Review article

Ribosomal frameshifting on viral RNAs

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Introduction

Accurate maintenance of the translational reading frame by ribosomes is essential for the production of functional proteins and unsurprisingly, errors in frame maintenance have been estimated to occur at rates probably lower than $5 \times 10^{-5}$ per codon (Kurland, 1992). However, an increasing number of examples have been documented where a purposeful shift in reading frame is programmed into the mRNA and serves an essential function (Gesteland et al., 1992; Atkins & Gesteland, 1995). Highly efficient ribosomal frameshifting is an example of such a programme frameshift site. In response to certain signals in the mRNA, ribosomes are induced to move into the $-1$ reading frame (in a 5' direction) at a specific point and continue translation in the new reading frame. The phenomenon was first described in 1985 as the way in which the Gag-Pol polyprotein of the retrovirus Rous sarcoma virus (RSV) is expressed from the overlapping gag and pol open reading frames (ORFs) (Jacks & Varmus, 1985) and to date, most examples of frameshifting come from virus systems. In this article, I will describe the nature and role of frameshift signals in viruses, review our current understanding of the mechanism of the frameshift process and discuss the prospects for using frameshift sites as targets for antiviral intervention.

Occurrence and nature of $-1$ ribosomal frameshift signals

Since the inaugural RSV report, related frameshift signals have been characterized in several other retroviruses, a number of eukaryotic positive-strand RNA viruses, dsRNA viruses of yeast, some plant RNA viruses and certain bacteriophage (see Table 1). The phenomenon however is not restricted to viruses; frameshift signals of the 'retrovirus-type' occur in a number of Escherichia coli insertion elements (Chandler & Fayet, 1993) and in a conventional cellular gene, the dnaX gene of E. coli (see Tsuchihashi & Brown, 1992). In most of the systems studied to date, frameshifting is involved in the expression of replicases. In retroviruses, it allows the synthesis of the Gag-Pol and Gag-Pro-Pol polyproteins from which reverse transcriptase is derived, and for most other viruses, frameshifting is required for expression of RNA-dependent RNA polymerases. Correspondingly, frameshifting in the E. coli insertion elements regulates the expression of transposases and in the dnaX gene, production of the $\gamma$ subunit of DNA polymerase III. However, in the bacteriophage examples studied, frameshifting is involved in the production of virion structural components. In certain yeast retrotransposons (see Pande et al., 1995 and references therein) and in a recently identified acyclovir-resistant isolate of herpes simplex virus type 1 (Hwang et al., 1994), $+1$ ribosomal frameshifts have been documented. The available evidence suggests that $+1$ frameshift sites differ both structurally and mechanistically from $-1$ frameshift sites and will not be discussed further in this review.

Much of our understanding of the nature of $-1$ frameshift signals comes from an in vitro translation analysis of the RSV gag-pol frameshift signal carried out by Jacks et al. (1988a). The coding sequence of pol lies downstream of and overlaps gag and is in the $-1$ reading frame with respect to gag. Synthetic mRNAs containing the RSV frameshift signal, when translated in the rabbit reticulocyte lysate system (RRL), produced a translation product terminated at the gag stop codon and an additional Gag-Pol fusion protein at about 5% of the level of Gag alone. This was shown to result from ribosomes frameshifting prior to encountering the gag stop codon and continuing to translate pol. Further analysis using specific point mutations and deletions identified two essential components of the RSV frameshift signal; a homopolymeric 'slippery' sequence of nucleotides (AAAAUUA) and a region of RNA secondary structure located a few nucleotides downstream. Nucleotide sequence comparisons of several other retro-
viruses revealed that in almost all cases, a hepta-nucleotide stretch containing two homopolymeric triplets (XXXXYYYZ) is present at the gag-pro, gag-pol or pro-pol overlap regions. These observations led to the proposal of a simultaneous slippage model of frameshifting in which two adjacent ribosome-bound tRNAs in the zero reading frame (X-XX-YYN) slip back simultaneously by one nucleotide during the frameshift such that both tRNAs are in the -1 phase (XXX-YYY) and are base-paired to the mRNA in at least two out of three anticodon positions (Jacks et al., 1988a). Following transfer of peptidyl-tRNA to the ribosomal P site, the next aminoacyl-tRNA decodes the -1 frame and translation continues as normal to generate the fusion protein. In those systems in which the amino acid sequence of the transframe protein has been determined, it has been shown that the frameshift occurs within the slippery sequence at the second codon of the tandem slippery pair (X-XXX-YYZ); i.e. at that codon decoded in the ribosomal A site (Hizi et al., 1987; Jacks et al., 1988a, b; Nam et al., 1993).

