CellPress

An Acetyldegron Triggers CRBN to Take Down the "Q"

Sajjan Koirala[1] and Patrick Ryan Potts[1,*]
[1]Department of Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38105-3678, USA
*Correspondence: ryan.potts@stjude.org
http://dx.doi.org/10.1016/j.molcel.2016.03.003

In this issue of *Molecular Cell*, Nguyen et al. (2016) show that p300/CBP-mediated acetylation of glutamine synthetase (GS) triggers recognition by the CRL4^{CRBN} E3 ubiquitin ligase, resulting in its ubiquitylation and degradation in response to high glutamine concentrations.

Amino acids play many essential roles in a wide variety of metabolic processes including protein synthesis and energy production. Glutamine is the most abundant free amino acid in our body, comprising more than 20%–40% of free amino acid in certain tissues (DeBerardinis and Cheng, 2010). Besides being required for synthesis of proteins, glutamine is also necessary for the de novo synthesis of nucleotides, fatty acids, and glutathione. Additionally, numerous studies have shown that dysregulation of glutamine is common in many diseases, including cancer (DeBerardinis and Cheng, 2010). Despite glutamine's wide-ranging impact on metabolism and pathophysiology, the signaling networks regulating glutamine synthesis, transport, and consumption are poorly understood. In this issue of *Molecular Cell*, Nguyen et al. (2016) provide new insights into how the glutamine biosynthetic enzyme, glutamine synthetase (GS), is regulated.

Glutamine synthetase is conserved from bacteria to humans. It catalyzes an ATP-dependent condensation reaction of glutamate and ammonia to produce glutamine (Crook and Tomkins, 1978). GS is especially critical in the brain, kidneys, and liver for the detoxification of excess ammonia and to maintain pH balance (Taylor and Curthoys, 2004). Past studies have established that cellular GS levels are tightly regulated by glutamine through transcriptional and posttranscriptional mechanisms (Arad et al., 1976; Crook and Tomkins, 1978). For example, high concentrations of glutamine trigger degradation of GS in a proteasome-dependent manner (Crook and Tomkins, 1978). However, the mechanistic details of this regulation, including the identity of E3 ubiquitin ligases involved, have remained elusive. One report showed that the tissue-restricted ZNRF1 E3 ubiquitin ligase is involved in degradation of GS in Schwann cells (Saitoh and Araki, 2010), but how GS is more broadly regulated in other cell types has remained unclear. In the current study, Nguyen et al. (2016) took an unbiased SILAC-based quantitative IP-MS approach to identify GS as a novel binding partner of the immunomodulatory drug (iMiD) binding protein, Cereblon (CRBN). CRBN is an integral substrate receptor protein of the CUL4-DDB1-RBX1 E3 ubiquitin ligase complex (referred to as CLR4^{CRBN}) that functions as a specificity factor to target specific proteins for ubiquitylation by CRL4. Through a number of elegant studies, Nguyen et al. (2016) characterized the molecular mechanism of how CLR4^{CRBN} regulates GS degradation that led to several unanticipated insights, including the identification of an acetyldegron.

First, Nguyen et al. (2016) validated their IP-MS findings and showed that GS is a direct binding partner of CRBN. GS recognition by CRBN led to its polyubiquitylation by CLR4^{CRBN} and degradation by the proteasome (Figure 1). After establishing that CRBN mediates GS ubiquitylation, Nguyen et al. (2016) tested whether CLR4^{CRBN} is the elusive E3 ligase responsible for degradation of GS in response to high glutamine concentrations. Indeed that was the case, as knockdown of CRBN significantly impaired glutamine-induced degradation of GS in a variety of cell lines. Furthermore, they found increased GS levels in $Crbn^{-/-}$ mice and this led to a 25% increase in serum glutamine/glutamate ratio. However, it should be noted that in CRBN$^{-/-}$ cells, GS was not completely stable under high glutamine conditions, suggesting that additional regulatory mechanisms likely exist. Thus, cells employ a multi-layered approach to regulate GS and glutamine levels, which is not surprising given the plethora of important GS and glutamine functions.

Next, Nguyen et al. (2016) characterized the GS-CRBN interaction and found an N-terminal GS-fragment that was necessary and sufficient for CRBN binding and CRL4^{CRBN}-mediated degradation. They carried out extensive mutagenesis and mass spectrometry experiments to uncover a posttranslational modification, acetylation, on GS that was necessary and sufficient for glutamine-induced, CRL4^{CRBN}-mediated GS degradation (Figure 1). In this intriguing finding, Nguyen et al. (2016) identified two lysine residues (K11 and K14) in the N terminus of GS that were acetylated by p300/CBP in response to high glutamine concentrations. These acetylation marks served as a degron to allow CRBN binding and CLR4^{CRBN}-mediated ubiquitylation and degradation of GS in response to high glutamine concentrations. Thus, this acetyldegron is functionally similar to phosphodegrons often required for recognition of substrates by the related CLR1^{FBOX}/SCF ubiquitin ligases. Additionally, N-terminal acetylation has also previously been shown to promote degradation of proteins through the N-end rule pathway (Hwang et al., 2010). The discovery that CRBN also senses an acetyldegron opens up a number of interesting questions, including whether acetyldegrons are found in additional CRBN substrates and whether other CRL4 adaptors may also recognize acetyldegrons. Furthermore, a big unknown is how high glutamine concentrations trigger p300/CBP-mediated acetylation of GS (Figure 1). Nguyen et al. (2016) propose that high glutamine concentrations trigger a conformational change in GS to expose the

