Revelations into resveratrol's mechanism

Resveratrol, 'the red wine chemical', has received much attention for its multitude of purported positive effects on health. At the forefront of this interest in resveratrol is its ability to mimic calorie restriction, which has been suggested to account for the observations that it can extend lifespan in yeast and decrease the effects of aging and metabolic disease in mice. However, it has been unclear exactly how resveratrol mediates these downstream effects. One hypothesis is that resveratrol works by activating the sirtuin SIRT1, as sirtuins have also been linked to modulation of lifespan and calorie restriction. Park *et al.*[1] now delineate a pathway in which resveratrol directly inactivates phosphodiesterase enzymes (PDEs), leading to a signaling cascade that activates SIRT1. We asked three experts to comment on how these new findings affect understanding the beneficial effects of resveratrol on health and the implications for the development of therapeutics to treat aging-related or metabolic diseases.

Steve Percival / Photo Researchers, Inc.

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Leonard Guarente

More than 300 years ago, John Ray recorded the old English proverb "there are more ways to kill a dog than hanging," which has since been updated to involve a feline creature and an even more ghastly demise. It may find a still more *au courant* phrasing when the final story is told of how resveratrol, a polyphenol found in red wine, provides benefits to metabolic health.

> "The mechanism of SIRT1 activation by small molecules is of great relevance to developing medicines for diseases of aging."

Indeed, the mechanism of resveratrol action has been hotly debated. Howitz *et al.*[2] showed direct activation of the SIRT1 NAD-dependent deacetylase by resveratrol *in vitro*, and subsequent studies demonstrated SIRT1 activation *in vivo*. Others, however, claimed that activation of SIRT1 was an artifact, and resveratrol's health benefits and sirtuins were not connected[3]. Now a new study[1] shows that resveratrol inhibits PDEs to increase cyclic AMP (cAMP) levels and activate SIRT1 indirectly in a mechanism involving Ca^{2+}, AMP kinase and NAD.

Joseph A Baur

Resveratrol produces substantial metabolic benefits in rodent models, and there have been positive indications in several small human trials, yet the mechanism of action for this compound remains controversial. Although resveratrol has multiple direct targets in mammalian cells, and many of these may ultimately prove relevant to health, the bulk of the controversy has centered around its effects on the deacetylase SIRT1. Resveratrol was shown to activate SIRT1 in an *in vitro* assay[2], and many of its effects in cultured cells are abolished when SIRT1 is inhibited. However, the validity of the *in vitro* SIRT1 activation assay, and hence the evidence for a direct effect of resveratrol on SIRT1, has been challenged by several groups.

> "The discovery that resveratrol inhibits PDEs is an important new piece in a puzzle that is far from complete."

Park *et al.*[1] now provide an alternative explanation for the metabolic benefits of resveratrol and indicate that PDEs are previously unidentified direct targets of the compound. They further elucidate details of a complex downstream pathway that ultimately stimulates SIRT1 activity. Interestingly, the path to SIRT1 activation leads through AMP-activated protein kinase (AMPK), an enzyme

So do these new findings settle the question of whether SIRT1 activation by resveratrol is direct? A central tenet of studying biological systems is that redundancy rules. Just because one mechanism is in play does not mean that another does not occur. In fact, the most recent biochemical data show that SIRT1 can be directly activated by a large number of compounds that have been developed for therapeutic applications[4]. We should also keep in mind that resveratrol is a natural product, and evolution may have selected for multiple ways in which this compound can activate SIRT1 orthologs, thereby maximizing the range of control over this key hub of physiology.

But this new study does throw down the gauntlet to those who envisage direct activation of SIRT1 by small molecules. It will be fascinating to see whether concrete mechanisms of activation by small molecules that hold up to biochemical and structural scrutiny are shown in the near future. The mechanism of SIRT1 activation by small molecules is of great relevance to developing medicines for diseases of aging. John Ray might be amazed how lessons from medieval times resonate in an era electrified by science and technology.

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known to be required for many of resveratrol's metabolic effects. SIRT1 and AMPK positively influence each other, but in support of the view that AMPK is upstream in the response to resveratrol, stimulation has been observed even in cells lacking SIRT1 (ref. 5). The model proposed by Park *et al.*[1] thus reconciles many previous observations.

Does this paper close the book on resveratrol's mechanism of action? Not quite yet. Like most mechanistic studies to date, this study employs concentrations of resveratrol *in vitro* that exceed those achieved *in vivo*, and the specificity of the compound is not thoroughly tested in animals. The authors' experiments with rolipram, a PDE inhibitor, provide compelling evidence that this intervention can mimic some of resveratrol's effects in mice. However, all of these changes are ostensibly downstream of AMPK activation, which could be achieved through alternative mechanisms during resveratrol treatment *in vivo*. Indeed, resveratrol enhances nitric oxide production[6] and, in one study, stimulated adenylyl cyclase[7], either of which could mimic PDE inhibition. Moreover, the hypothesis that direct or indirect SIRT1 stimulation contributes to AMPK activation has not been tested *in vivo*, and it remains to be seen whether targeting PDEs can mimic other salient features of resveratrol treatment, such as the amelioration of fatty liver or improvements in vascular function. The discovery that resveratrol inhibits PDEs is an important new piece in a puzzle that is far from complete.

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After the discovery that resveratrol can extend lifespan in budding yeast, by mimicking calorie restriction and by activating SIRT1 (ref. 2), great efforts have been made to study the possible effects of resveratrol in cancer, inflammation, neurodegeneration and cardiovascular, metabolism and age-related diseases. Nevertheless, the molecular mechanism of action of resveratrol has been elusive. Resveratrol was initially shown to activate SIRT1 in a fluorescent assay in which the substrate used was a peptide covalently linked to a fluorophore, but when an unlabeled substrate was used SIRT1 activation by resveratrol could not be demonstrated[8]. Thus, it remained controversial whether resveratrol directly activated SIRT1, as the observed effect seemed to be strongly dependent on the structural features of the peptide used in the assay.

> "It is time to move from the concept of 'one drug, one target' toward 'smart' drugs that can simultaneously modulate multiple targets."

Park *et al.*[1] now identify PDEs as direct targets of resveratrol and propose that resveratrol indirectly activates SIRT1 through a signaling cascade involving cAMP, Epac1 (a cAMP effector protein) and AMPK. Notably, in mice on a high-fat diet, the authors show that administration of the PDE4 inhibitor rolipram induces similar beneficial metabolic effects to resveratrol, including prevention of diet-induced obesity. Despite this evidence by Park *et al.*[1] that PDE can be directly inhibited by resveratrol, a further direct effect of SIRT activators such as active regulator of SIRT1 (AROS)[9] on SIRT1 should not be ruled out.

A number of more potent SIRT1 activators than resveratrol have been developed as promising therapeutic agents[10], and some of them are in phase 1 or 2 clinical trials for the treatment of metabolic, inflammatory or cardiovascular diseases. However, it is worth bearing in mind that these highly complex diseases involve a wide variety of altered cellular pathways and signals, and many reductionist single-target chemotherapy approaches have been largely fruitless. Therefore it is time to move from the concept of 'one drug, one target' toward 'smart' drugs that can simultaneously modulate multiple targets, which may lead to successful treatment of many of these challenging diseases.

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