The CCA-adding enzyme (ATP(CTP):tRNA nucleotidyltransferase) synthesizes and regenerates the 3'-terminal CC of tRNA. Approximately 65% of mature human U2 small nuclear RNA (snRNA) ends in 3'-terminal CCA, as do all mature tRNAs; the other 35% ends in 3' CC or possibly 3' C. The 3'-terminal A of U2 snRNA cannot be encoded because the 3' end of the U2 snRNA coding region is CC/CC, where the slash indicates the last encoded nucleotide. The first detectable U2 snRNA precursor contains 10–16 extra 3' nucleotides that are removed by one or more 3' exonucleases. Thus, if 3' exonuclease activity removes the encoded 3' CC during U2 snRNA maturation, as appears to be the case in vitro, the cell may need to build or rebuild the 3'-terminal A, CA, or CCA of U2 snRNA. We asked whether homologous and heterologous class I and II CCA-adding enzymes could add 3'-terminal A, CA, or CCA to human U2 snRNA lacking 3'-terminal A, CA, or CCA. The naked U2 snRNAs were good substrates for the human CCA-adding enzyme but were inactive with the Escherichia coli enzyme; activity was also observed on native U2 snRNPs. We suggest that the 3' stem/loop of U2 snRNA resembles a tRNA minihelix, the smallest efficient substrate for class I and II CCA-adding enzymes, and that CCA addition to U2 snRNA may take place in vivo after snRNP assembly has begun.

The CCA-adding enzyme builds and repairs the 3' terminus of tRNA. Approximately 65% of mature human U2 small nuclear RNA (snRNA) ends in 3'-terminal CCA, as do all mature tRNAs; the other 35% ends in 3' CC or possibly 3' C. The 3'-terminal A of U2 snRNA cannot be encoded because the 3' end of the U2 snRNA coding region is CC/CC, where the slash indicates the last encoded nucleotide. The first detectable U2 snRNA precursor contains 10–16 extra 3' nucleotides that are removed by one or more 3' exonucleases. Thus, if 3' exonuclease activity removes the encoded 3' CC during U2 snRNA maturation, as appears to be the case in vitro, the cell may need to build or rebuild the 3'-terminal A, CA, or CCA of U2 snRNA. We asked whether homologous and heterologous class I and II CCA-adding enzymes could add 3'-terminal A, CA, or CCA to human U2 snRNA lacking 3'-terminal A, CA, or CCA. The naked U2 snRNAs were good substrates for the human CCA-adding enzyme but were inactive with the Escherichia coli enzyme; activity was also observed on native U2 snRNPs. We suggest that the 3' stem/loop of U2 snRNA resembles a tRNA minihelix, the smallest efficient substrate for class I and II CCA-adding enzymes, and that CCA addition to U2 snRNA may take place in vivo after snRNP assembly has begun.

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† The abbreviations used are: snRNA, small nuclear RNA; nt, nucleotide(s); SRP, signal recognition particle.
substrate, we show here that the human but not the E. coli CCA-adding enzyme can build and repair the 3’-terminal CCA sequence of human U2 snRNA. The human CCA-adding enzyme is also active on native U2 snRNPs. The yeast (48) and vertebrate CCA-adding enzymes (49, 50) are known to be present in the nucleus as well as the cytoplasm, consistent with the possibility that the human CCA-adding enzyme may build the 3’ end of immature U2 snRNPs in the cytoplasm or nucleus or repair the 3’ end of mature nuclear U2 snRNPs.

**EXPERIMENTAL PROCEDURES**

**In Vitro Transcription and Purification of U2 snRNA Substrates—**To generate templates for in vitro transcription of U2 snRNAs lacking 3’-terminal A, CA, and CCA, we performed PCR using plasmid pU2-T7 as template (51) and appropriate combinations of 5’ and 3’ primers. The three different 5’ primers (T7U2-1, T7U2-111, T7U2-147) each contained the T7 RNA polymerase promoter sequence (underlined below) just upstream from the U2 snRNA sequence; T7U2-1 generated a template for in vitro transcription of full-length U2 snRNA, T7U2-111 generated a template for in vitro transcription of 3’-terminal stem/loops III and IV, and T7U2-147 generated a template for in vitro transcription of 3’-terminal stem/loop IV. The six different 3’ primers (U2-N, U2-NC, U2-NCC, U2-NCCA, U2-NCU, and U2-NCUA, where N is the discriminator base) each contained a 

