Breaking Down the COP9 Signalsome in the Heart
How Inactivating a Protein Ubiquitin Ligase Increases Protein Ubiquitylation and Protects the Heart

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Cellular function depends on protein homeostasis, also known as proteostasis. Proteostasis requires the efficient folding of nascent proteins, as well as the maintenance of mature protein folding. Overseeing proteostasis is a quality control process involving the surveillance of protein-folding status and the subsequent degradation of proteins that are not properly folded. Imbalanced proteostasis resulting from the accumulation of misfolded and unfolded proteins can lead to proteotoxicity, cell death, and ultimately organ failure. Many diseases, including neurodegenerative, hepatic, endocrine, and cardiovascular disorders, are thought to be associated with, if not caused by the organ failure resulting from impaired protein folding. For example, in the heart, impaired protein folding is associated with hypertrophic and dilated cardiomyopathies, as well as ischemic heart disease. Therefore, a better appreciation of the mechanisms governing the recognition and degradation of misfolded proteins will improve our understanding of cardiac physiology and pathology.

UPS plays an important role in proteostasis in the heart and because E3 ubiquitin ligases catalyze the rate-limiting step of the UPS, the E3 ubiquitin ligases, such as the muscle specific ubiquitin ligases, atrogin-1, MuRF1, MuRF3, CHIP, Mdm2, and Trim 32 have attracted interest as potential targets for the design of novel therapies for heart disease.

The E3 ubiquitin ligases, of which there are hundreds, fall into 2 main categories, depending on the existence of either a HECT or a RING finger domain in the catalytic sites. In humans there are ≈40 genes encoding HECT domain E3 ubiquitin ligases and ≈400 genes encoding RING finger domain E3 ubiquitin ligases. Thus, the RING finger domain proteins constitute the vast majority of E3 ubiquitin ligases. Among the RING finger domain E3 ubiquitin ligases are the cullin-RING family of ubiquitin ligases (CRLs), which are by far the most abundant gene products in the RING finger protein family. In fact, CRLs are responsible for the ubiquitylation of as much as 20% of proteins that are targeted for proteasome-mediated degradation. Therefore, it is important to understand the mechanisms by which the activities of the CRLs are regulated because CRL-mediated ubiquitylation of misfolded proteins in cardiac myocytes may play an important role in maintaining proteostasis and thus, proper heart function.

The constitutive photomorphogenic 9 signalsome (COP9 signalsome or CSN) is a critical regulator of CRL activity, but until recently, it had not been studied in the heart. The CSN was identified first in Arabidopsis thaliana as a complex protein comprised of 8 subunits. In Arabidopsis, the CSN was shown to regulate light-dependent development. Subsequently, the CSN has been found in many other species and is evolutionarily conserved from yeast to humans. The CSN plays critical roles in numerous processes, including development, DNA repair, and cytokine signaling. The main biochemical activity of the CSN is neddylation, which is the removal of neural precursor cell expressed developmentally downregulated protein 8 (NEDD8) from neddylated proteins. NEDD8 is an 81-amino acid ubiquitin-like protein that shares ≈60% amino acid sequence identity with ubiquitin. Like ubiquitylation, neddylation of proteins, which requires NEDD8-specific E1 activating, E2 conjugating, and E3 ligases, has been shown to regulate many processes, including transcription, signal transduction, autophagy, and cell death. Dysregulation of neddylation has been linked to a variety of diseases, including heart failure. Even though CRLs must be neddylated to be active, paradoxically it is by removing NEDD8 from CRLs that the CSN increases CRL-mediated protein ubiquitylation (Figure). This paradox can be explained by evidence suggesting that CRLs cannot engage a new substrate for ubiquitylation unless they recycle through the deneddylation/reneddylation process; the CSN is required for that process.

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(Circ Res. 2015;117:914-916. DOI: 10.1161/CIRCRESAHA.115.307664.) © 2015 American Heart Association, Inc.
Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/CIRCRESAHA.115.307664

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Because proteotoxicity from misfolded proteins can cause cardiomyopathy and impaired heart function and because the CRLs, and their major regulator, the CSN were shown in other cell types to enhance the degradation of misfolded proteins, the effects of CSN deletion in the heart were examined initially in the laboratory of Xuejun (XJ) Wang. Germline ablation of any of the CSN subunits had previously been shown to be embryonic lethal; thus, in the laboratory of Wang, Su et al10 studied the roles for the CSN in the mouse heart using a conditional gene targeting approach in which a critical subunit of the CSN, CSN8, was deleted in mouse hearts soon after birth. However, these mice prematurely died of dilated cardiomyopathy and heart failure at ≈30 days of age. Although these findings showed that CSN8 and thus CSN activity are required for normal postnatal cardiac development and function, the premature death of the mice precluded an examination of roles for the CSN in the ubiquitylation and degradation of misfolded proteins in the adult mouse heart. Accordingly, to address this question, in this issue of Circulation Research, Su et al11 engineered a mouse model in which CSN activity was attenuated but not completely inactive; this involved the preparation of a new line of mice in which 1 allele of CSN8 was deleted in a cardiac-specific manner. Initial characterization showed that these CSN8 hypomorphic mice exhibited an ≈80% reduction of CSN8 in the heart and that they survived into adulthood. Moreover, CRL neddylation was increased in the hearts of the CSN8 hypomorphic mice, consistent with roles for the CSN in CRL neddylation.

