LABORATORY OF STEM CELL BIOLOGY

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DNA is constantly damaged from both endogenous and exogenous sources. One of the most important challenges for all living organisms is to prevent genome instability that can lead to cell malfunction. Our group is interested in the strategies that cells use to protect themselves from alterations in the genome. To date, much information has been gained from various model organisms and tissue culture cells, and we are beginning to learn that the choice of genome-maintenance strategies taken by a cell depends on the cell type, cell cycle- and it's developmental stage. Our focus is on the genome maintenance mechanisms of embryonic stem cells, and their roles during differentiation and reprogramming processes.

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"). Pluripotent cells exist only transiently and are lost as development proceeds. However, ES cells are capable of proliferating indefinitely when given an appropriate culturing condition. Curiously, ES cells proliferate with truncated gap phases while lengths of S (DNA replication) and M (mitosis) phases are similar to other cell types. ES cells also appear to lack some of the mechanisms that ensure genome integrity (*i.e.*, checkpoint mechanisms). The significance of these phenomena remains a mystery.

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 We have now established several protocols to synchronize ES cells (Tsubouchi *et al.*, Cell, 2013; unpublished), which has allowed us to investigate specific stages of the ES cell cycle.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 populations.

II. Genome instability during nuclear reprogramming

In order to gain a deeper understanding of the relationship between the choice of genome maintenance mechanisms and pluripotency, we are investigating the behavior of factors involved in genome maintenance mechanisms during nuclear reprogramming towards pluripotency.

Specifically, 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. 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 from within a few hours to 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 instabilities, 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-specific events, we are in the process of setting up a system to isolate and track a single fused cell through live-imaging (Figure 1).

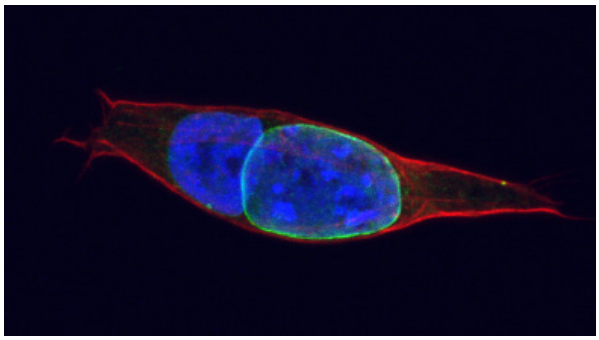

Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus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

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 altering it's cellular identity (through differentiation, reprogramming *etc.*) is 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 uncover the nature of such genetic instability and to gain a comprehensive understanding of the mechanisms that maintain genome integrity.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).