The Human Homolog of *Escherichia coli* Orn Degradates Small Single-stranded RNA and DNA Oligomers

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We report here the identification of human homologues to the essential *Escherichia coli* Orn protein and the related yeast mitochondrial DNA-escape pathway regulatory factor Ynt20. The human proteins appear to arise from alternatively spliced transcripts, and are thus identical, except the human Ynt20 equivalent contains an NH2-terminal extension that possesses a predicted mitochondrial protease cleavage signal. *In vitro* analysis revealed that the smaller human protein exhibits a 3’ to 5’ exonuclease activity for small (primarily ≤5 nucleotides in length) single-stranded RNA and DNA oligomers. We have named this human protein Sfn for small fragment nuclease to reflect its broad substrate range, and have termed the longer protein hSfnα. Sfn prefers Mn2+ as a metal cofactor and displays a temperature-resistant (to 50 °C) nuclease activity. Kinetic analysis indicates that Sfn exhibits a similar affinity for small RNAs and DNAs (Km of ~1.5 μM), but degrades small RNAs ~4-fold more efficiently than DNA. Mutation of a conserved aspartate (Asp 136) to alanine abolishes its ribonuclease activity, while requiring further processing to mononucleotides (6). This ribonuclease activity, YjeR was renamed Orn for oligonucleosome activity (6). Data base mining revealed a human EST homologue to YjeR, indicating an evolutionary conserved role for the encoded protein (5).

ORN homologue is also present in yeast *Saccharomyces cerevisiae* (5, 9). This YNT20 gene encodes a larger protein that has been shown to localize to the mitochondria (9). YNT20 plays a role in a *S. cerevisiae* mitochondrial DNA escape pathway, a process that involves the transfer of genetic material from the mitochondria to the nucleus (9). It was suggested, based on sequence comparisons, that the *S. cerevisiae* Ynt20 protein is potentially capable of hydrolyzing both DNA and RNA (9). Although it has been reported that the *E. coli* Orn protein exhibits detectable DNase activity on double stranded T7 chromosomal DNA (10), its ability to degrade short deoxyribonucleotides apparently has not been examined. We set out to examine the substrate specificity of the human Orn equivalent, with a particular interest in its activity for DNA molecules, and demonstrate here that this protein cleaves not only short RNAs but also short DNAs in a 3’ to 5’ direction. Thus, we suggest that the human enzyme be named Sfn for small fragment nuclease. We discuss our results as they may relate to the cellular role of this protein in nucleic acid metabolism.

Nucleases are critical components of DNA and RNA metabolism, carrying out functions in DNA repair, replication and recombination, and RNA processing and degradation. Several candidate nucleases were recently classified as members of the 3’ to 5’ exonuclease superfamily (1). This superfamily includes RNases (such as RNase T and D), the proofreading domains of pol I family DNA polymerases, and DNases that exist as independent proteins (such as *Escherichia coli* Exo1) or as domains within larger polypeptides (such as a region within the Werner’s syndrome helicase) (1–3). Homology within the superfamily is centered around three conserved exonuclease motifs (Exo I, II, and III) (2). The crystal structure of the Klenow fragment of *E. coli* DNA polymerase I and complementing site-directed mutagenesis studies indicate that the Exo I, II, and III motifs are clustered around the active site and contain four conserved negatively charged residues that are critical for coordinating the two metal ions involved in phosphodiester bond cleavage (reviewed in Ref. 4).

Based on conservation of the Exo motifs, the protein encoded by the *E. coli* open reading frame YjeR was placed into the 3’ to 5’ exonuclease superfamily (5). This protein was subsequently shown *in vitro* to be an exonuclease specific for RNA molecules shorter than about 5 nucleotides (nt) in length (6). Based on this ribonuclease activity, YjeR was renamed Orn for oligonucleosome activity (6). Data base mining revealed a human EST homologue to YjeR, indicating an evolutionary conserved role for the encoded protein (5).

ORN is one of eight distinct 3’ to 5’ exoribonucleases present in *E. coli* (7). It is a processive enzyme that initiates attack at a free 3’ hydroxyl group on single-stranded RNA, releasing 5’-mononucleotides in a sequential manner (6). Notably, unlike previously characterized *E. coli* ribonucleases, ORN is an essential gene. Experiments using a temperature-sensitive ORN construct and pulse-chase experiments with radiolabeled RNA revealed an accumulation of small RNA oligonucleotides at the non-permissive temperature (8). These observations suggested a role for Orn in the final steps of mRNA degradation, since mRNA-degrading enzymes such as RNase II and polynucleotide phosphorylase generate small oligonucleotide fragments that require further processing to mononucleotides (8).

