Cellular Dynamics Laboratory
Chief Scientist: Naoko Imamoto (Ph.D.)

(0) Research field

CPR Subcommittee**:** Biology

Keywords:
nucleocytoplasmic transport, importin, Hikeshi, nuclear pore complex, cellular stress

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

The prime feature of eukaryotic cells is the separation of the intracellular space into two compartments, the nucleus and the cytoplasm. Active nuclear transport is crucial for the maintenance of this separation. Our long-term goal is to understand the physiological relevance of nucleocytoplasmic transport at the molecular level. There are no questions arguing the importance of nucleocytoplasmic transport in the regulation of various cellular functions, as it is a key step in regulating gene expression. The molecular mechanisms of nucleocytoplasmic transport have been described in detail; however, some important questions remain unsolved, hindering our understanding of how nucleocytoplasmic transport engages in distinct cellular processes at the molecular level. We focus on the diversity of transport pathways and hope to understand and highlight the importance of nucleocytoplasmic transport in biological areas.

(2) Current research activities (FY2021) and plan (until Mar. 2024)

(A) Methods to separate nuclear soluble fractions reflecting localizations in living cells

To date, many studies on nuclear protein functions have been conducted using nuclear fractionation, because to understand various intranuclear functions, it is important to know when, what, and how proteins enter the nucleus. Although many methods and commercial kits for nuclear fractionation have been developed, there are still no methods for obtaining a complete nuclear proteome, because soluble nuclear proteins are often lost during fractionation. We developed a new method for separating the nuclear soluble fraction from other cytoplasmic fractions much more precisely than those reported previously, overcoming the above problems. First, we optimized the conditions, including the treatment temperature for the selective permeabilization of the plasma membranes using digitonin (Fig1). Second, we identified an inhibitor WGA (wheat germ agglutinin) that prevented leakage of small proteins from the nucleus. Therefore, supplementation of WGA during the cell permeabilization of digitonin, the loss of soluble and small proteins from the nuclear fractions was remarkably minimized compared to conventional methods. Our method allow 10 million cells to be separated into the cytoplasmic and nuclear soluble fractions more precisely in a 1.5-mL test tube. By using sample preparation for the isobaric tags for relative and absolute quantitation (iTRAQ)-based proteome, we showed that WGA supplementation significantly contributed to the preservation of small nuclear proteins in the nucleus of cells with permeabilized plasma membranes (Fig2). We also compared our method with commercially available kit, and showed the advantages of our methods. Because of the simple protocols and easy application for multiple samples, our methods are expected to be applied to various studies on spatiotemporal changes of dynamic nuclear proteins, such as signal transduction.

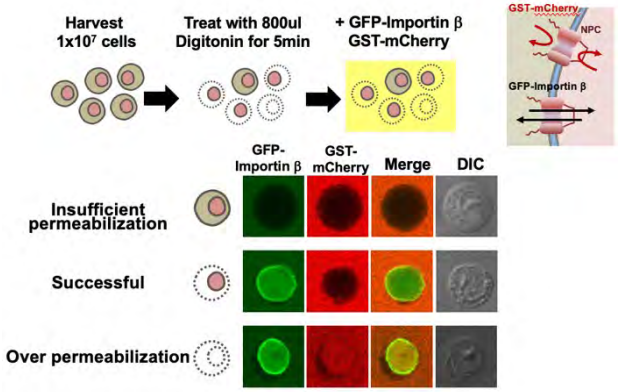

Fig.1 Illustration of the digitonin treatment procedure(upper), and for optimization, cells treated with digitonin were classified as insufficient, successfully or over permeabilized cells.

