2000; Rodgers and Shiozawa, 2008). By demonstrating that phenylalanyl-tRNA synthetases can misacylate $tRNA^{Phe}$ with L-DOPA, Moor et al. (2011) provide a fourth pathway for incorporating L-DOPA into proteins (Figure 1). This new pathway may be particularly relevant to proteins synthesized by the mitochondria, given the inability of the mitochondrial phenylalanyl-tRNA synthetase to hydrolyze misacylated $tRNA^{Phe}$.

Incorporation of L-DOPA through misacylation of $tRNA^{Phe}$ is distinct from the other known mechanisms for incorporating L-DOPA into proteins, as it replaces a phenylalanine residue, whereas in the other three mechanisms L-DOPA replaces tyrosine. As a result, it should be possible to quantify the extent to which misacylation of $tRNA^{Phe}$ results in the incorporation of L-DOPA into proteins in vivo. Furthermore, since the efficiency of tRNA misacylation by L-DOPA appears to be similar for the phenylalanyl- and tyrosyl-tRNA synthetases, misincorporation of L-DOPA in place of phenylalanine may also provide an estimate for the frequency with which L-DOPA incorporation is due to misacylation of $tRNA^{Tyr}$. Given that L-DOPA is currently the most commonly prescribed drug for Parkinson's disease, it is imperative to determine the extent to which tRNA misacylation is responsible for the elevated levels of L-DOPA-containing proteins found in patients treated with this drug (Rodgers et al., 2006). The article by Moor et al. (2011) in this issue of *Chemistry & Biology* provides a way to address this question.

REFERENCES

Fu, S., Davies, M.J., Stocker, R., and Dean, R.T. (1998). Biochem. J. *333*, 519–525.

Klipcan, L., Moor, N., Kessler, N., and Safro, M.G. (2009). Proc. Natl. Acad. Sci. USA *106*, 11045–11048.

Molnár, G.A., Nemes, V., Biró, Z., Ludány, A., Wagner, Z., and Wittmann, I. (2005). Free Radic. Res. *39*, 1359–1366.

Moor, N., Klipcan, L., and Safro, M.K. (2011). Chem. Biol. *18*, this issue, 1221–1229.

Reynolds, N.M., Ling, J., Roy, H., Banerjee, R., Repasky, S.E., Hamel, P., and Ibba, M. (2011). Proc. Natl. Acad. Sci. USA *107*, 4063–4068.

Rodgers, K.J., and Dean, R.T. (2000). Int. J. Biochem. Cell Biol. *32*, 945–955.

Rodgers, K.J., and Shiozawa, N. (2008). Int. J. Biochem. Cell Biol. *40*, 1452–1466.

Rodgers, K.J., Hume, P.M., Morris, J.G., and Dean, R.T. (2006). J. Neurochem. *98*, 1061–1067.

Roy, H., Ling, J., Irnov, M., and Ibba, M. (2004). EMBO J. *23*, 4639–4648.

Roy, H., Ling, J., Alfonzo, J., and Ibba, M. (2005). J. Biol. Chem. *280*, 38186–38192.

Green-Red Flashers to Accelerate Biology

Konstantin A. Lukyanov[1,*]
[1]Institute of Bioorganic Chemistry, Miklukho-Maklaya 16/10, 117997 Moscow, Russia
*Correspondence: kluk@ibch.ru
DOI 10.1016/j.chembiol.2011.10.003

Photoactivatable fluorescent proteins are now widely used for cell and protein tracking and super-resolution optical imaging. In this issue, Adam et al. (2011) report a general approach to introduce photochromism into green-to-red photoconvertible proteins and describe new photoactivatable protein with a complex four-state flasher-like behavior and advanced characteristics.

Green fluorescent protein (GFP) from jellyfish *Aequorea victoria* and its numerous mutants and homologs from diverse marine creatures possess a unique type of posttranslational modifications—a chromophore group formed via self-catalyzed reactions from its own internal amino acids (Chudakov et al., 2010). As a result, GFP-like fluorescent proteins (FPs) become fluorescent by themselves, with no help of external enzymes or cofactors except molecular oxygen. This extraordinary ability makes it possible to use FPs as fully genetically encoded labels to mark specific cells, cell organelles, and proteins and monitor process in live systems. Nowadays, they are an indispensable toolkit in experimental biology, which is used in thousands of studies each year.

