Research

Jonathan Loh's research

Pluripotency and Stemness

Pluripotent stem cells display a remarkable capacity to form differentiated cell types in laboratory cultures. The ability to derive multiple lineages from ESCs opens exciting new opportunities for its use as unlimited source of cells for the treatment of degenerative diseases such as diabetes and Parkinson's disease. The unique properties of pluripotent cells are controlled by genetic and epigenetic factors. We had sought to identify, characterize and understand the role of transcription regulators and chromatin-modifying enzymes in regulating gene expression programs in pluripotent embryonic stem cells. We have detailed the regulatory relationships between the master regulators of ESCs, Oct4, Sox2 and Nanog (Loh et al. 2006; Chew et al. 2005). This has enhanced our understanding of the transcriptional regulatory network and how they orchestrate early cell fate decisions and establish the transcriptional landscape essential for self-renewal and pluripotency. Furthermore we have uncovered novel transcription factors such as *Esrrb* and *Rif1* which regulate self-renewal, pluripotency and differentiation of ES cells (Figure 1) (Loh et al. 2006).

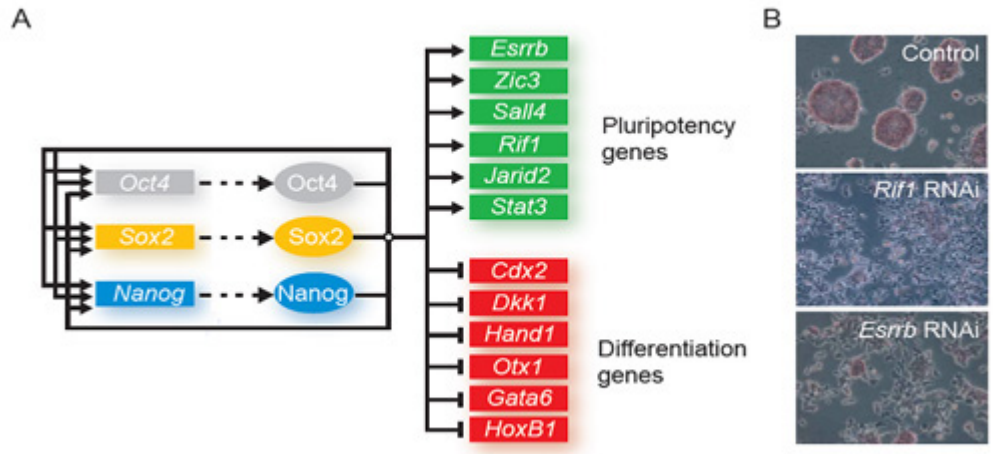

Figure 1: (A) Transcription circuitry regulating stem cell pluripotency. (B) Knockdown of Esrrb or Rif1 led to differentiation of ES cells. Cells were stained for alkaline phosphatase (pink), which is a characteristic of non-differentiated cells. (Adapted from Boyer et al 2005; Loh et al. 2006; Loh et al. 2011)

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Epigenetic and Cell Identity

We have elucidated the novel link between transcriptional circuitry and epigenetic regulation of ESCs chromatin. We showed that Oct4 controls the expression of genes which encode for histone H3 lysine 9 histone demethylases (Jmjd1a and Jmjd2c) that are important for maintaining the ESCs state through their regulation of the H3K9me status at the promoters of pluripotency genes such as *Tcl1* and *Nanog* (Loh et al. 2007, Loh et al. 2008).

