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Efficient synthesis, termination and release of RNA polymerase III transcripts in *Xenopus* extracts depleted of La protein

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La proteins are conserved, abundant and predominantly nuclear phosphoproteins which bind to the 3′-U termini of newly synthesized RNA polymerase III transcripts. The human La protein has been implicated in the synthesis, termination and release of such transcripts. Here we examine the potential transcriptional properties of La in *Xenopus laevis*, using a homologous tRNA gene as template. Immunodepletion of La from cell-free extracts leads to the formation of tRNA precursors lacking 3′-U residues. This shortening can be uncoupled from RNA polymerase III transcription, indicating that it results from nuclease degradation rather than incomplete synthesis. Extracts containing <1% of the normal La protein content synthesize tRNA precursors just as well as complete extracts, with no change in termination efficiency, and the vast majority of these full-length transcripts are not associated with the template or with residual La protein. Hence, *Xenopus* La seems not to function as an initiation, termination or release factor for RNA polymerase III. Consistent with the recently discovered role of La in yeast tRNA maturation *in vivo*, recombinant *Xenopus* La prevents 3′-exonucleolytic degradation of tRNA precursors *in vitro*. A conserved RNA chaperone function may best explain the abundance of La in eukaryotic nuclei.

Keywords: nuclease/RNA chaperone/transcription/tRNA gene/tRNA processing

Introduction

The existence of eukaryotic La proteins was first realized because the 47 kDa human version is a frequent autoantigen in systemic lupus erythematosus and Sjögren's syndrome patients (Mattioli and Reichlin, 1974; Alspaugh and Tan, 1975). Since the cloning of human La (Chambers *et al.*, 1988), homologues have been found in other mammals (Chan *et al.*, 1989; Topfer *et al.*, 1993), frogs (Scherly *et al.*, 1993), insects and yeasts (Yoo and Wolin, 1994; Lin-Marq and Clarkson, 1995; Van Horn *et al.*, 1997). It is now clear that La proteins are highly conserved, mostly nuclear phosphoproteins. They are also abundant, being present at ~2×10^7 copies per mammalian cell; they are particularly abundant in *Xenopus laevis* oocytes, in which they accumulate to ~8×10^{11} copies. The extent of this stockpiling during oogenesis strongly suggests that La has a crucial function during early embryogenesis. Despite these indications of their importance, La proteins are not needed for yeast viability (Yoo and Wolin, 1994; Lin-Marq and Clarkson, 1995; van Horn *et al.*, 1997).

La proteins are RNA-binding proteins, showing a preference for RNAs with 3′-terminal U residues (Stefano, 1984). This is a characteristic feature of most nascent transcripts produced by RNA polymerase III (pol III), suggesting that La may be involved in pol III transcription termination. Indeed, evidence has been presented over the years that human La may function as a pol III termination, release and even initiation factor (Gottlieb and Steitz, 1989a,b; Maraia *et al.*, 1994; Maraia, 1996; Fan *et al.*, 1997; Goodier *et al.*, 1997). Other reports suggest, however, that accurate transcription termination and transcript release can occur with purified vertebrate pol III without the need for additional proteins (Cozzarelli *et al.*, 1983; Watson *et al.*, 1984; Campbell and Setzer, 1992), and there is no evidence to suggest that yeast La proteins have functions in pol III transcription (Yoo and Wolin, 1997).

Every pol III transcription unit requires TFIIIB, most require TFIIIC, and 5S RNA genes also need a third factor, TFIIIA, for accurate and efficient initiation (reviewed in White, 1994). For efficient termination, an accessory factor might be needed by those pol III transcription units with an intrinsically weak terminator. For *X.laevis* 5S RNA genes, an efficient termination site comprises a stretch of four or more T residues embedded in G–C-rich DNA in the non-coding DNA strand (Bogenhagen and Brown, 1981). These two features are not found at the end of every pol III gene, however, even in *X.laevis*. For example, termination efficiency is increased rather than decreased by a mutation that makes the sequences surrounding the T cluster behind a *X.laevis* $tRNA^{Lys}$ gene more A–T-rich (Mazabraud *et al.*, 1987). A second exception is provided by a *X.laevis* $tRNA^{Phe}$ gene. Its natural terminator is a T4 stretch with three G–C base pairs (bp) on each side, yet, as shown below, significant read-through occurs from this site *in vitro*.

We have used this $tRNA^{Phe}$ gene as a paradigm of poorly terminating pol III transcription units to determine whether *X.laevis* La protein increases the efficiency of termination and/or transcript release at its T4 site in homologous cell-free extracts. Our results provide no evidence for such activities, or for a pol III transcription initiation function, but they are consistent with *X.laevis* La having a chaperone function in tRNA processing comparable with that recently described for the yeast La homologues (van Horn *et al.*, 1997; Yoo and Wolin, 1997).

Results

Reagents

The cloned $tRNA^{Phe}$ gene used here is normally found together with seven other tRNA genes on a tandemly

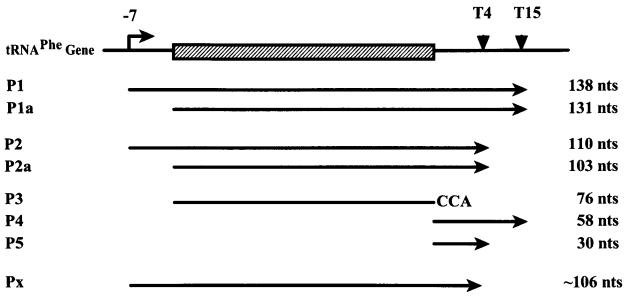

Fig. 1. Schematic diagram of the *X.laevis* tRNA^{Phe} gene and its pol III transcripts. Transcription initiation (arrowed) occurs 7 nt upstream of the mature tRNA coding region (boxed) and terminates within T4 or T15 tracts. The lengths of the primary transcripts P1 and P2 and their processing products are shown to the right.

repeated 3.18 kb fragment of *X.laevis* DNA (Müller *et al.*, 1987). In homologous S-100 extracts (Weil *et al.*, 1979), pol III initiates transcription from this gene with a pppA located seven nucleotides (nt) upstream of the mature tRNA^{Phe} sequence. Most of the primary transcripts terminate with three or four U residues within a downstream T4 tract (P2; Figures 1 and 2B). Read-through products stop within a T15 tract, the natural terminator of a *X.laevis* tRNA^{Tyr} gene (Gouilloud and Clarkson, 1986), which was placed further downstream to act as a 'fail-safe' terminator (P1; Figures 1 and 2B). The 5′ leaders of these primary transcripts are removed by RNase P to yield processing intermediates (P1a and P2a); these in turn are cleaved by a 3′ endonuclease to yield 3′ trailers (P4 and P5) and, after 3′-CCA addition, the mature-length tRNA^{Phe} (P3).

All experiments made use of S-100 extracts from which the La protein had been progressively removed by immunodepletion. For this approach, rabbit polyclonal antibodies were raised against recombinant *X.laevis* La protein that had been expressed in *Escherichia coli* and partially purified by heparin–Sepharose chromatography. The anti-La antibodies were mixed with protein A–Sepharose beads; the beads were washed with transcription buffer, incubated with extract, and the immobilized antigen–antibody complexes were removed by centrifugation. The partially depleted extracts were routinely subjected to at least two further rounds of immunodepletion.

