Individual subprojects:

Project 1 - Investigating the Allosteric Regulation of Human RNR by Nucleotide-Induced Oligomerization

Scientific Background and Significance- Human RNR (hRNR) is a multi-subunit enzyme that catalyzes the conversion of ribonucleotides to 2'-deoxynucleotides, which is the rate determining step of dNTP synthesis (Fig. 1A). hRNR is specifically regulated to maintain an active form of the enzyme during S-phase of the cell cycle (Fig. 1B). hRNR consist of a large catalytic subunit (α) that houses a catalytic site (C-site), a specificity site (S-site) and an allosteric site (A-site) (Fig. 1C), and a small subunit (β) that delivers an essential free radical required for catalysis. The allosteric activator ATP and inhibitor dATP both induce α to form hexamers (Fig. 1D) and the mechanistic basis for this functional difference remains a major question. A previous 28 Å negative stain EM structure from our lab revealed ATP and dATP dependent hexamerization of α subunit and different conformations for each. To achieve the level of resolution necessary to reveal the molecular nature of these differences we are using cryo-EM to compare the ATP and dATP conformations and characterize differences in their interaction with the β subunit. Samples of hexamers formed in dATP are stable, monodisperse, and provide high quality images and 2D class averages (Fig 1E). Results to date have proven groundbreaking in revealing for the first time the presence of the β subunit in the α hexamer at moderate resolution (Fig. 1F), as well as a high-resolution (2.8 Å) structure of the inactivated hexameric enzyme (Fig. 1G).

Goals and Objectives- Structures of high-order oligomerization states of α-β complexes will define the subtle conformational changes induced by ATP versus dATP binding. Our initial focus is determining how the dATP-induced hexamers are inactivated and the complexes formed with the β subunit. Next, we will seek to understand how the concentration and identification of dNTP effectors and substrate binding affects RNR hexamerization. These complexes are being pursued biochemically in parallel and are yielding additional samples that will guide cryo-EM analysis. Moreover, the determined structure (Fig. 1) will be used to design anticancer molecules for inhibiting hRNR. Recent evidence suggests that a set of nucleoside drugs developed in the Dealwis group cause hRNR to form inactive hexamers analogous to the allosteric inhibitor dATP. Hence, dissecting how structural arrangement leads to inactivity will provide further clues to designing more potent and selective inhibitors for the treatment of cancer.

Project 2 – Structure, Function, and Inhibition of Bacterial Ribonuclease P Enzymes

Scientific Background and Significance- RNase P occurs ubiquitously in biology and catalyzes the 5' end maturation of tRNAs and other RNA precursors (Fig. 2A). Most RNase P enzymes are ribonucleoproteins with RNA subunits (P RNAs) that contain the active site and a protein that binds substrate 5' leader sequences. In addition to pre-tRNA, Bacterial RNase P cleaves multiple mRNAs and other small RNAs, although a complete accounting of substrate specificity is unavailable. To learn how a highly specific RNase accommodates variation in sequence and structure among its targets we determined the structure of the *E. coli* RNase P using cryo-EM. Preliminary data demonstrates the ability to generate stable, uniform RNase P ribonucleoprotein particles (Fig. 2B). The resulting structure model reveals the compact folded P RNA and the smaller protein subunit (Fig. 2C) and the binding pocket for tRNA and other substrates. While the enzymes from *E. coli* and *B. subtilis* are well studied, the P RNA subunit can vary in regions known to contact substrates (Fig. 2D). Understanding the differences in structure and function among RNase P enzymes from pathogenic bacteria could thus provide information relevant to inhibitor development as well as revealing basic principles of macromolecular recognition.

Goals and Objectives- Significant differences in P RNA structure are seen throughout phylogeny, including pathogens, which may provide inroads for small molecule inhibition. We expressed, purified, and are characterizing the RNase P enzymes from over ten species including important human pathogens (Fig. 2E). Multiple variants that form stable, active complexes amenable for immediate cryo-EM analysis. Importantly, transcriptomic experiments identified multiple alternative substrates for RNase P, however, the recognition of these substrates, the contacts formed, and the similarities and differences between the contacts they form with RNase P will be pursued in the next phase of the project. Similar to Project 1, the combination of cryo-EM, enzymology, and biochemistry will provide synergistic information for necessary for bridging the gap in our understanding between the structure of RNase P and its biological functions. This information in turn will enhance the pace and scope of inhibitor discovery for this key antimicrobial target.

