Leucyl-tRNA Synthetase-dependent and -independent Activation of a Group I Intron

Michal T. Boniecki, Seung Bae Rho, Mikhail Tukalo, Jennifer L. Hsu, Eliana P. Romero, and Susan A. Martinis

From the Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

Leucyl-tRNA synthetase (LeuRS) is an essential RNA splicing factor for yeast mitochondrial introns. Intracellular experiments have suggested that it works in collaboration with a maturase that is encoded within the bI4 intron. RNA deletion mutants of the large bI4 intron were constructed to identify a component folded intron for biochemical analysis. The minimized bI4 intron was active in RNA splicing and contrasts with previous proposals that the canonical core of the bI4 intron is deficient for catalysis. The activity of the minimized bI4 intron was enhanced in vitro by the presence of the bI4 maturase or LeuRS.

Although the aminoacyl-tRNA synthetases (aaRSs) are best known for their role in protein synthesis, many have functionally expanded and are essential to a wide range of other cellular activities that are unrelated to tRNA aminoacylation (1). The class I aaRSs, leucyl-(LeuRS or NAM2) and tyrosyl-tRNA synthetase (TyrRS or CYT-18) are required for RNA splicing of cognate group I introns in the mitochondria of certain lower eukaryotes (2). In yeast, processing of two related group I introns called bI4 and aI4 (Fig. 1) from the cob and cox1 genes, respectively, require yeast mitochondrial LeuRS (3, 4). Likewise, expression of Neurospora crassa mitochondrial genes, such as those for the large ribosomal RNA, is dependent on TyrRS for excising group I introns (5).

LeuRS facilitates RNA splicing in concert with a bI4 maturase that is encoded within the bI4 intron. Genetic investigations showed that an inactivated bI4 maturase resulting in deficient splicing activity of the bI4 and aI4 group I introns can be rescued by a suppressor mutation of LeuRS to restore mitochondrial respiration (4, 6). In addition, the splicing defect can be compensated by a mutant aI4e DNA endonuclease that is closely related to the bI4 maturase (7, 8).

Previously, we used intracellular three-hybrid assays to demonstrate that LeuRS and bI4 maturase can independently bind to the bI4 intron and stimulate RNA splicing activity in the non-physiological yeast nucleus compartment (9). RNA-dependent two-hybrid assays also supported that the bI4 intron could simultaneously bind both the bI4 maturase and LeuRS. In this case, the RNA was co-expressed with LeuRS and bI4 maturase that was fused to either LexA or B42 to generate a two-hybrid response. This suggested that the bI4 intron was bridging these two protein splicing factors. In either the RNA-dependent two-hybrid or three-hybrid assays, bI4 intron splicing occurred only in the presence of LeuRS or bI4 maturase or both.

We hypothesized that the bI4 maturase and LeuRS bind to distinct sites of the bI4 intron to form a ternary complex and promote efficient splicing activity. However, the functional basis of the collaboration between these two splicing cofactors or how either of them promotes RNA splicing remains unclear.

We sought to characterize the respective splicing roles of the bI4 maturase and LeuRS via biochemical investigations. Previous attempts to develop an in vitro splicing assay for the bI4 intron or its closely related aI4e intron have failed (10, 11). It was hypothesized that the long length of the bI4 intron (~1600 nucleotides) and its highly A:U-rich content (~80%) hindered RNA folding in vitro as well as stabilization of its competent structure.

Efforts to produce an active form of the bI4 intron have relied on building chimeric group I introns by interchanging RNA domains with the more stable Tetrahymena thermophila group I intron (11). Based on these results, it was proposed that the catalytic core of the bI4 group I intron was inherently defective (11). In this case, the group I intron would be expected to be completely dependent on its protein splicing factors similar to the bI3 intron that relies on the bI3 maturase and Mrs1 for activity (12). Thus, it was hypothesized that the bI4 maturase and/or LeuRS splicing factors aided the bI4 group I intron by targeting its core region to compensate for these deficiencies.

