Obliterating Obstacles to an Odyssey

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Why is reprogramming to generate induced pluripotent stem cells (iPSCs) a protracted and inefficient odyssey? In this issue of *Cell Stem Cell*, Mor et al. (2018) hypothesize that reprogramming factors paradoxically activate and inhibit pluripotency gene expression and show that eliminating Gatad2a (a NuRD corepressor complex subcomponent) rapidly and efficiently reprograms multiple cell types into iPSCs.

Homer's *The Odyssey* details how Odysseus meandered across distant lands, navigating dangerous side journeys and avoiding entrapment by all types of monstrosities to find his way home to Ithaca. This in many ways parallels the arduous odyssey of cells undergoing reprogramming into iPSCs, which is usually inefficient and lengthy (typically occurring at several percent efficiency over multiple weeks) and contains diversions into unintended termini such as misprogrammed "pre-iPSCs."

Perhaps the greatest surprise is that, analogous to how fickle gods alternately aided and antagonized Odysseus on his journey, the iPSC reprogramming transcription factors (TFs) conspire to incongruously both promote and impede reprogramming (Loh and Lim, 2013). Reprogramming TFs Oct4, Sox2, Klf4, and c-Myc activate pluripotency genes by recruiting coactivators. However, Oct4 and Klf4 also inadvertently silence such genes by recruiting the Nucleosome Remodeling and Deacetylase (NuRD) chromatin repression complex (Rais et al., 2013); likewise, c-Myc recruits other corepressors (Zhuang et al., 2018) to inhibit pluripotency genes (Figure 1A). As shown in this issue of *Cell Stem Cell*, Mor et al. (2018) therefore posited that eliminating NuRD might enable reprogramming factors to exclusively activate pluripotency genes, consequently enhancing reprogramming.

However, suppressing NuRD subcomponent Mbd3 has led to contradictory effects on iPSC reprogramming (dos Santos et al., 2014; Rais et al., 2013). In retrospect these discrepancies are unsurprising. *Mbd3* elimination slows proliferation of, or kills, the starting cells at early stages of reprogramming, necessitating careful (incomplete or later-stage) *Mbd3* suppression (dos Santos et al., 2014; Mor et al., 2018; Rais et al., 2013).

To circumvent this confounding issue, Mor et al. focused on another NuRD subcomponent, Gatad2a. Gatad2a and Mbd3 proteins form a heterodimer that bridges ATP-dependent chromatin remodeler Chd4 to the core NuRD complex (comprising Hdac1/2-Mta1/2/3-Rbbp4/7 proteins) (Torrado et al., 2017). Strikingly, the authors show that suppressing *Gatad2a* significantly enhances the efficiency of, and accelerates, iPSC reprogramming without the unintended consequence of being toxic to the starting fibroblasts (Mor et al., 2018) (Figure 1B). The authors demonstrate this through three independent means—siRNA knockdown, constitutive genetic ablation ($Gatad2a^{-/-}$ cells), and conditional genetic ablation (Cre-treated $Gatad2a^{fl/fl}$ cells)—and verify that re-expressing *Gatad2a* reverses the phenotype. Ultimately, ~90%–100% of $Gatad2a^{-/-}$ cells reprogram in 8 days, of which a ~5- to 10-fold enhancement in reprogramming efficiency relative to normal cells can be ascribed to *Gatad2a* loss (Mor et al., 2018).

An enduring mystery at the crux of this work is why suppressing distinct NuRD subcomponents should lead to diverging phenotypes. First, $Mbd3^{-/-}$ and $Chd4^{-/-}$ mouse embryos perish days earlier than $Gatad2a^{-/-}$ mice (Mor et al., 2018 and references therein). Second, *Mbd3*, *Chd4*, or *Gatad2a* knockdown enhances reprogramming, yet a reduction in other NuRD components (*Hdac1/2* or *Mta2*) is innocuous (Mor et al., 2018; Rais et al., 2013; Zhuang et al., 2018). This may reveal non-NuRD functions of specific subcomponents: Mbd3 may have a role in mitosis (Sakai et al., 2002) and Chd4 in DNA repair, perhaps underpinning why their knockdowns are deleterious to the starting fibroblasts (Mor et al., 2018). Deleting *Gatad2a* dislodges Chd4 from the NuRD complex (Mor et al., 2018), perhaps preventing Chd4 recruitment to NuRD target genes while ostensibly allowing Chd4 to exercise non-NuRD functions integral to fibroblast viability. Another explanation for these diverging phenotypes is that NuRD subcomponents do not coordinately work toward the same objective.