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EXPERIMENTAL PROCEDURES

Identification of SFN and SFN\(a\) cDNAs—The EST database was searched using the TBLASTN program to identify human cDNA clones that encode a protein homologous to the \(E. coli\) Orn protein (GenBank accession number P39287). IMAGE clone 663460 was chosen, sequenced (11), and found to contain an open reading frame encoding a 205-amino acid protein. This protein is identical to CGI-114 protein (accession number is AF151872). The SFN\(a\) cDNA was found in the GenBank database (accession number AL110239).

Chromosomal Localization of the Human SFN Gene—The sequence at the 5' end (accession number AA224296) or the 3' end (accession number AA224194) of the SFN gene, when compared with the "Sequenced Tagged Site" database at National Cancer Biology Institute, identified STS-H98169 as the genetic locus of SFN. This sequence-tagged site is positioned at chromosome 11 in a region between markers D11S1347 and D11S939 (110.3–117.9 centimorgan), which corresponds to the region 11q23.1–11q23.2.

Buffers and Reagents—All reagents were purchased from Sigma unless otherwise indicated. Restriction enzymes were purchased from New England Biolabs. Labeled nucleotides were from Amersham Pharmacia Biotech. Spectrophotometric grade glycerol was obtained from Fisher. Olio(dT)\(4\)–\(22\) Ladder was purchased from Life Technologies, Inc. and consists of single-stranded DNA oligos from 4 to 22 nt in length, increasing by 1-nucleotide increments. DNA oligos were obtained from Operon Biotechnologies (Alameda, CA). Synthetic RNA was obtained from Dharmacon Research (Boulder, CO). L buffer (Lysis buffer) con-

Fig. 1. Comparison of amino acid and DNA sequences of the human SFN. A, the amino acid sequence of the SFN subfamily. Amino acids in black are identical, gray are conserved, and white are non-conserved. This alignment was produced with the Boxshade program. hSFN is the \(H. sapiens\) Sfn protein sequence (accession number Q9Y3B8); hSFN\(a\), \(H. sapiens\) Ynt20 (CAB53690); mSFN, \(M. musculus\) Sfn; EcORN, \(E. coli\) Orn (P39287); CeSFN, \(C. elegans\) Sfn (AAC77855); SpSFN, \(S. pombe\) Sfn (CAB37438); and ScYNT20, \(S. cerevisiae\) Ynt20 (P54964). The conserved exonuclease motifs of the 3' to 5' exonuclease superfamily (Exo I, II, and III) are shown as boxes. The stars indicate the catalytically important tyrosine and the four conserved negatively charged residues that are involved metal ion binding (1, 4). The nuclear localization signal (NLS), KKKK, is boxed (18). l is used to mark the putative mitochondrial targeting signal, ARGVR, for hSFN\(a\) (17). B, sequences of the putative splice site in hSFN (accession number AF151872) and hSFN\(a\) (AL110239) mRNAs. The first nucleotide of the respective protein coding sequence is designated as +1. The first codon of hSFN\(a\) is boxed. The differences in the two sequences are underlined.
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The SFN cDNA, Genomic Location, and mRNA Expression

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Analytical Gel Filtration Chromatography—Sfn-His protein was separated on a 7.8 × 300-mm BioSil SEC 125–5 gel filtration column from Bio-Rad. The running buffer was 50 mM Tris-HCl, pH 7.9, 100 mM NaCl, 20% glycerol, 5 mM imidazole, and 0.1 mM phenylmethylsulfonyl fluoride. The apparent molecular mass of Sfn-His protein was calculated to be 66 kDa when dialyzed against L buffer containing 0.1 mM dithiothreitol, and the resin was washed 2 times each with 20 ml of L buffer, followed by 4 washes each with 10 ml of W40 buffer. Sfn-His proteins were then separated on a 300-mm BioSil SEC 125–5 gel filtration column from Bio-Rad. The running buffer was 50 mM Tris-HCl, pH 7.9, 100 mM ammonium sulfate, 5% glycerol, 0.1 mM EDTA, and 0.1 mM dithiothreitol. The flow rate was 0.25 ml/min. Size markers used for calibration were thyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B-12 (1.35 kDa). Proteins were detected by ultraviolet absorbance at 280 nm.

