ACS chemical biology

Spotlight

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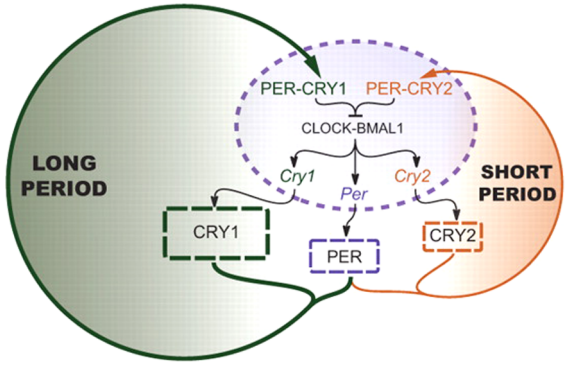
A COMPOUND THAT STOPS THE CLOCK

Circadian rhythms are biological processes that oscillate on roughly a 24 h time scale, such as sleep, body temperature, and hormone production. Dysregulation of the circadian clock, the main mechanism that controls circadian rhythms, is associated with a variety of disorders including insomnia, depression, and metabolic diseases. A network of pathways has been implicated in controlling the circadian clock, and small molecules that modulate clock regulation could have therapeutic potential for circadian-related disorders. Using a cell-based circadian screen, Hirota *et al.* (*Science*, published online July 12, 2012, DOI: 10.1126/science.1223710) report the identification and characterization of a small molecule that specifically targets the key clock protein cryptochrome.

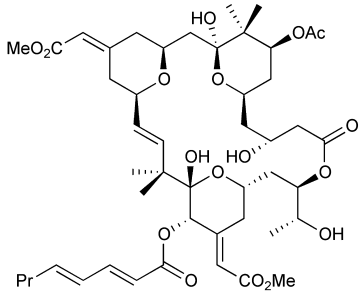


From Hirota, M., *et al.*, Science, July 12, 2012, DOI: 10.1126/science.1223710. Reprinted with permission from AAAS.

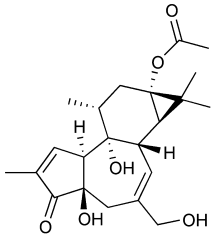
Cryptochrome is a nuclear protein that helps control the periodic expression of genes involved in rhythmic processes. From a screen of 60,000 compounds, a small molecule called KL001 was found to lengthen the rhythms of a transcription factor involved in regulating the expression of clock genes. Using affinity capture and liquid chromatography–tandem mass spectrometry analysis, the target of KL001 was identified as cryptochrome. Exploration into how KL001 lengthens the circadian period revealed that it stabilizes cryptochrome by protecting it from ubiquitin-dependent degradation. Mathematical modeling offered additional insight into the mechanism of KL001 by suggesting that cryptochrome stabilization occurs in the nucleus and that the two isoforms of the protein have similar roles, hypotheses that were confirmed experimentally. Intriguingly, KL001 prevented hormone-induced glucose generation in mouse liver cells, consistent with its ability to stabilize cryptochrome since the protein is known to negatively regulate genes involved in glucose production. The small molecule identified in this study is an exciting new molecular tool for exploration of circadian rhythms and could be a starting point for the design of drugs targeting circadian disorders. **Eva J. Gordon, Ph.D.**

HUNTING DOWN CELLS HARBORING HIV

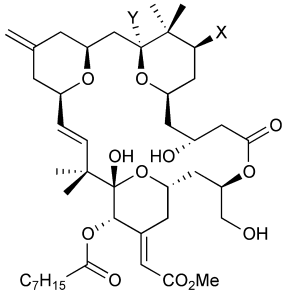
A daunting challenge in the treatment of AIDS is combatting the ability of the virus to persist in a latent form in certain T cells. Latent virus is resistant to drugs targeting active virus but can emerge at any time and reestablish infection, a behavior that precludes individuals from ever being cured of the disease. Development of agents that induce the reactivation of latent virus, which can then be eradicated with current HIV drugs, is an exciting potential strategy for curing individuals of the disease once and for all. Bryostatin, a marine natural product in clinical trials for the treatment of cancer and Alzheimer's disease, has also been shown to reactivate latent HIV due to its ability to activate the signaling molecule protein kinase C (PKC). However, access to this structurally complex macrolide lactone is a significant challenge, as isolation from natural sources is extraordinarily inefficient, and known chemical syntheses require approximately 40 steps. In addition, the compound has side effects that diminish its potential as a clinical agent. Toward tackling these challenges for the development of bryostatin as a therapeutic agent, DeChristopher *et al.* (*Nat. Chem.*, advance online publication July 15, 2012; DOI: 10.1038/nchem.1395) now report a more accessible synthesis of potentially clinically viable bryostatin analogues.



