La Protein and its Associated Small Nuclear and Nucleolar Precursor RNAs

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After transcription by RNA polymerase (pol) III, nascent Pol III transcripts pass through RNA processing, modification, and transport machineries as part of their posttranscriptional maturation process. The first factor to interact with Pol III transcripts is La protein, which binds principally via its conserved N-terminal domain (NTD), to the UUU-OH motif that results from transcription termination. This review includes a sequence Logo of the most conserved region of La and its refined modeling as an RNA recognition motif (RRM). La protects RNAs from 3′ exonucleolytic digestion and also contributes to their nuclear retention. The variety of modifications found on La-associated RNAs is reviewed in detail and considered in the contexts of how La may bind the termini of structured RNAs without interfering with recognition by modification enzymes, and its ability to chaperone RNAs through multiple parts of their maturation pathways. The CTD of human La recognizes the 5′ end region of nascent RNA in a manner that is sensitive to serine 366 phosphorylation. Although the CTD can control pre-tRNA cleavage by RNase P, a rate-limiting step in $tRNA^{Ser}_{UGA}$ maturation, the extent to which it acts in the maturation pathway(s) of other transcripts is unknown but considered here. Evidence that a fraction of La resides in the nucleolus together with recent findings that several Pol III transcripts pass through the nucleolus is also reviewed. An imminent goal is to understand how the bipartite RNA binding, intracellular trafficking, and signal transduction activities of La are integrated with the maturation pathways of the various RNAs with which it associates.

tRNA processing   RNA modification   RNase P   RNA recognition motif (RRM)
Protein structure modeling   Transcription   Nucleolus   Autoantigen   5′-ppp
Walker A motif   Lhp1   Sla1

RNA polymerase (pol) III synthesizes many small RNAs including 5S ribosomal (r)RNA and transfer (t)RNAs, as well as a variety of less abundant, small nuclear (sn), small nucleolar (sno), and small cytoplasmic (sc) RNAs. Newly synthesized Pol III transcripts undergo a maturation process that involves cleavage and/or modification and, in some cases, addition of nucleotides. Some Pol III transcripts undergo little processing while others must endure an elaborate maturation process (e.g., tRNAs).

Unlike the case for pre-mRNAs, which are transcribed by Pol II, neither RNA processing nor modification has been demonstrated to occur during Pol III transcription. Therefore, maturation of a Pol III transcript would appear to begin after synthesis is completed and the nascent RNA is released from the transcription complex. The final stage of transcription occurs in response to an oligo(dA) tract found at the 3′ ends of all pol III-dependent genes, which causes Pol III to terminate synthesis (16). Because the proximal part of the oligo(dA) signal is transcribed, all nascent Pol III transcripts share the common motif, oligo(rU)-OH, at their 3′ termini. Termination within the oligo(dA) tract generates heterogeneous ends, usually U(1-4)U-OH (hereafter referred to as UUU-OH), the distribution of which may vary depending on oligo(A) length and context (21,41,89,90,103,105, 106,145). Because terminal uridylates comprise a high-affinity binding site for the La protein, all nascent Pol III transcripts are bound, at least transiently,

by La (122). Indeed, the first protein that can be detected in association with newly synthesized Pol III transcripts is La.

La was first identified as an autoantigen in patients suffering from rheumatic disorders such as systemic lupus erythematosus and Sjogren's syndrome, although homologues have been identified in every eukaryote examined, including protozoa (3), trypanosomes (87,137), yeasts (79,131,143), insects (10,99, 143), amphibians (113), and mammals (24,127). Nascent Pol III transcripts that are known to associate with La are the precursors to tRNAs, 5S rRNA, U6 snRNA, 7SL (SRP) scRNA, 7SK snRNA, hY scRNAs, 7-2 (MRP) snoRNA, RNase P RNA, 4.5S I RNA, B1-Alu, Alu RNAs, and others, including *Euplotes* telomerase RNA (3,25,35,51,77,80,82,89,103, 105,106,117) (see below).

Presumably by binding to UUU-OH, La can facilitate clearance of the newly terminated RNA from the transcription complex (40,41,86). Human La has also been shown to act as an ancillary factor that stimulates Pol III transcription in some in vitro systems, promoting efficient recycling of Pol III (27,34,38-41,84,86), although this is controversial (35,78,136, 144). Another proposed activity of La, involvement in the translation of viral and cellular mRNAs that contain internal ribosome entry sites, has been reviewed previously (11,56,85), and will not be discussed here.

La has been considered as a molecular chaperone in multiple contexts: (i) that it may stabilize RNAs in the correct conformation (i.e., an activity related to RNA folding or RNA structure), (ii) that it can stabilize associated transcripts against exonuclease digestion or other degradative processes, and (iii) that it can serve as a platform on which associated transcripts may be recognized by modification and processing factors that contribute to the maturation of the RNA (i.e., that it accompanies transcripts during modification) (33,57,83,85,97,98,144). Examples of each of these will be considered below.

La also associates with certain RNAs that are synthesized by pol II. The first of these to be described were the leader transcripts generated from vesicular stomatitis virus and a U1 snRNA precursor in HeLa cells (70,81), followed by reports of others including the 5′ regions of viral and cellular mRNAs, several of which were represented by probes that contained terminal uridylates [reviewed in (85)]. La associates with precursor intermediates of U1-U5 snRNAs as well as U3 snoRNA, in large part via short tracts of terminal uridylates that result from incomplete trimming of their 3′ ends (69,142). La was also found to associate with an intermediate in a histone mRNA decay pathway whose 3′ processing occurred at a po-

sition that is predicted to expose terminal uridylates (91). Thus, for many of the above transcripts, binding appears to be driven primarily by recognition of 3′ terminal uridylates. Although recently published evidence suggests that La can also recognize RNAs that contain a CACAA sequence, via an RNA binding activity that will be considered in the context of pre-tRNA$_e^{Met}$ stability in a later section, the elements in La responsible for this binding also reside in the NTD and overlap and/or cooperate with UUU-OH recognition (4,64). Thus, substantial evidence indicates that the major mode of RNA binding, recognition of UUU-OH, is mediated by the most highly conserved region of La, the N-terminal domain (NTD) (85).

As diagrammed in Figure 1 and discussed in more detail in a later section, the human La protein has been shown to recognize the 5′-ppp end region of RNA, a mode of binding that is clearly distinct from the NTD-mediated interaction with UUU-OH, as this 5′ end binding activity is mediated by the C-terminal domain (CTD) of La [(33,57), reviewed in (85)].

