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Reining in H_2O_2 for Safe Signaling

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Mammalian cells use hydrogen peroxide (H_2O_2) not only to kill invading pathogens, but also as a signaling modulator. Woo et al. (2010) now show that the local inactivation of a H_2O_2-degrading enzyme ensures that the production of this oxidant is restricted to the signaling site.

The notion that hydrogen peroxide (H_2O_2) acts as a signaling molecule in mammalian cells was first proposed over a decade ago but remains controversial. Skepticism stems from the apparent paradox between the specificity that is required for a signaling molecule and the damaging properties of this oxidant, which is normally inactivated in cells by detoxifying enzymes, such as peroxiredoxins (D'Autréaux and Toledano 2007). In phagocytic cells, NADPH oxidase generates H_2O_2 for the direct purpose of killing invading microbes. So how does the mammalian cell tame the toxicity of H_2O_2 to make it useful as a signaling molecule? In this issue, Woo et al. (2010) help to resolve this paradox. They show that localized inactivation of a peroxiredoxin enzyme allows low concentrations of H_2O_2 to mediate tyrosine kinase receptor signaling in specific cellular subdomains, while preventing the toxic accumulation of H_2O_2 elsewhere in the cell.

The details of how H_2O_2 is produced and how it affects signaling after activation of receptor tyrosine kinases are becoming clearer. Engagement of a receptor tyrosine kinase with its ligand results in the production of H_2O_2, which is catalyzed by an NADPH oxidase in the plasma membrane of many types of mammalian cells (Lambeth 2004) (Figure 1). Produced close to the activated receptor tyrosine kinases, H_2O_2 helps to sustain the nascent signal because H_2O_2 inactivates nearby protein tyrosine phosphatases, which normally shut down signaling by dephosphorylating pathway components (Tonks 2006). Protein tyrosine phosphatases have an active site cysteine residue with a low pKa value, making them susceptible to oxidation by H_2O_2 and hence inactivation.

Although the involvement of H_2O_2 in membrane receptor signaling is well established, major gaps remain in the current model. First, it is difficult to fathom how a build up of H_2O_2 at the levels required for effective oxidation, and hence inactivation of protein tyrosine phosphatases, occurs in the presence of cellular peroxiredoxins, which reduce H_2O_2 to water (Figure 1). Considering that both cellular concentrations and reactivity toward H_2O_2 are orders of magnitude greater for peroxiredoxins than for protein tyrosine phosphatases, oxidation of protein tyrosine phosphatases by H_2O_2 is highly unlikely (D'Autréaux and Toledano 2007). So, how does the cell override this redox barrier while still preventing the toxic accumulation of H_2O_2?

In their new work, Woo and colleagues provide an interesting, albeit indirect, answer to this problem. They build upon the notion that H_2O_2 production begins only when the NADPH oxidase complex assembles within discrete plasma membrane subdomains near activated receptors (Ushio-Fukai 2006) (Figure 1). The authors identify peroxiredoxin PrxI as being integral to the precision of H_2O_2 signaling in mammalian cells. They show that, upon receptor engagement, this enzyme becomes phosphorylated on a tyrosine residue (Tyr[194]) by a Src family kinase. In vitro peroxidase assays demonstrated that phosphorylation inactivates PrxI by decreasing the reactivity of its catalytic cysteine residue. They then show that PrxI phosphorylation correlates with H_2O_2 production and with the strength of the intracellular signal induced by the engaged receptor. Most importantly, the authors find that, whereas the majority of unmodified PrxI protein is found in the soluble fraction of cell extracts, phosphorylated PrxI, which accounts for about 0.3% of the total PrxI in the cell, is exclusively confined to the membrane-associated fraction that is known to contain c-Src, NADPH oxidase and receptor tyrosine
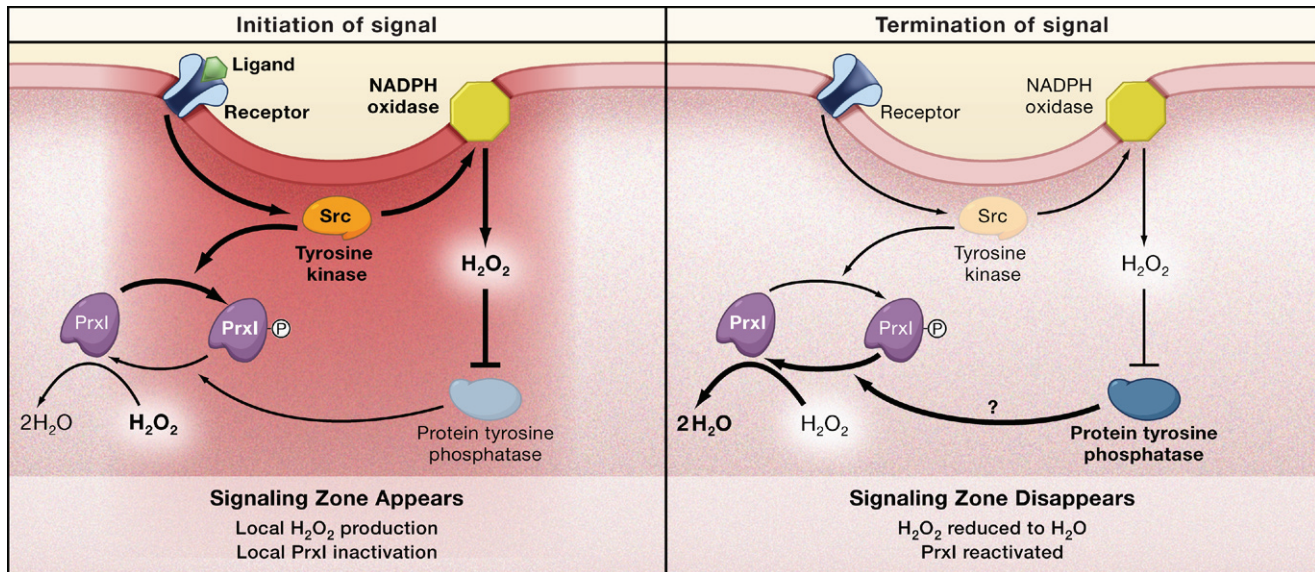
Figure 1. H$_2$O$_2$ and Receptor Signaling

