論 文 の 内 容 の 要 旨

論文題目　Contribution of RNA degradation and posttranscriptional regulation to gene expression

（ＲＮＡ発現に対するＲＮＡ分解および転写後制御の寄与）

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The eventual RNA abundance is regulated at multiple levels that include: transcription, mRNA processing, nuclear export, translation and degradation. Many large-scale functional genomics projects have focused on elucidating the transcription initiation through chromatin immunoprecipitation sequencing (ChIP-seq) and the eventual RNA abundance through RNA sequencing (RNA-seq). In comparison, the understanding of RNA decay is far from complete. There have been reports that use the ChIP-seq datasets of chromatin modifications to predict the eventual RNA abundance, which concluded that they were able to predict the eventual RNA abundance by using the ChIP-seq data, with a certain level of accuracy, suggesting that the RNA decay does not play significant roles in regulating the eventual RNA abundance. However, in majority of reports, the rate of RNA degradation is not directly assayed, therefore can only be inferred through other assays, due to the difficulty in obtaining RNA decay data without interrupting the physiology of the cell.

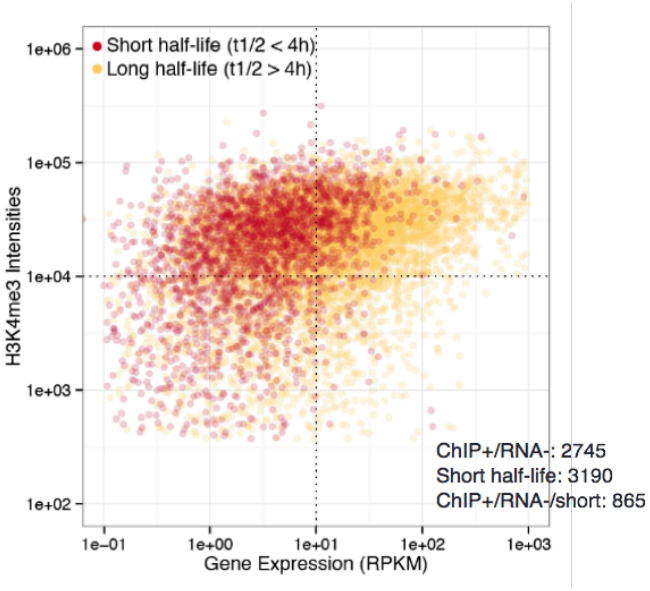

Figure 1 ChIP+/RNA- genes have shorter RNA half-life
Genes that have shorter half-lives have tendencies to be in ChIP+ / RNA- quadrant.

In this thesis, I have broken down into two components; analysis of RNA decay in steady-state and the contribution of RNA decay upon a stimulus, by using 5-bromouridine immunoprecipitation chase sequencing (BRIC-seq). In short, BRIC-seq uses 5'-bromouridine (BrU) as a nucleoside analogue to conduct the pulse-chase to determine the RNA remaining, and through the relative abundance of RNA in a time-course, the rate of RNA decay can be determined.

GO: ID	GO: term	Number	FDR
GO:0003677	DNA binding	216	5.19E-58
GO:0006351	Transcription, DNA-templated	192	1.37E-37
GO:0006355	Regulation of transcription, DNA-templated	147	5.13e-34

Table 1 Gene ontology enrichment for ChIP+/RNA-/short RNA half-life genes. Strong enrichment to the transcription factors suggest that mRNA of transcription factors are regulated through RNA decay.

To understand the contribution of transcription initiation on the eventual RNA abundance, I conducted ChIP-seq for histone H3K4me3(K4me3) and RNA polymerase II (pol2) and compared against the eventual RNA abundance estimated through the RNA-seq data, in HeLa cells. When these datasets were directly compared, there was a general positive correlation between ChIP-seq and RNA-seq data (r^2=0.50, p-value < 2.2×10^{-16}), suggesting that for majority of genes transcription initiation is a major determinant of RNA abudance. However, I identified 2745 genes that have discrepancies between the K4me3 intensity and RNA-seq data, where there are sufficient ChIP-seq signal to suggest transcription (ChIP+/RNA-); however, fail to observe the RNA expression from the RNA-seq data. When RNA decay was compared to ChIP-seq and RNA-seq data, I observed that those ChIP+/RNA- genes had significantly shorter RNA half-life (6.0 hours) than genes where the RNA abundance followed the linear regression of ChIP-seq signal (10.9 hours). Out of 2745 ChIP+/RNA- genes, 865 genes had RNA half-life shorter than 4 hours and these

