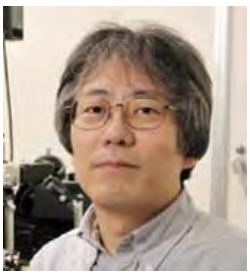
Cellular Informatics Laboratory
Chief Scientist: Yasushi Sako (D.Sci.)



(0) Research field

CPR Subcommittee**:** Biology

Keywords:

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

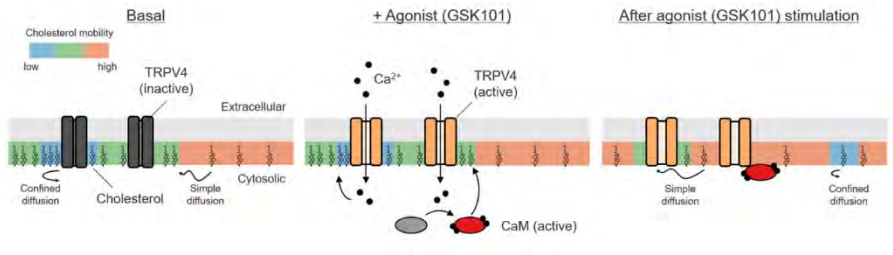
(1) Long-term goal of laboratory and research background

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 (FY2023) and plan

Ion channel specific Ca^{2+} influx induces remodeling of the plasma membrane

The activities of the transient receptor potential vanilloid 4 (TRPV4), a Ca^{2+}-permeable non-selective cation channel, are controlled by its surrounding membrane lipids (*e.g.*, cholesterol, phosphoinositides). The transmembrane region of TRPV4 contains a cholesterol recognition amino acid consensus (CRAC) motif and its inverted (CARC) motif located in the plasmalemmal cytosolic leaflet. TRPV4 localizes in caveolae, a bulb-shaped cholesterol-rich domain at the plasma membrane. We visualized the spatiotemporal interactions between TRPV4 and cholesterol at the plasma membrane in living cells by dual-color single-molecule imaging using total internal reflection fluorescence microscopy (TIRFM). To this aim, we labelled cholesterol at the cytosolic leaflets of the plasma membrane using a cholesterol biosensor, D4H. Our single-molecule tracking analysis showed that the TRPV4 molecules colocalize with D4H-accessible cholesterol molecules mainly in the low fluidity membrane domains in which both molecules are highly-clustered. Colocalization of TRPV4 and D4H-accessible cholesterol was observed both inside and outside of caveolae. Agonist-evoked TRPV4 activation remarkably decreased colocalization probability and association rate between TRPV4 and D4H-accessible cholesterol molecules. Interestingly, upon TRPV4 activation, the particle density of D4H-accessible cholesterol molecules was decreased and the D4H-accessible cholesterol molecules in the fast-diffusing state were increased at the plasma membrane. The introduction of skeletal dysplasia-associated R616Q mutation into the CRAC/CARC motif of TRPV4, which reduced the interaction with cholesterol clusters, could not alter the D4H-accessible cholesterol dynamics. Mechanistically, TRPV4-mediated Ca^{2+} influx and the C-terminal calmodulin-binding site of TRPV4 are essential for modulating the plasmalemmal D4H-accessible cholesterol dynamics. We propose that TRPV4 remodels its surrounding plasmalemmal environment by manipulating cholesterol dynamics through Ca^{2+} influx. (Kuwashima et al. 2024)

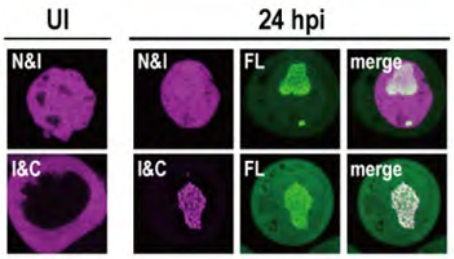


A model of cholesterol remodeling regulated by the Ca^{2+} influx through TRPV4 channel.

An insect virus protein IE1 controls its focus formation

Baculovirus IE1 is a multifunctional protein that is essential for both DNA replication and RNA transcription of the virus. Prior to viral DNA replication, IE1 promotes early gene transcription in its *hr*-dependent focal distribution. During viral DNA replication, IE1 foci expand and fuse to generate the

virogenic stroma (VS) where IE1 forms the VS reticulum. To explore IE1 structural features for the coordinated localization, we constructed various IE1 mutants based on a putative IE1 domain structure (N, I, and C domains) and revealed that BDI located in the intrinsic disorder region (IDR) between the N and I domains acts as a nuclear localization signal, while BDII and HLH in the C domain are required for its VS localization in infected cells and for chromosomal association in uninfected mitotic cells. Deletion of the SLiM (short linear motif) located in the I domain restrains both nuclear- and VS localization. Intra-molecular fluorescence resonance energy transfer (FRET) probes of IE1 mutants revealed a conformational change of the I&C two-domain fragment during infection, which was inhibited by aphidicolin, suggesting that IE1 undergoes a stage-dependent conformational change. The IE1 SLiM was required for homo-dimerization of the I domain as well as the stage-dependent conformational change. While both SLiM mutants, S291A and S291E, retained the activities for VS localization and chromosomal association, they exhibited differences in *hr*-dependent focus formation activity. These results suggest that coordinated IE1 localization is controlled by the SLiM-dependent conformational change and may be switched by the phosphorylation states of the SLiM. (Nagamine and Sako, 2023)



Focus formation by the fragments of IE1

In addition, this year, we studied functions of membrane lipids in collaboration with Strasburg University. We have revealed the distribution of a glycolipid, GM3 in the cell membrane depends on the cell density (Murate et al. 2023) and the surface protein of HIV recognizes membrane rafts for infection into the host cells (Tomishige et al. 2023). We will continue studies on the cell signaling protein dynamics in relation to the cell structure.

(3) Members as of March, 2024

(Chief Scientist)
Yasushi Sako

(Senior research scientist)
Akihiro Yamamoto

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

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

(Student Trainee)
Yutaro Kuwashima

(Part-time Worker)
Itsumi Ota

(4) Representative research achievements

1. “TRPV4-dependent Ca^{2+} influx determines cholesterol dynamics at the plasma membrane”, Kuwashima, Y., Yanagawa, M., Maekawa, M., Abe, M., *Sako, Y., and *Arita, M. **Biophys. J.** in press (2024). *co-corresponding
2. “HIV-1 Gag targeting to the plasma membrane reorganizes sphingomyelin and cholesterol rich lipid domains”, Tomishige, N., Nasim, M. B., Murate, M., Pollet, B., Didier, P., Godet, J., Richert, L., Sako, Y., Mély, Y., and Kobayashi, T. **Nat. Commn.** 14, 7353 (2023).
3. “A SLiM-dependent conformational change in baculovirus IE1 controls its focus formation ability”, Nagamine T. and Sako Y. **J. Gen. Virol.** 104, doi: 10.1099/jgv.0.001910 (2023)
4. “Cell density-dependent membrane distribution of ganglioside GM3 in melanoma cells”, Murate, M., Yokoyama, N., Tomishige, N., Richert, L., Humbert, N., Pollet, B., Makino, A., Kono, N., Mauri, L., Aoki, J., Sako, Y., Sonnino, S., Kaneko, M. K., Kato, Y., Inamori, K-i., Iokuchi, J-i. Mély, Y., Iwabuchi, K., and Kobyashi, T. Cell. **Mol. Life Sci.** 80, 167 (2023).

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