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Cell Cycle Rules Pluripotency

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Stem cell self-renewal is intrinsically associated with cell cycle control. However, the precise mechanisms coordinating cell fate choices and cell cycle remain to be fully uncovered. Now in *Cell*, Gonzales et al. (2015) and colleagues demonstrate that factors controlling the G2/M phase are necessary to block pluripotency upon induction of differentiation.

Stem cells are defined by their ability to proliferate almost indefinitely while maintaining their capacity to differentiate into several cell types. The coordination of these two properties, self-renewal and multipotency, is essential to ensure proper embryonic development, organogenesis, organ homeostasis, and tissue repair upon injury. Furthermore, uncontrolled proliferation of stem cells could play a major role in diseases such as cancer. Thus, understanding the interplay between cell-cycle regulation and cell fate decisions represents a major interest for the stem cell field. Nonetheless, the study of these mechanisms has been restricted by the technical difficulties impairing investigations of cell-cycle regulations in vivo and also by the lack of appropriate in vitro model systems. Now in *Cell*, Huck-Hui Ng and colleagues demonstrate that pluripotency of human embryonic stem cells (hESCs) is controlled by factors necessary for the transition of the G2/M phase of the cell cycle. This study provides new insights into the mechanisms by which stem cells exploit cell-cycle machinery to control their cell fate decisions (Gonzales et al., 2015).

The cell cycle can be divided into four different phases: the G1 phase, during which a cell decides to engage in a new division; the S phase, when DNA is replicated; the G2 phase, which allows DNA repair mechanisms; and the M phase, at the end of which cells divide. The G1 phase has been the focus of a broad number of studies on stem cells since cell fate choices seems to occur or at least be initiated during this part of the cell cycle. Indeed, several reports have shown that stem cells can perceive differentiation signals specifically in G1. Of particular interest, the early G1 phase is permissive for endoderm differentiation in hESCs while the late G1 phase is only permissive for neuroectoderm specification (Pauklin and Vallier, 2013). This divergent capacity of differentiation is established by CyclinD/CDK4-6, which are expressed during the late G1 phase. These cell-cycle regulators inhibit the Activin/Nodal signaling pathway, which is known to block neuroectoderm differentiation of hESCs while being necessary for endoderm differentiation. Importantly, the importance of the G1 phase and CyclinD/CDK4-6 appear to be conserved in adult stem cells (Lange et al., 2009; Mende et al., 2015). Thus, the cell-cycle machinery could directly orchestrate initiation of differentiation during the G1 phase progression in a diversity of cell types.

The importance of S/G2 in stem cell control is by far less explored. Regulators of these phases of cell cycle such as Cyclin B1 are necessary for cell survival and their absence often results in cell death and/or major genomic anomalies. For these reasons, the importance of the G2 phase in stem cell self-renewal and differentiation remains to be fully uncovered. The report by Gonzales et al. (2015) remedies this shortfall by revealing that the G2 transition could also have a key function in the mechanisms directing hESC differentiation.

The authors first performed an siRNA screen to identify factors that could delay differentiation induced by the absence or inhibition of TGFβ/Activin/Nodal and FGF signaling, both of which are known to be essential for the pluripotent state of hESCs (Thomson et al., 1998; Vallier et al., 2004). This screen revealed that several epigenetic mechanisms such as histone acetylation and chromatin remodeling are essential for the transition between pluripotency and differentiation to

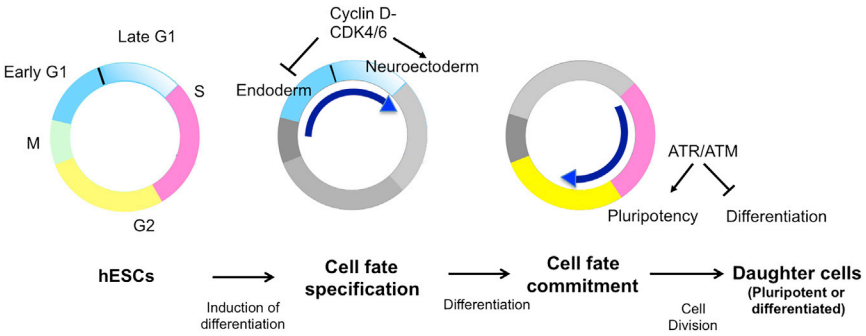

Figure 1. Cell Fate Specification Starts in the G1 Phase When hESCs Can Sense Differentiation Signals

