(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Ahmad Luqman Bin Abdul Fatah
Thesis Title	The interferon-stimulated gene product HELZ2 destabilizes human LINE-1 RNA to inhibit LINE-1 retrotransposition and the associated type I interferon response		

(Thesis Summary)

Human retrotransposon Long INterspersed Element-1 (LINE-1 or L1) amplifies its sequence by a copy-and-paste mechanism termed retrotransposition. L1 retrotransposition can cause genome mutation and several host factors potently inhibit L1 retrotransposition to maintain genome integrity. L1 encodes two proteins: ORF1p and ORF2p. ORF1p, an RNA binding protein is required for L1 ribonucleoprotein (RNP) complex formation, which is necessary for L1 retrotransposition. ORF2p has endonuclease and reverse transcriptase activities. To identify unknown host factors regulating L1 retrotransposition and the RNP formation, the author generated ten L1 ORF1p mutants and characterized an RNA-binding mutant ORF1p that is stably expressed, has an impaired retrotransposition efficiency, and fails to form L1 RNPs. The mutant was used in the host factors screening by ORF1p immunoprecipitation-coupled mass spectrometry.

To search for L1 RNP-specific host factors, the author performed a differential label-free quantitative mass spectrometry, comparing the wild type and the RNA-binding mutant ORF1p complexes. Gene ontology and gene set enrichment analyses of the protein hits enriched in the wild type L1 fraction (L1 RNP-specific host factors) suggest that L1 is regulated by an innate immune response that leads to the associated interferon-stimulated gene (ISG) expression. Furthermore, the author found a group of antiviral ISGs that interact together with L1 RNPs; four of the ISGs have been reported to inhibit L1 retrotransposition while the other 16 have not. Among the 16 ISGs, the author identified three proteins that inhibit L1 retrotransposition: 2'-5'-oligoadenylate synthetase-like (OASL), HECT and RLD domain containing E3 ubiquitin-protein ligase 5 (HERC5), and helicase with zinc finger 2 (HELZ2). The author demonstrated that these proteins inhibit L1 retrotransposition at different stages of the L1 replicating cycle. OASL impairs the ORF1p cytoplasmic foci formation and HERC5 reduces steady state levels of ORF1p, while HELZ2 recognizes structures and/or sequences within the L1 5' untranslated region (UTR) to reduce L1 RNA, ORF1p, and ORF1p cytoplasmic foci levels. Furthermore, the author characterized HELZ2 domains by showing that the helicase domains are indispensable for L1 retrotransposition inhibition.

In agreement with previous studies, the author's results suggest that type I interferon expression is induced by individually over-expressing WT and reverse-transcriptase deficient L1s, which was abolished by simultaneous HELZ2 over-expression. Notably, type I interferon expression is enhanced when the ORF1p RNA-binding mutant was overexpressed, suggesting that the ORF1p RNA-binding shields L1 RNA from "triggering" the type I IFN upregulation. Taken together, these results suggest a negative feedback regulation of L1 retrotransposition by ISG proteins through different mechanisms.

(Form 2)

（Thesis Evaluation Summary）

Although L1 retrotransposition is long known to cause genetic mutation and DNA damage, the retrotransposition-independent effects of L1 have just recently gained attention, as it has been found that the cytoplasmic accumulation of L1 intermediates upregulates innate immune responses possibly leading to inflammatory phenotypes and aging. L1 intermediates including both cDNA and RNA were shown to be detected by the pathogen recognition receptors, i.e., DNA sensor (cGAS) and RNA sensors (RIG-I, MDA5, TLR7, and TLR8) respectively, suggesting that L1 intermediates are potential endogenous immunogens. Using HEK293T cells that have a relatively low cGAS amount and a reverse transcriptase deficient L1, the author showed that L1 RNA can induce an innate immune response. On top of that, the author's results suggest an unreported function of ORF1p, which is to "protect" L1 RNA from the RNA sensors.

In this thesis, the author hypothesized that a network of ISG proteins directly regulates L1 RNA/RNP, to counteract the immunogenicity of L1 RNA/RNP in the form of a negative feedback loop. The author arrived at this idea following a series of bioinformatics analyses of the ORF1p complex mass spectrometry, which revealed a group of antiviral/innate immune-related proteins that interact with L1 RNP. The author showed that some ISG proteins identified in this study have been reported to inhibit L1 but proceeded to identify three ISG proteins as unreported L1 retrotransposition inhibitors. Among the three, HELZ2 was shown to destabilize L1 RNA through L1 RNA 5' UTR recognition that subsequently blunts the innate immune response. This thesis comprehensively demonstrates L1 regulation by the immune pathway and the ISGs.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of genome biology, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On January 26th, 2023, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

(Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) : mm dd , yyyy