Poster Presentation

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Investigation of exponential RNA amplification by the Qβ replicase complex

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Positive-stranded RNA viruses are common among human pathogenic viruses, which often cooperate with host proteins to fulfill essential functions during infection. One function is replication of the viral genome. The Qβ phage is a positive-stranded RNA virus that infects E.coli. The Qβ replicase holo enzyme comprises the phage-encoded RNA-dependent RNA polymerase (β-subunit) and the host-encoded translation elongation factors, EF-Ts and EF-Tu as well as the ribosomal protein S1. The Qβ replicase has an extraordinary ability to exponentially amplify RNA in vivo and in vitro. A prerequisite for this is release of product and template RNA as single strands that can serve as new templates in subsequent rounds of replication. The role of S1 in the Qβ replicase is not clear. Recently, S1 was found to promote release of single-stranded product in Qβ replicase–mediated RNA synthesis. We have undertaken NMR spectroscopy and crystallization trials to improve our understanding of distinct S1 domains in solution as well as the ribosome- and replicase-binding properties of S1. Expression of distinct S1 domains for NMR spectroscopy has been optimized by use of autoinduction and results in high yields of [13C15N]-labelled protein fragments. These have proven very suitable for NMR studies and spectra revealed both ordered and disordered regions in the protein. Studies are ongoing. The structure of the Qβ core complex was recently determined at 2.5Å resolution. Thus, co-crystallization of the Qβ core in complex with S1 domains was undertaken and different crystal forms were obtained. These initial crystals diffracted to 3.2Å resolution and data processing as well as further optimization of the crystals is ongoing. S1 is thought to bind the β-subunit close to a region lined with basic amino acids, which potentially could facilitate interactions with the template RNA backbone and split it from the product strand. We demonstrate that neutralization of these basic amino acids indeed decrease or abolish infectivity of the Qβ phage. However, only one mutation, R503A affects the exponential replication in vitro. Crystallization of the Qβ holo enzyme bound to a truncated legitimate RNA template will be the next step for investigation of the mechanism of exponential RNA amplification by Qβ replicase.

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