As revealed by immunoblotting, most of the La protein is removed from S-100 extracts by two rounds of anti-La immunodepletion, whereas two rounds of treatment with pre-immune serum have little effect (Figure 2A, compare α-La2, PI2 and NT). A third round of immunodepletion (α-La3) reduces La levels even further. From immunoblots of diluted extracts (for example Figure 5A), we estimate that 1–3% of the original La protein remains after three rounds of immunodepletion. Additional rounds of immunodepletion remove some, but not all, of the remainder (data not shown).

tRNA^{Phe} precursors are more heterogeneous in length in La-depleted extracts

These immunodepleted and mock-depleted extracts are able to transcribe the tRNA^{Phe} gene template but both quantitative and qualitative differences are evident when compared with untreated S-100 extracts. First, there is an inverse relationship between the number of pol III transcripts and the number of rounds of depletion.

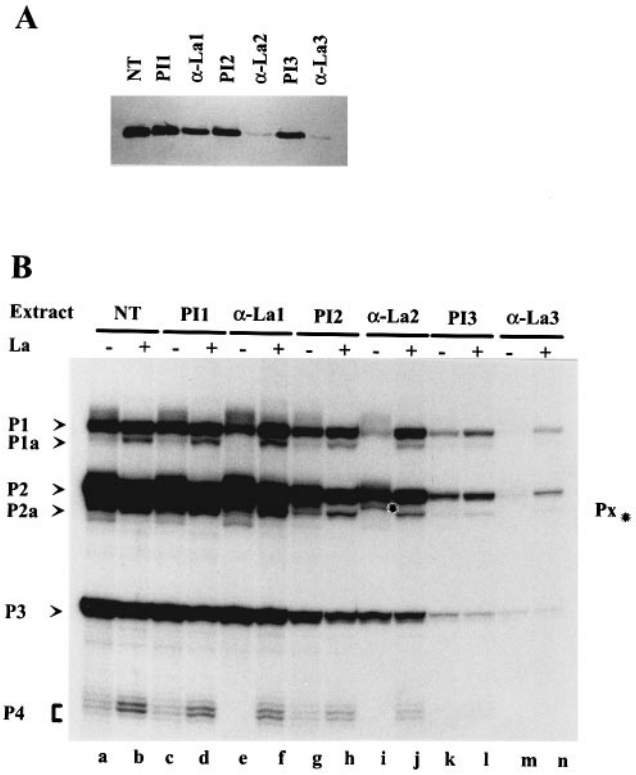

Fig. 2. Transcription of the tRNA^{Phe} gene in S-100 extracts progressively immunodepleted of La protein. (**A**) Western blot analysis of the extracts probed with a polyclonal anti-La antibody. S-100 extracts were not treated (NT) or subjected to one, two or three rounds of depletion with immobilized anti-La antibody (α-La1, 2, 3) or pre-immune serum (PI1, 2, 3). (**B**) Transcription and processing products of the tRNA^{Phe} gene during a 2 h incubation at 20°C with the indicated extracts. Reactions contained [α-^{32}P]GTP and either no additional La protein (–) or 500 ng of recombinant *X.laevis* La (+). Transcripts were purified and resolved on a 12% denaturing polyacrylamide gel. Arrows indicate the tRNA precursors and mature tRNA shown schematically in Figure 1. The bracket indicates the 3′ trailer P4 containing three or four 3′-U residues. The star indicates Px, the pol III transcript found in La-depleted extracts without supplementary La protein.

Although most noticeable with La-depleted extracts, this effect also occurs with mock-depleted extracts (Figure 2B, compare lanes c, g and k) and is due to the dilution of the extract that occurs at each round of depletion, which in turn reduces transcriptional ability. This effect can be overcome by concentrating the depleted extracts (see below).

Addition of an excess of recombinant La protein to the extracts stabilizes the transcripts and/or slows down RNA processing, in particular 3′ processing. This is also a general effect, as shown by the increased levels of the 5′ processed precursor P1a and reduced levels of mature tRNA^{Phe} P3, in untreated as well as depleted extracts (Figure 2B, compare lanes a and b, c and d, e and f). In apparent contradiction with this, the 3′ trailer RNAs P4 are also more prominent in La-supplemented extracts. These 3′ trailers are fully immunoprecipitable by anti-La antibodies (data not shown). Together, these results suggest that bound La protein inhibits the degradation of these processing products.

The major effect specific to La immunodepletion is that the tRNA^{Phe} precursors are more heterogeneous in length

than normal. Some are longer and some are normal length, while others are shorter. Although some transcripts longer than P1 are also evident in untreated and mock-depleted extracts, those found in La-depleted extracts are more heterogeneous in length and represent a more substantial fraction of the precursors (Figure 2B, compare lanes a, c and e); their formation is prevented by addition of recombinant La protein to the extracts (Figure 2B, lanes b, d and f). Transcripts longer and shorter than P2 are seen most clearly after two rounds of immunodepletion; they are not found in the corresponding La-supplemented extract, which instead generates the normal P1 and P2 $tRNA^{Phe}$ precursors and 5′ processing intermediates (Figure 2B, compare lanes i and j).

The P1 precursors made in untreated extracts terminate within a T15 tract; most of them possess $UUUU_{OH}$ at their 3′-terminus, the remainder having three or five U residues (data not shown). Thus, in principle, the RNAs longer than P1 could result from pol III transcription extending further into the T15 tract. The P2 precursors terminate within a T4 tract and have UUU_{OH} or $UUUU_{OH}$ at their 3′-terminus (see below). Any extension of these RNAs by pol III would imply transcription beyond the T4 tract into G–C-rich DNA. We consider it more likely that the transcripts longer than P2 represent pol III primary transcripts that are extended by another polymerase. Such elongated, labelled RNAs are found when unlabelled P2 precursor is incubated with La-depleted extract in the presence of [α-^{32}P]UTP but not [α-^{32}P]GTP (data not shown). This may indicate addition of U residues to the 3′-terminal (U)UUU_{OH} by a poly U polymerase (Andrews and Baltimore, 1986). These longer transcripts have not been investigated further.

A truncated $tRNA^{Phe}$ precursor is made in La-depleted extracts

The most prominent shorter transcript found in La-depleted extracts is a novel RNA with an electrophoretic mobility intermediate between that of the P2 primary transcript and its 5′ processed product P2a (Figure 2B, Px, lane i). To examine this RNA in more detail, we incubated the $tRNA^{Phe}$ gene template in a La-depleted extract in the presence of [α-^{32}P]UTP, gel purified the Px transcript and then compared its RNase T1 fingerprint with that of the P2 precursor. Apart from changes in some sub-molar spots that may be due to post-transcriptional modification or contaminating RNAs, the major difference is the absence of two spots (24 and 25) from the Px fingerprint (Figure 3). These correspond to $CUUU_{OH}$ and $CUUUU_{OH}$, respectively, which are normally found at the 3′ end of the P2 $tRNA^{Phe}$ precursor, at the base of a potential stem–loop structure (Figure 3C). This analysis cannot distinguish between the presence or absence of the C residue but a spot corresponding to CU_{OH} is absent from the Px fingerprint, indicating that all 3′-U residues are missing. The question naturally arises as to whether these shorter transcripts are the result of incomplete transcription by pol III, or of nucleolytic attack on the fully synthesized P2 precursor.

