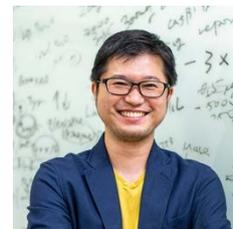


**RNA Systems Biochemistry Laboratory**  
**Chief Scientist: Shintaro Iwasaki (Ph.D.)**



**(0) Research field**

CPR Subcommittee: Biology

**Keywords:**

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

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

“The central dogma of molecular biology”, which represents information flow from DNA to RNA to protein, has been a most basic principle in life. Recent quantitative and comprehensive analysis revealed that the amount of RNA could not simply correlate with protein abundance in cells, suggesting that “translation control” significantly contributes to gene expression more generally than we previously expected. Our laboratory tackles to unveil the unknown mechanisms of translation control, through the combination of next-generation deep sequencing and classical biochemistry. Especially we harness a technique called ribosome profiling, which enables us to measure cellular translation status in a genome-wide manner. Applying this technology to a variety of living organisms, we aim to reveal diverse biological phenomena controlled by protein synthesis regulations.

**(2) Current research activities (FY2023) and plan (until Mar. 2025)**

**Boric acid-mediated translational control**

Plants sense cytoplasmic boron concentrations and alter the synthesis of certain proteins in order to increase the accumulation of transporters for the absorption of boron. The borate transporter *NIP5;1*, which is responsible for the absorption of boron from soil, is a key player. The translation of *NIP5;1* is tightly regulated by the borate concentration through the minimal uORF consisting only of a start and end codons (AUG-UAA, hereafter referred to as AUG-stop) in the 5' UTR. However, the molecular mechanism of how the increased cytoplasmic boron concentration affects the translation process was not clear.

To understand the translational regulation on *NIP5;1* minimal uORF, we applied ribosome profiling (or 80S footprinting) and TCP-Seq (or 40S footprinting) to the reporter mRNA translated in an *in vitro* translation system. These analyses unveiled that borate stalls 80S ribosomes on the AUG-stop, reducing the ribosome supply to the downstream main ORF. On the other hand, under limited borate, 80S ribosomes assembled on the AUG-stop could slide downstream and then reinitiate the translation from the start codon from main ORF. Our results provided the first example of the functional involvement of 80S sliding in the regulatory mechanism of expression. We also solved the cryo-EM structure of stalled 80S on the AUG-stop. This work was a wonderful collaboration with Ito lab from RIKEN BDR and was published in Nature Chemical Biology (Tanaka, Yokoyama, Saito *et al. Nat Chem Biol* 2024)

**Development of a method for translational knockdown**

A system involved in bacterial immunity called CRISPR-Cas is currently the focus of much attention. Recently, a protein called Cas13 was discovered as part of this system: Cas13 is an enzyme that, when bound to a short RNA called guide RNA, specifically recognizes and cleaves RNA complementary to that sequence. Therefore, Cas13 was expected to have a more specific knockdown effect than RNA interference. However, once Cas13 binds to a target RNA, it nonspecifically cleaves nearby RNAs, making it difficult to perform specific gene knockdown using Cas13.

Here, taking advantage of the high specificity of Cas13 in RNA binding, we have developed a new method, CRISPR $\delta$  (CRISPR delta, delta stands for DEpLetion of Translation by blockAde), which causes translation inhibition rather than RNA degradation. A Cas13 mutant (dead Cas13 or dCas13), which has lost its RNA-cleaving activity, is tightly bound in the middle of mRNA, interferes with the access of scanning ribosome to the start codon, and inhibits protein synthesis from the target mRNA. Strikingly, genome-wide ribosome profiling showed that CRISPR $\delta$  is highly mRNA-specific. This work was published in Nature Communications (Apostolopoulos *et al. Nat Commun* 2024).

**Future direction**

Ribosome profiling has been a powerful technique for understanding translational regulations. However, simultaneously, we noticed the technical hurdles in this method, such as in sensitivity, resolution, throughput, and data analysis. We are actively working on breaking through these analytical poses.

