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Article Addendum

# A hydrogen peroxide detoxification system in the nucleus of wheat seed cells

## Protection or signaling role?

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**Aerobic metabolism inevitably produces reactive oxygen species (ROS), including hydrogen peroxide, which may cause damage to the cell. Besides this toxic effect, hydrogen peroxide has an important signaling function in plant development and response to environmental stimuli. So, the balance of toxic and signaling effects of hydrogen peroxide is highly dependent on mechanisms to adjust its level in the different cell compartments. We recently described a redox system, formed by NADPH thioredoxin reductase (NTR) and 1-Cys peroxiredoxin (1-Cys Prx), able to use the reducing power of NADPH to reduce hydrogen peroxide. This system is localized in the nucleus of wheat seed cells and probably has an important antioxidant function in aleurone and scutellum cells, which suffer oxidative stress during seed development and germination. We discuss here the possibility that the control of the level of hydrogen peroxide in the nucleus may be important to balance redox regulation of gene expression and cell death in cereal seed cells.**

## Introduction

Life in aerobiosis is challenged by the production of reactive oxygen species (ROS) including hydrogen peroxide. In plants, different environmental stimuli increase ROS production; however, there are tissues that naturally suffer oxidative stress during plant growth and development. This is the case of the cereal seed that suffers oxidative stress provoked by the massive loss of water at late stages of development, and after resumption of respiration following germination.[1-4] Though during seed development different tissues undergo programmed cell death (PCD),[5-7] aleurone and scutellum cells play an essential role in germination and therefore have to survive this oxidative stress. Germination of cereal seeds is activated by gibberellins which induce in aleurone cells the expression of genes encoding hydrolytic enzymes that allow the mobilization of the storage components of the starchy endosperm.[8] Once this process is completed, gibberellins activate aleurone PCD,[9] which progression takes place after cytoplasmic ROS detoxification systems are down-regulated.[10] We have recently described that NADPH thioredoxin reductase (NTR) supports the antioxidant activity of 1-Cys peroxiredoxin (1-Cys Prx) using NADPH as source of reducing power. Interestingly, this novel redox system accumulates in the nucleus of seed cells suffering oxidative stress.[11]

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## Signaling versus Toxic Effect of Hydrogen Peroxide in the Nucleus of Wheat Seed Cells

The finding that the NTR/1-Cys Prx system accumulates in the nucleus of seed cells suffering oxidative stress, and in vitro assays showing that this system is able to use NADPH to reduce hydrogen peroxide provide evidence of a mechanism to control the oxidant environment of the nucleus. A primary function of this system may be to avoid damage to DNA and nuclear structures, which is probably important taking into account the recent demonstration of ROS production in nuclei of plant cells.[12] DNA protection assays in vitro support this detoxification role of the nuclear-localized hydrogen peroxide scavenging system.[13,14]

However, the control of the level of hydrogen peroxide in the nucleus probably has a signaling function which may involve redox regulation of transcription, as shown in yeast and animal cells.[15] In *S. pombe* the expression of genes of the antioxidant response is regulated by a bZIP transcription factor, Pap1, activated by the formation of an internal disulfide bridge upon $H_2O_2$ treatment.[16] Although the available information on redox regulation of gene expression in plants is still scarce,[17] the DNA binding activity of several transcription factors depends on the redox environment.[18-20] In the context of gene expression in cereal seeds, it was shown that P1, a R2R3-type MYB transcription factor from maize, requires reducing conditions for DNA binding.[21,22] This finding is interesting because a masterpiece of gene regulation in response to gibberellins in aleurone cells from cereal seeds, GAMYB,[23] is a R2R3-type MYB transcription factor. Though no redox regulation of its activity has been reported so far, an Arabidopsis line overexpressing 1-Cys Prx showed lower

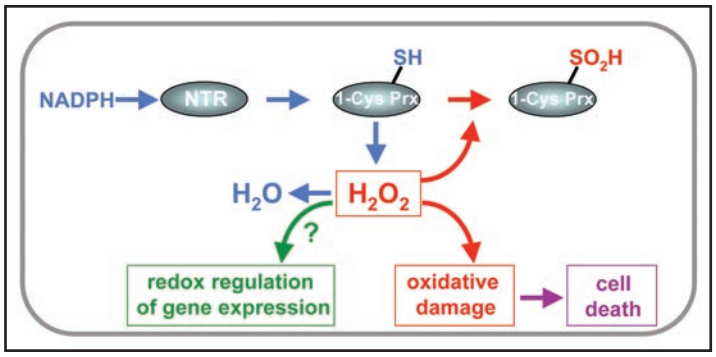


Figure 1. The nuclear-localized redox system formed by NTR and 1-Cys Prx is able to use NADPH to detoxify hydrogen peroxide in cereal seeds suffering oxidative stress. This system may control the oxidant conditions in the nucleus, which is probably important for redox regulation of gene expression in germinating seed cells. As 1-Cys Prx is progressively inactivated by overoxidation, the increase of the oxidant conditions in the nucleus promote oxidative damage and cell death.

germination efficiency than wild type,[14] suggesting that the nuclear redox environment plays an important role in the activation of germination.

A remarkable feature of the nuclear NTR/1-Cys Prx system is the sensitivity of the 1-Cys Prx to oxidant conditions, which provoke overoxidation of the peroxidatic Cys residue to sulfinic acid, thus inactivating the enzyme.[11] Inactivation by overoxidation is a well-described characteristic of eukaryotic 2-Cys Prxs,[24] important for hydrogen peroxide-dependent signaling in eukaryotes.[25,26] Whilst the overoxidation of 2-Cys Prx to sulfinic acid is reversible,[27] overoxidation of 1-Cys Prx seems to be irreversible.[28] Therefore, the progressive overoxidation of the nuclear 1-Cys Prx will probably increase the oxidant environment of the nucleus, according to the scheme depicted in Figure 1. The level of hydrogen peroxide in the nucleus may influence gene expression as shown for the redox regulation of the yeast Pap1 transcription factor, which is mediated by a 2-Cys Prx at low concentration of $H_2O_2$[29,30] but not at high concentration of $H_2O_2$ because the 2-Cys Prx becomes overoxidized.[30] Therefore, in germinating seeds the nuclear NTR/1-Cys Prx redox system would control the level of hydrogen peroxide allowing gene expression. Then, as 1-Cys Prx is progressively inactivated by overoxidation, the nuclear environment is likely to become more oxidant, thus favouring cell death[9] (Fig. 1). In yeast and animal cells hydrogen peroxide promotes DNA cleavage mediated by toposimerase I and II.[31,32] Whether the increase of hydrogen peroxide provokes cell death in cereal seeds through the damage of nuclear structures or by the regulation of signal transduction events, as occurs in animal cells,[33] is not yet known.

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