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SNAP-Shots of Hydrogen Peroxide in Cells

Amy E. Palmer[1,*] and Philip J. Dittmer[1]
[1]Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA
*Correspondence: Amy.palmer@colorado.edu
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Long regarded as a toxic byproduct, hydrogen peroxide is increasingly recognized as an important cellular signal. Efforts at defining the spatiotemporal nature of hydrogen peroxide production recently got a boost by the development of a series of organelle-targeted fluorescent probes by Srikun et al. (2010).

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) are generally thought of as unwanted and detrimental byproducts, produced accidentally as a result of cellular metabolism. There are many good reasons for this bad rap. After all, organisms have evolved exquisite mechanisms for detoxifying ROS through enzymes such as catalase, superoxide dismutase, glutathione peroxidases, and peroxiredoxins, which serve to limit the buildup of ROS and are generally thought of as cellular protective agents. Without question, excessive generation of ROS such as H_2O_2 are toxic to cells, leading to oxidative stress, apoptosis or necrosis, and cell senescence (Finkel and Holbrook, 2000). However, H_2O_2 can be produced deliberately and in a regulated manner by the NADPH oxidase (NOX) and Dual oxidase (Duox) family of enzymes (Bedard and Krause, 2007). A classic example is the oxidative burst utilized by professional phagocytes such as macrophage and neutrophils to protect a host against invasion by pathogens. While this is a specialized example, NOX family enzymes exist in a wide range of nonphagocytic cells, suggesting that deliberate production of H_2O_2 plays a fundamental role in cell biology (Bedard and Krause, 2007).

Mounting evidence suggests that H_2O_2, produced by NOX extracellularly, can act in both an autocrine and paracrine fashion (Figure 1). For autocrine signal transduction, H_2O_2 is widely becoming recognized as a bona fide second messenger. Bursts of H_2O_2 are produced in response to a variety of stimuli, including growth factors, cytokines, hormones, calcium, and neurotransmitters (Bedard and Krause, 2007). The primary action of H_2O_2 as a signaling molecule is the oxidation of proteins to modulate their function. H_2O_2 can oxidize cysteine residues to sulfenic acid (Cys-S-OH) that can be readily reversed by cellular reductants such as glutathione and thioredoxin. However, H_2O_2 does not specifically oxidize any Cys-containing protein because the Cys must be deprotonated at physiological pH, and hence have a low pKa. Thus, H_2O_2 acts on select sites, including those found in a number of transcription factors and protein tyrosine phosphatases (Rhee, 2006). H_2O_2 can also modify histidine and methionine residues. By modulating the function of intracellular protein targets, H_2O_2 has been found to affect gene transcription, cell proliferation, differentiation, metabolism, and migration (Bedard and Krause, 2007). Lastly, Niethammer et al. (2009) recently provided convincing evidence that H_2O_2 produced by Duox serves as a paracrine signal for recruitment of leukocytes to wounds in the vertebrate zebrafish.

A paradigm is emerging that when and where H_2O_2 is produced has a profound impact on downstream cellular consequences. The ability to monitor the spatiotemporal nature of H_2O_2 production and clearance in real time would be an invaluable tool in elucidating H_2O_2 biology. Toward this end, Srikun et al. (2010) have now generated a family of H_2O_2 sensitive fluorescent probes targeted to various cellular organelles. These localized probes should help provide insight into the spatial heterogeneity of H_2O_2 signaling.

To generate organelle-targeted probes, Srikun et al. (2010) combined the power of a small molecule fluorescent indicator, namely the Peroxy Green probe previously developed by the same research group (Miller et al., 2007), with the genetic targetability of the SNAP-tag technology pioneered by Keppler et al. (2004) (Figure 1). Peroxy Green consists of a boronate-modified Tokyo Green fluorophore; reaction with H_2O_2 liberates the boronate, resulting in an increase in fluorescence and hence a ''turn-on'' signal. To be compatible with the SNAP-tag technology, this basic probe was modified to incorporate a moiety that could serve as a substrate for AGT (O^6-alkylguanine-DNA alkyltransferase). Two different Peroxy Green probes were synthesized; one conjugated to the traditional benzylguanine substrate (referred to by the authors as SPG1) and another linked to a benzyl-2-chloro-6-aminopyrimidine substrate