We focused our efforts on re-designing the bI4 intron to develop a minimized molecule that might be competent for splicing. Because both the bI4 and aI4e group I introns rely on the bI4 maturase and LeuRS for their splicing activity, we compared their secondary structures to identify and eliminate peripheral regions outside of their catalytic cores. A small active derivative of the bI4 intron, comprised of just 380 nucleotides primarily from the canonical core, was generated. Thus, we...
**bl4 Intron Splicing Activity**

show that, in and of itself, the canonical core of this group I intron is competent for splicing. Both the bl4 maturase and LeuRS enhance the splicing activity of the minimized bl4 intron. However, it is possible that protein-dependent splicing of the bl4 intron represents an intermediate evolutionary step in which the RNA activity is becoming increasingly dependent on its protein splicing factors.

**EXPERIMENTAL PROCEDURES**

RNA Production—Plasmids were constructed as described under supplemental information and then isolated from *Escherichia coli* DH5α using a QIAprep minikit, followed by restriction digest with either BamH1 or Sall restriction enzymes. RNA was transcribed using a T7 MEGAscript II kit (Ambion, TX) and concentrations estimated optically. The intron was transcribed using a T7 MEGAscript II kit (Ambion, TX) and 1 mM MgCl2 at 50 °C for 2 min, followed by incubation at 37 °C for 2 min. The intron RNA was re-folded in 1 mM MgCl2 at 50 °C for 2 min, followed by incubation at 32 °C for 10 min (13).

Protein Purification—LeuRS was prepared as described (14). Because KCl enhances yeast LeuRS enzymatic activity (15), 150 mM KCl was incorporated during the purification procedure. The bl4 maturase was expressed with an N-terminal glutathione S-transferase tag from pGEX2T-MAT2 (16) by isopropyl-1-thio-β-D-galactopyranoside induction (1 mM) for 2 h at 37 °C. The cells were suspended in 10 ml 1× PBS with 0.1 mM phenylmethylsulfonyl fluoride, followed by sonication on ice for 3 min. The clear supernatant was incubated with 0.25 g of glutathione-agarose affinity resin (Sigma) (pre-equilibrated in 1× PBS) for 4 h and then washed with 1× PBS. The resin-bound bl4 maturase was cleaved from the GST fusion protein with 18 units of thrombin protease overnight at room temperature with slow mixing. The cleaved bl4 maturase protein was eluted with 1× PBS and stored in 50% glycerol.

Binding Assays—Nitrocellulose membranes were preincubated in binding buffer (10 mM Tris-HCl pH 7.5, 100 mM NaCl, 5 mM MgCl2, 5 mM dithiothreitol, 1 mM EDTA, 100 µg/ml bovine serum albumin, 10% glycerol) that contained 1 mg/ml ribosomal RNA. An aliquot of 100 nm [α-32P]UTP-labeled RNA was incubated with increasing concentrations (0 to 500 nm) of bl4 maturase or LeuRS in 250 µl of binding buffer for 1 h at 37 °C (17). Solutions were filtered on nitrocellulose under vacuum pressure as described (18), air-dried, and visualized using a phosphorimager. The *Kd* was calculated by fitting the data in Equation 1,

\[
Y = \frac{K_d + B_{\text{max}} + X - \sqrt{(K_d + B_{\text{max}} + X)^2 - 4 \times B_{\text{max}} \times X}}{2 \times B_{\text{max}}} \quad \text{(Eq. 1)}
\]

where *Bmax* is maximal binding (GraphPad Prism 4, San Diego, CA).

