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 (FY2020) and plan (until Mar. 2025)

(A) Characterization of importin pathways in human cells

The structures of nuclear transport machinery, such as the nuclear pore complex (NPC), are well understood, while complex cellular components that use this machinery are much less understood. We recently developed an experimental system to comprehensively identify cargoes for individual nuclear transport receptors (NTRs) and showed that cargoes of the same receptor are functionally related to one another, and that the predominant protein group in the cargo cohort differs among the receptors, indicating that each NTRs is linked to distinct biological processes by their cargoes. At the same time, we realized that each NTRs can be expected to carry two-three hundred different cargoes that do not share common consensus sequences. In FY 2020, like 2019, we aim to approach open issue of what determines NTR-cargo specificity. Addressing this issue, we generated mutants of transportin- (Trn)SR, of which many cargoes lack a consensus NLS, and mutants of Imp13, where no consensus NLS has been defined, based on crystal structures and evolutionary trace analysis (Fig 1). We analyzed their binding to as many as 40 cargo candidates that we previously identified by a nuclear import reaction-based method. The cargoes bind differently to the NTR mutants, suggesting that positions on an NTR contribute differently to the binding of respective cargoes (under revision).

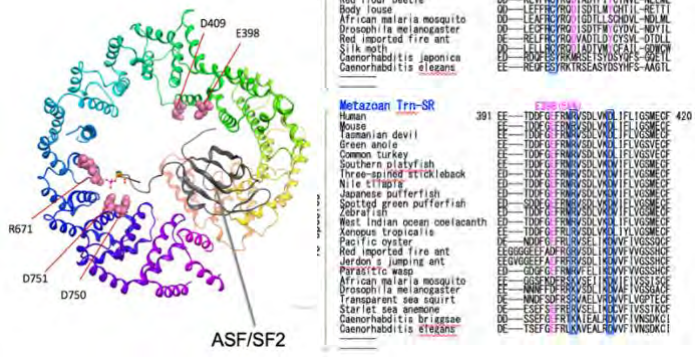

Fig.1 Cargo contact sites. Right: Residues conserved across species within either TrnSR or Imp13 orthologs but not between them were identified via an evolutionary trace analysis of about 70 metazoan TrnSR and Imp13 sequences. Left: residues exposed on the concave surface facing the cavity of the TrnSR or Imp13 structure were considered candidate sites for mutational analysis.

Plan We hope to characterize examples of large fraction of the soluble proteome that can be subject to nucleocytoplasmic transport associated with different physiological processes, such as cellular differentiation and aging. For this, we will reveal nuclear transport pathways that are differentially regulated during cellular differentiation and aging by using several model cellular systems, and analyze how the affected cargoes contribute to these physiological processes.

(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 does not belong to the importin β family. We noticed that dysfunction of Hikeshi influence various biological events, both at cellular level and organism level. At cellular level, Hikeshi knockout disrupt normal heat stress response, at organism levels, Hikeshi knockout mice are lethal and a missense mutation in the human Hikeshi gene is linked to human genetic disease. To get hint on why Hikeshi dysfunction cause these various effects, we examined gene expression profile of Hikeshi knockout cells using RNAseq. This analysis showed that Hikeshi knockout upregulates activities many of HSF1 target genes (Fig2). HSF1 is a master transcription factor regulating protein homeostasis under normal condition. When we carefully reviewed subcellular localization of Hsp70 in cells under normal condition, we noticed, Hikeshi mediates nuclear import of Hsp70, not only at stress condition but also at normal condition. When cells were transfected with basic conventional nuclear localization tagged Hsp70, expressions of HSF1 target genes that were upregulated in Hikeshi knockout cells, were strongly down regulated. Also, we confirmed nuclear Hsp70, supplied by Hikeshi, suppresses activity of HSF1 under normal condition. These results suggest, Hikeshi knockout weakly perturb protein homeostasis as result of loss of nuclear Hsp70 under normal condition.

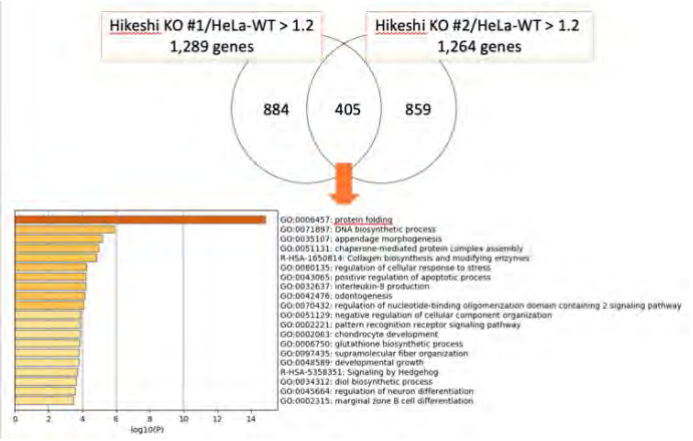

Fig.1 Hikeshi knockout up-regulates HSF1 target genes under normal conditiom. More than 1000 proteins are weakly upregulated in different Hikeshi KO cells, and GO analysis showed common genes are enriched with protein folding genes.

Plan We hope to reveal function of Hikeshi by identifying the nuclear target of Hsp70 that cause upregulation of HSF1, and molecular mechanism that switches on the Hikeshi nuclear transport, using cell biological, molecular biological and biochemical approaches. We will perform genome wide screening to identify genes that interacts with Hikeshi gene to get further information on Hikeshi function. We also aim to reveal molecular mechanism of Hikeshi dysfunction at organismal level through establishing disease model mouse.

(3) Members

as of March, 2021

(Chief Scientist)
Naoko Imamoto

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

(Research scientist)
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(Technical Staff)
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(Student Trainee)
Sakie Yoshioka

(Assistant)
YukoTokuhisa, Masae Takano

(4) Representative research achievements

1. Imamoto N, Kose S “Functional Analysis of Nuclear Transport Factor Hikeshi” in Symposium, a new frontier in stress-responsive signal transduction 第43回日本分子生物学会年会 (MBSJ2020)
2. Ogawa Y, Patent application: 09215-JP、発明の名称: 核可溶性タンパク質の新規分画法、出願番号: 2020-200641、出願日: 2020/12/2

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