Patterns of microRNA biogenesis and expression in the process of Unfolded Protein Response

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Abstract. Abnormal protein folding could have a dangerous consequence for a cell and whole organism. Nearly all membrane and secretory proteins fold into endoplasmic reticulum (ER). The overload of newly synthesized unfolded proteins leads to insufficient capacity of ER and triggers global cellular response called unfolded protein response (UPR). UPR have three parallel pathways of response mediated by signal transducers proteins ATF6, PERK and IRE1. UPR mediates short-term attenuation of ER protein loading and long-term increase of ER protein-folding capacity and activation of UPR target genes. Recent researches demonstrated that microRNAs contributes to the regulation of different steps of UPR pathways. Here we profile microRNA and mRNA expression in UPR-stressed and control Jurkat cell line. We report genome-wide downregulation of microRNAs compared to other classes of small RNAs (e.g. snoRNAs, snRNAs and tRNAs), and downregulation of the main proteins constituent microRNA biogenesis pathway. Despite global downregulation, there exists a class of microRNAs with increased expression. MicroRNA fate could be regulated through different kinds of modifications, which are emergence as new layer of regulation of microRNA stability and targeting. Here we observe unique patterns of microRNA 3'-modifications with 40% increased uridinilation and 20% decreased adenylation.

Keywords: microRNA, unfolded protein response, expression, modification

Increased protein load in ER could lead to a massive misfolding of proteins. Inability to handle this situation and to recover homeostasis leads to a numerous human disease (Walter et al., 2011) and cancers (Chen et al., 2014). UPR is the global cellular response aimed to recover homeostasis of the protein-folding process, or to trigger apoptosis otherwise. UPR constitutes three main branches mediated by stress sensors ATF6, PERK and IRE1 (Ron et al., 2008). Transcription factor ATF6, that transduces signal through regulated proteolysis, activates UPR target genes that increase protein-folding capacity of ER (e.g. chaperons,). ATF6-mediated pathwat constitutes transcriptional response to the stress. PERK transmembrane kinase senses unfolded proteins in ER lumen and oligomerizes and phosphorilates itself followed by

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phosphorilation of eIF2a. Phosphorilation of eIF2a globally reduces translation. However translation of some proteins increased, among them transcription factor ATF4 which activates apoptosis-associated genes. The third, and the most ancient branch of UPR, activates IRE1 kinase-RNAase. IRE1 mediates nonconventional splicing of transcription factor XBP1 that activates genes associated with protein-folding capacity and ER-associated degradation (ERAD). IRE1 is also capable to modulate promiscuous co-localized mRNA degradation through endonuclease activity.

Recent researches hint that microRNAs regulate different steps of these pathways (Chitnis et al., 2013). MicroRNAs are small 18-26 nt non-coding RNAs that regulate gene expression. MicroRNAs, associated with RISC, mediate post-transcriptional downregulation of mRNAs through complementary binding preferentially to 3'UTR. While their impact only modest in homeostatic conditions (Baek et al., 2008), they seem contribute critially to developmental and stress transitions (Stark et al., 2005).

Among UPR-associated microRNAs, miR-211 and miR-204 regulate PERK pathway through downregulation of CHOP mRNA, miR-30c regulates Xbp1 mRNA and a number of microRNAs regulate IRE1-pathway. We thus conduct small RNA sequencing in cells treated with DTT, that induces UPR, and control in order to monitor changes of small RNAs abundance and biogenesis. In parallel, we profile mRNA expression using Affimetrix microarrays.

We conduct sequencing of the fraction of small RNAs with two replicates of control and DTT-treated cell, and obtain 10-13 million reads per sample. Row reads are mapped using Bowtie to different classes of small RNAs: microRNAs, tRNAs, snRNAs, snoRNAs. We observe global decrease of microRNAs expression relative to other classes of small RNAs with 40% reads mapped to microRNAs in control compared to 25% in UPR-stressed cells. In agreement, we observe that downregulated mRNAs, profiled using Affymetrix microarrays, are highly enriched in genes associated with microRNA biogenesis. Despite global downregulation, we observe a class of microRNAs that are upregulated in stressed cells.

MicroRNA biogenesis is tightly regulated. Besides downregulation of the main biogenesis enzymes (for example Drosha, Dicer, Argonaute familly), modifications are newly arised regulatory layer of microRNA expression, stability and targeting. The most prominent modifications are variation of 5' and 3' ends of mature microRNA, editing and strand selection. MicroRNA editing, strand selection and 5' end modifications primarily change pool of microRNA targets. And we don't observe any of these events in UPR-stressed cells. In contrast, 3' end modifications are associated with stability and localization of microRNAs and represent fine-tuned level of regulation of microRNA stability. We observe decrease of adenylated from 35% to 28% and increase of uridinilaton from 20% to 28% upon stress induction. Addition of adenine is commonly associated with increased stability of microRNAs, whereas addition of uracile is associated with degradation (Yates et al., 2013). Sequencing of small RNAs captures microRNAs associated with Ago1-Ago4 as well as free cytoplasmic microRNAs. While length of mature microRNAs could vary substantially, loading to each of Ago proteins require specific length. We investigate distribution of length of microRNA fragments in control and UPR-stressed conditions. We take into account only modified microRNAs because 3' modification determines precise 3'end of mi-

croRNA. We observe statistically significant increase of microRNA length, as well as less dispersed distribution in UPR-stressed cells compared to control. This could be explained by variation in preferences of Ago loading proteins or degradation rates upon UPR. In agreement with the first statement, we observe differential mRNA expression changes of four Argonauts.

We next investigate, whether changes in microRNA expression leads to detectable changes in mRNA expression. Using several well known computational and experimental approaches, we predict mRNAs targets of microRNAs. Computational and experimental methods of microRNA binding sites detection has significant variation, we thus use three different approaches to ensure robustness of the results: TargetScan, mirSVR and miRTarBase (the database of experimentally verified targets). We observe that changes of microRNA expression positively correlate with changes of mRNA expression (spearman=0.23, $p<10^{-4}$). While the expected correlation is negative, we could hypothesis that this result attributes to a complex feedback loops during the stress.

Overall, for the first time we investigate genome-wide coupled expression profile of microRNAs and mRNAs. We observe global downregulation of microRNA-mediated machinery, while some microRNAs is upregulated. UPR-stressed cells show shift of microRNA biogenesis patterns reflected in 3' modification and distribution of mature length. Whether this physiological changes have functional consequences remains an open question.

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