

MINIREVIEW

Recognition of Nascent RNA by the Human La Antigen: Conserved and Divergent Features of Structure and Function

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La is a conserved RNA-binding phosphoprotein that interacts with a large variety of ligands. The most ubiquitous function of La is association with newly synthesized RNA polymerase (Pol) III transcripts via their common UUU-OH 3' termini and stabilization of these against exonucleolytic digestion. Accumulating evidence also indicates an activity for La in internal ribosome entry site-mediated translation in mammalian cells and in the metabolism of a subset of 3'-processed snRNA intermediates that end in uridylates but are synthesized by Pol II. The most highly conserved region of La resides in the N-terminal domain (NTD), and this appears to mediate high-affinity UUU-OH recognition. As critically reviewed here by comparison to a consensus core RNA recognition motif (RRM) structure, the NTD can be modeled into a pair of tandem RRM. In addition to the conserved NTD, human La (hLa) protein contains a C-terminal domain (CTD) that harbors a third RRM and a potential Walker A motif that appears to recognize the 5'-ppp ends of nascent RNAs. The resulting bipartite mode of RNA binding can account for previously unexplained observations and may underlie a unifying principle of La function. While a role for hLa in transcription remains controversial, its presence in a Pol III holoenzyme suggests a role reminiscent of the CTD of Pol II, as an integrator of transcriptional and posttranscriptional activities that include 5'- and 3'-RNA metabolism. Evidence that the 5'-end-RNA recognition activity of hLa can be modulated by phosphorylation provides mechanistic insight into the signal transduction function of this protein.

La was first described as a human autoantigen more than 20 years ago and has since been identified in many eukaryotes from yeasts to humans (79, 143). La has been implicated in several cellular and viral RNA-associated processes, the most ubiquitous of which is the binding to and stabilization of newly synthesized RNA polymerase (Pol) III transcripts (6, 17, 41, 44, 58, 67, 88, 103, 143, 153, 154). These transcripts are the precursors to tRNAs, 5S rRNA, U6 snRNA, 7SL (SRP) RNA, 7SK snRNA, hY scRNAs, 4.5S I RNA, B1-Alu, Alu RNAs, and other RNAs (24, 43, 76, 81, 86, 89, 113–115). Binding occurs via the common UUU-OH 3'-terminal motif which results from transcription termination within the Pol III ter-

mination signal, oligo(dT) (58, 89, 113, 115, 136). As a result of UUU-OH binding and other activities, human La (hLa) also appears to function in transcription termination and reinitiation by Pol III (29, 42, 44–47, 87, 88), although this remains controversial (43, 82, 146, 154).

La proteins have been differentially expanded in the mass and domain complexity of their C-terminal domains (CTD) and utilize different pathways of nuclear import in different species. La protein of the yeast *Saccharomyces cerevisiae* is imported into the nucleus via a pathway that is different from the pathway used by the fission yeast, *Schizosaccharomyces pombe*, and higher eukaryotes (117). The type of nuclear localization signal (NLS) as well as the transport factors that mediate nuclear import of *S. cerevisiae* La is different from the NLS type and the import factors used in fission yeast and other eukaryotes (117). Thus, the NLS resides in the conserved N-terminal domain (NTD) of *S. cerevisiae* La but has been relegated to the CTD in all other eukaryotes examined, removed from the RNA-binding domain (117). It was suggested previously that this reorganization was associated with the acquisition of additional activities of La during evolution (117). In any case, it should be kept in mind that, while all identifiable La proteins share a highly conserved NTD, the *trans*-acting factors that recognize these proteins and their biological functions may differ significantly.

hLa is a 47-kDa phosphoprotein that is present at approximately 2×10^7 molecules per cell, mostly nuclear, although nucleolar (11, 36, 37, 48, 58) and cytoplasmic (54, 58, 93) localization has also been documented. The determinants in La responsible for nucleolar localization have not been elucidated (36, 37, 48, 58). Most La is extractable in a readily soluble form while a significant amount appears to be tightly associated with the nuclear matrix or other complexes (54). In addition to an NLS in the CTD, La also contains sequence elements that retain it in the nucleus (131). Accordingly, La can mediate transport of some RNAs into the nucleus and, for these and others, can retain them there, provided that they retain their 3' UUU termini (15, 49, 132). Recently, *Euplotes* telomerase holoenzyme was found associated with a protein homolog of La. Since *Euplotes* telomerase RNA is a Pol III product that retains its UUU terminus as a small nuclear RNA, this La homolog may function as an ancillary factor to chaperone or retain telomerase RNA in the nucleus (reference 2 and references therein).

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In addition to nascent Pol III transcripts, La also associates with other small RNAs. The first of this class to be described were the leader transcripts generated from vesicular stomatitis virus (78), although there are now reports of binding to the 5' regions of the transcripts of many viruses (see below). Accumulating evidence indicates that La also associates with precursor intermediates of U1 to U5 snRNAs as well as U3 snoRNA, which are synthesized by Pol II (77, 85, 150). Apparently, the ends of these intermediates are exonucleolytically trimmed to expose 3' uridylates prior to La binding (77, 85, 150). La was also found associated with an intermediate in a histone mRNA decay pathway whose 3' end was processed at a position that would be expected to expose terminal uridylates (91). Thus, for many if not all of the above transcripts, binding appears to depend on La's best-known RNA-binding activity, recognition of 3'-terminal uridylates.

Many if not all of the above transcripts would presumably also share a triphosphate moiety on their initiating ends. Phosphatase treatment, which would remove the 5' phosphates, has been shown to decrease La's affinity for UUU-OH-containing pre-tRNA in a functionally relevant manner (41). Direct binding as well as RNase P protection assays using a panel of mutant La proteins suggests that recognition of the 5'-ppp-RNA motif may be mediated by a Walker A motif (WAM) in the CTD of hLa (41). The modulation of this RNA recognition activity by phosphorylation and its apparent role in RNA expression in vivo (41, 67) will be reviewed below.

