mRNA is modified co-transcriptionally at the 5' end by the addition of an inverted guanosine cap structure which can be methylated at several positions. The mRNA cap recruits proteins involved in gene expression and identifies the transcript as being cellular or ‘self’ in the innate immune response. Methylation of the first transcribed nucleotide on the ribose 2'-O position is a prevalent cap modification which has roles in splicing, translation and provides protection against the innate immune response. In this review, we discuss the regulation and function of CMTR1, the first transcribed nucleotide ribose 2'-O methyltransferase, and the molecular interactions which mediate methylated 2'-O ribose function. © 2017 Wiley Periodicals, Inc.

How to cite this article:
WIREs RNA 2017, 8:e1450. doi: 10.1002/wrna.1450
discussion on the mammalian capping enzymes. In mammals, a single enzyme, RNGTT (RNA guanylyltransferase and 5’ triphosphatase) possess both triphosphatase and guanylyltransferase activities. Guanosine cap 7′-N methyltransferation, creating m7G(5′)ppp(5′)N, is catalyzed by RNMT (RNA guanine-7 methyltransferase), which is found in a complex with its activating subunit RAM (RNMT-activating miniprotein). Methylation of the first and second transcribed nucleotides at the ribose 2′-O position is catalyzed by CMTR1 and CMTR2, respectively. While analyses are in agreement that m7G(5′)ppp(5′)N is the most abundant mRNA cap structure, the relative proportion of the different cap methyl groups is an area of active research. Previously, mass spectrometric analyses of cap structures were restricted to cell lines and large organs. With recent improvements in chromatography and mass spectrometry, analysis of mRNA caps in an increasing array of primary tissues and cells will be possible.

mRNA CAP FUNCTION

Following its discovery, the mRNA cap was found to protect mRNA from exonucleases, and to recruit protein complexes involved in RNA processing, nuclear export and translation and initiation. In the nucleus, the binding of CBC (Cap Binding Complex) to the mRNA cap promotes pre-mRNA splicing, nuclear export and influences pre-mRNA 3′ end processing and nonsense mediated decay. elf4E and the elf4F complex also bind to the cap and promote nuclear export and translation initiation. Over the last decade, formation of the mRNA cap has been recognized to be regulated in a gene-specific manner by the influence of transcription factors, signalling pathways and developmental pathways. Regulation of mRNA cap formation results in alterations in gene expression and subsequent changes in cell function and fate. Recently CMTR1 has been recognized to be regulated in the innate immune response, and to have a critical role in the recognition of self-RNA. This has reinvigorated interest in 2′-O methylation of the first nucleotide ribose.

First Nucleotide 2′-O-Methylation in mRNA Translation

The 7-methyl guanosine cap is critical for elf4E binding and translation initiation. elf4E binds to the scaffold protein elf4G and helicase elf4A forming the elf4F complex, which recruits the 40S ribosomal subunit to mRNA. First nucleotide 2′-O methylation was recognised to have a role in translation shortly after its discovery; it was demonstrated to enhance ribosome binding and translation. In vivo, first nucleotide 2′-O methylation was demonstrated to be important for translation during Xenopus or sea urchin development. In 1985, Caldwell and Emerson reported significant upregulation of first nucleotide 2′-O methylation in maternal mRNA following the fertilization of sea urchin embryos. Further studies by the Ritcher lab reported that first nucleotide 2′-O methylation is upregulated during Xenopus oocyte maturation. Of note, c-mos mRNA translation and resultant oocyte maturation was found to be dependent upon first nucleotide O-2 methylation.
**CMTR1: FIRST NUCLEOTIDE 2'-O-METHYLTRANSFERASE**

First nucleotide 2'-O methyltransferase activity was initially characterized in vaccinia virus and HeLa cells. However, the methyltransferase responsible for first nucleotide 2'-O methylation in mammalian cells, CMTR1 (cap methyltransferase 1), was only identified recently by Belanger et al. (Figure 1). In vitro, CMTR1 catalyses methylation of ribose 2'-OH group on the first transcribed nucleotide of guanosine-capped RNA. Although CMTR1 requires an NLS (nuclear localization signal), a cap guanosine structure (GpppN) in its substrates, it acts independently of N-7 methylation. Belanger et al. also reported that knock-down of CMTR1 does not impact on global translation as measured by 35S methionine incorporation, although it is a possibility that the remaining CMTR1 may be sufficient to maintain translation. As discussed above, first nucleotide 2'-O methylation has been linked to mRNA translation in several eukaryotic systems and deletion of the first and second transcribed nucleotide 2'-O-methyltransferase in Trypanosomes results in a significant reduction in global translation.