Formation of the truncated $tRNA^{Phe}$ precursor is separable from pol III transcription

To try to distinguish between these possibilities, we inserted the *X.laevis* $tRNA^{Phe}$ gene into a plasmid downstream of a promoter for T7 RNA polymerase (T7 pol) and inserted an A4 tract after the T4 termination signal for the pol III-generated P2 primary transcripts. After digestion with the restriction enzyme *Dra*I (cut site TTT↓AAA), T7 pol synthesizes a transcript that mimics the P2 $tRNA^{Phe}$ precursor in that the 5′ leader of 14 nt starts with a triphosphorylated purine and the 3′ trailer contains the potential hairpin followed by a stretch of U residues. We then followed the fate of this T7P2 RNA, labelled with [α-^{32}P]GTP, after incubation in S-100 extracts that had been through three rounds of anti-La immunodepletion or mock-depletion.

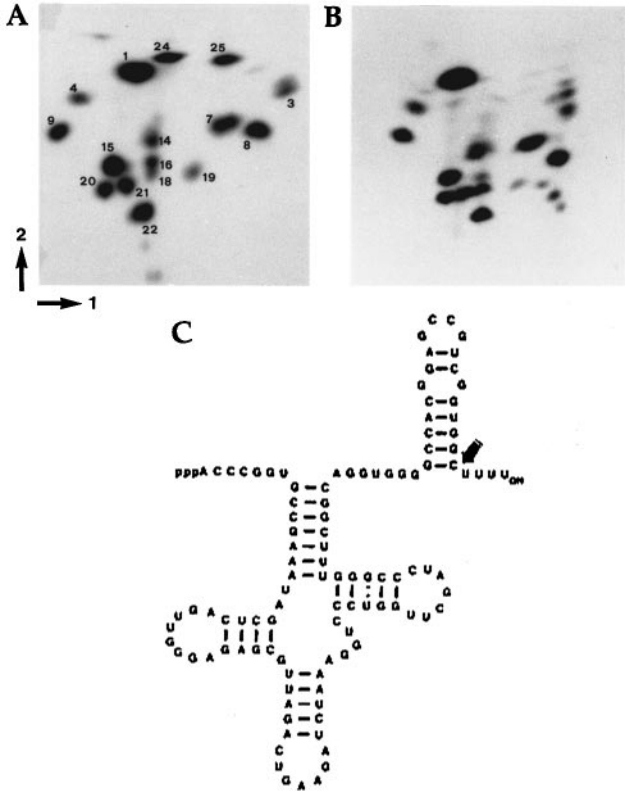

Fig. 3. Sequence of the $tRNA^{Phe}$ gene P2 and Px transcripts. (**A**, **B**) RNase T1 fingerprints of the P2 and Px transcripts, respectively, labelled with [α-^{32}P]UTP. Arrowed numbers indicate the direction of the first and second dimensions. The 3′-terminal spots 24 and 25, $CUUU_{OH}$ and $CUUUU_{OH}$, respectively, are missing from the Px fingerprint. (**C**) Cloverleaf model of the P2 primary transcript with the potential hairpin in its 3′ trailer. The arrow indicates the likely 3′ end of the Px transcript found in La-depleted extracts.

In both kinds of extracts, as with genuine pol III transcripts, processing of T7P2 RNA by RNase P precedes 3′ processing. Thus, the 5′ processed precursor P2a is the only processing intermediate to appear (Figure 4). Again, as with pol III transcription reactions, addition of recombinant La protein stabilizes the transcripts and/or inhibits their 3′ processing. Most importantly, the T7P2 RNAs migrate more rapidly after incubation in La-depleted extracts. Initially these RNAs are quite heterogeneous in length, but with longer incubation they are converted to a discrete T7Px transcript or processed to the mature $tRNA^{Phe}$ P3. Length heterogeneity and T7Px formation are completely suppressed by addition of recombinant La to the La-depleted extracts (Figure 4B).

These results demonstrate that Px RNA can be formed

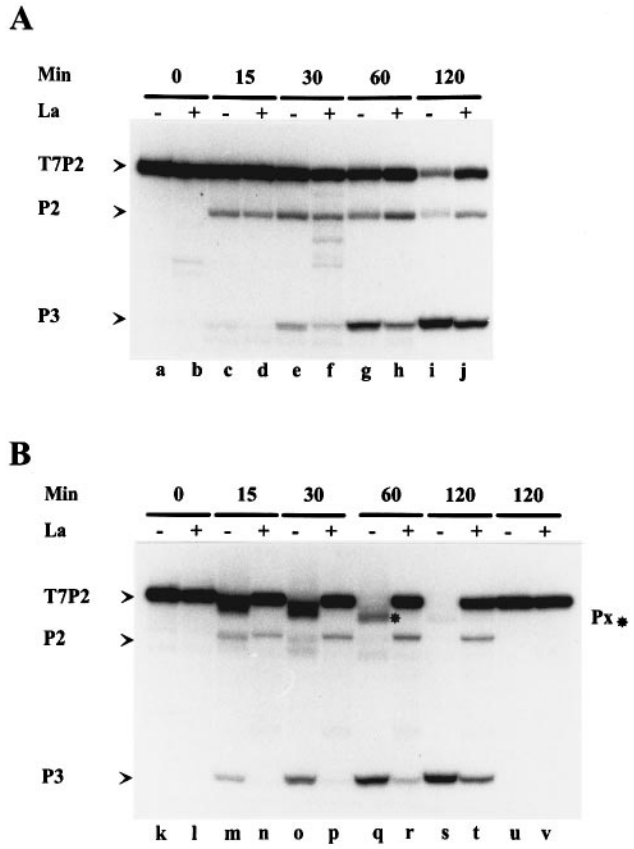

Fig. 4. Stability of a synthetic tRNA^{Phe} precursor in mock-depleted and La-depleted extracts. (**A**, **B**) RNA mimicking the tRNA^{Phe} precursor P2 was synthesized by T7pol and incubated at 20°C for the indicated times with S-100 extracts that had undergone three rounds of depletion with pre-immune serum or immobilized anti-La antibody, respectively. Reactions contained either no additional La protein (–) or 500 ng of recombinant *X.laevis* La (+). RNAs were purified and resolved on a 12% denaturing polyacrylamide gel. The star indicates T7Px, the T7pol transcript found in the La-depleted extract without supplementary La protein.