### (3) Members

as of March, 2024

#### (Chief Scientist)

Shintaro Iwasaki

#### (Research Scientist / tenured)

Yuichi Shichino

#### (JSPS PD Researcher)

Hiroataka Toh

Naohiro Kawamoto

#### (Visiting Researcher)

Peixun Han

#### (Technical Staff I)

Mari Mito

#### (Junior Research Associate)

Hironori Saito

#### (RIKEN Student Researcher M)

Kotaro Tomuro

#### (Student Trainee)

Taisei Wakigawa

Yuma Tsukada

#### (Special Temporary Employee)

Rie Yokoyama

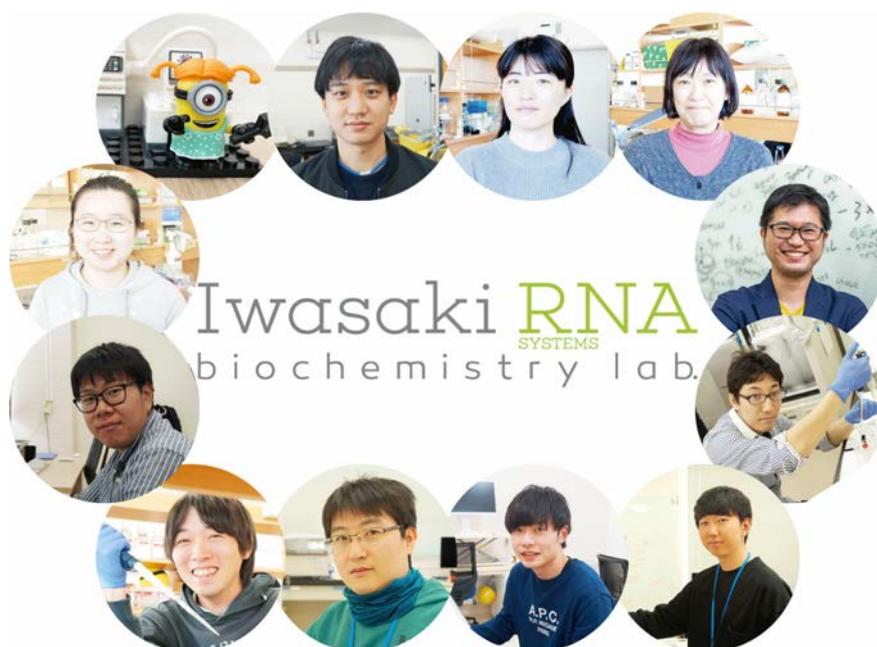
#### (Administrative Part-time Worker I)

Miho Tsunashima

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

1. Apostolopoulos A, Kawamoto N, Chow SYA, Tsuji H, Ikeuchi Y, Shichino Y\*, and **Iwasaki S\***. dCas13-mediated translational repression for accurate gene silencing in mammalian cells. *Nat Commun.* 15(1):2205 (2024) DOI: 10.1038/s41467-024-46412-7
2. Tanaka M#, Yokoyama T#, Saito H#, Nishimoto M, Tsuda K, Sotta N, Shigematsu H, Shirouzu M, **Iwasaki S\***, Ito T\*, and Fujiwara T\*. Boric acid intercepts 80S ribosome migration from AUG-stop by stabilizing eRF1. *Nat Chem Biol.* (2024) DOI: 10.1038/s41589-023-01513-0
3. Teyssonniere EM#, Shichino Y#, Mito M, Friedrich A, **Iwasaki S\***, and Schacherer J\*. Translation variation across genetic backgrounds reveals a post-transcriptional buffering signature in yeast. *Nucleic Acids Res.* 52(5):2434-2445 (2024) DOI: 10.1093/nar/gkae030
4. Zhao X#, Ma D#, Ishiguro K#, Saito H, Akichika S, Matsuzawa I, Mito M, Irie T, Ishibashi K, Wakabayashi K, Sakaguchi Y, Yokoyama T, Mishima Y, Shirouzu M, **Iwasaki S**, Suzuki Ta\*, and Suzuki Ts\*. Glycosylated queuosines in tRNAs optimize translational rate and post-embryonic growth. *Cell.* 186(25):5517-5535.e24 (2023) DOI: 10.1016/j.cell.2023.10.026
5. Nagao A\*, Nakanishi Y, Yamaguchi Y, Mishina Y, Karoji M, Toya T, Fujita T, **Iwasaki S**, Miyauchi K, Sakaguchi Y, and Suzuki T\*. Quality control of protein synthesis in the early elongation stage. *Nat Commun.* 14(1):2704 (2023) DOI: 10.1038/s41467-023-38077-5

### Supplementary



### Laboratory Homepage

[https://www.riken.jp/en/research/labs/chief/rna\\_sys\\_biochem/index.html](https://www.riken.jp/en/research/labs/chief/rna_sys_biochem/index.html)

<http://iwasakirna.com/>