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Finding a Target for Resveratrol

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Despite resveratrol's well-documented health benefits, its mechanism of action remains controversial. In particular, the direct molecular target of resveratrol has been elusive. Park et al. now show that resveratrol directly inhibits cAMP-dependent phosphodiesterases, triggering a cascade of events that converge on the important energy-sensing metabolic regulators AMPK, SIRT1, and PGC-1α.

For nearly 75 years, calorie restriction (CR) has been the most consistent behavioral intervention capable of extending life span and protecting against age-associated metabolic disease. Since the report that resveratrol—a polyphenol found in the skin of grapes—mimics the life-span-extending effects of CR in budding yeast (Howitz et al., 2003), this compound has been studied intensely. Debate about the direct targets, downstream effectors, and molecular mechanism by which resveratrol improves health span has ensued. In this issue, Park et al. (2012) identify phosphodiesterase (PDE) enzymes as direct targets of resveratrol and propose that resveratrol indirectly activates the sirtuin SIRT1 through a signaling cascade involving cAMP, Epac1, and AMPK.

Resveratrol burst into the news nearly 20 years ago, when it was proposed to account for the unique effects of red wine on life span and health. Subsequently, resveratrol was linked to myriad physiological benefits, including protection against cardiovascular disease, cancer, age-related deterioration, and the pathological consequences of high-fat diets (Baur, 2010). Resveratrol was reported to exert its effects by directly activating the yeast Sir2 protein and its mammalian homolog SIRT1 (Howitz et al., 2003). These members of the sirtuin family catalyze NAD^+-dependent deacetylation reactions. The resveratrol-sirtuin connection sparked a torrent of excitement, in part because sirtuins had been independently linked to life span regulation in budding yeast (Kaeberlein et al., 1999).

Over the last decade, fundamental roles for mammalian sirtuins in numerous cellular processes that impact metabolism, genomic stability, and aging-related disease have been demonstrated (Nakagawa and Guarente, 2011). Among these functions, SIRT1 deacetylates and activates PGC-1α, a master transcriptional regulator of genes involved in energy control, ultimately leading to improved mitochondrial function and protection against metabolic disease (Lagouge et al., 2006). Thus, the notion that resveratrol acts as a CR mimetic by activating sirtuins seemed promising.

However, the observed activation of SIRT1 by resveratrol in vitro now appears to be an artifact of the assay used, casting doubt on the direct resveratrol-SIRT1 connection (Baur, 2010). This raises two important questions: How does resveratrol lead to SIRT1 activation in vivo, and what is the direct cellular target of resveratrol? A series of studies provided some initial answers by showing that resveratrol can activate SIRT1 indirectly through AMPK, another energy-sensing enzyme that is required for many of the adaptations triggered by CR (Cantó et al., 2010; Um et al., 2010). AMPK promotes the activation of PGC-1α by SIRT1 through several mechanisms, including a priming phosphorylation that is required for its deacetylation by SIRT1 and an increase in NAD^+ concentration, which is rate limiting for SIRT1 activity (Cantó and Auwerx, 2010).

Until now, AMPK activation was the most upstream signaling event known to be triggered by resveratrol, but like SIRT1, AMPK did not appear to be a direct resveratrol target. Now, Park and colleagues (Park et al., 2012) seem to have found the missing link between resveratrol and AMPK. The authors provide evidence that resveratrol directly inhibits several PDE enzymes, and they systematically delineate the steps that lead from PDE inhibition to AMPK activation (Figure 1). These steps include increased levels of the ubiquitous second messenger cAMP, activation of the cAMP-dependent guanine nucleotide exchange factor Epac1, and increased intracellular calcium to activate CamKKβ, which phosphorylates and activates AMPK. Importantly, the PDE4 inhibitor rolipram phenocopies the cellular signaling events induced by resveratrol, including increased cAMP levels, Epac1-dependent activation of AMPK, increased NAD^+, and increased deacetylation of PGC-1α.

Park et al. complement this comprehensive pathway dissection with compelling in vivo experiments in mice. When fed to mice on a high-fat diet, rolipram induced a gene expression pattern that mirrors that induced by resveratrol in multiple mouse tissues. And like resveratrol-treated mice, rolipram-treated mice showed improved mitochondrial function, increased physical endurance, increased basal metabolism, and protection against diet-induced obesity and glucose intolerance.

