

Conservation of RNA chaperone activity of the human La-related proteins 4, 6 and 7

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ABSTRACT

The La module is a conserved tandem arrangement of a La motif and RNA recognition motif whose function has been best characterized in genuine La proteins. The best-characterized substrates of La proteins are pre-tRNAs, and previous work using tRNA mediated suppression in *Schizosaccharomyces pombe* has demonstrated that yeast and human La enhance the maturation of these using two distinguishable activities: UUU-3'OH-dependent trailer binding/protection and a UUU-3'OH independent activity related to RNA chaperone function. The La module has also been identified in several conserved families of La-related proteins (LARPs) that engage other RNAs, but their mode of RNA binding and function(s) are not well understood. We demonstrate that the La modules of the human LARPs 4, 6 and 7 are also active in tRNA-mediated suppression, even in the absence of stable UUU-3'OH trailer protection. Rather, the capacity of these to enhance pre-tRNA maturation is associated with RNA chaperone function, which we demonstrate to be a conserved activity for each hLARP *in vitro*. Our work reveals insight into the mechanisms by which La module containing proteins discriminate RNA targets and demonstrates that RNA chaperone activity is a conserved function across representative members of the La motif-containing superfamily.

INTRODUCTION

The La-motif (LAM) is a highly conserved domain similar in structure to the winged-helix fold commonly present in DNA-binding transcription factors but found instead in a family of RNA-binding proteins (1–4). The best studied LAM containing members are the genuine La proteins, which in addition to a LAM also contain an adjacent RNA recognition motif (RRM or RRM1) that combine

to form a tandem arrangement known as a La domain or a La module (1,2). La proteins are conserved from yeast to humans and have important functions in the processing and maturation of polymerase III transcripts, such as pre-tRNAs (2,5). La proteins bind these, in part, by sequence specific recognition of the UUU-3'OH motif found at the end of all pol III transcripts. In addition to a well-defined role in pre-tRNA processing in the nucleus, human La (hLa) has also been proposed to enhance the translation of certain viral or cellular mRNAs containing internal ribosome entry sites or upstream open reading frames in the cytoplasm (6–13), even though the mechanism(s) by which La engages transcripts that do not contain UUU-3'OH are less well understood. Thus, the La module is associated with a diversity of functions for a number of RNA targets, consistent with this RNA-binding arrangement displaying significant plasticity in ligand binding (2,5,9,14,15).

Recent structural and biochemical work has revealed that in addition to a UUU-3'OH-dependent binding mode, La also engages pre-tRNAs via a UUU-3'OH-independent mode and that these modes map to distinct RNA interaction surfaces on La (14,16). La engagement of UUU-3'OH occurs largely via conserved amino acids on the LAM (15,17,18), whereas the main body of the tRNA is bound via RNA structure-dependent contacts through the RRM and amino acids immediately C-terminal to this (14,16,19). These binding modes are hypothesized to promote pre-tRNA maturation by distinguishable activities. Specifically, La engagement of UUU-3'OH protects pre-tRNA trailers from 3' exonuclease digestion, causing accumulation of nascent pre-tRNA transcripts before endonucleolytic trailer cleavage by RNase Z (20–22). In addition to 3'-end protection, La also plays an important role in pre-tRNA folding (23–25), and contacts attributed to the RRM plus amino acids C-terminal to this are hypothesized to be required for this function via RNA chaperone activity (14,19, 25,26). Consistent with this, the isolated hLa LAM is inactive in fluorescence resonance energy transfer (FRET)-based assays testing for RNA strand annealing and dissociation (19), and LAM point mutants display

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defects in assays testing for UUU-3'OH-dependent binding (15,25), whereas point mutants in the RRM show normal affinity for UUU-3'OH but are defective in RNA chaperone assays (19,25). Notably, both the LAM and RRM classes of mutations are associated with defects in La-dependent enhancement of tRNA maturation *in vivo*, as measured by tRNA-mediated suppression and accumulation of pre-tRNA intermediates in *Schizosaccharomyces pombe* cells (14,19,25), suggesting that both 3'-end protection and RNA chaperone function have a role in La-dependent pre-tRNA processing. Evidence that RNA chaperone activity may also have important functions for non-UUU-3'OH-containing targets has emerged recently from the discovery that hLa promotes multiple turnover of the RISC complex by promoting strand separation of the siRNA/cleaved mRNA complex in an ATP-independent fashion (27).

The study of LAM-containing proteins is increasingly expanding into the La-related proteins (LARPs). Phylogenetic analysis segregates these into conserved LARP families, many of which maintain residues in the LAM associated with UUU-3'OH recognition (1). The degree of conservation in the adjacent RRM, however, is more variable (Figure 1). Although this RRM is apparent in genuine La proteins and members of the LARP7 family, it is replaced by RRM-like domains in members of the LARP6 and LARP4 families and can be absent entirely in some members of the LARP1 family and LAM-containing proteins from yeast. The best-characterized human LARP is LARP7 (hLARP7, also known as PIP7S), which recognizes the 7SK RNA, a polymerase III transcript [Figure 1; (28–30)]. This binding is associated with enhancing the stability of the 7SK RNA, and by extension that of the 7SK RNP, an inhibitory complex for the cell-cycle-associated transcriptional activator P-TEFb.

Consistent with a role in cell-cycle regulation, the *Drosophila* hLARP7 homolog was identified as a tumour suppressor (31), and mutations to hLARP7 associated with human gastric cancer have been shown to cause decreased 7SK RNA stability with a concomitant enhancement of P-TEFb activity (28,32). Notably, the La module of the LARP7 family is most similar to that of genuine La proteins (1,2), with the amino acids required for UUU-3'OH completely conserved with those of hLa except for the conservative substitutions Y to F at hLARP7 residue 44 (equivalent to hLa Y23) and Y to W at hLARP7 residue 45 (equivalent to hLa Y24) (Figure 1B).

The LARP4 and LARP6 families are not as well understood, and recent computational analyses indicate that some members of these have acquired the capacity to engage cytoplasmic poly-A binding protein via the acquisition of a PAM2 (or variant PAM2) motif; these same family members (but not ones lacking the PAM2 motif) also show decreased homology in LAM amino acids associated with UUU-3'OH-dependent RNA binding (33). Thus, human LARP6 (hLARP6) lacks such a PAM2 motif and contains 100% conservation with hLa LAM amino acids associated with terminal uridylylate binding, despite the only known RNA target of this protein being an internal stem loop in the 5'UTRs of the $\alpha 1$ and $\alpha 2$ collagen mRNAs (34). Conversely, the hLARP4 and hLARP4b proteins both harbour variant PAM2 motifs, engage cytoplasmic PABP and enhance the translation of a wide spectrum of messages (35,36). They also diverge the most significantly among the human LARPs at amino acids associated in hLa with UUU-3'OH binding, with hLARP4 containing two significant variations at hLARP4 residues C130 and M160 (Figure 1; hLa equivalent residues Y24 and F55, respectively). The LARP4 family is also different from the other