A particularly interesting and useful group of FPs is photoactivatable FPs (PAFPs) capable of drastically increasing their fluorescence in a specific spectral region in response to illumination with light of specific wavelength and intensity (Chudakov et al., 2010). There are two main types of such proteins—irreversible and reversible PAFPs. Proteins of the first type photoconvert once from one state to another due to irreversible photochemical reactions of the chromophore (e.g., extension of its conjugated π-system) and/or surrounding amino acids (e.g., decarboxylation of a Glu residue) (Mizuno et al., 2003; Chudakov et al., 2010). PAFPs of the second type can be photoconverted multiple times due to *cis-trans* isomerization of the chromophore and/or reversible reactions such as protonation-deprotonation and hydration-dehydration (Chudakov et al., 2003, 2010; Brakemann et al., 2011).

One of the first irreversible PAFPs was green-to-red photoconvertible FPs called Kaede (after the Japanese word for maple leaf) (Ando et al., 2002). This green FP becomes red after illumination

with UV or violet light (at around 400 nm). A number of Kaede-like proteins were generated, namely, EosFP, Dendra, mKikGR, and their improved variants. In 2008, a mutant of EosFP called IrisFP was created (Adam et al., 2008). IrisFP shows complex spectral behavior combining both reversible and irreversible photoconversions (Figure 1). In its initial green fluorescent state, IrisFP can be reversibly switched off and on by cyan (approximately 490 nm) and violet (approximately 400 nm) light, respectively. Strong illumination with violet light results in irreversible conversion of IrisFP into red fluorescent state. Red IrisFP in turn undergoes reversible photoswitching between fluorescent and dark states upon illumination with green (approximately 540 nm, On-to-Off) and blue (approximately 440 nm, Off-to-On) light.

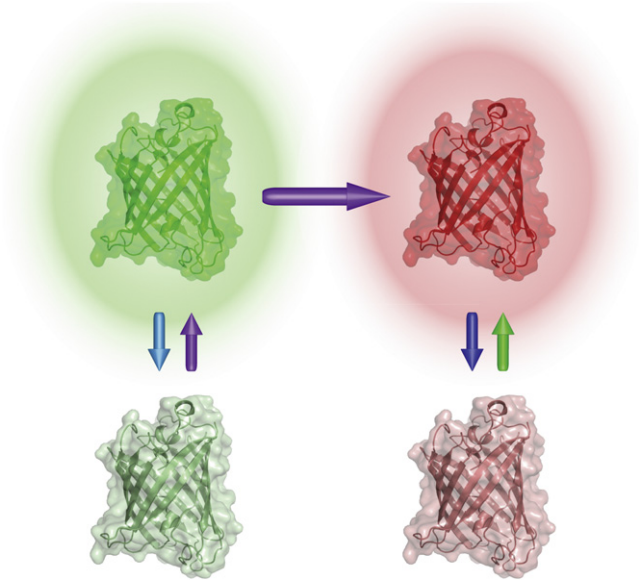

Figure 1. An Outline of Photoconversions of IrisFP-Like Fluorescent Proteins

Fluorescent protein β-barrels are colored correspondingly to fluorescence of different states, switching between which can be achieved by illumination with light of specific wavelength and intensity (arrows). These four-state photoswitchable proteins resemble an emergency flasher with blinking green and red beacons.