The primers for PCR amplification were as follows: T7U2-1, 5’-CA-

The human CCA-adding enzyme can add 3’-terminal A, CA, and CCA to full-length U2 snRNA substrates. A, secondary structure of human U2 snRNA. The Sm binding site is overlined. The 3’-terminal A of mature U2 snRNA is not encoded in the gene and must be added posttranscriptionally (see text). B, CCA addition in vitro to full-length U2 snRNA substrates lacking 3’-terminal CCA, CA, or A (A185, A185C, and A185CC, where A185 corresponds to the discriminator base); the natural m3,2,7Gppp cap is replaced by 5’ GpppG in the T7 transcript. Asterisks indicate the labeled nucleotide. Reactions were resolved by denaturing 6% PAGE; the length of the product is indicated in parentheses.

![Image](https://example.com/image.png)
zyme was expressed as described previously (13) with a slight modification. *E. coli* BL21-codonPlus(DE3)-RIL (Stratagene) was transformed by the overexpression plasmid (13), inoculated into LB broth at 37 °C in the presence of 30 μg/ml kanamycin and 34 μg/ml chloramphenicol, and grown to an A_{590} of 0.8. Isopropyl-1-thio-β-D-galactopyranoside (0.1 mM) was added to induce expression, and growth continued at room temperature for 3 h. The bacterial pellet was lysed by sonication in buffer A containing 20 mM Tris-HCl, pH 7.8, 10 mM MgCl₂, 500 mM KCl, 6 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 5% glycerol. The cleared lysate was loaded onto a nickel-NTA resin (Qiagen) column, and the column was washed extensively with buffer A plus 20 mM imidazole. Histidine-tagged protein was eluted with buffer A plus 150 mM imidazole and dialyzed against buffer A containing 0.25 M KCl and 10% glycerol with or without 0.1 mM phenylmethylsulfonyl fluoride.

Constructs expressing the *E. coli* and *Sulfolobus shibatae* CCA-adding enzymes have been described previously (3, 12). Proteins were expressed in *E. coli* BL21(DE3) for 4 h at 37 °C following induction with 1 mM isopropyl-1-thio-β-D-galactopyranoside, but purification was otherwise identical.

The CCA-adding enzyme genes of the other archaea (*Methanobacterium thermoautotrophicum*, *Methanococcus januschii*, and *Pyrococcus furiosus*) were amplified from genomic DNA by PCR. The wild type proteins are 454 (M. thermoautotrophicum), 449 (M. jannaschii), and 453 residues long (P. furiosus). The *M. thermoautotrophicum* gene was inserted between the NdeI and HindIII sites of pET22b (+) (Novagen); the *M. jannaschii* and *P. furiosus* genes were inserted between the NdeI and XhoI sites. The initiator ATG of the *M. thermoautotrophicum* and *P. furiosus* enzymes was retained, but the *M. jannaschii* initiator GTG encoding valine was changed to ATG. All three enzymes have additional C-terminal residues including a hexahistidine tag; the *M. thermoautotrophicum* TGA stop codon was changed to AAG (lysine) to generate the C-terminal sequence LAAALEH₆. The *M. jannaschii* and *P. furiosus* stop codons (TGA and TAA, respectively) were changed to CTC (leucine) to generate the C-terminal sequence LEH₆. These constructs were used to transform *E. coli* BL21(DE3) carrying an argU plasmid encoding a minor arginine tRNA. Transformants were inoculated into LB broth containing 100 μg/ml ampicillin and 30 μg/ml kanamycin, proteins were induced with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside, and growth continued at 37 °C for 12 h. Histidine-tagged proteins were purified as described above, and stored at −20 °C in buffer A containing 100 mM KCl and 50% glycerol.

