News and Commentary

Powering stem cell decisions with ubiquitin

Achim Werner^{*,1} and Michael Rape^{1,2}

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Many types of pluripotent stem cells exist in a quiescent state within specialized hypoxic niches of the metazoan body. Upon receiving appropriate inputs, these progenitor cells can start to proliferate or commit to differentiation programs that give rise to the different cell types of the developing organism.¹ Stem cells and their derivatives tailor their metabolic program to the demands given by their respective cellular states:² while stem cells rely on anaerobic glycolytic energy production to account for hypoxic conditions in the niche and to minimize production of toxic reactive oxygen species, differentiating cells switch to aerobic metabolism to increase energy production. To achieve this metabolic switch, stem cells regulate the biogenesis and function of mitochondria, the cell's powerhouses for anaerobic energy production, yet how this process is regulated is not well understood.

In an article in *Nature Cell Biology*,³ Donato *et al.* reveal a critical function for the ubiquitin-proteasome system in regulating mitochondrial mass expansion during mouse embryonic stem cell (mESC) differentiation. Key target of this pathway is the Kif1-binding protein (KBP) that together with the mitochondrial-associated kinesin KIF1B α controls microtubule–mitochondria interactions. The ubiquitin-dependent degradation of KBP in mESCs limits mitochondrial biogenesis for faithful stem cell maintenance, while inhibition of KBP turnover and its subsequent accumulation at the onset of differentiation allows for expansion of the mitochondrial network for proper differentiation (Figure 1).

The investigators uncovered this pathway by setting out to study the stem cell-specific F-box protein FBXO15, one of ~70 substrate adaptors for SCF (Skp1-Cul1-F-box) ubiquitin E3 ligase complexes. FBXO15 is preferentially expressed in undifferentiated cells, and indeed, had previously been used as a marker for induced pluripotent stem cells.⁴ Through proteomic, biochemical, and genetic analyses, the authors identified KBP as substrate of SCF^{FBXO15}-mediated proteasomal degradation and determined the molecular basis underlying KBP ubiquitylation. While many F-box proteins recognize their targets following substrate phosphorylation,⁵ FBXO15 relies on prior acetylation of a lysine residue within a conserved degron motif in KBP. Substitution of the critical lysine residue with arginine rendered KBP insensitive to SCF^{FBXO15}-mediated degradation. Thus, SCF^{FBXO15} targets acetylated KBP for proteasomal degradation, rendering

FBXO15 one of very few first known acetylation-dependent E3 ligases.

As KBP is turned over in mESCs, but not during differentiation, the acetylation of this important cellular regulator is likely regulated during development. To identify the relevant enzymes mediating KBP acetylation, the authors took a candidate approach. These experiments pointed to the mitochondrial acetyltransferase GCN5L1 and L-threonine dehydrogenase (TDH), an enzyme known to produce mitochondrial acetyl-coA in mESCs,⁶ as factors required for KBP acetylation. Indeed, siRNA-mediated depletion or pharmacological inhibition of GCN5L1 or TDH dampened the acetylation of KBP and consequently stabilized this protein in mESCs. Thus, GCN5L1 and TDH, through mediating the acetylation of KBP, are essential for SCF^{FBXO15}-dependent turnover of KBP in mESCs (Figure 1).

Previous studies had suggested roles for microtubules in mitochondrial biogenesis⁷ and for the KBP–KIF1B α complex in connecting mitochondria to microtubules.⁸ Based on these observations, the authors wondered whether interfering with KBP degradation in mESCs affects microtubule–mitochondria interactions and mitochondrial biogenesis. Confocal microscopy and quantitative image analyses revealed that expression of degradation-resistant KBP resulted in elevated co-localization of mitochondria and tubulin in mESCs and a significant increase in the number and volume of mitochondria per cell. In line with these findings, impeding the proteolytic circuitry by depleting FBXO15, deleting the *FBXO15* gene, or inhibiting TDH, fueled mitochondrial biogenesis in mESCs. As a consequence, mESCs lacking functional SCF^{FBXO15} or expressing stabilized KBP consumed more oxygen, produced more ATP, and generated increased amounts of reactive oxygen species, while showing decreased proliferation rates. These findings indicated that KBP degradation by SCF^{FBXO15} restricts mitochondrial biogenesis and preserves the fitness of mESCs, likely by limiting the production of harmful reactive oxygen species during cellular respiration.

