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SPOTLIGHT

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RNA Splicing under the Microscope

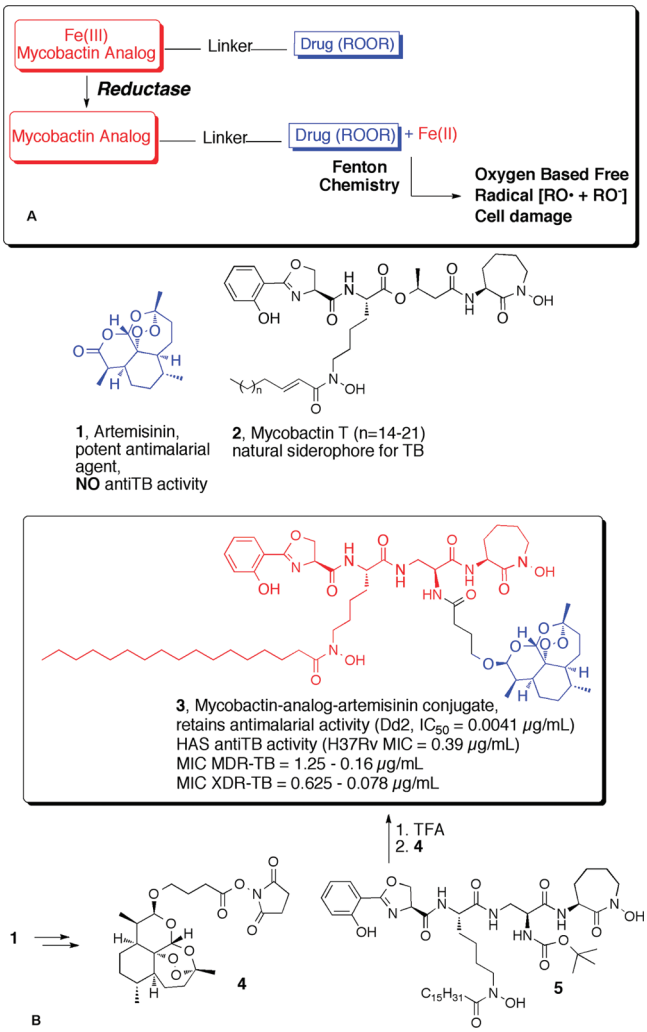
Among the largest macromolecular machines in the cell is the spliceosome, a complex of proteins and small RNAs that both decodes splice site signals in a pre-mRNA and precisely removes the intronic sequences to generate the mRNA. Studying splicing with detailed mechanistic tools has often proved difficult for researchers since experiments are not carried out in a purified system. Instead, a nuclear or whole cell extract is used to provide the hundred proteins and five RNAs required for the reaction. To get around these difficult challenges, a new study by Hoskins *et al.* (*Science* 2011 *331*, 1289−1295) borrowed from yeast genetics and fluorescence microscopy to yield an elegant new way to study spliceosome assembly in a single-molecule fashion.

Picture credit: Diana Katharine Hunt.

The technique, called CoSMoS, for colocalization single-molecule spectroscopy, was used to observe yeast pre-mRNAs immobilized on a glass surface. The researchers then employed extracts from budding yeast where individual, endogenous spliceosome proteins could be specifically labeled with fluorescent moieties by virtue of chemical tags, dihydrofolate reductase (DHFR) and O^6-alykylguanine *S*-transferase (SNAP). In a painstaking tour de force, experiments were carried out with proteins labeled in each of four major subcomplexes known to associate during the splicing cycle, the U1 and U2 snRNPs, the U4/U5/U6 tri-snRNP and the NTC, or Nineteen complex. Fluorescent versions of each complex, in addition to the pre-mRNA harboring a fluorescent label, allowed for real-time observation of the order of assembly and on−off rates of the various splicing complexes with their substrate RNA. The results indicate that the on rates are relatively similar, and that the spliceosome does follow the ordered assembly pathway that was previously postulated, where U1 joins the RNA first, followed by U2, then the tri-snRNP and finally the NTC. Significantly, binding of the subcomplexes was found to be reversible and commitment of the pre-mRNA to splicing increased as the spliceosome assembled. This method unlocks a new direction in the study of splicing mechanism and how the splicing complexes could be regulated, a central question that is still largely untouched in the alternative splicing field. **Jason G. Underwood, Ph.D.**

