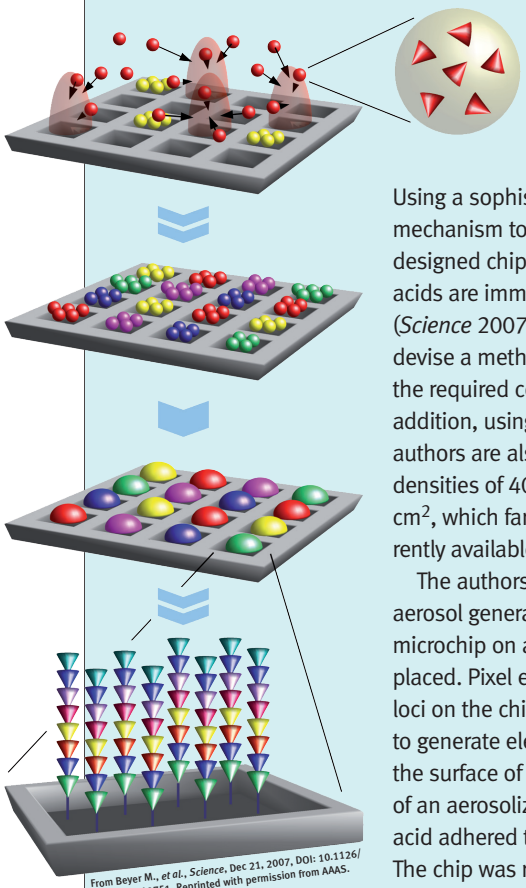
Spotlight

New and Improved Peptide Chips

Peptide arrays are useful for dissecting interactions between peptides composed of different amino acids and their targets. Until now, a stumbling block in the construction of peptide arrays was that each individual monomer had to be fixed to the solid support by chemical coupling. So, if 20 different amino acids were fixed in the same relative position in different spots, exactly 20 coupling cycles would be required—one for each reaction. This put severe practical limitations on the complexity of the peptide arrays used.



From Beyer M., *et al.*, *Science*, Dec 21, 2007, DOI: 10.1126/science.1149751. Reprinted with permission from AAAS.

Using a sophisticated electric field mechanism to highlight pixels on designed chips to which amino acids are immobilized, Beyer *et al.* (*Science* 2007, *318*, 1888) now devise a method that reduces the required coupling cycles. In addition, using this technique, the authors are also able to achieve densities of 40,000 peptide spots/cm^2, which far exceeds the currently available 22 peptides/cm^2.

The authors designed an aerosol generator into which a microchip on a circuit board was placed. Pixel electrodes at specific loci on the chip were switched on to generate electric fields above the surface of the chip. Particles of an aerosolized activated amino acid adhered to the "lit up" pixels. The chip was removed and placed in another aerosol generator with another set of amino acid particles, and a different pixel pattern was switched on. Iterations allowed deposition of different amino acids without any requirement for intermediate melting or washing. Only one coupling cycle is needed for each layer, so an array with nine randomized amino acids in peptide spots would require only nine coupling cycles. Because of the remarkable level of complexity now possible, constructing peptide arrays using this new method might allow entire proteomes to be analyzed all at once. **Anirban Mahapatra, Ph.D.**

Banking on DrugBank

Most online drug resources focus on either the clinical aspects of drugs, targeting pharmacists, physicians, and consumers, or the chemical aspects of drugs, targeting chemists, biochemists, and molecular biologists. Established in 2006, the online drug resource DrugBank (www.drugbank.ca) sets itself apart from other drug databases by incorporating both clinical and chemical information, and thus it is arguably the most comprehensive online drug resource available. Wishart *et al.* (*Nucleic Acids Res.*, published on Nov 29, 2007, DOI: 10.1093/nar/gkm958) review the numerous additions and enhancements incorporated in the latest version of DrugBank (release 2.0), available online in January 2008.