Previous reports had shown that FBXO15 and TDH are transcriptionally silenced at the onset of differentiation.^{6,9} This would allow restriction of KBP acetylation and degradation to the pluripotent state and enable accumulation of KBP during early differentiation when mitochondrial biogenesis is activated. Consistent with this model, the authors found that

¹Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720, USA and ²Howard Hughes Medical Institute, University of California at Berkeley, Berkeley, CA 94720, USA

*Corresponding author: A. Werner, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720, USA. Tel: (510) 642-0468; E-mail: a.werner@berkeley.edu

Figure 1 Upper panel: in stem cells, the E3 ubiquitin ligase SCF^{FBXO15} recognizes acetylated KBP, thereby inducing its ubiquitylation and proteasomal degradation. This limits mitochondrial biogenesis. Lower panel: in differentiating cells, acetylation of KBP and expression of FBXO15 are reduced, resulting in KBP stabilization and enhanced mitochondrial biogenesis

reduction of FBXO15 and TDH during early differentiation correlated with increased KBP levels. Moreover, failure to accumulate KBP levels by ectopic expression of FBXO15 or deletion of *KBP* reduced mitochondrial mass and cellular respiration, and impaired differentiation. Hence, accumulation of KBP is necessary to ensure mitochondrial biogenesis for faithful differentiation.

Taken together, the results by Donato *et al.* identify the microtubule cytoskeleton protein KBP as a key regulator of mitochondrial biogenesis in stem cells and uncover a novel ubiquitin-dependent pathway that ensures faithful stem cell proliferation and differentiation (Figure 1). At the heart of this regulatory pathway is the ubiquitin E3 ligase SCF^{FBXO15}, which mediates KBP proteolysis in a manner that requires substrate acetylation by the mitochondrial enzymes GCN5L1 and TDH. These findings raise intriguing questions and open novel avenues for future investigation. How does KBP regulate the microtubule cytoskeleton to allow the growth of mitochondria? KBP binds to the kinesin KIF1B α and increases microtubule–mitochondria interactions and mitochondrial biogenesis, suggesting that KBP–KIF1B α complexes might connect mitochondria to a microtubule platform that allows for mitochondrial growth. In light of findings that KBP is an inhibitor of kinesin motor activity,¹⁰ determination of the molecular mechanism by which KBP–KIF1B α regulates mitochondrial biogenesis will be an interesting avenue for future research. Moreover, the degradation of KBP by SCF^{FBXO15} relied on its prior acetylation, a process that required mitochondrial acetyl-coA produced by TDH and the

acetyltransferase GCN5L1. This implies that SCF^{FBXO15}-dependent KBP degradation could be a metabolic sensing mechanism to couple mitochondrial acetyl-coA production to mitochondrial biogenesis. It is intriguing to speculate that changes in the pool of acetyl-coA during differentiation could act in concert with the transcriptional downregulation of FBXO15 and TDH to induce mitochondrial growth during differentiation. Finally, F-box proteins frequently use the same binding mode to recognize more than one target.⁵ Searching for other acetylated proteins containing the FBXO15 degron motif of KBP might be a useful approach to identify novel substrates of this stem cell-specific ubiquitin ligase. Studying those substrates could reveal additional roles of SCF^{FBXO15} or of protein acetylation in different aspects of stem cell biology.

Conflict of Interest

MR is cofounder and consultant to Nurix, a company working in the ubiquitin space. WA declares no conflict of interest.

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