PhGPx, a beginning story

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PhGPx is an antioxidant enzyme

- PhGPx, a selenium dependent enzyme, detoxifies hydroperoxides by reducing them to alcohols.
- Example hydroperoxides are:
  - phospholipid hydroperoxides
  - fatty acid hydroperoxides
  - cholesterol hydroperoxides

PhGPx is a member of the glutathione peroxidase (GPx) family

- GPx 1: cGPx
- GPx 2: GI GPx
- GPx 3: plasma GPx
- **GPx 4: phospholipid hydroperoxide GPx (PhGPx)**
- GPx 5: secretory GPx

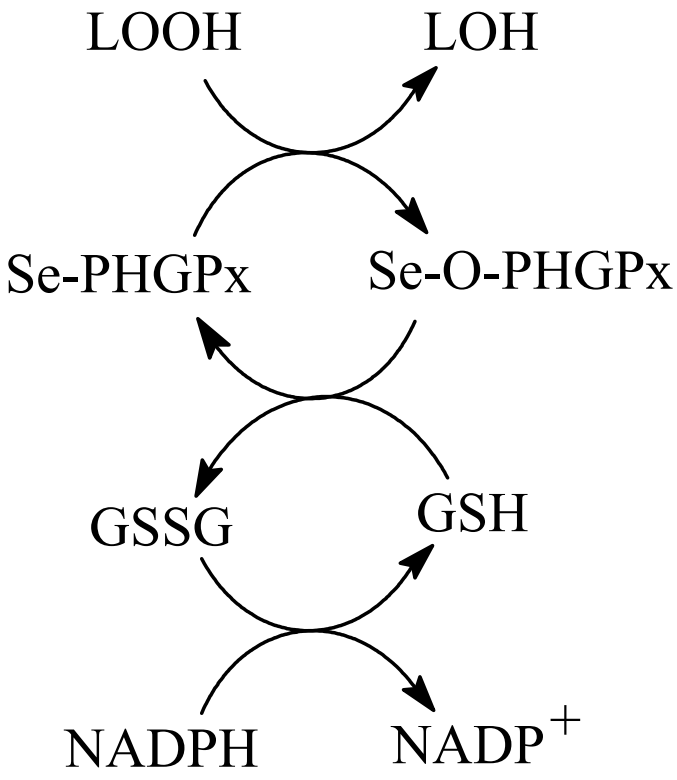
Biochemical mechanism of PhGPx in reducing lipid hydroperoxide

Ping-Pong mechanism

(1) the selenol moiety of PhGPx is first oxidized by hydroperoxides; and

(2) then, it reduced back by two GSHs.

LOOH: lipid hydroperoxide
LOH: alcohol derivative
GSH: glutathione
GSSG: glutathione disulfide



PhGPx reacts fast with hydroperoxides

- Rate Constants:

	k (M^{-1} s^{-1})	
Substrates	**PhGPx**	**cGPx**
hydrogen peroxide	3.2×10^{7}	4.8×10^{8}
linoleic acid hydroperoxide	3.0×10^{8}	3.8×10^{8}
phosphatidylcholine hydroperoxide (used in activity assay)	1.7×10^{8}	-

(Maiorino M. *et al.* 1990 *Method Enzymol.* 186: 448-457)

PhGPx: Some Facts

PhGPx is:

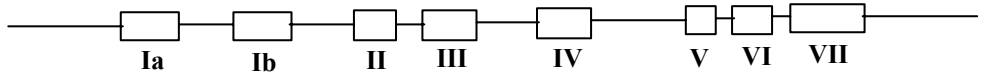
- the second selenoenzyme discovered in 1982;
- monomeric enzyme containing one selenium atom at the active site as selenocysteine;
- expressed in most tissues: testis, kidney, heart, skeletal muscle, liver, brain, lung, spleen;
- present in cytoplasm, mitochondria, as well as plasma and nuclear membranes.

(Ursini F, *et al.* 1985 *Biochim. Biophys. Acta* **839**:62-70; and
Godeas C, *et al.* 1994 *Biochim. Biophys. Acta* **1191**:147-150)

The PhGPx Gene

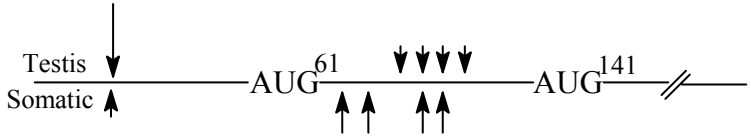
- PhGPx gene is localized on human chromosome 19p13.3;
- It consists of 7 exons, and spans 2.8 kb.
- It has two windows of transcription start sites, which results in two populations of mRNA.

