【Grant-in-Aid for Specially Promoted Research】

**Biological Sciences**

**Title of Project : Novel approach focusing on the tight junction-based paracellular barrier combined with the apical barrier toward understanding and manipulating epithelial barriers**

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## 【Purpose and Background of the Research】

Epithelial cell sheets that form barriers between two biological compartments are a basic feature of metazoans. The cells in these sheets are adjoined by a specialized cell-cell adhesion apparatus called the Tight Junction (TJ) to form paracellular barriers in vertebrates. Although the TJ molecular composition is well studied, questions remain about the organization and function of the TJ-paracellular barrier and its relationship to the apical barrier, which forms at the apical side of epithelial cells. Based on our previous achievements in TJ research, our current project comprises two subjects. One aim is to resolve the *in vivo* TJ molecular structure at the amino acid level using a highly advanced technique, single particle cryoelectron microscopy(CryoEM). The other aim is to establish how the TJ-paracellular barrier is integrated with the apical barrier by the "TJ-Apical Complex," a system consisting of TJs, the apical cytoskeleton, and apical membranes, to organize the epithelial barrier. These analyses will be performed at the molecular, cellular, tissue/organ, and animal levels. Our project explores broader roles of the TJ-paracellular and apical barriers in forming the total epithelial barrier, which plays critical roles in biological systems.

## 【Research Methods】

(1) Establishment of the hypothetical "Antiparallel double row model" for TJs *in vivo*. How claudins polymerize to form TJs *in vivo* remains unknown. We seek to resolve the *in vivo* TJ structure by high-resolution cryoEM using various TJ preparations.

(2) TJ-Apical Complex analysis at the molecular~animal level. We unbiasedly screened the TJ fractions to identify four novel factors that bridge the TJ and apical cytoskeleton termed TJ microtubule (MT)-associating proteins(TJMAPs). These proteins localize to the TJ and bind to MTs and actin filaments (AFs) (and possibly intermediate filaments) to organize apical functions. We seek to clarify how TJs are integrated with apical functions to organize the epithelial barrier.

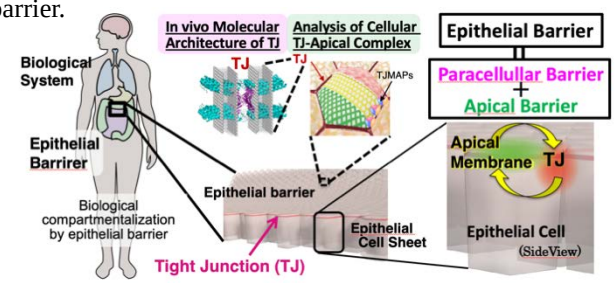


Figure 1 Epithelial barrier-based biological systems and projects in this study

## 【Expected Research Achievements and Scientific Significance】

We will pursue two major lines of study. One is a structural physiological study using the highly advanced cryoEM to obtain a detailed molecular model for the organization and function of the TJ-paracellular barrier. The other is a cell biological study in which we establish our proposed concept, "The TJ-Apical Complex," a functional cytoskeletal-signaling system that integrates the structure and function of the TJ and apical components. The data obtained will lead to a systematic understanding of biological functions governed by the TJ and TJ-Apical Complex in normal and disease states, which may provide a conceptual platform for new health management strategies, therapeutic approaches, and bioengineered tissues.

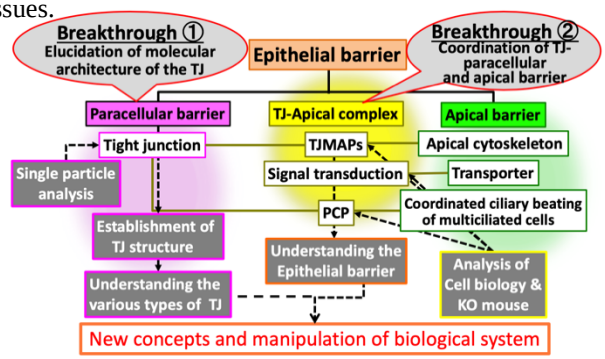


Figure 2 Molecular~mouse level analyses on epithelial barrier-based biological systems

## 【Publications Relevant to the Project】


- Kunimoto,K., …… , and Tsukita,S. Coordinated ciliary beating requires *Odf2*-mediated polarization of basal bodies via basal feet. *Cell* 148, 189-200 (2012).
- Saitoh,Y., Suzuki, S., Tani,K., ……, Tsukita,S., and Fujiyoshi,Y. Structural insight into tight junction disassembly by *Clostridium perfringens* enterotoxin. *Science* 347, 775-778 (2015).
- Tsukita,S., Tanaka,H., and Tamura,A. The claudins: from tight junctions to biological systems. *Trends in Biochem. Sci.* 44, 141-152 (2019).


## 【Term of Project】 FY2019-2023

## 【Budget Allocation】 431,000 Thousand Yen

## 【Homepage Address and Other Contact Information】

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