OVERCOMING CONFORMATIONAL PARADOX: TEMPLATE CIRCULARIZATION MIGHT PREVENT THE FORMATION OF DOUBLE STRANDS DURING RNA REPLICATION

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Replication of single-stranded nucleic acids encounters with a problem: for a complementary strand be synthesized according to the Watson-Crick rules it must base pair with a template, i.e., be a part of the double helix. However, to enable further replication, the template and the complementary copy must remain single stranded, i.e., unpaired. The hypothetical replicases of the ancient RNA World must have encountered with this conformational paradox as well [1]. To date, it is shown that ribozymes can copy the template resulting in a double stranded RNA, which is the end (dead) synthesis product [2]. At this point replication stops, and the problem of RNA replication by ribozymes has not yet been solved.

The paradox would have been solved if during replication the template and the product strand existed as circles. This would impose topological constrains on the formation of a double helix: the nascent strand and the template would be able to form a short duplex only, after which they would be forced to unwind. Considering RNA replication by Qβ replicase, the RNA-directed RNA polymerase of bacteriphage Qβ, Charles Weissmann suggested that the product strand could be secured in a circular conformation if the replicase can simultaneously bind both its 3' and 5' ends [3]. The possiblirty that a template strand might also form a circle was proposed by Spiegelman before any data on the primary structures of Qβ replicase templates were obtained; he suggested that template could form a circle if it possessed complementary terminal sequences capable of base-pairing [4]. Such a structure, now referred to as "panhandle", was demomstrated for many viral RNAs but not for the Qβ replicase templates. Nevertheless, the analysis of predicted secondary structures of known Qβ replicase templates has shown that each of them may potentially have their ends base-paired to form a short stalk of the molecule which can be stabilized by continuous stacking with an adjacent hairpin.

To check if this structure might have any functional significance, the structure of RQ135 RNA, a ligitimate Qβ replicase template, was manipulated in a number of ways to clarify which elements of the RNA structure were needed for replication. It turned out that defects at the 5' end of the template drastically decreased the initial rate of RNA synthesis and enhanced its dependence on the concentration of initiator nucleotide, GTP, as well as decreased the rate and yield of the initiation step and the stability of the post-initiation replicative complex [5]. It follows that the 5' end of RQ135 RNA interacts with its 3' end during the initiation of RNA synthesis and after it and, hence, that this RNA can form a circle during replication.

References

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