Genome-wide translational profiling of the inflammatory responses of macrophages to LPS

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ABSTRACT

Introduction: Exercise causes both inflammation and anti-inflammation and the details are complex. Genome-wide analysis, including proteomics or RNA sequencing (RNA-seq), is beneficial to reveal the complexity and to screen novel factors. However, there are some difficulties. Proteomics shows low reproducibility and analytical bias (eg., hydrophilic and hydrophobic proteins). Although RNA-seq or microarray present robust reproducibility, it has been known that the correlation of mRNA and protein levels is low (R^2 = 0.17 ~ 0.41). This is due to translational regulation. Considering translational efficiency, the correlation is boosted to R^2 = 0.95.

In 2009, newly introduced technique based on RNA-seq platform, called ribosome profiling, showed the ability to quantify translational efficiency. It conducts deep-sequencing of mRNA fragments encompassed by ribosomal complex (ie., translationally active mRNA region). Since ribosome profiling is based on highly reproducible RNA-seq and considers translational regulation, it is possible to carry out more precise and accurate measurements of cellular dynamics and to discover novel factors.

Here we conducted ribosome profiling to understand the impact of translational regulation and to discover novel candidates in the responses of macrophages to lipopolysaccharide (LPS).

Method: RAW264 macrophages ($2.5x10^5$/ml) were incubated for 24 hr. Then, LPS (100ng/ml) was added, followed by 30 min incubation. Cycloheximide (100 μ /ml) was added to stall translating ribosome. Cells were lysed and total RNA was extracted, followed by RNaseIf digestion and sucrose cushion to extract monosome (for ribosome profiling) or poly-A RNA extraction (for RNA-seq). Poly-A RNA was alkaline-digested and gel-purified to collect 25 ~ 45bp regions. For ribosome profiling, monosome was gel-purified to collect ribosome-protected mRNA fragments (26 ~ 32bp), followed by ribosomal RNA removal. These were further gone through 3'-dephosphorylation and 5'-phosphorylation and sequence library preparation using Ion total RNA-seq kit v2 (Ion torrent). Sequencing was conducted using Ion PGM (Ion torrent). The mapped sequences were analyzed by NOISeq to detect differential gene expression (DGE).

Results: Comparing basal state and LPS-activated state, only small fractions of mRNA levels were changed and called as DGE in RNA-seq. However, more DGEs were observed in ribosome profiling. Translational rates of each mRNA were highly varied even in the basal state.

Conclusions: Translational changes seem to be more sensitive to LPS compared to transcriptional regulation. Also, large fractions of mRNA are under translational regulation even in the basal condition as well as in the activated state. Translational regulation may play an important role in the inflammatory responses of macrophages.