AC-DCC

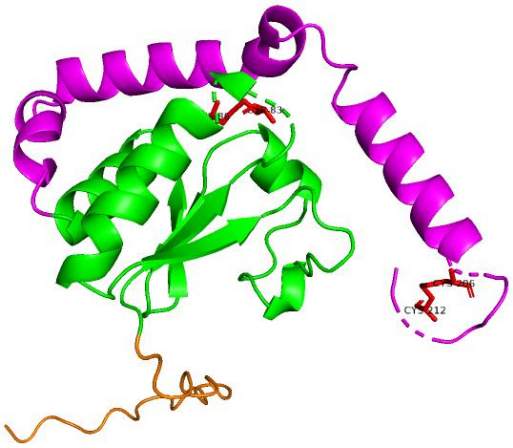

Figure 1. *Structure tridimensionnelle de la forme mature de la protéine DCC1 provenant de la plante terrestre Arabidopsis thaliana. En orange, l'extrémité N-terminale ; en vert, le domaine « thioredoxin-like » en en magenta, l'extension C-terminale. Les 4 cystéines sont colorées en rouge.*

Attribute a role to the Conserved and unassigned DCC proteins

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LabEx partners: ASIA

Thematic action concerned: WP1

Context **—**
Photosynthesis and respiration are two essential metabolic pathways in photosynthetic organisms controlling their energy balance. Like most metabolic processes, photosynthesis and respiration are finely regulated by reduction/oxidation (redox) mechanisms. However, among the hundreds of proteins regulated by redox exchanges, a large number of proteins have no assigned functions to date. In proteins, the combination of a CXXC motif (two reactive cysteines separated by two other amino acids X) and a thioredoxin folding characteristic of thiol:disulfide oxidoreductases is particularly suited to dithiol-disulfide exchange reactions. The presence of a CXXC motif in proteins with unknown functions and predicted to be located in the chloroplast or the mitochondria therefore constitutes an interesting observation for a possible involvement in redox metabolism.

Objectives **—**
Among the proteins of unknown function in *Populus trichocarpa*, we identified a small family of three proteins that we named DCC1-3, due to the presence of a conserved DXXCXXC motif, and which possess a potential sequence for the chloroplast or the mitochondria. The AC-DCC project aims to carry out a biochemical study of DCC proteins with the aim of characterizing new pathways with redox activity in plants.

Approaches **—**
In order to characterize the role of DCC proteins, a three-step approach has been initiated. The aim is to produce recombinant proteins corresponding to the mature forms of DCC proteins in order to undertake their biochemical and structural characterization. Then, the isolation of the protein partners of the DCCs followed by the characterization of in vitro and in vivo interactions will make it possible to define the protein interaction network of the DCCs.

Key results —
- DCC1 exhibits a weak reductase activity but no oxidase activity was detected.
- Two other forms of the protein have been produced and purified: the "thioredoxin-like" (TRX-like) domain alone (see Fig.1, in green) and the TRX-like domain with the N-terminus (in green and orange in Fig. 1 respectively).
- The purified “thioredoxin-like” domain alone exhibits the highest reductase activity of the three forms of DCC1 obtained.
- The three forms obtained are oxidized by two physiological oxidizing agents (H_2O_2 and GSNO) and this oxidation is reversible thanks to the action of physiological reducing systems.

Main conclusions including key discussion points —
Biochemical analysis of the mature form of DCC1 shows that it is sensitive to two oxidizing agents and that this oxidation is reversible by thioredoxin and glutaredoxin systems. Nevertheless, the functional analysis did not make it possible to assign a function to it. Thus, two other forms of the protein were produced and purified. One contains only the characteristic thioredoxin fold and the second contains the thioredoxin fold with the N-terminus extension. The results show that the TRX-like domain alone exhibits reductase activity. However, in the presence of the N-terminus, this activity decreases.

Perspectives —
The TRX-like domain of DCC1 would have a reductase activity but the N-terminal end of the protein influences this activity. In order to better understand the role of the predicted as unstructured N-terminal part of DCC1, functional analyses should be pursued. Analysis of the DCC1 interaction network should help to understand the place of DCC1 in plant metabolism. For this, a protein-protein interaction approach (i.e: Turbo-ID) is planned in order to identify DCC1 protein partners.

Valorization —
The results obtained were published in a special issue of "Free radical biology and medicine" following the selection of the abstract for a talk at the "Redox Biology Congress" conference in August 2022 in Ghent (Belgium). The results were also presented on the occasion of the "doc-postdoc day" of the UMR1136 IAM in the form of a poster in September 2022 by Natacha Donnay, the doctoral student recruited.

Leveraging effect of the project —
Through the AC-DCC project, a collaboration with Dr. Hakim Mireau (IJPB, Versailles) was undertaken with the aim of establishing the DCC1 interaction network.