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BIOCHEMICAL STUDIES OF U7 snRNPs AND OF HISTONE RNA 3' PROCESSING.

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3' end processing of histone pre-mRNAs is controlled by two cis-acting sequence elements, a dyad symmetry element immediately preceding the processing (cleavage) site and a purine-rich spacer element located a few nucleotides further downstream. Three trans-acting factors have been recognised and partly characterised: the U7 snRNP which interacts with the spacer element by RNA:RNA base pairing; a hairpin binding factor (HBF) which binds to the dyad symmetry element; and a heat-labile activity (HLA) for which no specific interactions are known. Histone RNA 3' processing is down-regulated in G1/G0 arrested cells due to a specific defect in HLA. This mechanism plays a major role in replication-dependent histone gene regulation.

We are currently characterising these factors by biochemical fractionation of nuclear extracts and by studying the interactions of such partly purified components with various synthetic pre-mRNA substrates, with DNA or RNA oligonucleotides, or with synthetic U7 RNA. To date, the most purified U7 snRNP preparations have been obtained by affinity chromatography on an anti-m3G antibody column followed by MonoQ anion exchange chromatography. These fractions are active in complementation assays with micrococcal nuclease treated or anti-m3G depleted extracts. Although these preparations still contain some U5 and U11 snRNPs, our results strongly suggest that U7 is the only snRNP participating in histone RNA 3' processing, but that other non-snRNP factors participate in the reaction.

In another approach we are studying the interactions of processing components partially purified by ion exchange chromatography with synthetic DNA or RNA molecules corresponding to various parts of the histone pre-mRNA. DNA oligonucleotides corresponding to either the dyad symmetry or spacer elements have no effect on the processing reaction. However an oligonucleotide fully complementary to the first 16 nucleotides of U7 RNA is strongly inhibitory and can be used to decorate U7 snRNPs on native polyacrylamide gels. Synthetic pre-mRNA molecules form a set of complexes on such gels whose specificity is, however, not yet fully established. A further study of these interactions is expected to lead to the identification of specific polypeptides associated with the processing reaction.

Attempts to assemble U7 RNA in vitro into functional snRNPs or into complexes reacting with anti-Sm antisera have, so far, not been successful. However, preliminary experiments suggest that northwestern blotting and UV crosslinking techniques may be more successful in identifying potential U7-specific proteins. Microinjection experiments with X. laevis oocytes have been initiated to study U7 snRNP assembly in vivo.

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