Blood vessels form a critical interface between the environment and the organism by supplying nutrients, oxygen and macromolecules to all tissues and cells in the body. Endothelial cells (ECs) line the inner surface of the hierarchybranched blood vessel network and integrate functionally into different organs to support tissue growth and function in development, physiology, and disease. In healthy postnatal tissues, ECs are mostly quiescent and rarely migrate or proliferate. However, ECs possess a remarkable phenotypic plasticity in terms of being able to proliferate rapidly in response to vascular injury, tissue ischemia, or other stress conditions. This phenotypic plasticity progressively declines when ECs age and/or are permanently exposed to metabolic (eg, hyperglycemia) and environmental stressors (eg, oxidative stress) and coincides with the development of endothelial senescence.1 Cellular senescence is a stress response that is characterized by a robust inhibition of cell proliferation, which often becomes irreversible and independent of the initial stress signal.2 Senescence can be observed in most mammalian cells after extended propagation in culture and is related to the attrition of telomeres after repetitive cycles of cell divisions.3 In addition to telomere shortening, other stressors such as DNA damage, genomic instability, and oxidative stress can induce a similar growth arrest and trigger senescence.2 Senescent cells display characteristic alterations in cell morphology and gene expression that may weaken cellular functions. In ECs, these changes cause a phenotype that is proinflammatory, prothrombotic, and proatherosclerotic, which also negatively affects the vasodilatory, angiogenic, and regenerative properties of ECs and, thus, accelerates the development of several cardiovascular diseases.1 Despite advances in understanding the molecular mechanisms of endothelial senescence and vascular aging, the signaling networks governing the development and progression of EC senescence remain less well defined. In this issue of Circulation Research, a new report from Zu et al describe an intricate signaling mechanism whereby sirtuin (SIRT1), a NAD+-dependent protein deacetylase that mediates adaptive responses to a variety of stresses, exerts its senescence protective effects on the vascular endothelium.3

SIRT1 belongs to the family of sirtuins, which have gained considerable attention for their impact on several important physiological processes associated with metabolism, stress resistance, and aging (for a comprehensive overview on sirtuins, see elsewhere4,5). SIRT1 acts as a NAD+-dependent protein deacetylase that adjusts cellular responses to the energetic state of the cell. By deacetylating transcription factors, cofactors, and histones, SIRT1 has been shown to promote resistance to metabolic, hypoxic, and genotoxic stress, thereby controlling cell metabolism, survival, proliferation, and cell fate.4,5 More recent studies highlighted important homeostatic functions of SIRT1 in the vascular endothelium, where it modulates vascular growth, shape and function.6 Consistent with these findings, Zu et al report that SIRT1 prevents cell culture-induced EC senescence in vitro as well as stress-induced senescence in mice.3 Interestingly, the authors found that SIRT1 mRNA and protein levels progressively declined during the development of endothelial senescence, whereas the protein levels of the stress-responsive serine/threonine kinase LKB1 were inversely regulated and robustly increased. The enhanced expression of LKB1 was also associated with an increased activity of the downstream target of LKB1, AMP-activated protein kinase (AMPK), a sensor of cellular energy status and master regulator of metabolism. The authors further showed that signaling by LKB1 and AMPK retarded endothelial proliferation and accelerated endothelial senescence development.3 The concept that SIRT1 prevents endothelial senescence is not new. Indeed, previous studies showed that SIRT1 prevents hydrogen peroxide-induced premature senescence of ECs by deacetylat ing the tumor suppressor p53.7 Nevertheless, it is the first time that the antisenescent activity of SIRT1 has been linked to deregulated LKB1/AMPK signaling. The important role of the LKB1/AMPK pathway as a SIRT1 target is highlighted by results showing that LKB1- and AMPK-induced senescence of EC are prevented by increasing the levels of SIRT1. Conversely, knocking down SIRT1 in ECs induced endothelial senescence and elevated the protein levels of LKB1, as well the phosphorylation of AMPK at threonine 172,1 a site that marks activation of AMPK by LKB1.