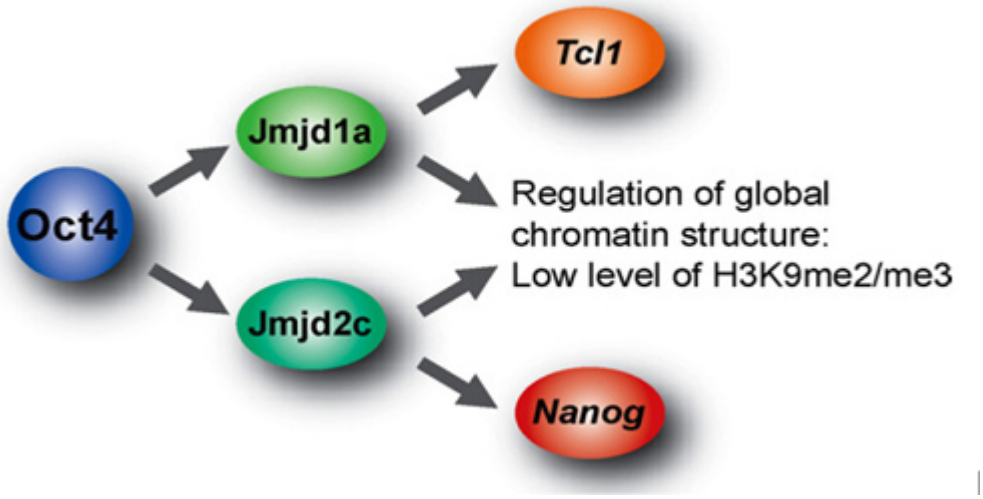

Figure 2: Transcription Network modulation. Transcription factors and epigenetic factors interact to modulate the ESC regulatory network. Several ESC-specific epigenetic factors are regulated by the core ESC transcription factors. For example, Oct4 activates the expression of histone demethylases, Jmjd1a and Jmjd2c, which in turn modify global chromatin H3K9 methylation and regulate the expression of *Tcl1* and *Nanog* (Loh et al. 2007) (Adapted from Ng et al. 2008).

Endogenous Retroviral Elements and Stemness

ESCs repress the expression of exogenous proviruses and endogenous retroviruses (ERVs). We have systematically dissected the cellular factors involved in provirus repression in embryonic carcinomas (ECs) and ESCs by a genome-wide siRNA screen (Yang et al. 2015). Histone chaperones (Chaf1a/b), sumoylation factors (Sumo2/Ube2i/Sae1/Uba2/Senp6) and chromatin modifiers (Trim28/Eset/Atf7ip) are key determinants that establish provirus silencing. We uncovered the roles of Chaf1a/b and sumoylation modifiers in the repression of ERVs using RNA-seq analysis. We also demonstrated direct recruitment of Chaf1a and Sumo2 to ERVs by ChIP-seq analysis. We illustrated a model where Chaf1a reinforced transcriptional repression via its interaction with members of the NuRD complex (Kdm1a, Hdac1/2) and Eset, while Sumo2 orchestrated the provirus repressive function of the canonical Zfp809/Trim28/Eset machinery by sumoylation of Trim28. Our study represent the first genome-wide atlas of functional nodes that mediate proviral silencing in ESCs, and illuminates

the comprehensive, interconnected and multi-layered genetic and epigenetic mechanisms by which ESCs repress retroviruses within the genome.