THE FUNCTIONAL REPERTOIRE OF La EXTENDS TO 5′ END METABOLISM OF PRE-tRNA

As detailed previously, a Walker A motif (WAM)-like sequence in the CTD of human La contributes to pre-tRNA 5′-ppp end recognition and control of processing by RNase P (85). This mode of recognition could potentially explain how La can protect a variety of pre-tRNA species from 5′ processing despite little if any conservation in their leader sequences, because the pppG/A moiety represents a unique determinant on each transcript (33). The BLOCKS program identifies aa 348–368 of hLa as a potential phosphate binding site (PBS), just downstream of the WAM, that is rich in conserved basic residues (53) (Fig. 1). Further support for this mode of interaction came from in vivo studies, which revealed that only when the WAM and the PBS sequences were intact was there protection of pre-tRNA from 5′ processing (57). The bipartite mode of 3′ and 5′ end binding by La also explains data obtained in vivo. Pre-tRNA 3′ end stabilization is mediated by La proteins that lack RRM-3 or downstream sequences, provided that they have an intact NTD, while 5′ end stabilization occurs only if the WAM-PBS and RRM-3 are also intact (33,57).

SIGNAL TRANSDUCTION THROUGH SERINE 366 PHOSPHORYLATION OF THE ACCESSORY CTS OF HUMAN La

The 5′-ppp end recognition activity of La and its modulation by serine 366 phosphorylation has been
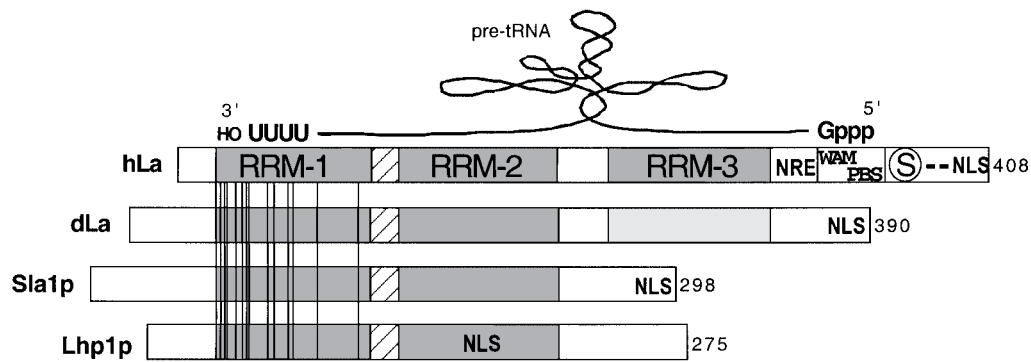
Figure 1. Schematic representation of human La, its apparent mode of bipartite binding to a nascent precursor tRNA, and comparison to other La proteins. Cartoon alignment of the La proteins of human (hLa), *D. melanogaster* (dLa), *S. pombe* (Sla1p), and *S. cerevisiae* (Lhplp). Their lengths, in aa, are indicated at the right. RRM-1, -2 and -3 domains are shaded and labeled for hLa only. Invariant residues in 13 sequences available in the database are shown as vertical lines; note that these are limited to RRM-1. The invariant residues are numbered according to hLa: Q20, E22, Y24, F25, N29, D33, F35, L36, G45, V47, F55, R57, A71, and R91. A putative Walker A motif (WAM) and phosphate binding site (PBS), and serine 366 (encircled S), are shown (see text). Positions of the NLS are indicated in bold font according to Rosenblum et al. (109). An element that functions as a nuclear retention element (NRE, see text) (119) is also shown. A schematized pre-tRNA has been modeled above to illustrate its proposed orientation and bipartite mode of binding, according to prior work (33,57) as described in the text. Reproduced from Maraia and Intine (85).

shown to play a functional role of tRNA expression in the intact cell [reviewed in (85)]. Indeed, human La is faithfully phosphorylated, specifically on S366 in fission yeast cells, and this modification promotes tRNA maturation, indicating the potential for regulation of tRNA processing in widely divergent species (57). Note that this mode of binding need not be limited to pre-tRNAs because all nascent Pol III transcripts initiate with 5′-ppp and terminate with UUU-OH (33,57). The CTD could afford La the ability to retain a nascent transcript (or its 5′ end region) in a stable form until it receives a signal (i.e., in the form of S366 phosphorylation) to release it (33). This feature, could potentially be used to control the handing off of a nascent RNA from La to another protein as occurs during RNP assembly, a period during which uncO chaperoned RNAs can succumb to exonucleolytic digestion as discussed above (69,97,142).

A CLASS OF La MOTIF-CONTAINING (LMC) POLYPEPTIDES THAT ARE STRUCTURALLY AND FUNCTIONALLY DISTINCT FROM GENUINE La PROTEINS

The most highly conserved region of the La proteins of many species resides in the N-terminal part of the protein, in a ~80- amino acid region referred to as the La motif or RRM-1 (85). The N-terminal position of the La motif is a characteristic that appears to distinguish genuine (i.e., Pol III transcript-associated) La proteins from other polypeptides that contain homology to the La motif, in which cases the

homology region is located at a more central or C-terminal position (121). As reviewed previously and summarized below, this region of La proteins appears to comprise an RNA recognition motif (RRM) that mediates UUU-OH recognition (85), suggesting a similar mode of RNA binding by the La motif-containing (LMC) proteins. Two of the LMC proteins have been identified in yeast, Sro9p and Slflp, and homologs have been identified in other species (121). Unlike genuine La proteins, the LMC proteins appear not to be involved with nascent Pol III transcripts but instead were associated with translating ribosomes (121). Indeed, genetic analyses have shown that the functions of these LMC proteins do not appear to overlap with the function of the genuine yeast La protein, Lhp1p (121). Sro9p has been identified as a suppressor of a variety of mutations, suggesting involvement in a wide range of genetic interactions [reviewed in (121)]. The ability of high copy Sro9p to suppress a mutation in a subunit of RNA polymerase II led Woychik and colleagues to the conclusion that this LMC protein increased general mRNA levels by increasing transcription as well as transcript stability (124). Although the characterization of the LMC class of proteins is still in its early stages, it seems clear that these proteins function independently of La and in a distinct functional pathway. For the remainder of this review, the focus will be on the genuine La proteins, all of which contain a La motif (a.k.a. RRM-1) near their N-terminus and many of which (*Drosophila*, human, rodent, *S. cerevisiae, S. pombe, X. laevis*) have been characterized to be

UUU-OH binding proteins that associate with nascent Pol III transcripts.

IN THE ABSENCE OF DETERMINED STRUCTURE, CONSIDER A MODEL OF THE CONSERVED NTD OF La

Although the capacity of the La motif to form a RRM-like structure has been detailed before (85), the present review includes a new analysis of the predicted secondary structure of this region and a new perspective provided by the sequence Logo (115). We believe that this analysis and perspective will be useful both for sequence comparisons and targeted mutagenesis of yet undiscovered La proteins as well as LMC proteins, as new sequences become available. In addition, this analysis has led us to propose here that the lack of solvent-exposed aromatic residues in the β3 region of the La RRM-1 motif (see below) may be a specific solution to the problem of having to recognize a short tract of pyrimidines [i.e., oligo(U)], because exposed aromatic side chains might select for purines as a result of greater stacking potential (below).

