# OVERCOMING EPIGENETIC ROADBLOCKS

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## Standfirst

Induced pluripotent stem cells (iPSCs) are invaluable tools both for research into pluripotency and development, as well as in the fields of regenerative and personalised medicine. They represent a unique resource, as they can be generated from a minimally invasive tissue sample obtained from an individual. However, much is still unclear about the molecular processes underlying this reprogramming and efficiencies remain low. The process of creating iPSCs requires resetting the epigenome of somatic cells toward a pluripotent chromatin state. A new chemical screen identifies epigenetic and signalling roadblocks for reprogramming of human somatic cells, with the inhibition of these roadblocks resulting in a more permissive epigenome for reprogramming. These findings shed light onto the complex epigenetic mechanisms underlying cellular reprogramming and are of high importance towards a more reliable production of iPSCs.

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During development, pluripotent stem cells differentiate toward all specialised cell types of a mature organism [2]. A well-known metaphor of cellular differentiation is Waddington's epigenetic landscape. Essentially, embryonic stem cells start atop a steep hill early in development. Over the course of subsequent development, these cells roll down the hill while taking various routes, symbolising lineage commitments. In the end, the cells will have reached one of the many valleys surrounding the central mountain and are stuck here. In 2006, this dogma was challenged by showing that forced expression of a select combination of transcription factors induced somatic cells to revert to a pluripotent state, essentially representing a climb back up Waddington's epigenetic landscape (Figure 1) [3]. Cells reprogrammed in this way are known as induced pluripotent stem cells, and these have since been used to study the biological underpinnings of pluripotency and differentiation. Furthermore, their great potential in personalised and regenerative medicine is becoming increasingly clear (e.g. for patient-specific disease modelling and drug screening) [4]. However, reprogramming of somatic cells remains inefficient (between 0.1–3% of all cells successfully reprogram), and numerous important questions underlying the reprogramming process have remained unanswered, such as which features make donor cells refractory to reprogramming [5, 6]. In a recent study published in *Nature Chemical Biology*, Kim *et al.* show that chemical inhibition of epigenetic and signalling factors results in a chromatin state that is more permissive for reprogramming (Figure 1) [7]. This permissiveness not only improves reprogramming efficiencies but also broadens the scope of transcriptional regulators that are able to effectuate reprogramming.

Establishment and maintenance of cell identity are governed by epigenetic modifications. These include post-translational histone modifications and DNA methylation, which affect transcription factor binding and accessibility of genes, thereby regulating gene expression [8]. As such, cellular differentiation is associated with a widespread reorganisation of the epigenome. In view of the stability of the epigenome of somatic cells, the pre-existing epigenome is expected to pose a substantial barrier for cellular reprogramming toward pluripotency. To overcome this barrier, Kim *et al.* applied an extensive chemical screen targeting epigenetic and signalling pathways to identify the main roadblocks for inducing pluripotency of human fibroblasts [7]. Chemical inhibitors that drastically improved reprogramming efficiencies targeted five main pathways: (I) DOT1L-mediated mono- and demethylation of H3K79*; (II) LSD1-mediated H3K4 and H3K9 demethylation; (III) HDAC-mediated histone deacetylation; (IV) TGF-β signalling; and (V) DNMT-mediated DNA methylation—see figure legend for abbreviations. Notably, sequential administration of these inhibitors displayed a synergistic effect, revealing some of the complex hierarchical epigenetic dynamics that occur during reprogramming. In particular, inhibitors of DOT1L represented the most effective compounds in facilitating reprogramming. Besides an expected strong reduction in H3K79me1/2, inhibition of DOT1L consistently resulted in reduced global levels of H3K27me1/2. This is a remarkable observation, as there is no established crosstalk between H3K79me1/2 and Polycomb Repressive Complex 2, the catalytic protein complex mediating methylation of H3K27. Together, these results show that H3K79me1/2, possibly together with H3K27me1/2, represent a major barrier in reprogramming.

Despite the profound similarity on structural, spatiotemporal, and functional levels, only one of eight known OCT factors, namely OCT4, can complement the reprogramming factors SOX2, KLF4, and c-MYC (SKM) in facilitating reprogramming. Kim *et al.* attribute this to the differential genomic binding between OCT4 and the other OCT proteins, including OCT4-exclusive binding at the enhancers of pluripotency factors [7]. Interestingly, the more permissive epigenomic state resulting from DOT1L inhibition allowed for the substitution of OCT4 in the reprogramming cocktail by other OCT factors. The authors hypothesise that this might be facilitated by increased binding of these OCT proteins at canonical OCT4 genomic binding sites, including at the enhancers for important pluripotency-associated genes like *NANOG* and *OCT4*. These enhancers likely become accessible for binding of these OCT proteins due to loss of H3K27me2, as H3K27me2 keeps enhancers poised [9]. Additional factors that were found to be able to substitute OCT4 during reprogramming from a