Gene Structure



Kelner *et al.* BBRC, 249: 53, 1998

Two Transcription Start Sites



Pushpa-Rekha *et al.* JBC, 270:26993, 1995

Two forms of PhGPx

The two populations of mRNA give rise two different sizes of PhGPx protein:

- Mitochondrial PhGPx (L-PhGPx): 23 kDa,
- Non-mitochondrial PhGPx (S-PhGPx): 20 kDa.

(Pushpa-Rekha TR, *et al.* 1995 *J. Biol. Chem*. **270**:26993-26999)

L-PhGPx

- L-PhGPx is a 197-amino acid protein containing a 27-amino acid mitochondrial leader sequence;
- L-PhGPx is localized to the intermembrane space of mitochondria;
- L-PhGPx is expressed abundantly in testis tissue.

S-PhGPx

- S-PhGPx is the same protein as L-PhGPx minus the 27-amino acid leader sequence; thus, it is a 170-amino acid protein.
- S-PhGPx is expressed in most somatic tissues.
- It is localized in cytosol and is bound to plasma membrane.

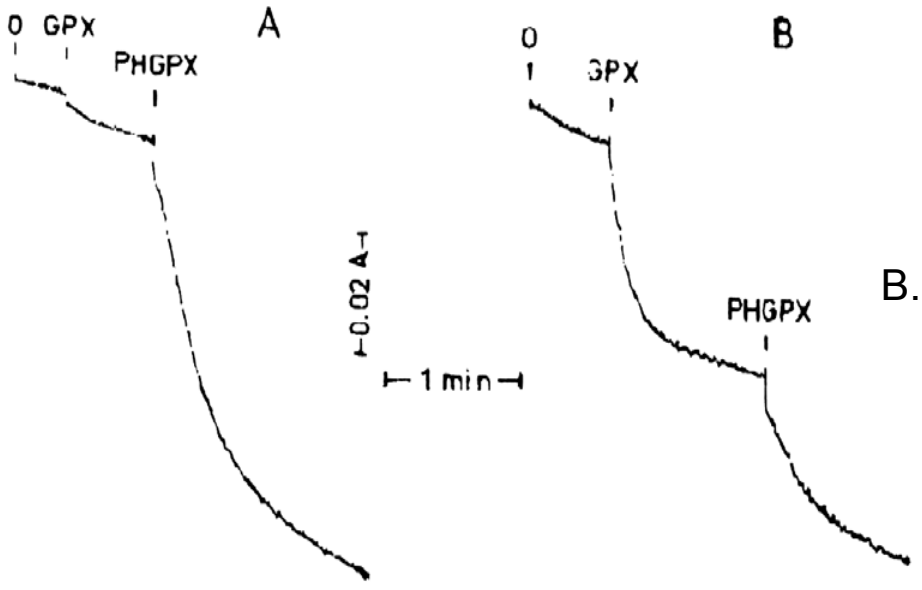
PhGPx can be structural protein in specific situations.

PhGPx is involved in spermatid differentiation where it functions as a structural protein.

PhGPx exists as a soluble peroxidase in spermatids, but persists in mature spermatozoa as an enzymatically inactive, oxidatively cross-linked, insoluble protein. It represents at least 50% of the capsule material that is associated with the helix of the mitochondria.

(Ursini F. *et al*. 1999 *Science* **285**: 1393-1396)