La belongs to a large family of proteins that contain an ~80 amino acid domain known as the RNA recognition motif (RRM) (13,19,65,132). The canonical RRM in human La that was initially noted (65) is now referred to as RRM-2, because two additional RRMs, designated RRM-1 and RRM-3, have been identified in the human La protein (13,64). The most highly conserved feature of all known La proteins is an ~80 aa region located near the N-terminus, referred to as the La motif or RRM-1, which appears to be critical for UUU-OH binding (64,85). Although there is not yet a crystal structure for La, the structures of other RRMs have revealed a globular domain with a β1-α1-β2-β3-α2-β4 topology (28,31,49,55, 96,116). In the RRM structure, the four β strands form a platform that contacts RNA on one surface while the other surface is packed against by the α helices, contributing to a hydrophobic core that is important for structural integrity of the RRM. A hallmark of the RRM is the presence of two short conserved sequence motifs, RNP-1 and RNP-2, which reside on β3 and β1, respectively (1,65,111), although some RRM proteins do not contain consensus RNP motifs (13,28,65). Some of the amino acids in the RNP motifs are oriented inward, toward and contributing to the hydrophobic core, while others face outward, exposed to the solvent, to contact RNA. In general, the buried hydrophobic residues in the RNP motifs alternate with the solvent-exposed residues that contact RNA (13,65).

In Figure 2, the RRM-1 regions of 13 La sequences were aligned and displayed as a sequence Logo (115). In this display, the single letter aa designations are stacked on top of each other for each position in the aligned sequence, the height of each letter being proportional to its frequency, and the letters are sorted with the most common one on top. The height of each stack is then adjusted by the Logo program, according to the information content (i.e., apparent conservation), and plotted on a logarithmic scale (base 2) on the Y axis, in bits (114). A sequence Logo reveals the consensus sequence as the top letter at each position, as well as the frequency of residues and the information content at each position (114). In the sequence Logo in Figure 2, single letters comprise the 14 tallest stacks, reflecting their complete invariance among the RRM-1 sequences (85).

The Logo output generated for La RRM-1 was annotated for Figure 2 in the following way: (i) differential shading of letters, (ii) placement of asterisks above or below certain positions, and (iii) addition of secondary structure elements that corresponded to the β1-loop1-α1-loop2-β2-loop3-β3-loop4-α2-loop5-β4 RRM topology as previously described (85). With regard to the latter, all of the α helices and connecting loops were previously predicted in the corresponding regions of the La sequences, while the regions of only three of the four putative β strands were predicted to contain an extended β strand (85). Examination of the consensus produced by Logo revealed an extended β strand in the β3 region that could not be predicted by previous methods (32) (data not shown), providing novel evidence to indicate that all of the secondary structure elements conforming to the RRM topology, β1-loop1-α1-loop2-β2-loop3-β3-loop4-α2-loop5-β4, have now been predicted for La RRM-1 as reflected in Figure 2.

Asterisks were placed below positions that correspond to all of the conserved residues that contribute to the hydrophobic core of the generic RRM structure, as discerned from examination of 70 RRMs (13,85). Although several of the hydrophobic core residues were described simply as hydrophobic, a hierarchy of sequence conservation at these positions had been noted. Some positions that contribute to the hydrophobic core were noted to be heavily represented by a single residue (e.g., the G found in loop 2 of 75–90% of the 70 RRMs analyzed), while identity at other core positions were moderately conserved (e.g., D or E in α1 occurs in 44% of the 70 RRM sequences) (13). Some of the conserved core RRM residues are thought to make defined contributions to the RRM structure; for example, the highly conserved G found in the loop 2 regions of 83% of 70 RRMS (invariant in La), may be required for the
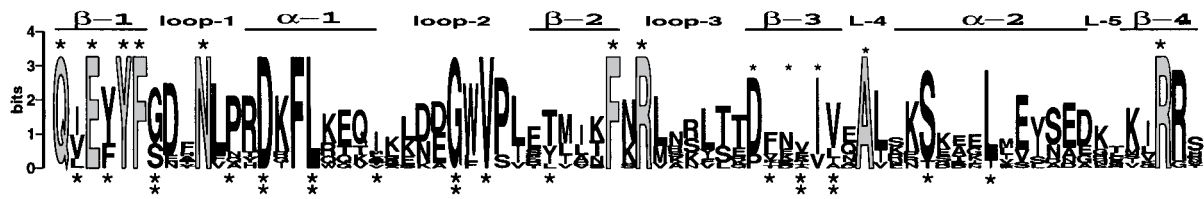
Figure 2. A sequence Logo and predicted structural features provide a model of the conserved RRM-1 of La protein. Sequences representing the RRM-1 regions of 13 La proteins were aligned and a sequence Logo was generated (114,115). The sequence Logo shown corresponds to residues 20–92 of hLa. The RRM topology, β1-loop1-α1-loop2-β2-loop3-β3-loop4-α2-loop5-β4 as proposed for La RRM-1, was placed above the sequence Logo (see text). Candidate residues potentially involved in RNA recognition are light shaded and topped with a bold asterisk. The putative RNP-2 and RNP-1 reside on β1 and β3, respectively (see text). Asterisks were placed below the positions corresponding to the conserved RRM core structure; two asterisks indicate a match to the consensus at that position and one asterisk indicates similarity (see text) (13). Apparent insertions in some of the La sequences (e.g., A.t. loop-3) were deleted prior to input into the Logo program. The sequences and accession numbers are: *Homo sapiens*, P05455; *Bos taurus*, P10881; *Rattus norvegicus*, P38656; *Mus musculus*, P32067; *Xenopus laevis*, P28049; *Xenopus laevis*, P28048; *Aedes albopictus*, Q26457; *Caenorhabditis elegans*, T30953; *Drosophila melanogaster*, P40796; *Trypanosoma brucei*, AAF34598.1; *Schizosaccharomyces pombe*, AAB 82145.1; *Saccharomyces cerevisiae*, P33399; *A. thaliana*, AAF09063.

turn into β2 (13). Therefore, to assimilate this information into Figure 2, the number of asterisks under the hydrophobic core positions was varied; two asterisks were placed where a match between the top letter in the sequence Logo and the conserved residue in the core consensus was observed. This revealed that a significant fraction of the invariant and conserved residues in La RRM-1 correspond to conserved identity structural core positions (two asterisks below the Logo). This analysis suggests that the significance of a substantial portion of the sequence information in the most conserved region of La is that it specifies structural integrity of the RRM-1 domain. In addition, there is also a very good match between La RRM-1 and the RRM core residues that are distinguished simply by the shared hydrophobicity of their side chains (one asterisk below the Logo). Overall, this further indicates that the conserved regions of La proteins have conserved the capacity to form RRM-like structures.

Several highly conserved and invariant positions in the RRM-1 sequence do not correspond to core residues (and therefore do not have asterisks under them). Because these may not correspond to conserved core residues, it was reasoned that their conservation may reflect a role in RNA recognition, and these are indicated by a light shaded letter topped by an asterisk in Figure 2. This display suggests that residues topped by asterisks might project from one surface of RRM-1, toward the RNA ligand, while the residues with asterisks below would project toward the hydrophobic core. This perspective is generally consistent with the putative RNP motifs described in the next paragraph.

