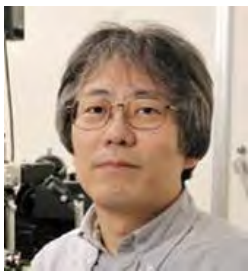
Cellular Informatics Laboratory
Chief Scientist: Yasushi Sako (D.Sci.)



(0) Research field

CPR Subcommittee**:** Biology

Keywords:

Biophysics, Biomembranes, Cell signaling, Membrane receptors, Protein dynamics

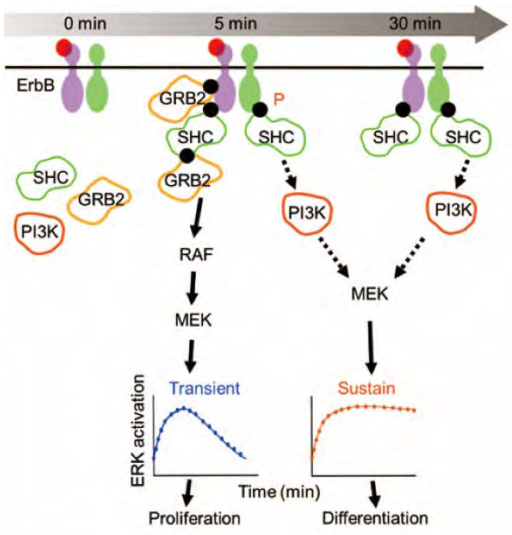
(1) Long-term goal of laboratory and research background

The aim of us is to understand the principles of signal processing carried out by biological systems in the classes of proteins, protein networks, and cells. We are studying how biomolecules assemble to process the intra- and extra-cellular information and express flexible higher-order cellular responses. In these studies, we develop and use techniques of single-molecule measurements, optical microscopy, cell engineering, reconstruction of biosignaling systems, as well as mathematical analysis and computer simulations. The recent main target of us is an intracellular protein reaction network called the ERBB-RAS-MAPK system. This system is responsible for cell fate decisions including cell proliferation, differentiation, and apoptosis. In addition, we are investigating the functions and dynamics of proteins, including GPCRs, which is also involved in cell signaling and fate decision. We are analyzing how diverse dynamics of reaction systems, which lead to higher-order biological function, emerged from the accumulations of elemental protein reactions.

(2) Current research activities (FY2021) and plan

Regulation of cell signaling dynamics

p52SHC (SHC) and GRB2 are adaptor proteins involved in the RAS/MAPK (ERK) pathway mediating signals from cell-surface receptors to various cytoplasmic proteins. To further examine their roles in signal transduction, we studied the translocation of fluorescently-labeled SHC and GRB2 to the cell surface, caused by the activation of ERBB receptors by heregulin (HRG). We simultaneously evaluated activated ERK translocation to the nucleus. Unexpectedly, the translocation dynamics of SHC were sustained when those of GRB2 were transient. The sustained localization of SHC positively correlated with the sustained nuclear localization of ERK, which became more transient after SHC knockdown. SHC-mediated PI3K activation was required for the sustained ERK in a GRB2 and RAF-independent manner. In cells overexpressing ERBB1, SHC translocation became transient and the HRG-induced cell fate shifted from a differentiation to a proliferation bias. Our results indicate that SHC and GRB2 functions are not redundant but that SHC plays a critical role in the temporal regulation of ERK activation. (Yoshizawa et al. 2021)

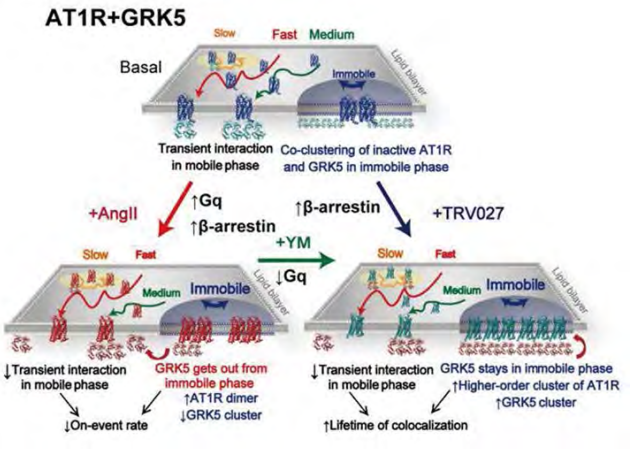


GRB2 and SHC differently regulate MAPK dynamics via RAF and PI3K, respectively.

