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Commentary

# An Application on Metabolic Labeling of RNA

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## ABOUT THE STUDY

In eukaryotes, metabolic tagging of RNAs with chemically active non-canonical nucleosides, followed by chemo-selective conjugation with imaging probes or enrichment tags, has emerged as a potent approach for examining RNA transcription and breakdown. Metabolic RNA labeling, on the other hand, is not suitable to prokaryotes, where the intricacy and uniqueness of gene regulation are still being investigated. 2′-deoxy-2′-azidoguanosine (AzG) is a non-canonical nucleoside that may be used to metabolically mark bacterial RNAs. We developed AIR-seq (azidonucleoside-incorporated RNA sequencing), with AzG, which allows for genome-wide study of transcription in *E. coli* under heat stress. Furthermore, when AIR-seq is used in conjunction with pulse-chase labeling, it is possible to do a worldwide investigation of bacterial RNA degradation. Finally, we show that in living mice, RNAs from the gut microbiota may be metabolically tagged with AzG.

AzG-enabled metabolic RNA labeling should have a wide range of applications in RNA biology research in a variety of bacterial species. The interaction of RNA transcription, processing, and destruction results in cellular RNA levels. In addition to studies on total RNAs, it is desirable to study nascent RNAs selectively to understand these tightly controlled processes. Several complementary approaches with nascent RNA-specificity have been developed, considerably assisting in the comprehension of gene regulatory networks. Chemically active nucleoside analogues (i.e. non-canonical nucleosides) can be employed as surrogates for natural nucleosides and for RNA production in live cells, according to one of the ways. Chemically conjugating non-canonical nucleoside-incorporated RNAs with fluorophores for imaging or affinity tags for enrichment and sequencing follows.

This approach enables for the study of transcription by separating nascent RNAs from pre-existing populations since non-canonical nucleosides can only be integrated into newly transcribed RNAs. Furthermore, by monitoring the decay of pulse-labeled RNAs, RNA degradation may be measured and profiled by using non-canonical nucleosides in pulse-chase labeling experiments. For metabolic RNA labeling in diverse eukaryotic cells, a variety of nucleoside analogues have been created. 4-thiouridine (4SU) and 5-ethynyluridine (EU) are two of the most common non-canonical nucleosides that may be conjugated using thiol coupling and click chemistry, respectively. Although metabolic RNA labeling has proved useful in the study of RNA dynamics in eukaryotes, it is not relevant to bacteria.

For a long time, prokaryotic transcriptases were thought to be considerably easier to study. As a result, until recently, whole-transcriptase investigations in bacteria trailed behind those in eukaryotes). Transcriptomics has reshaped our understanding of the complexity, dynamics, and regulatory mechanisms of bacterial transcriptase's during the last two decades. For example, hundreds of short noncoding RNAs (ncRNAs) are now known to influence bacterial mRNAs, which matches the extent of microRNA regulation in eukaryotic cells. Furthermore, through regulating transcription start and regulatory elements such as riboswitches and RNA thermometers, mRNA expression in bacteria varies dynamically in response to cellular and environmental stimuli. However, there are few ways for studying bacterial nascent RNAs. The development of a metabolic RNA labeling technique for understanding bacterial RNA biology is thus of tremendous importance.

The non-canonical nucleoside 2′-deoxy-2′-azidoguanosine (AzG) is described here as a non-canonical nucleotide for bacterial labelling. AIR-seq (azidonucleoside-incorporated RNA sequencing), in which bacterial RNAs are metabolically tagged with AzG, click-labeled, and selectively selected for analysis, is made possible by AzG. In *E. coli*, we use AIR-seq to perform a genome-wide study of transcription in response to heat stress. Heat shock-induced transcripts are revealed by AIR-seq, which were previously undetectable by RNA-sequencing (RNA-seq) examination of total RNAs. In addition, AIR-seq in combination with the pulse-chase labeling technique allows for a worldwide investigation of mRNA degradation in *E. coli* without the necessity for transcription inhibition. The findings classify bacterial transcripts based on their degradation rates. Finally, metabolic RNA tagging with AzG may be used on a variety of bacterial species as well as the gut micro-biotas of live mice.

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