Splicing Assay—Each in vitro splicing reaction contained 1 µM radiolabeled bl4 intron RNA (0.13 µCi/µl) resuspended in binding buffer (above), 1 mM guanosine, and 1.0 µM bl4 maturase, and/or LeuRS. Reactions were carried out at 37 °C and 10-µl aliquots quenched with 30 µl of 5 M urea, 30 mM EDTA and mixed with 10 µl of RNA-loading dye. The mixture was incubated for 10 min at 65 °C and then electrophoresed on a 6% denaturing polyacrylamide gel overnight at 10 mA current. The radiolabeled bands were visualized and integrated using a phosphorimager. Bands representing the precursor RNA, excised intron, and ligated exons were normalized for each time point by determining the fraction of each relative to the sum intensity of the three bands. In addition, background was subtracted based on the zero time point. Rates and extent of reaction (product/total bands in lane) were calculated using GraphPad Prism 4 via its single exponential function.

**RESULTS**

Comparative Analysis of the bl4 and al4α Group I Introns—The bl4 and al4α introns are closely related group I introns that are dependent on LeuRS and bl4 maturase for excision in vivo (3, 4, 6). Previously, the secondary structures of the canonical cores for both of these group I introns were predicted (Fig. 1) and identified as a subclass called BI1 (19). The bl4 and al4α group I introns also contain small and large RNA inserts. We investigated these inserts in more detail to determine if they might be important to the intron splicing activity or contribute to interactions with either of the protein splicing factors.

Each of the group I introns contains a large insert (>700 nucleotides) at the end of the P8 helix (Fig. 1), which encodes in part, a conserved open reading frame. The bl4 maturase and al4α DNA endonuclease are expressed respectively from the P8 insert of the bl4 and al4α group I introns. However, a limited number of mutations can functionally interchange the activities of these closely related proteins (7, 8). A second shorter 31-nucleotide insert at the end of the bl4 intron P6 stem appears to have diverged compared with the smaller insert at this site in the al4α intron.

The bl4 intron P5 helix contains two inserts, which are missing in the al4α intron structure. The smaller 21-nucleotide insert forms a short hairpin that we hypothesized is involved in a long-range “kissing interaction” (20) with the loop at the end of the P9 helix (Fig. 1). This tertiary interaction that is specific to the bl4 intron might be compensated in the al4α intron by a longer P9 stem that provides greater stability for this domain in close proximity to the splice site. The second 120-nucleotide insert called “hj” within the P5 helix can be folded into its own domain and appears to be stabilized independently by a self-contained long range interaction (Fig. 1).

The 1627-nucleotide long bl4 intron contains the core features that are required for group I intron self-splicing activity (Fig. 1). Its peripheral regions could be important to RNA-protein interactions with the LeuRS or bl4 maturase splicing partners, RNA folding, or a combination of both. To distinguish between these possibilities, we used the comparative structure of the al4α group I intron to systematically construct and characterize a series of bl4 intron mutations that deleted parts or all of these peripheral regions.

A Large Peripheral Domain of the bl4 Intron Is Dispensable for Interactions with Its Splicing Partners—Much of the large size of the bl4 intron is due to the 1016-nucleotide insert into the P8 loop, which encodes the bl4 maturase gene (Fig. 1). To begin mapping RNA-protein interactions we targeted this large maturase gene insert in the bl4 intron for reduction of the P8...
peripheral domain by deletion analysis. The catalytic core of the bI4 intron sequence overlaps with 96 nucleotides of the 5’-end of the predicted gene that encodes the bI4 maturase. Thus, we constructed a series of downstream internal deletions that eliminated the remainder of the maturase open reading frame, which would encode the C-terminal end of the protein. These constructions included reducing the 1016 nucleotide insertion to 712 (bI4Δ304), 463 (bI4Δ553), and 214 (bI4Δ802) nucleotides (Fig. 2A).