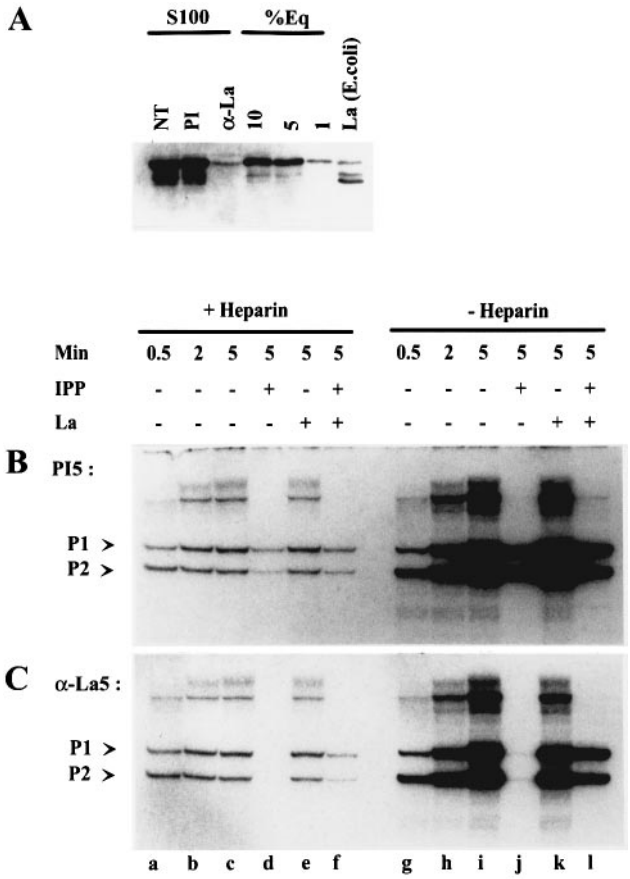

Fig. 5. Transcription of the tRNA^{Phe} gene under single- and multiple-round conditions in mock-depleted and La-depleted extracts. (**A**) Western blot analysis of the extracts probed with a polyclonal anti-La antibody. S-100 extracts were not treated (NT) or subjected to five rounds of depletion with immobilized pre-immune serum (PI) or anti-La antibody (α-La) then concentrated by ultrafiltration. The equivalents of 10%, 5% and 1% of non-treated S-100 extract were loaded on the same gel, together with 50 ng of recombinant *X.laevis* La protein. (**B**, **C**) Transcription products of the tRNA^{Phe} gene during the indicated times at 20°C with mock-depleted and La-depleted extracts, respectively. Reactions contained [α-^{32}P]GTP and either 300 μg/ml heparin (+ Heparin) or no heparin (– Heparin), and either no additional La protein (La –) or 500 ng of recombinant *X.laevis* La protein (La +). Aliquots of the 5 min reactions were also immunoprecipitated with fresh anti-La antibody (IPP +). Transcripts were purified and resolved on a 12% denaturing polyacrylamide gel.

in La-depleted extracts in the absence of any transcription. They strongly suggest that the lack of 3′-terminal U residues from this truncated precursor is the result of nucleolytic degradation of the full-length precursor. The length heterogeneity seen at early incubation times further suggests that this nuclease is a 3′-exonuclease. We suggest that its action is retarded when it encounters the base of the putative stem–loop (Figure 3C), thereby accounting for the Px transcript. Consistent with this interpretation, Px is not detected as a discrete RNA if the tRNA^{Phe} gene template is modified by a trinucleotide substitution that disrupts base-pairing in the putative stem (data not shown).

We conclude that there is no necessity to invoke a role for the *X.laevis* La protein in the synthesis of the 3′-terminal U residues of pol III transcripts. More positively, this protein is able to protect U-ending RNAs from 3′-exonucleolytic attack.

La-depleted extracts perform multiple rounds of pol III transcription

To determine if *X.laevis* La has a transcript release function, we examined the efficiency of pol III transcription in La-depleted extracts in the presence and absence of heparin to distinguish single from multiple rounds of transcription (Kassavetis *et al.*, 1989). Several factors were varied in preliminary experiments to optimize this approach. In the final protocol, the S-100 extracts were stabilized by addition of bovine serum albumin (BSA) to 0.1 mg/ml, endogenous NTPs were severely depleted by centrifugation through Sephadex G-50 spin columns and extracts were subjected to five rounds of anti-La immunodepletion or mock-depletion and then concentrated ~2.5-fold by centrifugation through ultrafiltration units to regain the original protein concentration. This protocol removes >99% of the La protein originally present in the S-100 extract (Figure 5A).

In other preliminary experiments, the concentration of the DNA template was varied to find the point at which pol III transcription reached a plateau; this was achieved at ~4 nM. Keeping the total DNA concentration constant at this level, decreasing amounts of the template were then mixed with pBR327 plasmid DNA in order to engage

the maximum number of template molecules in productive pol III transcription. A combination of 10 ng template (3.34 fmol) and 200 ng pBR327 (94 fmol) generated the same number of $tRNA^{Phe}$ gene transcripts as 300 ng template (100 fmol) in 25 μl reactions (data not shown).

The DNA template was also modified by inverting the T–A base pair located 1 bp upstream of the mature $tRNA^{Phe}$ coding sequence. With this template, the first UMP to be incorporated into the primary transcript is at position 15. Thus, in principle, pol III reactions done in the presence of ATP, CTP and [α-^{32}P]GTP, but not UTP, should generate labelled 14 nt stalled transcripts. In practice, we always detected some longer products, perhaps because of incomplete removal of the endogenous NTPs or the presence of a cytidine deaminase in the extracts. Formation of these longer products could be prevented by including 3′-dUTP in the reactions. These conditions generated ~1 fmol of 3′-blocked 15 nt RNAs, which is very similar to the number of precursors made in the presence of heparin (see below).

In the experiment shown in Figure 5, preinitiation complexes were allowed to assemble for 3 h in the presence of 0.2 mM ATP (Bieker *et al.*, 1985). CTP, UTP, [α-^{32}P]GTP and additional ATP were then added in the presence or absence of 300 μg/ml heparin and in the presence or absence of recombinant La protein. The RNAs made in 0.5, 2 and 5 min were recovered for gel electrophoresis and phosphorimager analysis. Aliquots of the 5 min reactions were also challenged with anti-La antibodies before RNA extraction.

In the presence of heparin, comparable numbers of P1 and P2 RNAs are synthesized in La-depleted and mock-depleted extracts (1.13 and 1.07 fmol, respectively). Most of this synthesis occurs within 0.5 min and is complete within 2 min (Figure 5, lanes a and b). These results indicate that transcription is limited to a single round and that 33–35% of the DNA template is committed. In contrast with the RNAs made in the mock-depleted extract, none of the precursors made in the La-depleted extract is immunoprecipitable by anti-La antibodies (Figure 5, lanes c and d). The presence of recombinant La protein has no effect on transcript levels but precursors are now immunoprecipitable from both extracts (lanes c–f).

In the absence of heparin, transcripts accumulate in a time-dependent manner in both kinds of extract (Figure 5, lanes g–i). After 5 min, the La-depleted extract contains 29 fmol of P1 and P2 RNA; the corresponding value for the mock-depleted extract is 30 fmol. Since heparin inhibits reinitiation, these transcript levels imply that each committed DNA template undergoes ~25–28 rounds of transcription in 5 min. Many of the RNAs made in the mock-depleted extract are immunoprecipitable by anti-La antibodies, whereas only a very minor fraction of the precursors made in the La-depleted extract appears to be immunoprecipitable, and even this may be a gel artefact (Figure 5B, lanes i and j). As with the single-round conditions, transcription efficiency is unaffected by the presence of recombinant La, but most newly synthesized RNAs are now immunoprecipitable when challenged with anti-La antibodies (Figure 5B, lanes i–l).

These results demonstrate that *X.laevis* cell-free extracts containing <1% of the normal La protein content are capable of efficient pol III transcription under both single-round and multiple-round conditions. Few, if any, of the transcripts made in depleted extracts under multiple-round conditions are associated with La. This strongly suggests that the residual La protein in such extracts is not functioning to strip nascent transcripts from their template.

Nascent pol III transcripts are released from template DNA in La-depleted extracts

To test the latter suggestion more directly, we incubated La-depleted and mock-depleted extracts with a DNA template immobilized on magnetic streptavidin beads and then asked if the newly synthesized pol III transcripts are associated with the template. For the experiment shown in Figure 6, extracts were again subjected to five rounds of anti-La immunodepletion or mock-depletion, concentrated, and incubated with the immobilized template and 0.2 mM ATP for 3 h. The preinitiation complexes were then either rinsed or not with transcription buffer (which contains 70 mM KCl). After 1 h of transcription in the presence of [α-^{32}P]GTP, newly synthesized RNAs contained in the total reactions, supernatants and attached to the beads were analysed on a denaturing gel. Aliquots of the supernatant fractions were also challenged with anti-La antibodies before RNA extraction.

