Finally, the ability of VCE-capped RNA to be translated was examined. RNA encoding *Renilla* luciferase was produced via IVT using an AmpliScribe™ T7-*Flash*™ Transcription Kit. The RNA was capped with VCE in the absence or presence of SAM, poly(A)-tailed with A-Plus™ Poly(A) Polymerase, and used to transfect HeLa cells in culture. Twenty-four hours post transfection, the cells were harvested and lysed. Lysates were assayed for luciferase activity and normalized to total protein content. The relative translation efficiencies are shown below in Table 1. Maximum signal intensity was produced with cap 0-capped, poly(A)-tailed RNA.

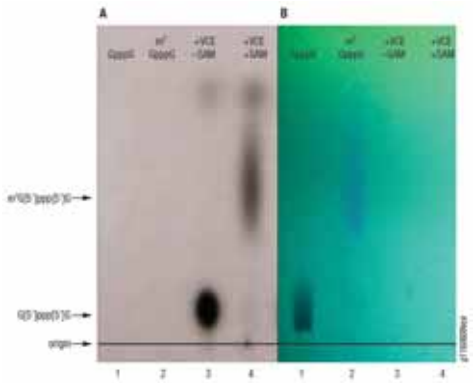

FIG 4. Complete guanine-7-methyl transfer activity of VCE. Digestion products were subjected to thin layer chromatography on PEI-F cellulose plates developed in 0.4 M LiCl and 1 M Formic acid. The plates were visualized under short-wave UV light (**B**) and then subjected to autoradiography (**A**). Cap analog standards G[5']ppp[5']G (**Lane 1**) and m^{7}G[5']ppp[5']G (**Lane 2**) were also run. The cap analog m^{7}G[5']ppp[5']G appears as a smear with blue fluorescence under these conditions.

Conclusion

EPICENTRE's new ScriptCap™ m^{7}G Capping System featuring Vaccinia Virus Capping Enzyme (ScriptCap™ Capping Enzyme), provides a convenient, highly efficient, single-enzyme capping system to quantitatively produce Cap 0 structures on any amount of RNA *in vitro*.

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Table 1. Translation efficiency of RNA capped with VCE.

Post-transcription Treatment	Relative Translation Efficiency
No RNA	0%
Poly(A)-tailed, non-capped	~0%
Poly(A)-tailed, capped without SAM	10%
Poly(A)-tailed, capped with SAM	100%

EPICENTRE Products utilized in this work (see www.EpiBio.com for more details).

- ✓ ScriptCap™ m^{7}G Capping System
- ✓ ScriptCap™ Capping Enzyme
- ✓ Tobacco Acid Pyrophosphatase
- ✓ A-Plus™ Poly(A)-Polymerase
- ✓ RNase I
- ✓ APex™ Heat-Labile Alkaline Phosphatase
- ✓ AmpliScribe™ T7-*Flash*™ Transcription Kit

www.EpiBio.com/scriptcapvce.asp

ScriptCap™ m^{7}G Capping System

SCCE0625 25 Reactions

Contents: ScriptCap™ Capping Enzyme, 10X Capping Buffer, ScriptGuard™ RNase Inhibitor, 20 mM SAM, 10 mM GTP, RNase-Free Water

New!

Improve the Translation Efficiency of any 5'-Capped mRNA with the NEW ScriptCap™ 2'-O-Methyltransferase

Ronald Meis and Judith E. Meis, EPICENTRE Biotechnologies

Introduction

Various classes of eukaryotic cap structures, differentiated by their state of methylation, have been identified. Cap 0 structures contain an N7 methyl group on the capping guanosine nucleotide (see previous article) and are found in lower eukaryotes (m^{7}GpppNpNpN...).[1] Cap 1 structures contain an additional methyl group at the 2'-O position of the penultimate nucleotide (m^{7}Gppp[m$^{2'\text{-}O}$]NpNpN...) (FIG 1), while Cap 2 structures contain yet an additional methyl group at the 2'-O position of the antepenultimate nucleotide (m^{7}Gppp[m$^{2'\text{-}O}$]Np[m$^{2'\text{-}O}$]NpN...). Cap 1 and Cap 2 structures are found in higher eukaryotes.[1]