Each of the bI4 intron deletions were tested in three-hybrid assays as described previously (9, 21) and retained binding to either LeuRS or bI4 maturase that was fused to LexA (Fig. 2B). RT-PCR analysis of RNA extracted from the yeast cells demonstrated that each of the bI4 intron deletion mutants retained protein-dependent RNA splicing activity (data not shown) as previously found for the full-length LeuRS (9, 21). The three P8 insert deletion mutants were also transcribed in vitro and RNA-protein interactions measured via nitrocellulose filter binding assays. Although the KD values for interactions with bI4 maturase were within 2-fold of the wild type bI4 intron (33 ± 15 nM, Table 1), only a small amount of the bI4 intron appeared to fold competently such that it could form an RNA-protein complex (Fig. 2C). In contrast, in the presence of LeuRS, a much larger fraction of the in vitro transcribed wild type and mutant bI4 intron RNA assembled into an RNA protein complex (Fig. 2D). It is possible that the LeuRS protein splicing factor interacts with or captures a region of the group I intron deletion mutants that is more prone to forming a native structure. The KD for each deletion mutant was similar to the wild type bI4 intron interactions with LeuRS (12 ± 4 nM, Table 1). In either the case of the bI4 maturase or the LeuRS, the P8 insert peripheral domain is dispensable to formation of the RNA-protein complex.

bI4 Intron Minimization Increases Its Intron Activity in Vitro—

We used the bI4Δ802 construct to target two smaller peripheral domains for deletion. We hypothesized that a short 31-nucleotide extension of the P6b helix, which is not found in the aI4 group I intron (Fig. 1) was dispensable for protein-dependent splicing activity and replaced it with a GAAA tetraloop (bI4Δ1048; Fig. 3A). A larger 120-nucleotide insert (hj) into the P5 stem was predicted to fold into a co-axially stacked pair of helices as well as a small stem loop (Fig. 1) and is completely missing in the aI4 intron. This entire region was deleted along with the P6b extension and replaced with a GAAA tetraloop to yield the smallest bI4 intron construct (bI4Δ1168). The small loop that replaced the 1016 nucleotide insertion at the end of the P8 helix was also replaced by a GUAA tetraloop in each of these deletion mutants.

Binding of bI4Δ1048 and bI4Δ1168 mutants to LeuRS was largely unaffected by the additional deletions yielding KD, that were similar to the wild-type bI4 intron-LeuRS interaction (Table 1). Both of the smallest bI4 intron deletion mutants exhibited increased complex formation with the bI4 maturase (Fig. 3C) accompanied by small 2–3-fold increases in the KD (Table 1). We hypothesize that deletion of these peripheral domains aided folding of the canonical bI4 intron core into a competent more native-like structure for protein interactions.
Previous attempts to develop an in vitro bI4 intron splicing assay that was dependent or independent of its protein splicing factors have been unsuccessful (10, 11). We hypothesized that this large group I intron failed to fold effectively into a competent structure that would confer splicing activity. We radiolabeled the smallest bI4/H90041168 deletion mutant during in vitro transcription and re-folded it by heating to 50 °C for 2 min followed by cooling to 32 °C for 10 min in the presence of 1 mM Mg2+/H11001. The re-folded bI4/H90041168 was incubated for 90 min at 37 °C in the presence of 1 mM guanosine and analyzed via gel electrophoresis (Fig. 4). Multiple bands emerged over the bI4/H90041168 incubation time.

To identify authentic group I intron splicing products, a customized RNA ladder (see supplemental information) was used to estimate the size of the expected RNA bands and identify products of the bI4 intron splicing reaction. Two bands were predicted to be the excised intron (234 bases) and ligated exons (146 bases). Both bands were probed by Northern analysis to confirm their identities (data not shown). The junction site of the ligated exons was also confirmed by amplifying the band at 150 bases via RT-PCR (see supplemental information). DNA sequencing showed that the exons were ligated accurately at the correct junction. It is possible that deletions of the bI4 intron resulted in some aberrant RNA splicing activity. However, the minimized bI4/H90041168 group I intron yielded major bands that accurately represented the expected ligated exons and excised intron.

