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Lights on Iron-Sulfur Clusters

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The biogenesis of iron-sulfur proteins within a living cell is traditionally investigated by enzyme activity measurements and radiolabeling techniques. In this issue of *Chemistry and Biology*, Hoff et al. introduce a non-invasive fluorescence-based method to trace the formation of iron-sulfur clusters at high sensitivity.

Iron-sulfur (Fe/S) clusters are among the most ancient and versatile cofactors in nature, performing important roles in electron transfer, enzyme catalysis, and environmental conditions sensing. Although their significance was recognized more than half a century ago, it was not until the late 1990s that it became evident that the assembly of Fe/S proteins is not a spontaneous but rather a catalyzed process of surprisingly high complexity (Lill and Kispal, 2000; Zheng et al., 1998). This realization stimulated a new, vibrant avenue of Fe/S proteins research that led to the discovery of more than 15 bacterial and 25 eukaryotic biogenesis components in the past few years, with no end in sight. These proteins assist distinct steps during the de novo biosynthesis of the Fe/S clusters and their insertion into apoproteins (Fontecave and Ollagnier-de-Choudens, 2008; Johnson et al., 2005; Lill, 2009), and functional characterization of the key components enabled elucidation of some basic biogenesis principles. Virtually all biological systems use a cysteine desulfurase (e.g., bacterial IscS or eukaryotic Nfs1) as a sulfur donor, thus converting cysteine to alanine and creating an enzyme-bound persulfide. The persulfide is transferred to a Fe/S scaffold protein (e.g., bacterial IscU or eukaryotic Isu1), facilitating the de novo synthesis of the Fe/S cluster on conserved cysteine residues of this protein. By virtue of specific transfer factors, the preassembled Fe/S cluster is released from the scaffold and inserted into apoproteins. Some Fe/S proteins might require additional specialized factors for the synthesis of their clusters.

For the assessment of the formation and/or disassembly of Fe/S clusters, researchers hitherto have mainly employed invasive methods; for example, the use of cell extracts for the analysis of Fe/S-dependent enzyme activities (such as aconitase, succinate dehydrogenase, or respiratory complex I), Fe/S protein stability (glutamine phosphoribosylpyrophosphate amidotransferase [GPAT]), or the capability of apoproteins to bind to RNA (iron-regulatory protein 1 [IRP1]) (Pierik et al., 2009). Radiolabeling with ^{55}Fe has proven to be a powerful assay capable of determining not only the steady-state levels of Fe/S proteins but also of estimating their de novo generation in a living cell. In this assay, the assembled ^{55}Fe/S proteins are purified from cell extracts by immunoprecipitation or affinity methods followed by quantitative scintillation counting. This technique has so far been mainly used in yeast cells due to their ability to grow under iron-limiting conditions and thus maintain a sufficiently high specific radioactivity. Circular dichroism (CD) spectroscopy provides a sensitive in vitro method for monitoring Fe/S cluster formation and incorporation as the conversion of an apoprotein to a holoprotein is commonly accompanied by structural changes (Bonomi et al., 2005). Other spectroscopic methods, such an EPR or Mössbauer spectroscopy, are far too insensitive for following biogenesis of Fe/S proteins.

In their article, Hoff et al. (2009b) introduce a fluorescence-based method for the noninvasive estimation of Fe/S cluster formation in a living cell. This extends the already impressive list of fluorescent tools for intracellular metal detection, including Ca, Cu, Zn, and Fe (Domaille et al., 2008). The authors use dithiol glutaredoxin, Grx2, which was previously shown to carry a [2Fe-2S] cluster bridged between two Grx2 monomers (Lillig et al., 2005), as a model Fe/S protein and a variant of the yellow fluorescent protein, Venus, as a fluorescent reporter. Grx2 was fused to two complementary fragments of this fluorescent protein with the idea that Fe/S cluster-driven dimer formation of Grx2 would bring the two fragments in intimate vicinity, thus inducing chromophore maturation resulting in a fluorescent protein formation (Figure 1). Increased fluorescence intensity was indeed observed upon synthesis of these fusion proteins in *E. coli*. Evidence for the presence of the Fe/S cluster was provided by CD spectroscopy and by the fact that the fluorescence changes were not observed when the [2Fe-2S] cluster-coordinating Cys residue of Grx2 was mutated to Ala. While it became clear from these pioneering experiments that the fluorescence increase is correlated with the formation of the Fe/S cluster, the removal of the cofactor by chemical methods hardly changed the fluorescence signal, indicating an irreversible association of the two Venus fragments. Hence, the technique, in principle, allows the identification of newly formed Fe/S clusters even if they are labile or transiently bound. However, the current approach cannot be used to monitor their reversible dissociation. Extending the strategy to allow this important measurement to be made would require further engineering of the fluorescent reporter molecules. Nonetheless, it is an important next step to make, as it would give insights into physiological conditions underlying Fe/S cluster disassembly (e.g., under oxidative stress conditions or low iron availability).