The slippery sequence alone is insufficient for efficient frameshifting and a stimulator is required in the form of a downstream RNA structure (Jacks et al., 1987, 1988a; Brierley et al., 1989). In some cases a stem–loop structure is present, but more often the downstream stimulator is an RNA pseudoknot, a structure formed when nucleotides in the loop region of a hairpin–loop base-pair with a region elsewhere in the mRNA to create a quasi-continuous double helix joined by single-stranded connecting loops (see ten Dam et al., 1992 for a review; Fig. 1). The involvement of RNA pseudoknots in the frameshift process was first noted for the signal at the 1a–1b overlap of the avian coronavirus IBV (Brierley et al., 1989) and such structures have been predicted to occur at many other frameshift sites (Brierley et al., 1989; ten Dam et al., 1990). Details of the established -1 ribosomal frameshift signals are provided in Table 2 and a subset of the sites is shown in Fig. 1. A variety of slippery sequences are employed by viruses, but G- or C-rich codons are avoided in the portion of the slip site that is decoded in the ribosomal A site. It has been speculated that such codons would form mRNA–tRNA contacts which are too stable to break during tRNA slippage and would thus prevent frameshifting (Jacks et al., 1988a). Indeed, several authors have reported the non-functionality of such slippery sequences.

With respect to the downstream RNA structures at frameshift sites, a number of features emerge. With the exception of the T7 10A–10B (Condron et al., 1991a) and TGEV orf1a-orf1b (Eleouet et al., 1995) frameshift sites, the distance between the slippery sequence and the downstream stimulator appears to be constrained to between 5 and 8 nucleotides. That this precise spacing must be maintained for efficient frameshifting to occur has been demonstrated both in RRL (Brierley et al.,

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Table 1. Occurrence of established -1 ribosomal frameshift signals in viral RNAs*

<table>
<thead>
<tr>
<th>Family/Group</th>
<th>Genus</th>
<th>Virus</th>
<th>Gene overlap</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Retroviridae</em></td>
<td>Lentivirus</td>
<td>Human immunodeficiency virus type 1 (HIV-1)</td>
<td>gag-pol</td>
<td>Jacks et al. (1988a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feline immunodeficiency virus (FIV)</td>
<td>gag-pol</td>
<td>Morikawa &amp; Bishop (1992)</td>
</tr>
<tr>
<td></td>
<td>ALSV</td>
<td>Rouss sarcoma virus (RSV)</td>
<td>gag-pol</td>
<td>Jacks &amp; Varms (1985)</td>
</tr>
<tr>
<td></td>
<td>B-type</td>
<td>Mouse mammary tumour virus (MMTV)</td>
<td>gag-pro</td>
<td>Moore et al. (1987); Jacks et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>D-type Simian retrovirus type 1 (SRV-1)</td>
<td>gag-pro</td>
<td>ten Dam et al. (1994)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HTLV-1</td>
<td>Human T cell leukaemia virus type I (HTLV-I)</td>
<td>gag-pro</td>
<td>Nam et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>HTLV-II</td>
<td></td>
<td>pro-pol</td>
<td>Nam et al. (1993)</td>
</tr>
<tr>
<td><em>Coronaviridae</em></td>
<td>Coronavirus</td>
<td>Infectious bronchitis virus (IBV)</td>
<td>orf1a-orf1b</td>
<td>Brierley et al. (1987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse hepatitis virus (MHV)</td>
<td>orf1a-orf1b</td>
<td>Bredenbeek et al. (1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human coronavirus (HCV)</td>
<td>orf1a-orf1b</td>
<td>Herold et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transmissible gastroenteritis virus (TGEV)</td>
<td>orf1a-orf1b</td>
<td>Eleouet et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Totiviridae</td>
<td>Berne virus (BEV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arterivirus†</td>
<td>Equine arteritis virus (EAV)</td>
<td>orf1a-orf1b</td>
<td>den Boon et al. (1991)</td>
</tr>
<tr>
<td><em>Astroviridae</em></td>
<td></td>
<td>Human astrovirus serotype-1 (HAst-1)</td>
<td>orf1a-orf1b</td>
<td>Marczinke et al. (1994)</td>
</tr>
<tr>
<td><em>Totiviridae</em></td>
<td>Totivirus</td>
<td>Giardia lambia virus (GLV)</td>
<td>orf1-orf2</td>
<td>Wang et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saccharomyces cerevisiae dsRNA virus L-A (ScV/L-A)</td>
<td>cap-pol</td>
<td>Diamond et al. (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. cerevisiae dsRNA virus L1 (ScV/L1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Podoviridae</em></td>
<td>T7 phage</td>
<td>Bacteriophage T7</td>
<td>10A–10B</td>
<td>Condron et al. (1991a)</td>
</tr>
<tr>
<td><em>Siphoviridae</em></td>
<td>λ phage group</td>
<td>Bacteriophage λ</td>
<td>gp6-T</td>
<td>Levin et al. (1993)</td>
</tr>
<tr>
<td><em>Luteoviridae</em></td>
<td></td>
<td>Barley yellow dwarf virus (BYDV)</td>
<td>39K–60K</td>
<td>Brault &amp; Miller (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beet western yellow mosaic virus (BWYM)</td>
<td>orf2–orf3</td>
<td>Garcia et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potato leaf roll virus (PLRV)</td>
<td>orf2–orf2b</td>
<td>Prüfer et al. (1992)</td>
</tr>
<tr>
<td><em>Dianthoviridae</em></td>
<td>Dianthovirus</td>
<td>Red clover necrotic mosaic virus (RCNMV)</td>
<td>p27–p27</td>
<td>Xiong et al. (1993)</td>
</tr>
</tbody>
</table>

