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REVIEW

Phases of reprogramming

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Abstract Despite advances in the field of somatic cell reprogramming, an understanding and exploration of the underlying mechanisms governing this process are only recently emerging. It is now increasingly apparent that key sequential events correlate with the reprogramming process; a process previously thought to be random and unpredictable is now looking, to a greater extent, defined and controlled. Herein, we will review the key cellular and molecular events associated with the reprogramming process, giving an integrative and conciliatory view of the different studies addressing the mechanism of nuclear reprogramming.

Contents

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Introduction

Since the discovery that somatic cells could be reprogrammed to induced pluripotent stem cells (iPSCs) (Takahashi and

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Yamanaka, 2006), many different pathways have been created based on Waddington's adaptation of the "epigenetic landscape", the model used to illustrate cell differentiation during development (Waddington, 1954). The somatic cell reprogramming and the process of transdifferentiation, further expanded the boundaries of cell plasticity giving rise, for example, to a non-hierarchical model of cell fate transition, represented by an "epigenetic disk" in which the ball of cell fate could assume any cell fate, provided that the master transcription factors were sufficiently expressed (Ladewig et al., 2013). While there is little doubt that such cell fate conversions are reproducible, a major hurdle that precludes further study of the reprogramming process is their low efficiency (Ho et al., 2011; Stadtfeld and Hochedlinger, 2010). To overcome this, secondary systems were implemented, and the resultant transgenic fibroblast could be reprogrammed through inducible expression of Oct4, Klf4, Myc and Sox2 (OKMS) (Carey et al., 2010; Maherali et al., 2008; Nagy, 2013; Stadtfeld et al., 2010; Woltjen et al., 2009). These improved reprogramming systems usually utilize doxycycline-inducible reprogramming factors. This allows temporally-controlled induction of expression of the reprogramming factors as well as a higher degree of homogeneity. As will be discussed during this review, the majority of the studies addressing the mechanism of reprogramming have made use of a secondary system.

First milestone: iPS cells are equivalent to ESC and can be obtained from the reprogramming of any adult cell

The first and more important questions that needed to be addressed were whether iPSCs were in fact identical to ESCs, and whether all cells were amenable to reprogramming. It was evident that iPSCs were not only morphologically and functionally equivalent to ESCs, but were also similar both transcriptionally and epigenetically (Maherali et al., 2007; Mikkelsen et al., 2008; Okita et al., 2007; Takahashi et al., 2007; Wernig et al., 2007). While some studies found differences between ESCs and iPSCs, others that investigated a broader array of samples showed that the heterogeneity between ESC and iPSC lines was mainly due to the method used to derive them (Yamanaka, 2012).

Subsequently, the "reprogramming technology" needed to prove that iPSCs were the result of reprogrammed cells, and not the selection of novel uncharacterized tissue-specific pluripotent cells. This was achieved by reprogramming cells with specific traceable genetic characteristics, such as the albumin promoter in hepatocytes, insulin promoter in pancreatic beta cells or the recombined immunoglobulin locus of B lymphocytes (Aoi et al., 2008; Hanna et al., 2008; Stadtfeld et al., 2008a). Indeed, Hanna and colleagues demonstrated that iPSCs could emerge from daughter cells from any given cell of a starting population, provided that the cells were still viable and the four reprogramming factors could maintain expression for extended periods (Hanna et al., 2009).

The finding that the timing of faithful reprogramming varies widely among cells, suggests that at least one event driving the reprogramming process is likely to be stochastic. A priori the steps leading to successful reprogramming may involve one or several stochastic events and could be divided by: a) the nature of the molecular events taking place during this process, which raises the question of whether reprogramming can be achieved through different molecular pathways (Fig. 1A) and b) the order of these key events: is there a hierarchy or can they be acquired independently? (Fig. 1B). Finally, if these events transpire in an orderly fashion we will be able to unveil them. If on the contrary, these events were acquired "accidently" in nature and timing, their time of occurrence will remain largely unknown and highly susceptible to variability (Fig. 1C).

In the subsequent sections we attempt to consolidate and discuss recent findings that have emerged from the study of the reprogramming process. Primarily, this is composed of three phases: initiation, maturation and stabilization and are discussed in greater detail below (Fig. 2).

Second milestone: unveiling the reprogramming pathway

Early events — initiation phase — first wave

Different molecular transitions during reprogramming were first documented by the laboratories of R. Jaenisch and K. Hochedlinger in 2008, when they described distinct molecular events occurring at defined times during the reprogramming process (Brambrink et al., 2008; Stadtfeld et al., 2008b). These events ranged from downregulation of fibroblast-specific surface markers and the concomitant upregulation of genes associated with the pluripotency network, as well as reactivation of telomerase activity. Based on an extensive transcriptomic profiling time course during reprogramming of fibroblasts in bulk cultures, the reprogramming process was subsequently grouped in to three phases by the laboratory of J. Wrana: the initiation phase, maturation phase and stabilization phase (Samavarchi-Tehrani et al., 2010). This early period also correlated with changes in morphology, such that fibroblast cells (the main somatic cell model used for reprogramming studies) undergo a mesenchymal-to-epithelial transition (MET). Molecularly, this is characterized by a loss of the somatic cell signature, for example the loss of the transcription factors Snai1/2 or Zeb1/2, which was also described in previous studies (Mikkelsen et al., 2008; Sridharan et al., 2009; Stadtfeld et al., 2008b), and the gain of an epithelial signature, such as the expression of Cdh1, Epcam or epithelial-associated miRNA-200 family (Li et al., 2010; Samavarchi-Tehrani et al., 2010). The importance of these cellular changes was further highlighted by the demonstration that the cell shape itself can trigger epigenetic modifications regulating reprogramming (Downing et al., 2013). In this study, MEFs seeded on microgrooved surfaces for 3 days that entered a MET, therefore enhancing the reprogramming efficiency (Downing et al., 2013). Accordingly, in a kinase shRNA screen attempting to lift barriers of mouse reprogramming, top hits were 2 kinases blocking cytoskeletal rearrangement: TESK1 and LIMK2 (Sakurai et al., 2014). Interestingly, TESK1 siRNA led to enhanced reprogramming in human fibroblasts as well (Sakurai et al., 2014). Aside from MET-associated changes,