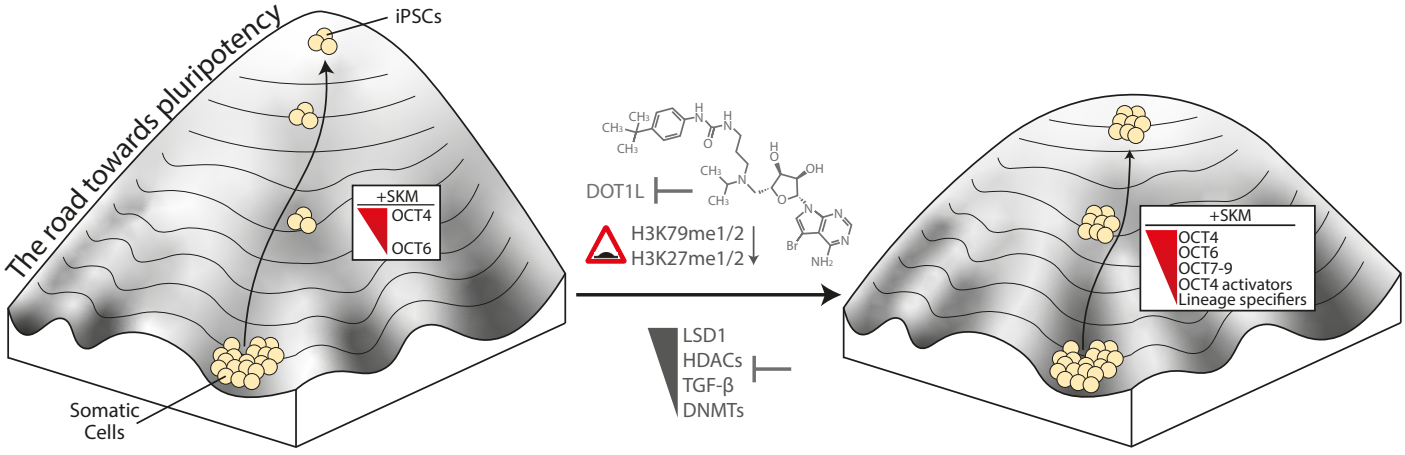


***Figure 1: Targeted chemical inhibition alters the epigenome of somatic cells toward a more permissive state for reprogramming.***
*In these epigenetic landscapes of Waddington, the slopes represent barriers faced during the reprogramming of somatic cells toward induced pluripotent stem cells (iPSCs). Following chemical inhibition of key epigenetic and signalling pathways, major epigenetic roadblocks (including H3K79me1/2 and H3K27me1/2 by inhibition of DOT1L) are removed. As a result, the landscape adopts a more permissive state in which barriers for reprogramming are lowered. Consequently, the reprogramming efficiency improves, while also allowing identification of low-competent reprogramming factors. DOT1L, disruptor of telomeric silencing 1-like; LSD1, lysine-specific histone demethylase 1; HDAC, histone deacetylase; TGF-β, transforming growth factor-beta; DNMT, DNA methyltransferase; OCT, octamer-binding transcription factor; SKM, the reprogramming factors SOX2 (Sex determining region Y box 2), KLF4 (Kruppel-like factor 4) and c-MYC.*

permissive epigenomic state include the lineage specifying transcription factors PAX4, FOXA2, and SIX3, confirming previous reports that these types of proteins can fulfil unexpected roles in reprogramming [10]. Together, these results provide exciting new insights into the mechanisms of reprogramming by showing that pre-existing epigenetic barriers such as post-translational histone modifications can limit the potential of such factors in inducing reprogramming.

In their article, Kim *et al.* provide important tools to improve the efficiency of reprogramming substantially [7]. However, low efficiencies remain a bottleneck. Therefore, an outstanding question is whether screening a broader range of (combinations of) inhibitors targeting epigenetic enzymes will reveal efficient chemical cocktails for reprogramming. With regard to the cells used, Kim *et al.* provide a proof of principle for their reprogramming screens in human fibroblasts [7]. Another important future question concerns whether there is a specificity of the epigenetic inhibitors with regard to cell type, and, if so, what are the associated epigenomic barriers for reprogramming of other types of donor cells. More mechanistically, the observations in the current study lay down the groundworks for in-depth analysis of the interactions between pre-existing epigenomes of donor cells and the transcription factors responsible for the activation of the pluripotency networks during reprogramming.

In conclusion, the pioneering study by Kim *et al.* provides invaluable insights into epigenetic and signalling roadblocks encountered during reprogramming [7]. This will pave the way toward more efficient reprogramming methods for human somatic cells, possibly by chemical compounds only, as has been demonstrated for mouse somatic cells [11].

**These abbreviations refer to specific histone posttranslational modifications and should be read as such: H3 refers to the histone H3 protein (which is one of the subunits that make up the histone octamer at the center of nucleosomes), K79 refers to the lysine residue (K) at the 79th amino acid position of the histone protein, and any further notations refer to a functional group that is covalently bound to the residue (i.e. me1 and me2 for mono- and dimethylation, yielding H3K79me1/2).*

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