CMTR1 had previously been identified as KIA0082/ISG95, a protein implicated in the response to interferon treatment and viral infection. Following these studies CMTR1 was characterized as a multi-domain protein with a nuclear localization signal, G-patch domain, a RrmJ/FtsJ methyltransferase domain, a nonfunctional cap guanylyltransferase-like domain and a WW domain. Deletion of the domains C-terminal to the methyltransferase (guanylyltransferase-like and WW domains) reduces the activity of CMTR1 in vitro and therefore these domains are likely to contribute to substrate recognition and/or structural configuration. The CMTR1 WW domain interacts with the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNA Pol II). Whether CMTR1 has enhanced affinity for a particular CTD phosphorylation state, remains to be determined. Since first nucleotide 2'-O-methylation does not inhibit or enhance 7'-N methylation of the cap guanosine it is not clear whether RNMT or CMTR1 methylates the cap first, or indeed whether they have an order of action.

Structural analysis of the CMTR1 catalytic domain resolved a Rossman-like fold methyltransferase domain and determined the mechanism of guanosine cap recognition. In the CMTR1 active site, the methylated guanosine (m7G) is accommodated in a deep pocket and the transcribed nucleic acids adopt an L shape with nucleotide 1 located at the bend. Analysis of the positioning of the RNA in the exit of the active site suggests that substrate binding and methylation are sequence-independent. However, whether the domains N- and C-terminal to the methyltransferase domain interact with RNA and/or other proteins, potentially providing substrate specificity, will require further exploration. As discussed above, CMTR1 has other domains (G-patch, WW and guanylyltransferase-like) which have been demonstrated to influence activity. The N- and C-terminal domains of CMTR1 are intrinsically unstructured, which to date has impaired structural characterisation of the full-length enzyme.

**CMTR1 AND 2'-O-METHYLATION IN ‘SELF’-RNA DISTINCTION**

In recent years, the cap 1 structure (m7GpppNm) has emerged as a key factor in ‘self-RNA’ identification during the innate immune response (Figure 2). ‘Non-self’ RNA intermediates from viruses are recognized by the cellular innate immune system as foreign, triggering cellular mechanisms which protect

![Figure 1](image-url)
The role of cap1 in the innate immune response was revealed by studying the viral first nucleotide ribose 2′-O methylation, which prevents transcript recognition by RIG-I or MDA5. In the absence of ribose 2′-O methylation, RIG-I or MDA5 elicit the interferon response which includes expression of IFIT proteins. IFIT1 binds to transcripts unmethylated on ribose 2′-O to inhibit translation. m7GpppN, 7′-N cap (N, first nucleotide); m, ribose 2′-O methylation; Green lines, activity or permissive effect; Black line, binding and repression; Grey dotted lines, absence of translation.

FIGURE 2 | ‘Self’-RNA recognition and immune tolerance to 2′-O methylated RNA. CMTR1 and viral methyltransferases (Mtases) catalyse first nucleotide ribose 2′-O methylation, which prevents transcript recognition by RIG-I or MDA5. In the absence of ribose 2′-O methylation, RIG-I or MDA5 elicit the interferon response which includes expression of IFIT proteins. IFIT1 binds to transcripts unmethylated on ribose 2′-O to inhibit translation. m7GpppN, 7′-N cap (N, first nucleotide); m, ribose 2′-O methylation; Green lines, activity or permissive effect; Black line, binding and repression; Grey dotted lines, absence of translation.

