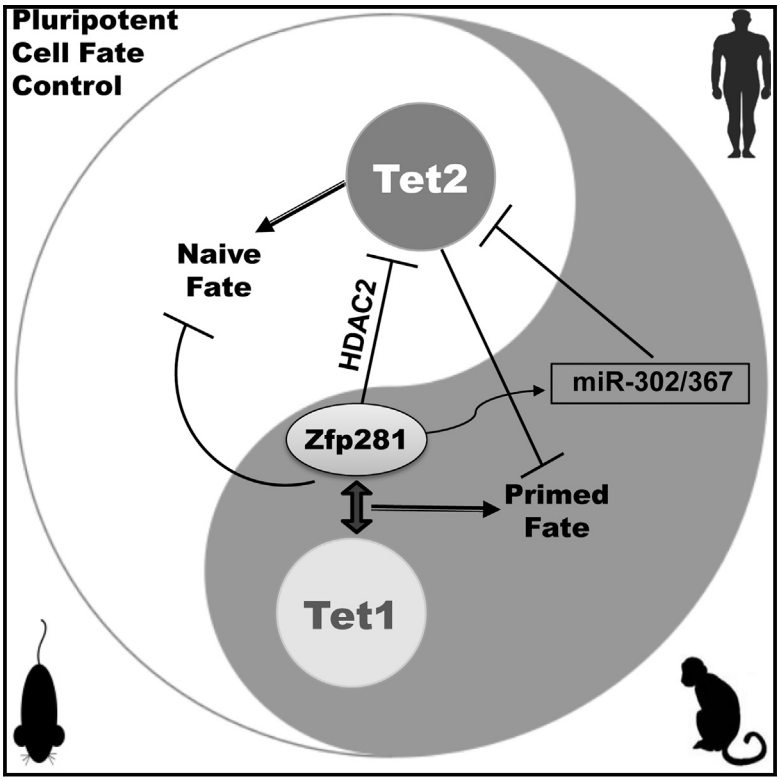
Article

Cell Stem Cell

Zfp281 Coordinates Opposing Functions of Tet1 and Tet2 in Pluripotent States

Graphical Abstract



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In Brief

Wang and colleagues define an evolutionarily conserved pluripotent cell fate (PCF) gene signature that functionally distinguishes naive from primed cells. An RNAi screen for PCF gene regulators led to the discovery of opposing functions of Tet1 and Tet2 mediated by Zfp281 in transcriptional and post-transcriptional control of pluripotent states.

Highlights

- Identification of gene expression signatures for alternative pluripotent states
- Requirement of Zfp281 for the establishment and maintenance of primed pluripotency
- Opposite functions of Tet1 and Tet2 for primed and naive pluripotency
- Transcriptional and post-transcriptional repression of *Tet2* for primed pluripotency

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Zfp281 Coordinates Opposing Functions of Tet1 and Tet2 in Pluripotent States

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SUMMARY

Pluripotency is increasingly recognized as a spectrum of cell states defined by their growth conditions. Although naive and primed pluripotency states have been characterized molecularly, our understanding of events regulating state acquisition is wanting. Here, we performed comparative RNA sequencing of mouse embryonic stem cells (ESCs) and defined a pluripotent cell fate (PCF) gene signature associated with acquisition of naive and primed pluripotency. We identify Zfp281 as a key transcriptional regulator for primed pluripotency that also functions as a barrier toward achieving naive pluripotency in both mouse and human ESCs. Mechanistically, Zfp281 interacts with Tet1, but not Tet2, and its direct transcriptional target, miR-302/367, to negatively regulate Tet2 expression to establish and maintain primed pluripotency. Conversely, ectopic Tet2 alone, but not Tet1, efficiently reprograms primed cells toward naive pluripotency. Our study reveals a molecular circuitry in which opposing functions of Tet1 and Tet2 control acquisition of alternative pluripotent states.

INTRODUCTION

Cell-fate decisions involve coordinated gene regulation at transcriptional and post-transcriptional levels, but the precise mechanisms underlying these complex processes are still poorly defined. Notably, manipulation of key signaling pathways is sufficient to force specific cell types to undergo global transcriptional changes in order to acquire new identities, including pluripotency (Chou et al., 2008). A great interest has thus emerged to understand how these alternative pluripotent identities are regulated (Weinberger et al., 2016; Wu and Izpisua Belmonte, 2015). In mouse embryonic stem cells (ESCs), standard culture conditions containing serum and leukemia inhibitory factor (LIF) (hereafter "SL") support the self-renewal and maintenance of a heterogeneous or metastable pluripotent state (Marks et al., 2012). This in vitro cell identity can be reprogrammed into two inter-convertible and defined pluripotent states by activating distinct signaling pathways. Specifically, serum-free medium containing MEK and GSK3β kinase inhibitors and LIF (hereafter "2iL") supports naive pluripotency that mimics the naive inner cell mass (ICM) of the blastocyst (Nichols and Smith, 2009). Alternatively, a more committed primed pluripotent state resembling post-implantation epiblast cells can be induced in serum-free medium containing the cytokines fibroblast growth factor 2 (Fgf2) and Activin A (hereafter "FA") (Guo et al., 2009; Han et al., 2010).

The functional conservation of pluripotency hallmarks, i.e., unlimited self-renewal and differentiation into all somatic cell types, among these three pluripotent states (naive, metastable, and primed) suggests that common regulatory networks may participate in the pluripotent cell fate (PCF) determination. Considerable progress has been made in identifying transcriptional and epigenetic regulators required for maintenance of gene expression patterns associated with pluripotency identity, mainly under SL and 2iL conditions (Hackett and Surani, 2014). However, our knowledge regarding the molecular players responsible for rewiring the epigenome for proper transcriptional and epigenetic control of naive and primed cell fate acquisition is still limited.

Manipulation of specific signaling cues to directly induce SL ESCs toward naive (2iL) or primed (FA) pluripotent states provides an opportunity to dissect the molecular mechanisms underlying PCF determination. Here, by using this experimental system, we identified the expression pattern of gene sets associated with naive and primed cell identities, referred to as PCF gene signature hereafter, which was found to be evolutionarily conserved in mammals. Using this PCF gene signature as the discovery tool, we performed an RNAi screen to search for transcriptional and epigenetic regulators that control the two pluripotent state transitions. We report here our findings of opposing functions of DNA dioxygenases Tet1 and Tet2 in regulating primed and naive pluripotency, respectively, and of Zfp281 as a key pluripotency factor that coordinates Tet1 and Tet2 functions, transcriptionally and post-transcriptionally, in activation of primed genes and repression of naive genes for primed pluripotency.