# 乳酸菌中のヘム濃度恒常性維持に関与する転写調節因子の構造生物科学


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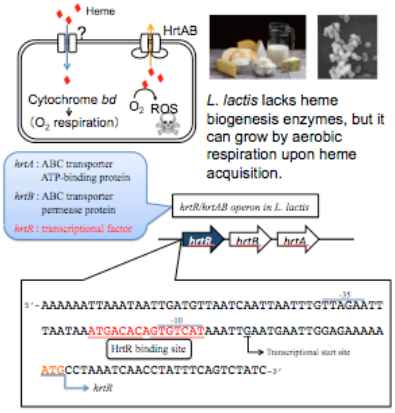
Though heme is a crucial element for many biological processes including respiration, heme homeostasis should be regulated strictly due to the cytotoxicity of free heme molecules. Numerous lactic acid bacteria, including *Lactococcus lactis*, acquire heme molecules exogenously to establish an aerobic respiratory chain. A heme efflux system plays an important role for heme homeostasis to avoid cytotoxicity of acquired free heme, but its regulatory mechanism is not clear.

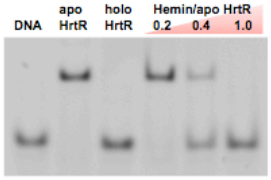
Here, we report that the transcriptional regulator HrtR senses and binds a heme molecule as its physiological effector to regulate the expression of the heme-efflux system responsible for heme homeostasis in *L. lactis*. To elucidate the molecular mechanisms of how HrtR senses a heme molecule and regulates gene expression for the heme efflux system, we determined the crystal structures of the apo-HrtR/DNA complex, apo-HrtR, and holo-HrtR at a resolution of 2.0, 3.1, and 1.9 Å, respectively. These structures revealed that HrtR is a member of TetR family of transcriptional regulators. The residue pair Arg46 and Tyr50 plays a crucial role for specific DNA-binding through hydrogen-bonding and a CH-π interaction with the DNA bases. HrtR adopts a unique mechanism for its functional regulation upon heme-sensing. Heme-binding to HrtR causes a coil-to-helix transition of the α4 helix in the heme-sensing domain, which triggers a structural change of HrtR causing it to dissociate from the target DNA for derepression of the genes encoding the heme efflux system. HrtR uses a unique heme-sensing motif with bis-His (His72 and His149) ligation to the heme, which is essential for the coil-to-helix transition of the α4 helix upon heme-sensing.

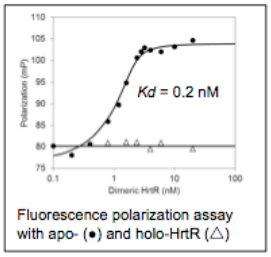






*L. lactis* lacks heme biogenesis enzymes, but it can grow by aerobic respiration upon heme acquisition.

## 1. DNA-binding of HrtR




Fluorescence polarization assay with apo- (•) and holo-HrtR (△)

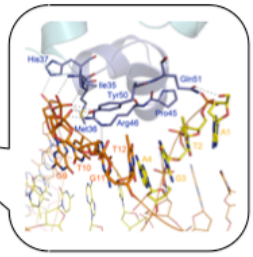
Apo HrtR binds to the target DNA, but holo HrtR does not.

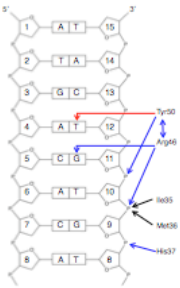
DNA-bound HrtR is dissociated from the target DNA upon heme binding.

## 2. Structure of the HrtR/DNA Complex

The global fold of HrtR is similar to that of TetR family transcriptional regulators. The DNA-binding domain and the heme-sensing (heme-binding) domain consist of $\alpha_N$, α1, α2, and α3 helices, and α4, α5, α6, α7, α8, α9, and $\alpha_C$ helices, respectively.

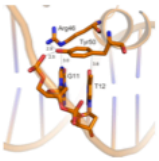
## 3. Interaction between HrtR and DNA

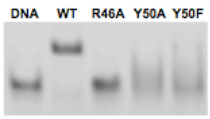





## 4. Pairwise Interactions of Arg46 and Tyr50 for the Specific DNA-binding

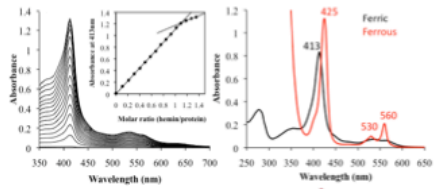
Arg46 (εNH) forms a hydrogen bond with N7 atom of G11, and Tyr50 forms a CH-π interaction with T12. Arg46 and Tyr50, whose orientations are fixed in pairs by the hydrogen bond between them, are required for the specific DNA-binding of HrtR. Arg46 and Tyr50 are essential for the DNA-binding of HrtR.

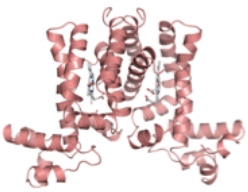




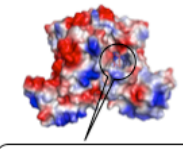

Gel-shift assay of R46A, Y50A and Y50F Mutants

## 5. Heme-sensing by HrtR



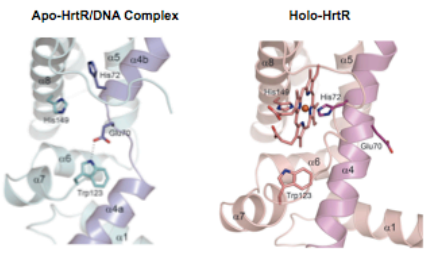



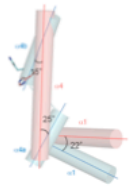
Structure of Holo-HrtR



Heme is accommodated in a large cavity, in which His72 and His149 are the axial ligands of the heme.

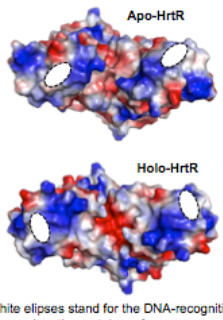
## 6. Coil-to-Helix Transition Induced by Heme-sensing



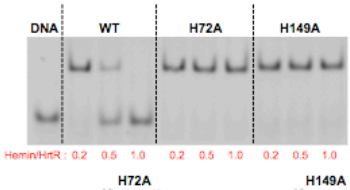
Upon heme-binding, a coil-to-helix transition occurs in the intervening loop between the α4a and α4b helices, which results in the formation of a long α4 helix in holo-HrtR. This coil-to-helix transition results in a change in the distance and relative orientation between the DNA-recognition helices, as shown in Fig. 7.

## 7. Change in the Relative Orientation of DNA-Recognition Helices upon Heme-binding




White elipses stand for the DNA-recognition helices exposed on the protein surface.

## 8. Crucial Role of His72 and His149 for Heme-dependent Transcriptional Regulation




While H72A and H149A specifically bind a heme, mutation of His72 or His149 results in the loss of the heme-dependent regulation for DNA-binding.