Cap1 functions to prevent the aberrant activation of innate immune response readers, RIG-I and MDA5. RIG-I (Retinoic Acid Inducible Gene-I), an innate immune receptor, is a cytoplasmic protein which detects triphosphate RNA (pppRNA) and induces cellular signaling responses that ultimately drive to an interferon response. The impact of individual cap modifications, including cap guanosine 7′-N methylation and first nucleotide 2′-O methylation, on RIG-I activation was analyzed using synthetic RNA.24,46 First nucleotide 2′-O methylation was found to prevent RIG-I activation, in a mechanism utilizing a highly conserved histidine residue (H830). In cells, experimental interference with this ‘key-lock’ mechanism elicits an interferon response similar to that triggered by viral infection. The RIG-I H830A mutation results in stimulation of RIG-I by endogenous mRNA. Furthermore, suppression of CMTR1 results in RIG-I stimulation in primary human fibroblasts and induction of IFN-beta mRNA in primed A549 cells. MDA-5, another RIG-like receptor, has also been linked to the induction of type I interferon by viruses deficient in 2′-O-methyltransferase activity. MDA-5 recognizes the internal duplex structure of long dsRNA.47 Whether the composition of the viral and endogenous mRNA cap structure has a role in this interaction remains an open question.48 Upon sensing of viral RNA, the cellular proteins RIG-I or MDA5 induce type I interferon (IFN) secretion, resulting in upregulation of antiviral IFN-induced proteins (IFIT) in the infected and neighboring cells.49 These IFIT proteins include those which inhibit virus replication or protect against new infection.

When the innate immune response is triggered, the expression of effector proteins protect the host from the external agent.49 IFIT1 is a key effector of the innate immune response, which blocks the translation of viral mRNA by competing with the eIF4F complex for binding to the cap. Over-expression of human IFIT1 in cells or reticulocyte lysates reduces translation significantly.50 Specific features of mRNAs were indicated to influence their sensitivity to IFIT1.51 Structural analysis of IFIT1
revealed that it forms a tight interaction with ribose 2'-hydroxyls of first and second nucleotides. Cellular mRNAs methylated at these positions are not recognized by IFIT1 thus restricting IFIT1 activity to unmethylated viral mRNAs. Importantly, methylation of the first nucleotide alone is not sufficient to protect all endogenous mRNAs from IFIT1. 2'-O methylation of the second transcribed nucleotide also contributes to inhibition of IFIT1 action on cellular RNA, particularly on susceptible RNA sequences and at high IFIT1 concentrations. This suggests a role for CMTR2, the second transcribed nucleotide 2'-O methyltransferase in innate immunity. Furthermore, RNA sensors may also have functional interactions with the second nucleotide, as single 2'-O methylation of the second transcribed nucleotide partially abolished RIG-I activation.

REGULATION OF CMTR1

Since CMTR1 has important roles in translation and innate immunity, its regulation is of interest. Analysis of mouse tissues revealed differences in the extent of first nucleotide 2'-O methylation suggesting tissue specific regulation of CMTR1 expression or activity. CMTR1 has several domains through which subcellular localisation, activity or expression may be regulated, potentially by protein:protein interactions or by post-translational modifications. The 7'-N cap methyltransferase, RNMT-RAM, has been demonstrated to be regulated at the level of expression, recruitment to chromatin and activity.

CMTR1 expression has been observed to be upregulated during the innate immune response. In four independent studies where an interferon response was elicited, the expression of CMTR1 was upregulated approximately threefold. Despite differences in model cell lines or organisms, upregulation of CMTR1 expression is transient and efficiently cleared after the initial anti-viral response. In human fibroblasts, CMTR1 knockdown was sufficient to elicit an interferon-like innate immune response.

FUTURE PERSPECTIVES: RIBOSE O-2 METHYLATION AND DISEASE

A causative effect for CMTR1 in human diseases has yet to be elucidated. However, significant changes in CMTR1 mRNA levels in patients experiencing asthma exacerbations have been observed, suggesting a role for CMTR1 in the pathogenesis of asthma exacerbations. Highly pathogenic viruses include RNA genome-based viruses which give rise to zoonotic and epidemic diseases. Inactivation of flavivirus, coronavirus and poxvirus families 2'-O MTases increased sensitivity to antiviral actions of type I IFN. Therapeutic targeting of 2'-O MTases may offer a new avenue to treat some of these viral infections.

ACKNOWLEDGMENTS

We thank Rupert Fray for advice. VHC and FIV are funded by an MRC Senior Fellowship MR/K024213/1, Lister Institute Prize Fellowship and a Wellcome Trust Centre award 097945/Z/11/Z.