RESULTS

The SFN cDNA, Genomic Location, and mRNA Expression Pattern—A cDNA containing the SFN coding region was obtained from the EST data base (see “Experimental Procedures”). By blasting the sequence of the 5’ end (accession number AA224296) or the 3’ end (accession number AA224194) of the SFN cDNA against the NCBI UniGene database, we found

Site-directed Mutagenesis of the SFN cDNA—Mutagenesis was performed using the overlapped PCR method as described (14). The site-specific mutant oligo used was DJ36A, 5′-TATAGAATTTCAGCCAGACT-3′, with the mutated codon underlined. We chose to mutate Asp136 because a structurally equivalent mutation in E. coli DNA pol I (D501A) caused a 13,000-fold decrease in 3′-5′ exonuclease activity (15). The final SFN mutant PCR product was digested with NcoI and HindIII and subcloned into the same restriction sites of pET28d. This construct was sequenced as described (11).

Nuclease Assays—Various DNA and RNA substrates were labeled at the 5′ end by T4 polynucleotide kinase with [γ-32P]ATP (16). RNA5 is 5′-GAGUCG-3′. DNA5 is 5′-GATCG-3′. DNA8 is 5′-CAGAGGCC-3′. 100 fmol of nucleic acid substrate was incubated with 100–2000 fmol of Sfn-His protein at 37°C in 10 μl of 50 mM HEPES-KDH, pH 7.4, 10% glycerol, 50 mM KCl, 10 mM MnCl2 (unless otherwise noted), 0.01% Triton X-100, and 0.1 mM dithiothreitol. The reactions were stopped with 10 μl of 90% formamide dye solution, heated at 80°C for 3 min, and then fractionated on a 20 or a 22.5% x 8 urea polyacrylamide gel. The gel was exposed to x-ray film. Visualization of the labeled substrate on the gels was also achieved using a Molecular Dynamics (Sunnyvale, CA) STORM 860 Phosphorimager and quantitative analysis was performed using Molecular Dynamics ImageQuant v1.11 software.

Kinetics of Sfn Exonuclease Activity on Single-Stranded DNA and RNA Substrates—Nuclease assays were performed under standard buffer conditions as described above at 37°C, with Sfn at a final concentration of 10 nM. The reaction was incubated for 5 min with RNA5 or 40 min with DNA5, and was within the linear range of enzymatic activity (i.e. ≤15% of the substrate was converted to product). The substrate concentration range was 0.1–3.2 μM. The apparent Michaelis-Menten constant (Km) and maximal velocity (Vmax) were obtained from a double-reciprocal Lineweaver-Burk plot. A linear plot of 1/[S] versus 1/V ([where S is the substrate concentration in μM and V is the velocity in μM min⁻¹ units) produces a slope of Km/Vmax and a y intercept of 1/Vmax*Et, where Vmax was calculated from the equation (kcat)R = Vmax, where kcat is the total concentration of enzyme in the assay. Linear regression analysis was performed using CricketGraph software (Cricket Software, Philadelphia, PA).

Northern Blot Analysis—Northern blots were prehybridized for 3 h at 65°C in ExpressHyb Solution (CLONTECH, Palo Alto, CA). The cDNA probe (SFN PCR product described above) was labeled using the Megaprime DNA Labeling System (Amersham Pharmacia Biotech) and [α-32P]dCTP (Amersham Pharmacia Biotech), and hybridized in ExpressHyb Solution at 65°C for 2–3 h. Blots were washed once at room temperature for 30 min and twice at 50°C for 30 min with 50 mM NaPO4, pH 7.4, 0.5% SDS, and 1 mM EDTA. Images were exposed on Kodak Bio-Max MS film for 16 h and developed. Blots were normalized with β-actin transcripts.

Fig. 2. Expression pattern of SFN mRNA in fetal and adult human tissues. Shown are Northern blot hybridization signals detected by autoradiography. Developmental stage, tissue type, and transcript type are indicated. β-Actin transcript was used for normalization. Panel A is an mRNA blot of fetal tissues. Panel B shows three mRNA blots of adult tissues.

Table 1. Primers Used for PCR

| Primer Name   | Sequence                  |
|---------------|---------------------------|
| NCO5          | 5′-TATAGAATTTCAGCCAGACT-3′ |
| HYJER         | 3′-TATAGAATTTCAGCCAGACT-5′ |

NcoI and HindIII sites of pET28d (Novagen, Madison, WI) to generate pET28d. This construct was sequenced as described (11).