The hLa antigen. Autoantigenic epitopes correspond to three major regions of the hLa protein, RRM-2 > RRM-1 > CTD (22, 25), and subpopulations of antibodies directed to these can vary during the course of autoimmune disease (23, 135). It should be noted that autoantigenic epitopes can differ from epitopes recognized during experimental immunization. La is targeted for proteolysis during infection by certain viruses and in the early phase of apoptosis, and recent evidence suggests that this may result in its autoantigenicity in some patients (19, 118). Accordingly, proteolytically exposed epitopes recognized during this phase of the immune response may be inaccessible in the native protein. Although some anti-La sera recognize more than one antigen, monospecific sera, e.g., in recognition of a single antigen by immunoblotting and/or other assays, are also available (e.g., see references 25 and 58).

The highly conserved NTD of La can be modeled into a tandem RRM domain. After cloning of the cDNAs encoding La and other RNA-binding proteins, La was recognized as a founding member of a family of RNA-binding proteins that contain one or more versions of a domain known as the RNA recognition motif (RRM; also known as the RNA-binding domain) (22, 26, 112). A hallmark of the RRM is the presence of two short sequence motifs, RNP-2 and RNP-1 (1, 73, 119). Although attempts in multiple labs have not yet led to a crystal or nuclear magnetic resonance structure for La, the structures of other proteins have revealed the RRM as a globular domain with a $\beta 1$ - $\alpha 1$ - $\beta 2$ - $\beta 3$ - $\alpha 2$ - $\beta 4$ topology (31, 38, 55, 61, 102, 122). In the RRM structure, the four β strands form a β sheet that contacts RNA on one surface while the other surface is packed by the two α helices, contributing to a hydrophobic core structure. The two RNP motifs occupy central locations on the β sheet, one each on the two internal β strands (RNP-2 on $\beta 1$ and RNP-1 on $\beta 3$). Some of the amino acids in the RNP motifs

are oriented toward and contribute to the hydrophobic core, while others face outward as solvent-exposed residues involved in RNA recognition. Residues in the loops that connect the strands and helices have also been found to contact RNA (95, 144). RRM-containing proteins that harbor atypical RNP-1 and/or RNP-2 motifs are also known, although these can be more difficult to identify by sequence search programs, the majority of which presently rely on the RNP motifs (14, 73). For example, the polypyrimidine tract-binding protein contains four RRMs with little similarity to the prototypical RNPs (31, 73). Several proteins harbor multiple RRMs, each comprising a globular domain separated by a linker (31, 38, 55), the length of which can be a determinant of RNA binding cooperativity between the RRMs (123). In the solved structures of some of these, adjacent RRMs form a trough or binding cleft for their RNA ligands and therefore appear to form one functional RNA-binding unit from two RRMs (4, 38, 55).

The highest degree of identity among all known La homologs is concentrated in a region that corresponds to residues 20 to 92 of human La (121, 143, 153). Kenan's proposal 5 years ago that this region would adopt an RRM structure (72) is critically reviewed here with the aid of several new La sequences from diverse organisms, a program that predicts secondary structure from a set of aligned sequences, and, most importantly, comparison to a consensus RRM core structure that was previously derived from 70 RRM sequences. Thirteen sequences representing the La proteins of plants, trypanosomes, yeasts, insects, amphibians, and rodents and other mammals, including humans, allow us to identify the invariant and highly conserved residues in these sequences and examine the potential for RRM structure (Fig. 1). Although the hLa structure is represented by three RRMs in addition to other motifs, only the putative RRM-1 will be reviewed in detail here since this is the most highly conserved region and, as will be discussed below, appears to be involved in UUU-OH recognition. Alignments of RRM-2 and, for metazoans, RRM-3 have been reported previously (8, 14, 73, 83, 117, 121, 143, 155). In the alignment in Fig. 1, invariant residues are in black rectangles, with the amino acid (single-letter designation) in white. Conserved residues, i.e., side chains with similar chemical groups, are in gray rectangles.

The uppermost line of Fig. 1, labeled "QL w/hom," reflects the output of a program that predicted the secondary structure elements of the set of 13 sequences in the alignment below it. In this output, H indicates α helix, E indicates extended β strand, and C indicates nonhelix and nonstrand. Below this line are indicated the proposed β strands, α helices, and connecting loops for La RRM-1 according to the RRM topology (14). This reveals general correspondence between the predicted structure of the α helices, β strands, and connecting loops and the consensus RRM topology. A noticeable lack of correspondence was found only in the $\beta 3$ region in which no extended β strand was predicted for the set of sequences. However, it must be noted that examination of the individual La sequences by a sister program (QL without homologs; see Fig. 1 legend) revealed one or more predicted extended β strands for six of the La sequences, those from *Homo sapiens*, *Bos taurus*, *Rettus norvegicus*, *Mus musculus*, *Aedes albopictus*, and *Trypanosoma brucei* (i.e., one less than required for consensus), in the $\beta 3$ region (data not shown). Therefore, it is highly possible that

