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Review

How reactive oxygen species and proline face stress together

Kilani Ben Rejeb [a,b], Chedly Abdelly [b], Arnould Savouré [a,*]

[a] Université Pierre & Marie Curie (UPMC), Univ. Paris 6, APCE, URF5, Case 156, 4 place Jussieu, 75252 Paris cedex 05, France
[b] Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cedria (CBBC), BP 901, Hammam-Lif 2050, Tunisia

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ABSTRACT

Reactive oxygen species (ROS) are continuously generated as a consequence of plant metabolic processes due to incomplete reduction of O_2. Previously considered to be only toxic by-products of metabolism, ROS are now known to act as second messengers in intracellular signalling cascades to trigger tolerance of various abiotic and biotic stresses. The accumulation of proline is frequently observed during the exposure of plants to adverse environmental conditions. Interestingly proline metabolism may also contribute to ROS formation in mitochondria, which play notably a role in hypersensitive response in plants, life-span extension in worms and tumor suppression in animals. Here we review current knowledge about the regulation of proline metabolism in response to environmental constraints and highlight the key role of ROS in the regulation of this metabolism. The impact of proline on ROS generation is also investigated. Deciphering and integrating these relationships at the whole plant level will bring new perspectives on how plants adapt to environmental stresses.

1. Introduction

Growing in their native environments plants encounter a variety of biotic and abiotic constraints (Atkinson and Urwin, 2012). Plants commonly accumulate reactive oxygen species (ROS) in response to environmental stresses. The excessive production of ROS in plant cells is potentially harmful to nucleic acids, proteins and lipids, which can lead to cell injury and death (Gill and Tuteja, 2010). To survive such constraints, plants have a variety of mechanisms that allow them to perceive and transduce the external signals in order to trigger adaptive responses and it has become apparent that ROS are important signal molecules involved in the stress responses (Miller et al., 2011).

In many plants, free proline accumulates in response to a wide range of biotic and abiotic stresses. Today it is clear that proline has multifunctional roles (for review, see Szabados and Savouré, 2010). Other than being an osmoprotectant, proline can act as a potent nonenzymatic antioxidant. As a singlet oxygen quencher (Alia et al., 2001) and scavenger of hydroxyl radicals (Smirnoff and Cumbes, 1989), when it accumulates in plant tissue proline may be important in preventing oxidative damage caused by ROS. Proline may stabilize DNA, membranes and protein complexes, and provide a source of carbon and nitrogen for growth after stress relief. Proline metabolism is involved in the regulation of intracellular redox potential and the storage and transfer of energy and reducing power (Szabados and Savouré, 2010; Sharma et al., 2011; Gilberti et al., 2014). Changes in proline metabolism may be more beneficial for plant tolerance to environmental stresses than properties of the amino acid itself.

Although the harmful effects and the signalling functions of ROS are well documented, readers are referred to recently published excellent reviews (Petrov and Van Bresegem, 2012; Miller et al., 2011; Suzuki et al., 2012), only limited knowledge is available on the relationship between ROS and proline metabolism.

In this paper, we reviewed the relationship between ROS signalling and proline metabolism in the context of plant adaptation to adverse environments with a special emphasis on how proline metabolism is regulated by these compounds. The impact of proline synthesis on ROS generation is also addressed.

2. Proline and ROS

2.1. Regulation of proline metabolism

Free proline accumulates in plants in response to a wide range of abiotic and biotic stresses. Plant stress has been defined by Lichtenthaler (1996) as any unfavorable environmental conditions that alter plant growth, development and metabolism, and even may lead to plant death. Salt and soil drought conditions are major

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* Corresponding author. Tel.: +33 (0)1 44 27 26 72; fax: +33 (0)1 44 27 61 51.
E-mail address: arnould.savoure@upmc.fr (A. Savouré).

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abiotic stress factors that limits crop productivity (Lawlor, 2002). Most of the data on the regulation of proline metabolism have been obtained from plants grown under salt and osmotic stress conditions.

