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Epigenetic Role of Nuclear S6K1 in Early Adipogenesis

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Abbreviations: S6K1, ribosomal protein S6 kinase 1; mTOR, mammalian target of rapamycin,; EZH2, enhancer of zeste homolog 2; H2BS36p, histone H2B serine 36 phosphorylation; H3K27me3, histone H3 lysine 27 trimethylation

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Abstract

S6K1 is a key regulator of cell growth/size and metabolism. In contrast to the established role of cytosolic S6K1 in these cellular processes, the function of S6K1 in the nucleus remains poorly understood. Our recent study revealed that S6K1 is translocated into the nucleus upon adipogenic stimulus, directly binding to and phosphorylating H2B at serine 36. This phosphorylation promotes EZH2 recruitment and subsequent histone H3K27 trimethylation on the promoter of its target genes including *Wnt6*, *Wnt10a*, and *Wnt10b*, leading to the repression of their expression. The S6K1-mediated suppression of the *Wnt* genes facilitates adipogenic differentiation through the expression of adipogenic transcription factors, *PPARγ* and *Cebpa*. Consistently, white adipose tissues from S6K1-deficient mice exhibit the marked reduction in H2BS36 phosphorylation (H2BS36p) and H3K27 trimethylation (H3K27me3), leading to the enhanced expression of the *Wnt* genes. In addition, levels of H2BS36p and H3K27me3 are highly elevated in white adipose tissues from mice fed on a high-fat diet or from obese humans. These findings describe a novel role of S6K1 as a transcriptional regulator controlling an epigenetic network initiated by phosphorylation of H2B and trimethylation of H3, acting to shut off *Wnt* gene expression in early adipogenesis.

Main Text

Ribosomal protein S6 kinase 1 (S6K1), a key downstream effector of the mammalian target of rapamycin (mTOR), plays a critical role in protein synthesis, lipid synthesis, cell growth, and aging. To understand the molecular mechanism of S6K1-mediated cellular processes, a majority of S6K1 research has continuously focused on identifying downstream target of S6K1. Recent reports revealed that S6K1 phosphorylates CAD (carbamoyl-phosphate

synthetase 2, aspartate transcarbamylase, and dihydroorotase), which explicates S6K1-dependent cell proliferation and DNA synthesis. Another unique substrate of S6K1 is BMAL1, a circadian transcription factor, which associates with translational machinery in the cytosol and promotes protein synthesis by S6K1-mediated phosphorylation. While most substrates of S6K1 have been found in the cytoplasm, nuclear substrate of S6K1 has not yet been clearly identified. Although there is some evidence of nuclear translocation of p70 S6K1, the nuclear function of S6K1 has little experimental support. Limited reports revealed that S6K1 phosphorylates transcription factors including cAMP-response-element modulator τ (CREMτ) and oestrogen receptor α at serine 167. Furthermore, our recent paper demonstrated that nuclear S6K1 acts as a direct histone modifier that phosphorylates histone H2B at serine 36. The level of phosphorylated H2B at serine 36 was increased upon adipogenic stimulus, accompanied by the activation and nuclear translocation of S6K1. Even though, the mechanism behind the translocation of S6K1 into the nucleus is still unknown, our study has clarified a nuclear substrate of S6K1 for the first time.

It has been reported that many signaling kinases phosphorylate histone molecules. MSK1/2, PKB/Akt, and RSK2/p90 S6K2, which belong to AGC kinase family, phosphorylate histone H3. Phosphorylation at each distinct residue of histone by these kinases affects other histone modifications thereby generating an epigenetic network. Interestingly, p70 S6K1, another member of AGC kinase family, has a similar target motif with the histone kinases. Based on our data, like other AGC kinase family members, S6K1 also phosphorylates H2B at serine 36 during adipogenesis. This phosphorylation is required for EZH2 recruitment, which facilitates further methylation related with gene suppression. In this manner, a combination of histone modifications constitutes a code for binding of other proteins, which alter chromatin structure and regulate gene transcription.

Histone crosstalk falls into two categories. First, it is frequently observed that histone phosphorylation has an impact on adjacent modifications within the same histone tail (‘cis’ effects). For instance, phosphorylation of histone H3 at serine or threonine residue promotes acetylation of nearby lysine residue of histone H3, regulating gene transcription. The second type of histone crosstalk occurs between two different histone molecules (‘trans’ effects). For example, modification of histone H2B by monoubiquitination induces methylation of histone H3, specifically H3K4 and H3K79. While histone phosphorylation is known to mainly induce intra-histone crosstalk, we showed that phosphorylation of histone H2B at serine 36 by S6K1 induces methylation of histone H3 at lysine 27, thereby suppressing the transcription of EZH2 target genes. These results suggest the possible involvement of histone phosphorylation in inter-histone crosstalk as well as intra-histone crosstalk.

It has been demonstrated that S6K1-deficient mice are lean at birth and resistant to high fat diet (HFD)-induced obesity. This anti-obesity phenotype of S6K1-deficient mice stems from impaired early adipogenesis. However, the molecular mechanisms underlying the S6K1-mediated promotion of the commitment stage of adipogenesis had been unknown. Our findings demonstrated that stimulus for adipogenic commitment promotes S6K1 activation and nuclear translocation. Activated S6K1–mediated H2BS36p and H3K27me3 suppress the expression of *Wnt6*, *Wnt10a*, and *Wnt10b* genes, eventually facilitating early adipogenesis. Given that obesity results from the increase of number and size of adipocyte, our study suggests S6K1-mediated epigenetic network as a therapeutic target for treatment of obesity.

