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HIGHLIGHTS ADVISORS

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HARVARD MEDICAL SCHOOL, BOSTON, MA, USA

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INSTITUT PASTEUR, PARIS, FRANCE

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UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PA, USA

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SAMUEL LUNENFELD RESEARCH INSTITUTE, TORONTO, CANADA

NORBERT PERRIMON
HARVARD MEDICAL SCHOOL, BOSTON, MA, USA

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YALE UNIVERSITY, NEW HAVEN, CT, USA

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THE BURNHAM INSTITUTE, LA JOLLA, CA, USA

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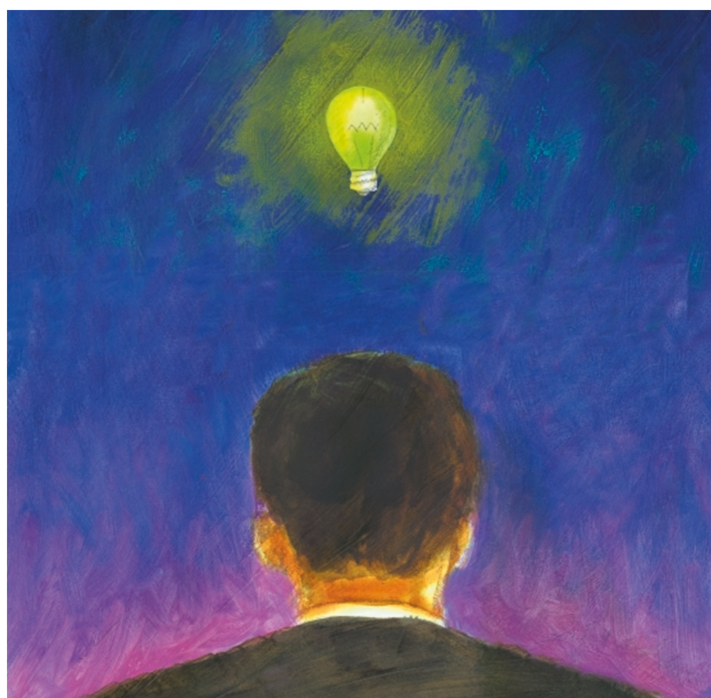
BEATSON INSTITUTE FOR CANCER RESEARCH, GLASGOW, UK

JOHN WALKER
MRC DUNN HUMAN NUTRITION UNIT, CAMBRIDGE, UK

TECHNIQUE

A bright idea



The intrinsic brightness of green fluorescent protein (GFP) and the fact that it can be used to make fusion-protein constructs make it an invaluable tool for studying biological processes *in vivo*. However, its value would be significantly greater if it could be used to selectively mark proteins through photoactivation — a goal that has now been achieved thanks to work published by Patterson and Lippincott-Schwartz in *Science*. These authors had the bright idea of making a GFP that remains 'off' until it's switched 'on'.

GFP normally exists in two forms — a 'neutral' and an 'anionic' form, which produce major and minor absorbance peaks, respectively. Intense illumination with ~400-nm light converts GFP mainly to the anionic form, which results in an increase in minor peak absorbance, as well as a subsequent threefold increase in fluorescence after excitation at 488 nm.

Patterson and Lippincott-Schwartz therefore decided to search for a GFP variant with a reduced minor absorbance peak. They hoped that if they could find such a variant, it would mean that photoconversion with ~400-nm light would produce a larger increase in minor peak absorbance, and therefore a more marked increase in fluorescence after 488-nm excitation.

Because a previously reported GFP mutation at threonine 203 resulted in reduced absorbance at 488 nm without affecting the major absorbance peak, the authors studied various substitutions at this position and found what they were looking for in the form of a histidine substitution. They called this stable GFP variant photoactivatable GFP (PA-GFP), because they found that it had virtually undetectable absorbance at the minor peak and that irradiation with ~400-nm light resulted in a large increase in minor peak absorbance. They also found that 488-nm excitation of photoconverted PA-GFP produced an ~100-fold increase in fluorescence.

So, how useful is PA-GFP for studies in living cells? When the authors studied cells expressing PA-GFP, they found that, in contrast to cells expressing wild-type GFP, 488-nm excitation produced very little fluorescence before photoconversion. Furthermore, after photoconversion with ~400-nm light, they saw that 488-nm excitation produced a more than 60-fold increase in fluorescence for PA-GFP-expressing cells, compared with only an ~2.6-fold increase for wild-type-GFP-expressing cells.

Patterson and Lippincott-Schwartz concluded their report by highlighting some of the biological applications of PA-GFP. They showed that it can be used both as a free protein to study protein dynamics (they looked at protein diffusion across the nuclear envelope) and as a chimeric construct to study membrane dynamics (they used it to monitor interlysosomal membrane exchange). All in all, the stability and optical contrast of PA-GFP, combined with the fact that signals can be obtained from it rapidly and specifically, mean that searching for PA-GFP was a very bright idea indeed.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Patterson, G. H. & Lippincott-Schwartz, J. A photoactivatable GFP for selective photolabelling of proteins and cells. *Science* **297**, 1873–1877 (2002)

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