* A number of other viruses are suspected to use frameshifting but this remains unproven. Most of these are listed in ten Dam et al. (1990).
† The currently unclassified arteriviruses are grouped with the *Coronaviridae* on the basis of the similarities of their frameshift signals (see Table 2).
1992) and in vivo (Kollmus et al., 1994) and probably directly affects the mechanism of the frameshift process (see below). Secondly, almost all examples of efficient frameshifting come from animal viruses; the plant virus frameshift sites are generally of low efficiency (see Rohde et al., 1994 for a review on plant virus frameshifting). Finally, it is clear that the majority of frameshift sites contain an RNA pseudoknot structure rather than a hairpin-loop and this seems to be reflected in an increased efficiency of the signal, at least in viruses of higher eukaryotes. Although it is possible to categorize sites in terms of the presence of a stem–loop or pseudoknot, only limited information exists on the precise folding of the downstream stimulators and even within categories there is considerable diversity, as is illustrated in Fig. 1.

At those sites with a hairpin–loop stimulator, the stems can be relatively simple, as in HAst-1 la–1b overlap (Marczinke et al., 1994), or potentially complex as in RSV gag–pol (Jacks et al., 1988a) and RCNMV p27–p57 (Kim & Lommel, 1994). The RSV and RCNMV structures are computer predictions. The boxed nucleotides in the RSV structure indicate nucleotide stretches which may base-pair to form an RNA pseudoknot (see text).
is possible to identify at least three types of stimulator. The first is the small pseudoknot, epitomized by BWYV orf2–orf3, which stimulates only low-level frameshifting (Garcia et al., 1993). The second class is the large pseudoknot found in the Coronaviridae. These are typified by the possession of a long (10–14 bp) stem 1 and have the ability to stimulate frameshifting at a variety of slippery sequences (Brierley et al., 1992), although in nature they are usually found associated with UUUAAC. In two members of this group, HCV 229E (Herold & Siddell, 1993) and TGEV (Eleouet et al., 1995), an additional stem is present, which may stack onto stem 2 of the pseudoknot to generate theoretically a quasi-continuous double-stranded helix of some 24–25 bp. The extra stem is essential for efficient frameshifting in HCV, but apparently is not present merely to compensate for the very long loop 2 in this virus (Herold & Siddell, 1993). The final class has become distinctive following a detailed study of the MMTV gag–pro signal (Chen et al., 1995). RNA structure mapping and site-directed mutagenesis experiments have shown that the presence of a bulged A nucleotide between the pseudoknot stems is essential for efficient frameshifting. The bulge may result in the pseudoknot adopting a conformation which allows this shorter pseudoknot to promote frameshifting at levels approaching those seen with the larger coronavirus pseudoknots. It seems likely that a bulged nucleotide will also prove to be important in FIV (Morikawa & Bishop, 1992) and in SRV-1 (ten Dam et al., 1994, 1995). Like MMTV gag–pro, frameshifting at these sites is highly efficient (20–30%) and the predicted pseudoknot structures are very similar (Chen et al., 1995).