In both mock-depleted and La-depleted extracts containing <1% of the normal La protein content, the vast majority of the transcripts are found in the supernatant fraction, i.e. released from the template, irrespective of whether or not the preinitiation complexes are rinsed (Figure 6, compare lanes a, b and d; e, f and h; i, j and l; m, n and p). Most of the precursors made from unrinsed preinitiation complexes in mock-depleted extracts are immunoprecipitable with anti-La antibodies (Figure 6, lanes b and c) whereas this is true of only a minor fraction of precursors made in the comparable La-depleted extract (Figure 6, lanes j and k). Rinsing the preinitiation complexes made in La-depleted extracts abolishes this minor fraction without affecting the efficiency of transcript release (Figure 6, lanes m, n and o). It is interesting to note that some mature tRNA is found under these conditions but not after rinsing the preinitiation complexes from mock-depleted extracts (Figure 6, compare lanes m and n with lanes e and f). This is consistent with the earlier observation (Figures 2 and 4) that processing is more rapid in the absence of La. More importantly, these results provide no evidence that *X.laevis* La functions as a pol III transcript release factor.

Discussion

La and pol III transcription

The preferential binding of human La to 3′-U residues (Stefano, 1984), the hallmark of most nascent pol III transcripts, was an early indicator that this protein may be involved in pol III transcription termination. Subsequent work with human cell-free extracts has lent support to that idea and has resulted in some specific proposals, namely that La not only binds to the 3′-U residues of nascent pol III transcripts but is also required for their synthesis (Gottlieb and Steitz, 1989a) and for the subsequent release of the transcripts from the DNA template (Gottlieb and Steitz, 1989b; Maraia *et al.*, 1994). Most recently, human La has been invoked as a pol III initiation

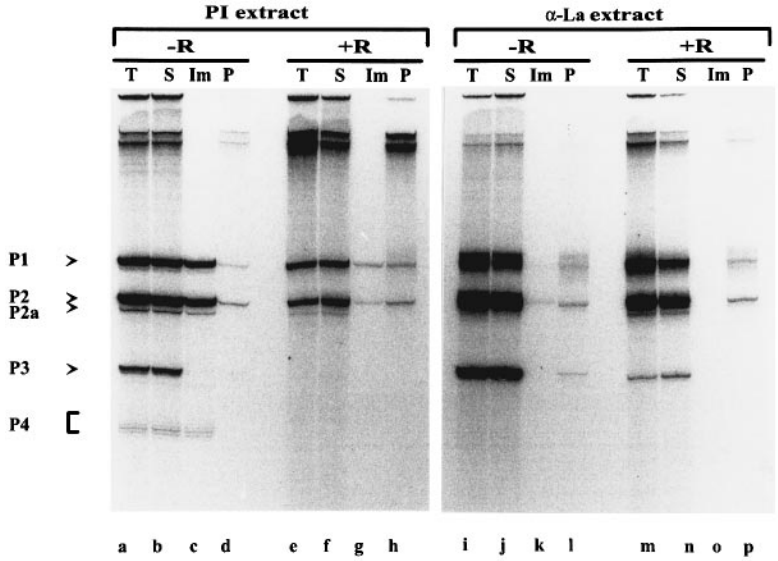

Fig. 6. Transcription of the tRNA^{Phe} gene immobilized on magnetic streptavidin beads in mock-depleted and La-depleted extracts. S-100 extracts were subjected to five rounds of depletion with immobilized pre-immune serum (PI) or anti-La antibody (α-La) and then were concentrated by ultrafiltration. The La-depleted extract contained <1% of the normal content of La protein (as Figure 5A, data not shown). Preinitiation complexes were either rinsed or not with transcription buffer (+R, –R) and pol III transcription was allowed to proceed for 1 h at 20°C in the presence of [α-^{32}P]GTP. Transcripts found in the total reaction (T), supernatant (S) and pellet (P) were recovered. A fourth aliquot was immunoprecipitated with fresh anti-La antibody (Im). Transcripts were purified and resolved on a 12% denaturing polyacrylamide gel.

factor (Maraia, 1996) whose activity depends on a particular residue, Ser366, being dephosphorylated (Fan *et al.*, 1997) and on the integrity of a nearby basic region of the protein (Goodier *et al.*, 1997).

Our interest in the *X.laevis* La protein also stemmed from the hope that it would function as a pol III termination factor, but the experiments reported here provide no support for that view. Progressive immunodepletion of La from *X.laevis* S-100 extracts reduces pol III transcriptional activity (Figure 2). This effect is not due to the loss of La, however, but rather to the inevitable dilution of the extracts that occurs at each round of immunodepletion, which can be overcome by concentrating the extracts. A pol III transcript lacking 3′-U residues is found in La-depleted extracts (Figures 2 and 3), but the 3′-U residues of a presynthesized T7pol transcript are also missing after incubation in La-depleted extracts (Figure 4). This strongly suggests that the 3′ truncated pol III transcript results from nucleolytic degradation of the primary transcript rather than incomplete synthesis on a stalled transcription complex. Extracts containing <1% of the normal content of La protein sustain very efficient levels of pol III transcription, under both single- and multiple-round conditions (Figure 5), with the vast majority of these full-length tRNA precursors being released from the template without being associated with La (Figure 6). Similarly, HeLa extracts depleted of La are competent for transcription of the adenovirus VAI RNA gene and the resulting transcripts are not precipitable with anti-La antibodies (Francoeur and Mathews, 1982). In addition, both *X.laevis* and calf thymus pol III seem capable of accurate and efficient termination, polymerase recycling and transcript release in the absence of other proteins (Cozzarelli *et al.*, 1983; Watson *et al.*, 1984; Campbell and Setzer, 1992). Together, these results provide no evidence for La functioning as a pol III initiation, termination or release factor in *X.laevis*, and they question such proposed roles for La in other vertebrates.

In vitro studies also provide no evidence for La functioning as a pol III transcription factor in *Saccharomyces cerevisiae* (Kassavetis *et al.*, 1995; Dieci and Sentenac, 1996). In particular, in the presence of TFIIIB and TFIIIC, limiting quantities of pol III preferentially reinitiate on tRNA gene templates that have already undergone transcription (Dieci and Sentenac, 1996). This facilitated recycling pathway also generates multiple-round transcripts of the yeast U6 gene; the template in this case requires only recombinant TFIIIB and highly purified pol III. If an accessory factor were needed for each transcript made, and not just for each template used, it should be present in substantial amounts in the polymerase fraction, yet highly purified yeast pol III preparations show little sign of even sub-stoichiometric amounts of additional polypeptides (Huet *et al.*, 1996). More specific to La, there is no difference in pol III activity between extracts of *S.cerevisiae* strains carrying wild-type or chromosomally disrupted La genes (Yoo and Wolin, 1997). As noted earlier, La is not needed for viability in either *S.cerevisiae* or *Schizosaccharomyces pombe*, so no essential pol III gene can depend on La for its expression in either yeast species. In *S.cerevisiae*, efficient pol III termination requires longer T tracts than are found in vertebrates (Allison and Hall, 1985), perhaps eliminating the need for an accessory termination factor.

