La Protein and the Trafficking of Nascent RNA Polymerase III Transcripts

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Several small nuclear and cytoplasmic RNAs are synthesized by RNA polymerase (Pol) as nascent transcripts that must then migrate through RNA processing, modification, and transport machinery on their trek to becoming mature, functional molecules. The first factor to interact with newly synthesized Pol III transcripts is the La protein. Evidence that this molecular chaperone accompanies precursor tRNAs through various RNA modification and processing events and facilitates the assembly of other cursor tRNAs through various RNA modification and transport machinery on their trek to becoming mature, functional molecules. The first factor to interact with newly synthesized Pol III transcripts is the La protein. Approximately 1–2 × 10^5 molecules of pre-5S rRNA (1–2% of mature 5S rRNA), in addition to a comparable number of pre-tRNAs, are associated with La (Rinke and Steitz, 1982; Steitz et al., 1988). Since the number of newly made transcripts detected by the direct visualization approach (10^4) (Pombo et al., 1999) is significantly exceeded by the number of pre-5S and pre-tRNAs, it would seem as if nuclei contain a reservoir of La-associated transcripts, consistent with a chaperone function for La (Pannone et al., 1998).

Nuclear Retention of RNA by La

The nuclear localization signal (NLS) at the COOH terminus (amino acids 382–408) of human La mediates nuclear import, but is not sufficient for nuclear accumulation, since additional elements are responsible for nuclear retention (Simons et al., 1996a). Available data suggest that La can sequester RNAs in the nucleus, provided that they retain their oligo(U) 3′ trailer and is associated with La in the nucleus, but exits to the cytoplasm after 3′ end processing as a La-free scB1 RNP (Maraia et al., 1988). 5S rRNA is transported to the cytoplasm only after dissociation from La (Guddat et al., 1990; Steitz et al., 1988). Similar results were obtained for human Y1 RNA (Simons et al., 1994, 1996b). Euplotes telomerase appears to represent an example of how association with La may provide an RNA and associated proteins with nuclear residence. Although this protein has not yet been shown to associate with a wide range of nascent Pol III transcripts, it does contain a highly conserved

La Is the First Protein to Interact with Nascent Pol III Transcripts

Newly made Pol III transcripts have been visualized directly in HeLa nucleoplasm and it was calculated that they reside at 1–2,000 discernible sites (distinct from Pol II transcription loci), containing ~5 nascent Pol III transcripts per site (Pombo et al., 1999). Transcription termination by Pol III produces a common motif, 3′ oligo(U), that is a high affinity binding site for an abundant nuclear phosphoprotein known as La that resides mostly in the nucleoplasm (Deng et al., 1981; Hendrick et al., 1981; Stefano, 1984). Accordingly, the first protein that can be detected in association with newly synthesized Pol III transcripts is La. Approximately 1–2 × 10^5 molecules of pre-5S rRNA (1–2% of mature 5S rRNA), in addition to a comparable number of pre-tRNAs, are associated with La (Rinke and Steitz, 1982; Steitz et al., 1988). Since the number of newly made transcripts detected by the direct visualization approach (10^4) (Pombo et al., 1999) is significantly exceeded by the number of pre-5S and pre-tRNAs, it would seem as if nuclei contain a reservoir of La-associated transcripts, consistent with a chaperone function for La (Pannone et al., 1998).
La motif positioned at its NH2-terminal end, followed by a region with significant homology to the characterized La proteins of other species (e.g., trypanosome and insect) in the RRM-2 region, making it a very likely genuine La homologe (analyzed by the BLAST 2 Sequences program available at http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html; data not shown). As a Pol III product that retains its UUU 3' terminus, *Euplotes* telomerase RNA is an integral component of telomerase reverse transcriptase that remains associated with La as a stable RNP (Aigner et al., 2000). This La homologue might function both in the stabilization and nuclear retention of telomerase in this ancient eukaryote (Aigner et al., 2000).

