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 (FY2019) 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 (Fig1). At the same time, we realized that each NTRs can be expected to carry two-three hundred different cargoes. Although the specificity of NTR-cargo interactions has been thought to involve consensus sequence of cargoes that can interact with specific NTRs,

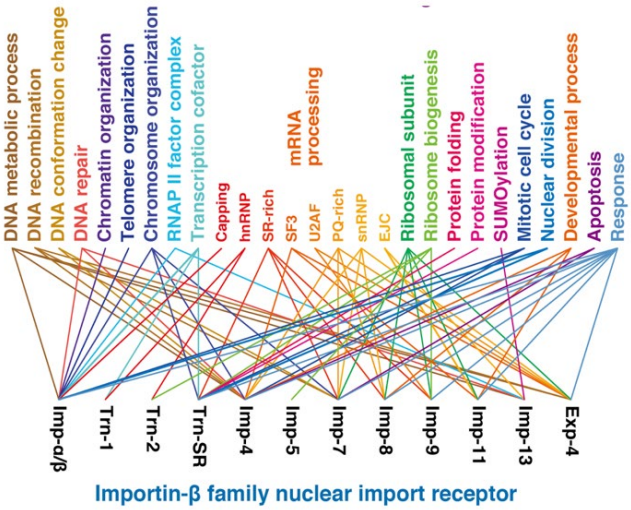

Fig.1 Division of role of Importin β family

we could not find any novel motifs that may serve as NTR-binding sites on the identified cargoes using the uncapped motif search method of MEME. In FY 2019, we aim to approach open issue of what determines NTR-cargo specificity. We took advantage of our previous study that showed large scale identification of cargo candidates of 12 importin-ß family NTRs, which enabled analysis of binding between the NTRs and many specific cargoes. We focused on transportin- (Trn)SR and importin 13 (Imp13), which are close paralogous NTRs that do not share common cargoes, and proposed configurations of NTR-cargo interactions are more widely diversified than expected (submitted).

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. 1st, we will further examine how a single protein (individual importin □ family member) can specifically bind to a variety of different cargoes.

Our analysis involves a bioinformatic approach and requires evolutionary aspects. 2nd, we hope to 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, i.e., Hikeshi knockout mice died within 48hr after birth, and a missense mutation in the human Hikeshi gene is linked to human genetic disease. Hikeshi was initially identified as an NTR for heat stress-induced nuclear import of Hsp70s, but now we noticed that Hikeshi also mediates nuclear import of Hsp70s even under normal condition. Protein structure of Hikeshi and its unique property to bind Hsp70 strongly suggest that Hsp70/Hsc70 is the sole transport cargo for Hikeshi, which in turn, allow us to consider that phenomena associated by Hikeshi dysfunction is due to loss of nuclear function of Hsp70, both under stress condition and non-stressed condition.

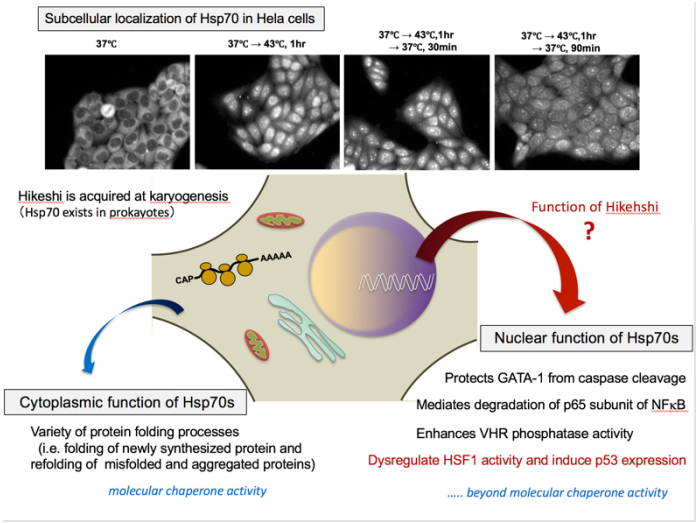

Fig.2 Cytoplasmic and nuclear role of Hsp70

Notably, Hsp70 is a major molecular chaperone whose cytoplasmic function has been extensively studied, but its nuclear function has remained unknown. The functions of Hsp70 may extend beyond molecular chaperone activity in the nucleus and regulate nuclear-specific roles such as regulation of transcription. Our study shows an intriguing example of the same molecule functioning in the different compartments differently, the nucleus and the cytoplasm, and emphasizes the importance of nucleocytoplasmic transport to understand the entire process (Fig.2). In FY2019, we are perusing nuclear target of Hsp70 through RNAseq analysis of Hikeshi knockout cells. We expect nuclear Hsp70 is essential for nuclear proteostasis, a process that have not been carefully investigated before.

Plan We hope to reveal function of Hikeshi by identifying the nuclear target of Hsp70, 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, 2020

(Chief Scientist)
Naoko Imamoto

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

(Research scientist)
Yutaka Ogawa

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

(Student Trainee)
Sakie Yoshioka

(Assistant)
YukoTokuhisa, Masae Takano

(4) Representative research achievements

1. "Nuclear import of IER5 is mediated by a classical bipartite buclear localization signal and is required for HSF1 full activation.", Yamano, Sh$., Kimura, M$., Chen, Yu., Imamoto, N#., Ohki, R#., *Exp. Cell Res.* 386 (2020) 111686. $ equal contribution, # equal correspondence
2. "Editorial overview: Cell nucleus", Imamoto, N, Larson, D., *Curr. Opin. Cell Biol.* Jun;58:iii-iv (2019)

3. “Analysis of Function of Hikeshi” Imamoto N., NT Nucleocytoplasmic Transport “Airlie” meeting. Peebles Hydro, Scotland, August 25 – 30, 2019, invited talk

Laboratory Homepage
https://www.riken.jp/en/research/labs/chief/cell_dyn/index.html
http://www2.riken.jp/celldynamics/english/index.html