The polymerase slips and PIPO exists

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Plant viruses that contain plus-sensed single-stranded RNA genomes are highly abundant in nature. As the equivalents of large mRNAs, these viral genomes utilize a wide variety of gene expression strategies for the production of their encoded proteins. The potyviruses, which are among the most agriculturally important members in this category, contain a single large open reading frame (ORF) coding for a polyprotein that is processed into functional units. For many years, the products derived from the full-length polyprotein were thought to be the only functional viral proteins. However, this notion was dispelled when an additional essential viral ORF, PIPO, was discovered encoded in an alternative reading frame. Since then, the PIPO protein—P3N-PIPO, which mediates virus movement in plants—has been intensively studied, but its mode of expression remained elusive until now. Two articles, one in this issue of *EMBO Reports*, now report that slippage of the viral polymerase during viral genome replication is responsible for shifting PIPO into a translated reading frame, thereby allowing for production of P3N-PIPO [1,2]. This mechanism of gene expression represents a novel strategy for plant viruses.

See also: A Olspert et al (August 2015) and B Rodamilans et al (July 2015)

The potyviruses comprise a large plant virus genus that has been studied extensively [3]. It was long thought that their plus-strand RNA genomes encode only a single large ORF that is translated into a polyprotein and subsequently cleaved into ten mature proteins (Fig 1A and B). It was therefore surprising when an additional functionally relevant, overlapping ORF, PIPO, was discovered through comparative genomic analysis of multiple potyviral sequences, which revealed its conservation and a pattern of non-synonymous and synonymous changes consistent with evolution of a protein-coding sequence (Fig 1A) [4]. The size of the PIPO protein that accumulates in potyviral infections is consistent with it being the C-terminal portion of a fusion with the N-terminal region of P3; consequently, it was termed P3N-PIPO (Fig 1B) [1]. Also, a conserved GAAAAAA (GAA) motif located at the beginning of PIPO was identified and proposed to mediate the frameshift event required for its expression (Fig 1A), either by a −1 ribosomal frameshifting process during translation of the polyprotein ORF or by gaining a single adenylate (+1A) through slippage of the viral RNA-dependent RNA polymerase during viral genome replication [4].

Since its discovery, the activities of P3N-PIPO have been intensely investigated. It is known to colocalize to plasmodesmata, where it acts in conjunction with another potyviral protein, CI, to mediate cell-to-cell spread of the virus [5]. Thus, its major function appears to be facilitating the dispersal of the infection within host plants. This movement role of P3N-PIPO is mediated by an interaction with plasma membrane protein PCaP1, and knockout of this host protein inhibits intercellular viral trafficking [6]. Although the basic role of P3N-PIPO has been elucidated [3], its mode of expression remained unclear.

Two recent studies provide compelling evidence that potyviruses use a viral polymerase slippage mechanism for P3N-PIPO production [1,2]. Both Olspert et al [1] and Rodamilans et al [2] used high-throughput sequencing of viral genomic RNAs isolated from different potyvirus infections to interrogate the GAA motif in progeny genomes for insertions or deletions. In both cases, a +1A insertion within the A-tract was detected in a small fraction of progeny viral genomes (0.8–2.1%), consistent with polymerase slippage shifting PIPO into the translated reading frame (Fig 1C). Olspert et al [1] extended their investigation by constructing and assessing mutant potyviral genomes that were engineered to distinguish between the two candidate expression strategies. Mutant *Turnip mosaic virus* (TuMV) genomes containing modifications in their GAA motifs designed to inhibit ribosome frameshifting were able to express P3N-PIPO, spread from inoculation sites and systemically infect plants [1]. In contrast, a corresponding mutant designed to compromise polymerase slippage exhibited none of the aforementioned phenotypes. Accordingly, these in vivo results supported only the polymerase slippage mechanism for P3N-PIPO expression.

In TuMV-infected cells, P3N-PIPO is produced at an extremely low level, which nonetheless suffices for movement-related functions, and correlates well with the minute levels of polymerase slippage observed [1]. This is not the case for a second candidate potyviral frameshift protein of unknown biological relevance, termed P1N-PIPO, which has so far only been identified in sweet potato potyviruses [7]. Sequence analyses of progeny genomes from *Sweet potato feathery mottle virus* (SPFMV) infections suggested that this protein, a C-terminal fusion with the N-terminal half of the viral P1 protein, is also produced by a +1A polymerase slippage event at a GAA motif (Fig 1A and B) [2]. However, the predicted PISPO slippage frequency is nearly 12%—compared to 1.2% for PIPO in SPFMV [2]—indicating that a tenfold difference in slippage activity can be achieved by the same viral polymerase. Given that PIPO is likely to be functionally relevant, this would be the system of choice for detailed mechanistic studies due to superior sensitivity.

The results above beg the question of what determines slippage efficiency. In SPFMV, the same viral polymerase is responsible for both slippage events; therefore, differences in the RNA template are likely responsible
for the observed disparity. Accordingly, it will be interesting to determine the RNA structural features (proximal and/or distal to the slip sites) that modulate this process. In other instances, regulation could be mediated by the viral polymerase. Although slippage may be a constitutive and unmodulated property of the polymerase, this activity could also be conferred or regulated through covalent modifications or interaction with cofactors. The occurrence of such events at defined times during an infection would also allow for temporal regulation. Indeed, P3N-PIPO in TuMV infections is detectable only at later time points during infections [1], which could potentially be the consequence of time-modulated control of slippage. Thus, the contribution of the viral polymerase and possible cofactors to the timing and amplitude of this process also warrants investigation.

Finally, it is worth considering the origin and classification of the viral RNAs that are produced by the slippage event. Slippage sequences presumably arise randomly by polymerase error or recombination, which then allow for sampling of products from alternative reading frames and, if beneficial, retention. Whether the retained slippage events occur during synthesis of the minus-strand RNA replicative intermediate or the plus-strand progeny genome remains unclear. These two steps in viral genome replication are mechanistically distinct; therefore, one may be more amenable to slippage for translation of a complete polyprotein from which P3N-PIPO-encoding mRNAs are not thought to be amplified [1], due to the requirement for translation of a complete polyprotein from a viral genome to confer replication competency [9]. This property, along with being synthesized by polymerase transcription during infections and mediating the expression of a specific viral protein, is consistent with classifying P3N-PIPO-encoding mRNAs as a novel type of sg mRNA that, unlike typical sg mRNAs, is larger than the parental genome (albeit by a single nucleotide). If this concept is deemed to be valid, then polymerase slippage should be considered a new mechanism for production of sg mRNAs in plus-strand RNA viruses.

Although polymerase slippage has been reported previously for hepatitis C virus [10], its biological relevance in infections remains to be established. Therefore, the work by Olspert et al [1] and Rodamilans et al [2] represents the first compelling evidence for this activity being functionally significant in plus-strand RNA virus infections and lays the foundation for investigating both mechanistic details of the process and its prevalence in other viral systems. Plus-strand RNA virologists can now proclaim with confidence that this elusive polymerase-based expression strategy is no longer giving us the slip!

References

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