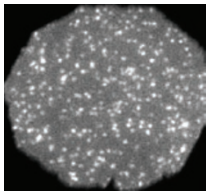
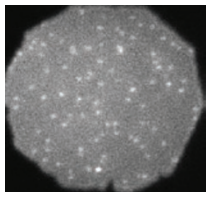
Seeing single spliceosomes





The spliceosome is a complex macromolecular machine made up of 5 small nuclear RNAs (snRNAs) and ~100 core proteins that act on precursor messenger RNAs (pre-mRNAs) to remove introns and join exons together. Previous work has shown that individual spliceosome subcomplexes associate with pre-mRNA in an ordered and sequential way to form functional spliceosomes. To directly examine the kinetics of subcomplex association with pre-mRNAs, Moore, Gelles, Cornish and colleagues labeled individual subcomplexes with different fluorophores and analyzed their assembly by using a previously published multiwavelength fluorescence technique—colocalization single-molecule spectroscopy (CoSMoS). Experiments were carried out in yeast whole-cell extracts, and the single fluorophore labeled pre-mRNA was tethered to a glass surface. In this system, the appearance and disappearance of fluorescent spots that colocalize with the surface represent the comings and goings of individual spliceosome subcomplexes. The authors found that commitment of an individual pre-mRNA to splicing increases as the assembly process proceeds and that each step seems to be reversible. Future studies using single-molecule systems such as this could lead to new insights into the mechanism of pre-mRNA splicing and perhaps alternative splicing as well. (*Science* **331**, 1289–1295, 2011) *BK*

ABA remastered

Some plant biologists feel there is a bias against their field because their findings are often not deemed directly relevant to human and animal models. Such perceived limitation can be turned to one's advantage, as recently described by Crabtree and colleagues, who co-opted molecular events in a plant signaling pathway to control the proximity of proteins in mammalian cells. Abscisic acid (ABA) is a plant hormone that controls seed dormancy and responses to environmental stress. Resolution of the ABA pathway has seen recent breakthroughs, with the identification of ABA receptors and the development of a mechanistic understanding of the molecular events involved. ABA binds to members of the PYL-PYR-RCAR family of proteins, triggering a conformational change that results in the recruitment and inhibition of phosphatases of the PP2C family such as ABI. This is essentially a dimerization event controlled by ABA. Fu-Sen Liang working in the Crabtree lab fused fragments of the ABA receptor PYL1 and the phosphatase ABI containing the complementary surfaces to different target proteins that would be in proximity only in the presence of ABA. Similar systems already exist; for example, rapamycin (Rap) can induce the proximity of proteins containing a domain from FKBP and Frb. However, because the ABA pathway does not exist in mammalian cells, there are no endogenous binding proteins that would compete for its availability. Also, unlike rapamycin, ABA has no detectable toxicity and in fact is readily found in fruits and vegetables that we consume. The authors directly compared the Rap and ABA systems in controlling gene activation, and they found a faster and more linear response with ABA. Moreover, ABA had the added benefit of being easier to wash off from cells than Rap. Both Rap and ABA systems can be used in the same cell to control different biological processes increasing the potential applications of both techniques. The authors also assessed the ABA system for controlling protein localization (with one of the fusion partners targeted to the different cellular compartments) and signal transduction events (by directing the membrane localization of Son of Sevenless and hence the activation of GTPase Ras). A catalytically inactive ABI mutant also worked well in this system, which should circumvent any potential issues due to extraneous phosphatase activity in the cell. Finally, ABA is quite inexpensive and was found to be stable in cultured mammalian cells and in mouse serum, even after oral administration; thus, in addition to its value as an experimental tool in cells, the ABA system has the potential to be used in animal models and perhaps even therapeutically. (*Sci. Signal.* **4**, rs2, 2011) *IC*

Trimming TLRs

Toll-like receptors (TLRs) 3, 7 and 9 detect invading microorganisms by binding non-methylated CpG motifs, single-stranded RNA and double-stranded RNA, respectively. However, they also have the potential to recognize self nucleic acids, and TLR7 and TLR9 have both been implicated in autoimmune diseases. As a result, TLR activity is thought to be tightly regulated through trafficking and proteolytic activation. Recent results have been conflicting over whether asparagine endopeptidase (AEP) or members of the cysteine cathepsin family are responsible for proteolytic activation of TLR9, and whether TLR7 is even regulated in this manner. Barton and colleagues now reveal that both AEP and cathepsins are involved in a two-step process to activate TLRs. The first step involves cleavage of TLR9's N terminus by either AEP or cathepsins; inhibiting both was necessary to fully prevent this cleavage. The second step involves further N-terminal trimming of TLR9 by cysteine cathepsins only. This step is necessary for full stimulation of TNF production in response to CpG, which in fact could be mostly blocked by pharmaceutical inhibition of cathepsins alone. Complete inhibition of TNF production required targeting of both AEP and cathepsins. Processing of TLR precursors involving both AEP and cathepsins also extended to TLR3 and TLR7, as combined proteolytic inhibition decreased the ability of TLR3 and TLR7 to induce TNF production. Further research will be needed to determine exactly how proteolytic cleavage frees TLRs for activation, but this evidence argues strongly that both AEP and cysteine cathepsins are integral in this process. Additional investigation of the activity of AEP and cysteine cathepsins in autoimmune diseases will also be interesting, as targeting of these proteases may inform patient treatment. (*J. Exp. Med.* doi:10.1084/jem.20100682, published online 14 Mar 2011) *SM*

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