Purification of Recombinant Sfn-His Protein—The SFN coding region was PCR amplified using primers NCO5/HYJER (5′-GCGGCGAGGGAGGAGCAT) and HIND3/YJER (3′-GCGAATAGCTTACTCAGGTCTTCTCAT), and subcloned after digestion into the NcoI and HindIII sites of pET28d (Novagen, Madison, WI) to generate phyjeR-His. This construct allows for expression of six histidine residues on the carboxyl terminus under the control of a T7 RNA polymerase promoter. phyjeR-His plasmid was sequenced and no PCR errors were found.

The phyjeR-His plasmid was transformed into BL21(DE3)/pLysS E. coli strain (Novagen). An overnight culture of 100 ml was grown at 37°C in LB (1% bactotryptone, 0.5% bactoyeast extract, and 1% NaCl) with 50 μg/ml chloramphenicol. The overnight ware, Philadelphia, PA).
that the human SFN gene mapped to chromosome 11 at position 11q23.1–11q23.2. Four additional genes also map within this vicinity: apolipoprotein A-I (apoA-I), human serotonin receptor 3 (HTR3), human nicotinamide N-methyltransferase (NNMT), and zinc finger protein ZNF259 (PLZF).

The SFN cDNA codes for a 205-amino acid protein of 23,754 daltons, with a theoretical pI of 5.6. SFN belongs to the YxeR/ORN family, which is a distinct subgroup of the 3’ to 5’ exonuclease superfamily (1, 5). Fig. 1 shows a comparison of the amino acid sequence of the Sfn-like proteins from bacteria, yeast, plant, worm, mouse, and human. The human Sfn protein is ∼50% identical to its E. coli counterpart, the Orn protein (5). Sfn and E. coli Orn possess the three characteristic sequence motifs, termed Exo I, II, and III (2), of the 3’ to 5’ exonuclease superfamily (1) (Fig. 1A). Four conserved negatively charged residues within these three Exo motifs (shown in Fig. 1A) are involved in positioning of the two divalent cations required for catalysis and phosphodiester bond cleavage (reviewed in Ref. 4).

Upon further examination of the GenBank data base, an identical protein to hSfn, but with an extended NH2-terminal domain, was identified (Fig. 1A). This protein, which we propose to call Sfnα, appears to be the human equivalent to the yeast Ynt20 protein and to have arisen from an alternative RNA splicing event where two additional nucleotides were introduced (Fig. 1B). Using the PSORT II computer search program, a putative consensus cleavage site motif for mitochondrial processing proteases (ARGVR) (17) was identified within the unique NH2-terminal portion of Sfnα. Both Sfn and Sfnα contain a consensus nuclear localization signal (KKRRK) (18) (Fig. 1A). It seems logical that Sfn would be targeted to the nucleus and that Sfnα would translocate predominantly to the mitochondria.

Northern blotting revealed that the SFN cDNA probe detects a single transcript of ∼1 kilobase in all fetal and adult tissues examined (Fig. 2). Comparatively, relatively low mRNA levels are observed in adult lymph nodes, brain, lung, liver, spleen, and thymus, with highest levels observed in heart. Since the human SFN and SFNα cDNAs appear to differ by only two nucleotides, it was not possible to distinguish the two transcripts here. Of the 10 human ESTs found in the data base, 8 contained a sequence identical to the SFN cDNA and two maintained a sequence identical to Sfnα.

_purification of overproduced SFN-His fusion protein_—We have characterized here Sfn, and expect that Sfnα will exhibit similar substrate specificity as it maintains the same core nuclease domain (Fig. 1A). The SFN gene was subcloned into the pET28d plasmid to produce a COOH-terminal-tagged Sfn-His fusion protein, and the recombinant protein was purified as described under “Experimental Procedures.” From 1 liter of induced bacterial culture (about 5 g of wet cell pellet), we obtained ∼4 mg of Sfn-His fusion protein of >95% purity (Fig. 3A). As shown in Fig. 3B, the Sfn-His fusion protein migrates as a single symmetrical peak on an analytical gel filtration column that corresponds to a globular protein with a molecular mass of ∼90 kDa. Since the calculated molecular weight of Sfn is 24,000, Sfn-His fusion protein appears to migrate as a tetramer. However, since the elution position on a gel filtration column is dependent on size and shape of the protein, it is possible that the Sfn-His fusion protein exists in solution as a rod-shaped protein of lower oligomeric state, since rod-shaped proteins are known to migrate slower than expected for their molecular weight.2 Unlike the human Sfn-His protein, the E. coli Orn protein was reported to be a dimer based on its gel filtration chromatographic profile, although it should be noted that the experimental conditions differed slightly from those used here (6).

