Mouse and Human La Proteins Differ in Kinase Substrate Activity and Activation Mechanism for tRNA Processing

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The La protein interacts with a variety of small RNAs as well as certain growth-associated mRNAs such as Mdm2 mRNA. Human La (hLa) phosphoprotein is so highly conserved that it can replace the tRNA processing function of the fission yeast La protein in vivo. We used this system, which is based on tRNA-mediated suppression (TMS) of *ade6-704* in *S. pombe*, to compare the activities of mouse and human La proteins. Prior studies indicate that hLa is activated by phosphorylation of serine-366 by protein kinase CK2, neutralizing a negative effect of a short basic motif (SBM). First, we report the sequence mapping of the UGA stop codon that requires suppressor tRNA for TMS, to an unexpected site in *S. pombe ade6-704*. Next, we show that, unlike hLa, native mLa is unexpectedly inactive for TMS, although its intrinsic activity is revealed by deletion of its SBM. We then show that mLa is not phosphorylated by CK2, accounting for the mechanistic difference between mLa and hLa. We found a PKA/PKG target sequence in mLa (S199) that is not present in hLa, and show that PKA/PKG efficiently phosphorylates mLa S199 in vitro. A noteworthy conclusion that comes from this work is that this fission yeast system can be used to gain insight into differences in control mechanisms used by La proteins of different mammalian species. Finally, RNA binding assays indicate that while mutation of mLa S199 has little effect on pre-tRNA binding, it substantially decreases binding to a probe derived from Mdm2 mRNA. In closing, we note that species-specific signaling through La may be relevant to the La-dependent Mdm2 pathways of p53 metabolism and cancer progression in mice and humans.

Key words: tRNA processing; *ade6-704*; tRNA-mediated suppression; CK2; Protein kinase A; La motif; Mdm2; C5 RNA

INTRODUCTION

La protein (also known as La antigen or SS-B) is a target of autoantibodies in patients suffering from Sjögren's syndrome, systemic lupus erythematosus, and neonatal lupus, and is found associated with a variety of small nuclear RNAs in the form of ribonucleoprotein (RNP) complexes [(17,32), reviewed in (34)]. La sequence homologs are present in all of the

eukaryote genomes examined, and La proteins have been characterized in ciliates, yeasts, flies, frogs, mice, rats, and humans (6,37,53,56). La associates with nascent RNAs synthesized by RNA polymerase (pol) III, which include the precursors to tRNAs, 5S rRNA, U6 snRNA, 7SL (SRP) RNA, and RNase P RNA; and in metazoa, 7SK snRNA, Y scRNAs, Alu and other SINE RNAs, and others [reviewed in (36)]. All of these RNAs are attracted to La by their com-

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mon high-affinity La binding motif, 3' UUU-OH, that results from transcription termination (46,47,50). A well-established and conserved function of La is to protect the 3' ends of its ligands from digestion, which promotes their processing and maturation [reviewed in (37,38,56)]. Indeed, human La (hLa) can replace the fission yeast La protein (Sla1p) in its capacity to promote functional tRNA maturation in vivo (20,24,27).

In addition to function in small RNA metabolism, vertebrate La proteins have been shown to modulate the translation of mRNAs that contain 5' regulatory sequences found on internal ribosome entry sites, Mdm2 mRNA, and the 5'-terminal oligopyrimidine (5'-TOP)-containing mRNAs that encode ribosomal proteins (7,44,49,54,56). Yeast La is also found associated with mRNAs that encode ribosome proteins in *S. cerevisiae* (23), suggesting a conserved function distinct from UUU-OH 3' end binding-related activity.

While La is not required for growth of the yeasts *S. cerevisiae* and *S. pombe*, both of which clearly use La to promote tRNA maturation (20,24,27,57), mouse embryos cannot survive without La (41), perhaps reflecting the more complex structure of the mammalian La protein (Fig. 1A).