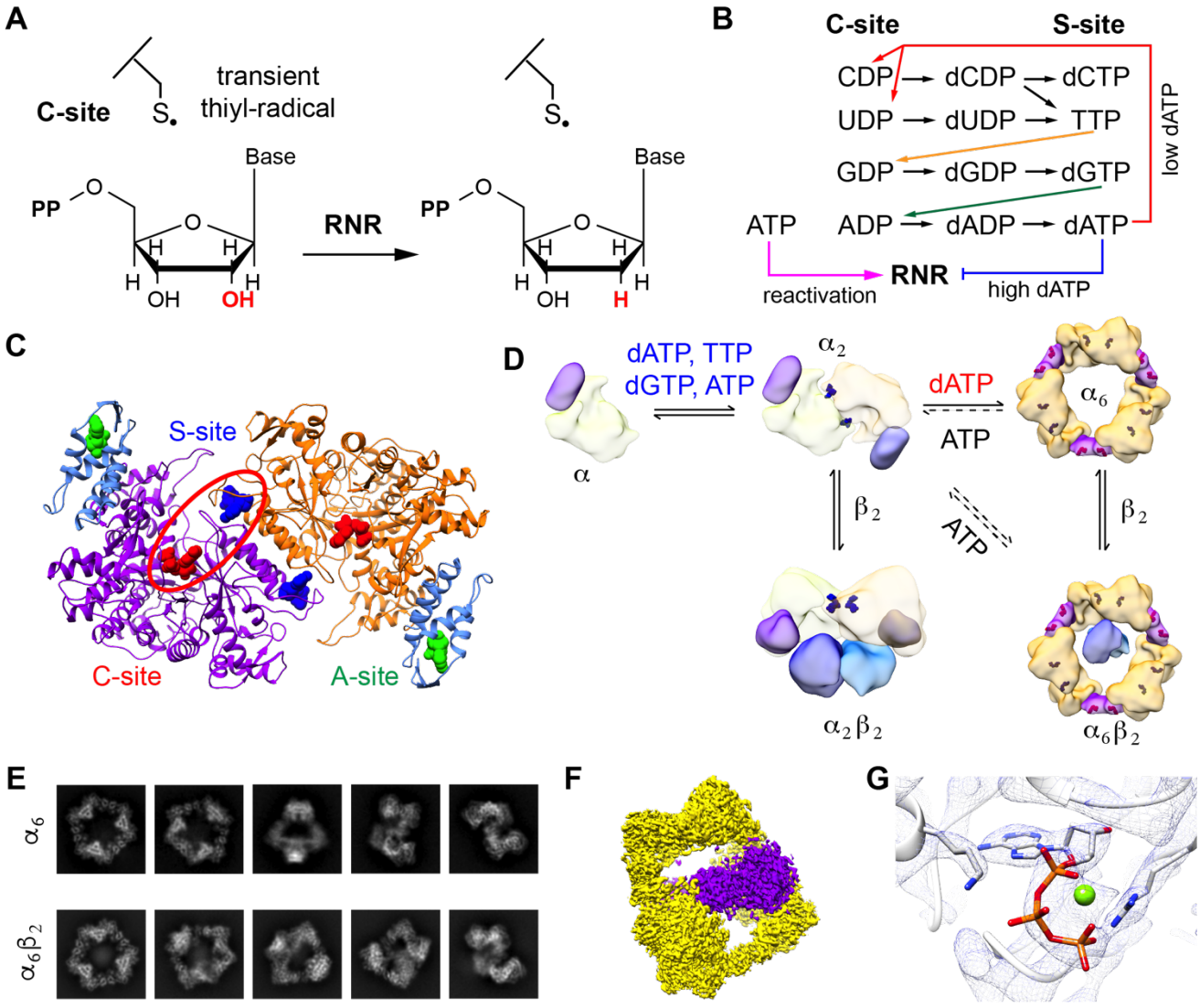

Figure 1. Structure and dynamics of human ribonucleotide reductase (RNR). (**A**) RNRs catalyze the reaction to replace the 2' hydroxyl group of a ribonucleotide by hydrogen. The reaction is initiated by a transient thiyl radical generated by one-electron oxidation of a cysteine residue in the catalytic site (C-site). (**B**) The specific regulation of a substrate in the active site is coupled with the effector at the specificity site (S-site). The coupling between C-site and S-site allows RNR to sense the ribonucleotide pool to regulate its own activity dynamically. (**C**) Structure of an hRNR dimer. The ligands in the C-site, S-site and allosteric site (A-site) are shown in red, blue and green, respectively. (**D**) Dynamic regulation of the RNR complex assemblies by sensing levels of different nucleotide. (**E**) Representative 2D class average of hRNR α6 (top) and α6β2 (bottom) complexes. (**F**) Cryo-EM density map of the α6β2 hRNR complex with α and β subunit in yellow and purple respectively. (**G**) High-resolution cryo-EM density map enables unambiguous assignment of the dATP nucleotide in the binding pocket.