Exonuclease Activity of Sfn-His Protein—Short fragments of ≤5 ribonucleotides were shown to be the optimal substrate for E. coli Orn protein (6, 10). Using a 5-nucleotide RNA (RNA5), labeled at the 5’ end, as the substrate, we determined the divalent metal cation preference of the human Sfn. At metal concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mM, only Mg2+ and Mn2+ had any stimulatory effect on Sfn-His exonuclease activity, with Mg2+ being 10-fold less effective than Mn2+ at 10 mM (Fig. 4A and data not shown). Both human Sfn-His and E. coli Orn enzymatic activities have a strong dependence on

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2 Dr. R. Burgess, University of Wisconsin-Madison, personal communication.
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(a preference for) Mn$^{2+}$ (Fig. 4A; Ref. 10). The RNase activity of Sfn-His also has a temperature optimum around 50 °C (Fig. 4B), similar to that of E. coli Orn protein (10). Thus both proteins are quite thermostable. The RNA-specific degradation activity of Sfn at 37 °C is ~2-fold less than that at 50 °C (Fig. 4B). Since we observed degraded 4-mer products prior to detecting mononucleotide products (Fig. 4C), Sfn-His appears, as expected, to be a 3’ to 5’ exonuclease.

To determine whether short single-stranded DNA can serve as substrate for Sfn-His, we used a single-stranded oligo(dT) ladder from 4 to 22 bases in length, and single-stranded DNA oligos of 5 and 8 nt. As shown in Fig. 5, Sfn-His is capable of degrading short single-stranded DNA, although with less efficiency than 5-mer RNAs. Furthermore, its DNase activity is inversely related to the length of the DNA oligo, as seen for the RNase activity of E. coli Orn (19). The data above is also consistent with the lack of DNase activity reported for Orn on double stranded T7 chromosomal (large) DNA (10).

**The DNase and RNase Activities Are Intrinsic to Sfn-His**—To confirm that the observed DNase and RNase activities are intrinsic to the Sfn-His protein, a catalytically inactive Sfn-His mutant was constructed. Amino acid residue Asp$^{136}$ (Fig. 1, in Exo III motif) was selected because an aspartate to alanine mutation at the structurally equivalent residue (5) in E. coli DNA pol I causes a 13,000-fold decrease in 3’ to 5’ exonuclease activity (14). The D136A Sfn-His mutant was purified using the same procedure as described for wild type Sfn-His protein (Fig. 6A). When compared with wild type Sfn-His, D136A Sfn-His mutant displayed a ~50-fold reduced exonuclease activity for both RNA5 and DNA5 substrates (Fig. 6B). These data are consistent with both nuclease activities being intrinsic to the Sfn-His protein, and not being the result of protein contaminants. The results also demonstrate the importance of this acidic residue in the enzymatic function of the 3’ to 5’ exonuclease family members (1).

We subsequently examined Sfn nuclease activity on DNA structures known to be products/substrates of DNA repair processes. As might be expected from the results above, in the presence of Mn$^{2+}$ or Mg$^{2+}$, we did not detect any protein-dependent degradation of 42-base pair double-stranded DNAs of different configurations, including gapped, nicked, 4-nucleotide recessed or overhanged 3’ ends, or flap structures (data not shown; substrates described in Ref. 20).
FIG. 6. The effect of a D136A mutation on the DNase and RNase activities of Sfn-His. A, purity of D136A Sfn-His mutant as determined on a 15% polyacrylamide SDS gel stained with Coomassie Blue R-250 dye. Lanes 1 and 5 are protein markers; and lanes 2–4 are 2.5, 5, and 10 μg of Ni-affinity purified D136A Sfn-His. Protein marker sizes in kilodaltons are indicated to the right. B, exonuclease activity of D136A Sfn-His mutant with RNA5 and DNA5 single-stranded substrates. Lanes 1–9 are with RNA5, and lanes 10–18 are with DNA5 substrates. The substrate concentration is 200 nM. Lanes 1 and 10 are the no protein control. Lanes 2–5 and 11–14 are 12, 24, 48, and 96 nM of purified wild type Sfn-His (WT). Lanes 6–9 and 15–18 are 12, 24, 48, and 96 nM of purified D136A Sfn-His mutant. The reactions were incubated at 37 °C for 20 min.