In the current issue of *Chemistry & Biology*, Adam et al. (2011) demonstrate that such on/off photochromism can be introduced in virtually any green-to-red photoconvertible fluorescent protein by rational point mutagenesis at key positions. This work represents a logical continuation of previous investigations of mechanisms of reversible photoconversions in PAFPs. When the first reversibly switchable PAFPs, a chromoprotein asFP595 from sea anemone *Anemonia sulcata* and its variants such as KFP1, were discovered, it was found that photoswitching between dark and red fluorescent states is mainly controlled by a few residues around chromophore, suggesting *cis-trans* isomerization of the chromophore during photoconversion (Chudakov et al., 2003). Further mutagenesis and structural studies with different fluorescent proteins confirmed and extended this concept. Importance of additional residues was revealed (Adam et al., 2008). Although exact influence of each amino acid substitution still cannot be predicted, construction of a few mutants was sufficient to find variants with IrisFP-like phenotype (Adam et al., 2011). In particular, single F173S mutation in Dendra2 resulted in excellent photochromic behavior. This protein named NijiFP, after the Japanese word for rainbow, possesses advanced characteristics, such as monomeric state, efficient green-to-red photoconversion and reversible photoswitching in both states, high fluorescence brightness, and high photostability. NijiFP appears to be a very promising PAFP for advanced imaging applications.

How to take advantage of PAFPs in imaging? The most straightforward application is to track movements of objects of interest (cells, cell organelles, or proteins). Light beam can be strictly controlled in space and time giving an opportunity of precise photolabeling with PAFPs. Second major field of PAFPs' applications emerged recently with introduction of super-resolution fluorescence microscopy technologies, which enable imaging down to 10–20 nm scale resolution (Chudakov et al., 2010). PAFPs can be also used to extend possibilities of imaging with multiple labels since PAFPs can be discriminated by their photoconversion behavior (Chudakov et al., 2003; Andresen et al., 2008), to establish photochromic FRET (Forster resonance energy transfer) with reversibly switchable acceptor (Subach et al., 2010), to track the degradation of a protein of interest (Zhang et al., 2007), or to discriminate target signal from noise (Marriott et al., 2008). As demonstrated by Adam et al. (2011) the newly introduced NijiFP can be successfully used to track diffusion of a target protein and obtain super-resolution images. Moreover, its four-state spectral behavior allows combining both tracking and super-resolution imaging for the same object. NijiFP is likely well suitable for photochromic FRET as its absorption spectra for both green and red states can be reversibly changed, although this is yet to be demonstrated. Again, due to combination of irreversible green-to-red conversion and reversible photoswitching, it is tempting to propose the use of NijiFP for simultaneous observation of target protein movements and its interaction with other proteins. Generally, sophisticated photoactivation scheme of NijiFP calls for new ideas and new questions to be solved by advanced microscopy.

REFERENCES

Adam, V., Lelimousin, M., Boehme, S., Desfonds, G., Nienhaus, K., Field, M.J., Wiedenmann, J., McSweeney, S., Nienhaus, G.U., and Bourgeois, D. (2008). Proc. Natl. Acad. Sci. USA *105*, 18343–18348.

Adam, V., Moeyaert, B., David, C.C., Mizuno, H., Lelimousin, M., Dedecker, P., Ando, R., Miyawaki, A., Michiels, J., Engelborghs, Y., and Hofkens, J. (2011). Chem. Biol. *18*, this issue, 1241–1251.

Ando, R., Hama, H., Yamamoto-Hino, M., Mizuno, H., and Miyawaki, A. (2002). Proc. Natl. Acad. Sci. USA *99*, 12651–12656.

Andresen, M., Stiel, A.C., Fölling, J., Wenzel, D., Schönle, A., Egner, A., Eggeling, C., Hell, S.W., and Jakobs, S. (2008). Nat. Biotechnol. *26*, 1035–1040.

Brakemann, T., Stiel, A.C., Weber, G., Andresen, M., Testa, I., Grotjohann, T., Leutenegger, M., Plessmann, U., Urlaub, H., Eggeling, C., et al. (2011). Nat. Biotechnol. 10.1038/nbt.1952.

Chudakov, D.M., Matz, M.V., Lukyanov, S., and Lukyanov, K.A. (2010). Physiol. Rev. *90*, 1103–1163.

Chudakov, D.M., Feofanov, A.V., Mudrik, N.N., Lukyanov, S., and Lukyanov, K.A. (2003). J. Biol. Chem. *278*, 7215–7219.

Marriott, G., Mao, S., Sakata, T., Ran, J., Jackson, D.K., Petchprayoon, C., Gomez, T.J., Warp, E., Tulyathan, O., Aaron, H.L., et al. (2008). Proc. Natl. Acad. Sci. USA *105*, 17789–17794.

