In This Issue

Crosstalk: Of Telomeres and Telomerase

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The 2009 Nobel Prize in Physiology or Medicine was awarded to three scientists, Elizabeth Blackburn, Jack Szostak, and Carol Greider, for their contribution to unraveling the role of telomeres and the enzyme telomerase. In this issue, David R. Corey provides an engaging discussion of the field, placing contributions of the three Nobel laureates in historical perspective, and then taking a step further to elaborate on some aspects of telomere and telomerase biology that are closer to the interests of chemical biology, especially focusing on exploring the prospect of developing anti-telomerase agents as drugs.

In Brief: Alkaloid Biosynthesis with New Substrates

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Biosynthetic pathways can be hijacked to yield novel compounds by introduction of unnatural starting substrates, a strategy known as precursor-directed biosynthesis. Use of alternate heterocycles in precursor-directed biosynthesis has not been widely demonstrated, and successful incorporation of starting substrate analogs containing the aza-indole functionality has not been previously reported. Notably, aza-indoles are important pharmacophores that are isosteric to the indole moiety but display improved water solubility and unique hydrogen bonding properties. Here, Lee et al. show that two aza-tryptamine substrates can be successfully incorporated into the alkaloid products of the medicinal plant *Catharanthus roseus*, known widely as Madagascar periwinkle. This work serves as a starting point to explore fermentation of aza-alkaloids from other tryptophan- and tryptamine-derived natural product pathways.

IspE, Mycobacterial Achilles' Heel

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The nonmevalonate pathway for isoprenoid biosynthesis constitutes an important metabolic pathway in a number of pathogens that are completely absent in humans. Thus, enzymes from this pathway constitute a group of potentially valuable drug targets. Eoh et al. now describe an allelic disruption of mycobacterial *ispE* gene from the nonmevalonate pathway, confirming the prediction that its gene product, IspE protein, is essential for bacterial survival. The authors go further to enzymatically characterize IspE from four pathogenic bacterial species (*Mycobacterium tuberculosis, Burkholderia mallei, Salmonella enterica serovar typhimurium,* and *Vibrio cholera*). Thus, this elucidation of the biochemical and kinetic properties of IspE from human pathogens provides the groundwork for development of high throughput screening (HTS) assays and, potentially, identification of novel antimicrobials.

mTOR Pathway Inhibitors Full Steam Ahead!

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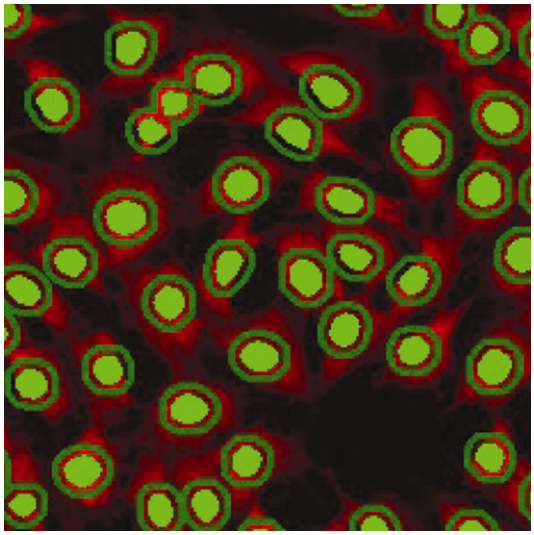


The mammalian target of rapamycin (m*TOR*) is a hub of a signal transduction pathway that regulates cellular growth and proliferation and represents a vibrant area of research for drug discovery. These ongoing research activities aimed at discovery of new mTOR inhibitors have recently yielded few promising compounds with therapeutic potential. One of the factors that might have limited the pace of search is the current absence of viable high throughput assay that would allow in vivo monitoring of the mTOR-mediated phosphorylation. Here, Livingstone et al. describe such a cell-based chemical genetic screen, based on the nuclear accumulation of eIF4E, which occurs in a 4E-BP-dependent manner specifically upon inhibition of mTOR signaling. The authors apply the assay to identify several compounds not previously known to inhibit mTOR signaling, demonstrating the usefulness of the method.