(A) Signal initiation favors the production of hydrogen peroxide (H$_2$O$_2$) by activating the pathways shown with bold arrows. When a receptor tyrosine kinase is stimulated by binding of ligand, NADPH oxidase in the plasma membrane is activated, resulting in the local production of H$_2$O$_2$. Src kinase phosphorylates and inhibits peroxiredoxin I (PrxI), which normally degrades H$_2$O$_2$. Thus, the levels of H$_2$O$_2$ rise (red shading), leading to the inactivation of neighboring protein tyrosine phosphatases and sustained tyrosine receptor signaling. Because H$_2$O$_2$ is produced close to the activated receptors, it can act as a signaling molecule but does not accumulate to toxic levels inside the cell (Woo et al., 2010). Any H$_2$O$_2$ that escapes from regions where it is locally produced will be degraded by active PrxI in the cytosol.

(B) During signal termination, H$_2$O$_2$ levels decrease as H$_2$O$_2$-reducing pathways are activated (bold arrows). As suggested by Woo et al. (2010), phosphorylated PrxI might resume catalysis after a lag time, thereby decreasing H$_2$O$_2$ levels and initiating a cascade that contributes to the termination of the signaling process. It is not known how PrxI is dephosphorylated or how protein tyrosine phosphatase is reactivated.

In both panels, the shading of the Src kinase and the protein tyrosine phosphatase indicates the relative activity of each enzyme; dark shading signifies high activity, and light shading signifies low activity.

kinases. The authors confirm these results in different mammalian cell lines, each stimulated by a different extracellular ligand, and also in vivo in a mouse model of wound healing.

The authors conclude that, during signaling, H$_2$O$_2$ production appears to be confined to a discrete membrane subdomain that contains phosphorylated PrxI. The colocalization of inactive PrxI and H$_2$O$_2$ production permits even low concentrations of H$_2$O$_2$ to shut off protein tyrosine phosphatases and enhance signaling (Figure 1). In addition, the spatial restriction of PrxI close to the plasma membrane insulates the rest of the cell from the unwanted effects of H$_2$O$_2$, as the majority of PrxI in the cytosol is still active and capable of neutralizing H$_2$O$_2$.

This new model also helps to explain how the H$_2$O$_2$ signal might terminate. Interestingly, phosphorylation inactivates PrxI by causing a lag time in catalysis that is inversely proportional to the concentration of H$_2$O$_2$. This suggests that reactivation of PrxI occurs automatically when H$_2$O$_2$ levels rise beyond a certain threshold. Thus, H$_2$O$_2$ not only is sequestered in

specific locations within the cell but also is kept in check by PrxI. Localized production of H$_2$O$_2$ also provides the basis of H$_2$O$_2$-substrate specificity, as protein tyrosine phosphatases can be specifically recruited to the signaling zone (and inactivated by H$_2$O$_2$) through attachment to the phosphorylated domains of activated receptors (Meng et al., 2002).