bI4 Intron Splicing in Vitro Is Stimulated by LeuRS and bI4 Maturase—Based on precursor RNA disappearance, the bI4/H90041168 mutant was processed with a $k_{obs}$ of 6 ± 2 x 10^{-3} min^{-1} (Table 2) concomitant with production of the expected bands for excised bI4 intron and ligated B4-B5 exons at about 230 and 140 nucleotides, respectively. We used the splicing active bI4/H90041168 group I intron to determine if we could also probe for biochemical effects of its protein splicing partners. We introduced 1 µM bI4 maturase and 1 µM LeuRS into the splicing reaction for bI4/H90041168, which resulted in a clear 9-fold stimulation of splicing activity (Fig. 5). Under these in vitro conditions, the proteins promoted processing of the group I intron with a $k_{obs}$ of 54 ± 8 x 10^{-3} min^{-1} (Table 2). Significantly, the $k_{obs}$ for the production of ligated B4-B5 exons (50 ± 5 x 10^{-3} min^{-1}) and excised introns (52 ± 8 x 10^{-3} min^{-1}) was similar to processing of the bI4/H90041168. This correlates to previous results with the full-length bI4 intron, which showed that LeuRS and bI4 maturase stimulated splicing activity of the bI4 intron in RNA-dependent two-hybrid assays (9).
The precursor RNA decayed nearly to completion (93%). However, as found for the protein-independent bI4/H9004 splicing reaction, gel analysis (Fig. 5 and supplemental information) indicated that in addition to formation of the expected spliced RNA products, other bands were produced during protein-dependent RNA processing. To probe for nonspecific decay or nuclease activity of the protein preparations, LeuRS and bI4 maturase were incubated with other RNAs such as 5S rRNA (supplemental information) and tRNA (data not shown), which resulted in no visible RNA degradation. We hypothesized that the bI4/H9004 deletion mutants might be prone to aberrant RNA splicing in the absence of certain RNA peripheral domains.

Because we had previously shown in three-hybrid experiments that either LeuRS or bI4 maturase could independently promote RNA splicing (9, 21), we tested these proteins individually using in vitro assays with the active minimized bI4 intron. Protein-dependent splicing of the bI4-Δ1168 group I intron was stimulated by either LeuRS or bI4 maturase, although more significantly by the latter (Fig. 6). However, the addition of both proteins indicated that LeuRS also plays a role in the bI4

TABLE 2

| Pre-RNA | B4-B5 | bI4 Intron |
|---------|-------|-----------|
| kobs × 10⁻³ min⁻¹ | kobs × 10⁻³ min⁻¹ | kobs × 10⁻³ min⁻¹ |
| No proteins | 6 ± 0.2 | ND| ND |
| LeuRS | 29 ± 7 | 25 ± 8 | 51 ± 7 |
| bI4 Maturase | 97 ± 15 | 40 ± 5 | 47 ± 8 |
| LeuRS + bI4 maturase | 54 ± 8 | 50 ± 5 | 52 ± 4 |

a Not determined because of low activity.

The precursor RNA decayed nearly to completion (93%). However, as found for the protein-independent bI4-Δ1168 splicing reaction, gel analysis (Fig. 5 and supplemental information) indicated that in addition to formation of the expected spliced RNA products, other bands were produced during protein-dependent RNA processing. To probe for nonspecific decay or nuclease activity of the protein preparations, LeuRS and bI4 maturase were incubated with other RNAs such as 5S rRNA (supplemental information) and tRNA (data not shown), which resulted in no visible RNA degradation. We hypothesized that the bI4-Δ1168 deletion mutants might be prone to aberrant RNA splicing in the absence of certain RNA peripheral domains.

Because we had previously shown in three-hybrid experiments that either LeuRS or bI4 maturase could independently promote RNA splicing (9, 21), we tested these proteins individually using in vitro assays with the active minimized bI4 intron. Protein-dependent splicing of the bI4-Δ1168 group I intron was stimulated by either LeuRS or bI4 maturase, although more significantly by the latter (Fig. 6). However, the addition of both proteins indicated that LeuRS also plays a role in the bI4

FIGURE 3. bI4 intron P5 and P6 insert deletion mutants. A, bI4Δ802 deletion mutant (See Fig. 2) was used to successively construct deletion mutants in the P6 (bI4Δ1048) and P5 (bI4Δ1168) domain inserts. The abbreviation TL indicates loops where a GAAA (P6 and P5) or GUAA (P8) tetraloop was introduced. Normalized binding curves for bI4 intron wild type and deletion mutants complexed with LeuRS (B) and bI4 maturase (C). Standard error bars represent measurements that were reproduced in at least triplicate.
maturase-dependent splicing reaction. This suggests that the two protein splicing partners play distinct roles.