**Enzyme Assays—CCA-adding enzyme assays were performed as described previously (53).** The 10-μl reactions contained 100 mM glycine/NaOH (pH 9.0), 10 mM MgCl₂, 1 mM dithiothreitol, 500 μM CTP, 1 mM ATP, 2 μM U2 snRNA transcript, 0.05 μM [α-32P]CTP or [α-32P]ATP (3,000 Ci/mmol; Amersham Biosciences, Inc.), and 10 ng of purified recombinant CCA-adding enzymes. Reactions were terminated by the addition of 5 μl of 95% formamide containing 20 mM sodium EDTA (pH 8.0), xylene cyanol (0.2%), and bromphenol blue (0.2%). Products were resolved by 6% or 12% denaturing PAGE and quantified using a PhosphorImager (Molecular Dynamics).

**Native U2 snRNPs—CCA addition to native U2 snRNPs was assayed by incubating 5 μl of HeLa nuclear extract (Promega; 18.2 mg/ml protein) with the human CCA-adding enzyme (50 ng) for 15 min at 37 °C in 100 mM glycine/NaOH (pH 9.0), 10 mM MgCl₂, 1 mM dithiothreitol, 500 μM CTP, 2 mM ATP, and 0.1 μM [α-32P]CTP or [α-32P]ATP (3,000 Ci/mmol; Amersham Biosciences, Inc.) in 50-μl total reaction volume.** After phenol extraction and ethanol precipitation, RNAs were fractionated by denaturing 6% PAGE, and the gel was subjected to autoradiography. To reveal endogenous 3′ exonucleases capable of acting on U2 snRNP, the nuclear extract was subjected to multiple freeze/thaw cycles before assaying for CCA addition to U2 snRNP; for each cycle, extract was thawed on ice for 2 h and refrozen at −70 °C for 2 h. To increase the fraction of U2 snRNPs with defective 3′ ends, nuclear exosome was added to the extract as described.
extract was treated with venom 3′ exonuclease as described previously (53). A 20-μl reaction containing 100 mM Tris-HCl (pH 9.0), 100 mM NaCl, 15 mM MgCl2, 5 μl of HeLa nuclear extract (18.2 mg/ml) and snake venom phosphodiesterase I (1.2 units/25 μl; Sigma) was incubated for 1 h at 20 °C, and the enzyme was then inactivated by addition of 50 mM EDTA (54–56).

RESULTS

The Human CCA-adding Enzyme Can Add 3′-terminal A, CA, and CCA to U2 snRNA Substrates—To ask whether the human CCA-adding enzyme can build or repair the 3′-terminal CCA sequence of U2 snRNA, we generated three sets of U2 snRNA substrates (full-length U2 snRNA, 3′-terminal stem/loops III and IV, and stem/loop IV only), each with three different 3′-terminal sequences (N, NC, and NCC, where N is the discriminator base). Using full-length U2 snRNA lacking CCA, CA, or A as substrates (Fig. 1A), we found that the human CCA-adding enzyme incorporates only CTP into U2 snRNA substrates lacking CCA or CA and only ATP into U2 snRNA substrates lacking A (Fig. 1B). Thus, the human CCA-adding enzyme could be responsible for adding 3′-terminal A, CA, and CCA to U2 snRNA processing intermediates or to mature U2 snRNA in need of repair.

To identify the minimum structure or sequence required for CCA addition by the human enzyme, we assayed 3′-terminal stem/loops III and IV of U2 snRNA (Fig. 2A, lanes 1–3), but equivalent substrates with mutant or mature 3′ ends (A185CU, A185CUA, and A185CCA, where A185 is the discriminator base) were not (Fig. 2, B, lanes 4–6). The 3′-terminal stem/loop IV of U2 snRNA alone was also a good substrate (Fig. 2, C and D), suggesting that the 3′ end of U2 snRNA resembles a tRNA minihelix (57, 58) and that the T+C loop contributes marginally, if at all, to substrate recognition (12).