REFERENCES

1. Furuichi Y. Proc Jpn Acad Ser B Phys Biol Sci 2015, 91:394–409.
2. Shuman S. RNA 2015, 21:735–737.
3. Meyer KD, Jaffrey SR. Annu Rev Cell Dev Biol 2017.
4. Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, Linder B, Pickering BF, Vasseur JJ, Chen Q, et al. Nature 2017, 541:371–375. http://doi.org/10.1146/annurev-cellbio-100616-060758
5. Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, Dai Q, Di Segni A, Salmon-Divon M, Clark WC, et al. Nature 2016, 530:441–446.
6. Jiao X, Doamekpor SK, Bird JG, Nickels BE, Tong L, Hart RP, Kiledjian M. Cell 2017, 168:1015–1027, e10.
7. Yue Z, Maldonado E, Pillutla R, Cho H, Reinberg D, Shatkin AJ. Proc Natl Acad Sci USA 1997, 94:12898–12903.
8. Yamada-Okabe T, Doi R, Shimm O, Arisawa M, Yamada-Okabe H. Nucleic Acids Res 1998, 26:1700–1706.
9. Tsukamoto T, Shibagaki Y, Niikura Y, Mizumoto K. Biochem Biophys Res Commun 1998, 251:27–34.
10. Pillutla RC, Yue Z, Maldonado E, Shatkin AJ. J Biol Chem 1998, 273:21443–21446.
11. Gonatopoulos-Pournatzis T, Dunn S, Bounds R, Cowling VH. Mol Cell 2011, 44:585–596.
12. Belanger F, Stepinski J, Darzynkiewicz E, Pelletier J. J Biol Chem 2010, 285:33037–33044.
13. Werner M, Purta E, Kaminska KH, Cymerman IA, Campbell DA, Mittra B, Zamudio JR, Sturm NR, Jaworski J, Bujnicki JM. *Nucleic Acids Res* 2011, 39:4756–4768.

14. Abdelhamid RF, Plessy C, Yamauchi Y, Taoka M, de Hoon M, Gingeras TR, Isobe T, Carninci P. *PLoS One* 2014, 9:e102895.

15. Kruse S, Zhong S, Bodi Z, Button J, Alcocer MJ, Hayes CJ, Fray R. *Sci Rep* 2011, 1:126.

16. Wetzel C, Limbach PA. *Analyst* 2016, 141:16–23.

17. Ramanathan A, Robb GB, Chan SH. *Nucleic Acids Res* 2016, 44:7511–7526.

18. Gonatopoulos-Pournatzis T, Cowling VH. *Biochem J* 2014, 457:213–224.

19. Topisirovic I, Svitkin YV, Sonenberg N, Shatkin AJ. *WIREs RNA* 2011, 2:277–298.

20. Cowling VH, Cole MD. *Mol Cell Biol* 2007, 27:2059–2073.

21. Cole MD, Cowling VH. *Oncogene* 2009, 28:1169–1175.

22. Aregger M, Kaskar A, Varshney D, Fernandez-Sanchez ME, Inesta-Vaquera FA, Weidlich S, Cowling VH. *Mol Cell* 2016, 61:734–746.

23. Grasso L, Suska O, Davidson L, Gonatopoulos-Pournatzis T, Williamson R, Wasmus L, Weidlich S, Peggie M, Stavridis MP, Cowling VH. *Cell Rep* 2016, 16:1352–1365.

24. Schubert-Wagner C, Ludwig J, Bruder AK, Herzner AM, Zillinger T, Goldeck M, Schmidt T, Schmid-Burgk JL, Kerber R, Wolter S, et al. *Immunity* 2015, 43:41–51.