Together, these data paint a detailed picture of how resveratrol activates AMPK and SIRT1 to produce metabolic benefits, with some interesting mechanistic and clinical implications. Resveratrol is thought to produce its health benefits by mimicking CR. Does CR produce beneficial health effects by activating the same signaling cascade as

resveratrol (cAMP-Epac1-CamKKβ-AMPK)? Can this network of proteins be exploited to develop CR mimetics with higher specificity and efficacy? A number of sirtuin-activating compounds (STACs) have been developed as promising therapeutic agents, and like resveratrol, these STACs appear to influence SIRT1 activity indirectly (Baur, 2010). Do any of these molecules also function by directly inhibiting PDEs? Alternatively, by identifying PDEs as direct resveratrol targets, the authors' findings (Park et al., 2012) may open the door to new uses for previously identified pharmacologic agents. For example, many of the players in the resveratrol-responsive signaling cascade also play anti-inflammatory and neuroprotective roles (Nakagawa and Guarente, 2011), and highly selective PDE inhibitors are currently being investigated as treatments for a wide range of pathologic conditions including psychiatric and neurodegenerative diseases as well as inflammatory disorders such as chronic obstructive pulmonary disease (Houslay et al., 2005).

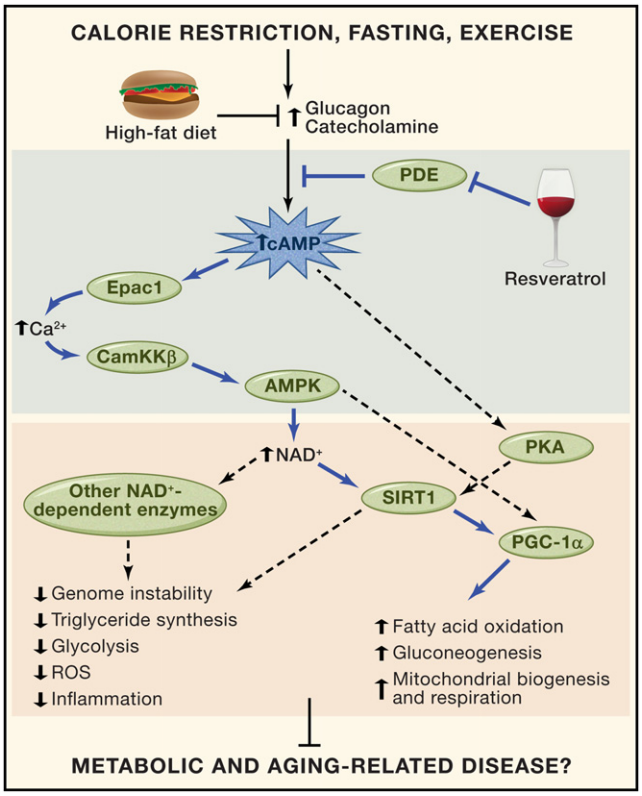

Figure 1. Dietary, Behavioral, and Pharmacologic Inputs Converge on cAMP-AMPK-SIRT1 Signaling to Produce Metabolic Benefits

Resveratrol inhibits PDEs, leading to increased cAMP levels, Epac1 activation, elevated intracellular calcium, and AMPK activation. Calorie restriction and other behavioral inputs also activate AMPK. Downstream of AMPK, an increase in NAD^+ levels leads to SIRT1 activation, which promotes beneficial metabolic changes primarily through deacetylation and activation of PGC-1α. In a parallel pathway, increased cAMP levels activate PKA, which directly phosphorylates and activates SIRT1. SIRT1 activation by either pathway, as well as potential activation of other NAD^+-dependent enzymes, can lead to numerous physiologic outputs. Blue box (top) highlights findings reported by Park et al. (2012); orange box (bottom) highlights pathway components previously identified that reside downstream of those identified by Park et al., as well as some of the reported and theoretical outputs of the signaling pathways detailed above. Blue arrows indicate the linear pathway proposed by Park et al.; dashed lines indicate molecular connections previously reported. PDE, phosphodiesterase; cAMP, cyclic AMP; Epac1, cAMP-regulated guanine nucleotide exchange factor 1; CamKKβ, calcium/calmodulin-dependent kinase kinase beta; AMPK, AMP-activated protein kinase; PKA, protein kinase A; NAD^+, nicotinamide adenine dinucleotide; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; ROS, reactive oxygen species.