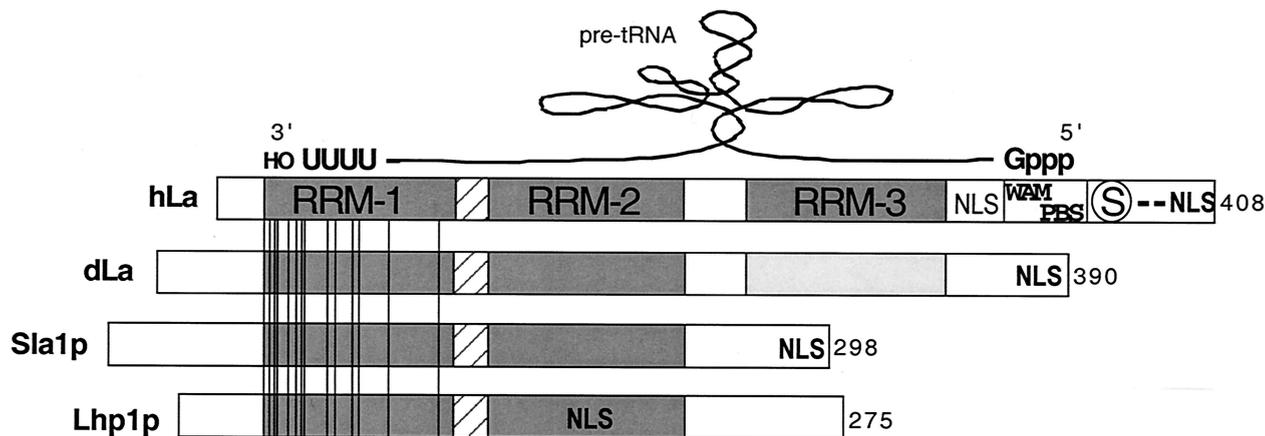


FIG. 2. Schematic representation of hLa and its apparent mode of bipartite interaction with a model precursor tRNA and comparison to three other La proteins. Cartoon alignment of the La proteins of human (hLa), *D. melanogaster* (dLa), *S. pombe* (Sla1p), and *S. cerevisiae* (Lhp1p), representatives of four phylogenetic branches according to the work of Rosenblum et al. (117). Note that these represent four polypeptides that have been characterized as UUU-OH-binding proteins that associate with nascent Pol III transcripts in vivo (83, 136, 139, 153). Their lengths, in amino acids, are indicated at the right. RRM-1, -2, and -3 domains are shaded and labeled for hLa only. Invariant residues in the 13 sequences are shown as vertical lines; note that these are limited to RRM-1. The invariant residues (numbered according to hLa) are Q20, E22, Y24, F25, N29, D33, F35, L36, G45, V47, F55, R57, A71, and R91. The linker between RRM-1 and RRM-2 is hatched. A putative WAM and PBS and serine 366 (encircled S) are shown (see text). Positions of the NLS are indicated in boldface according to reference 117. A consensus NLS in hLa, as noted previously (131), is shown in lightface. A schematized pre-tRNA has been imposed above the hLa cartoon to illustrate its proposed orientation according to a model based on prior work (41, 67) as described in the text.

(14). It is also noteworthy that the mammalian La proteins all contain V at position 67, precisely matching the V in the RRM core, and that all of the vertebrate La proteins contain a phenylalanine (F65 in hLa) in the $\beta 3$ region. Lack of invariant residues in the predicted $\beta 3$ would suggest that any contacts with RNA might be through the main chain protein backbone (or via stacking) which is known to occur for $\beta 3$ (31, 102). A71, which is invariant in all La proteins, as well as the all-hydrophobic amino acids at positions 72, 79, 80, 82, and 89, would also be expected to contribute to the core structure (Fig. 1) (14, 73). Curiously, the nearly invariant S75 of hLa (T in *Arabidopsis thaliana*) occupies a position predicted at the beginning of $\alpha 2$, precisely where a serine resides in RRM-1 of the Sex-lethal protein (55). In addition to conservation of hydrophobic core residues, the superimposition of the invariant residues in and around $\beta 1$ and loop 1, as well as $\beta 2$ -loop 3 and $\beta 4$, onto this core RRM structure suggests the potential for RNA recognition as indicated by comparison to documented RRM-RNA structures (see Fig. 1 in reference 31).

In summary, the analysis represented by Fig. 1 suggests that the most conserved region of the La protein (Q20 to S92; numbered according to *H. sapiens* La) can adopt an RRM fold (14). Three characteristics support this: (i) invariant and highly conserved hydrophobic residues that are positioned to provide a core RRM structure; (ii) predicted topology for five out of six of the α helices and β strands, as well as the connecting regions found in other RRMs; and (iii) invariant and highly conserved residues positioned in regions known to participate in RNA recognition in other RRMs.

A phylogenetic tree developed from a prior sequence alignment of nine La homologs revealed four major branches (117). Figure 2 shows a schematic alignment of four La proteins that have been characterized as bona fide UUU-OH-binding proteins (83, 136, 139, 153), each representing one of the four

major branches of the La phylogenetic tree (117). In this schematic, the thin vertical bars connecting the four proteins represent the 14 invariant residues identified in our 13 sequences. As shown above, all of the invariant residues reside in the RRM-1 region. The region corresponding to aa 110 to 190 of hLa represents a canonical RRM, and its conservation in La proteins from diverse organisms has been noted elsewhere (73). This RRM, designated RRM-2, is connected to RRM-1 by a linker of 15 aa in all vertebrates and insects, and 12, 10, and 8 aa in *A. thaliana*, *S. pombe*, and *T. brucei*, respectively. Thus, RRM-1 and -2 are arranged in tandem in La proteins, with the first residing close to the N terminus (133). Despite the overall conservation of RRM-2 in the La sequences, which includes identifiable RNP-1 and RNP-2 motifs as well as other conserved residues (23, 79, 83, 121, 140, 143, 153), no invariant residues were found in this region. This degree of conservation is consistent with the idea that RRM-2 might contribute to overall affinity for RNA more so than uridylyate recognition per se (see below).

Features of UUU-OH recognition. Although La will bind to almost any RNA (as will most RRM proteins), affinity increases as the number of terminal uridylylates increases to a maximum of four (136). While as few as one or two terminal U's can contribute to La binding, three and four U's constitute progressively more stable interactions, demonstrating that the optimal number of U's for La binding matches the template requirement for termination by Pol III (136) and, accordingly, the number of terminal U's at the 3' ends of nascent Pol III transcripts (47, 90, 115, 156).

Alteration of the terminal 3'-OH group to a phosphate has been shown to decrease RNA binding by yeast, insect, amphibian, and human La proteins (136, 139, 153). The high degree of conservation of this unique feature suggests (i) that it is critical to function and (ii) that some of the invariant residues of La

might contribute to a specific binding site for the terminal ribose 3'-OH, which cannot as easily accommodate a phosphate group. Therefore, although predictions indicate an RRM as a likely structure in the NTD, the actual structure would be expected to exhibit two features to accommodate the unique mode of RNA binding by La: (i) recognition of three to four U's specifically and (ii) sensitivity to the state of the 3' group on the terminal ribose.

In the context of hLa, high-affinity RNA binding requires RRM-1 and RRM-2, as RRM-1 alone does not bind RNA (28, 44, 72). Deletion of the most N-terminal residues of RRM-1, as in the mutants hLa 22-408 and 26-408, decreases affinity for RNA (28, 44, 72), although this can be overcome by increasing the concentration of hLa in the binding reaction mixture (44). Since deletion of N-terminal amino acids from La leads to a dramatic loss of binding to UUU-OH-containing RNAs and since the UUU-OH motif comprises a high-affinity binding site for La, it would appear that the N-terminal amino acids in La mediate UUU-OH binding. This reasoning is consistent with experiments that showed that, even at very high concentrations, hLa lacking only the first 25 aa of RRM-1 binds to RNA but cannot efficiently protect nascent UUU-OH-containing RNAs against 3'-exonuclease digestion while the full-length protein can do so at lower concentrations (41, 44). The data suggest that, while RRM-1 does not provide high-affinity binding on its own, it contributes significantly to UUU-OH recognition, consistent with a contact surface of 4 to 6 nucleotides per RRM (38, 55, 122). RRM-2 would serve to increase affinity for RNA, although a role in uridylyte recognition cannot yet be ruled out for RRM-2 (28, 44, 72). Thus, it would appear as if RRM-1 and RRM-2 of La work as a pair of RRMs to mediate high-affinity binding to nascent RNAs that contain UUU-OH.