PhGPx inhibits membrane-damaging lipid peroxidation

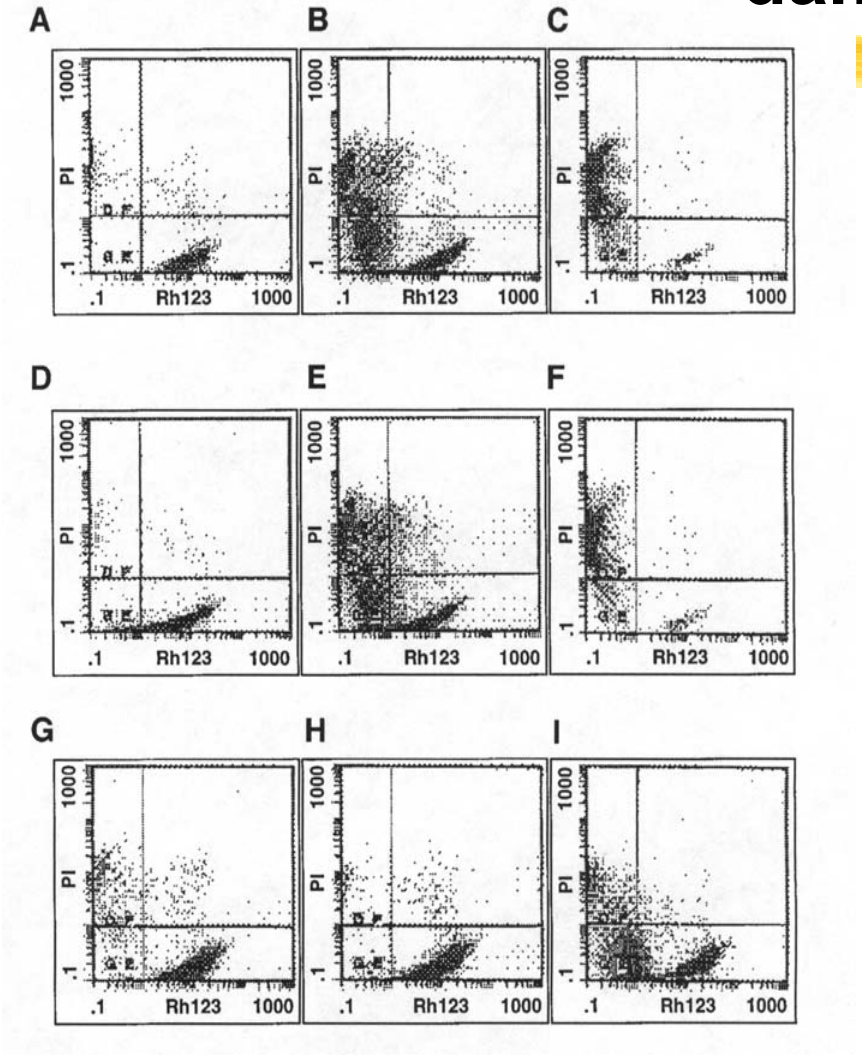


(Thomas JP *et al.* 1990 *J. Biol. Chem.* **265**: 454-461)

A. The system consisted of isolated membranes that were photoperoxidized with rose bengal, a singlet oxygen-generating dye. GPx alone caused little if any lipid hydroperoxide (LOOH) loss. When added after GPx, PhGPx caused an immediate and rapid decrease of LOOH.

B. The peroxidized membranes were treated with $CaCl_2$/PLA_2 before being analyzed. GPx produced a sizable decrease of LOOH. Subsequent addition of PhGPx resulted in another decrease, the magnitude of which was about 2/3 of that produced by GPx.

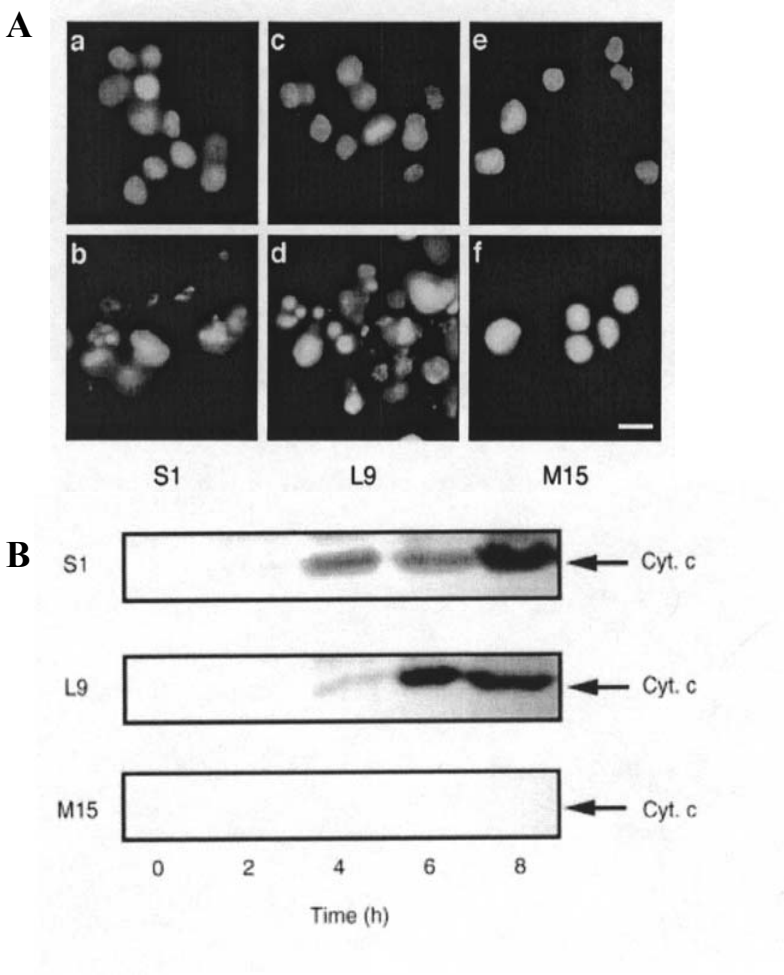
L-PhGPx protects against mitochondrial damage



Flow cytometry analysis of changes in mitochondrial membrane potential and in the integrity of plasma membranes. Parental RBL-2H3 cells (A-C), S-PhGPx transfected cells (D-F), L-PhGPx transfected cells (G-I). Cells were double stained with Rh123 and PI after the incubation with 25 mM KCN for 0 h (A, D, G), 2 h (B, E, H), and 4 h (C, F, I).