Release 2.0 of DrugBank includes several new features and significantly expanded content not available in release 1.0. For example, DrugBank now contains all drugs that have been approved in North America, Europe, and Asia. In addition, withdrawn drugs (those that have been withdrawn from the market) and illicit drugs (those that are legally banned) have been added to the database. Moreover, the number of drug targets included in the database has been substantially increased. In addition to the extensive links with most major bioinformatics, biomedical, drug, and pharmaceutical databases, DrugBank is now also reciprocally linked to many of these sites and other widely used sites, such as Wikipedia. Several data fields have also been added, such as drug brand names and food–drug interactions, and a number of improvements have been made to the text search and chemical structure search functionalities. Finally, the data handling capabilities have been streamlined with the development of automated text and web-mining tools, including BioSpider, a web spider that automatically gathers biological, chemical, and pharmacological data from trusted web sites, and PolySearch, a text-mining tool designed to mine data from abstracts in PubMed. With new releases planned twice a year, DrugBank provides a drug resource everyone can bank on! **Eva J. Gordon, Ph.D.**

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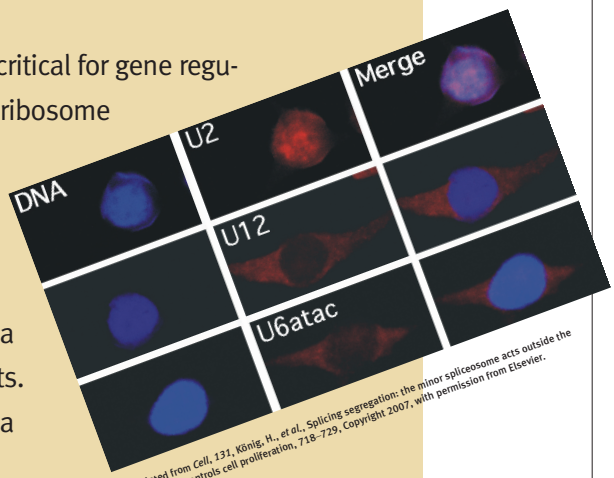
Another Dogma Broken?

In eukaryotes, being in the right place at the right time is critical for gene regulation. Messenger RNAs (mRNAs) are not delivered to the ribosome in the cytoplasm until the noncoding introns are removed in the nucleus. This compartmentalization has long been viewed as essential because the translation of unspliced messages could wreak havoc in the cell. To deal with the occasional splicing errors, the cell even has a built-in surveillance mechanism to look for such transcripts. Now, a recent study bends the compartment dogma with a groundbreaking hypothesis, splicing in the cytoplasm.

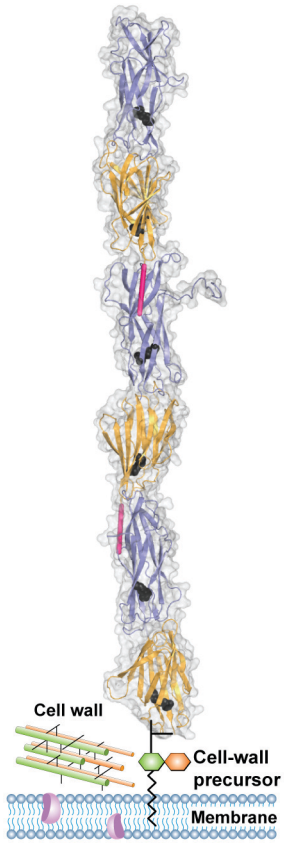


Reprinted from *Cell*, 131, König, H., *et al.*, Splicing segregation: the minor spliceosome acts outside the nucleus and controls cell proliferation, 718–729, Copyright 2007, with permission from Elsevier.

