論文の内容の要旨

Regulation of *fbp1*$^{+}$ long non-coding RNA stability before and during glucose starvation
（グルコース飢餓時の遺伝子応答に関わる長鎖非コードRNAの安定性制御）

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The importance of long non-coding RNAs (lncRNA) in controlling various cellular processes has been proven repeatedly over the past decade. Previous research in our lab has uncovered the existence of a novel type of sense RNA polymerase II-dependant lncRNA which reads through the fructose-1,6-bisphosphatase (*fbp1*$^{+}$) gene, the transcription of which is essential for chromatin remodeling and full induction of *fbp1*$^{+}$ mRNA during glucose starvation in *Schizosaccharyces pombe* (Hirota *et al.* 2008). In this study, we redefine these new lncRNAs as 'metabolic stress-induced long non-coding RNAs' (mlonRNA). Strand-specific RNA sequencing also revealed the presence of a non-coding antisense RNA which overlaps the entire *fbp1*$^{+}$ region and promptly disappears upon glucose starvation (Arisa Oda, unpublished results). This study aims to elucidate the respective characteristics of *fbp1*$^{+}$ antisense lncRNA (as-lncRNA) and sense mlonRNA before and during glucose starvation and the mechanisms involved in the regulation of their stability.

I have found that mlonRNA and as-lncRNA are much less stable than *fbp1*$^{+}$ mRNA. Furthermore, all of these transcripts are 5'-capped and sensitive to the nuclear exosome cofactor Rrp6-mediated 3' to 5' degradation. This degradation did not appear to require the TRAMP complex (Trf4/Air2/Mtr4p Polyadenylation complex), as demonstrated by the lack of effect in a *cid14Δ* mutant. Intriguingly, disruption of the core exosome by a cold-sensitive mutation resulted in the complete loss of the starvation-specificity of the induction, as *fbp1*$^{+}$ mRNA was fully induced even in glucose-rich media. The kinetics of *fbp1*$^{+}$ as-lncRNA remained however comparable to the wild-type. The core exosome is therefore expected to play a more complex role in the starvation response.

Because sense mlonRNA and as-lncRNA overlap the same genomic region, it is reasonable to expect that they may form stretches of double-stranded RNA (dsRNA), which may be targeted by Dicer and enter the siRNA pathway. Moreover, RNA secondary structure prediction software revealed a strong hairpin structure in the 5’ region of mlonRNA transcripts (a) and (b). mlonRNA transcript (b) in particular seemed very slightly stabilized in a Dicer deletion mutant. However, the deletion of part or whole of the potential structure had no major effect on the stability of this transcript. Since *fbp1*$^{+}$ mlonRNA and mRNA induction proceeded fairly normally in a Dicer mutant, I expect that siRNA does not play a major role in glucose starvation at this locus.

Although mlonRNA and as-lncRNA are degraded in the nucleus to some extent, polysome fractionation results show that the majority of them are exported and bound by multiple ribosomes. Moreover, as-lncRNA and

mlonRNA are differentially regulated in the cytoplasm. Both of these RNA species harbor an unusual number of small open reading frames (ORFs) compared to the average *S. pombe* mRNA 5' untranslated region (UTR), meaning that they could be targets of the Upf1-mediated nonsense-mediated decay (NMD) pathway. Interestingly, the glucose-rich-specific as-lncRNA were sensitive to NMD, whereas the glucose starvation-specific mlonRNA were resistant. Since small ORFs in the 5' region of mlonRNA are relatively enriched in rare codons compared to the *fbp1*$^{+}$ coding region, I hypothesize that instead of NMD, mlonRNA may be the first natural target of the no-go decay pathway (NGD), which targets transcripts on which translation is slowed down or arrested by the presence of secondary structure or rare codons.

Overall, as illustrated below, these results reveal that *fbp1*$^{+}$ as-lncRNA, mlonRNA and mRNA, although originating from the same locus and sharing many characteristics such as a 5' cap, poly(A)-tail, ORFs, and subcellular localization, are specifically regulated by distinct RNA surveillance systems.