Intricacies of ribosomal frameshifting

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Folded structures in mRNAs can stimulate reprogramming of ribosomes to make one protein from two different reading frames. The first crystal structure of a frameshift stimulatory RNA pseudoknot reveals remarkable features.

Textbooks typically depict mRNA as a straight line coming out of the nucleus and running through the hazy translation apparatus. Messenger RNA, however, is not just a linear structure to be simply read as a tape. Folded structures within the coding regions of mRNA play crucial but widely unappreciated roles in directing the traffic of translating ribosomes. In particular, pseudoknots, which are stem-loops with downstream sequences paired back to the loop, are found at key locations in mRNAs, where they stimulate specific redirection of ribosome reading frame or alter the meaning of stop codons. Since the sequences forming these pseudoknot structures often encode amino acids, they must ultimately be unfolded and read out by the ribosome. While the mechanisms by which pseudoknots alter ribosome behavior are still unclear, dissection of the structures themselves proves to be fascinating. The 1.6 Å resolution X-ray crystal structure of the pseudoknot in beet western yellows virus (BWYV) reported in this issue[1] reveals intriguing features of one such pseudoknot.

This pseudoknot is just downstream of a specific 'shifty' sequence in the BWYV viral mRNA at which ribosomes are prone to perform a doublet instead of a triplet translocation, causing a shift to the -1 frame and allowing synthesis of the viral polymerase. The combination of innate slipperiness of the shift site and stimulation by the pseudoknot results in ~1% of the ribosomes shifting frame[2], considerably above the very low level of error frameshifting that is on the order of 1 in 10^4.

A pseudoknot was first shown to be important for programmed frameshifting by Brierley and colleagues in their studies on the coronavirus known as infectious bronchitis virus (IBV)[3,4]. In addition, pseudoknots found near the 3' end of retroviral *gag* genes, which encode the major core proteins of the virus, were predicted to be important for many cases of frameshifting[5]. Later studies on mouse mammary tumor virus (MMTV)[6], simian retrovirus (SRV)[7,8] and several others confirmed this prediction for retroviruses. Nevertheless, while pseudoknots are common 3' stimulators of programmed -1 frameshifting, other structures such as simple stem loops[9,10], three way junctions[11] and even combinations of distant as well as nearby sequences[12,13] are also utilized in certain naturally occurring cases.

The use of pseudoknots for stimulating -1 frameshifting is not confined to animal and plant viruses. Extensive mutational analyses have confirmed the importance of a pseudoknot that stimulates -1 frameshifting in the decoding of L-A, a double-stranded RNA virus of the yeast *Saccharomyces cerevisiae*[14,15]. A pseudoknot has also been implicated in the bacterial insertion sequence IS 3 -1 frameshifting[16] and antizyme +1 frameshifting[17].

How pseudoknots stimulate frameshifting is not clear. The BWYV pseudoknot, whose X-ray crystallographic structure is reported in this issue[1], stimulates frameshifting but at a level about sixfold less than its MMTV *gag*, or IBV counterparts[3]. At least when tested in a heterologous system (rabbit reticulocyte lysate), the BWYV psuedoknot is considerably less stimulatory for framshifting than the IBV psuedoknot. The crucial structural features that modulate the efficiency are not understood. Additional structural studies, coupled with functional measurements of mutant pseudoknots, are necessary to understand their function.

Pseudoknot structure

Within the past four years, NMR experiments have led to proposed structures for frameshift stimulatory pseudoknots from MMTV[18,19], SRV[20] and human endogenous retrovirus-K10 (HERV; Wang, Y., Wills, N.M., Du, Z., R.F.G. & Hoffman, D.W., submitted). There are marked structural differences among the frameshift stimulatory pseudoknots, but it remains to be determined what distinguishes frameshifting pseudoknots from pseudoknots that do not act on the ribosome.

An expectation from structures of simple pseudoknots is that extra stability is obtained by coaxial stacking of the two stems. However, NMR analysis of a functional derivative of the MMTV *gag* pseudoknot revealed that the two stems are at an angle of ~60°. The bend is a consequence of an unpaired nucleotide between the two stems and constraints imposed by the number and stacking of nucleotides in loop 2, which connects the bottom of stem 1 and the bottom of stem 2[18]. An extrapolation of these findings is that a bent conformation may be a distinguishing feature of frameshift stimulatory pseudoknots. This was challenged by the report that stacking interactions were found between the stems of the SRV pseudoknot[20], but whether the stems are actually coaxial is unclear. In any case these experiments show that a pronounced bend of the order of ~60° is not a requisite feature for function as a frameshift stimulator. Nor does a bend of ~60° seem to be a requirement for high level frameshift stimulation, as the efficiencies of SRV and MMTV frameshifting are similar (~23%).