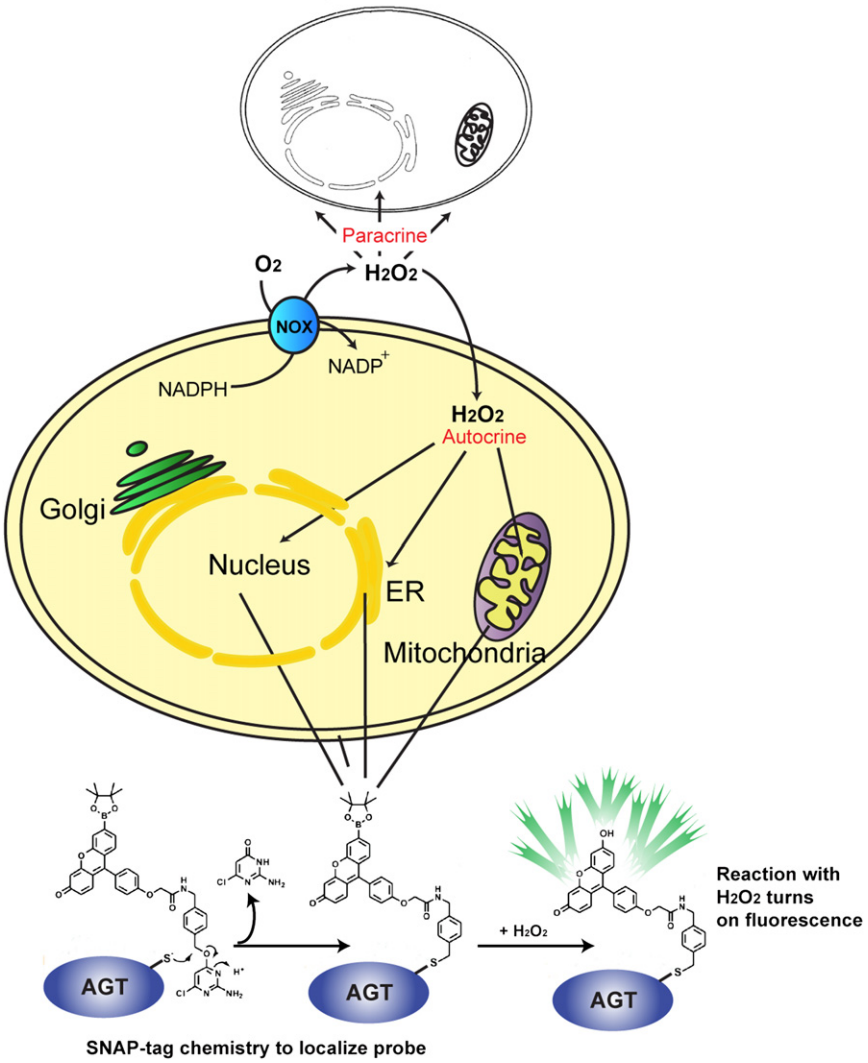

Figure 1. Organelle- and Membrane-Targeted Peroxy Green Probes Will Enable Detection of Autocrine and Paracrine H_2O_2 Signaling

(SPG2). Unexpectedly, SPG1 was not membrane permeable, while SPG2 successfully labeled multiple intracellular targets. Previous studies have demonstrated that the attachment of benzylguanine can alter cell permeability of small molecule fluorophores, sometimes forcing researchers to synthesize derivatives of different fluorophores to achieve intracellular labeling (Keppler et al., 2006). The work of Srikun et al. (2010) highlights an alternative solution that involves modification of the AGT substrate. The differential permeability of SPG1 and SPG2 allowed the authors to efficiently and selectively label both intracellular and extracellular sites, which for H_2O_2 may be especially useful in examining autocrine versus paracrine signaling.

One particularly exciting aspect of this work was the use of SNAP-tag to genetically target Peroxy Green to distinct cellular organelles that are sensitive to or capable of generating local H_2O_2, including the nucleus, mitochondria, plasma membrane, and endoplasmic reticulum. Organelle targeting is achieved by fusing AGT to a protein or signal sequence that directs the protein to these locations. This precise localization can only be accomplished by genetic targeting. In addition, Srikun et al. (2010) demonstrate the utility of these probes by demonstrating their ability to detect H_2O_2 in each location.

There is one example of a genetically encoded hydrogen peroxide sensor (HyPer), based on the insertion of a circular permuted yellow fluorescent protein into the regulatory domain of a prokaryotic H_2O_2-sensing protein (Belousov et al., 2006). Both HyPer and SPG2 can be localized within cells and each possesses different strengths. HyPer is ratiometric (its excitation spectrum shifts upon reaction with H_2O_2). This is beneficial for quantitative imaging; however, it does come at a cost of dynamic range. The maximum ratio change of HyPer is 3x, whereas the maximum signal change for SPG2 is 32x. HyPer has the advantage of being reversible, allowing dynamics of H_2O_2 production and consumption to be monitored in real time. However, circular permuted fluorescent protein probes are sensitive to pH fluctuations, as the mechanism of detection involves modification of the protonation state of the chromophore, rendering these probes susceptible to pH artifacts. But both these sensor platforms (HyPer and SPG2) provide an excellent starting point for sensitive, selective real-time imaging of H_2O_2 and will likely be invaluable tools for exploring the spatiotemporal patterning of H_2O_2 signals.

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