Kinetic Parameters of the Sfn Nuclease Activities—To determine the reason(s) for the different nuclease efficiencies of Sfn-His, we determined the Michaelis-Menten constant (Km), maximal velocity (Vmax), and the apparent rate of catalysis (kcat) for comparable RNA and DNA substrates (Fig. 7). The Km of RNA5 (Km = 1.56 μM) is essentially identical to that of DNA5 (Km = 1.51 μM). In contrast, the Vmax and kcat values were ~4-fold higher for RNA5 (Vmax = 0.015 μM min⁻¹, kcat = 1.5 min⁻¹) than for DNA5 (Vmax = 0.004 μM min⁻¹, kcat = 0.39 min⁻¹). Our Km value for RNA5 is consistent with the micromolar range reported for E. coli Orn on p(A)ₙ single-stranded RNA substrates (19).

DISCUSSION

The human SFN gene belongs to the YjeR/ORN subfamily of the 3’ to 5’ exonuclease superfamily previously described (1, 5). Some of the members of this superfamily, most notably the Werner syndrome gene product and the polymysitis-scleroderma overlap syndrome 100-kDa autoantigen (PM-Scl 100), are associated with human disease (21, 22). While a connection of SFN to a specific human disease is not obvious, the ubiquitous expression of its transcript may suggest a general and essential role for the encoded protein in mammalian cells. Notably, the human SFN gene maps to chromosome position 11q23.1–11q23.2, a region that undergoes translocation events in several leukemias (23, 24), although none of these translocation breakpoints have been finely mapped to SFN.

The unique NH₂ terminus of hSFN contains a consensus cleavage site pattern for mitochondrial processing proteases (17), whereas both SFNa and SFn possess a nuclear targeting signal in their COOH-terminal domains. This observation suggests that an alternative splicing event has evolved to give rise to a mitochondrial (SFna) and a nuclear (SFn) version of the Ynt20/ORN equivalents in human. However, at present, the genomic DNA sequence of the entire hSFN gene is not available, and thus the alternatively spliced products cannot be confirmed. Notably, in the mouse EST data base, there are also multiple transcripts, suggesting that alternatively spliced murine SFN transcripts exist as well. Future studies will need to address whether there is tissue-specific expression of the mRNAs splice variants found in mammals. Interestingly, while S. cerevisiae maintains a homologue to hSFNα (called Ynt20 or REX2), we were unable to find a homologue to SFN in the NCBI data base using the Blastp search program.

A knockout of the E. coli SFN homologue, the ORN gene, results in cellular lethality (7). An E. coli temperature-sensitive mutant is not only lethal at the nonpermissive temperature (i.e. where Orn is inactive), but accumulates small oligoribonucleotides, indicating that Orn maintains an essential activity to degrade RNA (8). Three possibilities were provided as to why ORN deletion mutants are inviable (8): 1) accumulation of oligoribonucleotides results in a depletion of cellular mononucleotides; 2) accumulated oligoribonucleotides inhibit certain enzymes and interfere with essential metabolic processes; or 3) Orn has an additional unknown function that is responsible for the growth cessation. Since SFn is capable of degrading both RNA and DNA, we propose that the human protein operates to remove not only short RNAs (likely resulting from RNA degradation processes) (25, 26), but also short single-stranded DNAs that might arise as products of DNA repair and recombination. Thus, by extension (8), Sfn would function globally to recycle nucleotides or to remove nucleic acids that may interfere with essential cellular processes. How
the nuclease activities of the Orn/Ynt20 proteins would function in mitochondrial DNA escape, a process that involves translocation of mitochondrial DNA to the nucleus (9) and is possibly linked to cellular aging or senescence (27), is presently unclear. Furthermore, although unlikely, whether the NH2-terminal differences between these proteins affect substrate specificity will need to be determined.

Not surprisingly, Sfn-His, which shares ~50% identity in amino acid sequence to its bacterial counterpart, has very similar biochemical properties to Orn (5). In particular, both amino acid sequence to its bacterial counterpart, has very specific terminal differences between these proteins affect substrate; temperature optimum of a tetramer, in contrast to matography suggests that the human Sfn-His fusion protein is thermore, previous kinetic data of differing activities as well.

Observations raise questions of how the substrate length or the nucleic acid chemistry influences Sfn/Orn enzymatic activity. To answer these questions will require determining which step(s) is influenced by nucleotide length or nucleic acid composition. High resolution structural data of Sfn, alone and in complex with RNA and DNA, would also shed light on the recognition and catalytic mechanisms of these proteins. Lastly, our studies emphasize that future experiments should pay particular attention to the potential range of substrate diversity recognized by the other 3’ to 5’ exonuclease superfamily members.

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