Fighting Tuberculosis with a Trojan Horse

Malaria and tuberculosis are two of the most prevalent and deadly diseases in the world, collectively killing millions of people each year. Drug resistant strains of both *Plasmodium falciparum*, the parasite responsible for causing malaria, and *Mycobacterium tuberculosis*, the agent that causes tuberculosis, are rapidly emerging, urgently calling for the development of new drugs. Miller *et al.* (*J. Am. Chem. Soc.* 2010, *133*, 2076−2079) present an innovatively designed conjugate of artemisin, an antimalarial agent, and mycobactin T, a mycobacterial-specific siderophore that exhibits potent and selective activity against drug-resistant strains of both of these lethal microbial pathogens.



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The success of this clever approach is based on the importance of iron for both the survival of the organisms and the mechanism of action of the drug. Artemisin is a natural product that contains

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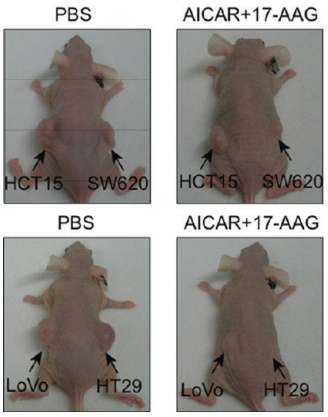
a peroxide functionality. Once it is inside *P. falciparum*, the peroxide moiety is reductively cleaved by iron, generating oxygen radicals that are key players in the demise of the parasite. *M. tuberculosis*, on the other hand, requires iron for its virulence, and utilizes specific siderophores, or iron chelators, such as mycobactin T, to maintain adequate iron levels. The authors hypothesized that linking the *P. falciparum* drug artemisin, which has no activity against *M. tuberculosis* on its own, with mycobactin T might result in a "Trojan-horse"-like agent with unique selectivity for both pathogens. Indeed, the conjugate maintained activity against several drug-resistant strains of *P. falciparum,* and was also highly potent and selective against *M. tuberculosis*. Exploration into the mode of action of the conjugate in *M. tuberculosis* suggested that the mycobactin T portion of the molecule facilitates generation of an iron species capable of reducing the peroxide moiety of the artemisin, thus generating the toxic radicals that help kill the mycobacteria. Notably, the conjugate was not active against numerous other bacteria and mycobacteria. **Eva J. Gordon, Ph.D**.

Attacking Aneuploidy

Though the specific effects of aneuploidy, an abnormal number of chromosomes, depend on the organism and which chromosomes are involved, the condition generally causes either disease or death. Examination of aneuploidy at the cellular level has revealed cell proliferation defects, but paradoxically this ill-fated condition is also associated with cancer, a disease characterized by undeterred cell growth. Compounds that selectively affect aneuploid cells over cells with normal chromosome numbers could be used as molecular tools to probe the mechanisms responsible for the varied effects of aneudploidy. Now, Tang *et al.* (*Cell* 2011, *144*, 499−512) report the identification of small molecules that selectively synergize with aneuploidy to prevent cell growth.



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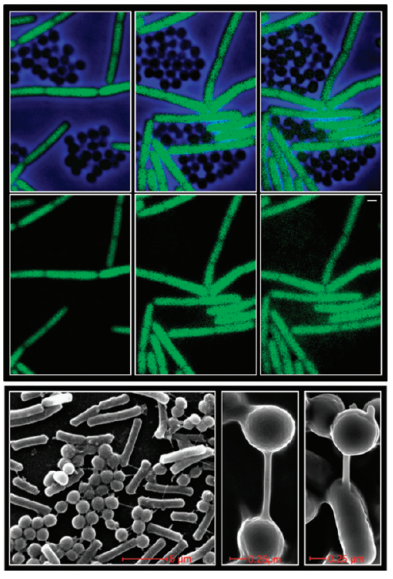
In addition to causing proliferation defects, aneuploidy induces proteotoxic and energy stress on cells. A collection of compounds known to cause similar stresses was screened for the ability to selectivity inhibit the growth of mouse cells containing three (instead of the normal two) copies of either chromosome 1, 13, 16, or 19, over control cells. Three compounds, the energy stress inducer AICAR, the Hsp90 inhibitor 17-AAG, and the autophagy inhibitor chloroquine, were synergistic with the aneuploid state of the cells in impairing growth. Exploration of their mechanism of action revealed that these compounds selectively induce apoptosis in trisomic cells through pathways involving activation of AMPK, an enzyme involved in energy homeostasis, and p53, a tumor suppressor protein. Further studies suggested that the compounds may aggravate the already stressed quality control systems in aneuploid cells that monitor the protein folding and chromosome segregation processes. Finally, the three compounds also selectively inhibited the growth of highly aneuploid cancer cells over cancer cells possessing fewer aneuploidies. This selectivity implicates aneuploidy as an intriguing new anticancer target. **Eva J. Gordon, Ph.D**.