Bovine serum albumin and also isoleucyl-tRNA synthetase (IleRS), which is closely related to LeuRS, were incorporated into bI4/H90041168 splicing assays, but failed to stimulate RNA splicing activity (Fig. 6 and supplemental information). Thus, even though IleRS has a similar domain architecture including a CP1-editing domain that was shown for LeuRS to be critical to RNA splicing (21), specific features of the LeuRS are required to promote protein-dependent bI4 intron splicing. We also tested the Mss116p helicase, which has been reported to be important for splicing multiple yeast mitochondrial group I and II introns in vivo (22). However, Mss116p did not simulate intron activity (supplemental information), either in the presence or absence of 1 mM ATP, supporting that the bI4 intron splicing activity is cognate to the bI4 maturase and LeuRS.

We calculated the extent of the reaction. The bI4 introns were excised at similar yields whether bI4 maturase, LeuRS, or both protein splicing factors were present (Fig. 7). Exon ligation appeared to be somewhat more efficient if bI4 maturase was present. As indicated above for protein-independent splicing of the bI4/H90041168 group I intron, processing of the precursor RNA also yielded alternate bands, which have not been identified.

Mg\(^{2+}\) Inhibits Splicing Activity of the bI4/H90041168 Intron—Cations, especially Mg\(^{2+}\), are typically incorporated into in vitro reactions to shield the negatively charged phosphate backbone and promote folding of RNA. We tested a range of concentrations of Mg\(^{2+}\) to determine if it would enhance splicing activity of the bI4/H90041168. Although Mg\(^{2+}\) is required for group I intron splicing, concentrations that were higher than 5 mM began to impede activity of the bI4/H90041168 (Fig. 8).
Mg$_{2+}$/H$_{11001}$-dependent inhibition also occurred for protein-dependent splicing activity of the minimized bI4 intron in the presence of the bI4 maturase and LeuRS (Fig. 8). At 100 mM Mg$_{2+}$/H$_{11001}$, protein-dependent splicing was inhibited (data not shown). These results parallel analysis of the CYT-18 splicing factor, which is also inhibited by increasing Mg$^{2+}$ concentrations (23, 24).

**DISCUSSION**

The aaRSs are an ancient family of enzymes that would be expected to have emerged at the origins of protein synthesis (1). This revolution in biology has been hypothesized to serve as a critical transition from an early RNA world to a contemporary cell that is governed by both proteins and RNA molecules (25). It is likely that this evolutionary period capitalized on molecules that were already available. As a result, structural and functional remnants of aaRSs can be found throughout many diverse biochemical pathways (26). More recently, the broad impact of the aaRSs in unusual functions has been highlighted by their connection to heritable diseases (27, 28).

The dependence of some mitochondrial group I intron splicing reactions on LeuRS and TyrRS may reflect this biological transition that encompassed significant evolutionary change and adaptation. For example, TyrRS is an important splicing factor to group I introns that are missing or have lost their P5abc domain (29). This nuclear-encoded housekeeping protein in *Neurospora crassa* has acquired short novel peptides that are critical to its splicing activity (30–34). The idiosyncratic peptides provide a new RNA binding face for TyrRS that is independent of interactions with tRNA and specifically targets the group I intron (34). TyrRS is a dimer, and the intron binds across both subunits. As predicted by extensive footprinting analysis in previous experiments (35, 36), the x-ray co-crystal structure of TyrRS with the bacteriophage Twort orf142-I2 group I intron, shows that the protein interacts with the catalytic core of the intron that is comprised of the P4-P6 and P3-P9 helical domains (34). It does not interact with any RNA peripheral domains outside of the catalytic core.