It is interesting to note the crystal structure of *Drosophila melanogaster* Sex-lethal (Sxl) protein complexed with its target ligand, the UGUUUUUUUU sequence of *tra* precursor mRNA (55). In the cocrystal structure, the first of a tandem pair of Sxl RRMs recognizes the 3' region of the target RNA, which is composed exclusively of U residues, while the second RRM recognizes the 5' nucleotides of the target ligand. Among the contacts with the uracil bases are hydrogen bonds formed by Asn in RNP-2, as well as Gln, Asn, and other residues in Sxl RRM-1 (55). It is noteworthy to recall here that, in addition to the invariant aromatic residues within the RNP-2 motif of La RRM-1, RNP-2 is also flanked by two invariant amino acids, Gln20 and Asn29, residues capable of side chain hydrogen bonds to uracil-specific nucleotides (55). The effect of deleting Gln20, as in hLa 22-408 and 26-408, a profound loss of RNA binding and RNA 3' protection, is consistent with a role for this invariant residue in UUU-OH recognition. The arrangement of Sxl, namely, that RRM-1 of a tandem RRM protein recognizes the 3' U-rich end of its ligand and confers U-specific recognition while RRM-2 recognizes the 5' end of the ligand, is similar to the UUU-OH binding arrangement proposed for hLa (Fig. 2) (72).

UUU-OH recognition and biological function. As noted earlier, yeast La associates with certain Pol II-synthesized RNA intermediates via their terminal uridylytes (77, 150). Although binding of La to a U1 snRNA precursor in HeLa cells was previously documented (85), it will be important to determine whether La binding to the Pol II snRNAs is more prevalent in

yeast than in other eukaryotes. Since *S. cerevisiae* snRNA intermediates do not appear to cycle through the cytoplasm for maturation as they do in mammalian cells (see Discussion in reference 150) and since *S. cerevisiae* La uses a different nuclear import pathway from that of mammalian cells (see above), the nuclear retention-transport function of La and involvement in snRNA metabolism could have evolved differently in yeasts and higher eukaryotes (49).

While Pol III transcription is not a prerequisite for association with La, a transcription termination signal that encodes the UUU-OH motif ensures that all Pol III transcripts will be bound by La (136, 139, 153). Pol III termination-dependent positioning affords La the opportunity to serve as a primary determinant in the posttranscriptional processing and/or the ordered assembly of these transcripts into specific ribonucleoproteins (24, 27, 53, 56, 86, 137, 139). Identification of the yeast homolog of La set the stage for a most important phase of La research, genetic manipulation and elucidation of function *in vivo* (83, 153). With regard to Pol III transcripts, an essential tRNA^{Ser} gene with a mutation that would disrupt base pairing in the anticodon stem of the tRNA was selected in a synthetic-lethal screen of La-deficient *S. cerevisiae* (154). In this case, the defect was at the level of pre-tRNA stability and processing. Another mutation isolated by the synthetic-lethal screen was in Lsm8p, a polypeptide component of a U6 snRNP (103). In this case, U6 precursors were unstable and inefficiently incorporated into U6 RNPs. Independent genetic analyses that are sensitive to alterations in tRNA expression revealed a role for La in stabilizing nascent pre-tRNA^{Met} (6, 17). These and another *in vivo* study indicate that La acts to stabilize various Pol III transcripts at the precursor stage only, maintaining them through a critical maturation process, after which time the mature species can accumulate and function in the absence of La (6, 17, 67, 103, 154). The necessity for this type of precursor-specific stabilizing activity may be explained by the possibility that, while some sequences may be optimal for function in the mature transcript, these same sequences may be suboptimal for stability in the context of a longer precursor transcript. Alternatively, precursor transcripts may contain sequences that target them for digestion that are masked by La. In any case, it appears that La serves as a molecular link between Pol III termination and posttranscriptional processing and as a chaperone that protects nascent transcripts during the time between synthesis and maturation (103). Stabilization by La could also provide a quality control function that would ensure that RNA-modifying enzymes or other factors (e.g., those that influence transcript stability) would have ample time to act on the precursor substrates (41).

Emergence of an accessory CTD in metazoan La proteins. As mentioned in the introduction, an NLS was relegated from the N-terminal RNA-binding domain to the CTD, and the domain complexity of the CTD of La was expanded during evolution (Fig. 2). A third RRM, represented by aa 220 to 300 of hLa, was apparently acquired by the metazoan La proteins; this motif was identified as part of a general search of the protein database for atypical RRMs (14). The RRMs of this class were found to be enriched in metazoan proteins involved in RNA metabolism (14), consistent with the absence of RRM-3 in the yeast La proteins Sla1p and Lhp1p (Fig. 2). The RRM-3 is highly conserved in the vertebrate La proteins, while

the three insect, one plant, and one trypanosome sequences exhibit significantly less similarity in this region. Functional involvement of RRM-3 in pre-tRNA processing has been demonstrated elsewhere for hLa (67).

The most significant variability among the vertebrate La proteins is in the region beyond RRM-3. Residues 316 to 332 of hLa conform perfectly to a consensus bipartite NLS, the sequence of which is well conserved only in the vertebrates. This is followed by a basic region (aa 328 to 363) in which 40% of the residues are positively charged, an acidic region (aa 366 to 390), and a terminal NLS (Fig. 2). The C-terminal NLS of hLa (aa 390 to 402) is functional, while the more fitting bipartite consensus NLS at aa 316 to 332 of hLa appears nonfunctional or masked in *Xenopus laevis* oocytes, although the effect of the consensus NLS at aa 316 to 332 was not examined in tissue culture cells (131).