Distinct 2'-O-methyltransferases are responsible for the methylation of the penultimate and antepenultimate nucleotides of the cap structures. These are sometimes referred to as Cap 1 methyltransferase and Cap 2 methyltransferase.[2] Although the exact function of these additional methylation events is still unclear, a growing body of evidence suggests that the Cap 1 methylation serves, at least in part, to increase the translation efficiency of the mRNA.[3] Cap 1-capped RNA cannot be produced through the use of a dinucleotide cap analog in an *in vitro* transcription system.[4] In this article, we introduce EPICENTRE Biotechnologies' new ScriptCap™ 2'-O-Methyltransferase derived from Vaccinia Virus Cap 1 methyltransferase[5-7] (VMT) for the preparation of completely Cap 1-capped RNA from any source of Cap 0-capped RNA, whether it be from the ScriptCap m^{7}G Capping System (see previous article; EPICENTRE), or cap analog-based transcription kits such as the AmpliCap-Max™ High Yield Message Maker Kits (EPICENTRE).

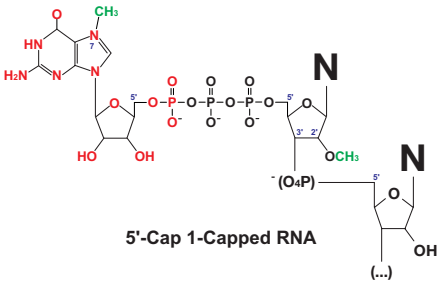

FIG 1. 5'-Cap 1-Capped RNA. Red atoms are derived from GTP, green atoms are derived from SAM.

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Improve Translation Efficiency. . . *Cont'd from page 5*

Methods and Results

FIG 2 shows both the substrate specificity and qualitative 2'-O-methyltransferase activity of VMT. A 51 nucleotide RNA transcript was either untreated or cap 0-capped using the ScriptCap™ m^{7}G Capping System (producing a 52 nucleotide RNA product). A mixture of uncapped and cap 0-capped RNA (lane 2), or just cap 0-capped RNA (lane 3), was incubated with VMT and ^{14}C-methyl-S-adenosyl-methionine (SAM; methyl group donor). Only cap 0-capped RNA served as a substrate for VMT.

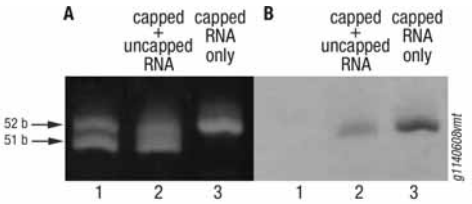

FIG 2. Substrate specificity and qualitative 2'-O-methyltransferase activity of VMT. A total of 0.1 µg of RNA from each treatment was run on a denaturing polyacrylamide gel. **A**. Stained with ethidium bromide. **B**. Dried and subjected to autoradiography. The 52 and 51 nucleotide RNA markers are shown in **Lane 1**.

Complete 2'-O-methylation by VMT is demonstrated in FIG 3. A 335 nucleotide transcript was capped by simultaneous treatment with both the ScriptCap m^{7}G Capping System and ScriptCap™ 2'-O-Methyltransferase with α-[^{32}P]-GTP in the absence (lane 2) or presence (lane 3) of SAM. A control ScriptCap m^{7}G Capping System reaction which lacked VMT is shown in lane 1. After capping, the RNA was digested to completion with RNase I and APex™ Heat-Labile Alkaline Phosphatase. The only RNase-resistant products are either the 5'-cap dinucleotide, due to its 5' to 5' linkage, or the 5'-cap trinucleotide, due to the 5' to 5' linkage and the 2'-O-methylation of the penultimate nucleotide. The lane 1 capping reaction produced cap 0-capped RNA, which resulted in a dinucleotide RNase-protected product. The lane 3 capping reaction produced cap 1-capped RNA, which resulted in a trinucleotide RNase protected product. The lane 2 capping reaction produced unmethylated capped RNA, which resulted in a dinucleotide RNase-protected product. The lack of an RNase-resistant dinucleotide in lane 3 indicates that the RNA was completely 2'-O-methylated by VMT. Reactions can be scaled up to quantitatively treat any amount of cap 0-capped RNA (data not shown).