Proline accumulation upon stress depends on both activation of its biosynthesis and inhibition of its degradation (Fig. 1). Housekeeping proline biosynthesis occurs in the cytosol of plant cells, but it is possible that production moves to chloroplasts upon stress (Székely et al., 2008). Under osmotic stress, proline is synthesized mainly from glutamate (Fig. 2). In this pathway, the bifunctional pyrroline-5-carboxylate synthetase (P5CS) reduces glutamate to glutamyl-5-semialdehyde (GSA), which is spontaneously converted to pyrroline-5-carboxylate (P5C). P5C is reduced to proline by P5C reductase (P5CR). Degradation of proline takes place in mitochondria via the sequential action of proline dehydrogenase (ProDH) and P5C dehydrogenase. The rate-limiting steps in proline biosynthesis and degradation in Arabidopsis are catalyzed by P5CS and ProDH respectively. Two closely related *P5CS* genes were identified in *Arabidopsis thaliana*. *P5CS1* is induced by drought and salt stress (Savouré et al., 1995; Yoshiba et al., 1995), while the expression of *P5CS2* is observed in dividing cells in cell suspension (Strizhov et al., 1997), in meristematic and reproductive tissues (Székely et al., 2008), and in response to biotic stress such as plant-pathogen incompatible interactions (Fabro et al., 2004). Similarly, two genes *ProDH1* and *ProDH2* were identified in *A. thaliana* (Funck et al., 2010; for review see Servet et al., 2012). *ProDH1* is considered to encode the main isoform involved in proline degradation. The expression of *ProDH1* is down regulated by water stress and up regulated by proline (Kiyosue et al., 1996) (Fig. 1). Although the regulation of proline metabolism is still poorly characterized, it is clear that tissue proline content depends on the tight regulation of biosynthesis and catabolism. Characterization and comparison of the signalling pathways involved in the regulation of proline metabolism have been more intensively investigated in two closely related species, *A. thaliana* and *Thellungiella salsuginea*, which is considered as an extremophile exhibiting high tolerance to salinity, drought, and cold. In contrast to *A. thaliana*, *T. salsuginea* accumulates high levels of proline in response to salt stress and also even in its absence (Ghars et al., 2008), which indicates that this species somehow anticipates stressful conditions. In addition, while mild salt stress (75 mM NaCl) reduced the growth of *A. thaliana*, growth of *T. salsuginea* was not affected by salinity up to 300 mM NaCl (Ghars et al., 2008). Recent reports have indicated that under normal growth conditions phospholipase D (PLD) functions as a negative regulator of proline biosynthesis in Arabidopsis (Thiery et al., 2004) and that plants present a higher proline responsiveness to hyperosmotic stress when this regulator is abolished (Fig. 1). By contrast, calcium signalling and phospholipase C (PLC) trigger *P5CS1* transcription and proline accumulation during salt stress (200 mM NaCl) (Parre et al., 2007). Interestingly, these lipid signalling pathways function in an opposite manner in the halophyte *T. salsuginea* (Ghars et al., 2012). This opposite regulation may partially explain why stress responses are upregulated in *T. salsuginea*, conferring it a very high capacity to withstand severe environmental constraints.

In Arabidopsis, *P5CS1* expression has also been found to be stimulated by light (Hayashi et al., 2000) and nitric oxide (NO) (Zhao et al., 2009) (Fig. 1). Under osmotic and salt stresses, ABA induces *AtP5CS1* expression through the *cis*-acting ABA responsive (ABRE) element (Strizhov et al., 1997). Savouré et al. (1997) showed that stress induction of *P5CS1* occurs independently of ABA upon cold and osmotic stresses. This is supported by a recent report indicating that there is significant ABA-independent expression of *P5CS1* at low water potential demonstrated by comparing ABA-deficient (*aba2-1*), proline accumulation deficient (*p5cs1-4*) and double (*p5cs1-4*/*aba2-1*) mutants (Sharma and Verslues, 2010).

From these data, proline metabolism appears to be tightly regulated by environmental and endogenous signals. ROS, key signalling molecules produced upon environmental constraints, may play an important role in the regulation of this pathway.

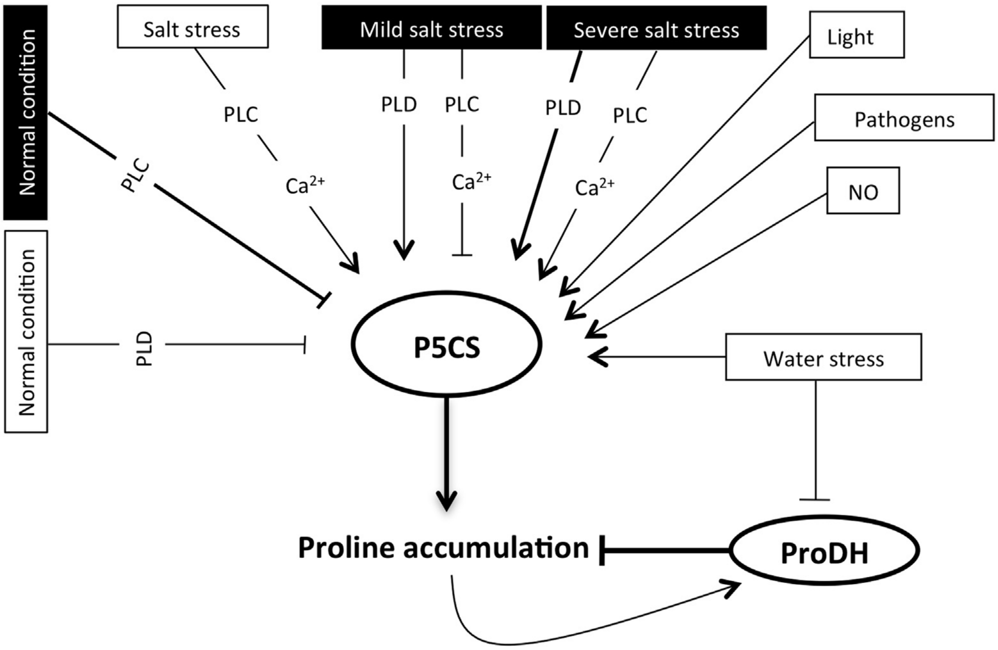

Fig. 1. Regulation of proline metabolism in plants. See text for details of the description of proline metabolism and its regulation. Most data were obtained using *Arabidopsis thaliana* and *Thellungiella salsuginea*. Circles designate enzymes and rectangles the environmental context; open and black boxes correspond to the signalling pathways demonstrated in *A. thaliana* and *T. salsuginea*, respectively. Abbreviations: P5CS, pyrroline-5-carboxylate synthetase; ProDH, proline dehydrogenase; PLC, phospholipase C; PLD, phospholipase D; ROS, reactive oxygen species.