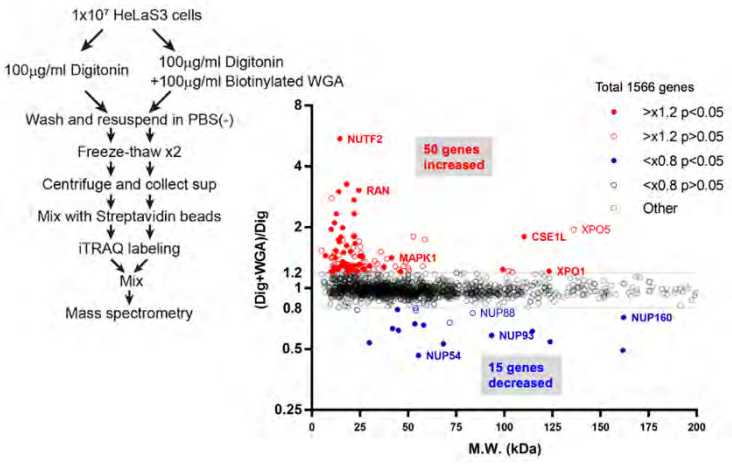

Fig.2 A flowchart of sample preparation for the isobaric tags for relative and absolute quantitation(iTRAQ)-based proteome （left）. Differences of abundances relative to molecular weights plotted (right). Red and blue points proteins expressions increased or decreased by 20% or more, respectively.

Plan We hope to use presently developed fractionation methods for soluble proteome to reveal physiological processes that is associated with nucleocytoplasmic transport, such as cellular differentiation and aging. For this, we are currently trying to reveal nuclear transport pathways that are differentially regulated during cellular differentiation and aging by using several model cellular systems, and our developed methods should contribute to identification of the affected cargoes.

(B)Investigation of the role of the Hikeshi pathway

We identified a protein named Hikeshi, that mediates the nuclear import of the molecular chaperone Hsp70 under stress conditions. Hikeshi is an evolutionarily conserved protein that exist in most of eukaryotic cells. We noticed that dysfunction of Hikeshi influences various biological events, both at cellular level and organism level, in mouse and human. However, function of Hikeshi has not been analyzed in any of the organisms. Although our lab mainly study function of Hikeshi using human cells, we planned to identify synthetic lethal genes with Hikeshi in yeast to get comprehensive information. For this, we used high-throughput genetic interaction mapping on the fission yeast Schizosaccharomyces pombe (PEM system: Roguev 他、Cold Spring Harb. Protoc. 2018）.

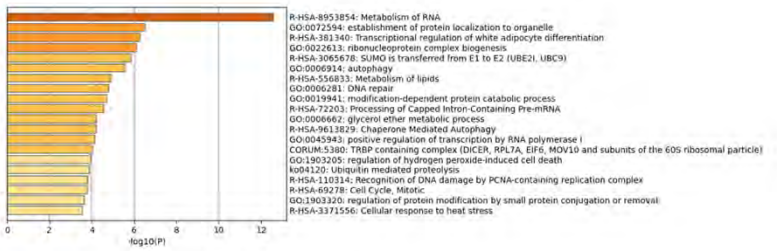

Fig.3 GO analysis of 238 synthetic lethal genes identified (Metascape)

We obtained two Hikeshi knockout haploid strains and mated with approximately 4000 non-essential haploid genes. According to drug selections mentioned in the protocol, we first picked-up 334 candidate genes. After confirming disrupted gene of library and reproducibility of our screening results, we finally identified 238 synthetic lethal genes of Hikeshi. We confirmed that many of these genes have human orthologue. GO analysis revealed that these genes are enriched with RNA metabolite-related genes (Fig3).

Plan We hope to analyze the identified synthetic lethal genes in human cells. We will finalize the analysis of regulatory mechanis of HSF1 function mediated by nuclear Hsp70. We hope to get into the mechanism that switches on the Hikeshi nuclear transport, and how Hikeshi dysfunction cause human disease.

(3) Members as of March, 2022

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(Senior research scientist)
Shingo Kose, Masatoshi Takagi, Makoto Kimura, Takeshi Mizuno

(Research scientist)
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(Technical Staff)
Ai Watanabe, Hisae Yoneno, Yoshiko Hosono

(Student Trainee)
Sakie Yoshioka

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(4) Representative research achievements

1. Kimura M, Imai K, Morinaka Y., Hosono-Sakuma Y., Horton P., Imamoto N. “Mutations in importin-β family nucleocytoplasmic transport receptors transpotin-SR and importin-13 differentially affect binding to respective cargoes.” ***Sci. Rep.*** 11, 15649, 2021.
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4. Mizuno T., Hirabayashi K., Miyazawa S., Kobayashi Y., Shoji K., Kobayashi M., Hanaoka F., Imamoto N., Torigoe H. “The Intrinsically disordered N-terminal region of mouse DNA polymerase alpha mediates its interaction with POT1a/b at telomeres” ***Genes Cells*** 26, 360-380, 2021.

Laboratory Homepage
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