CrossMark

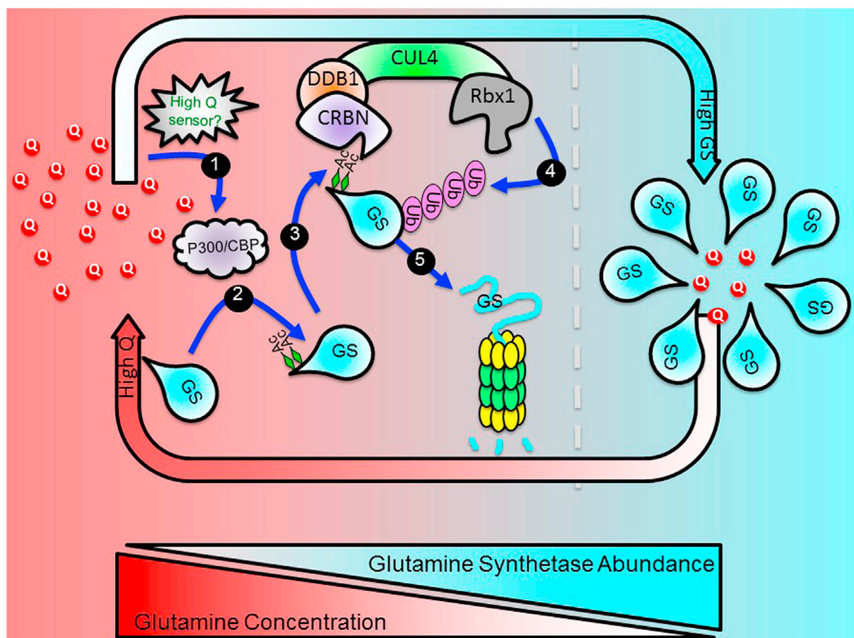

Figure 1. Model Depicting Major Steps in Glutamine-Induced Glutamine Synthetase Degradation

Glutamine (Q) concentration and glutamine synthetase (GS) protein levels are inversely correlated in cells to achieve proper glutamine concentrations. This is in part achieved by the following mechanism for degradation of GS under glutamine replete conditions:

(1) In high cellular glutamine concentrations, an unknown sensor/mechanism relays "high Q" status to p300/CBP.

(2) This triggers p300/CBP-mediated acetylation of GS K11 and K14 creating an acetyldegron.

(3) The acetyldegron of GS is recognized by the CRL4 substrate adaptor CRBN.

(4) $CUL4^{CRBN}$ E3 ubiquitin ligase ubiquitylates GS.

(5) Ubiquitylated GS is degraded by the proteasome, and this establishes low GS levels in cells with high glutamine concentrations.

critical lysines for acetylation by p300/CBP. Alternatively, acetylation is dependent on the availability of the acetyl-CoA metabolite and glutamine can produce acetyl-CoA via gutaminolysis or reductive carboxylation. Thus, elevated glutamine levels may result in increased available acetyl-CoA for acetylation of GS by p300/CBP, leading to GS degradation and decreased glutamine levels. Further studies toward testing these and other models will be important.

The CRBN substrate adaptor has gained much recent attention after the landmark discovery that it is an important target of IMiD drugs, such as thalidomide and lanelidomide. Nguyen et al. (2016) report that unlike MEIS2, the binding of GS to CRBN is not inhibited by thalidomide but is actually enhanced. Detailed mutagenic studies suggested that the acetyldegron of GS binds adjacent to the IMiD pocket. Structural studies will be necessary to reveal the precise mechanism of binding and how IMiDs enhance binding to GS. Furthermore, these studies raise additional questions regarding whether IMiDs regulate GS levels and whether this contributes to their activities. Additionally, several HDAC inhibitors that alter the global acetylation profile of cells are currently in clinical use. The results presented by Nguyen et al. (2016) and others raise new questions about whether these drugs may in part exert their clinical benefit through promoting degradation of specific proteins by the $CRL4^{CRBN}$ ligase.

In summary, the study by Nguyen et al. (2016) has not only answered long-standing questions about GS degradation, but also raises interesting new questions for future research. Given that glutamine addiction is quite common in cancer cells (DeBerardinis and Cheng, 2010), it will be interesting to examine whether the $CRL4^{CRBN}$ GS regulatory mechanism is perturbed in tumors to provide for this addiction. Consistent with this idea, pathogenic mutations exist in p300/CBP and CRBN (Fischer et al., 2014; Iyer et al., 2004). In the future, it will be interesting to determine whether these mutations affect GS stability, glutamine abundance, and tumor growth.

REFERENCES

Arad, G., Freikopf, A., and Kulka, R.G. (1976). Cell *8*, 95–101.

Crook, R.B., and Tomkins, G.M. (1978). Biochem. J. *176*, 47–52.

DeBerardinis, R.J., and Cheng, T. (2010). Oncogene *29*, 313–324.

Fischer, E.S., Böhm, K., Lydeard, J.R., Yang, H., Stadler, M.B., Cavadini, S., Nagel, J., Serluca, F., Acker, V., Lingaraju, G.M., et al. (2014). Nature *512*, 49–53.

Hwang, C.S., Shemorry, A., and Varshavsky, A. (2010). Science *327*, 973–977.

Iyer, N.G., Ozdag, H., and Caldas, C. (2004). Oncogene *23*, 4225–4231.

Nguyen, T.V., Lee, J.E., Sweredoski, M.J., Yang, S.J., Jeon, S.J., Harrison, J.S., Yim, J.H., Lee, S.G., Handa, H., Kuhlman, B., et al. (2016). Mol. Cell *61*, this issue, 809–820.

Saitoh, F., and Araki, T. (2010). J. Neurosci. *30*, 1204–1212.

Taylor, L., and Curthoys, N.P. (2004). Biochem. Mol. Biol. Educ. *32*, 291–304.