Protein Kinases in Pluripotency—Beyond the Usual Suspects

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http://dx.doi.org/10.1016/j.jmb.2017.04.013
Edited by Patrick Griffin

Abstract

Post-translational modification of proteins by phosphorylation plays a key role in regulating all aspects of eukaryotic biology. Embryonic stem cell (ESC) pluripotency, defined as the ability to differentiate into all cell types in the adult body, is no exception. Maintenance and dissolution of pluripotency are tightly controlled by phosphorylation. As a result, key signalling pathways that regulate pluripotency have been identified and their functions well characterised. Amongst the best studied are the fibroblast growth factor (FGF)-ERK1/2 pathway, PI3K-AKT, the leukemia inhibitory factor (LIF)-JAK-STAT3 axis, Wnt-GSK3 signalling, and the transforming growth factor (TGF)β family. However, these kinase pathways constitute only a small proportion of the protein kinase complement of pluripotent cells, and there is accumulating evidence that diverse phosphorylation systems modulate ESC pluripotency. Here, we review recent progress in understanding the overarching role of phosphorylation in mediating communication from the cellular environment, metabolism, and cell cycle to the core pluripotency machinery.

Introduction

Pluripotency is a fundamental of metazoan biology and is defined as the theoretical capability of a single cell to differentiate into any lineage in the developing organism [1]. The potential of pluripotent cells to form any tissue or organ in the body has pushed pluripotency research to the fore in the field of regenerative medicine. Pluripotent cells were initially isolated from developing embryos as embryonic stem cells (ESCs) [2–4]. More recently, induced pluripotent stem cells (iPSCs) have been derived by developmentally reprogramming somatic cells, and these closely resemble ESCs at the molecular level [5–7].

Pluripotency comprises at least two molecularly distinct states, which differ according to species and developmental context [8]. Naïve pluripotency is a developmental "ground state" characteristic of cells from the preimplantation mouse embryonic epiblast [mouse ESCs (mESCs)]. Primed pluripotency is characteristic of post-implantation mouse epiblast stem cells (EpiSCs) and ESCs isolated from human embryos (hESCs), although human naïve pluripotent cells were recently derived from primed hESCs or human embryos [9–16]. An intermediate state termed formative pluripotency was recently described [17], which represents the initial acquisition of developmental characteristics by naïve cells. Molecular distinctions and definitions of pluripotent states have been extensively reviewed elsewhere [18].

Pluripotency acquisition and maintenance are intrinsically linked to the expression of a pluripotency gene regulatory network, particularly the core transcription factor triumvirate of OCT4/PO5F1, SOX2, and NANOG [19–21]. OCT4 is a "governor" of pluripotency and is expressed in both naïve and primed pluripotent states via distinct enhancer elements [22]. OCT4 also controls lineage allocation following pluripotent exit [23]. SOX2 and NANOG are key regulators of naïve and primed pluripotency,

although Nanog is expressed at a reduced level in primed cells. Other transcriptional regulators of pluripotency are differentially expressed in naïve and primed cells. The naïve state is marked by the expression of the key NANOG effector *Esrrb*, Krueppel-like factors (*Klf2/4*), *Rex1*, *Fgf4*, and *Nr0b1* [24]. As the term suggests, primed pluripotent cells express markers of lineage priming, including the de novo DNA methyltransferases *Dnmt3a/b* [25], the embryonic epiblast marker *Fgf5*, and lineage-specific transcription factors such as *Brachyury* [24].

Pluripotency gene regulatory networks are under strict control of extrinsic and intrinsic signalling networks. Dynamic flow of signalling information between and within cells is dependent upon networks of reversible post-translational modifications, particularly protein phosphorylation [26]. Thus, pluripotent states can be accessed and stabilised by modulating activities of protein kinases. Specifically, cytokines, growth factors, and selective protein kinases inhibitors can be exploited to manipulate pluripotency pathways. Pluripotent mESCs were initially captured [24] using a combination of bone morphogenetic protein (BMP) to activate the SMAD1 inhibitor of differentiation pathway and leukemia inhibitory factor (LIF) to activate JAK-STAT3 signalling. Accessing naïve "ground state" mESC pluripotency, however, requires the inhibition of two protein kinases: MEK1/2, which phosphorylates and activates ERK1/2 MAP kinase; and GSK3, an antagonist of Wnt signalling [27,28]. Primed pluripotency in human and mouse is supported by fibroblast growth factor (FGF) and Activin [29–31], whilst human naïve pluripotency can be accessed using distinct combinations of growth factors and inhibitors [9–16].