The finding that SIRT1 opposes signaling by the LKB1/AMPK pathway is perhaps the most unexpected finding by Zu et al, because previous studies in skeletal muscle cells have shown that AMPK enhances SIRT1 activity by elevating cellular NAD+ levels.8,9 Why should the LKB1/AMPK pathway boost its own inactivation through SIRT1? A possible explanation for such a negative-feedback loop might be...
downregulating LKB1 activity. The regulation of LKB1 by SIRT1 for deacetylation and degradation, thereby providing evidence that LKB1 is an acetylated protein, which is noteworthy that several other senescence-regulating molecules are modulated by reversible acetylation and are targeted by SIRT1 such as p53, endothelial nitric oxide synthase and Foxo, and E2F14,5 as well as by microRNAs such as miR-34, miR-199, miR-217, and miR-92a.17–20 Interestingly, miR-217, which inhibits SIRT1 expression through a miR-217-binding site within the 3' untranslated region of the SIRT1 mRNA, is induced in aging ECs and promotes a premature senescence-like phenotype.19 Understanding the upstream factors that control the expression of SIRT1 will be essential to further unravel the relevance and function of the SIRT1/LKB1/AMPK signaling circuitry in ECs in development, homeostasis, and disease. Further investigations will also have to establish whether the modulation of the LKB1/AMPK pathway by SIRT1 is relevant in cardiovascular methylation to alter protein activity, subcellular localization or stability. The findings by Zu et al suggest that deacetylation of LKB1 by SIRT1 leads to increased LKB1 ubiquitylation and proteasomal degradation. Because both acetylation and ubiquitylation occur on lysine residues, deacetylation of LKB1 by SIRT1 might control the accessibility of these residues for ubiquitylation and thereby alter its stability in ECs. In other cell lines, however, SIRT1-mediated deacetylation of LKB1 does not induce its degradation but instead increases its cytoplasmic localization and activity.11,12 How can these opposing findings be explained? Besides tissue-specific functions of SIRT1 and LKB1, differences in the acetylation pattern of LKB1 might be a plausible explanation. Lan et al demonstrated that acetylation of LKB1 occurs on several specific lysines, among which lysine 48 appears to be critical for mediating the effects of SIRT1 on LKB1 in HEK293 and HepG2 cells.11 Although Zu et al did not characterize the site-specific acetylation of LKB1 in ECs, it is possible that the acetylation signature of LKB1 is different in ECs, e.g., as a result of differential expression and/or activity of lysine acetyltransferases (KATs). Regardless of this, it is noteworthy that several other senescence-regulating molecules are modulated by reversible acetylation and are targeted by SIRT1 such as p53, endothelial nitric oxide synthase and Foxo transcription factors.7,13–16 These observations raise the possibility that changes in lysine acetylation might provide an important regulatory switch in the complex processes of senescence and aging. By deacetylating several targets within the signaling network governing endothelial senescence, SIRT1 may exert robust control over this multistep process and thereby execute its protective effects on the vascular endothelium (Figure 2).

A key question arising from this work is how SIRT1 expression is itself regulated in ECs. SIRT1 expression is positively and negatively regulated by several transcription factors and transcriptional cofactors including HIC1, CtBP, p53, Foxo, and E2F14,5 as well as by microRNAs such as miR-34, miR-199, miR-217, and miR-92a.17–20 Interestingly, miR-217, which inhibits SIRT1 expression through a miR-217-binding site within the 3’ untranslated region of the SIRT1 mRNA, is induced in aging ECs and promotes a premature senescence-like phenotype.19 Understanding the upstream factors that control the expression of SIRT1 will be essential to further unravel the relevance and function of the SIRT1/LKB1/AMPK signaling circuitry in ECs in development, homeostasis, and disease. Further investigations will also have to establish whether the modulation of the LKB1/AMPK pathway by SIRT1 is relevant in cardiovascular methylation to alter protein activity, subcellular localization or stability. The findings by Zu et al suggest that deacetylation of LKB1 by SIRT1 leads to increased LKB1 ubiquitylation and proteasomal degradation. Because both acetylation and ubiquitylation occur on lysine residues, deacetylation of LKB1 by SIRT1 might control the accessibility of these residues for ubiquitylation and thereby alter its stability in ECs. In other cell lines, however, SIRT1-mediated deacetylation of LKB1 does not induce its degradation but instead increases its cytoplasmic localization and activity.11,12 How can these opposing findings be explained? Besides tissue-specific functions of SIRT1 and LKB1, differences in the acetylation pattern of LKB1 might be a plausible explanation. Lan et al demonstrated that acetylation of LKB1 occurs on several specific lysines, among which lysine 48 appears to be critical for mediating the effects of SIRT1 on LKB1 in HEK293 and HepG2 cells.11 Although Zu et al did not characterize the site-specific acetylation of LKB1 in ECs, it is possible that the acetylation signature of LKB1 is different in ECs, e.g., as a result of differential expression and/or activity of lysine acetyltransferases (KATs). Regardless of this, it is noteworthy that several other senescence-regulating molecules are modulated by reversible acetylation and are targeted by SIRT1 such as p53, endothelial nitric oxide synthase and Foxo transcription factors.7,13–16 These observations raise the possibility that changes in lysine acetylation might provide an important regulatory switch in the complex processes of senescence and aging. By deacetylating several targets within the signaling network governing endothelial senescence, SIRT1 may exert robust control over this multistep process and thereby execute its protective effects on the vascular endothelium (Figure 2).
diseases such as atherosclerosis, in which the vasculature must withstand increased levels of oxidative and metabolic stress. Lastly, SIRT1, LKB1, and AMPK play additional roles in blood vessels beyond those in endothelial senescence. For example, SIRT1, LKB1, and AMPK have been implicated in the regulation of angiogenesis, which is the formation of new blood vessels from existing vasculature. Thus, studying the molecular interplay of this signaling circuitry might become a fertile ground for future investigations in several aspects of cardiovascular biology.

Altogether, the study by Zu et al sheds new light on our understanding of endothelial senescence and vascular aging by illustrating the interdependent regulation of key energy- and stress-responsive regulators of EC homeostasis: SIRT1, LKB1, and AMPK. Although future studies will have to address the therapeutic usefulness, these results also suggest potential new pharmacological strategies to counteract age-related diseases of the cardiovascular system.

Acknowledgments
The author apologizes to those authors whose relevant works were not cited because of space restrictions.

Sources of Funding
Supported by grants from the Deutsche Forschungsgemeinschaft (PO1306/1-1, SFB 834/A6, and Exc 147/1).

Disclosures
None.

References

Key Words: SIRT1 ■ LKB1 ■ AMPK ■ endothelial cells ■ senescence
An Energy-Sensor Network Takes Center Stage During Endothelial Aging
Michael Potente

_Circ Res._ 2010;106:1316-1318
doi: 10.1161/CIRCRESAHA.110.219352

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/106/8/1316

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/