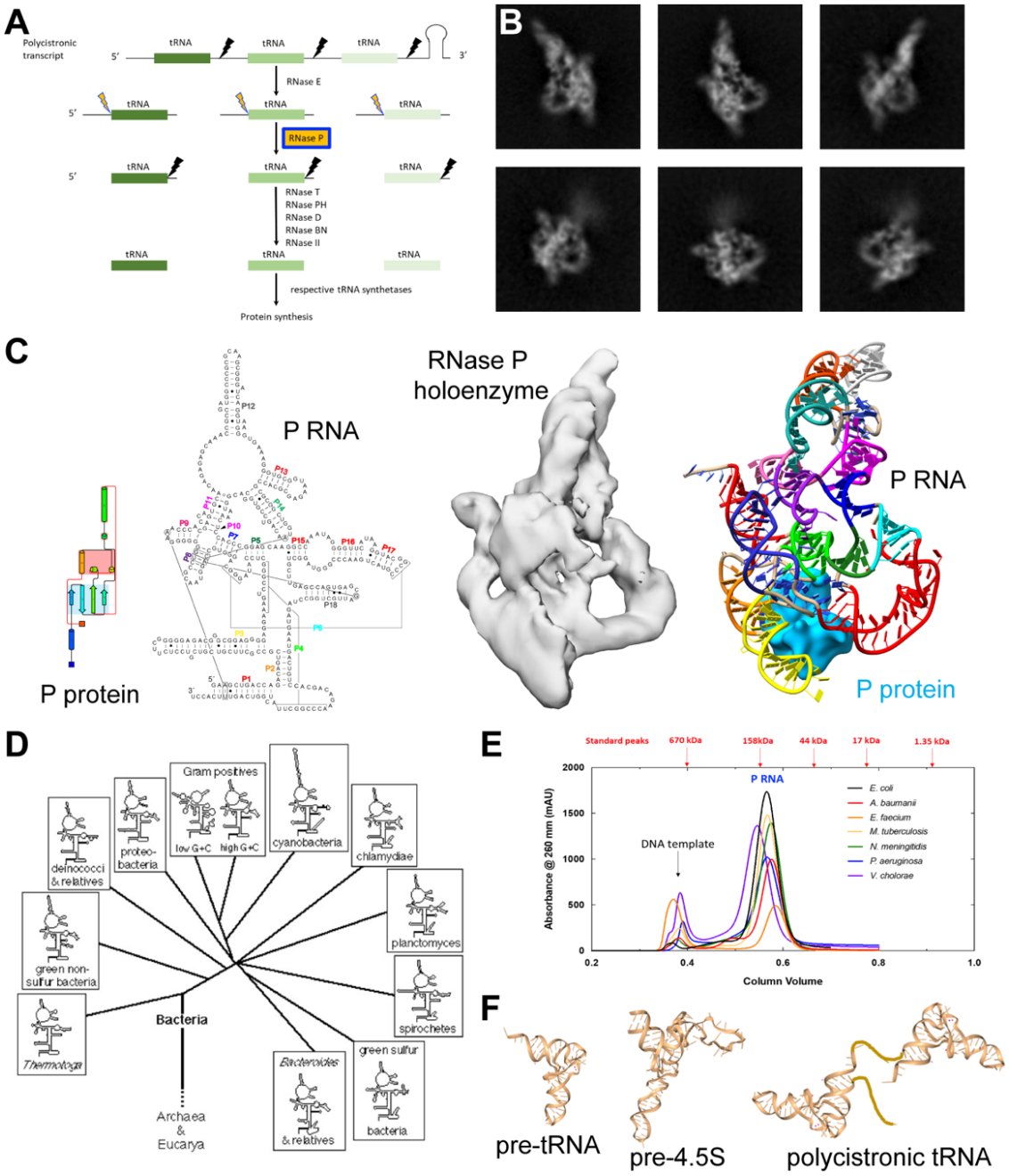

Figure 2. The structural plasticity of Ribonuclease P (RNaseP) and the structure determinant of their substrate specificity. (**A**) RNaseP is a ribozyme that process the 5' sequence of tRNA. (**B**) 2D class averages of *E. coli* RNaseP holoenzyme. (**C**) Secondary structure model of the P RNA from *E. coli* (left), cryo-EM density map of the ribonucleoprotein complex (middle) and preliminary 3D structural model of *E. coli* RNaseP holoenzyme. (**D**) Distinct phylogenetic variation of P RNAs are able to recognize a very similar pre-tRNA substrate. (**E**) Size exclusion chromatography of various P RNAs from various pathogenic bacteria generated from the Harris lab. (**F**) Compared to pre-tRNA with known 3D structure model for its binding to RNase P, two additional substrates, pre-4.5S and polycistronic tRNA, have limited structural information on their recognition and specificity.