論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Deubiquitinases USP5 and USP13 are recruited to and regulate heat-induced stress granules by deubiquitinating activities
著者(和文)	Xie Xuan
Author(English)	Xuan Xie
出典(和文)	学位:博士(理学), 学位授与機関:東京工業大学, 報告番号:甲第10909号, 授与年月日:2018年6月30日, 学位の種別:課程博士, 審査員:加藤 明,太田 啓之,岩﨑 博史,中村 信大,中戸川 仁
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第10909号, Conferred date:2018/6/30, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	生体システム　専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（　理学　）
学生氏名： Student's Name	XIE XUAN	指導教員（主）： Academic Supervisor(main)	駒田　雅之
		指導教員（副）： Academic Supervisor(sub)	加藤　明

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

[Introduction]

When cells are exposed to various stresses, including heat stress and oxidative stress, translation of most mRNAs is inhibited. Polysomes are then disassembled, and released mRNAs are associated with various RNA-binding proteins. These RNA-protein complexes cause the formation of stress granules (SGs). SGs are non-membranous cytoplasmic compartments, and contain non-translating RNAs and many RNA-binding proteins, as well as non-RNA-binding regulatory proteins. The function of SGs is proposed to modulate mRNA translation and stress responses. When stress is relieved, SGs are disassembled. However, the mechanisms of SG assembly and disassembly are largely unknown.

It has been reported that ubiquitin is present in SGs induced by some stressors. Here, I investigated regulatory mechanisms of ubiquitin chain levels in SGs, and roles of ubiquitin chains in SG assembly and disassembly. I found that ubiquitin-specific protease 5 (USP5) and USP13, two deubiquitinases (DUBs) with same domain structures, are recruited to heat-induced SGs. USP5 exhibits isopeptidase specificity toward unanchored ubiquitin chains, while USP13 exhibits isopeptidase activity toward ubiquitin chains conjugated to substrate proteins. I provided evidence suggesting that the stability of SGs is regulated by ubiquitin chains and their hydrolysis by these DUBs.

[Results]

1. USP5, USP13 and ubiquitin chains are localized to SGs

In HeLa cells, SGs can be formed by the incubation at 44°C for 1 h (heat stress). By immunofluorescence analysis, I found that a fraction of USP5 as well as USP13 co-localized with heat-induced SGs. SGs are also formed by other stressors including an oxidative agent arsenite, a proteasome inhibitor MG132 and a mitochondrial membrane-depolarizing agent carbonyl cyanide m-chlorophenyl hydrazine (CCCP). However, USP5 and USP13 did not co-localized with SGs induced by these stressors. These results indicated that USP5 and USP13 are preferentially recruited to SGs induced by heat stress. I also examined the localization of ubiquitin chains, and found that ubiquitin chains are also preferentially recruited to SGs induced by heat stress, not by other stressors. Super-resolution microscopy analysis demonstrated that small portion of USP5 and USP13 within heat-induced SGs co-localized with ubiquitin. It raised the possibility that these DUBs transiently associate with and hydrolyze ubiquitin chains in the SGs.

I also analyzed the mechanisms of the recruitment of USP5 and USP13 to heat-induced SGs. The experiments using a nuclear export inhibitor leptomycin B showed that a fraction of these DUBs in the SGs are translocated from the nucleus. Furthermore, I tried to determine the domain in these DUBs responsible for the recruitment to SGs. The experiments using USP13 deletion mutants showed that the USP domain itself has the ability to recruit USP13 to heat-induced SGs.

2. Depletion of USP5 or USP13 increases ubiquitin levels in SGs, accelerates SG assembly, and represses the disassembly

siRNA-mediated depletion of USP5 or USP13 increased the levels of ubiquitin in heat-induced SGs, suggesting that USP5 and USP13 hydrolyze the ubiquitin chains in heat-induced SGs.

I also evaluate the effects of the depletion of USP5 or USP13 on the assembly of heat-induced SGs. Depletion of USP5 or USP13 increased the percentage of cells with SGs after 45 min at 44°C. These results indicated the acceleration of SG assembly.

Both in control cells and knockdown cells, the percentage of cells with SGs was elevated to 90% or more after 1 h at 44°C. Cells were then returned to at 37°C (recovery incubation) to induce SG disassembly. In control cells, most of SGs disappeared after the recovery incubation for 1 h. Depletion of USP5 or USP13 increased percentage of cells with SGs at this time point. These results indicated the delay of SG disassembly.

3. Accumulation of unanchored ubiquitin chains represses disassembly of SGs

USP5, but not USP13, selectively hydrolyzes unanchored ubiquitin chains. I examined the effect of excess unanchored ubiquitin chains on the SG disassembly. Cells were transfected with plasmids encoding ubiquitin with a mutation in the C-terminal di-glycine motif (e.g., ubiquitinG75A/G76A). This mutant ubiquitin can form unanchored ubiquitin chains in cells. The cells were incubated at 44°C for 1 h, and then returned to 37°C. UbiquitinG75A/G76A overexpression increased the percentage of cells with SGs. It indicated that accumulation of unanchored ubiquitin chains represses disassembly of heat-induced SGs.

[Discussion]

Taken together with other data, this study suggests that:

- Heat-induced SGs contain ubiquitin chains much more than SGs induced by other stressors.
- USP5 and USP13 are preferentially recruited to heat-induced SGs, possibly through the interaction between their USP domain and ubiquitin chains in SGs.
- In SGs, USP5 hydrolyzes unanchored ubiquitin chains and USP13 hydrolyzes protein-conjugated ubiquitin chains. Both reactions are required for the efficient destabilization of SGs.

An abnormal formation of SGs is implicated in the pathogenesis of various diseases including neurodegenerative diseases. Thus, it is of not only cell biological but also medical importance to elucidate the precise molecular mechanisms of USP5 and USP13-mediated destabilization of SGs.