The Human CCA-adding Enzyme Repairs U2 snRNA Faithfully—To examine the fidelity of CCA addition by the human enzyme to U2 snRNA, we assayed the addition of ATP alone, CTP alone, or ATP and CTP together to uniformly labeled U2 snRNAs with incomplete or mature 3′ ends (Fig. 2E). As expected, U2 snRNAs lacking CCA or CA are substrates for CTP addition; U2 snRNA lacking A is a substrate for ATP addition; U2 snRNAs lacking CCA or CA are substrates for both CTP and ATP addition; and neither CTP nor ATP is added to U2 snRNA with a mature CCA end. Importantly, the human CCA-adding enzyme does not exhibit poly(C) polymerase activity on any of these 3′-terminally defective U2 snRNA substrates, even in the absence of ATP (Fig. 2E, middle panel), when the enzyme is assayed at catalytic levels (enzyme to substrate ratio, 1:250) instead of the more nearly stoichiometric levels (1:10 or 1:20) used by others (57, 59).
adding Enzyme—Unlike in vitro transcripts, natural U2 snRNA is capped, 2'-O-methylated, and pseudouridylation (60, 61). Although none of these modifications fall within the 3'-terminal stem/loops III and IV that serve as minimal substrates for the human CCA-adding enzyme, it was nonetheless important to establish that natural U2 snRNA, purified from HeLa nuclear extract, would function as substrate for the human CCA-adding enzyme. CTP addition to natural U2 snRNA was robust, but ATP addition was relatively weak (Fig. 4A, compare lanes 1).

Weak ATP addition could mean that U2 snRNA is not a natural substrate for the human CCA-adding enzyme or that the distribution of U2 3' ends in this extract is different from that observed previously (45); alternatively, C addition may be less specific than A addition because U1 snRNA and 5 S rRNA were also able to accept one or more 3'-terminal Cs (Fig. 4A, lane 1). We therefore asked whether U2 snRNA lacking A might be a substrate for the human SRP (or 7SL RNA) adenylation enzyme; this enzyme is homologous to the mRNA poly(A) polymerase but is thus unlikely to play any role in U2 snRNA metabolism.

When the human CCA-adding enzyme (Fig. 4A, lanes 1), the SRP 7SL RNA adenylation enzyme failed to add ATP to natural U2 snRNA, purified from HeLa nuclear extract, would function as substrate for the human CCA-adding enzyme. CTP addition to natural U2 snRNA was robust, but ATP addition was relatively weak (Fig. 4A, compare lanes 1).

Heterologous CCA-adding Enzymes Can Add 3'-terminal CCA to U2 snRNA—We next asked whether heterologous CCA-adding enzymes belonging to class I and class II could add CCA to human U2 snRNA substrates. We tested class I enzymes from archaea (M. jannaschii, M. thermoautotrophicum, P. furiosus, and S. shibatae) and class II enzymes from Gram-positive (B. subtilis and B. stearothermophilus) and Gram-negative (E. coli) eubacteria, with the human class II enzyme as control. Interestingly, only one class I enzyme (M. thermoautotrophicum) and two class II enzymes (Homo sapiens and B. stearothermophilus) were fully active for CCA addition to U2 snRNA; the other enzymes were either very weakly active (S. shibatae, M. jannaschii, and P. furiosus) or inactive (E. coli and B. subtilis) (Fig. 3A). Although fully active on tRNA substrates (data not shown), the E. coli class II enzyme was also inactive on U2 snRNA substrates lacking CA or A (Fig. 3B).

Natural U2 snRNA Is a Substrate for the Human CCA-
CCA Addition to U2 snRNPs—Mature U2 snRNPs consist of a 5’-terminal domain (stem/loops I, IIa, and IIb) that binds the SF3a and SF3b splicing factors and a 3’-terminal domain (the Sm site and stem/loops III and IV) that binds Sm antigens and the U2-specific proteins A’ and B’ (63–65) (Fig. 1A). Sm proteins assemble onto newly exported U2 snRNPs before 3’ trimming takes place (34, 66–69), but it is not yet clear when U2 snRNA acquires the A’ and B’ proteins (38, 42).

To determine whether assembly of U2 snRNA into a ribonucleoprotein particle might inhibit or facilitate CCA addition by the human enzyme, we performed CCA addition assays in HeLa nuclear extract (Fig. 5). Consistent with the results for naked human U2 snRNA (Fig. 4A), CTP addition to endogenous U2 snRNPs was much more efficient than ATP addition (compare Fig. 5A, lane 2, with Fig. 5B); moreover, CTP stimulated ATP addition to tRNA and also, albeit much more weakly, to U2 snRNA (Fig. 5B). Although active on naked RNAs (Fig. 4B), the SRP 7SL adenylylating enzyme did not appear to recognize U2 snRNPs or any other RNP substrates in nuclear extract (Fig. 5A, lane 1). These results suggest that endogenous 3’ exonucleases remove CA or CCA from mature U2 snRNA and tRNA and that the human CCA-adding enzyme can repair these species.