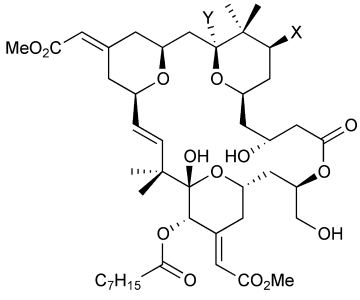
Bryostatin 1



Prostratin



1 X = Y = H
2 X = OH, Y = H
3 X = OAc, Y = H
4 X = OAc, Y = OH



5 X = Y = H
6 X = OAc, Y = H
7 X = OAc, Y = OH

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Key to their highly convergent synthetic strategy for synthesizing bryostatin analogues is a Prins macrocyclization, which involves coupling between an aldehyde and a homoallylic

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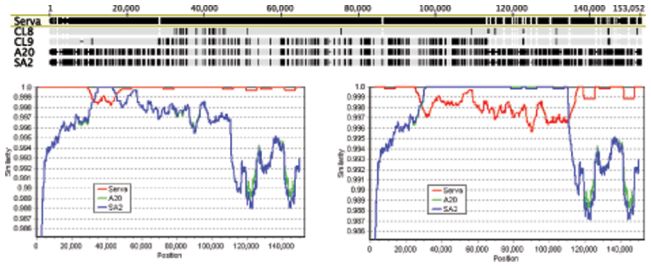
alcohol to generate a tetrahydropyran-containing macrocycle. Seven bryostatin analogs were efficiently synthesized and tested for their ability to activate PKC as well as their capacity to induce HIV production in cells harboring latent virus. The compounds were up to 1000 times more potent than current candidates for latent virus induction, and did not exhibit toxic effects. **Eva J. Gordon, Ph.D.**

RECKLESS RECOMBINATION

Live but somewhat impaired, or attenuated, viruses are commonly developed as vaccines for both humans and animals. Infectious laryngotracheitis virus (ILTV) is a herpesvirus that causes respiratory disease in chickens, and several attenuated forms of the virus have been made into vaccines. For example, SA2 and A20 are two closely related ILTV vaccines originating from Australia, while the Serva vaccine is another variety that originated in Europe. Though the possibility that attenuated herpesvirus vaccines might somehow recombine in the field and generate a virulent strain has been suggested, infection of animals or humans by such a strain has never been reported. Now, Lee *et al.* (*Science*, 2012, *337*, 188) find that the emergence of two new classes of virulent ILTV is the result of recombination between the European and Australian ILTV vaccines.

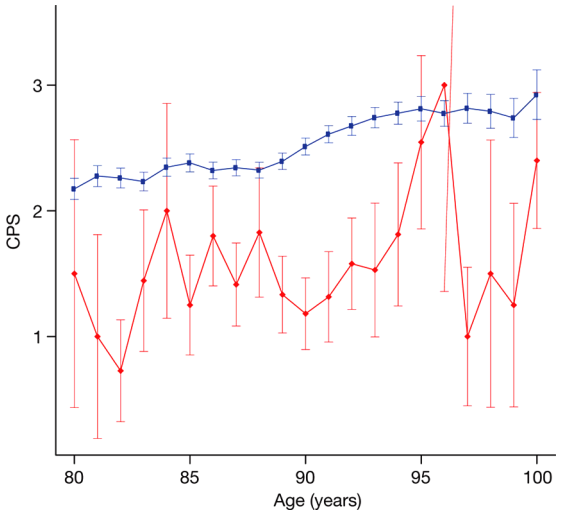


From Lee, S.-W., *et al.*, *Science*, 2012, *337*, 188. Reprinted with permission from AAAS.

Using high throughput and traditional DNA sequencing methods and sequence analysis techniques, the researchers found significant sequence overlap between the new viral classes and Serva, SA2, and A20. Their findings are highly suggestive of an interspecies recombination between the Australian and European vaccines that resulted in the emergence of virulent new strains in the field. Indeed, testing in chickens revealed that the recombinant strains were much more virulent than their parent strains. This study confirms apprehensions that, though rare, recombination between attenuated viral strains can result in strains with restored virulence. This discovery has important implications for the use of attenuated viruses as vaccines and will likely influence future vaccine development strategies. **Eva J. Gordon, Ph.D.**

A DECLINE IN COGNITIVE DECLINE

Across the globe, over 35 million people suffer from dementia, approximately two-thirds of which have Alzheimer's disease. This devastating condition, characterized by memory loss and cognitive decline, is caused by deposition of amyloid-β peptide aggregates in the brain. The amyloid-β peptide is formed through a series of proteolytic processing events within the amyloid-β precursor protein (APP), one of which is performed by aspartyl protease β-site APP cleaving enzyme 1 (BACE1). Analyzing whole-genome sequence data from 1795 Icelanders, Jonsson *et al.* (*Nature*, advance online publication July 11, 2012; DOI: 10.1038/nature11283) now report the identification of a genetic variant of APP that is resistant to cleavage by BACE1 and protects against the development of Alzheimer's disease.