La proteins are phosphorylated on multiple sites in humans and yeasts (4,10,33,55). In human cells, the majority of hLa is phosphorylated adjacent to a short basic motif (SBM) on serine-366, by the highly conserved protein kinase, CK2 (4,10,49). Although a role for phosphorylation of yeast La in *S. cerevisiae* has not been determined (33), faithful S366 phosphorylation of hLa occurs in the fission yeast, *S. pombe*, and this promotes La-dependent tRNA processing (9,27). Specifically, nonphosphorylatable hLa mutants inhibit tRNA processing while S366 phosphorylation relieves this inhibition (9,27). Deletion of either RNA recognition motif 2 (RRM2) or the SBM of hLa also relieves pre-tRNA processing inhibition (9,27). Phosphorylation of S366 is sufficient to relieve inhibition in the context of full-length hLa (9,27), suggesting that controlling the negative influence of the SBM is a determinant of La activity. The results in fission yeast, showing that phospho-hLa is isoform active for tRNA-related activity, fit with data from human cells, in which phospho-hLa is nucleoplasmic and associated with pre-tRNAs, while nonphospho-hLa is not concentrated in the nucleoplasm and is not associated with pre-tRNAs (26).

S366 phosphorylation is also linked to other aspects of hLa. Nonphospho-hLa is concentrated in nucleoli, associated with nucleolin at sites of rRNA processing (25), and was found at pol III-transcribed genes in vivo (8). Nonphospho-hLa also resides in

the cytoplasm associated with 5'-TOP mRNAs (25, 26). Increasing the ratio of nonphospho- to phospho-hLa led to increase in the 5'-TOP mRNA bound to La and decrease in polysome association (49). The cumulative data are consistent with a model in which the C-terminal domain (CTD) can control the complex activities of La proteins in mammals. We have begun to characterize mouse La protein (mLa), which contains an insertion in the SBM relative to hLa (53), and lacks an equivalent of S366 (Fig. 1B).

Using tRNA-mediated suppression (TMS) in fission yeast, we show that mLa contains an autoinhibitory motif in its CTD, similar to hLa. Unlike hLa, however, full-length mLa is not activated in fission yeast. We show that mLa is not a substrate for CK2. We show that cAMP-dependent protein kinase A (PKA) and cGMP-dependent protein kinase G (PKG) phosphorylate mLa on S199 in vitro whereas hLa does not contain PKA/PKG phosphorylation sites. Finally, RNA binding assays indicate that mutation of S199 has little effect on pre-tRNA binding but decreases binding to a probe representing a La binding site in Mdm2 mRNA, a previously identified ligand of mLa in vivo.

MATERIALS AND METHODS

tRNA-Mediated Suppression and La Expression in S. pombe

cDNA encoding mLa provided by J. McCluskey (University of Melbourne) (53) was amplified by PCR, cloned into the *Bam*HI site of the *S. pombe* expression vector pRep4X, and used to transform ySK5 cells to the *ura4*⁺ phenotype; suppression was assayed on EMM plates lacking uracil and containing 10 mg/L adenine (27). Analysis by immunoblotting was as described using anti-La (Go) serum (27).

Purification

Full-length or mutated mLa cDNA was amplified by PCR and subcloned into the *Nco*I and *Xho*I sites of pET-28 (Novagen, with C-terminal His6). Mutagenesis was by QuickChange (Stratagene). All constructs were confirmed by sequencing. Recombinant pET plasmids were expressed in BL21(DE3)LysS cells (Invitrogen) and the proteins purified by nickel-affinity chromatography (Qiagen).