A reasonably good RNP-2 motif can be fit to β1, with invariant residues projecting putatively toward the RNA interaction surface, in general alternating

with buried core residues. Arguing for the assignment of a RNP-1 motif appears weaker than for RNP-2 because solvent-exposed aromatic residues, a hallmark feature of prototypic RNP-1 motifs, are lacking in the β3 region, and those aromatic residues that are apparent correspond to buried positions. However, this should not necessarily detract from the RRM-1 model because some RRMs do not have prototypic RNP-1 motifs (13,28,65). Moreover, avoidance of aromatic residues in RNP-1 may be beneficial to the type of RNA binding exhibited by La proteins. Recall that aromatic residues on RNP-1 motifs of other RRM proteins contribute to RNA recognition via stacking, an interaction that is more favorable for purines than for pyrimidines (31,49,96,132). Thus, it should not be surprising that the putative RNP-1 would lack solvent-exposed aromatic residues because their inclusion might bias toward purine recognition. Therefore, the absence of solvent-exposed aromatic residues on RNP-1 might be a useful discriminatory factor when specificity must be limited to three or four uridylates.

Assignment of the hydrophobic core residues in β3 (13) leads to the putative designation of the conserved D and I residues in β3 along with the other position topped by a small asterisk in this region, as the solvent-exposed residues, because these alternate with the core positions. We suspect that these residues might contribute to RNA binding via protein backbone interactions with RNA as seen for other RNP-1 motifs (28,96).

In summary, the sequence Logo in Figure 2 reveals the most conserved residues in all La proteins, and should therefore be useful for comparisons of candidate La proteins including mutants. Additionally, the sequence and predicted secondary structure were evaluated in terms of the potential fit as a RRM. Our

conclusion is that the fit is moderately good with the expectation that substantial differences between the predicted and real structures of the La RRM-1 region will be revealed upon knowing the atomic coordinates of a crystal structure. With these limitations, the proposed model would be most useful if tested by structure probing methods, mutagenesis, and functional studies.

Pol III TERMINATION-MEDIATED RECRUITMENT OF La PROTECTS NASCENT TRANSCRIPTS FROM 3′ EXONUCLEASE DIGESTION

Although mechanisms exist that allow nascent Pol III transcripts to endure in the absence of La, when present, it increases the efficiency of expression (i.e., increases the fraction of newly synthesized RNA that is converted to a functional product) of a variety of RNAs, especially under certain conditions (20,57,79, 97,144). The number of Us required for optimal La binding matches the template requirement for termination by Pol III (122) and, accordingly, the number of terminal Us on nascent Pol III transcripts (41,90,106,145). Alteration of the terminal 3′-OH group to a phosphate decreases RNA binding by yeast, insect, amphibian, and human La proteins (122,125,143). This remarkably high degree of conservation of this otherwise subtle feature suggests that (i) recognition of the very end of a nascent transcript is critical to La function and (ii) some of the conserved residues of La might contribute specific recognition of the terminal ribose 3′-OH moiety. The high degree of conservation of 3′-OH discrimination by La discussed above, in conjunction with other experimental data, indicates that La's conserved function is to cap the 3′ ends of nascent RNAS, protecting them from 3′-5′ exonucleolytic digestion (33,38,78, 86,144).

Because the principal recognition site for La is produced by transcription termination by pol III, it seems reasonable to suspect that these transcripts are directed to La as a result of a link between La and Pol III termination or because they would otherwise be vulnerable, as newly terminated transcripts, to 3′ exonuclease digestion. In either case, Pol III termination leads to recruitment of La to the transcripts. Therefore, it seems worthwhile to consider a hypothetical thread that can putatively link La, Pol III termination, and RNA 3′ nucleolytic cleavage, which is suggested by recent but otherwise disparate observations. Pol III cleaves its associated nascent RNA at the 3′-OH end under certain circumstances, similar to bacterial RNA polymerase and pols I and II upon encountering pauses or other blocks to elongation (14,15,17,58, 104,129,138). A small subunit of pol III, RPC11, facilitates termination by stimulating this 3′-OH cleavage activity (26). Thus, it is a remarkable coincidence that La protein, a factor shown to function in Pol III termination, exhibits an activity that can protect nascent RNAs from a polymerase-intrinsic 3′ cleavage reaction that is important for termination. Indeed, La has been found to copurify with a human Pol III holoenzyme, suggesting intimate association with the synthetic machinery (135). It will be intriguing in the future to consider La in the context of the RPCII-associated termination activity.

RNA 3′ cleavage is certainly not limited to the hydrolytic activity of RNA polymerases, as exonucleases abound in nuclei, and it seems likely that protection of nascent transcripts after release from the transcription complex is a most important function of La (33,38,69,78). Indeed, Pol III termination is not a prerequisite for La binding, because, as mentioned above, La can protect snRNAs synthesized by pol II (69,142). Nonetheless, because Pol III produces 3′ oligo-U, there is an inextricable link between termination and La binding.

Normal cells rely on an orderly passage of nascent RNA from La to the next protein in a pathway, as the RNA matures. While the pathway of pre-tRNA maturation is quite elaborate (141), 5S rRNA maturation may appear less complex but nonetheless involves La. Evidence from mammalian and *Xenopus* cells indicated that 5S rRNA is first bound by La and then by the ribosomal protein L5 (or TFIIIA in *Xenopus* oocytes) prior to further transport (47,123). Similar involvement of La was suggested by studies of 7-2 (MRP) RNA, U6 snRNA, and B1-Alu RNA (51,82,125). Genetic evidence from yeast mutants indicates that La stabilizes newly synthesized U6 snRNA, increasing its chances for transfer or assembly into a Lsm8p-containing U6 snRNP (97). Indeed, multiple genetic interactions have been demonstrated between yeast La and the Lsm2–Lsm8 complex, to stabilize nascent U6 snRNA (98). Additional mutants indicate a similar chaperone activity of La for precursors of U3 snoRNA and U4 snRNA, even though they are synthesized by pol II (69,142). It is noteworthy that the proteins that replace La on U3 and U4 precursors may also stabilize the 3′ ends of the RNAs from digestion (69,98,142).

PRE-tRNA MODIFICATIONS OCCUR ON La-RNPs

It is becoming clear that facilitating RNA assembly into stable RNPs is not the extent of La's function as

a chaperone (97), because some evidence suggests that La may facilitate RNA folding and other evidence suggests that RNAs appear to remain associated with La during transient recognition by other proteins. Steitz and colleagues showed that La-associated transcripts contain a variety of modifications, including ribose and base methylation, dihydrouridylation, and pseudouridylation (52,75,105). As it was later shown that most La-associated RNAs were transient precursors to 5S rRNA and tRNAs (106), it would appear that the pre-tRNAs were modified before or while they were associated with La. Because, as discussed above, La appears to bind the RNAs before or immediately after transcription termination by Pol III, the cumulative evidence suggests that modifications occur while the nascent transcripts are associated with La [recently reviewed in (83)].