More efficient addition of CTP than ATP may reflect the 50-fold higher \( K_m \) for ATP than for CTP (Fig. 6). Kinetic parameters for the human CCA-adding enzyme with both U2 snRNA and tRNA substrates were determined by measuring the addition of \( [\alpha-\text{32P}]\text{CTP} \) to tRNA-DC and U2 snRNA lacking 3’-terminal CA in the presence of saturating ATP (Fig. 6A) and the addition of \( [\alpha-\text{32P}]\text{ATP} \) to tRNA-DCC and U2 snRNA lacking 3’-terminal A (Fig. 6B). The observed Michaelis constants (\( K_m \)) were 10 \( \mu M \) for CTP and 200 \( \mu M \) for ATP with the tRNA-DCC substrate and 33 \( \mu M \) for CTP and 1.5 \( mM \) for ATP with the U2 snRNA substrate. The substrate dependence of \( K_m \) values for CTP and ATP could reflect a conformational change in the proteins (70) or collaborative templating by substrate and enzyme together (5).

In an effort to uncover endogenous 3’ exonuclease activities that might act on U2 snRNA, we subjected HeLa nuclear extract to prolonged incubation at room temperature or to multiple freeze/thaw cycles; however, CCA addition to U2 snRNA was unaffected by these treatments. We also treated HeLa nuclear extract with snake venom 3’ exonuclease to determine whether the 3’ end of U2 snRNA is accessible to exonuclease attack, but CCA addition was unaffected by this treatment as well. These negative results (data not shown; see “Experimental Procedures”) suggest that U2 snRNAs with incomplete CCA termini do not reflect degradation of mature U2 snRNPs but are generated by RNA processing or 3’ exonuclease activity before addition of the Sm and/or A’ and B’ proteins to U2 snRNA.

DISCUSSION

All vertebrate U snRNAs belonging to the Sm class of U snRNPs and some small nucleolar RNAs such as U3 small nucleolar RNA apparently use the same 3’ end formation apparatus: specialized U snRNA promoter elements drive transcription by a form of RNA polymerase II that allows recognition of a highly conserved 3’ end formation signal (3’ box) located 6–25 nt downstream from the U snRNA coding (20).
Whether the 3′ box functions as a transcription termination signal or as an RNA processing signal is still unknown (25); however, the first detectable U snRNA precursors have 3′-terminal tails that extend nearly to the 3′ end of the U2 snRNA gene (22, 31, 32, 35, 36, 71) and are trimmed by cytoplasmic nucleases that may be related to the yeast multienzyme complex known as the exosome (10, 35, 36, 38).

The 3′-terminal sequence of most U snRNAs can be aligned with the corresponding 3′ sequencese, suggesting that nucleases are capable of generating the mature 3′ end of most U snRNA precursors. However, ~65% of mature human U2 snRNA ends in 3′-terminal CCA; the other 35% ends in 3′ CC or possibly 3′ C (44, 45). The 3′-terminal A of U2 snRNA cannot be encoded by the 3′-terminal sequence of most U snRNA precursors. We cannot exclude template-instructed misincorporation, as occurs in certain RNA viruses (73), but the 3′-terminal A is also unlikely to be an untemplated addition by the transcribing RNA polymerase (72) because transcription must continue for >10 bp beyond the 3′ end to generate the U2+10 family of precursors. We cannot exclude template-instructed misincorporation, as occurs in certain RNA viruses (73), but this has never been observed for a cellular RNA polymerase.

