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Spotlight

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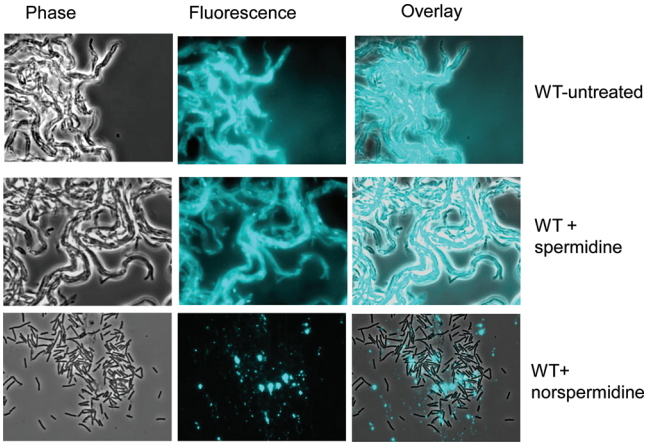
BREAKING UP BIOFILMS

Social creatures that they are, bacteria often reside in communities called biofilms. Made up of various polymeric biomolecules such as proteins, polysaccharides, and DNA, biofilms protect their communities from harmful substances such as detergents and antibiotics. They are an integral part, for good and for bad, of numerous agricultural, industrial, environmental, and clinical processes. For example, they can protect plants from microbial pathogens by forming on their roots but also comprise dental plaques and thus contribute to cavity formation. Biofilms have life cycles, but the molecular mechanisms that orchestrate biofilm construction and demolition are not fully understood. Now, Kolodkin-Gal *et al.* (*Cell* 2012, *149*, 684−692) report the discovery of a small molecule involved in the disassembly of the floating biofilm produced by *Bacillus subtilis* when grown in liquid culture.



Reprinted from *Cell*, *149*, Kolodkin-Gal, I., *et al.*, A Self-Produced Trigger for Biofilm Disassembly that Targets Exopolysaccharide, 684-692. Copyright 2012, with permission from Elsevier.

Previously studies had shown that two substances in conditioned medium from *B. subtilis* cultures prevented biofilm formation and that one of those substances was a mixture of D-amino acids. In the present study, a combination of chromatographic purification and structural analysis led to the identification of the other substance as the polyamine norspermidine. In addition, mutant bacteria unable to make D-amino acids and norspermidine formed long-lived biofilms, supporting the hypothesis that these compounds mediate biofilm deconstruction. Fluorescence microscopy, dynamic light scattering, and scanning electron microscopy experiments demonstrated that norspermidine interacts directly with exopolysaccharide during biofilm disassembly, as opposed to other biofilm components. Moreover, structure activity analysis showed that a motif comprised of three methylene groups flanked by two positively charged amino groups is important for biofilm disassembly activity. These insights into the structural and mechanistic properties of biofilm deconstruction will contribute to the development of methods to break up biofilms for various agricultural, environmental, and medical applications. **Eva J. Gordon, Ph.D.**

TRAPPING THE MESSENGER

MicroRNAs (miRNAs) are small, noncoding RNAs that posttranscriptionally regulate gene expression, typically through repressing mRNA (mRNA) translation or inducing mRNA degradation. Recent analysis suggests the existence of over 17,000 miRNAs, each potentially involved in regulating various aspects of biology such as cell development and disease progression. Identification of the mRNA targets of these thousands of miRNAs is a formidable challenge, and computational methods have been useful for predicting possible targets for experimental validation. However, current experimental methods are time-consuming, expensive, and technologically sophisticated. Baigude *et al.* (*Angew. Chem., Int. Ed.* 2012, advance online publication May 8, 2012, DOI: 10.1002/anie.201201512) now describe an efficient, benchtop-friendly process for the direct identification of miRNA targets in cells, which they appropriately named miRNA target RNA affinity purification (miR-TRAP).