Mechanism of signaling bias in GPCR

Recent studies have highlighted the dual roles of G-protein-coupled receptors (GPCRs) in activating signaling through heterotrimeric G proteins and β-arrestins, paving the way for the development of biased signaling drugs that discriminate a therapeutic signal from an on-target adverse signal. In vitro structural studies demonstrate a basis of preferential engagement of a specific transducer, accounting for GPCR bias. However, little is known about the mechanisms of transducer bias, a single effector that mediates different functions, in part due to a poor understanding of the regulation of GPCR kinase (GRK)-mediated GPCR phosphorylation. Here, by using the angiotensin II (Ang II) type-1 receptor (AT1R), we identified a unique role of the Gq heterotrimer as a determinant for GRK-subtype selectivity that regulates subsequent β-arrestin conformation and function. The β-arrestin-biased ligand TRV027 induced GRK5/6-dependent β-arrestin recruitment to AT1R, whereas Ang II recruited β-arrestin using both GRK2/3 and GRK5/6. Pharmacological inhibition or genetic loss of Gq shifted the GRK-subtype selectivity of Ang II to more closely resemble that of TRV027. The TRV027-induced or Gq inhibition-mediated GRK-subtype

selectivity differentially affected β-arrestin conformations and ERK signaling, whereas receptor internalization was indistinguishable. Using a dual-color, single-molecule-tracking analysis, we observed, upon Ang II stimulation, relocation of AT1R and GRK2 molecules, but not GRK5 molecules, to an immobile hotspot on the plasma membrane. In contrast, under the Gq-inactive, AT1R-stimulated conditions, AT1R and GRK5, but not GRK2, accumulated in the immobile hotspot. In addition, triple-color single-molecule imaging identified simultaneous co-localization of AT1R, Gq, and GRK5/6 molecules. Together, we propose a mechanism whereby (1) GRK-mediated AT1R phosphorylation occurs in the immobile hotspot, (2) active Gq repels GRK5/6 from the immobile hotspot, and (3) Gq-silence biased ligands alter GRK preference from GRK2/3 to GRK5/6. These findings uncover a previously unappreciated Gq-regulated mechanism that encodes GRK-subtype selectivity and imparts distinct phosphorylation barcodes directing downstream β-arrestin functions. (Kawakami, Yanagawa et al. 2022)



Angiotensin II (AngII) and TRV027 activate different cell signaling pathway inducing each specific motional dynamics of AT1R molecules.

(3) Members as of March, 2022

(Chief Scientist)
Yasushi Sako

(Senior research scientist)
Akihiro Yamamoto

(Research scientist)
Yukinobu Arata, Mitsuhiro Abe, Kenji Okamoto, Toshihiro Nagamine, Nobuhisa Umeki, Masataka Yanagawa, Ryo Yoshizawa, Michio Hiroshima

(Technical Staff)
Dong Kese, Maiko Minatohara, Mutsumi Nakanishi, Hiromi Sato, Miyoshi Suga

(Student Trainee)
Momoko Akiyama, Yutaro Kuwashima

(Part-time Worker)
Itsumi Ota, Hiroaki Toyoda

(4) Representative research achievements

1. "p52Shc regulates the sustainability of ERK activation in a RAF-independent manner", Yoshizawa, R., Umeki, N., Yamamoto, A., Okada, M., Murata, M., and Sako, Y. **Mol. Biol. Cell.** 32, 1838-1848 (2021).
2. "Comparative analysis of single-molecule dynamics of TRPV1 an TRPV4 channels in living cells",.Kuwashima, Y., *Yanagawa, M., Abe, M., Hiroshima, M., Ueda, M., Arita, M., and Sako, Y. **Int. J. Mol. Sci.** 22, 8473 (2021).
3. "PMP2/FABP8 induces PI(4,5)P_2-dependent transbilayer reorganization of sphingomyelin in the plasma membrane", Abe, M., Makino, A., Murate, M., Hullin-Matsuda, F., Yanagawa, M., Sako, Y., and Kobayashi, T. **Cell Rep.** 37, 109935 (2021).
4. "The origin of b-arrestin transducer bias: heterotrimeric Gq as a switch for GRK5/6 selectivity", *Kawakami, K., *Yanagawa, M., Hiratsuka, S., Yoshida, M., Ono, Y., Hiroshima, M., Ueda, M., Aoki, J., **Sako, Y., and **Inoue, A. **Nat. Comm.** 13, 487 (1-16),(2022).
5. "Assessing transfer entropy from biochemical data", Imaizumi, T., Umeki, N., Yoshizawa, R., Obuchi, T., Sako, Y., and Kabashima, Y. **Phys. Rev. E.** 105, 034403 (1-13), (2022).

Laboratory Homepage
https://www.riken.jp/en/research/labs/chief/cell_inf/index.html
http://www2.riken.go.jp/cell-info/en/