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One candidate for posttranscriptional addition of 3′-terminal

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**Fig. 7. Alignment of 3′-terminal region of U2 snRNA, cDNA, and gene sequences.** A, 3′-terminal stem/loops III and IV of human U2 snRNA, color coded as indicated in B. B, alignment of U2 snRNA, cDNA, and gene sequences from the small RNA database (mbcr.bcm.tmc.edu/smallRNA/Data base/U2). Alignments were generated by Clustal W (www.ebi.ac.uk/clustalw/) and adjusted manually. The bottom of stem/loop IV is shown as G147:C184 (human numbering), but an additional mispair C146:A185 or pair U146:A185 cannot be excluded because position 146 (boxed) is C in all sequences except pea, maize, and rice, where it is U. As described in the text and footnotes below, only some 3′ sequences have been determined directly; others are deduced or conjectural. The highly divergent Erythrobasidium hasegawianum (1, human RNA (H. sapiens, accession number X59360). Approximately 65% of U2 snRNA has 3′-terminal CCA, the other 35% has either 3′-terminal CC or possibly 3′-terminal C, as determined by pCp labeling methods (44, 45). 2, rat RNA (Rattus norvegicus, K00781). The 3′-terminal sequence of most U2 snRNAs can be aligned with the corresponding genes, suggesting that nucleases are capable of generating the mature 3′ end of most U snRNA precursors. However, ~65% of mature human U2 snRNA ends in 3′-terminal CCA; the other 35% ends in 3′ CC or possibly 3′-terminal A as determined by pCp labeling methods (44). 4, frog RNA (Xenopus laevis, K02457). Approximately 60% of U2 snRNA contains posttranscriptionally added 3′-terminal A (44). Note the C-richness of 3′-terminal A (44). 6, Thermus aquaticus (X59360). 7, bean (Phaseolus vulgaris, L29518A). Method not specified. 8, wheat (Triticum aestivum, K00781). The 3′-terminal sequence was determined by cloning poly(A)-tailed RNA, where (G) indicates 3′-terminal heterogeneity (86). 9, chicken (Gallus gallus, M12856). 13, green urchin DNA (Strongylocentrotus purpuratus, X69967). All accession numbers refer to GenBank. 10, mouse DNA (Mus musculus, X07913). 11, baboon DNA (Papio hamadryas, M31777). 12, chicken DNA (Gallus gallus, M12856). 13, green urchin DNA (Lytechinus variegatus, S64589). 14, purple urchin DNA (Strongylocentrotus purpuratus, M58447). 15, fly DNA (Drosophila melanogaster, X04245 and M15440). 16, worm DNA (Caenorhabditis elegans, X51381). 17, maize DNA (Zea mays, X16459). 18, rice DNA (Oryza sativa, U27085). 19, purple urchin cDNA (Strongylocentrotus purpuratus, X59360). Approximately 65% of U2 snRNA has 3′-terminal CCA, the other 35% has either 3′-terminal CC or possibly 3′-terminal C, as determined by pCp labeling methods (44, 45). 2, rat RNA (Rattus norvegicus, K00781). The 3′-terminal sequence of most U2 snRNAs can be aligned with the corresponding genes, suggesting that nucleases are capable of generating the mature 3′ end of most U snRNA precursors. However, ~65% of mature human U2 snRNA ends in 3′-terminal CCA; the other 35% ends in 3′ CC or possibly 3′-terminal A as determined by pCp labeling methods (44). 4, frog RNA (Xenopus laevis, K02457). Approximately 60% of U2 snRNA contains posttranscriptionally added 3′-terminal A (44). Note the C-richness of 3′-terminal A (44).
A to U2 snRNA is the SRP 7SL adenylating enzyme (62); another is the CCA-adding enzyme (tRNA nucleotidyltransferase), which builds and repairs the 3′-terminal CCA of tRNA. In fact, the 3′ end of U2 strongly resembles the minimal substrate for the CCA-adding enzyme: a tRNA minihelix (the “top half” of tRNA) in which the acceptor stem stacks on the TψC stem/loop. Not only is U2 stem IV 12 bp, the optimal length for CCA addition by the E. coli and S. shibatae enzymes, but the step is followed by CCA addition by the class II enzymes of both classes from all three kingdoms (3): class I CCA-adding enzymes from archaea (S. shibatae, M. jannaschii, and S. shibatae) class II enzymes from Gram-positive (B. steaothermophilus and B. subtilis) and Gram-negative (E. coli) eubacteria, and the human class II enzyme. All substrates were active with the human class II CCA-adding enzyme, including 3′-terminal stem/loop IV alone (Figs. 1–3), and CCA addition was faithful (Fig. 2E); however, the E. coli class II CCA-adding enzyme was inactive on the U2 snRNA substrates but fully active on tRNA substrates, consistent with the idea that CCA addition to U2 snRNA is specific. In contrast to CCA-adding enzymes, the SRP 7SL RNA adenylating enzyme nonspecifically added long 3′-terminal tails of >400 nt to U2 and other RNA substrates (Fig. 4). Thus, the SRP RNA adenylating enzyme behaves like a poly(A) polymerase and is unlikely to play a role in U2 snRNA biosynthesis. These results suggest that the 3′ stem/loop of U2 snRNA does in fact resemble a tRNA minihelix, the smallest efficient substrate for class I and II CCA-adding enzymes (12, 57), and that CCA addition to U2 snRNA could take place in vivo.