Identification of a WAM, ³³³GRRFKGKN₃₄₀, conforming to the consensus A/GXXXXGKX in hLa suggested the potential for ATP binding and helicase activity (140), although recent results suggest that this motif is not involved in helicase activity but is involved in 5'-ppp-RNA recognition (Fig. 2; also see below). A sequence tract that matches this consensus WAM is found in all vertebrate La proteins but not in those from other species. The rodent sequences contain a 16-aa expansion immediately upstream of the WAM. Farther downstream lies the major phosphorylation site of hLa, S366 (16, 42), which accentuates a transition from basic (aa 328 to 363) to acidic (aa 367 to 375) residues (42). The identity of the residues comprising and/or surrounding the S366 phosphorylation site in hLa exhibits significant differences in other species, although the residues are generally conserved (42). Sequences downstream of the acidic region of hLa are expanded in amphibian La (121). In summary, while the NTD of La is highly conserved, the CTD is not, being largely absent in yeasts and differentially expanded in metazoans.

A potential WAM in the CTD of hLa and its possible involvement in recognizing the 5'-ppp end of a nascent transcript. The unexpected ability of hLa to protect various precursor tRNAs from 5' processing led to a structure-function analysis that suggested that the WAM in the CTD could interact with the 5'-ppp end of a nascent transcript (41). This mode of interaction was suggested by an RNase P protection assay as well as direct binding studies that examined a collection of mutant La proteins and various forms of pre-tRNAs (41). Specifically, digestion of the RNA with phosphatase, which would remove the 5'-terminal phosphates, decreased interaction with La as monitored by direct binding and RNase P protection assays (41). 5'-ppp binding may also explain Stefano's observation (136) that even his best binding substrates (i.e., 5'-p-tRNA-UUUU-OH) formed complexes with La that were less stable than bona fide tRNA precursors. Thus, it was concluded that "the constructs used...lack some feature(s) recognized by the La protein on naturally occurring RNAs" (136). It must now be suspected that one outstanding feature was a 5'-ppp, which was missing on the mature 5'-p-tRNA-UUUU-OH ligand used in that study.

Interestingly, the BLOCKS program identifies aa 348 to 368 of hLa as a potential phosphate binding site (PBS), just downstream of the WAM, that is rich in conserved basic residues (59). The possibility that both of these motifs, the WAM and

the putative PBS, contribute to 5'-ppp-RNA recognition in vivo is consistent with functional analyses in which hLa constructs lacking either of these regions, residues 328 to 344 or residues 345 to 363, cannot stabilize pre-tRNA against 5' processing (67). We envisage that the WAM may recognize the initiating base, which is usually a purine in Pol III transcripts (58, 138, 157), while the PBS may recognize the 5' phosphates.

Although it was thought that the WAM would be involved in the helicase-ATPase activity that had been noted elsewhere for La (7, 65, 149), it was recently documented that double-stranded nucleic acid binding by La does not require either the WAM or a putative double-stranded RNA (dsRNA)-binding motif, and La appears not to unwind dsRNA (70). ATP hydrolysis has never been reported for La, and attempts to demonstrate this using purified La protein have been unsuccessful (72). Thus, while WAMs in other proteins contribute to ATP binding, it is tempting to speculate that, in hLa, this motif instead has been recruited to recognize the 5'-pppG/A moieties found at the initiating ends of nascent Pol III transcripts (41, 67).

A two-domain structure, as previously proposed for hLa (23), would allow bipartite RNA binding; the conserved NTD can interact with the UUU-OH 3' end of the RNA and the CTD can interact with the 5'-pppG/A end, both of which constitute unique determinants on all nascent Pol III transcripts. The predicted structures of many Pol III transcripts would place the 5' and 3' termini in close proximity, apparently well suited for bipartite interaction with La. According to the bipartite binding model, hLa would bind principally to the terminal regions of a pre-tRNA while leaving the body of the tRNA accessible to other factors, perhaps somewhat similar to what has been visualized elsewhere for EF-Tu and tRNA (41, 99, 100). That other proteins and/or modifying enzymes can recognize the RNA components of nascent RNA-La RNPs is indicated by modifications found on pre-tRNAs that coimmunoprecipitate with La (58).

A conserved role in precursor tRNA processing and maturation. Nascent pre-tRNAs must undergo 5'-end processing by RNase P, 3'-end processing, splicing (if befitting), base modifications, 3'-CCA addition, aminoacylation, and nuclear export prior to function in the cytoplasm (52, 64, 148). Various aspects of tRNA maturation, including splicing, aminoacylation, and nuclear transport, have been reviewed recently (52, 64, 148). Many of the tRNA maturation-related activities may be interrelated (66, 120, 124, 125) and/or integrated with other aspects of gene expression; for example, accumulation of unprocessed pre-tRNAs can induce a nutritional stress response in yeast, and independently, the unfolded protein response is controlled by a key catalytic component of the tRNA splicing machinery (32, 111, 128–130). As the first protein that interacts with pre-tRNAs and serves to determine their metabolism, the La phosphoprotein appears to have been integrated into some of these pathways.

La's role in the expression pathways for tRNAs has been examined in recent studies (6, 17, 41, 67, 143, 154). Removal of the pre-tRNA 5' leader is performed by RNase P, and in many cases, this appears to be the first step in the tRNA maturation pathway (5, 148). 3'-end formation can occur either by an endonuclease or by one or more exonucleases (21, 80, 97, 110, 154). The robust exonucleolytic digestion of the 3' ends of

nascent Pol III transcripts that occurs in La-depleted extracts and in La⁻ cells is prevented by La (41, 44, 82, 88, 154; also see reference 110). In the case of pre-tRNAs, protection from 3'-exonucleolytic digestion might be sufficient to promote default processing by the pre-tRNA 3' endonuclease, although recruitment of the endonuclease by La is also possible (154).