The complex narrative of how resveratrol works also provides exciting new directions for future research. For example, how does the cAMP-Epac1-CamKKβ-AMPK signaling cascade intersect with other well-characterized pathways induced in different physiologic contexts such as fasting, cold exposure, exercise, and acute stress, all of which can lead to a "fight-or-flight" β-adrenergic response? These physiologic triggers activate many of the same factors involved in the response to resveratrol but involve different inputs and connections, generating a seemingly tangled web of interconnected pathways. For example, β-adrenergic activation of the cAMP/PKA cascade by acute stress results in the rapid phosphorylation and activation of SIRT1 *independent* of both AMPK and NAD^+ concentration changes (Gerhart-Hines et al., 2011). Occurring within minutes, this signaling pathway allows increased fat utilization and adaptive thermogenesis in response to acute nutritional stress or cold exposure. Although the study by Park et al. (2012) focuses on the changes that occur over hours to weeks, it seems plausible that PDE inhibition by resveratrol might also be useful in modulating physiologic responses to acute stress. Questions of output specificity do, however, remain. For instance, given the numerous roles for cAMP and calcium in intracellular signaling, which downstream pathways are triggered by resveratrol? With the increase in NAD^+, are other NAD^+-dependent enzymes—such as the six other mammalian sirtuins and the many poly(ADP-ribose) polymerases (PARPs)—also downstream resveratrol effectors?

Finally, it will be interesting to learn whether the Epac1-AMPK-SIRT1 pathway operates in cell types besides muscle and white adipose tissue. For example, although PDEs regulate cAMP signaling in cardiac myocytes, resveratrol does not appear to alter PGC-1α acetylation or mitochondrial biogenesis in these cells (Lagouge et al., 2006), and inactivation of *Pde4* in mice results in heart defects such as progressive cardiomyopathy (Houslay et al., 2005), suggesting that tissue-varying responses might limit the efficacy of pharmacologic intervention. From molecular details to in vivo characterization, the biochemical circuit described by Park et al. (2012) provides important insight into the mechanism by which resveratrol promotes metabolic health. But in the intensely controversial, complex, and rapidly evolving field of resveratrol and sirtuins, the identification of PDEs as the putative "missing link" is certainly not the end of the story.

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Innate Immunity to TB: A Druggable Balancing Act

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Tobin and colleagues show that both inhibition and excessive production of the inflammatory mediator TNFα impact the pathogenesis of tuberculosis (TB) and the response to therapy. Identifying a critical role for the genetically determined balance between pro- and anti-inflammatory eicosanoids in regulating TNFα levels provides a roadmap to tailored TB treatment based on host genotype.

Mycobacterium tuberculosis (*Mtb*) has been our unwelcome companion for millennia, using humans to "walk" out of Africa, "sail" out of Europe and "canoe" across North America. In order to infect an estimated one-third of the world's population and cause 8.1 million new cases of active TB annually (World Health Organization, 2011), *Mtb* must withstand and exploit host immune responses to ensure its survival and transmission. In turn, the human immune response has been calibrated to optimize survival in the face of this intracellular threat, as exemplified by the immune control and absence of pathology that characterize latent TB infection. Infection develops into disease due to bacterial dissemination resulting from inadequate protective immunity, whereas tissue pathology is caused by an overly vigorous yet ineffective host response. Identifying pathways that regulate and potentially dissociate protection from pathology will aid rational development of new interventions for treatment and vaccination. In this issue of *Cell*, Tobin and colleagues dissect a pivotal genetic balance that maintains the host between the two extremes of failed immunity and damaging hyperimmunity (Tobin et al., 2012).

The inhibition of macrophage apoptosis and the concomitant induction of necrosis is currently emerging as a pathway that is important for *Mtb* virulence, allowing for bacterial spread between cells prior to the induction of an adaptive immune response. In contrast, avirulent mycobacteria induce macrophage apoptosis, resulting in prompt development of a protective immune response. Converging results from different labs have revealed that the arachidonic acid metabolites, eicosanoids, lipoxins, and leukotrienes influence cell death patterns following mycobacterial infection (Behr et al., 2010). Lipoxin A4 (LXA4), produced through the action of lipoxygenase enzyme, acts as an anti-inflammatory mediator of mycobacterial-induced necrosis of infected macrophages; its key role is evidenced by the greater resistance of LXA4-deficient 5-lipoxygense knockout (*Alox5*$^{-/-}$) mice to *Mtb* infection (Behar et al., 2011; Chen et al., 2008; Divangahi et al., 2010).

Studying the natural host-pathogen pair of *M. marinum* in zebrafish, the Ramakrishnan laboratory previously reported on the susceptibility to infection of mutant fish lacking the Leukotriene A4 (LTA4) hydrolase enzyme (Tobin et al., 2010), responsible for converting LTA4 to proinflammatory Leukotriene B4 (LTB4). This hypersusceptibility was due to increased LXA4 levels, which resulted in decreased transcription of TNFα and the promotion of an anti-inflammatory environment. Interestingly, humans heterozygous for SNPs in the Leukotriene A4 hydrolase (*lta4h*) gene were relatively protected against tuberculosis in comparison to either homozygote genotype. In the current report, Tobin and colleagues investigate the mechanisms underlying heterozygote advantage in the eicosanoid