We also found that the human CCA-adding enzyme is active on natural U2 snRNA purified from nuclear extract (Fig. 4) and on native U2 snRNPs (Fig. 5). In the mature 17S U2 snSNP, SF3a and SF3b splicing factors are bound to the 5′-terminal domain including stem/loops I, IIa, and IIb; Sm proteins are bound to the conserved Sm-binding sequence; and U2-specific proteins A′ and B′ are bound to stem/loop IV (62–64, 75, 76). However, assembly of Sm proteins onto U2-10 precursors occurs before formation of the mature 3′ end (34, 38), and U2 snRNA particles are the substrates for U2 snRNA 3′ processing (34, 67, 68). The activity of the human CCA-adding enzyme on native U2 snRNA is consistent with the observation that 3′-matured of U2 snRNA occurs in vivo after snRNP assembly has begun.

There are ample precedents for the ability of CCA-adding enzymes to recognize tRNA-like structures. tRNA consists of two structurally and functionally independent domains: a “top half” or minihelix consisting of the acceptor stem stacked on the TψC stem/loop, and a “bottom half” consisting of the DHU stem/loop stacked on the anticodon stem/loop (77, 78). Not only does a tRNA minihelix serve as substrate for CCA-adding enzymes (12, 57), but single-stranded RNA viruses often have 3′-terminal tRNA-like structures that serve as substrates for enzymes of tRNA metabolism including tRNA synthetases, the CCA-adding enzyme, and RNase P (11, 79–81). maize mitochondrial mRNAs also contain posttranscriptionally added 3′-terminal nucleotides that could be the product of a mitochondrial form of the CCA-adding enzyme (82).

Almost all U snRNAs have 3′-terminal stem/loops, presumably to confer stability on the RNAs (83), and some small RNAs may be stabilized by posttranscriptional adenylation (45, 46). Although direct RNA sequencing data are scanty, the U2 snRNA 3′-terminal stem/loop appears to be followed by CCA only in mammals (human and rat); even frog U2 snRNA lacks CCA (Fig. 7). The U2 sequences of other organisms are either cDNA or DNA sequences. The CDNA sequences are all derived from poly(A)-tailed RNA, so that it is impossible to distinguish posttranscriptional 3′-terminal A addition from the poly(A) tail. The DNA sequences almost all have C+A-Rich 3′-flanking regions; whether this C+A-Rich region plays a role in 3′-end formation remains to be seen (20, 25, 38), but it has already led to arbitrary assignment of U2 snRNA 3′ ends based solely on DNA sequence information. Because U2 snRNA sequences are strongly conserved from yeasts to mammals, failure to conserve the 3′-terminal CCA suggests that this sequence is not required for U2 snRNA function; however, mammalian U2 snRNA is shown to be stable (84), and CCA addition could serve to protect U2 snRNA from 3′ exonucleases. In fact, U2 might exploit the CCA-adding enzyme, which exists in nuclear, cytoplasmic, and mitochondrial forms (48), as the functional homolog of telomerase, to replenish 3′-terminal nucleotide sequences that are lost by attrition (76, 77).

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U2 Small Nuclear RNA Is a Substrate for the CCA-adding Enzyme (tRNA Nucleotidyltransferase)

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