nature biotechnology

Letters may be edited for space and clarity.
They should be addressed to:
Correspondence
Nature Biotechnology
345 Park Avenue South
New York, NY 10010-1707, USA
or sent by e-mail to biotech@natureny.com
Please include your telephone and fax numbers.

Protein tags enhance GFP folding in eukaryotic cells

To the editor:

The recent findings of Waldo et al.[1] demonstrate that the fusion of the green fluorescent protein (GFP) to insoluble proteins dramatically reduces its folding ability in prokaryotic cells. This was ingeniously exploited, and GFP was used as a reporter for the folding of upstream fusion proteins in E. coli.

Our findings demonstrate that fusion of GFP to amino acidic tags also affects GFP folding in eukaryotic cells. Remarkably, specific protein tags can not only reduce, but also dramatically enhance, the folding of GFP. Thus, fused polypeptides may help producing GFP variants with a more robust folding, e.g. when poorly folding spectral variants are used or when fusion to insoluble proteins is needed.

The three-dimensional structure of GFP demonstrates a similarity to that of streptavidin, both molecules possessing a compact β-barrel structure[2,3]. Schmidt et al.[4] selected a polypeptide that binds with high affinity to streptavidin and competes for the binding of biotin. This streptavidin-binding (sb) peptide demonstrates a stable two-turn helix conformation when bound to streptavidin, and caps one end of the β-barrel[3]. The sb-tag was successfully used as a C-terminal tag for recombinant proteins. Taken together, these findings suggested that the sb-tag might be efficiently used as a C-terminal tag for GFP. sb-tagged wild-type GFP (wtGFP) was produced by PCR and subcloned in the pRK-5 expression vector. GFP fused in frame to an N-terminal myc-tag[5] or to a C-terminal tag randomly generated by a polylinker sequence were also produced and expressed. An untagged wtGFP in pRK-5 (*NcoI*/*EcoRI* segment, i.e. devoid of the inhibitory 5′ untranslated region)[6] and a wtGFP in a CMV-driven expression vector were used as controls.

The GFP expression constructs were transiently transfected into human kidney 293T or COS-7 cells[7]. The amount of GFP protein (P) produced was quantified by western blot using rabbit anti-GFP antisera. The GFP fluorescence (F) was quantified by fluorescence spectroscopy of whole-cell lysates. wtGFP expressed in bacteria was purified to homogeneity and used as a standard in western blotting and spectrofluorimetry. The actual folding of bacterial GFP was determined as described[8], and the corresponding F/P ratio was used to estimate the percentage of correctly folded, functional molecules in mammalian cells.

Specific protein tags were found to profoundly affect the folding ability of GFP. The C-terminal sb-tag induces a striking threefold better folding of GFP. On the other hand, a random C-terminal tag does not significantly affect GFP folding, whereas the N-terminal myc-tag severely diminishes the folding efficiency of wtGFP. The different expression levels, translation rates, and protein stability of the various constructs did not significantly affect the generation of functional GFP chromophores indicating a direct effect of added amino acidic tags on the folding of GFP in mammalian cells.

The streptavidin-binding tag assumes a stable helical configuration[3]. Thus, it might nucleate to a folded domain, which may accelerate or stabilize the folding of the rest of the molecule, thereby acting as an intramolecular chaperone[9]. Interestingly, known intramolecular chaperones commonly are N-terminal[9], whereas the streptavidin-binding tag is C-terminal versus GFP. Thus, our findings do not support the view that protein folding rigidly proceeds in an N-terminal to C-terminal direction[10]. The downstream streptavidin-binding tag might also act as a "clamp" and prevent unfolding. However, this possibility appears unlikely, since no significant unfolding and loss of fluorescence of the clamp-less wtGFP-myc was observed over time. Quite to the contrary, the wtGFP-sb and wtGFP-myc demonstrate essentially identical fluorescence half-lives and decay curves after cycloheximide treatment.

Our findings demonstrate that a C-terminal streptavidin-binding tag efficiently augments the folding ability of GFP, and this might be usefully exploited in current experimental systems. Moreover, they indicate that a tagged GFP might be a novel experimental system to test fundamental theories of folding. The use of amino acidic tags to modulate folding has the significant advantage over mutagenesis methods of maintaining intact the protein structure under study.

Andrea Sacchetti and Saverio Alberti
Istituto di Ricerche
Consorzio, Mario Negri Sud
S.M. Imbaro (Chieti), Italy
alberti@cmns.mnegri.it

1. Waldo, G.S. et al. *Nat. Biotechnol.* **17**, 691–695 (1999).
2. Ormo, M. et al. *Science* **273**, 1392–1395 (1996).
3. Schmidt, T.G. et al. *J. Mol. Biol.* **255**, 753–766 (1996).
4. Schmidt, T.G. & Skerra, A. *Protein Eng.* **6**, 109–122 (1993).
5. Evan, G.I. et al. *Mol. Cell. Biol.* **5**, 3610–3616 (1985).
6. Chalfie, M. et al. *Science* **263**, 802–805 (1994).
7. Dell'Arciprete, R. et al. *J. Histochem. Cytochem.* **44**, 629–640 (1996).
8. Patterson, G.H. *Biophys. J.* **73**, 2782–2790 (1997).
9. Shinde, U., Fu, X. et al. *J. Biol. Chem.* **274**, 15615–15621 (1999).
10. Fedorov, A.N. & Baldwin, T.O. *J. Biol. Chem.* **272**, 32715–32718 (1997).

Facts and hypotheses

To the editor:

In their commentary on recent scientific studies of the potential ecological consequences of *Bt* crops and public discussion of those data, Anthony Shelton and Richard Roush ("False reports and the ears of men," *Nat. Biotechnol.* **17**, 829) apply the terms "false" and "rumor" no less than 10 times to interpretations of evidence that they don't regard as valid. Implicit in their borrowed Shakespearean rhetoric is the premise that an idea not yet scientifically proven to be true must be false.

A more accurate distinction would be between "facts" and "hypotheses," where the boundary may be harder to draw, but the tentative nature of new scientific knowledge is far more explicit. Debate over that distinction is the essence of good science. When science underlies important public policies, it is entirely legitimate for the media and the public to be interested in, report on, and participate in the debate. I believe the public, by and large, is sophisticated enough to understand that a hypothesis is not yet a fact, and that the policy implications of some hypotheses deserve discussion while research pursues better answers.

If we want good science to be the foundation for policy, we have to speak scientifically. If we want policy to acknowledge the tentative nature of new evidence, we have to call it a hypothesis, not try to discredit it as a "rumor." Calling something false when it's simply not yet agreed to be true is propaganda, not science.

Edward Groth III
Consumers Union of United States
Yonkers, NY
groted@consumer.org

Erratum

In the October Research News and Views entitled "Cheap DNA arrays—It's not all smoke and mirrors" (*Nat. Biotechnol.* **17**, 953, 1999), one of the authors' email addresses was given incorrectly. Alan Blanchard's correct email address is apb@abraxas.org