have shielded the charged groups of the inositol head group with butyrate and AM esters, which are subsequently hydrolyzed in the cell cytosol. Intriguingly, it would appear that, despite the inclusion of a novel phosphate on the 6' position, many PIP_3 binding and metabolizing proteins are capable of being recruited by the PIP_4 molecule. Laketa et al. (2009) show that PIP_4/AM is a more potent stimulator of several PIP_3-dependent processes in the cell than is the related derivative PIP_3/AM, suggesting that the novel PIP_4 is not as accessible to degradative enzymes (such as endogenous 3′ or 5′ phosphatases) as is PIP_3 once cytosolic hydrolases have removed the AM and butyrate groups from the inositol ring. Using this technique, they are able to bypass the requirement for growth factor stimulation and subsequent PI 3′-kinase activation, to look directly at a subset of PI dependent signaling in the cell, including their feedback onto growth factor receptors themselves. An exciting possibility is that the inclusion of the 6' phosphate group in PIP_4 will render this analog inaccessible to some PIP_3 binding proteins, thus becoming a growth factor-independent agonist of pathways such as the PDK1, AKT/PKB pathway, which is characterized in great detail in this article.

Use of this new molecule, in combination with other methods of manipulating PI metabolism, has the potential to untangle some of the complex web of protein modification and signaling feedback that growth factor signaling evokes in models of health and disease.

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A Versatile Actor Finds a New Role

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RNA stars in many roles within the cell. In a recent paper published in *Cell*, Loh et al. demonstrate a new mechanism of action for natural riboswitches and provide important insights into the regulation of virulence in a pathogenic bacterium.

Whether it's on the big screen or on the small one, in the lead role or in support, some actors seem to be able to play any type of role. Two of the most versatile actors of today, Christopher Walken and Meryl Streep, appeared together in the 1978 film "The Deer Hunter," for which Walken was awarded an Oscar for Best Supporting Actor, while Streep was nominated for Best Supporting Actress. Since then, both actors have gone on to appear in countless memorable roles, and I'm never surprised to see them in something unusual, such as Walken in "Hairspray," or Streep as Julia Child in "Julie and Julia."

Such versatility appears at the molecular level as well, where RNA is frequently discovered in new roles. Coincidentally, it was also in 1978 when Sidney Altman and coworkers showed that RNA plays an essential role in the activity of the enzyme RNase P (Stark et al., 1978). In the three decades since, we have begun to appreciate the versatility of RNA as it stars in an increasing variety of roles in biological systems. Far from being a bit player mediating translation, RNA appears in diverse starring roles in both eubacteria (Waters and Storz, 2009) and eukaryotes (Rana, 2007).

The bacterial kingdom also has its share of versatile players, including the pathogen *Listeria monocytogenes*, which excels at playing the bad guy. In addition to being a leading cause of food-borne illness in humans, *L. monocytogenes* can

cross the intestinal barrier, the blood-brain barrier, and the feto-placental barrier, all of which can lead to more serious diseases (Lecuit, 2005). Due to its relevance in disease and its historical role as a model for host-pathogen interactions, Toledo-Arana et al. (2009) recently carried out a comprehensive study of the *L. monocytogenes* transcriptome under a variety of conditions that mimic its infective cycle. In doing so, they identified over 50 small RNAs (sRNAs) that are expressed under different conditions, including two that are involved in virulence in a mouse model. In addition, the authors identified ~40 riboswitches, which are RNA sequences that directly bind to metabolites to mediate changes in gene expression without the need for additional protein cofactors.

In a new paper appearing in *Cell,* Loh et al. (2009) demonstrate how some of these riboswitches control the expression of virulence regulators in *L. monocytogenes*. In addition to providing new insights into the lifestyle of a pathogenic bacterium, Loh et al. (2009) demonstrate a new mechanism of action for riboswitches, which has broad implications for the control of gene expression in a range of bacteria and possibly other domains of life as well.

The word "riboswitch" entered the scientific vernacular in 2002, when it was demonstrated conclusively that mRNA sequences could directly bind small-molecule metabolites to control gene expression in bacteria (Nahvi et al., 2002). Over the past several years, a variety of mechanisms for riboswitch control have been identified, including those occurring both co- and posttranscriptionally (Roth and Breaker, 2009). While their specific mechanisms of action vary, all natural riboswitches identified thus far have been shown to act "*in cis*," which is to say that they mediate the expression of mRNAs that they are covalently linked to.

Loh et al. (2009) challenged this notion by considering the fate of 7 riboswitches that were predicted to respond to the ubiquitous metabolite *S*-adenosylmethionine (SAM). In Gram-positive bacteria, such as in *L. monocytogenes*, most SAM-responsive riboswitches terminate transcription of downstream genes upon binding SAM, leaving behind short, non-coding transcripts. Until now, it has been largely assumed that these truncated transcripts did not play further regulatory roles within the cell. Loh et al. (2009) questioned whether these truncated transcripts could mediate the expression of other genes *"in trans"*.

The authors began with the SAM-riboswitch SreA, which regulates the transcription of three downstream genes that encode an ABC-transporter complex. In nutrient-poor conditions, where SAM concentrations are expected to be low, the ABC-transporter is expressed, presumably leading to nutrient import. In nutrient-rich conditions, SAM is available to bind to the SreA riboswitch, which terminates transcription and leaves behind a 229 nucleotide RNA fragment. Loh et al. (2009) deleted the region of the 5′-UTR that coded for the riboswitch and looked for changes in the expression profiles of other genes. They discovered that 3 genes were upregulated, while 6 others were downregulated. Loh et al. (2009) were able to dissect the pathways by which a SAM-riboswitch could regulate these genes by focusing on one upregulated gene (*lmo2230*) and one downregulated gene (*lmo0049*), and by performing a great deal of molecular detective work.

Previous work had shown that expression of *lmo2230*, a protein homologous to a bacterial arsenate reductase, is activated by two regulators, the sigma factor σ^B and the virulence regulator PrfA (Milohanic et al., 2003). Through western blotting, the authors show that deletion of the SreA riboswitch had no effect on the levels of σ^B, but that expression of PrfA increased two fold. Through a variety of studies in *L. monocytogenes*, in an ectopic system in *E. coli*, and in vitro, Loh et al. (2009) make a compelling case that a 229 nucleotide RNA fragment left behind by a SAM riboswitch regulates the expression of PrfA *in trans*. Since PrfA is a master virulence regulator, many of its targets (including *lmo2230*) are thus regulated by SAM.

Interestingly, it was previously shown that the mRNA encoding PrfA has an RNA thermosensor in its 5′-UTR that prevents translation at low temperatures, but allows it at temperatures that permit infection (Johansson et al., 2002). Loh et al. (2009) show that the SreA riboswitch fragment only interacts with PrfA at higher temperatures, suggesting that the *Listeria* virulence program is activated most strongly under conditions that favor infection (e.g., body temperature, rich nutrients).

To be sure, there is much more to discover about the specific mechanisms that control the virulence programs in pathogenic bacteria. However, the work by Loh et al. (2009) answers many important questions, raises some new ones, and reminds us just how versatile an actor RNA is. Now that the world will be on the lookout for *trans*-acting riboswitches, I suspect that many other RNA "trans-actors" will be discovered to play critical roles in biology.

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