FASSBERG
SEMINAR SERIES

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NAD-capped RNA:
A cellular "Glue" for Specific RNA-protein linkage in bacteria

A hallmark in prokaryotic gene expression was the absence of 5′-capped RNA. In eukaryotes, the m7G-cap protects mRNA from degradation and modulates maturation, localisation or translation. Recently, we identified the ubiquitous redox cofactor nicotinamide adenine dinucleotide (NAD) to be covalently linked to bacterial RNA. Biochemical studies revealed that analogous to a eukaryotic cap, 5′-NAD modification stabilise RNA against 5′-processing by RppH and cleavage by RNase E. Moreover, we discovered the bacterial Nudix hydrolase NudC to act as an NAD-RNA decapping enzyme *in vitro* and *in vivo*. Crystal structures of *E. coli* NudC in complex with NAD and with cleavage product NMN reveal the catalytic residues lining the binding pocket and principles underlying molecular recognition of substrate and product. In *in vitro* competition experiments, NudC preferred NAD-RNA over NAD(H), suggesting that NAD-RNA is its primary biological substrate. Since our discovery of NAD-capped RNA in *E. coli*, NAD-RNAs were identified in higher eukaryotes as well. However, the biological relevance of the cap, despite RNA stabilization, remained largely unknown. To study the biological significance of this novel RNA modification we are interested in proteins that can "read" the NAD-cap. NAD itself is metabolized by various enzymes in the cell, e.g. ADP-ribosyltransferases (ADPRTs), which use the redox cofactor as a substrate to specifically ADP-ribosylate target proteins.

We can show that analogous to NAD, NAD-RNA serves as a substrate for ADPRTs. Prokaryotic and eukaryotic ADPRTs can catalyse the transfer of the ADP-ribose-RNA moiety of NAD-capped RNA to target proteins *in vitro* and *in vivo.* This novel reaction, which we term "RNAylation", describes the specific covalent attachment of an RNA to a protein using the NAD-cap as a "glue". Using Mass spectrometry as well as biochemical approaches we can show that ADPRTs of the Phage T4 specifically RNAylate bacterial proteins.

Our results not only challenge the established view on T4 phage infection, but also reveal a new direct interplay between 5′-RNA modification and posttranslational protein modification.

Host: Dirk Görlich

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Max Planck Institute for Biophysical Chemistry
Large Seminar Room / Administration Building