Phosphorylation of Recombinant La Proteins

A standard CK2 kinase assay (49) using recombinant CK2 (Calbiochem), 1 μ M of purified La, and kinase master mix of 0.1 mM GTP, 10 mM MgCl₂, 10 mM Tris-Cl (pH 7.5), 0.5 μ l of [³²P- γ]GTP (10

$\mu\text{Ci}/\mu\text{l}$; Perkin-Elmer), and 0.1–0.02 U of CK2. Assays using PKA catalytic subunit (New England Biolabs) or PKG, isoform I (Calbiochem) and 1 μM of purified La or Histone H1 (Sigma) were performed in reactions containing 0.2 mM ATP, 10 mM MgCl_2 , 50 mM Tris-Cl (pH 7.5), 0.5 μl of [^{32}P - γ]ATP (10 $\mu\text{Ci}/\mu\text{l}$; Perkin-Elmer), and 100–2500 units of PKA or 10–1000 units of PKG with 5 μM of cGMP (5). Aliquots of the master mix were added to the substrates and incubated at 30°C for 30 min, stopped with 2 $\times$ SDS sample buffer containing fresh β -mercaptoethanol, electrophoresed in 12% PAGE, and imaged with a Fuji PhosphorImager.

RNA Binding

Templates containing T7 RNA polymerase promoters were used to synthesize ^{32}P -pre-tRNA^{Met} (16) and Mdm2 exon-2 ^{32}P -RNA, GACCGGACACC CCUGGGGGAC CCUCUCGGAUCACC (54), using MAXI script (Ambion) with 0.5 mM ATP/CTP/UTP, and 50 μM GTP/0.5 mCi [^{32}P - α]GTP (MP Bio-medical). ^{32}P -RNAs were purified by 20% PAGE-urea. Electrophoretic mobility shift assay (EMSA) was as described (13); 10,000 cpm ^{32}P -RNA was incubated with La in 20 μl EMSA buffer and 10 ng *E. coli* tRNA (Sigma) for 30 min and analyzed by 6% native PAGE, followed by autoradiography, and quantified by PhosphorImager.

RESULTS

Our laboratory uses a suppressor tRNA^{Ser}UGA that suppresses a nonsense codon in *ade6-704* to study the role of *S. pombe* La protein, Sla1p, or human La protein, in tRNA biogenesis (15,21,24,27). Unsuppressed *ade6-704* results in red colonies, and full suppression results in white colonies. Mutations in pre-tRNA^{Ser}UGA cause dependency on Sla1p or hLa for maturation, either of which promote TMS in the *S. pombe* ySK5 strain, which is deleted for Sla1 (20,24,27). La-dependent processing is a primary determinant of mature tRNA^{Ser}UGA levels (21,27) and suppression is dependent on accumulation of mature tRNA^{Ser}UGA (15,20,24,27).

Because *ade6-704* is suppressed by the opal suppressor tRNA^{Ser}UGA encoded by *sup3-5*, *sup3-e*, and other alleles, it was presumed that the *ade6-704* contains a suppressible UGA stop codon, although the *ade6-704* mutation responsible for the tRNA^{Ser}UGA-mediated suppression phenotype had never been identified. It had been presumed that *ade6-704* contained a C to A mutation at nucleotide position 846, changing codon 282 in wild-type *ade6*⁺, to a UGA stop codon in *ade6-704* (48). Contrary to this expect-

tation, we identified no mutation at position 846 but instead detected a T to A mutation at nucleotide 645 in *ade6-704*, changing UGU codon 215 to UGA. tRNA^{Ser}UGA can be charged with and insert serine at UGA codons (31,45), thus indicating that active *ade6-704* protein contains Ser in place of Cys at amino acid 215 (Fig. 1C).

The CTD Is Autoinhibitory to the TMS Activity of mLa

Mouse La protein and mutated derivatives thereof were examined for TMS activity in strain ySK5, which is deleted of endogenous Sla1p, along with prior characterized constructs that served as controls (Fig. 1D). Sla1p and hLa were active while the empty vector, pRep4X, was not, and the nonphosphorylatable protein hLaA366 was less active than hLa, all as expected, demonstrating the specificity and range of this assay (Fig. 1D, sectors 1–4). Native mLa was inactive while mLa1-327, mLa1-332, and mLa Δ 340-387, deleted of the SBM, were more active (Fig. 1D, sectors 5–7). Native mLa and mLa1-327 were expressed in ySK5 at similar levels (Fig. 1E).