### The mechanism of the frameshift process

Although the cis-acting mRNA signals which specify frameshifting are reasonably well characterized, the precise mechanism of the process remains unknown. Any model for the frameshift mechanism must take into account the wide variation in nature and predicted stability of the RNA structures present at frameshift sites, the relative spacing distance between the stimulators and the slippery sequence and the magnitude of the frameshift induced at each site. Additionally, it must be remembered that hairpin–loops do not appear to be associated with efficient frameshifting; the induction of high levels of frameshifting requires an RNA pseudo-

### Table 2. Details of established –1 ribosomal frameshift signals in viral RNAs

<table>
<thead>
<tr>
<th>Virus</th>
<th>Overlap</th>
<th>Slip site</th>
<th>DS</th>
<th>SP</th>
<th>S1</th>
<th>HL</th>
<th>S2</th>
<th>L1</th>
<th>L2</th>
<th>RRL</th>
<th>Intact cells</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>gag-pol</td>
<td>UUUUAAC</td>
<td>SL</td>
<td>8</td>
<td>11</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>36%</td>
<td>0.63% (COS-7)</td>
<td>Parkin et al. (1992)</td>
</tr>
<tr>
<td>FIV</td>
<td>gag-pol</td>
<td>GGGGAAAC</td>
<td>PK</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>12%</td>
<td>30% (Insect cells)</td>
<td>Morikawa &amp; Bishop (1992)</td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td>gag-pol</td>
<td>AAUUAAC</td>
<td>PK?</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>17</td>
<td>5%</td>
<td>ND</td>
<td>Jacks et al. (1988a)</td>
<td></td>
</tr>
<tr>
<td>MMTV</td>
<td>gag-pro</td>
<td>AAAAAA</td>
<td>PK</td>
<td>7</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>20%</td>
<td>ND</td>
<td>Chamarro et al. (1992)</td>
</tr>
<tr>
<td>SRV-1</td>
<td>gag-pro</td>
<td>GGGGAAAC</td>
<td>PK</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>23%</td>
<td>ND</td>
<td>ten Dam et al. (1994)</td>
<td></td>
</tr>
<tr>
<td>HTLV-II</td>
<td>gag-pro</td>
<td>AAAAAAAC</td>
<td>SL</td>
<td>7</td>
<td>10</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5%</td>
<td>4.5% (RHK-21)‡</td>
<td>Falk et al. (1993)</td>
</tr>
<tr>
<td>IVB</td>
<td>orf1a-1b</td>
<td>UUUUAAC</td>
<td>PK</td>
<td>6</td>
<td>11</td>
<td>8</td>
<td>6</td>
<td>32</td>
<td>30%</td>
<td>30% (XO)</td>
<td>Brierley et al. (1989, 1991)</td>
<td></td>
</tr>
<tr>
<td>MHV</td>
<td>orf1a-1b</td>
<td>UUUUAAC</td>
<td>PK</td>
<td>5</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>2</td>
<td>30%</td>
<td>40% (HeLa)</td>
<td>Bredenbeek et al. (1990)</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>orf1a-1b</td>
<td>UUUUAAC</td>
<td>PK</td>
<td>5</td>
<td>12</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>16%</td>
<td>25% ND</td>
<td>Herold &amp; Siddell (1993)</td>
<td></td>
</tr>
<tr>
<td>TGEV</td>
<td>orf1a-1b</td>
<td>UUUUAAC</td>
<td>PK</td>
<td>3</td>