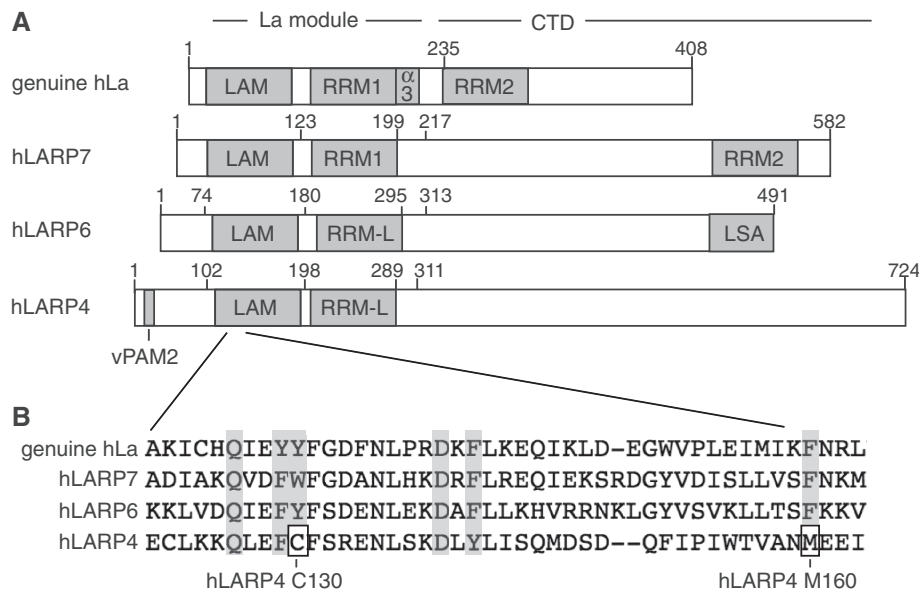


Figure 1. Alignment of hLa and human LARPs used in this study. (A) Conserved architecture of hLa and LARPs. LAM = La motif; RRM = RNA recognition motif; RRM-L = RNA recognition like motif; LSA = LAM and S1 associated motif; vPAM2 = variant PABP (poly-A binding protein) interacting motif. (B) Conservation of amino acids in the LAM associated in genuine La proteins with UUU-3'OH recognition and binding. Residues in the LAM important for UUU-3'OH recognition in genuine La, as well as their counterparts in the LARPs, are shaded in grey. Amino acids in hLARP4 102-289 mutated in this study (see Table 1 and 'Discussion' section) are boxed.

La motif containing family members in that its La module often has a uniquely shortened linker between the LAM and adjacent RRM-L domain.

Consistent with a role in the metabolism of nascent polymerase III transcripts, a subset of 7SK RNA associates with hLa, presumably before being subsequently displaced by hLARP7 (37). Given the high degree of conservation in the La motifs of the genuine La and LARP7 families, it is not surprising that hLa and hLARP7 show similarities in target recognition. We have previously assayed the ability of hLARP7 to engage pre-tRNA targets by testing its ability to rescue the maturation of a mutated suppressor tRNA in *S. pombe*, an assay in which full-length hLa or its La module is highly active (28). We found that the La module of hLARP7, but not the full-length protein, was active in this assay, suggesting that the hLARP7 La module in isolation is capable of engaging pre-tRNAs but that the rest of the protein may restrict its binding to the 7SK RNA.

In this work, we assess the capability of the La motifs, RRMs, La modules and C-terminal regions of the hLARPs 4, 6 and 7 for their capacity to participate in tRNA-mediated suppression in *S. pombe*. We show that the La modules of hLARP4, 6 and 7, but not their full-length counterparts, all show activity in this assay, despite evidence indicating that unlike hLa they do not participate in stable UUU-3'OH pre-tRNA trailer binding. Rather, the capacity of the hLARP La modules to participate in tRNA-mediated suppression is more likely related to RNA chaperone activity, as they (as well as their full-length counterparts) are active in a FRET-based *in vitro* RNA chaperone assay. Using this assay, we show that, similar to hLa, the LAMs of the hLARPs are inactive as RNA chaperones, and that maximal RNA chaperone activity associated with the RRMs or RRM-Ls requires amino acids immediately C-terminal to these domains. The data are consistent with a model in which the La modules of hLa and the hLARPs provide a general RNA-binding platform harbouring RNA chaperone activity, and that these factors may rely on regions external to the La module to modulate their binding and activity for their endogenous targets.

MATERIALS AND METHODS

Cloning

Full-length cDNAs for hLARP4 and hLARP6 were cloned from total RNA from HeLa cells and full-length or La modules of these were subcloned into pRep4 using the *SalI* & *BamHI* sites (hLARP4) or the *SalI* site (hLARP6) and confirmed by sequencing. Cloning of hLARP7 into pRep4 was described previously (28). All hLARPs cloned into pRep4 for expression in *S. pombe* had His tags (HHHHHH) added at their C-terminus for detection of proteins by western blot. Antibodies used were anti-6xHis (Clonotech catalogue #631212) and anti-actin (Abcam catalogue #ab8224). For expression and purification in *Escherichia coli*, the full length or La modules of hLARP4, 6 and 7 were cloned into the *NdeI* and *NotI* sites of pET28a. Site-directed mutagenesis was

performed by Quikchange (Stratagene) and confirmed by sequencing.

Protein purification

His-tagged proteins were expressed in BL21 Star (DE3) pLysS or RosettaBlue (DE3) pLysS. Protein production was induced with 0.1 to 1 mM IPTG for 3 h to overnight and purified using cobalt affinity chromatography (His-TRAP, ThermoFisher). Proteins were then concentrated and desalted into RNA chaperone buffer [50 mM Tris-HCl (pH 7.5), 1.5 mM MgCl₂ and 1 mM DTT] and quantified using SDS-PAGE and Coomassie staining.

FRET assays

RNA chaperone assays were performed as described (19). Cy5 and Cy3 labelled substrates containing the sequences Cy5-AUGUGGAAAUCUCUAGCAGU and Cy3-ACUGCUAGAGAUUUUCCACAU were synthesized by IDT. All fluorescence readings were obtained by a Cary Eclipse fluorimeter. FRET indexes and rate constants were by calculating a FRET index (emission at 680 nm versus emission at 590 nm) over time (s) in half-second time-points and normalizing these between 0 and 1 using Graphpad Prism 5.0. Histograms and tables with rate constants show the results of a minimum of three independent experiments.

tRNA-mediated suppression

tRNA-mediated suppression was performed as described (14). Briefly, plasmids encoding hLa and mutants cloned into the *SalI* and *BamHI* sites of pREP4 were transformed into ySH9 (25), and representative single colonies were selected and spotted on selective media containing 3 µg/ml adenine. To subclone hLARP6, which contains an endogenous *BamHI* site, this site was first mutated to a synonymous codon (I232 'ATC' to I232 'ATA') via Quikchange (Stratagene).

Northern Blots

Northern blots were performed as described (14). Probes for intron pre-tRNA^{Lys}CUU, trailer pre-tRNA^{Lys}CUU and U5 were CTTCTGATACCATTTCGTAAGAGTC, AAATTAACCTCCAAG and CTGGTAAAAGGCAA GAAACAGATACG, respectively.

RESULTS

tRNA-mediated suppression by hLARPs 4, 6 and 7

Previous work has established that the presence or absence of La can influence the order of pre-tRNA processing events in budding and fission yeast (20,38). For La-bound pre-tRNAs, 5' leader cleavage by RNase P precedes 3' endonucleolytic trailer cleavage by RNase Z. In the absence of La, exonucleolytic pre-tRNA trailer nibbling precedes 5' leader tRNA cleavage by RNase P. Notably, tRNA processing via the La-dependent pathway allows for the rescue of some mutated or defective pre-tRNAs that would otherwise be degraded via nuclear surveillance (19,21,23,25). tRNA-mediated suppression

in *S. pombe* is an established assay previously used to assess the capacity of yeast, mouse or hLa to enhance the maturation of pre-tRNAs *in vivo* (21,39). This assay uses a serine tRNA mutated in its anticodon such that it can now decode the stop codon UGA [tRNA^{Ser}UGA (C37:10)], causing suppression of a nonsense codon in the *ade6-704* allele. In a strain of *S. pombe* lacking its genuine La homolog (*slal-*), this mutated suppressor pre-tRNA is degraded, causing a block in the adenine biosynthetic pathway and red colonies when cells are plated on low adenine. However, white (high activity) or pink (intermediate activity) colony formation can be rescued by introducing plasmid encoded yeast or hLa (wild-type or mutants of these), which can rescue the mutated suppressor tRNA and suppress red colony formation (21,25).

Based on the high degree of conservation in the La modules of hLa and hLARP7, we have previously tested the functionality of hLARP7 in this assay (28). Although full-length hLARP7 was inactive (or marginally active) in tRNA-mediated suppression, its isolated N-terminal La module (hLARP7 1-217) was active, suggesting that the C-terminal region (CTD; 218-582) of hLARP7 may restrict its access to its endogenous target, the 7SK RNA (28). To expand this analysis of La module-dependent enhancement of tRNA-mediated suppression to the other LARP families, we tested the full-length versions as well as the LAMs, RRM (or RRM-like domains), La modules (LAM+RRM or RRM-L; both referred to hence as RRM for simplicity) as well as the CTDs of

hLARPs 4, 6 and 7 for their capacity to suppress red pigment formation similar to full-length hLa or the hLa La module in *S. pombe* cells (Figure 2). We included His-6 tags at the C-terminal ends of our constructs to compare expression levels of these via western blot (Supplementary Figure S1). We also tested full length and the La module of human LARP1 in this assay but found that these were not expressed to a significant extent in *S. pombe* and so did not pursue this further (data not shown). In the absence of high-resolution structures for the hLARPs, we relied on previously described secondary structure predictions (1,40) to clone these regions for expression in *S. pombe* (see Table 1 for summary of constructs used in this manuscript).