In this review, we train our focus away from these well-understood pluripotency signalling pathways to explore the role of emerging signalling networks and their impact upon the maintenance and dissolution of pluripotent states. Furthermore, we shed light on exciting but poorly appreciated roles for cell cycle, environmental, metabolic, structural, and stress-regulated phosphorylation networks in pluripotency regulation.

Novel Pluripotency Signalling Pathways

The human protein kinome consists of 538 kinases [†] [32] and includes some of the most studied enzymes in biology. However, vast swathes of the kinome have not yet been investigated, and understudied kinases likely perform key functions in biological processes such as pluripotency maintenance and dissolution [33]. Indeed, total and phosphoproteome analysis indicates that at least 300–400 kinases are expressed in pluripotent mESCs (Jens Hukelman and G.M.F., unpublished data) and hESCs [34]. In this section, we discuss technologies that have been employed to uncover new pluripotency kinase signalling pathways.

Phosphoproteomic profiling is a powerful method to identify novel mechanisms by which phosphorylation modulates pluripotency. Initial studies focussed on identifying novel targets of well-understood signalling pathways (e.g., FGF2 in hESCs) [35,36]. However, unbiased comparison of the phosphoproteomes from pluripotent hESCs and those from differentiating or somatic cells has uncovered new phosphorylated targets relevant for pluripotency regulation. In this manner, multiple receptor tyrosine kinases with previously unappreciated roles in hESC pluripotency were identified [37]. Furthermore, kinase-substrate motif prediction analysis of differentially phosphorylated proteins indicates that distinct families of kinases are active in hESCs compared to somatic cells [38]. Importantly, this approach suggests novel roles for cyclin-dependent kinases (CDKs), Aurora, p38, and c-Jun N-terminal kinases (JNKs) in pluripotency regulation [34] and reveals key hESC phosphorylation modules centred on CDK1/2 [39] and DNA methyltransferases [40]. Perhaps most excitingly, phosphoproteomic studies have the potential to reveal new pluripotency kinases, affirming phosphoproteomics as a core tool for systematic identification of novel mechanisms of pluripotency regulation.

Functional screening has also proven invaluable to uncover new pluripotency kinases. Global RNA interference screens have identified kinases that block mESC pluripotency maintenance [41] and reprogramming to pluripotency [42]. Interestingly, kinases such as TESK1 and LIMK2 identified in these studies have no previously described role in pluripotency or reprogramming. Future research will explore the molecular functions and regulation of these kinases in pluripotent cells. Similarly, cellular screening of kinase inhibitor libraries has elucidated new pluripotency pathways. A survey of selective kinase inhibitors uncovered a novel role for the ERK5 MAP kinase pathway in modulating mESC naïve-primed pluripotent transition [43]. In mESCs, ERK5 promotes the expression of a key network of naïve pluripotency factors, including *Klf2*, *Essrb*, and *Rex1* [43,44], which suppresses the transition of naïve cells to the primed state. Interestingly, both the ERK5 kinase and a C-terminal transcriptional activation domain are required for naïve maintenance [43]. ERK5 may therefore function in concert with transcription factors SP1 and MEF2 [45–47], which are required for *Klf2/4* expression in other developmental systems [44,46,48]. As with many emerging pluripotency pathways, identification of novel ERK5 substrates would shed light on the mechanisms by which ERK5 controls pluripotency. In addition, identifying factors that specifically activate ERK5 may have utility in capturing naïve pluripotency and/or reprogramming somatic cells. In this regard, BMP, LIF, and FGF activate ERK5 in mESCs and other cell types [44,49,50].

Identification of novel pluripotency kinase pathways thus represents an exciting niche within the ESC