RNA Systems Biochemistry Laboratory (2024)
Chief Scientist: Shintaro Iwasaki (Ph.D.)

(0) Research fields

PRI Subcommittee**:** Biology

Keywords:

Translation, RNA, translation inhibitor, RNA binding protein, next-generation sequencing

(1) Long-term goal of laboratory and research background

One of the most fundamental principles of biology is the "central dogma of molecular biology," which states that DNA is transcribed into RNA and RNA is translated into protein. Recent studies have revealed that RNA abundance does not simply correlate with protein abundance; instead, extensive regulation at the level of translation precisely controls the final amount of protein produced.

In our laboratory, we combine comprehensive analyses using next-generation sequencing with classical biochemical approaches to pursue a detailed understanding of translation, a core principle of biology. In particular, ribosome profiling is a powerful technique for globally measuring translation within cells. Building on this method, we aim to apply it broadly across biological systems and to elucidate the diverse translational regulatory mechanisms that underlie a wide range of biological phenomena.

(2) Current research activities (FY2024) and plan

Discovery of an mRNA sequence–specific translational inhibitor

The use of anticancer drugs is one of the major strategies for cancer treatment; however, severe side effects remain a significant concern. DMDA-PatA has been reported to exhibit high toxicity toward certain cancer cells while showing low toxicity in normal cells, suggesting that its cell-type–specific toxicity may offer a way to avoid the severe side effects associated with conventional anticancer drugs. Although DMDA-PatA has been reported to function as a translational inhibitor, its molecular mechanism of action remained unclear. We discovered that DMDA-PatA induces tight binding of the translation-related proteins eIF4A and DDX3 to specific GNG motifs on mRNAs, thereby creating steric obstacles that block the progression of scanning ribosomes searching for translation start sites. Through this mechanism, DMDA-PatA selectively represses the translation of specific mRNAs. This work was published in *Nature Communications* (Saito *et al. Nat Commun* 2024).

Mechanism of translational repression mediated by a translation-promoting factor

The translation initiation factor eIF4A has long been understood as a translation-promoting factor; however, its full range of functions has remained unclear. Using ribosome profiling, mass spectrometry, and related approaches, we discovered that eIF4A1—one of the eIF4A family members—binds strongly to TOP mRNAs via the RNA-binding protein LARP1. LARP1 is required for robust repression of TOP mRNA translation under nutrient-starved conditions, and we found that eIF4A1 enhances translational repression of TOP mRNAs by strengthening the interaction between LARP1 and TOP mRNAs. These findings were published in *Nature Structural & Molecular Biology* (Shichino *et al. Nat Struct Mol Biol* 2024).

A novel method for comprehensive kinetic analysis of translation

While ribosome profiling enables the calculation of *relative* translation levels, it does not reveal how many ribosomes are bound to a given mRNA molecule at any given time. Moreover, it cannot comprehensively measure the rate at which ribosomes are recruited to mRNAs (the translation initiation rate). To address these limitations, we developed the Ribo-Calibration method. Using this approach, we were able to discuss translation kinetics across the entire transcriptome, revealing that translation initiation occurs approximately once every 22 seconds, elongation proceeds at a rate of 4.1 seconds per codon, and each mRNA molecule is utilized about 1,800 times before degradation. These findings were published in *Nature Communications* (Tomuro, Mito *et al. Nat Commun* 2024).

Future direction

Ribosome profiling is a powerful method for understanding translational regulation. At the same time, however, it presents numerous technical challenges, including throughput, subcellular localization, and data analysis. To overcome these issues and trigger breakthroughs, we are actively engaged in the development of new technologies. Through these efforts, we aim to uncover novel biological phenomena

that could not be captured using pre-existing methods.