Metazoan La proteins have been expanded in their COOH-terminal domains (CTDs), presumably in associa-
tion with the acquisition of additional activities, some of which have been described for human, but not yeast, La (Rosenblum et al., 1998; Intine et al., 2000). The nuclear import system used by *Saccharomyces cerevisiae* La is different from the system used by other species (Rosenblum et al., 1998). An NLS resides within the NH2-terminal domain (NTD) of *S. cerevisiae* La, suggesting that RNA binding and nuclear import might be exclusive of each other, whereas this would not be expected in the other eukaryotes examined, which have an NLS at the COOH terminus (Rosenblum et al., 1998). A potential for vertebrate La to transport a synthetic RNA from the cytoplasm to the nucleus has been demonstrated (Grimm et al., 1997). It was noted that the *S. cerevisiae* La protein appeared to be exclusively nuclear, perhaps indicative of trafficking differences in *S. cerevisiae* and other eukaryotes (Rosenblum et al., 1998). Although the fraction of vertebrate La that resides in the cytoplasm is usually <20%, this is significantly increased under certain conditions (see Marai and Intine, 2001 and references therein).

La Chaperones Pre-tRNAs during Their Modification and at Subsequent Cleavage Events

It has been well documented in yeast that La protects precursors of U6 snRNA, U4 snRNA, and U3 snoRNA from degradation and facilitates their stable transfer to specific RNPs (Pannone et al., 1998; Kufel et al., 2000; Xue et al., 2000). It is also becoming clear that La’s function as a chaperone extends beyond RNP assembly since, as reviewed below, some RNAs appear to remain associated with La during transient recognition by other proteins. As reviewed in the next paragraph, three data sets can be reasonably interpreted to suggest, or indicate, that tRNA modifying enzymes act on their pre-tRNA substrates, albeit transiently, while the substrates are associated with La. After this, the data that pre-tRNAs are recognized transiently by RNase P and pre-tRNA 3' endonuclease while associated with La are also reviewed.

La’s involvement in pre-tRNA modification can be considered from three perspectives, (a) recent results linking La and pre-tRNAMet m1A58 methylation (Anderson et al., 1998; Calvo et al., 1999); (b) differences in the modifications found on a human pre-tRNAeMet that is stabilized by La, and its mature counterpart (Harada et al., 1984; Kenan, 1995); and (c) data that documented the modifications found on the nascent precursor and various intermediate species of tRNA\textsubscript{Tyr} (Nishikura and De Robertis, 1981). Methylation of A58 to m1A58 is one of the earliest modifications of pre-tRNA (Nishikura and De Robertis, 1981) and it was shown that yeast La cooperates with the m1A58 methylase (Anderson et al., 1998; Calvo et al., 1999).

Human pre-tRNA\textsubscript{Met} was initially identified as a tRNA precursor of unusually high stability (Harada et al., 1984). This pre-tRNA has been independently isolated as the most abundant La-associated transcript in HeLa cells (Ali et al., 2000). Pre-tRNA\textsubscript{eMet} contains six different modified bases (D, \(\Psi\), m1A, m5C, m2G, and Cm), whereas the mature tRNA\textsubscript{Met} species contains these plus three more modifications (Harada et al., 1984). This kinetic pattern is similar to that observed during the expression of tRNA\textsubscript{Tyr} (Nishikura and De Robertis, 1981). Specifically, the pre-tRNA\textsubscript{Tyr} species containing a 5' leader, 3' trailer, and intron bears several of the modifications found on pre-tRNA\textsubscript{eMet}, whereas others were found on the intron-containing species only after 5' and 3' end processing occurred (Nishikura and De Robertis, 1981).