A New Form of Bacterial Communication

Bacteria lack typical sensory organs to communicate with one another. They have, however, evolved "quorum sensing," a system by which they can communicate with other bacterial cells in their vicinity. This form of communication is achieved via the secretion and detection of small molecules called autoinducers. Quorum sensing typically aids bacteria in making important decisions with regards to their survival, such as the production of virulence factors. Another form of bacterial communication involves the packaging of cytoplasmic molecules into extracellular membrane vesicles that deliver these molecules via fusion to other bacterial cells in the vicinity. In a pioneering study, Dubey and Ben-Yehuda (*Cell* 2011, *144*, 590−600) identify a novel form of bacterial communication that could potentially provide new targets in the development of therapies against pathogenic bacteria.



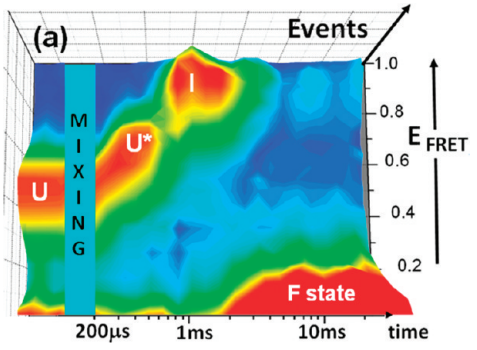
Reprinted from *Cell*, *144*, Dubey, G.P. and Ben-Yehuda, S. Intercellular Nanotubes Mediate Bacterial Communication, 590−600, Copyright 2011, with permission from Elsevier.

When grown on solid surfaces, the authors observed a transfer of cytoplasmic green fluorescent protein (GFP) from *Bacillus subtilis* strains carrying the reporter gene to strains that lacked the *gfp* reporter gene. Time-lapse microscopy captured the single-cell level transfer of GFP molecules from *gfp*+ to *gfp*− strains. The transfer of nongenetically encoded cytoplasmic calcein was also observed. The exchange of cytoplasmic content between adjacent cells brought to light the interesting possibility that bacterial cells may possess intercellular connections to transfer molecules. Using high-resolution scanning electron microscopy, tubular protrusions connecting *B. subtilis* cells were observed. Interestingly, these nanotubes, which

were frequently larger than 100 nm in width, appeared only when cells were grown on a solid medium. The authors went on to demonstrate the transfer of proteins and plasmids via these nanotubes. In a remarkable observation, *B. subtilis* was shown to transfer GFP to Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* cells. The discovery of bacterial communication that involves the transfer of both hereditary and nonhereditary molecules among diverse species of bacteria raises the troubling notion that virulence factors could potentially be transferred via nanotubes to commensal bacteria converting them into pathogens. **Jitesh A. Soares, Ph.D**.

Folding of a Single α-Synuclein

Protein folding is a complex, dynamic process that can be influenced by numerous factors, including the amino acid sequence of the protein, the local environment, and the presence of chaperones or binding partners. In fact, some proteins, like the central nervous system protein α-synuclein, are intrinsically disordered unless they are interacting with specific binding partners. Given the implication of misfolded α-synuclein in certain neurodegenerative disorders including Parkinson's disease, a better understanding of its folding process may offer insights into novel preventative or treatment strategies for these devastating conditions. To this end, Gambin *et al.* (*Nat. Methods* 2011, *8*, 239−241) investigate the kinetics of folding of α-synuclein by combining rapid microfluidic mixing with single-molecule fluorescence resonance energy transfer (smFRET) microscopy.



