mRNA Enrichment Techniques for Prokaryotes

Transcriptomics is useful for figuring out what genes are being expressed in an organism at a specific time, generally in response to a certain condition or stressor. This enables the discovery and description of processes within an organism and enables us to connect certain stressors to regulation of certain expressed genes.

Most transcriptomic studies have been done on Eukaryotic organisms. This was due to the fact that it was more difficult to perform messenger RNA (mRNA) enrichment on prokaryotes since they lack the characteristic eukaryote 3'-poly-A tail. Enrichment is generally necessary since mRNA comprises <5% of the total RNA. This combined with the idea that prokaryotic transcriptomes were much simpler than eukaryotic organisms (since they lack introns and are rarely alternatively spliced or changed) lead to relatively no research of prokaryote transcriptomes until recently.

New methods have come about that enable relatively easy mRNA enrichment of prokaryotes. Along with high-resolution tiling arrays or RNA-sequencing technologies the mRNA enrichmmnent techniques can reveal a lot about prokaryotes. The most widely used mRNA enrichment methods are: Ribosomal RNA (rRNA) capture, degradation of already processed RNA, ployadenylation of mRNA, and antibody capture of specific RNAs.

- Ribosomal RNA capture involves the use of probes that correspond to conserved regions of the 16S and 23S regions. The probes are attached to metallic beads, which are removed (along with the rRNA). This method removes most of the rRNA, but since the probes are specific it can vary from organism to organism. An example of a kit that has had success is the MICROBExpress kit by Ambion (Austin Texas). This kit cannot be used with archea, since the probes used are not compatible (drawback).
- Degradation of processed RNA involves an exonuclease that breaks down 5' monophosphate (5'P) RNA molecules, but not 5' triphosphate (5"PPP) mRNA molecules. This works due to the fact that most prokaryotic mRNA has a 5'PPP while rRNA and tRNA (processed molecules) only have a 5'P. An example of a kit that has had success is the mRNA-only prokaryotic mRNA isolation kit by Epicentre (Madison Wisconsin).
- Selective polyadenylation of the mRNAs uses *E.coli* poly(A) polymerase to polyadenylate the mRNAs, but not the rRNAs. Following the polyadenylation the mRNA can be captured by probes or transcribed with primers; oligo(dt) in both cases. A kit that has had success with this method is the MessageAMP II-Bacteria kit by Ambion (Austin Texas).
- Antibody capture uses immunoprecipitation to isolate RNAs that are associated with a certain protein. This has been used to capture rRNA and tRNA associated with the protein Hfq, which mediate between small RNA (sRNA) and mRNA targets.

(Sorek & Cossart, 2010).

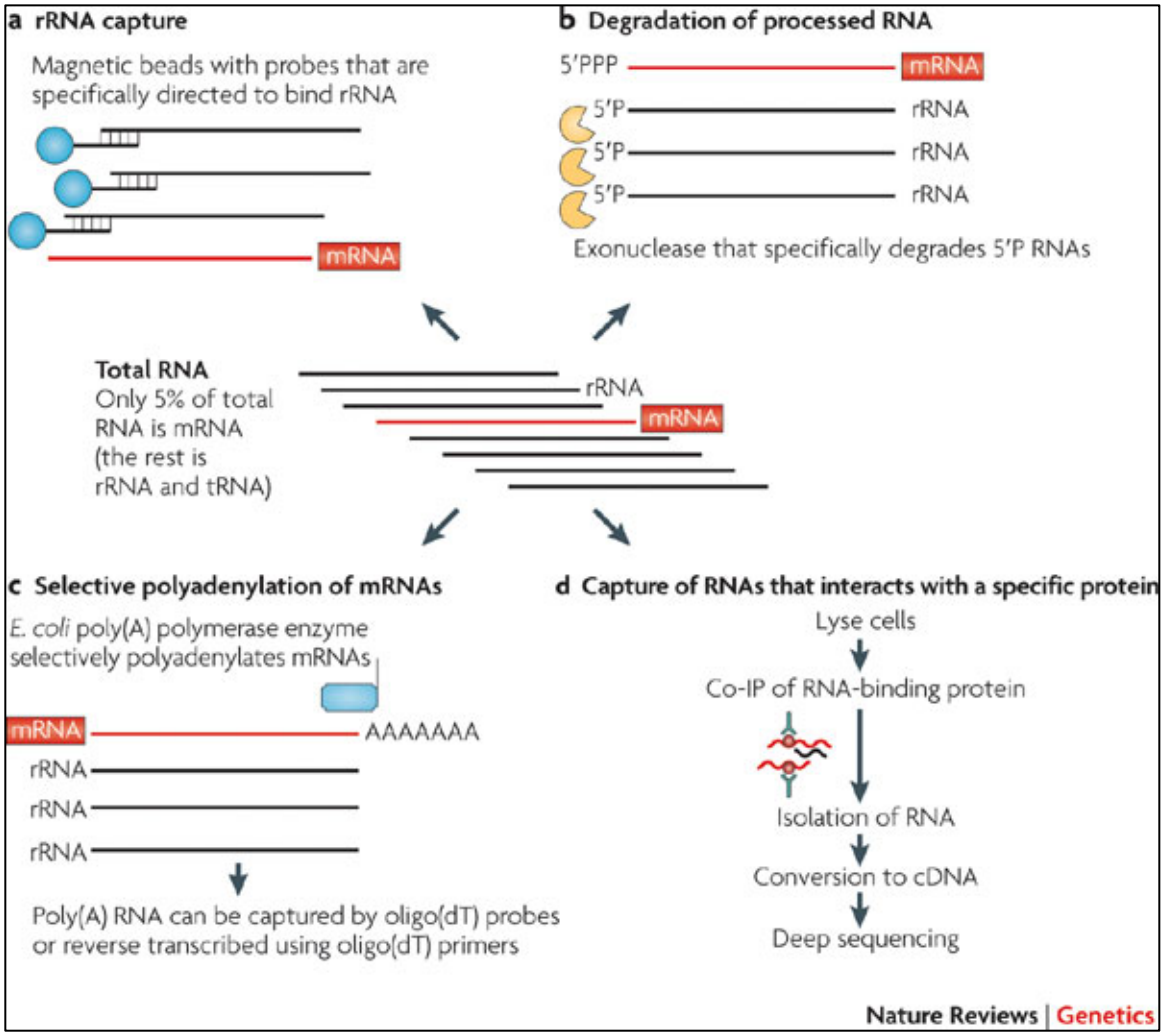

Figure 1 Different methods for mRNA enrichment. (Sorek & Cossart, 2010).

Sorek, Rotem, and Pascale Cossart. "Prokaryotic Transcriptomics: A New View on Regulation, Physiology and Pathogenicity." *Nat Rev Genet Nature Reviews Genetics* 11.1 (2010): 9-16. Web