# Structural basis for phosphorylation induced control of intracellular signaling

Fuyuhiko Inagaki, Yoshihiro Kobashigawa, Kenji Ogura and Satoru Yuzawa
*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.*
e-mail: finagaki@pharm.hokudai.ac.jp

Phosphorylation is a major control mechanism in intracellular signaling, but its implication on structural basis still remains elusive. Here, we report two structural studies to address this issue.

The phagocyte NADPH oxidase plays a crucial role in host defense against microbial infections by generating reactive oxygen species. It is composed of membrane-bound flavocytochrome $b_{558}$ (gp91 and p22) as well as cytosolic components, including p47, p67, p40 and Rac. When phagocytes are activated, the cytosolic components of the NADPH oxidase translocate to flavocytochrome $b_{558}$ by binding of the tandem SH3 domains of p47 to a proline-rich region (PRR) in p22. The translocation is induced by the phosphorylation of serine residues in the polybasic region (PBR) of p47. We revealed the structure of the tandem SH3 domains of p47 in the resting and activated states by X-ray and NMR (1-3). In the resting state, the tandem SH3 domains were enwrapped by the PBR into a compact structure, whereas, in the activated form, the p22 PRR bound to the groove formed by the SH3 domains. This suggests that the interaction between the tandem SH3 domains and PBR is loosened by the phosphorylation and finally the binding groove is open to bind to p22 PRR.

The other example is inactivation of Crk-II by tyrosine phosphorylation of inter SH3 region. Crk-II is an oncogenic adaptor protein composed of SH2-SH3-SH3. We studied Crk-II in unphosphorytaed and phosphorylated states. In the unphosphorylated state, the linker locked the three domains into the closed form that enables N-SH3 partially open to the target proteins. Upon tyrosine phosphorylation, SH2 binds to pTyr intramolecularlly that abrogates the physiological function of Crk-II.

(1) Yuzawa, S., Suzuki, N., Fujioka, Y., Ogura, K., Sumimoto, H., and Inagaki, F. (2004) A molecular mechanism for autoinhibion of the tandem SH3 domains of p47, the regulatory subunit of the phagocyte NADPH oxidase. *Genes Cells*, **9**, 443-456.
(2) Yuzawa, S., Ogura, K., Horiuchi, M., Suzuki, N., Fujioka, Y., Kataoka, M., Sumimoto, H., and Inagaki, F. (2004) Solution Structure of the tandem Src homology domains of p47 in an autoinhibited form. *J. Biol. Chem.*, **279**, 29752-29760.
(3) Ogura, K., Nobuhisa, I., Yuzawa, S., Takeya, R., Torikai, S., Saikawa, K., Sumimoto, H., and Inagaki, F. (2006) NMR solution structure of the tandem SH3 domains of p47 complexed with a p22-derived proline-rich peptide. *J. Biol. Chem.*, **281**, 3660-3668.