The yeast La homologues are significantly shorter than human La; the three proteins are most related at their N-termini and the yeast proteins may be missing some C-terminal residues implicated in transcription in the human protein (aligned in Van Horn *et al.*, 1997). The frog and human La sequences are much more related. In fact there are two distinct forms of La in *X.laevis*, the result of an ancient whole genome duplication and

subsequent sequence drift (Bisbee *et al.*, 1977). The two frog proteins are 90% identical (95% similar) and more than 61% identical (⩾74% similar) to human La (Scherly *et al.*, 1993). Both *X.laevis* La proteins contain a serine at position 366 (and also one at 367) corresponding to the phosphorylated residue in the human protein (Fan *et al.*, 1997). Given this high degree of relatedness, it is difficult to reconcile the disparate pol III transcription results obtained with the frog and human La proteins. Contributing factors could include the different DNA templates, extracts and experimental protocols used. We emphasize that the $tRNA^{Phe}$ gene used here does not possess an intrinsically strong termination site, that perhaps has no need of a factor required at less efficient sites. On the contrary, this gene was chosen because significant read-through occurs beyond its T4 termination site (Figure 2). Read-through at this site is neither increased by La depletion nor decreased by addition of recombinant, presumably unphosphorylated La protein (Figures 2, 5 and 6).

Hence, despite their sequence similarities, frog and human La proteins seem to have distinctly different properties with respect to pol III transcription. Alternatively, they may have similar properties and the residual <1% La protein remaining in La-depleted *X.laevis* extracts may be sufficient for a transcriptional function(s). We cannot exclude the latter possibility but the primary pol III transcripts made in La-depleted *X.laevis* extracts are released from the DNA template without being associated with La (Figures 5 and 6). This strongly suggests that the residual La in these extracts does not have a transcript release function. Moreover, if <1% of the normal La content is sufficient for a transcriptional function(s), the remainder is presumably needed for another, even more important purpose.

La and other functions

Other functions have indeed been ascribed to the La protein. For *X.laevis* La, these include retaining certain pol III transcripts in the nucleus (Boelens *et al.*, 1995; Simons *et al.*, 1996) and mediating RNA import from the cytoplasm to the nucleus (Grimm *et al.*, 1997). Human La has been implicated in regulating the activation of interferon-inducible protein kinase PKR (Xiao *et al.*, 1994), the stabilization of histone mRNAs (McLaren *et al.*, 1997) and in internal translation initiation of poliovirus (reviewed in Belsham and Sonenberg, 1996) and hepatitis C virus (Ali and Siddiqui, 1997). Evidence is accumulating that human and frog La proteins may have similar roles in the translation of some cellular mRNAs (McBratney and Sarnow, 1996; Peek *et al.*, 1996; Pellizzoni *et al.*, 1996).

Few of these functions readily account for the high concentration of La in eukaryotic nuclei. Recently, however, La has been found to be involved in yeast tRNA maturation *in vivo*. In both *S.cerevisiae* and *S.pombe*, binding of La to many tRNA precursors is needed for the correct endonucleolytic removal of their 3′ trailer sequences (Van Horn *et al.*, 1997; Yoo and Wolin, 1997). In the absence of La, these precursors are degraded by a 3′-exonuclease(s) (Yoo and Wolin, 1997) whereas the normal processing pattern can be re-established in *S.pombe* cells genetically depleted of La by introducing the human or yeast La cDNAs (Van Horn *et al.*, 1997). The experiments reported here are consistent with these results. Bona fide and synthetic tRNA precursors are subject to 3′-exonuclease attack in La-depleted *X.laevis* extracts (Figures 2–4). Addition of recombinant La prevents this exonucleolytic degradation of the precursors while stabilizing the endonucleolytically cut 3′ trailers (Figure 2). Overexpression of La in *S.pombe* appears to stabilize pol III transcripts and/or slow the rate of their processing (Van Horn *et al.*, 1997). The same effects are seen *in vitro* with an excess of recombinant *X.laevis* La, independent of pol III transcription (Figures 2 and 4).

In conclusion, the one function that appears to be shared between yeast, frog and human La proteins is an involvement in tRNA processing. Since La binding is not restricted to tRNA precursors, it may well be involved in the protection or maturation of other RNA species. A conserved RNA chaperone function may best explain the high nuclear abundance of these enigmatic proteins.

Materials and methods

Purification of recombinant LaB1

Full-length *X.laevis* LaB1 cDNA (Scherly *et al.*, 1993) was cloned behind the λ_{PL} promoter in the heat-inducible vector CBA (gift of B.Allet) and transfected into *E.coli* HB101 cells. 100 ml L-broth containing 5 μg/ml tetracycline was inoculated with a single colony and was shaken overnight at 28°C, then mixed with 300 ml L-broth and shaken for 3 h at 42°C. Cells were harvested and sonicated on ice several times in phosphate-buffered saline (PBS), then in PBS containing 6 M urea. The extract was progressively diluted with 25 mM Tris–HCl pH 8, 3 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM DTT (B buffer) containing 0.1 M KCl, until the urea concentration was <0.1 M. It was then loaded on a 1.5 ml CL4B heparin–Sepharose column (Pharmacia). The column was rinsed with 0.1 M KCl in B buffer and then step-eluted with increasing concentrations of KCl in B buffer. Full-length La protein (49 kDa) was eluted at 0.4 M KCl, together with two proteolytic fragments of ~42 and ~39 kDa (Figure 5A).

Immunodepletion

Polyclonal rabbit antibodies were raised against recombinant La protein in the heparin–Sepharose 0.4 M KCl fraction and purified on protein A–Sepharose columns (Bio-Rad). S-100 extracts (Weil *et al.*, 1979) were prepared from a *X.laevis* kidney cell line as described (Koski *et al.*, 1980). 0.1 ml of packed protein A–agarose beads (Boehringer Mannheim) was suspended in 0.4 ml of 30 mM HEPES pH 7.9, 3 mM $MgCl_2$, 140 mM KCl (S buffer), and the suspension was mixed gently with 0.1 ml pre-immune serum or anti-La IgG by end-over-end rotation for at least 2 h at 4°C. The beads were centrifuged for 3–4 s at 14 000 *g* and then washed three times with 1 ml S buffer. 0.4 ml S-100 extracts containing 0.1 mg/ml BSA was gently mixed with 0.1 ml antibody-coupled beads for 20 min at 4°C as above. This step was repeated two to four times and the extracts were then transferred to UFCMC ultrafiltration units (Millipore) and centrifuged at 2000 *g* for 1–1.5 h at 4°C to achieve an ~2.5-fold concentration.

Immunoprecipitation

Antibodies were coupled to protein A–agarose as described above in 50 mM Tris–HCl pH 8, 150 mM NaCl, 0.1% NP-40 (NET150 buffer). The beads were mixed with transcription reactions by end-over-end rotation for 1 h at 4°C, and then washed three times with NET150 buffer. Bead-associated RNAs were purified by phenol extraction and resolved on 12% denaturing polyacrylamide gels.

Immunoblotting

Proteins were resolved on 12.5% polyacrylamide–SDS gels and then transferred to nylon membranes (Boehringer Mannheim) in 25 mM Tris, 192 mM glycine, 20% methanol in a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad) for 16 h at 30 V and 4°C. Membranes were soaked for 1.5 h in 2% blocking reagent (Boehringer Mannheim) in 0.1 M Tris–HCl pH 7.5, 0.15 M NaCl (TBS), then incubated for 1 h with anti-La serum diluted to 1/250 with TBS containing 0.1% Tween 20. Blots were washed, then incubated for 0.5 h with an alkaline phosphatase-conjugated anti-rabbit antibody (Promega) diluted to 1/10

000 in the same buffer. The nylon sheets were incubated for 1 min with AMPPD (Boehringer Mannheim) diluted to 1/100 in 0.1 M Tris–HCl pH 9.5, 0.1 M NaCl, 0.05 M $MgCl_2$, dried briefly then incubated 15 min at 37°C before autoradiography.

