

Article Addendum

CPR5

A Jack of all trades in plants

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In our recent paper in *Journal of Experimental Botany*, we examined the effects of *cpr5/old1* mutations and *CPR5* overexpression on *Arabidopsis* growth and development.¹ We found that *CPR5* is important for early plant growth but promotes senescence at late development and hence proposed it as a senescence-regulatory gene as predicted by the Evolutionary Theory of Senescence derived from studies on animal ageing. One of the key unsolved issues is how *CPR5* contributes to the early plant growth and development. Here we discuss the possible cellular functions of *CPR5*.

The Pleiotropic Phenotypes of *cpr5* Mutations

CPR5 stands for CONSTITUTIVE EXPRESSER OF PR GENES5. The gene was initially identified in screens for mutants with enhanced disease resistance.^{2,3} Several more *cpr5* alleles were subsequently identified in screens for mutants with altered trichome development,⁴ dark induced senescence (*hys1* alleles)⁵ and ethylene-induced senescence (*old1* alleles).^{1,6} So far, described alterations in *cpr5* mutants include (1) enhanced constitutive expression of PR genes, (2) elevated salicylic acid and jasmonic acid levels, (3) hypersensitivities to sugar, ABA, ethylene and jasmonic acid, (4) accelerated leaf senescence, (5) spontaneous lesion mimic cell death, (6) defective cell proliferation, endoreduplication and trichome development, (7) reduced plant growth and reproduction fecundity.¹⁻⁷ Thus, *cpr5* mutations possess pleiotropic phenotypes indicating that *CPR5* is an essential-for-life gene in *Arabidopsis*.

How is *CPR5* related to all of these processes? Several double mutants were constructed to dissect how the altered phenotypes in *cpr5* mutants are related to the known signalling pathways such as salicylic acid, ethylene, jasmonic acid, sugar and ABA (Jing H-C, Dijkwel PP, unpublished data)^{8,9} Blocking glucose sensing

by knocking out *HXK1* (*hexokinase1*) could partially alleviate the glucose hypersensitive phenotype of the *cpr5* mutant, but did not affect the early onset of senescence and defect in trichome development.⁹ This is consistent to our results showing that blocking a particular signalling pathway did not affect the alterations in other signalling pathways in *cpr5* mutants.¹¹ For instance, *cpr5*-induced ethylene hypersensitivity was converted into ethylene insensitivity in *cpr5ein2* double mutants, but *cpr5ein2* seedlings still exhibited hypersensitivity to sugar, ABA and jasmonic acid. Similarly, *cpr5abi4* double mutants were sugar insensitive but were still hypersensitive to ethylene and jasmonic acid. Furthermore, all examined double mutants so far had no effects on *cpr5*-induced lesion mimic cell death and premature senescence as well as reduced cell proliferation and trichome development. It appears that *CPR5* independently controls multiple cellular processes.

Cellular Functions of *CPR5*: A Regulator of ROS Gene Network

There are at least two possible cellular mechanisms to explain the observed pleiotropy in *cpr5* mutants. *CPR5* can be a master regulator of a general signal transduction pathway that affects many processes. Alternatively, *CPR5* can independently interact with many signalling molecules. Defining the earliest alterations in cellular events in pre-symptom *cpr5* mutants may help understand the cellular functions of *CPR5*. We therefore examined the transcriptomic and proteomic profiles of pre-symptom *cpr5* mutants.^{10,11} Using 5-fold increase as a cut-off threshold three of the five universal ROS marker genes, 16 of the 27 genes induced by six ROS treatments and one third of the ROS-dependent putative transcription factors were upregulated in *cpr5* mutants. Proteins clearly exhibiting increased abundance were predominantly members of the detoxifying enzyme family of glutathione S-transferases (GSTs). Thus, pre-symptom *cpr5* mutants are under high cellular oxidative stress. Consistent with this, the presence of reactive oxygen species was recorded in *cpr5* mutants using nitro blue tetrazolium staining.²

These observations may link the various pleiotropic phenotypes in *cpr5* mutants to the alteration of cellular ROS production, scavenging and redox balance. ROS are by-products of essential cellular metabolic processes.¹² Recent evidence shows that ROS are a part of master signalling transduction pathways involved in the regulation of many growth and development as well as biotic and abiotic stress responses in plants.¹³ ROS have also been implicated in regulating

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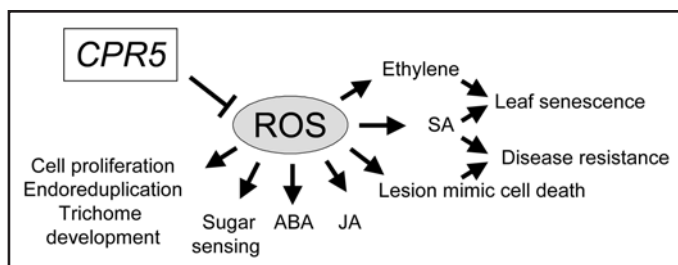


Figure 1. A diagram showing CPR5 as a master regulator of cellular ROS status and signalling.

responses to plant hormones such as auxin, ethylene, gibberellic acids, and ABA as well as cell death.^{14,15} We hence propose that CPR5 is a master regulator of cellular ROS status and/or signalling, which has close and complex interactions with other signalling networks to control cell proliferation, endoreduplication and trichome development, responses to ethylene, sugar, jasmonic acid and ABA, cell death and senescence as well as disease resistance (Fig. 1).

CPR5 Mode of Action: An Open Question

Arabidopsis CPR5 encodes a protein containing an amino terminus bipartite nuclear localisation signal and five transmembrane domains at the carboxy terminus. Blast search of currently available genome and protein databases identified putative *CPR5* homologues in higher plants as well as in mosses (*Physcomitrella patens*) which separated from plants 400 million years ago. Thus, *CPR5* is an ancient, plant unique gene.

The *CPR5* transcript is constitutively expressed throughout the plant and increases levels at late development.^{1,4,5} The amino acid sequence of CPR5 does not resemble any proteins of known function but its structure resembles several proteins in animals and bacteria.¹⁶⁻²⁰ Cellular localisation of CPR5 needs to be solved in order to accurately dissect how it acts in a plant cell. It is also necessary to identify the immediate interaction partners of CPR5 and domain swapping and chopping experiments should provide clues on the functional domains of CPR5. Knock-down/overexpression of *CPR5* in a developmental manner using inducible promoters may show how CPR5 changes functions during development.

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