We found that similar to what we had previously observed for the La module (1-217) of hLARP7 (28), the La modules of hLARP4 (102-311) and hLARP6 (71-313) showed moderate to high activity in the tRNA-mediated suppression assay (Figure 2; compare column 6 with the vector alone control in column 1). Also similar to hLARP7, the full-length versions of hLARP4 and hLARP6 were significantly less active in the assay (column 4), although full-length hLARP6 did not accumulate appreciably in cells as determined by western blot (see Supplementary Figure S1). However, neither the La motif (column 7) nor RRM (column 8) domains of hLARP4, 6 or 7, which in tandem form the La module, showed any activity in tRNA-mediated suppression when expressed in isolation, despite all of these individual

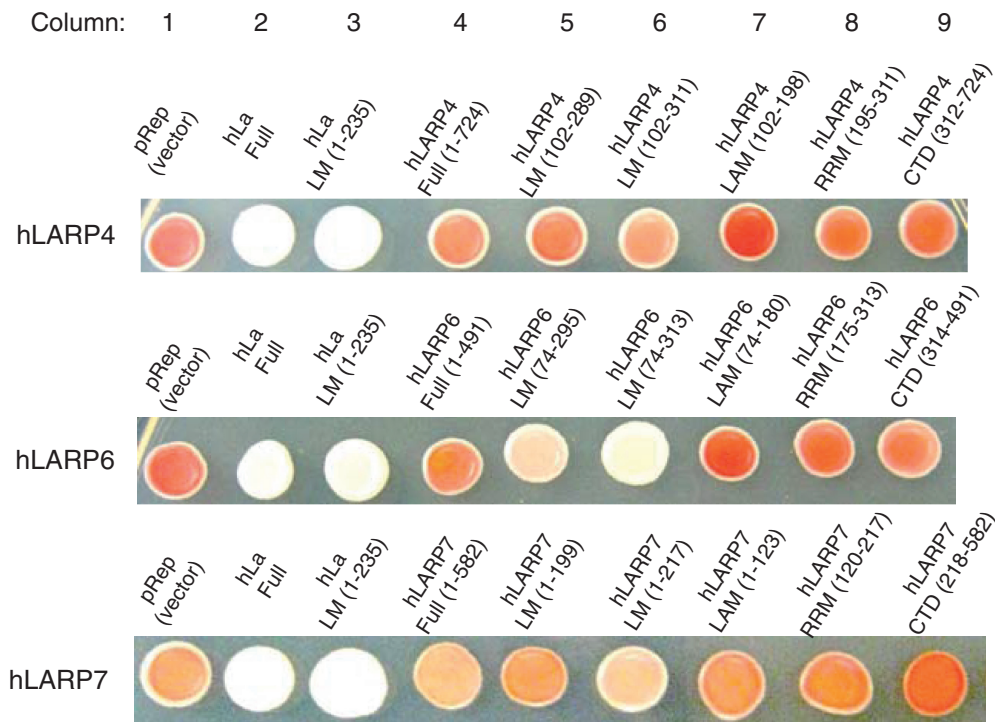


Figure 2. tRNA-mediated suppression by hLARP4, 6 and 7. Full-length and various deletion constructs of genuine La, hLARP4 (top row), hLARP6 (middle row) and hLARP7 (bottom row) were tested for their capacity to enhance tRNA-mediated suppression *in vivo* in the *S. pombe* strain ySH9 (25). Column 1: Vector alone (negative control; pRep4); Column 2: Full-length genuine hLa (positive control); Column 3: La module (LM) of hLa (positive control); Column 4: Full-length hLARPs; Column 5: hLARP La modules (LM); Column 6: hLARP La modules (LM) with C-terminal RRM extensions; Column 7: hLARP La motifs (LAMs); Column 8: hLARP RRM with C-terminal extensions; Column 9: hLARP C-terminal domains (CTDs).

Table 1. Constructs used in this work

No.	Name (protein)	<i>S. pombe</i> (pRep4 based)		<i>In vitro</i> (pET28a based)	
		tRNA-mediated suppression	Stable UUU-3'OH binding	Strand annealing	Strand displacement
1	hLa (full length)	++	++	+++	++
2	hLa LM (1–235)	++	++	++	++
3	hLARP4 full length(1–724)	–	–	++	++
4	hLARP4 LM (102–289)	–	–	+	+
5	hLARP4 LM (102–311)	+	–	++	+
6	hLARP4 LAM (102–198)	–	–	–	–
7	hLARP4 RRM (195–311)	–	–	+	++
8	hLARP4 CTD (312–724)	–	–	+	++
9	hLARP4 LM 102-289 C130Y	–	–	–	–
10	hLARP4 LM 102-289 M160F	–	–	–	–
11	hLARP4 LM 102-289 C130Y/M160F	–	–	–	–
12	hLARP4 LM 102-289/hLa loop 3	–	–	–	–
13	hLARP4 LM 102-289/hLa linker	–	–	–	–
14	hLARP4 LM 102–289 C130Y/M160F/hLa linker	–	–	–	–
15	hLARP4 LM 102–289 C130Y/M160F/hLa linker/hLa loop3	–	–	–	–
16	hLARP6 full length (1–491)	–	–	+++	++
17	hLARP6 LM (74–295)	+	–	+	++
18	hLARP6 LM (74–313)	++	–	+++	++
19	hLARP6 LAM (74–180)	–	–	–	–
20	hLARP6 RRM (175–313)	–	–	+	++
21	hLARP6 CTD (314–491)	–	–	+	++
22	hLARP7 full length (1–582)	–	+ ^a	+	++
23	hLARP7 LM (1–199)	–	–	+	++
24	hLARP7 LM (1–217)	+	–	+	++
25	hLARP7 LAM (1–123)	–	–	–/+	++
26	hLARP7 RRM (120–217)	–	–	–	+++
27	hLARP7 CTD (218–582)	–	–	++	++

^ahLARP7 full-length (1–582): stable accumulation of leader processed but trailer containing pre-tRNA intermediate (see Figure 3 and text).

domains being similarly expressed as measured by western blot (Supplementary Figure S1). Notably, expression of the LAMs of hLARP4 and hLARP6 reproducibly showed poorer tRNA-mediated suppression than even the vector alone control (compare column 7 with column 1), suggesting that when expressed in isolation, these domains are capable of interfering with the proportion of suppressor tRNAs that successfully mature even in the absence of genuine La. With the La module of hLARP4 showing higher activity in tRNA-mediated suppression than the full-length version, similar to what was demonstrated previously for hLARP7, the data are consistent with the hypothesis that regions external to the La modules may have a function in regulating the specificity or activity of these factors with respect to the engagement of potential RNA substrates.

tRNA-mediated suppression by human LARPs does not occur via stable pre-tRNA 3'-end protection

One hallmark of stable La pre-tRNA binding and 3'-end protection during La-dependent processing is the detection of leader and trailer containing pre-tRNA intermediates by northern blot, compared with their lower abundance in La null strains. To investigate the nature of hLARP-dependent activity in tRNA-mediated suppression further, we wanted to test whether the tRNA-mediated suppression associated with the La modules of the human LARPs also caused the accumulation of pre-tRNA processing intermediates similar to genuine La