ChIP+/RNA-/short RNA half-life genes were significantly enriched in gene ontology (GO) terms associated with transcription regulation, suggesting a control of transcriptional network through the mRNA decay of transcription factors. To test the generality of the pattern, I conducted similar analysis to identify ChIP+/RNA- genes from the ENCODE project and in 7 out of 8 cell-lines, ChIP+/RNA- genes were enriched in terms related to transcription factors. Furthermore, I conducted siRNA knockdown to three RNA decay factors: UPF1, STAU1 and EXOSC5 and identified their targets using BRIC-seq and RNA-seq. Out of 865 genes that were ChIP+/RNA-/short RNA half-life, 60 genes (8.5%) could be explained by the regulation through three RNA decay factors. In conclusion, the RNA decay factor mediated RNA stability contributions are important for numerous genes especially for transcription factors suggesting the presence of indirect feedback to the transcription initiation.

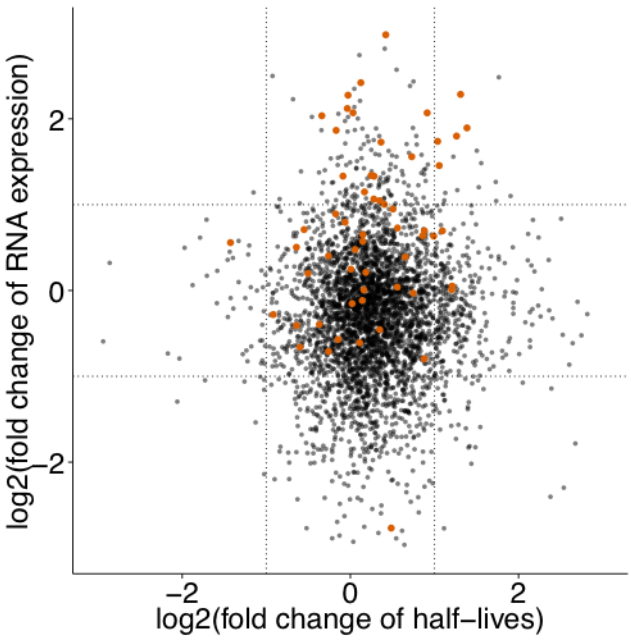

Figure 2 Scatterplot showing the relationship between changes in RNA expression against changes in RNA decay in hypoxia

Promoters of genes in orange are bound by HIF-1 according to ChIP-seq data

To understand the role of RNA decay in mediating changes to the RNA abundance, I used hypoxia (low oxygen potential) as a model. This is because the transcriptional regulation through HIFs (hypoxia inducible factor) have been widely studied, with HIF having a central role to modulate key genes involved in the Warburg effect, where the cells obtain the ATP through aerobic respiration instead of mitochondrial oxidative phosphorylation. I conducted BRIC-seq in hypoxia (1% O_2) and normoxia (20% O_2) and compared the RNA half-lives between these conditions,

in hypoxia resistant DLD-1 human colorectal cancer cell-line. I found that there is a statistically significant elongation of RNA half-life in hypoxia compared to normoxia, for genes that could be modelled onto the simplest decay model. For 321 genes that had their RNA stabilized by more than 2-fold in hypoxia, 301 genes did not change in the eventual RNA abundance, 20 and 38 genes showed up and down regulation by two-fold in RNA abundance, respectively. Interestingly, out of 66 genes with HIF1 binding sites, only 9 genes showed increase in RNA half-life, suggesting a different mode of regulation. Additionally, RNA that became stable without the changes in the eventual RNA abundance showed enrichment for gene ontologies in transcription factors, suggesting a tighter control of mRNA levels of genes encoding transcription factors to minimise the effect of mis-regulation of the transcriptional network.

Integration of RNA stability to other functional genomics datasets have started to reveal the mechanistic roles of transcriptional and post-transcriptional regulation in controlling gene expression. In particular, these data suggest that RNA degradation is gene specific and the transcription factors are tightly regulated through the mRNA stability. The characterisation of the additional layer in the gene expression mechanism is only going to aid to help to understand the mechanism behind gene expression that is essential in understanding cellular response to external conditions.