SENSORS AND PROBES

(Protein) timing is everything

Tandem fluorescent protein timers allow measurements of both protein turnover and trafficking in the cell.

To truly understand the dynamics of the proteome in a living cell, protein turnover and protein mobility must both be assessed. Typically this would require more than one method, but a new type of fluorescent protein timer now makes it possible to jointly follow turnover and movement.

Fluorescent timer proteins were introduced more than a decade ago; the original timers were fluorescent proteins that changed color over time—for example, from green to red. But technical issues have limited their widespread use.

Michael Knop and Elmar Schiebel of the University of Heidelberg and their colleagues now describe 'tandem' fluorescent timers, or tFTs, based on a fusion of two different colored fluorescent proteins. The two main requirements for constructing a tFT are that the fluorescent proteins have well-separated emission spectra and that they become fluorescent with different and well-characterized kinetics. The timer is attached to a protein of interest, whose communal age after translation can be determined by the ratio of the intensities of the two fluorescent moieties. The tFT concept is modular, allowing a variety of timers from fluorescent proteins with well-established properties to be constructed.

The Knop-Schiebel team designed timers made from the monomeric red fluorescent protein mCherry and a monomeric superfolder green fluorescent protein, sfGFP. mCherry has a maturation half-time of about 40 minutes, whereas sfGFP becomes fluorescent much faster, on the order of a few minutes. The construct therefore is mostly green right after synthesis but slowly becomes red over time.

The researchers showed a variety of uses for the mCherry-sfGFP tFT, which ranged from looking at how spindle pole bodies and nuclear pore complex components are inherited during mitosis in budding yeast, to performing a high-throughput screen to identify regulators of protein turnover. The mCherry-sfGFP tFT was useful for following proteins with half-lives between about 10 minutes and 8 hours. Given their modular design, tFTs could be constructed using faster- or slower-maturing red fluorescent proteins to report on fast events, such as transcriptional bursts, or slow events, such as differentiation.

By combining tFTs with genome-wide protein tagging, protein dynamics could be systematically analyzed across the proteome.

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RESEARCH PAPERS

Khmelinskii, A. *et al.* Tandem fluorescent protein timers for *in vivo* analysis of protein dynamics. *Nat. Biotechnol.* **30**, 708–714 (2012).