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LETTER TO THE EDITOR

A revised model for U4atac/U6atac snRNA base pairing

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The discovery of a second type of spliceosomal intron in the genomes of many multicellular eukaryotes raised the exciting possibility of comparing the structural and functional features of the two splicing systems with the hope of shedding light on important features of each (Tarn & Steitz, 1997). This hope was amply fulfilled with the discovery of the parallel roles played in the two systems by the respective sets of spliceosomal snRNAs. The initial identification of functions for the previously known U11 and U12 snRNAs (Hall & Padgett, 1994; Tarn & Steitz, 1996b) was magnified by the discovery of the novel U4atac and U6atac snRNAs (Tarn & Steitz, 1996a). The similarities of these latter two snRNAs to the U4 and U6 snRNAs of the major splicing system were apparent and led to detailed comparisons of the features of these RNAs and their interactions (Tarn & Steitz, 1996a). For both the U4/U6 pair and the U4atac/U6atac pair, a similar secondary structure was proposed involving two intermolecular base paired helical regions termed stem I and stem II, separated by an intramolecular stem-loop structure in both U4 and U4atac (Fig. 1A,B). Although the stem II interactions appeared to be very similar in both structures, the stem I interaction in the U4atac/U6atac structure was shorter and differently arranged than in the U4/U6 structure.

In the course of experiments on the function of U4atac, we noted that the sequence of the U4atac clone obtained from the Steitz laboratory differed in two places from the published sequence (Shukla & Padgett, 1999). The published sequence shows a C at position 60 and a G at position 61 whereas the clone contains a G at 60 and a C at 61 (see Fig. 1B,C). A BLAST search of the draft human genome sequence confirmed that the genomic sequence of U4atac matched the clone sequence and not the published sequence. Although this error has been corrected in the GenBank entry for U4atac snRNA (accession number U62822), the incorrect sequence has been reprinted numerous times in review articles (Tarn & Steitz, 1997; Burge et al., 1999; Wu & Krainer, 1999; Yu et al., 1999).

A further consequence of this error is that the published structure of stem I in the U4atac/U6atac structure is probably incorrect. When the correct sequence of U4atac is used, a longer stem I base pairing interaction is possible that closely resembles the stem I pairing seen in U4/U6 (Fig. 1C). As with the U4atac primary sequence, the original structure has been reprinted in several reviews of splicing (Tarn & Steitz, 1997; Burge et al., 1999; Wu & Krainer, 1999; Yu et al., 1999). Our goal here is to highlight this new model of the U4atac/U6atac interaction.

Using the corrected sequence, the U4atac/U6atac stem I can now be drawn with 7 bp instead of the original 4 bp (Fig. 1B,C) similar to the 8 bp in the U4/U6 stem I (Fig. 1A). In both models, the stem I structures now include equivalent regions of U6 and U6atac. The sequence in stem I, UUAGCA, is identical in U6 and U6atac (boxed residues in Fig. 1). These bases include residues involved in the helix Ia and Ib interactions with U2 and U12, respectively, including the highly conserved and functionally important U6 and U6atac AGC sequences. The stem I residues of U4 and U4atac, UGCUAA, are likewise identical in humans. Considerable evidence suggests that the unwinding of the U4/U6 and U4atac/U6atac stems are important steps in the activation of the spliceosome (Frilander & Steitz, 2001, and references therein). The close similarity of these two base-paired structures suggests that the same splicing factor(s) may be responsible for unwinding and activation in both splicing systems.