Strain yYH1 is wild-type for Sla1p, which allows it to reveal dominant negative activity of La mutants as demonstrated for hLaA366 (21). Prior data have shown that nonphosphorylated hLaA366 binds pre-tRNAs but impedes their processing, which leads to accumulation of unprocessed pre-tRNAs in *S. pombe* and a dominant negative effect on TMS in YH1 (9,21,27). Figure 1F shows that while additional ectopic Sla1p leads to more suppression in yYH1, native mLa and hLaA366 lead to less suppression than the empty vector. Thus, mLa is similar to hLaA366 in both ySK5 and yYH1. Full-length mLa caused darker color than hLaA366 in yYH1, indicating greater dominant negative effect on TMS than nonphosphorylatable hLaA366 (see the Discussion section). We conclude that the CTD of mLa has significant inhibitory effect on the intrinsic TMS activity of mLa. While the inhibitory effect of the hLa CTD is naturally relieved in fission yeast (by S366 phosphorylation), this appears not to be the case for mLa.

mLa Is Not a Substrate of Protein Kinase CK2

CK2 recognizes the consensus sequence S/TXXD/E. While the target site in hLa is 366-SDDE, the corresponding mLa sequence, DDDR, is not a CK2 consensus target (Fig 1B). Although it might appear that mLa contains a potential CK2 site 4 residues upstream, at RRTRFD, it has been noted that basic residues near the phosphorylatable residue inhibit CK2 activity (12,39). We performed in vitro phosphorylation of purified mLa, hLa, and hLaG366 (Fig. 2A).

Figure 1. (A) Representation of La proteins of *S. cerevisiae* (Lhp1p), *S. pombe* (Sla1p), human (hLa), and mouse (mLa). SBM (short basic motif) and NLS (nuclear localization sequence) are indicated. The $\alpha 3$ element has been observed as an α -helix in hLa (1) and is inferred in the other proteins (1); in Sla1p this corresponds to a nuclear retention element (NRE) (3), which is adjacent to RRM2 in hLa (28). (B) Sequence alignment of SBM and downstream regions of hLa, bovine (bLa), rat (rLa), and mLa proteins. hLa SBM is overlined. S366 is indicated above hLa and mLa sequence is numbered below. (C) Representation of *S. pombe* Ade6p, and the TGT $\rightarrow$ TGA substitution at nucleotide position 645 in *ade6-704*. An ATP-grasp homologous region is indicated. (D) TMS activities of pRep4X vector, Sla1p, hLa, hLaA366, mLla, mLla1-327, mLla1-332, mLla Δ 340-387 in *S. pombe* ySK5 cells. (E) Lanes 3–7 show immunoblot of La proteins. Lane 1: molecular weight markers; lane 2: purified mLla; lane 3: pRep4X empty vector; lane 4: mLla; lane 5: mLla1-327; lane 6: mLla1-332; lane 7: hLaA366. Molecular weights, in kDa, are indicated on the left. (F) TMS activities of pRep4X, Sla1p, mLla, and hLaA366 in strain yH1 (wild-type for Sla1p), which reveals dominant negative activity of ectopic hLaA366 mutant protein (21).