In vitro transcription

Clone pJ52 contains the *X.laevis* $tRNA^{Phe}$ gene in pBR322 with the natural T15 terminator of the TyrD gene (Gouilloud and Clarkson, 1986) placed behind the weaker T4 termination site of the $tRNA^{Phe}$ gene. Clone pJ53 is derived from pJ52 and it contains a T→A transversion immediately before the mature tRNA coding sequence so that the first U residue is incorporated at position +15. Clone pPheDra contains the *X.laevis* $tRNA^{Phe}$ gene behind the T7 promoter of the pGEM4 vector and a series of A residues downstream of the T4 tract.

For T7pol transcription, clone pPheDra was linearized after the T4 tract with *Dra*I and then incubated for 1 h at 37°C with T7pol in 40 mM Tris–HCl pH 7.5, 5 mM $MgCl_2$, 10 mM DTT, 1 mM each of ATP, CTP and UTP, 0.1 mM GTP, 10 µCi [α-^{32}P]GTP.

Pol III transcription was performed with S-100 somatic extracts in 20 mM HEPES–KOH pH 7.9, 70 mM KCl, 5 mM $MgCl_2$ (T buffer) containing 0.5 mM DTT, 10 mM creatine phosphate, one nucleoside α-^{32}P triphosphate at 25 µM and the remaining three NTPs at 200 µM, as described previously (Mazabraud *et al.*, 1987). Reactions were incubated at 20°C for the stated times. In some experiments, the template was incubated with extract previously passed through a G-50 spin column to remove endogenous NTPs for 3 h at 20°C in the presence of $MgCl_2$ and ATP to allow assembly of preinitiation transcription complexes (Bieker *et al.*, 1985). For single-round transcription conditions, reactions contained 300 µg/ml heparin to prevent reinitiation (Kassavetis *et al.*, 1989). RNAs were purified by phenol extraction and resolved on 12% denaturing polyacrylamide gels.

In vitro transcription with immobilized templates

Clone pJ52 was linearized with *Eco*RI, and the site filled in with the Klenow polymerase in the presence of Biotin-7dATP (Gibco-BRL) as described previously (Arias and Dynan, 1989). Non-incorporated NTPs were removed on G-50 Sephadex columns and, after digestion with *Sca*I, the 2079 bp fragment containing the $tRNA^{Phe}$ gene was incubated overnight at 4°C with magnetic streptavidin beads (Dynal) in 10 mM Tris–HCl pH 7.6, 1 M NaCl, 1 mM EDTA. The distance between the bead attachment site and the transcription start site is ~90 bp. After coupling, beads were washed three times with the above buffer, then three times with T buffer containing 10% glycerol and 250 µg/ml BSA. To form preinitiation complexes, 1.5×10^6 DNA coupled beads were incubated with 10 µl of S-100 extract for 2 h at 20°C in T buffer containing 0.5 mM DTT, 0.2 mM ATP and 250 µg/ml BSA in a total volume of 20 µl. When indicated, preinitiation transcription complexes were washed three times with 200 µl of T buffer with the supplements described above. Transcription was initiated with 25 µl of T buffer containing 0.5 mM DTT, 10 mM creatine phosphate, 200 µM ATP, 100 µM each of CTP and UTP, 25 µM GTP and 10 µCi [α-^{32}P]GTP. Reactions were performed for 1 h at 20°C. RNAs from the released and retained fractions were phenol extracted, precipitated and resolved on 12% denaturing polyacrylamide gels.

RNA sequence analyses

RNA from gel slices was digested with RNase T1, then fingerprinted by ionophoresis on cellulose acetate strips at pH 3.5 followed by homochromatography on polyethyleneimine–cellulose thin layers. These analyses and secondary analyses of the T1 oligonucleotides were performed as described previously (Koski and Clarkson, 1982).

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References

Ali,N. and Siddiqui,A. (1997) The La antigen binds 5′ noncoding region of the hepatitis C virus RNA in the context of the initiator AUG codon and stimulates internal ribosome entry site-mediated translation. *Proc. Natl Acad. Sci. USA*, **94**, 2249–2254.

Allison,D.S. and Hall,B.D. (1985) Effects of alterations in the 3′ flanking sequences on *in vivo* and *in vitro* expression of the yeast *SUP-4o* $tRNA^{Tyr}$ gene. *EMBO J.*, **4**, 2657–2664.

Alspaugh,M.A. and Tan,E.M. (1975) Antibodies to cellular antigens in Sjögren's syndrome. *J. Clin. Invest.*, **55**, 1067–1073.

Andrews,N.C. and Baltimore,D. (1986) Purification of a terminal uridyltransferase that acts as host factor in the *in vitro* poliovirus replicase reaction. *Proc. Natl Acad. Sci. USA*, **83**, 221–225.

Arias,J.A. and Dynan,W.S. (1989) Promoter-dependent transcription by RNA polymerase II using immobilized enzyme complexes. *J. Biol. Chem.*, **264**, 3223–3229.

Belsham,G.J. and Sonenberg,N. (1996) RNA–protein interactions in regulation of picornavirus RNA translation. *Microbiol. Rev.*, **60**, 499–511.

Bieker,J.J., Martin,P.L. and Roeder,R.G. (1985) Formation of a rate-limiting intermediate in 5S RNA gene transcription. *Cell*, **40**, 119–127.

Bisbee,C.A., Baker,M.A., Wilson,A.C., Hadji-Azimi,I. and Fischberg,M. (1977) Albumin phylogeny for clawed frogs (*Xenopus*). *Science*, **195**, 785–787.

Boelens,W.C., Palacios,I. and Mattaj,I.W. (1995) Nuclear retention of RNA as a mechanism for localization. *RNA*, **1**, 273–283.

Bogenhagen,D.F. and Brown,D.D. (1981) Nucleotide sequences in *Xenopus* 5S DNA required for transcription termination. *Cell*, **24**, 261–270.

Campbell,F.E. and Setzer,D.R. (1992) Transcription termination by RNA polymerase III: uncoupling of polymerase release from termination signal recognition. *Mol. Cell. Biol.*, **12**, 2260–2272.

Chambers,J.C., Kenan,D., Martin,B.J. and Keene,J.D. (1988) Genomic structure and amino acid sequence domains of the human La autoantigen. *J. Biol. Chem.*, **263**, 18043–18051.

Chan,E.K.L., Sullivan,K.F. and Tan,E.M. (1989) Ribonucleoprotein SS-B/La belongs to a protein family with consensus sequences for RNA binding. *Nucleic Acids Res.*, **17**, 2233–2244.

Cozzarelli,N.R., Gerrard,S.P., Schlissel,M., Brown,D.D. and Bogenhagen,D.F. (1983) Purified RNA polymerase III accurately and efficiently terminates transcription of 5S RNA genes. *Cell*, **34**, 829–835.

Dieci,G. and Sentenac,A. (1996) Facilitated recycling pathway for RNA polymerase III. *Cell*, **84**, 245–252.

Fan,H., Sakulich,A.L., Goodier,J.L., Zhang,X., Qin,J. and Maraia,R.J. (1997) Phosphorylation of the human La antigen on serine 366 can regulate recycling of RNA polymerase III transcription complexes. *Cell*, **88**, 707–715.