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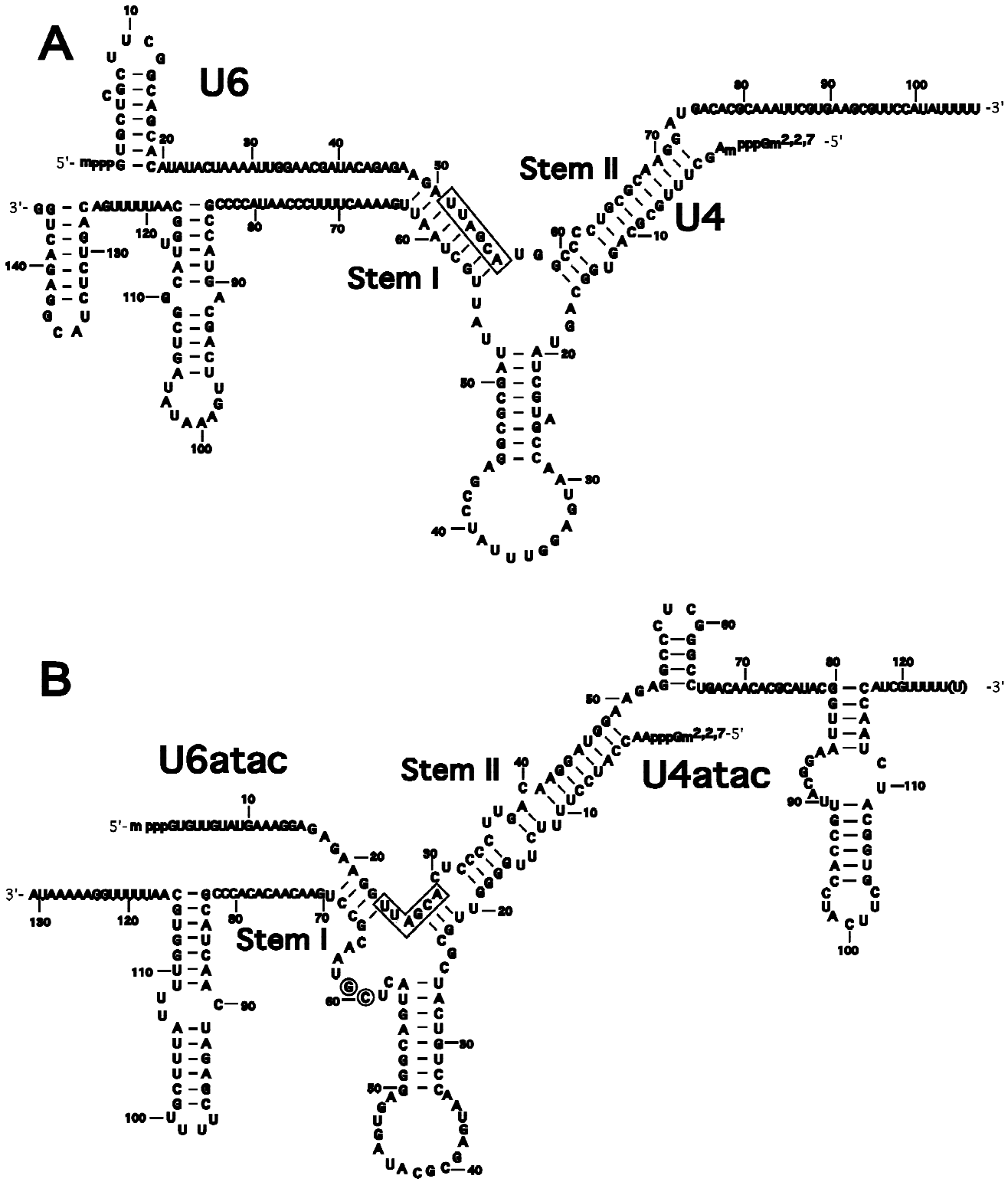

FIGURE 1. Sequences and secondary structures of U4/U6 and U4atac/U6atac snRNAs. **A:** Model of human U4 and U6 snRNA interactions adapted from Wersig and Bindereif (1990). **B:** Original sequences and model of human U4atac and U6atac interactions adapted from Tarn and Steitz (1996a). The incorrect bases at residues 60 and 61 are circled. **C:** Corrected sequences and proposed model of human U4atac and U6atac interactions. The corrected residues at positions 60 and 61 are circled. **D:** Model of *Drosophila melanogaster* U4atac and U6atac interactions. The putative snRNA sequences were identified in the *Drosophila* genome by Mount and Salz (2000). (*Figure continues on facing page.*)

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Although the pairing of these RNA sequences on paper is somewhat arbitrary, we also have experimental support for our new model of U4atac/U6atac interactions. We have described an in vivo mutant suppressor assay where U12-dependent splicing of a reporter minigene is dependent on coexpression of a U4atac snRNA gene modified in the stem II region to pair to a modified U6atac snRNA (Shukla & Padgett, 1999). Using this assay, we further modified the U4atac gene in the stem I region and tested the resulting mutants for their ability to support splicing in vivo. Two different double mutations that would be predicted to disrupt the stem I interaction shown in Figure 1C were inactive for splicing. One of these inactive mutations