Su et al11 went on to examine the effects of reduced CSN activity on cardiac pathology in a previously studied mouse model of proteinopathy induced by overexpression of a mutant form of the small heat shock protein CryAB, that is, CryABR120G. When CSN8 hypomorphic mice were crossed with transgenic mice that overexpress CryABR120G, their hearts exhibited increased CryAB-containing aggregates, which have been associated with cardiomyopathy. The CryABR120G-expressing CSN8 hypomorphic mouse hearts also had decreased levels of ubiquitylated proteins, impaired cardiac function, and increased mortality. Moreover, when examined in cultured cardiac myocytes, knockdown of CSN8 or chemical inactivation of CRLs decreased the ubiquitylation and degradation of CryABR120G but not native CryAB and increased cell death in response to CryABR120G expression. Thus, Su et al11 concluded that the CSN promotes the ubiquitylation and degradation of misfolded CryABR120G but not properly folded CryAB. Therefore, the inference is that the CSN protects against proteotoxicity caused by the misfolding of proteins in the heart (Figure).

Su et al11 focused their studies on the effects of CSN inactivation on the degradation of several model overexpressed misfolded proteins, such as CryABR120G; thus, it remains to be determined how many other proteins are affected by CSN
in the heart. However, because the CSN activates CRLs and because CRLs are responsible for widespread ubiquitylation of misfolded proteins in other cell types, it is reasonable to assume that via CRL activation, CSN has a similar widespread function in misfolded protein ubiquitylation in the heart. This assumption is supported by the study by Su et al that CSN deletion or hypomorphism reduced the levels of ubiquitylated proteins, in general. Moreover, Su et al showed that CSN is important for nutrient starvation stress- induced autophagy in cultured cardiac myocytes, which is believed to involve the misfolding of a broad spectrum of cardiac proteins. In addition, this study raises the question of whether the CSN plays a protective role in the ischemic and failing heart, in which function is impaired by protein misfolding. Future examination of the effects of CSN hypomorphism in myocardial infarction and pressure overload models of heart failure will begin to address this question.

In addition to marking proteins that become misfolded for proteasome-mediated degradation, the CSN has been shown to be involved in the selective stabilization and de-stabilization of key signaling proteins that are important in regulating their cellular functions. For example, CSN stabilizes some transcription factors (eg, MYC, p53, and HY5), cell cycle regulators (eg, cyclin E and p27), and signaling proteins (eg, β-catenin and Smad7) and destabilizes c-Jun and HIF1-α. Thus, in addition to targeting misfolded proteins for proteasome-mediated degradation, it is probable that in the heart, CSN is likely to regulate numerous other cellular processes.

The study by Su et al also revealed possible roles for neddylation as a regulator of cardiac myocyte function. Although neddylation has been shown to regulate many cellular functions in the settings of cancer, inflammation, immunodeficiency, and neurodegenerative diseases, little is known about the functions of neddylation in the heart. Substrates for neddylation, such as p53, Mdm2, and the epidermal growth factor receptor, have been examined in various non-cardiac cell types. Now, the study by Su et al provides the initial evidence that neddylation plays important roles in the heart by demonstrating that CSN-mediated deneddylation of CRLs is required for maintenance of optimal heart function.

Future studies on roles for the COP9 signalosome in the heart will undoubtedly reveal many new functions for this complicated, evolutionarily conserved multisubunit protein complex. The elegant experiments on the CNS in the heart that have been reported by Su et al have not only provided an important foundation of information but have also resulted in the generation of novel animal models that will facilitate future studies that are sure to reveal previously unappreciated roles for the COP9 signalosome in proteostasis in the heart.

Acknowledgments
We acknowledge Dr Shirin Doroudgar, Dr Jung-Kang Jin, Adrian Arrieta, Winston Stauffer, Erik Blackwood, and Amber Pentoney for insightful discussions.

Sources of Funding
This work was supported by National Institutes of Health (PO1 HL085577, R01 HL121539, and R01 HL127439).

Disclosures
None.

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Circ Res. 2015;117:914-916
doi: 10.1161/CIRCRESAHA.115.307644
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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