様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　元 年　 ６ 月 １７ 日現在

機関番号：１２１０２
研究種目：基盤研究(B)（一般）
研究期間：2016～2018
課題番号：１６Ｈ０４７４０

研究課題名（和文）RNA干渉におけるRISC形成への核内転写因子の関与

研究課題名（英文）Involvement of nuclear transcription factor in RISC formation in RNA interference

研究代表者
Ｌｉｕ　Ｑｉｎｇｈｕａ（Liu, Qinghua）

筑波大学・国際統合睡眠医科学研究機構・教授

研究者番号：９０７２３７９２

交付決定額（研究期間全体）：（直接経費）　　13,200,000円

研究成果の概要（和文）：RISCはRNAiにおいて中核的役割を果たすが、RISC形成の作用機序や活性制御の仕組みは未だ不明な点が残る。我々は近年、RLC（RISC-loading complex）の新規構成因子として、TAF11を同定し、TAF11がD2 ボディと呼ばれる細胞質内顆粒体で基地RLC因子のDicer-2-R2D2複合体と共存することを示した。本研究では、RLCの構造をクライオEMを用いて解析した。現在までのところ、高解像度のデータを取得できてはいないが、Dicer-2-R2D2がモノマーやダイマーの状態で存在すること、TAF11の結合によって、RLCのコンフォメーションが変化することが明らかになった。

研究成果の学術的意義や社会的意義
RNAi（RNA干渉）は遺伝子の発現制御機構のひとつであり、生命現象において非常に重要な役割を担っている。RISCはRNAiにおいて中核的役割を果たすが、RISC形成の作用機序や活性制御の仕組みは未だ不明な点を多く残す。単粒子解析クライオ電子顕微鏡は、原子分解能で構造を解明するために使用する手法である。2017年には、クライオ電子顕微鏡の開発に貢献した研究者がノーベル化学賞を受賞した。我々はこの最先端技術である単粒子解析クライオ電子顕微鏡を用いて、RISC形成において重要な役割を果たす複合体の構造解析を試みた。その結果、不完全ではあるが、RISC形成に関わる複合体の構造解析に成功した。

研究成果の概要（英文）：Recent technical advances in single particle cryo-EM data acquisition, classification, and reconstruction have caused the “EM resolution revolution” within the last few years. Thus, we have tried to reconstruct the three dimensional structures of the Drosophila RLC complex using cryo-EM. To date, we have obtained low resolution EM structure of Dicer-2-R2D2, Dicer-2-R2D2 complex with siRNA, and Dicer-2-R2D2 and TAF11 in complex with siRNA (RLC). Comparison of the EM maps reveals that Dicer-2-R2D2 complex exists in monomeric and dimeric states. The conformation of the RLC is clearly different from that of Dicer-2-R2D2-siRNA complex, suggesting that TAF11 binding results in significant conformational changes of the RLC, which may be critical for the RISC loading activity.

研究分野：生物学

キーワード：RISC　RNAi　Cryo-EM　miRNA

１．研究開始当初の背景

RISC is the catalytic engine of RNAi. RNA interference (RNAi) is a conserved dsRNA-induced gene-silencing mechanism in eukaryotes. First, RNase III Dicer initiates RNAi by processing long dsRNA into small interfering RNA (siRNA). Second, nascent siRNA duplex is assembled into the effector RNA-induced silencing complex (RISC). In an active RISC, single-stranded siRNA directs Ago2 endoRNase to catalyze sequence-specific cleavage of complementary mRNA.

1. **RISC loading.** We previously identified that Drosophila RISC loading complex (RLC) contains the Dicer-2 (Dcr-2)-R2D2 complex and coordinately recruits duplex siRNA to Ago2 to form an inactive pre-RISC (Liu et al., Science 2003; Pham et al., Cell 2004; Tomari et al. Cell 2004).

2. **RISC activation** is the conversion of pre-RISC (Ago2/duplex siRNA) into active RISC (Ago2/guide strand). First, Ago2 nicks the siRNA passenger strand into a 9-nt and a 12-nt fragment. Second, we showed that C3PO, a novel RNase and a complex of Translin and Trax, degrades the passenger fragments to activate RISC (Liu et al., Science 2009).

TAF11 assembles the RISC loading complex (RLC). We identified a novel function of TAF11, an annotated nuclear transcription factor, as the key missing factor of Drosophila RLC by forward genetic screen (Liang et al.,Mol. Cell 2015). By native agarose siRNA gel-shift assay, we showed that assembly of the RLC and RISC, but not the RDI -/- (R2D2-Dcr-2-initiator complex), was defective in taf11 mutant ovary extract, and that this defect could be fully rescued by addition of recombinant TAF11.