As mentioned above, the CTD of hLa unexpectedly interfered with 5'-end maturation of pre-tRNA (41), surprising because 5' cleavage is a necessary step in tRNA maturation. The finding that S366 phosphorylation could reverse the inhibitory effect of hLa and render the pre-tRNA susceptible to 5' processing by RNase P reconciled this paradox and suggested a mechanism by which tRNA maturation might be regulated (41). To examine whether this activity might be operational in vivo, a genetic system was developed using alleles of a nonessential tRNA^{Ser}UGA opal suppressor that suppress *ade6-704* and the accumulation of red pigment in fission yeast (67). This allowed examination of the expression pathway of a nonessential tRNA reporter gene by a colorimetric assay in vivo. Results obtained with this system confirmed the observations of Wolin and colleagues that hLa could substitute for fission yeast La in its ability to protect nascent pre-tRNAs from 3'-exonucleolytic digestion (143). However, hLa constructs bearing nonphosphorylatable residues at position 366 were found to block pre-tRNA processing at the 5'-end maturation step and were unable to support tRNA maturation and suppression (67, 143). Biochemical analyses confirmed that hLa is faithfully phosphorylated on S366 in fission yeast and allowed the conclusion that this modification promotes tRNA^{Ser}UGA maturation (67).

Since RNase P components, their pre-tRNA substrates, and La have each been localized to the nucleolus as well as the nucleoplasm, a challenge is to determine whether phosphorylation-mediated control of La function in pre-tRNA 5'-end maturation is compartment specific (13, 69, 71, 148).

The hLa phosphoprotein: signal transduction through the CTD. Approximately 80% of hLa isolated from HeLa cells is phosphorylated on serine 366 (42). Phosphorylation can also be detected at other sites, although only a very small fraction (probably <5%) of hLa is modified at these sites, and the significance is unknown (16). The phosphate on La exhibits a relatively short half-life in HeLa cells, indicating multiple cycles of phosphorylation and dephosphorylation (107). S366 of hLa constitutes a casein kinase II (CKII) target site that conforms to the consensus S/TXXD/E, although whether CKII phosphorylates this site in vivo has yet to be demonstrated (42, 67). A consensus CKII target site can also be identified in the homologous regions of the La proteins of other species, although amino acid identity is not highly conserved (42; R. Maraia, unpublished observations). While yeast La proteins are phosphorylated, the site of modification has not been reported (143). Although the phosphatase responsible for dephosphorylating hLa has not been identified, a PP2A-like activity is suspected (118).

The only activities of La that have been reported to be regulated by phosphorylation are those related to the synthesis and processing of Pol III-transcribed RNAs (41, 42, 67), as nuclear import, dsRNA binding, and inhibition of PKR activity have been shown to be unaffected by phosphorylation (16, 70).

As mentioned above, La is dephosphorylated during the early stages of induced apoptosis (16, 70).

The basic region from aa 328 to 363 of hLa includes the WAM and putative PBS and appears to recognize the 5'-ppp end of a nascent RNA, while phosphorylation of S366 negatively modulates this activity (41, 42). In the model depicted in Fig. 3, the phosphate group on S366 not only would alter the conformation of the CTD but might also compete for the PBS. A more detailed mechanism can also be envisaged. In the absence of a 5'-ppp-RNA, phosphoserine 366 and nearby acidic side chains might mimic 5'-ppp and interact with the PBS. This putative interaction could be stabilized by hydrophobic interactions contributed by conserved aromatic residues and could be intramolecular or intermolecular as the amino acids required for homodimerization overlap the basic region (33). The effect of phosphorylation on the dimerization of La has not been reported.

Extending the potential biological implications of 5'-ppp binding is the finding that 5'-end recognition can be modulated by phosphorylation and is consistent with the proposed role for La as a quality control factor (41, 67). The CTD could afford La the ability to retain any nascent Pol III 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 (41).

Evidence for and against a role for hLa in transcription by Pol III. HeLa cell extracts immunodepleted of La lost 99% of their Pol III transcription activity, and the few transcripts synthesized were foreshortened on their 3' ends, suggesting that La is required for termination and high-efficiency transcription (47). Foreshortening probably reflects exonuclease activity in the absence of La. Repletion with biochemically purified La restored transcript length but not transcription efficiency (46, 47). Experiments using transcription complexes isolated on immobilized DNA suggest that La stimulates Pol III recycling by increasing transcript release and the rate of reinitiation (87, 88). The use of a set of B1-Alu gene mutants that differ only in the sequence context of their terminator provided additional insight into the transcription activity of hLa (45). For some of these, the inability to engage La in a manner that protects the nascent transcript from 3'-exonucleolytic digestion appears also to render the transcription complex a poor substrate for reinitiation by Pol III (45). This line of study supports the idea that Pol III termination and reinitiation are linked through La.

A short basic region that includes the WAM is required for the Pol III reinitiation activity of hLa (44). Fractionation of native hLa into a phosphoserine 366-containing form and a nonphosphorylated form revealed that only the nonphosphorylated form of La stimulates Pol III recycling in vitro (42). The latter study also provided an explanation for the low transcription activity of biochemically purified La, as the fractionation scheme employed by Gottlieb and Steitz yields mostly phosphorylated La which is inactive for transcription (42, 46, 47). These structure-function analyses of hLa suggest a model in which the WAM and PBS act as positive determinants of 5'-pppG/A recognition and transcription factor activity. The downstream acidic region would act as a negative influence on the activity of the WAM and PBS, accentuated and enhanced by phosphorylation of S366 (Fig. 3).

Publications from three laboratories indicate a role for La in transcription while others contest this activity (29, 42, 44-47,