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The authors found that a single nucleotide polymorphism at position 673 of APP, in which alanine is replaced by a threonine, was more prevalent in elderly individuals that did not have Alzheimer's disease than those that did. Notably, this variant was also found to be enriched in elderly individuals who were cognitively in good health at the age of 85, supporting the notion that the mutation also protects against dementia in general. Position 673 of APP is near the BACE1 cleavage site, suggesting that the presence of the threonine residue somehow reduces APP cleavage. Indeed, several in vitro assays confirmed that cleavage of the A673T APP variant is reduced compared to wild type APP. Notably, this is in stark contrast to the increased cleavage observed in another variant at the same site, the alanine to valine mutation A673V. These findings indicate the critical importance of site 673 in APP processing, and uncover the first mutation conferring protection against Alzheimer's disease. These exciting results also support the reduction of BACE1 APP cleavage as a viable therapeutic strategy for preventing Alzheimer's disease and age-related cognitive decline. **Eva J. Gordon, Ph.D.**

THE BITE THAT KILLS BITES BACK

Over one million people die each year from malaria, an infectious disease caused by the unicellular parasite, *Plasmodium*. Unlike other killers like HIV, *Plasmodium* is not transmitted from person to person but instead depends on a sinister messenger to both mature and spread the organism. This messenger, the mosquito, ingests the gametocytes from one infected human's blood to begin a maturation process inside the insect. This cycle culminates in infectious sporozoites being deposited into mosquito saliva and, ultimately, into the next bite recipient. While insecticide tactics and antimalarial therapies have gained ground on *Plasmodium*, a new method takes the war against malaria inside of the insect host.

Wang *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, Epub ahead of print July 16, 2012, DOI: 10.1073/pnas.1204158109) genetically engineered strains of a symbiotic bacterium that normally lives in the mosquito midgut, a key incubator for the maturing malarial parasite. These strains of *Pantoea agglomerans*, all harbored the *E. coli* hemolysin system which facilitates secretion of proteins through a membrane pore. Each individual strain

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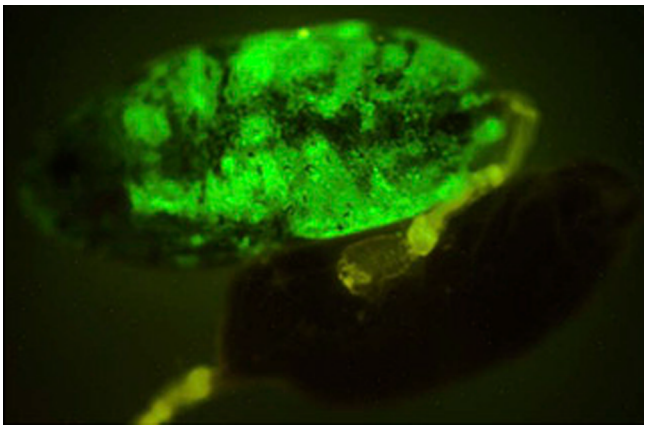

also expressed one or more known antimalarial proteins tagged for secretion. Taking advantage of the known life stage in the midgut, the various therapeutic proteins targeted the *Plasmodium* ookinete or the mosquito midgut players involved during invasion by the ookinete. After the researchers proved that the *Pantoea* strains displayed robust expression and secretion of the proteins, each were fed to mosquitos to colonize the midgut before administering a blood meal infected with *Plasmodium*. One week later, the mosquitos were assayed for the number of oocysts in the midgut, the next developmental stage after the ookinete which depends upon the insect host. The results showed that therapeutic strains of the *Pantoea* symbiote were successful in suppressing the transition to oocyst. Two of the strains displayed an impressive inhibition of over 97%. The same strains also showed therapeutic inhibition in a mouse model for malaria, *P. berghei*. Importantly, the authors showed that mosquito lifespan is not affected by having a genetically engineered symbiote instead of the wild type. They also discuss a hopeful future where this technique, known as paratransgenesis, could be implemented in the field so that mosquito soldiers can help fight the spread of this deadly parasite. **Jason G. Underwood, Ph.D.**