学位論文内容の要旨
（Summary of Dissertation）

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（Dissertation Title）Structural Analysis of Nuclear Stress Bodies Formed in Response to Thermal Stress

学 位 論 文 題 名: (熱ストレスに応答した核内ストレス体の構造解析)

Background and Purpose: Nuclear stress bodies (nSBs) are primate-specific membrane-less subnuclear structures that are formed in response to thermal and chemical stresses. The assembly of nSBs is initiated alongside HSF1-dependent transcription of the primate-specific highly repetitive satellite III (HSATIII) long noncoding RNA (lncRNA) and heat shock-induced HSF1 aggregation. HSATIII lncRNAs are retained on chromosomes near their own transcription sites for several hours and recruit various RNA-binding proteins (RBPs). It is still poorly understood why nSBs are assembled in primates' stress response events. Decoding the protein composition and structural organization of nSBs were our first approach to elucidate the nSBs functions in primates' stress response events. Recently, our lab has identified 141 proteins as components of nSBs, most of which are likely RBPs involved in RNA splicing, processing, modification, and export.

Here, we elucidate the existence of two distinct nuclear stress bodies containing different sets of RNA-binding proteins that are formed with HSATIII upon thermal stress exposure, nSB-M and nSB-S. In addition, we report a previously unrecognized thermal stress-induced recruitment of the N^6-methyleadenosine (m^6A) RNA modification machinery to nSBs. m^6A is the most abundant RNA modification in Eukaryotes and has been reported to have crucial regulatory roles in cancer proliferation, sex determination, cell differentiation, neuronal function, meiosis, circadian rhythm, and chromosomal silencing. Hence, m^6A machinery recruitment to nSBs proposes a novel nSBs-dependent regulatory function of m^6A in primates' stress response events.

Results: We observed adjacent localization of SAFB and HNRNPM, nSBs proteins, foci formed in response to thermal stress. First we confirmed that both entities utilize HSATIII lncRNAs as architectural scaffolds. Triple staining combined HSATIII RNA-FISH and immunofluorescence experiments have shown that both HNRNPM and SAFB foci recruit distinct RNA-binding proteins sets. We found that upon thermal stress exposure, HNRNPA1 and HNRNPH1 are strictly recruited to HNRNPM-foci, we named nSB-M, while SLTM, and NCOA5 are strictly recruited to SAFB-foci, we named nSB-S.

On the other hand, we detected a thermal stress-induced sequestration of the m^6A writers, METTL3 and WTAP, and the m^6A reader, YTHDC1, to nSBs. In addition, anti-m^6A modification antibody confirmed the localization of m^6A modified RNAs at nSBs. HSATIII knockdown was enough to abolish both stress-induced m^6A machinery recruitment and m^6A RNAs localization to nSBs. Moreover, we found that HSATIII transcripts are methylated and that at least HSATIII serves as target substrate for m^6A machinery at nSBs. RNA-MS analysis has identified GGAAU, the highly repetitive sequence of HSATIII, as a novel m^6A consensus motif. m^6A machinery showed a unique recruitment/enrichment pattern at nSBs. During stress, the m^6A machinery is recruited to nSBs and enriched upon stress removal 1h post-stress then releases from nSBs at 4h late recovery with exception of YTHDC1 that exhibit prolonged enrichment at nSBs. Finally, we found that TDP43 depletion dramatically inhibited nSBs-m^6A enrichment highlighting a novel regulatory role of TDP43 in stress-induced m^6A RNA modification.

Discussion: We identified two distinct nSBs (nSB-S and nSB-M) containing different sets of RNA-binding proteins that are formed with HSATIII arcRNAs upon thermal stress exposure. It is possible that nSB-S and nSB-M represent different stages of the synthesis and maturation of nSBs after thermal stress-induced HSATIII arcRNA synthesis. It

is also possible that HNRNP proteins with high affinity to RNAs may rapidly bind to HSATIII to form the dense core of nSBs, and then SAFB and other components subsequently join the surrounding area to form the larger nSB-Ss. On the other hand, we report a previously unrecognized thermal stress-induced recruitment of m^{6}A machinery to nSBs and m^{6}A RNAs localization to most HSATIII thermal stress-induced foci. To investigate the target RNAs of the m^{6}A machinery in nSBs, we re-purified HSATIII and found that HSATIII is enriched with m^{6}A modification. In addition, RNA-MS has identified the third A of GGAAU in the highly repetitive HSATIII sequence as the major m^{6}A sites. Moreover, RNA-MS has identified a small population of HSATIII m^{6}A consensus that exhibit two adjacent m^{6}A modification at AA of GGAAU. The known consensus motif of m^{6}A sites is GGACU which contains one base mismatch with m^{6}A sites in HSATIII, GGAAU lacking the C residue after A. The C residue in the m^{6}A consensus has been reported to be essential for m^{6}A deposition. Hence, shifting m^{6}A consensus motif from GGACU to GGAAU might be thermal stress-dependent. Meanwhile, we cannot rule out the possibility that m^{6}A machinery components and/or mechanism of action upon stress is distinct from normal condition. To elucidate the mechanism of action of the m^{6}A machinery at nSBs, we screened the dynamics of m^{6}A machinery components during stress/recovery phases. Remarkably, we found that m^{6}A machinery has a unique recruitment pattern to nSBs, in which m^{6}A machineries are recruited during stress and deposit m^{6}A modification at HSATIII with relative enrichment in the early post-stress recovery stage then released from nSBs by 4h post-stress recovery stage. Only YTHDC1, the m^{6}A reader, showed prolonged enrichment that might be due to additional non-m^{6}A related function in nSBs during the late recovery stage. Moreover, we detected unusual nSBs-m^{6}A tolerance upon METTL3/WTAP depletion suggesting the possibility that crucial components of the nSBs-m^{6}A machinery are yet to be identified that detect GGAAU m^{6}A sequence instead of the GGACU global consensus. TDP43 was reported to specifically bind to GGAAU RNA foci formed in SCA31 disease. TDP43 depletion dramatically downregulated nSBs-m^{6}A. Therefore, it raises the intriguing possibility that TDP43 acts as the adaptor to link the m^{6}A regulatory factors to HSATIII lncRNAs with GGAAU repeat sequences under stressed condition.

Conclusion:

- The nSBs components SAFB and HNRNPM exhibit adjacent localizations in response to thermal stress.
- Both SAFB-foci and HNRNPM-foci utilize HSATIII lncRNA as architectural scaffold.
- SAFB-foci (nSB-S) and HNRNPM-foci (nSB-M) sequestrate two distinct sets of RNA-binding proteins. SLTM and NCOA5 are strictly recruited to nSB-S while HNRNPA1 and HNRNPH1 are strictly recruited to nSB-M.
- m^{6}A RNA modification writers and readers' machineries are sequestrated to nSBs upon thermal stress.
- m^{6}A methylated RNAs are localized at HSATIII-based nSBs.
- HSATIII lncRNAs transcripts are the target substrates of m^{6}A machineries at nSBs.
- The m^{6}A consensus within HSATIII is the highly repetitive sequence GGAAU which is distinct from the global GGACU m^{6}A consensus.
- With exception of YTHDC1, all m^{6}A factors follow a consistent recruitment pattern at nSBs where they are gradually sequestrated during thermal stress (2h HS) and enriched at early recovery stage (1h post-stress) then rapidly released from nSBs at late recovery stage (4h post-stress)
- TDP43 is a possible modulator of m^{6}A methylation in nSBs.