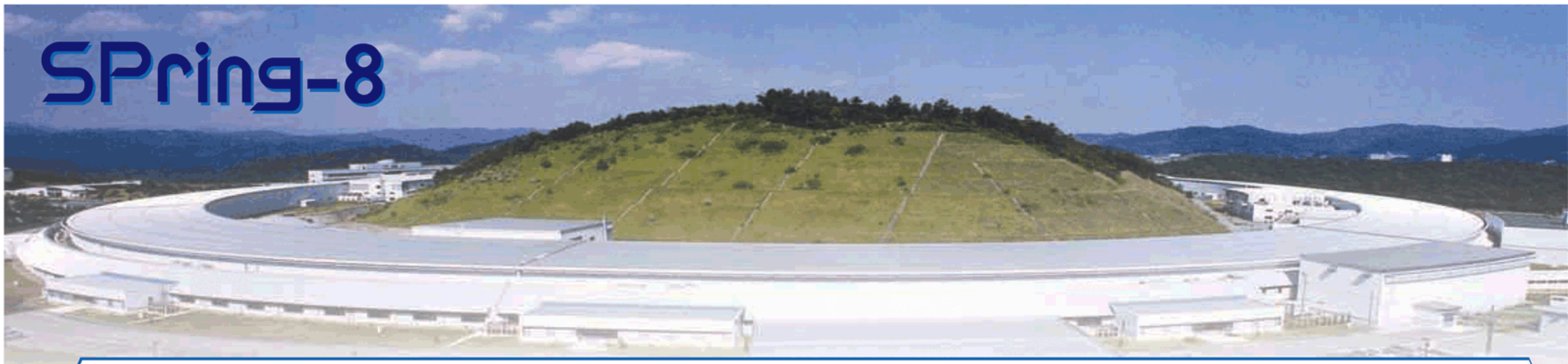
SPring-8



An Atomic-Level Analysis of the Mechanism of Protein Controlling the Heme Concentration inside a Cell

A research group under the direction of prof. Shigetoshi Aono of the Institute for Molecular Science (Okazaki Institute for Integrative Bioscience), National Institutes of Natural Sciences, together with a research group under the direction of chief scientist Yoshitsugu Shiro of the RIKEN SPring-8 Center, RIKEN Harima Institute, through biochemical experiments and X-ray crystal structural analysis, has successfully identified the protein that operate as a switch to regulate the expression of a heme efflux system upon sensing toxic free hemes, and the group has also identified the details of the mechanism whereby the intercellular heme concentration is kept constant.

The model organism chosen in this research was a lactic acid bacterium, Lactococcus lactis, which is used in making fermented dairy products such as cheese. Though this bacterium cannot biosynthesize heme due to a lack of heme biogenesis genes, it can uptake exogenous hemes to use a heme source for oxygen respiration. This research group showed that L. lactis cells contain the heme-sensor protein that senses free hemes to regulate the expression of the heme efflux system responsible for heme homeostasis (Fig. 1). This protein binds to DNA under heme deficient condition at the upstream of the gene encoding the heme efflux system to be repressed. When the intracellular concentrations of free hemes increase, this protein binds hemes to be dissociated from DNA, turning on the expression of the heme efflux system.

In order to further investigate the mechanism by which the sensor protein functions as a switch, the research group performed X-ray crystal structural analysis using SPring-8, the state-of-the-art large synchrotron radiation facility, to determine the structure of the protein in its three different forms, with heme, without heme, and the DNA/protein complex without heme. As a result, they discovered that when the sensor protein binds a heme, two molecules of histidine (His72 and His149 in Fig. 2) anchor down the heme. There is a coil-like structure of a row of atoms near one of the histidines (His72) when there is no heme inside, but this coil-like structure changes into a helix when a heme is taken in (the orange part of Fig. 2). This structural change lifts the portion of the protein that interacts with the gene (the green part of Fig. 2), making it impossible for the protein to bind to the gene.

This research may contribute to the development of new types of antibiotics that inhibit the growth of disease-causing bacteria by making them unable to avoid free heme toxicity.

