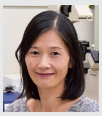
LABORATORY OF STEM CELL BIOLOGY



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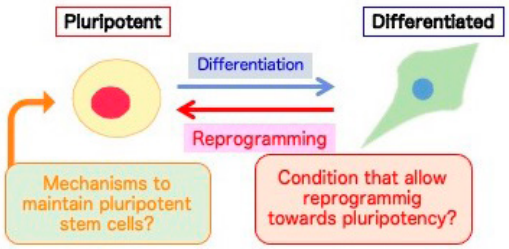
Pluripotent stem cells (PSCs) are defined as stem cells capable of producing all cell types that compose our body. *In vivo*, PSCs appear only transiently during the early stage of development and are lost as development proceeds. Molecular features that underlie pluripotency have been extensively studied, 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.



Visual overview of this lab's work.

I. Self-renewal of Embryonic Stem Cells and Their Genome-Maintenance Mechanisms

Embryonic stem (ES) cells are derived from the blastocyst stage of embryonic development, and are capable of differentiating into all of the cell types that compose our body (*i.e.*, ES cells are "pluripotent"). Recent reports suggest that, compared to other cell types, DNA replication machinery of ES cells proceed at lower speed, a feature generally infer presence of replication obstacles. However, the direct cause and the underlying mechanism remains to be uncovered. To date, studies on cell cycle regulation in ES cells have not been as straightforward compared to that of other cell types, as many commonly used cell-synchronization protocols are ineffective for ES cells. We have now established several protocols to synchronize ES cells (Tsubouchi *et al*., Cell, 2013; unpublished), which has allowed us to investigate specific stages of the ES cell cycle. So far, we have found that DNA replication is regulated differently in ES cells, to the extent that DNA replication of the whole genome is more accurate in ES cells. 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.

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 (Tsubouchi *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 repro-

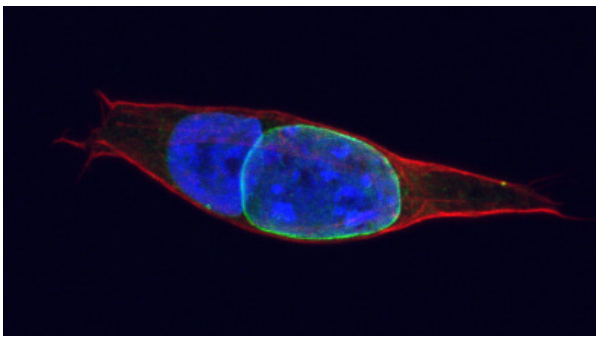


Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus (unpublished).

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〕

- Tsubouchi, T., and Pereira, C.-F. (2021). Reprogramming Stars #1: Genome Programming Through the Cell Cycle-An Interview with Dr. Tomomi Tsubouchi. Cell. Reprogram. *23*, 153–157. DOI: 10.1089/cell.2021.29039.tt