

# Efficient preparation and properties of mRNAs containing a fluorescent cap analog: Anthraniloyl-m<sup>7</sup>GpppG

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**Keywords:** capped mRNA, decapping assay, Fluorescence, imaging, RNA capping

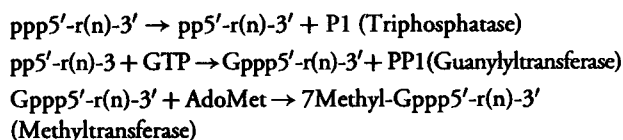
**Abbreviations:** Ant, Anthraniloyl; Ant-m<sup>7</sup>GTP, Anthraniloyl-7-methyl guanosine triphosphate; Ant-m<sup>7</sup>GMP, Anthraniloyl-7-methyl guanosine monophosphate; Ant-GTP, Anthraniloyl guanosine triphosphate; IVT, *In vitro* translation; OMP, Outer Membrane Protein; SAM, S-Adenosyl Methionine.

A method has been developed for synthesising fluorescently labeled capped mRNA. The method incorporates a single fluorescent molecule as part of the 5'-mRNA or oligonucleotide cap site. The fluorescent molecule, Ant-m<sup>7</sup>GTP is specifically incorporated into the cap site to yield Ant-m<sup>7</sup>GpppG-capped mRNA or oligonucleotide. Efficient capping was observed with 60–100% of the RNA transcripts capped with the fluorescent molecule. The Ant-m<sup>7</sup>G derivative, which has been previously shown to interact with the eukaryotic cap binding protein eIF4E, is shown in this paper to be a substrate for the *Vaccinia* capping enzyme and the DCP2 decapping enzyme from *Arabidopsis*. Further, the Ant-m<sup>7</sup>GTP-capped RNA is readily translated. This Ant-m<sup>7</sup>GTP-capped RNA provides an important tool for monitoring capping reactions, translation, and biophysical studies.

A distinctive feature found in eukaryotic mRNA and several small RNAs is the presence of a cap structure (m<sup>7</sup>GpppN, where N is any nucleotide) at the 5' terminus.<sup>1</sup> The cap structure serves as a multi-purpose modification that is recognized by many cellular proteins involved in pre-mRNA splicing, RNA export, translation initiation and RNA turnover.<sup>2</sup> Proteins that interact with the cap structure include the cap binding complex (CBC) that plays a major role in RNA processing by activating pre-mRNA splicing and nucleocytoplasmic transport of small snRNAs, eukaryotic translation initiation factors (eIFs) that initiate translation of processed mRNAs, as well as the Dcp1/Dcp2 complex which mediates the hydrolysis of the cap structure (decapping) prior to the 5' → 3' decay of RNA.<sup>3–7</sup>

Analogues of the mRNA cap have been instrumental in the study of cap-associated processes, in particular, cap-dependent translation.<sup>8–11</sup> It has been demonstrated that 7-methylguanosine, ribose and phosphoryl moieties are the minimal structural requirements for cap analogs.<sup>12</sup> Numerous cap analogs have been synthesized and have been instrumental in biophysical studies of cap binding, in assessing the determinants of translation inhibition, in the purification of a range of eIF4E proteins and in the priming of *in vitro* transcription reactions to synthesize capped RNA.<sup>8,13–17</sup>

*In vivo*, the cap structure is attached to an mRNA transcript, at the stage of 25–30 nucleotides by consecutive enzymatic reactions catalyzed by triphosphatase, guanylyltransferase, and methyltransferase activities.<sup>1,2,18–24</sup> The reactions are shown below:



This pathway seems to be conserved, although very few examples have been characterized in detail.<sup>25</sup> The consecutive enzymatic activities of capping have been primarily determined using the recombinant *Vaccinia* capping enzyme.<sup>18–20</sup> Capping involves the sequential hydrolysis of a monophosphate group from triphosphorylated RNA, transfer of a GMP moiety to the diphosphorylated RNA transcript and the acceptance of a single methyl group from S-adenosylmethionine to the seventh position of guanine moiety.<sup>18,19,25</sup> The recombinant *Vaccinia* capping enzyme has been used to synthesize radiolabeled capped RNA, primarily in studies on the capping and the decapping processes.<sup>6,18</sup>

In this study, we have demonstrated efficient fluorescent labeling at the cap of *in vitro* transcribed RNA, catalyzed by the

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Submitted: 07/03/2014; Revised: 09/30/2014; Accepted: 11/12/2014

<http://dx.doi.org/10.4161/21690731.2014.988538>