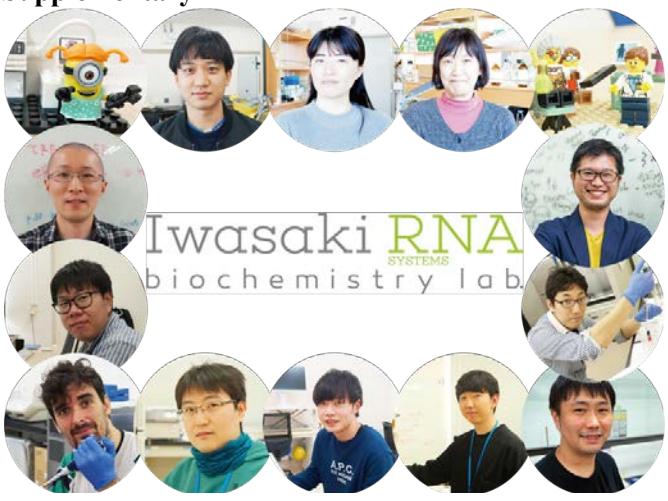
(3) Members as of March, 2024

(Chief Scientist)
Shintaro Iwasaki
(Research Scientist / tenured)
Yuichi Shichino
Tatsuaki Kurata
(RIKEN Special Postdoctoral Researcher)
Naohiro Kawamoto
(JSPS PD Researcher)
Hirotaka Toh
(Postdoctoral Researcher)
Akira Yamashita
(Visiting Researcher)
Elie Marcel Teyssonniere
(Technical Staff I)
Mari Mito
(Junior Research Associate)
Kotaro Tomuro
(Research Associate)
Taisei Wakigawa
(Student Trainee)
Yuma Tsukada
Margo Le Creff-Le Balc'h
(Assistant)
Miho Tsunashima
(Special Temporary Employee)
Rie Yokoyama

(4) Representative research achievements (#: equal contribution, *: correspondence)

1. Saito H, Handa Y, Chen M, Schneider-Poetsch T, Shichino Y, Takahashi M, Romo D, Yoshida M, Fürstner A, Ito T, Fukuzawa K, and **Iwasaki S***. DMDA-PatA mediates RNA sequence-selective translation repression by anchoring eIF4A and DDX3 to GNG motifs. *Nat Commun.* 15(1):7418 (2024) DOI: 10.1038/s41467-024-51635-9
2. Tomuro K#, Mito M#, Toh H, Kawamoto N, Miyake T, Chow SYA, Doi M, Ikeuchi Y, Shichino Y*, and **Iwasaki S***. Calibrated ribosome profiling assesses the dynamics of ribosomal flux on transcripts. *Nat Commun.* 15(1):7061 (2024) DOI: 10.1038/s41467-024-51258-0
3. Shichino Y*, Yamaguchi T, Kashiwagi K, Mito M, Takahashi M, Ito T, Ingolia NT, Kuba K, and **Iwasaki S***. eIF4A1 enhances LARP1-mediated translational repression during mTORC1 inhibition. *Nat Struct Mol Biol.* 31(10):1557-1566 (2024) DOI: 10.1038/s41594-024-01321-7
4. Fukuchi K#, Nakashima Y#, Abe N#*, Kimura S, Hashiya F, Shichino Y, Liu Y, Ogisu R, Sugiyama S, Kawaguchi D, Inagaki M, Meng Z, Kajihara S, Tada M, Uchida S, Li TT, Maity R, Kawasaki T, Kimura Y, **Iwasaki S**, and Abe H*. Internal cap-initiated translation provides efficient protein production from circular mRNA. *Nat Biotechnol.* XXX (2025) DOI: 10.1038/s41587-025-02561-8, Publisher Correction (2025) DOI: 10.1038/s41587-025-02758-x
5. Kaneko S, Miyoshi K, Tomuro K, Terauchi M, Tanaka R, Kondo S, Tani N, Ishiguro K, Toyoda A, Kamikouchi A, Noguchi H, **Iwasaki S**, and Saito K*. Mettl1-dependent m^7G tRNA modification is essential for maintaining spermatogenesis and fertility in *Drosophila melanogaster*. *Nat Commun.* 15(1):8147 (2024) DOI: 10.1038/s41467-024-52389-0

Supplementary



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