

HIGH-THROUGHPUT SCREENING OF CYTOCHROME C NOVEL PARTNERS IN CHLAMYDOMONAS REINHARTII

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Cytochrome *c* (*Cc*) has been described in mammals as a bifunctional protein, thus playing a double role in cell respiration as an electron carrier and in apoptosis as a triggering agent of the caspase-dependent apoptotic route [1]. In plants and green algae, the role of *Cc* in response to oxidative stress, or programmed cell death (PCD), is still unknown [2,3]. To identify novel targets of *Cc* in photosynthetic organisms, we have chosen the unicellular and flagellated alga *Chlamydomonas reinhardtii* as a simple model system. We have designed a proteomic approach starting with an affinity chromatography step, in which *Chlamydomonas Cc* is covalently bound to thiol-Sepharose 4B [4]. First, the protein extracts from the algal cell cultures grown under normal or oxidative stress conditions were loaded into the affinity column. Further, the proteins purified by such affinity chromatography were analyzed by the Linear Trap Quadrupole (LTQ) technique so as to identify novel proteins interacting with *Cc*. Currently, the physiological relevance of such potential *Cc* targets are being confirmed *in vivo* by using the bimolecular fluorescence complementation assay (BiFC) [5,6].

[1] Rodríguez-Roldán *et al.* A comparative kinetic analysis of the reactivity of plant, horse and human respiratory cytochrome *c* towards cytochrome *c* oxidase (2006) *Biochem. Biophys. Res. Commun.*

[2] Sanmartín M *et al.* Caspases. Regulating death since the origin of life (2005) *Plant Physiol.*

[3] Zuppin A *et al.* Programmed cell death and adaptation: two different types of abiotic stress response in a unicellular chlorophyte (2010) *Plant Cell. Physiol.*

[4] Azzi A *et al.* Affinity chromatography purification of cytochrome *c* binding enzymes (1982) *Proc. Natl. Acad. Sci. USA*

[5] Fujii *et al.* In vitro reconstitution of an abscisic acid signalling pathway (2009) *Nature*

[6] Gandia *et al.* Detection of higher-order G protein-coupled receptor oligomers by a combined BRET–BiFC technique (2008) *FEBS Lett.*