Molecular Science of Bio-Metal Dynamics: Understanding and Regulation of the Strategies of Metal Utilization in Living Cells

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Metals play important roles in sustaining life. Cells are mainly composed of water, proteins, and lipids, but they also contain small amounts of metals that help maintain health by being acquired from food. Those metals are used as active centers of enzymes that carry out functions essential to sustaining life, *e.g.* transport and storage of oxygen, energy production, gene synthesis, has been known for many years. However, the series of molecular mechanisms underlying metal dynamics in the body (absorption, sensing, transport, storage, and excretion of metals) (Figure1) and selectivity for individual metals remain unknown. Our group focuses on "iron," which is the most important metal among the essential metals for sustaining life of living things, focusing on various proteins that play a role in the selective absorption, sensing, and intracellular transport of iron in food. We are not only elucidating the structure of related proteins but also exploring their relationship with their functions in human cells.

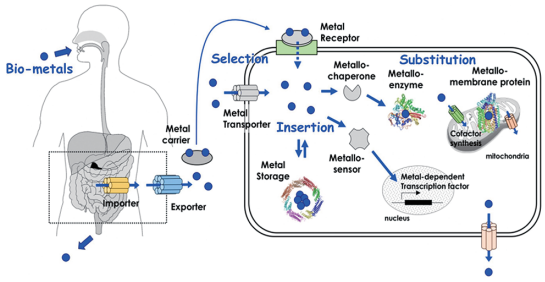

Figure 1. Our aim is to understand the uptake, trafficking, and regulation of "bio-metals" through the relay of protein–protein interactions.

Selected Publications

- M. Ganasen, H. Togashi, H. Takeda, H. Asakura, T. Tosha, K. Yamashita, K. Hirata, Y. Nariai, T. Urano, X. Yuan, I. Hamza, A. G. Mauk, Y. Shiro, H. Sugimoto and H. Sawai, "Structural Basis for Promotion of Duodenal Iron Absorption by Enteric Ferric Reductase with Ascorbate," *Commun. Biol.* **1**, 120 (2018). DOI: 10.1038/s42003-018-0121-8
- G. S. A. Wright, A. Saeki, T. Hikima, Y. Nishizono, T. Hisano, M. Kamaya, K. Nukina, H. Nishitani, H. Nakamura, M. Yamamoto, S. V. Antonyuk, S. Samar Hasnain, Y. Shiro and H. Sawai, "Architecture of the Complete Oxygen-Sensing FixL-FixJ Two-Component Signal Transduction System," *Sci. Signaling* **11**, eaaq0825 (2018). DOI: 10.1126/scisignal.aaq0825
- H. Sawai and K. Ishimori, "Integrated Bio-Metal Science: New Frontiers of Bio-Metal Science Opened with Cutting-Edge Techniques," *Biophys. Physicobiol.* **17**, 94–97 (2020).
- M. Nishinaga, H. Sugimoto, Y. Nishitani, S. Nagai, S. Nagatoishi, N. Muraki, T. Tosha, K. Tsumoto, S. Aono, Y. Shiro and H. Sawai, "Heme Controls the Structural Rearrangement of Its Sensor Protein Mediating the Hemolytic Bacterial Survival," *Commun. Biol.* **4**, 467 (2021). DOI: 10.1038/s42003-021-01987-5

1. Molecular Mechanism of Intracellular Fe^{2+} Regulation by the Iron-Delivery Chaperone PCBP

Iron is an essential "nutrient" for supporting life. In humans, iron nutrients (*e.g.*, heme iron and iron ions) ingested from food are absorbed from duodenum (upper small intestine) and after intracellular sensing, transport and storage, are eventually distributed as serum iron to organs and muscles throughout the body.[1] Iron ions can undergo redox, and although Fe^{2+} is water-soluble and easily accessible, it readily reacts with O_2 to produce reactive oxygen species, which are cytotoxic. On the other hand, Fe^{3+} is not a source of reactive oxygen species, but is less water-soluble. Therefore, the body uses a variety of proteins to avoid Fe^{2+} toxicity and strictly regulates iron homeostasis. Failure of this regulatory system to sustain iron homeostasis can lead to iron overload or deficiency. To help introduce the protein framework that dictates human iron dynamics, we focused on dietary iron dynamics via the intracellular iron-delivery chaperone, PCBP (poly rC binding protein)[2] (Figure 2).