The peripheral regions of the bI4 intron could also be deleted without sacrificing significant binding by its LeuRS and bI4 maturase splicing partners. While its not yet clear how LeuRS aids in RNA structure formation, it has been shown that TyrRS binds to a transient P4-P6 structure to stabilize an early step in the group I intron folding process (17, 35, 36). It is possible that LeuRS plays a similar role. However, since the monomeric LeuRS and dimeric TyrRS have very different architectures, it is likely that the protein-dependent mechanism that they each rely upon to promote RNA splicing is distinct.

As one example, LeuRSs do not require idiosyncratic features to promote RNA splicing. Rather, an ancient adaptation of LeuRS for tRNA aminoacylation is sufficient for RNA splicing (37). Significantly, LeuRSs that originate from organisms, which are devoid of group I introns, can stimulate splicing activity. LeuRS splicing activity is associated with the CP1 domain that is required for amino acid editing (21), although other domains of LeuRS appear to contribute to the RNA-processing role (14, 37, 38). In contrast, TyrRS does not have an editing domain. As discussed above, TyrRS enzymes that stimulate intron activity rely on small peptide insertions (30–34). These novel insertions form a new RNA binding surface for interactions with the final, folded form of the group I intron that are distinct from the tRNA binding site of TyrRS (34).
bI4 Intron Splicing Activity

We have demonstrated herein that the canonical core of the bI4 intron is inherently active similar to many other group I introns. This contrasts previous hypotheses that the bI4 intron core is defective for catalysis (11). There have been reports of introns, such as the Saccharomyces cerevisiae bI3 group I intron, that have become completely dependent on proteins for splicing activity (12). Similar to the bI4 intron (9), the bI3 intron relies on an intron-encoded protein (bI3 maturase). It also requires a nuclear-encoded protein called Mrs1 (12). However, in the case of the bI4 intron, at least in vitro, it can carry out splicing activity at detectable levels that are enhanced by one or both of its protein splicing factors. Based on rate and extent of reactions, it is clear that the bI4 maturase and LeuRS influence and enhance the splicing activity of the bI4 introns in different ways. This would be expected from these two completely different splicing partners, which appear to work collaboratively. It remains possible that the bI4 intron is becoming increasingly dependent on LeuRS and bI4 maturase and thus would represent an intermediate step in the transition from the RNA world.

Acknowledgments—We thank Drs. A. Lambowitz, S. Woodson, M. Caprara, M. Spies, E. Westhof, L. Jaeger, P. Perlman, and H.-R. Huang for experimental advice, Dr. S. Cusack for interest and support, and Drs. A. Delahodde and P. Perlman for providing plasmids. We thank C. Fernandez and C. Tran for early purification work.