Indeed, referring to a chromatin-modifying machine (e.g., NuRD) as a "complex" can imply that it is stable and singular, and in so doing is misleading. The appellation "NuRD" broadly refers to several protein assemblies, each with mutually exclusive subunit composition and function (Kloet et al., 2015 and references therein). Henceforth each complex should be distinguished with a separate name instead of the umbrella term "NuRD" and a new nomenclature for these varied complexes is needed. Much like building blocks, varied NuRD assemblies are dynamically fashioned in modular fashion from subcomplexes. One model is that there is a stable nucleosome repositioning module (Gatad2-Chd4-Doc1) and a separable histone deacetylation module (Rbbp-Mta-Mbd-Hdac): in isolation, each module executes its own enzymatic activity, but when they transiently coalesce they form *holo*-NuRD—capable of both nucleosome repositioning and histone deacetylation (Zhang et al., 2016). Further reinforcing its amorphous nature, NuRD can seemingly tolerate the absence of a "core" component (e.g., Chd4, which is omitted from certain complexes; Low et al., 2016) or the presence of cell-type-specific "accessory" components (e.g.,

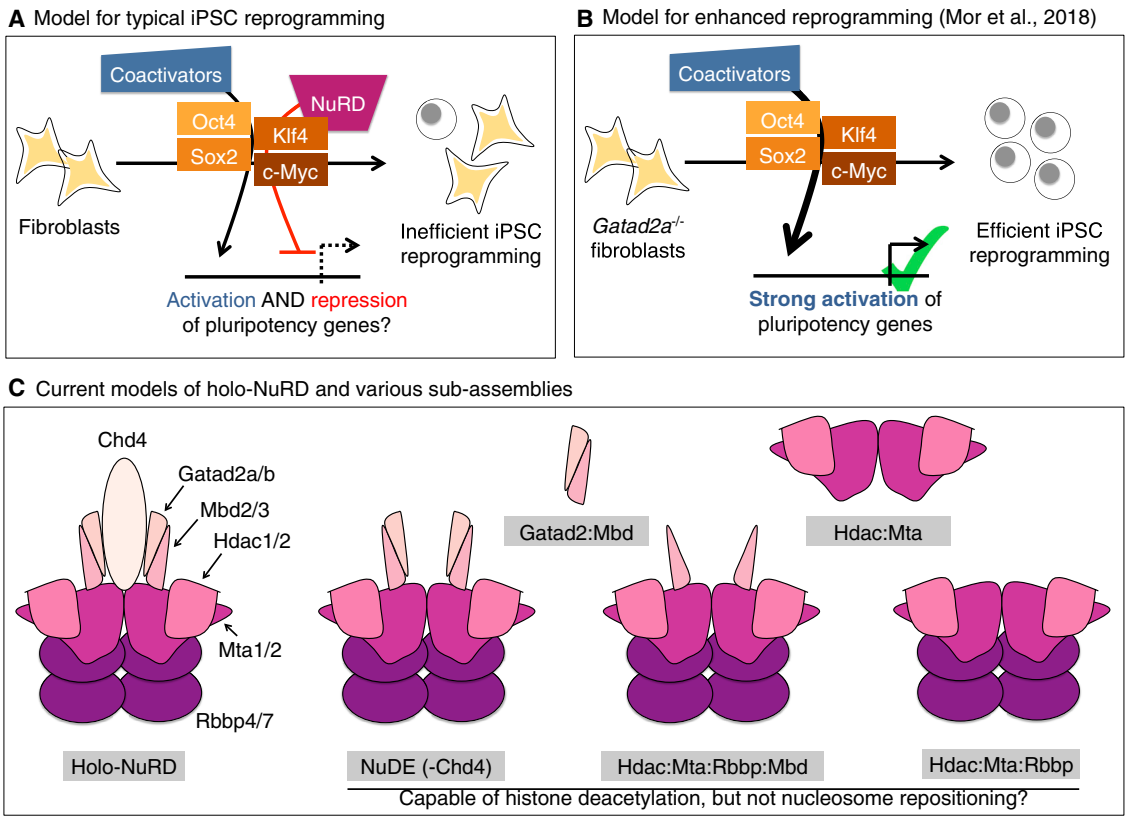

Figure 1. NuRD Assembly and Disassembly

(A) The reprogramming of cells into iPSCs is hypothesized to be inefficient because the reprogramming factors recruit both coactivators and corepressors (e.g., NuRD) to their target pluripotency genes (Mor et al., 2018; Rais et al., 2013; Zhuang et al., 2018).

(B) Deleting *Gatad2a* leads to partial NuRD complex disassembly. This is thought to enable reprogramming factors to exclusively activate their target pluripotency genes, thereby enhancing the efficiency and rapidity of iPSC generation (Mor et al., 2018).

(C) Proposed structures of varied NuRD assemblies, including the fully-fledged "*holo*-NuRD" complex, inferred from multiple types of experiments (Low et al., 2016; Torrado et al., 2017; Zhang et al., 2016). This emphasizes the modular, building-block nature of NuRD machines.