(Figure 3). Detection of the intron-containing pre-tRNA^{Lys}CUU using an intron-specific probe is an established method to assess La dependent 3'-end protection of pre-tRNA species (20,38), and therefore, we used such a probe on total RNA isolated from *S. pombe slal-* cells expressing genuine hLa, the hLa La module (1–235), various hLARP mutants or a vector alone control. Expression of hLa (either full-length or hLa 1–235) resulted in the characteristic accumulation of three pre-tRNA species: a prominent leader and trailer containing nascent transcript (top band) as well as the less abundant leader-processed (middle band) and leader and trailer processed intermediates (lower band), compared with the near lack of the nascent transcript band in the vector alone control. However, none of the hLARP La module constructs that were active in tRNA-mediated suppression showed the same pattern of pre-tRNA accumulation as hLa, with relative levels of pre-tRNA intermediates at the same or at only slightly elevated levels compared with the vector alone control, suggesting that these hLARP La modules do not stably bind pre-tRNAs. As previous work has suggested that stable 3'-end protection by La proteins is required for tRNA-mediated suppression (25), this represents the first example of factors that show activity in this tRNA-mediated suppression assay but do not stably engage pre-tRNAs. We did note that expression of the full-length but not the La module of hLARP7 in *S. pombe* led to significant accumulation of the leader processed but trailer containing pre-tRNA

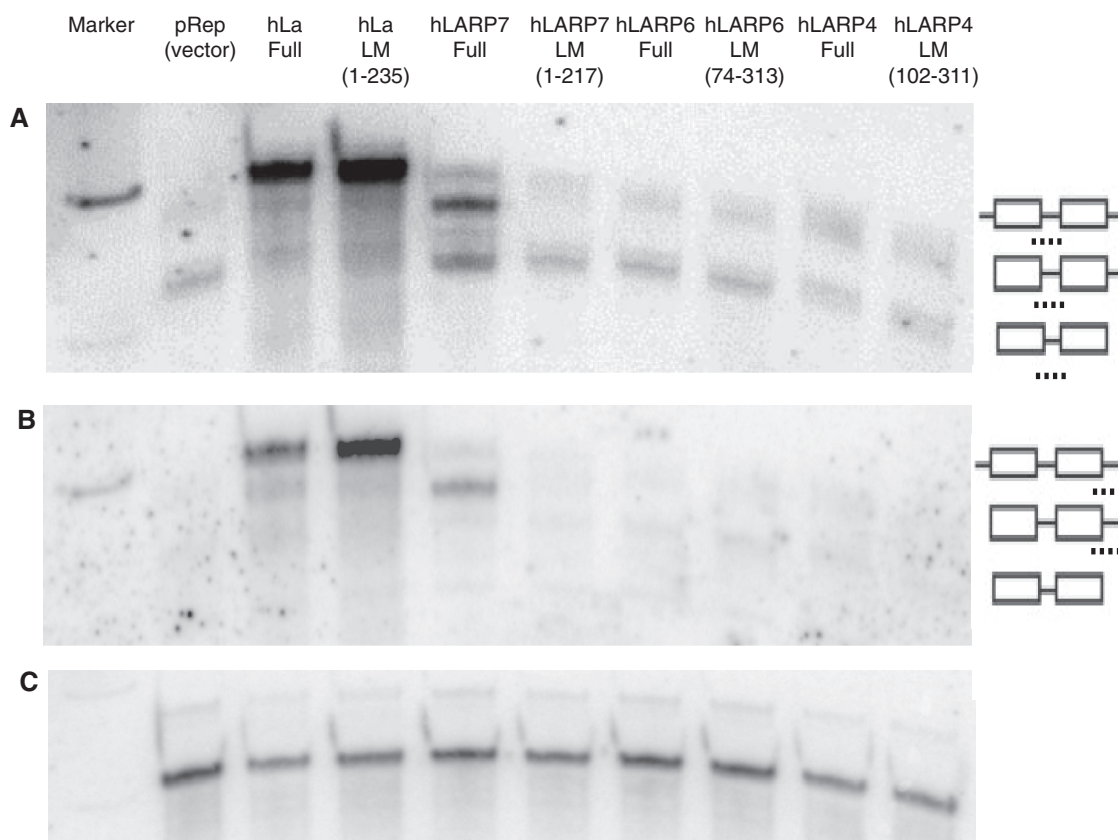


Figure 3. tRNA mediated suppression by the human LARPs does not correlate with UUU-3'OH dependent 3'-end protection. Northern analysis for pre-tRNA^{Lys}CUU (20,38) was performed to measure the capacity of the full-length and La modules of hLARP4, 6 and 7 to support the accumulation of pre-tRNA intermediates as has been previously shown for genuine human and yeast La. (A) probed using pre-tRNA^{Lys}CUU intron complementary probe, which detects newly transcribed, leader and trailer containing pre-tRNA (top band), leader processed pre-tRNA (middle) and leader and trailer processed (bottom) pre-tRNA (20,38). Locations of regions of complementarity of probes on pre-tRNAs indicated by dashed lines. (B) same blot probed using pre-tRNA^{Lys}CUU trailer complementary probe. (C) same blot probed for the U5 snRNA as a loading control.

species, a finding we confirmed by re-probing the blot using a probe complementary to the trailer sequence (Figure 3B), suggesting that full-length hLARP7 may be inactive in tRNA-mediated suppression by causing a block in pre-tRNA processing at this stage. In this context, the data are consistent with hLARP La module-dependent enhancement of tRNA-mediated suppression occurring via transient interactions with the suppressor tRNA substrate and through an activity independent of stable pre-tRNA 3'-end protection. We therefore attempted to assess whether these human LARPs, like yeast and hLa, harbour RNA chaperone activity.

hLARP4, 6 and 7 are RNA chaperones

Previous work has shown that human and *S. pombe* La can enhance the maturation of defective pre-tRNAs by two distinguishable activities: UUU-3'OH dependent 3'-end protection using amino acids mapping largely to the LAM and an activity independent of 3'-end protection that maps to the RRM (including amino acids C-terminal to the canonical RRM) and that is associated with RNA chaperone activity (19,25). It was also hypothesized for genuine La proteins that the second activity is dependent on the first: La proteins must first stably engage the pre-tRNA to function as RNA chaperones for these

substrates (25). Based on our data with the human LARPs, however, we considered the possibility that transient pre-tRNA interactions by the hLARP La modules may be sufficient for some degree of activity in the tRNA-mediated suppression assay should these factors, like genuine yeast and hLa, harbour RNA chaperone activity. Such a model would also be consistent with general models for RNA chaperones in which transient substrate interactions are permitted (and in some cases are required) for productive RNA remodelling (41). We have previously used a FRET-based assay to characterize the RNA strand annealing and strand dissociation activity of candidate RNA chaperones (19,42). The assay relies on RNA strands containing 21 nt of complementarity and labelled at their 5'-ends with either Cy5 (top strand) or Cy3 (bottom strand). When incubated in a heated cuvette or microplate reader at 37°C, the annealing of the two strands (Phase I) causes FRET (measured as an increase in FRET index) between the fluorophores, and the rate of annealing (k_{ann1}) can be compared in the absence or presence of a candidate RNA chaperone with strand annealing activity. In Phase II of the assay ($t = 180$ s after the initiation of Phase I), an excess of unlabelled competitor, corresponding to the bottom strand sequence but lacking the Cy3 fluorophore, is injected into

the cuvette. In the presence of an RNA chaperone with strand dissociation activity, the dissociation of the Cy5 and Cy3 annealed RNA substrates and their inability to reanneal in the presence of excess competitor causes a decrease in FRET index and an associated rate of strand displacement ($k_{S,D}$). In the absence of strand displacement, however, the increase in concentration of one partner of the annealing reaction causes an increase in strand annealing in Phase II, which is quantitated as k_{ann2} . We have used this assay previously to confirm that both yeast and hLa harbour both RNA strand annealing and strand displacement activity, compared with a previously established positive control protein, the bacterial RNA chaperone StpA, versus a no protein or BSA negative control (19).

To examine whether RNA chaperone activity is conserved in members of the LARP4, 6 and 7 families, as well the regions of the LARPs important for this, we cloned the LAMs, RRM, La modules (LAM + RRM), CTDs and full-length versions of hLARP4, hLARP6 and hLARP7 for expression and purification in *E. coli* (Table 1). As the intracellular concentration of the human LARPs is not known, we titrated the amounts of the full-length LARPs in the assay to determine their optimal concentration (Supplementary Figure S2) and found that all three LARPs were active (and in most cases, optimally active) at the same concentration (100 nM) previously examined for hLa (19), which is similar to the concentration of La estimated in human cells (43). Our negative control (BSA) showed no strand annealing or strand displacement activity at any tested concentration. We therefore used 100 nM as the concentration for all subsequent work using full-length and hLARP deletion constructs to compare the activity of these more directly. We found that like genuine La, each of the full-length human LARPs was active in both strand annealing and strand displacement (Figure 4).