Francoeur,A.M. and Mathews,M.B. (1982) Interaction between VA RNA and the lupus antigen La: formation of a ribonucleoprotein particle *in vitro*. *Proc. Natl Acad. Sci. USA*, **79**, 6772–6776.

Goodier,J.L., Fan,H. and Maraia,R.J. (1997) A carboxy-terminal basic region controls RNA polymerase III transcription factor activity of human La protein. *Mol. Cell. Biol.*, **17**, 5823–5832.

Gottlieb,E. and Steitz,J.A. (1989a) The RNA binding protein La influences both the accuracy and efficiency of RNA polymerase III transcription *in vitro*. *EMBO J.*, **8**, 841–850.

Gottlieb,E. and Steitz,J.A. (1989b) Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. *EMBO J.*, **8**, 851–861.

Gouilloud,E. and Clarkson,S.G. (1986) A dispersed tRNA gene from *Xenopus laevis* with high transcriptional activity *in vitro*. *J. Biol. Chem.*, **261**, 486–494.

Grimm,C., Lund,E. and Dahlberg,J.E. (1997) *In vivo* selection of RNAs that localize in the nucleus. *EMBO J.*, **16**, 793–806.

Huet,J., Manaud,N., Dieci,G., Peyroche,G., Conesa,C., Lefebvre,O., Ruet,A., Riva,M. and Sentenac,A. (1996) RNA polymerase III and class III transcription factors from *Saccharomyces cerevisiae*. *Methods Enzymol.*, **273**, 249–267.

Kassavetis,G.A., Riggs,D.L., Negri,R., Nguyen,L.H. and Geiduschek,E.P. (1989) Transcription factor IIIB generates extended DNA interactions in RNA polymerase III transcription complexes on tRNA genes. *Mol. Cell. Biol.*, **9**, 2551–2566.

Kassavetis,G.A., Nguyen,S.T., Kobayashi,R., Kumar,A., Geiduschek,E.P. and Pisano,M. (1995) Cloning, expression, and function of *TFC5*, the gene encoding the B′′ component of the *Saccharomyces cerevisiae* RNA polymerase III transcription factor TFIIIB. *Proc. Natl Acad. Sci. USA*, **92**, 9786–9790.

Koski,R.A. and Clarkson,S.G. (1982) Synthesis and maturation of *Xenopus laevis* methionine tRNA gene transcripts in homologous cell-free extracts. *J. Biol. Chem.*, **257**, 4514–4521.

Koski,R.A., Clarkson,S.G., Kurjan,J., Hall,B.D. and Smith,M. (1980) Mutations of the yeast *SUP4* $tRNA^{Tyr}$ locus: transcription of the mutant genes *in vitro*. *Cell*, **22**, 415–425.

Lin-Marq,N. and Clarkson,S.G. (1995) A yeast RNA binding protein that resembles the human autoantigen La. *J. Mol. Biol.*, **245**, 81–85.

Maraia,R.J. (1996) Transcription termination factor La is also an initiation factor for RNA polymerase III. *Proc. Natl Acad. Sci. USA*, **93**, 3383–3387.

Maraia,R.J., Kenan,D.J. and Keene,J.D. (1994) Eukaryotic transcription termination factor La mediates transcript release and facilitates reinitiation by RNA polymerase III. *Mol. Cell. Biol.*, **14**, 2147–2158.

Mattioli,M. and Reichlin,M. (1974) Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. *Arthritis Rheum.*, **17**, 421–429.

Mazabraud,A., Scherly,D., Müller,F., Rungger,D. and Clarkson,S.G. (1987) Structure and transcription termination of a lysine tRNA gene from *Xenopus laevis*. *J. Mol. Biol.*, **195**, 835–845.

McBratney,S. and Sarnow,P. (1996) Evidence for involvement of *trans*-acting factors in selection of the AUG start codon during eukaryotic translational initiation. *Mol. Cell. Biol.*, **16**, 3523–3534.

McLaren,R.S., Caruccio,N. and Ross,J. (1997) Human La protein: a stabilizer of histone mRNA. *Mol. Cell. Biol.*, **17**, 3028–3036.

Müller,F., Clarkson,S.G. and Galas,D.J. (1987) Sequence of a 3.18 kb tandem repeat of *Xenopus laevis* DNA containing 8 tRNA genes. *Nucleic Acids Res.*, **15**, 7191.

Peek,R., Pruijn,G.J.R. and van Venrooij,W.J. (1996) Interaction of the La(SS-B) autoantigen with small ribosomal subunits. *Eur. J. Biochem.*, **236**, 649–655.

Pellizzoni,L., Cardinali,B., Lin-Marq,N., Mercanti,D. and Pierandrei-Amaldi,P. (1996) A *Xenopus laevis* homologue of the La autoantigen binds the pyrimidine tract of the 5′ UTR of ribosomal protein mRNAs *in vitro*: implication of a protein factor in complex formation. *J. Mol. Biol.*, **259**, 904–915.

Scherly,D., Stutz,F., Lin-Marq,N. and Clarkson,S.G. (1993) La proteins from *Xenopus laevis*. cDNA cloning and developmental expression. *J. Mol. Biol.*, **231**, 196–204.

Simons,F.H.M., Rutjes,S.A., van Venrooij,W. and Pruijn,G.J.M. (1996) The interactions with Ro60 and La differentially affect nuclear export of hY1 RNA. *RNA*, **2**, 264–273.

Stefano,J.E. (1984) Purified lupus antigen La recognizes an oligouridylate stretch common to the 3′ termini of RNA polymerase III transcripts. *Cell*, **36**, 145–154.

Topfer,F., Gordon,T. and McCluskey,J. (1993) Characterization of the mouse autoantigen La. *J. Immunol.*, **150**, 3091–3100.

Van Horn,D.J., Yoo,C.J., Xue,D., Shi,H. and Wolin,S.L. (1997) The La protein in *Schizosaccharomyces pombe*: a conserved yet dispensable phosphoprotein that functions in tRNA maturation. *RNA*, **3**, 1434–1443.

Watson,J.B., Chandler,D.W. and Gralla,J.D. (1984) Specific termination of *in vitro* transcription by calf thymus RNA polymerase III. *Nucleic Acids Res.*, **12**, 5369–5384.

Weil,P.A., Segall,J., Harris,B., Ng,S.-Y. and Roeder,R.G. (1979) Faithful transcription of eukaryotic genes by RNA polymerase III in systems reconstituted with purified templates. *J. Biol. Chem.*, **254**, 6163–6173.

White,R.J. (1994) *RNA Polymerase III Transcription.* R.G.Landes, Austin, Texas, USA.

Xiao,Q., Sharp,T.V., Jeffrey,I.W., James,M.C., Pruijn,G.J.M., van Venrooij,W. and Clemens,M.J. (1994) The La antigen inhibits the activation of the interferon-inducible protein kinase PKR by sequestering and unwinding double-stranded RNA. *Nucleic Acids Res.*, **13**, 2512–2518.

Yoo,C.J. and Wolin,S.L. (1994) La proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae*: a yeast homolog of the La autoantigen is dispensable for growth. *Mol. Cell. Biol.*, **14**, 5412–5424.

Yoo,C.J. and Wolin,S.L. (1997) The yeast La protein is required for the 3′ endonucleolytic cleavage that matures tRNA precursors. *Cell*, **89**, 393–402.

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