miR-TRAP is based on covalently attaching miRNAs to their target mRNAs using photoactivatable cross-linkers. In designing the cross-linking agents, the authors chose psoralen, a natural product found in seeds of plants such as fig, celery, and parsley, due to its high photoreactivity and activation by relatively long wavelength radiation, which is less harmful to cells. Chemical conjugation of psoralen to a uridine residue of the miRNA provides the photoactivatable moiety, while attachment of biotin at the 3′ end provides a handle for purification of the complex after cross-linking. Exposure to UVA radiation induces cross-linking of the psoralen moiety to uridine on target mRNA, which can be isolated using biotin-streptavidin affinity purification. The mRNA target sequence can then be identified using quantitative PCR. Using targets predicted by computational methods as a guide, application of this experimental approach led to the identification of novel targets of miRNA-135b and miRNA-29a, two miRNAs involved in regulation of numerous processes including stem cell regulation. **Eva J. Gordon, Ph.D.**

SYNTHETIC GENETICS

DNA and RNA embody the genetic instructions for creating life as we know it. But are the structures of these miraculous biomolecules uniquely qualified to program life, or could other compounds be capable of transmitting genetic information and evolving the way that DNA and RNA do? Directly addressing this profound question, Pinheiro *et al.* (*Science*, 2012, *336*, 341−344) report the remarkable finding that synthetic nucleic acids capable of base-pairing with DNA, called xenonucleic acids (XNAs), and engineered polymerases can function together to store genetic information and evolve, just like regular DNA and RNA.

Six XNAs, each of which contains a structurally distinct five- or six-membered ring in place of the ribofuranose ring in DNA and RNA, were used in a selection strategy, called compartmentalized self-tagging (CST), with libraries of a

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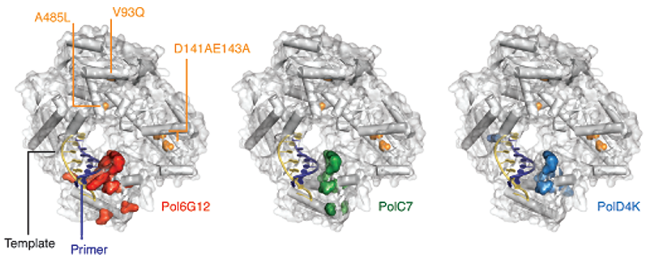


From Pinheiro, V. B., *et al., Science,* 2012, *336,* 341-344. Reprinted with permission from AAAS.

polymerase called TgoT. CST enabled generation of TgoT variants uniquely capable of transcription of XNA polymers from a DNA template. In addition, a strategy called statistical correlation analysis was used to help engineer polymerases capable of reverse transcription of XNAs back into DNA. To determine whether the XNA polymers were also capable of other inherent abilities of DNA and RNA, such as folding into specific three-dimensional structures and binding ligands, the authors attempted to generate XNA aptamers against two targets that had previously been used to generate DNA and RNA aptamers, the HIV trans-activating response RNA and hen egg lysozyme. Indeed, HNA (XNAs containing 1,5-anhydrohexitol nucleic acids) aptamers were created that selectively bound distinct regions of their respective targets. The strategies used in this study lay exciting groundwork for the generation and evolution of synthetic genetic polymers with unique chemical, physical, and functional properties for a variety of biotechnological applications. **Eva J. Gordon, Ph.D.**

SALICYLATE ACTIVATES AMPK

Although the medicinal effects of salicylate, an aspirin metabolite, were known to ancient physicians, scientists are still uncovering its many mechanisms of action. Long known to reduce pain and inflammation, more recent studies have shown that aspirin treatment reduces the risk of death from cancer. In a new study, Hawley *et al.* (*Science* 2012, *336,* 918−922) demonstrate an interaction between this small molecule and adenosine monophosphate activated protein kinase (AMPK) that may explain some of these effects.

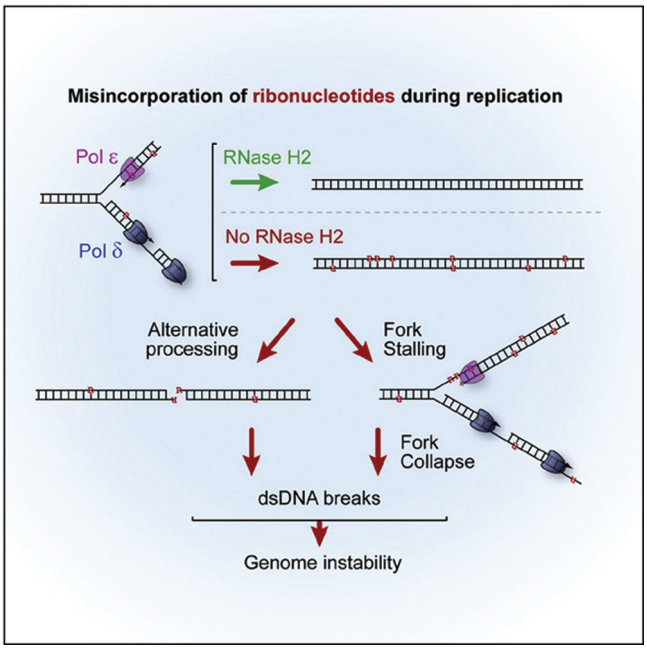
AMPK serves as a central metabolic switch in cells. When activated, it switches off processes that consume ATP, while switching on pathways producing it. AMPK senses cellular energy by direct binding of AMP, which causes allosteric activation, while binding of AMP or ADP causes additional activation by promoting phosphorylation of a threonine residue (Thr172).