<td>14</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>16%</td>
<td>20% ND</td>
<td>Eleouet et al. (1995)</td>
<td></td>
</tr>
<tr>
<td>BEV</td>
<td>orf1a-1b</td>
<td>UUUUAAC</td>
<td>PK</td>
<td>5</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>69%</td>
<td>25% 25% (HeLa)</td>
<td>Snijder et al. (1990)</td>
<td></td>
</tr>
<tr>
<td>EAV</td>
<td>orf1a-1b</td>
<td>GUUAAC</td>
<td>PK</td>
<td>6</td>
<td>11</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>69%</td>
<td>20% (HeLa)</td>
<td>den Boon et al. (1991)</td>
<td></td>
</tr>
<tr>
<td>HAST-1</td>
<td>orf1a-1b</td>
<td>AAAAAA</td>
<td>SL</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5%</td>
<td>ND</td>
<td>Marczinke et al. (1994)</td>
</tr>
<tr>
<td>GLV</td>
<td>orf1-orf2</td>
<td>CCUUUA</td>
<td>PK?</td>
<td>6</td>
<td>8</td>
<td>11</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>ND</td>
<td>2% (Giardia cells)</td>
<td>Wang et al. (1993)</td>
</tr>
<tr>
<td>ScV/L-A</td>
<td>gag-pol</td>
<td>GGGUUA</td>
<td>PK</td>
<td>5</td>
<td>13</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>ND</td>
<td>14% (Yeast cells)</td>
<td>Dimm &amp; Wickner (1992)</td>
</tr>
<tr>
<td>ScV/L1</td>
<td>cap-pol</td>
<td>GGGUUA</td>
<td>PK</td>
<td>5</td>
<td>13</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>2.5%¶</td>
<td>ND</td>
<td>Tzeng et al. (1992)</td>
</tr>
<tr>
<td>T7</td>
<td>I0A-10B</td>
<td>GGGGAAAG</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>Condron et al. (1991b)</td>
</tr>
<tr>
<td>λ</td>
<td>gp7-7</td>
<td>GGGGAAAG</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>Levin et al. (1993)</td>
</tr>
<tr>
<td>BYDV</td>
<td>39K-60K</td>
<td>GGGGUA</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>Braut &amp; Miller (1992)</td>
</tr>
<tr>
<td>BWYV orf2-orf3</td>
<td>gag-gag</td>
<td>GGGGAAAC</td>
<td>PK</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>14%</td>
<td>0.8% (E.coli)</td>
<td>Garcia et al. (1993)</td>
</tr>
<tr>
<td>PLRV-P orf2a-orf2b</td>
<td>UUUAUAU</td>
<td>PK 6</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1%</td>
<td>ND</td>
<td>Kajiwara et al. (1993)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLRV-G orf2a-orf2b</td>
<td>UUUAUAU</td>
<td>SL 5</td>
<td>13</td>
<td>9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1%</td>
<td>1% (Protoplats)</td>
<td>Prüfer et al. (1992)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCNMV p27-p57</td>
<td>GGGGAA</td>
<td>SL 5</td>
<td>25</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Low ND</td>
<td>Kim &amp; Lommel (1994)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: DS, downstream stimulator; SP, spacer distance (nt); S1, stem 1 length (bp); HL, hairpin-loop length (nt); S2, stem 2 length (bp); L1, loop 1 length (nt); L2, loop 2 length (nt); RRL, rabbit reticulocyte lysate; PK, pseudoknot; PK?, potential pseudoknot; SL, stem-loop structure; ?, downstream stimulator not established; ND, not determined; XO, Xenopus oocytes.

