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**Due Date August 22, 2014**

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**Contribution of the non-catalytic Subunits of Eukaryotic RNase H2 to Substrate Recognition and Protein Interactions**

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Eukaryotic RNases H2 comprise three different subunits. In this report we determine the composition and stoichiometry of the human RNase H2 complex by biochemical analysis and find it to be the same as described before by genetic studies. Human and *Saccharomyces cerevisiae* RNASEH2A/Rnh201p subunits contain the catalytic center and are similar to each other and to prokaryotic RNase HII, which is functionally active as a single polypeptide. Although the RNASEH2B and RNASEH2C subunits from human and *S. cerevisiae* share very little homology they both form soluble B/C complexes that may serve as a nucleation site for the addition of the catalytic RNase H2A protein, to form an active RNase H2, or for interactions with other proteins to support different functions. One such interaction, of very relevant significance, is with Proliferating Cell Nuclear Antigen (PCNA), a protein essential for replication and repair processes. Human RNase H2 interacts with PCNA via the PIP-box present in the RNase H2B subunits.

In an attempt to understand the function of the B/C subunits of RNase H2 and how defects in these proteins can induce Aicardi-Gouti**è**res Syndrome (AGS), we purified and characterized several AGS-related mutants, and found that most of these mutated proteins have almost normal RNase H activity.

Human RNase H2 purified from HeLa cells presents identical properties as the recombinant enzyme purified from *Escherichia coli*. Unlike *E. coli* RNase HII, eukaryotic RNase H2 can act in a variety of RNA/DNA hybrids with similar efficiencies, hydrolyzing these substrates in a processive manner, suggesting that the RNase H2 complex may participate in multiples cellular processes, some of which may involve long stretches of RNA/DNA hybrids or multiples single/short RNA sequences embedded in DNA. The fact that both types of eukaryotic RNases H are processive enzymes, while their prokaryotic counterparts are not, is very intriguing.