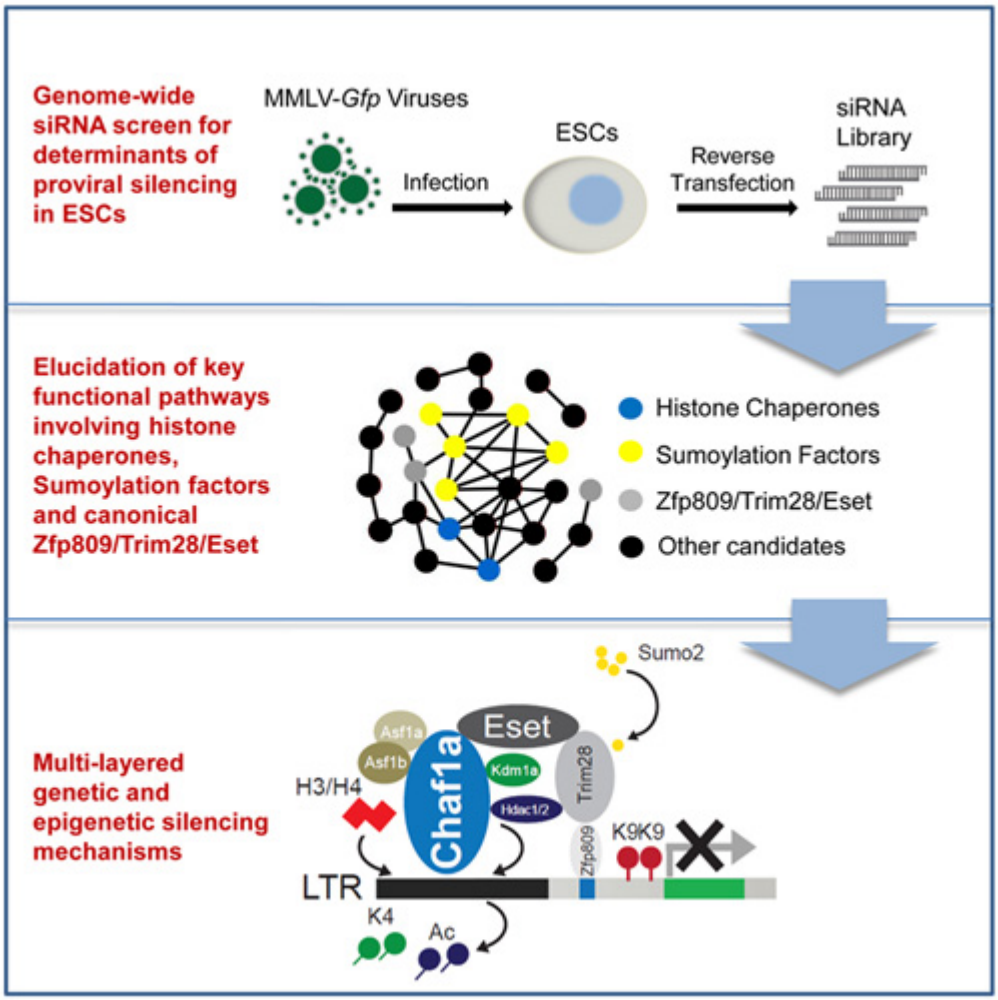

Figure 3: Work flow for systematic dissection of the cellular factors involved in retroviral repression in embryonic Stem cells by a genome-wide siRNA screen. Histone chaperones (Chaf1a/b), sumoylation factors (Sumo2/Ube2i/Sae1/Uba2/Senp6) and chromatin modifiers (Trim28/Eset/Atf7ip) are identified as key determinants. Sumo2 orchestrates viral silencing through sumoylation modification of Trim28. Chaf1a regulates provirus and ERVs via its interaction with Eset, Kdm1a and Hdac1/2 (Adapted from Yang et al. 2015).

Cell Fate Reversal and Reprogramming

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients can be a good model for studying human diseases and for future therapeutic regenerative medicine. Current initiatives to establish human iPSC (hiPSC) banking face challenges in recruiting large numbers of donors with diverse diseased, genetic, and phenotypic representations. We have pioneered novel techniques in establishing iPS cells from blood cells which are more

accessible and requiring lesser manipulations (Loh et al. 2009; Loh et al. 2010). More recently, we describe the efficient derivation of transgene-free hiPSCs from a single human finger-prick blood. Finger-prick sample collection can be performed on a “do-it-yourself” basis by donors and sent to the hiPSC facility for reprogramming. We show that single-drop volumes of finger-prick samples are sufficient for performing cellular reprogramming, DNA sequencing, and blood serotyping in parallel. Our novel strategy has the potential to facilitate the development of large-scale hiPSC banking worldwide (Tan et al. 2014).