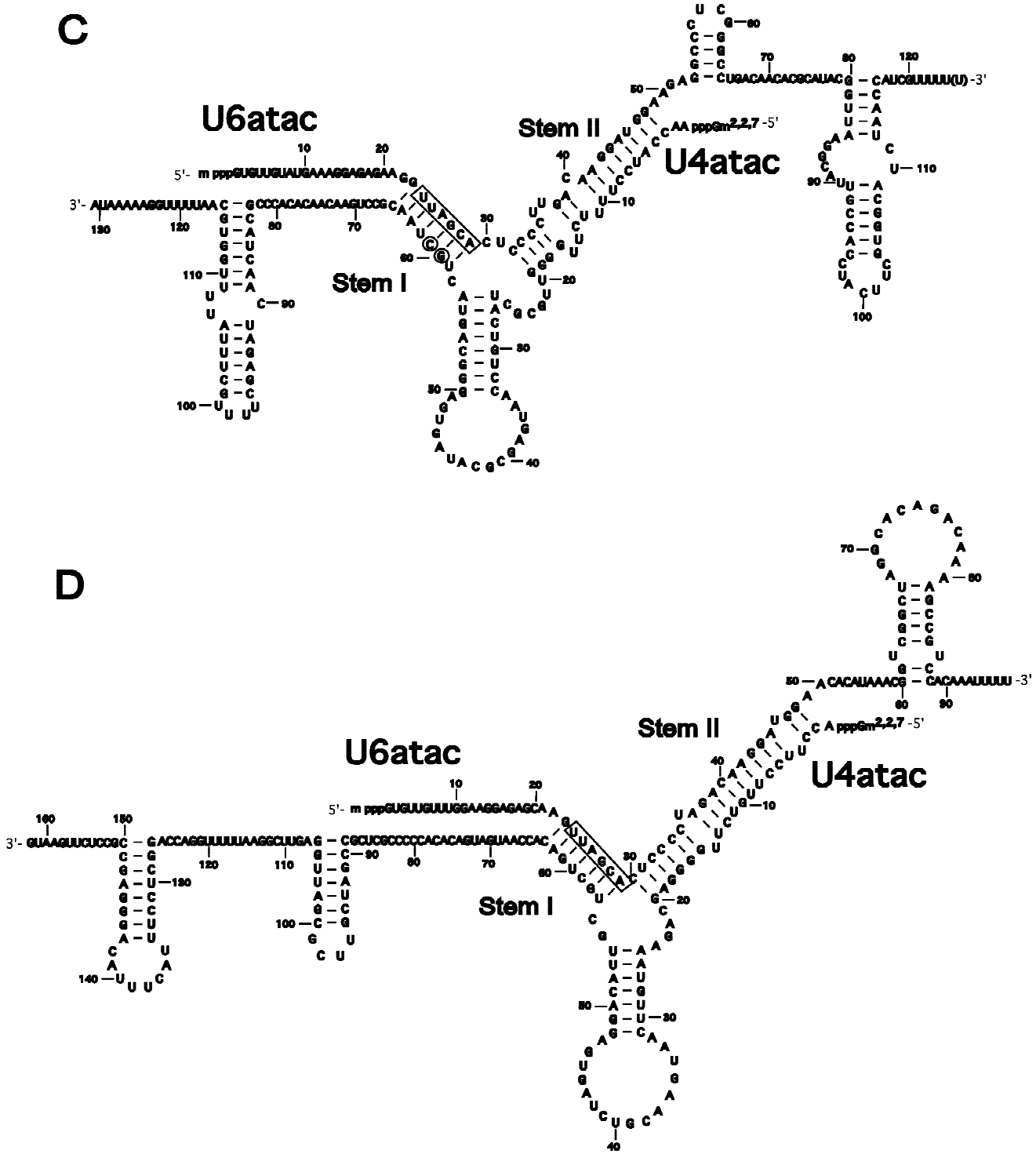

FIGURE 1. *Continued.*

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altered the U4atac snRNA sequence in this region to the originally published sequence (i.e., G_{60} C_{61} to C_{60} G_{61}). Another mutant specifically designed to disrupt the stem I interaction as originally proposed was, in contrast, fully functional in vivo (G. Shukla, A. Cole, R. Dietrich, and R. Padgett, in prep.).

Furthermore, there is phylogenetic conservation of this potential interaction in the apparent *Drosophila* homologs of U4atac and U6atac snRNAs (Fig. 1D). The only deviation from the human sequence in the stem I region is the substitution of a G-U base pair for an A-U base pair. These findings suggest that this revised model of the U4atac/U6atac secondary structure reflects a functional interaction that is required for pre-mRNA splicing by the U12-dependent spliceosome.

In passing, we also note that the published sequence of U6atac snRNA (accession number U62823; Tarn & Steitz, 1996a) differs at one position from the human genomic sequence identified by BLAST searches. This is at position 52, where an A in the clone sequence is a C in the genomic sequence. This could represent either a polymorphism in the U6atac snRNA gene or a mutation introduced during cDNA cloning. In any case, this deviation from the genomic sequence does not appear to have a large effect on function, as U6atac suppressor RNA constructs containing the A at 52 are active in vivo (Incorvaia & Padgett, 1998; Shukla & Padgett, 1999).

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