As it is generally accepted that the substrate for pre-tRNA 5' processing is complexed with La (Yoo and Wolin, 1997; Fan et al., 1998; Wolin and Matera, 1999; Intine et al., 2000), the above data indicate that multiple modifying enzymes act on the pre-tRNA-La RNP before recognition by RNase P. Data that indicate that yeast La facilitates endonucleolytic 3' processing, a cleavage event that normally occurs after 5' processing, provide further evidence that La chaperones its pre-tRNA ligands extensively through many parts of their maturation pathways (Yoo and Wolin, 1997; Wolin and Matera, 1999). It is also conceivable, under the conditions when pre-tRNA introns are removed before end processing, that a pre-tRNA-La RNP might be a substrate for the splicing machinery (Wolin and Matera, 1999). The cumulative results indicate that La protects transcripts during the time between their synthesis and incorporation into more stable RNPs, in some cases facilitating RNP assembly and in some cases supporting them during recognition by modification and cleavage enzymes.

Nascent Pol III Transcripts Move Transiently through the Nucleolus

Several Pol III transcripts have also been observed in the nucleolus; these include pre-tRNAs (Bertrand et al., 1998), U6 snRNA (Lange and Gerbi, 2000), and the RNA components of RNases P and MRP, as well as SRP RNA and SS rRNA (Jacobson et al., 1995, 1997; Bertrand et al., 1998; Jacobson and Pederson, 1998; Jarrous et al., 1999). When examined by time lapse methods, SRP RNA, RNase P RNA, and U6 snRNA were observed to pass transiently through the nucleolus, during what appears to be the early phases of their life cycles (Jacobson et al., 1997; Jacobson and Pederson, 1998; Lange and Gerbi, 2000). As indicated from kinetic comparisons, vertebrate U6 RNA appears to use a nucleolar localization system that is different from the box C/D and H/ACA snoRNAs (synthesized by Pol II) which remain more stably in the nucleolus (Lange and Gerbi, 2000). Thus, by contrast to the box C/D and H/ACA snoRNAs, the nucleolar localization of the majority of Pol III transcripts (with the ex-
ption of MRP RNA) is transient and limited to a fraction of molecules that most likely represent immature species. 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 (Tycowski et al., 1998; Ganot et al., 1999; Lange and Gerbi, 2000; see below).

**Proteins That Associate with Specific Pol III Transcripts and Localize to the Nucleolus**

As indicated above, La is a general Pol III nascent transcript-binding protein that has been observed in the nucleolus. Because Pol III transcripts pass through the nucleolus during a (early) phase of their life cycle, when they are associated with La but might otherwise be susceptible to exonucleases, it might seem that La would be involved in their nucleolar transit and protection in this subdivision. However, it appears that this need not be so for 5S, MRP, RNase P, and SRP RNAs, since as reviewed below, more specific proteins would appear to be better candidates for their nucleolar localization.

After transient association with La, 5S rRNA is bound by the ribosomal protein L5 (Steitz et al., 1988; Guddat et al., 1990), the latter of which has been shown to contain a signal that would appear to direct 5S rRNA to the nucleolus (Michael and Dreyfuss, 1996; Steitz et al., 1988). Mammalian RNase P RNA and MRP RNA are related by sequence homology and each associate with a shared set of specific proteins, including Rpp38, which reacts with To/Th autoimmune sera, that presumably direct them to nucleoli (Jacobson et al., 1995; Jarrous et al., 1999). Consistent with this scenario, previous studies suggested that To/Th antigen binding to MRP (7-2) RNA appears to occur after La dissociates from the RNA (Hashimoto and Steitz, 1983). In mammalian cells, the SRP RNA–associated proteins SRP19, SRP68, and SRP72 each have been observed to localize to the nucleolus, as has SRP RNA itself, directed in part by helix 8 and the Alu domain of the RNA (Jacobson and Pederson, 1998; Politz et al., 2000). These data indicate that multiple determinants direct the transient nucleolar localization of SRP RNA. Because UUU-OH comprises a predominate binding site for La (for review see Maraia and Intine, 2001), the finding that the terminal uridylylates of SRP RNA are removed and the 3′ terminus is adenylated before nucleolar accumulation argues that La is not associated with the SRP RNA that is localized to the nucleolus (Chen et al., 1998). Thus, for SRP RNA, 5S rRNA, MRP RNA, and RNase P RNA, it would appear that La would not be directly involved in transporting them to the nucleolus. Yet for pre-tRNAs and pre-U6 snRNA there is no protein other than La that is known to associate with them that has also been observed in the nucleolus (Deng et al., 1985; Hendrick et al., 1981). Finally, it is noteworthy here that although it was proposed that yeast La might reside in the nucleolus (Wolin and Matera, 1999), it has not yet been identified there (Rosenblum et al., 1998). Therefore, given the differences noted above regarding the species-specific nuclear import of La, it will be important to determine if the difference also extends to nucleolar trafficking.