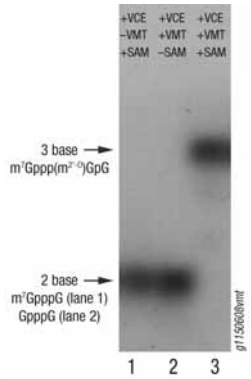

FIG 3. Complete 2'-O-methyltransfer by VMT. Digestion products were run on a denaturing polyacrylamide gel, dried and subjected to autoradiography.

Table 1. Translation efficiency of cap 1-capped RNA produced with VMT.

Means of Cap 0 Production	ScriptCap™ 2'-O-Methyltransferase Treatment	Final mRNA Cap Structures Formed	Translation Efficiency relative to non-treated mRNA
No RNA	no	none	0%
ScriptCap™ m^{7}G Capping System (Capping Enzyme)	no	m^{7}GpppN (Cap 0)	100%
ScriptCap™ m^{7}G Capping System (Capping Enzyme)	yes	m^{7}Gppp[m$^{2'-O}$]N (Cap 1)	147%
AmpliCap-Max™ High Yield Message Maker Kit (Standard Cap Analog)	no	m^{7}GpppN * (Cap 0)	100%
AmpliCap-Max™ High Yield Message Maker Kit (Standard Cap Analog)	yes	m^{7}Gppp[m$^{2'-O}$]N * (Cap 1)	148%
AmpliScribe™ T7-*Flash*™ Transcription Kit (ARCA Cap Analog)	no	m$_2^{7,3'-O}$GpppN * (Cap 0)	100%
AmpliScribe™ T7-*Flash*™ Transcription Kit (ARCA Cap Analog)	yes	m$_2^{7,3'-O}$Gppp[m$^{2'-O}$]N * (Cap 1)	128%

* Represents the predominant cap structure formed in the reaction (see previous article).

Since cap 0-capped mRNA can be produced post-transcriptionally with a capping enzyme, or co-transcriptionally with a cap analog-based transcription kit, we examined the translation efficiency of mRNA produced by both means after subsequent conversion to cap 1-capped mRNA with VMT. Cap 0-capped *Renilla* luciferase mRNA was produced, 2'-O-methylated, poly(A)-tailed with A-Plus™ Poly(A) Polymerase, and used to transfect HeLa cells in culture. Twenty-four hours post transfection, the cells were harvested and lysed. Lysates were assayed for luciferase activity and normalized to total protein content. The means of cap 0 production, final cap structures produced, and relative translation efficiencies are shown in Table 1. Treatment with ScriptCap 2'-O-Methyltransferase improved the luciferase expression irrespective of the means of cap 0-cap production.

Conclusion

EPICENTRE's new ScriptCap™ 2'-O-Methyltransferase provides a convenient and effective method for preparing cap 1-capped RNA from any source of cap 0-capped RNA. Treatment may be done simultaneously with the ScriptCap™ m^{7}G Capping System, or sequentially following cap analog-based transcription reactions.

References

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EPICENTRE Products Utilized in this Work (see www.EpiBio.com for more details).

- ✓ ScriptCap™ 2'-O-Methyltransferase
- ✓ ScriptCap™ m^{7}G Capping System
- ✓ AmpliCap-Max™ High Yield Message Maker Kits
- ✓ RNase 1
- ✓ APex™ Heat-Labile Alkaline Phosphatase
- ✓ A-Plus™ Poly(A)-Polymerase
- ✓ AmpliScribe™ T7-*Flash*™ Transcription Kit
- ✓ ARCA Cap Analog

www.EpiBio.com/scriptcapvmt.asp

ScriptCap™ 2'-O-Methyltransferase

SCMT0625 25 Reactions
Contents: ScriptCap™ 2'-O-Methyltransferase, 10X Capping Buffer, 20 mM SAM