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Research article

The cellular redox state in plant stress biology — A charging concept

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ABSTRACT

Different redox-active compounds, such as ascorbate, glutathione, NAD(P)H and proteins from the thioredoxin superfamily, contribute to the general redox homeostasis in the plant cell. The myriad of interactions between redox-active compounds, and the effect of environmental parameters on them, has been encapsulated in the concept of a cellular redox state. This concept has facilitated progress in understanding stress signalling and defence in plants. However, despite the proven usefulness of the concept of a redox state, there is no single, operational definition that allows for quantitative analysis and hypothesis testing.

1. Introduction

Due to their sessile life-style, plants have to endure a range of abiotic stress conditions. A large number of studies have demonstrated the stress-induced formation of reactive oxygen species (ROS). These reactive molecules have traditionally been associated with non-specific damage to macromolecules such as DNA, proteins and lipids (as reviewed by Pitzschke et al. [97]); damage which potentially can result in death of the cell and even the organism (Fig. 1, *left*). In this traditional view, the role of antioxidants was simply mopping up ROS, hence decreasing ROS-mediated damage [81]. However, during the last decade substantial evidence has been amassed, showing that many stress-induced responses are tightly regulated, specific processes, rather than unavoidable, non-specific consequences of ROS-mediated damage [79]. ROS are produced as a consequence of the disruption of cellular metabolism, or can be actively generated by, for example, NADPH-oxidases [2]. Notwithstanding the confusing, and often conflicting use of terms such as ROS-mediated "stress", "damage" and "signalling" [32], there is now considerable evidence to support a redox-regulated aspect of cell homeostasis, involving individual ROS, ROS-producing enzymes, antioxidants, their oxidised forms, and/or oxidation/reduction states, and its role in mediating plant responses to abiotic stress conditions. Redox regulation has been demonstrated in processes as diverse as stress regulated gene expression [35,38], stomatal closure [15,135], disease resistance [127], signal transduction [60,61], hormone signalling [60,89], development of vegetative and reproductive organs [5] and control of plant architecture [103,104]. Yet, these redox-regulated systems are best exemplified in the (stress-induced) onset of cell death. Instead of the cells simply dying due to uncontrolled oxidative damage, programmed cell death is a highly regulated process based upon a feedback amplification loop involving ROS, salicylic acid, the intervention of transcription factors such as Lsd1, WRKY75 and NIM1/NPR1, and MAPK signalling paths [38,51].

The idea of a redox-regulated aspect of cell homeostasis has been translated into the concept of a "cellular redox state" (Fig. 1, *right*). Essentially, the cellular redox state is envisaged as the sum of reducing and oxidising redox-active molecules, as well as redox capacities in the cell. This redox state maintains general cell homeostasis, and in particular, the ability of the cell to deal with redox events (such as oxidative stress) [30,31]. As such, redox states are not just control points in plant stress responses, but rather play a far more fundamental role in every living cell. Indeed, redox control is involved with regulation of non-stress-related processes such as the expression of large numbers of genes, energy production in mitochondria and chloroplasts, oxidation and reduction reactions, as well as many reactions in primary and secondary metabolism.

Abbreviations: ASC, ascorbate; DHA, dehydroascorbate; Grx, glutaredoxin; GSH, reduced glutathione; GSSG, oxidised glutathione; MDHA, monodehydroascorbate; NTR, NADPH thioredoxin reductase; Prx, peroxiredoxin; roGFP, redox-sensitive green fluorescent protein; ROS, reactive oxygen species; rx-YFP, redox-sensitive yellow fluorescent protein; Trx, thioredoxin.

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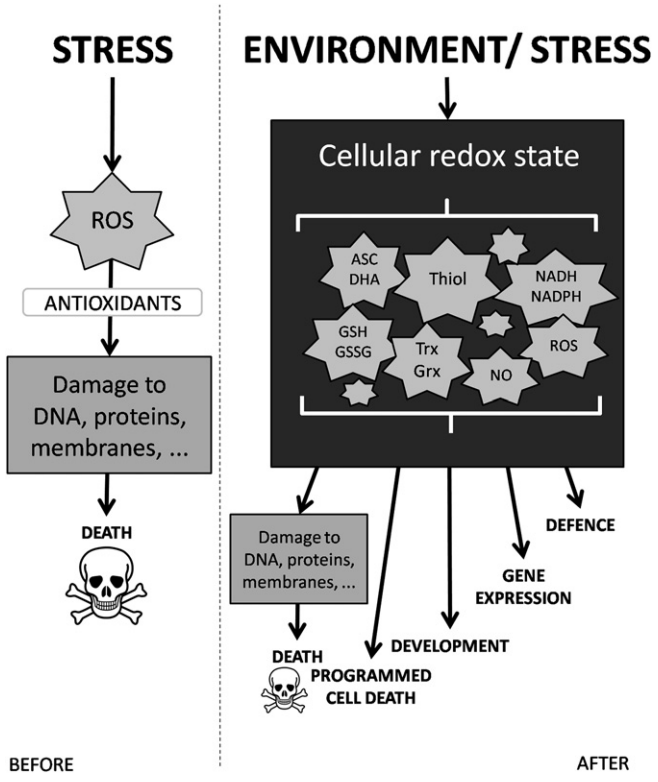

Fig. 1. Changing views on the concept of stress. Whereas at the outset of plant stress physiology ROS were simply considered as damaging the cell components, leading to either a need for repair and defence to the cause of cell death (left hand side), the view nowadays has shifted to ROS being an integrative part of cell signalling metabolism, modulated by the cellular redox state (and in turn modulating the cellular redox state itself), leading to different responses. Cellular redox state is represented here as a mechanism consisting of a multitude of possible players, interacting with one another. The best known among those players have been indicated.