To investigate the interaction of salicylate and AMPK, the researchers initially used human embryonic kidney (HEK-293) cells. At concentrations observed in humans taking aspirin, salicylate boosted AMPK activity. In studies with purified AMPK, it had effects similar to those of A-769662, a synthetic activator derived from a high-throughput screen. Both molecules allosterically activated AMPK and inhibited Thr172 dephosphorylation. Both agents only activated AMPK complexes containing the β1 subunit isoform, and a point mutation in β1 also blocked their effects. These results suggest that salicylate and A-769662 have overlapping binding sites.

Overall this study adds AMPK activation to the list of activities of salicylate and aspirin in vivo. Experiments in mice showed that salicylate activates fat oxidation in mice via a mechanism dependent on AMPK, consistent with earlier studies that showed that obese rats treated with aspirin have lower circulating lipid levels and are more responsive to insulin. This activity also overlaps with that of metformin, a drug for diabetes that activates AMPK and has also been linked to a lower incidence of cancer. **Sarah A. Webb, Ph.D.**

RNA'S DEADLY GUEST APPEARANCE

While DNA and RNA share many properties, that extra 2′ hydroxyl group on an RNA's ribose chain leaves the neighboring phosphodiester bond highly susceptible to degradation. In contrast, the deoxyribose backbone of DNA is far less reactive and thus serves as an excellent polymer for storing the cell's library of genetic information. But, what happens if a DNA polymerase misincorporates an RNA base during genomic replication? Eukaryotic cells utilize a special surveillance mechanism to seek and repair RNA bases that could otherwise be vulnerable sites for DNA breaks. The RNase H family of enzymes cut the RNA strand within an RNA-DNA hybrid. In particular, RNase H2 enzymes can cleave 5′ of a single RNA base within double-stranded DNA, thus activating a repair pathway. While previous studies in yeast showed that RNase H2 was not essential, a new study in mammals indicates a completely different tale.



Reprinted from *Cell,* Reijns, M. A., *et al.,* Enzymatic Removal of Ribonucleotides from DNA Is Essential for Mammalian Genome Integrity and Development., DOI: 10.1016/j.cell.2012.04.011. Copyright 2012, with permission from Elsevier.

Reijns *et al.* (*Cell* 2012, advance online publication May 9, 2012, DOI: 10.1016/j.cell.2012.04.011) bred a knockout mouse strain lacking one of the three RNase H2 subunits and demonstrated that this gene is essential to the developing embryo. Mice that were homozygous for the deletion showed normal early embryogenesis like their wild-type littermates up until day 6.5 but then rapidly declined in progress and viability. While extracts from wild type embryos could cleave a double-stranded DNA substrate with a single embedded RNA base, the mutant embryo extracts could not, indicating that a compensatory activity from other cellular enzymes is not present at significant levels. The researchers went on to use microarrays of the matched wildtype and mutant embryos, uncovering an altered expression of genes in the p53-response pathway. The p53 transcriptional regulator switches on a host of DNA damage response factors in RNase H2 null embryos, leading to proliferation defects and growth arrest. Finally, the

genomic DNA from RNase H2 null embryos was subjected to a variety of simple, yet powerful biochemical assays to measure ribonucleotide incorporation, yielding a calculated rate of 1 RNA base in every 7600 nucleotides. The large scale chromosomal instability uncovered by this study demonstrates that this misincorporation rate is not tolerated in a mammal and RNase H2 plays a critical role in keeping the cell's genome safe. **Jason G. Underwood, Ph.D.**