According to a bipartite binding model (Fig. 1), interactions with La would be limited principally to the terminal regions of a pre-tRNA, leaving the remainder of the tRNA accessible to modification enzymes and processing factors (33). As briefly reviewed previously (83) and described in greater detail below, the extent to which physical association with La may support or interfere with the activities of pre-tRNA modification and processing enzymes can be considered from perspectives afforded by five data sets: (i) recent results linking La and pre-tRNA$_i^{Met}$ m1A58 modification (6,20), (ii) differences in the modifications found on a human pre-tRNA$_e^{Met}$ that is stabilized by La, and its mature counterpart (50,64), (iii) results of Nishikura and DeRobertis who documented the modifications found on the nascent precursor and various intermediate species of tRNA^{Tyr} (94), (iv) the finding that La specifically modulates access of the pre-tRNA substrate to RNase P in a manner that is sensitive to serine 366 phosphorylation in the intact cell (57), and (v) evidence that association of pre-tRNA with La protects against 3′ exonucleolytic digestion and thereby facilitates 3′ endonucleolytic cleavage (144).

With regard to (i) above, data indicate that La cooperates with the m1A58 modifying enzyme and that m1A58 is one of the earliest modifications of pre-tRNAs (6,20). Because La associates with many if not all pre-tRNAs, we expect similar cooperation with other modifying enzymes. Scrutiny of preexisting data also suggests that La may interfere with certain modifications (below).

With regard to (ii) above, human pre-tRNA$_e^{Met}$ was initially identified as a tRNA precursor of unusually high stability (50). This pre-tRNA has been independently isolated as the most abundant La-associated transcript in HeLa cells (64) [see (4)]; this characteristic, in conjunction with its length of 86 nt and modi-

fication profile, suggests that it may be the "hb" RNA previously reported by Hendrick and colleagues (52). This stable pre-tRNA$_e^{Met}$ contains the modified bases, D, T, m1A, m5C, M$^2_2$G, and Cm, which are found mostly on the outer aspect of the elbow (D and T loops) and the lower half of the tRNA structure (Fig. 3), while the mature tRNA$_e^{Met}$ species contains these plus two M^{2}G residues (M^{2}Ci6 & M^{2}G1O, indicated as M^{2}G in the oval in Fig. 3) in the acceptor stem and D stem, respectively, and i^{6}A37 adjacent to the anticodon (50). With regard to (iii), this pattern of modifications is similar to that observed during the expression of pre-tRNA^{Tyr} (94). Specifically, the pre-tRNA^{Tyr} species containing a 5′ leader, 3′ trailer, and intron bears several of the modifications found on pre-tRNA$_e^{Met}$, while additional modifications were found on the intron-containing species only after end processing occurred (94).

We have superimposed these data onto a model of pre-tRNA structure. For this, we added non-base paired 5′ leader and 3′ trailer regions onto a preexisting model for tRNA^{Ser} (Fig. 3) (68). The following observations suggest that it is not inappropriate to propose that pre-tRNAs that resemble the overall structure depicted in the Figure 3 model are bound by La. A La-pre-tRNA is known to be a substrate for RNase P, the latter of which recognizes folded pre-tRNA (5,33,63,68,74,139-141,144). La has been proposed to stabilize the correctly folded form of a pre-tRNA (144). While the sequences of the 5′ leaders and 3′ trailers are not strictly conserved among eukaryotic pre-tRNAs, their potential ability to base pair most often involves the terminal uridylates in naked RNA (73), which would otherwise be bound by La in vivo. Finally, eukaryotic tRNA genes are usually flanked by A+T-rich DNA and extensions tend not to interfere with formation of tRNA structure [(2,36), R.M., unpublished observation]. When considering this model, recall that while the specificity elements recognized by La are localized at the 5′ and 3′ ends of a pre-tRNA, these are thought to include the entire leader and trailer regions (Fig. 1).

For both pre-tRNA$_e^{Met}$ and pre-tRNA^{Tyr}, the m^{2}G modifications (depicted in oval on right side of Fig. 3) appeared only after removal of the La binding sites, the 5′ and 3′ extensions, from the pre-tRNAs. Because these modifications were found on the intron-containing pre-tRNA^{Tyr} species, they almost certainly occurred in the nucleus (94). One interpretation is that La sterically hinders access to these modification sites, preventing their addition until after the leader and trailer, and La, are removed. This would not be too unlike what has been observed for La and RNase P (33). This is consistent with the model depicted in Figure 3, which suggests that the m^{2}G modi-
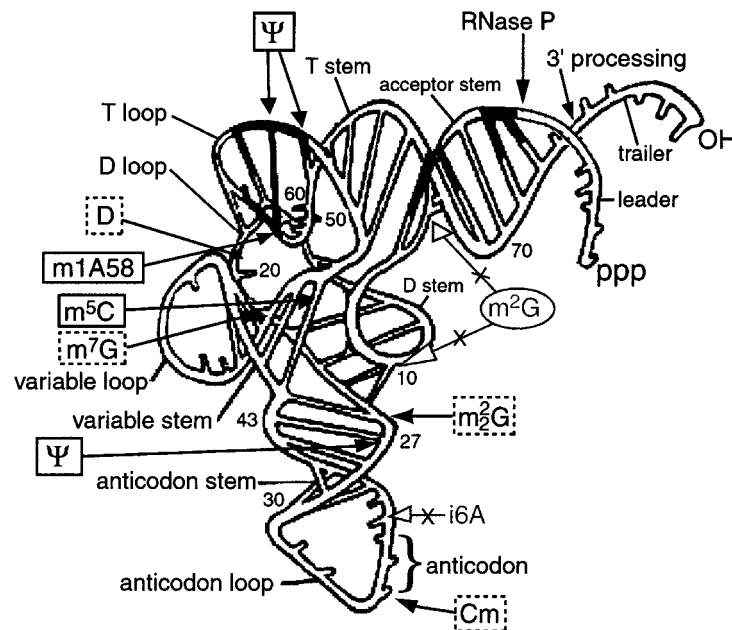
Figure 3. Model of a pre-tRNA with a summary of modifications found on precursors that have not yet had their 5′ leader or 3′ trailer removed, as previously described (50,94). Modifications in solid boxes were found on both pre-tRNA$_e^{Met}$ and pre-tRNA^{Tyr}, modifications in dashed boxes were found on pre-tRNA$_e^{Met}$ (see text), and the m^{2}G6 and m^{2}G10 modifications (in the oval) were not present on pre-tRNA$_e^{Met}$ or pre-tRNA^{Tyr}, but were detected after the 5′ leaders and 3′ trailers were removed from the precursors. The data are consistent with the possibility that stable association with La specifically precludes access of the #6 and #10 position to the m^{2}G modification machinery (see text). The blackened areas on the pre-tRNA backbone depict possible contact points for RNase P as previously reported (68). The model was modified from Krupp (68) and reproduced with permission.

fication enzyme must access the confined inner aspect of the tRNA structure, which may be hindered by La.