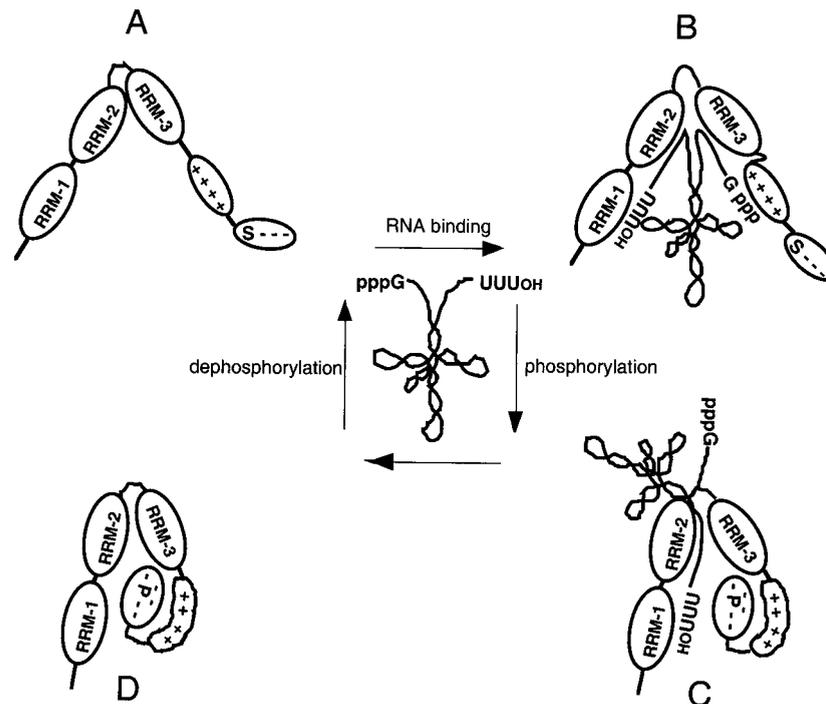


FIG. 3. A model of phosphorylation-regulated, bipartite precursor tRNA binding by the hLa protein. The model shown here depicts bipartite interactions with a nascent pre-tRNA according to previous data (41, 67) and the model in Fig. 2. The tandem RRM-1 and -2 of the NTD mediate high-affinity binding to UUU-OH-containing RNAs. The CTD of hLa contains a basic region and an acidic region, shown as +++ and ---, respectively; residues 328 to 344 conform to a WAM, and residues 348 to 368 represent a putative PBS (see Fig. 2 and text). Together, the WAM and PBS can recognize the 5'-pppG/A motif that comprises the 5' ends of nascent Pol III transcripts (41, 67). Serine 366, which resides in a region that demarcates a transition from basic to acidic residues, is shown as S in unphosphorylated La and as P in the phosphoserine form.

82, 87, 88, 154). In HeLa cells, La accumulates to $\sim 2 \times 10^7$ molecules/cell while Pol III appears to accumulate to only $\sim 10^4$ molecules/cell (109). Incomplete depletion might leave a surplus of La relative to Pol III and may also reflect La's association with a Pol III holoenzyme (82, 145, 146). Thus, while recent results raise significant doubt as to a role for La in Pol III transcription, the above concerns illuminate the need to address the differences noted in the different transcription systems (82, 145, 146), especially considering the intriguing correlations reviewed in the next paragraph.

The sensitivity of the 5'-ppp-RNA-binding activity to a panel of mutations in hLa was indistinguishable from the sensitivity of the transcription initiation factor activity of La to the same collection of mutations (41, 44). This correlation was strengthened by the finding that both activities are negatively modulated by phosphorylation of hLa S366 and strengthened the idea that they are mechanistically linked, i.e., that the transcription initiation factor activity of hLa depends on recognition of the initiating 5'-pppG/A nucleotide of the nascent transcript (42, 44). The presence of La in a human Pol III holoenzyme independently suggests involvement in RNA production (145). These observations provide a compelling reason to continue to consider the transcription factor activity of hLa seriously. We envisage that, just after transcription initiation, as the transcript emerges from the polymerase, La that is associated with the Pol III holoenzyme would capture the 5'-pppG/A end of the elongating RNA. As the transcript nears completion and the 3' terminus is formed, hLa would exchange

its contact with the holoenzyme for high-affinity contact with UUU-OH, dissociating from the enzyme complex with the nascent RNA securely attached. Unphosphorylated La would remain attached to both ends of the RNA (41, 67). Phosphorylation would initiate the dissociation of the nascent RNA and La. Accordingly, a phosphatase would then prepare phospho-La for involvement in the next round of RNA synthesis (42).

The ability of hLa to recognize both the 5' end and 3' end of a nascent Pol III transcript, the presence in a Pol III holoenzyme, and modulation by phosphorylation is reminiscent of the role of the CTD of Pol II in mRNA expression. The Pol II CTD recruits factors that modify both the 5' and 3' ends (5' capping and 3' cleavage and polyadenylation) of nascent Pol II transcripts, and it contributes to transcription efficiency by a mechanism that is regulated by phosphorylation and dephosphorylation (75, 98). A major challenge for the future is to develop a genetic or other *in vivo* system that can also be used for *in vitro* biochemistry with which to examine the transcription factor activity of hLa in more detail.

Interaction with the internal ribosome entry sites (IRES) and other elements in viral and cellular mRNAs. As mentioned above and previously reviewed, La appears to function in a pathway of translation initiation that is used by viral and cellular mRNAs that contain IRES elements (12, 62). Although first described for poliovirus mRNA (105), the list of IRES-containing mRNAs has been expanded to cellular mRNAs that encode cell cycle-dependent transcripts as well as

other mRNAs (31a, 63, 84, 96, 101, 110a, 142, 152). Of the cellular mRNAs, some have recently been found to interact with La (12, 62). Viral mRNAs that have been reported to interact with La are not limited to those containing IRES and include mRNAs from poliovirus, hepatitis C virus, rubella virus, vesicular stomatitis virus, encephalomyocarditis virus, Sindbis virus, and human immunodeficiency virus (28, 40, 57, 68, 74, 78, 104, 108, 134).

La has also been reported to interact with the U-rich 5' regions of ribosomal protein mRNAs *in vitro* and to stimulate their translation *in vivo* in a *Xenopus* tissue culture system (34, 106). Because many of the reported interactions between La and mRNAs occur near the 5' end of the RNAs, it seems noteworthy that 5'-pppG/A recognition [or m⁷G(5')ppp (5')-cap recognition] may be involved, since the region required for this activity includes the WAM and because La was found in close proximity (i.e., could be cross-linked) to a 5'-cap structure on a U1-like snRNA (41, 49). Consistent with this, residues within the region of aa 293 to 348 of hLa, which leads to dimerization, contain the WAM and are required for La's activity in IRES-mediated translation (33).

Fruit fly larvae carrying a null allele of La die as an apparent result of gastrointestinal defects that are associated with the loss of Ultrabithorax (*Ubx*) mRNA (9), suggesting that *Drosophila* La may influence *Ubx* mRNA metabolism, perhaps involving the IRES of *Ubx* (152).

Alternative isoforms of La mRNA, in addition to the major isoform that encodes nuclear La, have been identified in lymphocytes (18, 141). Some isoforms are tissue specific and, when expressed in recombinant form, give rise to La proteins that differentially localize to the nucleus or cytoplasm (50, 51, 60). Intriguingly, these La mRNA isoforms contain functional IRES, a finding that led to the proposal that synthesis of La may occur under conditions when cap-dependent translation is compromised, such as inflammation, apoptosis, or certain viral infections (18).

