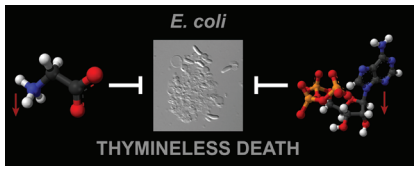
In this ISSUE

Preventing a Thymineless Death

Antifolates, compounds that interfere with folate biosynthesis or activity, have important therapeutic applications as antibiotics and anticancer agents. Folate derivatives are key intermediates in the biosynthesis of certain amino acids, DNA, and RNA, but the metabolic consequences of antifolate activity are not well understood. Using a metabolomics approach, Kwon *et al.* (DOI: 10.1021/cb100096f) investigate the effects of the antifolate antibiotic trimethoprim on metabolic pathways in the bacteria *E. coli*.

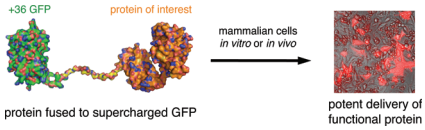
When lots of nutrients are around, trimethoprim causes thymineless death, in which bacteria die due to lack of thymine for DNA synthesis. However, in minimal media, the drug simply halts cell growth, and the cells enter a stable phase called stasis. Using high performance liquid chromatography-tandem mass spectrometry, it was discovered that in minimal media, glycine is depleted first, which rapidly activates the stringent response to stop cell growth. Purine depletion follows, which maintains the cell growth stoppage that enables long-term stasis. These mechanisms circumvent thymineless death and illustrate a clever method devised by *E. coli* for surviving folate depletion.



Protein Delivery: It is Positively the Charge

Biomolecules of all sizes can be powerful tools for understanding biology, and they also can have diverse therapeutic applications. However, while many small molecules can penetrate the cell membrane unimpeded to exert their desired effect inside the cell, proteins typically cannot. Some methods have been devised to facilitate protein entry into cells, but they are generally inefficient, especially for delivery in an *in vivo* setting. Cronican *et al.* (DOI: 10.1021/cb1001153) now report a novel method for delivering proteins into mammalian cells using a "supercharged" variant of green fluorescent protein (GFP).

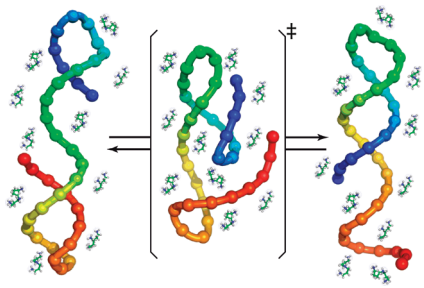
The method relies on the generation of a fusion protein comprised of the protein of interest and a GFP containing a net positive charge of 36 (+36 GFP). Proteins fused to +36 GFP entered the cell and could access the cytosol, as was demonstrated with ubiquitin, and the nucleus, as was the case for a recombinase enzyme. Furthermore, when injected *in vivo* into the subretinal space of mice, the recombinase fusion protein was delivered to the mouse retinae. This versatile approach offers a promising new method for the delivery of exogenous proteins into cells for numerous applications.



Refolding RNA

En route to a final, stable structure, RNA has been known hang out in more than one metastable state. These states have distinct dynamics and functions, but little is known about their structures or the mechanisms that govern the transitions between them as well as their transition to the final functional RNA fold. The transitions, which occur through a process known as RNA refolding, are profoundly affected by positively charged ions such as magnesium and polyamines. Fürtig *et al.* (DOI: 10.1021/cb100025a) now examine the influence of such ions on the structure and dynamics of refolding between two metastable states of a bistable RNA.

Using a cleverly designed photolabile caged RNA, one state of the bistable RNA could be selectively stabilized. Upon exposure to light, however, the RNA undergoes refolding to the other state. Kinetic and structural studies with CD and NMR spectroscopy in the presence of varying concentrations of magnesium or the polyamine spermidine demonstrated that different positively charged compounds utilize distinct mechanisms to modulate RNA refolding. These insights enabled the creation of a model of the transition states utilized by RNA during the refolding process.



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