While the stable pre-tRNA$_e^{Met}$ was found to contain m$^2_2$G26 as well as two dihydrouridines in the D loop, these modifications were not acquired by pre-tRNA^{Tyr} until after end removal, although they did occur on the intron-containing species (50,94). These findings are not necessarily contradictory if we consider the differences in the two pre-tRNA substrates in the context of their relationship to La. As recently described, pre-tRNA$_e^{Met}$ contains a CACAA motif, near its 3′ terminus, which is closely related to the sequence that constitutes a high-affinity binding site that was identified by RNA SELEX experiments (4,64). Because this CACAA sequence includes the pre-tRNA$_e^{Met}$ 3′ processing site (CA↓CAA), association with La may preclude efficient processing and contribute to the remarkable stability of this precursor (50,64). Thus, we speculate that the m$^2_2$G26 and dihydrouridines found on pre-tRNA$_e^{Met}$ but not pre-tRNA^{Tyr} may be somewhat aberrant due in part to the longer half-life of the pre-tRNA$_e^{Met}$. The presence of m$^2_2$G26 on the pre-tRNA$_e^{Met}$ would further suggest that the stable precursor La-RNP circulates through the nucleus because the m$^2_2$G enzyme appears to re-

side at the nuclear periphery (107,108). Yet, neither of the two m^{2}G modifications on mature tRNA$_e^{Met}$ was detected on the stable precursor (50).

In summary, 12 modifications were found on the stable pre-tRNA$_e^{Met}$ while three additional modifications were found on the mature tRNA$_e^{Met}$: m^{2}G6, m^{2}G1O, and i^{6}A37 (50). In the time course study by Nashimoto and DeRobertis, the i^{6}A37 modification did not appear to be impeded by the 5′ leader or 3′ trailer because the end-processed, intron-containing species also remained unmodified, while the mature tRNA^{Tyr} species contained i^{6}A37 (94). Consistent with this, pre-tRNA$_e^{Met}$ does not contain an intron nor the i^{6}A37 modification that is found on the mature species (50). These observations suggest that failure to acquire the i^{6}A37 modification is probably not due to association with La, and may require another type of downstream signal or compartmentalization.

Although other interpretations exist, the data are consistent with the possibility that stable association with La specifically precludes access of the pre-tRNA G6 and G1O modification sites to the m^{2}G methyltransferase (Fig. 3). Modification studies that employ transcripts corresponding to unprocessed species versus end processed may shed light on the possible influences of La on pre-tRNA modifications (45).

La CHAPERONES OTHERWISE VULNERABLE TRANSCRIPTS

It is becoming clear that La can stabilize certain imperfect pre-tRNAs that might not otherwise endure the maturation process. This is suggested by La's effects on a mutant pre-tRNA$^{Ser}_{CGA}$ that carries a destabilizing mismatch in the anticodon stem (144), a pre-tRNA$^{Met}_i$ that is hypomethylated at A58 (6,20), and a pre-tRNA$^{Ser}_{UGA}$M species with suboptimal base pairing between the anticodon and intron (57). Thus, it seems that La stabilizes Pol III transcripts predominantly at the precursor stage, supporting them through a critical maturation process, while they might otherwise be vulnerable to degradation by exonucleases or other degradative processes (6,20,57, 97,144). The absence of La has also been associated with decreased steady-state levels of several other Pol III transcripts, namely tRNA$^{Ser}_{CGA}$, NME1 RNA, and RPR1 RNA (the latter are components of RNases P and MRP, respectively) when combined with mutants in the nuclear protein, Gcdl0p-Gcdl4p, the 1-methyladenosine (m1A) methyltransferase (7,20). The lack of m1A methyltransferase activity in a $gcd10\Delta$ strain can be overcome by increasing expression of pre-tRNA$^{Met}_i$. This suggests that the tRNA$^{Met}_i$ m1A58 modification is most important for correct precursor processing because the defective unmethylated but otherwise matured tRNA$^{Met}_i$ can function at the ribosome (6,20). Because La can rescue certain $gcd10$-$gcd14$ mutants but not a $gcd10\Delta$ null strain, it may function by supporting increased levels of pre-tRNA$^{Met}_i$ (6,20). It is not yet clear for the class of $gcd10$-$gcd14$ mutants that La can rescue, whether La protects pre-tRNA$^{Met}_i$ and this allows methylation by a low activity (i.e., mutant) methylase, or if La promotes maturation of the unmethylated precursor, because neither the level of methylase activity nor the level of the tRNA$^{Met}_i$ m1A58 modification in the mutant strains has been reported. In any case, it does seem that La protects against degradation (6,20).

Cumulative results indicate that the conserved function of La is to protect transcripts during the time between synthesis and incorporation into more stable RNPs, in some cases facilitating their assembly into mature RNPs and in other cases supporting them during modification; both processes would be expected to increase the stability of the RNA. While the period of transcript vulnerability would appear to begin upon termination of synthesis, when this period ends probably varies for different transcripts. La's participation in the maturation pathways of the U RNAs occurs early in their lifetimes (69,142). While processing by RNase P of a La-associated pre-tRNA is thought to be among the earliest RNA cleavage events in the tRNA maturation pathway (57,141,144), pre-tRNA m1A58 methylation (and other modifications) appears to occur even earlier (50,94) (see Fig. 3). With regard to (iv) from the previous section, data that indicate La also affects pre-tRNA 3′ processing provide further evidence that La chaperones its pre-tRNA ligands extensively, through many parts of their maturation pathways (33,57,144) (Fig. 4).

Because modifications are thought to contribute to the stability of higher order structure, it would be consistent that these occur early in the lifetime of a pre-tRNA. This suggests that the ability of La to support tRNA modifications may explain, at least in part, La's ability to facilitate pre-tRNA folding (144) and is consistent with La's ability to cooperate with tRNA modifying enzymes.