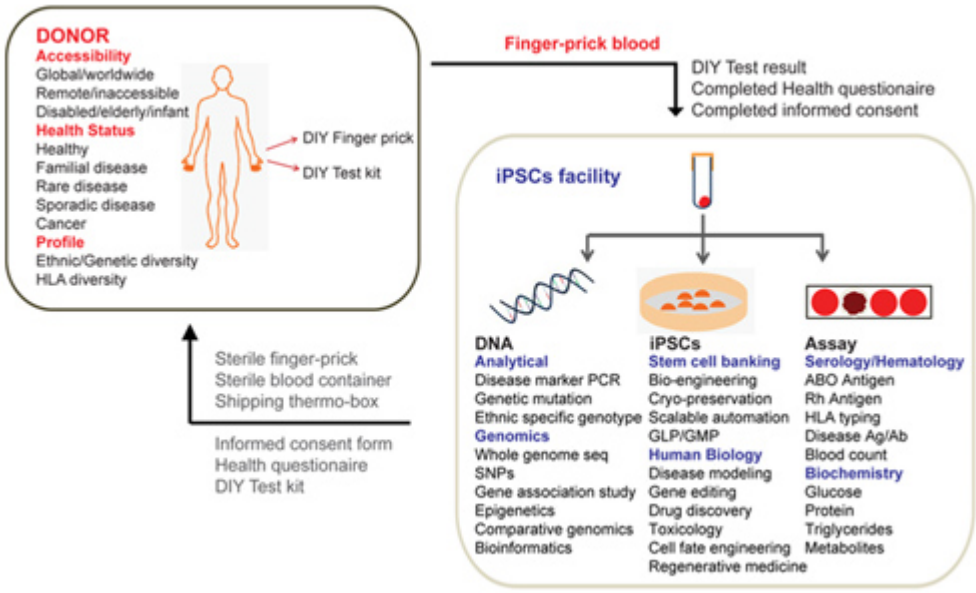

Figure 4: Integrative strategy for human induced pluripotent stem cells (hiPSC) banking. Illustration of the integrative strategy for hiPSC banking. Using finger-prick (FP) blood reprogramming, hiPSC facilities can recruit a diverse cohort of donors worldwide. hiPSC facility provides a kit containing sterile finger-prick and blood container to the donor. The donors will complete the informed consent form and the health questionnaire and return them back to the facility together with their FP blood. The facility is able to do a series of DNA sequencing, serological assays, as well as hiPSC derivation from a single drop of FP blood sample. Abbreviations: Ag/Ab, antigen/antibody; DIY, do-it-yourself; HLA, human leukocyte antigen; iPSC, induced pluripotent stem cell; GLP, good laboratory practice; GMP, good manufacturing practice; PCR, polymerase chain reaction; seq, sequencing; SNP, single-nucleotide polymorphism. (Adapted from Tan et al. 2014).

Deciphering the Mechanisms of Cell Fate Reprogramming

Incomplete knowledge of the mechanisms at work continues to hamper efforts to maximize reprogramming efficiency. We established a systematic genome-wide RNAi screen to determine the global regulators during the early stages of human reprogramming. Our screen identifies functional repressors and effectors that act to impede or promote the reprogramming process. Repressors and effectors form close interacting networks in pathways, including RNA processing, G protein signaling, protein ubiquitination, and chromatin modification. Combinatorial knockdown of five repressors (SMAD3, ZMYM2, SFRS11, SAE1, and ESET) synergistically resulted in 85% TRA-1-60-positive cells. Removal of the novel splicing factor SFRS11 during reprogramming is accompanied by rapid acquisition of pluripotency specific spliced forms. Mechanistically, SFRS11 regulates exon skipping and mutually exclusive splicing of transcripts in genes involved in cell differentiation, mRNA splicing, and chromatin modification. Our study provides insights into the reprogramming process, which comprises comprehensive and multi-layered transcriptional, splicing, and epigenetic machineries (Toh et al. 2016)