The model proposed by Woo et al. (2010) also supports the current "floodgate" hypothesis, which proposes that peroxiredoxin enzymes are temporarily shut down during H$_2$O$_2$ signaling (Wood et al., 2003). However, here peroxiredoxins are inactivated locally by phosphorylation and not by the reversible hyperoxidation of their catalytic cysteine residue, as proposed in the "floodgate" hypothesis. Indeed, Woo et al. find no evidence that PrxI is hyperoxidized during receptor-dependent signaling, even when H$_2$O$_2$ production is boosted by overexpression of NADPH oxidase. Woo and colleagues go further, showing that, of the two cytosolic enzymes, PrxI is prone to tyrosine phosphorylation, whereas the closely related PrxII is not. Conversely,

PrxII is more sensitive than PrxI to hyperoxidation when H$_2$O$_2$ concentrations reach subtoxic levels. Thus, the authors attribute distinct functions to these two enzymes: PrxI plays a regulatory role in signaling, whereas PrxII serves as a general defense against peroxide stress.

The elegant study by Woo and coworkers confirms that PrxI is integral to proper H$_2$O$_2$ signaling from membrane receptors and strongly supports the idea of localized H$_2$O$_2$ production within cellular subdomains. The study also raises many new questions regarding how multiple types of peroxiredoxins work together to mediate H$_2$O$_2$ signaling. For example, what is the purpose of the unphosphorylated PrxII enzyme found in the membrane-associated cellular fractions with the other signaling proteins? The authors suggest that the relatively low peroxidase activity of PrxII exempts it from requiring inactivation within signaling subdomains. However, other studies report that PrxII-deficient mice display site-specific enhancement of platelet-derived growth factor receptor phosphorylation, decreased protein

tyrosine phosphatase activity at membranes, and abnormal smooth muscle cell proliferation, suggesting that PrxII does play an active role in H_2O_2 signaling (Choi et al., 2005). Future studies with mice that lack either or both PrxI and PrxII may help to illuminate how these two enzymes coordinate their activity to optimize H_2O_2 signaling at particular receptor kinases, while preventing the toxic effects of H_2O_2.

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Rac in the Act of Forgetting

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Forgetting has been thought to occur as a result of the natural decay of the neuronal changes induced by learning or because of interference from other cognitive functions. In this issue, Shuai et al. (2010) find that the small G protein Rac may function as a switch for remembering versus forgetting.

Forgetting is undervalued. Although our forgetful nature is often a source of irritation, our lives would be chaos without forgetting given the mass of information that impinges on us daily. It is reasonable to think that our forgetfulness is passive, caused by the simple reversibility of molecular and cellular processes engaged when memories are first laid down in our brains. Alternatively, forgetting could be active, controlled by molecular and cellular mechanisms that erase unused or unwanted memories.

Research and thought in psychology over most of the 20th century have viewed forgetting in these two lights—as due to passive decay or to active interference from other mental activities that may rub out existing memories (Wixted, 2004; Jonides et al., 2008). In this issue, Shuai et al. (2010) have crystallized the notion that the brain mechanisms for remembering are balanced by a mechanism for active forgetting. Moreover, they have

ushered in a completely new line of research—the cell biology of active forgetting—by identifying the G protein Rac as critical for active forgetting.

The investigators take advantage of the fruit fly *Drosophila*, pairing a robust learning assay with sophisticated genetic tools for restricting the expression of developmentally deleterious transgenes of *rac* (McGuire et al., 2004) to only the adult phase. Flies are particularly good at learning about odors that are paired with a mild electric shock (Davis, 2005). In the initial experiments, transgenic flies that express a dominant-negative Rac, *Drac1(N17)*, in all adult neurons are found to have a more persistent memory of the odor:shock pairing compared to controls. In contrast, expression of a constitutively active form of Rac, *Drac1(V12)*, has the opposite effect. It accelerates memory decay after learning. Other experiments show that these effects are due to changes in the stability of memory rather than the level of learning (acquisition) about the

odor:shock association. Thus, Rac acts a rheostat for the stability of memory that can be dialed up or down, rather than functioning as a filter for memory acquisition.

Rac is a member of the Rho family of GTPases with roles in multiple signaling pathways that control transcription, cytoskeletal organization, vesicle trafficking, and cellular proliferation (Bustelo et al., 2007). This prompts the question, which of the many signaling pathways that utilize Rac underlie its memory functions? Cofilin is a potent actin-depolymerizing molecule that is inhibited by phosphorylation through the sequential activation of Rac, p21-activated kinase (PAK), and LIM-domain-containing protein kinase (LIMK) (Figure 1). Flies expressing a dephosphorylated and thus persistently active mutant of cofilin, the product of the *twinstar* gene *(tsr)*, exhibit enhanced memory similar to flies expressing dominant-negative Rac. This suggests that actin/cytoskeletal dynamics are at the heart of the function of Rac in memory.