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TECHNIQUES

Exploring protein–RNA interactions with RNA Tagging

> RNA Tagging could, at least in some cases, be used to distinguish high-affinity protein–RNA interactions

Current methods for studying protein–RNA interactions rely on the purification of protein–RNA complexes by immunoprecipitation, which is sometimes combined with UV crosslinking. These *in vitro* approaches do not always distinguish physiological from non-physiological interactions. A new method, termed RNA Tagging, has now been developed to identify protein–RNA interactions *in vivo*.

The new method relies on the production of a chimeric protein composed of an RNA-binding protein (RBP) of interest coupled to a poly(U) polymerase (PUP) that does not bind to RNA per se. Expression of this chimeric protein in cells leads to the addition of a string of uridines (the 'U-tag') specifically to the RNAs that are bound by the RBP. High-throughput sequencing of the cellular transcriptome after lysis enables easy identification of the U-tagged RNAs.

The researchers tested the new method by expressing the Puf3p protein fused to PUP-2 in *Saccharomyces cerevisiae*, which led to the identification of 467 mRNAs that are probable physiological targets of Puf3p *in vivo*. The identified target mRNAs were also highly enriched in Puf3p-binding motifs in their 3′ UTRs and were also enriched for mitochondrial functions, in line with the known physiological roles of Puf3p.

Interestingly, the length of the U-tag correlated with the binding affinity of the Puf3p protein to its targets. Therefore, RNA Tagging could, at least in some cases, be used to distinguish high-affinity protein–RNA interactions that support a regulatory action for the tested RBP, from brief, low-affinity interactions that would indicate 'sampling' of RNAs by the RBP but no biological effect *in vivo*.

The authors next investigated the RNA partners of Bfr1p, a protein that does not possess defined RNA-binding elements (unlike Puf3p). Bfr1p is involved in the secretory pathway, but previous analyses of Bfr1p–RNA interactions using other methods did not reveal an enrichment in RNA targets with roles in this pathway. RNA Tagging, by contrast, led to the identification of a set of targets that is enriched in cytoplasmic translation and membrane-associated functions.

These findings indicate that RNA Tagging is a practical and effective method to explore the functional roles of protein–RNA networks *in vivo*.

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