 activity regulates aspects of the coupling between nuclear gene expression machines.

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