

MUTATIONS DEFINE ESSENTIAL AND NONESSENTIAL U2 RNA STRUCTURES

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To understand snRNP structure and function, we must understand snRNA structure and its role in the mechanism of snRNP action, snRNP biogenesis, and the regulation of snRNP activity. We have examined the structure of yeast U2 by genetic methods, in an attempt to derive a structure model useful for interpreting the effect of U2 mutations on snRNP protein binding, splicing complex formation, and splicing.

Phylogenetic comparisons of U2 snRNA sequences (Guthrie & Patterson, 1988; Ares & Igel, 1989) do not distinguish among three secondary structures (Fig. 1), one of which includes a pseudoknot. Using site-directed mutagenesis, we constructed U2 mutations designed to disrupt potential helices, in order to test the models and eliminate one or more from consideration. Mutations were made singly and in pairs, designed such that the combination of the two would restore complementarity using a different primary sequences. Additional mutations were made to delete structural domains which appeared to tolerate multiple base substitutions. The mutant U2 genes were introduced into yeast and loss of the wild type gene was selected by a plasmid shuffling technique. Cells unable to grow after losing the wild type gene are presumed to carry lethal U2 mutations.

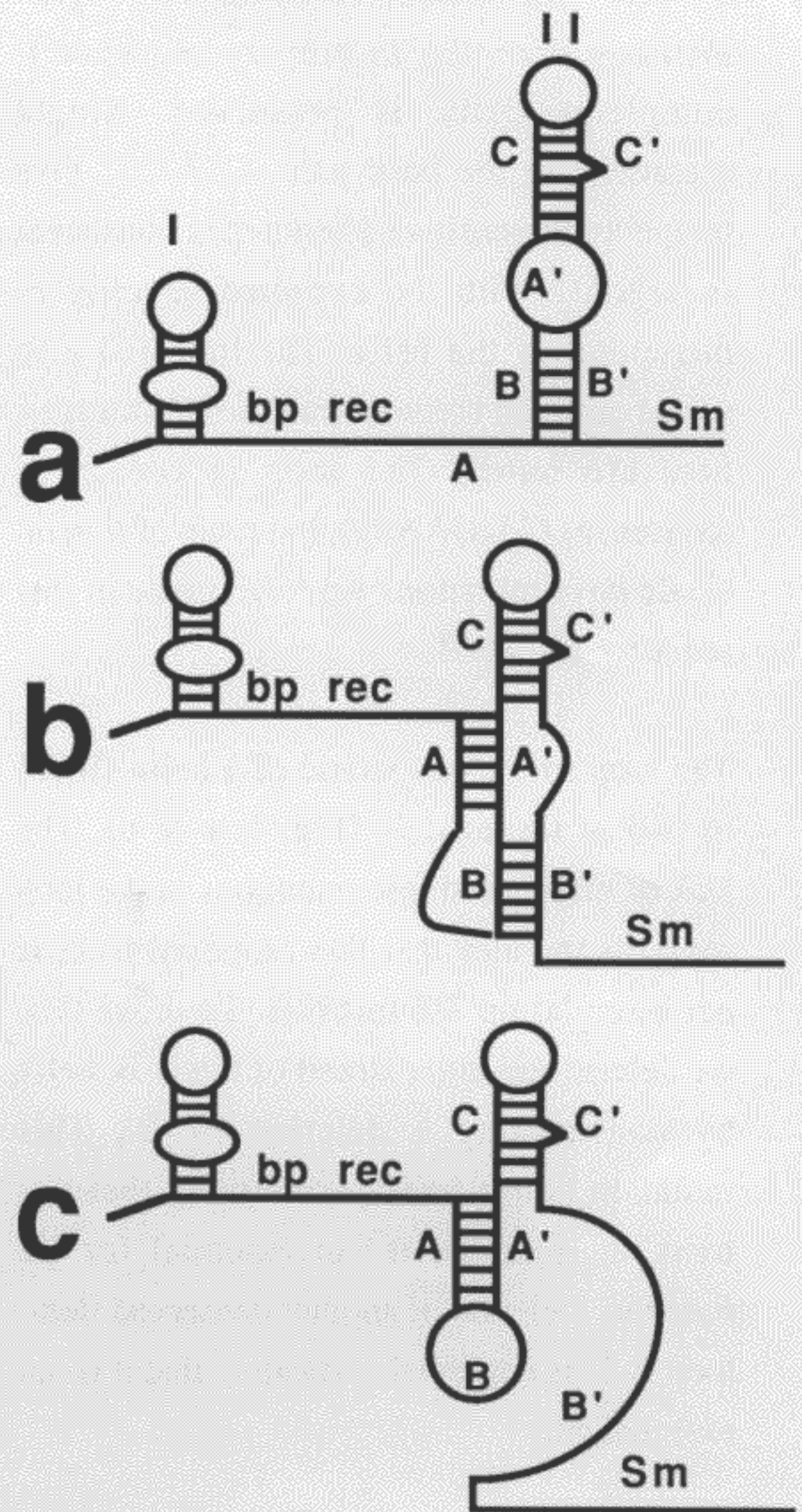


FIGURE 1. Phylogenetically consistent U2 structure models. (a) Keller & Noon (1985). (b) pseudoknot. (c) alternative.