König *et al.* (*Cell* 2007, *131*, 718–729) were investigating the minor spliceosome, a secondary machinery present in plants, animals, and even some single-celled protists. The minor spliceosome catalyzes removal of the rare U12-type introns, which contain different splicing signals at their intron termini than the U2-type introns removed by the major spliceosome. The first clue of a cytoplasmic function came from *in situ* hybridization to localize spliceosomal RNAs from both the major and minor spliceosomes in zebrafish. The U12 and U6atac small nuclear RNAs showed dramatic cytoplasmic staining, and this phenomenon held true in mouse cells as well. By using cellular fractionation techniques, the authors found that there were indeed pre-mRNAs that escaped the nucleus with an intact U12-type intron. Blocking the minor spliceosome with an antisense approach caused higher accumulation of U12-containing pre-mRNAs in both the nucleus and cytoplasm. Next, the authors asked whether the mitotic state of the cell influenced the activity of the minor spliceosome. Mitosis is known to down-regulate numerous nuclear processes, including splicing, but splicing in the cytoplasm might be expected to escape this regulation. The authors found that minor splicing was unaffected by mitosis and they even observed a general effect of minor splicing on cell cycle progression. Inhibiting the minor spliceosome inhibited entry into S phase of the cell cycle in fish and mammals. The conservation of minor introns in certain gene families, coupled with these results on cell proliferation, indicates that the minor spliceosome may mastermind an unforeseen regulatory mechanism. New biochemical efforts will certainly follow this study to track the putative cytoplasmic spliceosome and to elucidate how these unspliced RNAs evade decay. **Jason G. Underwood, Ph.D.**

Pili Go from Strength to Strength

Many bacterial species attach themselves to surfaces using thin hairlike appendages known as pili. Although both Gram-negative and Gram-positive bacteria possess these extensions, there are major structural differences in the subunits of pili from either group. For example, subunits of Gram-negative pilin are associated through noncovalent interactions; in contrast, the link between subunits of Gram-positive pilin is strengthened by covalent isopeptide bonds. These bonds are formed as part of the assembly reaction catalyzed by transpeptidase enzymes known as sortases. In general, Gram-positive pili have been difficult to study, in part because they may only be one-pilin-subunit thick. Now, for the first time, Kang and colleagues (*Science* 2007, *318*, 1625–1628) elucidate the crystal structure of the backbone pilin subunit of the Gram-positive pathogenic bacterium *Streptococcus pyogenes* at 2.2 Å resolution.



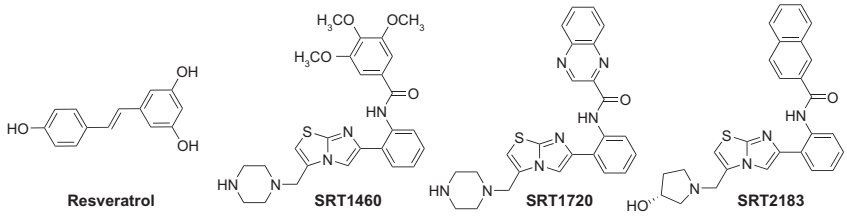
From Kang H. J., *et al.*, *Science*, Dec 7, 2007, DOI: 10.1126/science.1145806. Reprinted with permission from AAAS.

The authors observed that each unit of the pilin protein piles one after the other, lengthwise. The authors showed that a crucial lysine residue was required for isopeptide bonds between subunits. Perhaps even more surprising was the discovery of isopeptide bonds within the same subunit. To further study these bonds, the authors mutated residues thought to be involved in isopeptide bond formation and examined both wild-type and mutant proteins through crystallographic and mass spectrometric analysis. The authors also pointed out that these residues are conserved in all sequenced variants of this pilin subunit. Intrigued by the possibility that these intramolecular isopeptide bonds might be found in other proteins, the authors performed a comprehensive analysis of known sequences and structures and found other candidate surface proteins that might have intramolecular isopeptide bonds. Taking these studies together, the authors hypothesized that these bonds might play key roles in the stabilization of surface proteins in Gram-positive bacteria. Ultimately, the conserved structural elements of this pilin subunit that the authors describe are attractive targets that might be useful in the design of antibacterial drugs. **Anirban Mahapatra, Ph.D.**