Nascent Pol III Transcripts Are Synthesized in the Nucleoplasm

Newly made Pol III transcripts have been visualized directly and calculated to reside at ~2000 discernible sites (distinct from pol II transcription loci) containing ~5 nascent Pol III transcripts per site in HeLa nucleoplasm (102). Considering the quantity of newly synthesized Pol III transcripts in nuclei, there is a very large discrepancy between this number, 10^4 (102), and the much larger number of La molecules present in HeLa cells, 2×10^7. One consideration that could reconcile some of the discrepancy is that La may remain associated with a pool of transcripts, such that the number of RNAs associated with La is greater than the number of instantaneously labeled transcripts. It was noted that newly synthesized Pol III transcripts appeared unable to move away from their transcription sites, suggesting that a soluble factor that may normally mediate transcript clearance and/or transport was lost from the permeabilized cells (102). Thus, it is plausible that in living cells La would be involved in the clearance of nascent transcripts from their sites of synthesis and that La remains associated with a large reservoir of premature transcripts that would result from multiple rounds of synthesis per gene. This is consistent with the estimate that approximately $1–2 \times 10^5$ molecules of pre-5S RRNA are associated with La in HeLa cells (representing 1–2% of the amount of mature 5S rRNA) (106,123), transcribed from far fewer 5S rRNA genes, the latter estimated to be 2×10^3 per human haploid genome (120). Considering the possibility that an equally large number of precursor tRNAs and other premature transcripts remain bound to La, this would still lead to the conservative estimate that only about one tenth of La molecules would be associated with nascent RNA. Although this estimate is consis-
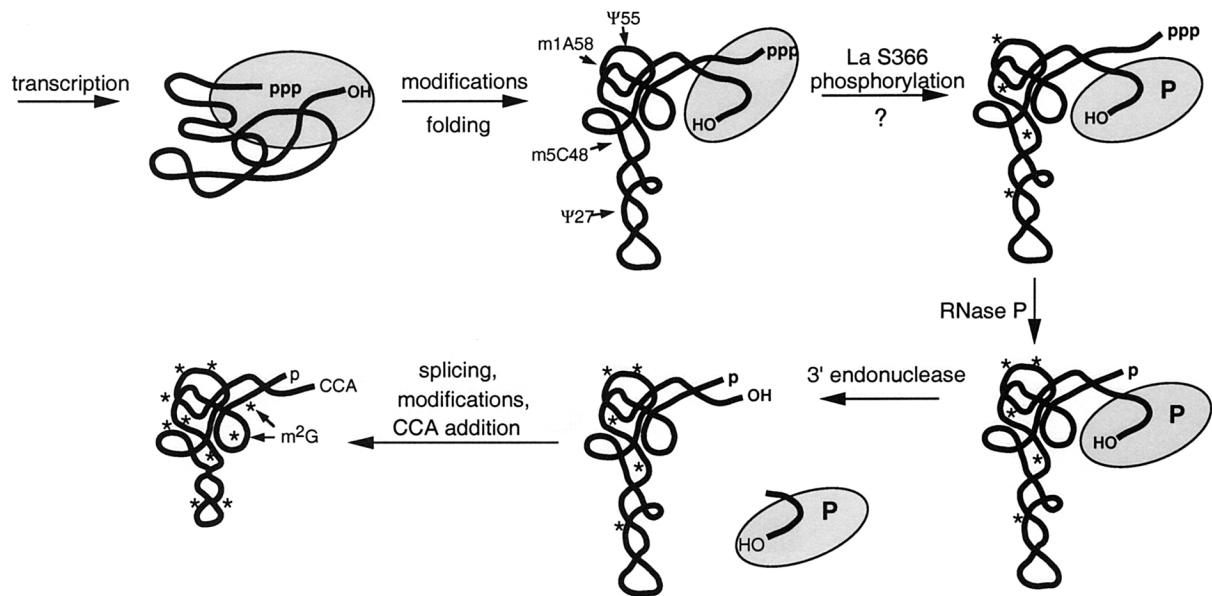
Figure 4. Cartoon view of the maturation pathway of a pre-tRNA. Asterisks represent modifications, some of which occur before end processing and some of which occur later (see text). La is represented by the yellow oval. Although it is unclear whether phosphorylation of serine 366 of human La occurs while associated with RNA or whether, in some cases, it may be phosphorylated prior to RNA binding, it is shown here. In any case, the data indicate that only the phosphorylated form of human La can support efficient cleavage by RNase P (see text). Although casein kinase 2 (CK2) is thought to be the kinase that phosphorylates serine 366 (31), the arrow indicates uncertainty as to *which* kinase mediates this activity.

tent with that of Habets et al., who noted that a substantial fraction of nuclear La may not be associated with Pol III transcripts (48), it is possible that the remaining La is either associated with other RNAs or free.

A point that has not been considered with regard to this issue is that approximately 15% of La in HeLa nuclear extract is unphosphorylated, a form that exhibits RNA binding characteristics subtly distinguishable from the phosphoprotein (33,57). Therefore, determination of the molar amounts of RNA associated with the phosphorylated and unphosphorylated forms of La is an issue that remains to be clarified.

Intracellular Trafficking of La

Although the majority of La is nuclear, a significant amount also resides in the cytoplasm and nucleolus as revealed by immunostaining (29,30,42,52). Most nuclear La appears to be freely soluble and readily distributes between cytoplasmic and nuclear fractions upon cellular disruption and biochemical preparation (48,52,105). Although mammalian La has been shown to reside in part in the nucleolus (22,30,42,52), identifying the determinants responsible for this localization has confirmed and extended our studies of the biology of La (manuscript in preparation). Species-specific differences in intracellular

trafficking and the ability of La to serve as a nuclear retention factor for its RNA ligands have been reviewed recently (83).

Accumulating evidence indicates that the nuclear cytoplasmic partitioning of La can be disturbed under certain conditions, suggesting that subcellular localization may be used to regulate La's activities. Infection with poliovirus and other viruses leads to an increase in the cytoplasmic staining of La [(9,92), also see (18)]. Poliovirus protease 3C cleaves La to leave a 358 amino acid (aa) protein (full length human La is 408 aa) that lacks its C-terminal nuclear localization signal (NLS), and this is associated with decrease of La in the nucleus and increase in the cytoplasm (118). However, because the amount of cleaved La appeared disproportionately lower than the amount of cytoplasmic accumulation (110), the data suggest that protease 3C cleavage alone may not be sufficient to explain the redistribution of La to the cytoplasm. This is consistent with the finding that poliovirus affects nuclear cytoplasmic redistribution in a global manner (134). Induced apoptosis also leads to cleavage of La, at or near residue 374, and is associated with cytoplasmic accumulation (8,110). In this case again, the amount of cytoplasmic accumulation is disproportionately higher than the amount of La that is cleaved, suggesting that the relationship between proteolytic cleavage and cytoplasmic accumulation is complex. Pruijn and colleagues showed that

proteolysis of La, although limited to a fraction of molecules, is associated with dephosphorylation of phosphoserine 366 early in apoptosis, although phosphorylation alone does not appear to affect the nuclear cytoplasmic distribution of La (18,110).

An isoform of La mRNA, distinct from the form that encodes nuclear La, has been identified in lymphocytes (23,128). Some isoforms are tissue specific and when expressed in recombinant form give rise to proteins that distribute differentially to the nucleus or cytoplasm (43,44,54). Intriguingly, these La mRNA isoforms may be differentially expressed during inflammation, apoptosis, or certain viral infections (23).