We then tested whether the La modules of the hLARPs harboured RNA chaperone activity, as we have previously demonstrated for hLa. Indeed, we found that the La modules for each human LARP were active in the assay, with hLARP4 (102–311), hLARP6 (74–313) and hLARP7 (1–217) all capable of both strand annealing and strand displacement. We had previously demonstrated that amino acids C-terminal to the canonical RRM of hLa were important for RNA chaperone activity in the La module of hLa (19), and therefore we also truncated the La modules of the human LARPs closer to (but not into) the predicted RRM domain boundary (1) to observe the effect of this in the assay. We observed a significant drop in strand annealing in all the hLARP La modules owing to trimming of the predicted C-terminal disordered region (compare hLARP4 102–311 with 102–289, hLARP6 74–313 with 74–295 and hLARP7 1–217 with 1–199), although the effect for hLARP7 was more modest. To test the effect of these truncations further, we compared these same La module variants with the more C-terminal extended modules in tRNA-mediated suppression (Figure 2). We found that the truncated variants all showed less activity in this assay (compare columns 5 and 6), although it should be taken into consideration

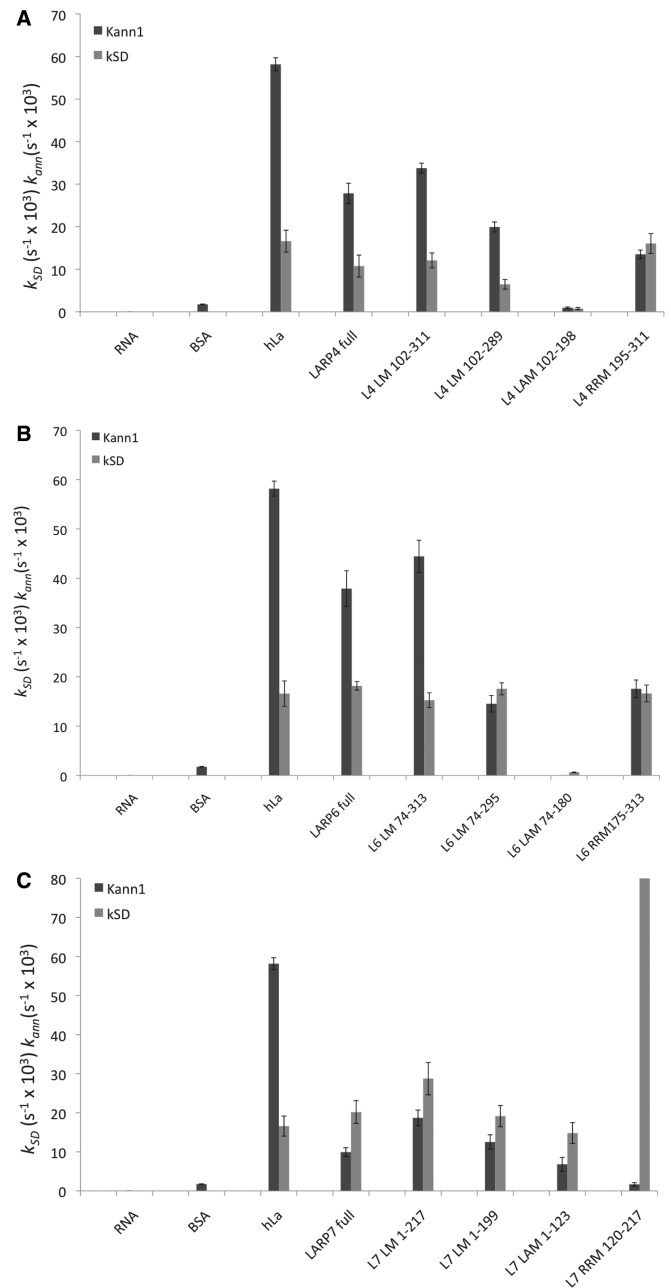


Figure 4. The human LARPs 4, 6 and 7 are RNA chaperones. Full-length and various domains of hLARP4, 6 and 7 were tested for strand annealing and dissociation activity, compared with full-length hLa. Dark grey bars: strand annealing activity. Light grey bars: strand displacement activity. Negative controls: RNA alone and BSA protein added at the same concentration as proteins tested (100 nM). Positive control: hLa. (A) hLARP4 constructs; (B) hLARP6 constructs; (C) hLARP7 constructs. LM: La module; LAM: La motif; RRM: RNA recognition motif.

that the truncated form of hLARP7 was underexpressed in *S. pombe* compared with its C-terminal extended counterpart (see Supplementary Figure S1). The truncated form of hLARP4 was expressed at a higher level than its C-terminal extended counterpart, suggesting that the lower activity of this mutant was not due to its lower expression, whereas both the truncated and C-terminal

extended versions of the La module of hLARP6 were similarly expressed.

We then examined the individual domains of the La module to see whether, as we had previously demonstrated for hLa, RNA chaperone activity in the La module is associated with the RRM and amino acids C-terminal to this. Similar to hLa (19), we found that the La motifs of hLARP4 and hLARP6 were completely devoid of RNA chaperone activity, whereas the La motif of hLARP7 had a low level of both strand annealing and strand displacement. As the RNA chaperone activity associated with the La module of hLARP7 also showed less dependence on amino acids C-terminal to the RRM compared with hLa, hLARP4 and hLARP6 [Figure 4C and (19)], these data suggest that hLARP7 may harbour a compensatory region in or around the LAM that may substitute for the C-terminal RRM extension. As regions implicated in RNA chaperone activity are often highly charged with basic residues, one such candidate region in hLARP7 (see Supplementary Figure S3) could be a unique insertion immediately N-terminal to the LAM (amino acids 19–32: KKKEVEKKKRSRVK; pI = 10.73), which was included in our LAM construct owing to its proximity to amino acids known to be important for the native fold of the LAM and in UUU-3'OH binding [i.e. amino acids Q41, F44, W45, F46 (2–4)].

We have previously demonstrated that although the hLa LAM does not itself harbour RNA chaperone activity, it may serve as an accessory RNA chaperone domain by assisting the adjacent RRM in binding RNA substrates (19). This was demonstrated by showing that the hLa RRM has higher activity in the context of the intact La module compared with when it is tested in isolation, and that increasing the concentration of hLa RRM tested can partially compensate for this decrease in activity. We therefore also tested the hLARP RRM domains in isolation and found that, like we had observed for hLa, each harboured both strand annealing and strand displacement activity and that the level strand annealing activity of the RRM was lower in isolation than the respective degree of activity we observed by the RRM in the context of the La module. As the LAM for each factor was inactive (hLa, hLARP4 and 6) or minimally active (hLARP7) in RNA chaperone activity in isolation, these data are consistent with a general role for the LAM of helping to bring RNA targets into proximity to the RNA chaperone activity harboured by the RRM. Interestingly, we also found that there was less of an effect of the presence or absence of the LAM on strand displacement activity by the RRM, and that in the case of hLARP7, the RRM in isolation had significantly higher strand displacement activity than with the LAM in the context of the La module. Although the reason for this enhancement for hLARP7 is unclear, we hypothesize that this could be due either to variations in higher-order structure of its RRM in isolation compared with when it is paired with its LAM in the context of the La module, or alternatively could more related to the capacity of the La module to possibly sequester the competitor RNA used during the strand displacement phase compared with the isolated RRM.

In sum, the data are consistent with RNA chaperone activity being a conserved feature of the La modules of several diverse members of the La motif containing superfamily. Furthermore, as was found previously for hLa, this activity centres around the RRM domain and amino acids immediately C-terminal to this, with the La module's LAM being largely devoid of activity but potentially acting as an accessory structure that helps recruit RNA to the adjacent RRM in the assay.

