Translational termination–re-initiation in viral systems

Michael L. Powell1, T. David K. Brown and Ian Brierley
Division of Virology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, U.K.

Abstract
Viruses have evolved a number of translational control mechanisms to regulate the levels of expression of viral proteins on polycistronic mRNAs, including programmed ribosomal frameshifting and stop codon readthrough. More recently, another unusual mechanism has been described, that of termination-dependent re-initiation (also known as stop–start). Here, the AUG start codon of a 3′ ORF (open reading frame) is proximal to the termination codon of a uORF (upstream ORF), and expression of the two ORFs is coupled. For example, segment 7 mRNA of influenza B is bicistronic, and the stop codon of the M1 ORF and the start codon of the BM2 ORF overlap in the pentanucleotide UAAUG (stop codon of M1 is shown in boldface and start codon of BM2 is underlined). This short review aims to provide some insights into how this translational coupling process is regulated within different viral systems and to highlight some of the differences in the mechanism of re-initiation on prokaryotic, eukaryotic and viral mRNAs.

Re-initiation of translation
Re-initiation of translation following the termination of a uORF has been shown to occur in prokaryotic cells and is often utilized by bacteriophages. This mechanism is employed to access downstream ORFs with weak SD (Shine–Dalgarno) sequences (ordinarily used to recruit de novo ribosomes to the mRNA), which would not be translated efficiently under normal conditions. In such cases, translation of the uORF circumvents the need to recruit ribosomes de novo by placing the ribosome within close proximity of the SD sequence, allowing an interaction based on weak base-pairing between the ribosome and the mRNA (for a review, see [1], also discussed further below).

Translation re-initiation has also been demonstrated to occur in eukaryotes, although re-initiation will generally occur efficiently only when the uORF is short (<13 codons; [2]). As with most other termination–re-initiation events, most of the ribosomes dissociate from the mRNA following termination at the uORF stop codon. As a result, re-initiation occurs with a relatively low frequency (~5–10%), and so the presence of short uORFs in the 5′-UTR (5′-untranslated region) of mRNAs is typically used as a means of down-regulating expression, for example, of the HER-2 (human epidermal growth factor receptor 2) oncogene [3]. The presence of more than one uORF in a eukaryotic mRNA can also be used as a means of repressing the synthesis of the downstream ORF, except when the availability of the ternary complex [eIF2 (eukaryotic initiation factor 2)-GTP-Met-tRNAi (initiator methionyl-tRNA)] is limiting, for example, under stress conditions. Here, the low availability of ternary complexes allows post-termination 40S subunits to scan through the uORFs, providing the opportunity to reach the downstream ORF start codon. This strategy has been best described in the GCN4 (general control non-derepressible 4) [4] and ATF4 (activating transcription factor 4) [5,6] mRNAs from yeast and higher eukaryotes respectively, which are expressed under circumstances when global translation is repressed. There is little information as to what allows ribosomes to resume scanning after translation of the uORF; in vitro studies suggest an involvement of eIF4F (or minimally the central third of eIF4G), as mRNAs that are translated in a cap-independent manner are unable to support re-initiation [7]. Furthermore, re-initiation does not occur on capped transcripts in eIF4G-depleted lysates. The fact that only short ORF's support re-initiation is thus thought to be due to the ability of the 80S ribosomes to retain eIF4F, and providing that the ribosome can complete translation of the uORF before the interaction is disrupted, scanning will resume post-termination [7].

Termination–re-initiation of viral mRNA translation is distinct from the eukaryotic termination–re-initiation mechanism described above in that the uORF encodes a functional protein and there is no apparent constraint on its size. Termination at the first ORF stop codon is an absolute requirement for re-initiation on the second ORF, distinguishing this process from IRES (internal ribosome entry site)-mediated regulation [8–11]. Typically, the uORFs and downstream ORFs overlap and lie in different reading frames.
The diversity of stop–start signals between and within virus families

(a) Schematic diagrams of the subgenomic RNAs of RHDV and FCV. In each case, VP10 and VP2 are accessed by termination-re-initiation. Primary sequences that are known to be required are shown. Motif 1 and motif 2 form the TURBS (see the text for details), which is likely to be important in tethering of the 40S subunit to the RNA, post-termination. The stop–start overlap is also shown and differs between the two viruses. (b) Diagram of segment 7 RNA of influenza B, which encodes both M1 and BM2. Regulated expression of these proteins is known to occur by stop-start, although little is known about the mechanism. However, there are two motif 1 homologues (as described above) upstream of the UAAUG overlap. As yet, it is not known if either of these plays a role in regulation of stop–start in influenza B. (c) Representation of the bicistronic M2 mRNA of the pneumo- and meta-pneumoviruses, which encodes M2 and M2-2. The putative stop-start overlap regions are highly diverse, and frequently contain more than one in-frame AUG for initiation on the M2-2 ORF (all of which can be used efficiently [8]).
VP2 in FCV [10,12,14] and M2-2 of the paramyxoviruses RSV (respiratory syncytial virus) [8,15,16], APV (avian pneumovirus) and the PVM (pneumovirus of mice) [16] (Figure 1).
has been suggested that eIF3 may bind upstream of the stop–start overlap, and promote dissociation of the 60S ribosomal subunit following termination, speeding the rate of ribosome recycling and allowing re-initiation on the downstream ORF [12]. The bound eIF3 may then play a further role in recruiting initiation factors for re-initiation on the downstream ORF, although this is yet to be demonstrated experimentally. Interestingly, mutation of 3 nt of motif 1 (thought to interact with the 18S rRNA) also reduces binding of the viral RNA to eIF3 [12]. While these data may complicate the interpretation of Luttermann and Meyers [10], namely that motif 1 is important in tethering the 40S subunit to the viral RNA, it is entirely possible that the upstream sequence plays a dual role, both in ribosome dissociation/recycling, and 40S tethering.