Derived from this concept of a cellular redox state are the organellar redox states, such as that of the mitochondria [129], chloroplast [91] and cell wall [19,93]. On a larger organisation scale, the literature contains references to tissue and even organ redox states. The latter includes studies of the redox state in flower buds [121], leaves [122] and roots [36].

However, in spite of the elegant concept of a cellular redox state (and the commonly used practice in publications to use this term), there is little agreement between authors when it comes to a universally accepted definition and protocols to measure and quantify the cellular redox state. Methods of assessing a cell's redox state range from measuring a single redox-sensitive component, a combination of several of these, to measurements of specific fluorescent signals. Here, we examine more closely the concept of a "cellular redox state", attempts to quantify this parameter, and its future in plant-redox research. We will do so by tackling the concept both from the viewpoint of its physical constituents, their response to stress conditions, and their role in signalling.

2. Components of the cellular redox state

The cellular redox state is made tangible in terms of the redox state of the individual redox-active molecules in a cell (Fig. 1, *right*). For each redox-active molecule its redox state can be defined as the proportion of reduced molecules relative to the total pool size, or alternatively as the ratio between reduced and oxidised molecules within a pool. It is relatively easy to measure the redox states of a single, purified metabolite under in vitro conditions. However, the problem that arises with the concept of the cellular redox state is that cells contain large numbers of different redox-active compounds which interact with each other. For example, aside from the "big three" antioxidants, ascorbate (ASC), glutathione (GSH) and the pyridine nucleotides NADH and NADPH [106], plants contain many distinct redox-active phenolics, quercetin and kaempferol-glycosides, carotenoids [119], cytochromes [4], tocopherols and tocotrienols [49], polyamines [66] and proteins carrying redox-active S-groups [25]. Plants also contain large numbers of low molecular weight, secondary metabolites, most of which are redox active [42]. To complicate matters even further; levels of many of these redox-active metabolites vary depending on developmental stage, growth conditions, sub-cellular location, stress exposure and so on [20,48,119,128]. The relationships between metabolite concentration and stress are complex. For example, UV-B induces at the same time a transient increase in polyamine concentration, transient decreases in ascorbate and GSH concentrations that are followed by increased accumulation, and gradual increases in flavonoid concentration [52]. Consequently, the concept of a cellular redox state, which regulates stress responses, is inherently associated with the kinetics of stress exposure, and especially the balance between damage and acclimation responses. Stress also alters the oxidation/reduction state of metabolites, and again this may either constitute damage or rather an active acclimation response resulting, for example, in production of H_2O_2 by NADPH-oxidases and/or class III peroxidases [2,78].

This dynamic plethora of redox-active compounds and their oxidation/reduction states creates a *hypothetical* cellular redox state (Fig. 2, *right*), which is having *tangible* effects on, among others, gene expression, enzyme activities, or hormone reception. In order to create a picture of the cellular redox state, we will

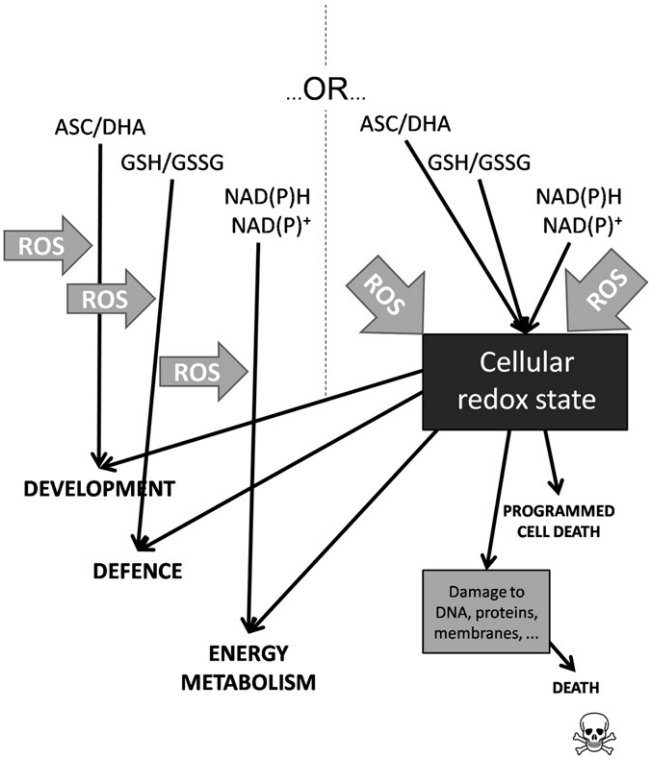

Fig. 2. On the role and interaction of the antioxidants. Given the sometimes very specific involvement of certain redox-sensitive compounds such as the ASC/DHA pair or the GSH/GSSG pair, the question should be asked whether one should emphasize the impact of the individual antioxidant (and hence, also the redox state of the individual antioxidant, each impacted on its own terms by ROS) or its contribution to the encompassing cellular redox state, on its whole modulated by oxidative influences such as ROS.