The described technique also appears to be applicable in eukaryotic cells. This is impressively demonstrated for two different compartments of human cells. Grx2-Venus fusion proteins expressed in either mitochondria or the cytosol, both native locations of this protein

(Lönn et al., 2008), showed the fluorescence increase expected for Fe/S cluster-mediated Grx2 dimerization (Figure 1). Importantly, the fluorescence increase depended on the function of two core Fe/S protein biogenesis components, the cysteine desulfurase Nfs1 and the scaffold protein, Isu1. These findings convincingly establish that the observed increase in fluorescence intensity is correlated with the Fe/S cluster-mediated dimerization of Grx2, as it facilitates the combination of the two fluorescent fragments.

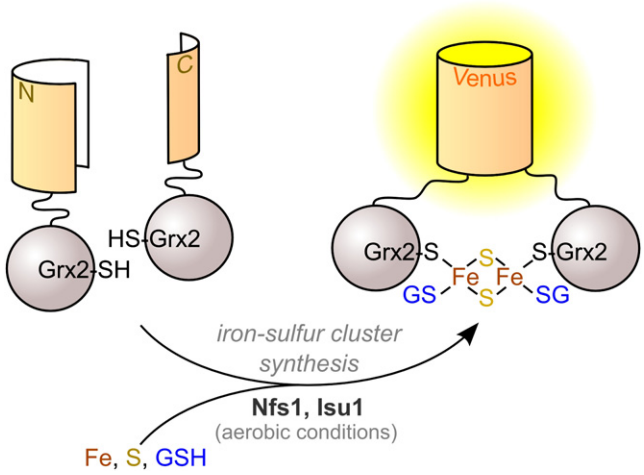

Figure 1. Principle of the Fluorescence-Based Detection of Fe/S Cluster Formation in a Living Cell

The glutathione (GSH)-dependent oxidoreductase Grx2 is fused independently to the N- and C-terminal fragments of a fluorescent protein (Venus). Upon formation of a bridging [2Fe-2S] cluster between two Grx2 monomers, the Venus fragments are located in immediate vicinity, increasing their chance to combine and generate the fluorescent conformation of Venus. The latter reaction requires the presence of molecular oxygen. The correlation between fluorescence emission and Fe/S cluster formation is demonstrated by the requirement of the cysteine desulfurase Nfs1 and the scaffold protein Isu1, both members of the mitochondrial ISC assembly machinery (Lill, 2009).

Another publication by the same team shows further examples of how this new technology might be used in future research for the analysis of Fe/S proteins both in vitro, and even more interestingly, in vivo (Hoff et al., 2009a). In this work, the authors analyze the monomer-dimer equilibrium of a GFP-Grx2 fusion protein, and find a significant fluorescence decrease upon Fe/S cluster-dependent dimerization of Grx2. Apparently, the presence of the Fe/S cluster quenched the fluorescence emission of the attached GFP molecules. Even though the transition from a dimeric to a monomeric state was slow in vitro (on the order of hours), one may envision that engineering of the system will allow faster rates, and thus enable the intracellular tracking of Fe/S cluster assembly and dissociation reactions in real time and with high sensitivity.

One may foresee that these novel approaches to monitor Fe/S clusters by fluorescence will find widespread applications, such as in systematic screens for the identification of new Fe/S biogenesis components, assaying diseases in Fe/S protein biogenesis (Rouault and Tong, 2008), and monitoring the suspected damaging effects of oxidative reagents on Fe/S clusters within a living cell. Such analyses so far have been hampered by the lack of rapid, real-time methods to follow the Fe/S cluster dynamics. The noninvasive character of the new method might therefore be a key property facilitating such studies of Fe/S protein dynamics in vivo. This technique may soon become a key tool in the workshop of Fe/S researchers, efficiently complementing the classical approaches for analyzing Fe/S proteins. Needless to say, the Fe/S community has a wish list for further lucent tools including those for the detection and discrimination of (nonbridging) [2Fe-2S] and [4Fe-4S] clusters.

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