† Datum from Chen et al. (1995).
‡ Datum from Kollmus et al. (1994).
§ These pseudoknots form an additional stem (stem 3, see citations for details).
¶ The BEV pseudoknot can form an alternative structure; the most likely form is shown.
pseudoknot cannot be replaced functionally by other
knot. If the IBV pseudoknot is replaced by a simple
stem-loop containing a base-paired stem of the same
length and base-pair composition as the stacked stems of
the pseudoknot, frameshifting is reduced by 30-fold
(Brierley et al., 1991). Similarly, the MMTV gag-pro
t pseudoknot cannot be replaced functionally by other
stable RNA structures (Chen et al., 1995).
Currently, the most appealing model for frameshifting
is the pausing model (Jacks et al., 1988a). In this model,
the RNA structure downstream of the slippery sequence
acts as a barrier to translation, pausing ribosomes over
the slippery sequence. The pausing increases the like-
lihood that the ribosome-bound tRNAs can realign on
the slippery sequence in the –1 phase (Jacks et al.,
1988a). Experimental evidence for pausing of ribosomes
at RNA pseudoknots has been obtained in the case of the
frameshift signals of ScV/L1 cap-pol (Tu et al., 1992)
and IBV 1a–1b (Somogyi et al., 1993). The mechanism of
ribosomal pausing is uncertain, but may involve a direct
interaction of a (ribosomal) protein with the pseudoknot.
Alternatively, pausing may reflect the inability of an 80S
ribosome-associated RNA helicase to unwind an RNA
pseudoknot as effectively as a standard hairpin. So far,
no cellular factors have been identified which may act at
RNA pseudoknots to bring about frameshifting. Indeed,
frameshifting at the SRV-1 gag-pro junction in RRL is
uninfluenced by the presence of a significant molar excess
of short RNAs containing the SRV-1 pseudoknot (ten
Dam et al., 1994). This observation suggests that if such
a factor(s) exists, it may well be associated with the
elongating ribosome and not easily titrated. Recently
however, a number of mutants of yeast cellular genes
have been isolated which alter the level of frameshifting
at the ScV/L-A site (Dinman & Wickner, 1994). Their
characterization may well provide important mechanistic
insights.

Role of frameshifting in virus systems

Why some viruses use ribosomal frameshifting to
express their replicases is not fully understood, but there
are a number of likely possibilities. In retroviruses,
frameshifting generates the Gag–Pro, Gag–Pol or Gag–
Pro–Pol polyproteins from which essential replication
enzymes are derived. By producing Pol as a fusion with
Gag, these enzymes can be incorporated directly into
virus particles during assembly. Frameshifting may also
serve to ensure that the correct ratio of structural (Gag)
to non-structural (Pol) proteins is maintained in the
cytoplasm. There is evidence to support the idea that this
ratio is crucial; disruption of the stoichiometry of the
Gag:Gag–Pol ratio has been shown to prevent virion
production in Moloney murine leukaemia virus
(Felsenstein & Goff, 1988) and in HIV-1 (Park &
Morrow, 1991). The clearest demonstration that modu-
lation of frameshift efficiency can dramatically influence
virus viability comes from studies with the yeast dsRNA
virus ScV/L-A (Dinman & Wickner, 1992). These
experiments exploited a satellite virus of ScV/L-A, M1,
whose replication and propagation in yeast cells can be
supported by the provision of ScV/L-A proteins
expressed from a full-length ScV/L-A cDNA clone.
When M1 replication was measured in cells containing
ScV/L-A cDNA variants with altered frameshift
efficiencies (from 0.3 to 12%; wild-type 19%), it was
found that an increase or decrease in the frameshift
efficiency of more than twofold disrupted M1 propa-
gation. The explanation for this observation concerns
the pathway of M1 genome packaging and particle
formation. When Gag is in excess, many particles may
assemble that do not contain the M1 genome, since
genome packaging in ScV/L-A requires the ssRNA-
binding activity of Pol (Fujimura et al., 1992). When the
level of Gag–Pol is artificially high, many incomplete
capsids may be formed, since the Gag–Pol polyprotein is
thought to prime capsid polymerization (Fujimura &
Wickner, 1988).
Frameshifting may also be a strategy to avoid the
packaging of defective RNAs. In retroviruses, the
packaging signal is located 3' to the splice donor such
that only unspliced RNAs are packaged. If synthesis of
the Gag–Pol polyprotein occurred from an RNA in
which gag and pol were fused into the same reading
frame by a splicing event, this RNA could be packaged
into virions and be non-viable (since it would not be able
to produce the Gag and Gag–Pol products in the correct
stoichiometric amounts) unless an essential component
of the packaging signal was also spliced out. Why
coronaviruses employ a frameshift strategy remains to be
determined, since the protein products of the 1a and 1b
ORFs are only poorly characterized, but there are a
number of possibilities. The most obvious is the likely
requirement for production of a defined ratio of 1a:
1a–1b products; the putative RNA-dependent RNA
polymerase is located in 1b and may be required in a
lower relative amount. All coronavirus proteins, with the
exception of those encoded by 1a and 1b, are expressed
from subgenomic mRNAs; the discovery that the MHV
1b ORF contains at least a component of the genomic
packaging signal (van der Most et al., 1991) provides a
possible explanation for the absence of a subgenomic
mRNA for 1b. Such an mRNA might compete for
packaging with the full-length virus genome and result in
defective virions. Coronaviruses may produce 1b by
frameshifting in order to avoid this predicament.