Oct4; Rais et al., 2013). Finally, "core" components (e.g., Rbbp4/7) are not long-term tenants but are rapidly switched in and out (Kloet et al., 2015). While construing a massive macromolecular machine like NuRD as a static "complex" may have been conceptually helpful at one point, this belies the reality that it has no singular obligatory composition and there are transient interactions between constituent substructures (Figure 1C). To graphically illustrate this complexity, we must capture high-resolution structures of *holo*-NuRD and its partially disassembled cousins.

The idea that NuRD proteins dynamically associate with one another and other non-NuRD partners is illuminating, with implications for NuRD deployment to specific target genes by client TFs. When ectopically overexpressed in fibroblasts, Oct4 interacts with NuRD to silence pluripotency genes during reprogramming; yet in its native habitat (naive pluripotent cells), Oct4 does not detectably interact with NuRD (Mor et al., 2018; Rais et al., 2013). This paradox can be reconciled if we do not view NuRD as a stable "complex" with a pre-set geometry and composition but rather as a dynamic machine that can incorporate or reject partner proteins (e.g., Oct4) based on conformational changes, post-translational modifications, concentrations of partner proteins, or cell-type-specific gatekeeper proteins that stabilize or exclude certain partners.

This dynamic association with chromatin-modifying complexes demands a more nuanced view and a more precise use of TFs as instruments of cell-fate change (e.g., reprogramming) informed by mechanism. Reprogramming often entails screening TFs expressed in a given cell type and then ectopically overexpressing them in unrelated cells, with little regard to the fact that these TFs can inadvertently recruit corepressors to undermine their own success. To rationally improve reprogramming, we must discover which chromatin-modifying complexes various TFs interact with, whether these complexes are friend or foe to reprogramming, and what their target genes across multiple stages of reprogramming are. Reprogramming to various cell types (including non-pluripotent lineages) occurs with variable efficiencies and we have scarce understanding as to why. Perhaps in the more successful cases, reprogramming TFs do not accidently recruit corepressors to their target genes. To generally augment reprogramming, we could engineer enhanced instruments that selectively recruit desired chromatin-modifying complexes to desired target genes (e.g., mutant Oct4 unable to interact with NuRD that exclusively activates its target genes to generate iPSCs). Odysseus's woes were

caused in part by capricious gods sending mixed signals: strategically employing reprogramming factors that do not inadvertently recruit corepressors, or engineering them to this end, could expedite the odyssey, perhaps broadly availing reprogramming into diverse lineages.

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Revert the SIRT: Normalizing SIRT1 Activity in Myelodysplastic Stem Cells

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Myelodysplastic syndromes are hematologic malignancies with few treatment options and a propensity to transform to acute myeloid leukemia. In this issue of *Cell Stem Cell*, Sun et al. (2018) report that low SIRT1 levels in myelodysplastic stem cells contribute to aberrant self-renewal through enabling hyperacetylation and reduced activity of TET2.

The seven mammalian sirtuins form a highly conserved family of nicotinamide adenine dinucleotide $(NAD)^+$-dependent histone deacetylases with wide-ranging activities and subcellular localizations (Anderson et al., 2014). SIRT1, the best-studied mammalian sirtuin, serves to deacetylate many targets in the nucleus and cytoplasm including histones, metabolic enzymes, transcription factors, and DNA repair proteins. Its activity counters the development of inflammation and insulin resistance, as well as age-associated conditions such as heart disease, diabetes, and neurodegeneration, at least in part through sensing nutritional stress. In cancer, SIRT1 has variably been reported to have both tumor suppressing and tumor promoting activities in a context-dependent manner (Chalkiadaki and Guarente, 2015).

In this issue of *Cell Stem Cell*, Sun and colleagues (Sun et al., 2018) report that in human myelodysplastic syndrome (MDS) stem and progenitor cells, SIRT1 protein levels are very low and that genetic and pharmacological SIRT1 activation counters their abnormal self-renewal. SIRT1 is therefore a novel therapeutic target in this difficult-to-treat disease. The human myelodysplastic syndromes are a diverse group of clonal myeloid malignancies characterized clinically by ineffective hematopoiesis leading to anemia, leucopenia, and/or thrombocytopenia. Patients often require blood product and/or growth factor support, and there is a frequent propensity for evolution to acute myeloid leukemia (AML). Currently available therapies including hypomethylating agents (e.g., azacitidine) or allogeneic transplantation have only limited efficacy or applicability.

Interestingly, previous studies have found SIRT1 overexpression in other myeloid malignancies compared with its presence in normal hematopoietic stem and progenitor cells (HSPCs), and they have done so to functional effect. In primary human chronic myeloid leukemia (CML) HSPCs, genetic or pharmacological inhibition of SIRT1 impaired growth and enhanced apoptosis through increasing the acetylation and activity of