With either 0.1 or 0.02 U of CK2 (Fig. 2B, lanes 1–3 and 4–6, respectively), mLla was not phosphorylated (lanes 1 and 4), while hLa was readily phosphorylated (lanes 2 and 5). hLaG366 was labeled at a low level, as previously noted, probably due to phosphorylation of Thr-389 (R396 in mLla) in the sequence 387-EETDKEE (10).

mLa Is Phosphorylated by PKA and PKG In Vitro

The program PROSITE (22,43) identified PKA and PKG consensus target sites R/KR/KXS/T at Ser-199, Thr-301, and Thr-318 in the mLla sequence (53). The corresponding three target sites do not exist in hLa because they lack either the phosphorylatable

Figure 2. (A) 1 μ M of purified mLa (lanes 1 and 4), hLa (lanes 2 and 5), or hLaG366 (lanes 3 and 6) was incubated with [32 P]- γ GTP and 0.1 U (lanes 1–3) or 0.02 U (lanes 4–6) of CK2. (B) The purified La proteins used as substrates in (A) were separated by SDS/PAGE and stained with Coomassie blue. Positions of molecular weight markers are indicated on the left, in kDa.

residue or a required basic residue (Fig. 3A). We examined the *in vitro* substrate activity of our purified recombinant proteins at three concentrations of enzymes (Fig. 3B, C), and also included purified histone H1, a test substrate for PKA and PKG (42). mLa was a better substrate than hLa for both PKA and PKG.

PKA and PKG Phosphorylate mLa on Ser-199 *In Vitro*

In order to test if mLa can be phosphorylated on the predicted PKA and PKG target sites, we mutated serine-199, threonine-301, and threonine-318 to alanine to produce mLaS199A, mLaT301A, and mLaT318A, and tested them as substrates for PKA and PKG (Fig. 3E, F). The purified substrates are shown in the Coomassie-stained gel in Figure 3D.

While PKA phosphorylated mLa, mLaT301A, and mLaT318A with high efficiency, phosphorylation of mLa S199A was at background levels, comparable to hLa (Fig. 3E). The data indicate that mLa was phosphorylated efficiently on S199, while little if any phosphorylation occurred on T301 or T318, by PKA.

mLaS199A was decreased relative to mLa for

phosphorylation by PKG (Fig. 4C, lanes 1 and 2). mLaT301A and mLaT318A also exhibited decreased substrate activity relative to mLa for PKG, although they were more active than mLaS199A (Fig. 3F). These differences were reproducible in reactions using varying conditions and PKG concentrations (not shown). The data suggest that each of the predicted sites may be phosphorylated by PKG *in vitro* and that failure to phosphorylate any of the sites has a negative influence on PKG activity for mLa. In any case, mutation of S199 to alanine causes a dramatic decrease in the substrate activity of mLa. The results demonstrated that S199 is highly favored over the other PKA/PKG sites in mLa.

Differential Binding of mLaS199A to Mdm2 RNA and pre-tRNA

Recombinant mLa protein was previously shown to bind poly(U)-RNA (53). The SBM in hLa resides within a larger basic tract that is followed by acidic residues, with phospho-serine 366 at the transition point (Fig. 1B). The SBM is thought to interact with RNA whereas phospho-S366 decreases binding (9,40). mLa has an interruption in the SBM and the acidic region is less extensive than in hLa (Fig. 1B). We therefore wanted to compare hLa and mLa for binding to two different classes of RNA ligands: the 3' UUU-OH-containing pre-tRNA^{Met}, also known as C5 RNA, a high-affinity ligand of hLa (2,16), and a 35 nt previously characterized probe that represents the La binding site within the 5' UTR of Mdm2 mRNA (Fig. 4A, B) (54). While binding to this Mdm2-derived RNA had been examined by UV cross-linking (54), that approach did not provide a sense of binding affinity or of how Mdm2 mRNA binding would compare to pre-tRNA binding. We employed EMSA, which has been used previously in our lab for La (9,10,14,20).

mLaS199A, mLaS199D, mLaS199E, and mLa1-332 exhibited similar binding to pre-tRNA^{Met} at three concentrations of each protein (Fig. 4C). mLaS199A and mLa1-332 were slightly decreased for binding (Fig. 4C).