Whether viral or cellular factors can influence the level
of frameshifting during the course of a virus infection is
not known. Certainly, no such factors have been
described to date. However, the possibility has been raised that certain retroviruses may be able to influence frameshifting by altering the level of anticodon nucleoside modification in infected cells (Hatfield et al., 1989, 1992). Of the codons which occur in the portion of the slippery sequence decoded in the ribosomal aminoacyl (A) site prior to tRNA slippage (XXXYYYN), only seven are known to function with substantial efficiency, namely AAA, AAC, AAU, UUA UUC and UUU in eukaryotes and AAG in prokaryotes (see Brierley et al., 1992 and references therein). Six of these contain a highly modified base in the anticodon loop. In tRNA\textsuperscript{Ace} (AAA, AAG), the wobble base is 5-methoxycarbonylmethyl-2-thiouridine (eukaryotes) or 5-methylaminomethyl-2-thiouridine (prokaryotes); in tRNA\textsuperscript{Ann} (AAC, AAU), the wobble base is queuosine (Q) and in tRNA\textsuperscript{Anhe} (UUC, UUU), wybutoxine (Y) is present just 3' of the anticodon. A highly modified anticodon base is lacking in tRNA\textsuperscript{An} (UUA). Hatfield et al. (1992) have suggested that 'hypomodified' variants of these tRNAs (and the already hypomodified tRNA\textsuperscript{Anhe} (UUC, UUU)) are more 'shifty' tRNAs, since such variants will have a considerably less bulky anticodon and be more free to move around at the decoding site.

Support for this theory comes from an examination of the modification status of the anticodons of those aminoacyl-tRNAs which are required for translation at and around the frameshift sites of HIV-1, HTLV-I and bovine leukaemia virus (BLV; Hatfield et al., 1989). It was found that in HIV-1-infected cells, most of the tRNA\textsuperscript{Anhe} lacks Y base and in HTLV-I and BLV-infected cells, most of the tRNA\textsuperscript{An} lacks Q base. At present, the hypothesis that virus-induced hypomodification of tRNA influences frameshifting is not supported experimentally. Recent studies have indicated that in T lymphoid cells (Cassan et al., 1994) or CD4-expressing human 293 cells (Reil et al., 1994), the level of frameshifting at the HIV-1 slip site is not altered by HIV-1 infection, although the modification status of the infected cell tRNAs was not monitored in these studies.

Prospects for using frameshift sites as targets for antiviral intervention

Frameshifting appears to be an essential component of the virus life-cycle and as such is a candidate target for antiviral intervention. The design of rational antiframeshift strategies is limited by our inadequate understanding of the mechanism of frameshifting, but it is highly likely that treatments which significantly perturb the efficiency of the process will have a detrimental effect on virus replication. It may be possible to target oligonucleotides to frameshift sites in such a way as to reduce or enhance the effectiveness of the downstream stimulator. It has been reported that 2' -O-methyl oligonucleotides which bind specifically to sequences immediately downstream of the HIV-1 stem–loop can enhance frameshifting up to sixfold in RRL, although no effect was seen \textit{in vivo} (Vickers & Ecker, 1992).

A second possibility is to identify peptides which recognize specifically stem–loops or pseudoknots and to test their ability to modulate frameshifting. This approach would not be without precedent. Kollmus and colleagues (cited in Farabaugh, 1993) have replaced the HIV-1 stem–loop with the iron-responsive element (IRE) from the human ferritin H chain (Klausner et al., 1993) and measured frameshifting in tissue culture cells under conditions where the cellular IRE-binding protein (IRE-BP) binds to the IRE and stabilizes the stem–loop structure. It was found that binding of the IRE-BP stimulated frameshifting.

Past optimism regarding the use of -1 frameshift sites as an antiviral target was based on the absence of any cellular examples of this class of frameshift in higher eukaryotes. Whilst this is still the case, the recent discovery and characterization of a pseudoknot-dependent +1 frameshift signal in the rat ornithine decarboxylase antisense gene (Gesteland et al., 1992; Rom & Kahana, 1994; Matsufuji et al., 1995) raises the distinct possibility that examples of -1 frameshift sites in mammalian systems will emerge.

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References