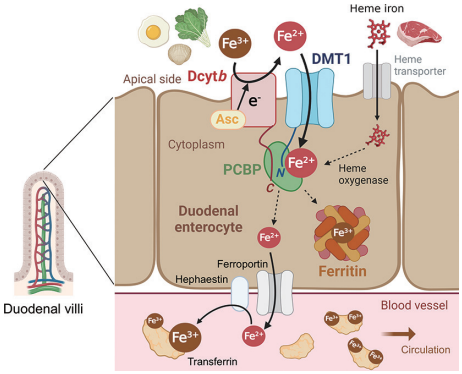

Figure 2. Dietary iron absorption in duodenum enterocyte. The first step of duodenal iron absorption is the transport of Fe^{2+} by DMT1 (divalent metal transporter 1) at the apical plasma membrane of duodenal enterocytes. Reduction of the predominant Fe^{3+} by Dcyt*b* (duodenal cytochrome b_{561}) using cytoplasmic electron doner, Asc (ascorbate), is necessary for Fe^{2+} transport by DMT1.

PCBP has been proposed to play a role in supplying iron ions to the iron storage protein ferritin,[3] but the molecular mechanism of iron trafficking from PCBP to ferritin remains elusive. To unveil these mechanisms, we prepared recombinant human PCBP and investigated its properties. Iron binding assays using apo-PCBP revealed that one molecule of apo-PCBP can bind one Fe^{2+}, while Fe^{3+} does not bind, clearly showing PCBP can only bind Fe^{2+}. The complexation of Fe^{2+}-bound PCBP and ferritin H- or L-chain were investigated by SEC-SAXS (size-exclusion chromatography integrated small-angle X-ray scattering) and mass photometry (Figures 3 and 4). Based on these results, we proposed that Fe^{2+}-bound PCBP preferentially binds the ferritin H-chain compared to the L-chain. Since the ferritin H-chain acts as a ferroxidase, converting Fe^{2+} to Fe^{3+} for the safe storage of iron in ferritin, PCBP thus mediates safe and effective iron trafficking to ferritin in the cytoplasm.

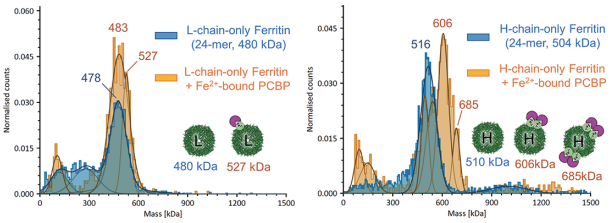

Figure 3. Mass photometry of ferritin and its mixture with Fe^{2+}-bound PCBP.

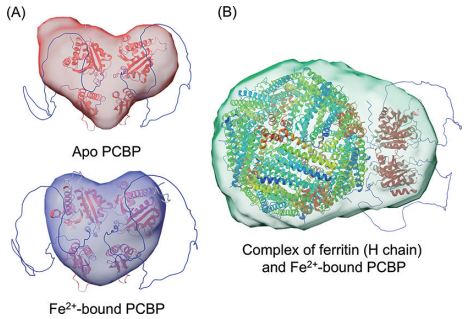

Figure 4. Space-filling models and pseudoatomic models of PCBPs (A) and its complex with H-chain only ferritin (B). Space-filling models were created based on SAXS data. Pseudoatomic models of PCBP in apo and Fe^{2+}-bound forms were predicted by AlphaFold3.

2. Cooperative Fe^{2+} Transfer between Dcyt*b* and DMT1 in the Plasma Membrane of Intestinal Living Cells

Because Fe^{2+} produced by the enzymatic reaction of Dcyt*b* is a source of reactive oxygen species, for safe and efficient trafficking of Fe^{2+}, Dcyt*b* must be close to or form a complex with DMT1 and transfer the produced Fe^{2+} to DMT1 as quickly as possible. To demonstrate that, we confirmed the localization of Dcyt*b* and DMT1 by immunofluorescence observation and proximity ligation assay between Dcyt*b* and DMT1 using Caco2-kh cells,[3] a human intestinal model cell. We further found that the *C*-terminal region of Dcyt*b* also binds to PCBP by biochemical analysis of complex formation using wild-type and deletion mutant of the *C*-terminal loop of Dcyt*b*. These data imply that not only the interaction between Dcyt*b* and DMT1 in the apical membrane of the cells, but also PCBP interacts with these membrane proteins to ensure safe and efficient Fe^{2+} transport. Therefore, it is possible that Dcyt*b*-DMT1-PCBP forms a ternary complex to play a cooperative function for the Fe^{2+} transport in the cell. Further studies at molecular and cellular levels will provide new insights into the mechanisms of ternary complex formation for dietary iron absorption in human duodenum.

References

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2) I. Yanatori *et al.*, *Biochim. Biophys. Acta, Gen. Subj.* **1864**, 129685 (2020).
3) H. Shi *et al.*, *Science* **320**, 1207–1210 (2008).
4) H. Fujishiro *et al.*, *J. Toxicol. Sci.* **42**, 559–567 (2017).