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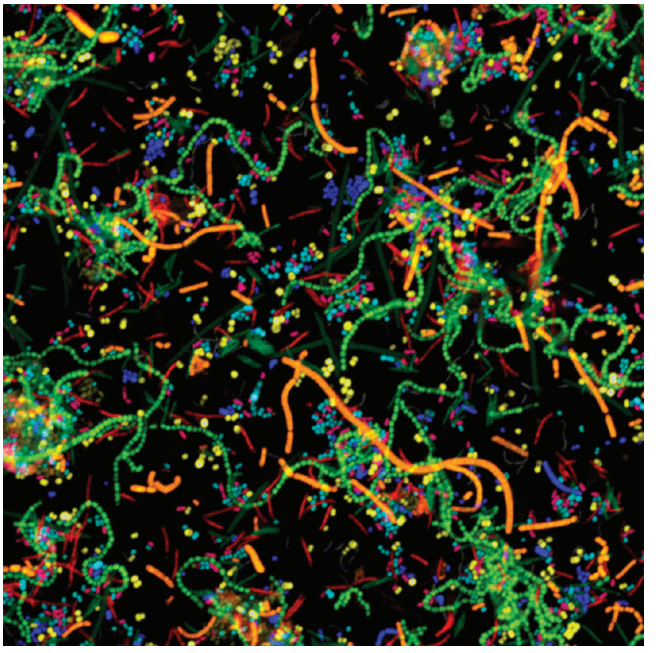
Single-molecule methods such as smFRET are useful for probing folding pathways because they provide galleries of observations on individual proteins. In addition, the use of a kinetic mixing apparatus, which rapidly triggers conformational transitions, enables a unique view into the sequence of binding and folding steps. However, existing rapid mixers, including microfluidic devices, use flow velocities that are too high for smFRET detection. In order to monitor folding with the submillisecond time resolution of smFRET, a microfluidic device was custom built to perform high-speed mixing followed by flow slow enough to enable smFRET detection. Using this innovative device, the kinetics of α-synuclein binding, folding, dissociation, and unfolding could all be interrogated. It was found that rapid mixing of α-synuclein with a binding partner initiated a sequence of folding steps that proceeded through transient structures. Interestingly however, rapid dilution to promote dissociation between the protein and the binding partner resulted in a simple two-state transition back to the unfolded state. This combination of a novel microfluidic mixer and smFRET opens a new path for interrogation of protein folding, and can be expanded to other complex transient processes such as the dynamics of weak molecular interactions. **Eva J. Gordon, Ph.D**.

Fluorescence Technique Decodes Patterns in Dental Plaque

In nature, microbes often cluster into complex communities of varied organisms to form biofilms. Though their diversity is well-known, scientists have not had available techniques that would allow them to understand the structural organization of these networks. Now Valm *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 2011, *108*, 4152−4157) have developed an innovative new combinatorial labeling and imaging technique based on fluorescence in situ hybridization (FISH) that allowed them to simultaneously visualize and identify 15 different types of microbes within a sample of a common biofilm, human dental plaque.



Picture credit: Alex Valm.

The researchers took advantage of advances in fluorescence imaging and linear unmixing algorithms that untangle overlapping fluorescence spectra to develop a technique called Combinatorial Labeling and Spectral Imaging FISH (CLASI-FISH). The strategy takes up to 8 different fluorophore probes and attaches them to the 5′-end of oligonucleotides that target the small subunit of the microbial ribosome. Labeling each group of microbes with a unique combination of two fluorescent molecules expands the color palette.

Initially Valm *et al.* labeled *E. coli* with these probes to demonstrate that the imaging technique would work. They divided the *E. coli* into 28 different batches, labeling each batch with two different fluorescent probes. They then combined these populations, measured the fluorescence of the bacteria. The unmixing algorithms allowed them to correctly identify the 28 different probe combinations and to quantify the mix of label types with 98% accuracy. The researchers then used the same strategy to analyze a laboratory mixture of 15 different microbes found in human dental plaque. They developed 15 different

sequences of probe DNA and created two probes for each microbe. They added these probes to the known mixture of microbes and analyzed them, identifying and quantifying them within the mixed sample. Finally they used the probes developed for the dental microbes to characterize samples of human dental plaque. The imaging technique allowed them to measure the relative amounts of these microbes within the sample and analyze the spatial relationships of these microbes based on which color combinations glommed together in the sample.

This work extends the number of species that researchers can identify simultaneously using fluorescence from a few to more than a dozen. This clever approach also offers the opportunity for biologists to examine organelles and other complex molecular arrangements within cells to better understand their structural organization. **Sarah A. Webb, Ph.D**.

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