TAF11 enhances RNAi efficiency. We showed that Dcr-2-R2D2 complex exhibited a sigmoidal siRNA-binding curve, whereas TAF11 enhances siRNA affinity of Dcr-2-R2D2 by ten-fold, and converts it into a hyperbolic siRNA-binding curve (Liang et al., Mol. Cell 2015). We hypothesize that at low concentration, Dcr-2-R2D2 complex exists as heterodimer with low affinity for siRNA. At high concentration, Dcr-2-R2D2 complex dimerizes to form heterotetramer with higher affinity for siRNA. TAF11 facilitates the formation of Dcr-2-R2D2 heterotetramer, such that the higher order complex binds siRNA as one entity with ten-fold higher affinity. TAF11 also enhances the RISC loading activity of Dcr-2-R2D2 complex (Liang et al., Mol Cell 2015).

TAF11 co-localizes with Dcr-2/R2D2 in D2 bodies. We collaborated with Dr. Mikiko Siomi (University of Tokyo) to show that GFP-TAF11 co-localized with endogenous Dcr-2 or R2D2 proteins in distinct cytoplasmic foci called D2 bodies in Drosophila S2 cells (Liang et al., Mol Cell 2015). We observed three different localization patterns of GFP-TAF11: nucleus, D2 body, or both, suggesting a dynamic shuttling of TAF11 proteins between the nucleus and cytoplasmic D2 bodies. Furthermore, GFP-TAF11 could not form any cytoplasmic foci following RNAi depletion of Dcr-2 (data not shown), suggesting that TAF11 localizes to cytoplasmic D2 bodies through its interaction with Dcr-2-R2D2 complex. These genetic, biochemical, and cell biological studies allow us to hypothesize that the TAF11-positive D2 bodies represent in vivo hotspots for dynamic RLC and RISC assembly.

２．研究の目的

RISC（RNA-induced silencing complex）は RNAi において中核的役割を果たすが、RISC 形成の作用機序や活性制御の仕組みは未だ不明な点を多く残す。我々は近年、遺伝学的スクリーニングを行い、RISC 形成の初期反応で重要な役割を担う複合体、RLC（RISC-loading complex）の新規構成因子として、TATA-binding protein associated factor 11（TAF11）を同定し、TAF11 が D2 ボディと呼ばれる細胞質内顆粒体で基地 RLC 因子の Dicer-2-R2D2 複合体と共存することを示した。本研究では、遺伝学的、生化学的、および細胞生物学的アプローチにより、TAF11 の活性型 RISC 形成メカニズムへの機能的関与の詳細を、分子レベルで解明することを目指す。

３．研究の方法

The detail experimental procedures for miRNA experiments are described in our published paper (Liu et al., 2016, Mol Cell). About the Cryo-EM, we haven't published yet (Liu et al., unpublished).

４．研究成果

Recent technical advances in single particle cryo-EM data acquisition, classification, and reconstruction have caused the "EM resolution revolution" within the last few years. Thus, we have been collaborating with Dr. Qiu-xing Jiang at UT Southwestern to reconstruct the three dimensional structures of the Drosophila RLC complex using cryo-EM. To date, we have obtained low resolution EM structure of Dicer-2-R2D2, Dicer-2-R2D2 complex with siRNA, and Dicer-2-R2D2 and TAF11 in complex with siRNA (RLC). Comparison of the EM maps reveals that Dicer-2-R2D2 complex exists in monomeric and dimeric states. The conformation of the RLC is clearly different from that of Dicer-2-R2D2-siRNA complex, suggesting that TAF11 binding results in significant conformational changes of the RLC, which may be critical for the RISC loading activity. However, we encountered the difficulty of protein aggregation and conformation heterogeneity during the cryo-EM reconstruction. We are making good progress to optimize the condition to achieve high-resolution EM reconstructions of Dicer-2-R2D2-siRNA and Dicer-2-R2D2-TAF11-siRNA complexes by obtaining more homogeneous samples, increasing the size of EM dataset, and reducing conformation heterogeneity by sorting EM images (unpublished).

Not only RNAi, but we also studied microRNA. We purified histone H1 as a primary microRNA (pri-miRNA)-binding protein from nuclear extracts of Drosophila S2 cells by biochemical fractionation. We showed that knockdown of H1, but not other histones, specifically reduced expression of mature miRNAs, but did not affect the levels of pri-miRNAs. Furthermore, we identified HP1BP3, one of 13 human H1-like chromatin proteins, as a pri-miRNA binding protein that specifically associates with the Microprocessor (Drosha-DGCR8 complex). Knockdown of HP1BP3, but not other H1 variants, compromised pri-miRNA processing by resulting in the premature release of pri-miRNA transcripts from the chromatin. Chromatin immunoprecipitation (ChIP) studies reveal genome-wide colocalization of HP1BP3 & Drosha and HP1BP3-dependent Drosha binding to actively transcribed miRNA loci. HP1BP3 exhibits a novel pri-miRNA binding activity and promotes the Drosha-pri-miRNA association in vivo. Together, these studies suggest that HP1BP3 promotes co-transcriptional miRNA processing through chromatin retention of nascent pri-miRNA transcripts (Liu et al. Molecular Cell, 2016).