25. Will CL, Luhrmann R. *Cold Spring Harb Perspect Biol* 2011, 3:a003707.

26. Pan ZQ, Prives C. *Genes Dev* 1989, 3(12A):1887–1898.

27. Segault V, Will CL, Sproat BS, Luhrmann R. *EMBO J* 1995, 14:4010–4021.

28. Yu YT, Shu MD, Steitz JA. *EMBO J* 1998, 17:5783–5795.

29. Domnez G, Hartmuth K, Luhrmann R. *RNA* 2004, 10:1925–1933.

30. Gu J, Patton JR, Shima S, Reddy R. *RNA* 1996, 2:909–918.

31. Muthukrishnan S, Moss B, Cooper JA, Maxwell ES. *J Biol Chem* 1978, 253:1710–1715.

32. Muthukrishnan S, Morgan M, Banerjee AK, Shatkin AJ. *Biochemistry* 1976, 15:5761–5768.

33. Caldwell DC, Emerson CP Jr. *Cell* 1985, 42:691–700.

34. Kuge H, Brownlee GG, Gershon PD, Gershon JD. *Nucleic Acids Res* 1998, 26:3208–3214.

35. Barbosa E, Moss B. *J Biol Chem* 1978, 253:7692–7697.

36. Langberg SR, Moss B. *J Biol Chem* 1981, 256:10034–10060.

37. Smietanski M, Werner M, Purta E, Kaminska KH, Stepinski J, Darzynkiewicz E, Nowotny M, Bujnicki JM. *Nat Commun* 2014, 5:3004.

38. Haline-Vaz T, Silva TC, Zanchin NI. *Biochem Biophys Res Commun* 2008, 372:719–724.

39. Zamudio JR, Mittra B, Campbell DA, Sturm NR. *Mol Microbiol* 2009, 72:1100–1110.

40. Su AI, Pezacki JP, Wodicka L, Brideau AD, Supekova I, Thimme R, Wieland S, Bukh J, Purcell RH, Schultz PG, et al. *Proc Natl Acad Sci USA* 2002, 99:15669–15674.

41. Geiss GK, Carter VS, He Y, Kwieciszewski BK, Holzman T, Korth MJ, Lazaro CA, Fausto N, Bumgarner RE, Katze MG. *J Virol* 2003, 77:6367–6375.

42. Guerra S, Lopez-Fernandez LA, Pascual-Montano A, Munoz M, Harshman K, Esteban M. *J Virol* 2003, 77:6493–6506.

43. Kato A, Homma T, Batchelor J, Hashimoto N, Imai S, Wakiguchi H, Saito H, Matsumoto K. *BMC Immunol* 2003, 4:8.

44. Leung DW, Amarasinghe GK. *Curr Opin Struct Biol* 2016, 36:133–141.

45. Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, Lin TY, Schneller S, Zust R, Dong H, et al. *Nature* 2010, 468:452–456.

46. Devarkar SC, Wang C, Miller MT, Ramanathan A, Jiang F, Khan AG, Patel SS, Maricatrigiano J. *Proc Natl Acad Sci USA* 2016, 113:596–601.

47. Wu B, Peisley A, Richards C, Yao H, Zeng X, Lin C, Chu F, Walz T, Hur S. *Cell* 2013, 152:276–289.

48. Zue W, Cervantes-Barragan L, Habjan M, Maier R, Neuman BW, Ziebuhr J, Szretter KJ, Baker SC, Barchet W, Diamond MS, et al. *Nat Immunol* 2011, 12:137–143.

49. Randall RE, Goodbourn J. *J Gen Virol* 2008, 89(Pt 1):1–47.

50. Kumar P, Sweeney TR, Skabkin MA, Skabkina OV, Hellen CU, Pestova TV. *Nucleic Acids Res* 2014, 42:3228–3245.

51. Young DF, Andrejeva J, Li X, Inesta-Vaquera F, Dong C, Cowling VH, Goodbourn S, Randall RE. *J Virol* 2016, 90:9446–9456. https://doi.org/10.1128/JVI.01056-16

52. Abbas YM, Laudenbach BT, Martinez-Montero S, Cencic R, Habjan M, Pichlmair A, Damha MJ, Pelletier J, Nagar B. *Proc Natl Acad Sci USA* 2017, 114:E2106–E2115.

53. Aregger M, Cowling VH. *Cell Cycle* 2012, 11:2146–2148.

54. Dahlin A, Denny J, Roden DM, Brilliant MH, Ingram C, Kitchner TE, Linneman JG, Shaffer CM, Weeke P, Xu H, et al. *Immun Inflamm Dis* 2015, 3:350–359.

55. Bray M. *Antiviral Res* 2008, 78:1–8.

56. Hyde JL, Diamond MS. *Virology* 2015, 479–480:66–74.