Termination–re-initiation in influenza B: parallels with caliciviral mechanisms

Influenza B is a segmented, negative-stranded RNA virus, and the M1 and BM2 ORFs are located on the segment 7 mRNA [9,21,22] (Figure 1b). BM2 is a functional homologue of influenza A M2 and encodes a proton channel important for virus uncoating during entry and also for virus assembly [23,24]. The BM2 ORF lies at the 3′-end of the segment 7 mRNA, and its initiation codon overlaps with the termination codon of M1 in the pentanucleotide UAAUG (stop codon of M1 is shown in boldface and start codon of BM2 is underlined), consistent with expression of BM2 by termination–re-initiation [9] (Figure 1b). However, little has been published about the mechanism of synthesis of BM2, only that there is no IRES element that is able to recruit ribosomes to the BM2 start codon directly. Indeed, termination of the uORF is absolutely required for synthesis of BM2, which does not fit with IRES-mediated mechanisms. Also, as with other termination–re-initiation events, the stop codon of the uORF and the start codon of the downstream ORF must lie within close proximity [9]. Our laboratory is currently investigating more fully the mechanisms involved in BM2 synthesis and we have observed a number of parallels with the calicivirus systems, the first example of such mechanisms operating in negative-stranded RNA viruses (M.L. Powell, I. Brierley and T.D.K. Brown, unpublished work). We are at present looking further into the role of interactions of 18S rRNA with the segment 7 mRNA in the process of influenza B termination–re-initiation.

Termination–re-initiation in the pneumoviruses and meta-pneumoviruses

Termination–re-initiation has also been demonstrated to regulate the synthesis of M2-2 of RSV [8,15], APV and PVM [16] (Figure 1c), although in comparison with the caliciviruses (see below), less is known about the mechanism(s). Similarly to the caliciviruses, it seems that there is a requirement for sequences upstream of the overlap region [15], although the extent of primary sequence required for full stop–start activ-

A common mechanism for termination–re-initiation in caliciviruses, influenza B and bacteriophage

Data from the calicivirus systems and our own work with influenza BM2 suggest a common mechanism for the stop–start process. The downstream ORF is unable to recruit 40S ribosomal subunits directly and, instead, translation and termination on the first ORF place the ribosome in the vicinity of the start codon of the second ORF (Figure 3a). At this point, subunit dissociation takes place and the 40S subunit is recruited to the AUG codon (Figure 3b). Both eIF3 and the 18S rRNA complementary region appear to play a role in this process, but how and when they act is uncertain. For example, we do not know whether eIF3 can bind to the upstream sequence prior to translation through the TURBS, or whether it is recruited later, perhaps while complexed with the terminating ribosome. One might expect the 80S ribosome to ‘knock off’ any prebound eIF3, arguing for the latter mechanism. However, another possibility is that translation through the minimal required sequence can remodel the RNA, inducing the upstream secondary structure to form, which would then allow eIF3 binding and promote ribosome dissociation (Figure 3b). It is thought that the structure could then act to tether the 40S subunit to the RNA by virtue of interactions with 18S rRNA (and possibly eIF3, which also binds the 18S rRNA [25,26]), while initiation factors are recruited allowing initiation on the downstream ORF (Figure 3c). This proposed mechanism has some parallels with stop–start processes in prokaryotes, with motif 1 of the FCV and RHDV TURBS mimicking the role of the vestigial SD sequence (for example that of bacteriophage f1 [27,28]). Importantly, many of the constraints on termination–re-initiation in the caliciviruses and influenza viruses also seem to hold true in bacteriophages. For example, initiation occurs in a window of approx. 40 nt surrounding the stop codon [11–13,29] and requires a sequence that may interact with 18S rRNA (SD or motif 1 of the TURBS) [10,13,29]. Also, and presumably due to the direct placement of the ribosome at the start of the second ORF following termination, there appears to be a relaxed requirement for the use of a canonical AUG start codon,
Figure 3 | A model for termination-re-initiation in the caliciviridae/ influenza B
(a) The 80S ribosome translates the uORF as normal. (b) As the ribosome approaches the overlap region, the stretch of RNA containing motif 1 is translated and may be remodelled. During termination, the secondary structure (indicated with an asterisk) including motif 1 is located in the exit channel of the ribosome. This translational remodelling may promote the binding of eIF3, which speeds dissociation of the ribosome after termination. (c) The secondary structure presents motif 1 of the TURBS to the solvent-accessible helix 26 of the 18S rRNA. This interaction may be stabilized by the presence of eIF3, also known to contact the 18S rRNA. These interactions act to tether the ribosome to the viral RNA, preventing its dissociation, allowing time for the recruitment of initiation factors and subsequent re-initiation on the downstream ORF.

with initiation occurring frequently at nearby GUG or even UUG codons [10,13,29]. Taken together, these parallels may suggest that similar mechanisms of stop–start are utilized in the caliciviruses, influenzaviruses and bacteriophages.

Our work on termination-re-initiation is supported by a BBSRC (Biotechnology and Biological Sciences Research Council) grant to I.B. and T.D.K.B. Many thanks to Sawsan Napthine (Division of Virology, Department of Pathology, University of Cambridge) for providing some of the data describing stop–start in MNV-1.

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Received 25 March 2008
doi:10.1042/BST0360717