Additionally, several pieces of evidence implicate that S6K1 is involved in not only adipogenesis but also glucose homeostasis, insulin sensitivity, and lipolysis. These findings suggest that S6K1 could regulate metabolic homeostasis in a complex way. Our recent findings, together with the previous evidence, allow us to refine the molecular model of

S6K1-dependent metabolic regulation (Figure 1). S6K1-mediated transcriptional regulation is apparently independent of its known functions in the cytoplasm. Dual roles of S6K1 in two distinct subcellular locations offer a potential therapeutic avenue for the treatment of obesity and insulin resistance, the hallmark of type 2 diabetes. Future studies on finding new nuclear targets of S6K1 might be able to explain other phenotypes of S6K1-deficient mice, including hypothalamic control of energy homeostasis, muscle atrophy, renal hypertrophy, memory impairment, and extended lifespan.

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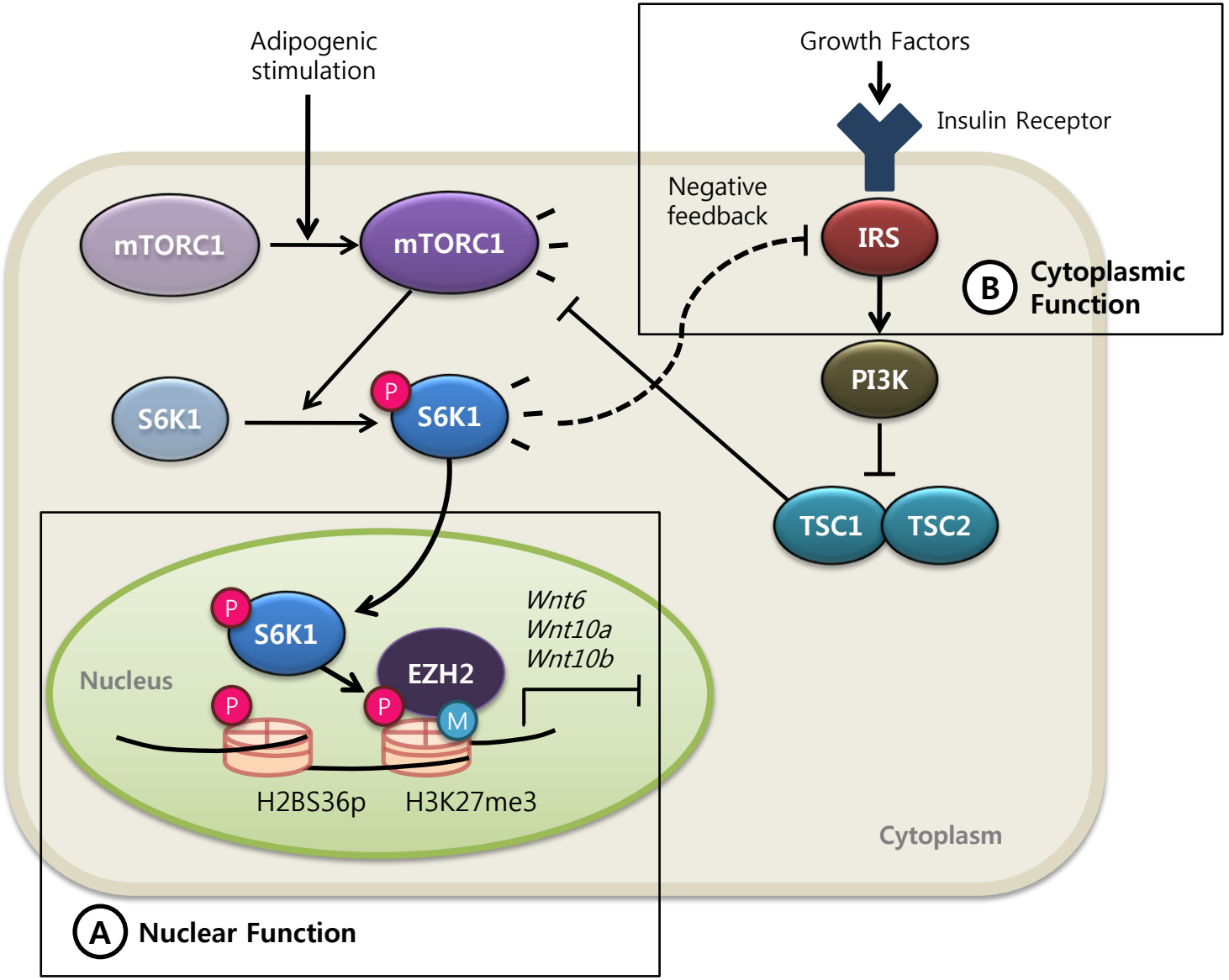
Figure 1. Schematic model of metabolic regulation by S6K1 in two ways

(A) Upon adipogenic stimulation, activated S6K1 moves into the nucleus and phosphorylates H2BS36, further inducing EZH2 recruitment and H3K27 trimethylation. This epigenetic network suppresses the expression of *Wnt6*, *10a*, and *10b* genes, leading to adipogenic commitment. (B) While growth factor-mediated activation of IRS activates mTOR-S6K1 pathway through a series of downstream signaling molecules, activated S6K1 phosphorylates and inhibits IRS operating a negative feedback loop in the cytoplasm.


Adipogenic stimulation
Growth Factors
Insulin Receptor
Negative feedback
mTORC1
mTORC1
IRS
B Cytoplasmic Function
PI3K
S6K1
P
S6K1
TSC1
TSC2
P
S6K1
Wnt6
Wnt10a
Wnt10b
Nucleus
EZH2
P
P
M
H2BS36p
H3K27me3
Cytoplasm
A Nuclear Function