５．主な発表論文等

〔雑誌論文〕（計　5 件）

(1) Z. Wang, J. Ma, C. Miyoshi, Y. Li, M Sato, Y. Ogawa, T. Lou, C. Ma, X. Gao, C. Lee, T. Fujiyama, X. Yang, S. Zhou, N. Hotta-Hirashima, D. Klewe-Nebenus, A. Ikkyu, M. Kakizaki, S. Kanno, L. Cao, S. Takahashi, J. Peng, Y. Yu, H. Funato, M. Yanagisawa, Q. Liu. Quantitative phosphoproteomic analysis of the molecular substrates of sleep need. *Nature*, 558:435-439 (2018) 査読有り

(2) Y. Wang, L. Cao, C-Y. Lee, T. Matsuo, K. Wu, G. Asher, L. Tang, T. Saitoh, J. Russell, D. Klewe-Nebenius, L. Wang, S. Soya, E. Hasegawa, Y. Chérasse, J. Zhou, Y. Li, T. Wang, X. Zhan, C. Miyoshi, Y. Irukayama, J. Cao, JP Meeks, L. Gautron, Z. Wang, K. Sakurai, H. Funato, T. Sakurai, M. Yanagisawa, H. Nagase, R. Kobayakawa, K. Kobayakawa, B. Beutler, Q. Liu. Large-scale forward genetics screening identifies Trpa1 as a chemosensor for predator odor-evoked innate fear

behaviors. *Nature Communications,* 9:2041 (2018) 査読有り

(3) K.L. Egerod, N. Petersen, P.N. Timshel, J.C. Rekling, Y. Wang, Liu Q, T.W. Schwartz, L. Gautron. Profiling of G protein-coupled receptors in vagal afferents reveals novel gut-to-brain sensing mechanisms. *Molecular Metabolism* 12:62-75 (2018). 査読有り

(4) H. Funato, et al., Q. Liu, K. Kume, S. Wakana, J.S. Takahashi, M. Yanagiaswa. Forward- genetics analysis of sleep in randomly mutagenized mice. *Nature* (Article), 539:378-383. (2016) 査読有り

(5) H. Liu, C. Liang, R.K. Kollipara, M. Matsui, X. Ke, B-C. Jeong, Z. Wang, K.S. Yoo, G.P. Yadav, L.N. Kinch, N.V. Grishin, Y. Nam, D.R. Corey, R. Kittler, Q.Liu. HP1BP3, a chromatin retention factor for co-transcriptional microRNA processing. Molecular Cell 63:420-432. PMCID4975613 (2016) 査読有り

〔学会発表〕（計　8 件）

1. Liu Q. "Quantitative phosphoproteomic analysis of molecular substrates of sleep need" The 9th Congress of Asian Sleep Research Society, Sapporo, Japan. 2018 (July 9-12) (Talk)
2. Liu Q. "Quantitative phosphoproteomic analysis of molecular substrates of sleep need" Sapporo Symposium on Biological Rhythm, Sapporo, Japan. 2018 (July 13-15) (Poster)
3. Liu Q. "TRPA1 is a novel chemosensor for predator odor-evoked innate fear behaviors" The 41th Japan Neuroscience Society annual meeting, Kobe, Japan. 2018 (July 26-29) (Talk)
4. Liu Q. "Molecular substrates of homeostatic sleep regulation" Hot Topic Symposium, Congress of European Sleep Research Society, Basel, Switzerland. 2018 (Sept 25-28) (Talk)
5. Liu Q. "Cumulative Phosphorylation of SNIPPs as a Function of Sleep Need" Gordon Research Conference on Sleep Regulation and Function, Galveston, USA. 2018 (March 18-23) (Talk)
6. Liu Q. "Quantitative phosphoproteomic analysis of molecular substrates of sleep need" 40th Annual meeting of the Japan Neuroscience Society, Chiba, Japan. 2017 (July 20-23) (Talk)
7. Liu Q. "Quantitative phosphoproteomic analysis of molecular substrates of sleep need" Asian Chronobiology Forum and 2nd Biennial conference of Chinese Society of Biological Rhythms, Hohhot, China 2017 (June 25-28). (Talk)
8. Liu Q. "Quantitative phosphoproteomic analysis of homeostatic sleep need" Cold Spring Harbor Asia meeting Francis Crick Frontier in Neuroscience, Suzhou, China. 2017 (May 8-12) (Talk)

〔図書〕（計　0 件）

〔産業財産権〕
○出願状況（計　0 件）

名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

○取得状況（計　0 件）

名称：
発明者：
権利者：
種類：

番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

(1)研究分担者
研究分担者氏名：

ローマ字氏名：

所属研究機関名：

部局名：

職名：
研究者番号（8 桁）：

(2)研究協力者
研究協力者氏名：
ローマ字氏名：

※科研費による研究は、研究者の自覚と責任において実施するものです。そのため、研究の実施や研究成果の公表等については、国の要請等に基づくものではなく、その研究成果に関する見解や責任は、研究者個人に帰属されます。