Reference: "Structural basis for the transcriptional regulation of heme homeostasis in *Lactococcus lactis*"
Hitomi Sawai, Masaru Yamanaka, Hiroshi Sugimoto, Yoshitsugu Shiro, Shigetoshi Aono
Journal of Biological Chemistry (J. Biol. Chem.), **287**, 30755-30768 (2012)

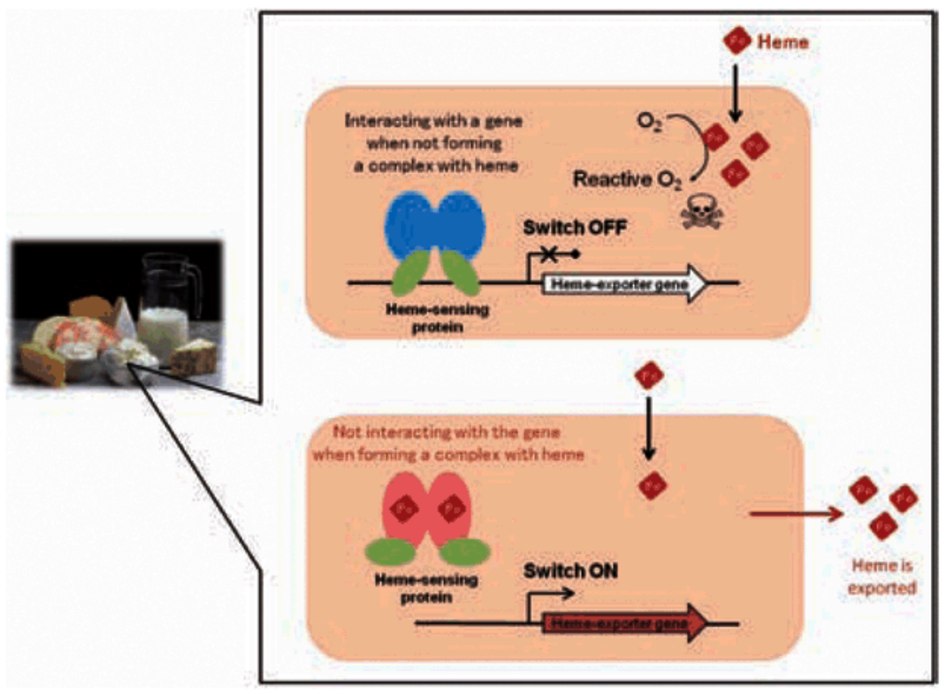


Fig.1. Mechanism that controls the intracellular heme concentration in Lactococcus lactis
The light pink rectangle represents a Lactococcus lactis cell.

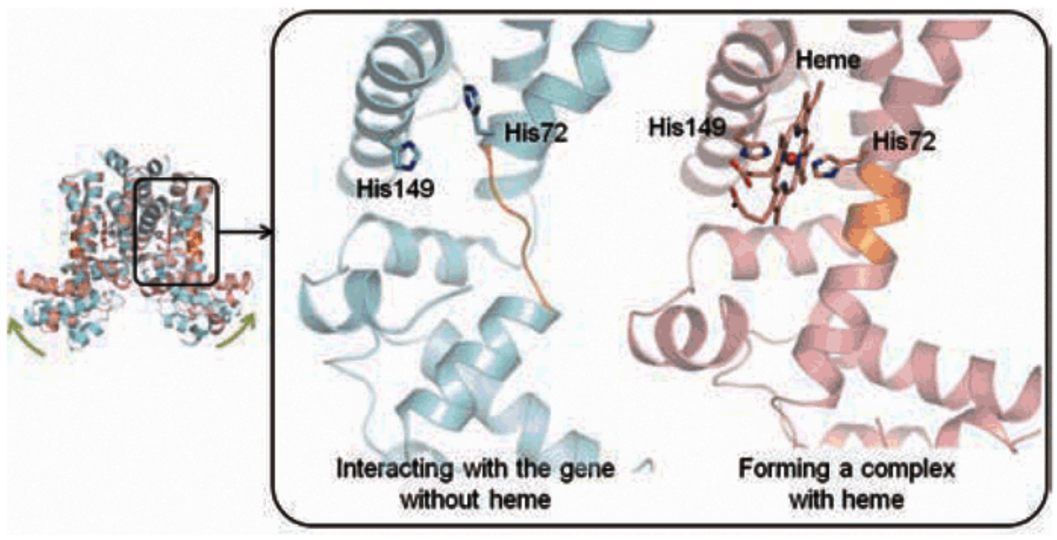


Fig.2. Difference in the 3-dimensional structure, upon heme binding
The figure on the left combines the total structure in both cases.