REFERENCES

1. Martinis, S. A., Plateau, P., Cavarelli, J., and Florentz, C. (1999) EMBO J. 18, 4591–4596
2. Lambowitz, A., Caprara, M., Zimmerly, S., and Perlman, P. (1999) in The RNA World, 2nd Ed. (Gesteland, R. F., Cech, T. R., and Atkins, J. F., eds), pp. 451–485, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Labouesse, M. (1990) Mol. Gen. Genet. 224, 209–221
4. Labouesse, M., Dujardin, G., and Slonimski, P. P. (1985) Cell 41, 133–143
5. Akins, R. A., and Lambowitz, A. M. (1987) Cell 50, 331–345
6. Labouesse, M., Herbert, C. J., Dujardin, G., and Slonimski, P. P. (1987) EMBO J. 6, 713–721
7. Goguel, V., Delahodde, A., and Jacq, C. (1992) Mol. Cell. Biol. 12, 696–705
8. Wenzlau, J. M., Saldanha, R. J., Butow, R. A., and Perlman, P. S. (1989) Cell 56, 421–430
9. Rho, S. B., and Martinis, S. A. (2000) RNA 6, 1882–1894
10. Gampel, A., and Tzagoloff, A. (1987) Mol. Cell. Biol. 7, 2545–2551
11. Tanner, N. K., and Sargueil, B. (1995) J. Mol. Biol. 252, 583–595
12. Bassi, G. S., de Oliveira, D. M., White, M. F., and Weeks, K. M. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 128–133
13. Rangan, P., and Woodson, S. A. (2003) J. Mol. Biol. 329, 229–238
14. Hsu, J. L., Rho, S. B., Vannella, K. M., and Martinis, S. A. (2006) J. Biol. Chem. 281, 23075–23082
15. Karkhanis, V. A., Boniecki, M. T., Poruri, K., and Martinis, S. A. (2006) J. Biol. Chem. 281, 33217–33225
16. Delahodde, A., Goguel, V., Becam, A. M., Creusot, F., Perea, J., Banroques, J., and Jacq, C. (1989) Cell 56, 431–441
17. Chen, X., Gutell, R. R., and Lambowitz, A. M. (2000) J. Mol. Biol. 301, 265–283
18. Patlak, H. B., Ghosh, S. K., Roberts, A. W., Sharma, S. D., Yoder, J. D., Arnold, J. J., Gohara, D. W., Barton, D. J., Paul, A. V., and Cameron, C. E. (2002) J. Biol. Chem. 277, 31551–31562
19. Michel, F., and Westhof, E. (1990) J. Mol. Biol. 216, 585–610
20. Westhof, E., Masquida, B., and Jaeger, L. (1996) Fold. Des. 1, 78–88
21. Rho, S. B., Lincecum, T. L., Jr., and Martinis, S. A. (2002) EMBO J. 21, 6874–6881
22. Huang, H. R., Rowe, C. E., Mohr, S., Jiang, Y., Lambowitz, A. M., and Perlman, P. S. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 163–168
23. Caprara, M. G., Myers, C. A., and Lambowitz, A. M. (2001) J. Mol. Biol. 308, 165–190
24. Guo, Q. B., Akins, R. A., Garriga, G., and Lambowitz, A. M. (1991) J. Biol. Chem. 266, 1809–1819
25. Deleted in proof
26. Schimmel, P., and Ribas De Pouplana, L. (2000) Trends Biochem. Sci. 25, 207–209
27. Martinis, S. A., and Joy Pang, Y. L. (2007) Chem. Biol. 14, 1307–1308
28. Park, S. G., Schimmel, P., and Kim, S. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 11043–11049
29. Mohr, G., Caprara, M. G., Guo, Q., and Lambowitz, A. M. (1994) Nature 370, 147–150
30. Mohr, G., Rennard, R., Cherniack, A. D., Stryker, J., and Lambowitz, A. M. (2001) J. Mol. Biol. 307, 75–92
31. Kämper, U., Kück, U., Cherniack, A. D., and Lambowitz, A. M. (1992) Mol. Cell. Biol. 12, 499–511
32. Cherniack, A. D., Garriga, G., Kittle, J. D., Jr., Akins, R. A., and Lambowitz, A. M. (1990) Cell 62, 745–755
33. Paukstelis, P. J., Coon, R., Madabus, L., Nowakowski, J., Monzingo, A., Robertus, J., and Lambowitz, A. M. (2005) Mol. Cell, 17, 417–428
34. Paukstelis, P. J., Chen, J. H., Chase, E., Lambowitz, A. M., and Golden, B. L. (2008) Nature 451, 94–97
35. Caprara, M. G., Lehnert, V., Lambowitz, A. M., and Westhof, E. (1996) Cell 87, 1135–1145
36. Caprara, M. G., Mohr, G., and Lambowitz, A. M. (1996) J. Mol. Biol. 257, 512–531
37. Houman, F., Rho, S. B., Zhang, J., Shen, X., Wang, C. C., Schimmel, P., and Martinis, S. A. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 13743–13748
38. Li, G. Y., Bécam, A. M., Slonimski, P. P., and Herbert, C. J. (1996) Mol. Gen. Genet. 252, 667–675