**What May Be the RNA Ligands and Function of Nucleolar La?**

In addition to nascent Pol III transcripts, yeast La has also been found associated with a U3 snoRNA precursor intermediate (Kufel et al., 2000) and it is possible that La holds off this transcript to the next proteins in the U3 RNP assembly pathway in the nucleolus. Reports of association with ribosomal subunits and involvement in translation (for review see Maraia and Intine, 2001) suggest the formal possibility that La may associate with translational machinery in the nucleolus (Pederson and Politz, 2000). Below, the possibility that La’s localization in the nucleolus is related to its action as a chaperone for nascent Pol III transcripts is considered.

Since MRP RNA is involved in the biogenesis (processing) of 5.8S rRNA and 5S rRNA is a ribosome component, these RNAs would naturally be found in the nucleolus (Pederson and Politz, 2000). By contrast, the unexpected finding that SRP RNA, RNase P RNA, and U6 snRNA were also found there raised interesting perspectives, including that they are modified, matured, or participate in a quality control step in the nucleolus (Jacobson et al., 1997; Pederson, 1998; Pederson and Politz, 2000). Although SRP RNA is not known to be modified other than at the 3′ end as mentioned above, the possibility that RPR1 RNA, a component of yeast RNase P, or its precursor, may be modified exists, although the enzyme implicated in the presumptive modification was not determined to be nucleolar (Calvo et al., 1999).

The snoRNAs that direct 2′-O-methylation and pseudouridylation of U6 snRNA almost certainly do so in the nucleolus (Tycowski et al., 1998; Ganot et al., 1999). Detailed analyses indicated the presence of several modified residues on the subset of U6 snRNA precursors that are associated with La (Rinke and Steitz, 1985). By reviewing the specific nucleotides that were modified in the La-associated U6 RNA in the context of recent data that identify the specific nucleotides modified by snoRNAs (Rinke and Steitz, 1985; Tycowski et al., 1998; Ganot et al., 1999), one can arrive at the tentative conclusion that it appears that modification of U6 may occur while associated with La in the nucleolus (data not shown). Specifically, several 2′-O-methylated and –pseudouridylated U6 nucleotides that were shown recently to be directed by specific snoRNAs (Tycowski et al., 1998; Ganot et al., 1999) appear to be present in La-associated pre-U6 RNA, because the corresponding oligonucleotides comigrated with the modified oligonucleotides isolated from mature U6 snRNA, although some of the La-associated oligonucleotides appeared to be undermodified (Rinke and Steitz, 1985). As noted earlier, U6 snRNA has been shown recently to transiently pass through the nucleolus (Lange and Gerbi, 2000). The cumulative data provide significantly compelling, albeit circumstantial, evidence to suggest that La travels with pre-U6 snRNA to the nucleolus.