Mizuno, H., Mal, T.K., Tong, K.I., Ando, R., Furuta, T., Ikura, M., and Miyawaki, A. (2003). Mol. Cell *12*, 1051–1058.

Subach, F.V., Zhang, L., Gadella, T.W., Gurskaya, N.G., Lukyanov, K.A., and Verkhusha, V.V. (2010). Chem. Biol. *17*, 745–755.

Zhang, L., Gurskaya, N.G., Merzlyak, E.M., Staroverov, D.B., Mudrik, N.N., Samarkina, O.N., Vinokurov, L.M., Lukyanov, S., and Lukyanov, K.A. (2007). Biotechniques *42*, 446, 448, 450.

Biology-Driven Library Design for Probe Discovery

James Inglese[1,*] and Samuel A. Hasson[1]
[1]NIH Center for Translational Therapeutics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: jinglese@mail.nih.gov
DOI 10.1016/j.chembiol.2011.10.004

Libraries of diverse small molecules are important to probe and drug discovery. The current trend toward building massive screening collections to support drug development, a special application of chemical biology, can limit their broader potential. Biology-driven construction methods (Wallace et al., 2011) are rapidly emerging to bring chemical libraries back on a viable path.

When the label of a prescription medicine reads "inactive ingredients 98%," it is telling you the vast majority of substances in the pills are inert, and that is an important and acceptable method of preparing a drug for administration. However, in compound libraries designed for chemical biology, inertness, with apt pharmacokinetics (PK)/pharmacodynamics or not, is an undesirable filler. Considerable effort is made to maximize the potential for bioactivity in modern screening libraries. Although no one claims to know exactly which compounds are bioactive in any particular system, data-driven models may help select the bright sparks from the dim bulbs.

The study conducted by Wallace et al. (2011 [this issue of *Chemistry & Biology*]) represents an important step toward establishing a dichotomy between therapeutic drug and biological probe discovery. For the most part, probe and drug development has historically been approached from the same canonical methodologies and central paradigms. However, with significantly different goals, it would seem logical to use divergent paths and starting points. For example, in library design, blanket application of Lipinski's rule of five (Ro5), and derivatives thereof, represents the status quo for compound library selection. Ro5, intended to maximize the proportion of bioavailable small molecule agents, has been applied to guide most commercial and academic library development (Dolle, 2011). The reality today is that scientists seeking small molecules as probes to study a target of interest are relying on compound libraries designed to maximize favorable PK in human/animal subjects. Compounds based on peptide-like sequences (Kodadek, 2010), privileged structures, and natural products (Welsch et al., 2010) are examples of useful chemical probes that generally fall outside of the Ro5 criteria.

Clearly, the Ro5 along with many other general compound attributes are desirable for therapeutic drug discovery (Overington et al., 2006). Who would want their drug screening results to be dominated by unstable molecules with poor PK? Models developed to guide the population of screening libraries are needed, if not marginally to simplify high-throughput screening (HTS) logistics and costs, then importantly to accommodate novel technology and methodology. In this respect, the design of chemical libraries is more valuable than the sheer size. The results in Wallace et al. (2011) demonstrate, not unexpectedly, that the Ro5 may not always be the ideal filter for compounds likely to be useful in chemical biology. A completely new set of compound selection principles may maximize the chemical space most relevant to non- or pretherapeutic applications. After all, the Wright brothers didn't include a pressurized cabin on their flying machine; why add drag before you even get off the ground? Wallace and colleagues move beyond generic property filters to develop models for bioactive molecule characteristics. This is an evolving concept in biology-driven library construction (Basu et al., 2011) that focuses on structural signatures instead of generic descriptors (such as calculated solubility partition coefficients, e.g., cLogP and molecular weight). In an examination of publicly available screening data sets, their Bayesian model showed promise in enriching for the most active hits.

Given the value of probe molecules to basic research, a fundamental rethinking of the methodologies used in such efforts is warranted. The Wallace et al. (2011) presents timely and immediately useful ideas for chemical biology. While the academic efforts to develop therapeutically relevant small molecules continue to show promise worldwide, their research to aid in the discovery of flexible and titratible tools (probes) that