RNA chaperone activity by the human LARPs is not dependent on UUU-3'OH binding

The hLARP4, 6 and 7 proteins have varying degrees of conservation in the amino acids associated in hLa with UUU-3'OH-dependent RNA binding, with hLARP6 and 7 having 100% similarity and near identity at these residues. We therefore tested the importance of UUU-3'OH-mediated binding in LARP-dependent RNA chaperone activity by comparing hLARP La domain associated rates for the original 21 nt substrates with rates where we substituted a modified 25 nt top strand ending in UUU-3'OH (Figure 5). We found that, similar to what we have described for hLa, there was no significant enhancement of strand annealing or strand dissociation with this modified substrate for hLARP4 or hLARP7, suggesting that UUU-3'OH-dependent binding is not required for their RNA chaperone activity, (19). Interestingly, we found that the strand annealing activity of hLARP6 was inhibited by this modification while the strand displacement activity was enhanced, although the effects were moderate. These data indicate that similar to hLa, UUU-3'OH-dependent RNA binding is not required for the RNA chaperone activities of the human LARPs.

DISCUSSION

Genuine La proteins bind nascent polymerase III transcripts, including pre-tRNAs, the 7SK RNA and the U6 snRNA, via sequence specific recognition of the UUU-3'OH motif found at the 3'-end of all polymerase III transcripts, and yet also associate with polymerase II transcripts that lack this motif (9,44). Consistent with genuine La proteins displaying a high degree of plasticity in RNA ligand binding, affinity measurements using RNA substrates carrying variations in the UUU-3'OH motif result in only modest (1.5–10-fold) decreases in La affinity, whereas mutations to the La motif amino acids associated with UUU-3'OH binding cause much more drastic changes, suggesting these amino acids may also have a role in the engagement of La targets that lack UUU-3'OH (15). Combined with the various RNA substrates found for the hLARPs, it would appear that the tandem La motif/RRM arrangement is associated with significant RNA ligand plasticity. In this work, we present evidence that the isolated La modules of the hLARPs 4, 6 and 7 are capable of participating in tRNA-mediated suppression. Furthermore, we present evidence that tRNA-mediated suppression by the hLARPs is likely related to RNA chaperone function, and that these hLARPs display RNA chaperone activity *in vitro*. A summary of the activities described for the full-length

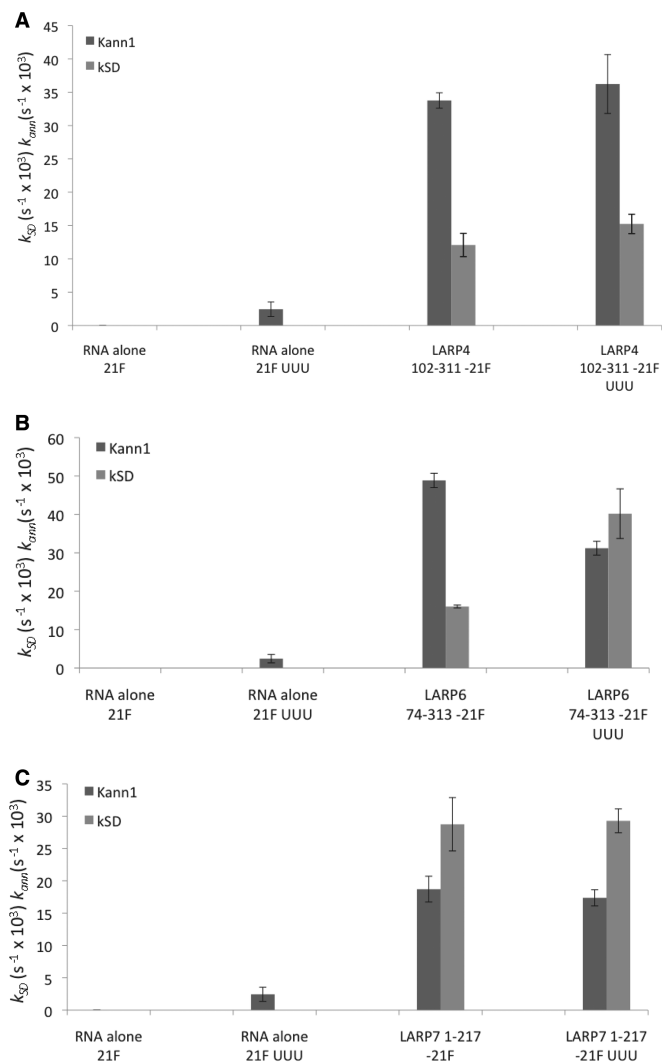


Figure 5. UUU-3'OH-dependent binding is not required for RNA chaperone activity by the La modules of human LARPs 4, 6 and 7. The presence of a UUU-3'OH on the Cy5 labelled strand did not significantly affect the RNA chaperone activity by hLARP7 and hLARP4 and had a minor effect on the strand annealing and strand displacement activity of hLARP6. (A) hLARP4, (B) hLARP6, (C) hLARP7.

hLARPs as well as the various constructs of these tested in this work is presented in Table 1.

In higher systems, LAM-containing proteins have been classified into five families based on sequence homology: the genuine La proteins, and the LARP1, LARP4, LARP6 and LARP7 families. In humans, hLARP7 is associated with the stability of the 7SK RNA, and by extension the 7SK RNP. hLARP4, 4b and 6 have been implicated in mRNA metabolism; hLARP4 and b engage poly-A binding protein and broadly enhance mRNA translation (35,36), whereas hLARP6 binds a conserved stem loop in the 5'UTR of the $\alpha 1$ and $\alpha 2$ collagen mRNAs and is hypothesized to coordinate their translation. (34). Human LARP1 has been found to associate with the cap-dependent translational apparatus and is associated with cell division and viral pathogenesis via

unknown mechanisms (45,46). We attempted to include the La module and full-length versions of hLARP1 in this study but found these were insoluble in both *E. coli* and yeast extracts.

That the La modules of hLARP4 and hLARP7 are more active than their full-length versions in tRNA-mediated suppression suggests that regions external to the La module may have important functions in RNA target binding/discrimination. We were not able to compare hLARP6 in this way, as its full-length version was poorly expressed in *S. pombe*. Similarly, RNA target discrimination by hLa for some non-UUU-3'OH containing targets, such as the Hepatitis C viral RNA and pri-/pre-miRNAs, has recently been shown to rely on cooperation between the hLa La module and the C-terminal RRM2 domain (47,48). We noted that the La module of hLARP4 gave the lowest level of tRNA mediated suppression and hypothesized that this may be due to it being the most divergent from that of hLa. In addition to the mutations already described, we therefore also substituted the linker between the hLARP4 LAM and RRM-like domain, the loop 3 joining the $\beta 2$ - $\beta 3$ strands of the RRM (14) or amino acids in the LAM associated with UUU-3'OH recognition (as well as combinations of these) to the equivalent linker, loop 3 or LAM amino acids found in hLa in the context of the C-terminal truncated La module (hLARP4 102–289) and tested these for tRNA-mediated suppression (see Table 1). As measured by western blot, these mutants accumulated to lower levels than the inactive hLARP4 102-289 but to higher levels than the active hLARP4 102-311 (data not shown); yet, none of these showed tRNA-mediated suppression, suggesting that it is the C-terminal extension of the hLARP4 RRM between amino acids 289–311 that is the most important determinant for activity in this assay.