**Does 5′ Processing of Pre-tRNA by RNase P Occur in the Nucleolus?**

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 (Jacob-
However, it has become a popular notion that 5′-pre-tRNA processing activity actually occurs there (Wolin and Matera, 1999; Lewis and Tollervey, 2000), although it should be noted that this has not been demonstrated. Although most of the RNase P RNA and intron-containing pre-tRNAs detectable in yeast appeared nucleolar (Bertrand et al., 1998), other studies reveal yeast pre-tRNAs in the nucleoplasm, as well as in the nucleolus (Sarkar and Hopper, 1998). In mammalian cells, a substantial amount of RNase P RNA is found in the nucleoplasm (Matera et al., 1995; Lee et al., 1996; Jacobson et al., 1997). In archaea, homologues 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 the possibility that eukaryotic pre-tRNAs may be similarly modified in the nucleolus (Omer et al., 2000), although this will be better addressed once the complete human genome has been analyzed for snoRNAs. One tRNA modification enzyme has been localized in the nucleolus as well as the nucleoplasm (Tolerico et al., 1999). Since transient nucleolar localization is common to several Pol III transcripts, pre-tRNAs and RNase P components may each be required to pass through this compartment without necessarily interacting there. In summary, while it is possible that RNase P functions in pre-tRNA processing in the nucleolus, the evidence for this remains circumstantial and alternative explanations may account for the localization of pre-tRNAs and RNAs P components there.

Phosphorylation and Signal Transduction through the CTD of La

The unexpected ability of human La to protect pre-tRNAs from 5′ processing led to a model that suggested that the evolutionarily conserved NTD of La recognizes 3′-oligo(U) and adjacent determinants, whereas the CTD recognizes the 5′-end region of the nascent RNA (Fan et al., 1998; Intine et al., 2000; Maraia and Intine, 2001). Evidence obtained from in vitro and in vivo studies suggest that 5′-end binding is significant only for RNAs that carry 3′-oligo(U), probably because the latter comprises a principal binding site for La (Fan et al., 1998; Intine et al., 2000; for review see Maraia and Intine, 2001). Unphosphorylated La binds both the 3′- and 5′-regions of a pre-tRNA, protecting the latter from processing at either end (Fan et al., 1998). Phosphorylated La attaches only to the 3′-end, allowing access to the 5′-end of a pre-tRNA by RNase P (Fan et al., 1998; Intine et al., 2000). A two-step binding and/or dissociation mechanism might provide La with versatility that would be useful as a chaperone for a wide variety of RNAs. A regulatable mode of binding might afford La the ability to retain a nascent transcript of its 5′-end region in a stable form until it receives a signal, i.e., in the form of S366 phosphorylation, to release it (Fan et al., 1998). This mode of binding activity and its modulation by phosphorylation is consistent with a role for La as a quality control factor (Fan et al., 1998; Intine et al., 2000). This feature could potentially be used to control the handing off of an RNA from La to another protein during RNP assembly, modification, cleavage, or transport. A challenge is to explain how the two-step RNA recognition mechanism, intracellular trafficking, and signal transduction functions of La are integrated with the maturation pathways of the various RNAs with which it associates.

Conclusion

La is an abundant, ubiquitous nuclear phosphoprotein that associates with and serves as a chaperone for a large variety of newly synthesized RNA Pol III transcripts. Accumulating evidence suggests that La maintains close contact with its ligands during maturation events that involve transient interactions between La RNPs and a variety of pre-tRNA and U6 snRNA modification enzymes. It is also becoming clear that while La may serve as a nuclear retention factor for associated RNAs and appears mostly nucleoplasmic, a fraction of La also resides in the nucleolus. Some precursor RNAs (e.g., pre-U6 snRNA), with which La is found physically associated, contain ribose and base modifications that recent evidence strongly suggest are added in the nucleolus, suggesting that La chaperones some transcripts during modification in the nucleolar compartment, although this remains to be investigated using more directed approaches. Although the NTD of human La binds the UUU-OH 3′ terminal motifs of nascent Pol III transcripts, recognition of the 5′-phosphoprotein end regions of these RNAs is mediated by the distinct CTD, a specific activity that can be modulated by phosphorylation of human La on serine 366. A challenge for the future is to decipher how this bipartite mode of nascent RNA binding, intracellular trafficking, and the signal transduction activities of La are integrated with the expression pathways for the variety of snRNA and snoRNA precursors with which it associates. An equally important goal is to understand how signal transduction through La may be integrated with other aspects of intermediary metabolism and cellular proliferation.

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