The results (Fig. 2) indicate that a highly conserved complementarity (A-A') between

positions 48-54 and 61-67 is essential for U2 function, and probably represents a helix.

Despite little natural variation, this region tolerates alteration in primary sequence if complementarity is preserved. Single mutations in the base pair G53-C62 give temperature sensitive phenotypes consistent in severity with the expected severity of disruption of the helix. The loop (B) also seems essential because multiple substitutions here are lethal. The loop is conserved (consensus G/UUAAPy), but none of several single base mutations we have made in this sequence are lethal.

The loop forms one strand of a helix (B-B') in two of the models (Fig. 1, a & b). The lack of effect of single mutations in the loop supports the idea that this proposed helix is not essential for U2 function. To prove this, we deleted the other strand of the B-B' helix by constructing a deletion of B'. This mutation is not lethal, confirming that this helix, if it exists, is not essential for U2 function. Deletion of another conserved stem-loop C-C' is not lethal, indicating that it is not essential either.

The only essential secondary structure element we have identified is the stem-loop A-B-A'. This structure could serve as a protein binding site or may play some role in branchpoint recognition or pre-mRNA catalysis. That conserved RNA structures are not essential does not imply that they do not

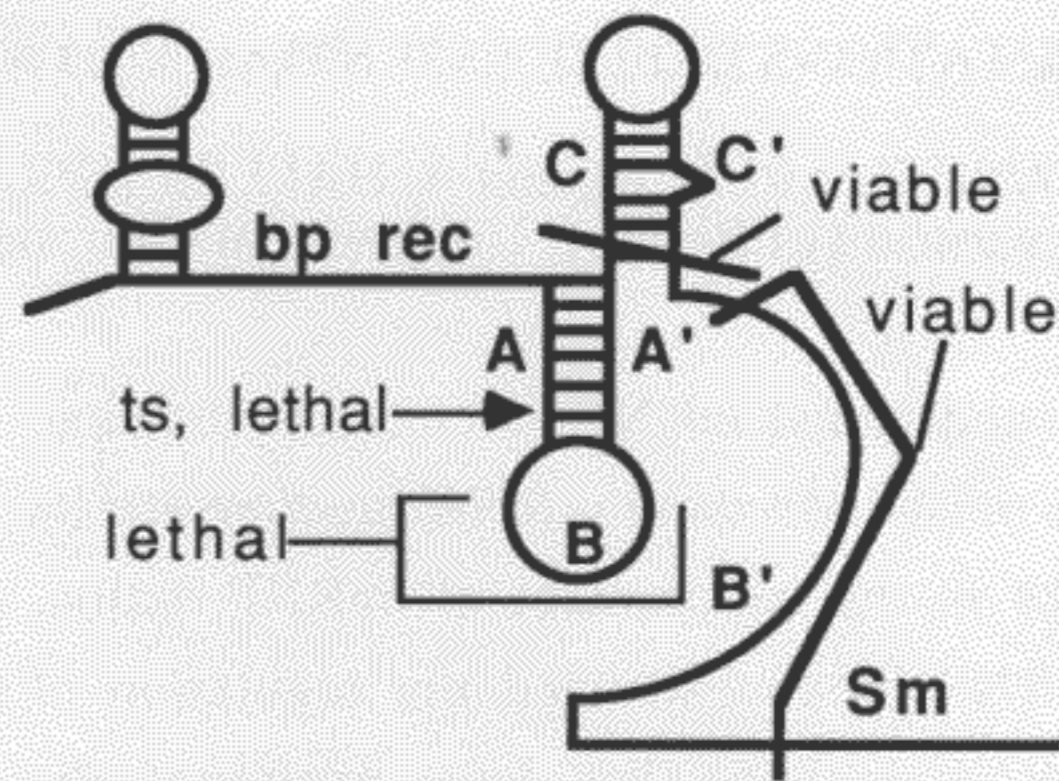


FIGURE 2. Phenotypes of mutations that disrupt U2 secondary structure elements.

have important functions, only that these functions are dispensable. Conservation could arise if sequence variation is disallowed by the potential of mutations in these nonessential regions to interfere with the formation of essential RNA structures, or the nonessential structures could confer some advantage measurable only after many generations of growth.

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