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Spotlight

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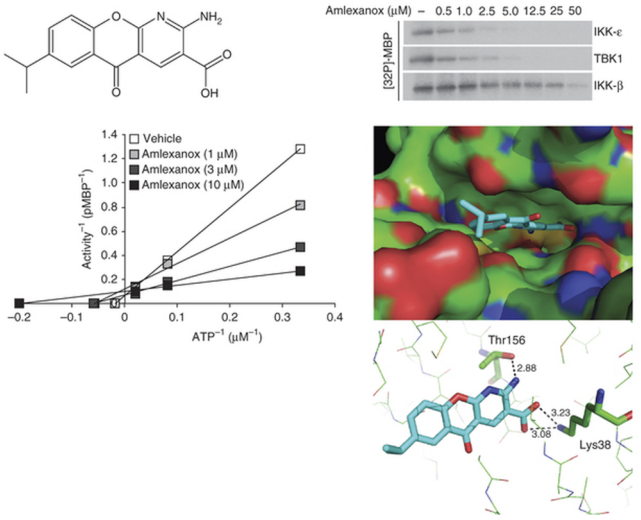
AN ALTERNATIVE FOR AMLEXANOX



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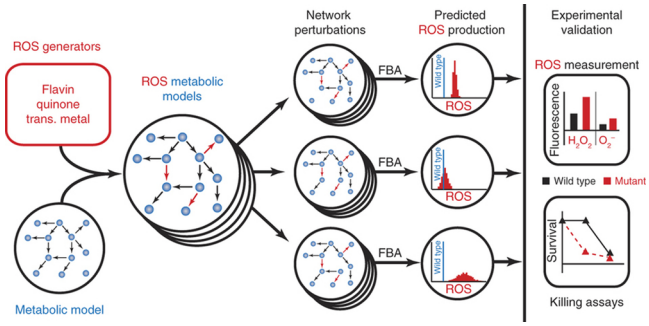
Over half of adults with diabetes are also obese, a rather staggering statistic. Increasing evidence incriminates inflammation as a likely culprit linking these two detrimental conditions, but the molecular mechanisms involved are unclear. It is known that, among other things, obesity promotes an increase in activity of two kinases, IKK-ε and TBK1, as a result of these inflammatory processes. This in turn can increase insulin resistance, setting the stage for development of type 2 diabetes. In a search for novel inhibitors of IKK-ε and TBK1 as potential therapeutics targeting this pathway, Reilly *et al.* (*Nat. Med.* advance online publication February 10, 2013; DOI: 10.1038/nm.3082) discover amlexanox, a drug already approved for ulcers, allergies, and asthma.

Amlexanox was identified from a screen of 150,000 small molecules for inhibitors of IKK-ε. The drug also inhibited TBK1 and was selective for these kinases over numerous others tested from a variety of kinase families. Mice fed a high fat diet and treated with amlexanox did not gain weight, and obese mice treated with amlexanox lost weight. Interestingly, amlexanox treatment did not affect overall food intake; rather, the drug appeared to employ increased energy expenditure as its mechanism for promoting weight loss. In addition, obese mice treated with amlexanox were more sensitive to insulin, had reduced inflammation, and had less fat in their livers. Combined with the excellent safety record in patients treated with amlexanox for other conditions, the data presented in this study suggest it as an exciting potential new candidate for the treatment of obesity and diabetes.

Eva J. Gordon, Ph.D.

MANIPULATING MICROBIAL METABOLISM



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Reactive oxygen species (ROS), chemically reactive substances such as hydrogen peroxide (H_2O_2) and superoxide anion radical (O_2^-), are normal byproducts of oxygen metabolism in organisms ranging from bacteria to humans. However, their high reactivity contributes to their destructive side, which manifests as damage to various biomolecules including DNA, RNA, proteins, and lipids. The destructive properties of ROS in microbes can be exploited to help kill pathogenic varieties, a skill that is desperately needed to combat the growing number of antibiotic-resistant bacteria that have emerged across the globe. However, the networks responsible for ROS production in bacteria are not well delineated. Now, Brynildsen *et al.* (*Nat. Biotechnol.* 2013, *31*, 160−165) use computational metabolic modeling to guide strategies for manipulating ROS production in bacteria for the purpose of enhancing the organism's susceptibility to toxic agents.