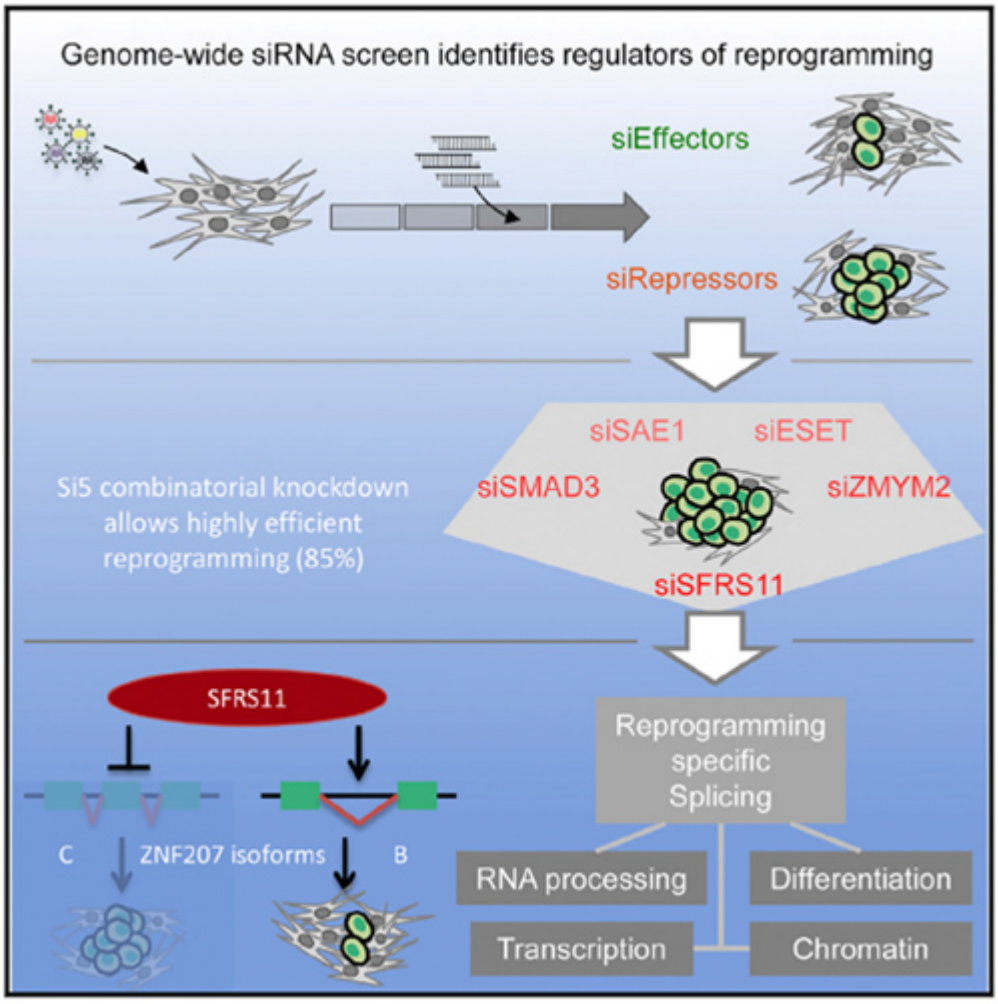

Figure 5: Identification of genome-wide functional regulators of the early stages of human somatic cell reprogramming in a phase-specific RNAi screen. Combinatorial depletion of the top repressors allows reprogramming to proceed unhindered at a near deterministic efficiency. SFRS11, an mRNA splicer, blocks reprogramming partially through the splicing of ZNF207 isoforms. (Adapted from Toh et al. 2016).

Our current research focuses on understanding the molecular mechanisms underpinning the process of cellular reprogramming and cell fate decision. Ongoing projects include: 1) Understanding the function of DNA and Histone modifiers in cell fate changes and 2) Epigenetic Reprogramming/Transdifferentiation using defined factors expression. Ultimately we want to harness these molecular switches to derive high-quality stem cells or differentiated cell types that can potentially be used for therapeutic applications.