A comparative study of affinity-tag based single-step methods for purifying ribosomes

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The ribosome, the biggest macromolecule present in cell, is a key player in protein synthesis. With a coordinated interaction of different proteins (translation factors) it translates the sequence of codon present in mRNA into a chain of amino acids called polypeptide. The structural, biochemical and genetic data gathered till date demonstrates ribosome as a complex ribonucleoprotein which is largely dependent on its RNA component (rRNA) for many of its functional activities, which includes decoding of codons, proofreading of the decoding process and peptide bond synthesis. To discover the underlying molecular mechanism of these steps, different conserved ribonucleotides with functional significance can be mutated and the effect can be tested in various standard translation assays. Unfortunately, due to their essential role in different steps in translation, these mutations are mostly lethal to cell viability. Hence, isolation of pure population of mutated ribosomes has been a bottleneck for ages. One of the methods used to overcome this hurdle is expressing the rRNA carrying such mutations with an affinity tag from a plasmid in a wild type background of non-mutated ribosomes to support cell growth. In the next step, the mutated ribosomes can be selectively purified in affinity chromatography using a well chosen matrix.

Two of the most widely used affinity purification methods for purifying ribosomes carrying lethal mutations are the MS2-affinity tag based method and the streptavidin binding tag based method. In the first approach, a small RNA sequence that binds to the coat protein of bacteriophage MS2 is inserted into a well characterized loop of large subunit rRNA (23S rRNA), in such a way that it is exposed optimally to interact with its binding partner. When passed through a suitable affinity column preloaded with a recombinant version of the MS2 protein, the ribosomes carrying the tag get sequestered from the untagged ones and can finally be eluated with a buffer containing a competitive binder. The second approach employs a streptavidin protein binding RNA tag, which is inserted into 23S rRNA and the ribosomes bearing the tag are purified over a streptavidin-sepharose matrix. Bound ribosomes are eluated out using a buffer saturated with biotin which has a relatively stronger affinity to streptavidin.

The objective of my study was to standardize and compare these two methods based on their final yield, its purity and activity in translational assays so that one of these techniques can be selected for further scaling up and expression of numerous functionally interesting mutations in large and small subunit rRNA. Though the initial data shows that the streptavidin affinity tag based method is more efficient in terms of yield, it needs to be further consolidated with follow up experiments to check purity and activity of tagged ribosomes.

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