Crystallization of chimeric proteins based on human glycyl-tRNA synthetase

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The translation initiation of entheroviral mRNA is occurred by cap-independent pathway, with the help of special structural elements - internal ribosome entry site (IRES). All enteroviruses have first type of IRES.
At the moment, there is no complete understanding of the mechanisms of the IRES functioning. Moreover, not all protein factors participating in translation initiation on type I IRES have been discovered. It was shown recently that human glycyl-tRNA synthetase (GARS) is one of these factors (IRES trans-acting factors – ITAFs). It interacts with an apical part of the domain V of poliovirus IRES and stimulates its activity.
We've obtained the anticodon-binding domain (ABD) of GARS in isolated form and showed its ability to specifically interact with IRES type I. However, working with isolated ABD is difficult because it's highly prone to aggregation and has low affinity to RNA for getting crystals of such complex. In order to solve this problem we decided to create a chimeric protein, containing ABD (specific recognition) and some small RNA-binding protein (strong binding abilities). In theory, affinity of the chimeric protein to IRES should be higher than that of the intact protein.
At present we have isolated, purified in a preparative scale and crystallized first chimeric protein. High-resolution diffraction data were collected at the ERSF in Grenoble. Currently, the structure determination and refinement is in the progress. The results of our work will help to understand the mechanisms of viral initiation translation.

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