Cell fate commitment is only achieved in G2/M, when pluripotency is dissolved through cell-cycle-dependent mechanisms. Control of G2/M factors and subsequently absence of ART/ATM is necessary for this process.

occur normally. The enrichment in cell-cycle genes controlling DNA replication and G2 phase was less expected. To further validate these observations, the authors used either shRNA or small-molecule inhibitors to block regulators of each phase of the cell cycle during spontaneous differentiation of hESCs provoked by the absence of TGFβ and FGF. These experiments showed that regulators of the S and G2 phases are necessary for rapid decrease of pluripotency upon induction of differentiation in these culture conditions. Knockdown of regulators of the G1 phase such as CDK4/6 or CyclinD had little effect on pluripotency markers, thereby confirming the S/G2 specificity of these results. Further investigations revealed that the DNA damage checkpoint factors ATR/ATM participate directly in these mechanisms by enhancing the activity of the TGFβ/Activin/Nodal pathway through p53 during S and G2. The upregulation of TGFβ then delays the decrease in the expression of pluripotency markers.

Considered together, these results imply that induction of differentiation occurs during the G1 phase while loss of pluripotency is achieved subsequently in S and G2. This model (Figure 1) could explain how cell fate specification and cell fate commitment can be achieved during cell-cycle progression upon differentiation. Indeed, hESCs could engage toward differentiation in early G1 and then decide only in G2 to fully commit toward a specific lineage by dissolving their pluripotent state. This model is in agreement with the notion that major epigenetic events occur during S phase. Indeed, inheritance of DNA methylation and histone marks in daughter cells occurs during the S and G2 phases, respectively. Thus, it would be rational for stem cells to change their cellular identity during these phases of the cell cycle when epigenetic marks are established.

However, these new results also raise several important questions. The involvement of a DNA repair checkpoint is intriguing since these mechanisms are associated with exposure to agents damaging DNA. The molecular link between these mechanisms, i.e., normal cell-cycle progression and cell fate decisions, remains to be fully uncovered. An intriguing possibility could be that incomplete differentiation signals activate the ATR/ATM response, which in turn reinforces pluripotency to avoid the production of "abnormal" cells. This hypothesis could be validated by the confirmation that the mechanisms described by the current study function during directed differentiation of hESCs into cells representative of the three germ layers. Furthermore, induction of differentiation and loss of pluripotency represent two aspects of the same process and are intrinsically linked. Indeed, differentiation of hESCs is orchestrated by the cooperation of pluripotency factors and developmental regulators (Radzisheuskaya et al., 2013; Teo et al., 2011), while signaling pathway such as TGFβ signaling are often necessary to maintain the transcriptional network characterizing both the pluripotent and differentiated state (Brown et al., 2011). Thus, the transition between pluripotent and differentiating cells is likely to be controlled by complex and interlinked molecular mechanisms during cell-cycle progression upon differentiation. Finally, an important challenge will be to demonstrate that such mechanisms are relevant for natural development and adult stem cells. Indeed, further studies will be necessary to demonstrate that perturbation of the ATR/ATM pathway can modify the differentiation capacity of adult stem cells. If true, these mechanisms could provide a new approach to control stem cell potency in vivo.

Thus, the current report represents an important step toward the understanding of the interplays between cell cycle regulation and cell fate decisions in stem cells. Such knowledge could be essential to control stem cell potency not only in vitro but also in vivo in the context of organ regeneration and disease.

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