Human or *S. pombe* La-dependent enhancement of tRNA-mediated suppression has been shown to depend on two distinguishable activities that map to different La module RNA-binding surfaces: UUU-3'OH-dependent binding via the La motif, and a second activity independent of 3'-end protection that maps to the canonical RRM RNA-binding surface (25). Previous work has shown that mutation of the RRM RNA-binding surface causes defects in La-dependent rescue of defective (mutated) tRNAs, but conversely enhances the maturation of non-defective pre-tRNAs (25). Accordingly, it has been hypothesized that the RRM1 RNA-binding surface provides a 'quality control' check that all pre-tRNAs sample but that only benefits defective pre-tRNAs. Consistent with the notion of a quality control check, mutagenesis of this binding surface, or of basic residues in the RRM1 $\beta 2$ - $\beta 3$ loop3, or of the non-canonical RRM1 $\alpha 3$ helix, results in defects in hLa dependent RNA chaperone activity (19). Using northern blots for the detection of pre-tRNA^{Lys}CUU intermediates, we have shown that the hLARP La modules can enhance tRNA-mediated suppression in the absence of stable pre-tRNA binding and associated pre-tRNA intermediate accumulation (Figure 3). It therefore seems likely that enhancement of tRNA-mediated suppression by the hLARPs is likely due to activities related more to the RRM1 associated activity of hLa, namely, RNA chaperone

activity, in the context of transient rather than stable pre-tRNA association. Interestingly, expression of full-length hLARP7 did result in accumulation of a trailer containing pre-tRNA intermediate, even in the presence of minimal activity in the tRNA-mediated suppression assay, suggesting that the full-length protein can bind pre-tRNAs but cannot promote normal processing. As previously noted, LARP7 family members share the greatest degree of homology with the genuine La proteins, and a subset of the hLARP7 endogenous substrate 7SK RNA is bound by hLa before being displaced by hLARP7. These data are reminiscent of a previously characterized mutant of hLa (hLa S366A), which binds pre-tRNAs but is refractory to their maturation due to a block to tRNA processing caused by restricting access to a processing enzyme [in that case, RNase P (21)]. Although a hLARP7-pre-tRNA complex has not been observed in human cells, it should be taken into consideration that the 7SK RNA is specific to metazoans (i.e. absent in the context of the *S. pombe* tRNA-mediated suppression assay) and that hLARP7 may not engage pre-tRNAs, or engage them to a much lower degree, in the presence of its endogenous target and/or in the presence of competing hLa (also absent in the context of the tRNA mediated suppression assay).

Consistent with an RNA chaperone function in tRNA-mediated suppression, we have used a FRET-based assay for RNA strand annealing and dissociation to show that the full length and La modules of hLARP4, 6 and 7 all harbour RNA chaperone activity. This represents, to our knowledge, the first demonstration of a conserved activity across the La motif containing superfamily, with representative members of all families having been tested with the exception of the LARP1 family. Based on our previous work indicating a role for amino acids C-terminal to the RRM having an important function in RNA chaperone activity, we wanted to also test the equivalent regions in the humanLARPs. Indeed, we found that deletion of regions C-terminal to the predicted RRM fold significantly inhibited strand-annealing activity for hLARP4 and hLARP6, and to a lesser extent, hLARP7, similar to what we have previously shown for the hLa RRM1 domain and suggesting that the structural determinants of RNA chaperone activity may be generally conserved across the LARP families. We also tested the C-terminal halves of the humanLARPs and found that, similar to hLa (19), these were also inactive in tRNA-mediated suppression (Figure 2) but capable of RNA chaperone activity (Table 1 and data not shown). These data are reminiscent of recent work showing that the *Tetrahymena thermophila* LARP7 family member p65 remodels its target, the polymerase III transcribed telomerase RNA, via a RRM2 domain C-terminal to the La module (see Figure 1) that also requires amino acids immediately C-terminal to the canonical RRM fold (49). Although the data here suggest that the capacity to function as an RNA chaperone is a conserved feature of the La module-containing proteins, it remains to be determined which regions of the humanLARPs are required for high-affinity binding and protection of their endogenous RNA targets.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

- Bousquet-Antonelli, C. and Deragon, J. (2009) A comprehensive analysis of the La-motif protein superfamily. *RNA*, **15**, 750–764.
- Bayfield, M.A., Yang, R. and Maraia, R.J. (2010) Conserved and divergent features of the structure and function of La and La-related proteins (LARPs). *Biochim. Biophys. Acta*, **1799**, 365–378.
- Dong, G., Chakshumathi, G., Wolin, S.L. and Reinisch, K.M. (2004) Structure of the La motif: a winged helix domain mediates RNA binding via a conserved aromatic patch. *EMBO J.*, **23**, 1000–1007.
- Alfano, C., Sanfelice, D., Babon, J., Kelly, G., Jacks, A., Curry, S. and Conte, M.R. (2004) Structural analysis of cooperative RNA binding by the La motif and central RRM domain of human La protein. *Nat. Struct. Mol. Biol.*, **7–14**, 7–12.
- Wolin, S.L. and Cedervall, T. (2002) The La protein. *Annu. Rev. Biochem.*, **71**, 375–403.
- Meerovitch, K., Svitkin, Y.V., Lee, H.S., Lejbkowitz, F., Kenan, D.J., Chan, E.K., Agol, V.I., Keene, J.D. and Sonenberg, N. (1993) La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *J. Virol.*, **67**, 3798–3807.
- Costa-Mattioli, M., Svitkin, Y. and Sonenberg, N. (2004) La autoantigen is necessary for optimal function of the poliovirus and Hepatitis c virus internal ribosome entry site in vivo and in vitro. *Mol. Cell. Biol.*, **24**, 6861–6870.
- Trotta, R., Vignudelli, T., Pecorari, L., Intine, R.V., Guerzoni, C., Santilli, G., Candini, O., Byrom, M.W., Goldoni, S., Ford, L.P. *et al.* (2003) BCR/ABL activates mdm2 mRNA translation via the La antigen. *Cancer Cell*, **13**, 145–160.
- Intine, R.V., Tenenbaum, S.A., Sakulich, A.S., Keene, J.D. and Maraia, R.J. (2003) Differential phosphorylation and subcellular localization of La RNPs associated with precursor tRNAs and translation-related mRNAs. *Mol. Cell*, **12**, 1301–1307.
- Sommer, G., Dittmann, J., Kuehnert, J., Reumann, K., Schwartz, P.E., Will, H., Coulter, B.L., Smith, M.T. and Heise, T. (2011) The RNA-binding protein La contributes to cell proliferation and CCND1 expression. *Oncogene*, **30**, 434–444.
- Schwartz, E., Intine, R.V. and Maraia, R.J. (2004) CK2 is responsible for phosphorylation of human La protein serine-366 and can modulate 5'TOP mRNA metabolism. *Mol. Cell. Biol.*, **24**, 9580–9591.
- Ali, N., Pruijn, G.J., Kenan, D.J., Keene, J.D. and Siddiqui, A. (2000) Human La antigen is required for the hepatitis C virus internal ribosome entry site (IRES)-mediated translation. *J. Biol. Chem.*, **275**, 27531–27540.
- Pudi, R., Srinivasan, P. and Das, S. (2004) La protein binding at the GCAC site near the initiator AUG facilitates the ribosomal assembly on the hepatitis C virus RNA to influence internal ribosome entry site-mediated translation. *J. Biol. Chem.*, **279**, 29879–29888.
- Bayfield, M.A. and Maraia, R.J. (2009) Precursor-product discrimination by La protein during tRNA metabolism. *Nat. Struct. Mol. Biol.*, **16**, 430–437.