By contrast to pre-tRNA^{Met}, mLaS199A and mLa1-332 were more deficient for binding to Mdm2 RNA, whereas mLaS199D and mLaS199E were comparable to mLa (Fig. 4D). While mLa, mLaS199D and mLaS199E bound ~95% of Mdm2 RNA at the highest concentration of protein, mLaS199A and mLa1-332 bound only ~5%.

Although we have not determined kDa values for these proteins, the data were quantified to reflect the ratio of Mdm2 to pre-tRNA^{Met} bound for each pro-

Figure 3. (A) Sequence alignment of hLa and mLa in two regions that contain PKA/PKG consensus sites (underlined). (B, C) Purified recombinant mLa (lanes 1, 4, and 7), hLa (lanes 2, 5, and 8), or histone H1 (lanes 3, 6, and 9) was incubated with [32 P]- γ ATP and either 2500, 1000, or 100 units of recombinant PKA (B) or 1000, 100, or 10 units of recombinant PKG (C), as indicated (note that PKA and PKG undergo autophosphorylation). (D) The purified mLa mutant proteins were separated by SDS/PAGE and stained with Coomassie blue. (E, F) 1 μ M of recombinant mLa (lane 1), mLaS199A (lane 2), mLaT301A (lane 3), mLaT318A (lane 4), hLa (lane 5), or histone H1 (lane 6) was incubated with [32 P]- γ ATP and either 2500 units of PKA or 500 units of PKG, as indicated. Positions of molecular weight markers are indicated on the right, in kDa.

tein. This ratio for mLa was set to 1.0 and the ratios of the other proteins were compared in Figure 4E. We conclude that mLaS199A and mLa1-332 are specifically deficient in binding to Mdm2 RNA.

DISCUSSION

La is an abundant multifunctional phosphoprotein that interacts with a large variety of RNAs, including small RNAs that end with UUU-OH and certain

mRNAs that lack this motif, as well as a limited number of proteins [(11,25), reviewed in (35)]. While several functions have been proposed for La, a conserved activity is 3' UUU-OH binding, mediated by its conserved N-terminal domain (NTD) (52). This binding mode serves the *in vivo* function of assisting pre-tRNAs and other RNAs during their maturation (35). While yeast La proteins are comprised of the NTD and a small CTD, mammalian La proteins contain expanded CTDs with an atypical RRM and a

Figure 4. (A, B) Purified La proteins were examined for binding to pre-tRNAⁱMet (A) and Mdm2 mRNA (B) in parallel. EMSA used increasing amounts of La (in pmol: indicated above the lanes). (C, D) EMSA as above using pre-tRNAⁱMet (C) and Mdm2 RNA (D) with buffer alone or increasing amounts of mLa or the mLa mutated proteins as indicated. (E) Graph of quantification of binding of mLa proteins, at 30 pmol, to pre-tRNAⁱMet and Mdm2 RNA. Ratio of bound Mdm2/pre-tRNAⁱMet obtained for mLa was arbitrarily set to 1.0; error bars reflect range of duplicate experiments.

SBM (28). Studies indicate that the hLa CTD can control the activity of the NTD, in a phosphorylation-sensitive manner (9,27,40). Other data indicate that the CTD interacts with certain mRNAs (2,19) and contributes to the translation function of La [(7,18), reviewed in (19,51,54)]. Accordingly, NTD- and CTD-mediated binding may represent different binding modes by which La can interact with different classes of RNAs. An intriguing possibility is that the tRNA-related and mRNA translation activities of La are coordinated through the CTD [(26), reviewed in (30)].

Functional Differences in the CTDs of Mammalian La Proteins

Even among mammals, the La CTD is quite variable, and in mLa this involves an insertion in the SBM. For the present work we took advantage of the phylogenetic differences in the CTDs of mLa and hLa by asking if these are associated with any functional consequences to the tRNA-related activity, and to their substrate specificities for protein kinases and RNA binding.