Alternative modes of RNA binding by La? As discussed above, a highly conserved feature of La RNA binding is recognition of a terminal 3'-OH group (136, 139, 153). High-affinity interaction has been reported for a small RNA probe (which ended in two U's) that was used to study the poliovirus IRES element (K_d , ~4 nM) (93), comparable to the affinity for UUU-OH-containing RNAs (28, 44). Yet, it is not clear that La would recognize native internal IRES elements via the UUU-OH mode of binding since these elements would not be expected to contain a 3'-OH group. A similar conundrum exists for TAR RNA (28). In an attempt to understand the apparent mechanism of recognition for TAR RNA, Chang et al. examined binding to various RNAs (28). Although more than a sixfold decrease in binding affinity was revealed by deletion of terminal uridylates, the analysis also suggested a mode of binding distinct from terminal uridylate recognition (28). Yet, the most N terminal of La residues, thought to be involved in UUU-OH recognition, contributed significantly as a primary determinant of binding in that study, regardless of which RNA was examined (28). In other studies, the RNA probes that contained terminal uridylates bound best to, or competed best for, La (3, 57, 92, 93) (note that *EcoRI* restriction leaves a 3' end that would encode two terminal U's in the runoff transcript). In general, it appears that if the only differ-

ence between two RNA probes is the presence of two or more terminal U's, this feature is a significant determinant of La binding, although it is not clear that such probes would best represent IRES elements. It should also be noted that, in the cases where the RNA probe ends with uridylates, some as yet unknown feature of internal sequences can further contribute to La binding, suggesting the existence of an alternative mode of binding in these cases (28, 57, 72). Further documentation and characterization of these features and the mechanism by which they contribute to the binding of viral and cellular mRNAs by La are outstanding issues.

La is targeted by viruses. Some viruses engage La either by binding their small RNAs or by modifying La by proteolysis, presumably to facilitate the execution of their own genetic programs. Adenovirus and Epstein-Barr virus encode small RNAs (VAI and EBER RNAs, respectively) that are synthesized at high levels by Pol III and are complexed with La (116). VAI RNA serves to inactivate the dsRNA-activated protein kinase (PKR) that allows viral mRNAs to be translated by the cap-dependent mechanism (147). Although EBER RNAs can also bind PKR, its role in the Epstein-Barr virus life cycle is less clear (155). Unlike most Pol III transcripts, which are only transiently associated with La, these RNAs remain associated with La as they accumulate to high levels, although the consequences of this are unknown. It has been proposed elsewhere that La can regulate PKR by virtue of an RNA-unwinding or helicase activity that was presumed to be dependent on ATP hydrolysis and the presence of the WAM (65, 149). However, as noted above, recent studies indicate that the WAM does not mediate this activity (70). Also, a region that resembles dsRNA-binding proteins that was thought to be responsible for dsRNA binding by La has subsequently been shown to be dispensable for dsRNA binding (70).

Finally, we note that poliovirus protease 3C cleaves hLa at residue 358 (127). This protease also cleaves and inactivates other factors involved in small RNA expression and causes apoptosis (10, 30, 35, 94, 126, 151). This cleavage would leave the dimerization and RNA-binding domains, including the 5'-ppp-interaction motif, intact (131). Thus, poliovirus causes La to accumulate in the cytoplasm, wherein the truncated protein would presumably be engaged in the IRES-dependent translation of poliovirus mRNA (see above). Induced apoptosis leads to a caspase-dependent cleavage of hLa at residue 371 or 374 (118). The role of La cleavage in apoptosis is unknown (19, 20, 118), although a function in the regulated expression of the X-linked inhibitor of apoptosis mRNA is suggested by recent work (62).

Conclusions. hLa antigen is a ubiquitous, abundant nuclear phosphoprotein that is found associated with a large variety of RNA ligands. La facilitates the expression of RNA Pol III-synthesized RNAs by stabilizing the precursor forms of these transcripts and can integrate activities that control the 5'- and 3'-end metabolism of these RNAs. A significant amount has been learned about the structural basis of RNA recognition by La and the relationship of this to activity. Although the atomic structure of La remains to be determined, its most conserved region, the NTD, can be readily modeled into a tandem pair of RRM with features consistent with UUU-OH recognition. While the NTD recognizes the UUU-OH 3'-terminal motif that results from transcription termination by Pol III, the CTD

of hLa recognizes the 5'-ppp-RNA motif that results from transcription initiation by Pol III. La is also found associated with certain precursor forms of snRNAs and other small RNAs in vivo, which are synthesized by Pol II, although in these cases it appears that posttranscriptional processing exposes 3'-terminal uridylates prior to La binding. For nascent Pol III transcripts, the 5'-ppp and UUU-OH-3' motifs are often found in close proximity in the structured RNA, affording high-affinity and specific, bipartite binding by La.

Although La is not essential in yeast, its presence in all eukaryotes and apparent essentiality in *D. melanogaster* suggest that it provides a function that has been repeatedly retained and perhaps refined during the radiation of eukaryotes. It is becoming increasingly clear that La is not simply a passive chaperone for Pol III transcripts but indeed serves a significant function in RNA expression. While all La proteins function by UUU-OH binding, it is not clear if the La proteins of yeast can mediate 5'-ppp-RNA recognition or if this was added to the repertoire of La activity during the evolution of higher eukaryotes. The 5'-end-RNA recognition activity of hLa can be modulated by phosphorylation of S366, and this can control tRNA maturation and perhaps other activities that might be mediated by this mode of RNA recognition.

Another activity of La is the apparent involvement in the expression of a subset of mRNAs, both viral and cellular, that rely on the IRES-mediated mechanism of translation, although the details of RNA recognition in these cases are not yet clear. Certain viruses engage La by employing its RNA Pol III-related activities, while others appear to relocalize it to the cytoplasm, apparently to direct IRES-dependent translation of the virus mRNA. A similar proteolysis-mediated localization mechanism may be used during apoptosis.

To date, only the Pol III-related functions of human La have been reported to be regulated by phosphorylation of S366, although rapid dephosphorylation occurs during the early phase of apoptosis. While an atomic structure for the conserved NTD will be highly informative, visualization of the structure of hLa's CTD will also be important.

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