Genome-scale metabolic models were used with a mathematical method for analyzing metabolic pathways, called flux balance analysis, to predict ROS production in the bacteria *Escherichia coli*. Metabolic pathways were perturbed *in silico* by systematically deleting genes one at a time, after which ROS production was quantitatively assessed. The genes whose deletions were most likely to increase ROS production *in silico* were identified and validated experimentally by generating mutant strains in *E. coli* with the same gene deletions. Indeed, the mutant bacteria exhibited increased ROS production, were more susceptible to killing by oxidants such as H_2O_2 and bleach, and were more sensitive to two different classes of antibiotics. This innovative approach for sensitizing bacteria to antibiotic activity could lead to the identification of new targets for drug discovery efforts and could have far-reaching ramifications in the current struggle with antibiotic resistance.

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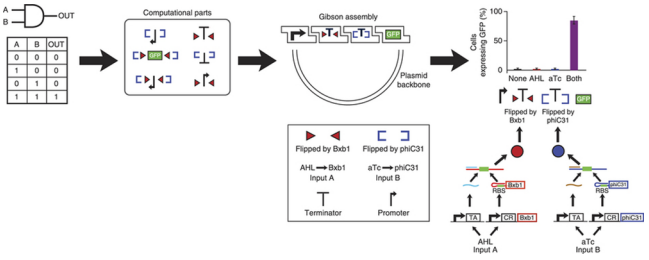
MAKING MEMORIES WITH SYNTHETIC CIRCUITS



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Synthetic biologists can exploit our understanding of natural biological pathways to create synthetic circuits. For example, synthetic genetic circuits can be designed with logic functions that dictate when a specific gene product is output, such as when either of two specific input signals is present, or only when both input signals are present, *etc.* Generation of increasingly complex computations within these circuits, however, will require the incorporation of both logic and memory. To this end, Siuti *et al.* (*Nat. Biotechnol.* advance online publication February 10, 2013; DOI:10.1038/nbt.2510) now report a strategy for building synthetic genetic circuits in live bacterial cells that employ recombinase enzymes to incorporate logic functions with DNA-encoded memory storage.

In their strategy, the small molecules *N*-acyl homoserine lactone (AHL) and anhydrotetracycline (aTc) serve as input signals for the circuit, and expression of green fluorescent protein is the output signal. The circuit is designed such that AHL and aTc drive the expression of the orthogonal serine recombinases Bxb1 and phiC31, which can invert or excise DNA based on the orientation of their surrounding recognition sites. This essentially enables them to implement logic functions and memory into the circuit through manipulation of promoter and terminator DNA sequences. The authors built all 16 possible two-input logic functions in *Escherichia coli* cells, and the states of the circuits were interrogated using flow cytometry to assess green fluorescent protein output and polymerase chain reaction to assess promoter and terminator direction. Notably, stable output memory was maintained for at least 90 cell generations after withdrawing the input signals. Two-bit digital-to-analog converters were also created using this approach, demonstrating that digital inducer inputs could be translated into stable analog gene expression outputs. This strategy of integrating logic and memory in synthetic circuits sets the stage for the creation of sophisticated, programmable cellular circuits with potential for various biotechnology and medical applications.

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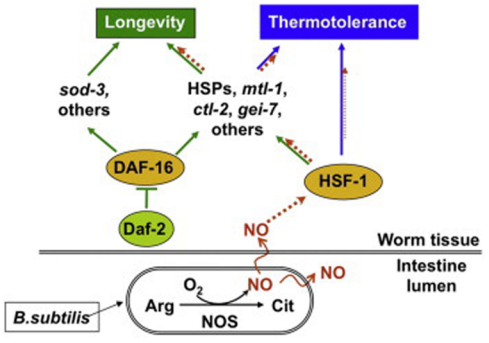
BACTERIAL NO EXTENDS LIFESPAN IN *C. ELEGANS*



Reprinted from *Cell*, *152*, Gusarov, I. *et al.*, Bacterial Nitric Oxide Extends the Lifespan of *C. elegans*, 818−830. Copyright 2013, with permission from Elsevier.

Nitric oxide (NO) regulates a whole host of signals in animals that relate to cardiovascular, immune, and neuronal function. In bacteria, NO buffers cells against oxidative stress and protects them from antibiotics. Although the roundworm *Caenorhabditis elegans* lacks genes for the NO synthases that produce this signaling molecule, Gusarov *et al.* (*Cell*, 2013, *152*, 818−830) have now shown that *C. elegans* can acquire NO from their bacterial foodstock. In addition, the NO interacts with signaling pathways that extend the lifespan of the worms.