This work revealed that the CTD of mLa is inhibitory to TMS, similar to hLa. The intrinsic activity of mLa for TMS was revealed by removal of its SBM. An unexpected conclusion is that the inhibitory effect of mLa CTD is not relieved in fission yeast as occurs for hLa. Indeed, mLa is not a substrate for CK2-mediated phosphorylation in vitro, consistent with lack of a CK2 target site in its sequence. Unlike hLa, which comes under the control of CK2 in yeast, there is no apparent mechanism for activating full-length mLa for TMS in fission yeast.

Another conclusion is that phylogenetic differences in the CTDs of rodent and human La proteins include PKA target sites in the former that are not present in the latter. The PROSITE program found three PKA/PKG consensus target sites in mLa but none in hLa. We showed that one of these, mLa S199, is phosphorylated by PKA in vitro. While it seems clear that mLa is not activated for TMS our S199 phosphomimetic mutants, mLaS199E and mLaS199D remained inactive for TMS (not shown), suggesting that even if phosphorylated this would not activate mLa. The other mutants, mLaT301A and mLaT318A, were also inactive for TMS (not shown).

Mutation in the Linker Between the NTD and CTD Differentially Affects Binding to Two Different Classes of La-Associated RNAs

We examined La binding to pre-tRNA^{Met}, which ends in UUU-OH, and a 35-nt region of Mdm2

mRNA, which ends in ACC-OH. It should be noted that pre-tRNA^{Met} (C5 RNA) is a highly stable precursor tRNA (16) that is bound to La in vivo and that exhibits high affinity for La (2,29). Our data suggest that binding to Mdm2 RNA is only about fivefold lower than pre-tRNA^{Met}. This showed that the La binding site in Mdm2 mRNA characterized by UV cross-linking (54) exhibits moderately high affinity for La.

By contrast to pre-tRNA^{Met} binding by wild-type mLa, mLaS199A and mLa1-332 were deficient for binding to the Mdm2 RNA probe, whereas mLaS199D and mLaS199E were comparable to mLa. Because mLaS199A was more deficient in binding to Mdm2 RNA than to pre-tRNA^{Met}, the data suggest that these RNAs are recognized by different binding modes. Reduced binding to mLa1-332 supports the idea that the CTD of mLa contributes to Mdm2 mRNA binding. The results also suggest that the identity of the amino acid at position 199 of mLa is more important for binding to Mdm2 mRNA than pre-tRNA^{Met}. By comparison to a hLa structure, S199 would be located beyond the α 3 helix that follows RRM1 (1). It should be noted that this is immediately adjacent to a peptide spanning residues 174–197 of hLa that was shown to bind a specific region of hepatitis C virus RNA and to affect its translation (44). Because S199 resides in the linker between the NTD and CTD, the data suggest that this linker may mediate functional interactions between the NTD and CTD. While future work will be necessary, the results are consistent with the possibility that alteration of S199 by phosphorylation may be more important for the mRNA-related activity of mLa than for its tRNA-related activity.

As noted above, mLa proteins mutated at position 199 were inactive for TMS in yeast and therefore exhibited no phenotypic difference from wild-type mLa. This is consistent with the finding that mLa-199-mutated proteins were not deficient for pre-tRNA binding. The defect in binding to Mdm2 mRNA that was observed for mLa-S199A suggests that future work should examine this protein for a defect in mRNA-related activity in vivo. Although mLa is required for early mouse development (41), a genetic system to assay mutated La proteins for a phenotype in mice is not presently available.

Phylogenetic variations in La CTDs reflect species-specific mechanisms for activation, and should caution against the expectation that rodent La and hLa will be interchangeable, including in systems used to examine Mdm2 expression (54).

Finally, it is noteworthy that this work shows for the first time that this in vivo fission yeast system can be used to gain insight into the differential regu-

lation of the La proteins of different mammalian (and probably other) species.

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