Structural diversity of frameshifting signals : reprogramming the programmed

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Chapter I

General introduction and outline

Translation is the process by which the genetic code is decoded by ribosomes and information (genes) is converted into functional products (proteins). In most cases, decoding follows the rule to decipher codon triplets one by one, however, alternative ways of decoding can expand the repertoire of gene expression. The term "recoding" initially proposed by R.F. Gesteland et al. in 1988 revealed the diversity of re-programmed genetic decoding utilized by organisms. Although the outcome of recoding events are diverse, the common feature in these processes is the requirement of cis-acting or recoding signals, including specific sequence motifs and RNA secondary structure elements embedded within mRNAs, to initiate non-linear translation. Most of these recoding events especially those focusing on −1 programmed ribosomal frameshifting (−1 PRF) are reviewed in Chapter 2.

It has long been believed that a given stem-loop structure cannot induce efficient -1 PRF although several hairpin-induced -1 PRF events have been reported in eubacteria and eukaryotic viruses. One of the reasons to assume that hairpins are not capable of stimulating -1 PRF is that the derivative stem-loop structure of the *Infectious bronchitis virus* (IBV) frameshifting pseudoknot cannot promote significant -1 PRF. We argued this dogma by demonstrating that the stem-loop derivative of the *Simian retrovirus type-1* (SRV-1) gag-pro frameshifting pseudoknot is very competent in inducing -1 PRF and as well as other artificial stem-loop structures. These results are presented in Chapter 3 of this thesis.

Antisense oligonucleotides (AONs) can form duplexes with mRNA, can mimic the stem region of a hairpin and induce substantial levels of frameshifting. Since a lot of modifications either on riboses or internucleotide linkages have been created to increase duplex stability or resistance to nuclease degradation, it is of interest and practical importance to know whether these nucleotide analogs can be used to promote -1 PRF *in vitro* and to shed light on the therapeutic potential in treating frameshifting diseases by AONs. In Chapter 4, we demonstrate that locked nucleic acid (LNA) is a promising modification for AONs to induce -1 PRF *in vitro*.

Although a simple hairpin can induce efficient -1 PRF, a pseudoknot is still considered to be a superior frameshifter because of its elevated stability contributed by tertiary interactions between loops and stems. Therefore, in Chapter 5 we decribe the design of AONs that simulate pseudoknot structures with the goal to enhance induced -1 PRF by AONs. Based on known tertiary interactions of the SRV-1 frameshifting pseudoknot, we rebuilt efficient pseudoknot-mimicking AONs. Furthermore, the correlation between stem length and the requirement of tertiary interactions for a frameshifting pseudoknot were also addressed.

A recently discovered riboswitch that regulates biosynthesis of nucleoside queuosine from GTP in bacteria drew our attention because of its high similarity to a

frameshifting pseudoknot in its ligand-bound state. Using our frameshift reporter assay, we identified queusoine riboswitches as efficient ligand-responsive frameshifters. By computer-assisted molecular dynamic (MD) simulation, we further characterized an unidentified residue playing an important role in ligand binding thereby affecting the overall stability of riboswitches from different species. These experiments were part of a collaborative effort with the Leiden Biophyical Structural Chemistry department and are presented in Chapter 6.

Finally, the main findings, discussions, and further ideas in relation to this thesis are described in Chapter 7.