The new crystal structure of BWYV[1] gives us a very detailed view of another functional pseudoknot that has novel and intriguing features. The orientation of the stems is very clear. They are not coaxially stacked but are bent at an angle of 25°. Strikingly, the stems are also rotated by 48° at the helical junction. The BWYV pseudoknot also has a remarkable quadruple-base interaction between the nucleotide in loop 1 and stem regions. Loop 1 traverses the major groove of stem 2 and links the top of stem 2 to the top of stem 1. However, previous mutational studies have shown that the identity of the loop 1 base is unimportant for SRV[8] and IBV frameshifting[4]. Whether the loop 1 base or any of the other structural features described here are important for frameshifting in the BWYV system is now being determined.

Perhaps the most surprising feature of the BWYV pseudoknot is the non-canonical interactions between nucleotides in

loop 2 and the minor groove of stem 1. Prior analyses in IBV and SRV showed that the identities of nucleotides in loop 2 are not important for frameshifting[4,8]. (In contrast, the identities of certain nucleotides of loop 2 of the pseudoknot that stimulates in-frame readthrough of stop codons are important in those RNAs, although the structural reason for this is unknown[21].) We eagerly await the results of mutational studies of the BWYV pseudoknot that will test the functional significance of the unusual interactions. Furthermore, since crystallography and NMR have different virtues, it would be particularly useful to have structures of the same pseudoknot by both methods.

Mechanism

The frameshift site for BWYV is a heptanucleotide G GGA AAC[2,22]. The shift occurs when the mRNA slips by one nucleotide so that tRNA anticodons within the ribosome pair with GGG AAA instead of the initial GGA AAC. This 'tandem shift' is somehow stimulated by the pseudoknot that in BWYV is six bases further downstream.

Pausing is clearly one way that pseudoknots can contribute to stimulation of frameshifting. Both the yeast L-A viral pseudoknot[23] and the IBV pseudoknot[24] cause ribosome pausing which may give a greater opportunity for tRNA-mRNA realignment and resultant frameshifting. However, when the role of pausing has been tested directly, it has been found not to be sufficient for stimulation[23,24]. Substitution of the pseudoknots of IBV, yeast L-A and MMTV with other stable RNA structures (for example, extended stem loops) greatly reduces frameshifting and in the cases tested still causes ribosomal pausing[4,23–25]. The implication is that the ribosome is slowed down by the structure but that the structure itself plays some more specific role in priming the ribosome for the frameshift event.

Beyond the pause, what does the pseudoknot do? We know very little about the environment around mRNA as a particular sequence enters a ribosome. In the case of BWYV we could reasonably guess that as the ribosome encounters the pseudoknot, the first base of the heptanucleotide shift site will not yet be in the A site of the ribosome. (About 40 mRNA nucleotides are encompassed by a ribosome and the decoding site is roughly in the middle of the ribosome; the mRNA has 13 nucleotides from the beginning of the shift site to the pseudoknot: seven nucleotides of the shift site plus six for the spacer.) Some accommodation of the ribosome or mRNA may be needed for the A and P sites to reach the shift site. The ribosome could possibly reach the shift site by engulfing the incoming pseudoknot structure intact, in which case the pseudoknot may stimulate frameshifting by specific interactions from within the ribosome. Alternatively, the ribosome could unwind at least part of the pseudoknot in order to access the shift site. In this case the pseudoknot may have already prepared the ribosome to frameshift before unfolding or perhaps it is the unfolded pseudoknot that causes the stimulation.

Whatever detailed model is considered, it seems likely that there are specific interactions that involve the structure of the pseudoknot. These could be directly between the pseudoknot and some element of the ribosome, or they could be through a protein intermediary that interacts with both the pseudoknot and the ribosome. So far, efforts to find specific pseudoknot binding proteins or titratable factors that may be involved have not been productive. This new structure of the BWYV pseudoknot will certainly stimulate efforts to look for the crucial interactions involved in stimulation of frameshifting and to examine other structures important for reprogramming translation.

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