15. Teplova, M., Yuan, Y.R., Phan, A.T., Malinina, L., Ilin, S., Teplov, A. and Patel, D.J. (2006) Structural basis for recognition and sequestration of UUU(OH) 3' termini of nascent RNA polymerase III transcripts by La, a rheumatic disease autoantigen. *Mol. Cell*, **21**, 75–85.
16. Kucera, N.J., Hodsdon, M.E. and Wolin, S.L. (2011) An intrinsically disordered C terminus allows the La protein to assist the biogenesis of diverse noncoding RNA precursors. *Proc. Natl Acad. Sci. USA*, **108**, 1308–1313.
17. Kotik-Kogan, O., Valentine, E.R., Sanfelice, D., Conte, M.R. and Curry, S. (2008) Structural analysis reveals conformational plasticity in the recognition of RNA 3' ends by the human La protein. *Structure*, **16**, 852–862.
18. Maraia, R.J. and Bayfield, M.A. (2006) The La protein-RNA complex surfaces. *Mol. Cell*, **21**, 149–152.
19. Naeeni, A.R., Conte, M.R. and Bayfield, M.A. (2012) RNA chaperone activity of the human La protein is mediated by a variant RNA recognition motif. *J. Biol. Chem.*, **287**, 5472–5482.
20. Van Horn, D.J., Yoo, C.J., Xue, D., Shi, H. and Wolin, S.L. (1997) The La protein in *Schizosaccharomyces pombe*: a conserved yet dispensable phosphoprotein that functions in tRNA maturation. *RNA*, **3**, 1434–1443.
21. Intine, R.V.A., Sakulich, A.L., Koduru, S.B., Huang, Y., Pierstorff, E., Goodier, J.L., Phan, L. and Maraia, R.J. (2000) Transfer RNA maturation is controlled by phosphorylation of the human La antigen on serine 366. *Mol. Cell*, **6**, 339–348.
22. Maraia, R.J. and Lamichhane, T.N. (2011) 3' processing of eukaryotic precursor tRNAs. *Wiley Interdiscip. Rev. RNA*, **2**, 362–375.
23. Chakshumathi, G., Kim, S.D., Rubinson, D.A. and Wolin, S.L. (2003) A La protein requirement for efficient pre-tRNA folding. *EMBO J.*, **22**, 6562–6572.
24. Copela, L.A., Chakshumathi, G., Sherrer, R.L. and Wolin, S.L. (2006) The La protein functions redundantly with tRNA modification enzymes to ensure tRNA structural stability. *RNA*, **12**, 644–654.
25. Huang, Y., Bayfield, M.A., Intine, R.V. and Maraia, R.J. (2006) Separate RNA-binding surfaces on the multifunctional La protein mediate distinguishable activities in tRNA maturation. *Nat. Struct. Mol. Biol.*, **13**, 611–618.
26. Belisova, A., Semrad, K., Mayer, O., Kocian, G., Waigmann, E., Schroeder, R. and Steiner, G. (2005) RNA chaperone activity of protein components of human Ro RNPs. *RNA*, **11**, 1084–1094.
27. Liu, Y., Tan, H., Tian, H., Liang, C., Chen, S. and Liu, Q. (2011) Autoantigen La promotes efficient RNAi, antiviral response, and transposon silencing by facilitating multiple-turnover RISC catalysis. *Mol. Cell*, **44**, 502–508.
28. He, N., Jahchan, N.S., Hong, E., Li, Q., Bayfield, M.A., Maraia, R.J., Luo, K. and Zhou, Q. (2008) A La-related protein modulates 7SK snRNP integrity to suppress P-TEFb-dependent transcriptional elongation and tumorigenesis. *Mol. Cell*, **29**, 588–599.
29. Krueger, B.J., Jeronimo, C., Roy, B.B., Bouchard, A., Barrandon, C., Byers, S.A., Searcey, C.E., Cooper, J.J., Bensaude, O., Cohen, E.A. et al. (2008) LARP7 is a stable component of the 7SK snRNP while P-TEFb, HEXIM1 and hnRNP A1 are reversibly associated. *Nucleic Acids Res.*, **36**, 2219–2229.
30. Markert, A., Grimm, M., Martinez, J., Wiesner, J., Meyerhans, A., Meyuhos, O., Sickmann, A. and Fischer, U. (2008) The La-related protein LARP7 is a component of the 7SK ribonucleoprotein and affects transcription of cellular and viral polymerase II genes. *EMBO Rep.*, **9**, 569–575.
31. Remillieux-Leschelle, N., Santamaria, P. and Randsholt, N.B. (2002) Regulation of larval hematopoiesis in *Drosophila melanogaster*: a role for the multi sex combs gene. *Genetics*, **162**, 1259–1274.
32. Mori, Y., Sato, F., Selaru, F.M., Olaru, A., Perry, K., Kimos, M.C., Tamura, G., Matsubara, N., Wang, S., Xu, Y. et al. (2002) Instability typing reveals unique mutational spectra in microsatellite-unstable gastric cancers. *Cancer Res.*, **62**, 3641–3645.
33. Merret, R., Martino, L., Bousquet-Antonelli, C., Fneich, S., Descombin, J., Billey, E., Conte, M.R. and Deragon, J.M. (2013) The association of a La module with the PABP-interacting motif PAM2 is a recurrent evolutionary process that led to the neofunctionalization of La-related proteins. *RNA*, **19**, 36–50.
34. Cai, L., Fritz, D., Stefanovic, L. and Stefanovic, B. (2010) Binding of LARP6 to the conserved 5' stem-loop regulates translation of mRNAs encoding type I collagen. *J. Mol. Biol.*, **395**, 309–326.
35. Schaffler, K., Schulz, K., Hirmer, A., Wiesner, J., Grimm, M., Sickmann, A. and Fischer, U. (2010) A stimulatory role for the La-related protein 4B in translation. *RNA*, **16**, 1488–1499.
36. Yang, R., Gaidamakov, S.A., Xie, J., Lee, J., Martino, L., Kozlov, G., Crawford, A.K., Russo, A.N., Conte, M.R., Gehring, K. et al. (2010) LARP4 binds poly(A), interacts with poly(A)-binding protein MLE domain via a variant PAM2w motif and can promote mRNA stability. *Mol. Cell Biol.*, **31**, 542–556.
37. Chambers, J.C., Kurilla, M.G. and Keene, J.D. (1983) Association between the 7 S RNA and the lupus La protein varies among cell types. *J. Biol. Chem.*, **258**, 11438–11441.
38. Yoo, C.J. and Wolin, S.L. (1997) The yeast La protein is required for the 3' endonucleolytic cleavage that matures tRNA precursors. *Cell*, **89**, 393–402.
39. Park, J.M., Intine, R.V. and Maraia, R.J. (2007) Mouse and human La proteins differ in kinase substrate activity and activation mechanism for tRNA processing. *Gene Expr.*, **14**, 71–81.
40. Katzman, S., Barrett, C., Thiltgen, G., Karchin, R. and Karplus, K. (2008) PREDICT-2ND: a tool for generalized protein local structure prediction. *Bioinformatics*, **24**, 2453–2459.
41. Rajkowitsch, L., Chen, D., Stampf, S., Semrad, K., Waldsich, C., Mayer, O., Jantsch, M.F., Konrat, R., Blasi, U. and Schroeder, R. (2007) RNA chaperones, RNA annealers and RNA helicases. *RNA Biol.*, **4**, 118–130.
42. Rajkowitsch, L. and Schroeder, R. (2007) Dissecting RNA chaperone activity. *RNA*, **13**, 2053–2060.
43. Gottlieb, E. and Steitz, J.A. (1989) The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription in vitro. *EMBO J.*, **8**, 841–850.
44. Inada, M. and Guthrie, C. (2004) Identification of Lhp1p-associated RNAs by microarray analysis in *Saccharomyces cerevisiae* reveals association with coding and noncoding RNAs. *Proc. Natl Acad. Sci. USA*, **101**, 434–439.
45. Karlas, A., Machuy, N., Shin, Y., Pleissner, K.P., Artarini, A., Heuer, D., Becker, D., Khalil, H., Ogilvie, L.A., Hess, S. et al. (2010) Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature*, **463**, 818–822.
46. Burrows, C., Abd Latip, N., Lam, S.J., Carpenter, L., Sawicka, K., Tzolovskiy, G., Gabra, H., Bushell, M., Glover, D.M., Willis, A.E. et al. (2010) The RNA binding protein Larp1 regulates cell division, apoptosis and cell migration. *Nucleic Acids Res.*, **38**, 5542–5553.
47. Martino, L., Pennell, S., Kelly, G., Bui, T.T., Kotik-Kogan, O., Smerdon, S.J., Drake, A.F., Curry, S. and Conte, M.R. (2012) Analysis of the interaction with the hepatitis C virus mRNA reveals an alternative mode of RNA recognition by the human La protein. *Nucleic Acids Res.*, **40**, 1381–1394.
48. Liang, C., Xiong, K., Szulwach, K.E., Zhang, Y., Wang, Z., Peng, J., Fu, M., Jin, P., Suzuki, H.I. and Liu, Q. (2013) Sjogren syndrome antigen B (SSB)/La promotes global microRNA expression by binding microRNA precursors through stem-loop recognition. *J. Biol. Chem.*, **288**, 723–736.
49. Stone, M.D., Mihalusova, M., O'Connor, C.M., Prathapam, R., Collins, K. and Zhuang, X. (2007) Stepwise protein-mediated RNA folding directs assembly of telomerase ribonucleoprotein. *Nature*, **446**, 458–461.