OAKs Hitting all the Grams, Positive and Negative

PAGE 1250

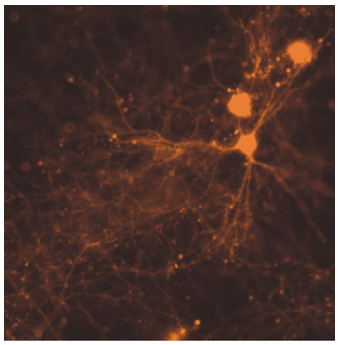
Oligomers of acyl-lysyl (OAKs) are chemical mimics of host defense peptides (HDPs) with selective antimicrobial and antitumor properties. Here, Livne et al. describe a screening strategy for selecting a new promising candidate as well as its detailed functional characterization, demonstrating the potential of this HDP-mimetic approach for the development of nonhemolytic broad-spectrum antibacterial OAKs. By virtue of its improved properties, the new OAK described here represents a potential candidate for a variety of antibacterial applications, including systemic treatment of resistant bacterial infections. Interestingly, functional data also provide mechanistic insights into how OAKs interact and affect Gram-positive and Gram-negative bacteria, suggesting that OAKs' interactions with the peptidoglycan layer of Gram-negative species retards the penetration to plasma membrane and shifts the mode of action from bacteriocidal to bacteriostatic in a time-dependent manner.

Hear It through a Quinolone Signal Grapevine

PAGE 1259

The multifactorial virulence of the opportunistic pathogen *Pseudomonas aeruginosa* is regulated via a sophisticated quorum-sensing network that incorporates both acylhomoserine lactone and alkylquinolone signal molecules. Here, Pustelny et al. discovered that a dioxygenase from an *Arthrobacter nitroguajacolicus* strain catalyzes the cleavage of the "*Pseudomonas* quinolone signal" [PQS, 2-heptyl-3-hydroxy-4(1H)-quinolone]. Addition of the enzyme to *P. aeruginosa* cultures downregulated the expression of key virulence factors and reduced in planta growth and plant tissue damage. The data highlight the potential of quenching alkylquinolone-dependent quorum sensing and hence virulence through the enzymatic degradation of alkylquinolones.

Optogenetic Probes for Brain Electrical Activity



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Electrical signals generated by nerve cells provide the basis of brain function. Perron et al. describe optogenetic probes with spectral properties designed for functional imaging of neuronal circuits in vivo. A family of fully genetically encoded, voltage-sensitive fluorescent proteins (VSFPs) was generated by molecular fusion of a transmembrane voltage sensor domain to red-shifted fluorescent protein operands. These indicator proteins resolve spontaneous and evoked activity of hippocampal neurons, at single-cell resolution, without temporal averaging.

Battle against the Proteasome

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Proteasomes, the major proteolytic enzymes in every eukaryotic cell and the targets of anticancer drugs, have three types of active sites: chymotrypsin-like, trypsin-like, and caspase-like. Chymotrypsin-like sites are the primary targets of inhibitors currently used in clinic and in research, with a caveat that the majority of these compounds also inhibit the other two sites. Whether or not coinhibition of the trypsin-like and caspase-like sites contributes to inhibitors' cytotoxicity has not been established. This study by Britton et al. uses newly developed specific inhibitors of caspase-like and chymtrypsin-like sites to demonstrate that the former sensitizes multiple myeloma cells to the letter and thus establishes caspase-like sites as cotargets of antineoplastic drugs.

Taming of a Lasso Peptide Capistruin

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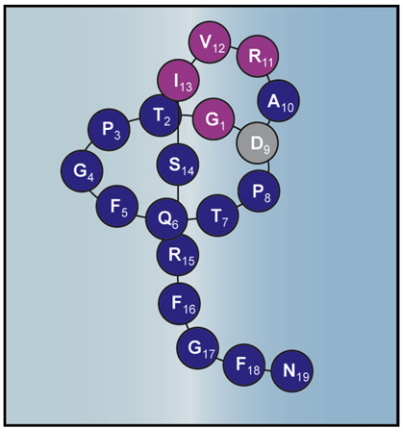
Lasso peptides are an emerging class of bacterial bioactive natural products, whose precursors are ribosomally assembled and matured by the action of two processing enzymes. In the present study, Knappe et al. investigate biosynthesis and stability of the recently discovered lasso peptide capistruin, using a global mutagenic approach of the precursor protein CapA and a heterologous production system for screening. The analysis reveals low overall specificity of the enzymatic machinery and establishes important structure/stability relationships of capistruin, which provides the foundation for future utilization of lasso peptides as robust peptide scaffolds for protein engineering efforts.



LIVE: Lights on Fe-S Clusters

PAGE 1299

An approach for in vivo detection of iron-sulfur clusters has been developed that is based on the complementation of fluorescent protein fragments fused to human glutaredoxin 2 (GRX2). Hoff et al. show that protein fluorescence depends on 2Fe2S coordination by GRX2, metallocluster coordination can be imaged in multiple cellular compartments, and Fe-S cluster assembly defects can be detected in living mammalian cells. These findings provide evidence that dithiol glutaredoxins dimerize through coordination of an Fe-S cluster in metazoans and implicate bimolecular fluorescence complementation as an effective strategy for imaging how 2Fe2S homeostasis is influenced by cell type, protein depletion, and pharmaceutical treatments.

