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**Ribosome Assembly and Transaction**

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Beamline(s): X28C

**ABSTRACT:** This study is aimed toward the use of synchrotron-generated hydroxyl radicals to study the structure-function relationships and three-dimensional folding of ribosomes. The advantages of synchrotron-generated radicals is (a) that they can be generated rapidly, on the millisecond time-scale, allowing for the first time real-time structural probing of ribosomal processes, and (b) synchrotron radiation penetrates into the interstices of the ribosome, generating radicals in molecular spaces to which conventional solvent-generated probes may have only limited access. Ribosomes or ribosomal subunits were reacted with hydroxyl radicals generated either chemical via the Fenton reaction using Fe(II)-EDTA, or by irradiation with synchrotron-generated X-rays at the X-28C beamline. Specific cleavage of the backbones of 16S and 23S rRNA was then assessed by primer extension following isolation of the RNA from the treated ribosomes or subunits. Cleavages were quantified using PhosphorImager analysis.

During this year, extensive calibration studies were carried out to establish the conditions under which hydroxyl radical probing can be carried out using synchrotron X-rays. The parameters that were studied included ribosome concentration, presence of 30S and 50S subunits, buffer types and concentrations, and times of irradiation. In addition, we addressed the possibility that synchrotron radiation causes oxidation of RNA in ribosomes due to activation of dissolved molecular oxygen; this potential problem was ruled out by showing that there were no detectable differences in RNA cleavage between samples saturated with oxygen vs. samples purged with helium. It was also observed that quenching of the radicals is significant at ribosome concentrations in the range that have been used for typical solution probing experiments. Therefore, experiments have to be carefully designed to ensure that the total mass of ribosomes is maintained at constant levels.

The first model experiment is to compare the protections against hydroxyl radical attack due to association of ribosomal subunits, using radicals generated by the synchrotron vs solution Fe(II)-EDTA methods. The results of these studies show that the cleavage patterns are similar, but not identical. The differences in the probing patterns may reflect different chemistries, or differences in the way the radicals are able to access the RNA surfaces within the ribosome. If the latter is the case, it may provide new information about molecular contacts at the ribosomal subunit interface, and about solvent accessibility to interstices of the ribosome. Experiments are being designed to distinguish between these possibilities.