A Fraction of Nascent Pol III Transcripts Localize to the Nucleolus

Several Pol III transcripts, including pre-tRNAs (12,46,66), U6 snRNA (71), and the RNA components of RNases P and MRP as well as SRP RNA and 5S rRNA have been observed in the nucleolus (12,59–62). Specific proteins known to be intimately associated with most of these RNAs have also been localized in the nucleolus (62,93,101). SRP RNA, RNase P RNA, and U6 snRNA pass transiently through the nucleolus (59,61,71). At least for one of these transcripts, U6, it is almost certain that some of its 2′-O-methylation and pseudouridylation modifications occur in the nucleolus [(37,71,130), reviewed in (83)].

Because components of RNase P, the endonuclease that matures the 5′ ends of pre-tRNAs, as well as pre-tRNAs themselves, have been visualized in the nucleolus (12,59,62), it has been proposed that 5′-pre-tRNA processing activity actually occurs there (67,76,141). Although most of the RNase P RNA and intron-containing pre-tRNAs detectable in yeast appeared nucleolar (12), other studies reveal yeast pre-tRNAs in the nucleoplasm as well as the nucleolus (46, 66,112). In mammalian cells, a substantial amount of RNase P RNA is found in the nucleoplasm (59,72, 88). Because transient nucleolar localization is common to several Pol III transcripts, including RNase P RNA, and associated proteins, pre-tRNAs and RNase P components may each be required to pass through this compartment without necessarily interacting there. In archaea, homologs of snoRNAs are associated with Archaeal fibrillarin and some of these exhibit complementarity to the regions of tRNAs known to be 2′-O-methylated, suggesting that eukaryotic pre-tRNAs may be similarly modified in the nucleolus (95). Some evidence suggests that other tRNA modifications may occur in the nucleolus (126). Indeed, our preliminary results in fission yeast,

using a La-dependent opal suppressor tRNA system, suggest that passage through the nucleolus has no effect on efficient 5′ processing by RNase P (manuscript in preparation). In summary, while it is possible that RNAse P functions in pre-tRNA processing in the nucleolus, the evidence for this is circumstantial and alternative explanations may account for the localization of pre-tRNAs and RNAs P components there.

What Is La Doing in the Nucleolus?

As alluded to above, earlier reports that La resides in part in the nucleolus have been confirmed by multiple approaches including direct visualization of La-GFP fusion proteins and colocalization with known nucleolar proteins (manuscript in preparation). Because pol III transcripts pass through the nucleolus during what appears to be an early phase of their life cycle, it might seem that La would be involved in their nucleolar transit perhaps to chaperone and/or protect them in this compartment. However as recently reasoned (83), this need not be so for several of the RNAs (5S, MRP, RNase P, SRP) as other nucleolar-localizing proteins have been identified that interact with these RNAs and may be better candidates for their nucleolar localization. However, for other Pol III nascent transcripts (e.g., pre-U6 RNA and pre-tRNA) there are no known proteins other than La that associates with them that are also found in the nucleolus.

Indeed, as reviewed recently, there is indirect evidence suggesting that La associates with pre-U6 snRNA in the nucleolus (83). The small nucleolar (sno)RNAs and other factors that direct 2′-O-methylation and pseudouridylation of U6 snRNA almost certainly do so in the nucleolus (37,130). Precursors to tRNAs and U6 snRNA that are associated with La contain methylated bases, pseudouridine, dihidrouridine, and some 2′-O-methylated ribose. For the subset of U6 snRNA precursors found associated with La, fingerprint analysis suggested the presence of several residues modified in U6 (105), which recent data strongly imply are modified in the nucleolus (37,130). Specifically, several 2′-O-methylated and pseudouridylated U6 nucleotides that were recently shown to be directed by specific snoRNAs (37,130) appear to be present in La-associated pre-U6 RNA. This was evidenced by co-migration of the corresponding oligonucleotides isolated from La-associated pre-U6 snRNA with the known modified oligonucleotides isolated from mature U6 snRNA (although some of the La-associated oligonucleotides did appear to be undermodified, providing additional evidence of their precursor status) (105). U6 snRNA

has recently been shown to transiently pass through the nucleolus (71). The cumulative data provide compelling yet indirect evidence that La travels with pre-U6 snRNA to the nucleolus.

As noted earlier, yeast La also associates with a U3 snoRNA intermediate and it is possible that La hands off this transcript to the next proteins in the U3 RNP assembly pathway in the nucleolus (69). Because biochemical evidence suggests that a fraction of La may be associated with a subset of small ribosomal subunits (100), we should also consider the possibility that La may join those subunits in the nucleolus. A fraction of La was observed in the nucleoli during the G1-early S phase of synchronized cells (29). It is noteworthy in this regard that Cfi1/Net1 sequesters Cdcl4 in the nucleolus during this time, contributing to the fidelity of the timing of mitotic exit (133). Although cumulative findings suggest several roles for La in the nucleolus, more definitive data are required before the function of nucleolar La is understood.

CONCLUSIONS AND PERSPECTIVES

La is a ubiquitous nuclear phosphoprotein that is found associated with the large variety of RNA ligands that end with 3′ terminal uridylates, the latter of which comprises the principal binding site for the conserved NTD of La proteins. The most clear and ubiquitous function of La is that it facilitates the expression of Pol III transcripts by stabilizing the nascent precursors that would otherwise be vulnerable to 3′ exonucleases and/or other degradative enzymes. Accumulating evidence suggests that La maintains close contact with its transcripts during several individual processing and/or modification events that involve transient interactions of the La-associated RNA with other factors. This proposal appears most reasonable for pre-tRNA modifying enzymes and RNase P. The yeast tRNA modifying enzyme, m1A58 methyltransferase, which acts early in tRNA maturation pathways, has been shown to cooperate with La in the expression of $tRNA_i^{Met}$, and La also appears to cooperate with RNAse P. La has also been described

as a chaperone that may help with RNA folding; because some modifications appear to limit the conformational flexibility of tRNA (i.e., stabilize certain isoforms over others), it is possible that La may affect tRNA folding in part by its ability to cooperate with tRNA modifying enzymes and support pre-tRNAs during modification. It is also becoming clear that La can serve as a nuclear retention factor for its RNA ligands.

Structure–function and molecular modeling analyses of a growing number of La protein sequences have strengthened the idea that a tandem pair of RRMs comprise the conserved NTD of La and work together to bind RNA. While the NTD recognizes the UUU-OH 3′ terminal motifs of nascent Pol III transcripts, the CTD recognizes the 5′-ppp end region that results from transcription initiation. The 5′-ppp end recognition activity of human La can be modulated by phosphorylation of S366. Although it remains unknown whether the yeast La phosphoproteins also exhibit 5′-ppp RNA recognition, the signal transduction function of La would appear to be conserved because human La is faithfully phosphorylated in fission yeast and this modification is transduced in the yeast to control tRNA processing and maturation (57). The challenge for the future is clear: to understand how the bipartite mode of nascent RNA binding, intracellular trafficking, and the signal transduction functions of La are integrated with the pathways of expression of the variety of small nuclear and nucleolar RNA precursors with which it associates. An additional challenge is to understand how the signal transduction through La may be integrated with other aspects of intermediary metabolism and cellular proliferation.

ACKNOWLEDGMENTS

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