A Molecular Quest

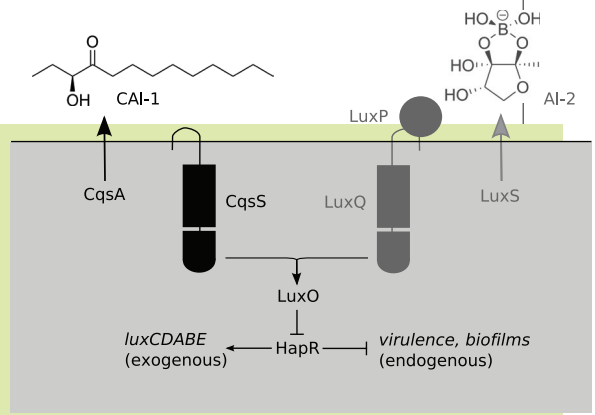
In the quest for small molecules that possess profound biological activities, those capable of extending the human lifespan are the holy grail. Calorie restriction is known to result in lifespan extension, likely from its beneficial effects on glucose homeostasis and insulin sensitivity. These metabolic changes are modulated by the NAD^+-dependent deacetylase, SIRT1, implicating this enzyme as a therapeutic target for diseases of aging such as type 2 diabetes. The small molecule resveratrol is a known SIRT1 activator that mimics the anti-aging effects of calorie restriction. Now, Milne *et al.* (*Nature* 2007, *450*, 712–716) report the discovery and characterization of several additional small-molecule activators of SIRT1 that are structurally unrelated to resveratrol.

Approximately 300,000 compounds were screened for their ability to activate human SIRT1 using a high-throughput fluorescence polarization assay, with promising structures optimized for *in vitro* enzyme activity using a high-throughput mass spectrometry assay. The authors then used a cell-based deacetylation assay to assess the functional activity of the lead compounds, which led to the identification of several potent SIRT1 activators. Investigation of the kinetics and the energetics of the interaction between the activators and SIRT1 provided valuable insights into the mechanism of activation, enabling the authors to propose that compound binding to a SIRT1–peptide substrate complex induces a conformational change that favors catalytic activity. In addition, SIRT1 truncation mutants were generated to identify the activator binding site. Finally, to evaluate their potential as therapeutics, the authors tested the SIRT1 activators in several *in vivo* rodent models of obesity and type 2 diabetes. Indeed, the compounds were found to improve insulin sensitivity, lower glucose levels, and increase mitochondrial capacity. The promising activity of these novel SIRT1 activators presents new leads for the treatment of diseases related to aging. **Eva J. Gordon, Ph.D.**



Cholera Gets a Taste of Its Own Medicine

Cells of the pathogen responsible for cholera, *Vibrio cholerae,* communicate through the use of small molecules in a process known as quorum sensing. This pathogen activates factors associated with virulence and biofilm formation under low cell densities, while limiting these traits in "crowded" communities. Interestingly, these traits are limited with shedding from hosts, which subsequently allows the pathogen to find new hosts and reinitiate infection. This entire process is remarkable, because many other pathogenic bacteria are not virulent at low cell density but rather at high cell densities. Responses to cell density in *V. cholerae* are mediated through two autoinducers that function together, cholerae autoinducer-1 (CAI-1) and autoinducer-2. Now, for the first time, Higgins *et al.* (*Nature* 2007, *450*, 883–886) identify and elucidate CAI-1.



The authors purified CAI-1 and identified it as (*S*)-3-hydroxytridecan-4-one, a previously unknown autoinducer. Overexpression of the *Vibrio* gene encoding the CAI-1 synthase in *Escherichia coli* allowed the biosynthesis of CAI-1. In a *tour de force*, the authors isolated this compound and then chemically synthesized it. The authors then demonstrated that the addition of synthetic CAI-1 to cultures of *V. cholerae* repressed the synthesis of a protein required for host colonization and virulence. The identification and elucidation of the CAI-1 molecule and the demonstration that it can check virulence indicate that this might help in developing drugs that treat cholera. **Ross Larue**

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