The puzzling lack of NO synthases in *C. elegans* led Gusarov *et al.* to wonder why and whether these worms could pick up this signaling molecule through the bacteria they ingest. In a series of experiments, they first looked at how NO affected the physiology of the nematodes. Worms that ate normal bacilli lived 15% longer than those that ingested bacteria that could not produce NO. Using fluorescence experiments, they observed the production of NO in ingested bacterial within the worms and its movement to the worm's tissues. When NO was added to the media outside of bacteria, the worms also lived longer. They also became more resistant to heat shock.

Gusarov *et al.* traced NO signaling to two gene pathways: the insulin-like pathway and the heat shock response, which are controlled by the daf-16 and hsf-1 transcription factors, respectively. Both factors were required for NO-mediated activation of 65 genes in *C. elegans* leading to life extension and heat resistance.

The results directly demonstrate the effects that a metabolite from a food source (the bacteria) can have on an organism that consumes it. Because NO can easily diffuse across membranes, it can directly interact with many proteins on the outside and inside of cells. That relatively free movement could have facilitated this NO-dependence between nematodes and bacteria and offers the possibility that similar transfer could happen between bacteria and the gastrointestinal tract in humans.

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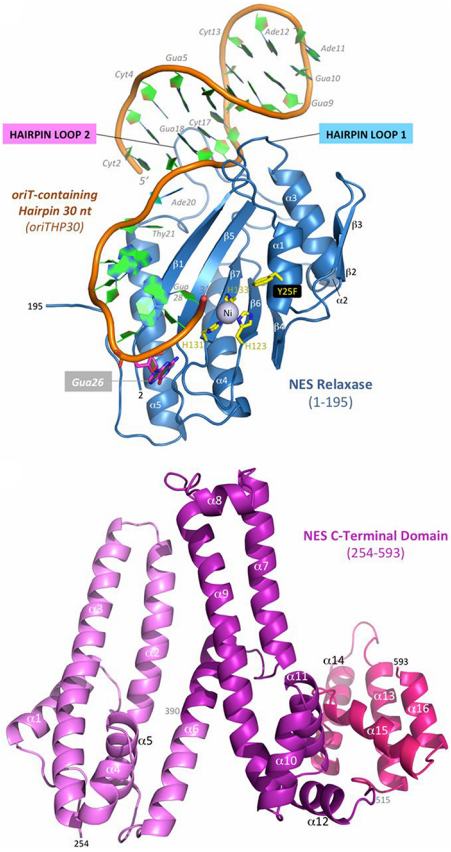
STAPH CONJUGATION MEETS VISUALIZATION



Edwards, J. S. *et al., Proc. Natl. Acad. Sci. U.S.A., 110,* 2804−2809. Copyright 2012 National Academy of Sciences, U.S.A.

The rise of pathogenic, antibiotic-resistant bacterial strains represents a significant health risk around the globe. These potent strains result from plasmid transfer where an antibiotic-resistance gene and the proteins required to propagate that gene between bacterial hosts are both carried on the same circular DNA molecule. Among the proteins, a relaxase enzyme binds and cuts DNA, initiating the process wherein a single strand of the plasmid is released for the transfer, termed conjugation. Now, a new study takes a closer look at a relaxase enzyme responsible for the multidrug-resistant *Staphylococcus aureus* that has appeared in clinics for a decade.

Edwards *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2013, *110,* 2804−2809) expressed the NES, or nicking enzyme in *Staphylococcus,* in N-terminal and C-terminal domains to determine the X-ray crystal structures. The N-terminal NES structure in complex with DNA showed that the protein adopts an interesting approach for locking onto its double-stranded substrate prior to catalysis. Two loops, one in the major groove and one in the minor groove of the DNA, form a total of 8 base-specific and five phosphate interactions. Together, these loops help position the DNA substrate for the nicking activity. Interestingly, mutating these loop regions or deleting them in the N-terminal protein actually shifted the cleavage-religation equilibrium of the nickase toward cleavage of the test substrate. This is in contrast to the full-length NES protein that rarely exhibits a cleavage above 10%. This indicated that the all-helical protein structure of the C-terminal portion, also solved in this study, was playing a role in modulating the protein's activity. In fact, without the C-terminal domain, the conjugation activity of NES is significantly reduced. Finally, using the structural information for how the enzyme recognizes DNA, the researchers designed a polyamide molecule that could effectively bind in the minor groove, competing with the loop structure's binding site. By *in vitro* inhibition studies, the polyamide that was specific for the GC-rich minor groove recognized by NES could effectively inhibit the protein with an IC_{50} in the low micromolar range. This study, and the structural coordinates deposited with it, should inspire researchers with new ideas for targeting nicking enzymes to dial back the rise of antibiotic-resistance strains.

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