Overexpression of L-PhGPx maintains the mitochondrial functions by reduction of hydroperoxides generated as a result of damage to the mitochondrial respiratory machinery. By contrast, overexpression of S-PhGPx is less efficient in protecting against the loss of mitochondrial membrane potential. (Arai M *et al*. 1999 *J. Biol. Chem.* **274**: 4924-4933)

L-PhGPx prevents apoptosis by blocking the release of cytochrome *c* from mitochondria

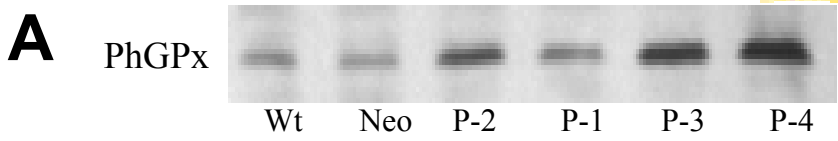


A. The nature of cell death caused by 2-deoxyglucose (2-DG) examined by fluorescence microscopy. Condensation of nuclei was observed in S1 (parental cells), L9 (S-PhGPx transfectant), no condensation of nuclei was observed in M15 (L-PhGPx transfectant).

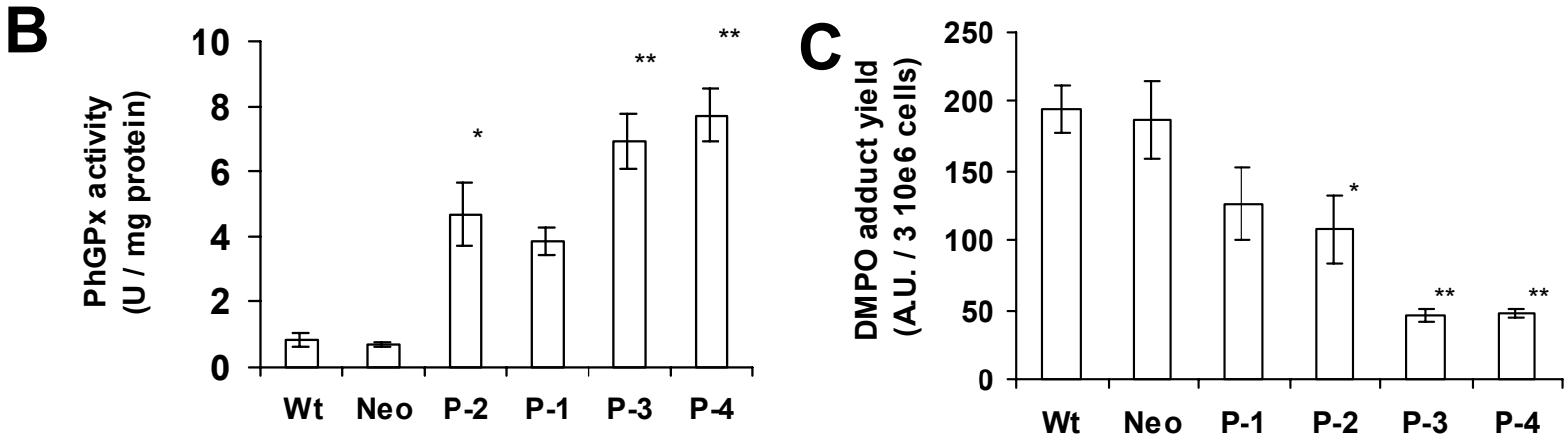
B. Cytochrome *c*, released from mitochondria, was detected in S1 and L9 cells 4 h after the start of exposure to 2-DG. No detectable cytochrome *c* was found in the cytosol of M15 cells.

(Nomura K *et al*. 1999 *J. Biol. Chem.* **274**: 29294-29302)

PhGPx inhibits photo-oxidative stress induced lipid-derived radical generation



(Wang HP *et al.* 2001
Free Radic. Biol. Med, **30:** 825-835)



MCF-7 cells were transfected with PhGPx. Different protein **(A)** and activity levels **(B)** of PhGPx are present in parental (wt), vector control (neo) and transfectants (P-1 to P-4). The lipid-derived radical adducts **(C)** of DMPO were observed using electron paramagnetic resonance spin trapping. Cells with low PhGPx activity produced higher levels of radicals.

Summary

- PhGPx is an important enzyme in regulating cellular peroxide levels.
- PhGPx can lower the peroxide tone, which might change the cellular redox environment and affect cell growth.
- L-PhGPx plays a central role in preventing mitochondrial damage.
- PhGPx may play a role in the resistance to oxidative stress-mediated anticancer therapy.
- PhGPx can also be a structural protein.