TECHNIQUE

Fluorescent timer

There are many ways to monitor the onset of gene expression, but so far it has been impossible to detect its down-regulation. This problem might have been solved now, as Terskikh and colleagues report in *Science* a simple method to follow promoter activity.

Last year, a red fluorescent protein (drFP583) was identified in tropical corals, further increasing the wide spectrum of possibilities to light up cells in different colours. Not satisfied with just one colour, Terskikh and colleagues introduced random mutations into drFP583, and found one mutant (called E5) that changes its fluorescence from green to red in a time-dependent manner. As E5 switches from green to red fluorescence over time, it can be used as a timer for gene expression. During the first hours of activity of a promoter, green fluorescence is predominant, whereas sustained activity of the promoter leads to a mixture of green and red fluorescence. A few hours after the promoter is turned off, only red fluorescence remains.

Terskikh and colleagues verified these predictions in three experimental systems. First they monitored up- and down-regulation of E5 expression in Tet-on and Tet-off mammalian expression systems. Then they followed the activity of a heat-shock promoter during heat-induced

stress of *Caenorhabditis elegans*. Last, they traced the expression of a homeobox gene involved in the patterning of anterior structures in *Xenopus laevis*. In all cases, green fluorescence correctly indicated the onset of gene expression and was replaced with red fluorescence when expression ceased. So after decades of blue-stained embryos, we'll now have to get used to seeing gene expression in green and red.

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References and links

ORIGINAL RESEARCH PAPER Terskikh, A. *et al.* "Fluorescent timer": protein that changes color with time. *Science* **290**, 1585–1588 (2000)

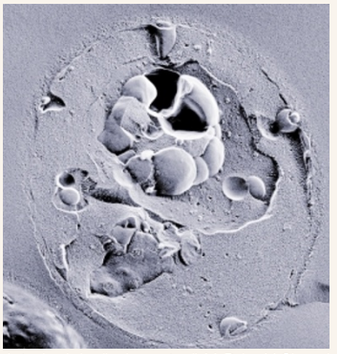
MEMBRANE DYNAMICS

Variation on a theme

When cells are starving, they can eat almost anything, even their own proteins and organelles. This desperate act — called autophagy — involves the engulfment of cytosol and organelles by a membrane that folds back onto itself, giving rise to an autophagosome.

Screens in the yeast *Saccharomyces cerevisiae* identified many Apg mutants defective in autophagy, and their characterization is progressing rapidly. About two years ago, Ohsumi and colleagues characterized a ubiquitin-like protein modification necessary for autophagy. Two papers from the same group now describe a second, more unusual, ubiquitin-like modification required for this process.

***Apg8* is essential for autophagy in yeast, and starvation increases its transcription. Its weak homology with the ubiquitin-like protein Apg12p suggests that Apg8p might also be a ubiquitin-like protein. Kirisako and colleagues showed that the carboxyl terminus of newly synthesized Apg8p is proteolytically cleaved by the cysteine protease Apg4p. The cleaved form of Apg8p is covalently modified on its terminal glycine and, as a consequence, becomes tightly membrane associated. Ichimura and colleagues determined the nature of this modification and found that it's not a protein, but the lipid phosphatidylethanolamine (PE). The sequence of events leading to the conjugation of Apg8p to PE is reminiscent of ubiquitylation, as Apg8p is first bound to the E1-type enzyme Apg7p, and then to the E2-type enzyme Apg3p, before being finally transferred to PE. The reaction is reversible, and Apg4p can cleave the amide bond.**



Autophagic bodies in yeast cell. M. Baba, Y. Ohsumi

So what is the function of 'Apg8ylation'? The authors speculate that Apg8p might be part of the fusion machinery involved in the formation of autophagosomes. Or could it be that, much like ubiquitin tags proteins for degradation in the proteasome, Apg8p tags PE-containing membranes for degradation through autophagy?

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References and links

ORIGINAL RESEARCH PAPERS Ichimura, Y. *et al.* A ubiquitin-like system mediates protein lipidation. *Nature* **408**, 488–492 (2000) | Kirisako, T. *et al.* The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J. Cell Biol.* **151**, 263–275 (2000)

WEB WATCH

Smart by name...

Modular protein domains are nature's solution for building versatile proteins from ready-made building blocks, but keeping track of how they've been shuffled is no easy task. Simple Modular Architecture Research Tool (SMART), written by Peer Bork's group at the European Molecular Biology Laboratory in Heidelberg, is a powerful tool for putting domains in context.

The simplest way to familiarize yourself with SMART's database of over 400 characterized domains is to click on an entry in the list. This categorizes the occurrence of each domain according to evolutionary distribution and cellular location, as well as including information on structure and function. You can also do sequence alignments.

More specific searches can be done on two levels: sequence or architecture. You can simply paste in a sequence or type in accession numbers from all the commonly used protein databases. If you already know what modular domains your protein of interest contains, you can use the architecture tool to search for proteins that contain the same combination of domains. You can also narrow your search down to a particular organism or group of organisms.

SMART can also represent groups of proteins as 'beads on a string' cartoons — an effective way to compare the architecture of a group of proteins. All the 'beads' in these cartoons are clickable, taking you through to that domain's page.

Data entry is a bit clumsy (for instance, the Boolean operators only work if you type them in upper case), but it's worth perservering; even if you don't have a specific query, half an hour spent playing with SMART will reveal nature's uncanny capacity to reuse the same module in many contexts.

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