Box C/D small nucleolar RNAs: Biogenesis, structure and utilization for in vivo ribozyme studies

Dmitry A Samarsky, *University of Massachusetts Amherst*

Abstract

Eukaryotic cells contain scores of small nucleolar RNAs (snoRNAs), which are required for maturation of pre-rRNA. Two large snoRNA families exist defined by vital box C/D and box H/ACA motifs. The goal of the present study was to gain new insights into the structure and biogenesis of the box C/D snoRNAs; the knowledge developed from this effort was then recruited for practical applications. The investigation was conducted with the phylogenetically conserved U14 and U3 box C/D snoRNAs, from the yeast Saccharomyces cerevisiae. The specific aims included: (1) identification of cis-elements sufficient for biogenesis of the U14 snoRNA; (2) development of a functional map for the U3 snoRNA, and; (3) development of a U3-based model ribozyme system for in vivo studies.^ Conclusions derived from the U14 biogenesis studies are: (1) production of U14 involves ordered folding of the precursor RNA, and this step is required for formation of the vital box C/D structure motif, and; (2) the active box C/D motif, which is now predicted to consist solely of the box C and D elements, is necessary and sufficient for both accumulation and targeting RNA to the nucleolus. A general model for box C/D snoRNA biogenesis is proposed.^ Functional mapping of U3 revealed that: (1) boxes C$\sp\prime$ and D and flanking helices are critical for U3 accumulation; (2) boxes B and C are not essential for U3 production, but are important for function, due most likely to binding of a trans-acting factor(s); (3) the 5$\sp\prime$ portion of U3 is required for function, but not stability, and; (4) the non-conserved hairpins, which account for 50% of the molecule, are not required for accumulation or function.^ Based on the knowledge obtained with U14 and U3, a model ribozyme system featuring chimeric U3:ribozyme RNAs, or "snorbozymes", was developed and tested in vivo. Remarkably, the cleavage efficiency by a hammerhead ribozyme, both in cis- and in trans-configurations, appears quantitative! Other advantages of the system are: (1) a final product is stable, and; (2) authentic in vivo cleavage can be easily distinguished from artifactual cleavages. Snorbozymes are predicted to be useful for targeting natural transcripts in any eukaryotes, for fundamental research or practical applications. ^

Subject Area

Molecular biology|Genetics|Cellular biology

Recommended Citation