CELL SIGNALLING

Letting H_2O_2 work

> “the fraction of PRXI that localizes at the membrane is locally inactivated by phosphorylation”

The oxidant hydrogen peroxide (H_2O_2) is a by-product of normal cellular processes that, as it is damaging to cells, is normally inactivated by detoxifying enzymes such as peroxiredoxins. In mammalian cells, however, H_2O_2 also functions as a signalling molecule; for example, H_2O_2 propagates growth factor signalling by inhibiting downstream proteinTyr phosphatases. How is this compatible with its toxicity? In *Cell*, Woo *et al.* show that the peroxiredoxin PRXI (also known as PRDX1) is locally inactivated to promote H_2O_2-mediated signalling at the cell membrane, whereas H_2O_2 is eliminated by peroxiredoxins in the rest of the cell. PRXI and PRXII (also known as PRDX2) are cytoplasmic, whereas other peroxiredoxins localize to specific organelles. The authors found that PRXI can be phosphorylated at Tyr194, which inhibits its peroxidase activity *in vitro*. Treatment of mammalian cells with epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) induced the phosphorylation of a small proportion of PRXI molecules (< 0.3%), but not of PRXII molecules, showing that PRXI is the primary target for growth factor-induced phosphorylation.

How is PRXI phosphorylation mediated and how does this affect growth factor signalling? Inhibiting the Src family of non-receptor protein Tyr kinases (which are activated downstream of EGF and PDGF receptors), using RNA interference or a specific Src inhibitor, blocked EGF- and PDGF-induced PRXI phosphorylation. Furthermore, phosphorylation of the PDGF receptor and one of its targets was substantially reduced in cells carrying a phosphorylation-resistant mutant PRXI. This suggests that EGF and PDGF induce Src-mediated PRXI phosphorylation and inactivation, and that this is required for signalling downstream of these receptors.

NADPH oxidases are activated at submembrane compartments and are responsible for the production of H_2O_2. Importantly, the isolation of cytoplasmic and membrane-bound fractions from EGF-stimulated cells, combined with immunofluorescence studies to visualize protein localization, revealed that, whereas non-phosphorylated (active) PRXI and PRXII localize to the cytosol, phosphorylated PRXI is membrane-associated (as is Src) and colocalizes with activated NADPH oxidases, which promotes H_2O_2-regulated signalling.

Thus, this study shows that, on growth factor stimulation, the fraction of PRXI that localizes at the membrane is locally inactivated by phosphorylation and allows H_2O_2-regulated signalling, whereas active PRXI and PRXII neutralize the toxic effects of H_2O_2 elsewhere in the cell.

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ORIGINAL RESEARCH PAPER Woo H. A. *et al.* Inactivation of peroxiredoxin I by phosphorylation allows localized H_2O_2 accumulation for cell signaling. *Cell* **140**, 517–528 (2010)