Pluripotent stem cells (PSCs) are defined as stem cells capable of producing all cell types that compose our body. In vivo, PSCs appear only transiently during the early stage of development and are lost as development proceeds. Molecular features that underlie pluripotency have been extensively studied, and accumulating evidences suggest that the unique cell cycle regulation of PSC is tightly linked to the maintenance of pluripotency. Curiously, they proliferate with truncated gap phases while lengths of the S (DNA replication) and M (mitosis) phases remain similar to non-pluripotent cell types. In particular, the G1 phase of PSCs needs to be kept short to maintain pluripotency: artificial elongation of the G1 phase makes them susceptible to differentiation. On the other hand, active proliferation with truncated gap phases can cause cellular stress as in some cancer cells, leading to genome instability. Our laboratory is interested in the role of cell cycle regulations in the maintenance of pluripotency and genome integrity of PSCs.

II. Induction of Pluripotency through Nuclear Reprogramming and Role of Cell Cycle Regulation

In mammals, PSCs are not maintained in a fully-developed organism. However, differentiated cells can regain pluripotency upon experimental trigger, albeit at a low efficiency. Factors that limit active reprogramming, and conditions that potentiate reprogramming, are the subjects under active investigation.

One of the main obstacles when investigating molecular mechanism underlying reprogramming is the time it takes to start seeing any sign of reprogramming. In order to overcome this problem, we are taking advantage of the cell-to-cell fusion approach, in which a target cell is fused to a pluripotent stem cell to induce pluripotency within a target nucleus, in a short time frame. The cell fusion system is a simple, versatile way to induce reprogramming towards another lineage, and is not limited to pluripotency. Using this system, the first sign of reprogramming can be detected within one day after fusion, thus allowing us to monitor the initial events of reprogramming after induction.

Using this system, we previously found that DNA synthesis is an important event for successful reprogramming (Tsoubuchi et al., Cell, 2013). Recent reports indicate that reprogramming may cause genetic instability, some of which are thought to arise as DNA replication errors. To investigate the nature of such errors and how they are linked to reprogramming, we are currently aiming to address how such differences are interlinked with pluripotency by carrying out side-by-side analyses between ES cells and differentiated cells.
gramming-specific events, we have set up a system to isolate and track a single fused cell (Figure 1) through live-imaging.

**III. Future Perspectives**

While the fundamental mechanisms that maintain genome integrity have been widely studied using various models, the danger a cell might face when its identity is being altered (through differentiation, reprogramming etc.) are largely unknown. Recent studies of cancer genome sequencing repeatedly identified mutations in the factors that govern cellular identities, leading us to hypothesize that cells may experience genome instability when their identity is unstable. Our goal is to gain a comprehensive understanding of how genome integrity is maintained in ES cells and cells undergoing reprogramming.

**Publication List:**

[Review article]